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Comparative *in silico* and *in vivo* Analgesic and Anti-Inflammatory Activities of Some Novel Schiff Bases of Isatin

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The objective of this study is to synthesize the different derivatives of isatin and pyrazole on Schiff base mechanism, which on subjected to docking study by AUTODOCK TOOL software. *in vitro* and *in vivo* analgesic and anti-inflammatory activities were performed on the compound showing the optimum docking results selected on the basis of binding energy.

Keywords: Isatin, Pyrazole, Schiff base, Analgesic activity, Anti-inflammatory activity, Docking study.

INTRODUCTION

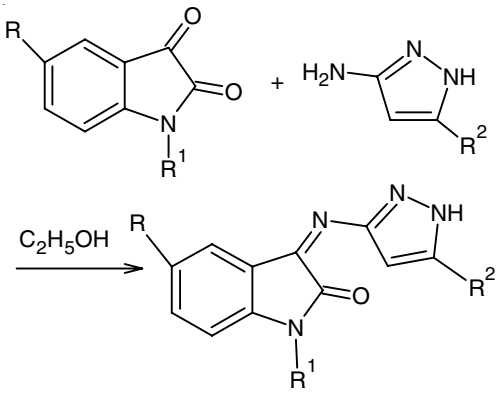
Isatin and pyrazole derivatives have achieved great importance due to the vast spectrum of pharmacological activities which are reflected by their use as antimicrobial, analgesic, anti-inflammatory and anticonvulsant activities [1-3]. Indomethacin having indole nucleus and phenylbutazone, a pyrazolone derivative are among the most widely used drug as NSAID [4]. The inhibition of cyclooxygenase enzyme has to be concerned either of the two nucleus. Even though indomethacin is a selective inhibitor of COX-1 enzyme, the changes in core indole moiety can change the selectivity towards COX-2 enzyme [5,6]. Cyclooxygenase (COX) enzyme is responsible for the conversion of arachidonic acid to prostaglandins, prostacyclins and thromboxanes which are keys for inflammation and analgesia. Non-steroidal anti-inflammatory drugs (NSAIDs) exert pharmacological action by inhibiting the cyclooxygenase [7].

In the present study, Schiff of isatin and pyrazole were prepared and analyzed by docking study. The compound showing optimum binding energy is then subjected to *in vivo* and *in vitro* analgesic and anti-inflammatory activities. Generally Schiff bases are prepared by the reaction between aldehydic/ketonic group of compound and primary amine in the presence ethanol and glacial acetic acid in different ratios, where glacial acetic acid provide the driving force for completion of reaction by maintaining anhydrous condition. For the preparation of Schiff bases strong acid catalyst (*e.g.* BF_3-etherate, anhydrous $ZnCl_2$ or $POCl_3$) is required for speed up the reaction. Generally, imino or azomethine group is formed by this mechanism shows analgesic and anti-inflammatory activities. In the present study, we compared the synthesized Schiff bases for analgesic activity, anti-inflammatory activity with the help of docking study [8]. Docking studies were done on a series of Schiff base of isatin derivatives on drug Discovery Studio by Accelerys and Auto dock tools 1.5.6 into the active sites of cyclooxygenase-2 (COX-2) [9]. The structure of enzyme cyclooxygenase-2 (prostaglandin synthase-2) was obtained from protein data bank (PDB code: 1CX2) [10] and used for docking. The docking model was demonstrated for inhibitor's conformation, protein interaction and hydrogen bonding.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using benzene:ethyl acetate (9:1) as eluent. IR spectra (KBr pellets) were recorded on Shimadzu FT-IR model 8010 spectrophotometer. ^{1}H NMR spectra (DMSO-d_6) were taken a Varian mercury spectrometer (model YH- 300 FT NMR) using TMS as internal standard and chemical shift are expressed in δ ppm. The potential energy, van der Waals energy, electrostatic energy and RMS gradient was checked for the protein before and after minimization.

