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Anti-tumor activity of a new series of benzoxazepine derivatives in breast cancer

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ABSTRACT

A series of new benzoxazepine derivatives substituted with different alkoxy and aryloxy group were synthesized comprising synthetic steps of Mitsunobu reaction, lithium aluminum hydride (LAH) reduction, followed by debenzylation and finally intramolecular Mitsunobu cyclization. The new benzoxazepines specifically inhibited growth of breast cancer cell lines, MCF-7 and MDA-MB-231, but lack cytotoxicity to normal HEK-293 cells. The cell growth inhibition induced by the active compounds was due to cell cycle arrest at G_0/G_1 phase. The active compound could cause significant reduction in tumor volume of MCF-7 xenograft tumor in nude mice model and their activity was comparable to that of tamoxifen citrate at 16 mg kg^{-1} dose at 30 days of treatment. The identified most active compounds of the series have specific advantages as anti-cancer agent in breast cancer than tamoxifen.

Currently, tamoxifen is most commonly used adjuvant drug for estrogen receptor (ER)-positive breast cancer.[1] Tamoxifen, classified under selective estrogen receptor modulators (SERM), competes with estrogen and downregulates estrogenic actions in breast tissue. As a result, tamoxifen is less effective in ER-negative breast cancer. Postmenopausal patients with ER-positive metastatic breast cancer exhibit a high response rate of more than 50% with mean duration of response of nine months,[2,3] whereas the response rate of premenopausal patients were reported in the range of 20–45%.[4–7] The safety of tamoxifen is controversial as several clinical trial reports revealed increase risk of uterine cancer in postmenopausal women.[8–10] Therefore, there is a need for anti-breast cancer agents with improved properties such as enhanced and specific activity against cancer with reduced uterine side effects.

In our ongoing effort toward searching new pharmacophores as anti-cancer breast agents, phenanthrene based compounds containing amino alkyl chains were reported to have promising activity.[11] Benzoxazepines are well-known pharmacophore in medicinal chemistry showing promising activity against various diseases such as antipsychotic, central nervous system activity along with anti-cancer profile against breast cancer cells.[12] One of these groups of benzoxazepines have been identified to target microtubule assembly as a way to induce anti-cancer activity.[12] However, amino acid based benzoxazepines were not explored much to evaluate their pharmacological activity. Syntheses of amino acid based polycycles were earlier reported from our laboratory.[13a–c] Easy availability of amino acid based benzoxazepines promoted us to evaluate them for anti-cancer breast activity. We designed new class of amino acid-derived benzoxazepine derivatives with alkyl amino ethyl chains and evaluated anti-tumor activity in human breast cancer cells and xenograft model.

The synthesis of benzoxazepines (Scheme 1) ring followed the methodology developed in our laboratory. *S*-Amino acid derived *N*-tosyl amino esters and substituted benzene derivatives were used as building blocks for the construction of benzannulated chiral heterocycles. The Mitsunobu reaction of *S*-amino acid derivatives **2a–h** with **1a–c** provided the esters **3a–h, 4a, 5a** in 60–75% yield. The lithium aluminum hydride (LAH) reduction of **3a–h, 4a, 5a** afforded the corresponding alcohols **6a–h, 7a, 8a** which on subsequent debenzylation by H_2/Pd (10% on carbon) gave **9a–h, 10a, 11a** containing free alcoholic and phenolic hydroxyl groups in 60–70% yield in two steps. Exposure of **9a–h, 10a, 11a** to Mitsunobu reaction conditions,[14] that is, diethylazodicarboxylate (DEAD), triphenylphosphine (TPP) at 0 °C resulted in the formation of desired enantiomerically pure benzoxazepine derivatives **12a–h, 13a** and **14a** in 63–76% yield. The biological result of the *S*-amino

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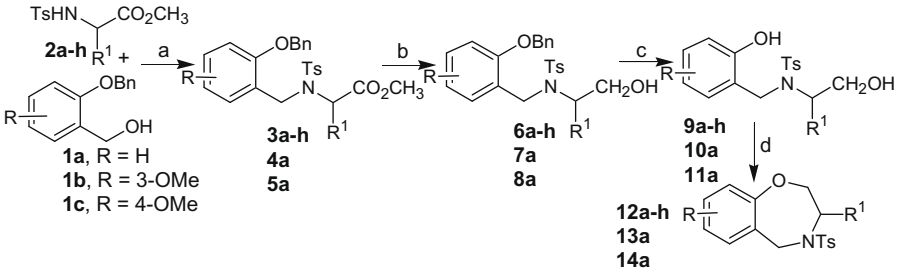
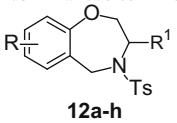
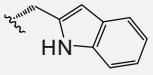
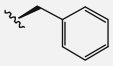
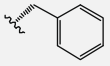
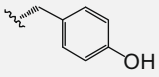
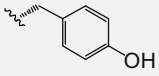
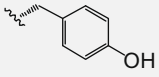


