pharmaceutics

Supplementary Materials : Pharmacophore-Based Discovery of Substrates of a Novel Drug/Proton-Antiporter in the Human Brain Endothelial hCMEC/D3 Cell Line

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A. Supplementary Tables

Table S1. List of compounds classified as substrate or non-substrate of the proton-antiporter according to direct evaluation (SUB, Substrate; N-S, Non-substrate; ND, Not documented;* our unpublished data).

Substrate*	Status	Non Substrate*	Status
Clonidine	SUB[1,2]	Morphine	ND
Desomorphine	ND	Nicotine	SUB[5]
Diphenhydramine	SUB[3,4]	Agmatine	N-S[14]
Heroine	ND	Choline	N-S[15]
Norbuprenorphine	ND	Cimetidine	N-S[16]
Tramadol	SUB[5,6]	Dihydromorphine	ND
Verapamil	SUB[7]	Dopamine	N-S[17]
6-monoacetylmorphine	ND	Ergothioneine	N-S[18]
Brimonidine	SUB[8]	Guanidine	N-S[19]
Cocaethylene	ND	Histamine	N-S[17]
Cocaine	SUB[9]	L-dopa	N-S[20]
Codeine	SUB[10]	L-carnitine	N-S[21]
Methadone	ND	Melatonin	ND
Norcocaine	ND	Milnacipran	N-S *
Oxycodone	SUB[11]	MPP	N-S[17]
Pyrilamine	SUB[11]	N-methylnaloxone	ND
Apomorphine	SUB[12]	Paraquat	N-S[22, 23]
MDMA	SUB[13]	Serotonin	N-S[17]
Naloxone	SUB[2]	Tetraethylammonium	N-S *
Hydromorphone	ND	Tyramine	ND

[1](Andre et al., 2009), [2](Chapy et al., 2015), [3](Auvity et al., 2016), [4](Sadiq et al., 2011), [5](Cisternino et al., 2013), [6](Kitamura et al., 2014), [7](Chapy et al., 2016), [8](Zhang et al., 2006), [9](Chapy et al., 2014), [10](Fischer et al., 2010), [11](Okura et al., 2008), [12](Okura et al., 2013), [13](Kuwayama et al., 2008), [14](Wang et al., 2014), [15](Allen and Smith, 2001), [16](Murakami et al., 2000), [17](Andre et al., 2012), [18](Shimizu et al., 2015), [19](Al-Khawaja et al., 2014), [20](Kageyama et al., 2000), [21](Berezowski et al., 2004), [22](Bartlett et al., 2011), [23](Vilas-Boas et al., 2014).

Table S2. Dataset used for the P1 pharmacophore validation and virtual screening based on the FLAP HOH similarity score descriptor**.**

Compound	Classification	Similarity Score	Compound	Classification	Similarity Score
Heroine	G	0.23	N-methylnaloxone	N-S	0.15
Tramadol	G	0.23	Brimodinine	M-H	0.14
Cocaethylene	M-H	0.23	Cimetidine	N-S	0.14
Cocaine	M-H	0.23	ergothioneine	N-S	0.14
Codeine	M-H	0.22	Dihydromorphine	N-S	0.13
Norcocaine	M-H	0.22	Verapamil	G	0.12
Methadone	M-H	0.21	L-dopa	N-S	0.12
Desomorphine	G	0.2	Clonidine	G	0.11
Diphenhydramine	G	0.2	Norbuprenorphine	G	0.11
Hydromorphone	W	0.19	Apomorphine	M-H	0.1
Pyrilamine	M-H	0.19	Choline	N-S	0.1
MDMA	M-H	0.18	Serotonin	N-S	0.1
L-carnitine	N-S	0.18	Dopamine	N-S	0.09
6-Monoacetylmorphine	M-H	0.17	Tyramine	N-S	0.08
Oxycodone	M-H	0.17	Agmatine	N-S	0.07
Naxolone	M-L	0.17	Histamine	N-S	0.07
Morphine	W	0.17	MPP	N-S	0.03
Nicotine	W	0.17	Paraquat	N-S	0.03
Melatonin	N-S	0.16	TEA	N-S	0.03
Milnacipran	N-S	0.16	Guanidine	N-S	0

Table S3. Dataset used for pharmacophore P2 validation and virtual screening based on the FLAP H*O*H similarity score descriptor.

