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Research Article

SYNTHESIS AND CHARACTERIZATION OF NEW ISATIN DERIVATIVES FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTACT

In afford to synthesize N1-(2-oxo2, 3-dihydro-1H-indol-3-yl) benzohydrazide derivatives different substituted isatins were coupled with benzohydrazide. The synthesized derivatives were characterized by means of chromatographic, IR, 1HNMR&MASS Spectral analysis. The synthesize derivatives were evaluated for In vivo anti-inflammatory activity by using induced rat paw edema method. Acute toxicity studies revealed that the compounds are non-toxic in rats upto 500mg/ kg (b.w) intraperitonially. The compounds VI a(R=H), VI d(R=5-Cl), Vie(R=5-F), VIh(R=6-Br) were found to be have moderate potent activity

Keywords: Isatin derivatives, Benzimidazole anti-inflammatory.

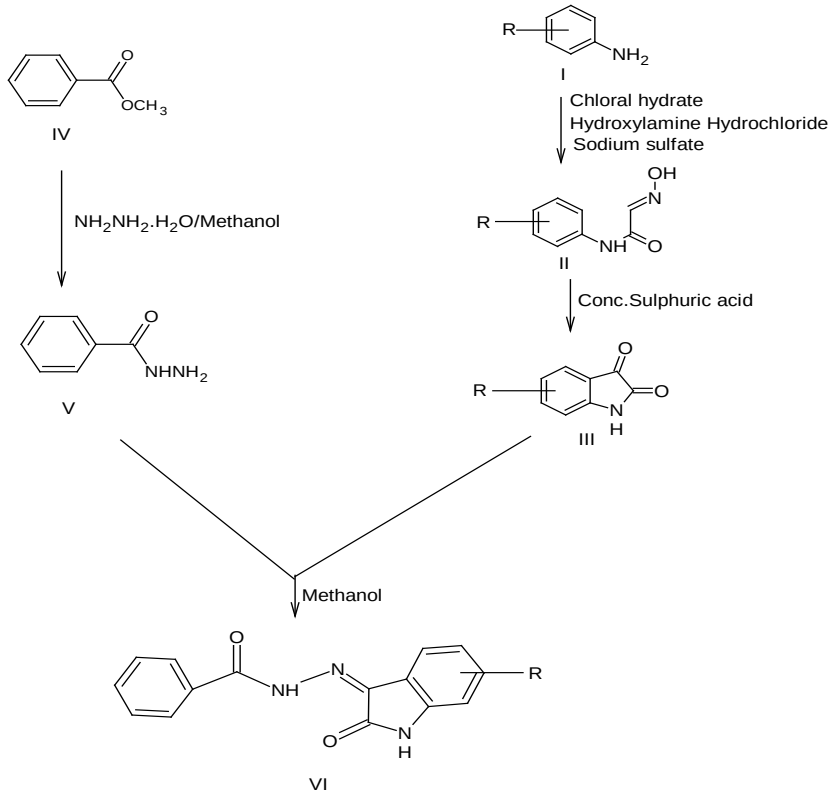
INTRODUCTION

Indole is the most beneficial heterocyclic nucleus which has gained prominence in medicinal chemistry due to its diverse biological activities such as antimicrobial [1-7], anticancer, antioxidant [8-12], antipyretic, analgesic and anti-inflammatory [14-15] activities.

In recent years, research is focused on existing molecules and their modifications in order to reduce their side effects and to explore their other pharmacological and biological effects. Most of anti-inflammatory drugs exert their beneficial effect by inhibiting either release of lysosomal enzymes or by stabilizing lysosomal membrane which is one of the major events responsible for the inflammatory process. The stabilization of lysosomal membranes is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release Some NSAIDs like indomethacin and acetylsalicylic acid are known to possess membrane stabilizing properties [16, 17] which may contribute to the potency of their anti-inflammatory effect.

