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Scaffold Repurposing of Nucleosides (Adenosine Receptor Agonists): Enhanced Activity at the Human Dopamine and Norepinephrine Sodium Symporters

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Abstract

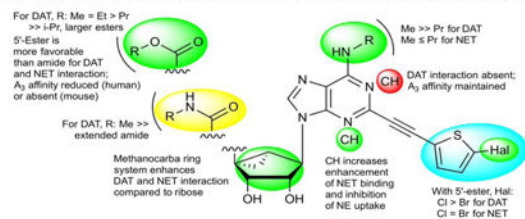
We have repurposed (N)-methanocarba adenosine derivatives (A_3 adenosine receptor (AR) agonists) to enhance radioligand binding allosterically at the human dopamine (DA) transporter (DAT) and inhibit DA uptake. We extended the structure-activity relationship of this series with small N^6 -alkyl substitution, 5'-esters, deaza modifications of adenine, and ribose restored in place of methanocarba. C2-(5-halothien-2-yl)-ethynyl 5'-methyl **9** (MRS7292) and 5'-ethyl **10** (MRS7232) esters enhanced binding at DAT ($EC_{50} \sim 35$ nM) and at norepinephrine transporter (NET). **9** and **10** were selective for DAT compared to A_3 AR in the mouse, but not human. At DAT, binding of two structurally dissimilar radioligands was enhanced; NET binding of only one radioligand was enhanced; SERT radioligand binding was minimally affected. **10** was more potent than cocaine at inhibiting DA uptake ($IC_{50} = 107$ nM). Ribose analogues were weaker in DAT interaction than corresponding bicyclics. Thus, we enhanced the neurotransmitter transporters activity of rigid nucleosides while reducing A_3 AR affinity.

Graphical abstract

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Supporting Information **Available:** NMR analysis and mass spectra of selected synthesized compounds, additional synthetic schemes and procedures, and off-target (PDSP) screening results.

Nucleosides as Novel Allosteric Modulators of Dopamine and Norepinephrine Transporters



Keywords

Dopamine transporter; G protein-coupled receptor; scaffold repurposing; adenosine derivatives; norepinephrine transporter; structure activity relationship

Introduction

In the course of examining off-target activities of potent A₃ adenosine receptor (AR) agonists for their novel application to the treatment of chronic neuropathic pain,^{1,2} we discovered a new class of allosteric ligands with low nM affinity at the human (h) dopamine (DA) transporter (DAT, SLC6A3).^{3,4} DAT function is inhibited by cocaine, which increases the levels of DA at the synapse by a combination of mechanisms.⁵ Micromolar activity of the same substituted nucleosides at the structurally related norepinephrine transporter (NET, SLC6A2) and weaker interactions with the serotonin transporter (SERT, SLC6A4) were also noted. These transporters are important drug targets for treatment of depression and other behavioral disorders and belong to the much larger class of sodium symporters, which transport diverse solute molecules across the cell membrane. X-ray structures of members of this family of transmembrane proteins have been determined, thus shedding light on the nature of their gating and substrate/inhibitor recognition.^{4,6,7}

The medicinal chemistry of diverse classes of DAT modulators has been explored toward the goal of treating drug abuse.⁸ Conventional inhibitors of DAT in the CNS are psychostimulants that are of interest for the treatment of addiction to cocaine, heroin or nicotine, and for obesity, narcolepsy and ADHD, but there is a potential for their abuse as recreational drugs.⁹ Based on pharmacological¹⁰⁻¹² and genetic data,¹³ decreased dopaminergic signaling in the mesocorticolimbic pathway is suggested to play a role in a wide range of behavioral disorders: depression, mood disorders, anhedonia, post-traumatic stress disorder (PTSD), addiction, attention deficit hyperactive disorder (ADHD), autism and pain. Antidepressant treatments that include DAT inhibition, including triple reuptake inhibitors (TRIs) that combine inhibition of SERT and NET with DAT, are being developed because of their expected higher efficacy in treating depression.^{12,14-16} The classical inhibitors of DAT, such as methylphenidate, are being considered as co-therapy for treating depression, but there is a risk of side effects, such as inducing mania in patients with bipolar disorder.¹⁷ However, an allosteric inhibitor of DAT that has a more subtle action might have an advantage over stimulant drugs that are orthosteric DAT inhibitors in the treatment of behavioral disorders associated with DA deficiency.

