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Graphical Abstract

Discovery of Novel Taspine Derivatives as VEGFR-2 Inhibitors

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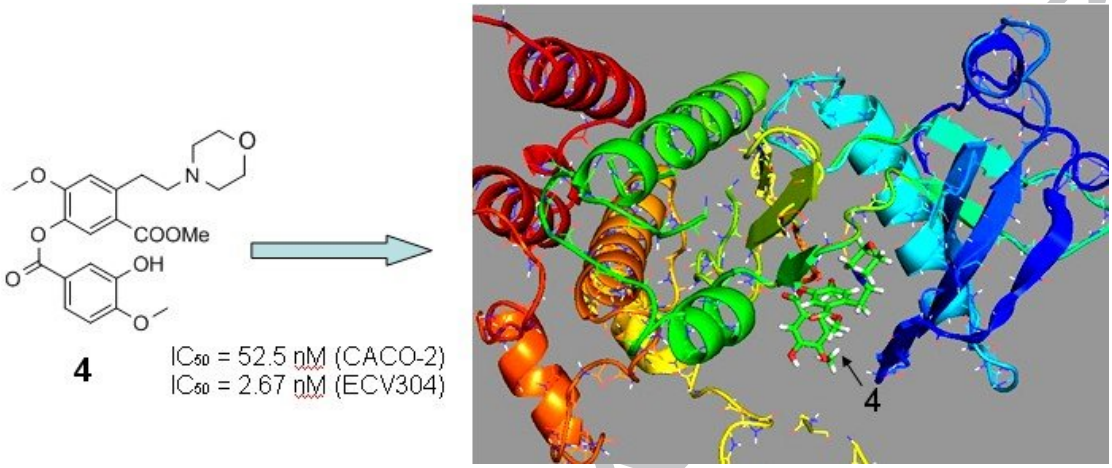

The discovery of novel inhibitors of VEGFR-2 is reported

Discovery of Novel Taspine Derivatives as Antiangiogenic Agents

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Abstract: VEGFR-2 plays a critical role in vasculogenesis and inhibitors of VEGFR-2 could be used in the treatment of cancer. Taspine was one of the active ingredients screened by using an endothelial cell membrane chromatography and showed inhibition against VEGFR-2. In our research, we explored how the lactone ring and biphenyl scaffold in taspine influence its potent *in vitro* anticancer and antiangiogenesis activities. Accordingly, we report the design, synthesis, and preliminary evaluation of four novel taspine derivatives as VEGFR-2 inhibitors. The preliminary biological test showed that one of the compounds showed much better inhibitory activities against CACO-2 (IC_{50} = 52.5 nM) and ECV304 (IC_{50} = 2.67 nM) than taspine. This result enlarges the interest in ring-opened taspine derivative skeleton in the search of new antiangiogenesis agents.

Keywords: Taspine Derivatives; VEGFR-2 Inhibitors; Tyrosine Kinase; Antiangiogenesis.

Angiogenesis is up-regulated in a wide range of diseases situations such as retinopathies, arthritis, endometriosis and cancer. Blockade of angiogenesis is an attractive approach for the treatment of such diseases especially for cancer treatment.[1] Vascular endothelial growth factor (VEGF) is one of the most important inducers of angiogenesis and exerts its cellular effects by interacting with VEGF receptors: VEGFR-1 and VEGFR-2. VEGFR-2 is the major positive signal transducer for endothelial cell proliferation and differentiation.[2] It has been proven that inhibition of VEGFR-2 activity reduces angiogenesis. For these reasons, VEGFR-2 appears to be an interesting target for design of anticancer agents.

