Synthesis, Characterization, Molecular docking studies of 5-substituted-3-(2-methoxy dibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-ones as potent antimicrobial and anticancer agents.

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ABSTRACT

We present here in the results of conventional method promoted N-alkylation of substituted Istain **I(a-f)**withAlkyl halides. The5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-one **[IV-3(a-r)]**were prepared by Schiff's base mechanism between compound II(a-r) with 3-amino-2-methoxydibenzofuran. Their chemical structures were confirmed by spectral analysis by means of IR, ^{1}H-NMR and Mass. The antimicrobial activity of the synthesized compounds was evaluated by Disc plate method and anticancer activity by MTT assay method against MCF-7 and SKOV3 cell lines. The compounds **IV-3c, IV-3d, IV-3e, IV-3g,IV-3h** and **IV-3l** are significantly active against bacteria and fungi. Anticancer activity reveals that compounds **IV-3c** active against MCF-7 cell line with $IC_{50}$of **0.043 µM** and **IV-3d** against SKOV3 cell line with $IC_{50}$of **0.058µM** respectively. Considerable binding affinity between EGFR and the hybridcompound 3a reported highest dock score of **-6.147** with Glide binding energy of -47.652 Kcal/mol. Dock scores of all the compounds ranged from -6.147 (compound 3a) to -2.34 (compound 3p). MET 769 and Asp 831 are the most common amino acids with H-bonds.

KEYWORDS: Isatin, 3-amino-2-methoxydibenzofuran, Antimicrobial and Anticancer activities, EGFR and Schrodinger suite.

INTRODUCTION:

The research-based pharmaceutical industry has increasingly employed modern medicinal chemistry methods, including molecular modelling, as powerful tools for the study of structure-activity relationships [1]. Cancer has become the second leading cause of deathworldwide over the past decades and is characterized byuntamed augmentation and

propagation of abnormal cells. According to World Health Organization, nearly 12 millionpeople around the world passed away because of cancer in the year 2020.Indole-2-one nucleus is utilized as privileged structural motif in the development of a wide range of drugs with interest in numerous areas [2-3]. Indole-2,3-dione(Isatin), its Schiff's bases and Mannich bases are reported to show a variety of biological activities such as antibacterial, antifungal, anthelmintic,anti-HIV,antiviral,anticonvulsant,anti-tubercular, anticancer and anti-inflammatory activities.

Protein-ligand interaction is comparable to the lock-and keyprinciple, in which the lock encodes the protein and thekey is grouped with the ligand. The major driving force forbinding appears to be hydrophobic interaction [4-6]. In silicotechniques helps identifying drug target via bioinformaticstools. They can also be used to explore the target structuresfor possible active sites, generate candidate molecules, dockthese molecules with the target, rank them according totheir binding affinities and further optimize the molecules toimprove binding characteristics.. Isatin, chemically known as1*H*-indole-2,3-dione, has become a popular topic due to its various uses. The chemistry of Istain andits derivatives is particularly interesting because of their potential application in medicinal chemistry.Isatins are very important compounds due to their biological activity. Schiff and Mannichbasesofisatin derivatives are reported to show variety of biological activities like antibacterial,antifungal, anticonvulsant, anti HIV, antidepressant, and anti-inflammatory activities. Similarly, dibenzofuran and their derivatives play important roles in medicinal, agriculturaland industrial fields. *N*-bridged heterocyclic derivatives derived from Indole-2-oneshow variedbiological activities[7−12].

These biological and chemical data prompted us to synthesized new Indole-2-one derivatives bearing dibenzofuran ring and the newly synthesized 5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-onecompounds were characterized by spectral analysis.

EXPERIMENTAL SECTION

Material and Methods:

Unless otherwise noted, all the reagents and chemicals were purchased are ofstandard grade . Melting point were determined by Thieles tube apparatus by using liquid paraffin as a solvent. Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 450-4000

cm^{-1} using KBr pellets, and ^{1}HNMR spectra were recorded on DPX-200 MHz NMR spectrometer exploiting DMSO-d6 and chemical shifts (δ) are recorded in parts per million downfield from internal reference Tetramethylsilane(TMS). Mass spectra were set down on Mass spectrophotometer (model Shimadzu) by LC-MS and the spectra were interpreted.

General Procedure:

Step: I: Synthesis of Substituted Isatin I(a-f)from substituted anilines: 9 gm of Chloral hydrate was taken into the round bottom flask and dissolved in 120 ml water. To that 13 gm of sodium sulphate, a solution of 5.4 gm of substituted aniline in 30 ml of water containing 5.12 gm of concentrated hydrochloric acid (4.34 ml) to dissolve the amine and solution of 11 gm of hydroxylamine hydrochloride in 50 ml of water were added. 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 reminder crystallized product with suction pump and air dried.

18.4 gm of concentrated sulphuric acid (10.0 ml) was warmed to 50^{0}C and 2.5 gm of dry nitrosoacetanilide was added in such a rate so as to keep the temperature between 60-70^{0}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly after the addition of isonitroso compound was finished. The solution was heated to 80^{0}C 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 ten times its volume of cracked ice. After standing for 90 mint, the final product was filtered with suction pump followed by washing with cold water to remove excess of sulphuric acid and dried in air.

Step.II:Synthesis of N-substituted Isatin derivatives: II(a-r): A flask equipped with a magnetic stirring bar was charged with DMF (100 ml) and Sodium hydroxide/KOH (13 mmol). The mixture was stirred at room temperature for 5 min., isatin (10 mmol) was then added and the stirring was continued for 45 min. Alkyl halide (11 mmol, chloride) was added to the reaction mixture and the stirring was continued at 80^{o}C for 4h. The mixture was then diluted with water (200 mL), extracted with ethyl acetate and dried over anhydrous sodium sulphate. The solvent was removed under vacuum, and the residue was purified by recrystallization using ethyl acetate and hexane (1:9) solvent system to get pure N-substituted isatins.

Step.III: Synthesis of 5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-oneIV-3(a-r): Compound II(a-r) (0.01 mol) was taken in a mixture of

3-Amino-2-methoxydibenzofuran (0.01 mol) , glacial acetic acid (5 ml) and Ethanol 30ml, then the reaction mixture was refluxing for 2hrs. The progress of the reaction was monitored by TLC (Hexane: EtoAc 7:3). The reaction mixture was cooled to room temperature. A solid was obtained, which was filtered off and washed with hexane and recrystallized from methanol to give crystalline product.

