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Original article

# Diastereoselective synthesis and bioactivity of long-chain *anti*-2-amino-3-alkanols

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ABSTRACT

An improved four-step approach for the stereoselective synthesis of long-chain *anti*-2-amino-3-alkanols is described. Using this method, the syntheses of antiproliferative (antitumoral) compounds, spisulosine (ES-285, **2**), clavaminols A and B (**3** and **4**), the deacetylated products of clavaminols H and N (**7** and **8**), as well as (2*S*,3*R*)-2-aminododecan-3-ol (**9**) and xestoaminol C (**10**), have been achieved in excellent diastereoselectivities. *In vitro* study showed that these compounds induced cell death and dose-dependently inhibited cell proliferation in human glioblastoma cell line SHG-44, indicating the anti-tumor property of this series of compounds.



## 1. Introduction

Sphingoid bases (also called sphingosines) are a class of naturally occurring long-chain 2-amino-3-alkanols. Since the first isolation of sphingosine (**1**) (Fig. 1), hundreds of sphingoid bases with considerable structural diversity have been isolated from plants and animals [1]. Due to the wide spectrum of biological activities, this family has attracted a lot of attention by both biologists and synthetic organic chemists [1–12]. Recently, marine organisms, ascidians (tunicates) and marine sponges have also been found to be the enriched sources of 1-deoxy-sphingoid bases [1]. In spite of their structural simplicity, these long-chain amino alcohols exhibit remarkable bioactivities, and are promising for the development of new anti-tumor agents [13]. 1-Deoxy-sphingoid bases have been shown to be more cytotoxic than sphingosine against HT29 cells [14]. In this context, spisulosine (**2**) (also referred as "ES-285"), isolated from the marine clam *Spisula polynyma* by Caudros *et al.* [15], is considered to be a novel anti-tumor compound and currently in Phase I clinical trial against solid tumor in Europe [16,17]. Specially, spisulosine has shown the inhibition of cell proliferation in numerous tumor cell lines due to the disruption of actin stress fibers through Rho signaling pathway [15]. It has also been found to activate caspases 3 and 12 and to modify the phosphorylation of p53 [18]. In addition, clavaminols A ~ N, a family of fourteen members isolated from the Mediterranean ascidian *Clavelina phlegraea*, also displayed biological cytotoxicity. Opposite to the 2*S*,3*R*-configuration of sphingosine, clavaminols A ~ N possess 2*R*,3*S*-configuration in the *anti*-2-amine-3-alkanol motif [19,20]. Clavaminols A–C and F were tested for their cytotoxic and pro-apoptotic properties and clavaminol A (**3**) was shown to be the most potent cytotoxic compound of this series in inducing cell death through activation of the apoptotic machinery. Interestingly, while clavaminols G–N have shown no significant cytotoxicity, the unnatural deacetylated product (**8**) of clavaminol N (**6**) showed a cytotoxic effect comparable to that of clavaminol A ($IC_{50} \approx 5$ μg/mL) on two different tumor cell lines, human lung adenocarcinomic epithelial cells A549 and human gastric adenocarcinoma epithelial cell AGS. Similarly, the deacetylated product (**7**) of clavaminol H (**5**) showed significant cytotoxic activity in AGS cells, while it has no effect on lung cancer cell line A549 [19,20]. Moreover, (2*S*,3*R*)-2-aminododecan-3-ol (**9**), the enantiomer of clavaminol A (**3**), is an antifungal agent isolated from the ascidian *Clavelina oblonga* in Brazil [21]. This compound displayed antifungal activity against *Candida albicans* ATCC 10231

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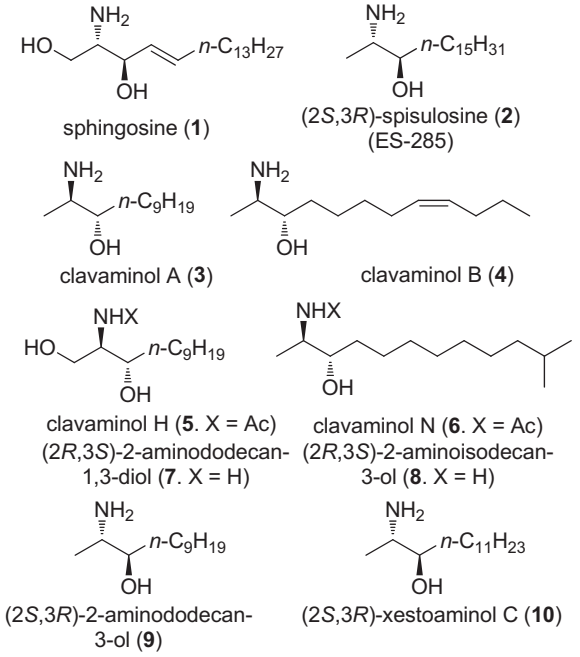



**Fig. 1.** Structure of long-chain *anti*-2-amino-3-alkanols.

and *Candida glabrata* with a MIC of 0.7 μg/mL and 30 μg/mL, respectively. Furthermore, xestoaminol C (**10**), isolated together with its congeners xestoaminols A and B from a Fijisponge *xestospongia*, sp., was extremely active in assay against reverse transcriptase with 95% inhibition at 1 mg/mL [22]. Apart from those mentioned above, many other bioactive long-chain 2-amino-3-alkanols have also been reported [23,24].

In recent years, several synthetic approaches to these amino alcohols have been reported [8,25–34], including seven for spisulosine (**2**) [25–31], two for the deacetylated products (**7**) of clavaminol H [28,32], and three for xestoaminol C (**10**) [8,33,34]. As a continuation of our longstanding interest in the synthesis of *N*-containing bioactive molecules [35–37], and in conjunction with our recent interest in the development of anti-tumor agents [38,39], we were engaged in the synthesis of above-mentioned long-chain amino alcohols. In present study, we used Reetz approach [40,41] with modification to synthesize sphingoid bases (Fig. 1), including spisulosine (**2**), clavaminols A and B (**3** and **4**), as well as the deacetylated products of clavaminols H and N (**7** and **8**), antifungal compound (**9**), and xestoaminol C (**10**). Moreover, we tested the effect of these synthetic compounds on cell toxicity and proliferation to elucidate their biological and pharmacological roles as anti-cancer agents.

