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Synthesis, Molecular Docking Studies, Antimicrobial, Anticancer and Antioxidant Activity of Some Novel Mannich Bases of Isatin Scaffold

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A series of novel Mannich bases of isatin derivatives (**VIIIa-VIIIt**) was synthesized and evaluated as potential antimicrobial, antioxidant, anticancer activities and molecular docking studies. Structure of all the isatin derivatives was evaluated by IR, ^{1}H NMR and mass spectral analysis. The antimicrobial activity results indicated that compounds **VIIIb**, **VIIIi**, **VIIIm** and **VIIIo** showed good activity in comparison to the activities of the standard molecules. Further, all isatin derivatives (**VIIIa-VIIIt**) have studied for their antioxidant activity by using ferric reducing antioxidant power assay (FRAP) method. Most of the synthesized compounds exhibited the significant antioxidant activities. The anticancer activity results suggested that the isatin derivatives **VIIIm** and **VIIIg** show the more activity against MCF-7 cells in comparison with doxorubicin as standard drug. Furthermore, the molecular docking studies of Mannich bases of isatin derivatives showed good agreement with the biological results when their binding pattern and affinity towards the active site of EGFR was inquisition.

Keywords: Iastin, Benzaldehyde, Ascorbic acid, Antimicrobial Antioxidant activity, Anticancer activity, Molecular docking.

INTRODUCTION

Isatin and its derivatives contain heterocyclic moieties, which are one of the most auspicious component that yielded many victorious biological drugs. Literature indicated that isatin and their substituted cyclic derivatives possessed antimicrobial, anticancer, antiviral, anthelmintic, analgesic, antihypertensive and vasodilating activities [1-3]. Furthermore, it was reported that many admixtures containing indole-2-one moiety possessed significant anticancer as well as other biological activities too [4-6]. Indole-2-one moiety fulfilled the minimum structural requirements which are habitual for the anticancer derivatives. The hetero atoms like nitrogen and sulfur containing heterocyclic rings are appraising as extraordinarily important, unique and valuable sources for prosper the new biological entities [7].

Mannich bases are also known to possess pharmacological activities like anti-inflammatory [8], anticancer [9] antimicrobial [10], antitubercular [11], antiparasitic [12], antimalarial [13], antiviral [14], fungicidal activities [15] anticonvulsant and anti-inflammatory activities. Indole and its derivatives like isatin are the class of heterocyclic moieties that have capability special attention because it belongs to a group of substances with activity in medicinal chemistry [16].

So, proportion to this investigation and in continuance of our heterocyclic synthesis of novel active compounds against some carcinoma cell lines, we aimed to synthesize novel Mannich derivatives of indole-2-one such as isatin compounds as promising anticancer agents towards a cell lines like breast cell lines and human ovary cancer cell line MCF7 using the MTT colorimetric test [17,18]. Build upon their molecular docking studies *via* Schiff and Mannich bases reaction mechanism of isatin and substituted isatin derivatives. The molecular docking investigation has been carried out in order to locate the viable protein ligand interactions of the dataset ligands and every docked complicated was once generated *via* Glide XP module [19,20].

EXPERIMENTAL

The innovation of isatin Mannich derivatives was screened for antimicrobial, anticancer and antioxidant activities. Fourier

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Transform IR spectrometer (Shimadzu 8700) in the range of 4000-400 cm^{-1} using KBr pellets. ^1H NMR spectra were recorded on DPX-200 MHz NMR spectrometer exploiting DMSO-d_6 and chemical shifts (δ) are prevalent in parts per million downfield from internal reference tetramethylsilane (TMS). The mass spectra were compiled on the mass spectrophotometer (Shimadzu) by LC-MS and the spectra were interpreted. Precoated silica gel G plates were used to observe the progress of reaction as well as to assessment the purity of the compounds using *n*-hexane:ethyl acetate (7:3) [21].

2D buildings of the compounds have been transformed to 3D the use of manageable algorithms and software of excessive environment friendly pressure fields. Initial geometrical optimization and strength minimization of molecules have been carried out through the the Ligprep device of Schrödinger suite. Various ionization states have been generated the usage of Ligprep module the use of exceptional software EPIK alongside with quite a number achievable conformers and tautomer's.

General procedures

Synthesis of nitrosoacetanilide from aniline (Step-1): Chloral hydrate (9 g) dissolved in 120 mL water was added to 13 g of sodium sulphate, Then, a solution of 5.4 g of aniline in 30 mL of water containing 5.12 g of conc. HCl (4.34 mL) to dissolve the amine and solution of 11 g of hydroxylamine hydrochloride in 50 mL of water were added. The round bottom flask was then heated vigorously until the reaction was completed. After it, the solution containing beaker was cooled in running water followed by the filtration of the crystallized product with suction pump and air dried.

