Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 1499–1505
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Syntheses and cytotoxicities of the analogues of the taxoid brevifoliol☆

Sunil K. Chattopadhyay*, Sarita Tripathi, Mahendra P. Darokar, Uzma Faridi, Brijesh Sisodia, Shubhra Negi, J. Kotesh Kumar, Suman P.S. Khanuja

Central Institute of Medicinal and Aromatic Plants (CIMAP), P.O. CIMAP, Lucknow-226015, India

Received 10 May 2007; received in revised form 8 August 2007; accepted 6 September 2007
Available online 15 September 2007

Abstract

Seven novel brevifoliol analogues have been synthesized by coupling brevifoliol and 2-monosubstituted-4-phenyl-1,3-oxazolidine carboxylic acid after removal of the protecting group with acid treatment. Brevifoliol and its synthesized analogues were tested for their cytotoxic activities against four different human cancer cell lines, oral (KB), breast (MCF-7), colon (CaCO2) and liver (HepG-2) as determined by MTT assay. The C-13 oxidized brevifoliol retained significant activity. Out of the seven analogues synthesized, C-13 oxidized brevifoliol-5-[*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine] analogue was interesting as it exhibited selective and potent cytotoxicity against liver cancer cell line predominantly.

Keywords: Brevifoliol analogues; Synthesis; Cytotoxicity; Selectivity

1. Introduction

Brevifoliol was originally isolated from the leaves of *Taxus brevifolia* [1]. Its correct structure was established by Georg et al. and Appendino et al. [2,3] and was assigned a 11(15 → 1)-abeo-taxa-4(20),11-diene skeleton. We have also isolated brevifoliol from the leaves of the Himalayan yew *Taxus wallichiana* with a yield of 0.012% and it was found to be the most abundant taxoid in the leaves of the plant [4]. In the process, we have also been able to isolate another naturally occurring analogue of brevifoliol, 2-acetoxy brevifoliol with a yield of 0.002% [4]. Brevifoliol was found to have significant cytotoxic activity against colon (CaCO2), oral (KB), breast (MCF-7) and liver (HepG-2) cancer cell lines [5].

In a previous publication, Georg et al. have prepared only one brevifoliol derivative 13-[*N*-benzoyl-(2′*R*,3′*S*)-3′-phenyl isoserinate] and it was tested for its activity in microtubule assembly and cytotoxicity assays [6]. In both the assays, the analogue showed little activity.

In a later publication, Tremblay et al. have prepared and characterized several acetyl, 2,2,2-trichloroethyl chloroformate (Troc), and bis-triethyl-silyl (Tes) derivatives of brevifoliol [7].

They have also described the preparation of brevifoliol derivatives with a 3-phenyl lactate at C-5 and C-13 positions of brevifoliol and also the same derivative brevifoliol-13-[*N*-benzoyl-(2′*R*,3′*S*)-3′-phenyl isoserinate] synthesized by Georg et al. Different derivatives of 5-acetyl brevifoliol were also synthesized via esterification with cinnamic acid, with both *S*-(−) and *R*-(+)-3-phenyllactic acid and with a [*N*-benzoyl-(2′*R*,3′*S*)-3′-phenyl isoserine at C-13.

The significant cytotoxic activity of brevifoliol [5] has prompted us to synthesize analogues and derivatives of the molecule which are not yet synthesized by others. The rationale behind synthesizing the analogues is based on making the cytotoxicity of brevifoliol selective towards certain tumors and increasing its cytotoxicity. As docetaxel (taxotere) is approximately two times more cytotoxic than paclitaxel (taxol) [8], we have decided to synthesize the analogues of brevifoliol

☆ CIMAP communication No.: 2007-51J.

* Corresponding author. Tel.: +91 522 2359628; fax: +91 522 2342666.
E-mail address: chattsk@yahoo.com (S.K. Chattopadhyay).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.09.002

and its closely related natural analogue 2-acetoxy brevifoliol in which the hydroxyl groups will be selectively esterified with the side chain [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine]. These types of analogues have not yet been synthesized.