Compound.IV-3a: 3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one. M.P. 168-169^{o}C; Mol.formula:$C_{21}H_{14}N_2O_3$; %Yield: 82%; IR (ν cm^{-1}); 3311(-NH *Str*, Isatin), 3083(-CH *Str*, Aromatic), 2909(-CH *Str*, Alkyl), 1707(-CO Str, Indole), 1680(-C=N, *Str*), 1518(-C=CH, *Str*), 1343(-C=C *Str*), 1108(-C-O *Str*, OCH_3),1037(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 12.0945(1H, s, -NH in Indole), 8.1009(1H, s, dibenzofuran Protons), 7.8909-7.7989(3H, t, Aromatic Protons), 7.6984-7.6854(2H, d, Aromatic Protons),7.6464-7.6287(2H, d, Aromatic Protons), 7.5798-7.5025(2H, t, Aromatic Protons), 7.4995(1H, s, dibenzofuran Protons), 3.6987(3H, s, methoxy protons in dibenzofuran). Mass (LC-MS): m/z 342.10(M), 343.21(M+1, 100%).

Compound.IV-3b:5-methyl-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one.

M.P. 143-145^{o}C; Mol.formula:$C_{22}H_{16}N_2O_3$; %Yield: 78%; IR (ν cm^{-1}); 3364(-NH *Str*, Isatin), 3118(-CH *Str,* Aromatic), 2918(-CH *Str*, Alkyl), 1703(-CO Str, Indole), 1632(-C=N, *Str*), 1591(-C=CH, *Str*), 1458(-C=C *Str*), 1155(-C-O *Str*, OCH_3),1030(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 12.0356(1H, s, -NH in Indole), 7.9984(1H, s, dibenzofuran Protons), 7.8675-7.8564(2H, d, Aromatic Protons), 7.8374(1H, s, Aromatic Protons in dibenzofuran),7.7234-7.7092(2H, t, Aromatic Protons), 7.6947-7.6783(2H, d, Aromatic Protons), 7.5999(1H, s, dibenzofuran Protons), 3.5989(3H, s, methoxy protons in dibenzofuran), 2.2075(3H, s, Ar-CH_3 proton). Mass (LC-MS): m/z 356.12(M), 357.21(M+1, 100%).

Compound.IV-3c: 5-chloro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one.

M.P. 257-259^{o}C; Mol.formula:$C_{21}H_{13}ClN_2O_3$; %Yield: 83%; IR(ν cm^{-1}); 3312(-NH *Str*, Isatin), 3007(-CH *Str*, Aromatic), 2980(-CH *Str*, Alkyl), 1700(-CO Str, Indole), 1547(-C=N, *Str*), 1485(-C=CH, *Str*), 1359(-C=C *Str*), 1238(-C-O *Str*, OCH_3),1025(-C-N *Str*), 745(-Cl *Str*, Ar-Cl). ^{1}H-NMR (DMSO) δ ppm: 11.8762(1H, s, -NH in Indole), 8.3123(1H, s, dibenzofuran Protons), 8.0987(1H, s, Aromatic Protons in dibenzofuran), 7.9389-7.9289(2H, t, Aromatic Protons),7.8375-7.8309(2H, d, Aromatic Protons), 7.7932-7.7876(2H, d, Aromatic Protons),

7.1098(1H, s, dibenzofuran Protons), 3.8654(3H, s, methoxy protons in dibenzofuran). Mass (LC-MS): m/z 376.06(M), 377.23(M+1, 100%), 378.15(M+2, 30%).

Compound.IV-3d: 5-nitro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one. M.P. 215-217^{o}C; Mol.formula:$C_{21}H_{13}N_3O_5$; %Yield: 81%; IR (ν cm^{-1}); 3240(-NH *Str*, Isatin), 3084(-CH *Str*, Aromatic), 2984(-CH *Str*, Alkyl), 1708(-CO Str, Indole), 1614(-NO_2*Str*, -Ar-NO_2),1580(-C=N, *Str*), 1477(-C=CH, *Str*), 1310(-C=C *Str*), 1267(-C-O *Str*, OCH_3),1039(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 12.1536(1H, s, -NH in Indole), 8.3712(1H, s, dibenzofuran Protons), 7.9508(1H, s, Aromatic proton in dibenzofuran), 7.8898(2H, d, Aromatic Protons), 7.8439(2H, d, Aromatic Protons),7.7984(2H, t, Aromatic Protons), 7.1485(1H, s, Aromatic Protons in dibenzofuran), 3.5837(3H, s, methoxy protons in dibenzofuran). Mass (LC-MS): m/z 387.09(M), 388.21(M+1, 100%).

Scheme-I

Compound.IV-3e:3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl-indolin-2-one. M.P. 137-139^{o}C; Mol.formula:$C_{22}H_{16}N_2O_3$; %Yield: 85%; IR (ν cm^{-1}); 3013(-CH *Str*, Aromatic), 2919(-CH *Str*, Alkyl), 1717(-CO Str, Indole), 1591(-C=N, *Str*), 1542(-C=CH, *Str*), 1337(-C=C *Str*), 1225(-C-O *Str*, OCH_3), 1095(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 7.8645(1H, s, dibenzofuran Protons), 7.6554-7.6034(2H, d, Aromatic Protons), 7.4598-7.4567(2H, d, Aromatic Protons),7.3685-7.3465(2H, t, Aromatic Protons), 7.3309-7.3098(2H, t, Aromatic Protons), 7.2323(1H, s, dibenzofuran Protons), 3.6832(3H, s, methoxy protons in dibenzofuran), 2.3087(3H, s, N-CH_3). Mass (LC-MS): m/z 356.12(M), 357.11

(M+1, 100%).

Compound.IV-3f:3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 159-161^{o}C; Mol.formula:$C_{23}H_{18}N_2O_3$; %Yield: 77%; IR (ν cm^{-1}); 3043(-CH *Str*, Aromatic), 2943, 2875(-CH *Str*, Alkyl), 1708(-CO Str, Indole), 1602(-C=N, *Str*), 1521(-C=CH, *Str*), 1322(-C=C *Str*), 1198(-C-O *Str*, OCH_3), 1025(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 8.2131(1H, s, dibenzofuran Protons), 7.8793-7.8320(2H, d, Aromatic Protons), 7.6785-7.6522(2H, d, Aromatic Protons),7.4532-7.3032(2H, t, Aromatic Protons), 7.298-7.1982(2H, t, Aromatic Protons), 7.0943(1H, s, dibenzofuran Protons), 3.5987(3H, s, methoxy protons in dibenzofuran), 3.2983(2H, q, N-CH_2-), 2.3293(3H, s, N-CH_3). Mass (LC-MS): m/z 370.13(M), 371.34(M+1, 100%).

