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Biology and pharmacological inhibition of homeodomain-interacting protein kinases

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Homeodomain-interacting protein kinases (HIPKs) represent a relatively underexplored sub-family of serine/threonine protein kinases. However, the recently published studies point to the role of HIPKs in the developmental biology and etiology of pathological states, in particular cancer, and potential therapeutic applications of targeting this kinase family. This review summarizes the biology of HIPKs and their heretofore published small-molecule inhibitors.

KEYWORDS

HIPK, kinase, inhibitor, selectivity, neurology, cancer

Introduction

Homeodomain-interacting protein kinases (HIPKs) are evolutionary conserved serine/threonine kinases (Kim et al., 1998; Manning et al., 2002). The four isoforms HIPK1-4 belong to the CMGC branch of the kinome, and form one of the three subunits of the DYRK kinase family (Figure 1). The isoforms HIPK1-3 were first described in 1998 (Kim et al., 1998), and HIPK4 in 2007 (Arai et al., 2007). HIPKs interact (acting as co-repressors) with homeobox proteins, which are prominent transcription factors. Unlike prototypical kinases, HIPKs act directly on transcription factors and other nuclear proteins. They play a role in terminal regulation rather than in activation of downstream signaling cascades (e.g., MAPK or PI3K/AKT) that consist of multiple sequential phosphorylation events (Pearson et al., 2001; Hemmings and Restuccia, 2012). In addition to their function as transcriptional repressors, HIPKs can also act as transcriptional activators influencing the expression of genes, depending on the status and requirements of the cell (Calzado et al., 2007). The highest concentrations of HIPK1-3 have been found in the nucleus, especially in interchromatin granules (nuclear speckles) (Kim et al., 1998; Rinaldo et al., 2008; Van Der Laden et al., 2015), while HIPK4 has been identified mainly in the cytoplasm (Arai et al., 2007). In contrast to HIPK1-3, which are present in all vertebrates, HIPK4 has been found only in mammalian cells (Schmitz et al., 2014).

Structure of HIPKs

HIPK1-3 have a high degree of structural homology (Figure 2). The salient feature of HIPK4 is the absence of the homeobox-interacting domain, in contrast to the other three isoforms (Rinaldo et al., 2008; Agnew et al., 2019).

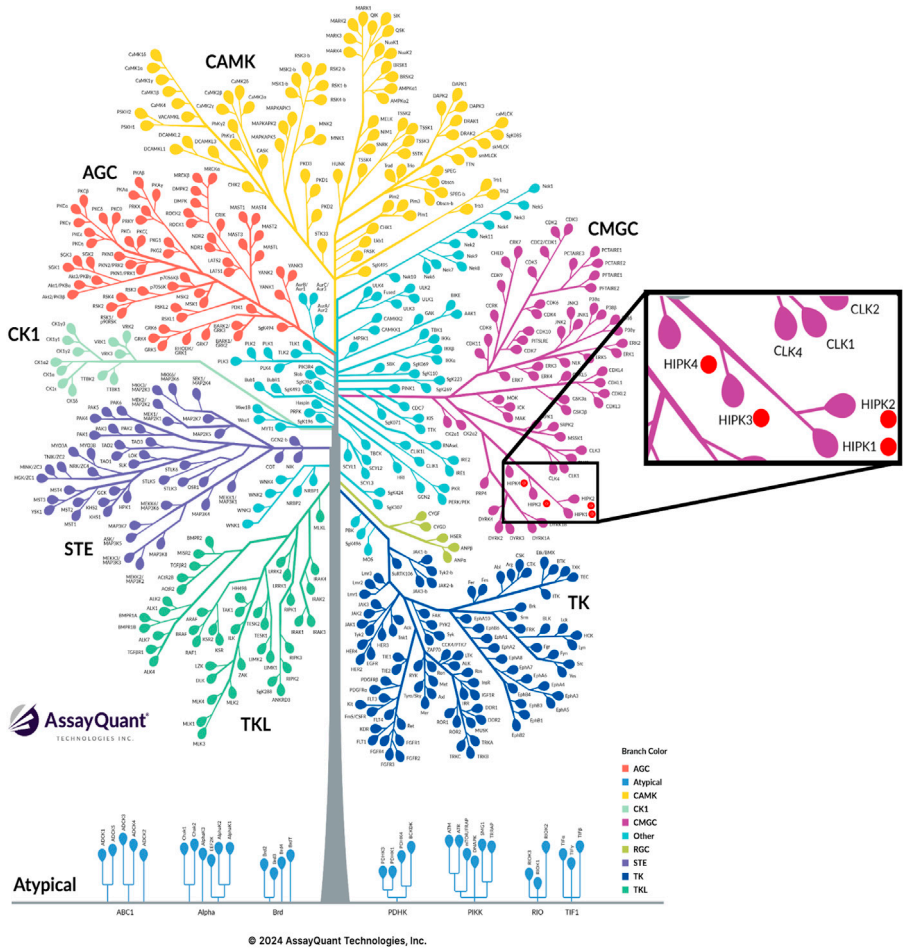


FIGURE 1
Graphical representation of the human kinome and the CMGC family. HIPKs are marked by red circles (Kinome Tree | Assayquant (29.10.2024)). https://www.assayquant.com/kinome-tree/#).

The HIPK structure features a standard arrangement consisting of smaller N-terminal lobe and larger C-lobe, connected by the hinge region (Figure 3) (Schmitz et al., 2014; Van Der Laden et al., 2015).

Of the HIPK isoforms, HIPK2 is the one most studied by X-ray crystallography; the published data include co-crystal structures of HIPK2 and several small-molecule inhibitors described below (Agnew et al., 2019; Němec et al., 2021).

As other kinases, HIPKs undergo various post-translational modifications (e.g., sumoylation, ubiquitinylation, and autophosphorylation) that regulate their activity, function and localization or mediate their degradation (Gresko et al., 2005; Hofmann et al., 2005; Winter et al., 2008; de la Vega et al., 2011; Saul et al., 2013).

Phosphorylation represents one of the most important post-translational modifications. Along this line, HIPK2 can be phosphorylated at multiple sites by Src kinase, and the resulting phosphorylated forms can be differentially re-distributed (Polonio-Vallon et al., 2014). HIPK autophosphorylation at tyrosine and serine residues also affects their activity (Van Der Laden et al., 2015), substrate specificity and the subcellular localization, and plays an important role in the regulation of various cellular processes, including apoptosis, DNA repair and responses to stressors (e.g., radiation or chemotherapeutics) (Saul et al., 2013; Siepi et al., 2013). In this regard, HIPKs are similar to their relatives in the CMGC kinase family, namely, the closely related DYRKs (Becker and Sippl, 2011; Van Der Laden et al., 2015).

