

Supplementary Information

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

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Table of Contents

- (1) The chemistry experimental and physical state of T1-T14**
- (2) The Mass Spectrometry (MS) and the ¹H-NMR Spectrum of T1-T14**
- (3) The Biological assays procedures of title compounds**

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(1) The chemistry experimental and physical state of T1-T14

General Chemical Methods: 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. The HPLC/MS conditions were as follows: a VP-ODS column (150 mm×2.0 mm i.d., 5 mm, Shimadzu Corporation, Kyoto, Japan), a mobile phase of methanol: water (65: 35, v/v), with 0.2 mL min⁻¹ flow rate, injection volume 1 μL, DAD detection over 20min. 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.

1.1 3-bromo-4,5-dimethoxybenzonitrile(2)

A mixture of (1) (20.70 g, 90 mmol), sodium formate (26.46 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, washed with water, and dried to give an off-white solid. A mixture of the obtained solid (13.51 g, 60 mmol), potassium carbonate (24.92 g, 180 mmol) and acetone (100 mL) was stirred at 50 °C for 30 min. Dimethyl sulfate (6.6 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 (2) (13.49 g, 94%).

1.2 3-bromo-4,5-dimethoxybenzamide(3)

To a solution of (2) (8.03 g, 33 mol) in ethanol (150 ml) was added NaOH (1.60 g, 40 mmol) and 30% H₂O₂(17 ml, 600 mol). The mixture was stirred at 60 °C for 1 h. After completion of reaction, the mixture was acidified with concentrated HCl. Ethanol was evaporated and residues was poured into water and filtered. The white solid obtain

was dried to give **(3)** (7.70 g, 89.5%).

1.3 3-bromo-4, 5-dimethoxyaniline (**4**)

Br₂ (0.9 mL) was added dropwise into a solution of NaOH (2.70 g, 68 mmol) in distilled water (54 mL) at -5 °C and was stirred for another 10 min. **(3)** (3.60 g, 14 mmol) was added into the above solution in batches and stirring continued for 20 min. The mixture was warmed to rt and kept for 30 min. Then the suspension was heated at 40 °C for 1 h. The mixture was cooled to rt and it was poured into water and extracted with AcOEt (50 mL × 3). The organic layer was collected and washed with water, brine, and dried over Na₂SO₄. Filtration and concentration in vacuo afford crude product. Further purification by silica gel flash chromatography (PE/AcOEt = 2:1) gave **(4)** (1.90 g, 60%) as yellow solid.

1.4 1-(4-bromo-2-methoxyphenyl)ethanone (**6**)

In a 250 mL round bottom flask **(5)** (5.49 g, 26 mmol) and anhydrous AlCl₃ (6.92 g, 51 mmol) was mixed thoroughly on oil bath at 160 °C for two hours. 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 Na₂SO₄. After filtration and concentration in vacuo, the residue was purified by silica gel flash chromatography (PE/AcOEt = 30:1) to give white solid. A mixture of the white solid (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 **(6)** (2.12 g, 75%) as yellow solid.

1.5 1-(5'-amino-2', 3, 3'-trimethoxybiphenyl-4-yl)ethanone (**8**)

A flask charged with Pd(pddf)Cl₂ (0.37 g, 0.5 mmol), KOAc (1.95 g, 20 mmol), and the bis(pinacolato)diboron (1.40 g, 5.5 mmol) and **(6)** (1.15 g, 5 mmol) was flushed with nitrogen. 1,4-dioxane (20 mL) was then added. After being stirred at 100 °C for 5 h under nitrogen atmosphere, the mixture was cooled to rt. The black oil mixture was not further purified **(7)**. **(4)** (0.81 g, 3.5 mmol), H₂O (6 mL) and 1,4-dioxane (5 mL)

were then added to the mixture. It was then refluxed overnight under nitrogen. The product was extracted with AcOEt (30 mL×3), washed with water, and dried over Na₂SO₄. After filtration and concentration in vacuo, the residues was purified by silica gel flash chromatography (PE/AcOEt= 3:1) gave (**8**) (0.98 g, 65%) as slight yellow solid.

1.6 *N*-(4'-acetyl-3',5,6-trimethoxybiphenyl-3-yl)-*N'*-{4-[2-(dimethylamino)ethoxy]phenyl}urea (**9**)

Triphosgene (0.36 g, 1.2 mmol) was dissolved in anhydrous CH₂Cl₂ (15 mL) and the mixture was stirred on the ice-bath for 15 min. A solution of the (**8**) (0.90 g, 3 mmol) in anhydrous CH₂Cl₂ (10 mL) was added dropwise to the above mixture and stirring continued for 20 min. Et₃N (0.36 mL, 2.58 mmol) diluted with CH₂Cl₂ (5 mL) was then added into the mixture. Stirring was continued for 20 min and a solution of Et₃N (0.36 mL, 2.58 mmol), 4-(2-(dimethylamino)ethoxy)aniline (0.54 g, 3 mmol) in anhydrous CH₂Cl₂(20 mL) was added. After completion of the action, the reaction was quenched with dilute Na₂CO₃. The organic layer was washed with water and brine, and dried over Na₂SO₄. After filtration and concentration in vacuo, the residues was purified by silica gel flash chromatography (CH₂Cl₂/MeOH= 30:1) gave as slight yellow solid (**9**).

1.7 *N*-{4-[2-(dimethylamino)ethoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea (**T1**)

Compound (**9**) (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 Na₂CO₃. The product was extracted with CH₂Cl₂ (20 mL×3), washed with water, and dried over Na₂SO₄. Filtration and concentration in vacuo afford **T1** (0.38 g, 88%) as white solid. mp: 155~158 °C.

Compounds **T2~T14** were also prepared by using the general procedure described above.

1.7.1 *N*-{4-[2-(diethylamino)ethoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea (**T2**) yield 89% as slight grey solid. mp: 122~

125 °C

1.7.2 *N*-{4-[3-(dimethylamino)propoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea(**T3**) yield 90% as slight yellow solid. mp: 167~170 °C

1.7.3 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-piperidin-1-ylethoxy)phenyl]urea (**T4**) yield 90% as white solid. mp: 117~120 °C

1.7.4 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-pyrrolidin-1-ylethoxy)phenyl]urea (**T5**) yield 85% as slight brown solid. mp: 132~135 °C

1.7.5 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-morpholin-4-ylethoxy)phenyl]urea (**T6**) yield 88% as slight yellow solid. mp: 165~168 °C

1.7.6 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(3-morpholin-4-ylpropoxy)phenyl]urea (**T7**) yield 93% as white solid. mp: 120~123 °C

1.7.7 *N*-{5-[3-(dimethylamino)propoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea(**T8**) yield 93% as yellow solid. mp: 190~192 °C

1.7.8 *N*-{5-[2-(diethylamino)ethoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea(**T9**) yield 95% as white solid. mp: 140~142 °C

1.7.9 *N*-{5-[3-(dimethylamino)propoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea(**T10**) yield 92% as grey solid. mp: 150~152 °C

1.7.10 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(2-piperidin-1-ylethoxy)phenyl]urea(**T11**) yield 92% as slight yellow solid. mp: 195~197 °C

1.7.11 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(2-pyrrolidin-1-ylethoxy)phenyl]urea(**T12**) yield 89% as slight yellow solid. mp: 172~175 °C

1.7.12 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-

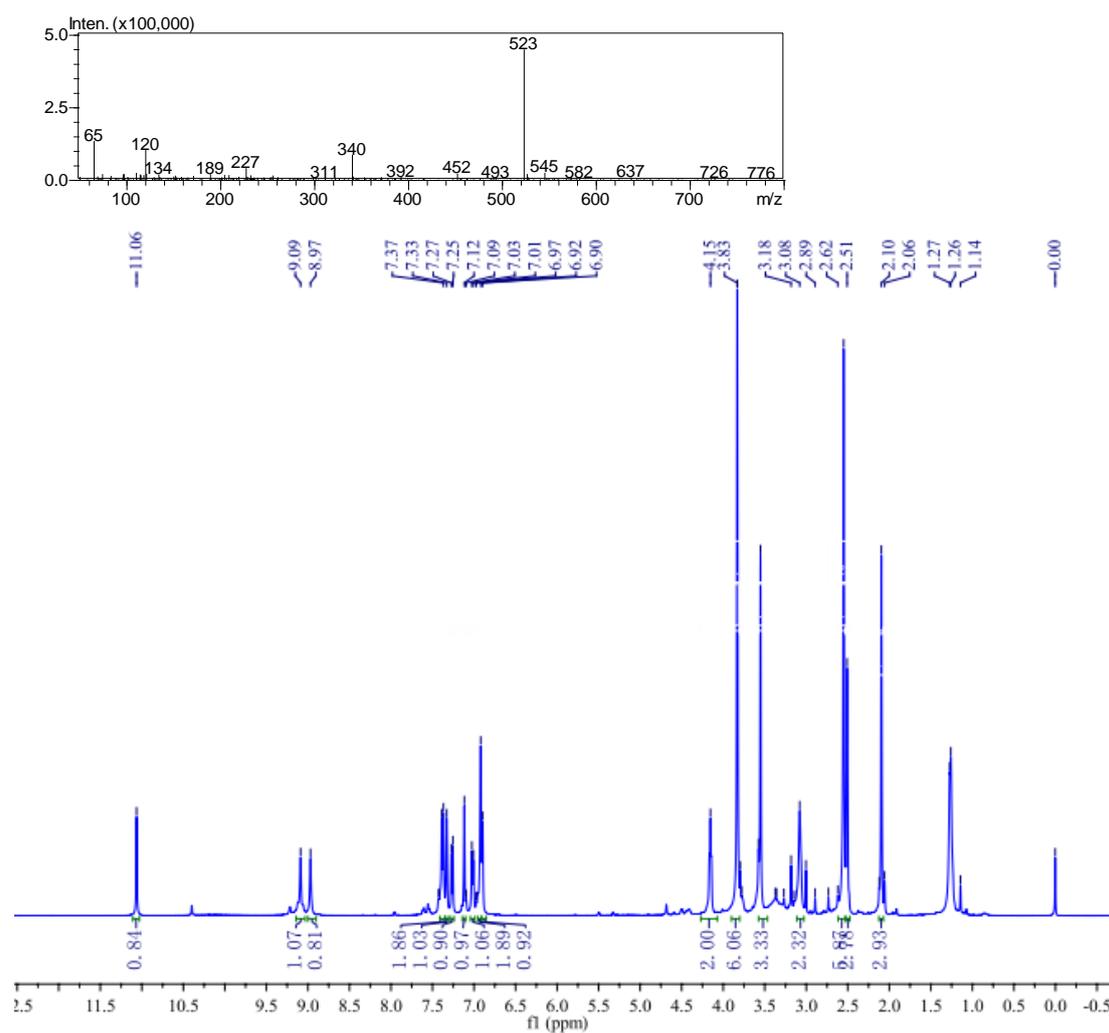
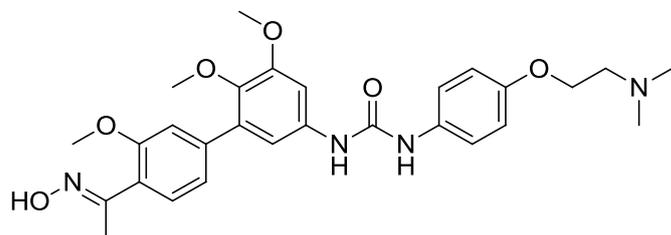
methyl-5-(2-morpholin-4-ylethoxy)phenyl]urea(**T13**) yield 84% as white solid.

