Synthesis and Cytotoxic Evaluation of Novel Symmetrical Taspine Derivatives as Anticancer Agents

Jie Zhang, Yanmin Zhang, Xiaoyan Pan, Sicen Wang and Langchong He*

College of Medicine, Xi'an Jiaotong University, No. 76, Yanta West Road, Xi'an, Shaanxi Province, 710061, P.R. China

Abstract: It has been demonstrated that taspine derivatives act as anticancer agents, thus we designed and synthesized a novel class of symmetrical biphenyl derivatives. We evaluated the cytotoxicity and antitumor activity of biphenyls against five human tumor and normal cell lines. The results indicated that the majority of the compounds exhibited anticancer activity equivalent to or greater than the positive control. Compounds (**11**) and (**12**) demonstrated the most potent cytotoxic activity with IC_{50} values between 19.41 μM and 29.27 μM. The potent antiproliferative capabilities of these compounds against ECV304 human transformed endothelial cells indicated that these biphenyls could potentially serve as antiangiogenic agents. We also reviewed the relationship between structure and activity based on the experimental results. Our findings provide a good starting point for further development of symmetrical biphenyl derivatives as potential novel anticancer agents.

Keywords: Symmetrical taspine derivatives, antiproliferative activity, cytotoxicity, anticancer agents, antiangiogenic agents; VEGFR-2.

1. INTRODUCTION

Cancer is a class of diseases in which a group of cells displays uncontrolled growth, causing a cell invasion that intrudes upon and destroys adjacent tissues, sometimes leading to metastasis and a spread to other locations in the body via lymph or blood. It is one of the leading causes of death, claiming more than one million victims every year [1]. Currently, cancer can be treated by chemotherapy, radiation therapy, surgery, immunotherapy, monoclonal antibody therapy, or other methods. Chemotherapy is a treatment of cancer through the use of anticancer cytotoxic drugs [2]. Although chemotherapy may carry significant side effects, many types of chemotherapy drugs have been developed. Currently, development of novel chemotherapeutic agents for cancer chemotherapy is a high priority for medicinal chemists and is an active area of research [3].

Natural products continue to play a highly significant role in the discovery and development of novel lead compounds and new chemical entities, and this is particularly evident in anticancer agent development [4]. Taspine (**1**, Fig. **1**) has been identified as an important bioactive constituent isolated from *Radix et Rhizoma Leonticis* (*Hong Mao Qi* in Chinese) [5]. It exhibits multiple pharmacological activities; for example, bacteriostatic, antibiotic, antiviral, anti-inflammatory, antiulcer, cytotoxic activity, and so on [6]. The anticancer and antiangiogenic properties of taspine have been previously demonstrated, and it has the potential to be an ideal candidate as a chemotherapeutic agent [7]. In a recent study, He *et al.* demonstrated that taspine was able to inhibit tumor angiogenesis, and suggested that one of its mechanisms might be to inhibit VEGFR-2 [8].

*Address correspondence to this author at the 54 mailbox, College of Medicine, Xi'an Jiaotong University, No. 76, Yanta West Road, Xi'an, Shaanxi Province, 710061, P.R. China; Tel/Fax: +86-29-82655451;
E-mail: helc@mail.xjtu.edu.cn

We are currently engaged in a project aimed at identifying novel biologically active small molecules that could serve as powerful anticancer therapeutic candidates [9]. Biphenyl compounds are known for various biological activities, including antibacterial, anti-inflammatory and anticancer activities. We recently synthesized a series of unsymmetrical biphenyls and evaluated their antiproliferative activity. These unsymmetrical biphenyls exhibited potent cytotoxicity against several human cancer cell lines. These findings indicated that biphenyl derivatives are suitable compounds for further optimization. Recently, we reported the synthesis of unsymmetrical biphenyl (**2**), a novel ring-opened biphenyl derivative of taspine [10]. During the synthesis of compound (**2**), a novel symmetrical biphenyl (**11**) was isolated as a byproduct and was also evaluated for biological activity (Fig. **1**). It was determined that compound (**11**) exhibited potent anticancer activity, which attracted considerable attention. To further investigate this finding, we aimed to enhance the structural complexity and diversity of compound (**11**) by generating novel biphenyls [11].

In this study, to further elucidate the relationship between structure and activity for biphenyl derivatives, we describe the synthesis of eighteen symmetrical biphenyl derivatives (**11**-**28**) and their cytotoxic activity against five human cancer and normal cell lines. Among them, two compounds (**11** and **12**) exhibited promising growth inhibitory effects against the tested cells with an IC_{50} range of 2.93-28.92 μM. Aniline that contains one or more halogen substituents (fluoro, chloro, brome and iodo) is useful for novel anticancer drug design, and in addition the presence of halogen in a molecule enhances drug persistence and lipid solubility [12].

2. CHEMISTRY

Eighteen symmetrical biphenyls were prepared by the general procedure described in Scheme **1**. We used commercially available isovanillin as the starting material. Initially,

