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Design, synthesis and cytotoxicity of novel 3′-*N*-alkoxycarbonyl docetaxel analogs

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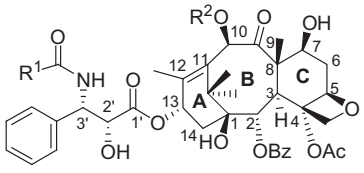
ABSTRACT

By-product **9a** exhibited potent cytotoxicity against both SK-OV-3 and A549 cell lines. The structure of **9a** was characterized using 1D and 2D NMR experiments and confirmed by synthesis to afford a diastereomeric mixture (**16a**) that was identical to **9a**, as well as a pair of diastereomers (*R*)-**16b** and (*S*)-**16c**. The preliminary SAR study demonstrated that analogs with an (*R*)-configuration were slightly more potent than analogs with an (*S*)-configuration. In addition, α,α-gem-dimethyl analogs **16g–i** were the most potent analogs in this series, exhibiting similar potency to docetaxel and greater potency than Taxol against the SK-OV-3 cell line. For the A549 cell line, analogs **16g–i** were more potent (>65-fold) than both docetaxel and Taxol.

Paclitaxel (Taxol, **1**) (Fig. 1) is a diterpenoid originally isolated from the bark of *Taxus brevifolia* in 1971[1] and is a promising anticancer agent for the treatment of ovarian and breast cancer, as well as lung, skin, neck and head cancers.[2] Paclitaxel is an antimitotic agent with a unique mode of action; by binding tubulin and stabilizing microtubule formation, it ultimately disrupts mitosis and causes cell death.[3] Docetaxel (Taxotere, **2**)[4] is a semi-synthetic derivative of paclitaxel with a similar mechanism of action. Docetaxel was approved by the FDA to treat breast cancer in 1996. Although both paclitaxel and docetaxel are potent antitumor agents, they have some drawbacks, such as poor aqueous solubility, multi-drug resistance (MDR) and low oral bioavailability.[5] Therefore, developing new paclitaxel-based anticancer agents retaining superior clinical antitumor activity while lacking these side effects remains a goal in drug discovery.

Extensive structure–activity relationship (SAR) studies of paclitaxel and docetaxel have been undertaken for decades and mainly focused on modifying the C-13 side chain, B and C ring.[2,6] The C-13 phenylisoserine side chain on paclitaxel is essential for the activity; analogs without a 3′-*N*-acyl group exhibited significantly decreased activity compared with paclitaxel.[7] Georg et al. reported that acylating *N*-debenzoyltaxol generated the *tert*-butylacetyl analog; this compound displayed comparable biological activity in the microtubule assembly assay, but demonstrated slightly diminished activity in the B16 melanoma cell line assay relative to Taxol.[8] Song and co-workers reported the synthesis of 3′-*N*-acyl-*N*-debenzoylpaclitaxel analogs that were highly cytotoxic. The compound bearing a *N*-cycloalkenoyl moiety was 20 times more potent than paclitaxel against the A549 and SK-OV-3 tumor cell lines in vitro and caused superior tumor growth inhibition (T/C = 212%) relative to paclitaxel (T/C = 200%) during in vivo experiments against ip implanted B16 melanoma.[9] Further modifications demonstrated that some analogs bearing *N*-alkylcarbonyl groups, such as *trans*-crotonyl, 2-butynoyl and *trans*-2-hexenoyl substituents, exhibited more potent inhibitory activity than Paclitaxel against five tumor cell lines.[10] Interestingly, except for 3′-*N*-ethoxycarbonyl[11] and a few small 3′-*N*-alkoxycarbonyl analogs, 3′-*N*-chiral alkoxycarbonyl substituted docetaxel analogs have not been reported so far.



1 paclitaxel R^1 = Ph, R^2 = Ac
2 docetaxel R^1 = *t*-BuO, R^2 = H

Figure 1.

