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Supporting Information

Probing the Chemical Space of Guanidino-Carboxylic Acids to Identify the First Blockers of the creatine-transporter-1

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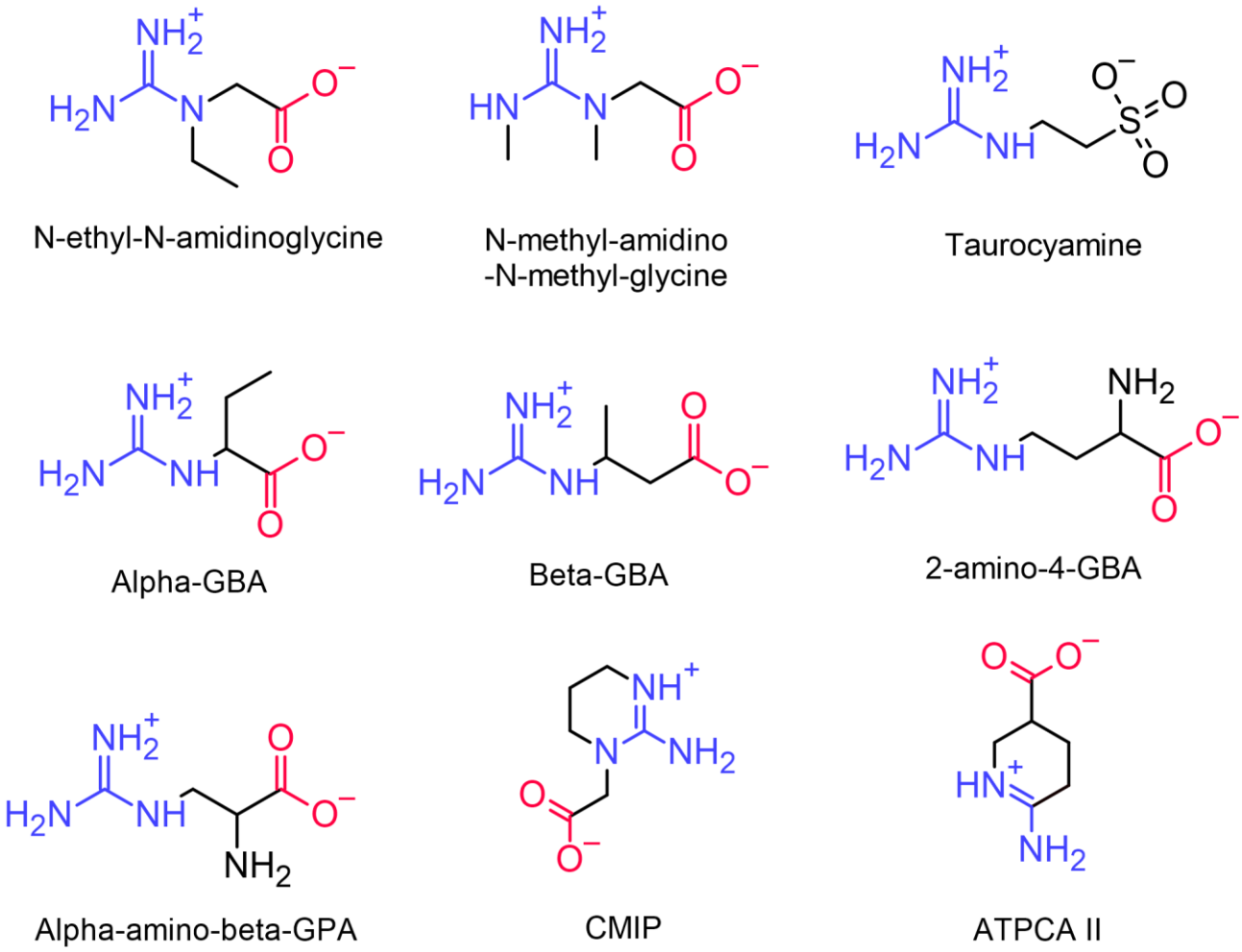
Table of contents:



