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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A novel and eco-friendly procedure for the synthesis of some pyrazolo-thiadiazolo-pyrimidinones and its in vitro anti-bacterial activity

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Abstract A novel series of 2-substituted-aminomethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones have been synthesized by treating 2-chloromethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-one with various nucleophiles. The chloromethyl derivative was prepared by treating 2-amino-3-mercapto pyrazolo[3,4-*d*]pyrimidine with one carbon donor, chloroacetic acid. The intermediate 2-amino-3-mercapto pyrazolo[3,4-*d*]pyrimidine was prepared by a novel, eco-friendly route starting from 2-amino-3-carbethoxy-1-phenyl-pyrazole. The target compounds exhibited broad-spectrum antibacterial activity (Kirby Bauer's Method).

Keywords Antibacterial activity · 2-Amino-3-mercapto-pyrazolo[3,4-*d*]pyrimidines · Bioisosteres · Eco-friendly route · Kirby Bauer's method · Thiadiazolopyrazolopyrimidines

Introduction

As of the day, several hundred of compounds belonging to various classes are available to combat bacterial infections. However, very few such compounds have found application in general medicinal practice, because in addition to the ability to combat infections, an antibiotic must possess other attributes like broad spectrum of activity, chemical stability, which is enough to be isolated, processed, and stored for a reasonable period of time without deterioration of potency, and should not produce undesirable side effects in the host, such as sensitivity or allergic reactions, damage or irritation to kidney or gastrointestinal tract; the rates of elimination of the antibiotic should be low enough to allow a convenient dosage form, yet rapid and complete enough to facilitate removal of drugs and its metabolites from the body soon after administration is discontinued, and more than everything the antibiotic should prevent the ready development of resistant forms of the parasite (John *et al.*, 2004). In order to overcome the resistance of strains, researchers look into finding novel drugs using the tool, bio-isosterism (http://www.cdc.gov/Drugresistance/community/anitbiotic-resistance.htm, 21 Jan 2010).

Thiadiazoloquinazolines, the biological analogs of purines, exhibit antibacterial activity (Soliman *et al.*, 1978). Thiadiazolopyrimidines have been reported to possess antibacterial (Robert *et al.*, 1974; Metzner and Heydehnauss, 1979; Niedlein and Menche, 1972), antifungal (Tokunaga *et al.*, 1987; Yadav and Sandhya, 1995), and herbicidal activities (Suiko and Mackawa, 1977; Kazayuki *et al.*, 1976). The concept of bio-isosterism has been exploited to design new types of condensed thiadiazolothienopyrimidines as isosteres of thiadiazoloquinazolines from our laboratories and has shown encouraging results (Raghu Prasad *et al.*, 2000, 2007).

Literature survey revealed that pyrazolopyrimidines possess various biological activities like antibacterial (Holla *et al.*, 2006; Shamourk *et al.*, 2005), antifungal (Rajesh and Leena, 2005; Wang Hong *et al.*, 2004; Giori *et al.*, 1985), and antiviral (Jyh-Haur *et al.*, 2004; El-Bendary and Badria, 2000) activities. However, few efforts have been made to fuse pyrazolopyrimidine ring system with other heterocyclic rings systems and test their efficacy on biological effect

