Supporting Information for:

Virtual Screening for Bioactive Molecules by Evolutionary De Novo Design

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EXPERIMENTAL SECTION

Biological assay. Activity of compounds was determined using a mammalian CHO cell line expressing human Kv1.5 (hKv1.5) voltage-gated potassium channel.[1] Potassium outward current was measured in the whole-cell configuration of the patch clamp technique.[2] The amplitude of the hKv1.5 current was assessed at the end of depolarizing steps to +40 mV from a holding potential of –70 mV at room temperature. Deactivation of tail current was recorded upon re-polarization to –50 mV. Pipette solution contained (mM): 130 KCl, 1 $MgCl_2$, 10 Hepes, 5 BAPTA, 3 Na_2ATP, 5 glucose; pH was adjusted to 7.2. External solution contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 Hepes, 5 glucose; pH was adjusted to 7.3.

Structure modeling. For prediction of favorable three-dimensional conformations the CORINA software was used.[3] Assignment of the absolute configuration was done according to

Castle and coworkers,[4] with (2S),(3S)-configuration of the biologically active enantiomer. Refinement of the structures was done with the modeling package MOLOC and the MAB force-field.[5] Automated pharmacophore matching was performed with MOLOC setting equal weights on charge, hydrogend-bond donor, and lipophile overlap. The resulting model was slightly manually refined.

Chemical synthesis. All reagents and chemicals were obtained commercially from Fluka Chemie AG and were used without further purification. 7-Methoxynaphthalene-2-sulfonyl chloride was prepared as described elsewhere.[6] NMR spectra were recorded with a Bruker 300- or 400-MHz spectrometer.

(2-Methoxy-phenyl)-(2-nitro-phenyl)-amine **1.** A mixture of 1.16 g (20 mmol) KF, 3.69 g (30 mmol) *o*-anisidine and 2.82 g (20 mmol) 1-fluoro-2-nitrobenzene was stirred at 165 °C for 24 h. The crude product was purified by chromatography on silica gel (5% *tert*-butyl methyl ether/hexane) to give 5.14 g (19.8 mmol, 99 %) of **1** as a red solid. ^{1}H – NMR ($CDCl_3$, 300 MHz): δ = 3.88 (*s*, 3H, OCH_3), 6.78 (*t*, 1H, ar), 7.03 – 7.42 (m, 6H, ar), 8.21 (*d*, 1H, ar); MS [M^+] = 244.

N–(2-Methoxy-phenyl)–benzene-1,2-diamine **2.** A mixture of 1.22 g (5 mmol) of **1**, 120 mg of 10% palladium on carbon, and 10 ml of methanol was stirred in an atmosphere of hydrogen for 12 h. The catalyst was filtered, the filtrate was concentrated in vacuo giving 1.05 g of an orange oil. The crude product was purified by chromatography on silica gel (10% EtOAc/hexane) to

give **2** (940 mg, 87.9%) as a colorless crystalline solid. ^{1}H – NMR ($CDCl_3$, 400 MHz): δ = 3.79 (*s*, 2H, NH_2), 3.92 (*s*, 3H, OCH_3), 5.68 (*s*, 1H, NH), 6.63 (*dd*, *J* = 2.0, 7.3 Hz, 1H, ar), 6.73 – 6.82 (m, 4H, ar), 6.87 (*dd*, *J* = 2.0, 7.3 Hz, 1H, ar), 7.02 (*dt*, *J* = 1.5, 7.8 Hz, 1H, ar), 7.14 (*dd*, *J* = 1.5, 7.8 Hz, 1H, ar); MS [M^+] = 214.

Naphthalene-2-sulfonic acid [2-(2-methoxy-phenylamino)-phenyl]-amide **3a**. To a solution of 150 mg (0.7 mmol) diamine **2** and 78 mg (0.7 mmol) DMAP in 5 ml CH_2Cl_2, naphthalene-2-sulfonyl chloride (158 mg, 0.7 mmol) was added. The homogeneous solution was stirred at room temperature for 12 h and diluted with methylene chloride (5 ml) and water (10 ml). The organic layer was separated, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude red oil was purified by chromatography over silica gel (10% EtOAc/hexane) yielding **3a** (117 mg, 42%) as a colorless crystalline solid. ^{1}H – NMR ($CDCl_3$, 400 MHz): δ = 3.78 (*s*, 3H, ar-OCH_3), 5.62 (*s*, 1H, NH), 6.20 (*dd*, *J* = 1.47, 7.83 Hz, 1H), 6.50 (*td*, *J* = 1.47, 7.34 Hz, 1H), 6.73 – 6.81 (m, 2H), 7.03 – 7.12 (m, 4H), 7.48 – 7.65 (m, 3H), 7.70 (*dd*, *J* = 1.47, 8.31 Hz, 1H), 7.86 (*t*, *J* = 9.29, 3H), 8.36 (*s*, 1H); MS [M^+-H] = 403. For preparation of **3b** alkaline treatment of the crude material of the condensation reaction was required to hydrolyse the *N,N*-bis-(7-methoxynaphthalene-2-sulfonyl)-amido derivative which had formed as major product.

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