Chem Biol Drug Des 2012

Research Article

doi: 10.1111/j.1747-0285.2012.01440.x

Synthesis and Kinetic Testing of Tetrahydropyrimidine-2-thione and Pyrrole Derivatives as Inhibitors of the Metallo-β-lactamase from *Klebsiella pneumonia* and *Pseudomonas aeruginosa*

Waleed M. Hussein[1,2], Samar S. Fatahala[1], Zainab M. Mohamed[1], Ross P. McGeary[2,3], Gerhard Schenk[2,4], David L. Ollis[5] and Mosaad S. Mohamed[1,*]

[1]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Helwan University, Ein Helwan, Helwan, Egypt*
[2]*School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Qld 4072, Australia*
[3]*School of Pharmacy, The University of Queensland, Brisbane, Qld 4072, Australia*
[4]*Department of Chemistry, National University of Ireland-Maynooth, Maynooth, Co. Kildare, Ireland*
[5]*The Australian National University Research School of Chemistry, Canberra, ACT 0200, Australia*
**Corresponding author: Mosaad S. Mohamed, doctor.mosaadmohamed@yahoo.com*

Metallo-β-lactamases (MBLs), produced by an increasing number of bacterial pathogens, facilitate the hydrolysis of many commonly used β-lactam antibiotics. There are no clinically useful antagonists against MBLs. Two sets of tetrahydropyrimidine-2-thione and pyrrole derivatives were synthesized and assayed for their inhibitory effects on the catalytic activity of the IMP-1 MBL from *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Nine compounds tested (1a, 3b, 5c, 6b, 7a, 8a, 11c, 13a, and 16a) showed micromolar inhibition constants (K_i values range from ~20–80 μM). Compounds 1c, 2b, and 15a showed only weak inhibition. *In silico* docking was employed to investigate the binding mode of each enantiomer of the strongest inhibitor, 5c (K_i = 19 ± 9 μM), as well as 7a (K_i = 21 ± 10 μM), the strongest inhibitor of the pyrrole series, in the active site of IMP-1.

Key words: inhibition assays, metallo-β-lactamases, pyrrole, tetrahydropyrimidine-2-thione

Received 14 March 2012, revised 13 June 2012 and accepted for publication 19 June 2012

The β-lactam antibiotics are broad-spectrum agents against many Gram-positive, Gram-negative, and anaerobic micro-organisms (1). β-Lactam antibiotics interfere with the synthesis of the peptidoglycan layer of bacterial cell walls (1), and β-lactamases are produced by a growing number of bacteria, enabling resistance to the effects of β-lactam antibiotics; their hydrolysis of the β-lactam ring renders these antibiotics inactive. Penams, carbapenems, and cephalosporins are examples of β-lactam antibiotics that are hydrolyzed by these enzymes. There are two families of β-lactamases: the serine-β-lactamases (SBLs), which operate via a nucleophilic serine residue in the active site, and metallo-β-lactamases (MBLs), which employ a metal-activated nucleophilic hydroxyl group (2,3). The development of inhibitors against SBLs and MBLs is urgent to retain the usefulness of β-lactam antibiotics. Potent inhibitors for SBLs are available and are in clinical use (e.g., clavulanic acid and its derivatives) (4), but no clinically useful antagonist of MBL activity has been reported.

The MBLs are members of the binuclear metallohydrolase family (5). A characteristic feature of the MBLs is the αββα protein fold with either one or two zinc (II) ions in their active sites. At least one of these zinc (II) ions plays an essential role in the catalytic hydrolysis of the β-lactam ring of the antibiotics (6–9). Of great concern for healthcare systems is that MBLs display a very broad range of utilizable substrates, including penams (e.g., benzylpenicillin), cephalosporins (e.g., cephalothin, cefoxitin), and carbapenems (e.g., meropenem and imipenem) (2,7). These observations, together with the ease with which MBLs may be spread between bacterial species through mobile genetic elements, make the development of new MBL inhibitors a very urgent task (10–13). Imipenemase-1 (IMP-1) is one of the best characterized MBLs and has been identified in, among others, the pathogenic microbes *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (6,7).

In our previous work (14), we designed and synthesized a set of thiosemicarbazide derivatives (K_i = 10–75 μM) as IMP-1 inhibitors based on *in silico* docking and fragment-based screening of a 500-compound Maybridge™ library (15). In another report, we assayed a series of pyrrole derivatives against IMP-1 that have K_i values ranging from ~10 to 30 μM (16).

The aim of this work was to find new candidate IMP-1 lead inhibitors with improved potency via the synthesis, evaluation, and *in silico* docking of two sets of compounds, pyrroles and thiopyrimidines.

Materials and Methods

Synthesis of lead compounds

All commercial chemicals used as starting materials and reagents in this study were purchased from Merck (Darmstadt, Germany) and were of reagent grade. All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Kyoto, Japan); IR spectra were recorded as potassium bromide pellets on a PerkinElmer 1650 spectrophotometer (Waltham, MA, USA). ^{1}H NMR spectra were recorded on a Varian Mercury (300 MHz) spectrometer (Varian, Crawley, UK), and chemical shifts are expressed as ppm against TMS as internal reference. Mass spectra were recorded at 70 eV (EI Ms-QP 1000 EX; Shimadzu). Microanalyses were operated using Vario, Elementar apparatus (Shimadzu, Japan). Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Compounds **7a–c**, **10b**, **13–16**, and **19** were prepared as reported in the literature (17–21). Compounds **8**, **10a**, **10c**, and **11–12** were prepared as reported in the literature (22).

General procedure for the synthesis of compounds 1a–e

A mixture of thiourea (0.76 g, 10 mmol), the appropriate aromatic aldehyde (10 mmol), and either acetylacetone or ethyl acetoacetate (10 mmol) in absolute ethanol (20 mL) containing 37% HCl (8 drops) was heated under reflux for 8 h, and the reaction mixture was allowed to cool and neutralized with 25% ammonia (0.5 mL). The precipitate formed was filtered off, washed with 50% ethanol, and recrystallized from ethanol to give compounds **1a–e**.

1-(6-Methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl)ethanone (1a)

Yield: 86%; m.p. 197–199 °C; IR (KBr) υ (per cm): 3366 (NH), 1680 (C=O); MS (EI) *m/z*: 246 (M^+, 77%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.60 (s, 3H, CH_3, pyrim), 2.70 (s, 3H, $COCH_3$), 5.26 (s, 1H pyrim), 7.23-7.33 (m, 5H, Ar-H), 9.60, 9.70 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{13}H_{14}N_2OS$: C, 63.39; H, 5.73; N, 11.37; S, 13.02%. Found: C, 63.15; H, 5.54; N, 11.24; S, 12.72%.

1-(6-Methyl-4-(4-methoxy-phenyl)-2-thioxo-1,2,3,4-tetrahydro pyrimidine-5-yl)ethanone (1b)

Yield: 88%; m.p. 179–181 °C; IR (KBr) υ (per cm): 3336 (NH), 1675 (C=O); MS (EI) *m/z*: 276 (M^+, 100%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.25 (s, 3H, CH_3, pyrim), 2.60 (s, 3H, $COCH_3$), 3.83 (s, 3H, OMe), 5.12 (s, 1H pyrim), 7.20 (dd, J = 8.1, J = 2.3 Hz, 4H, Ph-OCH_3), 9.69, 9.73 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{14}H_{16}N_2O_2S$: C, 60.85; H, 5.84; N, 10.14; S, 11.60%. Found: C, 60.59; H, 5.72; N, 9.98; S, 11.30%.

1-(6-Methyl-4-styryl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl)ethanone (1c)

Yield: 90%; m.p. 183–185 °C; IR (KBr) υ (per cm): 3279 (NH), 1686 (C=O), 1620 (C=S); MS (EI) *m/z*: 272 (M^+, 21%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, CH_3, pyrim), 2.30 (s, 3H, $COCH_3$), 5.00 (d, J = 8.1 Hz, 1H, pyrim), 6.20 (t, J = 7.1 Hz, 1H, CH=CH), 6.40 (d, J = 8.1 Hz 1H, CH=CH), 7.20–7.80 (m, 5H, Ar-H), 10.00 (s, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{15}H_{16}N_2OS$: C, 66.15; H, 5.92; N, 10.28; S, 11.77%. Found: C, 66.00; H, 5.73; N, 10.14; S, 11.49%.

Ethyl 6-methyl-4-(4-methoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1d)

Yield: 87%; m.p. 144–146 °C; IR (KBr) υ (per cm): 3390 (NH), 1683 (C=O), 1231 (C=S); MS (EI) *m/z*: 306 ($M^{+\cdot}$18%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.20 (t, J = 7.1 Hz, 3H, CH_3), 4.10 (q, 2H, J = 7.1 Hz, CH_2), 2.70 (s, 3H, CH_3), 3.40 (s, 3H, OCH_3), 5.10 (s, 1H, pyrim), 7.40 (dd, J = 8.2, J = 2.2 Hz, 4H, Ar-H), 9.70, 10.40 (2xs, 2H, 2NH D_2O exchangeable); Anal. Calcd for $C_{15}H_{18}N_2O_3S$: C, 58.80; H, 5.92; N, 9.14; S, 10.47%. Found: C, 58.47; H, 5.72; N, 9.42; S, 10.32%.

Ethyl 6-methyl-4-styryl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1e)

Yield: 85%; m.p. 205–207 °C; IR (KBr) υ (per cm): 3478 (NH), 1683 (C=O), 1220 (C=S); MS (EI) *m/z*: 302 (M^+, 100%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.10 (t, J = 7.5 Hz, 3H, CH_3), 4.01 (q, J = 7.5 Hz, 2H, CH_2), 2.30 (s, 3H, CH_3, pyrim), 5.10 (d, J = 8.2 Hz, 1H, pyrim), 6.30 (d, J = 8.2 Hz, 1H, CH=CH), 6.40 (t, J = 7.5 Hz, 1H, CH=CH), 7.20–7.40 (m, 5H, Ar-H), 9.70, 10.40 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{16}H_{18}N_2O_2S$: C, 63.55; H, 6.00; N, 9.26; S, 10.60%. Found: C, 63.21; H, 6.31; N, 9.00; S, 10.84%.

General procedure for the synthesis of compounds 2a–c

To a solution of chloroacetic acid (0.94 g, 10 mmol) and sodium hydroxide (10 mmol) in water (5 mL), a solution of **1a–c** (10 mmol) and sodium hydroxide (10 mmol) in water (10 mL) was added. The reaction mixture was stirred at room temperature for 3 h and then rendered acidic with 37% HCl. The precipitate formed was filtered off, washed with water and recrystallized from methanol to afford **2a–c**.

2-((5-Acetyl-6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidine-2yl)sulfanyl)acetic acid (2a)

Yield: 80%; m.p. 195–197 °C; IR (KBr) υ (per cm): 3340 (NH), 1681 (C=O); MS (EI) *m/z*: 304 (M^+, 5%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.21 (s, 3H, $COCH_3$), 2.28 (s, 3H, CH_3 pyrim), 4.21 (s, 2H, CH_2), 5.11 (s, 1H, pyrim), 6.98–7.21 (m, 5H, Ar-H), 9.82 (s, 1H, NH, D_2O exchangeable), 12.11 (s, 1H, CO_2H, D_2O exchangeable); Anal. Calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; S, 10.54%. Found: C, 59.43; H, 5.60; N, 9.51; S, 10.59%.

