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NOVEL ARMED PYRAZOLOBENZOTHIAZINE DERIVATIVES: SYNTHESIS, X-RAY CRYSTAL STRUCTURE AND POM ANALYSES OF BIOLOGICAL ACTIVITY AGAINST DRUG RESISTANT CLINICAL ISOLATE OF *STAPHYLOCOCCUS AUREUS*

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Novel structural hybrids of pyrazolobenzothiazine and triazole ring systems have been prepared to observe a synergistic effect of the two ring systems. The methodology involves condensation of pyrazolobenzothiazine rings with methyl chloroacetate, followed by hydrazide formation. The hydrazides were converted to triazoles through the formation of intermediate potassium salts of dithiocarbazate. The final compounds as well as intermediates were screened for their antibacterial activity against a multidrug resistant strain of *Staphylococcus aureus.* It was interesting to observe that dithiocarbazates (**7a, 7b**) and target triazoles (**8a, 8b**) exhibited antibacterial activity.

Keywords: pyrazolobenzothiazine dioxides; triazole; antibacterial pharmacophore site, Petra/Osiris/Molinspiration (POM) analyses.

1. INTRODUCTION

Staphylococcus aureus is the major substantial human infectious agent responsible equally for healthcare and community associated infections globally. *S. aureus* are cluster forming cocci classified as Gram positive bacteria. They are non-spore forming, non-motile facultative anaerobes, non-flagellates and catalase positive organisms [1]. *S. aureus* strains cause a wide range of infections such as furuncle, carbuncle, chronic furunculosis, boils, abscesses, impetigo, pneumonia, deep lesion, cellulites, endocarditis, osteomyelitis, scalded skin syndrome, toxic shock syndrome, and food poisoning [2].

Drug resistance in bacterial strains is becoming very common and is a severe health related issue that requires immediate attention. The outburst of methicillin resistant strains of *S. aureus* among patients is the major and serious problem of concern worldwide [3]. About 52 years ago, isolates of methicillin resistant *S. aureus* were reported. These isolates contain a gene named mecA, which produces β-lactamase enzyme that inactivates antibiotics [4]. This *S. aureus* strain is resistant to anti-microbial agents including macrolides, tetracycline, chloramphenicol, aminoglycosides and fluoroquinolones [5]. There is a constant need to discover new antibiotics because of the increasing number of bacterial strains that have developed a multidrug resistance.

As far as benzothiazines are concerned, there exist numerous compounds based on this family that act as antibacterial agents. Rufloxacin, a benzothiazine-containing antibiotic, was studied for its ability to inhibit *S. aureus* in the rat granuloma pouch model in comparison to ciprofloxacin. It was established that rufloxacin inhibited the bacterial growth

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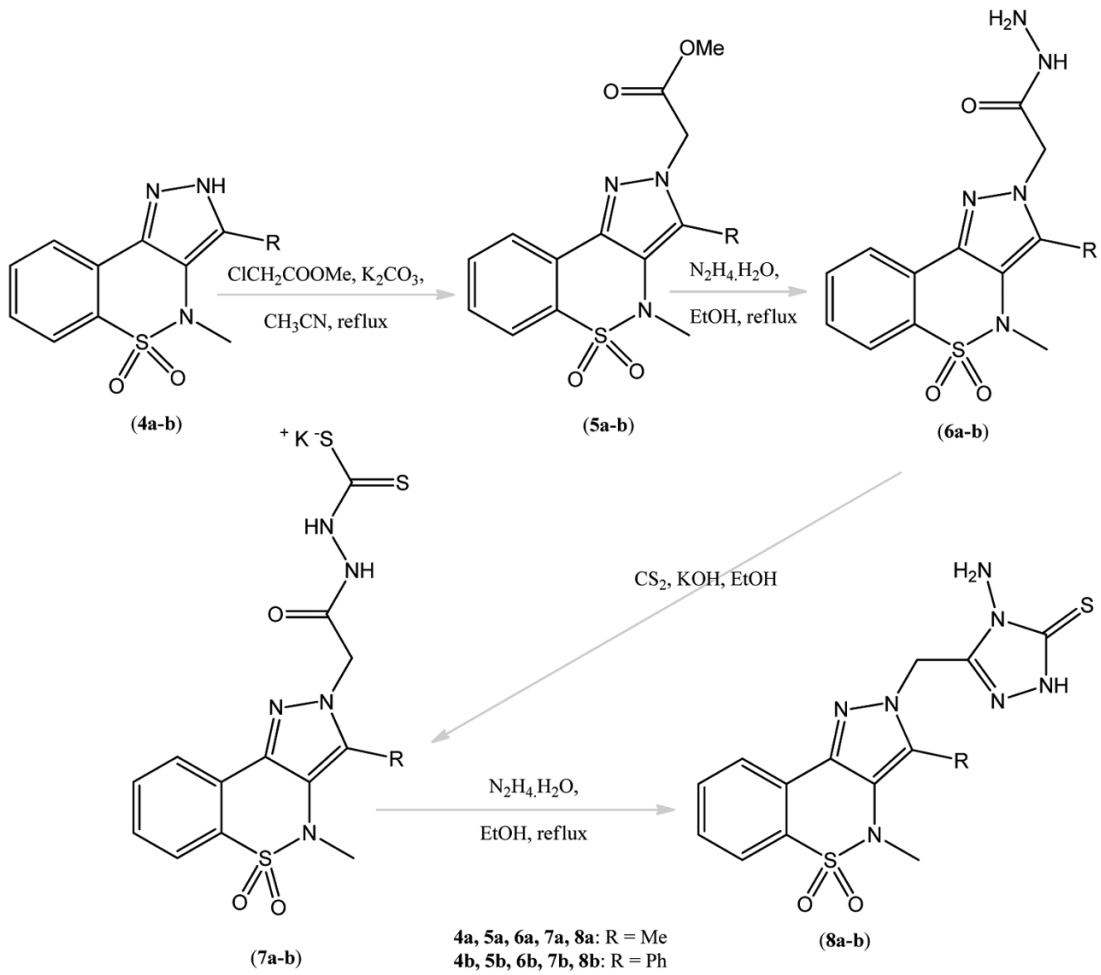