Compound.IV-3g: 5-methyl-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl-indolin-2-one. M.P. 211-213^{o}C; Mol.formula:$C_{23}H_{18}N_2O_3$; %Yield: 79%; IR(ν cm^{-1}); 3089(-CH *Str*, Aromatic), 2985, 2875(-CH *Str*, Alkyl), 1721(-CO Str, Indole), 1602(-C=N, *Str*), 1512(-C=CH, *Str*), 1343(-C=C *Str*), 1238(-C-O *Str*, OCH_3),1044(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 8.2343(1H, s, dibenzofuran Protons), 8.2763(1H, s, Aromatic proton), 7.9843-7.8543(2H, d, Aromatic Protons), 7.7865-7.6432(2H, d, Aromatic Protons),7.5643-7.4893(2H, t, Aromatic Protons), 7.3423(1H, s, Aromatic Protons in dibenzofuran), 3.6543(3H, s, methoxy protons in dibenzofuran), 3.2102(3H, s, -NCH_3), 2.1933(3H, s, Ar-CH_3). Mass (LC-MS): m/z 370.13(M), 371.34(M+1, 100%).

Compound.IV-3h: 5-chloro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl-indolin-2-one. M.P. 198-200^{o}C; Mol.formula:$C_{22}H_{15}ClN_2O_3$; %Yield: 87%; IR (ν cm^{-1}); 3098(-CH *Str*, Aromatic), 2977, 2854(-CH *Str*, Alkyl), 1703(-CO Str, Indole), 1619(-C=N, *Str*), 1514(-C=CH, *Str*), 1322(-C=C *Str*), 1229(-C-O *Str*, OCH_3),1054(-C-N *Str*), 812(-Cl Str,

Ar-Cl). ^{1}H-NMR (DMSO) δ ppm: 8.3452(1H, s, dibenzofuran Protons), 8.1982(1H, s, Aromatic proton), 7.8783-7.7673(2H, d, Aromatic Protons), 7.6563-7.5342(2H, d, Aromatic Protons),7.4387-7.3442(2H, t, Aromatic Protons), 7.1892(1H, s, Aromatic Protons in dibenzofuran), 3.8231(3H, s, methoxy protons in dibenzofuran), 3.3012(3H, s, -NCH_3). Mass (LC-MS): m/z 390.08(M), 391.21(M+1, 100%).

Compound.IV-3i: 5-nitro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl-indolin-2-one. M.P. 231-233^{o}C; Mol.formula:$C_{22}H_{15}N_3O_5$; %Yield: 86%; IR (ν cm^{-1}); 3098(-CH *Str*, Aromatic), 2978, 2856(-CH *Str*, Alkyl), 1708(-CO Str, Indole), 1632(-NO_2 Str, Ar-NO_2), 1612(-C=N, *Str*), 1532(-C=CH, *Str*), 1354(-C=C *Str*), 1222(-C-O *Str*, OCH_3),1043(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 8.3921(1H, s, dibenzofuran Protons), 8.1923(1H, s, Aromatic proton), 8.0322-8.002(2H, d, Aromatic Protons), 7.9832-7.8763(2H, d, Aromatic Protons),7.4673-7.3654(2H, t, Aromatic Protons), 7.2983(1H, s, Aromatic Protons in dibenzofuran), 3.6743(3H, s, methoxy protons in dibenzofuran), 3.2017(3H, s, -NCH_3). Mass (LC-MS): m/z 401.10(M), 402.19(M+1, 100%).

Compound.IV-3j: 5-nitro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 219-221^{o}C; Mol.formula:$C_{23}H_{17}N_3O_5$; %Yield: 78%; IR (ν cm^{-1}); 3098(-CH *Str*, Aromatic), 2965, 2899(-CH *Str*, Alkyl), 1711(-CO Str, Indole), 1630(-NO_2 Str, Ar-NO_2), 1609(-C=N, *Str*), 1543(-C=CH, *Str*), 1355(-C=C *Str*), 1219(-C-O *Str*, OCH_3),1029(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 8.1765(1H, s, dibenzofuran Protons), 8.0943(1H, s, Aromatic proton), 7.9843-7.8723(2H, d, Aromatic Protons), 7.7621-7.6732(2H, d, Aromatic Protons),7.5216-7.4895(2H, t, Aromatic Protons), 7.3092(1H, s, Aromatic Protons in dibenzofuran), 3.8632(3H, s, methoxy protons in dibenzofuran), 3.1902(2H, s, -NCH_2), 2.012(3H, s, -CH_3). Mass (LC-MS): m/z 415.12(M), 416.31(M+1, 100%).

Compound.IV-3k: 5-methyl-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 209-211^{o}C; Mol.formula:$C_{24}H_{20}N_2O_3$; %Yield: 83%; IR(ν cm^{-1}); 3076(-CH *Str*, Aromatic), 2988, 2893(-CH *Str*, Alkyl), 1715(-CO Str, Indole), 1639(-NO_2 Str, Ar-NO_2), 1614(-C=N, *Str*), 1523(-C=CH, *Str*), 1376(-C=C *Str*), 1222(-C-O *Str*, OCH_3),1012(-C-N *Str*). ^{1}H-NMR (DMSO) δ ppm: 8.3096(1H, s, dibenzofuran Protons), 8.2139(1H, s, Aromatic proton), 7.8796-7.8102(2H, d, Aromatic Protons), 7.6754-7.5943(2H, d, Aromatic Protons),7.3987-7.3210(2H, t, Aromatic Protons), 7.2893(1H, s, Aromatic Protons in dibenzofuran), 3.7832(3H, s, methoxy protons in dibenzofuran), 3.2093(2H, s, -NCH_2),

2.293(3H, s, -CH_3), 1.9833(3H, s, Ar-CH_3). Mass (LC-MS): m/z 384.15(M), 385.32(M+1, 100%).

Compound.IV-3l: 5-chloro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 155-157^oC; Mol.formula:$C_{23}H_{17}ClN_2O_3$; %Yield: 86%; IR (ν cm^{-1}); 3109(-CH *Str*, Aromatic), 2965, 2864(-CH *Str*, Alkyl), 1710(-CO Str, Indole), 1621(-C=N, *Str*), 1520(-C=CH, *Str*), 1367(-C=C *Str*), 1212(-C-O *Str*, OCH_3),1032(-C-N *Str*), 798(-Cl *Str*, Ar-Cl)..^{1}H-NMR (DMSO) δ ppm: 8.19826(1H, s, dibenzofuran Protons), 8.0832(1H, s, Aromatic proton), 7.7854-7.5873(2H, d, Aromatic Protons), 7.4984-7.4093(2H, d, Aromatic Protons),7.2983-7.2093(2H, t, Aromatic Protons), 7.1092(1H, s, Aromatic Protons in dibenzofuran), 3.87432(3H, s, methoxy protons in dibenzofuran), 3.1092(2H, s, -NCH_2), 2.192(3H, s, -CH_3), 1.9833(3H, s, Ar-CH_3). Mass (LC-MS): m/z 404.09(M), 405.13(M+1, 100%), 406.14(M+2, 30%).

