Supporting Information

A fluorescence quenching assay to discriminate between real and non-specific inhibitors of dengue virus protease

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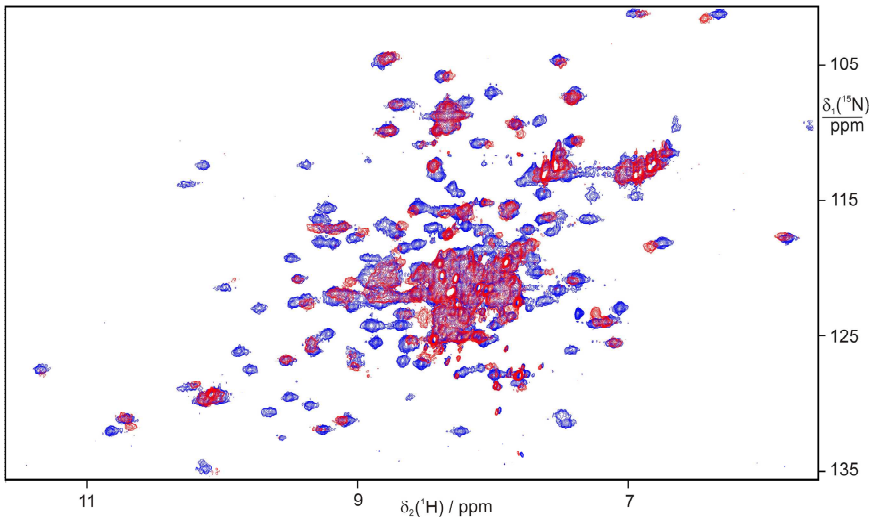
Table S1. Sequences of oligonucleotide primers used for site-directed mutagenesis.

Primer	Sequence
W5A-FOR	5'-GGAGTATTGGCGGATGTCCCTTCACCCCCA-3'
W5A-REV	5'-GACATCCGCCAATACTCCGGCCCC-3'
W50A-FOR	5' -CACAATGGCGCACGTCACACGTGGT-3'
W50A-REV	5'-GACGTGCGCCATTGTGTGGAATGTTCC-3'
W69A-FOR	5'-CCATCAGCGGCGGACGTCAAGAAAGACC-3'
W69A-REV	5'-GTCCGCCGCTGATGGTTCGATTCTCTTC-3'
W83A-FOR	5'-GGAGGCGCGAAGCTAGAAGGAGAATGG-3'
W83A-REV	5'-CTAGCTTCGCGCCTCCTCCATACGATA-3'
W89A-FOR	5'-GAAGGAGAAGCGAAGGAAGGAGAAGAAGTC-3'
W89A-REV	5'-TTCCTTCGCTTCTCCTTCTAGCTTCCA-3'

Mutated nucleotides are underlined.

Fig. S1. Superimposition of ^{15}N-HSQC spectra of 1 mM DENV2 NS2B/NS3pro in the absence (red) and presence (blue) of 1 mM compound **1**.



Method

The spectra were recorded on a Bruker 800 MHz NMR spectrometer. The sample conditions were 90% H_2O/10% D_2O, 50 mM Tris.HCl, pH 7.2, 2 mM DTT, 25 ^{o}C. The 1:1 complex was formed by adding 3 µl of a DMSO stock solution of compound **1** to 500 µl of protein solution.

Fig. S2. Global fit of compound **1**-protease interaction data. DENV2 NS2B/NS3pro was immobilized to a level of 7000 RU using ligand-thiol coupling chemistry. Compound **1** was injected at two-fold dilutions of a 12.5 µM solution. Sensorgrams are shown as coloured replicates of serial dilutions of **1**, as obtained are after double-referencing and solvent correction. Binding affinity was obtained by global fitting to a 1:1 Langmuir model (black lines) yielding k_a = 2.4 x 10^4 $M^{-1}s^{-1}$, k_d = 0.09 s^{-1}, K_D = 3.75 µM. Average values calculated from the sensorgrams in the shaded area were used for steady-state analysis. Inset shows the plot of steady-state response (RU) against analyte concentration (M). The mild increase in the response for the highest concentration injected may be due to aggregation.

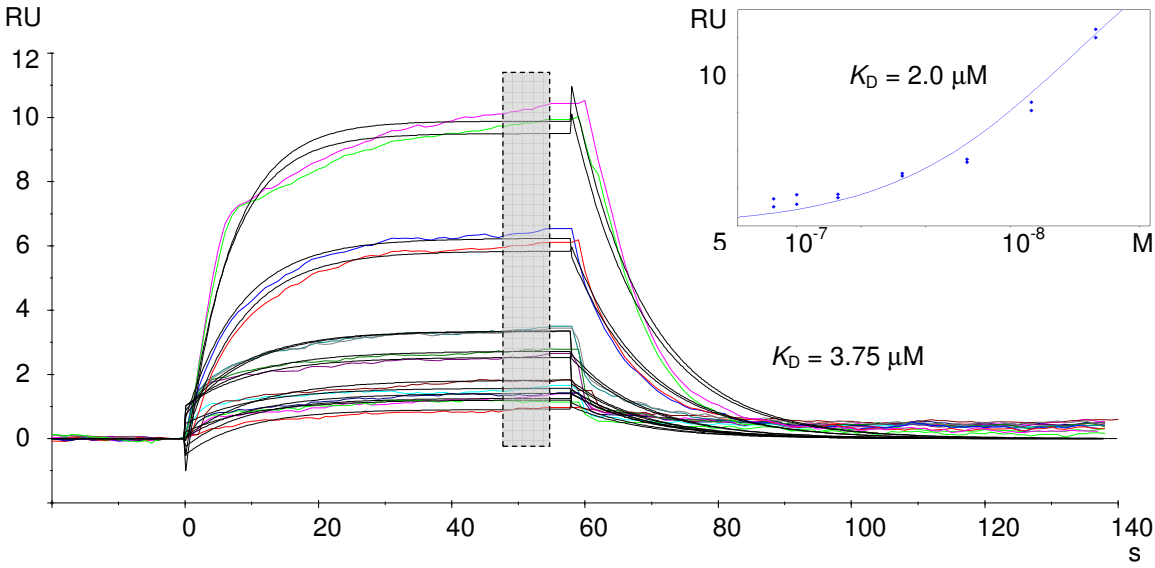


Fig. S3. Corrected sensorgram showing the competition assay between compound **1** (first co-injection) and BPTI (second co-injection). The binding response for compound **1** (50 μM) was completely abolished by BPTI (0.09 nM), as denoted by the absence of a continuation of response demarcated by the dotted line. This demonstrated that compound **1** bound on immobilized NS2B/NS3pro was replaced by co-injected BPTI.

