Asian Journal of Chemistry; Vol. 30, No. 4 (2018), 783-789

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.20992

in vitro Antimicrobial Activity and *in silico* Activity of 1-Thiocarbamoyl Substituted Pyrazole Derivatives

RAJA CHINNAMANAYAKAR[1], M.R. EZHILARASI[1,*], B. PRABHA[1] and KULANDHAIVEL[2]

[1]Department of Chemistry, Karpagam Academy of Higher Education, Eachanari, Coimbatore-641 021, India
[2]Department of Microbiology, Karpagam Academy of Higher Education, Eachanari, Coimbatore-641 021, India

*Corresponding author: E-mail: ezhilarasi.au@gmail.com

Received: 21 August 2017; *Accepted*: 24 October 2017; *Published online*: 28 February 2018; AJC-18784

A convenient synthesis of 1-thiocarbamoyl-3-phenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives (**2a-2g**) was carried out by the condensation reaction of (*E*)-3-(phenyl)-1-(biphenyl-2-yl)-3-arylprop-2-en-1-ones (**1a-1g**) with thiosemicarbazide, sodium hydroxide as a catalyst in the presence of ethanol as solvent. Further, (*E*)-3-(phenyl)-1-(biphenyl-2-yl)-3-arylprop-2-en-1-ones were synthesized by the Claisen-Schmidt condensation reaction of substituted aldehydes with 4-acetylbiphenyl in the presence of basic ethanolic solution. The structure of synthesized compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY. The antimicrobial susceptibility tests of synthesized compounds were screened against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa*. The docking studies also carried out by using 1UAG receptor for all the synthesized compounds (**2a-2g**).

Keywords: 4-Acetyl biphenyl chalcone, Thiosemicarbazide, Antimicrobial activity, Molecular docking studies.

INTRODUCTION

Infectious diseases caused by the bacteria and fungi are the most serious problems in many countries. The Gram-positive and Gram-negative pathogen are cause many infectious diseases globally [1]. So that, we search for a novel and new microbial resistance drugs nowadays. The morbidity is associated with gastroenteritis of humans caused mainly by the common pathogens like *Staphylococcus*, *Yersinia enterocolitica* and *Aeromonas hydrophila*. It is one of the major threats to public health worldwide [2]. In the synthesis of heterocyclic compounds, chalcone is used as a intermediate and it should have a good biological activity as well as it plays a principle role in medicinal chemistry [3-5]. The organo-nitrogen and sulphur compounds dominate much of synthetic, analytical and medicinal chemistry. Several small molecules like thiadiazole, triazole and oxadiazole based heterocycles have also been reported to possess potential bioactivity, including antiallergic, anticonvulsant, analgesic, anticancer, anti-microbial and anti-inflammatory [6-9]. Thiosemicarbazides and their derivatives exhibit good biological activities such as anticancer [10], anti-HIV [11], antibacterial [12], antiviral [13] and antifungal [14]. The complexes of nitrogen and sulphur donor ligands are having a special and specific effect against the pathogens such as small pox virus, influenza, fungi and cancer [15]. The docking studies are mainly used to understand the drug-receptor interaction. Herein, the docking studies were performed for six antimicrobial compounds with 1UAG receptor. In this receptor, all these active sites are used to docking studies (LEU 15, THR 16, THR 36, ARG 37, GLY 73, ASN 138 and HIS 183). Finally to evaluate the binding affinity values, types of interactions, 2D and 3D image of each compounds.

In this work, thiocarbamoyl substituted pyrazole derivatives were synthesized. The skeleton structures of the synthesized compounds confirmed by using IR, ^{1}H and ^{13}C NMR and ^{1}H-^{1}H COSY spectral values and the screening test against Gram-positive and Gram-negative pathogens like *Staphylococcus aureus, Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. Also carried out the docking studies that have briefly explained the structural activity relationship effectiveness of synthesized compounds against the 1UAG protein receptor which belongs to the origin of *Escherichia coli*.

EXPERIMENTAL

Thiosemicarbazide (25 g) was purchased from Sigma Aldrich. The melting points of the compounds were determined through open capillary method and values were uncorrected. The FT-IR spectrum (cm^{-1}) was recorded through KBr in a Fourier transform IR spectrometer (model Shimadzu 8400s) in the range of 4000-400 cm^{-1}. The ^{1}H, ^{13}C NMR spectra and ^{1}H-^{1}H Cosy were recorded by Brucker 400 MHz spectrometer

and chemical shifts are recorded in δ value (ppm) with tetra methylsilane (TMS) as internal standard, as well as $CDCl_3$ used as a solvent.

Synthesis of (*E*)-3-(phenyl)-1-(biphenyl-2-yl)-3-aryl-prop-2-en-1-one derivatives (1a-1g): 4-Acetyl biphenyl (1 mol) and various substituted aromatic aldehydes (1 mol) were taken in a beaker and added 30 mL of ethanol containing 2 g of NaOH pellets. The mixture was stirred well for 30 min in an ice cold bath, after it was poured into the crushed ice containing 500 mL beaker and this reaction mixture was kept into overnight at room temperature. The chalcone were precipitated out as solid. Then it was filtered, dried and recrystallized from ethanol. The purity of the compound was checked by TLC by using $CHCl_3$ as a solvent.

