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Synthesis and biological activity evaluation of 3-amino-9-chloro-8-fluoro-4-oxo-(2H)/aryl/heteryl-pyrazolo[3',4': 4,5]pyrimido [2,1-b] [1,3]benzothiazoles

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ABSTRACT

A new series of 3- amino -9- chloro-8- fluoro -4- oxo- (2H)/aryl/heteryl- pyrazolo[3',4' : 4, 5] pyrimido [2,1 –b] [1,3] benzothiazole and its derivatives were synthesized . The antimicrobial activity of the synthesized compounds were studied by disc diffusion method using various strains of microbes and compared with standard drug streptomycin. The antifungal activity was also evaluated against four fungal strains and compared with amphotericin –B as standard. The biological activity of the synthesized compounds were found to be good to moderate.

Key words: Benzothiazole derivatives, antimicrobial activity, antifungal activity, streptomycin, amphotericin – B

INTRODUCTION

Fused pyrimido benzothiazole compounds were reported to exhibit selective biological activities [1-4]. Benzothiazoles display anti-tumour properties that are modulated by substitutes at specific positions on the benzothiazole pharmacophore [5-8]. They were also found to exhibit anti-allergic, anti-diabetic, anticancer, anti-inflammatory and fungicidal activities [9-14].

In the present work, a novel synthesis and biological evaluation of 3- amino -9- chloro-8- fluoro -4- oxo-(2H)/aryl/heteryl- pyrazolo [3',4' : 4, 5] pyrimido [2,1 –b] [1,3] benzothiazole and its derivatives is described. The compounds were screened for antimicrobial & antifungal activities and compared with standard drug compounds.

MATERIALS AND METHODS

All melting points were determined in capillary tube and are uncorrected. IR spectra were recorded on Thermo Nicolet Nexus 670 FT-IR, ^{1}H-NMR Spectra on a FT Gemini 60(200MHZ) spectrometer with TMS as internal standard and Mass spectra on a FT VG-7070H Mass spectrometer using EI technique at 70 ev. All the reactions were monitored by TLC, carried out on 0.25mm thick gel –G plate using iodine vapour for detection.

Chemistry

2-amino-7-chloro-6-fluoro benzothiazole (0.01mole) and ethyl-2-cyano-3,3-bismethyl thioacrylate (0.01mole) was refluxed in the presence of dimethyl formamide (DMF) and a pinch of potassium carbonate for 4hr. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered,

washed with water and re-crystallized from DMF-ethanol mixture to give crystalline solid 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido (2, 1-b) (1, 3) benzothiazole **(3) (fig1).**

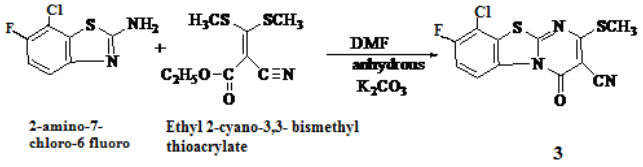


Figure1: Synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole

The structure of the compound **(3)** was established based on spectral analysis data.
IR (KBr): 2218 cm^{-1} (CN str.), 1680 cm^{-1} (C=O str.)
^{1}H- NMR in DMSO: δ 2.6 (s, 3H, SCH_3), δ 8.2 (d, 2H, Ar H)
MS (m/e): 327(M^{+2}, 33%), 325(M^+. 100%), 250, 224, 186, 160