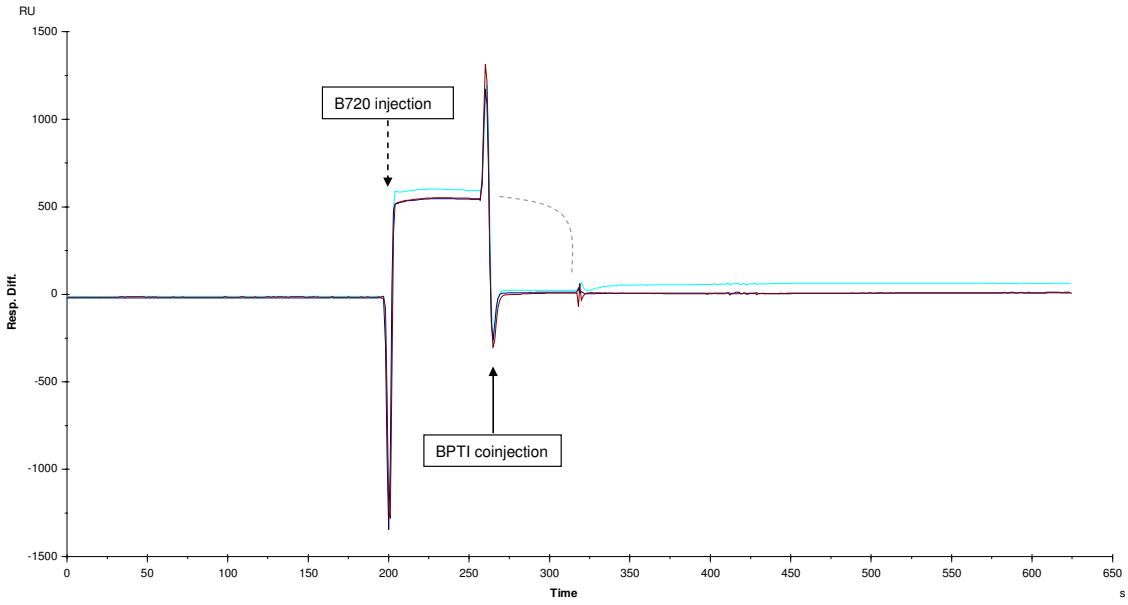


Fig. S4. Titrations of 15 μM N-acetyl-tryptophanamide with four compounds. Titration data were normalized by dividing the measured fluorescence (F) by the fluorescence measured in the absence of compound (F_0). Different titrations are represented as follow: compound **1** (■),compound **2** (□),compound **3** (○), compound **4** (▼).

