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Research Article

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Synthesis, Antiinflammatory and Antioxidant Activity of N-(Benzoxazol-2-Yl)-2-(2-Oxoindolin-3-Ylidine) Hydrazine Carbothioamides

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ABSTRACT

A series of seven new compounds were synthesized. Seven different isatins were utilized for condensing with N-(Benzoxazol-2-yl)hydrazine carbothioamide. The investigation of antiinflammatory activity revealed that the test compounds Vc (R=5-NO_2), Vd (R=5-Br) and Va (R=5-H) significantly reduced the inflammation, there by showed a promising antiinflammatory activity towards carrageenan induced paw edema rat model, when compared to the standard drug indomethacin. Among these compounds, compound Va (R=5-H) and Ve (R=5-CH_3) showed comparatively more antioxidant activity.

Keywords: Isatin; Benzoxazole; Antiinflammatory; Antioxidant

INTRODUCTION

Benzoxazole derivatives are biologically active compounds and are known to exhibit various biological activities such as anticancer, antimicrobial, anti-HIV etc. Targets containing the Benzoxazole moiety, either isolated from plants or accessed by total synthesis have remarkable biological activities. For example Gram-positive antibacterials polycyclic antibiotics**,** antiparasitic, anti-inflammatory, elastase inhibitors and H_2-antagonists containing the benzoxazole fragment.

Isatin and several of their derivatives have been generally associated with various biological and pharmacological properties. The synthesis of a large number of isatin derivatives have been described to obtain biologically potent compounds. A few even have clinical applications also.

This prominence aroused interest to several chemists and medicinal chemists to prepare day to day newer and newer potential benzoxazole derivatives by molecular conjunction with isatin and evaluating them for possible pharmacological actions [1-6].

MATERIALS AND METHODS

Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus. Expressed in °C and are uncorrected. The IR spectra (KBr pellets) were recorded on Elmer Spectrum BX-1 spectrometer for the compounds.1H NMR spectra were recorded for compounds on AV 300MHz NMR Spectrometer, using TMS as an internal standard. The Mass spectra were recorded on LCQ ion Mass spectrometer. The purity of the compounds were checked by Thin Layer

Chromatography(TLC) on Merck Silica gel 60 F254 pre coated sheet using Petroleum Ether and Ethyl acetate in 1:1 v/v.

EXPERIMENTAL METHOD

Synthesis of 2-Aminobenzoxazole (Ii)
To a solution of 2-amino phenol (0.1 mol) in toluene was added a solution of Cyanogen bromide (0.02 mol) in toluene with continuous stirring at room temperature and the stirring was continued for 3 hr. The completion of the reaction was monitored by TLC. The solid separated was filtered and washed with carbon tetrachloride (CCl4) and air dried to give a purple colored solid, and recrystallized from ethylacetate (yield 75%) m.p 116-118°C.

Synthesis of ISATIN

Isonitrosoacetanilides:
In a 5 L R.B. Flask were placed 90 g (0.54 mol) of chloral hydrate and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 g) followed by a solution of appropriate aniline (0.5 mol) in 300 ml of water, to which 51.2 g (43 ml, 0.52 mol) of concentrated hydrochloric acid has been added to dissolve the aniline. Finally, a solution of hydroxylamine HCl, 110g (1.58 mol) in 500 ml of water was added. The contents of the flask were heated on water bath so that vigorous boiling began in about 40 to 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period, some crystals of isonitrosoacetanilide separated out. On cooling the solution in running water, the remaining crystallized. It was filtered under suction and air dried [7].

ISATIN:
Sulphuric acid (600 g, 326 ml, sp.gr. 1.84) was warmed at 50°C in a 1 litre R.B. flask fitted with an efficient mechanical stirrer, and to this, 0.46mol of dry finely powdered appropriate isonitrosoacetanilide was added at such a rate so as to maintain the temperature between 60°C to 70°C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitrosoacetanilide was completed, the solution was heated to 80°C and maintained at that temperature for 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured up on 10 to12 times the volume of crushed ice while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water, several times to remove sulphuric acid. It was then air dried.

Synthesis of N-(benzoxazole-2-YL) hydrazine carbothioamide (IV):
2-Aminobenzoxazole (0.1 mol) was dissolved in ammonia solution (20 ml). Carbon disulfide (8 ml) was added gradually with stirring in ice bath. Ethanol (25 ml) was added and stirring was continued till carbon disulfide was completely dissolved. The reaction mixture was allowed to stand for 3 hours while stirring. Sodium chloro acetate solution (0.1 mol) was added followed by hydrazine hydrate (10 ml). The reaction mixture was stirred for 3 hours and allowed to stand overnight. Crystals separated were filtered and recrystallized from methanol (yield 70%).
The IR Spectrum (KBr) of the compound exhibited characteristic absorption bands (cm^{-1}) at: 3305(NH), 3203 (NH), 1639 (C=N).PMR spectrum (CDCl3) of the compound has been found to exhibit proton signals (δ ppm) at: 8.9 (s, 1H, NH), 8.5(s,1H,NH), 6.4-6.8(m, 4H,Ar-H), 4.1 (s, 2H, NH_2). Mass spectrum of compound (IV) recorded its molecular ion peak at m/z 209 [8].