Scheme 1. Synthesis of structural hybrids of pyrazolobenzothiazine and triazole ring systems.

for a longer period of time than did ciprofloxacin [6]. Various 1,4-benzothiazine derivatives were found to moderately inhibit the growth of *Escherichia coli* and *S. aureus* species [7]. Similarly, hydrazones derived from 2,1-benzothiazine dioxides and 1,2-benzothiazine hydrazides were also reported to moderately inhibit the bacterial growth of various strains [8, 9]. Among fused benzothiazines, pyrazolobenzothiazine derivatives, 2-[2-(3,5-dimethyl-1H-pyrazol-1-yl)-2-oxoethyl]-3,4-dihydro-2*H*-1,4-benzothiazin-3-ones and morpholinyl/2-hydroxyethylpiperazinylbenzothiazines exhibited activity comparable to that of chloramphenicol against *E. coli* and *Bacillus cereus* [10, 11].

In our previous work on pyrazolobenzothiazine based chalcones, several compounds showed marked activity against *E. coli* [12]. In the present article, we describe a synthetic layout for the formation of structural hybrids of the pyrazolobenzothiazine ring system with triazole ring (Scheme 1), which have been tested for antibacterial activity against drug resistant clinical isolate of *S. aureus*.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Precursor compounds **4a** and **4b** were prepared according to our previously reported methods [13 – 15] starting from sodium saccharine. A straight forward synthetic methodology was adopted for the structural hybridization with triazole ring. For this purpose, compounds **4a** and **4b** were treated with methyl chloroacetate in the presence of tetrabutylammonium bromide (TBAB) as a catalyst to afford compounds **5a** and **5b.** The use of TBAB resulted in reduction of the reaction times (Scheme 1). In ^{1}H NMR spectra, the protons of –COOMe groups were manifested by singlets at 3.79 and 3.27 ppm for **5a** and **5b,** respectively. Compounds **5a** and **5b** were treated with hydrazine hydrate to get respective hydrazides (**6a, 6b**). Then, potassium salts of dithiocarbamates (**7a, 7b**) were obtained by reaction of compounds **5a** and **5b** with carbon disulfide and potassium hydroxide, and subsequently converted to target triazoles **8a**

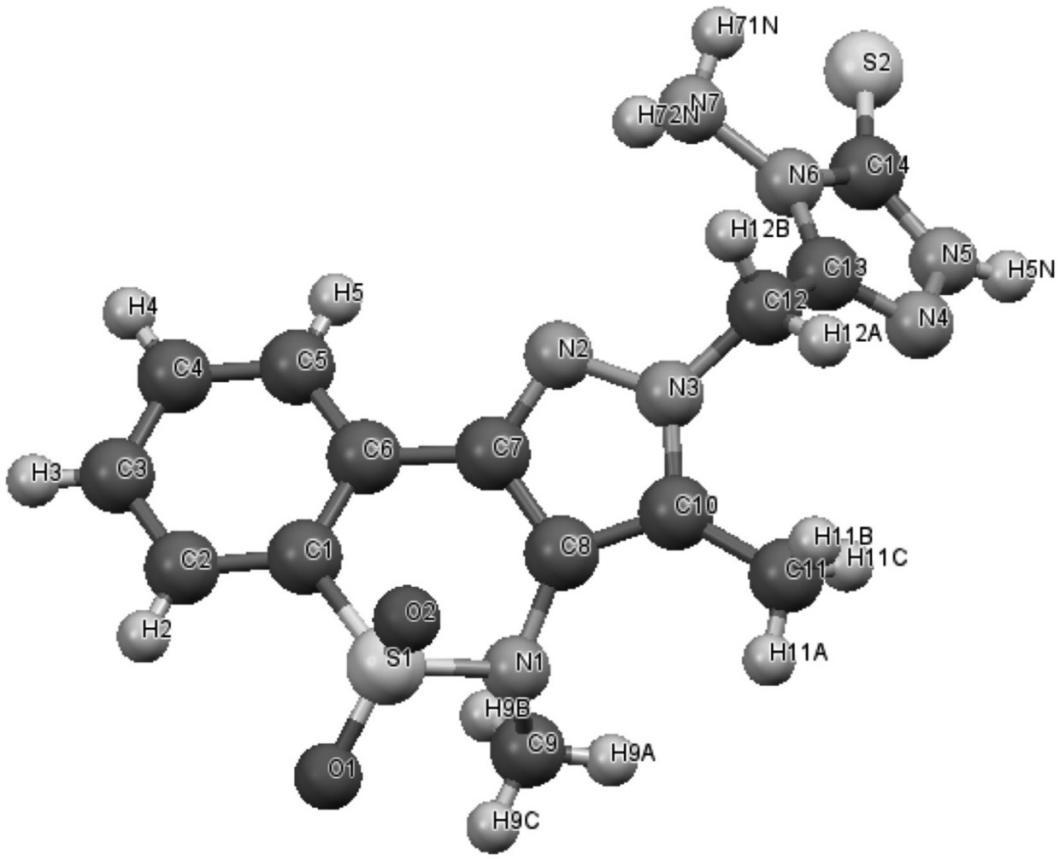

Fig. 1. Crystalline structure of compound **8a**. Displacement ellipsoids are drawn at the 50% probability level.

