



Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel Schiff's bases of 5-substituted Isatin

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Abstract

In the present study, a novel schiff bases were synthesized by condensation of 5-substituted imesatin with different substituted aromatic aldehydes. The chemical structures of the synthesized compounds were confirmed by means of IR, ¹H-NMR, ¹³C-NMR, Mass spectral and Elemental analysis. These synthesized compounds were investigated for analgesic (Tail-immersion method), anti-inflammatory (carrageenan- induced paw oedema method) and anti-bacterial activities by paper disc diffusion technique. The minimum inhibitory concentrations (MIC) of the compounds were also determined by Agar streak dilution method. Most of the synthesized compounds exhibited significant anti-bacterial and anti-fungal activities. Among the synthesized compounds **5c**, **5h**, **5i**, and **5b** exhibited remarkable analgesic and anti-inflammatory activities.

Key words: Isatin, schiff base, analgesic, anti-inflammatory, anti-microbial

Introduction

Bacterial infections often produce pain and inflammation. In normal practice, two group of agents (chemotherapeutic, analgesic and anti-inflammatory) are prescribed simultaneously. Isatin, a heterocyclic compound was identified in animals as a major component of the endogenous monoamine oxidase inhibitor. Isatin derivatives have gained unique importance due to the broad spectrum of pharmacological activities which are reflected by their use as anti-microbial [1] analgesic [2] anti-inflammatory [3] and anti-convulsant [4-7] The various substituent at 3rd position of the isatin which were reported various substituted phenyl ring moieties, heterocyclic rings and aliphatic system. In view of these facts and to develop novel series of Schiff bases of 5-substituted Isatin and N-acetyl isatin were synthesized (**5a-5i**, **6a-6c**) using different substituted aromatic aldehydes, and their chemical structure were confirmed by IR, ¹H-NMR, ¹³C-NMR, Mass spectral and the purity of these compounds was ascertained by elemental analysis. The synthesized compounds were evaluated for their analgesic (Tail-immersion method), anti-inflammatory (carrageenan- induced paw oedema method), and anti-

bacterial activity against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778), three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, and *Klebsiella pneumoniae* ATCC 11298) and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) activities by paper disc diffusion technique. The minimum inhibitory concentrations (MIC) of the compounds were also determined by Agar streak dilution method.

Results and Discussion

The analgesic activity data reveals that most of the synthesized novel isatin derivatives were exhibited significant activity (Table 1). Compounds **5b**, **5h**, and **5i** were found to possess equipotent analgesic and anti-inflammatory activity when compared with standard drugs (Pentazocin, 10 mg/kg, i.p and Indomethacin 20mg/kg respectively). Among synthesized compounds 5-fluoro substituted title compound **5i** showed promising analgesic and anti-inflammatory activity, this may be due to high lipophilicity imported by the versatile pharmacophore. Compounds **5a**, **5c**, **5d** and **5g** were found to exhibit moderate analgesic activity. Above results concluded that compounds substituted with electron withdrawing groups were found to possess promising analgesic activity, whereas **5e** exhibited lowest analgesic and anti-inflammatory activity.

