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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anti(myco)bacterial activity of novel 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives and a functionalized hexahydro-1*H*-pyrrolo[1,2-*c*]imidazole

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Abstract In this paper, five novel 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives were synthesized by stereoselective cycloaddition of *N*-diphenylmethylene-protected glycine methyl ester and methyl acrylate, and subsequent coupling with aroylisothiocyanates. The *cis*-stereochemistry of one of the heterocyclic thiourea derivatives was characterized by single crystal X-ray diffraction studies. The compounds showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Aeromonas hydrophila*, *Escherichia. coli* and *Acinetobacter baumannii* with minimum inhibitory concentration values in the range of 62.5–1000 µg/mL against these bacterial strains. Antimycobacterial activity of the compounds was investigated against the *M. tuberculosis* H37Rv strain and all compounds exhibited antimycobacterial activity with a minimum inhibitory concentration value of 80 µg/mL. Additionally, methyl 5,5-diphenylhexahydro-1-oxo-3-thioxo-1*H*-pyrrolo[1,2-*c*]imidazole-6-carboxylate was synthesized by cyclization reaction of the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives in the presence of hydrazine monohydrate and exhibited antibacterial activity with a minimum inhibitory concentration value of 62.5 µg/mL against the same bacterial strains and exhibited antimycobacterial activity with a minimum inhibitory concentration value of 80 µg/mL against the *M. tuberculosis* H37Rv strain.

Keywords 5,5-Diphenylpyrrolidine · Thiohydantoin · Antibacterial activity · Antimycobacterial activity · *M. tuberculosis* H37Rv

Introduction

Nitrogen and sulfur are among the most important elements, which are present in the structure of biologically important compounds. Many drug molecules, such as amoxicillin, quetiapine, olanzapine, pioglitazone, and duloxetine contain at least one nitrogen and sulfur atom in their structures (Ilardi et al. 2014). Thiohydantoins, containing nitrogen and sulfur atoms, represent an important class of compounds within pharmaceutical chemistry and are present in a wide range of pharmacologically active compounds exhibiting antimicrobial (Khurana et al. 2014), antiviral (Khodair et al. 2001), anti-inflammatory (Blanc et al. 1992), antitumor (Al-Obaid et al. 1996), and hypoglycemic (Yildiz and Bozdag-Dundar 2010) activities. Thiohydantoins have also been used as intermediates for the synthesis of biologically active compounds (Abdelaziz et al. 2015; Metwally and Abdel-Latif 2012).

N-Substituted-*N*′-aroyl(acyl)thiourea derivatives are gaining significance in pharmaceutical, coordination and analytical chemistry (Saeed et al. 2014b; Koch 2001). *N*-Substituted-*N*′aroyl(acyl)thiourea derivatives are known to show a wide range of biological properties, such as

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15-lipoxygenase inhibitory activity (Mahdavi et al. 2014), carbonic anhydrase inhibitor (Saeed et al. 2014a), antimicrobial (Saeed et al. 2013; Döndaş et al. 2006), antitumor (Saeed et al. 2010), and analgesic activity (Shoaib et al. 2014). In addition, some *N*-benzoyl-*N*′-ferrocenylthiourea derivatives exhibited cytokinin and auxin activities (Zhang et al. 2015). Furthermore, *N*-substituted-*N*′-aroyl(acyl) thioureas are also useful compounds for the synthesis of biologically important heterocyclic scaffolds, such as thiohydantoins (Nural et al. 2011) and thiazoles (Saeed et al. 2008). Ni(II) (Yang et al. 2012; Nural et al. 2009; Del Campo et al. 2004), Pt(II) (Cîrcu et al. 2009; Del Campo et al. 2004) and Co(III) (Tan et al. 2014; Del Campo et al. 2004) chelate complexes of *N*-Substituted-*N*′-aroyl(acyl) thioureas have been synthesized. In addition, these types of ligands are useful for analytical applications, such as potentiometric sensors for heavy metals (Otazo-Sanchez et al. 2002) or chemosensors for anions (Li et al. 2012; Hu et al. 2009). Moreover, some *N*-aroyl(acyl)thioureas were used for extraction, separation, and determination of heavy metals (Alkherraz et al. 2014; Luckay et al. 2009).

Heterocycles containing one or more nitrogen atoms are a priority area of drug research (Hernandez-Toribio et al. 2012). Pyrrolidines incorporate one of the most important scaffolds, possessing various pharmacological activities, such as antimicrobial (Schwartz et al. 1998), antiviral (Martín-Rodríguez et al. 2011; Nájera and Sansano 2009) andantitumor (Fujimoto et al. 1987) activity. Furthermore, the pyrrolidine ring is present in many synthetic or natural bioactive molecules, such as procyclidine (Ettinger et al. 2003),bepridil (Fujiki et al. 2003), elacomine (Pellegrini et al. 1996), anisomycin (Stocker et al. 2010), and hygrine (Rubioa et al. 2013). Although pyrrolidine derivatives have been intensively studied in the literature (Verma et al. 2016; Chen et al. 2013; Pandey et al. 2006; Dondas et al. 1996 2004 Döndaş et al. 2003), there are only a limited number of studies about their 5,5-diphenylpyrrolidine derivatives (Hut'ka et al. 2013; Kim et al. 2011; Tsubogo et al. 2008; Xue et al. 2008; Wang et al. 2008, Khlebnikov et al. 1997, 2001). Noteworthy, the *gem*-diphenyl group is present in various physiologically active nitrogen-containing compounds, such as methadone (Hassan et al. 2009), diphenhydramine (Ahmadi et al. 2012), clotrimazole (Prasanna et al. 2014), loperamide (Geyer et al. 2015), cinnarizine (Sassene et al. 2015), dextromoramide (Bye 1976), benzatropine (Othman et al. 2008), darifenacin (Pramanik et al. 2012), doxapram (Krasikovs 2015), modafinil (Okunola-Bakare et al. 2014), and phenytoin (Soman et al. 2014).

In this study, we designed and synthesized five novel 5,5-diphenylpyrrolidine *N*-aroylthioureas as precursors of a compound with a thiohydantoin moiety fused to a pyrrolidine and investigated their antibacterial and antimycobacterial activities.

Material and methods

Chemicals and general technical data

All chemicals used were high-grade commercial products purchased from Merck or Aldrich and all solvents provided from commercial suppliers as having reagent grade quality, and used without further purification.