Supplementary Figure 1. Structures of compounds previously reported to inhibit CRT-1 or creatine uptake. Guanidino groups (and the related group in ATPCA II) are highlighted in blue, carboxylate groups are visualized in red. Details and references are given in Supplementary Table 1. GBA = guanidinobutyrate; ATPCA II = 2-amino-3,4,5,6-tetrahydropyridine-5-carboxylic acid; CMIP = 1-carboxy-methyl-2-imino-hexahydro-pyrimidine. The structrures of the other previously reported inhibitors - i.e., GAA/guanidinoacetate, GPA guanidinopropionate, GBA/4-guanidinobutyrate; ATPCA/ 2-amino-1,4,5,6-tetrahydropyrimidine-5-carboxylic acid and cyclocreatine – are shown in Figure 1.

Supplementary TABLE 1.
Compilation of compounds, which were tested for inhibition of creatine uptake and inhibited uptake by ≥25%.

Compound		Tested cell/tissue preparation	[^{3}H]/[^{14}C]Cr µM	Uptake % of control	Reference
Name	**µM**				
1-carboxy-methyl-2-imino-hexahydro-pyrimidine (CMIP)	50	Rat skeletal muscle	0.21 µmol injected i.p	57	Fitch and Chevli, 1980
		Rat heart		58	
2-amino-1,4,5,6-tetrahydro-pyrimidine-5-carboxylic acid (ATPCA-II)	1000	tsA201 cells	0.2	25	Al-Khawaja et al., 2018
2-amino-4-guanidino-butyrate	1000	Astroglia rich culture	10	74	Möller and Hamprecht, 1989
α-amino-β-guanidino-propionate	1000	Astroglia rich culture	10	63	Möller and Hamprecht, 1989
	5000	Cos-7 cells	50	40	Guimbal and Kilimann, 1993
				49	Sora et al., 1994
β-guanidino-propionate	15	Brush border membrane vesicles from enterocytes	10	55	Tosco et al., 2004
	20	Chicken enterocytes	1	58	Peral et al., 2002
	50	Rat skeletal muscle	0.21 µmol injected i.p	50	Fitch and Chevli, 1980
		Rat heart		44	
		Brush border membrane vesicles from enterocytes	10	25	Tosco et al., 2004
	500	Brush border membrane vesicles from enterocytes	10	5	
		Uterine smooth muscle	50	12	Daly and Seifter, 1980
	1000	Rat skeletal muscle	100	17	Fitch et al., 1968
		Human erythrocytes	1.7 – 4.2	70	Ku and Passow, 1980
		Astroglia rich culture	10	4	Möller and Hamprecht, 1989
		Hela cells	0.5	16	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	5	Ohtsuki et al., 2002
		Chicken enterocytes	1	16	Peral et al., 2002
		Rat ileum	2	17	Peral et al., 2002
		Rat skeletal muscle giant sarcolemmal vesicles	10	25	Walzel et al., 2002
		TR-iBRB2 cells	18.2	9	Nakashima et al., 2004
		Hippocampal neurons	20	6	Dodd et al., 2010
	5000	Macrophages	21.7 - 43.4	20	Loike et al., 1986
		Cos-7 cells	50	10	Guimbal, and Kilimann, 1993
				6	Sora et al., 1994
		HEK293 cells	0.5	4	Dodd et al., 1999
	10000	Uterine smooth	1000	27	Daly and Seifter, 1980