During our initial work to improve the metabolic stability and pharmacokinetic profile of docetaxel, we synthesized a series of fluorinated docetaxel analogs. These analogs revealed improved metabolic stability, as expected.[12] When synthesizing fluorinated docetaxel analog **9** (Scheme 1), precursor **8** was obtained in 23% yield alongside an unexpected by-product (**8a**) in 18% yield; **8a** was converted into **9a** by removing the 7,10-troc protecting groups. The preliminary analysis of the NMR data for **9a** indicated

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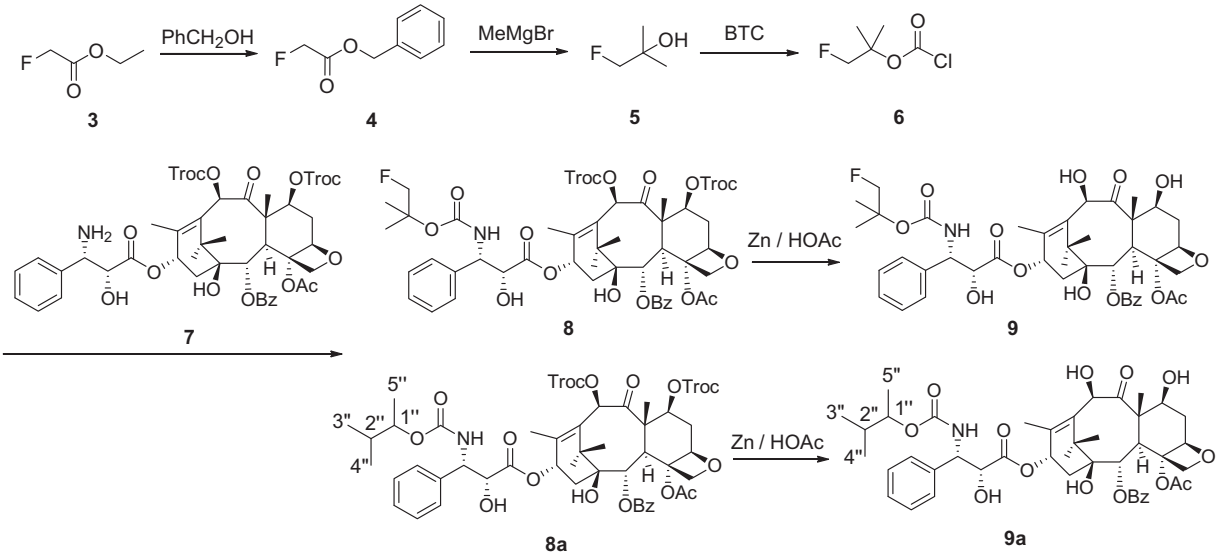
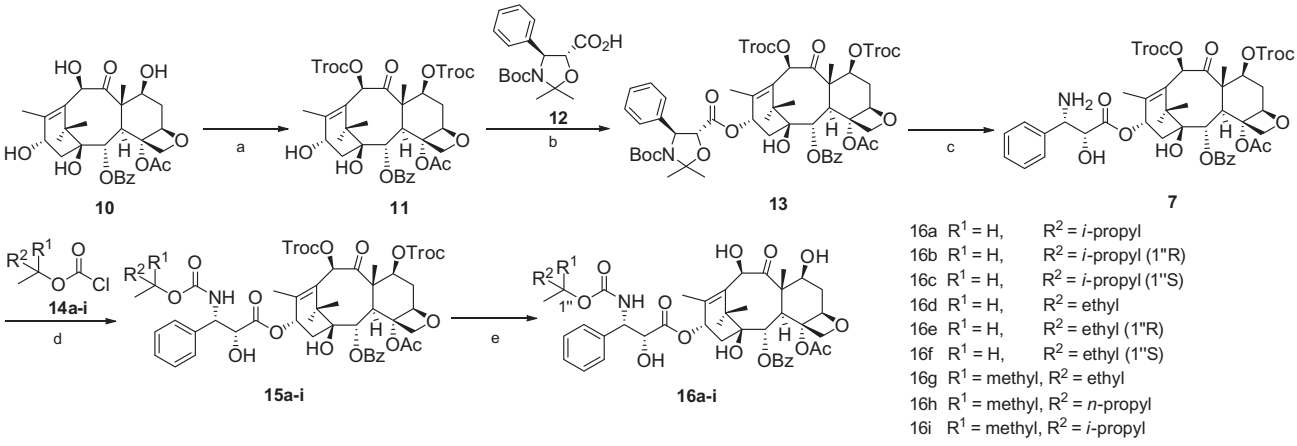