We have utilized a bicyclo[3.1.0]hexane ring (methanocarba) system in place of ribose in numerous nucleoside derivatives and noted that high affinity and selectivity at the A₃AR could be achieved with the retention of full agonism.^{2,18,19} Because this rigid, bicyclic ring system offers the opportunity for systematic substitution at multiple positions with defined geometry, we have explored its use as a component of a repurposed scaffold: shifting adenine nucleosides from ARs to other target proteins, such as receptors and transporters.^{3,20} For example, (*1S,2R,3S,4R,5S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxy-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (MRS5980, **1**, Chart 1), which displays >10,000-fold greater affinity for the hA₃AR (K_i = 0.70 nM) than at other ARs, also inhibited DA uptake with an IC₅₀ of 253 nM.³ Contrary to effects on uptake, however, **1** enhanced the binding of a tropane radioligand [¹²⁵I]methyl (1*R,2S,3S*)-3-(4-iodophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (RTI-55, **23**)³ to hDAT with an EC₅₀ of 35 nM. The closely related derivatives **2** and **3** differing only in the terminal aryl group enhanced binding of [¹²⁵I]**23** with EC₅₀ values of 9.1 and 70 nM, respectively. Compound **1** enhanced the affinity of radioligand binding at hDAT, with 100 nM of **1** reducing the K_d of [¹²⁵I]**23** from 1.3 nM to 0.39 nM. We now report the synthesis and *in vitro* structure-activity relationship (SAR) of an expanded class of nucleosides. Results indicate that disparate nucleoside structures are effective A₃AR and transporter ligands with a unique pharmacology.

Results

Chemical synthesis

The class of nucleosides initially examined was based on our earlier report that (*N*)-methanocarba nucleosides, containing a rigid C2-arylethynyl group and 5'-carbonyl and N⁶-methyl substitution, allosterically modulate hDAT.³ Compounds **4**,³ **6** and **8** - **16**, **22**, and **27** - **29** were synthesized by the routes shown in Schemes 1 - 3. Known nucleosides **1** - **3**, **5** and **7** were prepared as reported.^{2,21} The novel modifications included extended small N⁶-alkyl substitution (**7**, **28**), 5'-esters (**9** - **21**), 5'-carboxylate (**22**), 5' extended amide (**8**), deaza modifications of adenine (**5**, **6**, and **13**) and ribose restored in place of methanocarba (**27** - **29**), and combinations thereof (Table 1). For the (*N*)-methanocarba adenosine derivatives, 5'-ethyl ester intermediate **32** was prepared by a reported method,² which upon Sonogashira reaction with 5-chloro or 5-bromo-2-ethynylthiophene gave compounds **33** and **34** (Scheme 1). Deprotection of the acetonide group of compounds **33** and **34**, with 10% aqueous TFA provided the ethyl ester derivatives **10** and **12**. Compound **12** was subjected to transesterification with sodium methoxide to give compound **11**. Compound **12** was saponified to provide carboxylic acid **22** or aminolyzed with ethylenediamine to yield extended amide **8**. Acid derivative **22** was esterified with various alcohols in the presence of DCC and DMAP to afford 5'-esters **14** - **21**.

9-Riboside analogues were prepared as shown in Scheme 2 from the intermediate **35a**.^{22a} Protection of the 2' and 3'-hydroxy groups provided an acetonide derivative **35b**, which underwent C6 substitution with alkyl amines to give compounds **37** and **38**. PDC oxidation of the 5'-hydroxy group of compounds, **37** and **38**, and coupling of resulting carboxylic acids, **39** and **40**, with methylamine in presence of HATU afforded the methylamide

derivatives **41** and **42**. Compound **39** was also condensed with MeOH in presence DCC and DMAP to give the ester derivative **43**. Sonogashira coupling of compounds **41-43** with 5-chloro-2-ethynylthiophene and subsequent acid hydrolysis provided compounds **27-29**.

The 3-deazaadenine derivatives (**6** and **13**) were synthesized from key bicyclo[3.1.0]hexane intermediate **36**^{22b} as depicted in Scheme 3. A Mitsunobu condensation of compound **35** with 3-deaza-2-bromo-6-chloropurine gave compound **47**, which underwent a C6 substitution with MeNH₂ under microwave conditions at 125 °C to give compound **48**. Aminolysis of compound **48** with aqueous MeNH₂ solution gave the methylamide **49**, which was subjected to a Sonogashira coupling with 5-chloro-2-ethynylthiophene under microwave conditions at 130 °C to provide compound **51**, and subsequent acid hydrolysis afforded the 3-deaza derivative **6**. Acid hydrolysis of compound **48** gave the nucleoside derivative **50**, which also underwent a Sonogashira coupling with 5-chloro-2-ethynylthiophene under microwave condition to afford compound **13**.

