Design, Synthesis and Biological Evaluation of Morpholinated Isatin-Quinoline Hybrids as Potential Anti-Breast Cancer Agents.

Atamjit Singh (atamjitpharma.rsh@gndu.ac.in)  
Guru Nanak Dev University  https://orcid.org/0000-0001-5439-8032

Komalpreet Kaur  
Guru Nanak Dev University

Jaspreet Kaur  
Guru Nanak Dev University

Puja Gulati  
Guru Nanak Dev University

Amit Duggal  
Drug Control Wing, Chandigarh

Preet Mohinder Singh Bedi  
Guru Nanak Dev University

---

**Research Article**

**Keywords:** Isatin, Quinoline, Morpholine, Molecular hybridization, Breast cancer.

**DOI:** https://doi.org/10.21203/rs.3.rs-595432/v1

**License:** ☑️ This work is licensed under a Creative Commons Attribution 4.0 International License.  
[Read Full License]
Abstract

Keeping in view the emerging need of potent and safer anti-breast cancer agents as well as pharmacological attributes of isatin, quinoline and morpholine derivatives, novel hydrazone linked morpholinated isatin-quinoline hybrids has been designed, synthesized and evaluated as anti-breast cancer agents. Synthesized hybrid compounds were preliminary screened against two breast cancer cell lines (MCF-7 and MDA-MB-231). Almost all synthetics showed potent inhibitory potential against hormone positive MCF-7 cells while inactive against hormone negative MDA-MB-231 cells. Potent compounds were further evaluated against L929 (noncancerous skin fibroblast) cell line and found highly selective for MCF-7 cells over L929 cells. Cell cycle analysis confirmed that most potent compound AS-4 (MCF-7: GI50 = 4.36 µM) cause mitotic arrest at G2/M-phase. Due to higher selectivity toward estrogen receptor alpha (ERα) dependent MCF-7 cells various binding interactions of AS-4 with ERα are also streamlined, suggesting the capability of AS-4 in completely blocking ERα. Overall study suggest that, AS-4 can act as a potential lead for further development of potent and safer anti-breast cancer agents.

1. Introduction

Cancer remains the most difficult disease to treat with second leading cause of deaths around the globe, responsible for approximately 9.6 million deaths in 2018. In fact 1 out of 6 deaths is due to cancer and low as well as middle income nations have 70 % share in it [1]. Rapidly dividing cells of the organs like breast, skin and uterine are more fond to mutations as compared to the cells of other organs of human body thus at high risk to develop cancer. Breast cancer is most commonly occurring cancer type in women and cause significant morbidity and mortality [2]. In 2019, it was responsible for 41760 deaths among women and men in US alone [3]. In India situation is more alarming where BC has 25–32% share in all cancer cases. A survey report issued by Indian Council of Medical Research (ICMR) in 2016 estimated 14.5 million new cancer patients at that time which were estimated to be lifted up to 17.3 million in current 2020 [4].

Estrogen is a primary female sex hormone that play an essential role in the growth and development of mammary glands. Interaction of estrogen with its estrogen receptors (ERα and ERβ) are reported to play an important role in proliferation of mammary cells. All these facts resulted in the emergence of selective estrogen receptor modulators (SERMs) in drug development field. MCF-7 cells which are ERα dependent in nature and are sensitive to SERMs while MDA-MB-231 cells are ERβ dependent in nature in which the expression of estrogen, progesterone and HER2 receptors is absent thus also known as triple negative breast cancer cells [5–8]. US Food and Drug Administration (US-FDA) has already approved triarylethylene derivatives like raloxifene, tamoxifen and toremifene as SERMs with anti-breast cancer profile [9]. Among them, tamoxifen is a first line drug and widely prescribed for the treatment of breast cancer. It responds to approximately 70% of ERα cases while other need adjuvant therapy that generally relapse [10–12]. Additionally, tamoxifen adversely affect the endometrium leading to endometrial cancer while another SERM raloxifene has hot flashes, insomnia, dizziness, and melancholy like side effects [13]. Thus there is a global need to develop novel potent and safer anti-breast cancer agents.
Isatin (2,3-Indolinedione) is a well-known pharmacologically active scaffold with broad range of biological activities such as anticancer, antibacterial, antifungal, antidepressant, anticonvulsant, anti-HIV etc [14–17]. It is widely distributed in plants and marine based natural products including fungal metabolites [18]. Sunitinib III (sutent) is an isatin based drug that has been recently approved for clinical use by US-FDA for the treatment of gastrointestinal stromal tumors and advanced renal cell carcinoma [19]. Another isatin based candidate BIBF1120 II (triple angio-kinase inhibitor) is in phase III clinical trials for the diagnosis of non-small cell lung cancer [20]. Numerous reports (Fig. 1) are available that showcase the anti-breast cancer efficacy of isatin based hybrids including isatin-benzothiazole (1), Isatin-chalcone hybrids (2), Isatin-benzimidazole (3) and Isatin-benzoazine hybrids (4) [21–24].

Quinoline (benzopyridine) is a well known biologically active nucleus that has been widely distributed in natural products and represents a family of compounds called quinoline alkaloids. Some of the quinoline alkaloids are well known for their anticancer potential including berberine, camptothecin, chelidonine, chelerythrine, dictamine etc. Inspired from the anticancer potential of quinoline nucleus, synthetic anticancer analogues has been successfully developed by the researchers and are under clinical trial such as bosutinib, levatinib, cbazantinib and tipifarnib [25]. Halogen substituted quinoline compounds especially chloro substituted ones are currently gaining interest particularly due to their anticancer potential [26]. Some chloroquinoline based molecular hybrids (Fig. 1) has been reported as potential anti-breast cancer agents (5–8) [27–30].

Morpholine is a unique nucleus with nitrogen and oxygen embedded in it and highly popular in medicinal chemists due to its unique biochemical features. Oxygen atom present in the outer end significantly increase the affinity of morpholine ring with donor-acceptor type interactions with enzymatic receptors. Oxygen atom also decrease the basicity of nitrogen present in the ring via electronegative effect. Due to its unique biochemical properties, researchers are widely exploiting this nucleus for developing anticancer drugs with minimal or no side-effects. Morpholine containing drug named gefitinib has been approved by US-FDA for the treatment of metastatic non-small cell lung cancer while various other morpholine containing compound like GDC-0941, WAY-600, Foretinib, Copanlisib etc are under clinical trials [31]. Various hybrids molecules containing morpholine nucleus (Fig. 1) has been reported as potential anti-breast cancer agents (3, 8, 9) [23, 30, 32].

