Synthesis, molecular modelling and antibacterial activity of 4-aryl-thiosemicarbazides

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N-Substituted phenyl/cyclohexyl-2-(pyridine-4-carbonyl) hydrazine-1-carbothioamides (2a–r) were synthesized, characterized by spectral and analytical data. The compounds were evaluated for antibacterial activity by the disc diffusion method. Most of the compounds showed activity against Gram-positive bacteria. Compound 2h with 4-Sulfapyrimidine phenyl substitution was found to be the most promising candidate, active against Gram-positive and methicillin-resistant Staphylococcus aureus (MRSA) strains with minimum inhibitory concentration (MIC) of (2–7 μg/mL). From the docking study, we predicted that compounds (2r, 2g, 2h, 2o, 2p and 2e) possess better antibacterial activity by having a good binding affinity with target protein and they could be used as potential drugs as antimicrobials. Amongst all the docked compounds, the compound 2h presented near binding affinity & interaction docking score with DNA gyrase enzymes with reference to ciprofloxacin.

Keywords: Aryl-thiosemicarbazides, antibacterial activity, molecular docking, DNAGyrase.

INTRODUCTION

Antibiotic resistance of bacterial pathogens has been increased for many years, both in Gram-positive and Gram-negative bacteria. Therefore, many research groups have been working to improve the potency and spectrum of available drugs. There is always an urgent need of finding new chemical entities to curb drug resistance. In antimicrobial research, bacterial topoisomerasers have found importance since the discovery of Escherichia coli topoisomerase II (DNA gyrase). DNA gyrase and topoisomerase IV are attractive targets in the search for new antibiotics. During bacterial DNA replication and chromosome segregation, these two homologues enzymes play an important role. DNA gyrase and topoisomerase IV are structurally related to each other. Both the enzymes are heterotetramers. GyrA comprises the DNA binding, cleavage and relegation activity, while GyrB comprises the ATPase activity. Quinolones are highly effective antimicrobial drugs. Because of the widespread use of quinolones, there is an emergence of drug-resistant bacteria due to changes in the DNA gyrase or topoisomerase IV.

Thiosemicarbazides and their derivatives display interesting biological activities, including antifungal, antibacterial, anti-trypanosomal, antimalarial, anticancer, anticonvulsant, anti-mycobacterial, topoisomerase inhibition and anti-HIV. Two compounds, 4-benzoyl-1-(indol-2-yl)-carbonylthiosemicarbazide and 4-benzoyl-1-(4-methyl-imidazol-5-yl)-carbonylthiosemicarbazide were reported as a new class of topoisomerase IV inhibitors possessing good activity against Gram-positive opportunistic bacteria. 4-Arylthiosemicarbazides having indole moiety have been reported to possess good Gram-positive antibacterial activity as topoisomerase IV inhibitors. For the further optimization of novel antibacterial agents, these templates can be used as starting point.

To discover potent antibacterial compounds, a new set of thiosemicarbazides (2a–r) were synthesized, characterized and in vitro antibacterial activity was studied. Pyridyl ring, an important scaffold present in many bioactive molecules, has played an important role in the development of different medicinal agents. The thiosemicarbazide pharmacophore was preserved and indole moiety was replaced by pyridine moiety of the isoniazid (Fig. 1).

EXPERIMENTAL

Chemistry

All the solvents were procured from Merck. The purity of the compounds was determined by thin-layer chromatography (TLC) performed on Silica gel G coated plates (Merck). The FT IR spectra were determined in KBr pellets on a (Spectrum BX) Perkin Elmer FT-IR spectrophotometer. Gallenkamp melting point apparatus was used for the determination of the melting point of compounds and thermometer was uncorrected. NMR
Spectra were scanned in DMSO-$d_6$ on a Bruker NMR spectrophotometer operating at 500 MHz for $^1$H and 125.76 MHz for $^{13}$C at the Research Center, College of Pharmacy, King Saud University, Saudi Arabia. High-resolution mass of the compounds was determined by HRMS.

