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Original article

Synthesis and anticancer activity of chalcone-pyrrolobenzodiazepine conjugates linked via 1,2,3-triazole ring side-armed with alkane spacers

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ABSTRACT

Aiming to develop multitarget drugs for the anticancer treatment, a new class of chalcone-pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) conjugates linked through a 1,2,3-triazole moiety containing alkane spacers has been designed and synthesized. Combining these two core pharmacophore structures with modifications at *A*-C8/*C*-C2-position of PBD ring system yielded analogs with improved efficacy and have shown promising in vitro anticancer activity ranging from <0.1–2.92 μM. These PBD-conjugates caused G1 cell cycle arrest with effect on G1 cell cycle regulatory proteins such as Cyclin D1 and Cdk4. These conjugates also exhibited inhibitory effect on NF-kB, Bcl-XL proteins that play a vital role in breast cancer cell proliferation. These findings suggest that one of the compound **4d** among this series is most effective and has potential for detailed investigations.

1. Introduction

Cancer is a multifactor disease with superfluous and robust biological networks. It may require treatment with compounds that could target multiple intracellular components. Currently, cancer therapy interfering with a single biological molecule or pathway has been successfully utilized for the treatment in clinics [1–4]. There is general belief that agents modulating more than one target could have superior efficacy compared to single target drugs [5,6]. Therefore, modulating multiple targets simultaneously can be achieved by the combination of multiple drugs with different mechanisms or by single chemical entity that could modulate several targets of a multifactorial disease. As a result, there is increasing interest in the discovery of agents that concomitantly address more than one biological target for cancer treatment [7,8].

Chalcones represent an important group of natural products [9,10], that received significant attention for their antitumor properties, particularly in view of their similar mode of action to the structurally related natural combretastatin (**3**, Fig. 1) [11]. In addition, chalcones have been reported for a wide range of pharmacological activities including cytotoxicity [12] and anticancer activity [13,14]. Moreover, recent studies have shown that these chalcones induce apoptosis in a variety of cell types, including breast cancers [15,16]. The development of anticancer agents by performing structural modifications in chalcone scaffold led to the improvement of their bioavailability [17] and some synthetic chalcone derivatives are showing selective cytotoxicity against MCF-7 cells [18].

Over the past few years, 1,2,3-triazole molecules have been synthesized as useful chemotherapeutic agents for various diseases [19]. This class of heterocycles is known to exhibit interesting biological activities, such as antibiotic, antifungal, antehelmintic [20–23], and anticancer activity [24]. Whereas, the imine or carbinolamine-containing pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) are a family of low molecular weight antitumor antibiotics originally isolated from various *Streptomyces* species [25] and

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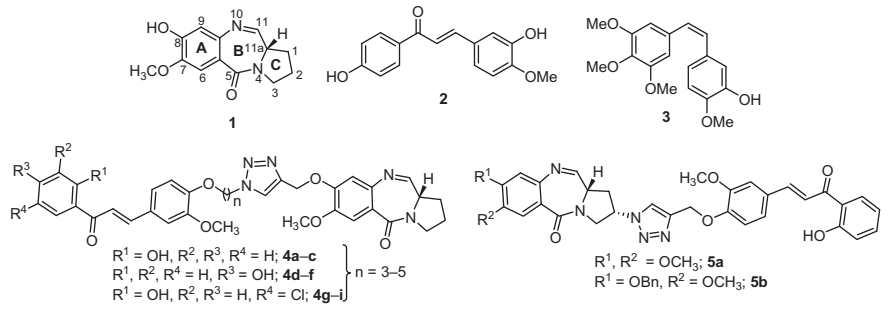
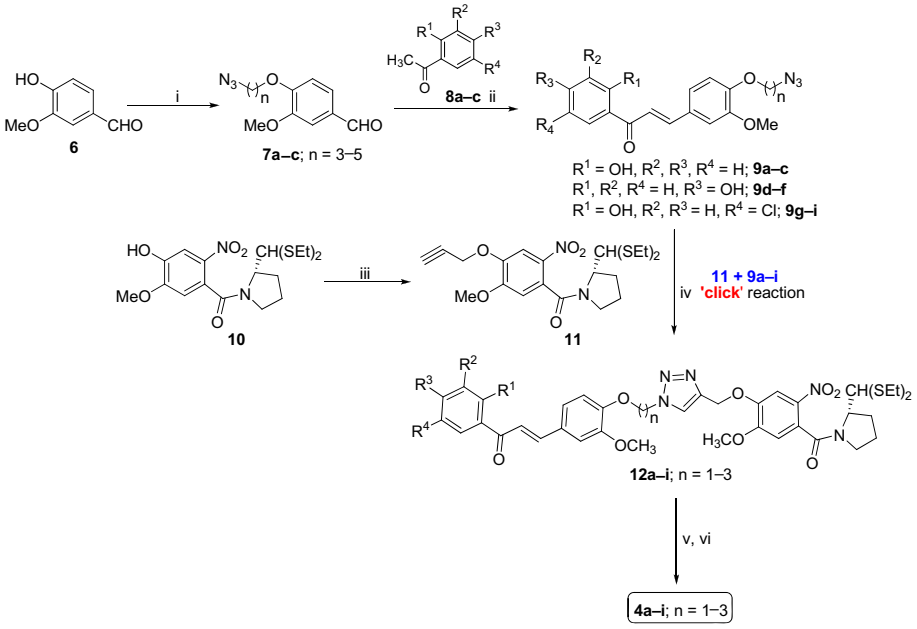
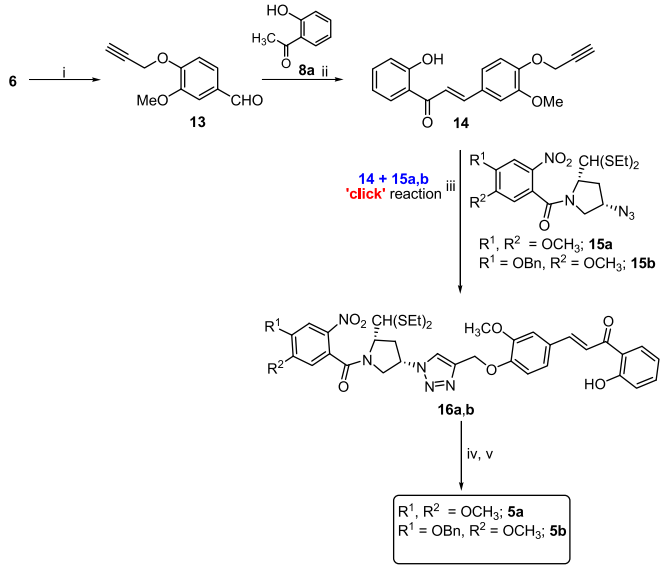


Fig. 1. Structures of DC-81 (1), skeleton of chalcone (2), combretastatin (3), 1,2,3- triazolo–chalcone-PBD hybrids (**4a**–**i** and **5a**,**b**).



Scheme 1. Reagents and conditions: (i) a) K_2CO_3, dibromo alkane spacers, dry DMF, rt, 12 h; b) NaN_3, (0.5 M in DMSO), 80 °C, 6 h; (ii) KOH/MeOH, rt, 12 h; (iii) K_2CO_3, propargyl bromide, dry DMF, rt, 12 h; (iv) $CuSO_4 \cdot 5H_2O$ (1 mol%), sodium ascorbate (5 mol%), *t*-BuOH/H_2O (1:1), rt, 12 h; (v) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 2 h; (vi) $HgCl_2$–$CaCO_3$, CH_3CN-H_2O (4:1), rt, 12 h.



Scheme 2. Reagents and conditions: (i) K_2CO_3, propargyl bromide, dry DMF, rt, 12 h; (ii) KOH/MeOH, rt, 12 h; (iii) $CuSO_4 \cdot 5H_2O$ (1 mol%), sodium ascorbate (5 mol%), *t*-BuOH/H_2O (1:1), rt, 12 h; (iv) $SnCl_2 \cdot 2H_2O$, MeOH, reflux, 2 h; (v) $HgCl_2$–$CaCO_3$, CH_3CN–H_2O (4:1), rt, 12 h.

examples of which include, DC-81 (**1**), anthramycin, tomaymycin and sibiromycin. These compounds bind selectively in the minor groove of DNA while a covalent aminal bond formation between the electrophilic C11-position of the PBD and the nucleophilic N2-amino group of a guanine base [26,27] results for their biological activity. Some representative structures of chalcone, DC-81 and their conjugates are illustrated in Fig. 1.

In the past few years, several conjugates in which known antitumor agents linked to PBD moiety have been designed, synthesized and evaluated for their biological activity [28–31]. Recently, Wang and co-workers have reported DC-81-indole and DC-81-enediyne conjugates as potential anticancer agents and a correlation between antitumor activity and apoptosis has been well explained [32,33]. In the last few years, we have not only been involved in the preparation of the PBD ring system by developing new synthetic strategies [34,35] but also in the design of structurally modified PBDs and their conjugates [36–40]. In continuation of these efforts, we herein describe the synthesis and biological evaluation of chalcone-PBD conjugates linked through the 1,2,3-triazole ring side-armed with alkane spacers. In view of the promising anticancer activity observed by these new PBD conjugates, it was considered of interest to examine their role by cell proliferation and apoptosis by using the breast carcinoma cell line MCF-7. Detailed biological assays revealed that these conjugates

Table 1
GI50 values[a] (in μM) for compounds **4a**–**i** and **5a** in selected human cancer cell lines.

Compound	Zr-75-1[b]	MCF 7[b]	HOP 62[c]	Gurav[c]	A549[c]	A2780[d]	DWD[e]	KB[e]	Colo-205[f]	Pc3[g]	SiHa[h]
4a	2.20	2.40	2.92	2.31	2.33	0.17	NA	2.19	0.15	2.48	NA
4b	0.15	0.14	0.16	0.12	<0.16	0.10	<0.13	0.17	NA	0.13	0.14
4c	0.17	2.10	2.56	0.16	0.17	0.14	2.30	0.18	0.14	2.04	2.71
4d	0.17	0.16	0.17	0.13	0.15	0.12	0.12	2.03	NA	0.16	0.18
4e	0.16	0.16	0.19	0.13	<0.15	0.11	<0.16	0.16	0.11	0.15	2.52
4h	0.19	0.16	2.50	1.66	0.19	0.13	<0.17	2.66	<0.1	0.16	NA
4i	2.02	0.18	2.28	0.19	2.09	0.15	2.30	NA	0.10	0.19	NA
5a	NA	2.35	NA	2.51	2.78	0.15	2.70	NA	0.11	2.23	NA
ADR[i]	1.79	0.17	0.14	0.17	7.25	0.16	0.10	0.17	0.14	1.81	0.17
DC-81	2.37	0.17	0.15	0.16	0.16	0.14	1.49	0.17	0.11	0.20	0.17

[a] 50% growth inhibition and the values are mean of three determinations and are reported as mean ± standard error of the mean.
[b] breast cancer.
[c] lung cancer.
[d] ovarian cancer.
[e] oral cancer.
[f] colon cancer.
[g] prostate cancer.
[h] cervix cancer.
[i] ADR (adriamycin).

show G1 cell cycle arrest and effect cell cycle regulatory proteins such as Cyclin D1 and Cdk4. This study also revealed that the inhibitory activity of these conjugates on NF-kB (p65) protein there by controlling active cell proliferation in MCF-7 cells.

2. Chemistry

The synthesis of azido-chalcone intermediates **9a**–**i** was carried out by employing commercially available substituted-acetophenones (**8a**–**c**) and vanillin (**6**) [41] as starting materials. Etherification of vanillin (**6**) with dibromoalkanes followed by azidation [42] of bromine using NaN_3 produces the corresponding azido precursors **7a**–**c**. Then, the aldol condensation reaction was performed by reacting **7a**–**c** with a variety of acetophenones (**8a**–**c**) using KOH as the base to afford the azido-chalcone key intermediates **9a**–**i**. The synthesis of *A*-C8 chalcone linked PBD conjugates (**4a**–**i** and **5a**,**b**) that are attached through a triazole containing alkane spacer was carried out from 4-hydroxy nitrothioacetal **10**, which was synthesized by the methods reported in the previous studies [40,42,43]. The etherification of compound **10** with propargyl bromide in presence of K_2CO_3 provides one of the key alkyne intermediate **11** [42]. At this stage the 'click' chemistry protocol is introduced between alkyne **11** and azides **9a**–**i** by employing a $CuSO_4 \cdot 5H_2O$ catalyst (1 mol%) with sodium ascorbate (5 mol%) to afford nitro intermediates **12a**–**i**. Later, the nitro functionality was reduced with $SnCl_2 \cdot 2H_2O$ followed by deprotective-cyclization with $HgCl_2$-$CaCO_3$ to afford the target compounds **4a**–**i** as depicted in Scheme 1.

