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Biphenyl derivatives incorporating urea unit as novel VEGFR-2 inhibitors: Design, synthesis and biological evaluation

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ABSTRACT

A series of novel biphenyl urea derivates were synthesized and investigated for their potential to inhibit vascular endothelial growth factor receptor-2 (VEGFR-2). In particular, **A7**, **B3** and **B4** displayed significant enzymatic inhibitory activities, with IC_{50} values of 4.06, 4.55 and 5.26 nM. Compound **A7** exhibited potent antiproliferative activity on several cell lines. SAR study suggested that the introduction of methyl at *ortho*-position of the biphenyl urea and tertiary amine moiety could improve VEGFR-2 inhibitory activity and antitumor effects. Molecular docking indicated that the urea moiety formed four hydrogen bonds with DFG residue. These biphenyl ureas could serve as promising lead compounds for further optimization.

1. Introduction

Angiogenesis plays an important role in tumor progression and metastasis of the great majority of solid tumors. Antiangiogenesis suppresses the growth of many types of tumor.[1] It has been studied intensively for a few decades as an effective treatment of many types of cancer.[2] Angiogenesis is regulated by many growth factors. The vascular endothelial growth factors (VEGFs) and receptor (VEGFR-2) play essential role in angiogenesis. VEGFR-2 is the critical receptor for tumor angiogenesis. Overexpression of VEGFR-2 has been closely implicated in the angiogenesis of solid tumors.[3] Therefore, VEGFR-2 inhibitors are capable of treating angiogenesis-related cancers. At present, numbers of small molecules had been identified as VEGFR-2 inhibitors. There are various structure elements of VEGFR-2 inhibitors (Fig. 1) such as urea (sorafenib),[4] **ABT-869**;[5] 3-substituted indolinones (sunitinib);[6] 4-anilinoquinazolines (ZD4190).[7] Type I inhibitors constitute the majority of ATP-competitive inhibitors and recognize mainly the active conformation of VEGFR-2. Meanwhile, type II inhibitors recognize the inactive DFG (Asp-Phe-Gly)-out conformation of VEGFR-2. Considerable effort has been made to identify potent VEGFR-2 inhibitors as therapeutic agents against cancers.

Natural alkaloid taspine could inhibit angiogenesis by inhibition of VEGFR-2. Structural optimization of taspine afforded novel biphenyl derivatives with potent antiangiogenic activity.[8] Further study disclosed that these biphenyls could significantly inhibit VEGFR-2 and subsequently reduce the angiogenesis.[9] SAR exploration demonstrated that biphenyl played a significant role in the maintenance of biological activity (Fig. 2).[10] Moreover, NMR-based screening also identified biphenyl as a promising scaffold to develop potential lead compound.[11]

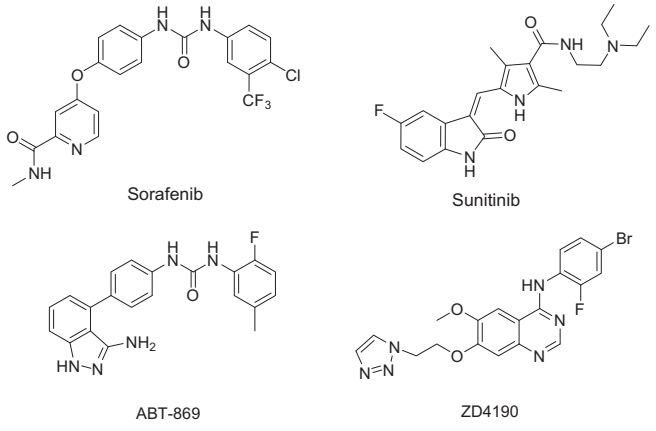

Figure 1. VEGFR-2 inhibitors currently approved or in clinical trials.

Further structural optimization was performed to search for novel VEGFR-2 inhibitors. It was known that urea moiety was an important pharmacophore in type II VEGFR-2 inhibitors.[12] It could form critical hydrogen bonds with the DFG domain of VEGFR-2. Therefore, urea unit was incorporated to biphenyl scaffold to improve biological activity (Fig. 2). According to previous results,[8] the biphenyls possessed poor water solubility. Therefore, tertiary

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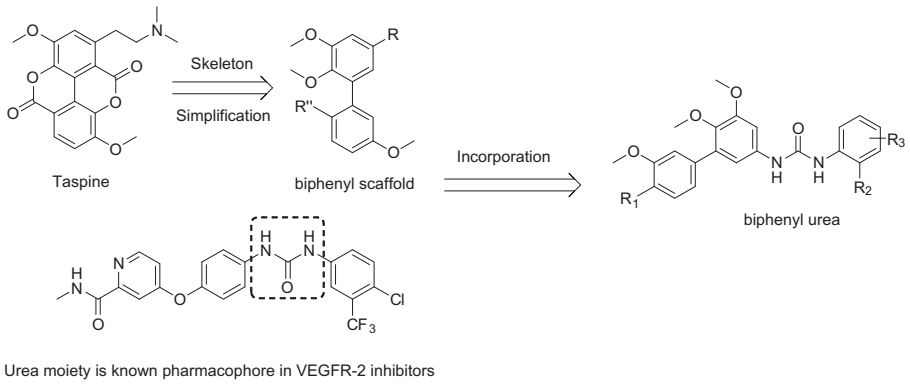


Figure 2. Discovery of biphenyl scaffold and structures of novel biphenyl ureas.

amine was introduced to terminal aryl to improve hydrophilicity. In present study, a series of novel biphenyl ureas were designed, synthesized and evaluated as VEGFR-2 inhibitors. These biphenyl derivatives bearing urea unit exhibited potent antitumor activity and could be considered as promising lead compounds.