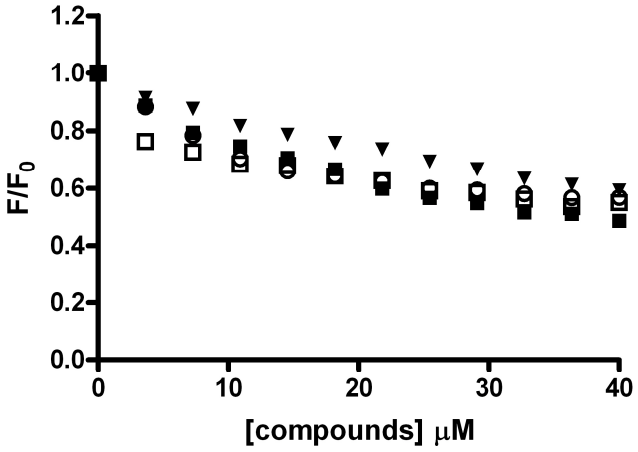
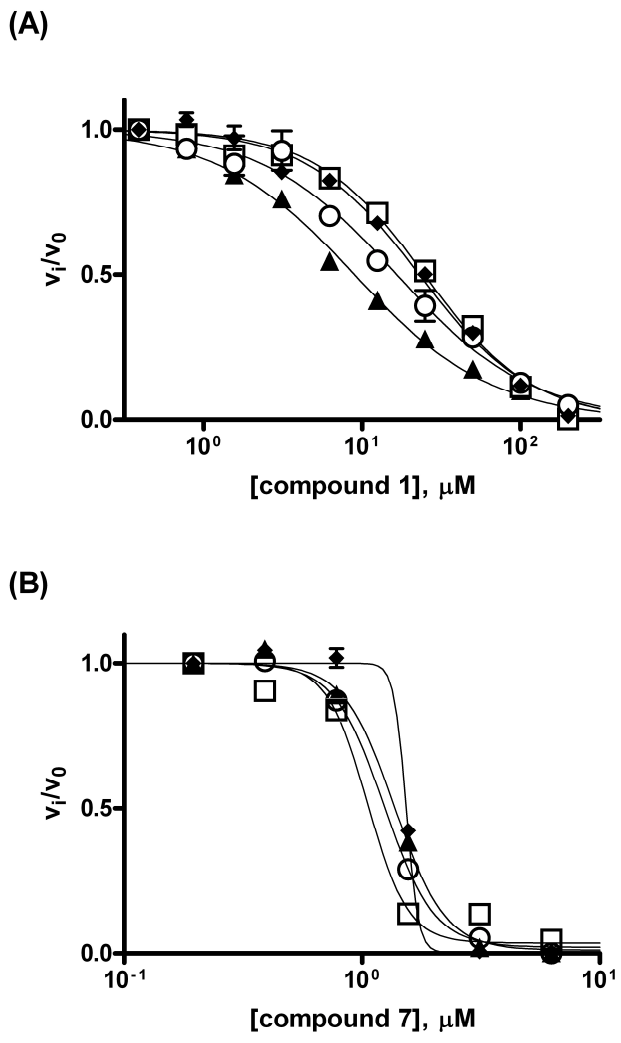
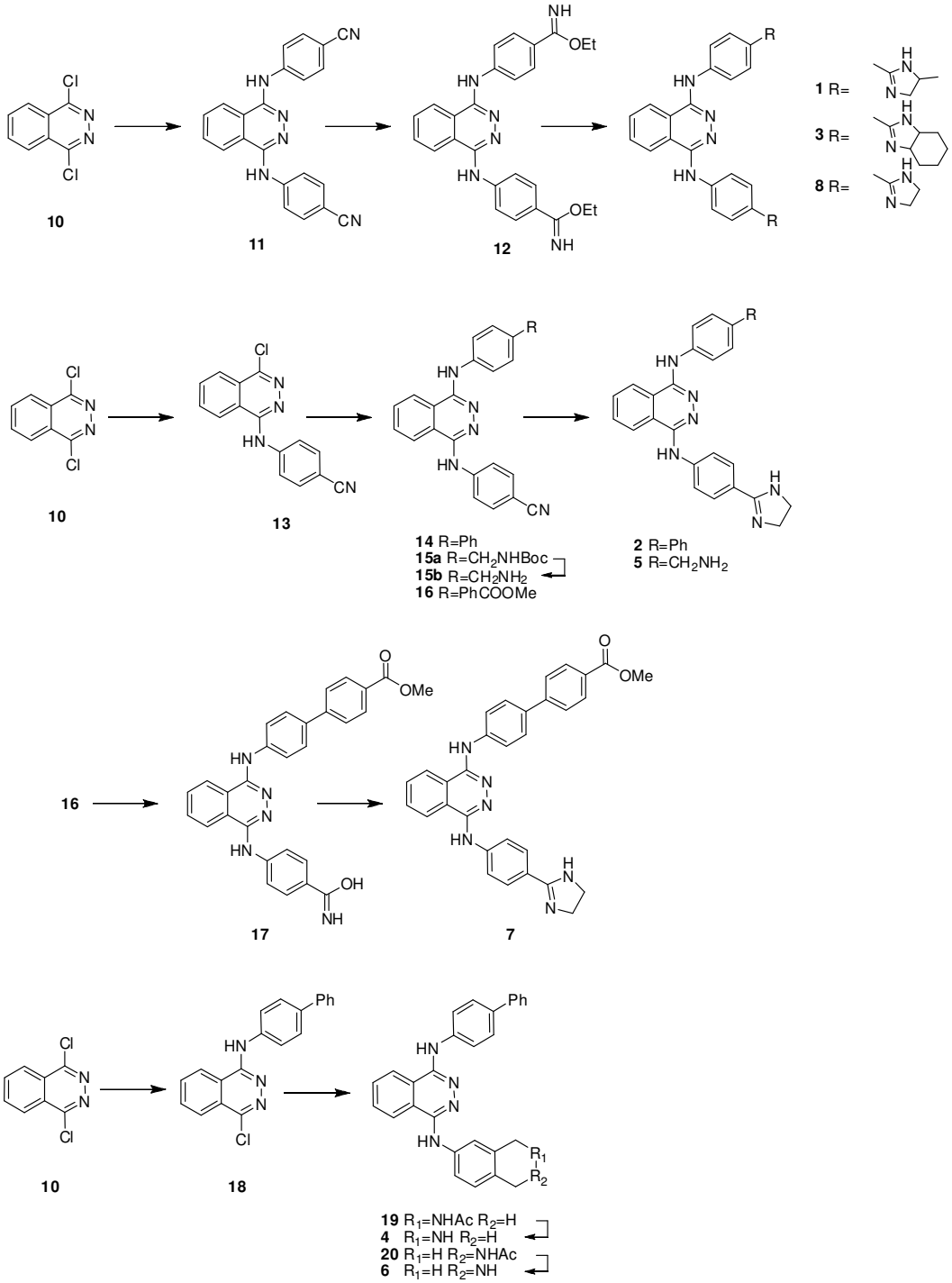


Fig. S5. Results from *in vitro* testing of compounds **1** and **7** on DENV1-4 proteases using substrate Bz-Nle-Lys-Arg-Arg-AMC. The IC_{50} curves were normalized as the ratio of initial velocity (v_i) over the initial velocity in absence of compound (v_0). Each IC_{50} measurement comprised 40nM enzyme incubated with serially diluted compound **1** or **7** from 0-100μM. IC_{50} values and Hill slopes were obtained by fitting calculated initial velocities to a non-linear regression curve using GraphPad Prism software. Each data point was measured in duplicate wells. Inhibition curves are represented as follow: DENV1 (□), DENV2 (▲),DENV3 (○), DENV4 (ŧ)



Chemistry


10
11
12
1 R=
3 R=
8 R=
10
13
14 R=Ph
15a R=CH2NHBoc
15b R=CH2NH2
16 R=PhCOOMe
2 R=Ph
5 R=CH2NH2
16
17
7
10
18
19 R1=NHAc R2=H
4 R1=NH R2=H
20 R1=H R2=NHAc
6 R1=H R2=NH

