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Synthesis and anti-inflammatory effects evaluation of 1,3 substituted isatin derivatives

Farshid Hassanzadeh[1], Elham Jafari[1], Nahal Khayambashi[1], Valiollah Hajhashemi[2]

[1]Department of Medicinal Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran, [2]Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Corresponding Author:
Elham Jafari,
Department of Medicinal Chemistry and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran. E-mail: Jafari@pharm.mui.ac.ir

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ABSTRACT

Objectives: Inflammation is a pathophysiological response of the body to a variety of agents including infections and physical injury leading to local accumulation of plasma fluid and blood cells. Isatin as a promising heterocyclic molecule has shown interesting biological profile. In this study, some derivatives of isatin containing both Schiff and mannich base fragments were synthesized in a three-step procedure. **Methods:** Benzohydrazide derivatives were prepared by reaction of benzoat derivatives with hydrazine hydrate. The Schiff bases were formed by treatment isatin with the benzo hydrazide derivatives. The corresponding mannich bases have been synthesized by the reaction of the Schiff bases with amine derivatives in the presence of formaldehyde and trifluoroacetic acid. In the case of 2-hydroxybenzohydrazide, final product was synthesized by condensation the corresponding mannich base derivative with 2-hydroxybenzohydrazide. Final compounds were evaluated for anti-inflammatory activity using the croton oil-induced ear edema test. **Results:** Among tested compounds, compound b_3 which included only mannich base fragment reduced inflammation very significantly. Compounds C_2 and C_3 containing both Schiff and mannich base groups showed moderate anti-inflammatory activity. **Conclusion:** It can be considered that the existence and nature of substituents at the 1 position of isatin nucleus play an important role in modulating their anti-inflammatory properties.

Keywords: Anti-inflammatory, benzohydrazide, isatin, mannich base, schiff base

INTRODUCTION

Inflammation is a protective and natural response of the body to tissue damage caused by physical trauma, chemical and microbial agents which is characterized by release and function of various inflammatory mediators. During the inflammation procedure, the body provides the stage and conditions for tissue repair by destroying and inactivating invasive agents.[1-3] Non –steroidal anti-inflammatory drugs are prescribed for inflammation as usual, however, these drugs aren't ideal because of significant unwanted effects, particularly, in elder.[3,4] Indole-1H-2,3-diones or isatins are well-known natural products and endogenous biological regulators which were found in the brain, peripheral tissues, and body fluids of humans and animals.[5] The importance of this scaffold in medicinal chemistry derives from its prominent biological effects including anti- microbial,[6-9] anti-inflammatory,[10-13] anticancer[14-16], and anticonvulsant[17,18] properties. Various chemical modifications and the synthesis of isatin derivatives through Schiff base formation, N-alkylation/acylation, and mannich base can lead to promising pharmacological effects. A number of Schiff and mannich bases of isatin are documented for pharmacological activities including antimicrobial and anti-inflammatory effects.[3,19-23] Structure-activity relationship studies of isatin derivatives have revealed that substitution at the 1-position with alkyl chains not longer than two carbon atoms can produce an egregious rise in the biological activities.[24] Reported results on isatin-based anti-inflammatory derivatives encouraged us to the synthesis of some new isatin-derivatives which could be considered as anti-inflammatory agents.

MATERIALS AND METHODS

Materials

All starting materials were purchased either from Merck or Sigma-Aldrich companies. Melting points were determined in open capillaries using electrothermal 9200 melting point apparatus (England) and are uncorrected. Infrared (IR) (KBr discs) was recorded with aWQF-510FT-IR spectrophotometer (China). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Bruker 400 MHz spectrometers (Germany) using tetramethylsilane as internal standard and either DMSO-d_6 or $CDCl_3$ as solvents. Mass spectra were registered on Agilent Technologies 5975C mass spectrometer (USA).

Synthesis

Synthesis of benzohydrazide derivatives (a_1, a_2)

Benzoat derivatives (ethyl benzoate or ethyl 2-hydroxy benzoate) (0.07 mol) were added dropwise to a solution of hydrazine hydrate (0.14 mol) in ethanol (30 mL). The solution was refluxed for 6 h. After the completion of reaction, the ethanol was removed by vacuum distillation. On cooling the resultant mixture, white needle crystals of benzohydrazide derivatives began to separate. It was filtered, and recrystallized from ethanol[25] [Scheme1].