Similarly, in order to explore their diversity at *C*-C2-position of the PBD ring system, the chalcone linked PBD (**5a**,**b**) conjugates are attached through a 1,2,3-triazole obtained from compounds **15a**,**b**, which were prepared from the previously reported method [40]. Etherification of the vanillin (**6**) with propargyl bromide followed by aldol condensation using hydroxy acetopheonone (**8a**) affords the alkyne precursor **14**. The two key intermediates **14** and **15a**,**b** were subjected to 'click' chemistry to produce **16a**,**b**. Finally, the nitro reduction followed by diethanethiol deprotective-cyclization provide the target compounds (**5a**,**b**) as shown in Scheme 2.

3. Results and discussion

3.1. Evaluation of biological activity

3.1.1. Anticancer activity

These PBD conjugates **4a**–**i** and **5a**,**b** were evaluated for their anticancer activity in selected human cancer cell lines of lung, breast,

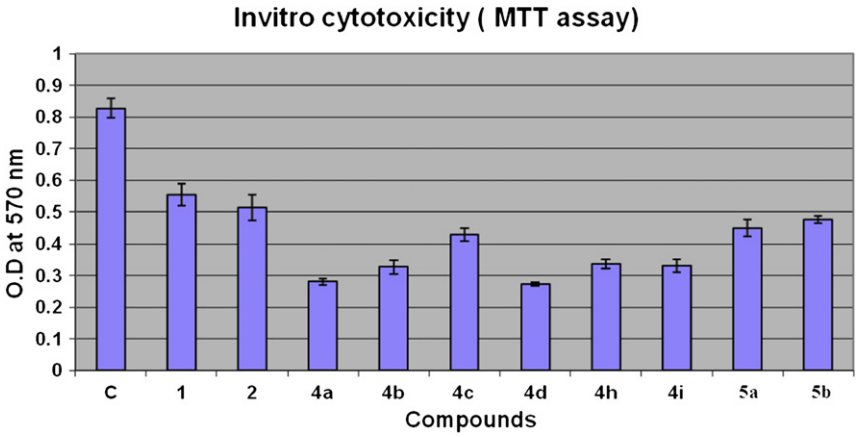


Fig. 2. Cell viability observed after the treatment with 1,2,3-triazolo chalcone-PBD compounds at 4 μM concentration by MTT assay. MCF-7 breast carcinoma cells were treated with various compounds **1**, **2**, **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **5a** and **5b** at 4 μM concentration as indicated for 24 h in 96 well plates at 10,000 cells per well. Compound **1** and **2** were used as starting materials in this experiment. Control indicates the untreated cells. Each experiment was repeated three times and Standard Deviations were derived from three independent experiments.

Table 2
FACS analysis of cell cycle distribution of MCF-7 cells after treatment with compounds (**1, 2, 4a, 4b, 4c, 4d, 4h, 4i, 5a** and **5b**) at 10 μM[a] concentration for 24 h. Compound **1** and **2** were used as starting materials in this experiment.

Compound	G0	G1	S	G2/M
Control	1.27 ± 0.08	47.64 ± 1.49	12.92 ± 1	38.15 ± 1.068
1	7.62 ± 0.32	61.81 ± 1.71	9.67 ± 0.62	20.89 ± 1.05
2	10.15 ± 0.29	51.61 ± 2.21	6.55 ± 0.5	31.5 ± 2.14
4a	12.06 ± 0.35	64.08 ± 1.04	9.52 ± 1.04	14.32 ± 0.5
4b	10.79 ± 0.42	77.47 ± 0.76	4.99 ± 0.21	7.07 ± 0.88
4c	7.81 ± 0.94	46.39 ± 0.87	9.89 ± 0.43	35.88 ± 1.37
4d	13.67 ± 0.63	72.51 ± 1.32	6.48 ± 0.66	7.33 ± 0.75
4h	10.51 ± 0.83	50.63 ± 1.18	7.74 ± 0.38	31.07 ± 1
4i	10.75 ± 0.3	60.56 ± 1.32	7.28 ± 0.26	22.06 ± 0.07
5a	5.19 ± 0.3	50.9 ± 1.82	8.71 ± 0.66	35.19 ± 1.05
5b	5.21 ± 0.37	54.61 ± 0.94	6.65 ± 0.35	32.06 ± 2.53

[a] Values represent the mean ± SEM of three determinations.

oral, colon, prostate, ovarian and cervix by using sulforhodamine B (SRB) method. The compounds that exhibit $GI_{50} \leq 10^{-5}$ M have been considered to be active on the respective cell lines and the results are illustrated in Table 1. All the compounds (**4a**–**i** and **5a**,**b**) showed significant anticancer activity with GI_{50} values ranging from <0.1 to 2.92 μM, while the positive controls, adriamycin and DC-81 (**1**) demonstrated the GI_{50} in the range of 0.1–7.25 μM and 0.1–2.37 μM, respectively in the cell lines employed. Interestingly, all the PBD conjugates showed promising anticancer activity, particularly against A2780, Gurav, MCF-7, Colo-205 and A549 cell lines, and some of the compounds like **4a**, **4b**, **4d, 4h** and **4i** have shown significant anticancer activity as illustrated in Table 1

MTT assay was also carried out to identify the cytotoxic effect of some of these chalcone linked PBD conjugates (**4a**–**d, 4h, 4i, 5a**, and **5b**) in MCF-7 cells at 4 μM concentration. The response of cells at 2 μM was not that effective in producing cytotoxic activity. Moreover, the anticancer activity of DC-81 (**1**) and compound **2**, which are the starting materials of this series was also examined to substantiate the activity of these new chalcone-PBD conjugates. It is interesting to observe that some of the compounds (**4a**, **4b** and **4d**) possess improved cytotoxic effect compared to the naturally occurring **1** and **2** as shown in Fig. 2.

3.1.2. *Effect of conjugates on cell cycle in MCF-7 cell line*

To investigate the mechanism underlying the anti-proliferative nature, the cell cycle distribution of MCF-7 cells was analyzed by flow cytometry. MCF-7 cells were treated with compounds **1, 2, 4a**–**d, 4h, 4i, 5a** and **5b** at 4 μM concentration. The majority of control cells treated with DMSO showed 50% of cells in G0/G1 phase. The starting materials such as **1** and **2** have shown 71.10% and 64.11% cells in G0/G1 phase. Compounds **4a**–**d, 4h, 4i, 5a** and

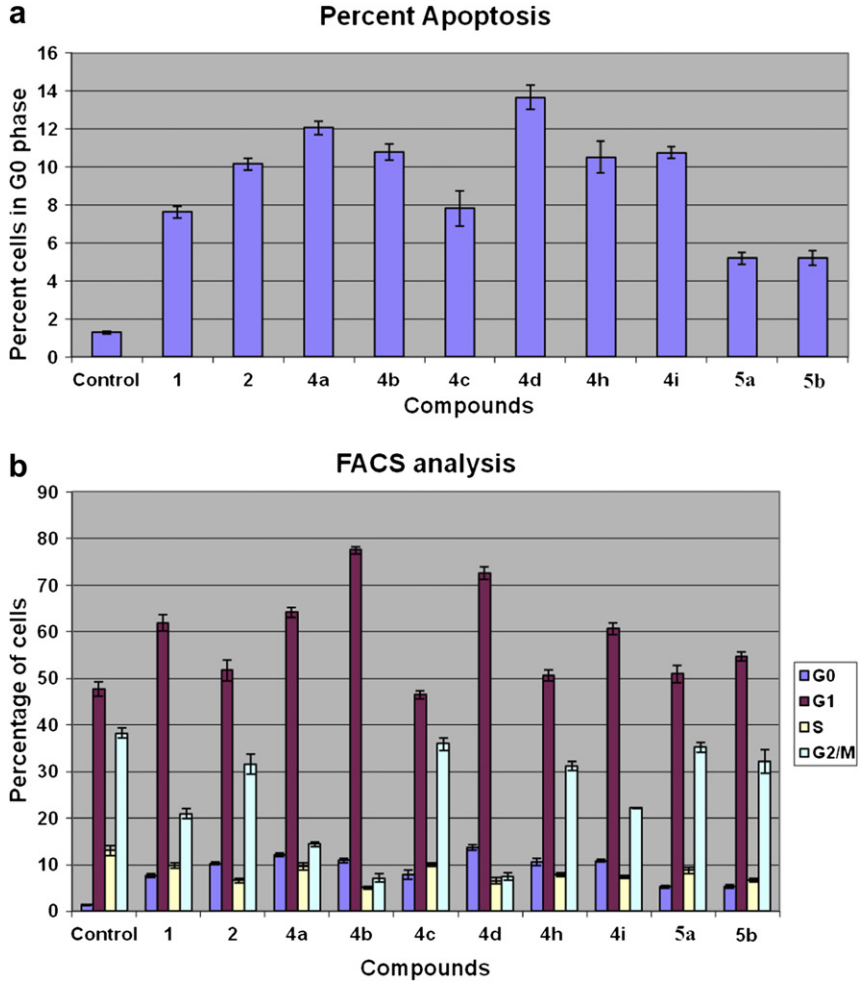


Fig. 3. a. Histogram of cell cycle distribution of MCF-7 cells after treatment with compounds (**1**, **2**, **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **5a** and **5b**) at 4 μM concentration for 24 h. Compound **1** and **2** were used as starting materials in this experiment. Each experiment was repeated three times and Standard Deviations were derived from three independent experiments. b. Histogram depicting the percentage of apoptotic cells of MCF-7 cells after treatment with compounds (**1**, **2**, **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **5a** and **5b**) at 4 μM concentration for 24 h.

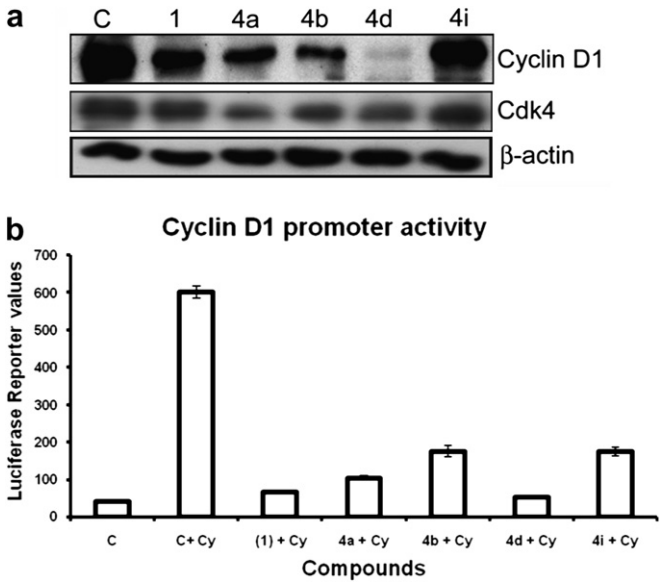


Fig. 4. a. Effect compounds on the expression of Cell cycle regulated (Cyclin D1 and Cdk4) proteins. MCF-7 Cells were treated with 4 μM concentration of compounds (**1**, **4a**, **4b**, **4d**, and **4i**) for 24 h. The cell lysates were collected and observed for expression levels of proteins such as Cyclin D1 and Cdk4 using specific antibodies. β-actin was used as loading control. C: control (untreated). b. Effect compounds on the Cyclin D1 promoter (−1745 Luc) activity. MCF-7 Cells were transfected with Cyclin D1 promoter (Cy) for 24 h followed by compound treatments (**1**, **4a**, **4b**, **4d**, and **4i**) at final concentration of 4 μM for further 24 h. Here Cy represents Cyclin D1 promoter transfection. C: represents the value of promoter activity without any transfection and can be considered as negative control. The C + Cy, represents the control untreated cells transfected with Cyclin D1 promoter (without any treatment) and can be considered as positive control. X + Cy: represents Cyclin D1 promoter transfection followed by compound treatments. X may be **1**, **4a**, **4b**, **4d**, and **4i**.

5b showed 76.87%, 89.10%, 56.23%, 87.70%, 61.38%, 60.18%, 55.24%, and 59.79% of cells in G0/G1 phase respectively. It is observed from this data that **4b** and **4d** was highly effective in causing G0/G1 arrest (Table 2 and Fig. 3a). The data obtained from the FACS analysis about apoptotic cell death (G0 phase) is comparable to the in vitro cytotoxicity data (Fig. 3b). It is observed from the data of cell cycle analysis that PBD conjugates show G1 arrest and whereas chalcone based compounds are known to cause G2/M cell cycle arrest. However, there are evidences that chalcones (HTMC-chalcone) cause G1 arrest in A549 adenocarcinoma cells [44] and accumulation of cells in sub G1 phase of cell cycle in MDA-MB-231 breast cancer cells [45]. Whereas in the present study G1 cell cycle arrest is clearly seen in case of PBD-chalcone conjugates.