		muscle			

Supplementary TABLE 1. Continued

Compound		Tested cell/tissue preparation	[^{3}H]/[^{14}C]Cr μM	Uptake % of control	Reference
Name	**μM**				
cyclo-creatine	1000	Chicken enterocyte	1	50	Peral et al., 2002
		Rat ileum	2	54	Peral et al., 2002
		Rat skeletal muscle giant sarcolemmal vesicles	10	51	Walzel et al., 2002
		Hippocampal neurons	20	24	Dodd et al., 2010
	5000	HEK293 cells	0.5	16	Dodd et al., 1999
dipyridamol	20	Human erythrocytes	1.7-4.2	55	Ku and Passow, 1980
DL-α-guanidino-butyrate	50	Rat skeletal muscle	0.21 μmol injected i.p	69	Fitch and Chevli, 1980
		Rat heart		63	
DL-β-guanidino-butyrate	50	Rat skeletal muscle	0.21 μmol injected i.p.	47	Fitch and Chevli, 1980
		Rat heart		49	
γ-guanidino-butyrate	15	Brush border membrane vesicles from enterocytes	10	90 *	Tosco et al., 2004
	50			75	
		Rat skeletal muscle	0.21 μmol injected i.p.	98 *	Fitch and Chevli, 1980
		Rat heart		109*	
	500	Brush border membrane vesicles from enterocytes	10	40	Tosco et al., 2004
	1000	Human erythrocytes	1.7-4.2	100 *	Ku and Passow, 1980
		Hela cells	0.5	42	Saltarelli et al., 1996
		TR-iBRB2 cells	18	28	Nakashima et al., 2004
		Hippocampal neurons	20	22	Dodd et al., 2010
	5000	Cos-7 cells	50	10	Guimbal, and Kilimann, 1993
				31	Sora et al., 1994
		HEK293 cells	0.5	26	Dodd et al., 1999
guanidino-acetate	50	Rat skeletal muscle	0.21 μmol injected i.p.	71	Fitch and Chevli, 1980
		Rat heart		95 *	
	1000	Rat skeletal muscle	100	74	Fitch et al., 1968
		Human erythrocytes	1.7-4.2	100 *	Ku and Passow, 1980
		Astroglia rich culture	10	47	Möller and Hamprecht, 1989
		Hela cells	0.5	69	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	30	Ohtsuki et al., 2002
		Rat skeletal muscle giant sarcolemmal vesicles	10	73	Walzel et al., 2002
		Hippocampal neurons	20	38	Dodd et al., 2010
	4000	Macrophages	21.7-43.4	59	Loike et al., 1986
	5000	Cos-7 cells	50	30	Guimbal and Kilimann, 1993
		Cos-7 cells	50	41	Sora et al., 1994
		HEK293 cells	0.5	41	Dodd et al., 1999
N-methyl-amidino-N-methyl-glycine	50	Rat skeletal muscle	0.21 μmol injected i.p.	57	Fitch and Chevli, 1980
Phloretin	1000	Human erythrocytes	1.7-4.2	20	Ku and Passow, 1980
Phlorizin	1000	Human erythrocytes	1.7-4.2	70	Ku and Passow, 1980
Taurocyamine	1000	Astroglia rich culture	10	47	Möller and Hamprecht, 1989

Compounds were classified as active, if they were reported to inhibit uptake of [^{3}H]- or [^{14}C]-labeled creatine ([^{3}H] /[^{14}C]Cr) by ≥25%.

* In these experiments, the compounds (i..e γ-guanidino-butyrate and guanidino-acetate) did not cause any inhibition of creatine uptake. However, because other reports showed inhibition of creatine, these two compounds were classified as active and included in this table.

Supplementary TABLE 2.
Compilation of compounds, which were tested for inhibition of creatine uptake and failed to suppress uptake by ≥20%.

Compound		Tested cell/tissue preparation	[^{3}H]/[^{14}C]Cr μM	Uptake % of control	Reference
Name	μM				
1-methyl-hydantoin	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
2-4'-amino-phenyl-6-methyl-benzene-thiazol-3',7-disulfonic acid	1000	Human erythrocytes	1.7-4.2	100	Ku and Passow, 1980
2,4-dinitro-phenol	170	Uterine smooth muscle	50	105	Daly and Seifter, 1980
2-amino-4-thiazole acetic acid	100	Xenopus oocytes	20	100	Dai et al., 1999
3-amino-1-2,4-triazole-5-carboxylic acid	100	Xenopus oocytes	20	100	Dai et al., 1999
4,4'-diisothio-cyano-dihydro-stilbene-2,2'-disulfonic acid	1000	Human erythrocytes	1.7-4.2	reported as non-inhibitory	Ku and Passow, 1980
α-amino-gamma-guanidino-butyrate	1000	Hela cells	0.5	91	Saltarelli et al., 1996
Alanine	1000	Hela cells	0.5	77	Saltarelli et al., 1996
	1000	Astroglia rich culture	10	90	Möller and Hamprecht, 1989
	2000	Uterine smooth muscle	50	81	Daly and Seifter, 1980
	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Arginine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Astroglia rich culture	10	76	Möller and Hamprecht, 1989
		Hela cells	0.5	104	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	80	Ohtsuki et al., 2002
		TR-iBRB2 cells	18.2	86	Nakashima et al., 2004
		Hippocampal neurons	20	100	Dodd et al., 2010
	2000	Uterine smooth muscle	50	80	Daly and Seifter, 1980
	4000	Macrophages	21.7-43.4	65	Loike et al., 1986
	5000	Cos-7 cells	50	100	Guimbal and Kilimann, 1993
β-Alanine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Chicken enterocytes	1	87	Peral et al., 2002
Betaine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Chicken enterocytes	1	97	Peral et al., 2002
Canavanine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968