2. Chemistry

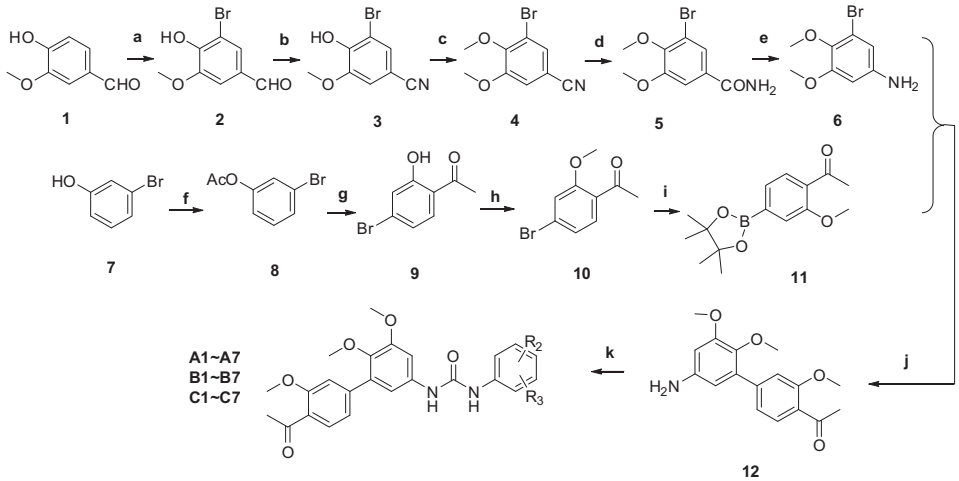
The synthetic route was outlined in Scheme 1**.** Commercial available vanillin was converted into bromoaldehyde (**2**) by reaction with bromine.[8] Compound (**3**) was obtained by reaction of (**2**) with sodium formate and hydroxylamine sulfate in formic acid.[13] Aniline (**6**) was synthesized from (**3**) by methylation with dimethyl sulfate,[14] hydrolysis with NaOH[15] and Hofmann rearrangement. Compound (**9**) was obtained by acylation of 3-bromophenol with acetic anhydride and Fries rearrangement.[16] Methylation of compound (**9**) with dimethyl sulfate in the presence of K_2CO_3 afforded (**10**). The Pd(dppf)Cl_2-catalyzed cross-coupling reaction of the pinacol ester of diboronic acid [$(Me_4C_2O_2)B–B(O_2C_2Me_4)$] with (**10**) in the presence of KOAc gave (**11**).[17] Critical intermediate biphenyl (**12**) was prepared from (**11**) and (**6**) by classical Suzuki coupling reaction.[18] Various substituted anilines were treated with triphosgene (BTC) to produce various isocyanates. Finally, these isocyanates were reacted with different anilines affording various biphenyl ureas.[19]

3. Results and discussion

The compounds were initially evaluated for their inhibitory activity against VEGFR-2 with sorafenib (free base) as positive control. The results were summarized in Table 1. Most compounds displayed moderate to high inhibitory activity with IC_{50} values ranging from 4.06 to 179 nM. Among them, compound **A7**, **B3**, **B4** potently inhibited VEGFR-2 at nanomolar IC_{50} values.

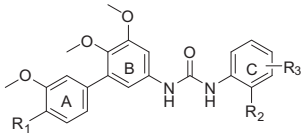
Compound (**A7**) was the most potent in a series of *para* substituted phenylureas with an IC_{50} value of 4.06 nM, while (**A3**) and (**A4**) showed moderate inhibitory activities with IC_{50} values of 17.5 and 12.8 nM, respectively. The presence of 3-morpholinopropoxy at C-ring may be critical for the activity of these biphenyls. **B** series compounds were afforded by replacement of hydrogen with methyl. Five compounds exhibited more potent inhibitory activity compared than their counterparts. Compounds (**B3**) and (**B4**) displayed significant inhibitory activities with IC_{50} values of 4.55 and 5.26 nM, respectively. Compounds of **C** series were yielded by incorporation of the R_3: alkoxyamino group at 5-position of terminal aniline. Only two compounds exhibited slightly improvement of activity compared to corresponding analogues. Compound (**C2**) was the most potent with IC_{50} value of 16.7 nM.

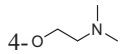
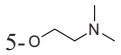
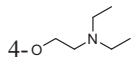
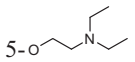
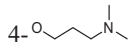
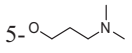
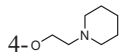
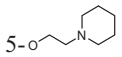
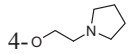
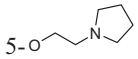
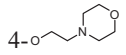
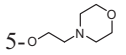
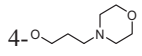
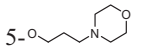
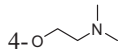
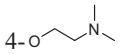
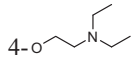
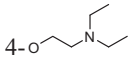
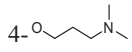
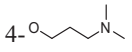
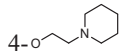
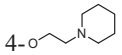
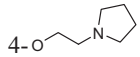
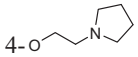
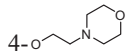
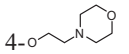
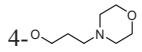
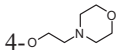
In general, the enzymatic inhibitory activities of **B** series compounds were better than **A** series. The results suggested that the methyl substitution at 2-position could slightly enhance the



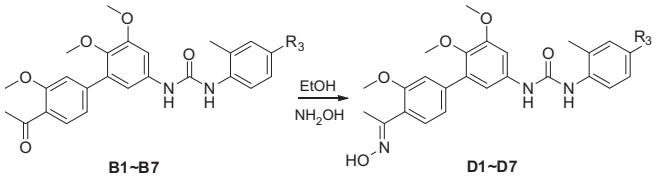
Scheme 1. Synthetic route of biphenyl ureas. Reagents and conditions**:** (a) Br_2, AcOH, AcONa, Fe, rt; (b) HCOOH, HCOONa, $(NH_2OH)_2{\cdot}H_2SO_4$, 90 °C; (c) $(CH_3O)_2SO_2$, K_2CO_3, Me_2CO, 50 °C; (d) NaOH, H_2O_2, EtOH, 60 °C; (e) Br_2, NaOH; (f) Py, Ac_2O, rt; (g) $AlCl_3$,160 °C; (h) $(CH_3O)_2SO_2$, K_2CO_3, acetone, 50 °C; (i) bis(pinacolato)diboron, dioxane, Pd(pddf)Cl_2, KOAc, 100 °C; (j) Pd(pddf)Cl_2, Na_2CO_3, H_2O, dioxane,100 °C; (k) triphosgene (BTC), anilines, Et_3N, DCM, rt.

Table 1
Structure and VEGFR-2 inhibitory activity of biphenyl ureas



	R_1	R_2	R_3	IC_{50} (nM)	*C*log*P*		R_1	R_2	R_3	IC_{50} (nM)	*C*log*P*
A1	$COCH_3$	H		>300	2.99	**C1**	$COCH_3$	CH_3		71.4	3.48
A2	$COCH_3$	H		>300	3.67	**C2**	$COCH_3$	CH_3		16.7	4.16
A3	$COCH_3$	H		17.5	3.10	**C3**	$COCH_3$	CH_3		17.6	3.59
A4	$COCH_3$	H		12.8	3.73	**C4**	$COCH_3$	CH_3		>300	4.21
A5	$COCH_3$	H		179	3.31	**C5**	$COCH_3$	CH_3		69.9	3.80
A6	$COCH_3$	H		>300	2.59	**C6**	$COCH_3$	CH_3		23.9	3.08
A7	$COCH_3$	H		4.06	2.70	**C7**	$COCH_3$	CH_3		33.3	3.19
B1	$COCH_3$	CH_3		>300	3.48	**D1**	$CNOHCH_3$	CH_3		ND*	3.87
B2	$COCH_3$	CH_3		128	4.16	**D2**	$CNOHCH_3$	CH_3		>300	4.55
B3	$COCH_3$	CH_3		4.55	3.59	**D3**	$CNOHCH_3$	CH_3		14.3	3.98
B4	$COCH_3$	CH_3		5.26	4.21	**D4**	$CNOHCH_3$	CH_3		ND	4.60
B5	$COCH_3$	CH_3		12.8	3.80	**D5**	$CNOHCH_3$	CH_3		40.9	4.18
B6	$COCH_3$	CH_3		ND	3.08	**D6**	$CNOHCH_3$	CH_3		129	3.47
B7	$COCH_3$	CH_3		23.8		**D7**	$CNOHCH_3$	CH_3		81.2	3.58
Sorafenib				1.06							

* ND = not Determined.



