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Synthesis, Characterization and Evaluation of Novel *N*-(1*H*-Benzimidazol-2-Yl)-2-Isatinylidene-Hydrazinecarboxamide Derivatives as Anti-Inflammatory Agents

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Abstract

A series of isatin derivatives were synthesized by condensation of N-(1H-benzimidazol-2-yl)-hydrazine carboxamide with various isatin derivatives and evaluated for in vivo (rat paw edema) for their anti-inflammatory activity using carrageenan induced rat paw edema model. The synthesized compounds 6a-6h were characterized by spectral data (IR, ^{1}H-NMR and mass) and elemental analysis. All the novel isatin derivatives exhibited mild to moderate activity. Compound 6b, 5-methyl isatin derivative exhibited better anti-inflammatory activity compared with control and other test compounds with percentage inhibition of 63.15.

Keywords: Isatin, Benzimidazole, anti-inflammatory agents, carrageenan induced rat paw edema.

INTRODUCTION

Asthma is a complex, chronic inflammatory disease of airways, which affects more than 100 million people worldwide, making it a serious global health problem [1]. This disorder involves multiple inflammatory mediators that are released from mast cells as a consequence of immunological response in the airways [2]. Cyclooxygenase (COX) and lipoxygenase (LOX) produce two groups of arachidonic acid metabolites, prostaglandins (COX products) and leucotrienes (LOX products), that play a key role in inflammation. The classical nonsteroidal antiinflammatory drugs (NSAIDs) act via the inhibition of the COX-1 isoenzyme or the combined inhibition of COX-1 and COX-2 isoenzymes. For example, aspirin is a COX-1 selective inhibitor, whereas indomethacin and naproxen are COX-1/COX-2 inhibitors. Because

COX-1 is mainly responsible for mucus formation in the gastrointestinal (GI) tract, COX-1 inhibition is implicated for inducing GI irritation, the main undesired side effect of such agents [3]. Another side effect, mild bleeding diathesis also results from the selective inhibition of the COX-1 catalyzed synthesis of the platelet aggregation factor, thromboxane A_2 [4].

COX-2 isoenzyme is evident to be over-expressed during inflammatory conditions and was also found to exhibit a protective role in asthma [5]. Literature reports reveal that COX isoenzymes are the attractive molecular targets for the development of NSAIDs [6]. Thus, inhibition of these enzymes leads to a decreased production of prostaglandins and thromboxanes which in turn accounts for the beneficial effects of NSAIDs (e.g. anti-inflammatory, antipyretic, analgesic and cardiovascular effects) as well as their undesirable side effect profiles (e.g. GI irritation). It has been hypothesized that there might be other forms of the COX enzyme yet to be discovered. The failure of COX-1 and COX-2 selective inhibitors evoked the concept that inflammation be considered as a multifactor process and all biochemical pathways should be taken into account [7]. Inhibition of leukocyte function and/or lipid mediator biosynthesis could be an important therapeutic intervention in inflammatory diseases and it may lead to the discovery of new drugs as alternative approaches to conventional anti-inflammatory agents possessing a high incidence of severe side-effects [8].

Isatin (1H-indol-2, 3-dione) analogues have proved to be versatile starting materials for the synthesis of heterocyclic compounds with potential biological activities [9] such as antibacterial [10, 11], antifungal [11, 12], anti-HIV [13, 14] and anticonvulsant [15].

The ring system in which a benzene ring is fused to the 4, 5-positions of imidazole is designated as Benzimidazole [16]. Benzimidazole is one of the most promising heteroaryl moiety that yielded many successful drugs [17]. Wide variety of pharmacological activities have been reported by benzimidazole moiety itself [18] and its derivatives [17]. The current literature indicates that benzimidazole derivatives possess diverse pharmacological activities, including antimicrobial, antiviral, antineoplastic, antihypertensive, and vasodilating activities [19]. Furthermore, it had been reported that many compounds containing benzimidazole moiety possess significant analgesic as well as anti-inflammatory activity [20]. Benzimidazole moiety fulfills the minimum structural requirements that are common for anti-inflammatory compounds [21-23]. In the past, many benzimidazole derivatives showed potential for anti-inflammatory and analgesic activity in animal models of inflammation and pain [24-33].

