



## Supporting Information

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Supplementary Online Material.

### **Library synthesis:**

As outlined in Figure 3, the starting crown, TFA·NH<sub>2</sub>-polystyrene lantern (37 μmol/lantern) **1**, was treated with a dimethylformamide/triethylamine mixture to deliver the free amine which was reacted with 4-(4'-Carboxybutyloxy)-2-methoxybenzaldehyde, TBTU and N-methylmorpholine to afford lantern-bound **2**. Lantern-bound **2** was then reductively aminated with the first building block and sodiumcyanoborohydride to yield the lantern-bound amine **3** which was acylated with 3-nitro-4-fluorobenzoic acid to yield lantern-bound o-nitrophenylfluoride **4**. When the amine component of the reaction mixture was replaced by methanol, lantern-bound alcohol **9** was obtained. This was transformed into lantern-bound ester **10** which enabled synthesis of carboxylic acid derivatives. At this stage the second building block was introduced by reacting the lanterns with the second amine. The obtained lantern-bound o-nitroaniline **5** was then reduced to the lantern-bound o-phenylenediamine **6**. Several published procedures based on heavy metal catalyzed reductions were unsuccessfully tried. Results were difficult to reproduce and products were always contaminated with a benzimidazole, arising from incorporation of dimethylformamide or dimethylacetamide during reduction. After numerous attempts we found two distinct procedures allowing clean and reproducible reduction of **5** to **6**. Tetrabutylammonium hydrogen sulfide in a toluene/ethanol mixture at 80 degrees or NaBH<sub>2</sub>S<sub>3</sub> in tetrahydrofuran at 60 degrees proved useful. The final lantern-bound benzimidazole **7** was obtained from **6** by reaction with an aldehyde and dichlorodicyanoquinone. Upon treatment with trifluoroacetic acid in dichloroethane, the final crude trisubstituted benzimidazoles **8** were isolated from the lanterns. After evaporation of the solvent, the final products were dissolved in DMA and purified by RP-HPLC/MS.

### **Base Liberation:**

Procedure:

Double lanterns were covered with a mixture of DMF/Et<sub>3</sub>N 7:3 and left 3 hrs at room temperature. The solvent was removed and the lanterns were washed with DMF (3 times) and with DCM (3 times). The lanterns were dried 1h under high vacuum.

### **Linker attachment:**

4-(4'-Carboxybutyloxy)-2-methoxybenzaldehyde (0.2M) was added to a solution of HOBt (0.2M), DIC (0.2M) and NMM (0.2M) in DMF. Lanterns were added to the mixture and left for 24 hrs at room temperature. The solvent was removed and the lanterns were washed with DMF (3 times), DCE at 50 deg. for 2h and with DCM (3 times). The lanterns were dried 1h under high vacuum.

### **Linker reduction:**

Lanterns were added to a mixture of MeOH (0.4M), NaBH<sub>3</sub>CN (0.1M) in Ti(OiPr)<sub>4</sub> / DCE 1:1. The mixture was left at 60 deg for 6 hrs in closed vessels. The solvent was removed and the lanterns were washed with DCM (2

times), DCE at 50 deg. for 2h, MeOH/NMM 8:2 at 50 deg. for 2h and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Linker reductive amination:**

Lanterns and NaBH<sub>3</sub>CN (0.1M) were added to a mixture of a building block (0.4M) in Ti(OiPr)<sub>4</sub> / DCE 1:1. The mixture was left at 60 deg for 6 hrs in closed vessels. The solvent was removed and the lanterns were washed with DCM (2 times), DCE at 50 deg. for 2h, MeOH/NMM 8:2 at 50 deg. for 2h and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Step 1 : Reaction with 3-nitro-4-fluoro benzoic acid.**

Lanterns were added to a mixture of 3- nitro-4-fluoro benzoic acid (0.2M), HATU (0.2M) and NMM (0.3M) in DMF. The mixture was left at room temperature for 24 hrs in closed vessels.

The solvent was removed and the lanterns were washed with DMF (2 times), DCE at 50 deg. for 2h, and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Step 2 : Amination.**

Lanterns were added to a mixture of a building block (0.3M) in DMSO. The mixture was left at 50 deg for 48 hrs in closed vessels. The solvent was removed and the lanterns were washed with DMSO (2 times), MeOH/Et<sub>3</sub>N 8:2 at 50 deg. for 2h, DCE at 50 deg. for 2h and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Step 3 : Reduction.**

Lanterns were added to a mixture of NaBH<sub>4</sub> (0.3M) and S<sub>8</sub> (0.9M) in THF. The mixture was left at 60 deg. for 18 hrs in closed vessels. The solvent was removed and the lanterns were washed with THF (2 times), Toluol/EtOH 9:1 (3 times) and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Step 4 : Cyclisation.**

Lanterns were added to a mixture of a building block (0.2M) and DDQ (0.1M) in DMA/H<sub>2</sub>O 9:1. The mixture was left at room temperature for 24 hrs in closed vessels. The solvent was removed and the lanterns were washed with DMF (2 times), DCM/Et<sub>3</sub>N (dry) 9:1 (2 times), DCE at 50 deg. for 2h and with DCM (3 times). The lanterns were dried 1h under high vacuum.

#### **Step 5 : Cleavage.**

Lanterns were added to a solution of 0.05M Anisol in TFA. The mixture was left at room temperature for 18 hrs in closed vessels. Lanterns were removed and the solvents evaporated to yield the crude product which was subjected to preparative HPLC/MS chromatography.

