Bioorganic & Medicinal Chemistry Letters 23 (2013) 5733–5739

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and study of benzothiazole conjugates in the control of cell proliferation by modulating Ras/MEK/ERK-dependent pathway in MCF-7 cells

Ahmed Kamal [a,*], Shaikh Faazil [a], M. Janaki Ramaiah [b], Md. Ashraf [a], M. Balakrishna [a], S.N.C.V.L. Pushpavalli [b], Nibedita Patel [b], Manika Pal-Bhadra [b,*]

[a] Medicinal Chemistry and Pharmacology, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India
[b] Chemical Biology, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

ARTICLE INFO

Article history:
Received 29 April 2013
Revised 12 July 2013
Accepted 30 July 2013
Available online 8 August 2013

Keywords:
Ras/MEK/ERK
Cell cycle
Caspase-9
Benzothiazole–pyrrole hybrid

ABSTRACT

By applying a methodology, a series of benzothiazole–pyrrole based conjugates (**4a**–**r**) were synthesized and evaluated for their antiproliferative activity. Compounds such as **4a**, **4c**, **4e**, **4g**–**j**, **4m**, **4n**, **4o** and **4r** exhibited significant cytotoxic effect in the MCF-7 cell line. Cell cycle effects were examined for these conjugates at 2 μM as well as 4 μM concentrations and FACS analysis show an increase of G2/M phase cells with concomitant decrease of G1 phase cells thereby indicating G2/M cell cycle arrest by them. Interestingly **4o** and **4r** are effective in causing apoptosis in MCF-7 cells. Moreover, **4o** showed down regulation of oncogenic expression of Ras and its downstream effector molecules such as MEK1, ERK1/2, p38 MAPK and VEGF. The apoptotic aspect of this conjugate is further evidenced by increased expression of caspase-9 in MCF-7 cells. Hence these small molecules have the potential to control both the cell proliferation as well as the invasion process in the highly malignant breast cancers.

Amplification of Ras proto oncogene and activating mutations that lead to the expression of constitutively active Ras proteins are observed in approximately 30% of human cancers.[1] ERK/MAPK signaling promotes cell proliferation, cell survival and metastasis along with the overwhelming frequency in which this pathway is aberrantly activated in cancer, in particular by upstream activation by the epidermal growth factor receptor (EGFR) and Ras small guanosine triphosphatases (GTPases).[2] Extra cellular-signal-regulated kinases (ERK1/2) are Serine/Threonine kinases and their activities are positively regulated by phosphorylation mediated by MEK1 and MEK2.[3] It is well known that phosphorylated ERK (pERK) is a key downstream component of the RAF/MEK/ERK signaling pathway. It can be translocated to the nucleus after phosphorylation, where it leads to changes in gene expression by phosphorylating and regulating various transcription factors such as Ets family transcription factors (Elk-1).[2] The Ras/Raf/MEK/ERK signaling cascades play a critical role in the transmission of signals from growth factor receptors to regulate gene expression and prevent apoptosis.[4] Because the expression is often aberrant in tumours it is a popular target for small molecule inhibition.[5] Over the years two main strategies have been vigorously pursued to identify anti-Ras inhibitors. First approach has been the development of inhibitors of Ras downstream effector signaling, with efforts focused on the ERK MAPK pathway. The second approach focused in blocking the post-translational modifications that promote Ras membrane association.[2] There has been considerable impetus for developing inhibitors of PI3K/AKT/mTOR and Ras/MEK/ERK pathways as these

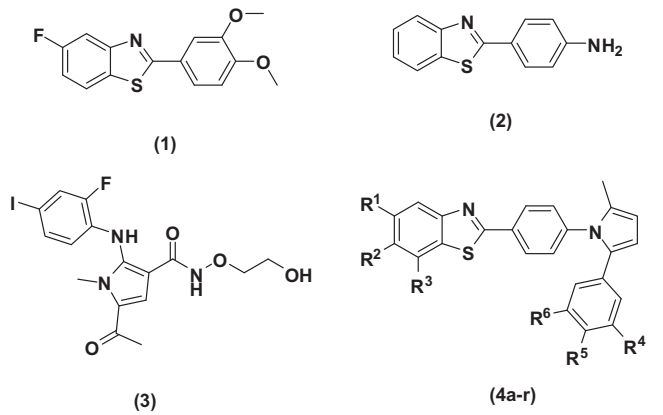
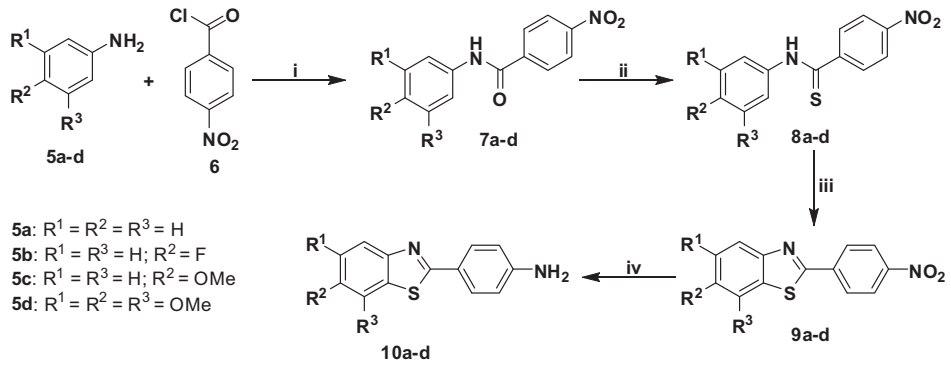


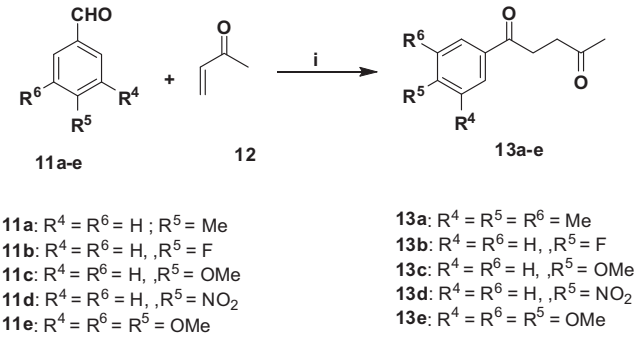
Figure 1. Chemical structure of 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (**1**), 2-(4-aminophenyl)benzothiazoles (**2**), 5-acetyl-2-(2-fluoro-4-iodophenylamino)-*N*-(2-hydroxyethoxy)-1-methyl-1*H*-pyrrole-3-carboxamide (**3**), benzothiazole–pyrrole hybrid derivatives (**4a**–**r**).

