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NOVEL PYRIMIDINE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS: SYNTHESIS AND BIOLOGICAL EVALUATION

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Keywords:

Pyrimidine derivatives, Spectral studies, Antibacterial activity, Antifungal activity

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ABSTRACT: The pyrimidine scaffold found in many bioactive drugs; some commercially available drugs containing at least one pyrimidine ring in their structure. 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. 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. Novel functionalized pyrimidine derivatives and their derived bicyclic pyrazolo thiazolo pyrimidine compounds were synthesized by cyclocondensation of 3-cyano-2- methylthio-4-oxo-4H-substituted thiazolo [1,2-a] pyrimidines with hydrazine hydrate gave novel series of pyrimidine derivatives in one step, and the chemical structures 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. The current research is being directed towards the synthesis and improvement of biological activity of the pyrazolopyrimidine derivatives.

INTRODUCTION: Fused pyrimidine derivatives have attracted the attention of numerous researchers over many years due to their important biological and chemotherapeutic activities. Nitrogen containing six membered heterocycles and their fused derivatives such as pyrimidine are reported to show a wide range of biological activities including antibacterial, analgesic, anti-inflammatory, anticancer, antitumor *etc* [1].

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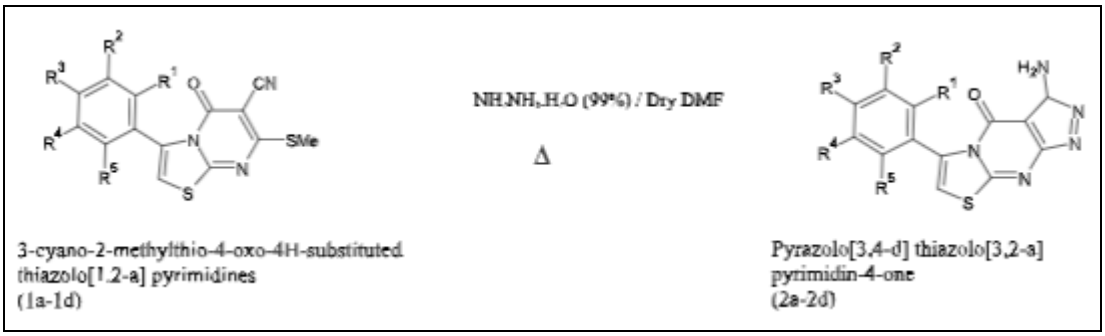
As prompted by these claims and in continuing synthetic studies on bioactive heterocycles, a novel series of pyrazolo-thiazolo-pyrimidine-4- one have been synthesized via hydrazine hydrate catalyzed cyclization of 3-cyano-2- methylthio-4-oxo-4H-substituted thiazolo [1,2-a] pyrimidines with the aim of evaluating their antibacterial and antifungal activities [3].

MATERIALS AND METHODS:

Instruments and Materials: All the chemicals used in the work were AR grade and LR grade purchased from Loba, Merk and Fisher Scientific fine chemicals. The melting point of the synthesized compound were determined in open capillary using LABHOSP melting point apparatus and recorded in ˚C and are uncorrected.

Progress of the reaction was controlled by thin-layer chromatography using petroleum ether: ethyl Acetate and n-hexane: ethyl acetate as solvent system on silica gel 60 G plates. The Infrared Spectra were recorded using SHIMADZU FT-IR spectrophotometer using potassium bromide pellet technique. The UV Spectra were recorded using SHIMADZU-1800 UV Spectrophotometer using ethanol as solvent. ^{1}H NMR Spectra were obtained using an Avance 300 MHz Spectrophotometer in dimethyl sulfoxide (DMSO)- d6 with tetramethylsilane (TMS) as an internal standard. In all cases chemical shifts are in ppm downfield to TMS. Mass Spectra were recorded on water UOLC-TQD (ESI-MS and APCIMS).

Synthetic Scheme:



SCHEME I: SYNTHESIS OF PYRAZOLO [3,4-D] THIAZOLO [3, 2-A] PYRIMIDIN-4-ONE DERIVATIVES (3A- 3D) [5]

Reaction 3 – cyano – 2 – methylthio – 4 – oxo - 4H substituted thizolo pyrimidine with hydrazine hydrate in the presence of dry dimethylformamide by flushing dry nitrogen to maintain anhydrous condition and was refluxed for 5 hours. It afforded the pyrazolo [3,4-d] thiazolo[1,2-a] pyrimidine-4-one derivatives. The solvent was removed and the residue was treated with chloroform and extracted with water. The chloroform layer was separated out and concentrated. After complete evaporation of chloroform, compounds were obtained as colourless crystalline solids.

