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SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF NEW BENZOXAZOLE DERIVATIVES AS ANTIBACTERIAL AGENTS

Anusha P[*1], J. Venkateshwar Rao[2] and G. Krishna Mohan[3]

[1]Bojjam Narasimhulu Pharmacy College for Women, Hyderabad, Telangana, India
[2]Talla Padmavathi college of Pharmacy, Warangal, Telangana, India
[3]Centre for Pharmaceutical Sciences, JNTU-H, Hyderabad, Telangana, India

*Corresponding Author Email: anushapharma01@gmail.com

ABSTRACT

Benzoxazole derivatives are very useful compounds with well-known biological activity. Various novel benzoxazole derivatives were synthesized by reacting with succinic anhydride and aromatic aldehydes. They are structurally characterized by IR, 1H-NMR, Mass spectroscopy methods and further investigated for antibacterial activity by using cup plate method against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and proteus vulgaris using Amphicillin sodium as a standard. Among all the compounds IXd and IXf were more effective against bacteria.

KEY WORDS

Anti-bacterial activity, Benzoxazole derivatives, IR, 1HNMR, Mass spectroscopy.

INTRODUCTION

Phenyl ring fused with Oxazole is considered as Benzoxazole moiety which has molecular formula C_7H_5NO. Biologically active benzoxazole derivatives have been known for long time, they are the isosteres of cyclic nucleotides and easily interact with the biopolymers of the organisms [1]. Targets containing the benzoxazole moiety either isolated from plants or accessed by total synthesis have remarkable biological activities like antibacterial [2,3], anti-inflammatory [4,5], antifungal [6], antitumor [7], antihistaminic [8] and ant tubercular activities [9]. Here in the present study attempt was made to synthesize novel benzoxazole derivatives with good biological activity and less toxic effects.

The title compounds were synthesized by treating the 2-(5-(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl) thio acetohydrazide with appropriate aromatic aldehydes to get a new series of N′-benzylidene-2-(5-(3,6-dioxotetrahydropyridazine-1-carbonyl) benzoxazole-2-yl) thio)acetohydrazide (IXa – IXf).

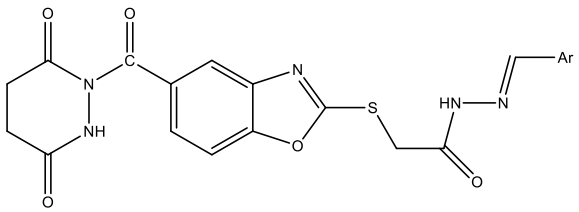


Figure 1: N′-benzylidene-2-(5-(3,6-dioxotetrahydropyridazine-1-carbonyl) benzoxazole- 2-yl) thio) acetohydrazide

MATERIALS AND METHODS

All the reagents and solvents used were of laboratory grade. The melting points of synthesized compounds were determined by open capillaries using cintex melting point apparatus, expressed in °c and were uncorrected. The IR spectra of the compounds were recorded using KBR pellets on perkin Elmer 337 spectrophotometer. 1HNMR spectra were recorded on Avance-300 MHz spectrophotometer using DMSO as solvent and TMS as an internal standard (chemical shifts in δ, ppm). Mass spectra were recorded on liquid chromatography Mass spectrophotometer.

SYNTHESIS AND CHARACTERIZATION OF COMPOUNDS

I. Synthesis of 4-carbomethoxy-2-nitrophenol (II)

To a solution of aluminium nitrate (40 g) in acetic acid acetic anhydride (1:1) mixture (160 ml), was added an appropriate phenol (I, 40g) in small portions, while cooling and shaking, occasionally. The reaction mixture was left at room temperature for 1.5 hours while shaking the contents, intermittently to complete the nitration. The resulting brown solution was diluted with ice-cold water (500 ml) and acidified with concentrated nitric acid (40 ml) to get a bulky, yellow precipitate. It was filtered washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline solid m.p.73°C, yield 85%.