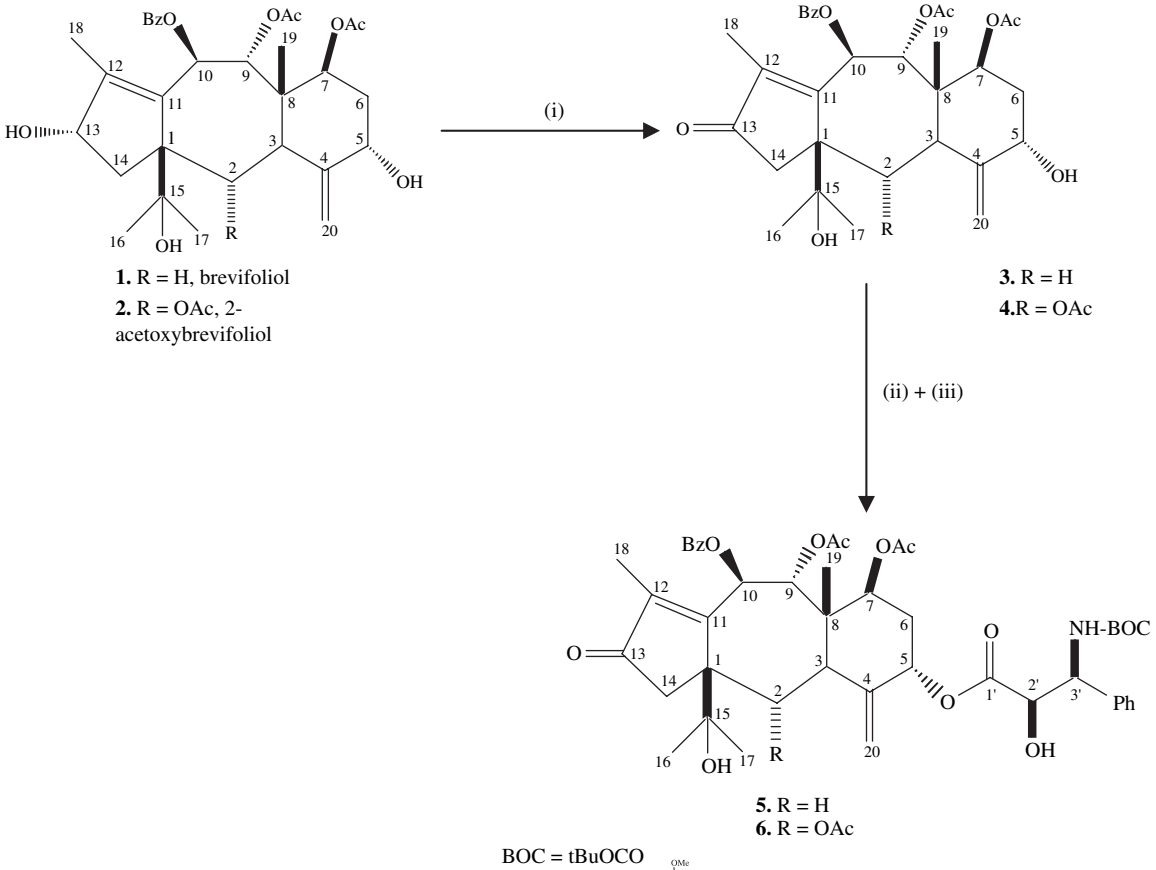
In this communication, we would like to report the selective oxidation of C-13 allylic hydroxyl groups of brevifoliol **1** and 2-acetoxy brevifoliol **2** and esterification of the remaining hydroxyl group in the oxidized products of both the molecules with the docetaxel side chain [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine]. Moreover, brevifoliol itself was converted into three different analogues in which its C-5, C-13 and both C-5 and C-13 hydroxyls were esterified with the side chain [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine]. The cytotoxic activity of all the new molecules were evaluated and included in this publication.

2. Results and discussion

2.1. Chemistry

We began our synthesis with the selective oxidation of brevifoliol **1** (Scheme 1). Brevifoliol has three types of hydroxyl groups-allylic at C-13, secondary at C-5 and tertiary at C-15. Reaction of brevifoliol with MnO_2 was tried as MnO_2 was used by Chordia and Kingston to oxidize the C-13 hydroxyl group of 10-deacetyl baccatin-III [9]. We found that the reaction of brevifoliol with MnO_2 in acetone at room temperature selectively oxidized the C-13 allylic hydroxyl group to produce the corresponding oxidized brevifoliol **3** cleanly. As compared to brevifoliol, the ^{1}H NMR of **3** was broad at room temperature. However, the spectra were well resolved at low temperature (−20 °C) and all the signals were assigned.

In the low temperature (−20 °C) ^{1}H NMR spectrum of oxidized brevifoliol **3**, the chemical shift of the H-10 underwent a downfield shift and appeared at δ 6.81 as compared to its chemical shift at δ 6.41 which indicated that the oxidation of C-13 hydroxyl group of brevifoliol has taken place. ^{13}C NMR spectrum of the compound **3** also supported the presence of an enone type carbonyl (δ 207.7). Moreover, in the HMBC spectrum of **3**, the C-18 methyl signal at δ 2.1 was correlated to the carbonyl signal at δ 207.7 thus confirming the oxidation of C-13 hydroxyl of brevifoliol. Direct coupling of the oxidized brevifoliol with 2-monosubstituted-4-phenyl-1,3-oxazolidine carboxylic acid [10] in presence of dicyclohexylcarbodiimide (DCC), and dimethyl aminopyridine (DMAP) in toluene gave the condensed product at room temperature. Acid mediated cleavage of the oxazolidine ring of the condensed product in methanol afforded the desired product, oxidized brevifoliol-5-[*N*-*tert*-butoxycarbonyl



Scheme 1. Reagents and conditions: (i) MnO_2, acetone (5 ml), 25 °C, 30 h, 80%; (ii) DCC (3 equiv), DMAP (0.2 equiv), toluene (3 ml), 25 °C, 1 h, 70%; (iii) *p*-TSA (1.1 equiv), MeOH (5 ml), 25 °C, 2 h, 51%.

amino-(2′*R*,3′*S*)-3′-phenyl isoserine] **5** in 51% isolated yield. Again, the NMR spectra of **5** were broad at room temperature (23 °C) which was well resolved at low temperature (−20 °C). Oxidation of 2-acetoxy brevifoliol **2** with MnO_2 at room temperature gave **4**. The diagnostic feature in the ^{1}H NMR of **4** was the small coupling constant (3.2 Hz) between H-9 and H-10 which suggested a chair/boat conformation for the B/C ring [11]. Search in literature revealed that this molecule has been isolated as a natural taxoid, taxuspinane B from the stems of the plant *Taxus cuspidata* by Morita et al. [11]. 2-Acetoxy brevifoliol **2** was converted into **6** following the sequence of reactions as described in Scheme 1. Georg et al. has reported that condensation of brevifoliol with β-lactam (precursor of the taxol side chain) gave exclusively brevifoliol-13-[*N*-benzoyl-(2′*R*,3′*S*)-3′-phenylisoserinate] [6]. Reaction of brevifoliol itself with 2-monosubstituted-4-phenyl-1,3-oxazolidine carboxylic acid [10] in presence of dicyclohexylcarbodiimide (DCC), and dimethyl aminopyridine (DMAP) in toluene gave three condensed products as expected. Cleavage of the oxazolidine ring of the condensed products with acid gave three products **7**, **8** and **9** in a ratio of 6:3:1 in which 5, 13 and both 5 and 13 hydroxyl groups were esterified with *N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine. As compared to the ^{1}H NMR spectrum of the oxidized brevifoliol **3**, line broadening in the NMR spectra of **7** was moderate and full assignment of the ^{1}H and ^{13}C NMR spectra by 1D and 2D techniques (COSY, HSQC and HMBC) could be achieved at room temperature.