Pyrazolo [3,4-d] thiazolo [1,2-a] pyrimidine-4-one (2a). Yield-89%, MP-168-170˚C; IR Spectrum: (Vmax cm^{-1}): (pyrimidine) 549 cm^{-1}, (thiazole) 457 cm^{-1}, (pyrazole) 921 cm^{-1}, (-NH) 3534 cm^{-1}, (C=O)1478 cm^{-1}, (C=N)1764 cm^{-1}, (C=S)745 cm-1, (NH2) 3464 cm-1; 1H-NMR: (DMSO-d6): δ = 3.7 (br s, 2H NH2), δ = 7.18 (s, 1H, 5H-thiazole), δ = 7.38-7.86 (m, 4H, Ar-H), δ = 8.36 (s, 1H, NH). 6-(3-Nitrophenyl) 1H pyrazolo [3,4d] thiazolo[3,2a] pyrimidine 4-one (2b). Yield 84%, MP-164-166; IR Spectrum: (Vmax cm-1): (pyrimidine) 472 cm^{-1}, (thiazole) 597 cm^{-1} (pyrazole) 1307 cm^{-1}, (-NH) 3566.38 cm^{-1}, (C=O)1716.65 cm^{-1}, (C=N)1950.3 cm^{-1}, (C=S) 774cm^{-1}, (NH2) 3437.15 cm^{-1}, (NO2) 1338 cm^{-1}; 1H-NMR: (DMSO-d6): δ = 4.82 (br s, 2H NH2), δ= 7.12 (s, 1H, 5H-thiazole), δ= 7.38-7.86 (m, 4H, Ar-H), δ= 8.36 (s, 1H, NH). 6-(4-Aminophenyl) 1H pyrazolo [3,4d] thiazolo[3,2a] pyrimidine 4-one (2c). Yield 88%, MP-157-159; IR Spectrum: (Vmax cm^{-1}): (pyrimidine) 472 cm^{-1}, (thiazole) 597 cm^{-1} (pyrazole) 1257 cm^{-1}, (-NH) 3426.38 cm^{-1}, (C=O)1612.65 cm^{-1}, (C=N) 1890cm-1, (C=S) 764cm^{-1}, (NH2) 3537.15 cm^{-1}; 1H-NMR: (DMSO-d6): δ = 3.82 (br s, 2H NH2), δ= 7.21 (s, 1H, 5H-thiazole), δ= 7.38-7.86 (m, 4H, Ar-H), δ= 8.36 (s, 1H, NH). 6-(4-Bromophenyl) 1H pyrazolo [3,4d] thiazolo [3,2a] pyrimidine 4-one (2d). Yield 79%, MP-178-180; IR Spectrum: (Vmax cm^{-1}): (pyrimidine) 542 cm^{-1}, (thiazole) 497 cm^{-1} (pyrazole) 924 cm^{-1}, (-NH) 3526.38 cm^{-1}, (C=O)1522.65 cm^{-1}, (C=N)1780cm^{-1}, (C=S)787cm^{-1}, (NH2) 3564 cm^{-1}; 1H-NMR: (DMSO-d6): δ = 4.82 (br s, 2H NH2), δ= 6.8 (s, 1H, 5H-thiazole), δ= 7.38-7.86 (m, 4H, Ar-H), δ= 8.36 (s, 1H, NH).

Antimicrobial Activity: *In-vitro* antibacterial and antifungal activities of synthesized compounds were tested by agar diffusion method. All pathogenic strains of bacteria and fungi were procured from Priyadarshini J. L. College of Pharmacy, Nagpur. The compounds 3a-3d were evaluated for antibacterial and antifungal activity against some human pathogenic bancteria *viz*. *Bacillus subtilis* (MTCC 1789) and Escherichia coli (MTCC 1650) and fungi *viz*. *Aspergillus niger*