Chemistry

Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus. Expressed in ^{0}C and are uncorrected. The IR spectra (KBr pellets) were recorded on Elmer Spetrum BX-1 spectrometer for the compounds.1H NMR spectra were recorded for compounds on AV 300MHz NMR Spectrometer, using TMS as 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.



VIa)R=H VIf) R=5-NO_2; VIb) R=5-CH_3 VIg) R=7-CH_3; VIc) R= 5-Br VIh) R=6-Br; VId) R =5-Cl VIi) R=7-Cl; VIe) R =5F VIj) R=7-F

MATERIALS AND METHODS

I. Synthesis of Indole-2, 3-diones (Isatins, III)

a) Isonitrosoacetanilides (II) – General Procedure

In a 5 lit. R.B. flask were placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate (1300 g) followed by a solution of an appropriate aromatic amine in 300 ml of water and concentrated hydrochloric acid (0.52mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was added. The contents of the flask were heated over a wire-guage by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

b) Indole-2,3-diones (III) – General Procedure

Sulphuric acid (600g, d, 1.84, 326 ml) was warmed at 50^{o}C in a one liter RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (II, 0.46 mol) was added at such a rate so as to maintain the temperature between 60^{o}C to 70^{o}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 isonitroso compound was completed the temperature of the solution was raised to 80^{o}C and maintained at that temperature for 10 minutes, to complete the reaction. Then the reaction mixture was cooled to room temperature and poured on crushed ice (2.5 kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by the recrystallization from methanol.

II. Preparation of N'-(2-oxo-2, 3-dihydro-1H-indol-3-yl) benzohydrazide (VII)

II). Preparation of Benzo hydrazide

Methyl Benzoate (IV, 0.1mole) was refluxed with this 0.1 mole of hydrazine hydrate 99% (0.2mole) in 20 ml of methanol (0.2mole) for 2 hrs. The reaction mixture was poured unto ice cold water and product was collected by filtration, dried and purified with methanol.m.p:125^{0}C.Yield: 80%.

III. Preparation of N'-(2-oxo-2, 3-dihydro-1H-indol-3-yl) benzohydrazide (VI)

A mixture of isatin (III, 0.01mole) and N'-(2-oxo-2, 3-dihydro-1H-indol-3-yl) benzohydrazide (VII, 0.01 mole) in 20 ml methanol containing traces of acetic acid was refluxed for 3 hrs. The solvent was evaporated the residue was poured onto crushed ice. The product obtained was filtered, washed with cold water, dried and purified dry column chromatography.

Adopting these procedure 10 isatin derivatives were prepared.

SPECTRAL DATA

1. Benzohydrazide (V)

IR (KBr) cm^{-1}: 3300.76 (NH), 1617.65 (C = O), 884.60 (Ar).

2. N'-(2-oxo-2,3-dihydro-1H-indol-3-yl)benzohydrazide (VIa): yield88. m.p.285-287. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) 7.9(d, Ar-2H), 7.6(m, 4H) 7.3(t,1H) 7.1(t,2H),6.8-8 (m, 9H, Ar-H). **MASS SPECTRA**: The molecular ion peak was observed at 266(m+1): **IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O), 1535.18(C=N).

3. *N*'-(5-methyl-2-oxo-2,3-dihydro-1*H*-indol-3-yl) benzohydrazide (VIb) yield85.m.p.290-292. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (S,1H,N-H),11.2 (S,1H,N-H),7.9(d, Ar-2H), 7.8(t, Ar-1H), 7.6(t, Ar-2H), 7.7(d, Ar-1H), 7.4(d, Ar-1H), 7.2(s, Ar-1H) and 2.5 (S, 3H, CH_3). **MASS SPECTRA**: The molecular ion peak was observed at 280(m+1): **IR (KBr) cm^{-1}**: 3250 (NH), 1677.41 (C = O), 1629.49 (C = O), 1529.18(C=N), 817 (Ar).