The 2-aminobenzimidazole ring system represents the core structure of a number of biologically significant molecules and its derivatives have been found to possess a wide spectrum of biological activity. Particularly, alkyl benzimidazole-2-carbamates show potent fungicide [34] and anti-helmintic activity [35, 36], being Carbendazim® a good example of a successful market fungicide.

Therefore, search for better and safer anti-inflammatory agents from the above observation synergistic activity can be assessed by introducing five membered heterocyclic systems on isatin and their derivatives for antiinflammatory activity.

RESULTS AND DISCUSSION

The target compounds were synthesized according to the representative scheme 1. The required starting material, 2-aminobenzimidazole **2** was prepared in good yield by condensation of o-Phenylene diamine with cyanogen bromide [37, 38]. The subsequent N-carbamate formation by the reaction of **2** with ethyl chloroformate in mild alkali medium gave the known ethyl 1*H*-benzimidazol-2-ylcarbamate **(3)**, which on hydrazinolysis afforded the corresponding of *N*-(1*H*-benzimidazol-2-yl)hydrazinecarboxamide**(4)**. The title compounds 6a-6h was finally synthesized by treated with different substituted isatin derivatives 5a-5h in the presence of catalytically amount of glacial acetic acid resulted in the formation of schiff's bases of title compounds 6a-6h. The yields of all the synthesized compounds were found to be in the range of 60-70%.

Assignments of selected characteristic IR band positions provide significant indication for the formation of *N*-(1*H*-benzimidazol-2-yl)-2-isatinylidene-hydrazinecarboxamide derivatives. All products were confirmed by IR (KBr, cm^{-1}) spectral data showing characteristic two peaks in the range 3202-3393 cm^{-1} indicating the presence of two –NH groups and sharp peak at 1510-1518 indicated the presence of C=N group in the products. The ^{1}HNMR spectra showed singlet in the range δ 12.00 – 12.12 correspond to CONH lactam of isatin and at δ 10.18 – 10.32 NH-N= proton appeared as singlet. The NHCO proton appeared in the range of δ 9.38 – 9.78 and the benzimidazole ring NH proton appeared as singlet at δ 8.38 -8.58. The aryl protons are appeared as multiplate in the range of δ 6.64 – 7.62, methyl proton are appeared at δ 2.26 – 2.38 as singlet and the carboxlic acid protons showed singlet at δ 12.60 – 12.74. Mass spectrum exhibited a molecular ion $[M^{+}]^{+1}$peak corresponding to its molecular weight. The mass spectral data of all the titled compounds were found to be in correlation with the expected structure.

All the title compounds were screened for their *in vivo* anti-inflammatory activity using the carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced edema (Fig. I and Table II). The protection ranged up to 63%, while the reference drug (diclofenac sodium) showed 81.05%. Among all the tested compounds, 6b with 5-methyl substituent showed remarkable anti-inflammatory activity compare to other derivatives.

MATERIALS AND METHODS

Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by open capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm-1 using KBr discs. ^{1}H-NMR spectra were recorded at 400 MHz in DMSO-d_6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany). All chemicals were purchased from Merck, Spectrochem or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were

characterized by IR spectroscopic analysis and Elemental Analysis for CHN. In the elemental analysis, the observed values were within ±0.4% of the calculated values. Final compounds were characterized by ^{1}H NMR and FAB mass spectrometry (MS). The final yields and the physicochemical data of the compounds 6a to 6h are presented in Table 1.

Synthesis of 2-aminobenzimidazole[37,38] (2):
Cyanogen bromide (3.5 g., 0.034 mole) was added in small portions, with shaking, to a suspension of o-phenylenediamine (4.2 g. , 0.34 mole) in water (40 ml), the exothermic reaction mixture was cooled to room temperature and it was stirred for 36hrs. The solution was filtered after standing overnight. Sodium hydroxide (1.4 g., 0.034 mole) in 30 ml of water was then added and the solution was evaporated. The solid thus obtained was filtered and recrystalized from water. Yield 85%, m.p. 228 - 230°C

