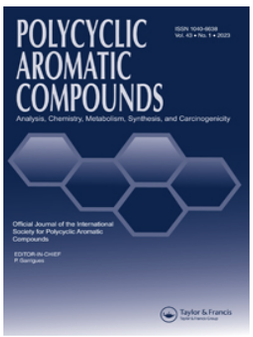

Polycyclic Aromatic Compounds

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Synthesis, Antimicrobial Assay and SARs of Pyrazole Included Heterocyclic Derivatives

N. Mahaboob Basha[a#], B.C. Venkatesh[b*], Guda Mallikarjuna Reddy[c#], Grigory V. Zyryanov[c,d], Munagapati Venkata Subbaiah[e], Jet-Chau Wen[e,f], and Anjani R.K. Gollakota[f]

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ABSTRACT

As pyrazoles are familiar for showing various biological properties, a series of novel isocoumarin tethered carbothioamide linked pyrazole derivatives were prepared successfully in the presence of environmental favor conditions and evaluated for their pathogenic resistance against four bacteria and two fungi, showing significant activity against different microbial except compound **5d**. Among the active compounds, **5f** displayed highest antimicrobial property. In fact, **5b** and **5l** motifs showing good inhibitory activity. Further, a detailed structure activity relationship also discussed, which revealed that the presence of electron withdrawing -NO_2 group in **5f** compound may delivered highest biological property, while the amino group attached in compound **5d** delivered inactive antibacterial property. Moreover, computer aided studies of biologically effective compounds to determine the interactions and docking score with the crystal structure of *Escherichia coli* 24 kDa DNA gyrase subunit B in complex with clorobiocin. It was observed that the amino acid residues present in the active binding site of the protein1KZN can frequently interact with targets.

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KEYWORDS
Pyrazoles; isocoumarin; antimicrobial assay; docking studies; SARs

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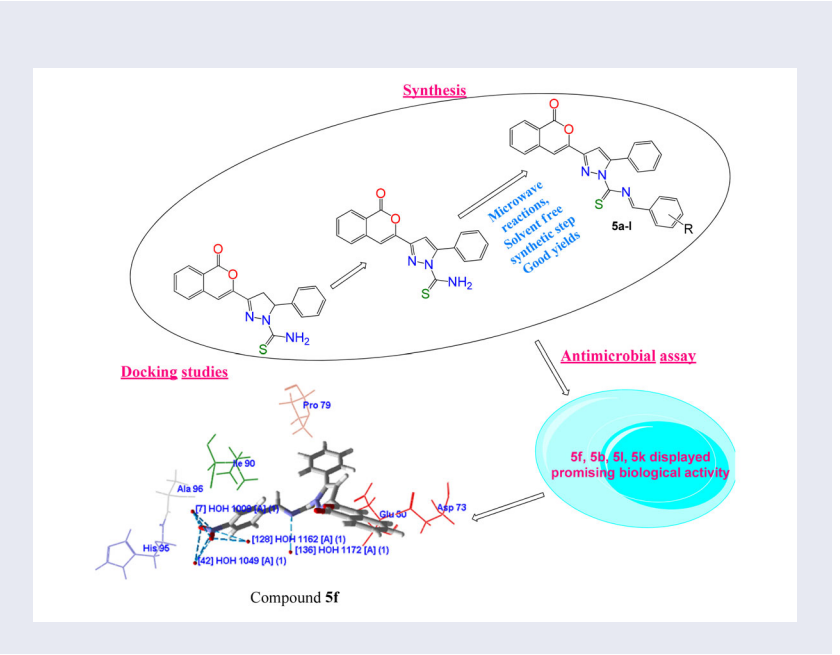


Introduction

The most privileged scaffold pyrazole derivatives hold its own valuable biological properties and are existing in many natural products. Numerous drugs like Fomepizole,[1] celecoxib (Celebrex), sildenafil (Viagra),[2] Tartrazine,[3] Ceftolozane[4] which contained pyrazole nuclei as a main core shows the prominence of pyrazole motif (Figure 1). Recently, Sanad *et al.* and Zang *et al.* conveyed that pyrazole derivatives exhibited promising antimicrobial activities against some bacteria.[5–7] In other study, pyrazole incorporated moieties displayed profound anticancer activity,[8] cyclooxygenase inhibiting activity,[9] tyrosine kinase hindrance,[10] anti-inflammatory activity,[11] antioxidant activities,[12] anticonvulsant,[13] analgesic,[14] antidiabetic,[15] and other activities.[16–18]

On the other hand, extreme usage of antibiotics causes the drug resistance of the microorganisms increased to hazardous level around the globe.[19,20] Therefore, the word "superbugs" become dangerous term in the society. Now a days, superbugs are challenging and cause severe risk to contemporary health care.[21–24] Usually, most of the Gram-positive and Gram-negative superbugs are developing their resistance power against all known drugs. For example, the Gram-negative superbug *Escherichia coli* infections in the society often resist antimicrobial actions with third generation cephalosporins.[25] Many antibiotic drugs like fluoroquinolones and carbapenem are opposed by *Klebsiella pneumonia* pathogen.[26,27] In addition, infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* are opposed to their related drugs.[28–31] Hence, to reduce the resistance power of superbugs or at least to overcome the opposed influences, some immediate activities like fresh antibiotic invention, combination of antibiotic drugs makes new powerful antibiotic agents, reduce the usage of heavy dosage of antibiotics.[32–35] In all the above, invention of novel antimicrobial drugs is very essential in this contemporary life. In this direction, the present work, synthesis of pyrazole linked with carbothioamide in combination with isocoumarin has been taken up. In addition, antimicrobial screening was done for all the produced analogues and discussed their structure activity relationship (SARs).