#### **Purification and analysis of the library.**

Introduction of samples occur by automated injection into the preparative HPLC column from a Gilson 233XL autosampler with an injection loop of 1 mL. The autosampler accomodates two mega Racks holding 5.5 mL glass tubes. Separation are done by linear gradient elution of 5 min. from 5% aqueous acetonitrile to 95% aqueous acetonitrile both containing 0.1% trifluoroacetic acid. Samples are eluted on a 20x50 mm C18 Waters Symmetry column using 20 mL/min. flow rates. The target compounds are identified by electrospray ionization and collected by the automatic detect-before-collect routine. Fractions are collected using a Gilson 204 fraction collector accomodating 2 mega racks. The expected product from each sample present in the input Rack is collected in one fraction (maximum 5.5 mL, tared glass tubes), based on mass detection, and placed at the same position in the output Rack.

The system automatically reports the purity of a target compound in each well, based on peak area ratio measurements taken from the combined mass traces sampled over the complete fraction collection time<sup>10</sup>.

Solvents from all collected fractions are evaporated in a Genevac Atlas evaporation system and the dried samples are weighed in order to record the amount of purified compound.

### Resynthesis of Hits:

A typical procedure is illustrated for the synthesis of AEX205:

#### **2-(4-Chloro-phenyl)-1H-benzimidazole-5-carboxylic acid methyl ester**

4-chlorobenzaldehyde (1 g, 7.11 mmoles) was dissolved in ethanol (25 mL) and after addition of an aqueous solution of NaHSO<sub>3</sub> (0.8 g, 7.11 mmoles) the mixture was stirred for 1 h at room temperature. The solvent was evaporated. The residue was taken up in DMA (30 mL) and methyl-3,4-diaminobenzoate (1 eq., 1.2g) was added. The mixture was heated to 110 degrees overnight. The solvent was evaporated and the residue crystallized from acetone/hexane.

Yield: 1.4216g brown solid

TLC analysis: DCM/Methanol (95:5)

<sup>1</sup>H NMR (CDCl<sub>3</sub>): 3.3 (1H, bs, NH), 3.9 (3H, s, OMe), 7.65 (2H, d, 9Hz, 2x ArH), 7.70 (1H, d, 9Hz, ArH), 7.85 (1H, d, 9Hz, ArH), 8.15 (1H, s, ArH), 8.2 (2H, d, 9Hz, 2x ArH), 13.3 (1H, bs, NH)

MS (ESI) 287.1 (MH<sup>+</sup>)

#### **2-(4-Chloro-phenyl)-1H-benzimidazole-5-carboxylic acid**

2-(4-Chloro-phenyl)-1H-benzimidazole-5-carboxylic acid methyl ester (536 mg, 1.87 mmoles) was dissolved in ethanol (4 mL) and after addition of NaOH (299 mg, 4 eq.) dissolved in water the mixture was stirred for 4h at 80 degrees. The solvent was evaporated and the residue taken up in water and neutralized dropwise with 2N HCl. The product precipitated and was filtered off and washed with water. The product was suspended in DCM/Methanol (9:1), sonicated to obtain a light brown powder and filtrated. The powder was washed two times with ethyl acetate.

Yield: 333 mg off white solid

TLC analysis: DCM/Methanol (95:5)

<sup>1</sup>H NMR: 3.3 (1H, bs, NH), 7.7 (3H, d, 9Hz, 3x ArH), 7.85 (1H, d, 9Hz, ArH), 8.15 (1H, s, ArH), 8.2 (2H, d, 9Hz, ArH), 12.75 (1H, bs, COOH)

MS (ESI) 273.1 (MH<sup>+</sup>) 271.1 (MH)

### Biological Evaluation

The MN9D cell line that shows dopaminergic neuronal properties was used as the parental cell line. MN9D is a clonal hybrid cell line derived from the ventral mesencephalon of embryonic mice by somatic fusion with a murine neuroblastoma cell line. To establish a Nurr1 screening cell line, MN9D cells were first stably transfected with a Nurr1 expressing plasmid. Shortly, mouse Nurr1 was cloned into a pTRACER vector. In this vector the expression of Nurr1 is under the control of a CMV promoter while a second SV40 promoter drives the expression of a zeocin-GFP fusion gene which allows the selection of stably transfected, zeocin-resistant cells. The expression of biologically functional Nurr1 was confirmed by measuring the activity of tyrosine hydroxylase (TH), an enzyme whose expression is regulated by Nurr1. A clone showing about a 4-fold increase in TH activity was chosen for the generation of the screening cell line. This was achieved by transfecting the cells with a reporter plasmid in which firefly luciferase expression was controlled by multiple copies of a Nurr1 specific DNA binding element. To demonstrate that luciferase expression is indeed under the control of Nurr1, a control cell line was established in which a retinoic acid receptor (RXRalpha) is also overexpressed. RXRalpha can form heterodimers with Nurr1 so that Nurr1 responsive elements can become retinoic acid inducible. In fact, the luciferase reporter gene activity in the resulting RXRalpha/Nurr1 overexpressing cell line was strongly induced by 9-cis retinoic acid, in contrast of the Nurr1 expressing lines, thus showing that the reporter gene was under the control of Nurr1 response elements (not shown). As a negative control to distinguish between Nurr1 selective versus general inducers, a renilla luciferase control plasmid (Promega Corp.) was co-transfected. This plasmid expressed renilla luciferase under the control of a thymidine kinase minimal promoter and does not contain any specific Nurr1 specific DNA binding sites.