* Corresponding authors. Tel.: +91 40 27193157; fax: +91 40 27193189.
E-mail address: ahmedkamal@iict.res.in (A. Kamal).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.07.068



Scheme 1. Reagents and conditions: (i) pyridine, reflux, 2 h; (ii) Lawesson's reagent, toluene, reflux, 8 h; (iii) $K_3Fe(CN)_6$, aq NaOH, 90 °C, 3 h; (iv) $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 2 h.

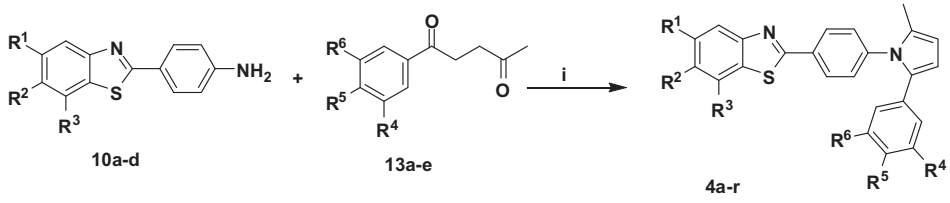


Scheme 2. Reagents and conditions: (i) 3-ethyl-5(2-hydroxyethyl)-4-methylthiazolium bromide, triethyl amine, EtOH, reflux, 6–8 h.

are considered to be the central transducers of oncogenic signals in solid tumors.[6]

Benzothiazoles is a privileged heterocyclic system with extensive pharmaceutical importance due to its diverse chemotherapeutic potential including versatile antineoplastic properties. Molecules containing the benzothiazole moiety have been recently reported to have anticancer activities through inhibition of the Raf/MEK/ERK signal cascade.[7,8] Literature survey revealed that these simple structures possess remarkable and intriguing anti-leukemic properties,[9,10] and their biological profile is unlike that of any known new anticancer agent. A variety of benzothiazole derivatives were synthesized with structural modifications and were studied for their diverse biological activities, like antimicrobial,[11] antiallergenic,[12] anti-inflammatory,[13] immunosuppressive,[14] anticonvulsant,[15] and antitumor activities through inhibiting different types of enzymes that play important roles in cell division.[16]

It is reported that some of the substituted 2-phenylbenzothiazole derivatives have revealed good anticancer activity.[17,18] Moreover, modifications on the benzothiazole nucleus have resulted in a large number of compounds having diverse pharmacological activities. Earlier studies on modified benzothiazoles demonstrated interesting pharmacological activities and thus represent an important scaffold of drugs.[19,20] Among them, imidazobenzothiazoles, as well as polymerized benzothiazoles and other substituted benzothiazoles such as 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (**1**) exhibited potent cytotoxicity (GI_{50} <0.1 nM). 2-(4-Aminophenyl)benzothiazoles (**2**) the original lead compound from the benzothiazole series exhibited nanomolar activity against certain breast cancer cell lines.[21] Similarly, pyrrole based series (**3**) are reported[22] as selective MEK kinase inhibitors show promising



4a: $R^1 = R^2 = R^3 = R^4 = R^6 = H$; $R^5 = Me$
4b: $R^1 = R^2 = R^3 = R^4 = R^6 = H$; $R^5 = F$
4c: $R^1 = R^2 = R^3 = R^4 = R^6 = H$; $R^5 = OMe$
4d: $R^1 = R^2 = R^3 = R^4 = R^6 = H$; $R^5 = NO_2$
4e: $R^1 = R^2 = R^3 = H$; $R^4 = R^5 = R^6$ = 3,4,5- trimethoxy
4f: $R^2 = F$; $R^1 = R^3 = R^4 = R^6 = H$; $R^5 = Me$
4g: $R^2 = R^5 = F$; $R^1 = R^3 = R^4 = R^6 = H$
4h: $R^1 = R^3 = R^4 = R^6 = H$; $R^2 = F$; $R^5 = OMe$
4i: $R^1 = R^3 = R^4 = R^6 = H$; $R^2 = F$; $R^5 = NO_2$
4j: $R^1 = R^3 = H$; $R^2 = F$; $R^4 = R^5 = R^6$ = 3,4,5-trimethoxy
4k: $R^2 = OMe$; $R^1 = R^3 = R^4 = R^6 = H$; $R^5 = Me$
4l: $R^2 = OMe$; $R^1 = R^3 = R^4 = R^6 = H$; $R^5 = F$
4m: $R^2 = R^5 = OMe$; $R^1 = R^3 = R^4 = R^6 = H$
4n: $R^2 = OMe$; $R^1 = R^3 = R^4 = R^6 = H$; $R^5 = NO_2$
4o: $R^1 = R^3 = H$; $R^2 = OMe$; $R^4 = R^5 = R^6$ = 3,4,5-trimethoxy
4p: $R^1 = R^2 = R^3$ = 3,4,5-trimethoxy; $R^4 = R^6 = H$; $R^5 = Me$
4q: $R^1 = R^2 = R^3$ = 3,4,5-trimethoxy; R^4 = R6 = H; $R^5 = F$
4r: $R^1 = R^2 = R^3$ = 3,4,5-trimethoxy; $R^4 = R^6 = H$; $R^5 = OMe$

Scheme 3. Reagents and conditions: (i) $KHSO_4$, EtOH, reflux, 1 h.