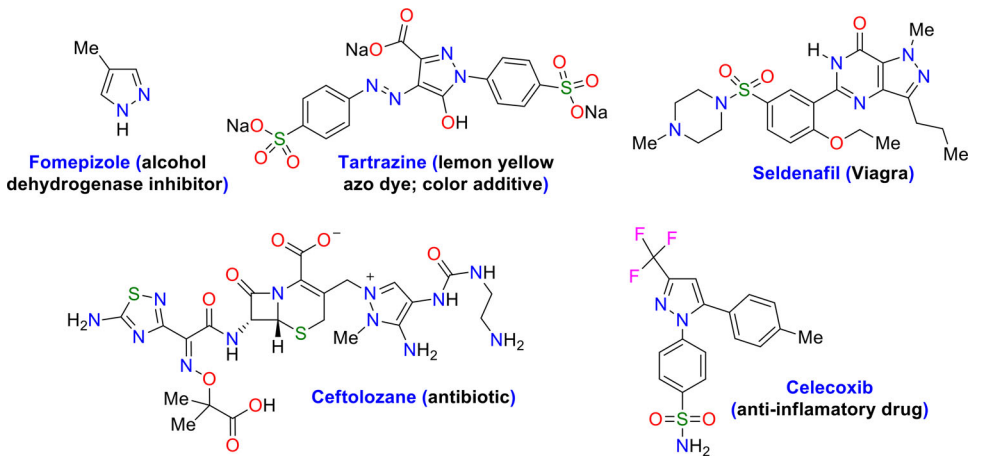


Figure 1. Imp ortant pyrazole containing drugs.

Experimental

Materials and instrumentation

All starting catalyst, reagents and solvents were pure and readily purchasable for use. Melting point data was recorded with micro melting point machine and were uncorrected. The IR spectra were recorded on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on Bruker® Avance 400 MHz spectrometer in deuterated solvents ($CDCl_3$/DMSO-d_6). ^{1}H NMR chemical shifts were reported in parts per million (ppm) (δ) with TMS as an internal standard (δ 0.00), and ^{13}C NMR chemical shifts with solvent reference were reported ($CDCl_3$, δ 77.00 ppm; DMSO-d_6, δ 39.52 ppm). All synthetic routes were conducted in a catalyst-4R microwave oven. The intermediate one (**1**) was prepared according to the literature procedure.[36,37] The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer.

Procedure for the intermediate compound (2)

In a microwave fitted round bottom flask (RB), methanol (5 mL) and NaOH (1.5 mmol) were taken. To this thiosemicarbazide (1 mmol) and compound **1** (1 mmol) were added and irradiated at 80 °C (250 W) for 15–20 min. The product formation was checked by the TLC. After completion of the reaction, the crude mixture was poured onto crushed ice. The precipitated solid was collected by filtration and recrystallized with 2-propanol resulted in compound **2**.

5-Phenyl-3-(1-oxo-1H-isochromen-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (**2**). Pale yellow solid; Yield 83%; Melting point 169–171 °C; IR (KBr): 3412, 3290, 3082, 2946, 1762, 1654, 1632, 1528 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (bs, 2H, NH_2), 7.02 − 7.80 (m, 10H, Aromatic and $C_{4'}$−H), 5.29 (dd, H_A, 1H, J_{AX} = 6.8 H_Z, J_{AM} = 14.7 H_Z), 3.86 (dd, H_M, 1H, J_{MX} = 11.0 H_Z, J_{AM}= 14.7 Hz), 3.16 (dd, H_X, 1H, J_{MX} = 11.0 H_Z, J_{AX} = 6.8 H_Z), ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.5 (C = O–NH_2), 179.2 (C = O), 157.3 (C–3), 120.4, 121.3, 123.5, 124.9, 127.5, 127.9, 130.2, 132.5, 133.3, 135.2, 139.9, 142.3, 66.5 (C–5), 42.1 (C–4) ppm,

Procedure for the preparation of the compound (3)

The round bottom flask was irradiated at 250 W (130 ^{0}C) which contained the mixture of intermediate motif **2** (1 mmol) and chloranil (2 mmol) in xylene (7 mL). the reaction progress was

monitred by TLC. Upon completion of the reaction, the mixture was wahsed with 5% sodium hydroxide solution followed by the separation of organic part, dried with anhydrous soduim sulfate and organic solvent was removed *in vacuo*. The obtained solid was treated with 2-propanol resulted in motif **3**.

5-Phenyl-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**3**). Color solid; Yield 84%; Melting point 170–172 °C; IR (KBr): 3415, 3295, 3073, 2951, 1765, 1645, 1632, 1532 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): 8.50 (bs, 2H, NH_2), 7.28 − 7.72 (m, 11H, Aromatic and $C_{4'}$−H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.2 (C = O-NH_2), 176.5 (C = O), 156.2 (C − 3), 119.3, 120.5, 121.2, 123.4, 124.9, 126.0, 128.3, 129.5, 130.2, 132.4, 134.6, 137.5, 143.1, 145.2.

Compounds 5(a–l) preparation method

The final intermediate compound **3** (1 mmole) was taken in a RB. To this, benzaldehyde (**4**, 1 mmole) was added and used the same reaction conditons mentioned in above step. After product formation, the reaction mixture was purified by using column chromatography. For this EtOH-Hexane solvent mixture was used as eluant to get the pure compound **5a**. Remaining analoges were prepared using the same method of **5a**.

5-Phenyl-(E)-N-benzylidene-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5a**). Color solid; Yield 80%; Melting point 199–201 °C; IR (KBr): 3060, 2965, 1770, 1655, 1628, 1535 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 8.75 (s, 1H, HC = NCO), 7.25–**7.92** (m, 16H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{18}N_3O_2S$ $(M+H)^+$ 436.1120; Found 436.1118; ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.3 (CARBONYL CARBON BETWEEN N), 170.8 (OXYGEN ATTACHED CARBONYL), 161.3 (HC=NH), 111.4, 113.5, 116.3, 121.4, 122.5, 123.2, 123.9, 125.6, 126.9, 127.4, 128.1, 128.8, 129.3, 130.4, 132.4, 140.6, 143.4, 145.2, 147.1 ppm; MS(m/z): 435.1075 $[M^{+\cdot}]$: C = 71.71; H = 3.93; N = 9.65. Found: C = 71.77; H = 3.91; N = 9.62.

5-phenyl-(E)-N-(4-chlorobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5b**). Color solid; Yield 79%; Melting point 183–185 °C; IR (KBr): 3072, 2942, 1768, 1643, 1625, 1520 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.85 (s, 1H, HC = NCO), 6.91–**7.84** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{17}ClN_3O_2S$ $(M+H)^+$ 470.0730; Found 470.0726; ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.4 (CARBONYL CARBON BETWEEN N), 171.4 (OXYGEN ATTACHED CARBONYL), 164.4 (HC=NH), 109.3, 113.5, 117.0, 121.4, 123.3, 124.1, 125.6, 125.9, 126.3, 128.2, 130.3, 132.4, 133.0, 136.4, 141.3, 144.5, 146.4, 149.9, 152.3 ppm. MS(m/z): 469.0652 $[M^{+\cdot}]$: C = 66.45; H = 3.43; N = 8.94. Found: C = 66.39; H = 3.47; N = 8.91.

