Asian Journal of Chemistry; Vol. 35, No. 1 (2023), 217-223

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.24004

Synthesis, Characterization and Molecular Docking Studies of Novel Mannich Bases of Indole Analogs as Potent Antibacterial and Anticancer Agents

V. PADMAJA[1,*], M. SUMAKANTH[1] and P. SHASHIKALA[2]

[1]Department of Pharmaceutical Chemistry, RBVRR Women's College of Pharmacy, Hyderabad-500027, India
[2]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad-500007, India

*Corresponding author: E-mail: pv.oct29@gmail.com

Received: 28 July 2022; *Accepted*: 22 December 2022; *Published online*: 27 December 2022; AJC-21101

A series of a novel indole analogs (5-substituted-1-methyl/ethyl-3-((5-methyl-1-(morpholino/piperazinmethyl)-1*H*-pyrazol-3-yl)-imino)indolin-2-one (**5a-l**) were synthesized *via* Schiff base and Mannich base mechanism. The structures of synthesized compounds were confirmed by IR, ^{1}H NMR and mass spectral data. The antibacterial activity by was measured by agar diffusion method. Some of the analogs (**5b, 5c, 5h, 5i** and **5j**) showed excellent antibacterial recreation against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, *Salmonella paratyphi*, *Pseudomonas* and compounds **5f, 5g**, **5i** showed good activity against MCF-7 cell line through MTT assay method. The molecular docking studies of novel indole analogs revealed that compound **5i** suggests perfect dock rating of -5.826 with glide binding strength of -38.76 Kcal/mol. Dock results of all the compounds were ranged from -5.826 (compound **5i**) to -2.792 (compound **5d**).

Keywords: 5-Methyl-3-amino pyrazole, Antibacterial and Anticancer activities, MCF-7, EGFR.

INTRODUCTION

Indole and its analogs contain one of a kind heterocyclic rings, which are most auspicious elements that found many triumphant pharmacological activities. Many literatures indicated that indole and their derivatives possessed anticancer, antiviral, anthelmintic, analgesic and antibacterial activities [1-3]. Further, many admixtures containing indole and its analogues were mentioned as tremendous anticancer as properly as other biological things to do [4-6]. Nowadays, about 67% of approved drugs contain at least one heterocycle ring with one or two hetero atoms. Among nitrogen containing heterocycles, indole and its analogs are exceedingly interesting compounds for their biological, pharmacological and agricultural applications [7]. The presence of different fused heterocyclic rings linked with indole or isatin help them to display various biological activities. Indole or its analogs like indole-2,3-dione (isatin) with imine linkage systems is a favourable structural element in medicinal chemistry and has wide-ranging appeal in the drug discovery and development process. Indole is also known as benzopyrrole which contains benzene nucleus and has 10π-electrons (two from lone pair on nitrogen and double bonds provide eight electrons), which make them aromatic in nature. Comparably to the benzene ring, an electrophilic substitution occurs readily on indole due to excessive π-electrons delocalization [8,9].

The Mannich bases are also known to possess biological activities such as antimicrobial, antiparasitic, antimalarial, anti-inflammatory, anticancer and anticonvulsant activities [10]. Cancer disease is a most growing problem in contemporary medicine and the use of anticancer drugs is common across the world. Most of the reported synthetic methods have multistep reactions, inherent limitations like limited substrate availability and cohesive chemicals are used. It recommends that the reactions of Mannich base are more attractive with amines and carbonyl compounds. In view of synthetic methods, unique Mannich bases of indole analogs have been synthesized and their antibacterial, anticancer activites were assessed. The molecular docking study in addition facilitated to identify the feas-ible mechanism between the target receptor and synthesized novel Mannich bases of indole analogs. Here in the existing work, a strive has been made to synthesize novel Mannich base of

indole derivatives, with the expectation to achieve new molecules with good pharmacological activities.

EXPERIMENTAL

All the reagents and chemicals have been purchased from commercial carriers and were used as such. Melting points were dedected using Thieles tube apparatus by the usage of liquid paraffin as a solvent. IR spectra were recorded the usage of Thermo-Nicolet Nexus 670 FTIR spectrometer and ^{1}H NMR spectra have been recorded on Bruker DPX-200 MHz NMR spectrometer using DMSO-d_6. Mass spectra had been recorded by the use of Shimadzu LCMS-8030 mass spectrophotometer.

General procedures

Step-I: Synthesis of substituted isatin derivatives from substituted anilines: Chloral hydrate (9 g) was taken into the round bottom flask and dissolved in 120 mL water. Then, sodium sulphate (13 g), a solution of 5.4 g of substituted aniline in 30 mL of water containing 5.12 g of conc. HCl (4.34 mL) to dissolve amine and solution of hydroxylamine hydrochloride (11 g) in 50 mL of water were added. Flask was then heated vigorously for 30-45 min until the reaction was completed which was monitored by TLC. Later, the solution was cooled under running water followed by the filtration of crystallized product with suction pump and air dried [11].

Conc. H_2SO_4 (10 mL) was warmed to 50 ºC and 2.5 g of dry nitrosoacetanilide was added in portion wise manner and keep the reaction temperature between 60-70 ºC. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was completed. The solution was heated to 80 ºC and maintain at this temperature for about 10 min to complete the reaction. The reaction mixture was cooled to room temperature and poured into ten times its volume of crushed ice. After standing for 90 min, the final product was filtered with suction pump followed by thorough washing with cold water to remove excess of sulphuric acid and dried in air.

