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Research paper

Synthesis of targeted dibenzo[b,f]thiepines and dibenzo[b,f]oxepines as potential lead molecules with promising anti-breast cancer activity

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ABSTRACT

A targeted library of substituted dibenzo[b,f]thiepines and dibenzo[b,f]oxepines (prototypes **I**, **II** and **III**), and structurally analogous to tamoxifen have been synthesized as a new class of anti-breast cancer agents. All the prototype molecules exhibited potential antiproliferative activity against ER + *ve* and ER- *ve* breast cancer cell lines. Dibenzo[b,f]thiepine prototypes were found to be more active. Of all the compound tested, **14b** exhibited potent *in-vitro* antiproliferative activity at **1.33 μM** and **5 μM** concentration in MCF-7 and MDA-MB-231 cell lines and was devoid of any cytotoxicity in normal HEK cells even at **50 μM**. Cell cycle analysis showed that the compound **14b** inhibited cell proliferation due to G0/G1 arrest in MCF-7 cells. Annexin-V FITC and PI staining experiments confirmed that the cell inhibition was primarily due to apoptosis and not by necrosis, which was also supported by LDH release assay experiment. Molecular docking studies showed better binding interaction of the new dibenzo[b,f]thiepine analogue **14b** with the estrogen receptor (ER) as compared to 4-hydroxy-tamoxifen and this enhanced binding might be responsible for its estrogen antagonistic activity that induces cell cycle arrest, apoptosis and inhibition of breast cancer cells.

1. Introduction

Breast cancer is a major health concern worldwide and the leading cause of death among women in economically developed as well as in developing countries. An estimated 1.7 million women will be diagnosed with breast cancer in 2020 – a 26% increase from current levels [1]. In India, almost 100,000 women are diagnosed with breast cancer every year, and a rise to 131,000 cases is predicted by 2020 [2,3]. Estrogens are a group of steroid hormones and key regulators of growth, differentiation and function of the female reproductive organs including the breast, uterus and ovaries. In addition, they also help in the regulation of cholesterol, lipid levels and the functioning of skeletal, central nervous and cardiovascular systems [4]. The biological actions of estrogens are mediated through intracellular transcription factors called estrogen receptors (ER) which belong to the nuclear receptor superfamily. In spite of these beneficial effects, estradiol stimulation also influences the pathological processes of hormone-dependent cancers of breast, endometrial, prostrate, ovary and many other type of malignant tumors [5,6]. The important finding of the hormone responsiveness of ER + *ve* breast cancers, triggered the development of molecules which can compete with estradiol at its receptor and counterbalance its proliferative action [7]. The discovery of non-steroidal antiestrogen, tamoxifen as the therapy of choice for patients with ER + *ve* breast cancer [8] revolutionized the approach to regulate the endocrine system by disrupting the signaling cascade. However, it was later discovered that tamoxifen exists as two geometrical stereoisomers which had opposite actions [9,10]. This pharmaceutical limitation steered the medicinal chemists towards development of nonisomerizable antiestrogenic compounds such as, nafoxidine, containing a cyclic hexene ring with a fixed double bond configuration [11,12] and some ring fused analogues like benzocycloheptene [13,14], benzoxepine [15] and benzothiepines [16] with stereochemical and endocrinological features similar to

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that of tamoxifen (Fig. 1).

The discovery of these compounds solved the issue of isomerization, but were devoid of any potential antiproliferative or antiestrogenic activity. In our continuous endeavor towards the search and development of estrogen antagonists as potential anti-breast cancer agents [17], we report in this communication, synthesis of some new hetrocyclic compounds having a planar tricyclic core to which is attached a pendant phenyl ring with basic tert.aminoalkoxy group (side chain) perpendicularly oriented in the desired antiestrogenic conformation, an essential requisite for the antiproliferative activity. Following prototypes of molecules **I**, **II** and **III** were successfully synthesized for the structure – activity relationship studies and biologically screened for anti-proliferative activity against breast cancer cell lines (Fig. 2).

2. Results and discussion

2.1. Chemistry

The target prototype molecules were synthesized as shown in Scheme 1 to **6**. The key precursors, dibenzo[b,f]thiepinone **3** and dibenzo[b,f]oxepinone **9** required for the synthesis of prototype **I** molecules were prepared by some modification of the reported methods [18,19]. Hetero-Ullmann coupling of thiophenol with methyl 2-(2-iodophenyl)acetate formed the thioether **1**, which on basic hydrolysis followed by intramolecular cyclization gave the desired cyclic ketone **3** in 78% yields (Scheme 1).

The dibenzo[b,f]oxepinone **9** was synthesized following the procedure shown in Scheme 2. Nucleophilic substitution of 2-fluoro-benzaldehyde with phenol [20] formed the 2-phenoxybenzaldehyde **4**, which on reduction with sodium borohydride gave the corresponding alcohol **5.** Reaction with thionyl chloride yielded the 2-phenoxy benzyl chloride **6** followed by its subsequent conversion to 2-phenoxy benzyl cyanide **7.** Alkaline hydrolysis of cyanide **7** furnished the desired 2-phenoxy phenyl acetic acid **8** which on intramolecular Friedel–Crafts acylation formed the dibenzo [b,f]oxepin-10(11H)-one scaffold **9** in good yields.

Grignard reaction of the cyclic ketones **3** and **9** with 4-methoxyphenylmagnesium bromide formed the 7-arylated products **10** and **11** in low yields. Alternatively, reacting the ketones with 4-bromoanisole and *n*-butyllithium at −78 °C, formed the carbinols which on acidic dehydration furnished the desired compounds **10** and **11** in good yields [21]. Subsequent demethylation with boron tribromide yielded the phenolic compounds **12** and **13** which were O-alkylated with different tert-aminoalkyl halides to form the prototype **I** molecules, 10-(4-ethoxyaminophenyl) dibenzo[b,f] thiepines, **14a-d** and 10-(4-ethoxyamino phenyl)dibenzo[b,f]oxepines **15 a-d** respectively (Scheme 3).

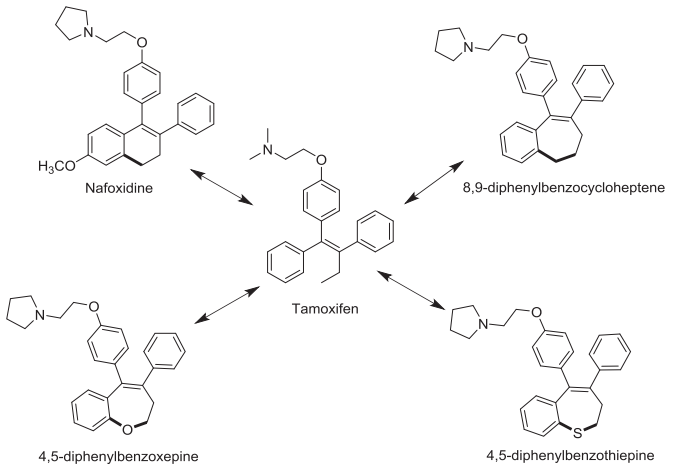


Fig. 1. Fused analogues of tamoxifen.

To mimic the biological effect of the ethyl group present in tamoxifen on the anti-breast cancer activity, prototype **II** molecules with an ethyl substituent at 6-position were synthesized as shown in Schemes 4 and 5. Alkylation at the α-position of 2-(2-(phenylthio)phenyl)butanoic acid **2** with ethyl iodide using freshly prepared lithium diisopropyl amide under strict anhydrous conditions furnished the product **16** in good yields. Subsequent cyclization of **16** to ketones **17** & **18** using either PPA, $AlCl_3$-$(COCl)_2$, or CH_3SO_3H was sluggish giving very low yields of the ketones. However, successful use of Eaton's reagent (PPA and CH_3SO_3H in 1:1 ratio) resulted in the formation of cyclized product 11-ethyl dibenzo[b,f] thiepin-10(11H)-one **17** in more than 60% yields. With dibenzo[b,f] oxepin-10(11H)-one **9,** α-ethylation was successfully achieved using PTC conditions at room temperature to accomplish the ethyl substituted dibenzo[b,f]oxepinone **18** [22], (Scheme 4).

The target molecules of prototype **II,** 10-ethyl-11-(4-ethoxyaminophenyl)dibenzo[b,f] thiepines **23a-d** and 10-ethyl-11-(4-ethoxyaminophenyl) dibenzo [b,f]oxepines **24a-e** respectively (Scheme 5) were synthesized following the same protocol of arylation at 7-position, demethylation and O-alkylation of compounds **17** and **18** as described for compounds **14a-d** and **15a-d**.

Further advancement in the modification of the pharmcophore by replacing the ethyl substituent with an aryl ring and its effect on the biological activity led to the synthesis of tetra-aryl derivatives of prototypes **III,** which were synthesized as summarized in Scheme 6. Bromination of the double bond in compounds **10** and **11** with pyridinium hydrobromide perbromide readily accomplished the bromo compounds **25** and **26** which on Suzuki coupling with phenyl boronic acid, afforded the tetra-arylated compounds **27** and **28**. Subsequent demethylation with BBr_3 led to the phenolic derivatives **29** and **30** which were finally O-alkylated with different aminoalkyl halides furnishing the target prototype III molecules, 11-(4-aminoethoxyphenyl)-10-phenyldibenzo[b,f]thiepines **31a-e** and 11-(4-aminoethoxy-phenyl)-10-phenyldibenzo[b,f] oxepines **32a-d** in good yields.