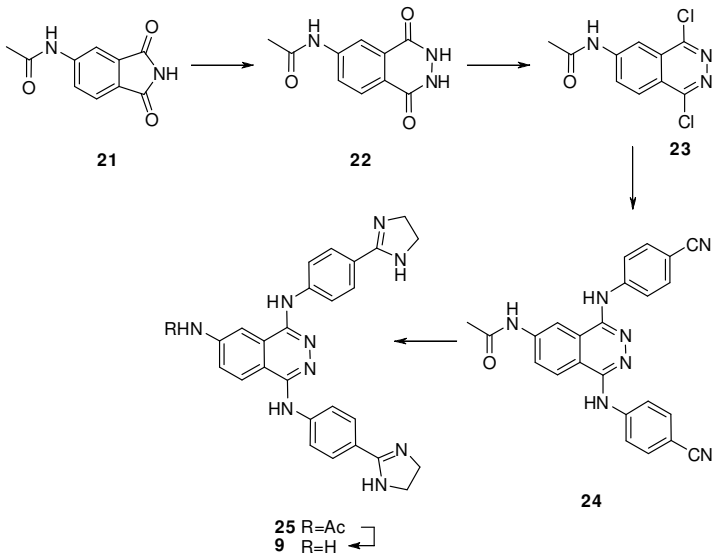


Fig. S6. Schematic representation of compound syntheses

General Methods. Chemicals from commercial sources were used without further purification. Reactions were routinely run under a dry nitrogen blanket using standard techniques. The progress of reactions was monitored using silica gel plates (Merck), which were visualized under UV light or with KMnO4 and Iodine stain. Depending on the scale, evaporation of solvents was performed on a rotary vacuum evaporator (Heidolph). ^{1}H NMR spectra of the synthetic intermediates and final products were obtained with Varian 400 MHz NMR with ATB and IDPFG probes (vnmrj 1.1d version software) in $CDCl_3$ or DMSO-d_6 and a Varian 300MHz Mercury NMR. Characterization of the purity and identity was carried out by liquid chromatography-mass spectrometry (LC-MS). Analytical LCMS was performed on an Applied Biosystems LC1100 with Applied Biosystems API2000 MS, using a PHENOMENEX, Onyx Monolithic C18, Column 50 x 4.6 mm. Gradient was run using A (HPLC grade Acetonitrile), B (0.1% Formic acid in water) from 95:5 to 5:95 A:B. Analytical UPLC-MS was performed on a Waters Acquity UPLC-MS (ZQ2000) using a Acquity C18 Column, 2.1x50mm, 1.8µm at 30°C, gradient A (0.1% Formic acid in water), B (HPLC grade Acetonitrile) 95:5 to 5:95 A:B. Analytical HPLC was performed on an Agilent LC1100 HPLC using a Waters Symmetry Shield Rp18, 3.5µm, 4.6 x 150mm column at 30°C, gradient A (0.1% Formic acid in water), B (HPLC grade Acetonitrile) 95:5 to 5:95 A:B. Final compound purity was assessed using a Waters Acquity UPLC with an Acquity C18 Column, 2.1x50mm, 1.8µm at 30°C, gradient A (0.1% Formic acid in water), B (HPLC grade Acetonitrile) 95:5 to 5:95 A:B. Preparative HPLC was performed using a Waters PerpLC preparative HPLC with a Waters Atlantis dC18 OBD (19 x 250mm, 10um) column or a Waters XTERRA Prep RP18 10µm 30 x 300MM column. Direct MS analysis was carried out by Agilent make single Quadrapole with Atmospheric pressure chemical ionization (APCI).

N,N'-Bis-(4-cyano-phenyl)-phthalazine-1,4-diamine (11). A mixture of 1,4-dichloropthalazine (2.0 g 15.07 mmol) and 4-aminobenzonitrile (5.3 g, 45.22 mmol) in N-methylpyrrolidone was stirred at 120 °C for 4 h in a sealed tube. It was cooled and diluted with water (25 mL) and filtered. The residual solid was washed with water (2 × 15 mL) and dried under vacuum. The solid was triturated with diethyl ether to afford 5.1 g (94%) **11** as a pale yellow solid.

Rf: 0.4 (EtOAc:$CHCl_3$ 4:6), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.16 (br s, 2H), 8.61 (dd, *J* = 6.4, 3.2 Hz, 2H), 8.20 (dd, *J* = 6.4, 3.6 Hz, 2H), 7.84 (d, *J* = 8.4Hz, 4H), 7.83 (d, *J* = 9.2 Hz, 4H), MS (APCI) m/z 363.14 [M+H].

N, N'-Bis-(4-(imino-ethoxy-methyl)-phenyl)-phthalizine-1,4-diamine (12). A solution of N,N'-bis-(4-cyano-phenyl)-phthalazine-1,4-diamine **11** (1.0 g, 2.76 mmol) in dry EtOH was purged with HCl gas for 12 h and stirred at RT for 48 h. It was degassed with argon for 15 min and neutralized with saturated $NaHCO_3$ solution. The reaction mixture was filtered and washed with water (10 mL). The residual solid was triturated with diethyl ether to afford 900 mg (71%) of **12** as a pale yellow solid. R_f: 0.3 (EtOAc:$CHCl_3$ 6:4). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.14 (br s, 2H), 8.55 (dd, *J* = 6.4, 3.2 Hz, 2H), 8.53 (m, 2H), 8.04 (dd, *J* = 6.4, 3.2 Hz, 2H), 7.93 (d, *J* = 8.8 Hz, 4H), 7.81 (d, *J* = 8.4 Hz, 4H), 4.24 (br s, 4H), 1.34 (t, *J* = 7.0 Hz, 6H). MS (APCI): m/z 455.0 [M+H].

N,N-Bis-(4-(4-methyl-4,5-dihydro-1H-imidazol-2-yl-phenyl)-phthalazine-1,4-diamine (1). A mixture of N, N'-bis-(4-(imino-ethoxy-methyl)-phenyl)-phthalizine-1,4-diamine **12** (200 mg, 0.44 mmol) 1,2-diaminopropane (0.45 mL, 5.28 mmol) in dry EtOH (3 mL) was refluxed for 2 h. The reaction mixture was diluted with ice water, filtered and the precipitate was dried under vacuum. The crude solid was purified by column chromatography on neutral alumina using a 10-25% gradient MeOH/$CHCl_3$ as eluent to afford 65 mg (30%) **1** as a pale yellow solid. R_f *:* 0.28 (Methanol with 0.1% aqNH_3). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 9.47 (s, 2 H), 8.56 (dd, *J*=6.3, 3.4 Hz, 2 H), 8.43 (s, 2 H), 8.06 (dd, *J*=6.2, 3.2 Hz, 2 H), 7.96 - 8.04 (m, 4 H), 7.81 - 7.95 (m, 4 H), 4.13 - 4.32 (m, 2 H), 3.94 (t, *J*=10.8 Hz, 2 H), 3.38 (dd, *J*=11.4, 7.9 Hz, 2 H), 1.28 (d, *J*=6.2 Hz, 6 H). MS (APCI) m/z 477.0 [M+H]. HPLC purity: 98.5%

