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Synthesis and antimicrobial activity of novel series of benzoxazinone containing piperazine derivatives

Devikasubramaniyan[1]*, Rameshpetchi Rajendran[1], Vankudoth Aruna[1]

[1]Department of Pharmaceutical Chemistry, Bomma Institute of pharmacy, Affiliated to JNTUH, Allipuram, Khammam – 507318, Telangana, India.

***Corresponding Author: Devikasubramaniyan**
Email: devikasubramaniyan@gmail.com

ABSTRACT

The present work is concerned with the synthesis of some new compounds comprising different benzoxazinones 4-(3-(dimethylamino-propyl)-4H-benzo[b][1,4]oxazin-3-one containing secondary amines derivatives.. Their potential therapeutically significance tested against three strains of Gram positive bacteria (Staphylococcus aureus Staphylococcus epidermdisis and Bacillus subtilis) and three strains of Gram negative bacteria (Escherichia coli, Pseudomonas aeruginsoa and Klebsiella pneumoniae) by agar well diffusion method. The antibacterial activity of synthesized derivatives were compared to reference standard antibiotics Penicillin and streptomycin. The present study revealed that 4-(3-(dimethylamino-propyl)-4H-benzo[b][1,4]oxazin-3-ones possess good bactericidal activity against a panel of bacteria causing common bacterial diseases and therefore opens the possibility of finding latest clinically useful antibacterial compounds. Structures of the newly synthesized compounds were established by elemental analysis and spectral data.

Keywords: 4-(3-(dimethylamino-propyl)-4H-benzo[b][1,4]oxazin-3-one, Agar well diffusion method, Anti microbial activity.

INTRODUCTION

The 2H-1,4-benzoxazin-3-(4H)-one scaffold has been studied intensively as important heterocyclic systems for building natural [2] and designed synthetic compounds. The 2H-1,4-benzoxazin-3-(4H)-ones and 3,4-dihydro-2H-1,4-benzoxazines have been frequently utilized as suitable skeletons for the design of biologically active compounds, ranging from herbicides and fungicides to therapeutically usable drugs. [1-18] The piperazine scaffold and its analogues are important pharmacophores that can be found in biologically active compounds across a number of different therapeutic areas. These include anticancer [19], antifungal [20], antibacterial, antimalarial, antipsychotic [21] agents, HIV protease inhibitors [22, 23] and antidepressants. [24]

A literature survey indicates that benzoxazinones derivatives possess different pharmacological and biological activities, of which the most potent is antimicrobial, antifungal, antiulcer and anti-inflammatory activities. In view

of above literature survey, we thought to synthesize some new substituted benzoxazinone containing piperazine derivatives. [25-28] and screening of newly synthesized compounds for antimicrobial activity.

EXPERIMENTATION

In view of biological prominence of benzoxazinones it is planned to synthesize new benzoxazinones 4-(3-(dimethylamino-propyl)-4H-benzo[b][1,4]oxazin-3-one (figure.1) containing secondary amines derivatives and screened for antimicrobial activities.

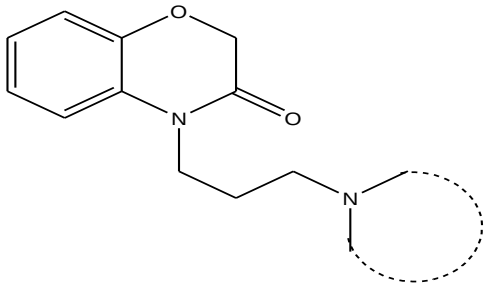


Figure1. 4-(3-(dimethylamino-propyl)-4H-benzo[b][1,4]oxazin-3-one

SYNTHESIS

Chemicals Required

Chloroacteyl chloride, Sodium bicarbonate, Tributyl ammonium chloride, Chloroform, dibromopropane, K_2CO_3, Tetra butyl ammonium iodide, Dimethyl formamide.

The title compounds were prepared by following steps.

Experimental procedure

Synthesis of 4H-benzo[b][1,4]oxazin-3(4H)-one(1)

Mechanism: Nucleophilic Substitution Reaction.

