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RESEARCH ARTICLE

Synthesis and Biological Evaluation of Benzoxazole Derivatives as New Anti-Inflammatory Agents

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ABSTRACT

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 anti-inflammatory activity by using carrageenan – induced rat paw edema method. The results showed that, compound VId was significantly ($p<0.001$) reduced the inflammation there by showed a promising anti-inflammatory activity; whereas the compounds i.e., VIa, VIc, VIe, VIb moderately reduced the inflammation. Only one compound VIf showed very poor anti-inflammatory activity after one hour of administration.

KEYWORDS

Benzoxazole Derivatives, IR, 1HNMR, Mass Spectroscopy, Anti-inflammatory Activity

INTRODUCTION

Recent observations suggest that targets containing benzoxazole moiety, have remarkable biological activities. For example, antimicrobial[1,2], anti-inflammatory[3,4], anti viral[5], antihistaminic[6], herbicidal[7], anti-helminthic[8], anticancer[9], hypoglycemic[10], antiparasitics[11], antifungal[12], antitubercular[13], elastase inhibitors[14], protein kinase inhibitors[15], steroid sulfatase inhibitors[16]. 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).

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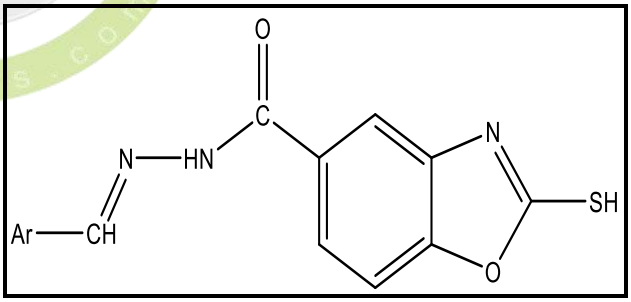


Figure 1: 2-Mercapto-N-(Substituted arylidene) benzoxazole-5- carbohydrazide

MATERIALS AND METHOD

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[17] to get a yellow crystalline solid .m.p. 73ºC, yield 85%.

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

4-Carbomethoxy-2-nitrophenol (II, 10 g) was dissolved in boiling alcohol (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. 143ºC, 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. 214ºC with a 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 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.

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).

^1H 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).

^1H 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).

^1H NMR (DMSO-d6): 9.5(s, 1H, NH), 7-8(d,8H,Ar-H,CH), 2.1(s,1H,SH).

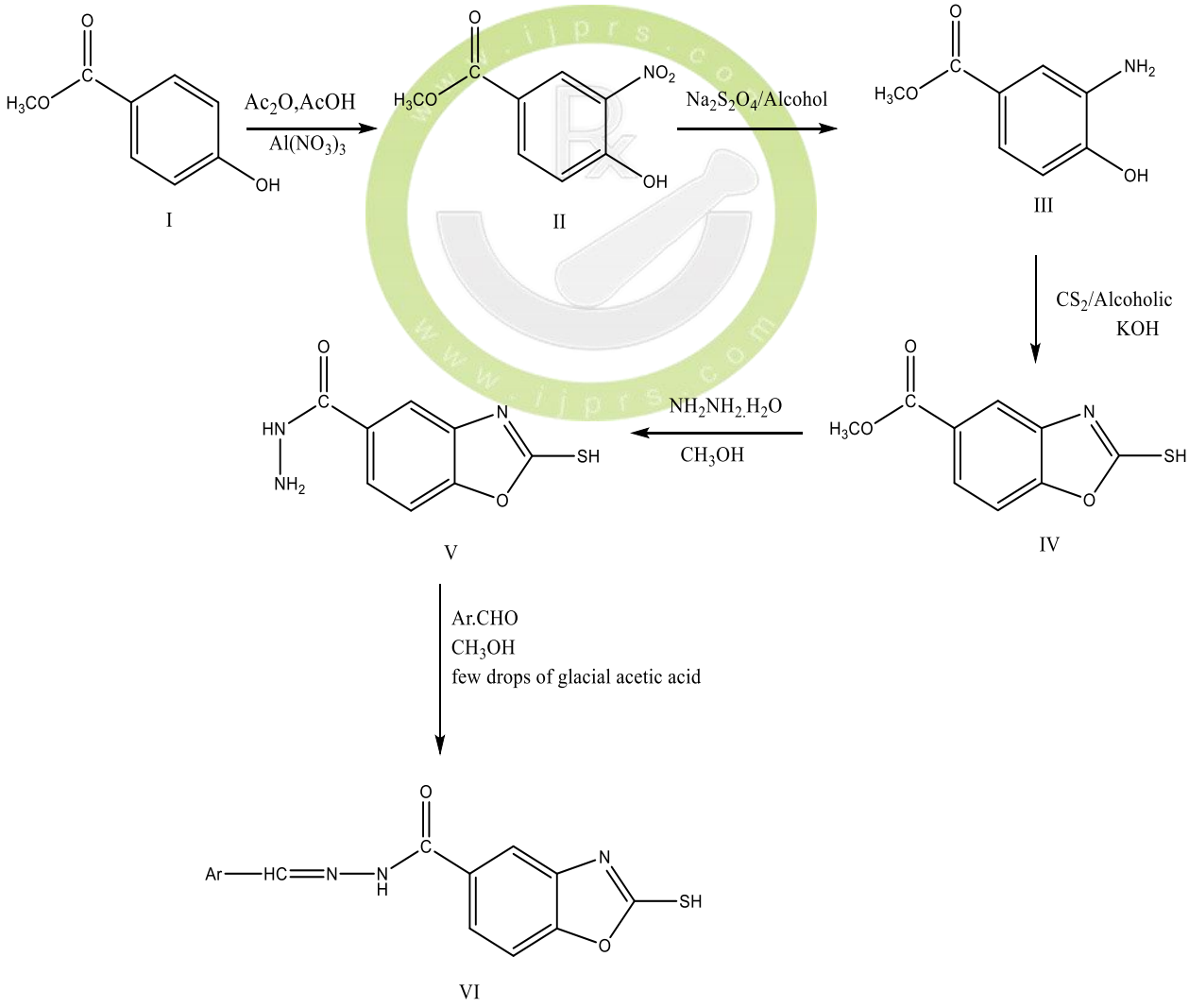
MS (*m/z*): M+ calculated 342, found 341.