SUMOylation affects the nuclear localization of HIPK2 and may be involved in both repression and activation of the kinase depending on the context of post-translational modifications. Reversible modification of HIPK2 at the Lys25 residue by SUMO-1 regulates the activation of c-Jun-NH2-terminal kinase (JNK), while deconjugation of SUMO-1 from HIPK2 mediated by the protease SuPr-1 increases the JNK activity (Hofmann et al., 2005). Binding of HIPKs to SUMO is mediated by the SUMO-interacting motif (SIM, Figure 4) - this interaction regulates the activity of HIPKs and their localization in nuclear speckles (de la Vega et al., 2011). Abnormal SUMOylation has been linked to the development of diverse pathological processes, including cancer (Han et al., 2018), and specifically, disrupted SUMOylation of mutated HIPK2 to acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) (Sung et al., 2019).

Another mechanism of positive regulation of HIPK2 is mediated by caspase 6, which in response to stress and DNA damage cleaves the auto-inhibitory domain of HIPK2 (Sombroek and Hofmann,

HIPKs amino acid residues

	10	20	30	40	50	60	70	80
HIPK 1	MASQLQVFSP	PSVSSSAFCS	AKKLKIEPSG	WDVSGQSSND	KYYTHSKTLP	ATQGQANSSH	QVANFNIPAY	DQGLLLPAPA
HIPK 2	MAPVYEGMAS	HVQVFSPHTL	QSSAFCSVKK	LKIEPSSNWD	MTGYGSHSKV	YSQSKNIPLS	QPATTTVSTS	LPVPNPSLPY
HIPK 3	MASQVLVYPP	YVYQTQSSAF	CSVKKLKVEP	SSCVFQERNY	PRTYVNGRNF	GNSHPPTKGS	AFQTKIPFNR	PRGHNFSLQT
HIPK 4	MSTIQSETDC	YDIIEVLGKG	TFGEVAKGWR	RSTGEMVAIK	ILKNDAYRNR	IIKNELKLLH	CMRGLDPEEA	HVIRFLEFFH
	90	100	110	120	130	140	150	160
HIPK 1	VEHIVVTAAD	SSGSAATSTF	QSSQTLTHRS	NVSLLEPYQK	CGLKRKSEEV	DSNGSVQIIE	EHPPLMLQNR	TVVGAAATTT
HIPK 2	EQTIVFPGST	GHIVVTSASS	TSVTGQVLGG	PHNLMRRSTV	SLLDTYQKCG	LKRKSEEIEN	TSSVQIIEEH	PPMIQNNASG
HIPK 3	SAVVLKNTAG	ATKVIAAQAQ	QAHVQAPQIG	AWRNRLHFLE	GPQRCGLKRK	SEELDNHSSA	MQIVDELSIL	PAMLQTNMGN
HIPK 4	DALKFYLVFE	LLEQNLFEFQ	KENNFAPLPA	RHIRTVTLQV	LTALARLKEL	AIIHADLKPE	NIMLVDQTRC	PFRVKVIDFG
	170	180	190	200	210	220	230	240
HIPK 1	TVTTKSSSSS	GEGDYQLVQH	EILCSMTNSY	EVLEFLGRGT	FGQVAKCWKR	STKEIVAIKI	LKNHPSYARQ	GQIEVSILSR
HIPK 2	ATVATATTST	ATSKNSGSNS	EGDYQLVQHE	VLCSMTNTYE	VLEFLGRGTF	GQVVKCWKRG	TNEIVAIKIL	KNHPSYARQG
HIPK 3	PVTVVTATTG	SKQNCTTGEG	DYQLVQHEVL	CSMKNTYEVL	DFLGRGTFGQ	VVKCWKRGTN	EIVAIKILKN	HPSYARQGQI
HIPK 4	SASIFSEVRY	VKEPYIQSRF	YRAPEILLGL	PFCEKVDVWS	LGCVMAELHL	GWPLYPGNNE	YDQVRYICET	QGLPKPHLLH
	250	260	270	280	290	300	310	320
HIPK 1	LSSENADEYN	FVRSYECFQH	KNHTCLVFEM	LEQNLYDFLK	QNKFSPLPLK	YIRPILQQVA	TALMKLKSLG	LIHADLKPEN
HIPK 2	QIEVSILARL	STESADDYNF	VRAYECFQHK	NHTCLVFEML	EQNLYDFLKQ	NKFSPLPLKY	IRPVLQQVAT	ALMKLKSLGL
HIPK 3	EVSILARLST	ENADEYNFVR	AYECFQHRNH	TCLVFEMLEQ	NLYDFLKQNK	FSPLPLKVIR	PILQQVATAL	KKLKSLGLIH
HIPK 4	AACKAHHFFK	RNPHPDAANP	WQLKSSADYL	AETKVRPLER	RKYMLKSLDQ	IETVNGGSVA	SRLTFPDREA	LAEHADLKSM
	330	340	350	360	370	380	390	400
HIPK 1	IMLVDPVRQP	YRVKVIDFGS	ASHVSKAVCS	TYLQSRYYRA	PEIILGLPFC	EAIDMWSLGC	VIAELFLGWP	LYPGASEYDQ
HIPK 2	IHADLKPENI	MLVDPSRQPY	RVKVIDFGSA	SHVSKAVCST	YLQSRYYRAP	EIILGLPFCE	AIDMWSLGCV	IAELFLGWPL
HIPK 3	ADLKPENIML	VDPVRQPYRV	KVIDFGSASH	VSKTVCSTYL	QSRYYRAPEI	ILGLPFCEAI	DMWSLGCVIA	ELFLGWPLYP
HIPK 4	VELIKRMLTW	ESHERISPSA	ALRHPFVSMQ	QLRSAHETTH	YYQLSLRSYR	LSLQVEGKPP	TPVVAAEDGT	PYYCLAEEKE
	410	420	430	440	450	460	470	480
HIPK 1	IRYISQTQGL	PAEYLLSAGT	KTTRFFNRDP	NLGYPLWRLK	TPEEHELETG	IKSKEARKYI	FNCLDDMAQV	NMSTDLEGTD
HIPK 2	YPGASEYDQI	RYISQTQGLP	AEYLLSAGTK	TTRFFNRDTD	SPYPLWRLKT	PDDHEAETGI	KSKEARKYIF	NCLDDMAQVN
HIPK 3	GALEYDQIRY	ISQTQGLPGE	QLLNVGTKST	RFFCKETDMS	HSGWRLKTLE	EHEAETGMKS	KEARKYIFNS	LDDVAHVNTV