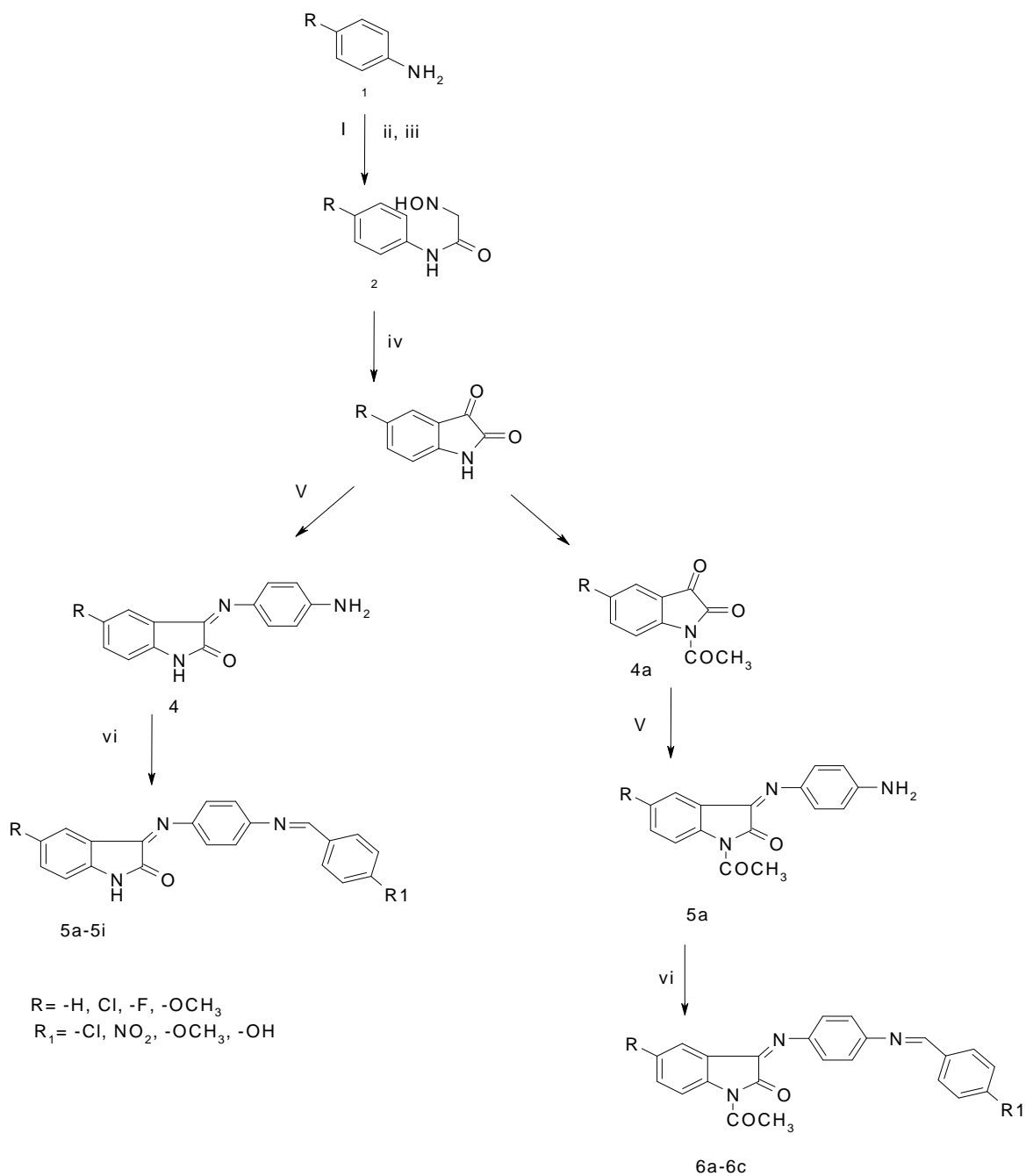
All the synthesized compounds were active against all the tested micro-organisms with the range of MIC values for *Staphylococcus aureus* (27.3-10.3 µg/ml), *Staphylococcus epidermidis* (31.3-11.3 µg/ml), *Micrococcus luteus* (36.7-12.1 µg/ml), *Bacillus cereus* (34.2-13.2 µg/ml), *Escherichia coli* (37.5-1.9 µg/ml), *Pseudomonas aeruginosa* (35.7-15.1 µg/ml), *Klebsiella pneumoniae* (37.2-10.7 µg/ml), *Aspergillus niger* (36.7-15.3 µg/ml) and *Aspergillus fumigatus* (38.4-16.2 µg/ml). 5-Fluoro-(4-(4-nitrobenzylideneamino)-phenylimino)indolin-2-one **5h** was found to exhibit the most potent *invitro* antimicrobial activity with the MIC of 10.3, 11.3, 12.1, 13.2, 11.9, 15.1, 10.7, 15.3 and 16.2 against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Aspergillus fumigatus* respectively. Compound **5b** also exhibited significant anti bacterial and anti fungal activity when compared to standard drugs Ciprofloxacin (100 µg/disc) and Ketoconazole (100 µg/disc). Other compounds, **5a**, **5c-5g**, **5i** and **6a-6c** showed moderate to potent anti microbial activity. These results revealed that the compound substituted with electron withdrawing groups was found to possess promising anti-microbial activity against tested micro-organisms. Whereas compound **5e** and **5f** though they contain both electron donating and electron withdrawing groups were observed to exhibit moderate anti-microbial activity. The interesting results which we found that the substitution of electron withdrawing groups at 5th position of isatin nucleus increases anti-microbial property. Whereas, compounds without substitution compounds like **6a** and **6c** exhibited least activity. Moreover, the substitution of electron withdrawing groups on isatin particularly at 5th position as well as 4th position of benzylideneamine moiety was found to increase the anti-microbial activity. From this study, it may be concluded that isatins substituted at 3rd position with different substituted aromatic aldehydes at meta and para position on the 2nd phenyl ring and substitution on isatin at 5th position enhances the analgesic, anti-inflammatory and anti-microbial activities and hence the study would deserve for future Investigation and derivatization

Materials and Methods

General method of synthesis 5a-5i /6a-6c

5-substituted isatin / N-acetyl isatin were prepared according to reported literature [8-11] and characterized. Equimolar quantities (0.01mol) of imesatin and various substituted aromatic aldehydes were dissolved in sufficient quantity of ethanol and refluxed for 8h, Then the product which separated out was filtered, dried and recrystallised from absolute ethanol.

Scheme 1



i) $NH_2OH.HCl$ (ii) Na_2SO_4 (iii) $CCl_3CHO.H_2O$ (iv) $Conc. H_2SO_4$ (v) p -phenylenediamine, CH_3COOH , ethanol (vi) Ethanol, $R-CHO$ (vii) $(CH_3CO)_2O$.

5-Chloro- 3-(4-(4-chlorobenzylideneamino)-phenylimino) indolin-2-one (5a).

Yield: 51 %; m.p. 272-274 °C; IR (cm⁻¹): 3332(Ar-CH), 1733 (C=O), 1592(C=C), 1516 (C=N), 826(C-Cl). ¹H-NMR(DMSO): δ 8.12 (s,1H, N=CH-), 7.80 (s,1H, N-H), 7.62 (s,1H, 4-H, Ar-H) 7.28-7.58 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H). ¹³C-NMR(DMSO): δ 164 (-C=N), 163.7 (N=CH), 160.0 (C=O), 151.7 (C-1',C-4'), 136.8 (C-9), 136.1 (C-4''), 131.4 (C-6), 130.4 (C-2'', C-6''), 129.6 (C-4), 129.5 (C-5), 129.3 (C-1''), 129 (C-3'', C-5''), 124.5 (C-8), 123.3 (C-2',C-3',C-5',C-6'), 121.9 (C-7). EI-MS (m/z, %): 394.25 (M⁺, 13), Anal. Calcd for C₂₁H₁₃Cl₂N₃O: C, 63.98; H, 3.32; N, 10.66. Found: C, 64.02; H, 3.36; N, 10.71.