2.2. Biological evaluation

2.2.1. Anti-proliferative activity against breast cancer

As the anti-estrogenic compounds act antagonistically on the breast cancer cells inhibiting their proliferation, all the targeted compounds were screened for their antiproliferative activity on breast cancer cell lines namely, MCF-7 (ER + *ve*) and MDA-MB-231 (ER-*ve*) cells using MTT assay. The results are shown in Table 1. Twelve compounds of the series, **14a**, **14b** and **14d**, **15d**, **24d**, **29**, **31a**, **31d** and **31e**, **32b**, **32c** and **32d** were found to inhibit growth of MCF-7 cells with IC_{50} less than 20 μM. In MDA-MB-231 cell lines, eighteen compounds **14b**, **14c** and **14d**, **13**, **15a** and **15d**, **23a**, **23b**, **23d** and **23e**, **29**, **31b**, **31c** and **31e**, **32a, 32b**, **32c** and **32d** inhibited cell proliferation with IC_{50} less than 20 μM. Among the active compounds, nine compounds, viz. **13**, **14b**, **14d**, **15d**, **24d**, **29**, **32b**, **c** and **d** showed activity against both ER + *ve* and ER-*ve* cell lines. Of all the active compounds, **14a, 14b** and **31e** showed maximum inhibition of cell growth in MCF-7 cells with an IC_{50} of 6.6 μM, 1.33 μM and 9.25 μM respectively, whereas, four compounds **14b**, **24d**, **31e** and **32d** showed maximum inhibitory effect on cell growth against MDA-MB-231 cells with an IC_{50} of 5 μM, 5.74 μm, 10.8 μM, and 7.02 μM respectively (Table 1). A close observation of the data showed that sulphur containing prototypes were more active than their oxygen counterpart. Further, of the three prototype molecules,

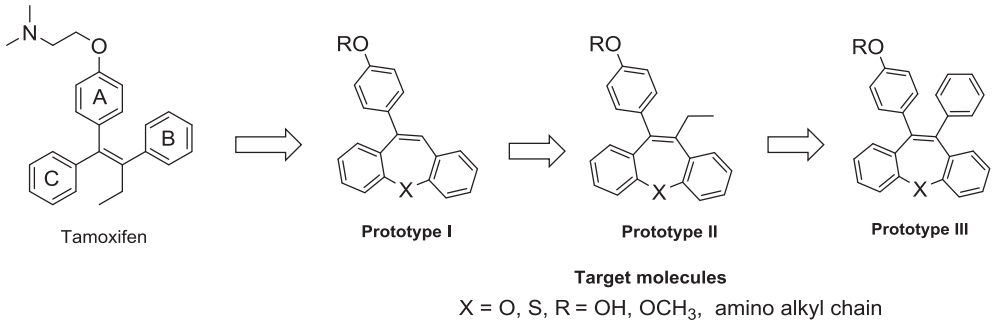
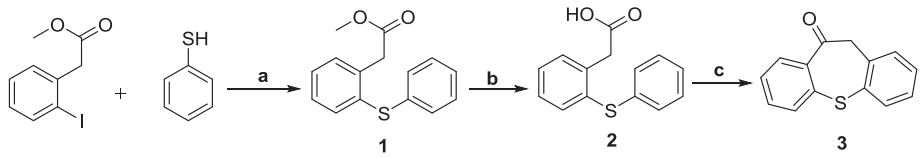
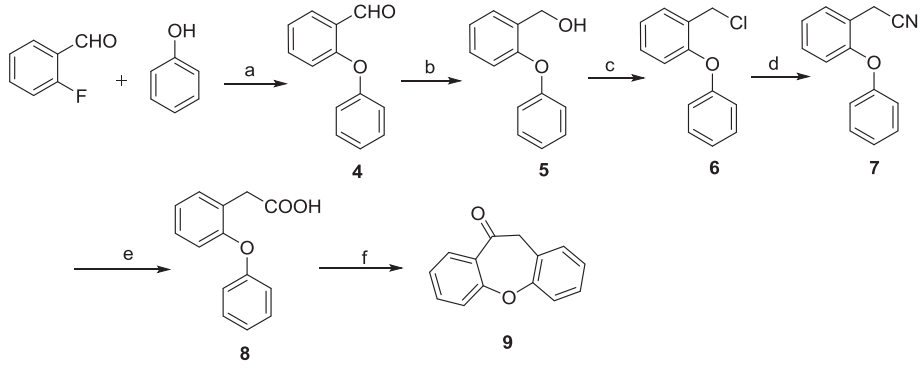


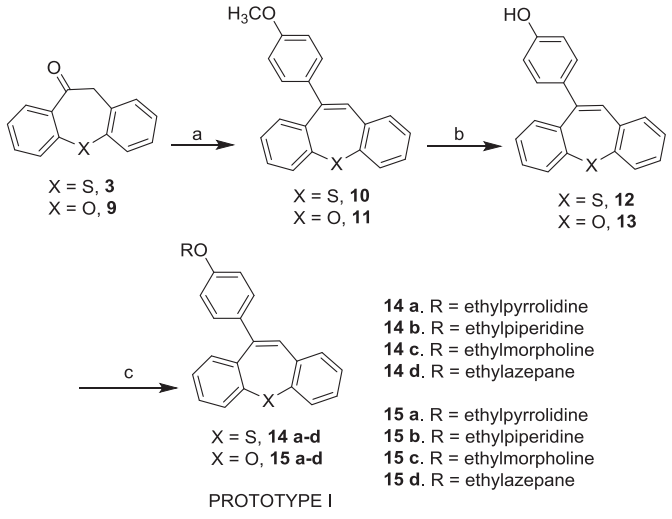
Fig. 2. Synthesized prototype molecules **I, II** and **III**.



Scheme 1. Reagents and conditions: (a) CuI, Neocuproine, dry toluene, 6 h; (b) KOH/EtOH (80% v/v), reflux 4 h; (c) (i) $(COCl)_2$, DMF, CH_2Cl_2, 30 min; (ii) $AlCl_3$, CH_2Cl_2, rt, 60 min.



Scheme 2. Reagents and conditions: (a) Cs_2CO_3, DMA, 150 °C, 2 h; (b) $NaBH_4$, methanol, 0 °C to rt; (c) $SOCl_2$, pyridine, benzene, reflux, 24 h; (d) NaCN, DMSO, rt, 24 h; (e) KOH, EtOH, H_2O, reflux, 4 h; (f) (i) $(COCl)_2$, DMF, CH_2Cl_2, 30 min; (ii) $AlCl_3$, CH_2Cl_2, rt, 60 min.

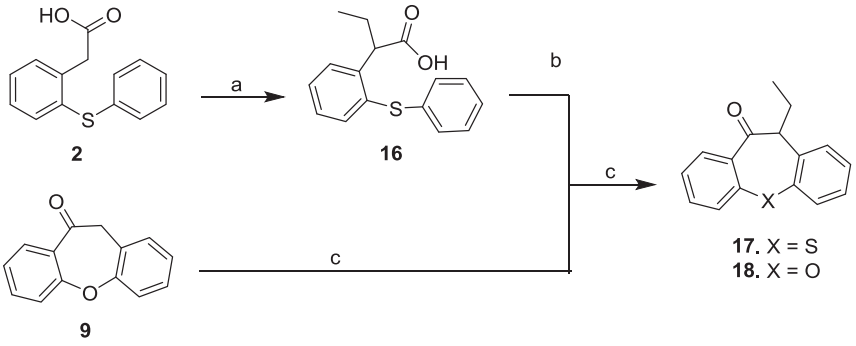


Scheme 3. Reagents and conditions: (a) (i) 4-bromoanisole, *n*-BuLi, THF, 0 °C-rt; (ii) 2-3 drops H_2SO_4, MeOH, 60 °C, 30 min; (b) BBr_3, CH_2Cl_2, −30 °C to rt, overnight; (c) aminoalkyl monohydrochlorides, anhyd. K_2CO_3, acetone, reflux, overnight.

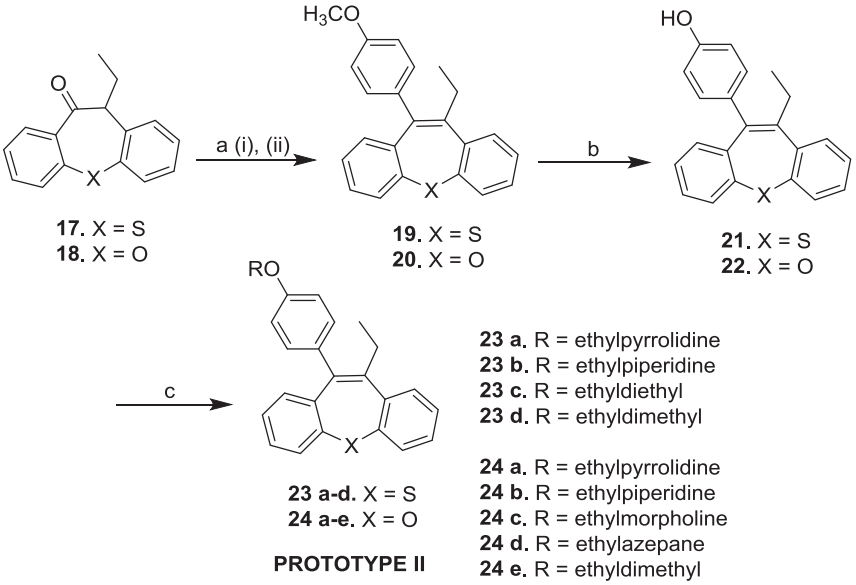
compound **14b** (prototype **I** with piperidino amino alkyl group) was the most active followed by compound **14a**. It was important to note that prototype **II** compounds with a α-ethyl substituent were devoid of any significant activity, whereas alpha arylated compounds exhibited better activity and compound **31e** (prototype **III**, with dimethyl amino group) was equiactive to tamoxifen in both the cell lines. Similarly compound **32b** also showed good activity but was found to be toxic. Though compound **14a** is more selective towards MCF-7 cells than MDA-MB-231 cells (6.6 μM versus 22.9 μM respectively), compound **14b** showed potent activity against both the breast cancer cell lines (1.33 μM and 5.0 μM respectively and was therefore chosen as lead molecule for further investigations.