mp:183~186 °C

1.7.13 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(3-morpholin-4-ylpropoxy)phenyl]urea(**T14**) yield 93% as slight green solid. mp:118~120 °C

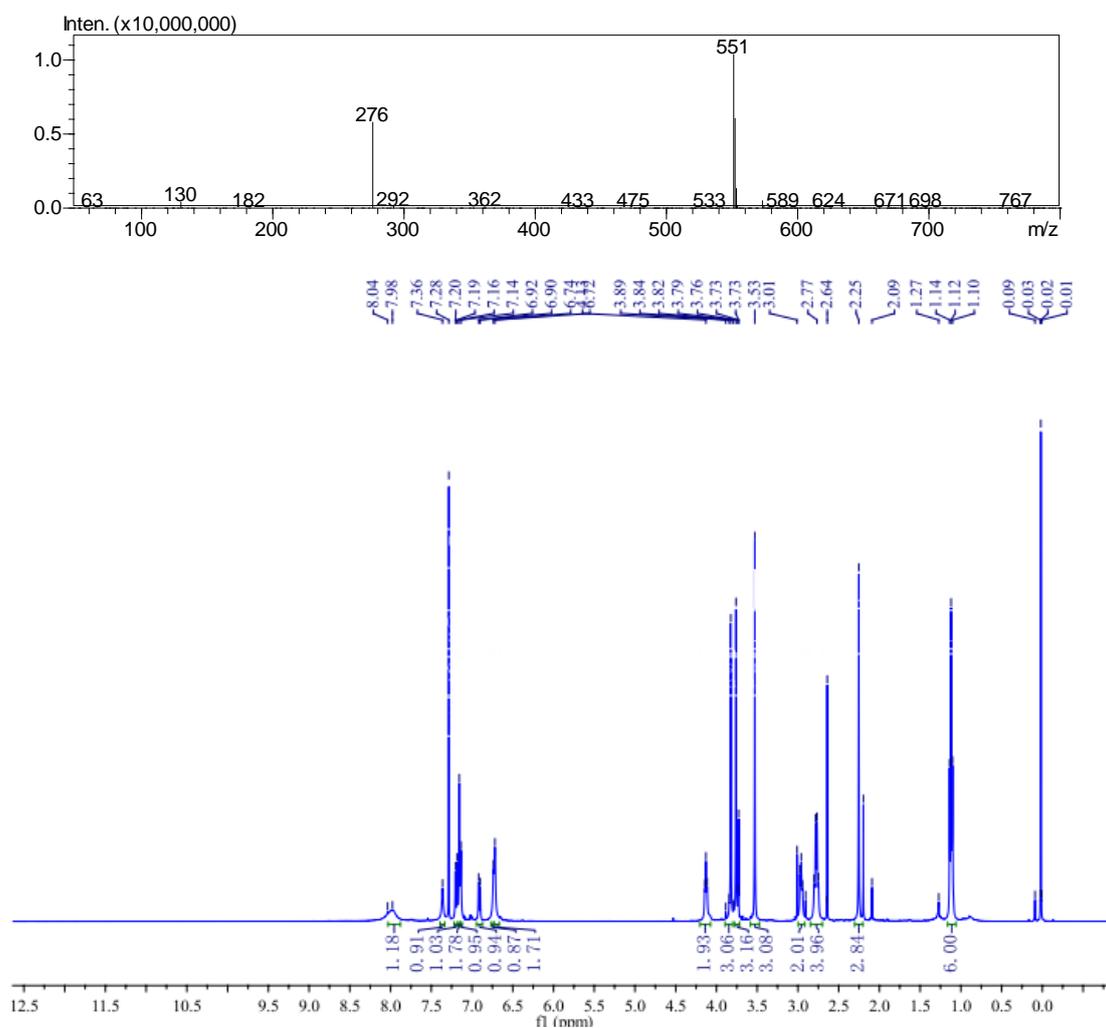
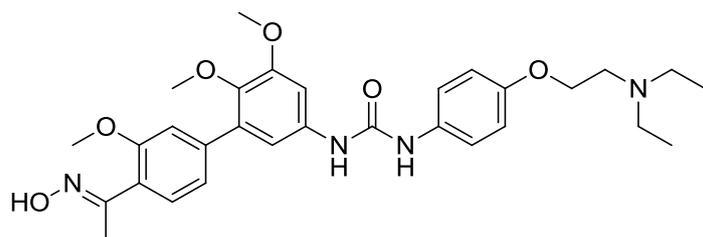
(2) The Mass Spectrometry (MS) and the ^1H Spectrum of T1-T14

T1 *N*-{4-[2-(dimethylamino)ethoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



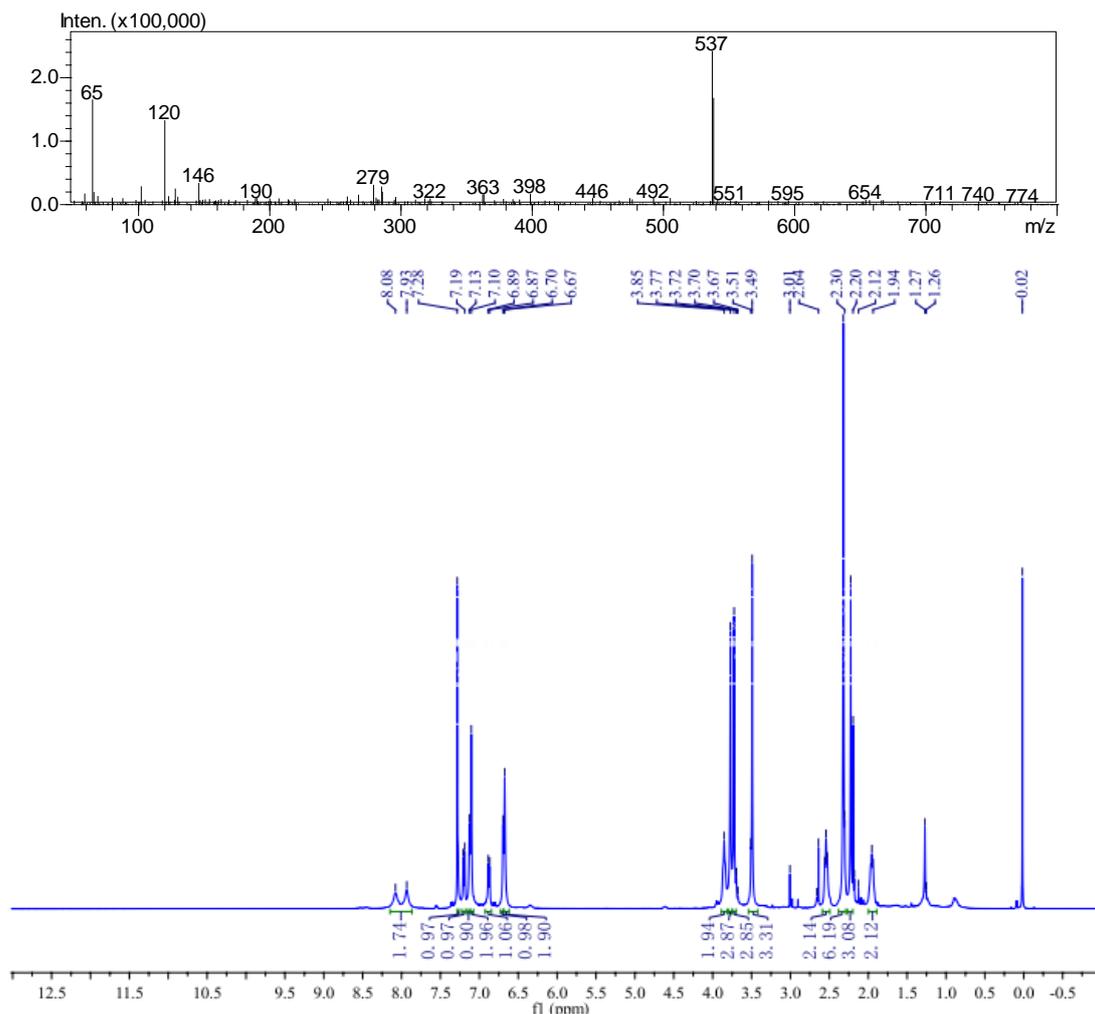
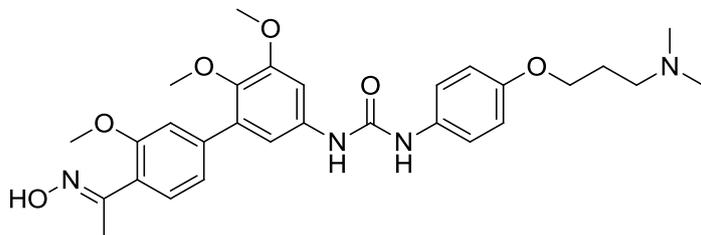
Yield: 88%, white solid, mp: 155~158 °C. LC-MS 99%, 7.47 min (20 minute LC-MS method), ESI-MS (m/z): 523 $[\text{M}+\text{H}]^+$. ^1H NMR (400MHz, $\text{DMSO}-d_6$): δ 11.06 (s, 1H), 9.09 (s, 1H), 8.97 (s, 1H), 7.38 (d, $J=8.0\text{Hz}$, 2H), 7.33 (s, 1H), 7.26 (d, $J=8.0\text{Hz}$, 1H), 7.12 (s, 1H), 7.02 (d, $J=8.0\text{Hz}$, 1H), 6.92 (s, 2H), 6.90 (s, 1H), 4.15 (s, 2H), 3.83 (s, 6H), 3.55 (s, 3H), 3.08 (s, 2H), 2.55 (s, 6H), 2.10 (s, 3H).