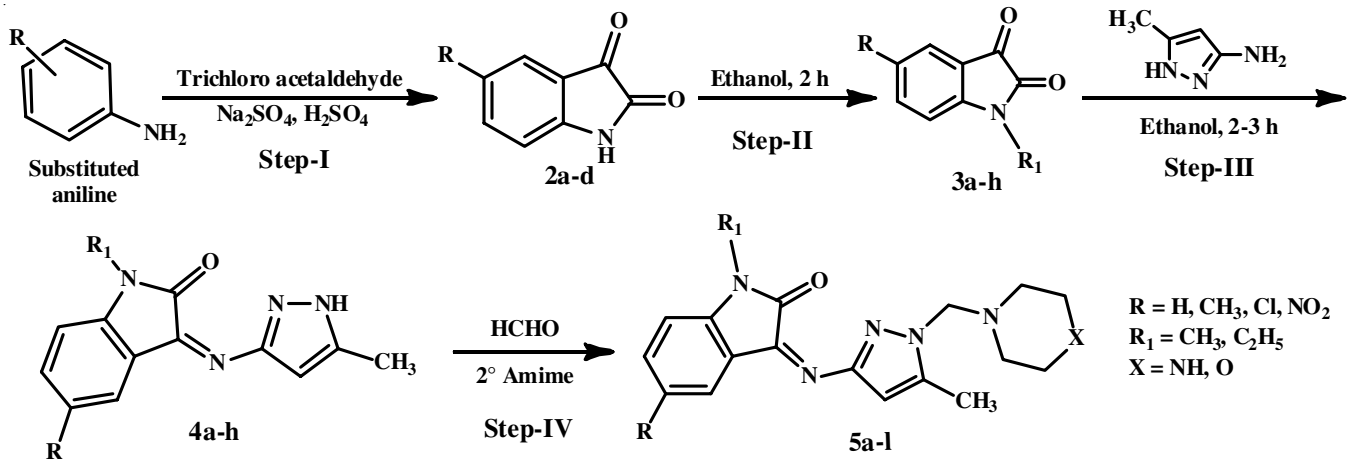
Step-II: Synthesis of *N*-substituted isatin derivatives (3a-d): A 250 mL flask equipped with a magnetic stirring bar was charged with DMF (100 mL) and NaOH (13 mmol). The mixture was stirred at room temperature for 5 min, substituted isatin (10 mmol) was then added and the stirring was continued for 45 min. Alkyl halide (11 mmol) was added to the reaction mixture and the stirring was continued at 80 ºC for 12 h. The mixture was then diluted with water (200 mL), extracted with ethyl acetate and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by recrystallization using ethyl acetate and hexane (1:9) to obain pure N-substituted isatin was obtained [12].

Step-III: 5-Substituted-1-ethyl/methyl-3-((5-methyl-1*H*-pyrazol-3-yl)imino)indolin-2-one (4a-h): A mixture of equimolar quantity of substituted isatin (**3a-d**) (0.01 mol) and 5-methyl-3-amino pyrazole (0.01 mol) was dissolved in 20 mL of ethanol, refluxed for 2-3 h in the presence of few drops of 2 mL glacial acetic acid. The progress of the reaction was monitored by TLC (*n*-hexane:EtOAc 7:3). The reaction mixture was cooled to room temperature overnight to get precipitate, which was filtered off and recrystallized from ethanol [13].

Step-IV: Synthesis of 5-substituted-1-methyl/ethyl-3-((5-methyl-1-(morpholino/piperazino methyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5a-l): Compound (**4a-h**) (10 mmol) dissolved in acetonitrile:dioxane (2:1) at room temperature was added to formaldehyde (37%, 0.5 mL) followed by the dropwise addition of morpholine/piperazine (10 mmol) in ethanol with vigorous stirring. The reaction mixture was stirred at room temperature for 2 h and left overnight [14]. The solid product was filtered and washed with ethanol and recrystallized from acetonitrile:dioxane (2:1) to yield title compound (**Scheme-I**).

1-Methyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5a): IR (KBr, ν_{max}, cm^{-1}): 3009 (C-H *str.,* Ar), 2939, 2839, 2736 (C–H *str.*, aliphatic), 1701 (C=O *str.*, indole), 1636 (-C=N *str.*), 1531 (C=CH *str.*), 1344 (C=C *str.*, Ar), 1046 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 7.79-7.77 (d, 2H, Ar-H, C_4H, C_7H), 7.69-7.67 (s, 1H, Ar-H, C_4H), 7.55-7.54 (t, 2H, Ar-H, C_5H, C_6H), 4.81 (s, 2H, -CH_2-N protones), 3.71 (m, J = 7.1 Hz, 4H in morpholin), 2.91 (m, J = 7.8 Hz, 4H in morpholin), 2.15 (s, 3H, N-CH_3), 1.99 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 339.17 (M), 339.32 (M + 1, 100%).

5-Methyl-1-methyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5b): IR (KBr,



Scheme-I

ν_{max}, cm^{-1}): 3082 (C-H *str.,* Ar), 2903, 2837 (C–H *str.*, aliphatic), 1701 (C=O *str.*, indole), 1616 (-C=N *str.*), 1406 (C=CH *str.*), 1309 (C=C *str.*, Ar), 1053 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 7.94-7.92 (d, 2H, Ar-H, C_4H, C_7H), 7.82 (s, 1H, Ar-H, C_6H), 7.81 (s, 1H, Ar-H C_4H), 4.44-4.40 (s, 2H, -CH_2-N protons), 3.7392 (m, *J* = 6.8 Hz, 4H in morpholin ring), 2.6914 (m, *J* = 6.4 Hz, 4H in morpholin ring), 2.26 (s, 3H, N-CH_3), 1.98 (s, 6H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 353.19 (M), 354.23 (M + 1, 100%).