Compound.IV-3m: 5-fluoro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one. M.P. 216-218^oC; Mol.formula:$C_{21}H_{13}FN_2O_3$; %Yield: 76%; IR(ν cm^{-1}); 3254(-NH Str, Indole), 3093(-CH *Str*, Aromatic), 2998(-CH *Str*, Alkyl), 1717(-CO Str, Indole), 1632(-C=N, *Str*), 1528(-C=CH, *Str*), 1343(-C=C *Str*), 1205(-C-O *Str*, OCH_3),1043(-C-N *Str*), 812(-F *Str*, Ar-F). ^{1}H-NMR (DMSO) δ ppm: 8.2983(1H, s, dibenzofuran Protons), 8.1982(1H, s, Aromatic proton), 7.9832-7.8532(2H, d, Aromatic Protons), 7.6543-7.6002(2H, d, Aromatic Protons),7.4873-7.4192(2H, t, Aromatic Protons), 7.2983(1H, s, Aromatic Protons in dibenzofuran), 3.6672(3H, s, methoxy protons in dibenzofuran). Mass (LC-MS): m/z 404.09(M), 405.13(M+1, 100%), 406.14(M+2, 30%).

Compound.IV-3n: 5-fluoro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl indolin-2-one. M.P. 227-229^oC; Mol.formula:$C_{22}H_{15}FN_2O_3$; %Yield: 81%; IR(ν cm^{-1}); 3102(-CH *Str*, Aromatic), 2976(-CH *Str*, Alkyl), 1712(-CO Str, Indole), 1621(-C=N, *Str*), 1531(-C=CH, *Str*), 1329(-C=C *Str*), 1212(-C-O *Str*, OCH_3),1028(-C-N *Str*), 811(-F *Str*, Ar-F). ^{1}H-NMR (DMSO) δ ppm: 8.4021(1H, s, dibenzofuran Protons), 8.3203(1H, s, Aromatic proton), 8.0321-8.0032(2H, d, Aromatic Protons), 7.9843-7.8743(2H, d, Aromatic Protons),7.5673-7.4984(2H, t, Aromatic Protons), 7.1983(1H, s, Aromatic Protons in dibenzofuran), 3.5985(3H, s, methoxy protons in dibenzofuran), 3.1093(3H, s, N-CH_3). Mass (LC-MS): m/z 374.11.09(M), 375.09(M+1, 100%), 376.43(M+2, 30%).

Compound.IV-3o: 5-fluoro-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 191-193^oC; Mol.formula:$C_{23}H_{17}FN_2O_3$; %Yield: 82%; IR(ν cm^{-1}); 3089(-CH *Str*, Aromatic), 2987(-CH *Str*, Alkyl), 1702(-CO Str, Indole), 1638(-C=N, *Str*), 1514(-C=CH,

Str), 1352(-C=C *Str*), 12014(-C-O *Str*, OCH_3),1033(-C-N *Str*), 809(-F *Str*, Ar-F). ^{1}H-NMR (DMSO) δ ppm: 8.2293(1H, s, dibenzofuran Protons), 8.1283(1H, s, Aromatic proton), 7.8944-7.7843(2H, d, Aromatic Protons), 7.6754-7.5873(2H, d, Aromatic Protons),7.3982-7.3002(2H, t, Aromatic Protons), 7.1982(1H, s, Aromatic Protons in dibenzofuran), 3.8643(3H, s, methoxy protons in dibenzofuran), 3.1092(2H, q, N-CH_2), 2.393(3H, d, -CH_3). Mass (LC-MS): m/z 388.12(M), 389.32(M+1, 100%), 389.10(M+2, 30%).

Compound.IV-3p: 5-bromo-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-indolin-2-one. M.P. 257-259^{o}C; Mol.formula:$C_{21}H_{13}BrN_2O_3$; %Yield: 82%; IR(ν cm^{-1}); 3309(-NH Str, Indole), 3087(-CH *Str*, Aromatic), 2989(-CH *Str*, Alkyl), 1715(-CO Str, Indole), 1609(-C=N, *Str*), 1534(-C=CH, *Str*), 1354(-C=C *Str*), 1229(-C-O *Str*, OCH_3),1055(-C-N *Str*), 821(-Br *Str*, Ar-Br). ^{1}H-NMR (DMSO) δ ppm: 8.3884(1H, s, dibenzofuran Protons), 8.2653(1H, s, Aromatic proton), 8.1203-8.0242(2H, d, Aromatic Protons), 7.9843-7.8734(2H, d, Aromatic Protons),7.3984-7.2893(2H, t, Aromatic Protons), 7.1553(1H, s, Aromatic Protons in dibenzofuran), 3.8754(3H, s, methoxy protons in dibenzofuran). Mass (LC-MS): m/z 420.01(M), 421.21(M+1, 100%), 422.32(M+2, 30%).

Compound.IV-3q: 5-bromo-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-methyl-indolin-2-one. M.P. 237-239^{o}C; Mol.formula:$C_{22}H_{15}BrN_2O_3$; %Yield: 83%; IR(ν cm^{-1}); 3082(-CH *Str*, Aromatic), 2977(-CH *Str*, Alkyl), 1723(-CO Str, Indole), 1615(-C=N, *Str*), 1528(-C=CH, *Str*), 1338(-C=C *Str*), 1238(-C-O *Str*, OCH_3),1048(-C-N *Str*), 818(-Br *Str*, Ar-Br). ^{1}H-NMR (DMSO) δ ppm: 8.2654(1H, s, dibenzofuran Protons), 8.1983(1H, s, Aromatic proton), 7.9807-7.8765(2H, d, Aromatic Protons), 7.7432-7.6364(2H, d, Aromatic Protons),7.4723-7.3342(2H, t, Aromatic Protons), 7.2983(1H, s, Aromatic Protons in dibenzofuran), 3.6923(3H, s, methoxy protons in dibenzofuran), 3.1023(3H, s, N-CH_3). Mass (LC-MS): m/z 434.03(M), 435.34(M+1, 100%), 436.21(M+2, 30%).

Compound.IV-3r: 5-bromo-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-ethyl-indolin-2-one. M.P. 209-211^{o}C; Mol.formula:$C_{23}H_{17}BrN_2O_3$; %Yield: 70%; IR(ν cm^{-1}); 3091(-CH *Str*, Aromatic), 2969(-CH *Str*, Alkyl), 1705(-CO Str, Indole), 1621(-C=N, *Str*), 1530(-C=CH, *Str*), 1343(-C=C *Str*), 1232(-C-O *Str*, OCH_3),10437(-C-N *Str*), 802(-Br *Str*, Ar-Br). ^{1}H-NMR (DMSO) δ ppm: 8.3302(1H, s, dibenzofuran Protons), 8.2134(1H, s, Aromatic proton), 8.1009-8.0932(2H, d, Aromatic Protons), 7.943-7.8954(2H, d, Aromatic Protons),7.6874-7.5425(2H, t, Aromatic Protons), 7.5673(1H, s, Aromatic Protons in dibenzofuran), 3.5865(3H, s, methoxy protons in dibenzofuran), 3.2093(2H, s, N-CH_2), 2.0953(3H, s, -CH_3). Mass (LC-MS): m/z 448.04(M), 449.03(M+1, 100%), 4450.27(M+2, 30%).