2.2.2. Cytotoxicity towards normal cells

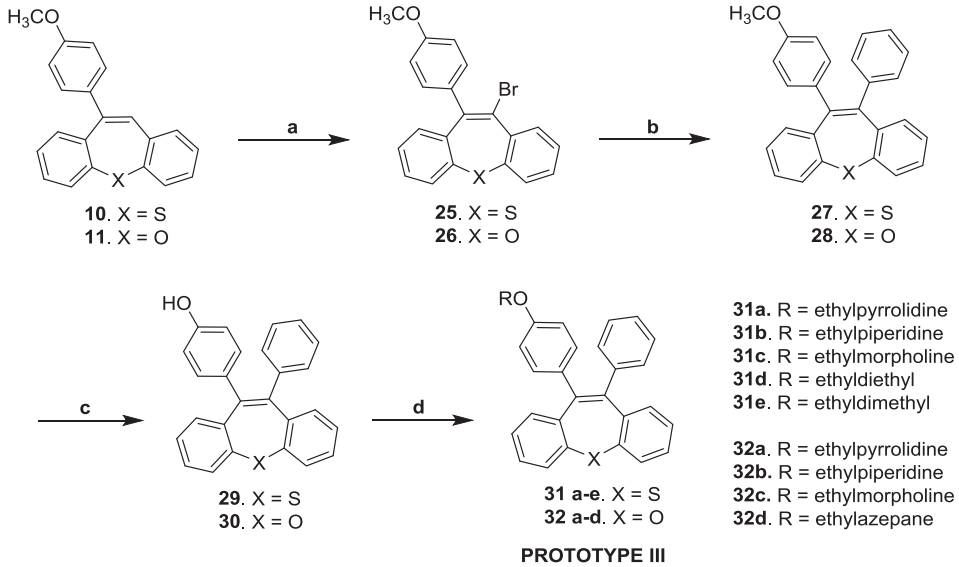
Specific killing of the cancer cells without affecting the normal cell growth is a key safety feature of cancer chemotherapy, therefore, all the compounds were evaluated for possible cytotoxicity in normal cells, HEK-293. Of the active molecules, two compounds, **24d** and **32d** induced significant cell death in HEK-293 cells. Significantly, compound **14b** showed no effect on the growth of HEK-293, suggesting that the molecule is specifically active against breast cancer cells, whereas compound **14a**, which is active against



Scheme 4. Reagents and conditions: (a) LDA, CH_3CH_2I, THF, −78 °C to rt; (b) Eaton's reagent, 1 h, rt; (c) TBAHS, NaOH, CH_3CH_2I, rt, 60 min.



Scheme 5. Reagents and conditions: (a) (i) 4-bromoanisole, n-BuLi, −78 °C-rt; (ii) H_2SO_4, CH_3OH, 30 min; (b) BBr_3, CH_2Cl_2, −20 °C-rt, overnight; (c) tert.aminoalkylmonohydrochlorides, K_2CO_3, dry acetone, reflux overnight.



Scheme 6. Reagents and conditions: (a) PyHBr, DCM, 0 °C-rt, overnight; (b) $Pd(OAc)_2$, $Pd_2(dba)_3$, Na_2CO_3, phenylboronic acid, THF, reflux, overnight; (c) BBr_3, CH_2Cl_2, −20 °C to rt, overnight; (d) tert.aminoalkylmonohydrochlorides, anhyd. K_2CO_3, acetone, reflux, overnight.

Table 1
Anti-proliferative activity of functionalized dibenzo[b,f]thiepines (**12**, **14a-d**, **23a-d**, **29**, **31a-e**) and dibenzo[b,f]oxepines (**13**, **15a-d**, **22**, **24a-e**, **30**, **32a-d**) derivatives.

Compd. No	IC_{50} (Mean ± SE, in μM)		
	MCF-7	MDA-MB-231	HEK 293
12	61.9±	20.0 ± 12.1	46 ± 18.0
13	16.5 ± 4.8	16 ± 7.30	26.6 ± 5.1
14a	**6.6 ± 2.2**	**22.9 ± 8.4**	**26.4 ± 13.1**
14b	**1.33 ± 3.4**	**5.0 ± 7.8**	**51.6 ± 7.23**
14c	27.1 ± 6.2	15.6 ± 9.4	41.8 ± 7.99
14d	15.8 ± 3.3	14.6 ± 10.6	20.1 ± 4.8
15a	33.6 ± 3.3	15.9 ± 16.2	6.68 ± 15.1
15b	39.2 ± 8.2	54.1 ± 11.0	Not done
15c	>100	>100	Not done
15d	13.2 ± 8.9	11.7 ± 11.3	19.6 ± 8.4
22	50.9±	>100	>100
23a	30.9 ± 8.6	18.2 ± 5.8	44.6 ± 17.6
23b	27.6 ± 13.9	16.2 ± 8.3	14.8 ± 7.2
23c	>100	41 ± 23.6	35.1 ± 10.0
23d	61.4 ± 7.5	43.4 ± 13.4	38.0 ± 8.3
24a	37.5 ± 11.5	26.9 ± 13.04	>100
24b	57.4 ± 7.6	27.1 ± 7.6	>100
24c	28 ± 11.4	24.3 ± 11.2	43.2 ± 7.7
24d	14.5 ± 4.4	**5.74 ± 4.9**	12.6 ± 6.4
24e	20.6 ± 7.6	13.4 ± 11.2	45.5 ± 8.35
29	19.5 ± 4.7	16.9 ± 7.2	18 ± 16.5
30	33.6 ± 15.0	>100	27.2 ± 5.6
31a	14.2 ± 4.7	>100	9.0 ± 4.4
31b	20.4 ± 6.5	16.3 ± 6.0	28.0 ± 4.9
31c	28.2 ± 6.4	12.5 ± 8.1	5.11 ± 5.66
31d	12.2 ± 4.5	41.8 ± 4.2	11.7 ± 6.4
31e	**9.25 ± 4.12**	**10.8 ± 5.6**	**32.7 ± 9.9**
32a	34.2 ± 16.5	15 ± 5.4	13.5 ± 13.1
32b	16.6 ± 6.1	13.4 ± 5.5	10.3 ± 6.5
32c	16.4 ± 21.8	13.5 ± 6.9	14.6 ± 4.73
32d	**14.8 ± 8.7**	**7.02 ± 4.1**	**15.8 ± 10.60**
Tamoxifen citrate	**9.6 ± 2.2**	**10.3 ± 1.7**	**18.2 ± 2.7**

Bold signifies better or comparable activity with the standard used.

ER + *ve* MCF-7 cells, was also found to be relatively safe against HEK-293 cells (Table 1).

*2.2.3. Morphological study of compound **14b** in bright field microscopy*

The ER + ve MCF-7 cells were treated with IC_{50} (1.33 μM), sub IC_{50} (0.87 μM) and higher than IC_{50} concentration(10.5 μM) of compound **14b** for 24 h along with untreated control and 5 μM of standard drug, tamoxifen in 24-well culture plates. Random fields under bright light inverted microscope were acquired after 24 h. On viewing the plates, visible rounding and shrinking of cell size was observed in the treated groups substantiating the inhibitory effect of compound **14b**. In addition, cell numbers were also found to be visibly reduced in higher concentration treatment possibly due to excessive cell death (Fig. 3).

*2.2.4. Analysis of the effect of compound **14b** on cell cycle distribution of MCF-7 cells*

For cell cycle analysis, MCF-7 cells grown in T25 cell culture flask were treated with IC_{50} (1.33 μM), sub IC_{50} (0.87 μM) and higher than IC_{50} (10.5 μM) concentration of compound **14b** for 24 h along with untreated control and 5 μM of standard drug, tamoxifen. Compound **14b** induced arrest of cells in G1 phase and reduction in S-phase cells in a dose dependent manner (Fig. 4). However, only higher concentration of 10.5 μM of compound **14b** could induce significant G1 arrest in comparison to untreated control and comparable to G1 arrest induced by 5 μM of tamoxifen. This indicates that cell growth inhibition by compounds **14b** is due to G0/G1 arrest in MCF-7 cells.

*2.2.5. Effect of compound **14b** on apoptosis of MCF-7cells*

In order to differentiate whether the resultant cell inhibition is due to necrosis or apoptosis, we carried out Annexin-V FITC and PI staining technique. Compound **14b** caused a significant decrease in live cells counts and increased early and late apoptotic cells counts at 10.5 μM concentration. Interestingly, compound **14b** did not increase necrotic cells in comparison to untreated control group (Fig. 5).

2.2.6. LDH release assay

When assessed for the percent cytotoxicity in terms of LDH release, **14b** showed less cytotoxity towards MCF-7 and HEK-293 cells. This suggested that at tested concentration and time point, **14b** did not disrupt cell membrane integrity for release of cytoplasmic LDH which supported the assumption that cell inhibition is due to apoptosis and not by necrosis (Fig. 6).

2.2.7. Molecular docking of three active compounds with ER alpha

The series of compounds were designed as potential estrogen antagonists for anti-breast cancer activity by their interaction with the estrogen receptor in the ER-α cavity of ER positive breast cancer cells. Therefore, to get an insight of the binding interactions and to validate the antagonistic activity profile of the three active compounds **14a, 14b** and **31e**, a comparative docking study of computationally energy minimized conformation of the three molecules was performed in comparison with the antiestrogenic drug, 4-hydroxy tamoxifen (OHT) as shown in Fig. 7. As can be clearly observed in the figure, the tricyclic core of all the three molecules occupies the same binding space in the ER pocket and is similarly oriented in the plane of the biphenyl stilbene residue of tamoxifen, (the superimposed structure of OH-TAM with compounds **14a, 14b and 31e** shown Fig. 7). Both the active compounds **14a**, **14b** and OH-TAM share significant homology (the binding energy and interacting residues of the compounds and OH-TAM are shown in Table 2) in interacting with critical amino acid residues (GLU353, GLY390, ILE326, ILE386, LEU387, LEU391, LEU403, LYS449), however, the tert.aminoalkoxy group (basic side chain) is placed in the opposite orientation to that of tamoxifen.

It has been demonstrated that molecular basis of antagonism is a complicated phenomenon and is a combination of several factors such as, spatial conformation within the ER cavity, specific interactions of the amino acid residues, hydrogen bonding, vander waal's forces that come into interplay with the ligand and the position of the basic side chain that may occupy either 7α-position or 11β-position to displace the helix 12 in forming the lid of the binding cavity. In compounds **14a** and **14b**, this chain probably orients itself in the 7α-position in contrast to tamoxifen (oriented in the 11-β position) thereby imparting the antagonistic activity to the molecules.