Biological testing

In vitro assays

DAT binding screen: The nucleoside derivatives were screened initially by the Psychoactive Drug Screening Program (PDSP) for binding interactions at diverse receptors, ion channels and transporters (Table 1, Supporting Information).²³ A fixed concentration of 10 μM was used in the primary screen for inhibition of radioligand binding at the hDAT. Because (N)-methanocarba nucleosides may enhance binding of a high affinity tropane derivative, [³H]methyl (1*R*,2*S*,3*S*)-3-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate (WIN35,428, **24**), at hDAT,³ a significant negative value for % inhibition of radioligand binding, i.e. greater binding than control, was also interpreted as a hit. The radioligands used for NET and SERT binding were [³H](*R,S*)-3-(2-methoxyphenoxy)-*N*-methyl-3-phenylpropan-1-amine (nisoxetine, **52**) and [³H](*R,S*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (citalopram, **53**), respectively. In the secondary screen by the PDSP, compounds that inhibited binding by >50% or significantly enhanced binding were characterized in full concentration response curves.

A secondary screen of selected binding enhancers allowed a more precise comparison of their interaction with hDAT and in few cases, other transporters. Figures 1 and 2 show the full concentration response curves for % enhancement of [³H]**24** binding by selected nucleosides (**6**, **8**, **10**, **11**, **14**, **15**, **22**, **27-29**) in comparison to known inhibitor **30**. EC₅₀ values for **7**, **8**, **15**, **22** and **28** were >10⁻⁶ M; 1.2 μM for **16**; 400 nM for **12**. Significant (>200%) enhancement of binding (% at 10 μM) was seen with compounds **6** (334%), **9** (352%), **10** (539%), **11** (370%), **12** (430%), **13** (354%), **14** (426%), **16** (285%), **27** (321%), and **29** (220%). *i*-Propyl (**15**) ester slightly inhibited binding of [³H]**24** at 10 μM, while 5'-propyl ester **14** enhanced binding; thus, branching of the α-carbon in 5'-esters was not tolerated. Among extended 5'-alkyl and 5'-alkylaryl esters (**17** - **21**), only the *i*-pentyl analogue **17** significantly interacted with hDAT (196% enhancement at 10 μM). 5'-Carboxylate (**22**) and *N*⁶-propyl riboside (**28**) interacted only weakly at hDAT at 10 μM, indicated by enhancement of [³H]**24** binding of <100%. 5'-*N*-(2-aminoethyl)-carboxamide **8**

(133% enhancement of [³H]**24** binding) was not comparable in efficacy to the corresponding 5'-methylamide **1** at hDAT. 5-Bromothien-2-yl 5'-ethyl ester derivative **12** appeared to be comparable to reference 5-chlorothien-2-yl derivative **1** in enhancing hDAT binding. Thus, 5-bromo is a suitable substitution for 5-chloro with respect to hDAT interaction (also evident in **2** compared to **1**).³ Curiously, benzyl ester **19** inhibited binding at SERT with a K_i value of 5.6 μM, and **28** inhibited NET binding with a K_i of 3.1 μM, and none of the other newly synthesized nucleosides inhibited binding at these two transporters with K_i < 10 μM.

Various 1- or 3-deaza derivatives (**5**, **6**, and **13**) were compared to probe the participation of these H-bond acceptor nitrogens of adenine for recognition at hDAT. The 1-deaza modification (**5a** and **5b**) prevented enhancement of [³H]**24** binding at hDAT, but not A₃AR activation.^{3,21} The corresponding 3-deaza 5'-methylamide **6** significantly enhanced [³H]**24** binding at hDAT, but with less efficacy than **1**. Thus, the N1, but not N3, nitrogen atom was essential for interaction with hDAT.

Ribosides **27** and **29** displayed % enhancement of [³H]**24** binding at 10 μM of 321% and 220%, respectively, which were less than the corresponding (N) methanocarpa analogues (**1** and **9**, with enhancement of 556% and 391%, respectively). Compound **28** inhibited NET binding with a K_i value of 3.1 μM (Supporting Information).

