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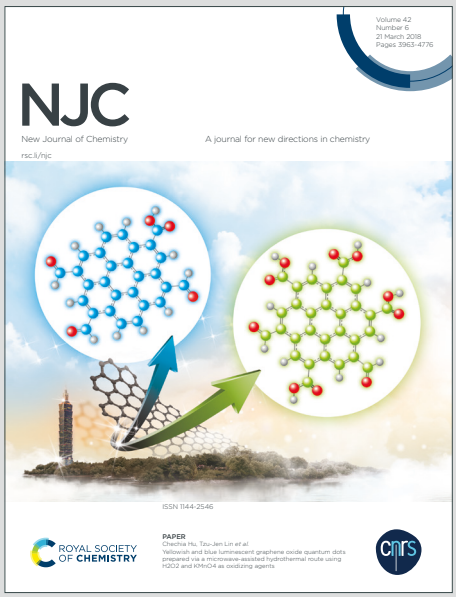

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ARTICLE

Design, Synthesis, and anti-proliferative evaluation of 1*H*-1,2,3-triazole grafted tetrahydro-β-carboline-chalcone/ferrocenylchalcone conjugates in Estrogen Responsive and Triple Negative Breast Cancer cells

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Abstract: A series of 1*H*-1,2,3 triazole grafted tetrahydro-β-carboline-chalcone/ferrocenylchalcone conjugates were synthesized and *invitro* evaluated against Estrogen Responsive (MCF-7) and Triple Negative (MDA-MB-231) breast cancer cells. Comparative analysis revealed the improvement of selectivity towards estrogen responsive cells with the inclusion of ferrocene core. The most potent compounds of the series **13l** (R = 4-F, n = 3) exhibited IC_{50} value of 10.33 μM against MCF-7 and was ~5 folds potent than standard drug Tamoxifen while **13d** (R = 2,3,4-trimethoxy, n = 5**)** exhibited IC_{50} value of 21.99 μM against MDA-MB-231 cells, being ~3 folds potent than Tamoxifen. The experimental results were further supported by molecular docking studies in ligand Binding Domain of ERα and greater binding affinity has been attributed to energetically favourable fit and balance between hydrophobic and hydrophilic interactions in ERα active site.

Introduction

Breast cancer is the second leading cause of mortality amongst females worldwide next to the lung cancer. There has been a rapid increase in the incidence rates of female breast cancer cases by 0.4% each year from 2006 to 2015. It is expected that approximately 42,260 people will die from breast cancer and 268,600 new cases of invasive breast cancer would arise in United States in 2019.[1] According to a survey conducted by Breast International Group (BIG), 40% of 2.1 million cases of the diagnosed breast cancer worldwide were found in Asia. The Health Ministry of India reported that breast cancer accounts for 25% of all cancer cases present in the country.[2,3] To tackle the outburst of this multiform catastrophe, therapeutic advancement *viz.* surgical procedures, chemotherapy, ionizing radiations, hormone dependent and targeted therapies has successfully entered the arena, but high cost of these treatments put socio-economic burden on under-developed countries.[4] Thus, the development of new chemotherapeutics from existing pharmacologically active natural scaffolds with minimal side effects are much needed.

Chalcone, belonging to naturally occurring flavonoid family, is a fascinating molecule with a wide range of antibacterial, anti-inflammatory, anti-plasmodial, antileishmanial, antiviral and anti-proliferative properties.[5] Substituted 4-amino chalcones **I** have been reported to possess anti-breast cancer potential *via* induction of apoptosis and p53 up-regulation in MCF-7 cell lines.[6] Thieno-pyrimidine-chalcones **II** induced apoptotic evasion in breast cancer cells *via* inhibiting Fas-activated serine/threonine kinase (FASTK).[7] β–carboline-chalcone conjugates **III** displayed anti-proliferative potential on lung carcinoma with IC_{50} values less than 10 μM *via* DNA intercalation.[8]

Natural plant based polycyclic indole tetrahydro-β–carboline, (THβC) is known to possess antifungal, anti-plasmodial and anti-cancer potential.[9] The exploration of Selective Estrogen Receptor Down-regulator (SERD) (AZD9496) **IV**, a THβC analogue, with equal potency as that of anticancer drug Fulvestrant, is currently under Phase I clinical investigation.[10] Another THβC analogue **V** showed anti-cancer effect by inhibition of breast cancer resistance protein ABCG2 with IC_{50} value of 0.2 μM.[11] Furthermore, tetrahydro-β-carboline-imidazolinium salt derivatives exhibited anti-proliferative potential against MCF-cell line *via* induction of apoptosis and G1 phase cell cycle arrest with IC_{50} value of 2.79 μM.[12] Recent report from our group revealed the anti-breast cancer potential of triazole linked tetrahydro-β-carboline-isatins with the most potent compound **VI** having IC_{50} value of 37.42 μM against MCF-7 cell line comparable to that of Tamoxifene (**Figure1**).[13]

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† ^{1}H and ^{13}C spectral data of the synthesized compounds along with Scanned ^{1}H and ^{13}C of 12a, 12b, 12c, 12e, 13c, 13e, 13f, 13g, 13h, 13j, 13k, 13l,13m, 13o

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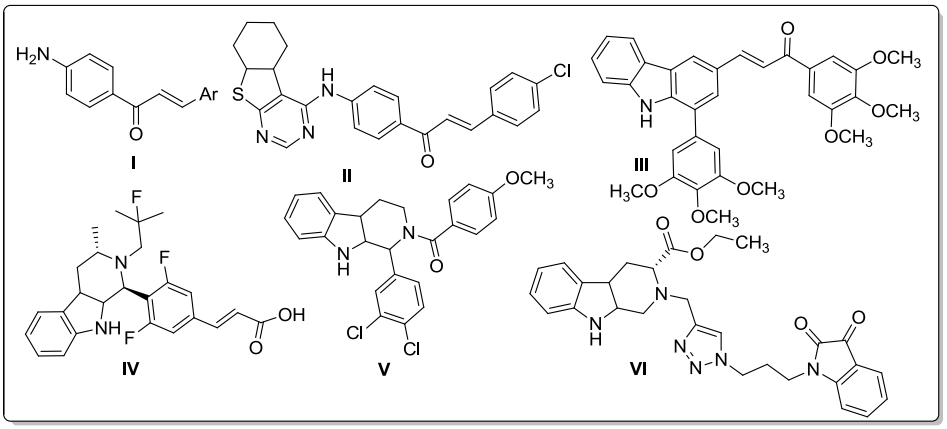


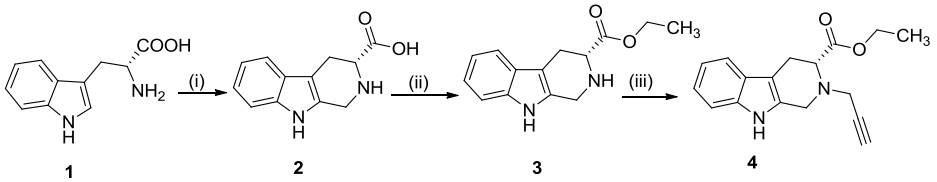
Figure 1: Typical structures of clinically approved drugs or synthetically prepared molecules possessing anti-breast cancer potential.

In continuation with the extension of our research on breast cancer,[14,15] the present manuscript pertains to amalgamating two pharmacologically active moieties *viz*. tetrahydro-β-carboline and chalcones/ferrocenylchalcones in a single framework *via* triazole linkage with an aim to investigate their anti-proliferative Structure-Activity Relationship against estrogen responsive MCF-7 and triple negative MDA-MB-231 cell lines. The introduction of ferrocenyl core among the designed hybrids could be rationalized by its ability to enhance the anti-proliferaive efficacy as evident by ferrocifens and ferrocephenols.[16] 1*H*-1,2,3 triazoles hold an exceptional importance as linker with unique features *viz*. ease of preparation, rigidity, metabolic stability and H-bonding interactions in biological environment.

Results and Discussion

Chemistry

The preparation of tetrahydro-β-carboline-chalcone/ferrocenylchalcone conjugates proceeded with synthesis of precursors *N*-propargylatedtetrahydro-β-carboline **4,** *O*-alkylazido-ferrocenylchalcones **10** and *O*-alkylazidochalcones **11**. Pictet Spengler condensation of L-tryptophan with 30% formalin solution in the basic medium at room temperature furnished 2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid **2** which upon esterification with thionyl chloride in absolute ethanol afforded corresponding ester **3.**[17] Base-promoted *N*-propargylation of **3** in acetonitrile at room temperature gave **4** as depicted in **Scheme 1**. The second set of precursors *viz*. *O*-alkylzido-ferrocenylchalcones **10** and *O*-alkylazidoaryl chalcones **11a-o** were synthesized *via* reported protocols[18] involving an initial base promoted alkylation of 4-hydroxyacetophenone **5** with dibromoalkane in dry DMF at 80°C. The treatment of **5** with sodium azide in dry DMF afforded **7**. The base promoted aldol condensation of **7** with ferrocene-Carboxaldehyde **8** and substituted aldehydes **9** in ethanol afforded corresponding *O*-alkylazido-chalcones **10** and **11**, respectively (**Scheme 2**). Cu-promoted azide-alkyne cycloaddition of **4** with **10** and **11** afforded **12a-e** and **13a-o** (**Scheme 3**). Structures to the synthesized compounds were assigned based on spectral techniques and analytical evidences. For example, compound **12a** exhibited molecular ion peak at $[M+H]^+$ 684.2173 in its High Resolution Mass Spectrum (HRMS). Its ^{1}H NMR exhibited characteristic singlets at δ 4.15 (5H); 4.46 (2H) and 4.57 (2H) corresponding to ferrocene ring protons along with doublet at δ7.73 (J = 15.3Hz) corresponding to olefinic proton and a singlet at δ7.68 (1H) corresponding to triazole ring proton. The presence of characteristic absorptions at δ14.3, 24.0, 49.9, 60.2, 60.8, corresponding to THβC ring carbons along with absorptions at δ172.7 and 188.4 corresponding to carbonyl carbons in ^{13}C NMR spectrum further corroborated the assigned structure.