Compound	Classification	Similarity Score	Compound	Classification	Similarity Score
Methadone	M-H	0.19	Clonidine	G	0.09
Cocaethylene	M-H	0.18	Desomorphine	G	0.09
Heroine	G	0.17	Brimodinine	M-H	0.09
Diphenhydramine	G	0.16	L-carnitine	N-S	0.09
Pyrilamine	M-H	0.16	Norbuprenorphine	G	0.08
Cocaine	M-H	0.15	Cimetidine	N-S	0.08
Naxolone	M-L	0.15	Milnacipran	N-S	0.08
Apomorphine	M-H	0.15	Morphine	W	0.07
Verapamil	G	0.14	Dihydromorphine	N-S	0.07
6-monoacetylmorphine	M-H	0.13	Choline	N-S	0.06
Oxycodone	M-H	0.12	L-dopa	N-S	0.05
Ergothioneine	N-S	0.12	Serotonin	N-S	0.05
Hydromorphone	W	0.11	Agmatine	N-S	0.04
N-methylnaloxone	N-S	0.11	Dopamine	N-S	0.04
Tramadol	G	0.1	Tyramine	N-S	0.04
Codeine	M-H	0.1	Histamine	N-S	0.03
Norcocaine	M-H	0.1	MPP	N-S	0.03
Nicotine	W	0.1	Paraquat	N-S	0.03
MDMA	M-H	0.1	TEA	N-S	0.03
Melatonin	N-S	0.1	Guanidine	N-S	0

Table S4. Substrate candidates after virtual screening on the Tropsha's database (A), the Recon2 Database (B) and the HMDB database (C), using pharmacophore P1 as a template.

Candidate	SMILES	Similarity score*
Database (A)		
Methixene	CN1CCCC(CC2C3=CC=CC=C3SC3=CC=CC=C23)C1	0.23
Dextromethorphan	[H][C@]12CC3=C(C=C(OC)C=C3)[C@@]3(CCCC[C@]13[H])CCN2C	0.23
Laudanosine-6′-bromo	COc1c(OC)cc(C[C@@H]2[NH+](C)CCc3cc(OC)c(OC)cc23)cc1	0.22
Cilazaprilat	C1C[C@@H](C(=O)N2[C@@H](CCCN2C1)C(=O)O)N[C@@H](CCC3=CC=CC=C3)C(=O)O	0.22
Benactyzine	CC[NH+](CC)CCOC(=O)C(O)(c1ccccc1)c1ccccc1	0.20
CHEMBL131459	CC(C)N(C[C@H](O)COc1c(C(=O)c2ccccc2)c(C)nn1C)C(C)C	0.20
Venlafaxine	OC1([C@H](c2ccc(OC)cc2)C[NH+](C)C)CCCCC1	0.19
N-[2-(3,4-dimethoxyphenyl)ethyl]-1-methylpyrrolidin-2-imine	COc1ccc(CC/[NH+]=C/2CCCN2C)cc1OC	0.19
CHEMBL339825	CC(C)N(C[C@H](O)COc1c(C(=O)c2ccccc2)c(C)nn1c1ccccc1)C(C)C	0.19
Oxybutynin	O[C@](C1CCCCC1)(C(=O)OCC#CCN(CC)CC)c1ccccc1	0.19
Database (B)		
Cocaine	C[NH+]1[C@H]2CC[C@@H]1[C@H]([C@@H](C2)OC(=O)c1ccccc1)C(=O)OC	0.26
(R)-4-phosphopantothenate	CC(C)(CO)C(/C(=N/CCC(=O)[O-])O)O	0.20
N-Acetylmethionine	[H][C@@](CCSC)(NC(C)=O)C(O)=O	0.20
Database (C)		
Oxycodone	[NH2+]([C@]1(C([C@@H]2C[C@H]1CC2)(C)C)C)C	0.38
Oxymorphone	O1[C@@H]2[C@]34[C@](O)([C@H]([NH+](CC3)C)Cc3c4c1c(O)cc3)CCC2=O	0.31
Naltrexone	O1[C@@H]2[C@]34[C@](O)([C@H]([NH+](CC3)CC3CC3)Cc3c4c1c(O)cc3)CCC2=O	0.27
Hydrocodone	O1[C@@H]2[C@]34[C@H]([C@H]([NH+](CC3)C)Cc3c4c1c(OC)cc3)CCC2=O	0.26
Naloxone	O1[C@@H]2[C@]34[C@](O)([C@H](N(CC3)CC=C)Cc3c4c1c(O)cc3)CCC2=O	0.26
Thebaine	C1[NH+](C)[C@H]2Cc3ccc(c4c3[C@]3(C2=CC=C([C@H]3O4)OC)C1)OC	0.23
6-O-Methylcodeine	O1[C@@H]2[C@H](C=C[C@H]3[C@@]42CC[NH+](C)[C@@H]3Cc2c4c1c(OC)cc2)OC	0.22
Nalbuphine	c12c3c(c(cc1)O)O[C@H]1[C@H](CC[C@]4([C@H]([NH+](CC[C@]314)CC1CCC1)C2)O)O	0.21
Norbutorphanol	C1CC[C@]2([C@H]3Cc4ccc(cc4[C@]2(C1)CC[NH2+]3)O)O	0.20
Butorphanol	O[C@@]12[C@]3(CC[NH+]([C@@H]1Cc1c3cc(O)cc1)CC1CCC1)CCCC2	0.19
Hydromorphone	O1[C@@H]2[C@]34[C@H]([C@H]([NH+](CC3)C)Cc3c4c1c(O)cc3)CCC2=O	0.19