The general synthetic approach involved condensation of an equimolar mixture of corresponding substituted indole-2, 3-dione (0.01 mol) and substituted pyrazole (0.01 mol) in absolute ethanol in the presence of 2,3-drops of glacial acetic acid for 3-4 h. On cooling, flakes separated out which were filtered and recrystallized from hot ethanol to give shining bright needles of Schiff base [11] (**Scheme-I**). All the synthesized compounds were characterized for their spectral studies.



Scheme-I

Spectral data

3-[(1*H*-Pyrazol-3-yl)imino]indolin-2-one (P01): Yield: 82 %, m.f.:$C_{11}H_8N_4O$, m.w. 212.21, m.p. 165-167 ºC. Elemental analysis (found) %: C, 62.26; H, 3.80; N, 26.40; O, 7.54. IR (KBr, ν_{max}, cm^{-1}): 3410 (NH *str.*), 1715 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.50 (d, 1H, *J* = 7.96 Hz, Ar-H), 7.43 (m, 1H, Ar-H), 7.60 (m, 1H, Ar-H), 7.75 (d, 1H, *J* = 7.4 Hz, Ar-H), 7.89 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 7.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 10.3 (s, 1H, N-H), 12.49 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-chloroindolin-2-one (P02): Yield: 68 %, m.f.: $C_{11}H_7N_4OCl$, m.w. 246.65, m.p. 166-167 ºC. Elemental analysis (found) %: C, 53.57; H, 2.86; Cl, 14.37; N, 22.72; O, 6.49. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.48 (d, 1H, *J* = 5.7 Hz, CH), 7.63 (m, 2H, Ar), 7.89 (d, 1H, *J* = 6.8Hz, Ar), 8.01(t, 1H, *J* = 7.77Hz, pyrazole Ar-H), 10.03(s, 1H, NH), 12.51(s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-bromoindolin-2-one (P03): Yield: 68 %, m.f.: $C_{11}H_7N_4OBr$, m.w. 291.11, m.p. 167-169 ºC. Elemental analysis (found) %: C, 45.39; H, 2.42; Br, 27.45; N, 19.25; O, 5.50. IR (KBr, ν_{max}, cm^{-1}): 3416 (NH *str.*), 2891 (C-H *str.*), 1709 (carbonyl *str.*), 1606 (NH bend.). ^{1}H NMR DMSO-d_6, 400 MHz) δ: 6.46 (d, 1H, *J* = 5.7Hz, CH), 7.62 (m, 2H, Ar), 7.88 (d, 1H, *J* = 6.8Hz, Ar), 8.02 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 10.01 (s, 1H, NH), 12.50 (s, 1H, pyrazole N-H).

