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Synthesis and Antimicrobial Screening of Novel 2-Thiopyrimidine Derivatives Bearing Pyrazole Moiety

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Abstract

2-Thiouracil-5-carbonitrile derivatives **2a,b** were fulfill starting material forsynthesis of the chloropyrimidines **3a,b** were prepared and then reacted with thiourea giving the pyrimidine dithione derivatives **4a,b**, which in turn were methylated yielding the methyl mercaptopyrimidines **5a,b**. Moreover, from **5a,b** a series of compounds **6a,b**, **7a,b** and **8a,b** were synthesized. All compounds were screened against select microorganisms to assess their antimicrobial activity. Compound **6a** demonstrated the best activity against all test microorganisms. In addition, compounds **4b**, **6a**, and **8b** showed the highest antibacterial activity. Thus, the three compounds were selected for a molecular docking study against the bacterial dihydropteroate synthase enzyme to which they revealed a good binding affinity.

Keywords: Thiopyrimidine-5-carbonitrile; antimicrobial; dihydropteroate synthase; molecular docking

1. Introduction

Searching for antimicrobial and antiviral medications is crucial since many infections have few or no therapeutic choices and are accompanied by antibiotic resistance. This emphasizes the necessity of creating novel medications that can be employed as antiviral and antibacterial treatments in addition to fighting resistant strains.[1] Due to its exceptional biological activity, thiouracil is regarded as one of the most well-known and frequently utilized thiopyrimidine compounds. The thiouracilcarbonitrile ring system is a wonderful paradigm for designing and synthesizing a novel class of chemotherapeutic compounds with promising pharmacological activity, according to findings in the literature.[1-3] In reality, triazolopyrimidine is the primary option of many researchers worldwide due to its various forms and strong action. Researchers have focused on developing novel compounds with anticancer, anti-inflammatory, antibacterial, antifungal, antiviral, anti-Alzheimer's, and antimalarial effects in a range of biochemical systems because of the interest in triazolopyrimidine structures.[4] First, because it interferes with the iodination of thyroxine precursors, Maloof and Soodak focused spot lights on it as an antithyroid agent.[5] Several research labs examined the anticancer potential of several thiouracils between 1964 and 2024.[6-14] Moreover, thiouracil derivatives demonstrated anti-infective properties, such as leishmanicidal, antibacterial, antifungal, and antiviral properties, in a number of communication papers.[15-17]

In addition to what has been mentioned about the medicinal value of thiouracils. Literature survey revealed that some 5-substituted thiouracils could act as MAO inhibitors in the brains of experimental animals.[18] 2-Thiouracil has a preventive effect on liver cirrhosis in rats,[18] it reduces the serum cholesterol levels in certain species of monkeys, and its effect in the treatment of angina pectoris and congestive heart failure has been investigated.[18]2-Thiouracil-5-carbonitriles are promising compounds that synthesized and evaluated especially in the last twenty years to have a diversity of biological activity as antibacterial, antifungal, antiviral, anticancer, antioxidant, antithyroid…etc.[18]Here we developed a program aimed to synthesize thiouracilcarbonitriles from the classic Biginilli condensation of three component condensation of aromatic aldehydes, thiourea and ethyl cyanoacetate hoping to have wide spectrum biological activities.

2. Results and discussion

2.1 Chemistry

The newly synthesized 2-thiouracil-5-cyano-6-pyrazole derivatives 2a,b were synthesized according to Biginelli condensation during one pot condensation involves of thiourea, aromatic aldehydes namely; 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde 1a or 1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde 1b and ethyl cyanoacetate in basic mediumof ethanolic potassium carbonate to afford 2a,b in satisfied yield. Different hypotheses have been proposed to explain the formation of these products. One acceptable mechanism involves the formation of aryeidene derivative between the aldehyde

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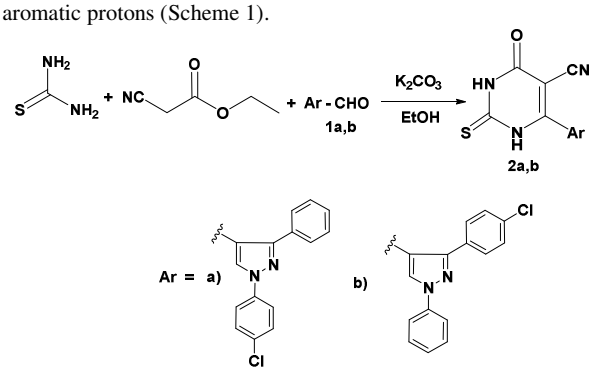
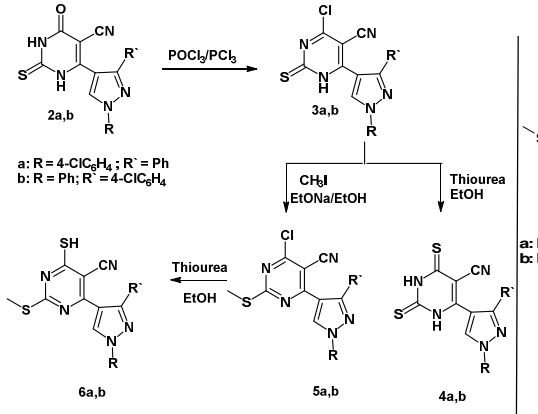
and ethyl cyanoacetate followed by the nucleophilic attack of thiourea to give a dihydropyrimidine intermediate followed by atmospheric oxidation to give a more stable pyrimidine derivative. The IR spectra of compounds 2a, as an example, showed well-defined absorption bands at ν 3319 , 3163 and 2230 cm-1 referring to 2NH and CN groups, beside absorption band at ν 1680 cm-1 for C=O group, its 1H NMR spectrum showed signals at δ 10.45, 11.36 ppm characteristic for 2NH groups (as a D2O exchangeable) beside to aromatic protons (Scheme 1).