RESULTS AND DISCUSSION:

Synthesis: Synthesis of5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-one **[IV-3(a-r)]**were carried out by conventional method. In step-II, substituted Istain derivatives I(a-f) were undergone N-alkylation with ethyl/methyl chloride to give a n-alkyl Istain derivatives II(a-r) and these on a Schiff's base reaction with 3-amino-2-methoxydibenzofuran gave title compounds. All the synthesized compound structures were confirmed by spectral analysis by means of IR, ^{1}H-NMR and Mass. The %yield of the synthesized compounds were ranged from 68-86%.

Spectral data: Spectral characterization of the title compounds was performed by IR, 1H-NMR and Mass spectroscopy. In IR spectra of synthesized compounds are showing the aromatic and aliphatic C-H stretching frequency, as expected is observed at around 3008-3097 cm^{-1} and 2902-2812 cm^{-1}. All the compounds have been show strong absorption in the region of 1701-1726 cm^{-1} is found to be presence of C=O(Indole) stretching frequency and in most of the compounds the C=NH stretching of the Imine is around 1602-1626cm^{-1}respectively. The Ar-Cl/F stretching is showing the strong absorption in the region 765-834 cm^{-1}.In the ^{1}H-NMR (DMSO-d6) spectra of novel Indole-2-one derivatives showing a singlet at 3.503-3.875 for methoxy protons in dibenzofuran. Few of the compounds are showing triplet at 2.032-2.394 for -CH_3proton in aromatic ring. The compounds have aromatic protons were found between δ 8.342-6.983 ppm as singlet, doublet and triplet protons.

Antimicrobial activity: All the synthesized 5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-one [IV-3(a-r)]were screened for antimicrobial activity by cup plate (agar diffusion) method[13-14]. The antibacterial activity of the were screened against*Bacillussubstillus,Staphylococcusaureus,Escherichia coli* and *Salmonella paratyphi*species.From the results, the compound **IV-3c, IV-3e, IV-3h** and **IV-3j** are expected to exhibit antibacterial activity and compounds **IV-3d, IV-3e, IV-3g, IV-3l** are showing good antifungal activity.

Table. No:01. Antibacterial activity of novel compounds IV-3(a-r)-Zone of inhibition (in mm)

Compounds	**Zone of Inhibition (in mm)**			
	Staphylococcus aureus	***Bacillus substiles***	***Escherichia Coli***	***Salmonella paratyphi***
IV-3a	21	18	17	10
IV-3b	20	17	09	10

IV-3c	**26***	19	**25***	**27***
IV-3d	09	12	10	13
IV-3e	10	**26***	**24***	**28***
IV-3f	09	15	16	17
IV-3g	20	20	09	12
IV-3h	**23***	**24***	**27***	13
IV-3i	10	09	12	10
IV-3j	**27***	**24***	16	19
IV-3k	19	09	14	13
IV-3l	16	11	09	09
IV-3m	09	14	17	20
IV-3n	11	19	14	10
IV-3o	17	19	20	21
IV-3p	14	10	17	20
IV-3q	10	09	09	21
IV-3r	14	12	09	17
Streptomycin	**30**	**32**	**32**	**34**

All the values are expressed as Zone of Inhibition in mm, bore size=6mm, * The compounds are showed maximum activity against respective bacteria: Zone size 09-11=poor activity, 12-18= Moderate activity; Concentration of the test compounds is 50µg/ml.

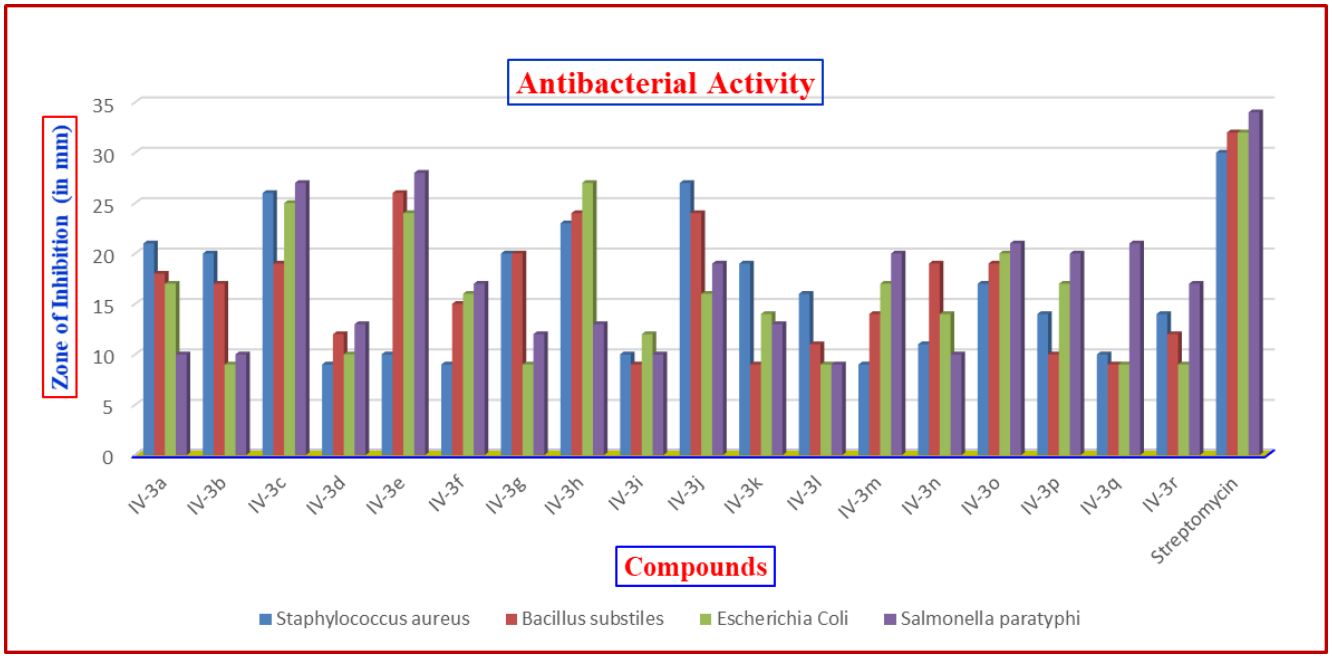


Fig.01: Graphical representation of antibacterial activity of Novel compounds IV-3(a-r)-Zone of inhibition

Table. No:02. Antifungal activity of novel compounds IV-3(a-r)-Zone of inhibition (in

mm)