*5-Phenyl-(E)-N-(2-chlorobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5c**).* Color solid; Yield 74%; Melting point 189–191 °C; IR (KBr): 3085, 2952, 1772, 1654, 1622, 1524 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.88 (s, 1H, HC = NCO), 6.61–**7.73** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{17}ClN_3O_2S$ $(M+H)^+$ 470.0730; Found 470.0728; ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.5 (CARBONYL CARBON BETWEEN N), 172.1 (OXYGEN ATTACHED CARBONYL), 160.8 (HC=NH), 109.2, 115.3, 118.9, 121.4, 122.8, 123.3, 124.6, 126.3, 127.2, 129.5, 130.1, 131.4, 132.7, 134.2, 135.6, 139.1, 141.5, 143.2, 145.4, 148.7, 151.2 ppm; MS(m/z): 469.0652 $[M^{+\cdot}]$: C = 66.45; H = 3.43; N = 8.94. Found: C = 66.41; H = 3.40; N = 8.97.

5-Phenyl-(E)-N-(2-aminobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5d**). Color solid; Yield 82%; Melting point 236–238 °C; IR (KBr): 3410, 3280, 3065, 2950, 1765, 1642, 1634, 1531 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.90 (s, 1H, HC = NCO), 6.90–**7.88** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{19}N_4O_2S$ $(M+H)^+$ 451.1229; Found 451.1225; ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.5 (CARBONYL CARBON BETWEEN N), 170.2 (OXYGEN ATTACHED CARBONYL), 161.3 (HC=NH), 113.2, 116.7, 118.9, 120.1, 121.7, 122.4, 125.2, 126.1, 128.2, 129.4, 130.1, 131.5, 133.4, 134.5, 135.2, 136.7, 141.3, 142.9, 144.6, 146.2, 149.3 ppm. MS(m/z): 450.1150 $[M^{+\cdot}]$: C = 69.32; H = 4.03; N = 12.44. Found: C = 69.27; H = 4.01; N = 12.50.

5-Phenyl-(E)-N-(4-aminobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5e**). Color solid; Yield 76%; Melting point 228–230 °C; IR (KBr): 3418, 3284, 3074, 2942, 1766, 1645, 1625, 1530 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.92 (s, 1H, HC = NCO), 6.72––**7.80** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{19}N_4O_2S$ $(M+H)^+$ 451.1229; Found 451.1226; ^{13}C NMR (75 MHz, DMSO-d_6): δ 180.4 (CARBONYL CARBON BETWEEN N), 171.5 (OXYGEN ATTACHED CARBONYL), 162.8 (HC=NH), 111.7, 114.2, 119.5, 121.5, 122.2, 123.4, 124.3, 125.8, 127.1, 128.7, 130.1, 131.3, 133.4, 136.3, 141.3, 142.7, 144.1, 146.5, 148.8 ppm. MS(m/z): 450.1150 $[M^{+\cdot}]$: C = 69.32; H = 4.03; N = 12.44. Found: C = 69.29; H = 4.06; N = 12.48.

5-Phenyl-(E)-N-(4-Nitrobenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5f**). Light yellow color solid; Yield 84%; Melting point 195–197 °C; IR (KBr): 3082, 2964, 1765, 1654, 1632, 1528, 1503, 1332 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.84 (s, 1H, HC = NCO), 7.14–**7.94** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{17}N_4O_4S$ $(M+H)^+$ 481.0971; Found 481.0966; ^{13}C NMR (75 MHz, DMSO- d_6): δ 180.4 (CARBONYL CARBON BETWEEN N), 170.9 (OXYGEN ATTACHED CARBONYL), 161.3 (HC=NH), 114.4, 116.7, 119.7, 120.2, 122.4, 123.5, 125.1, 126.1, 127.4, 129.2, 130.4, 131.5, 132.8, 134.1, 141.4, 142.8, 145.3, 147.3, 151.1 ppm. MS(m/z): 480.0892 $[M^{+\cdot}]$: C = 64.99; H = 3.36; N = 11.66. Found: C = 65.04; H = 3.30; N = 11.68.

5-Phenyl-(E)-N-(2-methylbenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5g**). Light yellow color solid; Yield 81%; Melting point 223–225 °C; IR (KBr): 3065, 2961, 1768, 1650, 1630, 1524 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.84 (s, 1H, HC = NCO), 6.84–**7.79** (m, 15H, Aromatic), 2.24 (s, 3H, CH_3) ppm; HRMS: *m/z* calcd for $C_{27}H_{20}N_3O_2S$ $(M+H)^+$ 450.1276; Found 450.1271; ^{13}C NMR (75 MHz, DMSO- d_6): δ 180.9 (CARBONYL CARBON BETWEEN N), 171.4 (OXYGEN ATTACHED CARBONYL), 161.5 (HC=NH), 116.8, 118.2, 121.3, 122.4, 123.5, 125.1, 126.5, 127.2, 128.5, 129.1, 129.5, 131.1, 132.4, 133.7, 135.0, 136.5, 139.9, 141.8, 143.3, 145.1, 147.8, 20.1 (CH_3) ppm. MS(m/z): 449.1198 $[M^{+\cdot}]$: C = 72.14; H = 4.26; N = 9.35. Found: C = 72.08; H = 4.29; N = 9.31.

5-Phenyl-(E)-N-(4-methylbenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5h**). Light yellow color solid; Yield 82%; Melting point 217–219 °C; IR (KBr): 3081, 2953, 1765, 1645, 1627, 1530 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.91 (s, 1H, HC = NCO), 6.88–**7.91** (m, 14H, Aromatic), 2.29 (s, 3H, CH_3) ppm; HRMS: *m/z* calcd for $C_{27}H_{20}N_3O_2S$ $(M+H)^+$ 450.1276; Found 450.1273; ^{13}C NMR (75 MHz, DMSO- d_6): δ 180.9 (CARBONYL CARBON BETWEEN N), 169.5 (OXYGEN ATTACHED CARBONYL), 160.7 (HC=NH), 112.3, 116.9, 119.3, 121.5, 123.4, 124.5, 125.7, 127.1, 128.2, 129.4, 130.5, 132.3, 134.1, 135.8, 138.3, 141.1, 143.4, 144.9, 147.1 18.9 (CH_3) ppm. MS(m/z): 449.1198 $[M^{+\cdot}]$: C = 72.14; H = 4.26; N = 9.35. Found: C = 72.10; H = 4.21; N = 9.32.