Scheme 1. Reagents and conditions: (a) **1a–c**, DEAD, PPh_3, THF, 0 °C (2 h) to rt (10 h), N_2, 60–75%; (b) LAH, THF, 0 °C, 1 h, 59–70%; (c) H_2, 10% Pd/C, MeOH, rt, 2 h, 50 psi, 61–75%; (d) DEAD, PPh_3, THF, 0 °C (1 h) to rt, (14 h), N_2, 63–76%.

Table 1
The in vitro cell growth inhibitory effect of benzoxazepine series of compounds in various cell lines with MTT assay **12a–h**, **13a**, **14a**



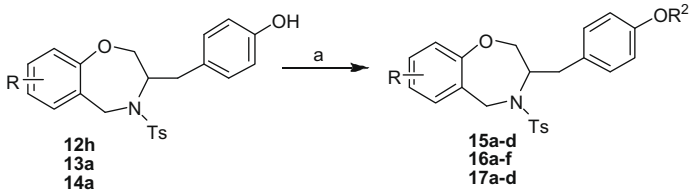
12a-h
13a
14a

Compound no.	R	R[1] [a]	Cell growth inhibition in terms of IC_{50} (μM)			
			MCF-7	MDA-MB 231	Ishikawa	DU-145
12a	H		NA[b]	NA	NA	NA
12b	H		NA	NA	NA	NA
12c	H		NA	NA	NA	NA
12d	H		NA	NA	NA	NA
12e	H		NA	NA	NA	NA
12f	H		NA	NA	NA	NA
12g	H		NA	NA	NA	NA
12h	H		29.1	>50	34	
13a	3-OMe		ND[c]	ND	ND	ND
14a	4-OMe		ND	ND	ND	ND
Tamoxifen citrate			10.2	12.3	12.5	15.25

Synthesized benzoxazepines, **12a–h**, **13a**, **14a**.
[a] R^1 means alkyl derivatives of different amino acids.
[b] NA means not active and having IC_{50} >50 μM.
[c] ND means not determined.

acid derived benzoxazepines showed the compounds having benzene ring in side chain exhibiting better activity and the compound derived from tyrosine was most potent, Table 1. This observation influenced us to further derivatize the tyrosine-based benzoxazepine compound. In this context we made various amino alkyl chain derivatives **15a–d, 16a–f, 17a–d**, Scheme 2 (Table 2).

Structure–activity relationships. Introduction of methoxy substituent in the benzene ring of benzoxazepine series of compounds did not increase the activity in vitro. Among compounds without methoxy substituent, the chains containing ethylamino group exhibited better activity. Compound **15b** had the highest cell growth inhibitory effect in MCF-7 cells and more active than tamoxifen citrate appeared from the IC_{50} value (6.53 μM vs 10.2 μM). Overall, within this series, **15b** and **15c** appear to be more effective in inhibiting cell growth of breast cancer cells than other type of cancer cells (endometrial cancer-Ishikawa and pros-

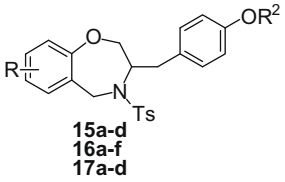


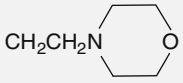
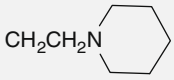
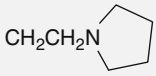
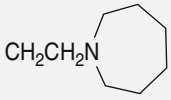
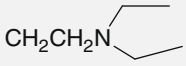
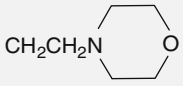
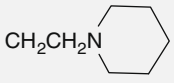
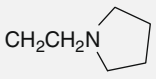
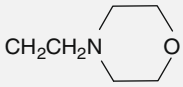
Scheme 2. Synthesis of amino ethyl alkyl chain derivatives with reagents and conditions: (a) anhyd K_2CO_3, R^2Cl, dry acetone, reflux, 5 h, 60–70%.

tate cancer DU-145, Table 1). Furthermore, **12h**, **15b** and **15c** showed time dependent inhibition of cell growth of MCF-7 and MDA-MB-231 cells at IC_{50} concentration (Fig. 1a and b).

