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Research Article

SYNTHESIS AND SCREENING OF ANTIINFLAMMATORY ACTIVITY OF INDOLE DERIVATIVES

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ABSTRACT

In the present work, some new 5-[2(3)-dialkylamino alkoxy] Indole 2, 3-diones and **5**-Hydroxyindole 3-semicarbazone 2-ones were prepared from 5-hydroxy isatin. The structures of the products were characterized by IR, NMR, MASS Spectral studies Thus synthesized and characterized targetted compounds were further screened for their anti-inflammatory activity by using Carrageenan - induced paw edema rat model. Among all the newly synthesized derivatives, Compounds IIIa-b and Compounds IVa-b were reduced the inflammation very significantly , thus these compounds showed promising anti-inflammatory activity and compounds IIIc-e & IVc-e showed moderate anti-inflammatory activity.

KEY WORDS

Synthesis, 5-[2(3)-dialkyl amino alkoxy] Indole 2, 3-diones, 5-Hydroxyindole 3-semicarbazone 2-ones, Antiinflammatory activity.

1. INTRODUCTION

Non–steroidal anti-inflammatory drugs are among the most widely used of all therapeutic agents. They are commonly prescribed for 'rheumatic' musculo skeletal complaints and are often taken without prescription for minor aches and pains. There are now more than 50 different NSAIDs on the market and none of these is ideal in controlling or modifying the signs and symptoms of inflammation. Virtually all currently available NSAIDs can have significant unwanted effects, especially in elders. Therefore, discovery of new safer NSAIDs represent challenging goal in the research area. In our ongoing medicinal chemistry research work, the substituted isatins have attracted much attention due to their prominent utilization as hypnotic [3], antibacterial [4-6] and MAO inhibitory [7] activity probably resulting from its planar and compact structure. Studies showed that these isatin moieties exerted there *in vivo* activity by inhibiting the synthesis of prostaglandin E_2. In the present study we have synthesized a series of novel derivatives with a view to screen the products for anti-inflammatory effect by carrageenan induced rat paw edema method. All the synthesized compounds were purified and the reactions were monitored by TLC. The chemical structures of the synthesized compounds (**Table 1**) were confirmed by IR, ^{1}H NMR and mass spectral analysis. The logP values of all the compounds were computed by using Graph pad prism 5.0.

2. MATERIALS AND METHODS

The compounds were mostly synthesized by conventional methods and described in experimental selection and also by the methods established in our laboratory.

2.1. Chemicals

Carragenan, Indomethacin, Dialkyl amino alkylhalides, semi carbazidehydrochloride purchased from Sigma- Aldrich Chemicals Private Limited, Hyderabad, India. p-amino phenol, hydroxylamine hydrochloride, sodium sulfate were purchased from Merck Chemicals Private Limited, Hyderabad, India.

2.2. Chemistry

Solvents were dried or distilled before use. Melting points were obtained on a Thoshniwall melting point apparatus in open capillary tubes and are uncorrected. The purity of the compounds were ascertained by TLC on silica gel –G plates(Merck).Infrared spectra(IR) were recorded with KBR pellet on a Perkin-Elmer BX series, Infrared spectrophotometer. Mass spectra were recorded by the direct inlet method on Thadmam-mass-quantam API 400H mass spectrophotometer.^{1}H NMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer in DMSO-$d_{6.}$ 5-hydroxy Isatin was synthesized from p- amino phenol by using Sandmayer[8] method It consists in the reaction of aniline with chloral hydrate and hydroxylamine hydrochloride in aqueous sodium sulfate to form an isonitrosoacetanilide, which after isolation, when treated with concentrated sulfuric acid, furnishes isatin in >75% overall yield.

