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Synthesis, antioxidant, and antimicrobial evaluation of novel 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives using K_2CO_3/glycerol as a green deep eutectic solvent

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Providing green methods for the synthesis of new heterocyclic compounds with biological properties is interesting for scientists. Through the multi-component reaction of aldehyde derivatives, methyl 2-cyanoacetate, and phenylhydrazine using K_2CO_3, and glycerol as a deep eutectic solvent, new derivatives of 2,3-dihydro-1H-pyrazole-4-carbonitrile were synthesized. Biological evaluation of the synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, including antioxidant activity on DPPH free radical and antimicrobial activity on a wide range of Gram-negative, Gram-positive bacterial, and fungal species, was done. In the antioxidant activity, IC_{50} value, and in the antimicrobial activity, IZD, MIC, MFC, and MBC parameters were assessed. The structure of the newly synthesized compounds was confirmed using ^{1}H NMR, ^{13}C NMR, and elemental analysis. 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives showed significant antioxidant and antibacterial activity and, in examining the results, a good relationship between the structure and biological activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives were found.

KEYWORDS

green chemistry, deep eutectic solvent, pyrazoles, antioxidant activity, antimicrobial activity

1 Introduction

Excessive accumulation of oxidants in the body leads to oxidative stress, which causes neurological disorders, Alzheimer's disease, kidney diseases, heart disease, etc. Carotenoids, vitamin C, vitamin E, etc., present in the diet, have antioxidant properties that lead to the neutralization of active oxidants in the body, but in some acute substances, it is necessary to use antioxidant agents (Nocella et al., 2019; Goshtasbi et al., 2022; Kumar et al., 2022).

Other pathogenic factors that affect human health include microbes. Microbes include bacteria, protozoa, viruses, and some fungi. Microbes cause diseases such as pneumonia, bacterial meningitis, and gastroenteritis. These microorganisms are sometimes eliminated by the body, but in some cases, antibiotic drugs are needed to stop them. Most drugs have organic and heterocyclic compounds in their structure (Lees and Aliabadi, 2002; Moser et al., 2019; Rajapaksha et al., 2019; Sarkar et al., 2022).

Celecoxib is a commercial drug that inhibits COX-2. This non-steroidal drug has anti-inflammatory properties. The pyrazole heterocyclic compound forms the core of this drug. Other medicines, such as rimonabant, sildenafil, and fomepizole, have pyrazole in their structure (Ansari, Ali, and Asif, 2017). The heterocyclic compound of pyrazole, with two nitrogens in its structure, is also found abundantly in nature (Kumar et al., 2013). Many synthetic compounds with the biological properties of antioxidant activity, antimicrobial activity, anticancer activity, etc., have been reported in pyrazole derivatives (Kumari, Paliwal, and Chauhan, 2018).

Pyrazoles can be synthesized through multicomponent reactions (MCRs) (Maddila et al., 2017; Becerra, Abonia, and Castillo, 2022). Today, these reactions are widely used for synthesizing organic and heterocyclic compounds based on the laws of green chemistry. In MCRs, three or more reagents react together, leading to the desired product that contains essential parts of all the components used. A reduction in reaction steps, high efficiency, less purification action, and cost-effectiveness are some advantages related to MCRs. The reaction conditions, including suitable solvents and catalysts, are the essential factors in multicomponent reactions (Singh and Chowdhury, 2012; Sharma et al., 2020; Mamaghani and Hossein Nia, 2021; Neto, Rocha, and Rodrigues, 2021).

