Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 4| Issue 4|OCT-DEC|2014|83-89

Research Article
Pharmaceutical Sciences

SYNTHESIS AND BIOLOGICAL EVALUATION OF BENZOXAZOLE DERIVATIVES AS NEW ANTI MICROBIAL AGENTS

P Anusha [*1], J Venkateshwar Rao [2]

[1] Bojjam Narasimhulu Pharmacy College for Women, Vinaynagar, Hyderabad-500079

[2] Talla padmavathi college of pharmacy, Warangal, Telangana, India-506002

*Corresponding Author Email: anushapharma01@gmail.com

ABSTRACT

Heterocyclic compounds containing oxazole moiety plays an important role in medicinal chemistry and exhibit wide range of biological activities. The main objective of the medicinal chemistry is to synthesize the compounds that show promising activity as therapeutic agents with lower toxicity. Benzoxazole derivatives are very useful compounds with well known biological activity. In the current research work, the title compounds 2-mercapto-N-(substituted arylidine) benzoxazole-5-carbohydrazide derivatives were synthesized by the reaction of Schiff bases of 2-mercapto benzoxazole-5-carbohydrazide with appropriate aromatic aldehydes. The synthesized compounds were confirmed structurally by means of IR, 1HNMR, Mass spectral analysis. Further, the synthesized compounds (VIa-VIf) were screened for antimicrobial activity by using agar diffusion method. The results showed that, compound VId showed promising antimicrobial activity; whereas the compounds i.e., VIb, VIa, VIc, VIf moderately showed antimicrobial activity. Only one compound VIe showed very poor antimicrobial activity.

KEY WORDS

Anti microbial activity, Benzoxazole derivatives, IR, 1HNMR, Mass spectroscopy.

INTRODUCTION

Targets containing the benzoxazole moiety either isolated from plants or accessed by total synthesis have remarkable biological activities. Benzoxazole finds use in research as a starting material for the synthesis of larger bioactive structures. 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].

Recent observations suggest that targets containing benzoxazole moiety, have remarkable biological activities. For example, antimicrobial [2,3], anti-inflammatory [4,5], anti viral [6], antihistaminic [7], herbicidal [8], anti helminthic [9], anticancer [10], hypoglycemic [11], antiparasitics [12], antifungal [13], antitubercular [14], elastase inhibitors [15], protein kinase inhibitors [16], steroid sulfatase inhibitors [17]. The novel antibacterial agent containing benzoxazole system is Boxazomycin B [18]. Benzoxazole ring containing antibiotic calcymicin [19] and the anti inflammatory agent Benzoxaprofen [20] are also obtained by synthetic methods. Zoxazolamine [21], an α-amino-5-chlorobenzoxazole is reported to possess muscle relaxant, sedative and uricosuric effect.

The title compounds were synthesized by treating the 2-mercapto benzoxazole-5-carbohydrazide with appropriate aromatic

aldehydes to get a new series of 2-mercapto -N- (substituted arylidene) benzoxazole -5- carbohydrazide (VIa – VIf).

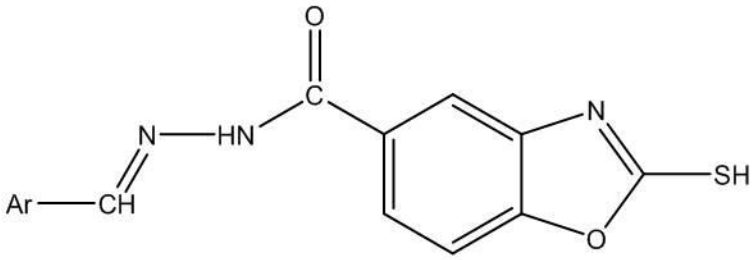


Figure 1: 2-Mercapto-N-(Substituted arylidene) benzoxazole-5- carbohydrazide

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 [22] 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 yields 70%.

V. Synthesis of 2-mercapto-N-(substituted arylidine) benzoxazole-5- (VI)

A mixture of an appropriate 2-mercapto benzoxazol-5-carboxylic acid hydrazide (V, 0.01 mol) 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 solvent(s). The physical data of these benzoxazole derivatives were given in **Table 1**.