Taspine was first isolated and characterized from *Leontice ewersmannii Bge* in 1956[3] and cecently found in small yield in blue cohosh, Caulophyllum thalictroides (L.) Michx.[4,5] Taspine was screened for the first time from *Radix et Rhizoma Leonticis* (Hong Mao Qi in Chinese, HMQ) using cell membrane chromatography by He *et al*.[6] Taspine has many pharmacological actions such as bacteriostasis, antibiosis, antivirus, treatment of wound healing, anti-inflammatory, antiulcer, and cytotoxic activity.[7] He *et al* screened taspine for the first time using cell membrane chromatography (CMC), and they found that taspine had good affinity characteristics in human umbilical vein endothelial cell membrane chromatography model.[8] In a further study, He *et al* assumed that taspine could inhibit tumor angiogenesis and one of its mechanisms may be that it inhibited VEGFR-2 and the proliferation of vascular endothelial cells.[9] Taspine also showed a significantly higher effect on acetylcholinesterase and selectively inhibited the enzyme in a long-lasting and concentration-dependent fashion.[10] However, in silico ADME prediction showed that taspine wasn't a compound with drug-like properties. And biological and cellular assays showed that it had very poor solubility in any solvent. Poor solubility was a major problem in the biological testing of compounds and it made formulating the compounds a challenge for *in vivo* studies, both for efficacy and pharmacokinetic experiments. Meanwhile, low solubility can cause low bioavailability or give rise to large fluctuations in the fraction absorbed in humans. Low solubility was also associated with stability problems.[11]

Extensive degradative work established taspine to possess the dilactonic, tertiary amine

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structure (**Fig. 1**). The planar tetracyclic compound consists of two linked lactone ring; each is condensed again with an aromatic ring that is substituted with a methoxy group.[12] Taspine is a tetracyclic natural product and may be more structurally complex than is necessary for optimal pharmacologic effects. A complex lead compound may have a simpler pharmacophore buried within its structure, and if this pharmacophore can be clearly defined, the resulting biological active, simpler molecule may have improved synthetic tractability and be more useful as a scaffold for further analogue design. To research the individual contribution of the lactone ring and biphenyl scaffold of taspine to the activity against cancer cells, we designed and synthesized four novel ring-opened target compounds by structure-based drug design (**Fig. 1)**. The novel ring cleavage compounds were designed in two pathways: cleavage of the C-C bond of diphenyl and ester bond of ring B and ring D.