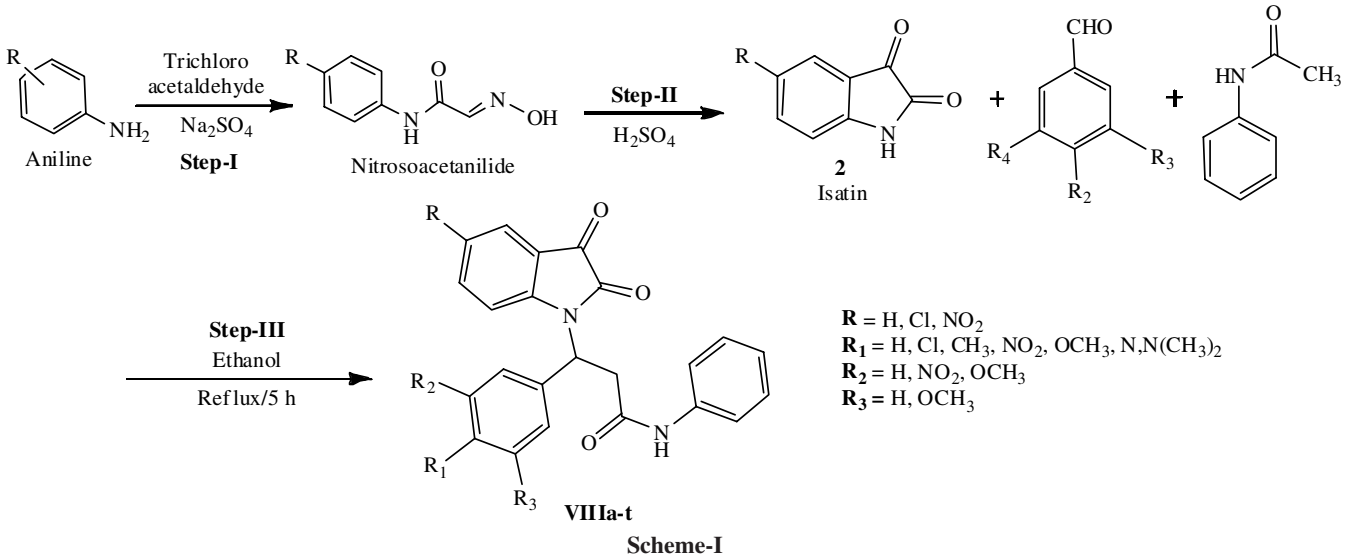
Synthesis of substituted isatin from nitrosoacetanilide (Step-2): Concentrated sulphuric acid (10.0 mL) was warmed to 50 ºC and 2.5 g of dry nitrosoacetanilide was added in such a rate so as to keep the temperature between 60-70 ºC. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound. The solution was heated to 80 ºC and kept at this temperature for about 10 min to complete the reaction. Then the reaction mixture was cooled to room temperature and poured it into 10 times its volume of ice. After standing for 90 min, the final product was filtered with suction pump followed by washing with cold water to remove sulphuric acid and dried in air.

Synthesis of novel Mannich base of isatin derivatives VIII(a-t) (Step-3): A reaction mixture of substituted benzaldehyde (0.01 mol), isatin (0.01 mol) and phenylacetamide (0.01 mol) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture was cooled and poured into the ice-cold water. The precipitate was collected by filtration, dried and recrystallized from absolute ethanol. The above procedure was followed by all the remaining compounds (**Scheme-I**).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-*N*-phenylpropanamide (VIIIa): Yield: 86%, m.p.: 216-217 ºC; m.f.: $C_{23}H_{18}N_2O_3$, IR (KBr, ν_{max}, cm^{-1}): 3403 (NH *str.*, -CO-NH-), 3042 (C-H *str.*, Ar), 2998, 2890 (C–H *str.*, aliph.), 1710 (C=O *str.*, indole), 1697 (C=O *str.*, amide), 1565 (C=CH *str.*), 1498 (C=C *str.*, Ar), 1090 (C-N *str.*). ^1H NMR (DMSO) δ ppm: 10.793 (s, 1H, acetamide), 7.993-7.881 (d, 2H, Ar-H), 7.695-7.639 (d, 2H, Ar-H), 7.636-7.568 (t, 3H, Ar-H), 7.489-7.438 (t, 3H, Ar-H), 7.437 (d, 2H, Ar-H), 7.395-7.300 (t, 2H, Ar-H), 4.274-4.283 (s, 2H, $-CH_2-$), 2.264 (s, 1H, -CH-). Mass (LC-MS): *m/z* 370 (M), 371 (M + 1, 100%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-*N*-phenylpropanamide (VIIIb): Yield: 79%, m.p.: 229-231 ºC; m.f.: $C_{23}H_{17}N_2O_3Cl$, IR (KBr, ν_{max}, cm^{-1}): 3405 (NH *str.*, -CO-NH-), 3038 (C-H *str.*, Ar), 2998, 2917, 2874 (C–H *str.*, aliph.), 1716 (C=O *str.*, indole), 1644 (C=O *str.*, amide), 1555 (C=CH *str.*), 1432 (C=C *str.*, Ar), 985 (C-N *str.*), 809 (Cl *str.*, Ar-Cl). ^1H NMR (DMSO) δ ppm: 10.930 (s, 1H, acetamide), 7.979-7.801 (d, 2H, Ar-H), 7.687-7.520 (d, 2H, Ar-H), 7.489-7.448 (t, 2H, Ar-H), 7.433-7.387 (d, 2H, Ar-H), 6.762 (t, 1H, Ar-H), 6.583 (d, 2H, Ar-H), 6.202-6.109 (t, 2H, Ar-H), 4.183-4.143 (s, 2H, $-CH_2-CO$), 2.284-2.169 (s, 1H, -CH-). Mass (LC-MS): *m/z* 404 (M), 405 (M + 1, 100%), 406 (M + 2, 30%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-dimethylamino)-*N*-phenyl propanamide (VIIIc): Yield: 81 %; m.p.: 168-170 ºC; m.f.: $C_{24}H_{22}N_2O_5$, IR (KBr, ν_{max}, cm^{-1}): 3406 (NH *str.*, -CO-NH-), 3096 (C-H *str.*, Ar), 2960, 2895 (C–H *str.*, aliph.), 1710 (C=O *str.*, indole), 1669 (C=O *str.*, amide), 1514