## 2. Chemistry

All the molecules shown in Fig. 1 are vicinal amino alcohols with an *anti* stereochemistry and with either (2*S*,3*R*) or (2*R*,3*S*) configuration. Biosynthetically they are originated from L or D-alanine or serine [19–21]. Thus α-amino acids would afford a convenient starting point to access these compounds and the homologues and analogs thereof via the addition of an organometallic reagent to the α-amino aldehydes (Scheme 1) [42]. Among the methods available for the conversion of α-amino acids into the corresponding vicinal *anti*-amino alcohols [43], the Reetz methodology of *anti*-diastereoselective addition with *N*,*N*-dibenzyl amino aldehydes appeared to be the ideal one [40,44–46]. Although this method has been applied for the enantioselective synthesis of vicinal *anti*-amino

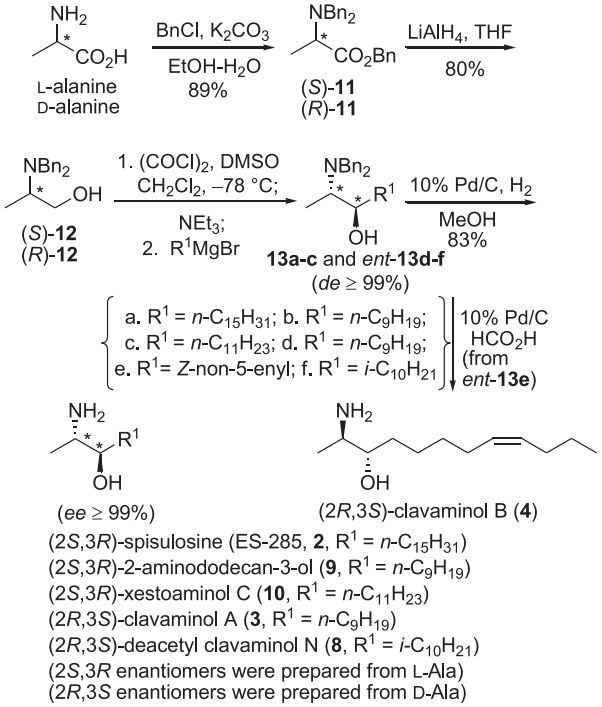
**Scheme 1.** *Anti*-diastereoselective addition of organometallic reagents to *N*-protected α-amino aldehydes.

alcohols [40,44–46], the lability of the α-amino aldehydes towards partial racemization and side reaction [43,47,48] made it worthwhile to modify to ensue a more reliable C–C bond formation with high enantioselectivity.

On the basis of these considerations, a four-step approach for the synthesis of 1-deoxy-sphingoid bases starting from unprotected L or D-alanine is developed and shown in Scheme 2. The method features a tandem Swern oxidation-organometallic reagent addition procedure [48–50], which will allow, on one hand, avoiding the racemization of the *N*,*N*-dibenzyl amino aldehyde, and on the other hand, simplifying a purification step.

For the synthesis of the natural products spisulosine (**2**), (2*S*,3*R*)-2-aminododecan-3-ol (**9**), and xestoaminol C (**10**) with (2*S*,3*R*) configuration, L-alanine was the required starting material (Scheme 2). Compounds (*S*)-**11** and (*S*)-**12** were prepared as previously described [25,44,46,51]. The key one-pot reaction was run by Swern oxidation [$(COCl)_2$, DMSO, $CH_2Cl_2$; $NEt_3$] at −78 °C, followed by addition of a solution of *n*-pentadecyl magnesium bromide in ether, which produced the amino alcohol **13a** in 68% yield. Removal of the protecting groups under catalytic hydrogenolytic conditions (10% Pd/C, $H_2$, MeOH, 28 h) afforded (2*S*,3*R*)-spisulosine (**2**) in 84% yield. The physical and spectral data of our synthetic compound agreed with those reported by Gálvez *et al.* [27] {$[\alpha]_D^{20}$ +24.2 (*c* 1.0, $CHCl_3$); lit. [27] $[\alpha]_D^{25}$ +24.0 (*c* 1.0, $CHCl_3$)}.

Using *n*-nonyl magnesium bromide in the tandem Swern oxidation-Grignard reagent addition of (*S*)-**12** led to the formation of **13b** in 67% yield. Catalytic hydrogenolysis (10% Pd/C, $H_2$, MeOH,




**Scheme 2.** Synthesis of 1-deoxy-sphingoid bases from L or D-alanine.

28 h) of the latter afforded (2*S*,3*R*)-2-aminododecan-3-ol (**9**) in 84% yield. The optical rotation data of the synthetic compound matched those reported {$[\alpha]_D^{20}$ +4.2 (*c* 1.5, MeOH); lit. [21] $[\alpha]_D^{29}$ +4.5 (*c* 0.22, MeOH)}, while differences existed in its $^1$H and $^{13}$C NMR data comparing with those of the natural product reported [21]. The structure of the synthetic compound was confirmed via the synthesis of its dibenzoyl derivative, whose $^1$H NMR data matched those reported [21].

Similarly, tandem Swern oxidation-undecyl magnesium bromide addition produced **13c** in 73% yield. Catalytic hydrogenolysis (10% Pd/C, $H_2$, MeOH, 28 h) of **13c** afforded (2*S*,3*R*)-xestoaminol C (**10**) in 90% yield. It is worthy to note that the spectral data of our synthetic compound agreed with those reported by Ooi *et al*. [33] The physical and spectral data of the diacetyl derivative matched those reported [34] {$[\alpha]_D^{20}$ −22.8 (*c* 0.8, MeOH); lit. [34] $[\alpha]_D^{25}$ −22.1 (*c* 0.17, MeOH)}.

