## Supporting Information

### A. Supplementary Tables

**Table S1.** Effects of selected organic compounds on [$^3$H]clonidine (A) and [$^3$H]naloxone (B) uptake in hCMEC/D3 cells**.**

**A**

| Compound | Concentration (mM) | Relative uptake [$^3$H]clonidine (%) |
|---|---|---|
| Control | | 100 ± 4.9 |
| TEA | 2 | 92.4 ± 2.0 |
| Nicotine | 0.1 | 59.6 ± 1.9** |
| Diphenhydramine | 0.05 | 35.4 ± 1.6*** |
| Verapamil | 0.05 | 14.5 ± 0.8*** |
| Codeine | 0.05 | 58.1 ± 2.2*** |
| Naloxone | 0.1 | 30.7 ± 12.0* |
| Oxycodone | 0.05 | 48.8 ± 7.4* |
| Cocaine | 0.05 | 43.1 ± 0.2*** |

**B**

| Compound | Concentration (mM) | Relative uptake [$^3$H]naloxone (%) |
|---|---|---|
| Control | | 100 ± 9.8 |
| TEA | 5 | 108.1 ± 10.3 |
| Carnitine | 5 | 105.3 ± 4.9 |
| Choline | 5 | 99.2 ± 9.9 |
| Nicotine | 0.1 | 75.0 ± 4.0*** |
| Clonidine | 0.05 | 49.7 ± 1.6 *** |
| Diphenhydramine | 0.05 | 31.3 ± 1.3 *** |
| Verapamil | 0.05 | 25.7 ± 1.7 *** |
| Codeine | 0.05 | 52.5 ± 2.0 *** |
| Oxycodone | 0.1 | 46.5 ± 2.1*** |
| Cocaine | 0.05 | 61.3 ± 3.6*** |

The inhibition of the transport of [$^3$H]clonidine and [$^3$H]naloxone (~2 nmol.L$^{-1}$) by the compounds above was evaluated in hCMEC/D3 cell an incubation time of 5 min in Krebs-HEPES buffer ($pH_e$ 7.40). Data are expressed as means ± SD (performed in triplicate). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ compared to the control group.

**Table S2.** Inhibition potency of selected organic compounds against [$^3$H]cocaine (A) and [$^3$H]naloxone (B) transport in hCMEC/D3 cells.

**A**

| Compound | $IC_{50}$ ($\mu mol.L^{-1}$) |
|---|---|
| Imipramine* | 0.20 ± 0.05 |
| Desipramine | 0.33 ± 0.05 |
| Amitriptyline | 0.42 ± 0.05 |
| Nortriptyline | 0.48 ± 0.12 |
| Clomipramine | 0.50 ± 0.07 |
| Methadone | 0.50 ± 0.05 |
| Doxepin | 0.71 ± 0.10 |
| Verapamil* | 1.48 ± 0.15 |
| Diphenhydramine | 1.60 ± 0.21 |
| Buprenorphine* | 4.67 ± 0.86 |
| MDMA | 5.02 ± 0.61 |
| Norbuprenorphine* | 5.75 ± 1.60 |
| Cocaethylene | 5.79 ± 0.60 |

**B**

| Compound | $IC_{50}$ (µM) |
|---|---|
| Imipramine* | 1.0 ± 0.3 |
| Amitriptyline | 1.1 ± 0.1 |
| Clomipramine | 1.1 ± 0.2 |
| Nortriptyline | 1.4 ± 0.4 |
| Desipramine | 1.4 ± 0.2 |
| Doxepine | 2.3 ± 0.5 |
| Methadone | 4.8 ± 0.8 |
| Diphenhydramine | 8.6 ± 2.1 |
| Norbuprenorphine* | 9.8 ± 1.6 |
| Buprenorphine* | 13.9 ± 1.5 |
| Cocaethylene | 15.5 ± 3.0 |
| Verapamil* | 15.5 ± 2.5 |
| MDMA | 16.0 ± 3.2 |

Determination of $IC_{50}$ for previously selected G-I compounds on [$^3$H]cocaine (A) and [$^3$H]naloxone (B) uptake in hCMEC/D3 cells. The uptake of the labeled drug was measured for 5 min in hCMEC/D3 cells and plotted against the inhibitor at 7 selected concentrations (0.1; 1, 10, 50; 100; 1000 and 2000 µM depending on the compound solubility) in Krebs-HEPES incubation medium ($pH_e$ 7.40; 37°C). Results were fitted according to equation 2.

* Compounds were tested at lower concentration due to lack of solubility: ≤500 $\mu mol.L^{-1}$ for buprenorphine and ≤1 $mmol.L^{-1}$ for norbuprenorphine, imipramine and verapamil. MDMA (3,4-methylene-dioxy-methamphetamine).

**Table S3.** Predicted pKa value and charge of the most abundant species at pH=7.4.

| Compound | pKa[(1)] | charge at pH=7.4 | Compound | pKa[(1)] | charge at pH=7.4 |
|---|---|---|---|---|---|
| Nortriptyline | 9.61(b) | positive | Clonidine | 8.52 | positive |
| Desipramine | 9.88(b), 5.04(b) | positive | Codeine | 8.40(b) | positive |
| Imipramine | 9.04(b), 4.66(b) | positive | Oxymorphone | 7.80(b), 9.39(a) | positive |
| Amitriptyline | 8.69(b) | positive | Hydromorphone | 8.35(b), 9.08(a) | positive |
| Quinine | 8.11(b), 4.18(b) | positive | Naloxone | 7.30(b), 9.39(a) | positive |
| Methadone | 8.61(b) | positive | Nicotine | 8.98(b), 3.11(b) | positive |
| Verapamil | 8.93 (b) | positive | Morphine | 8.40(b), 9.48(a) | positive |
| MDMA | 9.47(b) | positive | Dihydromorphine | 9.15(b), 9.91(a) | positive |
| Buprenorphine | 8.68(b), 9.87(a) | positive | Cotinine | 4.90(b) | neutral |
| Desomorphine | 9.19(b),10.25(a) | positive | Ecgonine | 10.24(b), 3.57(a) | zwitterion |
| Norbuprenorphine | 9.95(b),10.55(a) | positive | Tetraethylammonium | - | positive[(2)] |
| Diphenhydramine | 8.55(b) | positive | Benzoylecgonine | 9.66(b), 3.12(a) | zwitterionic |
| Cocaethylene | 8.23(b) | positive | Choline | - | Positive[(2)] |
| Norcocaine | 9.46(b) | positive | Carnitine | 3.85(a) | zwitterion |
| 6-Acetylcodeine | 8.09(b) | positive | Guanidine | 14.00(b) | positive |
| Hydrocodone | 8.48(b) | positive | MPP | - | positive[(2)] |
| Cocaine | 8.24(b) | positive | Histamine | 9.96(b), 5.96(b) | positive |
| 6-MAM | 8.09(b), 9.56(a) | positive | Serotonin | 10.06(b),10.65(a) | positive |
| Tramadol | 9.01(b) | positive | Dopamine | 9.76(b), 8.87(a) | positive |
| Nalbuphine | 8.14(b),10.35(a) | positive | Agmatine | 10.90 (b) | positive |
| Heroine | 8.09(b) | positive | L-dopa | 9.37(b), 2.27(a) | zwitterionic |
| Oxycodone | 7.80(b),16.60(a) | positive | Cimetidine | 6.52(b) | neutral |

