Bioorganic & Medicinal Chemistry Letters 23 (2013) 6816–6821

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Amino acids derived benzoxazepines: Design, synthesis and antitumor activity

Shailendra Kumar Dhar Dwivedi [a,*], Krishnananda Samanta [c], Manisha Yadav [b], Amit Kumar Jana [c], Abhishek Kumar Singh [b], Bandana Chakravarti [a], Sankalan Mondal [c], Rituraj Konwar [a], Arun Kumar Trivedi [b], Naibedya Chattopadhyay [a], Sabyasachi Sanyal [b], Gautam Panda [c,*]

[a] Division of Endocrinology, CSIR-Central Drug Research Institute, 10 Janakipuram Extension, Sitapur Road, Lucknow 226031, UP, India
[b] Division of Drug Target Discovery and Development, CSIR-Central Drug Research Institute, 10 Janakipuram Extension, Sitapur Road, Lucknow 226031, UP, India
[c] Medicinal and Process Chemistry Division, CSIR-Central Drug Research Institute, 10 Janakipuram Extension, Sitapur Road, Lucknow 226031, UP, India

ARTICLE INFO

Article history:
Received 23 April 2013
Revised 23 September 2013
Accepted 5 October 2013
Available online 11 October 2013

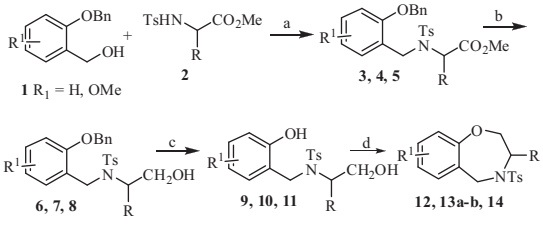
Keywords:
Amino acids
Benzoxazepine
Mitsunobu reaction
Anticancer activity
Capasase

ABSTRACT

Two series of new benzoxazepines substituted with different alkyl amino ethyl chains were synthesized comprising synthetic steps of inter and intramolecular Mitsunobu reaction, lithium aluminium hydride (LAH) reduction, debenzylation, bimolecular nucleophilic substitution (S_N2) reaction. The present study investigates the effect of a tyrosine-based benzoxazepine derivative in human breast cancer cells MCF-7 and MDA-MB-231 and in breast cancer animal model. The anti-proliferative effect of **15a** on MCF-7 cells was associated with G1 cell-cycle arrest. This G1 growth arrest was followed by apoptosis as **15a** dose dependently increased phosphatidylserine exposure, PARP cleavage and DNA fragmentation that are hallmarks of apoptotic cell death. Interestingly, **15a** activated components of both intrinsic and extrinsic pathways of apoptosis characterized by activation of caspase-8 and -9, mitochondrial membrane depolarization and increase in Bax/Bcl2 ratio. However, use of selective caspase inhibitors revealed that the caspase-8-dependent pathway is the major contributor to **15a**-induced apoptosis. Compound **15a** also significantly reduced the growth of MCF-7 xenograft tumors in athymic nude mice. Together, **15a** could serve as a base for the development of a new group of effective breast cancer therapeutics.

Breast cancer is the most frequently diagnosed life-threatening disease in women and more than 1 million women are diagnosed with breast cancer every year, accounting for 10% of all new cancers and 23% of all female cancer cases. In India the prevalence of this cancer is estimated around 2.5 million, with over 0.8 million new cases and 0.5 million deaths occurring each year.[1] Current available therapies suffer from several drawbacks including resistance and toxic side effects.[2,3] We have previously shown that the benzoxazepine derivatives are active against breast cancer and are non-toxic in normal cell lines tested[4] the present study is designed to investigate the effect of new compounds synthesized as lead optimization process.

Benzoxazepines are well-known pharmacophore in medicinal chemistry with promising activity against various diseases such as antipsychotic, central nervous system activity along with anticancer profile against breast cancer cells.[5] One of these groups of benzoxazepines has been identified to target microtubule assembly to induce anti-cancer activity.[5] In our previous study, we have synthesized and explored the amino acid based benzoxazepines[6a–d] with antitumor activity. Thus, in search of new lead molecule, we synthesized two different series of benzoxazepine (A, B) from amino acids. Easy availability of amino acid-based benzoxazepines promoted us to evaluate them for anticancer activity.

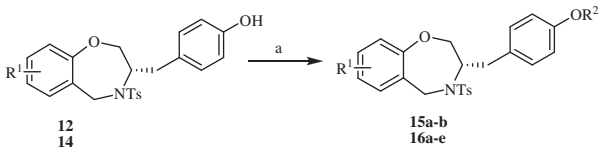


Scheme 1. Reagents and conditions: (a) **1**, **2**, DEAD, PPh_3, THF, 0 °C (2 h) to rt (10 h), N_2, 60–75%; (b) LAH, THF, 0 °C, 1 h, 59–70%; (c) H_2, 10% Pd/C, MeOH, rt, 2 h, 50 psi, 61–70%; (d) DEAD, PPh_3, THF, 0 °C (1 h) to rt, (14 h), N_2, 63–76%.

We followed the same methodology developed in our laboratory for the synthesis of benzoxazepines (Scheme 1). N-tosylated *S*-amino acid esters and substituted benzyl alcohol were used as building blocks for the construction of benzannulated chiral heterocycles. The intermolecular Mitsunobu reaction of *S*-amino acid

* Corresponding authors.
E-mail address: gautam.panda@gmail.com (G. Panda).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.10.013



Scheme 2. Reagents and conditions: (a) anhyd K_2CO_3, R^2Cl, dry acetone, reflux, 5 h, 60–70%.