N,N'-Bis-[4-(4,5-dihydro-1H-imidazol-2-yl)-phenyl]-phthalazine-1,4-diamine (8). A mixture of *N*, N'-bis-(4-(imino-ethoxy-methyl)-phenyl)-phthalizine-1,4-diamine **12** (150 mg, 0.33 mmol) and ethylenediamine (0.11mL, 0.98 mmol) in dry EtOH (3 mL) was refluxed for 2 h. The reaction mixture was diluted with ice water, filtered and the precipitate was dried under vacuum. The crude solid was purified by column chromatography on neutral alumina using a 10-15% gradient MeOH/DCM as eluent to afford 70 mg (79%) **8** as a pale yellow solid. R_f : 0.4 (Methanol with 0.1% TFA). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 2H), 8.56 (dd, *J* = 6.0, 3.2 Hz, 2H), 8.06 (dd, *J* =

6.0, 3.2 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 4H), 7.84 (d, *J* = 8.8 Hz, 4H), 3.74 (s, 8H). MS(APCI) m/z 449.2 [M+H]. HPLC purity: 96.5%.

N,N'-Bis-[4-(3a,4,5,6,7,7a-hexahydro-1H-benzoimidazol-2-yl)-phenyl]-phthalazine-1,4-diamine (3). A mixture of N,N'-bis-(4-(imino-ethoxy-methyl)-phenyl)-phthalizine-1,4-diamine **12** (200 mg, 0.4 mmol) and (±-trans-cyclohexane-1,2-diamine (0.6 mL, 5.2 mmol) in EtOH (5 mL) was stirred at 110 °C for 4h. The reaction mixture was poured onto ice and the resultant solid was filtered. The precipitate was washed with water and dried under vacuum. The crude compound was purified by column chromatography on silica gel (100-200 mesh) using 2% of MeOH-NH_3/THF as eluent to afford 172 mg (70%) of **3** as a yellow solid. R_f: 0.3 (MeOH-NH_3: THF 1:9). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.65 (s, 2H), 9.76 (s, 2H), 8.61 (dd, *J* = 6.4, 3.2 Hz, 2H), 8.30-8.15 (m, 6H); 8.01 (d, *J* = 8.4 Hz, 4H), 3.65-3.50 (m, 4H), 2.24 (br d, *J* = 10.8 Hz, 4H), 1.84 (br d, *J* = 7.2 Hz, 4H), 1.70-1.50 (m, 4H), 1.50-1.30 (m, 4H). MS (APCI) m/z 555.3 [M-H]. HPLC purity: 98.1%

4-(4-chloro-phthalazin-1-ylamino)-benzonitrile (13). A mixture of 1,4-dichlorophthalazine (**10**) (200 mg, 1.0 mmol) and 4-aminobenzontrile (83 mg, 0.7 mmol) in t-BuOH (0.5 mL) was heated at 100 ^{o}C in a sealed tube for 24h. The reaction mixture was diluted with water (10 mL), filtered and washed with water (2 × 5 mL) and dried under vacuum. The crude compound was purified by column chromatography on silica gel (100-200 mesh) using 1-5% MeOH: $CHCl_3$ as eluent to afford 200 mg (71%) of **13** as an off-white solid. R_f: 0.5 (EtOAc: $CHCl_3$ 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.78 (s, 1H), 8.68 (d, *J* = 7.2 Hz, 1H); 8.25-8.10 (m, 5H), 7.82 (d, *J* = 8.4 Hz, 2H); MS (APCI): m/z 281 [M+H], 283 [(M+2)+H]

4'-[4-(4-cyano-phenylamino)-phthalazin-1-ylamino]-biphenyl-4-carboxylicacid methyl ester (16). A mixture of 4-(4-chloro-phthalazin-1-ylamino)-benzonitrile **13** (340 mg, 1.07 mmol) and 4'-amino-biphenyl-4-carboxylic acid methyl ester (291 mg, 1.2 mmol) in NMP (1 mL) was heated at 120 ^{o}C for 4h in a sealed tube. The reaction mixture was cooled and diluted with water. The resulting solid was filtered, washed with water and dried under reduced pressure. The crude compound was triturated with 3% MeOH in Diethlyether to afford 542 mg (94%) of 4'-[4-(4-cyano-phenylamino)-phthalazin-1-ylamino]-biphenyl-4-carboxylicacid methyl ester **16** as yellow solid. R_f: 0.3 (EtOAc:$CHCl_3$ (3:7)). ^{1}HNMR (400Mz, DMSO-d6): δ 10.6 (br, 1H), 10.0 (br, 1H), 8.88 (d, *J*=7.6Hz, 1H), 8.75 (d, *J*=8.0Hz, 1H), 8.23 (m, 2H), 8.06 (d, *J*=8.4Hz, 2H), 7.96 (d, *J*=8.8Hz, 2H), 7.89 (d, *J*=8.4Hz, 4H), 7.81 (m, 4H), 3.89 (s, 3H). MS-APCI: m/z 470.3 [M-H]

4'-[4-(4-methoxycarbonimidoyl-phenylamino)-phthalazin-1-ylamino]-biphenyl-4-carboxylic acid methyl ester (17). 4'-[4-(4-cyano-phenylamino)-phthalazin-1-ylamino]-

biphenyl-4-carboxylic acid methyl ester **16** (350 mg, 0.74 mmol) in dry MeOH (20 mL) was purged with dry HCl gas and stirred at RT for 5h. The reaction was monitored by TLC and excess HCl was removed by degassing with N_2 gas. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was washed with water and brine solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude compound was triturated with 5% MeOH:Diethylether to afford 312 mg (83%) of 4'-[4-(4-methoxycarbonimidoyl-phenylamino)-phthalazin-1-ylamino]-biphenyl-4-carboxylic acid methyl ester **17** as pale yellow solid. R_f: 0.2 (MeOH:$CHCl_3$ (2: 8)). ^{1}HNMR (400Mz, DMSO-d6): δ 9.10 (d, *J*=7.6Hz, 2H), 8.55 (m, 2H), 8.03 (d, *J*=8.4Hz, 4H), 7.94 (d, *J*=8.4Hz, 2H), 7.88 (dd, *J*=13.4, 8.4Hz, 4H), 7.76 (d, *J*=9.2Hz, 2H), 3.88 (s, 3H), 3.79 (s, 3H). MS-APCI: 502.3 [M-H]