2-((5-Acetyl-6-methyl-4-(4-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine-2yl)sulfanyl)acetic acid (2b)

Yield: 87%; m.p. 207–209 °C; IR (KBr) υ (per cm): 3333 (NH), 1678 (C=O); MS (EI) *m/z*: 334 (M^+, 21%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, $COCH_3$), 2.29 (s, 3H, CH_3 pyrim), 3.73 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 5.20 (s, 1H, pyrim), 7.90 (dd, J = 8.2, J = 2.3 Hz, 4H, Ar-H), 9.84 (s, 1H, NH, D_2O exchangeable), 11.90 (s, 1H, CO_2H, D_2O exchangeable); Anal. Calcd for $C_{16}H_{18}N_2O_4S$: C, 57.47; H, 5.43; N, 8.38; S, 9.59%. Found: C, 57.86; H, 5.31; N, 8.54; S, 9.33%.

2-((5-Acetyl-6-methyl-4-styryl-1,2,3,4-tetrahydropyrimidine-2yl)sulfanyl)acetic acid (2c)

Yield: 77%; m.p. 204-206 °C; IR (KBr) υ (per cm): 3334 (NH), 1675 (C=O); MS (EI) *m/z*: 330 (M^+, 8%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.27 (s, 3H, CH_3, pyrim), 2.30 (s, 3H, $COCH_3$), 4.00 (s, 2H, CH_2), 5.02 (d, J = 8.1 Hz, 1H, pyrim), 6.23 (d, J = 8.1 Hz, 1H, CH=CH), 6.31 (t, J = 7.5 Hz, 1H, CH=CH), 7.35–8.21 (m, 5H, Ar-H), 9.70 (s, 1H, NH, D_2O exchangeable), 12.01 (s, 1H, CO_2H, D_2O exchangeable); Anal. Calcd for $C_{17}H_{18}N_2O_3S$: C, 61.80; H, 5.49; N, 8.48; S, 9.70%. Found: C, 61.49; H, 5.21; N, 8.54; S, 9.70%.

General procedure for the synthesis of compounds 3a–c

A solution of **1a–c** (10 mmol) in benzene (50 mL) was treated with malononitrile (10 mmol), ammonium acetate (10 g, 0.13 mol), and glacial acetic acid (15 mL). The reaction mixture was heated under reflux for 24 h using Dean-Starke water separator. On cooling to room temperature, it was diluted with benzene (100 mL) and washed successively with water (100 mL) and 5% aq. sodium carbonate (200 mL). Then the solvent was removed in vacuum on a steam bath and the residual oil crystallized out upon standing at room temperature to afford dark brown crystals of **3a–c**.

2-(1-(6-Methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl)ethylidene) malononitrile (3a)

Yield: 45%; m.p. 220–222 °C; IR (KBr) υ (per cm): 2223 (CN), 3427 (NH); MS (EI) *m/z*: 294 (M^+, 11%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.20 (s, 3H, CH_3, pyrim), 2.40 (s, 3H, CH_3), 5.21 (s, 1H, pyrim), 6.90–7.50 (m, 5H, Ar-H), 8.90, 10.40 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{16}H_{14}N_4S$: C, 65.28; H, 4.79; N, 19.03; S, 10.89%. Found: C, 65.12; H, 4.92; N, 19.25; S, 10.72%.

2-(1-(6-Methyl-4-(4-methoxyphenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-yl)ethylidene) malo- nitrile (3b)

Yield: 49%; m.p. 210–212 °C; IR (KBr) υ (per cm): 2227 (CN), 3322 (NH); MS (EI) *m/z*: 324 (M^+, 18%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, CH_3, pyrim), 2.41 (s, 3H, CH_3), 3.81 (s, 3H, OMe), 5.11 (s, 1H, pyrim), 7.40 (dd, J = 8.2, J = 4.1 Hz, 4H, Ar-H), 9.30, 10.30 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27; S, 9.88%. Found: C, 63.00; H, 5.20; N, 17.38; S, 10.10%.

2-(1-(6-Methyl-4-styryl-2-thioxo-1,2,3,4-tetrahyd ropyrimidine-5-yl)ethylidene)malono-nitrile (3c)

Yield: 50%; m.p. 170–172 °C; IR (KBr) υ (per cm): 2220 (CN), 3279 (NH); MS (EI) *m/z*: 320 (M^+, 10%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.25 (s, 3H, CH_3, pyrim), 2.42 (s, 3H, CH_3), 5.22 (d, J = 8.2 Hz, 1H, pyrim), 6.30 (t, J = 7.2 Hz, 1H, CH=CH), 6.50 (d, J = 8.2 Hz, 1H, CH=CH), 6.60–7.70 (m, 5H, Ar-H), 8.20, 10.40 (s, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{16}N_4S$: C, 67.47; H, 5.03; N, 17.49; S, 10.01%. Found: C, 67.63; H, 4.99; N, 17.70; S, 10.21%.

General procedure for the synthesis of compounds 4a–c

A mixture of pyrimidine derivatives **1a–c** (10 mmol) and the appropriate aromatic aldehyde (10 mmol) in 10% ethanolic sodium hydroxide solution (50 mL) was stirred at room temperature for 24 h. The mixture was then heated under reflux for 1 h, cooled, poured onto ice water (100 mL), and acidified with 37% HCl (0.5 mL). The formed precipitate was filtered off, dried, and recrystallized from aqueous DMF to give compounds **4a–c**.

1-(6-Methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5yl)-3-(2-methoxyphenyl)-prop-2en-1-one (4a)

Yield: 85%; m.p. 240–242 °C; IR (KBr) υ (per cm): 3403 (NH), 1749 (C=O); MS (EI) *m/z*: 364 (M^+, 83%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.37 (s, 3H, CH_3, pyrim), 3.75 (s, 3H, OMe), 4.99 (s, 1H, pyrim), 5.50 (dd, J = 8.2, J = 2.3 Hz, 2H, CH=CH), 6.83–7.67 (m, 9H, Ar-H), 9.65, 9.83 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{21}H_{20}N_2O_2S$: C, 69.21; H, 5.53; N, 7.69; S, 8.80%. Found: C, 69.00; H, 5.38; N, 7.45; S, 8.59%.

1-(6-Methyl-4-(4-methoxyphenyl)-2-thioxo-1,2,3, 4-tetrahydropyrimidine-5yl)-3-(2-methoxyphenyl) -prop-2en-1-one (4b)

Yield: 64%; m.p. 145–147 °C; IR (KBr) υ (per cm): 3357 (NH), 1696 (C=O); MS (EI) *m/z*: 394 (M^+, 23%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, CH_3, pyrim), 3.63 (s, 3H, OMe), 3.73 (s, 3H, OMe), 5.52 (dd, J = 8.1, J = 5.2 Hz, 2H, CH=CH), 5.25 (s, 1H pyrim), 6.94–7.64 (m, 8H, Ar-H), 10.05, 10.13 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{22}H_{22}N_2O_3S$: C, 66.98; H, 5.62; N, 7.10; S, 8.13%. Found: C, 66.94; H, 5.40; N, 7.31; S, 8.19%.

1-(6-Methyl-4-styryl-2-thioxo-1,2,3,4-tetrahy dropyrimidine-5yl)-3-(2-methoxyphenyl)-prop-2en-1-one (4c)

Yield: 75%; m.p. 148–150 °C; IR (KBr) υ (per cm): 3399 (NH), 1688 (C=O); MS (EI) *m/z*: 390 (M^+, 5%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.46 (s, 3H, CH_3, pyrim), 3.83 (s, 3H, OMe), 4.95 (d, J = 8.0 Hz, 1H pyrim), 5.09 (dd J = 8.0, J = 2.4 Hz, 2H, CH=CH), 6.19 (t, J = 7.0 Hz, 1H, CH=CH), 6.50 (d, J = 8.0 Hz, 1H, CH=CH), 6.94–7.69 (m, 9H, aromatic), 9.68, 9.73 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{23}H_{22}N_2O_2S$: C, 70.74; H, 5.68; N, 7.17; S, 8.21%. Found: C, 70.44; H, 5.39; N, 7.49; S, 8.44%.

General procedure for the synthesis of compounds 5a–c

To a solution of chalcone derivative **4a–c** (10 mmol) in dry ethanol (30 mL), phenyl hydrazine (4 mL) was first added, followed by glacial acetic acid (5 mL). The reaction mixture was heated under reflux for 5 h, cooled, and concentrated under reduced pressure; the precipitate formed was filtered, dried, and recrystallized from ethanol to give compounds **5a–c**.

5-(5-(2-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-4-phenyl-1,2,3,4-tetrahydropyrimidine-2(1*H*)-thione (5a)

Yield: 60%; m.p. 185–187 °C; IR (KBr) υ (per cm): 3393 (NH), 1622 (C=N); MS (EI) *m/z*: 452 (M^+, 59%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.30 (s, 3H, CH_3, pyrim), 3.80 (s, 3H, OCH_3), 5.09 (s, 1H pyrim), 7.07–8.22 (m, 15H, Ar-H), 9.73, 9.82 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{27}H_{24}N_4OS$: C, 71.66; H, 5.35; N, 12.38; S, 7.08%. Found: C, 71.83; H, 5.00; N, 12.00; S, 7.32%.

5-(5-(2-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-4-(4-methoxyphenyl)-1,2,3,4-tetrahydropyrimidine-2(1*H*)-thione (5b)

Yield: 92%; m.p. 130–132 °C; IR (KBr) υ (per cm): 3479 (NH), 1599 (C=N); MS (EI) *m/z*: 482 ($M^{+\cdot}$, 86%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.26 (s, 3H, CH_3, pyrim), 3.63 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 5.02 (s, 1H pyrim), 7.07–8.32 (m, 14H, Ar-H), 10.02, 10.31 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{28}H_{26}N_4O_2S$: C, 69.69; H, 5.43; N, 11.61; S, 6.64%. Found: C, 69.39; H, 5.70; N, 11.84, S, 6.34%.

5-(5-(2-Methoxyphenyl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-4-styryl-1,2,3,4-tetrahydropyrimidine-2(1*H*)-thione (5c)

Yield: 70%; m.p. 120–122 °C; IR (KBr) υ (per cm): 3462 (NH), 1622 (C=N); MS (EI) *m/z*: 478 (M^+, 65%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.33 (s, 3H, CH_3, pyrim), 3.84 (s, 3H, OCH_3), 5.23 (d, J = 8.3 Hz, 1H pyrim), 6.21 (t, J = 7.2 Hz, 1H, CH=CH), 6.82 (d, J = 8.3 Hz, 1H, CH=CH), 7.05–8.32 (m, 15H, Ar-H), 9.60, 10.40 (2xs, 2H, 2NH, D_2O exchangeable); Anal. Calcd for $C_{29}H_{26}N_4OS$: C, 72.78; H, 5.48; N, 11.71; S, 6.70%. Found: C, 72.54; H, 5.52; N, 11.93; S, 6.88%.

General procedure for the synthesis of compounds 6a–b

A mixture of pyrimidine derivative **1d**–**e** (10 mmol) and sulfadiazine (2.50 g, 10 mmol) in DMF (30 mL) was heated under reflux for 8 h. The solution was poured onto ice water, and the precipitate was filtered off and washed with water to give **6a**–**b**.

4-(4-Methoxyphenyl)-5-(sulfadiazinyl-amido)-6-methyl-1,2,3,4-tetrahydropyrimidine-2-thiones (6a)

Yield: 79%; m.p. 195–197 °C; IR (KBr) υ (per cm): 3380 (NH), 1720 (C=O); MS (EI) *m/z*: 510 (M^+, 10%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.35 (s, 3H, CH_3, pyrim), 3.75 (s, 3H, OCH_3), 5.23 (s, 1H, pyrim), 7.33–8.62 (m, 11H, Ar-H), 9.70, 10.80, 11.00, 11.23 (4xs, 4H, 4NH, D_2O exchangeable); Anal. Calcd for $C_{23}H_{22}N_6O_4S_2$: C, 54.10; H, 4.34; N, 16.46; S, 12.56%. Found: C, 53.86; H, 4.51; N, 17.00; S, 12.72%.

