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Design, Synthesis, In Silico Docking Studies, and Antibacterial Activity of Some Thiadiazines and 1,2,4-Triazole-3-Thiones Bearing Pyrazole Moiety

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Abstract—In view of developing new bioactive compounds, a series of 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines and 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones were synthesized in good yields. The compounds were confirmed by elemental analyses, mass spectrometry, FT-IR, ^{1}H, and ^{13}CNMR spectroscopy. To study the binding interactions of the derivatives with the receptor, they were docked with the prostaglandin D2 synthase (PGDS). The docking pose and non-covalent interactions gave insights into their plausible inhibitory action. They showed good antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Particularly, chloro, fluoro, dimethoxy, and dihydroxy substituted derivatives displayed good activity over other derivatives.

Keywords: thiadiazine, Schiff base, molecular docking, prostaglandin D2 synthase, antibacterial activity

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INTRODUCTION

Heterocyclic compounds are common structural units in marketed drugs and also in medicinal chemistry targets in the drug discovery process. The main reason behind this is the high prevalence of oxygen, sulfur, and especially nitrogen-containing rings in drug molecules [1]. In the last few decades the chemistry of *N*-bridged heterocycles derived from 1,2,4- triazole and their fused heterocyclic derivatives have received much attention owing to their synthetic and effective medical applications. These N-bridged heterocyclic compounds are known to possess significant activity, such as antibacterial [2], anti-inflammatory [3], anticancer [4], anti-allergic [5], antimicrobial [6], antitubercular [7], antiviral [8], antitumor [9], antioxidant [10], anthelmintic [11], anticonvulsant [12, 13], antifungal [14], analgesic [15], and antiparasitic [16] properties. Many compounds containing 1,2,4-triazole nucleus are used as drugs; for example, Loreclezole, Itraconazole, and Alprazolam, are the triazole-containing anticonvulsant and antifungal drugs and also drugs for panic disorder. Moreover, sulfur substituted 1,2,4-triazole ring systems represents an important group of bioactive compounds [17–20]. In current days non-steroidal drugs Vorozole, Letrozole, and Anastrozoleare also used for the treatment of cancer, as are many drugs containing pyrazoles, like pyrazofurin, phenylbutazone, novalgine, celecoxib, apixaban, and ramifenazone, which are already in the market. Therefore,1,2,4-triazole derivatives have the chance in cancer researches with good results. Keeping these observations in sight, synthesis of triazoles fused to one or more heterocyclic ring has attracted our attention due to their miscellaneous pharmacological applications.

Non-steroidal anti-inflammatory drugs (NSAIDs) represent one of the most useful clinical therapies for the treatment of pain, fever, and inflammation [21]. It is well documented that traditional NSAIDs exert their pharmacological effects through the inhibition of cyclooxygenase (COX) dependent prostaglandins biosynthesis. COXs are a class of bifunctional enzymes responsible for bis-oxygenation followed by reduction of arachidonic acid to generate prostaglandin H2 (PGH_2) [22]. COX-1, COX-2, and COX-3 are identified as three isoforms of COX enzyme [23]. Prostaglandins are lipid autacoids, which are derived from arachidonic acid by the action of cyclooxygenase (COX) isoenzymes. They perform a part of various homeostatic functions ofimmense physical importancein the central nervous system (CNS), cardiovascular, endocrine, immune, and genitourinary systems. They also mediate pathogenic mechanisms, including the inflammatory response. Prostaglandins play a key role in the generation of the inflammatory response [24]. The exact role of each prostaglandin is examined by their receptor expression profile and the cellular

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context. Prostaglandin D2 synthase (PGDS) catalyzes the reaction of isomerization of PGH2 to PGD2. The release of PGD2 results in a various set of responses ranging from sleep promotion and inhibition of the platelet aggregation to the attraction of bronchoconstriction and inflammatory cells [25]. Their biosynthesis is blocked by non-steroidal anti-inflammatory drugs (NSAIDs), including those selective for inhibition of COX-2. Despite the clinical potency of NSAIDs, prostaglandins also function in the promotion and resolution of inflammation.

All the above mentioned literature facts motivated us to design and synthesize derivatives of the novel and bioactive thiadiazines and Schiff bases. In the present work we report the synthesis of novel derivatives of 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*- [1, 2, 4]triazolo[3,4-*b*] [1,3,4]thiadiazine(**VIIa**–**f**) and 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione(**VIIIa**–**i**).

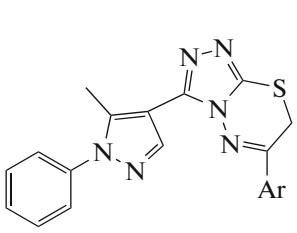
RESULTS AND DISCUSSION

The synthetic procedure for aimed derivatives (**VIIa**–**f**) and (**VIIIa**–**i**) is represented in Scheme 1. According to the literature procedure, we synthesized 5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole-2(3*H*)-thione (**V**) [26, 27]. Further, the intermediate (**V**) was treated with hydrazine hydrate in reflux condition with ethanol to get 4-amino-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**VI**). The resultant product was further refluxed with substituted phenacyl bromide in the presence of ethanol as a solvent to obtain the target derivatives 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*- [1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (**VIIa**–**f**). The same intermediate (**VI**) was refluxed with substituted benzylaldehyde in 10 mL of absolute ethanol in presence of catalytic amount of acetic acid to obtain the series of 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**VIIIa**–**i**). The structure of the compound was confirmed by FT-IR, ^{1}H and ^{13}C NMR, and mass spectroscopic techniques. Physical properties of synthesized compounds are listed in Table 1.

