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Research Article

Design, Synthesis, Molecular Docking Studies and Anticancer Activity of 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one Scaffolds

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ABSTRACT

A new series of 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one derivatives (3a-3l) are synthesized by conventional method. All synthesized compounds were characterized via IR, ^{1}H-NMR, ^{13}C-NMR and MASS spectral analysis. Compounds were evaluated for their anticancer activity against MCF-7 cell lines and antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, E. Coli and Salmonella paratyphi.* These results indicated that compound 3a, 3e, 3f and 3j showed good activity compared to the standard drug. Synthesized target molecules exhibited anticancer activity against breast cancer cell lines with IC_{50} values of range from 60.21. to 130.21 µg. Compound 3c (60.21µg) showed good activity compared with doxorubicin. Compounds were subjected to molecular docking studies with estrogen receptor (ER) epidermal growth factor receptor (EGFR) with PDB ID:3ERT. Among the docked ligands, compound 3a and 3e reported highest docking score (-8.512, -6.869) with glide binding energy (-43.785, -24.187).

Introduction

Isatin, the important class of nitrogen containing heterocyclic compound can be sourced from plants, human blood and tissue and acts as important moiety for the development of various heterocyclic compounds especially, indolic and quinolinic compounds.[1] The multicomponent reactions involving isatin as basic moiety are one of the demanding strategies for the one pot synthesis of spirooxindole fused heterocycles.[2] Isatin (1H-indole-2,3-dione) derivatives have wide spectrum of pharmacological activities such as anticancer, antibacterial, antifungal, antiviral, anti-inflammatory and anti-convulsing drugs.[3,4] The second leading cause of death worldwide over the past decades was dreadful cancer characterized by untamed augmentation and propagation of uncontrollable abnormal cells. According to World Health Organization, approximately 9.6 million people worldwide, passed away due to cancer in the year 2018 and one in six deaths was reported globally due to cancer.[5] Cytotoxocity is mainly caused due to non selectivity of chemotherapeutics, for replicating cells leading to hillarious side effects, so the major provocation to medicinal chemistry researchers in inventing or developing strange compact molecules with efficient activity as vigorous and selective anticancer agents needs to get fulfilled.[6]

Modern medicinal chemistry methods have been increasingly employed in the research-based pharmaceutical industry, including molecular modeling,

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as potent tools for structure-activity relationship studies.[7] The field has proceeded hand-in-hand with strengthened biomolecular spectroscopic methods like X-ray crystallography and nuclear magnetic resonance (NMR), which have enabled noticeable progress in structural and molecular biology and have provided crucial structural information about key macromolecular drug targets by the resolution of more than 100,000 three-dimensional protein structures.[8-10]

So as a part of our research in the category of hardback heterocyclic compounds containing indole moiety, the main focus was on the design and development of N-mannich and schiff's base reacions at C-3 of isatin with various secondary amines and heterocyclic amine analogues. Here in we report the design, synthesis of some novel 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one derivatives, their characterization, antibacterial, anticancer and molecular docking studies.

Materials and Methods

The Thieles tube method determined the melting points by using liquid paraffin as a solvent. IR spectra were recorded by Thermo Nicolet Nexus 670 FTIR spectrometer and ^{1}HNMR and ^{13}CNMR were recorded on Bruker DPX-200 Hz using DMSO-d_6 and chemical shifts (δ ppm) are recorded in parts per million downfield from internal reference tetramethylsilane (TMS). Mass spectra had been recorded by the use of Schimadzu LCMS-8030 Mass spectrophometer and all the spectra had been interpreted. The recoated silica gel G plates were used to find the progress of reaction and to assess the purity of the compounds: n-Hexane: Ethyl acetate (8:2). The molecular docking studies were carried out by using Schrodinger Suite.

General Procedure

Step-1: Synthesis of 2-amino-4-phenyl thiazole

A mixture of substituted acetophenone (0.1 mol), thiourea (0.2 mol) and Iodine (0.1 mol) was taken in a cleaned and dried beaker and were heated on a steam bath for 4 hours. After the completion of reaction time, the hydro iodide, thus separated, was filtered under suction, washed with ether and dried. The precipitate collected from the above step was dissolved in hot water completely, filtered while it is still hot and the clear solution was neutralized with a strong solution of ammonia. The solid separated was filtered with repeated water washes and the precipitate was collected. Finally, recrystallized from benzene to get the pure product.

Step-2: Synthesis of N-substituted Isatin derivatives

Substituted isatin (0.01 mol) is taken in alcohol (30 mL) and was stirred with aqueous formaldehyde (0.002 mol) at room temperature for 1-hour. The reaction mixture was then heated under reflux with an appropriate secondary amine (0.02mol) for about 3 hours. The alcohol was removed, the residue obtained was cooled and left overnight in a refrigerator. The resultant product was filtered under suction, washed 2–3 times with small portion of ice-cold water and dried completely. The precipitate thus obtained was recrystallized from aqueous alcohol to get the pure compound.