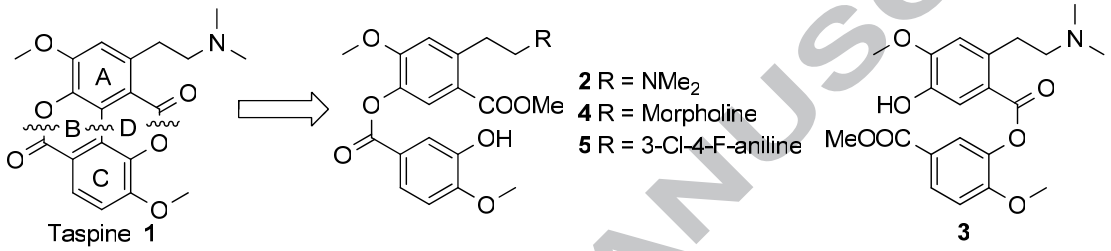
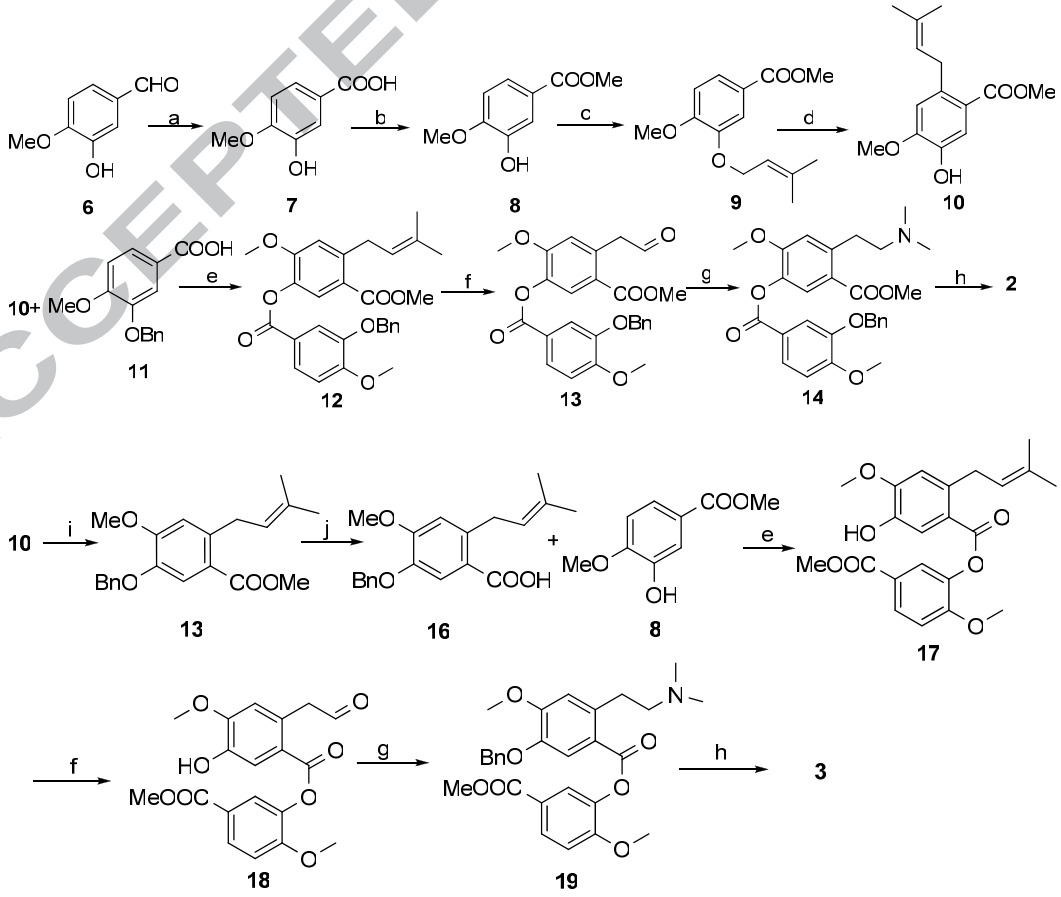


Figure 1. Design of ring-opened target compounds **2-5**.

In this paper, we described the design, synthesis and preliminary evaluation of four ring-opened derivatives of taspine from commercially available isovanillin (**4**) using dissection strategies. A molecular docking study of target compounds with VEGFR-2 was also performed using SYBYL to identify their binding mode with enzyme.



Scheme 1. Preparation of target compounds

Reagents and Conditions: (a) NaOH, KOH, H_2O, 84%; (b) CH_3OH, H_2SO_4, 90%; (c) prenyl bromide, K_2CO_3, acetone, 92%; (d) *N*,*N*-dimethylaniline, N_2, reflux, 71%; (e) anhydrous THF, DCC, DMAP, 78%; (f) OsO_4, $NaIO_4$, acetone/H_2O/t-BuOH, 32%; (g) dimethylamine/Morpholine/3-Cl-4-F-aniline, THF, CH_2Cl_2, $NaBH(OAc)_3$, 25-40%; (h) H_2, Pd/C, 97%; (i) BnCl, K_2CO_3, EtOH, 95%; (j) NaOH/H_2O, CH_3OH, 93%.