2.3. Preparation of 5-Hydroxyindole 3-semicarbazone 2-one

5-Hydroxyisatin was heated under reflux in methanol containing two or three drops of acetic acid with semicarbazide hydrochloride for half an hour. The product thus separated was filtered and purified by recrystalization from suitable solvent. (Yield 89%, m.p.270^{o}c)

2.4 Preparation of 5-[2(3)-dialkyl amino alkoxy] Indole 2,3 diones and 5-Hydroxyindole 3-semicarbazone 2-ones

A mixture of 5-hydroxyisatin/5-Hydroxyisatin-3-semicarbazone (0.01 Moles) and dialkylamino alkylhalide (0.01 Moles) placed in 10% alcoholic potassium hydroxide and this mixture was stirred at room temperature for 6 hours .The alcohol was reduced to half of its volume and cooled. The product separated was filtered, washed with small portions of cold alcohol repeatedly and dried .It was purified by recrystalisation from hydro alcoholic mixtures to get a crystalline solid. Similarly other 5-Hydroxy Isatin derivatives as shown in Scheme 1 were prepared and their melting points were determined in Open capillary tubes using Toshniwall melting point apparatus and are uncorrected. Purity of the compounds was checked by TLC. The physical data of the title compounds were presented in **Table 1**. The compounds were characterized by spectral data.

2.5. Spectral data

The compounds have been characterized by the spectral data IR, PMR and Mass.

IR spectrum (KBr) of compound **(III)** exhibited absorption bands (cm-1) 3421.47 (OH),
1630.08 (C = O), 1548 (Ar,C=C), 1282(C-O-C), 883.85-579.8 (Ar).

Its PMR spectrum (DMSO, **III**) showed characteristic peaks at (d ppm) 300 MHz 13.3 (s, 1H, OH), 10.36(s, 1H,-CONH), 6.65-7.29(m, 3 H, Ar-H).

Mass spectrum of compound III showed molecular ion(M+) base peak at m/z (164.1).

Compound (**IIIa)** showed characteristic IR peaks at 3276(NH), 1651.96 (C=O), 1569.82
(Ar, C=C), 1276(C-O-C), 1080(C-N), 2860(C-C), 807.93 (Ar).

Its PMR spectrum (DMSO, **IIIa**) showed characteristic peaks at (d ppm) 300 MHz 10.36(s, 1H,-CONH), 7.21(d, H,Ar-H), 7.26(d, H,Ar-H), 7.01(s, H,Ar-H),3.2 (t,2H,O-CH_2) ,2.9 (t,2H,N-CH_2), 1.36 (s,6H,N-$(CH_3)_2$).

Mass spectrum of compound **IIIa** showed molecular ion (M+) base peak at m/z 234 (100%).It also shows peak at m/z (72) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IIIb)** showed characteristic IR peaks at 3274(NH), 1681.53 (C=O), 1570.21
(Ar, C=C), 1243(C-O-C), 1084(C-N), 2890(C-C), 845.51 (Ar).

Its PMR spectrum (DMSO, **IIIb**) showed characteristic peaks at (d ppm) 300 MHz 10.25(s, 1H,-CONH), 7.22(d, H,Ar-H), 7.26(d, H,Ar-H), 7.11(s, H,Ar-H), 2.99 (t,2H,O-CH_2) ,2.72 (t,2H,N-CH_2) ,1.24 (s,4H,N-$(CH_2$-C$)_2$), 1.22 (s,6H,(N-C-$CH_3)_2$)

Mass spectrum of compound **IIIb** showed molecular ion (M+) base peak at m/z 252 (100%). It also shows peak at m/z (99) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IIIc)** showed characteristic IR peaks at 3274(NH), 1651.96 (C=O), 1579.72 (Ar, C=C), 1266(C-O-C), 805.91(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.46(s, 1H,-CONH), 7.21-7.49(m,3 H,Ar-H),2.84 (t,2H,O-CH_2),2.51 (m,2H, CH_2),2.48 (t,2H,N-CH_2), 1.25 (S,6H,N-$(CH_3)_2$).Mass spectrum of compound **IIIc** showed molecular ion (M+) base peak at m/z