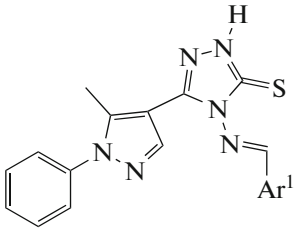
The formation of target compounds 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*- [1,2,4]triazolo[3,4-*b*] [1, 3, 4]thiadiazine (**VIIa**–**f**) was favored by their IR data. IR spectrum of compound (**VIId**) showed no absorption bands in the range of 2000–3500 cm^{-1} this indicated there is no –NH and –NH$_2$ groups in the targeted derivatives. The appearance of the band at 1592 cm^{-1} supported the presence of C=N group in pyrazoline and triazole ring. Also, bands appeared at 1453 and 1356 cm^{-1} supporting the presence of C=C and C=S functional groups and a sharp band near 760 cm^{-1} indicated the presence of C–Br in the derivative. Further, its ^{1}H NMR showed a singlet at 2.73 ppm for CH$_3$ protons. A sharp singlet at 4.00 ppm confirmed the presence of CH$_2$ protons in the derivative. The aromatic protons appeared as a multiplet in the range of 7.49–7.54 ppm. The protons of the 4-bromophenyl group appeared as two distinct doublets one at 7.67 ppm (J = 8.8 Hz) and other at 7.82 ppm (J = 8.8 Hz) correspondingly. A characteristic singlet appeared at 8.24 ppm was assigned to CH proton of pyrazole. The ^{13}C NMR spectrum of compound (**VIId**) showed the signal at 11.3 ppm corresponding to the CH$_3$ group of the target molecule. Moreover, it showed a signal at 25.4 ppm corresponding to the CH$_2$ proton, which clearly indicates the formation of the thiadiazine ring in (**VIId**). Aromatic carbons appeared in the range of 110.2–174.3 ppm. The mass spectrum provided support to the structure of the compound (**VIId**) as it displayed $(M + H)^+$ ion peak at m/z 451.07 for the molecular formula $C_{21}H_{15}BrN_6S$. The results obtained from elemental analysis are also in agreement with the experimental section. The IR, ^{1}H NMR, and ^{13}C NMR data of all other final targets were also in agreement with the formation of thiadiazine.

The formation of target compounds 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**VIIIa**–**i**) was also favored by their IR data. The IR spectrum of compound (**VIIIb**) exhibited absorption band at 1597 cm^{-1}, corresponding to the C=N group of the triazole and pyrazoline ring. The absorption band appeared at 1504 cm^{-1} due to the presence of the C=C group. Absorption bands seen at 3409 and 1249 cm^{-1} supported the presence of aromatic N–H and C=S groups. The observed IR bands supported the formation of the target compound. The ^{1}H NMR spectrum of compound (**VIIIb**) showed a singlet at 2.17 ppm for CH$_3$ protons. The two methoxy protons appeared at 3.94 and 3.98 ppm as two distinct singlets. –NH proton resonated at 4.82 ppm as a singlet. The fifth proton of 3,4-dimethoxyphenyl group resonated at 6.92 ppm as a doublet with a coupling constant 8.4 Hz. Sixth proton of the 3,4-dimethoxyphenyl group showed a doublet with a coupling constant of 8.4 Hz. The second proton showed a singlet at 7.24 ppm. Protons of the phenyl group appeared as a multiplet in the range of 7.44–7.67 ppm. The pyrazole proton resonated at 8.48 ppm as a singlet and another singlet signal appearing at 8.60 ppm was assigned to benzylidene proton. The ^{13}C NMR spectrum of the (**VIIIb**) showed signals at 171.4 and 172.8 ppm corresponding to C=N and C=S carbons, respectively. The signalappearing at 12.8 ppm indicated the presence of methyl in the derivative. The appearance of two signals at 55.95 and 55.97 ppm exhibited the presence of methoxy carbons. C-4 of the pyrazole appeared at 108.8 ppm. The remaining aromatic carbons appeared

Table 1. Characteristics of the newly synthesized 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazines (**VIIa–f**) and 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**VIIIa–i**)



(**VIIa–f**)



(**VIIIa–i**)

Compound no.	Ar/Ar^1	Chemical formula	Molecular weight, g/mole	Yield, %	mp, °C
(VIIa)	4-Me–C_6H_4	$C_{21}H_{18}N_6S$	386.47	80	180–182
(VIIb)	4-Cl–C_6H_4	$C_{21}H_{15}ClN_6S$	406.89	85	152–154
(VIIc)	4-F–C_6H_4	$C_{21}H_{15}FN_6S$	390.44	85	162–164
(VIId)	4-Br–C_6H_4	$C_{21}H_{15}BrN_6S$	451.34	85	146–148
(VIIe)	4-OMe–C_6H_4	$C_{21}H_{18}N_6OS$	402.47	80	138–140
(VIIf)	C_6H_5	$C_{20}H_{16}N_6S$	372.45	80	130–132
(VIIIa)	2,4-Cl_2–C_6H_3	$C_{19}H_{14}Cl_2N_6S$	429.33	85	156–158
(VIIIb)	3,4-$(OMe)_2$–C_6H_3	$C_{21}H_{20}N_6O_2S$	420.49	85	160–162
(VIIIc)	3,4,5-$(OMe)_3C_6H_2$	$C_{22}H_{22}N_6O_3S$	450.51	85	170–172
(VIIId)	4-F–C_6H_4	$C_{19}H_{15}FN_6S$	378.43	85	140–142
(VIIIe)	5-Br-2-OH C_6H_3	$C_{19}H_{15}BrN_6OS$	455.33	85	212–214
(VIIIf)	2,3-Cl_2–C_6H_3	$C_{19}H_{14}Cl_2N_6S$	429.33	85	162–164
(VIIIg)	4-OH–C_6H_4	$C_{19}H_{16}N_6OS$	376.43	85	228–230
(VIIIh)	3,4-$(OH)_2$–C_6H_3	$C_{19}H_{16}N_6O_2S$	392.43	85	228–230
(VIIIi)	3-Br-4-OH-5-MeO–C_6H_2	$C_{20}H_{17}BrN_6O_2S$	485.36	85	176–178

in the range of 110.7–168.3 ppm. The formation of (**VIIIb**) was further confirmed by the appearance of molecular ion peak at m/z 421.05 $[M + H]^+$, which is in agreement with the molecular formula $C_{21}H_{20}N_6O_2S$. The elemental analysis gave satisfactory values for the percentage of C, H, and N present in the synthesized molecule, and is presented in the experimental section.

In Silico Molecular Docking

Molecular docking is an important tool used in drug design. Structure-based drug design mainly aims at identification of drug candidates, which includes the design and optimization of a chemical structure with the goal of identifying a compound which is suitable for clinical testing. In silico molecular docking analysis has been undertaken to investigate the significant binding interactions of the target molecules with the binding pockets of prostaglandin D2 synthase [PDB ID: 2VCW], which is the principal requirement for producing the biological effect. The cDock energy of the compounds varied from −7.75 to −5.49 kcal/mol where the reference inhibitor gives a docking score of −5.247 kcal/mol.