T2 *N*-{4-[2-(diethylamino)ethoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



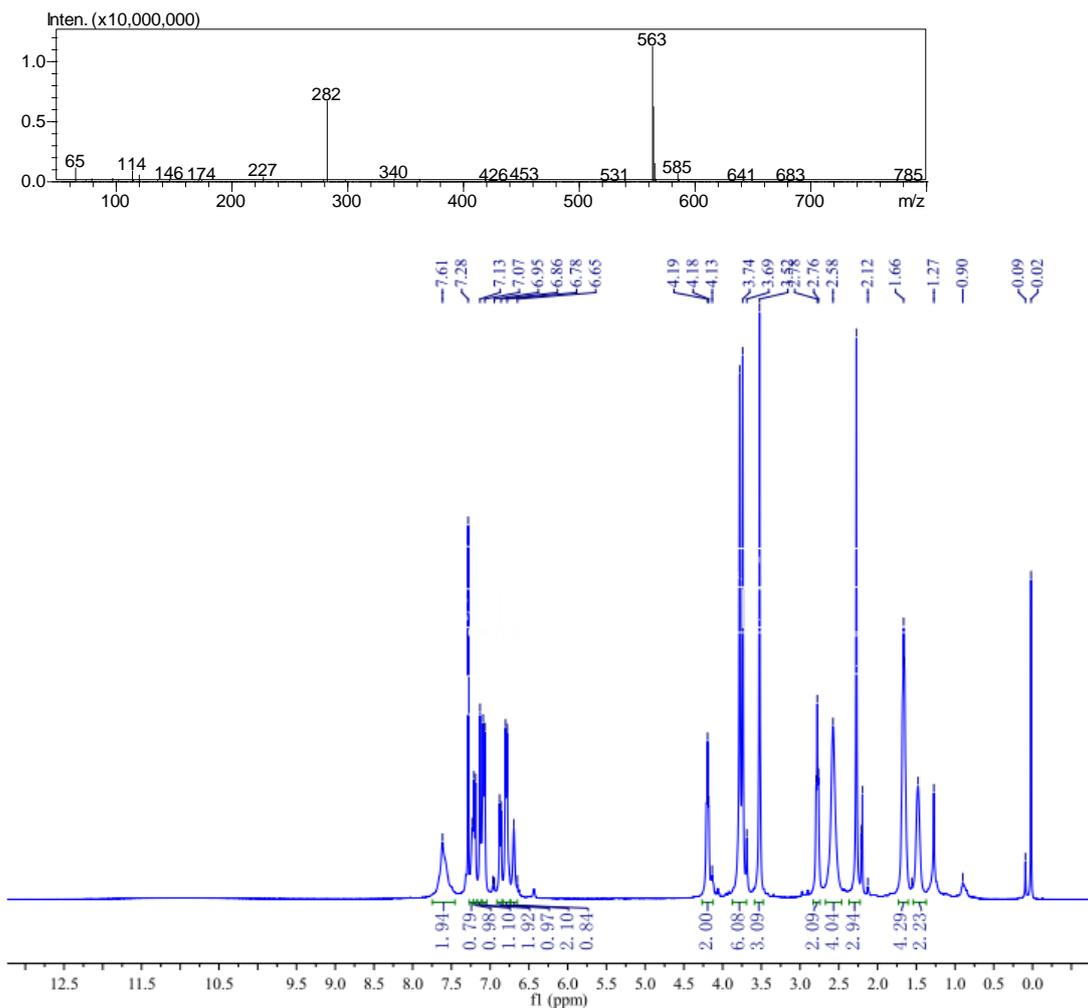
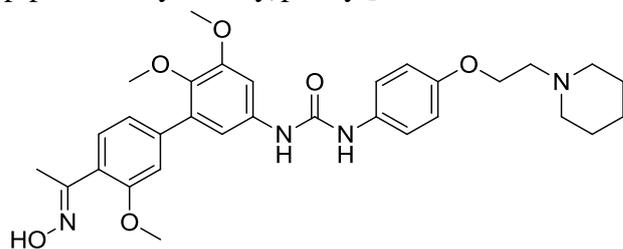
Yield: 89%, slight grey solid, mp: 122~ 125 °C. LC-MS 98%, 10.42 min (20 minute LC-MS method), ESI-MS (m/z): 551 $[M+H]^+$. 1H NMR (400MHz, $CDCl_3$) : δ 7.98 (s, 1H), 7.36 (s, 1H), 7.20 (d, $J=4.0$ H, 1H), 7.16 (s, 2H), 7.14 (s, 1H), 6.91 (d, $J=8.0$ Hz, 1H), 6.74 (s, 1H), 6.72 (s, 2H), 4.13 (t, $J=6.0$ Hz, 2H), 3.84 (s, 3H), 3.76 (s, 3H), 2.96 (t, $J=6.0$ Hz, 2H), 2.75~2.80 (m, 4H), 2.25 (s, 3H), 2.23 (s, 3H), 1.12 (t, $J=8.0$ Hz, 6H).

T3 *N*-{4-[3-(dimethylamino)propoxy]phenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



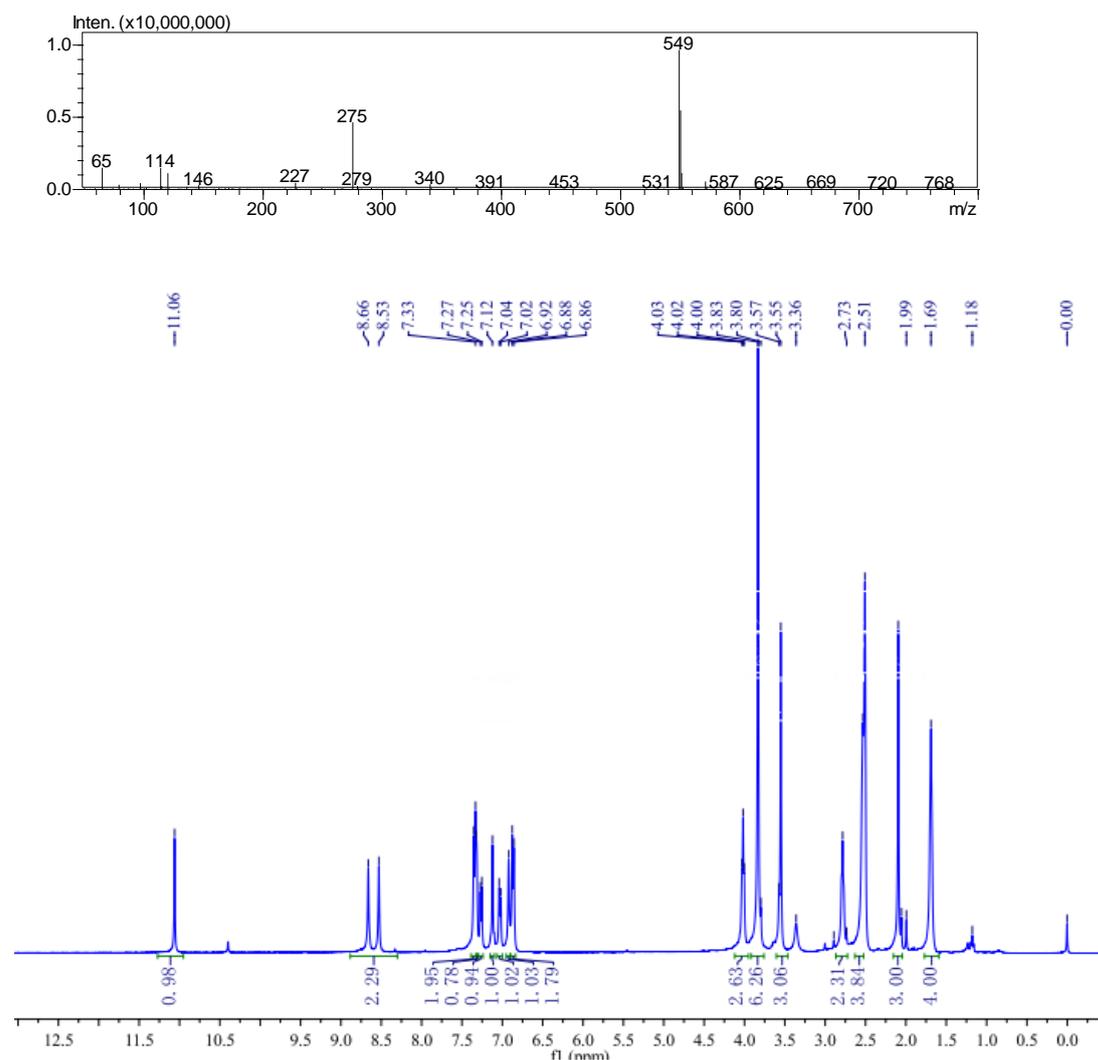
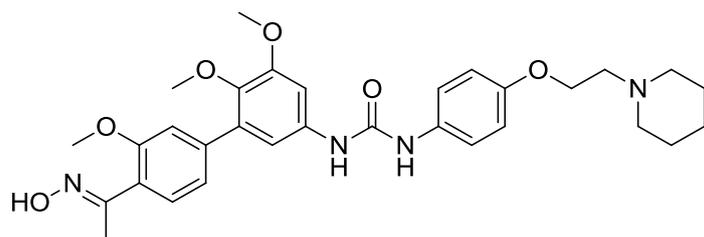
Yield: 90%, slight yellow solid, mp: 167~ 170 °C. LC-MS 99%, 7.20 min (20 minute LC-MS method), ESI-MS (m/z): 523 $[M+H]^+$. $^1\text{H NMR}$ (400MHz, CDCl_3): δ 8.08 (s, 1H), 7.93 (s, 1H), 7.28 (s, 1H), 7.20 (d, $J=8.0\text{Hz}$, 1H), 7.13 (s, 1H), 7.10 (s, 2H), 6.88 (d, $J=8.0\text{Hz}$, 1H), 6.70 (s, 1H), 6.67 (s, 2H), 3.85 (s, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 3.49 (s, 3H), 2.54 (t, $J=6.0\text{Hz}$, 2H), 2.32 (s, 6H), 2.23 (s, 3H), 1.95 (s, 2H).

