Supplemental information

Rigid adenine nucleoside derivatives as novel modulators of the human sodium symporters for dopamine and norepinephrine

Aaron Janowsky, Dilip K. Tosh, Amy J. Eshleman, Kenneth A. Jacobson
Transporter binding assays

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Supplemental Figure Legends

Figure S1. Effects of adenosine receptor agonists on [^{3}H]WIN 35428 binding to DAT, [^{3}H]citalopram binding to the SERT, and [^{3}H]nisoxetine binding to NET. Representative curves determined by PDSP (Besnard et al., 2012), Univ. of North Carolina, are shown.

Figure S1A: Compound **2**, MRS5474 (ATDP33618, PDSP 24974) inhibited radioligand ([^{3}H]WIN35,428) binding at the DAT, Ki = 1.69 μM (compared to standard GBR12909). Drugs did not displace radioligand binding at NET ([^{3}H]nisoxetine) or SERT ([^{3}H]citalopram), at concentrations up to 10 μM (data not shown).

Figure S1B: Compound **6**, MRS5697 (ATDP33619, PDSP 27859) enhanced radioligand binding to the DAT approximately 7 fold at 10 μM.

Figure S1C: Compound **6**, MRS5697 (ATDP33619, PDSP 27859) weakly inhibited [^{3}H]citalopram binding to the SERT, Ki = 1869 nM. The drug was inactive at the NET at 10 μM (data not shown).

Figure S1D: Compound **8**, MRS5917 (ATDP33621, PDSP 29884) inhibited [^{3}H]WIN 35428 binding to DAT, Ki = 755 nM. Compound **8** (10 μM) did not displace radioligand from the NET and SERT.

Figure S1E: Compound **9**, MRS5980 (ATDP33622, PDSP 30519) enhanced [^{3}H]WIN 35428 binding to DAT approximately 5 fold at 10 μM. Compound **9** (10 μM) had no effect on radioligand binding to the NET and SERT.

Figure S1F: Compound **11**, MRS7135 (ATDP33733, PDSP 34074) enhanced [^{3}H]WIN 35428 binding to the DAT by 330%, and had no effect on radioligand binding to the NET and SERT at 10 μM.

Figure S1G: Compound **22**, MRS5755 (ATDP33620, PDSP 27857) inhibited radioligand binding to the NET, Ki = 1.96 μM, but had no effect on radioligand binding to the DAT and SERT at 10 μM.

Figure S1H: Compound **27,** MRS7112 (PDSP 33692) inhibited radioligand binding to the DAT (Ki = 4.48 μM) but had no effect at the NET and SERT at 10 μM.

Figure S1I: Compound **28,** MRS7111 (PDSP 33691) inhibited radioligand binding to the DAT at single point determination (69%), but was inactive in a full concentration-response curve. At 10 μM, the drug did not displace radioligand binding from the NET or SERT.

Other drugs tested by PDSP (data not shown):

Compound 5. MRS5697 (ATDP33654, PDSP 27370)**:** Inhibited radioligand binding at the dopamine transporter, Ki = 2.26 μM. NET and SERT, inactive at 10 μM.

Compound 7. MRS5969 (ATDP33651, PDSP 30105)**:** Enhanced radioligand binding at the dopamine transporter by 290%. NET and SERT, inactive at 10 μM.
Compound 16. MRS7036 (ATDP33656, PDSP 33113)**:** DAT, enhanced 230%. NET and SERT, inactive at 10 μM.
Compound 17. MRS5914 (ATDP33653, PDSP 29883)**:** inhibited DAT, K_i 1.81 μM. NET and SERT, inactive at 10 μM.
Compound 19. MRS5972 (ATDP33652)**:** enhanced at DAT by 120% (average of 2 values). NET and SERT, inactive at 10 μM.

Figure S2. Radioligand Binding to VMAT2 (for structures of inhibitors, see Table S1). Compounds **5**, **6**, **8**, and **11** had no effect on [^{3}H]DHTB or on [^{3}H]Ketanserin binding to the VMAT2. Additional compounds tested that had a similar lack of effect on the binding of these radioligands were **2**, **4**, **7**, **9**, **16** , **17**, **19**, **20**, **22**, **23**, **24**, **25**, **26**, **31**, and **32** (data not shown). N= 2-3 for test compounds, 5 for Ro4-1284 and ketanserin. Data shown are mean ± sem.