Most of derivatives synthesized (compounds (**VIIa**), (**VIIc–f**), and (**VIIIa–i**)) showed good interactions with the target protein 2VCW. The N-2 of the pyrazole compound (**VIIIh**), which is a dihydroxy derivative, makes hydrogen bond as a donor with a water molecule and that water molecule makes hydrogen bond interaction with Thr159. The pyrazole ring exhibited π–π stacking interaction with Trp104. Hydroxy substituted phenyl group exhibited π–cation interaction with Arg14. Beside these interactions, hydroxyl groups of the derivatives also showed hydrogen bond interaction with Lys50 and Ile51 amino acids. Besides these interactions, the targeted molecule makes hydrophobic interaction with the residues around the binding

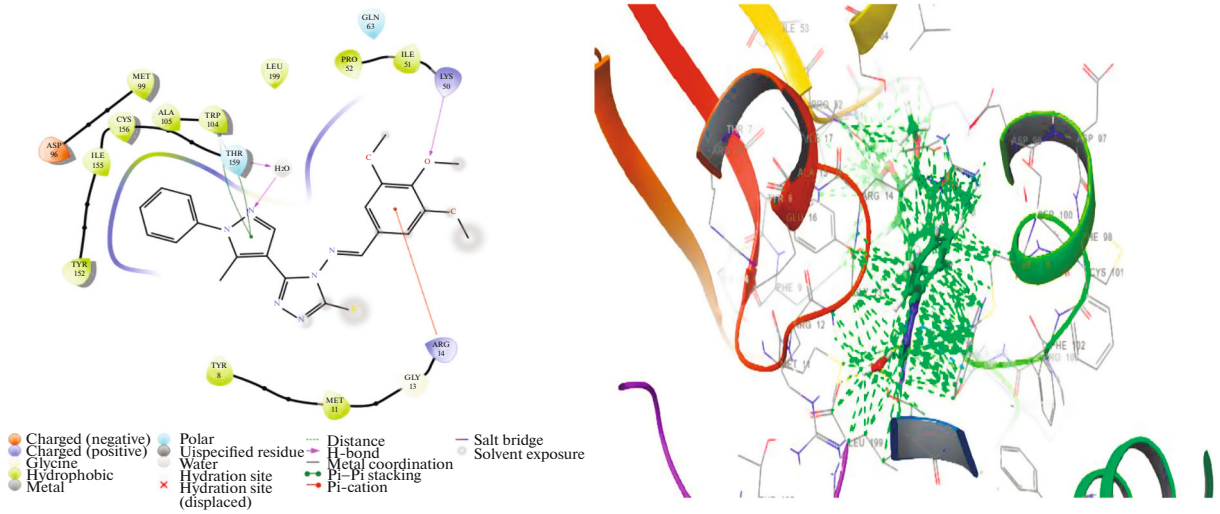


Fig. 1. Ligand interaction diagrams of compound (**VIIIc**) with human prostaglandin D2 synthase (PGDS).

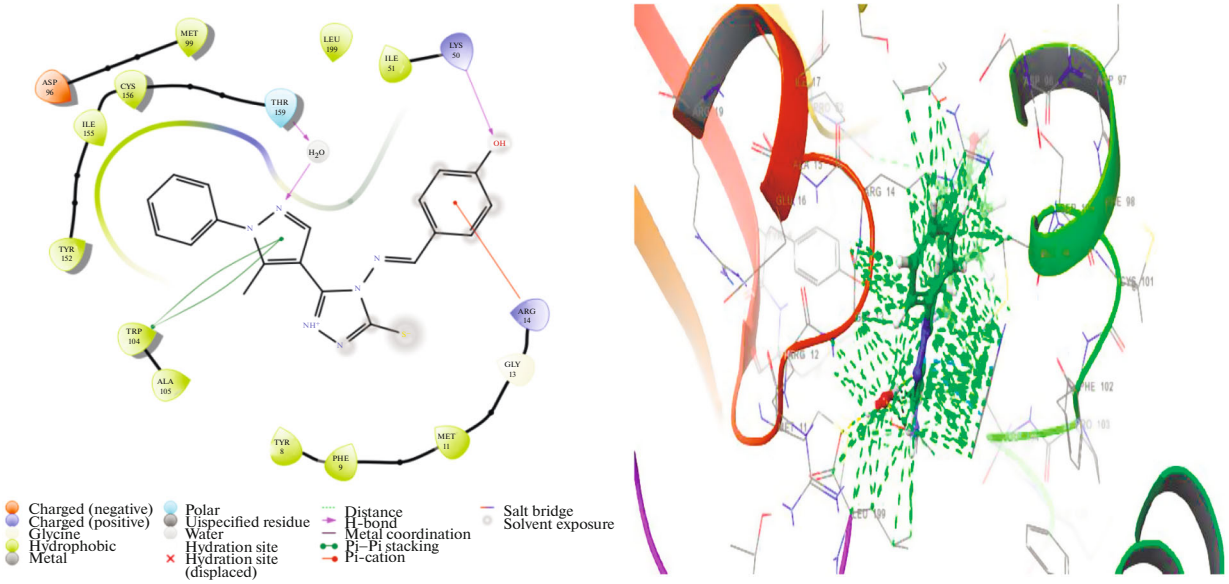


Fig. 2. Ligand interaction diagrams of compound (**VIIIg**) with human prostaglandin D2 synthase (PGDS).

pocket, such as Tyr152, Ile155, Cys156, Thr159, Ala105, Lys50, Ile51, and Gln63. All observed interactions revealed that the molecule has a great affinity towards the enzyme. Pharmacophore interaction of (**VIIIc**), (**VIIIg**), (**VIIIh**), and (**VIIIi**) are shown in Figs. 1–4.

Antibacterial Assay

Antibacterial potency of newly synthesized compounds was checked using the broth dilution method against four bacterial strains. *Staphylococcus aureus* and *Enterococcus faecalis* represented gram-positive strains and *Escherichia coli*, and *Pseudomonas aeruginosa*, gram-negative strains. Ciprofloxacin was used as a reference drug. The results of the preliminary antibacterial activity of compounds (**VIIa–f**) and (**VIIIa–i**) are presented in Table 2. The newly synthesized compounds possess moderate-to-excellent inhibition against the tested microorganisms. Most of the compounds exhibited excellent inhibiting activity against *E. coli* and *P. aeruginosa* bacterial strains with MIC values less than those of standard drug ciprofloxacin (2 μg/mL). Among the series, compounds (**VIIb**), (**VIIc**), (**VIIIb**), and (**VIIIh**) displayed the best antibacterial activity. A thiadiazinering-containing derivative (**VIIb**) (Ar = 4-chlorophenyl) showed MIC value of 1.56 μg/mL against *E. coli* and *P. aeruginosa*, but it