Table 1: Physical Data of 2-Mercapto-N-(substituted arylidene) benzoxazole-5-carbohydrazide.

S.No	Compound	Ar	Mol.Formula	Melting point (°C)	% yield
1	VI a	4-Chlorophenyl	$C_{15}H_{10}ClN_3O_2S$	230	79
2	VI b	4-Flourophenyl	$C_{15}H_{10}FN_3O_2S$	210	74
3	VI c	4-Hydroxyphenyl	$C_{15}H_{11}N_3O_3S$	237	88
4	VI d	4-Methoxyphenyl	$C_{16}H_{13}N_3O_3S$	235	82
5	VI e	2-Nitrophenyl	$C_{15}H_{10}N_4O_4S$	240	80
6	VI f	4-Dimethyl amino phenyl	$C_{17}H_{16}N_4O_2S$	207	82

RESULTS AND DISCUSSION

Compound VI a

IR (KBR, cm^{-1}): 3200(NH), 1710(C=O), 1635(C=N), 1294(C-O-C), 2575(SH).

^{1}H NMR (DMSO-d6): 9.6(s, 1H, NH), 7-8(d, 8H, Ar-H, CH), 2.2(s,1H,SH).

MS (*m/z*): M+ calculated 331, found 330.

Compound VI b

IR (KBR, cm^{-1}): 3284(NH), 1712(C=O), 1355(C-O-C), 700(C-H), 2610(SH).

^{1}H NMR (DMSO-d6): 9.7(s, 1H, NH), 7-8(d, 8H, Ar-H,CH), 2.5(s,1H,SH).

MS (*m/z*): M+ calculated 315, found 316.

Compound VI c

IR (KBR, cm^{-1}): 3445(OH), 3210(NH), 1690(C=O), 1560(C=N), 1265(C-O-C),2613(SH).

^{1}H NMR (DMSO-d6): 11.5(s,1H,OH), 9.8(s,1H,NH), 7-8(d,8H,Ar-H,CH), 2.6(s,1H,SH).

MS (*m/z*): M+ calculated 313, found 314.

Compound VI d

IR (KBR, cm^{-1}): 3210(NH), 1670(C=O), 1605(C=N), 1310(C-O-C), 2565(SH).

^{1}H NMR (DMSO-d6): 9.6(s,1H,NH), 7-8(d,8H,Ar-H,CH), 3.7(s,3H,CH_3), 2.5(s,1H,SH).

MS (*m/z*): M+ calculated 327, found 326.