Synthesis of 1-thiocarbamoyl-3-phenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives (2a-2g): One equivalent of (*E*)-3-(phenyl)-1-(biphenyl-2-yl)3-arylprop-2-en-1-one (**1a-1j**), two equivalents of thiosemicarbazide and 30 mL ethanol containing 3 g of NaOH solution were taken in a round bottom flask. The reaction mixture was refluxed for 32-40 h at 60 °C. After the completion of the reaction, the reaction mixture was poured into crushed ice and kept overnight at room temperature (**Scheme-I**). The crude product was filtered, dried and recrystallized from ethanol. The purity of the compound was checked by TLC by using petroleum ether and ethyl acetate (9:1) as a solvent.

Antimicrobial activity: The synthesized compounds were screened for antibacterial activity and calculate the minimal inhibitory concentrations (MICs). The newly synthesized compounds were tested against four different bacterial strains using agar disk diffusion method. The Gram-positive bacteria strains were *Staphylococcus aureus, Staphylococcus pyogenes* and Gram-negative bacteria strains were *Escherichia coli*, *Pseudomonas aeruginosa*. The drug ciprofloxacin was used as standard drug and the DMSO used as the solvent control in this activity.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration of the synthesized compounds was performed using broth dilution assay method. For assaying synthesized compounds, the starting concentration was kept at 8 mg/mL in the first tubes containing 1 mL of broth. Tubes were vortexed to make the initial standard concentration. This was serially diluted to other tubes and finally 1 mL was discard from the last tube hence making the dilution of 2, 1, 0.5, 0.23 mg/mL respectively. To all these test tubes, 0.1 mL of the long phase cultures of target microorganisms were added separately and incubated at 37 °C for 24-48 h. The tube was examined for visible turbidity after the incubation and plated on their respective media incubated at 37 °C for 24 h for bacteria and room temperature for yeast [16].

Molecular docking studies: The molecular docking studies were done through the Auto Dock Tools version is 1.5.6 and Auto dock version 4.2.5.1. The preparation of protein was done by literature method [17].

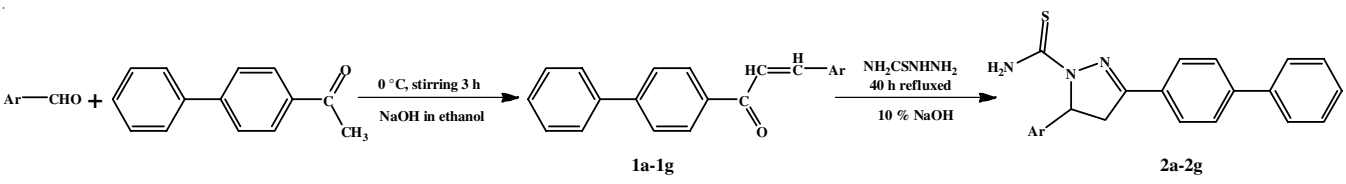
RESULTS AND DISCUSSION

The starting materials of the chalcone derivatives were prepared from known Claisen-Schmidt condensation and to form (*E*) form chalcone derivatives. Further, the chalcones were reacted with thiosemicarbazide in the presence of base medium; from this the thiocarbamoyl derivatives (**2a-2g**) were synthesized. The IR, ^1H, ^{13}C NMR and ^1H-^1H Cosy NMR spectral results were used to predict the chemical structure of the synthesized compounds **2a-2g**.

1-Thiocarbamoyl-3-phenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2a): Yield 87 %; m.p.: 248-250 °C; yellow solid; m.f.: $C_{22}H_{19}N_3S$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1587.42 (C=N), 1525.69 (C=C), 1462.04 (C-N), 3253.91, 3402.43 (N-H), 3059.10 (Ar-CH), 692.44, 731.02, 759.95, 837.11; ^1H NMR ($CDCl_3$), 400 MHz, δ, ppm (*J*, Hz): 3.27 (1H, dd, H_{4A}, $J_{4A,4B}$ = 17.6 Hz, $J_{4A,5X}$ = 3.6 Hz); 3.90 (1H,dd, H_{4B}, $J_{4B,4A}$ = 11.2 Hz, $J_{4B,5X}$ = 17.6 Hz); 6.09 (1H, dd, H_{5X}, $J_{5X,4A}$= 3.4 Hz, $J_{5X,4B}$ = 11.4 Hz); 7.27-7.83 (14H, m, Ar-H). ^{13}C NMR δ; 176.75 (C=S), 155.69 (C3 of pyrazole ring), 43.14 (C4 of pyrazole ring), 63.55 (C5 of pyrazole ring); 125.43, 127.09, 127.45, 127.50, 127.69, 128.09, 128.95, 128.99 (Ar-C); 143.82, 141.78, 139.92, 129.49 (Ipso carbon).