Carnitine	5000	Cos-7 cells	50	100	Guimbal and Kilimann, 1993
Choline	1000	Hela cells	0.5	117	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	75	Ohtsuki et al., 2002
		Chicken enterocytes	1	80	Peral et al., 2002

Supplementary TABLE 2. Continued

Compound		Tested cell/tissue preparation	[^{3}H]/[^{14}C]Cr μM	Uptake % of control	Reference
Name	**μM**				
Citrulline	1000	Hela cells	0.5	96	Saltarelli et al., 1996
	5000	Cos-7 cells	50	100	Guimbal and Kilimann, 1993
Creatinine	1000	Astroglia rich culture	10	92	Möller and Hamprecht, 1989
		Hela cells	0.5	117	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	83	Ohtsuki et al., 2002
		TR-iBRB2 cells	18.2	91	Nakashima et al., 2004
	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
	5000	Cos-7 cells	50	100	Guimbal, and Kilimann, 1993
Cyano-guanidine	50	Rat skeletal muscle	0.21 μmol injected i.p	115	Fitch and Chevli, 1980
		Rat heart		122	
Cysteine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
DL-α-guanidino-propionate	50	Rat skeletal muscle	0.21 μmol injected i.p	84	Fitch and Chevli, 1980
		Rat heart		81	
	2000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
DL-guanidino-succinate	50	Rat skeletal muscle	0.21 μmol injected i.p	97	Fitch and Chevli, 1980
		Rat heart		91	
Epoxy-creatine	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
GABA	1000	Astroglia rich culture	10	94	Möller and Hamprecht, 1989
		Hela cells	0.5	113	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	89	Ohtsuki et al., 2002
		Chicken enterocytes	1	80	Peral et al., 2002
		Rat ileum	2	85	
		TR-iBRB2 cells	18.2	94	Nakashima et al., 2004
		Hippocampal neurons	20	96	Dodd et al., 2010
	5000	Cos-7 cells	50	100	Guimbal, and Kilimann, 1993
Glutamic acid	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Glutamine	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
	1000	Hela cells	0.5	108	Saltarelli et al., 1996
Glucose	10000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
Glycine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Astroglia rich culture	10	82	Möller and Hamprecht, 1989
		Hela cells	0.5	109	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	81	Ohtsuki et al., 2002
		Chicken enterocytes	1	96	Peral et al., 2002
		Rat ileum	2	97	
	2000	Uterine smooth muscle	50	84	Daly and Seifter, 1980
	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Guanidine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
Guanidino-succinate	1000	Hela cells	0.5	110	Saltarelli et al., 1996
Hexanol	8000	Human erythrocytes	1.7-4.2	80	Ku and Passow, 1980
Histamine	4000	Macrophages	21.7/43.4	>90	Loike et al., 1986
Isopropyl-urea	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968