To the stirred solution of O-aminophenol (5 gm, 0.045mol) & benzyltriethyl ammonium chloride (10.45gm, 0.045mol) in 25 ml of chloroform and to this add finely powdered Sodium hydrogen carbonate (15.41 gm, 0.1834mol). The resultant mixture is cooled in an ice bath then a solution of chloroacteyl chloride (6.21 gm, 0.055mol) in chloroform is added drop wise over a period of 20 minutes. After the addition is completed the mixture is stirred at 0-5°C for 1 hour. Then it is heated at 55°C for 12 hours. The solvent was removed &water (40 ml) was added. The crude product obtained by suction was washed with water & required product was separated by column chromatography and recrystallised from ethanol. The melting point was 232-234 ^oC and percentage yield was 84 %.

Synthesis of 4-(3-(bromopropyl)-2H-benzo[b] [1, 4] oxazin-3(4H)-one (11)

Mechanism: Nucleophilic Substitution Reaction.

4H-benzo[b] [1, 4] oxazin-3(4H)-one (1) (3.40 gm, 0.022 mol) was taken in RBF. The compound was dissolved in 15ml of dimethyl formamide. To this K_2CO_3 (9.45gm, 0.068 mol) and tetra butyl ammonium iodide of (0.1335 mole) by continuous stirring for half an hour. Later to this 1, 3 dibromopropane was added and stirred for another 5 hours. The progress was monitored by TLC. Later the reaction mixture poured into water and extracted with ethyl acetate. The ethyl acetate layer was washed with water followed by brine and dried over Sodium Sulphate. The ethyl acetate layer was distilled under reduced pressures and crude product obtained was purified by column chromatography over silica gel. The melting point was 127-132 ^oC and percentage yield was 68 %.

Procedure for Synthesis of compounds (3 A-F)

4-(3-(bromopropyl)-2H-benzo[b] [1, 4] oxazin-3(4H)-one (11) of (200 mg, 0.0037moles) was taken in a round bottom flask and dissolved in anhydrous dimethyl formamide. To this potassium carbonate of (121mg, 0.00088 moles) and a pinch of tetrabutyl ammonium iodide was added. To this add compounds (3 A-F). (Figure 2).The mixture was heated at 60 °C for 12 hours. The progress of the reaction was monitored by thin layer chromatography. Later it was cooled to room temperature. The reaction mixture was extracted

with ethyl acetate and washed with water followed by brine and dried over anhydrous sodium sulphate. The ethyl acetate layer was distilled under reduced pressure and the crude product obtained was purified by column chromatography over silicagel. Physical characterization of synthesized compounds were reported in table1.