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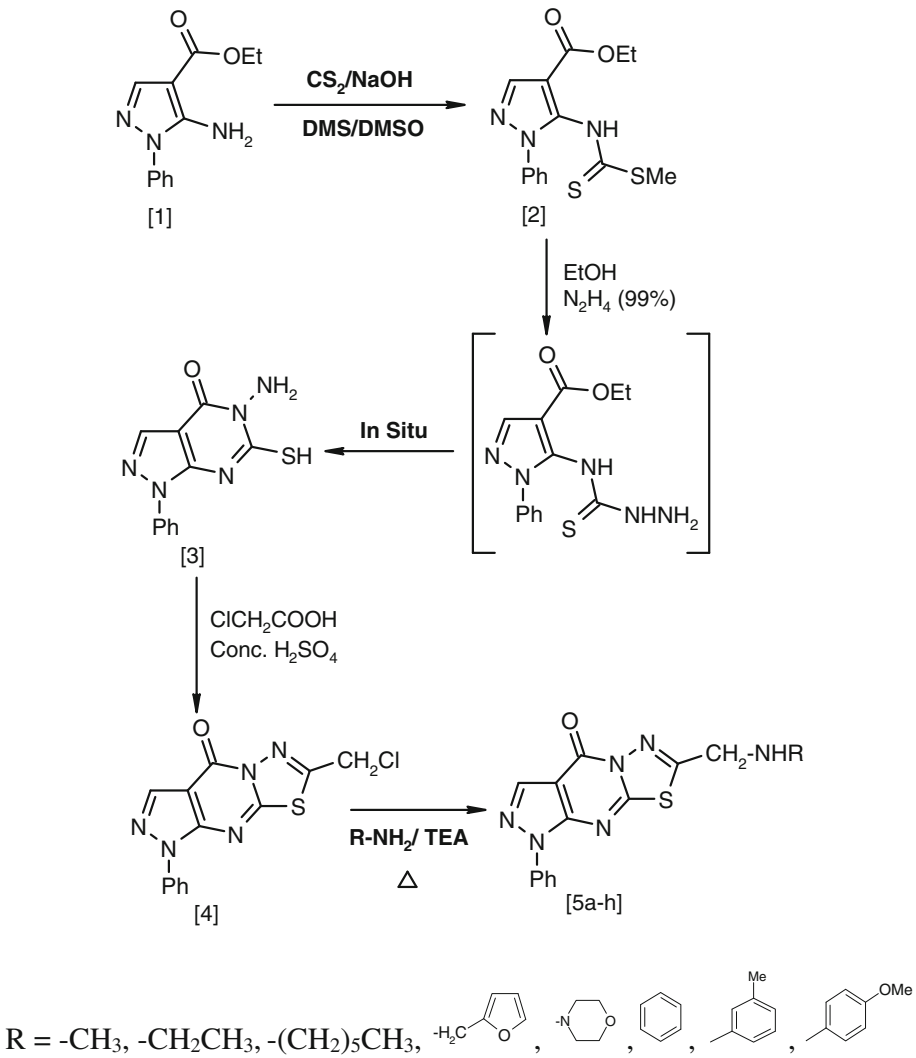
(Gatta *et al.*, 1993; Russo *et al.*, 1992, 1993; Gruccione *et al.*, 1996). This observation prompted us to fuse thiadiazole ring system to pyrazolopyrimidines, which in turn are the bio-isosteres of thiadiazolothienopyrimidines and thiadiazolo-quinazolines. Herewith, we are reporting the synthesis of some novel of 8-phenyl-2-substituted-aminomethyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones **5a–h** as antibacterial agents.

Results and discussion

The target compounds, 8-phenyl-2-substituted-amino-methyl-pyrazolo[3,4-*d*][1,3,4] thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones **5a–h**, were synthesized by following the route depicted in Scheme 1. The starting material, 5-amino-4-carbethoxy-1-phenyl-pyrazole **1,** was prepared as per the reported method (James *et al.*, 1987). A few reports are available in the literature for the synthesis of 2-amino-3-mercapto hetero-fused pyrimidinones (Modica *et al.*, 1997) using thiophosgene as reagent. However, this was not an attractive route as thiophosgene was non-eco-friendly and also resulted in less yields. Thus, an alternate, simple, eco-friendly route has been developed to synthesize the intermediate 2-amino-3-mercapto-pyrazolo[3,4-*d*]pyrimidine-4-ones **3** via dithio carbamate derivative **2**. The dithiocarbamate derivative **2** was obtained by reacting o-amino ester of pyrazole **1** with CS_2, NaOH, and dimethylsulfate in dimethylsulphoxide. Dimethylsulphoxide is a polar aprotic solvent, with high dielectric constant (high insulating properties). A polar solvent has both positive and negative ends and therefore can easily solvate anion or cation. Thus, a single charge on o-amino ester of pyrazole is surrounded by hundreds of solvent molecules; hence, area of charged surface is increased by thousand folds and the rate of reaction increased by million folds. Further, a high concentration of alkali (20 mol NaOH) was used to restrict amount of water, which otherwise would have



Scheme 1 Synthesis of 8-phenyl-2-substituted-aminomethyl-pyrazolo [3,4-*d*][1,3,4] thiadiazolo [3,2-*a*]pyrimidin-4(1*H*)-ones **5a–h**

caused hydration of **1** and would have hindered the rate of reaction. The other reason for using dimethylsulphoxide as a solvent may be that it increases the rate of alkylation at sulfur atoms.