247 (100%). It also shows peak at m/z (113) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IIId)** showed characteristic IR peaks at 3257(NH), 1679.64 (C=O), 1546.86 (Ar, C=C), 1245(C-O-C), 812.71(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.51(s, 1H,-CONH), 7.12-7.42(m,3 H,Ar-H), 2.76 (m, 2H,O-CH_2), 2.45(t,3H,R_1=CH_3), 2.31 (m,1H,N-CH), 1.44 (s,6H,N-$(CH_3)_2$).Mass spectrum of compound **IIId** showed molecular ion (M+) base peak at m/z 247 (100%). It also shows peak at m/z (113) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IIIe)** showed characteristic IR peaks at 3257(NH), 1689.46 (C=O), 1576.34 (Ar, C=C), 1228(C-O-C), 814.53(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.26(s, 1H,-CONH), 7.34-7.51(m,3 H,Ar-H),2.96 (t,2H,O-CH_2) ,2.82 (t,2H,N-CH_2), 1.35 (t, 2H,N-CH) ,1.21 (d,12H,C -$(CH_3)_2$).Mass spectrum of compound **IIIe** showed molecular ion (M+) base peak at m/z 291 (100%). It also shows peak at m/z(129) may be due to the fragmentation of the alkyl chain from the molecule ion.

Compound (**IVa)** showed characteristic IR peaks at 3276(NH), 1651.96 (C=O), 1569.82 (Ar, C=C), 1276(C-O-C), 807.93(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.36(s, 1H,-CONH), 7.01-7.29(m,3 H,Ar-H),3.2 (t,2H,O-CH_2) ,2.9 (t,2H,N-CH_2), 1.36 (s,6H,N-$(CH_3)_2$).Mass spectrum of compound **IVa** showed molecular ion (M+) base peak at m/z 291 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVb)** showed characteristic IR peaks at 3274(NH), 1681.53 (C=O), 1570.21 (Ar, C=C), 1243(C-O-C), 845.51(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.25(s, 1H,-CONH), 7.03-7.45(m,3 H,Ar-H),2.99 (t,2H,O-CH_2 s) ,2.72 (t,2H,N-CH_2) , 1.24 (m,6H,N-C-CH_3,1.12(t, 2H,N-CH_2), 7.41-7.46(d,2H, NH_2) ,11.36(s,1H, NH).Mass spectrum of compound **IVb** showed molecular ion (M+) base peak at m/z 317 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVc)** showed characteristic IR peaks at 3274(NH), 1651.96 (C=O), 1579.72 (Ar, C=C), 1266(C-O-C), 805.91(Ar). ^{1}H NMR (300 MHz, DMSO-d_6):10.46(s, 1H,-CONH), 7.21-7.49(m,3H,Ar-H),2.84 (t,2H,O-CH_2) , 2.51 (m,2H, CH_2), 2.48 (t,2H,N-CH_2), 7.41-7.46(d,2H, NH_2) ,11.36(s,1H, NH), 1.25 (s,6H,N-$(CH_3)_2$). Mass spectrum of compound **IVc** showed molecular ion (M+) base peak at m/z 363 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVd)** showed characteristic IR peaks at 3257(NH), 1679.64 (C=O), 1546.86(Ar ,C=C), 7.41-7.46(d,2H, NH_2) ,11.36(s,1H, NH) 1245(C-O-C), 812.71(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.51(s, 1H,-CONH), 7.12-7.42(m,3 H,Ar-H), 2.76 (m, 2H,O-CH_2), 2.45(t,3H,R_1=CH_3), 2.31 (m,1H,N-CH), , 7.41-7.46(d,2H, NH_2) ,11.36(s,1H, NH), 1.44 (s,6H,N-$(CH_3)_2$).Mass spectrum of compound **IVd** showed molecular ion (M+) base peak at m/z 363 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

Compound (**IVe)** showed characteristic IR peaks at 3257(NH), 1689.46 (C=O), 1576.34 (Ar ,C=C), 1228(C-O-C), 814.53(Ar). ^{1}H NMR (300 MHz, DMSO-d_6): 10.26(s, 1H,-CONH), 7.34-7.51(m,3 H,Ar-H),2.96 (t,2H,O-CH_2) , 7.41-7.46(d,2H, NH_2) ,11.36(s,1H, NH),2.82 (t,2H,N-CH_2), 1.35(m, 2H, N-CH),1.21(d,12H,C-$(CH_3)_2$)..Mass spectrum of compound **IVe** showed molecular ion (M+) base peak at m/z 347 (100%). The mass spectrum shows its base peak at m/z 77 (100%) may be due to the fragmentation of the semicarbazone from the molecule ion.