5-Chloro- 3-(4-(4-nitrobenzylideneamino)-phenylimino) indolin-2-one (5b).

Yield: 79.2 %; m.p. 271-273 °C; IR (cm⁻¹): 3111(Ar-CH), 1732 (C=O), 1598(C=C), 1535(C=N), 1343 (C-NO₂), 668(C-Cl). ¹H-NMR(DMSO): δ 8.10 (s,1H, N=CH-), 8.15 (s,1H, N-H), 7.64 (s,1H, 4-H, Ar-H), 7.28-7.90 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H). ¹³C-NMR(DMSO): δ 164.2 (-C=N), 163.5 (N=CH), 160.1 (C=O), 151.7 (C-1',C-4'), 150.7 (C-4''), 137.3 (C-1''), 136.8 (C-9), 131.4 (C-6), 129.9 (C-2'', C-6''), 129.6 (C-4), 129.5 (C-5), 124.5 (C-8), 123.7 (C-3'', C-5''), 123.3 (C-2',C-3',C-5',C-6'), 121.9 (C-7). EI-MS (m/z, %): 404.18 (M⁺, 8), Anal. Calcd for C₂₁H₁₃ClN₄O₃: C, 62.31; H, 3.24; N, 13.84. Found: C, 62.35; H, 3.20; N, 13.88.

5-Chloro-3-(4-(4-methoxybenzylideneamino)-phenylimino) indolin-2-one (5c).

Yield: 74.7 %; m.p. 275-277 °C; IR (cm⁻¹): 3157(Ar-CH), 1732 (C=O), 1607(C=C), 1617(C=N), 1259 (C-OCH₃), 628(C-Cl). ¹H-NMR(DMSO): δ 8.13 (s,1H, N=CH-), 8.16 (s,1H, N-H), 7.66 (s,1H, 4-H, Ar-H) 6.8-7.61 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 3.73 (s,3H, -OCH₃). ¹³C-NMR(DMSO): δ 164.2 (-C=N), 163.4 (N=CH), 160.2 (C=O), 164.3 (C-4''), 151.7 (C-1',C-4'), 136.8 (C-9), 131.4 (C-6), 130.0 (C-2'', C-6''), 129.6 (C-4), 129.5 (C-5), 124.5 (C-8), 123.5 (C-1''), 123.3 (C-2',C-3',C-5',C-6'), 121.9 (C-7), 114.2 (C-3'', C-5''), 56.0 (-OCH₃). EI-MS (m/z, %): 389.83 (M⁺, 10), Anal. Calcd for C₂₂H₁₆ClN₃O₂: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.82; H, 4.18; N, 10.74.

5-Chloro-3-(4-(2-hydroxybenzylideneamino)-phenylimino) indolin-2-one (5d).

Yield: 65.7 %; m.p. 272-275 °C; IR (cm⁻¹): 3157 (Ph-OH), 2997(Ar-CH), 1732 (C=O), 1608(C=C), 1610(C=N), 628(C-Cl). ¹H-NMR(DMSO): δ 8.10 (s,1H, N=CH-), 8.16 (s,1H, N-H), 7.63 (s,1H, 4-H, Ar-H) 6.76-7.61 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 6.10 (s, 1H, Ar-OH). ¹³C-NMR(DMSO): δ 163.9 (-C=N), 163.2(N=CH), 159.9 (C=O), 157.8 (C-2''), 151.7 (C-1',C-4'), 136.8 (C-9), 132.2 (C-4''), 131.4 (C-6), 130.4 (C-6''), 129.6 (C-4), 129.5 (C-5), 124.5 (C-8), 123.3 (C-2',C-3',C-5',C-6'), 121.9 (C-7), 121.2 (C-5''), 118.4 (C-1''), 115.8(C-3''), EI-MS (m/z, %): 375.81 (M⁺, 12). Anal. Calcd for C₂₁H₁₄ClN₃O₂: C, 67.12; H, 3.75; N, 11.18. Found: C, 67.16; H, 3.79; N, 11.22.

5-Methoxy- 3-(4-(4-chlorobenzylideneamino)-phenylimino) indolin-2-one (5e).

Yield: 60 %; m.p. 195-197 °C; IR (cm⁻¹): 3230(Ar-CH), 1746 (C=O), 1617(C=C), 1609(C=N), 1199 (C-OCH₃), 623(C-Cl). ¹H-NMR(DMSO): δ 8.12 (s,1H, N=CH-), 8.18 (s,1H, N-H), 7.40 (s,1H, 4-H, Ar-H) 6.78-7.36 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 3.73 (s,3H, -OCH₃). ¹³C-NMR(DMSO): δ 164.4 (-C=N), 163.1 (N=CH), 160.3 (C=O), 157.7 (C-5), 151.7 (C-1',C-4'), 136.1(C-4''), 131.1 (C-9), 130.4 (C-2'', C-6''), 129.3 (C-1''), 129.0(C-3'',C-5''), 124.1 (C-8), 123.3 (C-2',C-3',C-5',C-6'), 121.3 (C-7), 116.6 (C-6) 114.8 (C-4), 56.0(-OCH₃), EI-MS (m/z, %): 389.83 (M⁺, 10). Anal. Calcd for C₂₂H₁₆ClN₃O₂: C, 67.78; H, 4.14; N, 10.78. Found: C, 67.82; H, 4.18; N, 10.82.