Synthesis of ethyl 1*H*-benzimidazol-2-ylcarbamate (3):
To a solution of 2-aminobenzimidazole (2; 0.01 mole) in dry acetone (40 ml) anhydrous potassium carbonate (0.01 mole) was added and the ethyl chloroformate (0.01 mole) added drop wise. After the completion of addition, the mixture was refluxed for 12 hours and then allowed to cool to room temperature. The solid thus obtained was filtered and recrystalized from ethanol. Yield 70%, m.p. 122 °C

Synthesis of *N*-(1*H*-benzimidazol-2-yl)hydrazinecarboxamide(4):
To a mixture of ethyl benzimidazol-2-ylcarbamate (3, 0.01 mole) and hydrazine hydrate (0.02 mole), methanol (30 ml) was added and refluxed for 30 minutes. The excess of solvent was removed by evaporation under vacuum and the obtained solid was filtered then recrystallised from ethanol. The yield of the product was 74%, m.p. 204-206 °C

General method for the synthesis of *N*-(1*H*-benzimidazol-2-yl)-2-isatinylidene-hydrazine-carboxamide derivatives (6a - 6h):
A mixture of *N*-(1*H*-benzimidazol-2-yl)hydrazinecarboxamide (4, 0.01mol), the corresponding isatin (5a – 5h, (0.01mol) and catalytic amount of p-toluenesulphonic acid mol) in methanol (30ml) was heated and refluxed for 3-4 hrs. Progress of reaction was monitored by TLC. At the end of reaction, the solvent was evaporated to one quarter of its volume; then the obtained solid products were filtered, dried, purified via column chromatography (hexane:ethyl acetate; 1:0.5) to give compounds 6a–6h.

***N*-(1*H*-benzimidazol-2-yl)-2-isatinylidene-hydrazinecarboxamide (6a):**
Elemental analysis (Found): C,59.78 ; H,3.89 ; N,26.74 ; (Calculated): C, 60.00; H, 3.78; N, 26.24; Mol. Formula: $C_{16}H_{12}N_6O_2$; IR (KBr, cm^{-1}): 3393.71(N-H), 1687.95(C=O, lactam), 1652.78 (C=O), 1512.77 (C=N); ^{1}H NMR (δ ppm): 12.07(s, 1H, CONH lactam), 10.28(s, 1H, NH-N=), 9.5 (S, 1H, NHCO), 8.44(S,1H, NH, benzimidazole), 6.82-7.38 (m, 8H, Ar-H) ; MS: m/z $[M^+]^{+1}$ 321.

***N*-(1*H*-benzimidazol-2-yl)-2-(5-methyl-isatinylidene)hydrazinecarboxamide (6b):**
Elemental analysis (Found): C, 60.14; H,4.19 ; N,25.25 ; (Calculated): C, 61.07; H, 4.22 ; N, 25.14; Mol. Formula: $C_{17}H_{14}N_6O_2$; IR (KBr,cm^{-1}): 3202.11(N-H), 1690.75(C=O, lactam), 1662.28 (C=O), 1510.80 (C=N); ^{1}H NMR (δ ppm): 12.08(s, 1H, CONH lactam), 10.30(s, 1H,

NH-N=), 9.5 (S, 1H, NHCO), 8.43(S,1H, NH, benzimidazole), 6.80-7.40 (m, 7H, Ar-H), 2.26 (s, 3H, CH_3); MS: m/z $[M^+]^{+1}$ 335.

***N*-(1*H*-benzimidazol-2-yl)-2-(7-methyl-isatinylidene)hydrazinecarboxamide (6c):**
Elemental analysis (Found): C,60.59 ; H,4.27; N,25.09 ; (Calculated): C, 61.07; H, 4.22; N, 25.14; Mol. Formula: $C_{17}H_{14}N_6O_2$; IR (KBr,cm^{-1}): 3383.93(N-H), 1684.79(C=O, lactam), 1665.78 (C=O), 1513.27 (C=N); ^{1}H NMR (δ ppm): 12.06(s, 1H, CONH lactam), 10.26(s, 1H, NH-N=), 9.53 (S, 1H, NHCO), 8.50(S,1H, NH, benzimidazole), 6.78-7.48 (m, 7H, Ar-H), 2.38 (s, 3H, CH_3); MS: m/z $[M^+]^{+1}$ 335.

