Small Heterocyclic Molecules as Anticancer Agents: Design, Syntheses and Evaluation Against MCF-7 Cell Lines

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Abstract

Isatin and substituted isatin derivatives possess a broad spectrum of biological activities. These moieties also have a wide scope for substitution, particularly at positions N-1 and C-3. Therefore, the study focused at synthesizing some novel isatin/substituted isatin Schiff's bases and screening them for anti-cancer activity against MCF-7 cell lines. All the compounds were synthesized by conventional methods in a two-step synthesis. Mannich bases of isatin were synthesized by reacting secondary amines and 37% formalin, which were then reacted with primary aromatic amines in the presence of glacial acetic acid to yield the title compounds. All the compounds were characterized by physical, chromatographic and spectroscopic methods. The synthesized analogues showed good activity against MCF-7 cell lines when compared to the standard drug, Doxorubicin. At a concentration of 10 µg/ml, the analogues **IDF 3B**, **IDF 3G**, **IDF 3H** and **IDF 3I** showed percentage cell viability of 35.46227 ± 12.92 , 34.37412 ± 5.39 , 35.81035 ± 5.25 , 36.19748 ± 3.27 respectively compared to percentage cell viability of 22.36828 ± 2.35 for the standard. The obtained data indicated that the analogues were effective against breast cancer, and therefore the isatin moiety could serve as the lead to the development of new anti-breast cancer agents.

Introduction

Cancer is a collection/group of cells characterized by their uncontrolled growth and division. It is the second leading cause of death worldwide and exerts tremendous burden on the health care system [1, 2]. In Asian countries, more than 60% breast cancer cases are diagnosed as estrogen receptor α positive (ER α) cancers. In normal mammary gland development, ER α plays a significant role in breast cancer development [3]. Among all types of cancer, breast cancer is one of the most common cancers occurring especially in the females.

Isatin (1*H*-Indole-2,3-dione) is an indole oxidized derivative belonging to the class of heterocyclic molecules [4]. It possesses several biological activities *viz.* anti-fungal [5], anti-tubercular [6], anti-HIV, anti-viral [5], anti-cancer [7–9], anti-convulsant [10], anti-bacterial [11], anti-inflammatory [12], and anti-oxidant [13]. A number of isatin analogues may be obtained by substitutions at different positions to potentiate the cytotoxic and anticancer effectiveness [14].

Also, benzothiazole possesses a wide variety of biological activities [15–22]. 2-aminobenzothiazole has many applications in medicinal and bioorganic chemistry [23]. In addition, pyrimidine is a well-known bio-active scaffold [24–31]. Both the moieties are found to exhibit cytotoxic effect against various human cancer cell lines [32, 33]. These nucleuses in combination with particularly with isatin have been found active against breast cancer [34–36]. Considering the above-mentioned facts, it was decided to synthesize conjugates of isatin and benzothiazole/pyrimidine moieties, in anticipation that the new compounds may have synergistic effect against the breast cancer cell-lines.

Results And Discussion

A novel series of conjugates of isatin/substituted isatin derivatives and heterocyclic moieties were designed using molecular docking simulations. The most molecules displaying good binding affinity were selected. These were synthesized in wet-laboratory through a two-step reaction scheme as given in Scheme 1. The compounds obtained were purified by column chromatography and their physico-chemical parameters were studied. The structures of the synthesized derivatives were confirmed by spectral techniques [37] *viz.* FTIR spectroscopy, mass spectrometry, ^1H -NMR, ^{13}C -NMR spectroscopy, and elemental analysis.

Through endocrine study hyper-production of estrogen hormone has been of the leading causes for development of the breast cancer. Human being naturally contains two estrogen receptors, namely ER- α and ER- β . These predominantly are expressed in the mammary gland and uterus; and play critical role in metabolism, proliferation and maturation of the

breast cancer cells. The receptor, ER- α is well established to be involved in monitoring of the immune response, retarded apoptosis, in metastasis and in cell growth [38]. Hyper activity of estrogen may potentially lead to rise in the number of ER- α receptor in the mammalian cells which may lead to development of various types of breast cancers [39, 3].