1573-4064/11 $58.00+.00

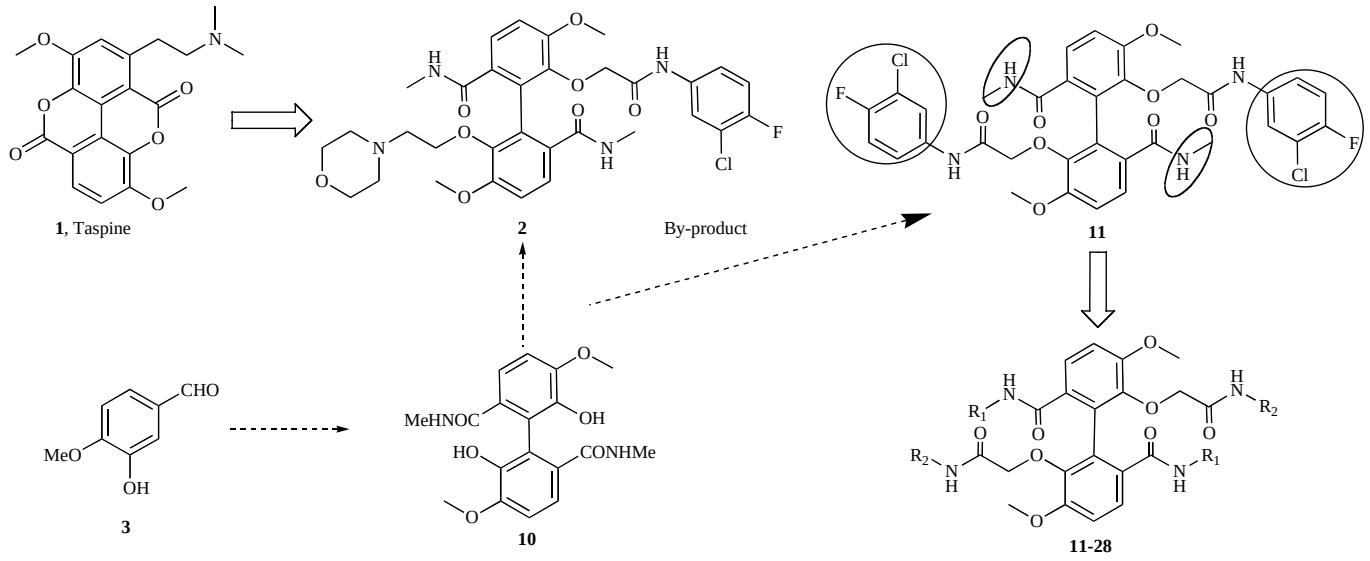
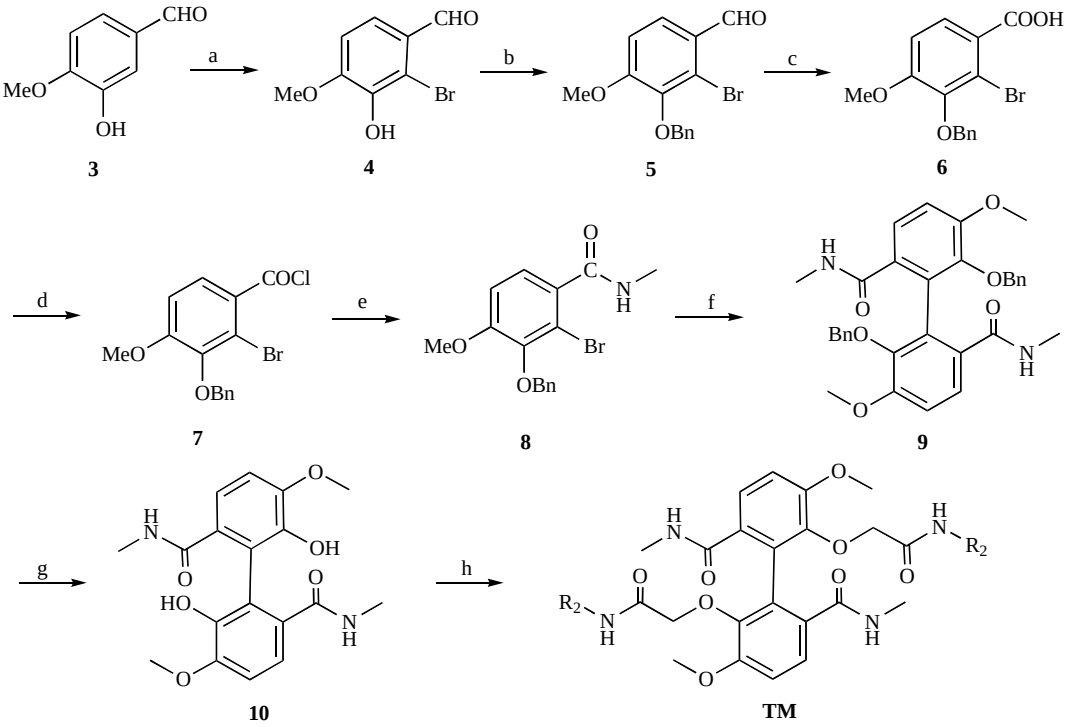


Fig. (1). The surprising discovery and structures of title biphenyl compounds (11-28).



Scheme 1. Preparation of target compounds (**11-28**)
Reagents and Conditions: (a) Fe, NaOAc, AcOH, Br_2, 83%; (b) BnCl, K_2CO_3, 96%; (c) NaH_2PO_4, $NaClO_2$, 30% H_2O_2, 95%; (d) $SOCl_2$, DMF(cat), CH_2Cl_2, 100%; (e) CH_2Cl_2, 30% CH_3NH_2 or diethylamine or isopropylamine, 80% ~ 87%; (f) Cu, DMF, 75%; (g) H_2, Pd/C; 98%; (h) K_2CO_3, anhydrous ethanol, (64%~85%).

isovanillin (**3**) was converted into 2-bromoisovanillin (**4**) by a reaction with bromine [13]. After protection of (**4**) as ether (**5**) [14], the aldehyde group in (**5**) was oxidized to the carboxyl group yielding (**6**) [15]. Amidation of (**6**) with methylamine yielded the monocyclic precursor (**8**) for the Ullmann reaction [16]. Biphenyl dimethane amide (**9**) was prepared by dimerization of (**8**) through a standard symmetrical Ullmann reaction [17]. Compound (**10**) was readily obtained by deprotection of benzamide (**9**) under catalysis with palladium/carbon [18]. Both of the hydroxyl groups in (**10**) were etherified with various alkyl halides in anhydrous ethanol in the presence of K_2CO_3 to give the title compounds in various yields [19]. The other target compounds were prepared in an identical manner, except for compound (**6**), which was amidated with a different fatty amine.

3. RESULTS AND DISCUSSION

It is well understood that potential activity does not necessarily guarantee acceptable bioavailability. The molecular properties regarding adsorption, distribution, metabolism, and excretion (ADME) are crucial for drug design, however,

Table 1. Volsurf Descriptors of Symmetrical Biphenyl Derivatives

Compound	Lipinski's Rule of 5				ADME Predicted (Volsurf)		
	MW	NRB[a]	NHA[b]	NHD[c]	logP[d]	PSA[e]	PPB%[f]
Taspine	369.37	5	7	0	0.68	74.30	29.22%
11	731.52	13	12	4	5.47	153.32	99.88%
12	762.65	15	12	4	5.19	153.32	99.85%
13	764.43	13	12	4	6.62	153.32	99.97%
14	686.71	15	14	4	3.56	171.78	98.66%
15	784.45	13	12	4	5.25	153.32	99.87%
16	662.64	13	12	4	3.09	153.32	97.46%
17	662.64	13	12	4	4.11	153.32	99.23%
18	626.65	13	12	4	3.28	153.32	97.67%
19	558.62	13	12	4	1.04	153.32	69.87%
20	664.61	13	14	4	2.32	179.10	98.61%
21	787.63	15	12	4	6.95	153.32	99.98%
22	818.76	17	12	4	6.67	153.32	99.98%
23	820.54	15	12	4	8.10	153.32	100.00%
24	742.81	17	14	4	5.04	171.78	99.81%
25	840.55	15	12	4	6.73	153.32	99.98%
26	720.72	15	14	4	3.80	179.10	99.63%
27	815.68	17	12	2	5.81	135.74	99.94%
28	846.81	19	12	2	5.53	135.74	99.93%

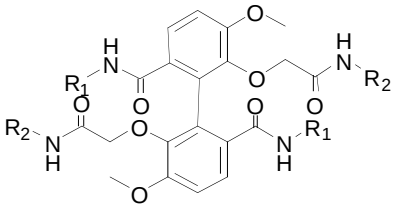
[a] NRB = number of rotatable bonds; [b] NHA = number of H-bond acceptors; [c] NHD = number of H-bond donors; [d] logP = log octanol-water partition coefficient; [e] PSA = polar surface areas ($Å^2$); [f] PPB% = Drug Binding to Plasma Proteins.

it is not always practical to perform the respective experimental measurements. Therefore, it is useful to rapidly predict certain molecular physicochemical properties such as LogP, polar surface areas (PSA), and plasma protein binding rate that may indicate bioavailability [20]. Prior to biological evaluation, we evaluated a broad range of molecular physicochemical properties that potentially impact drug-like ADME parameters based on chemical structure. All compounds were filtered using Lipinski's rule of 5 [21], and subsequently tested according to the Volsurf module (SYBYL-X 1.1) in order to calculate predicted ADME properties [22]. The ADME properties of compounds are summarized in Table **1,** including MW, logP, number of H-bond acceptors, donors and rotatable bonds, PSA and plasma protein binding rate. It is evident from Table **1** that the majority of the biphenyls were neutral compounds that primarily bonded to lipoproteins, and to a lesser extent to albumin. Compounds (**20**) and (**26**) were weak bases (base PKa < 8.5) and predominantly bonded to alpha1-acid glycoprotein and albumin. The molecular polar surface areas of biphenyl derivatives were much greater compared to taspine; in fact, the majority were above 140, indicating that these compounds exhibit poor cell membrane permeability. The maximum plasma protein binding rates of target compounds were over 90%; much higher in comparison to taspine. The results indicated that these compounds were easily absorbed in plasma. In general, when the bonded concentration is less than 90% of the total plasma concentration the plasma protein binding has little clinical importance. Plasma protein binding becomes important when it is greater than 90%.