1-Thiocarbamoyl-3-fluorophenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2b): Yield 75 %; m.p.: 242-248 °C; Yellow solid; m.f.: $C_{22}H_{18}N_3SF$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1572.05 (C=N), 1498.75 (C=C), 1469.82 (C-N), 3349.53, 3473.95 (N-H), 3048.62 (Ar-CH), 694.40, 730.09, 760.95, 833.28; ^1H NMR ($CDCl_3$), 400 MHz, δ, ppm, (*J*, Hz): 3.19 (1H, dd, H_{4A}, $J_{4A,4B}$ = 18 Hz, $J_{4A,5X}$=3.2 Hz); 3.93 (1H, dd, H_{4B}, $J_{4B,4A}$=11.4 Hz, $J_{4B,5X}$ = 18.2 Hz); 5.94 (1H, dd, H_{5X}, $J_{5X,4A}$=3.4 Hz, $J_{5X,4B}$= 11.4 Hz); 7.12-8.05 (13H, m, Ar-H).^{13}C NMR δ; 176.03 (C=S); 42.27 (C4 of pyrazole ring); 62.28 (C5 of pyrazole ring); 162.30 (C3 of pyrazole ring); 115.08,115.30, 126.70, 126.83, 127.38, 127.46, 127.75, 128, 129.02, 129.82 N(Ar-C); 139.11, 142.01, 154.64, 159.89 Ipso carbon).

1-Thiocarbamoyl-3-methylphenyl-5-diphenyl-4.5-dihydro-(1*H*)-pyrazole (2c): Yield 81 %; m.p.: 230-238 °C; yellow solid; m.f.: $C_{23}H_{21}N_3S$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1587.48 (C=N), 1513.22 (C=C), 1462.11 (C-N), 3244.41, 3431.51 (N-H), 3027.41 (Ar-CH), 651.00, 692.47, 724.30, 762.88. ^1H NMR ($CDCl_3$), 400 MHz, δ, ppm, (*J*, Hz): 3.25 (1H, dd, H_{4A}, $J_{4A,4B}$ = 16 Hz, $J_{4A,5X}$ = 4 Hz); 3.88 (1H, dd, H_{4B}, $J_{4B,4A}$ = 12 Hz, $J_{4B,5X}$ = 20 Hz); 6.06 (1H, dd, H_{5X}, $J_{5X,4A}$ = 4 Hz, $J_{5X,4B}$ = 12 Hz); 2.34 (CH_3 (H), S; 7.17-7.83 (13H, m, Ar-H).



Scheme-I: Synthesis of 1-thiocarbamoyl-3-phenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole derivatives

^{13}C NMR δ; 176.68 (C=S); 155.72 (C3 of pyrazole ring); 43.18 (C4 of pyrazole ring); 63.37 (C5 of pyrazole ring); 125.39, 1227.09, 127.45, 127.49, 128.09, 129, 129.56, 129.62 (Ar-C); 143.76, 139.94, 138.89, 137.35 (Ipso carbon).

1-Thiocarbamoyl-3-bromophenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2d): Yield 73 %; m.p.: 232-236 °C; yellow solid; m.f.: $C_{22}H_{18}N_3SBr$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1588.45 (C=N), 1523.83 (C=C), 1463.07 (C-N), 3258.87, 3404. 51 (N-H), 3029.34 (Ar-CH), 656.79, 692.47, 723.34, 765.77. ^{1}H NMR (CDCl3), 400 MHz, δ, ppm, (*J*, Hz): 3.23 (1H, dd, H_{4A}, $J_{4A,4B}$ = 16 Hz, $J_{4A,5X}$ = 4 Hz); 3.90 (1H, dd, H_{4B}, $J_{4B,4A}$ = 12 Hz, $J_{4B,5X}$ = 16 Hz); 6.04 (1H, dd, H_{5X}, $J_{5X,4A}$ = 4 Hz, $J_{5X,4B}$ = 12 Hz); 7.14-7.83 (13H, m, Ar-H). ^{13}C NMR, δ, ppm; 182.91 (C=S); 49.13 (C4 of pyrazole ring; 69.19 (C5 of pyrazole ring); 150.15 (C3 of pyrazole ring; 127.76, 133.27, 133.51, 133.63, 133.72, 134.32, 135.18, 135.43 (Ar-C); 150.15, 147.01, 146.03, 138.26 (Ipso carbon).

1-Thiocarbamoyl-3-methoxyphenyl-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2e): Yield 85 %; m.p.: 234-238 °C; yellow solid; m.f.: $C_{23}H_{21}N_3OS$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1579.77 (C=N), 1509.36 (C=C), 1473. 68 (C-N), 3427.65, 3475.88 (N-H), 3053.45 (Ar-CH), 695.37, 728.16, 764.81, 834.25. ^{1}H NMR ($CDCl_3$), 400 MHz, δ, ppm, (*J*, Hz): 3.84 (1H, dd, H_{4A}, $J_{4A,4B}$ = 10 Hz, $J_{4A,5X}$ = 7.6 Hz); 3.88 (1H, dd, H_{4B}, $J_{4B,4A}$ = 12.4 Hz, $J_{4B,5X}$ = 8 Hz); 6.09 (1H, dd, H_{5X}, $J_{5X,4A}$ = 11.4, $J_{5X,4B}$ = 4 Hz); 3.80 (3H, s, OCH_3); 7.42-7.84 (13H, m, Ar-H). ^{13}C NMR, δ, ppm; 172.26 (C=S); 153.78 (C3 of pyrazole ring); 50.01 (C4 of pyrazole ring; 57.82 (C5 of pyrazole ring); 109.02, 121.59, 121.83, 122.00, 122.19, 122.24, 122.63, 122.83, 123.73 (Ar-C); 150.62, 138.40, 134.74, 128.93 (Ipso carbon).

