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Synthesis and Screening of New Isatin Derivatives

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ABSTRACT

In view of the biological prominence of isatin derivatives and benzimidazole derivatives, it is planned to synthesize new isatin derivatives containing 1-H-benzimidazol-2yl methane-thiol moiety in one side chain at 3rd position. So, some new 2-[1H-benzimidazol-2-ylmethyl) sulfanyl]-N'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides (VIII) have been synthesized as depicted in scheme-I. The intermediates and final compounds were purified and their chemical structures have been confirmed by IR, ^{1}H NMR, Mass and by elemental analysis. Investigation of anti-inflammatory activity was done by carrageenan induced rat hind paw edema method. Among the compounds tested, Compounds (VIIIc) & (VIIId) with methyl group at 5th and 7th positions of the indole ring exhibited maximum activity with percentage inhibition of 68.56 and 61.41. Compounds VIIIi(R=5-COOH), VIIIg(R=5-Cl), VIIIh(R=7-Cl), VIIIb(R=5-Br), VIIIf(R=7-NO_2), VIIIe(R=5-NO_2) and VIIIa(R=H) were found to be next in the order of anti-inflammatory activity.

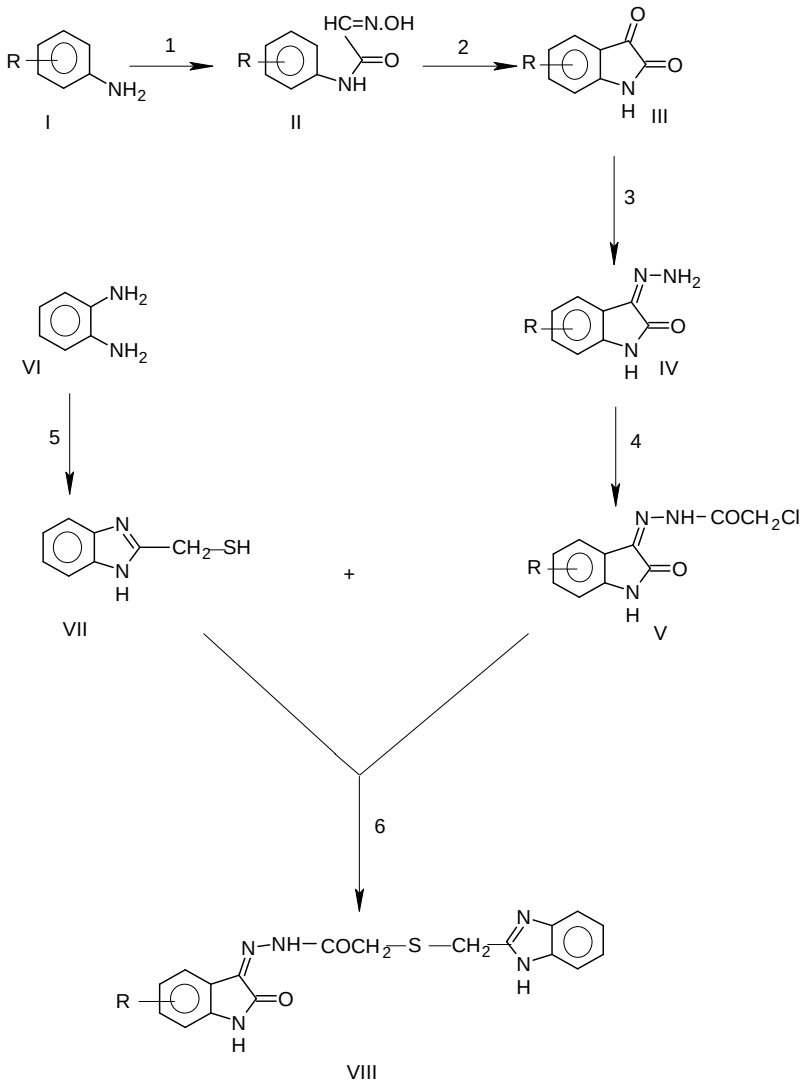
Keywords: Isatins, benzimidazoles, anti-inflammatory agents.

INTRODUCTION

It is evident from literature that isatin derivatives are known to be associated with broad spectrum of biological activity like antibacterial[1], antiinflammatory[2], analgesic[3], anti-viral [4], antifungal[5], anti-tubercular[6], anti-depressant[7]. Isatin hydrazones have been reported to possess anticonvulsant [7] activity also. In view of these facts and as a continuation of our work in the laboratory, prompted us to synthesize some new 2-[1H-benzimidazol-2-ylmethyl) sulfanyl]-N'-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides (VIII). All the synthesized compounds were screened for their anti-inflammatory activity.