The most common research screening method for anticancer agents employs a cytotoxicity test against a panel of cancer cell lines [23]. All target compounds were evaluated for cytotoxicity against several cancer cell lines *in-vitro* using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay with taspine and gefitinib serving as the positive controls. The structure and biological activity of all symmetrical biphenyls (**11**-**28**) are summarized in Table **2**.

The results (shown in Table **2**) indicated that most compounds exhibited potent cytotoxic activity comparable to or stronger than that of gefitinib. Two compounds (**11** and **12**) demonstrated a promising growth inhibitory effect against tumor and normal cells with an IC_{50} range of 2.93-28.92 μM. Compounds (**11** and **21**, 3-chloro-4-fluoroaniline substitution) exhibited a greater potency than the others, including gefitinib, against 7721 cells with IC_{50} value**S** of 19.41 and

Table 2. Cytotoxic Activities of Title Compounds on Human Cancer and Normal Cell Lines



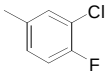
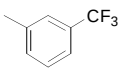
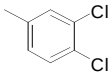
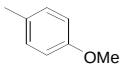
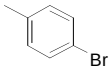
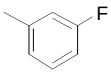
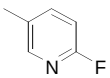
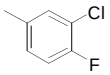
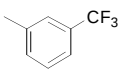
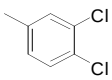
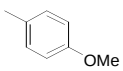
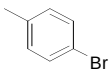
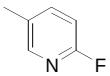
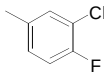
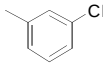
Compound	R_1	R_2	Cell Lines (IC_{50}, μM)				
			7721[a]	MCF-7[b]	LOVO[c]	A549[d]	ECV304[e]
Taspine	/	/	57.58	ND	ND	4.18	22.30
2	/	/	194.09	60.38	ND	240.51	72.17
11	CH_3		19.41	28.29	ND	26.70	2.93
12	CH_3		28.92	29.27	ND	28.69	4.08
13	CH_3		39.46	28.72	ND	29.16	18.94
14	CH_3		186.75	141.63	ND	142.84	72.11
15	CH_3		62.95	46.39	ND	42.46	68.07
16	CH_3		156.45	79.79	ND	110.41	66.64
17	CH_3		97.41	67.44	ND	94.39	67.13
18	CH_3		456.39	184.28	194.07	263.25	ND
19	CH_3		996.07	128.66	222.69	439.39	ND
20	CH_3		439.39	273.57	164.25	864.45	ND
21	isopropyl		25.41	76.55	34.86	30.53	ND
22	isopropyl		36.18	99.83	40.22	76.98	ND
23	isopropyl		24.14	93.15	55.51	32.15	ND
24	isopropyl		567.26	91.56	429.24	327.20	ND
25	isopropyl		59.68	345.96	66.16	239.31	ND
26	isopropyl		94.81	452.38	263.18	190.41	ND

Table 2. contd…

Compound	R_1	R_2	Cell Lines (IC_{50}, μM)				
			7721[a]	MCF-7[b]	LOVO[c]	A549[d]	ECV304[e]
27	diethyl		54.92	134.30	59.37	70.40	ND
28	diethyl		63.79	117.52	140.99	350.96	ND
Gefitinib	/	/	30.21	23.75	46.30	26.60	27.49

ND is not determined.
[a] 7721, human hepatocellular carcinoma cell line; [b] MCF-7, human breast cancer cell line; [c]LOVO, human colon cancer cell line; [d]A549, human lung adenocarcinoma epithelial cell line; [e]ECV304, human umbilical vein endothelial cell line.

25.41 μM, respectively. Compound (**11**) also exhibited potent antiproliferative activity against MCF-7 cells and A549 cells with IC_{50} values of 28.29 and 26.70 μM, respectively. Additionally, compound (**21**, 3-chloro-4-fluoroaniline substitution) exhibited the greatest growth inhibitory activity against LOVO cells with an IC_{50} value of 34.86, which was greater than gefitinib (IC_{50} = 46.30 μM).

As shown in Table **2**, biphenyls without halogen substitution (**14**, **18**, **19**, and **24**) were much less potent than those containing halogen. This finding indicated that the halogen substitution played a critical role in the activity of biphenyls. Halogen substitution could potentially improve the anticancer activity of biphenyl. The cytotoxicity of two pyridine-containing compounds (**20** and **26**) was too weak for further development.

We also evaluated the antiproliferative activity of compound (**11**) against several other human cancer cell lines using the MTT assay. Compound (**11**) exhibited a broad spectrum of growth inhibition activity against A431 (human epithelial carcinoma cell line, IC_{50} = 37.90 μM), A375 (human malignant melanoma cells, IC_{50} = 26.31 μM), HT-29 (human colon carcinoma cells, IC_{50} = 31.03 μM), Hela (human epithelial cervical cancer cells, IC_{50} = 33.07 μM), CACO-2 (human colonic carcinoma cell line, IC_{50} = 14.44 μM), and PANC-1 (human pancreatic carcinoma cell line, IC_{50} = 34.94 μM) cells.

The antiangiogenic activity of taspine has been previously tested using the chicken chorioallantoic membrane neovascularisation model *in vivo* and the human umbilical vein endothelial cell (HUVEC) proliferation and migration models *in vitro* [24]. In this study, we used antiangiogenesis screening to focus on the antiproliferative activity of the compounds against HUVEC *in vitro*. In order to investigate the mechanism of action of the biphenyls we also tested their antiproliferative activity against ECV304 human transformed endothelial cells. As shown in Table **2**, the majority of the title compounds exhibited potent growth inhibition of ECV304 proliferation in a dose-dependent manner, with an IC_{50} range of 2.93 - 72.17 μM.

Compounds (**11**) and (**12**) exhibited the greatest antiproliferative activity against ECV304 cells with IC_{50} values of 2.93 and 4.08 μM, respectively. The results indicated that these biphenyls could potentially serve as antiangiogenic agents.

