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Research Article

SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF SOME FUSED IMINO PYRIMIDO BENZOTHIAZOLE DERIVATIVES

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ABSTRACT

A new series of heterocyclic compounds 9-chloro-10-fluoro-15-imino-14-oxo-14H-benzothiazole [2, 3-b] pyrimido [5, 6-e] pyrimido [2, 3-b] benzothiazole and its substituted derivatives have been synthesized. The anti-bacterial activity of the synthesized compounds were studied by disc diffusion method using various strains of microbes and compared with standard drug Streptomycin. The anti-fungal 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.

Keywords: Benzothiazole derivatives, Anti-bacterial activity, Anti-fungal activity.

INTORDUCTION

Pyridine, iminopyrimidine and fused benzothiazoleheterocycles are found to be effective Pharmacophores[1-6] which exhibit a wide spectrum of activities. A survey of literature revealed that fused pyrimidobenzothiazoles display various biological activities like anti-tumor[7], phosphodiesterase inhibition[8], anti-allergic[9], anti-inflammatory[10] and anti-parkinsonism[11]. In view of various applications of this system, synthesis of such condensed system has attracted much attention in recent years. In the present work, a novel synthesis and biological activity evaluation of 9-chloro-10-fluoro-15-imino-14-oxo-14H-benzothiazole [2, 3-b] pyrimido [5, 6-e] pyrimido [2, 3-b] benzothiazole and its substituted derivatives is described. The compounds were screened for anti-bacterial & anti-fungal activities and compared with standard drugs.

2. MATERIALS AND METHODS

All melting points 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 (200 MHz) spectrometer with TMS as an internal standard and Mass spectra on a FT VG-7070 H Mass spectrometer using EI technique at 70 eV. All the reactions were monitored by TLC, carried out on 0.25 mm thick gel-G plate using iodine vapour for detection.

2.1. Synthesis of 9-chloro-3-cyano-8-fluoro-2-methylthio-4-oxo-4H-pyrimido [2, 1-b] [1, 3] benzothiazole (1)

A mixture of 2-amino-7-chloro-6-fluoro-benzothiazole (0.01mol) and ethyl-2-cyano-3, 3-bismethylthioacrylate (0.01mol) was refluxed in the presence of dimethylformamide (DMF) and a pinch of potassium carbonate (K_2CO_3)for 4 hrs. (Figure.1). 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 recrystallised 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**1**. The structure of compound **1** was established based on spectral analysis data.

2.2. Spectral Data

IR (KBr): 2218 cm^{-1} (CN Str.), 1680 cm^{-1} (C=O Str.)

^{1}HNMR 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.
Compound **1** possesses reactive methylthio group at 2 position and cyano group at 3 position. Hence, the compound **1** would become best precursor for the synthesis of 9-chloro-10-fluoro-15-imino-14oxo-14H benzothiazole [2, 3-b] pyrimido [5, 6-e] pyrimido [2, 3-b] benzothiazole and its substituted derivatives (**Figure**. **2**).
Accordingly substituted derivatives of compound **1** were prepared by reacting with selected compounds 2-aminobenzothiazole (**2a**)/ 2-amino-4-chloro benzothiazole (**2b**)/ 2-amino-6-nitro benzothiazole(**2c**)/ 2-amino-4,7-dimethyl benzothiazole (**2d**)/ 2-amino-5,6-dichlorobenzothiazole (**2e**) afforded the corresponding substituted compounds (**3a-e**).
IR spectra of these compounds showed absence of CN stretching absorption band in the region of 2190-2250cm^{-1} and presence of absorption band due to (=NH) group in the region of 3355-3425cm^{-1}. 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.

2.3. Anti-bacterial activity

Anti-bacterial activity of the synthesized compounds was evaluated by disc diffusion method. In this method thesensitivity 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.**

Method

The microbes selected for anti-bacterial studies are *B.substilis,E. coli,S.epidermidis, S.aureus* and *K.pneumonia.* 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.pneumonia* 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 (What man 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.

2.4. 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. cerevisiae, C.rugosa,Aspergillusniger*) 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 replicates.