Table 1
Anti-proliferative (GI_{50} values) activity of compounds (**4a–r**) against breast cancer cell line (MCF-7) by SRB assay

Compound	GI_{50} for MCF-7 (μM)	GI_{50} for MDA-MB-231 (μM)
Etoposide	0.87 ± 0.07	1.67 ± 0.08
4a	2.5 ± 0.12	4.4 ± 0.24
4b	2.62 ± 0.32	4.34 ± 0.37
4c	2.78 ± 0.23	3.99 ± 0.40
4d	2.54 ± 0.11	3.90 ± 0.27
4e	2.34 ± 0.26	3.45 ± 0.35
4f	2.15 ± 0.08	3.14 ± 0.06
4g	1.79 ± 0.17	3.03 ± 0.28
4h	2.12 ± 0.05	3.07 ± 0.25
4i	0.918 ± 0.06	1.67 ± 0.21
4j	1.41 ± 0.16	3.0 ± 0.453
4k	2.57 ± 0.25	4.01 ± 0.38
4l	2.48 ± 0.05	4.06 ± 0.19
4m	3.09 ± 0.24	4.12 ± 0.15
4n	3.17 ± 0.244	4.56 ± 0.26
4o	0.92 ± 0.04	1.76 ± 0.352
4p	2.91 ± 0.138	4.14 ± 0.65
4q	2.67 ± 0.062	3.94 ± 0.17
4r	1.23 ± 0.16	2.24 ± 0.18

activity against colon cancer (Fig. 1). Pyrrole nucleus is well known to exhibit anticancer activity[23–25] and their structurally modified derivatives have encouraged current attention as effective anticancer agents.[26,27] A number of mechanisms are involved in their cytotoxic action, like dihydrofolate reductase inhibitors,[27] cyclin dependant kinase inhibitors,[28] tyrosine kinase inhibitors.[29] Moreover, the ERK inhibition exhibited by selective compounds is rationalized by molecular docking investigations. Modified benzothiazole derivatives constitute a class of powerful antitumour agents particularly for the treatment of hepatocarcinoma by modulating signaling pathways and key molecules that regulate cell proliferation, oncogenesis, invasion and metastasis of liver cancer such as AP1(C-Jun, JunB), NF-kB and p38MAPK.[8,30] It is not only active against malignant cell but as well as useful for the treatment of solid tumors.[31]

In continuation of our efforts on the design of new anti-cancer agents[32–34] and keeping in mind the medicinal importance of benzothiazole moiety for its impressive anticancer profile and their effect on MAP kinases led us to synthesize new conjugates of benzothiazole. Herein we report the synthesis of benzothiazole–pyrrole conjugates and their evaluation as potential anticancer

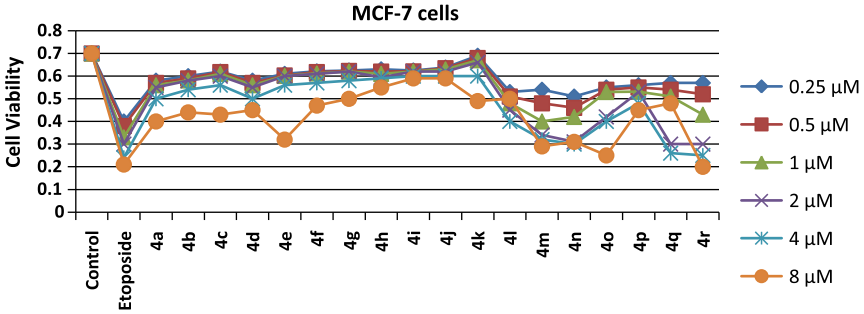


Figure 2. MCF-7 cells were treated with conjugates (**4a–r**) and etoposide (**Eto**) at 0.25–8 μM concentrations for 72 h. The MTT cell viability assay was conducted and optical density (O.D) was observed at 570 nm. Control indicates untreated cells.

Table 2
IC_{50} values for conjugates (**4a–r**) were derived by conducting MTT assay against tumor and nontumor cells

Compound	IC_{50} for MCF-7[a] (μM)	IC_{50} for MDA-MB-231[b] (μM)	IC_{50} for MCF-10A[c] (μM)	SI for MCF-7	SI for MDA-MB-231
Etoposide	1.2	1.5	36.26	30.21	24.17
4a	3.1	17.77	30.56	9.1	1.71
4b	3.181	11.76	40	12.57	3.4
4c	3.255	14.81	36.26	11.13	2.44
4d	3.11	12.12	38.80	12.47	3.2
4e	4.37	16	38.80	8.87	2.42
4f	2.97	16.66	33.58	11.30	2.01
4g	2.8	14.06	37.77	13.48	2.68
4h	2.545	19.04	37.80	14.85	1.98
4i	2.372	17.02	39	16.4	2.29
4j	2.362	15.38	40	16.93	2.60
4k	2.85	20.51	34	11.92	1.65
4l	2.80	13.79	38.3	13.67	2.77
4m	4.827	12.69	30.56	6.36	2.23
4n	4.516	12.70	34.87	25.3	2.74
4o	1.52	12.90	37.77	24.84	2.92
4p	3.11	12.30	42.5	13.66	3.45
4q	2.91	13.11	43.17	14.83	3.29
4r	2.2	12.50	43.87	19.945	3.5

SI = selective index and is obtained by dividing IC_{50} of the normal cells to IC_{50} of cancer cells; IC_{50} is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. IC_{50} values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.

[a] Estrogen positive breast cancer cells.
[b] Estrogen negative breast cancer cells.
[c] Normal mammary epithelial cells.