Since **15b** and **15c** showed better cell growth inhibition of breast cancer cell line than tamoxifen citrate, we tested cytotoxicity in normal cells (HEK-293). Data show that **15b** had no cell

Table 2
The in vitro cell growth inhibitory effect of benzoxazepine series of compounds in various cell lines with MTT assay **15a–e**, **16a–f**, **17a–d**



Compound no.	R	R^2	Cell growth inhibition in terms of IC_{50} (μM)			
			MCF-7	MDA-MB 231	Ishikawa	DU-145
15a	H		ND[a]	ND	ND	ND
15b	H		6.53	17.45	22.6	23
15c	H		9.34	13.01	23.8	30
15d	H		NA[b]	NA	NA	NA
16a	3-OMe		NA	NA	NA	NA
16b	3-OMe		NA	NA	NA	NA
16c	3-OMe		NA	NA	NA	NA
16d	3-OMe		NA	NA	NA	NA
16e	3-OMe		NA	NA	NA	NA
16f	3-OMe		NA	NA	NA	NA
17a	4-OMe		NA	NA	NA	NA
17b	4-OMe		NA	NA	NA	NA
17c	4-OMe		NA	NA	NA	NA
17d	4-OMe		NA	NA	NA	NA
Tamoxifen citrate			10.2	12.3	12.5	15.25

Synthesized alkyl amine ethyl chain derivatives of benzoxazepines **15a–d**, **16a–f**, **17a–d**.

[a] ND means not determined.

[b] NA means not active and having IC_{50} >50 μM.

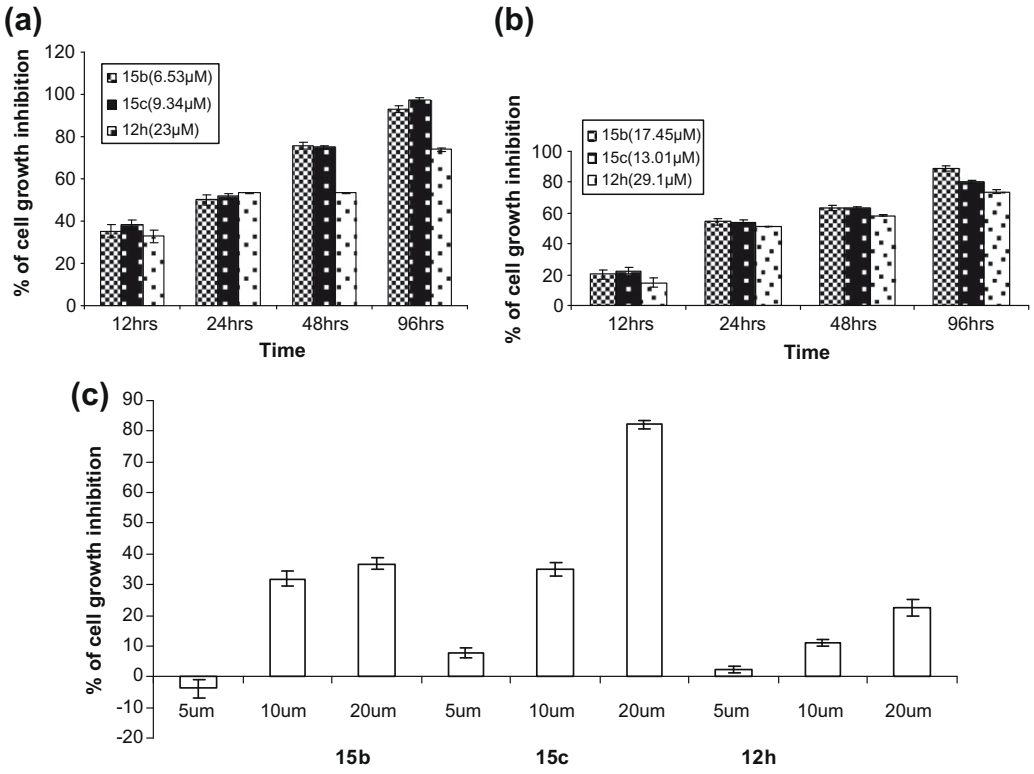


Figure 1. Percent cell growth inhibition of (a) MCF-7 cells, (b) MDA-MB-231 cells and (c) HEK-293 cells by various compounds.