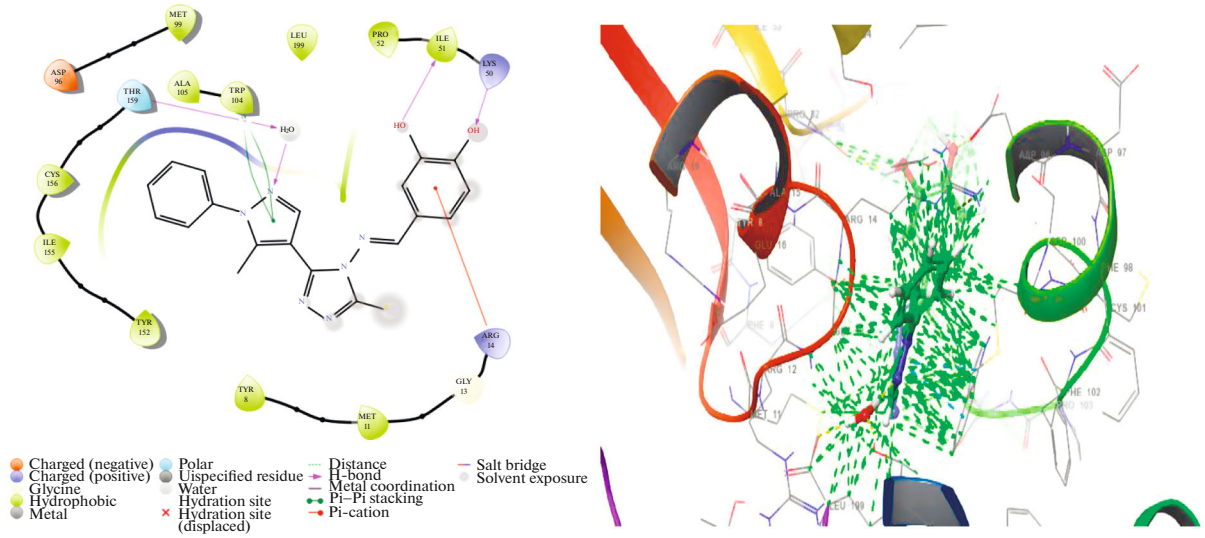


Fig. 3. Ligand interaction diagrams of compound (**VIIIh**) with human prostaglandin D2 synthase (PGDS).

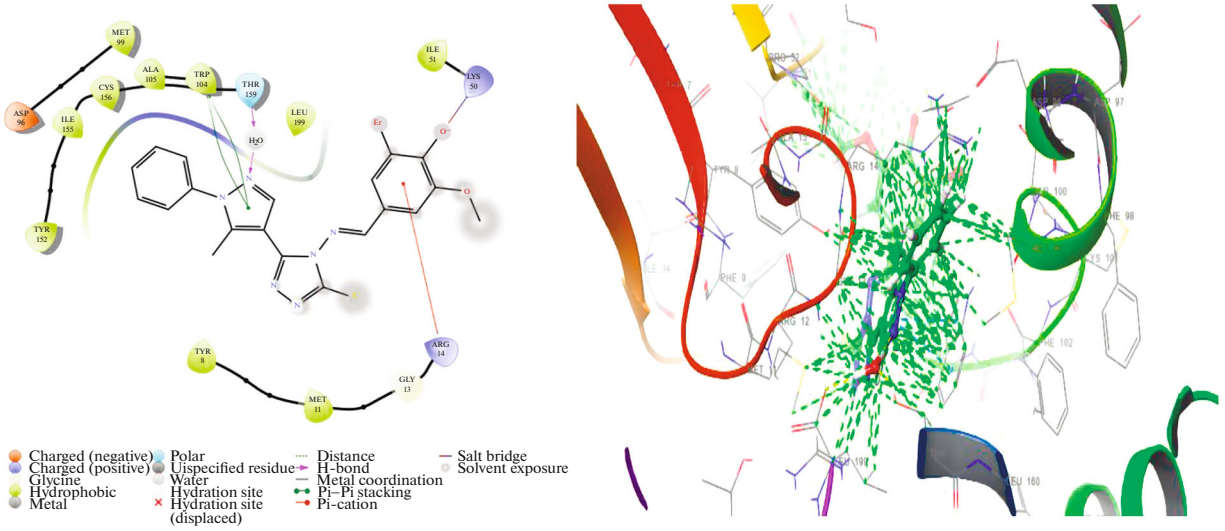


Fig. 4. Ligand interaction diagrams of compound (**VIIIi**) with human prostaglandin D2 synthase (PGDS).

showed moderate activity towards *S. aureus* and *E. faecalis* bacterial strains with MIC values of 3.125 and 12 μg/mL, respectively. On the other hand, replacing chloro group by fluoro one led to compound (**VIIc**) (Ar = 4-fluorophenyl), which retained the activity towards gram-positive *S. aureus* (1.56 μg/mL) and gram-negative *P. aeruginosa* (1.56 μg/mL) strain, but lost its activity against *E. faecalis* (12.5 μg/mL) and *E. coli* (12.5 μg/mL). Compound (**VIIIb**) (Ar^1 = 3,4-dimethoxyphenyl) displayed excellent inhibition against *E. faecalis* and *E. coli* with MIC 1.56 μg/mL, but it did not display good activity against the rest of the bacterial strains. Compound (**VIIIh**) (Ar^1 = 3,4-dihydroxyphenyl) illustrated significant activity against both gram-positive (*E. faecalis*) and gram-negative (*E. coli*) bacterial strains with MIC values of 1.56 μg/mL. Compounds (**VIIa**), (**VIId**), (**VIIf**), (**VIIIa**), (**VIIIe**), (**VIIIf**), and (**VIIIg**) were found active against all bacterial strains. Compound (**VIIa**) (Ar = 4-methylphenyl) showed good activity against *P. aeruginosa* (1.56 μg/mL) but failed to show activity against *S. aureus*, *E. faecalis*, or *E. coli* (MIC 6.25, 3.125, and 3.125 μg/mL, respectively). Similarly, derivatives (**VIId**) (Ar = 4-bromophenyl) and (**VIIf**) (Ar = 4-bromophenyl) showed good activity against *E. faecalis* (1.56 μg/mL) and *E. coli* (1.56 μg/mL), but lost its activity against remaining two bacterial strains. The series of Schiff base derivatives (**VIIIa**) (Ar^1 = 2,4-dichlorophenyl), (**VIIIf**) (Ar^1 = 2,3-dichlorophenyl), and (**VIIIg**) (Ar^1 = 4-hydroxyphenyl) showed good

Table 2. Antibacterial activity of synthesized compounds **(VIIa–f)** and **(VIIIa–i)** by broth dilution method