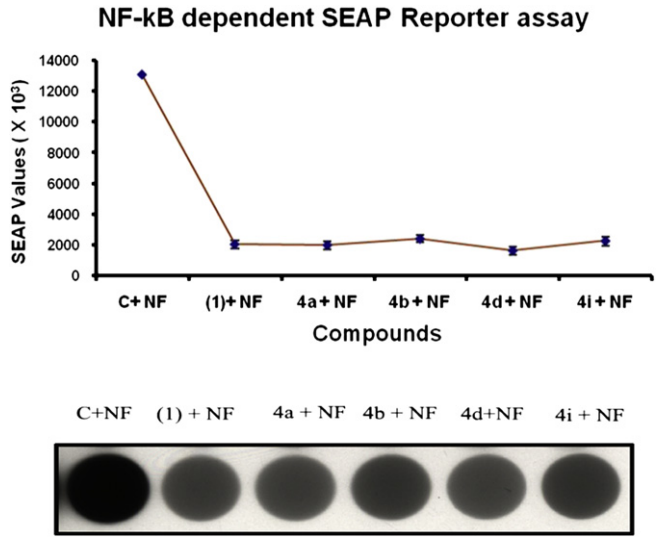


Fig. 5. Effect of compounds on NF-kB proteins. MCF-7 Cells were transfected with NF-kB SEAP Reporter by lipofectamine 2000. After 24 h the cells were treated with (**1**, **4a**, **4b**, **4d**, and **4i**) 4 μM concentration of compounds for 24 h. The cell lysates have been collected and studied the expression levels of protein NF-kB. C + NF: the Control cells transfected with NF-kB SEAP Reporter construct without any compound treatment. Y + NF: are the compound treatments after transfection with NF-kB SEAP Reporter. Y may be **1**, **4a**, **4b**, **4d**, **4i**.

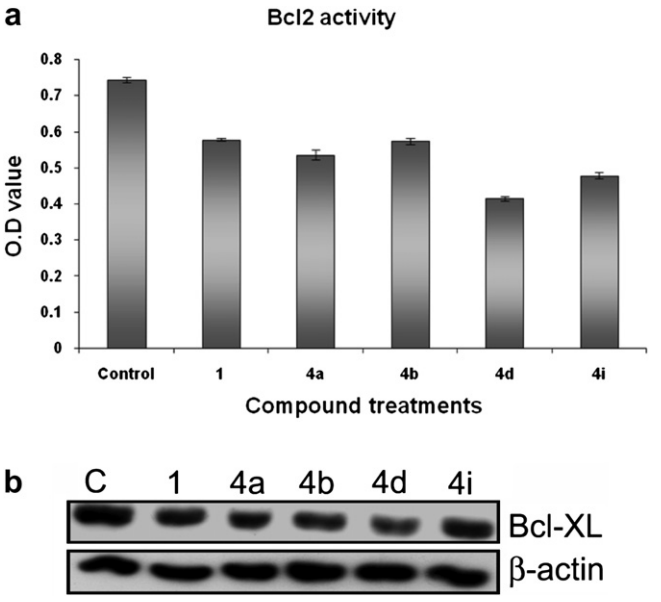


Fig. 6. a. Effect of compounds on the expression Bcl2 protein. MCF-7 cells treated with 4 μM concentration of compounds **1**, **4a**, **4b**, **4d**, and **4i** for 24 h. The cell lysates were collected and observed for expression level of Bcl2 protein using Bcl2 ELISA kit (Bender Med Systems, ALEXIS Biochemicals Company). b. Effect of compounds on the expression Bcl-xL protein. MCF-7 cells were treated with 4 μM concentration of compounds (**1**, **4a**, **4b**, **4d** and **4i**) for 24 h. The cell lysates were collected and observed for expression level of Bcl-xL protein using Bcl-xL specific antibody, β-actin was used as a loading control. C: control (untreated).

3.1.3. Effect of compounds on cell cycle regulator proteins at G1 phase of cell cycle

Cell cycle regulatory proteins such as Cyclin D1 and its Cyclin dependent kinase component (Cdk4) [46] are known to play a vital role in G1 to S-phase transition [47,48]. Therefore, we examined the effect of some of the promising compounds (results obtained from FACS analysis) on these proteins. Thus MCF-7 cells were treated with compounds **1, 4a, 4b, 4d,** and **4i** at 4 μM concentration. We observed a significant down regulation of Cyclin D1 for these conjugates revealing the involvement of G1 cell cycle proteins in this event. Similarly, Cdk4, an important protein associated with Cyclin D1 was down-regulated as illustrated in Fig. 4a. Further, we focused our efforts on the effect of these compounds on the Cyclin D1 promoter (−1745 Luc) activity. Interestingly we have observed down regulation of promoter activity (Fig. 4b) thus revealing the possible action of these compounds on Cyclin D1, the major regulator of cell proliferation in breast cancer [49].

3.1.4. Effect of compounds on NF-kB protein

It is well known that NF-kB protein regulates Cyclin D1 [50]. As a reduction in the level of cyclin D1 protein and its promoter activity is observed in the compound treated cells, the possibility of involvement of NF-kB protein in this event was anticipated. Interestingly upon ectopic expression (transfection) of NF-kB SEAP

reporter construct in MCF-7 cells followed by compound treatments resulted in significant reduction in NF-kB activity and was highly down-regulated in cells treated by **4d** (Fig. 5). Infact similar results were obtained in the literature for the studies against 3-hydroxy-4,3′,4′,5′-tetramethoxy chalcones [51].

3.1.5. Effect of chalcone-1,2,3-triazole–pyrrolobenzodiazepine conjugates on anti- apoptotic proteins

Bcl-xL and Bcl2 are the proteins that play an important role for causing rapid cell proliferation in breast cancer [52–54]. Thus, MCF-7 breast cancer cells were treated with compounds **1, 4a, 4b, 4d** and **4i** at 4 μM concentration and Western blot analysis against Bcl-XL and ELISA against Bcl2 was carried out. As expected a down regulation of these proteins resulting in apoptotic event, and this was more pronounced in case of **4d**. The possible action of these compounds on cell proliferation is illustrated in Fig. 6a and b.

4. Conclusion

In conclusion, a series of new *A*-C8/*C*-C2-chalcone–linked pyrrolo[2,1-*c*] [1,4]benzodiazepine conjugates connected through a 1,2,3-triazole side-armed with alkane spacers have been designed and synthesized by employing 'click' chemistry protocol. These conjugates have exhibited promising anticancer activity against selected human cancer cell lines. The effect of these chalcone-PBD conjugates on MCF-7 cells has been studied extensively. It is observed from the MTT proliferation assay that the conjugate **4d** is more effective as an anti-proliferative agent than the naturally occurring (DC-81) at 4 μM concentration. The FACS analysis showed more population in G0/G1 phase indicating that all these PBD conjugates have cell cycle regulatory properties. Involvement of G1 cell cycle proteins such as cyclin D1 and Cdk 4 has also been investigated. Detailed studies have revealed that these conjugates inhibit NF-kB protein as well as transcriptional activation of the NF-kB dependent genes such as (Cyclin D1 and Bcl-XL) that help in active cell proliferation [55]. Interestingly, these conjugates function as inhibitors of Cyclin D1 as well as NF-kB protein and such inhibitors are known to play a crucial role in breast cancer. The results obtained in this study has shown the functional importance of NF-kB and its dependent genes such as (Cyclin D1 and Bcl-XL) that help in cell cycle and control of cell proliferation [56,57]. The effect of these new conjugates on cell cycle proteins such as inhibition on Cyclin D1, Cdk4, NF-kB, Bcl-XL and Bcl2 protein observed and could be considered as promising leads against breast cancer. Hence from this data it can be concluded that linking of a chalcone moiety with PBD scaffold through a 1,2,3-triazole ring side-armed with alkane spacers has not only enhanced the anticancer activity but has provided an insight in the design of such conjugates.

5. Experimental section

5.1. General

All chemicals and reagents were obtained from Aldrich (Sigma–`Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 GF-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H spectra were recorded on Gemini Varian-VXR-unity (200 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI + software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. Melting points were determined with an Electro thermal melting point apparatus, and are uncorrected.

*5.1.1. General procedures for synthesis of 4-(azidoalkyloxy)-3-methoxybenzaldehyde (**7a**–**c**)*

To a stirred solution of compound **6a**–**c** (5 mmol) in DMF (20 mL), anhydrous K_2CO_3 (15 mmol) and dibromo alkane spacers (7.5 mmol) were added and the mixture was refluxed for 12 h. After completion of the reaction, K_2CO_3 was removed by filtration and solvent was evaporated under reduced pressure to give the crude products. This was further purified by column chromatography using ethyl acetate–hexane (10%) to afford the bromo compounds as white solids. Later, these compounds (2.5 mmol) dissolved in DMF (30 mL), NaN_3 (3 mmol) was added and the mixture was heated to 80 °C for 6 h. The residue was extracted with ethyl acetate in ice-water and organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under vacuum to afford the crude products. These were further purified by column chromatography using (15% EtOAc–hexane) as eluant to afford the pure compounds **7a**–**c**.

*5.1.1.1. 4-(3-Azidopropyloxy)-3-methoxybenzaldehyde (**7a**).* Yield 88%; Yellow solid; mp:137–140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.87 (s, 1H, aldehyde–**H**), 7.40–7.38 (m, 1H, –Ar**H**), 7.33 (s, 1H, –Ar**H**), 6.78 (d, J = 8.30 Hz, 1H, –Ar**H**), 4.11 (t, 2H, –OC**H**$_2$–), 3.92 (s, 3H, –OC**H**$_3$), 3.50 (t, 2H, –NC**H**$_2$–), 2.16–2.04 (m, 2H, –C**H**$_2$–): MS (ESI); m/z 236 (M + H)$^+$.

*5.1.1.2. 4-(4-Azidobutyloxy)-3-methoxybenzaldehyde (**7b**).* Yield 90%; Yellow solid; mp 144–148 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.81 (s, 1H, aldehyde–**H**), 7.39–7.36 (m, 2H, –Ar**H**), 6.88 (d, J = 8.30 Hz, 1H, –Ar**H**), 4.10 (t, 2H, –OC**H**$_2$–), 3.91 (s, 3H, –OC**H**$_3$), 3.40 (t, 2H, –NC**H**$_2$–), 2.02–1.77 (m, 4H, –C**H**$_2$–); MS (ESI): m/z 250 (M + H)$^+$.

*5.1.1.3. 4-(5-Azidopentyloxy)-3-methoxybenzaldehyde (**7c**).* Yield 83.2%; Yellow solid; mp 142–146 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.85 (s, 1H, aldehyde–**H**), 7.39–7.37 (m, 1H, –Ar**H**), 7.36 (s, 1H, –Ar**H**), 6.87 (d, J = 8.30 Hz, 1H, –Ar**H**), 4.04 (t, 2H, –OC**H**$_2$–), 3.90 (s, 3H, –OC**H**$_3$), 3.39 (t, 2H, –NC**H**$_2$–), 1.88–1.27 (m, 6H, –C**H**$_2$–); MS (ESI): m/z 264 (M + H)$^+$.

*5.1.2. General procedures for synthesis of (E)-3-(4-(azidoalkyloxy)-3-methoxyphenyl)-1-(substitute phenyl)prop-2-en-1-one(**9a**–**i**)*

Potassium hydroxide (1 mL of a 50% w/v aqueous solution) was added to a stirred solution of azido-benzaldehyde (**7a**–**c**, 2 mmol) and substituted acetophenone (**8a**–**c**, 2 mmol) in methanol (30 mL). The mixture was stirred overnight at room temperature. After completion of reaction, the mixture was acidified to pH 1 with 1 N aq. solution of HCl and extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, evaporated in vacuo and purified by column chromatography (15% ethyl acetate–hexane) to afford the pure chalcones.