TABLE 1. Crystallographic and Structure Refinement Data for Compound **8a**

Parameter	Value	Parameter	Value
CCDC No.	—	Theta range for data collection	3.7 to 27.5°.
Empirical formula	$C_{14}H_{15}N_7O_2S_2$	Index ranges	$-35 \le h \le 36$, $-12 \le k \le 12$, $-15 \le l \le 15$
Formula weight	377.45	Reflections collected	7276
Temperature	173(2) K	Independent reflections	3766 [R(int) = 0.024]
Wavelength	0.71073 Å	Completeness to theta = Temperature	99.4 % 27.5°
Crystal system	Monoclinic	Absorption correction	Multi-scan method
Space group	C2/c	Max. and min. transmission	0.953 and 0.927
Unit cell dimensions	Refinement method	Full-matrix least-squares on F^2	
a = 27.801(8) Å b = 9.884(2) Å c = 12.282(3) Å	a = 90° b = 101.270(14)° g = 90°		
Volume	3309.8(14) $Å^3$	Data / restraints / parameters	3766 / 0 / 237
Z	8	Goodness-of-fit on F^2	1.02
Density (calculated)	1.515 Mg/m^3	Final R indices [I > 2sigma(I)]	$R1$ = 0.035, wR2 = 0.086
Absorption coefficient	0.347 mm^{-1}	R indices (all data)	$R1$ = 0.042, wR2 = 0.090
F(000)	1568	Largest diff. peak and hole	0.33 and -0.44e. $Å^{-3}$
Crystal size	0.22 × 0.16 × 0.14 mm^3		

TABLE 2. Selected Geometric Parameters (Å) of Compound **8a**

S1-O1	1.4276(11)	N2-N3	1.363(2)
S1-O2	1.4350(12)	N3-C10	1.361(2)
S1-N1	1.6482(13)	N3-C12	1.464(2)
S1-C1	1.7617(16)	N4-C13	1.307(2)
S2-C14	1.6754(17)	N4-N5	1.380(2)
N1-C8	1.430(2)	N5-C14	1.342(2)
N1-C9	1.479(2)	N6-C13	1.369(2)
N2-C7	1.339(2)	N6-C14	1.376(2)
O1-S1-O2	119.91(7)	C7-N2-N3	103.94(12)
O1-S1-N1	109.05(7)	C10-N3-N2	113.72(12)
O2-S1-N1	106.50(7)	C10-N3-C12	126.79(13)
O1-S1-C1	108.51(7)	N2-N3-C12	119.17(12)
O2-S1-C1	106.85(7)	C13-N4-N5	103.86(13)
N1-S1-C1	105.06(7)	C14-N5-N4	113.78(13)
C8-N1-C9	116.84(13)	C13-N6-C14	109.04(13)
C8-N1-S1	109.95(9)	C13-N6-N7	124.29(13)
C9-N1-S1	117.29(10)	C14-N6-N7	126.63(14)
C1-S1-N1-C8	49.04(11)	S1-C1-C6-C7	4.19(19)
N1-S1-C1-C6	-35.60(14)	C1-C6-C7-C8	16.3(2)
S1-N1-C8-C7	-37.90(18)	C6-C7-C8-N1	2.0(2)

and **8b**. In compounds **8a** and **8b**, NH_2 protons signaled at 5.63 and 5.89 ppm, whereas NH peaks appeared at 13.74 and 13.72 ppm, respectively. In addition, the structure of compound **8a** was confirmed by x-ray crystallography (Fig. 1).

*2.2. X-ray Crystal Structure of Compound **8a***

In the structure of compound **8a**, the molecular dimensions are in agreement with the expected values (Table 1). The heterocyclic thiazine ring adopts a half-chair conformation, with S1 and N1 atoms displaced in the opposite directions by 0.424(3) and 0.330(3) Å, respectively, from the planes defined by the remaining ring atoms (Fig. 1). The five-member rings, N2/N3/C7/C8/C10 and N4/N5/N6/C13/C14 are essentially planar with RMS deviations of 0.0049 and 0.0042 for fitted atoms, and are inclined at 60.61(7)° with respect to each other (Table 2). The crystal structure is stabilized by strong N-H…S and N-H…N type intermolecular interactions; details are given in Table 3.