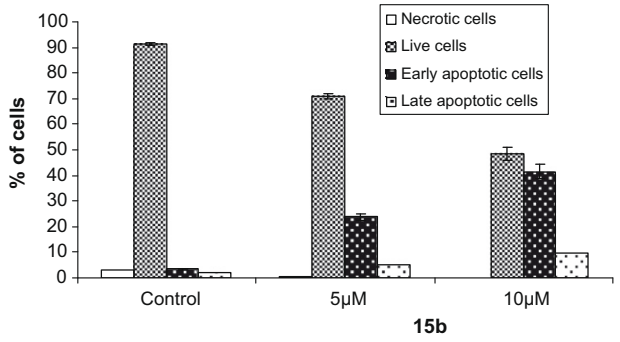


Figure 2. Negative cell population at 5 μM and 10 μM of **15b**; after 24 h, the cells were harvested, stained with Annexin-V and propidium iodide, and analyzed by flow cytometry. Data are expressed as% of total cell Count.

growth inhibitory effect in HEK-293 cells whereas, **15c** significantly inhibited cell growth (Fig. 1c). Because of its highest cytostatic effect in MCF-7 cells with no effect in normal cells, we took **15b** for subsequent experiments.

Flow cytometric evaluation of induction of apoptosis. Compound **15b** significantly (p >0.05) increased Annexin-V positive and PI negative cell population at 5 and 10 μM indicating induction of apoptosis compared with untreated control (Fig. 2).

Cell growth inhibition by benzoxazepine is due to G_0/G_1 arrest. Cell cycle analysis by PI staining showed that **15b** at 5 and 10 μM induced cell cycle arrest at G_0/G_1 phase suggesting that cell cycle arrest is responsible for cell growth inhibitory activity in MCF-7 cells (Fig. 3).

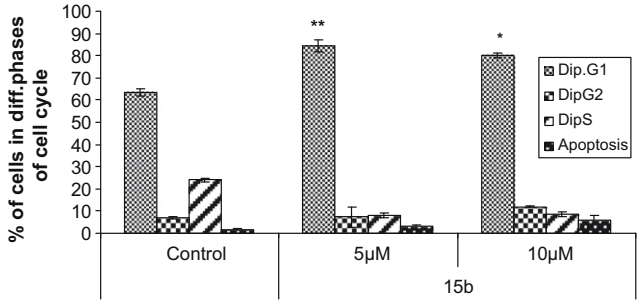


Figure 3. Apoptosis of MCF-7 by **15b** occurs due to G_0/G_1 arrest. MCF-7 cells were incubated in DMEM supplemented with 10% FBS and treated with 5 μM and 10 μM of **15b**. After 24 h, the cells were harvested, stained with PI, and analyzed by flow cytometry. Data are expressed as% of total cell count.

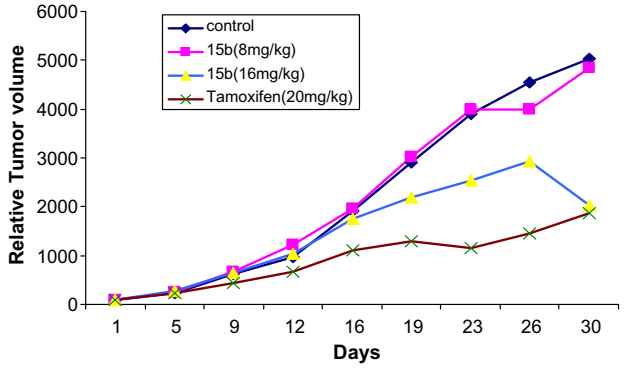


Figure 4. Regression in mean relative tumor volume in human breast cancer cell (MCF-7) xenograft model. Animals were treated with **15b** after tumor induction for 30 days. The data presented are average of relative tumor volume (n = 10 animals/group).

Anti-tumor effect in MCF-7 xenograft model. As shown in Figure 4, treatment of mice by **15b** (16 mg kg^{-1} dose) resulted in significantly higher relative tumor volume regression than the control group. At 30 days, **15b** (16 mg kg^{-1} dose) exhibited comparable tumor regression with that of tamoxifen citrate. Our data showed that out of 24 molecules, three (**12h, 15b**, and **15c**) exhibited significant cell growth inhibition when subjected to the MTT assay on a panel of three human cancer cell lines (IC_{50} ranging from 6.5 to 29.1 μM). However, only one compound **15b** showed significantly better IC_{50} values than tamoxifen citrate (p >0.05) in inhibiting cell growth of MCF-7 cells. Compound **15b** had no effect on the growth of HEK-293 cells, suggesting that this compound may be safe in normal cells. In flow cytometry analyses, **15b** was found to induce cell cycle arrest at G_0/G_1 phase. The in vitro ability to inhibit cell growth inhibition of MCF-7 cells by **15b** was confirmed in vivo in MCF-7 xenograft in nude mice model. Compound **15b** significantly regressed tumor volume that was comparable to that of tamoxifen citrate. Given its cytostatic effect in MCF-7 cells, in vivo anti-tumor activity in MCF-7 xenograft model, **15b** is a potential lead suitable for further improvement for developing as anti-cancer breast therapy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.115.

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