A Platinum Open Access Journal for Organic Chemistry

Paper

Free to Authors and Readers | DOAJ Seal | *Arkivoc* **2024** (8) 202412327

Synthesis, antimicrobial screening and computational study of some 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles

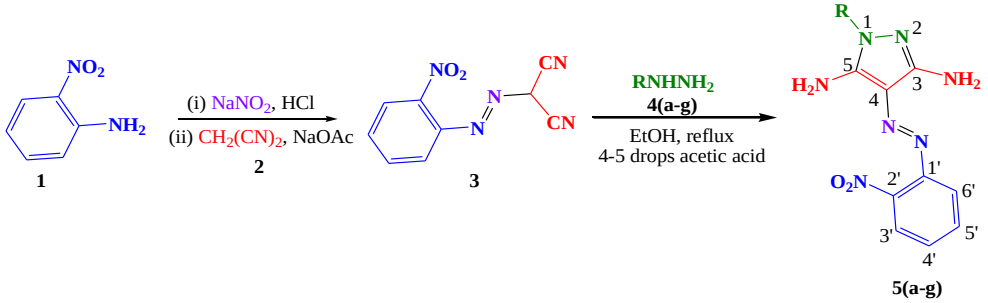
Virender Kumar[a], Anshul Bansal[b*], Ranjana Aggarwal[c], Mahavir Parshad[d], Naman Jain[e], Pooja Sethi[f], and Divya Mittal[g]

[a]Dpt of Chemistry, Khalsa Tri-Centenary, Government College, Ratia, 125051, Haryana, India ;
[b]Dpt of Chemistry, S. A. Jain College, Ambala City, 134003, Haryana, India;
[c]CSIR-National Institute of Science, Communication and Policy Research, New Delhi 110012, India;
[d]Dpt of Chemistry, Guru Jambheshwar University of Science & Technology, Hisar, 125001, Haryana, India;
[e]Dpt of Chemistry, Kurukshetra University, Kurukshetra-136119, Haryana, India;
[f]Dpt of Chemistry, Maharshi Markandeshwar (Deemed to be University), Mullana-133207, India;
[g]Dpt of Industrial Biotechnology, CCSHAU, Hisar.
Email: anshulbansal001@gmail.com

Received 11-19-2024 **Accepted** 12-22-2024 **Published on line** 12-26-2024

Abstract

In our quest for potent antimicrobial agents, we have developed a series of 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles 5(a-g) by refluxing aryl/heteroarylhydrazines 4(a-g) with 2-[(2'-nitrophenyl)hydrazono]malononitrile 3 in ethanol. The structural elucidation of all compounds was accomplished using IR, NMR (^{1}H and ^{13}C) and mass spectrometry analyses. Subsequently, these synthesized compounds were screened for their antimicrobial activity against two bacterial strains and one fungal strain. Remarkably, all compounds exhibited significant activity against the tested strains, displaying MIC values either better than or comparable to those of reference drugs. To further elucidate these findings, docking studies were conducted, reinforcing the substantial antibacterial and antifungal potential of the synthesized compounds, which in many instances surpassed that of the reference drugs.



Keywords: Antimicrobial, pyrazoles, screening, docking studies

Cite as *Arkivoc* **2024** (8) 202412327
DOI: https://doi.org/10.24820/ark.5550190.p012.327

Introduction

Antimicrobial drugs are fundamental to contemporary healthcare. The hike and dissemination of drug-resistant pathogens jeopardize our ability to treat frequent infections and conduct vital protocols such as anticancer therapy, abdominal deliveries, hip prostheses, organ replacements, and other surgical operations. A study indicates that by 2050, antibiotic-resistant infections could lead to 10 million fatalities each year and inflict economic harm similar to the 2008-2009 global financial crisis[1].

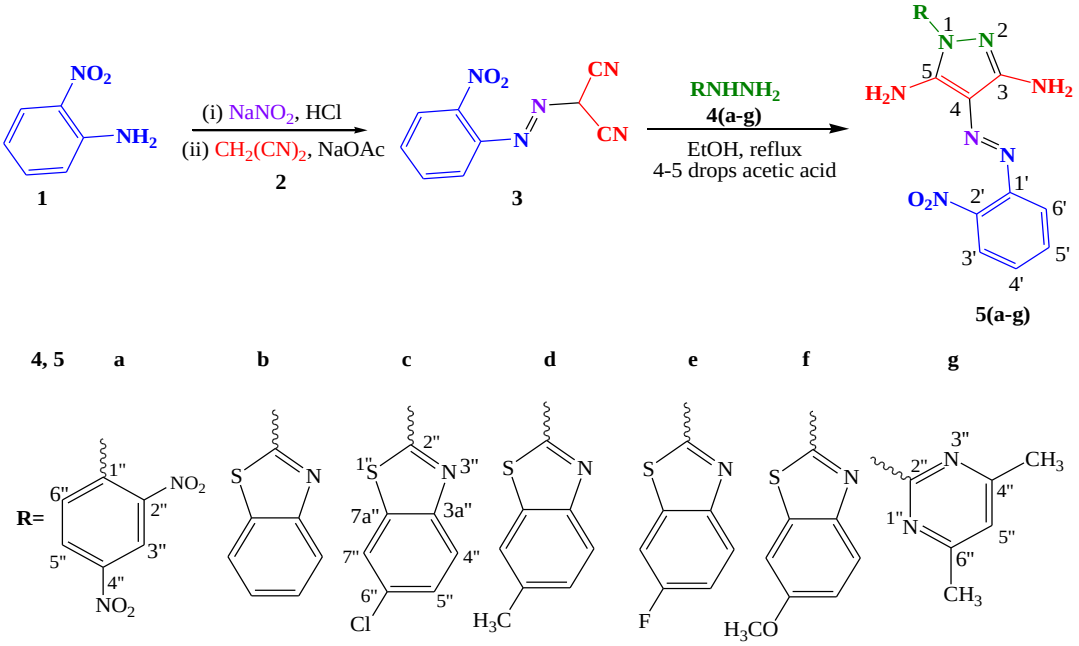
In the realm of medicinal chemistry, the pursuit of novel compounds with potent antimicrobial properties remains paramount in combating the ever-evolving threat of microbial infections. Pyrazole derivatives have garnered significant attention due to variety of pharmacological activities including anti-inflammatory[2-5], antimicrobial[6-9], anticancer[10-11], antipyretic[12], antidepressant[13-14], protein kinase inhibitors[15] etc. prompting researchers to explore their synthesis and biological potential extensively. Among these derivatives, 3,5-diamino-1-aryl/heteroarylpyrazoles emerged as a promising class, exhibiting notable antimicrobial properties[16-17] that warrant thorough investigation. The amalgamation of 3,5-diamino and 2'-nitrophenylazo functionalities within the pyrazole scaffold offers a unique molecular framework ripe for pharmacological exploration[4]. Such structural intricacies often confer desirable bioactivities, motivating synthetic chemists to devise efficient routes for their synthesis. Furthermore, the introduction of varied aryl and heteroaryl substituents at the 1-position of the pyrazole moiety introduces further structural diversity, potentially influencing both the physicochemical and biological properties of these compounds[18-19].