(1) pKa values were predicted using the MoKa 2.5 software. (2) ammonium salts, with permanent protonated nitrogen.

**Table S4.** Virtual screening selected hits from the Tropsha et al. database (Sedykh *et al.*, 2013).

| Compound | SMILES | Concatenated "TARGET" |
|---|---|---|
| **1-Phenethylpiperidine** | C(Cc1ccccc1)N1CCCCC1 | MDR1 |
| **Aminopentamide** | CC(CC(C(=O)N)(c1ccccc1)c2ccccc2)N(C)C | MDR1 |
| **Amitriptyline** | N(C)(C)CCC=C2C1=C(C=CC=C1)CCC3=C2C=CC=C3 | MDR1, BCRP, MRP2, OCT1 |
| **Antazoline** | N\1=C(\NCC/1)CN(c2ccccc2)Cc3ccccc3 | MDR1 |
| **Atropine** | O(C(=O)C(C1=CC=CC=C1)CO)C2CC3N(C)C(C2)CC3 | MDR1, BCRP, MRP2, ASBT,OCT1 |
| **Biperiden** | OC(CCN1CCCCC1)(C1CC2CC1C=C2)c1ccccc1 | MDR1 |
| **Butethamate** | CCC(C(=O)OCCN(CC)CC)c1ccccc1 | MDR1 |
| **Chlorpheniramine** | CN(C)CCC(c1ccc(cc1)Cl)c2ccccn2 | MDR1, OCT1 |
| **Chlorpromazine** | ClC2=CC=C1SC3=C(N(CCCN(C)C)C1=C2)C=CC=C3 | MDR1, BCRP, MRP2, OCT1 |
| **Chlorprothixene** | CN(C)CC/C=C\1/c2ccccc2Sc3c1cc(cc3)Cl | MDR1, BCRP, MRP2, OCT1 |

| | | |
|---|---|---|
| **Clemastine fumurate** | CN1CCC[C@@H]1CCO[C@](C)(C1=CC=CC=C1)C1=CC=C(Cl)C=C1 | MDR1, OCT1 |
| **Clindamycin** | CCC[C@@H]1C[C@H](N(C1)C)C(=O)N[C@@H]([C@@H]2[C@@H]([C@@H]([C@H]([C@H](O2)SC)O)O)O)[C@H](C)Cl | MDR1 |
| **Clofedanol** | CN(C)CCC(O)(c1ccccc1)c2ccccc2Cl | MDR1 |
| **Clomipramine** | ClC3=CC=C2C(N(CCCN(C)C)C1=C(C=CC=C1)CC2)=C3 | MDR1, OCT1 |
| **D-617 (metabolite of Verapamil)** | CNCCCC(C#N)(C(C)C)C1=CC(OC)=C(OC)C=C1 | MDR1 |
| **Delsoline** | CCN1C[C@@]2(CCC([C@@]34[C@@H]2[C@@H](C(C31)(C5(C[C@@H]([C@H]6C[C@@H]4[C@@H]5[C@H]6OC)OC)O)O)OC)O)COC | MDR1 |
| **Doxapram** | O4CCN(CCC3C(C1=CC=CC=C1)(C2=CC=CC=C2)C(=O)N(CC)C3)CC4 | MDR1 |
| **Doxepin** | O2C3=C(C(=CCCN(C)C)C1=C(C=CC=C1)C2)C=CC=C3 | MDR1 |
| **Doxylamine** | CC(c1ccccc1)(c2ccccn2)OCCN(C)C | MDR1 |
| **Gamfexine** | C2=C(C(C1CCCCC1)CCN(C)C)C=CC=C2 | MDR1 |
| **Homoclomin** | CN1CCCN(CC1)C(c2ccccc2)c3ccc(Cl)cc3 | MDR1 |
| **Imipramine** | N(C)(C)CCCN2C1=C(C=CC=C1)CCC3=C2C=CC=C3 | MDR1, OCT1 |

| | | |
|---|---|---|
| **Levomeprazine** | COc1ccc2Sc3ccccc3N(C[C@H](C)CN(C)C)c2c1 | MDR1 |
| **Lidocaine** | O=C(NC1=C(C)C=CC=C1C)CN(CC)CC | MDR1, OATP2B1, OCT1 |
| **Maprotiline** | N(C)CCCC13C4=C(C(C2=C1C=CC=C2)CC3)C=CC=C4 | MDR1, BCRP, MRP2 |
| **Mephentermine** | CC(C)(CC1=CC=CC=C1)NC | MDR1 |
| **Metergoline** | CN1CC(CNC(=O)OCc2ccccc2)CC2C1Cc1cn(C)c3cccc2c13 | MDR1 |
| **Methadone** | O=C(C(C1=CC=CC=C1)(C2=CC=CC=C2)CC(C)N(C)C)CC | MDR1 |
| **Methapyrilene** | S2C(CN(C1=NC=CC=C1)CCN(C)C)=CC=C2 | MDR1 |
| **Methixene** | CN1CCCC(C1)CC1c2ccccc2Sc2c1cccc2 | MDR1 |
| **Mitomycin C** | CO[C@]12[C@H]3N[C@H]3CN1C1=C([C@H]2COC(N)=O)C(=O)C(N)=C(C)C1=O | MDR1 |
| **N-[2-(3,4-dimethoxyphenyl)ethyl]-1-methylpyrrolidin-2-imine** | CN1/C(CCC1)=N\CCC2=CC=C(OC)C(OC)=C2 | MDR1 |
| **Naltrexone** | O5C4=C3C26C(O)(C(N(CC1CC1)CC2)CC3=CC=C4O)CCC(=O)C56 | MDR1, OCT1 |
| **N-Desmethyl-venlafaxine** | CNCC(C1=CC=C(OC)C=C1)C1(O)CCCCC1 | MDR1 |