Scheme1: Synthetic route to *N*-propargylatedtetrahydro-β-carboline ethyl ester **4** (i) 30% formaldehyde, NaOH, H_2O, 8 h, reflux (ii) $SOCl_2$, EtOH, 6 h, reflux (iii) propargyl bromide, acetonitrile, rt, 6–8 h

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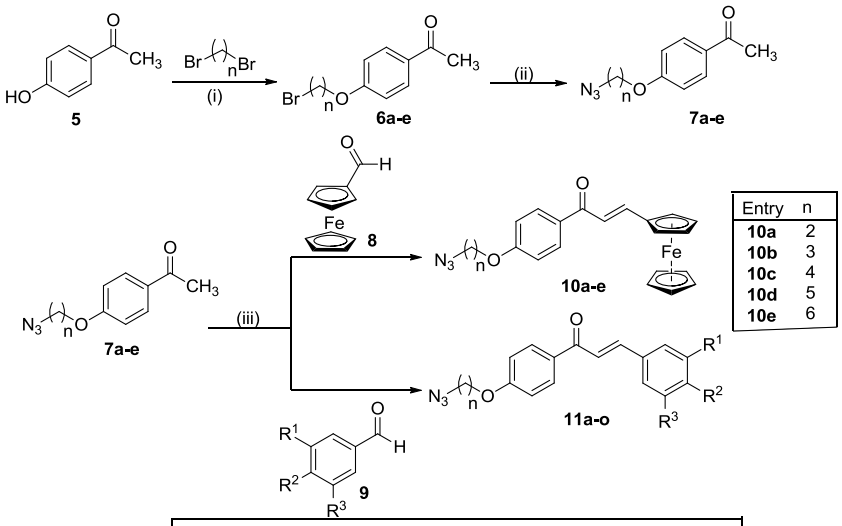
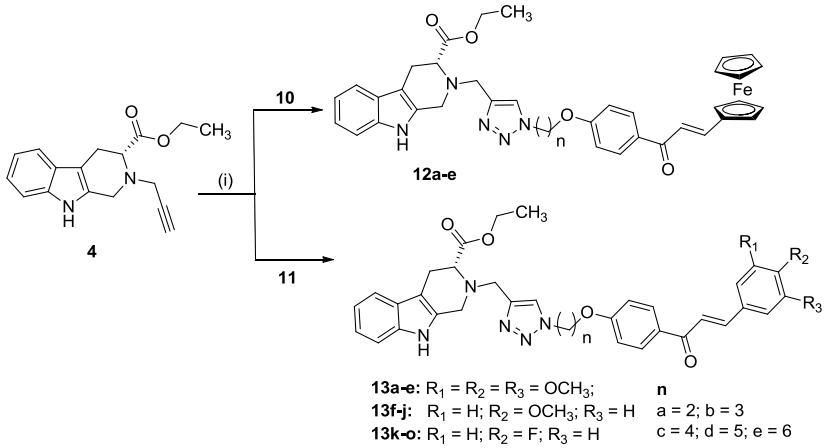


Entry	n
10a	2
10b	3
10c	4
10d	5
10e	6

Entry	n	R^1	R^2	R^3	Entry	n	R^1	R^2	R^3
11a	2	$-OCH_3$	$-OCH_3$	$-OCH_3$	**11i**	5	H	$-OCH_3$	H
11b	3	$-OCH_3$	$-OCH_3$	$-OCH_3$	**11j**	6	H	$-OCH_3$	H
11c	4	$-OCH_3$	$-OCH_3$	$-OCH_3$	**11k**	2	H	F	H
11d	5	$-OCH_3$	$-OCH_3$	$-OCH_3$	**11l**	3	H	F	H
11e	6	$-OCH_3$	$-OCH_3$	$-OCH_3$	**11m**	4	H	F	H
11f	2	H	OCH_3	H	**11n**	5	H	F	H
11g	3	H	$-OCH_3$	H	**11o**	6	H	F	H
11h	4	H	$-OCH_3$	H					

Scheme 2: Synthesis of *O*-alkylazidoferrocenylchalcones**10** and *O*-alkylazido organic Chalcones**11a-o** (i)K_2CO_3, dry DMF, 80 °C, 8h (ii) NaN_3, dry DMF, 60 °C, 2h (iii) 10% NaOH, EtOH, rt, 6h.



Scheme 3: Synthesis of tetrahydro-β–carboline-chalcone/ferrocenylchalcone conjugates**12/13**(i) $CuSO_4.5H_2O$, Sodium ascorbate, EtOH:H_2O (8:2), rt, 8h.

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Anti-proliferative evaluation of target compounds

The synthesized compounds were assessed for their anti-proliferative activities on Hormone responsive (MCF-7) and non-responsive (MDA-MB-231) cells. Six different concentrations (1, 5, 10, 20, 50, 100 μM) of test compounds were used to determine their percentage growth inhibition using plumbagin as positive control (**Figure 5**) and their corresponding IC_{50} values (concentration of test compounds causing 50 % inhibition of cell proliferation) have been enlisted in **Table 1**. As evident, the activities were found to depend on the nature of chalcone core (aryl/ferrocenyl), nature of substituent (on aryl ring) as well as the alkyl chain length. Analysis of SAR amongst conjugates **12a-e** and **13a-o** revealed that aryl chalcone based conjugates showed better anti-proliferative activities on both the cell lines tested. Amongst ferrocenylchalcone-THβC conjugates **12a-e**, compounds with longer even alkyl chain **12c** (n = 4) and **12e** (n= 6) were found to be inactive against both cell lines except **12a** (n = 2) which exhibited IC_{50} of 71.4 μM against MCF-7. The conjugates with odd alkyl chain length **12b (**n=3**)** and **12d (**n=5**)** exhibited selectivity on MCF-7 cells with IC_{50} values of 79.3 and 70.71 μM, respectively.

Among THβC-chalcone conjugates, the nature of substituent on phenyl ring of chalcone predominantly played an important role in enhancing the cytotoxicity on breast cancer cell lines whereas length of alkyl chain hardly affected the activities. Compounds with electron donating tri-methoxy substituents on phenyl ring **13a-e** displayed appreciable cytotoxicities on breast cancer cells as compared to compounds with mono-methoxy substituent **13f-j,** which were inactive on both breast cancer cell lines. Among tri-methoxylated conjugates, the compounds **13a** (n = 2) and **13d** (n = 5) exhibited IC_{50} values of 68.61 and 44.73 μM in MCF-7 cells. The compound **13d** with a pentyl chain as spacer displayed an IC_{50} value of 21.99 μM in MDA-MB-231 cells and was therefore ~3 folds potent than Tamoxifen. Among mono-methoxylated conjugates, the anti-proliferative activities were completely missing in MCF-7 cells, however the conjugate **13i** (n=5) proved to be selective against triple negative MDA-MB-231 cell line and displayed IC_{50} of 70.71 μM.

Interestingly, the compounds with electron withdrawing fluoro-substitution at phenyl ring were found to be the most active amongst all the synthesized conjugates. The compound **13k** (n = 2); and **13n** (n = 5) proved to be selective inhibitors of MCF-7 cells displaying IC_{50s} of 19.00 and 31.62 μM respectively. The compound **13l** (n=3) proved to be potent inhibitor of both Estrogen responsive as well as un-responsive cells exhibiting IC_{50} values of 10.33 and 70.71 μM against MCF-7 and MDA-MB-231 cells, respectively. The compound **13l**, therefore was ~5 folds potent than Tamoxifen in MCF-7 cells and comparable in MDA-MB-231 cells. The conjugates with butyl (13m) and hexyl (13o) chain lengths proved to be inactive against both the cells, confirming the influence of alkyl chain lengths on the activities among fluoro-substituted conjugates. The conjugates **13k**,**13l** and **13n** therefore are selective inhibitors of Estrogen-responsive cancer cells while the conjugate **13d** can act as a promising lead for the difficult-to-treat ER-cancer cells. The generalized SAR in the pictorial form has been depicted in **Figure 2**.