5-Chloro-1-methyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl) imino)indolin-2-one (5c): IR (KBr, ν_{max}, cm^{-1}): 3090 (C-H *str.,* Ar), 2994, 2894 (C–H *str.*, aliphatic), 1704 (C=O *str.*, indole), 1630 (-C=N *str.*), 1564 (C=CH *str.*), 1273 (C=C *str.*, Ar), 1030 (C-N *str.*), 851 (C-Cl *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.37 (s, 1H, Ar-H, C_6H), 7.95-7.84 (d, 2H, Ar-H, C_4H, C_7H), 7.79 (s, 1H, Ar-H, C_4H), 4.80 (s, 2H, -CH_2-N protons), 3.60 (m, *J* = 8.1 Hz, 4H in morpholin ring), 3.12 (m, *J* = 8.3 Hz, 4H in morpholin ring), 2.08 (s, 3H, N-CH_3), 1.87 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 373.13 (M), 374.21 (M + 1, 100%), 375.43 (M + 2, 30%).

5-Nitro-1-methyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5d): IR (KBr, ν_{max}, cm^{-1}): 3038 (C-H *str*., Ar), 2985, 2845 (C–H *str.*, aliphatic), 1700 (C=O *str.*, indole), 1657 (C-NO_2*str.*), 1549 (-C=N *str.*), 1485 (C=CH *str.*), 1285 (C=C *str.*, Ar), 1025 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 7.84 (s, 1H, Ar-H C_4H), 7.68-7.65 (d, 2H, Ar-H, C_4H, C_7H), 7.63 (s, 1H, Ar-H, C_4H), 4.90 (s, 2H, -CH_2-N protons), 3.85 (m, *J* = 7.3 Hz, 4H in morpholin ring), 2.85 (m, *J* = 6.9 Hz, 4H in morpholin ring), 2.26 (s, 3H, N-CH_3), 2.06 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 384.15 (M), 385.34 (M + 1, 100%).

1-Methyl-3-((5-methyl-1-(piperazin-1-ylmethyl)-1*H*-pyrazol-3-yl))indolin -2-one (5e): IR (KBr, ν_{max}, cm^{-1}): 3364 (-NH *str.*), 3018 (C-H *str.,* Ar), 2959, 2836 (C–H *str.*, aliphatic), 1703 (C=O *str.*, indole), 1591 (-C=N *str.*), 1458 (C=CH *str.*), 1273 (C=C *str.*, Ar), 1056 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 11.46 (s, 1H, NH in piperazine), 7.84 (s, 1H, Ar-H, C_4H), 7.69-7.65 (d, 2H, Ar-H, C_4H, C_7H), 7.63-7.58 (t, 2H, Ar-H, C_6H, C_5H), 4.25-4.23 (s, 2H, -CH_2-N protons), 3.64 (m, *J* = 8.3 Hz, 4H in piperazine ring), 3.07 (m, *J* = 7.9 Hz, 4H in piperazine ring), 2.23 (s, 3H, N-CH_3), 2.07 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 338.19 (M), 339.21 (M + 1, 100%).

5-Chloro-1-methyl-3-((5-methyl-1-(piperazin-1-ylmethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5f): IR (KBr, ν_{max}, cm^{-1}): 3098 (C-H *str.,* Ar), 2967, 2884, 2756 (C–H *str.*, aliphatic), 1712 (C=O *str.*, indole), 1532 (-C=N *str.*), 1498 (C=CH *str.*), 1302 (C=C *str.*, Ar), 1087 (C-N *str.*), 807 (C-Cl *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 7.90 (s, 1H, Ar-H, C_6H), 7.78-7.70 (d, 2H, Ar-H, C_4H, C_7H), 7.47 (s, 1H, Ar-H, C_4H), 4.87-4.80 (s, 2H, -CH_2-N protons), 3.84 (m, *J* = 6.7 Hz, 4H in piperazine ring), 3.09 (m, *J* = 7.4 Hz, 4H in piperazine ring), 2.43 (s, 3H, N-CH_3), 2.10 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 372.15 (M), 373.21 (M + 1, 100%), 374.05 (M + 2, 30%).

5-Nitro-1-methyl-3-((5-methyl-1-(piperazin-1-ylmethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5g): IR (KBr, ν_{max}, cm^{-1}): 3289 (-NH *str.*), 3084 (C-H *str.,* Ar), 2977, 2867, 2739 (C–H *str.*, aliphatic), 1721 (C=O *str.*, indole), 1643 (-N-NO_2 *str.*), 1565 (-C=N *str.*), 1489 (C=CH *str.*), 1298 (C=C *str.*, Ar), 1102 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.04 (s, 1H, Ar-H, C_6H), 7.98-7.89 (d, 2H, Ar-H, C_4H, C_7H), 7.54 (s, 1H, Ar-H, C_4H), 4.67-4.39 (s, 2H, -CH_2-N protons), 3.78 (m, *J* = 7.9 Hz, 4H in piperazine ring), 2.98 (m, *J* = 7.6 Hz, 4H in piperazine ring), 2.1293 (s, 3H, N-CH_3), 2.0093 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 383.17 (M), 384.21 (M + 1, 100%).