In view of the synthesis of clavaminol A (**3**) and clavaminol B (**4**) with 2*R*,3*S*-configuration, amino alcohol (*R*)-**12** was prepared from D-alanine by the procedure shown in Scheme 2 [44]. One-pot Swern oxidation-nonyl magnesium bromide addition gave the *anti*-amino alcohol *ent*-**13d** in 67% yield. 10% Pd/C-Catalyzed hydrogenolysis then afforded the clavaminol A (**3**) in 85% yield. The optical rotation of the synthetic compound matched that reported {$[\alpha]_D^{20}$ −4.2 (*c* 0.5, MeOH); lit. [19] $[\alpha]_D^{25}$ −4.25 (*c* 0.0094, MeOH)}. The spectral data of its diacetyl derivative matched those reported [19].

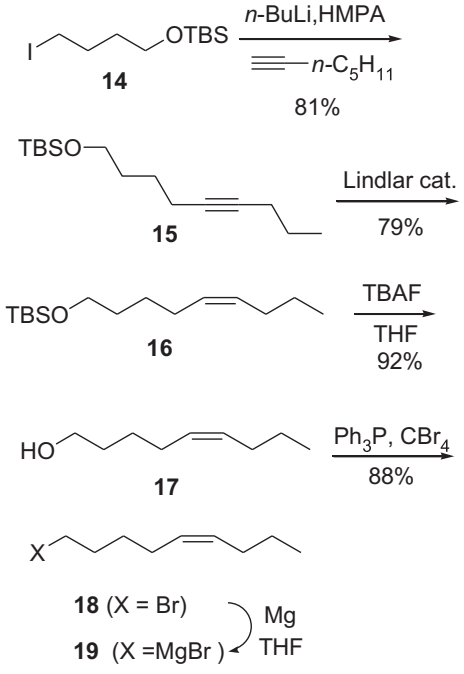
For the synthesis of clavaminol B (**4**), the Grignard reagent **19** was prepared (Scheme 3). Thus, deprotonation of *n*-hept-1-yne with *n*-butyl lithium followed by coupling of the resultant lithium heptylide with iodide **14** afforded the coupling product **15** in 81% yield. Lindlar catalyst-catalyzed partial hydrogenation of the alkyne **15** gave *cis*-olefin **16** in 79% yield. Unfortunately, compound **16** could not be converted directly into the corresponding bromide **18** with triphenylphosphine and bromine [52], as the addition of bromine to double bond would also happen. Finally, it was necessary that cleavage of the silyl group (TBAF, THF) yielded the alcohol **17**, which was treated with $PPh_3/CBr_4$ [53] to give bromide **18**. Tandem Swern oxidation and addition with the Grignard reagent (**19**) prepared from bromide **18** produced the *anti*-adduct **13e** in 70% overall yield (Scheme 2). For the chemoselective cleavage of the *N*,*N*-dibenzyl groups, the conventional catalytic hydrogenolytic condition could not be used due to the presence of the double bond. This was achieved smoothly under 10% Pd/C-catalyzed transfer hydrogenolytic conditions [54] ($HCO_2H$, $NEt_3$, MeOH), which provided the desired product **4** {$[\alpha]_D^{20}$ −8.3 (*c* 0.2, MeOH); lit. [19] $[\alpha]_D^{25}$ −5.0 (*c* 0.001, MeOH)} in 89% yield. The physical and spectral data, which were not reported for its synthetic compound or derivative so far, were similar to those of the obtained compounds described above.

As regarding the synthesis of compound **8**, the deacetylated product of clavaminol N (**6**), the required Grignard reagent **25** was prepared from 1,5-hexanediol in five steps (Scheme 4). Monosilylation of the diol **20** with TBSCl (imidazole, DMAP, $CH_2Cl_2$) followed by tosylation (*p*-TsOH, $NEt_3$, DMAP, $CH_2Cl_2$, r.t.) gave the desired tosylate **22** in 76% overall yield. CuI-Mediated coupling of *i*-butyl magnesium bromide with tosylate **22** smoothly produced the coupling product **23** in 90% yield. Treatment of the silyl ether **23** with triphenylphosphine and bromine [52] produced directly the bromide **24**, which was converted into the Grignard reagent **25**. One-pot Swern oxidation-Grignard addition of **25** then gave *anti*-amino alcohol **13f** in 72% yield (Scheme 2). Catalytic hydrogenolysis of **13f** produced compound **8** in 86% yield, of which the $^{13}$C spectral and physical data {$[\alpha]_D^{20}$ −4.5 (*c* 0.22, MeOH)} were not reported so far.

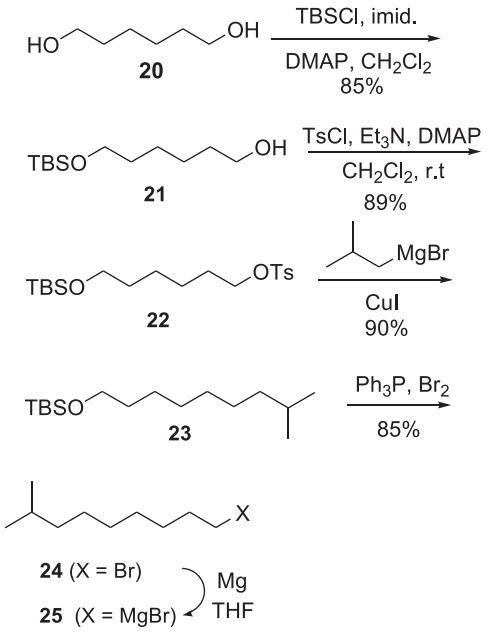
We next turned our attention to the synthesis of the deacetylated product (**7**) of clavaminol H (**5**). The synthesis was performed as shown in Scheme 5. Compounds **26**, **27**, **28** were prepared as previously described [45,55–58]. One-pot Swern oxidation-nonyl magnesium bromide addition gave the *anti*-amino alcohol **29** in 65% yield. 10% Pd/C-Catalyzed hydrogenation then afforded the deacetylated clavaminol H (**7**) in 88% yield. The optical rotation and spectral data of the synthetic compound matched those reported by Ferreira/Chemla [28] {$[\alpha]_D^{20}$ −6.3 (*c* 0.3, MeOH); lit. [28] $[\alpha]_D^{25}$ −6.0 (*c* 0.10, MeOH)}.