***N*-(1*H*-benzimidazol-2-yl)-2-(5-nitroisatinylidene)hydrazinecarboxamide (6d):** Elemental analysis (Found): C, 52.37; H,3.18 ; N, 25.14; (Calculated): C, 52.61; H, 3.04; N, 26.84; Mol. Formula: $C_{16}H_{11}N_7O_4$; IR (KBr,cm^{-1}): 3363.98(N-H), 1684.12 (C=O, lactam), 1655.78 (C=O), 1516.32 (C=N), 1468.15 (Ar-NO_2); ^{1}H NMR (δ ppm): 12.06(s, 1H, CONH lactam), 10.30(s, 1H, NH-N=), 9.45 (S, 1H, NHCO), 8.38(S,1H, NH, benzimidazole), 6.92-7.46 (m, 7H, Ar-H); MS: m/z $[M^+]^{+1}$ 366.

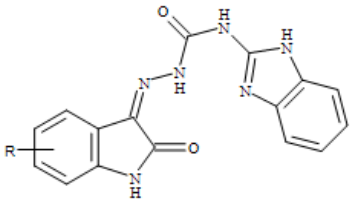
***N*-(1*H*-benzimidazol-2-yl)-2-(5-carboxylisatinylidene)hydrazinecarboxamide (6e):**
Elemental analysis (Found): C, 56.78; H,3.12 ; N,24.42 ; (Calculated): C, 56.05; H, 3.32; N, 23.07; Mol. Formula: $C_{17}H_{12}N_6O_4$; IR (KBr,cm^{-1}): 3353.93(N-H), 1679.72(C=O, lactam), 1655.16 (C=O), 1515.72 (C=N), 1382.75(Ar-COOH); ^{1}H NMR (δ ppm): 12.60 (s, 1H, COOH), 12.00(s, 1H, CONH lactam) 10.18(s, 1H, NH-N=), 9.78 (S, 1H, NHCO), 8.58(S,1H, NH, benzimidazole), 6.68-7.60 (m, 7H, Ar-H); MS: m/z $[M^+]^{+1}$ 365.

***N*-(1*H*-benzimidazol-2-yl)-2-(7-carboxylisatinylidene)hydrazinecarboxamide (6f):** Elemental analysis (Found): C, 56.76; H,3.08 ; N,2373 ; (Calculated): C, 56.05; H, 3.32; N, 23.07; Mol. Formula: $C_{17}H_{12}N_6O_4$; IR (KBr,cm^{-1}): 3355.56(N-H), 1672.76(C=O, lactam), 1651.06 (C=O), 1518.32 (C=N), 1378.25(Ar-COOH); ^{1}H NMR (δ ppm): 12.75 (s, 1H, COOH), 12.10(s, 1H, CONH lactam) 10.22(s, 1H, NH-N=), 9.58 (S, 1H, NHCO), 8.44(S,1H, NH, benzimidazole), 6.64-7.62 (m, 7H, Ar-H); MS: m/z $[M^+]^{+1}$ 365.

***N*-(1*H*-benzimidazol-2-yl)-2-(7-chloroisatinylidene)hydrazinecarboxamide (6g):**
Elemental analysis (Found): C, 54.01; H,3.16 ; N,23.56 ; (Calculated): C, 54.17; H, 3.13; N, 23.69; Mol. Formula: $C_{16}H_{11}ClN_6O_2$; IR (KBr,cm^{-1}): 3385.33(N-H), 1686.69(C=O, lactam), 1667.78 (C=O), 1513.27 (C=N); ^{1}H NMR (δ ppm): 12.06(s, 1H, CONH lactam), 10.28 (s, 1H, NH-N=), 9.46 (S, 1H, NHCO), 8.44(S,1H, NH, benzimidazole), 6.82-7.38 (m, 7H, Ar-H); MS: m/z $[M^+]^{+1}$ 355.