The present study demonstrates the synthesis, antimicrobial screening, and docking studies of a series of new 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles. Our synthetic approach aimed at the facile assembly of these compounds while maintaining synthetic accessibility and structural diversity. Subsequently, the synthesized compounds were subjected to comprehensive antimicrobial assays to elucidate their efficacy against a panel of pathogenic microorganisms. Moreover, molecular docking studies were conducted to gain insights into the potential binding interactions between the synthesized pyrazoles and their putative biological targets. Computational docking provides invaluable information regarding the binding modes and affinities of the compounds within the active sites of target enzymes or receptors, aiding in rationalizing their observed biological activities and facilitating structure-activity relationship (SAR) studies. Through the integration of synthetic chemistry, antimicrobial evaluation, and computational docking studies, this work aims to elucidate the structure-activity relationships governing the antimicrobial properties of 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles. Furthermore, the findings of this study hold promise in guiding the rational design and development of potent antimicrobial agents to address the persistent challenges posed by infectious diseases.

Results and Discussion

The pathway for the synthesis of the desired molecules 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles **5(a-g)** is summarised in Scheme 1[20-21]. The precursor 2-[(2'-Nitrophenyl)hydrazono]malononitrile **3** used for the synthesis of target compounds **5(a-g)** was obtained by the diazotization of o-nitroaniline **1** followed by reaction with malanonitrile **2**. Further reaction of 2-[(2'-Nitrophenyl)hydrazono]malononitrile **3** with appropriate hydrazines **4(a-g)** under reflux in ethanol with a catalytic quantity of glacial acetic acid, yielded desired compounds 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles **5(a-g)** in 75-88% yield. The reaction demonstrated excellent functional group

tolerance, accommodating a wide range of electron-donating and electron-withdrawing groups on hydrazine. The substrate scope of the given methodology is highlighted in Figure 1.