To a vigorously stirred solution of 5-amino-4-carbethoxy-1-phenyl-pyrazole in dimethylsulphoxide, carbon disulfide and aqueous sodium hydroxide was added drop wise at room temperature. After two and half hours, dimethylsulfate was added drop wise under cooling in an ice bath. Stirring was continued for one and half hour. Then, the reaction mixture was poured into ice-cold water mixture. The precipitate obtained was filtered, dried, and recrystallised from boiling ethanol. The IR data of this compound showed characteristic –NH– vibration at 3398 cm^{-1} and –C=N vibration at 1557.8 cm^{-1} and –C=O vibration at 1647.55 cm^{-1}. This indicated the formation of the product. ^{1}H NMR spectra of the synthesized compound also showed $SC\underline{H}_3$ signals as singlet at around $\delta = 2.67$ ppm, which clearly confirmed the formation of the product. The mass spectra showed (M^+–SMe) peak at $m/z = 275$ with a relative abundance of 35%, and the fragmentation pattern was in agreement with the structure.

Solution of methyl-*N*-[4-carbethoxy-1-phenyl-pyrazolyl] dithiocarbamate **2** in ethanol was treated with hydrazine hydrate (99%) and heated to reflux on a water bath until the methyl mercaptan evolution ceased. The solution was immediately filtered under hot condition. The solid obtained was collected, dried, and recrystallised from boiling ethanol to yield 5-amino 6-mercapto-1-phenyl-pyrazolo[3,4-*d*]-pyrimidine-4[3*H*]-one 4. The IR data of the synthesized compound showed characteristic $–NH_2$ vibration at 3333.3 cm^{-1} and 3204.5 cm^{-1}, and –C=O vibration at 1655.94 cm^{-1} and =C–H vibration at 2929.97 cm^{-1}. The ^{1}HNMR spectra of the synthesized compound showed $–N\underline{H}_2$ and –SH signals as singlets at around $\delta = 4.96$ and 4.20 ppm, SMe signal at $\delta = 2.67$ disappeared, which confirmed cyclization. The mass spectra showed (M^+) peak at $m/z = 259$ with a relative abundance of 90%, and the fragmentation pattern was in agreement with the structure.

2-Chloromethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-one **4** was obtained by fusion of 5-amino-6-mercapto-1-phenylpyrazolo[3,4-*d*]-pyrimidine-4[3*H*]-one **3** with chloroacetic acid in presence of concentrated sulfuric acid by heating the mixture on a water bath for 5 h (neat reaction). The compound was purified using column chromatography by gradient elution technique using chloroform and ethyl acetate as solvent system. The IR data of the synthesized compound showed characteristic =C–H aromatic vibrations at 3174.65 cm^{-1} and C=O vibrations at 1562.5 cm^{-1} and C=N vibrations at 1519.32 cm^{-1}, and $–CH_2–Cl$ vibrations at 1238.70 cm^{-1}. The appearance of characteristic $–CH_2–Cl$ vibrations and disappearance of NH_2 peak indicated the formation of cyclized compound. The NMR spectrum of the synthesized compound showed $–CH_2Cl$ signal as singlet at around $\delta = 4.87$ ppm, and the disappearance of $–NH_2$ and –SH signals at $\delta = 4.9$ and 4.2 ppm confirmed the formation of cyclized product. The mass spectrum showed the M^+ peak at $m/z = 317$ with a relative abundance of 100% and M^+2 peak at $m/z = 319$ (isotope effect due to the presence of chlorine) with relative abundance of 20%, and the fragmentation pattern was in agreement with the structure. The target compounds, 2-substituted-aminomethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones **5a–h**, were obtained by heating the mixture of 2-chloromethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-one 4, triethylamine and methylamine in dioxane. Then, the reaction mixture was cooled to room temperature and poured into ice-water mixture, and the excess amine was neutralized by dilute hydrochloric acid (10%). The resultant precipitate thus obtained was filtered, dried, and recrystallised from benzene. The formations of these new amino derivatives were confirmed by specific IR peaks, which showed the absence of $–CH_2Cl$ peak and the presence of –NH stretching and bending vibrations. The structure of the compounds has been further ascertained based on the ^{1}H NMR spectrum, which showed presence of –NH signal and the disappearance of $–CH_2Cl$ signal followed by the presence of molecular ion peak corresponding to their molecular weights in mass spectra.