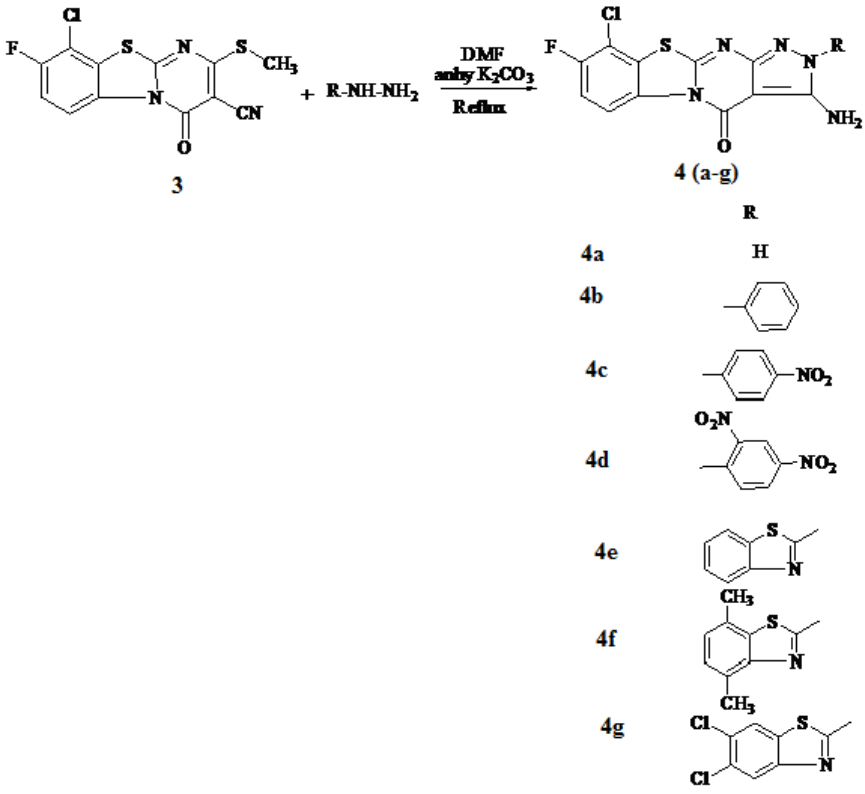


Figure 2: Synthesis of 3-amino-9-chloro-8-fluoro-4-oxo-2(H)/aryl/heteryl - Pyrazolo [3', 4': 4, 5] Pyrimido [2, 1-b] [1, 3] benzothiazoles

Compound **(3)** possesses reactive methylthio group at 2 position and cyano group at 3 position. Hence, the compound **(3**) would become best precursor for the synthesis of 3-amino-9-chloro-8-fluro-4-oxo-(2H)-pyrazolo [3', 4': 4, 5] pyrimido (2,1-b) (1,3) benzothiazole and its substituted derivatives **(4 a-g).**

General procedure for the synthesis of compounds (4a-g): A mixture of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4 H-pyrimido (2, 1-b) (1,3) benzothiazole (0.01 mole) and hydrazine hydrate/ aryl hydrazine / heteryl hydrazine (0.02mole) was refluxed in the presence of catalytic amount of anhydrous potassium carbonate and 20-25ml of dimethyl formamide (DMF) for 4 hr. The reaction mixture was cooled to room temperature and poured into ice-cold water. The separated solid product was filtered, washed with water and re-crystallized from DMF-ethanol mixture to give pure compounds **(4a-g) (fig .2).**

The structures of the newly synthesized compounds were confirmed on the basis of elemental analysis, IR, ^{1}H-NMR and Mass spectral data. The IR spectra of the compounds **(4a-g)** shown the absence of CN stretching absorption bands in the region 2190-2250 cm^{-1} and presence of absorption bands in the region 3300-3450 cm^{-1} which can be assigned to $–NH_2$ group. The presence of absorption band in the region 1680-1740 cm^{-1} can be assigned to C=O stretching. The NMR spectra exhibited peaks in the region δ7.3-7.7 and broad peak in the region δ4.0-5.2 which can be assigned to aromatic protons and $–NH_2$ protons respectively. Mass spectra of the compounds **(4a-g)** exhibited molecular ion peaks which correspond to respective molecular weights.

Antimicrobial activity

Antimicrobial activity of the synthesized compounds was evaluated by disc diffusion method. In this method the sensitivity of the compounds is measured by determining the zone of inhibition after placing the paper disc dipped in solution of compounds. These results were compared with the zone of inhibition produced after placing disc dipped in solution of standard antibiotic.

Preparation of medium

The glassware and other materials were sterilized. The nutrient agar medium was prepared by taking the ingredients as per the quantity given in **Table 1** for 1000ml of medium.

Table 1: Ingredients for preparation of nutrient agar medium

Ingredients	Quantity
1. Peptone	10 g
2. Sodium chloride	05 g
3. Meat extract	10 g
4. Agar agar powder	20 g
5. Distilled water	1000 ml.

Method

The microbes selected for antimicrobial studies are B.*substilis*, E.*coli*, S.*epidermidis*, S.*aureus* and K.*pneumoniae*. The nutrient agar medium was sterilized by autoclaving at the temperature of 121^{o}C at 15 lb/sq inch pressure for 20-25min. Then five different flasks were labeled as B.*substilis*, E.*coli*, S.*epidermidis*, S.*aureus* and K.*pneumoniae* containing nutrient agar medium maintained at temperature of 50-55^{o}C. Then 1ml of suspension of test organism i.e., B.*substilis*, E.*coli*, S.*epidermidis*, S.*aureus* and K.*pneumoniae* was poured in separately labeled flask and mixed thoroughly, maintaining the temperature of 50^{o}C. The medium was poured in to petri dishes to form a layer of about 3mm thickness and was allowed to solidify at room temperature. A filter paper disc (Whatman no.1) of 6mm diameter dipped in to 1ml of dimethyl formamide solution containing 5mg of test compound was placed with sterile foreceps on medium. Six discs were placed on a plate, one being served as a control to which disc dipped in plain dimethyl formamide solvent was placed. All the test compounds were applied in the same manner. After 24hrs of incubation at temperature of 37^{o}C, the plates were observed for zone of inhibition around the disc. The degree of sensitivity was determined by measuring zone of inhibition around the disc.