***N*-(1*H*-benzimidazol-2-yl)-2-(5-bromoisatinylideneylidene)hydrazinecarboxamide (6h):**
Elemental analysis (Found): C, 48.75; H,2.57 ; N,21.27 ; (Calculated): C, 48.14; H, 2.78; N, 21.05; Mol. Formula: $C_{16}H_{11}BrN_6O_2$; IR (KBr,cm^{-1}): 3202.11(N-H), 1690.35 (C=O, lactam), 1662.98 (C=O), 1510.80 (C=N); ^{1}H NMR (δ ppm): 12.12(s, 1H, CONH lactam), 10.32(s, 1H, NH-N=), 9.38 (S, 1H, NHCO), 8.50(S,1H, NH, benzimidazole), 6.82-7.38 (m, 7H, Ar-H); MS: m/z $[M^+]^{+1}$ 399.

Table I. Physico-chemical data of compounds 6a-6h.



Compd.	R	MF	MW	MP (°C)	%Yield	R^*_f
6a	H	$C_{16}H_{12}N_6O_2$	320	156-158	70	0.45
6b	5-CH_3	$C_{17}H_{14}N_6O_2$	334	140-144	65	0.47
6c	7-CH_3	$C_{17}H_{14}N_6O_2$	334	148-150	60	0.48
6d	5-NO_2	$C_{16}H_{11}N_7O_4$	365	142-144	62	0.42
6e	5-COOH	$C_{17}H_{12}N_6O_4$	364	240-242	60	0.50
6f	7-COOH	$C_{17}H_{12}N_6O_4$	364	146-148	64	0.52
6g	7-Cl	$C_{16}H_{11}ClN_6O_2$	354	116-118	68	0.46
6h	5-Br	$C_{16}H_{11}BrN_6O_2$	398	190-192	65	0.39

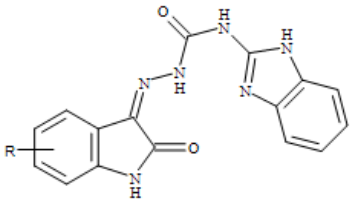
Hexane:Ethyl acetate (2:1)

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats. Wister strain albino rats of either sex weighing 180-250 g were used. Animals were housed in a group of six per cage. A 12:12 light: dark cycle was followed during the experiment. Animals had free access to food and water, however, food was withdrawn six hours before and during experiment. Diclofenac sodium and test compound were suspended in 1% sodium CMC in distilled water. The standard groups received diclofenac sodium 20 mg/kg body weight was administered as a standard drug for comparison and the test group received 100 mg/kg of 6a-6h were administered intraperitonially. The control animals received 1% sodium CMC in distilled water only. The test compounds and standard were administered one hour prior to the carrageenin injection. Acute inflammation was induced by injecting freshly prepared 0.1% w/v aqueous solution of carrageenin in the subplanter region of right hind paw. After carrageenin injection the paw volume was measured using the mercury displacement technique with the help of a plethysmograph after 1, 2 and 3 h of carrageenan injection by plathysmometer. Any significant reduction in the volume of the paw compared to the control group was considered as antiinflammatory response. The results are presented in Table 2 and graphically in Figure 1. Percent inhibition of inflammation after 3 h was calculated by using following formula.

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

Table II. Effect of 6a-6h on carrageenin induced paw edema in rat



Groups	Compd	R	Control	Test	Difference	% inhibition of paw edema volume
1	Contol		3.80	--	--	--
2	Diclofenac Sodium		3.80	0.72	3.08	81.05
3	6a	H	3.80	3.01	0.79	20.78
4	6b	5-CH_3	3.80	1.40	2.40	63.15
5	6c	7-CH_3	3.80	1.51	2.29	60.26
6	6d	5-NO_2	3.80	2.60	1.20	31.57
7	6e	5-COOH	3.80	2.50	1.30	34.21
8	6f	7-COOH	3.80	2.58	1.22	32.10
9	6g	7-Cl	3.80	1.82	1.98	52.10
10	6h	5-Br	3.80	1.90	1.90	50.00