IR spectra were recorded in the range of 4000–600 cm^{-1} by a Varian Scimitar Series 1000 FT-IR spectrophotometer, using horizontal Attenuated Total Reflectance. Nuclear magnetic resonance spectra and decoupling experiments were determined at 400 MHz on a Bruker Ultrashield Plus Biospin GmbH. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Spectra were determined in deuterochloroform. The following abbreviations are used; s = singlet, d = doublet, m = multiplet and brs = broad singlet. Flash column chromatography was performed using silica gel 60 (230–400 mesh). Kieselgel columns were packed with silica gel GF254. Melting points were determined on Stuart SMP3 hot stage apparatus and are uncorrected. Mass spectra were recorded by an Agilent 6460 Triple Quad LC/MS/MS mass spectrometer. High resolution mass spectra were recorded by an LC-MS TOF electrospray ionization technique. The X-ray crystal structure data were collected by a Rigaku R-Axis Rapid-S model X-ray diffractometer.

*Synthesis of cis-dimethyl 5,5-diphenylpyrrolidine-2,4-dicarboxylate (**3**)*

The compound **3** was prepared by modification of literature methods (Dondas et al. 1996, 2004, Döndaş et al. 2003). To a stirred solution of methyl 2-(diphenylmethyleneamino) acetate **1** prepared according to the literature (De Brabandere et al. 2014) (0.25 g, 1 mmol) in dry toluene (10 mL) was added a solution of Et_3N (0.17 g, 1.7 mmol) in dry toluene (10 mL) and Ag_2O (0.06 g, 0.25 mmol). After 5 min, methyl acrylate **2** (0.17 g, 2 mmol) in dry toluene (20 mL) was added. The mixture was stirred at room temperature for 18 h. After completion of the reaction, the mixture was quenched sequentially with saturated aqueous sodium chloride and aqueous ammonium chloride, and extracted with ethyl acetate. The crude mixture was purified by column chromatography (Et_2O:hexane/1:8) to give **3** (0.3 g, 89%) as colorless crystals. m.p.: 127–129 °C. IR (cm^{-1}): υ_{max} 3296, 3060, 2950, 1743, 1724. ^1H NMR (400 MHz, $CDCl_3$): δ 7.67–7.11 (m, 10H, Ar-H), 4.00 (dd, 1H, $J = 6.7$ Hz, 3.2 Hz, 2-H), 3.84 (dd, 1H, $J = 9.0$ Hz, 6.6 Hz, 4-H), 3.78 (s, 3H, OCH_3), 3.34 (s, 3H, OCH_3), 2.30–2.28 (m, 2H, 3-H, 3-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5 (C=O), 173.5 (C=O), 144.6, 143.8, 128.6 (2 × C), 128.2 (2 × C), 127.0, 126.9, 126.1 (2 × C), 126.0 (2 × C), 76.2, 58.4, 52.3

(2 × C), 51.5, 34.3. MS (ESI, M + H^+): *m/z* 340.2 (M + H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{20}H_{21}NO_4$ $[MH]^+$ 340.1549; found 340.1548.

*General procedure for the synthesis of cis-5,5-diphenylpyrrolidine N-aroylthiourea derivatives (**5a–e**)*

The novel 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **5a–e** were synthesized by modification of the literature methods (Nural et al. 2011, 2009; Döndaş et al. 2006; Arslan and Külcü 2003) from reaction of **3** and corresponding aroylisothiocyanates **4** in acetonitrile for 36 h. The solvent was evaporated under reduced pressure and the crude mixture was purified by column chromatography (Et_2O:hexane/1:6). The requisite aroylisothiocyanates **4** were synthesized by modification of the literature method (Arslan and Külcü 2003) via reaction of potassium thiocyanate and corresponding acyl chloride in acetone at reflux temperature for 2 h. Upon reaction completion, the solvent was removed and used without any further purification.

Cis-dimethyl 1-((benzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**5a**) Yellow crystals. Yield, 0.8 g, 95%. m.p.: 178–180 °C. IR (cm^{-1}): υ_{max} 3366, 3057, 2982, 1746, 1735, 1710, 1349, 1225, 1210. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (brs, 1H, NH), 7.93–6.86 (m, 15H, Ar-H), 5.39 (dd, 1H, J = 8.3 Hz, 8.3 Hz, 2-H), 4.04 (dd, 1H, J = 8.4 Hz, 8.4 Hz, 4-H), 3.88 (s, 3H, OCH_3), 3.38 (s, 3H, OCH_3), 2.44 (dd, 2H, J = 8.0 Hz, 8.0 Hz, 3-H, 3-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9 (C=S), 170.6 (C=O), 169.3 (C=O), 163.6 (C=O), 140.1, 135.7, 135.1, 133.4, 132.2, 130.5, 129.4 (2 × C), 129.0 (3 × C), 128.9 (2 × C), 128.7, 128.3, 127.8, 127.0 (2 × C), 79.8, 64.9, 61.1, 52.7, 52.1, 28.8. MS (ESI, M − H^+): *m/z* 501.4 (M − H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{26}N_2O_5S$ $[MH]^+$ 503.1641; found 503.1657.

Cis-dimethyl 1-((4-tert-butylbenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**5b**) Amorphous yellow solid. Yield, 0.5 g, 79%. m.p.: 87–89 °C. IR (cm^{-1}): υ_{max} 3385, 3062, 2952, 1736, 1685, 1346, 1267, 1195. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (brs, 1H, NH), 7.95–6.78 (m, 14H, Ar-H), 5.41(dd, 1H, J = 8.1 Hz, 8.1 Hz, 2-H), 4.03 (dd, 1H, J = 7.8 Hz, 7.8 Hz, 4-H), 3.88 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 2.43 (dd, 2H, J = 8.4 Hz, 8.1 Hz, 3-H, 3-H′), 1.25 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0 (C=S), 170.7 (C=O), 169.4 (C=O), 163.4 (C=O), 156.0, 140.0, 135.9, 130.4, 129.4 (2 × C), 129.0 (2 × C), 128.9 (2 × C), 128.6, 127.8 (2 × C), 126.9 (2 × C), 126.0, 125.2 (2 × C), 79.8, 65.0, 61.2, 52.6, 52.0, 34.9, 31.0 (3 × C), 28.7. MS (ESI, M − H^+): *m/z* 557.2 (M − H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{32}H_{34}N_2O_5S$ $[MH]^+$ 559.2267; found 559.2280.

Cis-dimethyl 1-((4-methoxybenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**5c**) Amorphous yellow solid. Yield, 0.3 g, 71%. m.p.: 85–87 °C. IR (cm^{-1}): υ_{max} 3348, 3057, 2955, 1736, 1688, 1354, 1253, 1207. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (s, 1H, NH), 7.94–6.60 (m, 14H, Ar-H), 5.40 (dd, 1H, J = 8.2 Hz, 8.2 Hz, 2-H), 4.04 (dd, 1H, J = 7.9 Hz, 7.9 Hz, 4-H), 3.88 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 2.43 (dd, 2H, J = 8.1 Hz, 8.1 Hz, 3-H, 3-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2 (C=S), 170.7 (C=O), 169.4 (C=O), 163.1 (C=O), 162.8, 140.09, 135.9, 129.4 (2 × C), 129.1 (2 × C), 129.0 (2 × C), 128.9 (2 × C), 128.6 (2 × C), 127.8 (2 × C), 125.7, 113.5 (2 × C), 79.7, 64.9, 61.1, 55.4, 52.6, 52.0, 28.7. MS (ESI, M − H^+): *m/z* 531.2 (M − H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{29}H_{28}N_2O_6S$ $[MH]^+$ 533.1746; found 533.1745.