One of the methods recently noticed in multicomponent reactions involves deep eutectic solvents. Deep eutectic solvents are a mixture of compounds containing hydrogen bond donors and hydrogen bond acceptors or metal salts. Deep eutectic solvents play an essential role in green chemistry. In reactions containing deep eutectic solvents, no catalyst is needed as the solvent also plays the role of a catalyst. They can be used as cheap non-toxic recyclable environmentally friendly materials to synthesize heterocyclic and organic compounds. In organic chemistry and the synthesis of heterocycles, these compounds can play a role as a Lewis and Lowry Bronsted acid and base. (Aryan et al., 2017; Xu et al., 2017; Beyzaei et al., 2018). Several reports of the use of deep eutectic solvents in the synthesis of heterocyclic compounds have been presented. For example, choline chloride and urea have been used as a deep eutectic solvent system in synthesizing 3-amino-1, 2, 4-triazole derivatives. The synthesized compounds in these reports were evaluated biologically, and the tests showed that the synthesized compounds have significant antimicrobial properties (Arab, Beyzaei, and Aryan, 2022). Glucose and urea are another system that has been reported for synthesizing pyrazole derivatives (Aryan et al., 2017; Aryan et al., 2019). Recently, glycerol and K_2CO_3 (potassium carbonate)/glycerol as a deep eutectic solvent have been used to synthesize heterocycles. For example, the above system was used to synthesize isoxazole derivatives with high efficiency and biological properties, such as antioxidant, antifungal, and antibacterial effects (Beyzaei et al., 2018).

One of the most important advantages of using deep eutectic solvents is their greenness and environmental friendliness. Green chemistry, which has been welcomed by scientists in the last two decades, leads to the safe synthesis of compounds and environmental protection. Deep eutectic solvents fully comply with the rules of green chemistry. For example, it is utterly consistent with safer solvents and auxiliaries (principle 5), less hazardous chemical syntheses (principle 3), prevention (principle 1), etc. (Chen et al., 2020; Ardila-Fierro and Hernández, 2021).

Given that the synthesis and reporting of new compounds with biological properties, as well as the reporting of green and environmentally friendly methods for heterocyclic compounds, are important, in the present study, K_2CO_3/glycerol was used as an active solvent in synthesizing new 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives. Next, the antioxidant properties of the synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives against DPPH free radicals were investigated. Finally, the inhibitory effects of the synthesized derivatives on a wide range of Gram-positive bacteria, Gram-negative bacteria, and fungal species based on IZD, MIC, MBC, and MFC, using clinical and laboratory standards institute guidelines, were tested.

2 Materials and methods

2.1 Solvents and raw materials

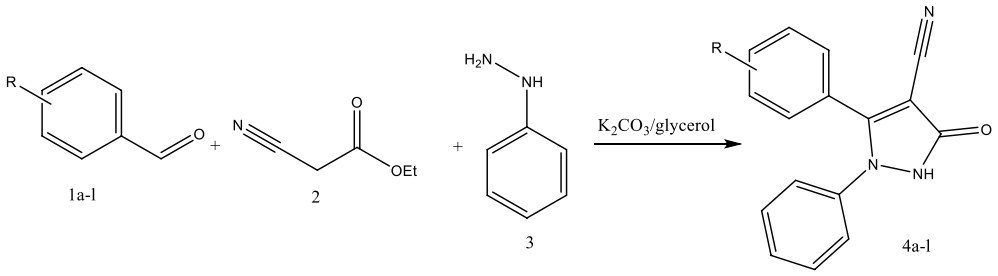
The raw materials and chemical compounds that were used to prepare deep eutectic solvent for synthesizing derivatives, such as K_2CO_3, glycerol, aldehyde derivatives, ethyl cyanoacetate, and phenylhydrazine, were obtained from Merck and Sigma.

2.2 Devices

The melting points of the compounds were measured using Kruss, KSP1N. PerkinElmer 2400 series II was used for the elemental analysis of derivatives. ^{1}H NMR (250 MHz, CDCl3) and ^{13}C NMR (75 MHz, CDCl3) spectra were obtained using a Bruker Ultra Shield-250. A Unico S2150 spectrophotometer was used to prepare the concentration of bacterial and fungal suspensions and measure the antioxidant properties.

2.3 Synthesis of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

For the synthesis of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, first, the deep eutectic solvent system was prepared. To achieve this, 0.01 mol K_2CO_3 and 0.04 mol glycerol were stirred



SCHEME 1
Synthesis of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives using aromatic aldehyde derivative K_2CO_3:glycerol as a green deep eutectic solvent.

for 2 h at 80°C. After a clear solution was observed, the mixture was used as a deep eutectic solvent to synthesize 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives.