3-[(1H-Pyrazol-3-yl)imino]-5-nitroindolin-2-one (04): Yield: 70 %, m.f.: $C_{11}H_7N_5O_3$, m.w. 257.21, m.p. 173-175 ºC. Elemental analysis (found) %: C, 51.37; H, 2.74; N, 27.23; O, 18.66. IR (KBr, ν_{max}, cm^{-1}): 3417 (NH *str.*), 2891 (C-H *str.*), 1711 (carbonyl *str.*), 1607 (NH bend.), 1510.8, 1401.1 (NO_2 *str.*). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.44 (d, 1H, *J* = 5.7 Hz, CH), 7.61(m, 2H, Ar), 7.83 (d, 1H, *J* = 6.6Hz, Ar), 8.01 (t, 1H, *J* = 7.69 Hz, pyrazole Ar-H), 10.03(s, 1H, NH), 12.45 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-fluoroindolin-2-one (P05): Yield: 71 %, m.f.: $C_{11}H_7N_4OF$, m.w. 230.20, m.p. 170-172 ºC. Elemental analysis (found) %: C, 57.39; H, 3.07; F, 8.25; N, 24.34; O, 6.95. IR (KBr, ν_{max}, cm^{-1}): 3411 (NH *str.*), 2892 (C-H *str.*), 1710 (carbonyl *str.*), 1604 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.48 (d, 1H, *J* = 5.7, CH), 7.66 (m, 2H, Ar), 7.80 (d, 1H, *J* = 6.8, Ar), 8.03 (s, 1H, Ar), 10.00 (s, 1H, NH), 12.51 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-methoxyindolin-2-one (P06): Yield: 64 %, m.f.: $C_{12}H_{10}N_4O_2$, m.w. 242.25, m.p.176-178 ºC. Elemental analysis (found) %: C, 59.50; H, 4.16; N, 23.13; O, 13.21. IR (KBr, ν_{max}, cm^{-1}): 415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.50 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.20 (d, 1H, *J* = 7.4 Hz, Ar-H), 8.89 (t, 1H, *J* = 7.77Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 10.4 (s, 1H, N-H), 12.5 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-1-methylindolin-2-one (P07): Yield: 64 %, m.f.: $C_{12}H_{10}N_4O$, m.w. 226.24, m.p.164-166 ºC. Elemental analysis (found) %: C, 63.71; H, 4.46; N, 24.76; O, 7.07. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.46 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 5.7 Hz, CH), 7.66 (m, 2H, Ar), 7.80 (d, 1H, *J* = 6.8 Hz, Ar), 7.90 (d, 1H, *J* = 7.1Hz, Ar), 8.03 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 12.54 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-chloro-1-methylindolin-2-one (P08): Yield: 71 %, m.f.: $C_{12}H_9N_4OCl$, m.w. 260.68, m.p. 166-167 ºC. Elemental analysis (found) %: C, 55.29; H, 3.48; Cl, 13.60; N, 21.49; O, 6.14. IR (KBr, ν_{max}, cm^{-1}): 3414 (NH *str.*), 2894 (C-H *str.*), 1708 (carbonyl *str.*), 1611 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.84 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 5.7 Hz, CH), 7.63 (m, 2H, Ar), 7.89 (d, 1H, *J* = 6.8Hz, Ar), 8.01 (t, 1H, *J* = 6.89 Hz, pyrazole Ar-H), 12.36 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-bromo-1-methylindolin-2-one (P09): Yield: 64 %, m.f.: $C_{12}H_9N_4OBr$, m.w. 305.14, m.p. 166-167 ºC. Elemental analysis (found) %: C, 47.24; H, 2.97; Br, 26.19; N, 18.36; O, 5.24. IR (KBr, ν_{max}, cm^{-1}): 3411 (NH *str.*), 2895 (C-H *str.*), 1711 (carbonyl *str.*), 1609 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.80 (s, 3H, CH_3), 6.41 (d, 1H, *J* = 5.7 Hz, CH), 7.62 (m, 2H, Ar), 7.89 (d, 1H, *J* = 6.8Hz, Ar), 8.0 (t, 1H, *J* = 7.72 Hz, pyrazole Ar-H), 12.16 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino)-1-methyl-5-nitroindolin-2-one (P10): Yield: 64 %, m.f.: $C_{12}H_9N_5O_3$, m.w. 271.24, m.p. 165-167 ºC. Elemental analysis (found) %: C, 53.14; H, 3.34; N, 25.82; O, 17.70. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.), 1510.8, 1401.1 (NO_2 *str.*). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.55 (s, 3H, CH_3), 6.44 (d, 1H, *J* = 5.7 Hz, CH), 7.61 (m, 2H, Ar), 7.83 (d, 1H, *J* = 6.6 Hz, Ar), 8.03 (t, 1H, *J* = 7.71 Hz, pyrazole Ar-H), 12.45 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-fluoro-1-methylindolin-2-one (P11): Yield: 70 %, m.f.: $C_{12}H_9N_4OF$, m.w. 244.23, m.p. 169-171 ºC. Elemental analysis (found) %: C, 59.02; H, 3.71; F, 7.78; N, 22.94; O, 6.55. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.66 (s, 3H, CH_3), 6.40 (d, 1H, *J* =

5.6 Hz, CH), 7.60 (m, 2H, Ar), 7.87(d, 1H, *J* = 6.7 Hz, Ar), 8.3 (t, 1H, *J* = 7.75Hz, pyrazole Ar-H), 12.01 (s, 1H, pyrazole N-H).