Supplemental References:
Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, Norval S, Sassano MF, Shin AI, Webster LA, Simeons FR, Stojanovski L, Prat A, Seidah NG, Constam DB, Bickerton GR, Read KD, Wetsel WC, Gilbert IH, Roth BL and Hopkins AL (2012) Automated design of ligands to polypharmacological profiles. Nature 492:215-20.

Figure S1: Effect of adenosine receptor agonists on [^{3}H]WIN 35428 binding to DAT, SERT and NET, PDSP results.

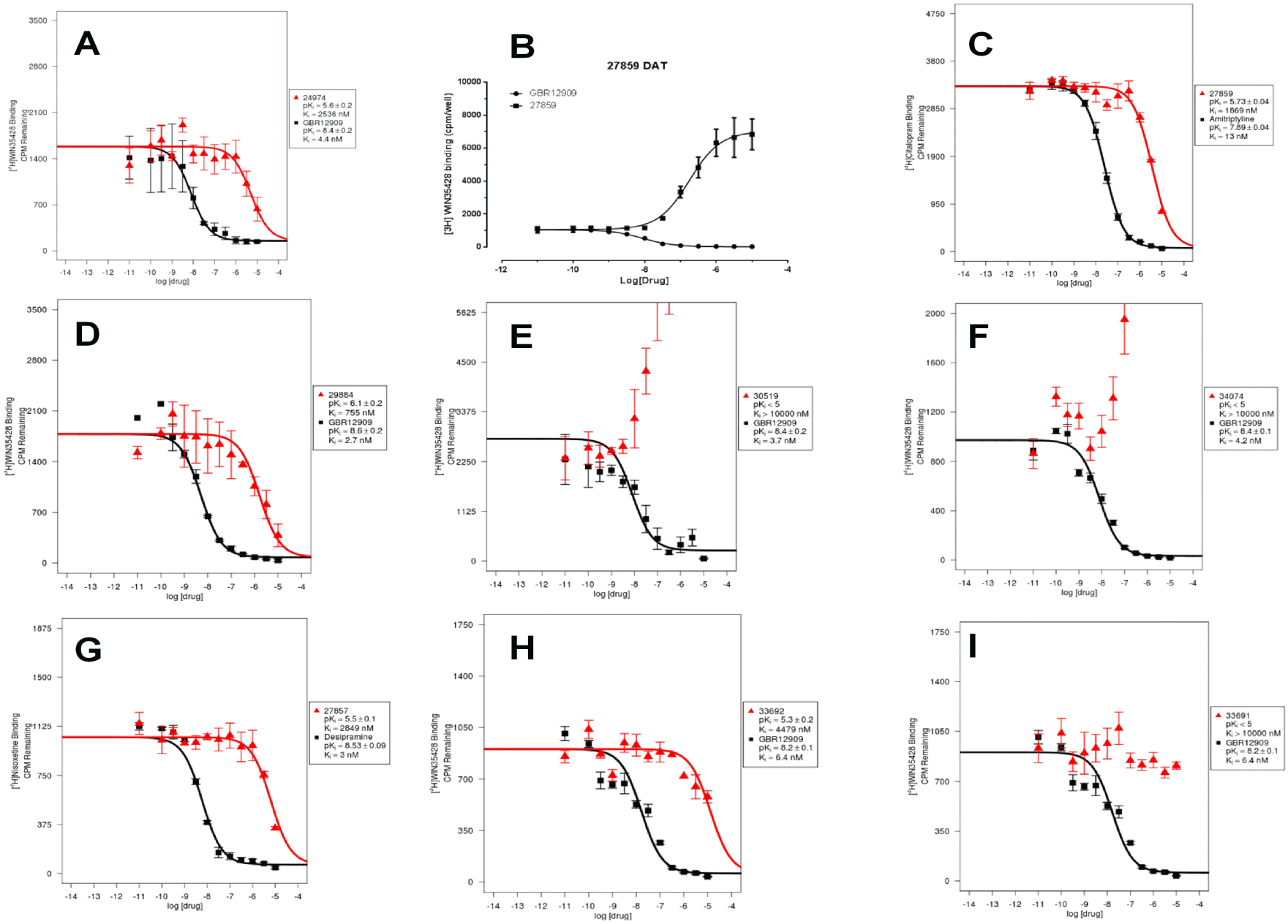
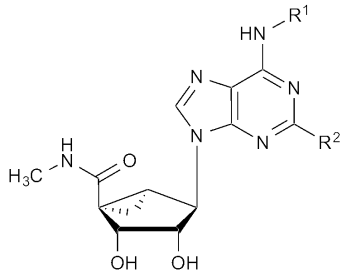
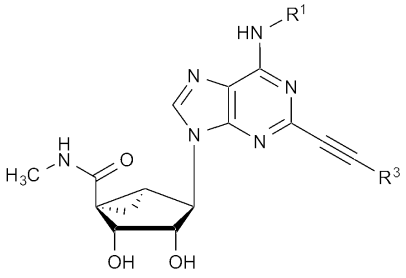