5-Phenyl-(E)-N-(4-methoxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5i**). Light yellow color solid; Yield 80%; Melting point 187–189 °C; IR (KBr): 3075, 2964, 1762, 1653, 1624, 1535 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.92 (s, 1H, HC = NCO), 6.74–**7.83** (m, 15H, Aromatic), 3.51 (s, 3H, OCH_3) ppm; HRMS: *m/z* calcd for $C_{27}H_{20}N_3O_3S$ $(M+H)^+$ 466.1225; Found 466.1221; ^{13}C NMR (75 MHz, DMSO- d_6): δ 181.7 (CARBONYL CARBON BETWEEN N), 170.3 (OXYGEN ATTACHED CARBONYL), 161.4 (HC=NH), 112.3, 118.2, 120.1, 121.3, 123.2, 124.1, 125.3, 126.8, 127.5, 128.2, 130.0, 131.3, 132.5, 133.3, 141.4, 143.4, 145.6, 147.1, 151.3 58.9 (OCH_3) ppm. MS(m/z): 465.1147 $[M^{+\cdot}]$: C = 69.66; H = 4.11; N = 9.03. Found: C = 69.61; H = 4.16; N = 9.01.

5-Phenyl-(E)-N-(2-methoxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5j**). Light yellow color solid; Yield 77%; Melting point 191–193 °C; IR (KBr): 3072, 2962, 1771, 1642, 1625, 1532 cm^{-1}; ^1H NMR (300 MHz, DMSO- d_6): δ 8.89 (s, 1H, HC = NCO), 6.94–**7.91** (m, 15H, Aromatic), 3.49 (s, 3H, OCH_3) ppm; HRMS: *m/z* calcd for $C_{27}H_{20}N_3O_3S$ $(M+H)^+$ 466.1225; Found 466.1223; ^{13}C NMR (75 MHz, DMSO- d_6): δ 180.6 (CARBONYL CARBON BETWEEN N), 169.3 (OXYGEN ATTACHED CARBONYL), 160.5 (HC=NH), 113.2,

115.2, 120.3, 121.1, 122.5, 124.8, 125.1, 126.3, 127.2, 129.1, 129.9, 130.5, 131.0, 132.7, 134.3, 135.2, 137.3, 141.5, 142.8, 145.1, 147.2, 59.7 (OCH_3) ppm. MS(m/z): 465.1147 [$M^{+\cdot}$]: C = 69.66; H = 4.11; N = 9.03. Found: C = 69.62; H = 4.13; N = 9.00.

5-Phenyl-(E)-N-(2-hydroxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5k**). Light yellow color solid; Yield 76%; Melting point 207–209 °C; IR (KBr): 3467, 3102, 2973, 1770, 1655, 1622, 1528 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.93 (s, 1H, HC = NCO), 6.72–**7.90** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{18}N_3O_3S$ $(M+H)^+$ 452.1069; Found 452.1066; ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.5 (CARBONYL CARBON BETWEEN N), 171.2 (OXYGEN ATTACHED CARBONYL), 161.3 (HC=NH), 111.4, 117.3, 119.1, 120.3, 121.2, 123.5, 124.7, 125.9, 127.3, 129.1, 131.5, 132.6, 133.8, 134.1, 136.3, 138.1, 140.2, 143.5, 145.3, 148.1, 149.5 ppm. MS(m/z): 451.0991 [$M^{+\cdot}$]: C = 69.17; H = 3.80; N = 9.31. Found: C = 69.21; H = 3.86; N = 9.33.

5-Phenyl-(E)-N-(4-hydroxybenzylidene)-3-(1-oxo-1H-isochromen-3-yl)-1H-pyrazole-1-carbothioamide (**5l**). Light yellow color solid; Yield 73%; Melting point 221–213 °C; IR (KBr): 3455, 3105, 2962, 1765, 1664, 1631, 1535 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.79 (s, 1H, HC = NCO), 6.75–**7.84** (m, 15H, Aromatic) ppm; HRMS: *m/z* calcd for $C_{26}H_{18}N_3O_3S$ $(M+H)^+$ 452.1069; Found 452.1068; ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.3 (CARBONYL CARBON BETWEEN N), 169.8 (CARBONYL CARBON ATACHED O), 160.7 (HC=NH), 112.4, 115.9, 120.2, 121.7, 123.4, 124.5, 125.2, 127.0, 128.4, 129.3, 130.5, 132.3, 134.5, 135.5, 139.8, 141.4, 143.2, 145.5, 147.2 ppm. MS(m/z): 451.0991 [$M^{+\cdot}$]: C = 69.17; H = 3.80; N = 9.31. Found: C = 69.20; H = 3.82; N = 9.29.

Biological experiment

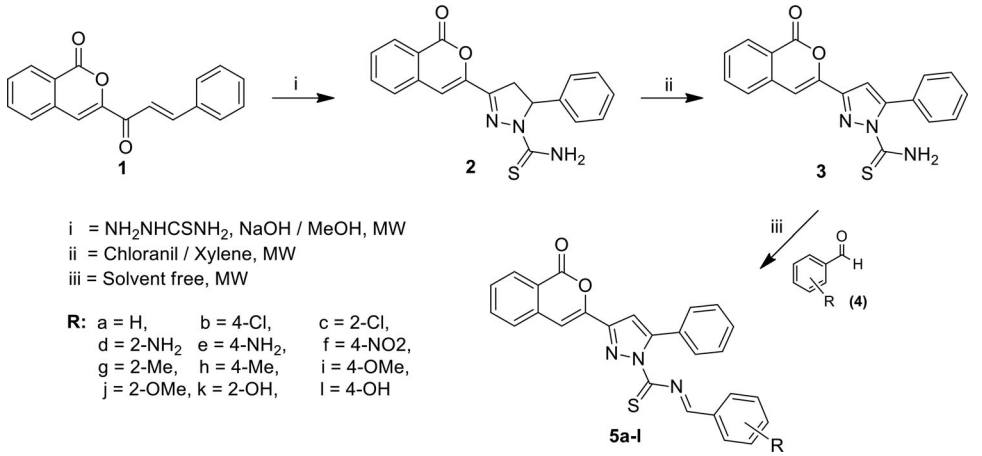
Antimicrobial activity

The antimicronial screening was carried out at two concentrations 25 and 50 μg/well, measuring the compounds antibacterial and antifungal inhibition zone values according to the reported procedure.[33]

Results and discussion

Chemistry

3-Cinnamoyl-1*H*-isochromen-1-one (**1**) was utilized as a precursor to attain a family of carbothioamide linked pyrazole and their analogs **5a–l**. The schematic representation of the final targets and their derivatives was depicted in Scheme 1. The reactive intermediate in this work



Scheme 1. Preparation of carbothioamide linked pyrazoles.