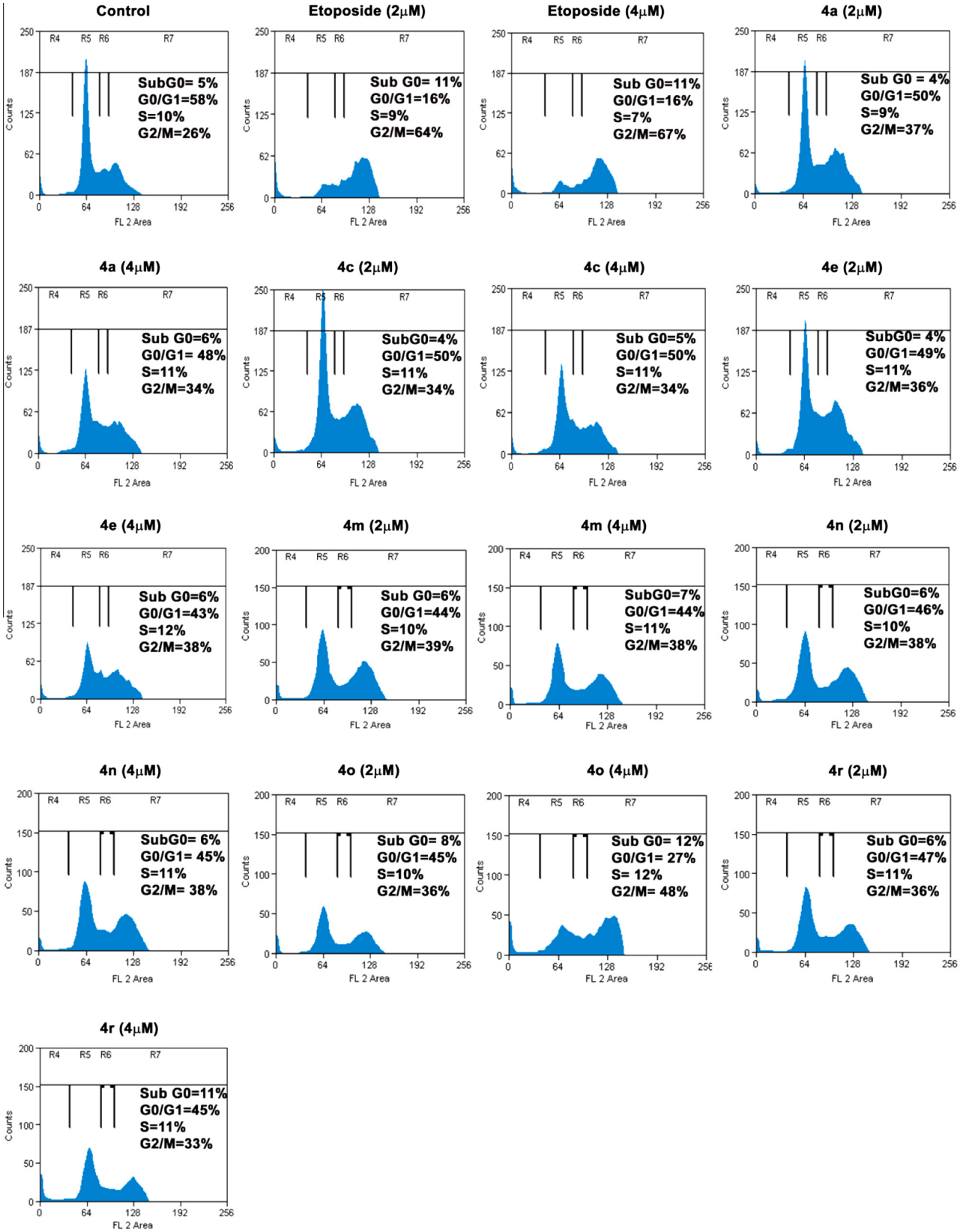


Figure 3. MCF-7 were treated with conjugates **4a**, **4c**, **4e**, **4m**, **4n**, **4o**, **4r** and etoposide at 2 and 4 μM concentrations for 24 h. The cells were further processed for FACS analysis and control indicates untreated cells.

agents. The synthesized compounds were evaluated for their effect on MEK1, ERK1/2, p38 MAPK and VEGF signaling. Few representatives of some biologically important benzothiazole and pyrrole related compounds have been illustrated in Figure 1.

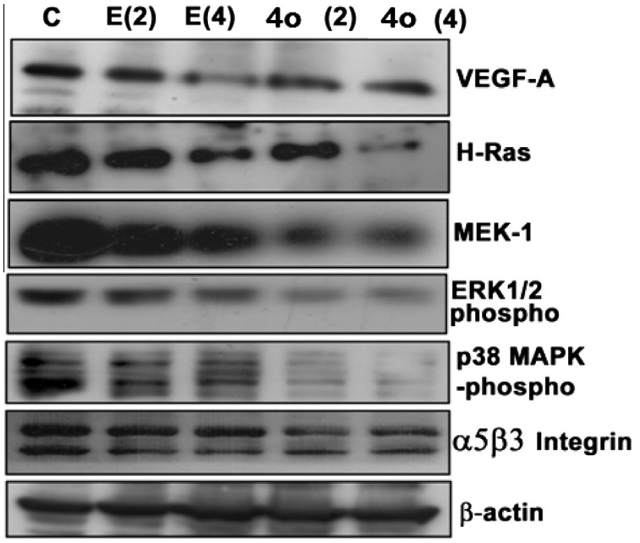


Figure 4. Treatment of MCF-7 cells with etoposide (**E**) and **4o** at 2 and 4 μM concentration for 24 h and the cell lysates were subjected to Western blot analysis using antibodies against VEGF-A, H-Ras, MEK1, phospho p38 MAPK, phospho ERK1/2 and integrin αVβ3 proteins. Beta-actin is used as loading control. **E** (**2**): Etoposide concentration for cells is 2 μM, **E** (**4**): Etoposide concentration for cells is 4 μM. Compound **4o** (**2**): compound (**4o**) concentration for cells is 2 μM, **4o** (**4**): compound (**4o**) concentration for cells is 4 μM.

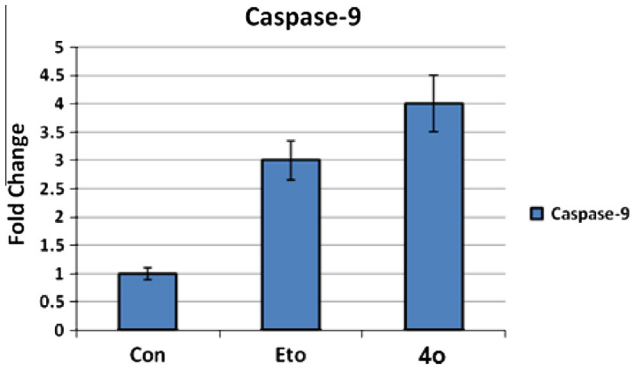


Figure 5. The effect of compounds etoposide (**Eto**) and **4o** on caspase-9 activity. MCF-7 cells were treated with etoposide (**Eto**) and **4o** at 2 μM concentrations for 24 h. The cell lysates were subjected to fluorescence based caspase-9 assay. The compound treatment resulted in increase in caspase-9 activity. Con: indicates untreated cells.

