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Design, synthesis and biological evaluation of biphenyl urea derivatives as novel VEGFR-2 inhibitors

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Abstract: VEGFR-2 plays a critical role in vasculogenesis and VEGFR-2 inhibitors have been broadly used in the treatment of cancer. In our continued efforts to search for potent and novel VEGFR-2 inhibitors as antitumor agents, we identified a potent lead compound (HMQ-16) bearing biphenyl scaffold. Rearragement and replacement of arylcarbamoyl in HMQ-16 with urea moiety generated a series of novel VEGFR-2 inhibitors. In order to enhance the affinity with VEGFR-2, 4'-acetyl group was converted to oxime group. Fourteen biphenyl urea derivatives were designed and synthesized as potent VEGFR-2 inhibitors. Six of them (**T2**, **T5**, **T7**, **T9**, **T11**, **T14**) exhibited potent VEGFR-2 inhibitory activity comparable to that of Sorafinib. Compound **T7** was the most potent with an IC_{50} value of 1.08 nM. The enzymatic and cellular assays suggested that **T7** has potential as a valuable lead compound for further optimization.

Keywords: Taspine, VEGFR-2, Biphenyl, Urea, Antiangiogenesis.

Angiogenesis is essential for solid tumor growth and dissemination. Blockade of angiogenesis is an attractive approach for the treatment of cancer.[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 (KDR). VEGFR-2 is the major positive signal transducer for endothelial cell proliferation and differentiation.[2] VEGFR-2 inhibition is considered to be important in regulating angiogenesis which is vital for the survival and proliferation of tumor cells. In recent years, VEGFR-2 inhibitors have attracted great attention in cancer therapy.[3]

There has been a lot of research focusing on the development of novel VEGFR-2 inhibitors. A number of VEGFR-2 inhibitors have been reported with potency to inhibit tumor angiogenesis.[4] Several small molecule VEGFR-2 inhibitors, such as Sorafenib (BAY-43-9006, Bayer), Sunitinib (SU-11248, Pfizer) and Vandetanib (ZD6474, AstraZeneca) have been clinically approved for the treatment of several cancers.[5] Vandetanib was approved by FDA in April 2011 for the treatment of late-stage (metastatic) medullary thyroid cancer. Moreover, numerous small molecules have progressed to the clinical evaluation stage. Linifanib (ABT-869, Abbott) is a potent inhibitor of members of the VEGFR-2 and PDGFR families. Linifanib is currently in phase III trials. The preclinical characteristics of Linifanib suggested that it may offer distinct advantages in cancer therapy. Linifanib is a novel, orally active multi-targeted receptor tyrosine kinase (RTK) inhibitor that exhibits potent antitumor and antiangiogenic activities against a broad spectrum of experimental tumors and malignancies in patients. Development of novel VEGFR-2 inhibitors continues to be an intense area of investigation in anticancer agents.[6]

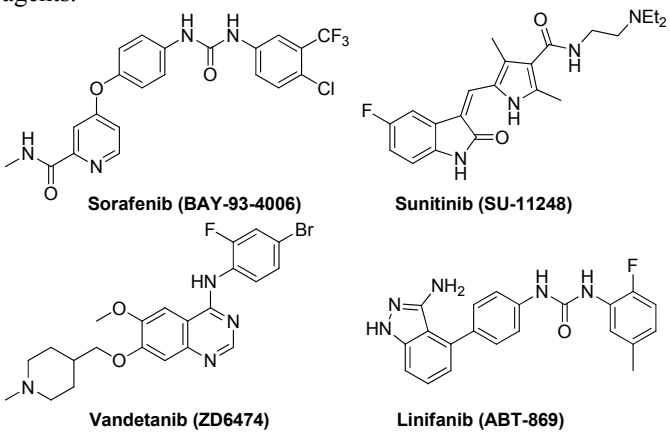


Figure 1. Structures of VEGFR-2 inhibitors.