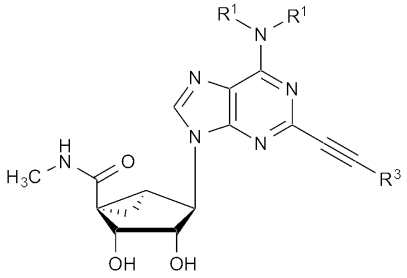
Table S1. Structures and binding modulation at human DAT[a] of (N)-methanocarba adenosine derivatives, including simple C2 derivatives **1**, **2** and **21**, alkyne compounds **3** - **20** (5'-amides) and **22** – **24** (truncated), and triazole derivatives **25**-**32**.



1



3 – 9 and **11 – 19**



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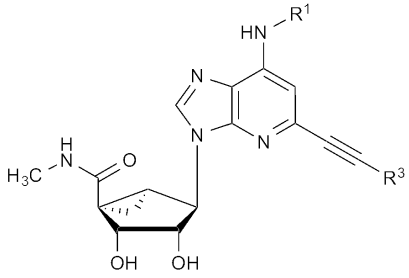

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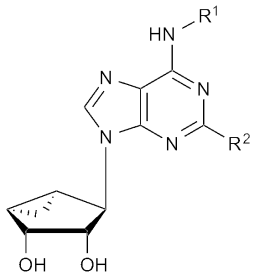
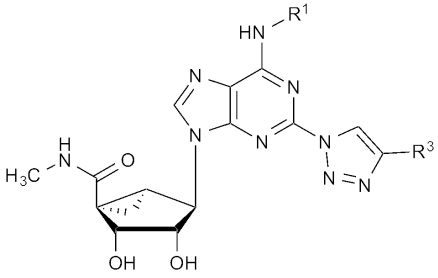
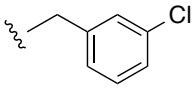
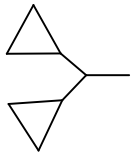
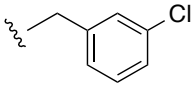
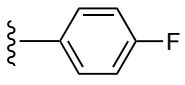
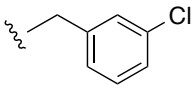
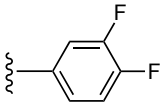
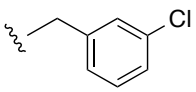
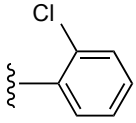
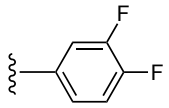
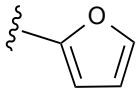
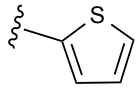
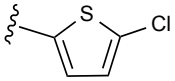
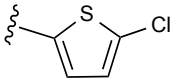
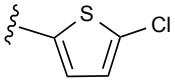
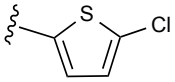
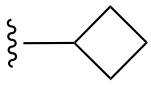
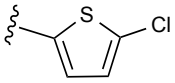
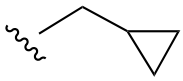
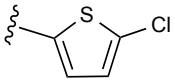
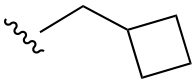
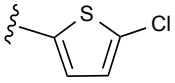
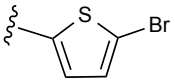
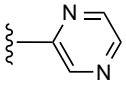
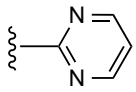
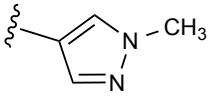
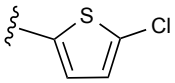