Detailed characterization at DAT, NET and SERT: The PDSP screen led to a selection of strongly enhancing compounds that were further characterized in greater detail at hDAT and the other transporters (e.g. binding of [¹²⁵I]**23** at DAT, NET and SERT and functional activity at each). Selected compounds were also characterized for their effect on binding of [³H](±)-5-(4-chlorophenyl)-3,5-dihydro-2*H*-imidazo[2,1-*a*]isoindol-5-ol (mazindol, **26**). The latter detailed pharmacological parameters for the novel compounds are reported in Table 2 in comparison to compounds **1**, **2**, **3** and **7**, which were characterized in our previous study.³

5'-Methyl (**9**) and ethyl (**10**) esters containing a 5-chlorothienyl moiety enhanced [¹²⁵I]**23** binding to hDAT equipotently to a maximal level ~450% of control binding (absence of drug), with EC₅₀ values of ~35 nM (Figure 3). Interestingly, **9** and **10** also enhanced [¹²⁵I]**23** binding to NET to a maximal level ~370% of control, with EC₅₀ values of 310 and 490 nM, respectively (Table 2). The effects of **9** and **10** on SERT binding of [¹²⁵I]**23** were small (inhibition at >10 μM). Contrary to their enhancing effects on radioligand binding to hDAT, compounds **9** and **10** were potent DA uptake inhibitors, with IC₅₀ values of 127 and 107 nM, respectively, compared with 274 nM for cocaine and 13.0 nM for **26**. Thus, the potencies of esters **9** and **10** in inhibiting DA uptake were ~2-fold greater than the corresponding methylamide **1**. Compounds **9** and **10** were less potent at inhibiting norepinephrine uptake by the NET with IC₅₀ values of 3.38 and 3.98 μM, respectively, compared with 221 nM for cocaine and 0.92 nM for **26**. The inhibitory effect of both compounds on 5HT uptake was estimated as >10 μM.

Compounds with intermediate potency in enhancing binding of [¹²⁵I]**23** at hDAT (in order of increasing EC₅₀, nM) were: 5-bromothienyl 5'-ethyl ester **11** (53.5), ribose 5'-methyl ester **29** (127), 3-deaza 5'-methylamide **6** (239) and 5'-propyl ester **14** (294). At NET, compounds **6**, **11** and **14** were roughly proportionately weaker at NET (EC₅₀ ~400 nM - 1

μM) than at hDAT, except the ribose derivative **29** was disproportionately weak with $\text{EC}_{50} > 1 \mu\text{M}$. However, the newly synthesized 5'-ester compounds tended to be more potent in enhancement of [^{125}I]**23** binding to NET than the previous set of 5'-methylamides **1 - 3**.

Five nucleoside derivatives were tested in binding and functional assays at the mouse (m) DAT expressed in HEK-293 cells (Table 3).²⁴ Cocaine was comparable in inhibiting binding at mDAT and at hDAT, but **26** was 12-fold more potent at hDAT than mDAT. The radioligand [^{125}I]**23** was less potent in binding to mDAT than hDAT with a 3-fold higher K_d value (3.9 vs 1.3 nM). Qualitatively, the nucleosides acted similarly at mDAT and hDAT in that they enhanced [^{125}I]**23** binding and inhibited [^3H]DA uptake. At the fixed concentration of radioligand, the percent of maximal binding enhancement by all of the compounds in the mDAT experiments was greater than at hDAT. However, all of the adenosine derivatives were less potent at mDAT than at hDAT in both enhancing binding and inhibiting uptake. The rank order of EC_{50} values (decreasing affinity in binding) was **9** > **2**, **10** > **1** > **6** at mDAT compared to **2** > **1**, **9**, **10** > **6** at hDAT. The factors for inhibition by the nucleosides of DA uptake, between hDAT and mDAT, were 7- to 26-fold. The two 5'-amide derivatives **1** and **2** displayed affinity and potency of 1 - 3 μM at mDAT. Thus, those compounds remain somewhat selective for the A_3AR compared to DAT in both human and mouse. However, two nucleoside 5'-esters **9** and **10** and one 5'-amide **6**, which were inactive in binding to the mA_3AR (<50% inhibition at 10 μM), still displayed considerable activity (1 - 4 μM) at mDAT. Thus, moderate DAT selectivity was indicated, at least in one species.

5'-Esters **9**, **10** and **29** were also compared for their effect on hDAT binding of a nontropane radioligand, [^3H]**26** (Figure 3). The enhancement of [^3H]**26** binding by riboside 5'-methyl ester **29** was weaker and less efficacious, with a 7-fold higher EC_{50} value and half the maximal effect, compared to the corresponding (N)-methanocarba analogue **9**. Furthermore, riboside **28** inhibited binding of nontropane radioligand [^3H]**52** to NET with a K_i value of 3.1 μM (data not shown).

