THE CHALLENGE POSED BY NEW SYNTHETIC OPIOIDS: PHARMACOLOGY AND TOXICOLOGY

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THE CHALLENGE POSED BY NEW SYNTHETIC OPIOIDS: PHARMACOLOGY AND TOXICOLOGY

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New Synthetic Opioids (NSOs), most of which are illegally produced and sold for recreational use, are posing a serious threat to the health of consumers. Due to the low cost of materials and equipment required for clandestine laboratories production with respect to the production cost of heroin, NSOs are climbing the illegal street and web drug market. Several of these drugs have been involved in a recent rise in acute intoxications and overdose deaths. Since NSOs offer enormous profit potential, and there is strong demand for their use, these drugs are being trafficked by organized crime and present major challenges for medical professionals facing intoxications and fatalities, law enforcement agencies fighting against their diffusion and policymakers trying to restrain the use and abuse of NSOs.

This Research Topic aimed to fill the gap on current knowledge on pharmacology and toxicology, health risks for adult and newborns of NSOs covering both basic scientific as well as epidemiological and clinical aspects. 3 reviews, 3 mini-reviews, 1 original article, 2 case reports and 1 opinion are here presented.

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EDITORIAL
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Editorial: The Challenge Posed by New Synthetic Opioids: Pharmacology and Toxicology

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Editorial on the Research Topic

The Challenge Posed by New Synthetic Opioids: Pharmacology and Toxicology

Diverted prescription opioid analgesics (e.g., oxycodone, hydrocodone, hydromorphone), failed opioid drug candidates (e.g., benzamide derivatives), and various legal and illegal fentanyl analogs (e.g., acetyl fentanyl, furanylfentanyl, carfentanil) constitute the class of New Synthetic Opioids (NSOs), which is currently posing a global public health threat (Pichini et al., 2018).

Due to the low cost of materials and equipment required for clandestine laboratory production and enormous profit potential, NSOs are establishing a strong position on the illegal drug market as stand-alone products, adulterants in heroin, or constituents of counterfeit prescription medications. Recently, NSOs have been involved in a significant spike of acute intoxications (classic opioid toxidrome) and overdose deaths in North America, challenging healthcare professionals, law enforcement agencies fighting against their diffusion, and policymakers trying to restrain their use (Marchei et al., 2018; Busardò et al., 2019).

Since there is little information available regarding the pharmacology and the toxicology of NSOs in abuse settings, the main purpose of this Research Topic was to fill the current knowledge gap. The topic covers basic scientific, epidemiological, and clinical aspects of NSOs and includes 3 reviews, 3 mini-reviews, 1 original article, 2 case reports, and 1 opinion.

The Research Topic begins with the opinion of Pichini et al. on the health risks entailed in the emergence of illicit fentanyl mixes onto the European drug market, following the recent spike in overdose deaths in North America. To fight against this incoming threat, the authors advocated for the improvement of epidemiological surveillance and data sharing through National and International Early Warning systems and various communication platforms, and the publication of analytical methodologies for the identification of fentanyl analogs and metabolites in ante- and post-mortem cases.

Indeed, Schifano et al. demonstrated that the occurrences of fentanyl misuse, abuse, dependence, and withdrawal-related adverse drug reactions increased over time on international databases, with the most represented adverse reactions being "drug dependence," "intentional product misuse," and "drug abuse" with most cases involving adult males and the concomitant use of other prescribing/illicit drugs.

This latter occurrence was addressed by Pérez-Mañá et al. who reviewed drug-drug interactions with NSOs through pharmacokinetic and pharmacodynamic mechanisms, and discussed the role of naloxone, an opioid receptor antagonist, as an antidote to the NSO toxidrome. The authors recommended that medical doctors prescribing potentially abused opioids should be aware of

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the life-threatening risks induced by drug-drug interactions with NSOs to prevent new cases of intoxication.

With respect to fatalities caused by fentanyl and derivatives (e.g., acetyl fentanyl, butyryl fentanyl, carfentanil, furanyl fentanyl) and non-traditional opioid agonists (e.g., AH-7921, MT-45, U-47700), Concheiro et al. reviewed the current data available on the post-mortem toxicology of synthetic opioids and their chemical and pharmacological properties. The review includes pharmacokinetic parameters (metabolism), post-mortem redistribution, and stability studies in post-mortem samples.

Two single post-mortem cases were then reported. In the first case, Cannaert et al. described a novel *in vitro* opioid activity reporter assay based on μ-opioid receptor activity and a sensitive bioanalytical method for the determination of carfentanil in a fatal intoxication, reporting the highest carfentanil concentrations in a post-mortem case: 92 ng/mL in whole blood, 2.8 ng/mL in urine, and 23 ng/mL in vitreous humor. In the second case involving a man with previous history of drug addiction, Gerace et al. detected U-47700, a strong μ-opioid agonist with a 7.5-fold higher potency than that of morphine, in blood (380 ng/mL), urine (10,400 ng/mL), and pubic hair (5.7 ng/mg) using a new ultra-performance liquid chromatography tandem mass spectrometry method.

Wilde et al. reviewed the metabolic profiles and pharmacological potencies of new fentanyl analogs. Since only limited to no information on the metabolism of fentanyl analogs is available, the authors hypothesized and anticipated the metabolism of new compounds taking into consideration the well-characterized metabolism of pharmaceutically or illicitly used analogs, which generally involves phase I reactions such as hydrolysis, hydroxylation (and further oxidation steps), N- and O-dealkylation, and O-methylation and phase II metabolic reactions such as glucuronide or sulfate conjugation.

Solimini et al. reviewed the available information on the pharmacological properties of non-fentanyl NSOs, including U-47700, U-49900, AH-7921, and MT-45, providing a better understanding of these compounds, particularly on the toxicity and dangerous adverse effects in users.

Further with respect to non-fentanyl derived NSOs, Cardia et al. summarized the pre-clinical and clinical characteristics of hydrocodone. Pharmacokinetic aspects (terminal half-life, maximum serum concentration, and time to maximum serum concentration) and the influence of metabolic genetic polymorphism in analgesic response to the drug has been illustrated and discussed.

Finally, Gilardi et al. reported the pre-clinical and clinical findings on the implications of parental exposure to NSOs for their offspring. The authors concluded that in utero exposure to opioids has an impact on the neuronal development of the offspring with long-term potentially transmissible repercussions. Additionally, they reported that opioid use before conception also influences the reactivity to opioids of the progeny and the subsequent generations, likely through epigenetic mechanisms.

In conclusion, this research topic provides updated studies and reviews concerning the pharmacology and the toxicology of NSOs as an eye opener of this incoming hazard to scientists and health professionals operating in the field of psychotropic drugs.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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OPINION
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European Drug Users at Risk from Illicit Fentanyls Mix

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Keywords: fentanyl derivatives, intoxications, fatalities, new synthetic opioids, health threat

The increase of overdose deaths involving licit and illicit fentanyl analogs (FAs), recently observed in North America, has shifted toward Europe posing a serious public health menace (Mounteney et al., 2015).

The widespread appearance of these synthetic opioids, between 50 and 100 times more potent than morphine and in many cases not approved for medical use, has been reported by the European Monitoring Centre for Drugs and Drug Addiction (European Drug Report, 2017)[1].

Indeed, in the last few years a number of FAs, (e.g., despropionyl-2-fluorofentanyl, furanylfentanyl, valerylfentanyl, acryloylfentanyl, carfentanyl, butyrfentanyls) has appeared for the first time on the European illicit market having caused more than 100 fatalities, when used alone or in association to other drugs (UNODC, 2017).

Due to the low cost of the required materials and equipment for producing these compounds in clandestine laboratories inside and outside Europe, they are sold by drug dealers in place of heroin or mixed with it as cutting agents. In this context, the possibility of fatal overdoses is extremely high because of the narrow range between a safe and a lethal dose and the manufacturing of quantitatively inaccurate and contaminated products (UNODC, 2017).

Current evidence suggests that both FAs availability on the illicit market and related acute and lethal intoxications are indisputably underestimated because of the analytical challenges caused by the structural difficulties in identifying unscheduled compounds. Often, overdoses caused by novel synthetic opioids are not fully investigated, difficult to report according to ICD 10 (especially in the way to distinguish different fentanyl analogs) and are simply classified as "heroin-related fatalities" (Swanson et al., 2017). Additional challenge could be also that the range of FAs being used is continuously changing and for health professionals, forensic toxicologists, pathologists, epidemiologists and lawmakers it is difficult to be up to date. Recently, Frank and Pollack commented on the threat to public health caused by widespread use of illegal fentanyl in US with the doubling of overdose deaths involving this drug (Frank and Pollack, 2017). The authors suggested to primarily implement policies of "harm reduction" redirecting user demand away from products containing fentanyl, reducing unintentional fentanyl consumption, increasing penalties on illegal fentanyl distributors and sellers and finally providing timely availability of naloxone and medication-assisted therapy. In this concern, there is a suggestion by health professionals to increase naloxone doses in case of overdoses by fentanyl analogs.

In addition to these recommendations, we wish to draw the attention of the whole scientific community on the importance of improving surveillance and data sharing of FAs through National and International Early Warning Systems (EWS) set up throughout Europe and a standardization in reporting FAs related deaths. Analytical methodologies for the identification of fentanyl analogs and eventual metabolites in ante mortem and post mortem cases should be developed, validated, and analytical data shared through different communication platforms (e.g., EWS and Europol).

[1]http://www.emcdda.europa.eu/edr2017 (last accessed July 13, 2017).

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We advocate for the correct identification of the phenomenon to implement contextual strategies in order to restrain the use and abuse of FAs and stop this incoming threat for health of European drug users.

AUTHOR CONTRIBUTIONS

FPB prepared the first daft of this opinion paper together with the other three coauthors, revising the existing literature and approved the last draft of the paper. SP, RP, and EM revised the existing literature concerning fentanyls, prepared the first draft of the opinion with FPB and approved the final version.

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CASE REPORT
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Activity-Based Detection and Bioanalytical Confirmation of a Fatal Carfentanil Intoxication

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Carfentanil, one of the most potent opioids known, has recently been reported as a contaminant in street heroin in the United States and Europe, and is associated with an increased number of life-threatening emergency department admissions and deaths. Here, we report on the application of a novel *in vitro* opioid activity reporter assay and a sensitive bioanalytical assay in the context of a fatal carfentanil intoxication, revealing the highest carfentanil concentrations reported until now. A 21-year-old male was found dead at home with a note stating that he had taken carfentanil with suicidal intentions. A foil bag and plastic bag labeled "C.50" were found at the scene. These bags were similar to a sample obtained by the Belgian Early Warning System on Drugs from a German darknet shop and to those found in the context of a fatality in Norway. Blood, urine and vitreous, obtained during autopsy, were screened with a newly developed *in vitro* opioid activity reporter assay able to detect compounds based on their μ-opioid receptor activity rather than their chemical structure. All extracts showed strong opioid activity. Results were confirmed by a bioanalytical assay, which revealed extremely high concentrations for carfentanil and norcarfentanil. It should be noted that carfentanil concentrations are typically in pg/mL, but here they were 92 ng/mL in blood, 2.8 ng/mL in urine, and 23 ng/mL in vitreous. The blood and vitreous contained 0.532 and 0.300 ng/mL norcarfentanil, respectively. No norcarfentanil was detected in urine. This is the first report where a novel activity-based opioid screening assay was successfully deployed in a forensic case. Confirmation and quantification using a validated bioanalytical procedure revealed the, to our knowledge, highest carfentanil concentrations reported in humans so far.

Keywords: synthetic opioids, untargeted screening, activity-based, bioassay, carfentanil, LC–MS/MS

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INTRODUCTION

Carfentanil, a very potent derivative of the pharmaceutical opioid fentanyl, was developed in 1974 by Janssen Pharmaceutica (Van Bever et al., 1976). It is one of the most potent opioids known at ∼10,000 times the potency of morphine and ∼30–100 times the potency of fentanyl in the tail withdrawal test in rats (Van Bever et al., 1976). Commercially, it is always sold in combination with the μ-opioid antagonist naloxone due to its extreme toxicity in humans. Carfentanil is used to immobilize large exotic wildlife and has been implicated in the 2002 Moscow theater hostage

crisis (Wax et al., 2003; Riches et al., 2012). Recently, carfentanil and other synthetic opioids have been reported as a contaminant in street heroin in the United States and Europe, and have been associated with an increased number of life-threatening emergency department admissions and deaths (EMCDDA and Europol, 2017; Papsun et al., 2017; Shanks and Behonick, 2017; Shulman et al., 2017). Here, we report on the application of a novel cell-based bioassay and a sensitive bioanalytical assay in the context of a fatal carfentanil intoxication, in which we found the highest carfentanil concentrations reported until now.

CASE PRESENTATION

A 21-year-old male was found dead at home along with a note stating that he had taken carfentanil with suicidal intentions, in addition notifying first responders that care should be taken, given the potency of the compound. A foil bag and plastic bag labeled "C.50" were found at the scene (**Figure 1A**), suggesting that up to 50 mg of carfentanil may have been insufflated by the decedent. Remarkably, during routine monitoring of new psychoactive substances (NPSs) present on darknet websites by the Belgian Early Warning System on Drugs, a carfentanil sample was obtained with strikingly similar packaging and handwriting as the packaging found on the scene of death in this toxicological case (**Figure 1B**). A similar bag with identical labeling in similar handwriting has also been reported in the context of a fatality in Norway (**Figure 1C**), where the powder was apparently ordered from a German darknet shop (Vevelstad and Drange, 2017). Based on this information, the vendor (or primary source) is most probably the same vendor as mentioned in other publications (Marlin and Hoyte, 2017; Vevelstad and Drange, 2017).

A swab of the plastic bag tested positive for carfentanil via GC–MS analysis. Biological matrices available were blood, urine and vitreous. Routine toxicological analyses were performed on peripheral blood and urine. This involved, in addition to immunological screening by EMIT and ELISA, the use of HPLC-diode-array detection (DAD) and GC–MS for screening and quantification of drugs and headspace-GC-FID for the determination of ethanol and other volatile compounds, essentially following procedures described before (Stove et al., 2013). GC–MS screening of blood and urine revealed the presence of caffeine, theobromine, propranolol, sertraline, and cannabinoids in non-toxic doses. Immuno-assay based screening for fentanyl (Fentanyl Direct Elisa Kit, Immunalysis, Pomona, CA, United States) was negative.

An additional opioid screening of the biological matrices was done with a new in-house developed opioid activity reporter assay. We recently reported on cell-based cannabinoid reporter assays for the activity-based detection of synthetic cannabinoids and their metabolites, demonstrating cannabinoid activity in authentic urine and blood samples (Cannaert et al., 2017). A similar bioassay using the μ-opioid receptor to screen for opioid activity in bulk materials and biological samples was set up and evaluated (Cannaert et al., 2018). The principle of the bioassay is activity-based, using an *in vitro* cell system, in which activation of the μ-opioid receptor leads to the recruitment of the cytosolic β-arrestin 2 (βarr2) protein, which results in functional complementation of a split NanoLuc luciferase, thereby restoring luciferase activity. In the presence of the substrate furimazine, this results in a bioluminescent signal, which can be read out with a standard luminometer.

In practice, expression vectors encoding human μ-opioid receptor or βarr2, fused via a flexible linker to the subunits of NanoLuc luciferase (LgBiT or SmBiT), were generated using standard molecular biology techniques, similar as in Cannaert et al. (2016). These constructs, with addition of a G-protein coupled receptor kinase 2, were used to transiently transfect human embryonic kidney (HEK) 293T cells, which were seeded in poly-D-lysine-coated 96-well plates at 5×10^4 cells/well and incubated overnight before performing the assay. On the day of the assay, the cells were washed twice with Opti-MEM® I reduced serum medium to remove any remaining fetal bovine serum, and 90 μL of Opti-MEM® I was added. The Nano-Glo Live Cell reagent, a non-lytic detection reagent containing the cell-permeable furimazine substrate, was prepared by diluting the Nano-Glo Live Cell substrate 20× using Nano-Glo LCS Dilution buffer, and 25 μL was added to each well. Subsequently, the plate was placed in a GloMAX96 plate reader (Promega, Madison, WI, United States). Luminescence was monitored during the equilibration period until the signal stabilized (30 min). For agonist experiments, we added 20 μl per well of test compounds, present as 6.75× stocks in Opti-MEM® I. Also for the analysis of biological extracts, 20 μL was added per well. These extracts were generated from 250 μL of matrix (blood, urine, or vitreous), which was added to 1000 μL of ice-cold acetonitrile, followed by shaking for 5 min at 1400 RPM and centrifuging for 20 min at 20,000 *g*. After evaporation of 1 mL of supernatant under nitrogen at 40°C, the extract was reconstituted in 100 μl of Opti-MEM® I. The luminescence was continuously detected (105 or 120 min).

Application of carfentanil and fentanyl solutions on the opioid activity reporter assay resulted in concentration-dependent curves and EC_{50} (95% confidence interval profile likelihood) values were determined for carfentanil [EC_{50} = 0.027 nM (0.021–0.035)] and fentanyl [EC_{50} = 4.32 nM (2.43–7.83)] as a measure of relative potency (**Figure 2A**). Although it is difficult to compare EC_{50} values from different assays (due to different experimental setups), our values are in line with those found in literature. Feasel (2017) stated in his dissertation an EC_{50} of 0.006 nM for carfentanil and 0.511 nM for fentanyl (PerkinElmer® LANCE Ultra cAMP Assay), which supports the significantly stronger potency of carfetanil, as also found here. Norcarfentanil, the major metabolite of carfentanil, was only able to generate low opioid activity at a high concentration (1 μM/326 ng/mL) (**Figure 2A**). All extracts from the three matrices (blood, urine, and vitreous) showed very strong opioid activity. Even application of 1 μL of urine sample from the presented case (without any sample preparation) on the bioassay was able to generate a clearly positive signal, easily distinguishable from negative control blank urine, in the opioid activity reporter assay (**Figure 2B**).

The screening results from the opioid activity reporter assay were confirmed with an LC–MS/MS method for carfentanil and

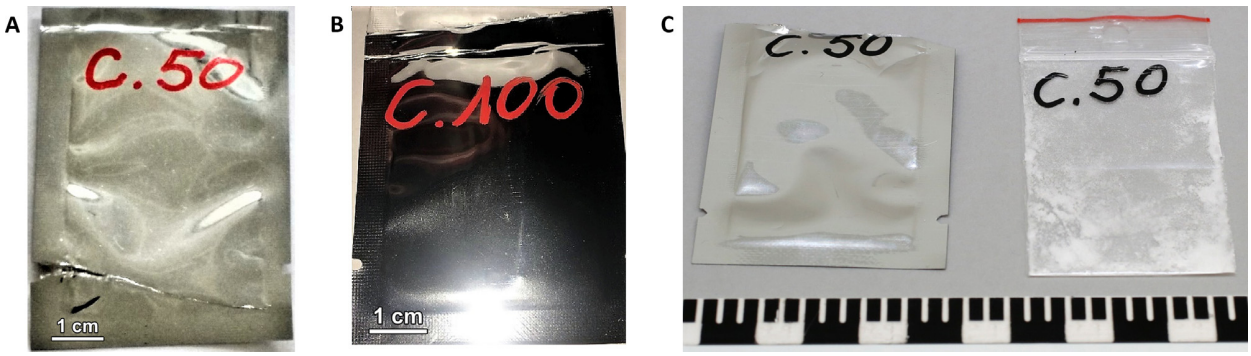

FIGURE 1 | (A) Foil bag found at the scene. **(B)** Foil bag obtained by the Belgian Early Warning System on Drugs. **(C)** Foil bag and plastic bag found in a fatality in Norway [image used with kind permission of the National Criminal Investigation Service/Photo (Norway)] (Vevelstad and Drange, 2017).

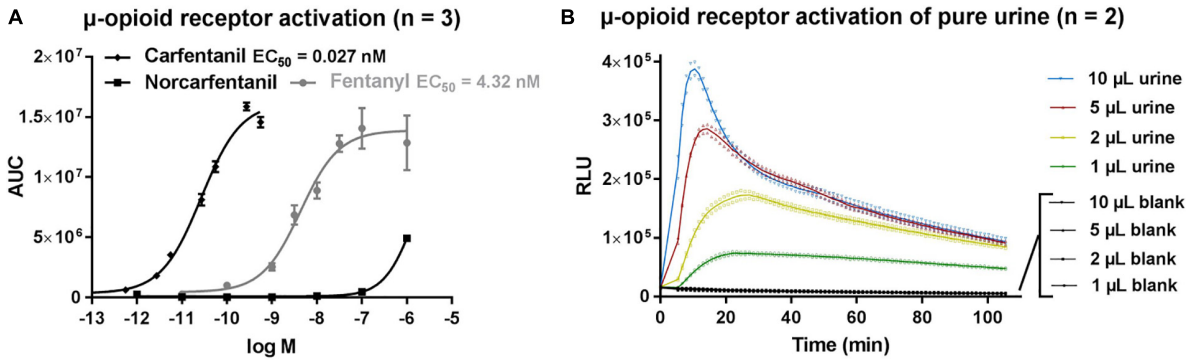

FIGURE 2 | (A) μ-opioid receptor activation by fentanyl, carfentanil and norcarfentanil. **(B)** μ-opioid receptor activation of pure urine without sample preparation. AUC, area under curve; RLU, relative light units.

norcarfentanil. To 250 μL sample (blood, urine, or vitreous), 10 μL of internal standard solution containing fentanyl-D_5 and norcarfentanil-D_5 (0.25 and 12.5 ng/mL, respectively) in methanol were added. Sample processing was as described above, except that reconstitution was with 55 μL acetonitrile, of which 50 μL were then mixed with 50 μL of mobile phase A (H_2O + 0.1% HCOOH) in an autosampler vial with 100 μL insert. For the analysis of carfentanil, the injection volume was 20 μL, whereas for the determination of norcarfentanil, 10 μL were injected. Chromatographic separation was achieved on a Kinetex Biphenyl column (50 mm × 2.1 mm, 2.6 μm) (Phenomenex, Utrecht, Netherlands) in a 3.7 min gradient using H_2O + 0.1% HCOOH and methanol + 0.1% HCOOH as mobile phases, at a flow rate of 0.6 mL/min. The following gradient was used: 0–0.2 min: 5%B, 0.25–0.35 min: 5–30% B, 0.35–1.5 min: 30–95% B, 1.5–2.5 min: 95% B, 2.5–2.51 min: 95–5% B, 2.51–3.7 min: 5% B. A QTRAP 5500 mass spectrometer (SCIEX, Nieuwerkerk aan den Ijssel, Netherlands) with positive electrospray ionization in multiple reaction monitoring mode was used for detection. For carfentanil, the following transitions were used: 395.2 > 246.1 [quantifier, declustering potential (DP): 70 V, collision energy (CE): 27 eV, collision cell exit potential (CXP): 12 V] and 395.2 > 146.2 (qualifier, DP: 70 V, CE: 37 eV, CXP: 9 V). For norcarfentanil, the transitions were 291.1 > 142.2 (quantifier, DP: 74 V, CE: 22 eV, CXP: 7 V) and 291.1 > 146.2 (qualifier, DP: 74 V, CE: 37 eV, CXP: 10 V). For fentanyl-D_5, 342.2 > 188.2 (DP: 110 V, CE: 32 eV, CXP: 10 V) was used. For norcarfentanil-D_5, the transition was 296.1 > 151.1 (DP: 75 V, CE: 38 eV, CXP: 8 V). The entrance potential was 10 V for all transitions; source temperature was set to 600°C, ion spray voltage to 2000 V, curtain gas to 35 psi, gas 1 to 40 psi and gas 2 to 50 psi.

The method was validated in whole blood. Eight-point calibration curves were set up for carfentanil (range: 0.0025–2.5 ng/mL, linear regression with $1/x^2$ weighting) and norcarfentanil (range: 0.025–25 ng/mL, linear regression with $1/x^2$ weighting). Quality control samples at 0.015/0.25 ng/mL for carfentanil and at 0.15/2.5 ng/mL for norcarfentanil were run in sixplicate on 4 days, yielding acceptable intra- and inter-run imprecision (intra-run: <8.8%, inter-run: <14%) and bias (< ±8.7%, n = 24 at two different concentrations). Matrix effects were assessed at the two above-mentioned concentrations by comparing the signal ratios of analyte to internal standard of post-extraction-spiked samples with those of standards spiked in neat injection solvent (n = 6). Matrix effects were 78% for carfentanil and 118% for norcarfentanil. Extraction efficiency, assessed by comparing the signal ratios of analyte to internal standard of pre- versus post-extraction-spiked samples, was 66% for carfentanil and 24% for norcarfentanil (n = 6, at the two

above-mentioned concentrations). Also, autosampler stability (change in concentration <9% for at least 3 days, n = 6, two different concentrations), specificity and carry-over (none within calibration range) were successfully evaluated. Dilution integrity was checked by spiking blood and aqueous samples with 100 ng/mL carfentanil and norcarfentanil, then diluting 1:1000 with blank matrix (n = 6) and comparing relative peak areas to control samples with 0.1 ng/mL (n = 6). Differences were $\leq \pm 13.5\%$.

The vitreous sample was quantified using a calibration curve in ultra-pure water. The urine sample was quantified by standard addition. To quantify carfentanil concentrations, blood and vitreous samples had to be diluted 1:1000 with blank blood and water, respectively, while the urine sample was diluted 1:100 with blank urine. For norcarfentanil, undiluted samples were analyzed. Carfentanil concentrations were 92 ng/mL in blood, 2.8 ng/mL in urine, and 23 ng/mL in vitreous. The blood and vitreous contained 0.532 and 0.300 ng/mL norcarfentanil, respectively. No norcarfentanil was detected in urine. It should be noted that carfentanil concentrations are typically in the sub-ng/mL range (Papsun et al., 2017: 0.1–14 ng/mL, median: 0.38 ng/mL; Shanks and Behonick, 2017: 0.0102–2 ng/mL, median: 0.0984 ng/mL; Hikin et al., 2018: 0.09–4 ng/mL, median: 0.234 ng/mL).

DISCUSSION

Given the continued emergence of novel synthetic opioids, the major disadvantage for their detection via immunoassays, GC–MS and LC–MS/MS analysis is that the methods are often targeted in nature or, for the latter two, limited by the availability of pre-established mass spectral libraries. Here in this case, the immunoassay for fentanyl did not pick up carfentanil, a fentanyl analog, due to the lack of cross-reactivity. Therefore, an alternative untargeted approach for the detection of (synthetic) opioids, not directly based on the structure of the opioids, but on their opioid activity, was applied. Such an approach may serve as a first-line screening tool, complementing the conventional analytical methods which are currently used.

The high ratio of carfentanil/norcarfentanil in blood and vitreous and the absence of norcarfentanil in urine can be explained by the presumably sudden death of the victim caused by the massive overdose. The detected concentrations of carfentanil are, to the best of our knowledge, the highest ever reported in a human being. Other intoxications always state sub-ng to low ng/mL levels of carfentanil (Müller et al., 2017; Papsun et al., 2017; Shanks and Behonick, 2017; Swanson et al., 2017; Elliott and Hernandez Lopez, 2018; Hikin et al., 2018). In conclusion, this is the first report in which a novel activity-based opioid screening assay was successfully deployed in a forensic case, where confirmation and quantification using a validated bioanalytical procedure revealed very high carfentanil concentrations.

ETHICS STATEMENT

We received permission from the Belgian Department of Justice to use the samples for this study.

AUTHOR CONTRIBUTIONS

AC was involved in the development and application of the bioassay and wrote the manuscript. LA worked on the development and validation of the LC–MS/MS method and wrote the manuscript. PB provided the carfentanil standard, gave additional information concerning the carfentanil package found at the scene, and checked the final version of the manuscript. CS was the forensic toxicologist in charge of the case and wrote the manuscript.

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frontiers in Pharmacology

MINI REVIEW
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Pharmacotoxicology of Non-fentanyl Derived New Synthetic Opioids

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A class of opioid agonists not structurally related to fentanyl, derived from research publications of pharmaceutical companies or patents within the United States and abroad are contributing to the current opioid epidemic. Novel synthetic opioids (NSOs) created to circumvent drug control laws such as U-47700, U-49900, AH-7921, or MT-45 have no recognized therapeutic use, are clandestinely manufactured and sold on conventional or dark web. We herein provide a review of the pharmacological properties available on most of these substances trying to provide a better knowledge on these compounds, particularly with respect to toxicity and dangerous adverse effects in users. Indeed, these NSOs share not only a great potency of action and receptor affinity with respect to natural or synthetic opiates (e.g., morphine, heroin, and methadone) but also a non-negligible toxicity leading to intoxications and fatalities, posing a serious harm to public health and society.

Keywords: novel synthetic opioids, U-47700, U-49900, AH-7921, MT-45, toxicity, health threat

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INTRODUCTION

Non-fentanyl derived novel synthetic opioids (NSOs) have initially emerged worldwide as non-illegal drugs diffused to replace heroin and thus circumvent prohibition laws, resulting in numerous abuse reports and overdose cases, especially across United States and Europe (Carroll et al., 2012; Armenian et al., 2017b; Baumann et al., 2017; Fabregat-Safont et al., 2017).

These NSOs are a broad family of analgesics and anesthetics, mainly synthesized in the 1970s, acting at the mu (μ) opioid receptor, but also at the delta (δ) and kappa (κ) ones. The power of physiological and psychological effects is different according to the specific synthetic opioid being used and the type of receptor that is activated or inhibited (Baumann et al., 2017; European Monitoring Centre for Drugs, and Drug Addiction [EMCDDA], 2017).

To precisely define this particular class of NSOs, it is worth mentioning that the alkaloid compounds naturally found in the opium poppy plant are defined opiates and include, among others, morphine, codeine, and thebaine as principal alkaloids.

Instead, substances such as hydromorphone, oxymorphone, and heroin are semisynthetic opioids made from morphine with pharmacological properties similar to those of opiates and affinity for one of the 7-transmembrane G protein-coupled opioid receptors (Raffa et al., 2018).

All the above reported opioids belong to the phenanthrene family, while the family of benzomorphans include, e.g., pentazocine phenazocine, dezocine, and eptazocine, developed through the modification of the basic phenanthrene structure of morphine (Cittern et al., 1986). Conversely, methadone is a phenylheptylamine agent whereas meperidine is a phenylpiperidine derivative (Knapp, 2002; Raffa et al., 2018).

The family of phenylpiperidines (characterized by a phenyl moiety directly linked to a piperidine) includes also the NSO fentanyl (synthesized by P. Janssen in the 1960s) and its analogs, up to 1000 times more potent as analgesic than meperidine and differing in structure from the latter for a phenethyl group on the piperidine nitrogen in place of a methyl group (Elbaridi et al., 2017; Raffa et al., 2018).

While extensive literature has been published in regards to pharmacology and toxicology of fentanyl and its illicit analogs (Bäckberg et al., 2015; Mounteney et al., 2015; Dwyer et al., 2017; Giorgetti et al., 2017; Guerrieri et al., 2017; Helander et al., 2017a; Pichini et al., 2017a,b; Shoff et al., 2017; Suzuki and El-Haddad, 2017), the pharmacological and toxicological properties of non-fentanyl derived NSOs have not yet been reviewed in detail.

Compounds such as U-47700, U-51754, U-49900, U-448800, AH-7921 from the chemical family of benzamide, U-50488 and U-51754 from the acetamide family and MT-45 from the piperazine family are the NSOs most recently reported as health threats for opioids consumers (Mohr et al., 2016; Amin et al., 2017; Baumann et al., 2017; Domanski et al., 2017; Fabregat-Safont et al., 2017; Prekupec et al., 2017; Marchei et al., 2018). Indeed, this new generation of derivatives has been involved in a number of recent overdose deaths worldwide (Drug Enforcement Administration [DEA], 2016; Baumann et al., 2017; Domanski et al., 2017; Fabregat-Safont et al., 2017).

Clandestine manufacturing of NSOs has been pirated from scientific literature or patent filings published by pharmaceutical companies attempting to search for new therapeutic drugs without addiction-related adverse effects (Logan et al., 2017).

In a similar manner to fentanyl derivatives, these NSOs are being partly used as heroin adulterants or as constituents of counterfeit pain pills and they can be bought directly by users from online vendors via conventional web or cryptomarket (European Monitoring Centre for Drugs, and Drug Addiction [EMCDDA], 2016; Armenian et al., 2017b; Baumann et al., 2017; Van Hout and Hearne, 2017).

Similarly to morphine and heroin (opiates) or to semi-synthetic opioids (like hydro- and oxycodone, hydro- and oxymorphone), these compounds produce CNS depressants effects such as respiratory depression, analgesia, hypothermia, sedation, euphoria, anxiety, sweating, disorientation, drowsiness, nausea, and miosis (Carroll et al., 2012; Guerrini et al., 2013; Hill and Thomas, 2016; Armenian et al., 2017b), and although the effects of tolerance and dependence may rapidly reach high levels, elevated risks of overdose and death are frequent for these compounds (United Nations Office on Drugs and Crime [UNODC], 2017b). Furthermore, the typical rewarding characteristics and the easy availability induce users to abuse of these opioids (Carroll et al., 2012).

The main NSOs AH-7921, MT-45, and U-47700 have been identified in Europe between 2013 and 2016, and over 40 deaths were reported to the European Monitoring Centre for Drugs and Drug Addiction in a short time after that AH-7921 and MT-45 were found out on the European drug market (EMCDDA) (European Monitoring Centre for Drugs, and Drug Addiction [EMCDDA], 2017). Moreover, in 2016 U-47700 has been the cause of at least 46 confirmed fatalities as well as the subject of 88 reports from forensic laboratories submissions in the United States (Fabregat-Safont et al., 2017).

Since the popularity of these substances is rapidly increasing and evolving over time, there is a great need to update all possible information, particularly with respect to their subjective and side effects and to tackle unsolved issues, including limited analytical methods to disclose and monitor different compounds (Katselou et al., 2015; Lucyk and Nelson, 2017).

To fill this gap, we here sought to report the latest information available on non-fentanyl derived NSOs U-47700, U-50488, U-51754, U-49900, U-48800, AH-7921, and MT-45 with particular regard to their pharmacotoxicology and adverse effects on users (see **Figure 1**).

Literature Search

A literature search was performed on the multidisciplinary research databases Scopus and Web of Science and on PubMed for biomedical literature, to identify all the relevant articles (up to March 2018). The search terms used in different combinations were: *new* or *novel synthetic opioid*, *designer opioid/drug, analgesics*, *narcotics, street drug, novel or new psychoactive substance/drug*. Articles related to fentanyl and its derivatives were excluded. Further studies were retrieved by hand search through the reference lists of the selected articles. Moreover, a search for reports was conducted on Institutional websites, to identify documentation published by international agencies or institutions such as World Health Organization (WHO), United Nations Office on Drugs and Crime (UNODC), United States Drug Enforcement Administration (DEA), and European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). Only articles or reports written in English were selected. All articles were screened independently by three of the authors to determine their relevance in the framework of the current review and only those selected at least by two of them were included.

NSOs of Benzamide Family

U-47700 and U-48800

U-47700 (3,4-dichloro-*N*-[(1R,2R)-2-(dimethylamino) cyclohexyl]-*N*-methylbenzamide), also known under the street names of Pinky (because impurities in its synthesis cause the drug powder to be slightly pink in color), U4 or Fake morphine, is an example of a non-fentanyl benzamide compound initially individuated as a heroin adulterant and as constituent of counterfeit analgesic pills, mimicking pharmaceutical opioids (Drug Enforcement Administration [DEA], 2016; World Health Organization [WHO], 2016; Baumann et al., 2017; Prekupec et al., 2017).

U-47700 is also actively being used as a legal substitute of illegally abused morphine, heroin, or fentanyl derivatives (Coopman et al., 2016).

It is a potent μ-opioid receptor agonist belonging to the *trans*-1,2-diamine class of analgesics and derived from another opioid analgesic compound, AH-7921 (Coopman et al., 2016; Domanski et al., 2017).

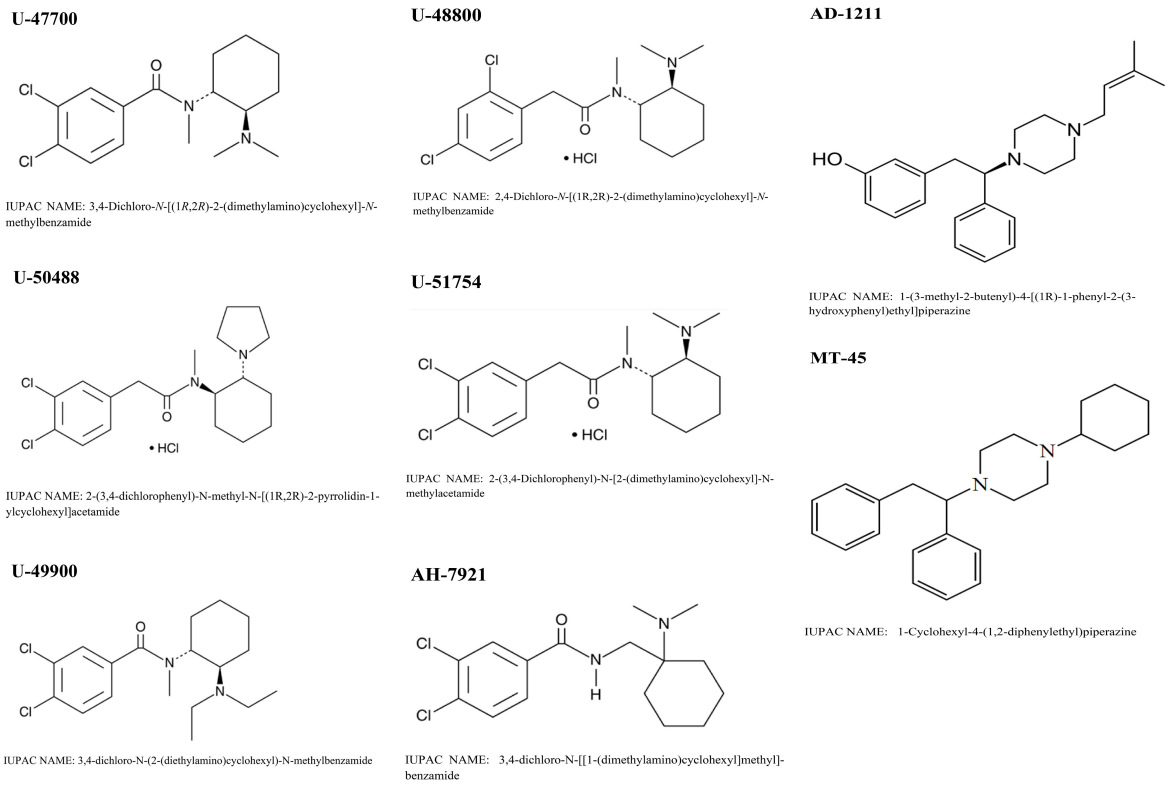

FIGURE 1 | Chemical structures of NSO_s reported in this review.

U-47700 was originally developed by the Upjohn Company in 1978 and is about 1/10 as potent as fentanyl and 7.5-fold more potent than morphine in animal models.

Up to now, the compound has never been studied in humans and it is not registered for medical use, but possibly induces typical opioid side effects, including respiratory depression, pinpoint pupils, cyanosis, depressed consciousness, and sedation (World Health Organization [WHO], 2016, 2017; Domanski et al., 2017; Prekupec et al., 2017).

It is likely to be used for its morphine-like pharmacological effects such as varying degrees of sedation, euphoria, a general lift in mood with desired effects being experienced in waves. Consumers also report having experimented a "cool, relaxed" effect (Elliott et al., 2016; Domanski et al., 2017).

The routes of administration, as referred by users in web forums, include the oral, insufflation, intravenous and rectal routes and via an inhaler which contains a liquid solution with a minty taste (World Health Organization [WHO], 2016, 2017). Naive information, always on websites, reports that light doses range from 5 to 7.5 mg, common doses from 7.5 to 15 mg and strong doses from 15 to 25 mg. Onset of action after oral administration is around 15 min, duration of subjective effects is 5–7 h and after effects 1–4 h. Similarly, in case of insufflation, onset of action is 15 min, duration of subjective effects is 3–4 h and hang-over period 1–4 h. Finally in case of intravenous use, onset of action is 0–1 min, duration of subjective effects 1–2 h and after effects 1–4 h (Zawilska, 2017).

In vitro metabolic profile of U-47700 was recently mapped for the first time using human liver microsomes (HLMs). Found metabolites were *in vivo* verified by analysis of urine specimens collected after five analytically confirmed cases of overdose from U-47700 consumption.

A total of four metabolites were identified in urine specimens. *N*-Desmethyl-U-47700 was recognized as the principal metabolite of U-47700, while the other detected metabolites were *N,N*-didesmethyl-U-47700, *N*-Desmethyl-hydroxyl-U-47700, and *N,N*-Didesmethyl-hydroxyl-U-47700. The study identified also similarities in metabolic transformation between U-47700 and its analog U-49900, resulting in a common metabolite 3,4-dichloro-*N*-(2-aminocyclohexyl)-*N*-methyl-benzamide and isomeric species (Krotulski et al., 2017).

The subjective effects of U-47700 makes it particularly appealing to users when compared to other substances. Indeed, consumers described it as producing more euphoric effects than other fentanyl analogs, more potent in its action than AH-7921, cheap, and easily available (Fabregat-Safont et al., 2017). Users also report the induction of tolerance and the emergence of withdrawal signs and symptoms upon discontinuing use of this compound, being this occurrence suggestive of physical dependence (World Health Organization [WHO], 2016, 2017).

However, abuse of the drug often happens unknowingly to the user, unaware of what he/she is consuming or in other cases the substance is encountered in combination with other drugs (heroin, fentanyl, and fentanyl analogs). Since substances like

U-47700 are often produced in illegal laboratories, the identity, purity, and effective dose of the product are unknown (Drug Enforcement Administration [DEA], 2016).

In Belgium a seizure of 'spice-like' incense found out, after toxicological analysis, the presence of U-47700 in the herbal mixture. This finding generated great concern, since users appeared to be not aware that they were consuming such a substance openly sold on the Internet as "legal high" (Coopman and Cordonnier, 2017).

Users abusing U-47700 appear to overlap with the individuals abusing prescription opioid analgesics, 'designer opioids' or heroin, as evidenced by drug use history documented in U-47700 fatal overdose cases (Drug Enforcement Administration [DEA], Department of Justice, 2016).

A number of fatalities and non-fatal intoxications from U-47700 have occurred in United States and Europe (Coopman et al., 2016; Elliott et al., 2016; Mohr et al., 2016; Armenian et al., 2017b; Domanski et al., 2017; Ellefsen et al., 2017; Jones et al., 2017; McIntyre et al., 2017; Rao and Nelson, 2017; Rambaran et al., 2017; Schneir et al., 2017; Seither and Reidy, 2017; Shoff et al., 2017), with concentrations varying widely, ranging from 7.6 to 1,460 ng/mL; while in other 16 confirmed fatalities across United States, blood concentrations ranged from 17 to 490 ng/mL (Mohr et al., 2016; Logan et al., 2017; World Health Organization [WHO], 2017). In 2016, U-47700 was identified for the first time in East and South-East Asia (United Nations Office on Drugs and Crime [UNODC], 2017a).

Reported symptoms of non-fatal intoxication included respiratory depression, agonal breathing, cyanosis, pinpoint pupils, bilateral pulmonary consolidation, atelectasis, anxiety, nausea, abdominal pain, shivering (Elliott et al., 2016; Armenian et al., 2017a; Domanski et al., 2017; Fleming et al., 2017).

Fatal intoxications have been mostly attributed to cardiac arrest, pulmonary and cerebral edema, cardiomegaly and to the depressant effect on the central nervous system, notably causing respiratory depression (Elliott et al., 2016; Fleming et al., 2017).

An additional compound analog of U-47700 recently emerged on the web is U-48800 (3,4-Dichloro-*N*-[(1R,2R)-2-(dimethylamino)cyclohexyl]-*N*-methylbenzamide). Available information is from drug fora (Bluelight, Reddit) or from research chemicals vendors on the web. Indeed, this agent is available as a research chemical of the opioid analgesic class to replace U-47700. U-48800 was also developed by the Upjohn company in the 1970s and it acts as a selective agonist of the μ-opioid receptor and has around 7.5-fold the potency of morphine in animal models.

U-48800 became the lead compound of selective kappa-opioid receptor ligands such as U-50488, U-51754 (containing a single methylene spacer difference) and U-69593, which share very similar structures. Although not used medically, the selective kappa ligands are used in research (Research chemical, 2018).

U-49900

U-49900 (3,4-dichloro-*N*-(2-(diethylamino)cyclohexyl)-*N*-methylbenzamide) is a structural analog of U-47700 with sparse clinical data available. The structural similarities with U-47700 raises concern regarding the risks associated with U-49900 use. Currently, no reported deaths have been associated with U-49900, but this agent is growing in popularity as a replacement or an alternative to the scheduled U-47700. Worldwide use is increasing as it has been specifically documented in some European countries such as Sweden and Spain (Alzghari et al., 2017).

Although both substance names are very similar, U-49900 does not pertain to the same Upjohn patent as the one already mentioned for U-47700 or U-51754, and it is in fact a completely new synthetic opioid (Fabregat-Safont et al., 2017).

Similarly to what happened with U-47700, Krotulski et al. (2017) mapped for the first time the generation of *in vitro* metabolic profile of U-49900 using HLMs. Metabolites were confirmed *in vivo* by analysis of human urine specimens collected after one case report of overdose following U-49900 ingestion. In urine specimens, five metabolites of U-49900 were overall identified. *N*-Desethyl-U-49900 was established to be the main metabolite of U-49900 following microsomal incubations, while *N,N*-didesethyl-*N*-desmethyl-U-49900 was the most abundant in another urine specimen: the other identified metabolites were *N,N*-Didesethyl-U-49900, *N*-Desethyl-hydroxyl-U-49900 and *N*-Desethyl-*N*-desmethyl-U-49900.