In HMBC spectrum of **7**, the signal at δ 5.46 (s, H-5) was correlated to the carbonyl signal at δ 170.8 which indicated that the C-5 hydroxyl group has been esterified with the side chain [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine]. The other two synthesized analogues **8** and **9** of brevifoliol displayed spectral data which were in agreement with their assigned structures.

2.2. Cytotoxic activities

The brevifoliol analogues thus obtained were tested for their cytotoxic activity against four different human cancer cell lines, oral (KB), breast (MCF-7), colon (CaCO2) and liver (HepG-2) as determined by MTT assay [12,13] (Table 1). From Table 1, it appears that although C-13 oxidized brevifoliol **3** was less active than brevifoliol, it still retained significant cytotoxic activity against the above cancer cell line except HepG-2 line. However, in case of C-13 oxidized 2-acetoxy brevifoliol **4**, it became less cytotoxic as compared to its precursor 2-acetoxy brevifoliol **2**. Analogue **5** was interesting as it exhibited selective and potent cytotoxicity against liver cancer cell line predominantly. Similar selectivity was observed with analogue **6** against oral cancer cell line with more cytotoxicity. The three other analogues **7**, **8**, and **9** synthesized from brevifoliol exhibited less cytotoxic activity as compared to brevifoliol **1** as shown in Table 1. The only brevifoliol analogue, 13-[*N*-benzoyl-(2′*R*,3′*S*)-3′-phenyl isoserinate] synthesized by Georg et al. showed cytotoxicity with a ED_{50} value which was greater than 10 μM (=8.23 μg/ml; highest concentration tested) as compared to 27 nM for paclitaxel [6]. However, in our case, out of the three brevifoliol analogues **7**, **8** and **9**, compound **9**, the diesterified analogue of brevifoliol exhibited selective and significant cytotoxicity against oral cancer cell line (KB) (IC_{50} and IC_{90} are 2.5 and 30 μg/ml).

Thus, from the result of cytotoxicity data for brevifoliol analogues **7**, **8** and **9** synthesized by us, we can say that the replacement of the side chain of the paclitaxel with that of docetaxel did not lead to the enhancement in cytotoxicity except in case of compound **9** when compared with the data reported by Georg et al. [6].

Thus, we have prepared and characterized C-13 oxidized brevifoliol, C-13 oxidized 2-acetoxy brevifoliol and their condensed products with [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine]. Also, C-5, C-13 and C-5 and 13 analogues of brevifoliol esterified with [*N*-*tert*-butoxycarbonyl amino-(2′*R*,3′*S*)-3′-phenyl isoserine] have been prepared. To our knowledge, the above analogues of brevifoliol have been prepared for the first time and some of them, e.g. **5** and **6**, possessed selective and significant cytotoxicity against liver and oral cancer cell lines, respectively. The selective and significant cytotoxicity of the oxidized brevifoliol **5** and **6** may be

Table 1
Cytotoxicity of brevifoliol and its analogues against human cancer cell lines by MTT assay (values in μg/ml)[a]

Compound	KB		MCF-7		CaCO2		HepG2	
	IC_{50} ± s.d.	IC_{90} ± s.d.	IC_{50} ± s.d.	IC_{90} ± s.d.	IC_{50} ± s.d.	IC_{90} ± s.d.	IC_{50} ± s.d.	IC_{90} ± s.d.
1	0.0031 ± 0.00047	0.6 ± 0.071	0.86 ± 0.023	6.16 ± 0.471	0.0025 ± 0.00041	1.32 ± 0.259	0.055 ± 0.0071	1.13 ± 0.262
2	0.316 ± 0.0471	7.5 ± 0.816	58.3 ± 4.71	91.6 ± 2.35	6.16 ± 1.027	21 ± 2.94	96.6 ± 2.35	100 ± 4.08
3	0.0078 ± 0.00023	1.13 ± 0.262	5.33 ± 0.471	22.6 ± 2.054	0.008 ± 0.0016	21 ± 2.94	100 ± 4.08	100 ± 4.08
4	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	96.6 ± 2.35	100 ± 4.08
5	100 ± 4.08	100 ± 4.08	8.5 ± 1.08	43.33 ± 4.71	100 ± 4.08	100 ± 4.08	0.065 ± 0.016	5.33 ± 0.85
6	0.105 ± 0.075	5.33 ± 0.471	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08
7	100 ± 4.08	100 ± 4.08	33.3 ± 2.35	55 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08
8	100 ± 4.08	100 ± 4.08	48.3 ± 4.71	68.3 ± 4.71	100 ± 4.08	100 ± 4.08	78.33 ± 4.71	91.6 ± 4.71
9	2.73 ± 0.21	33.3 ± 2.35	100 ± 4.08	100 ± 4.08	100 ± 4.08	100 ± 4.08	96.6 ± 2.35	100 ± 4.08
Paclitaxel	0.0013 ± 0.0004	0.048 ± 0.0012	0.008 ± 0.0016	0.86 ± 0.023	0.0078 ± 0.00023	0.065 ± 0.016	0.008 ± 0.0016	1.13 ± 0.262

s.d. is the standard deviation of three replicates.
[a] Highest concentration tested for each compound was 100 μg/ml.

due to the conformational changes in the taxane rings which helps in binding to the target of a particular cell (liver and oral cancer cell lines).

