

# **Discovery of S-Nitrosoglutathione Reductase Inhibitors: Potential Agents for the Treatment of Asthma**

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## **Contents:**

- **General Experimental**
- **Biological methods**
- **Synthesis of Selected Compounds and Intermediates**
- **Abbreviations**

**General:**

All reagents and solvents were of commercial quality and used without further purification unless indicated otherwise. <sup>1</sup>H NMR spectra were obtained on either a Bruker or a Varian 400 or 500 MHz spectrometer. Chemical shifts are reported in parts per million relative to TMS as internal standard. Samples provided for accurate mass measurement were taken up in acetonitrile/water (50/50). The solutions were analyzed by the use of electrospray ionization (ESI) on a mass spectrometer. Silica gel chromatography was carried out with a Biotage Isolera Chromatography system or ISCO CombiFlash Companion purification system using prepacked silica gel cartridges. The purity of all compounds tested in the biological assays is >95% as judged by LC-MS and NMR.

**S-Nitrosoglutathione reductase (GSNOR) assay:**

GSNOR expression and purification is described by Sanghani et al in *Biochemistry*, **2000**, 39, 10720-10729. GSNOR fermentation: Pre-cultures were started from a glycerol stock of a GSNOR expressing strain and grown in 2XYT media containing 100 µg/ml ampicillin overnight at 37 °C. Cells were then added to fresh 2XYT (4L) containing ampicillin and grown to an OD ( $A_{600}$ ) of 0.6-0.9 at 37 °C before induction of GSNOR expression with 0.1% arabinose overnight at 20 °C.

GSNOR Purification: *E. coli* cell paste was lysed by nitrogen cavitation and the clarified lysate was purified by Ni affinity chromatography on an AKTA FPLC (Amersham Pharmacia). GSNOR was eluted in 20 mM Tris pH 8.0/250 mM NaCl with a 0-500 mM imidazole gradient. Fractions containing the Smt-GSNOR fusion were digested overnight with Ulp-1 at 4 °C to remove the affinity tag then re-run on the Ni column under the same conditions. GSNOR was

recovered in the flow through fraction and, for crystallography, was further purified by Q-Sepharose and Heparin flow through chromatography in 20 mM Tris pH 8.0, 1 mM DTT, 10  $\mu$ M ZnSO<sub>4</sub>.

**GSNOR assay:** S-nitrosoglutathione (GSNO) and enzyme/NADH solutions were prepared fresh daily. The solutions were filtered and allowed to warm to room temperature. GSNO solution comprised of 100 mM sodium phosphate buffer (pH 7.4) and 0.480 mM GSNO. GSNO solution (396  $\mu$ l) was added to a cuvette followed by addition of 8  $\mu$ L of test compound in DMSO (or DMSO only for full reaction control) and mixed with the pipette tip. Compounds were made up at a stock concentration of 10 mM in 100% DMSO. 2 fold serial dilutions were done in 100% DMSO. Eight microliters of each dilution were added to an assay so that the final concentration of DMSO in the assay was 1%. The concentrations of compounds tested ranged from 100 to 0.003  $\mu$ M. Enzyme/NADH solution was comprised of 100 mM NaPO<sub>4</sub> (pH 7.4), 0.600 mM NADH and 1.0  $\mu$ g/ml GSNO reductase. The Enzyme/NADH solution (396  $\mu$ l) was added to the cuvette to start the reaction. The cuvette was placed in a Cary 3E UV/Visible Spectrophotometer and the change in 340 nm absorbance / min at 25 °C was recorded for 3 minutes. The assays were performed in triplicate for each compound concentration. IC<sub>50</sub>s for each compound were calculated using the standard curve analysis in the Enzyme Kinetics Module of SigmaPlot.

### **Crystallography:**

Crystals of S-nitrosoglutathione reductase (GSNOR) were grown in the presence of NAD<sup>+</sup> by vapor diffusion against a crystallization condition of 19% (w/v) PEG-8000/100 mM potassium phosphate pH 7.0/100  $\mu$ M zinc sulfate/1 mM DTT. These native GSNOR crystals

were soaked in 100  $\mu\text{M}$  GSNOR inhibitors to prepare crystals of GSNOR complexes with GSNOR inhibitors. The crystals were cryo-protected with a solution that contains crystallant solution with 25% ethylene glycol. X-ray diffraction data were collected on the GSNOR candidate complex crystals using synchrotron radiation at the BCDB beamline 5.0.1 at the Advanced Light Source (ALS; Berkeley, CA). The data were reduced with the HKL2000 package (Otwinowski, Z.; Minor, W. *Methods Enzymol.*, **1997**, 276, 307-326). The native structure of GSNOR (PDB ID 1M6H (Sanghani, P. C.; Robinson, H.; Bosron, W.F.; Hurley, T.D. *Biochemistry*, **2002**, 41, 10778-10786) containing  $\text{NAD}^+$ , zinc, potassium, and Tris was refined directly with REFMAC5 (Murshudov, G. N.; Vagin, A.A.; Dodson, E.J. *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, **1997**, 53, 240-255) against the X-ray diffraction data. Structures that showed ligand density were refined in iterative cycles of real space density fitting with COOT (Emsley, P.; Cowtan, K. *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, **2004**, 60, 2126-2132.) and reciprocal space refinement with REFMAC5.

### **Cytotoxicity:**

Selected GSNOR inhibitors were screened for *in vitro* cytotoxicity in the A549 epithelial lung cell line. GSNOR inhibitors were prepared as a 10 mM stock in DMSO and diluted to a starting concentration of 250  $\mu\text{M}$  GSNOR inhibitors in cell culture media (2.5% DMSO final concentration). At concentrations above this, significant toxicity is caused by the DMSO vehicle. Additional 1:2 serial dilutions were performed in cell culture media from 125  $\mu\text{M}$  to 1  $\mu\text{M}$ . Cells were exposed to GSNOR inhibitors for 48 hours prior to analysis of cell viability using an MTS assay (Promega CellTiter 96 AQueous One Solution Cell Proliferation Assay). No cytotoxicity was observed and the  $\text{IC}_{50}$  was  $> 100 \mu\text{M}$  for GSNOR inhibitors tested.

### Off-target panel screening of GSNOR inhibitors:

Cerep (Paris, France) panel screening assays were used to assess potential off-target effects to critical body functions (Cerep catalog #P1). The percent of inhibition of receptor radioligand binding was measured in the presence of 10  $\mu$ M of test compound. The majority of the 54 assays in the ExpressProfile panel are human recombinant receptors (see table below). A typical protocol for binding assays included a minimum of 6-control wells (non-specific and total binding) with or without vehicle for solvent-soluble compounds. Inhibition of 50% or greater was considered a positive response.

A1	CB1	H2	NTS1	5-HT5A	Cl channel (GABA)
A2a	CCK1	MC4	Dop Delta2	5-HT6	norepi channel
A3	D1	MT1	Kappa (KOP)	5-HT7	dopamine channel
Alpha 1	D2S	M1	Mu (MOP)	sst	5-HT transporter
Alpha 2	Eta	M2	NOP	VPAC1	
Beta1	GABA	M3	TP	V1a	
Beta2	GAL2	NK2	5-HT1A	ca+2 channel (L)	
AT1	CXCR2 (IL-8B)	NK3	5-HT1b	Kv channel	
BZD	CCR1	Y1	5-HT2A	Skca channel	
B2	H1	Y2	5-HT3	sodium channel	

**5-Day exploratory toxicity study in mice with GSNOR inhibitor IV:** Male CD-1 mice (6/group) were dosed intravenously (IV) with either vehicle (5% 2-hydroxypropyl-beta cyclodextrin in PBS) or GSNOR inhibitor (5, 30, or 60 mg/kg) on study days 0-4. Clinical observations of general physical condition and body weights of the animals were recorded daily. Additionally, animals were observed for evidence of penile erection following test article administration. On study day 5, each animal was bled for serum chemistry and hematology parameters and for PT/PTT analysis. A gross necropsy was then performed, and select organs

were collected, weighed, and placed in 10% NBF. Descriptive pathology observations for livers and adrenals were also recorded.

### **5-Day exploratory toxicity study in mice with GSNOR inhibitor PO:**

Male CD-1 mice (6/group) were dosed orally with 1% carboxymethyl cellulose (CMC) as vehicle or GSNOR inhibitor (20, 100, or 500 mg/kg) daily on study days 0-4. Clinical observations of general physical condition and body weights of the animals were recorded daily. Additionally, animals were observed for evidence of penile erection following test article administration. On study day 5, each animal was bled for serum chemistry and hematology parameters, and for PT/PTT analysis. A gross necropsy was then performed, and select organs were collected, weighed, and placed in 10% NBF. Descriptive pathology observations for livers and adrenals were also recorded.

### **Efficacy of GSNOR inhibitors in experimental animal models:**

#### Mouse model of ovalbumin (OVA) induced asthma:

OVA was complexed with alum as adjuvant and administered to 8 week old female Balb/c mice (N = 10 per group). Mice were systemically sensitized to OVA on day 1 via intraperitoneal injection of 100 µg OVA. Mice were then subjected to four pulmonary challenges with OVA on days 9, 16, 19, and 22 using doses of 250 µg on day 9 and 125 µg on all other days. GSNOR inhibitors were administered orally or intravenously at 0.0005 to 30 mg/kg at 30 minutes to up to 72 hours prior to assessment. Mice were dosed with oral or IV vehicle in a similar manner to the GSNOR inhibitors as controls. Combivent (Boehringer Ingelheim), a combination of the  $\beta$ 2-adrenergic agonist, albuterol, and the cholinergic antagonist, ipratropium

bromide, was used as the positive control for the model. Combivent was administered via inhalation at a dose of 0.9 mg/kg ipratropium bromide and 5 mg/kg albuterol at 48 hours, 24 hours, and again at 1 hour prior to assessment.

Airway responsiveness was measured under baseline conditions, saline aerosol, and 5, 20, and 50 mg/ml methacholine (MCh) aerosol challenges using whole body plethysmography (Buxco). Enhanced pause (Penh) was used as a descriptor of bronchoconstriction/airway hyper-responsiveness (AHR). Bronchoalveolar lavage was performed for assessment of the percent eosinophils in the bronchoalveolar lavage fluid (BALF) via light microscopy as a descriptor of pulmonary inflammation. Other white blood cell populations were also assessed. Statistical differences among groups were determined and considered to be significant at  $p < 0.05$ .