Molecular hybridization is a well-established stratagem in drug development in which two bioactive moieties are combined together with or without any linker to get a single hybrid molecule having properties of both parent moieties with higher potency, reduced toxicity and minimized resistance [18]. CUDC-907 is a well-known example for molecular hybridization which is a hybrid molecule of vorinostat (a potent histone deacetylase inhibitor approved by FDA in 2006 for treatment of T-cell lymphoma) and GDC-0941 (a phosphoinositide 3-kinase inhibitor) which was more effective in both in vitro and in vivo models with no systemic toxicity and resistance. This hybrid molecule has recently passed the phase I clinical trial for advanced solid tumor and lymphoma treatment and entered in phase II clinical trial. Thus molecular hybridization would be an efficient approach for the development of potent and safer drug candidates with minimum resistance [33].
Hydrazone is a versatile moiety in medicinal chemistry that has been widely employed in architecting broad range of pharmacologically active compounds including antibacterial, antifungal, anticonvulsant, analgesic and anticancer etc. Various isatin based hybrids linked to other biological moieties through hydrazone has been reported with admirable anticancer activity [24]. Due to dominant and favorable profile of hydrazone in anticancer area, it has been selected to be utilized into target hybrid molecules (10) to connect Quinoline with morpholinated isatin (Fig. 1).

Considering the alarming heath issue of breast cancer and lack of potent and safer anti-breast cancer agents, present study targets the synthesis of hydrazone linked morpholinated isatin-quinoline hybrids and evaluation against MCF-7 and MDA-MB-231 cell lines along with effect on cell cycle. Compounds with promising activity were evaluated for cytotoxicity against L929 (noncancerous skin fibroblast) cell line. Furthermore, the binding interactions of most potent compound with target ERα were explored via molecular docking studies.

2. Results And Discussion

2.1. Chemistry

Synthesis of targeted hybrids was conducted via a series of chemical reactions (Scheme 1), initiated from various substituted isatins (11). Isatins were reacted with various alkylated morpholines (12) with halogen on alkyl end in the presence of K$_2$CO$_3$ using DMF as solvent. Reaction was carried out at room temperature and monitored by TLC. After completion, reaction mixture was poured on crushed ice and precipitates so obtained were subjected to column chromatography to get pure 1-(2-morpholinoalkyl)indoline-2,3-diones (13). Simultaneously, 4,7-dichloroquinoline (14) was treated with hydrazine hydrate in ethanol under reflux, yielded 7-chloro-4-hydrazinylquinoline after washing and crystallization with ethanol itself (15) [34]. 1-(2-morpholinoalkyl)indoline-2,3-diones and 7-chloro-4-hydrazinylquinoline were further refluxed together in ethanol with few drops of glacial acetic acid and monitored for progress by TLC. After completion, reaction kept overnight at room temperature for precipitation. Precipitates obtained were further washed and recrystallized with ethanol to get desired morpholinated isatin-quinoline hybrids (AS-1 to AS-18). All chemical reactions were proceeded very smoothly and hybrids were obtained in decent yields. Chemical structures of targeted hybrids were characterized through $^1$H and $^{13}$C NMR along with elemental analysis and were accordance with assumed structures.

2.2. Biological evaluation

Synthesized compounds were evaluated for their cytotoxic activities on two breast cancer cell lines using MTT assay. One was MCF-7, which is ERα dependent and hormone positive cell line while another one was MDA-MB-231, which is ERβ dependent and hormone negative cell line. Preliminary screening of test compounds was performed by using initial concentration of 100 µM each. Compounds showing percentage growth inhibition (Table 1) greater than 60% were only considered as active and further
evaluated for GI\textsubscript{50} values (Table 2) using different concentrations of test compounds against sensitive breast cancer cell line along with L929 (noncancerous skin fibroblast) cell line. In preliminary screening all synthesized compounds showed potent growth inhibition potential against MCF-7 cell line while found inactive against MDA-MB-231 at 100 µM concentration that makes the hybrid molecules, selective inhibitors of hormone positive breast cancer. Among the screened hybrids compounds, compounds with two carbon alkyl chain between morpholine and isatin (AS-1 to AS-6) showed more sensitivity toward MCF-7 cells as compare to those with three (AS-7 to AS-12) and four carbon chain (AS-13 to AS-18). Suggesting that two carbon chain length between morpholine and isatin is most suitable for anticancer activity. Furthermore, only hybrids with two carbon chain length between morpholine and isatin (AS-1 to AS-6) were able to show growth inhibition more than 60 % thus explored for GI\textsubscript{50} values. GI\textsubscript{50} values generated an exclusive relationship between the activity and electronic environment at 5th position of isatin nucleus. AS-4 with fluoro substitution (R = F) was most potent among all synthesized compounds with GI\textsubscript{50} value of 4.36 µM, followed by AS-3 (GI\textsubscript{50} = 9.22 µM) with chloro (R = Cl) and AS-2 (GI\textsubscript{50} = 18.62 µM) with bromo (R = Br) substitution, which suggest that electronegative halogen groups at isatin are most suitable for the anticancer activity. On the other hand, in AS-5 and AS-6 i.e. methyl (R = CH\textsubscript{3}) and methoxy (R = OCH\textsubscript{3}) substitutions on isatin lowered the activity with GI\textsubscript{50} values of 24.23 and 22.19 µM that suggest that electropositive substitutions around isatin are unfavorable for the anticancer potential. Unsubstituted isatin in hybrid molecule (AS-1) give GI\textsubscript{50} values of 28.27 µM which is even lower than AS-5 and AS-6. Thus, the overall preference order for R becomes F > Cl > Br > OCH\textsubscript{3} > CH\textsubscript{3} > H and for carbon chain length between isatin and morpholine, it is n = 1 > 2 > 3 that generates a beautiful structure activity relationship. Active compounds when further evaluated against L929 cell line, most of them (AS-1, AS-2, AS-5 and AS-6) were found inactive with GI\textsubscript{50} values above 100 µM while AS-3 and AS-4 showed GI\textsubscript{50} values of 78.63 and 52.42 µM respectively. Most potent compound showed selectivity index of 12.03 between MCF-7 and L929 cell line that makes it highly selective for breast cancer cells over normal fibroblast cells.