Scheme 1. Synthesis of **9** and **9a**.

that it had the same core structure as docetaxel, but the structural details remained unknown at this stage. Nevertheless, **9a** was also tested for its cytotoxicity with **9**. Surprisingly, **9a** demonstrated more potent cytotoxicity (IC_{50} = 47 nM) than **9** (IC_{50} = 140 nM) and docetaxel (IC_{50} = 850 nM) against the A549 cell line. This result encouraged us to elucidate the structure of **9a**. Herein, we report the structure of **9a** and a follow-up SAR study based on the confirmed structure of **9a**.

Compound **9a** was obtained as a white powder. The molecular formula of **9a** was determined to be $C_{44}H_{55}NO_{14}$ through analysis of ESIMS, HRESIMS (*m*/*z* 844.3515 [M+Na]$^+$), ^{1}H NMR, ^{13}C NMR, and DEPT experiments. In the aromatic region of the ^{1}H NMR spectrum, ten protons were assigned to the two mono-substituted aromatic rings and confirmed by the ^{13}C NMR spectrum. The higher field portion of the ^{1}H NMR spectrum revealed four oxygen-bearing methine signals at δ = 5.67 (1H, d, *J* = 6.5 Hz, H-2), 4.22 (1H, m, H-7), 5.21 (1H, s, H-10), and 6.22 (1H, t, *J* = 8.7 Hz, H-13), as well as one pair of methylene signals at δ = 4.19 and 4.20 (2H, 2d, *J* = 8.0 Hz, 2H-20). These data suggested the presence of representative signals from the 10-deacetylbaccatin III (10-DAB) skeleton. Further analysis of the ^{1}H–^{1}H COSY, HMBC, and HMQC spectra of **9a** revealed the ester linkage between the C-1′ on the side chain and the C-13 from the 10-DAB. According to the molecular formula and the docetaxel skeleton, there was a 1″,2″-dimethyl-propoxycarbonyl group attached to the side chain. Interestingly, there were a pair of doublets (1.07 (1.5H, d, *J* = 6.6 Hz) and 1.00 (1.5H, d, *J* = 5.7 Hz)) in the high field region of the ^{1}H NMR spectrum. Meanwhile, there were two doublet peaks (0.84 (1.5H, d, *J* = 2.7 Hz) and 0.82 (1.5H, d, *J* = 2.7 Hz)) and one multiple peak (0.77 (3H, m)). These peaks suggested that **9a** might be a mixture of diastereomers at the 1″ site with a *R*/*S* = 1 to 1 ratio.

To confirm the proposed structure of **9a** unambiguously, the diastereomeric mixture (**16a**) and a separated pair of diastereomers (**16b** and **16c**) were synthesized. The synthesis of compounds **16a–c** began from commercially available 10-DAB (**10**), as illustrated in Scheme 2. First, the hydroxyl groups at the C7 and the C10 of **10** were protected using 2,2,2-trichloroethyl chloroformate (TrocCl) with pyridine to obtain 7,10-ditroc-10-deacetylbaccatin (**11**) in 91% yield.[13] Subsequently, **11** was coupled with enantiopure oxazolidine **12**[14] using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to generate **13** in 86% yield. After removing the 3′-*N*-Boc and acetonide protective group from **13** using 98% formic acid at room temperature, amino alcohol **7** was obtained in 55% yield.[15] With **7** in hand, intermediates



Scheme 2. Synthesis of **16a–i**. Reagents and conditions: (a) TrocCl, pyridine, rt, 30 min; (b) **12**, DCC, DMAP, toluene; (c) HCOOH, rt, 2 h; (d) **14a–i**, Et_3N, CH_2Cl_2; (e) Zn/HOAc, MeOH, 50 °C.