MATERIALS AND METHODS

SCHEME I



R=H, 5-Br, 5-CH_3, 7-$CH_3$5-NO_2, 7-NO_2, 5-Cl, 7-Cl, 5-COOH.

1. Chloral hydrate, hydroxyl amine hydrochloride & sodium sulphate.
2. Conc. sulphuric acid.
3. Hydrazine hydrate (99%) & methanol.
4. Chloroacetylchloride & dry benzene.
5. Thioglycollic acid, 4N HCl
6. Alcoholic KOH

Experimental Procedure:

1. Synthesis of isatins (III, indole-2, 3-dione):-

a) Synthesis of Isonitrosoacetanilides:

In a 5 lit. R.B.flask were placed chloral hydrate (0.54mol) and 1200ml of water. To this solution, were then added crystallized sodium sulphate (1300g) followed by a solution of an appropriate aromatic amine (0.5 mol) in 300ml of water and concentrated hydrochloric acid (0.52 mol). Finally a solution of hydroxylamine hydrochloride (1.58 mol) in 500ml of water was added. The contents of flask were heated over a wire-guage by a Meckner burner so that vigorous boiling begins in about 45min. After 1-2 min of vigorous boiling the reaction was completed. During the

heating period itself the crystals of isonitrosoacetanilide 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.

b) Synthesis of Indole-2, 3-dione (Isatins):
Sulphuric acid (600g,d,1.84,326ml) was warmed to 50ºC in a one-liter R.B.flask fitted with an efficient mechanical stirrer and to this, finely powdered an appropriate isonitrosoacetanilide (0.46mol) was added at such a rate so as to maintain the temperature between 60ºC and 70ºC, but not higher. External cooling was applied at this stage so that the reaction could be carried more rapidly. After the addition of isonitrosoacetanilide compound was completed, the temperature of the solution was raised to 80ºC 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 (2.5kg) 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 affected by recrystallization from methanol.

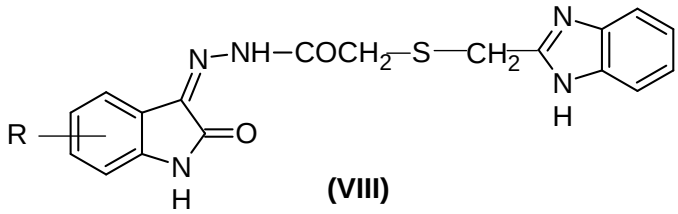
2. Synthesis of [3-hydrazono-1,3-dihydro-2H-indol-2-one] (IV):
An appropriate isatin (indole-2,3-dione) (III, 0.01 mol) was dissolved in alcohol (20 ml) and added hydrazine hydrate (99%, 0.015 mol) while shaking. The reaction mixture was stirred well, warmed on a water-bath for 10 min and left in the refrigerator for 3 h. The resultant yellow crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small quantity of cold alcohol. The product was dried and purified by recrystallization from chloroform. M.P; 220°C, Yield; 74.5%.

3. Synthesis of 2-chloro-N'-[2-oxo-1,2-dihydro-3H-indol-3- ylidene] acetohydrazide (V):
An appropriate amount of isatin hydrazone (IV) and chloroacetylchloride in dry benzene was taken in a round bottom flask and the solution was boiled under reflux for half an hour under anhydrous conditions using calcium chloride guard tube. The solution was cooled to room temperature and poured into petri dish and allowed for evaporation. The resultant reddish brown crystalline solid was purified by recrystallization from chloroform. M.P; 280°c, Yield; 70%.

4. Synthesis of 1H-benzimidazole-2-yl methanethiol (VII):
A mixture of 4gm Orthophenylene diamine, 36ml of 4N HCL and 3.4ml thioglycollic acid was taken in a round bottom flask and the solution was boiled under reflux for 3 hours until reaction completes which is checked by T.L.C analysis. Further the solution was cooled on ice and made alkaline by the addition of sodium hydroxide pellets. The precipitate formed was filtered, dried and recrystalized from suitable solvents. M.P; 154°-157°c; Yield; 65%.

5. Synthesis of 2-[(1H-benzimidazol-2-ylmethyl) sulfanyl]-N'-[2-oxo-1,2- dihydro-3H-indol-3-ylidene]aceto hydrazides (VIII):
A mixture of an appropriate amount of 2-chloro-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]acetohydrazide (0.01mole) and 1H-benzimidazole-2-yl methanethiol (0.01mole) in ethanolic potassium hydroxide(50ml) was refluxed for 3 hours at 70ºC until reaction completes which is checked by TLC analysis. The resultant solution was cooled to room temperature and poured into petri dish for evaporation of the solvent. The compound thus obtained was dried well and recrystallized from suitable solvents. The purity of the compound was checked by TLC and spectral data.