Synthesis of 5-sustituted-3-((2-(4- ubstitutedphenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one Derivatives

A mixture of equimolar quantity of substituted 4-phenyl-2-amino thiazol (0.01 mol) and substituted Isatin (0.01 mol) was dissolved completely in 30 mL of ethanol. To this mixture 2-3 ml of glacial acetic acid was added. Then the reaction mixture was refluxed for 2–3 hours using steam bath. TLC monitored the progress of the reaction (Hexane: EtOAc 8:2). After the completion of the reaction, n-hexane the reaction mixture was cooled at room temperature and refrigerated overnight for the formation of a precipitate. A solid thus obtained was filtered off and recrystallized from methanol/ethanol to get the pure crystalline solid. The schematic representation of synthesis was shown in Fig. 1 and the physical properties of tittled compounds are tabulated in Table 1.

3a:3-((2-thiazol-4-yl)imino)-1-(dimethyl amino-1-ylmethyl)indolin-2-one

M.P. 213-215°C; Mol. formula: $C_{20}H_{18}N_4OS$, yield 78%, IR (ν cm^{-1}): 3055(C-H *Str*, Ar), 2923, 2882(C–H *Str*, Aliphatic), 2380(C-S-C *Str*, thiazole), 1701(C=O *Str*, Indole), 1512(C=CH *Str*), 1453(C=C *Str*, Ar), 1099(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.446(s, 1H, thiazole proton), 8.1683-8.0593(d, 2H, Ar-H), 7.9696-7.5228(d, 2H, Ar-H),7.6990-7.5187(t, 2H, Ar-H), 7.5060-7.4307(t, 3H, Ar-H), 4.249-4.237(s, 2H, -N-CH_2-N), 2.737-2.730(s, 6H, -N$(CH_3)_2$. ^{13}C-NMR (DMSO) δ ppm: 171.80, 162.79, 160.1,157.0, 154.5, 141.94, 137.94, 135.30, 129.99, 128.1, 126.84,124.78,120.72,114.43, 109.76, 64.62, 24.63. Mass (LC-MS): m/z 362 (M), 363(M + 1, 100%).

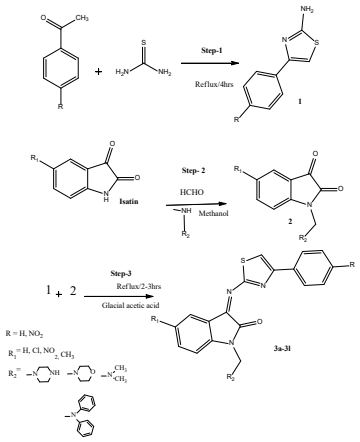

Fig. 1: Scheme

3b: 3-((2-thiazol-4-yl)imino)-1-(N,N-diphenyl)amono-1-ylmethyl)indolin-2-one

M.P. 203-205°C; Mol. formula: $C_{30}H_{22}N_4OS$, yield 72%, IR (ν cm^{-1}): 3055(C-H Str, Ar), 2955, 2817(C–H Str, Aliphatic), 2315(C-S-C Str, thiazole), 1728(C=O Str, Indole), 1507(C=CH Str), 1420(C=C Str, Ar), 1183(C-N Str). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.3638-8.3056(d, 2H, Ar-H), 8.4699(s, 1H, thiazole proton), 8.2738-8.040(d, 2H, Ar-H), 7.9595-7.9011(t, 2H, Ar-H), 7.8752-7.8552(d, 2H, Ar-H), 7.7980-7.7487(d, 2H, Ar-H), 7,6663-7.6497(t, 3H, Ar-H), 7.5013-7.3360(t, 3H, Ar-H), 7.2643-7.0970(t, 3H, Ar-H), 4.164-4.159(s, 2H, -N-CH_2-N). ^{13}C-NMR (DMSO) $\delta\delta$ ppm: 175.99, 153.14,150.83,145.79, 139.06, 138.95, 127.70, 126.67, 120.63, 114.40, 113.96, 112.41, 109.99, 49.99. Mass (LC-MS): m/z 386 (M), 387(M + 1, 100%).

3c: 5-nitro 3-((2-thiazol-4-yl)imino)-1-(piperidin-1-ylmethyl)indolin-2-one

M.P. 227-229°C; Mol. formula: $C_{23}H_{21}N_5O_3S$, yield 82%, IR (ν cm^{-1}): 3064(C-H Str, Ar), 2918, 2832, 2773.67(C–H Str, Aliphatic), 2364(C-S-C Str, thiazole), 1703.10(C=O Str, Indole), 1632(NO_2 Str, Ar-NO_2), 1501(C=CH Str), 1455(C=C Str, Ar), 1155(C-N Str). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4974(s, 1H, Ar-H), 8.3775(s, 1H, thiazole proton), 7.9733-7.8428(d, 2H, Ar-H), 7.7960-7.7813(d, 2H, Ar-H), 7.6020-7.4083(t, 3H, Ar-H), 4.548(s, 2H, -N-CH_2-N), 2.742(t, 4H, -CH_2-O-CH_2). 2.281(m, 6H, -Cycloalkanes –CH_2-). ^{13}C-NMR (DMSO) δ ppm: 173.01, 153.16, 150.86, 145.25, 141.79, 139.0, 138.98, 127.79, 126.61, 120.63, 114.42, 113.94, 112.49, 109.76, 45.09. Mass (LC-MS): m/z 447.14(M), 448(M + 1, 100%).