Minimum inhibitory concentration (MIC), μg/mL					
entry	Ar/Ar[1]	Gram-positive		Gram-negative	
		S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
(VIIa)	4-Me–C_6H_4	6.25	3.125	3.125	**1.56**
(VIIb)	4-Cl–C_6H_4	3.125	25	**1.56**	**1.56**
(VIIc)	4-F–C_6H_4	**1.56**	12.5	12.5	**1.56**
(VIId)	4-Br–C_6H_4	12.5	**1.56**	12.5	12.5
(VIIe)	4-OMe–C_6H_4	12.5	3.125	3.125	3.125
(VIIf)	C_6H_5	50	12.5	**1.56**	12.5
(VIIIa)	2,4-Cl_2–C_6H_3	50	25	3.125	**1.56**
(VIIIb)	3,4-$(OMe)_2$–C_6H_3	25	**1.56**	**1.56**	3.125
(VIIIc)	3,4,5-$(OMe)_3C_6H_2$	12.5	3.125	6.25	25
(VIIId)	4-F–C_6H_4	3.125	12.5	3.125	1.56
(VIIIe)	5-Br-2-OH C_6H_3	12.5	3.125	**1.56**	3.125
(VIIIf)	2,3-Cl_2–C_6H_3	3.125	3.125	3.125	**1.56**
(VIIIg)	4-OH–C_6H_4	25	3.125	3.125	**1.56**
(VIIIh)	3,4-$(OH)_2$–C_6H_3	12.5	**1.56**	**1.56**	3.125
(VIIIi)	3-Br,4-OH,5-MeO–C_6H_2	3.125	12.5	12.5	12.5
	Ciprofloxacin	6.25	6.25	3.125	6.25

activity against gram-negative bacteria strain *P. aeruginosa* but failed to show activity against remaining bacterial strains. The rest of the tested compounds exhibited weak-to-moderate activity against the tested organisms. Most of the compounds showed significant activity against gram-negative bacterial strains. Additionally, activity results revealed that halo, methoxy, and hydroxy substituents in the aryl part are essential for the antibacterial activity.

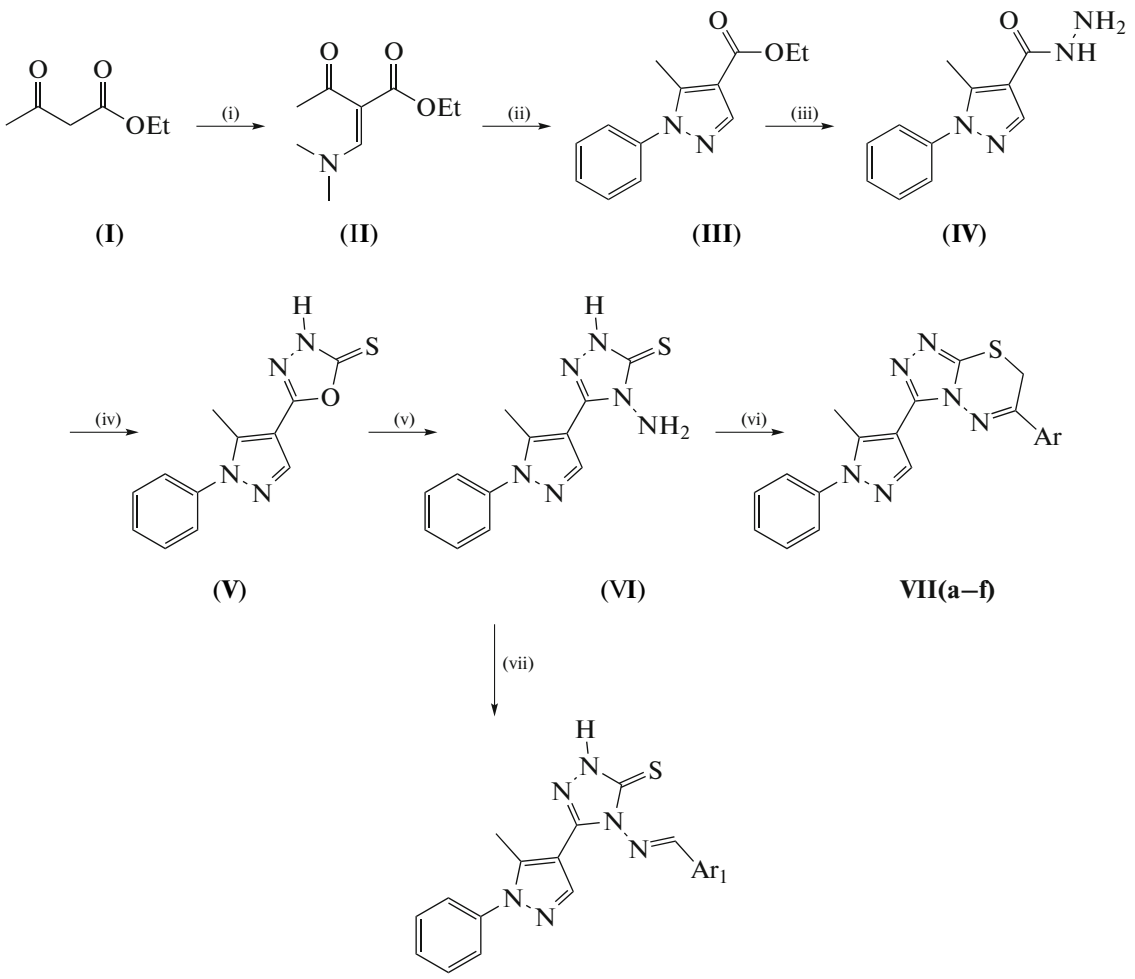
EXPERIMENTAL

All the chemicals and solvents of appropriate grade were obtained from Spectrochem Pvt. Ltd. (Bangalore, India) and Sigma-Aldrich (Bangalore, India) and were used without further purification. The purity of the synthesized compounds was checked by TLC (thin layer chromatography) on silica coated aluminum sheets (silica gel 60F254) using hexane and ethyl acetate mixtures and visualized under UV at 254 nm. The melting points of the new compounds were determined using open glass capillary tubes and left uncorrected. FT-IR spectra (ν_{max}, cm^{-1}) were recorded on a ShimadzuFT-IR157Spectrometer in KBr. NMR spectra were measured in $CDCl_3$ on a Bruker AvanceII 400 spectrometer operating at 400MHz for ^1H and at 100 MHz for ^{13}C nuclei, respectively, using tetramethylsilane (TMS) as an internal standard. The chemical shifts (δ) and the coupling constants (*J*) are reported in parts per million (ppm) and in Hertz, respectively. Mass spectra were recorded in an Agilent Technology LC-mass spectrometer with ESI ionization in positive mode. Elemental analysis was performed using CHNS ElementarVario EL III.