## 3. Pharmacology

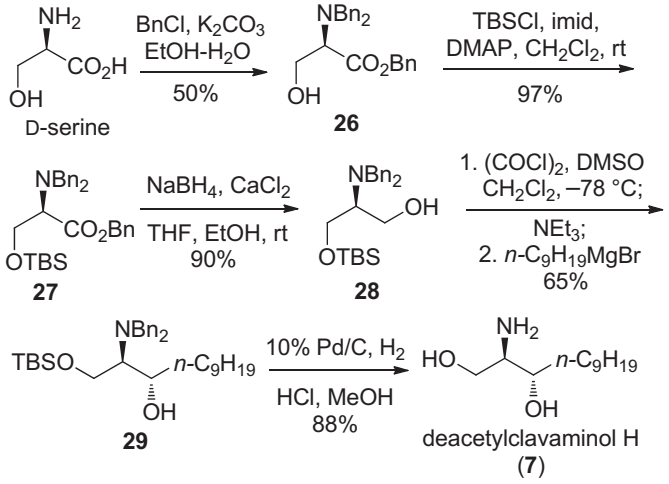
Cancer therapies predominantly focus on the anti-proliferation strategy as a number of studies have demonstrated and supported that tumor growth occurred upon imbalance between cell




**Scheme 3.** Preparation of the Grignard reagent **19** for the synthesis of clavaminol B.




**Scheme 4.** Preparation of the Grignard reagent **25** for the synthesis of compound (**8**).




**Scheme 5.** Synthesis of the deacetylclavaminol H (**7**).

proliferation and apoptosis. It has been suggested that marine organisms be the potential sources for antineoplastic agents [59] and several compounds isolated from marine crops have been portrayed as anti-cancer candidates [60–63]. Although recent reports showed that sphingosine and its derivatives can cause cell growth arrest or death in various tumor cell lines [16,64], their anti-tumor effects on glioma have not yet been reported. Gliomas, accounting for 30–35% of the primary malignant brain tumors in adults, are a line of the most aggressive and invasive tumors that lead to death in most cases. Treatments for human gliomas nowadays are still ineffective and mainly palliative [65,66]. Due to their poor prognosis and high mortality, it is urgent and necessary to develop an effective therapeutics for human gliomas. To test whether sphingosine derivatives effectively affect the proliferation of human gliomas, we used human glioblastoma cell line SHG-44 and Cell Count Kit-8 (CCK-8) assay to examine cell viability and toxicity in response to these compounds.

## 4. Results and discussion

In SHG-44 cells, all compounds were capable to induce cell toxicity at 50 μM and compounds **7**, **9**, **10** were found of the strongest effect among these compounds (10 μM of compound **7**, **9**, **10** inhibited cell growth with 60%, 80% and 100%, respectively) (Fig. 2). The extended studies showed that compound **7**, **9**, **10** demonstrated anti-proliferation property in SHG-44 cell with $IC_{50}$ of 4.41 μM, 7.96 μM and 4.5 μM, respectively (Fig. 3). In contrast, compounds **4** and **8** were lack of anti-proliferation activity, indicating that additional instauration and branched carbon chain were detrimental for the cytotoxic activities [67,68].

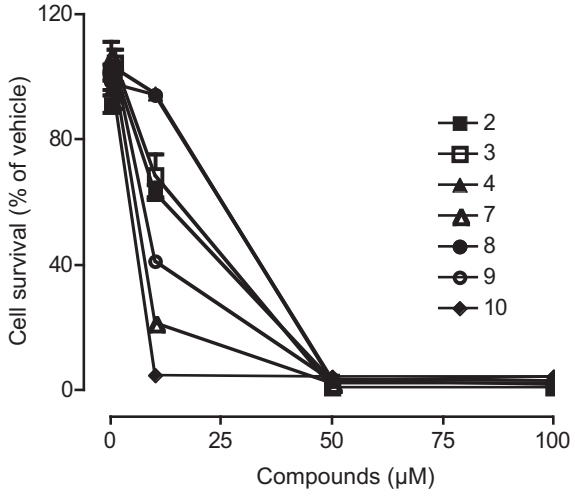



**Fig. 2.** Effect of synthetic compounds on cell death. Dose-dependent (0.1 μM, 1 μM, 10 μM, 50 μM and 100 μM) compounds induced cell death in SHG-44 cells. Compound **2**, closed square; **3**, open square; **4**, closed triangle; **7**, open triangle; **8**, closed circle; **9**, open circle; **10**, closed diamond.

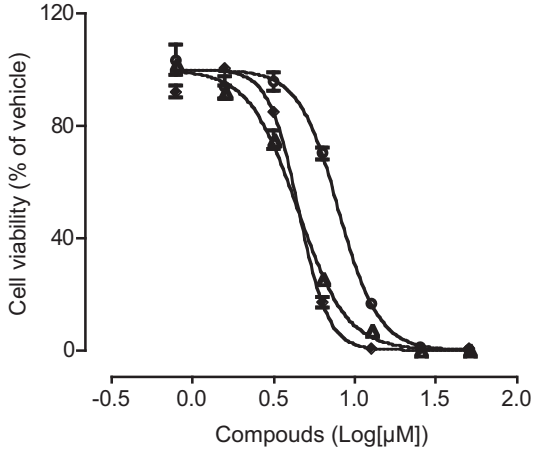



**Fig. 3.** Dose-dependent inhibition of cell proliferation by compound **7** (open triangle), **9** (open circle) and **10** (closed diamond) with $IC_{50}$ of 4.41 μM, 7.96 μM, and 4.5 μM, respectively.

The detection sensitivity of CCK-8 was greater than other tetrazolium salt methods such as MTT, XTT or MTS. Cell proliferation assay using CCK-8 correlated well with thymidine incorporation assay, the most common assay to detect chromosomal DNA synthesis during mitotic cell division. Thus, the CCK-8 assay may directly measure cell proliferation and determine cell division that has occurred in response to test agents. However, CCK-8 assay was unable to distinguish the two major cell biology pathways, i.e., cell arrest and apoptosis, which induced cell death. In the future study, we would like to carry out the specific assay to illustrate the mechanism of antitumor effect exerted by these compounds. To detect cell cytotoxicity, CCK-8 assay may minimize and facilitate the process to determine living cell number. Therefore, the cytotoxicity assay using CCK-8 was suitable for the preliminary screening of chemicals.