HIPK 4	AAGMGSVAGS	SPFFREEKAP	GMQRAIDQLD	DLSLQEAGHG	LWGETCTNAV	SDMMVPLKAA	ITGHHVPDSG	PEPILAFYSS
	490	500	510	520	530	540	550	560
HIPK 1	MLAEKADRRE	YIDLLKKMLT	IDADKRITPL	KTLNHQFVTM	THLLDFPHSN	HVKSCFQNME	ICKRRVHMYD	TVSQIKSPFT
HIPK 2	MTTDLEGSDM	LVEKADRREF	IDLLKKMLTI	DADKRITPIE	TLNHPFVTMT	HLLDFPHSTH	VKSCFQNMEI	CKRRVNMYDT
HIPK 3	MDLEGSDLLA	EKADRREFVS	LLKKMLLIDA	DLRITPAETL	NHPFVNMKHL	LDFPHSNHVK	SCFHIMDICK	SHLNSCDTNN
HIPK 4	RLAGRHKARK	PPAGSKSDSN	FSNLIRLSQV	SPEDDRPCRG	SSWEEGEHLG	ASAEPLAILQ	RDEDGPNIDN	MTMEAERPDP
	570	580	590	600	610	620	630	640
HIPK 1	THVAPNTSTN	LTMSFSNQLN	TVHNQASVLA	SSSTAAAATL	SLANSDVSLL	NYQSALYPSS	AAPVPGVAQQ	GVSLQPGTTQ
HIPK 2	VNQSKTPFIT	HVAPSTSTNL	TMTFNNQLTT	VHNQAPSSTS	ATISLANPEV	SILNYPSTLY	QPSAASMAAV	AQRSMPLQTG
HIPK 3	HNKTSLLRPV	ASSSTATLTA	NFTKIGTLRS	QALTTSAHSV	VHHGIPLQAG	TAQFGCGDAF	QQTLIICPPA	IQGIPATHGK
HIPK 4	ELFDPSSCPG	EWLSEPDCTL	ESVRGPRAQG	LPPRRSHQHG	PPRGATSFLQ			
	650	660	670	680	690	700	710	720
HIPK 1	ICTQTDPFQQ	TFIVCPPAFQ	TGLQATTKHS	GFPVRMDNAV	PIVPQAPAAQ	PLQIQSGVLT	QGSCTPLMVA	TLHPQVATIT
HIPK 2	TAQICARPDP	FQQALIVCPP	GFQGLQASPS	KHAGYSVRME	NAVPIVTQAP	GAQPLQIQPG	LLAQQAWPSG	TQQILLPPAW
HIPK 3	PTSYSIRVDN	TVPLVTQAPA	VQPLQIRPGV	LSQTWSGRTQ	QMLVPAWQQV	TPLAPATTTL	TSESVAGSHR	LGDWGKMISC
HIPK 4								
	730	740	750	760	770	780	790	800
HIPK 1	PQYAVPFTLS	CAAGRPALVE	QTAAVLQAWP	GGTQQILLPS	TWQQLPGVAL	HNSVQPTAMI	PEAMGSGQQL	ADWRNAHSHG
HIPK 2	QQLTGVATHT	SVQHATVIPE	TMAGTQQLAD	WRNTHAHGSH	YNPIMQQPAL	LTGHVTLPAA	QPLNVGVAHV	MRQQPTSTTS
HIPK 3	SNHYNSVMPQ	PLLTNQITLS	APQPVSVGIA	HVVWPQPATT	KKNKQCQNRG	ILVKLMEWEP	GREEINAFSW	SNSLQNTNIP
HIPK 4								
	810	820	830	840	850	860	870	880
HIPK 1	NQYSTIMQQP	SLLTNHVTLA	TAQPLNVGVA	HVVRQQQSSS	LPSKKNKQSA	PVSSKSSLDV	LPSQVYSLVG	SSPLRTTSSY
HIPK 2	SRKSKQHQSS	VRNVSTCEVS	SSQAISSPQR	SKRVKENTPP	RCAMVHSSPA	CSTSVTCGWG	DVASSTTRER	QRQTIVIPDT
HIPK 3	HSAFISPKII	NGKDVEEVSC	IETQDNQNSE	GEARNCCETS	IRQDSDSSVS	DKQRQTIIIA	DSPSPAVSVI	TISSDTDEEE
HIPK 4								
	890	900	910	920	930	940	950	960
HIPK 1	NSLVPVQDQH	QPIIPDTPS	PPVSVITIRS	DTDEEEDNKY	KPSSSGLKPR	SNVISYVTVN	DSPDSDSSLS	SPYSTDTLSA
HIPK 2	PSPTVSVITI	SSDTDEEEEQ	KHAPTSTVSK	QRKNVISCVT	VHDSPYSDSS	SNTSPYSVQQ	RAGHNNANAF	DTKGSLENHC
HIPK 3	TSQRHSLREC	KGSLDCEACQ	STLNIDRMCS	LSSPDSTLST	SSSGQSSPSP	CKRPNSMSDE	EQESSCDTVD	GSPTSDSSGH
HIPK 4								
	970	980	990	1000	1010	1020	1030	1040
HIPK 1	LRGNSGSVLE	GPGRVVADGT	GTRTIIVPPL	KTQLGDCTVA	TQASGLLSNK	TKPVASVSGQ	SSGCCITPTG	YRAQRGGTSA
HIPK 2	TGNPRTIIVP	PLKTQASEVL	VECDSLVPVN	TSHHSSSYKS	KSSSNVTSTS	GHSSGSSSGA	ITYRQQRPGP	HFQQQQPLNL
HIPK 3	DSPFAESTFV	EDTHENTELV	SSADTETKPA	VCSVVVPPVE	LENGLNADEH	MANTDSICQP	LIKGRSAPGR	LNQPSAVGTR
HIPK 4								
	1050	1060	1070	1080	1090	1100	1110	1120
HIPK 1	AQPLNLSQNQ	QSSAAPTSQE	RSSNPAPRRQ	QAFVAPLSQA	PYTFQHGSPL	HSTGHPHLAP	APAHLPSQAH	LYTYAAPTSA
HIPK 2	SQAQQHITTD	RTGSHRRQQA	YITPTMAQAP	YSFPHNSPSH	GTVHPHLAAA	AAAAHLPTQP	HLYTYTAPAA	LGSTGTVAHL
HIPK 3	QQKLTSAFQQ	QHLNFSQVQH	FGSGHQEWNG	NFGHRRQQAY	IPTSVTSNPF	TLSHGSPNHT	AVHAHLAGNT	HLGGQPTLLP
HIPK 4								
	1130	1140	1150	1160	1170	1180	1190	1200
HIPK 1	AALGSTSSIA	HLFSPQGSSR	HAAAYTTHPS	TLVHQVPVSV	GPSLLTSASV	APAQYQHQFA	TQSYIGSSRG	STIYTGYPLS
HIPK 2	VASQGSARHT	VQHTAYPASI	VHQVPVSMGP	RVLPSPTIHP	SQYPAQFAHQ	TYISASPAST	VYTGYPLSPA	KVNQYPYI
HIPK 3	YPSSATLSSA	APVAHLLASP	CTSRPMLQHP	TYNISHPSGI	VHQVPVGLNP	RLLPSPTIHQ	TQYKPIFPPH	SYIAASPAYT
HIPK 4								
	1210							
HIPK 1	PTKISQYSYL							
HIPK 2								
HIPK 3	GFPLSPTKLS							
HIPK 4								

FIGURE 2
The amino acid sequences of human HIPK1-4 (obtained from UniProt database). The ATP-binding sites of HIPK1-3 sharing common amino acid sequences are highlighted in red.