*5.1.2.1. (E)-3-(4-(3-Azidopropyloxy)-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one(**9a**).* Yield 90%; Yellow solid; mp 180–182 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.92 (s, 1H, –ArO**H**), 7.96–7.92 (m, 1H, –Ar**H**), 7.91 (d, 1H, J = 15.62 Hz, db–**H**), 7.54–7.45 (m, 1H, –Ar**H**), 7.50 (d, 1H, J = 15.62 Hz, db–**H**), 7.28–7.17 (m, 2H, –Ar**H**), 7.05–6.90 (m, 3H, –Ar**H**), 4.16 (t, 2H, –OC**H**$_2$–), 3.95 (s, 3H, –OC**H**$_3$), 3.57 (t, 2H, –NC**H**$_2$–), 2.20–2.04 (m, 2H, –C**H**$_2$–); MS (ESI): m/z 354 (M + H)$^+$.

*5.1.2.2. (E)-3-(4-(4-Azidobutyloxy)-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one(**9b**).* Yield 95%; Yellow solid; mp 174–176 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.81 (s, 1H, –ArO**H**), 7.95–7.90 (m, 1H, –Ar**H**), 7.89 (d, 1H, *J* = 15.62 Hz, db–**H**), 7.55–7.50 (m, 1H, –Ar**H**), 7.48 (d, 1H, *J* = 15.62 Hz, db–**H**), 7.21–7.16 (m, 1H, –Ar**H**), 7.12 (s, 1H, –Ar**H**), 6.98 (d, *J* = 8.49 Hz, 1H, –Ar**H**), 6.92–6.88 (m, 1H, –Ar**H**), 6.85 (d, *J* = 8.49 Hz, 1H, –Ar**H**), 4.07 (t, 2H, –OC**H**$_2$–), 3.92 (s, 3H, –OC**H**$_3$), 3.40 (t, 2H, –NC**H**$_2$–), 2.00–1.78 (m, 4H, –C**H**$_2$–); MS (ESI): *m*/*z* 368 (M + H)$^+$.

*5.1.2.3. (E)-3-(4-(5-Azidopentyloxy)-3-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one (**9c**).* Yield 95%; Yellow solid; mp 182–184 °C; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.81 (s, 1H, –ArO**H**), 7.91–7.87 (m, 1H, –Ar**H**), 7.84 (d, 1H, *J* = 15.89 Hz, db–**H**), 7.49–7.40 (m, 1H, –Ar**H**), 7.44 (d, 1H, *J* = 15.89 Hz, db–**H**), 7.23–7.17 (m, 1H, –Ar**H**), 7.12 (s, 1H, –Ar**H**), 6.98 (d, *J* = 8.32 Hz, 1H, –Ar**H**), 6.92–6.88 (m, 1H, –Ar**H**), 6.81 (m, 1H, –Ar**H**), 4.04 (t, 2H, –OC**H**$_2$–), 3.93 (s, 3H, –OC**H**$_3$), 3.31 (t, 2H, –NC**H**$_2$–), 2.04–1.49 (m, 6H, –C**H**$_2$–); MS (ESI): *m*/*z* 382 (M + H)$^+$.

*5.1.2.4. (E)-3-(4-(3-Azidopropoxy)-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**9d**).* Yield 95%; Yellow solid; mp 174–176 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.04 (s, 1H, –Ar**H**), 7.84 (d, *J* = 8.30 Hz, 2H, –Ar**H**), 7.68 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.39 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.15–7.09 (m, 2H, –Ar**H**), 6.95 (d, *J* = 8.30 Hz, 2H, –Ar**H**), 6.85–6.90 (m, 1H, –Ar**H**), 4.14–4.07 (m, 2H, –OC**H**$_2$–), 3.86 (s, 3H, –OC**H**$_2$–), 3.59 (m, 2H, –NC**H**$_2$–), 2.13–2.00 (m, 2H, –C**H**$_2$–); MS (ESI): *m*/*z* 354 (M + H)$^+$.

*5.1.2.5. (E)-3-(4-(4-Azidobutyloxy)-3-methoxyphenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (**9e**).* Yield 92.5%; Yellow solid; mp 170–172 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.51 (s, 1H, –ArO**H**), 7.97 (d, *J* = 8.30 Hz, 2H, –Ar**H**), 7.70 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.49 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.39–7.34 (m, 1H, –Ar**H**), 7.16 (d, *J* = 8.30 Hz, 1H, –Ar**H**), 7.11 (s, 1H, –Ar**H**), 6.89 (d, *J* = 8.30 Hz, 1H, –Ar**H**), 6.84 (d, *J* = 8.30 Hz, 1H, –Ar**H**), 4.12–4.05 (m, 2H, –OC**H**$_2$–), 3.91 (s, 3H, –OC**H**$_3$), 3.49 (t, 2H, –NC**H**$_2$–), 2.14–1.99 (m, 4H, –C**H**$_2$–); MS (ESI): *m*/*z* 368 (M + H)$^+$.

*5.1.2.6. (E)-3-(4-(5-Azidopentyloxy)-3-methoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (**9f**).* Yield 94%; Yellow solid; mp 176–178 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.93 (s, 1H, –ArO**H**), 7.91 (d, *J* = 8.30 Hz, 2H, –Ar**H**), 7.69 (d, *J* = 15.48 Hz, 1H, db–**H**), 7.32 (d, *J* = 15.48 Hz, 1H, db–**H**), 7.12 (d, *J* = 8.49 Hz, 1H, –Ar**H**), 7.10 (s, 1H, –Ar**H**), 6.85 (d, *J* = 8.30 Hz, 2H, –Ar**H**), 6.80 (d, *J* = 8.49 Hz, 1H, –Ar**H**), 4.00 (t, 2H, –OC**H**$_2$–), 3.86 (s, 3H, –OC**H**$_3$), 3.24 (t, 2H, –NC**H**$_2$–), 1.89–1.77 (m, 2H, –C**H**$_2$–), 1.67–1.45 (m, 4H, –C**H**$_2$–); MS (ESI): *m*/*z* 382 (M + H)$^+$.

*5.1.2.7. (E)-3-(4-(3-Azidopropoxy)-3-methoxyphenyl)-1-(5-chloro-2-hydroxyphenyl) prop-2-en-1-one (**9g**).* Yield 92.5%; yellow solid; mp 146–148 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.74 (s, 1H, –ArO**H**), 7.88 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.84–7.83 (m, 1H, –Ar**H**), 7.44–7.39 (m, 1H, –Ar**H**), 7.39 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.27–7.18 (m, 1H, –Ar**H**), 7.13 (s, 1H, –Ar**H**), 6.95 (d, *J* = 9.06 Hz, 1H, –Ar**H**), 6.90 (d, *J* = 8.30 Hz, 1H, –Ar**H**), 4.14 (t, 2H, –OC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 3.58 (t, 2H, –NC**H**$_2$–), 2.16–2.08 (m, 2H, –C**H**$_2$–); MS (ESI): *m*/*z* 388 (M + H)$^+$.

*5.1.2.8. (E)-3-(4-(4-Azidobutyloxy)-3-methoxyphenyl)-1-(5-chloro-2-hydroxyphenyl) prop-2-en-1-one (**9h**).* Yield 93%; Yellow solid; mp 144–146 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.75 (s, 1H, –ArO**H**), 7.89–7.85 (m, 1H, –Ar**H**), 7.83 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.42–7.36 (m, 1H, –Ar**H**), 7.36 (d, *J* = 15.10 Hz, 1H, db–**H**), 7.25–7.23 (m, 1H, –Ar**H**), 7.13 (s, 1H, –Ar**H**), 6.94 (d, *J* = 8.79 Hz, 1H, –Ar**H**), 6.84 (d, *J* = 7.91 Hz, 1H, –Ar**H**), 4.08 (t, 2H, –OC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 3.40 (t, 2H, –NC**H**$_2$–), 2.00–1.79 (m, 4H, –C**H**$_2$–); MS (ESI): *m*/*z* 402 (M + H)$^+$.

*5.1.2.9. (E)-3-(4-(5-Azidopentyloxy)-3-methoxyphenyl)-1-(5-chloro-2-hydroxyphenyl) prop-2-en-1-one (**9i**).* Yield 95%; Yellow solid; mp 148–150 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.84 (s, 1H, –ArO**H**), 7.87 (d, *J* = 15.67 Hz, 1H, db–**H**), 7.86 (s, 1H, –Ar**H**), 7.45–7.41 (m, 1H, –Ar**H**), 7.41 (d, *J* = 15.29 Hz, 1H, db–**H**), 7.28–7.24 (m, 1H, –Ar**H**), 7.17 (s, 1H, –Ar**H**), 6.99 (d, *J* = 8.87 Hz, 1H, –Ar**H**), 6.90 (d, *J* = 8.30 Hz, 1H, –Ar**H**), 4.09 (t, 2H, –OC**H**$_2$–), 3.96 (s, 3H, –OC**H**$_3$), 3.32 (t, 2H, –NC**H**$_2$–), 1.97–1.87 (m, 2H, –C**H**$_2$–), 1.77–1.56 (m, 4H, –C**H**$_2$–); MS (ESI): *m*/*z* 416 (M + H)$^+$.

*5.1.3. 3-Methoxy-4-(prop-2-ynyloxy)benzaldehyde (**13**)*

To a solution of compound **6** (760 mg, 5 mmol) in DMF (20 mL), anhydrous K_2CO_3 (2.07 g, 15 mmol) and propargyl bromide (0.54 mL, 6 mmol) were added and the mixture at room temperature for 12 h. After completion of the reaction, potassium carbonate was removed by filtration and solvent was evaporated under reduced pressure to get the crude product. This was further purified by column chromatography using ethyl acetate–hexane (10%) to afford the compound **13** as a yellow solid; Yield 93%; mp 105–108 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.84 (s, 1H, –Ar**H**), 7.42–7.39 (m, 2H, –Ar**H**), 7.09 (d, *J* = 7.55 Hz, 1H, –Ar**H**), 4.82 (d, 2H, *J* = 2.26 Hz, –OC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 2.50 (t, 1H, *J* = 2.26 Hz, alkyne–**H**); MS (ESI): *m*/*z* 191 (M + H)$^+$.

*5.1.3.1. (E)-1-(2-Hydroxyphenyl)-3-(3-methoxy-4-(prop-2-ynyloxy) phenyl)prop-2-en-1-one (**14**).* The compound **14** was prepared following the method described for the preparation of the compound **9a**, employing **13** (956 mg, 5 mmol) and 2-hydroxyacetophenone **8a** (0.52 ml, 5 mmol), and the crude product was purified by column chromatography (30% ethyl acetate–hexane) to afford the compound **14** as a yellow solid; Yield 88%; mp 145–147 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.77 (s, 1H, –ArO**H**), 7.92–7.82 (m, 1H, –Ar**H**), 7.56–7.42 (m, 1H, –Ar**H**), 7.50 (d, *J* = 15.10 Hz, 1H, –db**H**), 7.29–7.14 (m, 1H, –Ar**H**), 7.12 (s, 1H, –Ar**H**), 7.04–6.86 (m, 3H, –Ar**H**), 6.89 (d, *J* = 15.10 Hz, 1H, –db**H**), 4.80 (s, 2H, –OC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 2.48 (s, 1H, alkyne–**H**); MS (ESI): *m*/*z* 309 (M + H)$^+$.

*5.1.4. (2S)-N-[4-(Prop-2-ynyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidine-2-carbox-aldehyde diethylthioacetal (**11**)*

To a solution of compound **10** (400 mg, 1 mmol) in DMF (15 mL) was added, anhydrous K_2CO_3 (553 mg, 4 mmol), propargyl bromide (106 mL, 1.2 mmol) and the mixture was stirred at room temperature for 12 h. The reaction was monitored by TLC using EtOAc–hexane (1:1). After completion of the reaction as indicated by TLC, diluted in water and extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and evaporated under vacuum. The residue, thus obtained was purified by column chromatography using ethyl acetate and hexane (2:3) to afford compound **11** as yellow liquid; Yield 93%; ^{1}H NMR ($CDCl_3$, 300 Mhz): δ 7.84 (s, 1H, –Ar**H**), 6.81 (s, 1H, –Ar**H**), 4.82 (s, 2H, –OC**H**$_2$–), 4.74–4.62 (m, 1H, –SC**H**–), 4.17–4.04 (m, 1H, –NC**H**–), 3.96 (s, 3H, –OC**H**$_3$), 3.33–3.16 (m, 2H, –NC**H**$_2$–), 2.88–2.64 (m, 4H, –SC**H**$_2$–), 2.42–2.02 (m, 2H, –C**H**$_2$–), 2.01 (s, 1H, alkyne–**H**), 2.00–1.69 (m 2H, –C**H**$_2$–), 1.40–1.29 (m, 6H, –C**H**$_3$); MS(ESI): *m*/*z* 439 (M + H)$^+$.

