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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) & 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) & 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 inhibitorsand H_2-antagonistscontaining the benzoxazole fragment.

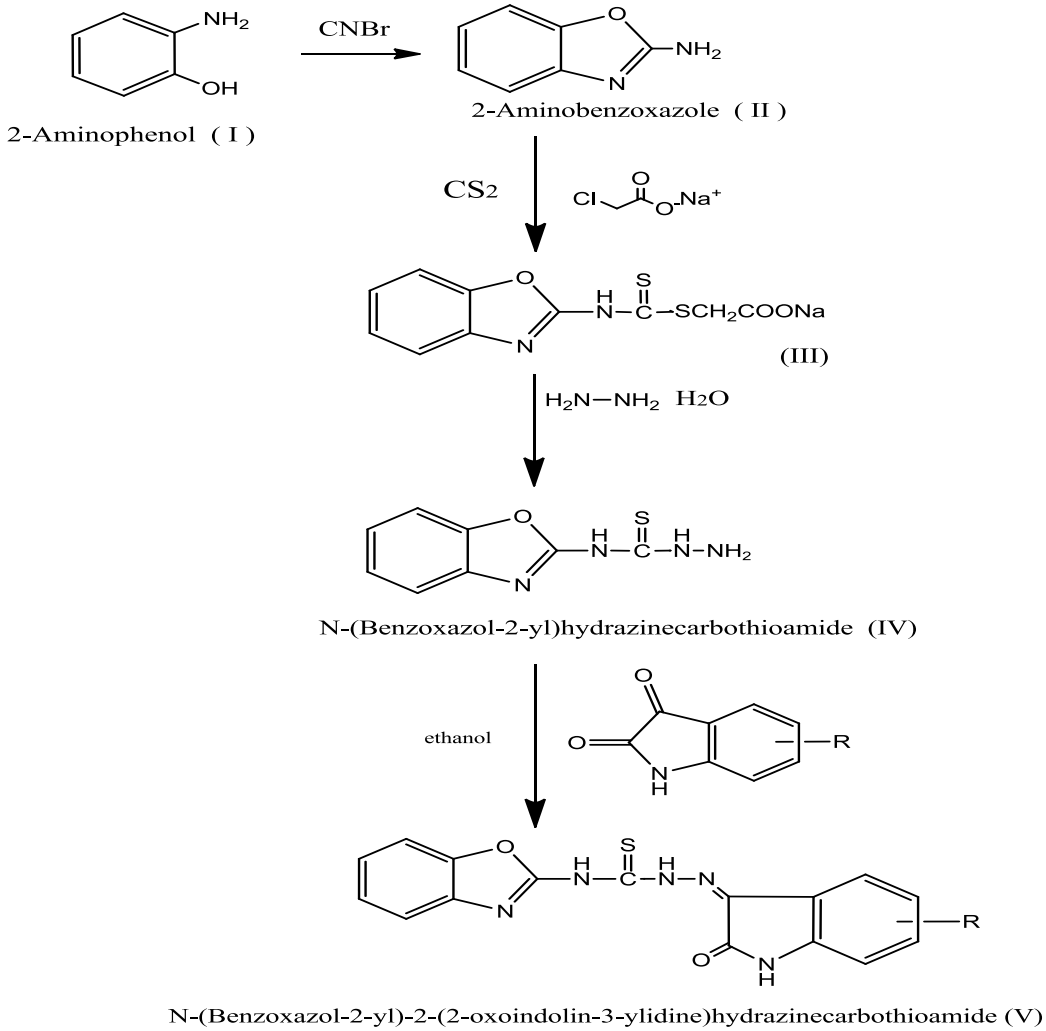
Isatin and several of their derivatives have been generally associated with various biological and pharmacological properties. The synthesis of a large number of isatin derivatives have been described to obtain biologically potent compounds. A few even have clinical applications also.

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.