Adopting this procedure as many as nine compounds were prepared and their physical data is presented in Table-I



(VIII)

Table – I: Physical data of 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides (VIII)

S.No	Compound	Substituent	Molecular formula	Melting Point(°C)	Molecular weight	Yield %
1	VIIIa	H	$C_{18}H_{15}O_2N_5S$	215-217	366	60
2	VIIIb	5-Br	$C_{18}H_{14}O_2N_5SBr$	310-312	444	50
3	VIIIc	5-CH_3	$C_{19}H_{17}O_2N_5S$	230-233	378	70
4	VIIId	7- CH_3	$C_{19}H_{17}O_2N_5S$	220-222	378	70
5	VIIIe	5-NO_2	$C_{18}H_{14}O_4N_6S$	315-318	409	40
6	VIIIf	7-NO_2	$C_{18}H_{14}O_4N_6S$	320-322	409	40
7	VIIIg	5-Cl	$C_{18}H_{14}O_2N_5SCl$	155-158	398	50
8	VIIIh	7-Cl	$C_{18}H_{14}O_2N_5SCl$	160-162	398	50
9	VIIIi	5-COOH	$C_{19}H_{15}O_4N_5S$	250-253	408	40

Spectral data:

1. 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-N'-[2-oxo-1,2 dihydro-3H-indol-3-ylidene]aceto hydrazides(VIIIa):

IR (KBr): 3359.82 (NH lactum); 3291.11(NH acid hydrazide); 3195.33 (NH imidazole); 2919.44(C-H str); 1664.52(C=O); 1612.35(C=N); 1464.62 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.3(s,1H, NH lactum);10.8(s,1H,NH acid hydrazide);6.9- 8.1(m, 8H, Ar-H); 1.2 (s, 2H, CH_2); 2.6(s, 2H, CH_2).; EI-MS: *m/Z* = 366 (M+).

2. **2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-N'-[2-oxo-5 bromo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides(VIIIb):**

IR(KBr): 3348.87(NH- lactum);3284.80(NH acid hydrazide); 3062.80 (NH imidazole);2917.95(C-H str);1678.99(C=O);1615.34(C=N); 1463.46(C=C Ar); 573.63(C-Br).; ^{1}H NMR (DMSO-d_6) (: δ 11.4(s,1H, NH lactum);10.9(s,1H,NH acid hydrazide);7.1- 8.2(m, 8H, Ar-H); 1.4 (s, 2H, CH_2); 2.7(s, 2H, CH_2).; EI-MS: *m/Z* = 444 (M+).

3. 2-((1H-benzo[d]imidazol-2-yl)methylthio)-N'-(5-methyl-2-oxoindolin-3-ylidene)acetohydrazide(VIIIc):

IR (KBr): 3401.25 (NH lactum); 3293.28(NH acid hydrazide); 3192.81 (NH imidazole); 2914.41(C-H str); 1661.76(C=O); 1619.38(C=N); 1462.70 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.4(s,1H, NH lactum);10.6(s,1H,NH acid hydrazide);6.8- 8.0(m, 8H, Ar-H); 1.2 (s, 2H, CH_2); 2.4(s, 2H, CH_2); 2.1(s, 3H, CH_3)

4. 2-((1H-benzo[d]imidazol-2-yl)methylthio)-N'-(7-methyl-2-oxoindolin-3-ylidene)acetohydrazide(VIIId):

IR (KBr): 3368.12 (NH lactum); 3280.23(NH acid hydrazide); 3193.51 (NH imidazole); 2911.49(C-H str); 1655.86(C=O); 1612.96(C=N); 1466.29 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ

11.2(s,1H, NH lactum);10.8(s,1H,NH acid hydrazide);7.0- 8.1(m, 8H, Ar-H); 1.2 (s, 2H, CH_2); 2.6(s, 2H, CH_2); 2.3(s, 3H, CH_3)

5. 2-((1H-benzo[d]382midazole-2-yl)methylthio)-N'-(5-nitro-2-oxoindolin-3-ylidene)acetohydrazide(VIIIe):
IR (KBr): 3349.86 (NH lactum); 3292.25(NH acid hydrazide); 3189.05 (NH imidazole); 2929.43(C-H str); 1669.50(C=O); 1611.39(C=N); 1460.52 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.3(s,1H, NH lactum);10.8(s,1H,NH acid hydrazide);6.8- 7.9(m, 8H, Ar-H); 1.1 (s, 2H, CH_2); 2.3(s, 2H, CH_2).

6. 2-((1H-benzo[d]imidazol-2-yl)methylthio)-N'-(7-nitro-2-oxoindolin-3-ylidene)acetohydrazide(VIIIf):
IR (KBr): 3389.03 (NH lactum); 3290.11(NH acid hydrazide); 3191.38 (NH imidazole); 2915.49(C-H str); 1634.50(C=O); 1622.85(C=N); 1462.62 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.4(s,1H, NH lactum);10.7(s,1H,NH acid hydrazide);6.9-8.1(m, 8H, Ar-H); 1.3 (s, 2H, CH_2); 2.5(s, 2H, CH_2).