Scheme 1

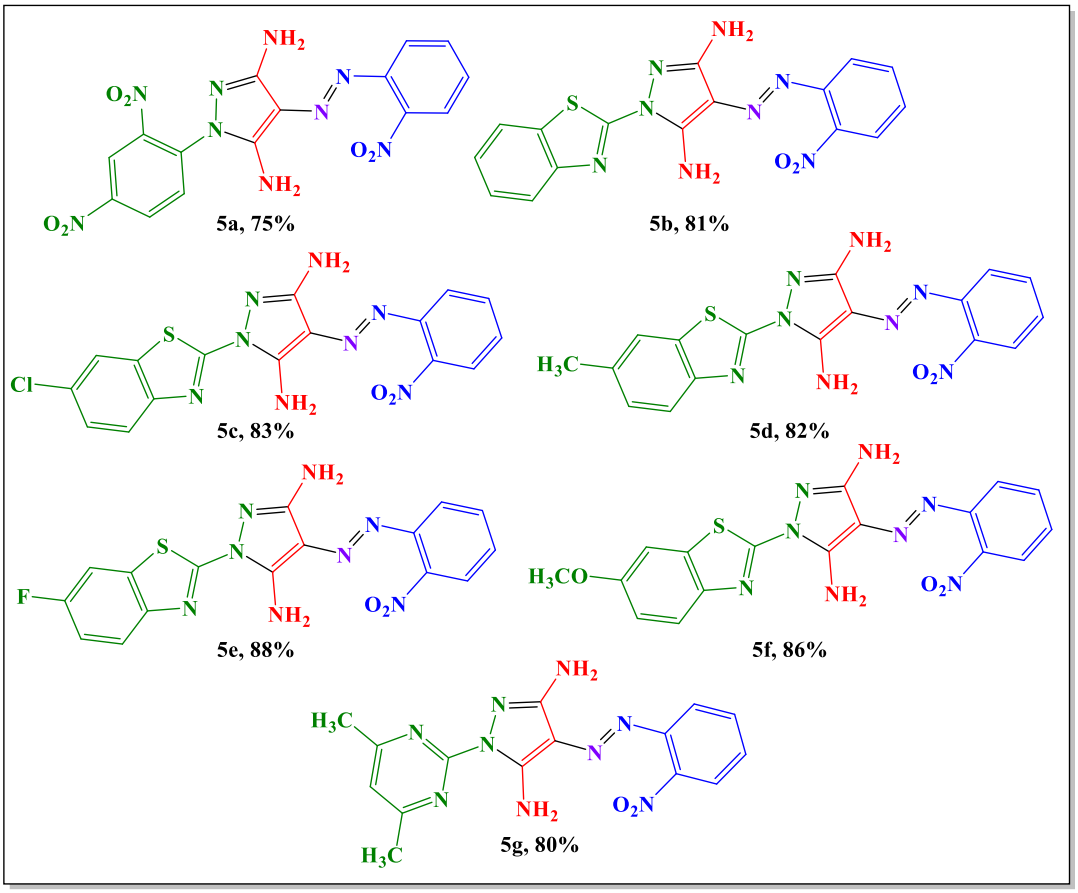


Figure 1. Substrate Scope.

All the products were unambiguously characterized by an integrated use of IR, ^{1}H, ^{13}C NMR, mass spectra and elemental analyses. The $–NH_2$ group in compounds **5(a-g)** was verified by IR spectroscopy, which displayed two distinct absorption peaks at 3250 cm^{-1} (symmetric) and 3400 cm^{-1} (asymmetric) attributed to NH_2 stretching, along with the absence of the CN stretch absorption band at 2222 cm^{-1} from compound **3**. Further, the peaks at 1550 and 1370 cm^{-1} in the IR spectra confirmed the existence of nitro group in the synthesized compounds **5(a-g)**. The ^{1}H NMR spectra of all the compounds **5(a-g)** displayed two broad singlets at δ 6.7 and δ 8.5 verifying the existence of two $–NH_2$ substituents in the compounds.

The singlets at δ 2.44 in **5d** and at δ 3.84 in **5f** integrated for three protons each and singlet due to six protons at δ 2.51 in **5g** confirmed the presence of methyl, methoxy and two methyl groups in these compounds. The structures of compounds **5(a–d)** were further validated by ^{13}C NMR spectra, which displayed signals for C-3, C-4, and C-5 at approximately 151, 114-117, and 148-150 ppm, respectively.

Biological assessment

Antibacterial efficacy: The in vitro antibacterial efficacy of all the synthesized pyrazoles **5(a-g)** was assessed using the agar well diffusion method. The screening was conducted against Staphylococcus aureus (MTCC 96) Gram-positive bacterium, and Escherichia coli (MTCC 1652), Gram-negative bacterium. The diameter of the growth inhibition zone and the minimum inhibitory concentration (MIC) of compounds **5(a-g)** against the aforementioned bacterial strains are represented in Tables 1 and 2.

Table 1. Antimicrobial range of synthesized pyrazoles

Pyrazole	Zone of inhibition (mm)		
	S. aureus	*E. coli*	*Candida strain*
5a	32±0.00	24±0.10	15±0.35
5b	35±0.20	29±0.30	19±0.20
5c	36±0.25	30±0.45	20±0.15
5d	33±0.55	28±0.20	18±0.35
5e	38±0.00	32±0.50	22±0.60
5f	35±0.40	26±0.65	17±0.45
5g	33±0.15	27±0.15	16±0.80
Ampicillin	32±0.55	25±0.50	-
Fluconazole	-	-	17±0.50

Table 2. Minimum Inhibitory Concentration Assessment

Pyrazole	MIC (µg/ml)		
	S. aureus	*E. coli*	*Candida strain*
5a	11	14	14
5b	08	07	08
5c	07	06	06
5d	08	09	10
5e	06	05	04
5f	09	10	09
5g	10	12	10
Ciprofloxacin	10	08	-
Fluconazole	-	-	09

The antibacterial effectiveness of these compounds was evaluated against that of the reference antibiotic, Ampicillin. The findings revealed that all the compounds demonstrated excellent activity against both the tested strains, with MIC values ranging from 5 to 14 µg/ml. Compound **5e** was found to be most active against both the tested bacterial strains with MIC values of 06 and 05 µg/ml, respectively against S. aureus and E. coli. Excellent activity, comparable to that of the reference drug ciprofloxacin, was also shown by all other compounds against both tested strains.

It was observed that better activity was exhibited by compounds **5(b-f)**, containing the benzothiazole moiety, compared to compounds **5a** and **5g**, which possess 2,4-dinitrophenyl and 4,6-dimethylpyrimidin-2-yl moieties, respectively. Furthermore, among the compounds containing the benzothiazole ring, better results were shown by compounds **5c** and **5e**, having electron-withdrawing substituents (F and Cl), than the unsubstituted compound **5b**, followed by compounds **5d** and **5f** with electron-releasing substituents.

Antifungal Efficacy: The antifungal properties of all the compounds **5(a-g)** were evaluated against a virulent fungal strain, Candida (MTCC 227). The diameter of the growth inhibition zones and the MIC values of compounds **5(a-g)** against the tested fungal strain are shown in Tables 1 and **2** respectively. Fluconazole was used to assess the antifungal efficacy of these compounds. The findings in the tables revealed that excellent antifungal activity was exhibited by all the compounds against the candida strain having MIC values ranging between 04 and 14 µg/ml. Better potency, as compared to the reference drug, was shown by compounds **5b**, **5c**, **5e**, and **5f**. Furthermore, as observed in the case of antibacterial activity, better inhibition was displayed by compounds **5(b-f)**, containing the benzothiazole moiety, compared to compounds **5a** and **5g**, which possess 2,4-dinitrophenyl and 4,6-dimethylpyrimidin-2-yl moieties, respectively.