3-(1-oxo-1*H*-isochromen-3-yl)-5-phenyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**2**) was synthesized by using 3-cinnamoyl-1*H*-isochromen-1-one (**1**). Reaction of compound **1** with thiosemicarbazide under microwave irradiation condition resulted in compound **2**. Aromatization of intermediate motif **2** with chloranil in xylene produced 3-(1-oxo-1*H*-isochromen-3-yl)-5-phenyl-1*H*-pyrazole-1-carboxamide (**3**) with excellent yield. The absance of AMX signals confirmed the formation of the product **3**. Finally, solvent free reaction was carried out between the compound **3** and benzaldehyde **4a** under microwave method, formed the related product 5-phenyl-(*E*)-*N*-benzylidene-3-(1-oxo-1*H*-isochromen-3-yl)-1*H*-pyrazole-1-carbothioamide (**5a**). In proton NMR spectra of **5a**, a sharp peak appeared at 8.75 ppm belongs to the hydrogen which is attached to benzaldehyde –C = N– carbon. In addition, in carbon NMR spectra, there was a peak at 161.3 ppm belongs to the carbon of –*C* = *N*– group. Similarly, a diverse sequence of pyrazoles linked with carbothioamide **5b–l** were synthesized in good yields by the condensation of **3** with proper aldehydes. The structures of this derivatives were recognized by considering their proton NMR, carbon NMR and mass spectral analysis. The microwave irradiation conditions and solvent free condition of final step were the main advantageous in this work. Due to the involvement of microwave irradiation gave the uniform irradiation to the reaction, lead to the formation the products in good yields.

Compound's inhibition effect on bacterial and fungal strains

Selectively, four different types of bacteria namely *P. vulgaris* (ATCC-29213), *E. coli* (ATCC-8739), *S. aureus* (ATCC-19433), and *B. subtilis* (ATCC-6633) and two fungal strains namely *A. flavus* (MTCC-1884) and *A. niger* (MTCC-1881) were chosen to evaluate the antimicrobial properties. In order to compare the compounds activity, Chloramphenicol and Ketoconazole were used as standard drugs. The summarized antibacterial results were depicted in Table 1. When compared to reference drugs, screened compounds provided reliable bacterial inhibition effect except compound **5d**. In fact, active compounds providing higher inhibition effects against American code ATCC-19433 bacteria, while against *E. coli* they displayed minimal antibacterial activity. Besides, Table 2 exposed the antifungal results of all target molecules. Most of the compounds delivered good antifungal behavior. Interestingly against *A. Niger* total compounds showed potent antifungal activity than other fungi. Among all, two pathogens were highly sensitive towards compounds **5f**, **5b**, and **5l**. In fact, compound **5h** showed zero antifungal action against *A. flavus* fungi (Figure 2).

Table 1. Amalgams **5(a–l)** and their *in-vitro* antibacterial effects.

	Zone of inhibition (mm)							
	Gram-positive				Gram-negative			
	S. aureus (ATCC-19433)		*B. subtilis* (ATCC-6633)		*P. vulgaris* (ATCC-29213)		*E. coli* (ATCC-8739)	
Samples	**25** μg/well	**50** μg/well	**25** μg/well	**50** μg/well	**25** μg/well	**50** μg/well	**25** μg/well	**50** μg/well
5a	20 ± 1	24 ± 2	15 ± 2	19 ± 3	11 ± 2	18 ± 2	09 ± 2	12 ± 2
5b	32 ± 2	37 ± 1	29 ± 1	30 ± 2	22 ± 2	28 ± 3	17 ± 2	23 ± 1
5c	17 ± 3	22 ± 1	14 ± 3	17 ± 1	10 ± 1	15 ± 2	07 ± 1	10 ± 2
5d	0	0	0	0	0	0	0	0
5e	25 ± 1	26 ± 3	18 ± 1	20 ± 1	14 ± 1	20 ± 3	10 ± 1	14 ± 3
5f	34 ± 1	38 ± 1	31 ± 2	33 ± 1	24 ± 1	30 ± 2	18 ± 1	25 ± 2
5g	16 ± 1	20 ± 2	12 ± 1	13 ± 2	08 ± 2	13 ± 2	05 ± 1	06 ± 3
5h	09 ± 2	14 ± 1	05 ± 1	06 ± 1	05 ± 2	07 ± 2	0	0
5i	14 ± 2	18 ± 2	08 ± 1	11 ± 3	0	10 ± 1	04 ± 2	05 ± 3
5j	27 ± 3	29 ± 2	21 ± 2	25 ± 1	16 ± 3	22 ± 2	11 ± 2	15 ± 1
5k	28 ± 1	31 ± 1	24 ± 2	26 ± 3	19 ± 1	24 ± 2	13 ± 3	18 ± 2
5l	31 ± 2	33 ± 1	27 ± 1	29 ± 1	21 ± 1	25 ± 1	15 ± 1	20 ± 1
Chloramphenicol	36 ± 1	40 ± 2	32 ± 1	35 ± 2	26 ± 2	31 ± 3	22 ± 2	27 ± 1
Control(DMSO)	–	–	–	–	–	–	–	–

Table 2. Amalgams **5(a–l)** and their *in-vitro* antifungal effects.