7. **2-((1H-benzo[d]imidazol-2-yl)methylthio)-N'-(5-chloro-2-oxoindolin-3-ylidene)acetohydrazide(VIIIg):**
IR (KBr): 3349.82 (NH lactum); 3202.46(NH acid hydrazide); 3180.39 (NH imidazole); 2918.41(C-H str); 1669.51(C=O); 1610.37(C=N); 1465.68 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.3(s,1H, NH lactum);10.9(s,1H,NH acid hydrazide);6.8-8.1(m, 8H, Ar-H); 1.2 (s, 2H, CH_2); 2.4(s, 2H, CH_2).

8. **2-((1H-benzo[d]imidazol-2-yl)methylthio)-N'-(7-chloro-2-oxoindolin-3-ylidene)acetohydrazide(VIIIh):**
IR (KBr): 3350.81 (NH lactum); 3292.91(NH acid hydrazide); 3193.74 (NH imidazole); 2939.41(C-H str); 1674.58(C=O); 1622.35(C=N); 1464.60 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.2(s,1H, NH lactum);10.9(s,1H,NH acid hydrazide);7.0-8.1(m, 8H, Ar-H); 1.3 (s, 2H, CH_2); 2.3(s, 2H, CH_2).

9. **3-(2-((1H-benzo[d]imidazol-2-yl)methylthio)acetoylimino)-2-oxoindoline-5-carboxylic acid(VIIIi):**
IR (KBr): 3329.84 (NH lactum); 3231.86(NH acid hydrazide); 3190.37 (NH imidazole); 2969.74(C-H str); 1634.51(C=O); 1632.37(C=N); 1474.60 (C=C Ar).; ^{1}H NMR (DMSO-d_6) (: δ 11.3(s,1H, NH lactum);10.8(s,1H,NH acid hydrazide);6.9-8.2(m, 8H, Ar-H); 1.4 (S, 2H, CH_2); 2.5(s, 2H, CH_2); 10.9(s,1H, COOH)

Anti-inflammatory activity by carrageenan induced rat hind paw edema method [8,9].
Wistar strain albino rats weighing between 180-250 gm fasted 24 hrs before the test, were divided into four groups of six animals each. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading compounds were tested in the dose of 50 mg/kg body weight. Diclofenac 20 mg/kg was used as standard. The compounds were administered as suspensions in sodium CMC (0.1% w/v) intraperitoneally 30 min 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 swelling produced after injection of the phlogistic agent was measured at hourly intervals for 6 hrs. Percentage inhibition of edema was calculated using the formula given below and the results are presented in the Table II.

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

Table –II: Anti-inflammatory activity of 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides(VIII)

Compound 50mg/kg	R	Control	Test	Difference	%Inhibition
VIIIa	H	3.9	2.919	0.981	25.15
VIIIb	5-Br	3.9	2.810	1.090	27.94
VIIIc	5-CH_3	3.9	1.226	2.674	68.56
VIIId	7- CH_3	3.9	1.505	2.395	61.41
VIIIe	5-NO_2	3.9	2.815	1.085	27.82
VIIIf	7-NO_2	3.9	2.810	1.090	27.94
VIIIg	5-Cl	3.9	2.620	1.280	32.82
VIIIh	7-Cl	3.9	2.790	1.110	28.46
VIIIi	5-COOH	3.9	2.210	1.690	43.33
Diclofenac sodium 20mg/kg		3.9	0.512	3.228	84.30

RESULTS, DISCUSSION AND CONCLUSION

The preliminary studies on anti-inflammatory activity of the new title compounds ie., 2-[(1H-benzimidazol-2-ylmethyl)sulfanyl]-N'-[2-oxo-1,2-dihydro-3H-indol-3-ylidene]aceto hydrazides (VIII) generated some interesting data. The test compounds were evaluated for anti-inflammatory activity and data are presented in Table II using diclofenac sodium (20 mg/kg) as the standard.

The close observation of anti-inflammatory activity of all the test compounds shows that all the test compounds showed mild to moderate anti-inflammatory activity. Compounds (VIIIc) & (VIIId) with methyl group at 5^{th} and 7^{th} positions of the indole ring exhibited maximum activity with percentage inhibition of 68.56 and 61.41. Compounds VIIIi(R=5-COOH), VIIIg(R=5-Cl), VIIIh(R=7-Cl), VIIIb(R=5-Br), VIIIf(R=7-NO_2), VIIIe(R=5-NO_2) and VIIIa(R=H) were found to be next in the order of anti-inflammatory activity.

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