He *et al* found that taspine had good affinity characteristics in human umbilical vein endothelial cell membrane chromatography model. In a further study, they identified that taspine could inhibit tumor angiogenesis through inhibition of VEGFR-2.[7] In our efforts to search for potent VEGFR-2 inhibitors with taspine as lead compound,[8] we developed a novel class of potent VEGFR-2 inhibitors bearing biphenyl scaffold (**Figure 2**).[9] We found that aniline which contains one or more halogen substituents (fluoro, chloro, bome) are useful for biological activity.The biological evaluation of these biphenyls inspired us to search for potent inhibitors with biphenyl scaffold targeting VEGFR-2.[10] HMQ-16 from this series was selected as novel lead compound for further structural optimization. In this manuscript, we described further optimization of biphenyl-based VEGFR-2 inhibitors in order to enhance the structural complexity and diversity.

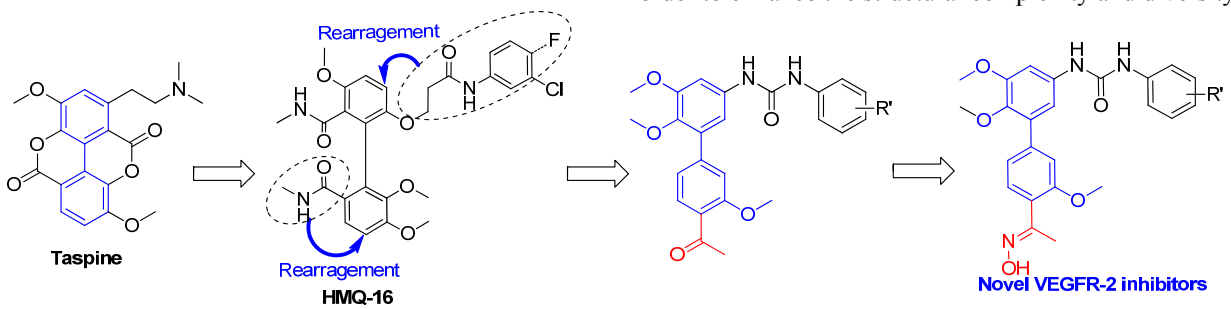
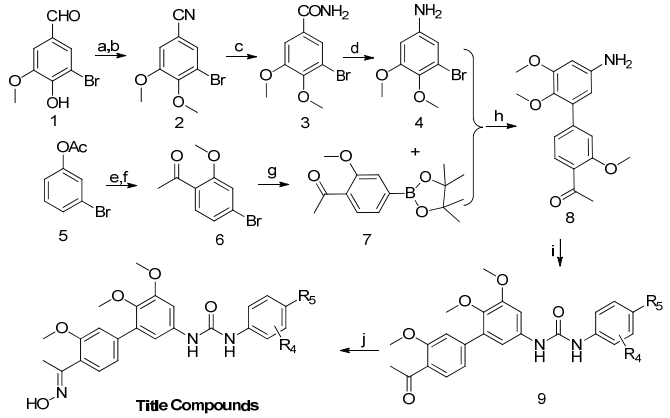


Figure 2. Structural optimization of HMQ-16 and strucutres of novel biphenyls.

According to the structural analysis, both Sorafenib and ABT-869 contain aromatic urea fragment. The structures of complexes between inhibitors and VEGFR-2 revealed that the urea unit interacted with conserved Glu885 and Asp1046 through hydrogen bonds. The terminal phenyl moiety occupies the hydrophobic pocket of receptor. Sufficient space was available for the introduction of various substitutes to the terminal aniline.[11] Firstly, rearragement of arylcarbamoyl at 2-position and amide at 2'-postion to the favorable position was performed. In order to enhance the affinity with receptor, the arylcarbamoyl was replaced with urea moiety and the acetyl was optimized to oxime. Moreover, we introduced a water-soluble tertiary amine side chain to terminal aniline. The incorporation of tertiary amine could increase the solubility of the compounds while retaining their biological activity. The tertiary amine also mimiced the *N,N*-dimethylaminoethyl side chain of taspine. Therefore, the preferable length of side chain between oxygen and nitrogen was two or three atoms. We further explored the influence of terminal side chain variations. The optimization of the substituents at the urea terminal phenyl ring afforded a series of biphenyl ureas as novel VEGFR-2 inhibitors (**Figure 2**). We directed structural diversity in various substituted aniline.

Based on our earlier work,[12] a general synthesis of biphenyl ureas followed the reaction pathway outlined in **Scheme 1**. We used commercially available isovanillin as the starting material.



