Synthesis, characterization, and analgesic activity of novel Schiff base of isatin derivatives

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DOI: 10.4103/0110-5558.72428

J. Adv. Pharm. Tech. Res.

Abstract

In the present study, a series of novel Schiff bases of isatin [5a-5l] were synthesized by condensation of imesan with different aromatic aldehydes. The imesanates were synthesized by the reaction of isatin with p-phenylenediamine. The chemical structures of the synthesized compounds were confirmed by means of Infrared (IR), Mass spectroscopy, and Elemental analysis. These compounds were screened for the analgesic activity by the tail-immersion method at a dose of 200 mg/kg body weight. Among the tested compounds 3-(4-(4-hydroxy-3-methoxybenzylideneamino)-phenylimino) indoline-2-one (5i) exhibited better analgesic activity when compared to standard pentazocine. From the above-mentioned results it may be concluded that compounds containing electron-donating groups exhibit better analgesic activity than the electron-withdrawing groups.

Key words: Analgesic activity, isatin, Schiff base

INTRODUCTION

Isatin (indole-2, 3-dione) is an endogenous compound, widely distributed in mammalian tissues and body fluids.[1] In the brain the highest levels have been found in the hippocampus[2] and an immunocytochemical staining revealed its specific localization within particular cells. In vitro isatin administration causes a range of dose-dependent behavioral effects,[3] including angiogenesis and increased water retention. In vitro, isatin is a potent inhibitor of both atrial natriuretic peptide (ANP)-stimulated, membrane-bound guanylate cyclase and nitric oxide-stimulated soluble guanylate cyclase.[4] It is an inhibitor of monoamine oxidase B (IC50=8 IM) and of atrial natriuretic peptide receptor binding (0.4 IM) at levels that may be in the physiological range.[5] Isatin is well known as a pharmacological agent having a range of action in the brain and it is protective against certain types of infections. Isatin derivatives are reported to show other biological activities, such as, antibacterial,[6-8] anti-fungal,[9-11] anti-viral,[12-14] anti-HIV,[15-17] anti-protozoa,[18,19] muscle relaxant,[20] anti-allergic,[21] and anti-inflammatory.[22] Schiff bases of isatin were synthesized using different aromatic aldehydes by condensation with imesan and their chemical structures were confirmed by IR, 1H-NMR, 13C-NMR, Mass spectroscopy, and Elemental analysis. These compounds were screened for their analgesic properties. The results of such studies are discussed in this article.

MATERIALS AND METHODS

The melting points were taken with the help of an open capillary tube and were uncorrected. The IR spectra of the compounds were recorded on ABB Bomem FT-IR spectrometer MB 104 with KBr pellets. The 1H (400 MHz) and 13C-NMR (400 MHz) spectra were recorded on a Bruker 400 NMR spectrometer (with TMS for 1H and DMSO-d6 for 13C as internal references). Mass spectroscopy was recorded on Shimadzu GC MS QP 5000. Microanalyses were obtained with an elemental analyses system GmbH VarioEL V300 element analyzer. The purity of the compounds was checked by TLC on pre-coated SiO2 gel (HF25O100 mesh) aluminium plates (E-Merck) using ethyl acetate : n-hexane (2 : 3) and

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visualized in a UV chamber. IR, 1H-NMR, 13C-NMR, mass spectroscopy, and elemental analysis were consistent with the assigned structures.

**General Method of Synthesis**
In the present study, aniline 1 is treated with chloral hydrate to form isonitrosoacetanilide 2. Then this intermediate undergoes cyclization with sulfuric acid to form isatin 3,[33] which is further reacted with p-phenylenediamine, resulting in the formation of imesatin 4. Compound 4 is subjected to reaction with various aromatic aldehydes in the presence of ethanol as a solvent to form Schiff bases (5a–5f, Figure 1). All the synthesized compounds are soluble in dimethylformamide.