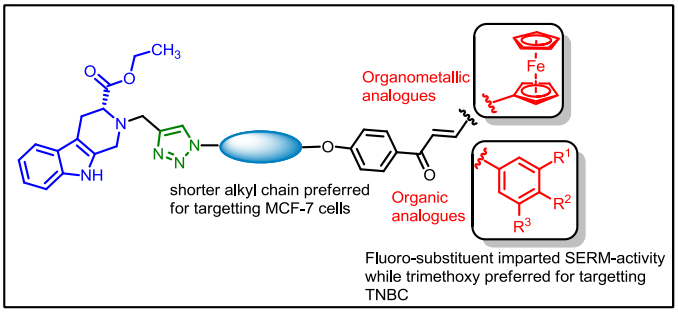


Figure2: Diagrammatic representation of generalized Structure Activity Relationship (SAR).

Molecular Docking Studies

Estrogen receptor subtypes ERα and ERβ are nuclear receptors and ligand-activated transcription factors which regulates the expression of genes controlling different physiological events in humans. The up-regulation and activation of ERα mediate cell proliferation in estrogen-responsive breast cancer. In contrast, the precise role of ERβ in breast cancer is still contentious, although accumulating evidence suggests bi-faceted anti-proliferative and pro-proliferative roles in estrogen-responsive and triple-negative breast cancers respectively.[19] Accordingly, molecular docking studies of compounds **13d**, **13k** and **13l** were conducted in the ligand-binding domain (LBD) of ERα to identify the pharmacophores furnishing the ligand-receptor interactions responsible for the observed anti-proliferative activities.

The docking results presented in **Table 2** show that the superior anti-proliferative activity of compound **13l** as compared to **13d** and **13k** is due to its favourable fit and stability in the receptor's LBD. The computed descriptor of binding affinity (ΔG_{bind}) is also lowest in compound **13l**. An analysis of compound **13l**-ERα complex (**Fig. 3/Fig. 4**) reveals both direct and water-mediated hydrogen bond (H-

b) interactions of the THβC and triazole moieties with residues crucial to anti-estrogenic activity, *i.e.*, Leu346, Thr347 and Asp351. The phenoxy ring, however afforded aromatic H-b interaction with Met522 as well as $\pi-\pi$ stacking with both Trp383 and Tyr526. Aromatic H-b also exists between the ethoxy oxygen of THβC and Trp383. Moreover, the potency of compound **13l** against MCF-7 can be attributed to its increased hydrophobic interactions with critical residues such as Met343, Leu354, Trp383 and Leu525 in the ligand-binding pocket (LBP).

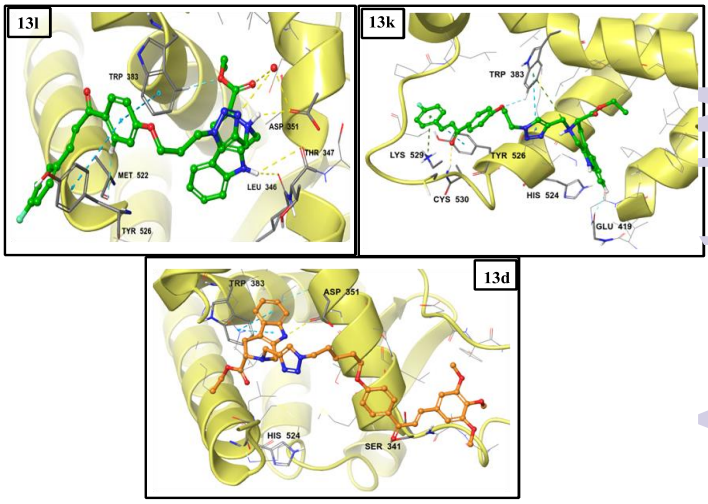
On the other hand, the binding profile of compound **13k** corroborates the 2-fold reduced potency relative to compound **13l**. The docked complex (**Fig. 3/ Fig. 4**) is characterized by just one H-b interaction of chalcone carbonyl oxygen with Cys530 along side $\pi-\pi$ stacking and π-cation interactions of the *p*-fluorophenyl ring with Tyr526 and Lys529, respectively. Similarly, the triazole ring and quaternary nitrogen of THβC are involved in $\pi-\pi$ stacking and π-cation interactions, respectively with Trp383. A robust network of hydrophobic interactions with amino acid residues in the LBP, including Met343, Thr347, Asp351, Trp383, Ile424 and Leu525 (**Fig. 6b**) also help stabilize the complex. Furthermore, the complex of compound **13d** shows H-b interactions of chalcone carbonyl oxygen and NH unit of THβC with Ser341 and Asp351, respectively, while His524 adopted an open conformation to accommodate the ligand's length. However, the bulkiness and steric restrictions of the 3,4,5-trimethoxy substituent seem to distort the ligand's conformation in the LBP, thus reducing the hydrophobic contacts and stability.

These results re-emphasize the significance of hydrophobic contacts to binding affinity and ERα antagonism. Also, an opposite balance between hydrophilic and hydrophobic interactions is crucial for energetically favourable fit and stability of long ligands in the LBD. This is seen in by the modest binding profile and consequently, the inferior potency of compound **13d** against estrogen-responsive breast cancer (MCF-7) cells.

Conclusion

A library of 1*H*-1,2,3-triazole tethered THβC-chalcone/ferrocenylchalcones with varied alkyl chain was synthesized and *in-vitro* evaluated for anti-breast cancer potential against MCF-7 and MDA-MB-231 cell lines. The comparative activity analysis of synthesized conjugates revealed the organic chalcone-linked conjugates to be more active against MCF-7 cell lines, though selectivity for MCF-7 cells was found to higher in ferrocenyl-chalcone linked conjugates. The compound **13l** with an optimum combination of electron withdrawing and lipophilic 4-fluoro substituent on phenyl ring of chalcone and propyl chain as spacer proved to be the most potent with IC_{50} value of 10.33 µM against MCF-7 while **13d** with electron donating trimethoxy substituent on phenyl ring of chalcone and pentyl as spacer proved to be the most active compound against MDA-MB-231 cells with IC_{50} value of 21.99 µM. In general, the results indicated that the introduction of electron withdrawing fluoro substituent on aryl ring with shorter alkyl chain as spacer improved their ability to act as SERMs while the presence of trimethoxy substituent along with longer alkyl spacer lengths improved their potential to target triple negative breast cancer.

Figure 3: 3D representation of predicted binding mode of Erα complexes of most active compounds **13l, 13k, 13d**. Binding interactions are shown as dashed lines: hydrogen bond (yellow), aromatic H-bond (light blue), pi-pi (cyan), pi-cation (green). Atoms: Water (red sphere), carbon (ligand, green; receptor, grey), nitrogen (blue), oxygen (red).



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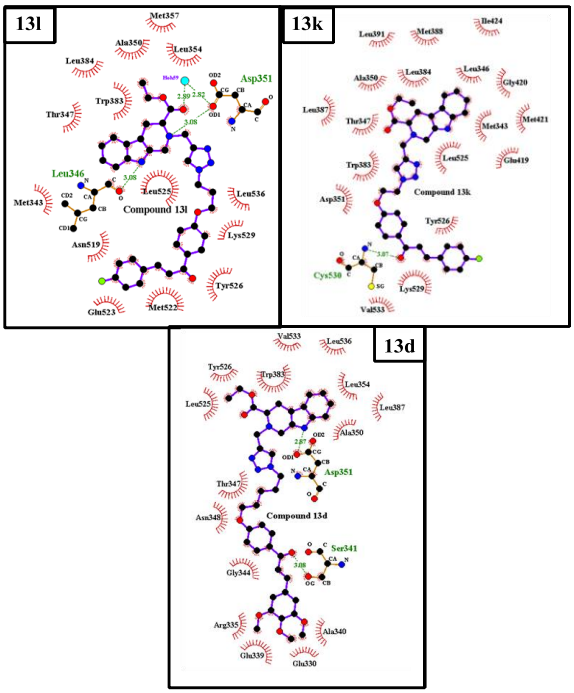

Figure 4: Two dimensional (2D) Ligand Protein interaction profile diagram of Erα complexes of most active compound **13d, 13k, 13l** Hydrogen bond (green), hydrophobic contacts (maroon).

Table 1: In-vitro anti-proliferative activities in terms of IC_{50} (μM) values of test compounds on MCF-7 and MDA-MB-231 cell lines

Entry	R^1	R^2	R^3	n	MCF-7	MDA-MB-231	Entry	R^1	R^2	R^3	n	MCF-7	MDA-MB-231
12a	-	-	-	2	71.4	>100	**13h**	H	OCH_3	H	4	>100	>100
12b	-	-	-	3	79.3	>100	**13i**	H	OCH_3	H	5	>100	70.71
12c	-	-	-	4	>100	>100	**13j**	H	OCH_3	H	6	>100	>100
12d	-	-	-	5	70.71	>100	**13k**	H	F	H	2	19	>100
12e	-	-	-	6	>100	>100	**13l**	H	F	H	3	10.33	70.71
13a	OCH_3	OCH_3	OCH_3	2	68.61	>100	**13m**	H	F	H	4	>100	>100
13b	OCH_3	OCH_3	OCH_3	3	>100	>100	**13n**	H	F	H	5	31.62	>100
13c	OCH_3	OCH_3	OCH_3	4	>100	>100	**13o**	H	F	H	6	>100	>100
13d	OCH_3	OCH_3	OCH_3	5	44.73	21.99	**4**					>100	>100
13e	OCH_3	OCH_3	OCH_3	6	>100	>100	**Plumbagin**					**3.5**	**4.4**
13f	H	OCH_3	H	2	>100	>100	**Tamoxifen**					**50**	**75**
13g	H	OCH_3	H	3	>100	>100							