Docking Studies

Over the years, molecular modeling has been recognized as a vital tool in the realm of drug discovery and development. Molecular docking is used to determine how effectively a ligand interacts with a receptor molecule by adjusting the receptor's conformation[22]. In this study, all synthesized compounds **5a-g** were docked with gram-positive bacteria *S. aureus*, gram-negative bacteria *E.coli*, and antifungal strain *Candida albicans* using Autodock Vina. The results were then visualized through Biovia Discovery Studio and compared with the reference drugs Ampicillin and Fluconazole. To prepare the protein receptor structures for docking, the X-ray crystal structures of *S. aureus* (PDB entry 4DXD)[23], *E.coli* (PDB entry 1Q8I)[24], and *Candida albicans*

(PDB entry 4YDO)[25] were taken from the Protein Data Bank (https://www.rcsb.org/). Water molecules and co-crystal ligands were removed, and polar hydrogens were added. Lastly, a grid box was created for the receptor proteins. After protein preparation, all synthesized compounds **5a-g** and the reference drugs were successfully docked with the aforementioned strains. The results, in terms of dock score, are highlighted in Table 3.

Table 3. Dock score of the synthesized compounds **5a-h** and reference drug Ampicillin with *S. aureus*, *E.coli* and C*andida albican*

Sr No.	Compound	*Staphylococcus aureus* (kcal/mol)	*E. coli* (kcal/mol)	*Candida albican* (kcal/mol)
1.	5a	-6.9	-7.8	-8.6
2.	5b	-8.2	-8.4	-8.8
3.	5c	-8.4	-8.7	-8.9
4.	5d	-8.0	-8.2	-8.0
5.	**5e**	**-8.5**	**-8.8**	**-9.2**
6.	5f	-7.8	-8.0	-7.9
7.	5g	-7.1	-7.4	-7.6
8.	Ampicillin	-7.3	-7.2	-
9.	Fluconazole	-	-	-6.9

The results indicate that the pyrazole nucleus linked with a benzothiazole substituent **5b-f** interacted more efficiently than the 2,4-dinitrophenyl **5a**, and pyrimidine **5g** substituents. Additionally, it was found that electron-withdrawing substituents on the benzothiazole ring increased the binding efficiency, whereas donating groups decreased it. Compound **5e** was found to be the most effective among all the tested pyrazole derivatives, exhibiting strong interactions with all three strains under evaluation. These findings suggest that the synthesized compounds possess significant antibacterial and antifungal potential, outperforming the reference drugs in most cases. The results obtained were visualized using Biovia Discovery Studio. The 2D and 3D poses of the most stable conformation of potential ligand 5c with S. aureus, E. coli, and Candida albicans are displayed in the Figure 2 given below.