Compounds	Zone of Inhibition (in mm)		
	Aspergillus niger	***Colletotricumcoffeanum***	***Pencilliumnotatum***
IV-3a	15	14	09
IV-3b	10	-	18
IV-3c	09	15	12
IV-3d	**21***	**24***	**25***
IV-3e	17	**24***	**25**
IV-3f	19	09	15
IV-3g	**23***	**25***	19
IV-3h	-	16	10
IV-3i	17	18	-
IV-3j	18	-	17
IV-3k	15	-	10
IV-3l	**24***	12	**25***
IV-3m	17	-	13
IV-3n	-	17	-
IV -3o	18	20	-
IV-3p	15	-	18
IV-3q	10	10	-
IV-3r	09	-	-
Griseofulvin	**29**	**32**	**34**

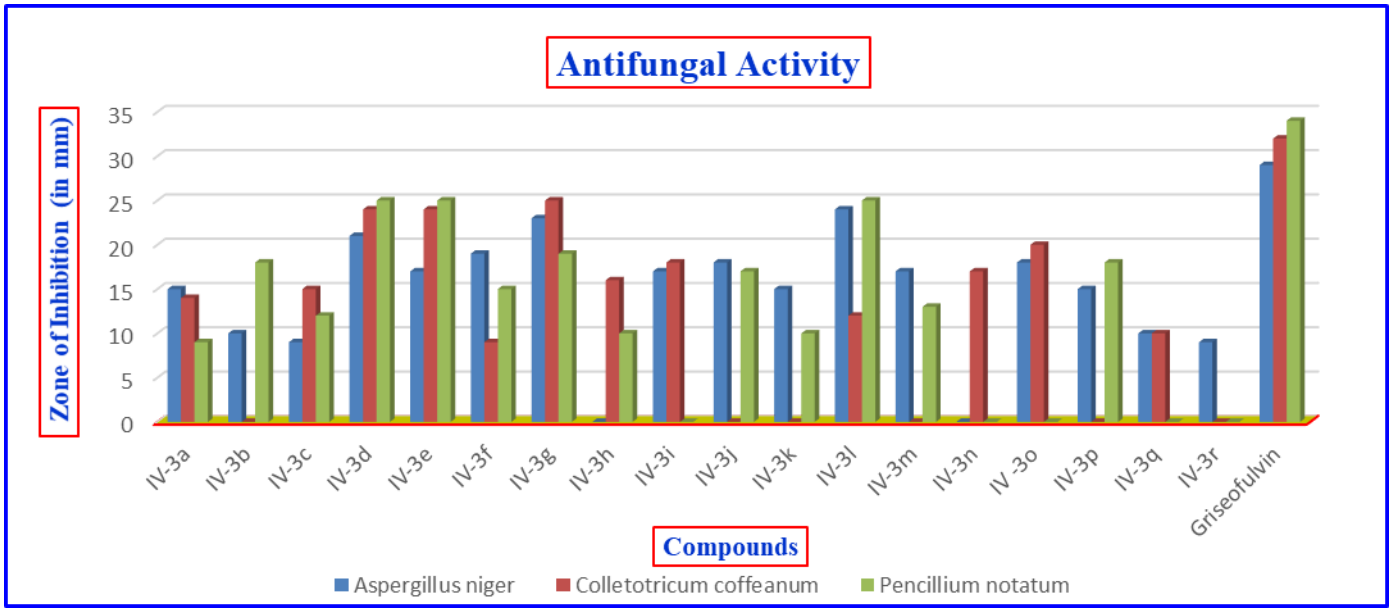


Fig.02: Graphical representation of antifungal activity of Novel compounds IV-3(a-r)-Zone of inhibition

Anticancer Activity:

5-substituted-3-(2-methoxydibenzo[b,d]furan-3-ylimino)-1-substituted-indolin-2-one **[IV-3(a-r)]**were screened for anticancer activity against MCF-7 and SKOV3 cell lines by MTT assay method[14]. All the results proposed that both cell lines were susceptible to the evaluated compounds showed IC_{50} values in the range of **0.043µM** to **0.143**µM against MCF-7 cell line and **0.058 µM** to **0.171 µM**against SKOV3 cell lines**.**Anticancer activity reveals that compounds **IV-3c**active against MCF-7 cell line with $IC_{50}$of **0.043 µM** and **IV-3d** against SKOV3cell line with $IC_{50}$of **0.058µM** respectively.

Table.3. Anticancer activity of Novel compounds [IV-3a, 3c, 3d, 3g, 3l and 3m]

S. No	SAMPLE NAME	MCF-7 IC_{50} (µM)	SKOV3 IC_{50} (µM)
1	IV-3a	0.056	0.118
2	IV-3c	0.043	0.076
3	IV-3d	0.067	0.058
4	IV-3g	0.143	0.152
5	IV-3l	0.056	0.116
6	IV-3m	0.125	0.171
7	Doxorubicin	0.02	0.042