A green chemistry protocol was employed for the synthesis of the target compounds, benzothiazole–pyrrole hybrid (**4a**–**r**) via Paal knorr pyrrole methodology. The synthesis of the final compounds was achieved by condensation reaction between aminobenzothiazoles and γ-diketones. Nitrobenzoyl chloride was added slowly to a solution of the appropriately substituted aniline (**5a**–**d**) in pyridine and the solution was stirred under reflux for 2 h to afford the benzanilides (**7a**–**d**) as solids. Treatment of benzanilides with Lawesson's reagent in refluxing toluene yielded substituted thiobenzanilide (**8a**–**d**), which on treatment with aqueous sodium hydroxide containing ethanol (3 mL) was added dropwise to a pre-heating solution of potassium ferricyanide in water at 90 °C over a period of 3 h, to afford substituted 2-(4-nitrophenyl)-benzothiazoles (**9a**–**d**). Further, substituted 2-(4-nitrophenyl)-benzothiazoles with tin(II) chloride dihydrate in ethanol under reflux reduced to amines (**10a**–**d**) as shown in Scheme 1. The γ-diketones (**13a**–**e**) were prepared by Stetter reaction,[35] a mixture of the appropriate benzaldehyde (**11a**–**e**), triethylamine,

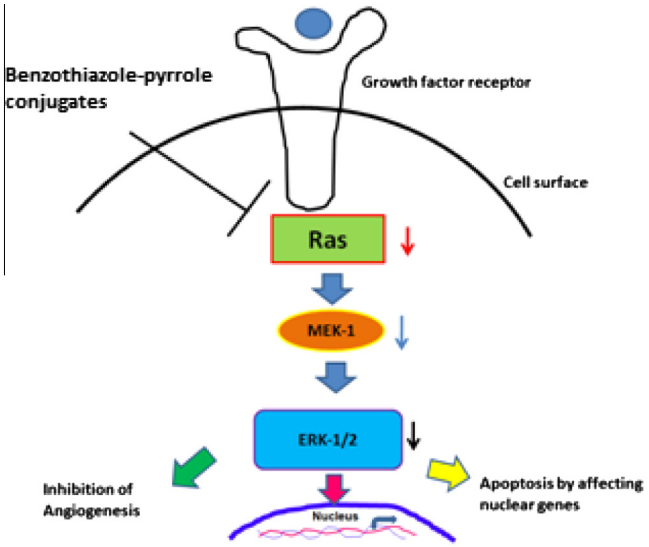


Figure 6. Model proposing the mode of action of compound **4o**.

methyl vinyl ketone (**12**) and 3-ethyl-5(2-hydroxyethyl)-4-methylthiazolium bromide was refluxed at 80 °C for 6 h, quenched with aqueous acetic acid to yield γ-diketones (**13a**–**e**) as shown in Scheme 2. These γ-diketones (**13a**–**e**) on treatment with different substituted 2-(4-aminophenyl)benzothiazoles (**10a**–**d**) by using catalytic potassium bisulphate ($KHSO_4$) in ethanol upon heating afforded the desired conjugates (**4a**–**r**) in good yields, as shown in Scheme 3.

All the synthesized compounds have displayed significant antiproliferative activity against breast cancer cell line (MCF-7) with GI_{50} values in the range of 1–4 μM. The results are summarized in Table 1. With a view to understand the cytotoxic nature of these compounds, MTT assay was performed by treating MCF-7 cells with compounds **4a**–**r** at different concentrations ranging from 1 to 8 μM for 72 h. It is observed that compounds **4o** and **4r** cause almost 50% of cell death at 4 μM. The cytotoxic effect of these conjugates was comparable to etoposide, the standard drug used in the study. It is found that most of the compounds induce cytotoxicity without effecting cell cycle. Although **4g**–**j** have shown the significant cytotoxicity, however they did not exhibit effect on cell cycle and thus these conjugates have not been evaluated for further studies. The IC_{50} values were deduced for MCF-7, MDA-MB-231 and MCF-10A (normal cells) to show the specificity of conjugates towards the breast cancer cells Figure 2, Table 2.

In order to understand the effect of these conjugates in cell cycle, FACS analysis was conducted for some representative compounds in MCF-7 breast cancer cells. The cells were treated for 24 h with compounds at 2 and 4 μM concentrations. It was observed that cells showed G2/M cell cycle arrest (i.e., cells accumulated in G2/M phase). One of the compounds **4o** has shown highest G2/M cell cycle arrest (i.e., 48%) whereas etoposide, a standard drug has shown 67% of G2/M cell cycle arrest. There was not much change in cell cycle arrest upon increasing the concentration of the compounds; however the percentage of apoptotic cells enhanced Figure 3.

Combretastatin-amidobenzothiazole affected the ERK activity as observed by protein activity studies in MCF-7 cells and docking studies.[7] Furthermore Mannich bases of 2-arylimidazo[2,1-*b*]benzothiazoles caused G2/M cell cycle arrest and affected the MAPK proteins in HepG2 hepatocarcinoma.[8] It is evident from studies on EphB4 protein the involvement of Ras/MEK/ERK dependent pathway in breast cancer and as well as endothelial cell

proliferation and invasion.[36] MCF-7 cells treated with compounds **4o** and etoposide caused decrease in the expression of oncogenic proteins such as Ras, MEK1 and phosphorylated forms of ERK1/2 and proteins that help in angiogenesis such as integrin αVβ3 and VEGF proteins. Similar to our results sorafenib (Nexavar) blocks the tumor cell proliferation and tumor angiogenesis by targeting Raf/MEK/ERK and VEGF signaling.[37] Thus the data obtained suggests the inhibitory role of these compounds on tumor proliferation and invasion Figure 4.

Raf/MEK/ERK and PI3K/Akt affect the phosphorylation of apoptotic regulatory molecules that exert their effects on mitochondrial membrane potential and thereby preventing apoptosis. Moreover the Ras/Raf/MEK/ERK pathway aids in the assembly of the protein translation complex responsible for the translation of 'weak' mRNAs (MCl-1) important in the prevention of apoptosis.[38] Thus we were interested to observe caspase-9 expression as a measure of cell death. Treatment of cells with **Eto** and **4o** for 24 h. The lysates were then subjected to caspase-9 assay. As expected we observed increased caspase-9 activity Figure 5.