*5.1.5. General procedure of synthesis (2S)-N-4-{(1-(3-(4-((E)-3-(phenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)alkyl)-1H-1,2,3-triazol-4-yl}methoxy)-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12a**–**i**)*

To a stirred solution of compound **11** (438 mg, 1 mmol) and (*E*)-3-(4-(azido alkyloxy)-3-methoxyphenyl)-1-(substituted phenyl)

prop-2-en-1-one **9a**–**i** (1.2 mmol) in *t*-BuOH:H_2O (1:1) (50 mL), $CuSO_4 \cdot 5H_2O$ catalyst (1 mol%) (1.36 g, 3 mmol) and sodium ascorbate (5 mol%) were added and this mixture was stirred for 12 h at room temperature. After completion of the reaction, quenched with NH_3 solution and n-butanol was removed under reduced pressure. Chloroform and water added to the above residue and stirred for another 30 min followed by celite filtration, extracted with chloroform, and organic solvent was evaporated under reduced pressure to afford the crude products. These were further purified by column chromatography using ethyl acetate–hexane (90%) provides the pure compounds **12a**–**i**.

*5.1.5.1. (2S)-N-4-{(1-(3-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12a**).* Yield 80%; Yellow solid; mp 125–127 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.90 (s, 1H, –ArO**H**), 7.95–7.85 (m, 2H, –Ar**H**), 7.87 (d, J = 15.86 Hz, 1H, db–**H**), 7.75 (m, 1H, triazole–**H**), 7.54 (d, J = 15.10 Hz, 1H, db–**H**), 7.53–7.42 (m, 1H, –Ar**H**), 7.24–7.18 (m, 1H, –Ar**H**), 7.06–6.83 (m, 5H, –Ar**H**), 5.32 (s, 2H, –OC**H**$_2$–), 4.86 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.73–4.70 (m, 1H, –NC**H**–), 4.70–4.64 (m, 2H, –OC**H**$_2$–), 4.15–4.03 (m, 2H, –NC**H**$_2$–), 3.96 (s, 3H, –OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 3.32–3.15 (m, 2H, –NC**H**$_2$–), 2.86–2.65 (m, 4H, –SC**H**$_2$–), 2.56–2.44 (m, 2H, –C**H**$_2$–), 2.35–1.73 (m, 4H, –C**H**$_2$–), 1.38–1.29 (m, 6H, –C**H**$_3$); MS (ESI): m/z 792 (M + H)$^+$.

*5.1.5.2. (2S)-N-4-{(1-(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12b**).* Yield 85%; Yellow solid; mp 122–124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.90 (s, 1H, –ArO**H**), 7.94–7.87 (m, 3H, –Ar**H**, triazole–**H**), 7.89 (d, J = 15.67 Hz, 1H, db–**H**), 7.51 (d, J = 15.98 Hz, 1H, db–**H**), 7.51–7.46 (m, 1H, –Ar**H**), 7.26–7.23 (m, 1H, –Ar**H**), 7.17 (s, 1H, –Ar**H**), 7.02 (d, J = 8.30 Hz, 1H, –Ar**H**), 6.96–6.91 (m, 1H, –Ar**H**), 6.86 (d, J = 8.30 Hz, 1H, –Ar**H**), 6.82 (s, 1H, –Ar**H**), 5.33 (s, 2H, –OC**H**$_2$–), 4.85 (d, J = 3.77 Hz, 1H, –OC**H**$_2$–), 4.72–4.66 (m, 1H, –OC**H**$_2$–), 4.54 (t, 2H, –OC**H**$_2$–), 4.11 (t, 2H, –NC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 3.93 (s, 3H, –OC**H**$_3$), 3.33–3.18 (m, 2H, –NC**H**$_2$–), 2.81–2.67 (m, 4H, –SC**H**$_2$–), 2.40–1.74(m, 8H, –C**H**$_2$–), 1.38–1.30(m, 6H, –C**H**$_3$); MS (ESI): m/z 806 (M + H)$^+$.

*5.1.5.3. (2S)-4-{(1-(5-(4-((E)-3-(2-Hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12c**).* Yield 86%; Yellow solid; mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.80 (s, 1H, –ArO**H**), 7.92–7.80 (m, 2H, –Ar**H**), 7.82 (d, J = 15.86 Hz, 1H, db–**H**), 7.64 (s, 1H, –Ar**H**), 7.47 (d, J = 15.10 Hz, 1H, db–**H**), 7.47–7.35 (m, 1H, –Ar**H**), 7.20 (d, J = 8.30 Hz, 1H, –Ar**H**), 7.12 (s, 1H, –Ar**H**), 6.99 (d, J = 9.06 Hz, 1H, –Ar**H**), 6.91–6.84 (m, 1H, –Ar**H**), 6.81 (d, J = 8.30 Hz, 1H, –Ar**H**), 6.78 (s, 1H, Ar**H**), 5.30 (s, 2H, –OC**H**$_2$–), 4.81 (d, J = 3.77 Hz, 1H, –SC**H**$_2$–), 4.70–4.61 (m, 1H, –NC**H**$_2$–), 4.41 (t, 2H, –OC**H**$_2$–), 4.07–4.05 (m, 2H, –NC**H**$_2$–), 3.92 (s, 3H, –OC**H**$_3$), 3.91 (s, 3H, –OC**H**$_3$), 3.29–3.16 (m, 2H, –NC**H**$_2$–), 2.83–2.65 (m, 4H, –SC**H**$_2$–), 2.34–1.74 (m, 8H, –C**H**$_2$–), 1.63–1.52 (m, 2H, –C**H**$_2$–), 1.38–1.30 (m, 6H, –C**H**$_3$); MS (ESI): m/z 820 (M + H)$^+$.

*5.1.5.4. (2S)-N-{4-{1-(3-(4-((E)-3-(4-Hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12d**).* Yield 82%; Yellow solid; mp 117–119 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.87 (s, 1H, –ArO**H**), 8.01 (d, J = 8.30 Hz, 2H, –Ar**H**), 7.89 (s, 1H, –Ar**H**), 7.83 (s, 1H, triazole–**H**), 7.75 (d, 1H, J = 15.10 Hz, db–**H**), 7.56 (d, 1H, J = 15.10 Hz, db–**H**), 7.46–7.41 (m, 2H, –Ar**H**), 6.95 (d, J = 8.30 Hz, 2H, –Ar**H**), 6.91–6.85 (m, 1H, –Ar**H**), 6.84 (s, 1H, Ar**H**), 5.35 (s, 2H, –OC**H**$_2$–), 4.89 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.75–4.67 (m, 1H, –NC**H**–), 4.55 (t, 2H, –OC**H**$_2$–), 4.17–4.10 (m, 2H, –NC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 3.93 (s, 3H, –OC**H**$_3$), 3.34–3.18 (m, 2H, –NC**H**$_2$–), 2.87–2.66 (m, 4H, –SC**H**$_2$–), 2.38–1.74 (m, 6H, –C**H**$_2$–), 1.39–1.29 (m, 6H, –C**H**$_3$); MS (ESI): m/z 792 (M + H)$^+$.

*5.1.5.5. (2S)-N-{4-{1-(4-(4-((E)-3-(4-Hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12e**).* Yield 80%; yellow solid; mp 123–125 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.81 (s, 1H, –ArO**H**), 7.94 (d, J = 8.30 Hz, 2H, –Ar**H**), 7.91 (s, 1H, –Ar**H**), 7.85 (s, 1H, triazole–**H**), 7.62 (d, 1H, J = 15.10 Hz, db–**H**), 7.47 (d, J = 15.10 Hz, 1H, db–**H**), 7.15–7.07 (m, 1H, –Ar**H**), 6.95 (d, J = 8.30 Hz, 2H, –Ar**H**), 6.93–6.85 (m, 3H, –Ar**H**), 5.34 (s, 2H, –OC**H**$_2$–), 4.87 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.73–4.67 (m, 1H, –NC**H**–), 4.54 (t, 2H, –OC**H**$_2$–), 4.15–4.10 (m, 2H, –NC**H**$_2$–), 3.93 (s, 3H, –OC**H**$_3$), 3.92 (s, 3H, –OC**H**$_3$), 3.34–3.18 (m, 2H, –NC**H**$_2$–), 2.87–2.66 (m, 4H, –SC**H**$_2$–), 2.39–1.75 (m, 8H, –C**H**$_2$–), 1.38–1.29 (m, 6H, –C**H**$_3$); MS (ESI): m/z 806 (M + H)$^+$.

*5.1.5.6. (2S)-N-{4-{1-(5-(4-((E)-3-(4-Hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)–5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12f**).* Yield 78%; Yellow solid; mp 122–124 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): 9.86 (s, 1H, –ArO**H**), 8.14 (d, J = 8.30 Hz, 2H, –Ar**H**), 7.89 (s, 1H, –Ar**H**), 7.83 (s, 1H, triazole–**H**), 7.75 (d, J = 15.62 Hz, 1H, db–**H**), 7.45 (d, J = 15.62 Hz, 1H, db–**H**), 7.25–7.15 (m, 1H, –Ar**H**), 6.94 (d, J = 8.30 Hz, 2H, –Ar**H**), 6.93–6.86 (m, 3H, –Ar**H**), 5.33 (s, 2H, –OC**H**$_2$–), 4.86 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.71–4.67 (m, 1H, –NC**H**–), 4.53 (t, 2H, –OC**H**$_2$–), 4.14–4.10 (m, 2H, –NC**H**$_2$–), 3.94 (s, 3H, –OC**H**$_3$), 3.93 (s, 3H, –OC**H**$_3$), 3.33–3.17 (m, 2H, –NC**H**$_2$–), 2.88–2.65 (m, 4H, –SC**H**$_2$–), 2.36–2.17 (m, 2H, –C**H**$_2$–), 2.16–1.74 (m, 4H, –C**H**$_2$–), 1.63–1.41 (m, 4H, –C**H**$_2$–), 1.38–1.28 (m, 6H, –C**H**$_3$); MS (ESI): m/z 820 (M + H)$^+$.

*5.1.5.7. (2S)-N-{4(1-(3-(4-((E)-3-(5-Chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)propyl)-1H-1,2,3-triazol-4-yl) methoxy}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12g**).* Yield 84%; Yellow solid; mp 120–122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.84 (s, 1H, –ArO**H**), 7.95–7.85 (m, 3H, –Ar**H**), 7.90 (d, J = 15.10 Hz, 1H, db–**H**), 7.46–7.43 (m, 1H, Ar**H**), 7.43 (s, 1H, d, J = 15.10 Hz, 1H, db–**H**), 7.29–7.25 (m, 1H, –Ar**H**), 7.18 (s, 1H, –Ar**H**), 6.98 (d, J = 9.06 Hz, 1H, –Ar**H**), 6.95–6.90 (m, 1H, db–**H**), 6.86 (d, J = 8.30 Hz, 1H, –Ar**H**), 6.84 (s, 1H, –Ar**H**), 5.35 (s, 2H, –OC**H**$_2$–), 4.86 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.73–4.67 (m, 1H, –NC**H**–), 4.53 (t, 2H, –OC**H**$_2$–), 4.10 (t, 2H, –NC**H**$_2$–), 3.97 (s, 3H, –OC**H**$_3$), 3.94 (s, 3H, –OC**H**$_3$), 3.32–3.19 (m, 2H, –NC**H**$_2$–), 2.85–2.67 (m, 4H, –SC**H**$_2$–), 2.40–1.74 (m, 6H, –C**H**$_2$–), 1.37–1.29 (m, 6H, –C**H**$_3$); MS (ESI): m/z 827 (M + H)$^+$.

*5.1.5.8. (2S)-N-{4(1-(4-(4-((E)-3-(5-Chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)butyl)-1H-1,2,3-triazol-4-yl) methoxy}-5-methoxy-2-nitrobenzoyl}pyrrolidine-2-carboxaldehyde diethylthioacetal (**12h**).* Yield 85%; Yellow solid; mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.83 (s, 1H, –ArO**H**), 7.94–7.84 (m, 3H, –Ar**H**), 7.89 (d, J = 15.10 Hz, 1H, db–**H**), 7.44–7.40 (m, 1H, Ar**H**), 7.42 (d, J = 15.86 Hz, 1H, db–**H**), 7.29–7.25 (m, 1H, –Ar**H**), 7.19 (s, 1H, –Ar**H**), 6.99 (d, J = 9.06 Hz, 1H, –Ar**H**), 6.89 (d, J = 8.30 Hz, 1H, –Ar**H**), 6.84 (s, 1H, –Ar**H**), 5.34 (s, 2H, –OC**H**$_2$–), 4.86 (d, J = 3.77 Hz, 1H, –SC**H**–), 4.73–4.67 (m, 1H, –NC**H**–), 4.54 (t, 2H, –OC**H**$_2$–), 4.12 (t, 2H, –NC**H**$_2$–), 3.96 (s, 3H, –OC**H**$_3$), 3.93 (s, 3H, –OC**H**$_3$), 3.32–3.19 (m, 2H, –NC**H**$_2$–), 2.85–2.67 (m, 4H, –SC**H**$_2$–), 2.40–2.21 (m, 2H, –C**H**$_2$–), 2.14–1.74 (m, 6H, –C**H**$_2$–), 1.37–1.30 (m, 6H, –OC**H**$_3$); MS (ESI): m/z 841 (M + H)$^+$.