2009). This modification, occurring in HIPK2 at the residues D916 and D977 (Figure 4), results in increased kinase activity (Sombroek and Hofmann, 2009) and enhances apoptotic signaling via phosphorylation of p53 at Ser46. This process, crucial for promoting the transcription of pro-apoptotic genes, is discussed in greater detail below.

Negative regulation of HIPK2 proceeds via the ubiquitination pathway: ubiquitin ligase Siah-1 binds to HIPK2, which leads to polyubiquitination at the lysine residue K1182 (Figure 4), transfer to the proteasome and subsequent degradation (Winter et al., 2008).

Role of HIPKs in developmental biology

Studies in *Drosophila* have demonstrated the importance of HIPK in the development of the eye as well as in neural and muscular development (Lee et al., 2009b; Blaquiere et al., 2014; Tettweiler et al., 2019; Wang et al., 2020).

Along this line, developmental defects were observed in mice with different degrees of HIPKs dysfunction; specifically, eye stunting was observed in 40% of Hipk1$^{+/-}$ Hipk2$^{-/-}$ mice (Inoue et al., 2010).

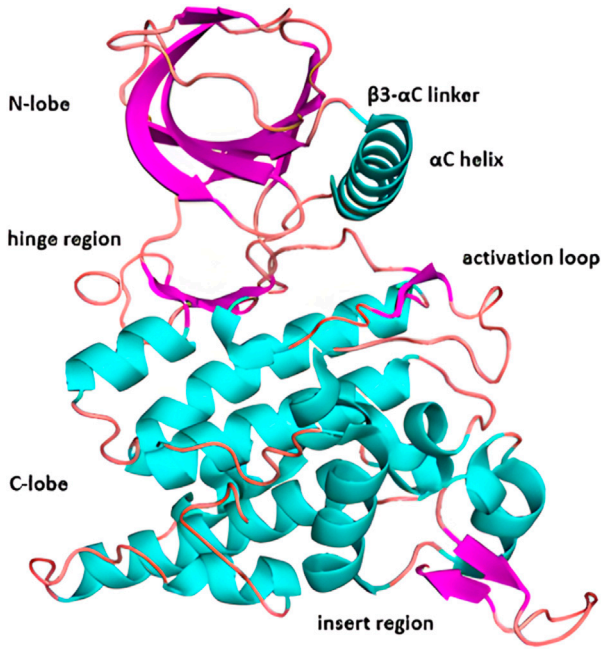


FIGURE 3
X-ray crystal structure of HIPK2 with highlighted major structural subunits (pdb code: 7NCF).

A degree of redundancy in HIPK1/2 functions has been observed in several *in vivo* studies. Absence of both HIPK1 and HIPK2 has been shown to cause early embryonic death due to insufficient development of the vascular and nervous systems (Aikawa et al., 2006; Isono et al., 2006). In this process, HIPK1/2-mediated phosphorylation of the p300 acetyltransferase appears to be essential (Aikawa et al., 2006). Another report revealed the role of HIPK1/2 downstream of the TGF-β-TAK1 signaling pathway regulating numerous angiogenic genes during early embryonic development (Shang et al., 2013).

HIPK1 was found to be important for proper spleen B cell function and homeostasis (Guerra et al., 2012). However, due to the dynamic cellular and tissue expression and high heterogeneity of the interacting molecules, the effects of HIPKs can be rather specific in a particular cellular context. In addition, HIPKs are involved in the regulation of several important cell signaling pathways in *Drosophila*–e.g., the Hippo pathway (SWH) responsible for the organ shape and size (Poon et al., 2012; Heidary Arash and Attisano, 2013). HIPK2 is involved in the regulation of the transcription factor IPF1/PDX1, which is critical for pancreatic development and proper β-cell function (Boucher et al., 2009). HIPKs are also involved in the regulation of the Wnt signaling pathway (through stabilization of the β-catenin arm) that is crucial in embryonic development and plays an important role in the etiology of cancer or type 2 diabetes (Lee et al., 2009a).

Biochemical studies demonstrated that HIPK3 participates in the regulation of the runt-related transcription factor 2 (Runx2) by the transcriptional regulators MINT+FGF_2 that are critical for mammalian development (Sierra and Towler, 2010).

The role of the most recently discovered isoform HIPK4 in developmental biology is still poorly understood. High-throughput RNAi screening analysis revealed its function as a suppressor of skin epithelial cell differentiation (Larribère et al., 2017). Recent study revealed HIPK4 to be essential for spermiogenesis and fertility–mice lacking HIPK4 showed sperm head defects, deformation and shortening of the sperm flagella, and overall impaired sperm function associated with the inability to bind to the oocyte (Crapster et al., 2020). Subsequent report demonstrated an essential role of HIPK4 in the process of sperm head shaping, which is essential for male fertility (Liu et al., 2022).

HIPKs in pathological states

Numerous studies link HIPKs to the mechanism of tumorigenesis (Conte et al., 2023). One of the key HIPK-interacting biomacromolecules is the protein p53 - one of the key DNA repair factors and tumor suppressor genes. Proper function of p53 is critical in cell aging and controlled cell death, and its mutation or deregulation is very common in a variety of malignancies (Kondo et al., 2003; Lavra et al., 2011). Mutations of p53 are associated with the genetic disorder Li-Fraumeni syndrome, characterized by the frequent occurrence of various malignancies early in life (Li and Fraumeni, 1969; Varley, 2003). HIPK2 has been reported to