Table 1
The ^{1}H NMR data for the 1″,2″-dimethyl-propoxycarbonyl side chain

	9a (300 MHz, $CDCl_3$)	**16a** (500 MHz, $CDCl_3$)	(*1″R*)-**16b** (500 MHz, $CDCl_3$)	(*1″S*)-**16c** (500 MHz, $CDCl_3$)
1″	4.51 (1H, m)	4.51 (1H, m)	4.52 (1H, m)	4.50 (1H, m)
2″	1.72 (1H, m)	1.69 (1H, m)	1.71 (1H, m)	1.70 (1H, m)
3″	0.84 (1.5H, d, *J* = 2.7 Hz)	0.84 (1.5H, d, *J* = 4.1 Hz)	0.76 (6H, br s)	0.84 (3H, d, *J* = 4.1 Hz)
4″	0.82 (1.5H, d, *J* = 2.7 Hz)	0.82 (1.5H, d, *J* = 3.2 Hz)		0.82 (3H, d, *J* = 3.2 Hz)
	0.77 (3H, m)	0.76 (3H, br s)		
5″	1.07 (1.5H, d, *J* = 6.6 Hz)	1.07 (1.5H, d, *J* = 6.3 Hz)	1.07 (3H, d, *J* = 6.3 Hz)	0.99 (3H, br s)
	1.00 (1.5H, d, *J* = 5.7 Hz)	0.99 (1.5H, br s)		

Table 2
Cytotoxicities (IC_{50}, nM) of the docetaxel analogs

Compd	R^1	R^2	SK-OV-3	A549
16a (9a)	H	*i*-Propyl	16 ± 3	47 ± 4
16b	H	*i*-Propyl (*R*)	13 ± 2	35 ± 3
16c	H	*i*-Propyl (*S*)	17 ± 3	60 ± 4
16d	H	Ethyl	22 ± 4	30 ± 2
16e	H	Ethyl (*R*)	13 ± 2	18 ± 2
16f	H	Ethyl (*S*)	27 ± 5	40 ± 3
16g	Methyl	Ethyl	5.9 ± 1.2	13 ± 2
16h	Methyl	*n*-Propyl	2.9 ± 0.5	10 ± 2
16i	Methyl	*i*-Propyl	2.7 ± 0.4	12 ± 3
Docetaxel	Methyl	Methyl	5.0 ± 0.8	850 ± 43
Taxol			35 ± 5	930 ± 52

15a–c were prepared by acylating the amino group using freshly prepared acyl chlorides **14a–c**; the acyl chlorides were synthesized by combining the corresponding alcohols with triphosgene in ether at low temperatures. Finally, analogs **16a–c** were obtained in desirable yields by removing 7,10-troc protecting groups from **15a–c** using zinc in acetic acid.

The peaks in ^{1}H NMR spectrum of **9a** were identical to those of (±)-**16a**. Further analysis of the high field portion of the ^{1}H NMR data for (*R*)-**16b** and (*S*)-**16c** revealed the strange peaks (Table 1). Therefore, the structure of **9a** was assigned as (±) 3′-*N*-De-*t*-butoxycarbonyl-*N*-[1″,2″-dimethyl-propoxycarbonyl]docetaxel, which is identical to (±)-**16a**.

The formation of **9a** was puzzling. After examining the preparation of the fluorinated *tert*-butyl alcohol, GC/MS and ^{1}H NMR data confirmed that 2-(±)-3-methyl-2-butanol was present after the Grignard reaction (Scheme 1), even though the mechanism for producing the 2-(±)-3-methyl-2-butanol remained unclear. Because the boiling points of the fluorinated *tert*-butyl alcohol and 2-(±)-3-methyl-2-butanol were very similar, they co-distilled and reacted with triphosgene to form the corresponding acyl chlorides, forming both the desired product **8** and the by-product **8a**.

According to the literature, the biological activity of 3′-*N*-ethoxycarbonyltaxol was 2.5-fold less potent than Taxol while inhibiting microtubule disassembly.[11] After accounting for the activity of **9a** (Table 2), we reasoned that the α-methyl group on the 3′-*N*-alkoxycarbonyl portion may be crucial for the increase in potency against both the SK-OV-3 and A549 cell lines. Therefore, a series of 3′-*N*-alkoxycarbonyldocetaxel analogs (**16d–i**) with a α-methyl or α,α-gem-dimethyl group were designed, synthesized and evaluated for their cytotoxicity.[16]