Synthesis of (2)-N'-(2-oxoindolin-3-ylidene) benzohydrazide (b_1)

A mixture of isatin (0.014 mol), benzohydrazide (0.014 mol) **($\mathbf{a}_1$),** and two or three drops of glacial acetic acid in methanol (40 mL) was heated under reflux on water bath for 6–7 h. Colored separated product was filtered and purified by recrystallization from methanol[12] [Scheme 1].

Synthesis of (z)-N'-(1-((dimethylamino) methyl)-2-oxoindolin-3-ylidene) benzohydrazide (C_1)

Dimethylamine (0.01 mol) was added to a slurry containing (2)-N'-(2-oxoindolin-3-ylidene) benzohydrazide (0.01 mol) (**$\mathbf{b}_1$**) and formaldehyde (0.02 mol) in 20 mL of absolute ethanol.[13] Two or three drops of trifluoroacetic acid were added to this mixture as catalyst and stirred for 9 h at room temperature. Upon completion, the mixture was filtered by suction. The solvent was evaporated under vacuum and residue recrystallized from methanol to give **$\mathbf{C}_1$** [Scheme 1].

Synthesis of (z)-N'-(1-((methylamino) methyl)-2-oxoindolin-3-ylidene) benzohydrazide (C_3)

Methylamine (0.01 mol) was added to a slurry containing (2)-N'-(2-oxoindolin-3-ylidene) benzohydrazide (0.01 mol) (**$\mathbf{b}_1$**) and formaldehyde (0.02 mol) in 20 mL of absolute ethanol.[13] Two or three drops of trifluoroacetic acid were added to this mixture as catalyst and stirred for 3h at room temperature. Upon completion, the mixture was filtered by suction. The solvent was evaporated under vacuum and residue stirred in a mixture of petroleom ether/chloroform system (60:40) then was filtered by suction to give **$\mathbf{C}_3$** [Scheme 1].

Synthesis of 1-((Dimethylamino)methyl)indoline-2,3-dione (b_2)

Isatin (0.013 mol) was dissolved in absolute ethanol (20 mL). Then, dimethylamine (0.013 mol), formaldehyde (0.026 mol)[15] and two or three drops of trifluoroacetic acid were added to this solution followed by stirring for 3 days at room temperature. As soon as the reaction is completed, filtered to give compound **$\mathbf{b}_2$ [**Scheme 1].

Synthesis of (z)-N'-(1-((Dimethylamino)methyl)-2-oxoindolin-3-ylidene)-2-hydroxybenzohydrazide (C_2)

A mixture of **$\mathbf{b}_2$** (0.014 mol), compound **$\mathbf{a}_2$** (0.014 mol) and two or three drops of glacial acetic acid in methanol (40 mL) was heated under reflux on water bath for 10 h.[12] After the completion of reaction, mixture was filtered and obtained precipitate was suspended in a mixture of petroleom ether/chloroform system (60:40) and stirred for 1 h, then filtered and resulting solid extracted with water/ethyacetate. The extracted ethyl acetate upon evaporation afforded the products **$\mathbf{C}_2$**([Scheme 1].

Synthesis of 1-((Ethylamino) methyl) indoline-2, 3-dione (b_3)

Isatin (0.013 mol) was dissolved in absolute ethanol (20 mL). Then, ethylamine (0.013 mol), formaldehyde (0.026 mol)[15] and two or three drops of trifluoroacetic acid were added to this solution followed by stirring for 4 days at room temperature. Reaction was monitored by TLC. Solvent removed by evaporation under vaccum. The residual brown solid was suspended in petroleum ether, filtered and solvent evaporated to give **$\mathbf{b}_3$** [Scheme 1].

Biological Assays

Croton oil-induced ear edema

Anti-inflammatory activity was evaluated using the Croton oil-induced ear edema test.

Male mice (25–30 g) were assigned to groups of six animals. At first, two doses of the final compound (100 and 200 or 200 and 400 mg/kg) were separately injected intra-peritoneal to each group of six animals 30 min prior to croton oil administration. Normal saline and indomethacin (10mg/kg) were used as control and positive analgesic groups respectively. 20µL of croton oil solution 2.5% (v/v) which was previously prepared in acetone topically speared with sampler to the inner surface of the right ear of mice. The left ear was considered as a blank. The mice were sacrificed 6 h later with ether in desiccatore. Six mm disks were separated from both ears of mice and then weighed. The weight difference between disjunct disks of right and left ears for each mice was taken as an anti-inflammatory effect index.[26]

RESULTS AND DISCUSSION

Benzohydrazide (a_1)