*According to the FLAP H*O*H descriptor

Table S5. Substrate candidates after virtual screening on the Tropsha's database (A), the Recon2 Database (B) and the HMDB database (C), using pharmacophore P2 as a template.

Candidate	SMILES	Similarity Score*
Database (A)		
Trihexyphenidyl	O[C@](CC[NH+]1CCCCC1)(C1CCCCC1)[C@H-]1[CH-][CH-][CH+][CH+][CH+]1	0.23
Doxylamine	O(CC[NH+](C)C)[C@](c1ncccc1)(c1ccccc1)C	0.22
Thenyldiamine	C[NH+](C)CCN(Cc1cscc1)c1ncccc1	0.19
Methadone	O=C(C(c1ccccc1)(c1ccccc1)C[C@H]([NH+](C)C)C)CC	0.19
D-617(metabolite of Verapamil)	CC(C)[C@](C#N)(c1cc(OC)c(OC)cc1)CCCNC	0.19
Doxapram	O=C1N(CC)C[C@@H](CC[NH+]2CCOCC2)[C@@]1([C-]1[CH-][CH-][CH-][CH-][CH-]1)[CH+]1[CH+][CH+][CH+][CH+][CH+]1	0.19
Chlorpromazine	Clc1cc2N(c3c(Sc2cc1)cccc3)CCC[NH+](C)C	0.18
Antazoline	N1CC[NH+]=C1CN(Cc1ccccc1)c1ccccc1	0.18
Methixene	C[NH+]1CCC[C@H](C[C@@H]2[C@@H-]3[CH-][CH-][CH-][CH-][C@@H-]3S[C@@H+]3[C@H+]2[CH+][CH+][CH+][CH+]3)C1	0.17
Zolamine	C[NH+](C)CCN(Cc1ccc(OC)cc1)c1nccs1	0.17
Methapyrilene	C[NH+](C)CCN(Cc1cccs1)c1ccccn1	0.17
Fenipramide	C1CC[NH+](CC1)CCC(C(=O)N)(c1ccccc1)c1ccccc1	0.17
Gamfexine	C[NH+](C)CC[C@H](C1CCCCC1)c1ccccc1	0.16
Laudanosine-6*f*-bromo	COc1c(OC)cc(C[C@@H]2[NH+](C)CCc3cc(OC)c(OC)cc23)cc1	0.16
Pheniramine	n1ccccc1[C@@H](c1ccccc1)CC[NH+](C)C	0.16
Imipramine	c1cc2c(cc1)CCc1c(cccc1)N2CCC[NH+](C)C	0.16
Promazine	S1c2ccccc2N(c2c1cccc2)CCC[NH+](C)C	0.16
Procyclidine	O[C@](c1ccccc1)(CC[NH+]1CCCC1)C1CCCCC1	0.16
Buflomedil	COc1cc(OC)c(C(=O)CCC[NH+]2CCCC2)c(OC)c1	0.16
Amitriptyline	c1cc2c(/C(=C/CC[NH+](C)C)c3c(cccc3)CC2)cc1	0.16
Ungerine	CO[C@H]1C=C2CC[NH+](C)[C@H]2[C@H]2[C@@H]1OC(=O)c1cc3c(OCO3)cc21	0.15
Levophacetoperane	CC(=O)O[C@@H]([C@H]1CCCC[NH2+]1)c1ccccc1	0.15
Chlorpheniramine	Clc1ccc(cc1)[C@@H](c1ncccc1)CC[NH+](C)C	0.15
ChemSpiderID23281049	O=C1C[C@H](C[NH+]2CCCCC2)Oc2ccccc12	0.15
Isopropamide	NC(=O)C(CC[NH+](C(C)C)C(C)C)(c1ccccc1)c1ccccc1	0.15
Xylometazoline	N1CC[NH+]=C1Cc1c(C)cc(cc1C)C(C)(C)C	0.15
Delcorine	O[C@@H]1[C@H]2[C@@]34[C@H]5C[C@@H]6[C@@H](OC)[C@H]5[C@]5(C[C@@H]6OC)OCO[C@@]15[C@@H]3N(CC)C[C@]2(COC)CC[C@@H]4OC	0.15
Protriptyline	c1cc2c(C=Cc3c(cccc3)C2CCC[NH2+]C)cc	0.14
NSC-664565	[NH+]1=C(NCC1)CN1[C@H](C)[C@H](C)C=C(C)c2ccccc12	0.14
Vincamine	O[C@]1(C[C@@]2(CC)CCC[NH+]3CCc4c([C@@H]23)n1c1ccccc41)C(=O)OC	0.14
Doxepin	O1c2ccccc2/C(=C/CC[NH+](C)C)c2c(cccc2)C1	0.14
Eburnamonine	CC[C@@]12CCC[NH+]3CCc4c([C@H]13)n(c1ccccc41)C(=O)C2	0.14