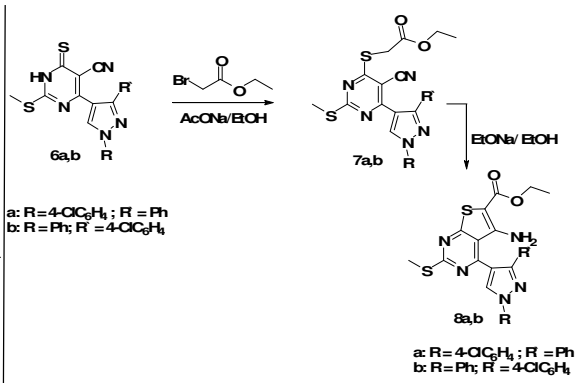
Scheme 1. Synthesis of (**2a,b**) by Biginelli condensation.

Thiouacil derivatives 2a,b were then chlorinated by a mixture of POCl3/PCl3 to give 4-chloropyrimidine derivatives 3a,b, their structures confirmed by IR spectrum which exhibited disappearance of two absorption bands for CO and NH groups of each compound. Chloropyrimidines 3a,b were heated with thiurea as a sulphurating agent producing dithioxopyrimidines 4a,b. 13C NMR spectrum of 4a displayed the presence of two C=S signals at δ 173.16, 178.20 ppm. Meanwhile, Chlorothiopyrimidines 3a,b could be S-alkylated by methyl iodide in basic medium to afford methyl thiopyrimidinethione **5a**, as an example, its structure confirmed by ^{1}H NMR which displayed the presence of a new singlet signal for methy protons at δ 2.13 ppm, and disappearance of signal corresponding to NH group. The latter *S*-methyl derivatives were sulphurated by thiourea to yield 4-mercapto-2-thiopyrimidines derivatives **6a,b**. Compounds **6a** showed the appearance of signal at δ 185.29 ppm in the ^{13}C NMR spectrum due to C=S group (Scheme 2).

In another pathway, compounds **6a,b** were 4-*S*-alkylated with ethylbromoacetate to yield primidines **7a,b**. Structure of **7a** for example, confirmed by IR spectrum, which showed a new absorption band at ν 1685 cm^{-1} for (CO) of ester group while, its ^{1}H NMR spectrum showed two signals of ester moiety at δ 1.47 and 4.18 ppm characteristic for the methyl and methylene groups. ^{13}C NMR spectrum showed a signal of (CO) groupat δ 167.12 ppm characteristic for the ester carbonyl group. Finally, cyclized by refluxing with sodium ethoxide in ethanol into thienopyrimidines **8a,b**. ^{1}H NMR spectrum of **8a**, as an example, showed the disappearance of the methylene signal and the appearance of exchangeable signal at δ 6.67 ppm NH_2 group confirmed the formation of product (Scheme 3).



Scheme 2. Synthesis of compounds from (**3a,b**) to (**6a,b**).



Scheme 3. Synthesis of compounds **(7a,b)** and **(8a,b).**

2.2 Antimicrobial assay results

2-Thiouracil itself has an antibiotic-like action against a variety of bacteria and microorganisms such as *Staphylococcus aureus, E. coli, L. arabinosus, L.casei, L. leichmannii* in addition to Influenza virus. It resembles thymine, cytosine and uracil which are the essential building blocks of nucleic acid DNA and RNA thus inhibiting nucleic acid of bacteria as an anti-metabolite. Several thiouracils were prepared and exhibited broad spectrum activities as antimicrobial agents. The cup diffusion method was used to assess the synthetic compounds antibacterial properties. The inhibition zone diameters (ZOI) of all newly synthesized compounds are depicted in (Table 1).

It has been found that compound 6a exhibited the highest antimicrobial activities against all test microbes with inhibition zone diameters of 18, 19, 17 and 21 mm for S. *aureus, E. coli, C. albicans* and *A. niger,* respectively. Compounds 4b, and 8b showed moderate antimicrobial activities with inhibition zone diameters of 20, 22, 20, and 23, 18, 12, mm for *S. aureus, E. coli* and *C. albicans*, respectively. While the rest of compounds doesn't showed a pleasant results (Figure 2). All tested compounds showed variable activity

against gram +ve & -ve bacteria. Compound **6a** exhibited comparable potent activity in respect to neomycin. It was active against *A. niger* in contrast to neomycin (Fig. 1 & Table 1).