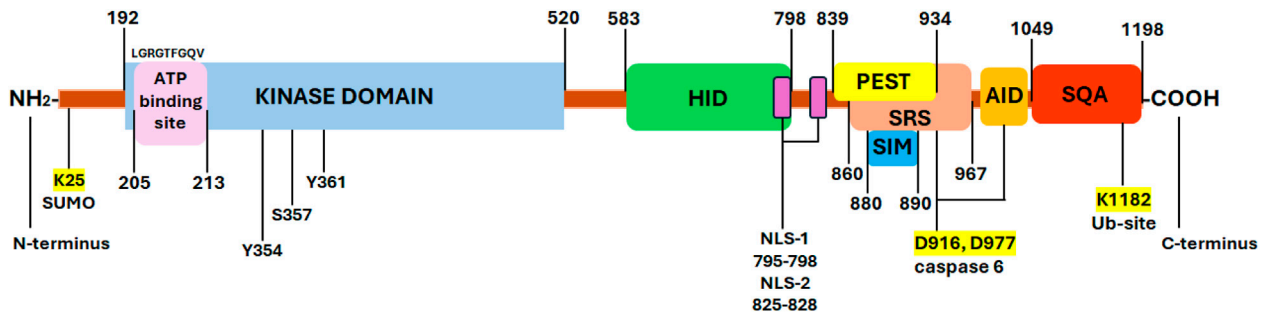


FIGURE 4
The schematic representation of the HIPK2 structure with the regions involved in the post-translational regulations and discussed in the text highlighted in yellow. (Homeobox-interacting domain: HID; speckle retention signal region: SRS; SUMO-interacting motive: SIM; auto-inhibitory domain: AID; serine, glutamine and alanine repeat region: SQA).

phosphorylate p53 at Ser46 (D'Orazi et al., 2002), and HIPK4 at Ser9 (Arai et al., 2007).

Phosphorylation of p53 is a crucial response of the cell to genotoxic stress (induced by, e.g., heat, ionizing radiation, xenobiotics or toxic metabolites) and the resulting DNA damage (Kuwano et al., 2016). Under normal conditions, the level of p53 is relatively low and the protein is gradually degraded through interaction with its negative regulator MDM2 (Ashcroft et al., 2000; Wade et al., 2010). Phosphorylation of p53 can lead to the protein stabilization and suppression of the MDM2-mediated degradation, and consequently increase the concentration of p53. This can halt the cell progression in the G1 phase, providing the cell sufficient time to repair DNA (Ashcroft et al., 2000; Oren, 2003). In case of severe DNA damage, apoptosis is induced via activated p53-mediated expression of pro-apoptotic genes such as Noxa, p53API1, Bax or PUMA (Oda et al., 2000a; Oda et al., 2000b; Fritsche et al., 2015). HIPK2 can control the mechanism of p53 degradation either directly through its phosphorylation, or indirectly by inactivating phosphorylation of MDM2 that promotes its export and degradation (Stefano et al., 2004; Di Stefano et al., 2005; Kuwano et al., 2016). This defines HIPK2 as an important regulatory factor, and further investigations will likely lead to better understanding of oncogenesis.

On the macroscopic level, HIPK1-deficient mice were found to be more susceptible to chemically induced skin cancer using DMBA initiator (Kondo et al., 2003).

However, HIPKs' role in carcinogenesis can vary, depending on the tumor type–in particular the role of HIPK 2 (Torrente et al., 2017; Blaquiere et al., 2018). Faster skin tumor growth and disease progression were observed in HIPK2 $^{-/-}$ mice (Wei et al., 2007). In acute myeloid leukemia (AML), the level of HIPK1/3 mRNA was found to be significantly increased, while the level of HIPK2 mRNA was comparatively low (Gu et al., 2004). Recently, HIPK1 has been identified as one of 3 MSI-1-associated genes in group 3 medulloblastoma, and defined as an attractive therapeutic target (Kameda-Smith et al., 2022). Low levels of the HIPK2 protein expression have been associated with poor prognosis in pancreatic (Qin et al., 2019), colorectal, and thyroid cancers (Lavra et al., 2011; Soubeyran et al., 2011). However, rather opposite trends have been found in the cases of HPV-positive throat and cervical cancers (Al-Beiti and Lu, 2008; Kwon et al., 2017), and brain malignancies (Deshmukh et al., 2008; Schulten et al., 2016) where poor prognosis was associated with HIPK2 protein and mRNA overexpression. In addition, HIPK2 overexpression in squamous cell carcinoma has been linked to higher resistance of the tumors to radio- and chemotherapy (Kwon et al., 2017).

Low levels of HIPK3 mRNA has been found in renal tumors and in that context it may be considered as a negative prognostic factor (Xiao et al., 2021). In contrast, significantly elevated levels of HIPK3 non-coding RNA circHIPK3 were observed in esophageal squamous cell carcinoma, correlating with tumor progression and extent of metastasis (Ba et al., 2020). However, the cancer cell lines HCT116 and SW480 stably overexpressing HIPK3 were found to exhibit retarded cell growth, migration, and increased sensitivity to fluorouracil (Tao et al., 2022).

Of other diseases, chronic kidney diseases associated with fibrosis have been associated with HIPK2 over-activity (Jin et al., 2012; Xiao et al., 2020; Overstreet et al., 2022; Zhong et al., 2022). Mechanistically, HIPK2 regulates the TGF-β/Smad3 signaling pathway that is often associated with the development of fibrosis, which suggests that this kinase could be an attractive candidate for targeted therapy (Hu et al., 2024; Lee et al., 2024).

In the area of neurology, it has been experimentally shown that HIPK2 expression within the central nervous system (CNS) decreases with age, except in the cerebellum (Anzilotti et al., 2015). Genetic ablation of HIPK2 in mice led to the neurodegenerative process characterized by significant loss of Purkinje cells in the cerebellum, neuromotor impairment and ataxia (Anzilotti et al., 2015). Absence of HIPK2 negatively affected neural development, specifically the number and survival rate of dopaminergic neurons during early postnatal programmed cell death phases, where HIPK2$^{-/-}$ mutant mice developed numerous severe psychomotor abnormalities (Zhang et al., 2007). These observations define HIPK2 as a neuroprotective factor (Zhang et al., 2007). Conversely, overexpression of HIPK2 was found to be beneficial in a rat model of spinal cord injury where it reduced the inflammatory response, oxidative stress, and apoptosis (Li et al., 2018). HIPK2 may contribute to the pathogenesis of Alzheimer's disease: soluble beta-amyloid peptides have been reported to be involved in HIPK2 degradation, thereby regulating the conformational state of p53 and the vulnerability to noxious stimuli, and triggering the amyloidogenic cascade (Lanni et al., 2010; Stanga et al., 2010). Finally, HIPK2 has been recently defined as a promising therapeutic target for the treatment of amyotrophic lateral sclerosis (ALS) – in the SOD1^{G93A} mouse model, loss of HIPK2 was found to be associated with later disease onset, lesser extent of cell death of spinal motor neurons, and improved overall survival (Lee et al., 2016).