4'-{4-[4-(4,5-dihydro-1H-imidazol-2-yl)-phenylamino]-phthalazin-1-ylamino}-biphenyl-4-carboxylic acid methyl ester (7) A mixture of 4'-[4-(4-methoxycarbonimidoyl-phenylamino)-phthalazin-1-ylamino]-biphenyl-4-carboxylic acid methyl ester **17** (305 mg, 0.61 mmol) and ethylenediamine (0.12 mL, 1.82 mmol) in ethanol (10 mL) was heated at 110 ^oC for 2h. The solvent was removed under reduced pressure and the remaining oil was diluted with water. The resulting solid was filtered, washed with water and dried under vacuum. The crude compound was triturated with 5%MeOH:diethylether to afford 262 mg (49%) of 4'-{4-[4-(4,5-dihydro-1H-imidazol-2-yl)-phenylamino]-phthalazin-1-ylamino}-biphenyl-4-carboxylic acid methyl ester **7** as a yellow solid. R_f: 0.2 (MeOH.NH_3:$CHCl_3$ (1: 9)). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.20 (s, 1H), 9.11 (s, 1H); 8.58 (m, 1H), 8.53 (m, 1H), 8.10-8.00 (m, 6H), 7.94 (d, *J*=8.8 Hz, 2H), 7.85 (d, *J*=8.4 Hz, 2H), 7.81 (d, *J*=9.2Hz, 2H); 7.76 (d, *J*=8.8Hz, 2H), 3.88 (s, 3H), 3.70 (s, 4H). MS (ESI): m/z 514.9 [M+H]. HPLC prurity: 99%

4-[4-(Biphenyl-4-ylamino)-phthalazin-1-ylamino]-benzonitrile (14). A mixture of 4-(4-chloro-phthalazin-1-ylamino)-benzonitrile (**13**) (200 mg, 0.7 mmol) and biphenyl-4-ylamine (144 mg, 0.8 mmol) in NMP (2 mL) was stirred in a sealed tube at 110 °C for 3h. The reaction mixture was diluted with water and the precipitate was filtered. The residue was washed with water and dried under reduced pressure. The crude compound was triturated with diethyl ether and dried under reduced pressure to afford 280 mg (94%) of **14** as a yellow solid, which was used without further purification. R_f: 0.30 (Diethyl ether).

N-Biphenyl-4-yl-N'-[4-(4,5-dihydro-1H-imidazol-2-yl)-phenyl]-phthalazine-1,4-diamine (2). A mixture of 4-[4-(biphenyl-4-ylamino)-phthalazin-1-ylamino]-benzonitrile (**14**) (200 mg, 0.48 mmol), sulfur (30 mg, 0.9 mmol) and ethylenediamine (0.3 mL, 4.8 mmol) were stirred at 110 °C for 2h. The reaction was diluted with water, and the resulting precipitate was filtered, washed with water and dried under reduced pressure. The crude compound was purified by column chromatography on neutral alumina using 5% of MeOH/$CHCl_3$ as eluent to afford 160 mg (72%) of **2** as a yellow solid. R_f: 0.30

(MeOH: $CHCl_3$ 1:9). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 9.06 (s, 1H), 8.60 (m, 1H), 8.52 (m, 1H), 8.05 (m 2H), 8.00 (t, *J* = 8.4 Hz, 4H), 7.84 (d, *J* = 9.2 Hz, 2H), 7.67 (t, *J* = 7.2 Hz, 4H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.32 (t, *J* = 7.6 Hz, 1H), 3.78 (s, 4H). MS (APCI): m/z 457.2 [M+H]. HPLC purity: 98.2%

{4-[4-(4-Cyano-phenylamino)-phthalazin-1-ylamino]-benzyl}-carbamic acid tert-butyl ester (15a). A mixture of 4-(4-chloro-phthalazin-1-ylamino)-benzonitrile (**13**) (200 mg, 0.71 mmol) and (4-amino-benzyl)-carbamic acid tert-butyl ester (158 mg, 0.71 mmol) in *N*-methylpyrrolidone (0.3 mL) was heated in a sealed tube at 120 °C for 3 h. The reaction mixture was diluted with water and filtered to obtain 300 mg of crude **15a** as a yellow solid. The crude compound was used as such in the next step without further purification. R_f: 0.2 (EtOAc:$CHCl_3$ 3:7).

4-[4-(4'-Aminomethyl-biphenyl-4-ylamino)-phthalazin-1-ylamino]-benzonitrile (15b). A solution of crude 4-[4-(4-cyano-phenylamino)-phthalazin-1-ylamino]-benzyl}-carbamic acid tert-butyl ester **15a** (300 mg, 0.64 mmol) in CH_2Cl_2 was treated with triflouro acetic acid (0.19 mL, 2.57 mmol) for 4 h at RT. After the reaction was completed the solvent was removed followed by neutralization with saturated $NaHCO_3$. The resulting solid was filtered and dried under vacuum to give 200 mg of (76%) of **15b** as a yellow solid. R_f: 0.3 (Aq. Ammonia:THF 1:9). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.40-9.80 (br s, 2H), 8.65 (d, *J* = 5.2 Hz, 1H), 8.56 (d, *J* = 5.6 Hz, 1H), 8.40-8.00 (m, 4H), 7.93 (d, *J* = 8.0 Hz, 2H), 7.80 (d, *J* = 7.2 Hz, 2H), 7.79 (*J* = 8.0 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 2H), 4.02 (d, *J* = 4.0 Hz, 2H); MS (APCI) m/z 367 [M+H].