## 5. Conclusion

In summary, by the development of a one-pot Swern oxidation-organometallic reagent addition procedure, the Reetz's method for the asymmetric synthesis of β-amino alcohols has been improved. Using this concise and versatile method, the asymmetric syntheses of seven naturally occurring or derived cytotoxic long-chain *anti*-2-amino-3-alkanols, including the first stereoselective synthesis of clavaminol B (**4**) and (2*R*,3*S*)-deacetylclavaminol N (**8**), have been achieved in ≥99% diastereoselectivities and ≥99% enantioselectivities. Bioactivity assay further confirmed in human glioblastoma cells their anti-proliferation effect, which was previously reported in other cancer cell lines. Future mechanism studies will be performed on those compounds that are potential candidates for anti-cancer drugs.

## 6. Experimental

### 6.1. Chemistry

#### 6.1.1. General methods

Melting points (M.p.) were determined on a Yanaco MP-500 micro melting point apparatus and were uncorrected. Infrared

spectra were measured with a Nicolet Avatar 360 FT-IR spectrometer using film KBr pellet techniques. $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ or $CD_3OD$ on a Bruker 400 spectrometer with tetramethylsilane as an internal standard. Chemical shifts are expressed in $\delta$ (ppm) units downfield from TMS. Mass spectra were recorded by a Bruker Dalton ESquire 3000 plus liquid chromatography-mass spectrum (direct injection). Optical rotations were measured with a Perkin–Elmer 341 automatic polarimeter. Diastereoselectivities and enantioselectivities were determined by chiral HPLC analysis using a Shimadzu LC-10AT VP series and a Shimadzu SPD-M10Avp photo diode array detector (190–370 nm) with a Chiralcel OJ-H column using *n*-hexane/*i*-PrOH (98:2, *v/v*) as a mobile phase. Flash column chromatography was carried out with silica gel (300–400 mesh). THF was distilled over sodium benzophenone ketyl under $N_2$.

#### 6.1.2. (2S,3R)-2-(Dibenzylamino)octadecan-3-ol (**13a**)

To a cooled (−78 °C) solution of $(COCl)_2$ (0.2 mL, 1.96 mmol) in $CH_2Cl_2$ (2 mL) was added DMSO (0.3 mL, 3.92 mmol). After stirring for 10 min, (*S*)-**12** [44] (200 mg, 0.78 mmol) in $CH_2Cl_2$ (2 mL) was carefully added. 30 min later, $Et_3N$ (0.6 mL, 3.92 mmol) was carefully added and the mixture was warmed to room temperature and stirred for 1 h. Then the mixture was cooled to −78 °C again and a solution of *n*-$C_{15}H_{31}MgBr$ (3.12 mmol in 6 mL of $Et_2O$) was added. The resultant mixture was stirred for 40 min. After warming to −40 °C and stirring for 20 min, the mixture was warmed to room temperature, stirred for 1 h, before quenching with a saturated $NH_4Cl$ (8 mL). The mixture was extracted with EtOAc (10 mL × 3). The organic phases were washed with brine, dried over $Na_2SO_4$, filtered and concentrate in vacuo. The residue was purified by flash chromatography on silica gel (eluent: EtOAc/PE 1:30) to give **13a** (248 mg, 0.53 mmol, 68%) as a colorless oil. $[\alpha]_D^{20}$ +17.4 (*c* 0.3, $CHCl_3$); IR (film) $\nu_{max}$: 3381, 2927, 2841, 1494, 1454, 1375, 1027 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.88 (t, *J* = 6.8 Hz, 3H), 1.10 (d, *J* = 6.8 Hz, 3H), 1.13–1.34 (m, 27H), 1.64–1.74 (m, 1H), 1.78 (s, 1H), 2.67–2.76 (m, 1H), 3.52 (d, *J* = 13.8 Hz, 2H), 3.56–3.63 (m, 1H), 3.76 (d, *J* = 13.8 Hz, 2H), 7.17–7.37 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 14.1, 22.7, 25.9, 29.4, 29.6, 29.7, 31.9, 34.3, 54.8, 57.3, 73.7, 126.9, 128.2, 128.8, 140.2; MS (ESI, *m/z*) 466.3 (M + H$^+$, 100). HRMS *m/z* calcd for $C_{32}H_{51}NO$ (M + H$^+$): 466.4043; found: 466.4041.

#### 6.1.3. (2S,3R)-2-(Dibenzylamino)dodecan-3-ol (**13b**)

Using the procedure described for the synthesis of the compound **13a**, reaction of (*S*)-**12** (500 mg, 1.96 mmol) with *n*-nonyl magnesium bromide (8.00 mmol in 16 mL of $Et_2O$) gave **13b** (519 mg, 1.31 mmol, 67%) as a colorless oil. $[\alpha]_D^{20}$ +28.0 (*c* 0.2, $CHCl_3$); IR (film) $\nu_{max}$: 3365, 2917, 2853, 1607, 1454, 1375, 1119 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.88 (t, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 1.14–1.42 (m, 15H), 1.62–1.74 (m, 1H), 1.79 (s, 1H), 2.67–2.74 (m, 1H), 3.47 (d, *J* = 13.8 Hz, 2H), 3.56–3.62 (m, 1H), 3.76 (d, *J* = 13.8 Hz, 2H), 7.19–7.35 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 14.1, 22.7, 25.9, 29.3, 29.6, 29.6, 29.7, 31.9, 34.4, 54.8, 57.3, 73.7, 126.9, 128.2, 128.8, 140.2; MS (ESI, *m/z*) 382.2 (M + H$^+$, 100). Anal. calcd for $C_{26}H_{39}NO$: C, 81.84; H, 10.30; N, 3.67. Found: C, 81.81; H, 10.18; N, 3.66.