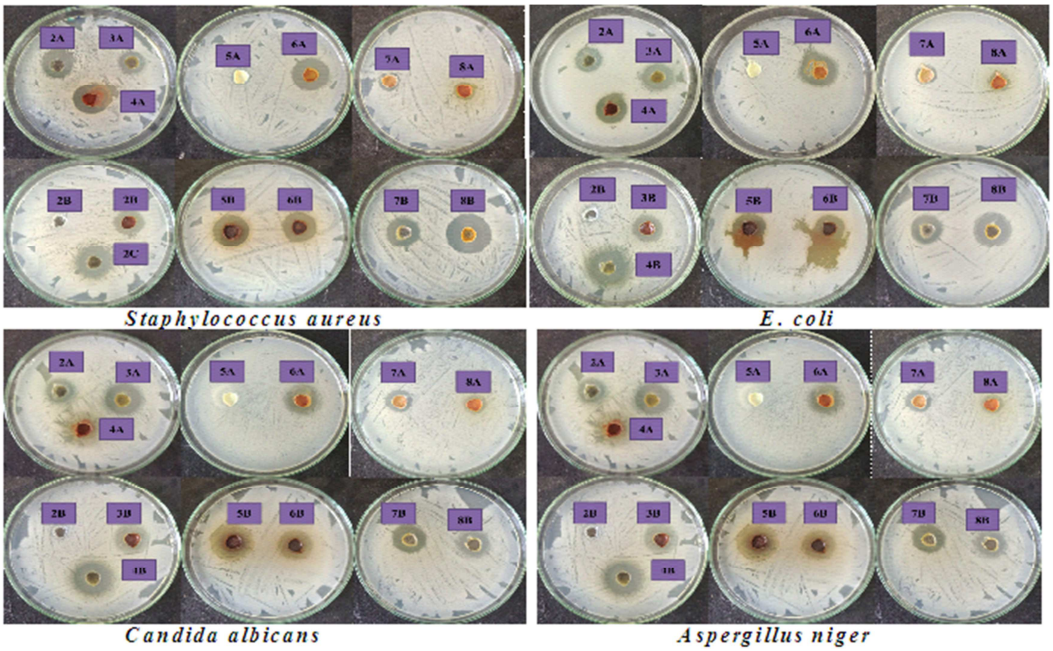


Figure 1: Estimation of antibacterial activity by Agar plate diffusion experiment carried on Staphylococcus aureus, E. coli, Candida albicans and Aspergillus niger with synthesized compounds Incubation conditions, 293°K, 24 h.

Table 1: Antimicrobial activity results the fourteen compounds against different Gram +ve, Gram -ve bacteria and fungi. *S. aureus, Staphylococcus aureus ATCC 6538-P; E. coli, Escherichia coli ATCC 25933; C. albicans, Candida albicans ATCC 10231 and A. niger, Aspergillus niger NRRL-A326; NA, not active.*

Compd. No.	***Gram +ve bacteria***	***Gram -ve bacteria***	***Fungi***	
	S. aureus	*E. coli*	*C. albicans*	*A. niger*
2a	17	14	12	16
2b	NA	NA	NA	NA
3a	13	15	17	25
3b	14	13	15	NA
4a	19	16	15	NA
4b	20	22	20	NA
5a	NA	NA	NA	NA
5b	18	14	23	NA
6a	18	19	17	21
6b	16	13	15	NA
7a	10	8	10	NA
7b	15	14	14	16
8a	10	NA	8	NA
8b	23	18	12	NA
Neomycin	25	21	27	NA
Cyclohexamide	NA	NA	NA	33

2.3 Minimum inhibitory concentration evaluation Compound **6a** was discovered to have the strongest antibacterial activity against microorganisms. Thus, the minimum concentrations (MIC), demanded to limit the growth was studied for **6a** showed potent values as determined and tabulated (Table 2).

Table 2: MIC of the most active compound by agar diffusion method

Compd. No.	Concentration	S. aureus	E. coli	C. albicanc	A. niger
6a	5 (µg/mL)	3.10 ± 0.17	6.20 ± 0.12	5.00 ± 0.10	6.70 ± 0.26
	10 (µg/mL)	8.53 ± 0.29	14.50 ± 0.29	12.70 ± 0.45	18.10 ± 0.60

2.4 Molecular docking studies

The dihydropteroate synthase (DHPS) enzyme catalyzes the formation of dihydropteroic acid from *p*-aminobenzoic acid (PABA), a key step in the synthesis of folic acid, which is essential for nucleic acid synthesis in bacteria. The enzyme is also the target of the sulfonamide antibacterial agents and upon binding to it, sulfonamides competitively inhibit folate synthesis leading to a bacteriostatic effect.[17-19] Among the *in vitro* screened compounds, three with the best activity against *E. coli* and *S. aureus*, **4b**, **6a**, and **8b**, were selected for a molecular docking study against the DHPS of each to assess their binding affinities. To validate the virtual procedures, the co-crystallized ligand to each enzyme was separated and docked into its active site, and the RMSD value versus the native co-crystallized ligand was calculated. The procedures were then implemented to dock compounds **4b**, **6a**, and **8b** into the active sites of both enzymes.