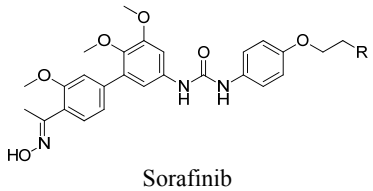
Scheme 1. Preparation of title compounds.

Reagents and Conditions: (a) HCOOH, HCOONa, $(NH_3OH)_2SO_4$, 98%; (b) $(CH_3)_2SO_4$, K_2CO_3, Me_2CO, 94%; (c) H_2O_2, NaOH, EtOH, 90%; (d) Br_2, NaOH, 60%; (e) $AlCl_3$, 80%; (f) $(CH_3)_2SO_4$, K_2CO_3, EtOH, 95%; (g) $[BO_2C_2(CH_3)_4]_2$, dioxane, Pd(pddf)Cl_2; (h) Pd(pddf)Cl_2, Na_2CO_3, H_2O, dioxane, 65%; (i) triphosgene, Et_3N, DCM, 50-85%; (j) EtOH, NH_4OH, H_2O, 88%.

The key intermediate **4** was prepared in a four-step sequence from 5-bromovanillin **1**. Firstly, the aldehyde group of **1** was converted to cyano via one-pot oxime formation and dehydration, and subsequent O-methylation to yield **2**. Subsequent oxidative hydration of nitrile afforded amide **3**. The amide group of **3** was converted to amine through Hoffmann's degradation. Another intermediate boronate **7** was prepared by coupling of bis(pinacolato)diboron with bromide **6**, derived from Fries rearrangement of 3-bromophenyl acetate and O-methylation. Without workup and further addition of palladium catalyst, in-situ Suzuki coupling with **4** afforded the key intermediate **8**. Subsequently, compound **8** was treated with triphosgene to produce isocyanate, followed by reacting with various substituted anilines in dichloromethane to yield ureas **9**. The title compounds **T1-T14** were prepared by subsequent condensation of **9** with hydroxylamine hydrochloride. All the title compounds were characterized by NMR, mass spectroscopy and melting point analysis and their purity were above 95% determined by LC-MS (**Supplementary Information**).

All synthesized compounds were screened for kinase inhibitory activity against VEGFR-2 and the results are summarized in **Table 1** and **Table 2**. Kinase enzymatic assays were performed utilizing the homogeneous time-resolved fluorescence (HTRF) protocol.[13] Most of them exhibited potent VEGFR-2 inhibitory activities. As shown in Tables, the IC_{50} values of title compounds ranged from 1.08 nM to 203 nM, while the IC_{50} value of Sorafinib was 1.06 nM. Compound **T7** displayed the greatest activity with IC_{50} value of 1.08 nM which was comparable to Sorafinib. Compounds **T2** and **T14** also exhibited potent VEGFR-2 inhibitory activities with IC_{50} values of 1.28 nM and 3.07 nM, respectively. The unexpected loss of potency of **T3** might be due to the poor solubility. The inhibitory rates of **T3** were 45.7% (0.032 nM), 64.0% (0.32 nM), 59.7% (3.2 nM), 45.7% (32 nM), 53.0% (320 nM) and 28.1 (3200 nM). The unexpected IC_{50} value of **T3** was calculated according to above inhibitory rates which were not concentration-dependent. The experimental measurements of physicochemical properties of title compounds are being performed to validate the hypothesis. Furthermore, changing dimethylamine to morpholine (**T7**) restored potency 5-fold greater than **T1**. **T7** also displayed the highest inhibitory rate against VEGFR-2 at 3.2 nM. The oxygen atom of morpholine might interact with the active site of VEGFR-2 as a hydrogen-bond donor. Moreover, morpholine could improve the physicochemical properties such as solubility. Unfortunately, the preliminary VEGFR-2 inhibitory activity of T6 was not concentration-dependent. The inhibition ratios at low concentration were higher. Therefore, the IC_{50} value of **T6** could not be correctly calculated according to corresponding inhibition ratios. Based on these results, we assumed that the potency of these biphenyl ureas might be affected by the length of side chain between benzene ring and nitrogen atom as well as the terminal side chain variations.