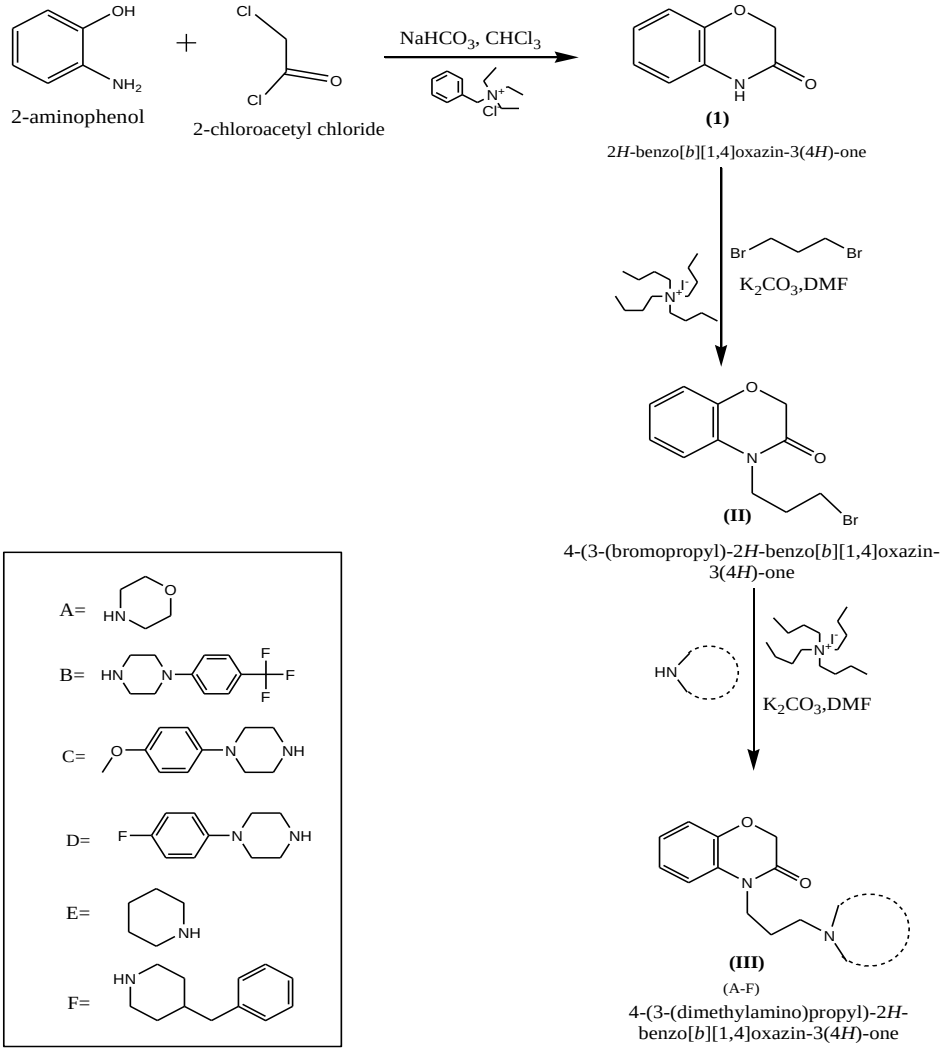
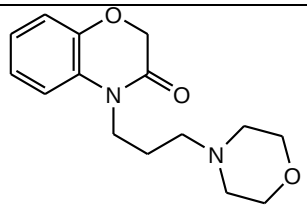
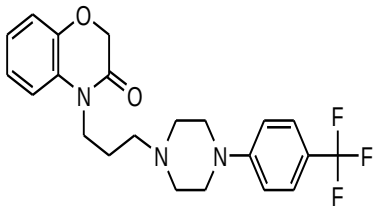
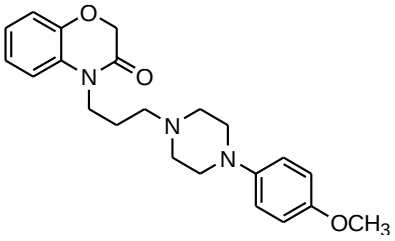
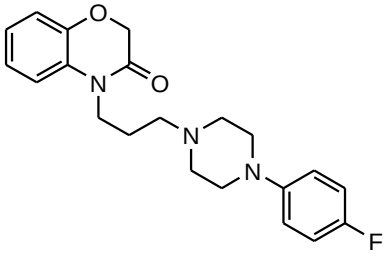
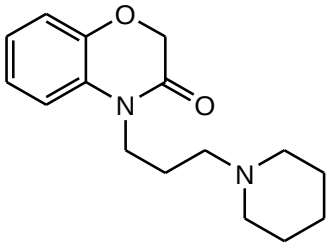
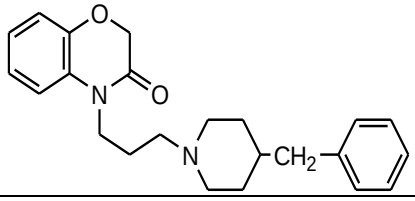


Figure.2. Scheme for synthesis of compounds (3 A-F)

Table No 1: Physical characterization of the synthesized compounds (3 A-F)

COMPOUND CODE	*COMPOUND*	*MOL. WEIGHT*	*MOL. FORMULA*	*% YIELD*
3A		*276*	$C_{15}H_{20}O_3N_2$	*82%*
3B		*419*	$C_{22}H_{24}O_2N_3F_3$	*78%*

				
3C		*381*	$C_{22}H_{27}O_3N_3$	*80%*
3D		*369*	$C_{21}H_{24}O_2N_3F$	*75%*
3E		*274*	$C_{16}H_{22}O_2N_2$	*70%*
3F		*364*	$C_{23}H28N2O2$	*79%*

ANTIBACTERIAL ACTIVITY

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial activity of the compounds was assessed by cup and plate method. The antibacterial tests were conducted on six common microorganisms which are the representative types of gram positive and gram-negative organisms as follows.Table.2

Table 2 : Microorganism used in determination of antimicrobial activity

Gram positive bacteria	Gram negative bacteria
Bacillus subtilis	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Escherichia coli*
Staphylococcus epidermdisis	*Klebsiella pneumoniae*

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar.