Docking of the ligands to the protein structural model was carried outwith the Glide module in Schrodinger. The receptor grid was generated at the site having lowest energy and using the ligand-docking panel, the molecular docking was carried out. Using the 2D sketcher the structures of the ligands were drawn and then optimized with Ligprep to generate the energy optimized 3D structures. The ionization states were generated at pH 7.0 ± 2 using Epik module in LigPrep with all other default options. The high resolution 3D structure of prostaglandin D2 synthase (PGDS) (PDB ID: 2VCW) protein was downloaded from protein data bank and was prepared with the Protein Preparation Wizard in Maestro using default options and the missing hydrogen's in side chains of amino acids of protein were added and any incorrect bond orders were corrected. The structure was finally minimized to release any possible strains. The prepared molecules were docked flexibly at the protein grid using extra precession (XP) mode. The protein–ligand complex was further analyzed and visualized for the ligand fitting and interactions using Maestro.

General procedure for the synthesis of 4-amino-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-

1,2,4-triazole-3-thione (VI). The synthetic scheme for target compounds (**VIIa–f**) and (**VIIIa–i**) is illustrated in Scheme 1. According to the literature procedure, we synthesized 5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1,3,4-oxadiazole-2(3*H*)-thione(**V**) [26, 27]. Further the intermediate (**V**) was treated with hydrazine hydrate under reflux condition for 5–6 hrs with ethanol. After the completion of reaction, reaction mixture was poured to crushed ice. The precipitated solid was filtered, washed with water, dried, and recrystallized from ethanol to get 4-amino-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**VI**).



Scheme 1. Outline for the synthesis. Reagents and conditions: (i) DMF–DMA, 90°C, 5–6 h. (ii) Phenylhydrazine, ethanol, reflux, 3 h. (iii) N_2H_4, reflux, 5–6 h. (iv) CS_2, KOH, EtOH, reflux 4 h. (v) N_2H_4, reflux, 5–6 h. (vi) Phenacyl bromide, ethanol, reflux, 5 h. (vii) Substituted benzaldehydes, AcOH, ethanol, reflux, 6 h.

General procedure for thesynthesis of 6-(substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazines (VIIa–f). A mixture of compound (**VI**) (0.01 mol) and substituted phenacyl bromides (0.01 mol) was prepared in ethanol. The reaction mass was refluxed on a water bath for about 5 h. The completion of the reaction was monitored by TLC. After completion, the reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried, and recrystallized from ethanol or methanol.

6-(4-Methylphenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (VIIa). Yield 80%; mp 180–182°C; IR: 1645 (C=N), 1588 (C=C), 1253 (C=S); ^{1}H NMR: 2.33 (3H, s,–C<u>H</u>$_3$), 2.72 (3H, s, –CH_3), 4.00 (2H, s, –CH_2), 7.50–7.75 (4H, m, Ar-H), 7.65(2H, d, methylphenyl, J =8.8 Hz), 7.81 (3H, d, 4-methylphenyl, J = 8.8 Hz), 8.24 (1H, s, pyrazole–CH); ^{13}C NMR: 11.9, 21.3, 36.3, 105.5, 124.9, 126.2, 127.0, 129.3, 131.0, 134.9, 140.7, 151.1, 159.0, 164.6; LCMS: m/z 387.13 (M + 1).

Anal. calcd. for $C_{21}H_{18}N_6S$: C, 65.26; H, 4. 69; N, 21.75. Found: C, 65.28; H, 4. 71; N, 21.79.

6-(4-Chlorophenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (VIIb). Yield 85%; mp 152–154°C; IR: 1622 (C=N), 1586 (C=C), 1261 (C=S), 760 (C–Cl); ^{1}H NMR: 2.14 (3H, s, $-CH_3$), 4.00 (1H, s, –NH), 7.44–7.55 (5H, m, Ar-H), 7.66–7.69 (2H, d, Ar-H), 7.79–7.83 (2H, d, Ar-H), 8.24 (1H, s, pyrazole–CH); ^{13}C NMR: 12.8, 23.4, 109.8, 113.7, 119.4, 125.3, 128.5, 129.3, 129.6, 139.4, 149.9, 152.3, 158.5, 163.1, 172.3, 174.7; LCMS: *m/z* 407.05 (*M* + 1), 408.05 (*M* + 2). Anal. calcd. for $C_{20}H_{15}ClN_6S$: C, 59.04; H, 3.72; N, 20.65. Found: C, 59.06; H, 3.75; N, 20.68.

6-(4-Fluorophenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (VIIc). Yield 85%; mp 162–164°C; IR: 1567 (C=N), 1532 (C=C), 1364 (C=S), 1092 (C–F); ^{1}H NMR: 2.72 (3H, s, $-CH_3$), 4.00 (2H, s, $-CH_2$), 7.49–7.54 (4H, m, Ar-H), 7.68 (2H, d, 4-fluorophenyl *J* = 8.8 Hz), 7.84 (3H, d, 4-fluorophenyl, *J* = 8.8 Hz), 8.25 (1H, s, pyrazole–CH); ^{13}C NMR: 12.3, 23.3, 105.5, 115.6, 124.9, 126.2, 129.3, 134.9, 139.7, 151.1, 159.0, 165.2 LCMS: *m/z* 391.11 (*M* + 1). Anal. calcd. for $C_{21}H_{15}FN_6S$: C, 61.52; H, 3.87; N, 21.52. Found: C, 61.55; H, 3.89; N, 21.56.

6-(4-Bromophenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (VIId). Yield 85%; mp 146–148°C; IR: 1582 (C=N), 1453 (C=C), 1356 (C=S), 760 (C–Br); ^{1}H NMR: 2.73 (3H, s, $-CH_3$), 4.00 (1H, s, –NH), 7.49–7.54 (4H, m, Ar-H), 7.67 (2H, d, *J* = 8.8 Hz Ar-H), 7.82 (2H, d, *J* = 8.8 Hz Ar-H), 8.24 (1H, s, pyrazole–CH); ^{13}C NMR: 11.3, 25.4, 110.2, 113.7, 119.5, 124.6, 127.9, 129.4, 129.8, 136.1, 139.4, 153.3, 159.4, 164.8, 171.5, 174.3; LCMS: *m/z* 452.02 (*M* + 1). Anal. calcd. for $C_{21}H_{15}BrN_6S$: C, 53.22; H, 3.35; N, 18.62. Found: C, 53.26; H, 3.38; N, 18.68.

6-(4-Methoxyphenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3, 4]thiadiazine (VIIe). Yield 80%; mp 138–140°C; IR: 1588 (C=N), 1513 (C=C), 1388 (C=S); ^{1}H NMR: 2.73 (3H, s, $-CH_3$), 3.78 (3H, s, $-OCH_3$), 4.00 (2H, s, $-CH_2$), 7.49–7.54 (4H, m, Ar-H), 7.51(2H, d, 4-methoxyphenyl *J* = 8.8 Hz), 7.62 (3H, d, 4-methoxyphenyl, *J* = 8.8Hz), 8.25 (1H, s, pyrazole–CH); ^{13}C NMR: 12.2, 36.3, 55.8, 105.5, 114.4, 124.9, 126.3, 129.3, 128.7, 134.9, 139.7, 151.1, 159.0, 162.9, 164.6; LCMS: *m/z* 403.13 (*M* + 1). Anal. calcd. for $C_{21}H_{18}N_6OS$: C, 62.67; H, 4. 51; N, 20.88. Found: C, 62.69; H, 4. 55; N, 20.90.