As previously mentioned, U-47700 and U-49900 metabolize to a common metabolite 3,4-dichloro-*N*-(2-aminocyclohexyl)-*N*-methyl-benzamide, and this is an important information for the analysts, especially in case of increasing prevalence of U-49900 in the street drug scenario (Krotulski et al., 2017).

U-49900 firstly appeared online in 2016 on a popular drug forum[1] by a drug user reporting its availability and asking other users about possible dangerous effects. Since the substance is a close analog to U-47700, users expressed concerns about its potential health risks, such as harms caused by nasal and rectal passages, damaged veins, severe withdrawals, and other serious side effects such as loss of taste, smell and of the sense of touch, pain upon insufflation, neurologic pain on the left side of the body and a foam-like discharge from the lungs. In a Swedish forum, a consumer reported usual opioid effects when using a U-49900 dose of 50 mg of the drug intravenously, while at lower doses of 5–10 mg other users informed on no effects. Conversely, in those latter amounts (5–10 mg) U-47700 is already active. Hence, U-49900 needs to be probably consumed at higher doses to have significant effects (Fabregat-Safont et al., 2017).

AH-7921

AH-7921 (3,4-dichloro-*N*-{[1-(dimethylamino)-cyclohexyl]methyl}benzamide) is an opioid structurally similar to U-47700 and firstly developed by Allen and Hanburys in the mid-1970s, with extensive *in vitro* and in animal studies, but it has never made available for medical use, because of its heavy addictive properties. AH-7921 was firstly identified in 2012 in samples of a product known as Doxylam which was used by Internet retailers as an alternative name for AH-7921. The name Doxylam could be easily confounded with the name of an antihistamine drug with sedative-hypnotic properties, doxylamine, present in several over-the-counter medicines. The accidental use of

[1] http://www.bluelight.org/vb/forum.php?

AH-7921/doxylam for the treatment of allergy or as a hypnotic might lead to serious health damages (Zawilska, 2017). There is therefore a concern that individuals looking for obtaining the unrelated hypnotic 'Doxylamine' might accidentally purchase AH-7921, mislabeled as 'Doxylam,' which could lead to unintentional drug overdoses (European Monitoring Centre for Drugs, and Drug Addiction [EMCDDA], 2014).

AH-7921 recently entered the illicit drug market as new psychotropic substance in countries such as Japan, United States and Europe, resulting in several fatalities and intoxications (Karinen et al., 2014; Kronstrand et al., 2014; Coppola and Mondola, 2015; Coopman et al., 2016; Armenian et al., 2017b; Dolengevich-Segal et al., 2017; Domanski et al., 2017; Fels et al., 2017). In fatalities occurred in European countries, the reported blood concentrations ranged from 31 to 1,449 ng/mL (Logan et al., 2017).

AH-7921 is an agonist of μ and κ opioid receptors, with a moderate selectivity toward μ opioid receptors, a narrow therapeutic window, and may cause dependence (Zawilska and Andrzejczak, 2015). It is 1.7-fold more potent than morphine at inducing respiratory depression in mice, suggesting greater risk for adverse effects in humans (Prekupec et al., 2017; Tracy et al., 2017).

AH-7921 can be purchased on the web market under the guise of being a research chemical 'not for human consumption' and it has also been detected in synthetic cannabinoid products (Karinen et al., 2014; Fabregat-Safont et al., 2017). Wohlfarth et al. (2016) comprehensively studied AH-7921 metabolism, by assessing HLM metabolic stability and determining AH-7921 metabolic profile after human hepatocytes incubation. Then, the findings in a urine case specimen were confirmed and results were compared to *in silico* predictions. Twelve AH-7921 metabolites after hepatocyte incubation were identified, mainly generated by demethylation, less dominantly by hydroxylation, and combinations of different biotransformations. The two major metabolites after hepatocyte incubation, also identified in the urine case specimen, were desmethyl and di-desmethyl AH-7921. Together with the glucuronidated metabolites, these are likely suitable analytical targets for documenting AH-7921 intake (Wohlfarth et al., 2016).

Users describe its effects to be similar to the classical opioids' ones including euphoria, mental relaxation, pleasant mood lift; while the side effects include sedation, miosis, nausea, vertigo, hypertension, tachycardia, respiratory depression, hypothermia, and withdrawal symptoms possibly worse than morphine due to its much longer half-life (Coppola and Mondola, 2015; Katselou et al., 2015; Fabregat-Safont et al., 2017).

The substance is sold in the form of capsules, tablets or powder and administration routes described in the web forums include mainly the oral, but also inhaled (vaporized), intravenous, intranasal, sublingual and intrarectal routes with a high risk of overdose (Coppola and Mondola, 2015; Katselou et al., 2015; Dolengevich-Segal et al., 2017). Light doses are from 5 to 10 mg, common doses from 10 to 25 and strong doses > 25 mg. Onset of action for oral administration is 15–45 min, duration 6–8 h and after effects 1–6 h (Zawilska, 2017).

In 2013, AH-7921 was detected in several cases of acute non-fatal intoxications and deaths in United States and European countries such as in Sweden, United Kingdom and Norway, combined with other substances such as cannabis, alcohol, synthetic cathinones, benzodiazepines, metoxetamine, or gabapentin. Lung edema was evidenced during the autopsy in most of the dead people (Coppola and Mondola, 2015; Katselou et al., 2015; Dolengevich-Segal et al., 2017).

In all the reported fatalities, cause of death could be attributed to respiratory depression. In a specific case, the autopsy revealed cerebral edema with moderate to increased intracranial pressure. Moreover, signs for an incipient pneumonia in the central lung sections were found (Fels et al., 2017). The absence of pharmacokinetic and pharmacodynamic information in humans makes the risk related to AH-7921 consumption combined with other central nervous system depressants unknown. Currently available information confirms that AH-7921 is a potent respiratory depressant with a high addictive potential (Coppola and Mondola, 2015).

NSOs of Acetamide Family

U-50488, U-51754

Information about U-50488 and U-51754 is quite scant. These acetamides are U-47700 related compounds, being part of the *trans*-1,2-diamine opioid analgesic chemical class synthesized by the Upjohn Company in the attempt to produce a non-addicting analgesic as potent as morphine (Mohr et al., 2016; Amin et al., 2017; Domanski et al., 2017; Fleming et al., 2017).

U-50488 (2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide) is a κ-opioid receptor agonist (KOR) with analgesic properties and some reported μ-opioid receptor respiratory antagonist effects (Mohr et al., 2016; Baumann et al., 2017; Domanski et al., 2017).

In animal models, U-50488 has been studied for its diuretic, antitussive, analgesic and anticonvulsant properties, but it is known to induce dysphoria and stress-like effects in rodents (Muschamp et al., 2012; Mohr et al., 2016; Amin et al., 2017). U-50488 abuse potential is unknown and at present this synthetic opioid is an uncontrolled substance available online from companies selling research chemicals (Mohr et al., 2016). Currently, information about the toxicological profile and toxicoepidemiology of U-50488 is poor, although the structural similarity of U-50488 to U-47700 poses users at potential health risks associated with its abuse and easy accessibility (Amin et al., 2017).

U-51754 (*trans*-3,4-dichloro-*N*-[2-(dimethylamino)cyclo hexyl]-*N*-methyl-benzeneacetamide) derived from the same Upjohn patent, has also recently appeared on the market. This substance is not as selective for KOR, and with respect to the effects, consumers report that it is more dysphoric and dissociating than U-47700 (Fabregat-Safont et al., 2017).

NSOs of Piperazine Family

MT-45

MT-45 (1-cyclohexyl-4-(1,2-diphenylethyl)piperazine) is a piperazine derivative chemically unrelated to other opioid

agonists, originally synthesized in the 1970s in a Japanese laboratory as an analgesic agent. It is an agonist of κ, μ and δ opioid receptors, with analgesic and sedative effects, with a potency nearly identical to morphine and highly addictive potential, although it has not been studied in human (Lindeman et al., 2014; Papsun et al., 2016; Baumann et al., 2017; Dolengevich-Segal et al., 2017; Logan et al., 2017). In animal studies MT-45 showed a high toxicity (Montesano et al., 2017).

MT-45 has been associated with a number of deaths in United States and Europe (especially in Sweden) (Bradley et al., 2016; Papsun et al., 2016; Baumann et al., 2017; Fels et al., 2017; Logan et al., 2017; Montesano et al., 2017). In 2016, MT-45 was identified for the first time in East and South-East Asia (United Nations Office on Drugs and Crime [UNODC], 2017a).

The light doses orally used range from 30 to 45 mg, the common from 45 to 60 mg and the strong > 60 mg. Onset of action is 30–45 min, duration is 4–6 h and after effects 2–3 h (Zawilska, 2017).

MT-45 blood concentrations in the reported deaths ranged from 8.3 to 1,989 ng/mL; while in a few non-fatal intoxications MT-45 was detected in blood at 6–157 ng/mL (Logan et al., 2017).

A recent study by Montesano et al. (2017), identified the chemical structures of 14 Phase I and II MT-45 metabolites, using primarily the prediction *in silico*, then the metabolites were confirmed by *in vivo* experiments. The detected metabolites are principally products of mono- or dihydroxylation, and *N*-dealkylation; in addition it was observed also a glucuronide conjugation of mono- and dihydroxylated metabolites. Hydroxylated MT-45 showed to be bioactive and may contribute to the overall pharmacotoxicological profile of MT-45 *in vivo*. The knowledge of Phases I and II MT-45 metabolite structure is necessary to develop analytical methods to detect MT-45 for clinical and forensic purposes (Montesano et al., 2017).

MT-45 surfaced on internet shops late 2012 (Fabregat-Safont et al., 2017) and was first reported as a new psychoactive substance (NPS) through the Early Warning System of the EMCDDA in December 2013 (Armenian et al., 2017b). Internet suppliers and retailers typically sell MT-45 in its dihydrochloride salt form. It has been seized mixed with other drugs, including synthetic cannabinoids or in combination with synthetic cathinones ("Wow") (Schifano et al., 2015; Papsun et al., 2016).

Users report a slow onset of action, which possibly increases the risk of toxic overdose from redosing before peak effect is reached. Intravenous administration of MT-45 is 11 times more lethal than morphine according to data observed in mice (Helander et al., 2014; Prekupec et al., 2017). MT-45 and novel fentanyls are probably similar in addictive potential and withdrawal effects (Tracy et al., 2017).

Clinical data from 12 analytically confirmed hospital cases of MT-45 poisoning, demonstrate that, similarly to other opioids, the main dangerous effects of MT-45, are respiratory depression, cognitive deficits, and loss of consciousness. A few users reported bilateral hearing loss and significant auditory symptoms with transient tinnitus, whilst a pronounced sensorineural hearing loss still present at 2 weeks follow-up affected one user. Hence MT-45 may be an ototoxic substance (Helander et al., 2014; Lindeman et al., 2014; Papsun et al., 2016; Dolengevich-Segal et al., 2017).

Other side effects, unclearly attributable solely on MT-45 or another contaminant, include folliculitis and dermatitis with hair loss, dry eyes, elevated liver enzymes, leukonychia striata (Mees' lines), typically found in thallium poisoning, and severe bilateral cataracts requiring surgery (Armenian et al., 2017b; Helander et al., 2017b). In a case report, autopsy revealed brain and hemorrhagic pulmonary edema and hyperemia of the internal organs (Fels et al., 2017).

Administration routes of MT-45 are typically oral or by nasal aspiration, but also intravenous, sublingual, intrarectal, or inhaled (vaporized). Typical doses reported by users are 15–30 mg for insufflation and 25–75 mg for oral administration; desired effects can last for up to 2 h (Zawilska and Andrzejczak, 2015). The effects sought by users is a sensation of well-being, relaxation and euphoria. In Switzerland 30 fatalities and several acute intoxications have been recently reported (Dolengevich-Segal et al., 2017).

Another piperazine, AD-1211 (1-(3-methyl-2-butenyl)-4-[(1R)-1-phenyl-2-(3-hydroxyphenyl)ethyl]piperazine), was also synthesized in the 1970s by the same Japanese laboratory which created MT-45. This compound has narcotic and analgesic antagonist activities with a physical dependence weaker than that of pentazocine (an opioid painkiller) (Natsuka et al., 1987). Information on this compound is limited and no pharmacotoxicological properties have been reported in the international literature.

CONCLUSION

Novel synthetic opioids were originally synthesized by pharmaceutical companies in their research for analgesic drugs without addictive properties. However, because of their toxicity or abuse potential, the NSOs reported in this review were never approved for medical use. Currently NSOs are mainly used by individuals who already used heroin, prescription opioids, or other illicit opioids looking for same opiates effects: relaxation, sedation, and euphoria. Psychonauts (from the Ancient Greek Ψυχή or psyche, which means "soul," "spirit," or "mind," and ναύτης or naútēs, which means "sailor" or "navigator," herein indicating a modern drug users seeking for altered mental states) appear also interested in experimenting the eventual peculiar effects of these NPSs.

Novel synthetic opioids are readily available on internet web sites and often used in association with other recreational drugs, leading to a public health danger in many countries worldwide. These substances have been causing severe intoxications and deaths pushing the United States and European governments to take the necessary measures to prevent their further spread.

Unfortunately, conventional drug tests do not currently detect the NSOs reported in this review. The growing number of acute intoxication cases, often associated with polyabuse, indicates that pharmacological, toxicological, and forensic research on these compounds is highly needed in order to determine

their pharmacokinetic profiles, long-term effects, and effective detection methods.

AUTHOR CONTRIBUTIONS

All the authors searched for bibliographic material, drafted different chapter of the manuscript, and contributed substantially to manuscript intellectual content and revision.

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Will Widespread Synthetic Opioid Consumption Induce Epigenetic Consequences in Future Generations?

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A growing number of evidence demonstrates that ancestral exposure to xenobiotics (pollutants, drugs of abuse, etc.) can perturb the physiology and behavior of descendants. Both maternal and paternal transmission of phenotype across generations has been proved, demonstrating that parental drug history may have significant implications for subsequent generations. In the last years, the burden of novel synthetic opioid (NSO) consumption, due to increased medical prescription of pain medications and to easier accessibility of these substances on illegal market, is raising new questions first in term of public health, but also about the consequences of the parental use of these drugs on future generations. Besides being associated to the neonatal abstinence syndrome, *in utero* exposure to opioids has an impact on neuronal development with long-term repercussions that are potentially transmitted to subsequent generations. In addition, recent reports suggest that opioid use even before conception influences the reactivity to opioids of the progeny and the following generations, likely through epigenetic mechanisms. This review describes the current knowledge about the transgenerational effects of opioid consumption. We summarize the preclinical and clinical findings showing the implications for the subsequent generations of parental exposure to opioids earlier in life. Limitations of the existing data on NSOs and new perspectives of the research are also discussed, as well as clinical and forensic consequences.

Keywords: opioids, transgenerational inheritance, epigenetics, parental exposure, prenatal exposure, vulnerability

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INTRODUCTION

The last decade is witnessing a huge increase in medical use and abuse of opioids, which is emerging as a major public health threat due to the concomitant dramatic rise in overdose morbidity and mortality (Humphreys, 2017; Kertesz, 2017). In the United States, epidemiological data indicate that the number of deaths involving opioids has more than quadrupled since 1999 (CDC, 2017), and the trend shows no sign of diminishing. The increase of opioid prescriptions to manage acute and chronic pain obviously contributed to generate this burden (Bedson et al., 2013; McCabe et al., 2017). In addition, opioid spread has been strongly favored by the easy

accessibility of a number of licit (pharmaceutical or counterfeit), and illicit opioids of synthesis, cheaply manufactured on industrial scale and distributed online (Pergolizzi et al., 2018). These opioids include fentanyl, firstly synthetized in 1960 and approved as anesthetic and for palliative use, fentanyl analogs and novel synthetic opioids (NSOs), such as AH-7921, U-47700 and MT-45 butyrylfentanyl (Armenian et al., 2017). Newer compounds are also produced by clandestine manufacturers at a fast pace, which makes difficult their analytical detection and legal regulation by international drug agencies (Armenian et al., 2017). Most of these molecules are potent agonists of the μ-opioid receptor, while they are less active on the κ and δ isoforms. Opioid receptors are distributed throughout the central nervous system and mediate the analgesic, but also the adverse effects including respiratory depression, constipation, rewarding properties, etc. (Cox, 2011; Pasternak and Pan, 2013). Notably, increasing evidence suggests that, besides its direct effect on treated individuals, drug exposure may induce lasting effects on subsequent generations (Vassoler et al., 2014). Nevertheless, information about the possible transgenerational consequences of opioid use is still very limited, with relevant consequences at regulatory level for prescription. This review summarizes the molecular mechanisms that underlie transgenerational inheritance of drug exposure and the available data on opioids, focusing on human data. Due to the scarcity of studies specifically addressing NSOs, data on opiates and opioids will also be included.

MOLECULAR MECHANISMS UNDERLYING THE IMPACT OF DRUGS ON FUTURE GENERATIONS

A family history of drug abuse correlates with increased risk of drug use in offspring (Yohn et al., 2015). However, only a small number of gene variants has been associated to drug addiction, indicating that genetics cannot provide the sole explanation. Indeed, environmental components, including drug consumption, may also influence the physiology and behavior of future descendants. The first demonstration of such impact referred to exposure to vinclozin, an agricultural fungicide, which can generate stable and heritable changes across several generations (Anway et al., 2005). Since then, many examples of both maternal and paternal phenotype transmission have been documented following prenatal stress (Morgan and Bale, 2011), diet variations (Kaati et al., 2002; Dunn and Bale, 2009; Champagne, 2010; Ng et al., 2010; Ost et al., 2014) and drug use/abuse (He et al., 2006; Novikova et al., 2008; Vassoler et al., 2014). In most cases, such transgenerational effects are mediated by epigenetic mechanisms. Epigenetics refers to all the molecular processes that regulate genome activity without changes in the DNA sequence (Skinner, 2011), which underlie, for instance, the ability of the same genome to produce multiple differentiated cell types in the same organism. Of note, epigenetic information responds to short- and long-term environmental inputs, allowing cells to adapt to new conditions, and such changes might be preserved during mitosis (Campos et al., 2014). Therefore, epigenetic remodeling events occurring in the germline can potentially persist through several generations, thus promoting effects also on individuals that were not exposed to the initial insult (Sharma and Rando, 2017).

Speaking about epigenetic inheritance, one important distinction relates to the type of exposure that can be **prenatal,** when occurring in a pregnant female or **parental,** if occurring prior to pregnancy (**Figure 1A**). In the first case, the possible effects are considered as true transgenerational inheritance, manifesting in the absence of any exposure, only if they are preserved at least in the third generation of descendants (F3) (Heard and Martienssen, 2014). By contrast, in case of parental exposure, we speak about transgenerational epigenetic alterations starting already in the F2 generation.

Epigenetic Mechanisms

Epigenetic changes are able to regulate the expression of specific genes by remodeling the structure of chromatin thus enabling the transition from open and transcriptionally active state to a condensed and transcriptionally repressed state (Margueron and Reinberg, 2010). At molecular level, the modifications involved in epigenetic inheritance include mainly post-translational modifications (PTMs) of histones and DNA methylation, but also non-coding and coding RNAs (Heard and Martienssen, 2014).

Histone N-terminal tails are targets of a number of covalent, but reversible modifications, such as acetylation, methylation, phosphorylation, crotonylation, succinylation, ubiquitylation, citrullination, and O-GlcNAcylation (Allis and Jenuwein, 2016; Sharma and Rando, 2017). The transcriptional effects of distinct histone PTMs are different (**Figure 1B**). While histone acetylation is associated with transcriptional activation, histone methylation is implicated in both activation and repression of transcription, depending on the residue involved and on the level of methylation (Teperino et al., 2010). In mammals, sperm alterations of histone H3 acetylation and methylation were reported in response to cocaine (Vassoler et al., 2013), hepatotoxin (Gapp et al., 2014), and low-protein diet (Carone et al., 2010), although it is still unclear if these PTMs are sufficient to convey instructive information for the progeny.

Another fundamental epigenetic process is DNA methylation, which occurs typically on the cytosine of CpG dinucleotides, enriched in the proximity of gene promoters and enhancers (**Figure 1B**) (Margueron and Reinberg, 2010). DNA methylation is mostly associated with transcriptional suppression and this mechanism underlies several examples of genomic regulation, such as genomic imprinting (genes whose expression is determined only by the paternal or maternal allele), X-chromosome inactivation and epigenetic memory maintenance (Bergman and Cedar, 2013). In sperm, the degree of DNA methylation at various loci is influenced by environmental factors including diet (Radford et al., 2014), alcohol (Govorko et al., 2012) and traumatic stress (Gapp et al., 2014; Bohacek et al., 2015) and similar aberrations were observed in the brain of the offspring. However, also in this case, the demonstration that parental DNA methylation alterations are causally contributing to specific traits in the descendants is

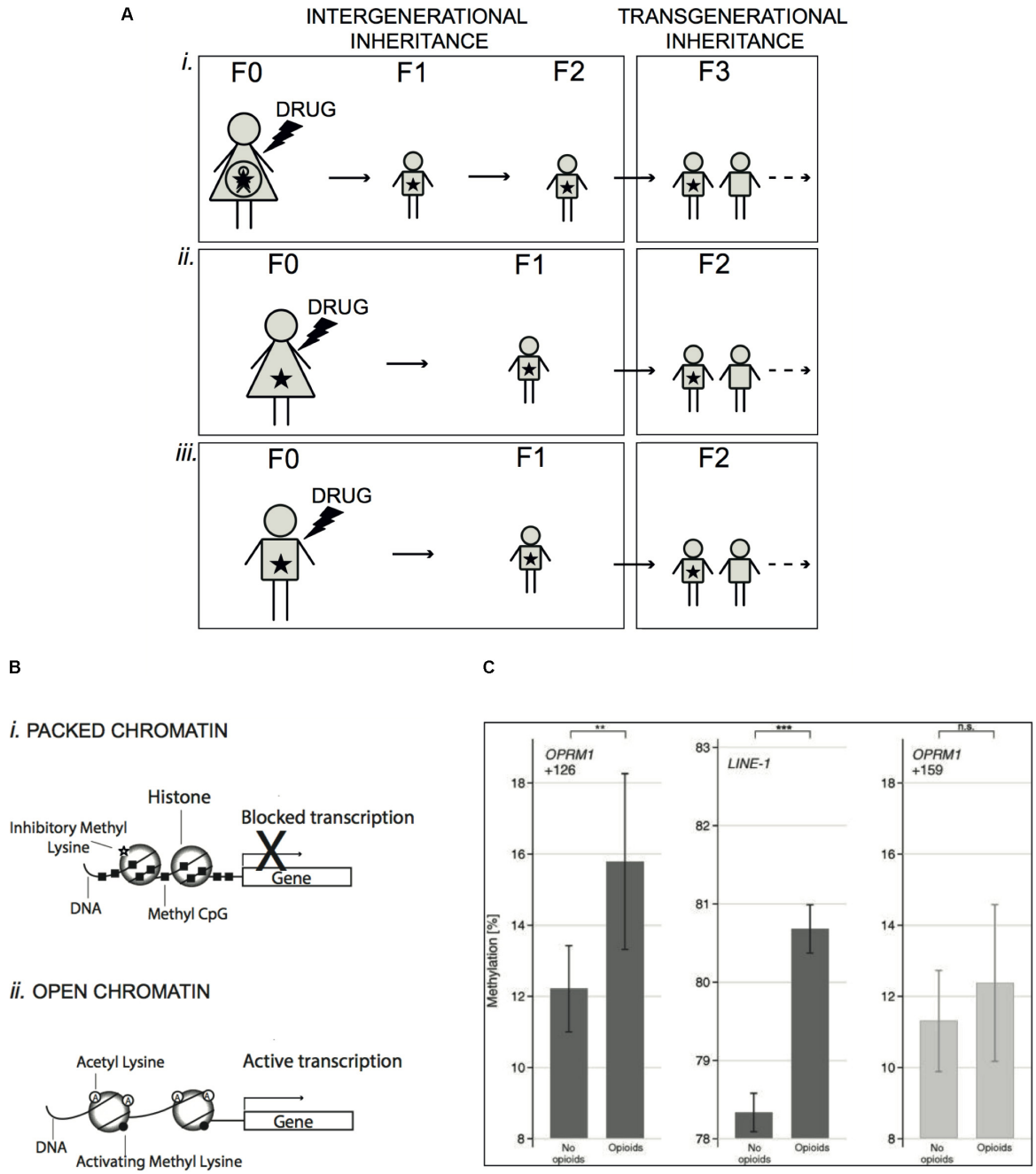

FIGURE 1 | Mechanisms of epigenetic inheritance induced by drug exposure. **(A)** Exposure of a pregnant female (F0) to a drug (prenatal exposure) implies also exposure *in utero* of the fetus and its own future germline (i). In this case, inherited epigenetic effects rising in the newborn (F1) and the direct descendants (F2) are considered as intergenerational, because cells of the future organism were directly exposed. Only epigenetic changes preserved in the following generations (F3 and after) are transgenerational, as manifesting in the absence of any exposure. On the other hand, exposure can occur in females (ii) or males (iii) prior to pregnancy (parental exposure), thus potentially touching the germline, which will produce the next generation (F1). In this case, inherited epigenetic alterations will be considered as transgenerational already in the F2 generation and beyond. **(B)** Schematic representation of epigenetic mechanisms. In chromatin, DNA is wrapped around individual histone proteins. DNA methylation at CpG dinucleotides (Methyl CpG) and methylation of histones at specific lysine residues are associated to a condensed chromatin structure, where DNA is less accessible to transcription factors, which results in silenced transcription (i). In contrast, acetylation of histones and specific histone methylation favors a more open structure of the chromatin, which allows the recruitment of transcription factors and activation of transcription (ii). **(C)** DNA methylation (means and 95% confidence intervals) at *OPRM1* gene (position +126 counted from the adenine of the start codon, left panel) and LINE-1 (long-interspersed nuclear elements – central panel) in a cohort of 132 chronic pain patients of whom 62 were treated with opioid analgesics for more than 1 year. Methylation was higher in the opioid-treated patients than in age-matched non-opioid-treated pain patients. The significances (**$P < 0.01$, ***$P < 0.001$) are the results of *t*-test comparisons between groups. For comparison of the specificity of the hypermethylation at position +126 of the *OPRM1* gene, a non-significant position (+159) is shown at right. Data shown in C panel are from Doehring et al. (2013) (License No. 4331930562761).

challenging, due to the difficulty to assign specific modifications to a given phenotype (Bohacek and Mansuy, 2015).

In mammals, most epigenetic changes arising in germline throughout life are actually erased during reproduction, which apparently leaves little chance for inheritance of epigenetic marks (Heard and Martienssen, 2014). Two reprogramming events of global DNA methylation take place in early embryonic development to promote cellular totipotency. However, at specific loci, some methylation and histone marks can escape this complete erasure (Bartolomei, 2009; Orozco et al., 2014; Sharma and Rando, 2017), which suggests that epigenetic modifications might be carriers of inheritable information. More recently, several reports described that sperm RNAs can convey the transfer of complex acquired phenotype from father to the offspring (Grandjean et al., 2015; Rodgers et al., 2015; Chen et al., 2016), likely through their ability to influence DNA methylation (Kiani et al., 2013). Although these findings pointed out a causal role of sperm RNAs in epigenetic germline inheritance, the underlying mechanisms remain unresolved so far.

CONSEQUENCES OF OPIOID PRENATAL EXPOSURE

According to recent reports, up to 1 in 5 women are taking an opioid medication at some point while pregnant (Desai et al., 2014). This is concerning because opioids are known to cross rapidly the placenta in concentrations consistent with maternal dose (de Castro et al., 2011) thus potentially triggering short and long-term vulnerabilities in the progeny. Prenatal opioid exposure can induce neonatal abstinence syndrome (NAS) in newborn infants, but knowledge about its long-term effects is limited, and information about possible transgenerational effects is even less abundant.

Neonatal abstinence syndrome is a true opioid withdrawal syndrome often requiring pharmacological treatment with replacement opioids and longer hospitalization to cope with symptoms including dehydration, diarrhea, fever, congestion, and diaphoresis (Practice and Medicine, 2017). A maintenance treatment with methadone or buprenorphine is the gold standard therapy for opioid-addicted pregnant women and NAS is estimated to occur in about 50% of infants chronically exposed to opioids (Klaman et al., 2017). This incidence corresponds to 5 out of 1000 live birth in United States (Patrick et al., 2015), with big health and economical implications, particularly because of the current inability to understand the factors associated to a severe NAS outcome. Indeed, despite multiple efforts aiming at modeling the contributions of maternal opioid dose and of the concurrent exposure to other medications or illicit drugs, the results remain so far inconclusive. Some genetic polymorphisms of genes related to dopamine and endogenous opioid systems such as prepronociceptin (*PNOC*) (Wachman et al., 2017), opioid receptors (Wachman et al., 2015) (*OPRM1*, *OPRK1,* and *OPRD1*), and catechol-*O*-methyltransferase (*COMT*) (Wachman et al., 2013), seem associated to a more severe NAS outcome, although further test on a larger scale are required to confirm these indications. Notably, one report showed that high methylation of three specific CpG sites of the *OPRM1* promoter is associated to a worse NAS outcome in newborn babies from mothers receiving methadone or buprenorphine during pregnancy, likely due to the subsequent lower expression of the receptor and a need for higher doses of opioid medication to control NAS symptoms (Wachman et al., 2014).

Regarding the long-term consequences of *in utero* opioid exposure, clinical studies in humans are extremely complicated by the huge amount of variables (i.e., doses and length of treatment) and of concurring risk factors that are often present, such as polysubstance use, stability, mother–child interaction, etc. Animal studies, performed mostly in rodents and in rigorously controlled experimental conditions, have helped to partially fill this gap. These studies highlighted broad neurodevelopmental effects of prenatal opioid exposure, including long-lasting changes in pre- and post-synaptic activity, altered opioid-mediated analgesia, reward-related behaviors, and impairment of hippocampal-based learning, in addition to alterations of the immune response (for recent review readers can refer to Byrnes and Vassoler, 2017). Unfortunately, these investigations almost completely referred to morphine, with only few exceptions examining oxycodone (Davis et al., 2010; Devarapalli et al., 2016), methadone (Hou et al., 2004; Vestal-Laborde et al., 2014; Wong et al., 2014; Chiang et al., 2015), and buprenorphine (Hung et al., 2013; Chiang et al., 2014; Wu et al., 2014). To the best of our knowledge, no report exists on long-term effects of prenatal administration of other synthetic opioids, such as fentanyl, in animal models. In addition, the heritability of such changes in the following generations was not really investigated. Alarmingly, in spite of converging animal data indicating possible long-term consequence of prenatal exposure to opioids, only few studies addressed the fate of exposed infants as they grow and enter adolescence and young adulthood. Young children born from women exposed to opioids during pregnancy show increased likelihood of problems related to motor skills, attention, and behavior regulation (Ornoy et al., 2001; Slinning, 2004; Melinder et al., 2013; Sundelin Wahlsten and Sarman, 2013). More divergent findings are reported concerning general cognitive abilities, with some study indicating an impairment of memory abilities in exposed children (Bunikowski et al., 1998; Hunt et al., 2008; Salo et al., 2009; Sundelin Wahlsten and Sarman, 2013), whereas others show no differences (Rosen and Johnson, 1985; de Cubas and Field, 1993; Melinder et al., 2013). Less information is available about adult offspring of opioid-dependent users, although few longitudinal studies reported deficits on several cognitive parameters (Konijnenberg et al., 2016; Nygaard et al., 2016, 2017). Collectively, however, these data must be taken with caution due to the heterogeneity of prenatal drug exposure and the difficulty to dissociate opioid effects from other risk factors to which they are often associated.

It is important to mention that, although most available studies refer to infants/young adult born to opioid-dependent women, many other patients are prescribed opioids during the pregnancy for pain control issues [severe migraine headache, myalgia, joint pain, low back, and pelvic pain (Bateman et al., 2014)]. A report referring to more than 1 million pregnant women with low socioeconomical status in the

United States, highlighted that 21.6% was dispensed at least once with prescription opioids during pregnancy and a significant increase was observed between the beginning (2000) and the end (2007) of the enrolment (Desai et al., 2014). The percentage was slightly lower, but still substantial (14%) in more affluent women (commercially insured) (Bateman et al., 2014). In Europe, data collected from a population-based registry covering the entire Norwegian population showed that, between 2004 and 2006, 6% of the pregnant women who ended the pregnancy filled at least one opioid prescription (Engeland et al., 2008). Chronic treatment with prescription opioids seems less diffused, as reported by a retrospective study on the period between 1998 and 2009, which recorded opioid use for more than 1 month during pregnancy in 6 out of 1000 deliveries (Kellogg et al., 2011). Regarding their prescription in pregnancy, most opioids were classified by the Food and Drug Administration under category C (**Table 1**), indicating that animal studies provided evidence for potential harm to the fetus, but human studies are lacking. Considered as overly simplistic, letter pregnancy categories were removed from drug labeling in 2015 and now risks for drug use during pregnancy, breast-feeding and in females and males of reproductive potential must be detailed. However, it remains difficult to infer from the available data if and at which doses/treatment conditions the use of opioids in pregnancy is safe. A comprehensive study highlighted an association between medical use of opioids in the first trimester of pregnancy and heart and neural tube birth defects (Interrante et al., 2017), while others refer to the third trimester of pregnancy (Coluzzi et al., 2014) and no study that we are aware of investigated the association with inheritable changes. Importantly, to counteract excessive opioid use, cannabinoids are emerging as alternative or combination treatment, due to the tight reciprocal interactions that exist between opioid and endocannabinoid signaling (Hurd, 2017). However, not even medical marijuana is devoid of risk of inducing hereditary effects (recently reviewed by Szutorisz and Hurd, 2018). Thus, it remains an urgent need of systematic longitudinal studies investigating the actual long-term impact of prenatal opioid exposure on the progeny and in subsequent generations.

TABLE 1 | Classification of opioid medications according to the FDA pregnancy category rule valid until June 2015.

Active substance	Common names of medication	Use in pregnancy category (FDA)
Buprenorphine	Belbuca, Bunavail, Buprenex, Buprenorphine, Butrans, Probuphine, Sublocade, Suboxone, Zubsolv	C
Codeine	Butalbital, Carisoprodol, Fioricit, Codeine, Fioricet, Fiorinal, Prometh VC, Synalgos, Tylenol	C
Fentanyl	Abstral, Actiq, Duragesic, Fentanyl, Fentora, Ionsys, Lazanda, Sublimaze, Subsys	C
Hydrocodone	Anexsia, Apadaz, Flowtuss, Hycofenix, Hydrocodone bitartrate, Hysingla, Norco, Reprexain, Rezira, Tussicaps, Tussigon, Vicodin, Vituz, Zohydro ER, Zutripro	C
Methadone	Dolophine hydrochloride, methadone hydrocloride, Methadose	C
Morphine	Apokyn, Arymo ER, Astramorph PF, Duramorph PF, Embeda, Infumorph, Kadian, Morphabond ER, MS Contin	C
Oxycodone	Oxaydo, Oxycet, Oxycodone, Oxycodone hydrochloride, Oxycontin, Percocet, Percodan, Roxicet, Roxicodone, Roxybond, Xtampza ER	B
Tapentadol	Nucynta, Tapentadol Hydrochloride	C
Tramadol	Conzip, Tramadol Hydrochloride, Ultracet, Ultram	C

Commercial medication names are listed in alphabetical order. ***Category B:*** *no evidence of harm to the fetus in animal studies, but no adequate and well-controlled studies in pregnant women.* ***Category C:*** *some evidence of adverse effect in animal studies OR no animal studies have been conducted AND no adequate and well-controlled studies in pregnant women.*

INHERITANCE LINKED TO OPIOID PARENTAL EXPOSURE

As mentioned above, epigenetic changes induced by drug exposure in the germline might be inherited by descendants. In term of public health, the potential ability of opioids to trigger transgenerational effects following drug exposure before pregnancy might generate considerable long-term consequences in the population. The μ-opioid receptor is expressed in sperm cells and β-endorfin, an endogenous opioid, is produced locally in male reproductive tract (Albrizio et al., 2006). Expression of all opioid receptors is also detected in oocytes and, interestingly, the pattern of μ- and κ-opioid receptors is changing during oocyte maturation, which points to a possible role of endorphins in this process (Agirregoitia et al., 2012). The presence of opioid receptors in both gamete types is suggestive not only of a contribution of endorphins to maintain gamete function, but also of possible epigenetic effects triggered by opioids on these cells that, in turn, could be transmitted to subsequent generations. Consistent with this hypothesis, opioid addiction increased DNA methylation at specific sites of the *OPRM* gene promoter in several cells, including sperm (Nielsen et al., 2009; Chorbov et al., 2011; Ebrahimi et al., 2018). Interestingly, an *in vitro* study showed that morphine inhibits cellular cysteine uptake thus altering the redox state of the cells, which results in reduced availability of *S*-adenosyl methionine (SAM), the principal methyl donor for DNA methylation (Trivedi et al., 2014). Accordingly, global reduction of DNA methylation was observed in cells treated with morphine, while an opposite effect was found in leukocytes of chronic pain patients treated with opioid analgesics (**Figure 1C**) (Doehring et al., 2013). The apparent lack of congruence of these results might be explained by cell specific responses induced by opioids. Nevertheless, these reports unequivocally demonstrate that opioids induce epigenetic changes. Accordingly, in mice, morphine reduced histone methylation (Sun et al., 2012) and augmented histone H3 acetylation in nucleus accumbens (Sheng et al., 2011) and basolateral amigdala (Wang et al., 2015), two brain regions involved in the reward control.

In humans, most reports highlighted opioid-induced epigenetic alterations only in the exposed generation, where they can mediate some of the observed behavioral effects, while there is not direct evidence of their transmission to subsequent generations. However, several studies in animals have started to investigate the impact of parental exposure to morphine at adolescence (F0) to the offspring (F1). Typically, adolescent female rats were treated with morphine and, after a wash out period, mated with drug-naïve males and tests were performed in adult F1 generation. Both male and female F1 rats showed enhanced locomotor activity when parents were exposed to morphine (Byrnes, 2005). In addition, males exhibited a more rapid development of morphine tolerance (Byrnes et al., 2011) and attenuated locomotor sensitization in association to increased expression of the dopamine D2 and κ-opioid receptors in nucleus accumbens (Byrnes et al., 2013). In contrast, in F1 females, parental morphine exposure altered anxiety-like behaviors (Byrnes et al., 2011), increased the sensitivity to opioid rewarding effects, likely due to sex-specific induction of the μ-opioid receptor (Vassoler et al., 2016), and lowered the levels of morphine self-administration (Vassoler et al., 2017). Thus, parental exposure to morphine induces neuroadaptation in both dopamine and opioid signaling and reshapes drug response in a sex-dependent manner. Moreover, alterations in hippocampal synaptic plasticity, with possible consequence on memory performance, were highlighted in the offspring of either F1 male or female (Sarkaki et al., 2008). Beyond the effects described in F1 generation, the first evidence of true transgenerational inheritance of opioid-induced effects came for the observation that F2 offspring from F0 morphine-exposed fathers exhibits decreased expression of synaptophysin and reduced synaptic connection (Vyssotski, 2011). Of note, changes in drug seeking behavior and drug tolerance were also observed in F2 generation from females exposed at adolescence (Byrnes et al., 2013; Vassoler et al., 2017), indicating that even limited exposure to opioids can have lasting effects across multiple generations. Whether these transgenerational repercussions are limited to opioid exposure during adolescence, when the reproductive system is still maturing, or if they are also present when exposure occurs in adults, remains to be verified.

CONCLUSION

Medical use and misuse of opioids have strongly increased in the last decades and the consequences for public health are numerous. Besides the main effects on the directly exposed individuals, converging evidence suggests that opioids can induce long-lasting transgenerational changes in subsequent generations, particularly concerning drug sensitivity and tolerance, with possible implications for drug abuse vulnerability. However, both preclinical and clinical studies are currently too limited to draw rigorous conclusions on the actual impact that the spread NSO use might have on future generations. One big limitation relies on data mostly referring to a small group of molecules such as morphine, methadone and buprenorphine, whose relevance is restricted to specific conditions (i.e., replacement therapy during pregnancy), while a gap of knowledge persists for other NSO medications used for pain control, also during pregnancy. Consequently, the doses of substances that can be considered as safe not only for the mother, but also for the child and future generation still remain an open question for lots of NSOs. Moreover, given the increasing alternative use of opioids together with cannabinoids, the study of possible effects of such combinations might be highly relevant. A second crucial point is the very limited amount of publications investigating the transmission across multiple generations of parental opioid exposure. Moreover, future studies should consider not only mother, but also father habits, as epigenetic transmission occurs also through paternal gametes. Another critical aspect depends on the complex interpretation of the clinical studies that tried to address opioid effects on the following generations, because of the co-occurrence of many confounding factors (polysubstance use, genetic component). Therefore, preclinical studies must be carefully designed to increase as much as possible the translational relevance of the results and help establishing cause-effect relationships and the role of epigenetics. For instance, all animal studies investigating the effects of parental exposure to morphine were performed in a similar experimental paradigm with exposure at adolescence. Thus, so far, we totally lack information about the potential transgenerational impact of opioid exposure at adulthood that corresponds to the age with major NSO consumption in humans.

In conclusion, a huge research effort is warranted to inform the regulatory measures that are needed to curb the spread of synthetic opioids and to keep the risk–benefit ratio of the medicinal use of opioids as low as possible.

AUTHOR CONTRIBUTIONS

FG wrote the manuscript. MA and AT conceived and edited the manuscript.

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CASE REPORT
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First Case in Italy of Fatal Intoxication Involving the New Opioid U-47700

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The drug commonly known as U-47700 is a strong μ-opioid agonist with an approximate potency 7.5 times higher than morphine. It has been available in Europe since 2014, where it is usually sold through the internet or black market as an abuse morphine-like substance. In the case reported here, a Caucasian man was found dead in his apartment. Next to the body, the police seized one transparent plastic bag containing a white powder and two amber glass bottles with nasal spray containing few milliliters of a transparent liquid. During the autopsy, no evidence of natural disease or trauma was found to account for the death. Blood, urine and pubic hair were collected and submitted for toxicological analysis. The content of the seized materials was also submitted to a general screening analysis in order to determine its composition. U-47700 was detected in blood, urine and hair samples using an UHPLC/MS-MS method purposely developed. The blood and urine concentrations were 380 and 10,400 ng/mL, respectively. No other drugs of abuse nor ethanol were found in blood and urine specimens. Pubic hair analysis revealed a frequent past exposure to U-47700. Finally, U-47700 was identified as the main component of the powder and the liquids contained in the nasal spray bottles. The combined circumstantial elements and toxicological results of the case revealed the occurrence of an acute intoxication produced by U-47700 abuse. To the best of our knowledge, this is the first fatal intoxication case reported on the Italian territory involving the synthetic opioid U-47700.

Keywords: U-47700, synthetic opioid, postmortem, intoxication, NPS

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INTRODUCTION

U-47700 (3,4-dichloro-N-[2-(dimethylamino)cyclohexyl]-N-methylbenzamide) is an opioid analgesic drug developed by the pharmaceutical company Upjohn in the 1970s and structurally related the earlier opioid AH-7921 (Belgian Early Warning System Drug, 2017). At the moment, it is controlled in 3 European countries (Sweden, Finland and United Kingdom) and in the USA. U-47700 is a strong μ-opioid receptor agonist, and reproduces all (or most of the) common effects of opiates such as morphine, including analgesia, pronounced euphoria, sedation and itching (Nikolaou et al., 2017). For these reasons, the compound is gaining popularity on drug user forums as a legal alternative to morphine/heroin (Zawilska, 2017). It is generally sold online as research chemical, not for human consumption, as a white or slightly pinkish powder or fine crystals under different names, including "U4", "Pink," or "Pinky." There is limited information available on the routes of administration and the doses of U-47700 used. It is taken by oral, nasal,

rectal routes, or by smoking, intravenous injection, or even by combinations of these routes (Nikolaou et al., 2017; Zawilska, 2017). Side effects, including overdose reactions, are presumed to be very similar to other opiates/opioids as well: depressed respiration (slow breathing), miosis (pinpoint pupils), constipation (World Health Organization, 2016; Baumann and Pasternak, 2018). Even if several cases of acute and lethal intoxication involving U-47700 were reported and reviewed in the literature (Rambaran et al., 2017; Gerace et al., 2018), little is still known about the correlation between its blood concentration and the observed effects. In this investigation, we report a case of fatal intoxication after U-47700 intake. The presence of the drug was confirmed in blood, urine and pubic hair specimens by means of mass spectrometry-based chromatographic methods.

BACKGROUND

Case History

A Caucasian man, with previous history of drug addiction, was found dead in his apartment, lay down on the floor. Two amber glass bottles with nasal spray, containing few mL of a transparent liquid, plus a plastic bag containing a white powder were found on a table near the decedent. Moreover, a package containing a vial of naloxone hydrochloride 0.4 mg/mL was also found on the table. No evidence of violence was observed in the room. The death was reported to the Public Prosecutor who took jurisdiction of the case. To investigate the cause of death, he ordered a post-mortem examination and toxicological analysis.

Post-mortem Examination

The findings were irrelevant, except for a general pulmonary edema. The body appeared well-nourished, and the internal examination presented no evidence of natural disease or trauma to account for his death. At the external examination of the body, no signs of injection was found. To execute the inherent toxicological analyses, heart blood, urine and pubic hair (length: 3 cm) specimens were collected during the post-mortem examination. Peripheral blood was not collected. All of the samples were stored at −20°C before the analysis.