3. RESULTS AND DISCUSSION

The screening of anti-bacterial activity of newly synthesized compound 9-chloro-10-fluoro-15-imino-14-oxo-14H- benzothiazole [2,3-b] pyrimido [5, 6-e] pyrimido [2, 3-b] benzothiazole and its substituted derivatives

has been carried out against *B.substilis, E.coli, S.epidermidis, S. aureus and K. pneumonia* species by disc diffusion method. The preliminary screening showed that the compounds exhibited zone of inhibition for the species understudy. Compound **3a** has exhibited zone of inhibition in the range of 7-8 mm in diameter for all the species. Compounds **3a-e** exhibited zone of inhibition 8-12mm in diameter for the species *E.coli*. Among these compounds **3b &3d** have exhibited maximum zone of inhibition of 16 & 12 mm in diameter against *B. substilis* and *E. coli* respectively which are nearer to the zone of inhibition of standard drug Streptomycin. The results of anti-bacterial activity are given in **Table 2**.

The screening of the newly synthesized compounds for anti-fungal activity against *C.rugosa*, *A.niger*, *C.albicans* and *S.cerevisiae* was carried out by Agar cup diffusion method. The preliminary screening showed that all the compounds exhibited zone of inhibition for *C.rugosa* in the range 13-19mm in diameter. Compounds **3b and 3e** exhibited maximum zone of inhibition of 19 and 18 mm respectively against *C.rugosa* and are comparable with the standard drug *Amphotericin-B*. The compounds are not active for other species viz., *A. niger, C.albicans* and *S. cerevisiae*. The results are shown in **Table.2**. A graphical representation of all the results (anti-bacterial & anti-fungal activities) along with standards is given in **Fig 2 & 3**.

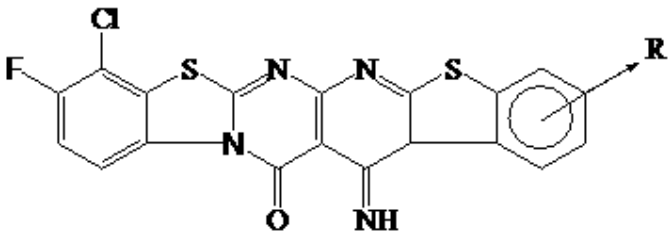
4. CONCLUSION

Newly synthesized compounds fused iminopyrimidobenzothiazole derivatives are biologically active. It is indicated from the results that the compounds possess a good anti-bacterial activity against several multidrug resistant pathogenic bacteria. Compound **3b &3d** are the most effective anti-bacterial amongst all the compounds synthesized which are comparable to the standard *Streptomycin* against *B.Substilis&E. coli* bacterial strain. Similarly, the newly synthesized compounds also possess anti-fungal activity against the species *C.rugosa*. Compounds **3b & 3e** have shown maximum activity against *C.rugosa* anti-fungal strain.

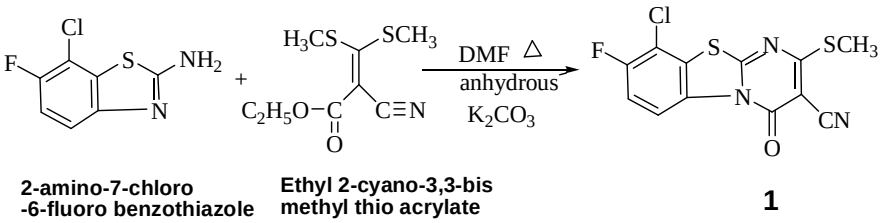
Table 1: Ingredients for preparation of nutrient agar medium

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

Table 2: Anti-bacterial and Anti-fungal activity of synthesized compounds

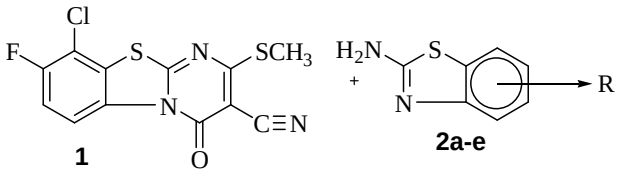


Comp. No.	R	Zone of Inhibition (Diameter in mm)								
		Anti-bacterial activity					Anti-fungal activity			
		B. substilis	E. coli	S. epidermidis	K. pneumoniae	S. aureus	C. rugosa	A. niger	C. albicans	S. cerevisiae
3a	-H	08	08	07	08	08	15	-	-	-
3b	1-Cl	16	08	15	13	-	19	-	-	-
3c	3-NO_2	-	10	13	10	-	17	-	-	-
3d	1,4-dimethyl	-	12	-	-	-	13	-	-	-
3e	2,3-dichloro	-	10	-	10	-	18	14	-	-
STD	Streptomycin	20	15	25	23	21	-	-	-	-
STD	Amphotericin-B	-	-	-	-	-	24	25	23.5	22