3-(5-Methyl-1-phenyl-1*H*-pyrazol-4-yl)-6-phenyl-7*H*-[1,2,4]triazolo[3,4-*b*] [1,3,4]thiadiazine (VIIf). Yield 80%; mp 130–132°C; IR: 1572 (C=N), 1495 (C=C), 1342 (C=S); ^{1}H NMR: 2.73 (3H, s, $-CH_3$), 4.00 (2H, s, $-CH_2$), 7.55–7.96 (10 H, m, Ar-H), 8.25 (1H, s, pyrazole–CH); ^{13}C NMR: 11.9, 36.3, 55.8, 105.5, 124.9, 126.2, 128.8, 131.0, 134.9, 139.7, 151.1, 159.0, 164.6; LCMS: *m/z* 403.13 (*M* + 1). Anal. calcd. for $C_{20}H_{16}N_6S$: C, 64.50; H, 4.33; N, 22.56. Found: C, 64.55; H, 4.37; N, 22.58.

General procedure for thesynthesis of 4-[(substituted-benzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (VIIIa–i). A mixture of compound **(VI)** (0.01 mol) and substituted aldehydes (0.01mol) in 10 mL of absolute ethanol in the presence of catalytic amount of acetic acid was refluxed for 4 h. After cooling, the precipitated product was filtered, washed with water, and dried. The solid product obtained was recrystallized from ethanol.

4-[(2,4-Dichlorobenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIa). Yield 85%; mp 156–158°C; IR: 3079 (N–H), 1680 (C=N), 1591 (C=C), 1462 (C=S), 761 (C–Cl); ^{1}H NMR: 2.38 (3H, s, $-CH_3$), 4.83 (1H, s, –NH), 7.26–7.52 (5H, m, phenyl), 7.91 (1H, d, 2,4-dichlorophenyl *J* = 8.4 Hz), 8.11 (1H, s, 2,4-dichlorophenyl), 8.16 (1H, d, 2,4-dichlorophenyl *J* = 8.4 Hz), 8.99 (1H, s, pyrazole –CH), 9.19 (1H, s, =CH); ^{13}C NMR: 12.9, 108.8, 110.7, 116.5, 123.9, 126.1, 127.3, 128.9, 130.7, 145.3, 149.4, 151.8, 154.1, 161.1, 167.8, 169.5; LCMS: *m/z* 428.15 (*M* + 1). Anal. calcd. for $C_{19}H_{14}Cl_2N_6S$: C, 53.15; H, 3.29; N, 19.57. Found: C, 53.17; H, 3.31; N, 19.59.

4-[(3,4-Dimethoxybenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIb). Yield 85%; mp 160–162°C; IR: 3409 (N–H), 1597 (C=N), 1504 (C=C), 1249 (C=S); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 3.94 (3H, s, $-OCH_3$), 3.98 (3H, s, $-OCH_3$), 4.82 (1H, s, –NH), 6.92 (1H, d, 3,4-dimethoxyphenyl *J* = 8.4 Hz), 7.11(1H, dd, 3,4-dimethoxyphenyl *J* = 8.4, 2 Hz), 7.24 (1H, s, 3,4-dimethoxyphenyl), 7.44–7.67 (5H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.8, 55.9, 55.9, 108.8, 110.7, 116.5, 123.9, 126.1, 127.3, 128.9, 130.7, 149.4, 151.8, 161.2, 168.3, 171.4, 172.8; LCMS: *m/z* 421.05 (*M* + 1). Anal. calcd. for $C_{21}H_{20}N_6O_2S$ C, 59.98; H, 4.79; N, 19.99. Found: C, 59.99; H, 4.83; N, 19.96.

5-(5-Methyl-1-phenyl-1*H*-pyrazol-4-yl)-4-[(3,4,5-trimethoxybenzylidene)amino]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIc). Yield 85%; mp 170–172°C; IR: 3289 (N–H), 1597 (C=N), 1504 (C=C), 1258 (C=S); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 3.95 (6H, s, $-OCH_3$), 3.98 (3H, s, $-OCH_3$), 4.82 (1H, s, –NH), 6.90 (2H, d, 3,4,5-trimethoxyphenyl *J* = 1.5 Hz), 7.44–7.66 (5H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 56.1, 60.8, 104.0, 114.6, 124.9, 126.3, 129.3, 139.7, 144.4, 148.9, 153.2, 154.1, 181.1; LCMS: *m/z* 451.15 (*M* + 1). Anal. calcd. for $C_{22}H_{22}N_6O_3S$: C, 58.65; H, 4.92; N, 18.65. Found: C, 58.68; H, 4.96; N, 18.68.

4-[(4-Fluorobenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIId). Yield 85%; mp 140–142°C; IR: 3289 (N–H), 1587 (C=N), 1453 (C=C), 1123 (C=S); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 4.82 (1H, s, –NH), 7.08 (2H, d, 4-F-phenyl J = 8.4 Hz), 7.33 (2H, d, 4-F-phenyl J = 8.4 Hz), 7.44–7.73 (5H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 114.6, 115.6, 124.9, 126.2, 129.3, 130.8, 139.7, 140.3, 144.4, 148.9, 154.1, 165.2, 181.1; LCMS: m/z 379.11 (M + 1). Anal. calcd. for $C_{19}H_{15}FN_6S$: C, 60.30; H, 4.00; N, 22.21. Found: C, 60.35; H, 4.04; N, 22.24.

4-[(5-Bromo-2-hydroxybenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIe). Yield 85%; mp 212–214°C; IR: 3189 (N–H), 1623 (C=N), 1512 (C=C), 1342 (C=S), 689 (C–Br); ^{1}H NMR:2.17 (3H, s, $-CH_3$), 4.82 (1H, s, –NH), 5.00 (1H, s, –OH), 6.9 (1H, d, 5-bromo-2-hydroxyphenyl, J = 8.4 Hz), 7.31–7.73 (7H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 110.5, 114.6, 119.3, 120.7, 124.9, 126.2, 129.3, 132.0, 135.3, 140.3, 143.3, 144.4, 148.9, 160.1, 181.1; LCMS: m/z 456.02 (M + 1). Anal. calcd. for $C_{19}H_{15}BrN_6OS$: C, 50.12; H, 3.32; N, 18.46. Found: C, 50.13; H, 3.43; N, 18.56.