5-Methoxy-3-(4-(4-nitrobenzylideneamino)-phenylimino) indolin-2-one (5f).

Yield: 76 %; m.p. 209-211 °C; IR (cm⁻¹): 2853(Ar-CH), 1725 (C=O), 1594(C=C), 1535(C=N), 1193 (C-OCH₃), 1343 (C-NO₂). ¹H-NMR(DMSO): δ 8.10 (s,1H, N=CH-), 8.16 (s,1H, N-H), 7.70 (s,1H, 4-H, Ar-H) 6.78-7.62 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 3.73 (s,3H, -OCH₃). ¹³C-NMR(DMSO): δ 163.9(-C=N), 163.4(N=CH), 160.6 (C=O), 157.7 (C-5), 151.7 (C-1',C-4'), 150.7 (C-4''), 137.3 (C-1''), 131.0 (C-9), 129.9 (C-2'', C-6''), 124.1 (C-8), 123.7(C-3'',C-5''), 123.3 (C-2',C-3',C-5',C-6'), 121.8 (C-7), 116.6 (C-6) 114.8 (C-4), 56.0(-OCH₃), EI-MS (m/z, %): 400.37 (M⁺, 9). Anal. Calcd for C₂₂H₁₆N₄O₄: C, 66.00; H, 4.03; N, 13.99. Found: C, 66.04; H, 4.07; N, 13.95.

5-Fluoro-3-(4-(4-chlorobenzylideneamino)-phenylimino) indolin-2-one (5g).

Yield: 84.6 %; m.p. 297-299 °C; IR (cm⁻¹): 3081(Ar-CH), 1734 (C=O), 1617 (C=C), 1607(C=N), 648 (C-Cl), 1096 (C-F). ¹H-NMR(DMSO): δ 8.12 (s,1H, N=CH-), 8.15 (s,1H, N-H), 7.34 (s,1H, 4-H, Ar-H) 6.98-7.25 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H). ¹³C-NMR(DMSO): δ 159.7 (-C=N), 163.2 (N=CH), 160.6(C=O), 157.8 (C-5), 151.7 (C-1',C-4'), 136.1 (C-4''), 134.3 (C-9), 130.4 (C-2'', C-6''), 129.3 (C-1''), 129(C-3'',C-5''), 124.7 (C-8), 123.3 (C-2',C-3',C-5',C-6'), 122.1 (C-7), 118.0 (C-6) 116.2 (C-4). EI-MS (m/z, %): 376.98 (M⁺, 15). Anal. Calcd for C₂₁H₁₃ Cl F N₃O: C, 66.76; H, 3.47; N, 11.12. Found: C, 66.80; H, 3.51; N, 11.16.

5-Fluoro-3-(4-(4-nitrobenzylideneamino)-phenylimino) indolin-2-one (5h).

Yield: 85 % ; m.p. 322-324 °C; IR (cm⁻¹): 3111(Ar-CH), 1733 (C=O), 1597 (C=C), 1617(C=N), 1343 (C-NO₂), 1104 (C-F). ¹H-NMR(DMSO): δ 8.13 (s,1H, N=CH-), 8.17 (s,1H, N-H), 7.32 (s,1H, 4-H, Ar-H) 6.98-7.23 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H). ¹³C-NMR(DMSO): δ 164.1 (-C=N), 163.3(N=CH), 160.5(C=O), 157.8 (C-5), 151.7 (C-1',C-4'), 150.7 (C-4''), 137.3 (C-1''), 134.3 (C-9), 129.9 (C-2'', C-6''), 124.7 (C-8), 123.7 (C-3'',C-5''), 123.3 (C-2',C-3',C-5',C-6'), 122.1 (C-7), 118.0 (C-6) 116.2 (C-4). EI-MS (m/z, %): 388.35 (M⁺, 10). Anal. Calcd for C₂₁H₁₃ F N₄ O₃: C, 64.95; H, 3.37; N, 14.43. Found: C, 64.91; H, 3.43; N, 14.47.

5-Fluoro-3-(4-(4-methoxybenzylideneamino)-phenylimino) indolin-2-one (5i).

Yield: 85 %; m.p. 279-281 °C; IR (cm⁻¹): 3119(Ar-CH), 1732 (C=O), 1617 (C=C), 1607(C=N), 1260 (C-OCH₃), 1019 (C-F). ¹H-NMR(DMSO): δ 8.10 (s,1H, N=CH-), 8.16 (s,1H, N-H), 7.30 (s,1H, 4-H, Ar-H) 6.8-7.28 (m,10H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 3.73 (s,3H, -OCH₃). ¹³C-NMR(DMSO): δ 169.8(-C=N), 162.9 (N=CH), 160.1 (C=O), 164.3 (C-4''), 157.8 (C-5), 151.7 (C-1',C-4'), 134.3 (C-9), 130.0 (C-2'', C-6''), 124.7 (C-8), 123.5 (C-1''), 123.3 (C-2',C-3',C-5',C-6'), 122.1 (C-7), 118.0 (C-6) 116.2 (C-4), 114.2 (C-3'',C-5''). EI-MS (m/z, %): 373.38 (M⁺, 12). Anal. Calcd for C₂₂H₁₆ F N₃ O₂: C, 70.77; H, 4.32; N, 11.25. Found: C, 70.73; H, 4.36; N, 11.29.