Table 1 Various morpholinated isatin-Quiloline hybrids with their percentage growth inhibition against breast cancer cell lines at 100 µM.
| Code | R | n | Percentage growth inhibition (100 µM) |
|------|---|---|-------------------------------------|
|      |   |   | **MCF-7**                           |
|      |   |   | Hormone positive breast cancer cell |
|      |   |   | line                                |
| AS-1 | H | 1 | 65                                  |
| AS-2 | Br| 1 | 79                                  |
| AS-3 | Cl| 1 | 85                                  |
| AS-4 | F | 1 | 93                                  |
| AS-5 | CH₃| 1 | 75                                  |
| AS-6 | OCH₃| 1 | 71                                  |
| AS-7 | H | 2 | 39                                  |
| AS-8 | Br| 2 | 48                                  |
| AS-9 | Cl| 2 | 53                                  |
| AS-10| F | 2 | 56                                  |
| AS-11| CH₃| 2 | 43                                  |
| AS-12| OCH₃| 2 | 47                                  |
| AS-13| H | 3 | 11                                  |
| AS-14| Br| 3 | 15                                  |
| AS-15| Cl| 3 | 14                                  |
| AS-16| F | 3 | 18                                  |
| AS-17| CH₃| 3 | 11                                  |
| AS-18| OCH₃| 3 | 13                                  |
|      |   |   | **MDA-MB-231**                      |
|      |   |   | Hormone negative breast cancer cell |
|      |   |   | line                                |
|      |   |   | 6                                   |
|      |   |   | 10                                  |
|      |   |   | 17                                  |
|      |   |   | 22                                  |
|      |   |   | 13                                  |
|      |   |   | 15                                  |
|      |   |   | .a                                  |
|      |   |   | 4                                   |
|      |   |   | 9                                   |
|      |   |   | 17                                  |
|      |   |   | .a                                  |
|      |   |   | .a                                  |
|      |   |   | .a                                  |
|      |   |   | .a                                  |
|      |   |   | 8                                   |
|      |   |   | 12                                  |
|      |   |   | .a                                  |
|      |   |   | .a                                  |

*aNo growth Inhibition detected*
Table 2 GL50 values of active morpholinated isatin-Quinoline hybrids against MCF-7 (Hormone positive breast cancer) and L929 (noncancerous skin fibroblast) cell lines along with selectivity index.

| Code | GI50 (µM) | MCF-7 | L929 | Selectivity Index (SI) |
|------|-----------|-------|------|------------------------|
| AS-1 | 28.27     | >100  | .c   |                        |
| AS-2 | 18.62     | >100  | .c   |                        |
| AS-3 | 9.22      | 79.63 | 8.64 |                        |
| AS-4 | 4.36      | 52.42 | 12.02|                        |
| AS-5 | 24.23     | >100  | .c   |                        |
| AS-6 | 22.19     | >100  | .c   |                        |
| Plumbagin | 3.5   | .b   | .c   |                        |
| Tamoxefene | 50   | .b   | .c   |                        |

aSI value > 3 is considered to be highly selective. bNot tested. cNot calculated

Effect of most potent compound AS-4 on cell cycle distributions is further evaluated on most sensitive MCF-7 cells. MCF-7 cells were treated with AS-4 with a concentration of 4.36 µM (GI50 value) for 24 hours. Results revealed that AS-4 cause significant accumulation of MCF-7 cells in G2/M-phase (50.1 %) at GI50 concentration as compare to control (28.04 %) while in reduced accumulation was observed in G0/G1-phase (24.1 %) and S-phase (18.06 %) as compare to control which was 36.2 % in G0/G1-phase and 28.8 % in S-phase (Fig. 2). Thus, overall results suggest that AS-4 hinders the proliferation of MCF-7 cells by arresting them at G2/M-phase that ultimately lead to cell death.

2.3. Molecular docking study

Among the synthesized compounds AS-4 was emerged as potent inhibitor of MCF-7 breast cancer cells which are hormone positive in nature and ERα dependent. On the other hand, compounds showed insignificant inhibition against MDA-MB-231 which is ERα negative cell line thus it can be concluded that the inhibitory pattern of by AS-4 may go through the inhibition of ERα. Thus molecular docking studies were performed to get insight into various molecular interactions possibly responsible for the modulation of ERα by AS-4. For that purpose, the X-ray crystallographic structure of human estrogen receptor alpha (ERα) in complex with its selective antagonist 4-hydroxytamoxifen (PDB entry: 3ERT; Resolution: 1.9 Å), was employed [REF PDB]. Accuracy of docking protocol was validated by docking co-crystallized ligand 4-hydroxytamoxifen into its binding site. The program was capable to reproduce best fit confirmation of 4-hydroxytamoxifen in chain A with root mean square deviation (RMSD) value of 0.7756, indicating the
reliability of docking protocol. After that AS-4 was docked into 4-hydroxytamoxifen binding site, and best pose with −10.1584 score was selected for discussion (Fig. 3).

Overall binding pattern of AS-4 with its binding site disclose that compound is well settled in the cavity which is stabilized through various electrostatic interactions. Major interactions of AS-4 with ERα include π-σ, π-π stacked, π-alkyl, salt bridge attractive charge, halogen interaction, C-H bond, π-donor hydrogen bond and conventional hydrogen bond interaction. Chloroquinoline moiety of AS-4 is well stabilized in the cavity formed by Ala350, Leu387, Met357, Trp383, Leu536 and Leu354 (hydrophobic residues). Chloroquinoline moiety is seemed to be sandwiched between Trp383, Ala350, Leu387 and Leu536 stabilized through π-σ, π-π stacked and π-alkyl type interactions. Long distanced (π-orbital; d = 2.843 Å) π-alkyl interaction of chloro group on quinoline with aromatic residue of Trp383 is also observed. Short distanced conventional hydrogen bond interaction (H-bond acceptor; d = 1.876 Å) is observed between the Asp351 and hydrazone linkage that proves importance of this linkage in strong binding of AS-4 with active site. Halogen type interaction was observed between flouro group at isatin and Met528. Additional π-donor hydrogen bond interaction was also observed between isatin and Cys530. Alkyl chain between isatin and morpholine seems to interact with Asp351 through C-H bond interaction. A salt bridge attractive charge interaction is also observed between nitrogen group of morpholine and carboxylic oxygen of Asp351. Additional C-H bond interactions are observed between Asp351 and morpholine moiety. Another conventional hydrogen bond (H-bond acceptor; d = 2.692 Å) is observed between the oxygen of morpholine and Met528. Overall study seems to propose that AS-4 has been adequately decorated with small, rigid and planer groups showcasing ideal scaffold that is able to complete the pharmacophoric need for ERα inhibition.

3. Conclusion

In the present study, hydrazone linked morpholinated isatin-quinoline hybrids has been designed as anti-breast cancer agents and synthesized in good yields that were characterized by using 1H and 13C NMR along with elemental analysis. All compounds were preliminarily screened against one hormone positive (MCF-7) and one hormone negative (MDA-MB-231) breast cancer cell line. Compounds showed good growth inhibition against hormone positive MCF-7 cells while were inactive against hormone negative MDA-MB-231 cells. Potent compounds were further evaluated for cytotoxicity against L929 (noncancerous skin fibroblast) cell line and found highly selective for MCF-7 over L929 cells. Cell cycle analysis confirm that compounds cause mitotic arrest at G2/M-phase. Since compounds showed potent activity and selectivity toward ERα dependent MCF-7 cells thus various binding interactions of most potent compound AS-4 (MCF-7: GI50 = 4.36 µM) with ERα are also streamlined. Overall study suggest that AS-4 can act as a hit lead for further development of potential and safer anti-breast cancer agents.