Compound VI e

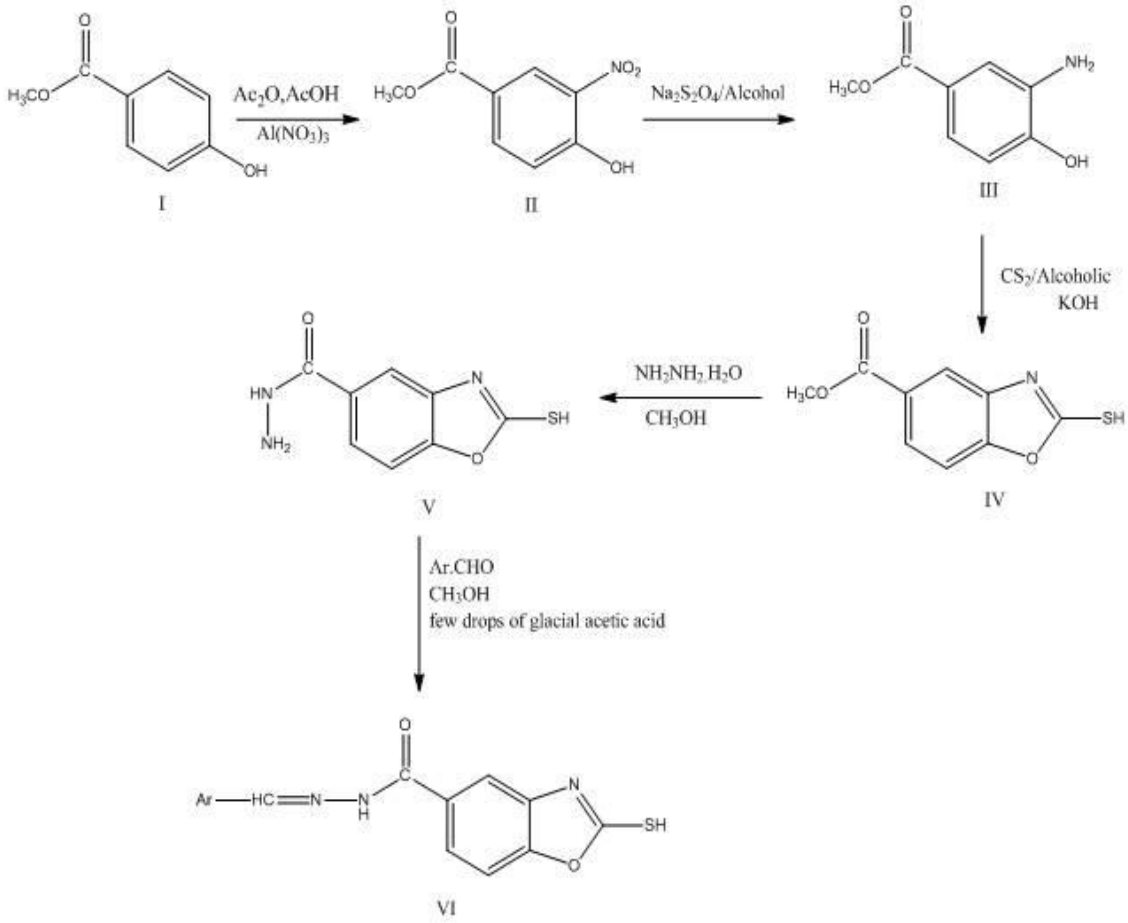
IR (KBR, cm^{-1}): 3250(NH), 1688(C=O), 1625(C=N), 1300(C-O-C), 2605(SH).

^{1}H NMR (DMSO-d6): 9.5(s, 1H, NH), 7-8(d, 8H, Ar-H,CH), 2.1(s,1H,SH).
MS (*m/z*): M+ calculated 342, found 341.

Compound VI f

IR (KBR, cm^{-1}): 3240(NH), 1700(C=O), 1620(C=N), 1255(C-O-C), 700(C-H), 2578(SH).
^{1}H NMR (DMSO-d6): 9.5(s,1H,NH), 7-8(d,8H,Ar-H,CH), 3.5(s,6H,CH_3), 2.6(s,1H,SH).
MS (*m/z*): M+ calculated 340, found 339.

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 extracts	**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 ± 1^{0}C 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 ± 1^{0}C 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 121^{0}C (15lb/sq.inch) for 15 minutes. Petri-plates, tubes and flasks plugged in cotton were sterilized in hot-air oven at 160^{0}C 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 ± 1^{0}C 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 **VI 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 **VI b, VI a, VI c, VI f, VI e**.

Table-2: Antimicrobial activity of N'- benzylidine benzoxazol - 5 - carbohydrazide derivatives (VI)

S. No	Derivatives		Conc^{n} in µg/ml	Zone of inhibition (in mm)			
	Compound No	Ar		B.subtilis	S.aureus	E.coli	P.vulgaris
1	VI a	4-Chlorophenyl	100	11	12	12	10
			150	10	11	10	10
			200	12	12	11	11
2	VI b	4-Fluorophenyl	100	12	12	11	10
			150	11	13	11	11
			200	13	14	12	11
3	VI c	4-Hydroxyphenyl	100	10	10	11	11
			150	11	9	9	10
			200	10	9	10	9
4	VI d	4-Methoxyphenyl	100	14	12	13	11
			150	15	12	14	13
			200	15	13	14	14
5	VI e	2-Nitrophenyl	100	7	8	8	7
			150	7	7	8	7
			200	8	7	6	8
6	VI f	4-Dimethylamino phenyl	100	10	9	8	9
			150	9	8	9	10
			200	9	9	8	11
Standard drug (Ampicillin)			10	18	20	19	18

CONCLUSION

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

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Talla Padmavathi College of Pharmacy, Warangal, Telangana, for providing laboratory facilities and financial support.

