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Short communication

Design, synthesis and anticancer activity evaluation of novel C14 heterocycle substituted epi-triptolide

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ABSTRACT

Two series of novel C14 heterocycle substituted epi-triptolide derivatives as potential anticancer agents were synthesized and tested for their cytotoxicity against SKOV-3 and PC-3 tumor cell lines. The introduction of C14β-aryl heterocycle aminomethyl substituent to the leading compound was found to be an effective modification method to retain the potent anticancer activity. Meanwhile, the series of epi-triptolide derivatives (**21**–**40**) with C14α-hydroxyl group, still retained the natural product's cytotoxicity. This is apparently challenges the classical structure–activity relationship of triptolide that considers the C14β-hydroxyl group to be essential for its anticancer activity.

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1. Introduction

Triptolide (1, Fig. 1), a diterpenoid triepoxide, was originally isolated from extracts of *Tripterygium wilfordii* Hook F (TWHF) [1]. It possesses potent antitumor, antiinflammatory, immunosuppressive, and antifertility activities [2–23]. At the cellular level, triptolide shows strong antiproliferative activity, and it inhibits the proliferation of all 60 cancer cell lines of US National Cancer Institute with IC_{50} values in a low nanomolar range (average $IC_{50} = 12$ nM). Meanwhile, triptolide interferes with a number of transcription factors including NF-κB, HSP70, p53, NF-AT and HSF-1 at the molecular level [8,19,23]. It also inhibits the growth of xenograft models formed by different solid tumor cells [11]. Compared with some conventional chemotherapeutic drugs, triptolide has similar or even superior anticancer activity, especially over p53 mutated or multi-drug resistant cells [14]. All the studies mentioned above greatly support its potential development as an antineoplastic agent. However, its clinical development has been hindered by its severe toxicity and poor water solubility.

Previous studies on the structure–activity relationship (SAR) of triptolide indicated that the hydrogen-bond between C9,C11-epoxide and C14β-hydroxyl group might play a key role in exerting its antitumor activity [2], and the configuration of the C14-hydroxyl group should be β orientation. Based on this principle, for a long time, the aim of C14 modification was limited to improve the natural compound's water-solubility by carboxylation of C14-hydroxyl group with water-solubility enhancing fragments. For example, C14-succinyl triptolide sodium salt (**4**, Fig. 1) and minnelide (**5**, Fig. 1) are both prodrugs that will convert to triptolide in serum and retain all the toxicity of triptolide [21,22]. However, Takaya and Li group respectively found that substitution of C14β-hydroxyl group with β-fluoride ((14*R*)-fluorotriptolide (**6**), Fig. 1) and a chiral epoxide group ((14*S*)-14, 21-epoxytriptolide (**7**), Fig. 1) could also retain the cytotoxicity of the natural compound [24–26]. So, the SAR of triptolide cannot be explained as simply as before.

The introduction of nitrogen is usually a useful tactic in the natural product modification. In fact, the synthetic drugs generally contain more nitrogen than the natural products, because nitrogen can carry a positive charge and act as a hydrogen bond acceptor or donor that strongly influence the interaction between the medicinal agent and its target molecule [27–29]. In addition, the p*K*a values of amines are often in the range of physiological pH, which is essential for improving the bioavailability of drugs [30]. Moreover, *N*-heterocycles are ubiquitous in a variety of natural products and biologically active molecules [31], and they have been assigned as privileged structures in drug development, because *N*-heterocyclic

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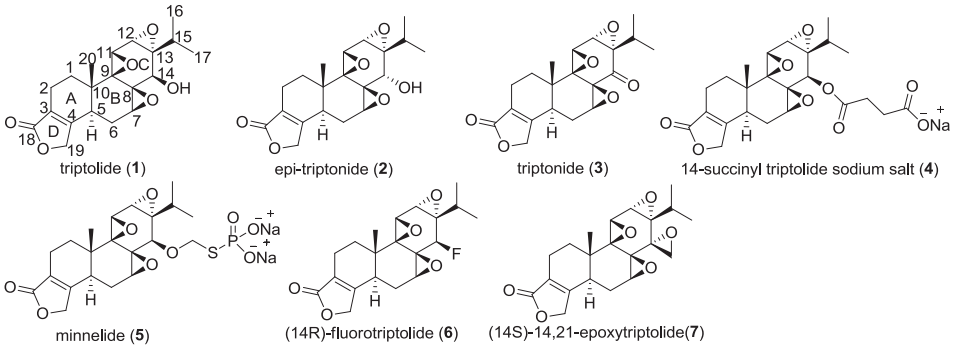
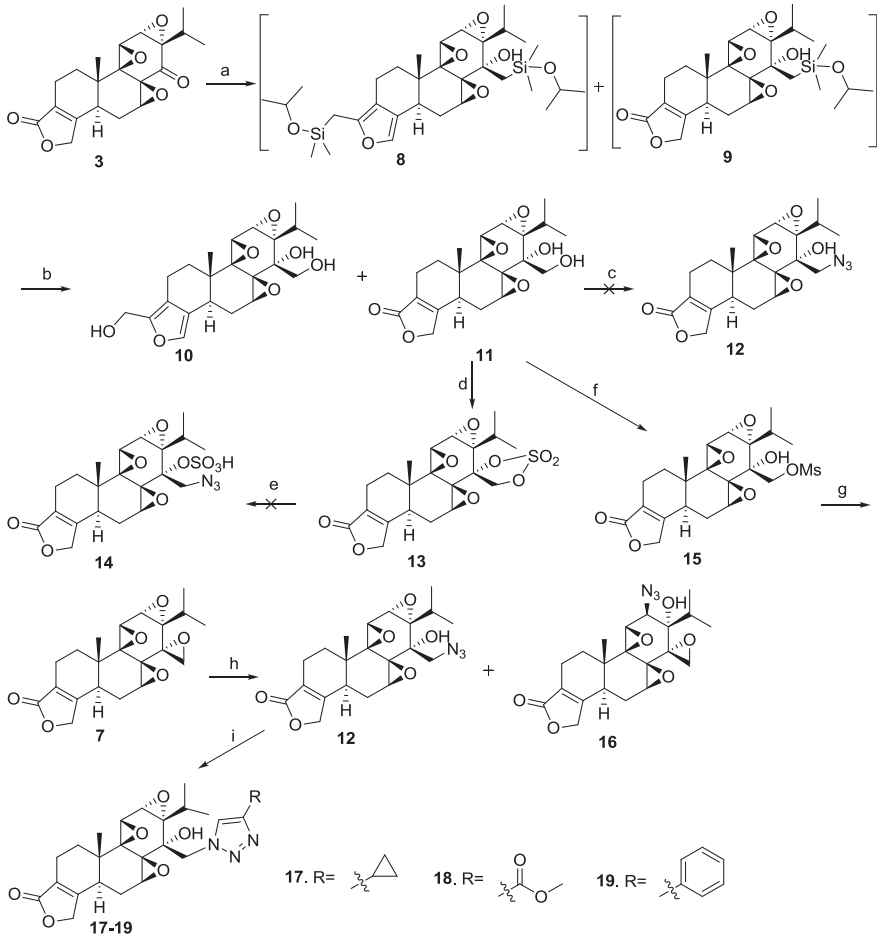


Fig. 1. Structure of triptolide, epi-triptolide, triptonide, 14-succinyl triptolide sodium salt, (14*R*)-fluorotriptolide, minnelide, and (14*S*)-14,21-epoxytriptolide.

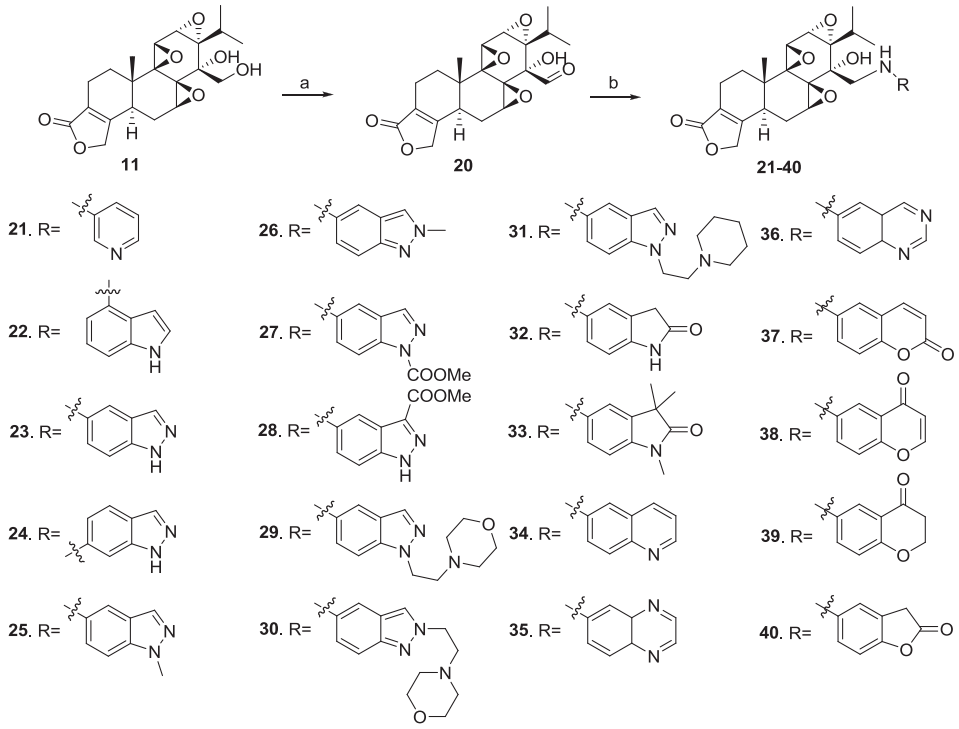
moieties often exhibit improved solubility and can facilitate the salt formation property, both of which are important for oral absorption and bioavailability [32]. Based on these principle, we designed two series of novel C14 heterocycle substituted epi-triptolide derivatives by introducing of 1H-1,2,3-triazolylmethyl substituents (compounds **17**–**19**, Scheme 1) and aryl heterocycle aminomethyl substituents (compounds **21**–**42**, Schemes 2 and 3). Their anticancer activities were evaluated by ovary (SKOV-3) and prostate (PC-3) tumor cell lines.