4. Experimental

4.1. Materials and measurements
All chemicals and reagents were procured form Sigma Aldrich, Spectrochem and CDH, India and utilized without any further purification. All yield mentioned are refer to the isolated compounds after purification process. Characterization of synthesized compounds was done via spectroscopic techniques such as $^1$H and $^{13}$C NMR along with elemental analysis. NMR spectra were recorded on Avance III HD 500 MHz Bruker Biospin and JOEL 400 MHz using DMSO-$d_6$. Chemical shifts in $^1$H NMR were reported in δ values relative to TMS as internal standard (0.00 ppm). Splitting pattern in obtained $^1$H NMR spectra are reported as s: singlet, d: doublet, m: multiplet, br: broad peak and coupling constants (J) in hertz (Hz).

**4.1.1. Procedure for synthesis of 1-(2-morpholinoalkyl)indoline-2,3-diones**

Isatin (1 equiv) was mixed with $\text{K}_2\text{CO}_3$ (1.5 equiv) and dissolved in DMF. Mixture was allowed to stir for 20 min. After 20 min, 4-(2-Chloroethyl)morpholine hydrochloride was added to the reaction mixture and allowed to stir further at room temperature until reaction was completed (monitored by TLC). On completion, reaction mixture was added in crushed ice and allowed to stand for a while (until the ice completely melts to form water and precipitates completely settled down). Precipitates formed were filtered and dried. Obtained product was further purified using column chromatography using petroleum ether/ethylacetate (9:1) to get 1-(2-morpholinoethyl)indoline-2,3-dione (16). Characterization data for 1-(2-morpholinoethyl)indoline-2,3-dione is as follows:

Yield 83%, $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, TMS = 0): 7.65–7.60 (1H, m), 7.52–7.50 (1H, m), 7.18 (1H, $J$ = 8, d), 7.08 (1H, $J$ = 8, t), 3.77–3.74 (2H, m), 3.47–3.45 (4H, m), 2.51–2.48 (2H, m), 2.40–2.36 (4H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, TMS = 0): 184.05, 158.67, 151.31, 138.80, 125.01, 123.70, 117.95, 111.55, 66.72, 55.27, 53.76, 37.47. Anal. Calcd for C$_{14}$H$_{16}$N$_2$O$_3$: C, 64.58; H, 6.20; N, 10.76; Found: C, 64.58; H, 6.21; N, 10.74.

The same procedure described above was utilized in the synthesis of remaining 1-(2-morpholinoalkyl)indoline-2,3-diones.

**4.1.2. Procedure for synthesis of 7-chloro-4-hydrazinylquinoline**

4,7-dichloroquinoline (1 equiv) was dissolved in absolute ethanol and hydrazine hydrate (15 equiv) added drop wise with stirring. Reaction mixture so obtained was refluxed for 3 hours. After that reaction mixture was cooled down to room temperature and kept overnight for precipitation. Yellow precipitates so obtained were filtered, washed with ethanol (10 mL) twice and further recrystallized using ethanol to get desired 7-chloro-4-hydrazinylquinoline with 80% yield and m.p. 223–225 ºC [34].

**4.1.3. Procedure for synthesis of morpholinated isatinquinoline hybrids (AS-1 to AS-18)**
1-(2-morpholinoalkyl)indoline-2,3-dione (1 equiv) and 7-chloro-4-hydarzinylquinoline (1 equiv) were dissolved in absolute ethanol and few drops of glacial acetic acid was added to it. Reaction mixture was allowed to reflux until both reactants are not completely consumed (monitored by TLC). On completion, reaction mixture was cooled down to room temperature and kept overnight for precipitation. Precipitates so obtained were filtered, washed with ethanol (10 mL) twice and further recrystallized using ethanol to get desired morpholinated isatin-quinoline hybrids. Characterization data of all synthesized morpholinated isatin-quinoline hybrids is as follows:

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(2-morpholinoethyl)indolin-2-one (AS-1): Yield 76%, $^1$H NMR (DMSO-$d_6$, 500 MHz, $\delta$, TMS = 0): 12.21 (1H, br), 8.53 (1H, J = 10, d), 8.43–8.41 (1H, m), 7.90 (1H, J = 10, d), 7.85 (1H, m), 7.50–7.47 (1H, m), 7.32–7.28 (1H, m), 7.15 (1H, J = 10, d), 7.08–7.04 (2H, m), 3.85–3.82 (2H, m), 3.50–3.48 (4H, m), 2.53–2.50 (2H, m), 2.42 (4H, s). $^{13}$C NMR (DMSO-$d_6$, 125 MHz, $\delta$, TMS = 0): 165.35, 143.48, 142.30, 140.17, 138.64, 136.77, 130.67, 127.45, 125.52, 120.93, 117.96, 109.18, 100.53, 66.71, 55.89, 55.83, 36.99. Anal. Calcd for C$_{23}$H$_{22}$ClN$_5$O$_2$: C, 63.37; H, 5.09; N, 16.07; Found: C, 63.34; H, 5.06; N, 16.05.

(Z)-5-bromo-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(2-morpholinoethyl)indolin-2-one (AS-2): Yield 68%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 12.94 (1H, br), 8.82–8.80 (1H, m), 8.06–8.05 (1H, m), 7.86–7.82 (3H, m), 7.73–7.72 (1H, m), 7.62–7.60 (1H, m), 7.33–7.31 (2H, m) 4.29–4.27 (2H, m), 3.80–3.78 (4H, m), 2.08–2.07 (4H, m), 1.94(2H, s). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 154.02, 153.89, 152.67, 152.41, 143.33, 139.53, 138.64, 138.60, 126.70, 119.50, 115.74, 113.84, 63.53, 58.58, 55.29, 51.88, 19.31. Anal. Calcd for C$_{23}$H$_{22}$BrClN$_5$O$_2$: C, 53.66; H, 4.11; N, 13.60; Found: C, 53.65; H, 4.08; N, 13.57.