4-Styryl-5-(sulfadiazinyl-amido)-6-methyl-1,2,3,4-tetrahydropyrimidine-2-thiones (6b)

Yield: 65%; m.p. 105–107 °C; IR (KBr) υ (per cm): 3390 (NH), 1655.90 (C=O); MS (EI) *m/z*: 506 (M^+, 2%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.33 (s, 3H, CH_3, pyrim), 5.01 (d, J = 8.1 Hz, 1H, pyrim), 6.70 (d, J = 8.1 Hz, 1H, CH=CH), 6.90 (t, J = 7.1 Hz, 1H, CH=CH), 7.04–8.30 (m, 12H, Ar-H), 9.50, 10.20, 10.40, 11.23 (4xs, 4H, 4NH, D_2O exchangeable); Anal. Calcd for $C_{24}H_{22}N_6O_3S_2$: C, 56.90; H, 4.38; N, 16.59; S, 12.66%. Found: C, 57.10; H, 4.21; N, 16.64; S, 12.57%.

General procedure for the preparation of compounds 8a–c

A mixture of **7** (10 mmol) in 37% HCl (10 mL) was cooled with stirring to 0–5 °C in ice, and cooled sodium nitrite solution (2.50 g, 36 mmol) in water (10 mL) was added to it dropwise during 30 min. The reaction mixture was then stirred for 30 min. Without separation, an ice-cold mixture of malononitrile (1.00 g, 15 mmol) and sodium acetate (4.10 g, 50 mmol) in ethanol (50 mL) was added dropwise with stirring during 15 min. Stirring was continued for 30 min on ice, and the reaction mixture was then left for 12 h at room temperature. The precipitate was filtered off and recrystallized from ethanol/H_2O to give **8**.

(1-Benzyl-3-cyano-4,5-diphenyl-*1H*-pyrrol-2-yl)carbonohydrazonoyl dicyanide (8a)

Yield 58%, m.p. 115–120 °C. IR (KBr) υ (per cm): 3280 (NH), 2330 (C≡N), 1585 (C=N). MS (EI) *m/z(%)*: 426 (M^+, 18%), 427 (M^++1, 5.5%), 428 (M^++2, 1.5%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 5.62 (s, 2H, CH_2), 6.90 (s, 1H, NH, hydrazone), 7.20–7.80 (m, 15H, Ar-H). Anal. Calcd for $C_{27}H_{18}N_6$ (426.47): C, 76.04; H, 4.25; N, 19.71. Found: C, 76.25; H, 4.41; N, 19.86.

(3-Cyano-1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-4,5-diphenyl-*1H*-pyrrol-2-yl)carbonohydrazonoyl dicyanide (8b)

Yield 48%, m.p. 153–157 °C. IR (KBr) υ (per cm): 3290 (NH), 2320 (C≡N), 1695(C=O), 1585 (C=N). MS (EI) *m/z(%)*: 522 (M^+, 18%), 523 (M^++1, 8.5%), 524 (M^++2, 0.8%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 2.20 (s, 2H, CH_2-C=O), 2.33 (s, 3H, CH_3), 3.12 (s, 3H, N-CH_3), 6.90 (s, 1H, NH, hydrazone), 7.30–7.90 (m, 15H, Ar-H). Anal. Calcd for $C_{31}H_{22}N_8O$ (522.559): C, 71.25; H, 4.24; N, 21.44; O, 3.06. Found: C, 71.48; H, 4.62; N, 21.55; O, 3.17.

(3-Cyano-1-(3,4-dichlorophenyl)-4-phenyl-*1H*-pyrrol-2-yl)carbon hydrazonoyl dicyanide (8c)

Yield 45%, m.p. 218–222 °C. IR (KBr) υ (per cm): 3300 (NH), 2310 (C≡N), 1565 (C=N). MS (EI) *m/z(%)*: 405 (M^+, ^{35}Cl, 19%), 407 (M^++2, ^{37}Cl, 12.2%), 409 (M^++4, 2*(^{37}Cl), 2.1%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 6.90 (s, 1H, NH, hydrazone), 7.00 (s, 1H, C_6-H), 7.20–7.80 (m, 8H, Ar-H). Anal. Calcd for $C_{20}H_{10}Cl_2N_6$ (405.24): C, 59.28; H, 2.49; Cl, 17.50; N, 20.74. Found: C, 59.41; H, 2.62; Cl, 17.65; N, 20.86.

2-Amino-1-(aryl)-4-phenyl-5-substituted-*1H*-pyrrole-3-carboxamide (10)

To a suspension of compound **7** (10 mmol) in a mixture of 37% HCl (5 mL) and glacial acetic acid (10 mL) at 0–5 °C, a solution of

sodium nitrite (4 g, 58 mmol) in water (10 mL) was added dropwise with continuous stirring during 30 min. After 8 h of stirring at room temperature, the foamy mixture was diluted with water and neutralized with aqueous ammonia. The solid was filtered off and recrystallized from EtOH/H_2O to give compound **10.** Compound **10c** prepared with this method is identical in all respects (physical and spectral data) as reported (18).

2-Amino-1-benzyl-4,5-diphenyl-*1H*-pyrrole-3-carboxamide (10a)

Yield 60%, m.p. 98–102 °C. IR (KBr) υ (per cm): 3350-3300 (NH_2), 1680 (C=O). MS (EI) *m/z(%)*: 367 (M^+, 27%), 368 (M^++1, 8.5%), 369 (M^++2, 1.3%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 5.62 (s, 2H, CH_2), 6.50 (s, 2H, NH_2), 7.20–8.00 (m, 15H, Ar-H and 2H, $CONH_2$). Anal. Calcd for $C_{24}H_{21}N_3O$ (367.443): C, 78.45; H, 5.76; N, 11.44; O, 4.35. Found: C, 78.26; H, 5.59; N, 11.32; O, 4.61.

2-Amino-1-(3,4-dichlorophenyl)-4-phenyl-*1H*-pyrrole-3-carboxamide (10c)

Yield 58%, m.p. 178–181 °C. IR (KBr) υ (per cm): 3330-3280 (NH_2), 1670 (C=O). MS (EI) *m/z(%)*: 346 (M^+, ^{35}Cl, 32%), 348 (M^++2, ^{37}Cl, 19%), 350 (M^++4, 2*(^{37}Cl), 5.6%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 6.60 (s, 2H, NH_2), 7.00 (s, 1H, C_6-H), 7.30–8.00 (m, 8H, Ar-H and 2H, $CONH_2$). Anal. Calcd for $C_{17}H_{13}Cl_2N_3O$ (346.211): C, 58.98; H, 3.78; Cl, 20.48; N, 12.14; O, 4.62. Found: C, 59.04; H, 3.96; Cl, 20.71; N, 12.47; O, 4.85.

7-Aryl-5,6-disubstituted-2-thioxo-2,3-dihydro-*1H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (11)

A mixture of compound **10** (10 mol) and thiourea (1.20 g, 20 mmol) was refluxed in dry ethanol (20 mL) for 10 h. The reaction mixture was evaporated under reduced pressure and the residue was recrystallized from methanol to give **11**.

7-Benzyl-5,6-diphenyl-2-thioxo-2,3-dihydro-*1H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (11a)

Yield: 80%, m.p. 92–94 °C. IR (KBr) υ (per cm): 3350 (NH), 1680 (C=O), 1615 (C=S). MS (EI) *m/z(%)*: 409 (M^+, 45%), 410 (M^++1, 13.5%), 411 (M^++2, 1.1%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 5.56 (s, 2H, CH_2), 7.10–7.80 (m, 15H, Ar-H), 12.20 (s, 1H, NH), 13.30 (s, 1H, NH). Anal. Calcd for $C_{25}H_{19}N_3OS$ (409.503): C, 73.32; H, 4.68; N, 10.26; O, 3.91; S, 7.83. Found: C, 73.49; H, 4.90; N, 10.42; O, 3.63; S, 7.86.

7-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-5,6-diphenyl-2-thioxo-2,3-dihydro-*1H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (11b)

Yield 74%, m.p. 132–137 °C. IR (KBr) υ (per cm): 3390 (NH), 1690 (C=O), 1630 (C=S). MS (EI) *m/z(%)*: 505 (M^+, 70.0%), 506 (M^++1, 22.17%), 507 (M^++2, 3.73%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 2.43 (s, 3H, CH_3), 3.12 (s, 3H, N-CH_3), 7.20–7.80 (m, 15H, Ar-H), 12.32 (s, 1H, NH), 13.14 (s, 1H, NH). Anal. Calcd for $C_{29}H_{23}N_5O_2S$ (505.590): C, 68.89; H, 4.59; N, 13.85; O, 6.33; S, 6.34. Found: C, 68.89; H, 4.59; N, 13.85; O, 6.33; S, 6.34.

7-(3,4-Dichlorophenyl)-5-phenyl-2-thioxo-2,3-dihydro-*1H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (11c)

Yield 75%, m.p. 138–140 °C. IR (KBr) υ (per cm): 3360 (NH), 1690 (C=O), 1605 (C=S). MS (EI) *m/z*: 387 (M^+, ^{35}Cl, 20%), 389 (M^++2, ^{37}Cl, 8.3%), 391 (M^++4, 2*(^{37}Cl), 7.5%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 7.00 (s, 1H, C_6-H), 7.30–8.00 (m, 8H, Ar-H), 12.20 (s, 1H, NH), 13.30 (s, 1H, NH). Anal. Calcd for $C_{18}H_{11}Cl_2N_3OS$ (388.270): C, 55.68; H, 2.86; Cl, 18.26; N, 10.82; O, 4.12; S, 8.26. Found: C, 55.89; H, 2.97; Cl, 18.58; N, 10.99; O, 4.23; S, 8.49.

7-Aryl-5,6-disubstituted-2-(ethylthio)-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (12)

Compound **11** (10 mmol) was added to a warmed alcoholic solution of sodium (0.56 g, 10 mmol) in ethanol (50 mL) and heating was continued for 20 min. The mixture was allowed to cool to room temperature, and alkyl halide (20 mmol) was added dropwise with continuous stirring during 30 min. The mixture was stirred under reflux for 5 h, monitored by TLC, allowed to cool to room temperature, and finally poured into cold water. The solid product was filtered off and washed with water. The residue was dried and recrystallized from methanol to give **12**.

7-Benzyl-2-(ethylthio)-5,6-diphenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (12a)

Yield 80%, m.p. 84–86 °C. IR (KBr) υ (cm-1): 3330 (NH), 1680(C=O). MS (EI) *m/z(%)*: 437 (M^+, 34%), 438 (M^++1, 10.28%), 439 (M^++2, 1.4%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 1.32 (t, 3H, J = 7.2 Hz, CH_3), 3.53 (q, 2H, J = 7.2 Hz, CH_2), 5.98 (s, 2H, CH2), 7.10–7.80 (m, 15H, Ar-H), 12.59 (s, 1H, NH). Anal. Calcd for $C_{27}H_{23}N_3OS$ (437.556): C, 74.11; H, 5.30; N, 9.60; O, 3.66; S, 7.33. Found: C, 74.41; H, 5.63; N, 9.80; O, 3.79; S, 7.56.

7-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-2-(ethylthio)-5,6-diphenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7*H)-one (12b)

Yield 64%, m.p. 145–147 °C. IR (KBr) υ (per cm): 3450 (NH), 1700 (C=O), 1675 (C=O). MS (EI) *m/z(%)*: 533 (M^+, 31.15%), 534 (M^++1, 11.2%), 535 (M^++2, 2.3%). ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): ^{1}H NMR (DMSO-d_6, 300MHz) δ (ppm): 1.46 (t, 3H, J = 7.5, CH_3), 2.43 (s, 3H, CH_3), 3.01 (q, 3H, J = 7.5, CH_3), 3.21 (s, 3H, N-CH_3), 7.00–7.80 (m, 15H, Ar-H), 12.49 (s, 1H, NH). Anal. Calcd for $C_{31}H_{27}N_5O_2S$ (533.643): C, 69.77; H, 5.10; N, 13.12; O, 6.00; S, 6.01. Found: C, 70.01; H, 5.38; N, 16.01; O, 6.25; S, 6.35.

