Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(3):1606-1611

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and biological activity evaluation of some fused pyrimido-benzothiazole derivatives

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ABSTRACT

New series of 2-substituted aryl amine, heteryl amine and active methylene group compound derivatives of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole are synthesized. The antimicrobial activity of the synthesized compounds were studied by disc diffusion method using various strains of microbes and compared with the 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 was found to be good to moderate .

Key words: Benzothiazole derivatives, antimicrobial activity, antifungal activity.

INTRODUCTION

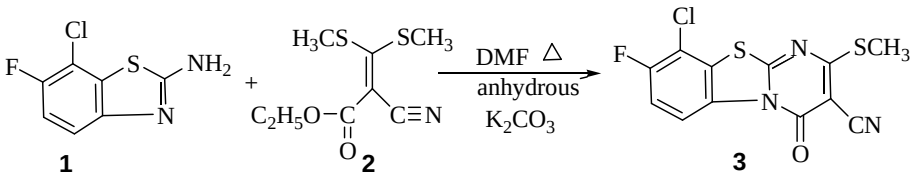
Compounds containing benzothiazole ring possess potent and selective biological activities [1-7]. Benzothiazoles display antitumor properties that are modulated by substituents at specific positions on the benzothiazole pharmacopore [8-11]. They are also found to exhibit anti-allergic, anti-diabetic, anti-cancer, anti-inflammatory and fungicidal activities [12-17].

In the present work, a novel synthesis and biological activity evaluation of 9-chloro-3-cyano-8-fluoro-2-methyl thio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole and its derivatives is described. The compounds were screened for anti-microbial & anti-fungal activities and compared with standard drug compounds.

EXPERIMENTAL SECTION

Procedure

A mixture of 2-amino-7-chloro-6-fluoro-benzothiazole (0.01 mol) (**1**) and ethyl-2-cyano-3, 3-bismethyl thioacrylate (0.01 mol) (**2**) was refluxed in dimethyl formamide (DMF) and a pinch of anhydrous potassium carbonate for 4 hours. The reaction mixture was cooled to room temperature and poured in ice cold water. The separated solid product was filtered, washed with water and recrystallized from DMF-ethanol mixture to give crystalline solid of compound **3**.



Spectral data
IR (KBr): 2218 cm^{-1} (CN str.), 1680 cm^{-1} (C=O str.); ^{1}H-NMR in DMSO: 2.6 (S, 3H, SCH_3) δ 8.2 (d, 2H, ArH); MS (m/e): 327 (M+2, 33%), 325 (M+ 100%), 250, 224, 186, 160

Compound **3** possess replaceable methyl thio group at 2-position, activated by the ring nitrogen atom and electron withdrawing cyano group at 3-position. Therefore, it is clear that the compound **3** would be susceptible for the synthesis of 2-substituted derivatives. Accordingly 2-substituted derivatives of compound **3** were prepared by reacting with selected nucleophiles like aryl amines, heteryl amines and active methylene group compounds to get corresponding substituted derivatives of **3** (**Fig.1**)