However, it is most surprising to note that compound **31e** having very similar conformation with OH-TAM and showing high docking energy is active at a higher concentration as compared to compounds **32d** or **14a** and **14b.** This difference in the activity of the compound **31e** gives credence to interplay of other factors critical for inhibitory activity.

3. Conclusion

A targeted series of constrained tricyclic compounds (three prototypes **I**, **II**, and **III**) of substituted dibenzo[b,f]thiepine and dibenzo[b,f]oxepines and structurally analogous to tamoxifen were prepared and evaluated for their antiproliferative activity on breast cancer cell lines. Among the evaluated compounds, sulphur analogues showed better anti-breast cancer activity than their oxygen analogues. Three compounds **14a**, **14b** and **31e** showed good

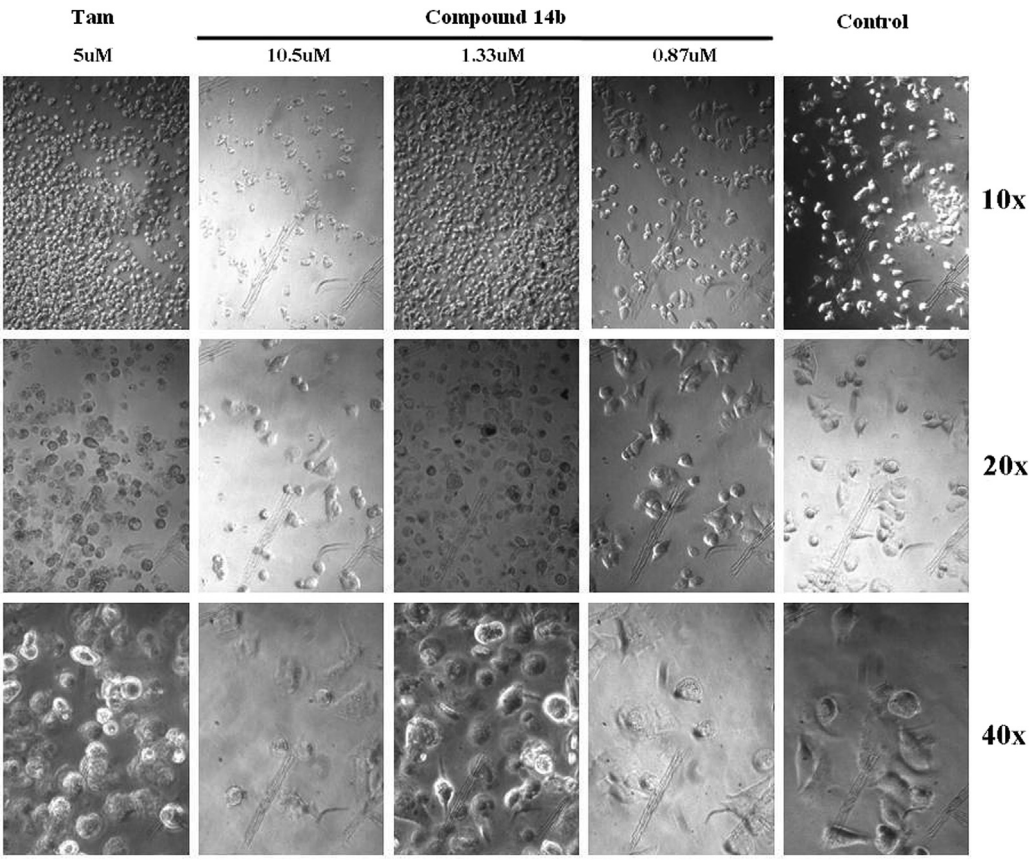


Fig. 3. White light image after treatment with compound **14b.**

activity, particularly compound **14b** which effectively inhibited both the breast cancer cell lines, MCF-7 with IC_{50} of 1.33 μM and MDA-MB-231 with IC_{50} of 5 μM. It was devoid of any cytotoxic effect on the normal HEK-293 cells at 50 μM concentration, which was more than 35 fold of their IC_{50} value and much better than the anti-breast cancer drug, tamoxifen. Further, molecular docking studies with estrogen receptor indicated ER-α as the plausible target of compound **14b** for exerting its anti-cancer action.

4. Experimental

4.1. Materials and methods

All the glass apparatus were oven dried prior to use. All chemicals and reagents were purchased from Sigma–Aldrich or ACROS ORGANICS and were used without further purification. Solvents THF, DCM, DMF, DMSO, diethyl ether, acetone were purified using standard methods. 100–200 mesh silica gel was used for column chromatography, and TLC was performed on Merck-precoated silica gel 60-F_{254} and aluminum oxide 60-F_{254} plates. Melting points were recorded with COMPLAB melting point apparatus and are uncorrected. The chromatographic solvents are mentioned as v/v ratios. All the synthesized compounds were fully characterized by ^{1}H, ^{13}C NMR, IR, and further confirmed through ESI-MS, ESI-HRMS analysis. IR spectra were recorded on a Perkin–Elmer FT-IR RXI spectrophotometer and values reported in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX-300 (300 MHz for ^{1}H and at 75 MHz for ^{13}C or DPX-200 (at 50 MHz for ^{13}C) spectrometers using $CDCl_3$, DMSO-d_6,CD_3OD or acetone-d_6 as solvents using tetramethylsilane as internal standard. Chemical shifts are reported in parts per million. ESI-MS spectra were obtained on an LCQ Advantage Ion trap mass spectrometer (Finnigan thermo fischer scientific) and High-resolution mass spectra (ESI-HRMS) were recorded on Agilent 6520 ESI-QTOP mass spectrometer. All products reported showed ^{1}H NMR and ^{13}C NMR spectra in agreement with the assigned structures. The purity of compounds was determined by HRMS, and all tested compounds yielded data consistent with a purity of at least 95% compared with the theoretical values.

4.2. Representative procedure for the preparation of thiepine derivatives (*14a-d* and *15a-d*)

A mixture of 4-(dibenzo[b,f]thiepin-10-yl)phenol **10** (302 mg, 1 mmol), 1-(2-chloro ethyl)pyrrolidine monohydrochloride (255 mg, 1.5 mmol), dry acetone (10 mL) and anhydrous K_2CO_3 (345 mg, 2.5 mmol) were refluxed overnight. On completion, the reaction mixture was cooled, filtered and washed with acetone (5 mL × 3). The filtrate was concentrated and the residue chromatographed over a column of basic alumina to furnish compounds **14a-d** and **15a-d** (prototype **I**).

4.2.1. 1-(2-(4-(Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl)pyrrolidine (compound *14a*)

Yellow solid, Yield 71%; Mp (76–78 °C); IR (KBr): 3427, 2925, 1602, 1458, 1248, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60–7.53 (m, 2H), 7.38–7.27 (m, 7H), 7.20–7.17 (m, 1H), 7.03 (d, $J = 1.32$ Hz, 1H), 6.94 (d, $J = 8.7$ Hz, 2H), 4.18 (t, $J = 5.96$ Hz, 2H), 2.96 (t, $J = 5.96$ Hz, 2H), 2.69 (m, 4H), 1.87–1.83 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.8, 145.5, 142.1, 136.8, 136.2, 132.9, 132.1, 130.9, 130.7, 130.1, 129.1, 128.3, 127.9, 127.7, 114.3, 67.0, 55.0, 54.7, 29.7, 23.5; MS (ESI) *m/z* 400 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{25}NOS + H]^{+}$ 400.1735, found 400.1769.

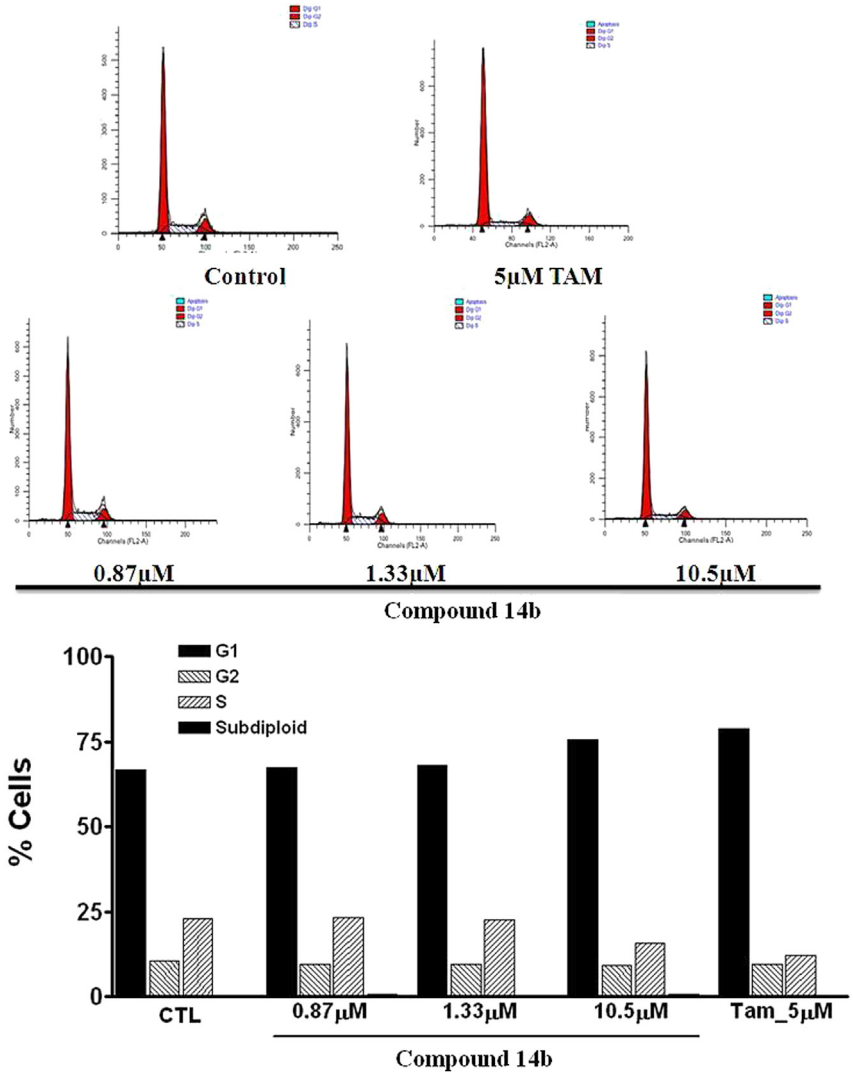


Fig. 4. Effect of Compound **14b** in cell cycle of breast cancer cells. MCF-7 cells were incubated in DMEM supplemented with 10% FBS and treated with IC_{50} (1.33 μM), sub IC_{50} (0.87 μM) and higher than IC_{50} (10.5 μM) concentration of compound **14b** for 24 h. After 24 h, the cells were harvested, stained with PI, and analyzed by flow cytometry. Data are expressed as % of total cell count.