N-(4-aminomethyl-phenyl)-N'-[4-(4,5-dihydro-1H-imidazol-2-yl)-phenyl]-phthalazine-1,4-diamine (5). A solution of 4-[4-(4-aminomethyl-phenylamino)-phthalazin-1-ylamino]-benzonitrile **15b** (200 mg, 0.54 mmol) in EtOH was purged with HCl gas for 18h at RT. The reaction mixture was degassed with argon for 15 min and neutralized with sat. aq $NaHCO_3$. The resulting solid was filtered and washed with water. The solid was triturated with diethyl-ether to afford of 150 mg (66%) of 4-[4-(4-aminomethyl-phenylamino)-phthalazin-1-ylamino]-benzimidic acid ethyl ester as yellow solid, which was immediately used without further purification. R_f: 0.2 (Aq.Ammonia : THF 2:8). MS (APCI)m/z 413 [M+1]. A mixture of 4-[4-(4-aminomethyl-phenylamino)-phthalazin-1-ylamino]-benzimidic acid ethyl ester (200 mg, 0.48 mmol) and ethylenediamine (0.13 mL, 1.94 mmol) in EtOH was refluxed at 90 ^{o}C for 4 h. The reaction mixture was concentrated, diluted with cold water (5 mL) and filtered. The remaining solid was washed with water. The crude residue was purified by column chromatography on neutral alumina using 15-20% MeOH/$CHCl_3$ as eluent to afford 50 mg (25%) of **5** as a yellow solid. R_f: 0.25 (Aq. Ammonia: THF 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (br s, 2H), 9.00 (br s, 1H), 8.60 -8.40 (m, 4H), 8.20 -7.60 (m, 7H), 7.36 (d, *J* = 8.0 Hz, 2H), 7.20 (m, 1H), 3.90-3.75 (m, 6H). MS (APCI) m/z 410 [M+H].

HPLC purity: 98.7%

Biphenyl-4-yl-(4-chloro-phthalazin-1-yl)-amine (18). A mixture of biphenyl-4-ylamine (100 mg, 0.59 mmol) and 1,4 dichloropthalazine (**10**) (196 mg, 0.98 mmol) in dry *tert*-butanol (2 mL) was refluxed for 2h. The reaction mixture was cooled, diluted with water and the resulting solid was filtered. The crude solid was purified over neutral alumina using 5-10% of ethyl acetate/pet ether as a eluent to afford 55 mg (20%) of **18** as a pale brown solid. R_f: 0.5 (EtOAc:Pet ether 4:6 in neutral alumina TLC plate). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H), 8.71 (d, *J* = 7.2Hz, 1H), 8.25-8.10 (m, 3H), 8.00 (d, *J* = 8.4Hz, 2H), 7.75-7.65 (m, 4H), 7.47 (t, *J* = 7.6Hz, 2H), 7.35 (t, *J* = 7.2Hz, 1H). MS (APCI) m/z 332 [M+H]

1-{7-[4-(Biphenyl-4-ylamino)-phthalazin-1-ylamino]-3,4-dihydro-1H-isoquinolin-2-yl}-ethanone (19). A mixture of 1-(7-amino-3,4-dihydro-1H-isoquinolin-2-yl)-ethanone (150 mg, 0.453 mmol) and **18** (112 mg, 0.59 mmol), in NMP (0.3 mL) in sealed tube was heated at 120 °C for 3h. Reaction mixture was diluted with water and the resulting solid was filtered and dried. The crude solid was purified by column chromatography on silica gel (100-200 mesh) using 0.5-5% gradient mixture of MeOH:$CHCl_3$ to afford 180 mg (82%) of **19** as an yellow solid. R_f: 0.4 (MeOH: $CHCl_3$ 1:9). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.92 (br s, 1H), 8.80 (br s, 1H), 8.51 (br s, 2H), 8.00 (br s, 2H), 7.91 (br d, 2H), 7.70-7.50 (m, 6H), 7.50-7.35 (m, 2H), 7.29 (br s, 1H), 7.11 (br s, 1H), 4.63 (s, 1H), 4.58 (s, 1H), 3.65 (br s, 2H), 2.90-2.65 (m, 2H), 2.08 (s, 3H). MS(APCI): m/z 486 [M+H]

N-Biphenyl-4-yl-N'-(1,2,3,4-tetrahydro-isoquinolin-7-yl)-phthalazine-1,4-diamine (4). Conc. HCl (2 mL) was added to a cold solution (0 °C) of **19** (180 mg, 0.37 mmol) in methanol (10 mL), and the resulting mixture was refluxed for 48h. The solvent was removed and the crude mixture was triturated with methanol and diethyl ether to afford 59 mg (36%) of **4** as a yellow solid. R_f: 0.1 (MeOH: $CHCl_3$ 2:8). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.43 (br 3H), 8.83 (br s, 2H), 8.24 (m, 2H), 7.79-7.60 (m, 5H), 7.60-7.40 (m, 4H), 7.40-7.20 (m, 3H), 4.27 (s, 2H), 3.40 (br s, 2H), 3.02 (br s, 2H), MS (APCI) m/z 444 [M+H]. HPLC purity: 98.2%

1-{6-[4-(Biphenyl-4-ylamino)-phthalazin-1-ylamino]-3,4-dihydro-1H-isoquinolin-2-yl}-ethanone 20. A mixture of 1-(6-amino-3,4-dihydro-1H-isoquinolin-2-yl)-ethanone (190 mg, 0.574) and **18** (120 mg, 0.63) in dry NMP was heated at 120 °C for 3 h. The reaction mixture was cooled to RT and diluted water. The resulting solid was filtered and dried under vacuum. The crude compound was filtered through a plug of silica gel (100-200 mesh) using 5% of MeOH/DCM as eluent to afford 78 mg (28%) of **19** as yellow solid, which was used without further purification. R_f: 0.40 (MeOH: $CHCl_3$ 1:9).