7-(3,4-Dichlorophenyl)-2-(ethylthio)-5-phenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (12c)

Yield 76%, m.p. 125–128 °C. IR (KBr) υ (cm-1): 3410 (NH), 1680 (C=O). MS (EI*) m/z (%)*: 416 (M+, 35Cl, 43.2%), 418 (M++2, 37Cl, 8.9%), 420 (M++4, 2*(37Cl), 2.1%). 1H NMR (DMSO-d_6, 300MHz) δ (ppm): 1.43 (t, 3H, J = 6.8,CH3), 3.23 (q, 3H, J = 6.8, CH3), 6.90 (s, 1H, C_6-H), 7.20–7.80 (m, 8H, Ar-H), 12.61 (s, 1H, NH). Anal. Calcd for $C_{20}H_{15}Cl_2N_3OS$ (416.324): C, 57.70; H, 3.63; Cl, 17.03; N, 10.09; O, 3.84; S, 7.70. Found: C, 57.93; H, 3.89; Cl, 17.26; N, 10.18; O, 3.96; S, 7.75.

7-(Aryl)-2-(chloromethyl)-5,6-disubstituted-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (17)

A mixture of **7** (10 mmol) and chloroacetyl chloride (5.65 g, 50 mmol) in dry dioxane (30 mL) was allowed to stir at room temperature overnight and then under reflux for 5 h. The solvent was evaporated, and the remaining mixture was triturated with ethanol. The solid product that formed was collected by filtration and recrystallized from ethanol/ H_2O to give **17**.

1-(4-Amino-1-benzyl-6-methyl-2,3-diphenyl-*1H*-pyrrolo[2,3-*b*]pyridin-5-yl) ethanone (17a)

Yield 64%, m.p. 130–135 °C. IR (KBr) υ (per cm): 3320 (NH), 1670 (C=O). MS (EI) *m/z*: 425 (M^+, 65%), 426 (M^++1, 15%), 427 (M^++2, ^{37}Cl, 23.5%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 3.60 (s, 2H, CH_2-Cl), 5.85 (s, 2H, CH_2), 7.00–7.60 (m, 15H, Ar-H), 11.14 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{26}H_{2\circ C}ClN_3O$ (425.909): C, 73.32; H, 4.73; Cl, 8.32; N, 9.87; O, 3.76. Found: C, 73.43; H, 4.86; Cl, 8.41; N, 9.93; O, 3.85.

2-(Chloromethyl)-7-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-5,6-diphenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (17b)

Yield 61%, m.p. 110–112 °C. IR (KBr) υ (per cm): 3335 (NH), 1700, 1680 (C=O). MS (EI) *m/z*: 521 (M^+, 44%), 522 (M^++1, 12%), 523 (M^++2, ^{37}Cl, 15.5%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 2.39 (s, 3H, CH_3), 3.21 (s, 3H, N-CH_3), 3.62 (s, 2H, CH_2-Cl), 7.00–7.60 (m, 15H, Ar-H), 12.24 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{30}H_{24}ClN_5O_2$ (521.997): C, 69.03; H, 4.63; Cl, 6.79; N, 13.42; O, 6.13. Found: C, 69.10; H, 4.84; Cl, 6.88; N, 13.15; O, 5.99.

2-(Chloromethyl)-7-(3,4-dichlorophenyl)-5-phenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (17c)

Yield 63%, m.p. 103–105 °C. IR (KBr) υ (per cm): 3330 (NH), 1680 (C=O). MS (EI) *m/z*: 404 (M^+, ^{35}Cl, 75%), 406 (M^++2, ^{37}Cl, 51%), 408 (M^++4, 2*(^{37}Cl), 17.5%), 410 (M^++6, 3*(^{37}Cl), 4.5%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 3.46 (s, 2H, CH_2-Cl), 6.70 (s, 1H, C_6-H), 7.00–7.50 (m, 8H, Ar-H), 12.04 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{19}H_{12}Cl_3N_3O$ (404.677): C, 56.39; H, 2.99; Cl, 26.28; N, 10.38; O, 3.95. Found: C, 56.12; H, 2.63; Cl, 26.09; N, 10.25; O, 3.72.

7-(Aryl)-2,5,6-trisubstituted-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (18)

To a solution of **17** (10 mmol), hydrazine hydrate (5 mL) and pyridine (2 mL) in ethanol (30 mL) were added. The reaction mixture was refluxed for 8 h (monitored by TLC). The mixture was then cooled to room temperature, poured onto crushed ice (25 g), and neutralized with HCl. The precipitate was filtered off and recrystallized from ethanol/H_2O to give **18**.

7-Benzyl-2-(hydrazinylmethyl)-5,6-diphenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (18a)

Yield 61%, m.p. 117–120 °C. IR (KBr) υ (per cm): 3350-3290 (NH, NH_2), 1670 (C=O). MS (EI) *m/z*: 421 (M^+, 32%), 422 (M^++1, 11%), 423 (M^++2, 1.5%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 3.02 (s, 2H, CH_2-NH), 4.92 (s, 2H, NH_2), 5.85 (s, 2H, CH_2), 6.80 (s, 1H, NH), 7.00–7.70 (m, 15H, Ar-H), 12.45 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{26}H_{23}N_5O$ (421.494): C, 74.09; H, 5.50; N, 16.62; O, 3.80. Found: C, 74.16; H, 5.63; N, 16.71; O, 3.96.

7-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-2-(hydrazinylmethyl)-5,6-diphenyl-*3H*-pyrrolo[2,3-*d*]pyrimidine-4(*7H*)-one (18b)

Yield 55%, m.p. 130–132 °C. IR (KBr) υ (per cm): 3380-3320 (NH, NH_2), 1690, 1665 (C=O). MS (EI) *m/z*: 517 (M^+, 35%), 518 (M^++1, 12%), 519 (M^++2, 2.8%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 2.59 (s, 3H, CH_3), 3.21–3.25 (s, 5H, N-CH_3 and CH_2-NH), 4.90 (s, 2H, NH_2), 6.80 (s, 1H, NH), 7.20–7.80 (m, 15 H, Ar-H), 12.44 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{30}H_{27}N_7O_2$ (517.581): C, 69.62; H, 5.26; N, 18.94; O, 6.18. Found: C, 69.79; H, 5.42; N, 19.08; O, 6.21.

7-(Aryl)-8,9-disubstituted-3,7-dihydro-*2H*-imidazo[1,2-*c*]pyrrolo[3,2-*e*]pyrimidine-2-one (20)

A mixture of **19** (10 mmol) and ethyl bromoacetate (50 mmol) in absolute ethanol (25 mL) containing pyridine (1–2 mL) was heated on a water bath for 15 h. The solid product obtained after cooling was collected by filtration and recrystallized from ethanol to give **20.**

7-Benzyl-8,9-diphenyl-3,7-dihydro-*2H*-imidazo[1,2-*c*]pyrrolo[3,2-*e*]pyrimidine-2-one (20a)

Yield 52%, m.p. 128–130 °C. IR (KBr) υ (per cm): 1695 (C=O). MS (EI) *m/z*: 416 (M^+, 18%), 417 (M^++1, 5.5%), 418 (M^++2, 2.25%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 3.68 (s, 2H, CO-CH_2), 5.88 (s, 2H, CH_2), 7.00–7.70 (m, 15H, Ar-H), 8.44 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{27}H_{20}N_4O$ (416.474): C, 77.87; H, 4.84; N, 13.45; O, 3.84. Found: C, 77.97; H, 4.99; N, 13.63; O, 3.95.

7-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl)-8,9-diphenyl-3,7-dihydro-*2H*-imidazo[1,2-*c*]pyrrolo[3,2-*e*]pyrimidine-2-one (20b)

Yield 51%, m.p. 142–144 °C. IR (KBr) υ (per cm): 1690, 1680 (C=O). MS (EI) *m/z*: 512 (M^+, 19%), 513 (M^++1, 5.6%), 514 (M^++2, 1.05%). ^1H NMR (DMSO-d_6, 500MHz) δ (ppm): 2.32 (s, 3H, CH_3), 3.21 (s, 3H, N-CH_3), 3.79 (s, 2H, CO-CH_2), 7.00–7.60 (m, 15 H, Ar-H), 8.26 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{31}H_{24}N_6O_2$ (512.561): C, 72.64; H, 4.72; N, 16.40; O, 6.24. Found: C, 72.77; H, 4.89; N, 16.63; O, 6.41.

7-(3,4-Dichlorophenyl)-9-phenyl-3,7-dihydro-*2H*-imidazo[1,2-*c*]pyrrolo[3,2-*e*]pyrimidine-2-one (20c)

Yield 48%, m.p. 280–282 °C. IR (KBr) υ (per cm): 1680 (C=O). MS (EI) *m/z*: 395 (M^+, ^{35}Cl, 22%), 397 (M^++2, ^{37}Cl, 6.5%), 399 (M^++4, 2*(^{37}Cl), 1.7%), 410 (M^++6, 3*(^{37}Cl), 4.5%). ^1H NMR (DMSO-d_6,

500MHz) δ (ppm): 3.65 (s, 2H, CO-CH_2), 6.80 (s, 1H, C_6-H), 7.00–7.70 (m, 8H, Ar-H), 8.26 (s, 1H, NH pyrimidine). Anal. Calcd for $C_{20}H_{12}Cl_2N_4O$ (395.241): C, 60.78; H, 3.06; Cl, 17.94; N, 14.18; O, 4.05. Found: C, 60.88; H, 3.28; Cl, 17.69; N, 14.05; O, 4.36.

Enzyme expression and purification

The IMP-1 enzyme, lacking the first 21 signal peptide amino acid residues, was expressed and purified using a previously published procedure (15,23).

Kinetic assays

Inhibition assays were performed in 96-well 400-μL multi-titer plates using a UV/Vis multi-plate spectrophotometer. The assay was performed in HEPES buffer (50 mM HEPES, 0.1 M NaCl, 100 μM $ZnCl_2$, pH 7.0). The substrate CENTA (Figure 1) was synthesized according to the previously published work (15,24) with final substrate concentrations ranging between 5–70 μM. The final concentration of IMP-1 in the assay was 2 nM. A final concentration of 20 μg/mL of bovine serum albumin was added to the assay. Three different concentrations of the compounds **1a**, **1c**, **2b**, **3b**, **5c**, **6b**, **7a**, **8a**, **11c**, **13a**, **15a**, and **16a** were used as illustrated in Figure 2 for compound **5c**. The inhibition data were analyzed by nonlinear regression using WinCurveFit (Kevin Raner Software) and eqn 1, where V_{max} is the maximum rate, K_M the Michaelis constant, and K_{ic} and K_{iuc} the inhibition constants for the competitive and uncompetitive inhibition modes, respectively (25).

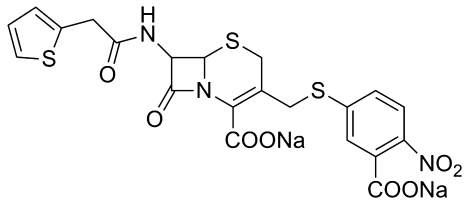


Figure 1: Chemical structure of CENTA.

