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Synthesis and anti-inflammatory activity of 5-substituted isatin derivatives

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ABSTRACT:

Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever. Isatin (1H-indole-2,3-dione) was first obtained by erdman and laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. The synthesis of isatins derivatives (iia-h) starting from substituted anilines (Ia-h) by using oxalyl chloride as the acylating agent and H-β zeoliteas a reusable catalyst in the presence 1,2-dichloroethane as solvent at 80°C under heterogeneous conditions, and all the synthesized derivative IIa-h scrrening for the anti-inflammatory activity of synthesized isatin derivatives were carried out using carrageenan induced rat hind paw edema method, indomethacin as standard drug at dose 3 mg/kg, volume of its displacement was measured using plethysmometer and the % inhibition of edema was calculated at the end of 3 hrs, synthesized compounds showed anti-inflammatory activity ranging from 27.58 to 62.06% inhibition of rat paw edema volume after 3 hours, whereas the standard drug indomethacin showed 62.06 % inhibition of rat paw edema volume after 4 hours, the compound IId was found to be nearly significant 57.50% inhibition.

Keywords: Inflammation,Isatin,Rathindpawedema method, Anti-inflammatory activity.

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INTRODUCTION

Inflammation is an important physiological reaction which occurs in response to a wide variety of injurious agents (e.g. bacterial infection, physical trauma, chemicals or any other phenomenon) ultimately aiming to perform the dual function of limiting damage and promoting tissue repair[1]. Inflammatory processes are required for immune surveillance, optimal repair, and regeneration after injury[2]. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign

substances and prevent infection[3]. However, sustained, excessive or inappropriate inflammation is the cause of numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease [4]. Inflammation is a major component of the damage caused by autoimmune diseases, and is a fundamental contributor of various infectious and non-infectious diseases such as cancer, diabetes, cardiovascular disease, rheumatoid arthritis, Alzheimer's and arteriosclerosis. Depending on the intensity of this process, mediators generated in the inflammatory site can reach the circulation and cause fever [5]. Inflammatory process has two phases: acute and chronic. Acute and chronic inflammations are known to be complicated processes induced by several different classes of chemical mediators, e.g. prostaglandins, leukotrienes and platelet-activating factor, etc. Anti inflammatory agents exert their effect through a spectrum of different modes of action [6].

Acute inflammatory response is characterized by an increase in vascular permeability and cellular infiltration leading to oedema formation as a result of extravasation of fluid and proteins and accumulation of leukocytes at the inflammatory site for short time [7]. *Chronic inflammation* is the reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and infiltration of neutrophils with exudation of fluid. It occurs by means of development of proliferative cells which can either spread or form granuloma. Inflammatory diseases are a major cause of morbidity of the work force throughout the world. These have been called the "King of Human Miseries" [8]. Pain is an objectionable sensory and emotional incident associated with actual or potential tissue inflammation. Pyrexia or fever is caused as a secondary impact of inflammation [9]. Inflammation, pain and fever are all associated with enhanced production of prostaglandins [10]. Thus, most anti-inflammatory agents are expected to possess analgesic and antipyretic activity [11].

Isatin (1*H*-indole-2,3-dione, Figure 1) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids [12]. The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. Three reviews have been published regarding the chemistry of this compound: the first by Sumpter, in 1954, a second by Popp in 1975, and the third on the utility of isatin as a precursor for the synthesis of other heterocyclic compounds [13].

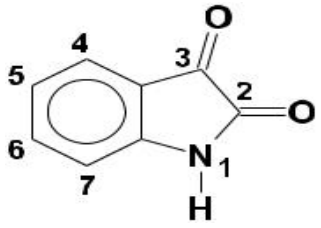
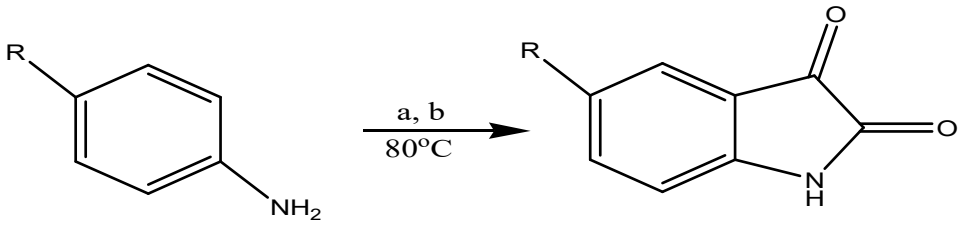


Figure 1: Structure of isatin

MATERIALS AND METHOD:

Synthetic scheme:



Ia-h IIa-h

a: *H*-beta Zeolite , **b:** 1, 2-dichloro-ethane, 32-36 Hrs, reflux

Compound Code	R
IIa	H
IIb	CH_3
IIc	Propyl
IId	Cl
IIe	F
IIf	OCH_3
IIg	NO_2
IIh	MeOOC

Procedure:

The synthesis of isatins **iia-h** starting from substituted anilines Ia-h by using oxalyl chloride as the acylating agent and H-β zeoliteas a reusable catalyst in the presence 1,2-dichloroethane as solvent at 80°C under heterogeneous conditions . The methodology thus demonstrates that H-β zeolite is superior catalyst as compared to homogeneous Lewis acid catalysts like $SnCl_4$ and $BF_3.Et_2O$[14]. The procedure requires simple filtration of the catalyst and evaporation of the solvent to obtain good yields of isatins 48–79%.

Screening of anti-inflammatory activity:

Animals

For the biological evaluation Albino wistar rats, (200-300 g), were used. The animals were kept in colony cages (6 rats each), maintained on a standard pellet diet with water, and left for 2 days for acclimatization before the experimental session [15]. They kept on fast for 16 hour before

the experiment, but free access to water. Experiments were carried out according to the ethical guidelines for the care of laboratory animals [16].