*4.2.2. 1-(2-(4-(Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl)piperidine (compound **14b**)*

Off white solid, Yield 68%; Mp (100–102 °C); IR (KBr) 3010, 2920, 1602, 1456, 1243, 1038, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.64–7.54 (m, 2H), 7.38–7.29 (m, 7H), 7.20–7.15 (m, 1H), 7.06 (d, J = 7.68 Hz, 1H), 6.94 (d, J = 8.58 Hz, 2H), 4.17 (t, J = 6.03 Hz, 2H), 2.82 (t, J = 6.03 Hz, 2H), 2.57–2.54 (m, 4H), 1.67–1.61 (m, 4H), 1.52–1.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 145.7, 142.1, 140.3, 136.9, 136.7, 136.1, 132.8, 132.1, 130.9, 130.9, 130.7, 130.1, 129.1, 128.3, 127.9, 127.7, 114.3, 66.0, 57.9, 55.1, 25.9, 24.2; MS (ESI) m/z 414 (M + H); HRMS (ESI) exact mass calcd for $[C_{27}H_{27}NOS + H]^+$ 414.1892, found 414.1892.

*4.2.3. 4-(2-(4-(Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) morpholine (compound **14c**)*

Solid, Yield 64%; Mp (84 °C); IR (KBr) 3020, 2923, 1600, 1450, 1248, 1038, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 6.93 Hz, 1H), 7.56–7.53 (m, 1H), 7.38–7.15 (m, 8H), 7.04 (d, J = 7.59 Hz, 1H), 6.93 (d, J = 8.61 Hz, 2H), 4.17 (t, J = 5.73 Hz, 2H), 3.77 (t, J = 4.56 Hz, 4H), 2.85 (t, J = 5.7 Hz, 2H), 2.62 (t, J = 4.47 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.4, 145.6, 142.1, 140.3, 137.0, 136.2, 132.9, 132.2, 130.9, 130.8, 130.2, 129.2, 128.4, 127.8, 114.4, 66.8, 65.7, 57.6, 54.1; MS (ESI) m/z 416 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{25}NO_2S + H]^+$ 416.1684, found 416.1695.

*4.2.4. 1-(2-(4-(Dibenzo[b,f]thiepin-10-yl)phenoxy)ethyl)azepane (compound **14d**)*

Viscous oil, Yield 71%; IR (KBr): 3012, 2920, 1602, 1452, 1240, 1036, 743 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.63–7.53 (m, 2H), 7.37–7.28 (m, 7H), 7.20–7.15 (m, 1H), 7.05 (d, J = 7.53 Hz, 1H), 6.93 (d, J = 8.55 Hz, 2H), 4.15 (t, J = 6.10 Hz, 2H), 3.02 (t, J = 6.09 Hz, 2H), 2.86–2.83 (m, 4H), 1.71–1.65 (m, 8H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.5, 145.6, 142.1, 140.3, 136.9, 136.1, 132.8, 132.1, 130.9, 130.1, 129.1, 128.3, 127.9, 114.3, 66.2, 56.3, 55.8, 27.4, 27.0; MS (ESI) m/z 428 (M + H).

*4.2.5. 1-(2-(4-(Dibenzo[b,f]oxepin-10-yl)phenoxy)ethyl)pyrrolidine (compound **15a**)*

Yellow solid, Yield 76%; Mp (80–82 °C); IR (KBr): 2930, 2770, 1604, 1453, 1234, 1036, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, J = 8.52 Hz, 2H), 7.34–7.23 (m, 5H), 7.18–7.06 (m, 1H), 7.03–6.93 (m, 5H), 4.18 (t, J = 5.97 Hz, 2H), 2.96 (t, J = 6.02 Hz, 2H), 2.67 (s, 4H), 1.84 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 158.5157.9, 141.7, 134.9, 132.2, 130.8, 130.5, 130.1, 130.0, 129.8, 129.2, 127.2, 124.8,

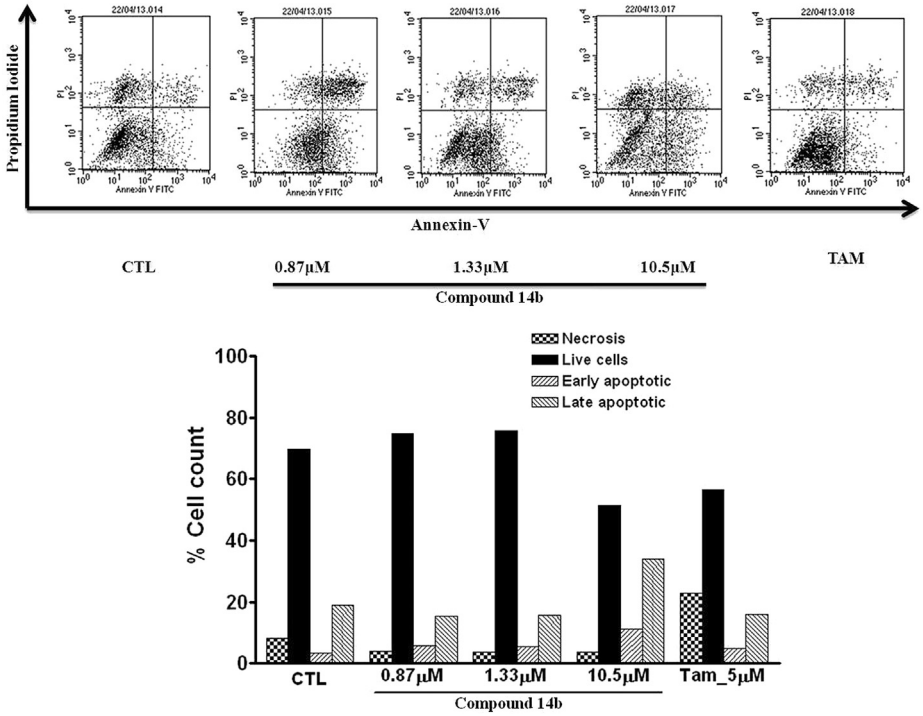


Fig. 5. Effect of compound **14b** treatment on apoptosis in terms of Annexin-V and PI staining of MCF-7 cells.

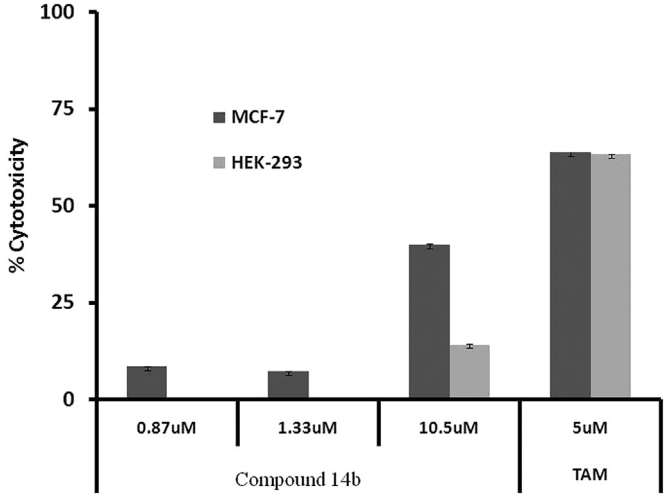


Fig. 6. % cytoxicity induced by compound **14b** in MCF-7 and HEK-293 cells in terms of LDH release.

124.5, 121.4, 120.8, 114.5, 114.4, 67.1, 55.1, 54.7, 23.5; MS (ESI) *m*/*z* 384 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{25}NO_2+H]^+$ 384.1964, found 384.1965.

*4.2.6. 1-(2-(4-(Dibenzo[b,f]oxepin-10-yl)phenoxy)ethyl)piperidine (compound **15b**)*

Yellow solid, Yield 50%; Mp (94–96 °C); IR (KBr): 2934, 2775, 1606, 1458, 1236, 1038, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, *J* = 6.72 Hz, 2H), 7.38–7.23 (m, 5H), 7.18–7.13 (m, 1H), 7.12–7.00 (m, 2H), 6.97–6.93 (m, 3H), 4.18 (t, *J* = 6.01 Hz, 2H), 2.83 (t, *J* = 6.06 Hz, 2H), 2.56–2.54 (m, 4H), 1.67–1.63 (m, 4H), 1.50–1.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 157.9, 141.7, 134.9, 132.1, 130.8, 130.1, 130.0, 129.8, 129.4, 129.2, 127.2, 124.8, 124.5, 121.4, 120.8, 114.5, 65.9, 57.9, 55.0, 25.8, 24.1; MS (ESI) *m*/*z* 398 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{27}NO_2+H]^+$ 398.2120, found 398.2115.

*4.2.7. 4-(2-(4-(Dibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) morpholine (compound **15c**)*

Yellow solid, Yield 86%; Mp (116 °C); IR (KBr): 2932, 2770, 1600, 1453, 1236, 1038, 757 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.34–7.31 (m, 2H), 7.29–7.15 (m, 6H), 7.09–7.04 (m, 1H), 7.00–6.84 (m, 4H), 4.16 (t, *J* = 5.4 Hz, 2H), 3.74 (t, *J* = 4.5Hz, 4H), 2.85 (t, *J* = 5.38 Hz, 2H), 2.64 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.4, 156.8, 140.6, 134.0, 131.1, 129.8, 129.4, 129.1, 128.8, 128.2, 127.3, 126.2, 124.1, 122.7, 122.0, 121.2, 119.7, 116.1, 115.8, 113.3, 65.9, 64.8, 56.6, 53.0; MS (ESI) *m*/*z* 400 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{25}NO_3+H]^+$ 400.1913, found 400.1900.