Since, this may be considered as one of the important molecular targets of development of anti-breast cancer drugs, therefore, it was decided to take this protein (Human estrogen receptor alpha, PDB ID 3ERT) and explore the molecular interactions with the synthesized ligand molecules. Another target protein, (Human progesterone receptor, PDB ID 4OAR) was also selected to dock the synthesized ligands and determine their binding scores. The hormone plays a key role in reproductive function in women. It is found to have diverse application in the female health and in treatment of hormone-related conditions including breast-cancer. The binding energy values for the synthesized compounds **IDF 3(A-L)** were found to lie majorly in the range of -12.13 to -8.05 kcal/mol. The binding energies for the fifty best conformer poses of compounds were evaluated, and the best pose of compounds was energetically filtered. The compound **IDF 3G** displayed the lowest binding energy. Hydrogen-bonding interactions between the pyrimidine ring and the amino acid residue Glu 695 and Ser 728, a pi-pi T-shaped interaction between the isatin ring and Val 726, Phe 778, and a pi-alkyl interaction between diphenyl ring system and Met 759, Leu 718, and a pi-sulfur interaction between benzothiazole and Arg 766 were the important bindings observed for the compounds against the target protein 4OAR. While for the protein 3ERT, a hydrogen-bond between hydrogen atom of pyrimidine ring and Asp 351 and Glu 380 was observed. Other important non-covalent interactions observed were pi-anion, pi-donor, pi-sigma, and pi-cation with Trp 383, Lue 525, Ala 350 and Lys 891. (Figure 2) The compounds **IDF 3C**, **IDF 3H** and **IDF 3I** displayed higher binding energies against the both target proteins, thereby suggesting their weaker interactions with the receptor site. Thus, the *in-silico* molecular docking studies helped to predict binding affinities of the compounds for the target proteins, and hence, the compounds were further studied for their *in-vitro* potentials.

The synthesized compounds were tested *in-vitro* for their anti-breast cancer activity against MCF-7 cell line by MTT assay (Table 1). All the tested compounds showed good to moderate activity against the cell line. The compounds **IDF 3B**, **IDF 3C**, **IDF 3G**, **IDF 3H** and **IDF 3I** were significantly more effective in decreasing cell viability of MCF-7 cell lines at all concentrations compared to rest of the tested compounds. (Figure 3) From the results obtained, it was found that the compound's ability to decrease the percentage of viable cells was in a concentration gradient manner.

Structure-activity relationship

The study also helped to established the structure-activity relationship in the series. It was concluded that the compounds bearing the bromoisatin as the primary nucleus, namely **IDF 3G**, **IDF 3H** and **IDF 3I**, were found to be more potent than the compounds containing the isatin nucleus, **IDF 3(A-D)**. The results also helped to conclude that the compounds (**IDF 3B**, **IDF 3H**) bearing piperazine at position N-1 and pyrimidine moiety at position C-3 of isatin/bromoisatin were more effective, with percentage cell viability of 35% each at a concentration of 10 μ g/mL, than the compounds bearing diphenylamine at position N-1 and pyrimidine moiety at position C-3 of the nucleus (**IDF 3D**, **IDF 3J**), with percentage cell viability of 48% and 50%, respectively at concentration of 10 μ g/mL.

Table 1
Percentage cell viability of MCF-7 on treatment with the synthesized compounds, **IDF 3(A-L)**.

Compounds	Concentration (µg/ml)				
	Control	1	2	5	10
Doxorubicin	99.4189±3.17071	66.3348±9.1622	53.9813±4.0154	30.8801±2.5096	22.3683±2.3539
IDF 3A	100.6812±1.4661	77.6218±1.6895	71.3351±5.4433	60.2499±8.8545	48.5854±7.7658
IDF 3B	101.0238±4.8264	75.0815±10.4512	72.6951±11.5036	63.8680±2.8346	35.4623±12.9189
IDF 3C	99.4329±1.8507	75.1841±5.9514	71.5147±10.1972	62.3593±8.1658	41.8698±8.0962
IDF 3D	96.5333±1.4661	78.7509±10.1766	74.9019±4.1369	68.8973±4.0644	48.8568±6.3225
IDF 3E	100.4337±1.5241	74.2604±3.5563	71.5917±6.6128	59.0770±5.3064	48.9595±3.5967
IDF 3F	101.8963±3.5926	93.8134±5.3707	89.0662±3.3749	80.0904±4.6532	68.6921±5.2619
IDF 3G	100.6848±1.4661	71.1075±10.1766	68.9446±4.1369	48.2261±4.0644	34.3741±5.3951
IDF 3H	101.0238±4.8264	65.1361±3.1249	58.1309±6.3225	44.1265±4.5806	35.8104±5.2540
IDF 3I	99.4329±5.8507	69.4214±7.6207	61.0004±3.7419	51.0487±2.9596	36.1975±3.2701
IDF 3J	99.5333±4.0644	77.2626±6.2929	70.6612±9.5186	57.3503±3.9301	50.2960±8.8654
IDF 3K	100.4337±1.5241	94.4835±6.2338	90.4277± 3.7419	81.9071±5.6693	74.7992±4.1006
IDF 3L	101.8963±3.5926	93.8134±5.0236	89.2459±9.5559	89.5538±6.6772	73.3109±2.5039

% Cell Viability Mean ± Sd

However, compounds with diphenylamine at N-1 but benzothiazole substitution at C-3 (**IDF 3C**, **IDF 3I**) were found to be more potent (% cell viability 41% and 36%, respectively). With exception, it was found that compound (**IDF 3G**) bearing piperazine at position N-1 and benzothiazole at position C-3 of bromoisatin exhibited highest effective with percentage cell viability of 34% at 10µg/mL.