N-acetyl-3-(4-(4-chlorobenzylideneamino)-phenylimino) indolin-2-one. (6a).

Yield: 47.73 %; m.p. 224-226 °C; IR (cm⁻¹): 3069(Ar-CH), 1668 (C=O), 1515 (C=C), 1603 (C=N), 1688 (-COCH₃), 754 (C-Cl). ¹H-NMR(DMSO): δ 8.12 (s,1H, N=CH-), 7.02-7.67 (m,12H, 4-H, 5-H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 2.4(s,3H,-COCH₃). ¹³C-NMR(DMSO): δ 166.2 (-COCH₃), 164.3 (-C=N), 163.1 (N=CH), 158.1 (C=O), 151.7 (C-1',C-4'), 141.3 (C-9), 136.1 (C-4''), 131.0 (C-6), 130.4 (C-2'', C-6''), 129.2 (C-4),129.3 (C-1''), 129.0 (C-3'', C-5''), 124.2 (C-5), 123.3 (C-2',C-3',C-5',C-6'),123.1 (C-8), 120.5 (C-7), 14.9 (-COCH₃). EI-MS (m/z, %): 401.97 (M⁺, 18). Anal. Calcd. for C₂₃H₁₆N₃ClO₂: C, 68.74; H, 4.01; N,10.46. Found: C, 68.71; H, 4.05; N, 10.42.

N-acetyl-3-(4-(4-nitrobenzylideneamino)-phenylimino) indolin-2-one (**6b**).

Yield: 73.1 %; m.p. 204-206 °C; IR (cm⁻¹): 3074(Ar-CH), 1669 (C=O), 1515 (C=C), 1601 (C=N), 1669 (-COCH₃), 1342 (C-NO₂). ¹H-NMR(DMSO): δ 8.10 (s,1H, N=CH-), 7.0-8.22 (m,12H, 4-H, 5-H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 2.39(s,3H,-COCH₃). ¹³C-NMR(DMSO): δ 166.1 (-COCH₃), 163.9 (-C=N), 163.4 (N=CH), 157.9 (C=O), 151.7 (C-1',C-4'), 150.7 (C-4''), 141.3 (C-9), 137.3 (C-1''), 131.0 (C-6), 129.9 (C-2'', C-6''), 129.2 (C-4), 124.2 (C-5), 123.7 (C-3'',C-5'') 123.3 (C-2',C-3',C-5',C-6'),123.1 (C-8), 120.5 (C-7), 14.9 (-COCH₃). EI-MS (m/z, %): 412.12(M⁺, 10), Anal. Calcd for C₂₃H₁₆N₄O₄: C, 66.99; H, 3.91; N, 13.59. Found: C, 66.94; H, 3.95; N, 13.63.

N-acetyl-3-(4-(4-methoxybenzylideneamino)-phenylimino) indolin-2-one (**6c**).

Yield: 16.9 %; m.p. 209-211 °C; IR (cm⁻¹): 3065(Ar-CH), 1669 (C=O), 1515 (C=C), 1603 (C=N), 1254 (C-OCH₃), 1669 (-COCH₃). ¹H-NMR(DMSO): δ 8.11 (s,1H, N=CH-), 6.80-7.67 (m,12H, 4-H, 5-H, 6-H, 7-H, 2'-H, 3'-H, 5'-H, 6'-H, 2''-H, 3''-H, 5''-H, 6''-H, Ar-H), 3.73 (s,3H, -OCH₃), 2.4(s,3H,-COCH₃). ¹³C-NMR(DMSO): δ 166.3 (-COCH₃), 164.3 (-C=N), 163.3 (N=CH), 158.1 (C=O), 164.3 (C-4''), 151.7 (C-1',C-4'), 141.3 (C-9), 131.0 (C-6), 130 (C-2'', C-6''), 129.2 (C-4), 124.2 (C-5), 123.5 (C-1''), 123.3 (C-2',C-3',C-5',C-6'),123.1 (C-8), 120.5 (C-7), 114.2 (C-3'', C-5''), 56.0 (-OCH₃), 14.9 (-COCH₃). EI-MS (m/z, %): 397.14 (M⁺, 15), Anal. Calcd for C₂₄H₁₉N₃O₃: C, 72.53; H, 4.82; N, 10.57. Found: C, 72.49; H, 4.86; N, 10.61.

Analgesic Activity (Tail-immersion method) [12, 13]

All protocols of animal experiments have been sanctioned by the Institutional Animal ethics Committee (IAEC) Reference No: IAEC/ XIII/ 08/ CLBMC/2007-2008. Dated: 10-10-2007.

Swiss mice (n=6) of either sex selected by random sampling technique was used for the study. Pentazocine 10 mg/kg i.p was administered as standard drug for comparison. The test compounds (**5a-5i**, **6a-6c**) at 2 dose levels (200 and 400 mg/kg) 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 dipped in a beaker of water maintained at 55±0.5°C.