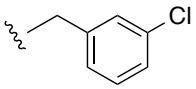
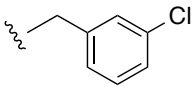
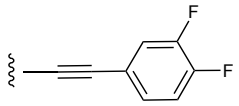
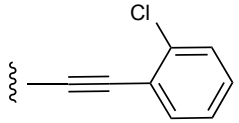
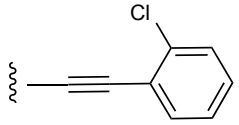
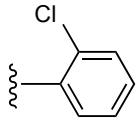
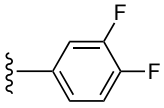
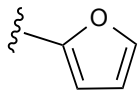
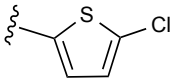
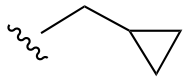
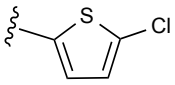
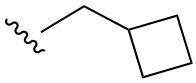
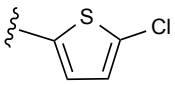
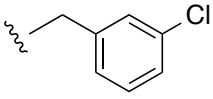
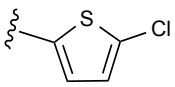
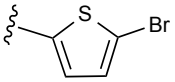
2, **21 – 24**



25 – 32

Compd., ATDP#[c]	R^1	R^2 or R^3	PDSP Results, % inhibition at 10^{-5} M [a]	PDSP Results **K_i,** μM [b]
1 MRS3558 NS		Cl	*3%*	ND
2 MRS5474 33618		Cl	*-12%*	**1.69**
3 MRS5678 NS			*21%*	ND
4[b,d] **MRS5698 33655**			*16%*	ND
5 MRS5697 33654			*67%*	**2.26**

6 MRS5676 33619	CH_3		*-320%*	ND
7 MRS5969 33651	CH_3		*-290%*	ND
8 MRS5917 33621	CH_3		*81%*	**0.76**[d]
9 MRS5980 33622	CH_3		*-560%*	ND
10 MRS7158 NS	N,N-di-CH_3		*25%*	ND
11 MRS7135 33733	C_2H_5		*-330%*	ND
12 MRS7154 33752	$(CH_2)_2CH_3$		*-159%*	ND
13 MRS7153 33751			*-137%*	ND
14 MRS7146 NS			*-10%*	ND
15 MRS7147 NS			*6%*	ND
16 MRS7036 33656	CH_3		*-230%*	ND
17 MRS5914 33653	CH_3		*69%*	**1.81**
18 MRS5913 NS	CH_3		*6%*	ND
19 MRS5972 33652	CH_3		*-120%*	ND
20 MRS7140 33734	CH_3		*1%*	ND

21 MRS5202 NS		H	*-2%*	ND
22 MRS5755 33620			*-2%*	ND
23 MRS5782 33737	CH_3		ND	ND
24 MRS5778 33739	C_2H_5		ND	ND
25 MRS7115 33740	CH_3		ND	ND
26 MRS7117 33735	CH_3		ND	ND
27 MRS7112 NS	CH_3		*75%*	**4.5**
28 MRS7111 NS	CH_3		*70%*	*(inactive in full curve)*
29 MRS7162 NS			*15%*	ND
30 MRS7163 NS			*35%*	ND
31 MRS7138 33736			ND	ND
32 MRS7119 33738	CH_3		ND	ND

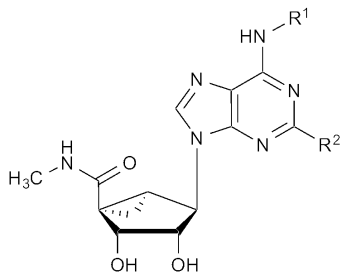
[a] Inhibition of [^{3}H]WIN35,428 binding at 10 μM. If there was an insignificant effect on DAT binding (<50%), a Ki value was not determined. A negative value indicates enhancement of radioligand binding over control.