Table 1. VEGFR-2 inhibitory activity of the biphenyl ureas (**T1-T7**)



Compound	R	VEGFR-2, IC_{50} (nM) (**n=5**)	Inhibitory rate (%)[b]
T1	$N(CH_3)_2$	5.77	20.7
T2	$N(CH_2CH_3)_2$	1.28	52.4
T3	$CH_2N(CH_3)_2$	203	59.7
T4	$N(CH_2)_5$	7.01	52.2
T5	$N(CH_2)_4$	4.08	55.9
T6	$N(CH_2)_4O$	ND[a]	51.0
T7	$CH_2N(CH_2)_4O$	1.08	63.3
Sorafinib	-	1.06	68.5

[a]ND is not determined; [b] Inhibitory rate determined at 3.2 nM.

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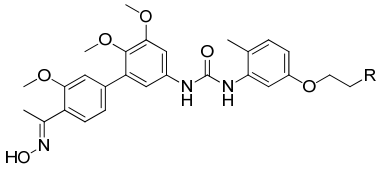
Table 2. VEGFR-2 inhibitory activity of the biphenyl ureas (**T8-T14**)



Compound	R	VEGFR-2, IC_{50} (nM) (**n=5**)	Inhibitory rate (%)[b]
T8	$N(CH_3)_2$	ND[a]	34.2
T9	$N(CH_2CH_3)_2$	3.90	54.7
T10	$CH_2N(CH_3)_2$	9.87	48.7
T11	$N(CH_2)_5$	3.77	29.5
T12	$N(CH_2)_4$	8.90	30.2
T13	$N(CH_2)_4O$	ND[a]	36.1
T14	$CH_2N(CH_2)_4O$	3.07	54.7
Sorafinib	-	1.06	68.5

[a]ND is not determined; [b] Inhibitory rate determined at 3.2 nM.

Based on the results of the VEGFR-2 enzymatic assay, six potent compounds were further evaluated in functional cell-based assays using a variety of cancer cell lines.[14] The results were shown in **Table 3**. Most of the title compounds displayed moderate antiproliferative activity against various cancer cell lines. Specially, compound **T7** showed the highest activity against 7901 cell with IC_{50} value of 6.80 μM which was much less than that of Sunitinib. Moreover, compound **T7** also exhibited the greatest activities against K562 and SY5Y with IC_{50} values of 0.29μM and 4.91μM, respectively. For MDA-MB-231 cell, compound **T2** was the most potent. However, the activity of these compounds against other cells was less potent than that of Sunitinib. The antiproliferative results indicated that these biphenyl ureas displayed higher selectivity against K562 cells with IC_{50} values ranging from 0.29 μM to 15.8μM.

Table 3. Antiproliferative activities of title compounds on cancer cells

Compound	Cancer Cell Lines, IC_{50} (μM) (**n=5**)								
	7901[a]	K562[b]	SY5Y[c]	MDA-MB-231[d]	LOVO[e]	SK-BR-3[f]	A549[g]	7721[h]	Hela[i]
Sunitinib	102	0.86	5.73	35.0	6.56	5.21	3.36	9.86	7.68
T1	34.2	8.58	6.20	35.2	ND	ND	ND	ND	63.2
T2	58.1	1.29	18.4	20.3	48.4	ND	11.7	36.4	13.4
T5	ND*	4.81	ND	ND	49.2	38.9	ND	ND	ND
T7	6.80	0.29	4.91	ND	ND	ND	11.7	9.88	92.3
T9	ND	1.25	43.5	ND	ND	14.7	11.2	ND	ND
T14	ND	15.8	18.4	49.2	ND	ND	23.7	33.3	243

[a]7901, human gastric cancer cell line; [b] K562, human immortalised myelogenous leukemia line; [c] SY5Y, human neuroblastoma cell line; [d]MDA-MB-231, human breast carcinoma cell line; [e]LOVO, human colon adenocarcinoma cell line; [f]SK-BR-3, humanbreast adenocarcinoma cell line; [g]A549, carcinomic human alveolar basal epithelial cells; [h]7721, human hepatocarcinoma cell line; [i]Hela, Human epithelial carcinoma cell line.

* ND is not determined.