3d: -((2-thiazol-4-yl)imino)-1-(piperidin-1-ylmethyl) indolin-2-one

M.P. 191-193°C; Mol. formula: $C_{23}H_{22}N_4OS$, yield 76%, IR (ν cm^{-1}): 3020(C-H *Str,* Ar), 2985, 2857(C–H *Str,* Aliphatic), 2409(C-S-C *Str,* thiazole), 1700(C=O *Str,* Indole), 1549(C=CH *Str*), 1485(C=C *Str*, Ar), 1238(C-N Str). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.3685(s, 1H, thiazole proton), 8.2970-8.1142(d, 2H, Ar-H), 7.8892-7.7714(t, 3H, Ar-H), 7.5513-7.4111(t, 2H, Ar-H), 7.1458-7.1098(d, 2H, Ar-H), 4.643-4.432(s, 2H, -NCH_2-N), 3.745-3.452(m, 4H, piperidin), 2.623-2.526(m, 6H, piperidin). ^{13}C-NMR (DMSO-d_6) δ ppm: 178.32, 155.26, 149.02, 143.65, 144.22, 136.2, 135.02, 126.32, 124.65, 122.62, 117.93, 115.912, 113.06, 110.54, 46.81, 32.83, 23.45. Mass (LC-MS): m/z 402.15(M), 403(M + 1, 100%).

3e:3-((2-thiazol-4-yl)imino)-1-(morpholine-1-ylmethyl) indolin-2-one

M.P. 235-237°C; Mol. formula: $C_{22}H_{20}N_4O_2S$, yield 77%, IR (ν cm^{-1}): 3073(C-H Str, Ar), 2932, 2838(C–H Str, Aliphatic), 2340(C-S-C Str, thiazole), 1705(C=O Str, Indole), 1512(C=CH Str), 1475(C=C Str, Ar), 1180(C-N Str). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.3685(s, 1H, thiazole proton), 8.2970-8.1142(d, 2H, Ar-H), 7.8892-7.7714(t, 3H, Ar-H), 7.5513-7.4111(t, 2H, Ar-H), 7.1458-7.1098(d, 2H, Ar-H), 4.4512-4.4311(s, 2H, -N-CH_2-N), 3.6393(t, 4H, morpholine). 2.5599(m, 4H, morpholine). ^{13}C-NMR (DMSO) δ ppm: 171.21, 158.44, 152.17, 140.43, 138.93, 137.32, 135.12, 129.34, 125.34, 120.99, 117.09, 115.43, 114.55, 47.33, 26.23, 22.04. Mass (LC-MS): m/z 405.13(M), 406.32(M + 1, 100%).

3f:5-methyl-3-((2-thiazol-4-yl)imino)-1-(morpholine-1-ylmethyl)indolin-2-one

M.P. 203–205°C; Mol. formula: $C_{23}H_{22}N_4O_2S$, yield 71%, IR (ν cm^{-1}): 3057(C-H *Str*, Ar), 2950, 2858, 2751(C–H *Str*, Aliphatic), 2348(C-S-C *Str*, thiazole), 1701(C=O *Str*, Indole), 1531(C=CH *Str*), 1434(C=C *Str,* Ar), 1122(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4532(s, 1H, Ar-H), 8.2310(s, 1H, thiazole proton), 7.9843-7.9326(d, 2H, Ar-H), 7.5743-7.4532(d, 2H, Ar-H), 7.4532-7.2854(t, 3H, Ar-H), 4.642-4.564(s, 2H, -N-CH_2-N), 3.564(t, 4H, morpholine). 2.875(m, 4H, morpholine), 2.212(s,3H, Ar-CH_3). ^{13}C-NMR (DMSO) δ ppm: 178.34, 157.51, 155.45, 145.09, 140.32, 136.03, 134.33, 128.23, 126.02, 123.54, 116.41, 112.83,

Table 1: Physical properties of compounds(3a-3l)

Sample Code	*Molecular Formula*	*R*	*R_1*	*R_2*	*Mol. Wt*	*M.P (^{0}C)*	*% Yield*	*R_f value*
3a	$C_{20}H_{18}N_4OS$	H	H	N,N-dimethyl amine	362	213-215	78	0.59
3b	$C_{30}H_{22}N_4OS$	H	H	N, N-diphenyl amine	386	203-205	72	0.66
3c	$C_{23}H_{21}N_5O_3S$	H	NO_2	Piperidine	447	227-229	82	0.64
3d	$C_{23}H_{22}N_4OS$	H	H	Piperidine	402	191-193	76	0.85
3e	$C_{22}H_{20}N_4O_2S$	H	H	Morpholine	404	235-237	77	0.66
3f	$C_{23}H_{22}N_4O_2S$	H	CH_3	morpholine	418	203-205	71	0.79
3g	$C_{20}H_{17}N_5O_3S$	NO_2	H	N,N-dimethyl amine	407	250-253	80	0.69
3h	$C_{22}H_{19}N_4O_2SCl$	Cl	H	morpholine	438	242-244	82	0.65
3i	$C_{23}H_{20}N_6O_5S$	NO_2	NO_2	Piperidine	492	261-263	73	0.71
3j	$C_{30}H_{21}N_5O_3S$	NO_2	H	N, N-diphenyl amine	531	191-193	81	0.67
3k	$C_{23}H_{21}N_5O_4S$	NO_2	CH_3	Morpholine	463	169-172	79	0.60
3l	$C_{22}H_{19}N_5O_4S$	NO_2	H	Morpholine	449	237-239	76	0.72

110.43, 41.33, 26.32, 21.89. Mass (LC-MS): m/z 418.15(M), 419.30(M + 1, 100%).