REFERENCES

1. Turker L., Sener E., Yalcin I., Akbulut U., Kayalidere I., QSAR of some antifungal- active benzoxazole using the quantum chemical parameters, Scientia Pharmaceutica, 58, 107-113, (1990)
2. Sultan Nacak, Seyban Ersan, Rukiye Berkem and Tancel Ozden. Arzneim-Forsch/Drugs Res. 41: 963, (1997).
3. M.Sarangapani and V.Malla Reddy (1994), Indian J. Pharm. Sci., 56, 174-179, (1994)
4. Dawid W. Dunwell, Delme Evans, Terrence A. Hicks, J. Med. Chem., 18, 1151-1157, (1975)
5. Unlu S, Baytas S, Kupeli E Archives der Pharmazie. 336 (6-7):310, (2003)
6. Johan Neyts, Erik De Clercq. Synthesis of Heterobicycle Coumarin conjugates and evaluation for Anti Hepatitis C. J. Med. Chem, 52, 1486-1490, (2009)
7. Yousuke Katsura, Yoshikazu Inoue, Signetaka Nishino, Masaaki Tomoi , Chem. Pharm. Bull., 40(6), 1424-1429, (1992)
8. Yuhan Zhou, Na Xue, Guowei wang. Synthesis and Herbicidal activity of Pyrazolyl benzoxazole derivatives. J. Heterocyclic Chem.,47, 15-21, (2010)

9. R.K.Saxena, Sadhana puri and Rachna Prakash, Synthesis and evaluation of 2-mercaptoacetylaminobenzoxazole-2-yl-thiadiazoles as potent Anti-helminthic agents, Indian J. Het. Chem., 13, 127-134, (2003)
10. Mireya L. Mckee, Sean M.Kerwin, Synthesis and evaluation of 2-(2'-hydroxyphenyl)benzoxazole analogues of UK-1 as anti cancer agents, Bioorg & Med. Chem., 6, 1775-1783, (2008)
11. Arakova K, Inamasu M. Masumoto M. (1997), Chem. Pharm. Bull. 45 (12), (1984).
12. Qian, Xuhong, Li, Zhibin, Sorg, Gonghua, Lizhorg (2001), J. Chem. Res. Synop. 4, 138.
13. Ismail Y, LKay O, Ozlem T, Acta Biochimica Polonical, 47, 481-486, (2000)
14. Klimensova V, Koci J, Waisser K, Kaustova J, Dahse M, Bioorganic and Medicinal Chemistry Letters, 12, 3275-3278, (2002)
15. Jalmira Mulchande, Rudi Oliveria, Marta Carrasca, New Benzoxazoles as potent Selective Inhibitors of Human Leukocyte Elastase , J. Med. Chem., 53, 241-253, (2010).
16. Sham M.Sondhi, Nirupama Singh, Ashok kumar, Olivier Lozach, Synthesis, anti-inflammatory,analgesic and kinase (CDK-1, CDK-2 and GSK-3) inhibition activity evaluation of benzimidazole/benzoxazole derivatives and some Schiff's bases , Bioorg. Med. Chem,14 (11), 3758-3765, (2006)
17. Billich, Andreas, Schreiner, Erwin Paul, Wolf-Winiski, A.G. Barlara Noverkis, GB 1999-27439 (1991), GB 2000-7511 (2001) Patent CA Section: 28 Section 1, 2, 32.
18. M.J.Suto and W.R. Turner, Tetrahed. Lett., 36 (1995) 7213-7215.
19. D.A. Evans, C.E. Sacks, W.A. Kleschick, T.R. Taser, J. Am. Chem. Soc., 101, 6789-6792, (1979)
20. D.W. Dunwell, D.Evans, T.A.Hicks, C.H. Cashin, A. Kitchen, J. Med. Chem., 18, 53-58, (1975)
21. Barton & Ollis, Comprehensive organic chemistry, Pergamon Press 4, 962, (1979)
22. Rajmohan k and Subba Rao NV, Indian. J. Chem. 11, 1076, (1973)

***Corresponding Author:**
P.Anusha*
Bojjam Narasimhulu Pharmacy College for Women, Hyderabad.
Email: anushapharma01@gmail.com