4-[(2,3-Dichlorobenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIf). Yield 85%; mp 160–162°C; IR: 3109 (N–H), 1612 (C=N), 1487 (C=C), 1289 (C=S), 769 (C–Cl); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 4.82 (1H, s, –NH), 7.36 (2H, dd, 2,3-dichlorophenyl J = 8.4, 8.4, 1.5 Hz), 7.50 (1H, dd, 2,3-dichlorophenyl J = 8.4, 1.5 Hz), 7.51–7.73 (5H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 114.6, 124.9, 126.2, 128.3, 129.3, 130.8, 132.5, 134.8, 139.7, 143.3, 144.4, 148.9, 181.1; LCMS: m/z 429.04 (M + 1), 430.03 (M + 2). Anal. calcd. for $C_{19}H_{14}Cl_2N_6S$: C, 53.15; H, 3.29; N, 19.57. Found: C, 53.18; H, 3.31; N, 19.59.

4-[(4-Hydroxybenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIg). Yield 85%; mp 228–230°C; IR: 3211 (N–H), 1625 (C=N), 1487 (C=C), 1162 (C=S); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 4.82 (1H, s, –NH), 5.00 (1H, s, –OH), 7.19–7.73 (9H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 114.6, 116.3, 124.9, 126.2, 129.3, 130.6, 140.3, 144.4, 148.9, 154.1, 160.8, 181.1; LCMS: m/z 377.11 (M + 1). Anal. calcd. for $C_{19}H_{16}N_6OS$: C, 60.62; H, 4.28; N, 22.33. Found: C, 60.66; H, 4.30; N, 22.36.

4-[(3,4-Dihydroxybenzylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIh). Yield 85%; mp 228–230°C; IR: 3211 (N–H), 1625 (C=N), 1487 (C=C), 1162 (C=S); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 4.82 (1H, s, –NH), 5.00 (2H, s, –OH), 6.7 (1H, d, 3, 4-dihydroxy phenyl, J = 8.4 Hz), 7.18–7.73 (7H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.7, 114.6, 116.3, 123.2, 124.9, 126.2, 129.3, 131.3, 139.7, 144.4, 149.6, 154.1, 181.1; LCMS: m/z393.11 (M + 1). Anal. calcd. for $C_{19}H_{16}N_6O_2S$: C, 58.15; H, 4.11; N, 21.42. Found: C, 58.18; H, 4.15; N, 21.46.

4-[(3-Bromo-4-hydroxy-5-methoxybenzylidene)-amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2, 4-dihydro-3*H*-1,2,4-triazole-3-thione (VIIIi). Yield 85%; mp 176–178°C; IR: 3189 (N–H), 1623 (C=N), 1512 (C=C), 1342 (C=S), 689 (C–Br); ^{1}H NMR: 2.17 (3H, s, $-CH_3$), 3.80 (3H, s), 4.82 (1H, s, –NH), 5.00 (1H, s, –OH), 6.9 (1H, d, 3-bromo-4-hydroxy-5-methoxyphenyl J = 1.5 Hz), 7.31–7.73 (6H, m, phenyl), 8.48 (1H, s, pyrazole–CH), 8.60 (1H, s, =CH); ^{13}C NMR: 12.6, 56.1, 111.1, 114.6, 122.6, 124.9, 126.2, 129.3, 140.3, 144.4, 148.9, 153.7, 181.1; LCMS: m/z 486.03 (M + 1). Anal. calcd. for $C_{20}H_{17}BrN_6O_2S$: C, 49.49; H, 3.53; N, 17.32. Found: C, 49.51; H, 3.56; N, 17.35.

Antibacterial activity of the compounds. Synthesized compounds **(VIIa–f)** and **(VIIIa–i)** were assayed for their antibacterial activity against four bacterial strains. *Staphylococcus aureus* and *Enterococcus faecalis* represented gram-positive bacterial strains and *Escherichia coli* and *Pseudomonas aeruginosa* represented gram-negative bacterial strains. Broth dilution method [28] was followed for the determination of antibacterial activity. Ciprofloxacin was used as a positive control for antibacterial activity. The MIC was defined as the lowest concentration without visible growth. The nine dilutions of each drug **(VIIa–f)** and **(VIIIa–i)** and ciprofloxacin were done with brain heart infusion (BHI) for MIC. In the initial tube, 2 μL of the drug DMSO solution was added into the 380 μL of BHI broth. For further dilutions, 200 μL of BHI broth was added into the next 9 tubes separately. Then from the initial tube, 200 μL was transferred to the first tube containing 200 μL of BHI broth. This was considered as 10-1 dilution. From 10-1 diluted tube, 200 μL was transferred to second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each drug. From the maintained stock cultures of essential organisms, 5 μL was taken and added into 2 mL of BHI broth. Successively in each diluted tube 200 μL of above culture suspension was added. The tubes were incubated at 37°C for 24 h for bacteria using NB as a control. In order to ensure that the solvent had no effect on growth, control without test samples and with solvent (DMSO) was assayed simultaneously. All the tubes were examined for their visible turbidity. The lowest concentration was noted as MIC at which no visible growth was observed.

CONCLUSIONS

6-(Substituted-phenyl)-3-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-7*H*- [1,2,4]triazolo[3,4-*b*] [1,3, 4]thiadiazines **(VIIa–f)** and 4-[(substituted-ben-

zylidene)amino]-5-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**VIIIa–i**) were synthesized in good yields and their potency to act as a ligand to the target protein 2VCW was also evaluated by in silico molecular docking. Synthesized derivatives were also tested for their antibacterial activity against four bacterial strains. In the series of derivatives, most of the compounds, particularly compounds (**VIIa**) and (**VIIIa–i**)), showed good hydrogen bond interactions and a π–π stacking interaction along with a good docking score with the target protein 2VCW. The docking studies indicate that the target compounds might have some pharmacological activity. Evaluation of antibacterial activity revealed that some of the synthesized compounds (like compounds (**VIIb**), (**VIIc**), (**VIIIb**), and (**VIIIh**)) can emerge as more potent antibacterial agents. Further, compounds (like compounds (**VIIa**), (**VIId**), (**VIIf**), (**VIIIa**), (**VIIIe**), (**VIIIf**), and (**VIIIg**)) displayed moderate antibacterial activity when compared with the standard. The investigation confirmed the design is successful in generating members in the antibacterial agent family.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performed by any of the authors.

SUPPLEMENTARY MATERIALS

Supplementary materials are available for this article at https://doi.org/10.1134/S1068162020010069 and are accessible for authorized users.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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