5-Methyl-1-methyl-3-((5-methyl-1-(piperazin-1-yl-methyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5h): IR (KBr, ν_{max}, cm^{-1}): 3384 (-NH *str.*), 3076 (C-H *str.,* Ar), 2987, 2852, 2743 (C–H *str.*, aliphatic), 1709 (C=O *str.*, indole), 1603 (-C=N *str.*), 1521 (C=CH *str.*), 1298 (C=C *str.*, Ar), 1092 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.01 (s, 1H, Ar-H, C_6H), 7.89-7.79 (d, 2H, Ar-H, C_4H, C_7H), 7.67 (s, 1H, Ar-H, C_4H), 4.55-4.43 (s, 2H, -CH_2-N protons), 3.66 (m, *J* = 8.3 Hz, 4H, -CH_2 in piperazine ring), 3.09 (m, *J* = 7.6 Hz, 4H, -CH_2 in piperazine ring), 2.08 (s, 3H, N-CH_3), 1.99 (s, 3H, CH_3 in pyrazole). Mass (LC-MS): *m/z* 352.20 (M), 353.03 (M + 1, 100%).

5-Methyl-1-ethyl-3-((5-methyl-1-(piperazin-1-yl-methyl)-1*H*-pyrazol-3-yl) imino)indolin-2-one (5i): IR (KBr, ν_{max}, cm^{-1}): 3289 (-NH *str.*), 3076 (C-H *str.,* Ar), 2965, 2893, 2761 (C–H *str.*, aliphatic), 1710 (C=O *str.*, indole), 1605 (-C=N *str.*), 1534 (C=CH *str.*), 1287 (C=C *str.*, Ar), 1105 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.15 (s, 1H, Ar-H, C_6H), 7.94-7.88 (d, 2H, Ar-H, C_4H, C_7H), 7.56 (s, 1H, Ar-H, C_4H), 4.49-4.30 (s, 2H, -CH_2-N protones), 3.77 (m, *J* = 8.3 Hz, 4H in piperazine ring), 2.99 (m, *J* = 8.1 Hz, 4H in piperazine ring), 2.84 (q, 2H, N-CH_2-), 2.00 (s, 3H, CH_3 in pyrazole), 1.98 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 366.22 (M), 367.19 (M + 1, 100%).

5-Chloro-1-ethyl-3-((5-methyl-1-(piperazin-1-yl-methyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5j): IR (KBr, ν_{max}, cm^{-1}): 3249 (-NH *str.*), 3054 (C-H *str.,* Ar), 2999, 2877, 2751 (C–H *str.*, aliphatic), 1723 (C=O *str.*, indole), 1621 (-C=N *str.*), 1565 (C=CH *str.*), 1253 (C=C *str.*, Ar), 1092 (C-N *str.*), 812 (C-Cl *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.09 (s, 1H, Ar-H, C_6H), 7.78-7.69 (d, 2H, Ar-H, C_4H, C_7H), 7.38 (s, 1H, Ar-H, C_4H), 4.66-4.54 (s, 2H, -CH_2-N protones), 3.89 (m, *J* = 8.5 Hz, 4H in piperazine ring), 3.09 (m, *J* = 8.5 Hz, 4H in piperazine ring), 2.99 (q, 2H, N-CH_2-), 2.23 (s, 3H, CH_3 in pyrazole), 2.09 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 386.16 (M), 387.32 (M + 1, 100%), 388.17 (M +2, 30%).

1-Ethyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5k): IR (KBr, ν_{max}, cm^{-1}): 3043 (C-H *str.,* Ar), 2967, 2839, 2778 (C–H *str.*, aliphatic), 1718 (C=O *str.*, indole), 1603 (-C=N *str.*), 1589 (C=CH *str.*), 1248 (C=C *str.*, Ar), 1078 (C-N *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz) δ, ppm: 8.28 (s, 1H, Ar-H, C_6H), 8.02-8.00 (d, 2H, Ar-H, C_4H, C_7H), 7.67 (s, 1H, Ar-H C_4H), 4.88-4.78 (s, 2H, -CH_2-N protons), 3.98 (m, *J* = 8.5 Hz, 4H in morpholin ring), 3.10 (m, *J* = 8.1 Hz, 4H in morpholin ring), 2.56 (q, 2H, N-CH_2-), 2.19 (s, 3H, CH_3 in pyrazole), 2.00 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 386.16 (M), 387.32 (M + 1, 100%), 388.17 (M +2, 30%).

5-Chloro-1-ethyl-3-((5-methyl-1-(morpholinomethyl)-1*H*-pyrazol-3-yl)imino)indolin-2-one (5l): 3071 (C-H *str.,* Ar), 2985, 2846, 2790 (C–H *str.*, aliphatic), 1715 (C=O *str.*, indole), 1610 (-C=N *str.*), 1571 (C=CH *str.*), 1302 (C=C *str.*,

Ar), 1102 (C-N *str.*), 798 (C-Cl *str.*). ^{1}H NMR (DMSO-d_6, 200 MHz): δ 8.39 (s, 1H, Ar-H, C_6H), 7.99-7.90 (d, 2H, Ar-H, C_4H, C_7H), 7.65 (s, 1H, Ar-H, C_4H), 4.76-4.57 (s, 2H, -CH_2-N protones), 3.87 (m, *J* = 7.8 Hz, 4H in morpholin ring), 3.21 (m, *J* = 7.9 Hz, 4H in morpholin ring), 2.87 (q, 2H, N-CH_2-), 2.30 (s, 3H, CH_3 in pyrazole), 1.90 (t, 3H, -CH_3). Mass (LC-MS): *m/z* 387.15 (M), 388.04 (M + 1, 100%), 389.18 (M +2, 30%).