Antibacterial activity

The antibacterial activities of compounds **5a–h** were tested against one strain each of a *Staphylococcus aureus* (Gram +ve bacteria), *Bacillus subtilis* (Gram +ve spore), *E. coli* (Gram −ve bacteria), and *Klebsiella* (Gram −ve capsule) using Ampicillin (0.005 mL, 50 μg) as a standard drug using Kirby Bauer's Method (Bauer *et al.*, 1966) (Table 1). The negative control did not show any zone of inhibition in any of the bacterial strains used for the study. Compounds **5e** (14.5 ± 1.414) and **5h** (14.0 ± 1.425) were the most active compounds of the series against *Klebsiella*. The same compound **5h** (11.5 ± 0.707) showed the best activity against *E. coli*, but compounds **5c** (20.5 ± 0.707) and **5d** (20 ± 1.421) showed selectivity toward *B. subtilis* and *S. aureus*.

Conclusions

Series of 2-substituted-aminomethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidin-4(1*H*)-ones **5a-h** have been synthesized by treating 2-chloromethyl-8-phenyl-pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-one **4** with various nucleophiles. The chloromethyl derivative

Table 1 Antibacterial activity of preparation of 8-phenyl-2-substituted-aminomethyl- pyrazolo[3,4-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidin-4(1*H*)-ones **5a–h**

Compounds	*Klebsiella* Gram (−ve)	*E.coli* Gram (−ve)	*S. aureus* Gram (+ve)	*B. subtilis* Gram (+ve)
5a	11.5 ± 0.500	11 ± 1.414	13.5 ± 0.707	6.5 ± 0.707
5b	12 ± 1.414	10.5 ± 0.707	18.5 ± 0.505	16 ± 1.440
5c	11.5 ± 0.707	11 ± 1.414	17.5 ± 0.707	20.5 ± 0.707
5d	13 ± 1.414	8.5 ± 0.707	20 ± 1.421	18.5 ± 0.407
5e	14.5 ± 1.414	09 ± 1.414	7.5 ± 0.707	07 ± 1.414
5f	12.5 ± 0.707	10.5 ± 0.707	14.5 ± 0.707	14.0 ± 1.414
5g	12 ± 1.414	9.5 ± 0.707	16.5 ± 0.707	17.0 ± 1.414
5h	14.0 ± 1.425	11.5 ± 0.707	15.5 ± 0.707	15.5 ± 0.707
Ampicillin	24.5 ± 0.707	18.5 ± 0.707	22.5 ± 0.707	23.3 ± 2.001

was prepared by treating 2-amino-3-mercapto pyrazolo[3,4-*d*]pyrimidine **3** with one carbon donor, chloroacetic acid. The intermediate 2-amino-3-mercapto pyrazolo[3,4-*d*] pyrimidine 3 was prepared by a novel, eco-friendly route starting from 2-amino-3-carbethoxy-1-phenyl-pyrazole **1**.

All the tested compounds were found to possess activity against all the microorganisms (broad-spectrum activity) with potency lesser than the standard drug. This observation indicates that further lead optimization is necessary to get compounds with better antibacterial activity. The efforts are in progress toward reaching the goal.

Experimental

General techniques

Analytical TLC was performed on silica gel F_{254} plates (Merck) with visualization by UV or iodine vapors. Melting points were determined in open capillaries on a Gallenkemp melting point apparatus and are uncorrected. The IR spectra (KBr, vMax, cm^{-1}) were run on Perkin Elmer FTIR spectrophotometer. ^{1}H-NMR (δ ppm $CDCl_3$/DMSO-d_6) spectra were recorded using AMX-400 with TMS as internal standard. MS spectra were recorded on Autospec. Elemental analyses were performed on Carlo Erba 1108 analyzer and were within ±0.4% of theoretical values. All the chemicals used were of analytical grade.

Chemistry

*Synthesis of methyl-n-[4-carbethoxy-1-phenyl-pyrazolyl]dithiocarbamate **2***

To a vigorously stirred solution of 5-amino-4-carbethoxy-1-phenyl-pyrazole 1 (2 g, 20 mmol) in dimethylsulphoxide (20 mL), carbon disulfide (3.2 mL, 26 mmol) and aqueous sodium hydroxide (3 mL, 20 mmol) was added drop wise at room temperature. After 2.5 h stirring, dimethylsulfate (6.3 mL, 25 mmol) was added drop wise under cooling in an ice bath. Stirring was continued for 1.5 h. Then, the reaction mixture was poured into ice-cold water mixture. The precipitate obtained was filtered and dried. The residue was sticky semisolid and was triturated with petroleum ether several times until the compound became free flowing. The free flowing solid was recrystallised from boiling ethanol to give white needle like compound.