Similarly, the zone of inhibition was observed for standard strepromycin against B.*substilis*, E.*coli*, S.*epidermidis*, S.*aureus* and K.*pneumoniae*. The diameter of the zone of inhibition in mm for various test compounds and standard drug were compared.

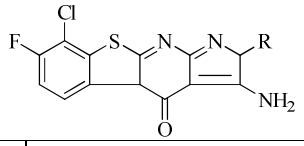
Antifungal activity

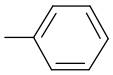
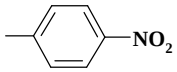
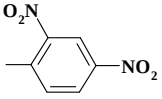
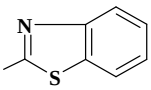
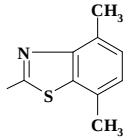
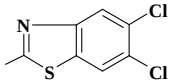
Antifungal activity was studied by Agar cup diffusion method. The ready-made potato dextrose agar (PDA) medium (Himedia, 398) was suspended in distilled water (100ml) and heated to boiling until it dissolved completely. The medium and petri-dishes were autoclaved at a pressure of 15 lb/sq inch for 20mins. The medium was poured in to sterile petri-dishes under aseptic conditions. When the medium in the plates solidified, 0.5ml of culture of test organism (C.*albicans*, S.*cerviseae*, C.*rugosa*, A.*niger*) was inoculated and uniformly spread over the agar surface. Solutions were prepared by dissolving the compound under study in DMSO. After the inoculation, cups were scooped out with 6mm sterile cork and the lids of the dishes were replaced. Controls were maintained with DMSO and Amphotericin-B. The treated and the controls were kept at room temperature for 48hrs. Inhibition zones were measured and the diameter was calculated in millimeters. Experiments were carried out in three to four replicates.

RESULTS AND DISCUSSION

The screening of antimicrobial activity of newly synthesized compounds **(4a-g)** has been carried out against B.*substilis*, E.*coli*, S.*epidermidis*, S.*aureus* and K.*pneumoniae* species by disc diffusion method. Compounds **(4a-g)** of the series 3-amino-9-chloro-8-fluoro-4-oxo-2H-pyrazolo [3', 4': 4, 5] pyrimido [2, 1-b] [1, 3] benzothiazole and its substituted derivatives exhibited zone of inhibition of 8-15mm in diameter and found more active against E.coli. Compound **4C** is found to be more active compared to other compounds against the species studied. The results of antimicrobial activity are given in **Table 2.**

Table 2: Antimicrobial activity by disc diffusion method of 3-amino-9-chloro-8-fluoro-4-oxo-2(H) pyrazolo [3', 4': 4, 5] pyrimido [2, 1-*b*] benzothiazole and its 2-substituted derivatives

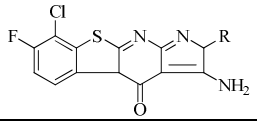


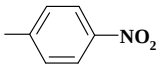
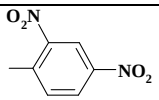
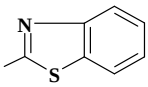
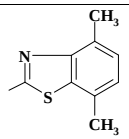
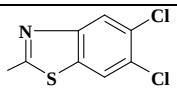
Compound. no	R	Zone of Inhibition (Diameter in mm)				
		B. substilis	*E. coli*	*S. epidermidis*	*K. pneumoniae*	*S. aureus*
4-a	—H	08	12	14	12	09
4-b		08	08	12	08	08
4-c		09	08	15	10	09
4-d		08	08	NA	10	08
4-e		08	08	14	08	09
4-f		08	08	11	08	09
4-g		10	NA	07	10	08
	Streptomycin	20	15	25	23	21

NA = Not Active

The screening of the newly synthesized compounds for their antifungal activity against C.*rugosa*, A. *niger*, C. *albicans* and S.*cerviseae* was carried out by Agar cup diffusion method. The preliminary screening shown that all the compounds **(4a-g)** exhibited zone of inhibition for C.*rugosa* species in the range 11-19mm in diameter. The compounds are not shown any activity against C.*albicans* and S.*cerevisiae* species. The results are given in **Table 3**. A comparative graphical representation of the results along with standards are also given in **Fig.3 & 4.**