Desipramine	c1cc2c(cc1)CCc1c(cccc1)N2CCC[NH2+]C	0.14
Promethazine	S1c2ccccc2N(c2c1cccc2)C[C@@H]([NH+](C)C)C	0.13
Aminopentamide	C[C@H](CC(C(=O)N)(c1ccccc1)c1ccccc1)[NH+](C)C	0.13
Disopyramide	NC(=O)[C@](c1ccccn1)(c1ccccc1)CC[NH+](C(C)C)C(C)C	0.13
Oxymetazoline	Oc1c(cc(C)c(CC2=[NH+]CCN2)c1C)C(C)(C)C	0.13
Maprotiline	CNCCCC12CCC(C3=CC=CC=C13)C1=CC=CC=C21	0.13
Database (B)		
Cocaine	C[NH+]1[C@H]2CC[C@@H]1[C@H]([C@@H](C2)OC(=O)c1ccccc1)C(=O)OC	0.14
6-Hydroxymelatonin	C/C(=N/CCc1c[nH]c2cc(c(cc12)OC)O)O	0.14
Database (C)		
Methadone	O=C(C(C[C@@H]([NH+](C)C)C)(c1ccccc1)c1ccccc1)CC	0.20
Norpropoxyphene	CCC(=O)O[C@@](Cc1ccccc1)([C@@H](C)C[NH2+]C)c1ccccc1	0.19
Hydromorphone-3-sulphate	C[NH+]1CC[C@]23[C@@H]4Oc5c(OS(=O)(=O)[O-])ccc(C[C@@H]1[C@H]2CCC4=O)c35	0.18
noracymethadol	CC[C@H](C(C[C@@H](C)[NH2+]C)(c1ccccc1)c1ccccc1)OC(=O)C	0.17
Isothipendyl	S1c2c(N(C[C@@H]([NH+](C)C)C)c3ncccc13)cccc2	0.17
Nor-Levomethadyl acetate	CC[C@@H](C(C[C@@H](C)[NH2+]C)(c1ccccc1)c1ccccc1)OC(=O)C	0.16
Ritalinic acid	c1ccc(cc1)[C@@H]([C@H]1CCCC[NH2+]1)C(=O)[O-]	0.16
Triprolidine	[NH+]1(CCCC1)C/C=C(/c1ccc(cc1)C)c1ncccc1	0.16
Hydrocodone	O1[C@@H]2[C@]34[C@H]([C@H]([NH+](CC3)C)Cc3c4c1c(OC)cc3)CCC2=O	0.15
(R)-Laudanidine	CN1CCc2cc(c(cc2[C@@H]1Cc1ccc(c(c1)O)OC)OC)OC	0.15
Sibutramine	Clc1ccc(C2(CCC2)[C@@H]([NH+](C)C)CC(C)C)cc1	0.15
Diphenhydramine	C(OCC[NH+](C)C)(c1ccccc1)c1ccccc1	0.14
Propylhexedrine	C[C@H](CC1CCCCC1)[NH2+]C	0.14
Amdinocillin	[C@@H]1([C@@H]2N(C1=O)[C@H](C(S2)(C)C)C(=O)[O-])/[NH+]=C/N1CCCCCC1	0.14
Doxepin	C1(=C\CC[NH+](C)C)/c2c(COc3c1cccc3)cccc2	0.14
Oxycodone	O1[C@@H]2[C@]34[C@](O)([C@H](N(CC3)C)Cc3c4c1c(OC)cc3)CCC2=O	0.14
Mecamylamine	[NH2+]([C@]1(C([C@@H]2C[C@H]1CC2)(C)C)C)C	0.14
3-Isothiocyanatomethyl-1-methoxy-1H-indole	COn1cc(C[NH+]=C=S)c2ccccc12	0.13
3-Methoxymorphinan	[C@@]123c4c(C[C@@H]([C@H]1CCCC3)[NH2+]CC2)ccc(c4)OC	0.13