*5.1.5.9. (2S)-N-{4(1-(5-(4-((E)-3-(5-Chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)pentyl)-1H-1,2,3-triazol-4-yl) methoxy}-5-methoxy-2-nitrobenzoyl pyrrolidine-2-carboxaldehyde diethylthioacetal (**12i**).* Yield 84.5%; Yellow solid; mp 121–123 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.86 (s, 1H, −ArO**H**), 7.89 (s, 2H, −Ar**H**), 7.89 (d, *J* = 14.88 Hz, 1H, db−**H**), 7.69 (s, 1H, triazole−**H**), 7.45–7.42 (m, 1H, −Ar**H**), 7.43 (d, *J* = 14.88 Hz, 1H, db−**H**), 7.28–7.24 (m, 1H, −Ar**H**), 7.18 (s, 1H, −Ar**H**), 6.97 (d, *J* = 8.62 Hz, 1H, −Ar**H**), 6.91 (d, *J* = 8.62 Hz, 1H, −Ar**H**), 6.84 (s, 1H, −Ar**H**), 5.34 (s, 2H, −OC**H**$_2$−), 4.86 (d, *J* = 3.13 Hz 1H, −SC**H**−), 4.73–4.68 (m, 1H, −NC**H**−), 4.42 (t, 2H, −OC**H**$_2$−), 4.08 (t, 2H, −NC**H**$_2$−), 3.96 (s, 3H, −OC**H**$_3$), 3.94 (s, 3H, −OC**H**$_3$), 3.41–3.18 (m, 2H, −NC**H**$_2$−), 2.88–2.66 (m, 4H, −SC**H**$_2$−), 2.41–1.75 (m, 8H, −C**H**$_2$−), 1.61–1.51 (m, 2H, −C**H**$_2$−), 1.37–1.31 (m, 6H, −OC**H**$_3$); MS (ESI): *m*/*z* 855 (M + H)$^+$.

*5.1.6. General procedure for synthesis of 7-methoxy-8-{(1-(n-(4-((E)-3-(substitute phenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy) alkyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4a**)*

To a stirred solution of compounds **12a**–**i** (1.0 mmol) in methanol (20 mL), $SnCl_2 \cdot 2H_2O$ (5 mmol) was added and refluxed for 2 h. The methanol was evaporated in vacuum and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ solution and then extracted with ethyl acetate (20–30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the amino diethylthioacetals, which due to potential stability problems proceeded for the next step. A solution of amino diethylthioacetal (1 mmol), $HgCl_2$ (2.26 mmol) and $CaCO_3$ (2.26 mmol) in CH_3CN–water (4:1) was stirred slowly at room temperature until TLC indicated complete loss of starting material (12 h). The reaction mixture was diluted with ethyl acetate (30 mL) and filtered through a Celite bed. The clear yellow organic supernatant was washed with saturated 5% $NaHCO_3$ (20 mL), brine wash (20 mL), and the combined organic phase was dried over anhydrous Na_2SO_4. The organic layer was evaporated in vacuum and purified by column chromatography (MeOH/$CHCl_3$, 5%) to give the final products **4a**–**i**.

*5.1.6.1. 7-Methoxy-8-{(1-(3-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4a**).* Yield 56%; Yellow solid; mp 100–102 °C; $[\alpha]_D^{25}$ + 118.5 (*c* = 0.1, CHCl3); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.90 (s, 1H, −ArO**H**), 7.96–7.91 (m, 1H, −Ar**H**), 7.88 (d, *J* = 14.84 Hz, 1H, db−**H**), 7.74 (s, 1H, triazole−**H**), 7.70 (d, *J* = 4.68 Hz, 1H, imine−**H**), 7.62 (s, 1H, −Ar**H**), 7.50 (d, *J* = 14.84 Hz, 1H, db−**H**), 7.47–7.40 (m, 1H, −Ar**H**), 7.18 (s, 1H, −Ar**H**), 7.10–6.86 (m, 5H, −Ar**H**), 5.30 (s, 2H, −OC**H**$_2$−), 4.63 (t, 2H, −OC**H**$_2$−), 4.08–4.00 (m, 2H, −NC**H**$_2$−), 3.95 (s, 3H, −OC**H**$_3$), 3.91 (s, 3H, −OC**H**$_3$), 3.86–3.48 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.53–2.27 (m, 4H, −C**H**$_2$−), 2.16–1.97 (m, 2H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 195.0, 163.6, 162.4, 152.3, 149.7, 149.1, 147.3, 145.5, 143.5, 136.2, 132.4, 129.6, 128.2, 127.0, 123.6, 123.4, 118.6, 118.0, 114.8, 113.8, 111.6, 111.3, 111.0, 109.9, 106.7, 65.4, 56.1, 55.8, 53.6, 46.9, 46.6, 44.5, 34.6, 29.6, 24.1; IR (KBr): 3348 (br), 2931, 1684, 1632, 1602, 1510, 1463, 1422, 1374, 1309, 1261, 1137, 1025, 816, 762, 659 cm^{-1}; MS (ESI): *m*/*z* 638 (M + H)$^+$; Anal. Calcd for: $C_{35}H_{35}N_5O_7$ C, 65.92; H, 5.53; N, 10.98; found: C, 65.85; H, 5.48; N, 9.71.

*5.1.6.2. 7-Methoxy-8-{(1-(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-me thoxyphenoxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4b**).* Yield 58%; Yellow solid; mp 95–97 °C; $[\alpha]_D^{25}$ + 159.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 12.92 (s, 1H, −ArO**H**), 7.95–7.90 (m, 1H, −Ar**H**), 7.88 (d, *J* = 15.10 Hz, 1H, db−**H**), 7.81 (s, 1H, triazole−**H**), **7**.65 (d, *J* = 4.53 Hz, 1H, imine−**H**), 7.53 (d, *J* = 15.86 Hz, 1H, db−**H**), 7.53 (s, 1H, −Ar**H**), 7.51–7.47 (m, 1H, −Ar**H**), 7.16 (s, 1H, −Ar**H**), 7.09–6.85 (m, 5H, −Ar**H**), 5.31 (s, 2H, −OC**H**$_2$−), 4.53–48 (m, 2H, −OC**H**$_2$−), 4.14–4.04 (m, 2H, −NC**H**$_2$−), 3.92 (s, 6H, −OC**H**$_3$), 3.85–3.48 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.41–2.28 (m, 2H, −C**H**$_2$−), 2.22–2.02 (m, 4H, −C**H**$_2$−), 1.92–1.81 (m, 2H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 191.6, 164.4, 163.5, 162.6, 150.7, 149.9, 149.4, 147.7, 145.4, 143.1, 136.1, 131.5, 129.4, 127.7, 127.0, 123.5, 121.5, 118.7, 117.8, 112.7, 112.3, 111.6, 111.2, 110.0, 109.6, 68.3, 62.8, 56.0, 53.6, 49.9, 46.6, 44.5, 29.4, 27.3, 25.7, 24.0; IR (KBr): 3354 (br), 2933, 1682, 1602, 1497, 1462, 1420, 1370, 1313, 1259, 1140, 1020, 818, 760, 655 cm^{-1}; MS (ESI): *m*/*z* 652 (M + H)$^+$; Anal. Calcd for: $C_{36}H_{37}N_5O_7$ C, 66.35; H, 5.72; N, 10.75; found: C, 66.30; H, 5.68; N, 10.71.

*5.1.6.3. 7-Methoxy-8-{(1-(5-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methox yphenoxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4c**).* Yield 55%; Yellow solid; mp 100–102 °C; $[\alpha]_D^{25}$ + 147.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.92 (s, 1H, Ar**OH**), 7.98–7.91 (m, 1H, −Ar**H**), 7.87 (d, *J* = 14.69 Hz, 1H, db−**H**), 7.67 (s, 1H, triazole−**H**), 7.64 (d, *J* = 3.37 Hz, 1H, imine−**H**), 7.53–7.40 (m, 2H, −Ar**H**), 7.53 (d, *J* = 14.69 Hz, 1H, db−**H**), 7.16 (s, 1H, −Ar**H**), 7.10–6.81 (m, 5H, −Ar**H**), 5.30 (s, 2H, −OC**H**$_2$−), 4.39 (t, 2H, −OC**H**$_2$−), 4.13–3.99 (m, 2H, −NC**H**$_2$−), 3.93 (s, 3H, −OC**H**$_3$), 3.91 (s, 3H, −OC**H**$_3$), 3.88–3.48 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.37–2.23 (m, 2H, −C**H**$_2$−), 2.13–1.82 (m, 6H, −C**H**$_2$−), 1.64–1.44 (m, 2H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 191.5, 164.3, 163.2, 162.5, 151.0, 149.8, 149.5, 147.5, 145.5, 143.2, 136.2, 131.4, 129.4, 127.8, 126.9, 123.3, 121.4, 118.4, 117.6, 112.5, 112.4, 111.5, 111.4, 110.1, 109.5, 68.2, 62.6, 55.8, 53.4, 49.7, 46.4, 44.2, 31.4, 28.3, 27.3, 25.7, 22.2; IR (KBr): 3364 (br), 2933, 1600, 1497, 1463, 1422, 1338, 1313, 1245, 1179, 1127, 1009, 865, 737, 653 cm^{-1}; MS (ESI): *m*/*z* 666 (M + H)$^+$; Anal. Calcd for: $C_{37}H_{39}N_5O_7$ C, 66.75; H, 5.90; N, 10.52; found: C, 66.70; H, 5.85; N, 10.46.

*5.1.6.4. 7-Methoxy-8(1-(3-(4-((E)-3-(4-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyr rolo [2,1-c] [1,4]benzodiazepine-5-one (**4d**).* Yield 55%; Yellow solid; mp 95–97 °C; $[\alpha]_D^{25}$ + 142.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.95 (s, 1H, −Ar**OH**), 7.92 (d, *J* = 8.81 Hz, 2H, −Ar**H**), 7.75 (s, 1H, triazole−**H**), 7.71 (d, *J* = 16.89 Hz, 1H, db−**H**), 7.65 (d, 1H, *J* = 3.90 Hz, imine−**H**), 7.50 (s, 1H, −Ar**H**), 7.40 (m, *J* = 16.16 Hz, 1H, db−**H**), 7.13 (m, 1H, −Ar**H**), 6.97 (s, 1H, −Ar**H**), 6.96 (d, *J* = 8.81 Hz, 2H, −Ar**H**), 6.79 (d, *J* = 8.08 Hz, 2H, Ar**H**), 5.29 (s, 2H, −OC**H**$_2$−), 4.62 (t, 2H, −OC**H**$_2$−), 4.08–3.94 (m, 2H, −NC**H**$_2$−), 3.92 (s, 3H, −OC**H**$_3$), 3.88 (s, 3H, −OC**H**$_3$), 3.77–3.44 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.52–2.23 (m, 4H, −C**H**$_2$−), 2.16–1.94 (m, 2H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 193.2, 163.3, 162.4, 156.4, 150.8, 149.4, 147.6, 145.4, 143.1, 140.2, 136.1, 129.4, 127.6, 127.0, 123.2, 121.4, 118.6, 117.7, 112.6, 112.2, 111.4, 111.1, 110.4, 109.4, 68.1, 62.6, 56.2, 53.4, 50.9, 46.4, 44.3, 30.0, 25.4, 24.2; IR (KBr): 3360 (br), 2930, 1678, 1632, 1497, 1463, 1422, 1338, 1313, 1245, 1179, 1127, 1009, 865, 737, 653 cm^{-1}; MS (ESI): *m*/*z* 638 [M + H]$^+$; Anal. Calcd for: $C_{35}H_{35}N_5O_7$ C, 65.92; H, 5.53; N, 10.98; found: C, 65.88; H, 5.48; N, 10.92.