3. PHARMACOLOGY

3.1 Anti-inflammatory activity

Carrageenan - induced rat paw edema method[9] was employed for evaluating the anti inflammatory activity of the synthesized compounds**.** Wister Albino rats of either sex weighing approx 200- 300 gm, were housed in clean polypropylene cages and kept under room temperature (25±2°C), and relative humidity 40-50% in a 12 h light-dark cycle. Food was withdrawn 12 h before and during experimental hours. In this study, the animals were divided into groups as shown in the **Table-2.** Acute inflammation was produced by sub plantar injection of 0.1ml of 1% suspension of Carrageenan in normal saline, in the right hind paw of the rats. After intra peritoneal administration of the test compounds,

the paw volume was measured Plethysmometrically at 1, 2, 3, and 4 h intervals. Indomethacin 5mg/kg in normal saline was used as standard drug.

Table 2: Antiinflammatory activity 5-[2(3)-dialkyl amino alkoxy] Indole 2,3di-ones (IIIa-IIIe) and 5-[2(3)-dialkyl amino alkoxy] Indole 3-semicarbazone-2-ones (IVa-IVe)

Compound	1hr	%inhibition	2hr	%inhibition	3hr	%inhibition	4hr	%inhibition
IIIa	0.53	34.30	0.55	47.81	0.47	62.7	0.46	73.6
IIIb	0.63	22.7	0.62	38.1	0.56	58.9	0.43	73.3
IIIc	0.72	16.5	0.68	30.7	0.55	57.6	0.53	70.5
IIId	0.71	13.2	0.67	30.8	0.63	52.5	0.55	69.4
IIIe	0.75	10.8	0.70	27.2	0.63	51.2	0.55	69.4
IVa	0.54	37.5	0.51	47.9	0.48	63.07	0.45	75
IVb	0.60	28.3	0.58	40.8	0.50	61.5	0.43	74.1
IVc	0.75	16.7	0.68	30.6	0.65	50	0.36	78.8
IVd	0.68	22.4	0.65	34.7	0.53	59.2	0.46	72.4
IVe	0.62	20	0.67	33.6	0.56	56.9	0.48	71.3
INDOMETHACIN	0.63	25.8	0.53	45.9	0.48	63.07	0.43	76.1
CONTROL	0.85	-	0.98	-	1.3	-	1.8	-

n=6 animals per each group, dose 100mg/kg body weight

4. RESULTS AND DISCUSSIONS

Physical data TLC, IR, ^{1}H NMR and mass spectra confirmed the structures and purity of the synthesized compounds. All the title compounds decomposed before melting. All the synthesized compounds were evaluated for their in vivo antiinflamatory activity. It was observed that compounds **IIIa, IVa, IIIb, IVb**, significantly reduced the inflammation, there by showed a promising anti-inflammatory activity, where as the compounds I**Vd, IVe IIIc, IVc, IIId, IIIc,IIIe** moderately reduced the inflammation towards carrageenan induced paw edema rat model, when compared to the standard drug indomethacin.

5. CONCLUSION

A new series of five 5-[2(3)-dialkyl amino alkoxy] Indole 2,3 dione derivatives were synthesized by reacting 5-hydroxyindole 2,3 diones with 2-N,N di alkylamino alkyl halides.Evaluation of these compounds as antiinflamatory activity revealed that the compounds **IVa**($R=CH_3$)**,IVb**($R=C_2H_5$)**, IIIa**($R=CH_3$) **and IIIb**($R=C_2H_5$) with a dimethyl and diethyl amino ethyl chain derivatives was found to be relatively superior in antiinflamatory activity and other compounds(**IIIc, IVc, IIId, IVd, IIIe, IVe**) are next in the order of activity.This study reports the successful synthesis of the title compounds in good yields and moderate to potent anti-inflammatory activity of these derivatives containing isatin moiety which is comparable with standard drug. It has been observed that the increased anti-inflammatory activity is attributed to the presence of pharmacologically active groups like semicarbazide side chain.

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