(Z)-5-chloro-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(2-morpholinoethyl)indolin-2-one (AS-3): Yield 81%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.63 (1H, br), 8.86–8.85 (1H, m), 8.09 (1H, s), 7.95–7.94 (1H, m), 7.81–7.80 (3H, m), 7.52–7.50 (1H, m), 7.34–7.33 (1H, m), 3.76 (2H, s), 3.54 (4H, s), 2.65 (4H, s), 2.38–2.37 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 155.16, 154.18, 151.32, 151.04, 142.83, 138.13, 138.05, 137.83, 127.31, 126.10, 118.91, 115.14, 62.93, 58.02, 54.79, 51.17, 18.97. Anal. Calcd for C$_{23}$H$_{21}$Cl$_2$N$_5$O$_2$: C, 58.73; H, 4.50; N, 14.89; Found: C, 58.69; H, 4.46; N, 14.85.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-fluoro-1-(2-morpholinoethyl)indolin-2-one (AS-4): Yield 73%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.64 (1H, br), 8.85 (1H, s), 8.09 (1H, s), 7.95–7.93 (1H, m), 7.81–7.79 (1H, m), 7.76–7.75 (1H, m), 7.63–7.61 (1H, m), 7.34–7.33 (2H, m), 4.07–4.06 (2H, m), 3.63 (4H, s), 2.57–2.53 (2H, s), 2.50 (4H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 157.13, 155.12, 152.66, 152.14, 143.27, 139.32, 139.13, 138.43, 129.12, 125.16, 119.07, 115.22, 114.23, 62.83, 58.62, 54.89, 51.78, 19.22. Anal. Calcd for C$_{23}$H$_{21}$ClF$_2$N$_5$O$_2$: C, 60.86; H, 4.66; N, 15.43; Found: C, 60.83; H, 4.62; N, 15.41.
(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-methyl-1-(2-morpholinoethyl)indolin-2-one (AS-5): Yield 83%, $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, TMS = 0): 13.65 (1H, br), 8.84–8.83 (1H, m), 8.08 (1H, s), 7.93–7.92 (1H, m), 7.80–7.78 (1H, m), 7.71–7.70 (1H, m), 7.58 (1H, s), 7.29–7.27 (1H, m), 7.20–7.18 (1H, m), 4.12 (2H, s), 3.61 (4H, s), 2.55 (5H, s), 2.38 (4H, s). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, TMS = 0): 157.22, 155.36, 152.64, 152.48, 143.87, 139.22, 138.79, 138.04, 128.79, 125.11, 119.16, 115.94, 114.29, 62.46, 58.36, 54.97, 51.49, 19.82, 18.23. Anal. Calcd for C$_{24}$H$_{24}$ClN$_5$O$_2$: C, 64.07; H, 5.38; N, 15.57; Found: C, 64.04; H, 5.36; N, 15.55.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-methoxy-1-(2-morpholinoethyl)indolin-2-one (AS-6): Yield 86%, $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, TMS = 0): 13.68 (1H, br), 8.84 (1H, m), 8.09 (1H, s), 7.96–7.92 (1H, m), 7.82–7.76 (2H, m), 7.36 (1H, s), 7.25–7.24 (1H, m), 7.06–7.00 (2H, m), 4.00 (3H, s), 3.77 (2H, s), 3.63 (4H, s), 2.65–2.64 (2H, m), 2.60–2.57 (4H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, TMS = 0): 157.16, 155.59, 152.49, 152.47, 143.49, 139.34, 138.47, 138.49, 128.99, 125.46, 119.49, 115.74, 114.97, 62.32, 58.49, 54.22, 51.79, 19.58, 21.64. Anal. Calcd for C$_{24}$H$_{24}$ClN$_5$O$_3$: C, 61.87; H, 5.19; N, 15.03; Found: C, 61.85; H, 5.16; N, 15.06.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(3-morpholinopropyl)indolin-2-one (AS-7): Yield 76%, $^1$H NMR (DMSO-$d_6$, 500 MHz, δ, TMS = 0): 12.22 (1H, br), 8.54 (1H, $J = 10$, d), 8.44–8.40 (1H, m), 7.91 (1H, $J = 10$, d), 7.86–7.84 (1H, m), 7.49–7.46 (1H, m), 7.31–7.26 (1H, m), 7.12 (1H, $J = 10$, d), 7.06–7.02 (2H, m), 3.88–3.84 (2H, m), 3.52–3.50 (4H, m), 2.51–2.48 (2H, m), 2.10 (4H, s), 1.96–1.94 (2H, m), 1.92 (2H, s). $^{13}$C NMR (DMSO-$d_6$, 125 MHz, δ, TMS = 0): 165.33, 143.47, 142.22, 140.37, 138.49, 136.79, 130.32, 126.51, 125.18, 120.95, 116.22, 109.79, 100.48, 66.39, 55.27, 55.66, 36.41, 24.16. Anal. Calcd for C$_{24}$H$_{24}$ClN$_5$O$_2$: C, 64.07; H, 5.38; N, 15.57; Found: C, 64.04; H, 5.34; N, 15.53.

(Z)-5-bromo-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(3-morpholinopropyl)indolin-2-one (AS-8): Yield 68%, $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, TMS = 0): 12.98 (1H, br), 8.81–8.83 (1H, m), 8.05–8.04 (1H, m), 7.85–7.81 (3H, m), 7.75–7.73 (1H, m), 7.60–7.58 (1H, m), 7.31–7.29 (2H, m), 4.28–4.26 (2H, m), 4.26–4.24 (2H, m), 3.82–3.80 (4H, m), 2.10–2.08 (4H, m), 1.96–1.94 (2H, m), 1.92 (2H, s). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, TMS = 0): 155.18, 156.32, 153.98, 151.49, 141.22, 139.16, 137.12, 136.27, 128.62, 126.80, 119.92, 115.92, 115.22, 113.84, 63.32, 58.49, 55.19, 51.17, 24.41, 19.32. Anal. Calcd for C$_{24}$H$_{23}$BrClN$_5$O$_2$: C, 54.51; H, 4.38; N, 13.24; Found: C, 54.47; H, 4.35; N, 13.20.