Equimolar quantities of (0.01 mol) isatin and p-phenylenediamine were dissolved in a sufficient quantity of methanol (30 mL) in the presence of acetic acid and refluxed for one hour, and then kept for two hours at room temperature (37°C). The product that separated out was filtered, dried in a vacuum, and recrystallized from absolute ethanol. Equimolar quantities (0.01 mol) of imesatin 4 and various aromatic aldehydes were dissolved in ethanol and refluxed for eight hours. After standing for one-to-two days at room temperature, the product of different substituted derivatives of isatin (5a–5f) separated out as a mixture of E and Z isomers, which was filtered, dried, and recrystallized from absolute ethanol. In the present study a novel series of various three-substituted isatin compounds were synthesized. The target compounds 5a–5f were synthesized according to Figure 1. Aniline and chloral hydrate were used as starting materials to produce the Schiff bases of substituted isatin via the intermediate imesatin (4) through a condensation reaction.

The condensation proceeds selectively on the carbonyl group in position 3 of the isatin ring. Reactions of imesatin with different aromatic aldehydes have been carried out in ethanol in the presence of glacial acetic acid, and a variety of Schiff base derivatives have been isolated according to the synthetic Figure 1. The method used for the preparation and isolation of the compounds has given materials of good purity, as evidenced by their spectral analyses and thin layer chromatography. The Schiff base derivatives are found to be soluble in chloroform, dimethyl sulfoxide, and dimethylformamide.

**Pharmacological Screening**

**Animals**
The animals used in the present study were Swiss albino mice weighing 20 – 25 gm, which were procured from the C. L. Baid Metha College of Pharmacy, Chennai, India. The animals were maintained in colony cages at 25±2°C, with relative humidity of 45 – 55%, under 12 hours light and dark cycle, and were fed with the standard animal feed and water *ad libitum*. The animals were maintained under standard conditions in an animal house approved by the committee for the purpose of control and supervision of experiments on animals (CPCSEA). The Institutional Animal Ethics Committee approved the experimental protocol. All the animals were acclimatized for a week before use.

**Acute Toxicity Studies**
The Acute toxicity test was performed for the entire synthesized compound, to ascertain the LD₅₀ values as per OECD guidelines.[34] The experimental dose was selected between the minimum effective dose and maximal non-lethal dose.

**Analgesic Activity (Tail Immersion Method in Mice)**
The analgesic activity[35] was determined by the tail-immersion method. Swiss mice (n = 6) of either sex, selected by the random sampling technique, were used for the study. Pentazocine at a dose of 10 mg/kg (i.p.) was administered as a standard drug for comparison, to check the centrally acting analgesic activity of the synthesized compounds. Pentazocine would produce excellent centrally acting analgesic action compared to other analgesic standards. Moreover, pentazocine is a synthetically prepared compound and known to act as an opioid-mixed agonist and antagonist.[36] The test compounds at 200 mg/kg dose level were administered orally. The animals were held in position by a suitable restrainer with the tail extending out.
and the tail (up to 5 cm) was taken, dipped in a beaker of water maintained at 55±0.5°C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The first reading (0 minute) was taken immediately after the administration of the test compound and subsequent the reaction time was recorded at 30, 60, 120, and 180 minutes after the administration of the compounds. A cut-off point of 15 seconds was observed to prevent tail damage. The percentage analgesic activity was calculated using the following formula, and the results are presented in Table 1.

PAA = [(T<sub>2</sub>-T<sub>1</sub>)/T<sub>1</sub>]*100

Where, T<sub>1</sub> and T<sub>2</sub> are the reaction times (in seconds) before and after treatment, respectively; PAA is the percentage analgesic activity.

RESULTS AND DISCUSSION

Chemistry

IR, 1<sup>H</sup>-NMR, 13<sup>C</sup>-NMR, Mass spectra, and Elemental analysis were consistent with the assigned structures.

3-(4-(benzylideneamino) phenylimino) indoline-2-one [5a]

Bright yellow crystals; Yield: 80%; mp. 322 – 324°C; IR: 1777 (N-H), 3050 (Ar-CH), 1690 (C=O), 1597 (C=N), 1580 (C=C) cm<sup>-1</sup>; 1<sup>H</sup>-NMR (DMSO): δ 8.29 (s, 1H, N-CH=), 8.02 (s, 1H, -NH), 7.01-7.68 (m, 13H, H-4, H-5 H-6, H-7, H-2', H-3', H-5, H-6, H-2'', H-3'', H-4'', H-5'', H-6'', Ar-H); 13<sup>C</sup>-NMR (DMSO): δ 167.2 (C-2), 163.5 (C-3), 160.3 (-N=CH=), 151.6 (C-1' and C-4'), 133.5 (C-9), 132.5 (C-6), 130.4 (C-8), 131.2 (C-4''), 129.5 (C-4), 129.2 (C-2'' and C-6''), 129.2 (C-5), 128.7 (C-3' and C-5''), 126.4(C-1''), 123.4 (C-2', C-3', C-5 and C-6'), 121.9 (C-7); EI-MS (m/z, %): 325(M<sup>+</sup>, 21), 235(14), 120(100), 105(24), 69(44); (Calcd. for C<sub>21</sub>H<sub>15</sub>N<sub>2</sub>O, 359.80); Anal. Calcd.