EXPERIMENTAL

Samples Preparation for Fluids and Pubic Hair

General screening analysis was executed according to a standard procedure employed in our laboratory (Gerace et al., 2014). Briefly, urine sample was extracted with tert-butyl methyl ether (TBME) at alkaline condition after a deconjugation with β-glucuronidase from *E. coli*. After mixing and centrifugation, the organic layer was separated and dried under a nitrogen flow. The residue was reconstituted with 50 μL of methanol and a 1 μL aliquot was injected into the gas chromatography/mass spectrometry (GC/MS) system. In addition, the blood sample was screened with a method for the detection of about ninety pharmaceutical drugs and metabolites routinely employed in our laboratory (Vincenti et al., 2013). For U-47700 quantitation in blood, 50 μL of samples were added with the internal standard (fentanyl-d5) at final concentration of 50 ng/mL and added with 950 μL of acetonitrile/methanol 80:20 (v/v), previously stored at −20°C, and then incubated at −20°C for 15 min. For quantitation in urine 50 μL of samples were added with the internal standard (fentanyl-d5) at final concentration of 50 ng/mL and added with 950 μL of water with formic acid 5 mM/acetonitrile 95:5 (v/v). In both cases, the sample was centrifuged at 14,000 rpm for 5 min and 100 μL of the organic phase was transferred into a new vial. Finally, 1 μL aliquot was directly injected into the UHPLC-MS/MS system operating in selected reaction monitoring (SRM) mode.

Pubic hair analysis was performed on the entire length of the hair lock (3 cm). Approximately 50 mg of hair was twice-washed with dichloromethane and methanol (3 mL each, vortex mixed for 3 min). After complete removal of the solvent wash, the hair was dried at room temperature by a gentle nitrogen flow and subsequently pulverized with a ball mill. For U-47700 quantitation, the hair sample was fortified with 3 μL of a fentanyl-d5 dilute solution used as the internal standard at a final concentration of 0.3 ng/mg. After the addition of 1 mL of methanol, the sample was incubated at 55°C for 15 h without stirring. Finally, the vial was centrifuged once more at 14,000 rpm for 5 min and 1 μL aliquot was directly injected into the UHPLC-MS/MS system operating in SRM mode.

The linear calibration model was checked by analyzing blank hair samples spiked with standard solutions at final concentration of 0, 0.01, 0.025, 0.05, 0.1, and 0.25 ng/mg. Whenever the effective drug concentration exceeded the calibration range, the samples were diluted to fit the quantitation interval considered in the curve.

Moreover, qualitative and quantitative hair analyses for the detection of (i) the most common drugs of abuse, (ii) synthetic cannabinoids and (iii) synthetic cathinones were performed by means of analytical methods used in our laboratory and described elsewhere (Di Corcia et al., 2012; Salomone et al., 2014, 2016).

Sample Preparation for Liquids and Powder Found on the Scene

The liquids and the powder found on the scene were subjected to systematic analysis for the detection of drugs and toxic substances. A 100 μL aliquot of liquid and 100 mg of the powder were dissolved in 5 mL of methanol. After sonication in an ultrasound bath for 1 h at 55°C, a 1 μL aliquot of methanolic solution was injected into the GC/MS system with the mass spectrometer acquiring the spectra in the full scan mode (40–650 amu).

Apparatus and Methods

Preliminary screening analyses for amphetamines, tricyclic antidepressants, barbiturates, benzodiazepines, cannabinoids, methadone, cocaine and opiates were performed on urine by the Enzyme Multiplied Immunoassay Technique (EMIT, Abbott Laboratories, IL, USA). The presence of ethanol in the blood was determined by headspace-GC-MS. Screening analysis for unknown substances was performed using a 6890N GC apparatus (Agilent Technologies, Milan, Italy) equipped with a HP−5

17 m fused-silica capillary column (J&W Scientific) with a 0.2-mm inner diameter and a 0.33–μm film thickness. Full scan spectra in the interval 40–650 amu were acquired using a 5,975 inert mass-selective detector (Agilent Technologies, Milan, Italy) operating in the EI mode at 70 eV. The qualitative identification of the underivatized compounds was performed by comparing the full scan spectra obtained with those recorded in the updated spectra libraries (PMWTox2, SWGDRUG version 3.0, AAFS2012, CaymanSpectraLib). For the U-47700 confirmation analysis, a dedicated UHPLC-MS/MS procedure was developed as follows. The chromatographic separation was performed using a Shimadzu LC-30A series system (Shimadzu, Duisburg, Germany) equipped with a CORTECS UPLC C18 column 1.6 μm × 2.1 mm × 100 mm (Waters Corporation, Italy). The elution solvents were water/formic acid 5 mM (solvent A) and acetonitrile/formic acid 5 mM (solvent B). After an initial isocratic condition at 95% A for 0.5 min, the mobile phase composition was varied by a linear gradient (A:B; v/v) from 95:5 to 45:55 in 4.0 min; followed by isocratic elution at 55% B for 0.5 min. The flow rate was 0.5 mL/min and the total run time was 6.0 min including re-equilibration at the initial conditions before each injection. Detection was carried out by an API 5500 triple quadrupole mass spectrometer (ABSCIEX, Foster City, CA, USA) equipped with turbo ion spray source, operating in the positive ionization mode. The SRM transitions used for the determination of U-47700 were 330.9→286.1 (quantifier) and 330.9→204.1 (qualifier), while for the internal standard the transitions 342.2→188.2 was chosen.

Validation of the LC-MS Confirmation Methods for U-47700 Quantitation

The method was validated by investigating the following parameters: selectivity, linearity, identification and quantitation limits (LOD and LOQ), precision, accuracy and matrix effect. The linear calibration model was checked by analyzing (three replicates) blank samples spiked with U-47700 standard solution at final concentrations of 0, 10, 25, 50, 100, and 250 ng/mL. Whenever the effective drug concentration exceeded the calibration range, the extract was diluted in order to fit the quantitation interval considered in the curve. Ten different blank samples were prepared as previously described to test the selectivity of the whole analytical procedure. The occurrence of possible interferences from endogenous substances was checked by monitoring the signal to noise ratio (S/N) for the U-47700 SRM transitions at the expected retention time. LOD values were estimated as the analyte concentration whose response provided a S/N value equal to 3, as determined from the least abundant transition. The S/N value at the lowest concentration was used to extrapolate the theoretical LOD. This calculated LOD was then experimentally confirmed by analyzing spiked samples at LOD concentration of U-47700. LOQ was calculated as three times the LOD. Within-batch precision (expressed as percent variation coefficient, CV%) and accuracy (expressed as bias %), were assessed by extracting and analyzing a series of ten blood samples fortified at 50 ng/mL. Matrix effect was evaluated by comparing the signal obtained when the analyte was added to the matrix extract with the response obtained from a methanolic solution containing the analyte at the same concentration. The percent difference represented either matrix suppression (value below 100%) or matrix enhancement (value above 100%).

RESULTS AND DISCUSSION

The calibration plot showed good linearity in the range 0–250 ng/mL, with a determination coefficient of 0.998. The SRM chromatograms from ten negative samples of blood showed no interfering signals (i.e., S/N ratio lower than 3) at the retention time of U-47700, indicating that the method is selective and free from matrix interferences. The calculated LOD was 0.6 ng/mL and the LOQ was fixed at 2 ng/mL. The results show a satisfactory within-batch precision (CV%: 3.1) and accuracy (bias%: 11.7) at 50 ng/mL. No significant matrix effect was observed (matrix effect 4.0%).

The presence of U-47700 was confirmed in all specimens. **Figure 1** depicts the SRM profiles obtained from the blood, urine and pubic hair samples for the detection of the target analyte. U-47700 was quantified at a concentration of 380 ng/mL in blood while higher amount of the drug was detected in urine (10,300 ng/mL, creatinine 156 mg/dL). No other drugs nor ethanol were detected in the body fluids. Pubic hair analysis revealed past exposure to U-47700 (5.7 ng/mg). Moreover, pubic hair turned out negative for the presence of traditional drugs of abuse, synthetic cathinones and synthetic cannabinoids. U-47700 was also identified as the main component of the white powder (purity 99%) and of the liquid content of the nasal spray bottles (0.1 mg/ml). The presence of the substance in the nasal spray bottles together with the absence of injection signs on the body indicated that one of the consumption ways was presumably intranasal.

Presently, several intoxication cases related to the consumption of U-47700 alone, or in combination with other drugs, were recently reported (Coopman et al., 2016; Elliott et al., 2016; Mohr et al., 2016; Armenian et al., 2017; Dziadosz et al., 2017; Jones et al., 2017; McIntyre et al., 2017; Papsun et al., 2017; Vo et al., 2017; Koch et al., 2018), but reference toxic and lethal concentrations in body fluids and organs are not yet available for U-47700. Intoxication cases involving U-47700 are summarized in **Table 1**.

A brief description of the most significant cases is presented as follows.

A 41-year-old woman presented to the emergency department (ED) for altered mental status, pinpoint pupils and respiratory depression which reversed after 0.4 mg naloxone administration intravenously. The U-47700 concentration in the serum, recorded at the arrival at the ED, was 7.6 ng/mL (Armenian et al., 2017). Other relevant findings included fentanyl (15.2 ng/mL), hydrocodone (107.6 ng/mL), sertraline (15.7 ng/mL) and gabapentin (350.9 ng/mL).

A 26-year-old woman was found cyanotic and with respiratory depression after a nasal insufflation and injection of a product called "U4" (Jones et al., 2017). Toxicological analysis revealed the presence of U-47700 in serum and urine samples at

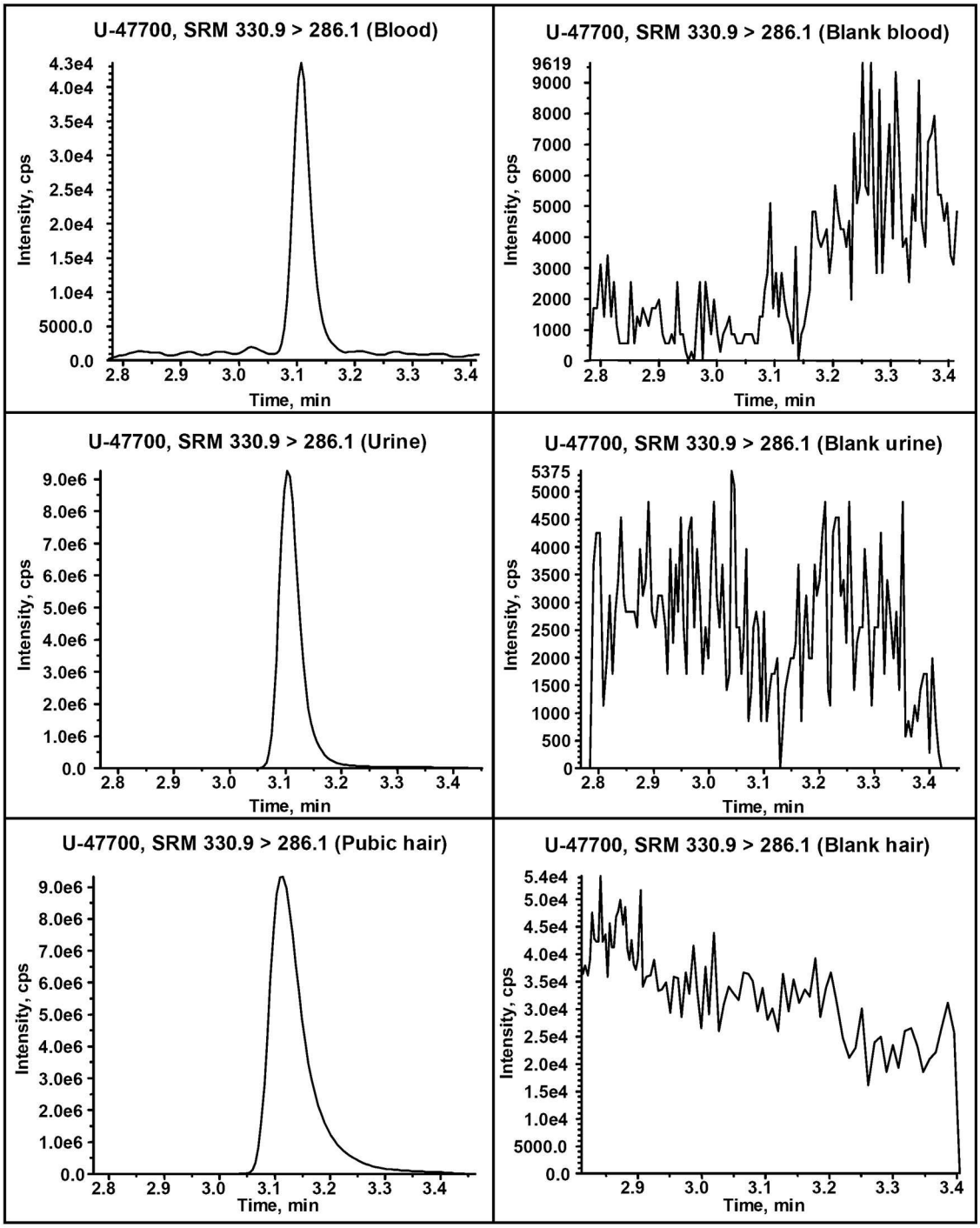

FIGURE 1 | Comparison between the UHPLC-MS/MS extracted ion chromatogram resulting from the detection of U-47700 in the victim's blood, urine and pubic hair (left) and a blank blood, urine and hair samples (right).

concentrations of 228 and 393 ng/mL, respectively. Also in this case the patient became more responsive after an intravenous naloxone administration.

Another case involving a 29-year-old man found unresponsive after the intravenous injection of U-47700 was described (Vo et al., 2017). The patient regained consciousness spontaneously before the transportation at the ED. Serum sample was positive for U-47700 and phenazepam at a concentrations of 240 ng/mL and 1.4 mg/L, respectively.

TABLE 1 | U-47700 blood concentration in intoxication cases.

Intoxication outcome	Country	Year	Blood (ng/mL)	Specimen[a] (site of sampling)	Other relevant findings (ng/mL)	References
Fatal	**Italy**	**2017**	**380**	**cb (heart)**	**None**	**Presented case**
Non-fatal	USA	2016	**7.6**	serum	Fentanyl (15.2), Hydrocodone (107.6), Sertraline (15.7), Gabapentin (350.9)	Armenian et al., 2017
Non-fatal	USA	2016	**228**	serum	None	Jones et al., 2017
Non-fatal	USA	2016	**240**	serum	Phenazepam (1,400)	Vo et al., 2017
Fatal	Germany	2017	**370**	serum	Flubromazepam (830)	Koch et al., 2018
Fatal	USA	2016	**190** **340**	pb (femoral) cb (heart)	Alprazolam (120), Doxylamine (300), Diphenhydramine (140), Carboxy-THC (2.4)	McIntyre et al., 2017
Fatal	UK	2016	**1,460**	pb (femoral)	Quetiapine, Amphetamine	Elliott et al., 2016
Fatal	Belgium	2016	**13.8**	pb (subclavian)	Fentanyl (10.9), Sertraline (180)	Coopman et al., 2016
Fatal	Germany	2017	**525** **1,347**	pb (femoral) cb (heart)	Diphenidine (1.7), Methoxiphenidine (26)	Dziadosz et al., 2017
Fatal	Germany	2017	**819** **1,043**	pb (femoral) cb (heart)	Diphenhydramine (45), Methylphenidate (2.5)	Dziadosz et al., 2017
Fatal	USA	2017	**189**	pb (femoral)	Oxycodone (67)	Papsun et al., 2017
Fatal	USA	2017	**547**	pb (femoral)	Etizolam	Papsun et al., 2017
Fatal	USA	2015–16	**382**	pb (n/a)	Amphetamine (12)	Mohr et al., 2016
Fatal	USA	2015–16	**217**	pb (femoral)	Mephedrone (22), Etizolam	Mohr et al., 2016
Fatal	USA	2015–16	**334**	pb (n/a)	None	Mohr et al., 2016
Fatal	USA	2015–16	**252**	pb (n/a)	Citalopram (43)	Mohr et al., 2016
Fatal	USA	2015–16	**453**	blood	None	Mohr et al., 2016
Fatal	USA	2015–16	**242**	pb (n/a)	Carboxy-THC (5.3)	Mohr et al., 2016
Fatal	USA	2015–16	**103**	n/a	Diphenhydramine (694)	Mohr et al., 2016
Fatal	USA	2015–16	**299**	cb (aorta)	Alprazolam (47), Lorazepam (11), 3-methoxyphencyclidine (180), Tramadol (<250)	Mohr et al., 2016
Fatal	USA	2015–16	**487**	cb (aorta)	Etizolam (86), diphenhydramine (250), chlorpheniramine (<250)	Mohr et al., 2016
Fatal	USA	2015–16	**311**	cb (aorta)	Oxycodone (11), Venlafaxine (2600), o-desmethylvenlafaxine (380)	Mohr et al., 2016
Fatal	USA	2015–16	**59**	cb (aorta)	None	Mohr et al., 2016
Fatal	USA	2015–16	**135**	cb (aorta)	Furanylfentanyl (26), Ethanol	Mohr et al., 2016
Fatal	USA	2015–16	**167**	cb (aorta)	Furanylfentanyl (56), Morphine (48), 6-monoacetylmorphine	Mohr et al., 2016
Fatal	USA	2015–16	**490**	cb (aorta)	Furanylfentanyl (76)	Mohr et al., 2016
Fatal	USA	2015–16	**105**	cb (aorta)	Furanylfentanyl (2.5)	Mohr et al., 2016
Fatal	USA	2015–16	**17**	pb (n/a)	Butyrylfentanyl (26), Ethanol (0.03 g/dL)	Mohr et al., 2016

[a]*pb, peripheral blood; c, central blood; s, serum; n/a, data not available.*

A fatal intoxication related to the effect of the U-47700 in combination with the benzodiazepine flubromazepam was recently reported (Koch et al., 2018). A 24 year-old man suffered apnoea and after reanimation and hospital admission, hypoxic cerebral damage and severe brain oedema were stated. Six days after admission mechanical ventilation was discontinued and the patient died. Serum sample collected at the admission to the hospital was positive for U-47700 and flubromazepam at a concentrations of 370 ng/mL and 830 mg/L, respectively.

McIntyre et al. described a fatal intoxication case related to the intake of powder containing U-47700, likely by nasal insufflations (McIntyre et al., 2017). The drug was detected in several post-mortem samples including peripheral blood (190 ng/mL) and the central blood (340 ng/mL). Further presence of U-47700 was determined in liver (1,700 ng/g), vitreous humor (170 ng/mL), urine (360 ng/mL) and gastric content at trace amount (<1 mg).

Another fatal intoxication case associated with the consumption of U-47700 was reported by Elliott et al. (2016). The U-47700 concentration in the femoral blood of a man found dead at home was 1,460 ng/mL.

The presence of U-47700 was found in blood and urine in a case of 30-year old man found dead in his home after

inhaling fumes of a powder burned on aluminum foil (Coopman et al., 2016). U-47700 was quantified in post-mortem blood and urine at concentrations of 13.8 and 71 ng/mL, respectively. Toxic levels of fentanyl were also measured in the subclavian blood (10.9 ng/mL). The dead was ascribable to the concomitant intake of U-47700 and fentanyl.

In two cases in which the cause of death was explained by the consumption of U-47700, the concentration of the drug in femoral blood was 525 ng/mL (case 1) and 819 ng/mL (case 2) (Dziadosz et al., 2017). The presence of U-47700 was quantitatively confirmed in additional specimens including heart blood (1,347 ng/mL in case 1 and 1,043 ng/mL in case 2), urine (1,393 and 1,848 ng/mL), kidney (2.7 and 1.4 ng/mg), liver (4.3 and 3.1 ng/mg), lung (3.2 and 2.4 ng/mg), and brain (0.97 and 1.1 ng/mg).

Other two fatal cases involving U-47700 were reported by Papsun et al. (2017). The concentrations of U-47700 in femoral blood were 189 and 547 ng/mL respectively. In the first case oxycodone was also found in blood at significant levels (67 ng/mL) and the death was ascribed to an acute oxycodone and U-47700 overdose. In the second case, the presence etizolam was found together with U-47700. The death was attributed to a U-47700 and etizolam intoxication.

The application of a LC-MS/MS method for the simultaneous analysis of U-47700, U-50488 and furanyl-fentanyl in blood specimens related to 20 postmortem cases, initially attributed to heroin or other opioid-related drug overdoses, was recently described (Mohr et al., 2016). The presence of U-47700 was confirmed in 16 of 20 cases. The U-47700 was the only opioid detected in 9 cases, while in two cases two prescription opioids were detected (tramadol and oxycodone) and in the remaining 5 cases the drug was found in combination with furanylfentanyl (3 cases), morphine and furanylfentanyl (1 case) and fentanyl (1 case). For U-47700, the mean concentration ($N = 16$) was 253 ng/mL, within a range of 17–490 ng/mL.

It is challenging to speculate about which U-47700 levels in blood might be fatal. The available literature deals with cases in which the molecule was detected by means of different analytical techniques in subjects with different characteristics. Furthermore, in most cases U-47700 was not used alone, thus other compounds might have been a contributing factor in the death. In particular, several authors reported the finding of other opioids, including fentanyl and analogs, which likely intensified the central nervous system and respiratory depression. Currently, the sporadic records in which U-47700 was the only detected toxic agent suggest to be cautious before any definitive lethal concentration is presented.

In the present case, the heart blood concentration of U-47700 was comparable with those reported in some other fatal cases previously described, where no other drugs were found to play a role in the intoxication (Mohr et al., 2016). As a matter of fact, the U-47700 blood concentration was also comparable with those recorded in some cases of non-fatal intoxication (Jones et al., 2017; Vo et al., 2017), although in the latter cases these values were obtained from peripheral blood. In one of these cases, there was a prompt resuscitation of the patient at the ED using naloxone (Jones et al., 2017), while in the second case the patient regained consciousness spontaneously (Vo et al., 2017). In the present case, the comparison of the U-47700 concentration in blood and urine (the latter showing a much higher value, also in light of the high density) suggests the occurrence of extensive drug excretion and possibly a long agony before death. Moreover, the presence of U-47700 in the pubic hair sample indicates that the decedent had previously been exposed to the same drug on more than one occasion.

CONCLUDING REMARKS

In the fatal case reported here, the intake of U-47700 was proved and suggests that its depressant effect on the central nervous system was likely to account for the consumer's death. U-47700 was present in body fluids at a concentration compatible with acute intoxication conditions, possibly leading to death. To the best of our knowledge, this is the first reported case of fatal intoxication involving U-47700 that occurred on the Italian territory. Intoxication cases involving NPS, including U-47700, continue to pose challenges for toxicologists. Deaths and intoxication cases consistent with opioid consumption, but negative to the traditional drug screenings, should be subjected to further testing for the detection of fentanyl analogs and novel opioid-like compounds. Moreover, in all fatalities involving opiates and opioids, where the toxic effect is related with acquired tolerance through frequent use, the interpretation of post-mortem drug concentration may be challenging. The absence of reference concentrations in post-mortem matrices together with the contribution of other drugs to the intoxication makes the interpretation even more problematic.

INFORMED CONSENT FOR PUBLICATION

This manuscript does not violate the privacy of the deceased, nor contain identifiable details and therefore the anonymity is maintained. The Institution represented by AS was informed and consequently waived the request for informed consent from the next of kin of the deceased.

AUTHOR CONTRIBUTIONS

All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. EG and AS: case study concept and design. EG, AS, CL, and DD: acquisition, analysis, or interpretation of data; EG, AS, and MV: drafting of the manuscript; All authors: critical revision of the manuscript for important intellectual content; All authors: study supervision.

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MINI REVIEW
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Preclinical and Clinical Pharmacology of Hydrocodone for Chronic Pain: A Mini Review

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Hydrocodone is one of the most prescribed oral analgesic drugs and it is one of the most abused drugs in general population. It is a mu-opioid agonist predominantly metabolized to the O-demethylated product hydromorphone and to the N-demethylated product norhydrocodone. The purpose of the study is to summarize the preclinical and clinical characteristics of hydrocodone. Pharmacokinetic aspect (terminal half-life, maximum serum concentration, and time to maximum serum concentration) of hydrocodone and the influence of metabolic genetic polymorphism in analgesic response to hydrocodone are also illustrated and commented. Literature on experimental preclinical pharmacology investigating analgesic activity in laboratory animals is furtherly discussed. Moreover, the authors discuss and comment on the updated data regarding safety profile and effectiveness of hydrocodone in the treatment of chronic pain. A bibliographic research was carried out (from February 01, 2018 to August 28, 2018) independently by two researchers (blinded to the authors and initially on results) in the major scientific databases and research engines of peer-reviewed literature on life sciences and biomedical topics, starting from January 1990 to August 2018. Analysis of results of clinical studies suggests that abuse-deterrent extended-release (ER) hydrocodone formulations can be effective and they are well tolerated in the treatment of chronic low back pain. Weaker is the evidence of the analgesic effectiveness of ER hydrocodone on other chronic pain syndromes and non-cancer non-neuropathic chronic pain. In these conditions, hydrocodone showed to have positive effects in non-controlled open studies and needs to be further studied to assess the real strength of results.

Keywords: hydrocodone, chronic pain, opioids, pain, analgesics

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INTRODUCTION

Opioids are the most potent drugs producing analgesia and their use is fundamental for the clinical pain management (Mercadante et al., 2014). The largest part of prescriptions regarding pain relievers opioid drugs are oxycodone, hydrocodone, morphine, codeine, methadone and transdermal morphine and fentanyl (Graziani and Nisticò, 2016). Hydrocodone is currently used

in the pain management, but risks related to its abuse and misuse raise an increasing problem for health (Kenan et al., 2012; Smith et al., 2013). Objective of this review is to summarize the principal preclinical and clinical characteristics of the opioid drug hydrocodone.

A bibliographic research was carried out (from February 01, 2018 to August 28, 2018) independently by two researchers (blinded to the authors and initially on results) in the major scientific databases and search engines of peer-reviewed literature on life sciences and biomedical topics (PubMed, Scopus, Embase, Web of Science, and Google Scholar) starting from January 1990 to August 2018. The investigators used the following keywords or combination of keywords: "hydrocodone," "hydrocodone" and "chronic pain," "hydrocodone" and "opioids." The analysis included all articles written in English language, published in peer-reviewed scientific journals, describing preclinical findings and clinical applications of hydrocodone. All the authors reviewed all the eligible articles and resolved by discussion any uncertainty regarding the content about hydrocodone to be discussed.

HYDROCODONE IN THE MEDICINAL PRODUCTS

Hydrocodone is a semi-synthetic phenanthrene opiate derivative with analgesic and antitussive effects. The chemical name of hydrocodone is (4R,4aR,7aR,12bS)-9-methoxy-3-methyl-1,2,4,4a,5,6,7a,13-octahydro-4,12-methanobenzofuro[3,2-e]isoquinoline-7-one, the drug name dihydrocodeinone was given when it was first marketed in Germany (Fraser and Isbell, 1950). Since the release, in 1943, of the first product, hydrocodone acquired growing popularity as a drug considered as a "middle-level" opioid (Covvey, 2015). The rescheduling of hydrocodone combination products has been discussed in the United States by Food and Drug Administration (FDA) in 2012. Currently, hydrocodone is listed in Schedule II of the Controlled Substances Act. Following a re-evaluation of the drug abuse-related data, hydrocodone combination products including analgesic and cough suppressant compounds were listed in Schedule III (Food and Drug Administration [FDA], 2012).

It was originally marketed as a single drug as immediate-release (IR) dosage forms indicated for the short management of acute pain and it was successively released in association with non-opioid drugs (Gould and Paul, 2015). Opioids and co-analgesics such as non-steroidal anti-inflammatory drugs are often used to improve pain control or to reduce opioids prescription or their dosage and decrease the risk for adverse events caused by opioids (Vardy and Agar, 2014). In particular, hydrocodone has been marketed in combination with different dosages of acetaminophen to increase analgesia and simultaneously to induce reduction of the intake of hydrocodone because of the acetaminophen side effects. It is well known that excessive doses of acetaminophen are the leading cause of acute liver failure in the developed world (Larson et al., 2005). This combination has been authorized in the United States with different amounts of acetaminophen (200, 325, 400, 500, 650, or 750 mg) with a presence of hydrocodone in the tablets of 2.5, 5, 7.5, or 10 mg, with a dosing interval from 4 to 6 h (Krashin et al., 2013; Gould and Paul, 2015).

Analgesic products containing hydrocodone in combination with the anti-inflammatory drug ibuprofen (hydrocodone bitartrate/ibuprofen 2.5–7.5 mg/200 mg) to be taken by oral administration and a long-acting formulation of hydrocodone not containing acetaminophen were also released (Krashin et al., 2013).

In February 2018, the FDA approved the association between the prodrug benzhydrocodone and acetaminophen (Mustafa et al., 2018). However, therapeutic indication for both these drugs is for the short-term management of acute pain.

Changing hydrocodone from schedule III to schedule II has been associated with an increase in the total amount of opioids filled in the initial prescription following surgery (Habbouche et al., 2018). However, hydrocodone prescriptions decreased, and prescriptions of oxycodone, another opioid drug widely used to alleviate moderate and severe acute and chronic pain, increased in frequency (Pantano et al., 2017; Tan et al., 2018). The necessity to reduce chronic pain produced an increase in use of opioids leading in turn to a growing opioid misuse, abuse and addiction associated with overdose deaths (Volkow and McLellan, 2016). It has been reported that only in United States of America in the year 2012 about 2 million persons were taking for the first time prescribed opioids. The same analysis reported that the most of misused prescription drugs in young people were pain relievers (Graziani and Nisticò, 2016).

Other novel synthetic opioids, such as U-47700, U-49900, AH-7921, or MT-45 have no recognized therapeutic use but emerged as non-illegal drugs diffused to circumvent prohibition laws, resulting in numerous abuse reports and overdose cases, especially across the United States and Europe (Solimini et al., 2018).

Abuse of hydrocodone seems to be lower than the oxycodone abuse. This is true for previously dependent opioid abusers, for non-dependent opioid abusers (Zacny and Gutierrez, 2009) and for non-physical dependent prescription opioid abusers (Walsh et al., 2008).

Hydrocodone is the second drug of abuse in the United States but the first preferred by women. Female preference could be due to the fake perception of a better safety profile deriving by the minor rate of overdose associated with hydrocodone (Cicero et al., 2013). This misconception has been successively denied by subsequent reports showing the increase of hydrocodone-related deaths and by the prevalence of female among the victims (Mowry et al., 2016; Gummin et al., 2017). The grown consumption raised concerning safety issues because, in 2011, use of hydrocodone was linked with about 97,000 drug-related emergency room visits caused by abuse/misuse. With the aim to attenuate this phenomenon regulatory acts were developed about hydrocodone products. After consultation of stakeholders and companies the agency FDA took note that products with hydrocodone or similar opioid products contained larger quantities of drugs in a single tablet to be taken over a short period (8–24 h). In this way, these products showed

attractiveness and had a strong potential for abuse and adverse reactions. On this basis, the FDA introduced a risk evaluation and mitigation strategy for extended-release (ER) long-acting opioid products (Covvey, 2015). Moreover, the FDA authorized for long-term management of pain two new oral ER products based only on hydrocodone bitartrate (10–90 mg) (Covvey, 2015). Abuse-deterrent formulations have been designed and marketed with the aim to obtain opioid-based products keeping effective analgesia but the decreasing behavior of abuse through the use of alternative routes of administration (Hale et al., 2016a,b).

ER opioid formulations are considered more suitable for appropriate management of pain in chronic patients. Oral intake of ER medicinal products produces higher plasmatic concentrations and lower peak-to-trough changes over the dosing interval, in comparison with IR products (Gudin, 2013; Nicholson, 2013). The first formulation having, according to FDA, abuse-deterrent characteristics contained only hydrocodone bitartrate was a once-daily hydrocodone bitartrate ER product, prescribed for the long-term opioid treatment of severe pain refractory to other analgesic strategies (Dhillon, 2016).

PRECLINICAL STUDIES

Metabolism of hydrocodone was studied in laboratory, animals and species differences have been observed. In rats, metabolic conversion of hydrocodone to hydromorphone is performed by the enzyme CYP2D1, homolog of the human CYP2D6. While hydrocodone mainly underwent O-demethylation and ketone reduction in rats to form hydromorphone and hydromorphone in the reduced form, in dogs it is metabolized prevalently by N-demethylation and N-oxidation (Li et al., 2013).

Rewarding and euphoric effects of hydrocodone, investigated by using the conditioned place preference (CPP) paradigm, have been compared in rats to those of morphine. In laboratory animals, hydrocodone is self-administered and produces an opiate-like subjective discriminative generalization profile and a withdrawal syndrome after sudden treatment cessation that was similar to morphine and/or oxycodone (Gauvin et al., 2015).

Hydrocodone and morphine injected intraperitoneally produce a CPP at the 5 mg/kg dose, but not the lower 1 mg/kg dose, suggesting similar rewarding properties, furthermore hydrocodone and morphine equally reduced phosphorylation levels of extracellular signal-regulated kinase (ERK) and cAMP response element-binding (CREB) proteins in the nucleus accumbens, thus indicating that these drugs cause their effects through signal transduction pathways involved in rewarding and reinforcing effects (Tenayuca and Nazarian, 2012).

Studies *in vivo* in rats by using the pain models "tail withdrawal test" and "formalin test" on opioids administered subcutaneously, shown that analgesia caused by hydrocodone is greater than codeine and lower than, in decreasing order, fentanyl, buprenorphine, oxycodone, and morphine (Meert and Vermeirsch, 2005). Sex differences in antinociceptive effects of opioids have been observed in rats. Male rats are more sensitive to the antinociceptive effects of morphine than female rats and this difference, as a lesser extent, is evident also with hydrocodone (Peckham and Traynor, 2006).

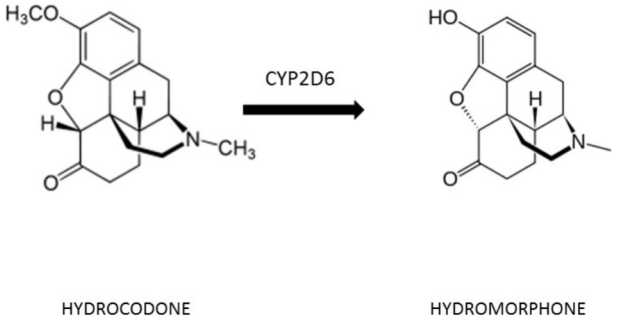

FIGURE 1 | Metabolic conversion of hydrocodone to hydromorphone.

PHARMACOKINETICS OF HYDROCODONE

After single oral ingestion, hydrocodone reaches maximum serum concentrations within 1 h and it shows to have an elimination half-life of 4–6 h (Otton et al., 1993). Following single oral doses of ER hydrocodone formulations, blood concentration reaches the peak (Cmax) at a median time (Tmax) of 14–16 h for the different doses (the range is 6–30 h). Hydrocodone steady state is reached in 2 days after taking once daily an ER hydrocodone formulation (Kapil et al., 2016). Hydrocodone and its metabolite norhydrocodone appear in the urine within 2 h after single drug administration (Cone et al., 2013).

The size of plasma protein-binding is unknown but it could be similar to semi-synthetic opiates such as hydromorphone, about 19% bound (Cone et al., 2015). The apparent volume of distribution after ER administration is 402 L (for an adult of 70 kg), thus indicating a large hydrocodone tissue distribution (Dhillon, 2016).

The principal metabolites of hydrocodone are norhydrocodone and hydromorphone. Hydrocodone is a prodrug (inactive), only through the bioconversion to its active metabolite hydromorphone it induces analgesia (Otton et al., 1993; **Figure 1**). Pain relief correlates with plasma hydromorphone but not with hydrocodone concentration, thus confirming that the ability to convert hydrocodone to its active drug is essential (Stauble et al., 2014). Hydrocodone is transformed to hydromorphone through O-demethylation catalyzed by the cytochrome P450 (CYP450) enzyme CYP2D6, influencing the metabolism of 25% of all drug therapies (Cone et al., 1978).

Hydromorphone undergoes phase II glucuronidation to be transformed in the predominate metabolite hydromorphone-3-glucuronide. Approximately 7% of the Caucasian population are poor metabolizers (PMs), causing a slower rate of conversion of hydrocodone to hydromorphone. Urinary hydromorphone after a single dose of hydrocodone was found at relatively small

amounts in both extensive metabolizers (EMs) and PMs but PMs were equally responsive to oral hydrocodone as EMs. The study demonstrated that although hydrocodone is less potent than hydromorphone, it clearly has its own agonist actions (Valtier and Bebarta, 2012).

Hydrocodone is also metabolized by CYP3A4, but the product of transformation is the inactive metabolite norhydrocodone (Hutchinson et al., 2004). Isoenzymes CYP2B6 and CYP2C19 may also be partially involved in the formation of norhydrocodone and hydromorphone (Dhillon, 2016).

Approximately 3% of Blacks, 1% of Asians are PMs of CYP2D6. The remainder of individuals in these populations produce functional levels of CYP2D6 and are labeled EMs (Bertilsson, 1995).

Patients who are ultra-rapid metabolizers may produce more hydromorphone, while subjects who are PMs of CYP2D6 may experience little to no analgesia from hydrocodone since they lack or have not sufficient activity of the enzyme to metabolize it (Lurcott, 1998; De Leon et al., 2003). PMs are able to produce only small amounts of the active metabolite hydromorphone, regardless of dose. After a 10 mg oral dose of hydrocodone, hydromorphone levels in EMs have been found to be about 5–10 times greater than in PMs (Otton et al., 1993). Besides CYP2D6 PMs, also patients in therapy with strong inhibitors or high-affinity substrates of CYP2D6 such as the antiarrhythmic quinidine and the antidepressants selective serotonin reuptake inhibitors, paroxetine and fluoxetine, are unable to metabolize hydrocodone into its active metabolites. Even if, prescription in patients of hydrocodone with antidepressant drugs has not been met with any poor analgesic issues in clinical practice, in these patients it can be recommended to prescribe hydrocodone in its active form, such as hydromorphone (Lurcott, 1998). The isoenzymes of P450 system CYP2B6 and CYP2C19 may also be partially responsible for the transformation in the metabolites norhydrocodone and hydromorphone (Pathan and Williams, 2012).

Other products of the hydrocodone metabolism are the glucuronate conjugated products to hydrocodone-3β-glucuronide and hydrocodone-6β-glucuronide. The metabolite hydromorphone is also glucuronidated to hydromorphone-3β-glucuronide and hydromorphone-6β-glucuronide (Larson et al., 2005). The 6β-metabolites but not 3β-metabolites are active and 6β-metabolites are more active than hydrocodone against pain (Fordyce, 1989).

Renal excretion is the principal way of elimination for hydrocodone and its metabolism products. Renal hydrocodone elimination in healthy subjects account for 6.5% and it reduces in values with the severity of the disease (Dhillon, 2016). Severe or moderate liver insufficiency, moderate to severe renal impairment or end-stage renal disease may cause higher plasmatic hydrocodone level. Age and gender seem to be not affecting the pharmacokinetics of hydrocodone (Dhillon, 2016).

Blood concentrations of hydrocodone and its metabolite dihydrocodeine are widely used to determine the cause of death. While concentrations in postmortem samples may not necessarily reflect the original drug concentration at the time of death, a study of liver to peripheral blood hydrocodone ratio suggests that hydrocodone is unlikely to undergo substantial postmortem distribution changes (Saitman et al., 2015).

PHARMACODYNAMICS OF HYDROCODONE

Hydrocodone produces its analgesic effects by activating mu-opioid receptors (MORs), it is a μ-opioid receptor agonist analgesic, even though when higher concentrations are reached it can bind with different opioid receptors. As the dose of opioids increases beyond typical starting doses, delta-opioid receptors and kappa-opioid receptors are activated. MORs are G-protein coupled receptors that inhibit cAMP production and activate G-protein mediated inwardly rectifying potassium channels. The analgesic effect appears to be associated with the latter signaling pathway. As the dose of hydrocodone increases over the starting doses, delta-opioid receptors and kappa-opioid receptors are also activated. In *in vitro* experiments, hydrocodone itself is a low efficacy agonist (Mitrovic et al., 2003; Gould and Paul, 2015).

The affinity measured using a single binding assay in a cell membrane preparation expressing recombinant human MOR of the product of conversion hydromorphone for the MOR has been reported to be over 100 times greater than that of hydrocodone, with Ki values of 0.36 nM for hydromorphone versus 41.58 nM for hydrocodone (Volpe et al., 2011; Vuilleumier et al., 2012).

Hydromorphone, but not hydrocodone, exerts analgesic effects (Boswell et al., 2013) and it possesses more potency (7–10 times) than morphine (Stauble et al., 2014).) Approximately 0.9–1.2 mg of hydromorphone is equianalgesic with 10 mg of morphine, with a similar incidence of side effects (Mahler and Forrest, 1975).

Hydromorphone had a similar effect on patient-perceived cancer pain intensity as described for oxycodone and morphine (Bao et al., 2016) and there is not sufficient evidence to support or refute the suggestion that hydromorphone is effective in neuropathic pain (Stannard et al., 2016). As it is for oxycodone and dihydrocodeine, the potency of hydrocodone is about 10 times than its parent molecule, codeine (Gould and Paul, 2015). It is less polar than codeine and thus has more rapid pharmacokinetic properties. Rapid pharmacokinetics influences reinforcing effects and potential of abuse of hydrocodone (Ko et al., 2002; Flugsrud-Breckenridge et al., 2007).

It has also been shown that hydrocodone, as well as morphine, is conditioning the locomotor response involved in the dopamine reward system to the D2/D3 dopamine receptor agonist quinpirole (Emery et al., 2015).

HYDROCODONE FOR CHRONIC PAIN: CLINICAL STUDIES

Opioids are used for moderate to severe chronic non-cancer pain in patients that are refractory to other analgesics such as acetaminophen and nonsteroidal anti-inflammatory drugs and when other opioids are not appropriate in patients because they experience unsupportable adverse effects (Chou et al., 2009).

In 2008, the American Society of Interventional Pain Physicians (ASIPP) released guidelines to provide guidance for opioids use for chronic non-cancer pain. According to these guidelines, hydrocodone and methadone were considered at level III of evidence, the level of evidence for transdermal fentanyl and sustained-release morphine was II-2, whereas for oxycodone the level of evidence was II-3. The level III of clinical evidence for hydrocodone is a weak level since was based on expert opinion (Trescot et al., 2008).

More recently, several studies have been performed to investigate on the effectiveness of ER hydrocodone in chronic pain. Chronic pain has been recognized as pain that persists past normal healing time and hence lacks the acute warning function of physiological nociception (Treede et al., 2015).

The authors collected 13 clinical studies on the effects of ER hydrocodone bitartrate administered alone in the treatment of chronic pain deriving from different pathologic conditions.

Five clinical studies were carried out with ER hydrocodone on chronic pain deriving from low back pain. Four of them were randomized controlled double-blinded clinical trials (RCTs), one was designed as a 22-week open study. The RCTs recruited 1246 patients; dose ranging was 15–120 mg of ER hydrocodone every 12 h, duration of treatment was 12 weeks in all the clinical trials. Similarly, conclusions were that ER hydrocodone is significantly more effective than placebo in alleviating chronic low back pain and shows a safety profile without the risk of liver toxicity associated with acetaminophen (Rauck et al., 2014; Hale et al., 2015a,b; Wen et al., 2015). The open study recruited 182 patients with chronic low back pain receiving ≥1 dose of abuse-deterrent ER hydrocodone (15–90 mg every 12 h), 170 entered open-label treatment for 22 weeks and 136 completed the study. ER hydrocodone was generally well tolerated and maintaining efficacy over the period of treatment (Hale et al., 2016a).

Three studies reported the effects of ER hydrocodone on non-cancer non-neuropathic moderate-to-severe chronic pain. They were long-term open-label designed studies recruiting a total number of 542 patients. Ranging dose of ER hydrocodone was 20–120 mg administered once daily for an elapsed period of 12–18 months. Adverse events reported were those normally associated to opioids: nausea, vomiting, constipation, dry mouth, hematemesis, abdominal pain, dizziness, dysgeusia, headache, myalgia, paresthesias, scratch, fatigue, sleep disorders, and hyperhidrosis (Kapil et al., 2016; Taber et al., 2016; Broglio et al., 2017).

Another open-label, long-term trial (1-year maintenance treatment) investigated on long-term safety and effectiveness of hydrocodone 20–120 mg tablets taken by 269 patients with moderate to severe chronic, non-malignant and non-neuropathic pain. Supplemental non-opioid pain medications were permitted. A total of 226 patients (84%) completed the 1-year maintenance period. Results showed a reduction in pain intensity consistently maintained during the entire treatment period (Lynch et al., 2014).

One study evaluated safety and effectiveness of a once-daily, single-entity, ER hydrocodone over a 52-week period in 97 patients with chronic non-cancer and non-neuropathic pain who required opioid rotation from IR oxycodone. Hydrocodone was well tolerated and produced effective analgesia; furthermore, use of opioids decreased (Pergolizzi et al., 2017).

Other three open studies investigated on the effectiveness of hydrocodone on chronic pain deriving from any origin. Dose ranging was 15–300 mg divided into two daily doses for a period of 3–13 months. Results showed moderate to substantial levels of pain relief associated with functional improvements in patients treated with ER hydrocodone (Argoff et al., 2015; Hale et al., 2015a,b,c).

Finally, Bartoli et al. (2015) showed that hydrocodone was effective in reducing pain intensity and in maintaining analgesia over time without the need for continued dose increase and with tolerability profiles similar to those of other opioid analgesics. Treatment showed positive effects also on the health-related quality of life (HRQL), although not in mental HRQL or sleep quality.

Data from this overview of clinical studies with ER hydrocodone suggest that this formulation can be used to relieve chronic pain. However, more relevant results were showed in RCTs conducted in patients with low back pain. On a lower step of importance are open studies on chronic non-cancer and non-neuropathic pain. All the studies showed a good tolerability of ER hydrocodone and the one long-term open study (Lynch et al., 2014) indicated that both effectiveness and tolerability could be maintained over time.