TABLE 3. Hydrogen Bonding Data for Compound **8a**

D-H…A	*d*(D-H)	*d*(H…A)	*d*(D…A)	<(DHA)
N7-H72N…S2#1	0.86(2)	2.56(2)	3.372(2)	160(2)
N5-H5N…N(2)#2	0.86(2)	2.15(2)	2.961(2)	156(2)

Symmetry transformations used to generate equivalent atoms: #1 -x+1,-y,-z+1; #2 x,-y, z-1/2

2.3. Antimicrobial Activity of Synthesized Compounds

Antimicrobial activity of the synthesized compounds was checked on a multidrug resistant strain of *S. aureus*. These extracts exhibited clear zones of growth inhibition around disks impregnated with test compounds. In a series of ten compounds studied, only four exhibited inhibition of *S. aureus* growth. The maximum inhibition zone of 30 mm was observed for compound **7b,** while the inhibition zone observed for compound **7a** was 14 mm wide. Some other compounds (**8a** and **8b)** gave the zones of inhibition greater than 10 mm, while the rest of compounds produced no inhibition of bacterial growth (Table 4).

The obtained data show interesting structure – activity relationships. The incorporation of carbodithiolate moiety into the side chain of pyrazolobenzothiazine ring system resulted in compounds having excellent activity (**7a, 7b**). This moiety is well known for its antibacterial studies. On the other hand, compounds **8a, 8b** bearing triazole rings were also active inhibitors of bacteria studied.

2.3.1. Minimum inhibitory concentration (MIC) of compounds 4(a, b) – 8(a, b). MIC is the lowest concentration of an antimicrobial agent that will hinder visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is indicative of a better antimicrobial agent. The MIC value is generally regarded as the most basic laboratory characteristic of the activity of an antimicrobial agent against a microbe. Potent compound **7b** exhibited a

TABLE 4. Zone of Growth Inhibition Produced by Compounds **4(a, b)** – **8(a, b)** and standard drugs **SD1 – SD5** against Multidrug Resistant *S. aureus* Isolates

Compd.	ZI[a] (mm)	MIC (mg/mL)	Compd.	ZI[a] (mm)	MIC (mg/mL)
4a	0.0	—	6b	0.0	—
4b	0.0	—	7a	14	—
5a	0.0	—	7b	30	0.0319
5b	0.0	—	8a	11	—
6a	0.0	—	8b	11	—
SD-1[b]	9	ND	SD-4[b]	14	ND
SD-2[b]	27	ND	SD-5[b]	9	ND
SD-3[b]	11	ND	—	—	—

[a] ZI: zone of Inhibition, mm; ND =not determined.[b] Concentrations of standard drugs: **SD1** = oxacillin (1 μg), **SD2** = ampicillin (10 μg), **SD3** = chloramphenicol (30 μg), **SD4** = ciprofloxacin (5 μg), **SD5** = sulfamethoxazole (25 μg).

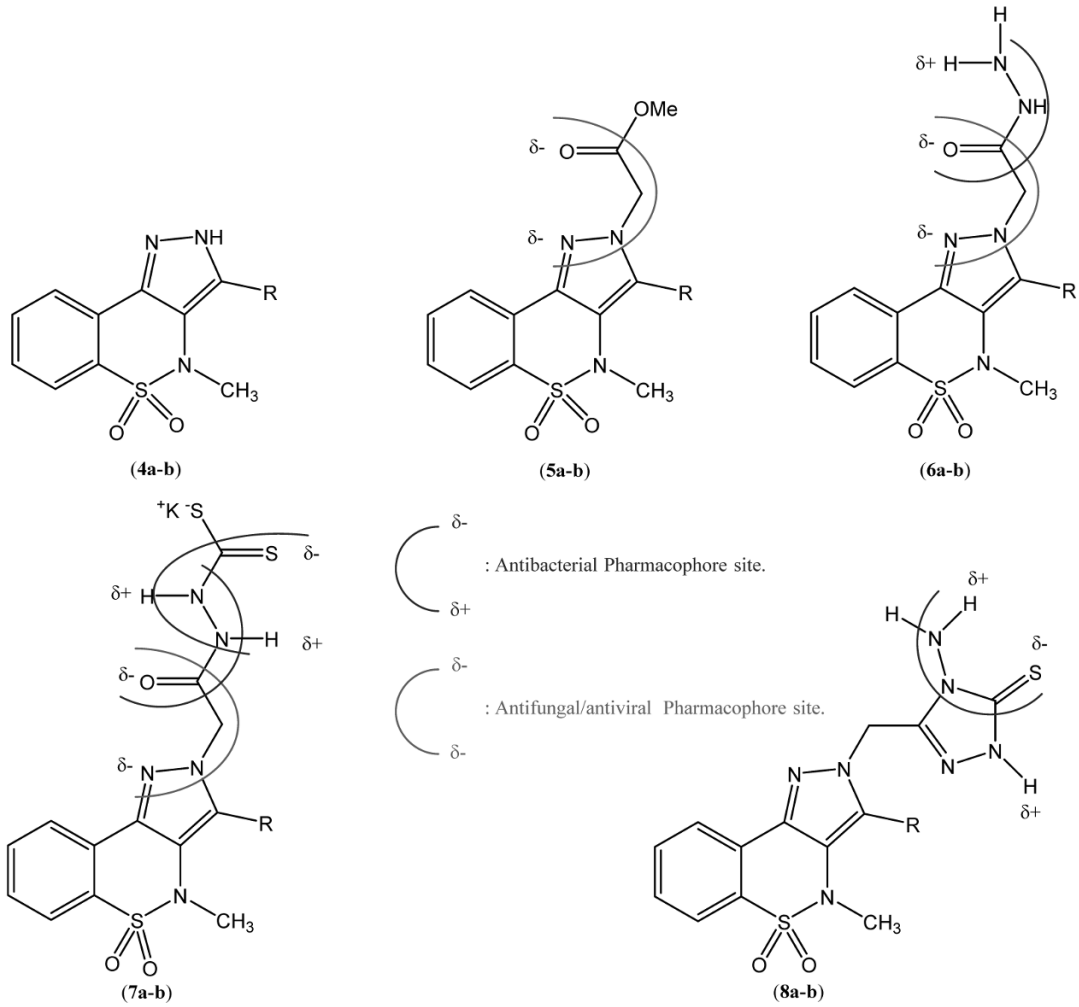

Fig. 2. Identification of pharmacophore sites of compounds **4(a, b)** – **8(a, b)**.