3-(4-(4-chlorobenzylideneamino) phenylimino) indoline-2-one [5b]

Pale yellow crystals; Yield: 75%; mp. 346 – 348°C; IR: 3130 (N-H), 2988 (Ar-CH), 1613 (C=N), 1595 (C=C), 744 (C-Cl) cm<sup>-1</sup>; 1<sup>H</sup>-NMR (DMSO): δ 8.36 (s, 1H, -NH), 7.92 (s, 1H, -NH<sub>2</sub>), 7.03-7.60 (m, 12H, H-4, H-5 H-6, H-7, H-2', H-3', H-5, H-6', H-2'', H-3'', H-5'', H-6'', Ar-H); 13<sup>C</sup>-NMR (DMSO): δ 166.9 (C-2), 164.2 (C-3), 160.1 (N=CH=), 151.2 (C-1 and C-4'), 136.2 (C-4''), 133.5 (C-9), 131.2 (C-8), 130.5 (C-2'' and C-6''), 129.8 (C-4), 129.1 (C-3' and C-5''), 126.6 (C-1'), 124.4 (C-5), 123.1 (C-2', C-3', C-5 and C-6'), 121.6 (C-7); EI-MS (m/z, %): 326(M+2, 26), 360(M+, 20), 264(22), 91(100), 77(22), 69(44); (Calcd. for C<sub>21</sub>H<sub>15</sub>N<sub>2</sub>O<sub>2</sub>, 395.80); Anal. Calcd.

Table 1: Analgesic activity of the synthesized compounds

| Compounds     | Dose (mg/kg) | 0 minute | Mean±SEM | 30 minutes | Mean±SEM | %       | 60 minutes | Mean±SEM | %       | 120 minutes | Mean±SEM | %       | 180 minutes | Mean±SEM | %       |
|---------------|-------------|----------|----------|-----------|----------|---------|-----------|----------|---------|-------------|----------|---------|-------------|----------|---------|
| 5a            | 200         | 3.83±0.31| 6.00±0.3*| 36.16     | 8.17±0.40*| 53.12   | 10.17±0.65*| 62.34    | 8.33±0.76*| 54.02       |
| 5b            | 200         | 4.00±0.34| 5.67±0.37*| 29.45     | 7.50±0.22| 46.66   | 10.83±0.48**| 63.06    | 7.00±0.37**| 42.85       |
| 5c            | 200         | 4.00±0.34| 7.50±0.43*| 46.67     | 8.50±0.43*| 52.44   | 10.50±0.43*| 61.90    | 8.83±0.79*| 54.70       |
| 5d            | 200         | 3.17±0.17| 6.83±0.60*| 53.58     | 7.50±0.67| 57.73   | 9.50±0.67*| 65.26    | 7.83±0.48*| 59.51       |
| 5e            | 200         | 4.00±0.34| 6.83±0.40*| 41.43     | 7.17±0.31*| 44.21   | 10.17±0.31**| 60.66    | 9.33±0.21**| 57.12       |
| 5f            | 200         | 3.50±0.23| 6.63±0.33*| 47.52     | 7.33±0.49*| 62.25   | 10.50±0.49*| 63.52    | 8.83±0.31*| 60.36       |
| 5g            | 200         | 4.00±0.34| 6.00±0.52*| 33.33     | 7.67±0.61| 47.84   | 11.67±0.56**| 65.72    | 8.50±0.76**| 52.94       |
| 5h            | 200         | 3.50±0.22| 7.02±0.50*| 50.00     | 9.00±0.58*| 61.11   | 10.50±0.67*| 66.66    | 9.50±0.56*| 63.15       |
| 5i            | 200         | 3.30±0.21| 6.33±0.33*| 47.86     | 8.50±0.76*| 61.17   | 11.00±0.57**| 70.00    | 8.83±0.31**| 62.62       |
| 5j            | 200         | 3.50±0.34| 5.83±0.40*| 39.96     | 6.50±0.56*| 46.15   | 9.00±0.45*| 61.11    | 7.83±0.60*| 55.30       |
| 5k            | 200         | 3.17±0.17| 6.33±0.21**| 49.92     | 7.83±0.40*| 59.51   | 9.50±0.43**| 66.63    | 8.00±0.58*| 60.37       |
| 5l            | 200         | 3.83±0.40| 7.67±0.56*| 50.06     | 8.17±0.79*| 53.12   | 10.50±0.67**| 63.52    | 7.83±0.54**| 51.08       |
| Pentaazocine  | 10          | 3.50±0.34| 8.67±0.21**| 59.63     | 11.00±0.40*| 68.18   | 13.50±0.34**| 74.07    | 10.33±0.49**| 66.11       |