Detailed molecular docking results against *E. coli* DHPS are presented in (Table 3 and Fig. 2). The docked co-crystallized ligand (2PH) exhibited a binding energy of -8.3 kcal/mol and an RMSD value of 0.356 versus the native co-crystallized one. The docked ligand interacted with the active site via a pi-pi stacking between the pterin moiety and PHE190, four hydrogen bonds between the pterin polar functionalities and ASN115, ASP185, and LYS221, and four hydrogen bonds between the diphosphate group and THR62, ASN22, ARG255, and HIS257 (Table 3 and Fig. 2A). All the interactions were the same as those of the native ligand except for an additional hydrogen bond between the diphosphate of the native ligand and ASN22. The binding energies of compounds **4b**, **6a**, and **8b** to the enzyme were relatively higher than that of the co-crystallized ligand and equal to -7.4, -7.7, and -6.7 kcal/mol, respectively (Table 3). While the chlorine atom of both **4b** (Fig. 2B) and **8b** (Fig. 2D) possessed electrostatic interactions (i.e., halogen bonds) with both ASN22 and ARG255, the nitrile and pyrimidine moieties of **6a** (Fig. 2C) possessed hydrogen bonds with THR62 and LYS221, respectively.

Table 3: Molecular docking results against *E. coli* DHPS.

Ligand Name	Binding Energy (kcal/mol)	Binding Interaction Type	Binding Groups	
			Ligand	Residue
2PH[a]	-8.3	Pi-pi stacking	Pterin Ring	PHE190 Ring
		Hydrogen bond	Pterin NH2	ASN115 C=O
		Hydrogen bond	Pterin NH2	ASP185 COO-
		Hydrogen bond	Pterin NH	ASP185 COO-
		Hydrogen bond	Pterin N	LYS221 NH3+
		Hydrogen bond	Diphosphate O	THR62 OH
		Hydrogen bond	Diphosphate O-	ASN22 NH2
		Hydrogen bond	Diphosphate O-	ARG255 NH2
		Hydrogen bond	Diphosphate C=O	HIS257 NH
4b	-7.4	Halogen bond	Cl	ASN22 NH2
		Halogen bond	Cl	ARG255 NH2, NH2+
6a	-7.7	Hydrogen bond	C≡N	THR62 NH
		Hydrogen bond	Pyrimidine N	LYS221 NH3+
8b	-6.7	Halogen bond	Cl	ASN22 NH2
		Halogen bond	Cl	ARG255 NH2+

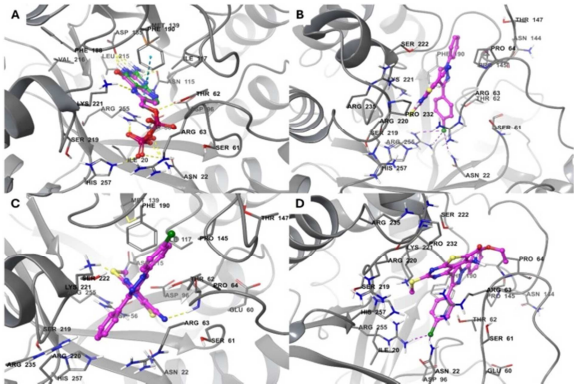


Figure 2: Binding modes to *E. coli* DHPS. (A) Native co-crystallized ligand (green) versus docked ligand (magenta). (B) Compound **4b.** (C) Compound **6a**. (D) Compound **8b**. Docked ligands are shown as magenta balls and sticks while active site residues are shown as grey tubes. Hydrogen bonds, pi-pi stackings, and halogen bonds are displayed as yellow, cyan, and purple dashed lines, respectively.

The *S. aureus* DHPS docking results are illustrated in (Table 4 and Fig. 3). The docked co-crystallized ligand (HH2) had a binding energy of -6.3 kcal/mol and an RMSD value of 1.901 relative to the native co-crystallized one. The interactions of the docked ligand to the active site involved: (i) a pi-pi stacking and (ii) a pi-cation interaction of the pterin moiety with PHE172 and ARG239, respectively, (iii) three hydrogen bonds of the pterin polar groups with ASP84, ASN103, and LYS203, (iv) two hydrogen bonds between the diphosphate moiety and ARG52 and HIS241 (Table 4 and Fig. 3A). The diphosphate also coordinated with the manganese ion (i.e., metal coordination) in the enzyme active site. Although the interactions were similar to those performed by the native co-crystallized

ligand, the interacting groups or residues sometimes varied. Compounds **4b**, **6a**, and **8b** exhibited lower binding energies of -7.0, -7.7, and -6.7 kcal/mol, respectively, than that of the co-crystallized ligand (Table 4). Compound **4b** performed a halogen bond, hydrogen bond, and metal coordination between its chlorine atom and LYS203, nitrile group and HIS241, and pyrazole nitrogen and the manganese ion, respectively (Fig. 3B). Similarly, the nitrile group of compound **6a** showed a hydrogen bond between its nitrile moiety and HIS241 (Fig. 3C). Compound **8b**. Docked ligands are shown as magenta balls and sticks, active site residues as grey tubes, and the manganese ion in violet. Hydrogen bonds, pi-pi stackings, pi-cation interactions, and halogen bonds are displayed as yellow, cyan, green, and purple dashed lines, respectively.