Howsoever, the compounds (**IDF 3E**, **IDF 3F**, **IDF 3K**, **IDF 3L**) that bore morpholine moiety at position N-1 of either isatin or bromoisatin irrespective of the type of substitution at position C-3, were potentially ineffective in decreasing cell-viability of the MCF-7 cells.

The molecular properties of all the synthesized compounds were prevised using Swiss-ADME [40] and admetSAR [41, 42] web servers (Table 1). The OSIRIS Property Explorer and LAZAR applications were used to predict any toxic potential associated with the compounds. The title compounds were found to present no risks of reproductive toxicity, carcinogenicity, irritancy and acute toxicity. (Table 2)

Conclusion

In the present study, design, syntheses and *in-vitro* anti-breast cancer activity of novel conjugates of isatin/substituted isatin with heterocyclic moieties have been carried out. All the analogues showed good to moderate activity against the tested MCF-7 anti-cancer cell line. The compounds, **IDF 3B**, **IDF 3G** and **IDF 3H** were found to be the most potent members in the series. The results obtained from the molecular docking study were also in sync with the *in-vitro* laboratory result reports. Also, none of the compounds were found to be toxic from the *in-silico* prediction. The study therefore concludes isatin moiety to be effective and safe, and hence can be used to design more new compounds as effective anticancer agents.

Materials And Method

Molecular Docking Studies

AutoDock with its GUI (graphical user interface), AutoDockTools was used to perform the docking simulations [43]. Crystal structures of the target protein was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). Open Babel GUI was used to convert ChemDraw structure of ligands to their 3D coordinates in the pdb format. Re-dock with the cocrystal ligand was performed to validate the docking calculations. Protein structure was prepared for docking by removal of water molecules and the co-crystallized ligand, followed by addition of polar hydrogen atoms and the partial charges (Kollman and Gasteiger charges), using AutoDockTools 1.5.6. Similarly, ligand structures were prepared, and the files saved in the pdbqt format. For molecular docking, AutoDock 4.2.6 was used. A maximum of fifty conformations were set for each compound using GA (Genetic Algorithm) Lamarckian. 3-D analysis of the obtained results was performed for interactions. Discovery Studio Client 2020 was used for the visualization (2D and 3D) of docked poses of the compounds within the protein target.

General information

The chemicals and reagents were procured from S.D. Fine Chemicals, Rankem, TCI, and Kemphasol. The pre-coated TLC sheets were purchased from Merck, Germany. The solvents were of reagent-grade which were purified and dried by standard procedures.

Table 2: Docking scores and *In-silico* ADME prediction data of the synthesized compounds, **IDF 3(A-L)**

Comp.	Docking scores		Molecular weight	TPSA (Å ²)	Rotatable bonds	HBA	HBD	Log S	GI absorption	P-gp substrate	CYP3A4 substrate	Synthetic accessibility	Violation of Rule of Five
	3ERT	4OAR											
IDF 3A	-9.28	-9.55	363.44	89.07	2	5	1	-4.64	High	No	Yes	3.54	No
IDF 3B	-9.16	-6.38	308.34	73.72	2	6	1	-2.06	High	Yes	No	3.23	No
IDF 3C	-11.55	-12.13	460.55	77.04	5	3	0	-8.43	High	No	Yes	3.8	No
IDF 3D	-9.58	-9.41	405.45	61.69	5	4	0	-5.85	High	No	Yes	3.44	No
IDF 3E	-9.46	-9.58	378.45	86.27	3	5	0	-4.78	High	No	Yes	3.39	No
IDF 3F	-8.91	-7.01	323.35	70.92	3	6	0	-2.19	High	Yes	No	3.03	No
IDF 3G	-10.42	-8.23	456.36	89.07	3	5	1	-5.26	High	Yes	Yes	3.5	No
IDF 3H	-9.97	-7.52	401.26	73.72	3	6	1	-2.68	High	Yes	Yes	3.19	No
IDF 3I	-10.75	-11.59	539.45	77.04	5	3	0	-9.15	Low	No	No	3.85	No
IDF 3J	-9.91	-8.77	484.35	61.69	5	4	0	-6.57	High	No	Yes	3.55	No
IDF 3K	-9.39	-9.04	457.34	86.27	3	5	0	-5.49	High	No	Yes	3.44	No
IDF 3L	-9.53	-8.05	402.25	70.92	3	6	0	-2.91	High	Yes	Yes	3.13	No
Doxorubicin	-9.28	-10.59	943.77	268.79	11	18	6	-8.63	Low	Yes	No	7.64	No
Std values	-	-	130-725	7-200	-	2-20	0-6	-	-	-	-	1 very easy; 10 very difficult	Max = 4