MATERIALS AND METHODS

Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus. Expressed in ^{0}Cand 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



2-Aminophenol (I)

2-Aminobenzoxazole (II)

N-(Benzoxazol-2-yl)hydrazinecarbothioamide (IV)

N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazinecarbothioamide (V)

SYNTHESIS OF 2-AMINOBENZOXAZOLE (II)

To a solution of2-amino phenol (0.1mol) in toluene was added a solution of Cyanogen bromide (0.02mol) in toluene with continuous stirring at room temperature and the stirring was continued for 3hr. 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^{0}c.

SYNTHESIS OF ISATIN: A. Isonitrosoacetanilides

In a 5 L R.B. Flask were placed 90g (0.54mol) 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 (0.5mol) in 300 ml of water, to which 51.2g(43ml, 0.52 mol) of concentrated Hydrochloric acid has been added to dissolve the aniline. Finally, a solution of hydroxylamine HCl, 110g (1.58mol) 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.

b. ISATIN – Sulphuric acid (600 g, 326 ml, sp.gr. 1.84) was warmed at 50^{0}C in a 1 itre 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 OFN-(BENZOXAZOLE-2-YL) HYDRAZINECARBOTHIOAMIDE (IV)2-Aminobenzoxazole (0.1mol) 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 stirringwas continued tillcarbon disulfidewas completely dissolved. The reaction mixture was allowed to stand for3 hours while stirring. Sodium chloro acetate solution (0.1 mol) was added followed by hydrazine hydrate (10 ml). The reaction mixture wasstirred for 3 hours

and allowed to stand overnight. Crystals separated were filtered and recrystallized frommethanol(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.

SYNTHESIS OFN-(BENZOXAZOL-2-YL)-2-(2-OXOINDOLIN-3-YLIDINE) HYDRAZINECARBOTHIOAMIDES (V)

The compoundN-(Benzoxazol-2-yl)hydrazine carbothioamide(IV, 0.1 mol) in absolute ethanol (20ml), isatin (0.1mol) was added, followed by a catalytic amount of glacial acetic acid (3drops). Then the mixture was refluxed for 3 hrs. Excess solvent was removed by distillation and poured on to crushed ice to give a solidrecrystallized from ethanol.

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 (CDCl3) of the compound has been found to exhibit protonsignals (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.

Antiinflammatory activity: Wistar Strain albino rats weighed between 250-300gm and fasted for 24hours 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 100mg/kg body weight. Indomethacin (5mg/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. 0.1ml 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 4hrs.

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

Antioxidant activity (DPPH) method: To 0.1ml of test sample/ascorbic acid, 2.5ml of methanol and 0.5ml of DPPH solutionwere added, mixed thoroughly and absorbance was measured at 517nm against blank, prepared in an identical waybut 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:

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

Table 1: Physical data ofN-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine)hydrazine carbothioamides (V).

S.NO	COMPOUND	R	MOLECULAR FORMULA	M.Wt	MELTING POINT(^{0}C)	%YIELD
1	Va	5-H	C16H11N502S	337	288-290	89
2	V b	5-Cl	C16H10N502SCl	371	172-174	84
3	V c	5-NO_2	C16H10N604S	382	206-208	85
4	V d	5-Br	C16H10N502SBr	416	182-184	78
5	V e	7-CH_3	C17H13N502S	351	210-212	80
6	V f	7-Cl	C16H10N502SCl	371	203-206	75
7	Vg	7-CH_3	C17H13N502S	351	232-234	86

Table 2: Antiinflammatoryactivity ofN-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V).

S. S.no	Compound	R	% inhibition of paw edema			
			1h	2h	3h	4h
1	Va	5- H	11.5	26.3	36.5	50.0
2	Vb	5-Cl	13.0	25.0	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	40.0
7	Vg	7-CH_3	11.5	23.6	32.9	41.1
8	**Indomethacin**		21.59	35.5	45.1	62.2