3g:3-((2-(4-nitrophenyl) thiazol-4-yl)imino)-1-(dimethyl amino-1-ylmethyl)indolin-2-one

M.P. 250–253°C; Mol. formula: $C_{20}H_{17}N_5O_3S$, yield 80%, IR (ν cm^{-1}): 3058(C-H *Str*, Ar), 2959, 2846, 2727(C–H *Str*, Aliphatic), 2400(C-S-C *Str*, thiazole), 1703(C=O *Str*, Indole), 1612(NO_2, *Str*), 1536(C=CH *Str*), 1344(C=C *Str,* Ar), 1236(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4719-8.3505(d, 2H, Ar-H), 8.0636-7.9201(d, 2H, Ar-H), 7.8287-7.7573(t, 2H, Ar-H), 7.1727-7.1418(s, 1H, thiazole proton), 6.8831-6.8661(d, 2H, Ar-H), 4.4424-4.4071(s, 2H, -N-CH_2-N), 2.675-2.647(s, 6H, -N(CH_3)$_2$). ^{13}C-NMR (DMSO) δ ppm: 173.31, 159.93, 158.22, 148.83, 144.21, 137.42, 136.03, 130.87, 128.94, 125.45, 118.66, 115.11, 113.45, 42.32, 28.82. Mass (LC-MS): m/z 408.11(M), 409.33(M + 1, 100%).

3h: 5-chloro-3-((2-thiazol-4-yl)imino)-1-(morpholine-1-ylmethyl)indolin-2-one

M.P. 242–244°C; Mol. formula: $C_{22}H_{19}N_4O_2SCl$, yield 82%, IR (ν cm^{-1}): 3097(C-H *Str*, Ar), 2982, 2933, 2831(C–H *Str*, Aliphatic), 2398(C-S-C *Str*, thiazole), 1692(C=O *Str*, Indole), 152(C=CH *Str*), 1381(C=C *Str,* Ar), 1270(C-N *Str*), 757(Ar-Cl *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4784(s, 1H, Ar-H), 8.3785(s, 1H, thiazole proton), 7.9508-7.8863(d, 2H, Ar-H), 7.8412-7.7999(d, 2H, Ar-H), 7.1439(t, 3H, Ar-H), 4.514(s, 2H, -N-CH_2-N), 3.647(m, 4H, morpholine). 2.507(m, 4H, morpholine). ^{13}C-NMR (DMSO) δ ppm: 173.98, 155.51, 153.82, 147.31, 145.34, 136.01, 133.28, 131.07, 129.32, 126.33, 120.19, 117.34, 115.87, 48.21, 32.73, 24.92. Mass (LC-MS): m/z 438.09(M), 439.52(M + 1, 100%), 440.18(M + 2, 30%).

3i:5-nitro-3-((2-(4-nitrophenyl) thiazol-4-yl)imino)-1-(piperidin -1-ylmethyl)indolin-2-one

M.P. 261–263°C; Mol. formula: $C_{23}H_{20}N_6O_3S$, yield 73%, IR (ν cm^{-1}): 3087(C-H *Str,* Ar), 2905, 2870(C–H *Str,* Aliphatic), 2398(C-S-C *Str,* thiazole), 1753(C=O *Str*, Indole), 16059-NO_2 *Str*), 1484(C=CH *Str*), 1457(C=C *Str*, Ar), 1187(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.2435(s, 1H, Ar-H), 8.0432(s, 1H, thiazole proton), 7.9087-7.8412(d, 2H, Ar-H), 7.7682-7.6573(d, 2H, Ar-H), 7.5421-7.4872(d, 2H, Ar-H), 4.564-4.482(s, 2H, -N-CH_2-N), 3.590-3.412(m, 4H, piperidin), 2.875-2.784(m, 6H, piperidin). ^{13}C-NMR (DMSO) δ ppm: 176.12, 158.04, 147.23, 148.44, 143.98, 134.38, 133.19, 132.18, 130.43, 127.43, 120.31, 118.52, 115.23, 113.78, 49.65, 36.33, 28.23. Mass (LC-MS): m/z 492.12(M), 493.43(M + 1, 100%).

3j: 3-((2-(4-nitrophenyl)-thiazol-4-yl) imino)-1-(N,N-diphenyl)amono-1-ylmethyl)indolin-2-one

M.P. 191–193°C; Mol. formula: $C_{30}H_{21}N_5O_3S$, yield 81%, IR (ν cm^{-1}): 3076(C-H *Str,* Ar), 2979, 2761(C–H *Str,* Aliphatic), 2349(C-S-C *Str,* thiazole), 1707(C=O *Str*, Indole), 16057(-NO_2 *Str*), 1532(C=CH *Str*), 1444(C=C *Str*, Ar), 1249(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.3771-8.2838(d, 2H, Ar-H), 8.1086(s, 1H, thiazole proton), 7.8868-7.8482(d, 2H, Ar-H), 7.6881-7.6359(d, 4H, Ar-H), 7.5801-7.5788(t, 2H, Ar-H), 7.5585-7.5173(t, 6H, Ar-H),7.1413-7.1186(d, 2H, Ar-H), 4.254-4.230(s, 2H, -N-CH_2-N). ^{13}C-NMR (DMSO) δ ppm: 177.43, 154.23, 144.03, 140.34, 139.21, 136.43, 135.76, 134.44, 133.17, 132.94, 129.32, 125.22, 123.22, 121.254, 119.45, 119.93, 118.75, 47.43. Mass (LC-MS): m/z 531.56(M), 532.76(M + 1, 100%).