2. Chemistry

The synthetic strategy followed for the preparation of the analogs **17**–**19** and **21**–**40** is depicted in Schemes 1–3. We used triptonide (**3**), which was extracted from TWHF of our region, as the starting material. Treatment of **3** with (isopropoxy-dimethylsilyl) methylmagnesium chloride in THF, and subsequently Tamao oxidation yielded a 1:2 mixture of triol **10** (28%) and diol **11** (59%) [25,33]. With diol **11** in hand, we then switched to the synthesis of



Scheme 1. Synthesis of compounds **17**–**19**. Reagents and conditions: (a) (isopropoxy-dimethylsilyl)methyl chloride, Mg, THF, reflux 45 min; (b) KF, $KHCO_3$, 30% H_2O_2, 0 °C, 2 h, **10** (28% over 2 steps), **11** (59% over 2 steps); (c) Ph_3P, DEAD, DPPA, CH_2Cl_2, rt, 8 h, decomposition of **11**; (d) (1) $SOCl_2$, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (2) $NaIO_4$, $RuCl_3{\cdot}3H_2O$, CH_3CN/H_2O = 4:1, rt, 15 min, 86%; (e) NaN_3, DMF, rt to 100 °C, decomposition of **13**; (f) MsCl, TEA, CH_2Cl_2, 0 °C to rt, 1.5 h, 97%; (g) K_2CO_3, CH_3OH, rt, 20 min, 90% or NaN_3, THF, rt, 30 min, 91%; (h) NaN_3, NH_4Cl, 2-methoxyethanol, 80 °C, 48 h, **16** (53%) and **12** (27%); (i) alkyne, sodium ascorbate, $CuSO_4{\cdot}5H_2O$, *t*-BuOH–H_2O 1:1, 50 °C. Ph_3P = Triphenylphosphane, DEAD = diethyl diazenedicarboxylate, DPPA = diphenylphosphoryl azide.

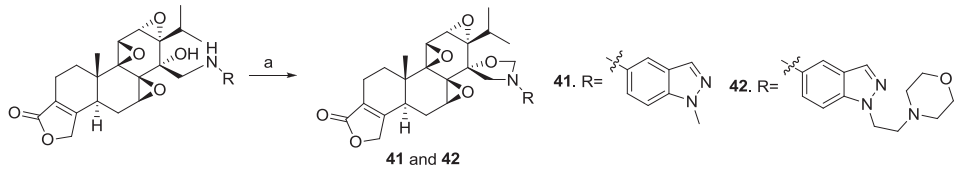


Scheme 2. Synthesis of derivatives **21**–**40**. Reagents and conditions: (a) TEMPO, Trichloroisocyanuric acid, CH_2Cl_2, 0 °C, 1 h, 81%; (b) NH_2R, $NaBH(OAc)_3$, CH_2Cl_2, rt; TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl.

the key azide compound **12**. Unfortunately, the transformation of diol **11** to azide **12** was found to be problematic. Initially, diol **11** treated directly with Ph_3P, DEAD and DPPA failed to yield the desired azide **12**. Only decomposition of starting material was observed under these conditions. Next, transformation of diol **11** to the corresponding cyclic sulfate **13**, followed by nucleophilic azide reaction, also failed to produce the desired product. Further, we tried the selective mesylation of the primary alcohol in **11**, followed by reaction with NaN_3, but none of the desired azide **12** was yielded. Instead, the intramolecular cyclization product (14*S*)-14,21-epoxytriptolide **7** was afforded in high yield (91%). From an inspection of a molecular model of **7**, it appeared that the nucleophilic substitution reaction may occur at the less-hindered C12,C13-epoxide and C14,C21-epoxide of **7**. Fortunately, upon reaction of **7** with azide sodium in 2-methoxyethanol at 80 °C, a 1:2 mixture of C12-azide **16** (53%) and C21-azide **12** (27%) were produced. Subsequently, reaction of **12** with various alkyne produced 1*H*-1,2,3-triazole analogs **17**–**19** in moderate to high yield. Then the focus was shifted to the synthesis of aryl heterocycle aminomethyl substituent analogs **21**–**40**. After surveying a variety of oxidation conditions, including Dess-Martin periodinane, TPAP/NMO, PCC, IBX, we determined that TEMPO/trichloroacetonitrile system was the most effective condition for oxidizing diol **11** to the corresponding aldehyde **20** (97%). Subsequently, the reductive amination of compound **20** with a variety of aryl heterocycle amine yielded analogs **21**–**40**. Further, protection of the C14-hydroxy group and C21-amino group of compounds **25** and **29** with methylene group produced C21-amino group conformation bonding compounds **41** and **42** in high yield.

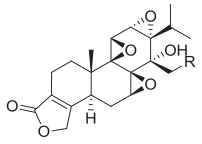
3. Results and discussion

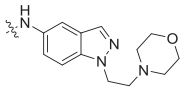
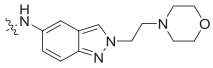
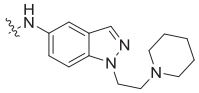
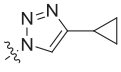
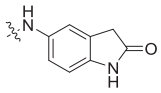
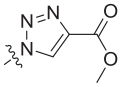
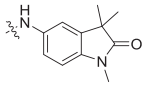
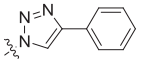
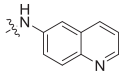
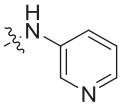
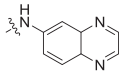
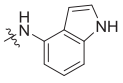
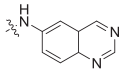
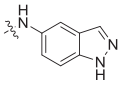
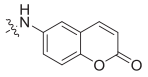
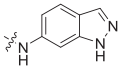
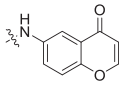
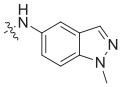
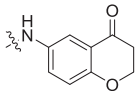
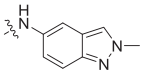
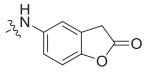
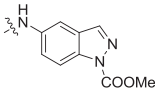
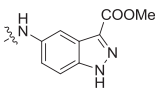
As shown above, we obtained two series of novel C14 heterocycle substituted epi-triptolide analogs. To examine whether the substitution affected their biological activities, we evaluated the anticancer effects of these target compounds (**17**–**19** and **21**–**42**) against two human tumor cell lines derived from ovary (SKOV-3) and prostate (PC-3) using sulforhodamine B (SRB) assays [34]. The result revealed that although there was no free C14β-hydroxyl group in our new derivatives, the series of C14β-aryl heterocycle aminomethyl substituted analogs **21**–**40** were all effective against these two cell lines, with IC_{50} values ranging from 11.7 to 2807 nM (Table 1). Among them, compound **33** exhibited the highest potency, with the lowest IC_{50} value (11.7 nM for SKOV-3 cells). Generally, electron rich aryl heterocycle amines substituted analogs were more potent than electron deficient aryl heterocycle amines substituted analogs, and as for the indazole aminomethyl substituted analogs **23**–**31**, there were no significant substituent effect. Moreover, the conformation bonding compounds **41** and **42**, both of which were impossible to form the similar hydrogen bond as that of triptolide, still retained the cytotoxic activity, albeit a little less potent. This indicated that the hydrogen bond of C21-amino



Scheme 3. Synthesis of derivatives **41** and **42**. Reagents and conditions: (a) 37% aqueous formaldehyde, 30% aqueous acetic acid, acetonitrile, rt.

Table 1
In vitro anticancer activity of triptolide derivatives in SKOV-3, and PC-3 cells.



Compound	R	IC_{50} (nM)		Compound	R	IC_{50} (nM)	
		SKOV-3	PC-3			SKOV-3	PC-3
1	–	6	20	**29**		36.4	36.6
2	–	790	1320	**30**		77	40.8
7	–	100	270	**31**		99	66.1
17		>10,000	>10,000	**32**		38.4	54.1
18		>10,000	>10,000	**33**		11.7	84.9
19		>10,000	>10,000	**34**		85	452
21		745.4	2807	**35**		368.7	157
22		53.8	97.8	**36**		274	781
23		17.4	25.3	**37**		87.2	353
24		49	188	**38**		367.9	341
25		15	132	**39**		32.1	172
26		22.3	97	**40**		12.5	67.9
27		102	188	**41**	–	79.7	365
28		35.8	264	**42**	–	152	253

group and C9,C11-epoxide might not play a critical role in retaining the potent anticancer activity. But, unfortunately, C14β-1*H*-1,2,3-triazolylmethyl substituted analogs **17**–**19** all lost their cytotoxicity against PC-3 and SKOV-3 cell lines. On the whole, our result showed that the introduction of C14β-aryl heterocycle aminomethyl substituent to the leading compound was an effective modification method to retain the potent anticancer activity. Meanwhile, our result apparently challenges the classical structure–activity relationship of triptolide (**1**) that considers the C14β-hydroxyl group to be essential for its anticancer activity [2]. And these indicate that the C14β-hydroxyl group of triptolide is not unchangeable even in order to generate analogs with potent anticancer activity.

4. Conclusions

Two series of novel derivatives of triptolide as potential anticancer agents were synthesized and tested for their cytotoxicity against two human tumor cell lines. The introduction of C14β-heterocycle aminomethyl substituent to the natural product was found to be an effective modification method to retain the potent anticancer activity. Meanwhile, in our study, the series of epitriptolide derivatives (**21**–**40**) with C14α-hydroxyl group, still retained the natural product's cytotoxicity. This will add new SAR to triptolide. And also, by introducing of nitrogen atom, the water solubility of leading compound may be improved and also facilitate the salt formation property, both of which are important for oral absorption and bioavailability. Furthermore, C14β-aryl heterocycle aminomethyl substituent analogs (**21**–**40**) generally possess strong fluorescence, which makes the series possible as molecular fluorescent probes to study the mechanism of triptolide with its target molecule.

5. Experimental

5.1. Chemistry

5.1.1. General

Mass spectra were obtained on Finnigan MAT 95 (EI), ThermoDFS (EI) or Finnigan MAT 900 (ESI) spectrometers, high resolution mass spectra on a Finnigan MAT 95 (HR-EIMS) or on a Finnigan MAT 900 (HR-ESI-MS). ^{1}H and ^{13}C NMR spectra were determined on Bruker AM-300, Bruker AM-400 instruments using tetramethylsilane as internal reference. Data are presented as follows: chemical shift, multiplicity (s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, t = triplet, m = multiplet), *J* = coupling constant in hertz (Hz). The signals of the ^{13}C NMR were assigned utilizing DEPT experiments and on the basis of literature data. Silica gel 60H (200–300 mesh) manufactured by Qingdao Haiyang Chemical Group Co. (China) was used for general chromatography.