3. Experimental protocols

IR spectra, KBr pellets, 600–4000 cm^{-1}, were recorded on PerkinElmer FTIR BX Spectrophotometer. ^1H NMR and ^{13}C NMR were recorded on a Bruker AVANCE 300 instrument at 300 MHz (chemical shifts are expressed as δ in parts per million relative to TMS as internal standard. The following abbreviations are used: singlet (s), broad singlet (br s), doublet (d), broad doublet (br d), double doublet (dd), distorted doublet (dist.d), triplet (t), broad triplet (br t), distorted triplet (dt), multiplet (m). Mass spectra (electro spray ionization in positive mode, ESI+) were recorded on an API 3000 (Applied Biosystem) mass spectrometer; chromatographic purifications were performed by silica gel 60–120 mesh (Loba Chemie) column chromatography. All the synthesized compounds gave satisfactory elemental analysis (carbon, hydrogen).

3.1. Synthesis

3.1.1. General procedure for synthesis of 3 and 4

To a solution of compound **1** (0.36 mmol) in acetone (5 ml) was added MnO_2 (1 g) and the mixture was stirred at 25 °C for 40 h. The reaction was filtered over a bed of celite and the organic phase was concentrated at reduced pressure. The residue thus obtained could not be induced to crystallization but solidified from hexane:acetone mixture to give compound **3**.

Similar procedure was repeated with compound **2** to get compound **4**.

3.1.1.1. Compound ***3***. Yield 80%, amorphous solid. Mass (ESI+) $[M+Na]^+$: *m/z* 577. IR (KBr): 3449, 2975, 2934, 1722, 1373 cm^{-1}. ^1H NMR ($CDCl_3$ at −20 °C): 2.6 (2H, m, 2-H), 2.63 (1H, m, 3-H), 4.33 (1H, br s, 5-H), 1.25 (1H, m, 6α-H), 2.17 (1H, m, 6β-H), 5.60 (1H, dd, $J = 5.0$ and 10.5 Hz, 7-H), 6.20 (1H, d, $J = 10.8$ Hz, 9-H), 6.81 (1H, d, $J = 10.8$ Hz, 10-H), 2.01 (1H, d, $J = 18.1$ Hz, 14 β-H), 2.55 (1H, d, $J = 18.1$ Hz, 14α-H), 0.96 (3H, s, 16-H), 0.88 (3H, s, 17-H), 1.37 (3H, s, 18-H), 1.22 (3H, s, 19-H), 4.78 (1H, br s, 20α-H), 5.14 (1H, br s, 20β-H), 1.75, 2.02 ($2 \times 3H$, s, $OCOCH_3$), 7.84 (2H, d, $J = 7.5$ Hz, *o*-Bz), 7.46 (2H, t, $J = 7.5$ Hz, *m*-Bz), 7.56 (1H, t, $J = 7.5$ Hz, *p*-Bz). ^{13}C NMR ($CDCl_3$ at −20 °C): 59.0 (C-1), 27.8 (C-2), 37.5 (C-3), 147.1 (C-4), 68.6 (C-5), 36.1 (C-6), 69.9 (C-7), 45.9 (C-8), 77.0 (C-9), 70.3 (C-10), 163.9 (C-11), 147.1 (C-12), 207.7 (C-13), 50.1 (C-14), 76.1 (C-15), 9.2 (C-16), 12.9 (C-17), 14.3 (C-18), 27.0 (C-19), 111.7 (C-20), 20.9, 21.6 ($2 \times CH_3$, $OCOCH_3$), 170.2, 170.3 ($2 \times CO$, $OCOCH_3$), 163.9 (CO–Bz), 128.8 (*o*-Bz), 129.9 (*m*-Bz), 133.8 (*p*-Bz), 129.6 (C-1 Bz).