The time in sec taken to withdraw the tail clearly out of water was taken as the reaction time. The first reading (0 min) was taken immediately after the administration of the test compound and subsequent reaction time was recorded at 30, 60, 120 and 180 min after the administration of compounds. A cut off point of 15 sec was observed to prevent the tail damage. The percentage analgesic activity was calculated using the following formula and the results are presented in table 1.

$$\text{PAA} = [(T_2 - T_1) / T_2] \times 100$$

Where,

T₁ is the reaction time (in sec) before treatment

T₂ is the reaction time (in sec) after treatment

PAA is the percentage analgesic activity

Table 1: Analgesic activity of the synthesized compounds

COMPOUN DS	DOSE (mg/kg)	0 min			30 min		60 min		120 min		180 min	
		MEAN \pm SEM	MEAN \pm SEM	%	MEAN \pm SEM	%	MEAN \pm SEM	%	MEAN \pm SEM	%		
6a	200	3.17 \pm 0.17	6.83 \pm 0.60*	53.58	7.50 \pm 0.67*	57.73	9.50 \pm 0.67*	65.26	7.83 \pm 0.48*	59.51		
	400	3.30 \pm 0.33	7.33 \pm 0.67 ^{NS}	54.97	9.33 \pm 0.71*	64.63	10.50 \pm 0.56**	68.57	9.17 \pm 0.98**	64.01		
6b	200	3.83 \pm 0.31	6.00 \pm 0.3*	36.16	8.17 \pm 0.40*	53.12	10.17 \pm 0.65*	62.34	8.33 \pm 0.76*	54.02		
	400	4.00 \pm 0.37	6.83 \pm 0.31 ^{NS}	41.43	10.17 \pm 0.79*	60.66	11.67 \pm 0.49*	65.72	8.83 \pm 0.75**	54.69		
6c	200	3.50 \pm 0.23	6.67 \pm 0.33*	47.52	7.33 \pm 0.49*	62.25	10.50 \pm 0.49*	63.52	8.83 \pm 0.31*	60.36		
	400	3.83 \pm 0.31	8.00 \pm 0.58*	52.12	9.67 \pm 0.76*	60.39	12.33 \pm 0.76*	66.25	10.00 \pm 0.43**	61.70		
5a	200	3.17 \pm 0.17	6.33 \pm 0.21*	49.92	7.83 \pm 0.40*	59.51	9.50 \pm 0.43**	66.63	8.00 \pm 0.58**	60.37		
	400	3.33 \pm 0.21	7.83 \pm 0.69*	57.47	9.83 \pm 0.40**	66.12	11.50 \pm 0.34*	71.04	8.50 \pm 0.43**	60.82		
5b	200	3.83 \pm 0.40	7.67 \pm 0.56*	50.06	8.17 \pm 0.79*	53.12	10.50 \pm 0.67**	63.52	7.83 \pm 0.54**	51.08		
	400	3.33 \pm 0.21	8.00 \pm 0.83*	58.37	9.67 \pm 0.76**	65.56	12.67 \pm 0.76**	73.71	8.50 \pm 0.62**	60.82		
5c	200	4.00 \pm 0.34	7.50 \pm 0.43*	46.67	8.50 \pm 0.43*	52.44	10.50 \pm 0.43*	61.90	8.83 \pm 0.79**	54.70		
	400	3.83 \pm 0.31	8.17 \pm 0.48 ^{NS}	53.12	9.17 \pm 0.31*	58.23	12.17 \pm 0.31**	69.53	10.83 \pm 0.54*	64.64		
5d	200	3.50 \pm 0.22	7.00 \pm 0.52*	50.00	9.00 \pm 0.58*	61.11	10.50 \pm 0.67*	66.66	9.50 \pm 0.56*	63.15		
	400	3.83 \pm 0.31	8.00 \pm 0.68*	52.12	10.50 \pm 0.43*	63.52	12.50 \pm 0.50**	69.36	9.83 \pm 0.60**	61.03		
5e	200	3.50 \pm 0.34	5.83 \pm 0.40 ^{NS}	39.96	6.50 \pm 0.56*	46.15	9.00 \pm 0.45*	61.11	7.83 \pm 0.60*	55.30		
	400	3.83 \pm 0.31	6.50 \pm 0.34*	41.07	8.83 \pm 0.70*	56.62	11.17 \pm 0.48*	65.71	9.50 \pm 0.56*	59.68		
5f	200	4.00 \pm 0.37	6.00 \pm 0.52*	33.33	7.67 \pm 0.61*	47.84	11.67 \pm 0.56**	65.72	8.50 \pm 0.76**	52.94		
	400	3.17 \pm 0.17	6.83 \pm 0.54*	53.58	8.50 \pm 0.48*	62.77	10.17 \pm 0.88*	68.82	9.00 \pm 0.97*	64.78		
5g	200	4.00 \pm 0.37	6.83 \pm 0.40*	41.43	7.17 \pm 0.31*	44.21	10.17 \pm 0.31**	60.66	9.33 \pm 0.21**	57.12		
	400	3.17 \pm 0.17	7.33 \pm 0.42*	56.75	8.50 \pm 0.72*	62.70	10.83 \pm 0.65*	70.72	9.83 \pm 0.48**	67.75		
5h	200	3.30 \pm 0.21	6.33 \pm 0.33*	47.86	8.50 \pm 0.76*	61.17	11.00 \pm 0.57**	70.00	8.83 \pm 0.31**	62.62		
	400	3.30 \pm 0.21	7.33 \pm 0.49*	54.97	9.83 \pm 0.79**	66.42	12.67 \pm 0.61**	73.95	9.17 \pm 0.70**	64.01		
5i	200	4.00 \pm 0.37	5.67 \pm 0.37*	29.45	7.50 \pm 0.22*	46.66	10.83 \pm 0.48**	63.06	7.00 \pm 0.37**	42.85		
	400	3.17 \pm 0.17	6.67 \pm 0.49*	52.47	9.83 \pm 0.60*	67.75	12.00 \pm 0.22**	73.58	8.17 \pm 0.48**	61.19		
Pentazocine	10	3.50 \pm 0.34	8.67 \pm 0.21**	59.63	11.00 \pm 0.40*	68.18	13.50 \pm 0.34**	74.07	10.33 \pm 0.49**	66.11		

Each value expressed as Mean \pm SEM (n= 6) in the tail flick method using Dunnet's t test followed by one way ANOVA and *P< 0.05, **P<0.01, NS (Non significant), % (Percentage of analgesic activity).

Anti-inflammatory Activity [14]

Anti-inflammatory activity was performed by carrageenan- induced paw oedema method in rats. Indomethacin (20 mg/kg, i.p) was administered as standard drug for comparison. The synthesized compounds (**5a-5i**, **6a-6c**) were administered at 2 dose levels (100 and 200 mg/kg) orally 30 min prior to the administration of 0.1ml/kg body weight of carrageenan in saline (1% w/v) into the lateral malleolus of the sub-planter region of the left hind paw. The paw volumes were measured using the mercury displacement technique with the help of a plethysmograph immediately before and 30 min, 1, 2 and 3h after carrageenan injection. The percentage inhibition of paw odema was calculated by using the following formula and the results are depicted in table 2.

$$\% \text{ protection} = [(\text{Control}-\text{Test})/\text{Control}] \times 100$$

Table2: Anti-inflammatory activity of the synthesized compounds

COMPOUNDS	DOSE (mg/kg)	30 min		60 min		120 min		180 min	
		MEAN±SEM	%	MEAN±SEM	%	MEAN±SEM	%	MEAN±SEM	%
6a	200	0.66±0.01*	7.74	0.74±0.01*	18.05	0.91±0.01*	36.72	0.85±0.02*	20.50
	400	0.61±0.02*	15.21	0.64±0.02*	28.34	0.78±0.01*	45.73	0.75±0.02*	29.82
6b	200	0.67±0.01*	7.33	0.76±0.01*	15.50	0.91±0.02*	36.93	0.78±0.01*	27.02
	400	0.62±0.01*	13.27	0.71±0.01*	21.37	0.82±0.02**	42.96	0.70±0.02*	34.48
6c	200	0.71±0.01 ^{NS}	0.82	0.80±0.01*	11.07	0.99±0.01*	31.18	0.77±0.02*	27.95
	400	0.65±0.01 ^{NS}	9.12	0.73±0.01*	18.38	0.91±0.01*	36.93	0.68±0.02*	35.97
5a	200	0.58±0.02*	18.81	0.70±0.02*	21.70	0.84±0.02**	41.58	0.68±0.02**	35.97
	400	0.52±0.02*	27.66	0.64±0.02**	28.34	0.73±0.02**	49.20	0.58±0.02**	45.66
5b	200	0.61±0.03*	14.66	0.68±0.01**	23.92	0.81±0.01**	43.65	0.60±0.02**	43.42
	400	0.56±0.02*	22.13	0.62±0.02**	31.00	0.72±0.02**	49.89	0.58±0.01**	45.29
5c	200	0.70±0.01*	2.76	0.81±0.01*	9.52	0.94±0.01*	34.85	0.75±0.02*	29.82
	400	0.64±0.02 ^{NS}	11.06	0.74±0.02*	17.71	0.86±0.01*	40.19	0.64±0.02*	39.70
5d	200	0.65±0.02*	10.09	0.77±0.02*	13.95	0.90±0.02*	37.42	0.75±0.01**	29.82
	400	0.60±0.01 ^{NS}	17.01	0.71±0.02*	21.37	0.83±0.02**	41.99	0.70±0.01**	34.76
5e	200	0.66±0.02*	7.74	0.74±0.02*	18.05	0.92±0.02**	35.75	0.81±0.02**	24.01
	400	0.59±0.01*	17.98	0.67±0.02*	25.47	0.77±0.02**	46.15	0.71±0.02**	33.83
5f	200	0.71±0.01*	0.82	0.78±0.01*	12.84	0.88±0.01*	38.8	0.74±0.02**	30.75
	400	0.66±0.01*	8.71	0.73±0.02*	19.15	0.76±0.01**	47.12	0.66±0.01**	38.21
5g	200	0.61±0.02*	14.66	0.66±0.01**	26.57	0.77±0.02**	46.15	0.64±0.01**	40.07
	400	0.53±0.02**	25.72	0.60±0.03**	33.22	0.71±0.02**	50.58	0.57±0.03**	46.87
5h	200	0.60±0.02*	16.59	0.65±0.02**	27.68	0.80±0.03**	44.07	0.70±0.01**	34.76
	400	0.54±0.02*	25.03	0.60±0.01*	33.22	0.72±0.01**	49.61	0.59±0.01**	44.36
5i	200	0.66±0.01*	7.74	0.72±0.01**	20.26	0.88±0.02*	38.53	0.76±0.02*	29.17
	400	0.58±0.02*	19.36	0.67±0.01*	25.47	0.81±0.02**	43.65	0.63±0.02**	41.28
Indomethacin	20	0.52±0.02**	27.66	0.58±0.02**	35.43	0.66±0.02**	53.77	0.54±0.02**	49.67
Control	-	0.72±0.01	-	0.90±0.02	-	1.44±0.03	-	1.07±0.03	-

Each value expressed as Mean ± SEM (n= 6) in the carrageenan paw oedema method using Dunnet's t test followed by one way ANOVA and *P< 0.05, **P<0.01, NS (Non significant), % (Percentage reduction of oedema)

Anti-microbial screening

The anti-bacterial activity of the synthesized compounds was tested against four Gram-positive bacteria (*Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 155, *Micrococcus luteus* ATCC 4698 and *Bacillus cereus* ATCC 11778), three Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, and *Klebsiella pneumoniae* ATCC 11298) and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) using Sabouraud dextrose agar medium (Hi-Media Laboratories, India).