Scheme-I

(C=CH *str.*), 1434 (C=C *str.*, Ar), 1058 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.013 (s, 1H, acetamide), 7.977-7.891 (d, 2H, Ar-H), 7.877-7.848 (d, 2H, Ar-H), 7.798-7.781 (d, 2H, Ar-H), 7.698-7.682 (d, 2H, Ar-H), 7.606-7.583 (t, 2H, Ar-H), 7.512-7.180 (t, 2H, Ar-H), 4.289-4.248 (s, 2H, -CH_2-CO), 2.528-2.500 (s, 6H, -$N(CH_3)_2$, 2.084-2.069 (s, 1H, -CH-). Mass (LC-MS): *m/z* 430 (M), 431 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-dimethoxy)-*N*-phenyl propanamide (VIIId): Yield: 81 %; m.p.: 231-233 ºC; m.f.: $C_{24}H_{20}N_2O_4$, IR (KBr, ν_{max}, cm^{-1}): 3424 (NH *str.*, -CO-NH-), 3083 (C-H *str.*, Ar), 2978, 2890 (C–H *str.*, aliphatic), 1701 (C=O *str.*, indole), 1678 (C=O *str.*, amide), 1523 (C=CH *str.*), 1421 (C=C *str.*, Ar), 1063 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.564 (s, 1H, acetamide), 7.930-7.903 (d, 2H, Ar-H), 7.794-7.701 (d, 2H, Ar-H), 7.683-7.589 (d, 2H, Ar-H), 7.437-7.328 (d, 2H, Ar-H), 7.298-7.109 (t, 2H, Ar-H), 7.042-7.001 (t, 2H, Ar-H), 4.453-4.290 (s, 2H, -CH_2-CO), 3.586-3.854 (s, 3H, -OCH_3, 2.010-2.146 (s, 1H, -CH-). Mass (LC-MS): *m/z* 400 (M), 401 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-hydroxy)-*N*-phenyl propanamide (VIIIe): Yield: 75 %; m.p.: 223-225 ºC; m.f.: $C_{23}H_{18}N_2O_4$, IR (KBr, ν_{max}, cm^{-1}): 3640 (OH *str.*, Ar-OH), 3420 (NH *str.*, -CO-NH-), 3091 (C-H *str.*, Ar), 2953, 2872 (C–H *str.*, aliph.), 1712 (C=O *str.*, indole), 1431 (C=C *str.*, Ar), 1082 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.532 (s, 1H, acetamide), 8.231-8.210 (d, 2H, Ar-H), 8.012-8.001 (d, 2H, Ar-H), 7.983-7.892 (d, 2H, Ar-H), 7.683-7.602 (d, 2H, Ar-H), 7.431-7.235 (t, 2H, Ar-H), 7.271-7.190 (t, 2H, Ar-H), 4.402-4.400 (s, 2H, -CH_2-CO), 2.172-2.102 (s, 1H, -CH-). Mass (LC-MS): *m/z* 386 (M), 387 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-nitro)-*N*-phenylpropanamide (VIIIf): Yield: 79 %; m.p.: 271-273 ºC; m.f.: $C_{23}H_{17}N_3O_5$, IR (KBr, ν_{max}, cm^{-1}): 3415 (NH *str.*, -CO-NH-), 3068 (C-H *str.*, Ar), 2977, 2863 (C–H *str.*, aliph.), 170 (1C=O *str.*, indole), 1671 (C=O *str.*, amide),1586 (-NO_2 *str.*), 1539 (C=CH *str.*), 1473 (C=C *str.*, Ar), 1097 (C-N *str.*).^{1}H NMR (DMSO) δ ppm: 11.090 (s, 1H, acetamide), 8.153-8.113 (d, 2H, Ar-H), 8.098-8.001 (t, 2H, Ar-H), 7.947 (t, 1H, Ar-H), 7.906-7.848 (d, 2H, Ar-H), 7.804-7.789 (d, 2H, Ar-H), 7.750-7.674 (d, 2H, Ar-H), 7.149-7.095 (d, 2H, Ar-H), 4.304-4.250 (s, 2H, -CH_2-), 2.252-2.206 (s, 1H, -CH-). Mass (LC-MS): *m/z* 415 (M), 416 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4,3-dimthoxyphenyl)-*N*-phenyl propanamide (VIIg): Yield: 84 %; m.p.: 237-239 ºC; m.f.: $C_{25}H_{22}N_2O_5$, IR (KBr, ν_{max}, cm^{-1}): 3412 (NH *str.*, -CO-NH-), 3032 (C-H *str.*, Ar), 2986, 2893 (C–H *str.*, aliph.), 1701 (C=O *str.*, indole), 1696 (C=O *str.*, amide), 1596 (C=CH *str.*), 1454 (C=C *str.*, Ar), 1097 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 10.912 (s, 1H, acetamide), 7.901 (s, 1H, Ar-H), 7.878-7.869 (d, 2H, Ar-H), 7.798-7.788 (d, 2H, Ar-H), 7.698-7.688 (d, 2H, Ar-H), 7.606-7.512 (s, 3H, Ar-H), 7.496-7.149 (t, 2H, Ar-H),4.489-4.880 (s, 2H, -CH_2-CO), 3.580 (s, 6H, -$(OCH_3)_2$, 2.185 (s, 1H, -CH-). Mass (LC-MS): *m/z* 430 (M), 431 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-bromophenyl)-*N*-phenyl propanamide (VIIIh): Yield: 74 %; m.p.: 247-249 ºC; m.f.: $C_{23}H_{17}N_2O_5$, IR (KBr, ν_{max}, cm^{-1}): 3413 (NH *str.*, -CO-NH-), 3028 (C-H *str.*, Ar), 2969, 2843 (C–H *str.*, aliph.), 1715 (C=O *str.*, indole), 1695 (C=O *str.*, amide), 1521 (C=CH *str.*), 1432 (C=C *str.*, Ar), 1061 (C-N *str.*), 821 (Br *str.*, Ar-Br). ^{1}H NMR (DMSO) δ ppm: 11.721 (s, 1H, acetamide), 7.893-7.821 (d, 2H, Ar-H), 7.673-7.601 (d, 2H, Ar-H), 7.473-7.402 (t, 3H, Ar-H), 7.382-7.301 (d, 2H, Ar-H), 7.202-7.021 (t, 3H, Ar-H), 6.934 (s, 1H, thiazole), 4.372-4.302 (s, 2H, -CH_2-). Mass (LC-MS): *m/z* 448 (M), 449 (M + 1, 100%), 450 (M + 2, 30%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(3,4,5-trimehoxyphenyl)-*N*-phenylpropanamide (VIIIi): Yield: 78 %; m.p.: ≥ 300 ºC; m.f.: $C_{26}H_{24}N_2O_6$, IR (KBr, ν_{max}, cm^{-1}): 3409 (NH *str.*, -CO-NH-), 3009 (C-H *str.*, Ar), 2958, 2815, 2767 (C–H *str.*, aliph.), 1737 (C=O *str.*, indole), 1673 (C=O *str.*, amide), 1511 (C=CH *str.*), 1437 (C=C *str.*, Ar), 1030 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.929 (s, 1H, acetamide), 8.363-8.2914 (s, 2H, Ar-H), 7.885-7.842 (d, 2H, Ar-H), 7.792-7.777 (d, 2H, Ar-H), 7.559-7.414 (t, 3H, Ar-H), 7.141-7.1078 (d, 2H, Ar-H), 7.135-6.815 (t, 3H, Ar-H), 4.247 (s, 2H, -CH_2-), 3.638-3.513 (s, 3H, (-$OCH_3)_2$, 2.102 (s, 1H, -CH-). Mass (LC-MS): *m/z* 460 (M), 461 (M + 1, 100%).