3k:5-methyl-3-((2-(4-nitrophenyl) thiazol-4-yl)imino)-1-(morpholine-1-ylmethyl)indolin-2-one

M.P. 169–172°C; Mol. formula: $C_{23}H_{21}N_5O_4S$, yield 79%, IR (ν cm^{-1}): 3091(C-H *Str*, Ar), 2987, 2854, 2731(C–H *Str*, Aliphatic), 2365(C-S-C *Str*, thiazole), 1711(C=O *Str*, Indole), 1623(-NO_2 *Str*), 1528(C=CH *Str*), 1401(C=C *Str,* Ar), 1198(C-N *Str*), 757(Ar-Cl *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4512(s, 1H, Ar-H), 8.2734(s, 1H, thiazole proton), 8.0932-8.0043(d, 2H, Ar-H), 7.8976-7.7685(d, 2H, Ar-H), 7.6756-7.5643(d, 2H, Ar-H), 4.409-4.387(s, 2H, -N-CH_2-N), 3.803(m, 4H, morpholine). 2.783(m, 4H, morpholine), 2.043(s, 3H, Ar-CH_3). ^{13}C-NMR (DMSO) δ ppm: 174.18, 159.91, 153.82, 150.22, 147.54, 139.39, 137.95, 135.12, 126.87, 124.29, 123.01, 120.33, 118.04, 47.79, 35.21, 26.32, 20.84. Mass (LC-MS): m/z 463.13(M), 464.32(M + 1, 100%).

3l:3-((2-(4-nitrophenyl) thiazol-4-yl)imino)-1-(morpholine -1-ylmethyl)indolin-2-one

M.P. 227–229°C; Mol. formula: $C_{22}H_{19}N_5O_4S$, yield 76%, IR (ν cm^{-1}): 3056(C-H *Str*, Ar), 2975, 2866, 2784(C–H *Str*, Aliphatic), 2365(C-S-C *Str*, thiazole), 1712(C=O *Str*, Indole), 1618(-NO_2 *Str*), 1543(C=CH *Str*), 1412(C=C *Str,* Ar), 1187(C-N *Str*). ^{1}H-NMR (DMSO-d_6) δ ppm: 8.4043-8.3908(d, 2H, Ar-H), 8.2675(s, 1H, thiazole proton), 8.1542-8.0543(d, 2H, Ar-H), 7.9843-7.7862(d, 2H, Ar-H), 7.7683-7.5423(t, 2H, Ar-H), 4.378-4.234(s, 2H, -N-CH_2-N), 3.654(m, 4H, morpholine). 2.865(m, 4H, morpholine). ^{13}C-NMR (DMSO) δ ppm: 178.43, 156.03, 152.89, 151.54, 148.64, 136.32, 133.04, 130.43, 125.76, 122.43, 120.76, 119.32, 123.61, 45.82, 34.12, 24.33. Mass (LC-MS): m/z 449.12(M), 450.05(M + 1, 100%).

Pharmacological Activity

Anticancer Activity

Cell viability measurement and cell proliferation forms the basis for numerous *in-vitro* assays of a cell population's response to external factors. The MTT cell proliferation assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, cell viability is reduced.[11] The cell viability was once appraised by means of the MTT Assay with three impartial experiments with six different concentrations of compounds in triplicates. Cells have been trypsinized and function the trypan blue assay to be aware of doable cells in cell lline suspension. Total no of cells had been counted

by using a hemocytometer and seeded at a density of 5.0×10^3 cells/properly in one hundred µL media in ninety-six properly plate subculture medium and incubated in a single day at 37°C. After incubation, remove the old media and add fresh media 100 µL with different concentrations of synthesized analogs in labelled wells in 96 plates. After 48 hours, discard the drug solution and add the fresh media with MTT solution (0.5 mg/mL) was added to each well, plates were incubated at 37°C for 3 hours. At the give up of incubation time, precipitates are fashioned as an end result of the discount of the MTT salt to chromophore Formosan crystals by way of the cells with metabolically energetic mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. Novel isatin derivatives were evaluated for cytotoxicity against human breast cancer cells (MCF7) using MTT assay, with Doxorubicin as standard drug. Results (Table 2) proposed that MCF cell lines were susceptible to the evaluated compounds. Graphical representation of anticancer activity was shown in Figs. 2 and 3

Antibacterial Activity

All the newly synthesized of 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one Derivatives (3a-3l) were screened for antimicrobial activity by agar diffusion-cup plate method against *Staphylococcus aureus, Bacillus subtilis* (Gram positive) and *Escherichia coli, Salmonella paratyphi, Pseudomonas* (Gram negative) bacteria. Finally, to determine the zone of inhibition in mm. The results were evaluated by comparing the zone of inhibition the synthesized compounds showed with standard drug (Streptomycin)[12] was tabulated in Table 3 along with graphical representation and Photograph of antibacterial activity was shown in Figs. 4 and 5 respectively.