5.1.2. (3bS,5S,5aS,6S,7S,8S,9S,9aS,9bS)-(5,5a)β,(7,8)β,(9,9a)α-Tris(epoxy)-6-hydroxyl-7-isopropyl-9b-methyl-3b,4,5,5a,6,7,8,9,9a,9b,10,11-dodecahydrophenanthro[2,1-c]furan-1,6-dimethanol (*10*) and (14S)-14β-(hydroxymethyl)epitriptolide (*11*)

Under Ar atmosphere, a portion of a solution of (isopropoxydimethylsilyl)methyl chloride (0.72 mL, 4.0 mmol) in anhydrous THF (7.0 mL) was added to Mg turnings (108 mg, 4.5 mmol). To the stirred mixture was added a few drops of 1,2-dibromoethane at 60 °C and an exothermic reaction started in several minutes. The remaining solution was added dropwise over 5 min. After the addition was completed, the gray mixture was refluxed for 45 min and then cooled to 0 °C. A solution of triptonide **3** (358 mg, 1.0 mmol) in anhydrous THF (10.0 mL) was added to the Grignard reagent (freshly prepared) at the same temperature over a few minutes. After stirring at 0 °C for 2 h, the mixture was quenched with a saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give a single adduct. To the stirred mixture of colorless crude product, MeOH (5.0 mL), THF (8.0 mL), $KHCO_3$ (416 mg, 4.2 mmol), and KF (464 mg, 4.9 mmol) was added H_2O_2 (30%, 1.1 mL, 9.71 mmol) dropwise at room temperature. The mixture was stirred at room temperature until starting material disappeared. Aqueous sat. $Na_2S_2O_3$ solution (50%) was added slowly to the mixture and stirred until a negative starch/iodide test was observed. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by chromatography on silica gel using ethyl acetate/cyclohexane (1/3 to 1/2) to provide **10** as a white solid in 28% yield (113 mg, 0.28 mmol) and **11** as a white solid in 59% yield (230 mg, 0.59 mmol).

10: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.10 (d, *J* = 1.7 Hz, 1H), 4.51 (s, 1H), 4.24 (dd, *J* = 11.6, 4.5 Hz, 1H), 3.84 (d, *J* = 3.2 Hz, 1H), 3.81 (d, *J* = 5.6 Hz, 1H), 3.66 (dd, *J* = 11.5, 8.8 Hz, 1H), 3.47 (d, *J* = 3.2 Hz, 1H), 3.22 (s, 1H), 2.98 (dd, *J* = 8.9, 4.7 Hz, 1H), 2.70 (ddd, *J* = 12.2, 6.4, 1.4 Hz, 1H), 2.59 (dd, *J* = 16.9, 4.7 Hz, 1H), 2.52–2.35 (m, 3H), 1.91 (dd, *J* = 15.3, 12.4 Hz, 1H), 1.72 (s, 1H), 1.50 (dd, *J* = 12.3, 4.0 Hz, 1H), 1.25–1.16 (m, 1H), 1.04 (s, 3H), 0.95–0.87 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 147.7, 136.7, 125.3, 116.7, 74.7, 67.6, 65.9, 65.1, 64.8, 57.2, 56.0, 55.6, 54.6, 37.4, 36.0, 31.0, 25.8, 25.5, 20.8, 18.6, 15.7, 13.0. MS (EI, 70 eV) *m*/*z* (%) 404 (M^+, 90), 387 (33), 357 (72), 71 (100), 55 (69); HRMS (EI) calcd. for $C_{22}H_{28}O_7$ (M^+): 404.1835, found 404.1833.

11: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.67 (s, 2H), 4.26 (d, *J* = 11.8 Hz, 1H), 3.87–3.80 (m, 2H), 3.64 (d, *J* = 11.5 Hz, 1H), 3.46 (d, *J* = 3.3 Hz, 1H), 2.76–2.64 (m, 1H), 2.45 (sept, *J* = 6.9 Hz, 1H), 2.37–2.25 (m, 1H), 2.23–2.04 (m, 2H), 1.89 (t, *J* = 14.1 Hz, 1H), 1.55 (dd, *J* = 12.6, 5.2 Hz, 1H), 1.25–1.13 (m, 1H), 1.07 (s, 3H), 0.91 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 160.2, 125.4, 74.4, 70.0, 67.5, 65.3, 65.2, 65.0, 56.5, 56.1, 54.4, 40.3, 36.0, 30.1, 25.5, 23.4, 20.9, 18.6, 17.1, 13.7. MS (EI, 70 eV) *m*/*z* (%) 391, ($[M + H]^+$, 2), 372 (1), 71 (100). HRMS (EI) calcd. for $C_{22}H_{28}O_7$ (M^+): 390.1679, found 390.1677. These assignments matched with those previously reported by our group [25].

5.1.3. (14S)-14-Spiro-14α, 21-sulfonyldioxytriptolide (*13*)

To a solution of compound **11** (78 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (6.0 mL) was added dry Et_3N (0.21 mL, 1.6 mmol) dropwise. The mixture was then cooled to 0 °C. Under Ar atmosphere, $SOCl_2$ (0.3 mL, 1.2 mmol) was added to the mixture carefully. After being stirred for 2 h, the mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the crude product was dissolved in CH_3CN (8.0 mL), then $NaIO_4$ (80 mg, 0.184 mmol), $RuCl_3 \cdot 3H_2O$ (12 mg, 0.056 mmol) and H_2O (2.0 mL) were added. The mixture was stirred at room temperature for 15 min. After removal of the solvent under reduced pressure, the residue was diluted with ethyl acetate and washed with water and brine and dried over anhydrous Na_2SO_4. The crude product was purified by chromatography on silica gel using acetate/cyclohexane (1/4) to provide **13** as a white solid in 86% yield (78 mg, 0.17 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.92 (d, *J* = 10.0 Hz, 1H), 4.72–4.63 (m, 3H), 3.87 (d, *J* = 2.9 Hz, 1H), 3.83 (d, *J* = 5.6 Hz, 1H), 3.65 (d, *J* = 3.0 Hz, 1H), 2.79–2.68 (m, 1H), 2.51 (sept, *J* = 6.8 Hz, 1H), 2.39–2.07 (m, 3H), 1.98 (t, *J* = 14.2 Hz, 1H), 1.53 (dd, *J* = 12.6, 5.2 Hz, 1H), 1.29–1.14 (m, 1H), 1.09 (s, 3H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H); 13C NMR ($CDCl_3$, 100 MHz) δ 172.9, 159.4, 125.6, 91.3, 73.4, 69.9, 66.3, 65.2, 61.9, 58.3, 55.7, 55.5, 40.3, 35.7, 30.2, 25.5, 23.2, 20.4, 18.3, 17.0, 13.4; ESIMS *m*/*z* 453.5

$[M + H]^+$; HRMS (ESI) calcd. for $C_{21}H_{25}SO_9$ $[M + H]^+$ 453.1219, found 453.1221. These assignments matched with those previously reported by our group [25].

*5.1.4. (14S)-14β-(Methylsulfonyloxy)epitriptolide (**15**)*

Compound **11** (0.50 g, 1.3 mmol) was dissolved in dry pyridine (4.0 mL, 49.3 mmol). The solution was cooled to 0 °C, then MsCl (0.61 mL, 7.7 mmol) was added to the solution dropwise and stirred for 10 min at room temperature. After removal of the solvent under reduced pressure, the residue was diluted with water, then extracted with ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. After concentration, the residue was purified by chromatography by ethyl acetate/cyclohexane (1/2) to provide **15** as a white solid in 97% yield (0.61 g, 1.3 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.70–4.57 (m, 4H), 3.80 (d, J = 3.2 Hz, 1H), 3.77 (d, J = 5.6 Hz, 1H), 3.47 (d, J = 3.2 Hz, 1H), 3.10 (s, 3H), 2.77–2.67 (m, 1H), 2.54 (sept, J = 6.9 Hz, 1H), 2.37–2.26 (m, 1H), 2.25–2.06 (m, 1H), 1.91 (dd, J = 14.8, 13.4 Hz, 1H), 1.54 (dd, J = 12.9, 5.1 Hz, 1H), 1.28–1.13 (m, 2H), 1.07 (s, 3H), 0.92 (d, J = 7.7 Hz, 3H), 0.89 (d, J = 7.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 159.4, 125.6, 91.3, 73.4, 69.9, 66.3, 65.2, 61.9, 58.3, 55.7, 55.5, 40.3, 35.7, 30.2, 25.5, 23.2, 20.4, 18.3, 17.0, 13.4; ESIMS m/z 469.5 $[M + H]^+$; HRMS (ESI) calcd. for $C_{22}H_{29}SO_9$ $[M + H]^+$ 469.1532, found 469.1534. These assignments matched with those previously reported by our group [25].

*5.1.5. (14S)-14, 21-Epoxytriptolide (**7**)*

To a solution of **15** (504 mg, 1.1 mmol) in CH_3OH (20.0 mL) was added K_2CO_3 (1.33 g, 9.6 mmol). The mixture was stirred for 20 min under room temperature. After removal of the solvent under reduced pressure, the residue was diluted with water and then extracted with ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. The crude product was purified via chromatography on silica gel using ethyl acetate/cyclohexane (1/4) to give **7** as a white solid in 90% yield (368 mg, 0.99 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.67 (s, 2H), 3.88 (d, J = 2.9 Hz, 1H), 3.52 (d, J = 3.1 Hz, 1H), 3.40 (d, J = 5.5 Hz, 1H), 2.84 (d, J = 5.2 Hz, 1H), 2.80–2.69 (m, 2H), 2.37–2.25 (m, 1H), 2.21–2.05 (m, 2H), 1.91–1.80 (m, 2H), 1.57 (dd, J = 12.4, 4.9 Hz, 1H), 1.29–1.17 (m, 1H), 1.06 (s, 3H), 0.86 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 160.2, 125.2, 69.9, 65.2, 65.0, 58.4, 56.8, 55.9, 55.6, 54.1, 47.9, 40.4, 35.6, 30.2, 23.4, 23.2, 19.8, 17.6, 17.0, 13.5; ESIMS m/z 373.4 $[M + H]^+$; HRMS (ESI) calcd. for $C_{22}H_{25}O_6$ $[M + H]^+$ 373.1651, found 373.1658. These assignments matched with those previously reported by our group [25].

*5.1.6. (14S)-14β-Azidomethylepitriptolid (**12**) and (12R,13R,14S)-12β-azido-13α-hydroxyl-14, 21-epoxytriptolide (**16**)*

To stirred solution of **7** (372 mg, 1.0 mmol) in 2-methoxyethanol (20.0 mL), H_2O (2 mL), NaN_3 (260 mg, 4.0 mmol), NH_4Cl (107 mg, 2.0 mmol) were added, and the mixture was stirred at 80 °C for 24 h, then cooled to room temperature. After removal of the solvent under reduced pressure, the residue was diluted with water and then extracted with ethyl acetate, washed with brine, and dried with anhydrous Na_2SO_4. The crude product was purified via chromatography on silica gel using ethyl acetate/cyclohexane (1/8 to 1/4) to give **12** as a white solid in 27% yield (112 mg, 0.27 mmol) and **16** as a white solid in 53% yield (220 mg, 0.53 mmol).