Angiogenesis is a key event of tumor progression and metastasis and hence a target for cancer chemotherapy [25]. Angiogenesis is primarily a receptor-mediated process in which growth factors cause signal transduction via receptor tyrosine kinase (RTK). Vascular endothelial growth factor (VEGF) has been implicated in tumor angiogenesis and it is a potent angiogenesis inducer *in vivo* and *in vitro* [26]. Inhibition of the VEGF RTK has provided a new paradigm in the treatment of tumors. It is well established that antiangiogenic agents also manifest significant inhibitory activity against VEGFR-2. To investigate the mechanism of antiangiogenesis and the possible binding modes of biphenyl derivatives, a molecular docking study of target compounds with VEGFR-2 was performed using SYBYL-X 1.1 [27]. Compounds (**11**) and (**12**) were docked into the kinase domain of VEGFR-2 (PDB code: 3CJF) (Fig. **2**) [28]. According to our docking simulation, shown in Fig. (**2**), methylamine hydrogen of (**11**) formed a hydrogen bond interaction with carbonyl oxygen of ARG 1030 with a distance of 1.96 Å. Regarding the binding mode of compound (**12**), the oxygen of carboxyl formed a H-bond to ASP 1044 with a distance of 2.20 Å, while the fluorine atom formed a hydrogen bond with ASN 921 with a bond length of 2.19 Å. The binding hypothesis potentially provides valuable information for the structure-based design of novel biphenyl derivatives.

In the present study, eighteen novel biphenyls were designed and synthesized. All of the compounds were evaluated and the majority demonstrated moderate to good anticancer activity. Of these compounds, (**11**) and (**12**) exhibited the highest antiproliferation activity against several cancer cell lines and ECV304.

4. CONCLUSION

In summary, a novel class of symmetrical biphenyls was investigated in this study. Preliminary bioassays indicated that the majority of these biphenyls demonstrated potent anticancer and antiangiogenic activity. The docking results and the antiproliferative activity against HUVECs suggested that these compounds could potentially serve as antiangiogenic agents, a promising indication of the importance of further medicinal chemistry efforts. A thorough biological evaluation and mechanistic study of these compounds are currently in progress and will be reported in due course.

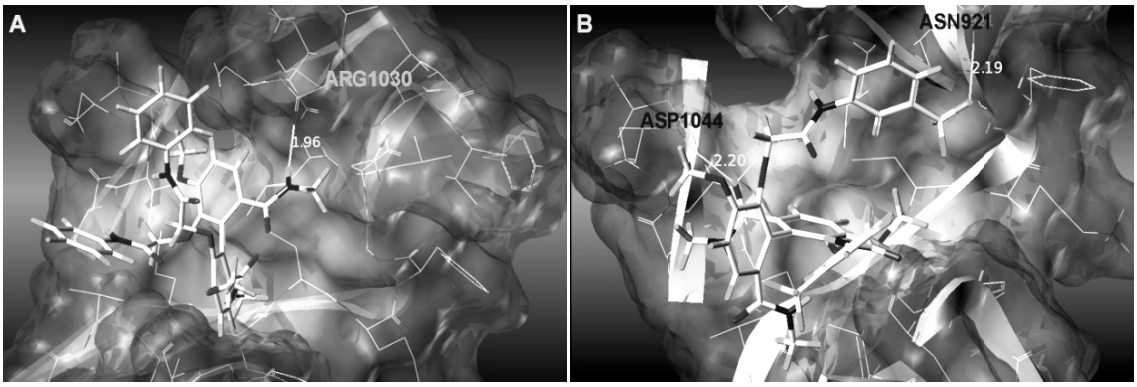


Fig. (2). Compound (**11**, **A**) and (**12**, **B**) was built and docked into the active site of VEGFR-2 (PDB code: 3CJF).

5. EXPERIMENTAL

5.1. Antiproliferative Activity of Taspine Derivatives

Growth inhibitory activity was evaluated on the following cell lines: 7721, MCF-7, LOVO, A549, ECV304, A431, A375, CACO-2, Hela, HT-29, and PANC-1. The effects of the compounds on cell viability were evaluated using the MTT assay [29]. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1×10^4 cells/well, and incubated for 24 h at 37°C. The respective cells in the wells were treated with target compounds at various concentrations for 48 h. Subsequently, 20 μL MTT (5 mg/mL) was added to each well and the cells were incubated for 4 h at 37°C. After the supernatant was discarded, 150 μL DMSO was added to each well, and the absorbance values were determined by a microplate reader (Bio-Rad Instruments) at 490 nm.

5.2. Chemistry: General Procedures

All reactions except those in aqueous media were conducted according to standard techniques for the exclusion of moisture. Solvents were purified before use according to standard procedures. All reactions were monitored by thin layer chromatography on 0.25-mm silica gel plates (60GF-254) and visualized with UV light. All melting points were determined on a Beijing micro-melting point apparatus and were uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AVANCF 400 MHZ instrument in $CDCl_3$ solution with TMS as internal standard. Mass spectra were performed on a Shimadzu GC-MS-QP2010 instrument.

5.2.1. 2-bromo-3-hydroxy-4-methoxybenzaldehyde (4).

To a mixture of (**3**) (10.0 g, 66.0 mmol), NaOAc (10.80 g, 0.132 mol) and iron powder (0.34 g, 6.0 mmol) was added glacial acetic acid (60 mL). The mixture was stirred at room temperature for 30 min. Br_2 (3.5 mL, 70.0 mol) in glacial acetic acid (15 mL) was added dropwised into above mixture at 23-25 ℃. The mixture was stirred at the same temperature for 3 h. Ice water (150 mL) was added to the mixture and stirred for another 1 h and filtered. The solid obtained as dried and recrystallized from EtOH to give (**4**) (12.65 g, 83%) as a gray solid. mp 206-207 ℃; EI-MS(m/z): 230.9 ($[M+H]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 4.01 (s, 3H), 6.07 (s, 1H), 6.93 (d, *J*=8.5 Hz, 1H), 7.58 (d, *J*=8.5 Hz, 1H), 10.26 (s, 1H).

5.2.2. 3-(benzyloxy)-2-bromo-4-methoxybenzaldehyde (5).

To a suspension of (**4**) (3.47 g, 15 mmol) in dehydrated alcohol (70 mL) was added anhydrous potassium carbonate (6.22 g, 45 mmol) and benzyl chloride (2.30 mL, 20 mmol). The mixture was refluxed for 4 h. Filtration and evaporation of alcohol was done in a vacuum. The residue was extracted with EtOAc (3×40 mL). The combined organic layers were washed with H_2O (3×30 mL), 2 M NaOH (3×30 mL), 2 M HCl (3×30 mL) and brine (2×30 mL), dried over Na_2SO_4, and concentrated to give (**5**) (4.62 g, 96%) as a colorless solid. mp 79-81 ℃; EI-MS(m/z): 320.9 ($[M+H]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 3.96 (s, 3H), 5.03 (s, 2H), 6.98 (d, *J*=9.2 Hz, 1H), 7.35-7.54 (m, 5H), 7.76 (d, *J*=8.4 Hz, 1H), 10.27 (s, 1H).

5.2.3. 3-(benzyloxy)-2-bromo-4-methoxybenzoic acid (6).