Pharmacological activity

Antibacterial activity: All the synthesized Mannich bases of indole analogs (5-substituted-1-methyl/ethyl-3-((5-methyl-1-(morpholino/piperazin methyl)-1*H*-pyrazol-3-yl)iminoindolin-2-one (**5a-l**) were screened for antimicrobial activity by agar diffusion (cup plate) method. This activity was assessed against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive) and *Escherichia coli*, *Salmonella paratyphi*, *Pseudomonas* (Gram-negative). The bacterial strains were cultured overnight at 37 ºC in Mueller-Hinton broth, the yeast was cultured overnight at 30 ºC in YEPDE agar for antibacterial tests. Test strains were marked in nutrient agar to give an ending density of 5 × 10^{-5} cfu/mL. All the plates were incubated at 37 ± 2 ºC for 24-48 h and finally determined the zone of inhibition. The results were evaluated by comparing the zone of inhibition shown by the synthesized compounds with standard drug streptomycin [15,16].

Anticancer activity: All the synthesized indole analogs cell viability was evaluated by the MTT assay with three independent experiments with six concentrations in triplicates. Cells were trypsinized and performed the trypan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 × 10^3 cells/well in 100 µL media in 96-well plate culture medium and incubated overnight at 37 ºC. Then take off the old media and added fresh media 100 µL with different concentrations of synthesized analogs in labelled wells in 96 plates after the incubation. After 48 h, discard the drug solution and the fresh media with MTT solution (0.5 mg/mL) was added to each well, plates were incubated at 37 ºC for 3 h. Finally, at end of incubation time, precipitates were formed as a result of the reduction of the MTT salt to chromophore formosan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO solvent was measured at 570 nm on a microplate reader. The % growth inhibition was calculated using standard formula and concentration of title compounds needed to inhibit cell growth by 50% values (IC_{50}) was generated from the dose- response curves for MCF-7 cell line using origin software [17,18].

Molecular docking: The synthesized Mannich bases of indole derivatives were docked into active site of the target. The epidermal growth factor receptor (EGFR) was retrieved from the Protein databank with PDB Id: 1M17 and the structure was optimized by deleting unbound water molecules which are over 1 Å, adding hydrogen atoms to satisfy the valencies, adding missing amino acids to stabilize side chains and energy of the whole structure was minimized using OPLS-2005 force field using Protein Preparation Wizard tool of Schrödinger Suite [19,20].

RESULTS AND DISCUSSION

The synthesized novel Mannich bases of indole derivatives (**5a-l**) were successfully prepared and their stuctures were well elucidated on the basis of FT-IR, ^{1}H NMR and mass spectral data. In this synthetic process, a series of novel Mannich bases of indole derivatives have been synthesized using substituted isatin with alkyl halides to obtain *N*-substituted isatin derivatives which further treated with 5-methyl-3-amino pyrazole to give Schiff bases. Further on, using Mannich base reaction with piperazine or morpholine, novel Mannich bases of indole analogs were obtained (**Scheme-I**).

The key IR bands in the synthesized compounds exhibit the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3000-3098 cm^{-1} and 2910-2732 cm^{-1}, respectively. Also, all compounds have showed a strong absorption in the region of 1725-1700 cm^{-1} confirmed the presence of C=O stretching frequency. Similarly, in most of the compounds, the C-C stretching of the aromatic ring was around in 1545-1535 cm^{-1} region. The Ar-Cl stretching showed the strong absorption in the region 825-790 cm^{-1}, whereas some compounds containing -NO_2 group exhibited the stretching peak at around 1650-1625 cm^{-1} respectively. The ^{1}H NMR (DMSO-d_6) spectra of the indole derivatives show a singlet at δ 4.344-4.6754 ppm for methylene (N-CH_2-N) protons. Whereas some compounds showed triplet at δ 3.40-3.87 ppm for -CH_2 in morpholine ring. All the synthesized compounds have aromatic protons between δ 8.65-6.80 ppm as singlet, doublet and triplet protons.

Antibacterial activity: The antibacterial activity of the synthesized compounds was performed by cup plate method. The streptomycin drug was used as a standard against *S. aureus*, *B. subtilis* (Gram-positive) and *S. paratyphi*, *Pseudomonas* , *E. coli* (Gram-negative). Most of the synthesized compounds (**5b, 5c**, **5h, 5i** and **5j**) showed significant antibacterial activity. It is interesting to observe that the presence of electron withdrawing group –Cl in the aromatic ring (**5c, 5j**) exhibited better activity against *Bacillus subtilis* (Table-1).

Anticancer activity: The IC_{50} values of the synthesized compounds were found to be in the range of 0.05 µM to 0.19 µM against the MCF7 cell lines (Table-2). However, compounds **5f** (0.07 µM), **5g** (0.09 µM), **5i** (0.05 µM) showed the best potency towards the MCF-7 cell lines, while rest of the compounds exhibit moderate anticancer activity. These results indicated that the most activity compounds (**5f** and **5i**) are those with pyrazol-3-yl)imino)indolin-2-one skeleton and the presence of hydrophobic and electron withdrawing substituent (–Cl, –NO_2, -C_2H_5) in the aromatic moiety.