#### 6.1.4. (2S,3R)-2-(Dibenzylamino)tetradecan-3-ol (**13c**)

Using the procedure described for the synthesis of the compound **13a**, reaction of (*S*)-**12** (519 mg, 2.02 mmol) with *n*-$C_{11}H_{23}MgBr$ (8.08 mmol in 16 mL of $Et_2O$) gave **13c** (604 mg, 1.48 mmol, 73%) as a colorless oil. $[\alpha]_D^{20}$ +20.4 (*c* 0.5, $CHCl_3$). IR (film) $\upsilon_{max}$: 3361, 2922, 2847, 1602, 1453, 1366, 1084 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.92 (t, *J* = 6.9 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.18–1.32 (m, 19H), 1.68–1.78 (m, 1H), 1.79 (s, 1H), 2.71–2.77 (m, 1H), 3.51 (d, *J* = 13.8 Hz, 2H), 3.59–3.67 (m, 1H), 3.80 (d, *J* = 13.8 Hz, 2H), 7.19–7.41 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 14.1, 22.7, 25.9, 29.4, 29.6, 29.6, 29.6, 29.6, 29.7, 31.9, 34.3, 54.8, 57.3, 73.7, 126.9, 128.2, 128.8, 140.2; MS (ESI, *m/z*) 410.2 (M + H$^+$, 100). Anal. calcd. for $C_{28}H_{43}NO$: C, 82.09; H, 10.58; N, 3.42. Found: C, 82.45; H, 10.33; N, 3.64.

#### 6.1.5. (2R,3S)-2-(Dibenzylamino)dodecan-3-ol (**13d**)

Using the procedure described for the synthesis of the compound **13a**, reaction of (*R*)-**12** [44] (252 mg, 0.96 mmol) with *n*-$C_9H_{19}MgBr$ (3.84 mmol in 8 mL of $Et_2O$) gave **13d** (260 mg, 0.66 mmol, 67%) as a colorless oil. $[\alpha]_D^{20}$ −28.0 (*c* 0.2, $CHCl_3$); IR (film) $\nu_{max}$: 3365, 2917, 2853, 1607, 1454, 1375, 1119 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.88 (t, *J* = 6.9 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 1.14–1.42 (m, 15H), 1.62–1.74 (m, 1H), 1.79 (s, 1H), 2.67–2.74 (m, 1H), 3.47 (d, *J* = 13.8 Hz, 2H), 3.56–3.62 (m, 1H), 3.76 (d, *J* = 13.8 Hz, 2H), 7.19–7.35 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 14.1, 22.7, 25.9, 29.3, 29.6, 29.6, 29.7, 31.9, 34.3, 54.8, 57.3, 73.6, 126.9, 128.2, 128.7, 140.2; MS (ESI, *m/z*) 382.2 (M + H$^+$, 100). Anal. calcd for $C_{26}H_{39}NO$: C, 81.84; H, 10.30; N, 3.67. Found: C, 81.81; H, 10.18; N, 3.66.

#### 6.1.6. (Z,2R,3S)-2-(Dibenzylamino)dodec-8-en-3-ol (**13e**)

Using the procedure described for the synthesis of the compound **13a**, reaction of (*R*)-**12** (519 mg, 2.02 mmol) with the Grignard reagent **19** (9.09 mmol in 18 mL of $Et_2O$) gave **13e** (561 mg, 1.48 mmol, 73%) as a colorless oil. $[\alpha]_D^{20}$ −22.6 (*c* 0.2, $CHCl_3$); IR (film) $\nu_{max}$: 3398, 2971, 2930, 2842, 1582, 1457, 1262, 1084, 1030 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.92 (t, *J* = 7.3 Hz, 3H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.18–1.31 (m, 5H), 1.31–1.47 (m, 6H), 1.63 (s, 1H), 1.61–1.82 (m, 1H), 2.70–2.78 (m, 1H), 3.49 (d, *J* = 13.8 Hz, 2H), 3.57–3.66 (m, 1H), 3.79 (d, *J* = 13.8 Hz, 2H), 5.35–5.42 (m, 2H), 7.21–7.53 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 13.8, 22.9, 25.6, 29.3, 29.7, 29.8, 34.2, 54.8, 57.3, 73.6, 126.9, 128.2, 128.8, 129.8, 129.9, 140.2; MS (ESI, *m/z*) 380.3 (M + H$^+$, 100). HRMS *m/z* calcd for $C_{26}H_{37}NO$ (M + H$^+$): 380.2947; found: 380.2941.

#### 6.1.7. (2R,3S)-2-(Dibenzylamino)-11-methyldodecan-3-ol (**13f**)

Using the procedure described for the synthesis of the compound **13a**, reaction of (*R*)-**12** (508 mg, 1.98 mmol) with the Grignard reagent **25** (7.92 mmol in 16 mL of $Et_2O$) gave **13f** (577 mg, 1.43 mmol, 72%) as a colorless oil. $[\alpha]_D^{25}$ −32.0 (*c* 0.1, $CHCl_3$); IR (film) $\nu_{max}$: 3396, 3024, 2951, 2927, 2850, 2808, 1942, 1722, 1607, 1494, 1451, 1360 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 0.92 (d, *J* = 6.6 Hz, 6H), 1.15 (d, *J* = 6.8 Hz, 3H), 1.19–1.23 (m, 2H), 1.26–1.37 (m, 11H), 1.58–1.20 (m, 1H), 1.68–1.80 (m, 1H), 1.82 (s, 1H), 2.71–2.81 (m, 1H), 3.52 (d, *J* = 13.8 Hz, 2H), 3.62–3.68 (m, 1H), 3.82 (d, *J* = 13.8 Hz, 2H), 7.21–7.53 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 8.7, 22.7, 26.0, 27.5, 28.0, 29.7, 29.7, 29.9, 34.3, 39.1, 54.8, 57.3, 73.7, 126.9, 128.3, 128.8, 140.2; MS (ESI, *m/z*) 396.3 (M + H$^+$, 100). HRMS *m/z* calcd for $C_{27}H_{41}NO$ (M + H$^+$): 396.3266; found: 396.3264.