Compound VI f

IR (KBR, cm^{-1}): 3240(NH), 1700(C=O), 1620(C=N), 1255(C-O-C), 700(C-H), 2578(SH).

^1H 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

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

Table 2: Anti-inflammatory activity of 2-Mercapto-N-(substituted arylidene) benzoxazole-5-carbohydrazide

S.No	Compound 50mg/kg	Ar	Paw volume in mL(mean ± S.D)			
			1 Hr	2 Hr	3 Hr	4 Hr
1	VI a	4-Cl_6H_4	0.15±0.05**	0.06±0.02	0.30±0.10	0.35±0.05
2	VI b	4-FC_6H_4	0.25±0.12	0.35±0.15	0.40±0.08	0.40±0.08*
3	VI c	4-OHC_6H_4	0.23±0.15*	0.31±0.12	0.36±0.10	0.26±0.10*
4	VI d	4-$OCH_3C_6H_4$	0.10±0.05***	0.20±0.05	0.35±0.05	0.30±0.05
5	VI e	2-$NO_2C_6H_4$	0.20±0.00	0.25±0.05	0.35±0.015	0.30±0.05
6	VI f	4-$N(CH_3)_2C_6H_4$	0.35±0.04	0.41±0.08*	0.43±0.05	0.05±0.05
7	Standard	Diclofenac sod.	0.13±0.05**	0.23±0.05**	0.25±0.05**	0.25±0.05***
8	Control	Sod.CMC	0.35±0.05	0.34±0.05**	0.36±0.05**	0.36±0.05**

Values are expressed as mean ± SD, n=5 animals per group, *P < 0.05, **P < 0.01, ***P < 0.001 as compared to control.

Table 3: Anti-inflammatory activity of 2-Mercapto-N-(substituted arylidene) benzoxazole-5-carbohydrazide

S.No	Compound 50mg/kg	Ar	% Inhibition of paw oedema			
			1 Hr	2 Hr	3 Hr	4 Hr
1	VI a	4-Cl_6H_4	54.65	62.45	36.43	40.68
2	VI b	4-FC_6H_4	33.67	21.51	16.44	18.32
3	VI c	4-OHC_6H_4	46.35	34.53	30.52	38.85
4	VI d	4-$OCH_3C_6H_4$	75.65	30.20	15.26	35.30
5	VI e	2-$NO_2C_6H_4$	40.10	34.05	10.15	34.22
6	VI f	4-$N(CH_3)_2C_6H_4$	9.25	15.53	10.30	12.27
7	Standard	Diclofenac sod.	61.40	56.53	50.50	46.38

Anti – Inflammatory Activity

Carrageenan-Induced Rat Paw Edema Method[18]

Wister strain albino rats weighing between 180-250gm, were housed in clean polypropylene cages and kept under room temperature (25±2°C) fasted 24 hours before the test, divided into eight groups of five animals each. Acute inflammation was produced by sub plantar injection of 0.1ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of rats. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading. Compounds were tested in the dose of 100mg/kg body weight. Diclofenac 20mg/kg was used as standard. The compounds were administered as suspensions in sodium CMC (0.1%w/v) intraperitonially 1 hr before the injection of carrageenan. Control group of animals received a suspension of sodium CMC only. 0.1ml of 1.0%w/v carrageenan suspension in normal saline was injected into the plantar region (aponeurosis) of the right hind paw. The swelling produced after injection of the phlogistic agent was measured at hourly intervals for 4 hrs. Percentage inhibition of edema was calculated and the results were presented in table 3.

All the newly synthesized benzoxazole derivatives were evaluated for anti inflammatory activity by using diclofenac sodium as standard for the period of four hours with one hour interval. The activity of the test compounds is comparable with the activity of the standard diclofenac sodium.

The most potent compound was found to be 4-methoxy phenyl with 75.65 percent inhibition of paw volume after one hour of administration. Compounds VIa, VIc, VIe, VIb were in the next order of inhibition of paw volume after an hour of administration. Compound VIf does not contain any anti inflammatory activity. It has showed that the compound VIa with 4-Chloro phenyl group showed 40.68 percent inhibition of rat paw volume after four hours, followed by the compounds VIc, VId, VIe with the percent inhibition of 38.85%, 35.30%, 34.22% respectively. The rest of the compounds were found to show moderate anti-inflammatory activity.

CONCLUSION

From the above results we can conclude that benzoxazole derivatives showed promising anti inflammatory activity. The most potent compound was found to be VId (4-methoxy phenyl) with an inhibition of paw volume of 75.65 percent after one hour of administration.

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