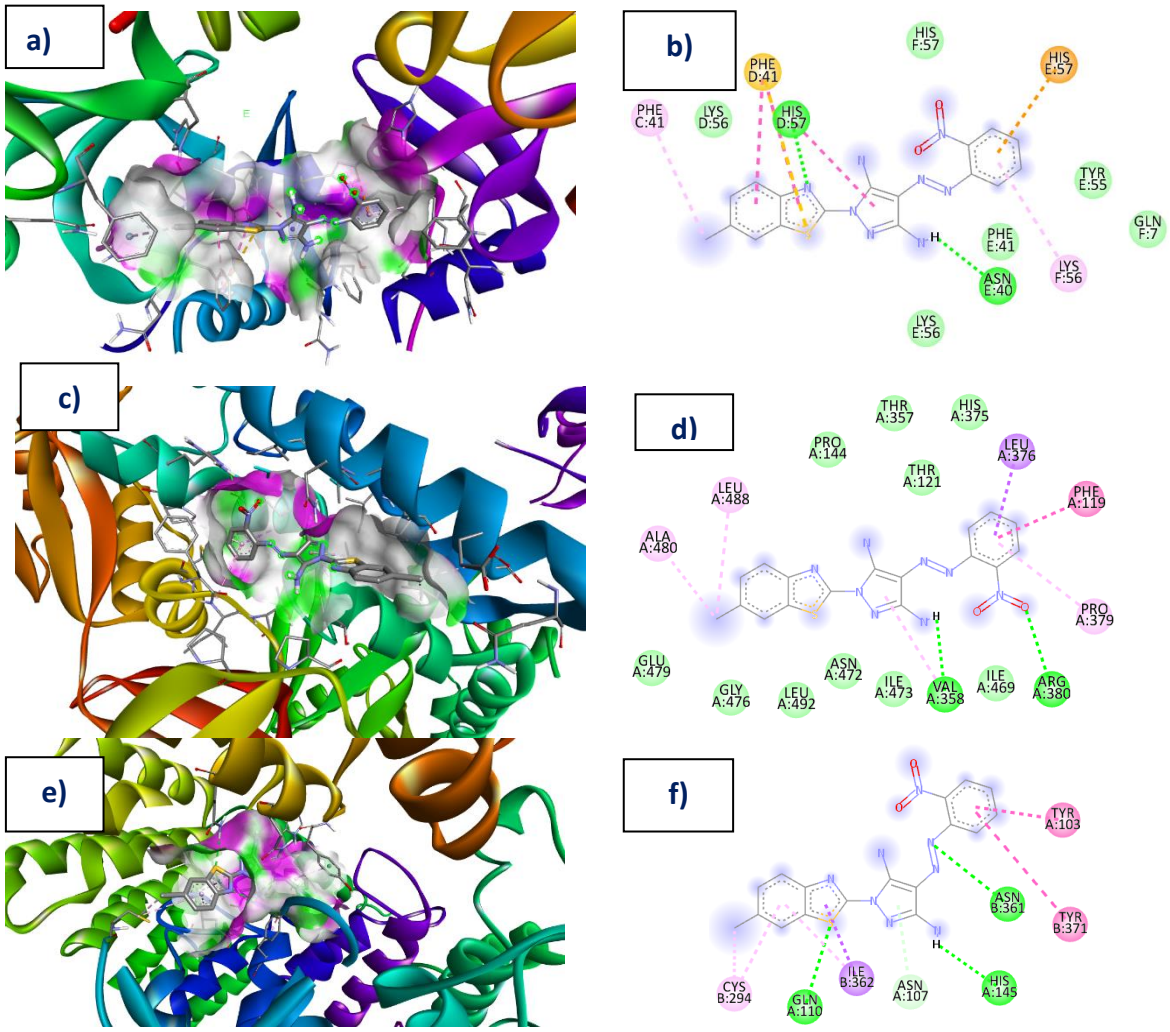


Figure 2. 2D and 3D poses of compound **5c** with *Staphylococcus aureus, E. coli and Candida albican* **a)** 3D pose of compound **5c** with *Staphylococcus aureus*; **b)** 2D pose of compound **5c** with *Staphylococcus aureus*; **c)** 3D pose of compound **5c** with *E. coli*; **d)** 2D pose of compound **5c** with *E. coli*; **e)** 3D pose of compound **5c** with *Candida albican*; **f)** 2D pose of compound **5c** with *Candida albican.*

The docking analysis conducted using Biovia Discovery Studio revealed that the derivative **5f** exhibited significant interactions with S. aureus, E. coli, and Candida albicans through various non-covalent interactions. Specifically, derivative **5f** was found to interact with S. aureus via conventional hydrogen bonding, van der Waals forces, π-cation, π-sulfur, and π-alkyl interactions. Its interaction with E. coli involved conventional hydrogen bonding, van der Waals forces, π-sigma, amide-π stacking, and π-alkyl interactions. In the case of Candida albicans, the derivative engaged through conventional hydrogen bonding, π-donor hydrogen bonding, π-sigma, π-π stacking, and π-alkyl interactions.

Conclusions

Some newly biologically active 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles 5(a-g) were synthesised by refluxing 2-[(2'-Nitrophenyl)hydrazono]malononitrile 3 with appropriate hydrazines 4(a-g) in

ethanol using glacial acetic acid as a catalyst. The antimicrobial potency of all the compounds was tested against three virulent strains namely S. aureus, E.coli, and Candida albicans respectively. It was found that the compounds bearing benzothiazole moiety have displayed better activity than the compounds bearing 2,4-dinitrophenyl and 4,6-dimethylpyrimidin-2-yl moieties. Further, in case of compounds possessing benzothiazole ring, the compounds having electron withdrawing substituents (F and Cl) have shown better results than the unsubstituted one followed by compounds having electron releasing substituents. Notably, all the compounds demonstrated substantial activity against the tested strains, achieving MIC values that were either superior to or on par with those of the reference drugs. Docking results revealed that the pyrazole nucleus linked with a benzothiazole substituent 5(b-f) interacts more efficiently than the 2,4-dinitrophenyl 5a and pyrimidine 5g substituents. Additionally, it was found that electron-withdrawing substituents on the benzothiazole ring enhance binding efficiency, while electron-donating groups decrease it. Among all the tested pyrazole derivatives, compound 5e was found to be the most effective exhibiting strong interactions with all three strains under evaluation.

Experimental Section

General. Melting points were recorded using an electrical apparatus with open capillaries and are reported without correction. The FTIR spectra of the compounds were obtained using Perkin Elmer Spectrum IR Version 10.6.2 using KBr pellets (vmax in cm^{-1}). ^{1}H and ^{13}C NMR spectra on a Avance III, Bruker instrument at 400 and 100 MHz, respectively in DMSO-d_6. HRMS values were recorded in the form of *m/z* on SCIEX TripleTOF 5600 and 5600+/SCIEX spectrometer.

Arylhydrazines 4a were purchased commercially. 2-[(2'-nitrophenyl)hydrazono]malononitrile[26] 3 and heteroarylhydrazines 4(b-g) were synthesized following the procedure described in the literature[27-30].

General method for preparing 2-[(2'-nitrophenyl)hydrazono]malononitrile 3. The *o*-nitroaniline (5 mmol) was dissolved in a mixture of water (30 mL) and hydrochloric acid (4.5 mL of 37% w/v soln). A solution of $NaNO_2$ (0.35 g, 5 mmol) in ice-cold water (5 mL) was added dropwise with stirring to the cooled amine solution (0-5 °C). The diazonium salt solution so formed was then added dropwise to a solution of malononitrile (0.5 g, 7.5 mmol) and NaOAc (12.5 g) in water (50 mL) with continuous stirring and cooling. After adding the diazonium salt, the reaction mixture was stirred and cooled for an additional 30 min before being placed in a refrigerator overnight. The following day, the precipitated hydrazone product was filtered, washed with water, and dried.
Mp. 142 °C (Lit. 144-146 °C)[26]

Standard procedure for synthesis of 3,5-diamino-4-(2'-nitrophenylazo)-1-aryl/heteroarylpyrazoles 5a-g. 2-[(2'-Nitrophenyl)hydrazono]malononitrile 3 (1.08 g, 0.005 mol) was dissolved in 40 ml of ethanol, followed by the addition of an equivalent amount of the respective hydrazines 4(a-g) (0.005 mol) and 4-5 drops of acetic acid. The mixture was then refluxed for 2 hours. The resulting crude product was filtered and recrystallized from methanol to obtain pure compounds 5(a-g).