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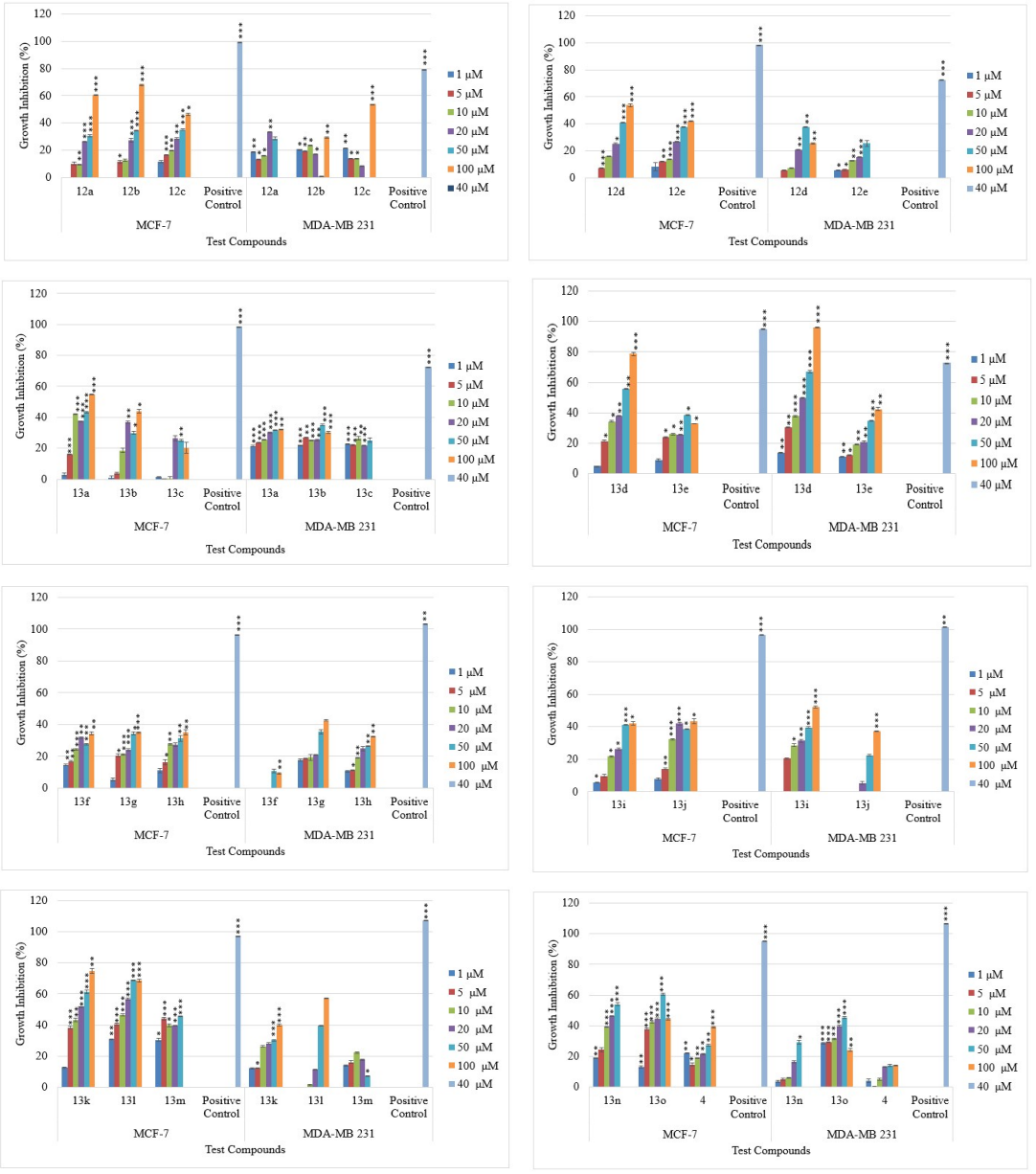
Table 2 Molecular docking results of selected compounds **13l, 13k, 13d** on ERα.

Compound	$IC_{50}\mu M$ (MCF-7)	Docking score	Glide score	Glide model	Glide energy (kcal/mol)	ΔG_{bind} (kcal/mol)
13l	10.33	-11.64	-11.65	-112.43	-65.92	-81.33
13k	19.00	-10.01	-10.02	-102.41	-64.71	-74.12
13d	44.73	-4.84	-4.86	-76.21	-56.25	-59.38

Figure 5: Representative graph comparing the percentage growth inhibition of MCF-7 and MDA-MB 231 cells at selected concentrations of test compounds. 40 µM Plumbagin was used as a positive control. Data are mean ± standard deviation S.D. (n=3, triplicates), where *p<0.05, **p<0.01 and ***p<0.001 significant difference to untreated control.



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Experimental section

General synthetic protocols

The standard protocols and techniques were used to carry out all the reactions. The column chromatography was carried out using silica gel (60–120 mesh) with ethyl acetate: hexane as eluent. Melting points were recorded by using open capillaries and are uncorrected. The spectra of ^{1}H NMR and ^{13}C NMR spectra were recorded on JEOL400 and 100 MHz spectrometers and were obtained as $CDCl_3$ solutions relative to tetramethylsilane (TMS) as an internal standard. Chemical shifs were reported in parts per million (ppm) and coupling constants J were indicated in hertz. Splitting patterns are indicated as s: singlet, d: doublet, t: triplet, m: multiplet, dd: double of doublet, ddd: doublet of a doublet of a doublet, and br: broad peak. Mass spectral data was assembled on BrukermicrOTOF QII equipment using ESI as the source.

General Procedure for synthesis of 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid 2

A mixture of L-tryptophan (0.5 mmol) and NaOH (0.5 mmol) was added sequentially to 200 mL water and stirred. After the solution became clear, 30% formalin (0.5 mmol) was added and the stirring was continued at room temperature for 3h with subsequent refluxing for 3h. After completion of reaction as monitored by TLC, the reaction mixture was neutralized with glacial acetic acid to pH = 5. The precipitates, thus obtained, were collected by filtration, washed with water (2 X 50 mL) and dried to obtain pale white solid which was used in next step without further purification.

General Procedure for synthesis of 2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (3)

To a well stirred solution of 2,3,4,9-tetrahydro-1*H*-β-carboline-3-carboxylic acid **2** (20 mmol) in 500 mL ethanol, a solution of thionyl chloride (20 mL) was added drop-wise at 0 ^{o}C. The mixture was brought to room temperature and subsequently refluxed for 2h. The resulting mixture was concentrated under vacuum, poured in H_2O (200mL) and extracted with ethylacetate (3 x 200 mL). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain 2,3,4,9-Tetrahydro-1*H*-β-carboline-3-carboxylic acid ethyl ester **3** as brown solid.

General Procedure for synthesis of2-Prop-2-ynyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (4)

To a mixture of **3** (3 mmoL) in acetone (15 mL), K_2CO_3 (3.6 mmoL) was added and resulting suspension was stirred at room temperature. After 30 mins, propargyl bromide (3.6 mmoL) was added and the stirring was continued at room temperature for 6-8h. After completion of reaction (TLC control), reaction mixture was filtered and the filtrate was concentrated under reduced pressure to obtain crude product which was purified by column chromatography on silica gel using EtOAc: Hexane (3:7) mixture.

General Procedure for synthesis of hybrids 12a-e /13a-o

To the stirred solution of azido-ferrocenylchalcone **10** (1mmol)/phenyl substituted ferrocenylchalcone **11** and 2-Prop-2-ynyl-2,3,4,9-tetrahydro-β-carboline-3-carboxylic acid ethyl ester **4** in a mixture of ethanol: water (85:15), was added $CuSO_4.5H_2O$ (0.055 mmol) and sodium ascorbate (0.143 mmol). The reaction mixture was allowed to stir at room temperature for 7–8 h. Upon completion of the reaction as monitored through TLC, the resulting mixture was extracted with Chloroform (2x30 mL) and water (2x25 mL). The organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the desired conjugates (**12a-e /13a-o**); which were purified *via* chromatography using Silica Gel (60-120 mesh) as stationary phase and ethyl-acetate: hexane (80: 20) as eluent mixture.

2-[1-(2-{4-[3-(ferrocenyl)-acryloyl]-phenoxy}-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (12a)

Red solid; mp 97-99 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, *J* = 7.1 Hz, 3H, -CH_3); 3.07-3.19 (m, 2H, -CH_2); 3.73 (d, *J* = 15.3 Hz, 1H, -CH_2); 3.87 (t, *J* = 5.2 Hz, 1H, -CH); 4.03-4.13 (m, 7H, -CH_2+-OCH_2+-NCH_2+-NCH_2); 4.15 (s, 5H, Cp); 4.46 (s, 2H, Cp); 4.57 (s, 2H, Cp); 4.73 (m, 2H, -OCH_2); 6.85 (d, *J* = 8.6 Hz, 2H, Ar-H); 7.03-7.12 (m, 3H, 2Ar-H+--CH=CH-); 7.31 (d, *J* = 7.7 Hz, 1H, Ar-H); 7.44 (d, *J* = 7.5 Hz, 1H, Ar-H); 7.68 (s, 1H, triazole-H); 7.73 (d, *J* = 15.3 Hz, 1H, -CH=CH-); 7.91 (d, *J* = 8.6 Hz, 2H, Ar-H); 8.26 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 24.0, 46.2, 49.3, 49.9, 60.2, 60.8, 66.5, 69.1, 69.8, 71.5, 79.2, 105.9, 111.0, 114.2, 117.8, 118.6, 119.2, 121.4, 124.1,127.0, 130.7, 131.2, 132.2, 136.3, 145.9, 146.7, 161.4, 172.7, 188.4 HRMS calcd.for $C_{38}H_{37}FeN_5O_4$: $[M+H]^+$ 684.2195, found : 684.2173.