3-(2,3-Dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(3-nitrophenyl)-*N*-phenylpropanamide (VIIIj): Yield: 75 %; m.p.: 211-212 ºC; m.f.: $C_{23}H_{17}N_3O_5$, IR (KBr, ν_{max}, cm^{-1}): 3432 (NH *str.*, -CO-NH-), 3027 (C-H *str.*, Ar), 2963, 2845 (C–H *str.*, aliph.), 1728 (C=O *str.*, indole), 1689 (C=O *str.*, amide), 1632 (NO_2 *str.*, Ar-NO_2), 1502 (C=CH *str.*), 1453 (C=C *str.*, Ar), 1072 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 12.721 (s, 1H, acetamide), 8.289-8.204 (s, 2H, Ar-H), 7.990-7.902 (d, 2H, Ar-H), 7.683-7.621 (d, 2H, Ar-H), 7.482-7.399 (t, 3H, Ar-H), 7.121-7.102 (d, 2H, Ar-H), 7.002-7.000 (t, 3H, Ar-H), 4.201 (s, 2H, -CH_2-), 2.281 (s, 1H, -CH-). Mass (LC-MS): *m/z* 415 (M), 416 (M + 1, 100%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(phenyl)-*N*-phenyl propanamide (VIIIk): Yield: 85 %; m.p.: 217-219 ºC; m.f.: $C_{23}H_{17}N_2O_3Cl$, IR (KBr, ν_{max}, cm^{-1}): 3401 (NH *str.*, -CO-NH-), 3051 (C-H *str.*, Ar), 2923, 2815 (C–H *str.*, aliph.), 1706 (C=O *str.*, indole), 1681 (C=O *str.*, amide), 1509 (C=CH *str.*), 1428 (C=C *str.*, Ar), 1053 (C-N *str.*), 809 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 11.383 (s, 1H, acetamide), 7.724 (s, 1H, Ar-H), 7.647-7.556 (d, 2H, Ar-H), 7.517-7.432 (d, 2H, Ar-H), 7.384-7.365 (t, 3H, Ar-H), 7.289-7.146 (d, 2H, Ar-H), 7.135-6.815 (t, 3H, Ar-H), 6.707 (s, 1H, thiazole), 4.286-4.277 (s, 2H, -CH_2-), 2.573-2.551 (s, 3H, -CH_3). Mass (LC-MS): *m/z* 404 (M), 405 (M + 1, 100%), 406 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-chloro-phenyl)-*N*-phenyl propanamide (VIIIl): Yield: 86 %; m.p.: 249-251 ºC; m.f.: $C_{23}H_{16}N_2O_3Cl_2$, IR (KBr, ν_{max}, cm^{-1}): 3389 (NH *str.*, -CO-NH-), 3072 (C-H *str.*, Ar), 2945, 2892 (C–H *str.*, aliph.), 1712 (C=O *str.*, indole), 1693 (C=O *str.*, amide), 1532 (C=CH *str.*), 1430 (C=C *str.*, Ar), 1084 (C-N *str.*), 819 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 11.043 (s, 1H, acetamide), 7.901 (s, 1H, Ar-H), 7.675-7.603 (d, 2H, Ar-H), 7.520-7.501 (d, 2H, Ar-H), 7.432-7.400 (t, 3H, Ar-H), 7.167-7.003 (d, 2H, Ar-H), 6.909-6.834 (t, 3H, Ar-H), 6.875 (s, 1H, thiazole), 4.189-4.102 (s, 2H, -CH_2-), 2.427-2.402 (s, 3H, -CH_3). Mass (LC-MS): *m/z* 439 (M), 440 (M + 1, 100%), 441 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-dimethylamino)phenyl)-*N*-phenylpropanamide (VIIIm): Yield: 84 %; m.p.: 233-235 °C; m.f.: $C_{25}H_{22}N_3O_3Cl$, IR (KBr, ν_{max}, cm^{-1}): 3420 (NH *str.*, -CO-NH-), 3073 (C-H *str.*, Ar), 2972, 2862, 2759 (C–H *str.*, aliph.), 1715 (C=O *str.*, indole), 1684 (C=O *str.*, acetamide), 1543 (C=CH *str.*), 1454 (C=C *str.*, Ar), 1032 (C-N *str.*), 805 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 12.092 (s, 1H, acetamide), 8.291 (s, 1H, Ar-H), 8.173 (d, 2H, Ar-H), 7.993 (d, 2H, Ar-H), 7.793 (d, 2H, Ar-H), 7.563 (d, 2H, Ar-H), 7.283 (t, 3H, Ar-H), 4.281 (s, 2H, -CH_2-CO), 2.209-2.200 (s, 6H, N(CH_3)$_2$, 2.125-2.100 (s, 1H, -CH-). Mass (LC-MS): *m/z* 447 (M), 448 (M + 1, 100%), 449 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-methoxy)-phenyl)-*N*-phenylpropanamide (VIIIn): Yield: 79 %; m.p.: 201-203 ºC; m.f.: $C_{24}H_{19}N_2O_4Cl$, IR (KBr, ν_{max}, cm^{-1}): 3443 (NH *str.*, -CO-NH-), 3039 (C-H *str.*, Ar), 2945, 2857, 2782 (C–H *str.*, aliph.), 1725 (C=O *str.*, indole), 1693 (C=O *str.*, acetamide), 1512 (C=CH *str.*), 1427 (C=C *str.*, Ar), 1064 (C-N *str.*), 816 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 12.263 (s, 1H, acetamide), 8.301 (s, 1H, Ar-H), 8.201 (d, 2H, Ar-H), 7.952 (d, 2H, Ar-H), 7.567 (d, 2H, Ar-H), 7.432 (d, 2H, Ar-H), 7.302 (t, 3H, Ar-H), 4.281 (s, 2H, -CH_2-CO), 3.652-3.761 (s, 3H, OCH_3), 2.033-2.003 (s, 1H, -CH-). Mass (LC-MS): *m/z* 434 (M), 435 (M + 1, 100%), 436 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-hydroxyphenyl)-*N*-phenylpropanamide (VIIIo): Yield: 72 %; m.p.: 237-239 °C; m.f.: $C_{23}H_{17}N_2O_4Cl$, IR (KBr, ν_{max}, cm^{-1}): 3638 (OH *str.*, -Ar-OH), 3472 (NH *str.*, -CO-NH-), 3009 (C-H *str.*, Ar), 2924, 2882 (C–H *str.*, aliph.), 1720 (C=O *str.*, indole), 1682 (C=O *str.*, amide), 1508 (C=CH *str.*), 1440 (C=C *str.*, Ar), 1152 (C-N *str.*), 814 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 11.736 (s, 1H, acetamide), 8.419 (s, 1H, Ar-H), 8.350 (d, 1H, Ar-H), 7.945-7.920 (d, 2H, Ar-H),7.828 (s, 1H, Ar-H), 7.812-7.757 (t, 3H, Ar-H), 7.172-7.141 (d, 2H, Ar-H), 6.880-6.861 (d, 2H, Ar-H), 4.367 (s, 2H, -CH_2-CO), 2.075 (s, 1H, -CH-). Mass (LC-MS): *m/z* 420 (M), 421 (M + 1, 100%), 422 (M + 2, 130%).