| | | |
|---|---|---|
| **N-methylquinidine** | C[N+]12CCC(CC1[C@H](C3=C4C=C(C=CC4=NC=C3)OC)O)C(C2)C=C | MDR1, OCT1 |
| **Nortriptyline** | N(C)CCC=C2C1=C(C=CC=C1)CCC3=C2C=CC=C3 | MDR1 |
| **NSC-664565** | CC1=CC(C)C(C)N(CC2=NCCN2)C3=CC=CC=C31 | MDR1 |
| **o-Desmethyl-venlafaxine** | CN(C)CC(c1ccc(O)cc1)C1(O)CCCCC1 | MDR1 |
| **Orphenadrine** | CN(C)CCOC(c1ccccc1)c1ccccc1C | MDR1, BCRP, OCT1 |
| **Parhexilene** | [H][C@@]1(NCCCC1)CC(C2CCCCC2)C3CCCCC3 | MDR1 |
| **Phenethyl isothiocyanate** | c1ccc(cc1)CCN=C=S | MDR1, BCRP, MRP1, MRP2 |
| **Pheniramine** | CN(C)CCC(C1=CC=CC=C1)C1=CC=CC=N1 | MDR1 |
| **Pinafide** | [O-][N+](=O)c1cc2C(=O)N(CCN3CCCC3)C(=O)c3cccc(c1)c23 | MDR1 |
| **Pridinol** | OC(CCN1CCCCC1)(C1=CC=CC=C1)C1=CC=CC=C1 | MDR1 |
| **Proadifen** | CCCC(C(=O)OCCN(CC)CC)(c1ccccc1)c1ccccc1 | MDR1 |
| **Procyclidine** | OC(CCN1CCCC1)(C1CCCCC1)c1ccccc1 | MDR1, BCRP, MRP2 |

| | | |
|---|---|---|
| **Profenamine** | CCN(CC)C(C)CN1C2=CC=CC=C2SC2=CC=CC=C12 | MDR1 |
| **Promazine** | S1C3=C(N(CCCN(C)C)C2=C1C=CC=C2)C=CC=C3 | MDR1, OCT1 |
| **Promethazine** | S1C3=C(N(CC(C)N(C)C)C2=C1C=CC=C2)C=CC=C3 | MDR1, OCT1 |
| **Propafenone_cmpd_reft_2d** | CCCNCCC1=C(CCC2=CC=CC=C2)C(C=CC=C3)=C3O1 | MDR1 |
| **Propafenone_deriv_ref44** | N1(CCOC2=C(CCC3=CC=CC=C3)C(C=CC=C4)=C4O2)CCCCC1 | MDR1 |
| **Propranolol** | O(C1=C2C(=CC=C1)C=CC=C2)CC(O)CNC(C)C | MDR1, BCRP, MRP2,OATP2B1, OCT1 |
| **Protriptyline** | CNCCCC1C2=CC=CC=C2C=CC2=CC=CC=C12 | MDR1 |
| **Quinine** | [H][C@]1(C[C@@H]2CC[N@]1C[C@@H]2C=C)[C@H](O)C1=CC=NC2=CC=C(OC)C=C12 | MDR1, BCRP, MRP1, ASBT,OCT1 |
| **Ramosetron** | CN1C=C(C2=CC=CC=C21)C(=O)[C@@H]3CCC4=C(C3)NC=N4 | MDR1 |
| **Rotoxamine** | [C@H](OCCN(C)C)(C1=CC=C(Cl)C=C1)C2=CC=CC=N2 | MDR1 |
| **Selegiline** | CC(CC1=CC=CC=C1)N(C)CC#C | MDR1 |
| **Sumatriptan** | CNS(=O)(=O)Cc1ccc2c(c1)c(c[nH]2)CCN(C)C | MDR1 |

| | | |
|---|---|---|
| **Thenyldiamine** | CN(C)CCN(CC1=CSC=C1)C1=CC=CC=N1 | MDR1 |
| **Thonzylamine** | COc1ccc(CN(CCN(C)C)c2ncccn2)cc1 | MDR1 |
| **Timolol** | CC(C)(C)NC[C@H](O)COc1nsnc1N1CCOCC1 | MDR1, BCRP, MRP2, OCT1 |
| **Tomoxetine** | CNCCC(Oc1ccccc1C)c1ccccc1 | MRP2 |
| **Trihexyphenidyl** | OC(CCN1CCCCC1)(C1CCCCC1)c1ccccc1 | OCT1 |
| **Trimipramine** | N(C)(C)CC(C)CN2C1=C(C=CC=C1)CCC3=C2C=CC=C3 | MDR1, OCT1 |
| **Venlafaxine** | COC1=CC=C(C=C1)C(CN(C)C)C1(O)CCCCC1 | MDR1, OATP2B1 |
| **Vincamine** | O=C(OC)[C@]3(O)n1c4c(c2ccccc12)CCN5CCC[C@](C3)(CC)[C@@H]45 | MDR1 |
| **Xylometazoline** | CC1=CC(=CC(C)=C1CC1=NCCN1)C(C)(C)C | MDR1 |
| **Zolamine** | COc1ccc(CN(CCN(C)C)c2nccs2)cc1 | MDR1 |

**Table S5.** Virtual screening selected compounds from the Recon 2 database.

| Compound | SMILES | CHARGE |
|---|---|---|
| **Thyrotropin releasing hormone** | OC(=N)[C@@H]1CCCN1C(=O)[C@H](CC1=CN=CN1)N=C(O)[C@@H]1CCC(O)=N1 | positive |
| **N-Methylserotonin**[(1)] | CNCCC1=CNC2=C1C=C(O)C=C2 | positive |
| ***Melatonin***[(2)] | CC(=O)NCCc1c[nH]c2c1cc(cc2)OC | *neutral* |
| ***5,6,7,8-Tetrahydrobiopterin***[(2)] | CC(O)C(O)C1CNc2nc(N)[nH]c(=O)c2N1 | *neutral* |

[(1)] Compound, although positively charged at pH 7.4, showed a low Glob-Prod similarity score, and thus was not classified as a possible G-I and selected to validate the pharmacophore

[(2)] Compounds in italics were filtered out by the charge-filter applied, but they were selected to validate the pharmacophore.