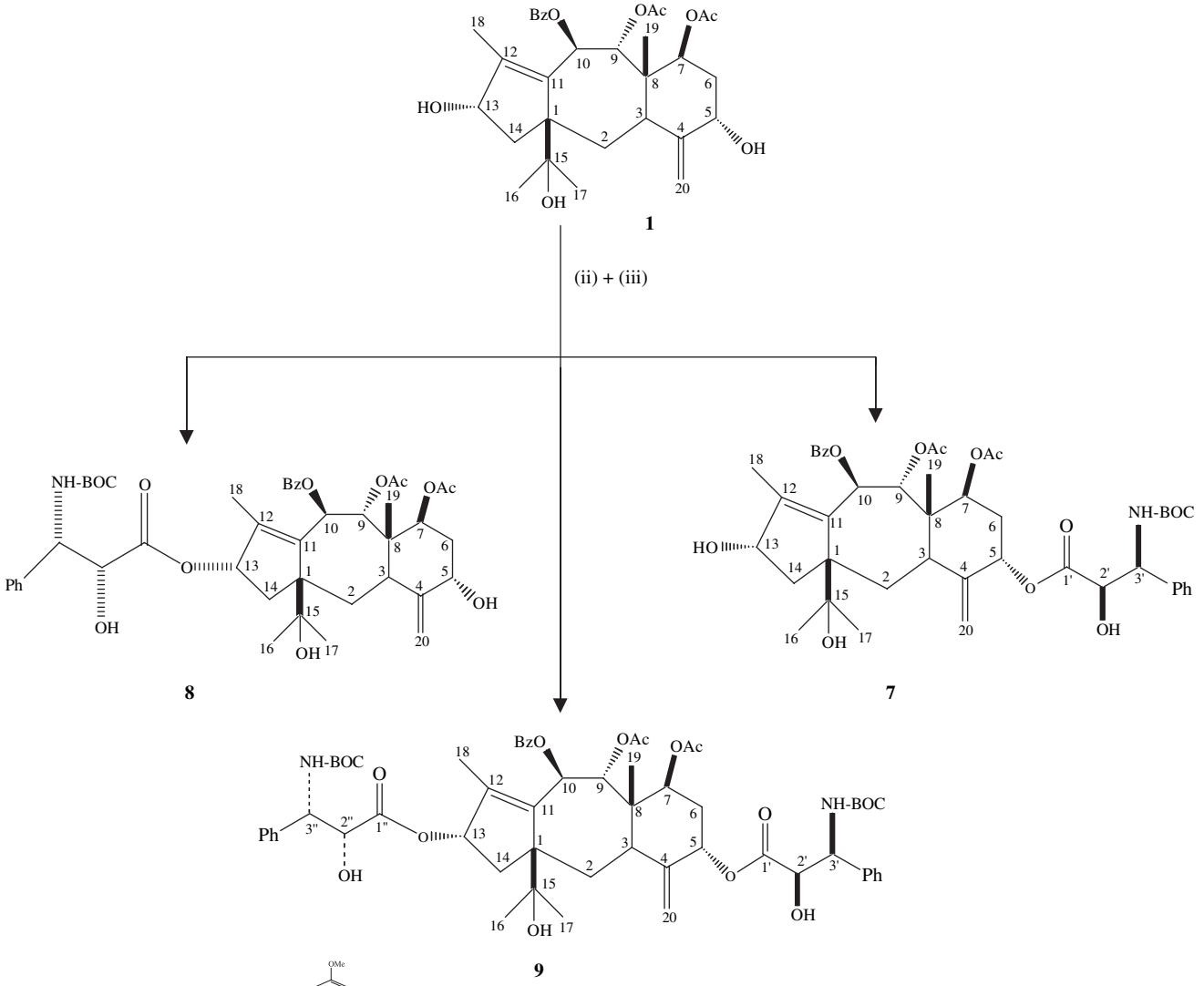
3.1.1.2. Compound ***4***. Yield 70%, amorphous solid. Mass (ESI+) $[M+Na]^+$: *m/z* 635. IR (KBr): 3451, 2933, 1717, 1372, 1244 cm^{-1}. ^1H NMR ($CDCl_3$ at 23 °C): 6.07 (1H, d, $J = 9.2$ Hz, 2-H), 2.77 (1H, d, $J = 9.2$ Hz, 3-H), 4.75 (1H, br t, 5-H), 1.18 (1H, m, 6α-H), 1.88 (1H, m, 6β-H), 4.84 (1H, t, $J = 8.8$ Hz, 7-H), 5.12 (1H, d, $J = 3.2$ Hz, 9-H), 6.32 (1H, d, $J = 3.2$ Hz, 10-H), 2.47 (1H, d, $J = 18.6$ Hz, 14 β-H), 2.77 (1H, d, $J = 18.6$ Hz, 14α-H), 1.15 (3H, s, 16-H), 0.82 (3H, s, 17-H), 2.00 (3H, s, 18-H), 1.84 (3H, s, 19-H), 4.79 (1H, br s, 20α-H), 5.49 (1H, s, 20β-H), 1.96, 1.97, 1.98 ($3 \times 3H$, s, $3 \times OCOCH_3$), 8.01 (2H, d, $J = 7.4$ Hz, *o*-Bz), 7.48 (2H, t, $J = 7.4$ Hz, *m*-Bz), 7.60 (1H, t, $J = 7.4$ Hz, *p*-Bz). ^{13}C NMR ($CDCl_3$ at 23 °C): 59.1 (C-1), 69.9 (C-2), 45.9 (C-3), 147.1 (C-4), 68.6 (C-5), 36.1 (C-6), 70.5 (C-7), 44.8 (C-8), 75.7 (C-9), 70.2 (C-10), 148.4 (C-11), 161.2 (C-12), 207.6 (C-13), 45.9 (C-14), 76.0 (C-15), 28.0 (C-16), 27.9 (C-17), 9.14 (C-18), 14.2 (C-19), 111.7 (C-20), 21.6, 20.9, 20.8 ($3 \times CH_3$, $OCOCH_3$), 170.2, 170.1, 169.7 ($3 \times CO$, $OCOCH_3$), 167.9 (CO–Bz), 133.80, 133.7 (*o*-Bz), 130.1, 129.9 (*m*-Bz), 129.1 (*p*-Bz), 129.7 (C-1 Bz).

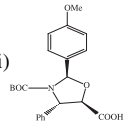
3.1.2. General procedure for synthesis of 5–9

The 2-monosubstituted-4-phenyl-1,3-oxazolidine carboxylic acid as mentioned in Schemes 1 and 2 was prepared by following a literature procedure [9]. A mixture of oxazolidine carboxylic acid (1 mmol), dicyclohexyldicarbodiimide (DCC, 1.01 mmol), compound **3** (0.33 mmol) and 4-dimethyl aminopyridine (DMAP, 0.1 mmol) in dry toluene (3 ml) were stirred for 2 h. The reaction mixture was concentrated and filtered with benzene (3×5 ml). The residue thus obtained was purified by column chromatography using a mixture of EtOAc:hexane (25:75) to obtain the condensed product. It was dissolved in MeOH (5 ml) and treated with *p*-TSA (1.1 equiv) at 25 °C for 1 h. The reaction mixture was concentrated and purified by preparative TLC (20×20 cm, mobile phase C_6H_6:Me_2CO (3:1)) to yield compound **5**.

Similar procedure was repeated with compound **4** to get compound **6** and with compound **1** to get three compounds **7**, **8** and **9**.