Chemistry

The title compounds were synthesized through a two-step efficient synthetic scheme. The synthetic route for the final compounds **IDI 3(A-L)**, along with their intermediates **IDI 2(A-F)**, is outlined in Scheme 1. The target compounds **IDI 3(A-L)** were then successfully prepared by treating Mannich bases with primary aromatic amines, in the presence of glacial acetic acid under refluxing condition. All the synthesized intermediate compounds were characterized by melting point, FTIR and mass spectrometry. However, complete physicochemical and spectral characterizations were performed for the title compounds. The spectral data obtained were found to be in concurrence with the proposed compounds.

General procedure for synthesis of compounds, **IDI 2(A-F)**

Mannich bases of isatin/5-bromoisatin were prepared as per established method [44], which involved refluxing a mixture of isatin/5-bromoisatin and secondary amine (**3-5**) and 37% formalin for about 8-10 hrs. The progress of the reaction

was monitored by TLC, using methanol: chloroform (0.5:9.5) as the solvent system. After completion of the reaction, the reaction mixture was refrigerated for 48 hrs. The solid obtained was filtered, washed with *n*-hexane, dried and recrystallized from ethanol.

Characterization of the synthesized intermediate compounds, IDI 2(A-F)

1-piperazin-1-ylmethyl-1H-indole-2,3-dione (IDI 2A)

Yellowish-orange solid, yield 77%, mp 208-210°C; IR (cm⁻¹): 3399.04 (N-H stretch), 1215.89 (C-N stretch), 3020.19 (C-H stretch (sp²), aromatic), 1630.55 (C=C aromatic). MS (m/z): 245 (M⁺).

1-[(diphenylamino)-methyl]-1H-indole-2,3-dione (IDI 2B)

Dark orange solid, yield 52.89%, mp 169-171°C; IR (cm⁻¹): 3566.87 (N-H stretch), 1319.42 (C-N stretch), 1595.18, 1441.47 (C=C aromatic), 1715.89 (C=O). MS (m/z): 328 (M⁺).

1-morpholin-4-ylmethyl-1H-indole-2,3-dione (IDI 2C)

Bright orange solid, yield 73.80%, mp 196-198°C; IR (cm⁻¹): 1079.60 (C-O), 1349.68 (C-N stretch), 1608.80, 1471.37 (C=C aromatic), 1715.89 (C=O). MS (m/z): 247 (M⁺).

5-bromo-1-piperazin-1-ylmethyl-1H-indole-2,3-dione (IDI 2D)

Brown solid, yield 76.89%, mp 198-200°C; IR (cm⁻¹): 3566.87 (N-H stretch), 1329.55 (C-N stretch), 1724.48 (C=O), 3184.50 (C-H stretch (sp²) aromatic), 1612.91, 1488.85 (C=C aromatic). MS (m/z): 323 (M⁺).

5-bromo-1-[(diphenylamino)-methyl]-1H-indole-2,3-dione (IDI 2E)

Brown solid, yield 86.60%, mp 169-171°C; IR (cm⁻¹): 1290.85 (C-N stretch), 3179.22 (C-H stretch (sp²) aromatic), 1612.10, 1468.97 (C=C aromatic), 1707.33 (C=O), 660.16 (C-Br). MS (m/z): 406 (M⁺).

5-bromo-1-morpholin-4-ylmethyl-1H-indole-2,3-dione (IDI 2F)

Dark orange solid, yield 86.27%, mp 197-198°C; IR (cm⁻¹): 1335.48 (C-N stretch), 1608.80, 1471.37 (C=C aromatic), 1730.47 (C=O), 1092.83 (C-O), 612.07 (C-Br). MS (m/z): 325 (M⁺).

General procedure for synthesis of compounds, IDF 3(A-L)

Equimolar ratio of the synthesized Mannich base, **IDI 2(A-F)**, and primary aromatic amine (**7, 8**) in the presence of catalytic amount of glacial acetic acid was refluxed for 15-18 hrs. The reaction progress was monitored by TLC using the solvent system chloroform: methanol (9:1). The reaction mixture was then cooled to obtain a solid. The solid product was filtered and recrystallized from absolute ethanol to obtain the title compound.