Docking studies: All the synthesized molecules had been docked in a phased manner the usage of XP mode. Glide dock ratings of the dataset ligands have been docked and the results are shown in Table-3. Among the docked ligands, compound **5i** said best possible dock score of -5.826 with Glide binding power of -38.76 Kcal/mol. Docking scores of all the compounds ranged from -5.826 (compound **5a**) to -2.792 (compound **5d**) and also halogen bond used to be discovered between compound **5c** and LYS 721 (Fig. 1). The docking results confirmed that van der Waals, electrostatics and desolvation energies play a

TABLE-1
ANTIBACTERIAL DATA OF SYNTHESIZED MANNICH BASES OF INDOLE ANALOGS (**5a-l**)

Compound	m.f.	R	R_1	X	Zone of inhibition (mm)				
					Staphylococcus aureus	*Bacillus subtilis*	*E. coli*	*Pseudomonas*	*Salmonella paratyphi*
5a	$C_{18}H_{21}N_5O_2$	H	H	O	14	14	13	9	15
5b	$C_{19}H_{23}N_5O_2$	CH_3	CH_3	O	13	18	12	**23***	15
5c	$C_{18}H_{20}N_5O_2Cl$	Cl	CH_3	O	10	**24***	10	12	12
5d	$C_{18}H_{20}N_6O_4$	NO_2	CH_3	O	16	10	16	10	13
5e	$C_{18}H_{22}N_6O$	H	CH_3	NH	12	14	10	16	09
5f	$C_{18}H_{21}N_6OCl$	Cl	CH_3	NH	19	13	12	10	14
5g	$C_{18}H_{21}N_7O_3$	NO_2	CH_3	NH	12	12	15	15	09
5h	$C_{19}H_{24}N_6O$	CH_3	CH_3	NH	09	15	12	**20***	**25***
5i	$C_{20}H_{26}N_6O$	CH_3	C_2H_5	NH	12	**21***	19	16	10
5j	$C_{19}H_{23}N_6OCl$	Cl	C_2H_5	NH	13	13	16	19	**23***
5k	$C_{19}H_{23}N_5O_2$	H	C_2H_5	O	12	9	12	10	13
5l	$C_{19}H_{22}N_5O_2Cl$	Cl	C_2H_5	O	16	16	14	15	17
Streptomycin	$C_{21}H_{39}N_7O_{12}$	–	–	–	30	32	32	29	32

TABLE-2
CYTOTOXIC ACTIVITY OF INDOLE DERIVATIVES ON MCF-7 CELLS

Sample description	Test parameters IC_{50} (μM)
5b	0.16
5c	0.13
5f	0.07
5g	0.09
5h	0.19
5i	0.05
Doxorubicin	0.02

key role in the binding. These elements are considered for designing new inhibitors for EGFR.

Conclusion

In this work, some Mannich bases of indole derivatives (**5a-l**) were synthesized and evaluated for antibacterial and anticancer activities. Compounds **5f**, **5g** and **5i** exhibited significant biological activities. Moreover, compound **5i** with highest dock score of -5.826 with Glide binding energy of -38.76 Kcal/mol and IC_{50} value of 0.05 μM. Due to the excellent biological importance, these novel indole derivatives containing pyrazole moiety with the assumption that these molecules might have more potential.

ACKNOWLEDGEMENTS

The authors thank Synteny Lifesciences Pvt. Ltd. for conducting the biological activities.

CONFLICT OF INTEREST

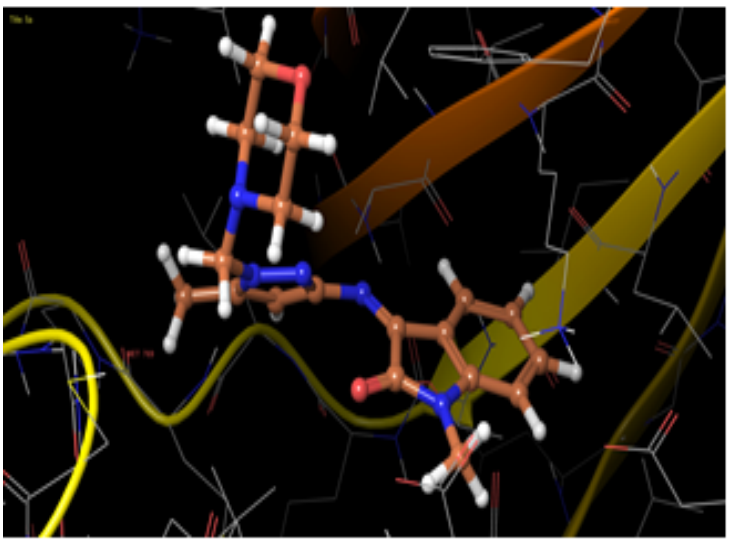
The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