Table 4: Molecular docking results against *S. aureus* DHPS

Ligand Name	Binding Energy (kcal/mol)	Binding Interaction Type	Binding Groups	
			Ligand	Residue
HH2[a]	-6.3	Pi-pi stacking	Pterin Ring	PHE172 Ring
		Pi-cation interaction	Pterin Ring	ARG239 NH2+
		Hydrogen bond	Pterin NH2	ASP84 COO-
		Hydrogen bond	Pterin NH2	ASN103 C=O
		Hydrogen bond	Pterin C=O	LYS203 NH3+
		Hydrogen bond	Diphosphate O	ARG52 NH
		Hydrogen bond	Diphosphate O-	HIS241 NH
		Metal coordination	Diphosphate C=O	MN269 (Mn2+)
4b	-7.0	Halogen bond	Cl	LYS203 NH3+
		Hydrogen bond	C≡N	HIS241 NH
		Metal coordination	Pyrazole N	MN269 (Mn2+)
6a	-7.7	Hydrogen bond	C≡N	HIS241 NH
8b	-6.7	Nil	Nil	Nil

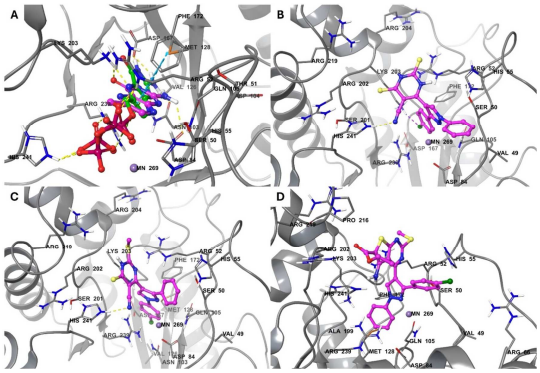


Figure 3: Binding modes to *S. aureus* DHPS. **(A)** Native co-crystallized ligand (green) versus docked ligand (magenta). (B) Compound **4b**. (C) Compound **6a**. (D) [a] Docked co-crystallized ligand However, compound **8b** did not display significant interactions with the active site residues other than hydrophobic ones (Fig. 3D).

2.4 Structure activity relationship

Structure activity relationship of our target compounds are substituted 2-thiouracil and are expected to have antimicrobial activity due to: the presence of 2-mercapto group which when introduced to many heterocyclic rings sustains the antimicrobial activity (Fig. 4). The tautomeric amid-imidol at position 4 gives 2-thiouracil a phenolic antimicrobial activity. The presence of cyano group at the5th position in drug design modulates the physicochemical and pharmacokinetic properties of drugs by improving bioavailability, selectivity and binding affinity to receptors by hydrogen bond interactions, covalent interaction, and polar interaction….etc. Incorporation of a pyrazole ring at 6th position which modulate the antimicrobial activity as DNA gyrase inhibitor active against gram +ve and gram -ve bacteria.

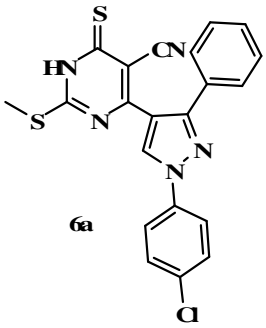


Figure 4: Similarity of the structures of thymine, cytosine and pyrimidine (**6a**).

3. Experimental

3.1 Chemistry

All melting points are uncorrected and were determined in capillary tube on a Boetius melting point microscope. Microanalyses were performed by the micro analytical unit at Cairo University. IR spectra were recorded as KBr pellets on a Beckmann infra spectrophotometer PU9712 using KBr discs. ^{1}HNMR spectra were determined on a Joel EX 270 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Finigan SSQ 7000 Mass spectrometer at 70 ev. All reactions were followed and checked by TLC using Chloroform/Methanol (3:1) and spots were examined under a UV-lamp.

Synthesis of 6-[1-aryl-3-phenyl-1H-pyrazol-4-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (2a,b)

A mixture of the appropriate aldehydes namely; 3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde **1a** or 1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde **1b** (2.8g, 0.01 mol), ethylcyanoacetate (1.1g, 0.01 mol), thiourea (0.76g, 0.01 mol) and potassium carbonate (0.01 mol) in 25 ml absolute ethanol was refluxed for 24 hour. The precipitate which formed after cooling and acidification was filtered off, dried and recrystallized from butanol to give **2a** and **2b** respectively.

6-[1-(4-Chlorophenyl)-3-phenyl-1H-pyrazol-4-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (2a). Yield: 71 %; Color: light yellow; m.p. 205-207 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3319, 3163 (2NH), 2230 (CN), 1680 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.56-8.01 (m, 5H), 7.56 (d, 2H, J = 8.1 Hz), 7.92 (d, 2H, J = 7.6 Hz), 8.32 (s, 1H), 10.45 (brs, 1H), 11.36 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 81.48, 113.53, 121.47, 124.58, 127.12, 128.12, 128.70, 128.95, 129.02, 130.16, 148.75, 152.17, 153.21, 154.77, 174.70, 185.27; MS (*m/z*): M$^+$405 (10.6 %). Anal. Calcd for $C_{20}H_{12}ClN_5OS$: C, 59.19; H, 2.98; N, 17.26; Found: C, 59.33; H, 2.78; N, 17.38.

6-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (2b). Yield: 73 %; Color: yellow crystals; m.p. 215-217 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3320, 3185 (2NH), 2235 (CN), 1687 (C=O); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.56-8.16 (m, 5H), 7.62 (d, 2H, J = 8.4 Hz), 7.88 (d, 2H, J = 7.8 Hz), 7.67 (s, 1H), 10.32 (brs, 1H), 11.38 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 88.29, 109.84, 110.06, 115.32, 118.77, 120.66, 120.89, 128.72, 128.77, 128.91, 130.03, 138.37, 140.09, 144.74, 151.79, 153.11, 171.76, 182.01; MS (*m/z*): M$^+$405(12.3 %); Anal. Calcd for $C_{20}H_{12}ClN_5OS$: C, 59.19; H, 2.98; N, 17.26. Found: C, 59.36; H, 2.77; N, 17.76.