Yield 50%, mp: 152–154°C; IR (KBr) ν_{max} 3388 (–N–H), 3182 (C–H aromatic), 1642 (C=O), 1552 (C=N), 748 (=C–H oop) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.18 (s, 1H), 7.91 (m, 2H), 7.52 (m, 2H), 7.42 (m, 1H), 4.26 (t, $J = 4.12$ Hz, 2H), 2.67 (s, 3H), 1.78 (q, $J = 4.14$ Hz, 3H). MS *m/z*: 275 (M^+). Anal. Calc. for $C_{14}H_{15}N_3O_2S_2$: C, 52.33; H, 4.67; N, 13.08. Found: C, 52.55; H, 4.78; N, 13.10.

*Synthesis of 5-amino 6-mercapto-1-phenyl-pyrazolo[3,4-d]pyrimidine-4[3H]-one **3***

Solution of methyl-*N*-[4-carbethoxy-1-phenyl-pyrazolyl] dithiocarbamate **2** (2 g, 10 mmol) in ethanol (10 mL) was treated with hydrazine hydrate (99%) (1.2 g/3.0 mL, 100 mmol) and heated to reflux on water bath until the methyl mercaptan evolution ceased (8 h). The solution was immediately filtered under hot condition. The solid obtained was collected, dried, and recrystallised from boiling ethanol.

Yield 50%, mp: 259–260°C; IR (KBr) ν_{max} 3333, 3204 (–NH_2), 2957 (C–H aromatic), 1655 (–C=O), 1581 (C=C, aromatic), 754 (=C–H oop) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.14 (s, 1H), 8.02 (d, $J = 4.06$ Hz, 2H), 7.48 (t, $J = 4.06$ Hz, 2H), 7.30 (t, $J = 4.06$ Hz, 1H), 4.96 (s, 2H), 4.20 (s, 1H). MS *m/z*: 259 (M^+). Anal. Calc. for $C_{11}H_9N_5OS$: C, 50.96; H, 3.47; N, 27.02. Found: C, 51.08; H, 3.75; N, 27.04.

*Synthesis of 2-chloromethyl-8-phenyl-pyrazolo [3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidin-4(1H)-one **4***

Fusion of 5-amino 6-mercapto-1-phenyl-pyrazolo[3,4-*d*] pyrimidine-4[3*H*]-one 3 (1 g, 10 mmol) with chloro acetic

acid (0.070 g, 10 mmol) was carried out in presence of concentrated sulfuric acid by heating the mixture on a water bath for 5 h. The reaction mixture was cooled and poured into ice water. The resultant precipitate was filtered and dried. The compound was purified using column chromatography by gradient elution technique using chloroform and ethyl acetate as solvent system, the extract was subjected to rotary flash evaporator, and the pure compound was isolated.

Yield 75%, mp: 220–221°C; IR (KBr) ν_{max} 3174 (C–H aromatic), 2931(–C–H stretch), 1562 (–C=O), 1519 (C=N), 1238 (–CH_2Cl), 818 (aromatic C–H oop), 710 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.4 (s, 1H), 8.03 (d, J = 4.16 Hz, 2H), 7.54 (t, J = 4.16, 2H), 7.40 (t, J = 4.26, 1H), 4.92 (s, 2H). MS *m/z*: 317 (M^+). Anal. Calc. for $C_{13}H_8N_5OSCl$: C, 49.21; H, 2.52; N, 22.08. Found: C, 49.45; H, 2.70; N, 22.12.

General procedure for the synthesis of 2-alkyl/aryl/hetroarylaminomethyl-8-phenyl-pyrazolo[3,4-d][1,3,4]thiadiazolo[3,2-a]pyrimidin-4(1H)-ones ***5a–h***

A mixture of 8-phenyl-2-chloromethyl-pyrazolo[3,4-*d*][1,3,4] thiadiazolo[3,2-*a*] pyrimidin-4(1*H*)-one 4 (3.17 g, 10 mmol), triethylamine (1.01 mL, 12 mmol), and various amine (12 mmol) in dioxan was heated to reflux for 8 h. Then, the reaction mixture was cooled to room temperature and poured into ice-water mixture, and the excess amine was neutralized by dilute hydrochloric acid (10%). The resultant precipitate thus obtained was filtered, dried, and recrystallised from benzene.