5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-nitrophenyl)-*N*-phenylpropanamide (VIIIp): Yield: 81 %; m.p.: 267-269 ºC; m.f.: $C_{22}H_{14}N_3O_5SCl$, IR (KBr, ν_{max}, cm^{-1}): 3427 (NH *str.*, -CO-NH-), 3089 (C-H *str.*, Ar), 2986, 2881, 2728 (C–H *str.*, aliph.), 1719 (C=O *str.*, indole), 1673 (C=O *str.*, acetamide), 1619 (-NO_2 *str.*, Ar-NO_2), 1570 (C=CH *str.*), 1434 (C=C *str.*, Ar), 1028 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 10.980 (s, 1H, acetamide), 8.476 (s, 1H, Ar-H), 8.371 (d, 2H, Ar-H), 7.950 (d, 2H, Ar-H), 7.889 (d, 2H, Ar-H), 7.843 (d, 2H, Ar-H), 7.148 (t, 3H, Ar-H), 4.150 (s, 2H, -CH_2-CO), 2.0523-2.002 (s, 1H, -CH-). Mass (LC-MS): *m/z* 435 (M), 436 (M + 1, 100%), 437 (M + 2, 30%).

5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-trimethoxy)-*N*-phenylpropanamide (VIIIq): Yield: 77 %; m.p.: 229-231 ºC; m.f.: $C_{25}H_{21}N_2O_5Cl$, IR (KBr, ν_{max}, cm^{-1}): 3409 (NH *str.*, -CO-NH-), 3062 (C-H *str.*, Ar), 2995, 2867, 2789 (C–H *str.*, aliph.), 1712 (C=O *str.*, indole), 1699 (C=O *str.*, acetamide), 1546 (C=CH *str.*), 1428 (C=C *str.*, Ar), 1032 (C-N *str.*). ^{1}H NMR (DMSO) δ ppm: 11.263 (s, 1H, acetamide), 8.320 (s, 1H, Ar-H), 8.102 (d, 2H, Ar-H), 7.990-7.892 (d, 2H, Ar-H), 7.673-7.591 (d, 2H, Ar-H), 7.530-7.490 (d, 2H, Ar-H), 7.293-7.210 (t, 3H, Ar-H), 4.289 (s, 2H, -CH_2-CO), 3.682-3.601 (s, 9H, -(OCH_3)$_3$), 2.032-2.006 (s, 1H, -CH-). Mass (LC-MS): *m/z* 464 (M), 465 (M + 1, 100%), 466 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-methylphenyl)-*N*-phenylpropanamide (VIIIr): Yield: 83%; m.p.: 223-227 ºC; m.f.: $C_{24}H_{14}N_2O_3Cl$, IR (KBr, ν_{max}, cm^{-1}): 3382 (NH *str.*, -CO-NH-), 3082 (C-H *str.*, Ar), 2925, 2825 (C–H *str.*, aliph.), 1725 (C=O *str.*, indole), 1623 (C=O *str.*, amide), 1519 (C=CH *str.*), 1437 (C=C *str.*, Ar), 1088 (C-N *str.*), 815 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 11.018 (s, 1H, acetamide), 8.493 (s, 1H, Ar-H), 8.370 (s, 1H, Ar-H), 7.977-7.781 (d, 2H, Ar-H), 7.698-7.682 (d, 2H, Ar-H), 7.606-7.583 (d, 2H, Ar-H), 7.512-7.480 (t, 3H, Ar-H), 4.500 (s, 2H, -CH_2-), 2.152-2.106 (s, 1H, -CH-), 2.302 (s, 3H, -CH_3). Mass (LC-MS): *m/z* 418 (M), 419 (M + 1, 100%), 420 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(4-trimethoxyphenyl)-*N*-phenylpropanamide (VIIIs): Yield: 84%; m.p.: 233-235 ºC; m.f.: $C_{25}H_{20}N_3O_7Cl$, IR (KBr, ν_{max}, cm^{-1}): 3406 (NH *str.*, -CO-NH-), 3039 (C-H *str.*, Ar), 2928, 2884 (C–H *str.*, aliph.), 1715 (C=O *str.*, indole), 1698 (C=O *str.*, amide), 1504 (C=CH *str.*), 1454 (C=C *str.*, Ar), 1031 (C-N *str.*), 820 (Cl *str.*, Ar-Cl).^{1}H NMR (DMSO) δ ppm: 11.324 (s, 1H, acetamide), 8.342 (s, 1H, Ar-H), 8.291 (s, 1H, Ar-H), 7.895-7.802 (d, 2H, Ar-H), 7.732-7.702 (d, 2H, Ar-H), 7.582-7.502 (d, 2H, Ar-H), 7.341-7.320 (t, 3H, Ar-H), 4.290-4.201 (s, 2H, -CH_2-), 3.602-3.590 (s, 3H, -(OCH_3)$_2$), 2.152-2.106 (s, 1H, -CH-). Mass (LC-MS): *m/z* 494 (M), 495 (M + 1, 100%), 496 (M + 2, 30%).