3-[(1*H*-Pyrazol-3-yl)imino]-5-methoxy-1-methylindolin-2-one (P12): Yield: 68 %, m.f.: $C_{13}H_{12}N_4O_2$, m.w. 256.27, m.p. 172-174 ºC. Elemental analysis (found) %: C, 60.93; H, 4.72; N, 21.86; O, 12.49. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2891 (C-H *str.*), 2822 (O-CH_3 *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 3.34 (s, 3H, CH_3), 3.77 (s, 3H, O-CH_3), 6.39 (d, 1H, *J* = 5.6 Hz, CH), 7.17 (d, 1H, *J* = 6.4 Hz, Ar), 7.60 (m, 1H, Ar), 7.87 (d, 1H, *J* = 6.7 Hz, Ar), 7.89 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 12.11 (s, 1H, pyrazole N-H).

3-[(5-Methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P13): Yield: 69 %, m.f.: $C_{12}H_{10}N_4O$, m.w. 226.24, m.p. 171-173 ºC. Elemental analysis (found) %: C, 63.71; H, 4.46; N, 24.76; O, 7.07. IR (KBr, ν_{max}, cm^{-1}): 3416 (NH *str.*), 2889 (C-H *str.*), 1711 (carbonyl *str.*), 1603 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 6.29 (s, 1H, pyrazole Ar-H), 6.50 (d, 1H, *J* = 7.88 Hz, Ar-H), 7.81 (m, 1H, Ar-H), 8.23 (d, 1H, *J* = 7.4 Hz, Ar-H), 8.70 (d, 1H, *J* = 7.51 Hz, Ar-H), 10.2 (s, 1H, N-H), 12.1 (s, 1H, pyrazole N-H).

5-Chloro-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P14): Yield: 69 %, m.f.: $C_{12}H_9N_4OCl$, m.w. 260.68, m.p. 168-170 ºC. Elemental analysis (found) %: C, 55.29; H, 3.48; Cl, 13.60; N, 21.49; O, 6.14. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.31 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 5.7 Hz, CH), 7.63 (m, 1H, Ar), 7.89 (d, 1H, *J* = 6.8Hz, Ar), 8.01 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 10.03 (s, 1H, NH), 12.51 (s, 1H, pyrazole N-H).

5-Bromo-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P15): Yield: 66 %, m.f.: $C_{12}H_9N_4OBr$, m.w. 305.14, m.p. 165-167 ºC. Elemental analysis (found) %: C, 47.24; H, 2.97; Br, 26.19; N, 18.36; O, 5.24. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2899 (C-H *str.*), 1710 (carbonyl *str.*), 1611 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.35 (s, 3H, CH_3), 6.46 (d, 1H, *J* = 5.7 Hz, CH), 7.62 (m, 1H, Ar), 7.88 (d, 1H, *J* = 6.8 Hz, Ar), 8.04 (t, 1H, *J* = 7.34 Hz, pyrazole Ar-H), 10.01 (s, 1H, NH), 12.50 (s, 1H, pyrazole N-H).

3-[(5-Methyl-1*H*-pyrazol-3-yl)imino]-5-nitroindolin-2-one (P16): Yield: 69 %, m.f.: $C_{12}H_9N_5O_3$,m.w. 271.24, m.p. 174-176 ºC. Elemental analysis (found) %: C, 53.14; H, 3.34; N, 25.82; O, 17.70. IR (KBr, ν_{max}, cm^{-1}): 3411 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.), 1510.8, 1401.1 (NO_2 *str.*). ^{1}H NMR (DMSO-d6, 400 MHz) δ: 2.18 (s, 3H, CH_3), 6.44 (d, 1H, *J* = 5.7 Hz, CH), 7.61 (m, 1H, Ar), 7.83 (d, 1H, *J* = 6.6Hz, Ar), 8.01 (t, 1H, *J* = 7.79 Hz, pyrazole Ar-H), 10.01 (s, 1H, NH), 12.11 (s, 1H, pyrazole N-H).