To synthesize 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, 1 mmol of aldehyde derivatives, 1 mmol of ethyl cyanoacetate, and 1 mmol of phenylhydrazine were added to 1 g of the synthesized deep eutectic solvent. The mixture was stirred at a temperature of 50°C. The reaction was monitored by thin-layer chromatography. After the completion of the reaction, 5 mL of water/ethanol mixture (1:1) was added, and the sediments obtained were separated and recrystallized in methanol.

2.4 Antioxidant properties of the synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

The DPPH method was used to evaluate the antioxidant activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives. An aliquot of 4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.004% (w/v) was added to 1 mL of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives at different concentrations (concentrations of 25, 50, 75 and 100 μg/mL of derivatives were prepared). The mixture was stirred for 30 min at room temperature in the dark. Then the absorbance of the mixture was measured at 517 nm. The following formula was used to measure the percentage inhibition of DPPH by 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives (Beyzaei et al., 2018; Hosseinzadegan et al., 2020; Hosseinzadegan, Hazeri, and Maghsoodlou, 2020):

% inhibition = [(Absorption DPPH—Absorption DDPH and sample)/(Absorption DPPH)] × 100.

By using the percentage of inhibition and the concentration of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, the linear equation was obtained, and the IC50 value was calculated (Beyzaei et al., 2018).

2.5 Antimicrobial properties of the synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

The antifungal and antibacterial species examined in this study were obtained from the American Type Culture Collection (ATCC). To investigate antimicrobial activities, the antifungal and antibacterial effects of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives were examined. Clinical and Laboratory Standards Institute guidelines were used in the tests. Guidelines M07-A9, M26-A, M02-A11, M44-A, and M27-A2 were used for measuring the parameters of MIC and MFC (in the evaluation of antibacterial activity) and the parameters of MIC and MBC (in the evaluation of antifungal activity) (Etemadi et al., 2016; Igei et al., 2016; Heidari Majd, Akbarzadeh, and Sargazi, 2017; Moghaddam-Manesh et al., 2020; Abdieva et al., 2022).

TABLE 1 Optimizing the ratio of solvent components in the synthesis of 5-(4-methoxyphenyl)-3-oxo-1-phenyl-2,3-dihydro-1H-pyrazole-4-carbonitrile (4j).

Entry	Ratio of K_2CO_3:glycerol	Reaction time[a]	Efficiency[b]
1	1:0	60	26
2	0:1	120	N.R
3	1:2	60	52
4	1:3	30	84
5	1:4	15	95
6	1:5	20	89
7	1:6	30	72
8	1:10	60	67

Temperature: 60°C.
[a]Reaction time: min.
[b]Efficiency: %.

TABLE 2 Temperature optimization in the synthesis of 5-(4-methoxyphenyl)-3-oxo-1-phenyl-2,3-dihydro-1H-pyrazole-4-carbonitrile (4j).

Entry	Temperature[a]	Reaction time[b]	Efficiency[c]
1	50	20	90
2	60	15	95
3	75	15	92
4	100	15	89

Ratio of K_2CO_3:glycerol: 1:4.
[a]Temperature: °C.
[b]Reaction time: min.
[c]Efficiency: %.