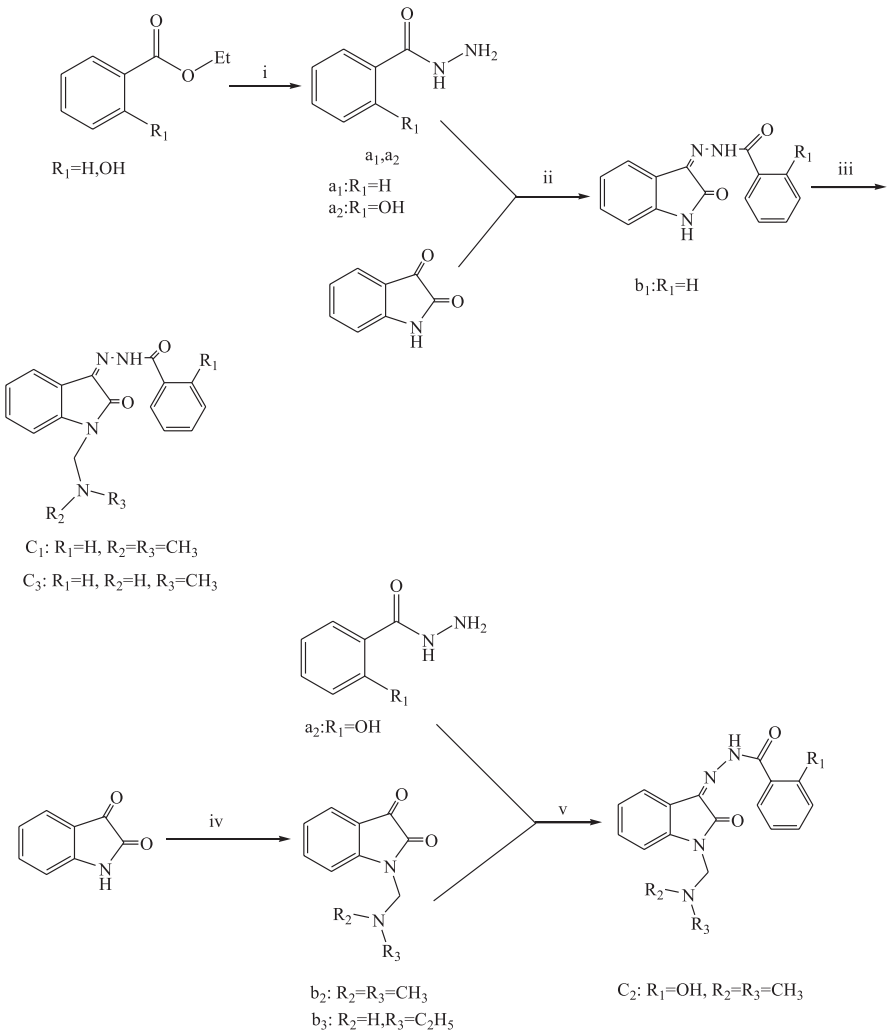
Yield: 90%, white solid, m.p. 113–115°C (lit: 113–115°C),[27] IR (KBr, cm^{-1}), 3213, 3300 (NH), 1666 (C=O); MW. 136.15 g/mol.

2-Hydroxybenzohydrazide (a_2)

Yield: 90%, white solid, m.p. 249–252°C (lit: 251–254°C),[11] IR (KBr, cm^{-1}),3270 (NH), 1643 (C=O); MW. 152.15 g/mol.

(z)-N'-(2-Oxoindolin-3-ylidene)benzohydrazide (b_1)

Yield: 85%, Orange solid, IR (KBr, cm^{-1}), 3372(NH), 3192(C-H, Ar), 1686 (C=O), 1555(NH), ^{1}HNMR: (400



Scheme 1: Synthetic route to final compounds (C_1-C_3), b_3. **(i)** NH_2NH_2, EtOH, 6 h reflux; **(ii)** glacialaceticacid, methanol 6 h reflux; **(iii)** $NH(CH_3)_2/NH_2$-CH_3, CH_2O, CF_3COOH, EtOH, room temperature; **(iv)** $NH(CH_3)_2/NH_2$-C_2H_5, CH_2O, CF_3COOH, EtOH, room temperature; **(v)** glacialacetic acid, methanol10 h reflux

MHz; $CDCl_3$): δ 7.94 (2H, d, *J*=8Hz, H-Ar), 7.81-7.79 (br, NH), 7.54 (1H, d, *J* = 8 Hz, H- Isatin), 7.46 (2H, t, *J* = 8 Hz, H-Ar), 7.30 (1H, t, *J*= 8 Hz, H-Ar), 7.14–7.24 (1H, m, H- Isatin), 7.09 (1H, t, *J* = 8Hz, H- Isatin), 6.85 (1H, d, *J* = 8Hz, H- Isatin); MW. 265.27 g/mol.