2-[1-(3-{4-[3-(ferrocenyl)-acryloyl]-phenoxy}-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (12b)

Red solid; mp 92-94 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, *J* = 7.2 Hz, 3H, -CH_3); 2.38-2.44 (m, 2H, -CH_2); 3.06-3.18 (m, 2H, -CH_2); 3.80 (d, *J* = 15.1 Hz, 1H, -CH_2); 3.88 (t, *J* = 5.4 Hz, 1H, -CH); 3.98-4.13 (m, 7H, -CH_2+-NCH_2+-OCH_2+-NCH_2); 4.16 (s, 5H, Cp); 4.46 (s, 2H, Cp); 4.54-4.58 (m, 4H, Cp+-OCH_2); 6.91 (d, *J* = 8.8 Hz, 2H, Ar-H); 7.03-7.12 (m, 3H, 2Ar-H+ -CH=CH-); 7.30 (d, *J* = 7.8 Hz, 1H, Ar-H); 7.44 (d, *J* = 7.5 Hz, 1H, Ar-H); 7.52 (s, 1H, triazole-H); 7.74 (d, *J* = 15.3 Hz, 1H, -CH=CH-); 7.95 (d, *J* = 8.8 Hz, 2H, Ar-H); 8.06 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 24.0, 29.7, 46.4, 47.0, 49.2, 60.1, 60.8, 64.3, 69.0, 69.8, 71.4, 79.3, 105.9, 111.0, 114.2, 117.8, 118.7, 119.2, 121.4, 123.5, 127.0, 130.7, 131.2, 131.8, 136.3, 145.5, 146.4, 162.0, 172.8, 188.3 HRMS calcd.for $C_{39}H_{39}FeN_5O_4$: $[M+H]^+$ 698.2351, found: 698.2382.

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2-[1-(4-{4-[3-(ferrocenyl)-acryloyl]-phenoxy}-butyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (12c)

Red solid; mp 85-87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 7.1 Hz, 3H, -CH_3); 1.78-1.85 (m, 2H, -CH_2); 2.07-2.14 (m, 2H, -CH_2); 3.08-3.20 (m, 2H, -CH_2); 3.84-3.90 (m, 2H, -CH + -CH_2); 4.02 (t, J = 5.8 Hz, 2H, -NCH_2); 4.07-4.20 (m, 10H, Cp+ -CH_2 + -OCH_2 + -NCH_2); 4.40 (t, J = 6.9 Hz, 2H, -OCH_2); 4.45 (s, 2H, Cp); 4.57 (s, 2H, Cp); 6.90 (d, J = 8.7 Hz, 2H, Ar-H); 7.05-7.13 (m, 3H, 2Ar-H+ -CH=CH-); 7.27 (d, J = 7.8 Hz, 1H, Ar-H); 7.44 (d, J = 7.5 Hz, 1H, Ar-H);7.56 (s, 1H, triazole-H); 7.72 (d, J = 15.3 Hz, 1H, -CH=CH-); 7.96 (d, J = 8.7 Hz, 2H, Ar-H); 8.14 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 23.9, 26.1, 27.1, 46.5, 49.1, 50.0, 60.1, 60.8, 67.1, 69.0, 69.8, 71.3, 79.4, 105.9, 111.0, 114.2, 117.8, 118.8, 119.2, 121.4, 122.9, 127.0, 130.7, 131.3, 131.5, 136.3, 145.7, 146.1, 162.3, 172.8, 188.2 HRMS calcd.for $C_{40}H_{41}FeN_5O_4$: $[M+H]^+$ 712.2508, found: 712.2562.

2-[1-(5-{4-[3-(ferrocenyl)-acryloyl]-phenoxy}-pentyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (12d)

Red solid; mp 79-81°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 1.39-1.42 (m, 2H, -CH_2); 1.69-1.71 (m, 2H, -CH_2); 1.94-2.01 (m, 2H, -CH_2); 3.07-3.19 (m, 2H, -CH_2); 3.85-3.91 (m, 2H, -CH + -H_2); 4.01 (t, J = 6.1 Hz, 2H, -NCH_2); 4.07-4.20 (m, 10H, Cp + -OCH_2 + -CH_2 + -NCH_2); 4.31 (t, J = 7.0 Hz, 2H, -OCH_2); 4.41 (s, 2H, Cp); 4.55 (s, 2H, Cp); 6.93 (d, J = 8.8 Hz, 2H, Ar-H); 7.04-7.15 (m, 3H, 2Ar-H +-CH=CH-); 7.24 (d, J = 7.9 Hz, 1H, Ar-H); 7.45 (d, J = 7.4 Hz, 1H, Ar-H); 7.55 (s, 1H, triazole-H); 7.71 (d, J = 15.3 Hz, 1H, --CH=CH-); 7.97 (d, J = 8.7 Hz, 2H, Ar-H); 8.04 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.7, 23.9, 26.4, 27.3, 46.4, 49.2, 50.1, 60.2, 60.9, 67.2, 69.3, 69.9, 71.4, 79.5, 105.8, 111.3, 114.5, 117.7, 118.1, 119.3, 121.4, 122.8, 127.1, 130.6, 131.2, 131.4, 136.2, 145.8, 146.2, 162.4, 172.9, 188.3 HRMS calcd.for $C_{41}H_{43}FeN_5O_4$: $[M+H]^+$ 726.2664, found : 726.2681.

2-[1-(6-{4-[3-(ferrocenyl)-acryloyl]-phenoxy}-hexyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (12e)

Red solid; mp 77-82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.1 Hz, 3H, -CH_3); 1.35-1.43 (m, 2H, -CH_2); 1.47-1.55 (m, 2H, -CH_2); 1.75-1.82 (m, 2H, -CH_2); 1.89-1.96 (m, 2H, -CH_2); 3.08-3.20 (m, 2H, -CH_2); 3.86-3.90 (m, 2H, -CH + -CH_2); 4.00 (t, J = 6.2 Hz, 2H, -NCH_2); 4.08-4.23 (m, 10H, Cp + -OCH_2 + -CH_2+ -NCH_2); 4.33 (t, J = 7.0 Hz, 2H, -OCH_2); 4.45 (s, 2H, Cp); 4.57 (s, 2H, Cp); 6.92 (d, J = 8.8 Hz, 2H, Ar-H); 7.06-7.14 (m, 3H, 2Ar-H + -CH=CH-); 7.26 (d, J = 7.9 Hz, 1H, Ar-H); 7.44 (d, J = 7.4 Hz, 1H, Ar-H); 7.53 (s, 1H, triazole-H); 7.72 (d, J = 15.3 Hz, 1H, --CH=CH-); 7.96 (d, J = 8.7 Hz, 2H, Ar-H); 8.05 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 23.9, 25.5, 26.2, 28.9, 30.2, 46.5, 49.1, 50.2, 60.0, 60.7, 67.9, 69.0, 69.8, 71.2, 79.5, 106.1, 110.8, 114.2, 117.9, 118.9, 119.3, 121.5, 122.7, 127.1, 130.6, 131.2, 131.3, 136.3, 145.6, 145.8, 162.6, 172.7, 188.2 HRMS calcd.for $C_{42}H_{45}FeN_5O_4$: $[M+H]^+$ 740. 2821, found: 740.2843.

2-[1-(2-{4-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenoxy}-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13a)

Pale yellow solid; mp 96-98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 3.04-3.10 (m, 2H, -CH_2); 3.70 (t, J = 5.4 Hz, 1H, -CH); 3.89 (s, 3H, -OCH_3); 3.94 (s, 6H, -OCH_3); 3.97 (t, J = 5.1 Hz, 2H, -NCH_2); 4.03-4.13 (m, 6H, -OCH_2+ -CH_2 + -NCH_2); 4.29 (t, J = 6.3 Hz, 2H, -OCH_2); 6.82 (s, 2H, Ar-H); 6.87 (d, J = 8.8 Hz, 2H, Ar-H); 7.03-7.09 (m, 2H, Ar-H + -CH=CH-); 7.22 (m, 1H, Ar-H); 7.37-7.41 (m, 2H, Ar-H); 7.54 (s, 1H, triazole-H); 7.71 (d, J = 15.3 Hz, 1H, -CH=CH-); 7.92 (d, J = 8.8 Hz, 2H, Ar-H); 8.54 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 24.0, 46.3, 49.3, 49.8, 56.3, 60.2, 60.7, 61.0, 66.5, 105.8, 105.9, 107.1, 110.9, 114.3, 117.8, 119.2, 121.1, 121.4, 124.1, 127.0, 130.4, 130.9, 131.2, 131.9, 136.3, 140.5, 144.8, 153.5, 161.6, 172.7, 189.0 HRMS calcd.for $C_{37}H_{39}N_5O_7$: $[M+H]^+$ 666.2849, found: 666.2872.