SAFETY PROFILE OF HYDROCODONE

Hydrocodone use can trigger the occurring of adverse reactions. Abuse, addiction, and adverse effects related to opioid drugs have been detected (Larochelle et al., 2015). At higher doses, hydrocodone can cause respiratory depression due to direct action on the brain stem centers. As well as occurs with other drugs acting on the central nervous system, hydrocodone may impair mental and/or physical abilities, such as driving a vehicle or operating machinery. The risk for respiratory depression and coma is more frequent at the beginning of therapy with or when the dose of the drug is increased (Cone et al., 2013).

There have been cases of self-reported severe to profound sensorineural hearing loss in people using hydrocodone-acetaminophen association. Sensorineural hearing loss is an ototoxic condition resulting in permanent, severe to profound auditory damage and it has been associated with the use of these combination products (Rigby and Parnes, 2008). Hearing loss does not resolve with withdrawn of hydrocodone or with the application of a steroidal therapy (Ho et al., 2007).

It has been suggested that opioids such as transdermal fentanyl, methadone, and oxycodone can be associated with increased odds of androgen deficiency. However, this kind of risk is lesser with hydrocodone use (Rubinstein and Carpenter, 2017).

CONCLUSION

Hydrocodone is one of the most prescribed and effective opioid analgesic drugs, however, the rate of its abuse raised a new health

problem. In particular, medicinal products based on association hydrocodone-acetaminophen released with the aim to enhance analgesic effects and at the same time to reduce the dose of hydrocodone caused abuse and addiction prevalently in young people. To fight this phenomenon, abuse-deterrent formulations ER long-acting opioid products were authorized, however, these deterrent forms remain abused orally. Analysis of results of clinical studies considered for this review suggests that abuse-deterrent ER hydrocodone formulations can be effective and they are well tolerated in the treatment of chronic low back pain. However, guidelines for the management of chronic pain still report for hydrocodone the level III of clinical evidence, corresponding only to expert opinion. Although it is important to keep in mind that several studies are from corporate sponsored articles, on the basis of the assessed effectiveness of RCTs investigating on long-term therapy with ER hydrocodone in patients affected by low back chronic pain. About the evidence of the analgesic effectiveness of ER hydrocodone on other chronic pain syndromes and non-cancer non-neuropathic chronic pain, hydrocodone showed to have positive effects in open non-controlled studies and needs to be further studied to assess the real strength of results.

AUTHOR CONTRIBUTIONS

GC and EM developed the project of the study, performed the analysis, and discussed preclinical and clinical data. LC, CrM, and DQ collected and discussed clinical data. VA, CaM, and FC collected and discussed data on pharmacokinetics and pharmacodynamics.

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REVIEW
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Drug Interactions With New Synthetic Opioids

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Fentanyl, fentanyl analogs, and other new synthetic opioids (NSO) have burst onto the illegal drug market as new psychoactive substances (NPS). They are often sold as heroin to unsuspecting users and produce euphoria through their agonist action on μ- opioid receptors. Their high consumption, often combined with other substances, has led to multiple intoxications during recent years. In some countries, such as the United States, the consumption of opioids, whether for medical or recreational purposes, has become epidemic and is considered a public health problem. Fentanyl analogs are more potent than fentanyl which in turn is 50 times more potent than morphine. Furthermore, some fentanyl analogs have longer duration of action and therefore interactions with other substances and medicines can be more serious. This review is focused on the potentially most frequent interactions of opioid NPS taking into account the drugs present in the reported cases of poly-intoxication, including other illegal drugs of abuse and medication. Substances involved are mainly antidepressants, antihistamines, antipsychotics, benzodiazepines, analgesics, anesthetics, psychostimulants, other opioids, alcohol, and illegal drugs of abuse. The interactions can be produced due to pharmacokinetic and pharmacodynamic mechanisms. Naloxone can be used as an antidote, although required doses might be higher than for traditional opioid intoxications. It is crucial that doctors who habitually prescribe opioids, which are often misused by patients and NPS users, be aware of designer opioids' potentially life-threatening drug-drug interactions in order to prevent new cases of intoxication.

Keywords: interaction, fentanyl, fentanyl analogs, new synthetic opioids, new psychoactive substances

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INTRODUCTION

New Synthetic Opioids as New Psychoactive Substances (Opioid NPS)

New psychoactive substances (NPS) are compounds designed to mimic classical illegal recreational drugs (Tracy et al., 2017). Synthetized by slightly tweaking the molecular structure of an existing illegal drug or a legally prescribed medication, they have spread rapidly in recent years. As new chemical substances they are considered legal by default until outlawed which, in most cases, may

take several years after reaching the market, for this reason, they are sometimes referred to as "legal highs." Some NPS have also been called "research chemicals," psychoactive substances which have played little or no role in scientific and medical studies.

According to the United Nations Office on Drugs and Crime (UNODC) NPS are substances of abuse, either in a pure form or as a preparation, that do not come under the 1961 Single Convention on Narcotic Drugs or the 1971 Convention on Psychotropic Substances, but which may pose a public health threat (United Nations Office on Drugs and Crime [UNODC], 2018a). Not all NPS are new substances, some were synthetized 40 years ago, but all of them have recently reached the market. NPS are usually obtained through internet or specialist establishments (head shops), sold under a broad range of names and brands, and generally consumed by experienced drug users.

The chemical diversity of these products and their exponential increase complicates monitoring. Among all the various reported NPS, the opioid group does not, however, include a large amount of chemical substances in comparison with others. Between 2009 and 2017, a total of 779 NPS were reported to the UNODC with the majority from 2016, 34 were opioids, including 26 fentanyl analogs (United United Nations Office on Drugs and Crime [UNODC], 2017; United Nations Office on Drugs and Crime [UNODC], 2018c). UNODC registered 72 first-time NPS in 2017, and according to the most recent European data, a total of 51 NPS were detected in 2017, 13 of which were new opioids (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018b).

Moreover, 16.0% of the total sample (13000 respondents) of the Global Drug Survey (2018) reported lifetime use of NPS, and 5% reported previous 12-month use. In addition, the drug effects that the NPS attempted to mimic were opioid-like in 8.9% of cases (Global Drug Survey, 2017).

In Europe, 3% of students aged 15–16 years admitted to previous year use of NPS and 4% lifetime use in 2015 (European School Survey Project on Alcohol and Other Drugs, 2015).

The term opioid applies to any substance, whether endogenous or synthetic, that produces morphine-like effects. Opiates are restricted to the natural plant alkaloids, such as morphine, codeine, thebaine, and many semisynthetic derivatives. Additionally, the term novel or new synthetic opioids (NSO) has been used to refer to emerging fentanyl analogs and non-fentanyl compounds with other chemical structures, all of them included in the group of NPS opioids.

In an online survey conducted in 2015 with 619 NPS international users, among the 1551 NPS consumed 3.3% were opioids. The most common drugs in this group were Kratom (56.6%), AH-7921 (9.4%), and 0-desmethyltramadol (5.7%) (Soussan and Kjellgren, 2016).

There is a growing supply and consumption of illicitly manufactured synthetic opioids such as fentanyl, fentanyl analogs, and NSO belonging to other structural groups. The following are non-fentanyl analogs: U-47700, U-448800, U-77891, U-50488, U-51754 AH-7921, MT-45, and O-desmethyltramadol (Prekupec et al., 2017; Ventura et al., 2018).

Regarding legalization, fentanyl is a Schedule II drug under the Controlled Substances Act in the United States (Drug Enforcement Administration, 2018). Substances and chemicals included in schedule II are defined as drugs with a high potential for abuse, with consumption potentially leading to severe psychological or physical dependence. Schedule II also encompasses pharmaceutical fentanyl analogs. Some non-pharmaceutical fentanyl analogs are in Schedule I, which contains non-medical substances with high abuse potential. On the other hand, fentanyl and its pharmaceutical analogs are Schedule I substances according to the United Nations Single Convention on Narcotic Drugs 1961, amended in 1972 (substances that are highly addictive and liable to abuse). In turn, some non-pharmaceutical analogs are schedule I–IV substances, while new compounds are being progressively incorporated into the lists (United Nations Office on Drugs and Crime [UNODC], 2018b).

Epidemiology of Opioid Overdose Crisis

A worrisome increase in overdose deaths involving synthetic opioids (licit and illicit fentanyl and its analogs, and also other NSO) has been detected mainly in the United States (US) in recent years and is spreading to other countries such as Canada, Australia, and Japan, in addition to Europe.

In the 1970–1980s, synthetic opioids, such as fentanyl and its analogs, first appeared combined with heroin (China White, Tango and Cash, synthetic heroin). Since 2014 resurgence of this phenomenon has been observed, in this case with fentanyl and fentanyl analogs produced clandestinely, and also with the introduction of NSO not intended for medical use.

Non-medical use (or misuse) of a drug refers to the use, whether obtained by prescription or otherwise, other than in the manner or for the time period prescribed, or by a person for whom the drug was not prescribed (United United Nations Office on Drugs and Crime [UNODC], 2017). With respect to opioids, parallel to their dramatic increase in prescription has been their non-medical use over the last two decades in the US and Canada. It is noteworthy, however, that although opioid prescriptions have decreased recently in these countries (van Amsterdam and van den Brink, 2015; Piper et al., 2018) opioid overdose death rates continue increasing. These epidemiological findings can be explained by the rise in illicitly manufacturated fentanyl (IMF) and NSO contaminating heroin and counterfeit pain pills, leading to an epidemic of poisonings.

In Europe, the medical use of opioids also increased during the previous decade although at a slower rate, while non-medical use has been rarely reported (van Amsterdam and van den Brink, 2015). Furthermore, changes in prescribed recommended doses, or routes of administration, raise overdose risk. As an example, some individuals misuse fentanyl by extracting it from patch formulations and then injecting it, without knowing the exact dose taken (Tharp et al., 2004).

The increase in opioid overdoses can thus be explained by a rise in the consumption of synthetic opioids from different origins and by various collectives. They can be illicitly manufactured or diverted from pharmaceutical fentanyl or derivatives, and deceased subjects may be heroin addicts looking for a substitute, patients previously treated for pain, and NPS

users. The contribution of each collective depends on the country and year, and incomplete data hinders the establishment of exact figures. The situation is evolving, nowadays overdoses driven by or involving illicitly manufactured fentanyl and fentanyl derivatives are the most common, particularly in the US and Canada.

Furthermore, constant changes in NPS opioids available on the market, and analytical difficulties in identifying them (common toxicology screens do not detect NPS opioids that have little structural similarities to morphine and other commonly tested opioids), make it difficult to blame fentanyl analogs and other NSO for overdoses, as a result, underreporting is probable (Pichini et al., 2017; Baumann and Pasternak, 2018).

United States

The opioid epidemic or opioid crisis initially focused on extended-release prescription opioids. Later, heroin (greater supply and use) and IMF (mixed with heroin or alone) expanded into the context of widespread opioid prescription misuse (O'Donnell et al., 2017a,b; Schnoll, 2018).

Opioids accounted for 66.4% of all overdose deaths in the US in 2016 (Seth et al., 2018). The recent rise in synthetic opioid overdoses is largely due to IMF, and fentanyl analogs are implicated in 17% of fentanyl-related deaths (Prekupec et al., 2017). As an example, between January and February 2017, in Ohio among 281 deaths, 90% tested positive for fentanyl, 48% for acrylfentanyl, 31% for furafentanyl, 8% for carfentanil, 6% for heroin, and 23% for pharmaceutical opioids (Daniulaityte et al., 2017).

Data from October 2017 report 68,400 overdose deaths in the previous year in the US: 40,149 related to opioids, 17,027 due to synthetic opioids analgesics other than methadone (fentanyl, tramadol), 14,984 due to heroin, 14,072 due to natural opioid analgesics (morphine and codeine) and semisynthetic opioids (oxycodone, hydrocodone, hydromorphone, and oxymorphone), and 3,343 due to methadone (Ahmad et al., 2018).

Regarding trends in medical use, since 2011, opioid prescription has been decreasing, with the exception of buprenorphine, employed to treat opioid use disorder. These data reflect favorable results from reduction prescription polices. The percentage change in fentanyl prescription in 2016 was −8.9% relative to 2015. The most prescribed drugs were oxycodone, followed by hydrocodone, morphine, and codeine (Piper et al., 2018).

Another important adopted measure has been to reduce the number of days in treatment, as it correlates with the maintenance of opioid treatment 1 year later. In fact, 6% of those with at least 1 day of treatment were on opioids 1 year later, while it increased to 30% in those with at least 31 prescription days (Shah et al., 2017).

Canada

Until 2012 opioid deaths were mainly related with medically prescribed opioids (modified release oxycocone formulations). Opioid-related overdose deaths rose from 2010 to 2016, and in 2016 fentanyl products represented from 60 to 2% of all opioids depending on the province (Fischer et al., 2018). In the same year there were 2,861 apparent opioid-related deaths in Canada. From January to June 2017, 74% involved fentanyl or its analogs, compared to 53% in 2016, and 74% of deaths occurred among males and 28% among individuals aged 30–39 years. No distinction was made between pharmaceutical and non-pharmaceutical opioids (Public Health Agency of Canada, 2017).

Australia

The most common class of drug identified in toxicology reports about drug-induced deaths is opioids. From 2011 to 2015, 3,601 individuals died from overdoses due to an opioid (1.6-fold increase from 2001 to 2005). In this period accidental deaths from oxycodone, morphine, and codeine were responsible for most opioid-related deaths, followed by heroin, fentanyl, tramadol, and pethidine. A significant increase was, however, observed in fatal overdoses due to fentanyl, with an 8-fold increase over 10 years. In 2015 70% of those who died from overdose were males aged 30–59 years (Penington, 2017).

There was a 4-fold increase in pharmaceutical opioid use between 1990 and 2014. Non-medical use of pharmaceutical opioids increased over time among individuals who injected drugs. Extra-medical use of fentanyl is suspected as only fentanyl deaths increased significantly taking into account prescriptions (Roxburgh et al., 2017).

Europe (EU)

Heroin remains the most commonly used illicit opioid, but a number of sources suggest that licit synthetic opioids are increasingly misused. Opioids reported by treatment centers include misused methadone, buprenorphine, fentanyl, codeine, morphine, tramadol, and oxycodone. In 2016, in 18 EU countries more than 10% of all opioid clients entering specialized services presented opioids other than heroin (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018a).

Recently, an overall increase in opioid-related overdose deaths, as well as rising reports of problems with NSO, has been detected. The average prevalence of high-risk opioid use among adults (15–64 years) is estimated at 0.4 % of the EU population, the equivalent of 1.3 million high-risk opioid users (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2017). Opioids are found in 81% of fatal overdoses. The role that synthetic opioids play in overdose deaths is difficult to quantify, but in many countries these substances are gaining importance, and in a few predominate. Overall, 25 new opioids have been detected since 2009, including 18 fentanyls. (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2017).

In Estonia an endemic problem with fentanyl has existed since the early 2000s with high rates of overdose in comparison with other EU countries. In 2010–2012 both fentanyl and 3-methylfentanyl were marketed as a replacement for heroin in EU countries affected by heroin shortages (e.g., Bulgaria, Slovakia). More recently (2006–2012), Germany, Finland, and the United Kingdom reported new outbreaks of fentanyl-related deaths (Mounteney et al., 2015).

Between 2015 and 2017 intoxications due to fentanyl and its analogs occurred mostly in Sweden, but also in Hungary,

Belgium, Switzerland, Poland, and the United Kingdom (Pichini et al., 2018).

Although in the EU, in contrast to the US, very few patients require specialized drug treatment for addiction to opioid pain medication, underreporting cannot be dismissed. In fact, among young people 4% of students aged 15–16 years reported lifetime use of painkillers to get high (European School Survey Project on Alcohol and Other Drugs, 2015).

Data from the Early Warning System reported 250 deaths in 2016–2017 linked to fentanyls. It was the first time new opioids were the single largest group of new substances to appear. Nowadays, 38 new opioids are being monitored, 28 of them highly potent fentanyl analogs (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018a).

Japan

Due to the low consumption rate of opioids in Japan they are not among the leading causes of overdose-related deaths which are benzodiazepines and barbiturates (Okumura et al., 2017). However, several cases of intoxication with fentanyl analogs have been recently reported (Takase et al., 2016; Yonemitsu et al., 2016).

Epidemiology of Opioid Consumption With Medication and Drugs of Abuse

In most opioid overdoses other substances are found or involved. As commented before, NPS opioids cannot be easily detected in routine screen tests, however, these tests can be helpful in detecting concomitant use of other drugs.

In 2016, at least half the people who died from an opioid overdose in the US were taking fentanyl, and 57% of those with positive tests for fentanyl or analogs were also positive for other drugs such as heroin (O'Donnell et al., 2017b). In Canada approximately 82% of apparent opioid-related deaths from 2016 to 2017 also involved one or more type of non-opioid substances (mainly alcohol, benzodiazepines, cocaine, and W-18) (Public Health Agency of Canada, 2017).

A study conducted in Boston compared toxicological findings in drug overdose fatalities due to illicit fentanyl with data from accidental fatalities (licit use). There were 55 cases of illicit use, 26 of licit use, and 26 of indeterminate use. Deaths associated with illicit use occur in younger people (40 *vs.* 62 years) with higher fentanyl concentrations (17.1 vs.4.4 ng/ml) and more frequent cocaine co-intoxication (65 vs. 12%). The presence of other opioids was higher in the group of licit fentanyl (81 vs. 55%). In illicit users the most common opioids were morphine, oxycodone, and methadone, while for licit ones it was mainly morphine. Ethanol, cannabinoids, diazepam, citalopram, and diphenhydramine were each detected at rates greater than 10% in the illicit fentanyl cases (Hull et al., 2007).

Other substances involved in NSO overdoses, taking into account the published cases of intoxication (Hull et al., 2007; Lee et al., 2016; McIntyre et al., 2017; Zawilska, 2017), are listed in **Table 1**.

Illicitally manufacturated fentanyl and its analogs can be stand-alone products, low cost additives to increase the potency of other illicit drugs including heroin, cocaine, and methamphetamine, or sold as counterfeit medicines, such as oxycodone, hydrocodone, and alprazolam.

On the other hand, patients treated with opioids for pain frequently have other comorbidities and 67% of them receive other prescription drugs. A retrospective analysis showed that among patients with chronic back pain on long-term opioids, prevalence of drug–drug interactions (DDI) was 27%. Metabolic DDI involving cytochrome P450 (CYP450) were among the most common (Pergolizzi and Raffa, 2017). Furthermore, in a systematic review of opioid-related problems including 105 publications, 30% describes opioid-associated DDI (Butts and Jatoi, 2011).

CLASSIFICATION

For the purpose of this review the included NPS opioids will be fentanyl, fentanyl analogs, and NSO that are non-fentanyl analogs.

Fentanyl

Fentanyl or *N*-(1-(2-phenethyl)-4-piperidinyl-*N*-phenyl-propanamide is a piperidine derivative used in severe pain and anesthesia. It was originally synthesized by Janssen Pharmaceutical in 1974. There are several formulations of pharmaceutical fentanyl: solutions for injection, transdermal patches and lozenges, and buccal tablets. It produces analgesia, drowsiness, and euphoria, the last effect less than heroin and morphine. Common side effects are nausea, dizziness, vomiting, fatigue, headache, constipation, and edema. Overdose causes respiratory depression, miosis, and sedation, and can lead to cardiac arrest.

Prescribed fentanyl can be "diverted" in a number of ways: it can be obtained inappropriately through the individual's own profession (e.g., healthcare workers), used for a non-medically intended purpose by someone who has been prescribed it, or by employing another person's prescription.

Non-prescribed fentanyl is misused by injection, oral ingestion of lozenges, and patches. Fentanyl powder and patches are also smoked and snorted. Furthermore, patches can be taken orally (sublingually) by sectioning them into doses which requires a solvent such as overproof ethanol for sufficient absorption (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018c). IMF is also sometimes mixed with other drugs to increase potency.

Fentanyl Analog or Derivatives

Fentanyl analogs have been developed by adding various substituents to the basic molecule to modify potency. Those that have recently appeared are generated by modification or replacement of the fenanyl propionyl chain or replacement of the ethylphenyl moiety. Furthermore, existing variants have been substituted with chloro, fluoro or methoxy substituents at the *N*-phenyl ring. Some fentanyl analogs are medically used but most lack medical indication.

TABLE 1 | Other substances involved in new synthetic opioid overdoses, taking into account the published cases of intoxication (Hull et al., 2007; Lee et al., 2016; McIntyre et al., 2017; Zawilska, 2017).

Group	Substances
Anticholinergics	Dicycloverine
Antihistamines	Hidroxycine, prometazine, diphenhydramine, chlorphenyramine, doxylamine
Antiinflammatory or analgesic drugs	Ibuprofen, acetaminophen, salicylic acid, naproxen
Antidepressants	Fluoxetine, mirtazapine, citalopram, sertraline, amitriptyline, venlafaxine
Antipsychotics	Clorprormazine, risperidone, quetiapine, olanzapine, doxepin, promethazine
Anticonvulsants	Pregabalin, clonazepam, gabapentin, carbamazepine
Barbiturates	Phenobarbital
Benzodiazepines	Diazepam, alprazolam, nordiazepam, chlordiazepoxide, etizolam, lorazepam
Stimulants	Ephedrine, cocaine (benzoylecgonine), amphetamine, nicotine (cotinine), mephedrone
Other opioids	Morphine, oxycodone, 6-acetylmorphine, methadone hydrocodone, meperidine (pethidine), tramadol,6-acetylmorphine, propoxyphene, hydromorphone, codeine, tramadol, buprenorphine, heroin, dextrometorphan,
Other drugs of abuse	Ethanol, delta-9-tetrahidrocannabinol, synthetic cannabinoids, ketamine,
Other substances	Levamisole, lidocaine

Pharmaceutical Fentanyl Derivatives

The fentanyl analogs with medical use (anesthesia) are sufentanil, alfentanil, and remifentanil. Carfentanyl is only approved for veterinary issues.

Non-pharmaceutical Fentanyl Derivatives or "Designer" Fentanyls

It should be noticed that the term non-pharmaceutical fentanyl is not only restricted to fentanyl analogs without medical use. It also includes illicitly produced pharmaceutical fentanyls (described in the previous section).

There is a long list of non-pharmaceutical fentanyl analogs. Those reported in published reviews of cases of intoxication (Suzuki and El-Haddad, 2017; Zawilska, 2017; Pichini et al., 2018) are the following: alpha-methyl-fentanyl, 3-methylfentanyl, acetylfentanyl, butyrylfentanyl, beta-hydroxy-thio-fentanyl, 4-fluorobuyrylfentanyl, furanylfentanyl, ocfentanil, acrylyolfentanyl, 4-methoxibutyrylfentanyl, tetrahydr ofuranfentanyl, beta-hydroxytihiofentanyl, para-fluoroisobut yrylfentanyl, cyclopentyfentanyl, 4-fluoroisobutyrylfentanyl, and 4-chloroisobutyrylfentanyl.

Fentanyl analogs reported to the UNODC Early Warning Advisory, 2012–2016 (United United Nations Office on Drugs and Crime [UNODC], 2017) are the following: 3-fluorofentanyl, 4-fluorobutyrfentanyl, 4-methoxybutyrfentanyl, acetylfentanyl, acrylfentanyl, beta-hydroxy-thiofentanyl, butyrfentanyl, despropionylfentanyl, despropionyl-2-fluorofentanyl, furanylfentanyl, isobutyrfentanyl, (iso)butyr-F-fentanyl *N*-benzyl analog, methoxyacetylfentanyl, ocfentanil, para-fluoroisobutyrfentanyl, tetrahydrofuranylfentanyl, and valerylfentanyl.

New Synthetic Opioid Non-fentanyl Analogs

The following NSO are structurally unrelated to fentanyl and have been reported in cases of intoxication: U-47700, AH-7921, MT-45, bromadoline, U-50488, U-51754, U-77891 (Suzuki and El-Haddad, 2017; Solimini et al., 2018). Regarding their chemical structure, U-47700 (from AH-7921/doxylam), U-448800, and U-77891 are benzamides, U-50488 and U-51754 are acetamides, and MT-45 is a piperazine (Solimini et al., 2018).

Other non-fentanyl analogs included in the group of NPS opioids are desomorphine and *O*-desmethyltramadol, (Ventura et al., 2018). Desomorphine is a morphine analog and a component of krokodil, a homemade opioid synthetized from codeine.

CLINICAL PHARMACOLOGY

Mechanism of Action and Pharmacodynamics

Most NSO have mechanisms of action and effects similar to established opioids. Their main effects are mediated through activation of μ-opioid receptors (analgesia, respiratory depression, euphoria, miosis, decreased intestinal motility, sedation, addiction, and dependence). As an example, U-47700 is a potent μ-opioid receptor agonist, AH-7921 is an agonist of μ and κ receptors, and MT-45 is an agonist of μ, κ and δ opioid receptors. Interestingly antinociceptive effects of U-47700, MT-45 and butyrylfentanil have been studied in mice. These compounds act as agonists of murine μ-opioid receptor 1 but *in vitro* finding affinity does not predict *in vivo* potency (Baumann et al., 2018).

Some others, however, have different mechanisms of action. U-50488 is mainly a κ-opioid receptor agonist that has been studied in animal models as an analgesic, antitussive, diuretic, and anticonvulsant. Its side effects include dysphoria and hallucinations, and it has been reported to present μ-opioid receptor antagonist effects. U-51754 is not as selective for KOR as the previous one (Solimini et al., 2018; Ventura et al., 2018).

New synthetic opioids overdoses are characterized by the presence of the following triad: respiratory depression, stupor-sedation, and miosis. The major cause of death is respiratory failure. Additional clinical features include bradycardia, cyanosis, hypotension, pulmonary edema, ileus, nausea, vomiting, and

pruritus. Atypical characteristics in overdoses with fentanyl and analogs have been described such as immediate blue discoloration of the lips, gurgling sounds with breathing, foaming at the mouth, confusion, seizure-like activity, and chest wall rigidity. For MT-45 bilateral hearing loss, low miotic effect, hair depigmentation and loss, folliculitis, dermatitis, dry eyes, liver enzyme alteration, leukonychia striata on the nails, and cataracts have been observed (Armenian et al., 2017; Prekupec et al., 2017; Solimini et al.,2018; Ventura et al., 2018). Desomorphine when injected can cause thrombophlebitis, ulcerations, and gangrene.

Fentanyl withdrawal symptoms include anxiety, diarrhea, shivers, abdominal cramps, and sweating. Addiction and tolerance are also quickly achieved with repeated use of NSO; U-50488 abuse potential is, however, unknown (Ventura et al., 2018).

In vivo potency is usually measured by analgesic activity in rodent species. Regarding potency, fentanyl is 50–100 fold more potent than morphine and 24–40 fold more potent than heroin. The enhanced *in vivo* potency of fentanyl is most likely related to its higher lipophilicity and brain penetration when compared to morphine. Some fentanyl analogs have higher potency than fentanyl. Sufentanil and carfertanil potency in relation to morphine are 500–1,000 and 10,000 fold, respectively. Non-pharmaceutical analogs have higher potencies than morphine: butyryl-fentanyl (1.5-7), acetylfentanyl and 4-fluorobutyrfentanyl (15.7), alpha-metyl-fentanyl (56.9), octafentanil (90), 3-methyl-fentanyl (48.5-569), and beta-hydroxy-3-methylfentanyl (6,300).

Several NSO with other chemical structures have lower potencies than fentanyl: 1.7 for AH-7921, 3.5 for MT-45, and 7.5 times for U-47700 in relation to morphine (Prekupec et al., 2017; Suzuki and El-Haddad, 2017). Desomorphine is a morphine analog 10 times more potent than morphine. In turn, *O*-desmethyltramadol is 2–4 times more potent than tramadol itself (Dickman, 2007), which is ten times less potent than morphine.

Pharmacokinetics

Fentanyl can be administered through intravenous, oral, epidural, intranasal, intrathecal, and transdermal routes. Different routes of administration have also been reported for fentanyl analogs and other NSO: oral, sublingual, nasal insufflation, nasal spray, inhalation, rectal, and intravenous injections. As an example, acetylfentanyl can be smoked, snorted and intravenously injected; for butyrylfentanyl, rectal, nasal, intravenous, transdermal, and sublingual routes have been reported; and AH-7921 administration can be nasal, intravenous, insuflated, and oral (Ventura et al., 2018). Additionally E-liquids containing fentanyls can be vaped using electronic cigarettes (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018a).

The half-life and duration of fentanyl effects depend on the route of administration. Fentanyl elimination half-life is 7 h for buccal and transmucosal routes, 219 min for intravenous, and 2.63–11.7 h for transdermal (Food and Drug Administration [FDA], 2018). Fentanyl pharmaceutical derivative half-lives are 90–111 min for alfentanil, 164 min for sufentanil, and 3–10 min for remifentanil (Food and Drug Administration [FDA], 2018). No pharmacokinetic data are available for non-pharmaceutical analogs and other NSO as they have not been experimentally administered to humans.

Most opioids are lipophilic, consequentially fentanyl and its analogs can pass easily through membranes, including the blood brain barrier. Fentanyl analogs have closer chemical structures to fentanyl than morphine so low oral bioavailability is predicted. In the case of intranasal fentanyl bioavailability is 89%, while oral transmucosal administration has a bioavailability of 50% (Armenian et al., 2017).

Fentanyl has a rapid onset (2–5 min) and a short duration of action (around 1–4 h) for transmucosal, insuflated, and buccal routes, and longer for transdermal ones (48–72 h). Fentanyl pharmaceutical derivatives such as alfentanil, sufentanil, and remifentanil are all administered by intravenous route in anesthesia and have very short onset and duration of action. For NSO that are non-fentanyl analogs data from self-administration describe longer effects (6–8 h for AH-7921, 4–6 h for MT-45, and 5–7 h for U-47700 by oral route). However, for U-47700 via the intravenous route the onset is 1 min and the duration of action 1–2 h, producing a strong urge for redosing (Zawilska, 2017; Ventura et al., 2018).

Chemical reactions metabolizing a drug generally make it more water soluble and can be classified in phase I (hydrolysis, oxidation, reduction) and phase II reactions (conjugation with glucuronide, sulfate, glycine, and glutation). Opioids undergo phase I metabolism by the cytochrome P450 system, phase II metabolism by conjugation, or both. Phase I metabolism of opioids mainly involves the CYP3A4 and CYP2D6 enzymes, and glucuronidation is catalyzed by uridine diphospate glucuronosyltransferase (UGT). Most oxidative metabolism of opioids is performed in the liver, but also in enterocytes responsible for first-pass metabolism mediated by CYP3A enzyme family (Gudin, 2012).

In the following paragraphs routes of metabolism for morphine, heroin, fentanyl and its analogs, and other NSO are described.

Morphine has minimal phase I metabolism, and follows glucuronidation via UGT2B7. Its metabolism may be altered by interactions with other drugs that are either substrates or inhibitors of this enzyme. Morphine is glucuronidated to two active metabolites: morphine-6-glucuronide and morphine-3-glucuronide, and also undergo minor routes of metabolism, including *N*-demethylation to normorphine or normorphine 6-glucuronide, diglucuronidation to morphine-3, 6-diglucuronide, and formation of morphine ethereal sulfate. A minor conversion to hydromorphone has also been described (Smith, 2009).

Heroin (diacetyl-morphine or diamorphine), in turn, is rapidly deacetylated to 6-monoacetylmorphine (6MAM), then further metabolized into morphine. Both 6MAM and morphine are bioactive metabolites. In fact recent evidence suggest that 6MAM could have a major role mediating heroin effects (Gottås et al., 2013). Hydrolysis of heroin and 6MAM is thought to be catalyzed by different types of esterases (Rook et al., 2006).

Fentanyl is mainly metabolized via the CYP3A4 isoenzyme in the liver and intestinal mucosa to norfentanyl through

N-dealkylation (norfentanyl has not been reported to be pharmacologically active in animal studies). Less than 1% is metabolized to despropionylfentanyl, hydroxyfentanyl, and hydroxynorfentanyl which also lack clinically relevant activity. It has no phase II metabolism. A small amount (5–10%) is renally and fecally cleared (Smith, 2009; Food and Drug Administration [FDA], 2018).

Alfentanil follows piperidine and amide *N*-dealkylation to noralfentanil and *N*-phenylpropionamide through CYP3A4 (Klees et al., 2005; Kharasch et al., 2011). It can be used as an *in vivo* probe for hepatic and first-pass CYP3A activity, although midazolam is the reference substance. In the case of alfentanil, pupil diameter change is a surrogate for plasma concentrations, leading to noninvasive assessment of CYP3A. Sufentanil and carfentanyl are also primarily metabolized via CYP3A4 generating *N*-dealkylated metabolites (Guitton et al., 1997; Feasel et al., 2016). Remifentanil, in turn, is mainly metabolized through non-CYP enzymes. It follows rapid hydrolysis through blood esterases and the *N*-dealkylated metabolite is minor (Bürkle et al., 1996).

Little information is available about the metabolism of non-medical fentanyl analogs. Alpha-methylfentanyl, 3-methylfentanyl, and isofentanyl are also metabolized to the nor-metabolite, in a similar manner to fentanyl (Watanabe et al., 2017). 3-methylfentanyl is metabolized in rats through CYP2D6, CYP2C19, CYP3A4, and CYP3A5 (Meyer et al., 2012).

The metabolic profile of acetylfentanyl, acrylfentanyl, 4-fluoro-isobutyrylfentanyl, and furafentanyl has also been studied *in vitro* with human hepatocytes and human urine samples after consumption. The first three were predominantly metabolized by *N*-dealkylation like fentanyl. In addition, each also has a hydroxyethyl and hydroxymethoxy metabolite. Additionally, human live microsomal and *in vivo* studies in rodents have demonstrated that acetylfentanyl is converted to acetylnorfentanyl through cytochrome P450 (Patton et al., 2014).

On the other hand, furanyfentanyl major metabolites are not generated by *N*-dealkylation. It undergoes amide hydrolysis with/without hydroxylation and dihydrodiol formation, while the nor-metabolite was undetected in urine samples (Watanabe et al., 2017).

Butyrylfentanyl metabolism was studied *in vitro* in liver microsomes and with recombinant cytochrome P450 enzymes and *in vivo* in urine samples. It also has different metabolites (carboxybutyrfentanyl, hydroxybutyrfentanyl, norbutyrfentnayl, and desbutyrfentanyl) generated by hydroxylation and carboxilation of the butyryl side chain in this case, and mainly CYP2D6 and 3A4 are involved (Steuer et al., 2017).

Non-fentanyl NSO analogs such as AH-7921 follow demethylation, as U-4770, for which *N*-desmethyl,*N,N*-didesmethyl, desmethylhydroxy and *N,N*-didesmethylhydroxy metabolites in urine have been detected (Wohlfarth et al., 2016; Jones et al., 2017; Fleming et al., 2017). Additionally, desomorphine is metabolized by CYP3A4 and UGT, leading to desomorphine glucuronide as the main metabolite (Ventura et al., 2018) while tramadol is metabolized through CYP2B6, CYP2D6, and CYP3A4, with *O*- and *N*-demethylated to five different metabolites. Of these, *O*-desmethyltramadol (desmetramadol) is the most relevant since it has 200 times the μ-affinity of (+)-tramadol. Desmetramadol is metabolized into the active metabolite *N,O*-didesmethyltramadol via CYP3A4 and CYP2B6. The inactive metabolite *N*-desmethyltramadol is metabolized into the active metabolite *N,O*-didesmethyltramadol by CYP2D6 (Gong et al., 2014).

Opioids metabolized through CYP450 or glucuronized can be affected by hepatic disease, and dose reductions may be necessary. Regarding fentanyl, its half-life is not affected significantly in the case of hepatic impairment (Haberer et al., 1982). Nevertheless, dose adjustments are recommended for transdermal fentanyl and, because of its long half-life, its use is not recommended in severe hepatic impairment. The same is applicable to fentanyl pharmaceutical analogs (Food and Drug Administration [FDA], 2018).

Regarding clearance of opioids, while the liver is responsible for the biotransformation of most of them, renal excretion is predominant. Fentanyl excretion in renal impairment is less affected than other opioids such as morphine. With morphine, a significant accumulation of glucuronide metabolites has been observed producing serious adverse effects (Smith, 2009).

NEW SYNTHETIC OPIOID DRUG INTERACTIONS

Opioids have a narrow therapeutic index, wide interindividual response variability, and potentially life-threatening toxicity. They can, therefore, lead to clinically relevant DDI.

Their strong potency in comparison to morphine, the prolongation of effects (for some NSO), and their frequent use in combination with other drugs increases the risk of serious drug interactions which can result in toxicity (respiratory depression) and opiate withdrawal symptoms (in the case of previously developed opioid tolerance).

Drug–drug interactions with opioids can be classified in two groups: pharmacodynamic (a) and pharmacokinetic (b) drug interactions.

(a) Pharmacodynamic DDI refer to interactions in which drugs influence each other's effects directly. In this case, when two drugs are co-administered the concentration-response curve of one or both is altered without a change in the object drug pharmacokinetics (Overholser and Foster, 2011).

Additive interaction means that the effect of the two chemicals is equal to the sum of the effect of the two drugs taken separately, while synergistic interaction means that the effect of two drugs taken together is greater than the sum of their separate effect at the same doses. On the other hand, an antagonistic interaction occurs when the effect of two drugs is actually less than the sum of the effect of the two drugs taken independently.

(b) Pharmacokinetic DDI occur when a drug A (precipitant drug) interferes in the absorption, distribution, metabolism, or excretion of drug B (object drug). Clinically relevant ones mainly affect opioid hepatic metabolism and P-glycoprotein (P-gp). Changes in concentrations due to pharmacokinetic DDI can translate into alterations in opioid effects (increase or reduction of therapeutic or toxic effects).

As most opioids are metabolized by one or more of the CYP450 isozymes (primarily by CYP3A4 and CYP2D6), they can potentially interact with prescription and over-the-counter medication, drugs of abuse, herbal remedies, and dietary supplements that are inducers or inhibitors of them. As an example, CYP3A4 can be induced by garlic and St. John's Wort while grapefruit inhibits CYP3A4 (Gudin, 2012). In turn, cocaine is a substrate and a weak inhibitor of CYP3A4, and also inhibits CYP2D6, while MDMA is a substrate and an inhibitor of CYP2D6 (O'Mathúna et al., 2008; Lindsey et al., 2012).

When opioids are metabolized active and non-active metabolites appear; the inhibition of this metabolism results in an increase in plasma parent opioid concentration and a reduction of metabolites. The rise in parent drug concentration can lead to an increase of drug therapeutic/toxic effects and intoxication. In the case of inhibiting the metabolism of a pro-drug (only metabolites are active) contrary effects can be observed. Furthermore, if several drugs have the same metabolizing isozyme in common, competitive inhibition can occur among them and result in higher concentrations of one or more. In general, however, if there is no competitive inhibition CYP450 is able to metabolize two substrates of the same isozyme at the same time. Other drugs can inhibit CYP450 isozymes by different mechanisms and even without being substrates. According to the most recent FDA Guidance for Industry of Drug interaction studies, a strong, moderate, and weak inhibitor can increase the area under the curve of concentrations of a sensitive index CYP substrate $\geq$ 5-fold, $\geq$2-to $<$ 5-fold, and $\geq$1.25- to $<$2-fold, respectively (Food and Drug Administration [FDA], 2017).

Induction of opioid metabolism results in decreased opioid plasma concentrations and may lead to reduced effects (unless the interfered drug is a pro-drug) (Pergolizzi and Raffa, 2017). As previously commented, fentanyl does not have clinically relevant active metabolites, as a result, metabolism inhibition can lead to intoxication and induction to a reduction of effects.

A strong inducer decreases the area under the curve of concentrations of a sensitive index by $\geq$80%, a moderate one by $\geq$50% to $<$80%, and a weak one by $\geq$20% to $<$50% (Food and Drug Administration [FDA], 2017). While inhibition can manifest itself immediately, induction requires increased enzyme formation (due to upregulation of enzyme expression) and takes from days to weeks to reach maximum effect and then disappear.

Those opioids metabolized by CYP3A4 have a high risk of DDI (for example, fentanyl), while those metabolized by CYP2D6 have an intermediate risk (for instance, codeine and hydrocodone). CYP2D6 does not respond to induction, consequently, DDI induction with opioids is limited to those metabolized through CYP3A and CYP2B6 (Overholser and Foster, 2011). Clinically relevant interactions are more frequent with strong-moderate inhibitors/inducers than weak ones.

On the other hand, opioids that undergo phase II conjugation, such as morphine, oxymorphone, tapentadol, and hydromorphone, have minimal pharmacokinetic interaction potential. However, interactions can also occur with other drug substrates of enzymes responsible for glucuronidation, such as UGT2B7, or due to polymorphisms in the genes coding those enzymes (Smith, 2009; Gudin, 2012).

In addition, genetic polymorphisms of cytochrome P450 isozymes and genetic variations of the receptors can modulate the effects of drug interactions. As an example, different CYP2D6 metabolizing phenotypes exist: ultrarapid metabolizer, extensive metabolizer, intermediate metabolizer, and poor metabolizer. It has been described that poor metabolizers can have fewer analgesic effects on pro-drugs such as codeine and tramadol which need O-demethylation into morphine, or *O*-desmethyltramadol mediated by CYP2D6, respectively.

As previously commented, pharmacokinetic drug interactions can also occur with drug absorption. P glycoprotein (P-gp) inhibitors (quinidine) can increase concentrations of fentanyl and morphine, while inducers (rifampin) can reduce morphine absorption (Feng et al., 2017).

Interactions of New Synthetic Opioids With Medication

Fentanyl, Morphine, and Pharmaceutical Fentanyl Analogs

Pharmaceutical opioids including morphine, fentanyl, and some fentanyl analogs can interact with a broad spectrum of drugs. **Table 2** depicts DDI described in the product label information of Sublimaze® (fentanyl), Duragesic® (fentanyl), Fentora® (fentanyl), Actiq® (fentanyl), Alfenta® (alfentanil), Rapifen® (alfentanil), Sufenta® (sufentanil), Ultiva® (remifentanil), Avinza® (morphine sulfate), Embeda® (morphine sulfate and naltrexone hydrochloride), and Duramorph® (morphine sulfate) (Food and Drug Administration [FDA], 2018). The table has been completed with information from reference literature on drug interactions (Mozayani and Raymon, 2004; Baxter, 2008; Preston, 2015; Karalliedde et al., 2016).

Regarding pharmacodynamic DDI, fentanyl and its pharmaceutical analogs interact with other central nervous system (CNS) sedative drugs such as antihistamines, benzodiazepines, barbiturates, tranquilizers, anesthetics, antipsychotics, and other opioids, increasing their effects.

On the other hand, precipitation of withdrawal can be observed with opioid non-selective antagonists including naloxone, naltrexone, and nalmefene and, in exceptional circumstances, with peripheral μ-opioid antagonists such as methylnaltrexone, naloxegol, and alvimopan (European Medicines Agency [EMA], 2018; Food and Drug Administration [FDA], 2018).

Co-administration of fentanyl with CYP3A4 inducers and inhibitors should be avoided; the same is applicable for pharmaceutical fentanyl analogs. Morphine, in turn, is mainly metabolized through UGT2B7 and minimal pharmacokinetic changes have been described with UGT inhibitors (Overholser and Foster, 2011).

Opioid-induced constipation is predominantly mediated by gastrointestinal μ-opioid receptors. Selective blockade of these peripheral receptors might relieve constipation without compromising the centrally mediated effects of

TABLE 2 | Drug–drug interactions with morphine, fentanyl and other pharmaceutical analogs (Mozayani and Raymon, 2004; Baxter, 2008; Preston, 2015; Karalliedde et al., 2016; Food and Drug Administration [FDA], 2018; European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2018a).

Opioid	Interacting medication	Type of drug–drug interaction	Result
Alfentanil Fentanyl Morphine Sufentanil	Anticholinergic drugs	PD: Additive or synergistic.	Increase the risk of urinary retention and severe constipation (paralytic ileus).
Fentanyl	Amiodarone	Uncertain.	Profound braycardia, sinus arrest, hypotension.
Fentanyl Morphine	Baclofen	PD: Additive or synergistic.	Enhances analgesic effect, sedation and risk of respiratory depression of opioid
Alfentanil Fentanyl Morphine Remifentanil Sufentanil	Benzodiazepines and other CNS depressants (barbiturates, tranquilizers, anesthetics, antypsychotics, other opioids, alcohol, antihistamines)	PD: Synergistic.	CNS and cardiovascular effects of opioids may be enhanced (hypotension, respiratory depression, profound sedation, coma, death)
Morphine	Cimetidine	PK: Possible decrease in morphine metabolism	Potentiate morphine effects and increase the risk of hypotension, respiratory depression, profound sedation, coma and death.
Alfentanil Fentanyl	Cimetidine	PK: Inhibition of opioid CYP34A mediated metabolism.	Cimetidine reduces opioid clearance, extending the duration of action
Alfentanil Fentanyl Sufentanil	CYP3A4 inhibitors (macrolide antibiotics, azole antifungical agents, HIV- protease inhibitors, grapefruit juice, dialtiazem)	PK: Inhibition of opioid CYP3A4 mediated metabolism.	Increase in plasma concentrations of opioid, increase or prolongation of effects. After stopping inhibitor, concentrations of opioid decrease, resulting in decreased efficacy or withdrawal in patients who have developed physical dependence.
Alfentanil Fentanyl Sufentanil	CYP3A4 inducers (rifampin, carbamazepine, phenytoin)	PK: Induction of opioid CYP3A4 mediated metabolism.	Reduction in plasma concentrations of opioid, decreased efficacy or onset of a withdrawal syndrome. After stopping inducer, concentrations of opioid increase, which could increase or prolong effects or adverse reactions of opioid, and may cause serious respiratory depression.
Fentanyl	Darafenib	PK: Induction of opioid CYP3A4 mediated metabolism.	Possible reduced concentrations of fentanyl.
Alfentanil Fentanyl Morphine Sufentanil	Diuretics	PD: Antagonism.	Reduction of diuretic effects because opioids induce release of antidiuretic hormone.
Morphine	Esmolol	PK: Unknown.	Increase in plasma concentrations of esmolol.
Morphine	Estrogens	PK: Increase metabolism of morphine (glucuronyl transferase).	Effect of morphine may be reduced, requiring higher doses.
Morphine	Ethanol	PK: Inhibition of morphine glucuronidation.	Increase in morphine plasma concentrations, potentially fatal overdose of morphine.
Alfentanil Fentanyl Morphine	Imatinib, nilotinib	PK: CYP3A4 and CYP2D6 inhibition (imatinib). CYP3A4 and P-gp inhibition (nilotinib)	Increase in plasma concentrations of opioid.
Alfentanil Fentanyl Morphine Sufentanil Remifentanil	MAOI (phenelzine, tranylcypromine, linezolid)	PD: Additive or synergistic. PK: MAOI inhibition of opioid metabolism (more common for morphine)	Opioid toxicity (respiratory depression, coma). Potentiation of MAOI effects (hypertension).
Morphine	Metformin	PK: Competition for renal tubular excretion.	Increase in metformin concentrations and risk of lactic acidosis.
Morphine	Metoclopramide	PK: Increase of gastric emptying with metoclopramide.	Increase speed of onset and effect of oral morphine.
Morphine	Mexiletine	PK: Reduction of mexiletine absorption due to a delay in gastric emptying produced by morphine.	Decreased plasma concentrations of mexiletine

(Continued)

TABLE 2 | Drug–drug interactions with morphine, fentanyl and other pharmaceutical analogs (Mozayani and Raymon, 2004; Baxter, 2008; Preston, 2015; Karalliedde et al., 2016; European Medicines Agency [EMA], 2018; Food and Drug Administration [FDA], 2018).