Computational studies were performed to investigate the potential binding mode of the title compounds with VEGFR-2. The most active compound **T7** was constructed and optimized using Powell's method with Tripos force field.[15] The convergence criterion was set at 0.05 kcal/(Å·mol), and assigned with Gasteiger-Hückel charge.[16] The docking study was performed using Sybyl/Surflex-Dock module, the residues in a radius of 6.5 Å around BAX 1500 of VEGFR-2 (PDB ID: 4ASD) were selected as the active site. The binding mode of **T7** into the active site of VEGFR-2 was shown in **Figure 3**. There were eight H-bond interactions between urea and oxime of **T7** and amino acid residues of the enzyme. The urea portion accessed the hydrophobic pocket adjacent to the hinge region, with the urea carbonyl oxygen forming a hydrogen bond with the NH of Asp1046 with length of 1.70 Å. The external NH of the urea unit formed two hydrogen bonds to the carboxylate of Glu885 with distance of 2.27 Å and 1.72 Å, respectively. The terminal oxime group acted as essential hydrogen bond acceptor and donor for Asp814 and Arg1027 in active site. The nitrogen of oxime acted as hydrogen bond acceptor, forming two hydrogen bonds to Arg1027 with distance of 2.21 Å and 2.83 Å, respectively. The oxygen formed a hydrogen bond to Arg1027 with distance of 2.56 Å while hydrogen formed two hydrogen bonds to Asp814 carboxylate (2.17 Å and 2.54 Å). These interactions between the urea moiety and VEGFR-2 were consistent with the reports of other VEGFR-2 inhibitors of the urea type. The capability to form hydrogen bonds of urea and oxime contributed significantly to the affinity with the receptor. Molecular insights based on molecular docking indicated favorable binding interactions of these biphenyl ureas with the active site of VEGFR-2.[17] This binding hypothesis could provide valuable information for structure-based design of VEGFR-2 inhibitors.

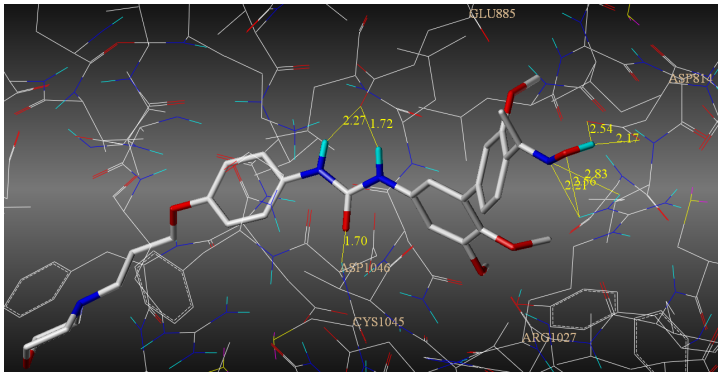


Figure 3. Binding conformation of **T7** with VEGFR-2 (PDB ID: 4ASD). Hydrogen bonds are shown with yellow dotted lines.

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In the current study, we described the design, synthesis and preliminary evaluation of novel biphenyl ureas as potent VEGFR-2 inhibitors. A molecular docking study was performed to identify their binding mode with the receptor. The results indicated that compound **T7** was nicely bound to VEGFR-2 with eight hydrogen bonds. The urea portion interacted with the conserved pocket as previous report. It also disclosed the importance of the urea and oxime for the potency of these inhibitors.

In summary, we indentified an alternative substitution pattern of biphenyl urea as novel VEGFR-2 inhibitors. Incorporation of urea unit and oxime moiety to biphenyl afforded a series of potent VEGFR-2 inhibitors. The molecular docking studies demonstrated that oxime played important role in its rationale interacting with VEGFR-2. Oxime group was identified to be capable of acting as both hydrogen bond donor and acceptor for the residues in active site. Compound **T7** was identified as a promising lead compound with novel scaffold. These compounds have strong potential to be further developed as novel VEGFR-2 inhibitors. The results suggest that they could therefore be a promising starting point for further medicinal chemistry efforts. Further modifications may be undertaken on the biphenyl core and terminal benzene ring of the urea unit. Our efforts in pursuing the optimization of the physicochemical and biological properties of the biphenyl ureas will be reported in due course.

Acknowledgments

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