Supplementary TABLE 2. Continued

Compound		Tested cell/tissue preparation	[^{3}H]/[^{14}C]Cr μM	Uptake % of control	Reference
Name	**μM**				
Leucine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Uterine smooth muscle	50	82	Daly and Seifter, 1980
	4000	Macrophages	21.7/43.4	>90	Loike et al., 1986
Lysine	4000	Macrophages	21.7/43.4	>90	Loike et al., 1986
Methionine	2000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
N-amidino-benzamide	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
N,N-dimethyl-guanidine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
N-ethyl-N-amidino-glycine	50	Rat skeletal muscle	0.21 μmol injected i.p.	78	Fitch and Chevli, 1980
		Rat heart muscle		86	
N-ethyl-guanidino-acetic acid	1000		100	reported as non-inhibitory	Fitch et al., 1968
N-methyl-N-amidino-β-alanine	50	Rat skeletal muscle	0.21 μM injected i.p.	91	Fitch and Chevli, 1980
		Rat heart		97	
N-methyl-glycine	1000	Human erythrocytes	1.7-4.2	reported as non-inhibitory	Ku and Passow, 1980
		Astroglia rich culture	10	100	Möller and Hamprecht, 1989
		Hela cells	0.5	92	Saltarelli et al., 1996
	2000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
N-methyl-guanidine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
		Human erythrocytes	1.7-4.2	100	Ku and Passow, 1980
Nipecotic acid	1000	Chicken enterocytes	1	72	Peral et al., 2002
	20			91	
	1000	Rat ileum	2	84	
Ornithine	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
	4000	Macrophages	21.7-43.4	95	Loike et al., 1986
Phenylalanine	2000	Uterine smooth muscle	50	86	Daly and Seifter, 1980
Phospho-creatine	1000	Astroglia rich culture	10	99	Möller and Hamprecht, 1989
	1000	Hela cells	0.5	98	Saltarelli et al., 1996
		TM-BBB4 cells	9.1	68	Ohtsuki et al., 2002
		Rat skeletal muscle giant sarcolemmal vesicles	10	102	Walzel et al., 2002
		TR-iBRB2 cells	18.2	58	Nakashima et al., 2004
	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Proline	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Propylurea	1000	Rat skeletal muscle	100	reported as non-inhibitory	Fitch et al., 1968
Serine	4000	Macrophages	21.7-43.4	>90	Loike et al., 1986
Taurine	1000	Astroglia rich culture	10	92	Möller and Hamprecht, 1989
		Hela cells	0.5	98	Saltarelli et al., 1996
		Chicken enterocytes	1	96	Peral et al., 2002
		Rat ileum	2	80	
		Rat skeletal muscle giant sarcolemmal vesicles	10	100	Walzel et al., 2002

Compounds were classified as non-inhibitory, if they were reported as non-inhibitory in the cited reference or if residual uptake in their presence was ≥80% of control creatine uptake (i.e., uptake measured in their absence). In some instances, individual references reported inhibition by >20%. In these cases, the classification as inactive is based on the average inhibition.