*According to the FLAP H*O*H descriptor

B. Supplementary Figure

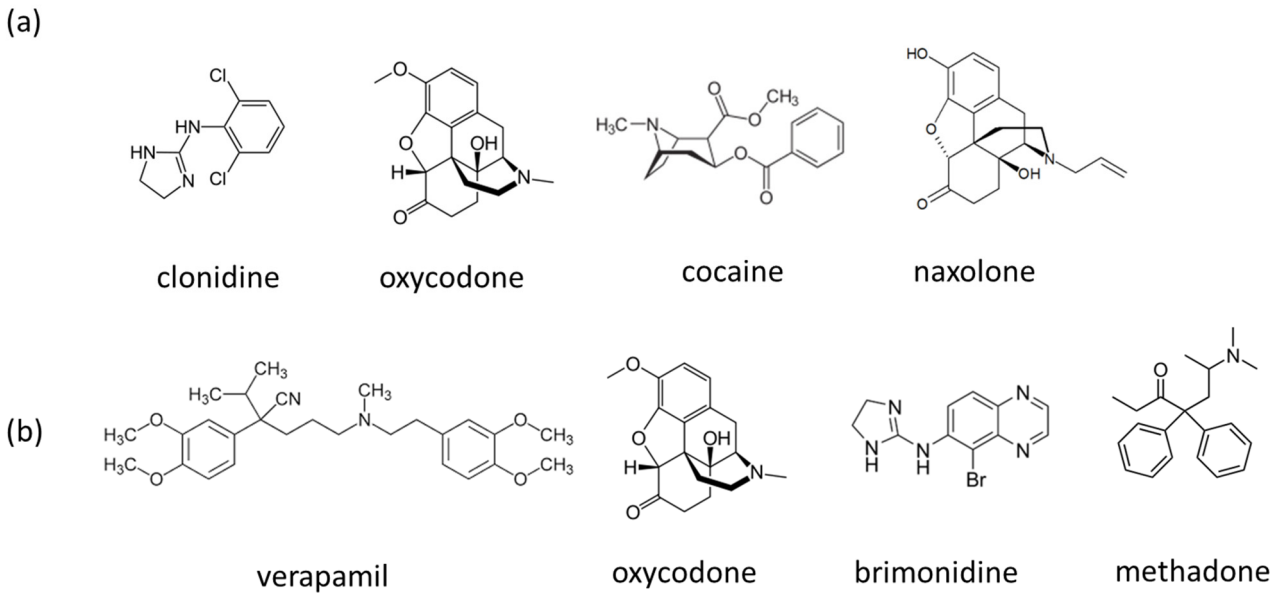

Figure S1: Chemical structures of drugs used for the generation of pharmacophore P1(**a**) and P2 (**b**).