The recent report by Zhang et al. points to another role of HIPK2 in neurodegenerative processes, namely, through modulation of mitochondrial function and affecting the Parkin-mediated pathway (Zhang et al., 2020). Loss of HIPK2 has been found to provide increased resistance towards mitochondrial toxins such as MPP+, rotenone or paraquat. Mechanistically, depletion of HIPK2 disrupts HIPK2-promoted Parkin degradation via proteasome-mediated mechanism, which leads to elevation of the Parkin protein level and higher mitochondrial durability (Zhang et al., 2020). These observations suggest that HIPK2 may influence the progression of neurodegenerative processes via regulation of mitochondrial resistance, and make HIPK2 a potential target for the treatment of Parkinson´s disease (Zhang et al., 2020).

Recent studies have demonstrated that in *Caenorhabditis elegans* the HIPK2 homolog HPK-1 plays a role in the response to stress (Berber et al., 2016), and is involved in two different molecular mechanism of proteostasis maintenance (Das et al., 2017). In one of them, HPK-1 prevents SUMOylation of the heat shock transcription factor HSF-1, which induces molecular chaperones upon thermal stress and enhances longevity (Das et al., 2017). In the other mechanism, HPK-1 induces autophagosome formation and autophagy gene expression upon dietary restriction or inactivation of TORC1 (Das et al., 2017). Along a similar line, HPK-1 was recently discovered to be the most broadly upregulated kinase in *C. elegans* during normal aging, and essential for preservation of integrity of the nervous system (Lazaro-Pena et al., 2023). In the aging nervous system, HPK-1 induction overlaps with key longevity transcription factors, and restoration

TABLE 1 List of biological targets modified by HIPK2.

Target	Modification	Biological role of the target	References
p63	Phosphorylation	Tumor suppression/apoptosis (p53 homologue)	Lazzari et al. (2011)
β-catenin	Phosphorylation	Wnt/β-catenin pathway	Kim et al. (2010)
Axin	Ternary axin/p53/HIPK2 complex-p53 phosphorylation supported	Wnt/β-catenin pathway (cell proliferation, differentiation)	Rui et al. (2004)
Notch1	Phosphorylation	Malignant progression	Ann et al. (2016)
CtBP	Phosphorylation	Transcription	Zhang et al. (2005)
Smad3	Protein/protein association (PPI)	TGF-β/Smad3 pathway (angiogenesis, fibrosis)	Liu et al. (2017)
c-Myb	Phosphorylation	Haematopoiesis	Kanei-Ishii et al. (2004)
Daxx	Phosphorylation	TGF-β/JNK activation (apoptosis)	Hofmann et al. (2003)
p300, AML1	Phosphorylation	Transcription activation	Aikawa et al. (2006)
HMGA1	Phosphorylation	DNA repair/transcriptional regulation	Zhang and Wang (2007)
HDAC3	Phosphorylation	Transcription modulation	Zhang et al. (2021)

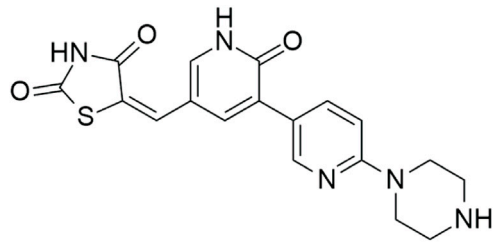


FIGURE 5
Structure of the compound **A64**.

of the HPK-1 neuronal expression rescues the premature age-associated decline and reduces thermotolerance of *hkp-1* null *C. elegans* (Lazaro-Pena et al., 2023). Specifically, the HPK-1 activity was found to be important for the heat shock response of serotonergic neurons, proper activity of GABAergic neurons, and proteome stability (Lazaro-Pena et al., 2023).

At the macroscopic level, HPK-1 overexpression in *C. elegans* increased the lifespan by up to 16% (Das et al., 2017). The recent study by Doering et al. identified HPK-1 as a central positive regulator of the nhr-49 dependent hypoxia response pathway, and revealed that null mutation of *hpk-1* caused a significant reduction of the survival rate under hypoxia (Doering et al., 2022).

Additional HIPK2 targets are summarized in Table 1.

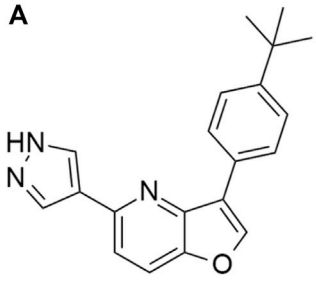
HIPK3 has been found to play a role in the metabolism of glucose and possibly in the pathogenesis of type 2 diabetes (Shojima et al., 2012). Specifically, mice lacking HIPK3 showed reduced beta cell proliferation and glucose-induced insulin secretion, associated with decreased phosphorylation of the kinase GSK3β and the transcription factor PDX1 (Shojima et al., 2012).

Collectively, the studies described above suggest that modulation of HIPK2 for therapeutic purposes may require finely balanced concentrations of HIPK inhibitors that would positively affect the maintenance of proteostasis and prevent age-induced neuronal cell death, but would not induce chronic stress response. Similarly delicately balanced scenario may be necessary for the development of potential anti-cancer drugs, where in some contexts inhibition of overexpressed HIPK2 (Al-Beiti and Lu, 2008; Kwon et al., 2017; Cao et al., 2021) could provide the desired anti-cancer effect, while in others it could negatively affect the p53 apoptotic function and promote tumorigenesis (D'Orazi et al., 2012). Similarly balanced regimes may be necessary for therapeutic targeting of the isoforms HIPK1, HIPK3 and HIPK4, whose biology (including post-translational modifications) is however comparatively less explored. In addition, modulation of the HIPK isoforms in specific compartments of the cell (Ritter and Schmitz, 2019) may require isoform-selective inhibitors.

Introduction to kinase inhibitors

The family of protein kinases contains >500 proteins that regulate numerous cellular signaling pathways in the cell. Targeting this class of enzymes represents one of the most dynamic areas of pharmaceutical research and >80 small-molecule kinase inhibitors approved for clinical use (Roskoski, 2024). Vast majority of them is represented by ATP-competitive inhibitors that are tightly anchored to the kinase hinge backbone. The ATP-binding site across the kinome is typically highly conserved, which makes identification of highly selective kinase inhibitors a non-trivial task (Breen and Soellner, 2015; Umezawa and Kii, 2021).

While sufficiently selective inhibitors have been identified for some kinases (and frequently served as the starting points for development of novel drugs), for many others that are only emerging as potential targets for pharmacological inhibition they are still to be discovered (Attwood et al., 2021).



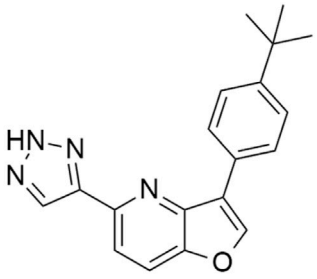


FIGURE 6
Structures of the compounds **MU135 (A)** and **MU1787 (B)**.