12: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.68 (s, 2H), 4.10 (d, J = 13.2, 1H), 3.80–3.77 (m, 2H), 3.62 (d, J = 13.2, 1H), 3.47 (d, J = 13.2, 1H), 2.75–2.57 (m, 2H), 2.44 (sept, J = 6.9 Hz, 1H), 2.31 (d, J = 13.8 Hz, 1H), 2.24–2.15 (m, 2H), 1.93 (t, J = 14.4 Hz, 1H), 1.55 (dd, J = 13.1 Hz, J = 5.1 Hz, 1H), 1.21–1.17 (m, 1H), 1.10 (s, 3H), 0.91 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 160.4, 125.2, 74.4, 69.9, 68.0, 65.1, 63.1, 56.3, 56.0, 55.4, 54.3, 40.1, 35.9, 29.7, 25.4, 23.5, 20.7, 18.5, 17.0, 13.4; MS (EI, 70 eV) m/z (%) 416 (M^+, 3), 387 (2), 372 (16), 243 (100), 71 (96); HRMS (EI) calcd. for $C_{21}H_{25}N_3O_6$ (M^+): 415.1743, found 415.1739.

16: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.70 (s, 2H), 3.90 (d, J = 5.7 Hz, 1H), 3.77 (d, J = 5.7, 1H), 3.45 (d, J = 5.7, 1H), 2.90–2.51 (m, 3H), 2.32 (d, J = 14.7 Hz, 1H), 2.20–2.07 (m, 2H), 1.98–1.89 (m, 2H), 1.76–1.64 (m, 2H), 1.34–1.28 (m, 1H), 1.07 (s, 3H), 0.97 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 161.2, 124.6, 75.4, 70.1, 66.3, 60.6, 58.9, 57.7, 57.4, 57.1, 47.7, 39.5, 34.9, 30.4, 27.7, 22.6, 17.2, 16.7, 16.3, 13.7; MS (EI, 70 eV) m/z (%) 416 (M^+, 2), 387 (3), 372 (19), 71 (100), 55 (31); HRMS (EI) calcd. for $C_{21}H_{25}N_3O_6$ (M^+): 415.1743, found 415.1736.

*5.1.7. General procedure for the synthesis of compounds (**17**–**19**)*

A solution of the azide **12** (0.1 mmol), the alkyne (0.11 mmol), 1 M aq $CuSO_4 \cdot 5H_2O$ (0.01 mmol) and 1 M aq. sodium ascorbate (0.01 mmol) in a t-BuOH–H_2O 1:1 mixture (3.0 mL) was heated at 50 °C until TLC showed no traces of the starting azide. The solvent was removed under reduced pressure, the residue was dispersed in EtOAc (10 mL) and the solution was washed with H_2O (3 × 15 mL), dried over $MgSO_4$, concentrated and purified by chromatography on silica gel to give the target analog.

*5.1.7.1. (14S)-14β-(4-Cyclopropyl-1H-1,2,3-triazol-1-yl)methylepitriptolide (**17**).* **17** (white solid, 26 mg, 55%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 (s, 1H), 5.12 (d, J = 13.8 Hz, 1H), 4.65 (d, J = 13.8 Hz, 1H), 4.63 (s, 2H), 3.86 (d, J = 3.0 Hz, 1H), 3.71 (s, 1H), 3.52 (d, J = 3.0 Hz, 1H), 3.39 (d, J = 5.7 Hz, 1H), 2.72–2.67 (m, 1H), 2.56 (sept, J = 6.9 Hz, 1H), 2.32 (d, J = 16.5 Hz, 1H), 2.24–1.94 (m, 5H), 1.68 (t, J = 13.8 Hz, 1H), 1.58 (dd, J = 12.3 Hz, J = 4.8 Hz, 1H), 1.21–1.06 (m, 3H), 0.98 (s, 3H), 0.97 (d, J = 4.5 Hz, 3H), 0.95 (d, J = 4.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 160.2, 150.5, 125.3, 122.4, 75.1, 69.9, 67.8, 65.6, 61.4, 56.0, 55.5, 53.6, 53.3, 40.5, 36.1, 29.6, 25.7, 23.3, 20.6, 18.6, 17.1, 13.3, 7.8 × 2, 6.6; MS (EI, 70 eV) m/z (%) 481 (M^+, 8), 359 (3), 337 (6), 94 (95), 59 (100); HRMS (EI) calcd. for $C_{26}H_{31}N_3O_6$ (M^+): 481.2213, found 481.2207.

*5.1.7.2. (14S)-14β-(4-(Methoxycarbonyl)-1H-1,2,3-triazol-1-yl) methylepitriptolide (**18**).* **18** (white solid, 36 mg, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (s, 1H), 5.24 (d, J = 13.8 Hz, 1H), 4.78 (d, J = 13.8 Hz, 1H), 4.67–4.54 (m, 2H), 3.93 (s, 3H), 3.89 (d, J = 3.0 Hz, 1H), 3.54 (d, J = 3.0 Hz, 1H), 3.39–3.35 (m, 2H), 2.70–2.66 (m, 1H), 2.53 (sept, J = 6.9 Hz, 1H), 2.15–2.06 (m, 2H), 1.75–1.65 (m, 2H), 1.57 (dd, J = 12.3 Hz, J = 4.8 Hz, 1H), 1.19–1.14 (m, 1H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 161.0, 160.0, 139.9, 130.1, 125.3, 75.2, 69.8, 67.7, 65.5, 61.5, 56.9, 55.6, 53.7, 53.6, 52.2, 40.4, 36.0, 29.5, 25.8, 23.2, 20.4, 18.6, 17.0, 13.2; MS (EI, 70 eV) m/z (%) 499 (M^+, 3), 468 (7), 141 (89), 83 (100), 82 (37); HRMS (EI) calcd. for $C_{25}H_{29}N_3O_8$ (M^+): 499.1955, found 499.1950.

*5.1.7.3. (14S)-14β-(4-Phenyl-1H-1,2,3-triazol-1-yl)methylepitriptolide (**19**).* **19** (white solid, 45 mg, 89%), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.93 (s, 1H), 7.84 (d, J = 7.6 Hz, 2H), 7.44–7.40 (m, 2H), 7.35–7.31 (m, 1H), 5.26 (d, J = 13.6 Hz, 1H), 4.77 (d, J = 14.0 Hz, 1H), 4.65–4.55 (m, 2H), 3.90 (d, J = 3.2 Hz, 1H), 3.68 (s, 1H), 3.55 (d, J = 3.2 Hz, 1H), 3.41 (d, J = 5.6 Hz, 1H), 2.71–2.67 (m, 1H), 2.65–2.58 (sept, J = 6.8 Hz, 1H), 2.31 (d, J = 13.8 Hz, 1H), 2.15–1.99 (m, 2H), 1.68 (t, J = 14.0 Hz, 1H), 1.60 (dd, J = 12.4 Hz, J = 4.8 Hz, 1H), 1.22–1.18 (m, 1H), 1.00 (d, J = 2.6 Hz, 3H), 0.98 (d, J = 2.9 Hz, 3H), 0.97 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 160.2, 147.8, 130.4 × 2, 128.9, 128.2, 125.6 × 2, 125.2, 122.4, 75.2, 69.9, 67.8, 65.7, 61.5, 56.5, 55.6, 53.7, 53.5, 40.5, 36.1, 29.6, 25.8, 23.2, 20.5, 18.6, 17.1, 13.4; MS (EI, 70 eV) m/z (%) 517 (M^+, 36), 500 (8), 460 (24), 105 (100), 59 (47); HRMS (EI) calcd. for $C_{29}H_{31}N_3O_6$ (M^+): 517.2213, found 517.2204.

*5.1.8. (14S)-14β-Formylepitriptolid (**20**)*

To a stirred solution of **11** (420 mg, 1.08 mmol) in dichloromethane (15.0 mL) at 0 °C, trichloroisocyanuric acid (376 mg, 1.62 mmol) and TEMPO (16 mg, 0.108 mmol) were added, and the mixture was stirred at 0 °C for 30 min, saturated Na_2CO_3 solution (1.0 mL) was added to quench the reaction. Then extracted with dichloromethane, washed with brine, and dried with anhydrous Na_2SO_4. The crude product was purified via chromatography on silica gel using ethyl acetate/cyclohexane (1/3) to give **20** as a white solid in 81% yield (40 mg, 0.87 mmol). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.03 (s, 1H), 4.76–4.59 (m, 2H), 3.97 (d, J = 3.0 Hz, 1H), 3.91 (s, 1H), 3.75 (d, J = 5.9 Hz, 1H), 3.60 (d, J = 3.0 Hz, 1H), 2.79–2.67 (m, 1H), 2.38–2.26 (m, 1H), 1.87 (dd, J = 14.7, 13.6 Hz, 1H), 1.58 (dd, J = 12.6, 4.0 Hz, 1H), 1.03 (s, 3H), 0.83 (d, J = 6.9 Hz, 3H), 0.79 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 198.3, 173.2, 160.1, 125.4, 81.7, 69.9, 65.9, 65.5, 62.1, 56.3, 55.8, 54.0, 40.4, 36.0, 30.2, 26.6, 23.3, 19.8, 17.3, 17.1, 13.6; MS (EI, 70 eV) m/z (%) 389 ([M + 1]$^+$, 4), 388 (M$^+$, 1), 343 (6), 327 (52), 299 (88), 71 (100). HRMS (EI) calcd. for $C_{21}H_{24}O_7$ (M$^+$): 388.1522, found 388.1525.

*5.1.9. General procedure for the synthesis of compounds **21**–**40***

A solution of **20** (0.1 mmol) and the amino (0.12 mmol), in dichloromethane (3.0 mL) under argon protect was stirred at room temperature for 30 min, then $NaBH(OAc)_3$ (0.15 mmol)was added and stirred at the same temperature until TLC showed no traces of the starting aldehyde **19**. The solvent was removed under reduced pressure, the residue was dispersed in EtOAc (10 mL) and the solution was washed with H_2O (3 × 15 mL), dried over $MgSO_4$ and concentrated to give a target analog.

*5.1.9.1. (14S)-14β-(Pyridin-3-ylamino)methylepitriptolide (**21**).* **21** (white solid, 30 mg, 65%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (s, 1H), 8.03 (s, 1H), 7.15 (dd, J = 8.4, 4.8 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 4.68 (s, 2H), 3.83–3.90 (m, 3H), 3.51 (d, J = 3.3 Hz, 1H), 3.46 (d, J = 13.2 Hz, 1H), 2.72 (d, J = 12.9 Hz, 1H), 2.48 (sept, J = 6.9 Hz, 1H), 2.32 (d, J = 15.6 Hz, 1H), 2.14–2.23 (m, 2H), 1.90 (t, J = 14.1 Hz, 1H), 1.56 (dd, J = 12.3, 4.8 Hz, 1H), 1.23–1.18 (m, 1H), 1.09 (s, 3H), 0.98 (d, J = 7.2 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.3, 144.3, 139.7, 136.8, 125.4, 123.9, 120.6, 73.8, 70.0, 68.1, 65.3, 64.5, 56.2, 55.8, 54.6, 49.6, 40.4, 36.1, 30.0, 25.8, 23.5, 21.1, 18.8, 17.2, 13.7; MS (EI, 70 eV) m/z (%) 466 (M$^+$, 3), 107 (100), 78 (14); HRMS (EI) calcd. for $C_{26}H_{30}N_2O_6$ (M$^+$): 466.2104, found 466.2095.