Cis-dimethyl 1-((4-chlorobenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**5d**) Amorphous yellow solid. Yield, 0.5 g, 72%. m.p.: 90–92 °C. IR (cm^{-1}): υ_{max} 3340, 3060, 2950, 1736, 1684, 1344, 1267, 1206. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (brs, 1H, NH), 7.93–6.77 (m, 14H, Ar-H), 5.38 (dd, 1H, J = 8.2 Hz, 8.2 Hz, 2-H), 4.04 (dd, 1H, J = 8.0 Hz, 8.0 Hz, 4-H), 3.88 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 2.44 (dd, 2H, J = 8.0 Hz, 8.0 Hz, 3-H, 3-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.8 (C=S), 170.5 (C=O), 169.3 (C=O), 163.0 (C=O), 140.1, 138.6, 135.7, 131.8, 129.5 (2 × C), 129.4, 129.0 (2 × C), 128.8, 128.7, 128.6 (2 × C), 128.5 (2 × C), 128.2, 127.8 (2 × C), 79.8, 65.0, 61.1, 52.6, 52.1, 28.7. MS (ESI, M − H^+): *m/z* 535.5 (M − H^+, 100), 537.5 (M − H^+, 35). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{25}ClN_2O_5S$ $[MH]^+$ 537.1251; found 537.1257.

Cis-dimethyl 1-((4-phenylbenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**5e**) Yellow crystals. Yield, 0.3 g, 80%. m.p.: 108–110 °C. IR (cm^{-1}): υ_{max} 3304, 3035, 2986, 1730, 1696, 1368, 1252, 1211. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (brs, 1H, NH), 7.96–6.92 (m, 19H, Ar-H), 5.41 (dd, 1H, J = 8.2 Hz, 8.2 Hz, 2-H), 4.04 (dd, 1H, J = 8.0 Hz, 8.0 Hz, 4-H), 3.89 (s, 3H, OCH_3), 3.39 (s, 3H, OCH_3), 2.45 (dd, 2H, J = 8.1 Hz, 8.1 Hz, 3-H, 3-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0 (C=S), 170.6 (C=O), 169.4 (C=O), 163.4 (C=O), 145.0, 140.1, 139.6, 135.8, 132.0, 129.4 (2 × C), 129.1, 129.0, 128.9 (4 × C), 128.7, 128.2, 127.8, 127.6 (2 × C), 127.2 (4 × C), 126.9 (2 × C), 79.8, 65.0, 61.1, 52.6, 52.1, 28.7. MS (ESI, M − H^+): *m/z* 577.5 (M − H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{34}H_{30}N_2O_5S$ $[MH]^+$ 579.1954; found 579.1975.

*The synthesis of cis-methyl 5,5-diphenylhexahydro-1-oxo-3-thioxo-1H-pyrrolo[1,2-c]imidazole-6-carboxylate (**6**)*

To a stirred solution of 5a (0.502 g, 1 mmol) in dry MeOH (20 mL) was added a solution of hydrazine monohydrate (0.055 g, 1.1 mmol) in dry MeOH (10 mL) and the mixture was stirred at reflux temperature for 12 h. After completion of the reaction, the solvent was evaporated under reduced pressure, quenched with saturated aqueous sodium chloride and extracted with ethyl acetate. The crude mixture was purified by column chromatography (ethyl acetate:hexane/1:4) to afford compound 6. Amorphous white solid. Yield, 0.28 g, 84%. m.p.: 172–174 °C. IR (cm^{-1}): υ_{max} 3202, 3069, 2921, 1746, 1716,1463, 1345, 1271, 1225.^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (bs, 1H, NH), 8.01–7.98 (m, 2H, Ar-H), 7.51–6.86 (m, 8H, Ar-H), 4.60 (dd, 1H, $J = 12.4$ Hz, 6.1 Hz, 7a-H), 4.24 (dd, 1H, $J = 12.3$ Hz, 7.3 Hz, 6-H), 3.25 (s, 3H, OCH_3), 2.90 (dt, 1H, $J = 12.6$ Hz, 12.3 Hz, 7-H),2.60 (ddd, 1H, $J = 13.1$ Hz, 7.0 Hz, 6.0 Hz, 7-H′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.5 (C=S), 171.2 (C=O), 169.7 (C=O), 142.6, 137.3, 128.7 (3 × C), 128.5 (3 × C), 128.2, 128.1, 127.6 (2 × C), 76.1, 66.1, 62.6, 52.2, 29.4. MS (ESI, M+H$^+$): *m/z* 367.1 (M+H$^+$, 100). HRMS (ESI-TOF-MS): calcd. for $C_{20}H_{18}N_2O_3S$ $[MH]^+$ 367.1116; found 367.1130.

Crystallography

For the crystal structure determination, single-crystal of **5a**, obtained from Et_2O:hexane 1:6 as crystallization solvent was used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area IP detector). Graphite-monochromated Mo-K_α radiation ($\lambda = 0.71073$ Å) and oscillation scans technique with $\Delta w = 5°$ for one image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2 > 2\sigma(F^2)$. Integration of the intensities, correction for Lorentz and polarization effects and cell refinement was performed using CrystalClear (2005) software. The structures were solved by direct methods using SHELXS-97and refined by a full-matrix least-squares procedure using the program SHELXL-97 (Sheldrick 1997). All non-hydrogen atoms were refined anisotropically and H atoms were positioned geometrically and refined using a riding model. The final difference Fourier maps showed no peaks of chemical significance. Crystal data for 5a: $C_{28}H_{26}N_2O_5S$, crystal system, space group: triclinic, *P*-1; (no:2); unit cell dimensions: $a = 14.190(2)$, $b = 13.043(2)$, $c = 14.261(2)$ Å, $\alpha = 90$, $\beta = 103.451(4)$, $\gamma = 90°$; volume: 2567.0(6) Å^3; $Z = 2$; calculated density: 1.30 g/cm^3; absorption coefficient: 0.167 mm^{-1}; $F(000)$: 1056; θ-range for data collection 3.1–25.9°; refinement method: full matrix least-square on F^2; data/parameters: 5040/328; goodness-of-fit on F^2: 0.95; final *R*-indices $[I > 2\sigma(I)]$: $R_1 = 0.068$, w$R_2 = 0.088$; largest diff. peak and hole: 0.179 and −0.203 e/Å^3.