Molecular Docking Studies

In molecular docking studies estrogen receptor(ER) epidermal growth factor receptor(EGFR) alpha was retrieved from the Protein databank internet site with PDB Id: 3ERT. And the deleting unbound water molecules which are over 1 Å, shape is to be optimized, 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 the usage of protein preparation Wizard device of Schrodinger Suite. Thus by utilizing Glide Xp docking protocol, structurally optimized protein configuration was once used to notice protein-ligand interactions of the dataset ligands. Initially, all the dataset ligands had been docked by setting up a 3D grid to the protein's binding pocket (active site).[13-15] Binding interactions and the binding efficiency have been calculated in phrases of Glide Score, which combines hydrophilic, hydrophobic, steel binding groups, Vander Waals energy and freezing rotatable bonds. Molecular docking research has been carried out to pinpoint the dataset ligands' viable protein ligand interactions. Additionally, assisted to track down the conformational adjustments of the ligand in the protein environment. Based on the E Model energy, solely one was once displayed in the result. Glide dock sores of the dataset ligands have been proven in Table 3 alongside with the interplay amino acids and quantity of amino acids.

Results and Discussions

Synthesis

Newly synthesiszed 5-sustituted-3-((2-(4-substitute-dphenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one derivatives(3a-3l) were prepared vi cyclization, N-alky/Benzylation and Schiff's base reactions. IR, ^{1}H-NMR and mass spectral data confirmed all the target molecular structures. In IR spectral data, all the compounds have aromatic C-H stretching frequency observed at around 3010–3098 cm^{-1} and aliphatic C-H stretching frequency observed around 2998–2725 cm^{-1}. The sharp absorption peak is observed at 232–2410 cm^{-1} indicates the presence of C-S-C stretching in thiazole ring. We are observed a single strong absorption peak at around 1695–1725 cm^{-1} is found to be presence of carbonyl group (C=O). Most of the compounds are given the C-C stretching of the aromatic ring is around 1505 to 1548 cm^{-1}, respectively. Some of the compounds are showing absorption peak around 785–823cm^{-1} is indicate C-Cl stretching. In ^{1}H-NMR(DMSO-d_6) spectral analysis of novel indole-2one derivatives showing multiple around at δ 3.782 to 2.520 for morpholine and piperidin protons. All the target molecules showing a singlet, doublet and triplet at δ 8.623 to 6.783 for aromatic protons and singlet pound at δ 4.865–4.453 for mannich base protons -N-CH_2-N). Few compounds are showed singles a singlet at δ 2.345–1.98 for methyl group (Ar-CH_3).

Anticancer Activity

All the target molecules were screened by MTT assay method. From these results (Table 3), the compounds 4a (80.50 µg), 4b (83.23 µg) and 4e (60.21 µg**)** showed good activity and all showed IC_{50}values in the range of 30.21 to 130.01 µg against MCF7 cell line. Compounds 7c and 7k showed good activity against the cell lines, whereas remaining synthesized compounds showed tolerable

Table 2: Cytotoxic Activity of Indole-2-one derivatives (3a, 3b, 3c, 3f and 3k) on MCF7 Cells

S. No	Sample Name	IC_{50} (µg)
1	3a	80.50 ± 0.318
2	3b	83.63 ± 0.4676
3	3c	60.21 ± 0.519
4	3f	87.95 ± 0.814
5	3k	130.01 ± 0.421
6	Doxorubicin	16.32 ± 0.142

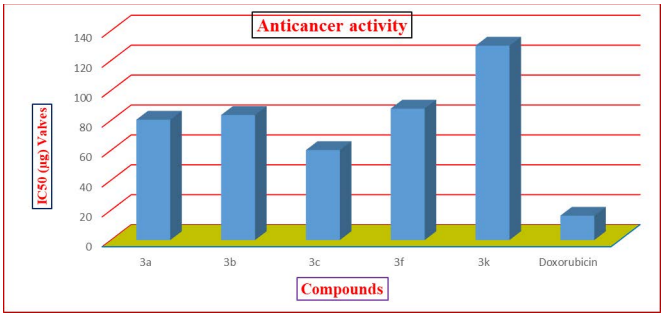

Fig. 2: Graphical representation of novel indole-2-one derivatives(3a, 3b, 3c,3f, 3k) on MCF-7 Cells

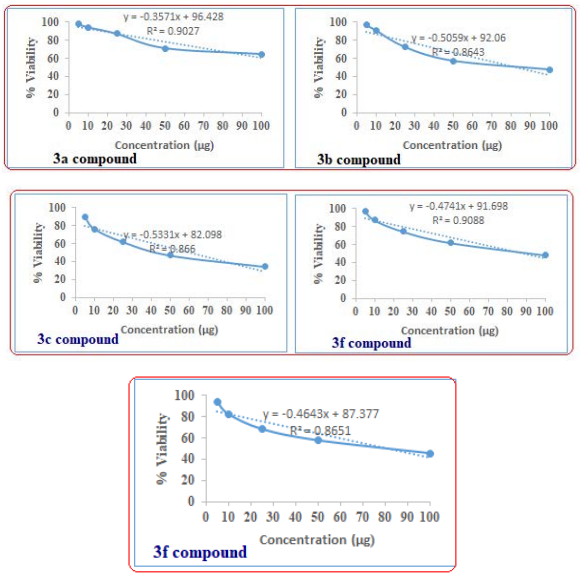

Fig. 3: Graphical representation of novel indole-2-one derivatives-IC_{50} values

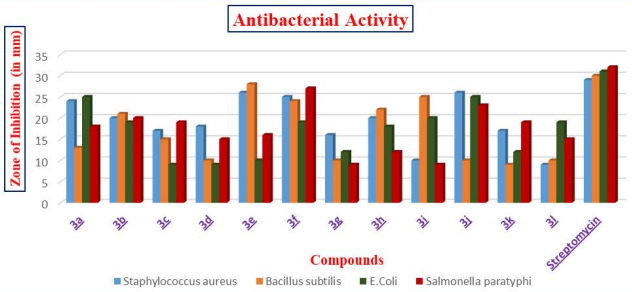

Fig. 4: Graphical representation of antibacterial activity of novel indole-2-one derivatives(3a-3l) –Zone of Inhibition.