**Table S6.**

Virtual screening selected hits from the HMDB database.

| Compound | SMILES |
|---|---|
| **(2S,2'S)-Pyrosaccharopine** | NC(CCCCN1C(CCC1=O)C(O)=O)C(O)=O |
| **1,4'-Bipiperidine-1'-carboxylic acid** | OC(=O)N1CCC(CC1)N1CCCCC1 |
| **1-Isothiocyanato-4-phenylbutane** | S=C=NCCCCC1=CC=CC=C1 |
| **2-Hydroxyclomipramine** | CN(C)CCCN1C2=CC=CC=C2CCC2=CC(O)=C(Cl)C=C12 |
| **2-hydroxydesipramine** | CNCCCN1C2=CC=CC=C2CCC2=CC(O)=CC=C12 |
| **2-hydroxyimipramine** | CN(C)CCCN1C2=CC=CC=C2CCC2=CC(O)=CC=C12 |
| **3-(Isothiocyanatomethyl)-1-methoxy-1H-indole** | CON1C=C(CN=C=S)C2=CC=CC=C12 |
| **3-Hydroxymonoethylglycinexylidide** | CCNCC(=O)NC1=C(C)C=CC(O)=C1C |
| **3-Phenylpropionylglycine** | OC(=O)CNCCC(=O)C1=CC=CC=C1 |
| **4-Hydroxyatomoxetine** | CNCC[C@@H](OC1=CC=C(O)C=C1C)C1=CC=CC=C1 |

| | |
|---|---|
| **5-Hydroxymethyl** tolterodine | CC(C)N(CC[C@H](C1=CC=CC=C1)C1=C(O)C=CC(CO)=C1)C(C)C |
| **8-Hydroxydesmethylclomipramine** | CNCCCN1C2=CC=C(O)C=C2CCC2=CC=C(Cl)C=C12 |
| **Acepromazine** | CN(C)CCCN1C2=CC=CC=C2SC2=C1C=C(C=C2)C(C)=O |
| **Aceprometazine** | CC(CN1C2=CC=CC=C2SC2=C1C=C(C=C2)C(C)=O)N(C)C |
| **Amitriptyline** | CN(C)CCC=C1C2=CC=CC=C2CCC2=CC=CC=C12 |
| **Antazoline** | C(N(CC1=CC=CC=C1)C1=CC=CC=C1)C1=NCCN1 |
| **Apraclonidine** | NC1=CC(Cl)=C(NC2=NCCN2)C(Cl)=C1 |
| **Aprindine** | CCN(CC)CCCN(C1CC2=CC=CC=C2C1)C1=CC=CC=C1 |
| **Asparaginyl-Tyrosine** | NC(CC(N)=O)C(=O)NC(CC1=CC=C(O)C=C1)C(O)=O |
| **Atherosperminine** | COC1=C(OC)C2=C(C=CC3=CC=CC=C23)C(CCN(C)C)=C1 |
| **Atomoxetine** | CNCC[C@@H](OC1=CC=CC=C1C)C1=CC=CC=C1 |
| **Biperiden** | OC(CCN1CCCCC1)(C1CC2CC1C=C2)C1=CC=CC=C1 |

| | |
|---|---|
| **Bromodiphenhydramine** | CN(C)CCOC(C1=CC=CC=C1)C1=CC=C(Br)C=C1 |
| **Brompheniramine** | CN(C)CCC(C1=CC=C(Br)C=C1)C1=CC=CC=N1 |
| **Calycanthidine** | CN1CCC2(C1NC1=C2C=CC=C1)C12CCN(C)C1N(C)C1=C2C=CC=C1 |
| **Carbinoxamine** | CN(C)CCOC(C1=CC=C(Cl)C=C1)C1=CC=CC=N1 |
| **Carteolol** | CC(C)(C)NCC(O)COC1=CC=CC2=C1CCC(=O)N2 |
| **Chlophedianol** | CN(C)CCC(O)(C1=CC=CC=C1)C1=CC=CC=C1Cl |
| **Chlorpheniramine** | CN(C)CCC(C1=CC=C(Cl)C=C1)C1=CC=CC=N1 |
| **Chlorpromazine** | CN(C)CCCN1C2=CC=CC=C2SC2=C1C=C(Cl)C=C2 |
| **Citalopram** | CN(C)CCCC1(OCC2=C1C=CC(=C2)C#N)C1=CC=C(F)C=C1 |
| **Clomipramine** | CN(C)CCCN1C2=CC=CC=C2CCC2=C1C=C(Cl)C=C2 |
| **Cocaethylene** | CCOC(=O)C1C2CCC(CC1OC(=O)C1=CC=CC=C1)N2C |
| **Cuscohygrine** | CN1CCCC1CC(=O)CC1CCCN1C |

| | |
|---|---|
| **Cyclobenzaprine** | CN(C)CCC=C1C2=CC=CC=C2C=CC2=CC=CC=C12 |
| **Cycrimine** | OC(CCN1CCCCC1)(C1CCCC1)C1=CC=CC=C1 |
| **Cysteinyl-Isoleucine** | CCC(C)C(NC(=O)C(N)CS)C(O)=O |
| **desmethylclomipramine** | CNCCCN1C2=CC=CC=C2CCC2=C1C=C(Cl)C=C2 |
| **Dicyclomine** | CCN(CC)CCOC(=O)C1(CCCCC1)C1CCCCC1 |
| **Diethylpropion** | CCN(CC)C(C)C(=O)C1=CC=CC=C1 |
| **Dimenhydrinate** | CN1C2=C([N-]C(Cl)=N2)C(=O)N(C)C1=O.C[NH+](C)CCOC(C1=CC=CC=C1)C1=CC=CC=C1 |
| **Dimethindene** | CC(C1=C(CCN(C)C)CC2=CC=CC=C12)C1=CC=CC=N1 |
| **Dimethylamphetamine** | CC(CC1=CC=CC=C1)N(C)C |
| **Dimethyltryptamine** | CN(C)CCC1=CNC2=CC=CC=C12 |
| **Doxapram** | CCN1CC(CCN2CCOCC2)C(C1=O)(C1=CC=CC=C1)C1=CC=CC=C1 |
| **Doxepin** | [H]C(CCN(C)C)=C1C2=CC=CC=C2COC2=CC=CC=C12 |