(Z)-5-chloro-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(3-morpholinopropyl)indolin-2-one (AS-9): Yield 81%, $^1$H NMR (DMSO-$d_6$, 400 MHz, δ, TMS = 0): 13.62 (1H, br), 8.85–8.84 (1H, m), 8.09 (1H, s), 7.91–7.89 (1H, m), 7.77–7.76 (3H, m), 7.51–7.49 (1H, m), 7.33–7.32 (1H, m), 3.74 (2H, s), 3.56 (4H, s), 2.63 (4H, s), 2.35–2.33 (2H, m), 1.93–1.91 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, δ, TMS = 0): 154.89, 154.12, 152.22, 151.32, 141.53, 137.93, 137.25, 136.83, 127.51, 126.19, 118.53, 115.59, 113.32, 62.22, 58.49, 53.21, 51.49, 23.16, 18.32. Anal. Calcd for C$_{24}$H$_{23}$Cl$_2$N$_5$O$_2$: C, 59.51; H, 4.79; N, 14.64; Found: C, 59.47; H, 4.75; N, 14.60.
(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-fluoro-1-(3-morpholinopropyl)indolin-2-one (AS-10): Yield 72%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.64 (1H, br), 8.87 (1H, s), 8.08 (1H, s), 7.95–7.93 (1H, m), 7.81–7.79 (1H, m), 7.76–7.75 (1H, m), 7.61–7.59 (1H, m), 7.31–7.29 (2H, m), 4.15–4.13 (2H, m), 3.51 (4H, s), 2.55–2.53 (2H, s), 2.50 (4H, m), 1.96–1.94 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 158.22, 155.79, 152.63, 152.05, 143.17, 139.49, 139.02, 138.65, 129.32, 125.27, 118.22, 115.37, 114.21, 62.49, 58.57, 54.34, 51.27, 24.59, 18.36. Anal. Calcd for C$_{24}$H$_{23}$ClFN$_5$O$_2$: C, 61.60; H, 4.95; N, 14.97; Found: C, 61.57; H, 4.92; N, 14.93.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-methyl-1-(3-morpholinopropyl)indolin-2-one (AS-11): Yield 86%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.63 (1H, br), 8.82–8.80 (1H, m), 8.09 (1H, s), 7.94–7.93 (1H, m), 7.81–7.79 (1H, m), 7.73–7.71 (1H, m), 7.57 (1H, s), 7.28–7.26 (1H, m), 7.21–7.19 (1H, m), 4.14 (2H, s), 3.63 (4H, s), 2.54 (5H, s), 2.35 (4H, s), 1.98–1.96 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 156.26, 155.17, 152.18, 152.07, 143.22, 139.36, 138.21, 138.17, 138.13, 128.51, 125.22, 119.37, 115.51, 114.41, 62.37, 58.16, 54.32, 51.48, 24.46, 19.32, 18.26. Anal. Calcd for C$_{25}$H$_{26}$ClN$_5$O$_2$: C, 64.72; H, 5.65; N, 15.09; Found: C, 64.69; H, 5.61; N, 15.05.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-methoxy-1-(3-morpholinopropyl)indolin-2-one (AS-12): Yield 81%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.62 (1H, br), 8.82 (1H, s), 8.07 (1H, s), 7.96–7.94 (1H, m), 7.80–7.78 (2H, m), 7.39 (1H, s), 7.23–7.21 (1H, m), 7.12–7.08 (2H, m), 4.12 (3H, s), 3.71 (2H, s), 3.65 (4H, s), 2.64–2.63 (2H, m), 2.59–2.57 (4H, m), 1.95–1.93 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 157.29, 155.78, 152.23, 152.17, 143.32, 140.29, 138.49, 138.13, 128.31, 125.42, 119.65, 116.41, 114.47, 62.79, 58.34, 54.47, 52.75, 24.87, 21.34, 19.35. Anal. Calcd for C$_{25}$H$_{26}$ClN$_5$O$_3$: C, 62.56; H, 5.46; N, 14.59; Found: C, 62.52; H, 5.43; N, 14.57.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(4-morpholinobutyl)indolin-2-one (AS-13): Yield 74%, $^1$H NMR (DMSO-$d_6$, 500 MHz, $\delta$, TMS = 0): 12.23 (1H, br), 8.51 (1H, J = 10, d), 8.45–8.43 (1H, m), 7.88–8.43 (1H, m), 7.78–7.45 (1H, m), 7.75–7.45 (1H, m), 7.31–7.45 (1H, m), 7.14 (1H, J = 10, d), 7.07–7.03 (2H, m), 3.81–3.79 (2H, m), 3.49–3.47 (4H, m), 2.86–2.83 (2H, m), 2.22 (4H, s), 1.75–1.73 (2H, m), 1.51–1.49 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 125 MHz, $\delta$, TMS = 0): 165.47, 143.32, 142.41, 140.47, 139.28, 135.51, 132.17, 127.32, 126.22, 121.17, 116.26, 109.69, 100.12, 66.51, 55.23, 55.47, 36.12, 25.27, 24.37. Anal. Calcd for C$_{25}$H$_{26}$ClN$_5$O$_2$: C, 64.72; H, 5.65; N, 15.09; Found: C, 64.69; H, 5.63; N, 15.07.

(Z)-5-bromo-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(4-morpholinobutyl)indolin-2-one (AS-14): Yield 71%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 12.95 (1H, br), 8.86–8.84 (1H, m), 8.07–8.06 (1H, m), 7.83–7.80 (3H, m), 7.76–7.74 (1H, m), 7.62–7.60 (1H, m), 7.33–7.31 (2H, m), 4.29–4.27 (2H, m), 3.80–3.79 (4H, m), 2.11–2.09 (4H, m), 1.95–1.93 (2H, m), 1.90 (2H, s), 1.55–1.53 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 155.32, 156.27, 153.41, 151.56, 141.37, 139.57, 137.48, 136.42, 128.63, 126.71, 119.64, 115.32, 113.59, 63.41, 58.79, 54.32, 51.48, 25.27, 24.63, 19.47. Anal. Calcd for C$_{25}$H$_{25}$BrClN$_5$O$_2$: C, 55.31; H, 4.64; N, 12.90; Found: C, 55.29; H, 4.63; N, 12.87.
(Z)-5-chloro-3-(2-(7-chloroquinolin-4-yl)hydrazono)-1-(4-morpholinobutyl)indolin-2-one (AS-15): Yield 79%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.64 (1H, br), 8.82–8.80 (1H, m), 8.09 (1H, s), 7.92–7.91 (1H, m), 7.76–7.73 (3H, m), 7.52–7.51 (1H, m), 7.34–7.33 (1H, m), 3.73 (2H, s), 3.57 (4H, s), 2.63 (4H, s), 2.35–2.33 (2H, m), 1.94–1.92 (2H, m), 1.53–1.50 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 154.93, 154.22, 152.49, 151.24, 141.47, 137.95, 137.32, 136.48, 127.12, 126.49, 118.27, 115.16, 113.41, 112.32, 58.10, 53.25, 51.47, 25.53, 23.36, 18.32. Anal. Calcd for C$_{25}$H$_{25}$ClN$_5$O$_2$: C, 60.25; H, 5.06; N, 14.05; Found: C, 60.22; H, 4.99; N, 13.98.

(Z)-3-(2-(7-chloroquinolin-4-yl)hydrazono)-5-fluoro-1-(4-morpholinobutyl)indolin-2-one (AS-16): Yield 76%, $^1$H NMR (DMSO-$d_6$, 400 MHz, $\delta$, TMS = 0): 13.66 (1H, br), 8.85 (1H, s), 8.09 (1H, s), 7.96–7.94 (1H, m), 7.83–7.81 (1H, m), 7.73–7.71 (1H, m), 7.63–7.61 (1H, m), 7.32–7.30 (2H, m), 4.27–4.25 (2H, m), 3.56 (4H, s), 2.56–2.51 (2H, s), 2.50 (4H, m), 1.95–1.93 (2H, m), 1.49–1.47 (2H, m). $^{13}$C NMR (DMSO-$d_6$, 100 MHz, $\delta$, TMS = 0): 158.36, 155.47, 152.32, 152.49, 143.34, 139.97, 139.43, 138.46, 129.05, 125.49, 118.34, 115.61, 114.34, 62.32, 58.49, 54.79, 51.18, 25.69, 24.63, 18.01. Anal. Calcd for C$_{25}$H$_{25}$ClFN$_5$O$_2$: C, 62.30; H, 5.23; N, 14.53; Found: C, 62.34; H, 2.19; N, 14.47.