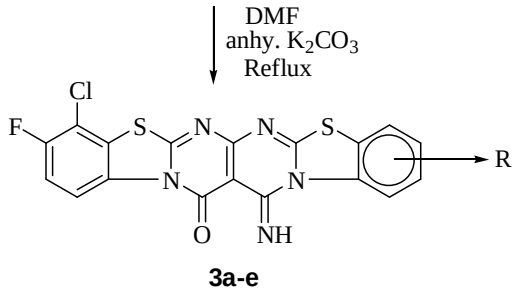


2-amino-7-chloro -6-fluoro benzothiazole **Ethyl 2-cyano-3,3-bis methyl thio acrylate** **1**

Fig. 1: Synthesis of compound 1



R	
2a	-H
2b	4-Cl
2c	6-NO_2
2d	4, 7-Dimethyl
2e	5, 6-dichloro



R	
3a	-H
3b	1-Cl
3c	3-NO_2
3d	1, 4-Dimethyl
3e	2, 3-dichloro

Fig. 2: Synthesis of 9-chloro-10-fluoro-15-imino-14oxo-14H- benzothiazole [2, 3-b] pyrimido [5, 6-e] pyrimido [2, 3-b] benzothiazole and its substituted derivatives (3a-e)

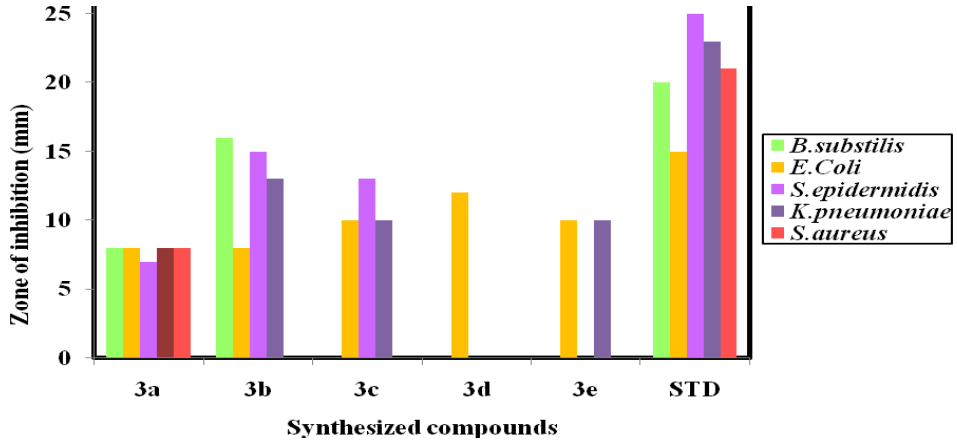


Fig. 3: Anti-bacterial activity of synthesized compounds

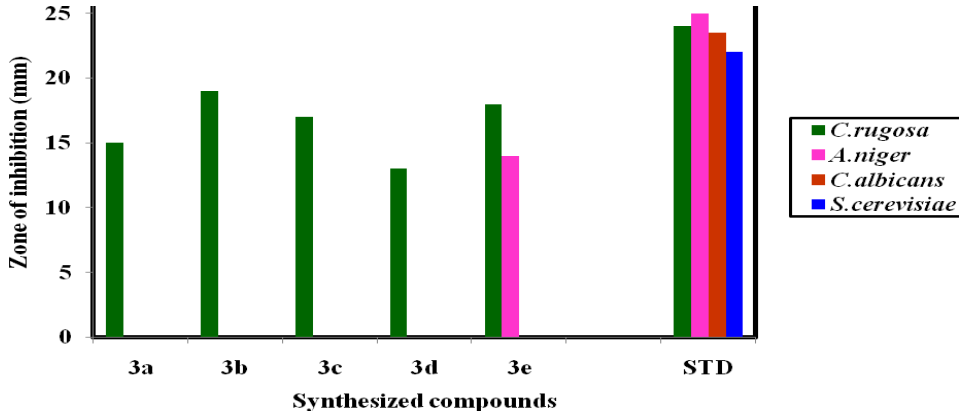


Fig. 4: Anti-fungal activity of synthesized compounds

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