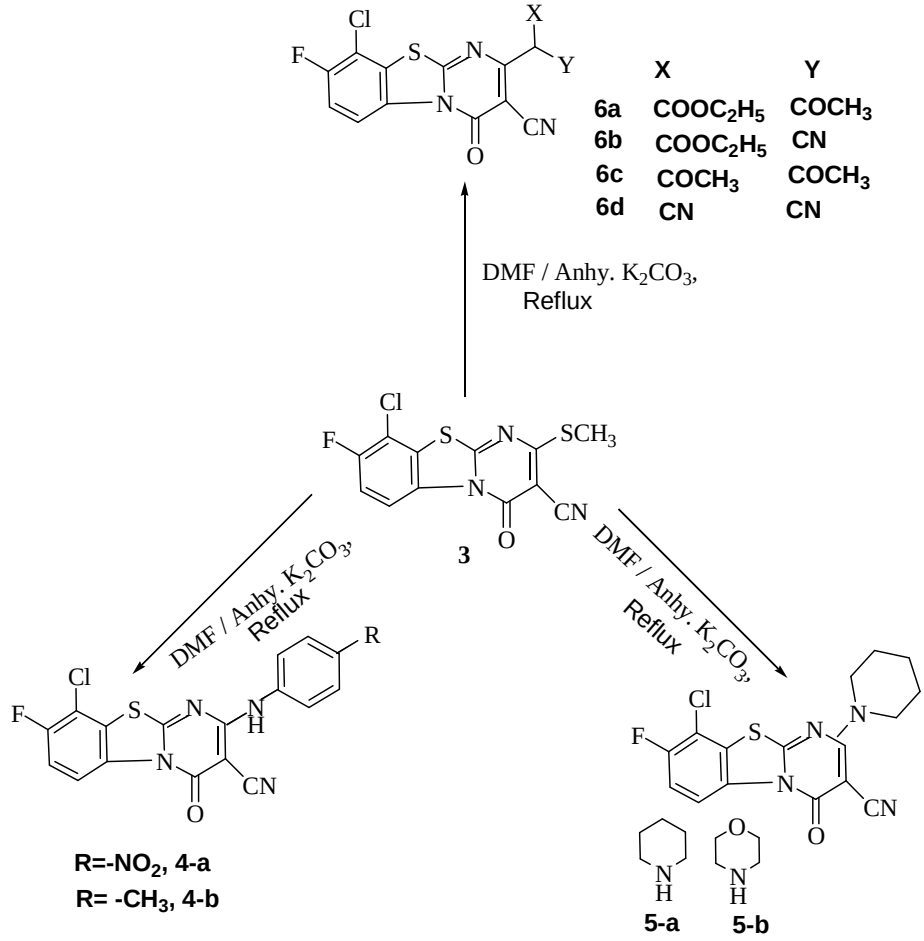


Figure 1: Synthesis of Derivatives of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1 b] [1, 3] benzothiazole

According to this method, compound **3** on reaction with p-nitroaniline in the presence of dimethyl formamide and anhydrous potassium carbonate afforded compound **4a**. similarly, compound **3** on reacting independently under

similar experimental conditions with p-toluidine/piperidine/morpholine/ethylacetoacetate/ethylcyanoacetate /acetylacetone/melanonitrile afforded respective substituted derivatives (**4b, 5a-b, 6a-d**). IR spectra of these compounds showed absorption bands in the range 2200-2226cm^{-1} which can be assigned to CN stretch and absorption bands in the range 1665-1675cm^{-1} due to C=O stretch. Mass spectra of compounds showed molecular ion peaks which correspond to their molecular weights.[1] H-NMR spectral data is also in agreement with the structures assigned to compounds.

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 zone of inhibition produced after placing disc dipped in the 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 for 1000ml of medium mentioned in **Table.1**

Table 1: Ingredients for preparation of nutrient agar medium

Ingredients	Quantity
Peptone	10g
Sodium Chloride	5g
Meat extract	10g
Agar powder	20g
Distilled water	1000ml

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°C at 15lb/sq.inch pressure for 20-25min. The five different flasks were labeled as *B. substilis, E. coli, S. epidermidis, S. aureus* and *K. pneumonia* containing nutrient agar medium maintained at temperature of 50-55°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°C. The medium was poured into petridishes to form a layer of about 3mm thickness and allowed to solidify at room temperature. A filter paper disc (Whatman no.1) of 6mm diameter-dipped in 1ml of dimethyl formamide solution containing 5mg of test compound was placed with sterile forceps 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°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 *streptomycin* against *B. substilis, E. coli, S. epidermidis, S. aureus* and *K. pneumonia*. The diameter of the zone of inhibition in mm for various test compounds and standard drug were compared.

Anti fungal activity

Method

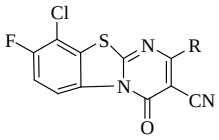
Anti fungal activity was studied by Agar cup diffusion method. The ready-made potato Dextrose Agar (PDA) medium (Himedia, 39g) was suspended in distilled water (100ml) and heated to boiling until it dissolved completely. The medium and petridishes were autoclaved at a pressure of 15lb/sq. inch for 20minutes. The medium was poured into sterile petridishes under aseptic conditions. When the medium in the plates solidified, 0.5ml of culture of test organism (*Candida albicans, S. cerviseae, C. rugosa, Aspergillus niger*) was innoculated 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 replicates.