Opioid	Interacting medication	Type of drug–drug interaction	Result
Alfentanil Fentanyl Morphine Sufentanil	Muscle relaxants	PD: Additive or synergistic.	Enhancement of muscle blocking action of muscle relaxants, increased degree of respiratory depression.
Fentanyl	Neuroleptics	PD: Additive or synergistic. Unexplained alterations in sympathetic activity following large doses.	Elevated blood pressure.
Alfentanil Fentanyl Sufentanil	Nitrous oxide	PD: Additive or synergistic.	Cardiovascular depression
Alfentanil Fentanyl Morphine Sufentanil Remifentanil	Opioids partial agonists or mixed agonist/antagonists (butorphanol, nalbuphine, pentazocine, buprenorphine)	PD: Antagonism.	Reduction of analgesic effect of full agonists, precipitation of withdrawal.
Opioid agonists	Opioid non-selective antagonists (naloxone, naltrexone, nalmefene)	PD: Antagonism.	Naloxone is indicated for treatment of opioid overdose and naltrexone for opioid use disorders. Antagonists can precipitate opiate withdrawal.
Opioid agonists	Peripherally acting μ-opioid receptor antagonists (methylnaltrexone, naloxegol, alvimopan)	PD: Antagonism	Methylnaltrexone and naloxegol are indicated for reduction of opioid constipation. May produce withdrawal and/or reduction of analgesic effect if blood brain barrier is altered. Alvimopan is indicated to accelerate the time to recovery following partial bowel resection surgery with primary anastomosis.
Morphine	P-glicoprotein inhibitors (quinidine)	PK: Inhibition of P-gp.	Increase the exposure to morphine 2-fold, and can increase risk of hypotension, respiratory depression, profound sedation, coma and death.
Alfentanil Fentanyl Morphine Sufentanil Remifentanil	Serotoninergic drugs (SSRIs, SNRIs (duloxetine), TCAs, MAOI, triptans, 5-HT3 receptor antagonists, mirtazapine, trazodone, tramadol, linezolid, intravenous methylene blue. lythium)	PD: Additive or synergistic.	Serotonin syndrome.
Fentanyl Morphine	TCAs	PK: Inhibition of CYP2D6-fentanyl metabolism. Unknown for morphine.	Increase in opioid plasma concentrations, may increase effects.

MAOI, monoamine oxidase inhibitors; PK, pharmacokinetic interaction; PD, pharmacodynamic interaction; SNRIs, Serotonin and norepinephrine reuptake inhibitors; SSRIs, selective serotonin reuptake inhibitors; TCAs, tricyclic antidepressants.

opioid analgesia or precipitating withdrawal. Two of these drugs are methylnaltrexone (Relistor®) and naloxegol (Movertig®), approved by the EMA (European Medicines Agency [EMA], 2018). Patients with blood-brain barrier alteration may have opioid withdrawal symptoms or experience reduction in the analgesic effects of opioids when using these peripheral antagonists. Alvimopan (Entereg®) is another peripheral antagonist approved by the FDA, indicated in this case for treatment of post-operative ileus induced by opioid agonists (Food and Drug Administration [FDA], 2018).

In abuse-deterrent oral preparations, a mixture of an agonist (oxycodone, buprenorphine) and an antagonist (naloxone, naltrexone) is employed in order to reduce the abuse of these combinations by intravenous route. An antagonist is added to be released upon manipulation and interfere with, reduce, or defeat the euphoria associated with abuse. For instance, naloxone is poorly absorbed when taken orally or sublingually and it is added to decrease the risk that the medication will be misused through injection (Papaseit et al., 2013; Argoff et al., 2014; Lee et al., 2017).

Non-pharmaceutical Fentanyl Analogs and Other NSO

Taking into account the DDI described for fentanyl and its pharmaceutical analogs, some interactions with other NPS opioids can be anticipated.

Theoretically, pharmacodynamic interactions can be observed with non-medical NSO and benzodiazepines and other CNS depressants (barbiturates, opioids, tranquilizers, anesthetics, antipsychotics, and antihistamines) due to their synergistic effects on CNS depression. Antagonistic effects can appear with NPS opioid-full agonists and other opioid-mixed agonists or mixed agonists/antagonists, and also with full antagonists, for instance, naloxone, naltrexone, and nalmefene.

Insufficient information is available about the metabolism of these substances, as a result, the pharmacokinetic interactions are more difficult to predict. In general, precaution is mandatory

when NSO are administered with CYP3A4/CYP2D6 inhibitors and CYP3A4 inducers.

Interactions of New Synthetic Opioids With Drugs of Abuse

Few experimental human data are available regarding interactions of NPS opioids with other drugs of abuse.

In general, the association of other opioids with heroin and alcohol increases CNS depression which can lead to serious side effects including respiratory distress, coma, and even death (Food and Drug Administration [FDA], 2018).

Frequently opiate abuse includes combinations with stimulants ("speedball" or "bombeta"). The most popular mixture among drug injectors is heroin with cocaine or amphetamines in the same syringe. A new trend is fentanyl-laced cocaine (adding fentanyl to cocaine) for the purpose of speedballing, to combine the rush of the stimulant (cocaine) with a drug that depresses the CNS (fentanyl) thus helping to ease the after effects. Furthermore it should be noticed that most users are inadvertently exposed to fentanyl or other NSO when cocaine or other drugs of abuse are laced with them. In this cases, a drug interaction can also occur and the user can have unexpected adverse effects.

Amphetamines when combined with opiates enhance the sense of euphoria (Atkinson and Fudin, 2014). Dexamphetamine and methylphenidate increase the analgesic effects of morphine and other opioids and reduce their sedative and respiratory depressant effects (Mozayani and Raymon, 2004; Baxter, 2008).

Additionally, amphetamine-like drugs with certain opioids may increase the risk of serotonin syndrome through effects on the serotonin transporter or serotonin receptors. Among reported cases, tramadol and fentanyl are the most frequently involved, while morphine is merely anecdotal and there are no cases with heroin (Rickli et al., 2018).

Heroin, Fentanyl, and Morphine

Heroin can interact with other drugs of abuse such as alcohol and psychostimulants. The combination of heroin and alcohol produce a sensation of greater drug pleasure and stronger "high" than the two drugs on their own. Furthermore, the inhibition of heroin metabolism by high doses of ethanol is suggested due to an increase in free morphine in the blood/total morphine in blood ratio (Polettini et al., 1999). In fact, ethanol inhibits two steps of the heroin metabolism, the hydrolysis of 6MAM to morphine, and the glucuronidation of morphine to morphine-3-glucuronide and morphine-6-glucuronide (Thaulow et al., 2014).

Heroin enhances cocaine-rewarding effects (Hemby et al., 1999) and reduces cocaine- induced anxiety or agitation while cocaine tempers opiate-induced sedation. In this case, a pharmacodynamic interaction seems to prevail as no changes in the ratio morphine in blood/total morphine in blood have been found (Polettini et al., 2005).

The combination of heroin and methamphetamine has been reported to produce a significant "high." Enhanced rewarding effects and higher stimulation of behavior than each drug on its own have been reported in animal models (Ranaldi and Wise, 2000; Trujillo et al., 2011). D-amphetamine increases the effects of heroin. In fact, opiate abusers employ amphetamines to increase the effects of poor quality heroin (Mozayani and Raymon, 2004).

Serious interactions can also occur between fentanyl combined with cocaine, heroin, and alcohol. At a pharmacodynamic level, fentanyl and alcohol exert a synergic depressive action on the cardio-circulatory system while cocaine does the opposite, exciting it, constricting the arteries, and inducing hyperkinetic cardiac arrhythmia (Ferrara et al., 1994). Furthermore, the use of alcohol with fentanyl may increase nervous system side effects such as drowsiness, dizziness, lightheadedness, difficulty concentrating, and impairment in thinking and judgment. In severe cases, low blood pressure, respiratory distress, fainting, coma, or even death may occur (Food and Drug Administration [FDA], 2018).

A recent study conducted in rats demonstrates that the effects of heroin plus fentanyl on brain oxygenation and temperature are enhanced when compared to the effects of either drug alone, providing evidence for synergism (Solis et al., 2018).

No human experimental studies have been identified studying the interaction of fentanyl and psychostimulants (cocaine, methamphetamine, and amphetamine).

Regarding morphine, it administration with cocaine in healthy volunteers increased cardiovascular and subjective effects, but less than predicted, and no changes were found in blood concentrations (Foltin and Fischman, 1992). In patients with postoperative and chronic malignant pain, the administration of cocaine with morphine did not increase analgesic response. Interaction effects were observed in terms of positive changes (cheerful, friendly) in postoperative patients, but negative ones (sad, serious) in patients with chronic pain (Kaiko et al., 1987).

Cannabis enhanced the effects of morphine in chronic pain (Lynch and Clark, 2003), smokers (nicotine), however, may require more opioid (morphine, fentanyl) analgesics for postoperative pain than non-smokers. It has been suggested that smoking may have an effect on pain perception and/or opioid response (Baxter, 2008).

On the other hand, administration of morphine with amphetamine to healthy volunteers showed an increase in opiate symptoms, liking and euphoria scale scores which were greater than the effects of either drug alone. Physiological effects were mutually antagonistic in pupillary effects, respiratory rate, temperature and pulse rate, though morphine had little or no effect on the amphetamine-induced blood pressure increases. The combination has a greater potential to be abused because of the additive euphoria and a lessening of side effects (Jasinski and Preston, 1986).

The use of two or more opioid μ-agonists can increase pharmacological and toxic effects whilst a μ-agonist with a μ-antagonist can reduce them. In fact, naloxone is the treatment of choice to address acute opioid toxicity. The simultaneous administration of a μ-agonist (such as fentanyl, morphine, or heroin) and a partial agonist (buprenorphine) or an antagonist can precipitate a withdrawal in addicted subjects. This can be observed with non-selective antagonists including naloxone, naltrexone, and nalmefene and, in exceptional

circumstances, with peripheral mu-opioid antagonists such as methylnaltrexone, naloxegol, and alvimopan (European Medicines Agency [EMA], 2018; Food and Drug Administration [FDA], 2018).

Pharmaceutical, Non-pharmaceutical Fentanyl Analogs, and Other NSO

Interactions between NPS opioids, other than fentanyl, and drugs of abuse can be extrapolated from those described for morphine, fentanyl, and heroin. Therefore, serious pharmacodynamic interactions can be anticipated between NSO mixed with heroin, alcohol, cocaine and other psychostimulants, and also with opioid antagonists.

Additionally, potential pharmacokinetic interactions can occur, with unknown clinical relevance, between cocaine, methamphetamine or MDMA, and NSOs using CYP2D6 as the main metabolic route. As previously mentioned, cocaine is a strong inhibitor of CYP2D6 (Lindsey et al., 2012) while MDMA acts as a high affinity substrate and a potent mechanism-based inhibitor of CYP2D6 (O'Mathúna et al., 2008). In turn, methamphetamine *N*-demethylation leading to amphetamine, and aromatic hydroxylation producing 4-hydroxymethamphetamine, are partially regulated by CYP2D6 (de la Torre et al., 2012).

CLINICAL ASSESSMENT AND TREATMENT

General recommendations to address the current opioid crisis are based on three main measures. The first is to reduce the supply of both licit and illicit opioids, the second to increase accessibility to evidence-based treatment of opioid use disorders (decrease the demand), and the last to provide overdose reversal medication (Schnoll, 2018).

Focusing on the misuse of non-prescribed opioids, over 20% of patients on chronic opioid therapy have a opioid use disorder (Boscarino et al., 2015). To reduce the risks associated with long-term opioid therapy, including opioid use disorder and overdose, several recommendations are available. Centers for Disease Control and Prevention guidelines for prescribing opioids for chronic pain advise clinicians to continue therapy only if there is a clinically meaningful improvement in pain and function that outweighs risks (Dowell et al., 2016).

There are some factors that increase the risk of developing addiction to analgesic opioids. They are related to individual aspects and the pharmacological characteristics of opioids. Among the individual factors are the following: (a) genetics: some variants in opioid receptor genes OPRM1, OPRK1, and OPRD1 and preproenkephalin that modulate the perception of pain, can increase the risk of further development of addiction (Khokhar et al., 2009); (b) a history of depression, anxiety (panic, social phobia, agoraphobia, posttraumatic stress disorder) and other substance use disorders (alcohol, benzodiazepines, cannabis, cocaine); (c) sociodemographics: women are at greater risk in relation to emotional and affective problems (e.g., depression), while men are at greater risk in relation to problematic and/or illegal behavior; also lower age is linked to more risk; (d) perception of pain: patients with more subjective pain, multiple complaints of pain, and greater limitations related to pain, have more risk. Finally, as previously described, among the pharmacological characteristics of opioids their fast absorption and/or short half-life multiply the risk of misuse. Although none of these factors alone increases the risk of aberrant behavior in a particular individual, when occurring simultaneously they can play a major role.

It is crucial to employ immediate-release opioids when commencing treatment instead of extended-release/long-acting opioids, because in the last formulations there are higher amounts of opioids. There is, however, contradictory information comparing short-acting and long-acting opioids due to different dosages administered (Chou et al., 2015). The lowest effective dosage should be prescribed. For chronic pain clinicians should evaluate benefits and harm within 1–4 weeks of initiation or dose escalation of treatment, and at least every 3 months after that. When opioids are used for acute pain, again the lowest effective dose of immediate-release opioids should be used, for 3 days or less in most occasions (Dowell et al., 2016). Other recommendations when prescribing opioids for pain treatment in patients with a history of substance use disorders, are to prescribe tamper-deterrent formulations (e.g., crush-resistant tablets) or combinations of ingredients (combination of agonist/antagonist), the use of a sequestered aversive agent, a pro-drug, and a novel delivery system (Papaseit et al., 2013; Argoff et al., 2014; Lee et al., 2017). There are contradictory results regarding the type of opioid prescribed, as some reviews recommend prescribing weaker ones, such as tramadol (Chou et al., 2015), other authors, however, found a higher risk of abuse with this type of medication compared to strong opioids (Higgins et al., 2018). Several tools are available that can help identify patients at risk. They include the following: the Opioid Misuse Measure (Melzer et al., 2011), the Opioid Misuse Measure (COMM) (Butler et al., 2010), the Opioid Risk Tool (Webster and Webster, 2005), the Screener and Opioid Assessment for Patients with Pain (Butler et al., 2008), and the Brief Risk Interview (Wu et al., 2006).

Furthermore, there are monitoring strategies to assess problematic use (misuse, abuse, addiction) in such patients including urine drug testing, medication counts, prescription drug monitoring programs, and blood level monitoring although their effectiveness has not yet been well-established (Voon et al., 2017).

Treatment of Overdoses: Naloxone

Regarding opioid overdose, clinical presentation is recognized by the classic opioid triad typically characterized by pinpoint pupils, unconsciousness, and respiratory depression which can lead to brain damage or even death. Currently, naloxone, a short-acting, broad opioid receptor antagonist, is the only useful pharmacological treatment when administered shortly after overdose (Boyer, 2012). In low doses naloxone can reverse opioid side effects without significantly reversing analgesia. At high doses, however, naloxone can block opioid analgesia causing precipitated opioid withdrawal (Levine et al., 1979).

Naloxone is available as a solution for intravenous, intramuscular, subcutaneous, and orotracheal injection, and as a spray for nasal administration (Narcan® Nasal Spray and Nyxoid®) as an alternative to intramuscular or subcutaneous auto-injection (Evzio®) (Food and Drug Administration [FDA], 2018; European Medicines Agency [EMA], 2018). The manufacturers recommended an initial dose of 2–4 mg intranasally or 0.4–2 mg intramuscularly/subcutaneously to be repeated after 2–3 min as needed. There is, however, no consensus or recommendation on which of the doses should be selected in a given case of opioid overdose. Because the half-life and the duration of naloxone effects after administration are short there is a risk of recurrence of respiratory depression or inadequate response following reversal with naloxone when treating the effects of long-acting, high-dose or potent NSOs (Rzasa Lynn and Galinkin, 2018). Naloxone should be administrated at a dose of 0.04 mg intravenously with upward titration to 10 mg for subjects with opioid-induced respiratory compromise (Hoffman et al., 2015). There is little evidence that naloxone doses required to treat fentanyl, its analogs and other NSO overdoses might be higher. In fact, empirical data from previous fentanyl epidemics in the US show that doses of naloxone up to 12 mg were required to rescue subjects intoxicated with fentanyl (Schumann et al., 2008).

American Heart Association (2015) incorporated new concepts regarding opioid overdose management, education, and naloxone training and distribution. It included empiric administration of intramuscular/intranasal naloxone to all unresponsive subjects of possible opioid-associated life-threatening emergencies as an adjunct to standard first aid and non-healthcare provider basic life support protocols. For patients with known or suspected opioid overdose presenting a definite pulse but no normal breathing or only gasping (i.e., a respiratory arrest), intramuscular or intranasal naloxone empiric administration by appropriately trained rescuers is also reasonable. Responders should not delay access to more advanced medical services while awaiting the patient's response to naloxone or other interventions (American Heart Association, 2015).

Additionally, nalmefene, an opioid antagonist analog of naltrexone now discontinued, was also approved through injection (Revex®) by the FDA for the management of known or suspected opioid overdose (Food and Drug Administration [FDA], 2018). At this moment it is approved orally by the EMA (Selincro®) to reduce alcohol consumption in alcohol dependence (European Medicines Agency [EMA], 2018).

Other possible drugs or strategies under research to treat respiratory depression are 5-hydroxytryptamine type 1A agonists, ampakines, and phrenic-nerve-stimulation devices. In addition, technology to detect overdose and autoinjected naloxone is being studied (Volkow and Collins, 2017).

New Synthetic Opioid Use Disorders and Treatment

At present, effective pharmacological treatment for opioid use disorders to reduce cravings and withdrawal symptoms include methadone (agonist) and buprenorphine (partial agonist), both of which are listed by the World Health Organization (WHO) as essential medicines, and naltrexone (an opioid antagonist) that blocks the effects of opioids (Volkow, 2018; Volkow et al., 2018). In addition, methadone and buprenorphine have been shown to increase adherence to antiretroviral therapy in HIV-infected drug users and treatment retention of opioid-dependent pregnant women (Bisaga et al., 2018). They are available as extended-release, transdermal, and long-lasting formulations (e.g., extended-release naltrexone, buprenorphine implant, long-acting injectable naltrexone).

Regarding psychosocial interventions, there is a lack of evidence on the efficacy of concurrent psychotherapy (mindfulness and cognitive behavioral treatments) in the management of chronic pain and opioid misuse (Eilender et al., 2016).

To combat the opioid crisis, besides optimizing the treatment of overdoses and continuing advances in the field of abuse deterrent formulations (10 approved by the FDA: 3 oxycodone, 1 oxycodone + naloxone, 1 oxycodone + naltrexone, 2 morphine, 1 morphine + naltrexone, and 2 hydrocodone products) the development of new pharmacological medication and strategies to manage opioid use disorders is crucial (Becker and Fiellin, 2017).

Current research is focused on brain-stimulation technologies, vaccines, and monoclonal antibodies, together with other drugs such as lorcaserin (5-HT2c antagonist) that have been shown to reduce opioid seeking in rodents, and lofexidine (α2A-adrenergic receptor agonist) to control withdrawal symptoms (Volkow and Collins, 2017).

CONCLUSION

New synthetic opioids are frequently used with other substances such as illegal drugs and medication. Their combination can lead to clinically relevant interactions and serious intoxications. Pharmaceutical fentanyl analog interactions with CYP3A4 inducers and inhibitors have been described. Pharmacokinetic interactions with non-medical fentanyl analogs and new compounds are, however, difficult to predict because there are limited data regarding their pharmacokinetics and metabolism. On the other hand, pharmacodynamic interactions between NSO and other drugs acting on the CNS can be anticipated. Effects are expected to be similar to those reported for heroin, morphine, and fentanyl, as they also act mainly as μ-opioid receptor agonists. In the case of opioid overdose due to a DDI, naloxone can be used as an antidote, taking into account the fact that the required doses might be higher than for traditional opioids.

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Postmortem Toxicology of New Synthetic Opioids

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One hundred fifteen Americans die every day from opioid overdose. These overdose fatalities have been augmented by the increased availability of potent synthetic opioids, such as fentanyl and its derivatives. The death rate of synthetic opioids, other than methadone, increased by 72.2% from 2014 to 2015, and doubled from 2015 to 2016, situating the USA in the midst of an opioid overdose epidemic. The analytical identification of these opioids in postmortem samples and the correct toxicological data interpretation is critical to identify and implement preventive strategies. This article reviews the current knowledge of postmortem toxicology of synthetic opioids and the chemical and pharmacological factors that may affect drug concentrations in the different postmortem matrices and therefore, their interpretation. These factors include key chemical properties, essential pharmacokinetics parameters (metabolism), postmortem redistribution and stability data in postmortem samples. Range and ratios of concentrations reported in traditional and non-traditional postmortem specimens, blood, urine, vitreous humor, liver and brain, are summarized in tables. The review is focused on fentanyl and derivatives (e.g., acetyl fentanyl, butyryl fentanyl, carfentanil, furanyl fentanyl, 4-methoxybutyrylfentanyl, 4-fluorobutyrylfentanyl, ocfentanil) and non-traditional opioid agonists (e.g., AH-7921, MT-45, U-47700). All of these data are critically compared to postmortem data, and chemical and pharmacological properties of natural opioids (morphine), semi-synthetic (oxycodone, hydrocodone, hydromorphone, and oxymorphone), and synthetic opioids (methadone and buprenorphine). The interpretation of drug intoxication in death investigation is based on the available published literature. This review serves to facilitate the evaluation of cases where synthetic opioids may be implicated in a fatality through the critical review of peer reviewed published case reports and research articles.

Keywords: opioids, synthetic opioids, fentanyl, postmortem toxicology, blood

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INTRODUCTION

Opioid overdose deaths continue to increase in the United States, killing more than 42,000 people in 2016. The opioids detected in these cases, in increasing order, were methadone, natural and semi-synthetic opioids (e.g., oxycodone, hydrocodone), heroin and synthetic opioids (e.g., fentanyl, fentanyl-analogs). Synthetic opioids (excluding methadone) and heroin deaths specifically experienced a sharp increase from 2015 to 2016 (20 and 100%, respectively) (Seth et al., 2018). Fentanyl and its derivatives have been increasingly present as adulterants mainly in heroin,

but also in other drugs such as cocaine and synthetic cannabinoids (Coopman and Cordonnier, 2017; Armenian et al., 2018), due to their ease of manufacturing and readily available precursors shipped from China (Armenian et al., 2018). In addition to being present in other drugs supply, fentanyl analogs have been also marketed as "research chemicals" and can easily be acquired over the internet. Due to their high potency and the increased use of heroin as an initiating opioid of abuse (8.7% in 2005 vs. 33.3% users in 2015) (Cicero et al., 2017; O'Donnell et al., 2017), the number of opioid-related deaths have drastically increased in the recent years. Given that opioid novices have limited tolerance to opioids, a slight imprecision in dosing inherent in heroin use and/or the presence of potent fentanyl and analogs, can be fatal.

Fentanyl, its analogs (e.g., acetyl fentanyl, 3-methylfentanyl, alphamethylfentanyl, furanyl fentanyl) and the new generation synthetic opioids (e.g., AH-7921, U-47700, MT-45) have a chemical core structure totally different from morphine, a naturally occurring opioid from *Papaver somniferum* and reference compound of the opioids group; but all of them act on the opioid receptor (mu-receptor) reducing the intensity of pain and showing a high addiction potential. These opioid receptor agonists also induce dose-dependent respiratory depression (Pattinson, 2008), which is the main reason for their life-threatening risk (Ujváry et al., 2017). Fentanyl is approximately 200 times more potent than morphine, and the potencies of its analogs are variable, from 7 times more potent than morphine for butyrfentanyl and furanyl fentanyl, to more than 4,000 and 10,000 times for sufentanil and carfentanil, respectively (UNODC, 2017). The new generation opioids AH-7921 and MT-45 show similar potency to morphine (Brittain et al., 1977; EMCDDA, 2015), and U-47700 about 7.5 times more potent (Cheney et al., 1985).

Synthetic opioids are widely regulated by the United States Controlled Substances Act of 1970 (CSA) in order to control their use and distribution. As new compounds arise and threaten public safety, compounds can be emergency scheduled by the DEA to slow production and use of these harmful substances and aid in prosecution of drug diverters for a temporary period until the formal procedures have gone through (US Drug Enforcement Administration, 2017). Substances are classified into schedules in the CSA based on their safety, medicinal use and potential for abuse. A Schedule I substance is classified as having no currently accepted medical use and a high abuse potential. Examples of synthetic opioids in Schedule I include furanyl fentanyl, U-47700, acetyl fentanyl and 3-methyl fentanyl. Schedule II classified opioids have a high potential for abuse but have current medicinal uses like fentanyl which is used as an anesthetic and analgesic, as well as carfentanil, remifentanil and sufentanil (US Drug Enforcement Administration, 2017). Most recently, the DEA issued a temporary scheduling order for all fentanyl –related substances (to include all analog modifications) in February of 2018, which cover all substances that were not already classified into Schedule I of the CSA in an aggressive attempt to regulate the manufacture and subsequent trafficking of new synthetic opioids into the United States (Drug Enforcement Administration, 2018).

The expansion of these new synthetic opioids constitutes an important challenge in forensic toxicology. First of all, most of these substances are not detected in the routine screening and confirmation methods in the laboratory. Also, due to the low doses employed of these highly potent drugs, the concentrations expected in the biological samples are in the low ng to pg/mL or ng to pg/g range, requiring extremely sensitive methods of analysis. Recently, Marchei et al. (2018) and Liu et al. (2018) reviewed the currently available screening and confirmation methods of new synthetic opioids in biological and non-biological samples. As indicated by Marchei et al. (2018), gas chromatography combined with mass spectrometry (GC-MS) and more frequently liquid chromatography tandem mass spectrometry (LC-MSMS) are the most common techniques due to their sensitivity and specificity. However, given the continued development of new derivatives, the major disadvantage of these target techniques, which employ quadrupole mass spectrometers, is that are limited by the reference standards available. High resolution mass spectrometry (time-of-flight, orbitrap) offers potential advantages to identify unknown compounds without the availability of a reference standard, but this technology is not readily available in most forensic laboratories (Marchei et al., 2018).

Regarding biological samples, most of these methods have been developed in blood or urine, and the target analytes are the parent compounds and rarely the metabolites (Marchei et al., 2018). In postmortem toxicology, other biological specimens such as vitreous humor, liver and brain are commonly analyzed. Unfortunately, fully validated methods for the determination of synthetic opioids in these specimens are lacking in the literature. This is in part due to the constant changes in illicit synthetic opioids being identified and laboratories being unable to justify the extensive time and cost associated with fully validating a method for a drug that may only be present in cases for a short time. Analytical methods in forensic toxicology are commonly validated in the corresponding biological sample following the guidelines published by the Scientific Working Group in Forensic Toxicology (SWGTOX) (Scientific Working Group for Forensic Toxicology, 2013) to guarantee the analytical quality of the measured concentrations. The analysis of metabolites in the different biological matrices may improve the interpretation of the results, extending the detection window and indicating if it was an acute or a delayed-death evaluating the metabolite-to-parent ratios. Recent publications about the identification of new metabolites of the synthetic opioids are available (Wohlfarth et al., 2016; Steuer et al., 2017; Watanabe et al., 2017; Krotulski et al., 2018a); however, its application to authentic samples is still scarce (Poklis et al., 2015; Staeheli et al., 2016; Martucci et al., 2017; Allibe et al., 2018).

Besides the analytical challenges associated with synthetic opioids, due to the scarcity of available postmortem data, the interpretation of the results is extremely difficult. Conducting postmortem toxicology interpretation provides a number of very significant challenges to the forensic toxicologist. The range of postmortem specimens (blood, urine, vitreous humor, tissues, hair), the lack of reference databases, the presence of other substances (e.g., benzodiazepines, alcohol), opioid tolerance,

and postmortem phenomena (postmortem redistribution and drug instability) complicates the interpretation of the analytical findings. Pichini et al. (2018) and Zawilska (2017) discussed non-fatal and lethal intoxications involving the new synthetic opioids, and Drummer (2018) focused his review on fatalities due to these compounds.

The present review is focused on fentanyl derivatives and new generation opioids due to the limited knowledge concerning these substances and their high prevalence in opioid-overdose related cases. This work complements the previously published literature reviewing the current knowledge of postmortem toxicology of synthetic opioids and the chemical and pharmacological factors that may affect drug concentrations in the different matrices and therefore, their interpretation in postmortem samples. These factors include key chemical properties, essential pharmacokinetics parameters, postmortem redistribution and stability data in postmortem samples. All of these data are critically compared to postmortem data of natural opioids (morphine), semi-synthetic (oxycodone, hydrocodone, hydromorphone, and oxymorphone), and synthetic opioids (methadone and buprenorphine). The interpretation of drug intoxication in death investigation is based on the available published literature. This review serves to facilitate the evaluation of cases where synthetic opioids may be implicated in a fatality through the review of peer reviewed published case reports and research articles.

METHODS

PubMed, Scopus and Google Scholar were searched for appropriate articles. Forensic case-reports and research articles of natural, semi-synthetic and synthetic opioids were reviewed up to May 2018. All articles were manually reviewed for content and references in each manuscript were further queried. Included articles were limited to peer-reviewed journals indexed by the Institute for Scientific Information (ISI) and published in English. Chemical properties were retrieved from the public databases PubChem (https://pubchem.ncbi.nlm.nih.gov/) and DrugBank (https://www.drugbank.ca/drugs).

CHEMICAL AND PHARMACOLOGICAL PROPERTIES

The chemical structure of the diverse synthetic opioids, including fentanyl and analogs, differs significally from the chemical structure of morphine and semi-synthetic opioids (e.g., oxycodone, hydrocodone, buprenorphine). **Figure 1** summarizes the chemical structure of selected classic opioids. Fentanyl is a piperidinyl derivative with moieties on the nitrogen and the 4-position (**Figure 2**). The different fentanyl derivatives show substitutions on the propionyl moiety (e.g., acetylfentanyl, acrylfentanyl, butyrfentanyl, furanyl fentanyl), phenethyl moiety (e.g., ohmefentanyl), N-phenyl ring (e.g., ocfentanil, 4-methoxy-butyrylfentanyl) and/or at the 4-piperidinyl-position (e.g., carfentanil). The chemical structures of the new generation synthetic opioids (AH-7921, U-47700, MT-45) are different from fentanyl. **Figure 3** shows 20 fentanyl derivatives and 3 new generation synthetic opioids not related to fentanyl. Due to the close chemical structure among fentanyl derivatives, some compounds, such as cyclopropyl fentanyl and crotonyl fentanyl, have exactly the same molecular formula, and therefore, the same molecular weight. As a consequence of this, special attention has to be paid in the development of the analytical methods for the determination of these compounds, and a complete chromatographic separation is required to guarantee their correct identification by gas or liquid chromatography coupled to mass spectrometry (GC-MS, LC-MSMS).

Chemically, opioids are predominantly basic drugs with pKa ranging from 7.5 to 10.9. The chemical parameter log P, the decimal logarithm of the partition coefficient Kp, is a useful

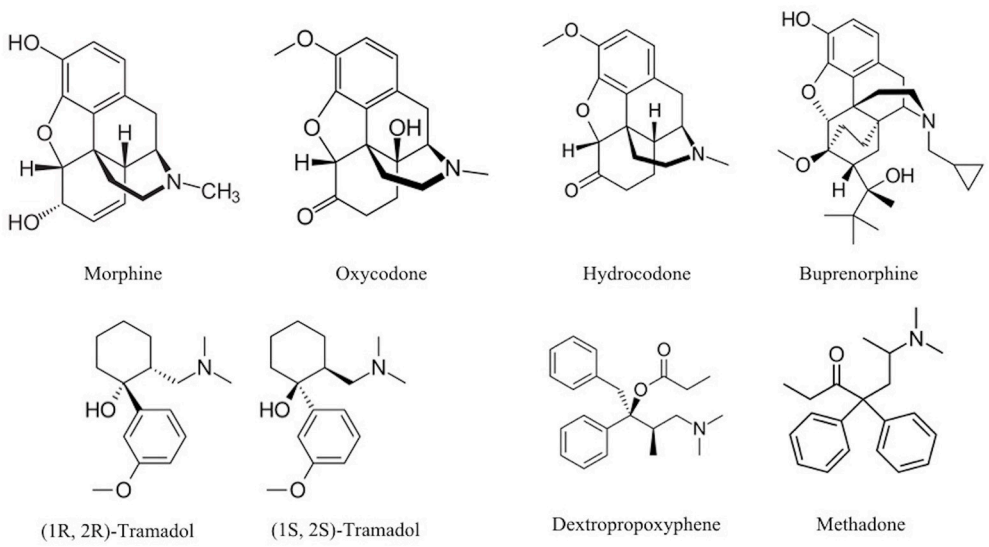


FIGURE 1 | Chemical structures of selected classic opioids.

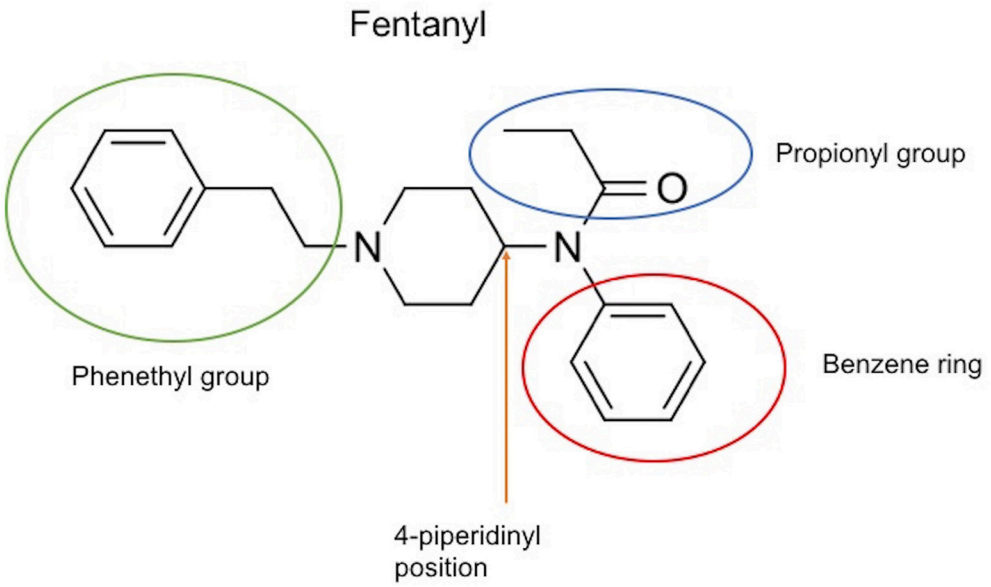


FIGURE 2 | Chemical structure of fentanyl.

indication of the lipophilicity of a compound. In the case of opioids, log P range is wide, from 0.8 (oxymorphone) to 5 (methadone). Morphine and related compounds show the lowest log *P* values (0.8–2). Fentanyl and analogs show a log P between 1.5 and 4.3. The high lipophilicity of fentanyl and its analogs enables rapid diffusion through membranes, including the blood-brain barrier. Also, this lipophilicity along with their basic characteristics make these group of drugs candidates to undergo postmortem redistribution. **Table 1** summarizes the molecular weight, pKa and log P of selected opioids.

Volume of distribution (Vd) and protein binding also help to predict the drugs that may exhibit postmortem redistribution. Vd is defined as the volume into which the total amount of the drug would have to be uniformly distributed to reach the concentrations measured in plasma. It is expressed in L/kg of body weight (amount of drug in the body divided by the plasma drug concentration). Drugs highly bound to plasma proteins but not to tissue components would be expected to have a small Vd, while those drugs which distribute into muscle, adipose tissue and other intracellular components will have a high Vd. Drugs with a Vd greater than 3 L/kg are considered to have a greater potential to undergo postmortem redistribution. **Table 2** summarizes the Vd and protein binding data currently available for selected opioids.

One of the critical issues related to fentanyl, its derivatives and the new synthetic opioids, is the low concentrations expected in the biological samples (ng to pg/mL or ng to pg/g range) due to their high potency. However, the potency of these type of drugs varies considerably within this group, and therefore the concentrations reported show a wide range, depending on the drug. **Table 2** summarizes the potencies relative to morphine for selected opioids.

METABOLISM

The identification and quantification of metabolites in postmortem samples may improve the interpretation of the analytical results. The determination of metabolites may extend the window of detection, and also can be employed to calculate metabolite-to-parent ratios in urine and other biological samples to differentiate acute or delayed death. In certain cases, as it happens in morphine and buprenorphine, metabolites can be pharmacologically active. Although this type of information is limited in the case of the synthetic opioids, fentanyl, sufentanil, and alfentanil's metabolites are inactive in the opioid system (Schneider and Brune, 1986).

Although the utility of metabolite determination in biological samples is known, its application to authentic specimens is still scarce in the case of synthetic opioids due to the limited data available about their metabolism (Poklis et al., 2015; Staeheli et al., 2016; Martucci et al., 2017; Allibe et al., 2018). Recent publications about the identification of new metabolites of the synthetic opioids *in vivo* and *in vitro* are available (Wohlfarth et al., 2016; Steuer et al., 2017; Watanabe et al., 2017; Krotulski et al., 2018a). While *in vitro* studies utilizing human liver hepatocytes or microsomes can identify multiple primary and secondary metabolites for a particular fentanyl derivative, actual human specimens typically show lower number and/or a different metabolite prevalence profile, so studies investigating the presence of the *in vitro* metabolites in authentic human samples are highly encouraged. **Table 3** summarizes recent publications about the identification of new metabolites of synthetic opioids *in vitro* and *in vivo*.

Fentanyl-derivatives metabolism studies showed similarities and differences from fentanyl metabolism pathways and

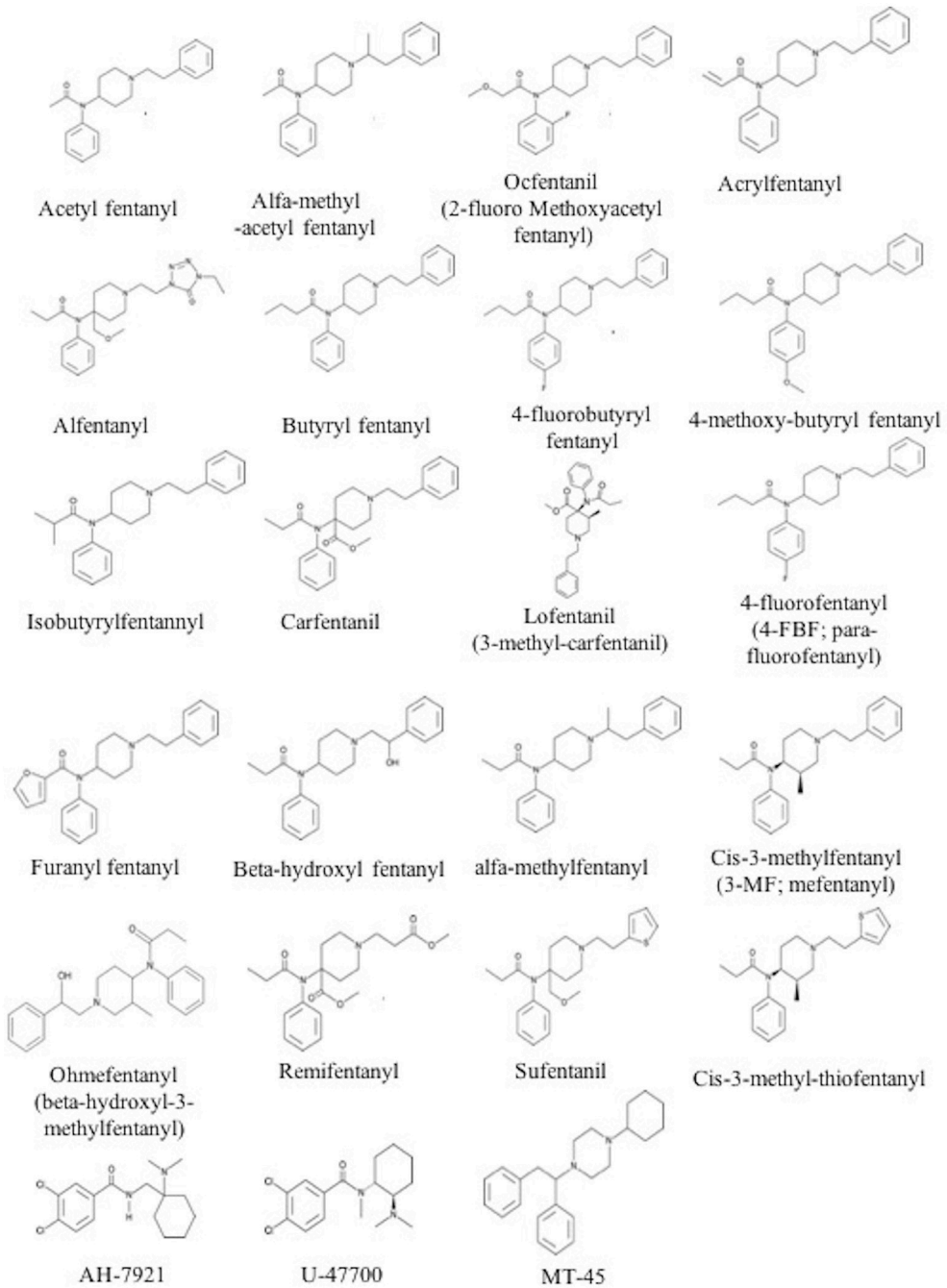


FIGURE 3 | Chemical structures of 20 fentanyl derivatives and 3 new generation opioids not related to fentanyl.

rates. These different metabolic pathways observed for certain derivatives, demonstrate the need to perform individual metabolism studies for each new compound. In the case of fentanyl, only less than 8% of fentanyl is excreted unchanged. Approximately 85% of the dose is excreted within 72 h in feces and urine, the majority as metabolites mainly as norfentanyl generated by N-dealkylation at the piperidine nitrogen (McClain and Hug, 1980). Minor fentanyl metabolites are despropionylfentanyl, also known as 4-ANPP, which is formed by carboxamide hydrolysis, and hydroxyfentanyl and hydroxynorfentanyl metabolites, both hydroxylated at the propionyl moiety (Goromaru et al., 1984; Mahlke et al., 2014).

Several synthetic opioids follow a similar metabolic pathway to fentanyl. Alfentanil undergoes piperidine N-dealkylation to noralfentanil (Meuldermans et al., 1988). Major alpha-methylfentanyl metabolites in rats were norfentanyl and

TABLE 1 | Monoisotopic molecular weight (g/mol), pKa and Log P of selected natural, semi-synthetic and synthetic opioids.

Group	Analyte	Monoisotipic molecular weight (g/mol)	pKa	Log P
Natural and semi-synthetic opioids	Morphine	285.136	8.2	0.9
	Codeine	299.152	9.2	1.3
	Hydrocodone	299.152	8.6	2.0
	Hydromorphone	285.133	8.6	1.6
	Oxycodone	315.147	8.2	1.0
	Oxymorphone	301.131	10.9	0.8
	Buprenorphine	467.300	7.5	4.5
Synthetic opioids	Fentanyl	336.220	8.8	3.8
	Methadone	309.445	9.1	5.0
	Tramadol	263.189	9.2	2.5
Synthetic opioids-Fentanyl derivatives	alphamethylacetylfentanyl; acetyl-alpha-methylfentanyl	336.220	9.01	3.5
	Alfentanil	416.253	7.5	2.8
	Butyryl fentanyl; butyr fentanyl	350.235	8.77	4.3
	Carfentanil	394.225	8.05	3.7
	3-methylcarfentanil; lofentanil	408.241	8.36	4.2
	4-fluorofentanyl; 4-FBF; para-fluorofentanyl	354.210	8.74	4.0
	beta-hydroxyfentanyl	352.215	8.28	2.9
	alpha-methylfentanyl	350.235	9	4.2
	cis-3-methylfentanyl; 3-MF; mefentanyl	350.235	9.08	4.3
	beta-hydroxy-3-methylfentanyl; ohmefentanyl	366.230	8.59	3.4
	Remifentanil	376.199	7.51	1.5
	Sufentanil	386.202	8.86	3.6
	3-methylthiofentanyl	356.192	9.07	4.2

hydroxypropionyl norfentanyl metabolites, exactly as fentanyl (Sato et al., 2010). Meyer et al. (2012) investigated the metabolism in rats of isofentanyl and 3-methyl fentanyl. After the administration of suspected recreational doses, the parent drugs could not be detected in urine and their common nor-metabolite was the predominant compound.