Effects of the nucleosides on uptake of radiolabeled neurotransmitters were measured in the cell lines expressing hDAT and hNET. The order of potency in inhibiting DA uptake was: **3**, **10**, **9** > **1**, **2**, **11** > **14**. The order of potency in inhibiting norepinephrine uptake was: **9**, **10**, **11** > **6**, **2** > **1**, **3**, and there was no significant inhibition of 5HT uptake. Thus, 5' esters **9** and **10** were among the most potent to inhibit uptake of both catecholamines. **10** was more potent than cocaine at inhibiting DA uptake ($\text{IC}_{50} = 107 \text{ nM}$).

A₃AR binding in two species: Because the reference compounds **1 - 3**, **5** and **7** were highly selective agonists for the hA_3AR with nM affinity and were nearly inactive at the other AR subtypes, we measured the binding affinity at recombinant hA_3AR (Table 1). The nucleosides were also tested at the mouse (m) A_3AR , because of the known phenomenon of species-dependent variation of affinity at this receptor.^{2,18} A standard agonist radioligand ([^{125}I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide **25**) binding assay was used,² and IC_{50} values were converted to K_i values as reported.²⁵

Among 5'-ester derivatives, considerable affinity was observed at the hA_3AR , but the mA_3AR affinity was greatly reduced compared to amides. For example, 5'-methylamide **1**

was 8-fold more potent than corresponding 5'-methyl ester **9** at the hA₃AR, but >300-fold at the mA₃AR (extrapolated K_i of **9** > 10 μM). Similarly, larger esters that contained the full complement of the adenine nitrogens **10** - **12** and **14** - **16** were weak or inactive at the mA₃AR. The *n*-propyl ester **14** displayed a K_i value of 2.81 μM at the mA₃AR.

Replacement of the adenine 3-nitrogen atom with a CH group in **6** prevented recognition at the mA₃AR but not at the hA₃AR, which registered only a 13-fold reduction of affinity compared to **1**. In general, adenine ring-modified analogues displayed affinity at the hA₃AR in the order: adenine > 1-deaza > 3-deaza.

This expanded set of nucleoside analogues also provided an opportunity to compare (N)-methanocarba nucleosides directly with the corresponding ribose analogues at the A₃AR in two species. In general, 2- to 5-fold greater hA₃AR affinity was associated with the methanocarba analogues compared to the equivalent ribose derivatives (cf. **1** versus **27**; **7** versus **28**; **9** versus **29**). At the mA₃AR, the differences in affinity were even greater, for example, 88-fold greater affinity of bicyclic 5'-amide **7** compared to ribose 5'-amide **28**. Curiously, in the ribose series, the mA₃AR affinity was at least one order of magnitude higher for a 5'-ester **29** than for the conventional 5'-methylamide agonists of this receptor, i.e. **27** and **28**. The same comparison in the (N)-methanocarba series showed the opposite; in contrast to the potent 5'-methylamide **1**, 5'-ester **9** was nearly inactive at the mA₃AR, but it still bound with high affinity at the hA₃AR (K_i = 5.38 nM). Thus, the dependence of mA₃AR affinity on the (N)-methanocarba modification vs. ribose was dramatically reversed between amides and esters.

In a functional assay of hA₃AR-mediated inhibition of cAMP formation (n=3),¹⁸ methyl ester **9** was a low efficacy partial agonist and 3-deaza ester **13** had no significant activity with maximal efficacies (at 10 μM) of 11.6±1.0% and 3.6±3.0%, respectively, compared to reference agonist adenosine-5'-*N*-ethyluronamide set at 100%. However, the 3-deaza analogue **6** of full A₃AR agonist **4** was also a full agonist (116.5±14.6%).

In vivo testing: Since some of these nucleosides still displayed moderate affinity at the A₃AR, although diminished affinity compared to reference compound **1**, several compounds were evaluated in the mouse chronic constriction injury (CCI) model of neuropathic pain²⁷ (Figure S2, Supporting Information). This activity was shown in our previous studies to associate closely with the activity of nucleosides as A₃AR agonists at peripheral and/or central sites.^{1,2,18-20} Mice were treated with an adenosine derivative at 7 d post-injury, i.e. the time of peak mechanoallodynia. Riboside analogues **27** and **28** (3 μmol/kg, p.o.) were fully efficacious with a long duration of action (>6 h) in increasing the paw withdrawal threshold (PWT), i.e. reducing pain.