Data represent Mean±S.E.M (Standard error mean) (n = 6); *P<0.05; **P<0.01; ***P<0.001; NS = Non-significant; PAA = Percentage analgesic activity.
3-(4-(4-methoxybenzylideneamino) phenylimino) indoline-2-one [5d]

Lemon yellow crystals; Yield: 79%; mp. 326 – 328°C; IR: 3146 (N-H), 3079 (Ar-CH), 1688 (C-O), 1647 (C-C), 1567 (C=N), 1270 (C-O-C) cm⁻¹; ¹H-NMR (DMSO): δ 8.39 (s, 1H, -N=C=H), 8.01 (s, 1H, -NH₂), 7.51 (d, J=6.3 Hz, 1H, C-6' Ar-H), 7.47 (d, J=5.9 Hz, 1H, C-2' Ar-H), 6.99-7.31 (m, 8H, H-4, H-5, H-6, H-7, H-2', H-3', H-5, H-6' Ar-H), 6.81 (d, J=7.2 Hz 1H, H-5' Ar-H), 6.77(d, J=6.5 Hz, 1H, H-3'' Ar-H), 3.70 (s, 3H, -OCH₃). ¹³C-NMR (DMSO): δ 167.6 (C-2), 163.5 (C-3), 163.1 (C-4'), 160.5 (N=CH₂), 151.64 (C-1' and C-4'), 133.6 (C-9), 131.2 (C-6), 130.6 (C-8), 130.2 (C-2' and C-6''), 129.4 (C-4), 126.1 (C-1''), 124.5 (C-5), 123.6 (C-2',C-3' and C-5), 121.5 (C-7), 114.3 (C-3'' and C-5''), 55.8 (-OCH₃); EI-MS (m/z, %): 355 (M⁺, 18), 282 (20), 191 (10), 94 (52), 59 (94). (Calcd. for C₂₇H₂₅NO₃: 355.38; Anal. Calcd. for C₂₇H₂₅NO₃: C, 74.35; H, 4.82; Found: C, 74.36; H, 4.80; N, 11.78).

3-(4-(4-nitrobenzylideneamino) phenylimino) indoline-2-one [5e]

Creamy crystals; Yield: 68%; mp. 338 – 340°C; IR: 3132 (N-H), 3012 (Ar-CH), 1690 (C=O), 1603 (C=O), 1590 (C=N), 1515 and 1310 (N(O)) cm⁻¹; ¹H-NMR (DMSO): δ 8.29 (s, 1H, -N=C=H), 8.21 (d, J=7.1 Hz, 1H, C-5' Ar-H), 8.17 (d, J=6.8 Hz 1H, C-3' Ar-H), 8.10 (s, 1H, -NH₂), 7.77(d, J=7.5 Hz, 1H, H-2' Ar-H), 7.69 (d, J=6.2 Hz 1H, H-6' Ar-H), 6.99-7.70 (m, 8H, H-4, H-5, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.51 (s, 1H, H-2'' Ar-H), 6.58 (s, 1H, H-6'' Ar-H), 3.70 (s, 3H, -OCH₃). (Calcd. for C₂₇H₂₅N₂O₂: 371.38; Anal. Calcd. for C₂₇H₂₅N₂O₂: C, 73.89; H, 4.43; N, 12.31; Found: C, 73.91; H, 4.45; N, 12.35).