To a solution of (**5**) (4.82 g, 15 mmol) in THF (60 mL) was added distilled H_2O (20 mL) and NaH_2PO_4 (1.08 g, 9 mmol). The mixture was stirred at room temperature for 10 min. $NaClO_2$ (4.49 g, 50 mmol) and 30% H_2O_2 (3.4 mL, 33 mmol) in distilled H_2O (15 mL) were added into the above mixture. The mixture was stirred at the same temperature for 3 h. THF was evaporated under vacuum and the residue was extrated with EtOAc (3×50 mL). The combined organic layers were washed with (3×20 mL) and the product was extracted with 2 M NaOH (5×20 mL). The aqueous phase was acidified with concentrated HCl and the solid obtained was collected by filtration and dried to give (**6**) (4.80 g, 95%) as a white solid. mp 159-161 ℃; EI-MS(m/z): 336.9 ($[M+H]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 3.94 (s, 3H), 5.03 (s, 2H), 6.93 (d, *J*=9.2 Hz, 1H), 7.37-7.56 (m, 5H), 7.87 (d, *J*=9.1 Hz, 1H).

5.2.4. 3-(benzyloxy)-2-bromo-4-methoxy-N-methylbenzamide (8).

To a suspension of (**6**) (5.06 g, 15 mmol) in CH_2Cl_2 (50 mL) was added thionyl chloride (4.37 mL, 60 mmol) and a catalytic amount of DMF. The mixture was stirred at room temperature for 2 h. The solvent was removed by evaporation *in vacuo* to give 5.34 g (100%) of the acid chloride (**7**).

To a solution of 30% aqueous methylamine (10.31 mL, 90 mmol) in THF (20 mL) was added a solution of the acid chloride (**7**) (5.34 g, 15 mmol) obtained above in CH_2Cl_2 (15 mL) dropwised with cooling by an ice-water bath. The mixture was stirred at room temperature for 2 h. The reaction

mixture was diluted with AcOEt, washed with aqueous saturated $NaHCO_3$, water and brine, and dried over $NaSO_4$. Filtration and concentration *in vacuo* and purification by silica gel flash chromatography ($CHCl_3$/MeOH = 30:1) gave 4.46 g (85%) of (**8**) as a white solid. mp 142-144℃; EI-MS(m/z): 350.9 ($[M+H]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 3.00 (d, *J*=4.3 Hz, 3H), 3.89 (s, 3H), 5.01 (s, 2H), 6.13 (br, 1H), 6.91 (d, *J*=7.2 Hz, 1H), 7.37-7.56 (m, 5H), 7.55 (d, *J*=7.0 Hz, 1H).

Another two intermediates were prepared in the same way except that compound (**6**) was amidated with isopropylamine or diethylamine.

5.2.5. 6,6'-bis(benzyloxy)-5,5'-dimethoxy-N,N'-dimethylbiphenyl-2,2'- dicarboxamide (9).

To a solution of (**8**) (7.00 g, 20 mmol) in anhydrous DMF (25 mL) was added freshly activated copper powder (12.8 g, 200 mmol) under nitrogen atmosphere, and the mixture was refluxed for 4 h. The mixture was filtered and the residual DMF was poured into 150 mL of ice-water. The residue was extracted with $CHCl_3$ (3×70 mL), and the combined organic layers were washed with 2 M HCl (3×50 mL) and brine (2×50 mL), dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel (PE/AcOEt = 1:1) to give (**9**) (4.06 g, 75%) as a white solid. mp 178-180℃; EI-MS(m/z): 540.1 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.65 (d, *J*=4.6 Hz, 6H), 3.87 (s, 6H), 4.73 (d, *J*=10.9 Hz, 1H), 4.82 (d, *J*=10.8 Hz, 1H), 6.94-7.19 (m, 12H), 7.33 (d, *J*=8.4 Hz, 2H).

5.2.6. 6,6'-dihydroxy-5,5'-dimethoxy-N,N'-dimethylbiphenyl-2,2'-dicarboxamide (10).

To a solution of (**9**) (5.41 g, 10.0 mmol) in anhydrous methanol (150 mL) was added 10% Pd/C (0.50 g) (10%) under hydrogen atmosphere. The mixture was stirred at room temperature until no starting material could be observed by TLC. Pd/C was filtered and washed with methanol (600 mL) and acetone (400 mL). Then the combined filtrates were evaporated under vacuum to give (**10**) (3.53 g, 98%) as a gray solid. mp 166-168℃; EI-MS(m/z): 360.1 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (d, *J*=4.2 Hz, 6H), 3.92 (s, 6H), 6.89 (d, *J*=8.4 Hz, 2H), 7.16 (d, *J*=8.5 Hz, 2H).

5.2.7. 6,6'-bis{2-[(3-chloro-4-fluorophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy- N,N'-dimethylbiphenyl-2,2'-dicarboxamide (11).

To a suspension of (24) (3.60 g, 10.0 mmol) in dehydrated alcohol (100 mL) was added anhydrous potassium carbonate (8.29 g, 60.0 mmol). The mixture was stirred at room temperature for 30 min and then 2-chloro-*N*-(3-chloro-4-fluorophenyl)acetamide (5.55 g, 25.0 mmol) was added. The reaction mixture was refluxed for 10 h. Filtration and evaporation of alcohol was done in a vacuum. The residue was extracted with EtOAc (3×50 mL). The combined organic layers were washed with H_2O (3×20 mL), 2 M NaOH (3×20 mL), 2 M HCl (3 ×20 mL) and brine (2×20 mL), dried over Na_2SO_4 and solvent was removed. The crude product was purified by silica gel column chromatography to give (**11**) (5.27 g, 72%) as a white crystalline powder, mp 199-201℃; EI-MS(m/z): 732.2 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (s, 3H), 2.76 (s, 3H), 3.86 (s, 6H), 4.20 (d, *J*=14.6 Hz, 2H), 4.40 (d, *J*=14.6 Hz, 2H), 6.88(d, *J*=8.5 Hz, 2H), 7.05-7.11 (m, 3H), 7.09 (d, *J*=8.4 Hz, 2H), 7.19-7.51 (m, 2H), 7.74-7.76 (m, 1H).

Compounds (**12**)–(**28**) were prepared using the same procedure described above.

5.2.8. 5,5'-dimethoxy-N,N'-dimethyl-6,6'-bis(2-oxo-2-{[3-(trifluoromethyl)phenyl] amino}ethoxy)biphenyl-2,2'-dicarboxamide (12).

Yield: 67%, mp 213-215℃; EI-MS(m/z): 762.1 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.73 (s, 3H), 2.75 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 4.22 (d, *J*=14.8 Hz, 2H), 4.41 (d, *J*=14.4 Hz, 2H), 6.78-6.86 (m, 4H), 7.22-7.33 (m, 5H), 7.50-7.53 (m, 3H).

5.2.9. 6,6'-bis{2-[(3,4-dichlorophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy-N,N'-dimethylbiphenyl-2,2'-dicarboxamide (13).

Yield: 64%, mp 199-201℃; EI-MS(m/z): 762.2 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (s, 3H), 2.75 (s, 3H), 3.80 (s, 3H), 3.82 (s, 3H), 4.21 (d, *J*=14.4 Hz, 2H), 4.40 (d, *J*=14.8 Hz, 2H), 6.81-6.86 (m, 4H), 7.13-7.15 (m, 2H), 7.30 (d, *J*=8.4 Hz, 2H), 7.48-7.51 (m, 2H).

5.2.10. 5,5'-dimethoxy-6,6'-bis{2-[(4-methoxyphenyl)amino]-2-oxoethoxy}-N,N'- dimethyl-biphenyl-2,2'-dicarboxamide (14).