3-(5-Chloro-2,3-dioxo-2,3-dihydro-1*H*-inden-1-yl)-3-(3-nitrophenyl)-*N*-phenylpropanamide (VIIIt): Yield: 86%; m.p.: 260-263 ºC; m.f.: $C_{23}H_{16}N_3O_5Cl$, IR (KBr, ν_{max}, cm^{-1}): 3410 (NH *str.*, -CO-NH-), 3020 (C-H *str.*, Ar), 2937, 2889 (C–H *str.*, aliph.), 1716 (C=O *str.*, indole), 1637 (C=O *str.*, amide), 1502 (C=CH *str.*), 1429 (C=C *str.*, Ar), 1092 (C-N *str.*), 815 (Cl *str.*, Ar-Cl). ^{1}H NMR (DMSO) δ ppm: 11.281 (s, 1H, acetamide), 8.302 (s, 1H, Ar-H), 8.291 (s, 1H, Ar-H), 7.830-7.812 (d, 2H, Ar-H), 7.702-7.700 (d, 2H, Ar-H), 7.374-7.302 (d, 2H, Ar-H), 7.189-7.178 (t, 3H, Ar-H), 4.432 (s, 2H, -CH_2-), 2.201-2.187 (s, 1H, -CH-). Mass (LC-MS): *m/z* 418 (M), 419 (M + 1, 100%), 420 (M + 2, 30%).

Pharmacological activity

Antimicrobial activity: The novel synthesized Mannich bases of isatin and their derivatives have been evaluated for antibacterial activity against Gram-negative bacteria *viz. Salmonella paratyphi*, *P. aeruginosa*, *E. coli*, *P. mirabilis* and some Gram-positive bacteria like *L. bacillus*, *S. pyrogenus*, were as antifungal activity against *A. nagram*, *P. notatum*, *C. coffeanum*, *A. tivatus* by the cup-plate method (agar diffusion disc method) for determining zone of inhibition, streptomycin and gentamycin were used as standard drugs for comparison, respectively [22,23].

Anticancer activity: The cell viability was once appraising by means of the MTT assay with three impartial experiments with six concentrations of compounds in triplicates [24]. Cells have been trypsinized and function the trypan blue assay

to be aware of doable cells in cellphone suspension. Cells had been counted by way of hemocytometer and seeded at a density of 5.0×10^3 cells/properly in 100 µL media in 96 properly plate subculture medium and incubated in a single day at 37 ºC. After incubation, take off the ancient media and add clean media a hundred µL with one of kind concentrations of the take a look at compound in labeled wells in 96 plates. After 48 h, discard the drug and added the clean medic with MTT (0.5 mg/mL) was once introduced to every plates incubated at 37 ºC for 3 h. At the period of incubation time, precipitates were shaped as an end result of the MTT salt to chromophore Formosan crystals by way of the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was used to measure at 570 nm on a microplate reader.

Antioxidant activity: Antioxidant activity is one of the best known mechanisms by which antioxidants inhibit lipid oxidation [25]. The total antioxidant activity can be measured for the all synthesized compounds by the ferric reducing antioxidant power assay (FRAP) method. Finally, the absorbance of test compounds and standard ascorbic acid were measured at 700 nm using spectrophotometer.

Molecular docking studies: The digital shape of the EGFR kinase area used to be retrieved from the protein databank internet site with PDB Id: 1M17 and the shape used to be optimized by deleting unbound water molecules which are over 1 Å, including hydrogen atoms to fulfil the valences, including lacking amino acids to stabilize facet chains and electricity of the complete shape was once minimized the usage of OPLS-2005 pressure discipline with the help of Protein Preparation Wizard device of Schrödinger Suite [26]. Thus structurally optimized protein shape was once used to observe protein-ligand interactions of the dataset ligands the use of Glide Xp docking protocol. Initially, a 3D grid used to be set up to the binding pocket (active site) of the protein, into which all the dataset ligands had been docked into. Binding interactions and effectively of the binding have been calculated in phrases of Glide Score, which is a mixture of hydrophilic, hydrophobic, steel binding groups, van der Waals energy, freezing rotatable bonds and polar interactions with receptor.

RESULTS AND DISCUSSION

Novel Mannich bases of isatin derivatives were synthesized by reaction between substituted isain derivatives, acetanilide and substituted benzaldehydes in ethanol to give final product respectively. The synthesized novel mannich bases (**VIIIa-VIIIt**) were screened for antibacterial, antioxidant and anticancer activities. The synthesized compounds were characterized as **VIIIa-VIIIt** based on satisfactory analytical and spectral data including IR, LC-MASS and ^{1}H NMR data.

In IR spectroscopy, virtually all the compounds have N-H stretching frequency observed at 3450-3400 cm^{-1}, the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3100-3000 and 2920-2750 cm^{-1}, respectively. Two strong absorption peaks observed at around 1740-1670 cm^{-1} is due to be presence of C=O stretching frequency in indole and acetamide carbonyl groups. Also in most of the compounds the C-C stretching of the aromatic ring is around 1553-1502 cm^{-1}. The C-Cl stretching is attributed to the strong absorption in the region 820-790 cm^{-1}. Few compounds showing asymmetric and symmetric bending observed at around 1265 and 1058 cm^{-1}, which indicates the presence of -OCH_3 group. Similarly, the ^{1}H NMR (DMSO-d_6) spectra of novel Mannich bases of isatin derivatives showing a singlet at δ 10-13.02 ppm for NH protons. All the compounds show a triplet and singlet at δ 4.200-4.630 ppm for acetamide protons, a singlet at δ 2.10-2.30 ppm for methylene group (Ar-CH_3). All the aromatic protons were found between δ 8.329-6.80ppm as singlet, doublet and triplet protons.

Antibacterial activity: Agar diffusion method used for evolution of antimicrobial potency for the novel synthesized Mannich bases of isatin derivatives, using the Gram-positive and negative microorganism. From Table-1, compounds **VIIIb**, **VIIIi**, **VIIIm** and **VIIIo** were found to be excellent towards the antibacterial and antifungal activities.