4. *N*'-(5-Bromo-2-oxo-2,3-dihydro-1*H*-indol-3-yl)benzohydrazide (VIc):yield88.m.p.295-297. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (S,1H,N-H),11.2 (S,1H,N-H),6.4-8.2(m, Ar,8H) .**MASS SPECTRA**: The molecular ion peak was observed at 345(m+1): **IR (KBr) cm^{-1}**: 3239.95 (NH), 1677.20 (C = O), 1618.43 (C = O), 1512.03 (C=N).

5. *N*'-(5-Chloro-2-oxo-2,3-dihydro-1*H*-indol-3-yl)benzohydrazide (VId) yield82%.m.p.288-289.^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) 6.6-8.3(8H, Ar-H). **MASS SPECTRA**: The molecular ion peak was observed at 300(m+1): **IR (KBr) cm^{-1}**: 3222.58 (NH), 1720.26 (C = O), 1675.74(C = O), 1535.08 (C=N).

6. *N*'-(5-fluoro-2-oxo-2,3-dihydro-1*H*-indol-3-yl)benzohydrazide (VIe) yield78%,m.p.293-295. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H),7.2-8.6(m, 8H, Ar-H).**MASS SPECTRA**: The molecular ion peak was observed at 283(m+1):**IR (KBr) cm^{-1}**: 3299.98 (NH), 1702.00 (C = O), 1676.94 (C = O), 1529.67 (C=N).

7. N'-(5-Nitro-2-oxo-2,3-dihydro-1H-indol-3-)benzohydrazide (VIf):yield75%,m.p.289-291. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) ,6.8-7.5 (m, 8H, Ar-H). **MASS SPECTRA**: The molecular ion peak was observed at 311(m+1). **IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O),1535.18(C=N).

8. N'-(7-Methyl-2-oxo-2,3-dihydro-1H-indol-3-yl)benzohydrazide (VIg) yield80%.m.p.290-292. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H),6.5-7.4 (m, 8H, Ar-H),. **MASS SPECTRA**: The molecular ion peak was observed at 280(m+1): **IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O), 1535.18(C=N).

9. N'-(6-Bromo-2-oxo-2,3-dihydro-1H-indol-3-yl)benzohydrazide (VIh) yield83%m.p.291-293. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) ,6.4-8.2(m, 8H, Ar-H).**MASS SPECTRA**: The molecular ion peak was observed at 345(m+1): **IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O),1535.18(C=N).

10. N'-(7-Chloro-2-oxo-2,3-dihydro-1H-indol-3-yl) benzo-hydrazide (VIi) yield75%.m.p.298-300. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) ,6.4-8.2(m, 8H, Ar-H). **MASS SPECTRA**: The molecular ion peak was observed at 300(m+1): **IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O), 1535. 18(C=N).

11. N'-(7-Fluoro2-oxo-2,3-dihydro-1H-indol-3-yl)benzohydrazide (VIj) yield79%.m.p.287-289.^{1}H NMR (300 MHz, DMSO): δ [ppm]: 14 (s,1H,N-H),11.2 (s,1H,N-H) ,6.4-8.2(m, 8H, Ar-H).**MASS SPECTRA**: The molecular ion peak was observed at 283(m+1):**IR (KBr) cm^{-1}**: 3233.33 (NH), 1693.65 (C = O), 1624.81 (C = O),1535.18(C=N).

METHODS

Anti-inflammatory activity

Carrageenan-induced rat paw edema method

The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced rat paw edema model described by Winter et al[18] Rats of either sex were treated orally with pyrazolone derivatives (400 mg/kg b.w.) and standard drug Indomethacin (10 mg/kg b.w.), 1 h prior to the 1% (w/v) solution injection of 0.1 ml carrageenan into plantar region of right hind paw (subcutaneously). Paw volume was measured by Plethysmometer at 0, 1, 2 and 3 h after carrageenan injection. The difference between the paw volume at 4th and 0 h measurement was calculated and taken as edema volume. Percentage inhibition in the paw was calculated by using the formula, percentage inhibition = 100 × (1 − V_t/V_c), where V_t = mean increase in paw volume of test, and V_c = mean increase in paw volume of control. Percentage inhibition shown by tested compounds is recorded.