Yield: 75%, mp 114-116℃; EI-MS(m/z): 681.9 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.72 (s, 3H), 2.73 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 4.20 (d, *J*=14.6 Hz, 2H), 4.39 (d, *J*=14.8 Hz, 2H), 6.87(d, *J*=8.8 Hz, 2H), 7.27-7.36 (m, 4H), 7.45-7.50 (m, 4H), 7.78-7.79 (m, 2H).

5.2.11. 6,6'-bis{2-[(4-bromophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy-N,N'- dimethylbiphenyl-2,2'-dicarboxamide (15).

Yield: 77%, mp 243-245℃; EI-MS(m/z): 684.0 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (s, 3H), 2.75 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 4.17 (d, *J*=14.4 Hz, 2H), 4.40 (d, *J*=14.4 Hz, 2H), 6.81-6.86 (m, 2H), 6.95-6.97 (m, 2H), 7.08-7.14 (m, 2H), 7.40-7.43 (m, 2H), 7.52-7.55 (m, 2H).

5.2.12. 6,6'-bis{2-[(4-bromophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy-N,N'- dimethylbiphenyl-2,2'-dicarboxamide (16).

Yield: 80%, mp 100-102℃; EI-MS(m/z): 661.9 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (s, 6H), 3.83 (s, 6H), 4.21 (d, *J*=14.8 Hz, 2H), 4.41 (d, *J*=14.8 Hz, 2H), 6.78-6.86 (m, 4H), 7.21-7.34 (m, 6H), 7.41-7.52 (m, 2H).

5.2.13. 6,6'-bis{2-[(3-fluorophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy-N,N'- dimethylbiphenyl-2,2'-dicarboxamide (17).

Yield: 78%, mp 108-110℃; EI-MS(m/z): 662.2 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.74 (s, 3H), 2.75 (s, 3H), 3.83 (s, 6H), 4.26 (d, *J*=14.8 Hz, 2H), 4.43 (d, *J*=14.4 Hz, 2H), 6.87(d, *J*=8.8 Hz, 2H), 7.26-7.46 (m, 8H), 7.85-7.87 (m, 2H).

5.2.14. 6,6'-bis(2-anilino-2-oxoethoxy)-5,5'-dimethoxy-N,N'-dimethylbiphenyl- 2,2'-dicarboxamide (18)

Yield: 85%, mp 110-112℃; EI-MS(m/z): 626.3 ($[M]^+$), ^1H NMR (300MHz, $CDCl_3$) δ ppm 2.70 (s, 3H), 2.71 (s, 3H),

3.80 (s, 3H), 3.81 (s, 3H), 4.24 (d, *J*=14.4 Hz, 2H), 4.42 (d, *J*=14.8 Hz, 2H), 7.09-7.14 (m, 6H), 7.35-7.49 (m, 4H), 7.68-7.75 (m, 4H).

5.2.15. 6,6'-bis[2-(isopropylamino)-2-oxoethoxy]-5,5'-dimethoxy-N,N'- dimethylbiphenyl-2,2'-dicarboxamide (19)

Yield: 76%, mp 168-170℃; EI-MS(m/z): 557.8 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.05 (t, *J*=6.7 Hz, 6H), 1.06 (t, *J*=6.7 Hz, 6H), 1.42 (m, 2H), 2.72 (s, 3H), 2.73 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 4.20 (d, *J*=14.6 Hz, 2H), 4.41 (d, *J*=14.8 Hz, 2H), 6.87(d, *J*=8.5 Hz, 2H), 7.12 (d, *J*=8.4 Hz, 2H).

5.2.16. 6,6'-bis{2-[(6-fluoropyridin-3-yl)amino]-2-oxoethoxy}-5,5'-dimethoxy- N,N'-dimethylbiphenyl-2,2'-dicarboxamide (20)

Yield: 65 %, mp 100-102℃; EI-MS(m/z): 664.1 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 2.73 (s, 3H), 2.74 (s, 3H), 3.82 (s, 6H), 4.22 (d, *J*=14.4 Hz, 2H), 4.44 (d, *J*=14.8 Hz, 2H), 6.87(d, *J*=8.8 Hz, 2H), 7.12 (d, *J*=8.4 Hz, 2H), 8.37-8.50 (m, 2H), 8.60-8.65 (m, 1H).

5.2.17. 6,6'-bis{2-[(3-chloro-4-fluorophenyl)amino]-2-oxoethoxy}-N,N'-diisopropyl- 5,5'-dimethoxybiphenyl-2,2'-dicarboxamide (21).

Yield: 79% mp 201-203℃; EI-MS(m/z): 786.1 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.07 (d, *J*=6.8 Hz, 12H), 1.32-1.35 (m, 2H), 3.88 (s, 6H), 4.27 (d, *J*=14.8 Hz, 2H), 4.46 (d, *J*=14.4 Hz, 2H), 6.92(d, *J*=8.5 Hz, 2H), 7.09-7.14 (m, 3H), 7.22 (d, *J*=8.4 Hz, 2H), 7.35-7.62 (m, 2H), 7.79-7.84 (m, 1H).

Compounds (**12**)–(**28**) were prepared using the same procedure described above.

5.2.18. 5,5'-dimethoxy-N,N'-dimethyl-6,6'-bis(2-oxo-2-{[3-(trifluoromethyl)phenyl] amino}ethoxy)biphenyl-2,2'-dicarboxamide (22).

Yield: 82%, mp 94-96℃; EI-MS(m/z): 817.8 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.06 (d, *J*=6.9 Hz, 12H), 1.30-1.33 (m, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 4.26 (d, *J*=14.6 Hz, 2H), 4.44 (d, *J*=14.8 Hz, 2H), 6.82-6.89 (m, 4H), 7.21-7.30 (m, 5H), 7.52-7.55 (m, 3H).

5.2.19. 6,6'-bis{2-[(3,4-dichlorophenyl)amino]-2-oxoethoxy}-5,5'-dimethoxy-N,N'-dimethylbiphenyl-2,2'-dicarboxamide (23).

Yield: 69%, mp 108-110℃; EI-MS(m/z): 820.2 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.03 (d, *J*=6.7 Hz, 12H), 1.32-1.35 (m, 2H), 3.81 (s, 3H), 3.82 (s, 3H), 4.24 (d, *J*=14.4 Hz, 2H), 4.41 (d, *J*=14.8 Hz, 2H), 6.87-6.92 (m, 4H), 7.15-7.19 (m, 2H), 7.29 (d, *J*=8.4 Hz, 2H), 7.42-7.45 (m, 2H).

5.2.20. N,N'-diisopropyl-5,5'-dimethoxy-6,6'-bis{2-[(4-methoxyphenyl)amino]-2 -oxoethoxy}biphenyl-2,2'-dicarboxamide (24).

Yield: 75%, mp 214-216℃; EI-MS(m/z): 741.9 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.02 (d, *J*=6.8 Hz, 12H), 1.29-1.32 (m, 2H), 3.80 (s, 6H), 4.22 (d, *J*=14.8 Hz, 2H), 4.38 (d, *J*=14.8 Hz, 2H), 6.85(d, *J*=8.7 Hz, 2H), 7.25-7.34 (m, 4H), 7.46-7.51 (m, 4H), 7.77-7.80 (m, 2H).