	Zone of inhibition (mm)			
	A. flavus (MTCC-1884)		*A. niger* (MTCC-1881)	
Samples	25 μg/well	50 μg/well	25 μg/well	50 μg/well
5a	18 ± 1	21 ± 2	23 ± 1	26 ± 2
5b	25 ± 1	31 ± 3	32 ± 2	38 ± 1
5c	16 ± 2	19 ± 3	20 ± 3	24 ± 1
5d	09 ± 1	11 ± 12	14 ± 1	16 ± 1
5e	19 ± 1	23 ± 1	24 ± 2	28 ± 2
5f	29 ± 1	32 ± 1	35 ± 1	40 ± 1
5g	14 ± 2	18 ± 1	18 ± 1	21 ± 3
5h	0	0	11 ± 2	13 ± 2
5i	11 ± 1	15 ± 3	17 ± 2	19 ± 2
5j	20 ± 1	25 ± 1	26 ± 1	31 ± 3
5k	22 ± 1	28 ± 2	29 ± 3	33 ± 3
5l	23 ± 2	29 ± 1	30 ± 1	35 ± 1
Ketoconazole	31 ± 1	34 ± 2	37 ± 3	41 ± 2
Control (DMSO)	–	–	–	–

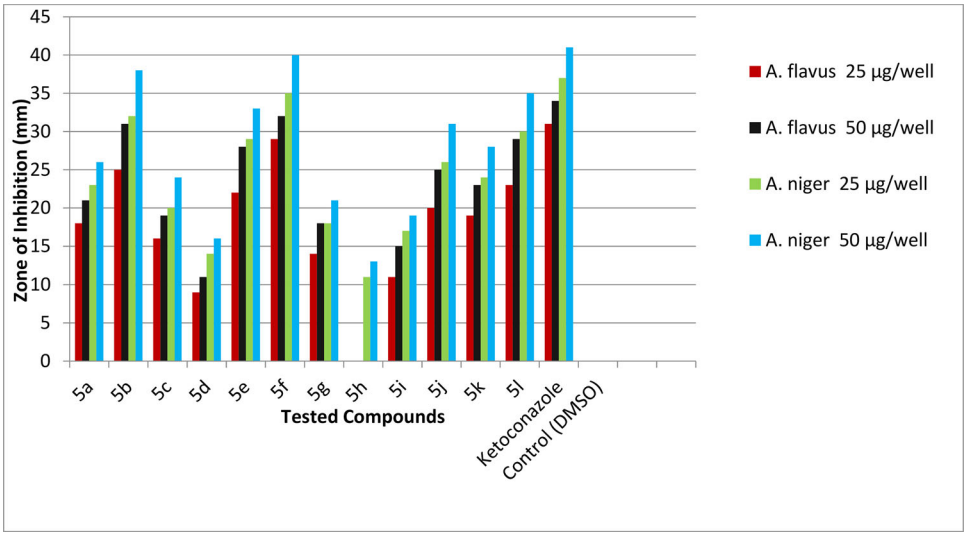


Figure 2. Antifungal property graph of compounds **5(a–l)**.

Structure activity relationship studies (SARs)

It was observed that **5f** derivative showed most activity against all bacteria and **5d** compound did not display any pathogenic opposition against targeted bacteria. Which clearly indicated that substituent on benzene ring of the compounds may influence the antibacterial properties. The presence of nitro group in compound **5f** may showed highest antibacterial activity while the amino group attached in compound **5d** may delivered inactive antibacterial property. In fact, the compound **5h** itself was also deficient antibacterial stuff due to the presence of methyl group in its core. Between the other active existences **5b** derivative holding chlorine substituent at para position of benzene caused a strong resistance effect on all bacteria, mainly in contrast to *S. aureus* pathogen (Figure 1). In addition, compounds **5k** and **5l** were displayed good antibacterial nature. While the electron rich substituent in **5e** and in **5j** had a medium influence on antibacterial effect and the compounds **5a** and **5c** were showed reliable antibacterial action. On the other hand, 2-methyl and 4-memthoxy groups linked to benzene ring of compounds **5g** and **5i** respectively, did

not have a noteworthy impact on the antibacterial effect on pathogens. In fact, appropriately the same interpretation was observed in the case of fungal activity. From the antimicrobial results as well as SARs, it was clearly observed that compounds that possess high antimicrobial properties hold the electron withdrawing substitution in its core.

Docking studies results and discussion

Molecular Docking investigations were achieved to detect the interactions of the highly biological effective amalgams viz., **5b**, **5l**, **5f**, and **5k** with the crystal structure of E. coli 24 kDa DNA gyrase subunit B in complex with clorobiocin (PDB ID:1KZN). The authentication of the docking technique was finished with the judgment between the co-crystallized active ligand and the docked active ligand (Figure 3).

Table 3 exposed the docking results of docked compounds **5b**, **5l**, **5f**, and **5k**. The ligand-protein interaction values, binding energy values, compounds rerank score, and molecular docking score are shown in Table 3. From the docking results it was identified clearly that the protein1KZN ligand contained the amino acid residues, which can normally interact with docked compounds. The protein 1KZN poses with the docked motifs are displayed in Figure 4, which

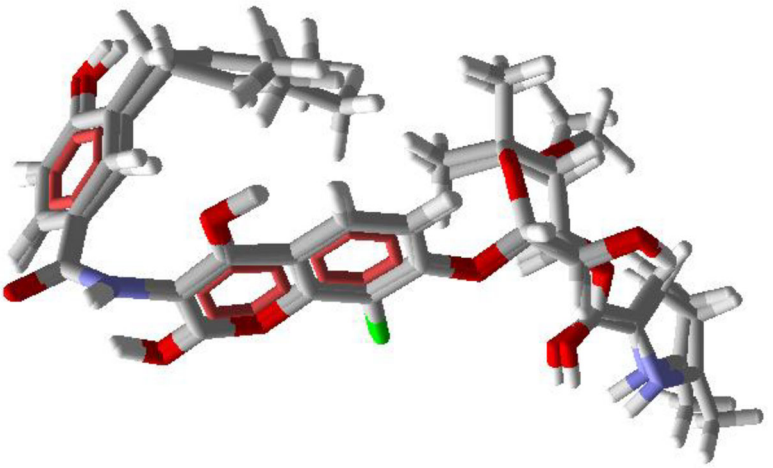


Figure 3. Active ligand and its validation of docking.

Table 3. Ligand-protein interactions of the most biologically potent products with the target 1KZN.

