Introduction

The advent of antibiotics completely changed the course of modern medicine by significantly reducing human morbidity and mortality. However, the recent global spread of deadly infectious diseases and the emergence of drug-resistant microorganisms have claimed millions of lives. According to the World Health Organization (WHO), lower respiratory tract infections are the fourth leading cause of death globally. The upsurge of multi-drug-resistant (MDR) Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and MDR *Streptococcus pneumoniae*, as well as MDR Gram-negative bacteria such as carbapenem-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are a major threat to healthcare systems. Moreover, recent reports of MDR *Candida auris* and mucormycosis fungal infections in critically ill coronavirus disease patients are an increasing health concern worldwide. Consequently, costly treatments due to drug resistance and their associated adverse reactions pose new challenges that prompt the development of novel antimicrobial lead compounds.

Azo (–N=N–) and related hydrazo (=N–NH–) groups are essential pharmacophores for the synthesis of antimicrobial agents. Furthermore, pyrazole derivatives are an interesting class of compounds showing a broad range of biological activities such as anticancer, antimalarial, antiviral, and antimicrobial.

Recently, a molecular hybridization method offered a promising strategy to design new potent hybrid molecules with multi-targeting synergistic effects. Previous studies showed that molecular hybrids containing pyrazole and azo (–N=N–)/hydrazo (=N–NH–) pharmacophores in a single molecular framework resulted in improved antimicrobial potentials. In continuation of our endeavors to develop potent and effective antimicrobial agents, this study deals with the optimization of pyrazole-azo/hydrazo molecular hybrid leads as potential antimicrobial agents.

Results and discussion

Chemistry

So far, the majority of previously reported syntheses of aryl diazenyl pyrazoles (AHPs) and arylhydrazono pyrazoles

**Synthesis and antimicrobial activity of aryl diazenyl/arylhydrazono pyrazoles**

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**Abstract**

We report the design, synthesis, and in vitro antimicrobial evaluation of functionalized pyrazoles containing a hydrazono/diazenyl moiety. Among these newly synthesized derivatives, 4-[2-(4-chlorophenyl)hydrazono]-5-methyl-2-[2-(naphthalen-2-yloxy)acetyl]-2,4-dihydro-3H-pyrazol-3-one is a promising antimicrobial agent against *Staphylococcus aureus* (minimum inhibitory concentration 0.19 μg mL⁻¹). Structure–activity relationship studies reveal that the electronic environment on the distal phenyl ring has a considerable effect on the antimicrobial potential of the hybrid analogues. Molecular docking studies into the active site of *S. aureus* dihydrofolate reductase also prove the usefulness of hybridizing a pyrazole moiety with azo and hydrazo groups in the design of new antimicrobial agents.