General method for the synthesis of 4-arylthiosemicarbazides

To a solution of appropriate aniline (0.01 mol) in absolute ethanol (25 mL), was added KOH (0.01 mol) and carbon disulfide CS$_2$ (0.75 mL). The mixture was stirred at 0–5 °C for one hour to form the potassium salt of substituted phenyl dithiocarbamate. To the stirred mixture, was added isoniazid (INH) (0.01 mol) and stirring was continued at 80 °C for one hour. Crashed ice was added to obtain 4-arylthiosemicarbazides.

$N$-phenyl-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2a)

Yield % 70; mp: 200–202 °C; FT IR spectrum ν cm$^{-1}$: 3487 (NH str.), 1664 (C=O str.), 1255 (C=S str.); $^1$H NMR spectrum, δ, ppm: 7.3–9.1 (m, 9H, Ar-H), 10.1 (s, 2H, NH, D$_2$O exchang.), 11.2 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.7, 181.5, 164.8, 150.5, 139.4, 128.3, 126.0, 125.5; HRMS: 272.1966 [M]$^+$, 254.0518 [M–18]$^+$. $^{13}$C NMR spectrum, δ, ppm: 1.9 (s, 6H, -CH$_3$), 7.0–7.4 (m, 7H, Ar-H), 8.5 (s, 2H, NH, D$_2$O exchang.), 14.5 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.0, 168.2, 150.5, 147.4, 136.2, 134.8, 132.5, 130.2, 128.9, 127.4, 126.4, 120.0, 30.6, 18.2, 17.8; HRMS: 281.9981 [M–18]$^+$. $N$-(3-ethylphenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2f)

Yield % 75; mp: 220–222 °C; FT IR spectrum ν cm$^{-1}$: 3414 (NH str.), 1607 (C=O str.), 1288 (C=S str.); $^1$H NMR spectrum, δ, ppm: 1.1 (t, 3H, -CH$_3$), 2.0 (q, 2H, -CH$_2$), 7.2–7.4 (m, 8H, Ar-H), 8.5 (s, 2H, NH, D$_2$O exchang.), 14.3 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.4, 169.1, 150.0, 148.3, 145.3, 133.1, 129.3, 127.7, 125.7, 121.8, 30.6, 27.7, 15.2; HRMS: 281.9821 [M–18]$^+$. $N$-(4-nitrophenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2g)

Yield % 70; mp: 238–240 °C; FT IR spectrum ν cm$^{-1}$: 3414 (NH str.), 1607 (C=O str.), 1288 (C=S str.); $^1$H NMR spectrum, δ, ppm: 6.8–7.4 (m, 8H, Ar-H), 8.9 (s, 2H, NH, D$_2$O exchang.), 14.1 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.4, 145.1, 136.6, 131.2, 124.3, 120.1, 114.4; HRMS: 299.3021 [M–18]$^+$. $2$-(pyridine-4-carbonyl)-N$'$(4-[phenyl methyl]sulfamoyl)phenyl)hydrazine-1-carbothioamide (2h)

Yield % 65; mp: 256–258 °C; FT IR spectrum ν cm$^{-1}$: 3424 (NH str.), 1653 (C=O str.), 1262 (C=S str.); $^1$H NMR spectrum, δ, ppm: 5.9–7.6 (m, 11H, Ar-H), 8.4 (s, 2H, NH, D$_2$O exchang.), 11.2 (s, 2H, CONH, SO$_2$NH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 158.2, 157.2, 153.0, 129.7, 124.8, 115.4, 121.1; HRMS: 429.0310 [M]$^+$.

$N$-(4-chlorophenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2c)

Yield % 70; mp: 210–212 °C; FT IR spectrum ν cm$^{-1}$: 3414 (NH str.), 1663 (C=O str.), 1395 (C=S str.); $^1$H NMR spectrum, δ, ppm: 7.3–8.7 (m, 8H, Ar-H), 9.9 (s, 1H, NH, D$_2$O exchang.), 10.8 (s, 1H, NH, D$_2$O exchang.), 11.1 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.0, 150.1, 128.5, 127.9, 121.6; HRMS: 279.0310 [M]$^+$.*

$N$-(4-methoxyphenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2d)