Scheme 2. Synthetic route of **D1**–**D7**.

activity. However, analogues belong to **C** series with the same methyl substitution exhibited weaker activity. The results demonstrated that 4-position of the urea may be privileged for the alkoxy group substitution. Various *N*,*N*-disubstituted propoxy amine had significant effects on the biological potency of biphenyl derivates. 3-*N*,*N*-dimethylpropoxy, 3-morpholinopropoxy, 2-piperidin-1-ylethoxy group were preferable substitutions and might be essential for the potency of compounds.

In order to improve the affinity with VEGFR-2, the acetyl group on the biphenyl core was converted to oximido.[20] Another seven derivatives were synthesized from (**B1**–**B7**) with the route as shown in Scheme 2. Newly prepared compounds were also evaluated for their inhibitory activities against VEGFR-2. The results were also summarized in Table 1. Unfortunately, the enzymatic inhibition activities of the newly synthesized compounds were not enhanced. However, the biological results confirmed our previous conclusion that the three *N*,*N*-disubstituted amines were beneficial for activity.

Table 2
Antiproliferative activity of (**A7**) against cancer cells

Compound	Cell lines (IC_{50}, μM)				
	SGC7901[a]	K562[b]	SH-SY5Y[c]	MDA-MB-231[d]	LOVO[e]
Sunitinib	101	0.86	5.73	35.0	6.56
A7	80.9	2.23	10.4	ND	11.1

ND: not determined.
[a] Human gastric cancer cells.
[b] Human immortalised myelogenous leukemia line.
[c] Human neuroblastoma cells.
[d] Human breast cancer cells.
[e] Human colon adenocarcinoma cell line.

In vitro antiproliferative activity of compound (**A7**) against various cancer cell lines was measured by 3-(4,5-dimethylthia-

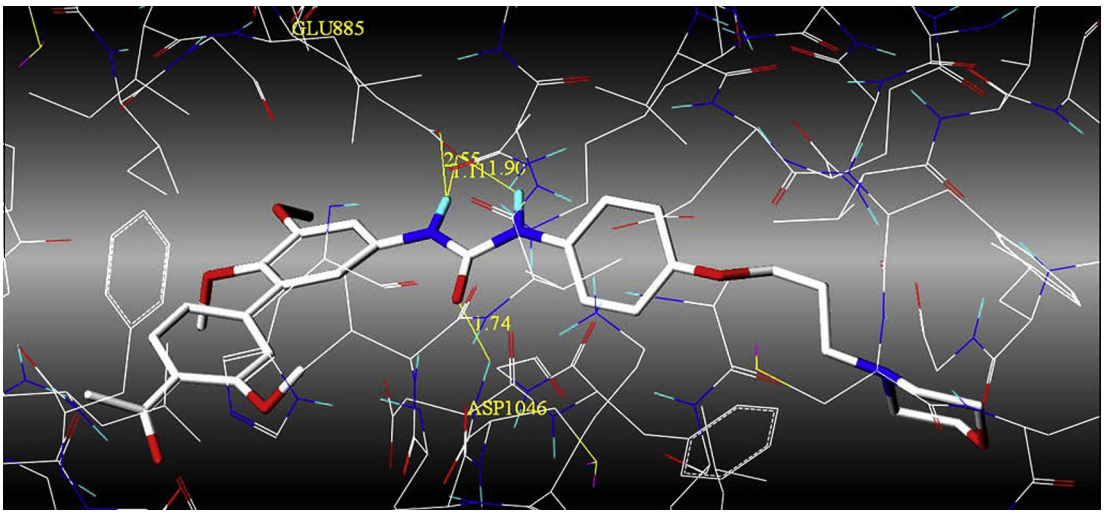


Figure 3. Docking model of **A7** bound to VEGFR-2 (PDB ID: 4ASD) active site.

zol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with sunitinib (free base) as positive control. As shown in Table 2, (**A7**) exhibited potent antiproliferative activity against four cancer cell lines comparable to that of sunitinib. In particular, it displayed more potent antiproliferation against SGC-7901 with IC_{50} value of 80.0 nM than that of sunitinib.

The most potent inhibitor (**A7**) was docked into the active site of VEGFR-2 (PDB ID: 4ASD) to understand its binding mode. The reason we chose 4ASD as receptor is that 4ASD complex contains sorafenib as a urea containing ligand. Molecular insights based on molecular docking indicated favorable binding interactions of compound (**A7**) with the active site of VEGFR-2 (Fig. 3). ^{1}N–H of the urea unit formed two hydrogen bonds with Glu885 with distance of 2.55 and 1.11 Å, respectively. ^{3}N–H could also bind to the the side chain carboxylate of Glu885 with distance of 1.90 Å. Carbonyl group identified to be involved in hydrogen bond with the backbone NH of Asp1046 with distance of 1.74 Å. Length of three hydrogen bonds was less than 2 Å and they could significantly improve the affinity with VEGFR-2.

4. Conclusion

In conclusion, twenty-eight novel biphenyl urea derivates were designed, synthesized and evaluated as novel VEGFR-2 inhibitors. Several derivatives exhibited potent inhibitory activity against VEGFR-2. Three of them (**A7**, **B3** and **B4**) displayed significant enzymatic inhibitory activities, with IC_{50} values of 4.06, 4.55 and 5.26 nM. Moreover, compound (**A7**) exhibited good antiproliferative activities against K562, SY5Y and LOVO cell lines. SAR study disclosed that methyl at 2-position of terminal aniline could improve biological activity. Furthermore, the favorable position for substitution of the aminoalkoxy group was the 4-position to the urea group. 3-*N*,*N*-Dimethylpropoxy, 3-morpholinopropoxy, 2-piperidin-1-ylethoxy groups were the most preferred substitutions on the phenyl group joined to urea group. Docking study showed four hydrogen bonds between urea unit and active site of VEGFR-2 as discussed above. These results confirmed as known that urea moiety played a vital role in the biological activity.[21–23] In summary, these biphenyl ureas have strong potential to be further developed as novel VEGFR-2 inhibitors. Further structural optimization of these promising anticancer agents will be reported in due course.

5. Experimental

5.1. Chemistry: general procedure

Solvents and reagents were purified according to the standard procedures. All reactions except those in aqueous media were carried out by standard techniques for the exclusion of moisture. Anhydrous reactions were carried out under nitrogen atmosphere. Reactions were monitored by thin layer chromatography on 0.25-mm silica gel plates (60GF-254) and visualized with UV light. Melting points were determined on electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured at 400 MHz on a Bruker Advance AC 400 instrument. Mass spectra were obtained on a Shimadzu HPLC–MS-QP2010 instrument.