2-[1-(3-{4-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenoxy}-propyl)-1H [1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13b)

Pale yellow solid; mp 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 2.39-2.45 (m, 2H, -CH_2); 3.06-3.18 (m, 2H, -CH_2); 3.79 (t, J = 5.3 Hz, 1H, -CH); 3.87 (s, 3H, -OCH_3); 3.90 (s, 6H, -OCH_3); 3.94 (t, J = 5.4 Hz, 2H, -NCH_2); 4.01-4.15 (m, 6H, -OCH_2 + -CH_2 + -NCH_2); 4.32 (t, J = 6.5 Hz, 2H, -OCH_2); 6.81 (s, 2H, Ar-H); 6.85 (d, J = 8.8 Hz, 2H, Ar-H); 7.01-7.08 (m, 2H, Ar-H + -CH=CH-); 7.21 (m, 1H, Ar-H); 7.39-7.43 (m, 2H, Ar-H); 7.53 (s, 1H, triazole-H); 7.69 (d, J = 15.3 Hz, 1H, -CH=CH-); 7.91 (d, J = 8.8 Hz, 2H, Ar-H); 8.58 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 23.9, 30.1, 46.4, 49.3, 50.1, 56.7, 60.3, 60.8, 61.1, 66.5, 105.7, 105.9, 107.2, 110.8, 114.2, 117.9, 119.1, 121.2, 121.5, 124.1, 127.1, 130.5, 130.9, 131.3, 131.9, 136.4, 140.1, 144.8, 153.6, 161.7, 172.7, 189.1 HRMS calcd.for $C_{38}H_{41}N_5O_7$: $[M+H]^+$ 680.3006, found: 680.3042.

2-[1-(4-{4-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenoxy}-butyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H–β–carboline-3-carboxylic acid ethyl ester (13c)

Pale yellow solid; mp 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (t, J = 7.1 Hz, 3H, -CH_3); 1.75-1.79 (m, 2H, -CH_2); 2.02-2.07 (m, 2H, -CH_2); 3.05-3.17 (m, 2H, -CH_2); 3.67 (m, 1H, -CH); 3.86 (s, 3H, -OCH_3); 3.88 (s, 6H, -OCH_3); 3.97 (t, J = 5.5 Hz, 2H, -NCH_2); 4.04-4.17 (m, 6H, -OCH_2+ -CH_2+ -NCH_2); 4.37 (t, J = 6.6 Hz, 2H, -OCH_2); 6.83 (s, 2H, Ar-H+ -CH=CH-); 6.89 (d, J = 8.7 Hz, 2H, Ar-H); 7.00-7.07 (m, 2H, Ar-H); 7.23 (m, 1H, Ar-H); 7.38-7.41 (m, 2H, Ar-H); 7.55 (s, 1H, triazole-H); 7.68 (d, J = 15.5 Hz, 1H, -CH=CH-); 7.98 (d, J = 8.8 Hz, 2H, Ar-H); 8.59 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 23.9, 28.7, 30.1, 46.4, 49.9, 50.2, 56.1, 60.2, 60.8, 61.2, 67.8, 105.6, 105.9, 106.7, 111.0, 114.2, 117.8, 119.1, 121.3, 121.5, 122.8, 127.1, 130.5, 130.8, 131.1, 131.5, 136.4, 140.1, 144.4, 153.4, 162.7,

172.1, 188.9 HRMS calcd.for $C_{39}H_{43}N_5O_7$: $[M+H]^+$ 694.3163, found: 694.3143.

2-[1-(5-{4-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenoxy}-pentyl)-1H-[1,2,3]triazol-4-methyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13d)

Pale yellow solid; mp 87-89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 1.40-1.44 (m, 2H, -CH_2); 1.78-1.82 (m, 2H, -CH_2); 2.04-2.09 (m, 2H, -CH_2); 3.02-3.15 (m, 2H, -CH_2); 3.69 (m, 1H, -CH); 3.87 (s, 3H, -OCH_3); 3.88 (s, 6H, -OCH_3); 3.98 (t, J = 5.7 Hz, 2H, -NCH_2); 4.06-4.19 (m, 6H, -OCH_2+ -CH_2+ -NCH_2); 4.32 (t, J = 6.8 Hz, 2H, -OCH_2); 6.83 (s, 2H, Ar-H); 6.90 (d, J = 8.8 Hz, 2H, Ar-H); 7.02-7.10 (m, 2H, Ar-H+- CH=CH-); 7.23-7.26 (m, 1H, Ar-H); 7.37-7.41 (m, 2H, Ar-H); 7.54 (s, 1H, triazole-H); 7.68 (d, J = 15.4 Hz, 1H, -CH=CH-); 7.97 (d, J = 8.8 Hz, 2H, Ar-H); 8.49 (s, 1H, -NH (exchangeable with D_2O)) ^{13}C NMR (100MHz, $CDCl_3$) δ 14.3, 22.6, 23.9, 26.4, 28.8, 30.4, 46.5, 50.1, 56.1, 60.1, 60.7, 61.3, 67.9,105.4, 105.9, 106.9, 111.1, 114.2, 117.7, 119.1, 121.3, 121.5, 122.8, 127.1, 130.7, 130.8, 131.3, 131.4, 136.5, 140.1, 144.4, 153.4, 162.7, 172.7, 188.9 HRMS calcd.for $C_{40}H_{45}N_5O_7$: $[M+H]^+$ 708.3319, found: 708.3342.

2-[1-(6-{4-[3-(3,4,5-Trimethoxy-phenyl)-acryloyl]-phenoxy}-hexyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β–carboline-3-carboxylic acid ethyl ester (13e)

Pale yellow solid; mp 76-78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 1.33-1.39 (m, 2H, -CH_2); 1.42-1.50 (m, 2H, -CH_2); 1.73-1.79 (m, 2H, -CH_2); 1.85-1.92 (m, 2H, -CH_2); 3.06-3.18 (m, 2H, -CH_2); 3.67-3.69 (m, 1H, -CH); 3.87 (s, 3H, -OCH_3); 3.89 (s, 6H, -OCH_3); 3.97 (t, J = 6.3 Hz, 2H, -NCH_2); 4.05-4.19 (m, 6H, -CH_2 + -OCH_2+ -NCH_2); 4.30 (t, J = 7.0 Hz, 2H, -OCH_2); 6.83 (s, 2H, Ar-H); 6.92 (d, J = 8.9 Hz, 2H, Ar-H); 7.03-7.09 (m, 2H, Ar-H+ -CH=CH-); 7.23-7.25 (m, 1H, Ar-H); 7.40 (d, J = 9.1 Hz, 1H, Ar-H); 7.43 (s, 1H, Ar-H); 7.53 (s, 1H, triazole-H); 7.69 (d, J = 15.5 Hz, 1H, -CH=CH-); 8.00 (d, J = 8.8 Hz, 2H, Ar-H); 8.45 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100MHz, $CDCl_3$) δ 14.3, 22.7, 23.9, 26.2, 28.9, 31.6, 46.5, 50.3, 56.0, 56.2, 60.0, 60.8, 61.1, 67.9, 105.5, 105.9, 106.8, 111.0, 114.3, 117.8, 119.2, 121.2, 121.4, 122.9, 127.0, 130.6, 130.9, 131.2, 131.4, 136.3, 140.2, 144.3, 153.5, 162.9, 172.8, 188.8 HRMS calcd.for $C_{41}H_{47}N_5O_7$: $[M+H]^+$ 722.3475, found: 722.3441.

2-[1-(2-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenoxy}-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13f)

Pale yellow solid; mp 98-99 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, J = 6.9 Hz, 3H, -CH_3); 3.07-3.16 (m, 2H, -CH_2); 3.70-3.75 (m, 1H, -CH); 3.81 (s, 3H, -OCH_3); 4.05-4.33 (m, 8H, -OCH_2 + -CH_2 + -NCH_2 + -NCH_2); 4.66 (m, 2H, -OCH_2); 6.81 (d, J = 8.2 Hz, 2H, Ar-H); 6.89 (d, J = 8.0 Hz, 2H, Ar-H); 7.03-7.06 (m, 2H, Ar-H); 7.35 (d, J = 15.5 Hz, 1H, -CH=CH-); 7.41-7.56 (m, 4H, Ar-H); 7.68 (s, 1H, triazole-H); 7.74 (d, J = 15.4 Hz, 1H, -CH=CH-); 7.92 (d, J = 8.0 Hz, 2H, Ar-H); 8.69 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 24.0, 46.3, 49.7, 55.3, 55.5, 60.1, 60.8, 66.4, 105.7, 111.1, 113.6, 114.3, 114.4, 117.8, 119.3, 121.3, 124.3, 127.0, 127.6, 130.3, 130.8, 131.3, 131.6, 132.0, 136.3, 144.4, 161.5, 161.7, 172.8, 189.0 HRMS calcd.for $C_{35}H_{35}N_5O_5$: $[M+H]^+$ 606.2638, found: 606.2656.