*5.1.9.2. (14S)-14β-(1H-Indol-4-ylamino)methylepitriptolide (**22**).* **22** (white solid, 40 mg, 80%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.23 (s, 1H), 7.14 (t, J = 2.7 Hz, 1H), 7.10 (t, J = 8.1 Hz, 1H), 6.93 (d, J = 8.1 Hz, 1H), 6.53 (m, 1H), 6.42 (d, J = 7.5 Hz, 1H), 4.68 (s, 2H), 4.02 (d, J = 12.9 Hz, 1H), 3.92 (s, 1H), 3.88 (d, J = 5.7 Hz, 1H), 3.84 (d, J = 3.3 Hz, 1H), 3.66 (d, J = 13.2 Hz, 1H), 3.52 (d, J = 3.3 Hz, 1H), 2.71 (s, 1H), 2.60 (sept, J = 6.9 Hz, 1H), 2.32 (d, J = 14.1 Hz, 1H), 2.14–2.24 (m, 2H), 1.92 (t, J = 13.8 Hz, 1H), 1.57 (dd, J = 12.3, 5.7 Hz, 1H), 1.22–1.19 (m, 1H), 1.11 (s, 3H), 1.03 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0, 161.3, 140.8, 136.3, 124.9, 122.7, 122.4, 117.8, 102.7, 100.1, 98.0, 73.7, 70.23, 68.4, 65.2, 64.4, 56.1, 55.6, 54.5, 49.2, 40.2, 35.9, 29.7, 25.5, 23.3, 21.0, 18.5, 16.9, 13.5; MS (EI, 70 eV) m/z (%) 504 (M$^+$, 26), 342 (21), 59 (100); HRMS (EI) calcd. for $C_{29}H_{32}N_2O_6$ (M$^+$): 504.2260, found 504.2268.

*5.1.9.3. (14S)-14β-(1H-Indazol-5-ylamino)methylepitriptolide (**23**).* **23** (white solid, 43 mg, 85%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.02 (s, 1H), 7.94 (s, 1H), 7.36 (d, J = 9.0 Hz, 1H), 7.02 (s, 1H), 6.96 (d, J = 8.7, 1.5 Hz, 1H), 4.69 (s, 2H), 4.21 (dd, J = 10.5, 4.2 Hz, 1H), 4.12 (s, 1H), 3.94 (dd, J = 12.9, 4.2 Hz, 1H), 3.88 (d, J = 5.7, 1H), 3.84 (d, J = 3.0 Hz, 1H), 3.52 (d, J = 3.0, 1H), 3.46 (t, J = 12.6 Hz, 1H), 2.71 (s, 1H), 2.51–2.61 (m, 1H), 2.32 (d, J = 15.0 Hz, 1H), 2.22–2.12 (m, 2H), 1.91 (t, J = 14.1 Hz, 1H), 1.57 (dd, J = 12.9, 5.1 Hz, 1H), 1.22–1.18 (m, 1H), 1.11 (s, 3H), 1.02 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 160.5, 142.5, 135.9, 133.8, 125.3, 123.9, 119.9, 110.7, 102.3, 73.3, 70.0, 68.5, 65.3, 64.7, 56.3, 55.8, 54.6, 51.1, 40.4, 36.0, 30.0, 25.7, 23.5, 21.3, 18.9, 17.1, 13.7; MS (EI, 70 eV) m/z (%) 505 (M$^+$, 2), 342 (20), 327 (21), 133 (100), 86 (64) 58 (70); HRMS (EI) calcd. for $C_{28}H_{31}N_3O_6$ (M$^+$): 505.2213, found 505.2215.

*5.1.9.4. (14S)-14β-(1H-Indazol-6-ylamino)methylepitriptolide (**24**).* **24** (white solid, 25 mg, 69%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.51 (s, 1H), 7.88 (s, 1H), 7.51 (d, J = 8.4 Hz, 1H), 6.71 (s, 1H), 6.62 (d, J = 8.7, 1H), 4.71–4.60 (m, 3H), 3.80–3.80 (m, 4H), 3.57–3.48 (m, 3H), 2.76–2.67 (m, 1H), 2.55 (sept, J = 6.6 Hz, 1H), 2.30 (d, J = 17.1 Hz, 1H), 2.16–2.07 (m, 1H), 1.86 (t, J = 13.8 Hz, 1H), 1.55 (dd, J = 12.6, 5.1 Hz, 1H), 1.22–1.19 (m, 1H), 1.08 (s, 3H), 0.99 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.4, 147.8, 141.8, 134.8, 125.4, 121.5, 117.1, 113.5, 90.4, 73.8, 70.0, 68.4, 65.3, 64.6, 56.3, 55.8, 54.6, 50.0, 40.4, 36.1, 30.0, 25.7, 23.5, 21.1, 18.8, 17.1, 13.7; MS (EI, 70 eV) m/z (%) 505 (M$^+$, 3), 342 (7), 145 (60), 133 (81), 55 (100); HRMS (EI) calcd. for $C_{28}H_{31}N_3O_6$ (M$^+$): 505.2213, found 505.2211.

*5.1.9.5. (14S)-14β-(1-Methyl-1H-indazol-5-ylamino)methylepitriptolide (**25**).* **25** (white solid, 37 mg, 72%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.26 (d, J = 8.7 Hz, 1H), 6.98–6.93 (m, 2H), 4.68 (s, 2H), 4.15 (s, 1H), 4.02 (s, 3H), 3.93 (d, J = 13.2 Hz, 1H), 3.87 (d, J = 5.6 Hz, 1H), 3.83 (d, J = 3.1 Hz, 1H), 3.51 (d, J = 3.0 Hz, 1H), 3.44 (d, J = 13.1 Hz, 1H), 2.71 (brs, 1H), 2.52 (sept, J = 6.8 Hz, 1H), 2.31 (d, J = 14.3 Hz, 1H), 2.24–2.09 (m, 2H), 1.90 (t, J = 14.4 Hz, 1H), 1.56 (dd, J = 12.4, 4.7 Hz, 1H), 1.23–1.16 (m, 1H), 1.10 (s, 3H), 1.01 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.5, 142.1, 135.9, 131.4, 125.3, 124.5, 119.4, 109.8, 102.6, 73.2, 70.0, 68.3, 65.3, 64.7, 56.2, 55.8, 54.5, 51.2, 40.3, 36.0, 35.6, 30.0, 25.7, 23.5, 21.2, 18.8, 17.1, 13.7; ESIMS m/z 520.6 [M + H]$^+$; HRMS (ESI) calcd. for $C_{29}H_{34}N_3O_6$ [M + H]$^+$ 520.2448, found 520.2445.

*5.1.9.6. (14S)-14β-(2-Methyl-2H-indazol-5-ylamino)methylepitriptolide (**26**).* **26** (white solid, 26 mg, 50%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.68 (s, 1H), 7.54 (d, J = 9.2 Hz, 1H), 6.85 (dd, J = 9.2, 2.1 Hz, 1H), 6.76 (d, J = 2.1 Hz, 1H), 4.65 (s, 2H), 4.21 (brs, 1H), 4.15 (s, 3H), 4.04 (s, 1H), 3.90–3.84 (m, 2H), 3.82 (d, J = 3.1 Hz, 1H), 3.50 (d, J = 3.1 Hz, 1H), 3.44 (d, J = 13.0 Hz, 1H), 2.69 (d, J = 13.1 Hz, 1H), 2.52 (sept, J = 6.9 Hz, 1H), 2.29 (d, J = 16.5 Hz, 1H), 2.20–2.09 (m, 2H), 1.87 (t, J = 14.1 Hz 1H), 1.54 (dd, J = 12.4, 4.5 Hz, 1H), 1.19–1.16 (m, 1H), 1.08 (s, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.5, 145.6, 142.4, 125.2, 122.5, 122.0, 121.2, 118.3, 98.4, 73.3, 70.0, 68.4, 65.3, 64.7, 56.2, 55.8, 54.5, 50.4, 40.3, 40.1, 36.0, 30.0, 25.7, 23.4, 21.3, 18.8, 17.1, 13.7; ESIMS m/z 520.6 [M + H]$^+$; HRMS (ESI) calcd. for $C_{29}H_{34}N_3O_6$ [M + H]$^+$ 520.2448, found 520.2444.

*5.1.9.7. (14S)-14β-((1-(Methoxycarbonyl)-1H-indazol-5-ylamino) methylepitriptolide) (**27**).* **27** (white solid, 35 mg, 63%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.05–8.02 (m, 2H), 7.04 (dd, J = 9.0 Hz, J = 2.4 Hz, 1H), 6.97 (d, J = 2.1 Hz, 1H), 4.68 (s, 2H), 4.50–4.48 (m, 1H), 4.10 (s, 3H), 3.87–3.83 (m, 2H), 3.76 (s, 1H), 3.61 (d, J = 7.2 Hz, 1H), 3.51 (d, J = 3.0, 1H), 2.72 (d, J = 12.6 Hz, 1H), 2.50 (sept, J = 6.6 Hz, 1H), 2.34–2.14 (m, 4H), 1.90 (t, J = 13.5 Hz, 1H), 1.57 (dd, J = 12.3, 5.4 Hz, 1H), 1.22–1.19 (m, 1H), 1.10 (s, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.4, 151.0, 145.1, 139.7, 134.5, 126.8, 125.3, 120.3, 115.0, 102.4, 73.5, 70.0, 68.2, 65.3, 64.6, 56.2, 55.8, 54.5, 54.3, 50.6, 40.4, 36.1, 30.0, 25.7, 23.5, 21.2, 18.8, 17.1, 13.7; ESIMS m/z 564.6, HRMS (ESI) calcd. for $C_{30}H_{34}N_3O_8$ [M + H]$^+$ 564.2346, found 564.2351.