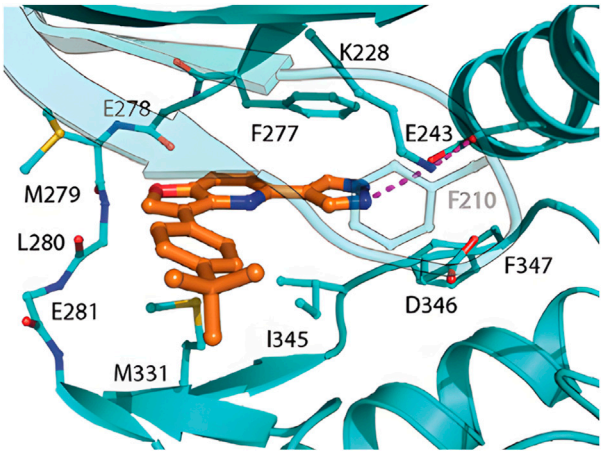


FIGURE 7
Crystal structure of **MU135** in HIPK2; adopted from (Němec et al., 2021).

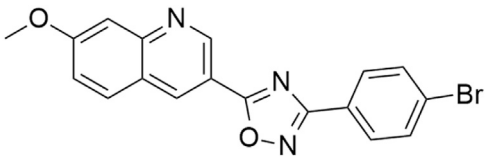


FIGURE 8
Structure of the compound **BT173**.

To our best knowledge, there are no clinically profiled inhibitors having HIPKs as primary targets. However, several classes of recently discovered HIPK inhibitors summarized below can serve as starting points for the pre-clinical identification of suitable candidates for pharmacological inhibition of these kinases.

Kinase inhibitors are classified according to their mode of interaction with the target kinase. Most common ones are ATP-competitive inhibitors, i.e., compounds that occupy the binding site of the natural substrate ATP (Roskoski, 2021; Roskoski, 2024). Type I ATP-competitive inhibitors bind to active conformation of the kinase (Wu et al., 2015), whereas type II inhibitors bind to inactive conformation and can also affect regions adjacent to the ATP binding site (Wu et al., 2015).

Allosteric inhibitors, on the other hand, inactivate the kinase indirectly, most frequently by changing its conformation or disrupting an association to a partner that is essential for the kinase function (Thomson et al., 2024). Most inhibitors interact with active or inactive forms of kinases through non-covalent interactions - hydrogen bonds, pi-stacking, dipole or lipophilic interaction (Łukasik et al., 2021). Historically less common (but currently growing) group includes covalent kinase inhibitors, e.g., ibrutinib and afatinib (Abdeldayem et al., 2020; Cheke and Kharkar, 2024).

Small-molecule inhibitors of HIPKs

High-throughput profiling of a library consisting of 118 compounds yielded the compound **A64** (Figure 5) (Miduturu et al., 2011). This commercially available compound (sold as the hydrochloride salt under the name Protein Kinase Inhibitor 1) inhibits HIPK1 and HIPK2 with the IC_{50} values of 136 nM and 74 nM, respectively (Miduturu et al., 2011). Recently, the compound **A64** has been applied as a research tool to inhibit HIPK2 in cells (using 74 nM concentration of its dihydrochloride PKI1H) and *in vivo* (with the 100 mg/kg dose, Liang et al., 2020; Zhou et al., 2021).

A recently reported class of HIPK inhibitors is based on the non-routine furo[3,2-*b*]pyridine scaffold, which was previously used as the basis of potent and highly selective inhibitors of cdc-like kinases (CLKs) (Němec et al., 2019). Expansion of the SAR in the sub-series of 3,5-disubstituted furo[3,2-*b*]pyridines with the focus on HIPK inhibition yielded the compounds **MU135** (HIPK1 IC_{50} = 248 nM; HIPK2 IC_{50} = 119 nM; HIPK3 IC_{50} = 476 nM) and **MU1787** (HIPK1 IC_{50} = 285 nM; HIPK2 IC_{50} = 123 nM; HIPK3 IC_{50} = 283 nM) (Figure 6) with remarkable kinome-wide selectivity (Němec et al., 2021).

Crystallographic studies revealed that **MU135** in HIPK2 adopts a rather unusual type-I binding mode: in contrast to standard type-I inhibitors, it features only a weak interaction of the furo[3,2-*b*] pyridine core to the hinge, combined with the hydrogen bond of the pyrazole with the salt bridge, and likely hydrophobic stacking with Phe 277 and Ile 345 in the back pocket (Figure 7).

In contrast to the ATP-competitive inhibitors described above, the compound **BT173** (Figure 8) binds to HIPK2 allosterically and

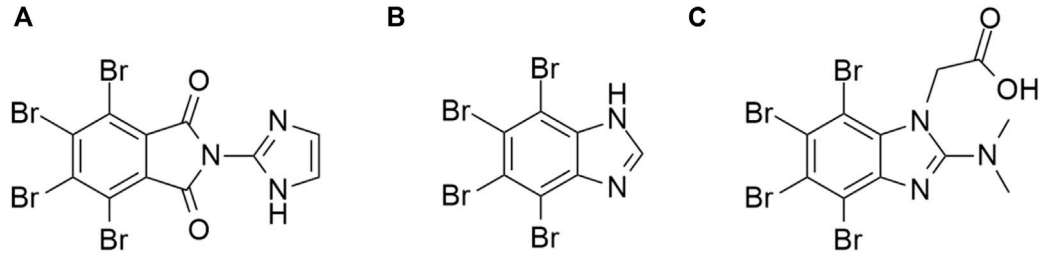


FIGURE 9
Structures of the compounds **TBID (A)**, **TBI (TBBz) (B)**, and **TMCB (C)**.

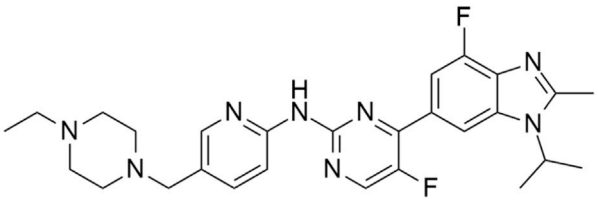


FIGURE 10
Structure of **abemaciclib**.

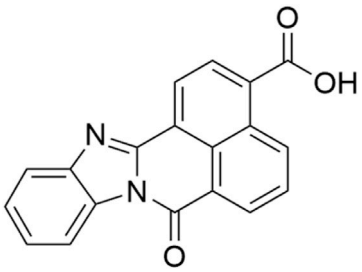


FIGURE 11
Structure of the compound **STO-609**.