Conjugate decreased the growth factor dependent expression of Ras and its downstream effectors of Ras such as MEK1 and ERK1/2 was decreased. The decreased level of MEK1 inhibited the phosphorylation of ERK1/2 which suppressed the rapid cell proliferation and induce apoptosis affecting both nuclear and mitochondrial genes (caspase-9). The schematic diagram proposing the mode of action of compound **4o** has shown in Figure 6.

In summary, the synthesis and evaluation of antiproliferative activity of a new series of benzothiazole–pyrrole conjugates has been investigated.[39] Most of these conjugates have exhibited potent antiproliferative activity against selected human cancer cell lines MCF-7 and MDA-MB-231. Cell cycle analysis indicated G2/M cell cycle arrest with increased cytoxicity at 2 as well as 4 μM concentrations. Thus, we provided the evidence that these newly synthesized hybrid molecules inhibited growth of human breast cancer MCF-7 cells by inducing apoptosis.[40] The apoptotic death was associated with down regulation of gene expression of oncogene such as Ras as well as activation of MEK1, ERK1/2, p38 MAPK and VEGF and integrin. In addition, we have observed increased expression of active caspase-9 protein in MCF-7 cells as observed by ELISA.

Acknowledgments

The authors (S.F., M.A. and M.B.) are thankful to Council of Scientific and Industrial Research (CSIR) for financial support under the XIIth Five year plan Project 'Affordable Cancer Therapeutics' and University Grant Commission (UGC) for the award of Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.068.