Four of the title compounds were synthesized by the route outlined in **Scheme 1**. We used commercially available isovanillin as the starting material. At first, Isovanillin (**6**) was oxidized to give isovanillic acid (**7**) with fused sodium hydroxide and potassium hydroxide. The methyl ester (**8**) was prepared to avoid side-reactions of the carboxylate group. Then refluxing of (**8**) with prenyl bromide in the presence of K_2CO_3 in anhydrous acetone afforded compound (**9**).[13] A solution of (**9**) in *N*,*N*-dimethylaniline was heated to reflux for 8h to give compound (**10**).[14] Prenyl group was moved into the *para*-position of hydroxyl in this Claisen rearrangement process. The next step was the coupling of (**10**) to the carboxyl of (**11**) by an ester bond with DCC and DMAP.[15] The oxidation of (**12**) with OsO_4/$NaIO_4$ produced aldehyde (**13**),[16] which reacted with dimethylamine followed by reduction with $NaBH(OAc)_3$ to give (**14**).[17] At last, benzyl deprotection of (**14**) with palladium-carbon in MeOH gave the target compound (**2**).[18] The other target compound (**3**), (**4**) and (**5**) were synthesized in the same way from isovanillin.[19]

Since it is not always practical to perform experimental measurements, it is useful to rapidly predict molecular physicochemical properties such as LogP, LogD, pKa, PSA, and solubility values.[20] Before biological evaluation, we evaluated a broad range of molecular physicochemical properties which impacted drug-like ADME parameters based on chemical structures with ACD/Labs (**Table 1**). From **Table 1**, we can see that solubility values of target compounds were 3-fold to 13-fold higher than taspine which had very poor solubility in any solvent.

Table 1. Prediction of physicochemical properties of compounds with ACD/Labs

	CLogP	LogD(pH=7.4)	pK_a	Solubility(g/L)(PH=7.4)	PSA[a]
Taspine	1.93	2.92	8.21	0.021	74.3
2	3.18	1.72	9.73; 8.75	0.14	94.53
3	3.18	1.97	9.55; 8.57	0.13	94.53
4	3.33	2.65	9.02; 7.28	0.27	103.76
5	6.06	6.56	9.01; 3.65	0.061	103.32

[a]: PSA = Polar Surface Area (Å^2)

All target compounds were tested in functional cell-based assays using a variety of cancer and normal cell lines.[21] **Table 2** summarized some of our findings. As shown in **Table 2**, all of the title compounds moderately inhibited the growth of A549 in nanomolar range which was less potent than the leading compound taspine. However, the activity of (**4**), a D-ring opened compound, was much better than that of taspine against CACO-2 (3-fold) and ECV304 (about 10-fold) with the IC_{50} values of 52.5 nM and 2.67 nM, respectively. Compound (**4**) appears to inhibit proliferation more potently in ECV304, a human umbilical endothelial cell line, and this may be an indication that the compound is acting through inhibition of VEGFR-2. If the antiangiogenic properties of (**4**) are confirmed, the compound could represent a novel hit for lead optimization efforts. The results demonstrated that the ester bond of B-ring was more important than that of D-ring in maintaining the biological activity of this compounds type.

Table 2. Antiproliferative activities of target compounds on human cancer and normal cells

Compound	Cell lines (IC$_{50}$, nM)		
	CACO-2[a]	A549[b]	ECV304[c]
Taspine	153	4.18	22.3
2	11003	453	ND
3	3100	111	ND
4	52.5	158	2.67
5	ND	180	61

ND is not determined.

[a] CACO-2, human epithelial colorectal adenocarcinoma cells; [b] A549, carcinomic human alveolar basal epithelial cells; [c] ECV304, human umbilical endothelial cell line.