(z)-N'-(1-((Dimethylamino) methyl)-2-oxoindolin-3-ylidene) benzohydrazide (C_1)

Yield: 40%, Yellow solid, m.p. >270°C, IR (KBr, cm^{-1}), 3459 (NH), 3186 (C-H, Ar), 1695,1676 (C=O). ^{1}HNMR: (400 MHz; $CDCl_3$): δ 7.90 (2H, d, *J*=8Hz, H-Ar), 7.61 (1H, d, *J*=8Hz, H- Isatin), 7.55 (1H, t, *J*=8Hz, H-Isatin), 7.43 (2H, t, *J*=8Hz, H-Ar), 7.29 (1H, t, *J*=8Hz, H-Ar), 7.04 (1H, d, *J*=8Hz, H-Isatin), 6.98 (1H, t, *J*=8Hz, H-Isatin), 5.28 (2H, d, *J*=8 Hz, CH_2), 1.48 (6H, s. CH_3); MW. 322.37 g/mol.

(z)-N'-(1-((Methylamino) methyl)-2-oxoindolin-3-ylidene) benzohydrazide (C_3)

Yield: 43%, Yellow solid, m.p. >270°C, IR (KBr, cm^{-1}), 3437 (NH), 3184 (C-H, Ar), 1671, 1683(C=O),^{1}HNMR: (400 MHz; $CDCl_3$): δ7.98 (2H, d, *J*=8Hz, H-Ar), 7.67-7.63 (2H, m, H-Isatin and H-Ar), 7.52 (2H, t, *J*=8Hz, H-Ar), 7.36 (1H, t, *J*=8Hz, H-Isatin), 7.12 (1H, d, *J*=8Hz, H-Isatin), 7.04 (1H, t, *J*=8Hz, H-Isatin), 5.36 (2H, d, *J*=8Hz, CH_2), 1.59 (3H, s, CH_3), MS (m/z): 308,(M^+), 265,281, 237; MW. 308.34 g/mol.

1-((Dimethylamino) methyl) indoline-2, 3-dione (b_2)

Yield: 80%, Orange solid, m.p. 107–109°C (lit: 108–110°C),[15] ^{1}HNMR: (400 MHz; DMSO; d_6): δ7.84 (1H, t, *J*=8Hz, H-Isatin), 7.72 (1H, d, *J*= 8Hz, H-isatin), 7.39 (1H, d, *J*=8Hz, H-Isatin), 7.31 (1H, t, *J*=8Hz, H-Isatin), 5.22 (2H, s, CH_2), 3.48 (6H, s, CH_3); MW 204.23 g/mol.

(z)-N'-(1-((Dimethylamino) methyl)-2-oxoindolin-3-yllidene)-2-hydroxybenzohydrazide (C_2)

Yield: 56.63%, Yellow solid, m.p. >270°C, IR (KBr, cm^{-1}), 3400 (OH), 3200 (NH), 3150 (C-H, Ar), 1721(C=O), ^{1}HNMR: (400 MHz; DMSO; d_6): δ 11.73 (1H, s, OH), 11.16 (1H, s, NH), 7.99 (1H, d, *J*=8Hz, H-Ar), 7.59 (1H, d, *J*=8Hz, H-isatin), 7.45 (1H, t, *J*=8Hz, H-Isatin), 7.37 (1H, t, *J*=8Hz, H-Ar), 7.10 (1H, t, *J*=8Hz, H-Ar), 6.96–7.03 (2H, m, Isatin),

6.93 (1H, d, *J*=8Hz, H-Ar), 1.24 (2H, s, CH_2) 2 (6H, s, CH_3), MS (m/z): 339 (M^+), 311, 281, 265; MW. 338.37 g/mol.

(1-((Ethylamino) methyl) indoline-2, 3-dione (b_3)

Yield:15.8%, Pale brown, m.p. >270°C, IR (KBr, cm^{-1}), 3200 (NH), 3150 (C-H, Ar), 2850 (C-H, Aliphatic), 1721(C=O), HNMR: (400 MHz; $CDCl_3$): δ 7.68-7.62 (2H, m, H-Isatin), 7.22-7.16 (2H, m, H-Isatin), 5.22 (2H, s, CH_2), 3.60 (2H, q, *J*=8 Hz, CH_2), 1.22 (3H, t, *J*=8Hz, CH_3), MS (m/z): 205(M^+); 190,176, 161, MW. 204.23 g/mol.