Antioxidant activity: The *in vitro* antioxidant activity of **VIIIa-VIIIt** was determined spectrophotometrically by ferric reducing antioxidant power assay (FRAP) method and the results are given in Table-2. The maximum and minimum antioxidant activities were observed for compounds **VIIk** and **VIIIe**, respectively.

Anticancer activity: Novel Mannich bases of isatin derivatives were evaluated for cytotoxicity against human breast cancer cells (MCF7) using MTT assay and compared with doxorubicin as standard. Results (Table-3) proposed that MCF cell lines were susceptible to the evaluated Mannich base compounds. The synthesized compounds showed the IC_{50} values in the range of 41.65 to 99.79 µM against MCF7 cell line. Compounds **VIIIe, VIIIg** and **VIIIm** showed good activity against the cell lines, whereas, remaining compounds showed the tolerable activity. All the results are expressed as a mean ± SEM of five concentrations.

Molecular docking studies: Molecular docking study has been carried out in order to locate the viable protein ligand interactions of the dataset ligands. Additionally, these additionally assisted in finding out the conformational adjustments of the ligand in the protein environment. About generates one hundred distinctive protein ligand complicated conformations for every docked complicated was once generated *via* Glide XP module (Fig. 1). Glide dock sores of the dataset ligands have been proven in Table-4 alongside with the interplay amino acids and quantity of amino acids. Among the docked ligands, compound **VIIIe** pronounced perfect dock rating of -8.104 with E mannequin strength of -43.785 Kcal/mol. Dock rankings of all the compounds ranged from -8.104 to -3.911.

Conclusion

In this work, novel Mannich bases of isatin derivatives were synthesized under conventional method. The yield of the synthesized compounds was found to be in the range from 67-84%. All the synthesized compounds showed biological and anticancer activities. Molecular docking studies were performed to determine the protein-ligand interactions and also to understand the conformational changes in the protein-ligand complex.

TABLE-1
ANTIBACTERIAL ACTIVITY OF MANNICH BASES OF ISATIN DERIVATIVES

Microorganism	Zone of inhibition (mm)					
	S. paratyphi	*P. auregunosa*	*E. coli*	*P. mirabilis*	*L. bacillus*	*S. pyrogenus*
VIIIa	11	14	15	12	15	11
VIIIb	23	12	15	19	22	17
VIIIc	11	16	14	11	16	13
VIIId	12	9	0	0	10	0
VIIIe	9	0	10	13	9	11
VIIIf	11	0	0	11	10	0
VIIIg	12	0	0	10	0	0
VIIIh	16	14	15	11	0	0
VIIIi	27	19	18	11	25	17
VIIIj	0	0	15	11	16	17
VIIIk	18	19	16	18	13	0
VIIIl	0	0	0	0	13	14
VIIIm	20	18	0	12	23	0
VIIIn	12	9	14	12	10	11
VIIIo	27	20	19	20	24	17
VIIIp	0	0	0	11	12	15
VIIIq	11	12	15	19	16	13
VIIIr	11	0	0	0	10	11
VIIIs	11	12	0	19	15	10
VIIIt	15	16	0	16	15	0
Streptomycin	30	32	32	33	28	30

TABLE-2
ANTIOXIDANT ACTIVITY OF MANNICH BASES OF ISATIN DERIVATIVES

Compound	Concentration (µg/mL)	Absorbance at 700 nm	Inhibition (%)
VIIIc	30	0.756	58.82
VIIId	20	0.514	50.35
	30	0.763	60.29
	40	0.872	63.29
VIIIf	5	0.174	61.11
	10	0.412	62.84
	20	0.568	66.08
	30	0.812	70.58
	40	0.924	73.03
VIIIh	10	0.398	57.31
	20	0.573	67.66
	30	0.812	70.58
	40	0.932	74.53
VIIIj	10	0.385	52.17
	20	0.543	58.77
	30	0.785	64.91
	40	0.923	72.84
VIIIk	30	0.781	64.11
	40	0.894	67.41
VIIIL	40	0.813	52.24

TABLE-3
CYTOTOXIC ACTIVITY OF MANNICH BASES OF ISATIN DERIVATIVES ON MCF-7 CELLS

Compound	IC_{50} values (µM)
VIIIb	86.52 ± 0.205
VIIIc	73.21 ± 0.102
VIIIe	62.76 ± 0.392
VIIIf	99.79 ± 0.281
VIIIg	54.13 ± 0.142
VIIIm	41.65 ± 0.391
VIIIn	78.21 ± 0.823
VIIIr	82.17 ± 0.563
Doxorubicin	16.32 ± 0.142

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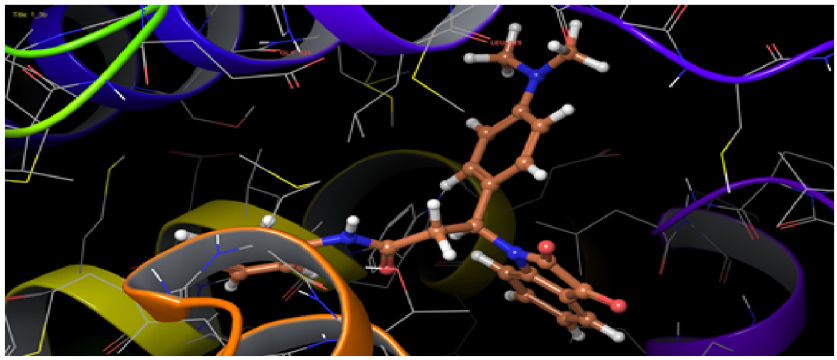
CONFLICT OF INTEREST

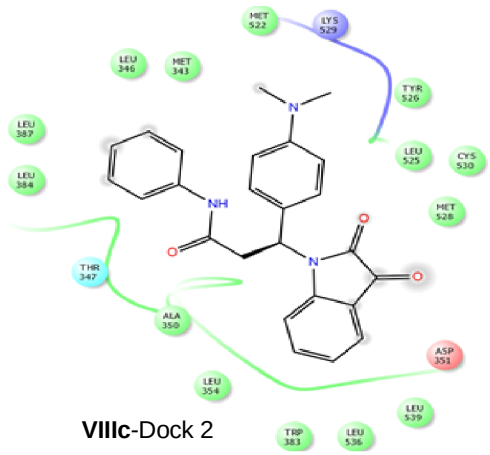
The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-4
In silico EGFR INHIBITION OF MANNICH BASES OF ISATIN DERIVATIVES-GLIDE DOCK SORES OF THE DATASET LIGANDS