Fig. 5: Photograph for antibacterial activity of novel indole-2-one derivatives.

Table. 3: Antibacterial activity of compounds(3a-3l)

	Zone of Inhibition (in mm)			
	Staphylococcus aureus	*Bacillus subtilis*	*E. coli*	*Salmonella paratyphi*
3a	24*	13	25*	18
3b	20	21	19	20
3c	17	15	09	19
3d	18	10	09	15
3e	26*	28*	10	16
3f	25*	24*	19	27*
3g	16	10	12	09
3h	20	22	18	12
3i	10	25*	20	09
3j	26*	10	25*	23*
3k	17	09	12	19
3l	09	10	19	15
Streptomycin	29	30	31	32

activity. All the results were shown in the table and expressed as a mean ± SEM of five concentrations each. The compounds confirmed reasonable activity via those with thiazole and indole-2-one skeleton and the presence of hydrophobic and electron-withdrawing substituent (–Cl, $–NO_2$, $-C_2H_5$) in the aromatic and fused heterocyclic moiety will increase their activity.

Antibacterial Activity

All the newly synthesized of 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-yl methyl) Indolin-2-one derivatives (3a-3l) were screened for antimicrobial activity by agar diffusion-cup plate method to determine the zone of inhibition in mm. The compound 3a (24* against *s. aureus*, 25* against *E. coli*), 3e (26* against *s. aureus*, 28* against *Bacillus substilis*), 3f (25* against *s. aureus*, 24* against *E. coli*, 27* against *Salmonella paratyphi*) and 3j (26* against *s. aureus*, 23* against *E. coli)*. The presence of electron-withdrawing group –Cl and $–NO_2$ in the aromatic/indole-2-one rings was found to be beneficial.

Structural Activity Relationship

Structural activity relationship of the synthesized thiazole fused novel Indole-2-one derivatives bearing substituted 2^0 amines like N, N-dimethyl amine, diphenylamine, piperidine, morpholine and electron-withdrawing, electron releasing groups at ortho/meta/para positions plays on magnificent role in the antibacterial, anticancer activities was shown in Fig. 6. Structure activity relationship for anticancer and antibacterial activity of synthesized novel Indole-2-one derivatives can be deduced as following.

- The novel indole-2-one analogs contain electron-withdrawing groups like Cl, NO_2 at para/ortho

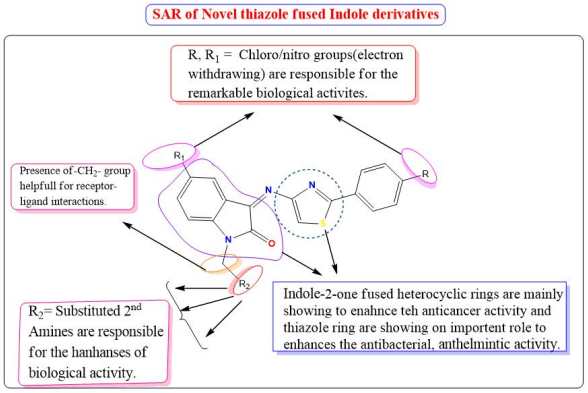

Fig 6: Structural Activity Relationship(SAR) of Novel Thiazole Fused Isatin Derivatives

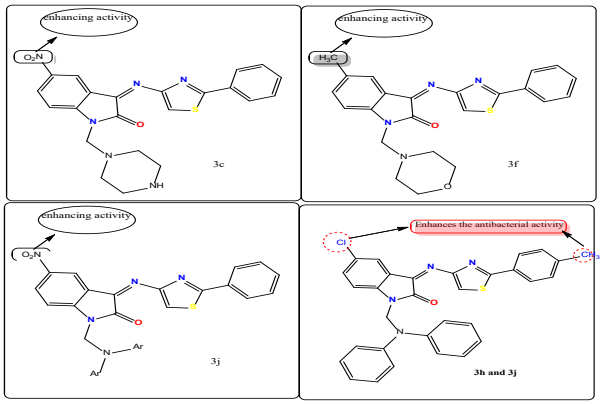

Fig 7: SAR- Electron withdrawing Groups Containing compounds Showing enhanced Pharmacological activity.

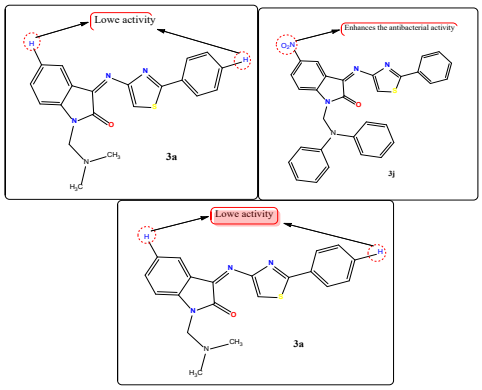

Fig 8: SAR- Substituted compounds showing good activity than the compounds with unsubstitution.

positions, enhances the antibacterial and anticancer activities.