5.1.1. 3-Bromo-4-hydroxy-5-methoxybenzaldehyde (2)

To a mixture of (**1**) (20.0 g, 0.13 mol), NaOAc (21.6 g, 0.26 mol) and iron powder (0.68 g, 0.01 mol) was added glacial acetic acid (120 mL). The mixture was stirred at room temperature for 30 min. Br_2 (7 mL, 0.14 mol) in glacial acetic acid (30 mL) was added dropwise into the above mixture at 23–258 °C. The mixture was stirred at the same temperature overnight. Ice-water (250 mL) was added to the mixture and stirred for another 1 h. The solid obtained was dried and recrystallized from EtOH to give (**2**) (24.6 g, 81%) as gray solid. Mp: 159–160 °C, MS (EI) $[M]^{+}$: $m/z = 230$, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.38 (s, 1H), 6.62 (s, 1H), 4.00 (s, 3H).

5.1.2. 3-Bromo-4-hydroxy-5-methoxybenzonitrile (3)

A mixture of (**2**) (20.7 g, 90 mmol), sodium formate (26.5 g, 300 mmol), and formic acid (150 mL) was heated to 90 °C. To the above mixture was added hydroxylamine sulfate (8.88 g, 54 mmol) in six equal portions at 30 min intervals, and the mixture was heated at 90 °C for 5 h. The reaction was cooled to room temperature and poured to a solution of sodium chloride (100 g) in water (400 mL). The resultant solid was collected by filtration and dried to give an off-white solid (**3**) (18.0 g, 86%). Mp: 142–144 °C, MS (EI) $[M]^{+}$: $m/z = 227$, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.07 (s, 1H), 6.42 (s, 1H), 3.98 (s, 3H).

5.1.3. 3-Bromo-4,5-dimethoxybenzonitrile (4)

A mixture of (**3**) (13.5 g, 60 mmol), potassium carbonate (24.9 g, 180 mmol) and acetone (100 mL) was stirred at 50 °C for 30 min.

Dimethyl sulfate (6.60 mL, 66 mmol) was added dropwise into the above mixture at 50 °C. After completion of reaction, the solid was filtered off and the solvent was evaporated. The solid obtained was dried to give off-white solid (**4**) (13.5 g, 94%). Mp: 122–123 °C. MS (EI) $[M]^+$: m/z = 243, ^{1}H NMR (400 MHz,$CDCl_3$) δ ppm: 7.50 (s, 1H), 7.12 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H).

5.1.4. 3-Bromo-4,5-dimethoxybenzamide (5)

To a solution of (**4**) (8.03 g, 33 mol) in ethanol (150 mL) was added NaOH (1.60 g, 40 mmol) and 30% H_2O_2 (17 mL, 600 mmol). The mixture was stirred at 60 °C for 1 h. After completion of reaction, the mixture was acidized with concentrated HCl. Ethanol was evaporated and residues was poured into water and filtered. The white solid obtain was dried to give (**5**) (7.70 g, 89.5%). Mp: 156–157 °C, MS (EI) $[M]^+$: m/z = 259, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.53 (s, 1H), 7.45 (s, 1H), 3.95 (s, 3H), 3.93 (s, 3H).

5.1.5. 3-Bromo-4,5-dimethoxyaniline (6)

Br_2 (0.9 mL) was added dropwise into a solution of NaOH (2.70 g, 68 mmol) in distill water (54 mL) at −5 °C and was stirred for another 10 min. (**5**) (3.6 g, 14 mmol) was added into the above solution in batches and stirring continued for 20 min. The mixture was warmed to room temperature and kept for 30 min. Then the suspension was heated at 40 °C for 1 h. The mixture was cool to room temperature, poured into water and extracted with AcOEt (50 mL × 3). The organic layer was collected and washed with water, brine, and dried over Na_2SO_4. Filtration and concentration in vacuo afforded crude product. Further purification by silica gel flash chromatography (PE/AcOEt = 2:1) gave (**6**) (1.90 g, 60%) as yellow solid. Mp: 90–91 °C, MS (EI) $[M]^+$: m/z = 233, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.48 (s, 1H), 6.23 (s, 1H), 3.83 (s, 3H), 3.79 (s, 3H).

5.1.6. 3-Bromophenyl acetate (8)

Compound (**7**) (10.0 g, 58 mmol) was dissolved in pyridine(30 mL) under ice-bath and was stirred for 30 min. Acetic anhydride (8.3 mL, 88 mmol) was added dropwise into above mixture at the same temperature. The ice-bath was removed after dropping and the mixture was stirred for 2 h. Concentrated HCl and ice-water (300 mL) was added to the above reaction solution to neutralize PH to 7. The mixture was extracted with AcOEt (100 mL × 3). The organic layer was collected and washed with brine and dried over Na_2SO_4. Filtration and concentration in vacuo yield yellow oil (**8**) (10.15 g, 74%).

5.1.7. 1-(4-Bromo-2-hydroxyphenyl)ethanone (9)

In a 250 mL round bottom flask (**8**) (5.49 g, 26 mmol) and anhydrous $AlCl_3$ (6.92 g, 51 mmol) was mixed thoroughly on oil bath at 160 °C for 2 h. Reaction mixture was poured into ice water and concentrated hydrochloric acid solution was added to break complex formed during reaction. The mixture was taken in AcOEt (50 mL × 3). The organic layer was combined and washed with water and brine, and dried over Na_2SO_4. After filtration and concentration in vacuo, the residues was purified by silica gel flash chromatography (PE/AcOEt = 30:1) gave (**9**) (3.85 g, 70%) as white solid. Mp: 36–38 °C, MS (EI) $[M]^+$: m/z = 213, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.60 (d, J = 8.0 Hz, 1H), 7.20 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 2.63 (s, 3H).

5.1.8. 1-(4-Bromo-2-methoxyphenyl)ethanone (10)

A mixture of (**9**) (2.14 g, 10 mmol), potassium carbonate (4.14 g, 30 mmol), and acetone (100 mL) was stirred at 50 °C for 30 min. Dimethyl sulfate (1.1 mL,12 mmol) was added dropwise into the above mixture at 50 °C. After completion of reaction, the solid was filtered off and the solvent was evaporated. The solid obtained was dried to give (**10**) (2.12 g, 75%) as yellow solid. Mp: 60–61 °C, MS (EI) $[M]^+$: m/z = 229, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.65 (d, J = 8.0 Hz, 1H), 7.18 (s, 1H), 7.15 (d, J = 4.0 Hz, 1H), 3.94 (s, 3H), 2.61 (s, 3H).