Synthesis of N-(benzoxazol-2-YL)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V):
The compound N-(Benzoxazol-2-yl)hydrazine carbothioamide (IV, 0.1 mol) in absolute ethanol (20 ml), isatin (0.1 mol) was added, followed by a catalytic amount of glacial acetic acid (3 drops). Then the mixture was refluxed for 3 hrs. Excess solvent was removed by distillation and poured on to crushed ice to give a solid recrystallized from ethanol (Table 1). The IR Spectrum (KBr) of the compound (V, R=H) exhibited characteristic absorption bands (cm^{-1}) at: 3292(NH), 3244(NH), 1691(C=O), 1618 (C=N), 1197 (C=S).

PMR spectrum ($CDCl_3$) of the compound has been found to exhibit proton signals (δ ppm) at: 10.06(s, 1H, indole CONH), 9.1(S, 1H, C=SNH), 8.7 (S, 1H, C=SNH), 7.5-7.6 (d, 1H, Ar-H), 7.45-7.5 (d, 1H, Ar-H), 7.1-7.2(d,1H,Ar-H), 6.7-7.01(m, 5H,Ar-H). Mass spectrum of compound (V) recorded its molecular ion peak at m/z 338 [9-16].

Antiinflammatory Activity

Wistar Strain albino rats weighed between 250-300 gm and fasted for 24 hours before the test. The animals were divided into seven groups with six animals in each group. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading. Compounds were tested in dose of 100 mg/kg body weight. Indomethacin (5 mg/kg) was used as standard. The compounds were administered as suspension in sodium CMC (0.1% W/V) orally one hour before the injection of carrageenan. Control group of animals received a suspension of sodium CMC only. 0.1 ml of 1.0% W/V carrageenan suspension in normal saline was injected into the plantar region of the right hind paw. The inflammation produced after injection of the phlogistic agent was measured at hourly intervals for 4 hrs (Table 2 and Figure 1).

$$\% \text{ inhibition of edema} = \frac{\text{Mean edema of Control groups} - \text{Mean edema of treated group}}{\text{Mean edema of control group}} \times 100$$

Antioxidant Activity (DPPH) Method

To 0.1 ml of test sample/ascorbic acid, 2.5 ml of methanol and 0.5 ml of DPPH solution were added, mixed thoroughly and absorbance was measured at 517 nm against blank, prepared in an identical way but without the test compound. The results were plotted on a graph and IC_{50} value was calculated.

The reduction in absorbance is calculated as percentage inhibition as follows:

$$\% \text{ Inhibition } = \frac{\text{Absorbance of blank } - \text{ Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Table 1: Physical data of N-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbothioamides (V)

S.No	Compound	R	Molecular Formula	M.Wt	Melting Point(°C)	%Yield
1	Va	5-H	$C_{16}H_{11}N_{50}2S$	337	288-290	89
2	V b	5-Cl	$C_{16}H_{10}N_{50}2SCl$	371	172-174	84
3	V c	5-NO_2	$C_{16}H_{10}N_{60}4S$	382	206-208	85
4	V d	5-Br	$C_{16}H_{10}N_{50}2SBr$	416	182-184	78
5	V e	7-CH_3	$C_{17}H_{13}N5_02S$	351	210-212	80
6	V f	7-Cl	$C_{16}H_{10}N_{50}2SCl$	371	203-206	75
7	Vg	7-CH_3	$C_{17}H_{13}N_{50}2S$	351	232-234	86

Table 2: Antiinflammatory activity of N-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbothioamides (V)

S.no	Compond	R	% inhibition of paw edema			
			1 h	2 h	3 h	4 h
1	Va	5- H	11.5	26.3	36.5	50
2	Vb	5-Cl	13	25	34.1	45.5
3	Vc	5-NO_2	20.2	35.5	45.1	61.1
4	Vd	5-Br	21.7	34.2	43.9	51.1
5	Ve	5-CH_3	10.1	22.3	32.9	46.6
6	Vf	7-Cl	14.5	21.1	31.7	40
7	Vg	7-CH_3	11.5	23.6	32.9	41.1
8	Indomethacin		21.59	35.5	45.1	62.2