Materials used

Sterilized Petri dishes, Pipettes and watch glasses,18-27 h old grown culture in dishes, Sterilized 6 mm cork borer, Sterilized inoculation loop, Sterilized test tubes, graduated nutrient broth Sterilized fine pointed forceps. Sterile tubercular syringes.

Preparation of Nutrient broth

Composition:

Peptone (Bacteriological)	20 g.
Beef extract (Bacteriological)	: 5 g.
Sodium Chloride	: 5 g.
Distilled water up to	: 1000 ml.

Nutrient broth is prepared by dissolving all these and steam for about 2 h, adjust the reaction mixture pH to about 7.2 and autoclave at 15 lbs pressure for 20 minutes. One day prior to the testing, the organisms obtained from the laboratory stock were subculture into sterile nutrient broth and incubated at 37 ^{o}C for 18-24 h. The culture growth thus obtained was used as inoculums for the antibacterial testing.

Preparation of nutrient agar media

The nutrient agar media was prepared by using the following ingredients.

Peptone (Bacteriological)	: 20 g.
Beef extract (Bacteriological)	: 5 g.
Sodium chloride (Bacteriological)	: 5 g.
Agar (Bacteriological)	: 20 g.
Distilled water up to	: 1000 ml.

Weighed quantities of peptone, beef extract were dissolved in distilled water by gentle warming, and then the specified amount of agar was dissolved by heating on boiling water bath. Then the pH of the above solution is adjusted by adding sodium chloride and the volume of final solution is made up to 1000 ml with distilled water. Then the above prepared nutrient agar media is sterilized by autoclave at 121 ^{o}C for 20 minutes at 15 lbs/in^2 pressure.

Preparation of test solution

20 mg of the test compound was dissolved in 20 ml of DMF, from this stock solution, 1 ml of solution was taken and further diluted to required concentration with DMF. These sample solution were made in suitably labeled sterilized test tubes.

Preparation of standard solution

The standard drug used for the comparison are Penicillin and Streptomycin, the solutions were prepared from sterile water soluble.

Method of testing

The above prepared nutrient agar media is cooled to 45 ^{o}C with gentle shaking to bring about uniform cooling. To this 0.5–0.6 ml of 18-24 h old culture was injected aseptically and mixed well by gentle shaking. This was poured onto the petridishes and was allowed to set for 1 hour.

Thereafter the cups were made by punching into the set agar with a sterile cork borer and scooping out the punched part of the agar. The diameter of each cup was 6 mm. To these cups 50 µl of the test compound was put, which was prepared in DMF. After adding the drug solution, it was allowed to diffuse for about 45 minutes, at room temperature. Then the plates were incubated at 37 ^{o}C for 24 h in an incubator. The minimum inhibitory concentration (MIC) is taken as a parameter of antibacterial activity and reports were recorded in Table3.

Table 3: Antibacterial activity of synthesized compounds (3_{A-F})

	Minimum Inhibitory Concentration (µg/ml)					
Compound Code	***Gram Positive Organism***			***Gram Negative Organism***		
	B.Subtilis	***S.aureus***	***S.epidermis***	***E.coli***	***P.aeroginosa***	***K.pneumoniae***
3_A	*23.15*	*150*	*17.10*	*75*	*150*	*18.0*
3_B	*18.60*	*75*	*22.50*	*14.55*	*13.0*	*75*
3_C	*24.75*	*4.65*	*75*	*75*	*17.75*	*16.75*
3_D	*24.50*	*14.25*	*1.16*	*75*	*75*	*29.35*
3_E	*75*	*30.15*	*75*	*150*	*150*	*75*

3_F	*21.60*	*75*	*85*	*13.50*	*75*	*150*
Penicillin	*1.526*	*6.25*	*3.125*	*7.81*	*12.50*	*6.25*
Streptomycin	*6.25*	*1.56*	*1.56*	*3.125*	*3.125*	*3.125*

Standard: Penicillin, Streptomycin.
Note: 20 µg/ml and above poor activity, 14-20 µg/ml moderate activity and 4-13 µg/ml significant activity.