Crystallographic data that were deposited in the Cambridge Structural Database under CCDC-1476027 registration number contain the supplementary crystallographic data for this Letter. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre (CCDC) via www.ccdc.cam.ac.uk/data_request/cif and are available free of charge upon request to CCDC, 12 Union Road, Cambridge, UK (fax: +441223 336033, e-mail: deposit@ccdc.cam.ac.uk).

Antibacterial activity

Stock solutions of **5a–e**, **6** were prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) and then diluting in Mueller–Hinton broth and Triptic soy broth to give an initial concentration of 2000 µg/mL. Further dilutions of the compounds and ampicillin, which was used as control drug in the test medium were prepared at the concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, and 1.9 µg/mL. To ensure that the solvents had no effect on microbial growth, a control test was performed containing inoculated broth supplemented with DMSO at the same dilutions used for the test compounds, and was determined to be inactive. MIC values for each compound were determined in duplicate against *Staphylococcus aureus* (ATCC 25925) and *Bacillus subtilis* (ATCC 6633) as Gram (+) bacterial strains and *Aeromonas hydrophila* (ATCC 95080), *Escherichia coli* (ATCC 25923) and *A. baumannii* (ATCC 02026) as Gram (−) bacterial strains obtained from the Refik Saydam Hıfzıssıhha Institute, Ankara, Turkey. The observed data on the antibacterial activity of **5a–e**, **6** and ampicillin are reported in Table 1.

Antimycobacterial activity

Agar proportion method

MIC values of synthesized compounds **5a–e**, **6** were determined by agar dilution in duplicate as recommended by the Clinical Laboratory Standards Institute (CLSI) (NCCLS -M24-A 2003; NCCLS -M24-T 2002). Positive and negative growth controls were run in each assay. Isoniazid (INH) (Sigma I3377) and ethambutol (EMB) (Sigma E4630) were used as control agents. *M. tuberculosis* H37Rv was used as the standard strain and was provided by the Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey. Stock solutions of **5a–e**, **6** and reference compounds were prepared in DMSO at a concentration of 1000 µg/mL. These solutions were then filtered through a 0.22-µm membrane filter (Millipore, USA). Middlebrook 7H10 agar medium

Table 1 The MIC values (μg/mL) of the tested compounds against the (myco)bacterial strains

Compound	*S. aureus*	*B. subtilis*	*A. hydrophila*	*E. coli*	*A. baumanii*	*M. tuberculosis H37Rv*
5a	125	62.5	125	125	62.5	80
5b	1000	250	500	1000	500	80
5c	125	500	250	125	125	80
5d	125	125	62.5	125	62.5	80
5e	500	500	500	500	500	80
6	62.5	62.5	62.5	62.5	62.5	80
Ampicillin	31.25	0.9	31.25	15.62	125	
Isoniazid						0.2
Ethambutol						5

MIC the minimal inhibitory concentrations determined in duplicate with deviations within one two-fold dilution

(BBL, Becton Dickinson and Company, Sparks, MD, USA) was supplemented with oleic acid-albumin-dextrose-catalase (OADC, BBL, Becton Dickinson and Company, Sparks, MD, USA). Compounds **5a–e**, **6** and control agents were added to obtain an appropriate final concentration in the medium. The final concentrations of INH and EMB were 0.2–1 and 5–10 μg/mL, respectively. Compounds **5a–e**, **6** were prepared at final concentrations of 5, 10, 20, 40, 80, and 160 μg/mL.Three milliliters of prepared medium without any references and synthesized compounds **5a–e**, **6** were dispensed into a sterile tube and it was used as a growth control. The DMSO concentration in the final solutions was not above 1% for antimycobacterial activity.

Inoculum preparation

H37Rv was maintained in Lowenstein–Jensen medium. A culture suspension was prepared by subculturing in Middlebrook 7H9 broth (BBL, Becton Dickinson and Company, Sparks, MD, USA) supplemented with 10% OADC at 37 °C for 7–10 days, until a density corresponding to 10^{-2} dilution was obtained from McFarland standard No. 1. Then 0.1 mL of the diluted suspension was inoculated onto the control and the other tubes with compounds **5a–e**, **6** in different concentrations. The tubes were incubated at 37 °C in an atmosphere of 5% CO_2 for 3 weeks. The MIC values were defined as the lowest concentration that inhibited more than 90% of the bacterial growth, and the results of INH and EMB were interpreted according to the CLSI. The MIC was considered the lowest concentration that showed no visible colonies in all dilutions. In case duplicates did not give an identical result and indicated different MIC values within one two-fold dilution, the highest of the different values was defined as the MIC.

Results and discussion

Cis-5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **5a–e** were synthesized from reaction of *cis*-5,5-diphenylpyrrolidine-2,4-dicarboxylate **3**, which was stereoselectively prepared as the sole and pure diastereomer by 1,3-dipolar *endo*-cycloaddition reaction of methyl 2-(diphenylmethyleneamino)acetate **1** (De Brabandere et al. 2014) and methyl acrylate **2**, and 4-substituted-benzoyl isothiocyanate **4**, prepared by reaction of potassium thiocyanate and the corresponding acyl chloride by modification of the literature method (Arslan and Külcü 2003), in good to excellent yields (Scheme 1). In the cycloaddition reaction, the formation of the *trans*-isomer of 5,5-diphenylpyrrolidine-2,4-dicarboxylate **3** was not observed.

The *cis*-methyl 5,5-diphenylhexahydro-1-oxo-3-thioxo-1H-pyrrolo[1,2-*c*]imidazole-6-carboxylate **6** was synthesized by cyclization reaction of the *cis*-5,5-diphenylpyrrolidine *N*-aroylthioureas **5a–e** with concomitant cleavage of the *N*-acyl group in the presence of hydrazine monohydrate in refluxing dry MeOH for 12 h (Scheme 1). The highest yield (84%) was obtained from the cyclization reaction of **5a** and the lowest yield (30%) was obtained from the cyclization reaction of **5c** in the presence of hydrazine monohydrate (Table 2). After all of these cyclization reactions, starting pyrrolidine **3** was found in the reaction mixture and could be recovered. The most recovery of pyrrolidine **3** (55%) was observed in the cyclization reaction of **5c**. Therefore, the decreasing yield can be explained by cleavage of the 4-substituted-*N*-thioformylbenzamide group. Structures of **3**, **5a–e** and **6** were fully characterized by ^{1}H and ^{13}C NMR, FT-IR, MS and HRMS studies. The stereochemistry was unambiguously determined for **5a** by single crystal X-ray diffraction studies (Fig. 1). Additionally, analogous ^{1}H NMR chemical shifts (2-H: $\delta = 5.39$–5.41 ppm, 3-H and 3′-H: $\delta = 2.43$–2.45 ppm, 4-H: $\delta = 4.03$–4.04 ppm) and the corresponding coupling constants (Table 3) of all derivatives **5** pointed out the *cis*-stereochemistry.