II. Synthesis of 4-carbomethoxy-2-aminophenol (III)

4-Carbomethoxy-2-nitrophenol (II, 10 g) was dissolved in boiling alochol (50%, 100 ml) and sodium dithionite was added to this boiling alcohol solution until it becomes almost colourless. Then the alcohol was reduced to one-third of its volume by distillation and the residual liquid was triturated with ice-cold water. The resulting colourless, shiny product was filtered, washed with cold water and dried. Its purification was effected by recrystallisation from benzene to get colourless,shiny scales m.p. 143°C, yield 60%.

III. Synthesis of 2-mercapto-5-carbomethoxy benzoxazole (IV)

4-Carbomethoxy-2-amino phenol (III, 0.01 mol) has been refluxed with potassium hydroxide (0.15 mol), carbon disulphide (0.15 mol), alcohol (95%) and water (45 ml) for 4 hours. The alcohol has been removed by distillation. The product obtained has been poured on to crushed ice and neutralized with acetic acid. The product thus separated has been dried and on purification by recrystallisation from methanol has resulted crystalline white solid, m.p. 214°C with an yield of 75%.

IV. Synthesis of 2-mercapto -benzoxazol-5-carboxylic acid hydrazides (V)

A mixture of an appropriate 2-mercapto-5-carbomethoxy benzoxazole (IV, 0.01 mol) in alcohol (25 ml) and hydrazine hydrate (99%, 0.015 mol) was heated under reflux, on water-bath for 4 hours. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and washed with small portions of cold alcohol first and then with cold water, repeatedly and dried. The product was purified by recrystallization from methanol. m.p.180°C yield 70%.

V. Synthesis of 1-(2-mercaptobenzoxazole-5-carbonyl)tetrahydro pyridazine-3,6-dione (VI)

A mixture of an appropriate 2-mercapto- benzoxazole -5-carboxylic acid hydrazides (V, 0.01 mol) and succinic anhydride (0.01mol) in glacial acetic acid was refluxed on water-bath for 4 hours. The product separated was filtered and washed with small portions of cold alcohol first and then with cold water, repeatedly and dried. The product was purified by recrystallization from methanol. m.p.195°C yield 65%.

VI.Synthesis of ethyl 2-(5-(3,6-dioxohexahydropyridazine-1-carbonyl)benzoxazole-2-yl)thio acetate (VII)

A mixture of 1-(2-mercaptobenzoxazole-5-carbonyl) tetrahydro pyridazine-3,6-dione (VI, 0.01 mol) and ethyl 2-chloroacetate(0.01mol) in KOH was refluxed on water bath for 3 hours. The resultant product was cooled , separated by filtration and washed with cold water and dried. The product was purified by recrystallization from methanol. m.p.205°C yield 65%.

VII. Synthesis of 2-(5-(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl) thio acetohydrazide (VIII)

A mixture of ethyl 2-(5-(3,6-dioxohexahydropyridazine-1-carbonyl)benzoxazole-2-yl)thio acetate (VII,0.01mol) in alcohol (25 ml) and hydrazine hydrate (99%, 0.015 mol) was heated under reflux, on water-bath for 4 hours. The alcohol was reduced to half of its volume and cooled. The product separated was filtered and washed with small portions of cold alcohol first and then with cold water, repeatedly and dried. The product was purified by recrystallization from methanol. m.p.210°C yield 70 %.

VIII. Synthesis of N'-benzylidene-2-(5(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl) thio acetohydrazide (IX)

A mixture of 2-(5-(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl) thio acetohydrazide (VIII, 0.01mol) and an appropriate aromatic aldehyde (0.015 mol) in alcohol (20 ml) with 2 to 3 drops of acetic acid, heated under reflux on a water bath for one hour. The product thus obtained was filtered, washed with water dried and purified by recrystallization from suitable solvents.

RESULTS AND DISCUSSION

Compound IX a

IR (KBR,cm^{-1}): 3219(NH), 1679(N-C=O), 1635(C=N), 1510(Ar, C=C), 2575(C-S), 768(Ar,CH), 1324(C-N).

^{1}H NMR (DMSO-d6):11.2(s,1H,NH), 11.07(s,1H,NH), 8.14(d,1H,Ar-H), 8.84(s,1H,Ar-H), 8.47(s,1H,Ar-H), 7.93(d,1H,Ar-H), 7.58(t,1H,Ar-H), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$).

M/z peak observed at 451 and M+1 peak observed at 452.