Data Analysis

Obtained data were expressed as mean ± SEM. The number of animals in each group was six (n = 6). Differences between groups were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan as the post hoc test. P < 0.05were considered to be statistically significant [Table1 and Figure 1].

Two doses of final compounds were separately injected intra-peritoneal to each group of six animals. Normal saline was used as control. 30 min later the croton oil solution was speared topically to the inner surface of the right ear of mice. From both ears of each mouse 6 mm disk was separated. The weight difference between two disks of right and left ear for each mice was taken as an inflammatory index.

Isatin –based derivatives have been known to possess potential biological properties.[6-11] Based on previous reports about isatin derivatives especially schiff and mannich bases with anti-inflammatory activity,[3,19,23] a number of isatin derivatives containing both schiff and mannich base moieties were synthesized. Primary and secondary aliphatic amines were employed as reagents in the mannich reaction. Anti-inflammatory activity was studied using the croton oil-induced ear edema test. *In vivo* anti-inflammatory evaluation of synthesized compounds is summarized in Table 1. Results of anti-inflammatory activity revealed that mannich-containing compound (b_3) was found to be the most active compound of the series. It has been observed that the increased anti-inflammatory activity is attributed to the presence of pharmacologically active groups like mannich group. Existence of substituents at the 1 position of isatin scaffold seemed to be necessary factors in providing higher anti-inflammatory activity.

It is no any significant difference between the activity of the compounds $C_1$and control. Compounds C_2 and C_3 containing both schiff and mannich bases groups exhibited moderate anti-inflammatory activity, in addition to, these compounds didn't show graded dose-response manner.

Comparison of the results of the two compounds C_1 and C_3 at dose of 200 mg/kg showed better effects for compound C_3. It can be concluded that the existence and nature of substituents at the 1 position of isatin nucleus play an important role in modulating their anti-inflammatory propertiesIn theses series of derivatives, the second type of amine showed better anti-inflammatory activity than the third type.

The comparison of results for compounds C_1 and C_2 showed that the presence of hydroxyl group could slightly improve anti-inflammatory activity.

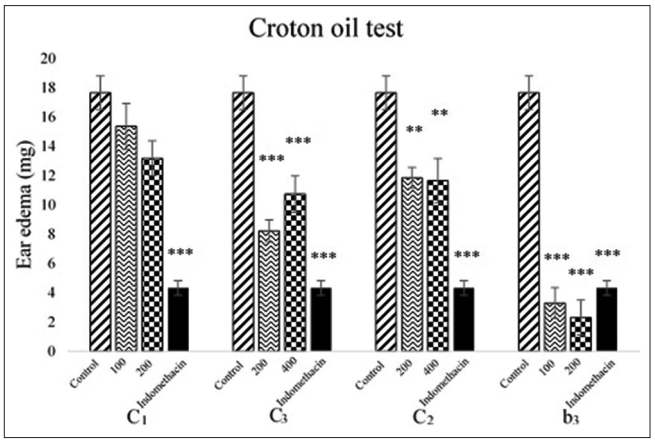


Figure 1: Anti-inflammatory effect of synthesized compounds on croton oil-induced ear edema in mice (n = 6)

Table 1: Anti-inflammatory effect of synthesized compounds on croton oil-induced ear edema in mice (n=6)

Groups	Dose (mg/kg)	Ear edema (mg) mean SEM
Control	–	17.66±1.15
Compound C_1	100	15.36±1.57
	200	13.18±1.19
Compound C_3	200	8.21±0.76***
	400	10.74±1.24***
Compound C_2	200	11.85±0.70**
	400	11.66±1.52**
Compound b_3	100	3.30±1.05***
	200	2.30±1.20***
Indomethacin	10	4.33±0.49***

**P<0.01; *** P<0.001 compared with control group

CONCLUSION

This study reports the successful synthesis of the final compounds in moderate yields. Compound b_3 which included mannich base group showed significant anti-inflammatory effects compared to the rest. Compounds **C_2**and **C_3** containing both schiff and mannich bases groups revealed moderate anti-inflammatory properties.

Although some of the synthesized compounds indicated significant anti-inflammatory effects, but they were not remarkably comparable to indomethacin**.**

CONFLICTS OF INTEREST

There are no conflicts of interest.

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