1-Thiocarbamoyl-3-(2,3-dichlorophenyl)-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2f): Yield 72 %; m.p.: 258-262 °C; yellow solid; m.f.: $C_{22}H_{17}N_3SCl_2$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1591.34 (C=N), 1519.97 (C=C), 1467.89 (C-N), 3255.98, 3403.54 (N-H), 3029.34 (Ar-CH), 621.11, 699.23, 726.73, 768.67. ^{1}H NMR ($CDCl_3$), 400 MHz, δ, ppm, (*J*, Hz): 3.17 (1H, dd, H_{4A}, $J_{4A,4B}$ = 18 Hz, $J_{4A,5X}$ = 6 Hz); 4.01 (1H, dd, H_{4B}, $J_{4B,4A}$ = 18 Hz, $J_{4B,5X}$ = 10 Hz); 6.41 (1H, dd, H_{5X}, $J_{5X,4A}$ = 4 Hz, $J_{5X,4B}$ = 12 Hz); 7.82-7.02 (11H, m, Ar-H). ^{13}C NMR, δ, ppm; 176.84 (C=S); 156.01 (C3 of pyrazole ring); 41.89 (C4 of pyrazole ring); 62 (C5 of pyrazole ring); 127.08, 127.44, 127.52, 127.73, 128.15, 128.99, 129.14, 129.59 (Ar-C); 144.01, 140.01, 140.90, 139.82 (Ipso carbon).

1-Thiocarbamoyl-3-(2-hydroxyphenyl)-5-diphenyl-4,5-dihydro-(1*H*)-pyrazole (2g): Yield 78 %; m.p.: 246-254 °C; yellow solid; m.f.: $C_{22}H_{19}N_3OS$; IR (KBr, ν_{max}, cm^{-1}): Pyrazole 1589.41 (C=N), 1521.90 (C=C), 1466.93 (C-N), 3405.47, 3357.91 (N-H), 3024.51, 3062.48 (Ar-CH), 652.90, 697.30, 727. 19, 765.77; ^{1}H NMR ($CDCl_3$), 400 MHz, δ, ppm, (*J*, Hz): 3.16 (1 H, dd, H4A, $J_{4A,4B}$ = 17.8 Hz, $J_{4A,5X}$ = 3.8 Hz); 3.96 ppm (1H, dd, H4B, $J_{4B,4A}$ = 11.6 Hz, $J_{4B,5X}$ = 18 Hz); 6.37 (1H, dd, H5X, $J_{5X,4A}$ = 3.8 Hz, $J_{5X,4B}$ = 11.4 Hz); 7.07-7.80 (11H, m, Ar-H). ^{13}C NMR, δ; 176.898 (C=S), 156.00 (C3 of pyrazole ring), 42.02 (C4 of pyrazole ring), 61.41 (C5 of pyrazole ring; 127.08, 127.28, 127.43, 127.48, 128.10, 128.82, 128.98, 129.36, 130.05 (Ar-C); 131.35, 138.64, 139.89, 143.90 (Ipso carbon).

2D NMR spectral analysis

^{1}H-^{1}H Cosy: The 2D ^{1}H-^{1}H COSY spectrum of compound **2a** shows that the signal at 3.27 ppm has one bond correlation with the doublet of doublet signals at 3.90 and 6.09 ppm. Similarly the signal at 3.90 ppm has one bond correlation with the signals at 3.27 and 6.09 ppm. The signal at 6.09 ppm has one bond also correlation with 3.27 and 3.90 ppm. From this observed the correlations of 2D NMR spectrum results, unambiguously assign that the doublet of doublets observed in the range of 3.27, 3.90 and 6.09 ppm are due to the presence of H_{4A}, H_{4B} and H_{5X} protons, respectively.