#### Mouse model of elastase induced chronic obstructive pulmonary disease (COPD):

COPD was induced in 8 week old female C57Bl/6 mice (N = 30 per group) by intratracheal administration of 80 µg porcine pancreatic elastase (PPE) and 20 U of papain in 0.1% NaCl for 8 days starting on study day 0 and continuing through study day 7. GSNOR inhibitors were administered orally at doses of 0.1, 1, or 10 mg/kg/day for either 7 days (study days 7 through 13) or 14 days (study days 7 through 20). Control groups of mice were treated in the same manner with either oral vehicle or, as a positive control, a combination of 220 µg/mouse/day inhaled Flovent (fluticasone, GlaxoSmithKline) and 50 mg/kg/day oral SCH CXCR2/R1 antagonist (inhibitor to neutrophil and monocyte recruitment, Schering-Plough). Pulmonary function and inflammation were assessed on study days 14 or 21 in a cohort of 10 mice per group at each time point. The third cohort of 10 mice per group was used for assessment of emphysema/airspace enlargement on study day 21.

Pulmonary function was assessed by measuring airway hyper-responsiveness (AHR) in the mice under baseline conditions, saline aerosol, and 5, 20, and 50 mg/ml methacholine aerosol challenges using whole body plethysmography (Buxco). The parameter enhanced pause (Penh) was calculated and used as a descriptor of bronchoconstriction/AHR. After the aerosol challenges, the animals were euthanized and bronchoalveolar lavage (BAL) was performed for assessment of the numbers and percents of white blood cells infiltrating the lung fluid (BALF) as measures of pulmonary inflammation. Emphysema was quantitated via morphometric assessment of the mean linear intercept (Lm) and average equivalent diameter (D2), a marker of early emphysema, in both lungs using light microscopy. Mice were weighed and observed clinically while on study to assess any gross adverse effects. Statistical differences among groups were determined and considered to be significant at  $p < 0.05$ .

#### Mouse model of dextran sodium sulfate (DSS) induced IBD:

Experimental IBD was induced by administration of DSS in the drinking water of male C57Bl/6 mice (N = 8 to 12 mice per group) for six consecutive days. In the acute model, GSNOR inhibitors were dosed orally at 1 or 10 mg/kg/day for 10 days starting two days prior to and continuing two days post DSS exposure. In the chronic IBD model, GSNOR inhibitors were dosed at 10 mg/kg for 14 days starting one day after DSS exposure. Efficacy was assessed in a blinded fashion via endoscopy and histopathology using a five point scale ranging from a score = 0 (normal tissue) through a score = 4 (ulcerative tissue damage and marked pathological changes). Levels of circulating cytokines involved in inflammatory pathways associated with IBD were also assessed. The effect of GSNOR inhibitors was compared to vehicle treated controls. The corticosteroid, prednisolone, was used as the positive control in this study and was

administered orally at 3 mg/kg/day for 10 or 14 days. Naïve mice (N = 5) were also assessed as a normal tissue control.

### **Mouse pharmacokinetic (PK) study:**

**Experimental model:** The mouse was used to determine the pharmacokinetics of compounds. This species is widely used to assess the bioavailability of compounds by administering both PO and IV test articles. Efficacy of the compounds was compared by assessing plasma exposure in male BALB/c mice either via IV or PO administration at the times of peak activity.

#### **Materials and methods:**

**IV administration of compounds:** The compounds were reconstituted in a phosphate buffered saline (PBS)/10% Solutol (HS 15) clear solution resulting in a concentration of 0.2 mg/ml and administered to mice (2 mg/kg) as a single IV dose. Animals were dosed via the lateral tail vein. Blood samples were collected at designated time points (0.083, 0.25, 0.5, 1, 2, 4, 8, 16, 24 hours) by cardiac puncture under isoflurane anesthesia (up to 1 ml blood per animal). The blood was collected into tubes containing Li-Heparin. The blood samples were kept on ice until centrifugation within approximately 30 minutes of collection. The plasma was transferred into labeled polypropylene tubes and frozen at -70 °C until analyzed by LC/MS/MS.

**PO administration of compounds:** The compounds were reconstituted in 40% Propylene Glycol/40% Propylene Carbonate/20% of a 5% Sucrose clear solution resulting in a concentration of 2 mg/ml and administered to mice (10 mg/kg) as a single oral dose via gavage. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, 16, 20 and 24 hours post dose by cardiac puncture under isoflurane anesthesia. The blood was collected in tubes containing Li-Heparin. The blood samples were kept on ice until centrifugation within approximately 30 minutes of

collection. The plasma was transferred into labeled polypropylene tubes and frozen at  $-70\text{ }^{\circ}\text{C}$  until analyzed by LC/MS/MS.

**LC/MS/MS Analysis:** Plasma samples at each time point were analyzed using a LC-MS/MS with a lower limit of quantification (LLOQ) of 1 ng/ml. Plasma was analyzed to determine the amount of the compound in each sample and regression curves were generated for each compound in the relevant matrixes.

WinNonlin analysis was used for calculating PK parameters for both the IV and PO administrations:

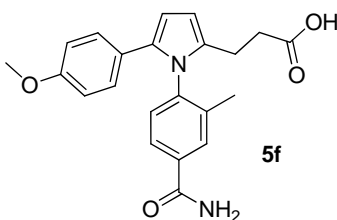
PK parameters for IV portion -  $\text{AUC}_{\text{last}}$ ;  $\text{AUC}_{\text{INF}}$ ;  $\text{T}_{1/2}$ ;  $\text{Cl}$ ;  $\text{V}_{\text{SS}}$ ;  $\text{C}_{\text{max}}$ ;  $\text{MRT}$

PK parameters for PO portion -  $\text{AUC}_{\text{last}}$ ;  $\text{AUC}_{\text{INF}}$ ;  $\text{T}_{1/2}$ ;  $\text{C}_{\text{max}}$ ;  $\text{Cl}$ ,  $\text{MRT}$ .

In addition to the above PK parameters, bioavailability (%F) was calculated.

### Synthetic procedures:

#### Representative procedures for Scheme 1: Synthesis of 3-[1-(4-Carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl]-propanoic acid (5f)



**Synthesis of (E)-3-Furan-2-yl-1-(4-methoxy-phenyl)-propenone:** A solution of 2-furaldehyde (5.85 g, 60.92 mmol) was added to a methanol solution (120 ml) of 4-methoxyacetophenone (8.5 g, 56.6 mmol), followed by the addition of sodium methoxide (3.1 g, 56.6 mmol). The reaction mixture was stirred at room temperature for 16 hours, followed by removal

of the solvent *in vacuo*. The resultant mixture was diluted with water (130 ml) and extracted with ethyl acetate (350 ml). The aqueous layer was re-extracted with ethyl acetate (100 ml). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* to obtain the product (E)-3-Furan-2-yl-1-(4-methoxy-phenyl)-propenone as an orange solid (12.6 g, 97%).

**Synthesis of 1-(4-Methoxy-phenyl)-decane-1,4,7-trione:** Conc. HCl (59 ml) was added to a solution of (E)-3-Furan-2-yl-1-(4-methoxy-phenyl)-propenone (12.6 g, 55.2 mmol) in ethanol (237 ml). The reaction mixture was heated under reflux for 16 hours, concentrated, and diluted with dichloromethane (250 ml), and the resultant organic layer was washed with water (25 ml). After phase separation, the organic layer was dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* to obtain the crude mixture, which was purified by silica gel flash chromatography to obtain 1-(4-methoxy-phenyl)-decane-1,4,7-trione (6.89 g, 43%).

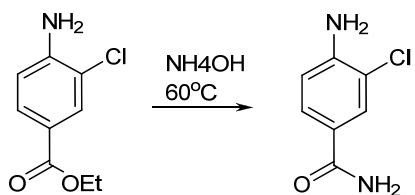
**Synthesis of 3-[1-(4-Carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl] propanoic acid ethyl ester:** 4-amino-3-methylbenzamide (180 mg, 1.2 mmol) was added to a solution 1-(4-methoxy-phenyl)-decane-1,4,7-trione (350 mg, 1.2 mmol) in ethanol (6 ml), followed by the addition of p-toluenesulfonic acid monohydrate (23 mg, 0.12 mmol). The reaction mixture was heated at reflux for 16 hours, and the solvent was removed *in vacuo* to obtain a crude product which upon purification by silica gel flash chromatography, gave 3-[1-(4-carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl] propanoic acid ethyl ester (147 mg, 30%).

**Synthesis of 3-[1-(4-Carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl]-propanoic acid:** 3-[1-(4-carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl] propanoic acid ethyl ester (86 mg, 0.216 mmol) was dissolved in ethanol (4 ml). Water

(0.5 ml) was added to the ethanolic solution followed by the addition of 1N NaOH (0.51 ml, 0.51 mmol). The reaction mixture was stirred at room temperature for 1 hour and then at 45 °C for an additional hour. After removal of the solvent *in vacuo*, the residue was diluted with water (6 ml) and extracted with ethyl acetate (2 x 6 ml). The pH of the aqueous layer was adjusted to 2 with 1N HCl and then extracted with ethyl acetate (6 ml). The combined organic layer was dried over anhydrous sodium sulfate and the solvent removed *in vacuo* to obtain 3-[1-(4-Carbamoyl-2-methyl-phenyl)-5-(4-methoxy-phenyl)-1H-pyrrol-2-yl]-propanoic acid **5f** (68 mg, 85%). <sup>1</sup>H NMR (DMSO, 400 MHz): δ 12.10 (s, 1H), 8.02 (s, 1H), 7.82 (d, J = 8 Hz, 1H), 7.78 (s, 1H), 7.48 (d, J = 8 Hz, 1H), 7.43 (s, 1H), 6.96 (d, J = 8 Hz, 2H), 6.72 (d, J = 8.5 Hz, 2H), 5.25 (d, J = 3.5 Hz, 1H), 5.06 (d, J = 3 Hz, 1H), 3.66 (s, 3H), 2.50 (m, 2H), 2.40 (m, 2H), 1.74 (s, 3H). MS (ESI): m/z 379.1 [M+H]<sup>+</sup>.

The synthesis of compounds **5a-5e** and **5g-5j** followed the same procedures as described above with commercially available starting materials except the following:

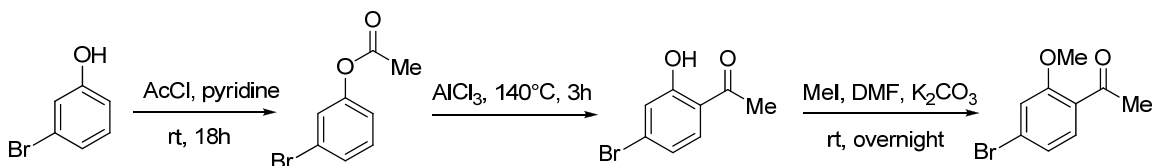
#### Synthesis of 4-Amino-3-chloro-benzamide:



A 18 ml vial was loaded with 4-amino-3-chloro-benzoic acid ethyl ester (350 mg, 1.88 mmol) and 5 ml of 30% NH<sub>4</sub>OH. The vial was sealed and the mixture was heated at 50 °C for 3 days. The mixture was cooled down and concentrated to half. The suspension was filtered and the solid was washed with water and dried to give 200 mg product in 62.3% yield.