*4.2.8. 1-(2-(4-(Dibenzo[b,f]oxepin-10-yl)phenoxy)ethyl)azepane (compound **15d**)*

Viscous oil, Yield 76.6%; IR (KBr): 2934, 2770, 1602, 1460, 1234, 1038, 753 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, *J* = 6.78 Hz, 2H), 7.37–7.26 (m, 5H), 7.23–713 (m, 1H), 7.06–7.02 (m, 2H), 7.00–6.93 (m, 3H), 4.21 (t, *J* = 5.98 Hz, 2H), 3.08 (t, *J* = 5.98 Hz, 2H), 2.92–2.98 (m, 4H), 1.75–1.64 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.6, 156.8, 140.7, 133.9, 131.1, 129.8, 129.0, 128.3, 126.1, 123.5, 123.5, 120.4, 119.7, 113.5, 113.3, 65.3, 55.3, 54.8, 52.3, 26.7, 26.0; MS (ESI) *m*/*z* 412 (M + H).

*4.3. Representative procedure for the synthesis of compounds **23a-d** and **24a-e** as described for compounds **14a-d** and **15a-d***

*4.3.1. 1-(2-(4-(11-Ethyldibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) pyrrolidine (compound **23a**)*

Yellow solid, Yield 54%; Mp (109–111 °C); IR (KBr): 3010, 2920, 2810, 1602, 1454, 1243, 1038, 747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.58–7.47 (m. 2H), 7.45–7.30 (m, 1H), 7.26–7.10 (m, 5H), 7.09–7.02 (m, 2H), 6.96–6.92 (m, 2H), 4.15 (t, *J* = 6.06 Hz, 2H), 2.94

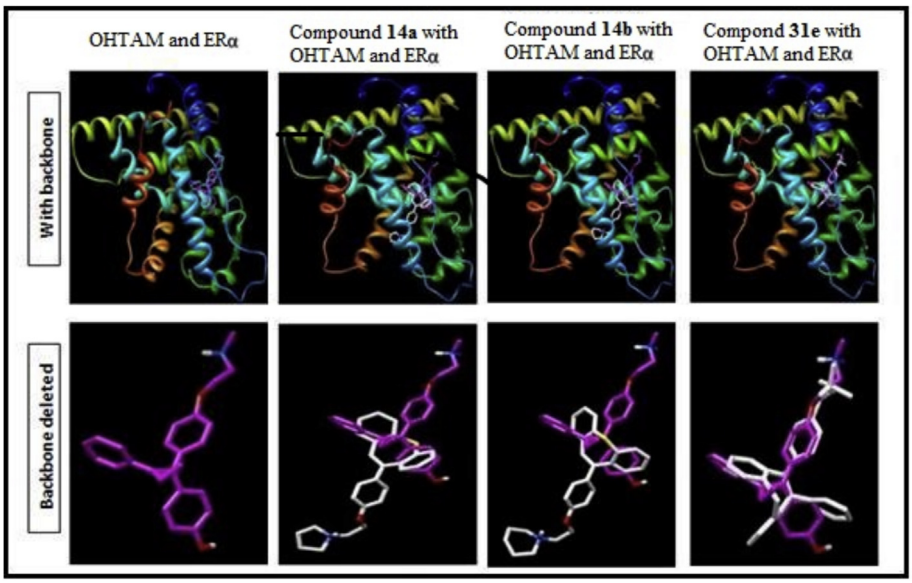


Fig. 7. Molecular docking showing orientation and interaction of active molecules within the ERα cavity in comparison to 4-hydroxy tamoxifen (OH-TAM).

Table 2
Comparative docking energy and interacting residues.

Comp.	Interacting residues	Docking energy
14a	LEU327,LEU346, LYS449, LEU525, GLU353, ARG394, MET343, LEU349, ALA350, GLY390, LEU391, ILE326, LEU387	−9.26
14b	LEU346, GLY390, LEU391, TRP383, ARG394, ILE326, PHE404,ALA350, LEU387, LYS449, GLU353, LEU403, ILE386	−9.62
31e	ILE326, PHE445, LEU391, ARG394, LEU403, GLU353, LEU387,LYS449, LEU349, PRO324, GLY390, GLU323, LEU327	−10.17
OH-TAM	ILE326, MET357, TRP393, PRO325, LEU387, ILE386, LYS449, GLU353, GLY390, GLU323, LEU327, LEU403, PHE404, LEU391	−7.59

(t, J = 6.0 Hz, 2H), 2.66–2.61 (m, 4H), 1.85–1.83 (m, 4H), 1.56 (q, J = 7.32 Hz, 2H), 0.65 (t, J = 6.85 Hz, 3H); ^{13}C NMR (75 MHz,$CDCl_3$):δ 157.7, 144.2, 142.6, 140.0, 139.5, 138.5, 136.2, 132.0.,131.9,131.3,128.2,127.131.9, 131.3, 128.2, 127.6,127.2,114.1, 66.9, 55.1, 54.7, 23.5, 13.9; MS (ESI) *m/z* 428 (M + H); HRMS (ESI) exact mass calcd for $[C_{28}H_{30}NOS + H]^+$ 428.2048, found 428.2040.

*4.3.2. 1-(2-(4-(11-Ethyldibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) piperidine (compound **23b**)*

Yellow solid, Yield 62%; Mp (92–94 °C); IR (KBr): 3020, 2928, 2810, 1600, 1456, 1248, 1038, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15–7.12 (m, 2H), 7.11 (d, J = 6.06 Hz, 1H), 7.07–6.92 (m, 6H), 6.91–6.79 (m, 3H), 4.08 (t, J = 5.91 Hz, 2H), 2.76 (t, J = 5.88 Hz, 2H), 2.51–2.46 (m, 4H), 1.59–1.56 (m, 4H), 1.43 (q, J = 6.98 Hz, 2H), 0.68 (t, J = 7.40 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.6, 144.3, 143.5, 142.5, 140.0, 139.8, 138.5, 136.5, 136.3, 131.9, 131.7, 13.

1.2, 128.2, 127.8, 127.6, 127.4, 114.1, 65.8, 58.0, 55.0, 29.6, 25.9, 24.1, 13.8; MS (ESI) *m/z* 442 (M + H); HRMS (ESI) exact mass calcd for $[C_{29}H_{32}NOS + H]^+$ 442.2204, found 442.2204.

*4.3.3. N,N-Diethyl-2-(4-(11-ethyldibenzo[b,f]thiepin-10-yl) phenoxy)ethanamine(compound **23c**)*

Yellow solid, Yield 57%; Mp (89 °C); IR (KBr): 3010, 2920, 2810, 1602, 1454, 1243, 1038, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (d, J = 7.74 Hz, 1H), 7.42–7.23 (m, 4H), 7.13–7.09 (m, 1H), 6.91–6.86 (m, 1H), 6.70–6.54 (m, 5H), 3.98 (t, J = 6.07 Hz, 2H), 2.85 (t, J = 6.36 Hz, 2H), 2.68–2.61 (m, 4H), 1.92–1.21 (m, 2H), 1.08 (t, J = 7.11 Hz, 3H), 0.95–0.82 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.8, 158.4, 152.3, 140.5, 137.3, 136.6, 135.7, 134.7, 133.2, 132.2, 131.8, 129.8, 129.4, 128.8, 126.3, 125.5, 125.0, 121.5, 120.9, 115.0, 66.7, 59.1, 46.7, 31.1, 14.0, 13.2; MS (ESI) *m/z* 430 (M + H); HRMS (ESI) exact mass calcd for $[C_{28}H_{31}NOS + H]^+$ 430.2205, found 430.2108.

*4.3.4. 2-(4-(11-Ethyldibenzo[b,f]thiepin-10-yl)phenoxy)-N,N-dimethylethanamine(compound **23d**)*

Yellow solid, Yield 59%; Mp (74–76 °C); IR (KBr): 3010, 2920, 2810, 1602, 1454, 1243, 1038, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.24–7.14 (m, 2H), 7.10 (d, J = 6.0 Hz, 1H), 7.18–7.02 (m, 6H), 6.98–6.82 (m, 3H), 4.03 (t, J = 5.34 Hz, 2H), 2.54 (t, J = 5.32 Hz, 2H), 2.43 (q, J = 7.29, Hz, 2H), 2.18 (s, 6H), 0.76 (t, J = 7.36 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.9, 157.6, 151.5, 139.7, 136.5, 135.8, 134.9, 133.9, 132.4, 131.4, 131.0, 129.0, 128.6, 128.0, 125.5, 124.7, 124.2, 120.7, 120.1, 114.2, 65.9, 58.3, 45.9, 30.3, 14.0; MS (ESI) *m/z* 402 (M + H); HRMS (ESI) exact mass calcd for $[C_{28}H_{28}NOS + H]^+$ 402.1869, found 402.1891.

*4.3.5. 1-(2-(4-(11-Ethyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) pyrrolidine(compound **24a**)*

Brown solid, Yield 65%; Mp (67 °C); IR (KBr): 3010, 2920, 2810, 1602, 1454, 1243, 1038, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36 (dd, J = 1.35, 7.92 Hz, 1H), 7.21–7.19 (m, 2H), 7.12–7.09 (m, 2H), 6.89–6.84 (m, 3H), 6.82–6.66 (m, 1H), 4.12 (t, J = 5.91 Hz, 2H), 2.91 (t, J = 5.85 Hz, 2H), 2.65–2.58 (m, 4H), 1.98 (m, 2H), 1.80–1.76 (m, 4H), 0.78 (t, J = 7.39 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 159.9, 158.8, 157.6, 139.7, 136.5, 133.9, 132.4, 131.4, 129.0, 128.6, 128.0, 124.7, 120.2, 120.1, 114.2, 66.5, 55.0, 33.8, 30.3, 29.7, 26.9, 23.4, 22.7, 14.0; MS (ESI) *m/z* 412 (M + H); HRMS (ESI) exact mass calcd for $[C_{28}H_{29}NO_2+H]^+$ 412.2277, found 412.2268.