***Selection of experimental animals**:*

Healthy Albino wistar male rats weighing between 200-300 g. were used for the evaluation of anti-inflammatory activity. The animals were obtained from Zydus research centre, Ahmedabad.

Laboratory conditions:

The rats were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top [17]. Environmental room should be 22°C (± 3°C) relative humidity was at least 30 % and preferably not exceed 70 % other than during room cleaning the aim was to maintain between 50-60% [18]. Lighting was to be artificial, the sequence being 12 hours light and 12 hours [19].

Anti-inflammatory activity:

The anti-inflammatory activity of synthesized isatin derivatives were carried out using carrageenan induced rat hind paw edema method [20], Animals used:-Albino wistar rats, No. of animals used per group:-6 rats, Dose of test compound:-3 mg/kg, Dose of standard drug:-3 mg/kg (Indomethacin) [21], Route of administration:-Intra peritoneal (suspended in 1% tween-80 solution)

Experimental design and procedure:

Weigh the animals and number them. Mark the animals with picric acid for individual animal identification. Divide rats into 5 groups of 6 rats each. Note the initial paw volume of each rat by dipping just beyond tibio-tarsal junction by mercury displacement method, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume [22]. The animals were deprived of food overnight (allowed free access to water) and synthetic compounds were administered once before 30 minutes the injection of carrageenan. Dose volume not exceeding 0.5ml/100gm intra peritoneal was administered [23]. Group I:-The solvent control received normal saline [24], Group II:-Positive control received Indomethacin (3 mg/kg) [25], Group III to VI:-Received isatin derivative-IIa-.IIh at a dose of 3 mg/kg suspended in 1%w/v tween-80 [26]. After 30 minutes of test compound administration, 0.1ml of 1%w/v of carrageenan in normal saline was injected in to the sub planter region of the left hind paw of rat. Immediately after the carrageenan injection, the volume of its displacement was measured using plethysmometer [27]. The reading was recorded at 0, ½, 1, 2, 3 hrs. The % inhibition of edema was calculated at the end of 3 hrs by using the formula [28].

Percent (%) inhibition = 1 – Vt/Vc X 100,

Where Vt: - edema volume in test group,

Vc: -edema volume in control group

Results were expressed as mean ± standard deviation.

RESULT AND DISCUSSION:

Table 1: physiochemical data of synthesized compound (iia-iih)

Compound Code	% yield	*Rf value	Mol. formula	Melting point range (in ^{O}C)
Iia	79.07	0.65	$C_8H_5NO_2$	112-113
Iib	61.27	0.71	$C_9H_7NO_2$	123-124
Iic	68.33	0.69	$C_{11}H_{11}NO_2$	145-146
Iid	71.01	0.81	$C_8H_4ClNO_2$	153-54
Iie	49.82	0.76	$C_8H_4FNO_2$	167-68
Iif	48.11	0.52	$C_9H_7NO_2$	158-59
Iig	54.42	0.49	$C_8H_4N_2O_4$	187-88
Iih	66.13	0.62	$C_{10}H_7NO_4$	176-78

*Ethyl acetate: chloroform (3:7)

Table 2: Screening of Anti-inflammatory activity in Albino wistar rat:

Compound code	Inhibition of inflammation in cm					% inhibition			
	0 hr	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	0.36±0.02	0.33±0.02	0.31±0.02	0.30±0.02	0.29±0.02				
Standard (Indomethacin)	0.31±0.02	0.27±0.02	0.17±0.02	0.14±0.09	0.11±0.009	18.18	16.12	53.33	62.06
Iia	0.33±0.02	0.28±0.02	0.26±0.02	0.22±0.02	0.18±0.02	15.15	16.12	26.66	37.93
Iib	0.34±0.07	0.30±0.02	0.21±0.008	0.17±0.08	0.21±0.01	09.09	32.19	43.33	27.58
Iic	0.34±0.07	0.30±0.02	0.21±0.008	0.17±0.08	0.21±0.01	14.09	15.19	40.29	30.50
Iid	0.32±0.02	0.31±0.1	0.21±0.02	0.14±0.02	0.10±0.01	13.12	18.09	50.33	**57.50**
Iie	0.32±0.02	0.32±0.02	0.25±0.01	0.15±0.007	0.13±0.02	13.13	20.13	27.77	37.58
Iif	0.33±0.02	0.33±0.02	0.26±0.01	0.18±0.007	0.14±0.02	15.13	19.13	30.12	39.62
Iig	0.31±0.02	0.32±0.02	0.27±0.01	0.19±0.007	0.13±0.02	16.20	19.50	33.25	**48.60**
Iih	0.31±0.02	0.30±0.02	0.28±0.01	0.19±0.006	0.18±0.02	18.07	23.16	46.10	42.23

No. of animals used in each Group (n) = 6, Values are expressed as Mean ± SEM
Dose of test compound = 3 mg/kg, Dose of Indomethacin = 3 mg/kg

CONCLUSION:

The pharmacological screening of the synthesized compounds showed anti-inflammatory activity ranging from 27.58 to 62.06% inhibition of rat paw edema volume after 3 hours, whereas the standard drug Indomethacin showed 62.06 % inhibition of rat paw edema volume after 4 hours. The compound IId was found to be nearly

significant 57.50% and indomethacin 62.06% inhibition which is used as standard drug. Except then above mention compound has shown less activity then indomethacin, compound which have possess 5-chloro (-Cl) and 5-nitro (-NO_2) group with isatin having pentacyclic ring responsible for activity.

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