The synthesis of compound **17** followed the similar procedures as described above only the starting materials were not commercial and were prepared as below:

### Synthesis of 1-(4-bromo-2-methoxyphenyl)ethanone:

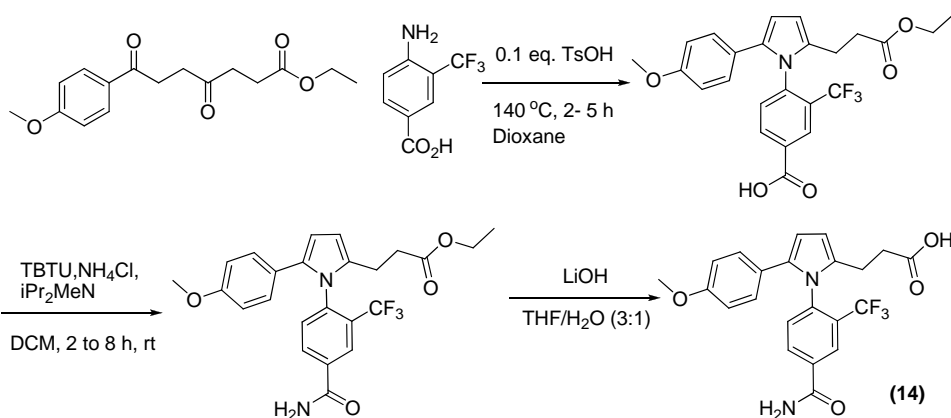


To a stirred suspension of 3-bromophenol (50 g, 0.29 mol) in pyridine (200 ml) and dichloromethane (100 ml) added was dropwise acetyl chloride (25 ml, 0.35 mol) at 0 °C and the mixture was stirred 18 hours at room temperature. LC-MS showed that the reaction was complete. Pyridine and dichloromethane was evaporated *in vacuo*. Water (600 ml) was added and the mixture was acidified with hydrochloric acid at pH 2. The reaction mixture was extracted with ethyl acetate (500 ml×3 ) and the organic phase was dried over anhydrous sodium sulfate, filtrated, concentrated and purified by column chromatography (PE/EA = 60:1) to afford compound 3-bromophenyl acetate as a colorless liquid (46 g, 74%).

A stirring suspension of 3-bromophenyl acetate (46 g, 0.21 mol) and anhydrous aluminum chloride power (57 g, 0.42 mol) was heated to 160 °C for 3 hours. The reaction mixture was cooled to room temperature and ice (200 g) and water (800 ml) was poured and acidified with hydrochloric acid at pH 7. The reaction was extracted with ethyl acetate (500 ml ×3) and the organic phase was washed with saturated sodium bicarbonate, dried over anhydrous sodium sulfate, filtrated, concentrated and purified by column chromatography (PE/EA = 60:1) to afford compound 1-(4-bromo-2-hydroxyphenyl)ethanone as a light green solid (35.1 g, 76 %).

To a suspensions of compound 1-(4-bromo-2-hydroxyphenyl)ethanone (25 g, 0.12 mol) and potassium carbonate (24 g, 0.18 mol) in anhydrous DMF ( 20 ml ) added was iodomethane (22.6 ml, 0.23 mol ) and the reaction mixture was stirred at room temperature overnight. LC-MS showed that the reaction was complete. Then water (300 ml) was poured and the mixture was extracted with ethyl acetate and the organic phase was washed with saturated sodium chloride, dried over anhydrous sodium sulfate, filtrated, concentrated to afford compound 1-(4-bromo-2-methoxyphenyl)ethanone as a colorless solid (26.1 g, 98 %).

**Synthesis of 3-(1-(4-carbamoyl-2-(trifluoromethyl)phenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid (14):**



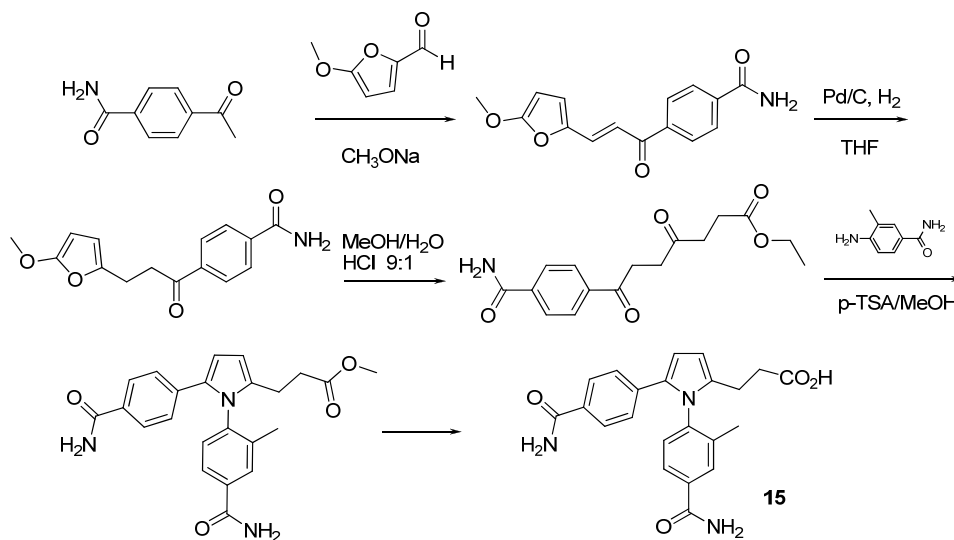
**4-(2-(3-Ethoxy-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-(trifluoromethyl)benzoic acid:** 4-amino-3-(trifluoromethyl)benzoic acid (69 mg) was mixed with ethyl 7-(4-methoxyphenyl)-4,7-dioxoheptanoate and p-toluenesulfonic acid (11mg) in dioxane (2 ml). The mixture was heated at 140 °C via microwave over 4 hours. The resultant solution was diluted with EtOAc (20 ml) and washed with 0.5 N HCl (8 ml), brine (8 ml) and dried over anhydrous sodium sulfate. After removal of solvents under reduced pressure, the residue was purified by flash silica gel column chromatography, eluted with 10% MeOH in

DCM, to afford 4-(2-(3-ethoxy-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-(trifluoromethyl)benzoic acid (111 mg, 89%) as oil.

**Ethyl 3-(1-(4-carbamoyl-2-(trifluoromethyl)phenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoate:** 4-(2-(3-Ethoxy-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-(trifluoromethyl)benzoic acid (50 mg) was treated with TBTU (50 mg), DPPA (10  $\mu$ L) and ammonium chloride (50 mg) in DCM (4 ml) over night at room temperature. The reaction mixture was diluted with EtOAc (10 ml), washed with 0.5 N HCl (8 ml), saturated NaHCO<sub>3</sub> (8 ml), and brine (8 ml). After removal of solvents, the residue was purified by flash silica gel chromatography to obtain ethyl 3-(1-(4-carbamoyl-2-(trifluoromethyl)phenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoate (40 mg, 80%) as a colorless solid.

**3-(1-(4-Carbamoyl-2-(trifluoromethyl)phenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid (14):** Ethyl 3-(1-(4-carbamoyl-2-(trifluoromethyl)phenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoate (40 mg) was treated with LiOH/H<sub>2</sub>O (40 mg) in THF (3 ml) and H<sub>2</sub>O (1.5 ml) over 12 hours at room temperature. The basic solution was acidified with 1N HCl and the aqueous layer was extracted with EtOAc (15 ml). After removal of the solvents under reduced pressure, it afforded the desired product **14** (32 mg, 80%) as colorless solids. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  8.26 (s, 1H), 8.25 (d, 1H), 7.66 (d, 1H), 6.95 (d, 2H), 6.70 (d, 1H), 6.25 (d, 1H), 6.10 (d, 1H), 3.7 (s, 3H), 2.55 (m, 4H). MS (ESI): m/z 433 [M+H]<sup>+</sup>.

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-carbamoylphenyl)-1H-pyrrol-2-yl)propanoic acid (15):**



**Synthesis of 4-[(E)-3-(5-methoxy-furan-2-yl)-acryloyl]-benzamide:** Sodium methoxide (in portions) was added, over a time interval of about three minutes, to 4-acetylbenzamide (1.38 g, 8.5 mmol) and 5-methoxy-furan-2-carbaldehyde (1.31g, 10.6 mmol) in a 1:1 mixture of methanol and DMF (16 ml). The resulting solution was placed under nitrogen and stirred overnight at 45 °C. The reaction mixture was then evaporated to dryness under vacuum, followed by the addition of water and brine (100 ml) to the resulting residue. The aqueous solution was extracted with ethyl acetate (125 ml x 3) and the combined organic layers were washed with brine (100 ml) and dried over MgSO<sub>4</sub>. The crude reaction mixture of 4-[(E)-3-(5-methoxy-furan-2-yl)-acryloyl]-benzamide and unreacted 4-acetyl acetamide was used without purification.

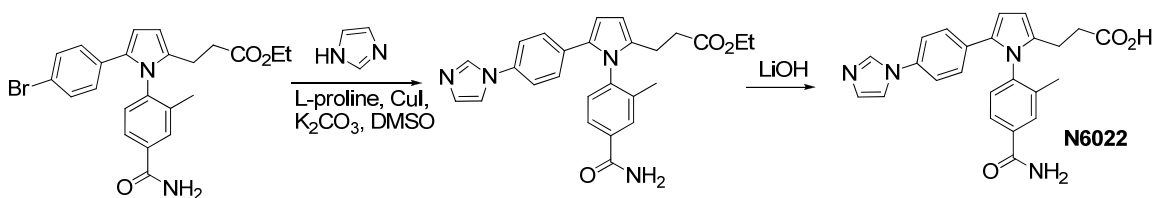
**Synthesis of 4-[3-(5-methoxy-furan-2-yl)propionyl]-benzamide:** 10% Pd/C (60 mg) was added to crude 4-[(E)-3-(5-methoxy-furan-2-yl)-acryloyl]-benzamide (180 mg) in THF (4 ml). The mixture was subjected to hydrogenation at 45 psi for 4 hours. After removal of the catalyst by filtration through Celite, the mixture was concentrated and carried forward without any purification.

**Synthesis of 7-(4-carbamoyl-phenyl)-4,7-dioxo-heptanoic acid:** Crude 4-[3-(5-methoxy-furan-2-yl-propionyl)-benzamide (175 mg) was dissolved in 10% aqueous methanol (3 ml) to which was added 0.2 N HCl (0.2 ml) to obtain a solution having a final pH = 3. The resulting solution was stirred for 30 minutes, evaporated to dryness and subjected to chromatographic purification using dichloromethane as the solvent. 53 mg of final product with 67% purity was obtained.