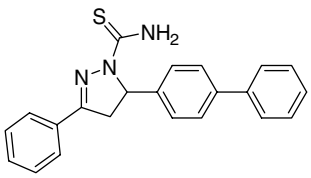
Docking studies

***in silco* Activity:** The 1UAG protein is responsible for the mechanism of cell wall synthesis. The synthesized compounds showed a good docking score and also have good interaction. Especially (compound **2e**) showed a good docking score (-8.4 kcal/mol) compared to other 6 compounds. Other compound docking scores are given by decreasing order -8.3, -8.3, -8.2, -8.1, -8.0 and -7.8 kcal/mol of **2f**, **2d**, **2b**, **2a**, **2c** and **2g**. The compound **2e** interacts with IUAG by forming conventional hydrogen bonding at LYS A: 319 and PHE A: 422. Other compounds (**2f**, **2d**, **2b**, **2a**, **2c**, **2g**) conventional hydrogen bonding at PHE A: 422; PHE A: 422; PHE A: 422, LYS A: 319; PHE A: 422; PHE A: 303, ARG A: 302, THR A: 297; ASN A:138,GLU A:157, LYS A:319. The benzene ring of the compound **2a** forms the alkyl and π-alkyl interaction with ALA A: 414; π-σ interaction with LEU A: 416; π-σ interaction in the pyrazole ring is ALA A: 414; C=S forms conventional hydrogen bond with PHE: 422. The benzene ring of the compounds **2b** forms the π-alkyl interaction with LEU A: 416; π-σ interaction with LEU A: 416, ALA A: 414; C=S forms the conventional hydrogen bond with PHE A: 422. The compound **2c** forms the alkyl and π-alkyl interaction with LYS A: 262; π-σ interaction with LYS A: 262; π-π stacked interaction with PHE A: 303. The benzene ring of the compound **2d** forms the alkyl and π-alkyl interaction with ALA A: 414; π-σ interaction with LEU A: 416; π-σ interaction in the pyrazole ring is ALA A: 414; C=S forms the conventional hydrogen bond with PHE A: 422. The benzene ring of the compound **2e** forms the alkyl and π-alkyl interaction with LYS A: 348, LEU A: 346; π-σ interaction with LEU A: 416, ALA A: 414; C=S forms the conventional hydrogen bond with PHE A: 422. The benzene ring of the compound **2f** forms the alkyl and π-alkyl interaction with LYS A: 348, ALA A: 414; π-σ interaction with LEU A: 416; π-σ interaction in the pyrazole ring is ALA A: 414; C=S forms the conventional hydrogen bond with PHE A: 422. The benzene ring of the compound **2g** show no alkyl and π-alkyl interaction; π-σ interaction with LEU A: 15; The π-π T-shaped interaction with PHE A: 422; C=S forms the conventional hydrogen bond with HIS A: 183.The binding value and conventional hydrogen bonds of the synthesized compounds are shown in Table-1. The 2-dimensional and 3-dimensional images of the synthesized compounds are shown in Fig. 1.

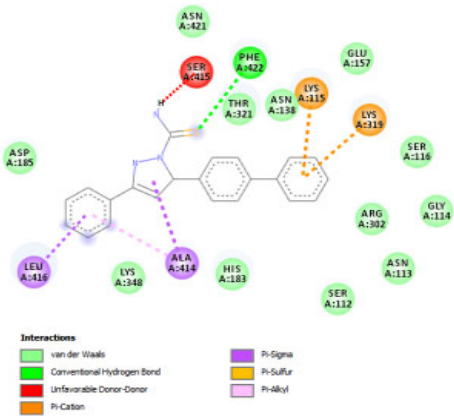
Antimicrobial activity: The *in vitro* antibacterial activities of the synthesized compounds are subjected at different concentrations (1.0, 0.5, 0. 25 mg/mL) are given in Table-2. From the screening results **2a**, **2d**, **2f** and **2g** showed a good

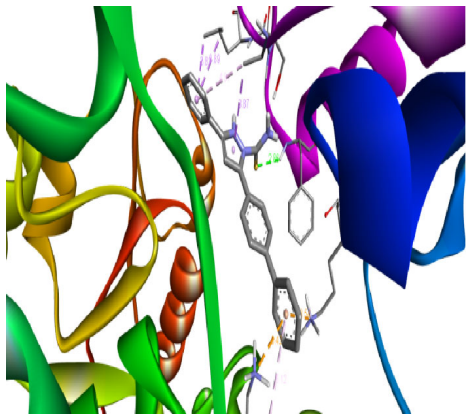
TABLE-1

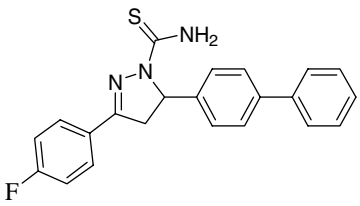
Compd. No.	Binding affinity (Kcal/mol)	Conventional hydrogen bond	Alkyl and π-alkyl bond	Other bond
2a	-8.1	PHE A:422	ALA A: 414	ALA A:414, LEU A:416, LYS A:115, LYS A:319
2b	-8.2	PHE A:422, LYS A:319	LEU A: 416	LEU A:416, ALA A:414
2c	-8.0	PHE A:303, ARG A:302, THR A:297	LYS A: 262	LYS A:26, LYS A: 262
2d	-8.3	PHE A:422	LEU A: 416, ALA A: 414	LYS A:115, LYS A:319, LEU A:416, ALA A:414
2e	-8.4	PHE A:422, LYS A: 319	LYS A:348, LEU A:346	LEU A:416, ALA A:414
2f	-8.3	PHE A:422	LYS A:348, LEU A:416, ALA A:414	ALA A:414, LEU A: 416
2g	-7.8	LYS A: 319, GLU A: 157, ASN A: 138	–	PHE A:422, LEU A 115