RESULTS AND DISCUSSION

The screening of antimicrobial activity of newly synthesized compound 9-chloro-3-cyano 8-fluoro-2 methylthio-4-oxo- 4H-Pyrimido [2, 1-b] [1,3] benzothiazole **(3)** and its derivatives (**4a-6d**) has been carriedout against *B.substilis*, *E. coli*, *S. epidermidis*, *S. aureus* and *K. pnemoniae* species by disc diffusion method. The preliminary screening showed that the compounds exhibited zone of inhibition for the species under study. The parent compound (**3**) has exhibited zone of inhibition in the range of 8-11 mm in diameter for all the species. Compounds **3, 5 a-b, 6a-d** exhibited zone of inhibition of 8-14mm in diameter for the species *E. coli*. Here, the compound **6c** has exhibited a maximum zone of inhibition of 14mm in diameter for the species *E. coli* which is almost equivalent to the zone of inhibition of standard drug *streptomycin* of 15mm in diameter. Therefore, the compound **6c** was found to be more active against *E. coli* and on paar with the standard drug *Streptomycin* activity. The results of antimicrobial activity are given in **Table. 2**

The screening of the newly synthesized compounds for their anti-fungal activity against *C. rugosa*, *A. niger*, C. *albicans* and *S. cervisiae* was carried out by Agar cup diffusion method. The preliminary screening showed that all the compounds exhibited zone of inhibition for *C. rugosa* species in the range of 10-19mm in diameter. Compounds **6a** and **6b** exhibited the maximum zone of inhibition of 19mm in diameter. Compounds **4a** and **4b** have not exhibited antifungal activity against the species under study. The results are shown in **Table.2.** A comparative graphical representation of the results along with standards is also given in **Fig. 2 & 3**.

Table 2: Anti microbial and Anti fungal activity of synthesized compounds



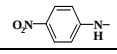
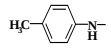
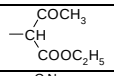
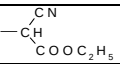
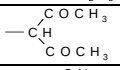
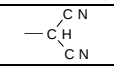
Comp. no.	R	Zone of Inhibition (Diameter in mm)								
		Anti microbial activity					Anti fungal activity			
		B. *Substilis*	**E. *coli***	**S. *epidermidis***	**K. *pneumoniae***	**S. *aureus***	**C. *rugosa***	**A. *niger***	**C. *albicans***	**S. *cerevisiae***
3	**-SCH3**	10	08	10	11	11	11	12	-	-
4a		-	-	10	-	09	-	11	-	-
4b		-	-	09	-	-	-	-	-	-
5a		-	09	-	-	-	10	-	-	-
5b		-	08	-	-	-	10	12.5	09	-
6a		10	08	11	10	-	19	-	-	-
6b		08	08	11	13	-	19	-	-	-
6c		07	14	-	11	-	16	-	-	-
6d		-	10	-	-	-	10.5	-	-	-
STD	**Streptomycin**	20	15	25	23	21	-	-	-	-
STD	**Amphotericin B**	-	-	-	-	-	24	25	23.5	22