[b] Values are expressed as the mean of 2 – 3 values. ND – not determined.

[c] Compound number corresponding to the main text, followed by MRS number, followed by ATDP number. NS – not submitted to the ATDP.

[d] Compound **8** enhanced binding of [^{125}I]RTI-55 (Table 1).

Table S2. Structures and binding at human ARs of (N)-methanocarba adenosine derivatives, including simple C2 derivatives **1**, **2** and **21**, alkyne compounds **3** - **20** (5'-amides) and **22** – **24** (truncated), and triazole derivatives **25-32**. Compounds for which AR affinity is not listed here will be reported elsewhere.



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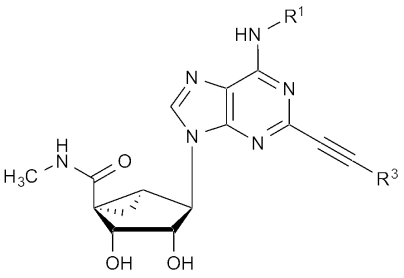
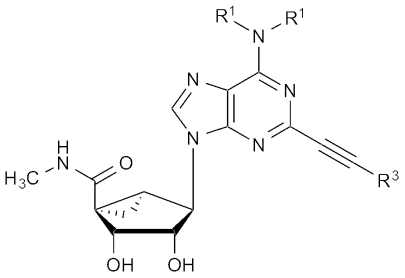
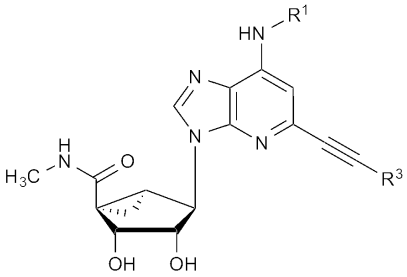


3 – 9 and **16 – 19**



10



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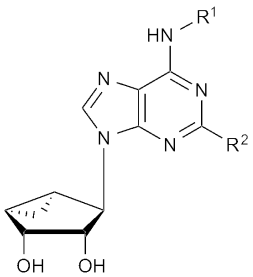
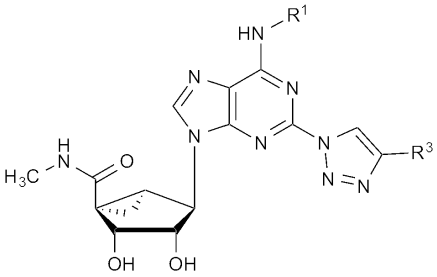
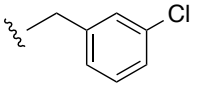
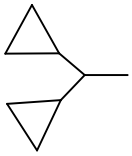
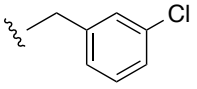
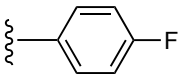
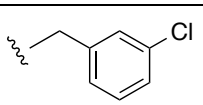
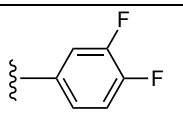
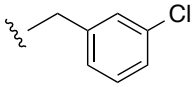
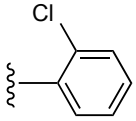
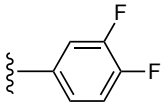
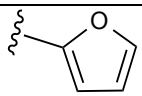
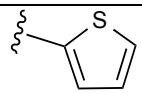
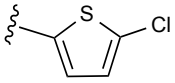
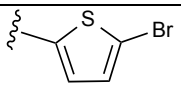
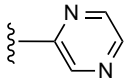
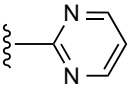
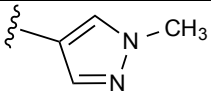
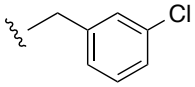
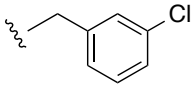
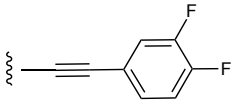
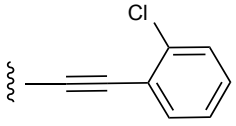
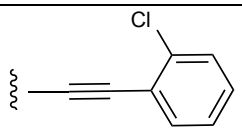
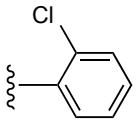
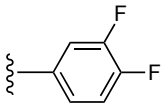
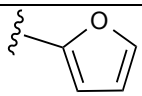