**Synthesis of 3-[1-carbamoyl-2-methyl-phenyl]-5-(4-carbamoyl-phenyl)-1H-pyrrole-2-yl]-propanoic acid methyl ester:** 4-amino-3-methyl benzamide (20 mg, 0.13 mmol) was added to 7-(4-carbamoyl-phenyl)-4,7-dioxo-heptanoic acid ethyl ester mixture (0.11 mmol) in EtOH, followed by the addition of p-toluenesulfonic acid (2 mg, 0.01 mmol). The resultant mixture was heated at 80 °C for 12 hours. The residue of the crude reaction mixture was dissolved in dichloromethane and then purified by silica-gel chromatography using 5% methanol in chloroform as the solvent system to obtain 5 mg of methyl 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-carbamoylphenyl)-1H-pyrrol-2-yl)propanoate.

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-carbamoylphenyl)-1H-pyrrol-2-yl)propanoic acid:** Methyl 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-carbamoylphenyl)-1H-pyrrol-2-yl)propanoate was hydrolyzed using the procedure described above in the final step of Scheme 2 (yield 4.8 mg). <sup>1</sup>H NMR (DMSO, 400 MHz): δ 8.05 (s, 1H), 7.84 (d, J = 8 Hz, 1H), 7.80 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.53 (d, J = 8 Hz, 1H), 7.46 (s, 1H), 7.22 (s, 1H), 7.07 (d, J = 8.5 Hz, 2H), 6.53 (d, J = 3.6 Hz, 1H), 6.15 (d, J = 3.6 Hz, 1H), 3.66 (s, 3H), 2.50 (m, 2H), 2.41 (m, 2H), 1.74 (s, 3H). MS (ESI): m/z 392.1 [M+H]<sup>+</sup>.

**Synthesis of 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoic acid (N6022) :**



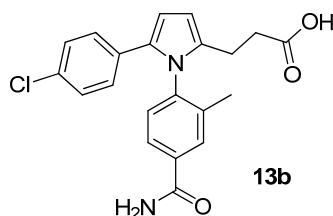
**Ethyl 3-(5-(4-bromophenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate** was prepared followed the similar procedure described for the synthesis of compound **5f**.

**Synthesis of ethyl 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate:** To a mixture of Ethyl 3-(5-(4-bromophenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (4.54 g, 10 mmol) and imidazole (2.04 g, 30 mmol) in DMSO (50 ml) added was L-proline (0.345 g, 3 mmol), CuI (1.14 g, 6 mmol) and K<sub>2</sub>CO<sub>3</sub> (2.76 g, 20 mmol). The resultant mixture was stirred under nitrogen at 100 °C overnight, cooled to room temperature, filtered, and concentrated *in vacuo*. The residue was dissolved in EA and sat. NaHCO<sub>3</sub> was added till pH = 8.5. The mixture was filtered and the resultant aqueous layer was extracted with EA (5 times). The combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, concentrated and purified by silica gel column chromatography (DCM/MeOH = 30/1-20/1) to afford ethyl 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate as a pale solid (1.6 g, 36%).

**Synthesis of 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoic acid:** To a solution of compound 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (22.0 g, 48.3 mmol) in THF/H<sub>2</sub>O (v/v

= 1/1, 220 ml) added was LiOH·H<sub>2</sub>O (4.15 g, 96.6 mmol). The reaction solution was stirred at room temperature for 5 hours. The THF was removed under reduced pressure and the aqueous solution was acidified with 10% HCl to pH = 5. The solid was filtered and recrystallized from 50% THF/water to afford 3-(5-(4-(1H-imidazol-1-yl)phenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoic acid **N6022** as a yellow solid (11.35 g, 55%). DSC Tm: 241.7 °C. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz): δ 8.20 (s, 1H), 8.07 (s, 1H), 7.86 (d, J = 7.5 Hz, 1H), 7.83 (s, 1H), 7.69 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.46 (m, 3H), 7.15 (d, J = 8.5 Hz, 2H), 7.05 (s, 1H), 6.48 (d, J = 3 Hz, 1H), 6.14 (d, J = 3 Hz, 1H), 2.43 (m, 3H), 1.78 (s, 3H). MS (ESI): m/z 415.2 [M+H]<sup>+</sup>.

**Representative procedure for Scheme 2: Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chlorophenyl)-1H-pyrrol-2-yl)propanoic acid (13b):**



**Synthesis of 3-methyl-4-(1H-pyrrol-1-yl)benzamide:** The 2,5-dimethoxy-tetrahydrofuran (106 g, 80 mmol) was added to the solution of 4-amino-3-methylbenzamide (100 g, 66.7 mmol) in AcOH (300 ml). The mixture was stirred at 80 °C for about 1.5 hours and then cooled to room temperature. The solution of sodium carbonate was added dropwise at 0 °C and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over sodium sulfate, concentrated and washed with petroleum ether. The resultant solid was filtrated and dried to afford 3-methyl-4-(1H-pyrrol-1-yl)benzamide as a pale solid (89.7 g, yield: 67%).

**Synthesis of 4-(2-formyl-1H-pyrrol-1-yl)-3-methylbenzotrile:** POCl<sub>3</sub> (65 g, 427 mmol) was added to DMF (34 ml) at 0 °C within 30 min. After addition, the mixture was stirred at room temperature for 1.5 hours, and then cooled to 0 °C. A solution of 3-methyl-4-(1H-pyrrol-1-yl)benzamide (42.7 g, 213.5 mmol) in DMF (150 ml) was added at 0 °C and the resultant mixture was stirred at room temperature for 20 min, and then heated to 80 °C for 1 hour. The solution was cooled to room temperature and then saturated sodium carbonate solution was added at 0 °C until pH = 8. The mixture was extracted with ethyl acetate three times. The combined organic layers were washed with sat. NaHCO<sub>3</sub> and brine, dried over sodium sulfate, concentrated and purified by silica gel column chromatography (PE/EA = 10/1) to afford 4-(2-formyl-1H-pyrrol-1-yl)-3-methylbenzotrile as a yellow solid (30.5 g, yield: 68%).

**Synthesis of ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)acrylate.**

**Method A:** The mixture of 4-(2-formyl-1H-pyrrol-1-yl)-3-methylbenzotrile (15 g, 71.4 mmol) and (carbethoxymethylene)-triphenylphosphorane (27.5 g, 78.6 mmol) in toluene was heated at 100 °C overnight. Then it was cooled to room temperature, concentrated and purified by silica gel column chromatography (PE/EA = 5/1) to afford ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)acrylate as a yellow oil (19.8 g, 98%).

**Method B:** To a mixture of 4-(2-formyl-1H-pyrrol-1-yl)-3-methylbenzotrile (24.5 g, 116.7 mmol), DMAP (2.9 g, 23.3 mmol) and potassium monoethyl malonate (99.2 g, 583.3 mmol) in DMF (600 ml) added was AcOH (35.0 g, 583.3 mmol) and piperidine (29.8 g, 350 mmol). The resultant mixture was heated to 80 °C and stirred for 48 hours and was then poured into cooled water and extracted with ethyl acetate (800 ml × 3). The combined organic layers were washed with sat. NaHCO<sub>3</sub> and brine, dried over sodium sulfate, concentrated and purified

by silica gel column chromatography (PE/EA = 5/1) to afford ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)acrylate as a yellow oil (21.8 g, 67%).

**Synthesis of ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate:** To a solution of ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)acrylate (8.0 g, 28.6 mmol) in ethanol added was 10% Pd/C (0.8 g). The mixture was stirred under 1 atm of H<sub>2</sub> for 30 min at room temperature and filtered. The resultant filtrate was concentrated to dryness affording the crude product of ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (7.5g), which was used for the next step without further purification.

**Synthesis of ethyl 3-(5-bromo-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate:** NBS (4.76g, 1 eq.) was added portion wise to a solution of ethyl 3-(1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate in DMF at 0 °C within 45 min. After addition, the mixture was stirred at room temperature for 30 min, then poured into water, and extracted with ethyl acetate for three times. The combined organic layers were washed with brine, dried over sodium sulfate, concentrated and purified by silica gel column chromatography (PE/EA = 15/1) to afford ethyl 3-(5-bromo-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate as a white solid.

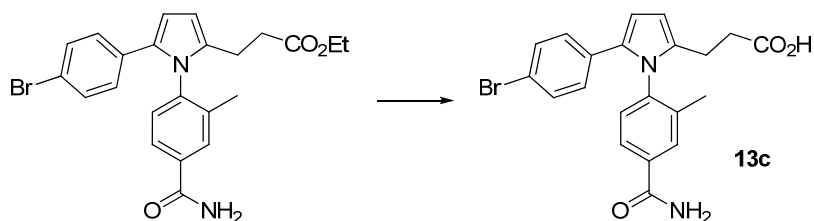
**Synthesis of ethyl 3-(5-(4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate** To a mixture of ethyl 3-(5-bromo-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (360 mg, 1 mmol), sodium carbonate (424 mg, 4 mmol) and 4-chlorophenylboronic acid (188 mg, 1.2 mmol) in the solvent (DME/H<sub>2</sub>O/Toluene/EtOH = 10/1/6/3, 3 ml) added was Pd(PPh<sub>3</sub>)<sub>4</sub> (116 mg, 0.1 mmol). After having been degassed and recharged with nitrogen, the mixture was heated at 80 °C overnight. TLC showed that the reaction was complete. Water (10 ml) was added and the mixture was extracted with ethyl

acetate (20 ml × 3). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (PE/EA = 10/1) to afford the product as a yellow solid (213 mg, 54%).

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chlorophenyl)-1H-pyrrol-2-yl)propanoic acid (13b):** To a mixture of ethyl 3-(5-(4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (213 mg) and potassium carbonate (112 mg, 0.815 mmol) in DMSO (1 ml) was added 30% aqueous H<sub>2</sub>O<sub>2</sub> (62 mg, 0.543 mmol). The resulting mixture was stirred at room temperature for 4 hours. TLC showed that the reaction was complete. Water (3 ml) was added and white solid precipitated. The suspension was centrifuged and the aqueous phase was discarded. The resulting solid was dried *in vacuo* to afford the amide as a yellow solid, which was used for the next step without further purification.