Figure 2: Antimicrobial Activity of Synthesized compounds

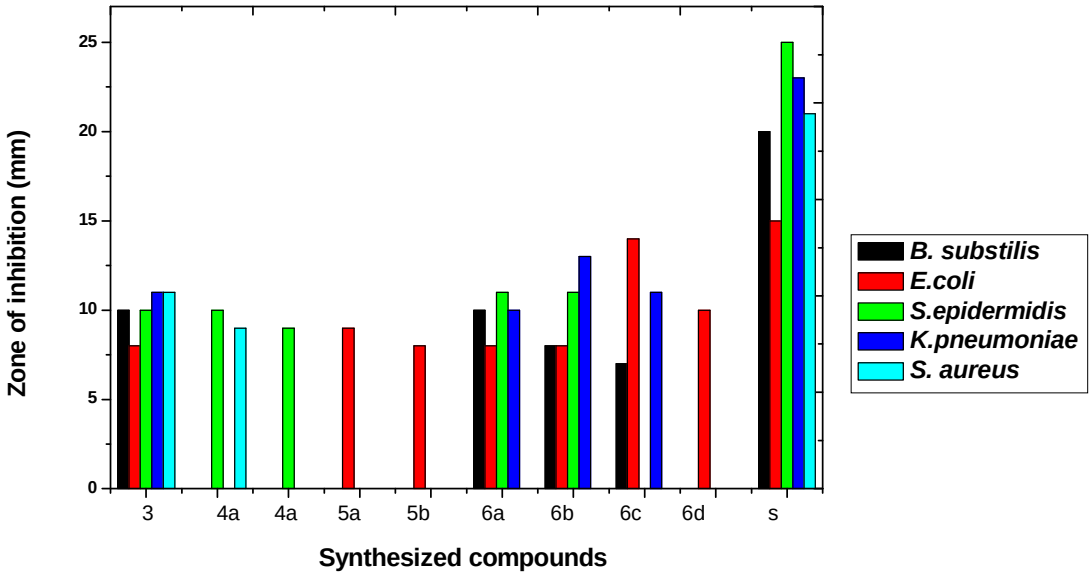
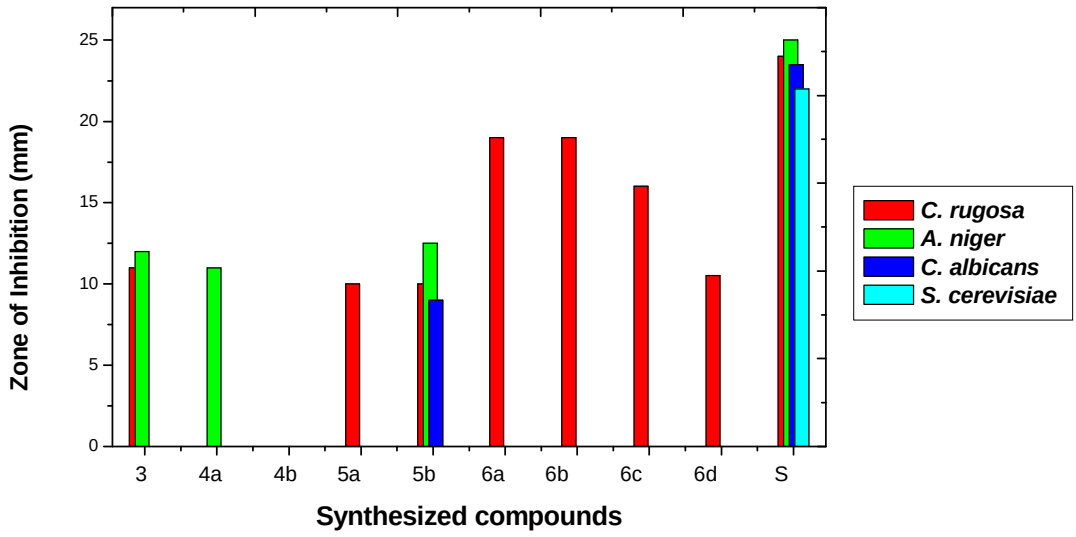


Figure 3: Antifungal Activity of Synthesized compounds



CONCLUSION

Newly synthesized 2-substituted aryl amine, heteryl amine and active methylene group compound derivatives of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole compounds are biologically active. The antimicrobial activity result indicated that the new series of synthesized compounds possess good activity against several multi-drug resistant pathogenic bacteria. Compound **6c** is the most effective anti microbial amongst all the compounds synthesized and found to be equivalent to the standard Streptomycin against *E. coli*

bacterial strain. Similarly, the newly synthesized compounds also possess antifungal activity against the species *C. rugosa*. Compounds **6a** and **6b** have shown maximum activity against *C. rugosa* antifungal strain.

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