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [19] which are aimed at host defense and usually activated in most disease conditions. These different reactions in the inflammatory response cascade are therapeutic targets which anti-inflammatory agents interfere with to suppress

exacerbated inflammatory responses usually invoked in such disorders such as rheumatoid arthritis, infection or injury.

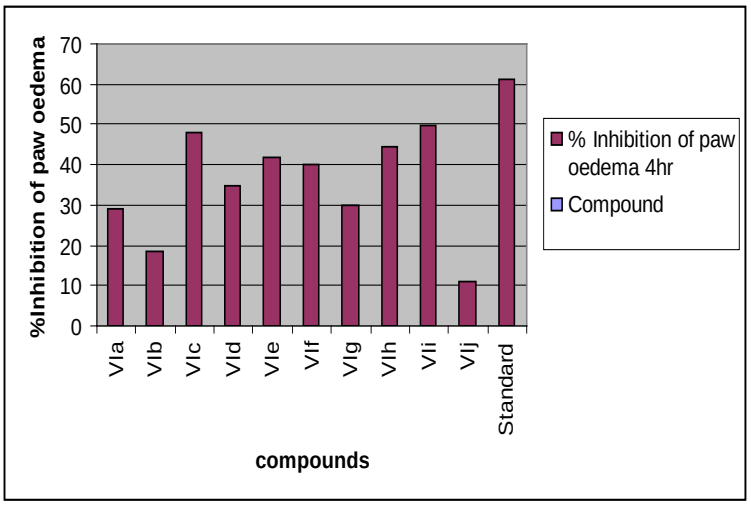
The non-steroidal anti-inflammatory drugs exert anti-inflammatory effect principally by inhibiting the synthesis of prostaglandin an eicosanoid mediator of the inflammatory response [20] .The most widely used primary test to screen new anti-inflammatory agent's ability to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The carrageenan test was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammation. The subplantar injection of carrageenan in rats leads to paw edema. The early phase (1–2 h) carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissues surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorph nuclear cells and prostaglandins produced by tissues macrophages [21]. The pyrazolone derivatives reduced the carrageenan-induced paw edema in rats. It may be due to inhibition of cyclooxygenase which activates prostaglandin synthesis followed by prevention of inflammatory mediator's release.

RESULTS AND DISCUSSION

The anti-inflammatory activity of the test compounds was evaluated by carrageenan-induced rat paw edema model described by Winter et al[18].Among all the derivatives R=7-Cl,R=5Br,an R=5F showed considerable anti-inflammatory activity when compared to the standard, which showed 61.37% of inhibition of paw edema.

Table 1: Anti inflammatory activity of N'-(2-oxo-2, 3-dihydro-1H-indol-3-yl) benzohydrazide (VI a-j)

S. No.	Compound	(R=)	%inhibition of paw edema after 4hrs.
1	VI a	H	29.26
2	VI b	5-CH_3	18.47
3	VI c	5-Br	48.16
4.	VI d	5-Cl	34.70
5	VI e	5-F	42.02
6	VI f	5-NO_2	40.09
7	VI g	7-CH_3	29.76
8	VI h	6-Br	44.50
9	VI i	7-Cl	49.84
10	VI j	7-F	11.22
11	Standard	Diclofenac sod.	61.37



CONCLUSION

Ten title compounds were synthesized (VIa-j) and were analyzed by FT-IR, NMR, MASS. Persentage yield of the compound ranges from 85-90.All the derivatives were found to be safe even up to a dose of 500mg/kg.(b.w.)i.p. in experimental animals.

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