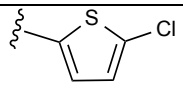
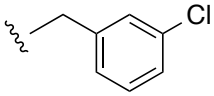
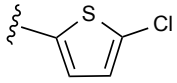
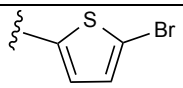
2, **21 – 24**



25 – 28, 31, 32

Compd[c]	R^1	R^2 or R^3	A_1AR % inhibition or K_i (nM)[a]	$A_{2A}AR$ % inhibition[c]	A_3AR % inhibition or K_i (nM)[a]
1		Cl	260 ± 60	2300 ± 100	0.29 ± 0.04
2		Cl	47.9±10.5	3950 ± 410	470 ± 15
3			*20%*	*42%*	2.16±0.34
4[b,d]			*6%*	*41%*	3.49±1.84

5			*19%*	*52%*	1.92±0.57
6	CH_3		*6%*	*6%*	1.65±0.08
7	CH_3		*30%*	*18%*	0.62±0.06
8	CH_3		*25%*	*20%*	0.57 ± 0.10
9	CH_3		*6%,*	*24%,*	0.70 ± 0.11
16	CH_3		*18%*	*19%*	0.44 ± 0.12
17	CH_3		*37%*	*25%*	1.76 ± 0.22
18	CH_3		*25%*	*27%*	1.97 ± 0.27
19	CH_3		*35%*	*12%*	2.34±0.29
21		H	*45%*	4520±830	4.9±0.7
22			*29%*	1350 ± 190	100 ± 30
23	CH_3		*35%*	*37%*	3.20±0.91
24	C_2H_5		*25%*	*17%*	5.80 ± 2.08
25	CH_3		*20%*	*31%*	0.95 ± 0.50
26	CH_3		*9%*	*39 %*	1.06 ± 0.10
27	CH_3		*43%*	*51%*	0.96 ± 0.09

28	CH_3		*27%*	*34%*	0.73 ± 0.10
31			*65%*	>10,000	9.02 ± 5.78
32	CH_3		*33%*	*7%*	0.58 ± 0.17

[a] Binding in membranes prepared from CHO or HEK293 (A_{2A} only) cells stably expressing one of three hAR subtypes, unless noted. The binding affinity for hA_1, A_{2A} and A_3ARs was expressed as K_i values (n = 3−4, unless noted), measured using agonist radioligands [^{3}H]N^6-R-phenylisopropyladenosine, [^{3}H]2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamido-adenosine, or [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide, respectively. Additional values designated (m) are for mouse ARs. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using NECA (10 μM). Values are expressed as the mean ± SEM. K_i values were calculated as reported.[24]

[c] Percent of inhibition at 10 μM. ND – not determined.

Figure S2. Radioligand Binding to VMAT2 (for structures of inhibitors, see Table S1).

As shown in Figure S2, compounds **5**, **6**, **8**, and **11** had no effect on [^{3}H]DHTB and [^{3}H]Ketanserin binding to the VMAT2. Additional compounds tested that had a similar lack of effect on the binding of these radioligands were **2**, **4**, **7**, **9**, **16** , **17**, **19**, **20**, **22**, **23**, **24**, **25**, **26**, **31**, and **32** (data not shown). N= 2-3 for test compounds, 5 for Ro4-1284 and ketanserin. Data shown are mean ± sem.