3.1.2.1. Compound ***5***. Yield 70%, amorphous solid. Mass (ESI+) $[M+H]^+$: *m/z* 840. IR (KBr): 3446, 2977, 1716, 1498, 1370, 1255 cm^{-1}. ^1H NMR ($CDCl_3$ at −20 °C): 2.65 (1H, m, 2α-H), 1.75 (1H, m, 2β-H), 2.70 (1H, br s, 3-H), 5.48 (1H, br s, 5-H), 2.20 (1H, m, 6α-H), 1.35 (1H, m, 6β-H), 5.55 (1H, t, $J = 9.3$ Hz, 7-H), 6.25 (1H, d, $J = 10.8$ Hz, 9-H), 6.91 (1H, d, $J = 10.8$ Hz, 10-H), 1.25 (1H, d, $J = 9.0$ Hz, 14α-H), 2.43 (1H, d, $J = 9.0$ Hz, 14β-H), 0.93 (3H, s, 16-H), 1.36 (3H, s, 17-H), 2.14 (3H, s, 18-H), 1.25 (3H, s, 19-H), 4.95 (1H, br s, 20α-H), 5.34 (1H, br s, 20β-H), 1.76, 2.08 ($2 \times 3H$, s, $2 \times OCOCH_3$), 4.26 (1H, br s, 2′-H), 5.12 (1H, d, $J = 10$ Hz, 3′-H), 1.39 (9H, s, *t*-Bu), 7.87 (2H, d, $J = 7.5$ Hz, *o*-Bz), 7.55 (1H, t, $J = 7.5$ Hz, *p*-Bz), 7.46 (2H, t, $J = 7.5$ Hz, *m*-Bz), 7.3–7.4 (5H, m, Ph). ^{13}C NMR ($CDCl_3$ at −20 °C): 59 (C-1), 28.07 (C-2), 30.0 (C-3), 139.3 (C-4), 70.01 (C-5), 33.8 (C-6), 74.19 (C-7), 45.5 (C-8), 76.2 (C-9), 71.0 (C-10), 163.7 (C-11), 145.2 (C-12), 207.7 (C-13), 50.13 (C-14), 75.5 (C-15), 9.10, 13.10, 27.0, 26.4 ($4 \times CH_3$), 21.5, 21.0 ($2 \times CH_3$, $OCOCH_3$), 115.0 (C-20), 169.8, 170.2 ($2 \times CO$,



Scheme 2. Reagents and conditions: (i)  DCC (3 equiv), DMAP (0.2 equiv), toluene (3 ml), 25 °C, 1 h, 70%; (ii) *p*-TSA (1.1 equiv), MeOH (5 ml), 25 °C, 2 h, 51%.

$OCOCH_3$), 171.6 (C-1′, −CO−), 164.5 (CO of Bz), 155.6 (CO of carbamate), 74.3 (C-2′), 57.2 (C-3′), 28.6 (CH_3, *t*-Bu), 80.13 (C−*t*-Bu), 129.0 (C-1 Bz), 129.2 (C-2,6 Bz), 127.2 (C-3,5 Bz), 133.8 (C-4 Bz), 128.0 (*o*-Ph), 129.1 (*m*-Ph), 129.0 (*p*-Ph), 139.3 (C-1 Ph).

3.1.2.2. Compound ***6***. Yield 60%, amorphous solid. Mass (ESI+) $[M + Na]^+$: *m/z* 898. IR (KBr): 3449, 2970, 1742, 1718, 1498, 1370, 1240 cm^{-1}. ^1H NMR ($CDCl_3$, 23 °C): 6.08 (1H, d, $J = 9$ Hz, 2-H), 2.82 (1H, d, $J = 9$ Hz, 3-H), 5.29 (1H, br s, 5-H), 2.10 (1H, m, 6α-H), 1.81 (1H, s, 6β-H), 5.68 (1H, dt, 7-H), 5.12 (1H, d, $J = 3.3$ Hz, 9-H), 6.22 (1H, d, $J = 3.3$ Hz, 10-H), 2.45 (1H, d, $J = 18.6$ Hz, 14α-H), 2.75 (1H, d, $J = 18.6$ Hz, 14β-H), 1.07 (3H, s, 16-H), 1.25 (3H, s, 17-H), 1.40 (3H, s, 18-H), 0.81 (3H, s, 19-H), 5.30 (1H, br s, 20β-H), 4.85 (1H, br s, 20α-H), 1.88, 1.95, 2.00 (3 × 3H, s, $OCOCH_3$), 4.45 (1H, br s, 2′-H), 5.30 (1H, br s, 3′-H), 1.30 (9H, s, *t*-Bu), 7.87 (2H, d, $J = 7.8$ Hz, *o*-Bz), 7.61 (1H, dt, *p*-Bz), 7.49 (2H, dt, *m*-Bz), 7.38−7.27 (5H, m, Ph). ^{13}C NMR ($CDCl_3$, 23 °C): 68.6 (C-1), 69.8 (C-2), 44.7 (C-3), 148.4 (C-4), 75.9 (C-5), 42.2 (C-6), 73.9 (C-7), 44.6 (C-8), 76.9 (C-9), 70.8 (C-10), 148.0 (C-11), 161.5 (C-12), 207.2 (C-13), 44.65 (C-14), 75.9 (C-15), 29.3 (C-16), 28.1 (C-17), 9.6 (C-18), 14.2 (C-19), 115.7 (C-20), 21.8, 21.2, 20.9 (3 × CH_3, $OCOCH_3$), 169.7 (3 × CO, $OCOCH_3$), 171.7 (C-1′, −CO−), 165.5 (CO of Bz), 155.5 (CO of carbamate), 72.8 (C-2′), 58.2 (C-3′), 28.2 (CH_3, *t*-Bu), 80.3 (C−*t*-Bu), 129.0 (C-1 Bz), 129.2 (C-2,6 Bz), 127.2 (C-3,5 Bz), 131.1 (C-4 Bz), 128.1 (*o*-Ph), 129.1 (*m*-Ph), 129.0 (*p*-Ph), 133.9 (C-1 Ph).