Synthesis of 4-chloro-6-aryl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (3a,b)

A mixture of **2a** or **2b** (3.8g, 0.0095 mol), $POCl_3$ (44g, 0.29 mol) and PCl_3 (2g, 0.015 mol) was refluxed on a water bath for 6 hrs. The reaction mixture was poured gradually on crushed ice and the solid that separated was filtered off, dried and recrystallized from DMF/water to give **3a** and **3b**, respectively.

4-Chloro-6-(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (3a). Yield: 75 %; Color: yellow crystals; m.p. 241-243 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3325 (NH), 2228 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.61 (d, 2H, J = 8.4 Hz), 7.92 (d, 2H, J = 8.1 Hz), 7.56-8.01 (m, 5H), 8.32 (s, 1H); 11.61 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 90.23, 120.85, 125.73, 126.46, 127.16, 127.91, 128.18, 128.73, 128.84, 128.93, 130.16, 152.25, 175.82; MS (*m/z*): M$^+$424 (7.34 %), M^{+2} 426 (2.44 %); Anal. Calcd for $C_{20}H_{11}Cl_2N_5S$: C, 56.61; H, 2.61; N, 16.51. Found: C, 56.77; H, 2.52; N, 16.67.

4-Chloro-6-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (3b). Yield: 70 %; Color: yellow crystals; m.p. 228-230 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3434 (NH), 2235 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.62 (d, 2H, J = 8.5 Hz), 7.93 (d, 2H, J = 7.9 Hz), 7.56-8.16 (m, 5H), 8.32 (s, 1H), 10.73 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 94.75, 127.15, 127.44, 128.15, 128.56, 128.72, 128.97, 130.08, 130.18, 149.71, 151.28, 153.26, 185.30; MS (*m/z*): M$^+$424 (9.32 %), M^{+2} 426 (3.10 %); Anal. Calcd for $C_{20}H_{11}Cl_2N_5S$: C, 56.61; H, 2.61; N, 16.51. Found: C, 56.77; H, 2.48; N, 16.39.

Synthesis of 6-aryl-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (4a,b)

To a solution of chloropyrimidine **3a** or **3b** (0.5g, 0.0012) in ethanol (25 mL), thiourea (0.9g, 0.012 mol) was added and the reaction mixture was heated under reflux for 10 hr. The solid obtained after cooling was filtered off, dried and recrystallized from DMF/water to give **4a** and **4b**, respectively.

6-(1-(4-Chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (4a). Yield: 69 %; Color: light yellow; m.p. 240-242 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3328, 3274 (2NH), 2227 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.61(d, 2H, J = 7.8 Hz), 7.74 (d, 2H, J = 7.5 Hz), 7.52-7.94 (m, 5H), 8.09 (s, 1H), 10.49 (brs, 1H), 11.29 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 98.85, 120.69, 126.35, 127.17, 127.26, 127.77, 128.18, 128.51, 128.84, 130.16, 133.27, 151.99, 173.16, 178.20; MS (*m/z*): M$^+$421 (21.21 %); Anal. Calcd for $C_{20}H_{12}ClN_5S_2$: C, 56.93; H, 2.87; N, 16.60. Found: C, 56.80; H, 2.68; N, 16.48.

6-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2,4-dithioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (4b). Yield: 70 %; Color: light yellow; m.p. 238-240 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 3335, 3288 (2NH), 2225 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.49 (d, 2H, J = 8.5 Hz), 7.88 (d, 2H, J = 8.2 Hz), 7.31-7.79 (m, 5H), 7.93 (s, 1H), 10.91 (brs, 1H), 14.11 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 120.87, 125.72, 126.46, 127.17, 127.92, 128.19, 128.73, 128.84, 128.95, 130.19, 152.26, 169.23, 175.83; MS (*m/z*): M$^+$421 (25.23 %); Anal. Calcd for $C_{20}H_{12}ClN_5S_2$: C, 56.93; H, 2.87; N, 16.60. Found: C, 56.77; H, 2.64; N, 16.51.

Synthesis of 4-chloro-2-(methylthio)-6-arylpyrimidine-5-carbonitrile (5a,b)

A solution of **3a** or **3b** (4.2g, 0.01 mol) and methyl iodide (2g, 0.015 mol) in ethanolic sodium ethoxide (2g, Na metal in 25 mL absolute ethanol) was refluxed for 2hr. The reaction mixture was cooled and poured onto ice-cold water. The solid obtained after cooling and acidification with HCl (dil.), filtered off, and recrystallized from DMF/water to give **5a** and **5b**, respectively.