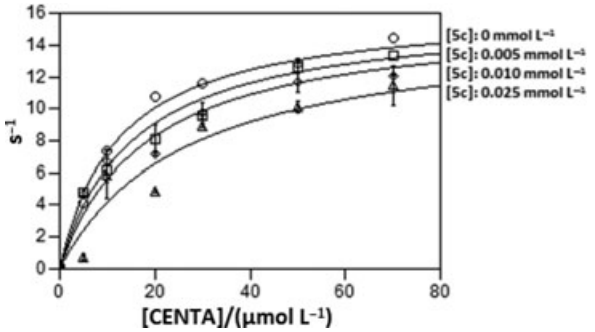


Figure 2: Determination of the inhibition constants for compound **5c** against IMP-1 MBL (2 nM) using CENTA as the substrate. Inhibitor concentrations are indicated on the right side. The experimental data were fit to eqn 1; error bars indicate standard errors.

$$v = \frac{V_{max}[S]}{[S]\left(1 + \frac{[I]}{K_{iuc}}\right) + K_M\left(1 + \frac{[I]}{K_{ic}}\right)} \quad (1)$$

Results and Discussion

Synthesis of lead compounds

The syntheses of compounds **1a–e** (26–30) were achieved by condensing thiourea, 1,3-dicarbonyl derivatives, and an aromatic aldehyde in refluxing ethanol containing a few drops of 37% HCl, to give the 5-acetyl tetrahydrothiopyrimidine-2-thione derivatives **1a–1c**, or the 5-ethoxycarbonyl derivatives **1d–e**, in very good yields, as shown in Figure 3.

As shown in Figure 4, tetrahydropyrimidine-2-thiones **1a–c** reacted with chloroacetic acid in aqueous sodium hydroxide to afford acetic acid derivatives **2a–c**. On the other hand, when **1a–c** were condensed with malononitrile in glacial acetic acid and ammonium acetate, they give ethylidene malononitrile derivatives **3a–c**. The reactions of **1a–c** with 2-methoxybenzaldehyde in ethanolic sodium hydroxide afforded chalcone derivatives **4a–c**, which were converted to pyrazole derivatives **5a–c** by their reactions with phenyl hydrazine.

The ethoxycarbonyl tetrahydropyrimidine-2-thiones (**1d**–**e**) reacted with sulfadiazine in DMF to afford amide derivatives **6a–b**, as shown in Figure 5.

The 2-amino-3-cyanopyrrole derivatives (**7a–c**) were prepared as reported in our previous works (17–21). These compounds were used for the preparation of pyrrole derivatives **8a–c** through the formation of diazonium chloride salt intermediates. Diazotization of 2-amino-pyrroles have been reported several times (31–34). Diazotization of **7a–c** using a mixture of sodium nitrite and concentrated HCl at 0–5 °C followed by the addition of an ethanolic solution of malononitrile in the presence of sodium acetate afforded the corresponding hydrazono derivatives **8a–c** (Figure 6). On the other hand, diazotization of **7a–c** using sodium nitrite in a mixture of acetic acid and concentrated HCl (2:1) at 0–5 °C afforded the carboxamide derivatives **10a–c**. This was unexpected, as there are many reports (31–37) that diazotization of compounds similar to **7** gives the corresponding triazine derivatives (**9**). The formation of compounds **10** can be explained by the hydrolysis of the cyano group in acidic medium into the amide without formation of the unstable diazonium salt from the amino group. The pyrrole derivatives **10a–10c** were converted to the corresponding pyrrolo[2,3-*d*]pyrimidine-2-thiones **11a–c** by heating (38) with thiourea in ethanol. Ethylation (39–41) of **11a–c** with ethyl iodide in the presence of base gave the corresponding S-ethyl pyrimidine derivatives **12a–12c** (Figure 6).

The 2-amino-3-cyanopyrrole derivatives (**7a–c**) were also used for the preparation of pyrrolopyrimidine derivatives **13–20**. Condensation of **7a–c** with formic acid afforded the corresponding pyrrolopyrimidine-4-ones (**13a–c**) (Figure 7) (17–21,42). Reacting **13a–c** (17–21,42) with phosphorous oxychloride gave their corresponding 4-chloro derivatives **14a–c**, which were subsequently reacted (17–21,42) with thiourea and hydrazine hydrate, separately, to give the

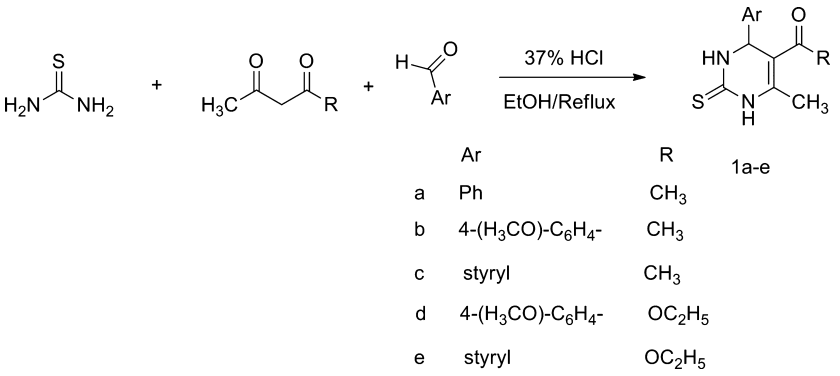


Figure 3: Synthesis of compounds **1a–e**.

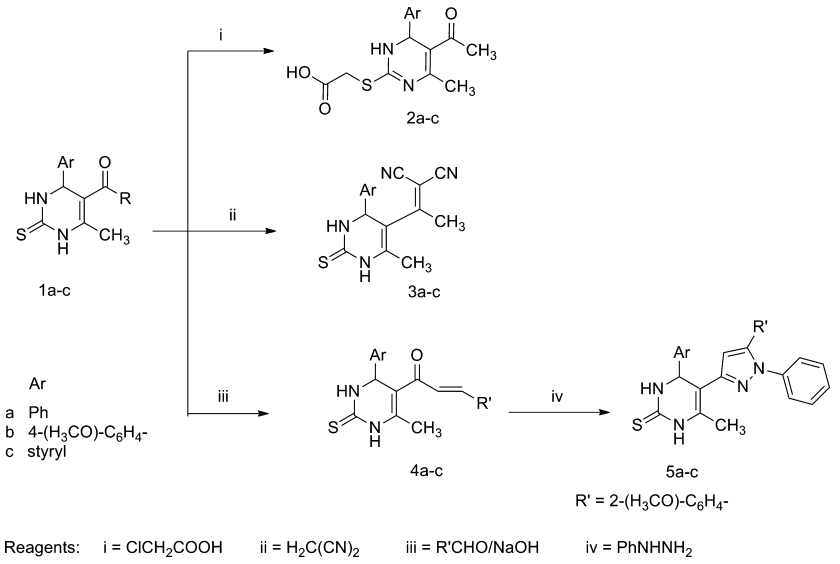


Figure 4: Synthesis of compounds **2–5**.

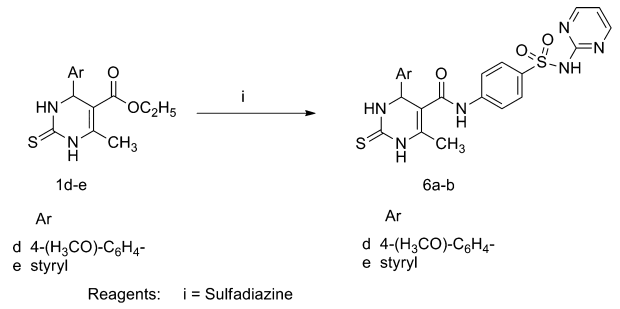


Figure 5: Synthesis of compounds **6a–b**.

corresponding pyrrolopyrimidine-4-thione (**15a–c**) and 4-hydrazino derivatives (**16a–c**), respectively.

Heating (43,44) **7a–c** with chloroacetyl chloride in refluxing dioxane afforded the pyrrolopyrimidine-4-one derivatives **17a–c**, which on reaction with hydrazine hydrate in refluxing ethanol gave the hydrazinomethyl derivatives **18a–c**. The enaminonitrile moiety in compound **7a–c** proved to be highly reactive toward other nitrogen nucleophiles. Thus, the reaction of **7a–c** with formamide (17–21) afforded the corresponding pyrrolopyrimidines **19a–c**. Heating **19a–c** with ethyl bromoacetate and pyridine in ethanol solution yielded the corresponding imidazopyrrolopyrimidine-2-ones **20a**–**c** (Figure 7).

Enzymatic inhibition assays

Based on the compounds' solubility under the assay conditions, the inhibitory effects of 10 thiopyrimidine derivatives **1a–e**, **2b**, **3b**, **4c**, **5c**, and **6b** (Table 1) and 17 pyrrole derivatives **7a–c**, **8a–b**, **10c**, **11c**, **13a–b**, **15a–c**, **16a–c**, **18a**, and **20b** (Table 2) on the catalytic activity of IMP-1 were initially assessed by measuring enzyme activity in the absence and presence of 10 μM of each compound. A previously developed assay based on the chromogenic cephalosporin substrate CENTA was used (15,24). The hydrolysis of CENTA by IMP-1 releases the chromophore 4-nitrophenolate, the formation of which can be measured spectrophotometrically with a plate reader at λ = 405 nm (at pH 7.0, ε = 6400/M/cm). The results are presented in Tables 1 and 2.

Twelve compounds, six thiopyrimidine derivatives (**1a**, **1c**, **2b**, **3b**, **5c**, and **6b**), and six pyrrole derivatives (**7a**, **8a**, **11c**, **13a**, **15a**, and **16a**) showed promising inhibition at a concentration of 10 μM.

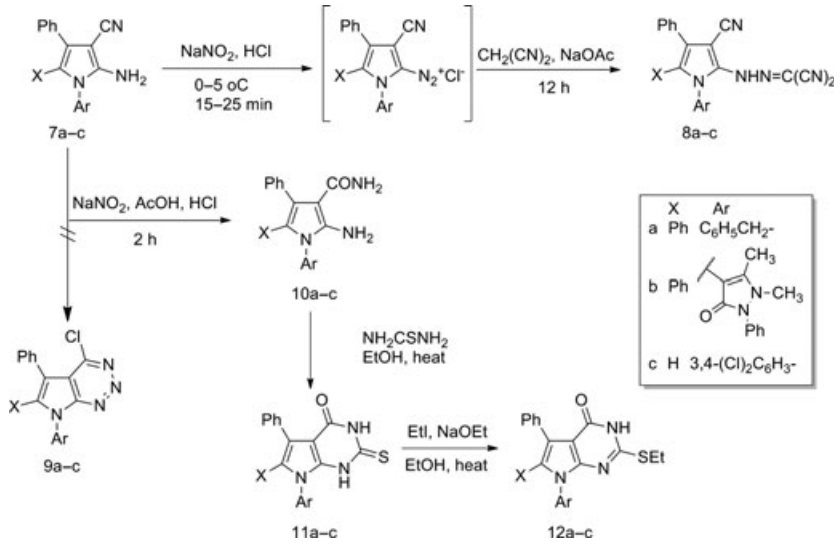


Figure 6: Synthesis of compounds **7–12**.