#### 6.1.8. (2S,3R)-Sphingosine (ES-285, **2**)

A suspension of compound **13a** (112 mg, 0.24 mmol) and 10% Pd/C (30 mg) in MeOH (3 mL) was stirred for 28 h under hydrogen atmosphere (1 atm) at room temperature. When the reaction was monitored to be complete by TLC analysis, the mixture was filtered and the solid phase was washed with methanol 5 times. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica gel (eluent: MeOH/$CH_2Cl_2$ 1:10) to give compound **2** [27] (56 mg, 0.20 mmol, 84%) as a white solid. M.p. 65–66 °C (EtOAc/PE) {lit. [27] M.p. 64.5–66 °C (EtOAc/PE)}; $[\alpha]_D^{20}$ +24.2 (*c* 1.0, $CHCl_3$) {lit. [27] $[\alpha]_D^{25}$ +24.0 (*c* 1.0, $CHCl_3$)}; IR (film) $\nu_{max}$: 3323, 2917, 2844, 1591, 1479, 1366, 1061 cm$^{-1}$; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 0.91 (t, *J* = 6.9 Hz, 3H), 1.05 (d, *J* = 6.6 Hz, 3H), 1.28–1.68 (m, 28H), 2.75–2.87 (m, 1H), 3.44 (m, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$: 14.5, 17.0, 23.8, 27.4, 30.6, 30.9, 30.9, 30.9, 30.9, 30.9, 30.9, 33.2, 34.1, 52.3, 76.3; MS (ESI, *m/z*) 286.2 (M + H$^+$, 100).

*6.1.9. (2S,3R)-2-Aminododecan-3-ol (**9**)*

Using the procedure described for the synthesis of the compound **2**, reaction of **13b** (65 mg, 0.17 mmol) with 10% Pd/C (20 mg) gave **9** (29 mg, 0.14 mmol, 84%) as a white solid. M.p. 63–64 °C (EtOAc/PE); $[\alpha]_D^{20}$ +4.2 (*c* 1.5, MeOH) {lit. [21] $[\alpha]_D^{29}$ +4.5 (*c* 0.22, MeOH)}; IR (film) $\nu_{max}$: 3335, 2914, 2847, 1582, 1481, 1390, 1079 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.90 (t, $J$ = 6.9 Hz, 3H), 1.05 (d, $J$ = 6.6 Hz, 3H), 1.28–1.60 (m, 16H), 2.82 (ddd, $J$ = 2.1, 4.2, 6.2 Hz, 1H), 3.44 (m, 1H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 12.5, 15.0, 21.7, 25.3, 28.5, 28.7, 28.8, 28.8, 31.1, 32.0, 50.2, 74.3; MS (ESI, *m/z*) 202.1 (M + $H^+$, 100). HRMS *m/z* calcd for $C_{12}H_{27}NO$ (M + $H^+$): 202.2171; found: 202.2170.

*6.1.10. (2S,3R)-Xestoaminol C (**10**)*

Using the procedure described for the synthesis of the compound **2**, reaction of **13c** (85 mg, 0.21 mmol) with 10% Pd/C (26 mg) gave **10** (43 mg, 0.19 mmol, 90%) as a white solid. M.p. 63–64 °C (EtOAc/PE); $[\alpha]_D^{20}$ + 12.0 (*c* 0.5, MeOH) {lit. [22] $[\alpha]_D^{20}$ +7.0 (*c* 0.14, MeOH)}; IR (film) $\upsilon_{max}$: 3432, 3336, 2913, 2843, 1582, 1462, 1379, 1042 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.85 (t, $J$ = 6.9 Hz, 3H), 0.98 (d, $J$ = 6.6 Hz, 3H), 1.28–1.58 (m, 20H), 2.75 (ddd, $J$ = 2.1, 4.2, 6.2 Hz, 1H), 3.35 (m, 1H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 14.2, 17.1, 23.5, 27.1, 30.0, 30.3, 30.5, 30.6, 30.6, 30.6, 32.9, 33.8, 51.9, 76.4; MS (ESI, *m/z*) 230.2 (M + $H^+$, 100); HRMS *m/z* calcd for $C_{14}H_{31}NO$ (M + $H^+$): 230.2484; found: 230.2480.

*6.1.11. (2R,3S)-Clavaminol A (**3**)*

Using the procedure described for the synthesis of the compound **2**, reaction of **13d** (98 mg, 0.24 mmol) with 10% Pd/C (29 mg) gave **3** (43 mg, 0.21 mmol, 84%) as a white solid. M.p. 63–64 °C (EtOAc/PE); $[\alpha]_D^{20}$ −4.2 (*c* 0.5, MeOH) {lit. [19] $[\alpha]_D^{25}$ −4.25 (*c* 0.0094, MeOH)}; IR (film) $\nu_{max}$: 3335, 2914, 2847, 1582, 1481, 1390, 1079 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.90 (t, $J$ = 6.9 Hz, 3H), 1.04 (d, $J$ = 6.7 Hz, 3H), 1.28–1.60 (m, 16H), 2.82 (ddd, $J$ = 2.1, 4.2, 6.2 Hz, 1H), 3.44 (m, 1H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 12.46, 15.09, 21.75, 25.28, 28.48, 28.75, 28.80, 28.84, 31.08, 31.98, 50.15, 74.36; MS (ESI, *m/z*) 202.1 (M + $H^+$, 100). HRMS *m/z* calcd for $C_{12}H_{27}NO$ (M + $H^+$): 202.2171; found: 202.2170.

*6.1.12. (2R,3S)-Clavaminol B (**4**)*

A solution of compound **13e** (76 mg, 0.20 mmol) in anhydrous MeOH (3.5 mL) and HCOOH (0.4 mL) was added to a suspension of 10% Pd/C (20 mg). The reaction mixture was stirred for 3 h at room temperature and under a nitrogen atmosphere. When the reaction was monitored to be complete by TLC analysis, the mixture was filtered and the solid phase was washed with methanol 5 times. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography on silica gel (eluent: MeOH/$CH_2Cl_2$ 1:10) to give **4** (36 mg, 0.18 mmol, 89%) as a white solid. M.p. 64–66 °C (EtOAc/PE); $[\alpha]_D^{20}$ −8.3 (*c* 0.2, MeOH) {lit. [19] $[\alpha]_D^{25}$ −5.0 (*c* 0.001, MeOH)}; IR (film) $\nu_{max}$: 3387, 2948, 2944, 2920, 1603, 1466, 1396, 1265, 1054, 798 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.83 (t, $J$ = 6.8 Hz, 3H), 1.01 (d, $J$ = 6.6 Hz, 3H), 1.21–1.45 (m, 12H), 2.85 (ddd, $J$ = 2.7, 3.8, 6.6 Hz, 1H), 3.45 (m, 1H), 5.38 (m, 2H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 11.4, 13.0, 20.7, 27.4, 27.7, 27.7, 30.1, 30.9, 49.2, 72.3, 132.9, 132.9; MS (ESI, *m/z*) 200.2 (M + $H^+$, 100). HRMS *m/z* calcd for $C_{12}H_{25}NO$ (M + $H^+$): 200.2014; found: 200.2012.