Yield % 70; mp: 217–218 °C; FT IR spectrum ν cm$^{-1}$: 3413 (NH str.), 1610 (C=O str.), 1324 (C=S str.); $^1$H NMR spectrum, δ, ppm: 2.0 (s, 3H, -OCH$_3$), 6.9–7.3 (m, 8H, Ar-H), 9.4 (s, 2H, NH, D$_2$O exchang.), 14.1 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 180.1, 156.4, 132.2, 126.0, 113.6, 55.2; HRMS: 288.3214 [M–14]$^+$. $N$-(3-methylphenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2j)

Yield % 75; mp: 278–280 °C; FT IR spectrum ν cm$^{-1}$: 3413 (NH str.), 1580 (C=O str.), 1241 (C=S str.); $^1$H NMR spectrum, δ, ppm: 2.0 (s, 3H, -CH$_3$), 7.1–7.4 (m, 8H, Ar-H), 9.4 (s, 2H, NH, D$_2$O exchang.), 14.1 (s, 1H, CONH, D$_2$O exchang.); $^{13}$C NMR spectrum, δC, ppm: 206.8, 179.0, 150.1, 136.0, 133.2, 131.0, 129.9, 128.5, 123.8, 121.8, 20.7; HRMS: 267.8779 [M–18]$^+$. $N$-(2-methylphenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2k)

Yield % 70; mp: 268–270 °C; FT IR spectrum ν cm$^{-1}$: 3413 (NH str.), 1597 (C=O str.), 1250 (C=S str.); $^1$H NMR spectrum, δ, ppm: 2.0 (s, 3H, -CH$_3$), 7.2–7.4 (m,
δ, ppm: 6.4–7.8 (m, 8H, Ar-H), 8.8 (s, 2H, NH, D₂O exchang.), 14.1 (s, 1H, CONH, D₂O exchang.); ¹³C NMR spectrum, δC, ppm: 150.8, 150.1, 137.0, 122.1, 122.0, 119.5, 116.6; HRMS: 379.9763 [M-18]⁺.

N-(2-nitrophenyl)-2-(pyridine-4-carbonyl)hydrazine-1-carbothioamide (2r)
Yield % 70; mp: 248–250 °C; FT IR spectrum v cm⁻¹: 3477 (NH str.), 1629 (C=O str.), 1242 (C=S str.); ¹H NMR spectrum, δ, ppm: 6.5–7.4 (m, 8H, Ar-H), 7.9 (s, 2H, NH, D₂O exchang.), 14.1 (s, 1H, CONH, D₂O exchang.); ¹³C NMR spectrum, δC, ppm: 206.4, 146.1, 135.6, 130.2, 125.3, 119.1, 115.4; HRMS: 299.7334 [M-18]⁺.

Antibacterial assay
The antibacterial activities for the compounds were tested against representative pathogenic Gram-positive strains by using the paper disc diffusion method². In this method, nutrient agar for bacteria was prepared and sterilized by autoclaving at 120 °C and 1.5 atm pressure for 20 minutes². The agar plates were poured, left to cool down and after solidification they were inoculated with the bacterial strains by streaking. The synthesized compounds were dissolved in methanol at a final concentration of 10 mg/mL and 5 μL of each compound was loaded on a sterile filter paper disc (5 mm diameter; 50 μg per disc). The filter paper disc was then transferred aseptically into the inoculated agar plates along with commercially available ciprofloxacin disc as positive controls and methanol as the negative control for comparison. After incubation, diameters of the inhibition zones around the paper discs were measured in mm as an indication of the antibacterial activities of the compounds.

Molecular docking
Docking for the synthesized compounds (2a–r) and references compound (ciprofloxacin) CIP was conducted via their 3D structures built and energy minimized using MMFF94x of Molecular Operating Environment energy minimization module (MOE, Version 2015, Chemical Computing Group Inc., Montreal, Quebec, Canada) that represents 3D crystal structure of DNA gyrase was selected from Protein Data Bank database (http://www.rcsb.org). The missing residues and side chains of the crystal structure were repaired, the 3D protonation of each complex was studied using the MOE’s Pose minimization module (MOE, Version 2015, Chemical Computing Group). (PDB ID: 4ZVI) using triangle matcher as placement method and Lone-pair donors as a scoring function. The obtained poses were subjected to force field refinement using the GBVI/WSA dG rescoring function. Thirty of the most stable
docking models for each ligand were retained with the best-scored conformation.