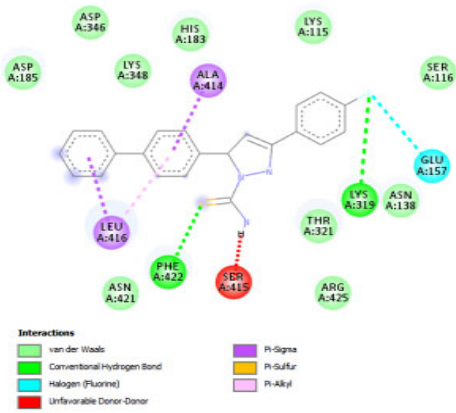
2a

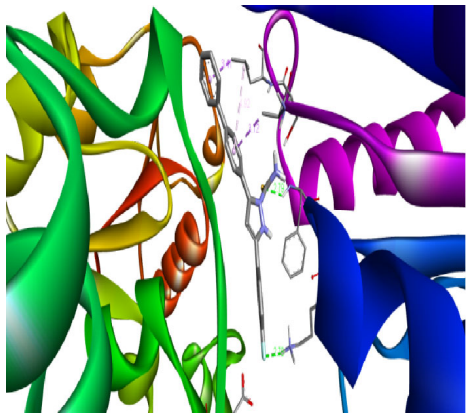


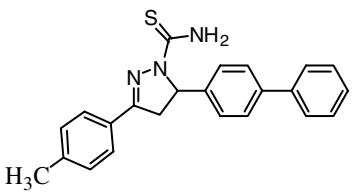




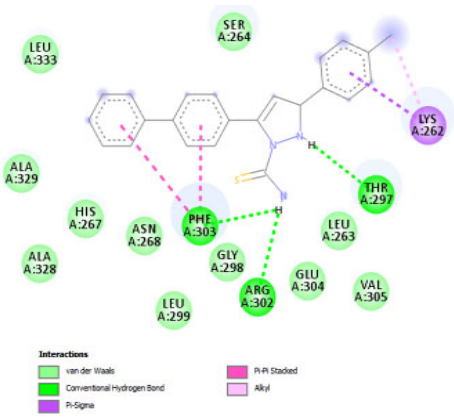
2b

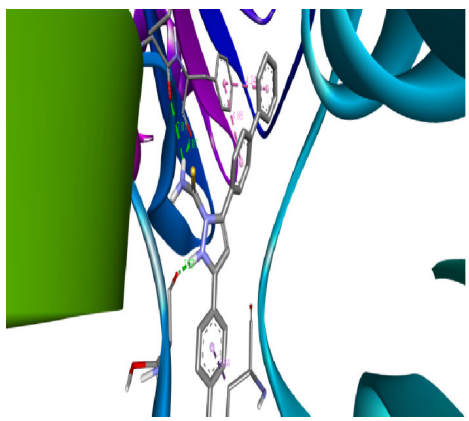






2c





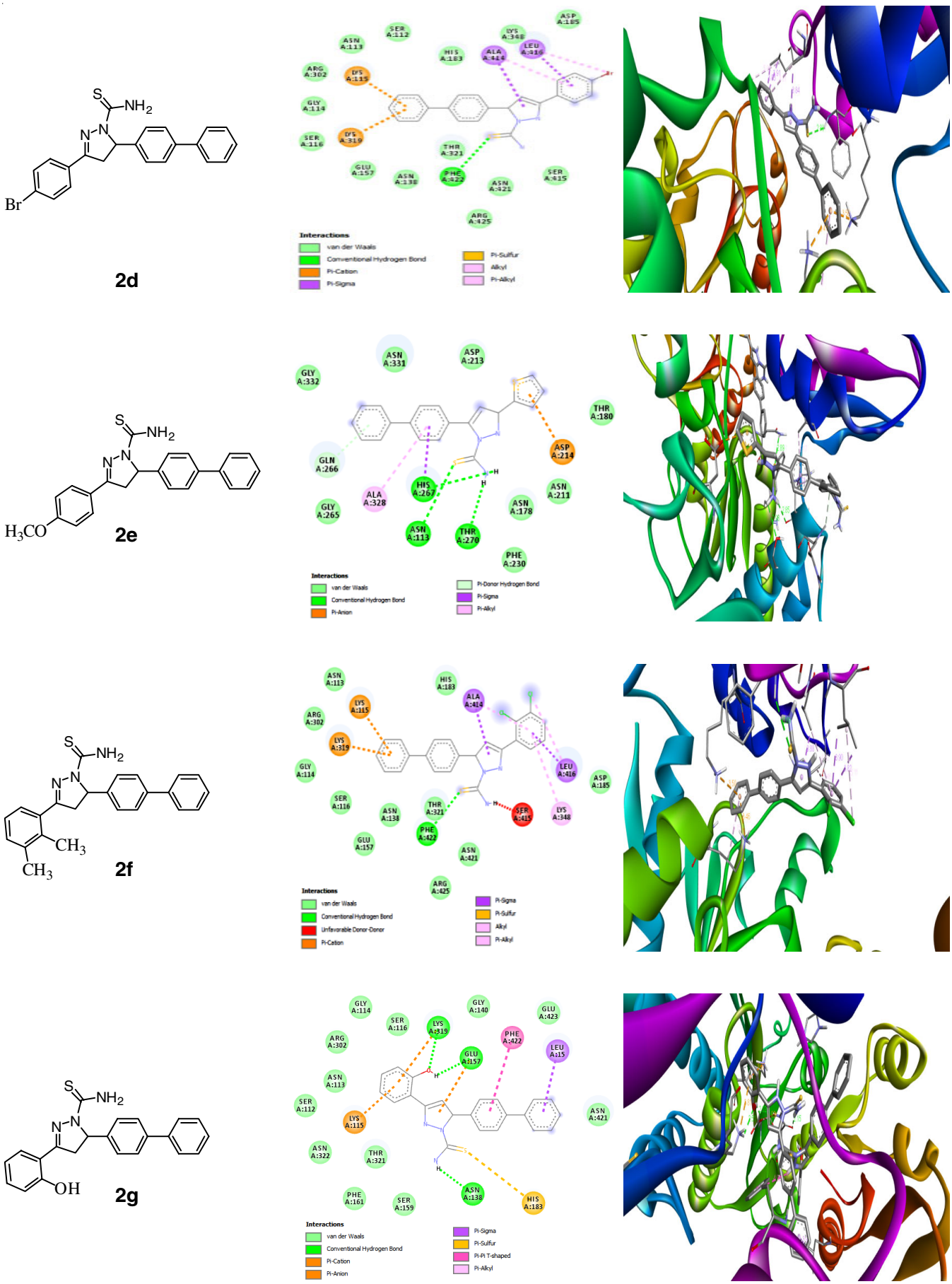


Fig. 1. 2D and 3D images of the synthesized 1-thiocarbamoyl substituted pyrazole derivatives (**2a-2g**)

TABLE-2
ANTIMICROBIAL SUSCEPTIBILITY AGAINST SYNTHESIZED
1-THIOCARBAMOYL SUBSTITUTED PYRAZOLE DERIVATIVES (**2a-2g**)

Compd. No.	Zone of inhibition (diameter in mm)											
	Staphylococcus aureus			*Streptococcus pyogenes*			*Escherichia coli*			*Pseudomonas aeruginosa*		
	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL
2a	16	13	> 10	16	15	10	17	12	10	20	13	> 10
2b	14	11	10	18	12	≥ 10	16	11	10	18	15	> 10
2c	15	10	> 10	21	16	12	18	11	10	16	13	> 10
2d	16	11	> 10	19	14	12	18	15	12	–	–	–
2e	13	> 10	> 10	15	13	10	13	> 10	> 10	10	> 10	> 10
2f	16	10	> 10	12	11	10	12	11	> 10	> 10	> 10	–
2g	16	15	10	22	20	13	24	14	14	25	15	11

TABLE-3
MINIMUM INHIBITORY CONCENTRATION OF SYNTHESIZED
1-THIOCARBAMOYL SUBSTITUTED PYRAZOLE DERIVATIVES (**2a-2g**)

Compd. No.	*Staphylococcus aureus*			*Streptococcus pyogenes*			*Escherichia coli*			*Pseudomonas aeruginosa*		
	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL	1.0 mg/mL	0.5 mg/mL	0.25 mg/mL
2a	–	–	++	–	–	++	–	–	++	–	–	++
2b	–	–	++	–	–	++	–	–	++	–	–	++
2c	–	++	++	–	–	–	–	++	++	–	–	++
2d	–	–	++	–	–	–	–	–	–	++	++	++
2e	–	++	++	–	–	++	–	++	++	++	++	++
2f	–	++	++	–	–	++	–	–	++	–	–	++
2g	–	–	++	–	–	++	–	–	–	–	–	++

The negative sign (–) indicates no growth on the plates; The positive sign (++) indicates growth on the plates.

