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Short communication

Synthesis and antimicrobial activity of novel pyrazolo[3,4-*d*]pyrimidin derivatives

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ABSTRACT

Pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one derivatives have been prepared by cyclocondensation of ethyl 2-cyano-3,3-bis(methylthio)prop-2-enoate with 2-amino-4-(substitutedphenyl)thiazole to give 3-cyano-2-methylthio-4-oxo-4*H*-6-(substitutedphenyl)thiazolo[3,2-*a*]pyrimidin (**2a**–**j**) and further reacting with hydrazine hydrate to yield the target compounds (**3a**–**j**). The chemical structure of the compounds was confirmed by IR and ^{1}H NMR spectral data. All the compounds of the series have been screened for their antibacterial and antifungal activity studies. The result revealed that all compounds showed significant antimicrobial activity.

1. Introduction

Emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global problem. Therefore, a substantial research for the discovery and synthesis of new classes of antimicrobial agents is needed [1,2].

Pyrazolopyrimidine and related heterocycles are found to possess a wide application in the field of medicine and agriculture. They exhibit diversified pharmacological activities like CNS depressant [3], neuroleptic [4], tuberculostatic [5], antihypertensive [6], antileishmanial [7], analgesic [8] and antimicrobial activities [9]. Some of the pyrazolopyrimidine derivatives are known to inhibit enzymes such as xanthine oxidase [10]. The current research is being directed towards the synthesis and improvement of biological activity of the pyrazolopyrimidine derivatives [11]. The literature survey reveals that replacement of 1H-pyrazole of pyrazolo[3,4-*d*]pyrimidine ring system by other bioactive moieties drastically alters its pharmacological properties [12]. Prompted by the varied biological activities of pyrazolopyrimidine derivatives, we envisioned our approach towards the synthesis and antimicrobial screening of a novel series of pyrazolo[3,4-*d*]pyrimidine derivatives.

2. Experimental

2.1. Materials and methods

All the chemicals used in the present study are of analytical grade purchased from Himedia chemical Co. TLC was run on the silica gel–G coated glass plates and visualized by iodine vapors. The IR spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer using KBr pellets. ^{1}H NMR spectra were obtained by using AVANCE 300 MHz spectrophotometer in DMSO-d_6 using TMS as an internal standard respectively. Melting points of synthesized compounds were determined by a Kofler micro melting point apparatus and were uncorrected.

2.2. Synthesis of 3-cyano-2–methylthio-4-oxo-4H–6-(substitutedphenyl)thiazolo[3,2-a] pyrimidin (*2a–j*)

A reaction mixture of equimolar quantities of 2-amino-4-(substitutedphenyl)thiazolo (1 mmol) (**1a**–**j**) and ethyl 2-cyano-3,3-bis(methylthio)prop-2-enoate (1 mmol) in 20 mL of dry DMF and 5 mL of triethylamine was refluxed for 3 h to obtain compounds **2a**–**j**. Progress of the reaction was monitored by TLC. The solvent was removed *in vacuo* and the residue was washed with diethylether

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Table 1
Spectral analysis of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one derivatives.

Name of compound	Spectral data
3–amino-6-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*d*] thiazolo[3, 2-*a*]pyrimidin-4-one (**3a**)	Yield-65%; M.P. 204 °C; IR (KBr υ max/cm^{-1}): 3455(–NH), 3340(NH_2), 3320 (–NH), 1660 (C═O), 1605 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.60 (bs, 2H, NH_2), 7.1 (s, 1H, 5H-thiazole), 7.31–7.64(m, 4H, Ar-H), 8.6 (s, 1H, NH).
3–amino-6-(2-hydroxyphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo [3,2–*a*]pyrimidin-4-one (**3b**)	Yield-66%; M.P. 215 °C; IR (KBr υ max/cm^{-1}): 3460(–NH), 3330 (–NH_2), 3316 (–NH), 3210(–OH), 1648 (C═O), 1608 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.85 (bs, 2H, NH_2), δ 7.08 (s, 1H, 5H-thiazole), 7.36–7.72 (m, 4H, Ar-H), 8.4 (s, 1H, NH), 11.56 (s,1H, OH)
3–amino-6-(5-chloro-2-hydroxyphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2–*a*]pyrimidin-4-one (**3c**)	Yield-64%; M.P. 220 °C; IR (KBr υ max/cm^{-1}): 3460(–NH),3442 (–NH_2), 3192 (–OH), 1649 (C═O), 1613 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.91 (bs, 2H, NH_2), 7.15 (s, 1H, 5H-thiazole), 7.31 (d, 1H, Ar-H), 7.54 (d, 1H, Ar-H),7.82 (s, 1H, Ar-H), 8.36(s, 1H, NH), 11.46 (s, 1H, OH).
3–amino-6-(3-bromo-5-chloro-2-hydroxyphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2–*a*]pyrimidin-4-one (**3d**)	Yield-65%; M.P. 195 °C; IR (KBr υ max/cm^{-1}): 3450(–NH), 3440(–NH_2), 3327.03(–NH), 3185 (–OH), 1655 (C═O), 1608 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.83 (bs, 2H, NH_2), 7.06 (s, 1H, 5H-thiazole), 7.50 (s, 1H, Ar-H), 7.81 (s, 1H, Ar-H), 8.51 (s, 1H, NH), 11.54 (s, 1H, OH).
3–amino-6-(4-nitrophenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo [3,2–*a*]pyrimidin-4-one (**3e**)	Yield-66%; M.P. 202 °C; IR (KBr υ max/cm^{-1}): 3442(–NH), 3398(–NH_2), 2098 (N═O), 1658 (C═O), 1610 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.85 (bs, 2H, NH_2), 7.12 (s, 1H, 5H-thiazole), 7.38–7.86 (m, 4H, Ar-H), 8.36 (s, 1H, NH).
3–amino-6-(4-bromophenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo [3,2–a]pyrimidin-4-one (**3f**)	Yield-6; M.P. 202 °C; IR (KBr υ max/cm^{-1}): 3432 (NH), 3340.15 (NH_2), 3210 (OH), 1660 (C═O), 1609 (C═N); ^{1}H NMR (DMSO-d_6): δ 5.10 (bs, 2H, NH_2), 7.16 (s, 1H, 5H-thiazole), 7.35–7.82 (m, 4H, Ar-H), 8.31 (s, 1H, NH).
3–amino-6-(4-chloro-2-hydroxy-5-methylphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2–*a*]pyrimidin-4-one (**3g**)	Yield-68%; M.P. 220 °C; IR (KBr υ max/cm^{-1}): 3334(NH), 3300 (NH_2), 3186 (–OH), 1665 (C═O), 1607.51 (C═N); ^{1}H NMR (DMSO-d_6): δ 2.15 (s, 3H, CH_3), 4.86 (bs, 2H, NH_2), 7.05 (s, 1H, 5H-thiazole), 7.81 (s, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 8.51 (s, 1H, NH), 11.54 (s, 1H, OH).
3–amino-6-(4,5-dichloro-2-hydroxyphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one (**3h**)	Yield-68%; M.P. 192 °C; IR (KBr υ max/cm^{-1}): 3410 (–NH), 3376 (–NH_2), 3195 (–OH), 1655 (C═O), 1604.51 (C═N);^{1}H NMR (DMSO-d_6): δ 4.91 (bs, 2H, NH_2), 7.16 (s, 1H, 5H-thiazole), 7.43 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H), 8.32 (s, 1H, NH), 11.82 (s, 1H, OH).
3–amino-6-(4-hydroxyphenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo [3,2–*a*]pyrimidin-4-one (**3i**)	Yield-67%; M.P. 241 °C; IR (KBr υ max/cm^{-1}): 3402 (–NH), 3360 (–NH_2), 3243 (OH), 1642 (C═O), 1660 (C═N);^{1}H NMR (DMSO-d_6): δ 5.05 (bs, 2H, NH_2), 6.22 (s. 1H, OH), 7.05 (s, 1H, 5H-thiazole), 7.41–86 (m, 4H, Ar-H), 8.41 (s, 1H, NH).
3-amino-6-(5-chloro-2-hydroxy-3-iodophenyl)-1*H*-pyrazolo[3,4-*d*]thiazolo[3,2–*a*]pyrimidin-4-one (**3j**)	Yield-68%; M.P. 210 °C; IR (KBr υ max/cm^{-1}): 3385(–NH), 3286 (–NH_2), 3192 (–OH), 1665 (C═O), 1606.51 (C═N); ^{1}H NMR (DMSO-d_6): δ 4.87 (bs, 2H, NH_2), 7.12 (s, 1H, 5H-thiazole), 7.85 (s, 1H, Ar-H), 7.51 (s, 1H, Ar-H), 8.52 (s, 1H, NH), 11.64 (s, 1H, OH).

(2 × 5 mL), recrystallized from ethanol and dried at room temperature with 70–80% yield.

2.3. Synthesis of 3-amino-6-(substitutedphenyl)-1H-pyrazolo[3,4-d]thiazolo[3,2-a]pyrimidin-4-one (**3a–j**)

A mixture of 3-cyano-2–methylthio-4-oxo-4*H*–6-(substituted-phenyl)thiazolo[3,2-*a*] pyrimidine (**2a–j**) (1 mmol) and hydrazine hydrate (99%) (5 mmol) in dry DMF (20 mL) was refluxed for 4 h to obtain compounds **3a–j**. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was extracted with diethylether (2 × 20 mL). The combined organic layers were dried over anhydrous sodium sulphate (Na_2SO_4) and the solvent was evaporated under reduced pressure. The residue was recrystallized from ethanol to afford the target compounds in 60–70% yields. This typical experimental procedure was used to prepare the other analogues of this series (Table 1). The spectral data confirm the structure of the compounds.

2.4. Antimicrobial activity

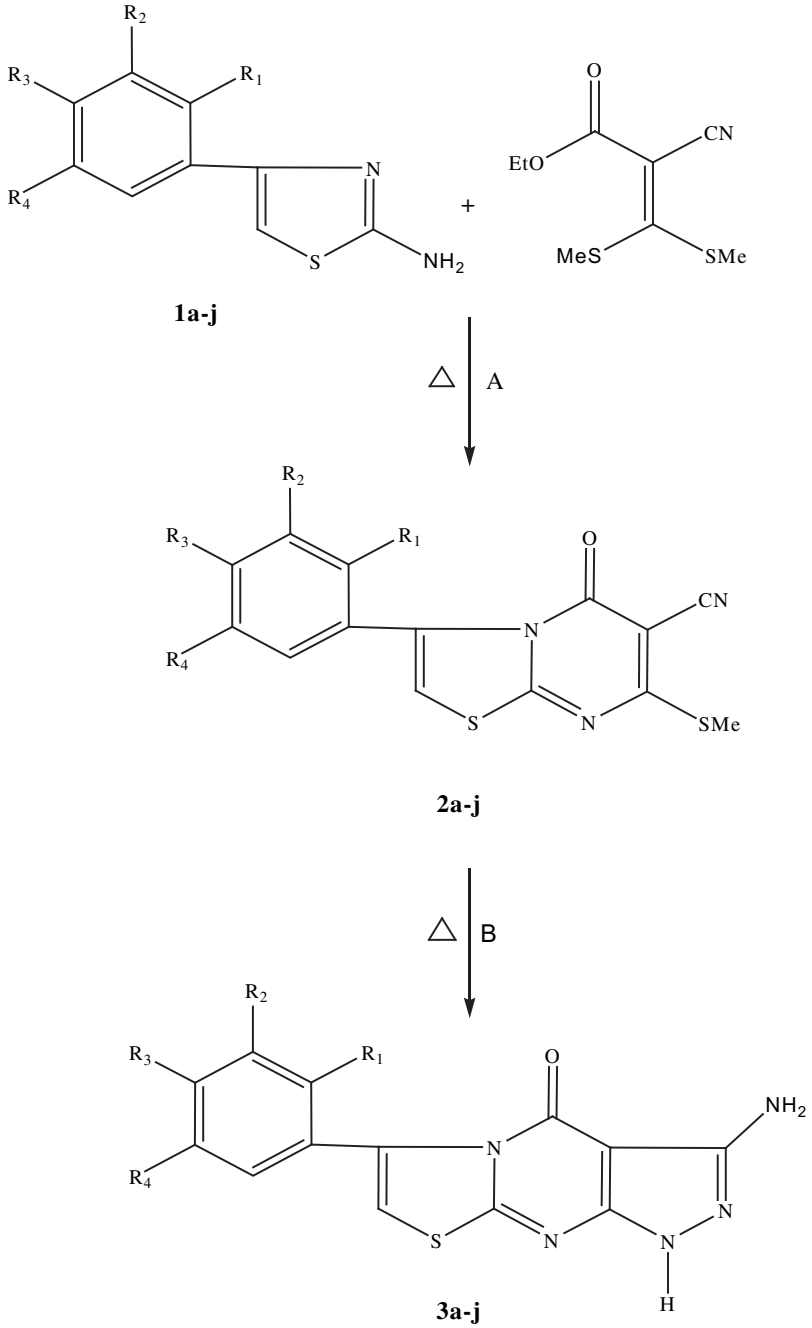
Antimicrobial activities of synthesized compounds were tested by agar diffusion method [13,14]. All Pathogenic strains of bacteria and fungi were procured from Institute of Microbial Technology

Table 2
Antimicrobial activity of pyrazolo[3,4-d]thiazolo[3,2-*a*]pyrimidin-4-one derivatives (Zone of Inhibition in mm).

Compound	Bacteria (MIC 50μg/mL)									Fungi (MIC 250 μg/mL)			
	EC	PA	PV	SA	KN	BS	BM	SM	AN	TV	PC	AF	CA
3a	–	–	±	–	15	10	–	21	12	20	26	23	13
3b	–	±	±	12	–	–	–	16	10	20	–	–	18
3c	–	±	9	31	13	10	12	18	16	27	25	11	12
3d	11	±	–	16	9	–	–	15	–	26	28	11	16
3e	10	±	–	31	13	–	–	16	–	29	32	16	21
3f	14	±	–	18	–	–	–	–	10	27	15	10	10
3g	12	±	–	–	±	–	–	–	9	27	32	12	17
3h	12	±	±	–	±	–	–	–	11	22	23	18	10
3i	–	25	14	30	11	12	–	17	16	26	29	21	–
3j	–	±	±	16	±	14	–	–	–	21	14	–	–
Tetracycline	–	32	20	25	17	20	27	15	–	–	–	–	–
Nystatin	–	–	–	–	–	–	–	–	14	18	17	14	17
Control	–	–	±	–	±	±	–	±	–	±	±	±	–

Data represent is mean of three replicates.
EC–*Escherichia coli* (MTCC 1650); PV -*Proteus vulgaris* (MTCC 1771); PA -*Pseudomonas aeruginosa* (MTCC 2488);
KN–*Klebsiella Pneumoniae* (NCIM 2957), SA -*Staphylococcus aureus* (MTCC 96);
BS–*Bacillus subtilis* (MTCC 1789) ; SM-*Serratia marcescens* (MTCC 86); BM - *Bacillus megaterium* (MTCC 1684);
AN–*Aspergillus niger* (MTCC 1781); AF- *Aspergillus flavus* (MTCC 2501),
PC–*Penicillium chrysogenum* (MTCC 1996); TV - *Trichoderma viridae* (MTCC 167); CA - *Candida albicans* (MTCC 227)
Not detected -; Trace activity ±.

(IMTech) Chandigarh and National Collection of Industrial Microorganisms (NCIM) Pune, India. The compounds **3a–j** were evaluated for antimicrobial activity against some human pathogenic bacteria viz. *Bacillus megaterium* (MTCC 1684), *Bacillus subtilis* (MTCC 1789), *Klebsiella pneumoniae* (NCIM 2957), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 2488), *Proteus vulgaris* (MTCC 1771), *Escherichia coli* (MTCC 1650) and *Serratia marcescens* (MTCC 86) and fungi viz. *Trichoderma viridae* (MTCC 167), *Penicillium chrysogenum* (MTCC1996), *Aspergillus flavus* (MTCC 2501), *Aspergillus niger* (MTCC 1781) and *Candida albicans* (MTCC 227). Stock solutions of compounds were diluted in dimethyl sulfoxide (DMSO) to give final concentrations ranging from 50 to 1000 μg/mL. For antifungal activity, different fungal spore suspensions in sterile distilled water were adjusted to give a final concentration of 10^6 cfu/mL. An inoculum of 0.1 mL spore suspension of each fungus was spreaded on Sabourauds Dextrose agar plates. For antibacterial activity, Muller Hinton agar was used. It was seeded with 0.1 mL of respective bacterial culture strains suspension prepared in sterile saline (0.85%) of 10^5 cfu/mL dilution. The wells of 6 mm diameter were filled with 0.1 mL of each compound dilution separately for each test of fungi and bacterial strain. The DMSO alone was used as control. The antibiotic nystatin (30 μg/mL) and tetracycline (10 μg/mL) are used as reference antifungal and antibacterial substance respectively for comparison.



Scheme 1. General synthetic pathway followed in the preparation of compounds **3a–j**. (A): DMF, TEA; (B) $NH_2NH_2.H_2O$ (99%), DMF. a: $R_1 = R_2 = R_4 = H$, $R_3 = Cl$, b: $R_1 = OH$, $R_2 = R_3 = R_4 = H$, c: $R_1 = OH$, $R_2 = R_3 = H$, $R_4 = Cl$, d: $R_1 = OH$, $R_2 = Br$, $R_3 = H$, $R_4 = Cl$, e: $R_1 = R_2 = R_4 = H$, $R_3 = NO_2$, f: $R_1 = R_2 = R_4 = H$, $R_3 = Br$, g: $R_1 = OH$, $R_2 = H$, $R_3 = Cl$, $R_4 = CH_3$, h: $R_1 = OH$, $R_2 = H$, $R_3 = R_4 = Cl$, i: $R_1 = R_2 = R_4 = H$, $R_3 = OH$, j: $R_1 = OH$, $R_2 = I$, $R_3 = H$, $R_4 = Cl$.

Inoculated plates were incubated for 24 h at 37 ± 0.5 °C for antibacterial activity and 48 h at 28 ± 0.2 °C for antifungal activity. The antimicrobial activity was measured in terms of the zone of inhibition in mm. Minimum inhibitory concentration (MIC) was determined as the lowest concentration of compound which completely inhibit the fungal and bacterial growth after incubation time. The investigation of antimicrobial screening data revealed that all the tested compounds showed moderate to good antimicrobial inhibition (Table 2).

3. Results and discussion

Synthesis of a series of pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one derivatives was carried out according to the reported procedure [15,16]. Scheme 1 illustrates the way used for the preparation of target compounds. The reaction mixture containing an equimolar quantities of 2-amino-4-(substituted phenyl)thiazoles **1a–j** and cyanoketene dithioacetal was refluxed in dry dimethylformamide (DMF) in presence of triethylamine (TEA) for 3 h to yield the corresponding 3-cyano-2–methylthio-4-oxo-4*H*–thiazolo[3,2-*a*]pyrimidin **2a–j** derivatives. The solvent was removed from the reaction mixture and solid was treated with chloroform, washed with water and dried over sodium sulphate. After complete evaporation of chloroform, target compounds **3a–j** were obtained as colorless crystalline solid. All the compounds were isolated in 60–70% yield after recrystallization. The structures of the compounds were elucidated by IR and ^{1}H NMR spectral data. The IR spectra of all the compounds show absorption band at 1600–1700 cm^{-1} for C═O, 1600–1610 cm^{-1} for C═N stretching mode. A distinct band at 2210–2220 cm^{-1} for C≡N stretching mode in **2a–j** and between 3400 and 3500 cm^{-1} for NH and 3300–3500 cm^{-1} for NH_2 in **3a–j** stretching mode confirms the pyrazolo ring formation. The ^{1}H NMR spectra of the compounds are taken in DMSO-d_6 solution. NH proton of pyrazolo ring was seen at singlet in between 8.4 and 8.6 ppm and 4–5 ppm for NH_2. All the compounds showed a common OH, 5H-thiazole proton at range 11–12 and 7–8 ppm respectively as singlet (Table 1).

For biological activity screening, all the test compounds were dissolved in 1% DMSO while DMSO without test compound was used as control, giving more or less zone of inhibition against different microbial strains as summarized in Table 2. The results revealed that compounds **3c** and **3i** displayed a good zone of inhibition (10–31 mm) against all the selected bacterial strains thereby exhibits interesting antibacterial activity. The remaining compounds **3a**, **3b**, **3d**, **3e, 3f**, **3j** were found to have moderate activity, while the compounds **3g** and **3h** were less active. Out of eight selected bacterial strains *S. aureus* and *S. marcescens* were more sensitive to the compounds **3b**, **3c**, **3d**, **3e** and **3i** as gives maximum zone of inhibition at MIC 50 μg/mL. Compounds **3a**, **3f** and **3j** were found to be less active while, **3g** and **3h** were totally inactive to both the strains. Compounds **3a**, **3c**, **3d**, **3e**, **3f**, **3g**, **3h**, **3i** and **3j** displayed a slight activity towards the *E. coli, K. pneumonia* and *B. subtilis*, while they were totally resistant towards **3b**. *B. megaterium*, *P. vulgaris* and *P. aeruginosa* were resisting to most of the compounds at this concentration. The results were found to be comparable with the standard tetracycline.

Further, the antifungal activity of all the synthesized compounds was determined against pathogenic fungi viz. *A. niger*, *A. flavus*, *P. chrysogenum*, *T. viridae* and *C. albicans*. Most of the compounds were showed a significant level of antifungal activity at MIC 250 μg/mL concentrations as compared to nystatin. Compounds **3c**, **3d**, **3e**, **3f**, **3g**, **3i** showed good activity against *T. viridae* (26–29 mm). Similar level of activity was displayed by the compounds **3a**, **3b**, **3h** and **3j** (20–22 mm) as compared to standard. *P. chrysogenum* was affected more by the compounds **3a**, **3c**, **3d**, **3e**, **3g** and **3i** with maximum zone of inhibition (25–32 mm) than **3f** and **3j**. Compound **3b** was totally inactive against *P. chrysogenum*.

A moderate activity was given by the compounds against remaining fungi. *A. flavus* and *A. niger* were more sensitive towards the compounds **3a**, **3c**, **3f**, **3g**, **3h** and **3i** as compare to **3b**, **3d**, **3e.** Both of the strains were totally resistant towards the compound **3j**. *C. albicans* was showed good activity against the entire synthesized compound except **3i** and **3j** at MIC 250 μg/mL concentration as shown in Table 2.

In general, 100% inhibition of the fungal strains was achieved by the compounds **3a**, **3c**, **3f**, **3g**, and **3h** while remaining compounds (**3b**, **3d**, **3e**, **3i** and **3j**) gives only 60–80% inhibition. The structure activity relationship suggested that fused pyrimidines containing imidazo and pyrazolo rings showed higher antibacterial and antifungal activities than the corresponding other moieties (like triazolo, halo, sulfonyl groups) [16]. Here, thiazolo moiety is introduced along with pyrazolo rings for activity reinforcement. Different substitutions (halo, methyl, hydroxyl, nitro) on phenyl ring affect the antimicrobial activity of the synthesized compounds drastically and substitution of hydroxyl groups revealed to be crucial for antimicrobial activity. The activity is further increased with halo substitution, particularly –Cl found to be beneficial followed by –Br and –I. Substitutions of $–NO_2$ and $–CH_3$ group gives moderate activity.

4. Conclusion

In summary, the synthesized pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidin-4-one derivatives exhibit promising antimicrobial activity. Substitution of hydroxyl and halo groups emerged as active in both antibacterial and antifungal screening. Hence it is concluded that there is enough scope for further study in the developing these as good lead compounds. Moreover, this preliminary study is encouraging to further explore their broad spectrum pharmacological activities particularly enzyme inhibition.

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