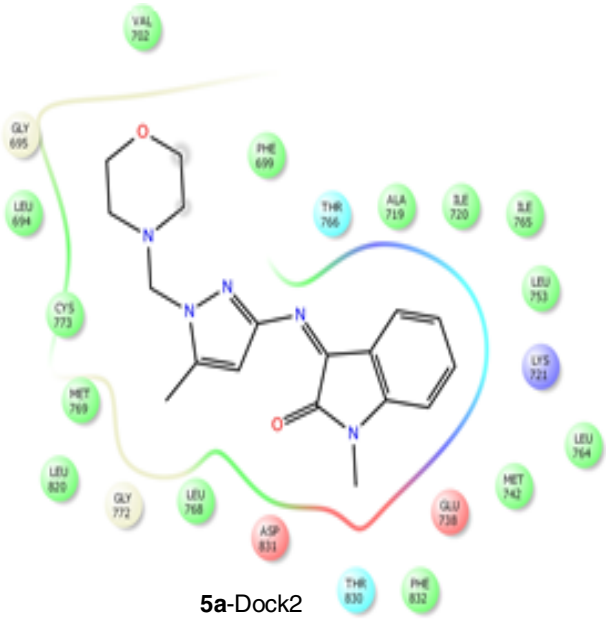
1. T.P. Singh and O.M. Singh, *Mini Rev. Med. Chem.*, **18**, 9 (2018); https://doi.org/10.2174/1389557517666170807123201
2. Y. Ouyang, J. Li, X. Chen, X. Fu, S. Sun and Q. Wu, *Biomolecules*, **11**, 894 (2021); https://doi.org/10.3390/biom11060894
3. B. Salehi, C. Quispe, I. Chamkhi, N. El-Omari, A. Balahbib, J. Sharifi-Rad, A. Bouyahya, M. Akram, M. Iqbal, A.O. Docea, C. Caruntu, G. Leyva-Gómez, A. Dey, M. Martorell, D. Calina, V. López and F. Les, *Front. Pharmacol.*, **11**, 592654 (2021); https://doi.org/10.3389/fphar.2020.592654
4. M. Kucerova-Chlupacova, V. Vyskovska-Tyllova, L. Richterova-Finkova, J. Kunes, V. Buchta, M. Vejsova, P. Paterova, L. Semelkova, O. Jandourek and V. Opletalova, *Molecules*, **21**, 1421 (2016); https://doi.org/10.3390/molecules21111421
5. M. Bhat, G.K. Nagaraja, P. Divyaraj, N. Harikrishna, K.S.R. Pai, S. Biswas and S.K. Peethamber, *RSC Adv.*, **6**, 99794 (2016); https://doi.org/10.1039/c6ra22705h
6. P. Kavitha and K. Laxma Reddy, *Arab. J. Chem.*, **9**, 640 (2016); https://doi.org/10.1016/j.arabjc.2013.06.018

TABLE-3
In silico EGFR INHIBITION OF NOVEL INDOLE DERIVATIVES-GLIDE DOCK SORES OF THE DATASET LIGANDS

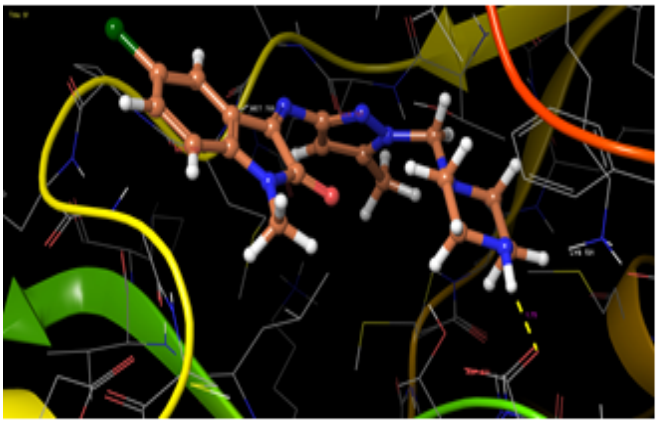
Compound	Dock score XP Gscore	No. of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy	Glide energy
5i	-5.826	1	ASP 831	1.76	-51.346	-38.760
5g	-5.687	1	ASP 831	1.75	-55.291	-38.498
5f	-5.642	1	ASP 831	1.75	-51.123	-37.630
5a	-5.429	0	–	–	-47.409	-39.615
5c	-5.009	1	MET 769	1.86	-48.272	-38.808
5k	-4.924	0	–	–	-47.994	-32.832
5l	-4.163	1	LYS 721	2.09	-59.622	-41.751
5d	-2.792	2	MET 769, LYS 721	2.05, 1.82	-61.327	-41.432
Doxorubicin	-6.781	4	LYS 721, ASP 831, CYS 773, ASP 776	2.14, 1.94, 1.93, 1.85	-55.203	-46.358