5.2.21. 6,6'-bis{2-[(4-bromophenyl)amino]-2-oxoethoxy}-N,N'-diisopropyl-5,5'-dimethoxybiphenyl-2,2'-dicarboxamide (25).

Yield: 81%, mp 207-209℃; EI-MS(m/z): 840.0 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.04 (d, *J*=6.7 Hz, 12H), 1.32-1.35 (m, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 4.26 (d, *J*=14.8 Hz, 2H), 4.44 (d, *J*=14.8 Hz, 2H), 6.78-6.83 (m, 2H), 6.95-7.12 (m, 4H), 7.47-7.58 (m, 4H).

5.2.22. 6,6'-bis{2-[(6-fluoropyridin-3-yl)amino]-2-oxoethoxy}-N,N'-diisopropyl-5,5' -dimethoxybiphenyl-2,2'-dicarboxamide (26)

Yield: 84 %, mp 145-146℃; EI-MS(m/z): 719.9 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 1.00 (d, *J*=6.7 Hz, 12H), 1.28-1.33 (m, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 4.21 (d, *J*=14.8 Hz, 2H), 4.47 (d, *J*=14.8 Hz, 2H), 6.88(d, *J*=8.7 Hz, 2H), 7.13 (d, *J*=8.7 Hz, 2H), 8.50-8.58 (m, 1H), 8.69-8.75 (m, 2H).

5.2.23. 6,6'-bis{2-[(3-chloro-4-fluorophenyl)amino]-2-oxoethoxy}-N,N,N',N'- tetraethyl-5,5'-dimethoxybiphenyl-2,2'-dicarboxamide (27).

Yield: 85% mp 179-181℃; EI-MS(m/z): 814.2 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 0.97 t, *J*=6.7 Hz, 6H , 1.05 (t, *J*=6.7 Hz, 6H), 3.00 (q, *J*=7.0 Hz, 2H), 3.09 (q, *J*=7.2 Hz, 2H), 3.90 (s, 6H), 4.29 (d, *J*=14.8 Hz, 2H), 4.44 (d, *J*=14.8 Hz, 2H), 6.94(d, *J*=8.6 Hz, 2H), 7.07-7.12 (m, 3H), 7.24 (d, *J*=8.6 Hz, 2H), 7.37-7.57 (m, 2H), 7.77-7.85 (m, 1H).

5.2.24. 5,5'-dimethoxy-N,N'-dimethyl-6,6'-bis(2-oxo-2-{[3-(trifluoromethyl)phenyl] amino}ethoxy)biphenyl-2,2'-dicarboxamide (22).

Yield: 81%, mp 76-78℃; EI-MS(m/z): 846.8 ($[M]^+$), ^{1}H NMR (300MHz, $CDCl_3$) δ ppm 0.99 t, *J*=6.9 Hz, 6H , 1.09 (t, *J*=6.9 Hz, 6H), 3.09 (q, *J*=7.2 Hz, 2H), 3.27 (q, *J*=7.4 Hz, 2H), 3.87 (s, 6H), 4.27 (d, *J*=14.8 Hz, 2H), 4.45 (d, *J*=14.4 Hz, 2H), 6.83-6.92 (m, 4H), 7.23-7.35 (m, 5H), 7.57-7.65 (m, 3H).

5.3. Molecular Modeling

The molecular docking method was frequently applied to predict the binding conformation of the ligands if no cocrystal structure was available. Molecule docking was performed using the Sybyl/Surflex (Tripos Inc.) based on the crystal structures of VEGFR-2 taken from the Protein Data Bank (PDB code 3CJF). The 3D-structures of docking compounds were constructed with the Sybyl/Sketch module and optimized using Powell's method. Energy minimization was performed using the Tripos force field with a convergence criterion of 0.005 kcal/(Å·mol) and a maximum of 1000 iterations and Gasteiger-Hückel charges. A non-bonded cutoff distance of 8 Å was used to determine the intramolecular interaction [30]. The VEGFR-2 was prepared using the Protein Preparation tool in the Sybyl-X Biopolymer module. The ligand within the active site and all of the water molecules were removed while the inhibitor (SAV) was extracted. The residues within a radius of 6.5 Å around the SAV (the ligand of VEGFR-2 in the crystal structure 3CJF) in VEGFR-2 were selected as the active sites. Other docking parameters of the program were maintained at a default set-

ting [31]. The virtual docking of VEGFR-2 in complex with the inhibitors has provided a basis for further studies aimed at identifying inhibitors of VEGF-induced angiogenesis, and has provided valuable information regarding the structure-based design of novel biphenyl VEGFR-2 TK inhibitors.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation (*NNSF*) of China (Grant Number 30901839 and 30730110).

DISCLOSURE

Part of information included in this article has been previously published in 'European Journal of Medicinal Chemistry Volume 45, Issue 7, Pages 2798-2805'.

REFERENCES

[1] Printz, C. BATTLE to personalize lung cancer treatment. Novel clinical trial design and tissue gathering procedures drive biomarker discovery. *Cancer*, **2010**, *116*, 3307-3308.

[2] Yang, A.S.; Chapman, P.B. The history and future of chemotherapy for melanoma. *Hematol. Oncol. Clin. North. Am.*, **2009**, *23*, 583-597.

[3] Ozkay, Y.; Işikdağ, I.; Incesu, Z.; Akalin, G. Synthesis of 2-substituted-*N*-[4-(1-methyl-4,5-diphenyl-1*H*-imidazole-2-yl)phenyl]acetamide derivatives and evaluation of their anticancer activity. *Eur. J. Med. Chem*., **2010**, *45*, 3320-3328.

[4] Wu, G.; Guo, H.F.; Gao, K.; Liu, Y.N.; Bastow, K.F.; Morris-Natschke, S.L.; Lee, K.H.; Xie, L. Synthesis of unsymmetrical biphenyls as potent cytotoxic agents. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 5272-5276.

[5] Li, Y.; Hu, Z.; He, L. An approach to develop binary chromatographic fingerprints of the total alkaloids from Caulophyllum robustum by high performance liquid chromatography/diode array detector and gas chromatography/mass spectrometry. *J. Pharm. Biomed. Anal.*, **2007**, *43*, 1667-1672.

[6] Perdue, G.P.; Blomster, R.N.; Blake, D.A.; Farnsworth, N.R. South American plants II: taspine isolation and anti-inflammatory activity. *J. Pharm. Sci.*, **1979**, *68*, 124-126.

[7] Zhao, J.; Zhao, L.; Chen, W.; He, L.C.; Li, X. Taspine downregulates VEGF expression and inhibits proliferation of vascular endothelial cells through PI3 kinase and MAP kinase signaling pathways. *Biomed. Pharmacother.*, **2008**, *62*, 383-389.

[8] Zhang, Y.; He, L.; Zhou, Y. Taspine isolated from Radix et Rhizoma Leonticis inhibits growth of human umbilical vein endothelial cell (HUVEC) by inducing its apoptosis. *Phytomedicine.*, **2008**, *15*, 112-119.

[9] Zhang, J.; Zhang, Y.; Zhang, S.; Wang, S.; He, L. Discovery of novel taspine derivatives as antiangiogenic agents. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 718-721.