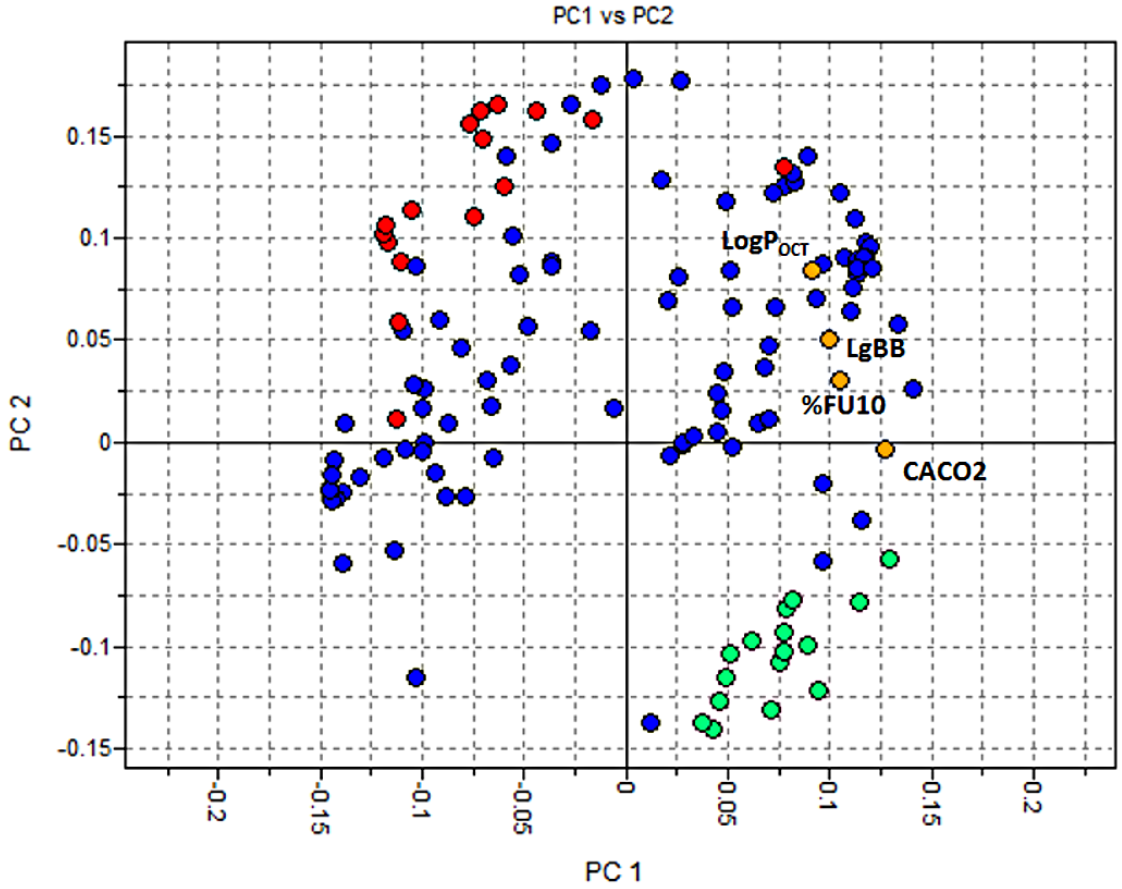

Figure S2: Loading plot for the PCA model generated for substrates and non-substrates of the proton antiporter. Hydrophilic descriptors (W1-W8, CW1-CW8) are red colored, while hydrophobic descriptors (D1-D8, CD1-CD8) are green colored. Additional descriptors discussed in the manuscript are highlighted in orange color. The other VolSurf+ descriptors are shown in blue. A detailed description of the descriptors has been reported elsewhere (Chapy et al., 2015).