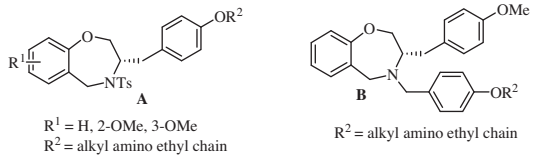
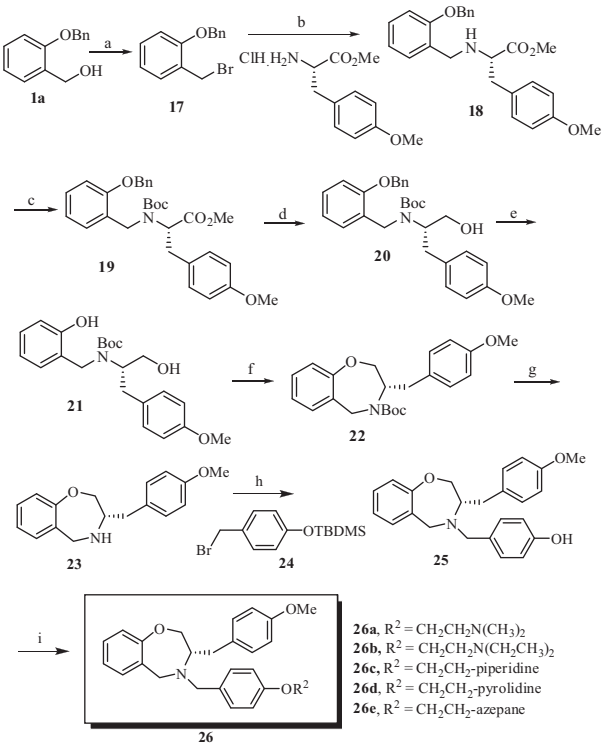


Figure 1. Two different types of benzoxazepine.

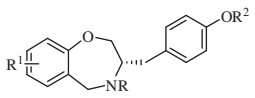


Scheme 3. Reagents and conditions: (a) PPh_3, CBr_4, DCM; (b) K_2CO_3, DMF, 2 h, 70% in two steps; (c) $NaHCO_3$, Boc_2O, EtOH, 74%; (d) $LiBH_4$, THF, 85%; (e) H_2, 10% Pd–C, 50 psi, 75%; (f) PPh_3, DEAD, 2 h, 75%; (g) 6 N HCl, MeOH, 70 °C, 2 h, 70%; (h) K_2CO_3, DMF, 74%; (i) R^2Cl, K_2CO_3, acetone, 70–80%.

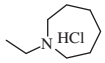
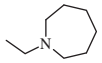
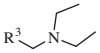
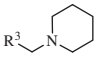
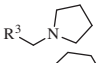
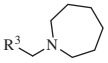
derivatives **2** with **1** provided the esters **3**, **4**, **5** in 60–75% yield. The Lithium aluminium hydride (LAH) reduction of **3**, **4**, **5** afforded the corresponding alcohols **6**, **7**, **8** which on subsequent debenzylation by H_2/Pd (10% on carbon) gave **9**, **10**, **11** containing free alcoholic and phenolic hydroxyl groups in 60–70% yield in two steps. Exposure of **9**, **10**, **11** to Mitsunobu reaction conditions[7], that is, diethylazodicarboxylate (DEAD), triphenylphosphine (TPP) at 0 °C in tetrahydrofuran as solvent resulted in the formation of desired enantiomerically pure benzoxazepine derivatives **12**, **13** and **14** in 63–76% yield.

The biological results of the *S*-amino acid derived benzoxazepines showed the compounds having benzene ring in side chain exhibiting better activity and the compound derived from tyrosine

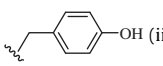
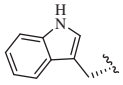
Table 1
IC_{50} (μM) value of benzoxazepine series of compounds in breast cancer cell lines using MTT assay after 24 h treatment



Benzoxazepine

Compd no.	R	R^1	R^2	MCF-7	MDA-MB-231
15a	Ts	H		6.78	7.2
15b	Ts	H		9.62	10.2
13a	Ts	3-OMe	R^4	Inactive	Inactive
13b	Ts	3-OMe	R^5	Inactive	Inactive
14	Ts	2-OMe	R^3	Inactive	Inactive
16a	Ts	2-OMe		Inactive	Inactive
16b	Ts	2-OMe		Inactive	Inactive
16c	Ts	2-OMe		Inactive	Inactive
16d	Ts	2-OMe		Inactive	Inactive
16e	Ts	2-OMe		Inactive	Inactive
22	Boc	H	Me	Inactive	Inactive
23	H	H	Me	Inactive	Inactive
25	R^3	H	Me	10.34	13.21
26a		H	Me	Inactive	Inactive
26b		H	Me	14.5	18.6
26c		H	Me	Inactive	Inactive
26d		H	Me	12.45	15.67
26e		H	Me	Inactive	Inactive
4OH-T				7.2	10.4

Results were calculated from three independent experiments with triplicate of each observation.
(i) Compound with IC50 more than 20 μM were considered inactive

R^3 =  (iii) R^4 =  (iv) $R^5 = CH_2CHMe_2$.

was most potent.[6d] This observation influenced us to further derivatize the tyrosine-based benzoxazepine compound. In this context we made various amino alkyl chain derivatives **15a–b**, **16a–e** (see Scheme 2).

Table 2
IC_{50} value (μM) of compounds in MCF-7 and MDA-MB-231 cells

Treatment	Time (h)	MCF-7	MDA-MB-231
15a	24	6.78	7.2
	48	3.55	5.45
	72	1.83	4.6
15b	24	9.62	10.2
	48	4.31	5.12
	72	3.23	4.2