T4 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-piperidin-1-ylethoxy)phenyl]urea



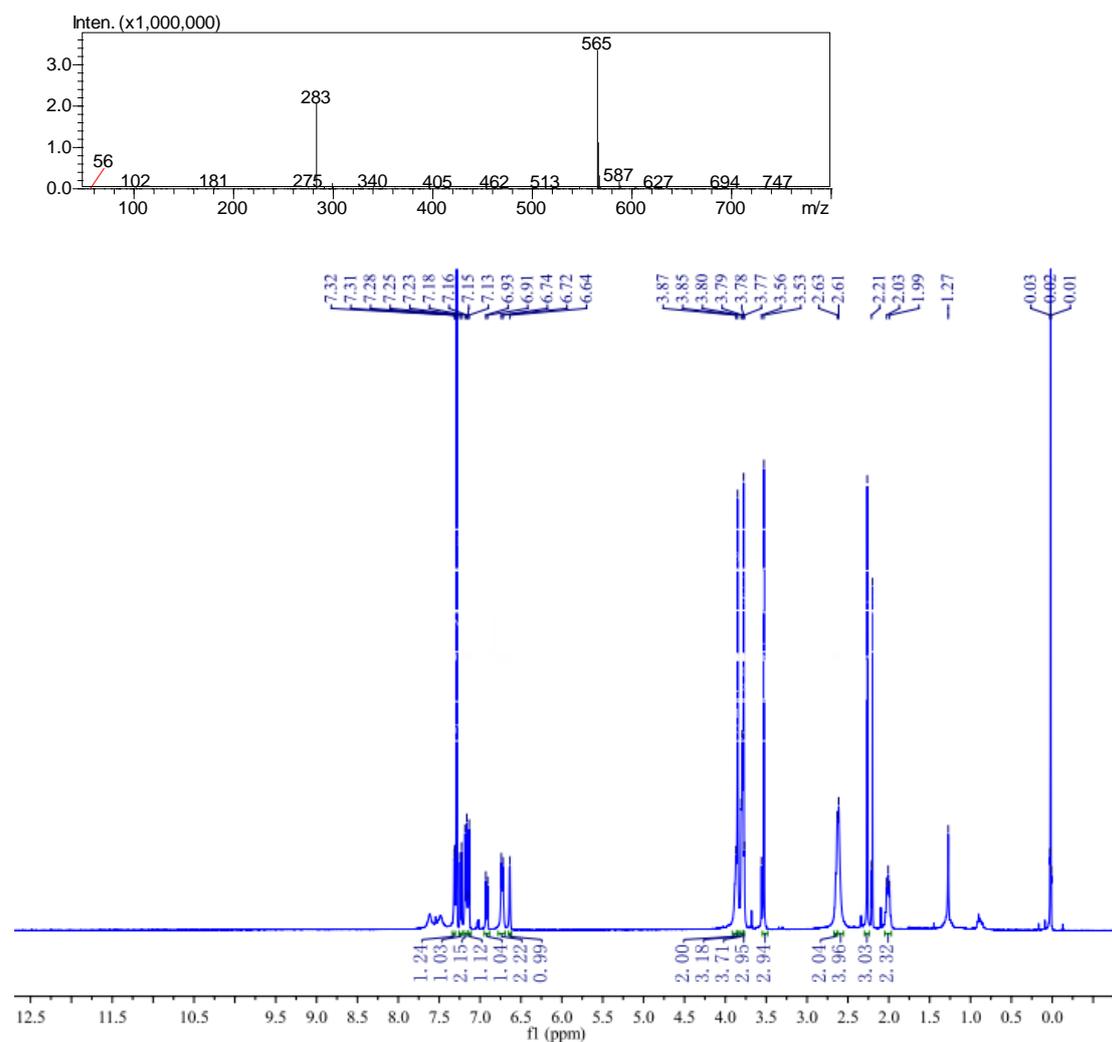
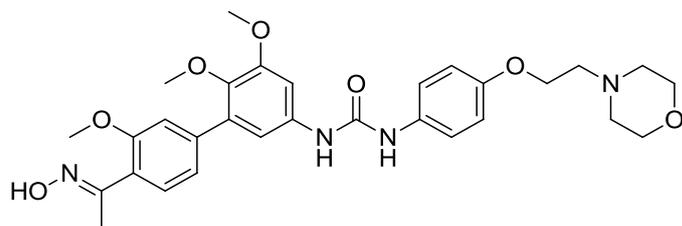
Yield: 90%, white solid, mp: 117~120 °C. LC-MS 99%, 12.07 min (20 minute LC-MS method), ESI-MS (*m/z*): 563 [M+H]⁺. ¹H NMR (400MHz, CDCl₃): δ 7.61 (s, 2H) 7.23 (s, 1H), 7.20 (d, *J*=8.0Hz, 1H), 7.13 (s, 1H), 7.08 (d, *J*=8.0Hz, 2H), 6.87 (d, *J*=8.0Hz, 1H), 6.79 (d, *J*=8.0Hz, 2H), 6.65 (s, 1H), 4.18 (t, *J*=6.0Hz, 2H), 3.78 (s, 3H), 3.74 (s, 3H), 3.52 (s, 3H), 2.78 (t, *J*=6.0Hz, 2H), 2.58 (s, 4H), 2.27 (s, 3H), 1.66 (s, 4H), 1.48 (s, 2H).

T5 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-pyrrolidin-1-ylethoxy)phenyl]urea



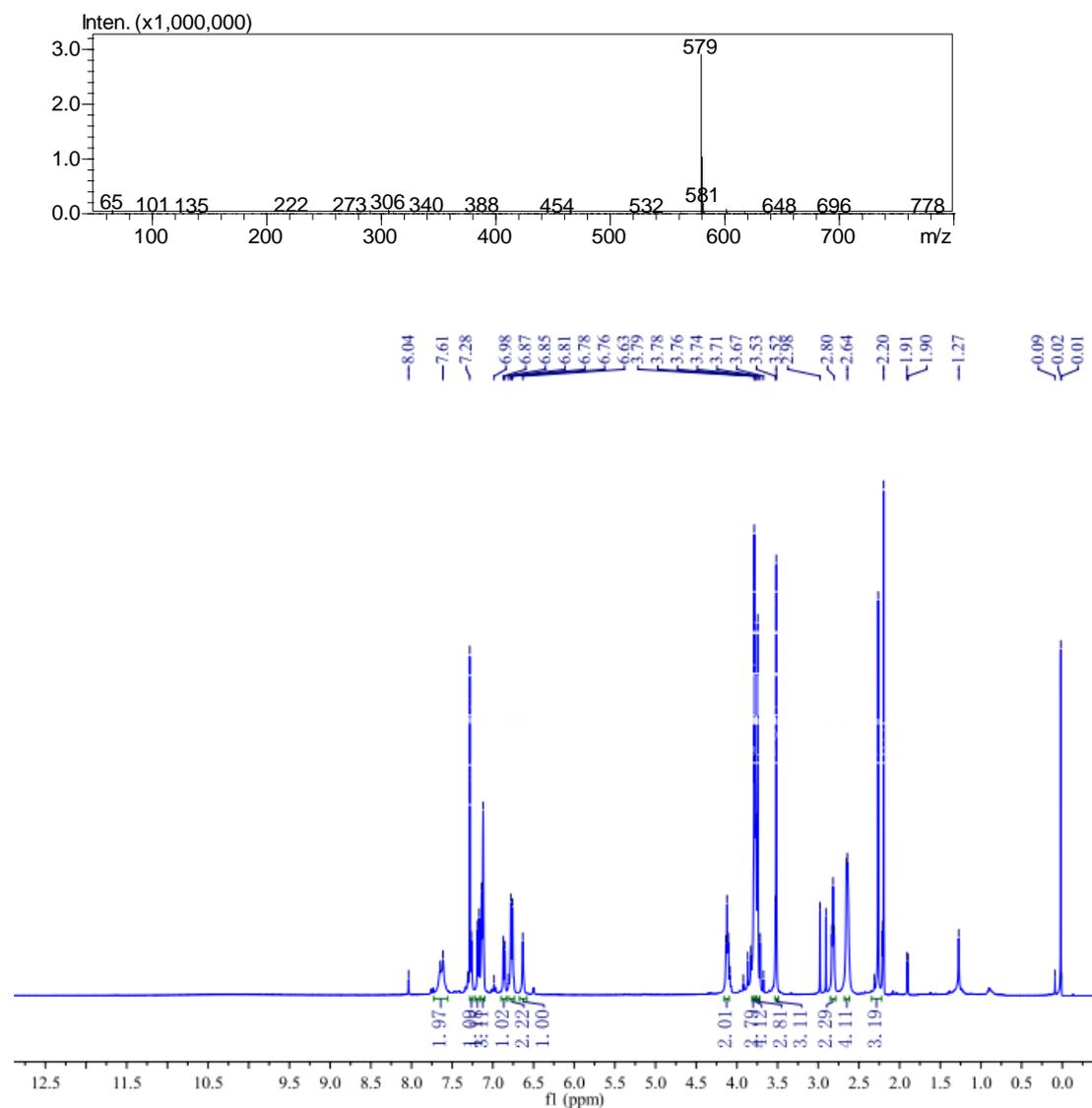
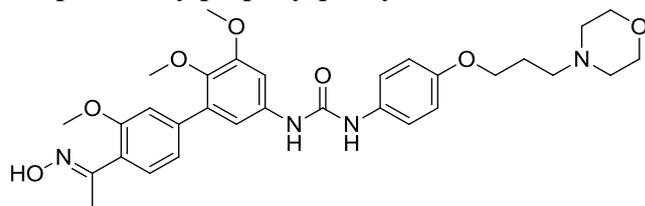
Yield: 85%, slight brown solid, mp: 132~ 135 °C. LC-MS 99%, 8.97 min (20 minute LC-MS method), ESI-MS(*m/z*): 549 [*M*+*H*]⁺. ¹H NMR(400MHz, DMSO-*d*₆): δ 11.06 (s, 1H), 8.66 (s, 1H), 8.53(s, 1H), 7.34 (d, *J*=8.0H, 2H), 7.32 (s, 1H), 7.26 (d, *J*=8.0H, 1H), 7.12 (s, 1H), 7.03 (d, *J*=8.0H, 1H), 6.92 (s, 1H), 6.87 (d, *J*=8.0H, 2H), 4.02 (t, *J*=6.0Hz, 2H), 3.83 (s, 6H), 3.55 (s, 3H), 2.78 (s, 2H), 2.53 (s, 4H), 2.06 (s, 3H), 1.69 (s, 4H).