Characterization of the synthesized title compounds, IDF 3(A-L)

3-(Benzothiazol-2-ylimino)-1-piperazin-1-yl-1,3-dihydro-indol-2-one (IDF 3A)

Dark brown solid, yield 81%, mp 161-163°C. IR (cm⁻¹): 3345.28 (N-H stretch), 1694.46 (C=N stretch), 1599.1, 1449.45 (C=C aromatic), 1383.11 (C-N stretch). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.312 (6H s of benzylidenimine), 7.018 (5H, s, CH benzylidenimine), 7.622 (4H, s, CH benzylidenimine), 2.488 (2H, d, CH₂ piperazinyl), 1.903 (4H, s, NH). ¹³C NMR

(DMSO- d_6 , 75 MHz, δ ppm): 125.38 (5C, s, benzothiazole), 152.80 (3'C, benzothiazole), 165.70 (3C, C=N), 124.67 (5C, s, benzylidenimine), 120.77 (7C, s, indole 2,3-dione), 56.02 (2C, d, piperazinyl). MS (m/z): 377 [M]⁺. Anal. Calcd. for C₂₀H₁₉N₅OS: C, 63.64; H, 5.07; N, 18.55. Found: C, 65.42; H, 4.093; N, 17.71.

1-Piperazin-1-ylmethyl-3-(pyrimidin-2-yl-imino)-1,3-dihydro-indol-2-one (IDF 3B)

Brown sticky mass, yield 81.47%. IR (cm⁻¹): 3332.49 (N-H stretch), 1641.13 (C=N stretch), 1462.92 (C-H bend), 1716.56 (C=O). ¹H NMR (DMSO- d_6 , 300 MHz, δ ppm): 7.300 (6H, s, benzylidenimine), 7.054 (5H, s, benzylidenimine), 4.141 (1H of methylene), 2.488 (2H, d, CH₂ piperazinyl), 2.12 (4H, s, NH¹³C NMR (DMSO- d_6 , 75 MHz, δ ppm): 157.3 (4C, d, pyrimidine), 164.97 (3C, C=N), 50.81 (3C, d, piperazinyl), 124.67 (5C, benzylidenimine), 122.76 (3'C, benzylidenimine). MS (m/z): 323 [M+1]⁺. Anal. Calcd. for C₁₇H₁₈N₆O: C, 63.89; H, 6.55; N, 24.83. Found: C, 64.32; H, 5.93; N, 25.87.

3-(benzothiazol-2-ylimino)-1-[(diphenylamino)-methyl]-1,3-dihydro-indol-2-one (IDF 3C)

Brown solid, yield 74.28%, mp 187-189°C. IR (cm⁻¹): 3010.98 (C-H aromatic), 1644.76 (C=N stretch), 1595.18, 1449.47 (C=C aromatic), 1285.33 (C-N). ¹H NMR (DMSO- d_6 , 300 MHz, δ ppm): 8.210 (4H, s, benzothiazole), 7.317 (6H, s, benzylidenimine), 7.550 (5H, d, benzothiazole), 7.601 (4H, s, benzylidenimine), 4.40 (1H, s, methylene), 6.547 (4H, benzene). ¹³C NMR (DMSO- d_6 , 75 MHz, δ ppm): 164.32 (3C, imine), 117.76 (2C, d, 1-benzene), 118.14 (4C, s, 1-benzene), 120.85 (7C, benzylidenimine), 125.44 (5C, s, benzothiazole), 133.83 (7'C, benzothiazole), 138.16 (7'C, benzylidenimine). MS (m/z): 460 [M]⁺. Anal. Calcd. for C₂₈H₂₀N₄OS: C, 63.02; H, 4.38; N, 12.17. Found: C, 62.30; H, 3.91; N, 13.00.

Table 3
In-silico toxicity prediction data of the synthesized compounds, IDF 3(A-L).

Compound	Toxicity Prediction			
	Osiris Property Explorer			LAZAR*
	Tumorigenic	Irritant	Reproductive effect	Carcinogenic Potency
IDF 3A	None	None	None	Non-carcinogenic
IDF 3B	None	None	None	Non-carcinogenic
IDF 3C	None	None	None	Non-carcinogenic
IDF 3D	None	None	None	Non-carcinogenic
IDF 3E	None	None	None	Non-carcinogenic
IDF 3F	None	None	None	Non-carcinogenic
IDF 3G	None	None	None	Non-carcinogenic
IDF 3H	None	None	None	Non-carcinogenic
IDF 3I	None	None	None	Non-carcinogenic
IDF 3J	None	None	None	Non-carcinogenic
IDF 3K	None	None	None	Non-carcinogenic
IDF 3L	None	None	None	Non-carcinogenic
* The maximum recommended daily dose (human) and acute toxicity could not be predicted as the software could not find similar substances for threshold 0.2 with experimental data in the training dataset.				