zone of inhibition against *Staphylococcus auresus* (Gram-positive); Compounds **2g** exhibited a good zone of inhibition against *Streptococcus pyogenes* (Gram-positive); Compounds **2g** exhibited a good activity against *Escherichia coli* (Gram-negative); Compounds **2g** showed a good zone of inhibition against *Pseudomonas aeruginosa* (Gram-negative).

The minimum inhibitory concentration of the synthesized compounds are subjected at different concentrations (1.0, 0.5, 0.25 mg/mL) are given in Table-3. From this screening result the compound **2c** and **2d** exhibit a good inhibition at minimum concentration (0.25 mg/mL against *Streptococcus pyogenes* (Gram-positive) strain. In *Escherichia coli* (Gram-negative), the synthesized compound **2d** and **2g** shows a good inhibition at minimum concentration (0.25 mg/mL). The compound **2a**, **2b**, **2d** and **2g** shows a good minimum inhibitory concentration (0.5 mg/mL) against *Staphylococcus aureus* (Gram-positive). The synthesized compound **2a**, **2b**, **2d**, **2f** and **2g** show a good minimum inhibitory concentration (0.5 mg/mL) against *Pseudomonas aeruginosa* (Gram-negative).

The electron negativity substitution group (Br) have a good inhibition against *Streptococcus pyogenes* and *Escherichia coli* and the electron releasing group (CH_3) have a efficient inhibition against *Streptococcus pyogenes* at 0.25 mg/mL. The same bromo substitutions have the good inhibition against *Staphylococcus aureus* at 0.5 mg/mL. Including base and the other substitutions such as fluoro have a better inhibition against *Pseudomonas aeruginosa* at the concentration 0.5 mg/mL. The hydroxy group also show a good MIC at 0.25 mg/mL against *Escherichia coli* and *Streptococcus pyogenes*.