References and notes

1. Stirewalt, D. L.; Kopecky, K. J.; Meshinchi, S.; Appelbaum, F. R.; Slovak, M. L.; William, C. L. *Blood* **2001**, *97*, 3589.
2. Roberts, P. J.; Der, C. J. *Oncogene* **2007**, *26*, 3291.
3. Chang, F.; Lee, J. T.; Novalanic, P. M. *Leukemia* **2003**, *17*, 590.
4. Mc Cubrey, J. A.; Steelman, L. S.; Chappel, W. L.; Abrams, S. L.; Wong, E. W. T.; Chang, F.; Lehmann, B.; Terrian, D. M.; Milella, M.; Tafuri, A.; Stivala, F.; Libra, M.; Basecke, J.; Camilla, E.; Alberto, M. M.; Franklin, R. A. *Biochim. Biophys. Acta* **2007**, *1773*, 1263.
5. Ripple, M. O.; Kim, N.; Springett, R. *J. Biol. Chem.* **2013**, *288*, 5374.
6. Elina, J.; Niina, L.; Jussi, P. K. *BMC Cancer* **2012**, *12*, 612.
7. Kamal, A.; Mallareddy, A.; Ramaiah, M. J.; Pushpavalli, S. N. C. V. L.; Paidakula, S.; Chandan, K.; Murty, J. N. S. R. C.; Rao, N. S.; Sowjanya, G.; Anthony, A.; Manika, P. E. *J. Med. Chem.* **2012**, *56*, 166.
8. Ravindra, M. K.; Kumar, K. V.; Ramaiah, M. J.; Tulshiram, D.; Pushpavalli, S. N. C. V. L.; Debasmita, M.; Divya, B.; Devi, T. A.; Umesh, K.; Manika, P. E. *J. Med. Chem.* **2011**, *46*, 4258.
9. Odlo, K.; Hentzen, J.; Fournier dit Chabert, J.; Ducki, S.; Gani, O. A. B. S. M.; Sylte, I.; Skrede, M.; Florenes, V. A.; Hansen, T. V. *Bioorg. Med. Chem.* **2008**, *16*, 4829.
10. Zhang, Q.; Peng, Y.; Wang, X. I.; Keeman, S. M.; Aurora, S.; Welsh, W. J. *J. Med. Chem.* **2007**, *50*, 749.
11. Bradshaw, T. D.; Wrigley, S.; Shi, D.-F.; Schultz, R. J.; Paull, K. D.; Stevens, M. F. G. *Br. J. Cancer* **1998**, *77*, 745.
12. Bradshaw, T. D.; Shi, D.-F.; Schultz, R. J.; Paull, K. D.; Kelland, L.; Wilson, A.; Fiebig, H. H.; Wrigley, S.; Stevens, M. F. G. *Br. J. Cancer* **1998**, *78*, 421.
13. Bryson, M.; Fulton, B.; Benfield, P. *Drugs* **1996**, *52*, 549.
14. (a) Al-Tel, T. H.; Al-Qawasmeh, R. A.; Zaarour, R. *Eur. J. Med. Chem.* **1874**, *2011*, 46; (b) Palkar, M.; Noolvi, M.; Sankangoud, R.; Maddi, V.; Gadad, A.; Nargund, L. V. *Arch. Pharm.* **2010**, *343*, 353.
15. Ager, I. R.; Barnes, A. C.; Danswan, G. W.; Hairsine, P. W.; Kay, D. P.; Kennewell, P. D.; Matharu, S. S.; Miller, P.; Robson, P.; Rowlands, D. A. *J. Med. Chem.* **1988**, *31*, 1098.
16. el-Shorbagi, A. N.; Sakai, S.; el-Gendy, M. A.; Omar, N.; Farag, H. H. *Chem. Pharm. Bull.* **1989**, *37*, 2971; (b) Papadopoulou, C.; Geronikaki, A.; Hadjipavlou-Litina, D. *Il Farmaco* **2005**, *60*, 969.
17. Kisak, E. T.; Coldren, B.; Evans, C. A.; Boyer, C.; Zasadzinski, J. A. *Curr. Med. Chem.* **2004**, *11*, 1241.
18. Kok, S. H. L.; Gambari, R.; Chui, C. H.; Yuen, M. C. W.; Lin, E.; Wong, R. S. M.; Lau, F. Y.; Cheng, G. Y. M.; Lam, W. S.; Chan, S. H.; Lam, K. H.; Cheng, C. H.; Lai, P. B. S.; Yu, M. W. Y.; Cheung, F.; Tanga, J. C. O.; Chana, A. S. C. *Bioorg. Med. Chem.* **2008**, *16*, 3626.
19. Mizoule, J.; Meldrum, B.; Mazadier, M.; Croucher, M.; Ollat, C.; Uzan, A.; Legrand, J. J.; Gueremy, C.; Le Fur, C. G. *Neuropharmacology* **1985**, *24*, 767.
20. Tanaka, T.; Umekawa, H.; Saitoh, M.; Ishikawa, T.; Shin, T.; Ito, M.; Kawamatsu, Y.; Sugihara, H.; Hidaka, H. *Mol. Pharmacol.* **1986**, *29*, 264.
21. Shi, D. F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. *J. Med. Chem.* **1996**, *39*, 3375.
22. Mark, E. A.; Michael, B. W.; Toufike, K.; Nicholas, S.; O'Connell, S. M.; Hiroshi, M.; Lihong, S.; Petro, H.; Lilly, Z.; Qing, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 241.
23. Hanauske, A. R.; Chen, V.; Paoletti, P.; Niyikiza, C. *Oncologist* **2001**, *6*, 363.
24. Lee, M.; Rhodes, A. L.; Wyatt, M. D.; DIncalci, M. D.; Forrow, S.; Hartley, J. A. *J. Med. Chem.* **1993**, *36*, 863.
25. Wyatt, M. D.; Garbiras, B. J.; Haskell, M. K.; Lee, M.; Souhami, R. L.; Hartley, J. A. *Anti-Cancer Drug Des.* **1994**, *9*, 511.
26. Bennasroune, A.; Gardin, A.; Aunis, D.; Cremel, G.; Hubert, P. *Crit. Rev. Oncol. Hematol.* **2004**, *50*, 23.
27. Moriarty, K. J.; Koblish, H. K.; Garrabrant, T.; Maisuria, J.; Khalil, E.; Ali, F.; Petrounia, I. P.; Crysler, C. S.; Maroney, A. C.; Johnson, D. L.; Galemmo, R. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5778.
28. Choi, H. S.; Wang, Z.; Richmond, W.; He, X.; Yang, K.; Jiang, T.; Karanewsky, D.; Gu, X. J.; Zhaou, V.; Liu, Y.; Che, J.; Lee, C. C.; Caldwell, J.; Kanazawa, T.; Umemura, I.; Matsuura, N.; Ohmori, O.; Fonda, T.; Gray, N.; He, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2689.
29. Rosowsky, A. A.; Chen, H.; Fu, H.; Queener, S. F. *Bioorg. Med. Chem.* **2003**, *11*, 59.
30. Weng, C. J.; Chau, C. F.; Hsieh, Y. S.; Yang, S. F.; Yen, G. C. *Carcinogenesis* **2008**, *29*, 147.
31. Nobuaki, A.; Yukitaka, I.; Mayumi, Y.; Sadao, K.; Katsunori, T.; Kiyohiro, S.; Akira, M.; Masafumi, K.; Masayuki, S. *Cancer Lett.* **2006**, *238*, 119.
32. Kamal, A.; Srikanth, Y. V. V.; Khan, M. N. A.; Ashraf, Md.; Reddy, M. K.; Sultana, F.; Kaur, T.; Chashoo, G.; Suri, N.; Sehar, I.; Wani, Z. A.; Saxena, A.; Sharma, P. R.; Bhushan, S.; Mondhe, D. M.; Saxena, A. K. *Bioorg. Med. Chem.* **2011**, *19*, 7136.
33. (a) Kamal, A.; Sultana, F.; Ramaiah, M. J.; Srikanth, Y. V. V.; Viswanath, A.; Kishore, C.; Sharma, P.; Pushpavalli, S. N. C. V. L.; Anthony, A.; Bhadra, M. P. *ChemMedChem.* **2012**, *7*, 292; (b) Kamal, A.; Khan, M. N. A.; Reddy, K. S.; Srikanth, Y. V. V.; Sridhar, B. *Chem. Biol. Drug Des.* **2008**, *71*, 78.
34. (a) Kamal, A.; Reddy, K. S.; Khan, M. N. A.; Shetti, R. V. C. R. N. C.; Ramaiah, M. J.; Bhadra, M. P. *Bioorg. Med. Chem.* **2010**, *18*, 4747; (b) Kamal, A.; Khan, M. N. A.; Srikanth, Y. V. V.; Shetti, R. V. C. R. N. C. *Chem. Biol. Drug Des.* **2009**, *73*, 687.
35. Stetter, H. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 639.
36. Xiao, Z.; Carrasco, R.; Kinneer, K.; Sabol, D.; Jallal, B.; Coats, S.; Tice, D. A. *Cancer Biol. Ther.* **2012**, *13*, 630.
37. Liu, L.; Cao, Y.; Chen, C.; Zhang, X.; Mc Nabola, A.; Wilkie, D.; Wilhelm, S.; Lynch, M.; Carter, C. *Cancer Res.* **2006**, *24*, 11851.
38. Steelma, L. S.; Chappell, W. H.; Abrams, S. L.; Kempf, R.; Long, J.; Laidler, P.; Mijatovic, S.; Maksimovic-Ivanic, D.; Stivala, F.; Mazzarino, M. C.; Donia, M.; Fagone, P.; Malaponte, G.; Nicoletti, F.; Libra, M.; Milella, M.; Tafuri, A.; Bonati, A.; Basecke, J.; Cocco, L.; Evangelisti, C.; Martelli, A. M.; Montalto, G.; Cervello, M.; Mc Cubrey, J. A. *AGING* **2011**, *3*, 192.
39. *General method for the synthesis of compounds* ***4a–r***: A mixture of substituted 2-(4-aminophenyl)benzothiazole (**10a–d**) (0.5 mmol), diketone (**13a–e**) (1 equiv) in 50 mL of ethanol with a catalytic amount (25 mol %) of potassium bisulfate was heated under reflux for 2 h. The reaction was monitored by TLC. After the completion of the reaction, the solvent was removed under vaccum, water was added to reaction mixture and extracted with ethyl acetate (2× 30 mL). The solvent was evaporated under the reduced pressure to afford the crude product which was purified by flash chromatography on silica gel eluting with EtOAc and hexane to give the pure compounds. *2-(4-(2-Methyl-5-p-tolyl-1H-pyrrol-1-*