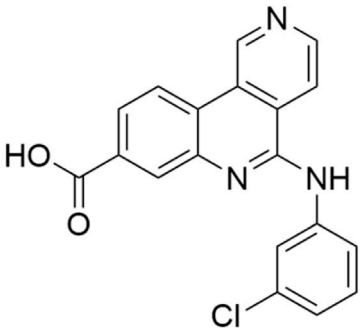


FIGURE 12
Structure of silmitasertib (**CX-4945**).

does not directly inhibit its kinase activity. However, at 10 μM concentration, it blocks the ability of HIPK2 to associate with the protein Smad3 in 293T cells (Liu et al., 2017), thereby regulating the TGF-β1/Smad3 pathway, and attenuating renal fibrosis (Sato et al., 2003; Zhang et al., 2010; Chen et al., 2014; Liu et al., 2017; Lee et al., 2024). The compound has been found to be effective also *in vivo*–in the Tg26 mouse model, it ameliorated proteinuria and kidney fibrosis at the dose of 20 mg/kg (Liu et al., 2017).

Kinase inhibitors with HIPK off-target activity

First reported class of inhibitors with attractive off-target HIPK activity consists of polyhalogenated (benz)imidazoles, exemplified by the compounds **TBI**, **TBiD** and **TMCB** (Figure 9). The compound **TBI** (also referred to as **TBBz**) was firstly reported as a CK2 inhibitor (Zień et al., 2003). Subsequent studies identified this compound as an antiprotozoal agent effective against *Acanthamoeba castellanii,* and also as a HIPK inhibitor with HIPK2 IC_{50} = 0.7 μM (Kopańska et al., 2004; Pagano et al., 2008; Cozza et al., 2014). The compound **TBID** is more potent (HIPK2 IC_{50} = 0.33 μM), capable of inhibiting phosphorylation of p53 at serine 46 in the cell at 50 μM concentration (Cozza et al., 2014). Both compounds exhibit moderate activity towards the kinase CK2: **TBI** CK2 IC_{50} = 0.6 μM and **TBID** CK2 IC_{50} = 5.5 μM (Cozza et al., 2014). The commercially available compound **TMCB** is comparatively less potent (HIPK2 IC_{50} = 15.25 μM) and targets several other kinases (namely, CK2, ERK8, PIM1) with higher potency (Pagano et al., 2008; Cozza et al., 2010; Janeczko et al., 2012) – it has been used as a research tool in that context (Schneider et al., 2012; Bollacke et al., 2016; Wadey et al., 2017; Kim et al., 2023). **TBI** iodinated analogues were also prepared and studied (Gianoncelli et al., 2009).

Abemaciclib (Figure 10) is a small-molecule inhibitor developed by Eli Lilly, sold under the name **Verzenio**. The compound was approved by the FDA in 2015 for the treatment of metastatic breast cancer and later for other types of breast cancers (Finn et al., 2009; Coates et al., 2010; Lu, 2015; Palumbo et al., 2019; Johnston et al., 2021; Rugo et al., 2022; Wekking et al., 2023). The primary targets of abemaciclib are CDK4/6; however, the compound was found to possess notable activity towards HIPK2 and HIPK3 with the IC_{50} values of 668 nM and 467 nM, respectively (Poratti and Marzaro, 2019; Kaltheuner et al., 2021). The compound is significantly less

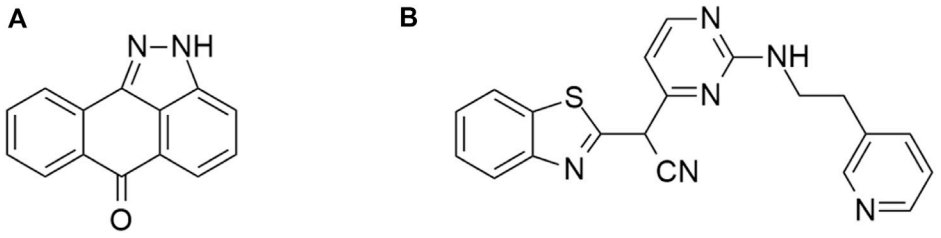


FIGURE 13
Structures of the compounds **SP600125 (A)** and **AS601245 (B)**.

active against HIPK1 and HIPK4 (HIPK1 IC_{50} = 4.53 μM; HIPK4 IC_{50} = 10.36 μM; Kaltheuner et al., 2021).

The compound **STO-609** (Figure 11) is currently commercially available as a research tool for inhibition of calmodulin-dependent protein kinase kinase (CaM-KK) (Tokumitsu et al., 2002; Kukimoto-Niino et al., 2011; Hou et al., 2021; Wang et al., 2021; 2022). Profiling of **STO-609** in a panel of 72 kinases revealed off-target inhibition of HIPK2 (65% inhibition at 1 μM) and HIPK3 (32% inhibition at 1 μM) (Bain et al., 2007).

Silmitasertib also known as **CX-4945** (Figure 12) is a small-molecule inhibitor of casein kinase 2 (CK2), currently undergoing clinical trials focused on the treatment for bile duct cancer and medulloblastoma (Pierre et al., 2011; Purzner et al., 2018; Borad et al., 2021; 2023). It sparked interest during the COVID-19 pandemic due to its antiviral effect (Bouhaddou et al., 2020; Gordon et al., 2020; Naik et al., 2022), possibly caused by modulation of CK2-mediated extracellular matrix remodeling (Bouhaddou et al., 2020). The compound was found to be significantly active against HIPK3 isoform with the IC_{50} value of 45 nM (Pierre et al., 2011).

Off-target activity against HIPK2 was observed in case of the c-Jun NH2-terminal protein kinase (JNK) inhibitors **SP600125** and **AS601245** (Figure 13) (Slouka et al., 1982; Bain et al., 2003; Gaillard et al., 2005). The compound **SP600125** is a broad-spectrum inhibitor of serine/threonine kinases and inhibits 65% of HIPK2 at 1 μM, while the more selective compound **AS601245** 71% at the same concentration (Bain et al., 2007).

Conclusion

HIPKs represent a relatively underexplored kinase family (Attwood et al., 2021). However, the biological studies published in the past decade (and summarized in this review) suggest that targeting HIPKs could bring therapeutic benefit. Development of sufficiently potent and selective small-molecule HIPK inhibitors therefore represents an attractive research area as it will likely afford quality chemical biology probes that would serve as valuable tools for further exploration of potential therapeutic relevance of targeting HIPKs. Those compounds will be likely useful also as the starting points for the development of clinical candidates. However, modulation of HIPKs for optimal therapeutic outcome may require finely balanced concentrations of HIPK inhibitors (in some cases those possessing sufficient isoform selectivity), especially in the areas of neurology and oncology. In addition, exploration and linking of the HIPK biology to new therapeutic applications could expand the medicinal use of the already approved drugs that inhibit HIPKs as off-targets.

Author contributions

AŠ: Data curation, Writing–original draft, Writing–review and editing. KP: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and editing.

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DeepL Pro and Grammarly (v.1.2.131.1585) were used to check grammar and search for more appropriate phrases or synonyms.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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