3,5-Diamino-1-(2'', 4''-dinitrophenyl)-4-(2'-nitrophenylazo)pyrazole (5a). Mp 162 °C; Yield 75%; IR (KBr, cm-1): 1492.05 (N=N), 3450.18 (NH_2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.38-7.41 (m, 1H,), 7.62-7.65 (m, 1H), 7.76 (m, 1H), 8.08-8.10 (m, 1H), 8.27-8.29 (m, 1H), 8.82 (m, 1H), 10.01 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ:110.18, 114.59, 115.98, 119.40, 123.89, 125.89, 125.98, 128.38, 130.08, 135.10, 135.84, 138.57, 149.27.; HRMS (*m/z*): 415.21 $[M+1]^+$; Elemental analysis: Calcd. for $C_{15}H_{11}N_9O_6$: N, 30.50; Found: N, 30.47.

3,5-Diamino-1-(benzothiazol-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5b). Mp 332 °C, Yield 81%.; IR (KBr, cm^{-1}): 1488.92 (N=N), 3313.90, 3438.48 (NH2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.76 (bs, 2H, NH_2, exchangeable with D_2O), 7.37-7.46 (m, 1H), 7.49-7.53 (m, 2H), 7.71-7.75 (m, 1H), 7.89-7.97 (m, 2H), 8.05-8.06 (m, 2H), 8.58 (bs, 2H, NH_2, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ:117.07, 117.55, 121.69, 122.55, 124.65, 127.17, 128.09, 131.43, 133.52, 145.48, 149.90, 151.28; HRMS (*m/z*): 381.08 $[M+1]^+$; Elemental analysis: Calcd. for $C_{16}H_{12}N_8O_2S$: N, 29.46; Found: N, 29.41.

3,5-Diamino-1-(6''-chlorobenzothiazol-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5c). Mp 343 °C; Yield 83%; IR (KBr, cm^{-1}): 1488.66 (N=N), 3313.06, 3438.64 (NH_2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.76 (bs, 2H, NH_2, exchangeable with D_2O), 7.39-7.48 (m, 1H), 7.50-7.52 (m, 2H), 7.72-7.75 (m, 1H), 7.90-7.97 (m, 2H), 8.05-8.07 (m, 1H), 8.58 (bs, 2H, NH_2, exchangeable with D_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ:117.56, 121.70, 122.57, 124.67, 127.18, 128.11, 131.45, 133.49, 145. 48, 151.28, 161.18; HRMS (*m/z*): 415.21 $[M+1]^+$ Elemental analysis: Calcd. for $C_{16}H_{11}ClN_8O_2S$: N, 27.01; Found: N, 26.98.

3,5-Diamino-1-(6''-methylbenzothiazol-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5d). Mp 302 °C; Yield 82%; IR (KBr, cm^{-1}): 1466.15 (N=N), 3446.80 (NH2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.44 (s, 3H, CH3), 6.49 (bs, 2H, NH_2, exchangeable with D_2O), 7.32-7.34 (m, 1H), 7.46-7.50 (m, 1H), 7.71-7.75 (m, 3H), 7.85-8.09 (m, 2H), 8.54 (bs, 2H, NH_2, exchangeable with D2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.48, 117.54, 121.32, 122.22, 124.67, 128.07, 128.45, 131.53, 133.54, 134.30, 135.96, 149.26. HRMS (*m/z*): 395.11 $[M+1]^+$; Elemental analysis: Calcd. for $C_{17}H_{14}N_8O_2S$: N, 28.41; Found: N, 28.35.

3,5-Diamino-1-(6''-fluorobenzothiazol-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5e). Mp 324 °C; Yield 88%; IR (KBr, cm^{-1}): 1460.91 (N=N), 3452.76 (NH_2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.75 (bs, 2H, NH_2, exchangeable with D_2O), 7.39-7.46 (m, 1H), 7.48-7.50 (m, 1H), 7.72-7.75 (m, 1H), 7.96-7.98 (m, 1H), 8.00-8.08 (m, 3H), 8.55 (bs, 2H, NH_2, exchangeable with D_2O); HRMS (*m/z*): 399.07 $[M+1]^+$; Elemental analysis: Calcd. for $C_{16}H_{11}FN_8O_2S$: N, 28.13; Found: N, 28.06.

3,5-Diamino-1-(6''-methoxylbenzothiazol-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5f). Mp 288 °C; Yield 86%; IR (KBr, cm^{-1}): 1474.71 (N=N), 3459.70 (NH_2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.84 (s, 3H, OCH_3), 6.46 (bs, 2H, NH_2, exchangeable with D_2O), 7.12-7.13 (m, 1H), 7.47-7.49 (m, 1H), 7.67-7.75 (m, 3H), 7.85-8.09 (m, 2H), 8.50 (bs, 2H, NH_2, exchangeable with D_2O); HRMS (*m/z*): 411.09 $[M+1]^+$; Elemental analysis: Calcd. for $C_{17}H_{14}N_8O_3S$: N, 27.30; Found: N, 27.23.

3,5-Diamino-1-(4'',6''-dimethylpyrimidin-2''-yl)-4-(2'-nitrophenylazo)pyrazole (5g). Mp 352 °C; Yield 80%; IR (KBr, cm^{-1}): 1464.74 (N=N), 3253.82, 3451.55 (NH_2 str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.51 (s, 6H, CH3), 7.06 (s, 1H), 7.48-7.56 (m, 1H), 7.76-7.78 (m, 1H). 7.96-8.05 (m, 1H). 8.45-8.46 (m, 1H). HRMS (*m/z*): 354.14 $[M+1]^+$; Elemental analysis: Calcd. for $C_{15}H_{15}N_9O_2$: N, 35.68; Found: N, 35.59.