*6.1.13. (2R,3S)-2-Amino-11-methyldodecan-3-ol (**8**)*

Using the procedure described for the synthesis of the compound **2**, reaction of **13f** (91 mg, 0.23 mmol) with 10% Pd/C (27 mg) gave **8** (43 mg, 0.20 mmol, 86%) as a white solid. M.p. 65–67 °C (EtOAc/PE); $[\alpha]_D^{25}$ −4.5 (*c* 0.22, MeOH); IR (film) $\nu_{max}$: 3389, 2923, 2853, 2521, 1747, 1463, 1383 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.88 (d, $J$ = 6.6 Hz, 6H), 1.05 (d, $J$ = 6.6 Hz, 3H), 1.11–1.22 (m, 2H), 1.22–1.45 (m, 11H), 1.46–1.63 (m, 2H), 2.74–2.87 (m, 1H), 3.37–3.51 (m, 1H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 14.8, 21.0 (2C), 25.2, 26.5, 27.2, 28.8, 29.0, 32.0, 38.3, 50.2, 74.2; MS (ESI, *m/z*) 216.2 (M + $H^+$, 100). HRMS *m/z* calcd for $C_{12}H_{26}NO$ (M + $H^+$): 216.2327; found: 216.2320.

*6.1.14. (2R,3S)-1-(tert-Butyldimethylsilyloxy)-2-(dibenzylamino) dodecan-3-ol (**29**)*

Using the procedure described for the synthesis of the compound **13a**, reaction of **28** [58] (302 mg, 0.78 mmol) with *n*-$C_9H_{19}MgBr$ (3.12 mmol in 7 mL of $Et_2O$) gave **29** (258 mg, 0.51 mmol, 65%) as a colorless oil. $[\alpha]_D^{20}$ +12.8 (*c* 1.0, $CHCl_3$); IR (film) $\nu_{max}$: 3457, 2930, 2850, 1597, 1448, 1250, 1088 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$: 0.09 (s, 3H), 0.11 (s, 3H), 0.86–0.98 (m, 12H), 1.18–1.36 (m, 15H), 1.68–1.79 (m, 1H), 2.63–2.69 (m, 1H), 2.97 (s, 1H), 3.62 (d, $J$ = 13.8 Hz, 2H), 3.82–3.91 (m, 3H), 3.94–4.05 (m, 2H), 7.19–7.35 (m, 10H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$: −5.6, −5.6, 14.1, 18.1, 22.7, 25.5, 25.9 (2C), 29.4, 29.6, 29.6, 29.7, 31.9, 34.5, 55.3, 61.3, 72.3, 126.9, 128.2, 128.8, 140.1; MS (ESI, *m/z*) 512.3 (M + $H^+$, 100). HRMS *m/z* calcd for $C_{32}H_{53}NO_2Si$ (M + $H^+$): 512.3918; found: 512.3915.

*6.1.15. (2R,3S)-Aminoldodecan-1,3-diol (**7**·**HCl**)*

Using the procedure described for the synthesis of the compound **2**, reaction of **29** (100 mg, 0.19 mmol) and a few drops of 6 N HCl with 10% Pd/C (30 mg) gave **7**·HCl (59 mg, 0.14 mmol, 70%) as a white solid. M.p. 78–80 °C (EtOAc/PE); $[\alpha]_D^{20}$ −6.3 (*c* 0.3, MeOH) {lit. [28] $[\alpha]_D^{20}$ −6.3 (*c* 0.3, MeOH)}; IR (film) $\nu_{max}$: 3394, 3312, 2922, 2847, 1587, 1472, 1381, 1060 $cm^{-1}$; $^1H$ NMR (400 MHz, $CD_3OD$) $\delta$: 0.90 (t, $J$ = 6.9 Hz, 3H), 1.26–1.59 (m, 16H), 1.61–2.81 (m, 1H), 3.48 (dd, $J$ = 10.8, 7.7 Hz, 1H), 3.52–3.68 (m, 1H), 3.73 (dd, $J$ = 10.8, 4.0 Hz, 1H); $^{13}C$ NMR (100 MHz, $CD_3OD$) $\delta$: 15.2, 24.5, 27.8, 31.2, 31.3, 31.5, 33.8, 35.0, 59.3, 59.7, 71.1; MS (ESI, *m/z*) 218.1 (M + $H^+$, 100).

*6.2. Pharmacology*

*6.2.1. Cell culture*

The human glioblastoma cell SHG-44 was purchased from ATCC (Shanghai, PRC) and cultured in DMEM medium (Thermo Scientific, Shanghai, PRC) supplemented with 10% fetal bovine serum, penicillin (50 U/mL) and streptomycin (50 μg/mL). Cells were maintained in 37 °C incubator with a 5% $CO_2$ humidified atmosphere. For viability assay, cells were plated onto 96-well plate with a density of 5000/well and cultured in DMEM medium containing 1% fetal bovine serum.

*6.2.1. Cell viability assay*

Cell viability was assessed by using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan) following the manufacture instruction. Briefly, 5000 cells/well in 96-well plate were cultured overnight, then incubated with compound with different concentrations (0.1–100 μM) and vehicle (0.1% Dimethyl sulfoxide, DMSO). After 24 h incubation, 10 μl of CCK-8 solution was added and incubated at 37 °C for 2 h. Plate was measured for absorbance at 450 nm using a microplate reader (Kehua Bio-engineering, Shanghai, PRC). Cell viability data was obtained from triplicate experiments and expressed as the percentage of compound-treated relative to vehicle-treated.

## Conflict of interest

None.

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.09.010.

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