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Der Chemica Sinica, 2016, 7(3):26-29



Antibacterial activity of [1,4]benzoxazino[2,3-*b*]phenoxazine derivatives

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ABSTRACT

Derivatives of [1,4]benzoxazino[2,3-*b*]phenoxazines **3(a-d)** were synthesized according to previously established literature. The antibacterial activity of the synthesized compounds were evaluated according to agar disc diffusion method against gram-negative bacteria *Escherichia coli*, *Salmonella paratyphi*, *Klebsiella pneumonia* and gram-positive bacteria *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus* with standard drug Streptomycin.

Key words: [1,4]Benzoxazino[2,3-*b*]phenoxazine, Antibacterial activity, Streptomycin, Inhibition zone.

INTRODUCTION

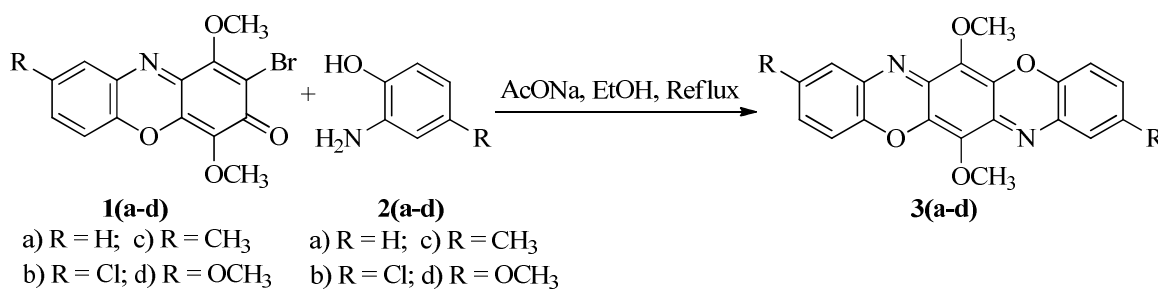
[1,4]Benzoxazino[2,3-*b*]phenoxazine or triphenodioxazine belongs to the family of phenoxazines. These are polycyclic aromatic compounds containing phenoxazine moiety, a linear pentacyclic system that consists of two oxazine rings with alternating double bonds and quinone coupling due to which these compounds shows good chromogenic properties. They form an important class of dyes of oxazine series [1-6] and exhibit biological activities [7a-c].

The discovery and development of triphenodioxazines (TPDOs) and their derivatives are useful for colouring plastics, varnishes, lacquers, viscose, rubber, and paper in different shades [8-10]. Their sulfonated derivatives have been widely used as direct cotton dye stuffs possessing high fastness against photochemical degradation [11]. These have also been useful as potential pharmaceutical agents [12a,b] such as antibacterial [13,14], antifungal [15-17], muscle relaxant and hypnotic agents [17], tranquillizers [18], antipsychotic [19], antihistamine [20], antiinflammatory [21] and antitumor [22,23] principles and in addition to some recent applications regarding the preparation of semiconductors [24,25], nonlinear optical wave guiding polymer films [26-28] bearing a unique photovoltaic property [29].

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

MATERIALS AND METHODS

General procedure for the synthesis of [1,4]benzoxazino[2,3-*b*]phenoxazine derivatives (3a-d): 15 ml of ethanol was taken into R.B flask and added 1.5 mmol of substituted *ortho*-aminophenol **2(a-d)** followed by 1.5 mmol anhydrous sodium acetate. The mixture was stirred at room temperature for 15 minutes. Then added 1.5 mmol of substituted 2-bromo-1,4-dimethoxy-3*H*-phenoxazin-3-one **1(a-d)** and heated at reflux temperature for 3-5 hours. After completion of the reaction, cooled to room temperature, poured into water and extracted with EtOAc. The organic layers were washed with brine and dried over anhydrous sodium sulfate. Evaporation of solvent obtained the crude product and it was purified by silica gel column chromatography using variants of ethyl acetate petroleum ether mixture. Evaporation of solvent afforded the pure products **3(a-d)** (Scheme 1). Structural characterization of the compounds was carried out by the use of literature [30].



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 °C. For bioassays, a suspension of approximately 1.5×10^8 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 °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**.

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

S. No	Compound	Zone of inhibition (in mm)																	
		<i>E. coli</i>			<i>Salmonella paratyphi</i>			<i>Klebsiella pneumoniae</i>			<i>Staphylococcus aureus</i>			<i>Micrococcus luteus</i>			<i>Bacillus cereus</i>		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	3a	13	14	14	-	10	12	12	12	13	12	13	14	-	-	10	14	14	15
2	3b	16	18	18	17	17	15	13	16	16	14	14	14	16	17	17	16	16	16
3	3c	10	10	11	10	16	16	10	12	13	-	10	11	-	-	-	12	15	15
4	3d	10	15	15	13	14	15	10	14	15	12	15	15	12	14	14	16	20	19
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.

Almost All the compounds **3(a-d)** showed moderate to good inhibitory activity against gram-negative and gram-positive bacteria. Compound **3b** is found to be showed good activity against *E.coli* and compound **3d** is found to exhibit good activity against *Bacillus cereus*. Compounds **3a** and **3c** not showed any activity against *Micrococcus luteus*.

CONCLUSION

The molecules synthesized as the part of present investigation with active groups as substituents are subjected to antibacterial screening. The compounds exhibited moderate to good activities. All microorganisms tested were sensitive to compounds (**3a-d**), except for *Micrococcus luteus* which is insensitive to compounds **3a** and **3c** as exhibited by the zone of inhibition. It has been found that chloro and methoxy substituted 6,13-dimethoxy[1,4]benzoxazino[2,3-*b*]phenoxazines (**3b**, **3d**) showed good antibacterial activity in comparison with unsubstituted (**3a**) and methyl substituted (**3c**) 6,13-dimethoxy[1,4]benzoxazino[2,3-*b*]phenoxazines.

Acknowledgements

We are thankful to the Department of Chemistry, Govt. Degree College, Peddapalli and the Department of Chemistry Satavahana University, Karimnagar for providing necessary facilities and encouragement for the execution of present research work. We are sincerely thankful to the Department of Microbiology, Kakatiya University, Warangal for providing antibacterial screening facility.

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