**Keywords**

antimicrobial, azo, hydrazo, one-pot synthesis, pyrazoles

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(ADPs) were carried out in two steps. The first step involved the reactions of aryl diazonium salts with acetylacetone/ethyl acetoacetate via the Japp–Klingemann reaction, followed by cyclo-condensation with aryl hydrazides in the second step. These procedures require extended reaction time at reflux and afford low yields.\textsuperscript{13,14,16} We have reported a fast and expedient method for the preparation of AHPs and ADPs using one-pot, three-component condensations of ethyl acetoacetate/acetylacetone, aryl hydrazides, and aryl diazonium salts under solvent-free conditions. We demonstrated that the catalyst, triethylamine, being basic in character, facilitated proton removal from the active methyl enone compounds and increased the reaction rate.\textsuperscript{17} Using this method, herein we report the one-pot synthesis of new AHPs 1a–f (75%–84% yield) and ADPs 2a–f (72%–85% yield). The synthetic protocols for the preparation of AHPs 1a–f and ADPs 2a–f are outlined in Scheme 1. All the prepared compounds were fully characterized by IR, NMR, and elemental analyses. The spectral data of new compounds are in full agreement with the previously reported spectra.\textsuperscript{17} Literature revealed that the AHPs exist in different tautomeric forms whose relative stabilities depend on the influence of the substituents and on the medium. In the solid state, such compounds exist largely in the aryldiazenyl (OH) or the aryldiazonii (NH) forms with the latter predominating. Moreover, it is noteworthy that the 5-oxo group on the pyrazole nucleus and the carbonyl substituent at the N-1 position were reported to participate in hydrogen-bonding interactions. Compound 1a showed a low-frequency C=O band at 1668 cm\(^{-1}\) may be strongly attributed to its conjugation with the C=N group as well as in-molecular hydrogen bonding with the NH group. A separate carbonyl frequency at 1692 cm\(^{-1}\) due to amide carbonyl vibration and absence of N=N stretching vibration ruled out the aryldiazonii (OH) form. The AHPs are therefore assigned in the hydrazono form which is consistent with our previous reports. The \(^1\)H NMR spectra of prototype compound 1a showed two shielded singlets at \(\delta\) 4.70 and \(\delta\) 2.11 due to methylene and methyl protons, respectively. In addition, a one deshielded singlet at \(\delta\) 11.55 was due to the –NH group. The 12 aromatic protons resonated between \(\delta\) 7.35 and \(\delta\) 7.83. The \(^13\)C NMR spectrum of compound 1a showed five deshielded signals at \(\delta\) 141.55, 146.85, 155.57, 166.67, and 170.14 due to two \(\text{C} = \text{N}\) one \(\text{O} – \text{C}\) (ether), and two carbonyl carbons, respectively. Two signals in the aliphatic range were observed at \(\delta\) 11.62 and \(\delta\) 64.55 due to methyl and methylene carbons, respectively. The remaining resonances in the aromatic region at \(\delta\) 106.95, 107.28, 115.18, 115.65, 118.47, 123.80, 125.10, 126.48, 127.52, 128.10, 128.72, 129.60, and 134.10 along with the upfield and downfield signals confirm the presence of 22 carbons in compound 1a.

**Biological activity**

The newly synthesized compounds 1a–f and 2a–f were evaluated for their antibacterial activity against \(S.\) \(aureus\) (ATCC-25923) and \(E.\) \(coli\) (ATCC-25922), along with their antifungal activity against \(A.\) \(niger\) (ATCC9029) and \(C.\) \(albicans\) (ATCC-90028) in Dimethylformamide (DMF) using the serial plate dilution method. All the results are presented as minimum inhibitory concentration (MIC), which is the lowest concentration of antimicrobial agent that results in the complete inhibition of visible growth of microorganisms (Table 1). The standard antibiotic ofloxacin, belonging to the quinolone class, and antifungal drug fluconazole, belonging to theazole class, were used for comparison of the MIC values. The pharmacophores AHP and ADP, which were reported to have promising antimicrobial activity in our previous studies, were the lead compounds in the design of these hybrid molecules (Figure 1).\textsuperscript{13,14} We hypothesized that replacement of the quinoline and benzothiazole nucleus with a more lipophilic naphthyl ring would further enhance the antimicrobial activity of these AHP and ADP analogues. Finally, substituents were installed on the terminal phenyl ring to study the stereo-electronic effects of different functional groups on the antimicrobial activity of the pyrazole derivatives. The results of the antibacterial activity studies of the AHPs revealed that most of these compounds demonstrated excellent to moderate microbial growth inhibition, with the exception of compound 1b (\(R = 4\text{-CH}_3\), MIC >100 mg mL\(^{-1}\)). Among the Gram-positive and Gram-negative bacterial strains, \(S.\) \(aureus\) was found to be most sensitive to the AHPs. Compound 1d (\(R = 4\text{-Cl}\), MIC 0.19 mg mL\(^{-1}\)) was found to be the most potent compound of the series and was equipotent with the standard drug ofloxacin (MIC 0.19 mg mL\(^{-1}\)) against the \(S.\) \(aureus\) strain. Compounds 1e (\(R = 4\text{-Br}\), MIC 6.25 mg mL\(^{-1}\)) and 1f (\(R = 4\text{-NO}_2\), MIC 12.5 mg mL\(^{-1}\)) also showed moderate antibacterial effects, whereas compounds 1a (\(R = \text{H}\), MIC 50 mg mL\(^{-1}\)) and 1c (\(R = 4\text{-OCH}_3\), MIC 50 mg mL\(^{-1}\)) showed

**Scheme 1.** One-pot synthesis of AHPs 1a-f and ADPs 2a-f.
the lowest antibacterial effects against *S. aureus*. AHPs were less effective against the Gram-negative *E. coli* bacterial strain. The potency of compound 1d (R = 4-Cl, MIC 0.78 μg mL\(^{-1}\)) was only fourfold lower than that of the reference drug ofloxacin (MIC 0.19 μg mL\(^{-1}\)) against *E. coli*. The remaining AHPs (1a, 1c, 1e, and 1f) showed moderate to lower antibacterial activity (MIC 12.5-100 μg mL\(^{-1}\)) against *E. coli*. The antibacterial activity of the ADPs showed that only compound 2d (R = 4-Cl, MIC 6.25 μg mL\(^{-1}\)) had moderate antibacterial activity, whereas compounds 2e (R = 4-Br, MIC 25 μg mL\(^{-1}\)) and 2f (R = 4-NO\(_2\), MIC 25 μg mL\(^{-1}\)) showed lower antibacterial effects, in fact being 131 times less potent than ofloxacin.

The AHPs and ADPs were also evaluated for their antifungal activity. Among these, AHP analogues 1d (R = 4-Cl, MIC 6.25 μg mL\(^{-1}\)) and 1e (R = 4-Br, MIC 6.25 μg mL\(^{-1}\)) showed moderate antifungal activities against both *A. niger* and *C. albicans*, respectively. The standard drug fluconazole showed antifungal growth inhibition with MIC values of 1.56 and 0.19 μg mL\(^{-1}\) against *A. niger* and *C. albicans*, respectively. The remaining compounds were minimally effective against these fungal strains (MIC 25 to >100 μg mL\(^{-1}\)).

The structure–activity relationship of the AHP analogues showed antibacterial potency in the following order: 4-Cl > 4-Br, 4-NO\(_2\) > H, 4-OCH\(_3\) > 4-CH\(_3\), whereas the ADP analogues had antibacterial potency in the order: 4-Cl > 4-Br, 4-NO\(_2\) > H > 4-CH\(_3\), 4-OCH\(_3\). Thus, it was clearly observed that compounds with electron-withdrawing groups on the phenyl ring were more effective than those with electron-releasing substituents. Moreover, it is evident that the AHPs were more effective than the ADPs.

Furthermore, the partition coefficient (log P) is an imperative physicochemical property affecting penetration into microbial cells. The partition coefficients of all the compounds were calculated using ChemDraw to establish the correlation between Clog P and antimicrobial activity. The results showed that the lipophilicity of the compounds increased with lipophilic electron-withdrawing groups, for example, Cl (ClogP 5.88–6.66) and Br (ClogP 6.03–6.81), which consequently enhanced the antimicrobial potencies of the AHPs and ADPs.

### Table 1. Antimicrobial activity of the AHPs and ADPs.

| Compound | R | ClogP\(^a\) | MIC (μg mL\(^{-1}\)) |
|----------|---|-------------|---------------------|
|          |   |             | *S. aureus* (Gram +ve) | *E. coli* (Gram −ve) | *A. niger* | *C. albicans* |
| 1a       | H | 3.48        | 50                  | 50                  | 50        | 50        |
| 1b       | CH\(_3\) | 5.66        | >100                | >100                | >100      | >100      |
| 1c       | OCH\(_3\) | 5.08        | 50                  | 100                 | 100       | 100       |
| 1d       | Cl | 5.88        | 0.19                | 0.78                | 6.25      | 6.25      |
| 1e       | Br | 6.03        | 6.25                | 12.5                | 6.25      | 6.25      |
| 1f       | NO\(_2\) | 4.91        | 12.5                | 12.5                | 25        | 25        |
| 2a       | H | 5.86        | 100                 | 100                 | 100       | 100       |
| 2b       | CH\(_3\) | 6.36        | >100                | >100                | >100      | >100      |
| 2c       | OCH\(_3\) | 6.04        | >100                | >100                | >100      | >100      |
| 2d       | Cl | 6.66        | 6.25                | 6.25                | 25        | 25        |
| 2e       | Br | 6.81        | 25                  | 25                  | 25        | 25        |
| 2f       | NO\(_2\) | 5.78        | 25                  | 25                  | 25        | 25        |
| Ofloxacin|   |            |                     |                     |           |           |
| Fluconazole| |            |                     |                     | 1.56      | 0.19      |

AHP: arylhydrazono pyrazole; ADP: aryldiazenyl pyrazole; MIC: minimum inhibitory concentration.

\(^{a}\)Calculated using ChemDraw version 14.0.0.117.

### Figure 1. Design strategy and lead optimization of the new AHP and ADP antimicrobial agents.
Molecular docking studies

To gain further support regarding the antibacterial effects of the most promising hybrid AHP 1d against *S. aureus*, a molecular docking study was carried out on the dihydrofolate reductase (DHFR) enzyme (PDB ID: 3SRQ) of an *S. aureus* bacterial strain using the AutoDock program. AutoDock 4.2.2 with a Lamarkian genetic algorithm-implemented program suite was employed to identify appropriate binding modes and the conformations of the ligand molecules. DHFR catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, which is essential for the synthesis of purines, some amino acids, and thymidine required for bacterial growth and proliferation. Thus, DHFR represents an attractive antifolate drug target which produces antibacterial action by selectively disrupting the folate pathway. The docking protocol was validated by redocking the co-crystallized ligand 7-aryl-2,4-diaminoquinazoline at the active site (root-mean-square deviation (RMSD) 0.12). The results of docking studies clearly showed that compound 1d fits nicely into the active site and formed hydrogen bonds, van der Waals, π-σ, and π-alkyl interactions with the active site residues (Figures 2–4). The binding free energy of compound 1d was found to be −10.4 kcal mol⁻¹, indicating sufficient affinity between the hybrid analogue and the enzyme. The 3D and 2D docked conformations of the ligand 1d bound to the active site of DHFR are shown in Figures 3 and 4. The hydrazo −NH group of compound 1d formed a hydrogen bond with Thr47, whereas the carbonyl oxygen formed a hydrogen bond with the Asp28 active site residue. Lipophilicity also appears to play a crucial role in the antibacterial activity of 1d, as the naphthyl ring positioned itself into the lipophilic pocket of the binding site and formed π-σ and π-alkyl interactions with the Leu55, Leu29, and Val32 residues. This validates our hypothesis that the optimization of previous lead molecules by changing the quinoline and benzothiazole rings to a lipophilic naphthyl moiety greatly improved the antibacterial potential of these hybrid analogues.

Conclusion

In summary, we have synthesized 12 hybrid azo/hydrazo-pyrazole derivatives as potential antimicrobial agents. Most of the compounds demonstrated promising antimicrobial activities. The results revealed that compound 1d (ClogP 5.88) was the most potent antibacterial agent against *S. aureus*, with an MIC value of 0.19 µg mL⁻¹. This study provides a novel optimized azo/hydrazo-pyrazole hybrid as a potential lead molecule for the development of promising antimicrobial agents.

Experimental procedure

All the chemicals used in this study were purchased from Sigma-Aldrich and used without further purification. The progress of the reactions was monitored on pre-coated silica gel 60 Thin-layer chromatography (TLC) plates on a support of aluminum (CAMAG®). Melting points were determined in open capillary tubes in a Buchi automated melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1720 FTIR spectrometer using KBr pellets. ¹H NMR spectra were obtained on a JEOL ECA 500-II 500 MHz NMR (125 MHz ¹³C) spectrometer using tetramethylsilane (TMS) as an internal reference (chemical shifts in δ ppm). The aryldiazonium salts were prepared by following previously reported methods. The amines (0.01 mol) were added to HBF₄ (5 mL, Sigma 48%), cooled to 0 °C, and diazotized by the dropwise addition of a solution of NaNO₂ (0.02 mol) in water (5 mL). Colorless shiny crystals of the diazonium salt were cooled on ice, collected, washed with ether, and used without purification.

General procedure for the synthesis of aryldiazono/aryldiazenyl pyrazoles (1a-f and 2a-f)

An equimolar amount of aryldiazonium salts (1 mmol), ethyl acetooacetate/acetilacetone (1 mmol), and 2-(1-naphthoxy)acetohydrazide (1 mmol) was mixed in the presence of 2–3 drops of triethylamine. The mixture was heated in an oil bath at 90 °C for 6–8 h. The completion of the reaction was ascertained by TLC using ethyl acetate/hexane (3:7) as the mobile phase. The resulting reaction mixture was poured onto crushed ice and the precipitate was filtered, washed with water, and recrystallized from ethanol.
5-Methyl-2-[(4-[(2-naphthalen-2-yloxy)acetyl]-2,4-dihydro-3H-pyrazol-3-one (1a): Yield: 0.32 g (84%); pale yellow solid; m.p. 107 °C–111 °C; IR (KBr): 1067, 1661, 1698, 3405 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆): δ = 11.68 (s, 1H, NH), 7.58–8.27 (m, 11H, ArH), 4.78 (s, 2H, OCH₂), 2.36 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆): δ = 170.64, 167.36, 156.14, 147.10, 136.57, 134.57, 129.92, 129.19, 128.04, 127.27, 127.00, 124.32, 118.98, 117.75, 117.23, 115.38, 107.84, 107.48, 66.55, 55.93, 12.14. Anal. Calcd for C₂₂H₁₇N₄O₃: C, 62.84; H, 4.10; N, 13.75. Found: C, 62.15; H, 3.97; N, 16.23. Conclusions: C, 61.32; H, 4.01; N, 16.17.

1-f3, 5-Methyl-2-(4-chlorophenyl)hydrazono-1H-pyrazol-1-yl)-2-(naphthalen-2-yl)oxacyetyl-2,4-dihydro-3H-pyrazol-3-one (1d): Yield: 0.34 g (82%); white solid; m.p. 152 °C–154 °C; IR (KBr): 1074, 1663, 1697, 3425 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆): δ = 11.54 (s, 1H, NH), 7.42–7.84 (m, 11H, ArH), 4.78 (s, 2H, OCH₂), 2.37 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆): δ = 170.13, 166.62, 156.52, 146.9, 141.53, 129.31, 128.68, 127.52, 126.75, 126.48, 123.19, 118.69, 118.47, 117.37, 116.75, 107.33, 107.29, 106.97, 66.64, 11.63. Anal. Calcd for C₂₇H₂₃N₄O₃: C, 72.69; H, 4.07; N, 13.31. Found: C, 62.84; H, 4.10; N, 13.43.

4-[2-(4-Bromophenyl)hydrazono]-5-methyl-2-[(4-naphthalen-2-yl)oxacyetyl]-2,4-dihydro-3H-pyrazol-3-one (1e): Yield: 0.37 g (80%); white solid; m.p. 190 °C–192 °C; IR (KBr): 1067, 1661, 1698, 3405 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆): δ = 11.57 (s, 1H, NH), 7.22–7.82 (m, 11H, ArH), 4.72 (s, 2H, OCH₂), 2.36 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆): δ = 170.74, 167.13, 156.31, 147.42, 141.56, 134.62, 132.83, 132.69, 129.84, 129.12, 128.02, 127.24, 126.94, 124.22, 119.06, 118.24, 117.64, 107.44, 66.64, 12.17. Anal. Calcd for C₂₇H₂₂BrN₂O₃: C, 56.79; H, 3.68; N, 17.17. Found: C, 56.82; H, 3.74; N, 17.25.

5-Methyl-2-[(4-methoxyphenyl)hydrazono]-2,4-dihydro-3H-pyrazol-3-one (1f): Yield: 0.32 g (75%); yellow solid; m.p. 103 °C–105 °C; IR (KBr): 1060, 1658, 1697, 3406 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆): δ = 11.68 (s, 1H, NH), 7.58–8.27 (m, 11H, ArH), 4.78 (s, 2H, OCH₂), 2.42 (s, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-d₆): δ = 170.64, 167.36, 156.13, 147.75, 143.75, 143.58, 129.92, 129.19, 128.04, 127.27, 127.00, 124.32, 118.98, 117.75, 117.23, 115.38, 107.84, 107.48, 66.55, 55.93, 12.14. Anal. Calcd for C₂₇H₂₁N₂O₃: C, 66.34; H, 4.84; N, 13.45. Found: C, 66.39; H, 4.91; N, 13.38.

Figure 4. 2D binding interactions of compound Id at the active site of DHFR.

[Diagram of molecular interactions]
1-[(3,5-Dimethyl-4-(p-toluidazinyl)-1H-pyrazol-1-yl)-2-(naphthalen-2-yl)oxy]ethan-1-one (2b): Yield: 0.29 g (75%); white solid; m.p. 176 °C–178 °C; IR (KBr): 1043, 1620, 1691 cm⁻¹. 1H NMR (500 MHz, DMSO-d₆): δ = 7.79 (d, J = 7.5 Hz, 2H, ArH), 7.75 (d, J = 8.0 Hz, 1H, ArH), 7.58 (d, J = 8.0 Hz, 1H, ArH), 7.40–7.45 (m, 2H, ArH), 7.31 (t, J = 7.5 Hz, 1H, ArH), 7.27 (d, J = 8.0 Hz, 1H, ArH), 7.19–7.22 (m, 2H, ArH), 7.15 (d, J = 2.0, 7.0 Hz, 1H, ArH), 4.75 (s, 2H, OCH₂), 2.43 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 2.36 (s, 3H, CH₃). 13C NMR (125 MHz, DMSO-d₆): δ = 170.06, 155.61, 151.03, 139.40, 139.17, 134.03, 130.00, 129.62, 129.34, 128.64, 127.47, 126.69, 126.42, 123.75, 121.30, 118.41, 116.29, 106.97, 64.55, 26.34, 20.50, 11.82. Anal. Calcd for C₂₃H₁₉ClN₄O₂: C, 69.95; H, 4.64; N, 13.38. Found: C, 69.67; H, 5.39; N, 13.46.

1-[(4-{4-Methoxyphenyl)diazenyl]-3,5-dimethyl-2-(naphthalen-2-yl)oxy]ethan-1-one (2e): Yield: 0.33 g (80%); brown solid; m.p. 165 °C–167 °C; IR (KBr): 1040, 1651, 1685 cm⁻¹. 1H NMR (500 MHz, DMSO-d₆): δ = 7.82 (d, J = 8.5 Hz, 2H, ArH), 7.77 (d, J = 8.0 Hz, 1H, ArH), 7.69 (dd, J = 2.0, 8.5 Hz, 1H, ArH), 7.53 (dt, J = 2.5, 9.0 Hz, 1H, ArH), 7.43 (t, J = 7.0 Hz, 1H, ArH), 7.33 (t, J = 7.0 Hz, 1H, ArH), 7.24 (d, J = 2.5, 1H, ArH), 7.18 (dd, J = 2.5, 11.5 Hz, 1H, ArH), 7.04 (dd, J = 2.0, 7.0 Hz, 1H, ArH), 7.00 (dd, J = 2.0, 9.0 Hz, 1H, ArH), 4.78 (s, 2H, OCH₂), 3.81 (s, 3H, OCH₃), 2.42 (s, 3H, CH₃), 2.38 (s, 3H, CH₃). 13C NMR (125 MHz, DMSO-d₆): δ = 170.65, 156.14, 147.67, 135.66, 134.57, 132.93, 129.89, 129.17, 128.02, 127.25, 129.67, 123.47, 118.98, 113.89, 115.35, 114.79, 107.45, 65.07, 55.95, 20.97, 12.39. Anal. Calcd for C₂₃H₁₉N₅O₄: C, 64.33; H, 4.46; N, 13.31. Found: C, 64.37; H, 4.50; N, 16.24.

Antimicrobial activity

The newly synthesized compounds 1a–f and 2a–f were evaluated for their antibacterial activity against S. aureus (ATCC-25923) and E. coli (ATCC-25922), and their antifungal activity against A. niger (ATCC-9029) and C. albicans (ATCC-90028) in DMF using the serial plate dilution method. A microbial broth was freshly prepared in normal saline using Mueller–Hinton agar and Sabouraud dextrose agar media for the antibacterial and antifungal activity, respectively. Compounds were serially diluted as 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, and 0.19 µM/mL concentrations. The microtiter plates were inoculated with 0.1 mL inoculums of 1 × 10⁵ cfu/mL and incubated at 37°C for 24h. The lowest concentration of the compounds that completely inhibited visible microbial growth (no turbidity) was assigned as the MIC. Ofloxacin and flucloxazole were used as the standard drugs for antibacterial and antifungal activity, respectively.

Molecular docking studies

Molecular docking studies were performed using AutoDock v. 4.2.2 to identify appropriate binding modes and the conformation of the ligand molecule. The crystal structure of S. aureus dihydrofolate reductase complexed with novel 7-aryl-2,4-di-aminooquinazolines (PDB code: 3SRQ) was retrieved from the RCSB protein data bank in PDB format. The structures of all the ligands were drawn using ChemDraw Ultra 13.0 and converted into 3D structures using HyperChem Pro 8.0 software (www.hyper.com). Autodock Tools (ADT) version 1.5.6 (www.autodock.scrips.edu) was used for the molecular docking. The active site was considered as a rigid molecule, while the ligands were treated as being flexible. Using default parameters, grid-based docking studies were carried out and docking was performed on all compounds using standard ligand 7-aryl-2,4-diaminoquinazolines. The best binding conformation was selected from the docking log (.dlg) file for
each ligand and further interaction analysis was performed using PyMol and Discovery Studio Visualizer 4.0.

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References
1. Ventola CL. Pharm Ther 2015; 40: 277–283.
2. Afshinnekoo E, Bhattacharya C, Burguete-Garcia A, et al. Lancet Microbe 2021; 4: e135–e136.
3. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global regional and national morbidity mortality and aetiologies of lower respiratory infections in 195 countries 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 2018; 18: 1191–1210.
4. Mwangi J, Hao X, Lai R, et al. Zool Res 2019; 40: 488–505.
5. Chowdhary A, Tarai B, Singh A, et al. Emerg Infect Dis 2020; 26: 2694–2696.
6. Al-Sheikh M, Medrasi HY, Sadek KU, et al. Molecules 2014; 19: 2993–3003.
7. Popiolek L. Med Chem Res 2017; 26: 287–301.
8. Saleh NM, El-Gazzar MG, Aly HM, et al. Front Chem 2020; 7: 917.
9. Kumar G, Tanwar O, Kumar J, et al. Eur J Med Chem 2018; 149: 139–147.
10. Ouyang G, Cai XJ, Chen Z, et al. J Agric Food Chem 2008; 56: 10160–10167.
11. Sharshira EM and Hamada NM. Molecules 2012; 17: 4962–4971.
12. Hassan MZ, Alsayari A, Osman H, et al. Acta Pol Pharm 2019; 76: 1029–1036.
13. Manojkumar P, Ravi TK and Gopalakrishnan S. Eur J Med Chem 2009; 44: 4690–4694.
14. Jois HSV, Kalluraya B, Babu M, et al. Ind J Heterocycl Chem 2015; 25: 7.
15. Amir M, Javed SA and Hassan MZ. Ind J Chem 2013; 52B: 1493–1499.
16. Ojha AC and Singh CP. J Ind Chem Soc 1979; 56: 1233–1236.
17. Alsayari A, Muhsinah AB, Asiri YI, et al. Lett Org Chem 2020; 17: 772–778.
18. Li X, Hilgers M, Cunningham M, et al. Structure-based design of new DHFR-based antibacterial agents: 7-aryl-24-diaminoquinazolines. Bioorg Med Chem Lett 2011; 21: 5171–5176.
19. Hawser S, Locuro S and Islam K. Biochem Pharmacol 2006; 71: 941–948.
20. Swain CG and Rogers RJ. J Am Chem Soc 1975; 97(4): 799–800.
21. Barry AL. Procedure for testing antimicrobial agents in agar media. In: Corian VL (ed.) Antibiotics in laboratory medicine. Baltimore, MD: Williams and Wilkins, 1980, pp. 1–23.
22. Mac Lowry JD, Jaqua MJ and Selepak ST. Appl Microbiol 1970; 20: 46–53.
23. Arrington-Skaggs BA, Moltely M, Warnock DW, et al. J Clin Microbiol 2000; 38: 2254–2260.
24. Verma RS, Khan ZK and Singh AP. Antifungal agents: past, present, future prospects. Lucknow, India: National Academy of Chemistry and Biology, 1998.