T6 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(2-morpholin-4-ylethoxy)phenyl]urea



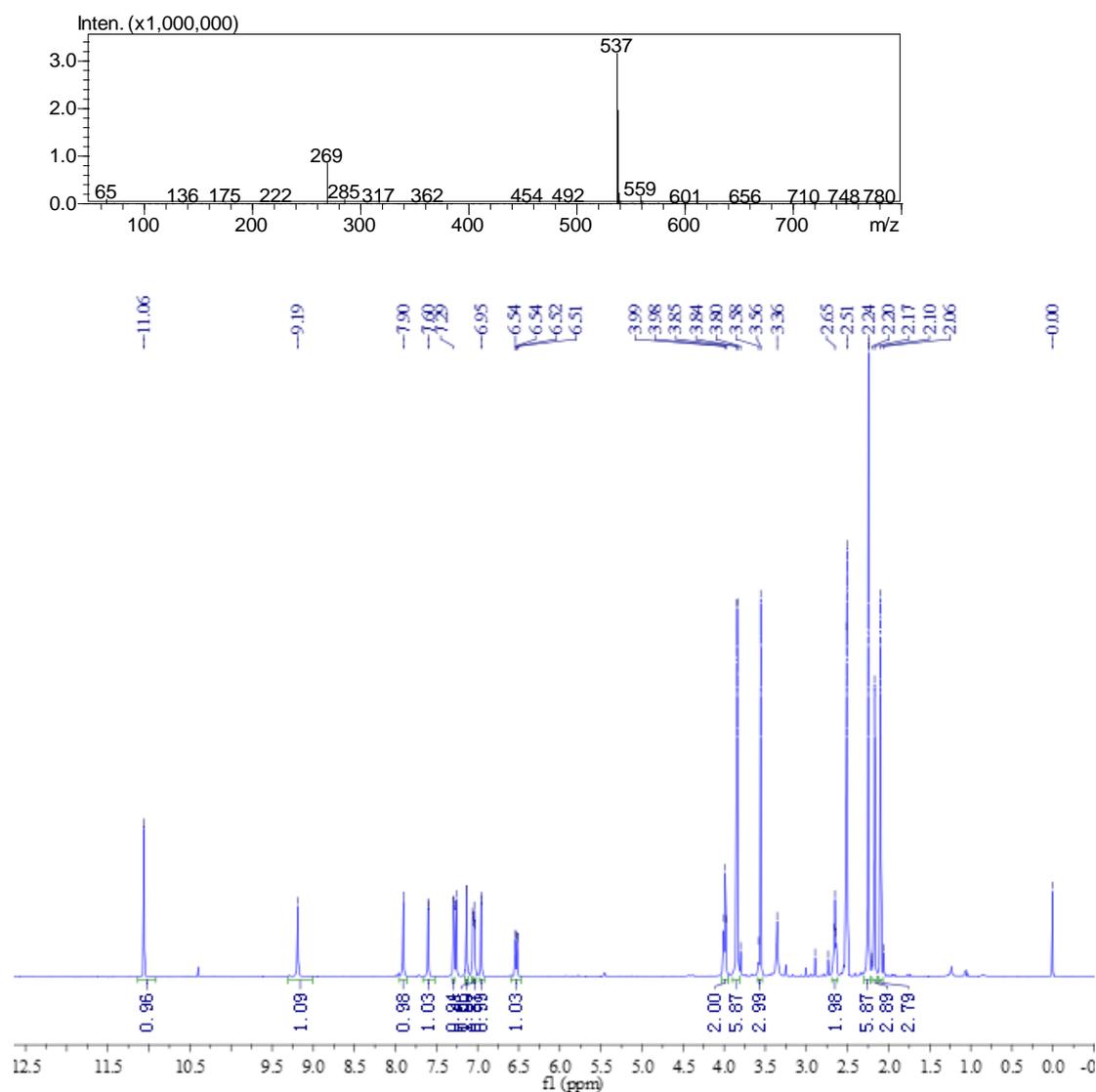
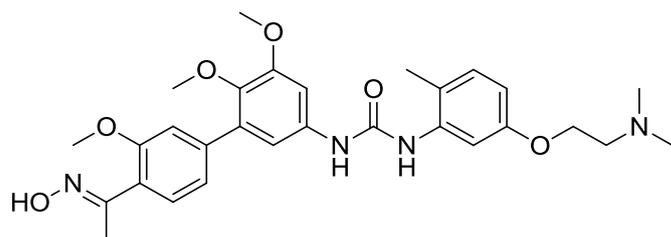
Yield: 88%, slight yellow solid, mp: 165~ 168 °C. LC-MS 97%, 7.48 min (20 minute LC-MS method), ESI-MS (*m/z*): 565 [M+H]⁺. ¹H NMR (400MHz, CDCl₃): δ 7.26 (s, 1H), 7.18 (d, *J*=8.0H, 1H), 7.14 (s, 1H), 7.12 (s, 2H), 6.86 (d, *J*=8.0H, 1H), 6.77 (d, *J*=8.0H, 2H), 6.63 (s, 1H), 4.12 (t, *J*=4.0Hz, 2H), 3.79 (s, 3H), 3.78 (t, *J*=6.0Hz, 4H), 3.74 (s, 3H), 3.52 (s, 3H), 2.82 (t, *J*=4.0Hz, 2H), 2.64 (s, 4H), 2.26 (s, 3H).

T7 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[4-(3-morpholin-4-ylpropoxy)phenyl]urea



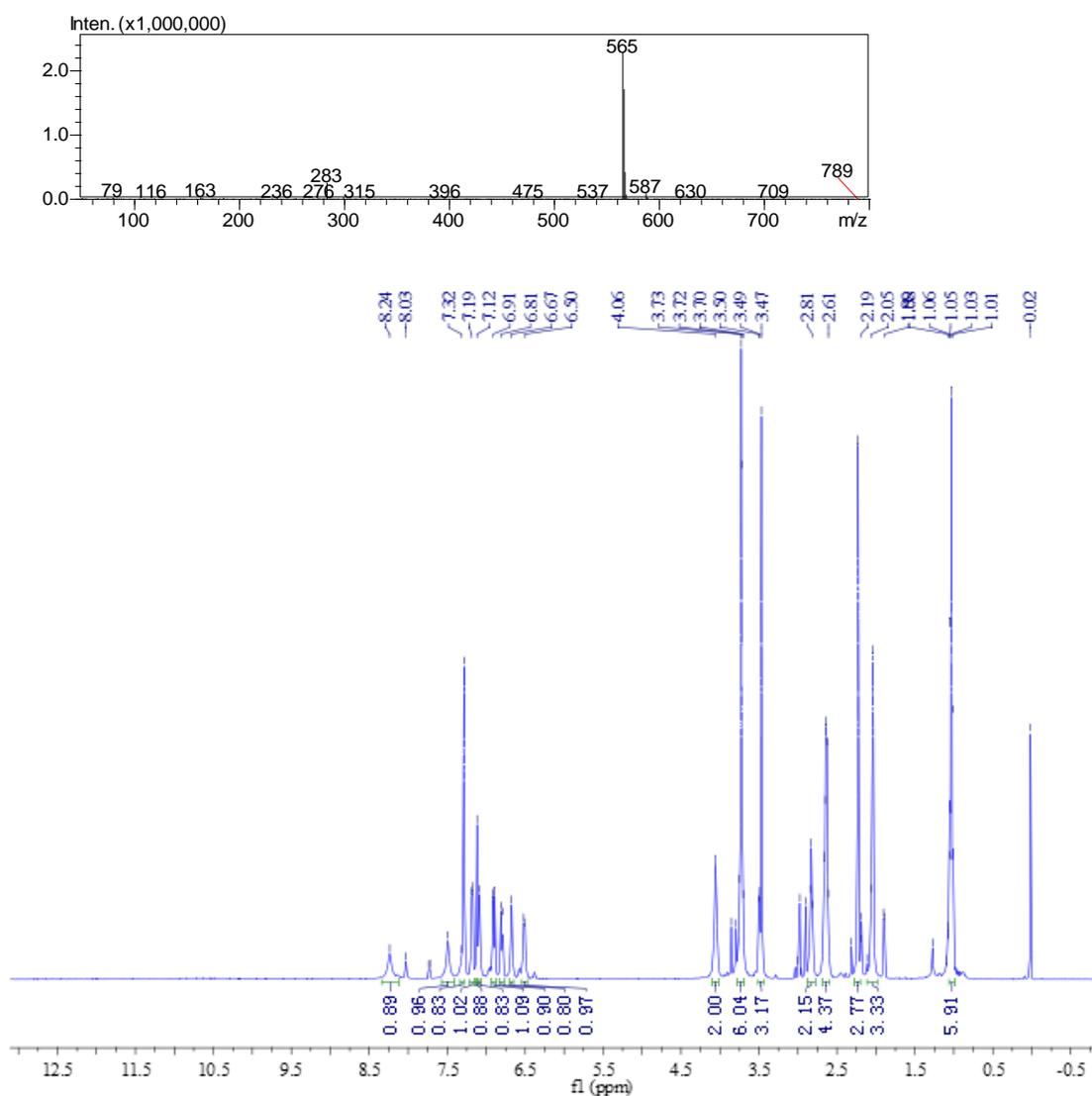
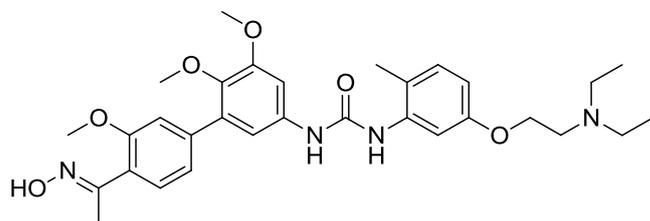
Yield: 93%, white solid, mp: 120~ 123 °C. LC-MS 98%, 12.75 min (20 minute LC-MS method), ESI-MS (*m/z*): 579 [*M*+*H*]⁺. ¹H NMR (400MHz, CDCl₃): δ 7.61 (s, 2H), 7.31 (d, *J*=8.0H, 1H), 7.24 (d, *J*=8.0H, 1H), 7.17 (d, *J*=8.0H, 2H), 7.13 (s, 1H), 6.92 (d, *J*=8.0H, 1H), 6.73 (d, *J*=8.0H, 2H), 6.64 (s, 1H), 3.87 (s, 2H), 3.85 (s, 3H), 3.79 (t, *J*=4.0Hz, 4H), 3.53 (s, 3H), 2.63 (s, 2H), 2.61 (s, 4H), 2.26 (s, 3H), 1.99~2.03 (m, 2H).

T8 *N*-{5-[3-(dimethylamino)propoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



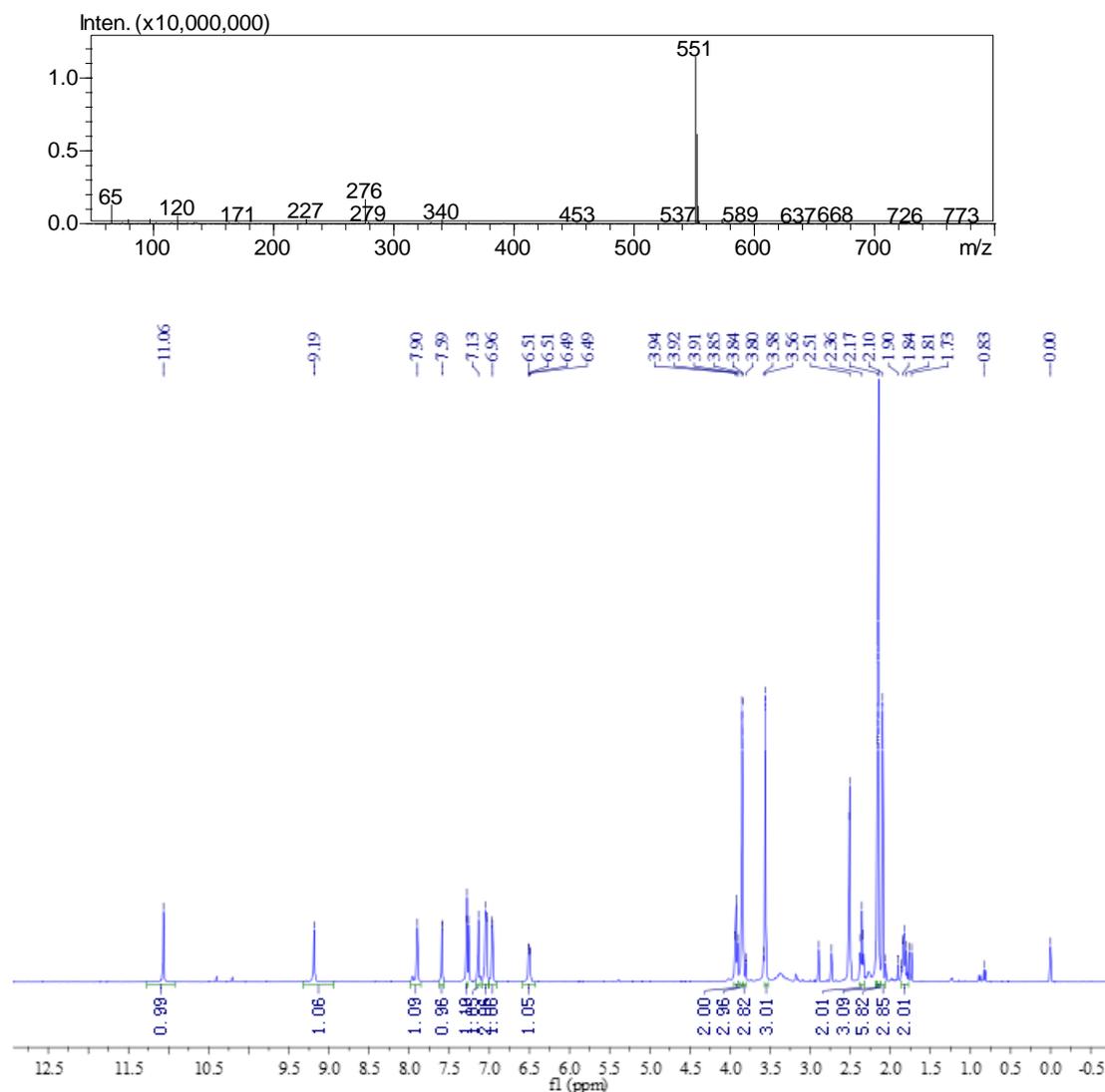
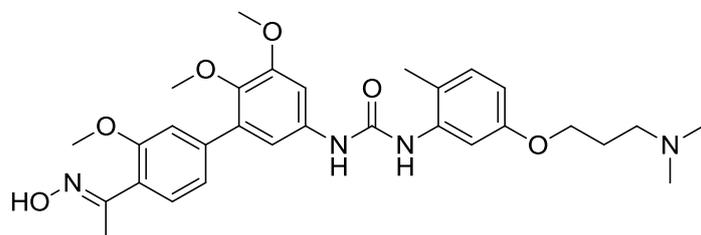
Yield: 93%, yellow solid, mp: 190~ 192 °C. LC-MS 97%, 8.05 min (20 minute LC-MS method), ESI-MS (m/z): 537 $[M+H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 11.06 (s, 1H), 9.19 (s, 1H), 7.90 (s, 1H), 7.60 (s, 1H), 7.29 (s, 1H), 7.14 (s, 1H), 7.06 (d, $J=4.0$ Hz, 1H), 7.04 (d, $J=4.0$ Hz, 1H), 6.95 (d, $J=4.0$ Hz, 1H), 6.53 (d, $J=8.0$ Hz, 1H), 3.99 (t, $J=4.0$ Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.57 (s, 3H), 2.73 (t, $J=4.0$ Hz, 2H), 2.24 (s, 6H), 2.17 (s, 3H), 2.10 (s, 3H).

T9 *N*-{5-[2-(diethylamino)ethoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



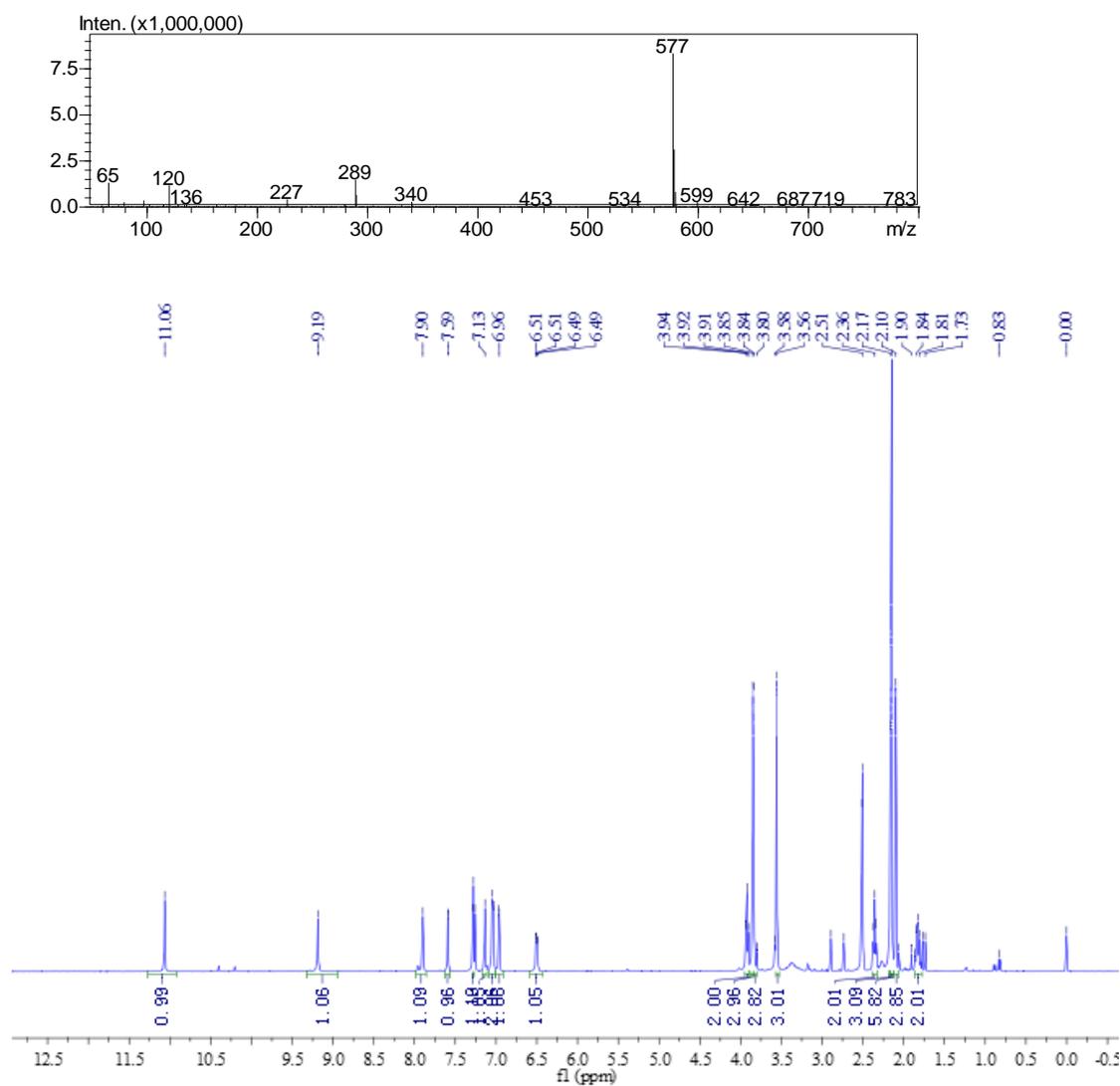
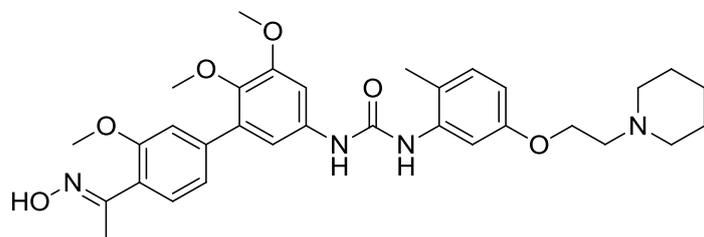
Yield: 95%, white solid, mp: 140~142 °C. LC-MS 98%, 15.02 min (20 minute LC-MS method), ESI-MS (*m/z*): 565 [M+H]⁺. ¹H NMR (400 MHz, CDCl₃): δ 8.24(s, 1H), 7.32 (s, 1H), 7.18 (d, *J*=8.0Hz, 1H), 7.12 (s, 1H), 7.09 (s, 1H), 6.90 (d, *J*=8.0Hz, 1H), 6.80 (d, *J*=8.0Hz, 1H), 6.67 (s, 1H), 6.51 (d, *J*=8.0Hz, 1H), 4.06 (s, 2H), 3.73 (s, 6H), 3.47 (s, 3H), 2.83 (s, 2H), 2.61~2.66 (m, 4H), 2.23 (s, 3H), 1.03 (t, *J*=8.0Hz, 6H).

T10 *N*-{5-[3-(dimethylamino)propoxy]-2-methylphenyl}-*N'*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}urea



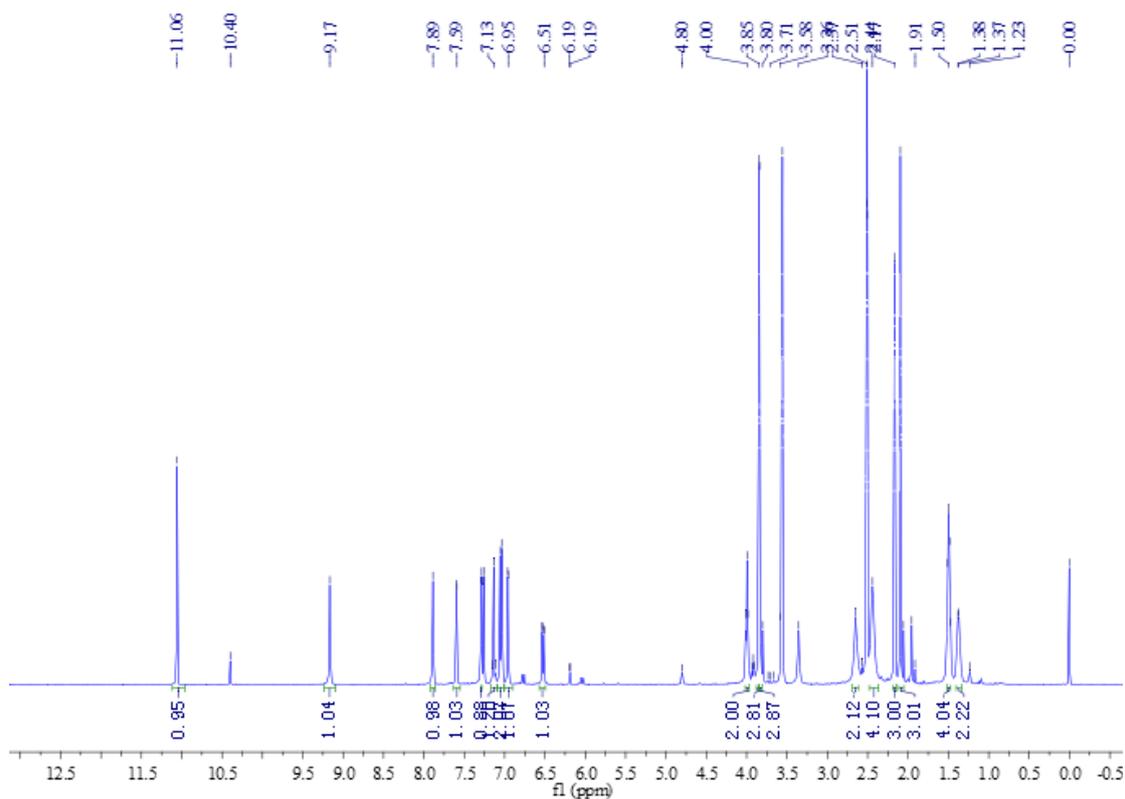
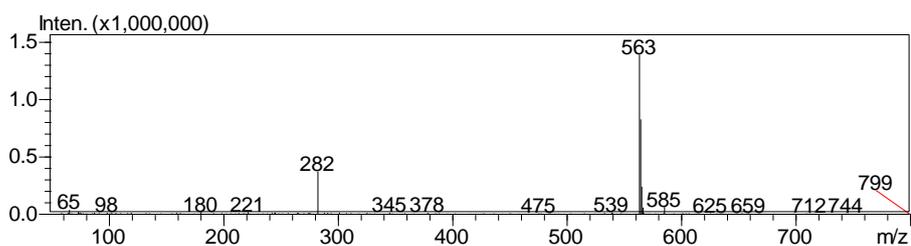
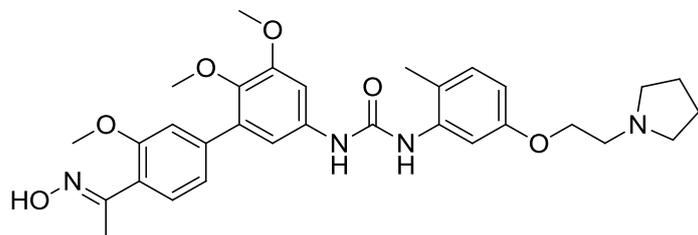
Yield: 92%, grey solid, mp: 150~152 °C. LC-MS 99%, 10.80 min (20 minute LC-MS method), ESI-MS (*m/z*): 551[M+H]⁺. ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.06 (s, 1H), 9.19 (s, 1H), 7.90 (s, 1H), 7.59 (s, 1H), 7.28 (d, *J*=4.0Hz, 1H), 7.13 (s, 1H), 7.04 (d, *J*=4.0Hz, 2H), 6.96 (d, *J*=4.0Hz, 1H), 6.50 (d, *J*=4.0Hz, 1H), 3.92 (t, *J*=6.0Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.56 (s, 3H), 2.36 (s, 2H), 2.17 (s, 3H), 2.15 (s, 6H), 2.10 (s, 3H), 1.81~1.86 (m, 2H).

T11 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(2-piperidin-1-ylethoxy)phenyl]urea



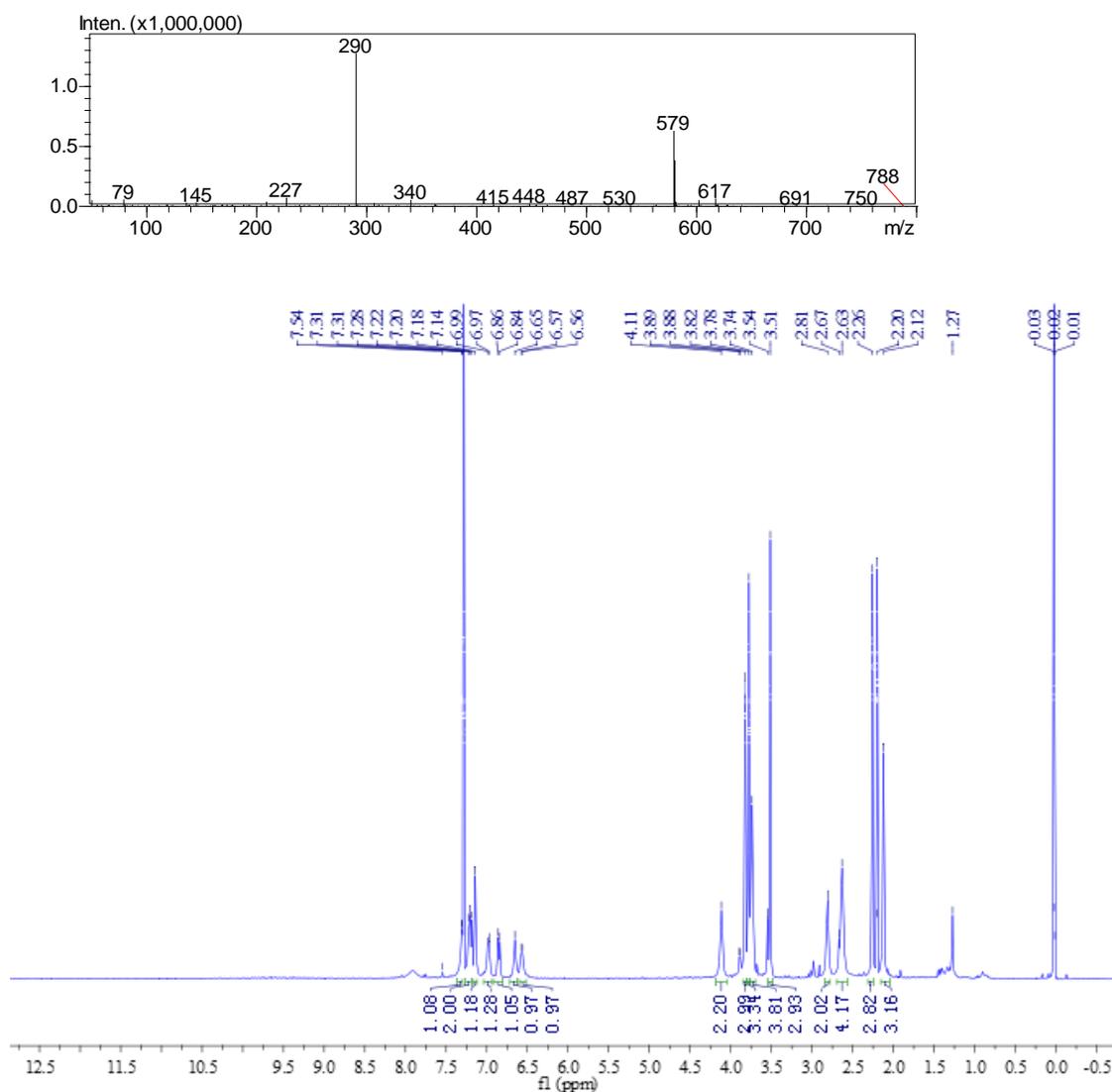
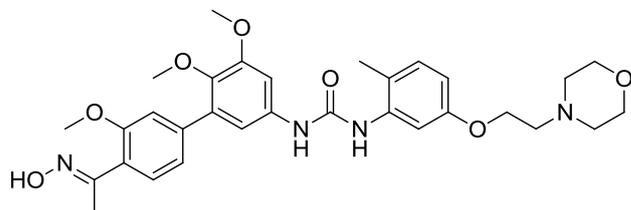
Yield: 92%, slight yellow solid, mp: 195~197 °C. LC-MS 99%, 10.91 min (20 minute LC-MS method), ESI-MS (*m/z*): 577 [*M*+*H*]⁺. ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.06 (s, 1H), 9.19 (s, 1H), 7.89 (s, 1H), 7.59 (d, *J*=4.0Hz, 1H), 7.28 (d, *J*=4.0Hz, 1H), 7.13 (s, 1H), 7.05 (d, *J*=8.0Hz, 2H), 6.96 (d, *J*=4.0Hz, 1H), 6.52 (d, *J*=4.0Hz, 1H), 4.00 (t, *J*=6.0Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.56 (s, 3H), 2.65 (s, 2H), 2.44 (s, 4H), 2.17 (s, 3H), 2.10 (s, 3H), 1.47~1.51 (m, 4H), 1.37~1.38 (m, 2H).

T12 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(2-pyrrolidin-1-ylethoxy)phenyl]urea



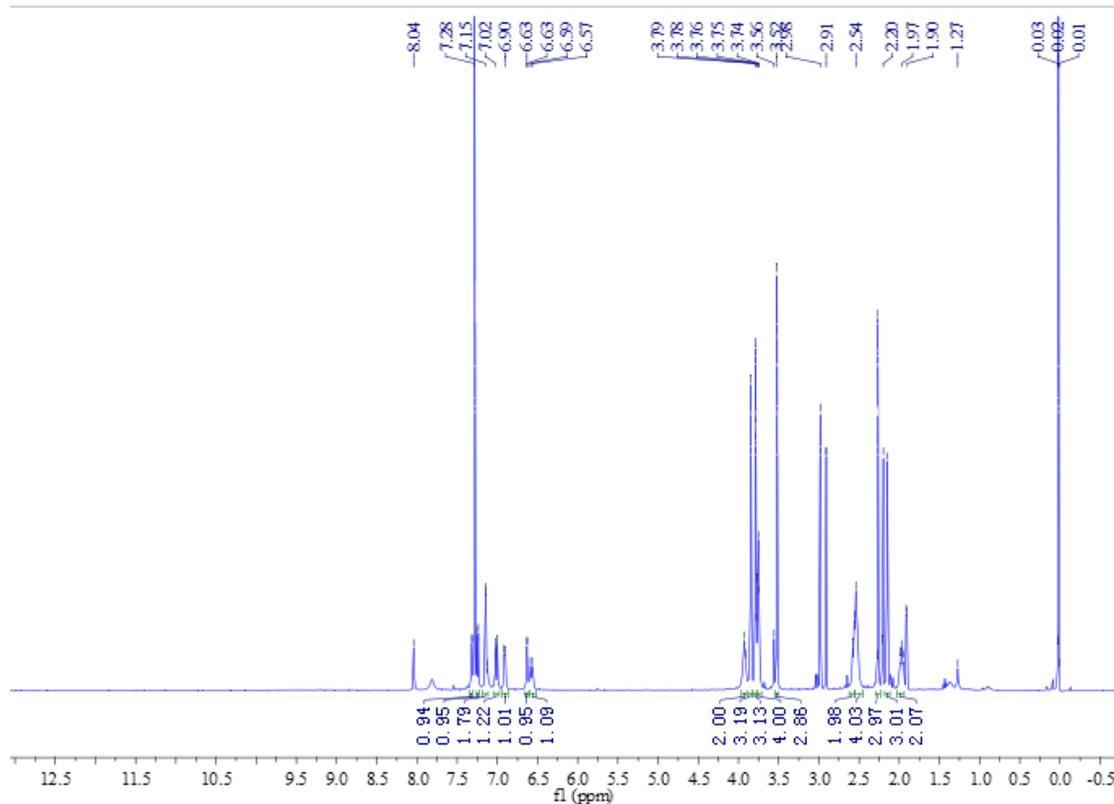
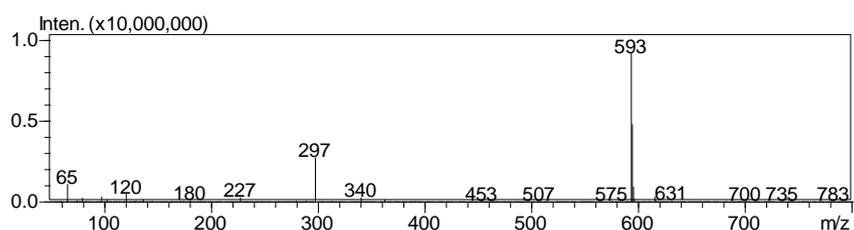
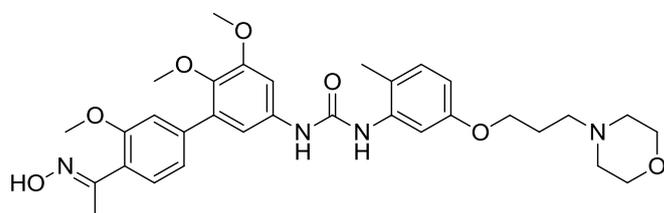
Yield: 89%, slight yellow solid, mp: 172~175 °C. LC-MS 99%, 17.91 min (20 minute LC-MS method), ESI-MS (m/z): 563 $[M+H]^+$. 1H NMR (400 MHz, DMSO- d_6): δ 11.06 (s, 1H), 9.17 (s, 1H), 7.90 (s, 1H), 7.59 (d, $J=2$ Hz, 1H), 7.29 (s, 1H), 7.14 (s, 1H), 7.05 (d, $J=8.0$ Hz, 2H), 6.95 (d, $J=4.0$ Hz, 1H), 6.52 (d, $J=8.0$ Hz, 1H), 4.00 (t, $J=6.0$ Hz, 2H), 3.85 (s, 3H), 3.84 (s, 3H), 3.56 (s, 3H), 2.78 (s, 2H), 2.53 (s, 4H), 2.17 (s, 3H), 2.10 (s, 3H), 1.68 (s, 4H).

T13 *N*-{4'-[(*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(2-morpholin-4-ylethoxy)phenyl]urea



Yield: 84%, white solid, mp: 183~ 186 °C. LC-MS 99%, 6.43 min (20 minute LC-MS method), ESI-MS (*m/z*): 579 [M+H]⁺. ¹H NMR (400MHz, CDCl₃): δ 7.31 (s, 1H), 7.21 (d, *J*=8.0Hz, 1H), 7.18 (s, 1H), 7.14 (s, 1H), 6.98 (d, *J*=8.0Hz, 1H), 6.85 (d, *J*=8.0Hz, 1H), 6.65 (s, 1H), 6.56 (d, *J*=4.0Hz, 1H), 4.11 (s, 2H), 3.89 (s, 3H), 3.78 (s, 3H), 3.74 (s, 4H), 3.51 (s, 3H), 2.81 (t, *J*=4.0Hz, 2H), 2.63 (s, 4H), 2.26 (s, 3H), 2.12 (s, 3H).

T14 *N*-{4'-[(1*E*)-*N*-hydroxyethanimidoyl]-3',5,6-trimethoxybiphenyl-3-yl}-*N'*-[2-methyl-5-(3-morpholin-4-ylpropoxy)phenyl]urea



Yield: 93%, slight green solid, mp: 118~ 120 °C. LC-MS 99%, 7.75 min (20 minute LC-MS method), ESI-MS (m/z): 593[M+H]⁺. ¹H NMR (400MHz, CDCl₃): δ 7.31 (s, 1H), 7.25 (d, J =8.0Hz, 1H), 7.14 (s, 2H), 7.01 (d, J =8.0Hz, 1H), 6.91 (d, J =8.0Hz, 1H), 6.63 (s, 1H), 6.58 (d, J =8.0Hz, 1H), 3.93 (t, J =4.0Hz, 2H), 3.85 (s, 3H), 3.79 (s, 3H), 3.75 (t, J =4.0Hz, 4H), 3.52 (s, 3H), 2.56 (t, J =8.0Hz, 2H), 2.54 (s, 4H), 2.27 (s, 3H), 2.15 (s, 3H) 1.95~1.99 (m, 2H).

(3) The Biological assays procedures of title compounds

3.1 Kinase assay

The ability of compounds to inhibit the phosphorylation of a peptide substrate by KDR kinase was evaluated in a microtiter plate format using homogeneous time-resolved fluorescence (HTRF). Firstly, 2 μL kinase ($K_m=0.003767 \text{ ng}/\mu\text{L}$) and 2 μL substrate ($K_m=121.4 \text{ nM}$) were separately added to a 384-well plate, and variable concentrations of compounds (diluted in kinase buffer) were then added to the assay plate. ATP (2 μL , $K_m=1.332 \mu\text{M}$) 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 615nm (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) $\times 10^4$

3.2 Antiproliferative activity of biphenyl urea derivatives

Growth inhibitory activities were evaluated on the following cell lines: 7901, K562, SY5Y, MDA-MB-231, LOVO, SK-BR-3, A549, 7721, HeLa. 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 μL MTT (5 mg/mL) was added to each well and incubated for 4h at 37 °C. Supernatant was discarded, and 150 μL DMSO was added to each well. Absorbance values were determined by a microplate reader (Bio-Rad Instruments) at 490 nm.