| | |
|---|---|
| **Doxylamine** | CN(C)CCOC(C)(C1=CC=CC=C1)C1=NC=CC=C1 |
| **Duloxetine** | CNCC[C@H](OC1=CC=CC2=CC=CC=C12)C1=CC=CS1 |
| **Dumetorine** | CN1CCCCC1CC1CC(C)=CC(=O)O1 |
| **E-10-Hydroxyamitriptyline** | CN(C)CC\C=C1/C2=CC=CC=C2CC(O)C2=CC=CC=C12 |
| **Emedastine** | CCOCCN1C(=NC2=CC=CC=C12)N1CCCN(C)CC1 |
| **Ethopropazine** | CCN(CC)C(C)CN1C2=CC=CC=C2SC2=CC=CC=C12 |
| **Fencamfamine** | CCNC1C2CCC(C2)C1C1=CC=CC=C1 |
| **Fenoldopam** | OC1=CC=C(C=C1)C1CNCCC2=C(Cl)C(O)=C(O)C=C12 |
| **Fluoxetine** | CNCCC(OC1=CC=C(C=C1)C(F)(F)F)C1=CC=CC=C1 |
| **gamma-Glutamylcysteinylserine** | NC(CCC(=O)NC(CS)C(=O)NC(CO)C(O)=O)C(O)=O |
| **Glutaminyl-Phenylalanine** | NC(CCC(N)C(NC(C(O)=O)CC1=CC=CC=C1)=O)=O |
| **Hydroxyclomipramine** | CN(C)CCCN1C2=CC=CC=C2CC(O)C2=C1C=C(Cl)C=C2 |

| | |
|---|---|
| **Ibopamine** | CNCCC1=CC(OC(=O)C(C)C)=C(OC(=O)C(C)C)C=C1 |
| **Imipramine** | CN(C)CCCN1C2=CC=CC=C2CCC2=CC=CC=C12 |
| **Imiquimod** | CC(C)CN1C=NC2=C1C1=CC=CC=C1N=C2N |
| **Indecainide** | CC(C)NCCCC1(C(N)=O)C2=CC=CC=C2C2=CC=CC=C12 |
| **Isomugineic acid** | OC(CN1CCC1C(O)=O)C(NCCC(O)C(O)=O)C(O)=O |
| **Isothipendyl** | CC(CN1C2=CC=CC=C2SC2=C1N=CC=C2)N(C)C |
| **Lepidine B** | OC1=CC=CC(CC2=NC=CN2)=C1OC1=CC=CC(CC2=NC=CN2)=C1 |
| **Lepidine C** | COC1=CC=C(OC2=CC=CC(CC3=NC=CN3)=C2)C(CC2=NC=CN2)=C1 |
| **Lepidine D** | OC1=CC=C(OC2=CC=CC(CC3=NC=CN3)=C2)C(CC2=NC=CN2)=C1 |
| **Lepidine F** | OC1=CC=C(C(CC2=NC=CN2)=C1)C1=C(O)C=CC(CC2=NC=CN2)=C1 |
| **Levomethadyl Acetate** | CC[C@H](OC(C)=O)C(C[C@H](C)N(C)C)(C1=CC=CC=C1)C1=CC=CC=C1 |
| **Lofexidine** | CC(OC1=C(Cl)C=CC=C1Cl)C1=NCCN1 |

| | |
|---|---|
| **L-phenylalanyl-L-hydroxyproline** | NC(CC1=CC=CC=C1)C(=O)N1CC(O)CC1C(O)=O |
| **Maprotiline** | CNCCCC12CCC(C3=CC=CC=C13)C1=CC=CC=C21 |
| **Mepyramine** | COC1=CC=C(CN(CCN(C)C)C2=NC=CC=C2)C=C1 |
| **Mesoridazine** | CN1CCCCC1CCN1C2=CC=CC=C2SC2=C1C=C(C=C2)S(C)=O |
| **Methadone** | CCC(=O)C(CC(C)N(C)C)(C1=CC=CC=C1)C1=CC=CC=C1 |
| **Methadyl Acetate** | CCC(OC(C)=O)C(CC(C)N(C)C)(C1=CC=CC=C1)C1=CC=CC=C1 |
| **Methylphenidate** | COC(=O)C(C1CCCCN1)C1=CC=CC=C1 |
| **Mitomycin** | CO[C@]12[C@H]3N[C@H]3CN1C1=C([C@H]2COC(N)=O)C(=O)C(N)=C(C)C1=O |
| **mono-isopropyl-disopyramide** | CC(C)NCCC(C(O)=N)(C1=CC=CC=C1)C1=CC=CC=N1 |
| **N-(1-Deoxy-1-fructosyl)tryptophan** | OC[C@H]1OC(O)(CN[C@@H](CCC2=CNC3=C2C=CC=C3)C(O)=O)[C@@H](O)[C@@H]1O |
| **N-(1-Deoxy-1-fructosyl)tyrosine** | OC[C@H]1OC(O)(CN[C@@H](CC2=CC=C(O)C=C2)C(O)=O)[C@@H](O)[C@@H]1O |
| **N-(Carbomethoxyacetyl)-4-S-chlorotryptophan** | COC(=O)C\C(O)=N\C(CC1=CNC2=CC=CC(Cl)=C12)C(O)=O |