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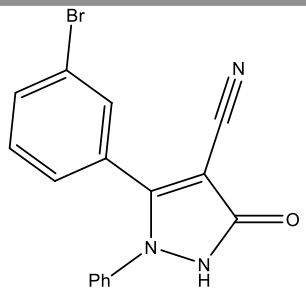
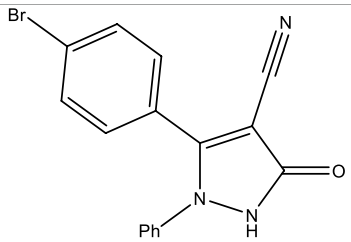
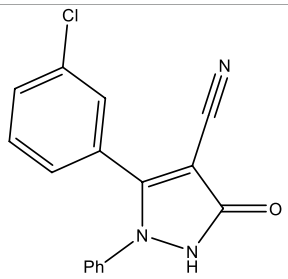
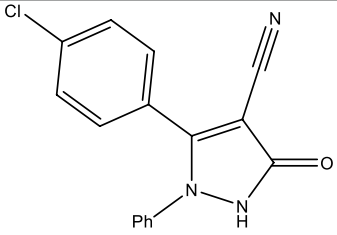
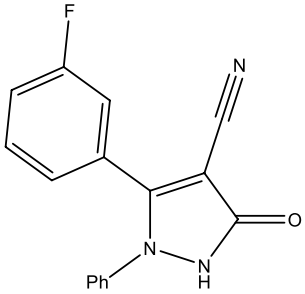
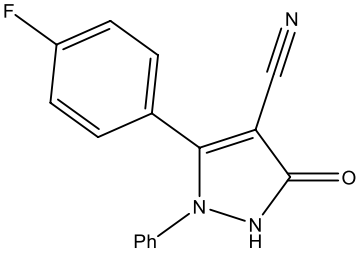
TABLE 3 Synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives in optimum conditions.

Entry	Structure	Reaction time [a]	Melting point [b]	Efficiency [c]
4a		20	162–164	89
4b		15	168–170	93
4c		18	149–153	91
4d		15	160–162	94
4e		17	135–138	90
4f		15	132–134	91

(Continued on following page)

TABLE 3 (*Continued*) Synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives in optimum conditions.

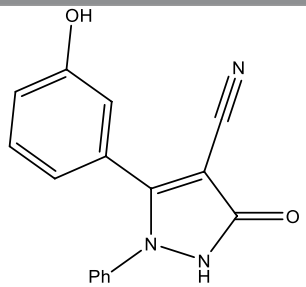
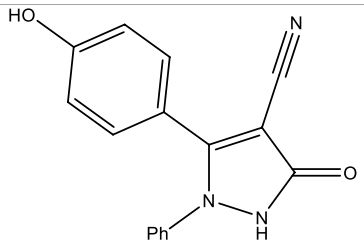
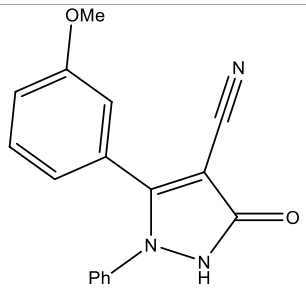
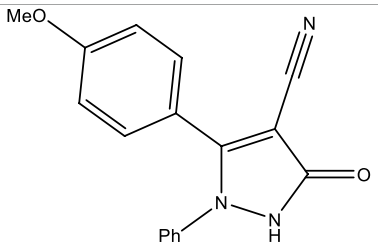
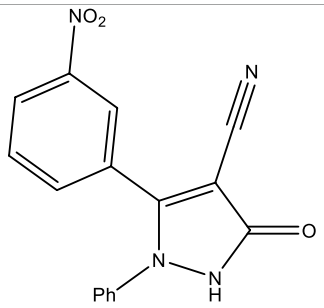
Entry	Structure	Reaction time [a]	Melting point [b]	Efficiency [c]
4g		20	191–192	85
4h		20	201–203	90
4i		20	183–186	87
4j		15	177–180	95
4k		17	166–168	93

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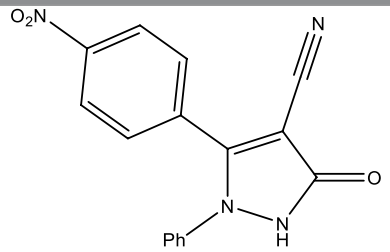
TABLE 3 (*Continued*) Synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives in optimum conditions.

Entry	Structure	Reaction time [a]	Melting point [b]	Efficiency [c]
4L		15	156–157	95

[a]Reaction time: min.
[b]Melting point: °C.
[c]Efficiency: %.

TABLE 4 Antioxidant activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives.

		2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives											
Inhibition percentage in the final concentration of derivatives		4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4L
	5	41	40	42	42	40	41	44	42	43	41	42	40
	10	46	43	45	46	47	45	47	44	45	44	46	43
	15	53	55	55	54	56	55	53	51	56	55	55	52
	20	71	72	69	73	70	69	71	70	71	68	71	72
Calculated IC_{50}[a]		12.53	12.51	12.58	12.21	12.37	12.63	12.31	12.88	12.25	12.80	12.32	12.75

[a]IC_{50}: μg/mL.