50% inhibition of *S. aureus* growth, and the corresponding MIC value was 0.0319 mg/mL.

2.4. POM Analysis

The analysis of theoretical toxicity risks for the series of compounds **4 – 8** using the Osiris program showed that compound **4a** and probably the rest of series **4 – 8** (Table 5) are less toxic than standard clinical drugs oxacillin **(SD1**) and chloramphenicol **(SD3**), so that series **4 – 8** can be used as antibiotics with some restriction. From the data in Table 5, it follows that five of the ten structures are supposed to be non-mutagenic when run through the mutagenicity assessment of coordinated system and, as far as irritating and reproductive effects are concerned, all the compounds are at low risk comparable with standard drugs **SD1** and **SD3**. The hydrophilic character of each compound has been expressed in terms of the cLog*P* value. It has been established that the absorption or permeation is greatly affected by the hydrophilicity (cLog*P* value). Accordingly, when cLog*P* is higher than 5, the absorption or permeation decreases. On this basis, most of compounds **4 –– 8** have cLog*P* values within the acceptable limits, but some other crucial parameter should be taken in consideration. This does not concern the geometric conformation of pharmacophore because it is fixed for all compounds **4 – 8**. The absorption, distribution, and bioactivity characteristics were proved to be dependent on the geometric parameter and aqueous solubility of each compound. Further, Table 6 shows drug-likeliness of compounds **4 – 8**, not in the comparable zone with standard drugs used. We have calculated overall drug score (DS) values for compounds **4 – 8** and compared them to those of standard drugs **SD1 – SD5**. The DS combines drug-likeliness, cLog*P*, log*S*, molecular weight, and toxicity risks, in one handy value that may be used to judge on the compound's overall potential to qualify for a drug. The reported compounds **4 – 8** showed moderate to excellent DS as compared with standard drugs **SD1** – **SD5** (Tables 5 and 6). In addition, the pharmacophore sites in title molecules were identified as shown in Figure 2.

3. EXPERIMENTAL PART

*3.1. Synthesis of Compounds **4 – 8***

Precursor compounds **4a** and **4b** were synthesized as reported elsewhere [13 – 15].

3.1.1. General method for the synthesis of methyl 2-(3,4-dimethyl/4-methyl-3-phenyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4*H*)-yl)acetates 5a and 5b. Compound **4a**or **4b** (0.01 mole), methyl chloroacetate (0.015 mole), potassium carbonate (0.02 mole), TBAB (0.1g) and acetonitrile (50 mL) were mixed in a round-bottom flask and the mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue was poured into 100mL distilled water. The precipitate was filtered and washed with excess water. Finally, precipitate was recrystallized from methanol.

Methyl-2-(3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo[4, 3-c][1,2]thiazin-2(4*H*)-yl)acetate **(5a)**: white powder; yield, 80%; m.p., 180°C; FT-IR (KBr; ν, cm^{-1}): 3061, 2968, 1695, 1341, 1173; ^{1}H NMR ($CDCl_3$; 400 MHz; δ, ppm): 2.32(s, 3H, CH_3), 3.04 (s, 3H, NCH_3), 3.79 (s, 3H, OCH_3), 4.91 (s, 2H, NCH_2), 7.51 (t, 1H, J = 7.7 Hz, ArH), 7.63 (t,1H, J = 7.7 Hz, ArH), 7.90(d, 1H, J = 7.7 Hz, ArH), 7.97 (d, 1H, J = 7.7 Hz, ArH). ^{13}C NMR ($CDCl_3$; 400 MHz; δ, ppm): 8.6, 38.7, 51.3, 52.4, 122.5, 123.4, 124.4, 127.7, 129.2, 131.5, 133.4, 134.4, 136.8, 167.6; MS (*m/z*): 321.2(M^+).