The structure of **5a** was fully characterized by single crystal X-ray diffraction study. Compound **5a** crystallized as yellow prisms and the structure was solved in the triclinic centrosymmetric space group $P\bar{1}$ with one molecule in the asymmetric unit (Fig. 1). There are two stereogenic carbon

Scheme 1 Synthesis of the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **5** and hexahydro-1*H*-pyrrolo[1,2-*c*]imidazole **6**

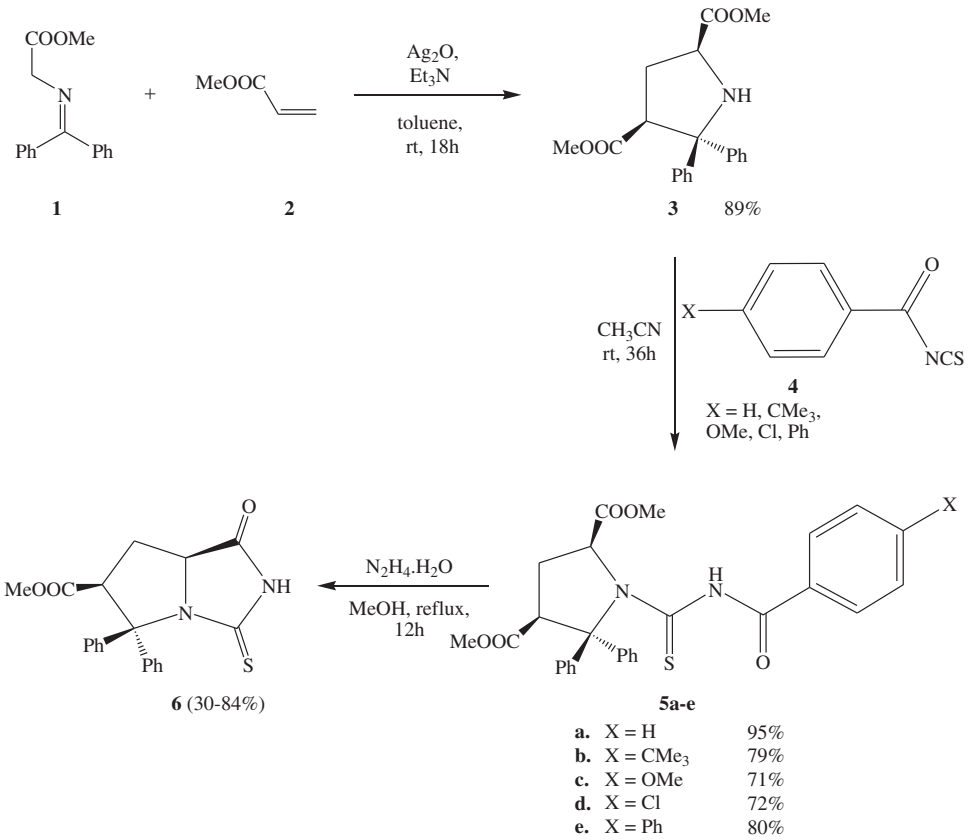

Table 2 Synthesis of hexahydro-1*H*-pyrrolo[1,2-*c*]imidazole **6**

Entry	*X*	Yield (%)
5a	H	84
5b	CMe3	42
5c	OMe	30
5d	Cl	82
5e	Ph	46

atoms and both have *S*-configuration (C14 and C16). The bond length of N1-C21 = 1.351(5) Å, N2-C21 = 1.393(5) Å and N2-C22 = 1.387(5) Å are shorter than typical N–C single bond values. Whereas, the bond S–C(21) = 1.640(4) Å and O(1)–C(22) = 1.203(5) Å are considerably longer than C=S and C=O double bonds, respectively. The bond values are consistent with the literature and these results show us the existence of a partial electron delocalization in the N–C(S)–NH–C(O) structural fragment (Saeed et al. 2010; Döndaş et al. 2006; Del Campo et al. 2004).

Antibacterial and antimycobacterial activities

Antibacterial activity studies of the compounds **5a–e**, **6** were performed against five standard bacterial strains. The compounds **5a–e** inhibited the growth of bacteria at minimum inhibitory concentrations (MIC) of 62.5 to 1000 µg/mL (Table 1). **5a**, **5c**, and **5d**, containing H, OMe and Cl as substituent at the 4-position of the aromatic ring, respectively, exhibited antibacterial activity against the *S. aureus* strain with a MIC value of 125 µg/mL. For the same bacterial strain, **5b** containing CMe_3 and **5e** containing Ph as substituent at the 4-position of the aromatic ring exhibited antibacterial activity with a MIC value of 1000 and 500 µg/mL, respectively. Ampicillin exhibited activity with a MIC value of 31.25 µg/mL against the *S. aureus* strain. Compared to ampicillin, **5a–e** showed moderate antibacterial activity against the *B. subtilis* strain. Compared to the other compounds, **5d** showed the best activity against the *A. hydrophila* strain with a MIC value of 62.5 µg/mL, while MIC values of the other compounds were observed in the range of 125–500 µg/mL. Compounds **5a**, **5c**, and **5d** exhibited antibacterial activity against the *E. coli* strain with a MIC value of 125 µg/mL, whereas the other compounds **5b** and **5e** exhibited bioactivity with a MIC value of 1000 and 500 µg/mL, respectively. The compounds **5a**, **5d** showed higher activity than the control against the *A. baumanii* strain, with a MIC value of 62.5 µg/mL. Compound **5c** showed activity with a MIC value of 125 µg/mL similar to ampicillin. Finally, derivatives **5b** and **5e** showed activity with a MIC value of 500 µg/mL against the *A. baumanii* strain.

Compound **6** exhibited antibacterial activity against the tested ATCC strains with a MIC value of 62.5 µg/mL. Thus,