*4.3.6. 1-(2-(4-(11-Ethyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) piperidine (compound **24b**)*

Brown solid, Yield 87%; Mp (90–92 °C); IR (KBr): 2934, 2775, 1606, 1458, 1236, 1038, 757 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.49 (m, 1H), 7.12–7.32 (m, 7H), 6.88–7.01 (m, 3H), 6.72–6.80 (m, 1H), 4.18 (t, J = 6.00 Hz, 2H), 2.84 (t, J = 6.02 Hz, 2H),

2.68 (q, J = 7.35 Hz, 2H), 2.62–2.52 (m, 4H), 1.59–1.71 (m, 4H), 1.42–1.54 (m, 2H), 0.87 (t, J = 7.41 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 159.9, 158.8, 157.7, 139.7, 136.6, 134.9, 133.9, 132.4, 131.4, 131.0, 129.0, 128.6, 128.0, 124.7, 124.2, 120.7, 120.1, 114.4, 114.3, 65.8, 58.0, 55.0, 29.7, 26.9, 25.8, 24.1, 14.0; MS (ESI) m/z 426 (M + H); HRMS (ESI) exact mass calcd for $[C_{29}H_{31}NO_2+H]^+$ 426.2433, found 426.2427.

*4.3.7. 4-(2-(4-(11-Ethyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) morpholine(compound **24c**)*

Solid, Yield: 72%; Mp (86–88 °C); IR (KBr): 3018, 2926, 2810, 1609, 1458, 1246, 1036, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48–7.41 (m, 1H), 7.31–7.14 (m, 7H), 7.00–6.89 (m, 3H), 6.79–6.73 (m, 1H), 4.18 (t, J = 5.64 Hz, 2H), 3.82–3.73 (m, 4H), 2.87 (t, J = 5.64 Hz, 2H), 2.71–2.60 (m, 6H), 0.87 (t, J = 7.39 Hz, 3H);^{13}C NMR (75 MHz, $CDCl_3$): δ 159.9, 158.9, 157.6, 139.8, 136.5, 135.1, 133.9, 132.4, 131.4, 130.9, 129.0, 128.7, 128.0, 124.7, 124.2, 120.7, 120.2, 114.3, 66.8, 65.7, 57.7, 54.1, 29.7, 26.9, 14.0; MS (ESI) m/z 428 (M + H); HRMS (ESI) exact mass calcd for $[C_{28}H_{29}NO_3+H]^+$ 428.2226, found 428.2224.

*4.3.8. 1-(2-(4-(11-Ethyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) azepane (compound **24d**)*

Yellow oil, Yield: 64%; IR (KBr): 3016, 2905, 2800, 1608, 1460, 1250, 1038, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47–7.43 (m, 1H), 7.30–7.20 (m, 7H), 6.97–6.95 (m, 3H), 6.76–6.73 (m, 1H), 4.37 (t, J = 4.95 Hz, 2H), 3.27 (t, J = 4.89 Hz, 2H), 3.15–3.12 (m, 4H), 2.78 (t, J = 4.93 Hz, 2H), 1.72 (m, 6H), 0.87 (t, J = 7.24 Hz, 3H);^{13}C NMR (75 MHz, $CDCl_3$): δ 160.9, 159.1, 157.7, 140.2, 137.3, 135.1, 133.5, 132.0, 131.9, 130.3, 129.0, 129.2, 127.8, 124.7, 124.0, 120.6, 114.3, 66.8, 56.7, 55.1, 29.7, 26.9, 14.2; MS (ESI) m/z 440 (M + H); HRMS (ESI) exact mass calcd for $[C_{30}H_{33}NO_2+H]^+$ 440.2590, found 440.2584.

*4.3.9. 2-(4-(11-Ethyldibenzo[b,f]oxepin-10-yl)phenoxy)-N,N-dimeth-ylethanamine(compound **24e**)*

Brown viscous oil, Yield 64%; IR (KBr): 3010, 2928, 1612, 1458, 1245, 1036, 747 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ (dd, J = 7.08 Hz, 1H), 7.17–7.01 (m, 7H), 6.87–6.77 (m, 4H), 6.65–6.62 (m, 1H), 4.00 (t, J = 4.00 Hz, 2H), 2.65 (t, J = 5.76 Hz, 2H), 2.55 (q, J = 7.24, 14.67 Hz, 2H), 2.25 (s, 6H), 0.74 (t, J = 7.39 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.9, 157.6, 151.5, 139.7, 136.5, 135.8, 134.9, 133.9, 132.4, 131.4, 131.0, 129.0, 128.6, 128.0, 125.5, 124.7, 124.2, 120.7, 120.1, 114.2, 65.9, 58.3, 45.9, 30.3, 14.0; MS (ESI) m/z 386 (M + H); HRMS (ESI) exact mass calcd for $[C_{26}H_{27}NO_2+H]^+$ 386.2120, found 428.2127.

*4.4. Representative procedure for the synthesis of target molecules **31a-e** and **32a-d** as described for compounds **23a-d** and **24a-e***

*4.4.1. 1-(2-(4-(11-Phenyldibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) pyrrolidine (compound **31a**)*

Yellow solid, Yield 67.3%; Mp (111–113 °C); IR (KBr): 3010, 2920, 1602, 1456, 1243, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61–7.48 (m, 1H), 7.46–7.44 (m, 1H), 7.29–7.05 (m, 12H), 6.96–6.84 (m, 1H), 6.75–6.71 (m, 2H), 4.10 (t, J = 2.89 Hz, 2H), 2.97 (t, J = 3.27 Hz, 2H), 2.75 (m, 4H), 1.88–1.85 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.1, 155.6, 140.1, 138.0, 137.7, 132.6, 131.8, 130.1, 129.2, 129.0, 127.8, 126.0, 124.8, 122.9, 119.0, 112.0, 65.2, 53.5, 53.1, 21.9; MS (ESI) m/z 476 (M + H); HRMS (ESI) exact mass calcd for $[C_{32}H_{29}NOS + H]^+$ 476.2048, found 476.2006.

*4.4.2. 1-(2-(4-(11-Phenyldibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) piperidine (compound **31b**)*

Yellow viscous oil, Yield 69.28%; IR (KBr): 3030, 2928, 1609, 1458, 1246, 1030, 757 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.63–7.56 (m, 1H), 7.49–7.45 (m, 1H), 7.29 (m, 12H), 6.99–6.90 (m, 1H), 6.75–6.67 (m, 2H), 2.73 (t, J = 6.1 Hz, 2H), 2.50 (M, 4H), 1.63–1.60 (m, 4H), 1.47–1.45 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 157.1, 142.0, 139.7, 134.2, 133.4, 132.9, 131.6, 130.8, 130.6, 130.5, 129.3, 127.6, 126.3, 124.5, 120.6, 113.5, 65.7, 57.9, 55.0, 25.9, 24.2; MS (ESI) m/z 490 (M + H); HRMS (ESI) exact mass calcd for $[C_{33}H_{31}NOS + H]^+$ 490.2205, found 490.2241.

*4.4.3. 4-(2-(4-(11-Phenyldibenzo[b,f]thiepin-10-yl)phenoxy)ethyl) morpholine (compound **31c**)*

White solid, Yield: 59%; Mp (97–99 °C); IR (KBr): 3020, 2910, 1609, 1458, 1243, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.23 (m, 4H), 7.19–7.14 (m, 4H), 7.11–6.94 (m, 7H), 6.47–6.35 (m, 2H), 3.96 (t, J = 5.71 Hz, 2H), 3.68 (t, J = 4.59 Hz, 4H), 2.69 (t, J = 5.71 Hz, 2H), 2.47 (t, J = 4.64 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.1, 156.9, 142.1, 141.2, 139.2, 136.3, 134.8, 134.1, 133.2, 131.4, 130.6, 129.5, 127.6, 126.8, 125.1, 124.0, 120.8, 112.9, 66.1, 64.9, 58.2, 53.7; MS (ESI) m/z 492 (M + H); HRMS (ESI) exact mass calcd for $[C_{32}H_{29}NO_2S + H]^+$ 492.1997, found 492.2002.

*4.4.4. N,N-Dimethyl-2-(4-(11-phenyldibenzo[b,f]thiepin-10-yl) phenoxy)ethanamine (compound **31d**)*

Yellow oil, Yield 52%; IR (KBr): 3032, 2926, 1606, 1450, 1242, 1030, 757 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.24 (m, 4H), 7.21–7.16 (m, 4H), 7.14–6.99 (m, 7H), 6.72 (d, J = 6.03 Hz, 2H), 4.03 (t, J = 5.98 Hz, 2H), 2.86 (t, J = 6.03 Hz, 2H),2.88–2.72 (m, 4H), 1.08–0.89 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.2, 141.5, 139.3, 139.0, 132.9, 132.6, 131.9, 130.8, 130.4, 130.0, 128.6, 127.9, 127.0, 124.1, 120.2, 119.8, 114.5, 68.7, 56.2, 13.8; MS (ESI) m/z 478 (M + H); HRMS (ESI) exact mass calcd for $[C_{32}H_{32}NOS + H]^+$ 478.2205, found 478.2225.

*4.4.5. N,N-Dimethyl-2-(4-(11-phenyldibenzo[b,f]thiepin-10-yl) phenoxy)ethanamine (compound **31e**)*

Off white solid, Yield 59%; Mp (98–100 °C); IR (KBr): 3010, 2920, 1602, 1456, 1243, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.28 (m, 4H), 7.26–7.17 (m, 4H), 7.14–6.98 (m, 7H), 6.71 (d, J = 8.7 Hz, 2H), 4.07 (t, J = 5.88 Hz, 2H), 3.0 (t, J = 5.92 Hz, 2H), 2.88–2.85 (m, 4H);^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 141.7, 139.6, 139.2, 133.3, 132.9, 131.6, 130.6, 130.6, 130.5, 129.3, 127.5, 126.3, 124.5, 120.6, 120.5, 113.5, 68.1, 56.2, 55.7, 27.0; MS (ESI) m/z 450 (M + H); HRMS (ESI) exact mass calcd for $[C_{30}H_{28}NOS + H]^+$ 450.1892, found 450.2002.