5-Fluoro-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P17): Yield: 70 %, m.f.: $C_{12}H_9N_4OF$, m.w. 244.23, m.p. 169-171 ºC. Elemental Analysis: C, 59.02; H, 3.71; F, 7.78; N, 22.94; O, 6.55. IR (KBr, ν_{max}, cm^{-1}): 3419 (NH *str.*), 2889 (C-H *str.*), 1715 (carbonyl *str.*), 1625 (NH bend.). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.30 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 5.7 Hz, CH), 7.66 (m, 1H, Ar), 7.80 (d, 1H, *J* = 6.8 Hz, Ar), 8.03 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 10.00 (s, 1H, NH), 12.51 (s, 1H, pyrazole N-H).

5-Methoxy-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P18): Yield: 71 %, m.f.: $C_{13}H_{12}N_4O_2$, m.w. 256.28, m.p. 161-163 ºC. Elemental analysis (found) %: C, 60.93; H, 4.72; N, 21.86; O, 12.49. IR (KBr, ν_{max}, cm^{-1}): 3416 (NH *str.*), 2891 (C-H *str.*), 1711 (carbonyl *str.*), 1603 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 6.50 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.20 (m, 1H, Ar-H), 7.70 (d, 1H, *J* = 7.4 Hz, Ar-H), 8.21 (t, 1H, *J* = 7.77 Hz, pyrazole Ar-H), 10.4 (s, 1H, N-H), 12.5 (s, 1H, pyrazole N-H).

1-Methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]indolin-2-one (P19): Yield: 72 %, m.f.: $C_{13}H_{12}N_4O$, m.w. 240.27, m.p. 163-165 ºC. Elemental analysis (found) %: C, 64.99; H, 5.03; N, 23.32; O, 6.66. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2888 (C-H *str.*), 1710 (carbonyl *str.*), 1612 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.46 (s, 3H, CH_3), 6.44 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.20 (d, 1H, *J* = 7.4 Hz, Ar-H), 8.89 (t, 1H, *J* = 7.77Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 12.5 (s, 1H, pyrazole N-H).

5-Chloro-1-methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]-indolin-2-one (P20): Yield: 67 %, m.f.: $C_{13}H_{11}N_4OCl$, m.w. 274.71, m.p. 164-166 ºC. Elemental analysis (found) %: C, 56.84; H, 4.04; Cl, 12.90; N, 20.40; O, 5.82. IR (KBr, ν_{max}, cm^{-1}): 3411 (NH *str.*), 2891 (C-H *str.*), 1712 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.46 (s, 3H, CH_3), 6.45 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.89 (t, 1H, *J* = 6.9 Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 12.5 (s, 1H, pyrazole N-H).

5-Bromo-1-methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)-imino]indolin-2-one (P21): Yield: 65 %, m.f.: $C_{13}H_{11}N_4OBr$, m.w. 319.16, m.p. 165-167 ºC. Elemental analysis (found) %: C, 48.92; H, 3.47; Br, 25.04; N, 17.55; O, 5.01. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2898 (C-H *str.*), 1710 (carbonyl *str.*), 1600 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.46(s, 3H, CH_3), 6.47 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.89 (t, 1H, *J* = 7.71Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 12.5 (s, 1H, pyrazole N-H).

1-Methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]-5-nitro-indolin-2-one (P22): Yield: 68 %, m.f.: $C_{13}H_{11}N_5O_3$, m.w. 285.26, m.p.170-172 ºC. Elemental analysis (found) %: C, 54.74; H, 3.89; N, 24.55; O, 16.83. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.), 1510, 1401 (NO_2 *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (s, 3H, CH_3), 3.46 (s, 3H, CH_3), 6.48 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.89 (m, 1H, Ar-H), 8.89 (t, 1H, *J* = 7.70 Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.51 Hz, Ar-H), 12.5 (s, 1H, pyrazole N-H).