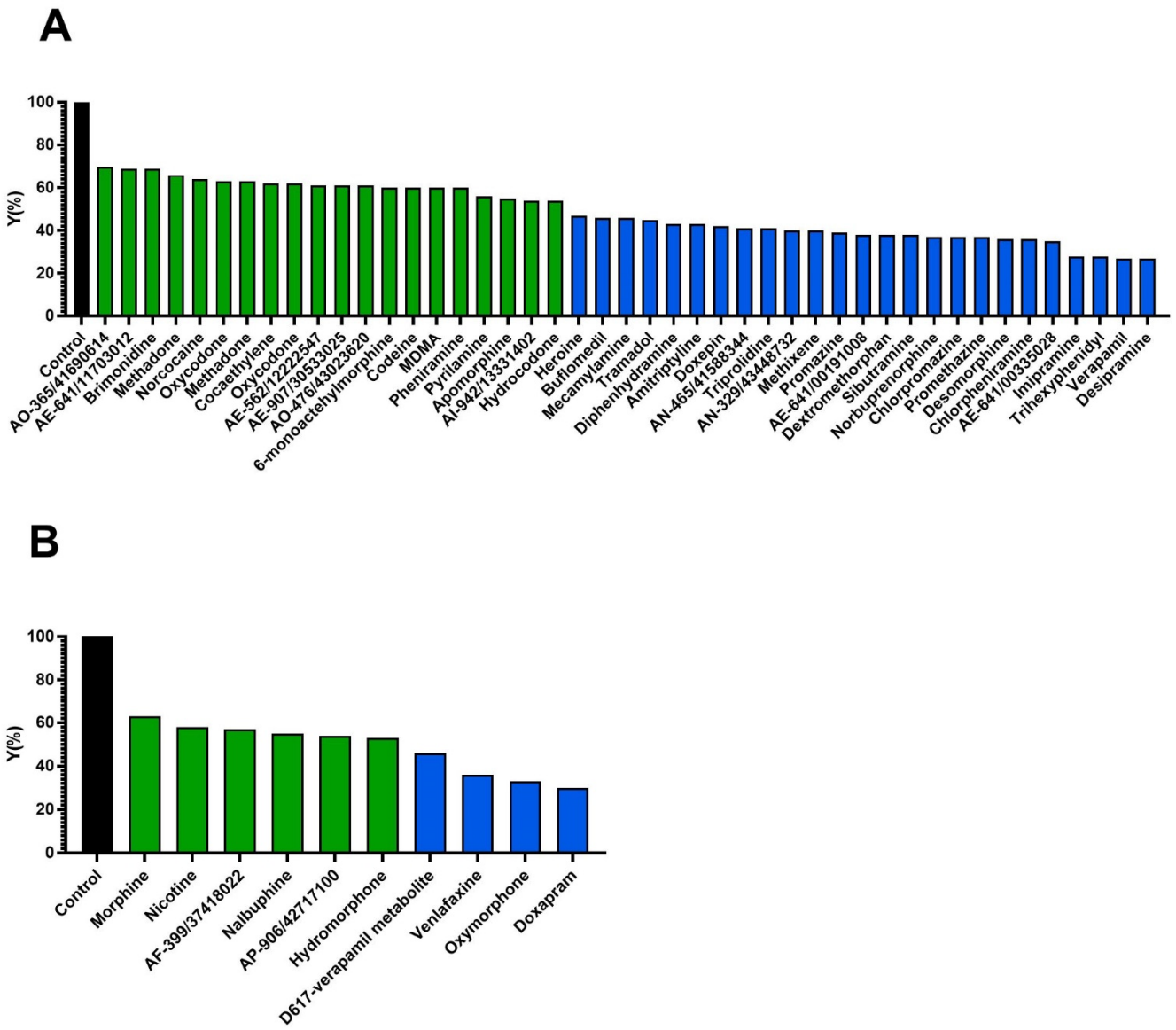

Figure S3: Substrate candidates Y(%) measured at 10 or 100μM by the [^{3}H]-clonidine trans-stimulation experiments in hCMEC/D3 cells; (**A**) Y is the percent of intracellular [^{3}H]-clonidine compared to control experiment (without unlabelled drug) obtained with subtrate candidate at 10 μM (blue, "Good" substrate; green, "Medium-high" substrate); (**B**) Y is the percent of intracellular [^{3}H]-clonidine compared to control experiment (without unlabelled drug) obtained with substrate at 100 μM (blue, "Weak" substrate; green, "Medium-low" substrate).