| | |
|---|---|
| **N,O-Didesmethylvenlafaxine** | CNCC(C1=CC=C(O)C=C1)C1(O)CCCCC1 |
| **N-Acetylhistidine** | CC(=O)NC(CC1=CNC=N1)C(O)=O |
| **N-Dealkylated tolterodine** | CC(C)NCC[C@H](C1=CC=CC=C1)C1=C(O)C=CC(C)=C1 |
| **N-Demethyl orphenadrine** | CNCCOC(C1=CC=CC=C1)C1=CC=CC=C1C |
| **N-Desmethyl tapentadol** | CC[C@H]([C@@H](C)CNC)C1=CC(O)=CC=C1 |
| **N-desmethylalmotriptan** | CNCCC1=CNC2=C1C=C(CS(=O)(=O)N1CCCC1)C=C2 |
| **N-Desmethylcitalopram** | CNCCCC1(OCC2=C1C=CC(=C2)C#N)C1=CC=C(F)C=C1 |
| **N-Desmethylpromazine** | CNCCCN1C2=CC=CC=C2SC2=CC=CC=C12 |
| **N-Desmethylvenlafaxine** | CNCC(C1=CC=C(OC)C=C1)C1(O)CCCCC1 |
| **Norpropoxyphene** | CCC(=O)OC(CC1=CC=CC=C1)(C(C)CNC)C1=CC=CC=C1 |
| **Orphenadrine** | CN(C)CCOC(C1=CC=CC=C1)C1=CC=CC=C1C |
| **Oxymetazoline** | CC1=CC(=C(O)C(C)=C1CC1=NCCN1)C(C)(C)C |

| | |
|---|---|
| **Oxynarcotine** | CNCCC1=CC2=C(OCO2)C(OC)=C1CC(=O)C1=C(C(O)=O)C(OC)=C(OC)C=C1 |
| **Perhexiline** | C(C(C1CCCCC1)C1CCCCC1)C1CCCCN1 |
| **Phendimetrazine** | CC1C(OCCN1C)C1=CC=CC=C1 |
| **Pheniramine** | CN(C)CCC(C1=CC=CC=C1)C1=CC=CC=N1 |
| **Phentolamine** | CC1=CC=C(C=C1)N(CC1=NCCN1)C1=CC(O)=CC=C1 |
| **Phygrine** | CN1CCCC1CC(=O)CC1CCC(CC(C)=O)N1C |
| **Procyclidine** | OC(CCN1CCCC1)(C1CCCCC1)C1=CC=CC=C1 |
| **Prolintane** | CCCC(CC1=CC=CC=C1)N1CCCC1 |
| **Prolyl-Valine** | CC(C)C(NC(=O)C1CCCN1)C(O)=O |
| **Promazine** | CN(C)CCCN1C2=CC=CC=C2SC2=CC=CC=C12 |
| **Promazine 5-sulfoxide** | CN(C)CCCN1C2=CC=CC=C2S(=O)C2=CC=CC=C12 |
| **Promethazine** | CC(CN1C2=CC=CC=C2SC2=CC=CC=C12)N(C)C |

| | |
|---|---|
| **Propiomazine** | CCC(=O)C1=CC2=C(SC3=CC=CC=C3N2CC(C)N(C)C)C=C1 |
| **Propoxyphene** | CCC(=O)OC(CC1=CC=CC=C1)([C@H](C)CN(C)C)C1=CC=CC=C1 |
| **Propylhexedrine** | CNC(C)CC1CCCCC1 |
| **Protriptyline** | CNCCCC1C2=CC=CC=C2C=CC2=CC=CC=C12 |
| **Quinidine** | [H][C@@]12CCN(C[C@@H]1C=C)[C@]([H])(C2)[C@@H](O)C1=C2C=C(OC)C=CC2=NC=C1 |
| **Rizatriptan** | CN(C)CCC1=CNC2=C1C=C(CN1C=NC=N1)C=C2 |
| **Ropivacaine** | CCCN1CCCC[C@H]1C(=O)NC1=C(C)C=CC=C1C |
| **Sibutramine** | CC(C)CC(N(C)C)C1(CCC1)C1=CC=C(Cl)C=C1 |
| **Sumatriptan** | CNS(=O)(=O)CC1=CC2=C(NC=C2CCN(C)C)C=C1 |
| **Tertatolol** | CC(C)(C)NCC(O)COC1=CC=CC2=C1SCCC2 |
| **Thalicpureine** | CNCCC1=C(OC)C(OC)=C(OC)C2=C1C=CC1=C2C=C(OC)C(OC)=C1 |

**Table S7.**

Predicted P-gp inhibition effect for the proton-antiporter good-inhibitors according to the VolSurf+ PLS-DA model.

| Name | Predicted P-gp inhibition(LV2) | Literature P-gp inhibition[(1)] |
|---|---|---|
| Verapamil | I | I |
| Compound 3* | I | |
| Buprenorphine | I | |
| Chlorpromazine | I | I |
| Clomipramine | I | I |
| Amitriptyline | N-I | |
| Quinine | N-I | I |
| Compound 6* | N-I | |
| Imipramine | N-I | N-I |
| Methadone | N-I | |
| Compound 1* | N-I | |
| Doxepin | N-I | |
| Pheniramine | N-I | N-I |
| Cocaethylene | N-I | |
| Compound 2* | N-I | |
| Diphenhydramine | N-I | N-I |
| Nortriptyline | N-I | N-I |
| Norbuprenorphine | N-I | |
| Compound 4* | N-I | |
| Compound 9* | N-I | |
| Desipramine | N-I | N-I |
| Compound 5* | N-I | |
| Desomorphine | N-I | |
| MDMA | N-I | |
| Tryptamine | N-I | |

**[(1)](Broccatelli *et al.*, 2011) and references cited therein. * Specs compounds see Table 3**

## B. Supplementary Figure

**Figure S1.**

*Passive and carrier-mediated flux of naloxone in hCMEC/D3 cells*. Total uptake (nmol/min/mg; black dashed line) was measured in hCMEC/D3 cells and plotted against total naloxone concentration in the Krebs-HEPES (KH) incubation buffer at $pH_e$ 7.40. The blue straight dotted line represents the passive transport of clonidine ($K_{passive}$ 3.4 ± 0.3 µL.min$^{-1}$.mg$^{-1}$ at pH 7.40). The red solid line represents the curve obtained by subtracting the passive flux from the total flux and fitting to the carrier-mediated Michaelis-Menten term (see Eq. 1) by nonlinear least-square regression. Estimated parameters for naloxone transport in hCMEC/D3 cells are: $K_m$, 0.19 ± 0.05 mmol.l$^{-1}$, $V_{max}$, 2.9 ± 0.5 nmol min$^{-1}$.mg$^{-1}$. Data represent means ± SD of experiments performed in triplicate.