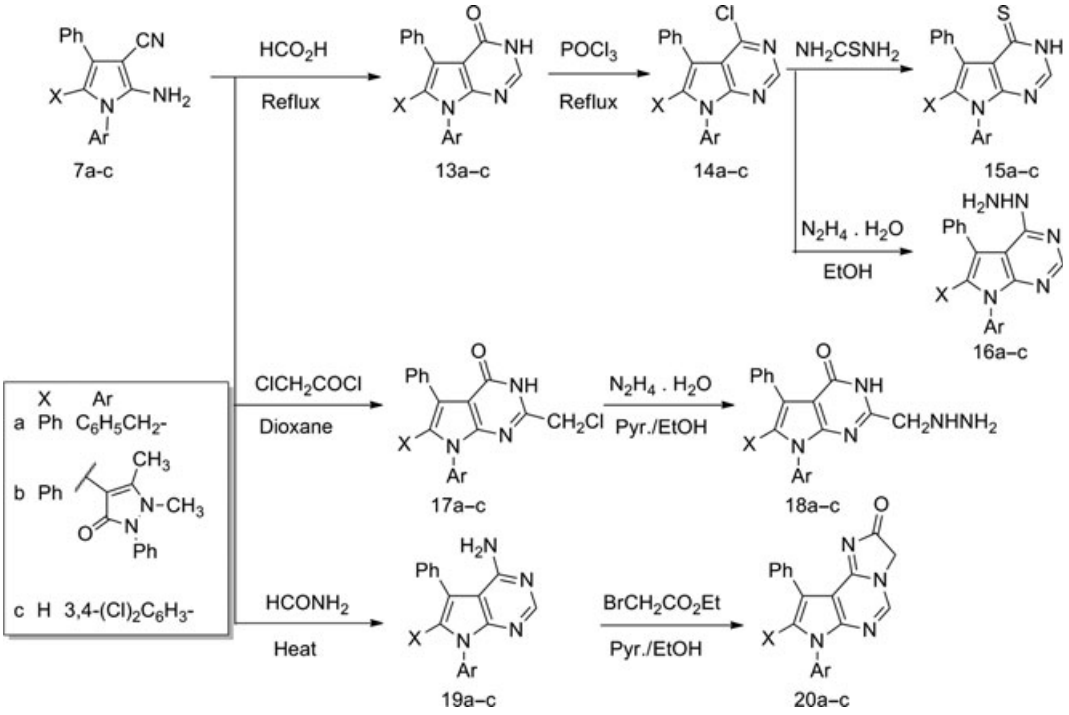
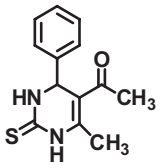
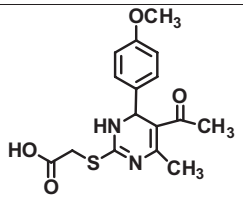
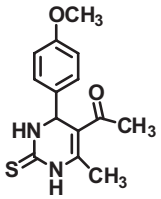
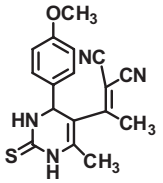
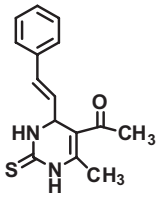
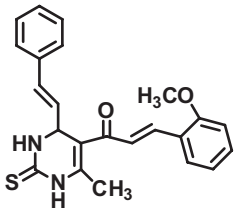
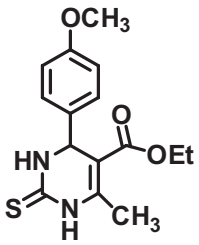
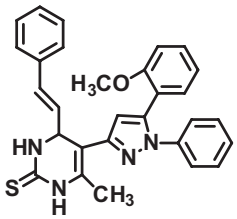
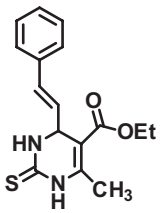
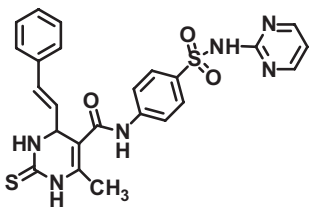


Figure 7: Synthesis of compounds **13–20**.

Therefore, they were selected for further analysis to determine their mode of inhibition and the magnitude of the corresponding inhibition constant(s) (K_i values).

As shown in Table 3, compounds **1a**, **5c**, **7a**, **8a**, and **11c** are the strongest inhibitors for IMP-1; all have inhibition constants of ~20 μM. The remaining compounds in Table 3 are also good inhibitors with inhibition constants ranging from 36 to 148 μM. Compounds **1a**, **5c**, **6b**, and **7a** are purely competitive inhibitors in this group with all the others displaying a mixed mode of inhibition, thus implying alternative possible binding interactions with the enzyme. Compound **5c** is the most potent inhibitor of IMP-1, with a K_{ic} of 19 μM. For comparison, the K_{ic} of the known MBL inhibitor L-captopril is 12.5 ± 2.4 μM (15), and those of compounds discovered from a fragment-based screening approach range from ~500 μM to ~1600 μM (15). The inhibition constants for the compounds reported here are also of comparable efficiency to a range of pyrrole derivatives reported previously (44). It is noted that, in general, the weaker the inhibitory effect is, the larger is the error associated with the corresponding inhibition constants (Table 3).

Table 1: Percentage inhibition of IMP-1 MBL (2 nM) at pH 7.0 by thiopyrimidine derivatives using CENTA (70 μM) as substrate

Compound	Structure	Inhibition % (10 μM)	Compound	Structure	Inhibition % (10 μM)
1a		29	**2b**		12
1b		0	**3b**		20
1c		27	**4c**		0
1d		0	**5c**		17
1e		0	**6b**		12

MBL, metallo-β-lactamase.

To investigate the possible binding modes of the most potent inhibitor **5c** to IMP-1, *in silico* docking was employed using Molegro Virtual Docker (45). The chemical structures of the two enantiomers of **5c** were drawn in ChemBioOffice, and their energies were minimized, and the resulting structures were saved in SYBL format (46,47). Molegro Virtual Docker was also used to modify the charge of the sulfur atom of the thiopyrimidine ring in **5c** to be negative (48), and the three bonds in the thiourea linkage in the same ring were modified to be delocalized bonds. The IMP-1 crystal structure (PDB Code: 1JJT) (47) was used in these docking studies. The crystal structure was modified for docking simulations by allowing residues Glu60, Val61, Asn62, Gly63, and Trp64 in a loop close to the active site to be flexible (49).

Table 2: Percentage inhibition of IMP-1 MBL (2 nM) at pH 7.0 by pyrrole derivatives using CENTA (70 μM) as substrate

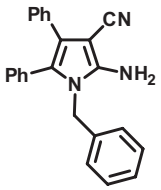
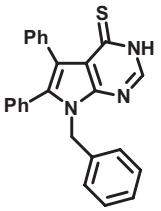
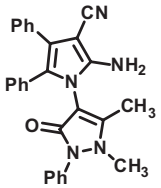
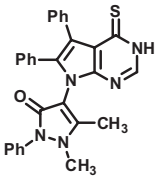
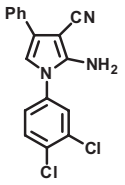
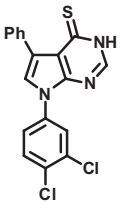
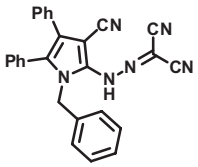
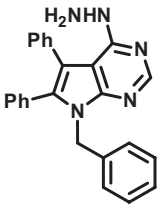
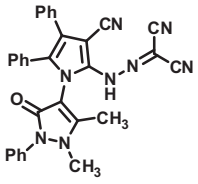
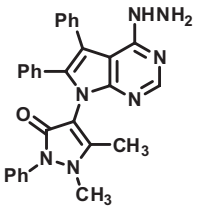
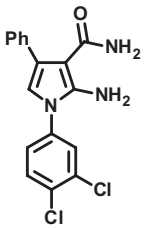
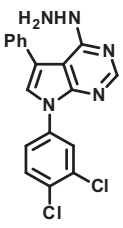
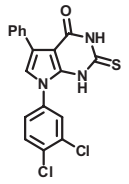
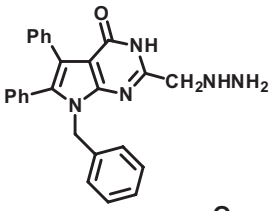
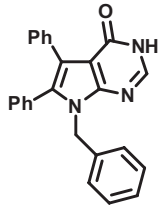
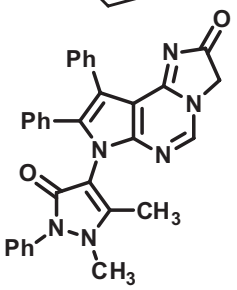
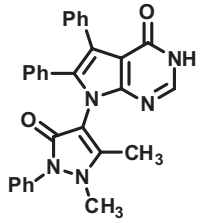
Compound	Structure	Inhibition % (10 μM)	Compound	Structure	Inhibition % (10 μM)
7a		17	**15a**		11
7b		0	**15b**		0
7c		7	**15c**		0
8a		57	**16a**		17
8b		6	**16b**		5
10c		0	**16c**		11

Table 2: Continued

Compound	Structure	Inhibition % (10 μM)	Compound	Structure	Inhibition % (10 μM)
11c		15	**18a**		6
13a		15	**20b**		0
13b		0			

MBL, Metallo-β-lactamase.

The lowest energy-binding orientation of (*R*)-**5c** in the active site of IMP-1 is shown in Figure 8. The modeling indicates that (*R*)-**5c** binds to the two zinc ions via the thiolate anion with sulfur–metal distances of 2.2 Å (Zn1) and 2.0 Å (Zn2). Apart from these interactions with the metal ions, one of the nitrogen atoms of the thiopyrimidine ring forms hydrogen bond to the nitrogen atom of the side chain of Lys224. Furthermore, there are hydrophobic interactions between the isopropyl group of Val67 and the methoxybenzene moiety. Furthermore, the interactions between the benzene ring of Phe87 and the isopropyl group of Val61 with the benzene ring of the styryl group also contribute to inhibitor binding (Figure 9).

In contrast, the lowest energy conformation of (*S*)-**5c** occupies the active site, close to the two zinc ions, but without any significant binding between the inhibitor and either of the two zinc ions (Figure 10). However, two hydrogen bonds are observed between the inhibitor and the enzyme-one between the oxygen of the methoxy group and the backbone N-H of Asn233, and the second between one of the N atoms of the thiopyrimidine ring of **5c** and the nitrogen atom of the side chain of Lys224. Furthermore, there are hydrophobic interactions between both the isopropyl group of Val61 and the benzene ring of residue Phe87 in IMP-1 and the benzene ring attached to the nitrogen atom of the pyrazole moiety in **5c**, between the isopropyl group of Val67 and the benzene ring of the styryl moiety, and also between the imidazole ring of His118 and the benzene ring attached to the methoxy group (Figure 11).There is a large difference in the docking score between the two enantiomers of **5c**, which indicates the more favorable binding energy for the (*R*) isomer.

To investigate the binding mode of the pyrrole series, the most potent inhibitor of pyrrole derivatives against IMP-1, **7a** with a K_{ic} of 21 μM, was docked into the active site of IMP-1 by using Molegro Virtual Docker as discussed before. The modeling illustrates that **7a** binds within the active site, close to the two zinc atoms. The nitrogen atom of the amino group of **7a** is located close to both metal ions in the active site (Zn1: 4.0 Å; Zn2: 3.3 Å) (Figure 12).

One of the protons on the amino group of **7a** forms a hydrogen bond with the nitrogen atom on the imidazole ring of His197 (N–N distance 3.0 Å) (Figure 13). Apart from this interaction, two hydrophobic interactions between **7a** and Val67 and Phe87 of IMP-1 may play an important role in enhancing the binding affinity of the inhibitor (Figure 13). The binding mode shown in Figures 10 and 11 represents competitive inhibition, as the close proximity of compound **7a** to the metal ions implies that the access of a substrate to the catalytically relevant binuclear metal center is blocked.