- Novel indole-2-one analogs has methyl or methoxy (electron donating) group at ortho/para position of the aromatic rings (Isatin or Phenyl) enhances the anticancer, anthelmintic and antibacterial activity.
- Indole-2-one derivatives having electron withdrawing groups like –F, -Cl, -Br and $-NO_2$ at para position and electron releasing group like $-CH_3$, $-OCH_3$ at ortho position enhancing the biological activities.
- SAR of novel Indole-2-one derivatives (3a-j) revealed that anthelminthic, antibacterial and anticancer activity dependence is necessary on the attached R and R_1 at 4th positions of indole and thiazole rings (3c, e, f and j) showed remarkable activities as shown in Figs 7 and 8.
- The synthesized compounds with para methyl substitutes (3f) showed more antibacterial activity than unsubstituted analogs (3a).
- Some of the derivatives have hydrophobic groups like chlorine/methyl at the 4th position of the phenyl ring (3f, h and k), which is assumed to be crucial, leading to remarkable increases in cytotoxicity and antibacterial activities.
- All the synthesized analogue's bearing the para-substituted phenyl group (3a-l) results in increasing anticancer activity (3c), and antibacterial activity (3f and j), especially on the MCF-7 cell line.
- The synthesized compounds with para methyl substitutes (3f) showed more antibacterial activity than unsubstituted analogs (3a) as shown in Fig 8.

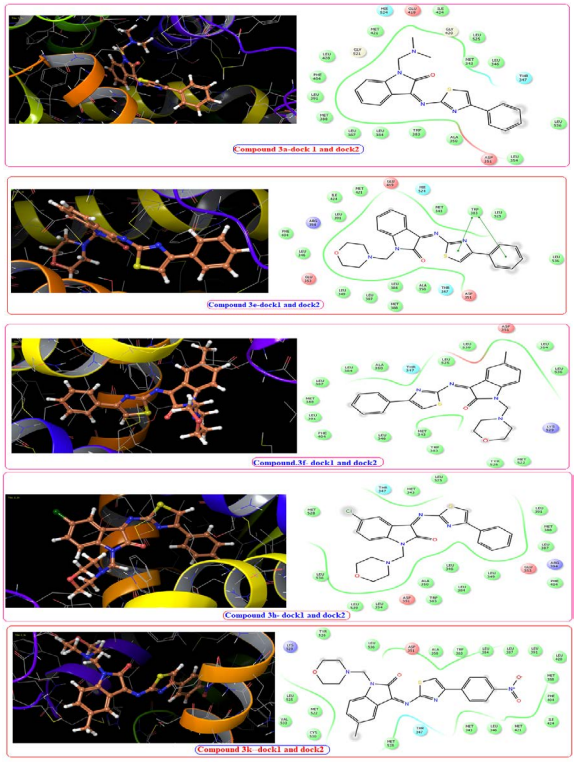

Fig. 9: Docking pose between the ligand(3a, 3c, 3e, 3f, 3h and 3k) and the protein (dock1 and dock-2).

Molecular Docking Studies

Molecular docking studies were performed in order to find the possible protein-ligand interactions of the dataset ligands. Additionally, these also assisted in identifying the conformational changes of the ligand in the protein environment. About generates 100 different protein-ligand complex conformations for each docked complex were generated through Glide XP module. Based on the E Model

Table 4: *In-silco* EGFR Inhibition of Indole-2-one Derivatives-Glide Dock Sores 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*
3a	-8.512	0	-	-	-63.653	-43.785
3e	-6.869	0	TRP 383	-	-2.161	-24.187
3f	-6.656	0	-	-	-31.603	-37.904
3k	-6.445	0	-	-	-61.887	-43.745
3h	-6.128	0	-	-	-27.836	-35.949
3g	-5.626	0	-	-	-55.105	-38.838
3c	-3.142	0	-	-	-34.187	-36.142

energy, only one was displayed in the result. Glide dock sores of the dataset ligands were shown in Table 4 along with the interaction amino acids and number of amino acids. Among the docked ligands, compound 3a reported highest dock scores of -8.512 with Glide binding energy of -43.785 Kcal/mol. Dock scores of all the compounds ranged from -8.512 (compound 3a) to -3.142 (compound 3c).

Conclusion

In the present investigation is focused on the synthesis, characterization of 5-sustituted-3-((2-(4-nitrophenyl) Thiazol-4-yl) Imino)-1-(substituted-1-ylmethyl) Indolin-2-one derivatives (3a-3l) and biological activities (antibacterial and anticancer). The compound 3a, 3e and 3f showed good antibacterial activity against *S. aureus, E. coli* bacteria. compound 7c and 7k please include:Compound 3a and 3c has shown good anticancer activity against MCF-7 cell lines, whereas, the remaining synthesized compounds showed tolerable activity. Further, the molecular docking study of the target data set of indole-2-one derivatives was performed by Schrodinger Suite. In this study, we used estrogen receptor (ER) epidermal growth factor receptor (EGFR) alpha with PDB Id: 3ERT. Molecular docking results showed that compound 3a reported the highest dock score of -8.512 with glide binding energy of -43.785 Kcal/mol with moderate to better anticancer activity potency towards cancer cell line within the binding pocket of the receptor and docking pose between the ligand and the protein was shown in Fig. 9.

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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.

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