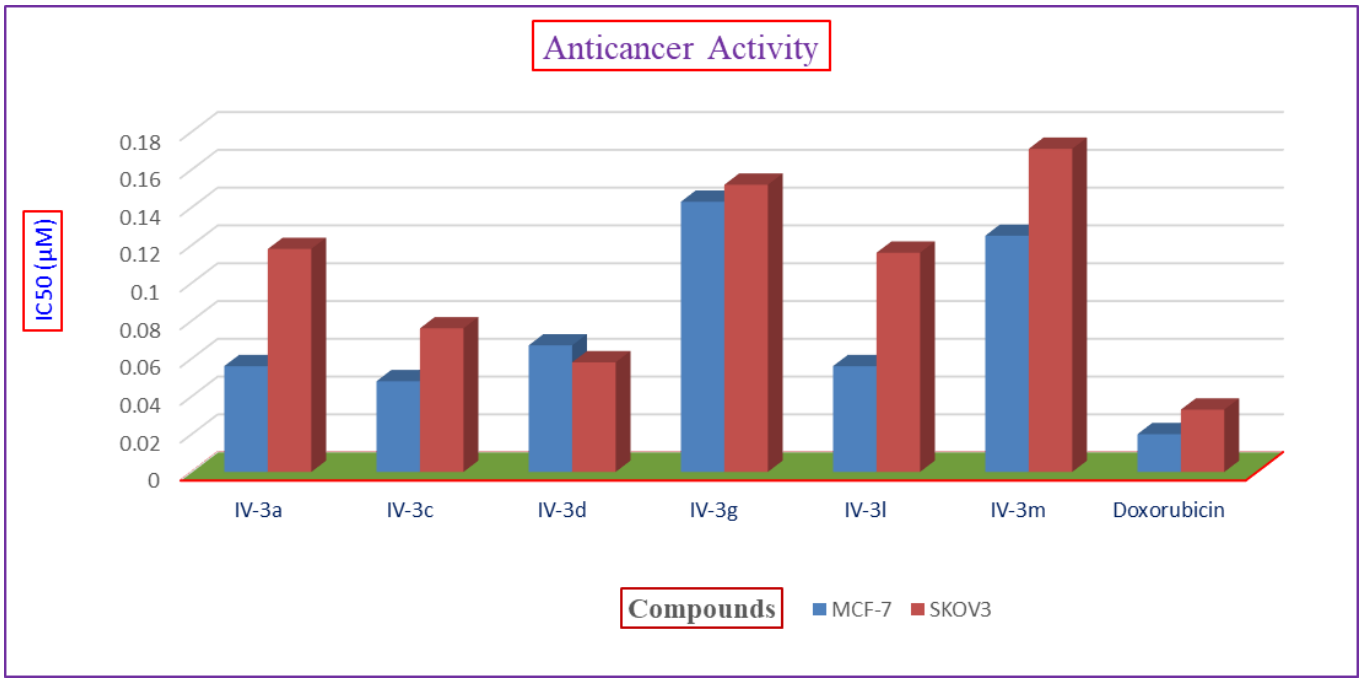


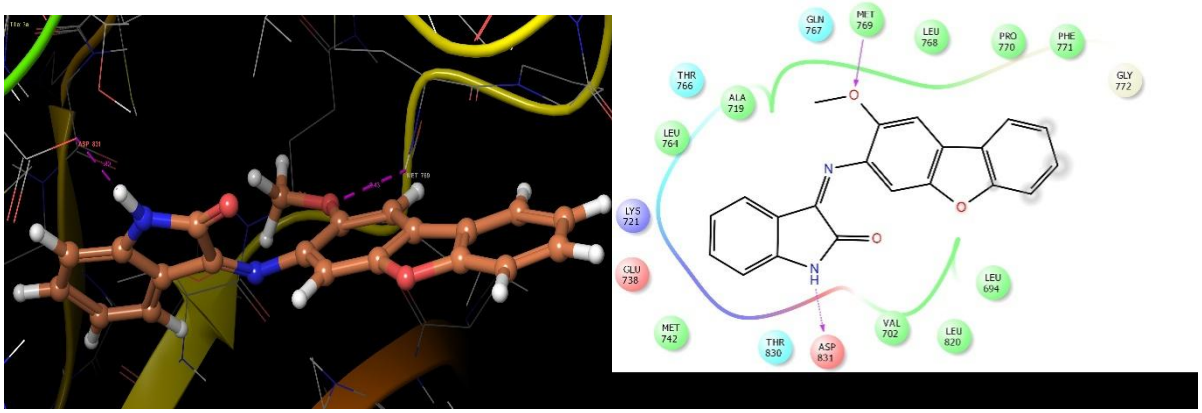
Figure.3. Graphical representation of novel compounds on MCF-7 and SKOV3 Cell lines.

Molecular Docking Studies: The molecular docking studies were carried out using of Schrodinger Suite[15]. I have docked the synthesized novel indole-2-one derivatives into active site of the Epidermal growth factor receptor which was retrieved from the Protein databank website with PDB Id: 1M17.The compound **3a** reported highest dock score of **-6.147** with Glide binding energy of -47.652 Kcal/mol. Dock scores of all the compounds ranged from -6.147 (compound 3a) to -2.34 (compound 3P). MET 769 and Asp 831 are the most common amino acids with H-bonds.

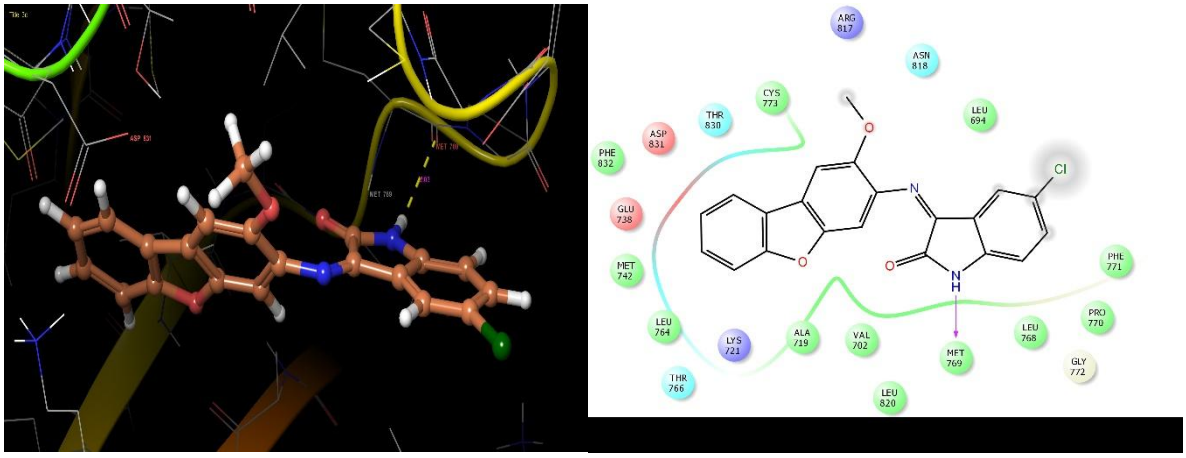
Table.No.4. Insilico EGFR inhibition of Novel Indole-2-one derivatives-Glide dock score of the dataset ligands.

Compound No	Dock score XP GScore	No of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy	Glide energy
IV-3a	-6.147	2	ASP 831 MET 769	1.80 2.43	-53.497	-47.652
IV-3d	-5.332	1	ASP 831	1.93	-48.683	-45.63
IV-3c	-5.262	1	MET 769	2.03	-48.737	-42.88
IV-3g	-5.046	0	-	-	-54.386	-43.232
IV-3f	-5.012	0	-	-	-38.432	-45.12
IV-3k	-5.007	0	-	-	-42.231	-46.323
IV-3h	-4.974	1	MET 769	1..98	-47.674	-40.873
IV-3b	-4.894	1	MET 769	2.04	-50.276	-44.321