Compound	Dock score XP GScore	No of H-bonds	Interacting amino acids	H-bond lengths	E model energy	Glide energy
VIIIe	-8.104	1	GLY 521	2.18	-69.316	-43.18
VIIId	-7.161	0	-	-	-32.765	-27.23
VIIIo	-6.011	1	LEU 525	2.09	-54.758	-45.802
VIIIc	-5.944	0	-	-	-38.549	-41.159
VIIIm	-5.58	0	-	-	-59.394	-43.885
VIIIr	-5.449	1	ASP 351	2.01	-45.488	-39.568
VIIIg	-4.589	0	-	-	-31.836	-25.003
VIIIf	-3.911	1	ASP 351	1.82	-52.8	-35.313



VIIIc-Dock 1



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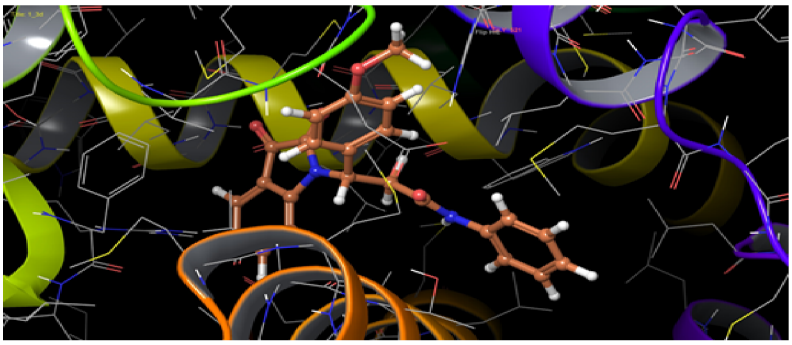
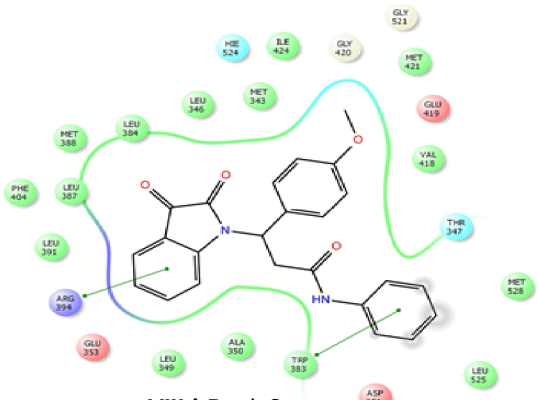
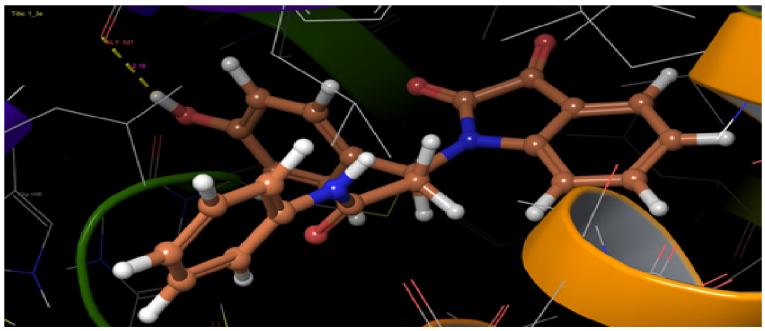


VIIId-Dock 1



VIIId-Dock 2



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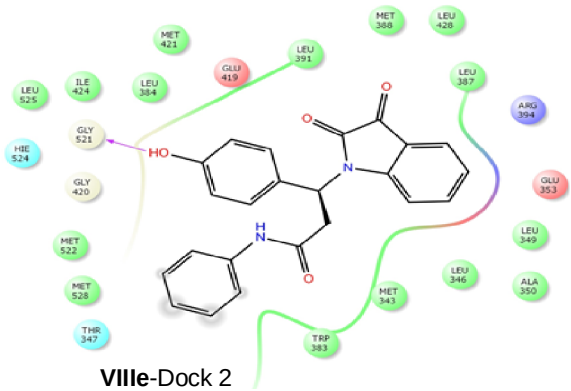

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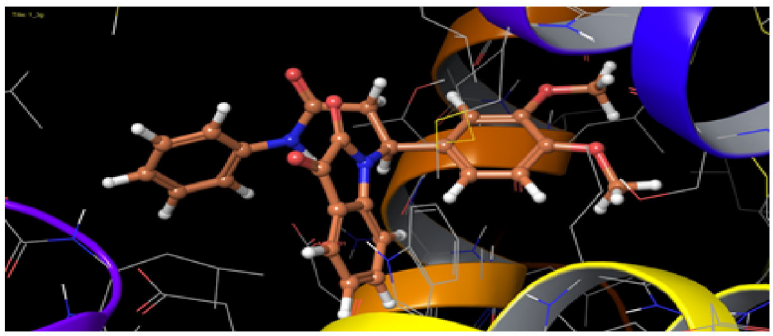

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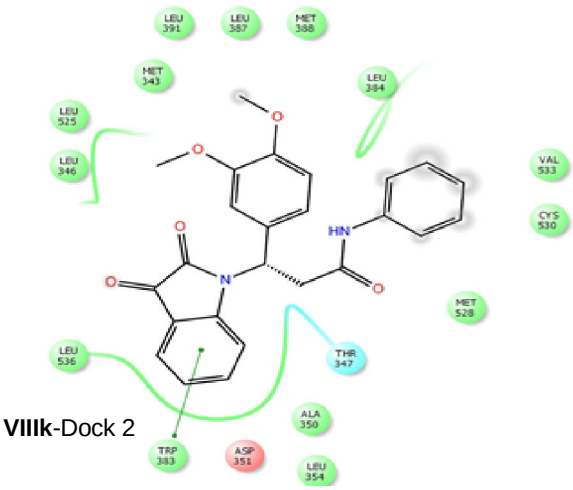

VIIIk-Dock 2

Fig. 1. Docking pose between the ligand and the protein

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