4-Chloro-6-(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-2-(methylthio)pyrimidine-5-carbonitrile (5a). Yield: 75 %; Color: darkorange; m.p. 254-256 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 2229 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.13 (s, 3H), 7.62 (d, 2H, J = 8.2 Hz), 8.01 (d, 2H, J = 7.7 Hz), 7.60-8.03 (m, 5H), 8.49 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 21.53, 121.47, 124.53, 127.12, 128.12, 128.70, 128.95, 129.02, 130.16, 148.75, 152.17, 153.21, 154.77; MS (*m/z*): M$^+$437 (14.22 %), M^{+2} 439 (4.74 %); Anal. Calcd for $C_{21}H_{13}Cl_2N_5S$: C, 57.54; H, 2.99; N, 15.98. Found: C, 57.38; H, 2.82; N, 15.79.

4-Chloro-6-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(methylthio)pyrimidine-5-carbonitrile (5b). Yield: 72 %; Color: light yellow; m.p. 264-266 ^{o}C; IR (KBr, υ_{max}, cm^{-1}): 2225 (CN); ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.02 (s, 3H), 7.37 (d, 2H, J = 7.9 Hz), 7.89 (d, 2H, J = 7.6 Hz), 7.16-7.65 (m, 5H), 7.90 (s, 1H); ^{13}C NMR (500 MHz, DMSO- d_6): δ 28.33, 120.84, 125.68, 126.46, 127.14, 127.91, 128.18, 128.71, 128.83, 128.97, 130.16, 152.23; MS (*m/z*): M$^+$437.54 (11.22 %), M^{+2} 439 (3.74 %); Anal. Calcd for

$C_{21}H_{13}Cl_2N_5S$: C, 57.54; H, 2.99; N, 15.98. Found: C, 57.44; H, 2.71; N, 15.73.

Synthesis of 4-mercapto-2-(methylthio)-6-aryl pyrimidine-5-carbonitrile (6a,b)

Amixture of **5a** or **5b** (0.5g, 0.0012 mol) and thiourea (0.09g, 0.0012 mol) in ethanol (25 mL) was refluxed for 10 hr, the solid obtained after cooling was filtered off, dried and recrystallized from DMF/water to give **6a** and **6b**, respectively.

4-(1-(4-Chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-2-(methylthio)-6-thioxo-1,6-dihydropyrimidine-5-carbonitrile (6a). Yield: 70 %; Color: yellow crystals; m.p. 248-250 ^oC; IR (KBr, υ_{max}, cm^{-1}): 3234 (NH), 2220 (CN); ^1H NMR (500 MHz, DMSO-d_6): δ 1.93 (s, 3H), 7.00 (d, 2H, *J* = 8.5 Hz), 7.55 (d, 2H, *J* = 8.2 Hz), 7.96-8.02 (m, 5H), 8.03 (s, 1H), 10.17 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 34.72, 121.47, 126.60, 127.13, 127.44, 128.14, 128.58, 128.73, 128.99, 130.17, 135.74, 153.25, 185.29; MS (*m/z*): M^+435 (17.27 %), M^{+2} 437 (5.75 %); Anal. Calcd for $C_{21}H_{14}ClN_5S_2$: C, 57.86; H, 3.24; N, 16.06. Found: C, 57.91; H, 3.36; N, 16.12.

4-(3-(4-Chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(methylthio)-6-thioxo-1,6-dihydropyrimidine-5-carbonitrile (6b). Yield: 71 %; Color: yellowish; m.p. 262-264 ^oC; IR (KBr, υ_{max}, cm^{-1}): 3367 (NH), 2217 (CN); ^1H NMR (500 MHz, DMSO-d_6): δ 2.46 (s, 3H), 7.06 (d, 2H, *J* = 8.3 Hz), 7.37 (d, 2H, *J* = 7.9 Hz), 7.29-7.64 (m, 5H), 7.92 (s, 1H), 14.08 (brs, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 25.29, 125.42, 131.13, 132.00, 132.54, 132.95, 133.30, 133.59, 134.92, 156.72, 182.99; MS (*m/z*): M^+435 (33.37 %), M^{+2} 437 (14.45 %); Anal. Calcd for $C_{21}H_{14}ClN_5S_2$: C, 57.86; H, 3.24; N, 16.06. Found: C, 57.83; H, 3.33; N, 16.15.

Synthesis of ethyl{[5-cyano-2-(methyl thio)-6-aryl-5,6-dihydropyrimidin-4-yl] thio} acetate (7a,b)

A mixture of **6a** or **6b** (0.8g, 0.0019 mol), sodium acetate (0.035 mol) and ethylbromoacetate (0.3g, 0.0019 mol) in ethanol (20 mL) was refluxed for 3hr, the precipitate that formed on cooling was filtered off and recrystallized from DMF/water to give **7a** and **7b**, respectively.

Ethyl 2-(6-(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-5-cyano-2-(methylthio)pyrimidin-4-ylthio)acetate (7a). Yield: 75 %; Color: light brown crystal; m.p. 253-255 ^oC; IR (KBr, υ_{max}, cm^{-1}): 2221 (CN), 1685 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 1.47 (t, 3H, *J* = 7.3 Hz), 2.37 (s, 3H), 4.18 (q, 2H, *J* = 7.3 Hz), 4.83 (s, 2H), 7.53 (d, 2H, *J* = 8.1 Hz), 7.94 (d, 2H, *J* = 7.7 Hz), 7.57-8.01 (m, 5H), 8.48 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 19.40, 31.19, 53.20, 64.01, 101.14, 120.73, 126.36, 127.18, 127.78, 128.19, 128.55, 128.84, 130.17, 133.41, 149.74, 152.01, 154.08, 167.12; MS (*m/z*): M^+521 (18.32 %), M^{+2} 523 (6.10 %); Anal. Calcd for $C_{25}H_{20}ClN_5O_2S_2$: C, 57.52; H, 3.86; N, 13.42. Found: C, 57.44; H, 3.72; N, 13.32.