In an effort to elucidate the binding modes of these compounds with VEGFR-2, the most active compound (**4**) was constructed with Sybyl/Sketch module and optimized using Powell's method with the Tripos force field with convergence criterion set at 0.05 kcal/(Å mol), and assigned with Gasteiger-HŰckel method.[22] The docking study performed using Sybyl/FlexX module, the residues in a radius of 6.5 Å around XIN 1172 in the VEGFR-2 (PDB ID: 3C7Q) were selected as the active site. Other docking parameters implied in the program were kept default (**Fig. 2**). Docking of compound (**4**) in the active site of VEGFR-2 showed four H-bond interactions between oxygen of the inhibitor and amino acid residues of the enzyme. We found that one oxygen of carboxylate group in ring A formed two hydrogen bonds to Asn923 with distance of 1.88 Å and 2.21 Å. The oxygen of phenolic hydroxyl group in ring B formed a hydrogen bond to Arg 1032 with distance of 1.78 Å while oxygen of morpholine formed hydrogen bonds to Cys 919 with hydrogen length of 1.90 Å. Morpholine played an important role in the binding of inhibitor with VEGFR-2. Although the computed information partially supported our assumption, the ligand bound structure of these taspine derivatives with VEGFR-2 will be definite confirmation of the binding mode described and efforts are underway. This binding hypothesis could provide valuable information for structure based design of analogs.

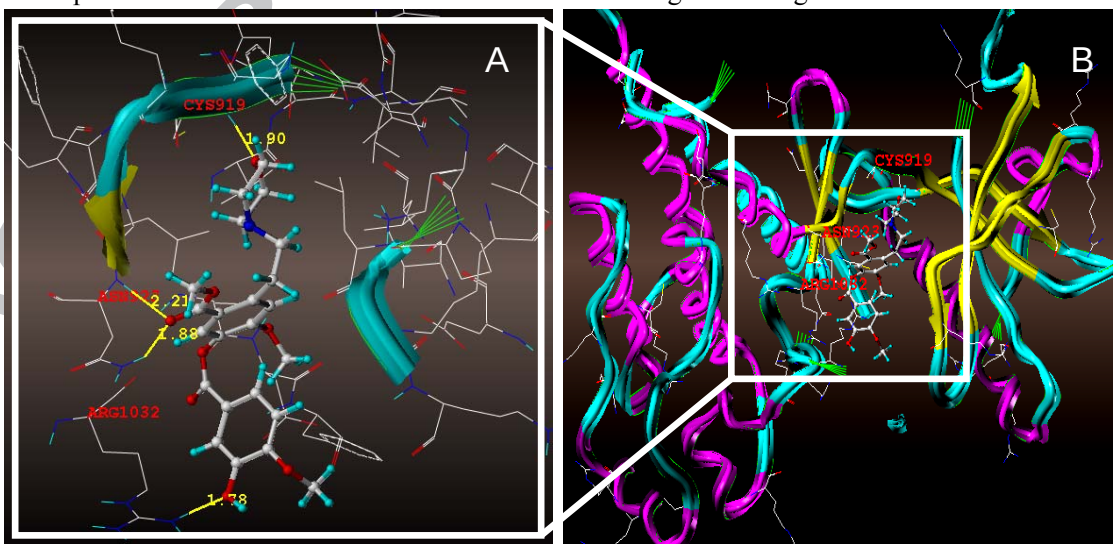


Figure 2. FlexX docked conformation of compound (**4**) in the active site of VEGFR-2 (PDB ID: 3C7Q). (A) Compound (**4**) reacting with the amino acids residues of the active site of VEGFR-2, Hydrogen bonds between the inhibitor and residues are shown with yellow dotted lines. (B) Kinase inhibitor-protein interactions are depicted by ribbon structure.

In summary, our research has led to further insights in the structure activity relationship of

taspine analogs. We have demonstrated that the lactone ring B is important for activity, while the lactone ring D can be opened retaining and even improving the antiproliferative properties of taspine. The data suggest that these compounds could also exhibit an antiangiogenic mechanism of action, similarly to taspine, and could therefore be a promising starting point for further medicinal chemistry efforts. Our efforts in pursuing the optimization of the physicochemical and biological properties of taspine analogs will be reported in due course.