Methyl-2-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4, 3-c][1,2]thiazin-2(4*H*)-yl)acetate **(5b)**: off-white powder; yield, 89%; m.p.,160°C; FT-IR (KBr; ν, cm^{-1}): 3078; 2965; 1685; 1339; 1275; 1168; ^{1}H NMR ($CDCl_3$; 400 MHz; δ, ppm): 2.91 (s, 3H, $N\text{-}CH_3$), 3.27 (s, 3H, $O\text{-}CH_3$), 5.77 (s, 2H, $N\text{-}CH_2$), 7.44 (t, 3H, J = 7.6Hz, Ar-*H*), 7.63 – 7.67 (dd, 1H, J_1 = 2.4 Hz, J_2 = 7.8 Hz, Ar-*H*), 7.75 – 7.79 (dd, 1H, J_1 = 3.2 Hz, J_2 = 8.0 Hz, Ar-*H*), 7.94 – 8.01 (dt, 2H, J_1 = 2.8 Hz, J_2 = 7.8 Hz, J_3 = 14.8 Hz, Ar-*H*), 8.2 (d, 2H, J = 7.6 Hz, Ar-*H*); ^{13}C NMR (DMSO-d_6; 400 MHz; δ, ppm): 39.8 ($N\text{-}CH_3$), 51.4 ($O\text{-}CH_3$), 53.9 ($N\text{-}CH_2$), 122.3 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 124.9 (Ar-C), 125.9 (Ar-C), 126.0 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 130.2 (Ar-C), 130.4 (Ar-C), 130.6 (Ar-C), 132.4 (Ar-C), 134.29 (Ar-C), 134.41 (Ar-C), 139.0(N=C, Ar-C), 167.8 (C=O); MS (*m/z*): 383.06 (M^+).

3.1.2. General method for the synthesis of 2-(3,4-dimethyl/4-methyl-3-phenyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4*H*)-yl)acetohydrazides (6a and 6b). Compound **5a** or **5b** (0.01 mole) and hydrazine hydrate (2 mL) were dissolved in ethyl alcohol (25 mL) and the mixture was refluxed for 5 h. The solvent was removed under vacuum, 100 mL distilled water was added to the residue, and pH of the solution was maintained at 5 – 6 by using 5% HCl. The precipitates formed were filtered and recrystallized from ethanol.

TABLE 5. Osiris Calculations for Compounds **4 – 8** and Standard Drugs **SD1 – SD4**

Compd.	R	Toxicity risks[a]				Drug score[b]			
		MUT	TUMO	IRRI	REP	CLP	S	DL	DS
4a	Me	+++	+++	+++	+++	0.68	-2.69	4.38	0.92
4b	Ph	+++	+++	+++	+++	2.04	-4.10	4.46	0.79
5a	Me	+++	+++	+++	+++	0.26	-2.49	2.18	0.86
5b	Ph	+++	+++	+++	+++	1.61	-3.90	2.12	0.73
6a	Me	+++	-/-/-	+++	+++	-1.33	-2.72	0.55	0.44
6b	Ph	+++	-/-/-	+++	+++	0.02	-4.13	0.49	0.37
7a	Me	+++	+++	+++	-/-/-	-0.26	-4.32	4.84	0.42
7b	Ph	+++	+++	+++	-/-/-	1.09	-5.73	4.78	0.30
8a	Me	-/-/-	+++	+++	-/-/-	-1.21	-3.45	4.43	0.30
8b	Ph	+++	+++	+++	+++	0.14	-4.86	4.37	0.23
SD1 [c]	—	-/-/-	+++	+++	-/-/-	2.78	-3.76	9.71	0.27
SD2 [c]	—	+++	+++	+++	+++	-1.66	-1.57	-10.72	-0.91
SD3 [c]	—	-/-/-	-/-/-	-/-/-	-/-/-	-0.42	-2.37	-4.61	-0.06
SD4 [c]	—	+++	+++	+++	+++	-1.53	-3.22	2.07	0.82
SD5 [c]	—	+++	+++	+++	+++	0.20	-3.40	-7.90	-0.44

Toxicity rating:(-/-/-) higly toxic;(+) slightly toxic;(+++) nontoxic. [a] MUT = mutagenic; TUMO = tumorigenic; IRRI = irritant; REP = reproductive effective. [b] CLP = cLogP; S = solubility, DL = drug likliness; DS = drug score. [c] Standard drugs: **SD1** = oxacillin, **SD2** = ampicillin, **SD3** = chloramphenicol, **SD4** = ciprofloxacin, **SD5** = sulfamethoxazole.

2-(3,4-Dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]-thiazin-2(4*H*)-yl)acetohydrazide **(6a):** white powder; yield, 72%; m.p., 187 – 189°C; IR (KBr; ν, cm^{-1}): 3451, 1678, 1346, 1150; ^{1}H NMR (DMSO-d_6; 300 MHz; δ, ppm): 2.26 (s,3H, CH_3), 2.95 (s, 3H, NCH_3), 4.35 (s, 2H, NH_2), 4.83 (s, 2H, NCH_2), 7.62(t, 1H, J = 7.5 Hz, ArH), 7.77 (t, 1H, J = 7.5 Hz, ArH), 7.85 – 7.92 (dd, 2H, J = 13.8, 7.78 Hz, ArH), 9.45 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6; 300 MHz; δ, ppm): 8.7, 38.9, 51.3, 122.3, 123.3, 124.3, 127.9, 128.8, 129.0, 131.4, 133.3, 136.4, 165.4; MS (*m/z*): 321.0(M^+).

2-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4*H*)-yl)acetohydrazide **(6b)**: off-white powder; yield, 75%; m.p., 185°C; IR (KBr; ν, cm^{-1}): 3468; 3010; 1675; 1337, 1165; ^{1}H NMR (DMSO-d_6; 400MHz; δ, ppm): 2.90 (s, 3H, N-CH_3), 4.39 (s, 2H, NH_2), 5.78 (s, 2H, N-CH_2), 7.49 (t, 3H, J = 7.6 Hz, Ar-*H*), 7.59 – 7.61 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.4 Hz, Ar-*H*), 7.71 – 7.73 (dd, 1H, J_1 = 2.8 Hz, J_2 = 8.4 Hz, Ar-*H*), 7.83 (t, 2H, J = 8.4 Hz, Ar-H), 8.11 (d, 2H, J = 7.8Hz, Ar-*H*) 11.94 (br-s, 1H, NH); ^{13}C NMR (DMSO-d_6; 400MHz; δ, ppm): 39.8 (N-CH_3), 53.9 (N-CH_2), 122.2 (Ar-C), 124.2 (Ar-C), 124.7 (Ar-C), 124.9 (Ar-C), 125.9 (Ar-C), 126.0 (Ar-C), 127.2 (Ar-C), 128.6 (Ar-C), 130.2 (Ar-C), 130.4 (Ar-C), 130.6 (Ar-C), 132.4 (Ar-C), 134.2 (Ar-C), 134.4 (Ar-C), 138.9 (N=C, Ar-C), 165.6 (C=O); MS (*m/z*): 383.07 (M^+).

3.1.3. General method for the synthesis of potassium 2-(2-(3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]-thiazin-2(4*H*)-yl)acetyl)hydrazinecarbodithioates 7a and 7b. Compound **6a** or **6b** (0.01 mole), carbon disulfide (0.05 mole), and potassium hydroxide (0.01 mole) were mixed in ethanol (30 mL). The mixture was stirred overnight and the precipitate was filtered and dried. Compounds **7a** and **7b** were used for further reaction without any characterization.

3.1.4. General method for the synthesis of 4-amino-3-((3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4*H*)-yl)methyl)-1H-1,2,4-triazole-5(4*H*)-thiones 8a and 8b. Compound **7a** or **7b** (0.01 mole), hydrazine hydrate (2 mL), and distilled water (2 mL) were mixed in ethanol (30 mL) and the mixture was refluxed for 3 h. The solvent was removed at reduced pressure, the residue was mixed with distilled water, pH of the solution was maintained at 6 using 5 % HCl, and the precipitate was filtered, washed with water, and dried.

4-Amino-3-((3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo-[4,3-c][1,2]thiazin-2(4*H*)-yl)methyl)-1H-1,2,4-triazole-5(4*H*)-thione **(8a):** white crystalline material; yield, 41%; m.p., 240°C; ^{1}H NMR (DMSO-d_6; 300 MHz; δ, ppm): 2.42 (s, 3H, C-CH_3), 2.95 (s, 3H, N-CH_3), 5.49 (s,2H, N-CH_2), 5.63 (s, 2H, N-NH_2), 7.63 (t,1H, J = 6.9 Hz, Ar-*H*), 7.76 (t, 1H, J = 7.2 Hz, Ar-*H*), 7.79 – 7.91 (dd, 2H, J_1 = 9.0 Hz, J_2 = 16.2 Hz, Ar-*H*), 13.74 (s, 1H, NH); MS (*m/z*): 377.2 (M^+).

TABLE 6. Molinspiration Property Calculations for Compounds **4 – 8** and Standard Drugs **SD1 – SD5**.

Compd.	Physicochemical properties[a]					Drug likeliness[b]					
	TPSA	O/NH	VIOL	ROTB	VOL	GPC	ICM	KI	NRL	PI	EN
4a	66	1	0	0	203	-0.36	-0.33	-0.64	-0.76	-0.41	-0.47
4b	66	1	0	0	258	-0.02	-0.16	-0.10	-0.17	-0.00	-0.19
5a	82	0	0	3	264	-0.27	-0.57	-0.56	-0.49	-0.03	-0.32
5b	82	0	0	4	319	-0.07	-0.33	-0.26	-0.20	0.03	-0.17
6a	110	3	0	2	263	-0.48	-0.92	-0.64	-0.94	-0.12	-0.44
6b	110	3	0	3	317	-0.24	-0.62	-0.33	-0.57	-0.05	-0.26
7a	96	2	0	4	306	-0.37	-0.80	-0.81	-0.92	-0.11	-0.44
7b	96	2	0	5	361	-0.19	-0.54	-0.52	-0.61	-0.09	-0.29
8a	115	3	0	2	297	-0.49	-0.94	-0.88	-1.04	-0.46	-0.57
8b	115	3	0	3	351	-0.31	-0.58	-0.62	-0.63	-0.28	-0.37
SD1[c]	116	1	0	4	350	-0.06	-0.27	-0.72	-0.14	-0.30	-0.14
SD2 [c]	113	4	0	4	315	0.04	-0.47	-0.71	-0.61	-0.87	0.25
SD3 [c]	115	3	0	6	249	-0.22	-0.28	-0.38	-0.41	-0.21	-0.00
SD4 [c]	75	2	0	3	285	O.12	-0.04	-0.07	-0.19	-0.21	0.28
SD5 [c]	98	3	0	3	205	-0.42	-0.62	-0.48	-1.06	-0.47	-0.25

[a] TPSA = Total polar surface area; O/NH: O–HN interaction; VIOL = number of violations; VOL = volume. [b] ICM = ion channel modulator; KI = kinase inhibitor; NRL = nuclear receptor ligand; PI = protease inhibitor; EI = enzyme inhibitor.[c] Standard Drugs: **SD1** = oxacillin; **SD2** = ampicillin, **SD3** = chloramphenicol, **SD4** = ciprofloxacin, **SD5** = sulphamethoxazole.