Evaluation of antibacterial activity: initial screening

The antimicrobial activity was determined using the agar well diffusion method[31]. A 24 h bacterial culture in broth was diluted with sterilized water to obtain a proportion of about 108 colony forming units (CFU/ml). Six to eight equidistant wells, each 7 mm wide, were punched in each plate by means of a sanitized cork borer. The compounds under testing were solubilized in Dimethyl Sulfoxide (DMSO) before being evaluated for their antimicrobial effects. Each well was injected with 1 ml of the test solution at a concentration of 1 mg/ml. The plates were maintained at 37 °C for 48 h. Antimicrobial efficacy was assessed by gauging the zones of bacterial growth inhibition around the wells following 24 and 48 hours. Ampicillin and Chloramphenicol (10 µg/ml) were used as positive antibacterial controls, while DMSO served as the negative control.

Assessment of the minimum inhibitory concentration (MIC)

The MIC of each compound, which produced a zone of antimicrobial activity at a concentration of 1 mg/ml, was also assessed using an adapted agar well diffusion technique[32-33]. Various concentrations of a single

compound, extending from 1000 to 1 µg/ml, were dispensed into several wells on the agar plates. 1 ml of each dilution was pipetted into the wells. All test plates were then maintained at 37°C for 48 hours. The concentration of each compound that produced a clear inhibition zone was designated as the MIC. The measurements were conducted in triplicate, and the outcomes were averaged.

In-vitro antifungal screening

Agar-well diffusion method was employed to assess the antifungal efficacy of the titled compounds[34]. The molds were cultivated on Potato Dextrose Agar (PDA) at 25°C for a week and used as inocula. Fifteen millilitres of molten PDA (at 45°C) were mixed with 50 µl of each compound was dispensed into sterilized petri dishes and allowed to solidify at room temperature. The compounds under testing as well as standards were solubilized in DMSO to obtain a concentration of 2000 µg/ml. Double dilutions of the compounds from this stock solution were applied to the corresponding wells. 8 mm fungal discs from actively growing cultures were placed in the centre of the solidified poisoned agar plates, which were then maintained at 25°C for 7 days. DMSO served as the negative control, while Fluconazole was utilized as the reference standard. The investigations were carried out in triplicate.

Supplementary Material

Copies of ^{1}H, ^{13}C NMR and HRMS of compounds **5a-g** are available in the Supplementary Material file associated with this manuscript.

References

1. Antimicrobial Resistance Collaborators. *The Lancet* **2022**, 399(10325): P629-655. https://doi.org/10.1016/S0140-6736(21)02724-0
2. TK, S. M.; Das, A.; Revanasiddappa, B. C.; Sreeraj, K.; Shijith, K. V., *Journal of Molecular Structure* **2022**, 1255, 132415. https://doi.org/10.1016/j.molstruc.2022.132415
3. Shaker, A. M.; Shahin, M. I.; AboulMagd, A. M.; Aleem, S. A. A.; Abdel-Rahman, H. M.; Abou El Ella, D. A. *Bioorganic Chemistry* **2022**, *129*, 106143. https://doi.org/10.1016/j.bioorg.2022.106143
4. Vashisht, K.; Sethi, P.; Bansal, A.; Gupta, S.; Dar, M.O.; Umar, A.; Kumar, R.; Kumar, S.; Kumar, H.; Alkhanjaf, A.A.M.; Ibrahim, A.A. *Journal of Molecular Structure* **2024**, p 139109. https://doi.org/10.1016/j.molstruc.2024.139109
5. Aggarwal, R.; Bansal, A.; Rozas, I.; Kelly, B.; Kaushik, P.; Kaushik, D. *European Journal of Medicinal Chemistry* **2023**, *70*, 350. https://doi.org/10.1016/j.ejmech.2013.09.052
6. Kumar, P.; Sood, S.; Kumar, A.; Verma, A.; Kumar, L.; Kumar, S.; Kumar, V.; Kumar, A.; Singh, K. *Polycyclic Aromatic Compounds* **2023**, *43*, 2960. https://doi.org/10.1080/10406638.2022.2058968
7. Aggarwal, R.; Bansal, A.; Rozas, I.; Diez-Cecilia, E.; Kaur, A.; Mahajan, R.; Sharma, J. *Medicinal Chemistry Research* **2014**, *23*, 1454. https://doi.org/10.1007/s00044-013-0751-9