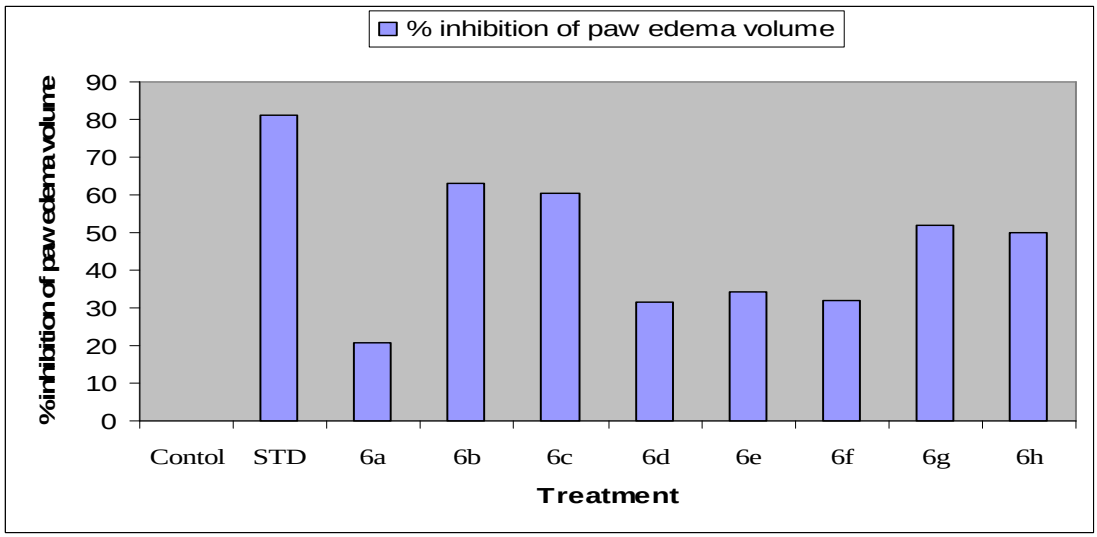
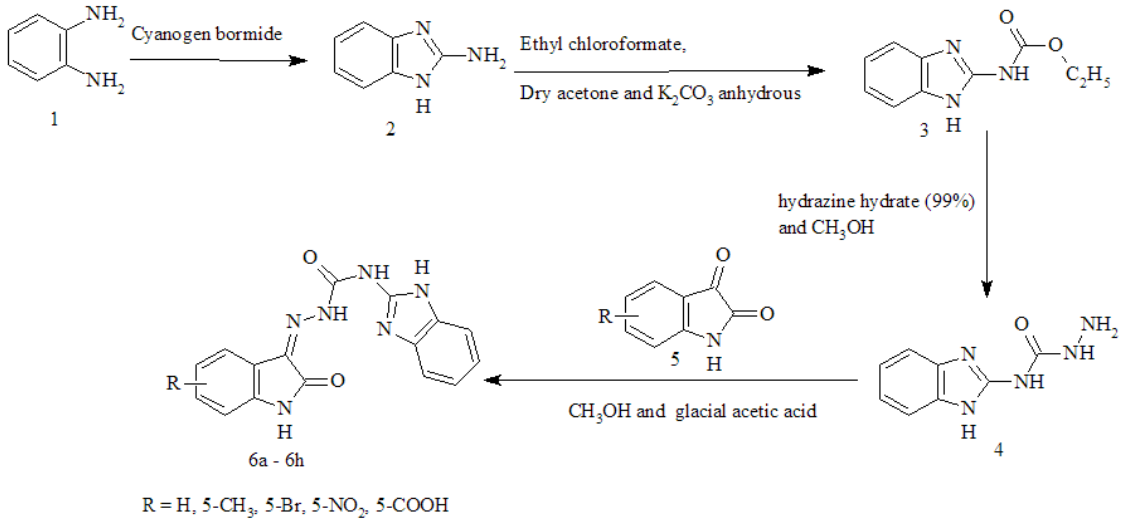


Figure I: % inhibition in Paw edema

Scheme-I: Synthetic scheme for the title compounds



CONCLUSION

In conclusion, a series of novel isatinylidene-hydrazinecarboxamide derivatives were synthesized, characterized and evaluated for *in vivo* antiinflammatory activity. The results of anti-inflammatory data revealed that the compounds possess significant activity which is on a par with the standard ligand. It is convincing that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of anti - inflammatory agents. Further studies to acquire more information concerning structural activity relationships are in progress.

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