(MTCC 1781) and *Candida albicans* (MTCC 227) using disc diffusion sensitivity test 9,16. Muller-Hinton agar media were sterilized (25 min at 120˚C) and poured into plates to a uniform depth of 6 mm and allow it to solidify. The microbial suspension (1.2×108 cfu/mL) was streaked over the surface of media using a sterile cotton swab (20 min at 160˚C) to ensure confluent growth of organisms. The tested compound was dissolved in DMF and diluted with ethanol to get a solution 100-600 $\mu g/mL^{-1}$ concentration. The disc measuring 5 mm in diameter (whatman filter paper) were infused with prepared solution of compounds (3a-3d) by 1 mL of the chemical solution. For antifungal activity, different fungal spore suspensions in sterile distilled water were adjusted to give a final concentration of 106 cfu/mL. An inoculum of 0.1mL spore suspension of each fungus was spreaded on Sabourauds Dextrose agar discs. For antibacterial activity, Muller Hinton agar was used. It was seeded with 0.1mL of respective bacterial culture strains suspension prepared in sterile saline (0.85%) of 105 cfu/mL dilution. The wells of 6 mm diameter were filled with 0.1mL of each compound dilution separately for each test of fungi and bacterial strain. Inoculated discs were incubated for 24 h at 37˚C for antibacterial activity and 48 h at 28°C for antifungal activity. The antibiotic amoxicillin and fluconazole was chosen as standard drug at a concentration of 10µg/ml-1. Amoxicillin is antibiotic that inhibits both gram +ve and gram –ve bacteria, and therefore a useful broad spectrum antibiotic. Fluconazole is antifungal that used to inhibit infection caused by different kinds of fungus. The antibacterial and antifungal activities 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 bacterial and fungal growth after incubation time. As it can be concluded from the data in **Table 1**, the compound bearing 3-NO2-C6H6 (3b) substituent has shown the highest sensitivity against *S. subtilis* and *A. niger*. Compound with other substituent 3-NH4-C6H6 (3c) exhibits moderate activity against *A. niger* and *C. albicans* while compound (3d) with substituent 4-Br-C6H6 found to be least active. Therefore, the remarkable activity can be attributed to the presence of group 3- nitro which is substistuted on phenyl ring which is directly attached to the thiazole system.

RESULT

Anti-Microbial Activity:

TABLE 1: ANTI- MICROBIAL ACTIVITY OF SYNTHESIZED COMPOUND (ZONE OF INHIBITION IN MM, IN 100UG/ML)

Compoundcode	Antibacterial Activity		Antifungal Activity	
	Gram +ve	*Gram -ve*	*CandidaAlbicans*	*Aspergillusniger*
	B. Subtilis	*E. Coli*		
2a	16± 0.63	20± 0.89	18 ±0.75	15± 0.89
2b	22± 0.75	20 ±0.89	21± 0.63	23± 0.63
2c	18± 0.75	19 ±0.89	16 ±0.63	18 0.75
2d	18± 0.63	14 ±0.75	11± 0.63	19 ±0.89
Amoxicillin	25± 0.89	27± 0.63	-	-
Fluconazole	-	-	29 ±0.89	32± 0.63

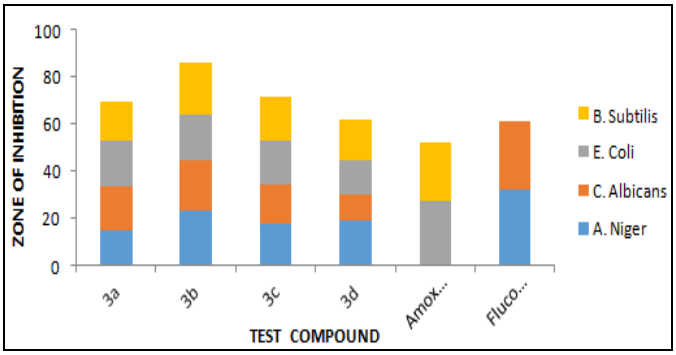


FIG. 1: GRAPHICAL REPRESENTATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF SYNTHESIZED COMPOUNDS

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. The structures of the compounds were elucidated by IR spectrum and 1H NMR spectral data. The IR spectra of all the compounds show absorption band at 1600 1700 cm for C-O, 1600-1610 cm for C-N stretching mode. A distinct band at 2210-2220 cm for CN stretching mode in 2a-2d and between 3400 and 3500 cm for NH; In 3a-3d stretching mode confirms the pyrazolo ring formation. The 1H NMR spectra of the compounds are taken in DMSO-d6 solution. NH proton of pyrazolo ring was seen at singlet in between 8.4 and 8.6 ppm and 4-5 ppm for NH2. All the compounds showed a common OH, SH-thiazole proton at range 11-12 and 7-8 ppm respectively as singlet.

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. The results revealed that compounds 3b displayed at good zone of inhibition (10-31 mm) against all the selected bacterial strains thereby exhibits interesting antibacterial activity. The remaining compounds 2a and 2c were found to have moderate activity, while the compounds 2d were less active. bacterial strains *Bacillus subtilis* and *Escherichia coli* were more sensitive to the compound 2b as gives maximum zone of inhibition at MIC 50 mg/mL. Compounds 2a and 2c were found to be less active while compound 2d were totally inactive to both the strains. 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. Aspergillus niger* and *Candida albicans*. The compound 2b shows the significant level of antifungal activity at MIC 250 mg/mL concentrations as compared to nystatin. compounds 2a and 2c shows moderate activity while compound 2d shows less activity. The results were found to be comparable with the standard Nystatin.

CONCLUSION: In summary, the synthesized pyrazolo [3.4-d] thiazolo[3,2-a] pyrimidin-4-one derivatives exhibit promising antimicrobial activity. Substitution of m-Nitro group 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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CONFLICTS OF INTEREST: Nil

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