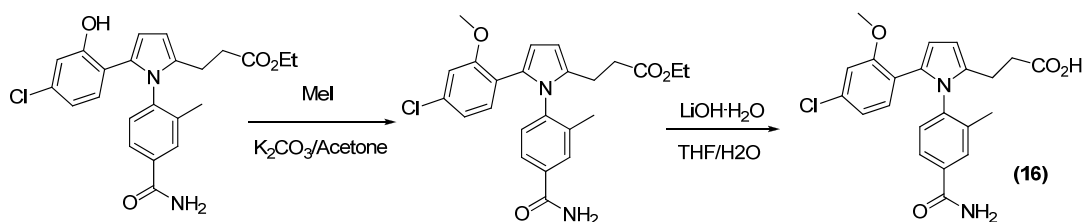
To the mixture of the amide in THF/H<sub>2</sub>O (2 ml, v/v = 1/1) added was lithium hydroxide monohydrate (19 mg, 0.762 mmol). The mixture was stirred at room temperature overnight. THF was evaporated *in vacuo*. The residue was acidified to pH = 5 with 5% hydrochloric acid, concentrated and purified by prep-HPLC to afford the product as a yellow solid **13b** (55 mg, 48%). <sup>1</sup>H NMR (MeOD, 500 MHz): δ 7.82 (d, J = 8 Hz, 1H), 7.78 (s, 1H), 7.46 (d, J = 8 Hz, 1H), 7.11 (d, J = 9 Hz, 2H), 7.03 (d, J = 9 Hz, 2H), 6.40 (d, J = 3.5 Hz, 1H), 6.17 (d, J = 3 Hz, 1H), 2.61 (m, 2H), 2.47 (m, 2H), 1.84 (s, 3H). MS (ESI): m/z 383.0 [M+H]<sup>+</sup>.

**Synthesis of 3-(5-(4-bromophenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoic acid (13c) :**



Following the similar procedure in the last step in Scheme 1, hydrolysis of ethyl 3-(5-(4-bromophenyl)-1-(4-carbamoyl-2-methylphenyl)-1H-pyrrol-2-yl)propanoate afforded final product **13c**. <sup>1</sup>H NMR (MeOD, 500 MHz): δ 7.83 (d, J = 8.5, 1H), 7.78 (s, 1H), 7.47 (d, J = 8 Hz, 1H), 7.25 (d, J = 8 Hz, 2H), 6.97 (d, 2H), 6.42 (d, J = 3.5 Hz, 1H), 6.16 (d, J = 3.5 Hz, 1H), 2.58 (m, 2H), 2.49 (m, 2H), 1.84 (s, 3H). MS (ESI): m/z 427.1, 429.1 [M+H]<sup>+</sup>.

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid (16) :**

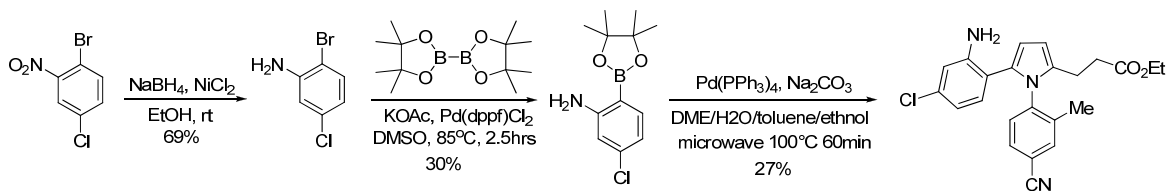


**Synthesis of ethyl 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-methoxyphenyl)-1H-pyrrol-2-yl)propanoate:** Ethyl 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-hydroxyphenyl)-1H-pyrrol-2-yl)propanoate (300 mg, 0.704 mmol) was dissolved in acetone, potassium carbonate (146 mg, 1.056 mmol) and methyl iodide (299 mg, 2.112 mmol) were added and stirred at room temperature overnight. When TLC indicated that the reaction was complete, the mixture was filtered, evaporated *in vacuo*. The residue was partitioned between ethyl acetate (20 ml) and water (5 ml). The organic phase was separated and dried with magnesium sulfate, filtered and concentrated to afford ethyl 3-(1-(4-carbamoyl-2-

methylphenyl)-5-(4-chloro-2-methoxyphenyl)-1H-pyrrol-2-yl)propanoate as a yellow oil (295 mg, yield: 95%).

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid (16):** Hydrolysis of ethyl 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-methoxyphenyl)-1H-pyrrol-2-yl)propanoate following final step of Scheme 2 afforded compound **16**. <sup>1</sup>H NMR (MeOD, 500 MHz): δ 7.58 (m, 2H), 7.21 (d, J = 8, 1H), 6.94 (d, J = 7.5 Hz, 1H), 6.68 (m, 2H), 6.09 (d, J = 3.5 Hz, 1H), 6.02 (d, J = 3 Hz, 1H), 3.05 (s, 3H), 2.49 (m, 2H), 2.36 (m, 2H), 1.80 (s, 3H). MS (ESI): m/z 413.0 [M+H]<sup>+</sup>.

**Synthesis of ethyl 3-(5-(2-amino-4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate:**



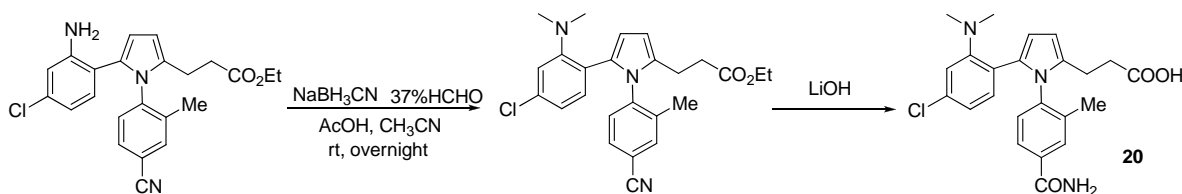
To a solution of 1-bromo-4-chloro-2-nitrobenzene (6.50 g, 27.66 mmol) and NiCl<sub>2</sub> (7.80 g, 55.3 mmol) in EtOH (50 ml) added was NaBH<sub>4</sub> (5.60 g, 138.3 mmol) slowly. The resultant mixture was stirred at 0 °C for 2 hours, filtered and concentrated under reduced pressure. The residue was dissolved with ethyl acetate (200 ml), washed with water (50 ml × 3), dried over sodium sulfate, concentrated and purified by silica gel column (PE/EA = 5:1) to afford 2-bromo-5-chloroaniline as a dark solid (3.778 g, yield: 67%).

A solution of 2-bromo-5-chloroaniline (3.778 g, 18.43 mmol), bis(pinacolato)diboron (8.5 g, 33.17 mol), KOAc (3.2 g, 36.86 mmol) and Pd(dppf)<sub>2</sub>Cl<sub>2</sub>·DCM (500 mg, 0.92 mmol) in DMSO (50 ml) was stirred at 85 °C for 2.5 hours. TLC showed that the reaction was complete.

Water (60 ml) was added and the mixture was extracted with ethyl acetate (60 ml × 3). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by silica gel column (PE/EA = 10/1) to afford 5-chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline as a yellow solid (5.0 g, yield: 100%).

To a solution of 5-chloro-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (7.0 g, 27.7 mmol), sodium carbonate (11.75 g, 110.8 mmol) and (ethyl 3-(5-bromo-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (10 g, 21.4 mmol) in DMSO (30 ml) added was Pd(PPh<sub>3</sub>)<sub>4</sub> (3.0 g, 8.31 mmol). After having been degassed and recharged with nitrogen, the reaction mixture was stirred at 80 °C overnight. TLC showed the reaction was complete. After cooling to room temperature, water (50 ml) was added and extracted with ethyl acetate (50 ml × 4). The combined organic layers were dried over sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (PE/EA = 3 : 1) to afford ethyl 3-(5-(2-amino-4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate as a yellow solid (3.10 g, yield: 27%).

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-(dimethylamino)phenyl)-1H-pyrrol-2-yl)propanoic acid (20) :**

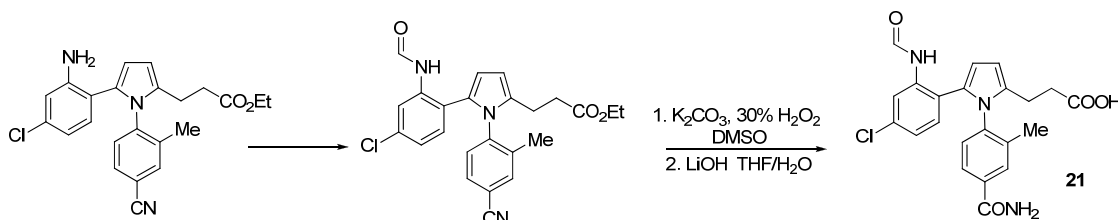


To a mixture of ethyl 3-(5-(2-amino-4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (200 mg, 0.419 mmol), NaBH<sub>3</sub>CN (53 mg, 0.838 mmol), 37% HCHO (1.5 ml, 2.095 mmol) in CH<sub>3</sub>CN (5 ml) added was AcOH (0.5 ml). After stirred at room

temperature overnight, the solution was concentrated and diluted with water (15 ml), extracted with ethyl acetate (10 ml  $\times$  4). The organic layers were combined and dried, purified with prep-TLC (PE/EA = 1/1) to afford ethyl 3-(5-(4-chloro-2-(dimethylamino)phenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate as a yellow oil (97 mg, 49%).

**3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-(dimethylamino)phenyl)-1H-pyrrol-2-yl)propanoic acid (20).** Hydrolysis of ethyl 3-(5-(4-chloro-2-(dimethylamino)phenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate following final step of Scheme 2 afforded compound **20**.  $^1\text{H}$  NMR (MeOD, 500 MHz):  $\delta$  7.63 (m, 2H), 7.28 (d,  $J = 8$ , 1H), 7.16 (d,  $J = 8$  Hz, 1H), 6.82 (m, 1H), 6.61(s, 1H), 6.17 (m, 2H), 2.60 (m, 2H), 2.41 (m, 2H), 2.40 (s, 6H), 1.86 (s, 3H). MS (ESI):  $m/z$  426.1  $[\text{M}+\text{H}]^+$ .

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-formamidophenyl)-1H-pyrrol-2-yl)propanoic acid (21) :**

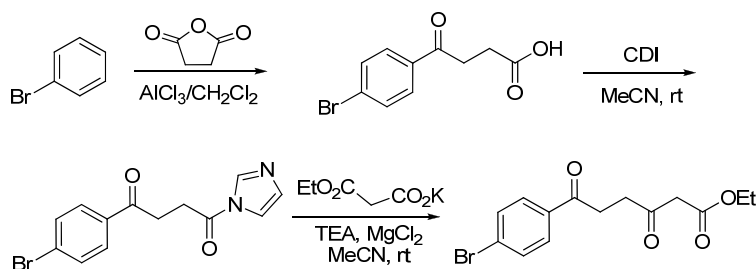


**Synthesis of ethyl 3-(5-(4-chloro-2-formamidophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate:** A mixture of  $\text{Ac}_2\text{O}$  (301 mg, 2.948 mmol) and  $\text{HCO}_2\text{H}$  (226 mg, 4.914 mmol) was stirred at 55  $^\circ\text{C}$  for 5 min. The mixture was added to the solution of ethyl 3-(5-(2-amino-4-chlorophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (300 mg, 0.737 mmol) in THF (6 ml) and stirred at 55  $^\circ\text{C}$  for 10 min. TLC showed the reaction was complete. The volatiles were removed under reduced pressure, and the residue was dissolved in EA (50

ml), washed with saturated NaHCO<sub>3</sub> (10 ml × 3) and brine (10 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated to afford the crude product as a yellow solid (320 mg, yield: 99%) which was used for the next step directly.