Compound IX b

IR (KBR,cm^{-1}): 3226(NH), 1664(N-C=O), 1630(C=N), 1510(Ar, C=C), 2565(C-S), 768(Ar,CH), 1324(C-N), 1010(C-Br)

^{1}H NMR (DMSO-d6): 11.2(s,1H,NH), 11.07(s,1H,NH), 8.14(d,1H,Ar-H), 8.84(s,1H,Ar-H), 8.47(s,1H,Ar-H), 7.93(d,1H,Ar-H), 7.58(t,1H,Ar-H), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$).

M/z peak observed at 530 and M+1 peak observed at 531.

Compound IX c

IR (KBR,cm^{-1}): 3445(OH), 3224(NH), 1685(N-C=O), 1615(C=N), 1505(Ar, C=C), 2576(C-S), 778(Ar,CH), 1324(C-N).

^{1}H NMR (DMSO-d6): 11.5(s,1H,OH), 11.2(s,1H,NH), 11.07(s,1H,NH), 8.24(d,1H,Ar-H), 8.74(s,1H,Ar-H), 8.47(s,1H,Ar-H), 7.93(d,1H,Ar-H), 7.52(t,1H,Ar-H), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$).

M/z peak observed at 467 and M+1 peak observed at 468.

Compound IX d

IR (KBR,cm^{-1}): 3116(NH), 1623(N-C=O), 1615(C=N), 1510(Ar, C=C), 2575(C-S), 768(Ar,CH), 1324(C-N).

^{1}H NMR (DMSO-d6): 11.2(s,1H,NH), 11.07(s,1H,NH), 8.14(d,1H,Ar-H), 8.84(s,1H,Ar-H), 8.47(s,1H,Ar-H), 7.93(d,1H,Ar-H), 7.58(t,1H,Ar-H), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$), 3.81(s,3H,OCH_3).

M/z peak observed at 481 and M+1 peak observed at 482.

Compound IX e

IR (KBR,cm^{-1}): 3215(NH), 1626(N-C=O), 1615(C=N), 1510(Ar, C=C), 2575(C-S), 768(Ar,CH), 1324(C-N).

^{1}H NMR (DMSO-d6): 11.2(s,1H,NH), 11.07(s,1H,NH), 8.14(d,1H,Ar-H), 8.84(s,1H,Ar-H), 8.47(s,1H,Ar-H), 7.97(d,1H,Ar-H), 8.27(d,1H,Ar-H),8.41(s,1H,CH), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$),

M/z peak observed at 496 and M+1 peak observed at 497.