yl)phenyl)benzo[d]thiazole (**4a**) Yellow solid; Yield 86%, 162 mg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (d, 2H, *J* = 8.30 Hz), 7.89 (d, 1H, *J* = 8.67 Hz), 7.82 (d, 1H, *J* = 8.30 Hz), 7.43 (t, 1H, *J* = 8.3, 6.73 Hz), 7.36 (t, 1H, *J* = 8.3, 6.73 Hz), 7.25 (d, 2H, *J* = 8.30 Hz), 7.07 (d, 2H, *J* = 8.67 Hz), 6.96 (d, 2H, *J* = 8.60 Hz), 6.26 (d, 1H, *J* = 2.83 Hz), 5.94 (d, 1H, *J* = 2.89 Hz), 2.13 (s, 3H), 1.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 13.55, 17.10, 96.65, 96.98, 99.28, 105.84, 115.79, 125.26, 127.67, 128.03, 128.60, 130.18, 132.27, 132.92, 137.68, 138.01, 153.72, 155.25, 159.95, 160.30, 166.73; ESI–MS: *m*/*z* 381 $[M+H]^+$. *2-(4-(2-(4-Fluorophenyl)-5-methyl-1H-pyrrol-1-yl)phenyl)benzo[d]thiazole* (**4b**) Yellow solid; Yield 90%, 173 mg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.19 (d, 2H, *J* = 8.30 Hz), 7.88 (d, 1H, *J* = 8.67 Hz), 7.85 (d, 1H, *J* = 8.30 Hz), 7.47 (t, 1H, *J* = 8.3, 6.73 Hz), 7.38 (t, 1H, *J* = 8.3, 6.73 Hz), 7.27 (d, 2H, *J* = 8.30 Hz), 7.07–6.94 (4H,m), 6.29 (d, 1H, *J* = 2.83 Hz), 5.98 (d, 1H, *J* = 2.89 Hz), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 14.13, 97.25, 97.48, 105.49, 116.35, 124.41, 125.99, 128.17, 128.78, 132.87, 133.43, 137.66, 138.38, 153.12, 153.18, 154.28, 154.24, 155.68, 160.38, 167.17; ESI–MS: *m*/*z* 385 $[M+H]^+$. *2-(4-(2-(4-Methoxyphenyl)-5-methyl-1H-pyrrol-1-yl)phenyl)benzo[d]thiazole* (**4c**) Yellow solid; Yield 182 mg, 91%, mp 140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (d, 2H, *J* = 8.3 Hz), 7.9 (d, 1H, *J* = 8.6 Hz), 7.8 (d, 1H, *J* = 8.3 Hz), 7.41 (t, 1H, *J* = 8.3, 6.7 Hz), 7.3 (t, 1H, *J* = 8.3, 6.7 Hz), 7.2 (d, 2H, *J* = 8.3 Hz), 7.02 (d, 2H, *J* = 8.6 Hz), 6.9 (d, 2H, *J* = 8.6 Hz), 6.2 (d, 1H, *J* = 2.8 Hz), 5.9 (d, 1H, *J* = 2.8 Hz), 3.8 (s, 3H), 2.1 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 12.11, 55.65, 97.05,97.43, 114.82, 116.22, 118.78, 125.67, 127.64, 128.12, 128.72, 129.56, 133.00, 133.21, 137.60, 1378.47, 146.12, 154.18, 155.58, 160.38; ESI-MS: *m*/*z* 397 $[M+H]^+$. The detail spectral data for other compounds are available in Supplementary data.

40. Evaluation of in vitro anti-cancer activity

Cell culture: Human breast carcinoma cells (MCF-7), MDA-MB-231 and MCF-10 were purchased from American Type culture collection was maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen), supplemented with 2 mM glutamax (Invitrogen), 10% fetal calf serum and 100 U/ml Pencillin and 100 mg/ml streptomycin sulfate (Sigma). The cell line was maintained at 37 °C in a humidified atmosphere containing 5% CO_2 in the incubator.

MTT assay: 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. MCF-7 cells were seeded in a 96-well plate at a density of 10,000 cells/well. After overnight incubation MCF-7 (breast cancer cells) were treated with **4a**–**r** at 1–8 μM concentration and incubated for 72 h. Medium was then 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 (OD) was read at 570 nm with micro plate reader (Multi-mode Varioskan instrument-Themo Scientific).

FACS analysis: 5×10^5 MCF-7 were seeded in 60 mm dish and were allowed to grow for 24 h. Further compounds (**4a**,**4c**,**4e**,**4m**,**4n**,**4o**,**4r**) were added at a final concentration of 2 and 4 μM 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 RNase A solution (Sigma) at 37 °C for 30 min. Cells were collected by centrifugation at 2000 rpm for 5 min and further stained with 250 mL 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]. These cells were analysed to observe the changes in the cellcycle pattern and apoptotic death. The DNA contents of 20,000 events were measured by flow cytometer (DAKO CYTOMATION, Beckman Coulter, Brea, CA). Histograms were analyzed using Summit Software.

Western blot analysis: Total cell lysates from cultured MCF-7 cells were obtained by lysing the cells in ice-cold RIPA buffer (1× PBS, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) and containing 100 mg/mL PMSF, 5 mg/mL, Aprotinin, 5 mg/mL leupeptin, 5 mg/mL pepstatin and 100 mg/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 (GE 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, primary antibody was added and incubated at 4 °C overnight (O/N). VEGF-A, H-Ras, MEK-1, ERK1/2 phospho, p38 MAPK phospho, αVβ3 integrin were purchased from Cell Signalling, Abbiotec and Millipore companies. The membrane was incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:2000) (Santa Cruz) at room temperature for 1 h. 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.

Caspase-9 assay: The Apoalert caspase-9/6 fluorescent assay kit (**Clonetech**, **CA**, **USA**) was used according to the manufacturer's recommendations. MCF-7 cells were treated with compounds Etoposide (**Eto**), **4o** at 2 μM. Here the substrate used is LEHD–AMC, which was added to the cell lysates, and incubation was carried out at 378 °C for 1 h. Readings were taken at λ excitation 400 nm and λ emmission 505 nm.