**Synthesis of 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-formamidophenyl)-1H-pyrrol-2-yl)propanoic acid (21):** To a mixture of ethyl 3-(5-(4-chloro-2-formamidophenyl)-1-(4-cyano-2-methylphenyl)-1H-pyrrol-2-yl)propanoate (320 mg, 0.734 mmol) and potassium carbonate (152 mg, 1.10 mmol) in DMSO (2 ml) added was 30% aqueous H<sub>2</sub>O<sub>2</sub> (83 mg, 0.73 mmol). The resultant mixture was stirred at 30 °C for 4 hours. TLC showed the reaction was completed. Water (20 ml) was added to precipitate the final product as a white solid. The suspension was centrifuged and the aqueous phase was discarded. The resultant solid was dried *in vacuo* and purified by silica gel column chromatography (PE/EA = 1/2) to afford 3-(1-(4-carbamoyl-2-methylphenyl)-5-(4-chloro-2-formamidophenyl)-1H-pyrrol-2-yl)propanoic acid (21) as a white solid (198 mg, yield: 59%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 400 MHz): δ 9.44 (d, J = 3, 1H), 8.29 (s, 1H), 8.15 (s, 1H), 7.98 (s, 1H), 7.70 (m, 2H), 7.50 (d, J = 15 Hz, 1H), 7.43 (s, 1H), 6.95 (s, 1H), 6.31 (d, J = 7 Hz, 1H), 6.20 (d, J = 7 Hz, 1H). MS (ESI): m/z 425.9 [M+H]<sup>+</sup>.

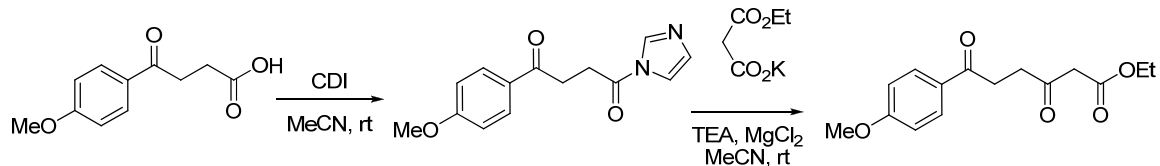
#### Synthesis of ethyl 6-(4-bromophenyl)-3,6-dioxohexanoate:



**Synthesis of 4-(4-bromophenyl)-4-oxobutanoic acid:** Anhydrous aluminum trichloride (29.1 g, 218 mmol) was suspended in dichloromethane (120 ml) and cooled to 0 °C, bromobenzene (35.1 g, 224 mmol) was added carefully. When the addition was complete, succinic anhydride (10.0 g, 100 mmol) was added in ten portions carefully. Then the mixture was warmed to room temperature and stirred for 4 hours. TLC showed the reaction was complete, 6N HCl (50 ml) was added dropwise. The solid was filtered, washed with distilled water (10 ml × 2) and dried *in vacuo* to afford 4-(4-bromophenyl)-4-oxobutanoic acid as a white solid (22 g, yield: 82%).

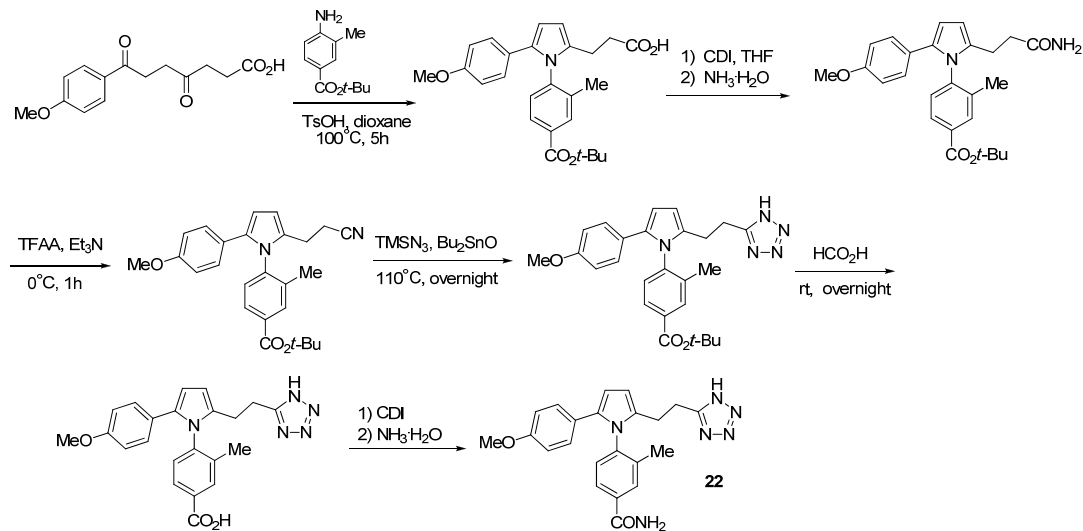
**Synthesis of ethyl 6-(4-bromophenyl)-3,6-dioxohexanoate:** To a solution of 4-(4-bromophenyl)-4-oxobutanoic acid (5.00 g, 18.6 mmol) in anhydrous MeCN (50 ml) added was CDI (3.91 g, 24.2 mmol). The solution was stirred at room temperature for 2 hours and turned red to give 1-(4-bromophenyl)-4-(1H-imidazol-1-yl)butane-1,4-dione, which was used for the next step without any purification. To a suspension of potassium 3-ethoxy-3-oxopropanoate (6.32 g, 37.2 mmol) in anhydrous MeCN (200 ml), TEA (5.63 g, 55.8 mmol) and anhydrous magnesium dichloride (5.3 g, 55.8 mmol) was added was at 0 °C. The mixture was stirred at room temperature for 2 hours, to which the solution of 1-(4-bromophenyl)-4-(1H-imidazol-1-yl)butane-1,4-dione was added in portions. The mixture was stirred at room temperature overnight. The volatiles were removed under reduced pressure and the residue was dissolved in EA (250 ml), washed with water (50 ml × 2) and brine (30 ml), dried over sodium sulfate, filtered, concentrated and purified by silica gel column chromatography (PE/EA = 3/1) to afford ethyl 6-(4-bromophenyl)-3,6-dioxohexanoate as a brown solid (5.3 g, yield: 88%).

### Synthesis of ethyl 6-(4-methoxyphenyl)-3,6-dioxohexanoate:



**Synthesis of ethyl 6-(4-methoxyphenyl)-3,6-dioxohexanoate:** 4-(4-methoxyphenyl)-4-oxobutanoic acid (9.5 g, 45.673 mmol) was converted to ethyl 6-(4-methoxyphenyl)-3,6-dioxohexanoate following the same procedure described in step 2 and 3 of the synthesis of above procedures, yielding ethyl 6-(4-methoxyphenyl)-3,6-dioxohexanoate as a brown solid (10.15 g, 80%).

### Synthesis of 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzamide (22):



**Synthesis of 3-(1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid:** 7-(4-methoxyphenyl)-4,7-dioxoheptanoic acid (550 mg, 2.083 mmol) and tert-butyl 4-amino-3-methylbenzoate (431 mg, 2.083 mmol) was dissolved in

anhydrous dioxane (5 ml). TsOH (35 mg, 0.208 mmol) was added and the reaction mixture was heated to 100 °C for 8 hours. TLC and LC-MS showed no starting material. The mixture was diluted with water (10 ml) and EA (10 ml). The organic layer was separated and the aqueous phase was extracted with EA (10 ml × 3). The combined organic layers were dried with magnesium sulfate, filtered, concentrated and purified by silica gel column chromatography to afford compound 3-(1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid as a brown oil (556 mg, 61%).

**Synthesis of tert-butyl 4-(2-(3-amino-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** 3-(1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-5-(4-methoxyphenyl)-1H-pyrrol-2-yl)propanoic acid (500 mg, 1.15 mmol) was dissolved in anhydrous THF (10 ml). CDI (230 mg, 1.38 mmol) was added in five portions and stirred at room temperature for 2 hours. TLC showed no starting material. The yellow solution was added dropwise to 25% ammonium hydroxide (10 ml) at 0 °C. After the addition was complete, the mixture was allowed to warm to room temperature and stirred for 2 hours. The solution was extracted with ethyl acetate (10 ml × 3) and the combined organic layers were washed with 5% hydrochloric acid, saturated sodium bicarbonate and brine separately, dried over MgSO<sub>4</sub>, filtered and concentrated to obtain the title compound tert-butyl 4-(2-(3-amino-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a yellow solid (320 mg, 65%).

**Synthesis of tert-butyl 4-(2-(2-cyanoethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate.** Tert-butyl 4-(2-(3-amino-3-oxopropyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate (355 mg, 0.818 mmol) was dissolved in anhydrous THF (2 ml), triethylamine (206 mg, 2.05 mmol) was added and the mixture was cooled to 0 °C. TFAA was added dropwise and stirred at this temperature for 30 min. TLC showed that the reaction was complete. The

reaction mixture was diluted with ethyl acetate (10 ml) and washed with brine. The organic phase was separated and dried with magnesium sulfate, filtered, concentrated and purified by column chromatography to afford compound tert-butyl 4-(2-(2-cyanoethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a brown oil (280 mg, 82%).