5-Fluoro-1-methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)imino]-indolin-2-one (P23): Yield: 69 %, m.f.: $C_{13}H_{11}N_4OF$, m.w. 258.26, m.p. 165-167 ºC. Elemental analysis (found) %: C, 60.46; H, 4.29; F, 7.36; N, 21.69; O, 6.20. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 6.40 (d, 1H, *J* = 7.90 Hz, Ar-H), 7.88 (m, 1H, Ar-H), 8.88 (t, 1H, *J* = 7.71Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.52 Hz, Ar-H), 12.5 (s, 1H, pyrazole N-H).

5-Methoxy-1-methyl-3-[(5-methyl-1*H*-pyrazol-3-yl)-imino]indolin-2-one (P24): Yield: 66 %, m.f.: $C_{14}H_{14}N_4O$, m.w. 270.23, m.p.163-165 ºC. Elemental analysis (found) %: C, 62.21; H, 5.22; N, 20.73; O, 11.84. IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*), 2890 (C-H *str.*), 1710 (carbonyl *str.*), 1605 (NH bend.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 3.48 (s, 3H, CH_3), 3.77 (s, 3H, O-CH_3), 6.40 (d, 1H, *J* = 7.90 Hz, Ar-H),

7.88 (m, 1H, Ar-H), 8.12 (t, 1H, *J* = 7.71Hz, pyrazole Ar-H), 8.91 (d, 1H, *J* = 7.52 Hz, Ar-H), 12.1 (s, 1H, pyrazole N-H).

The ligand synthesis included 2D-3D conversions, correcting structures, generating variations of these structures, verifying and optimizing the structures. All these tasks were performed using Marvin Sketch (ChemAxon) for drawing, displaying and characterizing chemical structures and sub-structures [12].

Protein preparation: The protocol prepares the proteins by inserting missing atoms in incomplete residues, modeling missing loop regions based on information, deleting alternate conformations, removing waters, standardizing atom names using the Discovery Studio by Accelerys and Auto dock tools 1.5.6.

Anti-inflammatory activity by protein albumin denaturation method [13]: All the synthesized compounds were screened for anti-inflammatory activity by using *in vitro* method reported earlier by Muzushima and Kabayashi with slight modification [14]. Accordingly, inhibition of albumin denaturation technique was studied, a 5 mL reaction mixture was prepared consisting of 0.2 mL of egg albumin, 2.8 mL phosphate buffered saline (pH: 6.4) and 2.0 mL of varying concentration of test compounds so that final concentrations become 25, 50, 100, 200 µg/mL. Similar volume of double distilled water served as control. Then the mixtures were incubated at 37± 2 ºC in an incubator for 15 min and then heated at 70 ºC for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Indomethacin with final concentration of 50 and 100 µg/mL was used as reference drug and treated similarly for determination of absorbance. The percent inhibition of protein denaturation was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

***in vivo* Analgesic activity:** Analgesic activity test was performed following the method of Eddy *et al*. [15]. Analgesic activities of all synthesized compounds were quantified *in vivo* by Eddy's hot plate method using analgesiometer. All the test compounds were suspended in 0.5 % of CMC and administered orally. The albino wistar rats were treated with synthesized derivatives (100 mg/kg, p.o.) and standard drug indomethacin (100 mg/kg, p.o.). The animals were individually placed on the hot plate maintained at 55 ºC, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response. The relative increase in reaction time was measured at an interval of 0, 30, 60 and 90 min in the individual animal of control, test and the standard group (Table-2). The percent increase in reaction time was calculated using following formula:

$$\text{Increase in reaction time (I, \%)} = \frac{I_t - I_o}{I_o} \times 100$$

where, I_t = reaction time at time t and I_o = reaction time at time zero (0 min).

RESULTS AND DISCUSSION

The research work was oriented towards the finding of newer derivatives of isatin with enhance anti-inflammatory and analgesic activities. The different derivatives were synthesized and characterized. The synthesized derivatives showed very good anti-inflammatory and analgesic activities against reported isatin derivatives (Tables 1 and 2). The results obtained from the docking study (Table-3) it was observed that *in silico* study gives good prediction of biological activity which does minimizes the lots of effort to synthesize the library of different derivatives.