5.1.9. 1-(5′-Amino-2′,3,3′-trimethoxybiphenyl-4-yl)ethanone (12)

A flask charged with Pd(pddf)Cl_2 (0.37 g, 0.5 mmol), KOAc (1.95 g, 20 mmol), and the pinacol ester of diboron (1.40 g, 5.5 mmol) and (**10**) (1.15 g, 5 mmol) was flushed with nitrogen. 1,4-dioxane (20 mL) were then added. After being stirred at 100 °C for 5 h under nitrogen atmosphere, the mixture was cooled to room temperature. Compound (**6**) (0.81 g, 3.5 mmol), H_2O (6 mL) and 1,4-dioxane (5 mL) were then added and the mixture was refluxed overnight under nitrogen. The product was extracted with AcOEt (30 mL × 3), washed with water, and dried over Na_2SO_4. After filtration and concentration in vacuo, the residues was purified by silica gel flash chromatography (PE/AcOEt = 3:1) gave (**12**) (0.98 g, 65%) as slight yellow solid. Mp: 116–117 °C,MS (EI) $[M]^+$: m/z = 301, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81 (d, J = 8.0 Hz, 1H), 7.27 (s, 1H), 7.15 (d, J = 8.0 Hz, 1H), 6.35 (s, 1H), 6.30 (s, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.52 (s, 3H), 2.67 (s, 3H).

5.1.10. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[2-(dimethylamino)ethoxy] phenyl}urea (A1)

Triphosgene (0.36 g, 1.2 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and the mixture was stirred on the ice-bath for 15 min. A solution of the (**12**) (0.90 g, 3 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to the above mixture and stirring continued for 20 min. Et_3N (0.36 mL, 2.58 mmol) diluted with CH_2Cl_2 (5 mL) was then added into the mixture. Stirring was continued for 20 min and a solution of Et_3N (0.36 mL, 2.58 mmol), 4-(2-(dimethylamino)ethoxy)aniline (0.54 g, 3 mmol) in anhydrous CH_2Cl_2 (20 mL) was added. After completion of the action, the reaction was quenched with dilute Na_2CO_3. The organic layer was washed with water and brine, and dried over Na_2SO_4. After filtration and concentration in vacuo, the residues was purified by silica gel flash chromatography (CH_2Cl_2/MeOH = 30:1) yielding (**A1**). yield 33%, mp: 107–110 °C, MS (ESI) $[M+H]^+$: m/z = 508, ^{1}H NMR (400 MHz,$CDCl_3$) δ ppm: 7.71 (d, J = 8.0 Hz, 2H), 7.38 (s, 1H), 7.25 (s, 1H), 7.21 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.90 (s, 1H), 6.63 (d, J = 8.0 Hz, 2H), 4.14 (s, 2H), 3.88 (s, 3H), 3.84 (s, 3H), 3.51 (s, 3H), 3.27 (s, 2H), 2.75 (s, 6H), 2.64 (s, 3H).

5.1.11. Compounds A2–A7, B1–B7 and C1–C7 were also prepared by using the general procedure described above

5.1.11.1. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[2-(diethylamino)ethoxy]phenyl}urea (A2). Yield 35%, mp: 107–109 °C. MS (ESI) $[M+H]^+$: m/z = 536, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.73 (d, J = 8.0 Hz, 1H), 7.43 (s, 1H), 7.32 (d, J = 8.0 Hz, 2H), 7.23 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 6.62 (d, J = 8.0 Hz, 2H), 4.28 (s, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.52 (s, 3H), 3.36 (s, 2H), 3.19 (s, 4H), 2.64 (s, 3H),1.27 (s, 6H).

5.1.11.2. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[3-(dimethylamino)propoxy]phenyl}urea (A3). Yield 40%, mp: 132–134 °C, MS (ESI) $[M+H]^+$: m/z = 522, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:7.76 (d, J = 8.0 Hz, 1H), 7.47 (s, 1H), 7.36 (d, J = 8.0 Hz, 2H), 7.25 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 6.63 (d, J = 8.0 Hz, 2H), 3.92 (s, 3H), 3.90 (s, 3H), 3.71 (t, J = 8.0 Hz, 2H), 3.55 (s, 3H), 3.15 (t, J = 8.0 Hz, 2H), 2.80 (s, 6H), 2.66 (s, 3H), 2.21–2.23 (m, 2H).

5.1.11.3. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[4-(2-piperidin-1-ylethoxy)phenyl]urea (A4). Yield 37%, mp: 114–116 °C, MS (ESI) $[M+H]^+$: m/z = 548, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81(d, J = 8.0 Hz,1H), 7.53 (s, 1H), 7.32 (s, 1H), 7.27 (s, 2H),

7.18 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 4.11 (t, J = 6.0 Hz, 2H), 3.91 (s, 6H), 3.56 (s, 3H), 2.81 (t, J = 6.0 Hz, 2H), 2.70 (s, 3H), 2.56 (s, 4H), 1.65 (s, 4H), 1.48 (s, 2H).

5.1.11.4. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[4-(2-pyrrolidin-1-ylethoxy)phenyl]urea (A5). Yield 41%, mp: 113–115 °C, MS (ESI) $[M+H]^+$: m/z = 534, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.80 (d, J = 8.0 Hz, 1H), 7.51 (s, 1H), 7.36 (s, 1H), 7.26 (d, J = 4.0 Hz, 2H), 7.08 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 6.63 (s, 1H), 4.12 (t, J = 6.0 Hz, 2H), 3.92 (s, 6H), 3.56 (s, 3H), 2.96 (t, J = 6.0 Hz, 2H), 2.71 (s, 4H), 2.69 (s, 3H), 1.85 (s, 4H).

5.1.11.5. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[4-(2-morpholin-4-ylethoxy)phenyl]urea (A6). Yield 35%, mp: 123–126 °C, MS (ESI) $[M+H]^+$: m/z = 550, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.81 (d, J = 8.0 Hz, 1H), 7.57 (s, 1H), 7.44 (s, 1H), 7.31 (s, 1H), 7.27 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.86 (d, J = 8.0 Hz, 2H), 6.59 (s, 1H), 4.13 (t, J = 6.0 Hz, 2H), 3.91 (s, 6H), 3.79 (t, J = 4.0 Hz, 4H), 3.55 (s, 3H), 2.87 (t, J = 6.0 Hz, 2H), 2.71 (s, 3H), 2.67 (s, 4H).

5.1.11.6. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[4-(3-morpholin-4-ylpropoxy)phenyl]urea (A7). Yield 39%, mp: 107–109 °C, MS (ESI) $[M+H]^+$: m/z = 564, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.82 (d, J = 8.0 Hz, 1H), 7.55 (s, 1H), 7.31 (s, 1H), 7.27 (s, 1H), 7.15 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 2H), 6.59 (s, 1H), 4.00 (t, J = 6.0 Hz, 2H), 3.92 (s, 6H), 3.77 (t, J = 4.0 Hz, 4H), 3.56 (s, 3H), 2.70 (s, 3H), 2.58 (t, J = 8.0 Hz, 2H), 2.54 (s, 4H), 1.97–2.02 (m, 2H).