8. Aggarwal, R.; Kumar, V.; Gupta, G.K.; Kumar, V. *Medicinal Chemistry Research* **2013**, *22*, 3566-3573. https://doi.org/10.1007/s00044-012-0343-0
9. Bansal, A. *Mini-Reviews in Organic Chemistry* **2020**, *17*, 197. https://doi.org/10.2174/1570193X16666190122162920
10. Theodore, C.E.; Anusuya, A.M.; Sivaiah, G.; Jain, R.; Kumar, C.A.; Prasad, S.B.; Raghu, M.S.; Alharti, F.A.; Prashanth, M.K.; Jeon, B.H. *Journal of Molecular Structure* **2023**, 1288, p 135765. https://doi.org/10.1016/j.molstruc.2023.135765
11. Bennani, F. E.; Doudach, L.; Cherrah, Y.; Ramli, Y.; Karrouchi, K.; Faouzi, M. E. A. *Bioorganic Chemistry* **2020**, 97, 103470. https://doi.org/10.1016/j.bioorg.2019.103470
12. do Carmo Malvar, D.; Ferreira, R.T.; de Castro, R.A.; de Castro, L.L.; Freitas, A.C.C.; Costa, E.A.; Florentino, I.F.; Mafra, J.C.M.; de Souza, G.E.P.; Vanderlinde, F.A. *Life Sciences* **2014**, *95*, 81. https://doi.org/10.1016/j.lfs.2013.12.005
13. Abdel-Aziz, M.; Abuo-Rahma, G. E. D. A.; Hassan, A. A.; *European Journal of Medicinal Chemistry* **2009**, *44*, 3480. https://doi.org/10.1016/j.ejmech.2009.01.032
14. Tripathi, A.C.; Upadhyay, S.; Paliwal, S.; Saraf, S.K. *Medicinal Chemistry Research* **2018**, *27*, 1485. https://doi.org/10.1007/s00044-018-2167-z
15. Yevale, D. B.; Teraiya, N.; Lalwani, T. D.; Ameta, R. K.; Sangani, C. B.; *Bioorganic Chemistry* **2023**, *141*, 106901. https://doi.org/10.1016/j.bioorg.2023.106901
16. Lusardi, M.; Spallarossa, A.; Brullo, C. *International Journal of Molecular Sciences* **2023**, *24*, 7834. https://doi.org/10.3390/ijms24097834
17. Helal, M. H.; Salem, M. A.; Aly, H. M. *Journal of Heterocyclic Chemistry* **2017**, *54*, 2614-2626. https://doi.org/10.1002/jhet.2858
18. Khan, K.A.; Faidallah, H.M. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2016**, *31*, pp 619-627. https://doi.org/10.3109/14756366.2015.1057717
19. Hussain, S.; Kaushik, D. *Journal of Saudi Chemical Society* **2015**, *19*, pp 274-281. https://doi.org/10.1016/j.jscs.2012.04.002
20. Pawar, D.C.; Gaikwad, S.V.; Kamble, S.S.; Gavhane, P.D.; Gaikwad, M.V.; Dawane, B.S. *Chem. Methodol*, **2022**, *6*, 677-90. https://doi.org/10.22034/chemm.2022.343572.1538
21. Şener, N.; Sener, I.Z.Z.E.T.; Yavuz, S.; Karci, F.I.K.R.E.T. *Asian Journal of Chemistry* **2015**, *27*, 3003-3012. http://dx.doi.org/10.14233/ajchem.2015.18769
22. Bouamrane, S.; Khaldan, A.; Hajji, H.; El-Mernissi, R.; Alaqarbeh, M.; Alsakhen, N.; Maghat, H.; Ajana, M.A.; Sbai, A.; Bouachrine, M.; Lakhlifi, T. *Molecular Diversity* **2023**, *27*, 2111-2132. https://doi.org/10.1007/s11030-022-10546-x
23. Sun, N.; Lu, Y.J.; Chan, F.Y.; Du, R.L.; Zheng, Y.Y.; Zhang, K.; So, L.Y.; Abagyan, R.; Zhuo, C.; Leung, Y.C.; Wong, K.Y. *Frontiers in Microbiology* **2017**, *8*, 855. https://doi.org/10.3389/fmicb.2017.00855
24. Guo, J.; Zhang, W.; Coker, A.R.; Wood, S.P.; Cooper, J.B.; Ahmad, S.; Ali, S.; Rashid, N.; Akhtar, M. *Acta Crystallographica Section D: Structural Biology* **2017**, *73*, 420. https://doi.org/10.1107/S2059798317004090

25. Shah, R.; Katouah, H.; Sedayo, A.A.; Abualnaja, M.; Aljohani, M.M.; Saad, F.; Zaky, R.; El-Metwaly, N.M. *Journal of Molecular Liquids* **2020**, *319*, 114116. https://doi.org/10.1016/j.molliq.2020.114116
26. Nesterov, V.N.; Timofeeva, T.V.; Duerksen, G.; Clark, R.D. *Journal of Molecular Structure* **1998**, 444(1-3), 135-146. https://doi.org/10.1016/S0022-2860(97)00374-8
27. Papke; Pohloudek-fabin, R. Pharmaize **1967**, *22*, 229 C*hem. Abstr.* **1968**, *68*, 12586n.
28. Sawhney S. N.; Tomer, R. K.; Prakash, O.; Prakash, I.; Singh, S. P. *Indian J. Chem.* **1981**, 20B:314-316.
29. Kosolapoff, G.M. and Roy, C.H., *The Journal of Organic Chemistry* **1961**, *26*, 1895. https://doi.org/10.1021/jo01065a049
30. Jois, V.H.; Kalluraya, B. *Indian Journal of Heterocyclic Chemistry* 2015, *24*, 271.
31. Perez, L.; Pauli, M.; Bazequre, P. *Journal of Actabiology* **1990**, *15*, 113.
32. Okunji, C.O.; Okeke, C.N.; Gugnani, H.C.; Iwu, M.M. *International Journal of Crude Drug Research* **1990**, *28*, 193. https://doi.org/10.3109/13880209009082811
33. Rios, J.L.; Recio, M.C.; Villar, A. *Journal of Ethnopharmacology* **1988**, *23*, 127. https://doi.org/10.1016/0378-8741(88)90001-3
34. Sadashiva, M.P.; Mallesha, H.; Hitesh, N.A.; Rangappa, K.S. *Bioorganic & Medicinal Chemistry* **2004**, *12*, 6389. https://doi.org/10.1016/j.bmc.2004.09.031