3 Results

3.1 Multicomponent synthesis of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives using K_2CO_3/glycerol as a green deep eutectic solvent

Glycerol and K_2CO_3 were used as a deep eutectic solvent in the three-component synthesis of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives using aldehyde derivatives, ethyl cyanoacetate, and phenylhydrazine (Scheme 1).

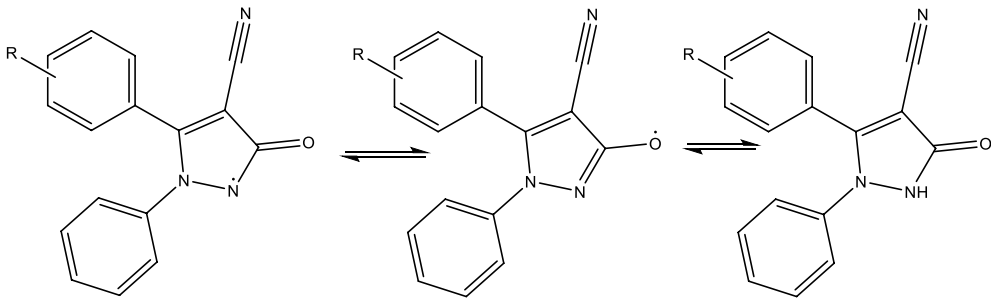
At first, the temperature was kept constant at 60°C, and different ratios of K_2CO_3/glycerol were investigated according to Table 1 (For synthesis 4a). This work aimed to obtain the best ratio of K_2CO_3 and glycerol for synthesizing 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives under suitable conditions and high efficiency.

According to Table 1, the ratio of 1:4, K_2CO_3 and glycerol, respectively, was the most efficient. As mentioned in previous studies, it has also been proven that a ratio of less than 1:4 of K_2CO_3:glycerol does not create a homogeneous and transparent mixture (Becerra, Abonia, and Castillo, 2022).

In the next step, the temperature was optimized. For this purpose, the reaction was tested at 50°C, 60°C, 75°C, and 100°C (Table 2). At temperatures below 50°C, owing to the high solvent viscosity, it was not possible to carry out the reaction.

As proven by the results shown in Table 2, the best temperature for the synthesis of 4a was 60 °C, and this temperature was used as the optimal temperature for the synthesis of other 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives. Then, using the obtained optimal conditions, including the ratio of 1:4 K_2CO_3:glycerol and temperature of 60°C, the 12, 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives shown in Table 3 were synthesized.

As shown in Tables 3, 6, 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, including 4a, 4e, 4f, 4g, 4h, and 4i, were novel derivatives, and their synthesis is reported for the first time. So far, only one method for synthesizing 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives has been reported. A study of previous research showed that the only method presented for synthesizing the compounds featured in this study is the use of sulfated alumina tungstic acid as a catalyst under reflux conditions in ethanol (Rather, Khan, and Siddiqui, 2020). Among the advantages of using the deep eutectic solvent examined in this research compared with the previously presented method, there is no need for a catalyst, synthesis can be carried out at lower temperatures, and novel 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives can be synthesized.



SCHEME 2
Antioxidant activity mechanism of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives and DPPH free radical stability.

TABLE 5 Antifungal activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives.

Derivatives	Fungi								
	ATCC 1022			ATCC 7601			ATCC 2091		
	Investigated parameters								
	IZD [a]	MIC [b]	MFC [b]	IZD	MIC	MFC	IZD	MIC	MFC
4a	- [c]	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-	-
4c	21	64	128	18	32	64	22	32	64
4d	23	32	64	19	32	64	21	16	32
4e	14	512	1024	-	-	-	13	512	512
4f	15	1024	2048	-	-	-	13	512	1024
4g	17	128	128	15	128	256	20	64	128
4h	18	128	256	14	256	256	20	128	256
4i	18	256	512	13	512	1024	15	256	512
4j	16	256	512	15	512	512	18	256	256
4k	21	64	128	17	64	128	20	32	64
4L	19	64	128	17	64	128	21	64	64
Drug A	-	-	-	-	-	-	-	-	-
Drug B	25	32	32	17	64	128	23	32	64

[a]IZD: mm.
[b]MIC and MFC: μg/mL.
[c]-: no effect; drug A: tolnaftate; drug B: terbinafine.