1-[(diphenylamino)-methyl]-3-(pyrimidin-2-ylimino)-1,3-dihydro-indol-2-one (IDF 3D)

Brown solid, yield 80.54%, mp 181-183°C. IR (cm⁻¹): 1698.68 (C=N stretch), 1725.97 (C=O), 1535.16, 1451.92 (C=C aromatic), 1349.55 (C-N stretch). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 4.89 (2H, d, 1-benzene), 7.04 (3H, d, 1-benzene), 7.023 (5H, s, benzylidenimine), 7.618 (4H, s, benzylidenimine), 7.380 (5H, s, pyrimidine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 159.37 (2C, amide), 164.95 (3C, C=N), 184.43 (2C, s, pyrimidine), 129.12 (4C, d, 1-benzene), 124.65 (5C, s, benzylidenimine), 138.37 (7'C, benzylidenimine), 117.06 (2C, d, 1-benzene), 129.12 (4C, s, benzylidenimine). MS (m/z): 405 [M]⁺. Anal. Calcd. for C₂₅H₁₉N₅O: C, 74.06; H, 4.72; N, 12.27. Found: C, 72.06; H, 4.42; N, 11.07.

3-(benzothiazol-2-ylimino)-1-morpholin-4-ylmethyl-1,3-dihydro-indol-2-one (IDF 3E)

Orange solid, yield 70.28%, mp 176-178°C. IR (cm⁻¹): 2998.11 (C-H stretch), 1652.76 (C=N stretch), 1595.18, 1441.47 (C=C aromatic), 1285.33 (C-N stretch). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.613 (4H, s, benzylidenimine), 7.008 (5H, s, benzylidenimine), 4.165 (1H, methylene), 7.312 (6H, s, benzylidenimine), 8.386 (4H, s, benzothiazole), 2.379 (2H, d, tetrahydro-1,4-oxazine) 3.672 (3H, d, tetrahydro-1,4-oxazine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 164.94 (3C, C=N), 158.93 (2C, amide), 122.74 (4C, s, benzothiazole), 120.77 (7C, s, benzylidenimine), 122.74 (4C, s, benzothiazole), 125.38 (5C, s, benzothiazole), 133.56 (7'C, s, benzothiazole). MS (m/z): 379 [M]⁺. Anal. Calcd. for C₂₀H₁₈N₄O₂S: C, 63.47; H, 4.79; N, 14.80. Found: C, 63.13; H, 4.44; N, 13.50.

1-morpholin-4-ylmethyl-3-(pyrimidin-2-ylimino)-1,3-dihydro-indol-2-one (IDF 3F)

Orange solid, yield 50.25%, mp 197-199°C. IR (cm⁻¹): 1657.85 (C=N stretch), 1383.07 (C-N stretch), 2925.26 (C-H stretch), 1062.16 (C-O). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 8.269 (4H, d, pyrimidine), 4.123 (1H, methylene), 2.370 (2H, d, tetrahydro-1,4-oxazine), 3.621 (3H, d, tetrahydro-1,4-oxazine), 7.283 (6H, s, benzylidenimine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 157.85 (4C, d, pyrimidine), 164.94 (3C, C=N), 158.96 (2C, amide), 123.27 (3'C, s, benzylidenimine), 124.23 (5C, s, benzylidenimine), 138.7 (7'C, s, benzylidenimine). MS (m/z): 323 [M]⁺. Anal. Calcd. for C₁₇H₁₇N₅O₂: C, 63.15; H, 5.30; N, 21.66. Found: C, 66.28; H, 4.73; N, 20.48.

3-(benzothiazol-2-ylimino)-5-bromo-1-piperazin-1-ylmethyl-1,3-dihydro-indol-2-one (IDF 3G)

Dark brown solid, yield 81.79%, mp 154-155°C. IR (cm⁻¹): 3356.66 (N-H stretch), 1649.51 (C=N stretch), 1725.62 (C=O), 662.53 (C-Br). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 8.210 (4H, s, benzothiazole), 7.601 (7H, s, benzylidenimine), 7.478 (6H, s, benzylidenimine), 2.488 (2H, d, piperazine), 7.550 (5H, d, benzothiazole), 2.68 (3H, d, piperazine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 159.36 (2C, amide), 163.58 (3C, C=N), 157.99 (2C, benzothiazole), 124.68 (5C, s, benzothiazole), 117.81 (5C, benzylidenimine), 122.76 (7C, s, benzylidenimine); MS (m/z): 456 [M]⁺. Anal. Calcd. for C₂₀H₁₈BrN₅OS: C, 52.64; H, 3.98; N, 15.35. Found: C, 52.06; H, 4.82; N, 16.04.