3.1.2.3. Compound ***7***. Yield 60%, amorphous solid. Mass (ESI+) $[M + Na]^+$: *m/z* 842. IR (KBr): 3427, 2977, 2933, 1738, 1370, 1260 cm^{-1}. ^1H NMR ($CDCl_3$): 1.40 (1H, m, 2α-H), 2.4 (1H, m, 2β-H), 2.75 (1H, d, $J = 8.4$ Hz, 3-H), 5.46 (1H, br s, 5-H), 2.1 (1H, m, 6α-H), 1.21 (1H, m, 6β-H), 5.53

(1H, dt, 7-H), 6.0 (1H, d, $J = 10.5$ Hz, 9-H), 6.60 (1H, d, $J = 10.5$ Hz, 10-H), 4.47 (1H, br t, 13-H), 1.25 (1H, m, 14α-H), 2.42 (1H, m, 14β-H), 0.98 (3H, s, 16-H), 1.30 (3H, s, 17-H), 2.13 (3H, s, 18-H), 0.88 (3H, s, 19-H), 5.27 (1H, br s, 20α-H), 4.89 (1H, br s, 20β-H), 1.73, 2.03 (2×3H, s, $2 \times OCOCH_3$), 4.45 (1H, br s, 2′-H), 5.20 (1H, br s, 3′-H), 1.30 (9H, s, *t*-Bu), 7.84 (2H, d, $J = 7.3$ Hz, *o*-Bz), 7.52 (1H, t, $J = 7.3$ Hz, *p*-Bz), 7.40 (2H, t, $J = 7.4$ Hz, *m*-Bz), 7.2–7.3 (5H, m, Ph). ^{13}C NMR ($CDCl_3$): 63.7 (C-1), 29.6 (C-2), 39.6 (C-3), 151.4 (C-4), 75.8 (C-5), 34.2 (C-6), 70.2 (C-7), 45.2 (C-8), 77.5 (C-9), 71.2 (C-10), 139.7 (C-11), 145.6 (C-12), 77.6 (C-13), 47.5 (C-14), 75.3 (C-15), 13.3 (C-18), 12.1 (C-19), 114.6 (C-20), 21.6, 21.0 ($2 \times CH_3$, $OCOCH_3$), 170.2 ($2 \times CO$, $OCOCH_3$), 170.8 (C-1′, –CO–), 75.3 (C-2′), 60.7 (C-3′), 155.99 (CO of carbamate), 28.7 (CH_3, *t*-Bu), 80.2 (C–*t*-Bu), 164.5 (CO of Bz), 129.7 (C-1 Bz), 129.8 (C-2,6 Bz), 127.3 (C-3,5 Bz), 133.6 (C-4 Bz), 127.9 (*o*-Ph), 128.6 (*m*-Ph), 129.1 (*p*-Ph), 139.65 (C-1 Ph).

3.1.2.4. Compound ***8***. Yield 30%, amorphous solid. Mass (ESI+) $[M + Na]^+$: *m/z* 842. IR (KBr): 3445, 2977, 2935, 1739, 1703, 1502, 1370, 1260 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.44 (br d, 1H, 2α-H), 2.44 (dd, $J = 5$ and 14 Hz, 1H, 2β-H), 3.02 (d, $J = 9.0$ Hz, 1H, 3-H), 4.32 (br s, 1H, 5-H), 2.03 (m, 1H, 6α-H), 1.76 (m, 1H, 6β-H), 5.73 (dd, $J = 11.0$ and 5.2 Hz, 1H, 7-H), 5.96 (d, $J = 10.4$ Hz, 1H, 9-H), 6.73 (d, $J = 10.4$ Hz, 1H, 10-H), 5.54 (dt, 1H, 13-H), 2.49 (m, 1H, 14β-H), 1.22 (m, 1H, 14α-H), 1.06 (s, 3H, 16-H), 1.31 (s, 3H, 17-H), 2.16 (s, 3H, 18-H), 0.87 (s, 3H, 19-H), 5.07 (br s, 1H, 20α-H), 4.67 (br s, 1H, 20β-H), 1.76, 2.06 (2s, 2×3H, $OCOCH_3$), 4.53 (br s, 1H, 2′-H), 5.32 (d, $J = 10.5$ Hz, 1H, 3′-H), 5.40 (br s, 1H, NH), 1.34 (s, 9H, *t*-Bu), 7.87 (d, $J = 7.3$ Hz, 2H, *o*-Bz), 7.55 (t, $J = 7.3$ Hz, 1H, *p*-Bz), 7.38 (t, $J = 7$ Hz, 2H, *m*-Bz), 7.45–7.33 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$): 64.8 (C-1), 29.2 (C-2), 36.1 (C-3), 151.0 (C-4), 72.6 (C-5), 36.1 (C-6), 70.6 (C-7), 45.6 (C-8), 77.3 (C-9), 71.0 (C-10), 138 (C-11), 146 (C-12), 82.8 (C-13), 43.7 (C-14), 75.7 (C-15), 27.2 (C-16), 25.11 (C-17), 12.4 (C-18), 13.4 (C-19), 110.8 (C-20), 21.7, 21.0 ($2 \times CH_3$, $OCOCH_3$), 170.3 ($2 \times OCOCH_3$), 173.1 (C-1′), 72.6 (C-2′), 55.88 (C-3′), 28.59 (CH_3, *t*-Bu), 80.6 (C–*t*-Bu), 155.63 (CO of carbamate), 164.6 (CO of Bz), 129.7 (C-1 Bz), 129.9 (C-2,6 Bz), 127.2 (C-3,5 Bz), 133.6 (C-4 Bz), 128.1 (*o*-Ph), 129.1 (*m*-Ph), 129.0 (*p*-Ph), 139.7 (C-1 Ph).