[10] Zhang, J.; Zhang, Y.; Shan, Y.; Li, N.; Ma, W.; He, L. Synthesis and preliminary biological evaluation of novel taspine derivatives as anticancer agents. *Eur. J. Med. Chem.*, **2010**, *45*, 2798-2805.

[11] Pizzirani, D.; Roberti, M.; Grimaudo, S.; Di Cristina, A.; Pipitone, R.M.; Tolomeo, M.; Recanatini, M. Identification of biphenyl-based hybrid molecules able to decrease the intracellular level of Bcl-2 protein in Bcl-2 overexpressing leukemia cells. *J. Med. Chem.*, **2009**, *52*, 6936-6940.

[12] Daly, J.W.; Guroff, G.; Udenfriend, S.; Witkop, B. Hydroxylation of alkyl and halogen substituted anilines and acetanilides by microsomal hydroxylases. *Biochem. Pharmacol.*, **1968**, *17*, 31-36.

[13] Knesl, P.; Röseling, D.; Jordis, U. Improved synthesis of substituted 6,7-dihydroxy-4-quinazolineamines: tandutinib, erlotinib and gefitinib. *Molecules.*, **2006**, *11*, 286-297.

[14] Bermejo, A.; Andreu, I.; Suvire, F.; Leonce, S.; Caignard, D.H.; Renard, P.; Pierre, A.; Enriz, R.D.; Cortes, D.; Cabedo, N. Syntheses and antitumor targeting G1 phase of the cell cycle of benzoyldihydroisoquinolines and related 1-substituted isoquinolines. *J. Med. Chem.*, **2002**, *45*, 5058-5068.

[15] Wang, J.Q.; Gao, M.; Miller, K.D.; Sledge, G.W.; Zheng, Q.H. Synthesis of [11C]Iressa as a new potential PET cancer imaging agent for epidermal growth factor receptor tyrosine kinase. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4102-4106.

[16] Palmer, B.D.; Wilson, W.R.; Atwell, G.J.; Schultz, D.; Xu, X.Z.; Denny, W.A. Hypoxia-selective antitumor agents. 9. Structure-activity relationships for hypoxia-selective cytotoxicity among analogues of 5-[*N*,*N*-bis(2-chloroethyl)amino]-2,4-dinitrobenzamide. *J. Med. Chem.*, **1994**, *37*, 2175-2184.

[17] Montoya-Pelaez, P.J.; Uh, Y.S.; Lata, C.; Thompson, M.P.; Lemieux, R.P.; Crudden, C.M. The synthesis and resolution of 2,2'-, 4,4'-, and 6,6'-substituted chiral biphenyl derivatives for application in the preparation of chiral materials. *J. Org. Chem.*, **2006**, *71*, 5921-5929.

[18] Svenson, S.; Thompson, D.H. Facile and Efficient Synthesis of Bolaamphiphilic Tetraether Phosphocholines. *J. Org. Chem.*, **1998**, *63*, 7180-7182.

[19] Wu, Y.; Yang, Y.Q.; Hu, Q. A facile access to chiral 4-isopropyl-, 4-benzyl-, and 4-phenyloxazolidine-2-thione. *J. Org. Chem.*, **2004**, *69*, 3990-3992.

[20] Naruse, I.; Ohmori, T.; Ao, Y.; Fukumoto, H.; Kuroki, T.; Mori, M.; Saijo, N.; Nishio, K. Antitumor activity of the selective epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) Iressa (ZD1839) in an EGFR-expressing multidrug-resistant cell line in vitro and in vivo. *Int. J. Cancer.*, **2002**, *98*, 310-315.

[21] Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settingsof outstanding interest. *Adv. Drug. Deliv. Rev.*, **1997**, *23*, 3-25.

[22] Falcó, J.L.; Lloveras, M.; Buira, I.; Teixidó, J.; Borrell, J.I.; Méndez, E.; Terencio, J.; Palomer, A.; Guglietta, A. Design, synthesis and biological activity of acyl substituted 3-amino-5-methyl-1,4,5,7-tetrahydropyrazolo[3,4-b]pyridin-6-ones as potential hypnotic drugs. *Eur. J. Med. Chem.*, **2005**, *40*, 1179-1187.

[23] Chen, L.; Zhang, Y.; Kong, X.; Peng, S.; Tian, J. Synthesis and biological evaluation of nitric oxide-releasing derivatives of oleanolic acid as inhibitors of HepG2 cell apoptosis. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 2979-2982.

[24] Zhang, Y.; He, L.; Meng, L.; Luo, W. Taspine isolated from Radix et Rhizoma Leonticis inhibits proliferation and migration of endothelial cells as well as chicken chorioallantoic membrane neovascularisation. *Vascul. Pharmacol.*, **2008**, *48*, 129-137.

[25] Schulz, P.; Scholz, A.; Rexin, A.; Hauff, P.; Schirner, M.; Wiedenmann, B.; Detjen, K. Inducible re-expression of p16 in an orthotopic mouse model of pancreatic cancer inhibits lymphangiogenesis and lymphatic metastasis. *Br. J. Cancer.*, **2008**, *99*, 110-117.

[26] Staehler, M.; Rohrmann, K.; Haseke, N.; Stief, C.G.; Siebels, M. Targeted agents for the treatment of advanced renal cell carcinoma. *Curr. Drug. Targets.*, **2005**, *6*, 835-846.

[27] Usui,T.; Ban, H.S.; Kawada, J.; Hirokawa, T.; Nakamura, H. Discovery of indenopyrazoles as EGFR and VEGFR-2 tyrosine kinase inhibitors by in silico high-throughput screening. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 285-288.

[28] Harris, P.A.; Boloor, A.; Cheung, M.; Kumar, R.; Crosby, R.M.; Davis-Ward, R.G.; Epperly, A.H.; Hinkle, K.W.; Hunter 3rd, R.N.; Johnson, J.H.; Knick, V.B.; Laudeman, C.P.; Luttrell, D.K.; Mook, R.A.; Nolte, R.T.; SRudolph, K.; Szewczyk, J.P.; Truesdale, A.T.; Veal, J.M.; Wang, L.; Stafford, J.A. Discovery of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (Pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor. *J. Med. Chem.*, **2008**, *51*, 4632-4640.

[29] Kiss, T.; Fenyvesi, F.; Bácskay, I.; Váradi, J.; Fenyvesi, F.; Iványi, R.; Szente, L.; Tósaki, A.; Vecsernyés, M. Evaluation of the cytotoxicity of beta-cyclodextrin derivatives: evidence for the role of cholesterol extraction. *Eur. J. Pharm. Sci.*, **2010**, *40*, 376-380.

[30] Sivan, S.K.; Manga, V. Molecular docking and 3D-QSAR studies on triazolinone and pyridazinone, non-nucleoside inhibitor of HIV-1 reverse transcriptase. *J. Mol. Model.*, **2010**, *16*, 1169-1178.

[31] Gupta, P.; Roy, N.; Garg, P. Docking-based 3D-QSAR study of HIV-1 integrase inhibitors. *Eur. J. Med. Chem.*, **2009**, *44*, 4276-4287.

Received: November 25, 2010 Revised: February 17, 2011 Accepted: April 06, 2011