The syntheses of **16a–i** followed the same procedure as was used for **16a**. Subsequently **16d–i**, as well as (*R*)-**16b** and (*S*)-**16c**, were tested in vitro for cytotoxicity against the SK-OV-3 and A549 cell lines (Table 2 and Fig. 2).[17] Although there were no significant differences between the racemic and optically pure analogs (**16a** vs (*R*)-**16b** and (*S*)-**16c**; **16d** vs (*R*)-**16e** and (*S*)-**16f**), both (*R*)-**16b** and (*R*)-**16e** were slightly more potent than (*S*)-**16c** and (*S*)-**16f** against both cell lines. Interestingly, single digit nanomolar values (**16g–i**) were achieved when the mono-methyl substitution at α-position was changed to a gem-dimethyl group. Analogs **16g–i** exhibited similar potency (IC_{50} = 2.7 – 5.9 nM) to docetaxel (IC_{50} = 5.0 nM) and were more potent than Taxol (IC_{50} = 35 nM) against the SK-OV-3 cell line. Additionally, **16g–i** were more potent (>65-fold) than both docetaxel and Taxol against the A549 cell line.

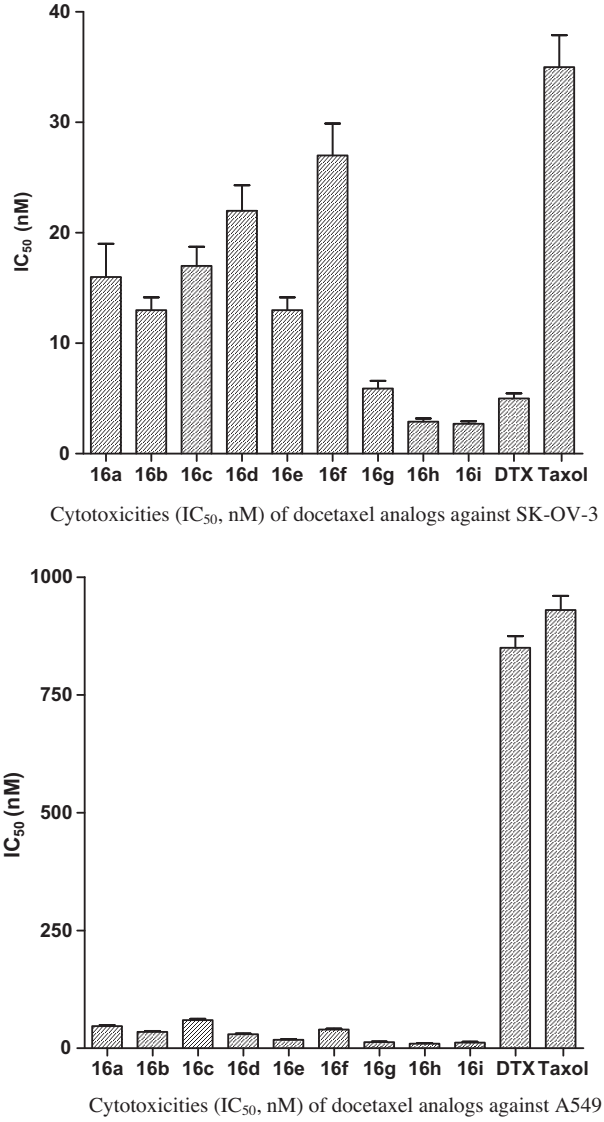


Figure 2.

By-product **9a** exhibited potent cytotoxicity against both the SK-OV-3 and A549 cell lines. The structure of **9a** was carefully characterized with 1D and 2D NMR and was confirmed using

synthesis to afford a diastereomeric mixture (**16a**) that was identical to **9a** and separate diastereomers (*R*)-**16b** and (*S*)-**16c**. A preliminary SAR study revealed that analogs with an (*R*)-configuration were slightly more potent than analogs with an (*S*)-configuration. In addition, the α,α-gem-dimethyl analogs **16g–i** were the most potent analogs in this series, exhibiting similar potency to docetaxel and exceeding the potency of Taxol against the SK-OV-3 cell line. For the A549 cell line, **16g–i** were all more potent (>65-fold) than docetaxel and Taxol. Based on these findings, an additional SAR study was carried out in our laboratory and will be reported in due course.