*4.4.6. 1-(2-(4-(11-Phenyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) pyrrolidine (compound **32a**)*

White solid, Yield 81.69%; Mp (93–95 °C); IR (KBr): 3010, 2920, 1602, 1456, 1243, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.24 (m, 4H), 7.21–7.16 (m, 4H), 7.14–6.99 (m, 7H), 6.72 (d, J = 6.03 Hz, 2H), 4.03 (t, J = 5.98 Hz, 2H), 2.86 (t, J = 6.03 Hz, 2H), 2.61 (m, 2H), 1.82 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.6, 156.1, 140.7, 138.5, 138.2, 133.1, 132.3, 130.6, 129.7, 129.5, 128.3, 126.5, 125.3, 123.4, 119.5, 112.5, 65.7, 54.0, 53.6, 22.4; MS (ESI) m/z 460 (M + H); HRMS (ESI) exact mass calcd for $[C_{32}H_{29}NO_2+H]^+$ 460.2277, found 460.2285.

*4.4.7. 1-(2-(4-(11-Phenyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) piperidine (compound **32b**)*

Off white solid, Yield 76%; Mp (112–114 °C); IR (KBr): 3030, 2928, 1610, 1460, 1248, 1038, 746 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29–7.26 (m, 4H), 7.21–7.19 (m, 4H), 7.15–6.99 (m, 7H), 6.70 (d, J = 8.94 Hz, 2H), 4.02 (t, J = 6.09 Hz, 2H), 2.72 (t, J = 6.09 Hz, 2H), 2.49 (m, 4H), 1.65–1.57 (m, 4H), 1.48–143 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 157.1, 142.0, 139.7, 134.2, 133.4, 132.9, 131.6, 130.8, 130.6, 130.5, 129.3, 127.6, 126.3, 124.5, 120.6, 113.5, 65.7,

57.9, 55.0, 25.9, 24.2; MS (ESI) *m/z* 474 (M + H); HRMS (ESI) exact mass calcd for $[C_{33}H_{31}NO_2+H]^+$ 474.2433, found 474.2426.

4.4.8. 4-(2-(4-(11-Phenyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) morpholine (compound **32c**)

Yellow solid, Yield 71%; Mp (122–123 °C); IR (KBr): 3018, 2926, 1602, 1458, 1245, 1036, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.19–7.17 (m, 4H), 7.15–7.10 (m, 4H), 7.05–6.89 (m, 7H), 6.39–6.31 (m, 2H), 3.93 (t, *J* = 5.76 Hz, 2H), 3.64 (t, *J* = 4.63 Hz, 4H), 2.66 (t, *J* = 5.76 Hz, 2H), 2.47 (t, *J* = 4.60 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.7, 157.0, 141.8, 139.6, 139.2, 135.8, 134.3, 133.4, 132.9, 131.7, 130.6, 129.5, 127.6, 126.3, 125.5, 124.5, 120.6, 113.6, 66.9, 65.5, 57.7, 54.1; MS (ESI) *m/z* 476 (M + H); HRMS (ESI) exact mass calcd for $[C_{32}H_{29}NO_3+H]^+$ 476.2226, found 476.2222.

4.4.9. 1-(2-(4-(11-Phenyldibenzo[b,f]oxepin-10-yl)phenoxy)ethyl) azepane (compound **32d**)

Brown oil, Yield 65%; IR (KBr): 3010, 2920, 1602, 1456, 1243, 1038, 746 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.31–7.28 (m, 4H), 7.26–7.17 (m, 4H), 7.14–6.98 (m, 7H), 6.71 (d, *J* = 8.7 Hz, 2H), 4.07 (t, *J* = 5.88 Hz, 2H), 3.0 (t, *J* = 5.92 Hz, 2H), 2.88–2.85 (m, 4H), 1.72–1.63 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.6, 141.7, 139.6, 139.2, 133.3, 132.9, 131.6, 130.6, 130.6, 130.5, 129.3, 127.5, 126.3, 124.5, 120.6, 120.5, 113.5, 68.1, 56.2, 55.7, 27.0; MS (ESI) *m/z* 488 (M + H).

5. Materials and methods

5.1. Cells and cell culture condition

MCF-7 (estrogen receptor positive breast cancer cell line), MDA-MB- 231 (estrogen receptor negative breast cancer cell lines) cells were originally obtained from ATCC, USA and maintained in laboratory. HEK-293 (human embryonic kidney epithelial cell line) cells were obtained from Institutional repository of CSIR-CDRI. Cells were routinely sub-cultured in DMEM medium with 10% fetal bovine serum (FBS), 100U/ml penicillin and 100 U/ml streptomycin incubated at 37 °C with 5% CO_2. FBS supplement was reduced to 2% during MTT assays.

5.2. MTT assay

The antiproliferative activities of the series of compounds were determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay 1×10^4 cells/well (MCF-7, MDA-MB-231 and HEK-293) were seeded in 200 μl DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 h at 37 °C in CO_2 incubator. Compound stock solutions were prepared in DMSO and were diluted to the desired concentrations in culture medium. Cells cultured in 96-well plate were treated with various concentrations of test compounds for 24 h. After the period of incubation, media were removed and to each well 20 μl of MTT (5 mg/mL) was added and the plates were further incubated for 4 h. Supernatant from each well was carefully removed, formazon crystals were dissolved in 200 μl of DMSO and absorbance at 540 nM wavelength was recorded. Percentage cell inhibition was calculated by comparing test compound absorbance versus untreated control group absorbance. Percent cell inhibition was plotted against various concentrations of each compound and IC_{50} concentration of test compounds were calculated using Graphpad Prism software [17b].

5.3. Morphological analysis of lead compound treated cells

MTT assay quantitatively measures live cells based upon treatment of test compounds based on activity of mitochondrial enzyme. In order to see how the lead compounds are affecting breast cancer cells in a concentration dependent manner, we observed the compound treated cells under bright field microscope. Briefly, cells were seeded at a density of 80,000 cells in 6-well plate and incubated for 24 h. Then, the medium was replaced, and cells were treated with desired concentrations of lead compounds for 24 h. For untreated control group in the experiment, cells were treated with vehicle (0.001% DMSO in culture medium). After the period of treatment, random fields of cells from each group were captured under bright field inverted microscope (Leica, Germany) [23].

5.4. Cell cycle analysis assays

The lead compounds based on activity against breast cancer cells and cytotoxicity towards normal HEK-293 cells were identified and subjected to cell cycle analysis. Briefly, MCF-7 cells were treated with compounds, at the end of the incubation adherent cells were trypsinized and combined with any floating cells present and then washed with cold PBS. Cells were fixed in 70% ethanol and incubated with RNase A and stained with 50 g/ml propidium iodide (PI) for 30 min before acquiring the flow cytometry reading (FACScan, BD Biosciences, USA) [24].

5.5. Morphological analysis for apoptosis using Hoechst staining

Hoechst is fluorescent dye and binds specifically to DNA and therefore used to visually asses the status of nuclear DNA in treated cancer cells. Briefly, cells were seeded at a density of 10,000 cells over 18-mm coverslips placed at the bottom of 6-well cell culture plates and incubated for 24 h. Then, the medium was replaced, and cells were treated with desired concentrations of lead compounds for 24 h. Cells treated with vehicle (0.001% DMSO in culture medium) were included as controls for all experiments. After the period of treatment, Hoechst 33342 (Sigma–Aldrich) stain were added to medium at a concentration of 0.5 g/ml containing 4% paraformaldehyde under low light condition. After incubation for 30 min at 37 °C in dark condition, the coverslips from each well were mounted on slides and random fields of cells from each group were captured under fluorescent microscope (Leica, Germany) using blue filter [25].

5.6. LDH release assay

Release of lactate dehydrogenase (LDH) is an indicator of membrane integrity and hence can be used to determine cellular membrane injury. LDH assay was performed to assess the LDH release to the media following compounds treatment on HEK-293 cells for 24 h as per the manufactures' protocols (Roche, Germany) [26].

5.7. Molecular docking

The X-ray crystallographic structure of human estrogen receptor with ligand binding domain (LBD) in complex with the endogenous estrogen, 4-hydroxytamoxifen and reference protein coordinates used for structural alignment was downloaded from Protein Data Bank (PDB Id: 3ERT) [1]. The energy minimized, 3D structure of the all the three compounds 5, 10, 11 and OH-tamoxifen were generated from PRODRG2 server. MGL Tools 1.5.4 was used to perform flexible docking. A grid spacing of 0.374 Å with 60 × 60 × 60 grid points

were prepared. The docking was performed for 150 population size for 100 runs applying Lamarckian genetic algorithm (LGA). Rest all the parameters were set default for the analysis. The top ranked complex with minimum scoring energy was selected as best docking complex [27].

5.8. Evaluation of apoptosis with flow cytometry

MCF-7 cells (1×10^6) were plated in six well plate and allowed to grow overnight. The medium was then replaced and cells were treated with desired concentrations of lead compounds for 24 h. Cells treated with vehicle (0.001% DMSO in culture medium) were included as controls for all experiments. After 24 h of treatment, cells from supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS at 300 g. Then the cells (1×10^5) were stained with Annexin V-FITC and propidium iodide using the Annexin-V-PI apoptosis detection kit (Sigma). Flow cytometry was performed using a FACScan (Becton Dickinson) equipped with a single 488-nm argon laser. Annexin V-FITC were analyzed using excitation and emission settings of 488 nm and 535 nm (FL-1channel) whereas PI was recorded with 488 nm and 610 nm (FL-2 channel). Debris and clumps were gated out using forward and orthogonal light scatter [28].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.035.

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