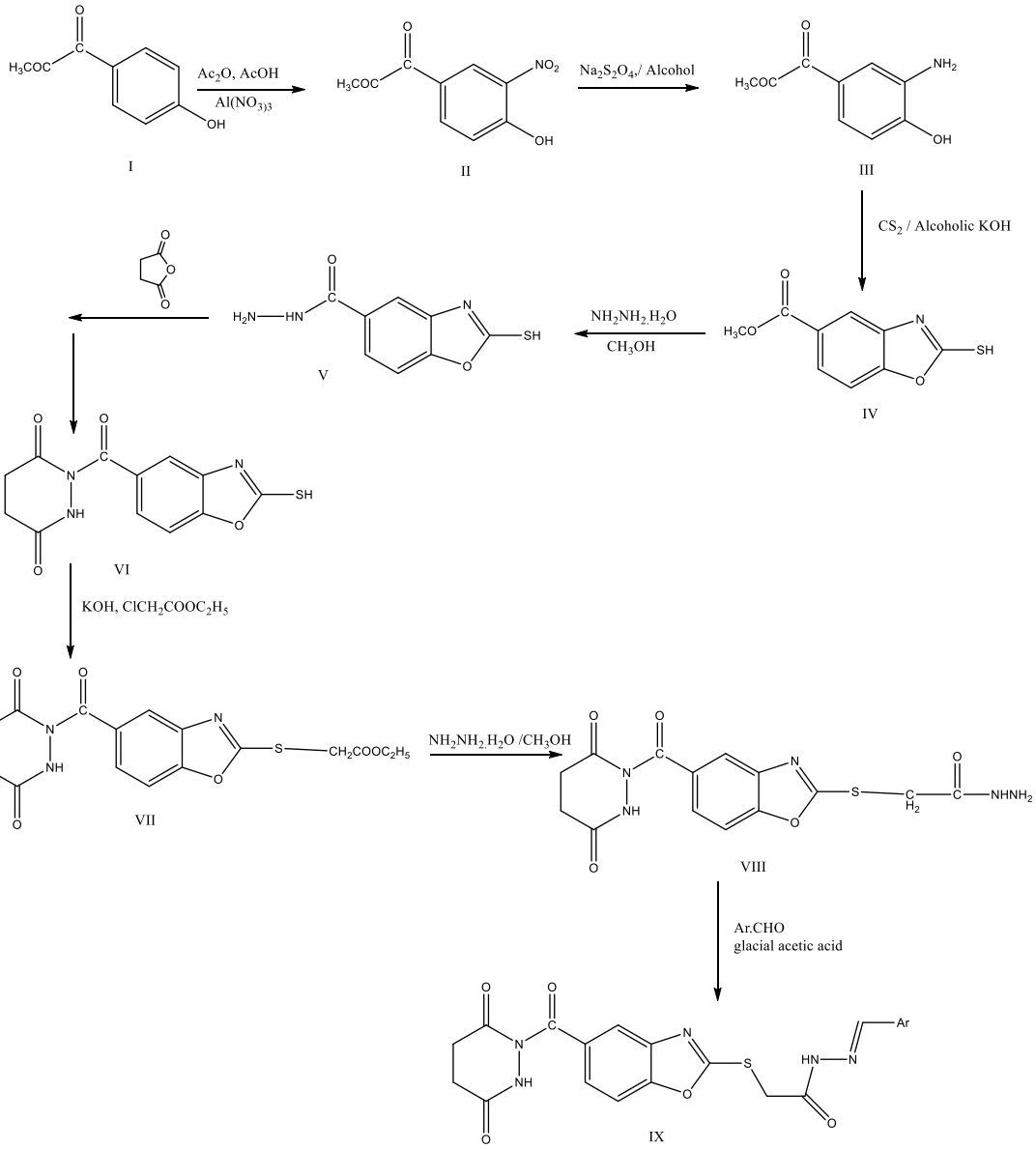
Compound IX f

IR (KBR,cm^{-1}): 3232(NH), 1634(N-C=O), 1623(C=N), 1510(Ar, C=C), 2575(C-S), 768(Ar,CH), 1324(C-N), 1573(N-O).

^{1}H NMR (DMSO-d6): 11.2(s,1H,NH), 11.07(s,1H,NH), 8.10(d,1H,Ar-H), 8.64(s,1H,Ar-H), 8.47(s,1H,Ar-H), 6.82(d,1H,Ar-H), 8.24(d,1H,Ar-H),8.41(s,1H,CH), 4.00(s,2H,CH_2), 2.27(s,4H,$(CH_2)_2$), 3.02(s,6H,$2CH_3$).

M/z peak observed at 494 and M+1 peak observed at 495.

SCHEME OF SYNTHESIS



ANTIBACTERIAL ACTIVITY

The antibacterial activity of title compounds was assayed against four different strains of bacteria by agar diffusion method.

Two Gram-Positive Bacteria: *Bacillus subtilis* and Staphylococcus *aureus*

Two Gram-Negative Bacteria: *Escherichia coli* and Proteus *vulgaris*

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. The bacterial inhibition can be measured by two methods: one is serial dilution method and the other is diffusion method. The serial dilution method is very useful for the determination of antimicrobial activity. It is not much useful for the quantitative detection tests and for the evaluation of large number of compounds. The agar diffusion is of three types.

1. Cup-plate method (disc method)
2. Filter-paper strip method
3. Gradient plate method

The method adopted in this investigation was cup-plate method. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of zone of inhibition was measured.

CULTURED MEDIUM

Nutrient broth was used for the preparation of inoculums of the bacteria and the nutrient agar used for the screening method.

Composition of Medium, nutrient agar:

Peptone	5.0gm
Sodium chloride	5.0gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water	1000ml
pH	7.4±0.2

The test organism was sub cultured using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strain. After incubation at 37 ± 1ºC for 24 hours, they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of stock culture to nutrient broth. The flasks were incubated at 37 ± 1ºC for 48 hours before the experimentation.