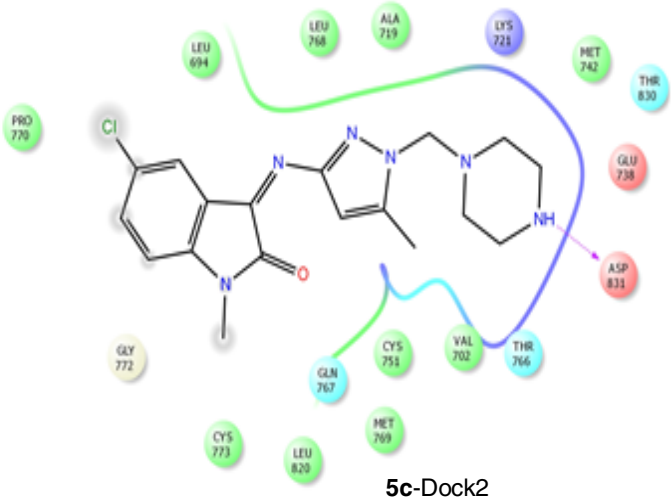
5a-Dock1



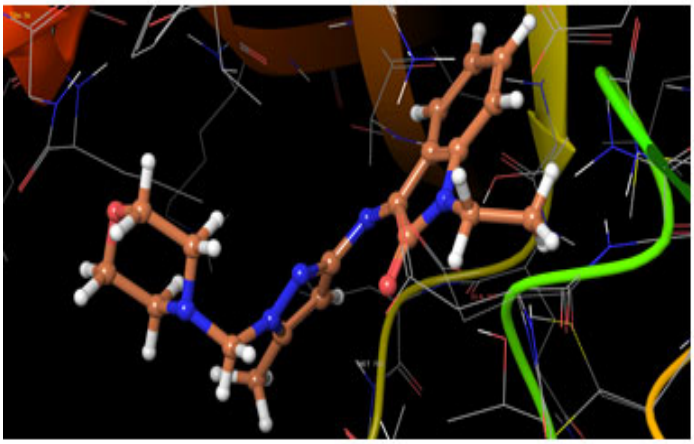
5a-Dock2



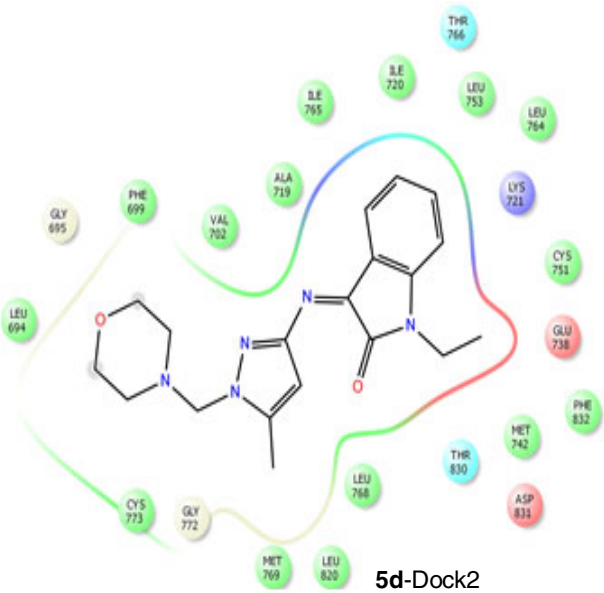
5c-Dock1



5c-Dock2



5d-Dock1



5d-Dock2

Fig. 1. Docking pose between the ligand and the protein (Dock1 and Dock-2)-compound **5a**, **5c** and **5d**

7. H. Keypour, M. Mahmoudabadi, A. Shooshtari, M. Bayat, E. Soltani, R. Karamian and S.H.M. Farida, *Chem. Data Collect.*, **26**, 100354 (2020); https://doi.org/10.1016/j.cdc.2020.100354
8. D.-H. He, Y.-C. Zhu, Z.-R. Yang and A.-X. Hu, *J. Chin. Chem. Soc.*, **56**, 268 (2009); https://doi.org/10.1002/jccs.200900039
9. W.R. Tully, C.R. Gardner, R.J. Gillespie and R. Westwood, *J. Med. Chem.*, **34**, 2060 (1991); https://doi.org/10.1021/jm00111a021
10. S. Bala, N. Sharma, A. Kajal, S. Kamboj and V. Saini, *Int. J. Med. Chem.*, **2014**, 191072 (2014); https://doi.org/10.1155/2014/191072
11. R. Gudipati, R.N. Reddy Anreddy and S. Manda, *J. Enzyme Inhib. Med. Chem.*, **26**, 813 (2011); https://doi.org/10.3109/14756366.2011.556630
12. P. Saluja, J.M. Khurana, K. Nikhil and P. Roy, *RSC Adv.*, **4**, 34594 (2014); https://doi.org/10.1039/C4RA02917H
13. R. Soman, S. Sujatha and C. Arunkumar, *J. Fluor. Chem.*, **163**, 16 (2014); https://doi.org/10.1016/j.jfluchem.2014.04.002
14. L.M. Aroua, *Asian J. Chem.*, **32**, 1266 (2020); https://doi.org/10.14233/ajchem.2020.22472
15. A. Padmaja, T. Payani, G.D. Reddy and V. Padmavathi, *Eur. J. Med. Chem.*, **44**, 4557 (2009); https://doi.org/10.1016/j.ejmech.2009.06.024
16. M. Prabhaharan, A.R. Prabakaran, S. Gunasekaran and S. Srinivasan, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **123**, 392 (2014); https://doi.org/10.1016/j.saa.2013.12.056
17. J.B. Foresman and M. Frisch, Exploring Chemistry with Electronic Structure Methods, Gaussian Inc., Pittsburgh, edn 2 (1996).
18. W.S. Hamama, M.A. Gouda, M.H. Badr and H.H. Zoorob, *Med. Chem. Res.*, **22**, 3556 (2013); https://doi.org/10.1007/s00044-012-0336-z
19. B.V. Subrahmanya, L.Y.R. Prasad and A.B. Shaik, *Indian J. Pharm. Educ. Res.*, **51(4S)**, S679 (2017); https://doi.org/10.5530/ijper.51.4s.99
20. R. Kikkawa, I. Hatanaka, H. Yasuda, N. Kobayashi, Y. Shigeta, H. Terashima, T. Morimura and M. Tsuboshima, *Diabetologia*, **24**, 290 (1983); https://doi.org/10.1007/BF00282716