5.1.11.7. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[2-(dimethylamino)ethoxy]-2-methylphenyl}urea (B1). Yield 32%, mp: 141–143 °C, MS (ESI) $[M+H]^+$: m/z = 522, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.78 (d, J = 8.0 Hz, 1H), 7.47 (s, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.82 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.60 (d, J = 4.0 Hz, 1H), 4.06 (t, J = 6.0 Hz, 2H), 3.91 (s, 3H), 3.90 (s, 3H), 3.54 (s, 3H), 2.76 (t, J = 6.0 Hz, 2H), 2.67 (s, 3H), 2.37 (s, 6H), 2.27 (s, 3H).

5.1.11.8. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[2-(diethylamino)ethoxy]-2-methylphenyl}urea (B2). Yield 33%, mp: 137–139 °C, MS (ESI) $[M+H]^+$: m/z = 550, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.73 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 7.41(d, J = 8.0 Hz, 1H), 7.24 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 6.58 (s, 1H), 6.55 (d, J = 8.0 Hz, 1H), 4.29 (s, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.53 (s, 3H), 3.31 (s, 2H), 3.14–3.19 (m, 4H), 2.65 (s, 3H), 2.16 (s, 3H), 1.33 (t, J = 6.0 Hz, 6H).

5.1.11.9. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{4-[3-(dimethylamino)propoxy]-2-methylphenyl}urea (B3). Yield 35%, mp: 137–139 °C, MS(ESI) $[M+H]^+$: m/z = 536, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.78 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.06 (d, J = 8.0 Hz, 1H), 6.79 (s, 1H), 6.76 (d, J = 8.0 Hz, 1H), 6.60 (s, 1H), 3.99 (t, J = 4.0 Hz, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.54 (s, 3H), 2.66 (s, 3H), 2.49 (t, J = 6.0 Hz, 2H), 2.29 (s, 6H), 2.27 (s, 3H), 1.97 (t, J = 6.0 Hz, 2H).

5.1.11.10. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-4-(2-piperidin-1-ylethoxy)phenyl]urea (B4). Yield 36%, mp: 181–182 °C, MS (ESI) $[M+H]^+$: m/z = 562, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.66 (d, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.34 (s, 1H), 7.24 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 6.96 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 3.96 (s, 2H), 3.93 (s, 3H), 3.83 (s, 3H), 3.54 (s, 3H), 3.36 (s, 4H), 2.57 (s, 3H), 2.35 (t, J = 12.0 Hz, 2H), 2.20 (s, 3H), 2.15 (s, 4H), 1.81–1.85 (m, 2H).

5.1.11.11. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-4-(2-pyrrolidin-1-ylethoxy)phenyl]urea (B5). Yield 45%, mp: 161–164 °C, MS (ESI) $[M+H]^+$: m/z = 548, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.72 (d, J = 8.0 Hz, 1H), 7.44 (s, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.95 (s, 1H), 6.56 (s, 1H), 6.52 (d, J = 8.0 Hz, 1H), 4.23 (s, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.71 (s, 2H), 2.52 (s, 3H), 3.39 (s, 2H), 2.99 (s, 2H), 2.64 (s, 3H), 2.13 (s, 3H), 2.06 (s, 4H).

5.1.11.12. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-4-(2-morpholin-4-ylethoxy)phenyl]urea (B6). Yield 44%, mp: 110–113 °C, MS (ESI) $[M+H]^+$: m/z = 564, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.50 (s, 1H), 7.36 (d, J = 12.0 Hz, 1H), 7.24 (s, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.81 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.58 (s, 1H), 4.11 (t, J = 6.0 Hz, 2H), 3.91 (s, 3H), 3.90 (s, 3H), 3.77 (t, J = 4.0 Hz, 4H), 3.54 (s, 3H), 2.85 (t, J = 6.0 Hz, 2H), 2.67 (s, 3H), 2.62 (s, 4H), 2.28 (s, 3H).

5.1.11.13. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-4-(3-morpholin-4-ylpropoxy)phenyl]urea (B7). Yield 30%, mp: 130–133 °C, MS (ESI) $[M+H]^+$: m/z = 578, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:7.42 (s, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.08(d, J = 8.0 Hz, 1H), 6.92 (s, 1H), 6.79 (s, 1H), 6.75 (d, J = 8.0 Hz, 1H), 6.65 (d, J = 4.0 Hz, 1H), 4.00(t, J = 6.0 Hz, 2H), 3.92 (s, 3H), 3.92 (s, 3H), 3.81 (t, J = 4.0 Hz, 4H), 3.53 (s, 3H), 2.70 (t, J = 8.0 Hz, 2H), 2.29 (s, 3H), 2.08 (s, 4H), 2.02–2.06 (m, 2H).

5.1.11.14. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{5-[2-(dimethylamino)et-oxy]-2-methylphenyl}urea (C1). Yield 47%, mp: 119–121 °C, MS (ESI) $[M+H]^+$: m/z = 522, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.74 (d, J = 8.0 Hz, 1H), 7.26 (s, 2H), 7.15 (d, J = 8.0 Hz, 1H), 7.12 (s, 1H), 7.07 (s, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.37 (d, J = 8.0 Hz, 1H), 4.31 (s, 2H), 3.92 (s, 3H), 3.85 (s, 3H), 3.54 (s, 3H), 3.18 (s, 2H), 2.66 (s, 3H), 2.22 (s, 3H), 1.34 (s, 6H).

5.1.11.15. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{5-[2-(diethylamino)ethoxy]-2-methylphenyl}urea(C2). Yield 42%, mp: 95–97 °C, MS (ESI) $[M+H]^+$: m/z = 550, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.68 (s, 1H), 7.66 (s, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.09 (t, J = 6.0 Hz, 2H), 7.02 (s, 1H), 6.57 (d, J = 12.0 Hz, 1H), 4.27 (s, 2H), 3.93 (s, 3H), 3.84 (s, 3H), 3.55 (s, 3H), 3.37 (s, 2H), 3.15 (s, 4H), 2.57 (s, 3H), 2.09 (s, 3H), 1.22 (s, 6H).

5.1.11.16. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-{5-[3-(dimethylamino)propoxy]-2-methylphenyl}urea (C3). Yield 40%, mp: 103–105 °C, MS (ESI) $[M+H]^+$: m/z = 536, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.67(s,1H), 7.60(s,1H), 7.34(d, J = 8.0 Hz,1H), 7.24 (s, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.04 (d, J = 8.0 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.50 (d, J = 8.0 Hz, 1H), 3.94 (s, 5H), 3.85 (s, 3H), 3.55 (s, 3H), 2.57 (s, 3H), 2.46 (s, 2H), 2.24 (s, 6H), 2.18 (s, 3H), 1.85–1.88 (m, 2H).

5.1.11.17. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-5-(2-piperidin-1-ylethoxy)phenyl]urea (C4). Yield 31%, mp: 111–113 °C, MS (ESI) $[M+H]^+$: m/z = 562, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.70 (s, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.23 (s, 1H), 7.09 (t, J = 4.0 Hz, 2H), 7.01 (d, J = 8.0 Hz, 1H), 6.57 (d, J = 8.0 Hz, 1H), 4.30 (s, 2H), 3.94 (s, 3H), 3.84 (s, 3H), 3.55 (s, 3H), 3.40 (s, 4H), 3.03 (s, 2H),2.57 (s, 3H), 2.22 (s, 3H), 1.76 (s, 4H), 1.53 (s, 2H).