Ligand	MolDock Score	Rerank Score	H-Bond Energy	Protein-Ligand Interactions
5b	−157.64	−134.80	0.00	Val71, Asp73, Pro79, HOH1172[A]
5l	−160.39	−135.65	−2.50	Ala96, Ile90, Pro79, Val71, Asp73, HOH1009[A], HOH1049[A], HOH1172[A]
5f	−165.62	−135.84	0.00	His95, Ala96, Ile90, Glu50, Pro79, Asp73, HOH1162[A], HOH1049[A], HOH1009[A], HOH1172[A]
5k	−165.57	−137.06	−1.283	Asn46, Ile90, Glu50, Pro79, Asp73, HOH1162[A], HOH1172[A]
Chloramphenicol	−118.43	−100.30	−0.343	Asn46, Glu50, Ile76, Asp73, Val167, HOH1066[A], HOH1172[A], HOH1001[A]

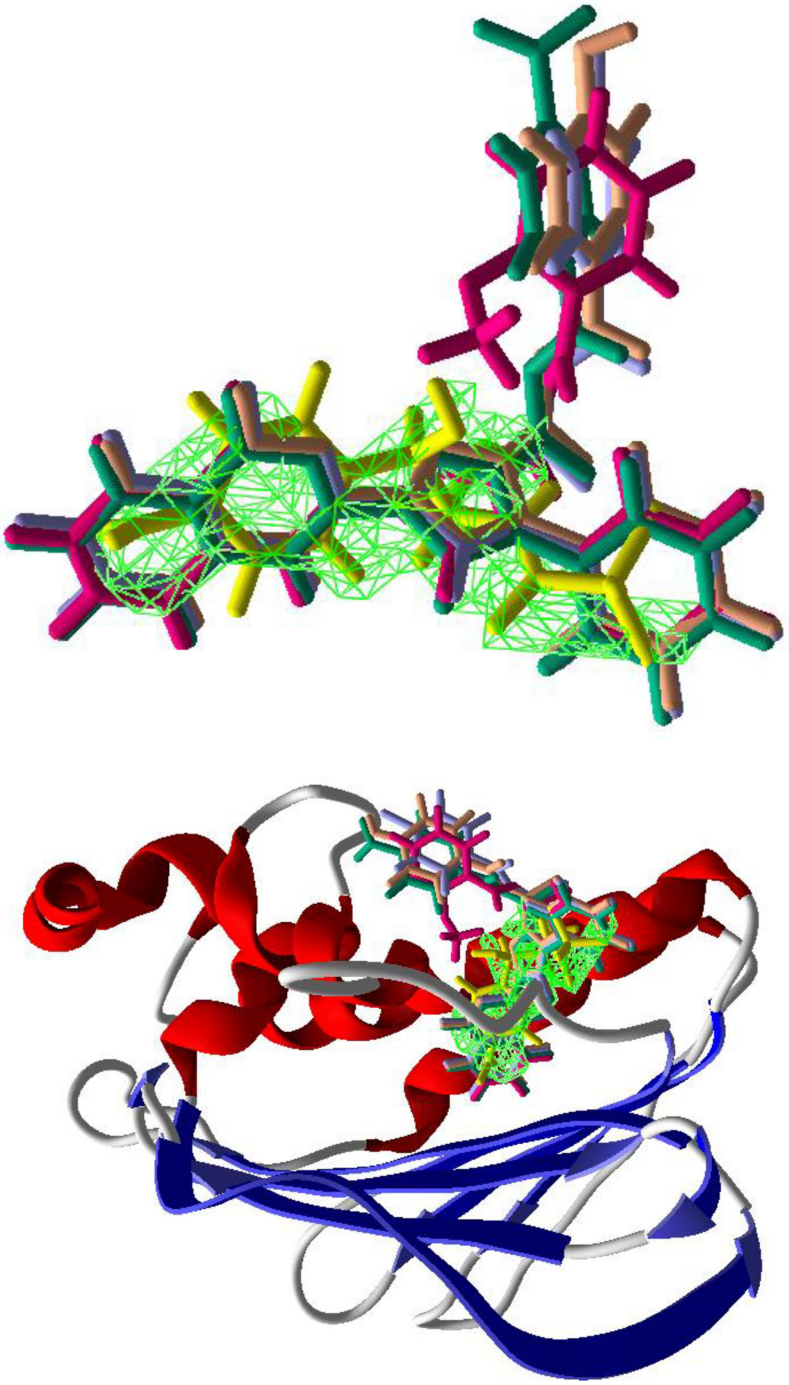
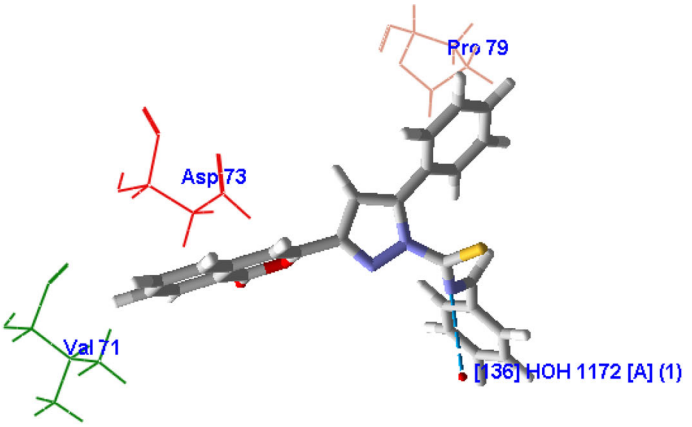


Figure 4. All the potent docked ligand poses with the target protein 1KZN.

evidently reveals the binding spots of ligands with the protein. Investigation of the receptor ligand composite representations produced after effective molecular docking of the produced amalgams with the 1KZN was completed based on the parameters like hydrogen bond energy, docked



5b

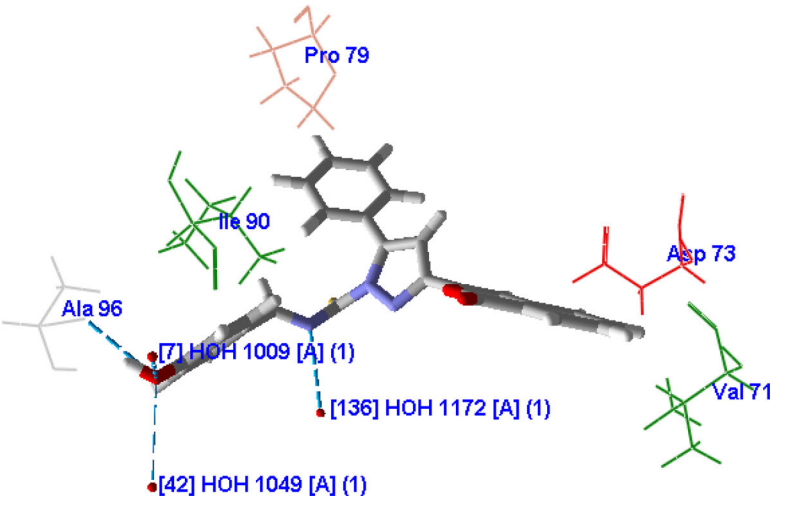


Figure 5. Protein-Ligand contacts of the active motifs with 1KZN.

amalgams rerank score, molecular docking score, and ligand-protein contacts. In addition, interaction of specific amino acid residue with ligand were showed in Figure 5. Ligand-protein contacts like binding energy, electrostatic contacts, and steric interactions between the receptor active site and ligand were found to be accountable for facilitating the biotic activity.