Conclusion

We have described a straightforward and efficient synthesis of thiopyrimidine and pyrrole derivatives that act as inhibitors of the MBL

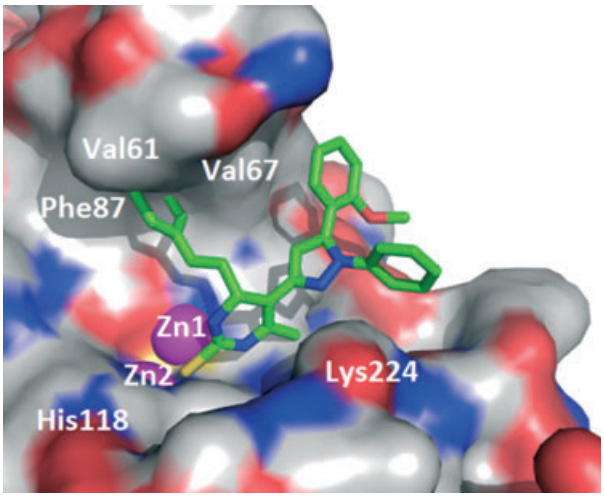


Figure 8: Surface view of the IMP-1 active site with the lowest energy conformation of (*R*)-**5c** docked into the active site. Atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1); green – carbon, yellow – sulfur (on inhibitor); magenta – zinc active site metals. The flexible loop consisting of residues Asn62, Gly63, Trp64, and Asn233 has been omitted for clarity.

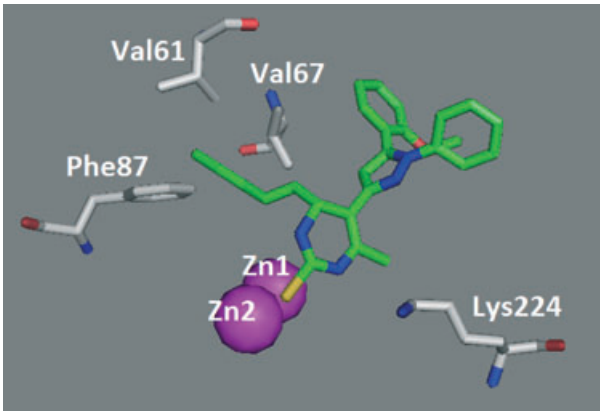


Figure 9: Surface view of the IMP-1 active site for the highest docking score conformation of IMP-1 with (*R*)-**5c** docked into the active site shows (i) the hydrogen bond (3.2 Å) between the amino group of (*R*)-**5c** and the nitrogen atom of the side chain of Lys224, (ii) the hydrophobic interaction between the isopropyl group of Val67 and the methoxybenzene moiety, and (iii) the hydrophobic interaction between the benzene ring of Phe87 and the isopropyl group of Val61 with the benzene ring of the styryl group of (*R*)-**5c**. Atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1), green – carbon (on inhibitor), magenta – zinc ions.

IMP-1. The inhibitory effects of 27 compounds **1a–e**, **2b**, **3b**, **4c**, **5c**, **6b**, **7a–c**, **8a–b**, **10c**, **11c**, **13a–b**, **15a–c**, **16a–c**, **18a**, and **20b** were tested against IMP-1. The thiopyrimidine derivatives **1b**, **1d**, **1e**, and **4c** and the pyrrole derivatives **7b**, **10c**, **13b**, **15b**, **15c**, and **20b** had no activity. In thiopyrimidine derivatives, compound **5c** showed the most potent inhibitory effect (K_{ic} = 19 ± 9 μM) with a purely competitive mode of inhibition. The

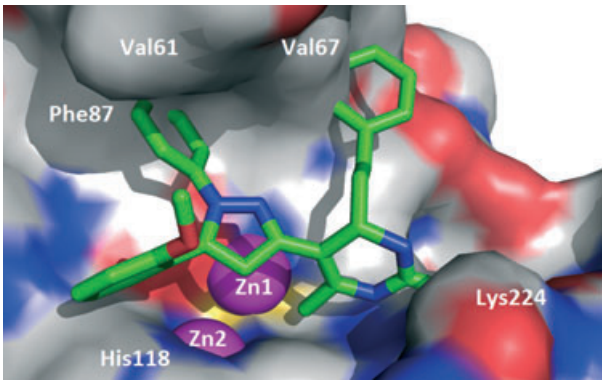


Figure 10: Surface view of the IMP-1 active site with the lowest energy conformation of (*S*)-**5c** docked into the active site. Atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1); green – carbon, yellow – sulfur (on inhibitor); magenta – zinc active site metals. The flexible loop consisting of residues Asn62, Gly63, Trp64, and Asn233 has been omitted for clarity.

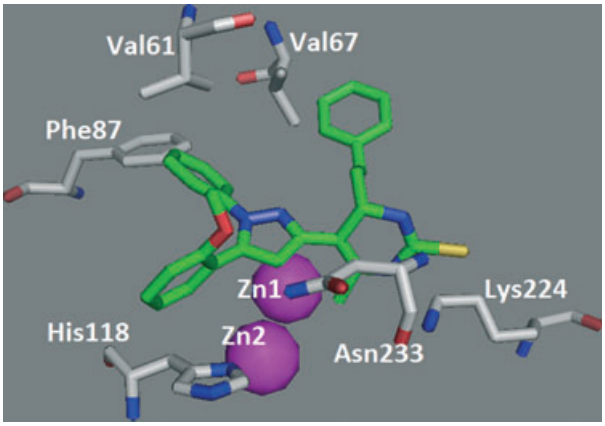


Figure 11: Surface view of the IMP-1 active site for the highest docking score conformation of IMP-1 with (*S*)-**5c** docked into the active site shows (i) the hydrogen bond (3.1 Å) between the oxygen of the methoxy group of (*S*)-**5c** and the backbone N-H of Asn233, (ii) the hydrogen bond (2.6 Å) between one of the N atoms of the thiopyrimidine ring of (*S*)-**5c** and the nitrogen atom of the side chain of Lys224, (iii) the hydrophobic interactions between both the isopropyl group of Val61 and the benzene ring of Phe87 in IMP-1 and the benzene ring attached to the nitrogen atom of the pyrazole moiety in (*S*)-**5c**, (iv) the hydrophobic interaction between the isopropyl group of Val67 and the benzene ring of the styryl moiety of (*S*)-**5c**, and (v) the hydrophobic interaction between the imidazole ring of His118 and the benzene ring attached to the methoxy group of (*S*)-**5c**. Atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1), green – carbon (on inhibitor), magenta – zinc ions.

inhibitory potency of **5c** is similar to the best known MBL inhibitors, L-captopril (K_{ic} = 12.5 ± 2.4 μM) and a pyrrole compound we recently reported (K_{ic} ~ 10 μM) (16). *In silico* docking of the (*R*) and

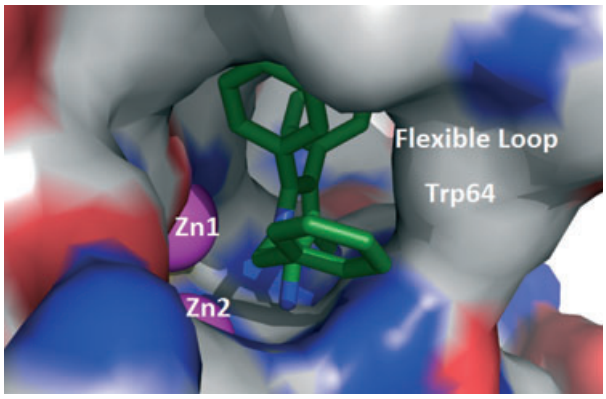


Figure 12: Surface view of the IMP-1 active site for the highest docking score conformation of IMP-1 with **7a** docked into the active site. For clarity, atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1), green-carbon (on inhibitor), magenta-zinc active site metals.

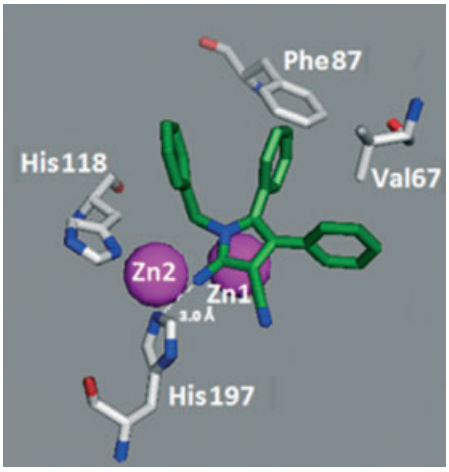


Figure 13: Surface view of the IMP-1 active site for the highest docking score conformation of IMP-1 with **7a** docked into the active site shows (i) the hydrogen bond (3.0 Å) between the amino group of **7a** and the nitrogen atom on the imidazole ring of His197, (ii) the hydrophobic interaction between the benzene ring attached to position 4 of pyrrole and the isopropyl group on Val67, and (ii) the hydrophobic interaction between the benzene ring attached to position 5 of the pyrrole moiety and both the isopropyl group on Val67 and the benzene ring of Phe87. Atom colors are as follows: blue – nitrogen, red – oxygen, white – carbon (on IMP-1), green – carbon (on inhibitor), magenta – zinc ions.

(*S*) configurations of **5c** in the active site of IMP-1 indicated that the two zinc ions in the active site play an important role in inhibitor binding through the thiolate anion of the (*R*) isomer only, with sulfur–metal distances of 2.2 Å (Zn1) and 2.0 Å (Zn2). The structure–activity relationship (SAR) indicates that the acetyl group in **1a** and **1c** is better than the ethoxy carbonyl group in **1d**–**e** for MBL inhibition. The introduction of the methoxybenzene group in **1b** dramatically decreases the inhibitory effect. The introduction of the dicyanide moiety (*i.e.,* as in **3b**) results in mixed-mode inhibition kinetics (**3b**: K_{ic} = 38 ± 59 μM and K_{iuc} = 82 ± 141 μM). The introduction of a sulfadiazine group in **6b** also results in considerable improvement in the inhibitory effect while maintaining a competitive mode of inhibition (**6b**: K_{ic} = 38 ± 59 μM). In contrast, the introduction of an acetic acid moiety to the sulfur atom in **2b** improves the inhibitory effect, but the uncompetitive mode of inhibitor binding becomes dominant (**2b**: K_{ic} = 235 ± 665 μM and K_{iuc}=70 ± 25 μM). Resolution of the two enantiomers of **5c** and their individual testing in these assays will allow us to validate the modeling described here, with our prediction that the (*R*)-enantiomer will be a much more potent MBL inhibitor than the (*S*)-enantiomer.

Table 3: Competitive (K_{ic}) and uncompetitive (K_{iuc}) inhibition constants for the inhibition of IMP-1 MBL by the most potent inhibitors

Compound	K_{ic} (μM)	K_{iuc} (μM)	Compound	K_{ic} (μM)	K_{iuc} (μM)
1a	29 ± 16	–	**7a**	21 ± 10	–
1c	176 ± 332	110 ± 58	**8a**	50 ± 28	21 ± 4
2b	235 ± 665	70 ± 25	**11c**	23 ± 8	47 ± 12
3b	38 ± 59	82 ± 141	**13a**	58 ± 64	58 ± 25
5c	19 ± 9	–	**15a**	148 ± 248	39 ± 10
6b	74 ± 38	–	**16a**	36 ± 20	50 ± 26
L-Captopril	12 ± 2.4	–			

MBL, metallo-β-lactamase.
Experiments were carried out in a duplicate mode.

On the other hand, in the pyrrole series, compound **7a** showed the most potent inhibition against IMP-1 (K_{ic} 21 ± 10 μM). The analysis of the highest docking score conformation of **7a**, in the active site of IMP-1, shows an interaction between the nitrogen atom of the amino group to the two zinc ions, along with further contributions involving hydrogen bonds and hydrophobic interactions between the inhibitor and various amino acid side chains in the active site. Based on K_i values, *in silico* docking, and SAR, we propose that the presence of 2-amino-3-cyanopyrrole nucleus, illustrated by **7a**, is crucial for achieving tight inhibitor binding. Introducing further substituents to the benzyl group and the two phenyl groups, the potency of **7a** against IMP-1 may be significantly enhanced. Steps toward substantiating these hypotheses are currently in progress. In summary, thiopyrimidine and pyrrole compounds have been shown to be promising leads in the development of novel MBL inhibitors.