5.1.11.18. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-5-(2-pyrrolidin-1-ylethoxy)phenyl]urea (C5). Yield 37%, mp: 102–104 °C, MS (ESI) $[M+H]^+$: m/z = 548, ^{1}H NMR

(400 MHz,$CDCl_3$) δ ppm: 7.43 (s, 1H), 7.26 (d, J = 4.0 Hz, 1H), 7.24 (s, 1H), 7.08 (d, J = 8.0 Hz, 2H), 7.03 (d, J = 4.0 Hz, 1H), 6.99 (d, J = 4.0 Hz, 1H), 6.34 (d, J = 4.0 Hz, 1H), 4.12 (t, J = 6.0 Hz, 2H), 3.92 (s, 3H), 3.91 (s, 3H), 3.56 (s, 3H), 2.98 (t, J = 6.0 Hz, 2H), 2.76 (s, 4H), 2.67 (s, 3H), 2.20 (s, 3H), 1.85 (s, 4H).

5.1.11.19. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-5-(2-morpholin-4-ylethoxy)phenyl]urea (C6). Yield 40%, mp: 120–124 °C, MS (ESI) $[M+H]^+$: m/z = 564, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.77(d, J = 8.0 Hz, 1H), 7.39 (s, 1H),7.33 (s, 1H), 7.24 (s, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.97 (d, J = 4.0 Hz, 1H), 6.75 (d, J = 4.0 Hz, 1H), 6.52 (d, J = 4.0 Hz, 1H), 4.19 (t, J = 6.0 Hz, 2H), 3.90 (s, 3H), 3.88 (s, 3H), 3.85(t, J = 4.0 Hz, 4H), 3.55 (s, 3H), 3.01 (s, 2H), 2.86 (s, 4H), 2.67 (s, 3H), 2.19 (s, 3H).

5.1.11.20. *N*-(4′-Acetyl-3′,5,6-trimethoxybiphenyl-3-yl)-*N*′-[2-methyl-5-(3-morpholin-4-ylpropoxy)phenyl]urea (C7). Yield 38%, mp: 97–100 °C, MS (ESI) $[M+H]^+$: m/z = 578, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.58 (s, 1H), 7.42(d, J = 4.0 Hz, 1H), 7.31 (s, 1H), 7.24 (s, 1H), 7.03 (d, J = 8.0 Hz, 2H), 6.60 (d, J = 4.0 Hz, 1H), 6.55 (d, J = 4.0 Hz, 1H), 3.99 (t, J = 6.0 Hz, 2H), 3.89 (s, 6H), 3.73 (t, J = 4.0 Hz, 4H), 3.54 (s, 3H), 2.68 (s, 3H), 2.52 (d, J = 8.0 Hz, 2H), 2.49 (s, 4H), 2.20 (s, 3H), 1.93–1.99 (m, 2H).

5.1.12. *N*-{4-[2-(Dimethylamino)ethoxy]-2-methylphenyl}-*N*′-{4′-[(1*E*)-N-hydro-xyl-ethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}urea (D1)

Compound (**B1**) (0.40 g, 0.69 mmol) was dissolved in ethanol 20 mL and the solution was heated to 50 °C. After being stirred for 15 min, hydroxylamine hydrochloride was added to the above mixture and stirring continued for 1 h. The mixture was poured into cool water and neutralized with Na_2CO_3. The product was extracted with CH_2Cl_2 (20 mL × 3), washed with water, and dried over Na_2SO_4. Filtration and concentration in vacuo to afford (**D1**) (0.38 g, 88%). Mp: 155–157 °C, MS(ESI) $[M+H]^+$:m/z = 537, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.61 (d, J = 8.0 Hz, 1H), 7.33 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.12 (s, 1H), 7.01 (d, J = 4.0 Hz, 1H), 6.95 (s, 1H), 6.84 (s, 1H), 6.78 (d, J = 4.0 Hz, 1H), 4.23 (s, 2H), 3.83 (s, 6H), 3.08 (s, 2H), 3.55 (s, 3H), 2.70 (s, 6H), 2.24 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.14, 154.41, 153.85, 153.74, 153.21, 140.78, 140.32, 136.99, 134.92, 131.63, 131.05, 129.30, 126.58, 123.99, 121.25, 116.78, 112.56, 111.24, 103.40, 63.30, 60.82, 56.11, 56.04, 55.99, 53.73, 43.51, 18.79, 15.76.

5.1.13. *N*-{4-[2-(Diethylamino)ethoxy]-2-methylphenyl}-*N*′-{4′-[(1*E*)-*N*-hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}urea (D2)

Yield 85%, mp: 155–157 °C, MS (ESI) $[M+H]^+$:m/z = 565, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.20 (d, J = 8.0 Hz, 2H), 7.15 (s, 1H), 7.08 (s, 1H), 6.88 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.0 Hz, 2H), 6.69 (s, 1H), 4.16 (t, J = 6.0 Hz, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 3.53 (s, 3H), 2.91 (t, J = 6.0 Hz, 2H), 2.71–2.76 (m, 4H), 2.26 (s, 3H), 2.06 (s, 3H), 1.12 (t, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.17, 155.07, 154.41, 153.63, 153.26, 140.90, 140.26, 136.81, 134.88, 131.41, 130.63, 129.31, 126.58, 124.52, 121.28, 116.53, 112.58, 112.28, 111.38, 103.37, 67.21, 60.81, 56.12, 55.98, 51.82, 47.46, 18.49, 15.76, 12.23.

5.1.14. *N*-{4-[3-(Dimethylamino)propoxy]-2-methylphenyl}-*N*′-{4′-[(1*E*)-*N*-hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}urea (D3)

Yield 89%, mp: 139–141 °C, MS (ESI) $[M+H]^+$: m/z = 551, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.29 (s, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.11 (s, 1H), 6.86 (d, J = 8.0 Hz, 2H), 6.71 (s, 1H), 6.61 (s, 1H), 3.97 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 3.75 (s, 3H), 3.50 (s, 3H), 2.50 (t, J = 6.0 Hz, 2H), 2.29 (s, 6H), 2.24 (s, 3H), 2.15 (s, 3H), 1.96–2.01 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.18, 155.26, 154.40, 153.72, 153.25, 140.88, 140.29, 136.91, 134.87, 131.48, 130.61, 129.30, 126.59, 124.60, 121.28, 116.49, 112.60, 112.23, 111.39, 103.38, 66.29, 60.80, 56.19, 56.11, 55.98, 45.68, 27.47, 18.51, 15.74.