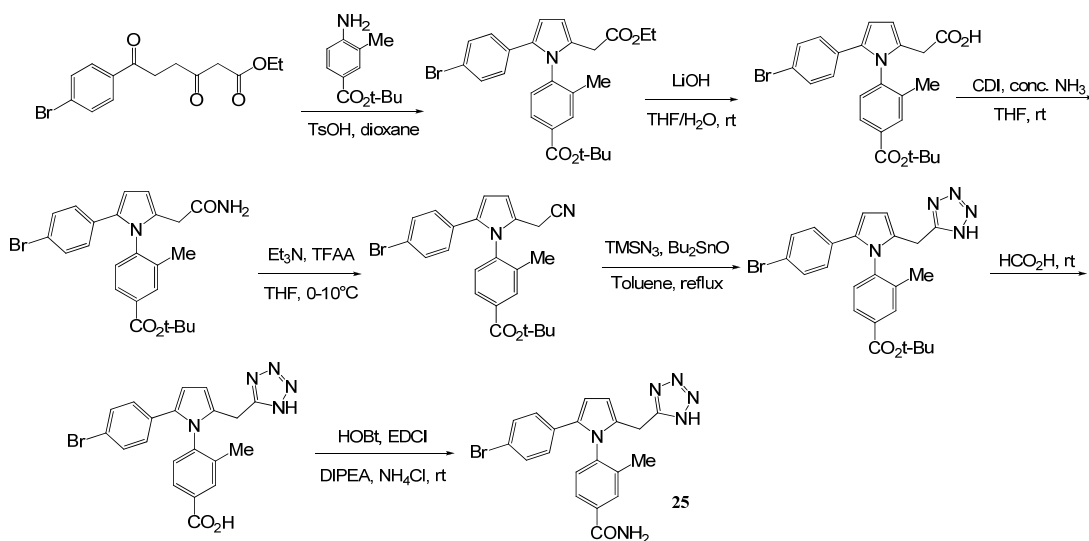
**Synthesis of tert-butyl 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** Tert-butyl 4-(2-(2-cyanoethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate (280 mg, 0.673 mmol) was dissolved in toluene (3 ml), Bu<sub>2</sub>SnO (30 mg, 0.135 mmol) and TMSN<sub>3</sub> (1.32 g, 12.1 mmol) were added and the mixture was heated at reflux overnight. Toluene was evaporated *in vacuo* and the residue was purified by silica gel column chromatography to afford tert-butyl 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a yellow oil (265 mg, 86%).

**Synthesis of 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid:** Tert-butyl 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoate (265 mg) was dissolved in formic acid (3 ml) and stirred at room temperature overnight. LC-MS showed that the reaction was complete. Formic acid was evaporated and the residue was purified by prep-TLC to afford 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid as a yellow solid (130 mg, 56%).

**Synthesis of 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzamide:** 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid (130 mg, 0.332 mmol) was dissolved in anhydrous THF (3 ml), CDI (68 mg, 0.419 mmol) was added in three portions at room temperature and stirred at this temperature for 1 hours. The resultant yellow solution was added dropwise to 25% ammonium hydroxide (3 ml) at 0 °C. After the addition was complete, the mixture was allowed to warm to room temperature

and stirred for 2 hours. The mixture was evaporated *in vacuo* and the residue was purified by prep-HPLC to afford 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-methoxyphenyl)-1H-pyrrol-1-yl)-3-methylbenzamide (**22**) as a yellow solid (7 mg, 5.4%). <sup>1</sup>H NMR (MeOD, 500 MHz): δ 7.82 (d, J = 8 Hz, 1H), 7.76 (s, 1H), 7.42 (d, J = 7.5 Hz, 1H), 6.98 (d, J = 8 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 6.26 (d, J = 3.5 Hz, 1H), 6.08 (d, J = 3.5 Hz, 1H), 3.70 (s, 3H), 3.11 (m, 2H), 2.84 (m, 2H), 1.80 (s, 3H). MS (ESI): m/z 403.2 [M+H]<sup>+</sup>.

**Synthesis of 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzamide (25):**



**Synthesis of tert-butyl 4-(2-(4-bromophenyl)-5-(2-ethoxy-2-oxoethyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** Ethyl 6-(4-bromophenyl)-3,6-dioxohexanoate (5.0 g, 15.0 mmol), *tert*-butyl-4-amino-3-methylbenzoate (3.105 g, 15.0 mmol) and 4-toluenesulfonic acid (258 mg, 1.500 mmol) in anhydrous dioxane was refluxed for 12 hours. The solvent was evaporated *in vacuo* and the residue was extracted with ethyl acetate (200 ml × 2), washed with water (100 ml) and brine (50 ml), dried over sodium sulfate, concentrated and purified by silica gel column

chromatography (PE/EA = 4/1) to afford tert-butyl 4-(2-(4-bromophenyl)-5-(2-ethoxy-2-oxoethyl)-1H-pyrrol-1-yl)-3-methylbenzoate as brown oil (4.1 g, yield: 55%).

**Synthesis of 2-(5-(4-bromophenyl)-1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-1H-pyrrol-2-yl)acetic acid:** tert-Butyl 4-(2-(4-bromophenyl)-5-(2-ethoxy-2-oxoethyl)-1H-pyrrol-1-yl)-3-methylbenzoate (2.0 g, 4.024 mmol) was converted to 2-(5-(4-bromophenyl)-1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-1H-pyrrol-2-yl)acetic acid, following the same procedure described in Scheme 2 to give 2-(5-(4-bromophenyl)-1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-1H-pyrrol-2-yl)acetic acid (1.30 mg, yield: 69%) as a brown powder.

**Synthesis of tert-butyl 4-(2-(2-amino-2-oxoethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** To a solution of 2-(5-(4-bromophenyl)-1-(4-(tert-butoxycarbonyl)-2-methylphenyl)-1H-pyrrol-2-yl)acetic acid (1.30 g, 2.772 mmol) in anhydrous THF (30 ml) added was CDI (0.525 g, 3.603 mmol) in portions. The mixture was stirred at room temperature for 2 hours. Then it was added to con.NH<sub>3</sub>H<sub>2</sub>O (60 ml), stirred at room temperature for 2 hours. The volatiles were removed under reduced pressure and the residue was extracted with ethyl acetate (50 ml × 2), washed with water (30 ml) and brine (20 ml), dried over sodium sulfate, concentrated to afford tert-butyl 4-(2-(2-amino-2-oxoethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a brown oil (1.30 g, yield: 100%).

**Synthesis of tert-butyl 4-(2-(4-bromophenyl)-5-(cyanomethyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** To a solution of tert-butyl 4-(2-(2-amino-2-oxoethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate (1.30 g, 2.776 mmol) in THF (20 ml) added was Et<sub>3</sub>N (2.805 g, 27.778 mmol) dropwise, then TFAA (2.5 ml) added was dropwise at 0-10 °C. The mixture was stirred at room temperature for 0.5 hour. The volatiles were removed under reduced pressure and the residue was extracted with ethyl acetate (50 ml × 2), washed with water (30 ml) and brine (20

ml), dried over sodium sulfate, concentrated and purified by silica gel column chromatography (PE/EA = 5/1) to afford tert-butyl 4-(2-(4-bromophenyl)-5-(cyanomethyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a yellow solid (730 mg, yield: 58%).

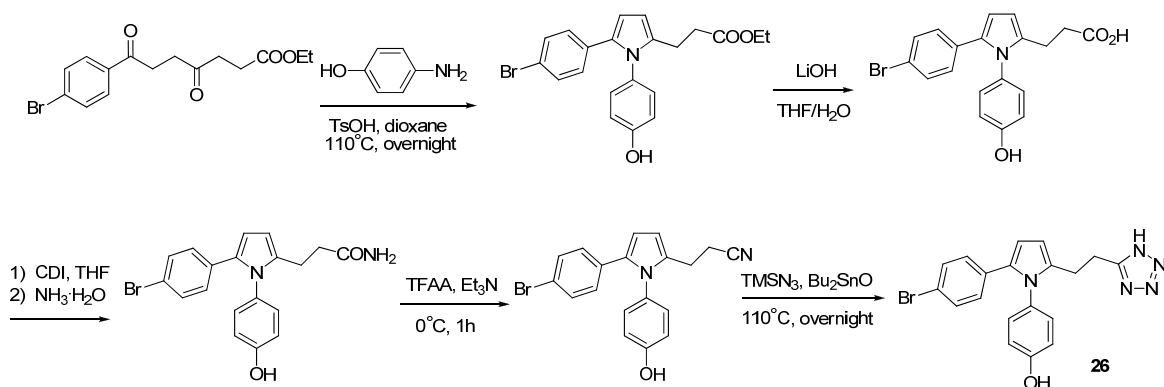
**Synthesis of tert-butyl 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate:** To a solution of tert-butyl 4-(2-(4-bromophenyl)-5-(cyanomethyl)-1H-pyrrol-1-yl)-3-methylbenzoate (730 mg, 1.662 mmol) in toluene (20 ml), added was TMSN<sub>3</sub> (3.731 g, 32.44 mmol) in portions. The mixture was stirred at 110 °C overnight. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate (50 ml × 2), washed with water and brine, dried over sodium sulfate, concentrated and purified by silica gel column chromatography (PE/EA = 1:2) to afford tert-butyl 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate as a yellow powder (520 mg, yield: 80%).

**Synthesis of 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid:** The solution of tert-butyl 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoate (730 mg, 1.662 mmol) in formic acid (10 ml) was stirred at room temperature for 24 hours. The solvent was removed with toluene under reduced pressure to afford 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid as a yellow powder (500 mg, yield: 100%).

**Synthesis of 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzamide:** To a solution of 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzoic acid (200 mg, 0.458 mmol) in anhydrous DMF (3 ml) added was HOBt (74 mg, 0.549 mmol) and EDCI (132 mg, 0.686 mmol). The mixture was stirred at 20 °C and checked by TLC until the starting material consumed. Then DIPEA (158 mg, 1.143 mmol) and NH<sub>4</sub>Cl (122 mg, 2.286 mmol) was added, stirred overnight. The crude product was purified

by preparative HPLC to afford 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)-3-methylbenzamide as a white powder **25** (68 mg, yield: 34%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 500 MHz): δ 16.0 (b, 1H), 8.04 (s, 1H), 7.80 (d, J = 8 Hz, 1H), 7.72 (s, 1H), 7.48 (s, 1H), 7.42 (d, J = 8 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 6.97 (d, J = 8.5 Hz, 2H), 6.49 (d, J = 3.5 Hz, 2H), 6.21 (d, J = 3.5 Hz, 1H), 3.99 (m, 2H), 1.63 (s, 3H). MS (ESI): m/z 437.3, 439.4 [M+H]<sup>+</sup>.

### Synthesis of 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (**26**)



**Synthesis of ethyl 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanoate:** Ethyl 7-(4-bromophenyl)-4,7-dioxoheptanoate (4.0 g, 11.76 mmol) was dissolved in anhydrous dioxane, 4-aminophenol (1.30 g, 11.76 mmol) and TsOH (203 mg, 1.18 mmol) was added and the mixture was heated to 110 °C overnight. TLC and LC-MS showed that the reaction was complete. Dioxane was evaporated *in vacuo* and the residue was purified by silica gel column chromatography to afford ethyl 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanoate as a brown solid (2.90 g, 60%).

**Synthesis of 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl) propanoic acid:** Ethyl 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanoate (1.0 g, 2.42

mmol) was dissolved in THF/H<sub>2</sub>O (10 ml, v/v = 1:1), lithium hydroxide monohydrate (254 mg, 6.05 mmol) was added and the mixture was stirred at room temperature overnight. TLC showed the starting material was consumed. THF was evaporated *in vacuo* and the residue was acidified to pH = 4 with 5% hydrochloric acid. White precipitate was filtered and dried *in vacuo* to afford 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanoic acid as a yellow solid (900 mg, 97%).