Acknowledgments

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    All key intermediates provided acceptable MS data and ^{1}H-NMR spectra that exhibit no discernible impurities. **Methyl 5-(3-(benzyloxy)-4-methoxybenzoyloxy)-4-methoxy-2-(3-methylbut-2-enyl)benzoate (13):** mp 115.0-116.0℃. EI-MS(m/z): 489.9 (M^+), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.67 (s, 6H), 3.76 (s, 3H), 3.77 (s, 3H), 3.71 (d, 2H, *J*=6.0 Hz), 3.89 (s, 3H), 5.13 (s, 2H), 5.23 (t, *J*=6.0 Hz, 1H), 6.79 (s, 1H), 6.89 (d, *J*=8.5 Hz, 1H), 7.22-7.33 (m, 5H), 7.39 (s, 1H), 7.69 (s, 1H), 7.79 (d, *J*=8.5 Hz, 1 H).
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19. All new compounds **2-5** provided acceptable MS data and ^{1}H-NMR spectra that exhibit no discernible impurities. **Methyl 2-(2-(dimethylamino)ethyl)-5-(3-hydroxy-4-**

methoxybenzoyloxy)-4-methoxybenzoate (2): ^{1}H-NMR, (300MHz, $CDCl_3$) δ ppm 2.52(s, 6H), 2.71 (t, *J*=7.3 Hz, 2H), 3.62 (t, *J*=7.0 Hz, 2H), 3.84 (s, 3H), 3.89 (s, 3 H), 3.98 (s, 3H), 6.89 (s, 1H), 7.08 (d, *J*=8.7Hz, 1H), 7.54 (d, *J*=9.0 Hz, 1H), 7.67 (s, 1H), 7.81 (s, 1H); EI-MS(m/z): 403.1 [M^+]; mp 112.5-114.5℃. **2-Methoxy-5-(methoxycarbonyl)phenyl-2-[2-(dimethylamino)ethyl]-5-hydroxy-4-methoxybenzoate (3).** mp 107.0-109.5 ℃, EI-MS(m/z): 402.9 (M^+), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 2.62 (s, 6H), 3.55 (t, *J*=6.6 Hz, 2H), 3.83 (s, 3H), 3.85 (s, 3 H), 3.98 (s, 3H), 4.01 (t, *J*=6.9 Hz, 2H), 6.66 (s, 1H), 7.13 (d, *J*=9.0 Hz, 1H), 7.72 (s, 1H), 7.86 (d, *J*=9.0 Hz, 1H), 7.93 (s, 1H).

Methyl 5-[(3-hydroxy-4-methoxybenzoyl)oxy]-4-methoxy-2-(2-morpholin-4-ylethyl) benzoate (4). mp 121.0-124.5 ℃, EI-MS(m/z): 445.2 (M^+), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 2.55-2.63 (m, 4H), 2.82 (t, *J*=8.0 Hz, 2H), 3.42-3.49 (m, 4H), 3.71 (t, *J*=7.8 Hz, 2H), 3.82 (s, 3H), 3.90 (s, 3 H), 3.97 (s, 3H), 6.76 (s, 1H), 7.00 (d, *J*=9.0Hz, 1H), 7.42 (d, *J*=8.7 Hz, 1H), 7.73 (s, 1H), 7.87 (s, 1H).

Methyl 2-{2-[(3-chloro-4-fluorophenyl)amino]ethyl}-5-[(3-hydroxy-4-methoxybenzoyl) oxy]-4-methoxybenzoate (5). mp 101.0-103.5 ℃, EI-MS(m/z): 503.7 (M^+), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 2.49 (t, *J*=7.5 Hz, 2H), 3.52 (t, *J*=7.2 Hz, 2H), 3.81 (s, 3H), 3.87 (s, 3 H), 3.95 (s, 3H), 6.88 (d, *J*=7.5 Hz, 1H), 6.94 (s, 1H), 6.97-7.10 (m, 2H), 7.19-7.21 (m, 1H), 7.54 (d, *J*=9.0 Hz, 1H), 7.68 (s, 1H), 7.77 (s, 1H).

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