3.1.2.5. Compound ***9***. Yield 10%, amorphous solid. Mass (ESI+) $[M + Na]^+$: *m/z* 1105. IR (KBr): 3428, 2971, 2931, 1736, 1726, 1498, 1369, 1258 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.44 (1H, m, 2α-H), 2.39 (1H, m, 2β-H), 2.86 (1H, d, $J = 6.3$ Hz, 3-H), 4.41 (1H, s, 5-H), 2.31 (1H, br s, 6α-H), 1.65 (1H, br s, 6β-H), 5.52–5.66 (3H, m, 7 + 13 + 3″-H), 6.13 (1H, d, $J = 10.3$ Hz, 9-H), 6.76 (1H, d, $J = 10.3$ Hz, 10-H), 1.25 (1H, m, 14α-H), 2.42 (1H, m, 14β-H), 1.12 (3H, s, 16-H), 1.34 (3H, s, 17-H), 2.16 (3H, s, 18-H), 0.85 (3H, s, 19-H), 4.89 (1H, br s, 20α-H), 5.31 (1H, br s, 20β-H), 1.73, 2.07 (2×3H, s, $2 \times OCOCH_3$), 4.35 (1H, m, 2′-H), 4.84 (1H, m, 2″-H) 5.12 (1H, m, 3′-H), 1.34 (9H, s, *t*′-Bu), 1.36 (9H, s, *t*″-Bu) 7.86 (2H, d, $J = 7.5$ Hz, *o*-Bz), 7.55 (1H, t, $J = 7.5$ Hz, *p*-Bz), 7.43 (2H, t, $J = 7.5$ Hz, *m*-Bz), 7.22–7.33 (2×5H, m, Ph′ + Ph″). ^{13}C NMR ($CDCl_3$): 62.8 (C-1), 29.7 (C-2), 39.3 (C-3), 146.8 (C-4), 74.4 (C-5), 34.1 (C-6), 69.8 (C-7), 45.4 (C-8), 77.7 (C-9), 70.1 (C-10), 137.7 (C-11), 145.1 (C-12), 80.5 (C-13), 43.8 (C-14), 75.9 (C-15), 27.6 (C-16), 25.6 (C-17), 12.0 (C-18), 13.2 (C-19), 114.2 (C-20), 21.5, 20.9 ($2 \times CH_3$, $OCOCH_3$), 170.2, 171.2 ($2 \times CO$, $OCOCH_3$), 169.8 (C-1′, –CO–), 173.3 (C-1″, –CO–), 76.1 (C-2′), 73.7 (C-2″), 57.5 (C-3′), 54.5 (C-3″), 155.8 (CO of carbamate′), 155.7 (CO of carbamate″), 28.7 (CH_3, *t*′-Bu), 28.6 (CH_3, *t*″-Bu), 81.8 (C–*t*′-Bu), 80.1 (C–*t*″-Bu), 164.5 (CO of Bz), 131.1 (C-1 Bz), 129.9 (C-2,6 Bz), 128.9 (C-3,5 Bz), 129.0 (C-4 Bz), 128.9 (*o*-Ph′), 128.7 (*o*-Ph″), 127.9 (*m*-Ph′), 127.3 (*m*-Ph″), 129.0 (*p*-Ph′), 128.9 (*p*-Ph″), 137.0 (C-1 Ph′), 137.0 (C-1 Ph″).

3.2. Biology

The human tumor cell lines KB, MCF-7, CaCO2, HepG-2 were obtained from the National Centre for Cell Sciences (NCCS), Pune, India.

3.2.1. Cytotoxic assay

IC_{50} values were determined and the cell survival was measured by using the MTT assay described by Mosman [13]. Briefly, 0.5 or 1×10^5 cells/ml cells at the exponential growth phase were taken in a flat-bottomed 96-well polystyrene-coated plate and were incubated for 24 h in CO_2 incubator at 5% CO_2 and 37 °C. Compound was added in concentrations of 100, 10, 1, 0.1, 0.01, and 0.001 μg/ml medium. After 48 h incubation, 10 μl/well MTT (stock solution 5 mg/ml PBS) was added for 4 h and formazan crystals so formed were dissolved in 100 μl DMSO. The plates were read immediately in a microplate reader (Spectramex, 190 Molecular Devices Inc., USA) operating at 570 nm. Wells with complete medium, compound, and MTT, but without cells were used as blanks. IC_{50} and IC_{90} values were expressed as micrograms of compound concentration per milliliter that caused a 50% and 90% inhibition of growth compared with controls. Paclitaxel was used as positive controls in every experiment.

References

[1] F. Balza, S. Tachibana, H. Barrios, G.H.N. Towers, Phytochemistry 30 (1991) 1613–1614.
[2] G.I. Georg, S.R. Gollapudi, G.L. Grunewald, C.W. Gunn, R.H. Himes, B.K. Rao, X.Z. Liang, Y.W. Mirhom, L.A. Mitscher, D.G.V. Velde, Q.M. Ye, Bioorg. Med. Chem. Lett. 3 (1993) 1345–1348.
[3] G. Appendino, L. Barboni, P. Gariboldi, E. Bombordelli, B. Gabetta, D. Viterbo, J. Chem. Soc., Chem. Commun. (1993) 1587–1589.
[4] S.K. Chattopadhyay, G.C. Saha, M. Kulshrestha, R.P. Sharma, S. Kumar, Indian. J. Chem. 35B (1996) 175–177.
[5] S.P.S. Khanuja, T.R. Santha Kumar, A. Garg, R.K. Misra, S.K. Chattopadhyay, S. Srivastva, A.S. Negi, United States Patent Publication No. 20040127561 A1, 2002.

[6] G.I. Georg, Z.S. Cheruvallath, D.V. Velde, Q.M. Ye, L.A. Mitscher, R.H. Himes, Bioorg. Med. Chem. Lett. 3 (1993) 1349–1350.
[7] S. Tremblay, C. Soucy, N. Towers, P.J. Gunning, L. Breau, J. Nat. Prod. 67 (2004) 838–845.
[8] L.R. Kelland, G. Abel, Cancer Chemother. Pharmacol. 30 (1992) 444–450.
[9] M.D. Chordia, D.G.I. Kingston, Tetrahedron 53 (1997) 5699–5710.
[10] E. Didier, E. Fouque, I. Taillepied, A. Commercon, Tetrahedron Lett. 35 (1994) 2349–2352.
[11] H. Morita, A. Gonda, L. Wei, Y. Yamamura, K. Takeya, H. Itokawa, J. Nat. Prod. 60 (1997) 390–392.
[12] A.C. Beekman, A.R.W. Barensten, H. Woerdenbag, W.V. Uden, N. Pras, J. Nat. Prod. 60 (1997) 325–330.
[13] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.