4.2. Biological evaluation

4.2.1. Cytotoxicity screening

Cell culturing: Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% of fetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin mixture was used to maintain cell lines (MCF-7, MDA-MB-231 and L929: acquired from American Type Culture Collection, ATCC, USA) at 37°C in humidified (containing 5% CO$_2$) atmosphere.
**MTT assay**

96-well plates seeded with cells (5 × 10³ cells/well) were treated with test compounds (100 µM) for 24 hours under same conditions used in maintaining cells. After 24 hours of incubation, MTT reagent (5 mg/mL) was added to each well of the plate. DMSO (100 µL) was added to solubilize formazan crystals [35, 36]. Absorption changes were noted down using microplate reader at 490 nm. Cell viability was calculated using following equation.

\[
\text{Cell viability} = 100 \times \frac{\text{Optical density of treated wells} - \text{Optical density of blank wells}}{\text{Optical density of control wells} - \text{Optical density of blank wells}}
\]

Compounds showing cell viability less than 60 % relative to untreated control cells were subjected to GI_{50} determination by treating cells with different concentrations (1, 10, 25, 50, 100 µM) of test compounds using same procedure described above and interpreting concentration response curve.

### 4.2.2. Cell cycle analysis

Most potent compound AS-4 against MCF-7 cell line was subjected to cell cycle phase distribution analysis [37] and was performed using BD Cycletest plus DNA Kit (BD Biosciences) according to manufacturer’s instructions. MCF-7 cells (4×10⁵ cells/well) were seeded in 6-well plates allowed for attachment. After 24 h, cells were treated with GI_{50} concentration of AS-4. After treatment, floating as well as adhered cells were collected in falcon tubes (15 mL) and subjected to centrifugation for 5 minutes. The cell pellet obtained after centrifugation was washed twice with PBS. After this, cell pellet was fixed by using ethanol (70%, 1 mL) and kept at -20°C for 2 hr. After that, cells were washed with PBS again. Then 250 µL of solution A (trypsin buffer) was added to each tube and allowed to stand for 10 minutes at room temperature followed by the addition of 200 µL of solution B (trypsin inhibitor and RNase buffer). After 10 minutes, 200 µL of cold solution C (PI stain solution) was added and incubated for 1 hour in dark on ice. The stained cells were analyzed using BD Accuri software by flow cytometry (BD Accuri C6 Flow Cytometer, BD Biosciences).

### 4.3. Molecular docking study

Crystal structure of human estrogen receptor alpha (ERα) in complex with its selective antagonist 4-hydroxytamoxifen (PDB entry: 3ERT; Resolution: 1.9 Å) was retrieved from Protein Data Bank [38]. Preparation of structures was done using the drug design platform LeadIT [39]. Co-crystalized ligand 4-hydroxytamoxifen from chain A was used to define the binding site in ERα with the radius of 6.50 Å. Structure of AS-4 was drawn on ChemDraw Ultra (2013), and its energy was minimized by using MM2 force field in Chem3D Ultra software (Cambridge, USA) [40]. Prepared AS-4 structure was used as protonated in aqueous solution and docked into prepared binding site of ERα using the FlexX docking module in LeadIT. All FlexX solutions yielded were scored by using a consensus scoring function (CScore) and ranked accordingly. Top best pose with the highest score was selected for further investigation of the interactions [18]. 3D enzyme-hybrid and monomer-hybrid interactions were visualized using Discovery Studio Visualizer [41].
Declarations

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

Acknowledgments

Authors are grateful to the University Grants Commission for providing funds under National Fellowship for Other Backward Classes (NFOBC) to AS, RUSA Component 4.0. The authors are also thankful to Guru Nanak Dev University, Amritsar for providing various facilities to carry out the research work.

References

1. https:// (Accessed on 24/05/2021)
2. DeSantis CE, Fedewa SA, Goding SA, Kramer JL, Smith RA, Jemal A (2016) Breast cancer statistics, 2015: Convergence of incidence rates between black and white women. CA Cancer J Clin 66(1):31–42
3. https:// (Accessed on 24/05/2021)
4. https:// (Accessed on 24/05/2021)
5. Palmieri C, Cheng GJ, Saji S, Zelada-Hedman M, Warri A, Weihua Z, Van NS, Wahlstrom T, Coombes RC, Warner M, Gustafsson JA (2002) Estrogen receptor beta in breast cancer. Endocr Relat Cancer 9(1):1–13
6. Deroo BJ, Korach KS (2006) Estrogen receptors and human disease. J Clin Invest 116(3):561–570
7. Magarian RA, Overacre LB, Singh S, Meyer KL (1994) The medicinal chemistry of nonsteroidal antiestrogens: a review. Curr Med Chem 1:61–104
8. Mitlak BH, Cohen FJ (1999) Selective estrogen receptor modulators. Drugs 57:653–663
9. Descoteaux C, Leblanc V, Belanger G, Parent S, Asselin E, Berube G (2008) Improved synthesis of unique estradiol-linked platinum(II) complexes showing potent cytocidal activity and affinity for the estrogen receptor alpha and beta. Steroids 73(11):1077–1089
10. Adsule S, Banerjee S, Ahmed F, Pandhye S, Sarkar FH (2010) Hybrid anticancer agents: isothiocyanate-progesterone conjugates as chemotherapeutic agents and insights into their cytotoxicities. Bioorg Med Chem Lett 20(3):1247–1251
11. Ellmen J, Hakulinen P, Partanen A, Hayes DF (2003) Estrogenic effects of toremifene and tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res Treat 82(2):103–111
12. Taras TL, Wurz GT, DeGregorio MW (2001) In vitro and in vivo biologic effects of ospemifene (FC-1271a) in breast cancer. J Steroid Biochem Mol Biol 77(4–5):271–279
13. Kaur G, Mahajan MP, Pandey MK, Singh P, Ramisetti SR, Sharma AK (2014) Design, synthesis and evaluation of ospemifene analogs as anti-breast cancer agents. Eur J Med Chem 86:211–218
14. Singh H, Singh JV, Gupta MK, Saxena AK, Sharma S, Nepali K, Bedi PMS (2017) Triazole tethered isatin-coumarin based molecular hybrids as novel antitubulin agents: Design, synthesis, biological investigation and docking studies. Bioorg Med Chem Lett 27(17):3974–3979

15. Bhagat K, Bhagat J, Gupta MK, Singh JV, Gulati HK, Singh A, Kaur K, Kaur G, Sharma S, Rana A, Singh H, Sharma S, Bedi PMS (2019) Design, synthesis, antimicrobial evaluation, and molecular modelling studies of novel indolinedione-coumarin molecular hybrids. ACS Omega 4(5):8720–8730