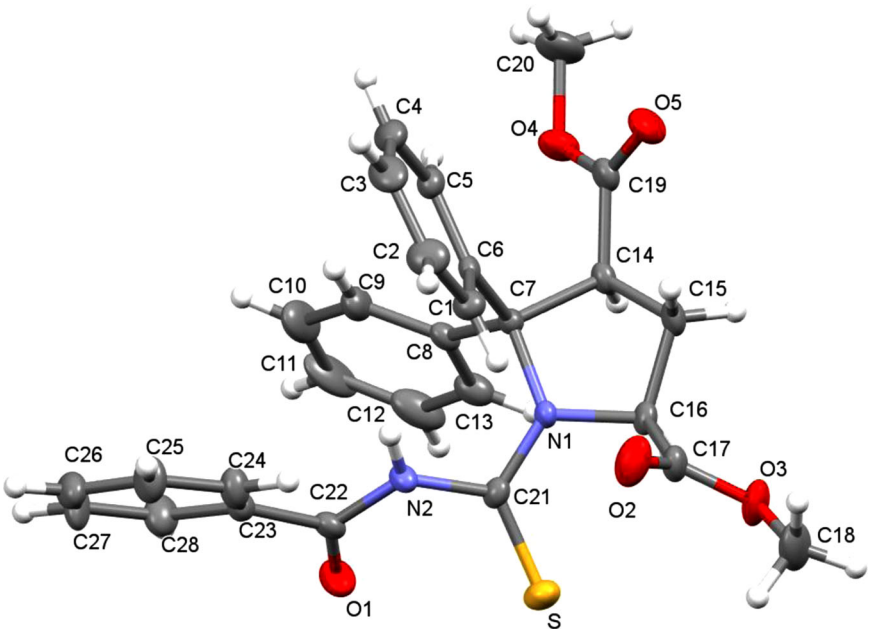

Fig. 1 Crystal structure of **5a**. Selected bond lengths (Å) and angles: S–C21 1.640(4), O(1)–C (22) = 1.203(5), N1–C7 1.511 (5), N1–C16 1.458(5), N1–C21 1.351(5), N2–C21 1.393(5), N2–C22 1.387(5), S–C21–N1 123.6(3), S–C21–N2 123.1(3), C22–N2–C21 128.4(3), N1–C7–C14 99.1(5)

Table 3 Selected ^{1}H NMR chemical shifts and coupling constants of pyrrolidines **5**

Compound	2-H		3-H, 3′-H		4-H	
	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
5a	5.39	8.3	2.44	8.0	4.04	8.4
5b	5.41	8.1	2.43	8.4, 8.1	4.03	7.8
5c	5.40	8.2	2.43	8.1	4.04	7.9
5d	5.38	8.2	2.44	8.0	4.04	8.0
5e	5.41	8.2	2.45	8.1	4.04	8.0

the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives possessed a broad spectrum of antibacterial activity, and it can be said that **5a** and **5d** showed a better pharmacological activity than the other derivatives. The pyrrolidine-fused thiohydantoin **6** showed better antibacterial activity than the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **5** against the tested ATCC strains.

Antimycobacterial activity studies of the compounds **5a–e**, **6** were performed against the *M. tuberculosis* H37Rv strain, and all compounds exhibited activity with a MIC value of 80 µg/mL against the *M. tuberculosis* H37Rv strain (Table 1). It can be said that the presence of the *tert*-butyl, chloro, methoxy, and phenyl substituent on para position of the phenyl ring of the benzoyl group has no effect on bioactivity.

Conclusion

In summary, we have demonstrated the synthesis of a pyrrolidine-fused thiohydantoin as a single stereoisomer by cyclization reaction of the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives in the presence of hydrazine monohydrate. These type of structures are very useful in pharmaceutical studies because the different functional groups of the synthesized hexahydro-1*H*-pyrrolo[1,2-*c*] imidazole compound can be easily modified. Although all compounds **5a–e** and **6** showed antibacterial activity, the final compound **6** exhibited better activity and its antibacterial activity is promising for future studies. Furthermore, the synthesized novel 5,5-diphenylpyrrolidine, *N*-aroylthioureas and the hexahydro-1*H*-pyrrolo[1,2-*c*]imidazole compounds showed a considerable antimycobacterial activity against the *M. tuberculosis* H37Rv strain.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Abdelaziz MA, El-Sehrawi HM, Mohareb RM (2015) Synthesis, cytotoxicity and toxicity of thieno[2,3-d]pyrimidine derivatives derived from 2-amino-3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene. Med Chem Res 24:3932–3948

Ahmadi A, Khalili M, Hajikhani R, Safari N, Nahri-Niknafs B (2012) Anti-inflammatory effects of two new methyl and morpholine derivatives of diphenhydramine on rats. Med Chem Res 21:3532–3540

Alkherraz AM, Lusta ZI, Zubi AE (2014) Synthesis and use of thiourea derivative (1-phenyl-3-benzoyl-2-thiourea) for extraction of cadmium ion. Int J Chem Mol Nucl Mater Metall Eng 8:108–110

Al-Obaid AM, El-Subbagh HI, Khodair AI, Elmazar MMA (1996) 5-Substituted-2-thiohydantoin analogs as a novel class of antitumor agents. Anticancer Drugs 7:873–880

Arslan H, Külcü N (2003) Synthesis and characterization of copper(II), nickel(II) and cobalt(II) complexes with novel thiourea derivatives. Transit Met Chem 28:816–819

Blanc M, Cussac M, Boucherle A, Leclerc G (1992) Synthesis of 2-thiohydantoin derivatives with potential immunomodulating and anticancer activities. Eur J Med Chem 27:267–275

Bye E (1976) Crystal structures of synthetic analgetics. V. Dextromoramide. Acta Chem Scand B 30:95–100

Chen G, Yang J, Gao S, Zhang Y, Hao X-J (2013) Theoretical study of the regioselectivity of the Huisgen reaction. Res Chem Intermed 39:1245–1250

Cîrcu V, Ilie M, Ilis M, Dumitrascu F, Neagoe I, Pasculescu S (2009) Luminescent cyclometallated platinum(II) complexes with *N*-benzoyl thiourea derivatives as ancillary ligands. Polyhedron 28:3739–3746

Clinical and Laboratory Standards Institute (CLSI) (formerly NCCLS) (2002) Antimycobacterial susceptibility testing for M. tuberculosis: Tentative Standard NCCLS Document M24-T. CLSI, Villanova, Pennsylvania

CrystalClear software (2005) Rigaku/MSC, Inc. The Woodlands, TX

De Brabandere S, Mangelinckx S, Kadam ST, Nural Y, Augustyns K, Van der Veken P, Törnroos KW, De Kimpe N (2014) Synthesis of γ,δ-aziridino α-amino acid derivatives and their stereoselective ring transformation to 2-(aminomethyl)-1-aminocyclopropanecarboxylic acid derivatives. Eur J Org Chem 2014:1220–1226

Del Campo R, Criado JJ, Gheorghe R, Gonzalez FJ, Hermosa MR, Sanz F, Manzano JL, Monte E, Rodriguez-Fernandez E (2004) *N*-Benzoyl-*N*′-alkylthioureas and their complexes with Ni(II), Co (III) and Pt(II) - crystal structure of 3-benzoyl-1-butyl-1-methylthiourea: activity against fungi and yeast. J Inorg Biochem 98:1307–1314

Döndaş HA, Nural Y, Duran N, Kilner C (2006) Synthesis, crystal structure and antifungal/antibacterial activity of some novel highly functionalized benzoylaminocarbothioyl pyrrolidines. Turk J Chem 30:573–583

Dondas HA, Grigg R, Thornton–Pett M (1996) Spiro(pyrrolidinyl-2,3′-benzodiazepines) related to MK-329. Tetrahedron 52:13455–13466