TABLE-1
in vitro ANTI-INFLAMMATORY ACTIVITY

S. No.	Compound	Absorbance	Inhibition of denaturation (%)
1	**P01**	0.0402	61.71
2	**P02**	0.0409	61.04
3	**P03**	0.0360	65.71
4	**P04**	0.0473	54.95
5	**P05**	0.0372	64.57
6	**P06**	0.0323	69.23
7	**P07**	0.0366	65.14
8	**P08**	0.0402	61.71
9	**P09**	0.0343	67.33
10	**P10**	0.0368	64.95
11	**P11**	0.0407	61.23
12	**P12**	0.0430	59.04
13	**P13**	0.0270	74.28
14	**P14**	0.0399	62.00
15	**P15**	0.0323	69.23
16	**P16**	0.0402	61.71
17	**P17**	0.0409	61.04
18	**P18**	0.0278	73.52
19	**P19**	0.0473	54.95
20	**P20**	0.0372	64.57
21	**P21**	0.0297	71.71
22	**P22**	0.0366	65.14
23	**P23**	0.0402	61.71
24	**P24**	0.0343	67.33
25	Indomethacin	0.0150	85.71

TABLE-2
in vivo ANALGESIC ACTIVITY

S. No.	Compound	% Increase in reaction Time (min)		
		30	60	90
1	**P01**	20.51	30.76	61.53
2	**P06**	28.20	35.89	43.58
3	**P13**	53.84	71.79	76.92
4	**P18**	58.97	71.79	74.35

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-3
DOCKING ANALYSIS

S. No.	Compound	Amino acid	Bond	Binding energy	Inhibitory constant	Inter molecular energy	Electrostatic energy
1	**P01**	ASN43; ARG44	HN; HN	-6.99	7.51	-7.59	-0.09
2	**P02**	CYS41; CYS41	HO; HO	-6.91	8.66	-7.5	-1.34
3	**P03**	GLU67; TYR55	HO; HO	-5.97	41.94	-6.57	-1.93
4	**P04**	VAL165; LYS166	HN; HN	-2.87	7.88	-3.46	00
5	**P05**	–	–	-5.45	100.69	-6.05	-1.65
6	**P06**	LYS56	HN	-5.43	105.32	-6.32	-2.09
7	**P07**	GLN42	–	-6.14	31.38	-6.74	-0.84
8	**P08**	LYS166	–	-6.4	20.25	-7.00	-0.77
9	**P09**	UNKO	HN	-6.4	20.47	-6.99	-0.64
10	**P10**	VAL165; LYS166	HN; HN	-2.87	7.85	-3.47	00
11	**P11**	GLN42	–	-6.14	31.54	-6.74	-0.49
12	**P12**	GLN54	–	-5.84	52.5	-6.73	-1.62
13	**P13**	ARG44	HH	-7.64	2.49	-8.24	-0.82
14	**P14**	–	–	-5.93	44.72	-6.53	-0.79
15	**P15**	GLN54	HH	-6.74	11.46	-7.34	-1.53
16	**P16**	VAL165; LYS166	HN; HN	-2.88	7.79	-3.47	00
17	**P17**	UNKO; UNKO	HO; HN	-6.67	12.98	-7.26	-1.66
18	**P18**	UNKO	HN	-7.32	4.32	-7.62	-0.21
19	**P19**	TYR55	HO	-6.67	12.94	-7.27	-1.75
20	**P20**	UNKO	NH	-5.69	66.98	-6.29	-1.75
21	**P21**	UNKO	HN	-7.14	5.88	-7.73	-1.81
22	**P22**	VAL165; LYS166	HN; HN	-2.87	7.91	-3.46	00
23	**P23**	GLN54; TYR55	HO; HN	-5.96	42.48	-6.56	-1.54
24	**P24**	MET163; VAL165	HO; HN	-6.89	8.96	-7.78	-0.66
25	Indomethacin	CYS57	NH	-6.31	23.78	-7.5	-0.31

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