*2-[{(Methyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a] pyrimidin-4(1H)-one (**5a**)* Yield 70%, mp: 269–271°C; IR (KBr) ν_{max} 3059 (C–H aromatic), 2926 (–C–H stretch), 1582 (–C=O), 1502 (C=N), 800 (aromatic C–H oop), 704 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.37 (s, 1H), 8.22 (s, 1H), 7.96 (d, J = 4.56 Hz, 2H), 7.50 (t, J = 4.56 Hz, 2H), 7.37 (t, J = 4.56 Hz, 1H), 4.78 (s, 2H), 2.37 (s, 3H). MS *m/z*: 312 (M^+). Anal. Calc. for $C_{14}H_{12}N_6OS$: C, 53.84; H, 3.84; N, 26.92. Found: C, 54.12; H, 4.10; N, 26.95.

*2-[{(Ethyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a]pyrimidin-4(1H)-one (**5b**)* Yield 75%, mp: 238–240°C; IR (KBr) ν_{max} 3059 (C–H aromatic), 2926 (–C–H stretch), 1582 (–C=O), 1502 (C=N), 800 (aromatic C–H oop), 704 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.38 (s, 1H), 8.23 (s, 1H), 7.96 (d, J = 4.12 Hz, 2H), 7.52 (m, 2H), 7.37 (t, J = 4.14 Hz 1H), 4.79 (s, 2H), 4.66 (q, J = 5.02 Hz, 2H), 2.18 (t, J = 5.06 Hz, 3H). MS *m/z*: 326 (M^+). Anal. Calc. for $C_{15}H_{14}N_6OS$: C, 55.21; H, 4.29; N, 25.76. Found: C, 55.48; H, 4.62; N, 25.76.

*2-[{(Hexyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a]pyrimidin-4(1H)-one (**5c**)* Yield 72%, mp: 252–254°C; IR (KBr) ν_{max} 3088 (C–H aromatic), 2918 (–C–H stretch), 1562 (–C=O), 1509 (C=N), 815 (aromatic C–H oop), 705 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.37 (s, 1H), 8.23 (s, 1H), 7.96 (d, J = 4.02 Hz, 2H), 7.52 (t, J = 4.02 Hz, 2H), 7.37 (t, J = 4.04 Hz, 1H), 4.79 (s, 2H), 4.66 (t, J = 5.42 Hz, 2H), 2.24 (m, 11H). MS *m/z*: 382 (M^+). Anal. Calc. for $C_{19}H_{22}N_6OS$: C, 59.68; H, 5.75; N, 21.98. Found: C, 59.75; H, 5.96; N, 22.02.

*2-[{(Furfuryl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a] pyrimidin-4(1H)-one (**5d**)* Yield 78%, mp: 228–230°C; IR (KBr) ν_{max} 3075 (C–H aromatic), 2922 (–C–H stretch), 1570 (C=O), 1509 (C=N), 744 (aromatic C–H oop), 705 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.36 (s, 1H), 8.22 (s, 1H), 7.95 (m, 3H), 7.50 (m, 3H), 7.35 (s, 2H), 4.79 (s, 2H), 4.65 (s, 2H). MS *m/z*: 378 (M^+). Anal. Calc. for $C_{18}H_{14}N_6O_2S$: C, 57.14; H, 3.70; N, 22.22. Found: C, 57.28; H, 3.88; N, 22.25.

*2-[(Morpholino)methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a]pyrimidin-4(1H)-one (**5e**)* Yield 65%, mp: 245–246°C; IR (KBr) ν_{max} 3074 (C–H aromatic), 2978 (–C–H stretch), 1591 (C=O), 1504 (C=N), 746 (aromatic C–H oop), 709 (–C–S) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.26 (s, 1H), 8.24 (m, 1H), 7.97 (m, 2H), 7.52 (m, 2H), 4.78 (s, 2H), 4.15 (d, J = 6.72 Hz, 4H), 3.24 (d, J = 6.74 Hz, 4H). MS *m/z*: 368 (M^+). Anal. Calc. for $C_{17}H_{16}N_6O_2S$: C, 55.43; H, 4.34; N, 22.82. Found: C, 55.65; H, 4.62; N, 22.86.