Among the docked molecules, compound **5f** was displayed higher MolDock score of 165.62, while the value 165.57 belongs to the motif **5k.** In fact, compound **5b** and **5l** displayed 157.64 and 160.39 MolDock score, respectively. On the other hand, the highest binding energy displayed the compound **5l.**

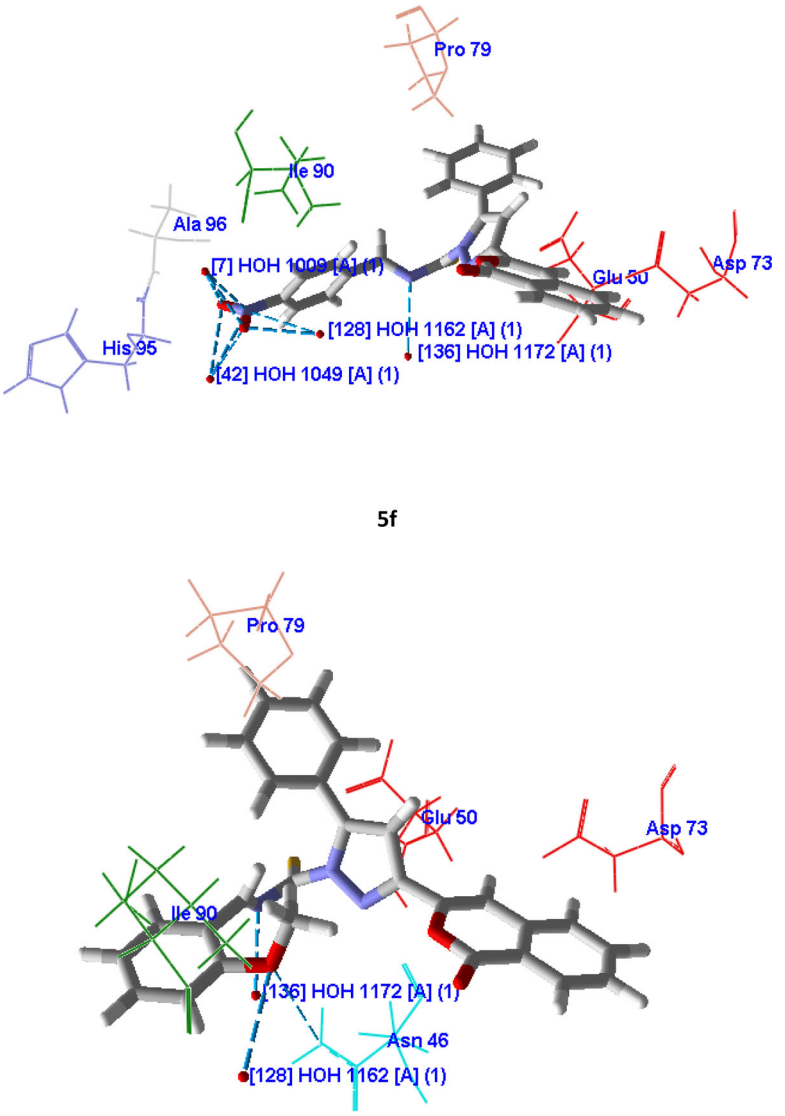


Figure 5. Continued.

Conclusions

A novel set of isocoumarin tethered carbothioamide linked pyrazole analogs were prepared successfully *via* microwave irradiation method under solvent free conditions. Newly synthesized compounds formed with excellent yield and were screened for their bacterial and fungal inhibition activity. Most of the compounds displayed significant activity towards the tested microbes. Among the active compounds, **5f** displayed highest antimicrobial activity. Also, **5b** and **5l** motifs showed good antimicrobial activity. Moreover, docking studies were formed for biologically effective compounds to determine the interactions and docking score with the crystal structure of *E. coli* 24 kDa DNA gyrase subunit B in complex with clorobiocin. It was observed that the synthesized target molecules with mixed chromophores strongly interacting with the amino acid residues present in the active binding site of the protein1KZN.

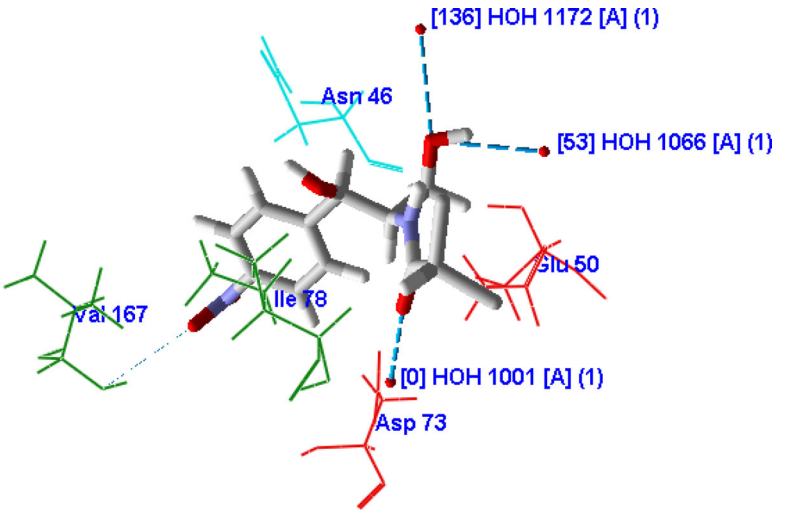


Figure 5. Continued.

Acknowledgements

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