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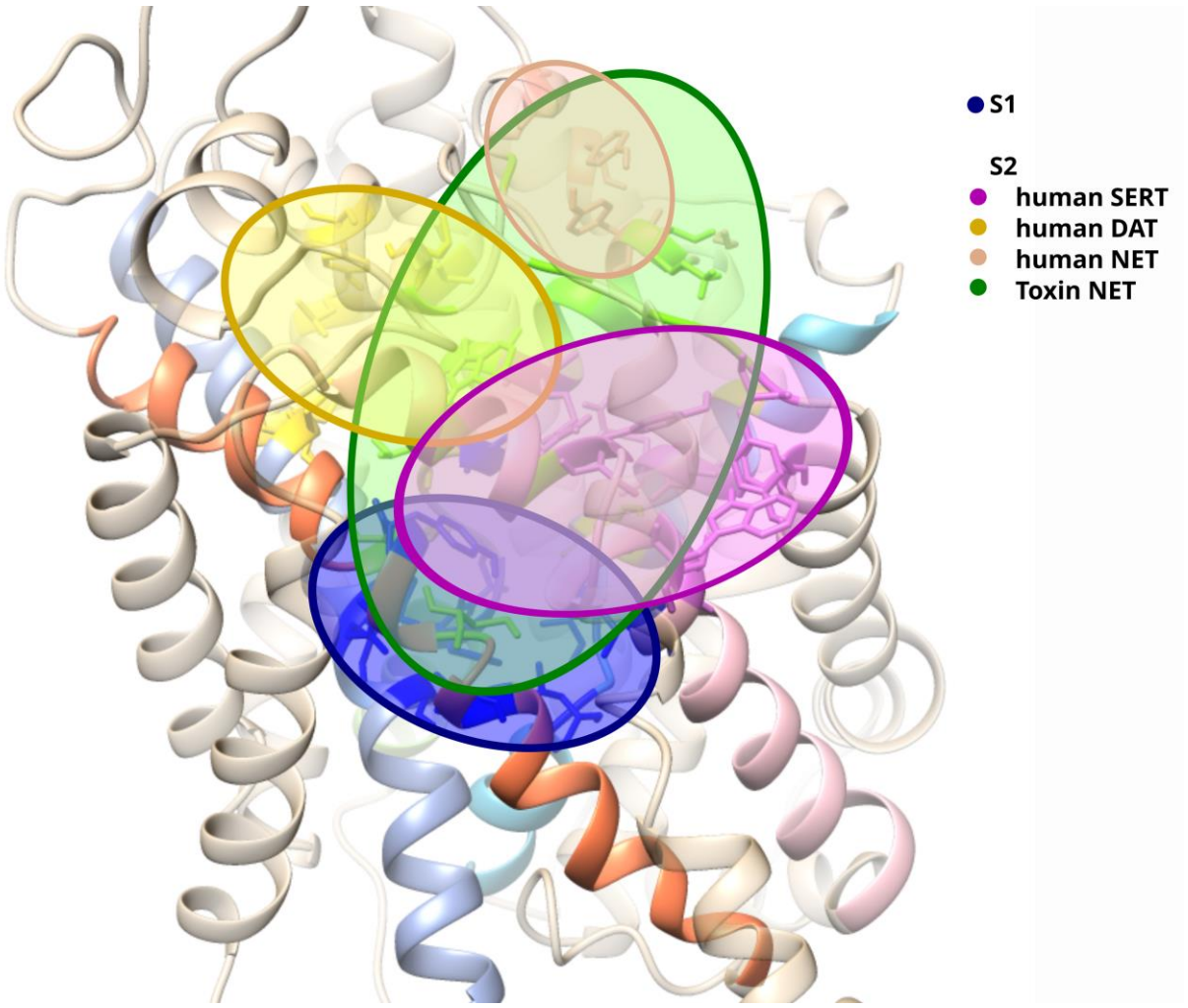

Supplementary Figure 2. Mapping of ligand interaction sites, which were identified in the monoamine transporters of the human SLC6 family, onto the structure of the human creatine transporter-1. The homology model structure of human CRT-1 is based on the structure of human SERT (Clarke et al., 2024). The helices are colored as in Fig. 3A. The blue ellipsoid indicates the orthosteric S1 site; the orchid, gold, salmon and green ellipsoids label the regions within the extracellular vestibule (collectively referred to as S2 site), where allosteric binding occurs in human SERT (PDB ID: 5I73, 5I75; Coleman et al., 2016; DOI: 10.1038/nature17629; PDB ID: 7LI9, 7LIA, 7MGW; Yang and Gouaux 2021; PDB ID: 7LWD; Plenge et al. 2021), in human DAT (PDB ID: 8VBY; Srivastava et al., 2024), in human NET (accessory site for norepinephrine in salmon and χ-conotoxin MrlA analog in green; PDB ID: 8WGX, 8HFG, 8HFF, 8WTV, 8WTW; Hu et al., 2024), respectively. The pertinent amino acid side chains of CRT-1, which reside in each of these sites, are highlighted as appropriately colored sticks.

References to supplementary Figure 2:

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PBD files:

1) hCRT1_Homology_Model.pdb. – Coordinates of the homology model of human CRT1

2-11) PDB-files for docking of all compounds shown in Figures 3 and 4:

2) ATPCA.pdb

3) Compound1.pdb

4) GiDi1254.pdb

5) GiDi1257.pdb

6) MIPA572_R.pdb

7) MIPA572_S.pdb

8) MIPA573_R.pdb

9) MIPA573_S.pdb

10) MIPA574_R.pdb

11) MIPA574_S.pdb