RESULTS AND DISCUSSION

All the synthesized compounds were screened for antibacterial activity studies against Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pnenmoniae by cup-plate technique on nutrient agar media, Penicillin and Streptomycin used as standard against Gram positive and Gram-negative bacteria.

The data in the **Table 3and Figure 3** indicate that, Compound $\mathbf{3_C}$ show significant activity and compound $\mathbf{3_D}$ shows moderate activity against staphylococcus aureus. Compound $\mathbf{3_D}$ also shows moderate activity against staphylococcus epidermidis. Compound $\mathbf{3_B}$ shows moderate activity against Escherichia coli.Compound $\mathbf{3_B}$ and Compounds $\mathbf{3_C}$ shows moderate activity against Pseudomonas aeruginosa. Compound $\mathbf{3_A}$ and Compounds $\mathbf{3_C}$ shows moderate activity against Klebsiella pneumoniae. And rest of the compounds were found to exhibit poor activity when compared to the standard Penicillin and Streptomycin.

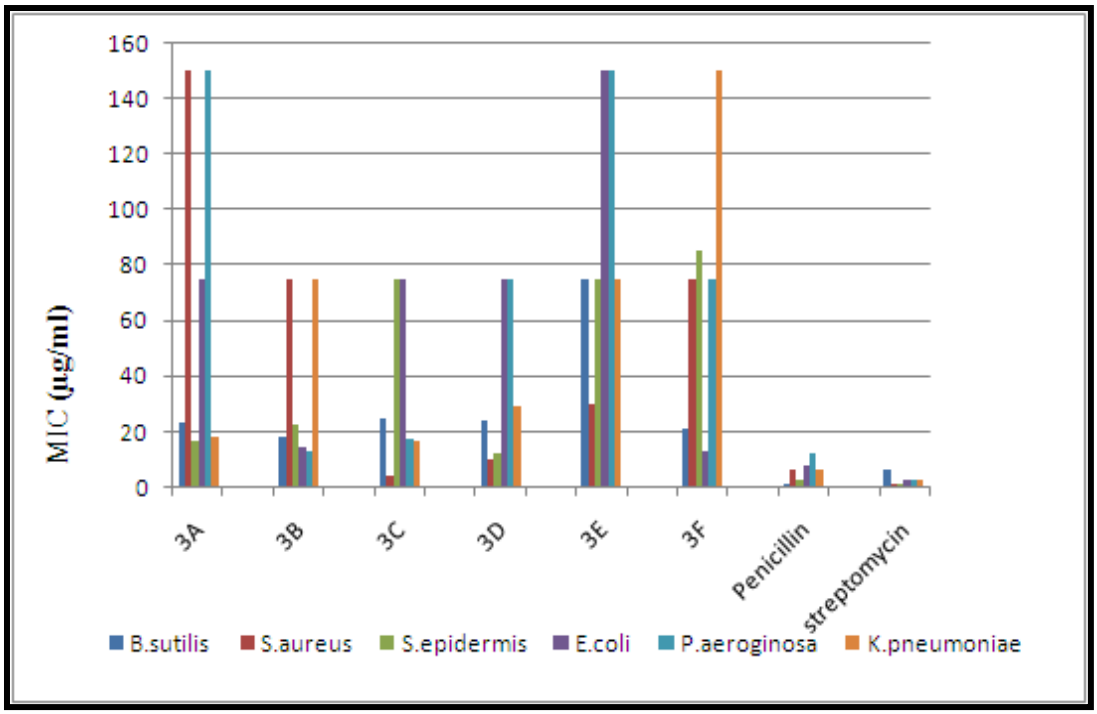


Fig No.3 Antibacterial activity of synthesized compounds (3_{A-F})

CONCLUSION

It is clearly concluded that the synthesized benzoxazinone derivatives were found to be moderate to weak antibacterial agents. When the two moieties are fused or combined and screened for antibacterial studies they showed moderate to weak antibacterial activity against Gram (+ve) and Gram (-ve) bacteria. The substituted benzoxazinone derivatives are already known for different biological activity. Further the detail structure activity relationship studies are required along with the molecular manipulation i.e. molecular modeling may give better drugs and further toxicological study is needed. Molecules prepared for the biological testing do not always turn out as potential new molecules, but may be intended to serve as models for evaluation of the hypothesis.

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