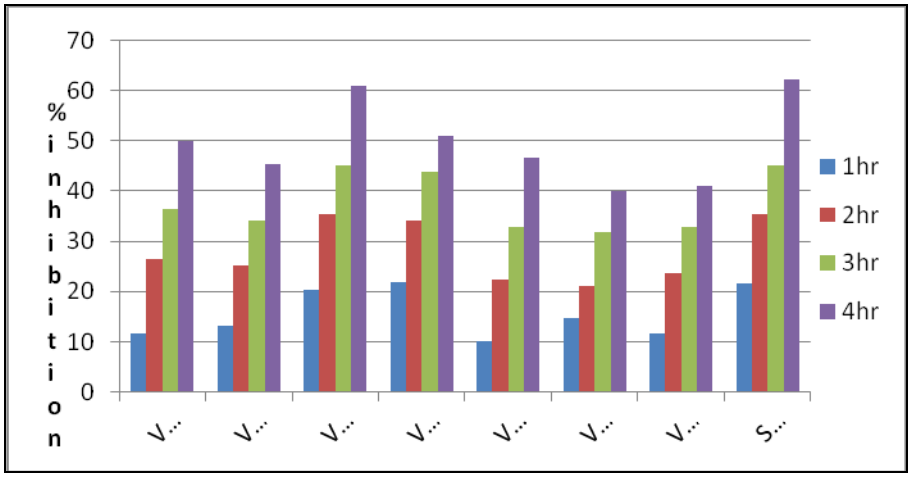

Figure 1: Graph showing anti-inflammatory activity of N-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbothioamides (V)

Table 3: Antioxidant activity of N-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbaothioamides (V)

s.no	Compound	R	IC_{50}(µM)
1	Va	5-H	30.71
2	Vb	5-Cl	80.46
3	Vc	5-NO_2	86.48
4	Vd	5-Br	63.7
5	Ve	5-CH_3	50.43
6	Vf	7-Cl	89.34
7	Vg	7-CH_3	70.32
8	STANDARD	Ascorbic acid	8.64

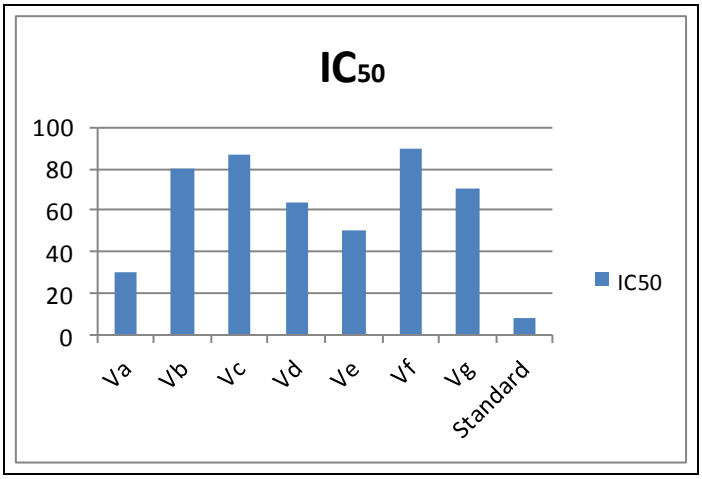

Figure 2: Graph showing antioxidant activity of N-(benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbaothioamides (V)

RESULTS AND DISCUSSION

The preliminary studies on antiinflammatory activity of the new N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V) have generated some data. An attempt has been made to infer the ultimate out-come of the present studies basing on this data.

Anti-inflammatory

All the synthesized new N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V) were evaluated for their antiinflammatory activity by using the standard indomethacin for the period of four hours with one hour interval [17].

All the synthesized compounds were tested at the concentration of 0.1 mole and the results were compared with standard indomethacin at concentration of 0.1 mole.

The investigation of antiinflammatory activity revealed that the test compounds Vc (R=5-NO_2), Vd (R=5-Br) and Va (R=5-H) significantly reduced the inflammation, there by showed a promising antiinflammatory activity, whereas the compounds Ve (R=5-CH_3), Vb (R=5-Cl), Vg (R=7-CH_3) and Vf (R=7-Cl) moderately reduced the inflammation towards carrageenan induced paw edema rat model, when compared to the standard drug indomethacin.

Antioxidant

All the seven new N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V) were evaluated for *in vitro* antioxidant activity by DPPH method. The results of the evaluation have been compared with the standard ascorbic acid and the IC50 values (concentration of the test compound require to scavenge the 50% free radical, DPPH) of all the compounds were shown in Table 3 and Figure 2 [18-20].

All these synthetic compounds produced a concentration dependent scavenging of free radical, DPPH. The IC_{50} values of all synthetic test compounds were found between 30.71 µM and 89.34 µM. Among these compounds, compound Va(R=5-H) and Ve (R=5-CH_3) showed comparatively more antioxidant activity. Vd (5-Br) showed moderate antioxidant activity. Compounds Vg (R=7-CH_3), Vb (R=5-Cl), Vc (R=5-NO_2) and Vf (R=7-Cl) showed poor antioxidant activity.

CONCLUSION

All the new compounds N-(Benzoxazol-2-yl)-2-(2-oxoindolin- 3- ylidine) hydrazine carbothioamides(V) showed antiinflammatory activity in varied degrees. Compounds Vc (R=5-NO_2), Vd (5-Br) and Va (R=5-H) were found to be the potent compounds among all the compounds towards carrageenan induced rat paw edema model, when compared to the standard drug indomethacin. N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-

ylidine)hydrazinecarbothioamides(V) showed promising antioxidant activity. Compounds Va (R=5-H) and Ve (R=5-CH_3) were found to be potent antioxidant compounds among all the test compounds.

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