Döndaş HA, Grigg R, Kilner C (2003) X=Y=ZH systems as potential 1,3-dipoles. Part 58: cycloaddition route to chiral conformationally constrained (*R*)-pro-(*S*)-pro peptidomimetics. Tetrahedron 59:8481–8487

Dondas HA, Fiswick CWG, Grigg R, Kilner C (2004) 1,3-dipolar cycloaddition of stabilised and non-stabilised azomethine ylides derived from uracil polyoxin C (UPoC): access to nikkomycin analogues. Tetrahedron 60:3473–3485

Ettinger U, Kumari V, Zachariah E, Galea A, Crawford TJ, Corr PJ, Taylor D, Das M, Sharma T (2003) Effects of procyclidine on eye movements in schizophrenia. Neuropsychopharmacology 28:2199–2208

Fujiki A, Tsuneda T, Sugao M, Mizumaki K, Inoue H (2003) Usefulness and safety of *bepridil* in converting persistent atrial fibrillation to sinus rhythm. Am J Cardiol 92:472–475

Fujimoto K, Oka T, Morimoto M (1987) Antitumor activity of a novel antitumor antibiotic, quinocarmycin citrate (KW2152). Cancer Res 47:1516–1522

Geyer M, Wellner E, Jurva U, Saloman S, Armstrog D, Tacke R (2015) Can silicon make an excellent drug even better? An *in vitro* and *in vivo* head-to-head comparison between Loperamide and its silicon analogue sila-Loperamide. ChemMedChem 10:911–924

Hassan HE, Myers AL, Coop A, Eddington ND (2009) Differential involvement of P-glycoprotein (ABCB1) in permeability, tissue distribution, and antinociceptive activity of methadone, buprenorphine, and diprenorphine: *in vitro* and *in vivo* evaluation. J Pharm Sci 98:4928–4940

Hernandez-Toribio J, Padilla S, Adrio J, Carretero JC (2012) Catalytic asymmetric synthesis of α-quaternary proline derivatives by 1,3-dipolar cycloaddition of α-silylimines. Angew Chem Int Ed 51:8854–8858

Hu S, Guo Y, Xu J, Shao S (2009) Tunability of anion binding strength based on acyl-thiourea receptors containing isatin group. Spectrochim Acta A 72:1043–1046

Hut'ka M, Tsubogo T, Kobayashi S (2013) Synthesis of glutamic acid and highly functionalized pyrrolidine derivatives by utilizing tunable calcium catalysts for chemoselective asymmetric 1,4-addition and [3+2] cycloaddition reactions. Adv Synth Catal 355:1561–1569

Ilardi EA, Vitaku E, Njardarson JT (2014) Data-mining for sulfur and fluorine: An evaluation of pharmaceuticals to reveal opportunities for drug design and discovery. J Med Chem 57:2832–2842

Khlebnikov AF, Novikov MS, Khlebnikov VA, Kostikov RR (2001) Prototropic generation of azomethine ylides from esters of *N*-benzylideneaminoacids, and 1,3-dipolar cycloaddition thereof. Russ J Org Chem 37:507–512

Khlebnikov AF, Novikov MS, Kostikov RR (1997) Ylides from dihalocarbenes and esters of *N*-benzhydrylidene amino acids: halogen-dependent reaction pathways. Mendeleev Commun 7:145–146

Khodair AI, El-Barbary AA, Abbas YA, Imam DR (2001) Synthesis, reactions and conformational analysis of 5-arylidene-2-thiohydantoins as potential antiviral agents. Phosphorus Sulfur 170:261–278

Khurana SJM, Sharma C, Aneja KR (2014) Chemoselective *N*-benzylation of 2-thiohydantoins and 2-thiobarbituric acids catalyzed by PEG-stabilized Ni nanoparticles and their anti-microbial activities. Med Chem Res 23:4595–4606

Kim HY, Li JY, Kim S, Oh K (2011) Stereodivergency in catalytic asymmetric conjugate addition reactions of glycine (ket)imines. J Am Chem Soc 133:20750–20753

Koch KR (2001) New chemistry with old ligands: *N*-alkyl- and *N*,*N*-dialkyl-*N*′-acyl(aroyl)thioureas in co-ordination, analytical and process chemistry of the platinum group metals. Coord Chem Rev 216-217:473–488

Krasikovs A (2015) Enantioselective formal synthesis of doxapram. Chem Heterocycl Compd 51:385–388

Li S, Cao X, Chen C, Ke S (2012) Novel salicylic acid-oriented thiourea-type receptors as colorimetric chemosensor: synthesis, characterizations and selective naked-eye recognition properties. Spectrochim Acta A 96:18–23

Luckay RC, Sheng X, Strasser CE, Raubenheimer HG, Safin DA, Babashkina MG, Klein A (2009) Competitive bulk liquid membrane transport and solvent extraction of some metal ions using $RC(S)NHP(X)(O\mathit{i}Pr)_2$(X=O, S) as ionophores. Formation of the polynuclear complex of $[Ag(N{\equiv}C{-}NP(S)(O\mathit{i}Pr)_2)]_n$. Dalton Trans 39:8227–8236

Mahdavi M, Shirazi MS, Taherkhani R, Saeedi M, Alipour E, Moghadam FH, Moradi A, Nadri H, Emami S, Firoozpour L, Shafiee A, Foroumadi A (2014) Synthesis, biological evaluation and docking study of 3-aroyl-1-(4-sulfamoylphenyl)thiourea derivatives as 15-lipoxygenase inhibitors. Eur J Med Chem 82:308–313

Martín-Rodríguez M, Nájera C, Sansano JM, De Cózar A, Cossío FP (2011) Chiral gold(I) vs chiral silver complexes as catalysts for the enantioselective synthesis of the second generation GSK-hepatitis C virus inhibitor. Beilstein J Org Chem 7:988–996

Metwally MA, Abdel-Latif E (2012) Thiohydantoins: synthetic strategies and chemical reactions. J Sulfur Chem 33:229–257

Nájera C, Sansano JM (2009) 1,3-Dipolar cycloadditions: applications to the synthesis of antiviral agents. Org Biomol Chem 7:4567–4581

National Committee for Clinical Laboratory Standards (2003) Susceptibility testing of Mycobacteria, Nocardia, and other aerobic actinomycetes: Approved Standard NCCLS Document M24-A. NCCLS, Wayne, Pennsylvania

Nural Y, Döndaş HA, Grigg R, Şahin E (2011) Polysubstituted fused ring bicyclic thiohydantoins from aminocarbo-*N*-thioylpyrrolidines derived from azomethine ylide 1,3-dipolar cycloadditions. Heterocycles 83:2091–2114

Nural Y, Kilincarslan R, Dondas HA, Cetinkaya B, Serin MS, Grigg R, Ince T, Kilner C (2009) Synthesis of Ni(II), Pd(II) and Cu(II) metal complexes of novel highly functionalized aroylaminocarbo-*N*-thioyl pyrrolidines and their activity against fungi and yeast. Polyhedron 28:2847–2854