References

1. Holten K.B., Onusko E.M. (2000) Appropriate prescribing of oral beta-lactam antibiotics. Am Fam Physician;62:611–620.
2. Felici A., Amicosante G., Oratore A., Strom R., Ledent P., Joris B., Fanuel L., Frere J.M. (1993) An overview of the kinetic-parameters of class-B beta-lactamases. Biochem J;291:151–155.
3. Lassaux P., Hamel M., Gulea M., Delbruck H., Mercuri P.S., Horsfall L., Dehareng D., Kupper M., Frere J.M., Hoffmann K., Galleni M., Bebrone C. (2010) Mercaptophosphonate compounds as

broad-spectrum inhibitors of the metallo-beta-lactamases. J Med Chem;53:4862–4876.
4. Ohsuka S., Arakawa Y., Horii T., Ito H., Ohta M. (1995) Effect of pH on activities of novel beta-lactamases and beta-lactamase inhibitors against these beta-lactamases. Antimicrob Agents Chemother;39:1856–1858.
5. Mitic N., Smith S.J., Neves A., Guddat L.W., Gahan L.R., Schenk G. (2006) The catalytic mechanisms of binuclear metallohydrolases. Chem Rev;106:3338–3363.
6. Bebrone C. (2007) Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol;74:1686–1701.
7. Crowder M.W., Spencer J., Vila A.J. (2006) Metallo-beta-lactamases: novel weaponry for antibiotic resistance in bacteria. Acc Chem Res;39:721–728.
8. Moran-Barrio J., Gonzalez J.M., Lisa M.N., Costello A.L., Dal Peraro M., Carloni P., Bennett B., Tierney D.L., Limansky A.S., Viale A.M., Vila A.J. (2007) The metallo-β-lactamase GOB is a mono-Zn(II) enzyme with a novel active site. J Biol Chem;282:18286–18293.
9. Paul-Soto R., Bauer R., Frere J.M., Galleni M., Meyer-Klaucke W., Nolting H., Rossolini G.M., de Seny D., Hernandez-Valladares M., Zeppezauer M., Adolph H.W. (1999) Mono- and binuclear Zn2+-beta-lactamase – role of the conserved cysteine in the catalytic mechanism. J Biol Chem;274:13242–13249.
10. Li J., Nation R.L., Turnidge J.D., Milne R.W., Coulthard K., Rayner C.R., Paterson D.L. (2006) Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis;6:589–601.
11. Livermore D.M. (2002) Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis;34:634–640.
12. Yong D., Toleman M.A., Giske C.G., Cho H.S., Sundman K., Lee K., Walsh T.R. (2009) Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother;53:5046–5054.
13. Zheng B., Tan S., Gao J., Han H., Liu J., Lu G., Liu D., Yi Y., Zhu B., Gao G.F. (2011) An unexpected similarity between antibiotic-resistant NDM-1 and beta-lactamase II from Erythrobacter litoralis. Protein Cell;2:250–258.
14. Faridoon, Hussein W.M., Vella P., Ul Islam N., Ollis D.L., Schenk G., McGeary R.P. (2012) 3-Mercapto-1,2,4-triazoles and N-acylated thiosemicarbazides as metallo-β-lactamase inhibitors. Bioorg Med Chem Lett;22:380–386.
15. Vella P., Hussein W.M., Leung E.W.W., Clayton D., Ollis D.L., Mitic N., Schenk G., McGeary R.P. (2011) The identification of new metallo-beta-lactamase inhibitor leads from fragment-based screening. Bioorg Med Chem Lett;21:3282–3285.
16. Mohamed M.S., Hussein W.M., McGeary R.P., Vella P., Schenk G., Abd El-hameed R.H. (2011) Synthesis and kinetic testing of new inhibitors for a metallo-β-lactamase from Klebsiella pneumonia and *Pseudomonas aeruginosa*. Eur J Med Chem;46:6075–6082.
17. Mohamed M.S., Kamel R., Fatahala S.S. (2010) Synthesis and biological evaluation of some thio containing pyrrolo 2,3-d Pyrimidine derivatives for their anti-inflammatory and anti-microbial activities. Eur J Med Chem;45:2994–3004.
18. Mohamed M.S., Kamel R., Fatahala S.S. (2011) New condensed pyrroles of potential biological interest Syntheses and structure-activity relationship studies. Eur J Med Chem;46:3022–3029.
19. Mohamed M.S., Rashad A.E., Adbel-Monem M., Fatahalla S.S. (2007) New anti-inflammatory agents. Z Naturforsch C: Biosci;62:27–31.
20. Mohamed M.S., Rashad A.E., Zaki M.E.A., Fatahala S.S. (2005) Synthesis and antimicrobial screening of some fused heterocyclic pyrroles. Acta Pharm;55:237–249.
21. Rashad A.E., Mohamed M.S., Zaki M.E.A., Fatahala S.S. (2006) Synthesis and biological evaluation of some pyrrolo 2,3-d pyrimidines. Arch Pharm;339:664–669.
22. Mohamed M.S., Kamel R., Fatahala S.S. (2011) Synthesis of new pyrroles of potential anti-inflammatory activity. Arch Pharm;344:830–839.
23. Laraki N., Franceschini N., Rossolini G.M., Santucci P., Meunier C., de Pauw E., Amicosante G., Frere J.M., Galleni M. (1999) Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo-beta-lactamase IMP-1 produced by *Escherichia coli*. Antimicrob Agents Chemother;43:902–906.
24. Bebrone C., Moali C., Mahy F., Rival S., Docquier J.D., Rossolini G.M., Fastrez J., Pratt R.F., Frere J.M., Galleni M. (2001) CENTA as a chromogenic substrate for studying beta-lactamases. Antimicrob Agents Chemother;45:1868–1871.
25. Segel I.H. (1993) Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibria and Steady-State Equilibria. New York: John Wiley and Sons.
26. Biginelli P. (1893) Aldureides of ethylic acetoacetate and ethylic oxalacetate. Gazz Chim Ital;23:360–416.
27. De S.K., Gibbs R.A. (2005) Ruthenium(III) chloride-catalyzed one-pot synthesis of 3,4-dihydropyrimidin-2-(1H)-ones under solvent-free conditions. Synthesis Stuttg;2005:1748–1750.
28. Narsaiah A.V., Basak A.K., Nagaiah K. (2004) Cadmium chloride: an efficient catalyst for one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones. Synthesis Stuttg;2004:1253–1256.
29. Peng J., Deng Y. (2001) Ionic liquids catalyzed Biginelli reaction under solvent-free conditions. Tetrahedron Lett;42:5917–5919.
30. Srinivas K.V.N.S., Das B. (2004) Iodine-catalyzed one-pot synthesis of 3,4-dihydropyrimidin-2(1H)-ones and thiones: a simple and efficient procedure for the Biginelli reaction. Synthesis Stuttg;2004:2091–2093.
31. Diana P., Barraja P., Lauria A., Montalbano A., Almerico A.M., Dattolo G., Cirrincione G. (2002) Pyrrolo 2,1-c 1,2,4 triazines from 2-diazopyrroles: synthesis and antiproliferative activity. Eur J Med Chem;37:267–272.
32. Diana P., Barraja P., Lauria A., Montalbano A., Almerico A.M., Dattolo G., Cirrincione G. (2003) Pyrrolo 2,1-d 1,2,3,5 tetrazine-4(3H)-ones, a new class of azolotetrazines with potent antitumor activity. Bioorg Med Chem;11:2371–2380.
33. Diana P., Stagno A., Barraja P., Montalbano A., Martorana A., Carbone A., Dattolo G., Cirrincione G. (2009) Pyrido 2′, 3′:4,5 pyrrolo 2,1-d 1,2,3,5 tetrazine-4(3H)-ones, a new class of temozolomide heteroanalogues. Arkivoc;177–186.
34. Lauria A., Diana P., Barraja P., Montalbano A., Cirrincione G., Dattolo G., Almerico A.M. (2002) New tricyclic systems of biological interest. Annelated 1,2,3-triazolo 1,5-a pyrimidines through domino reaction of 3-azidopyrroles and methylene active nitriles. Tetrahedron;58:9723–9727.

35. Abdelrahman A.E., Awad I.M.A., Bakhite E.A. (1991) Synthesis of some new heterocyclic-compounds containing thieno 2,3-b quinoline moiety. Phosphorus Sulfur;60:189–199.
36. Ismail Z.H., Abdel-Gawad S.M., Abdel-Aziem A., Ghorab M.M. (2003) Synthesis of some new biologically active sulfur compounds containing pyrazolo 3,4-d pyrimidine moiety. Phosphorus Sulfur;178:1795–1805.
37. Robins R.K. (1956) Potential purine antagonists .1. synthesis of some 4,6-substituted pyrazolo 3,4-d pyrimidines. J Am Chem Soc;78:784–790.
38. El-Dean A.M.K., Abdel-Moneam M.E. (2002) Synthesis of pyrimidines, thienopyrimidines, and pyrazolopyrimidines. Phosphorus Sulfur;177:2745–2751.
39. El-Emary T.I., Abdel-Mohsen S.A. (2006) Synthesis and antimicrobial activity of some new 1,3-diphenylpyrazoles bearing pyrimidine, pyrimidinethione, thiazolopyrimidine, triazolopyrimidine, thio- and alkylthiotriazolop-yrimidinone moieties at the 4-position. Phosphorus Sulfur;181:2459–2474.
40. El-Gazzar A.B.A., Hassan N.A. (2000) Synthesis of polynuclear heterocyclic compounds derived from thieno 2,3-d pyrimidine derivatives. Molecules;5:835–850.
41. El-Gazzar A.B.A., Hussein H.A.R., Aly A.S. (2006) Synthesis and reactions of polynuclear heterocycles: azolothienopyrimidines and thienothiazolopyrimidines. Phosphorus Sulfur;181:2771–2784.
42. Traxler P.M., Furet P., Mett H., Buchdunger E., Meyer T., Lydon N. (1996) 4-(Phenylamino) pyrrolopyrimidines: potent and selective, ATP site directed inhibitors of the EGF-receptor protein tyrosine kinase. J Med Chem;39:2285–2292.
43. Elgazzar A.B.A., Gafaar A.M., Hafez H.N., Aly A.S. (2006) Novel syntheses and reactions of polynuclear heterocyclic derivatives derived from thioxopyridopyrimidine, with a new ring system. Phosphorus Sulfur;181:1859–1883.
44. Mohamed M.S., El-Domany R.A., Abd El-Hameed R.H. (2009) Synthesis of certain pyrrole derivatives as antimicrobial agents. Acta Pharm;59:145–158.
45. Thomsen R., Christensen M.H. (2006) MolDock: a new technique for high-accuracy molecular docking. J Med Chem;49:3315–3321.
46. Kerwin S.M. (2010) ChemBioOffice Ultra 2010 Suite. J Am Chem Soc;132:2466–2467.
47. Toney J.H., Hammond G.G., Fitzgerald P.M.D., Sharma N., Balkovec J.M., Rouen G.P., Olson S.H., Hammond M.L., Greenlee M.L., Gao Y.-D. (2001) Succinic acids as potent inhibitors of plasmid-borne IMP-1 metallo-β-lactamase. J Biol Chem;276:31913–31918.
48. Lienard B.M.R., Garau G., Horsfall L., Karsisiotis A.I., Damblon C., Lassaux P., Papamicael C., Roberts G.C.K., Galleni M., Dideberg O., Frere J.-M., Schofield C.J. (2008) Structural basis for the broad-spectrum inhibition of metallo-β-lactamases by thiols. Org Biomol Chem;6:2282–2294.
49. Moali C., Anne C., Lamotte-Brasseur J., Groslambert S., Devreese B., Van Beeumen J., Galleni M., Frere J.-M. (2003) Analysis of the importance of the metallo-β-lactamase active site loop in substrate binding and catalysis. Chem Biol;10:319–329.