*5.1.6.5. 7-Methoxy-8(1-(4-(4-((E)-3-(4-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrr olo [2,1-c] [1,4]benzodiazepine-5-one (**4e**).* Yield 56%; Yellow solid; mp 94–96 °C; $[\alpha]_D^{25}$ + 112.0 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.95 (s, 1H, −Ar**OH**), 7.92 (d, *J* = 8.81 Hz, 2H, −Ar**H**), 7.74 (m, 1H, triazole−**H**), 7.73 (d, *J* = 15.60 Hz, 1H, db−**H**), 7.64 (d, *J* = 4.10 Hz, 1H, imine−**H**), 7.50 (s, 1H, −Ar**H**), 7.42 (d, *J* = 15.60 Hz, 1H, db−**H**), 7.13 (s, 1H, −Ar**H**), 7.09 (s, 1H, −Ar**H**), 6.97–6.91 (m, 4H, −Ar**H**), 5.28 (s, 2H, −OC**H**$_2$−), 4.61

(t, 2H, −OC**H**$_2$−), 4.02 (t, 2H, −NC**H**$_2$−), 3.90 (s, 3H, −OC**H**$_3$), 3.86 (s, 3H, −OC**H**$_3$), 3.77–3.46 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.52–1.80 (m, 8H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 193.4, 163.4, 162.5, 156.5, 150.9, 149.5, 147.8, 145.5, 143.0, 136.0, 129.6, 127.8, 127.1, 123.4, 121.5, 118.8, 117.8, 112.7, 112.3, 111.6, 111.3, 110.5, 109.6, 68.3, 62.8, 56.0, 53.6, 50.1, 46.6, 44.4, 29.6, 27.4, 25.7, 24.0; IR (KBr): 3352 (br), 2934, 1684, 1632, 1599, 1509, 1462, 1422, 1375, 1311, 1260, 1138, 1024, 815, 756, 659, 565 cm^{-1}; MS (ESI): *m*/*z* 652 [M + H]$^+$; Anal. Calcd for: $C_{36}H_{37}N_5O_7$ C, 66.35; H, 5.72; N, 10.75; found: C, 66.28; H, 5.66; N, 10.69.

*5.1.6.6. 7-Methoxy-8(1-(5-(4-((E)-3-(4-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4f**).* Yield 56%; Yellow solid; mp 100–102 °C; $[\alpha]_D^{25}$ + 159.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): *δ* 9.82 (s, 1H, −Ar**OH**), 7.92 (m, 2H, −Ar**H**), 7.71 (d, *J* = 15.60 Hz, 1H, db−**H**), 7.78–7.64 (m, 1H, triazole−**H**), 7.64 (m, 1H, imine−**H**), 7.51 (s, 1H, −Ar**H**), 7.44–7.36 (m, 1H, −Ar**H**), 7.40 (d, *J* = 15.60 Hz, 1H, db−**H**), 7.19–7.11 (m, 1H, −Ar**H**), 7.07 (s, 1H, −Ar**H**), 7.02–6.86 (m, 3H, −Ar**H**), 5.21 (s, 2H, −OC**H**$_2$−), 4.47–4.28 (m, 2H, −OC**H**$_2$−), 4.18–4.00 (m, 2H, −NC**H**$_2$−), 3.97 (s, 3H, −OC**H**$_3$), 3.90 (s, 3H, −OC**H**$_3$), 3.84–3.53 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.53–2.23 (m, 2H, −C**H**$_2$−), 2.11–1.38 (m, 8H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 192.5, 163.2, 162.3, 156.8, 149.9, 147.7, 145.4, 143.1, 142.6, 136.1, 129.5, 127.8, 127.0, 123.3, 121.4, 118.7, 118.0, 112.6, 112.2, 111.5, 111.1, 109.1, 105.6, 68.1, 62.7, 55.9, 53.5, 49.8, 46.5, 44.3, 29.4, 27.3, 26.7, 25.6, 23.2; IR (KBr): 3355 (br), 2934, 1684, 1632, 1597, 1509, 1462, 1422, 1375, 1312, 1260, 1155, 1025, 815, 758, 659, 565 cm^{-1}; MS (ESI): *m*/*z* 666 [M + H]$^+$; Anal. Calcd for: $C_{37}H_{39}N_5O_7$ C, 66.75; H, 5.90; N, 10.52; found: C, 66.68; H, 5.82; N, 10.44.

*5.1.6.7. 7-Methoxy-8(1-(3-(4-((E)-3-(5-chloro-2-hydroxyphenyl)-3-oxo prop-1-enyl)-2-methoxyphenoxy)propyl)-1H-1,2,3-triazol-4-yl) methoxy}-(11aS)-1,2,3,11a-tetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiaepine-5-one (**4g**).* Yield 56%; Yellow solid; mp 104–106 °C; $[\alpha]_D^{25}$ + 130.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): *δ* 12.87 (s, 1H, −Ar**OH**), 7.95–7.82 (m, 2H, triazole−**H,** −Ar**H**), 7.88 (d, *J* = 15.10 Hz, 1H, olifinic−**H**), 7.64 (d, *J* = 4.15 Hz, 1H, imine−**H**), 7.57 (s, 1H, −Ar**H**), 7.56–7.37 (m, 2H, −Ar**H**), 7.54 (d, *J* = 15.10 Hz, 1H, olifinic−**H**), 7.18 (s, 1H, −Ar**H**), 7.10–6.84 (m, 3H, −Ar**H**), 5.31 (s, 2H, −OC**H**$_2$−), 4.62 (t, 2H, −OC**H**$_2$−), 4.09–4.00 (m, 2H, −NC**H**$_2$−), 3.94 (s, 3H, −OC**H**$_3$), 3.92 (s, 3H, −OC**H**$_3$), 3.86–3.48 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.53–2.27 (m, 4H, −C**H**$_2$−), 2.16–1.97 (m, 2H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 192.5, 164.5, 162.7, 161.8, 151.0, 149.4, 147.5, 146.5, 143.3, 140.4, 135.6, 128.4, 127.3, 126.0, 123.6, 123.1, 120.0, 118.5, 116.7, 112.6, 112.0, 111.3, 111.0, 110.3, 109.4, 68.2, 62.5, 55.8, 53.4, 49.9, 46.4, 43.8, 29.2, 27.6, 23.8; IR (KBr): 3357 (br), 2933, 1685, 1632, 1600, 1567, 1510, 1466, 1428, 1338, 1313, 1245, 1179, 1136, 1024, 820, 753, 645 cm^{-1}; MS (ESI): *m*/*z* 672 (M + H)$^+$; Anal. Calcd for: $C_{35}H_{34}ClN_5O_7$ C, 62.54; H, 5.10; N, 10.42; found: C, 66.50; H, 5.06; N, 10.38.

*5.1.6.8. 7-Methoxy-8(1-(4-(4-((E)-3-(5-chloro-2-hydroxyphenyl)-3-oxo prop-1-enyl)-2-methoxyphenoxy)butyl)-1H-1,2,3-triazol-4-yl) methoxy}-(11aS)-,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4h**).* Yield 54%; Yellow solid; mp 106–108 °C; $[\alpha]_D^{25}$ + 125.5 (*c* = 0.1, CHCl3); ^{1}H NMR (CDCl3, 300 MHz): *δ* 12.85 (s, 1H, −Ar**OH**), 7.90 (d, *J* = 15.10 Hz, 1H, db−**H**), 7.88 (s, 1H, Ar**H**), 7.82 (s, 1H, triazole−**H**), 7.65 (d, *J* = 4.15 Hz, 1H, imine−**H**), 7.52 (s, 1H, Ar**H**), 7.47–7.38 (m, 2H, −Ar**H**), 7.43 (s, *J* = 15.10 Hz, 1H, db−**H**), 7.18 (s, 1H, −Ar**H**), 7.04–6.84 (m, 3H, −Ar**H**), 5.30 (s, 2H, −OC**H**$_2$−), 4.56–4.47 (m, 2H, −OC**H**$_2$−), 4.16–4.03 (m, 2H, −NC**H**$_2$−), 3.94 (s, 3H, −OC**H**$_3$), 3.92 (s, 3H, −OC**H**$_3$), 3.87–3.50 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.38–2.28 (m, 4H, −C**H**$_2$−), 2.23–2.01 (m, 2H, −C**H**$_2$−), 1.94–1.83 (m, 2H, −C**H**$_2$−); ^{13}C NMR (CDCl3, 75 MHz): 192.5, 164.6, 162.6, 161.8, 149.8, 149.4, 147.6, 146.6, 143.1, 140.3, 135.8, 128.6, 127.3, 126.2, 123.8, 123.3, 120.0, 118.7, 117.2, 112.7, 112.4, 111.5, 111.1, 110.5, 109.6, 68.1, 62.9, 56.0, 53.6, 50.1, 46.6, 44.0, 29.4, 27.3, 25.6, 24.0; IR (KBr): 3357 (br), 2932, 1691, 1632, 1600, 1567, 1510, 1466, 1428, 1428, 1336, 1313, 1264, 1181, 1136, 1024, 820, 753, 645 cm^{-1}; MS (ESI): *m*/*z* 686 (M + H)$^+$; Anal. Calcd for: C36H36ClN5O7 C, 63.02; H, 5.29; N, 10.21; found: C, 63.06; H, 5.25; N, 10.15.

*5.1.6.9. 7-Methoxy-8(1-(5-(4-((E)-3-(5-chloro-2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)pentyl)-1H-1,2,3-triazol-4-yl) methoxy}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**4i**).* Yield 52%; Yellow solid; mp 93–95 °C; $[\alpha]_D^{25}$ + 159.5 (*c* = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): *δ* 12.86 (s, 1H, −Ar**OH**), 7.92 (s, 1H, −Ar**H**), 7.89 (d, *J* = 15.38 Hz, 1H, db−**H**), 7.70 (s, 1H, Triazole−**H**), 7.64 (d, 1H, *J* = 4.35 Hz, imine−**H**), 7.53 (s, 1H, Ar**H**),7.46–7.40 (m, 1H, −Ar**H**), 7.42 (d, *J* = 15.38 Hz, 1H, db−**H**), 7.32–7.24 (m, 1H, −Ar**H**), 7.18 (s, 1H, −Ar**H**), 7.03–6.86 (m, 3H, −Ar**H**), 5.31 (s, 2H, −OC**H**$_2$−), 4.42–4.37 (m, 2H, −OC**H**$_2$−), 4.07 (t, 2H, −NC**H**$_2$−), 3.95 (s, 3H, −OC**H**$_3$), 3.93 (s, 3H, −OC**H**$_3$), 3.86–3.53 (m, 3H, −NC**H**$_2$−, −NC**H**−), 2.41–2.30 (m, 4H, −C**H**$_2$−), 2.12–1.50 (m, 6H, −C**H**$_2$−); ^{13}C NMR ($CDCl_3$, 75 MHz): 192.7, 167.0, 162.1, 161.9, 150.0, 149.6, 147.6, 146.7, 143.1, 140.8, 135.9, 128.6, 127.5, 126.3, 123.9, 123.4, 121.6, 120.6, 118.8, 116.6, 112.3, 111.3, 111.0, 110.9, 109.7, 68.6, 65.2, 56.1, 52.4, 50.7, 44.6, 42.7, 29.8, 28.2, 24.5, 23.3, 22.6; IR (KBr): 3357 (br), 2932, 1690, 1633, 1567, 1511, 1467, 1428, 1338, 1254, 1179, 1127, 1020, 820, 753, 648 cm^{-1}; MS (ESI): *m*/*z* 700 [M + H]$^+$; Anal. Calcd for: $C_{37}H_{38}ClN_5O_7$ C, 63.47; H, 5.47; N, 10.00; found: C, 63.40; H, 5.40; N, 10.08.

*5.1.7. General procedure for synthesis of substituted-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-thoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-nitrobenzoyl}pyrrolidine-5-carboxaldehyde diethylthioacetal (**16a**,**b**)*

To a stirred solution of alkyne **14** (1.0 mmol) and PBD-C2-azide **15a,b** (1.2 mmol) in *t*-Butanol-water (1:1) (50 mL), $CuSO_4 \cdot 5H_2O$ catalyst (1 mol%) (3 mmol) and sodium ascorbate (5 mol%) were added and the mixture was refluxed for 12 h. After completion of the reaction, quenched with NH_3 solution and butanol was removed under reduced pressure. Chloroform, water added to the above residue and stirred for another 30 min followed by celite filtration, extraction with chloroform, and organic solvent was evaporated under reduced pressure to get the crude product. These were further purified by column chromatography using ethyl acetate–hexane (90%) to afford the compounds **16a,b**.