Other off-target binding: Other off-target interactions at receptors and channels were detected for the derivatives in the PDSP screening (Figure S1, Supporting information). Compounds **11** (K_i = 258 nM), **13** (K_i = 395 nM), **15** (K_i = 430 nM) and **29** (K_i = 3.24±1.05 μM) bound to the h5HT_{2C} serotonin receptor. By analogy to other methanocarba nucleoside derivatives,^{20,28} we expect these compounds to act as 5HT_{2C} antagonists. At the α-adrenergic receptors (α-Rs) ribosides but not the corresponding methanocarba analogues

bound weakly to $h\alpha_{2A}$ -R (**27**), $h\alpha_{2B}$ -R (**29**) or both (**28**) with K_i values in the 5-10 μM range. K_i values at all other α -adrenergic receptors were $>10 \mu\text{M}$. Compounds **7** ($K_i = 1.44 \mu\text{M}$) bound to the $h\beta_3$ adrenergic receptor. Compounds **13** also bound to hM_5 muscarinic ($K_i = 3.8 \mu\text{M}$) and rat σ_1 ($K_i = 1.04 \mu\text{M}$) and rat σ_2 ($K_i = 1.90 \mu\text{M}$) receptors; **20** bound to σ_2 ($K_i = 5.9 \mu\text{M}$) receptors.

Discussion

Adenosine derivatives have been designed as agonists and in some cases partial agonists or antagonists of the ARs. $A_3\text{AR}$ agonists are being developed for treatment of cancer, inflammation and pain,^{1,29-31} but they also display off-target activities at higher concentrations.^{3,9} We have explored and enhanced the off-target activity of nucleosides, especially those having rigid methanocarba rings, and found them to serve as a privileged scaffold³² for a variety of targets. Targets other than neurotransmitter symporters that appear to be binding sites of moderate affinity in this chemical series are GPCRs for biogenic amines, TSPO and sigma receptors.²⁰

The SAR in this study extends our previous findings that (N)-methanocarba adenosine derivatives enhance DAT radioligand affinity and inhibit DA uptake. Most of the compounds synthesized here contain C2-(5-halothien-2-yl)-ethynyl and N^6 -methyl substitution, which were determined to promote DAT interaction in the previous study.³

The three transporters for neurotransmitters examined in this study possess closely related structures, each having 12 transmembrane helices.^{6,7,33} The cocaine-competitive tropane ligands that display high affinity at DAT also tend to interact with NET and SERT.³⁴ Our previous study of the off-target activity of (N)-methanocarba nucleoside derivatives at DAT was consistent with allosteric modulation of radioligand binding.³ We found similar results in this study, in which potent DAT modulation remained while the AR potency of key analogues was reduced in comparison to **1**, by structural changes. Percent enhancement of binding at DAT was similar using two different tropane radioligands. These nucleosides had differing interactions with the transporters: at DAT they enhanced both [¹²⁵I]**23** and [³H]**26** binding, at NET only [¹²⁵I]**23** binding was enhanced, while they had minimal effect on radioligand binding to SERT. Thus, as is characteristic of allosteric modulators, there was a probe-dependence of the interaction of these nucleosides at DAT and NET. Allosteric modulation of SERT is well validated structurally,³³ and that of NET has been described; for example, a peptide inhibitor was suggested to bind to the extracellular vestibule of the transporter.^{35,36}

Nevertheless, many of the compounds still interact with the $A_3\text{AR}$, as evident from their binding affinity and protective effects, as agonists, in a pain model. Reference compounds **1** - **3**, **5** and **7** were shown previously to be potent $A_3\text{AR}$ agonists and to reduce chronic pain in the CCI model, an effect of $A_3\text{AR}$ agonists that was shown to depend entirely on the $A_3\text{AR}$ as demonstrated using $A_3^{-/-}$ mice.^{1,21} However, in this study we have characterized the simultaneous activity at transporters of various $A_3\text{AR}$ ligands of nM affinity. The potency of **1**, for example, as an allosteric enhancer of hDAT binding is 50-fold weaker than its potency at the $hA_3\text{AR}$. One objective of this study was to preserve or enhance DAT potency while

decreasing A₃AR affinity, so we modified the nucleosides at positions that might be predicted to lower the A₃AR affinity. This was accomplished for the 5'-ester series, for which the DAT potency was maintained, and the A₃AR affinity was reduced (human) or absent (mouse). 5'-Esters reduced A₃AR affinity in general, compared to 5'-monoalkyl amides.³⁷ Consistently, compound **9** was 8-fold less potent than **1** at the hA₃AR, and the difference was even greater at the mA₃AR. Also, the ribose 5' binding region of ARs is limited sterically, based on X-ray structures.³⁸ Thus, the extended, charged 5'-(2-aminoethyl)-amide **8** displayed only moderate affinity at the hA₃AR and was completely inactive at the mA₃AR. However, it interacted only weakly with DAT.