3-(4-(3-(4-benzylideneamino)phenylimino)phenylimino)indoline-2-one [5f]

Yellow crystals; Yield: 65%; mp. 318 – 320°C; IR: 3467 (Ar-CH), 3210 (N-H), 3065 (Ar-CH), 1678 (C-O), 1649 (C=C), 1575 (C=N) cm⁻¹; ¹H-NMR (DMSO): δ 8.22 (s, 1H, -N=C=H), 7.06-7.67 (m, 8H, H-4, H-5, H-6, H-7, H-2', H-3', H-5', H-6' Ar-H), 6.75-7.40 (m, 4H, H-3', H-4', H-5' and H-6' Ar-H), 6.01 (s, 1H, -NH₂), 5.20 (s, 1H, Ar-CH₂). ¹³C-NMR (DMSO): δ 167.2 (C-2), 163.2 (C-3), 161.2 (C-2''), 160.2 (N=CH₂), 151.7 (C-1' and C-4'), 133.8 (C-9), 132.3 (C-4''), 131.4 (C-6), 130.1 (C-8), 130.5 (C-6''), 129.3 (C-4), 126.2 (C-1''), 124.5 (C-5), 123.5 (C-2', C-3', C-5 and C-6), 121.5 (C-7), 121.3 (C-5''), 116.0 (C-3''). EI-MS (m/z, %): 341 (M⁺, 36), 282 (26), 242 (34), 130 (89), 89 (26), 77 (30). (Calcd. for C₂₇H₂₅N₂O₂: 341.38; Anal. Calcd. for C₂₇H₂₅N₂O₂: C, 73.89; H, 4.43; N, 12.31; Found: C, 73.91; H, 4.45; N, 12.35).

3-(4-(4-methylbenzylideneamino)phenylimino)indoline-2-one [5g]

Creamy solid; Yield: 72%; mp. 314 – 316°C; IR: 3175 (N-H), 3055 (Ar-CH), 1686 (C=O), 1650 (C=N), 1652 (C=O), 1491 and 1373 (C=NO₂) cm⁻¹; ¹H-NMR(DMSO): δ 8.55 (s, 1H, H-2' Ar-H), 8.23 (d, J=8.1 Hz, 1H, H-4'' Ar-H), 8.19 (s, 1H, -NH₂), 8.10 (s, 1H, -NH₂), 8.03 (d, J=6.5 Hz, 1H, H-6'' Ar-H).
A general and convenient method was developed to synthesize Schiff base derivatives having unsubstituted, substituted with electron-donating and electron-withdrawing groups of isatin containing Schiff bases. Thus, 12 new Schiff base and indoline-2-one [5l] were synthesized in good yield. The analgesic activity of these compounds was measured at time intervals of 30, 60, 120, and 180 minutes. Among the compounds synthesized, the compound-bearing electron-withdrawing, nitro-substituent, such as 3-(4-(4-nitrobenzylideneamino)phenylimino)indoline-2-one [5i] and 3-(4-(3, 4, 5-trimethoxy benzylideneamino)phenylimino)indoline-2-one [5h], exhibited comparable analgesic activity at 200 mg/kg b.w. Compounds 3-(4-(4-dimethylaminobenzylideneamino)phenylimino)indoline-2-one [5k] and 3-(4-(4-methoxybenzylideneamino)phenylimino)indoline-2-one [5d] exhibited moderate analgesic activity. Among the compounds synthesized, in general and convenient method was established for the new synthesis of the heterocyclic compounds containing both electron-donating groups of isatin containing Schiff bases. Thus, 12 new Schiff base derivatives having unsubstituted, substituted with electron-donating and electron-withdrawing groups were synthesized in good yield. The analgesic activity results showed that Schiff bases bearing electron-donating substituents produced potent results, and therefore, might serve as lead molecules to obtain more clinically useful, novel entities in the future.

CONCLUSION

It is known that the heterocyclic compounds containing both Schiff base and isatin rings have diverse pharmacological properties.[32-31] A general and convenient method was established for the new synthesis of the heterocyclic compounds containing both Schiff base and isatin rings. It's known that the heterocyclic compounds containing both Schiff base and isatin rings have diverse pharmacological properties. It's known that the heterocyclic compounds containing both Schiff base and isatin rings have diverse pharmacological properties.

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