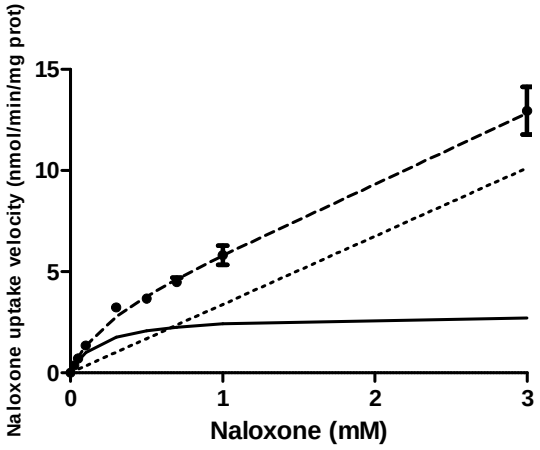


**Figure S2.**

*Second ranked pharmacophore model generated from a different alignment of imipramine, methadone and buprenorphine.* **A.** Chemical structures of the selected compounds. **B.** Alignment obtained for imipramine (purple), methadone (cyan), and buprenorphine (green). **C.** Pharmacophore obtained upon alignment in terms of common atom-centered pharmacophoric pseudo fields (pseudoPIFs): the green areas represent the hydrophobic moieties, the blue area represents the H-bond donor region, the red areas represent the H-bond acceptor regions, and the light-blue wireframe surface defines the shape of the Pharmacophore. The green and blue points represent the most relevant common pharmacophoric points at the centroid of the pseudoPIFs.

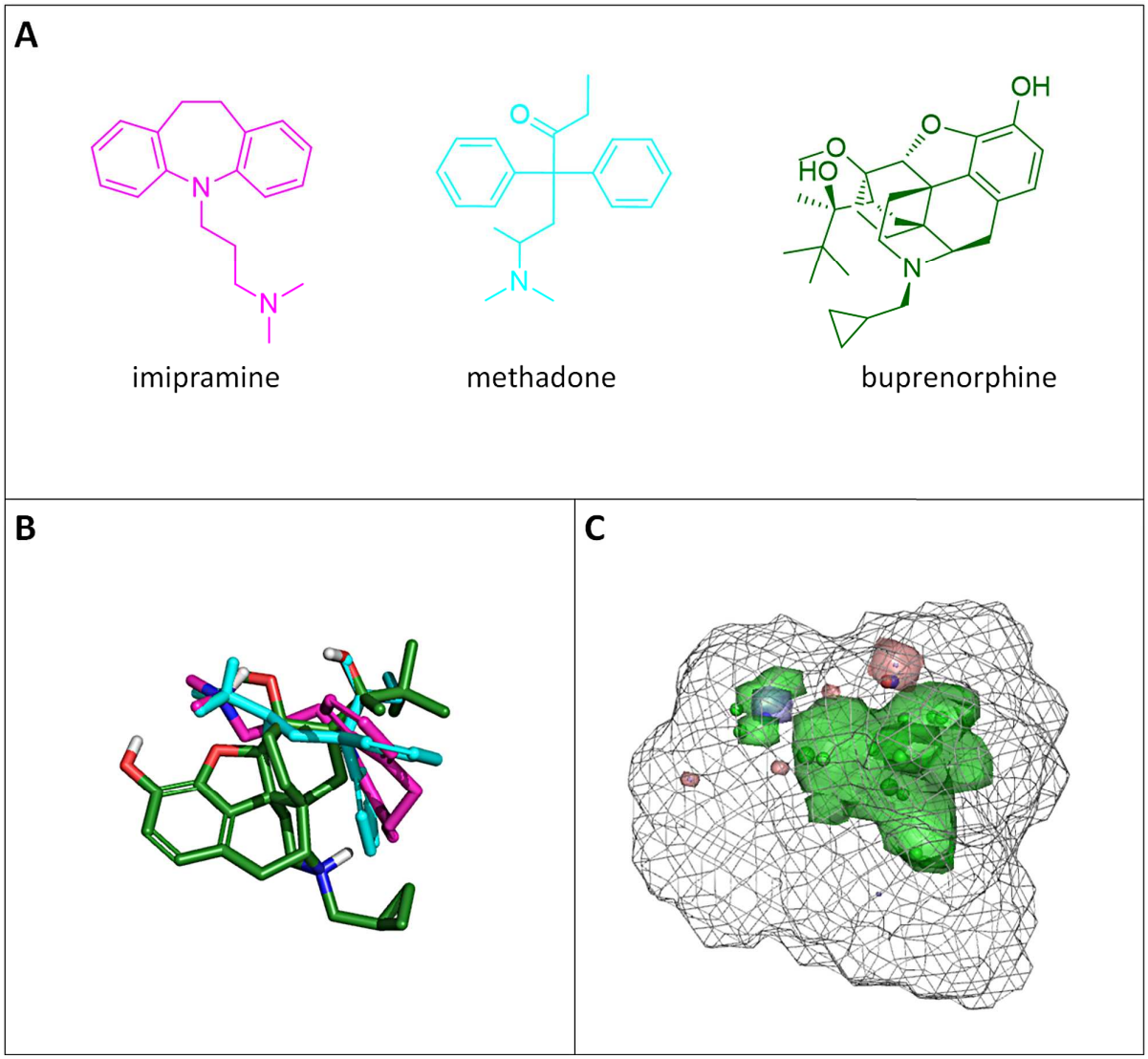


**Figure S3.**

*Selection of other three drugs based on the PCA t1-t2 score plot for the inhibitors and non-inhibitors of the proton antiporter, generated using the VolSurf+ descriptors* (G-I, red circles; M-I, blue circles; W-I, black circles; N-I, green circles). The background color refers to the LogP descriptor space (blue=high LogP and red = low LogP value). It suggests that strong inhibitors have preferentially high logP and %FU10 values (LogP mean value for G-I= 3.6, for N-I= -1.0; %FU10 mean value for G-I= 78, for N-I= 22). AMI=amitriptyline, DIP=diphenhydramine, QUI=quinine.