Table 3: Anti-fungal activity of 3-amino-9-chloro-8-fluoro-4-oxo-2H-pyrazolo [3', 4':4, 5] pyrimido [2, 1-*b*] benzothiazole and its 2 substituted derivatives



Comp. no.	R	Zone of Inhibition (Diameter in mm)							
		C. rugosa		A. niger		C. albicans		S. cerevisiae	
		100µg	150µg	100µg	150µg	100µg	150µg	100µg	150µg
4-a	—H	10	14	NA	NA	NA	NA	NA	NA
4-b		09	12	NA	NA	NA	NA	NA	NA
4-c		16	11	08	10	NA	NA	NA	NA
4-d		14	19	NA	NA	NA	NA	NA	NA
4-e		12	15	NA	NA	NA	NA	NA	NA
4-f		10	11	09	NA	NA	NA	NA	NA
4-g		13	17	NA	NA	NA	NA	NA	NA
STD	Amphotericin B	24		25		23.5		22	

NA = Not Active

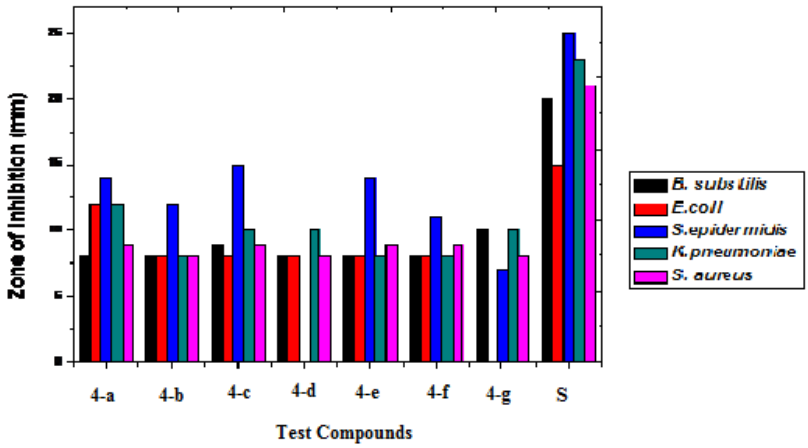


Figure 3: Bar graph representation of antimicrobial activity of 3-amino-9-chloro-8-fluoro-4-oxo-2(H) pyrazolo [3', 4': 4, 5] pyrimido [2, 1-*b*] benzothiazole and its derivatives

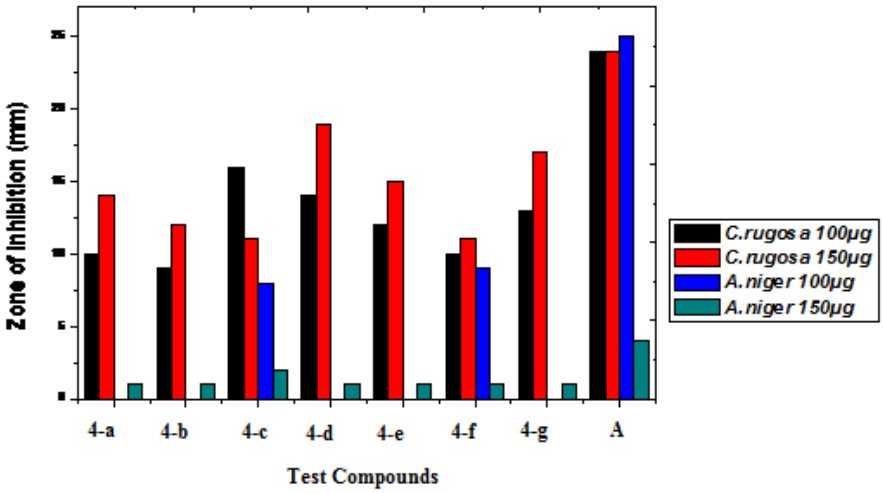


Figure 4: Bar graph representation of anti-fungal activity of 3-amino-9-chloro-8-fluoro-4-oxo-2H-pyrazolo [3', 4':4, 5] pyrimido [2, 1-*b*] benzothiazole and its derivatives

CONCLUSION

Newly synthesized compounds are found to be biologically active. The antimicrobial activity results indicate that the new series of synthesized compounds possesses good activity against multi drug resistant pathogenic bacteria. Compound **4C** is the most effective anti-microbial amongst all the compounds synthesized. Similarly, the newly synthesized compounds also possess antifungal activity against the species C.*rugosa*. Compounds **4d** and **4g** have shown maximum activity against C.*rugosa* antifungal strain.

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