Furthermore, dimethylation of the exocyclic amine in **4**, as noted previously,³ was more detrimental to DAT interaction than to A₃AR affinity. The deaza analogues displayed differential effects at the two primary targets compared in this study. The 1-deaza (**5**) modification is a means of introducing A₃AR selectivity compared to DAT with no loss of A₃AR affinity.²¹ The 3-deaza (**6**) modification of 5'-methyamide **1** decreased A₃AR affinity (13-fold lower than **1** at human and nearly inactive at mouse), while at DAT significant binding enhancement was present. The 3-deaza modification in the 5'-amide series (**6**) improved NET potency, but reduced DAT potency 7-fold (cf. **1**). The EC₅₀ of **6** for [¹²⁵I]**23** binding at DAT was only 6.8-fold higher than **1** and 4.2-fold higher for [³H]**26** binding. Thus, the 1-deaza (**5**), but not the 3-deaza (**6** and **13**) modifications are a means of eliminating DAT activity in this series. We speculate that N1 might serve as an energetically important H-bond acceptor in DAT.

5'-Methyl, ethyl and *n*-propyl esters, but not larger esters, maintained efficacious DAT interaction. The substitution of a 5-bromo in the C2-thienylethynyl group (**11**) maintained NET and DAT potency in the 5'-ester series, but did not improve DAT potency as it did in the amides (cf. **2**). The enhancing activity at mDAT of selected compounds was examined for a comparison with the A₃AR within the same species.³⁹ 3-Deaza 5'-amide **6**, 5'-methyl ester **9** and 5'-ethyl ester **10** were selective for the enhancement of binding at the mDAT with respect to the mA₃AR. These compounds were nearly inactive at mA₃AR, indicating that the 5'-ester group is not tolerated in general (except for bromo derivative **12** and propyl ester **14**). The potency of N⁶-propyl derivative **7** was slightly enhanced compared to **1** at NET, but greatly reduced at DAT. Therefore, at DAT there appear to be sterically limited binding subpockets surrounding the 5'-alkylamide, 5'-alkyl ester and N⁶-alkyl substituents. Future studies of DAT modeling will distinguish the specific amino acids and molecular interactions involved in radioligand binding as compared to transporter function.

At the A₃AR, we established importantly that the (N)-methanocarba modification enhances affinity in direct comparison to the riboside. However, there was no advantage to the DAT interaction upon restoring the ribose moiety, because the potency of ribosides was also diminished, by ~5-fold, at the DAT and NET. Our present results establish that various 5'-ester derivatives with extended C2 substituents can display potent affinity at the hA₃AR, which was not anticipated from our earlier report on 2-Cl derivatives.⁴⁰ Although the ester derivatives **9-16** bound at the hA₃AR with K_i values of 5 - 43 nM (**13**: 169 nM), two of them (**9** and **13**) were shown to have little or no ability to activate the receptor, suggesting that they may be A₃AR antagonists. Similarly, several weakly binding adenosine 5'-ester

derivatives (methanocarba series) were shown to be either partial agonist or antagonist at the hA₃AR²⁸; thus, 5'-amides but not 5'-esters tend to fully activate the hA₃AR.

Additional structural modification would likely be required before this nucleoside series could be considered for therapeutic use. We reported that mixed DAT enhancer/A₃AR agonist **1** at 10 mg/kg did not significantly stimulate locomotor activity in the mouse, and at 30 mg/kg reduced locomotor activity.⁴¹ The observed locomotor depression can be attributed to a peripheral action of **1** at a peripheral A₃AR. Our earlier study showed that decreased physical activity with high doses A₃AR agonists in the mouse correlated with histamine release by peripheral mast cells upon A₃AR activation.⁴¹ Also, we predict that nucleoside **1** (MW 459, total polar surface area, tPSA = 121.9 Å²) and related congeners will display a low brain concentration that is typical of peripherally-administered nucleosides.⁴² Thus, in order to study the CNS effects²⁶ of these modulators, especially **9** and **10** (both having tPSA = 119.1 Å²) that are DAT-selective in the mouse, it would be more informative to infuse them intracerebroventricularly in rodents.

Peripherally-selective inhibitors of DA and norepinephrine uptake might be useful as an emergency treatment of lung edema, to act as an inotropic agent in the heart and to increase kidney/splanchnic circulation, similar to currently used injection of biogenic amine receptor agonists, notably DA at medium doses.⁴³⁻⁴⁵ Alternatively, an allosteric modulator of DAT that did act centrally might have less evident behavioral stimulant effects than orthosteric inhibitors.