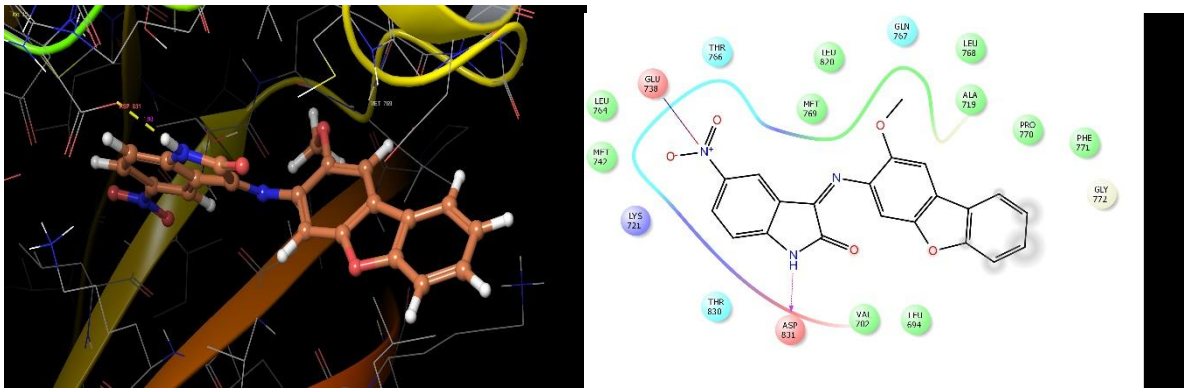
IV-3e	-3.982	1	ASP 831	1.87	-44.387	-39.03
IV-3j	-3.763	1	LYS 721	2.12	-49.943	-38.432
IV-3i	-3.564	0	-	-	-51.127	-37.367
IV-3l	-3.283	0	-	-	-46.321	-40.233
IV-3r	-3.198	0	-	-	-39.94	-43.43
IV-3m	-3.069	2	LYS 721 CYS 773	2.42 1.96	-50.114	-40.409
IV-3q	-2.984	1	MET 769	1.76	-45.854	-42.123
IV-3n	-2.763	0	-	-	-37.093	-48.43
IV-3o	-2.56	0	-	-	-43.432	-47.31
IV-3p	-2.341	1	ASP 831	2.03	-48.982	-41.43



Compound-IV-3a-dock1 and dock-2



Compound-IV-3c-dock1 and dock-2



Compound-IV-3d-dock1 and dock-2

Figure.4. Docking Pose between the Ligand and the Protein (Dock1 and Dock-2)- Compound IV-3a, IV-3c and IV-3d

CONCLUSION:A series of heterocyclic hybrids possessing dibenzofuran and Indole-2-one motifs, IV-3(a-r) has been synthesized by employing condensation followed by N-alkylation and Schiff's base reactions. The structure of the final compounds has been confirmed by spectral and physical methods. All these compounds exhibited significant biological activities like antibacterial, antifungal and anticancer activities.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES:

1. Sharma K, Shrivastava A, Mehra RN, et al. Synthesis of novel Benzimidazole acrylonitriles for inhibition of Plasmodium falciparum growth by dual targetinhibition. *Arch Pharm (Weinheim)*. 2018;351:1700251.
2. Schübler, M., Sadek, B., Kotler, T., Weizel, L., and Stark, H, Synthesis, molecular properties estimations, and dual dopamine D2 and D3 receptor activities of benzothiazole-based ligands. Front. Chem. 5:64-73(2017).
3. M. Kidwai, D. Bhatnagar, P. Mothsra, A. K. Singh, S. Dey, "Molecular Iodine as a Versatile Reagent for Hantzsch Synthesis of 2-Aminothiazole Derivatives," Journal of Sulfur Chemistry. 30(1), 29-36, (2009).

4. Ali, W.A. Wani, A. Khan, A. Haque, A. Ahmad, K. Saleem, N. Manzoor, Synthesis and synergistic antifungal activities of a pyrazoline based ligand and its copper(II) and nickel(II) complexes with conventional antifungals. Microb. Pathogen,53: 66–73 (2016).
5. Tonelli M, Gabriele E, Piazza F, et al. Benzimidazole derivatives endowed withpotentantileishmanial activity. *J Enzyme Inhib Med Chem*. 2018;33:210–226.
6. Cheretaev IV, Korenyuk II, Nozdrachev AD. Neurotropic, psychoactive, andanalgesic properties of benzimidazole and its derivatives: physiological mechanisms.*NeurosciBehav Physiol*. 2018;48:848–853.
7. Cheong JE, Zaffagni M, Chung I, et al. Synthesis and anticancer activity ofnovel water soluble benzimidazole carbamates. *Eur J Med Chem*.2018;144:372–385.
8. Rashid N, Kiran A, Ashraf Z, et al. Synthesis, characterization, antitumor, antibacterialand urease inhibitory activity of a small series of N-tosylbenzimidazoles.*JChemSoc Pakistan*. 2018;40:366–375.
9. Carbone, A., Parrino, B., Di Vita, G., Attanzio, A., Spano` , V.,Montalbano, A., Barraja, P., Tesoriere, L., Livrea, M.A., Diana,P., Cirrincione, G., 2015. Synthesis and anti-proliferative activity ofThiazolyl-bis-pyrrolo[2,3-b]pyridines and Indolyl-thiazolyl-pyrrolo[2,3-c]pyridines, Nortopsentin analogues. Mar. Drugs 13,460–492.
10. Carbone, A., Pennati, M., Barraja, P., Montalbano, A., Parrino, B.,pano` , V., Lopergolo, A., Sbarra, S., Doldi, V., Zaffaroni, N.,Cirrincione, G., Diana, P., 2014. Synthesis and anti-proliferativeactivity of substituted 3[2-(1H-indol-3-yl)-1,3-Thiazol-4-yl]-1HPyrrolo[3,2-b]Pyridines, marine alkaloid Nortopsentinanalogues.Curr. Med. Chem. 21, 1654–1666.
11. Carbone, A., Pennati, M., Parrino, B., Lopergolo, A., Barraja, P.,Montalbano, A., Spano, V., Sbarra, S., Doldi, V., De Cesare, M.,Cirrincione, G., Diana, P., Zaffaroni, N., 2013. Novel 1HPyrrolo[2,3-b]pyridine derivative Nortopsentin analogues: synthesisand antitumor activity in peritoneal mesothelioma experimentalmodels. J. Med. Chem. 56, 7060–7072.
12. Galal, S. A.; Khairat, S. H. M.; Ali, H. I.; Shouman, S. A.; Attia,Y. M.; Ali, M. M.; Mahmoud, A. E.; Abdel-Halim, A. H.; Fyiad, A. A.;Tabll, A.; El-Shenawy, R.; El Abd, Y. S.; Ramdan, R.; El Diwani, H. I.Part II: New candidates of pyrazole-benzimidazole conjugates ascheckpoint kinase 2 (Chk2) inhibitors. Eur. J. Med. Chem. 2018, 144,859−873.

13. Reddy, T. S.; Kulhari, H.; Reddy, V. G.; Bansal, V.; Kamal, A.;Shukla, R. Design, synthesis and biological evaluation of 1,3-diphenyl-1H-pyrazole derivatives containing benzimidazole skeleton aspotential anticancer and apoptosis inducing agents. Eur. J. Med.Chem. 2015, 101, 790−805.
14. Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y.; Alaizari,F.; Ansar, M. h. Synthesis and pharmacological activities ofpyrazole derivatives: a review. Molecules 2018, 23, 134.
15. Manju PT, Smith AA, Padmaja V, et al. In silico design, synthesis and in vitroanti-tubercular and anti-microbial screening of novel benzimidazolederivatives.*Int J PharmaceutSci Res*. 2018;9:3705–3711.