5-bromo-1-piperazin-1-ylmethyl-3-(pyrimidin-2-ylimino)-1,3-dihydro-indol-2-one (IDF 3H)

Orange solid, yield 76.34%, mp 182-184°C. IR (cm⁻¹): 3337.11 (N-H stretch), 1648.33 (C=N stretch), 1217.85 (C-N stretch), 652.44 (C-Br). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.477 (6H, s, benzylidenimine), 8.210 (4H, d, pyrimidine), 7.620 (7H, s, benzylidenimine), 4.33 (1H, methylene), 2.494 (2H, d, piperazine), 2.77 (4H, N-H). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 119.64 (5C, s, pyrimidine), 184.47 (1C, s, pyrimidine), 164.96 (3C, C=N), 159.40 (2C, amide), 132.67 (4C, s, benzylidenimine), 117.81 (5C, s, benzylidenimine), 122.77 (7C, s, benzylidenimine), 51.26 (3C, d, piperazine). MS (m/z): 401 [M]⁺. Anal. Calcd. for C₁₇H₁₇BrN₆O: C, 50.89; H, 4.27; N, 20.94. Found: C, 52.06; H, 4.46; N, 19.05.

3-(benzothiazol-2-ylimino)-5-bromo-1-[(diphenylamino)-methyl]-1,3-dihydro-indol-2-one (IDF 3I)

Orange solid, yield 74.19%, mp 156-158°C. IR (cm⁻¹): 2934.96 (C-H stretch), 1645.36 (C=N stretch), 1201.49 (C-N), 648.29 (C-Br). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 8.210 (4H, s, benzylidenimine), 4.47 (1H, s, methylene), 7.431 (6H, s, benzylidenimine), 7.541 (5H, d, benzothiazole), 6.530 (4H, d, 1-benzene), 8.195 (7H, s, benzothiazole). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 126.88 (6C, s, benzothiazole), 138.12 (7'C, s, benzothiazole), 164.24 (3C, C=N), 158.98 (2C, amide), 125.38 (5C, benzothiazole), 117.71 (2C, d, 1-benzene), 133.77 (7'C, s, benzothiazole). MS (m/z): 540 [M]⁺. Anal. Calcd. for C₂₈H₁₉BrN₄OS: C, 62.34; H, 3.55; N, 10.39. Found: C, 61.46; H, 3.24; N, 11.71.

5-bromo-1-[(diphenylamino)-methyl]-3-(pyrimidin-2-ylimino)-1,3-dihydro-indol-2-one (IDF 3J)

Brown solid, yield 55.60%, mp 167-169°C. IR (cm⁻¹): 2998.25 (C-H stretch), 1642.27 (C=N stretch), 1201.56 (C-N stretch), 646.84 (C-Br). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.437 (6H, s, benzylidenimine), 7.445 (5H, s, pyrimidine), 6.554 (4H, d, 1-benzene), 4.348 (1H, s, methylene), 7.651 (7C, s, benzylidenimine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 157.99 (4C, d, pyrimidine), 119.73 (5C, s, pyrimidine), 183.20 (1C, s, pyrimidine), 164.25 (3C, C=N), 158.98 (2C, amide), 133.78 (6C, benzylidenimine), 119.57 (4C, 1-benzene). MS (m/z): 484 [M]⁺. Anal. Calcd. for C₂₅H₁₈BrN₅O: C, 61.99; H, 3.75; N, 14.46. Found: C, 62.76; H, 4.02; N, 13.77.

3-(benzothiazol-2-ylimino)-5-bromo-1-morpholin-4-ylmethyl-1,3-dihydro-indol-2-one (IDF 3K)

Brown sticky mass, yield 81.79%. IR (cm⁻¹): 1386.22 (C-N stretch), 1114.31 (C-O stretch), 2937.03 (C-H stretch), 1654.34 (C=N stretch). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.551 (5H, d, benzothiazole), 7.430 (6H, benzylidenimine), 7.675 (7H, benzylidenimine), 3.667 (3H, d, tetrahydro-1,4-oxazine), 2.40 (2H, d, tetrahydro-1,4-oxazine), 4.032 (1H, s, methylene). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 125.47 (5C, s, benzothiazole), 133.77 (7'C, s, benzothiazole), 164.24 (3C, C=N), 125.36 (3'C, s, benzylidenimine), 118.77 (5C, s, benzylidenimine), 56.03 (2C, d, tetrahydro-1,4-oxazine). MS (m/z): 456 [M]⁺. Anal. Calcd. for C₂₀H₁₇BrN₄O₂S: C, 52.52; H, 3.75; N, 12.25. Found: C, 52.16; H, 5.07; N, 12.97.