*5.1.7.1. 7,8-Dimethoxy-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)}-2-nitrobenzoyl}pyrrolidine-5-carboxaldehyde diethylthioacetal (**16a**).* Yield 85%; Yellow solid; 120–122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 12.75 (s, 1H, −ArO**H**), 7.98–7.94 (d, 3H, −Ar**H**), 7.84 (d, *J* = 15.38 Hz, 1H, db−**H**), 7.75 (s, 1H, −Ar**H**), 7.43 (d, *J* = 15.38 Hz, 1H, db−**H**), 7.45 (s, 1H, −Ar**H**), 7.29–7.24 (m, 1H, −Ar**H**), 7.15 (s, 1H, −Ar**H**), 6.96–6.86 (m, 3H, −Ar**H**), 5.01 (s, 2H, −OC**H**$_2$−), 4.96–4.92 (m, 1H, −NC**H**−), 4.86 (d, 1H, −SC**H**−), 4.80–4.68 (m, 1H, −NC**H**−), 3.99 (s, 3H, −OC**H**$_3$), 3.97 (s, 3H, −OC**H**$_3$), 3.90 (s, 3H, −OC**H**$_3$), 3.20–3.15 (t, 2H, −NC**H**$_2$−), 2.87–2.65 (m, 4H, −SC**H**$_2$−), 2.11–2.04 (m, 2H, −C**H**$_2$−), 1.38–1.33 (t, 6H, −C**H**$_3$); MS (ESI): *m*/*z* 764(M + H)$^+$.

*5.1.7.2. 8-(Benzyloxy)6-methoxy-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)}- 2-nitrobenzoyl}pyrrolidine-5-carboxaldehyde diethylthioacetal (**16b**).* Yield 86%; Yellow solid; 118–120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): *δ* 12.75 (s, 1H, −ArO**H**), 7.98 (d, *J* = 9.06 Hz, 1H, −Ar**H**),

7.84 (d, J = 16.10 Hz, 1H, db–**H**), 7.82 (s, 1H, –Ar**H**), 7.76–7.52 (m, 3H, –Ar**H**), 7.42 (d, J = 15.86 Hz, 1H, db–**H**), 7.47 (s, 1H, –Ar**H**), 7.45–7.32 (m, 6H, –Ar**H**), 7.15 (s, 1H, –Ar**H**), 6.96–6.84 (m, 2H, –Ar**H**), 5.19 (s, 2H, –OC**H**$_2$–), 5.05 (s, 2H, –OC**H**$_2$–), 4.99–3.92 (m, 1H, –NC**H**–), 4.86 (d, 1H, –SC**H**–), 4.78–4.67 (m, 1H, –NC**H**–), 3.97 (s, 3H, –OC**H**$_2$–), 3.90 (s, 3H, –OC**H**$_2$–),3.88–3.80 (m, 2H, –NC**H**$_2$–), 2.86–2.65 (m, 4H, –SC**H**$_2$–), 2.15–2.04 (m, 2H, –C**H**$_2$–), 1.40–1.33 (t, 6H, –C**H**$_3$); MS (ESI): m/z 840 $(M + H)^+$.

*5.1.8. General procedure for synthesis of substituted-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-thoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**5a**,**b**)*

The compounds **5a**,**b** ware prepared according to the method described for the compounds **4a**–**i**, employing the compounds **16a**,**b** (1.0 mmol). The crude product was purified by column chromatography (MeOH/$CHCl_3$, 5%) to afford the pure compounds **5a,b**.

*5.1.8.1. 7,8-Dimethoxy-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxy phenoxy)methyl)-1H-1,2,3-triazol-1-yl)}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**5a**).* Yield 58%; Yellow solid; mp 114–116 °C; $[\alpha]_D^{25}$ + 145.0 (c = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.87 (s, 1H, –ArO**H**), 7.96–7.78 (m, 2H, –Ar**H**), 7.76 (d, J = 15.40 Hz, 1H, db–**H**), 7.65 (d, J = 4.15 Hz, 1H, imine–**H**), 7.65–7.50 (m, 2H, –Ar**H**), 7.42 (d, J = 16.10 Hz, 1H, db–**H**), 7.30–7.05 (m, 3H, –Ar**H**), 7.03–6.84 (m, 3H, –Ar**H**), 5.33 (s, 2H, –OC**H**$_2$–), 5.31–5.28 (m, 1H, –NC**H**–), 4.56–4.47 (m, 3H, –NC**H**$_2$–, –NC**H**–), 3.94 (s, 3H, –OC**H**$_3$), 3.92 (s, 6H, –OC**H**$_3$), 2.36–2.24 (m, 2H, –C**H**$_2$–); ^{13}C NMR ($CDCl_3$, 75 MHz): 192.5, 163.4, 162.3, 151.7, 149.7, 149.1, 147.4, 145.3, 143.5, 136.6, 132.4, 129.6, 128.1, 127.0, 123.8, 123.4, 118.6, 118.0, 114.8, 113.8, 111.6, 111.3, 111.0, 109.9, 105.2, 69.9, 59.7, 56.5, 56.1, 50.4, 40.1, 32.4, 28.0; IR (KBr): 3385 (br), 2927, 1685, 1634, 1577, 1510, 1458, 1428, 1377, 1342, 1311, 1258, 1145, 1027, 815, 763, 661, 567 cm^{-1}; MS (ESI): m/z 610 $[M + H]^+$; Anal. Calcd for: $C_{33}H_{31}N_5O_7$ C, 65.02; H, 5.13; N, 11.49; found: C, 64.98; H, 5.08; N, 11.45.

*5.1.8.2. 8-(Benzyloxy)7-methoxy-2(4-(4-((E)-3-(2-hydroxyphenyl)-3-oxoprop-1-enyl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)}-(11aS)-1,2,3,11atetrahydro-5H-pyrrolo [2,1-c] [1,4]benzodiazepine-5-one (**5b**).* Yield 56%; Yellow solid; mp 120–122 °C; $[\alpha]_D^{25}$ +129.5 (c = 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 12.88 (s, 1H, –ArO**H**), 7.94–7.80 (m, 3H, –Ar**H**), 7.83 (d, J = 15.60 Hz, 1H, db–**H**), 7.66 (d, J = 4.15 Hz, 1H, –Ar**H**), 7.58–7.50 (m, 2H, –Ar**H**), 7.42 (d, J = 15.80 Hz, 1H, db–**H**), 7.26–7.16 (m, 6H, –Ar**H**), 7.11–6.81 (m, 4H, –Ar**H**), 5.35 (s, 4H, –OC**H**$_2$–), 5.31–5.18 (m, 1H, –OC**H**$_2$–), 4.35–4.19 (m, 3H, –NC**H**$_2$–, –NC**H**–), 3.93 (s, 6H, –OC**H**$_3$), 3.27–2.81 (m, 2H, –C**H**$_2$–); ^{13}C NMR ($CDCl_3$, 75 MHz): 192.8, 168.4, 164.9, 162.4, 150.7, 149.5, 148.8, 146.9, 145.4, 144.6, 142.3, 135.4, 135.0, 129.9, 129.1, 127.6, 127.2, 126.7, 122.8, 121.9, 119.1, 117.9, 117.3, 116.6, 115.4, 112.8, 111.5, 110.2, 105.2, 69.9, 61.7, 56.1, 55.1, 50.4, 39.6, 31.4, 28.6; IR (KBr): 3385 (br), 2923, 1695, 1635, 1607, 1574, 1510, 1467, 1426, 1374, 1258, 1202, 1143, 1023, 848, 812, 757, 697, 659, 562 cm^{-1}; MS (ESI): m/z 686 $[M + H]^+$; Anal. Calcd for: $C_{39}H_{35}N_5O_7$ C, 68.31; H, 5.14; N, 10.21; found: C, 68.25; H, 5.08; N, 10.15.

5.2. Cell culture

The MCF-7 (breast carcinoma cells) were incubated by using DMEM media, supplemented with 10% fetal calf serum, 100 μg/mL pencillin-G and 100 μg/mL streptomycin sulfate. The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in the incubator.

5.3. In vitro evaluation of cytotoxicity

Cell viability was assessed by the MTT assay, a mitochondrial function assay. It is based on the ability of viable cells to reduce the MTT to insoluble formazan crystals by mitochondrial dehydrogenase. In this assay MCF-7 cells were seeded in a 96-well plate at a density of 10,000 cells/well. After overnight incubation cells were treated with compounds **1**, **2**, **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **5a** and **5b** at 4 μM concentration and incubated for 24 h. Then the medium was discarded and replaced with 10 μL MTT dye. Plates were incubated at 37 °C for 2 h. The resulting formazan crystals were solubilized in 100 μL extraction buffer. The optical density (O.D) was read at 570 nm with micro plate reader.

5.4. Cell cycle analysis

5×10^5 MCF-7 cells were seeded in 60 mm dish and were allowed to grow for 24 h, 4 μM concentration of **1**, **2**, **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **5a** and **5b** compounds were added to the culture media, and the cells were incubated for an additional 24 h. Cells were harvested with Trypsin–EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, washed with PBS and incubated with 1 mg/ml RNAase solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 μL of DNA staining solution [10 mg of Propidium Iodide (PI), 0.1 mg of trisodium citrate, and 0.03 mL of Triton X-100 were dissolved in 100 mL of sterile MilliQ water at room temperature for 30 min in the dark]. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

5.5. Protein extraction and western blot analysis

MCF-7 cells were seeded in 60 mm dish and were allowed to grow to attain 80% confluency for 24 h, 4 μM concentration of **1**, **4a**, **4b**, **4d**, **4i** compounds were added to the culture media, and the cells were incubated with compounds for 24 h. After 24h total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1XPBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 μg/mL PMSF, 5 μg/mL Aprotinin, 5 μg/mL leupeptin, 5 μg/mL pepstatin and 100 μg/mL NaF. After centrifugation at 12,000 rpm for 10 min, the protein in supernatant was quantified by Bradford method (BIO-RAD) using Multimode varioskan instrument (Thermo-Fischer Scientifics). Fifty micrograms of protein per lane was applied in 12% SDS-polyacrylamide gel. After electrophoresis, the protein was transferred to polyvinylidine difluoride (PVDF) membrane (Amersham Biosciences). The membrane was blocked at room temperature for 2 h in TBS + 0.1% Tween20 (TBST) containing 5% blocking powder (Santacruz). The membrane was washed with TBST for 5 min, and primary antibody was added and incubated at 4 °C overnight (O/N). Rabbit polyclonal antibodies Cyclin D1 (37 kDa) was purchased from Santacruz biotech company. Rabbit polyclonal Cdk4 (34 KDa) antibody was purchased from Prosci company. NF-kB SEAP reporter was purchased from Clone tech Company. Rabbit polyclonal antibodies Bcl-xL (29 kDa), β-actin (38 kDa) was purchased from Imgenex company. Membranes were washed with TBST three times for 15 min and the blots were visualized with chemiluminescence reagent (Thermo-Fischer Scientifics Ltd.). The X-ray films were developed with developer and fixed with fixer solution (from Kodak Company).

5.6. Cyclin D1 promoter assay

The Cyclin D1 promoter (−1745 Luc) was obtained from Richard. G. Pestell containing NF-kB binding site. Here MCF-7 cells were transfected with Cyclin D1 promoter tagged with Luciferase gene as reporter. This is followed by compound (**1, 4a, 4b, 4d, 4i)** treatments for 24 h. The lysates obtained after 24 h time period were subjected to luciferase assay by using Lumino meter as an option in Varioskan Reader (Thermo-Fischer Company).

5.7. NF-kB SEAP reporter assays

The NF-kB SEAPorter assay was conducted by transiently transfecting MCF-7 cells with NF-kB SEAP plasmid (6767 bp) obtained from Imgenex Company. SEAP is a secreted alkaline phosphatase tagged downstream to NF-kB promoter. After transfecting NF-kB SEAP reporter construct the cells were treated with compounds(**1, 4a, 4b, 4d, 4i)** at a concentration of 4 μM for 24 h, the cell lysates were subjected to Chemiluminiscent based reagent obtained from Clone Tech (Takara). The amount of the expression was measured using luminometry and as well as exposing the X-ray film kept on the top surface of the plate for 30 min.

5.8. Bcl2-ELISA

Bcl2 ELISA was conducted with Alexis Biochemicals BCl2 ELISA kit. Here MCF-7 cells were treated with compounds (**1, 4a, 4b, 4d, 4i)** for 24 h at 4 μM concentration. The cell lysates were isolated and added to the micro wells which contains Bcl2 antibody. After the addition of lysates 100 μL of biotin conjugated anti-human Bcl2 monoclonal antibody was added. After the incubation period and washing steps the bound Bcl2 was detected by using 150 μL Streptavidin-HRP secondary antibodies. The colored product obtained was detected by O.D at 450 nm. O.D is directly proportional to the amount of Bcl2 protein in the sample.

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