Ethyl 2-(6-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-cyano-2-(methylthio)pyrimidin-4-ylthio)acetate (7b). Yield: 71 %; Color: light yellow; m.p. 273-275 ^oC; IR (KBr, υ_{max}, cm^{-1}): 2219 (CN), 1697 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 1.06 (t, 3H, *J* = 7.2 Hz), 1.97 (s, 3H), 4.23 (q, 2H, *J* = 7.1 Hz), 5.27 (s, 2H), 7.18 (d, 2H, *J* = 8.1 Hz), 7.55 (d, 2H, *J* = 7.7 Hz), 7.51-7.87 (m, 5H), 7.88 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 19.41, 32.72, 52.01, 63.42, 99.84, 121.05, 126.85, 126.97, 127.14, 128.13, 128.20, 128.55, 128.97, 130.07, 149.70, 152.22, 164.02; MS (*m/z*): M^+521 (17.92 %), M^{+2} 523 (5.12 %); Anal. Calcd for $C_{25}H_{20}ClN_5O_2S_2$: C, 57.52; H, 3.86; N, 13.42. Found: C, 57.67; H, 3.65; N, 13.35.

Synthesis of ethyl-5-amino-2-(methylthio)-4-aryl-4,4a-dihydrothieno[2,3-*d*] pyrimidine-6-carboxylate (8a,b)

To a solution of **7a** or **7b** (0.002 mol) in absolute ethanol (20 mL), sodium ethoxide solution (50 mg sodium in 25 mL absolute ethanol) was added drop wise and the reaction mixture was heated. The solid that formed while hot was collected and the reaction mixture was heated under reflux for 30 min., cooled, filtered off and re-crystallized from DMF/water to give **8a** and **8b**, respectively.

Ethyl 5-amino-4-(1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-4-yl)-2-(methylthio)thieno[2,3-d]pyrimidine-6-carboxylate (8a). Yield: 67 %; Color: yellowish grey; m.p. 285-287 ^oC; IR (KBr, υ_{max}, cm^{-1}): 3319 (NH_2), 1683 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 1.42 (t, 3H, *J* = 7.4 Hz), 2.19 (s, 3H), 4.21 (q, 2H, *J* = 7.3 Hz), 6.67 (brs, 2H), 7.34-8.14 (m, 5H), 7.41 (d, 2H, *J* = 8.4 Hz), 7.75 (d, 2H, *J* = 7.8 Hz), 8.33 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 21.67, 34.22, 65.25, 94.74, 121.06, 127.00, 127.13, 128.13, 128.51, 128.95, 130.09, 130.43, 133.35, 148.55, 152.08, 166.65; MS (*m/z*): M^+521 (18.32 %), M^{+2} 523 (5.12 %); Anal. Calcd for $C_{25}H_{20}ClN_5O_2S_2$: C, 57.52; H, 3.86; N, 13.42. Found: C, 57.68; H, 3.74; N, 13.33.

Ethyl 5-amino-4-(3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-2-(methylthio)thieno[2,3-d]pyrimidine-6-carboxylate (8b). Yield: 65 %; Color: light brown crystal; m.p. 270-272 ^oC; IR (KBr, υ_{max}, cm^{-1}): 3378 (NH_2), 1688 (C=O); ^1H NMR (500 MHz, DMSO-d_6): δ 1.06 (t, 3H, *J* = 7.2 Hz), 2.00 (s, 3H), 4.13 (q, 2H, *J* = 7.1 Hz), 6.42 (brs, 2H), 7.30 (d, 2H, *J* = 8.5 Hz), 7.47 (d, 2H, *J* = 7.8 Hz), 6.92-7.11 (m, 5H), 7.93 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 19.23, 33.95, 65.02, 95.86, 121.09, 126.91, 127.13, 128.13, 128.51, 128.95, 130.09, 130.43, 133.35, 148.55, 152.08. 21.67, 34.22, 65.25, 94.74, 121.06, 127.03, 127.17, 128.17, 128.51, 128.94, 130.10, 133.33, 149.70, 152.20, 168.50; MS (*m/z*): M^+521 (22.72 %), M^{+2} 523 (18.62 %); Anal. Calcd for $C_{25}H_{20}ClN_5O_2S_2$: C, 57.52; H, 3.86; N, 13.42. Found: %. C, 57.66; H, 3.65; N, 13.57.

4. Conclusions

A series of novel 2-thiopyrimidine derivatives bearing a pyrazole moiety were successfully synthesized and characterized starting from the classic Biginelli condensation reaction. All compounds showed variable activity against gram +ve & gram -ve bacteria and some showed activity against fungi. Compound **6a** exhibited comparable potent activity to neomycin. It was also active against *A. niger* in contrast to neomycin. Compounds **4b**, **6a**, and **8b** displayed similar binding affinities to the *E. coli* and *S. aureus* DHPS. The order of the compounds in terms of binding affinity to both enzymes was found to be: **6a > 4b > 8b**.

5. Conflicts of interest

There are no conflicts to declare.

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