*5.1.9.8. (14S)-14β-((3-(Methoxycarbonyl)-1H-indazol-5-ylamino) methylepitriptolide) (**28**).* **28** (white solid, 40 mg, 71%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.08 (s, 1H), 7.48 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 1.8 Hz, 1H), 7.00 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.69 (s, 2H), 4.47 (d, *J* = 5.4 Hz, 1H), 4.03 (s, 3H), 3.97 (d, *J* = 13.5, 1H), 3.86 (m, 3H), 3.56 (m, 2H), 2.72 (s, 1H), 2.58 (sept, *J* = 6.9 Hz, 1H), 2.32 (d, *J* = 13.8 Hz, 1H), 2.20 (m, 2H), 1.93 (t, *J* = 13.8 Hz, 1H), 1.58 (dd, *J* = 14.4, 4.5 Hz, 1H), 1.22–1.19 (m, 1H), 1.11 (s, 3H), 1.05 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 163.6, 160.5, 144.5, 137.1, 134.6, 125.3, 123.7, 120.0, 112.2, 101.9, 73.5, 70.0, 68.4, 65.3, 64.7, 56.3, 55.9, 54.6, 51.9, 50.5, 40.4, 36.1, 30.0, 25.8, 23.50, 21.3, 18.9, 17.2, 13.8; MS (EI, 70 eV) *m*/*z* (%) 564 (M^+, 2), 342 (23), 327 (21), 203 (76), 191 (89), 84 (100); HRMS (EI) calcd. for $C_{30}H_{33}N_3O_8$ (M^+): 563.2268, found 563.2274.

*5.1.9.9. (14S)-14β-(1-(2-Morpholinoethyl)-1H-indazol-5-ylamino) methylepitriptolide (**29**).* **29** (white solid, 50 mg, 81%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.28 (d, *J* = 9.7 Hz, 1H), 6.97–6.90 (m, 2H), 4.65 (s, 2H), 4.44 (t, *J* = 6.9 Hz, 2H), 3.89 (d, *J* = 13.2 Hz, 1H), 3.84 (d, *J* = 5.6 Hz, 1H), 3.81 (d, *J* = 3.2 Hz, 1H), 3.67–3.61 (m, 4H), 3.49 (d, *J* = 3.1 Hz, 1H), 3.43 (d, *J* = 9.6 Hz, 1H), 2.84 (dd, *J* = 7.9, 5.9 Hz, 2H), 2.69 (d, *J* = 11.2 Hz, 1H), 2.56–2.39 (m, 5H), 2.28 (d, *J* = 17.6 Hz, 1H), 2.21–2.04 (m, 2H), 1.87 (t, *J* = 14.4 Hz, 13.6 Hz, 1H), 1.53 (dd, *J* = 12.5, 4.4 Hz, 1H), 1.20–1.17 (m, 1H), 1.07 (s, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.5, 142.2, 135.4, 131.9, 125.2, 124.5, 119.4, 109.8, 102.4, 73.2, 69.9, 68.3, 66.7 × 2, 65.2, 64.6, 57.6, 56.2, 55.8, 54.5, 53.6 × 2, 51.0, 46.5, 40.29, 36.0, 29.9, 25.6, 23.4, 21.2, 18.8, 17.1, 13.7; ESIMS *m*/*z* 619.7 [M + H]$^+$; HRMS (ESI) calcd. for $C_{34}H_{43}N_4O_7$ [M + H]$^+$ 619.3132, found 619.3130.

*5.1.9.10. (14S)-14β-(2-(2-Morpholinoethyl)-2H-indazol-5-ylamino) methylepitriptolide (**30**).* **30** (white solid, 37 mg, 60%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (s, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 6.86 (dd, *J* = 9.2, 2.0 Hz, 1H), 6.75 (d, *J* = 1.8 Hz, 1H), 4.65 (s, 2H), 4.48 (t, *J* = 6.3 Hz, 2H), 3.91–3.83 (m, 2H), 3.82 (d, *J* = 3.2 Hz, 1H), 3.71 (s, 4H), 3.50 (d, *J* = 3.1 Hz, 1H), 3.47–3.41 (m, 1H), 2.95 (t, *J* = 6.3 Hz, 2H), 2.67 (brs, 1H), 2.61 (s, 1H), 2.58–2.41 (m, 4H), 2.28 (d, *J* = 18.6 Hz, 1H), 2.20–2.08 (m, 2H), 1.87 (t, *J* = 14.4 Hz, 1H), 1.54 (dd, *J* = 12.3, 4.4 Hz, 1H), 1.19–1.16 (m, 1H), 1.08 (s, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.5, 145.3, 142.5, 125.3, 122.1, 121.9, 121.7, 118.3, 98.2, 73.4, 70.0, 68.3, 66.7 × 2, 65.3, 64.6, 58.5, 56.2, 55.8, 54.5, 53.7 × 2, 50.6, 50.3, 40.3, 36.0, 30.0, 25.7, 23.4, 21.3, 18.8, 17.1, 13.7; ESIMS *m*/*z* 619.7 [M + H]$^+$; HRMS (ESI) calcd. for $C_{34}H_{43}N_4O_7$ [M + H]$^+$ 619.3132, found 619.3128.

*5.1.9.11. (14S)-14β-(1-(2-(Piperidin-1-yl)ethyl)-1H-indazol-5-ylamino)methylepitriptolide (**31**).* **31** (white solid, 30 mg, 49%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.79 (s, 1H), 7.27 (d, *J* = 8.8 Hz, 1H), 6.93–6.90 (m, 2H), 4.62 (s, 2H), 4.42 (t, *J* = 7.2 Hz, 2H), 4.22 (brs, 1H), 3.88–3.83 (m, 2H), 3.78 (d, *J* = 3.0 Hz, 1H), 3.47 (d, *J* = 3.0 Hz, 1H), 3.42 (brs, 1H), 2.76 (t, *J* = 7.2 Hz, 2H), 2.66 (d, *J* = 12.4 Hz, 1H), 2.51 (sept, *J* = 13.7, 1H), 2.43–2.40 (m, 4H), 2.25 (d, *J* = 14.5 Hz, 1H), 2.17–2.06 (m, 2H), 1.83 (t, *J* = 14.0 Hz, 1H), 1.58–1.46 (m, 5H), 1.42–1.34 (m, 2H), 1.18–1.11 (m, 1H), 1.05 (s, 3H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 160.4, 142.1, 135.3, 131.6, 125.1, 124.4, 119.2, 109.9, 102.2, 73.1, 69.9, 68.2, 65.1, 64.5, 58.0, 56.1, 55.7, 54.6 × 2, 54.4, 51.0, 46.7, 40.2, 35.9, 29.8, 25.7 × 2, 25.6, 24.0, 23.3, 21.1, 18.8, 17.0, 13.6; ESIMS *m*/*z* 617.7 [M + H]$^+$; HRMS (ESI) calcd. for $C_{35}H_{45}N_4O_6$ [M + H]$^+$ 617.3339, found 617.3335.

*5.1.9.12. (14S)-14β-(2-Oxoindolin-5-ylamino)methylepitriptolide (**32**).* **32** (light yellow solid, 39 mg, 75%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.40 (s, 1H), 6.74–6.71 (m, 2H), 6.63 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.68 (s, 1H), 4.04 (s, 1H), 3.88–3.82 (m, 3H), 3.50–3.48 (m, 3H), 3.36 (d, *J* = 13.3 Hz, 1H), 2.72 (d, *J* = 13.3 Hz, 1H), 2.47 (sept, *J* = 6.9 Hz, 1H), 2.32 (m, 1H), 2.22–2.13 (m, 2H), 1.89 (t, *J* = 13.8 Hz, 1H), 1.55 (dd, *J* = 12.3, 4.7 Hz, 1H), 1.19–1.17 (m, 1H), 1.09 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.2, 173.3, 160.4, 143.7, 135.4, 126.6, 125.3, 114.3, 112.8, 110.1, 73.2, 70.0, 68.3, 65.3, 64.6, 56.2, 55.8, 54.6, 51.3, 40.4, 36.5, 36.0, 30.0, 25.7, 23.5, 21.2, 18.8, 17.1, 13.7; MS (EI, 70 eV) *m*/*z* (%) 520 (M^+, 20), 342 (63), 327 (42), 148 (100), 72 (55); HRMS (EI) calcd. for $C_{29}H_{32}N_2O_7$ (M^+): 520.2210, found 520.2213.

*5.1.9.13. (14S)-14β-(1,3,3-Trimethyl-2-oxoindolin-5-ylamino)methylepitriptolide (**33**).* **33** (white solid, 34 mg, 60%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (d, *J* = 2.0 Hz, 1H), 6.70–6.67 (m, 2H), 4.68 (s, 2H), 4.07 (s, 2H), 3.90–3.85 (m, 2H), 3.83 (d, *J* = 3.2 Hz, 1H), 3.50 (d, *J* = 3.1 Hz, 1H), 3.37 (d, *J* = 13.3 Hz, 1H), 3.17 (s, 3H), 2.79–2.66 (m, 1H), 2.48 (sept, *J* = 6.9 Hz, 1H), 2.30 (d, *J* = 13.8 Hz, 1H), 2.24–2.09 (m, 2H), 1.90 (t, *J* = 14.7, 13.6 Hz, 1H), 1.55 (dd, *J* = 12.4, 4.2 Hz, 1H), 1.34 (s, 6H), 1.22–1.14 (m, 1H), 1.09 (s, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.93 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 180.8, 173.3, 160.4, 144.1, 137.1, 135.7, 125.3, 113.3, 111.4, 108.4, 73.1, 70.0, 68.3, 65.3, 64.7, 56.3, 55.8, 54.6, 51.3, 44.5, 40.4, 36.0, 30.0, 26.2, 25.7, 24.4 × 2, 23.5, 21.2, 18.8, 17.1, 13.7; ESIMS *m*/*z* 563.7 [M + H] $^+$; HRMS (ESI) calcd. for $C_{32}H_{39}N_2O_7$ [M + H]$^+$ 563.2757, found 563.2750.

*5.1.9.14. (14S)-14β-(Quinolin-6-ylamino)methylepitriptolide (**34**).* **34** (light yellow solid, 45 mg, 88%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.66 (d, *J* = 4.2 Hz, 1H), 7.92 (m, 2H), 7.29 (dd, *J* = 8.4, 4.2 Hz, 1H), 7.20 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.86 (s, 1H), 4.78 (d, *J* = 9.3, 1H) 4.67 (s, 2H), 3.95 (dd, *J* = 13.2, 3.9 Hz, 1H), 3.87 (s, 1H), 3.86 (s, 1H), 3.57 (m, 3H), 2.70 (s, 1H), 2.57 (sept, *J* = 6.9 Hz, 1H), 2.32 (d, *J* = 14.7 Hz, 1H), 2.13–2.22 (m, 2H), 1.91 (t, *J* = 15.6 Hz, 1H), 1.57 (dd, *J* = 12.0, 4.8 Hz, 1H), 1.25–1.23 (m, 1H), 1.11 (s, 3H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 160.3, 146.9, 146.0, 143.7, 134.1, 130.4, 129.7, 125.4, 122.0, 121.5, 105.1, 74.0, 70.0, 68.2, 65.3, 64.6, 56.2, 55.8, 54.6, 49.7, 40.4, 36.1, 30.0, 25.8, 23.5, 21.2, 18.8, 17.2, 13.7; MS (EI, 70 eV) *m*/*z* (%) 516 (M^+, 5), 157 (65), 144 (100), 128 (54); HRMS (EI) calcd. for $C_{30}H_{32}N_2O_6$ (M^+): 516.2260, found 516.2268.