16. Zhen X, Peng Z, Zhao S, Han Y, Jin Q, Guan L (2015) Synthesis, potential anticonvulsant and antidepressant effects of 2-(5-methyl-2,3-dioxoindolin-1-yl)acetamide derivatives. Acta Pharm Sin B 5(4):343–349

17. Meleddu R, Distinto S, Corona A, Tramontano E, Bianco G, Melis C, Cottiglia F, Maccioni E (2017) Isatin thiazoline hybrids as dual inhibitors of HIV-reverse transcriptase. J Enzyme Inhib Med Chem 32(1):130–136

18. Singh A, Singh JV, Rana A, Bhagat K, Gulati HK, Kumar R, Salwan R, Bhagat K, Kaur G, Singh N, Kumar R, Singh H, Sharma S, Bedi PMS (2019) Monocarbonyl curcumin-based molecular hybrids as potent antibacterial agents. ACS Omega 4(7):11673–11684

19. Prenen H, Cools J, Mentens N, Folens N, Schuurs A, Schoffski P, Van OA, Marynen P, Debiec-Rychter M (2006) Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer res 12(8):2622–2627

20. Roth GJ, Heckel A, Colbatzky F, Handschuh S, Kley J, Lehmann-Lintz T, Lotz R, Tontsch-Grunt U, Walter R, Hilberg F (2009) Design, synthesis, and evaluation of indolinones as triple angiokinase inhibitors and the discovery of a highly specific 6-methoxycarbonyl-substituted indolinone (BIBF 1120). J Med Chem 52(4):4466–4480

21. Solomon VR, Hu C, Lee H (2009) Hybrid pharmacophore design and synthesis of isatin-benzothiazole analogs for their anti-breast cancer activity. Bioorg Med Chem 17:7585–7592

22. Karthikeyan C, Solomon VR, Lee H, Trivedi P (2013) Design, synthesis and biological evaluation of some isatin-linked chalcones as novel anti-breast cancer agents: A molecular hybridization approach. Biomed Prev Nutr 3(4):325–330

23. Taher AT, Khalil NA, Ahmed EM (2011) Synthesis of novel isatin-thiazoline and isatin-benzimidazole conjugates as anti-breast cancer agents. Arch Pharm Res 34(10):1615–1621

24. Abdel-Aziz HA, Eldehna WM, Keeton AB, Piazza GA, Kadi AA, Attwa MW, Abdelhameed AS, Atta MI (2017) Isatin-benzoazine molecular hybrids as potential antiproliferative agents: synthesis and in vitro pharmacological profiling. Drug Des Devel Ther 9(11):2333–2346

25. Jain S, Chandra V, Jain PK, Pathak K, Pathak D, Vaidya A (2019) Comprehensive review on current developments of quinoline-based anticancer agents. Arab J Chem 12:4920–4946

26. Katariya KD, Shah SR, Reddy D (2020) Anticancer, antimicrobial activities of quinoline based hydrazone analogues: Synthesis, characterization and molecular docking. Bioorg Chem 94:103406

27. Zhang H, Solomon VR, Hu C, Ulibarri G, Lee H (2008) Synthesis and in vitro cytotoxicity evaluation of 4-aminoquinoline derivatives. Biomed Pharmacother 62(2):65–69
28. Solomon VR, Hu C, Lee H (2010) Design and synthesis of chloroquin analogs with anti-breast cancer property. Eur J Med Chem 45(9):3916–3923
29. Solomon VR, Hu C, Lee H (2010a) Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach. Bioorg Med Chem 18(4):1563–1572
30. Solomon VR, Pundir S, Lee H (2019) Design and synthesis of 4-piperazinyl quinoline derived ureas/thioureas for anti-breast cancer activity by a hybrid pharmacophore approach. J Enzyme Inhib Med Chem 34(1):620–630
31. Arshad F, Khan MF, Akhtar W, Alam MM, Nainwal LM, Kaushik SK, Akhter M, Parvez S, Hasan SM, Shaquiquzzaman M (2019) Revealing quinquennial anticancer journey of morpholine: A SAR based review. Eur J Med Chem 167:324–356
32. Singh H, Singh JV, Bhagat K, Gulati HK, Sanduja M, Kumar N, Kinariwala N, Sharma S (2019) Rational approaches, design strategies, structure activity relationship and mechanistic insights for therapeutic coumarin hybrids. Bioorg Med Chem 27:3477–3510
33. Saeed S, Rashid N, Jones PG, Ali M, Hussain R (2010) Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur J Med Chem 45:1323–1331
34. Al-Sha’alan NH (2007) Antimicrobial activity and spectral, magnetic and thermal studies of some transition metal complexes of a schiff base hydrazone containing a quinoline moiety. Molecules 12:1080–1091
35. Zi C-T, Yang L, Xu F-Q, Dong F-W, Yang D, Li Y, Ding Z-T, Zhou J, Jiang Z-H, Hu J-M (2018) Synthesis and anticancer activity of dimeric podophyllotoxin derivatives. Drug Des Devel Ther 12:3393–3406
36. Singh H, Kumar M, Nepali K, Gupta MK, Saxena AK, Sharma S, Bedi PMS (2016) Triazole tethered C5-curcuminoid-coumarin based molecular hybrids as novel antitubulin agents: Design, synthesis, biological investigation and docking studies. Eur J Med Chem 116:102–105
37. Sanduja M, Gupta J, Singh H, Pagare PP, Rana A (2020) Uracil-coumarin based hybrid molecules as potent anti-cancer and anti-bacterial agents. J Soudi Chem Soc 24:251–266
38. https:// (Accessed on 24/05/2021)
39. LeadIT version 2.3.2; BioSolveIT GmbH, Sankt Augustin, Germany, 2017
40. ChemDraw U 6.0 and Chem3D Ultra, Cambridge Soft Corporation, Cambridge, USA
41. Dassault Systemes BIOVIA, Discovery Studio Modeling Environment; Release 2017, San Diego, Dassault Systemes: 2016

Figures
Figure 1

A. Various hybrid molecules containing isatin (1-4, 6, 7), chloroquinoline (5-8) and morpholine (3, 8-9) nucleus having anti-breast cancer activity; B. Designed morpholinated isatin-quinoline hybrids (10).
Figure 2

Flow cytometric analysis of compound AS-4 on MCF-7 cells showcasing cell cycle arrest at G2/M phase (Results represents triplicate experiment and provided as mean values).

Figure 3

A. Human estrogen receptor alpha (ERα) in complex with its selective antagonist 4-hydroxytamoxifen (PDB entry: 3ERT; Resolution: 1.9 Å); B. AS-4 docked on binding site of ERα; C. 3D view of interactions of AS-4 with residues of binding site of ERα; D. 2D view of interactions of AS-4 with residues of binding site of ERα.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- schema1.png
- Graphicalabstract.pptx
- Supplimentary.docx