3.2 Biological activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

3.2.1 Antioxidant activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

To evaluate the antioxidant activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, the inhibition percentage and IC_{50} were calculated (Table 4).

Based on the structure of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives, the hydrogen attached to the nitrogen of the pyrrole ring plays a role in the stability of the DPPH free radical. Table 6 shows that the antioxidant properties of the derivatives are very similar to each other. The IC_{50} calculated for the derivatives was in the range of 12.21–12.88 μg/mL. Therefore, antioxidant effects were not dependent on aldehyde derivatives.

TABLE 6 Antibacterial activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives against Gram-positive bacteria.

Derivatives	Gram-positive bacteria														
	ATCC 6939			ATCC 14579			ATCC 23235			ATCC 10745			ATCC 13813		
	Investigated parameters														
	IZD [a]	MIC [b]	MBC [b]	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC
4a	10	1024	1024	- [c]	-	-	-	-	-	10	1024	2048	-	-	-
4b	11	1024	2048	-	-	-	-	-	-	10	2048	2048	-	-	-
4c	19	16	32	19	2	4	18	4	8	19	16	32	19	16	32
4d	19	8	16	20	2	4	20	4	4	20	16	32	19	8	16
4e	11	512	1024	-	-	-	10	512	512	13	512	1024	-	-	-
4f	13	512	1024	-	-	-	10	512	2048	14	512	512	-	-	-
4g	15	128	256	13	32	32	14	64	64	14	64	128	16	256	256
4h	14	128	256	13	64	128	13	64	64	12	128	256	13	256	512
4i	-	-	-	13	256	512	12	256	512	14	256	256	14	512	1024
4j	-	-	-	12	256	256	11	128	256	13	128	256	15	512	512
4k	16	32	64	17	4	8	15	16	32	18	32	64	15	64	128
4L	15	64	128	17	8	16	13	16	64	14	32	64	16	128	128
Drug C	18	4	8	19	2	8	20	2	4	19	16	16	-	-	-
Drug D	-	-	-	-	-	-	19	8	16	17	16	32	21	8	16

[a]IZD: mm.
[b]MIC and MBC: μg/mL
[c]-: no effect; drug C: gentamicin; drug D: cefazolin.

Based on the results, the mechanism shown in Scheme 2 is proposed for the antioxidant activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives.

3.2.2 Antifungal and antibacterial activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives

The antimicrobial activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives was studied for three fungal species (*Aspergillus fumigatus* Fresenius [ATCC 1022], *Fusarium oxysporum* [ATCC 7601], and *Candida albicans* [ATCC 2091]), five Gram-positive bacterial species (*Rhodococcus equi* [ATCC 6939], *Bacillus cereus* [ATCC 14579], *Staphylococcus aureus* [ATCC 23235], *Streptomyces fradiae* [ATCC 10745], and *Streptococcus agalactiae* [ATCC 13813]), and five Gram negative bacterial species (*Proteus mirabilis* [ATCC 12453], *Acinetobacter baumannii* [ATCC 17978], *Klebsiella pneumoniae* [ATCC 13883], *Escherichia coli* [ATCC 25922], and *Yersinia enterocolitica* [ATCC 23715]). The activity results on fungal species and Gram-positive and Gram-negative strains are shown in Tables 5, 6, 7, respectively.

The order of effect of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives on fungal and bacterial species was as follows:

4d>4c>4k>4l>4g>4h>4j>4i>4e>4f>4a>4b.