*5.1.9.15. (14S)-14β-(Quinoxalin-6-ylamino)methylepitriptolide (**35**).* **35** (light yellow solid, 40 mg, 77%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.65 (d, *J* = 1.7 Hz, 1H), 8.54 (d, *J* = 1.7 Hz, 1H), 7.84 (d, *J* = 9.1 Hz, 1H), 7.21 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.04 (d, *J* = 2.2 Hz, 1H), 5.25 (dd, *J* = 8.3, 3.2 Hz, 1H), 4.66 (s, 2H), 3.95 (dd, *J* = 13.1, 3.4 Hz, 1H), 3.87 (d, *J* = 3.1 Hz, 1H), 3.84 (d, *J* = 5.6 Hz, 1H), 3.65 (dd, *J* = 13.0, 8.6 Hz, 1H), 3.54 (d, *J* = 2.9 Hz, 1H), 3.29 (brs, 1H), 2.73 (d, *J* = 14.2 Hz, 1H), 2.58 (sept, *J* = 6.8 Hz, 1H), 2.30 (d, *J* = 15.0 Hz, 1H), 2.22–2.09 (m, 2H), 1.90 (t, *J* = 14.4 Hz, 1H), 1.57 (dd, *J* = 12.3, 4.6 Hz, 1H), 1.21–1.19 (m, 1H), 1.20 (m, 1H), 1.09 (s, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.91 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.2, 160.2, 149.2, 145.1, 144.9, 140.8, 138.3, 130.1, 125.40, 123.0, 104.7, 74.5, 70.0, 68.1, 65.3, 64.5, 56.2, 55.8, 54.6, 49.2, 40.3, 36.1, 29.9, 25.8, 23.5, 21.1, 18.7, 17.1, 13.7; ESIMS *m*/*z* 518.6 [M + H]$^+$; HRMS (ESI) calcd. for $C_{29}H_{32}N_3O_6$ [M + H]$^+$ 518.2291, found 518.2287.

*5.1.9.16. (14S)-14β-(Quinazolin-6-ylamino)methylepitriptolide (**36**).* **36** (light yellow solid, 36 mg, 69%), ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.16 (s, 1H), 9.06 (s, 1H), 7.82 (d, *J* = 9.1 Hz, 1H), 7.36 (d, *J* = 9.0 Hz, 1H), 6.83 (s, 1H), 5.18 (dd, *J* = 7.8, 3.3 Hz, 1H), 4.67–4.63 (m, 2H), 3.89–3.82 (m, 3H), 3.62–3.52 (m, 2H), 2.71 (brs, 1H), 2.56 (sept, *J* = 6.9 Hz, 1H), 2.31 (d, *J* = 14.6 Hz, 1H), 2.22–2.15 (m, 2H), 1.91 (t, *J* = 14.4 Hz, 1H), 1.57 (dd, *J* = 12.2, 4.7 Hz, 1H), 1.20–1.18 (m, 1H), 1.09

(s, 3H), 1.00 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 160.2, 157.8, 152.1, 147.0, 145.2, 129.1, 126.7, 126.6, 125.4, 102.4, 74.6, 69.9, 68.1, 65.4, 64.4, 56.3, 55.9, 54.6, 49.4, 40.3, 36.1, 29.9, 25.8, 23.5, 21.0, 18.7, 17.1, 13.7; ESIMS m/z 518.6 $[M + H]^+$; HRMS (ESI) calcd. for $C_{29}H_{32}N_3O_6$ $[M + H]^+$ 518.2291, found 518.2293.

*5.1.9.17. (14S)-14β-(2-Oxo-2H-chromen-6-ylamino)methylepitriptolide (**37**).* **37** (white solid, 38 mg, 72%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, J = 9.6 Hz, 1H), 7.19 (d, J = 9.0 Hz, 1H), 6.96 (dd, J = 9.0 Hz, 2.7 Hz, 1H), 6.78 (d, 2.7 Hz, 1H), 6.38 (d, J = 9.6, 1H), 4.68 (s, 2H), 4.46 (dd, J = 9.6 Hz, 4.2 Hz, 1H), 3.91–3.84 (m, 3H), 3.62–3.57 (m, 3H), 3.52 (d, J = 3.3 Hz, 1H), 2.69 (d, J = 12.9 Hz, 1H), 2.49 (sept, J = 6.9 Hz, 1H), 2.34–2.07 (m, 2H), 1.90 (t, J = 14.4 Hz, 1H), 1.56 (dd, J = 12.3, 4.8 Hz, 1H), 1.21–1.18 (m, 1H), 1.06 (s, 3H), 0.99 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.3, 161.1, 160.3, 147.6, 144.9, 143.3, 125.3, 119.5, 119.2, 117.5, 116.9, 110.6, 73.7, 70.0, 68.2, 65.3, 64.5, 56.3, 55.8, 54.5, 50.5, 40.3, 36.0, 30.0, 25.7, 23.4, 21.1, 18.8, 17.1, 13.7; MS (EI, 70 eV) m/z (%) 533 (M^+, 3), 342 (11), 133 (100), 77 (52); HRMS (EI) calcd. for $C_{30}H_{31}NO_8$ (M^+): 533.2050, found 533.2044.

*5.1.9.18. (14S)-14β-(4-Oxo-4H-chromen-6-ylamino)methylepitriptolide (**38**).* **38** (white solid, 23 mg, 43%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.80 (d, J = 5.9 Hz, 1H), 7.36 (d, J = 2.7 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 7.11 (dd, J = 9.0, 2.7 Hz, 1H), 6.28 (d, J = 5.9 Hz, 1H), 4.79 (d, J = 5.5 Hz, 1H), 4.67 (s, 2H), 3.90 (dd, J = 13.0, 3.9 Hz, 1H), 3.85–3.83 (m, 2H), 3.56–3.49 (m, 2H), 2.79–2.69 (m, 2H), 2.52 (sept, J = 6.9 Hz, 1H), 2.31 (d, J = 16.3 Hz, 1H), 2.23–2.09 (m, 2H), 1.89 (t, J = 14.7 Hz, 1H), 1.56 (dd, J = 11.6, 5.0 Hz, 1H), 1.20–1.18 (m, 1H), 1.08 (s, 3H), 0.99 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.6, 173.3, 160.4, 154.9, 150.4, 145.6, 125.5, 125.3, 122.4, 119.1, 111.9, 105.8, 74.0, 70.0, 68.2, 65.3, 64.5, 56.2, 55.8, 54.6, 49.9, 40.3, 36.0, 29.9, 25.7, 23.4, 21.0, 18.8, 17.1, 13.8; MS (EI, 70 eV) m/z (%) 533 (M^+, 7), 342 (23), 327 (25), 189 (53), 161 (100), 79 (49); HRMS (EI) calcd. for $C_{30}H_{31}NO_8$ (M^+): 533.2050, found 533.2059.

*5.1.9.19. (14S)-14β-(4-Oxochroman-6-ylamino)methylepitriptolide (**39**).* **39** (light yellow solid, 29 mg, 55%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.21 (d, J = 2.4 Hz, 1H), 6.98 (dd, J = 8.8, 2.6 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 4.67 (s, 2H), 4.47 (t, J = 6.4 Hz, 2H), 4.21 (dd, J = 10.1, 3.8 Hz, 1H), 3.90–3.83 (m, 4H), 3.50 (d, J = 2.7 Hz, 1H), 3.44–3.30 (m, 1H), 2.80–2.59 (m, 3H), 2.47 (sept, J = 6.9 Hz, 1H), 2.30 (d, J = 16.9 Hz, 1H), 2.22–2.13 (m, 2H), 1.89 (t, J = 14.1 Hz, 1H), 1.55 (dd, J = 12.3, 4.6 Hz, 1H), 1.22–1.16 (m, 1H), 1.08 (s, 3H), 0.98 (d, J = 6.9 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.0, 173.3, 160.4, 155.8, 142.2, 125.3, 124.8, 121.3, 118.8, 110.1, 73.4, 70.0, 68.2, 67.0, 65.2, 64.6, 56.2, 55.8, 54.6, 50.6, 40.3, 37.8, 36.0, 30.0, 25.7, 23.4, 21.1, 18.8, 17.1, 13.7; MS (EI, 70 eV) m/z (%) 535 (M^+, 4), 342 (25), 327 (25), 175 (100), 163 (93), 79 (74); HRMS (EI) calcd. for $C_{30}H_{33}NO_8$ (M^+): 535.2206, found 535.2204.

*5.1.9.20. (14S)-14β-(2-Oxo-2,3-dihydrobenzofuran-5-ylamino)methylepitriptolide (**40**).* **40** (white solid, 26 mg, 50%), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.93 (d, J = 8.6 Hz, 1H), 6.72 (s, 1H), 6.67 (dd, J = 8.6, 2.5 Hz, 1H), 4.67 (s, 2H), 4.23 (brs, 1H), 3.92–3.73 (m, 4H), 3.68 (s, 2H), 3.49 (d, J = 3.1 Hz, 1H), 3.38 (d, J = 13.3 Hz, 1H), 2.72 (d, J = 13.3 Hz, 1H), 2.46 (sept, J = 6.9 Hz, 1H), 2.29 (d, J = 14.0 Hz, 1H), 2.23–2.09 (m, 2H), 1.89 (dd, J = 14.7, 13.6 Hz, 1H), 1.55 (dd, J = 12.5, 4.2 Hz, 1H), 1.21–1.16 (m, 1H), 1.08 (s, 3H), 0.97 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 173.3, 160.4, 147.9, 145.0, 125.3, 124.0, 114.9, 111.3, 111.1, 73.3, 70.0, 68.2, 65.3, 64.6, 56.2, 55.8, 54.5, 51.0, 40.3, 36.0, 33.5, 30.0, 25.7, 23.4, 21.2, 18.8, 17.1, 13.7; MS (EI, 70 eV) m/z (%) 521 (M^+, 36), 342 (19), 327 (17), 162 (100); HRMS (EI) calcd. for $C_{29}H_{31}NO_8$ (M^+): 521.2050, found 521.2053.

*5.1.10. General procedure for the synthesis of compounds **41** and **42***

A solution of the amine (0.1 mmol), 37% aqueous formaldehyde (1.0 mmol) and 30% aqueous acetic acid (1.0 mmol) in acetonitrile a (3.0 mL) was stirred at room temperature until TLC showed no traces of the starting amine. Then the solvent was removed under reduced pressure, the residue was dispersed in EtOAc (10 mL) and the solution was washed with H_2O (3 × 15 mL), dried over $MgSO_4$, concentrated and purified by chromatography on silica gel to give the target analog.