5-bromo-1-morpholin-4-ylmethyl-3-(pyrimidin-2-ylimino)-1,3-dihydro-indol-2-one (IDF 3L)

Brown sticky mass, yield 76.47%. IR (cm⁻¹): 1303.52 (C-N stretch), 1114.29 (C-O stretch), 2937.75 (C-H stretch), 1645.08 (C=N stretch). ¹H NMR (DMSO-*d*₆, 300 MHz, δ ppm): 7.426 (6H, s, benzylidenimine), 7.419 (5H, pyrimidine), 7.608 (7H, s, benzylidenimine), 2.369 (2H, d, tetrahydro-1,4-oxazine), 4.126 (1H, s, methylene), 3.668 (3H, d, tetrahydro-1,4-oxazine). ¹³C NMR (DMSO-*d*₆, 75 MHz, δ ppm): 157.82 (4C, d, pyrimidine), 119.73 (5C, s, pyrimidine), 164.26 (3C, C=N), 132.28 (4C, s, benzylidenimine), 56.04 (2C, tetrahydro-1,4-oxazine), 118.81 (5C, s, benzylidenimine). MS (m/z): 401 [M]⁺. Anal. Calcd. for C₁₇H₁₆BrN₅O₂: C, 50.76; H, 4.01; N, 17.41. Found: C, 49.96; H, 4.52; N, 16.87.

Biological Activity: Determination of percentage cell viability of MCF-7 cells by MTT assay

The MCF-7 breast cancer cell lines were used as the cells of choice for this study. The MCF-7 cell lines were procured from NCCS, Pune. The received vials were trypsinised and transferred to a new flask. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Hi-Media) supplemented with 10% foetal bovine serum (FBS, Hi-Media) and 1% penicillin/streptomycin antibiotics (Hi-Media) at 37°C, along with supply of 5% CO₂ and appropriate levels of humidity. The cells were passaged at least thrice before performing the experiment in order to get maximum viability of the cells. The cells were then seeded at a density of 1x10⁵ cells per well on the flat bottom surface of 96 well polystyrene culture plate (Tarsons) containing 200 µL of the media.

The wells were regularly replaced with fresh media and cells adhered on the tissue culture plates were observed under a bright field microscope. Cells cultured in the wells without any treatment were taken as a control. All the synthesized compounds and the standard drug, Doxorubicin, were loaded at variable concentration of 1 µg/mL, 2 µg/mL, 5 µg/mL and 10 µg/mL. After incubation, the culture medium was removed from each well and a total of 100 µL solution of medium and MTT (5 mg/mL in PBS) solution was added to each well. Incubation for 4h with MTT resulted in the formation of Formazan crystals inside the wells. These were solubilized using a 100 µL dimethyl sulfoxide (DMSO, Hi-Media) solution and then the optical absorbance was measured at 570 nm on a multimode reader (Synergy H1 hybrid, Biotek, USA). The experiment was performed in triplicate.

Pharmacokinetic properties: In-silico predictions

As poor ADME (absorption, distribution, metabolism, and excretion) profile results in failure of most of the drug candidates in clinical trials, it may result in significant surging of the drug development cost. Detection of the pharmacokinetic profile of drug candidates at an early stage thus prove significantly useful in terms of both time and resources. The SwissADME [40] and admetSAR [41, 42] programs were used to predict ADME parameters, pharmacokinetic properties and drug likeness of the synthesized compounds.

Toxicity: In-silico predictions

A number of freely available web-based programs are available to determine *in-silico* the toxicity of the compounds. Two such programs, OSIRIS property explorer and LAZAR were used in this study. Virtual Computational Chemistry

Laboratory maintains OSIRIS property explorer which is part of Actelion's in-house substance registration system. The software predicts various ADME properties of compounds including, toxicity parameters and drug likeness. LAZAR is a computational tool that predicts toxicological aspects such as carcinogenicity, long-term toxicity, irritability, and reproductive toxicity.

Declarations

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Conflict of Interest

The authors declare no conflict of interest.

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Figures

Figure 1

Isatin scaffold containing various substitution sites.

Figure 2

Depiction of ligand-receptor interaction for the potent compound **IDF 3G** in the active site of the target proteins, PDB ID: 3ERT and PDB ID: 4OAR as depicted in fig. 1A and 1B, respectively.

Figure 3

Graph depicting percentage cell viabilities of the MCF-7 cells on treatment with variable concentrations of the compounds, **IDF 3(A-L)** and the standard drug, Doxorubicin against the control.

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