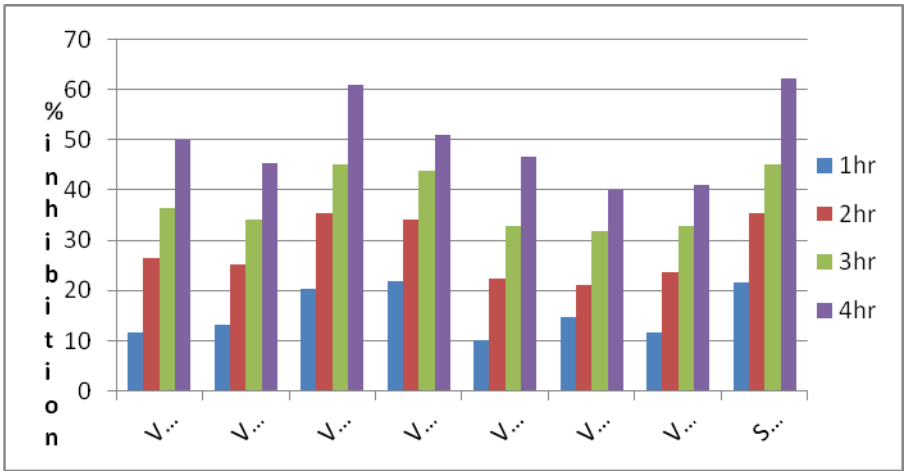


Figure 1: Graph showing Antiinflammatory activity ofN-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbothioamides (V).

Table 3: Antioxidantactivity ofN-(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	63.70
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

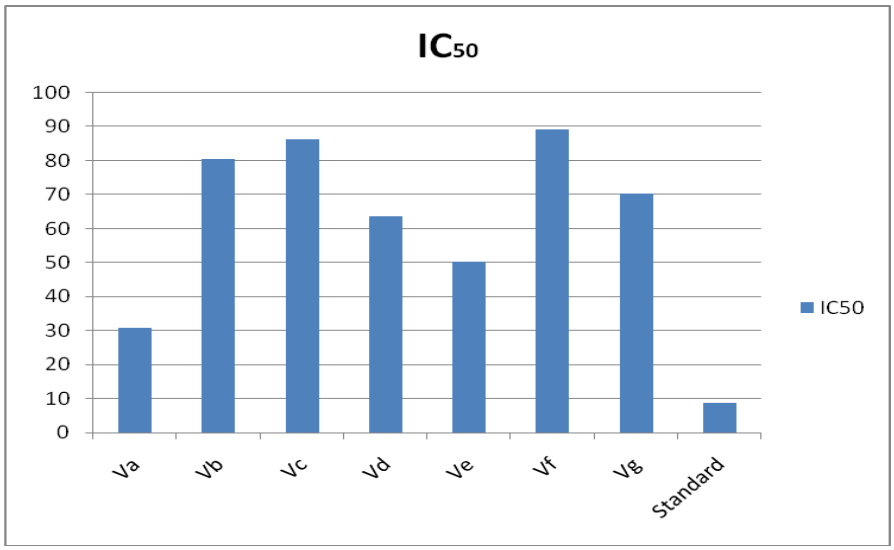


Figure 2: Graph showing Antioxidantactivity ofN-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazine carbaothioamides (V).

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 somedata. 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.

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

The investigation of antiinflammatory activity revealed that the test compounds Vc (R=5-NO_2), Vd (R=5-Br) & Va (R=5-H) significantly reduced the inflammation, there by showed a promising antiinflammatory activity, where as the compounds Ve (R=5-CH_3), Vb (R=5-Cl), Vg (R=7-CH_3) & 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 invitro 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.

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 30.71µM and 89.34 µM. Among these compounds, compound Va(R=5-H) & 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) & 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. CompoundsVc (R=5-NO_2), Vd (5-Br) & Va (R=5-H) were found to be thepotent compoundsamong all the compounds towards carrageenan induced rat paw edema model, when comparedtothe

standard drug indomethacin. N-(Benzoxazol-2-yl)-2-(2-oxoindolin-3-ylidine) hydrazinecarbothioamides (V) showed promising antioxidant activity. Compounds Va (R=5-H) & Ve (R=5-CH_3) were found to be potent antioxidantcompounds among all the test compounds.

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