*2-[{(Phenyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a] pyrimidin-4(1H)-one (**5f**)* Yield 62%, mp: 233–234°C; IR (KBr) ν_{max} 3355 (=C–H aromatic), 3204 (–NH– stretch), 2929 (–CH stretch), 1664 (–NH bend), 1559 (C=O), 1504 (C=N), 817 (aromatic –CH oop) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.39 (s, 1H), 8.00 (d, J = 4.25 Hz, 2H), 7.50 (t, J = 4.25 Hz, 2H), 7.34 (t, J = 4.25 Hz, 1H), 7.12 (t, J = 7.10 Hz, 2H), 6.68 (d, J = 6.95 Hz, 1H), 6.51(d, J = 7.05 Hz, 2H), 4.74 (d, J = 6.72 Hz, 2H), 4.55 (t, J = 6.72 Hz, 1H). MS *m/z*: 374 (M^+). Anal. Calc. for $C_{19}H_{14}N_6OS$: C, 60.96; H, 3.74; N, 22.45. Found: C, 61.18; H, 3.86; N, 22.45.

*2-[{(m-Tolyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a] pyrimidin-4(1H)-one (**5g**)* Yield 70%, mp: 240–242°C; IR (KBr) ν_{max} 3378 (=C–H aromatic), 3081 (–NH– stretch), 2962 (–C–H stretch), 1684 (–NH bend), 1590 (C=O), 1504 (C=N) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 8.39 (s, 1H), 8.03 (d, J = 4.72 Hz, 2H), 7.50 (t, J = 4.72 Hz, 2H), 7.36 (t, J = 4.72 Hz, 1H), 7.24 (t, J = 5.02 Hz, 2H), 6.74 (d, J = 5.02 Hz, 2H), 4.72 (d,

$J = 6.76$ Hz, 2H), 4.53 (t, $J = 6.76$ Hz, 1H) 2.28 (S, 3H). MS *m/z*: 388 (M^+). Anal. Calc. for $C_{20}H_{16}N_6OS$: C, 61.85; H, 4.12; N, 21.64. Found: C, 62.08; H, 4.27; N, 21.75.

*2-[{(p-Anisyl)amino}methyl]-8-phenyl-pyrazolo[3,4-d][1,3,4] thiadiazolo[3,2-a] pyrimidin-4(1H)-one (**5h**)* Yield 70%, mp: 277–279°C; IR (KBr) ν_{max} 3375 (=C–H aromatic), 3078 (–NH– stretch), 2974 (–CH stretch), 1656 (–NH bend), 1599 (C=O), 1506 (C=N) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 8.39 (s, 1H), 8.02 (d, $J = 4.72$ Hz, 2H), 7.50 (t, $J = 4.74$ Hz, 2H), 7.34 (t, $J = 4.74$ Hz, 1H), 7.12 (t, $J = 5.88$ Hz, 2H), 6.68 (d, $J = 5.88$ Hz, 2H), 4.74 (d, $J = 6.72$ Hz, 2H), 4.55 (t, $J = 6.72$ Hz, 1H), 3.28 (s, 3H). MS *m/z*: 404 (M^+). Anal. Calc. for $C_{20}H_{16}N_6O_2S$: C, 59.40; H, 3.96; N, 20.79. Found: C, 59.62; H, 4.04; N, 20.85.

Antibacterial activity–Kirby Bauer's method

Peptone water was prepared and autoclaved. Four broth cultures were prepared using peptone water containing one type of organism each from stock cultures. A sterile cotton swab was dipped into one of the broth cultures and used to inoculate a Mueller–Hinton agar plate. Incubation of the plate in this way ensured a lawn of bacterial growth after incubation. Repeated this inoculation procedure for four plates from four different broth cultures and the plates were labeled. After inoculation, the plates were allowed to dry for 15 min before proceeding to the next step. Into 250 mL beaker, 70% ethanol was poured. The forceps was dipped into the alcohol and then passed the forceps over Bunsen burner flame to sterilize it. The standard antibiotic disk Ampicillin (0.005 mL, 50 μg) was picked up and placed it in the centre of the plate. The Whatmann filter paper disk impregnated with newly synthesized drugs (0.005 mL, 50 μg) was picked up and placed it in the corners. The procedure was repeated for eight plates for eight newly synthesized drugs and incubated for 18 h at 35°C. The plates were examined for zone of inhibition. They were measured with millimetre ruler across the disk. The diameter of the zone to the nearest whole millimetre was recorded. The disk impregnated with solvent (DMSO) and evaporated to dryness was used as negative control.

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