Solution of test compounds was prepared by dissolving 10 mg each in dimethylsulfoxide (DMSO, 10ml). A reference standard for Gram-positive and Gram-negative bacteria was made by dissolving accurately weighed quantities of Ampicillin in DMSO (10µg/ml).

The nutrient agar medium was sterilized by autoclaving at 121ºC (15lb/sq.inch) for 15 minutes. Petri-plates, tubes and flasks plugged in cotton were sterilized in hot-air oven at 160ºC for an hour. Into each sterilized Petri-plate (10cm diameter), about 27ml of molten nutrient agar medium inoculated with the respective strain of bacteria (50µl of inoculum into each plate) was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, three discs of 6mm diameter were made with a sterile borer. These solutions at concentrations (200µg/ml, 150µg/ml, and 100µg/ml) was added to respective disc aseptically and labeled accordingly. The plates were kept undisturbed for 1 hour at room temperature to allow the diffusion of the solution properly in the nutrient agar medium. After incubation of the plates at 37 ± 1ºC for 24 hours, the diameter of zone inhibition surrounding each of discs was measured with the help of an antibiotic zone reader. All the experiments were carried out in triplicate. Simultaneously, controls were maintained employing 0.1ml of DMSO to observe the solvent effects.

The zone of inhibition values of the synthesized compounds against *Bacillus subtilis, Staphylococcus auresus, Escherichia Coli* and *Proteus vulgaris* bacteria were presented in Table 2.

Ampicillin was used for the reference for inhibitory activity against bacteria.

It has been observed that all the test compounds, showed mild to moderate activity against the bacteria, compound **IX d** (4-Methoxyphenyl) was more potent as anti- bacterial against both gram (+ve) and gram (-ve) organisms among all the test compounds. This was followed by compound **IX f**, **IX b, IX c.**

Table 1: Physical Data of N'-(5-(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl)thio acetohydrazide derivatives (IX)

S.No	Compound	Ar	Mol. Formula	Melting point (°C)	% yield
1	IX a	phenyl	$C_{21}H_{17}N_5O_5S$	210	79
2	IX b	4-Bromophenyl	$C_{21}H_{16}BrN_5O_5S$	214	73
3	IX c	4-Hydroxyphenyl	$C_{21}H_{17}N_5O_6S$	237	84
4	IX d	4-Methoxyphenyl	$C_{22}H_{19}N_5O_6S$	233	82
5	IX e	2-Nitrophenyl	$C_{21}H_{16}N_6O_7S$	240	76
6	IX f	4-Dimethyl amino phenyl	$C_{23}H_{22}N_6O_5S$	215	82

Table-2: Antimicrobial activity of N'-(5-(3,6-dioxohexahydropyridazine-1-carbonyl) benzoxazole-2-yl)thio acetohydrazide derivatives (IX)

S. No	Derivatives		Concⁿ in µg/ml	Zone of inhibition (in mm)			
	Compound No	Ar		B.subtilis	S.aureus	E.coli	P.vulgaris
1	IX a	phenyl	100	10	12	12	10
			150	11	11	10	10
			200	12	12	11	11
2	IX b	4-Bromophenyl	100	12	12	11	10
			150	11	13	11	11
			200	13	14	12	11
3	IX c	4-Hydroxyphenyl	100	10	10	11	11
			150	11	9	9	10
			200	10	9	10	9
4	IX d	4-Methoxyphenyl	100	15	13	14	11
			150	16	14	15	13
			200	17	14	15	14
5	IXe	2-Nitrophenyl	100	7	8	8	7
			150	7	7	8	7
			200	8	7	6	8
6	IX f	4-Dimethylaminophenyl	100	14	12	12	9
			150	16	13	14	10
			200	16	15	14	11
Standard drug (Ampicillin)			10	18	20	19	18

CONCLUSION

From the above results we can conclude that benzoxazole derivatives showed promising antibacterial activity. The most potent compound was found to be IXd (4-methoxy phenyl) with high zone of inhibition.

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***Corresponding Author:**
Anusha P*
Email: anushapharma01@gmail.com