From the order of observation, it can be concluded that 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives containing chlorine, nitro, hydroxyl, methoxy, fluorine, and finally, bromine were effective in that order. As mentioned, the highest effectiveness was related to 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives containing chlorine. Chlorine, which has antimicrobial properties (Wei et al., 2019), was introduced here as an agent for the best effect.

As the results of the table show, in the antifungal evaluation, tolnaftate and terbinafine were used as common drugs, and in the antibacterial evaluation, gentamicin and cefazolin were used as standard antibacterial drugs to compare the activity of the 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives. The results proved that all derivatives other than 4a and 4b have higher antifungal activity than standard tolnaftate against ATCC 1022 and ATCC 2091. In addition, derivatives other than 4a, 4b, 4e, and 4f have higher antifungal activity than standard tolnaftate against ATCC 7601. In terms of antibacterial activity, the derivatives, particularly 4d, 4a, 4k, and 4j, had higher effects than gentamicin and cefazolin, which are common drugs.

4 Conclusion

The new 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives were synthesized using K_2CO_3/glycerol as a deep eutectic

TABLE 7 Antibacterial activity of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives against Gram-negative bacteria.

Derivatives	Gram-negative bacteria														
	ATCC 12453			ATCC 17978			ATCC 13883			ATCC 25922			ATCC 23715		
	Investigated parameters														
	IZD [a]	MIC [b]	MBC [b]	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC	IZD	MIC	MBC
4a	-	-	-	13	512	512	-	-	-	-	-	-	-	-	-
4b	-	-	-	12	512	1024	-	-	-	-	-	-	-	-	-
4c	18	2	4	15	2	4	18	8	16	17	16	64	17	16	32
4d	19	1	2	15	2	4	19	4	8	16	16	32	19	8	16
4e	-	-	-	12	64	128	15	128	256	-	-	-	-	-	-
4f	-	-	-	11	64	128	13	256	512	-	-	-	-	-	-
4g	13	32	64	-	-	-	16	32	64	15	64	128	15	256	256
4h	12	64	256	-	-	-	11	64	128	13	128	128	15	256	512
4i	10	512	1024	-	-	-	-	-	-	13	128	256	11	1024	1024
4j	10	128	256	-	-	-	-	-	-	14	128	256	13	512	1024
4k	16	4	8	15	4	8	17	16	32	17	32	64	18	32	64
4L	14	8	16	13	4	8	16	32	32	14	64	128	17	64	256
Drug C	17	1	4	16	1	2	20	4	8	19	16	16	21	8	16
Drug D	19	2	4	-	-	-	18	1	2	19	8	16	18	16	32

[a]IZD: mm.
[b]MIC and MBC: μg/mL.
[c]-: No effect; drug C: gentamicin; drug D: cefazolin.

solvent. After optimizing the reaction conditions, 12 derivatives of 2,3-dihydro-1H-pyrazole-4-carbonitrile were synthesized with 85%–95% efficiency in 15–20 min. The synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives showed high antioxidant, antifungal, and antimicrobial activity. In the antioxidant activity assessment, the inhibition percentage of derivatives was tested and IC50 values for derivatives between 12.21 and 12.88 μg/mL were calculated. The IZD, MIC, MFC, and MBC were measured to assess antifungal and antibacterial activity. In the antifungal and antibacterial activity of derivatives, MIC values between 4 μg/mL and 2048 μg/mL were obtained. From the biological activity results, we can mention the superior effectiveness of 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives compared with some common drugs. In addition, the logical relationships between the structure of derivatives and their biological properties were observed. Among the novelties of this research, we can mention the synthesis of new 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives in green and easier conditions, compared with the previously presented method, and the bioactivities of the synthesized 2,3-dihydro-1H-pyrazole-4-carbonitrile derivatives. Therefore, the above deep eutectic solvent system can be extended to synthesize other heterocyclic compounds with biological properties.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization, AZ and IA; methodology, MK; software, MA; validation, RK; formal analysis, AK.; investigation, AZ; resources, EA; data curation, AZ; writing—original draft preparation, MK; writing—review and editing, IA, BR, and AA; visualization, AZ; supervision, MK; project administration, RK; funding acquisition, IA. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmats.2023.1196583/full#supplementary-material

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