Okunola-Bakare OM, Cao J, Kopajtic T, Katz JL, Loland CJ, Shi L, Newman AH (2014) Elucidation of structural elements for selectivity across monoamine transporters: novel 2-[(diphenylmethyl)sulfinyl]acetamide (modafinil) analogues. J Med Chem 57:1000–1013

Otazo-Sanchez E, Ortiz-del-Toro P, Estevez-Hernandez O, Perez-Marin L, Goicoechea I, Beltran AC, Villagomez-Ibarra JR (2002) Aroylthioureas: new organic ionophores for heavy metal ion selective electrodes. A nuclear magnetic resonance study. Spectrochim Acta A 58:2281–2290

Othman AA, Newman AH, Eddington ND (2008) The novel *N*-substituted benztropine analog GA2-50 possesses pharmacokinetic and pharmacodynamic profiles favorable for a candidate substitute medication for cocaine abuse. J Pharm Sci 97:5453–5470

Pandey G, Banerjee P, Gadre SR (2006) Construction of enantiopure pyrrolidine ring system via asymmetric [3+2]-cycloaddition of azomethine ylides. Chem Rev 106:4484–4517

Pellegrini C, Weber M, Borshberg HJ (1996) Total synthesis of (+)-elacomine and (−)-isoelacomine, two hitherto unnamed oxindole alkaloids from elaeagnus commutate. Helv Chim Acta 79:151–168

Pramanik C, Bapat K, Chaudhari A, Tripathy NK, Gurjar MK (2012) A New solvent system (cyclopentyl methyl ether–water) in process development of darifenacin HBr. Org Process Res Dev 16:1591–1597

Prasanna G, Ujwal A, Diliprajudominic S, Marimuthu T, Sarswathi NT (2014) A new pipeline to discover antimycotics by inhibiting ergosterol and riboflavin synthesis: the inspirations of Siddha medicine. Med Chem Res 23:2651–2658

Rubioa C, Strano-Rossib S, Taberneroc MJ, Anzillottib L, Chiarottib M, Bermejo AM (2013) Hygrine and cuscohygrine as possible markers to distinguish coca chewing from cocaine abuse in workplace drug testing. Forensic Sci Int 227:60–63

Saeed A, Al-Rashida M, Hamayoun M, Mumtaz A, Iqbal J (2014a) Carbonic anhydrase inhibition by 1-aroyl-3-(4-aminosulfonylphenyl)thioureas. J Enzyme Inhib Med Chem 29:901–905

Saeed A, Flörke U, Erben MF (2014b) A review on the chemistry, coordination, structure and biological properties of 1-(acyl/aroyl)-3-(substituted) thioureas. J Sulfur Chem 35:318–355

Saeed A, Zaib S, Pervez A, Mumtaz A, Shahid M, Iqbal J (2013) Synthesis, molecular docking studies, and in vitro screening of sulfanilamide-thiourea hybrids as antimicrobial and urease inhibitors. Med Chem Res 22:3653–3662

Saeed A, Zaman S, Maryam Jamil M, Mirza B (2008) Synthesis and antifungal activity of some novel *N*-(4-phenyl-3-aroylthiazol-2(3H)-ylidene) substituted benzamides. Turk J Chem 32:585–592

Saeed S, Rashid N, Jones PG, Ali M, Hussain R (2010) Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur J Med Chem 45:1323–1331

Sassene PJ, Mosgaard MD, Löbmann K, Mu H, Larsen FH, Rades T, Müllertz A (2015) Elucidating the molecular interactions occurring during drug precipitation of weak bases from lipid-based formulations: a case study with cinnarizine and a long chain self-nanoemulsifying drug delivery system. Mol Pharm 12:4067–4076

Schwartz RE, Liesch J, Hensens O, Zitano L, Honeycutt S, Garrity G, Fromtling RA, Onishi J, Monaghan R (1998) L-657, 398, a novel antifungal agent: fermentation, isolation, structural elucidation and biological properties. J Antibiot 41:1774–1779

Sheldrick GM (1997) SHELXS-97 and SHELXL-97, Program for Crystal Structure Solution and Refinement. University of Göttingen, Germany

Shoaib M, Shafi Ullah S, Bari AU, Tahir MN, Shah SWA (2014) Acute toxicity, brine shrimp cytotoxic, antioxidant and analgesic activities of synthesized 1-benzoyl-3-methyl thiourea. Pharmacologyonline 3:91–99

Soman SS, Soni JN, Patel TB (2014) Synthesis of new naphthoisoxazole amide derivatives and study of their biological evaluations. Med Chem Res 23:3803–3809

Stocker BL, Dangerfield EM, Win-Mason AL, Haslett GW, Timmer MSM (2010) Recent developments in the synthesis of pyrrolidine-containing iminosugars. Eur J Org Chem 2010:1615–1637

Tan SS, Al-abbasi AA, Tahir MIM, Kassim MB (2014) Synthesis, structure and spectroscopic properties of cobalt(III) complexes with 1-benzoyl-(3,3-disubstituted)thiourea. Polyhedron 68:287–294

Tsubogo T, Saito S, Seki K, Yamashita Y, Kobayashi S (2008) Development of catalytic asymmetric 1,4-addition and [3+2] cycloaddition reactions using chiral calcium complexes. J Am Chem Soc 130:13321–13332

Wang CJ, Liang G, Xue ZY, Gao F (2008) Highly enantioselective 1,3-dipolar cycloaddition of azomethine ylides catalyzed by copper(I)/TF-BiphamPhos complexes. J Am Chem Soc 130:17250–17251

Verma VA, Shore DGM, Chen H, Chen J, So S, Hackos DH, Kolesnikov A, Lyssikatos JP, Tay S, Wang L, Estrada AA (2016) α-Aryl pyrrolidine sulfonamides as TRPA1 antagonists. Bioorg Med Chem Lett 26:495–498

Xue MX, Zhang XM, Gong LZ (2008) The first organocatalytic enantio- and diastereoselective 1,3-dipolar cycloaddition of azomethine ylides with nitroalkenes. Synlett 2008:691–694

Yang W, Liu H, Li M, Wang F, Zhou W, Fan J (2012) Synthesis, structures and antibacterial activities of benzoylthiourea derivatives and their complexes with cobalt. J Inorg Biochem 116:97–105

Yildiz I, Bozdag-Dundar O (2010) Three-dimensional common-feature hypotheses for hypoglycemic flavonyl-2,4-thiazolidinedione derivatives. Med Chem Res 19:211–219

Zhang Q, Zhao B, Song Y, Hua C, Gou X, Chen B, Zhao J (2015) Synthesis and biological activity of *N*-aroyl (aryloxyacetyl)-*N*′-ferrocenyl thiourea derivatives. Heteroatom Chem 26:348–354