2-[1-(3-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenoxy}-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13g)

Pale yellow solid; mp 93-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 2.40-2.43 (m, 2H, -CH_2); 3.10-3.14 (m, 2H, -CH_2); 3.84 (s, 3H, -OCH_3); 3.87 (t, J = 5.4 Hz, 1H, -CH); 3.99-4.17 (m, 8H, -OCH_2+ -CH_2+ -NCH_2+ -NCH_2); 4.56 (t, J = 6.7 Hz, 2H, -OCH_2); 6.90 (d, J = 3.8 Hz, 2H, Ar-H); 6.92 (d, J = 3.7 Hz, 2H, Ar-H); 7.02-7.11 (m, 2H, Ar-H) 7.29 (d, J = 7.8 Hz, 1H, Ar-H); 7.38 (d, J = 15.5 Hz, 1H, -CH=CH-); 7.42-7.45 (m, 1H, Ar-H); 7.53 (s, 1H, triazole-H); 7.58 (d, J = 8.7 Hz, 2H, Ar-H); 7.77 (d, J = 15.6 Hz, 1H, -CH=CH-); 7.98 (d, J = 8.8 Hz, 2H, Ar-H); 8.03 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 23.9, 30.7, 46.5, 49.8, 55.2, 55.6, 60.1, 60.9, 66.5, 105.8, 111.2, 113.7, 114.4, 114.5, 117.9, 119.3, 121.1, 124.2, 127.0, 127.5, 130.1, 130.9, 131.2, 131.7, 132.1, 136.2, 144.5, 161.4, 161.8, 172.9, 188.9 HRMS calcd.for $C_{36}H_{37}N_5O_5$: $[M+H]^+$ 620.2795, found: 620.2772.

2-[1-(4-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenoxy}-butyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13h)

Pale yellow solid; mp 92-93°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 7.1 Hz, 3H, -CH_3); 1.78-1.83 (m, 2H, -CH_2); 2.08-2.13 (m, 2H, -CH_2); 3.09-3.18 (m, 2H, -CH_2); 3.83 (s, 3H, -OCH_3); 3.87-3.91 (m, 1H, -CH); 4.00 (t, J = 5.8 Hz, 2H, -NCH_2); 4.06-4.21 (m, 6H, -OCH_2 + -CH_2 + -NCH_2); 4.41 (t, J = 6.8 Hz, 2H, -OCH_2); 6.89-6.92 (m, 4H, Ar-H); 7.03-7.11 (m, 2H, Ar-H); 7.26 (d, J = 8.0 Hz, 1H, Ar-H); 7.38-7.44 (m, 2H, Ar-H + -CH=CH-); 7.58 (d, J = 8.6 Hz, 3H, Ar-H); 7.76 (d, J = 15.5 Hz, 1H, -CH=CH-); 7.98 (d, J = 8.8 Hz, 2H, Ar-H); 8.24 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.7, 23.9, 31.6, 46.5, 50.0, 55.3, 55.5, 60.1, 60.8, 67.1, 106.0, 110.9, 113.6, 114.2, 114.4, 117.8, 119.4, 121.5, 124.2, 127.0, 127.7, 127.9, 130.8, 131.3, 131.4, 131.5, 136.2, 144.1, 161.6, 162.4, 172.7, 188.9 HRMS calcd.for $C_{37}H_{39}N_5O_5$: $[M+H]^+$ 634.2951, found: 634.2978.

2-[1-(5-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenoxy}-pentyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13i)

Pale yellow solid; mp 88-90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 1.39-1.44 (m, 2H, -CH_2); 1.79-1.83 (m, 2H, -CH_2); 2.07-2.12 (m, 2H, -CH_2); 3.10-3.18 (m, 2H, -CH_2); 3.81 (s, 3H, -OCH_3); 3.87-3.91 (m, 1H, -CH); 4.02 (t, J = 5.8 Hz, 2H, -NCH_2); 4.07-4.23 (m, 6H, -OCH_2+ -CH_2 + -NCH_2); 4.42 (t, J = 6.8 Hz, 2H, -OCH_2); 6.90-6.94 (m, 4H, Ar-H); 7.02-7.10 (m, 2H, Ar-H+ CH=CH-); 7.28 (d, J = 8.0 Hz, 1H, Ar-H); 7.39-7.41 (m, 2H, Ar-H); 7.59 (d, J = 8.6 Hz, 3H, Ar-H); 7.78 (d, J = 15.5 Hz, 1H, -CH=CH-); 7.99 (d, J = 8.8 Hz, 2H, Ar-H); 8.21 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz,

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$CDCl_3$) δ 14.3, 22.7, 23.9, 26.9, 29.1, 46.7, 50.2, 55.4, 55.7, 60.2, 60.9, 67.2, 106.1, 110.9, 113.7, 114.1, 114.5, 117.9, 119.5, 121.6, 124.1, 127.1, 127.7, 127.9, 130.9, 131.2, 131.3, 131.5, 136.3, 144.1, 161.7, 162.4, 172.8, 188.9 HRMS calcd.for $C_{38}H_{41}N_5O_5$: $[M+H]^+$ 648.3108, found: 648.3145.

2-[1-(6-{4-[3-(4-Methoxy-phenyl)-acryloyl]-phenoxy}-hexyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13j)

Pale yellow solid; mp 74-76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (t, J = 7.1 Hz, 3H, -CH_3); 1.34-1.41 (m, 2H, -CH_2); 1.46-1.54 (m, 2H, -CH_2); 1.74-1.81 (m, 2H, -CH_2); 1.89-1.95 (m, 2H, -CH_2); 3.08-3.19 (m, 2H, -CH_2); 3.83 (s, 3H, -OCH_3); 3.99 (t, J = 6.2 Hz, 1H, -CH); 4.07-4.22 (m, 8H, -OCH_2 + -CH_2 + -NCH_2 + -NCH_2); 4.32 (t, J = 7.0 Hz, 2H, -OCH_2); 6.90 (d, J = 3.8 Hz, 2H, Ar-H); 6.92 (d, J = 3.9 Hz, 2H, Ar-H); 7.03-7.11 (m, 2H, Ar-H+ -CH=CH-); 7.39-7.44 (m, 3H, Ar-H); 7.54 (s, 1H, triazole-H); 7.58 (d, J = 8.7 Hz, 2H, Ar-H); 7.76 (d, J = 15.5 Hz, 1H, -CH=CH-); 8.00 (d, J = 8.7 Hz, 2H, Ar-H); 8.18 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.7, 23.9, 26.3, 28.9, 30.2, 46.5, 49.0, 55.3, 55.5, 60.0, 60.8, 67.9, 106.1, 110.9, 113.6, 114.3, 114.4, 117.9, 119.3, 119.5, 121.5, 124.3, 127.0, 129.2, 130.8, 131.2, 131.4, 131.5, 136.2, 143.9, 161.6, 162.8, 172.7, 188.9. HRMS calcd.for $C_{39}H_{43}N_5O_5$: $[M+H]^+$662.3264, found: 662.3223.

2-[1-(2-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenoxy}-ethyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13k)

Pale yellow solid; mp 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 3.07-3.19 (m, 2H, -CH_2); 3.75 (d, J = 15.2 Hz, 1H, -CH_2); 3.86 (t, J = 5.2 Hz, 1H, -CH); 4.07-4.15 (m, 5H, -OCH_2 + -CH_2 + -NCH_2); 4.39-4.43 (m, 2H, -NCH_2); 4.72-4.75 (m, 2H, -OCH_2); 6.87 (d, J = 8.7 Hz, 2H, Ar-H); 7.03-7.11 (m, 3H, 2Ar-H +- CH=CH-); 7.28 (d, J = 7.6 Hz, 1H, Ar-H); 7.38-7.44 (m, 3H, Ar-H); 7.58-7.62 (m, 2H, Ar-H); 7.69 (s, 1H, triazole-H); 7.74 (d, J = 15.6 Hz, 1H, -CH=CH-); 7.95 (d, J = 8.7 Hz, 2H, Ar-H); 8.21 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 24.0, 46.3, 49.3, 49.8, 60.2, 60.7, 66.5, 106.0, 110.9, 114.4, 116.0, 116.2, 117.8, 119.3, 121.4, 124.1, 127.0, 130.3, 130.4, 130.9, 131.2, 131.4, 131.9, 136.3, 143.3, 145.9, 161.7, 172.7, 188.7 HRMS calcd.for $C_{34}H_{32}FN_5O_4$: $[M+H]^+$ 594.2438, found: 594.2462.

2-[1-(3-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenoxy}-propyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β–carboline-3-carboxylic acid ethyl ester (13l)

Pale yellow solid; mp 85-87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 2.33-2.40 (m, 2H, -CH_2); 3.04-3.16 (m, 2H, -CH_2); 3.61 (d, J = 6.4 Hz, 1H, -CH_2); 3.85-4.17 (m, 8H, -OCH_2+ -CH +- CH_2 + -NCH_2 + -NCH_2); 4.53-4.56 (m, 2H, -OCH_2); 6.90 (d, J = 8.7 Hz, 2H, Ar-H); 7.03-7.10 (m, 5H, Ar-H); 7.36-7.46 (m, 2H, Ar-H); 7.54-7.62 (m, 3H, 2Ar-H+ -CH=CH-); 7.75 (d, J = 15.6 Hz, 1H, -CH=CH-); 7.97 (d, J = 8.7 Hz, 2H, Ar-H); 8.27 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 23.9, 29.7, 47.0, 49.1, 49.5, 60.0, 60.8, 64.3, 106.0, 110.9, 114.3, 115.2, 115.4, 116.0, 116.3, 117.8, 119.3, 121.4, 123.5, 127.0, 130.3, 130.4, 130.9, 131.1, 131.2, 131.4, 136.2, 143.1, 162.3, 172.7, 188.7 HRMS calcd.for $C_{35}H_{34}FN_5O_4$: $[M+H]^+$ 608.2595, found: 608.2561.