Paper disc diffusion technique [15]

The sterilized (autoclaved at 120 °C for 30min) medium (40-50 °C) was inoculated (1 mL/100 mL of medium) with the suspension (10^5 cfu mL⁻¹) of the micro-organism (matched to McFarland barium sulphate standard) and poured into a petridish to give a depth of 3-4 mm. The paper impregnated with the test compounds (μ g mL⁻¹ in dimethyl formamide) was placed on the solidified medium. The plates were pre-incubated for 1h at room temperature and incubated at

37°C for 24 and 48h for anti-bacterial and anti-fungal activities, respectively. Ciprofloxacin (100 µg/disc) and Ketoconazole (100 µg/disc) were used as standard drugs for anti-bacterial and anti-fungal activities respectively. The observed zone of inhibition is presented in table 3.

Minimum inhibitory concentration (MIC)

MIC [16] of the compound was determined by Agar streak dilution method. A stock solution of the synthesized compound (100 µg mL⁻¹) in dimethyl formamide was prepared and graded quantities of the test compounds were incorporated in specified quantity of molten sterile agar (nutrient agar for anti-bacterial activity and sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40-50 °C) containing the compound was poured into a petridish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism were prepared to contain approximately 10⁵ cfu mL⁻¹ and applied to plates with serially diluted compounds in dimethyl formamide to be tested and incubated at 37 °C for 24h and 48h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi in the plate. The observed MIC is presented in table 3.

Table 3: Antimicrobial activity of the synthesized compounds

compounds	In vitro activity - zone of inhibition (MIC 100 µg/ml)								
	S.aureus ATCC 9144	<i>S.epiderm idis</i> ATCC 155	<i>M.luteus</i> ATCC 4698	<i>B.cereus</i> ATCC 11778	<i>E.coli</i> ATCC 25922	<i>P.aeurigi nosa</i> ATCC 2853	<i>K.pneumo niae</i> ATCC 11298	<i>A.niger</i> ATCC 9029	<i>A.fumigatus</i> ATCC 46645
5a	21 (15.2)	21 (16.9)	19 (21.1)	19 (18.7)	22 (16.9)	16 (18.4)	18 (16.7)	19 (21.2)	20 (31.1)
5b	23 (11.6)	25 (12.3)	24 (13.2)	20 (14.8)	21 (13.2)	18 (16.3)	22 (11.7)	24 (16.8)	25 (18.3)
5c	16 (17.2)	16 (18.3)	15 (23.6)	17 (21.3)	17 (19.9)	15 (19.6)	17 (20.3)	18 (22.6)	17 (29.8)
5d	17 (21.6)	16 (23.7)	18 (29.1)	18 (31.6)	16 (34.8)	16 (26.2)	16 (30.1)	16 (31.6)	14 (34.3)
5e	16 (22.8)	18 (24.7)	16 (28.9)	17 (33.6)	15 (35.8)	15 (28.4)	14 (37.2)	15 (34.6)	19 (36.2)
5f	16 (18.8)	17 (17.6)	18 (24.5)	16 (22.1)	17 (23.2)	15 (19.3)	15 (24.4)	20 (25.6)	20 (27.3)
5g	22 (14.9)	23 (16.3)	20 (19.3)	19 (17.2)	21 (15.9)	18 (17.6)	17 (14.3)	24 (19.3)	25 (25.6)
5h	24 (10.3)	27 (11.3)	27 (12.1)	21 (13.2)	23 (11.9)	18 (15.1)	23 (10.7)	25 (15.3)	27 (16.2)
5i	20 (16.1)	22 (17.2)	19 (22.4)	20 (19.1)	20 (18.3)	17 (21.2)	17 (18.3)	19 (23.4)	22 (26.8)
6a	16 (25.1)	17 (29.8)	18 (31.7)	16 (34.2)	19 (36.7)	14 (32.6)	16 (36.7)	17 (32.6)	16 (38.4)
6b	18 (19.3)	18 (21.4)	19 (26.4)	15 (27.5)	19 (25.2)	16 (21.6)	16 (27.8)	17 (29.6)	17 (33.6)
6c	15 (27.3)	18 (31.3)	16 (36.7)	17 (25.4)	17 (37.5)	15 (35.7)	15 (37.2)	15 (36.7)	15 (41.2)
Ciprofloxacin (100µg/disc)	25 (0.2)	29 (0.35)	30 (0.1)	25 (0.3)	27 (0.2)	22 (0.2)	25 (0.1)	-	-
Ketoconazole (100µg/disc)	-	-	-	-	-	-	-	27 (6.1)	31 (6.3)
DMF	-	-	-	-	-	-	-	-	-

Acknowledgements

The authors are thankful to C.L.Baid Metha College of Pharmacy and its management for providing research facilities and the research coworkers those who helped us to complete this research.

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