RESULTS AND DISCUSSION

The synthesis of isoniazid derivatives was carried out in one pot as shown in (Scheme 1). Substituted anilines were reacted with carbon disulfide in presence of alcoholic KOH in equimolar quantities. Potassium salt of substituted phenyl dithiocarbamate was obtained by stirring at 0–5 °C for one hour. Equimolar quantity of isoniazid was added with stirring and refluxed for one hour at 80 °C. 4-Aryl-thiosemicarbazides were obtained by adding crushed ice to the mixture in good yield (60–80%). The purity of the compounds was confirmed by the elemental analysis and thin-layer chromatography (TLC). The compounds were identified by spectral data e.g., FTIR, $^1$H NMR, $^{13}$C NMR and HR MS. Spectral and analytical data of the synthesized compound were consistent with the composition of the compounds. The FTIR spectra of 4-aryl-thiosemicarbazides (2a–r) showed the appearance of NH, CO and C=S absorption bands in 3410–3487 cm$^{-1}$, 1580–1675 cm$^{-1}$ and 1225–1395 cm$^{-1}$ regions respectively. The $^1$H NMR (DMSO-d$_6$, 500 MHz) of compounds of this series revealed D$_2$O exchangeable singlet at 7.9–10.1 ppm and 10.5–14.5 ppm corresponding to NH proton and CONH proton respectively.

![Scheme 1. Synthetic route of compounds (2a–r)](image)

**Anti-bacterial activity**

All the compounds of this series were screened for anti-bacterial activity (Table 1). The synthesized compounds were evaluated against the strains of Gram-positive and methicillin-resistant *Staphylococcus aureus*. The microorganisms which were used in this study were: *Bacillus subtilis* ATCC 6633; *Bacillus pumilus* ATCC 14884; *Bacillus cereus* ATCC 10876; *Staphylococcus aureus* ATCC 6538 and methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA).

Initially, antibacterial activity of 4-aryl-thiosemicarbazides was screened based on growth inhibition zones by the disc diffusion method. Minimum inhibitory concentrations (MIC) were determined using the agar dilution method. Ciprofloxacin (25 μg) was used as standard drug for comparison of zone of inhibition of synthesized compounds. All compounds of this series were showing activity against Gram-positive strains. Compound 2h with sulfapyrimidine substitution was found to be effective against Gram-positive and MRSA strains. Compound 2r with 2-nitro substitution was found to be most active against MRSA ATCC 33591. Compound 2h was selected for minimal inhibitory concentration (MIC) calculation against all strains. Compound 2h was found to be the most promising candidate active against Gram-positive and MRSA strains with minimum inhibitory concentration MIC (2–7 μg/mL).

**Docking studies**

Considering the well obtained in vitro results, it was thought worthy to perform molecular docking studies, hence screening the compounds, inculcating both in silico and in vitro results. Considering DNA gyrase as the target receptor, comparative and automated docking studies with newly synthesized compounds were performed to determine the best in silico conformation. The native crystal structure of *E. coli* DNA gyrase B was obtained from Protein Data Bank (https://www.rcsb.org/structure/4ZVI) with the PDB ID 4ZVI. The active pocket consisted of amino acid residues as Asn 46; Val 43, 44, 71,120, 167; Ala 47; Ghu 42, 50; Gln 72 Asp 73; Arg 76, 136; Gly 75, 77; Ile 78, 94; Pro 79; Met 95, 166; and Thr 165. The synthesized ligand molecules minimized the energy of 3D structures and were further used for in silico protein–ligand docking. All the synthesized compounds (2a–r) were docked; (Fig. 2) shows the docked images of selected ligands.