5.1.15. *N*-{4′-[(1*E*)-*N*-Hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}-*N*′-[2-methyl-4-(2-piperidin-1-ylethoxy)phenyl]urea (D4)

Yield 93%, mp: 158–160 °C, MS (ESI) $[M+H]^+$: m/z = 577, ^{1}H NMR(400 MHz,$CDCl_3$) δ ppm: 7.35 (d, J = 8.0 Hz, 1H), 7.20(d, J = 8.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.12 (s, 1H), 6.87 (s, 1H), 6.70 (s, 3H), 3.97 (t, J = 6.0 Hz, 2H), 4.21 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 3.54 (s, 3H), 2.84 (t, J = 6.0 Hz, 2H), 2.63 (s, 4H), 2.26 (s, 3H), 2.16 (s, 3H), 1.65–1.68 (m, 4H), 1.48 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.18, 155.05, 154.40, 153.65, 153.26, 140.93, 140.26, 136.82, 134.87, 131.44, 130.67, 129.30, 126.60, 124.54, 121.28, 116.59, 112.60, 112.32, 111.41, 103.40, 66.03, 60.81, 57.81, 56.13, 55.99, 54.82, 25.93, 24.29, 18.49, 15.74.

5.1.16. *N*-{4′-[(1*E*)-*N*-Hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}-*N*′-[2-methyl-4-(2-pyrrolidin-1-ylethoxy)phenyl]urea (D5)

Yield 92%, mp: 140–142 °C, MS (ESI) $[M+H]^+$: m/z = 563, ^{1} H-NMR(400 MHz, $CDCl_3$) δ ppm:7.13–7.18 (m, 3H), 7.09 (s, 1H), 6.83 (d, J = 8.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 2H), 6.70 (s, 1H), 3.97 (t, J = 6.0 Hz, 2H), 4.22 (t, J = 6.0 Hz, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.54 (s, 3H), 2.95 (t, J = 6.0 Hz, 2H), 2.73 (s, 4H), 2.55 (s, 3H), 2.16 (s, 3H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.18, 155.09, 154.40, 153.70, 153.25, 140.90, 140.28, 136.88, 134.87, 131.50, 130.65, 129.30, 126.59, 124.61, 121.28, 116.53, 112.60, 112.24, 111.40, 103.40, 67.30, 60.80, 56.12, 55.98, 54.86, 54.47, 23.62, 18.51, 15.74.

5.1.17. *N*-{4′-[(1*E*)-*N*-Hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}-*N*′-[2-methyl-4-(2-morpholin-4-ylethoxy)phenyl]urea (D6)

Yield 89%, mp: 151–153 °C, MS (ESI) $[M+H]^+$: m/z = 579, ^{1} H-NMR (400 MHz, $CDCl_3$) δ ppm:7.32 (s, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.19 (d, J = 8.0 Hz, 1H), 7.16 (s, 1H), 6.90 (d, J = 4.0 Hz, 1H), 6.74 (d, J = 8.0 Hz, 2H), 6.66 (d, J = 4.0 Hz, 1H), 4.18 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 3.78 (s, 7H), 3.54 (s, 3H), 2.86 (t, J = 6.0 Hz, 2H), 2.68 (s, 4H), 2.27 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.18, 155.01, 154.41, 153.64, 153.26, 140.94, 140.26, 136.81, 134.88, 131.40, 130.70, 129.31, 126.60, 124.50, 121.28, 116.61, 112.60, 112.31, 111.41, 103.41, 66.63, 65.88, 60.81, 57.54, 56.13, 55.98, 54.10, 18.49, 15.74.

5.1.18. *N*-{4′-[(1*E*)-*N*-Hydroxyethanimidoyl]-3′,5,6-trimethoxybiphenyl-3-yl}-*N*′-[2-methyl-4-(3-morpholin-4-ylpropoxy)phenyl]urea (D7)

Yield 88%, mp: 154–156 °C, MS (ESI) $[M+H]^+$: m/z = 593, ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.52 (d, J = 8.0 Hz, 1H), 7.31 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.13 (s, 1H), 6.04 (d, J = 8.0 Hz, 1H), 6.94 (s, 1H), 6.78 (s, 1H), 6.72 (d, J = 8.0 Hz, 1H), 3.96 (t, J = 6.0 Hz, 2H), 3.83 (s,6H), 3.57 (t, J = 4.0 Hz, 4H), 3.55 (s, 3H), 2.41 (t, J = 8.0 Hz, 2H), 2.36 (s, 4H), 2.20 (s, 3H), 2.10 (s, 3H), 1.82–1.88 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.18, 155.24, 154.41, 153.65, 153.26, 140.94, 140.26, 136.81, 134.88, 131.44, 130.58, 129.31, 126.60, 124.57, 121.29, 116.55, 112.60, 112.28, 111.41, 103.42, 66.68, 66.28, 60.81, 56.13, 55.99, 55.37, 53.86, 26.42, 18.48, 15.74.

5.2. Kinase assay[24]

The ability of compounds to inhibit the phosphorylation of a peptide substrate by VEGFR-2 was evaluated in a microtiter plate

format using homogeneous time-resolved fluorescence (HTRF). Firstly, 2 μL kinase (K_m = 0.003767 ng/μL) and 2 μL substrate (K_m = 121.4 nM) were separately added to a 384-well plate, and variable concentrations of compounds (diluted in buffer) were then added to the assay plate. ATP (2 μL, K_m = 1.332 μM) was added and the reaction was allowed to proceed at 37 °C for 30 min. The TK-antibody labeled with Eu^{3+}-cryptate and streptavidin-XL665 were then added with EDTA to detect the phosphorylated product at room temperature for 1 h. Then the fluorescence was measured at 615 nm (cryptate) and 665 nm (XL665) using the Perkin-Elmer victor 2030 multilabel plate reader. Finally, the results were calculated as follows: ratio = (OD665 nm/OD615 nm) × 10^4.

5.3. Antiproliferative activity of biphenyl urea derivatives

Growth inhibitory activities were evaluated on the following cell lines: 7901, K562, SY5Y, LOVO. The effects of the compounds on cell viability were evaluated by the MTT assay. Exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1 × 10^4 cells/well, and then incubated for 24 h at 37 °C. The cells in the wells were treated with target compounds respectively at various concentrations for 48 h. Then, 20 mL MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Supernatant was discarded, and 150 mL DMSO was added to each well. Absorbance values were determined by a microplate reader (Bio-Rad Instruments) at 490 nm.

5.4. Molecular docking modeling

Molecule docking was performed using Sybyl/Surflex-dock based on the crystal structures of VEGFR-2 (PDB ID: 4ASD). Hydrogen was added and minimized using the Tripos force field and Pullman charges. Compound (**A7**) was depicted with the Sybyl/Sketch module (Tripos Inc.) and optimized applying Powell's method with the Tripos force field with convergence criterion set at 0.05 kcal/(Å mol), and assigned with the Gasteiger–Hückel method. The residues in a radius 5.0 Å around sorafenib (the ligand of VEGFR-2 in the crystal complex) were selected as the active site. Other docking parameters were kept at default.

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