4-Amino-3-((4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4*H*)-yl)methyl)-1H-1,2,4-triazole-5(4*H*)-thione **(8b):** light yellow substance; yield, 54%; m.p., 224°C; ^{1}H NMR (DMSO-d_6; 300 MHz; δ, ppm): 2.86 (s, 3H, N-CH_3), 5.67 (s, 2H, N-CH_2), 5.89 (s, 2H, N-NH_2), 7.44 (d, 1H, J = 7.2 Hz, Ar-*H*), 7.52 (t, 2H, J = 7.5 Hz, Ar-*H*), 7.77 (t, 1H, J = 7.2 Hz, Ar-*H*),7.88 (d, 1H, J = 7.5 Hz, Ar-*H*), 7.94 (d, 2H, J = 7.2 Hz, Ar-*H*), 8.01 – 8.09 (dd, 1H, J_1 = 7.2 Hz, J_2 = 16.2 Hz, Ar-*H*), 13.72 (s, 1H, NH); MS (*m/z*): 439.2 (M^+).

*3.2. X-ray Crystallographic Analysis of Compound **8a***

A colorless prismatic crystal of compound **8a** was coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber. All measurements were made on a Nonius Kappa CCD diffractometer using graphite-monochromated Mo-Kα radiation. Details of crystal data, data collection [16, 17], and structure refinement are presented in Table 1. The data were corrected for the Lorentz and polarization effects and for absorption using the multi-scan method [16]. The structure was solved by the direct methods [18] and expanded using Fourier techniques [19]. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms bonded to C-atoms were included at geometrically idealized positions and not refined, while those bonded to N atoms were allowed to refine. The final cycle of full-matrix least-squares refinement using SHELXL97 [20] converged with unweighted and weighted agreement factors, $R = 0.035$ and $wR = 0.090$ (all data), respectively, and goodness of fit, $S = 1.02$. The weighting scheme was based on counting statistics and the final difference map had no chemically significant features. The figures were plotted with the aid of ORTEPII [21]. Selected bond lengths [Å], angles [°] and torsion angles [°], and hydrogen bonding geometry [Å and °] for compound **8a** are given in Tables 2 and 3, respectively. The molecular structure of compound **8a**, showing the atom numbering scheme, is presented in Fig. 1.

3.3. Antimicrobial Activity Testing by Disc Diffusion Method

The antimicrobial susceptibility testing was performed by the disc diffusion method. At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a flask containing tryptone soy broth (TSB). The culture was incubated at 37°C overnight on a shaker. The inoculum should give semi-confluent growth of colonies after overnight incubation. Use of an inoculum that yields semi-confluent growth has the advantage that an incorrect inoculum can easily be observed. The Muller – Hilton agar (MHA) plates were prepared for disc assay. Inoculum suspensions were spread onto the MHA plates. The plates were allowed to dry for 3 – 5 minutes before the discs were applied. The disc diffusion technique and zone interpretation of antimicrobial agent were used in accordance with NCCLS guidelines. Standard drugs oxacillin (1 μg), ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg) and sulphamethoxazole (25 μg) were used for drug susceptibility testing. The plates were incubated at 37°C for 24 h and the results were interpreted according to NCCLS standards for antibiotic susceptibility testing.

3.3.1. Antibacterial analysis against drug resistant *Staphylococus aureus.*

The antibacterial activity of the synthesized compounds was determined on a multidrug resistant strain of *S. aureus.* Standardized inoculums were prepared and streaked. Sterile filter paper discs previously soaked in 10 ìL compound of known concentration were carefully placed at the center of the labeled plate. The plates were incubated aerobically at 37°C for 19 h and then examined for zones of inhibition.

3.3.2. Determination of minimum inhibitory concentration (MIC).

IC_{50} was determined by micro-dilution method using double serially diluted compounds according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The IC_{50} of extracts was determined by 2-fold dilution of each compound concentration 1mg/mL in 10 test tubes.

3.4. POM Analyses

One of the practical problems associated with synthetic drugs is the existence of various side effects. For a molecule to be potential drug, besides having a good biological activity, it must have good pharmacokinetic properties in biological systems. To access the pharmacokinetic profile of the synthesized molecules, we used well established *in silico* tools such as Osiris, Petra and Molinspiration (POM), which have been validated with almost 7000 drug molecules available on the market.

4. CONCLUSION

Compound dithiocarbazates **7a**, **7b** and **8a**, **8b** demonstrated significant antibacterial effects. Thus, it would be an attractive candidate series for treatment of various bacteria; further detailed studies may have to be undertaken in order to evaluate its clinical potential. The antibacterial effects of compounds **7** and **8** are partially attributed to the presence of two combined antibacterial pharmacophore sites [(N^{d-}–NH^{d+}) and (S^{d-}–N^{d+})] and an antifungal supplementary pharmacophore site (N^{d-}–O^{d-}) as has been shown in detail above (Fig. 2).

Results of the present POM investigation are in perfect agreement with experimental antibacterial screening. It has been further suggested that some functional groups, such asterminal thionyl (C=S) present in compounds **7a** and **7b**, play a crucial role in the biological activity; this group may be responsible for increased number of pharmacophore sites, hydrophobic character, and liposolubility of molecules. This, in turn, enhances the activity and biological absorbance of compounds. Thus, compounds **7a** and **7b** have good thera-

peutic properties and should also be tested against fungi because of the presence of antifungal site N^{d-}–O^{d-}.

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