2-[1-(4-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenoxy}-butyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13m)

Pale yellow solid; mp 69-70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 1.79-1.85 (m, 2H, -CH_2); 2.08-2.13 (m, 2H, -CH_2); 3.08-3.18 (m, 2H, -CH_2); 3.88 (d, J = 7.9 Hz, 1H, -CH_2); 4.00-4.17 (m, 8H, -OCH_2+ -CH + -CH_2+ -NCH_2+ -NCH_2); 4.41 (t, J = 6.9 Hz, 2H, -CH_2); 6.91 (d, J = 8.7 Hz, 2H, Ar-H); 7.02-7.10 (m, 5H, Ar-H); 7.42-7.46 (m, 2H, Ar-H); 7.59-7.62 (m, 3H, Ar-H); 7.74 (d, J = 15.6 Hz, 1H, -CH=CH-); 7.98 (d, J = 8.6 Hz, 2H, Ar-H); 8.28 (s, 1H, -NH(exchangeable with D_2O)) ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.3, 22.6, 23.9, 30.9, 47.0, 49.1, 49.6, 60.0, 60.8, 64.3, 105.7, 110.8, 114.3, 116.2, 116.3, 117.9, 119.0, 121.4, 121.7, 122.8, 127.2, 130.1, 130.3, 130.9, 131.2, 131.3, 131.4, 136.5, 142.6, 163.1, 172.9, 188.9 HRMS calcd.for $C_{36}H_{36}FN_5O_4$: $[M+H]^+$ 622.2751, found: 622.2719.

2-[1-(6-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenoxy}-pentyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13n)

Pale yellow solid; mp 62-63 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (t, J = 7.1 Hz, 3H, -CH_3); 1.39-1.43 (m, 2H,-CH_2); 1.78-1.83 (m, 2H,-CH_2); 2.04-2.09 (m, 2H, -CH_2); 3.09-3.17 (m, 2H, -CH_2); 3.90 (d, J = 7.9 Hz, 1H, -CH_2); 4.01-4.19 (m, 8H, -OCH_2+ -CH + -CH_2+ -NCH_2 + -NCH_2); 4.41 (t, J = 6.9 Hz, 2H, -OCH_2); 6.91 (d, J = 8.7 Hz, 2H, Ar-H); 7.02-7.10 (m, 5H, 4Ar-H+ -CH=CH-); 7.42-7.46 (m, 2H, Ar-H); 7.59-7.62 (m, 3H, Ar-H); 7.74 (d, J = 15.6 Hz, 1H, -CH=CH-); 7.98 (d, J = 8.6 Hz, 2H, Ar-H); 8.28 (s, 1H, -NH(exchangeable with D_2O)) ^{13} C NMR (100 MHz, $CDCl_3$) δ 14.3, 22.4, 23.9, 26.4, 30.1, 46.6, 49.1, 50.2, 60.0, 60.8, 67.8, 105.9, 110.9, 114.4, 116.1, 116.2, 117.9, 119.1, 121.5, 121.6, 122.9, 127.1, 130.2, 130.3, 130.9, 131.2, 131.3, 131.4, 136.4, 142.7, 163.0, 172.9, 188.9 HRMS calcd.for $C_{37}H_{38}FN_5O_4$: $[M+H]^+$ 636.2908, found: 636.2943.

2-[1-(6-{4-[3-(4-Fluoro-phenyl)-acryloyl]-phenoxy}-hexyl)-1H-[1,2,3]triazol-4-ylmethyl]-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid ethyl ester (13o)

Pale yellow solid; mp 58-61 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.1 Hz, 3H, -CH_3); 1.33-1.40 (m, 2H, -CH_2); 1.45-1.53 (m, 2H, -CH_2); 1.74-1.80 (m, 2H, -CH_2); 1.86-1.94 (m, 2H, -CH_2); 3.07-3.19 (m, 2H, -CH_2); 3.87 (t, J = 5.2 Hz, 1H, -CH); 3.98 (t, J = 6.3 Hz, 2H, -NCH_2); 4.06-4.20 (m, 6H, -OCH_2+ -CH_2+ -NCH_2); 4.31 (t, J = 7.1 Hz, 2H, -OCH_2); 6.92 (d, J = 8.9 Hz, 2H, Ar-H); 7.02-7.10 (m, 4H, 3Ar-H+ -CH=CH-); 7.24-7.26 (m, 1H, Ar-H); 7.42-7.47 (m, 2H, Ar-H); 7.53 (s, 1H, triazole-H); 7.58-7.62 (m, 2H, Ar-H+ -CH=CH-); 7.74 (d, J = 15.6 Hz, 1H, Ar-H); 8.00 (d, J = 8.8 Hz, 2H, Ar-H); 8.37 (s, 1H, -NH(exchangeable with D_2O)) ^{13} C NMR (100 MHz, $CDCl_3$) δ 14.3,

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23.9, 25.5, 26.3, 28.9, 30.2, 46.5, 49.0, 50.3, 60.0, 60.8, 67.9, 105.9, 110.9, 114.3, 116.0, 116.2, 117.8, 119.2, 121.4, 121.5, 122.8, 127.0, 130.3, 130.4, 130.9, 131.3, 131.3, 131.4, 136.3, 142.7, 163.0, 172.8, 188.6 HRMS calcd.for $C_{38}H_{40}FN_5O_4$: $[M+H]^+$ 650.3064, found: 650.3031.

Cell Culturing

MCF-7 cells (ECACC) were cultured in Dulbecco's modified eagle's media (DMEM)and supplemented with 10% (foetal bovine serum) FBS and 1% penicillin-streptomycin. MDA-MB 231 cells (ATCC) were cultured in 3:1 DMEM with HAMS F12 media supplemented with 10% FBS and 1% penicillin-streptomycin, both incubated at 37°C and 5% carbon dioxide (CO_2) to mimic *in-vivo* conditions.

MTT assay

MCF-7 and MDA-MB 231 cells were seeded at a density of 5000 cells per well in triplicate into a 96-well plate and incubated 37°C and 5% CO_2 for 24 hours (hrs). Test compounds were dissolved in dimethylsulphoxide (DMSO) and working solutions were diluted in DMEM. Cells were treated with a range of different concentrations (1, 5, 10, 20, 50, 100µM) of the various compounds for 24 hrs at 37°C and 5% CO_2. Subsequently, sterile 5µl of 5 mg/mL MTT (Sigma-Aldrich) dissolved in PBS was added to each well and incubated with cells for 2-3 hrs. Solubilisation solution (10% sodium dodecyl sulphate (SDS), 10mM hydrochloric acid (HCl)) of equal volume to the wells was then added and incubated for 16 hrs at 37 °C. The optical density of each well was read at 570 nm using a microtiter plate reader (Thermo Fisher Scientific Multiskan GO Microplate Reader, SkanIt™ software).[20]

Statistical analysis

The statistical analysis was performed using Excel® and IC_{50} values were estimated using GraphpadPrism5 software (Hearne Scientific Software). The experiments were performed in triplicates and at least two times for reproducibility. The statistical significance was calculated using student's t-test. A p-value of less than 0.05 was used to estimate the significance of the observations. A Z-factor was calculated for each 96-well plate and assays having Z-factor above > 0.6 were included in the statistical analysis.[21]

Molecular docking protocol

The 3D structure of selected ligands and minimized structure of ERα (PDB ID: 3ERT) were prepared using the standard protocols in LigPrep[22] and Protein Preparation Wizard[23] of Schrödinger Suite 2019-2. All calculations were performed using OPLS3e force field. Subsequently, docking simulations were performed using the induced-fit docking protocol.[24] The first round of glide docking involved a brief constrained refinement of the protein structure to an RMSD ≤ 0.18 Å and an 'auto-trimming' of up to 3 residues with B-factor > 40 Å2 and within 5Å of the active site. XP scoring function was selected for the redocking stage while other parameters were set at default. Finally, the binding affinity energies ΔGbind of the best pose in kcal/mol were computed with Prime Molecular Mechanics-Generalized Borne Surface Area.[24]

Conflicts of interest

The authors declare no conflicts of interest

Abbreviations

BC breast Cancer; TNBC Triple Negative Breast Cancer; ER estrogen responsive; SERMs Selective Estrogen Receptor modulator; THβC tetrahydro-β–carboline; IC_{50} 50 % inhibitory concentration; SAR Structure and Activity Relationship.

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Design, Synthesis and anti-proliferative evaluation of 1*H*-1,2,3-triazole grafted tetrahydro-β-carboline-chalcone/ferrocenylchalcone conjugates in Estrogen Responsive and Triple Negative Breast Cancer cells

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