Acknowledgments

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16. *Representative experimental procedure for 3′-N-de-t-butoxycarbonyl-N-[1″,1″,2″-trimethyl-propoxycarbonyl]docetaxel (***16i***):* To a solution of 1,1,2-trimethylpropanol (10 mmol) and triphosgene (3.33 mmol) in ether (10 mL) was added pyridine (11 mmol) in ether (8 mL) dropwise at −30 °C. The mixture was stirred for 2 h at this temperature before being slowly warmed to room temperature to be stirred further for 10 h. The resulting solution (**14i**) was used directly for the next step without purification. To a stirred solution of **7** (500 mg, 0.46 mmol) in anhydrous THF (10 mL) was added excess **14i** dropwise at 0 °C and, subsequently, triethylamine (0.13 mL, 0.93 mmol) in anhydrous THF (5 mL). The resulting mixture was stirred at room temperature for 1 h and extracted with ethyl acetate (20 mL × 3). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by silica gel flash chromatography column (petroleum ether/ethyl acetate: 2/1) to afford **15i** as a white solid. To a solution of **15i** (0.19 mmol) in methanol (10 mL) were added glacial acetic acid (4.60 mL) and zinc powder (0.46 g, 7.08 mmol). The resulting mixture was stirred at 50 °C for 1 h. The reaction mixture was filtered to remove the zinc and a solid formed. The solvent was removed by distillation, providing a white solid. The obtained solid was dissolved in ethyl acetate (60 mL), washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The obtained residue was purified by silica gel flash chromatography column (petroleum ether/acetone: 2/1) to furnish **16i** as a white solid. Mp 200–202 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75–0.87 (6H, m), 1.13 (3H, s), 1.24 (3H, s), 1.27 (3H, s), 1.28 (3H, s), 1.75(3H, s), 1.84 (1H, m), 1.85 (3H, s), 2.25 (1H, m), 2.26 (2H, m), 2.38 (3H, s), 2.58(1H, m), 3.46 (1H, s), 3.91 (1H, d, *J* = 6.9 Hz), 4.19 (1H, d, *J* = 8.7 Hz), 4.26 (1H, s), 4.27 (1H, m), 4.31 (1H, d, *J* = 8.7 Hz), 4.63 (1H, br s), 4.94 (1H, d, *J* = 9.3 Hz), 5.21 (1H, s), 5.27 (1H, m), 5.48 (1H, m), 5.67 (1H, d, *J* = 7.2 Hz), 6.22 (1H, t, *J* = 7.8 Hz), 7.38 (5H, m), 7.50 (2H, t, *J* = 7.5 Hz), 7.62 (1H, t, *J* = 7.5 Hz), 8.10 (2H, d, *J* = 7.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 9.4, 13.5, 16.7, 20.4, 22.1, 22.3, 22.4, 22.5, 26.1, 35.8, 35.9, 36.7, 43.2, 46.5, 57.0, 57.6, 71.2, 71.5, 74.2, 74.3, 75.1, 75.9, 77.7, 80.8, 83.6, 84.1, 127.1, 127.3, 128.3, 128.5, 129.9, 130.3, 133.1, 136.6, 137.5, 139.7, 155.3, 165.7, 170.0, 172.7, 210.5; ESI-MS *m*/*z* 858.6 $[M+Na]^+$; HRMS (MALDI) *m*/*z* calcd for $C_{45}H_{57}NO_{14}Na^+$ $[M+Na]^+$: 858.3708, found 858.3671.
17. The cytotoxic activity in vitro was measured using the MTT assay. The MTT solution in RPMI-1640 was added after cancer cells (SK-OV-3 and A549) were treated with the drug (100 μM, 10 μM, 1 μM, 0.1 μM, 0.01 μM, 1 nM, 0.1 nM) for 48 h, and cells were incubated for further 4 h at 37 °C. The purple formazan crystals were dissolved in 100 μL DMSO. After 5 min, the plates were read at 570 nm by an automated microplate spectrophotometer. The experiments were performed in triplicate. Inhibitory concentration (IC_{50}) was calculated and the results represent the mean ± SD of three independent experiments.