N-Biphenyl-4-yl-N'-(1,2,3,4-tetrahydro-isoquinolin-6-yl)-phthalazine-1,4-diamine (6). A solution of **20** (73 mg, 0.15 mmol) in conc.HCl/EtOH (1:3; 2 mL) was refluxed for 24h. The reaction mixture was concentrated under vacuum. The resulting solid was triturated with MeOH (4 mL), diethyl ether (5 mL) and dried under vacuum to afford 56 mg (84%) of **6** as an ash color solid. R_f: 0.30 (MeOH:$CHCl_3$ 3:7). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.45 (br s, 2H), 8.85 (s, 2H), 8.25 (m, 2H), 7.85-7.65 (m, 4H), 7.72 (d, *J* = 7.2Hz, 2H), 7.60-7.45 (m, 2H), 7.49 (t, *J* = 7.2Hz, 2H), 7.37 (t, *J* = 7.2Hz, 1H), 7.27 (d, *J* = 7.2Hz, 1H), 4.26 (s, 2H), 3.45-3.30 (m, 2H), 3.02 (m, 2H). MS (APCI) m/z 444 [M+1]. HPLC purity: 98.1%

N-(1,4-dioxo-1,2,3,4-tetrahydro-phthalazin-6-yl)-acetamide (22). A mixture of N-(1,3-dioxan-2,3-dihydro-1H-isoindol-yl)-acetamide (**21**) (1.2 g, 5.88 mmol) and hydrazine hydrate (6 mL) was heated at 80 ^{o}C for 1h. The reaction mixture was diluted with water and the pH was adjusted to 4 with acetic acid. The resulting precipitate was filtered, washed with water and dried under vacuum. The crude solid was triturated with diethyl ether to afford 1.1g (86%) of **22** as off-white solid. R_f: 0.55 (MeOH:$CHCl_3$ 1:4). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.46 (s, 1H), 8.37 (s, 1H), 8.05-7.90 (m, 2H), 2.12 (s, 3H); MS (APCI): m/z 220 [M+H]

N-(1,4-dichloro-pthalazin-6-yl)-acetamide (23). DIPEA (2.6 mL, 15.06 mmol) was added to a mixture of N-(1,4-dioxan-1,2,3,4-tetrahydro-pthlazin-6-yl)-acetamide (**22**) (1.1 g, 5.02 mmol) and $POCl_3$ (2.8 mL, 30.13 mmol) at RT and the resulting mixture was heated at 100 ^{o}C for 1h. The reaction mixture was poured into ice and the resulting precipitate was filtered, washed with water and dried under vacuum. The crude solid was triturated with diethyl ether to afford 850 mg (66%) of **23**. R_f: 0.6 (MeOH:$CHCl_3$ 2:8). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.90 (s, 1H), 8.76 (s, 1H), 8.33 (d, J=4.8 Hz, 1H), 8.22 (d, J=8.8Hz, 1H); 2.19 (s, 3H). MS (APCI): m/z 255 [M+H], 258 [(M+2)+H].

N-(1, 4-Bis-(4-cyano-phenylamino)-pthalazin-6-yl)-acetamide (24). A mixture of N-(1,4-dichloro-phthalazin-6-yl)-acetamide (**23**) (400 mg, 1.56 mmol) and 4-amino benzonitrile (737 mg, 6.24 mmol) in NMP (2.5 mL) was stirred at 140 ^{o}C in a sealed tube for 3h. The reaction mixture was cooled to RT and poured into water and filtered. The precipitate was washed with water and dried under vacuum. The crude solid was purified by column chromatography on silica gel (100-200 mesh) using 45-50% EtOAc-Hexane as eluent to afford 110 mg (17%) of **25** as pale yellow solid. R_f: 0.24 (EtOAc:Hexane (8:2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.57 (s, 1H), 9.50 (s, 1H), 9.44 (s, 1H), 8.56 (s, 1H), 8.48 (d, *J* = 8.8Hz, 1H), 8.10 (d, *J* = 8.8Hz, 1H), 8.04 (d, *J* = 9.2Hz, 2H), 7.80 (d, *J* = 8.8Hz, 2H), 7.74 (d, *J* = 8.4Hz, 2H), 7.71 (d, *J* = 8.4Hz, 2H), 2.16 (s, 3H). MS (APCI): 420.3 [M+H].

N-(1,4-Bis-(4-cyano-phenylamino)-phthalazin-6-yl)-acetamide (25). A mixture of **24** (150 mg, 0.36 mmol) and sulphur (69 mg, 2.15 mmol) in ethylenediamine (5 mL) was heated at 120 ^{o}C for 2 h. The reaction mixture was cooled to RT, methanol was added and the resulting precipitate was filtered. The crude solid was filtered through a plug of neutral alumina using 5-10% MeOH/$CHCl_3$ as eluent to afford 35 mg (18%) of **25** as pale yellow solid, which was used without further purification. R_f: 0.5 (MeOH: $CHCl_3$ 2:8).

N'1', N'4'-Bis-(4-(4, 5-dihydro-1H-imidaol-2-yl)-phenyl)-pthalazin-1, 4, 6-triamine (9). A solution of N-(1,4-bis-(4-cyano-phenylamino)-phthalazin-6-yl)-acetamide (**25**) (200 mg, 0.396 mmol) in ethanol (20 mL) was purged with HCl gas for 20 min and refluxed for 4h. The solvent was removed by distillation and the crude residue was stirred in MeOH/NH_3 for 10 min and concentrated. The crude residue was purified by column chromatography on neutral alumina using 10-20% MeOH/$CHCl_3$ as eluent to afford 75 mg (34%) of **9** as pale yellow solid. R_f: 0.25 (30% MeOH/$CHCl_3$/4 drops aq.NH_3). ^{1}H NMR (400 MHz, DMSO-d6): δ 9.45 (s, 1H), 9.41 (s, 1H), 8.24 (d, *J* = 8.4Hz, 1H), 8.03 (d, *J* = 8.8Hz, 2H), 8.00-7.90 (m, 4H), 7.85 (d, *J* = 8.0Hz, 2H), 7.27 (d, *J* = 9.2Hz, 1H), 7.23 (s, 1H), 6.32 (br s, 2H), 3.97 (s, 8H). MS (APCI) 462 [M-H]. HPLC: 98.1%