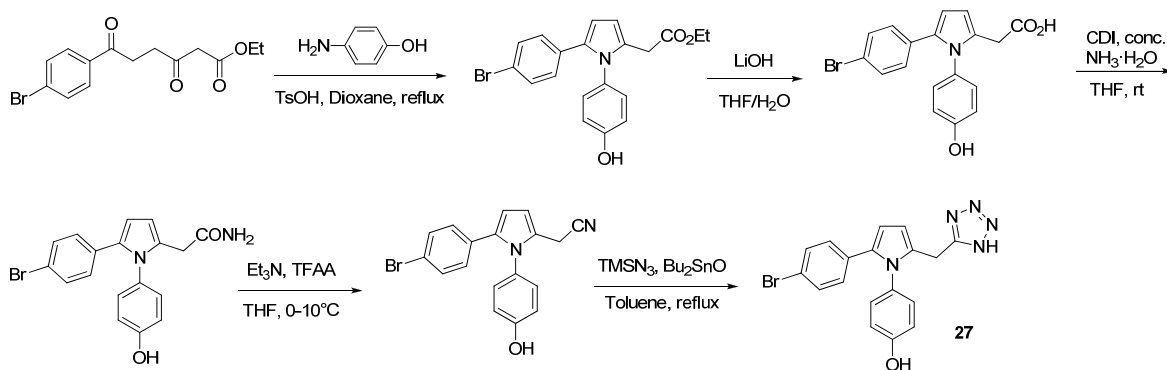
**Synthesis of 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanamide:** 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanoic acid was converted to 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanamide, following the same procedure described in for the synthesis compound **25** to give desired product as a yellow solid (96% yield).

**Synthesis of 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanenitrile:** 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanamide was converted to 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanenitrile by following the same procedure described for compound **25** to give desired product as a brown oil (160 mg, 38%).

**Synthesis of 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol:** 3-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)propanenitrile was converted to 4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (**26**) following the same procedure described for compound **25**, where purification was accomplished by prep-HPLC to afford desired product as a yellow solid (75 mg, 48%). <sup>1</sup>H NMR (MeOD, 500

MHz):  $\delta$  7.27 (d,  $J = 7$  Hz, 2H), 6.95 (m, 4H), 6.84 (d,  $J = 8.5$  Hz, 2H), 6.30 (d,  $J = 3.5$  Hz, 1H), 5.98 (d,  $J = 3.5$  Hz, 1H), 3.08 (m, 2H), 2.98 (m, 2H). MS (ESI):  $m/z$  410, 412  $[M+H]^+$ .

### Synthesis of 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (27):



### Synthesis of ethyl 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetate:

Ethyl 6-(4-bromophenyl)-3,6-dioxohexanoate (5 g, 15.0 mmol) was converted to ethyl 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetate, following the same procedure described for the synthesis of compound **26** with purification by silica gel column chromatography (PE/EA = 5/1) to afford ethyl 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetate as a yellow solid (3.2 g, yield: 52%).

### Synthesis of 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetic acid:

To a solution of ethyl 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetate (2.0 g, 5.013 mmol) in THF/H<sub>2</sub>O (v/v = 1/1, 20 ml) added was LiOH·H<sub>2</sub>O (632 mg, 15.04 mmol). The solution was stirred at room temperature for 3 hours. THF was evaporated *in vacuo* and the resultant aqueous solution was acidified with 10% HCl to pH = 3.0. The resultant precipitate was isolated by filtration, rinsed with water (50 ml) and dried *in vacuo* to afford 2-(5-(4-

bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetic acid (1.380 g, yield: 74%) as a brown powder.

**Synthesis of 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl) acetamide.**

To a solution of 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetic acid (1.3 g, 3.504 mmol) in anhydrous THF (30 ml) added was CDI (0.851 g, 5.256 mmol) in portions. The reaction was stirred at room temperature for 2 hours. Then it was added to concentrate ammonia hydroxide (80 ml) and stirred at room temperature for 2 hours. The volatiles were evaporated *in vacuo* and the residue was extracted with ethyl acetate (50 ml) twice. The combined organic layers were washed with water (30 ml × 2) and brine (20 ml), dried over sodium sulfate, filtered and concentrated to afford 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetamide as a brown oil (1.45 g, yield: 100%).

**Synthesis of 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl) acetonitrile:**

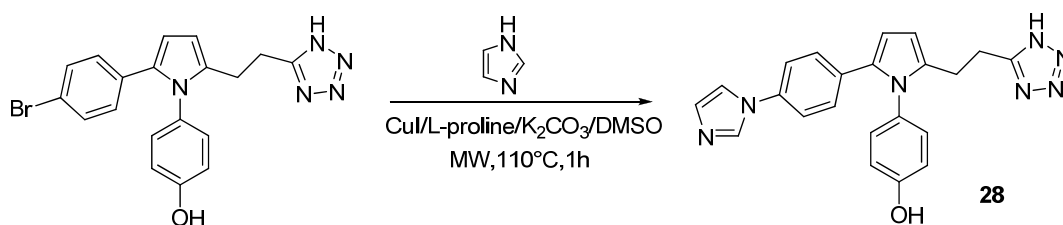
To a solution of 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetamide (1.45 g, 3.919 mmol) in THF (30 ml) added was Et<sub>3</sub>N (3.958 g, 39.189 mmol) dropwise, then TFAA (3 ml) added was dropwise at 0-10 °C. The mixture was stirred at room temperature for 0.5 hours. The volatiles were removed under reduced pressure and the residue was extracted with ethyl acetate (50 ml × 2), washed with water (30 ml) and brine (20 ml), dried over sodium sulfate, concentrated and purified by silica gel column chromatography (PE/EA = 4/1) to afford 2-(5-(4-bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetonitrile as a yellow solid (1.2 g, yield: 87%).

**Synthesis 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl) phenol:**

2-(5-(4-Bromophenyl)-1-(4-hydroxyphenyl)-1H-pyrrol-2-yl)acetonitrile (430 mg, 1.141 mmol) was converted to 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol,

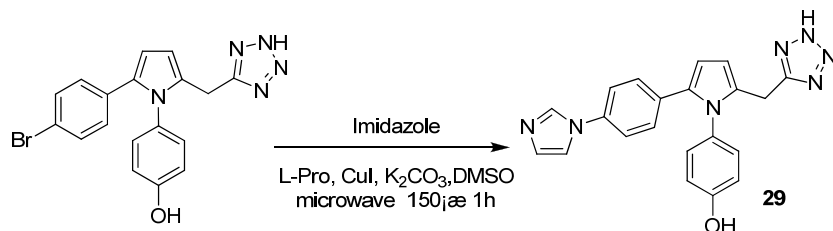
following the same procedure described in Scheme 2 with purification by silica gel column chromatography (PE/EA = 1/2) to afford 4-(2-((1H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (**27**) as yellow powder (393 mg, yield: 82%). <sup>1</sup>H NMR (MeOD, 400 MHz): δ 7.19 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 2H), 6.65 (d, J = 8.4 Hz, 2H), 6.30 (d, J = 3.6 Hz, 1H), 6.08 (d, J = 3.6 Hz, 1H), 4.09 (s, 2H). MS (ESI): m/z 396, 398 [M+H]<sup>+</sup>.

**Synthesis of 4-(2-(4-(1H-imidazol-1-yl)phenyl)-5-(2-(1H-tetrazol-5-yl)ethyl)-1H-pyrrol-1-yl)phenol (**28**) :**



4-(2-(2-(1H-tetrazol-5-yl)ethyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (**26**) (40 mg, 0.0978 mmol) and imidazole (10 mg, 0.1467 mmol) was dissolved in anhydrous DMSO (0.4 ml), CuI (6 mg, 0.0293 mmol), L-proline (6 mg, 0.0479 mmol) and potassium carbonate (27 mg, 0.1956 mmol) was added and purged with nitrogen for 5 min. Then the mixture was heated to 110 °C for 1 hour by microwave irradiation. The reaction mixture was diluted with THF (15 ml) and filtered. The filtrate was concentrated and purified by prep-HPLC to afford 4-(2-(4-(1H-imidazol-1-yl)phenyl)-5-(2-(1H-tetrazol-5-yl)ethyl)-1H-pyrrol-1-yl)phenol (**28**) (5 mg, yield: 12%). <sup>1</sup>H NMR (MeOD, 500 MHz): δ 9.36 (s, 1H), 8.00 (s, 1H), 7.72 (s, 1H), 7.51 (d, J = 9 Hz, 2H), 7.32 (d, J = 8 Hz, 2H), 7.04 (d, J = 9 Hz, 2H), 6.84 (d, J = 9 Hz, 2H), 6.44 (d, J = 3.5 Hz, 2H), 6.04 (d, J = 4 Hz, 1H), 3.00 (m, 2H). MS (ESI): m/z 398.2 [M+H]<sup>+</sup>.

**Synthesis of 4-[2-(4-Imidazol-1-yl-phenyl)-5-(2H-tetrazol-5-ylmethyl)-pyrrol-1-yl]-phenol (29) :**



To a mixture of 4-(2-((2H-tetrazol-5-yl)methyl)-5-(4-bromophenyl)-1H-pyrrol-1-yl)phenol (**27**) (280 mg, 0.712 mmol) and imidazole (97 mg, 1.424 mmol) in DMSO (2.0 ml) added was L-proline (49 mg, 0.428 mmol), CuI (41 mg, 0.214 mmol) and K<sub>2</sub>CO<sub>3</sub> (197 mg, 1.424 mmol). The resultant mixture was heated at 150 °C for 1 hour under microwave irradiation, then poured into THF (20 ml) and filtered. The filter cake was washed with EA (30 ml). The filtrate was concentrated and purified by prep-HPLC to afford 4-[2-(4-Imidazol-1-yl-phenyl)-5-(2H-tetrazol-5-ylmethyl)-pyrrol-1-yl]-phenol (**29**) as a white powder (60 mg, yield: 22%). <sup>1</sup>H NMR (MeOD, 500 MHz): δ 9.36 (b, 1H), 8.00 (b, 1H), 7.73 (b, 1H), 7.53 (d, J = 9 Hz, 2H), 7.36 (d, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 6.54 (d, J = 3.5 Hz, 1H), 6.25 (d, J = 3 Hz, 1H), 4.21 (s, 2H). MS (ESI): m/z 384.0 [M+H]<sup>+</sup>.

**Abbreviations:**

CDI	carbonyldiimidazole
DCM	dichloromethane
DIPEA	diisopropylethylamine
DMAP	N,N-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide

DPPA	diphenylphosphoryl azide
EA	ethyl acetate
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	hydroxybenzotriazole
NBS	N-bromosuccinamide
PE	petroleum ether
TEA	triethylamine
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilane