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Research Article

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Antibacterial activity of substituted 2-bromo-1,4-dimethoxy-3*H*-phenoxazin-3-ones

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ABSTRACT

Synthesized derivatives of 3H-phenoxazin-3-one ***3(a-d)*** *were tested for their antibacterial activity in comparison with the standard drug Streptomycin. These compounds have been found to possess antibacterial activity against both the bacterial stains.*

Key words: 3*H*-phenoxazin-3-one, Antibacterial activity, Streptomycin, Inhibition zone.

INTRODUCTION

The biological activity of a compound is attributed to be due to the result of interlinked chemical reactions or the observed manifestation of interference with a delicately balanced system of interdependent chemical and physical processes. The relationship between biological activity and chemical constitution in different series of compounds exhibiting different types of activity has been discussed by Sexton [1].

3*H*-phenoxazin-3-one belongs to the family of phenoxazines. These are polycyclic aromatic compounds containing a phenoxazine moiety, which is linear tricycle system that consists of two benzene rings joined by a 1,4-oxazine ring. They are widely distributed in natural world being found in mushrooms (St. George's mushrooms), *Agaricusbisporus imbatch* (edible brown mushroom), microorganisms [2-4] (*Actinomadura.sp, Actinomycetes*), lichens, and higher fungi and wood-rotting fungus [5] (*Pycnoporus cinnabarinus, Pycnoporus krast*).

3*H*-Phenoxazin-3-ones exhibit prominent pharmacological applications such as antiviral [6] anti-bacterial [7], antialgal [4], antifungal [4,8], antimicrobial [9], anticancer [4,10-12], cell growth stimulating [13], anticoccidal [14], sun induced antimelanogenesis [2], phytotoxic [15,16], anti-tubercular [17], antitumor [18] and antineoplastic [19] activities.

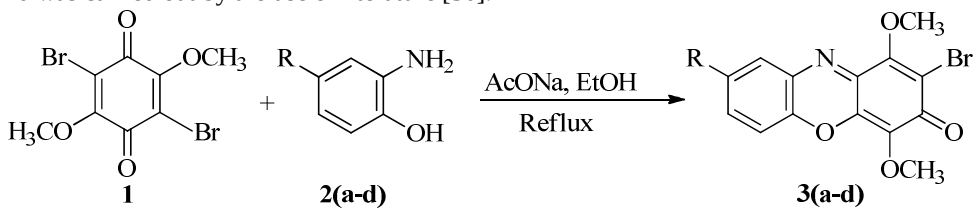
The chromophore of actinomycin and its analogues which are the antibiotics produced by *Actinomyces antibiotics* [20] and various species of *Streptomyces* [21, 22], is the 3*H*-phenoxazin-3-one system. In fact the variety of physiological properties of the system has actually stimulated interest in the synthetic and evaluation studies of the system. As the actinomycins show variable degrees of anticancer activity [23], several scientists have been trying their level best to enhance the activities of the heterocyclic system by making modifications to the chromophore

skeleton of the actinomycin. As the 3*H*-phenoxazin-3-one system shows variable degrees of anticancer effect scientists [24, 25] prepared a number of compounds by variation at N-substitution in the 2-amino and 2,7-diamino-3*H*-phenoxazin-3-one ring system. Synthetic study of several 3-N-arylimino-3*H*-phenoxazines [26-28] was done and some of those could exhibit antitubercular activity [29].

In view of the broad range of biological activities of 3*H*-phenoxazin-3-one system, we synthesized 3*H*-phenoxazin-3-one derivatives from 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone [30] and evaluated their biological activity.

EXPERIMENTAL SECTION

Preparation of substituted 2-bromo-1,4-dimethoxy-3*H*-phenoxazin-3-ones (3a-d): 6.135 mmol of substituted *ortho*-aminophenol **2(a-d)** was taken into 100 ml R.B flask and added 30 ml of ethanol followed by anhydrous sodium acetate (6.135 mmol). The mixture was stirred for 15 minutes at room temperature. After that 6.135 mmol of 2,5-dibromo-3,6-dimethoxy-1,4-benzoquinone **1** was added portion wise. Then the reaction mixture was heated at reflux temperature for 3-5 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature, poured into water and extracted with EtOAc thrice (3x20 ml). The combined organic layers were washed with brine dried over anhydrous sodium sulfate and the organic layer was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography using variants of ethyl acetate-petroleum ether mixture. Concentrated the fractions containing the compound by distilling out the solvent to obtain the pure products **3(a-d) (**Scheme 1). Structural characterization of the compound was carried out by the use of literature [30].



1 **2(a-d)** **3(a-d)**

a) R = H; b) R = Cl
c) R = CH_3, d) R = OCH_3

Scheme 1

Antibacterial activity

The antibacterial activity of the synthesized compounds **3(a-d)** were evaluated according to agar disc diffusion method [31] against gram-negative bacteria *Escherichia coli, Salmonella paratyphi, Klebsiella pneumonia* and gram-positive bacteria *Staphylococcus aureus, Micrococcus luteus, Bacillus cereus* with standard drug Streptomycin.

20 ml of the molted agar medium was poured in each of the sterilized petridishes and cooled to 45-48 ^{0}C. For bioassays, a suspension of approximately $1.5x10^8$ bacterial cells/ml was prepared as described by Forbes et al., [32] and 1.5 ml of it was uniformly spreaded on nutrient agar media. The plates were left to stand for 1 hour to solidify. After solidification of the medium, cups (wells) were made about 2 cm apart using sterile cork borer at equal distances. 0.2 ml of respective concentration of the test compound solution in dimethyl sulfoxide (DMSO) was added to each hole. The plates were allowed to stand at room temperature for one hour to allow the solution to diffuse into the medium and then incubated at 37 ^{0}C for 18 hours. After incubation period bioactivity was determined by measuring diameter of the inhibition zone (DIZ) in mm. Controls included the use of solvent without test sample. The experiment was performed three times with 400, 600 and 800 µg/ml concentrations.

RESULTS AND DISCUSSION

All the synthesized compounds **3(a-d)** were screened for their antibacterial activity according to agar disc diffusion method against gram-negative and gram-positive bacteria with standard drug Streptomycin. The results of the antibacterial activity of the synthesized compounds are presented in the **Table-1.** All the compounds **3(a-d)** showed inhibitory activity against gram-negative and gram-positive bacterial strains. Compound **3b** is found to be most active against *E.coli, Salmonella paratyphi, Klebsiella pneumonia* and showed good activity against *Staphylococcus*

aureus. Compound **3a** is found to exhibit good activity against *Klebsiella pneumoniae*, *Bacillus cereus* and good activity showed by compound **3d** against *E. coli*.

Table 1. Antibacterial activity of the compounds 3(a-d)

S. No	Compound	Zone of inhibition (in mm)																	
		E. coli			*Salmonella paratyphi*			*Klebsiella pneumoniae*			*Staphylococcus aureus*			*Micrococcus luteus*			*Bacillus cereus*		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	3a	16	18	18	17	17	17	16	19	19	16	16	16	16	17	17	16	19	19
2	3b	18	20	20	24	25	25	16	22	22	15	19	20	16	16	18	14	16	16
3	3c	18	18	18	15	16	17	14	15	15	16	17	17	15	15	15	15	16	16
4	3d	18	19	19	15	17	17	15	15	16	13	14	14	16	17	17	17	17	17
5	Streptomycin	30	32	32	27	28	29	29	29	30	28	30	32	28	30	31	28	28	30
Test solution and standard solution; A: 400 µg/ml; B: 600 µg/ml; C: 800 µg/ml.																			

CONCLUSION

The molecules synthesized as the part of present investigation with active groups as substituents are subjected to antibacterial screening. The compounds exhibited excellent to moderate activities. Whatever the case may be it is to be admitted that the investigations made in the present biological assay studies are modest and accurate and detailed information regarding the mode of action of these compounds can be arrived at only after identifying the active species of the compound and the site of its action. This would become possible only by further elaborate studies.

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