Patton et al. (2014) detected high concentrations of acetylfentanyl and acetyl norfentanyl (>16,500 ng/mL, 180 min post-dose) in urine samples from rats treated with a toxic dose of acetylfentanyl (3 mg/kg); however, Melent'ev et al. (2015), showed that the main pathway of the biotransformation of acetylfentanyl was hydroxylation by the phenylethyl moiety rather than N-dealkylation in authentic human samples. Melent'ev et al. (2015) and Watanabe et al. (2017) recommended as target analytes in human urine hydroxy-methoxy at phenylethyl moiety and monohydroxylated metabolites, although the reported hydroxylation position in both publications was different. In both publications, the parent compound acetylfentanyl was highly abundant in urine samples, indicating that the parent drug is a suitable target.

Acrylfentanyl underwent N-dealkylation at the piperidine nitrogen producing the major nor-metabolite (Watanabe et al., 2017). The parent compound was also detected at high concentrations in urine samples. N-Dealkylation and monohydroxylation of the piperidine ring were the dominant metabolic pathways for carfentanil *in vitro* (Feasel et al., 2016). In that study, the authors observed a slow parent depletion in the hepatocytes. For 4-fluoroisobutyrylfentanyl the main metabolites identified in urine were the nor-metabolite, and monohydroxy metabolites at the piperidine ring or at the ethyl linker, as well as the parent compound. In terms of specificity, Watanabe et al., recommended as target compounds in urine the monohydroxy metabolites and the hydroxymethoxy metabolite (Watanabe et al., 2017).

In the case of butyrfentanyl, hydroxylation of the butanamide side chain followed by subsequent oxidation to the carboxylic acid represented the major metabolic step (Steuer et al., 2017). Although the norbutyrfentanyl was not among the most abundant metabolites in human samples in that study, the authors suggested its inclusion as a recommended target analyte because it showed a high intensity in the *in vitro* experiment. In authentic postmortem blood and urine samples, butyrfentanyl was still detected at 66 and 1,000 ng/mL, respectively.

TABLE 2 | Critical pharmacological properties in postmortem toxicology, volume of distributon (Vd), protein bining and potency relative to morphine, of selected natural, semi-synthetic and synthetic opioids.

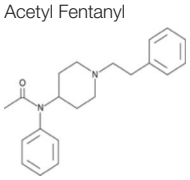
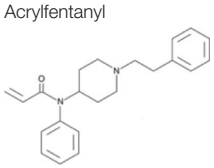
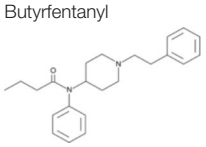
Group	Analyte	Vd (L/kg)	Protein binding (%)	Potency relative to morphine	References
Natural and semi-synthetic opioids	Morphine	1–6	30–40	1	Baselt, 2017
	Codeine	2.5–3.5	7–25	0.3	Baselt, 2017
	Hydrocodone	3.3–4.7	19–45	0.5–1	Patanwala et al., 2007; Baselt, 2017
	Hydromorphone	2.9	20	5–10	Bruera et al., 1996; Patanwala et al., 2007; Baselt, 2017
	Oxycodone	2.6	45	1	Patanwala et al., 2007; Al-Asmari et al., 2009
	Oxymorphone	3	10–12	10	Patanwala et al., 2007; Smith, 2009
	Buprenorphine	3–5	96	40	Dahan et al., 2005
Synthetic opioids	Fentanyl	3–8	80–85	224	Jumbelic, 2010
	Methadone	1–8	85–90	3–5	Patanwala et al., 2007; Baselt, 2017
	Tramadol	3	20	0.1	Christoph et al., 2007; Oertel et al., 2011
Synthetic opioids-Fentanyl derivatives	Acetylfentanyl	NA	NA	15	Higashikawa and Suzuki, 2008
	Acrylfentanil	NA	NA	170	Ujváry et al., 2017
	Alfentanil	0.4–1	92	72	Vardanyan and Hruby, 2014
	Butyryl fentanyl; butyr fentanyl	NA	NA	7	Higashikawa and Suzuki, 2008
	Isobutyrylfentanyl	NA	NA	1.3–6.9	Higashikawa and Suzuki, 2008
	Carfentanil	NA	NA	10,000	Van Bever et al., 1976
	Furanyl fentanyl	NA	NA	7	Higashikawa and Suzuki, 2008
	alpha-methylfentanyl	NA	NA	56.9	Higashikawa and Suzuki, 2008
	cis-3-methylfentanyl; 3-MF; mefentanyl	NA	NA	6000	Higashikawa and Suzuki, 2008
	Remifentanil	0.35	70	220	Wax et al., 2003
	Sufentanil	NA	NA	4,520	Niemegeers et al., 1976
Synthetic opioids-Not related to fentanyl	AH-7921	NA	NA	1	Hayes and Tyers, 1983
	U-47700	NA	NA	7.5	Cheney et al., 1985
	MT-45	NA	NA	1	EMCDDA, 2015

NA, not available.

Furanylfentanyl contains a furan group that affects its metabolic profile. This structure seemed to favor the amide hydrolysis, which is the main metabolite *in vitro* and *in vivo* (Watanabe et al., 2017). In terms of specificity of the target metabolites, Watanabe et al. (2017) recommended the dihydrodiol-metabolite and Goggin et al. (2017) recommended the same metabolite, as well as the sulfate of the metabolite that results from the amide hydrolysis. As it happened with butyrfentanyl (Steuer et al., 2017), the hepatocyte experiment also suggested high prevalence for the nor-metabolite, which was not significantly present in the authentic urine samples, illustrating the need to analyze human specimens. Furanylfentanyl parent compound was detected in authentic urine samples. For ocfentanyl, the predominant metabolite detected in blood, along with the parent drug, was the O-desmethylated metabolite (Allibe et al., 2018).

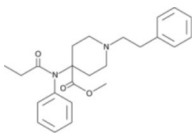
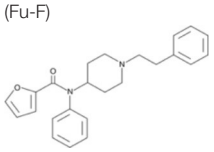
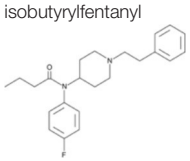
In the case of the new synthetic opioids not structurally related to fentanyl, different metabolic pathways has been reported. For AH-7921, the preferred metabolic sites were the amine function and the cyclohexyl ring. The two most dominant metabolites after hepatocyte incubation (also identified in a urine case specimen) were desmethyl and di-desmethyl AH-7921. Together with the glucuronidated metabolites, they were recommended as suitable analytical targets for documenting AH-7921 intake (Wohlfarth et al., 2016). In the case of MT-45, Montesano et al reported hydroxy-MT-45-glucuronide and di-hydroxy-MT-45-glucuronide as the most abundant metabolites in rat urine, while the parent drug was found at concentrations <10 ng/mL after 300 min (Montesano et al., 2017). Although similar in chemical structure, U-47700 and U-49900 showed specific metabolites. N-Desmethyl-U-47700 was identified as the major metabolite in human urine specimens,

TABLE 3 | *In vitro* and *in vivo* metabolism of synthetic opioids.

Compound	Study type	Matrix (species)	Total # phase I metabolites	Major metabolites (decreasing order of relative intensity)	Phase II metabolites	Recommended target analytes in urine	References
Acetyl Fentanyl 	*In vivo*	Urine (humans)	6	– Hydroxylated metabolite at phenylethyl ring – Hydroxy-methoxy metabolite at phenylethyl ring	Glucuronide of hydroxylated metabolites	– Hydroxylated metabolite at phenylethyl ring – Hydroxy-methoxy metabolite at phenylethyl ring – Acetyl fentanyl	Melent'ev et al., 2015
	In vitro	Pool human liver hepatocytes	7	– N-dealkylated metabolite at the piperidine moiety – Hydroxylated metabolites at the ethyl linker – Dihydroxylation at phenylethyl ring			Watanabe et al., 2017
	In vivo	Urine (human)	24	– Hydroxy-methoxy metabolite at phenylethyl ring – Hydroxy metabolite at the ethyl linker – N-dealkylated metabolite at the piperidine moiety	Glucuronides and sulfates of hydroxy-metabolites	– Hydroxy metabolite at the ethyl linker – Hydroxy-methoxy metabolite at phenylethyl ring – Acetyl fentanyl	
	In vitro	Pluripotent stem cell-derived hepatocytes	6	– N-dealkylated metabolite at the piperidine moiety – Hydroxylated metabolite at phenylethyl ring – Hydroxylated metabolites at the ethyl linker			Kanamori et al., 2018
Acrylfentanyl 	*In vitro*	Pool human liver hepatocytes	8	– N-dealkylated metabolite at the piperidine moiety – Hydroxylated metabolite at the piperidine moiety – Hydroxylated metabolite at the ethyl linker			Watanabe et al., 2017
	In vivo	Urine (human)	12	– N-dealkylated metabolite the piperidine moiety – Hydroxylated at the ethyl linker – Dihydroxylated metabolite at the piperidine and at the ethyl linker – Hydroxy-methoxy metabolite at phenylethyl ring	Glucuronides of hydroxy-metabolites	– Hydroxylated at the ethyl linker – Dihydroxylated metabolite at the piperidine and at the ethyl linker – Acrylfentanyl	
Butyrfentanyl 	*In vitro*	Human liver microsomes	36	– N-dealkylated metabolite – Hydroxy-metabolite at butanamide chain – Dihydroxy-metabolite at phenylethyl ring			Steuer et al., 2017

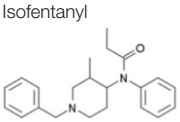
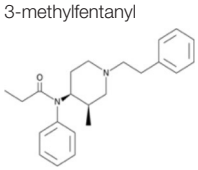
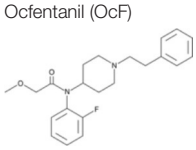
(Continued)

TABLE 3 | Continued

Compound	Study type	Matrix (species)	Total # phase I metabolites	Major metabolites (decreasing order of relative intensity)	Phase II metabolites	Recommended target analytes in urine	References
	In vivo	Urine (human)		– Carboxy-metabolite at butanamide chain – Hydroxy-metabolite at butanamide chain – Carboxy at butanamide chain and hydroxy at phenylethyl ring metabolite	Glucuronides of hydroxy-metabolites	– N-dealkylated metabolite – Hydroxy-metabolite at butanamide chain – Carboxy-metabolite at butanamide chain	
		Blood (human)		– Carboxy-metabolite at butanamide chain			
Carfentanil 	*In vitro*	Pool human liver hepatocytes	11	– Monohydroxylated metabolite at of piperidine ring N-dealkylated metabolite	Glucuronide of hydroxylated metabolite	– Monohydroxylated metabolite at of piperidine ring	Feasel et al., 2016
Furanylfentanyl (Fu-F) 	*In vitro*	Human hepatocytes Pooled human hepatocytes	13	– Amide hydrolysis – N-dealkylated metabolite – Dihydrodiol metabolite at furan group			Watanabe et al., 2017
	In vivo	Urine (human)	9	– Amide hydrolysis – Dihydrodiol metabolite at furan group – Dihydrodiol at furan group and hydroxy at ethyl linker metabolite	Glucuronide and sulfate of hydroxylated metabolites	– Dihydrodiol metabolite at furan group	
	In vivo	Urine (human)		– Amide hydrolysis – Dihydrodiol metabolite at furan group	Sulfate metabolite of amide hydrolysis metabolite	– Sulfate metabolite of amide hydrolysis metabolite – Dihydrodiol metabolite at furan group	Goggin et al., 2017
	In vitro	Human liver microsomes	17	– Despropnionyl fentanyl – Monohydroxylated metabolite – N-dealkylated metabolite			Gaulier et al., 2017
	In vitro	HepaRG cell Line	17	– Despropnionyl fentanyl – N-dealkylated metabolite – Dihydrodiol metabolite (at furan group)	Glucuronide hydroxylated metabolite		
4-Fluoro-isobutyrylfentanyl 	*In vitro*	Pooled human hepatocytes	9	– N-dealkylated metabolite of the piperidine moiety – Monohydroxy metabolite at the piperidine ring or at the ethyl linker – N-oxidation at the piperidine ring			Watanabe et al., 2017

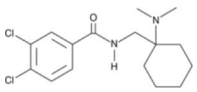
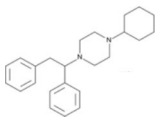
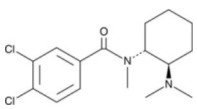
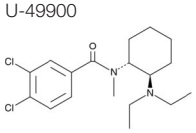
(Continued)

TABLE 3 | Continued

Compound	Study type	Matrix (species)	Total # phase I metabolites	Major metabolites (decreasing order of relative intensity)	Phase II metabolites	Recommended target analytes in urine	References
	In vivo	Urine (human)	13	– N-dealkylated metabolite of the piperidine moiety – Monohydroxy metabolite at the piperidine ring or at the ethyl linker – Hydroxymethoxy metabolite at phenylethyl ring	Glucuronide hydroxylated metabolites	– Monohydroxy metabolite at the piperidine ring or at the ethyl linker – Hydroxymethoxy metabolite at phenylethyl ring	
Isofentanyl 	*In vitro*	Urine (rats)	11	– N-dealkylation followed by hydroxylation of the alkyl and aryl moiety – Hydroxylation of the propanamide side chain followed by oxidation to the carboxylic acid – Hydroxylation of the benzyl moiety followed by methylation – N-oxidation	Glucuronides of hydroxy metabolites	– N-dealkylated metabolite	Meyer et al., 2012
3-methylfentanyl 	*In vivo*	Urine (rats)	9 /5	– N-dealkylation followed by hydroxylation of the alkyl and aryl moiety – Hydroxylation of the propanamide side chain followed by oxidation to the carboxylic acid – Hydroxylation of the benzyl moiety followed by methylation	Glucuronides of hydroxy metabolites	– N-dealkylated metabolite	Meyer et al., 2012
Ocfentanil (OcF) 	*In vitro*	Human liver microsomes	3	– O-desmethyl metabolite – Monohydroxylated metabolite at phenylethyl ring – O-desmethyl metabolite hydroxylated at phenylethyl ring	Glucuronide of O-desmethylated metabolite		Allibe et al., 2018
	In vivo	– Blood (human, $n = 1$) – Bile (human, $n = 1$)	3	– O-desmethyl metabolite – Monohydroxylated metabolite at phenylethyl ring – O-desmethyl metabolite hydroxylated at phenylethyl ring		– O-desmethylated-metabolite	

(Continued)

TABLE 3 | Continued

Compound	Study type	Matrix (species)	Total # phase I metabolites	Major metabolites (decreasing order of relative intensity)	Phase II metabolites	Recommended target analytes in urine	References
AH-7921 	*In vitro*	Human hepatocytes	11	– N-demethyl metabolite – N-dis-demethyl metabolite – N-demethyl metabolite hydroxylated at cyclohexyl	Glucuronide demethylated metabolite		Wohlfarth et al., 2016
	In vivo	Urine (human)	10	– N-demethylation – N-dis-demethyl metabolite	Glucuronide demethylated metabolite	– N-demethylation – N-dis-demethyl metabolite	
MT-45 	*In vitro*	Rat hepatocytes	10	– Hydroxy metabolite – Dihydroxy metabolite – 1-cyclohexyl-piperazine	Glucuronides of hydroxy metabolites		Montesano et al., 2017
	In vivo	Urine (rat)	10	– Hydroxy metabolite – Dihydroxy metabolite – 1-cyclohexyl-piperazine – OH-1-cyclohexyl-piperazine	Glucuronides of hydroxy metabolites	– Hydroxy metabolite – Dihydroxy metabolite	
U-47700 	*In vitro*	Human liver microsomes	4	– N-desmethyl-U-47700 – N,N-didesmethyl-U-47700 – N-desmethyl-hydroxy-U-47700 – N,N-didesmethyl-hydroxy-U-47700			Krotulski et al., 2018a
	In vivo	Urine (human, $n = 5$)	5	– N-desmethyl-U-47700 – N,N-didesmethyl-U-47700 – N-desmethyl-hydroxy-U-47700 – N,N-didesmethyl-hydroxy-U-47700 – N,N-didesmethyl-N-desmethyl-U-47700		– N-desmethyl-U-47700 – N,N-didesmethyl-U-47700	
U-49900 	*In vitro*	Human liver microsomes	5	– N-desethyl-U-49900 – N,N-didesethyl-U-49900; – N,N-didesethyl-N-desmethyl-U-49900 – N-desethyl-hydroxyo-U-49900 – N-desethyl-N-desmethyl-U-49900			Krotulski et al., 2018a
	In vivo	Urine (human, $n = 5$)	5	– N-desethyl-U-49900 – N,N-didesethyl-U-49900 – N,N-didesethyl-N-desmethyl-U-49900 – N-desethyl-hydroxyo-U-49900 – N-desethyl-N-desmethyl-U-49900		– N,N-didesethyl-N-desmethyl-U-49900	

and N,N-Didesethyl-N-desmethyl-U-49900 was identified as the most abundant metabolite present. Unlike U-47700 specimens, U-49900 was detected in low abundance in urine samples (Krotulski et al., 2018a).

As indicated by Watanabe et al. (2017), the target metabolites should generally be abundant, specific of the parent drug, and prevalent in most, if not all, case samples. Given the strong structural similarities among emerging designer fentanyls, many of them are coincidentally biotransformed to the exact same metabolite. This fact can make identification of the specific parent drug in a case difficult. The ability to identify minor metabolites that are unique and specific to the parent drug is therefore of considerable importance. 4-ANPP can be formed by fentanyl and other different fentanyl analogs metabolism, and it is also a precursor contaminant found in seized illicit fentanyl and analogs, so its presence is not particularly diagnostic. Other common metabolites are: acetylnorfentanyl from acetyl-alpha-methylfentanyl or acetylfentanyl (Watanabe et al., 2017); norfentanyl from fentanyl, beta-hydroxythiofentanyl and alpha-methyl-fentanyl (Sato et al., 2010); norcarfentanil from carfentanil, sufentanil and remifentanil (Feasel et al., 2016). 3,4-dichloro-N-(2-aminocyclohexyl)-N-methyl-benzamide is a common metabolite of U-47700 and U-49900, but it is not a major metabolite in urine for either compound (Krotulski et al., 2018a).

Another important aspect of the metabolism is the identification of the enzymes involved. Pharmacokinetic interactions may be produced due to the presence of other substances metabolized by the same enzymes, ultimately affecting the drug blood concentrations. Fentanyl, sufentanyl and alfentanil are mainly metabolized by CYP 3A4 (Feierman and Lasker, 1996; Guitton et al., 1997). Steuer et al., identified CYP 3A4 and CYP 2D6 as the isoforms involved in the metabolism of butyrfentanyl (Steuer et al., 2017). Meyer et al., reported that CYP 3A4, CYP 3A5 and CYP 2C19 are involved in the metabolism of 3-methylfentanyl and isofentanyl and, in the case of isofentanyl, additionally CYP2D6 (Meyer et al., 2012). Remifentanil is the only family member of this class found to be ∼95% metabolized in the blood and tissues by non-CYP enzymes, probably due to an easily accessible ester group allowing rapid hydrolysis by circulating blood esterases (Bürkle et al., 1996).

CONCENTRATIONS IN POSTMORTEM SPECIMENS AND OTHER FINDINGS

The concentrations determined in postmortem specimens varied considerably depending on the type of synthetic opioid detected. Derivatives with potencies relative to morphine of more than 170, showed concentrations in femoral blood in the low ng/mL or pg/mL range, while those derivatives with potencies similar to morphine showed concentrations of hundreds, and even thousands, of ng/mL. An exception happens with furanyl fentanyl, which is seven times more potent than morphine (Higashikawa and Suzuki, 2008), but the reported femoral concentrations were less than 50 ng/mL. Typical morphine postmortem concentrations in blood in fatalities are from 200 to 2,300 ng/mL, for methadone 400 to 1,800 ng/mL, for buprenorphine 1.1–29 ng/mL and norbuprenorphine (active metabolite) 0.2–13 ng/mL (Baselt, 2017), and for oxymorphone 23–554 ng/mL (Crum et al., 2013). The potency of the different drugs affects their lethal levels, but other important issues, such as the presence of other CNS depressant drugs, and developed opioids tolerance, have to be taken into account in the interpretation of the concentrations. The derivative with the highest number of published cases was acrylfetanyl, and with the lowest MT-45. **Table 4** summarizes the concentrations of the parent drugs found in case reports and articles where overdose due to a specific opioid was the cause of death.

In several cases, multiple synthetic opioids were detected. Acetylfentanyl and fentanyl were frequently found together (Pearson et al., 2015; Poklis et al., 2015; Dwyer et al., 2018). Other combinations were butyryl fentanyl and acetyl fentanyl (McIntyre et al., 2016b; Poklis et al., 2016), or U-47700 (Mohr et al., 2016); furanyl fentanyl and acetyl fentanyl (Papsun et al., 2017), acryl fentanyl (Butler et al., 2017), butyryl fentanyl (Mohr et al., 2016), fentanyl (Guerrieri et al., 2017a), or carfentanil (Shanks and Behonick, 2017); carfentanil and fentanyl (Shanks and Behonick, 2017); and tetrahydrofuran fentanyl and U-49900 (Krotulski et al., 2018b). The femoral concentrations reported in those combination cases were frequently below the range of the concentrations summarized in **Table 4**. Acetylfentanyl median and concentration range in multiple synthetic opioids cases were 9.4, 0.4–240 ng/mL ($n = 15$); acrylfentanyl 0.3 ng/mL ($n = 1$); butyrfentanyl 14.9, 0.3–58 ng/mL ($n = 4$); carfentanil 0.08, 0.05–0.1 ng/mL ($n = 2$); fentanyl 8.2, 1.1–38 ng/mL ($n = 14$); furanyl fentanyl 1.7, 0.6–6.1 ng/mL ($n = 4$) and U-47700 17 ng/mL ($n = 1$).

In all of the reports mentioned in **Table 4** and above, synthetic opioids were commonly detected with other drugs, especially other CNS depressants, such as benzodiazepines, ethanol and other opioids. This combination may produce a pharmacodynamic interactions and increase the risk of respiratory depression. This possible interaction between opioids, alcohol and benzodiazepines has been previously described for other opioids, such as buprenorphine (Häkkinen et al., 2012; Seldén et al., 2012), methadone (Jones et al., 2012; Pilgrim et al., 2013; Nielsen et al., 2015), oxycodone (Ogle et al., 2012), and heroin (Thaulow et al., 2014). Among the reviewed cases positive for synthetic opioids other than fentanyl, 44 reported as cause of death intoxication due to multiple drugs and 77 intoxication mainly due to one specific opioid. The manner of death was predominantly accidental ($n = 99$), and suicides were reported in 7 cases.

POSTMORTEM REDISTRIBUTION AND STABILITY

Postmortem changes in drug concentrations can happen via postmortem redistribution (PMR) from tissues of a higher to a lower concentration. Physicochemical and pharmacological properties of the analytes, such as pKa, log P, volume of

TABLE 4 | Postmortem concentrations in different biological samples for synthetic opioids (median, range, number of cases).

Analyte	Blood (ng/mL)				Vitreous humor (ng/mL)	Brain (ng/g)	Liver (ng/g)	Urine (ng/mL)
	Femoral	Cardiac	Subclavian	Non-specified				
3-Methylfentanyl	–	–	–	0.4 (0.3–0.9) $n = 3$	–	–	–	–
4–fluorobutyr fentanyl	–	–	–	91–112 $n = 2$	–	248 $n = 1$	902 $n = 1$	200 $n = 1$
Acetylfentanyl	223.5 (16–600) $n = 12$	270 (170–2,100) $n = 11$	220 $n = 1$	–	140–240 $n = 2$	620 $n = 1$	1,000–1,100 $n = 2$	2,660 (240–3,420) $n = 4$
Acrylfentanyl	0.2 (0.01–5) $n = 42$	–	–	–	–	–	–	–
Butyryl fentanyl	99 (66–145.2) $n = 3$	60.5 (39–220) $n = 3$	–	–	32 $n = 1$	93–200 $n = 2$	41–57 $n = 2$	64 $n = 1$
Carfentanil	0.2 (0.01–0.5) $n = 9$	0.1–0.2 $n = 2$	0.03 $n = 1$	–	–	–	–	–
Fentanyl	11 (1–60) $n = 207$	13 (1.8–139) $n = 81$	–	13 (2–383) $n = 66$	14.8 (8–20) $n = 4$	49 $n = 1$	78 (5.8–16,983) $n = 99$	97 (2.9–1,200) $n = 31$
Furanyl fentanyl	2.7 (0.4–42.9) $n = 13$	2.8 $n = 1$	–	–	–	–	–	–
Ocfentanyl	9.1 (3.7–15.3) $n = 3$	23.3 (3.9–27.9) n3	–	–	12.5 $n = 1$	37.9 $n = 1$	31.2 $n = 1$	6–480 $n = 2$
AH–7921	350 (30–9,100) $n = 13$	480–3,900 $n = 2$	–	–	190 $n = 1$	7,700 $n = 1$	530–26,000 $n = 2$	760–6,000 $n = 2$
MT–45	520–660 $n = 2$	1,300 $n = 1$	–	–	260 $n = 1$	–	24,000 $n = 1$	370 $n = 1$
U–47700	358 (189–1,460) $n = 12$	691.5 (260–1,347) $n = 4$	–	–	130 (90–170) $n = 2$	(0.9–380) $n = 3$	142.1 (3.1–1,700) $n = 4$	1620.5 (360–4,600) $n = 4$

3-Methylfentanyl references: (Ojanperä et al., 2006).
4-fluorobutyr fentanyl references: (Rojkiewicz et al., 2017).
Acetylfentanyl references: (Pearson et al., 2015; Poklis et al., 2015; Cunningham et al., 2016; Fort et al., 2016; McIntyre et al., 2016a; Takase et al., 2016; Yonemitsu et al., 2016; Dwyer et al., 2018).
Acrylfentanyl references: (Butler et al., 2017; Guerrieri et al., 2017b).
Butyryl fentanyl references: (Poklis et al., 2016; Staeheli et al., 2016).
Carfentanil references: (Shanks and Behonick, 2017; Swanson et al., 2017; Hikin et al., 2018).
Fentanyl references: (Anderson and Muto, 2000; Kuhlman et al., 2003; Martin et al., 2006; Coopman et al., 2007; Biedrzycki et al., 2009; Carson et al., 2010; Krinsky et al., 2011, 2014; Palamalai et al., 2013; Marinetti and Ehlers, 2014; McIntyre et al., 2014; Bakovic et al., 2015; Moore et al., 2015; Pearson et al., 2015; Poklis et al., 2015; Rodda et al., 2017; Dwyer et al., 2018).
Furanyl fentanyl references: (Mohr et al., 2016; Guerrieri et al., 2017a; Martucci et al., 2017; Papsun et al., 2017).
Ocfentanyl references: (Coopman et al., 2016; Dussy et al., 2016; Allibe et al., 2018).
AH-7921 references: (Karinen et al., 2014; Kronstrand et al., 2014; Vorce et al., 2014; Fels et al., 2017).
MT-45 references: (Papsun et al., 2016; Fels et al., 2017).
U-47700 references: (Elliott et al., 2016; Mohr et al., 2016; Dziadosz et al., 2017; Papsun et al., 2017; Rohrig et al., 2017).

distribution (Vd) and protein binding, may indicate drugs that experience this postmortem phenomenon. Lipophilic basic drugs with a Vd > 3 L/kg, such as fentanyl, may undergo PMR. Fentanyl has been reported to undergo extensive PMR (Luckenbill et al., 2008; Olson et al., 2010; Palamalai et al., 2013; Brockbals et al., 2018). In the case of the synthetic opioids, limited data is currently available about PMR, and as well as information about pKa, log P and Vd (**Tables 2**, **3**). Staeheli et al. (2016) reported postmortem concentration changes of butyrfentanyl and metabolites, suggesting these compounds were prone to PMR. PMR reports about other synthetic opioids are not currently available.

Based on currently published case reports and articles, the cardiac blood-to-femoral blood and liver-to-femoral blood ratios were calculated to predict candidates of PMR. Results are summarized in **Table 5**. Due to the scarce amount of data available (1–4 cases per analyte), no conclusions could be drawn. Synthetic opioids showed median cardiac-to-femoral ratios around 1, and a tendency to accumulate in the liver. Regarding the distribution to vitreous humor, it may be slow showing higher concentrations in blood. Other factors, such as time of death and sample collection, or rapid vs. delayed deaths, has not been taken into account in this analysis due to the limited data available.

PMR is still a controversial issue for classic opioids. Hargrove and Molina (2014) showed insignificant redistribution of morphine from central sites within 24 h after death in bodies kept at 4°C, while Staeheli et al. (2017) observed a significant increase of morphine concentration, although these changes were not relevant for forensic interpretation.

TABLE 5 | Postmortem concentration ratios in different biological samples for synthetic opioids (median, range, number of cases).

Analyte	Cardiac-to-femoral	Liver-to-femoral	Vitreous humor-to-femoral	References
Acetylfentanyl	1.2 (0.8–1.6) $n = 4$	3.8–5.7 $n = 2$	0.6–0.9 $n = 2$	Cunningham et al., 2016; Fort et al., 2016; McIntyre et al., 2016a; Yonemitsu et al., 2016
Butyryl fentanyl	0.6 (0.4–2.2) $n = 3$	0.4–0.9 $n = 2$	0.3 $n = 1$	Poklis et al., 2016; Staeheli et al., 2016
Fentanyl	(0.7–4.6) $n = 54$	6.6 (1.4–539.4) $n = 75$	1.5 (1.1–1.8) $n = 3$	Anderson and Muto, 2000; Krinsky et al., 2011, 2014; Palamalai et al., 2013; McIntyre et al., 2014; Bakovic et al., 2015
Furanyl fentanyl	1.5 $n = 1$	–	–	Martucci et al., 2017
Ocfentanyl	1.5 (1.1–3.1) $n = 3$	2 $n = 1$	0.8 $n = 1$	Coopman et al., 2016; Dussy et al., 2016; Allibe et al., 2018
AH–7921	0.4–1.1 $n = 2$	1.2–2.9 $n = 2$	0.4 $n = 1$	Vorce et al., 2014; Fels et al., 2017
MT-45	2 $n = 1$	36.4 $n = 1$	0.4 $n = 1$	Fels et al., 2017
U-47700	1.5 (0.7–2.6) $n = 4$	0.4 (0.003–8.9) $n = 4$	0.2–0.9 $n = 2$	Dziadosz et al., 2017; Rohrig et al., 2017

Morphine-derivatives, such us hydrocodone (Saitman et al., 2015), codeine (Frost et al., 2016), and oxycodone (Brockbals et al., 2018), are unlikely to undergo substantial PMR changes. More lipophilic opioids with higher Vd, like methadone (Jantos and Skopp, 2013; Holm and Linnet, 2015; Brockbals et al., 2018), may undergo PMR.

Several studies have been conducted to evaluate stability of fentanyl and some of its derivatives in fortified biological samples, such as blood, plasma and urine. Eleven fentanils (fentanyl, norfentanyl, carfentanil, norcarfentanil, sufentanil, norsufentanil, lofentanil, 3-methylfentanyl, alfa-methylfentanyl, ohmefentanyl, and remifentanil acid metabolite), were stable in urine samples stored at −20°C or below for at least 2 months. However, remifentanil in urine samples decreased by approximately 90% within 1 week at room temperature and by more than 50% in samples stored for 1 week at 4°C. Because of the instability of that analyte, the authors recommended to analyze the primary metabolite, remifentanil acid (Wang and Bernert, 2006). Fentanyl and its metabolites norfentanyl, despropionylfentanyl and hydroxynorfentanyl were stable in urine after 3 freeze-thaw cycles, and after storage at −20°C for 2 months (Mahlke et al., 2014).

Fentanyl, norfentanyl, acetyl fentanyl and acetyl norfentanyl spiked into whole blood were stable after three freeze-thaw cycles and at room temperature for 72 h (Poklis et al., 2015). No loss of fentanyl concentration could be observed after 3 months of storage at 4–8°C and −20°C in blood samples at 5 and 10 ng/mL (Andresen et al., 2012). However, another study showed fentanyl and its metabolites norfentanyl, despropionylfentanyl and hydroxynorfentanyl lose up to 51.6% after 3 freeze-thaw cycles, and fentanyl and despropionylfentanyl up to 34.8% after storage at −20°C for 2 months (Mahlke et al., 2014). Furanylfentanyl showed no significant degradation in blood samples at 5 and 10 ng/mL 48 h room temp and at 4°C 7 days (Guerrieri et al., 2017a) and up to 30 days (Mohr et al., 2016).

Regarding the new synthetic opioids not related to fentanyl, U-47700 was stable in blood refrigerated for up to 30 days (Mohr et al., 2016). AH-7921 was found to be stable for at least 21 days in blood and plasma at room temperature (Soh and Elliot, 2014). In the case of MT-45, a loss of 50% was observed after 12 months of storage (Papsun et al., 2016). Further studies are necessary to evaluate the stability of the different synthetic opioids and metabolites, and in additional biological samples of forensic interest, such as vitreous humor and tissues.

CONCLUSION

We performed a critical review of the currently available literature to assist in the toxicological interpretation of synthetic opioids postmortem cases. Synthetic opioids constitute a heterogenous group of compounds related or not to fentanyl, mostly basic and lipophilic, with a wide range of potencies related to morphine, from 1 to 10,000. Research has been conducted in the investigation of metabolic pathways and identification of target metabolites of fentanyl derivatives and non-structurally related synthetic opioids, showing similarities

and differences from fentanyl depending on the compound. Postmortem concentrations seemed to correlate with their potency, although the presence of other CNS depressants, such as ethanol and benzodiazepines has to be taken into account. Further research is guaranteed to investigate postmortem redistribution phenomena of this class of compounds, and stability issues in postmortem samples.

AUTHOR CONTRIBUTIONS

MC and GC contributed conception and design of the review. MC, RC, and JP searched, organized, reviewed and analyzed the case reports and research articles. MC wrote the first draft of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Assessing the 2004–2018 Fentanyl Misusing Issues Reported to an International Range of Adverse Reporting Systems

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Objective: A recent, global, increase in the use of opioids including the prescribing, highly potent, fentanyl has been recorded. Due its current popularity and the potential lethal consequences of its intake, we aimed here at analyzing the fentanyl misuse, abuse, dependence and withdrawal-related adverse drug reactions (ADRs) identified within the European Medicines Agency (EMA), the United Kingdom Yellow Card Scheme (YCS), and the United States Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) databases.

Methods: Descriptive analysis of both ADRs and related cases.

Results: The analysis of fentanyl-related misuse, abuse, dependence and withdrawal cases reported during years 2004–2018 to the EMA, the YCS, and the FAERS showed increasing levels overtime, specifically, EMA-related data presented two peaks (e.g., in 2008 and 2015), whilst the FAERS dataset was characterized by a dramatic increase of the ADRs collected over the last 18 months, and particularly from 2016. Some 127,313 ADRs (referring to n = 6,161 patients/single cases) related to fentanyl's misuse/abuse/dependence/withdrawal issues were reported to EMA, with 14,287 being judged by the reporter as "suspect." The most represented ADRs were: "drug dependence "(76.87%), "intentional product misuse" (13.06%), and "drug abuse" (7.45%). Most cases involved adult males and the concomitant use of other prescribing/illicit drugs. A range of idiosyncratic (i.e., ingestion/injection of transdermal patches' fentanyl) and very high-dosage intake cases were here identified. Significant numbers of cases required either a prolonged hospitalization (192/559 = 34.35%) or resulted in death (185/559 = 33.09%). Within the same time frame, YCS collected some 3,566 misuse/abuse/dependence/withdrawal ADRs, corresponding to 1,165 single patients/cases, with those most frequently reported being "withdrawal," "intentional product misuse," and "overdose" ADRs. Finally, FAERS identified a total of 19,145 misuse/abuse/dependence/withdrawal-related cases, being "overdose," withdrawal, and "drug use disorder/drug abuse/drug diversion" the most represented ADRs (respectively, 43.11, 20.80, and 20.29%).

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Conclusion: Fentanyl abuse may be considered a public health issue with significant implications for clinical practice. Spontaneous pharmacovigilance reporting systems should be considered for mapping new trends of drug abuse.

Keywords: opioids, fentanyl, prescription drug misuse, opioid-related deaths, new psychoactive substances

INTRODUCTION

The Current "Opioid Crisis"

During recent years, a massive, worldwide increase (United Nations Office on Drugs, and Crime [UNODC], 2018a) in the prescription of opioids for pain has been recorded (Guevremont et al., 2018). This has been associated with increasing risks of diversion, abuse, morbidity and mortality, with a rising number of deaths and treatment admissions observed. Such "opioid crisis" (Throckmorton et al., 2018) started in 2013 (National Institute on Drug Abuse [NIDA], 2017; O'Donnell et al., 2017), and in recent years has reached the magnitude level of a public health issue, being tramadol, fentanyl and oxycodone the most involved molecules (Stannard, 2012; Centers for Disease Control and Prevention [CDC], 2014; Van Amsterdam and Van den Brink, 2015; Helmerhorst et al., 2017; Jordan et al., 2017; Floyd and Warren, 2018). In the United States in 2016, nearly 4% of the population aged 12 years and older reported a non-medical, past-year, use of prescription opioids. Compared with heroin use, which has been increasing each year since 2007, the non-medical use of prescription opioids has shown a stable trend in the past 5 years. Even though the most commonly misused prescription opioids reported in the National Survey on Drug Use and Health in the United States are hydrocodone, oxycodone, codeine and tramadol, fentanyl appeared to be on the rise (World Drug Report, 2018). According to the European Monitoring Centre for Drug and Drug Addiction [EMCDDA] (2018a), in addition to heroin, other opioid products have been seized in European countries, including tramadol, buprenorphine, methadone, but also fentanyl derivatives, with figures respectively, being: 3,553, 3,523, 1,245, and 738 seizures, with overtime increasing levels of availability of the latter. In Europe, fentanyl issues seem to be particularly relevant in Estonia (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a). Considering data from the United States Unintentional Drug Overdose Reporting System (SUDORS), fentanyl has been detected in 56.3% of 5,152 opioid-related deaths during the months July–December 2016 (O'Donnell et al., 2017). In 2016, the United States synthetic opioid-related deaths accounted for 30.5% of all drug overdose fatalities and 45.9% of all opioid-related deaths, with a 100% increase in the rate of these fatalities compared with the previous year (Center for Disease Control and Prevention [CDC], 2018a,b; Seth et al., 2018; World Drug Report, 2018; News Release, 2018).

Although Europe does not seem to face a problem of the same scale of the United States (Van Amsterdam and Van den Brink, 2015), after a downward trend in opiate use since the late 1990s and until 2013, opiate use rates and drug-related deaths have started increasing again in Western and Central Europe (United Nations Office on Drugs, and Crime [UNODC], 2018b), In 2016, the use of opioids (e.g., heroin, but also: methadone, buprenorphine, fentanyl, codeine, morphine, tramadol and oxycodone) was reported as the main reason by 37% of all clients who entered European specialized drug clinics (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a). The United Kingdom, Spain, and Sweden present with the most significant levels of non-medical, opioid-based, prescription drug use (United Nations Office on Drugs, and Crime [UNODC], 2018b). In France, the national OPPIDUM ("Observation of illegal drugs and misuse of psychotropic medications") program of the French addictovigilance network (Frauger et al., 2017), anonymously collects information on drug abuse and dependence observed in patients recruited in specialized drug care centers. In 2015, OPPIDUM reported high percentages (77%) of opiate maintenance treatment among a number of 5,003 drug users, highlighting the emerging misuse of a range of synthetic opioids, such as tramadol, oxycodone, and fentanyl. In Germany, during years 2005–2014, a number of 242 fentanyl-related overdose fatalities were reported, with the onset of fentanyl-related deaths following the local launch of transdermal fentanyl matrix patches in 2004 (Sinicina et al., 2017).

The Emerging Threat of Illicit Fentanyl Products

In Europe, during 2016–2017 fentanyl has been involved in more than 250 fatalities (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a); this may have been associated with fentanyl derivatives' high potency, possible use by opioid-naive individuals, and recently increased drug availability levels. In the United Kingdom, in early 2017, fentanyl and its synthetic analogs, such as carfentanil, butyryl fentanyl, fluorobutyrylfentanyl, furanylfentanyl, and alfentanil, have been detected in 25 drug-related fatalities (Hikin et al., 2018).

Different fentanyl derivatives have been developed by the legitimate pharmaceutical industry by adding various substituents to the basic molecule in order to modify the potency (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015). The same approach has been mimicked by chemists in clandestine laboratories to produce new, illicit, fentanyl derivatives. In fact, an overall number of 38 new synthetic opioids have been detected in the European drug market since 2009, out of these, 28 pertained to the fentanyls' category (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a). The vast range of illicit fentanyls (Gladden et al., 2016; National Institute on Drug Abuse [NIDA], 2017; Armenian et al., 2018; Center for Disease Control and Prevention [CDC], 2018b; Pichini et al., 2018) are manufactured in a range of non-EU countries (Macmadua et al., 2017; European Monitoring Centre for Drug and Drug Addiction [EMCDDA],

2018b) and then made available from both the streets and the web to be typically self-administered either on their own or in combination with remaining psychoactives. Users are often unaware of the contents of the substance they are taking, which inevitably leads to a great number of fatal overdoses (Helander et al., 2017; Zawilska, 2017; Kuczyñska et al., 2018; World Drug Report, 2018).

Fentanyl, Clinical Pharmacological Issues

Fentanyl is an extremely fast-acting synthetic narcotic analgesic, first approved as an anesthetic in 1963 (Drummer, 2018; Food and Drug Administration [FDA], 2018). Currently it is available for intravenous (I.V.) and intramuscular (I.M.) injection, but also as transdermal patches, quick acting lozenges, and dissolving tablets and films. Fentanyl has a potency of at least 80 times that of morphine, and it is indicated for the treatment/management of chronic, malignant, and post-surgical pain conditions (Stanley, 2014; Drummer, 2018). Fentanyl is a narcotic analgesic acting predominately at the μ-opiate receptor (Drummer, 2018). Apart from the analgesic characteristics, the fentanyls as a group produce drowsiness, relaxation and euphoria, the latter being less pronounced than with heroin and morphine. The most common side effects include nausea, dizziness, vomiting, fatigue, headache, constipation, anemia, and peripheral oedema (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015; Prekupec et al., 2017). A range of severe toxicity effects, including muscle rigidity, seizures, overdoses, and death due to respiratory arrest, have been reported as well (Zawilska, 2017). Tolerance and dependence develop rapidly after repeated use. Characteristic withdrawal symptoms (sweating, anxiety, diarrhea, bone pain, abdominal cramps, shivers or "goose flesh") occur when use is stopped too quickly (Zawilska, 2017). Serious interactions can occur when fentanyls are mixed with heroin, cocaine, alcohol and other CNS depressants, e.g., benzodiazepines. Sudden fatalities may be related to a cardiac arrest or severe anaphylactic reactions. The estimated lethal dose of fentanyl in humans is 2 mg. The recommended serum concentration for analgesia is 1–2 ng/ml and for anesthesia it is 10–20 ng/ml. Blood concentrations of approximately 7 ng/ml or greater have been associated with fatalities where poly- substance use was involved (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015). Whilst fatalities have been reported after therapeutic use, many deaths have occurred as a result of the misuse of pharmaceutical products. Both used and unused fentanyl patches have been injected, smoked, snorted or taken orally with fatal consequences (Lilleng et al., 2004; Woodall et al., 2008; European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015).

Fentanyl and Derivatives' Misuse/Abuse Issues

The diversion of prescription fentanyl may involve individuals obtaining medication inappropriately through their profession, patients using their own prescribed fentanyl recreationally for a non-medically intended purpose, and subjects using a medication being prescribed to another person. Typical sources of medications included friends, family members, and online pharmacies (Novak et al., 2016).

Recreational fentanyl (also known by the street names "China White," "Synthetic Heroin," "Tango and Cash," etc., Zawilska, 2017) consumption seems to be often associated with the use of other drugs such as heroin, other opiate/opioid medicines, alcohol, cocaine, benzodiazepines, psychostimulants, and antidepressants, and may lead to fatal and non-fatal overdoses (Uusküla et al., 2015; National Institute on Drug Abuse [NIDA], 2016; Alcohol and Drug Foundation [ADF], 2018; Armenian et al., 2018; Drummer, 2018; Kuczyñska et al., 2018).

Even under medical surveillance, the risk of overdose when injecting fentanyl would be significantly higher than when injecting heroin (Frisoni et al., 2018). Fentanyl overdoses may start suddenly, with a potentially lethal respiratory depression possibly being reached within 2 min as opposed to some 20–30 min after heroin use (Abdulrahim et al., 2018); moreover, the high rate of fentanyl deaths may be explained as well by the molecule polydrug consumption. Naloxone, often in repeated doses, followed by a significant amount of post-emergency clinical observation time (Greene et al., 2018; Santos et al., 2019) is used to treat fentanyl's overdoses (Prekupec et al., 2017; Zawilska, 2017; Armenian et al., 2018; European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a), although at times this proves unsuccessful (Kuczyñska et al., 2018). A range of harm-reduction strategies have been implemented both in Europe (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2016) and in the United States (New York City Health, 2017).

The improper use of transdermal patches, either by applying multiple patches on the body, or injecting/insufflating/inhaling (after volatilization) the contents of a discarded patch has been reported (National Institute on Drug Abuse [NIDA], 2017; Sinicina et al., 2017; Drummer, 2018; Jones et al., 2018; Kuczyñska et al., 2018). The fentanyl's rewarding effects are increased when the drug is injected or self-administered with nasal sprays/e-liquids, which are vaped using electronic cigarettes (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a).