### Table 1. The antimicrobial activities of the synthesized compounds expressed as inhibition zones of growth in mm against the used test organisms

| Compound | Test Organism* |
|----------|----------------|
|          | B.s. | B.p. | B.c. | S.a. | MRSA |
| 2a       | –    | –    | –    | –    | –    |
| 2b       | –    | –    | –    | –    | –    |
| 2c       | –    | –    | –    | –    | –    |
| 2d       | 7    | 9    | 7    | –    | –    |
| 2e       | –    | –    | –    | –    | –    |
| 2f       | –    | –    | –    | –    | –    |
| 2g       | –    | –    | –    | –    | –    |
| 2h       | 23   | 25   | 20   | 18   | 15   |
| 2i       | –    | –    | –    | –    | –    |
| 2j       | –    | –    | –    | –    | –    |
| 2k       | 14   | 9    | 13   | 9    | –    |
| 2l       | –    | –    | –    | –    | –    |
| 2m       | 13   | 12   | 12   | 17   | 11   |
| 2n       | 14   | 14   | 13   | 11   | –    |
| 2o       | –    | –    | –    | –    | –    |
| 2p       | 12   | 13   | 13   | 11   | 11   |
| 2q       | –    | –    | –    | –    | –    |
| 2r       | 15   | 16   | 13   | 11   | 22   |

Ciprofloxacin 25 μg 35 35 32 34 28

[Table 1. The antimicrobial activities of the synthesized compounds expressed as inhibition zones of growth in mm against the used test organisms]
| Comp.       | 3D structure |
|------------|--------------|
| Ciprofloxacin | ![Image](#) |
| 2r         | ![Image](#) |
| 2g         | ![Image](#) |
| 2h         | ![Image](#) |

**Figure 2.** Docking of synthesized compounds 2r, 2g, 2h and reference standard ciprofloxacin into the active site of DNA gyrase. The amino acids involved in hydrogen, and hydrophobic interactions
Table 2. Molecular docking studies of synthesized compounds (2a–r)

| Comp. | 2Dstructure |
|-------|-------------|
| Ciprofloxacin | ![Ciprofloxacin] |
| 2r | ![Image] |
| 2g | ![Image] |
| 2h | ![Image] |

including the considered standard drug i.e. ciprofloxacin. (Table 2) shows the binding energy of all compounds including the standard. In silico studies revealed that all the synthesized molecules showed good binding energy toward the target protein from derivatives 2e, 2g, 2h, 2o, 2p and 2r which showed hydrophobic interactions with the active site of gyrase. Compound 2r revealed...
that compounds (2r, 2g, 2h, 2o, 2p, 95, 166; and Thr 165 amino acid residues of the target 46; Val 43, 44, 71, 120, 167; Ala 47; Glu 42, 50; Gln 72 the hydrophobic pocket of DNA gyrase de
hydrogen bond through a phenyl-H of the 2g ring. Apart from hydrogen bonding, hydrophobic and van der Waals interactions were also detected between lead molecules 2h, 2o, 2p and 2e with DNA gyrase. The synthesized compounds were projected into the hydrophobic pocket of DNA gyrase defined by Asn 46; Val 43, 44, 71,120, 167; Ala 47; Glu 42, 50; Gln 72 Asp 73; Arg 76, 136; Gly 75, 77; Ile 78, 94, Pro 79; Met 95, 166; and Thr 165 amino acid residues of the target protein (Fig. 3). From the docking study, we predicted that compounds (2r, 2g, 2h, 2o, 2p and 2e) possess better antibacterial activity by having good binding affinity with target protein and it could be used as potential drugs as antimicrobial. Amongst all the docked compounds, the compound (2r) shows near binding affinity & interaction docking score of –6.61570501 with DNA gyrase enzymes with reference to ciprofloxacin.

CONCLUSION

In conclusion, in the present study, eighteen 4-aryl-thiosemicarbazides (2a–r) were synthesized by one-pot method in good yield from substituted anilines and were fully characterized by spectral data. The compounds were evaluated for their antibacterial activity against Gram-positive and MRSA strains. Amongst all the docked compounds, the compound 2h showed near binding affinity & interaction docking score of –6.28270531 with DNA gyrase enzymes with reference to ciprofloxacin. The present study revealed new lead compound 2h with significant activity and the same results were reflected in docking studies also. Compound 2h was found to be the most promising candidate active against Gram-positive and MRSA strains. Finally, the compound 2h represents a good lead for the development of pyridine-based aryl-thiosemicarbazides as antibacterial agent.

Data Availability

Samples of the compounds (2a–r) in pure form are available from the authors.

Conflict of interest

The authors declare that there is no conflict of interest.

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