*5.1.10.1. (14S)-3′-(1-Methyl-1H-indazol-5-yl)spiro[triptolide-14,5′-oxazolidine](**41**).* **41** (white solid, 50 mg, 95%), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.30 (d, J = 9.0 Hz, 1H), 6.83 (dd, J = 9.0, 1.9 Hz, 1H), 6.75 (d, J = 1.9 Hz, 1H), 5.21 (s, 1H), 4.65 (s, 2H), 4.57 (s, 1H), 4.02 (s, 3H), 3.93 (d, J = 5.5 Hz, 1H), 3.81 (d, J = 3.1 Hz, 1H), 3.76 (d, J = 9.9 Hz, 1H), 3.57–3.48 (m, 2H), 2.72 (brd, J = 12.7 Hz, 1H), 2.48 (sept, J = 6.9 Hz, 1H), 2.29 (brd, J = 17.5 Hz, 1H), 2.21–2.05 (m, 2H), 1.86 (t, J = 10.4 Hz, 1H), 1.54 (dd, J = 12.4, 4.7 Hz, 1H), 1.27–1.13 (m, 1H), 1.04 (s, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.88 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 160.3, 139.6, 135.2, 131.4, 125.2, 124.6, 116.4, 109.7, 102.2, 85.5, 84.3, 69.9, 68.0, 65.1, 64.6, 57.8, 55.8, 55.5, 55.0, 40.5, 35.7, 35.5, 30.0, 25.6, 23.6, 20.6, 18.7, 17.0, 13.4; ESIMS m/z 532.6 $[M + H]^+$; HRMS (ESI) calcd. for $C_{30}H_{34}N_3O_6$ $[M + H]^+$ 532.2448, found 532.2442.

*5.1.10.2. (14S)-3′-(1-(2-Morpholinoethyl)-1H-indazol-5-yl)spiro[triptolide-14,5′-oxazolidine](**42**).* **42** (white solid, 58 mg, 92%), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.33 (d, J = 9.0 Hz, 1H), 6.82 (dd, J = 9.0, 2.1 Hz, 1H), 6.73 (d, J = 1.7 Hz, 1H), 5.20 (s, 1H), 4.65 (s, 2H), 4.56 (s, 1H), 4.45 (t, J = 6.9 Hz, 2H), 3.93 (d, J = 5.5 Hz, 1H), 3.80 (d, J = 3.2 Hz, 1H), 3.75 (d, J = 9.9 Hz, 1H), 3.66–3.60 (m, 4H), 3.54–3.50 (m, 2H), 2.82 (t, J = 6.9 Hz, 2H), 2.72 (brd, J = 12.8 Hz, 1H), 2.52–2.41 (m, 5H), 2.28 (brd, J = 18.1 Hz, 1H), 2.21–2.01 (m, 2H), 1.91–1.79 (m, 1H), 1.53 (dd, J = 12.5, 4.6 Hz, 1H), 1.23–1.14 (m, 1H), 1.03 (s, 3H), 0.96 (d, J = 6.9 Hz, 3H), 0.87 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 160.3, 139.6, 134.8, 131.8, 125.2, 124.7, 116.4, 109.8, 102.3, 85.5, 84.3, 69.9, 68.0, 66.8 × 2, 65.1, 64.6, 57.8, 57.6, 55.7, 55.5, 55.0, 53.6 × 2, 46.6, 40.5, 35.7, 30.0, 25.6, 23.6, 20.6, 18.7, 17.0, 13.4; ESIMS m/z 631.7 $[M + H]^+$; HRMS (ESI) calcd. for $C_{35}H_{43}N_4O_7$ $[M + H]^+$ 631.3132, found 631.3135.

5.2. Biology

5.2.1. Cell lines and cell culture

Human prostate cancer PC-3, and ovarian cancer SKOV-3 cell lines were from Japanese Foundation of Cancer Research (Tokyo, Japan). These cell lines were maintained in RPMI 1640 medium (Gibco, Grand Island, NE) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Gibco), 2 mmol/L glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 10 mmol/L HEPS (pH 7.4) in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C.

5.2.2. Cytotoxicity assays

The cytotoxicity of triptolide derivatives was examined using a panel of human tumor cell lines with the methods described before [35]. Briefly, cells in 96-well plates were treated in triplicate with gradient concentrations of tested agents at 37 °C for 72 h, then assessed by the sulforhodamine B (Sigma) assay. The cytotoxicity was expressed as an IC_{50}, defined as the concentration required for 50% inhibition of cell growth compared with control cells and calculated with the logit method.

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References

[1] S.M. Kupchan, W.A. Court, R.G. Dailey Jr., C.J. Gilmore, R.F. Bryan, J. Am. Chem. Soc. 94 (1972) 7194–7195.
[2] S.M. Kupchan, R.M. Schubert, Science 185 (1974) 791–793.
[3] K.Y. Lee, W.T. Chang, D.-M. Qiu, P.N. Kao, G.D. Rosen, J. Biol. Chem. 274 (1999) 13451–13455.
[4] Y.L. Zheng, J.F. Lin, C.C. Lin, Y. Xu, Acta Pharm. Sin. 15 (1994) 540–543.
[5] J.R. Zheng, K.X. Gu, L.F. Xu, J.W. Gao, Y.H. Yu, M.Y. Tang, Acta Acad. Med. Sin. 13 (1991) 391–397.
[6] W.Z. Gu, R. Chen, S. Brandwein, J. McAlpine, N. Burres, Int. J. Immunopharmacol. 17 (1995) 351–356.
[7] S.X. Yang, H.L. Gao, S.S. Xie, W.R. Zhang, Z.Z. Long, Int. J. Immunopharmacol. 14 (1992) 963–969.
[8] D.M. Qiu, G.H. Zhao, Y. Aoki, L.F. Shi, A. Uyei, S. Nazarian, J.C.H. Ng, P.N. Kao, J. Biol. Chem. 274 (1999) 13443–13450.
[9] Q.S. Zhen, X. Ye, Z.J. Wei, Contraception 51 (1995) 121–129.
[10] J.R. Zheng, J.L. Fang, K.X. Gu, Y.Q. Yin, L.F. Xu, J.W. Gao, H.Z. Guo, Y.H. Yu, H.Z. Sun, Chung Kuo I. Hsueh Ko Hsueh Yuan Hsueh Pao 9 (1987) 323–328.
[11] L.A. Shamon, J.M. Pezzuto, J.M. Graves, R.R. Mehta, S. Wangcharoentrakul, R. Sangsuwan, S. Chaichana, P. Tuchinda, P. Cleason, V. Reutrakul, Cancer Lett. 112 (1997) 113–117.
[12] Y. Yang, Z. Liu, E. Tolosa, J. Yang, L. Li, Immunopharmacology 40 (1998) 139–149.
[13] B.J. Chen, Leuk. Lymphoma 42 (2001) 253–256.
[14] S. Yang, J. Chen, Z. Guo, X.M. Xu, L. Wang, X.F. Pei, J. Yang, C.B. Underhill, L. Zhang, Mol. Cancer Ther. 2 (2003) 65–72.
[15] P.E. Lipsky, X.L. Tao, Semin. Arthritis Rheum. 26 (1997) 713–723.
[16] T.M. Kiviharju, P.S. Lecane, R.G. Sellers, D.M. Peehl, Clin. Cancer Res. 8 (2002) 2666–26744.
[17] B.Z. Carter, D.H. Mak, W.D. Schober, T. McQueen, D. Harris, Z. Estrov, R.L. Evans, M. Andreeff, Blood 108 (2006) 630–637.
[18] T. Tengchaisri, R. Chawengkirttikul, N. Rachaphaew, V. Reutrakul, R. Sangsuwan, S. Sirisinha, Cancer Lett. 133 (1998) 169–175.
[19] W.T. Chang, J.J. Kang, K.Y. Lee, K. Wei, E. Anderson, S. Gotmare, J.A. Ross, G.D. Rosen, J. Biol. Chem. 276 (2001) 2221–2227.
[20] S. Frese, F. Pirnia, D. Miescher, S. Krajewski, M.M. Borner, J.C. Reed, R.A. Schmid, Oncogene 22 (2003) 5427–5435.
[21] J.M. Fidler, K. Li, C. Chung, K. Wei, J.A. Ross, M. Gao, G.D. Rosen, Mol. Cancer Ther. 2 (2003) 855–862.
[22] R. Chugh, V. Sangwan, S.P. Patil, V. Dudeja, R.K. Dawra, S. Banerjee, R.J. Schumacher, B.R. Blazar, G.I. Georg, S.M. Vickers, A.K. Saluja, Sci. Transl. Med. 4 (2012) 156ra139.
[23] S.D. Westerheide, T.L. Kawahara, K. Orton, R.I. Morimoto, J. Biol. Chem. 281 (2006) 9616–9622.
[24] A. Yutaka, H. Yukio, H. Tomoyo, M. Saki, F. Haruhiko, T. Koichi, A. Ritsuo, M. Takeshi, H. Shusuke, Bioorg. Med. Chem. Lett. 18 (2008) 2459–2463.
[25] Z. Li, Z.L. Zhou, Z.H. Miao, L.P. Lin, H.J. Feng, L.J. Tong, J. Ding, Y.C. Li, J. Med. Chem. 52 (2009) 5115–5123.
[26] A. Yutaka, H. Yukio, H. Tomoyo, F. Haruhiko, T. Koichi, A. Ritsuo, M. Takeshi, H. Shusuke, Bioorg. Med. Chem. Lett. 21 (2011) 3046–3049.
[27] R. Hili, A.K. Yudin, Nat. Chem. Biol. 6 (2006) 284–287.
[28] M. Feher, J.M. Schmidt, J. Chem. Inf. Comput. Sci. 43 (2003) 218–227.
[29] T. Henkel, R.M. Brunne, H. Muller, F. Reichel, Angew. Chem., Int. Ed. 38 (1999) 643–647.
[30] F. Collet, R.H. Dodd, P. Dauban, Chem. Commun. 34 (2009) 5061–5074.
[31] R.W. De Simone, K.S. Currie, S.A. Mitchell, J.W. Darrow, D.A. Pippin, Comb. Chem. High Throughput Screen. 7 (2004) 473–494.
[32] P.D. Leeson, B. Springthorpe, Nat. Rev. Drug Discov. 6 (2007) 881–890.
[33] K. Tamao, N. Ishida, Tetrahedron Lett. 25 (1984) 4245–4248.
[34] L.V. Rubinstein, R.H. Shoemaker, K.D. Paull, R.M. Simon, S. Tosini, P. Skehan, D.A. Scudiero, A. Monks, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1113–1118.
[35] W. Li, Y. Shao, L. Hu, X. Zhang, Y. Chen, L. Tong, C. Li, X. Shen, J. Ding, Cancer Biol. Ther. 6 (2007) 787–794.