Conclusion

A new series of thiocarbamoyl derivatives were synthesized and characterized by FT-IR, ^{1}H NMR and ^{13}C NMR techniques and ^{1}H-^{1}H COSY. The synthesized compounds were screened for antibacterial activity. From this result, the compound (**2a**, **2d**, **2f**, **2g**) shows good zone of inhibition against Gram-positive strain *(Staphylococcus aureus)*. In the *Streptococcus pyogenes* (Gram-positive strain), the studies on electron releasing group (CH_3) shows a excellent zone of inhibition; The compound (**2d**, **2g**) shows a good zone of inhibition against *Escherichia coli* (Gram-negative strain); The compound **2g** shows a good zone of inhibition against *Pseudomonas aeruginosa* (Gram-negative strain). The Br substitution exhibit a good inhibition at 0.25 mg/mL against *Streptococcus pyogenes* and *Escherichia coli*. It also has a better inhibition against *Staphylococcus aureus* at 0.5 mg/mL. Finally the molecular docking studies were carried out. From this prediction of the *in silco* activity of the synthesized compounds against on the MurD enzymatic proteins. Also this docking result the compound **2e** shows a good docking score (-8.4 kcal/mol) against 1UAG protein.

REFERENCES

1. S.Y. Abbas, M.A.M.S. El-Sharief, W.M. Basyouni, I.M.I. Fakhr and E.W. El-Gammal, *Eur. J. Med. Chem.*, **64**, 111 (2013); https://doi.org/10.1016/j.ejmech.2013.04.002.
2. M. Rani, M. Yusuf and S.A. Khan, *J. Saudi Chem. Soc.*, **16**, 431 (2012); https://doi.org/10.1016/j.jscs.2011.02.012.
3. K.S. Atwal, G.C. Rovnyak, S.D. Kimball, D.M. Floyd, S. Moreland, B.N. Swanson, J.Z. Gougoutas, J. Schwartz, K.M. Smillie and M.F. Malley, *J. Med. Chem.*, **33**, 2629 (1990); https://doi.org/10.1021/jm00171a044.

4. K.S. Nimavat, K.H. Popat, S.L. Vasoya and H.S. Joshi, *Indian J. Heterocycl. Chem.*, **12**, 217 (2003).
5. B.B. Chavan, A.S. Gadekar, P.P. Mehta, P.K. Vawhal, A.K. Kolsure and A.R. Chabukswar, *Asian J. Biomed. Pharm. Sci.*, **6**, 1 (2016).
6. A.R. Jalilian, S. Sattari, M. Bineshmarvasti, M. Daneshtalab and A. Shafiee, *IL Farmaco*, **58**, 63 (2003); https://doi.org/10.1016/S0014-827X(02)00029-0.
7. A.R. Shafiee, B. Jalilian and M. Rezaei, *J. Heterocycl. Chem.*, **37**, 1325 (2000); https://doi.org/10.1002/jhet.5570370551.
8. A. Varvaresou, T. Siatra-Papastaikoudi, A. Tsotinis, A. Tsantili-Kakoulidou and A. Vamvakides, *Farmaco*, **53**, 320 (1998); https://doi.org/10.1016/S0014-827X(98)00024-X.
9. M. Bineshmarvasti, M. Sharifzadeh, A.R. Jalilian, K. Soltaninejad and A. Shafiee, *Daru*, **11**, 74 (2003).
10. P. Yogeeswari, N. Menon, A. Semwal, M. Arjun and D. Sriram, *Eur. J. Med. Chem.*, **46**, 2964 (2011); https://doi.org/10.1016/j.ejmech.2011.04.021.
11. N. Siddiqui and O. Singh, *Indian J. Pharm. Sci.*, **65**, 423 (2003).
12. C. Biot, B. Pradines, M.-H. Sergeant, J. Gut, P.J. Rosenthal and K. Chibale, *Bioorg. Med. Chem.*, **17**, 6434 (2007); https://doi.org/10.1016/j.bmcl.2007.10.003.
13. C. Shipman, S.H. Smith, J.C. Drach and D.L. Klayman, *Antimicrob. Agents Chemother.*, **19**, 682 (1981); https://doi.org/10.1128/AAC.19.4.682.
14. A. Siwek, J. Stefañska, K. Dzitko and A. Ruszczak, *J. Mol. Model.*, **18**, 4159 (2012); https://doi.org/10.1007/s00894-012-1420-5.
15. S. Singhal, S. Arora, S. Agarwal, R. Sharma and N. Singhal, *World J. Pharm. Pharm. Sci.*, **2**, 4661 (2013).
16. K.V. Hridhya and M. Kulandhaivel, *Int. J. Pharmacog. Phytochem. Res.*, **9**, 1001 (2017).
17. J.A. Bertrand, *EMBO J.*, **16**, 3416 (1997); https://doi.org/10.1093/emboj/16.12.3416.