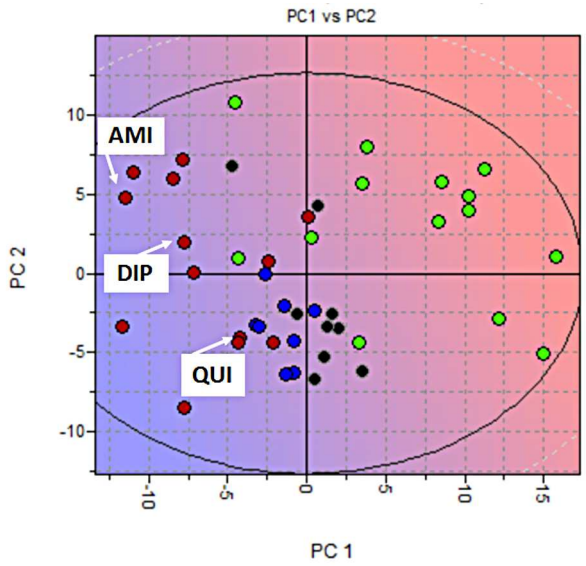


**Figure S4.**
*Pharmacophore for proton-antiporter inhibitor generated using FLAP compounds selected in Figure S3*. **A.** Chemical structures of the selected compounds. **B.** Alignment obtained for amitriptyline (orange), diphenydramine (pink), and quinine (grey). **C.** Pharmacophore obtained upon alignment in terms of common atom-centered pharmacophoric pseudo fields (pseudoPIFs): the green areas represent the hydrophobic moieties, the blue area represents the H-bond donor region, the red areas represent the H-bond acceptor regions, and the light-blue wireframe surface defines the shape of the Pharmacophore. The green and blue points represent the most relevant common pharmacophoric points at the centroid of the pseudoPIFs.

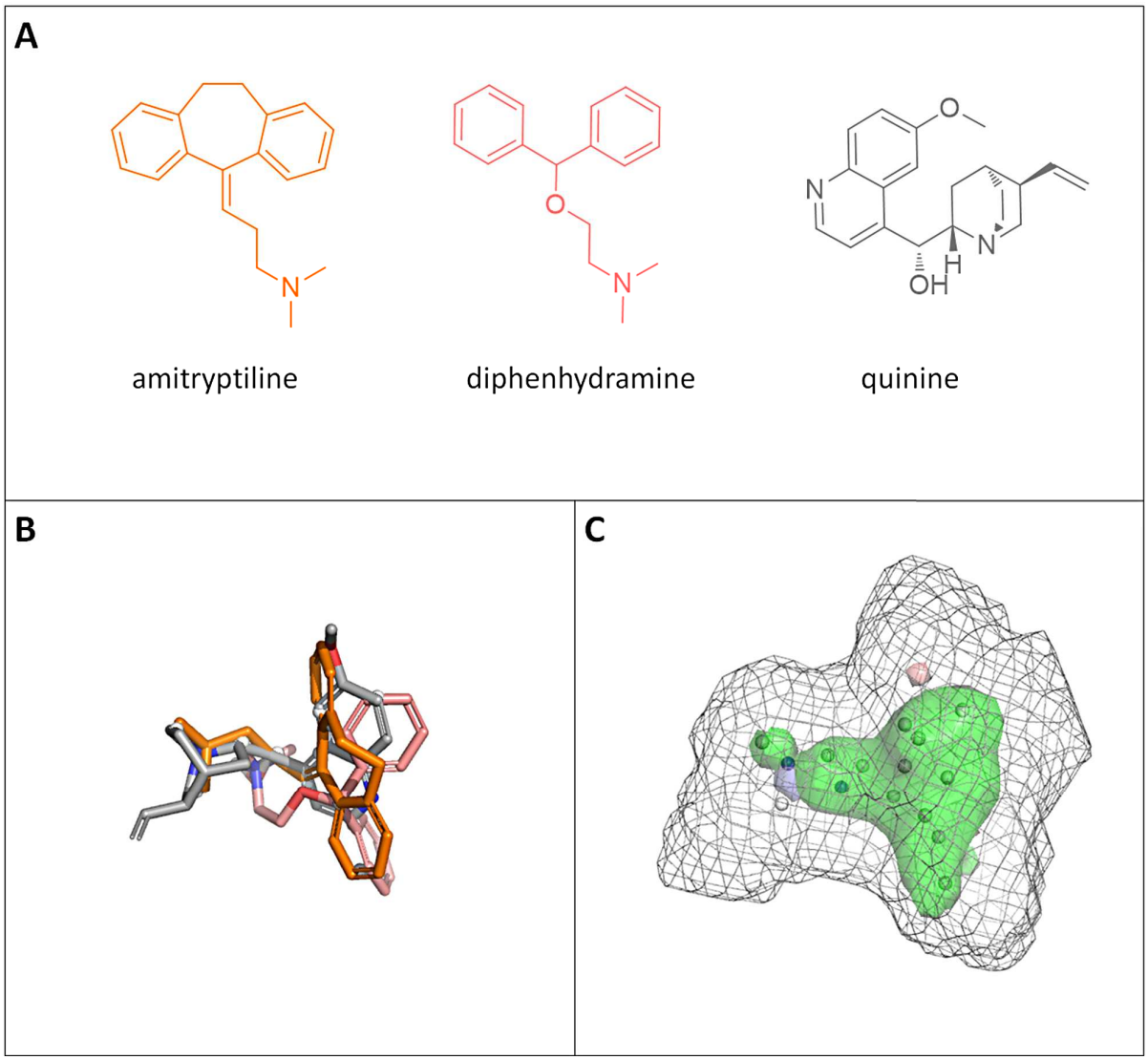
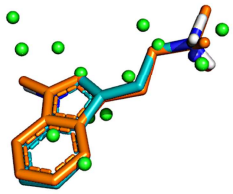


**Figure S5.**
*Alignment of dymethyl-tryptamine (orange) and tryptamine (cyano) on the pharmacophore*. The green spheres and the blue sphere represent the main features of the pharmacophore translated in atomic coordinates (green= hydrophobic; blue= H-bond donor).



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