Due to the growing fentanyl toxicity issues and their high abuse liability/dependence potential, all fentanyls approved for medical use are internationally controlled as Schedule II drugs under the Controlled Substance Act (National Institute on Drug Abuse [NIDA], 2018) and as a Class A drug under the Misuse of Drugs Act in the United Kingdom (GOV.UK, 1971). In 2017, also the fentanyl precursors 4-anilino-N-phenethylpiperidine (ANPP) and N-phenethyl-4-piperidone (NPP), have been added to **Table 1** of the 1988 United Nation Convention, and in 2018 have been included under the European drug monitoring regulations (Abdulrahim et al., 2018; European Commission (EC), 2018). Nonetheless, over the last few years there have been growing concerns regarding a range of illicitly manufactured fentanyl analogs being used as new/novel psychoactive substances (NPS) (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015, 2018a,b;

TABLE 1 | Data relating to fentanyl misuse/abuse/dependence/withdrawal-related ADRs reported to the European Medicines Agency (EMA), the Medicines and Healthcare products Regulatory Agency (MHRA), and the Food and Drug Administration (FDA) pharmacovigilance databases, 2004–2018.

Characteristics	EMA EV Data	MHRA YCS Data	FAERS Data
Fentanyl misuse/abuse/ dependence/withdrawal -related issues	127,313 (e.g., 6,161 Individual cases); 14,287 "suspect" (e.g., 559 CASES)	3,566 Reactions (e.g., 1,165 Individual cases)	19,145 Individual cases
Most frequently reported ADRS' issues	Drug dependence (76.9%), Intentional product misuse (13.1%), Drug abuse (7.5%)	Withdrawal (24.9%), Intentional product misuse and use issues (19.6%), Overdose (17.6%)	Overdose (42.1%), Withdrawal (20.5%), Drug abuse (20.0%)
Age (years)	Adult	Adult	N/A
	Age group 35–64 (229/559 = 41%)	Age group:	
		50–59 (164/1,165 = 14.1 %)	
		40–49 (144/1,165 = 12.4%)	
		60–69 (141/1,165 = 12.1 %)	
Gender	Male (M/F: 319/209 = 1.52)	F (M/F: 434/657 = 0.66)	N/A
Fentanyl as sole drug or in combination	Fentanyl sole drug: 307/559 = 54.9% cases.	N/A	N/A
	Concomitant drugs reported: other opioids (69.0%), cocaine (9.5%), benzodiazepines (6.8%), cannabis (5.6%), and ethanol (5.2%)		

National Institute on Drug Abuse [NIDA], 2017; Pichini et al., 2018; Ventura et al., 2018). Although their chemistry is similar to fentanyl, they are not routinely detected (O'Donnell et al., 2018) and may present with a higher/much higher potency than the parental compound. Most popular fentanyl derivatives include: carfentanil (approximately 10,000 times more potent than morphine), acetyl-fentanyl (about 15 times more potent than morphine), and butyrfentanyl (30 times more potent than morphine; Prekupec et al., 2017; Zawilska, 2017; Pichini et al., 2018).

Aims

To assess fentanyl misuse/abuse/dependence and withdrawal-related issues, we aimed here at analyzing the European Medicines Agency [EMA] EudraVigilance (EV) database, and comparing it with the United Kingdom Medicine and Healthcare products Regulatory Agency (MHRA) Yellow Card scheme (YCS), and the United States Food and Drug Administration [FDA] Adverse Event Reporting System [FAERS] databases.

MATERIALS AND METHODS

EudraVigilance (EV) Features

European Medicines Agency (EMA) data were collected through EV, i.e., the pharmacovigilance dataset which manages and analyses information on suspected adverse reactions to medicines which have been authorized in the European Economic Area (EEA) (European Medicines Agency [EMA], 2007). This dataset is a centralized database of all suspected adverse drug reactions (ADRs) submitted to EMA through Individual Case Safety Reports (ICSR), providing information related to an individual case of a suspected side effect due to a medicine. Specifically, an ADR is defined as "...a response which is noxious and unintended, and which occurs at doses normally used in humans ...f An ADR, contrary to an adverse event, is characterized by the suspicion of "...a causal relationship between the drug and the occurrence..." (European Medicines Agency [EMA], 2017). The ADRs here considered were, *per se*, spontaneous and unsolicited communications reported by both Regulatory Authorities of the EU Member States where the reaction occurred, and/or by the Marketing Authorization Holders for those ADRs occurring outside the EEA. Consistent with the EV Access Policy (European Medicines Agency [EMA], 2016), data were made available here after a formal, *ad hoc*, request regarding fentanyl, including the following molecules: "fentanyl," "fentanyl hydrochloride," and "fentanyl buccal," with all pharmaceutical combinations having been excluded. For each reported case, EV recorded Level 2A information, meaning: general information on the ADR (e.g., code number of the ADR, sender type, sender organization, type of report, date when the report was first received, primary source country, reporter qualification, seriousness of the case, and medical confirmation of the case), information on the patient (age, sex, weight, and height), type of reaction/event, drug information (e.g., type of drug, dosages, administration route, and duration), including concomitant licit and illicit drugs, medical history and comments, outcome of the reaction including death, literature references (European Medicines Agency [EMA], 2016, 2018). Each ADR was recorded according to the Medical Dictionary for Regulatory Activities (MedDRA) (Medical Dictionary for Regulatory Activities [MedDRA], 2017), and listed through Preferred Terms (PT). PTs are defined as "distinct descriptor (single medical concept) for a symptom, sign, disease diagnosis, therapeutic indication, investigation, surgical or medical procedure, and medical social or family history characteristic" (Medical Dictionary for Regulatory Activities [MedDRA], 2017).

In the data analysis of our study we included the ADR identified by the following PT: "dependence," "drug abuse," "drug abuser," "drug dependence," "drug diversion," "drug withdrawal syndrome," "intentional product misuse," "intentional product use issue," "intentional overdose," "overdose," "substance

use," "substance abuse," and "withdrawal syndrome" (Medical Dictionary for Regulatory Activities [MedDRA], 2017). The following ADRs were excluded from the analysis: "accidental exposure," "accidental overdose," "drug administration error," "drug prescribing error," "toxicity to various agents," "medication error," and "off-label use." ADRs' numbers differed from those referring to single patients, since different reporters/senders could have independently flagged the same ADR to EMA, or several ADRs (involving various organ classes and so identified with specific PT) related to the primary searched ADRs (abuse/misuse/dependence and withdrawal ADRs) for the same patient could have been reported as well. A descriptive analysis of ADRs and cases (which were unequivocally identified by an EV local number) was then performed, according to the information provided (Medical Dictionary for Regulatory Activities [MedDRA], 2017).

Access to the UK MHRA YCS and FAERS Pharmacovigilance Datasets

In order to obtain a better understanding of prescribing fentanyl misuse issues, publicly accessible, 2004–2018, data from both the UK MHRA YCS (Medicines and Healthcare products Regulatory Agency [MHRA], 2018b) and the FAERS (Food and Drug Administration [FDA] Adverse Event Reporting System [FAERS], 2018) were here analyzed as well. These data are made available via online public dashboards.

The YCS collects information on a range of ADRs spontaneously reported from healthcare professionals, members of the public, and pharmaceutical companies, this information are then entered onto the MHRA's ADR database by a team of safety experts to assess the likelihood of causal relationship between the drug and the reported reactions. The YCS publishes cumulative listings of all suspected ADRs received through interactive Drug Analysis Profiles (iDAPs) (Medicines and Healthcare products Regulatory Agency [MHRA], 2018a). After selecting the iDAP related to "fentanyl," a general overview of data relating to: age, gender, and type of reactions (organized by System Organ Class-SOC and MedDRA Preferred Terms – PTs) was made available online. A range of filters to the database were then applied here, with the time-frame and reactions selected being those used for the EV dataset (Medical Dictionary for Regulatory Activities [MedDRA], 2017).

The FAERS is a database that contains a range of voluntarily submitted adverse event reports (Food and Drug Administration [FDA] Adverse Event Reporting System [FAERS], 2018), with these events being coded using terms from the MedDRA dictionary (Medical Dictionary for Regulatory Activities [MedDRA], 2017). Searching for "fentanyl," "fentanyl hydrochloride," and "fentanyl buccal," we gained access to a range of FAERS-related data, which were then properly filtered according to the type of reaction, consistent with the above described EMA and YCS data extraction modalities.

Ethics Statement

Complying with the European Data Protection legislation (e.g., Regulation (EC) No 45/2001EMA, European Medicines Agency [EMA] (2016)), the protection of privacy and integrity of individuals is guaranteed. Thus, all EMA data are fully and completely de-identified/anonymized; therefore, any patient identifier is not being disclosed. Similarly, both the YCS data and FAERS data are completely anonymized and fully de-identified. The study has been approved by the University of Hertfordshire Ethics' Committee (reference number LMS/PGR/UH/03234, March 5, 2018).

RESULTS

The analysis of the two ADR-related datasets showed an overall increase in the number of reports since 2004 to 2018, with two peaks in the trend having been identified in the EV database, respectively, in 2008 (885 ADRs) and 2015 (1,081 ADRs). Conversely, YCS data remained broadly stable at relatively low levels, being slightly increasing from 2014, with 101 cases reported in 2017 (**Figure 1**).

Analysis of Data From the EV Database

During the period 2004–2018, the EMA EV system received a total of 127,313 ADRs (referring to n = 6,161 patients/single cases) relating to fentanyl misuse/abuse/dependence/withdrawal issues (**Table 1**). Out of these 127,313 ADRs, some 14,287 (corresponding to 559 patients) were identified following a further filtering exercise, whilst considering: (a) the PTs selected, and (b) the "suspect" fentanyl role in causing the index ADR case. The most represented ADRs were: "drug dependence" (10,982/14,287 ADRs, 76.87% of the total), "intentional product misuse" (1,866 ADRs, 13.06% of the total), and "drug abuse" (1,065 ADRs, 7.45%). Most reports (17.2%) were related to ADRs occurred in the United States and were posted by clinicians (10.82%). Out of these 559 individual cases, 429 (76.74%) were males (M/F: 319/209 = 1.52) in the 35–64 years-old age range; conversely, 10 subjects were younger than 12, and all had been diagnosed with an iatrogenic opioid withdrawal syndrome. Significant levels of ADR cases required either a prolonged hospitalization (192/559 = 34.35%) or resulted in death (185/559 = 33.09%).

Among the 185 fatal cases, the most reported causes of death were: toxicity to various agents (26/185 = 14.05%), drug abuse (19/185 = 10.27%), and overdose (18/185 = 9.73%). Although co-morbidity data went here typically unreported, chronic pain conditions (n = 36) were the most frequently mentioned medical conditions, whilst most typical psychiatric diagnoses included mood (55 cases) and anxiety (33 cases) disorders.

Although in most cases fentanyl was identified on its own (307/559 = 54.9% cases) concomitant drugs most typically mentioned in the EMA database included: remaining opiates/opioids (174/252 = 69%), cocaine (24/252 = 9.5%), benzodiazepines (17/252 = 6.8%), and cannabis (14/252 = 5.6%). Even though fentanyl's route of administration was infrequently reported (e.g., oral: 41/559 = 7.3%, and transdermal: 33/559 = 5.9%), a range of idiosyncratic ways of administration/high dosage intake were here described, e.g.: 23 cases of transdermal patches' ingestion, 10 cases of fentanyl nasal administration/inhalation, and 10 cases of

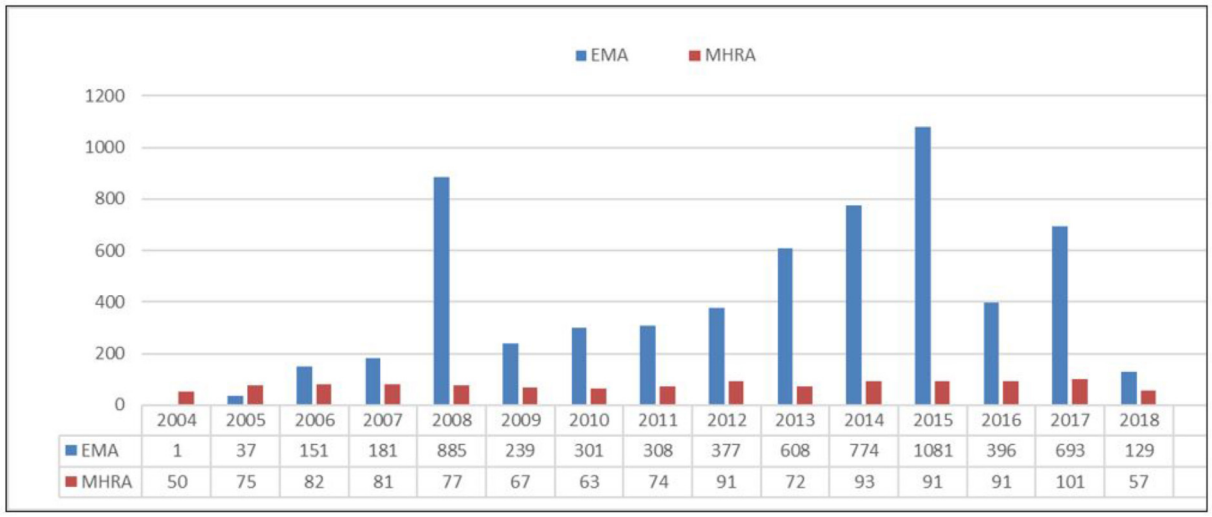

FIGURE 1 | 2004–2018 EMA and MHRA misuse/abuse/dependence/withdrawal-related fentanyl cases. EMA, European Medicines Agency; MHRA, Medicines and Healthcare products Regulatory Agency.

intravenous/parenteral use, with a case of tampered transdermal patches' injection on some 30 times a day having been reported. In terms of idiosyncratic dosages, a patient was here reported to be self-administering with 800 mcg 2–3 times/day, whilst another was daily self-administering with 11.56 mg of fentanyl transdermal patch. Where available, most typically reported blood toxicology screening results were in the range of: up to 10 ng/ml (7/559 cases), 10–50 ng/ml (16/559 cases), and in excess of 100 ng/ml (2/559 cases, in 1 case this was 313 ng/ml).

Analysis of the YCS and FAERS Databases

Analysis of the 2004–2018 fentanyl YCS iDAPs identified some 3,566 fentanyl misuse/abuse/dependence/withdrawal-related ADRs, corresponding to 1,165 single patients/cases (**Table 1**). ADR numbers showed an increase over time (**Figure 1**), with a peak in 2017 (101 reactions). Female subjects (F/M: 657/434 reports), aged 40–59 years (308 reports), were most typically involved. Out of all reactions, "withdrawal" (79 reactions), "intentional product misuse and use issues" (62 reactions), "overdoses" (56 reactions), and "addiction/dependence/drug dependence" (45 reactions) were the most typically represented ADRs (**Table 1**). Moreover, in the 2004–2018-time frame, the FAERS database identified a total (e.g., all causes ADRs) of 78,885 instances. After completion of the above-described filtering exercise, a total of 19,145 misuse/abuse/dependence cases/patients were here identified. Most frequently mentioned reports related to: "overdose" (n = 8,255/19,145, 43.11%), "withdrawal syndrome" (n = 3,983, 20.80%), "drug use disorder/drug abuse/drug diversion" (n = 3,886, 20.29%), "intentional product use issues/misuse" (n = 1,829, 9.55%), and "drug dependence" (n = 1,462, 7.64%) (**Table 1**).

DISCUSSION

This unprecedented, large scale, research study aimed at systematically identifying and analyzing a total of some 26,500 fentanyl misuse/abuse/dependence/withdrawal cases. Present data were extracted from a range of high-quality (Schifano and Chiappini, 2018) pharmacovigilance databases, such as the EV (providing description on a total of unfiltered 6,161 misuse/abuse/dependence/withdrawal cases), the United Kingdom YCS (1,165 cases), and the United States FAERS (19,145 cases). Indeed, these data seem to once again confirm that non-medical prescription high potency opioid use is a major public health concern both in Europe (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2015), and in the United States (Ali et al., 2017; Mital et al., 2018). According to European Monitoring Centre for Drug and Drug Addiction [EMCDDA] (2015) data, the main consumers in the EU in 2008 per million inhabitants per day were Belgium (13,601 Defined Daily Doses or S-DDD), Germany (13,341 S-DDD), and Austria (10,143 S-DDD). Moreover, even though illegally diverted fentanyl is a relatively marginal phenomenon in most EU countries, in Estonia as many as 70% of applicants for treatment services in 2009 reported fentanyl as their primary drug. Consistent with this, Han et al. (2017) in carrying out the 2015 National Survey on Drug Use and Health (NSDUH) exercise, analyzed data from 51,200 subjects who completed an *ad hoc* survey interview. They estimated that out of 91.8 million (37.8%) United States non-institutionalized adults who used prescription opioids, 11.5 million misused them, and 1.9 million had a use disorder. Being relief of physical pain the most commonly reported motivation for misusing prescription opioids (Han et al., 2017), developing new pain treatments would possibly reduce the access to opioids in

high-risk patients (National Institute on Drug Abuse [NIDA], 2017). A further challenge may be constituted by identifying new opioid formulations with physical or pharmacologic deterrents to reduce tampering (Stanos et al., 2012; Passik, 2014).

After many years of stability, fentanyl ADRs seemed to have peaked here over the last 10 years and in the United States even more dramatically just over the last 2 years or so. Although it is possible that high levels of reporting were facilitated by a recently growing awareness on fentanyl misuse, and may somehow mirror as well the increasing rates of worldwide availability of this medication (United Nations Office on Drugs and Crime [UNODC], 2017), present figures are in line with previous findings (Ali et al., 2017). However, increasing rates of fentanyl misuse/abuse issues may have been facilitated as well by the high number of pro drug websites/users' fora, where proper advice on how to both tamper fentanyl patches and best enjoy the related intake experience as well are given (Drugs-forum, 2017; The hive, 2018).

Possibly because of its high potency (Frisoni et al., 2018), fentanyl prescribing was here reported in a number of cases to be associated with iatrogenic dependence/withdrawal issues. It is a further reason of concern, however, that fentanyl was here self-administered either in idiosyncratic ways (i.e., parenteral, ingesting the transdermal patches) or at high/very high dosages to achieve significant blood levels (Frisoni et al., 2018). A large proportion of EMA ADR cases (e.g., roughly two out of three) was here associated either with a prolonged hospitalization or resulted in death. Fatalities related to novel synthetic opioids and fentanyls should be investigated using a multidisciplinary approach, aiming at framing each case and directing the investigations toward targeted toxicological analyses. This approach should be adopted routinely (Hikin et al., 2018), and especially in cases of death from uncertain or questionable causes (Lucyk and Nelson, 2017; Prekupec et al., 2017; Drummer, 2018). New approaches for the detection of potential unknown psychoactive substances, e.g., in the Emergency Departments, need to be developed, in order to identify and classify compounds that are new to the market and absent from existing chemical libraries (Assi et al., 2015; Guirguis et al., 2017; Calvo-Castro et al., 2018; Food and Drug Administration [FDA], 2018; News Release, 2018).

Although in some 54.9% of EMA ADRs a fentanyl intake was reported on its own, a range of both prescribing (e.g., remaining opiates/opioids, benzodiazepines), and recreational (e.g., cocaine and cannabis) psychotropics was here identified as well. Whilst these combinations are likely to lead to intoxication or death (Haukka et al., 2018), they may reflect the characteristics of clients prescribed with fentanyl, e.g., frequently affected by chronic pain conditions, anxiety, and depression, at times presenting as well with a history of drug misuse (Hughes et al., 2016).

Intake of high fentanyl dosages was possibly associated here with the need to relieve pain, whilst attempting to cope with the molecule's increasing levels of tolerance overtime (Han et al., 2017). Nonetheless, fentanyl recreational value (Frisoni et al., 2018) should not be overlooked (Drummer, 2018). High fentanyl dosages may be associated with respiratory arrest, pulmonary oedema, chest wall rigidity and apnoea. A reported uncommon intoxication symptom is chest pain, with non-specific T-wave changes on the electrocardiogram, mimicking an acute coronary syndrome (for a thorough review, see Frisoni et al., 2018).

LIMITATIONS

Even though the study of spontaneous reporting systems, such as EV, the YCS, and the FAERS, should be considered as a starting point for mapping the new trends of abuse, including the abuse of prescription drugs, the analysis of voluntarily reported ADRs may have some limitations. These pharmacovigilance database approach limitations include likely underreporting, reporting bias, and lack of access to the full range of available data. Some ADRs may be signaled several times by different reporters, therefore the number of suspected ADRs can be different to the number of cases as one individual case may refer to several suspected ADRs. Moreover, a report may describe different information, sometimes lacking useful data, such as medical history, dosages and route of administration, or cause of death when the outcome is fatal. Case reports of suspected ADRs do not confirm that a certain effect in a patient has been caused by a specific medicine (European Medicines Agency [EMA], 2011; Food and Drug Administration [FDA] Adverse Event Reporting System [FAERS], 2018; Medicines and Healthcare products Regulatory Agency [MHRA], 2018b), but may be used for detecting and assessing eventual safety issues to be investigated. Again, the worldwide fentanyl prescribing rates were not available here and, due to fentanyl's availability on the black and gray (semi-legal) markets, the true incidence of the misusing phenomenon may not be calculated.

Finally, because of only partial consistency of data collection of the datasets here examined, analyzing and comparing figures may prove problematic. Indeed, MHRA and FAERS public dashboards provided here only a portion of available data (e.g., excluding diagnoses, medical histories, fentanyl dosages and concomitant drugs ingested) relating to the cases reported.

CONCLUSION

Fentanyl abuse may be considered a public health issue, with enormous implications for the clinical practice. A national registry of patients to monitor and check opioid prescribing to high-risk patients would be helpful, this may improve the patient safety levels, whilst providing more focused epidemiological data regarding prescribing patterns (Mordecai et al., 2018). With the aim of reducing the number of people addicted to opioids, the FDA strategy is now to pose a control on prescription duration and doses for patients (Floyd and Warren, 2018; Food and Drug Administration [FDA], 2018).

In terms of prevention of the opiate epidemic and harm reduction strategies, it is here suggested that users should play an active role in helping drafting overdose education and abuse-deterrent strategies. Prompt referral/self-referral to treatment programs has been reported as being an effective intervention (Suzuki and El-Haddad, 2017;

Ralphs and Gray, 2018; Schiller and Mechanic, 2018; Younga et al., 2018). In Europe, a range of evidence-based prevention programs have been implemented over the last few years (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2017). Supervised drug consumption facilities and "take-home" naloxone programs, making the medication available to opioid users and their partners/peers/families, alongside with training in overdose recognition and response, appear to be helpful in preventing deaths (European Monitoring Centre for Drug and Drug Addiction [EMCDDA], 2018a) and should be encouraged (Uusküla et al., 2015). Moreover, the Welsh Emerging Drugs and Identification of Novel Substances [WEDINOS], 2018, the Loop in the United Kingdom (Wearetheloop.org, 2018), the Drug Information and Monitoring System in the Netherlands (Drug Information and Monitoring System [DIMS], 2018 and the MANchester DRug Analysis and Knowledge Exchange [MANDRAKE], 2018) are some drug awareness/harm reduction European projects aiming at monitoring the illegal drug market, identifying public health threats at an early stage.

Physicians should be educated and invited to a responsible prescribing of drugs with a diversion potential, whilst carefully evaluating the possibility for some clients (e.g., people with a personal history of drug abuse) to be more vulnerable to drug misuse (Han et al., 2017).

AUTHOR CONTRIBUTIONS

FS conceived the conceptual idea of the manuscript and the proof outline. SC performed the literature review and the analysis of data from EMA and drafted the initial version of the manuscript. AG and JMC supervised the manuscript and contributed to the final version of the manuscript. FS approved the final content of the manuscript.

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REVIEW
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Metabolic Pathways and Potencies of New Fentanyl Analogs

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Up to now, little is known about the metabolic pathways of new fentanyl analogs that have recently emerged on the drug markets worldwide with high potential for producing addiction and severe adverse effects including coma and death. For some of the compounds, limited information on the metabolism has been published, however, for others so far no information is available. Considering the well characterized metabolism of the pharmaceutically used opioid fentanyl and the so far available data, the metabolism of the new fentanyl analogs can be anticipated to generally involve reactions like hydrolysis, hydroxylation (and further oxidation steps), *N*- and *O*-dealkylation and *O*-methylation. Furthermore, phase II metabolic reactions can be expected comprising glucuronide or sulfate conjugate formation. When analyzing blood and urine samples of acute intoxication cases or fatalities, the presence of metabolites can be crucial for confirmation of the uptake of such compounds and further interpretation. Here we present a review on the metabolic profiles of new fentanyl analogs responsible for a growing number of severe and fatal intoxications in the United States, Europe, Canada, Australia, and Japan in the last years, as assessed by a systematic search of the scientific literature and official reports.

Keywords: novel synthetic opioids, fentanyl analogs, fentanyl biotransformations, *in vivo* and *in vitro* metabolism, metabolic profile, receptor binding affinity, toxicity

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INTRODUCTION

Opiates have been used for thousands of years to treat a broad variety of conditions. The first semi-synthetic opioids (such as heroin) were derived from the opium alkaloid morphine. In association with the discovery and deeper investigation of the opioid receptors numerous synthetic, structurally diverse opioids were developed by research chemists and pharmaceutical companies. Fentanyl has first been synthesized by Paul Janssen in 1959 (Janssen, 1965) and was derived from the synthetic opioid meperidine. Its pharmacological action is 50–100 times more potent than morphine and 25–40 times more than heroin, and it is commonly used in anesthesia and pain treatment (US Drug Enforcement Administration [US DEA], 2015; National Institute on Drug Abuse [NIDA], 2016). Fentanyl and its clinically used analogs are regarded as highly potent μ-opioid receptor agonists.

Besides the medicinal usage and progress in the therapeutic application of opioids, misuse of opioids has always been an issue. However, non-medical use of opioids often leads to health problems due to the high addictive potential of opioids and their severe acute side effects like

respiratory depression. Repeated opiate and opioid use leads to tolerance, a contributing factor to opioid dependence. Development of tolerance is a controversially discussed topic and not fully understood yet. However, there is a consensus that different mechanism are involved, among them pharmacodynamic tolerance (adaptive changes in networks or pathways in organs and tissues affected by drug interaction), behavioral tolerance, pharmacokinetic (metabolic) tolerance and tachyphylaxis (Bespalov et al., 2016). In both clinical use and misuse tolerance may lead to dose escalation and finally severe adverse effects.

Over the last few years a wave of highly potent synthetic opioids emerged on the market of new psychoactive substances (NPS). These 'new synthetic opioids' (NSO) are often derived from fentanyl (also known as 'designer fentanyls,' 'fentanyl derivatives,' or 'fentalogs') and available at a cheaper cost compared to heroin (Marchei et al., 2018; Rothberg and Stith, 2018). Fentanyl analogs have recently been encountered as cutting agents in seized heroin samples, in ready-to-use preparations like nasal sprays or as 'research chemicals' marketed via internet shops. These drugs have caused an increasing number of acute intoxications and fatalities in North America, as well as in Europe, Japan, Canada, and Australia (Pichini et al., 2017, 2018). Plenty of pharmacokinetic studies have been published evaluating and characterizing receptor binding and potency of fentanyl (Costa et al., 1992; France et al., 1995) and its clinically relevant analogs (Henriksen et al., 2005; Volpe et al., 2011). When comparing binding constants, it has to be kept in mind that variables like type of assay, choice of competitive ligand etc. significantly impact the experimental outcome and may lead to varying values for identical compounds. In contrast, information on pharmacological data – and in particular metabolism – of non-medically used fentanyl analogs is scant, with evident difficulties in identifying the molecules in biological fluids of the consumers in order to assess consumption (Armenian et al., 2018). In addition, ratios of parent compound and metabolite concentrations can help to examine the plausibility of specific scenarios in forensic toxicology (e.g., acute vs. slow accumulative poisoning). An early study assessing the opioid-like activity of several fentanyl metabolites in a guinea pig ileum assay found that norfentanyl, 4-ANPP (4-anilino-*N*-phenethylpiperidine) and 4-anilinopiperidine (metabolites of fentanyl) were less potent than either fentanyl or morphine by several orders of magnitude (Schneider and Brune, 1986). The only metabolite showing significant activity in this study was a phenolic derivative hydroxylated at the 4-position of the phenylethyl moiety of fentanyl, the activity of which was found to lie between morphine and pethidine.

Nevertheless, some of the fentanyl analog metabolites might retain opioid activity with clinical relevance. What has been documented for fentanyl metabolism typically translates to the new designer fentanyls, which also show an extensive metabolism, however, to varying degrees. This review article summarizes the current knowledge on pharmacological data with a focus on the metabolism of novel fentanyl analogs.

METHODS

Procedures for Assessment of Metabolic Profiles

To investigate the metabolism of a distinct compound, *in vivo* or *in vitro* approaches can be used: *in vivo* studies are performed in animals or humans, whereas *in vitro* approaches include the use of human liver microsome preparations, human hepatocytes or fungi as models for metabolism. In general, *in vivo* studies in humans would be the best choice due to limited transferability of animal data, but require ethical approval and are often not feasible. Human self-administration studies or the investigation of body fluids of death cases can serve as an alternative if available. However, such studies may show biased metabolic profiles due to health conditions or enzymatic phenotypes of study subjects. *In vitro* approaches generally do not reflect the full human metabolism, but are much easier to implement. Human hepatocytes are a commonly used model simulating human hepatic metabolism. However, due to varying factors like cell line and culture environment, the metabolic profile resulting from hepatocyte incubation may vary and does often not reflect the metabolic profile obtained *in vivo* sufficiently. Human liver microsomes or fungi like *Cunninghamella elegans* are further tools to produce *in vitro* metabolites. They are relatively easy to handle and cost-efficient, but may lack the ability to produce the whole human metabolic spectrum.

Analytical identification of metabolites is usually performed by mass spectrometric techniques like liquid chromatography-high resolution mass spectrometry (LC-HRMS) and use of different scan modes of tandem mass spectrometry. Differentiation of isomers often affords isolation of specific metabolites and nuclear magnetic resonance (NMR) spectroscopy analysis.

Literature Search

MEDLINE for biomedical literature and EMBASE for pharmacological literature as well as multidisciplinary databases such as Scopus and Web of Science were searched using the following combined terms: fentanyl analogs or analogs or derivatives or designer fentanyls or fentalogs, fentanyl, remifentanil, sufentanil, alfentanil, acetylfentanyl, acryloylfentanyl (or acrylfentanyl), α-methylfentanyl, butyr(-yl)fentanyl, carfentanil, cyclopropylfentanyl, cyclobutyl-fentanyl, cyclopentylfentany, cyclohexylfentanyl, 2,2,3,3-tetramethylcyclopropylfentanyl, crotonylfentanyl, 4-fluoro-isobutyr(-yl)fentanyl, isofentanyl, furanylfentanyl, methoxya-cetylfentanyl, ocfentanil, ortho-fluorofentanyl, tetrahydro-furanylfentanyl, metabolism, metabolic networks, metabolic pathways, μ-opioid receptor, opioid receptor binding. Further studies were retrieved by hand search through the reference lists of the selected articles. Moreover, a search for reports was conducted on Institutional websites, to identify documentation published by international agencies or institutions including the United States Drug Enforcement Administration (US DEA), United States National Institute on Drug Abuse (NIDA), World

Health Organization (WHO) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA).

GENERAL REMARKS

Opioid Receptors

Opioid receptors are membrane bound G-protein coupled receptors predominantly located at the synaptic complex in the central nervous system but are also found in peripheral tissues. In the 1960s and 1970s first binding studies were performed by Van Praag and Simon (1966), Ingoglia and Dole (1970), Simon et al. (1973), Terenius (1973) and Pert and Snyder (1973) locating the opioid receptors in different brain areas using radio-labeled ligand assays. First proof for the existence of multiple opioid receptors was published by Martin (1967) proposing three different types of the opioid receptor (μ, κ, and δ). The μ-opioid receptor (MOR) named by its agonist morphine is mainly located in brain tissue and the gastrointestinal (GI) tract. This receptor mediates many of the typical opiate effects like analgesia, euphoria, miosis, physical dependence, reduced GI-mobility and respiratory depression. Three subtypes of the μ-opioid receptor (μ_1, μ_2, and μ_3) have been identified, while μ_1 is characterized best (Pan et al., 2005). The κ- and δ-opioid receptors are both found primarily in the brain tissue. For the κ-receptor three subtypes and for the δ-receptors two subtypes have been identified (Rothman et al., 1989; Portoghese and Lunzer, 2003). In principal, the same central nervous effects are produced by activation of the κ receptors as for the μ receptors, but additionally κ receptor agonists can cause hallucination and dissociation. δ-Opioid receptors are believed to contribute to analgesia as well, but also modulate immune response of myenteric neurons (Poonyachoti et al., 2001). Fentanyl and its analogs have been specifically designed for the activation of the μ-opioid receptors and usually show high selectivity for this receptor type. This is one of the factors complicating a direct comparison of morphine and fentanyl potency. Considering potency regarding central nervous effects, transmission through the blood-brain barrier has to be taken into consideration, too.

FENTANYL AND FENTANYL ANALOGS

Fentanyl is a 2-phenylethyl-substituted 4-anilinopiperidine derivative carrying a propionylamide moiety linked to the aniline-nitrogen. In principle, there are four structural features which can potentially be modified, resulting in a huge variety of fentanyl analogs: (a) the piperidine ring, (b) the anilinophenyl ring, (c) the 2-phenylethyl substituent and (d) a carboxamide moiety linked to the anilino-nitrogen (**Figure 1**).

In the 1970s, Janssen Pharmaceutica patented a series of highly potent fentanyl derivatives, the *N*-4-substituted 1-(2-arylethyl)-4-piperidinyl-*N*-phenylpropanamides, such as the medically used Sufentanil and Carfentanil (Janssen, 1979). Carfentanil, which has been approved for veterinary use (Wildnil®) due to its extremely high potency, recently emerged as a designer drug on the recreational drug market, posing a huge health risk not only for users but also for first responders and law enforcement staff. Since the 1970s, a multitude of further analogs has been investigated (Brine et al., 1995; Wang et al., 1995; Vuckovic et al., 2009). One of the first fentanyl analogs on the designer drug market was the highly potent 3-methylfentanyl, methylated at the piperidine ring (a) (**Figure 1**) resulting in a pair of diastereomers (Van Bever et al., 1974). Several different substituents like halogen atoms, methyl or methoxy groups of the anilinophenyl ring (b) (**Figure 1**) have been published and some of these emerged on the designer drug market in recent years (United Nations Office on Drugs and Crime [UNODC], 2017). The 2-phenylethyl moiety (c) (**Figure 1**) substituted at the tertiary piperidinyl-nitrogen seems to improve receptor binding over non-substituted or methyl substituted compounds, presumably by fitting better into a hydrophobic cavity of the μ-opioid-receptor in close proximity to the active binding site (Jiang et al., 2000; Subramanian et al., 2000). Fentanyl analogs modified at this moiety like α-methylfentanyl have been reported to be involved in some fatal intoxication cases in the 1980s (Gillespie et al., 1982). The β-hydroxylated analog of 3-methylfentanyl, ohmefentanyl, has been well researched in the 1980s, showing extremely high potencies for some of the diastereomers (Subramanian et al., 2000). Modification of the propanamide moiety (d) (**Figure 1**) of fentanyl led to a huge variety of newly emerged fentanyl analogs in recent years (such as butyrfentanyl, furanylfentanyl, benzodioxole fentanyl, cyclopropylfentanyl, methoxyacetylfentanyl and many more). These derivatives are presently in the focus of research, since there is none or very little data available so far.

Fentanyl

Fentanyl is a medically used 4-Anilinopiperidine derivative like alfentanil, sufentanil, and remifentanil. These drugs are used in surgery as adjuncts to anesthesia, for sedation and for the treatment of acute and chronic pain (Van Bever et al., 1976; Van Daele et al., 1976; Kukanich and Papich, 2009).

First metabolism studies on fentanyl were conducted by Van Wijngaarden and Soudijn (1968) monitoring the parent compound and metabolites of radio-labeled fentanyl in urine and feces of rats after intravenous administration. In the late 1980s, Banks and Ferguson (1988) described fentanyl metabolism, indicating that several factors have to be taken into consideration in order to determine drug metabolism: administration routes (intravenous, subcutaneous, transdermal, transmucosal, and spinal), tissue chosen for analysis, isolation procedure and inter- and intra-individual variation that can influence metabolite formation and distribution (Streisand et al., 1991; Solassol et al., 2005).

Fentanyl (*N*-phenyl-*N*-[1-(2-phenethyl)-4-piperidinyl]propanamide) has several sites for metabolic transformation. It is a heterocyclic tertiary aliphatic amine containing two different phenyl rings and an aromatic amide function. Tertiary aliphatic amines are biotransformed through a reversible reaction into tertiary amine oxides. The tertiary amines also undergo *N*-dealkylation through the carbinolamine. When this process happens on the phenylethyl side chain, in addition to the secondary amine a phenylacetaldehyde

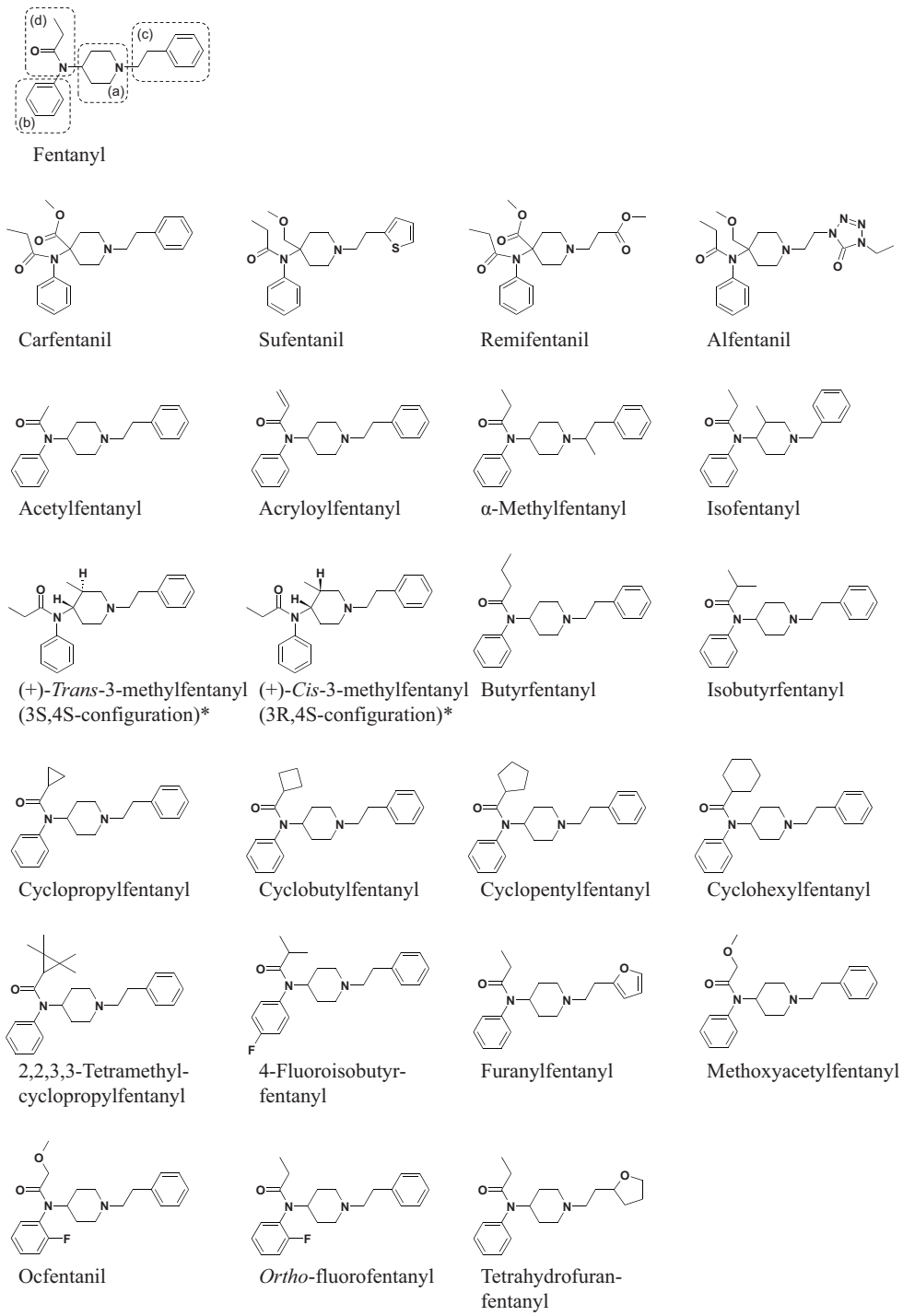

FIGURE 1 | Chemical structures of fentanyl and reviewed fentanyl analogs with data on metabolism and/or potency available in the scientific literature. The structures marked with '*' show only one of the two enantiomers.

is produced, which immediately oxidizes into phenylacetic acid. Oxidation at the 2-position of the piperidine ring generates a carbinolamine, which transforms into a more stable aminoaldehyde, resulting in ring cleavage. Aromatic rings undergo oxidation producing the equivalent phenolic derivatives. Furthermore, benzylic positions are more prone to oxidation. Amide functions usually undergo hydrolysis, and oxidation of the carbon chain is also frequent (Goromaru et al., 1984; Banks and Ferguson, 1988; Vardanyan and Hruby, 2014).

In humans, fentanyl is mainly metabolized in the liver by CYP3A4 into norfentanyl through oxidative *N*-dealkylation at the piperidine ring by hepatic CYP3A4 and 3A5 isoenzymes, which is the principal pathway of metabolism (Feierman and Lasker, 1996; Guitton et al., 1997; Labroo et al., 1997; Gudin, 2012; Bista et al., 2014; Armenian et al., 2018). The inactive metabolites and less than 10% of the intact molecule are mainly excreted in urine and feces (Mercadante, 2015; Kuip et al., 2017; Armenian et al., 2018) and less than 1% is metabolized by alkyl hydroxylation, combined *N*-dealkylation and hydroxylation or amide hydrolysis to the inactive compounds hydroxyfentanyl, hydroxy norfentanyl, and despropionylfentanyl (Kuip et al., 2017; Wu et al., 2017). The schematic human metabolic profile of fentanyl is depicted in **Figure 2**.

Fentanyl is also metabolized to norfentanyl in human duodenal microsomes; the mean rate is approximately half of the hepatic metabolism. Consequently, both intestinal and liver microsomes catalyze fentanyl metabolism and *N*-dealkylation

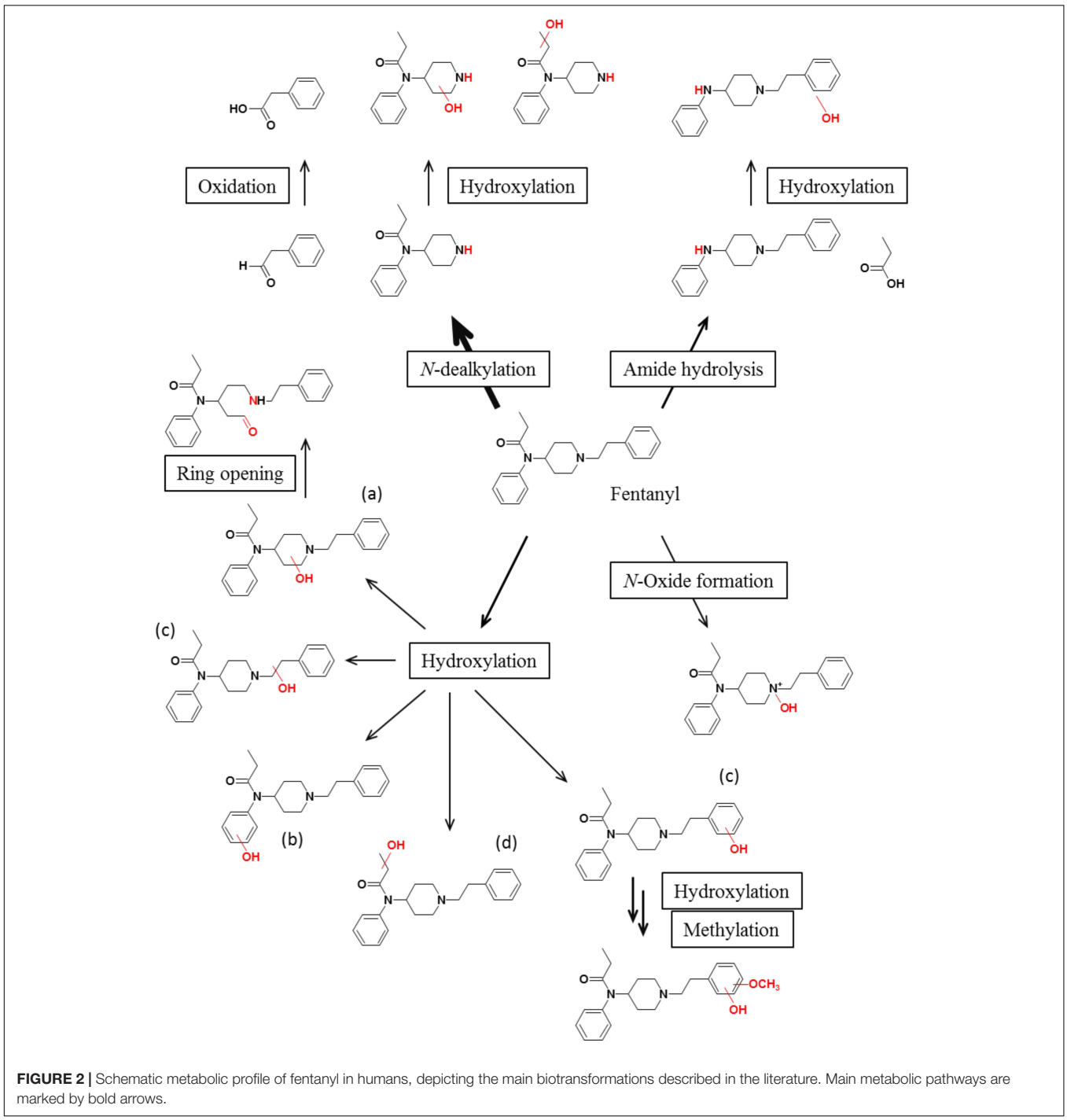

FIGURE 2 | Schematic metabolic profile of fentanyl in humans, depicting the main biotransformations described in the literature. Main metabolic pathways are marked by bold arrows.

by CYP3A4 is the principal active enzyme in both organs (Labroo et al., 1997). Hydroxylation occurs on the 2 or 3 position of the piperidine ring (a) (**Figure 1**), at the phenyl ring of the anilino moiety (b) (**Figure 1**), at the ethyl linker or the phenyl ring of the phenethyl moiety (c) (**Figure 1**), or along the amide alkyl chain (d) (**Figure 1**). The 4′-hydroxyfentanyl and other hydroxylated metabolites might be bioactive (Schneider and Brune, 1986), although the majority is believed to be inactive. The metabolite 4′-hydroxyfentanyl can undergo biotransformation via a second hydroxylation to allow a catechol that is then *O*-monomethylated to generate another metabolite. This reaction is probably catalyzed by the enzyme catechol-*O*-methyltransferase and presumably occurs at the 3′ position. This is technically a phase II metabolic product and can be detected in both hydrolyzed and non-hydrolyzed urine specimens due to its stability.

Minor metabolites such as hydroxypropionyl-fentanyl and hydroxypropionyl-norfentanyl are also created through different pathways without any relevant pharmacological activity. These metabolites have been detected in urine, stool and plasma (Bista et al., 2014).

Referring to despropionyl-fentanyl, another minor human metabolite, also known as 4-ANPP (Mahlke et al., 2014), results from carboxamide hydrolysis (Armenian et al., 2018).

Fentanyl is considered to be safer than morphine, in patients with liver and renal damage, because of a lack of metabolite accumulation (DePriest et al., 2015). Fentanyl activity can increase or decrease depending on genetic variations in the GI tract and in the liver, or through drugs which inhibit or induce CYP3A4. Fentanyl metabolism may be inhibited by macrolides, antifungal agents, and cimetidine (Bernard and Bruera, 2000). Serum fentanyl concentrations can vary significantly depending on liver function and the use of CYP3A4 inducers, therefore a model formula including these parameters has been provided, as a means to determine a transdermal fentanyl dose for the alleviation of cancer pain (Kokubun et al., 2012; Mercadante, 2015).

Bista and colleagues conducted a study to detect fentanyl and norfentanyl in plasma and saliva, showing that plasma and saliva had mean fentanyl concentrations of 0.785 and 3.335 μg/L, respectively. Similarly, in plasma and saliva the mean norfentanyl concentration was 0.53 and 0.517 μg/L, respectively. These data show that the concentration of fentanyl in saliva exceeds the concentration in plasma, suggesting an active transport into saliva. These data may in part be explained by the variable sample collection times with reference to time of dose, as distribution mechanisms will likely alter the saliva/plasma concentration ratio (Bista et al., 2014).

Alfentanil, Sufentanil, Remifentanil

In humans, the other fentanyl analogs frequently used in anesthesia – alfentanil, sufentanil, and remifentanil – are extensively metabolized and just a little percentage of the dose is excreted in urine without metabolic transformation. Metabolites such as norsufentanil and noralfentanil seem to be pharmacologically inactive (Valaer et al., 1997; Skulska et al., 2004).

Alfentanil (*N*-{1-[2-(4-ethyl-5-oxo-4,5-dihydro-1H-tetrazol-1-yl)ethyl]-4-(methoxymethyl)-piperidin-4-yl}-*N*-phenylpropanamide) and sufentanil (*N*-{4-(methoxymethyl)-1-[2-(thiophen-2-yl)ethyl]piperidin-4-yl}-*N*-phenylpropanamide) are also principally metabolized in the liver via the CYP3A4 hepatic pathway, which generates the same *N*-dealkylated inactive metabolite, making a forensic distinction impossible when only this metabolite is detected (Armenian et al., 2018).

Compared to fentanyl, alfentanil has a smaller volume of distribution, greater binding to plasma proteins, less binding to red blood cells, a shorter elimination half-life, a slower total body clearance, and is less lipid soluble – characteristics which suggest that alfentanil would be an appropriate drug to give by continuous i.v. infusion (Fragen et al., 1983).

Sufentanil is metabolized by the liver and enterocytes of the small intestines, catalyzed by the cytochrome P450 enzyme system (Donk et al., 2018). Sufentanil metabolites are excreted in the urine. *N*-Dealkylation of sufentanil leads to mostly inactive metabolites such as the metabolites formed by oxidative *N*-dealkylation at the piperidine ring (norsufentanil) or the phenylpropanamide nitrogen (leading to *N*-phenylpropanamide) and by aromatic hydroxylation (Lavrijsen et al., 1990; Tateishi et al., 1996; Koyyalagunta, 2007). Norsufentanil retains some activity, whereas the oxidative *O*-demethylation product (demethylsufentanil) is active retaining about 10% of the activity of sufentanil. However, it is produced in small quantities only and therefore not clinically relevant. The extensive metabolism of sufentanil in the GI tract is responsible for the low bioavailability following oral administration, so if a patient accidentally swallows a sublingual tablet this will result in under-dosing. Although the absence of clinically relevant metabolites makes sufentanil an option in mild-to-moderate renal impairment, there is insufficient data in patients with severe renal impairment, and hence careful patient monitoring is advised (Donk et al., 2018).

Remifentanil {methyl 1-(3-methoxy-3-oxopropyl)-4-[phenyl(propanoyl) amino]piperidine-4-carboxylate} is metabolized directly in the plasma by non-specific esterases, a hugely active group of enzymes found in blood and tissues throughout the body, resulting in an ultra-short duration of action (Rosow, 1999; Panzer et al., 2009). It is the only fentanyl analog that is 95% metabolized in the blood and tissues by non-CYP enzymes, because of an easily accessible ester group allowing for rapid hydrolysis by circulating blood esterases (Armenian et al., 2018). Its primary metabolite is remifentanil acid (a carboxylic acid derivative, GR90291), which has negligible pharmacological activity. Therefore, although remifentanil acid is excreted by the kidneys, remifentanil's action is not prolonged to a significant extent by renal injury or prolonged infusion in patients in intensive care (Panzer et al., 2009; Cascone et al., 2018). Experimental *in vivo* evaluations of the metabolic kinetics are presently not available (Cascone et al., 2018).

Acetylfentanyl

Acetylfentanyl (*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl] acetamide) is the acetyl amide analog of fentanyl. Relative potencies of several fentanyl analogs compared to fentanyl were

evaluated by Higashikawa and Suzuki (2008a) in an animal study using the Litchfield–Wilcoxon test after peroral administration of diluted solutions of the fentanyl analogs to mice. ED_{50} and LD_{50} values obtained for acetylfentanyl were 0.021 and 9.3 mg/kg, respectively, suggesting about 30% of the analgesic potency of fentanyl.

In general, acetylfentanyl is metabolized in a similar way to fentanyl. Acetylfentanyl has a major primarily inactive metabolite, acetyl norfentanyl, produced by *N*-dealkylation via CYP450 enzymes (Patton et al., 2014; Watanabe et al., 2017). Melent'ev et al. (2015) investigated metabolism of acetylfentanyl in urine samples collected from fatal intoxication cases with this fentanyl analog. In this study, besides the proposed main metabolite acetyl norfentanyl and the deacetylated acetylfentanyl metabolite (4-ANPP), primarily hydroxylated acetylfentanyl metabolites and their phase II conjugates were detected. Hydroxylated metabolites of acetylfentanyl were also identified after incubation with hepatocytes (Kanamori et al., 2018b), including a 4′-hydroxy-3′-methoxy-metabolite which has also been found as a metabolite of fentanyl and was also detected by Melent'ev et al. (2015) in the death cases involving acetylfentanyl. In an additional work Kanamori et al. (2018a) determined the involvement of different CYP isoenzymes in the formation of the metabolites of acetylfentanyl described above. Moreover, Watanabe et al. (2017) identified 31 metabolites of acetylfentanyl in human hepatocytes and authentic human urine samples, including the β-hydroxy and 4′-hydroxy-3′-methoxy metabolite, and several other phase I and phase II metabolites formed via various pathways such as glucuronidation, sulfation, dihydroxylation, monohydroxylation, carbonylation, and dihydrodiol formation.

Acryloylfentanyl

The fentanyl analog acryloylfentanyl (acrylfentanyl, *N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-acrylamide) differs from fentanyl only in dehydration in the 2,3-position of the propionylamide moiety. The competitive binding affinity of acryloylfentanyl was determined by Maryanoff et al. (1982) in rat brain using tritium-labeled naloxone. The IC_{50} value obtained was 1.4 nM and therefore similar to fentanyl (IC_{50} 1.6 nM). The analgesic properties of acryloylfentanyl were investigated by Essawi (1999) and it was found to be less potent than fentanyl (approximately 75% of fentanyl potency), but the analgesic effects persisted considerably longer. Though, the acrylamide moiety may lead to irreversible receptor binding and higher toxicity. However, LD_{50} values for acryloylfentanyl and fentanyl were 0.082 and 0.062 mg/kg, respectively, suggesting similar acute toxicity.

Similarly to fentanyl, acryloylfentanyl is lipophilic and expected to easily cross the blood-brain barrier. Distribution into fat and other tissues seems likely due to the presumably high volume of distribution (Ujváry et al., 2017). The metabolic pathway of acryloylfentanyl shows similarity with the pathways of fentanyl and acetylfentanyl. The main metabolites generated by human hepatocytes *in vitro* and of those detected in the urine in a few fatalities, caused by acryloylfentanyl, and their chemical structures were recently described by Watanabe et al. (2017). Overall, 14 biotransformation products, including major metabolites of acryloylfentanyl detected in human urine after hydrolysis of glucuronidated and/or sulfated phase II conjugates were identified in this work. The biotransformations involve an oxidative *N*-dealkylation, presumably catalyzed by cytochrome P450 (CYP450) enzymes, leading to the desphenethyl metabolite acryloylnorfentanyl which is biologically inactive. Furthermore, monohydroxylations were observed either at the alkyl linker of the phenylethyl moiety or at the piperidine ring. Dihydroxylation of the phenyl ring of the phenylethyl moiety resulting in a catechol structure followed by *O*-monomethylation were additional oxidative metabolic processes leading to similar metabolites as described for acetylfentanyl in the same work. Similar to fentanyl metabolism, amide hydrolysis (deacylation) results in a minor metabolite 4-ANPP, which is a common metabolite of fentanyl, acryloylfentanyl and several other fentanyl analogs. Acryloylfentanyl was also present in the urine of the deceased individuals.

With respect to acryloylfentanyl, the major human urinary metabolites identified *in vivo* (fatal cases) were acryloylnorfentanyl, as well as mono- and dihydroxylated derivatives and their conjugates (Watanabe et al., 2017).

α-Methylfentanyl and (*cis*/*trans*)-3-Methylfentanyl

As one of the mono-methylated fentanyl derivatives, α-methylfentanyl (*N*-Phenyl-*N*-[1-(1-phenyl-2-propanyl)-4-piperidinyl]propanamide) carries the additional methyl group at the 1-position of the ethyl bridge of the phenethyl moiety. The diastereomeric pairs of enantiomers *cis*-3-methylfentanyl and *trans*-3-methylfentanyl carry the additional methyl group at the 3-position of the piperidine ring. The analgesic activity of these derivatives proved to be similar to fentanyl or higher. In a study of Higashikawa and Suzuki (2008a) α-methylfentanyl showed a very similar ED_{50} value as fentanyl (0.0058 and 0.0061 mg/kg, respectively). However, toxic effects occurred at significantly lower doses of α-methylfentanyl when compared to fentanyl (LD_{50} values 8.6 and 62 mg/kg, respectively). Van Bever et al. (1974) synthesized the different isomers of α-methylfentanyl and 3-methylfentanyl and investigated their relative analgesic potencies. ED_{50} values for α-methylfentanyl (0.0085 mg/kg) obtained in this work were in agreement with the reported values of Higashikawa and Suzuki (2008a), although fentanyl showed a higher value here (0.011 mg/kg). The (±)-*trans*-3-methylfentanyl enantiomers (ED_{50} 0.0094 mg/kg) showed about the same effective dose as α-methylfentanyl, but the (±)-*cis*-enantiomers turned out to be even more potent and exhibited a significant difference between the (+)- and (−)-enantiomer. The most potent isomer was (+)-*cis*-3-methylfentanyl (ED_{50} 0.00058 mg/kg) being about 20 times more potent than fentanyl, whereas the (−)-*cis*-isomer showed only 20% of the potency of fentanyl.

The first reports about detection of a metabolite of α-methylfentanyl were published by Gillespie (Gillespie et al., 1982), who found the hydrolysis product despropionyl-α-methylfentanyl in several biological samples of fatal

intoxication cases. Higashikawa and Suzuki (2008b) investigated the metabolism of α-methylfentanyl in urine after administration to rats. The main metabolite formed by *N*-dealkylation in this study was norfentanyl, a metabolite shared with fentanyl. Furthermore, two metabolites in common with fentanyl were formed by further hydroxylation of the propionylamide moiety of norfentanyl [ω-hydroxypropionyl-norfentanyl and (ω-1)-hydroxypropionyl-norfentanyl]. However, four additional metabolites were identified enabling differentiation of α-methylfentanyl and fentanyl consumption resulting from mono- and dihydroxylation of α-methylfentanyl [ω-hydroxypropionyl-α-methylfentanyl, (ω-1)-hydroxypropionyl-α-methylfentanyl, *para*-hydroxyphenyl-α-methylfentanyl and *para*-hydroxyphenyl-ω-hydroxypropionyl-α-methylfentanyl].

Investigations in rat performed by Sato et al. (2010) confirmed the findings of Higasikawa and Suzuki regarding the metabolic spectrum and demonstrated the time-course of metabolite excretion as well as the proportions of metabolites excreted in rat urine over a 96 h time period. Non-specific metabolites of α-methylfentanyl were detectable up to 72 h after administration, whereas the specific metabolites were completely eliminated after 48 h and accounted for only 2–3% of the total amount of metabolites excreted in urine.

First detection of single metabolites of the methylated fentanyl analog 3-methylfentanyl was reported by Hammargren and Henderson (1988) who detected the dealkylated metabolite nor-3-methylfentanyl in urine of suspected drug users. A systematic investigation of the metabolism of 3-methylfentanyl was done by Meyer et al. (2012) proposing a metabolic pathway for this fentanyl analog and reporting 9 phase I and 5 corresponding phase II metabolites in rat urine after drug administration. In accordance to Hammargren and Henderson, the main metabolite detected was nor-3-methylfentanyl formed by oxidative *N*-dealkylation. Further oxidation of this metabolite led to formation of hydroxypropionyl-nor-3-methylfentanyl and hydroxyphenyl-nor-3-methylfentanyl. In addition, mono- and dihydroxylations were observed primarily at the phenylethyl and the propionylamide moiety followed by either further oxidative reactions leading to a carboxy-propionyl metabolite or methylation of the 3,4-dihydroxyphenyl metabolite leading to a 3-methoxy-4-hydroxy metabolite in analogy to previously reported fentanyl and fentanyl analog metabolites. Furthermore, Meyer et al. (2012) also reported phase II glucuronic acid conjugates detected for hydroxylated metabolites.

Isofentanyl

Isofentanyl (*N*-(1-benzyl-3-methylpiperidin-4-yl)-*N*-phenylpropanamide) was clandestinely synthesized to circumvent 3-methylfentanyl regulation.

Meyer and collaborators identified isofentanyl together with 3-methylfentanyl phase I and phase II metabolites in rat urine (Meyer et al., 2012). Isofentanyl is an isomer of fentanyl and shares some main fragment ions in MS analysis. Metabolites such as the nor-metabolite can help to unequivocally prove uptake of this compound. For isofentanyl 11 phase I and 4 phase II metabolites were identified. The following metabolic steps could be postulated: *N*-dealkylation resulting in a common metabolite with 3-methylfentanyl (nor-3-methylfentanyl = nor-isofentanyl) followed by hydroxylations of the alkyl and/or aryl moiety, hydroxylation of the propionylamide side chain followed by oxidation to the corresponding carboxylic acid, and hydroxylations of the benzyl moiety followed by methylation resulting in the corresponding 3-methoxy-4-hydroxy metabolite. In addition, *N*-oxidation of isofentanyl was also observed. Some hydroxylated metabolites were partly excreted as glucuronides. Using recombinant human isoenzymes, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 were found to be involved in the initial metabolic steps. The parent drugs could not be detected in urine. Their common nor-metabolite was suggested as a common target for urine screening for 3-methylfentanyl and isofentanyl. Targeting less abundant specific metabolites may enable differentiation of an uptake of either of the drugs (Meyer et al., 2012).

Butyrfentanyl and Isobutyrfentanyl

Butyrfentanyl (*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl] butanamide) belongs to the mono-methylated fentanyl derivatives carrying the additional methyl group at the ω-carbon of the propionyl amide resulting in a butyryl amide analog of fentanyl. Isobutyrfentanyl is the isomer carrying the additional methyl group at the α-carbon of the propionylamide moiety. Both compounds were included in the activity studies of Higashikawa and Suzuki (2008a). In a study from Alburges et al. (1992) the binding affinity of butyrfentanyl to the μ-opioid receptor was reported ($K_i = 32 \pm 4.1$ nM), which is about 32-fold lower than the binding affinity of fentanyl ($K_i = 1.03 \pm 0.15$ nM).

Metabolites of butyrfentanyl were detected and identified in a fatal poisoning described by Staeheli et al. (2016) with focus on the post mortem tissue distribution and redistribution, a phenomenon often observed when analyzing post mortem samples. The identified metabolites were norbutyrfentanyl, carboxybutyrfentanyl, hydroxybutyrfentanyl, and desbutyrfentanyl. In pursuit of elucidation of the metabolism of butyrfentanyl, blood and urine samples of the same fatal intoxication case in conjunction with *in vitro* studies producing phase I and phase II metabolites of butyrfentanyl were investigated by Steuer et al. (2017). Human liver microsomes and recombinant cytochrome P450 enzymes (CYP) were used for *in vitro* assays. Butyrfentanyl was shown to undergo extensive metabolism. In total, 36 metabolites were identified in this study. The postulated primary metabolic pathways were hydroxylations at the butanamide side chain (in two positions), the phenylethyl moiety and the piperidine ring, oxidative *N*-dealkylation, formation of *N*-oxides and hydrolysis of the acyl moiety. Besides that, combinations of these biotransformations and additional reactions were observed leading to, e.g., carboxylated metabolites by further oxidation of the ω-hydroxy-butanamide moiety or methylation of the 3,4-catechol moiety of dihydroxylated metabolites forming the respective 3-methoxy-4-hydroxy metabolites. Furthermore phase II conjugates were detected in the human post mortem samples for nine metabolites (eight glucuronic acid conjugates and one sulfate). The main metabolites detected in the *in vitro* studies, nor-butyrfentanyl,

butyrohydroxy-butyrfentanyl and phenylethyl-hydroxy-butyrfentanyl, were not in agreement with the main metabolites detected in authentic biological samples. The main metabolites detected *in vivo* were carboxy-butyrfentanyl in blood and carboxy-butyrfentanyl, butyrohydroxy-butyrfentanyl and carboxy-phenylethyl-hydroxy-butyrfentanyl in urine. Initial screening experiments with the most relevant CYPs indicated that mainly CYP2D6 and 3A4 were involved in the primary metabolic steps. Therefore, variability of phenotypes regarding these enzymes may have an influence on the metabolic profile *in vivo*. As a strategy to reach maximum detectability it seems advisable to include metabolites formed by different pathways as targets into analytical methods.

Carfentanil

Carfentanil [methyl 1-(2-phenylethyl)-4-[phenyl(propanoyl) amino]piperidine-4-carboxylate] is a member of the *N*-4 substituted fentanyl analogs carrying an additional methyl-carboxylate moiety at the 4-position of the piperidine ring. This group of fentanyl analogs turned out to be significantly more potent than their non-substituted analogs. Carfentanil is about 10,000 times more potent than morphine and shows 30–100 times the potency of fentanyl (Van Bever et al., 1976), thereby representing the most potent approved opioid drug. Receptor binding affinity and analgesic activity of this compound has been investigated extensively by many research groups, reporting ED_{50} values from 0.00032 nM up to 0.0017 (<0.01) nM and K_i values for the μ-opioid receptor of 0.024 nM up to 0.15 nM (Thompson et al., 1987; Costa et al., 1992; Maguire et al., 1992; Villemagne et al., 1994; Bi-Yi et al., 1999; Jewett and Kilbourn, 2004; Henriksen et al., 2005). Carfentanil is used in veterinary medicine as general anesthetic, for pain management, and to immobilize large animals (Kukanich and Papich, 2009).

Due to its extremely high potency studies assessing the metabolism of carfentanil in humans have not been performed yet and it seems unlikely that they would be approved by an Ethics Committee. Though, metabolites of carfentanil have only been detected in fatal intoxication cases so far, the most well-known case being the Moscow Theater hostage-taking (Riches et al., 2012). Riches et al. (2012) detected the *N*-dealkylated metabolite norcarfentanil in a donated urine sample. Norcarfentanil is a common (minor) metabolite of the fentanyl analog remifentanil. First and only studies assessing the metabolic pathways of carfentanil were performed by Feasel et al. (2016) using metabolism predictions software (Molecular Discovery's MetaSite software and Simulations Plus's ADMET Predictor) for first *in silico* prediction and human liver microsomes and hepatocytes as *in vitro* models.

In total, 12 metabolites were identified for carfentanil, 11 phase I metabolites and 1 phase II conjugate as glucuronide. The following metabolic reactions or combinations of these were observed: oxidative *N*-dealkylation, ester hydrolysis, hydroxylation and *N*-oxide formation. The most abundant metabolites reported were formed by *N*-dealkylation partly followed by ester hydrolysis or hydroxylation. Hydroxylations occurred at the propionylamide side chain, at the phenylethyl moiety or at the piperidine ring resulting in formation of eight hydroxylated metabolites, and five of them showed an additional biotransformation (ester hydrolysis, *N*-oxide formation or glucuronidation) or were further oxidized to ketones. Three *N*-oxide metabolites were reported with minor abundances, formed by oxidation of either the piperidine nitrogen or the anilino-nitrogen linked in the amide moiety. In contrast to studies concerning the metabolism of other fentanyl analogs so far, no amide hydrolysis metabolites or hydroxy-methoxy metabolites have been reported in this study.

Alicyclic Fentanyl Analogs: Cyclopropylfentanyl, Cyclobutylfentanyl Cyclopentylfentanyl, Cyclohexylfentanyl and 2,2,3,3-Tetramethylcyclopropyl-Fentanyl

This subgroup of newly emerging fentanyl analogs structurally differs in the aliphatic amide linked moiety, which is substituted by an aliphatic cyclic moiety in these compounds. Concerning the receptor binding affinities and potencies only cyclopropylfentanyl has been evaluated so far. *In vitro* studies using chinese hamster ovary (CHO) and rat cell preparations expressing the three types of opioid receptors were used for determination of binding affinities. Cyclopropylfentanyl binds selectively to the μ-opioid receptor (vs. [^{3}H]-DAMGO) with K_i values of 0.088 ± 0.027 nM for the μ-opioid receptor as well as 59.4 ± 3.0 nM and 36 ± 10 nM for the δ- and κ-opioid receptors, respectively. EC_{50} values were determined employing a [^{35}S]GTPγS binding assay, resulting in 10.8 ± 2.7 nM for cyclopropylfentanyl at the μ-opioid receptor compared to 32 ± 11 nM for fentanyl, showing a more or less similar (about threefold higher) potency to fentanyl (Drug Enforcement Administration–Veterans Affairs (DEA-VA) Interagency Agreement, 2017; European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2018b). For the other alicyclic analogs no literature on receptor binding affinities and potencies is available yet. Theoretically, these analogs may imitate the steric requirements for receptor binding of fentanyl. They probably show similar or lower potency than fentanyl, in analogy to butyr- and valerylfentanyl which have been reported to be less potent.

A study investigating metabolism of this group of compounds has been published very recently by Åstrand et al. (2018) using human hepatocytes. Seven metabolites were identified for cyclopropylfentanyl, and the most abundant metabolite was norcyclopropylfentanyl formed by oxidative *N*-dealkylation. Other metabolic reactions observed were monohydroxylation, dihydroxylation followed by subsequent methylation, dihydrodiol and *N*-oxide formation. The glucuronic acid conjugate of the most intense hydroxy metabolite (hydroxylated at the piperidine ring) was detected as well. Hydroxylation of the cyclopropyl moiety or amide hydrolysis has not been detected in this study. The main metabolite norcyclopropylfentanyl has also been detected by Maher et al. (2018) in urine samples of two death cases and Palaty et al. (2018) in several urine samples of patients with a substance use disorder from two canadian provinces.

The major metabolites detected for cyclobutylfentanyl by Åstrand et al. (2018) were also *N*-dealkylation and, in contrast to cyclopropylfentanyl, hydroxylation of the cyclobutyl moiety and amide hydrolysis leading to a metabolite found in common with fentanyl, 4-ANPP. Further mono- and dihydroxylated metabolites were identified, mainly hydroxylated at the cyclobutyl moiety, the piperidine ring or the phenylethyl moiety.

In agreement with findings of cyclobutylfentanyl, the most abundant metabolites found were hydroxylations of the cyclopentyl moiety and nor-cyclopentylfentanyl. Moreover, another monohydroxlated metabolite (at the piperidine ring) and two monohydroxylated normetabolites (both at the cyclopentyl ring), a dihydroxylated metabolite (at the piperidine ring and the cyclopentyl ring), the amide hydrolysis product 4-ANPP and two further oxidation products (*N*-oxide and carbonyl metabolite) were formed to a minor extent in this assay.

Incubation of cyclohexylfentanyl with hepatocytes mainly led to the amide hydrolysis product 4-ANPP, norcyclohexylfentanyl and two monohydroxlated metabolites (both modified at the cyclohexyl moiety). Again, further hydroxylated metabolites were detected, comprising monohydroxlation, dihydroxylation, and hydroxylations in a second metabolic step primarily at the cyclohexyl and the piperidine ring.

Substitution of the amide linked alkyl chain with a 2,2,3,3-tetramethylcyclopropyl moiety seemed to steer metabolic reactions to this part of the molecule. Except for the nor-2,2,3,3-tetramathylcyclopropylfentanyl metabolite, which was formed to a minor extent, all metabolites showed at least one biotransformation of the 2,2,3,3-tetramethylcyclopropyl moiety. Monohydroxylations and dihydroxylations and subsequent further oxidation steps resulting in the formation of, e.g., carboxylic acids have been reported by Åstrand et al. (2018).

4-Fluoroisobutyrfentanyl (4F-iBF, *Para*-Fluoroisobutyrfentanyl)

4-fluoroisobutyrfentanyl (4-fluoro-isobutylfentanyl (*N*-(4-fluorophenyl)-2-methyl-*N*-[1-(2-phenylethyl)-4-piperidinyl] -propanamide) is one of the fluorinated fentanyl analogs that emerged on the NPS drug market recently. For this analog no data on binding affinity and selectivity to the μ-opioid receptor is available. The potency of 4F-iBF might be similar or lower than the potency of butyrfentanyl/isobutyrfentanyl, following the evaluation of fluorinated derivatives by Higashikawa and Suzuki (2008a). Metabolism of 4F-iBF was investigated by Watanabe et al. (2017) using hepatocyte incubates and analyzing authentic urine samples. In total, 17 metabolites were found and the following biotransformations were observed: *N*-dealkylation, monohydroxylations, dihydroxylations and subsequent methylation and glucuronidation, dihydrodiol formation, amide hydrolysis, carbonylation and carboxylation. Nine metabolites were identified in the hepatocyte assay. The most abundant ones were nor-4F-iBF, and two hydroxylated metabolites (at the piperidine ring or the phenylethyl moiety). Analysis of the urine samples after conjugate cleavage revealed 11 metabolites, resulting in a similar metabolic profile as obtained from the hepatocyte incubation assay, although the hydroxy-methoxy metabolite was more dominant in the authentic urine sample. Two additional glucuronic acid conjugates were detected when analyzing the urine without hydrolysis prior to extraction.

Furanylfentanyl

Binding affinity studies on furanylfentanyl have been performed *in vitro* using CHO and rat cell preparations expressing the three types of opioid receptors. Furanylfentanyl binds selectively to the μ-opioid receptor (vs. [^{3}H]-DAMGO) with K_i values of 0.028 ± 0.008 nM for the μ-opioid receptor as well as 54 ± 15 nM and 59.2 ± 6.4 nM for the δ- and κ-opioid receptors, respectively. *In vitro* EC_{50} values were determined employing a [^{35}S]GTPγS binding assay, resulting in 2.52 ± 0.46 nM for furanylfentanyl compared to 17.9 ± 4.3 nM for fentanyl at the μ-opioid receptor, suggesting a sevenfold higher potency for furanylfentanyl over fentanyl (Drug Enforcement Administration–Veterans Affairs (DEA-VA) Interagency Agreement, 2016; European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2017). In the patent literature of furanylfentanyl an *in vivo* ED_{50} value (0.02 mg/kg) was reported after i.v. administration to mice, but comparative data of fentanyl or morphine was not reported (Huang et al., 1986).

Goggin et al. (2017) identified four metabolites of furanylfentanyl after analyzing 500 urine samples of opioid intoxication cases. The most pronounced metabolites detected in 42 out of 51 cases positive for furanylfentanyl was the hydrolysis product 4-ANPP and its sulfate conjugate. Moreover, a very unique metabolite formed by dihydrodiol formation of the heterocyclic furanyl moiety was detected in 86% of the cases. The *N*-dealkylated metabolite norfuranylfentanyl was detected in only four of the furanylfentanyl positive cases indicating that substitution of the amide linked moiety of the fentanyl analogs to a furanyl-carboxamide shifts the metabolic profile of this compound toward a hydrolytic reaction and biotransformation of the furanyl moiety. In accordance with these findings, Mohr et al. (2016) detected 4-ANPP in five out of eight fatal intoxications with furanylfentanyl and Martucci et al. (2018) reported detection and distribution of the hydrolysis metabolite 4-ANPP in various tissues of a fatal furanylfentanyl intoxication case. In total, 17 and 14 phases I and II metabolites of furanylfentanyl were identified in a more detailed *in vitro* approach by Richeval et al. (2017) and Watanabe et al. (2017) using human liver microsomes and hepatocytes. In contrast to the findings of Goggins and Martucci, the spectrum of metabolic reactions in these *in vitro* studies comprised several hydroxylations, *N*-oxide formation and glucuronidation besides the already mentioned amide hydrolysis (plus sulfate conjugation), dihydrodiol formation and *N*-dealkylation. The most abundant *in vitro* metabolites reported by both authors were the hydrolysis product 4-ANPP, a dihydrodiol metabolite and norfuranylfentanyl. Additionally, a metabolite formed by oxidative ring-opening of the furanyl ring (and further oxidation to a carboxylic acid) was reported by both groups. Since metabolism of furanylfentanyl has been studied by a couple of research groups it can be said, that *N*-dealkylation which often leads to main metabolites of fentanyl and fentanyl

analogs *in vitro* and *in vivo*, plays a minor role in the metabolism of furanylfentanyl, whereas amide hydrolysis and oxidative transformations of the furanyl moiety (such as dihydrodiol formation) are major biotransformation steps seen both *in vitro* and *in vivo* for this compound.

Methoxyacetylfentanyl

Methoxyacetylfentanyl is one of the numerous newly emerged fentanyl analogs differing from fentanyl by the modification of the *N*-acyl moiety. Structure activity relationships of methoxyacetylfentanyl and several other alkyloxy derivatives were investigated by Bagley et al. (1991) reporting an ED_{50} value of 0.053 mg/kg for methoxyacetylfentanyl. Compared to the ED_{50} of 0.018 mg/kg for fentanyl, about 30% of the potency of fentanyl can be assumed for this compound.

Metabolism of methoxyacetylfentanyl (2-methoxy-*N*-(1-phenethylpiperidin-4-yl)-*N*-phenylacetamide) was first examined *in vitro* using a human liver microsomal preparation (European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2018a). A main metabolic step for methoxyacetylfentanyl appears to be *O*-demethylation leading to 2-hydroxyacetamide metabolite. Further metabolic reactions were *N*-dealkylation, hydroxylations of the piperidine ring and the phenylethyl side chain, *N*-oxidation, as well as amide hydrolysis to 4-ANPP.

Mardal et al. (2018) investigated the *in vitro* and *in vivo* metabolic profiles of methoxyacetylfentanyl in the context of three case reports on deaths related to methoxyacetylfentanyl and by applying an additional *in vitro* study using human hepatocytes. A total of 10 methoxyacetylfentanyl metabolites were identified in hepatocyte incubates and biological samples. The metabolic pathways comprised mono- and dihydroxylations (at the phenylethyl ring or the anilinophenyl ring), *N*-dealkylation, *O*-demethylation, amide hydrolysis and combinations thereof as well as *O*-glucuronidation of the *O*-demethylated metabolite. The main metabolites both detected *in vitro* and *in vivo* were the *O*-demethylated metabolite and the hydrolysis product 4-ANPP. The findings of this study were consistent with unpublished data provided to the EMCDDA for risk assessment of methoxyacetylfentanyl (European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2018a).

Ocfentanil

This fentanyl analog has been developed and patented by Huang et al. (1986) and was evaluated for clinical application. Ocfentanil (*N*-(2-fluorophenyl)-2-methoxy-*N*-[1-(2-phenylethyl)-4-piperidinyl]acetamide) is the *ortho*-fluorinated analog of methoxyacetylfentanyl and has also been subject to the studies of Bagley et al. (1991). They determined an ED_{50} value of 0.0077 mg/kg for ocfentanil using the mouse hot plate test for evaluation of analgesic effects. Compared to fentanyl (0.018 mg/kg) the potency can be estimated to be about 2.5 times higher than for fentanyl. At the same time, ocfentanil showed less respiratory depression in animal studies. Fletcher et al. (1991) investigated dose-dependent pharmacologic effects in humans but were not able to draw conclusions from the study regarding a benefit of ocfentanil over fentanyl.

Allibe et al. (2018) performed metabolism studies on ocfentanil using human liver microsomes in addition to metabolism profiling in post mortem samples of a fatal intoxication case. Ocfentanil was found in all biological samples except nasal swab and concentrations were similar in peripheral blood and cardiac blood (Allibe et al., 2018). This observation is in contrast to results in two previously reported fatalities which observed significant deviations of the cardiac/peripheral blood concentration ratio (Coopman et al., 2016; Dussy et al., 2016).

Four metabolites were detected *in vitro* by Allibe et al. (2018) formed by hydroxylation (at the phenylethyl moiety), *O*-demethylation and combination of both reactions as well as the conjugation product of the *O*-demethyl metabolite with glucuronic acid. The main metabolite *in vitro* and *in vivo* clearly was *O*-demethyl ocfentanil, presumably even exceeding quantities of the parent compound (when comparing peak areas). In contrast, commonly seen biotransformations such as *N*-dealkylation and amide hydrolysis have not been detected in this work, suggesting that these metabolic reactions only play a minor role in metabolism of ocfentanil.

Ortho-Fluorofentanyl

Ortho-fluorofentanyl (*N*-(2-fluorophenyl)-*N*-[1-(2-phenylethyl)-4-piperidinyl]-propanamide) is a fluorinated fentanyl derivative. No data on receptor binding is available for this compound so far. However, the *para*-substituted analog was included in the studies performed by Higashikawa and Suzuki (2008a) and showed about 30% the potency of fentanyl determined by ED_{50} values. The LD_{50} values of 9.3 mg/kg for *p*-fluorofentanyl compared to 63 mg/kg for fentanyl indicate a higher toxicity of the fluorinated compound. The only study reporting metabolite identification of *ortho*-fluorofentanyl so far was a case report from Denmark by Andreasen et al. (2017). They detected the *N*-dealkylation product *ortho*-fluoro-norfentanyl in blood by HRMS techniques. Other potential metabolites like hydroxy-*ortho*-fluorofentanyl, hydroxy-*ortho*-fluoro-norfentanyl or the hydrolysis product *ortho*-fluoro-despropionylfentanyl were not detected in the authentic case sample. However, a urine sample was not part of the investigation, which could be the reason for the limited number of detected metabolites. The amide hydrolysis product *ortho*-fluoro-despropionylfentanyl has been reported to the EMCDDA as a fentanyl analog marketed independently, but further data on this compound is not available so far. A case report from Helland et al. (2017) focuses on the identification of *ortho*-fluorofentanyl and problems with the distinction of stereo-isomers. In this work the authors emphasize the necessity of integrating fluorinated analogs into general analytical screening procedures.

Tetrahydrofuranylfentanyl

The binding affinity of tetrahydrofuranylfentanyl (THFF, *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]oxolane-2-carboxamide) was determined by the United States Drug Enforcement Administration (DEA) using CHO and rat cell preparations for opioid receptor expression. K_i values for THFF were 0.95 ± 0.32 nM (μ-OR vs. [^{3}H]-DAMGO), compared to 741 ± 44 nM (vs. [^{3}H]-U-69593) and 1,730 ± 260 nM

TABLE 1 | Summary of the reviewed fentanyl analogs and their metabolites and metabolic pathways.

Compounds	Detected metabolites (metabolic pathways)	Estimated relative potencies to fentanyl
Alfentanil	Noralfentanil (*N*-dealkylation)	Approximately 0.3
Sufentanil	Norsufentanil and *N*-phenylpropanamide (*N*-dealkylation), demethylsufentanil (*O*-demethylation), hydroxy metabolites	Approximately 10
Remifentanil	Remifentanil acid (ester hydrolysis)	Approximately 1
Acetylfentanyl	Acetyl norfentanyl (*N*-dealkylation), 4-ANPP (amide hydrolysis), β-hydroxyacetylfentanyl and further hydroxy metabolites, 4'-hydroxy-3'-methoxy-acetylfentanyl (dihydroxylation + methylation) and phase II conjugates	0.3
Acryloylfentanyl	Acryloylnorfentanyl (*N*-dealkylation), 4-ANPP (amide hydrolysis), β-hydroxyacryloylfentanyl and further hydroxy metabolites, 4'-hydroxy-3'-methoxy-acryloylfentanyl (dihydroxylation + methylation) and phase II conjugates	Approximately 0.75
α-Methylfentanyl	Norfentanyl (*N*-dealkylation), Despropionyl-α-methylfentanyl (amide hydrolysis), alkyl/aryl hydroxy metabolites	Approximately 1
Cis-3-methylfentanyl *Trans*-3-methylfentanyl	Nor-3-methylfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites, carboxypropionyl-3-methylfentanyl (hydroxylation + oxidations), 4′-hydroxy-3′-methoxy-3-methylfentanyl (dihydroxylation + methylation) and phase II conjugates	20 (+) isomer 0.2 (−) isomer Approximately 1
Isofentanyl	Nor-3-methylfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites, carboxypropionyl-isofentanyl (hydroxylation + oxidations), 4′-hydroxy-3′-methoxy-isofentanyl (dihydroxylation + methylation), *N*-oxide formation and phase II conjugates	n.a.
Butyrfentanyl	Norbutyrfentanyl (*N*-dealkylation), carboxybutyrfentanyl (hydroxylation + oxidations), 4-ANPP (amide hydrolysis), alkyl/aryl hydroxy metabolites, 4′-hydroxy-3′-methoxy-butyrfentanyl (dihydroxylation + methylation), *N*-oxide formation and phase II conjugates	0.03–0.13
Isobutyrfentanyl	n.a.	0.13
Carfentanil	Norcarfentanil (*N*-dealkylation), alkyl/aryl hydroxy metabolites, carfentanil acid (ester hydrolysis), keto-carfentanil (hydroxylation + oxidation), *N*-oxide formation and phase II conjugates	30–100
Cyclopropylfentanyl	Norcyclopropylfentanyl (*N*-dealkylation), hydroxylations, dihydrodiol and *N*-oxide formation	3
Cyclobutylfentanyl	Norcyclobutylfentanyl (*N*-dealkylation), mainly alkyl hydroxy metabolites, 4-ANPP (amide hydrolysis), *N*-oxide and ketone formation	n.a.
Cyclopentylfentanyl	Norcyclopentylfentanyl (*N*-dealkylation), mainly alkyl hydroxy metabolites, 4-ANPP (amide hydrolysis), *N*-oxide and ketone formation	n.a.
Cyclohexylfentanyl	Norcyclohexylfentanyl (*N*-dealkylation), mainly alkyl hydroxy metabolites, 4-ANPP (amide hydrolysis), *N*-oxide and ketone formation	n.a.
2,2,3,3-Tetramethyl-cyclopropylfentanyl	Mainly alkyl hydroxy metabolites, Nor-2,2,3,3-tetramethylcyclopropylfentanyl (*N*-dealkylation), carboxy-2,2,3,3-tetramethylcyclopropylfentanyl (hydroxylation + oxidations)	n.a.
4-Fluoroisobutyrfentanyl	Nor-4-fluoroisobutyrfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites, 4-ANPP (amide hydrolysis), 4′-hydroxy-3′-methoxy-4-fluoroisobutyrfentanyl (dihydroxylation + methylation), dihydrodiol and ketone formation, carboxy-4-fluoroisobutyrfentanyl (hydroxylation + oxidations) and phase II conjugates	n.a.
Furanylfentanyl	Furano-dihydrodiol formation, 4-ANPP (amide hydrolysis), norfuranylfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites, ring opening of the furanyl ring and phase II conjugates	7
Methoxyacetylfentanyl	Demethylmethoxyacetylfentanyl (*O*-demethylation), 4-ANPP (amide hydrolysis), normethoxyacetylfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites and phase II conjugates	0.3
Ocfentanil	Demethylocfentanil (*O*-demethylation), alkyl/aryl hydroxy metabolites and phase II conjugates	2.5
Ortho-Fluorofentanyl	Nor-*ortho*-fluorofentanyl (*N*-dealkylation)	n.a.
Tetrahydrofuranylfentanyl	Nortetrahydrofuranylfentanyl (*N*-dealkylation), alkyl/aryl hydroxy metabolites, ring opening of the tetrahydrofuranyl ring and 4-ANPP (amide hydrolysis)	Approximately 0.2

Estimated relative potencies compared to fentanyl (set to 1) are also given (n.a., no data available).

(vs. [^{3}H]-DPDPE) for the δ- and κ-opioid receptors, respectively, showing high selectively for the μ-opioid receptor. EC_{50} values were determined *in vitro* employing an [^{35}S]GTPγS binding assay and resulted in 89 ± 16 nM for THFF at the μ-opioid receptor. The authors report a lower potency compared to fentanyl for this compound (European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2018c).

Data provided to the EMCDDA for risk assessment of THFF propose *N*-dealkylation to be the predominant metabolic step for THFF in human liver microsomal preparations, as in the case of fentanyl. Hydroxylation of the piperidine ring and the phenylethyl side chain, *N*-oxidation and amide hydrolysis to 4-ANPP were also observed (European Monitoring Centre for Drugs Drug Addiction [EMCDDA], 2018c).

Metabolic profiling of THFF was performed by Krotulski et al. (2018) to assist analytical identification of THFF in a fatality. Overall, seven metabolites were identified *in vitro* for THFF using pooled human liver microsomes. The hydroxylated metabolite species produced multiple, indistinguishable signals for hydroxylations at the tetrahydrofuranyl ring or the phenylethyl moiety. One of the major metabolites *in vitro* was nortetrahydrofuranylfentanyl formed by *N*-dealkylation, which proofed to be an applicable biomarker for THFF ingestion in biological samples. The hydroxylated species were also prominently detected in post mortem blood and urine samples. The hydrolysis product 4-ANPP was not unequivocally identified as a metabolite in this study (for analytical reasons), but may be considered as a possible minor metabolite since another hydroxylated metabolite (hydroxyl-4-ANPP) was also identified. Additionally, a biotransformation product presumably formed by oxidation of the tetrahydrofuranyl moiety and subsequent 'internal hydrolysis' under ring-opening was identified (Krotulski et al., 2018).

A short summary of the reviewed fentanyl analogs and their main metabolites (and metabolic pathways) described in the literature and estimated relative potencies (compared to fentanyl) are listed in **Table 1**.

A number of further fentanyl analogs have been reported to the EMCDDA (mainly referring to seizures by police or customs authorities or intoxication cases) and were included into the literature search. However, no data on potency, receptor binding or metabolism was available yet. For the sake of completeness these compounds will be listed here in alphabetical order:

2-fluoroisobutyrfentanyl, 2-methyl-acetylfentanyl, 3-methyl-crotonylfentanyl (senecionyl fentanyl), 3-fluorofentanyl, 3-phenylpropanoylfentanyl, 4-chloroisobutyrfentanyl, 4-fluoro-butyrfentanyl, 4-fluoro-cyclopropylbenzylfentanyl, 4-fluoro-fentanyl, 4-fluoroisobutyrfentanyl *N*-benzyl analog, 4-methoxybutyrfentanyl, α-methylfentanyl butanamide analog (2-methyl-*N*-phenyl-*N*-[1-(1-phenyl-propan-2-yl)piperidine-4-yl]propanamide), acetyl benzylfentanyl, benzodioxolefentanyl, benzoylbenzylfentanyl, benzoylfentanyl, benzylfentanyl, crotonylfentanyl, furanylbenzylfentanyl, furanylethylfentanyl, furanylfentanyl-3carboxamide isomer, thiophenefentanyl and valerylfentanyl.

AUTHOR CONTRIBUTIONS

RS, SP, RP, AT, FB, VA, and MW searched for bibliographic material, drafted different chapter of the manuscript, and contributed substantially to manuscript intellectual content and revision.

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