We have not yet studied the pharmacokinetics of 5'-esters that were shown here to be allosteric modulators of DAT. However, the in vivo stability of the corresponding 5'-ester group was shown for another member of this series of rigid (N)-methanocarba nucleosides in the context of 5HT_{2B}R antagonists.²⁸ Also, amide derivatives in the present set of compounds, such as orally administered **1**, were shown to be bioavailable and have long lasting in vivo effects in mice and are not rapidly metabolized.^{2,19} These rigid nucleosides, such as **1**, typically do not inhibit cytochrome P450 (5 isozymes) and are stable in the presence of liver microsomes, plasma and simulated body fluids.² A metabolomic study of **1** indicated that the compound was well tolerated in vivo.⁴⁶

In conclusion, following structural modification of our previous series of nucleoside modulators of DAT, we maintained or enhanced the activity of rigid nucleosides at DAT and NET while reducing A₃AR affinity. Two nucleoside 5'-esters **9** and **10** and one 5'-amide **6** that were inactive at the mA₃AR were shown to allosterically modulate mDAT in the μM range. These compounds, such as **9** and **10**, will be useful as research tools for studying the structure and function of DAT and NET.

Experimental Procedures

Chemical synthesis

Materials and instrumentation—All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). ¹H NMR spectra were obtained with a Bruker 400 spectrometer using CDCl₃, CD₃OD and DMSO as solvents. Chemical shifts are expressed in δ values

(ppm) with tetramethylsilane (δ 0.00) for CDCl_3 and water (δ 3.30) for CD_3OD . NMR spectra were collected with a Bruker AV spectrometer equipped with a z-gradient [^1H , ^{13}C , ^{15}N]-cryoprobe. TLC analysis was carried out on glass sheets precoated with silica gel F254 (0.2 mm) from Sigma-Aldrich. The purity of final nucleoside derivatives was checked using a Hewlett–Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μm analytical column (50 \times 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). Mobile phase: linear gradient solvent system, 5 mM tetrabutylammonium dihydrogenphosphate– CH_3CN from 80:20 to 0:100 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector at 230, 254, and 280 nm. All derivatives tested for biological activity showed >95% purity by HPLC analysis (detection at 254 nm). The nucleoside products tested in biological assays do not contain any functional groups associated with assay interference compounds,⁴⁷ and they display self-consistent potent structure activity relationships, with very few off-target hits in broad screening. Low-resolution mass spectrometry was performed with a JEOL SX102 spectrometer with 6-kV Xe atoms following desorption from a glycerol matrix or on an Agilent LC/MS 1100 MSD, with a Waters (Milford, MA) Atlantis C18 column. High resolution mass spectroscopic (HRMS) measurements were performed on a proteomics optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine, unless noted. Observed mass accuracies are those expected based on known performance of the instrument as well as trends in masses of standard compounds observed at intervals during the series of measurements. Reported masses are observed masses uncorrected for this time-dependent drift in mass accuracy. 6-Chloro-2-iodopurine-9-ribose **35a** was purchased from Matrix Scientific, Inc. (Columbia, SC). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO), Small Molecules, Inc. (Hoboken, NJ), Anichem (North Brunswick, NJ), PharmaBlock, Inc. (Sunnyvale, CA), Frontier Scientific (Logan, UT) and Tractus (Perrineville, NJ). Calculation of total polar surface area was performed using ChemDraw Professional (v. 15.1, PerkinElmer, Boston, MA).

(1S,2R,3S,4R,5S)-4-(6-((5-Chlorothiophen-2-yl)ethynyl)-4-(methylamino)-1H-imidazo[4,5-c]pyridin-1-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (6)—Compound **6** (90%) was prepared from compound **51** following the same method as for compound **50**. ^1H NMR (CD_3OD , 400 MHz) δ 8.11 (s, 1H), 7.20 (d, J = 4.0 Hz, 1H), 7.17 (s, 1H), 6.98 (d, J = 4.0 Hz, 1H), 4.81 (d, J = 6.4 Hz, 1H), 4.74 (s, 1H), 3.93 (d, J = 6.4 Hz, 1H), 3.10 (s, 3H), 2.87 (s, 3H), 2.25–2.21 (m, 1H), 1.94 (t, J = 5.2 Hz, 1H), 1.46–1.44 (m, 1H). HRMS calculated for $\text{C}_{21}\text{H}_{21}\text{N}_5\text{O}_3\text{SCl}$ (M + H)⁺: 458.1054; found 458.1058.

(1S,2R,3S,4R,5S)-N-(2-Aminoethyl)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxamide (8)—Ethylenediamine (1.5 mL) was added to a solution of compound **10** (13 mg, 0.027 mmol) in MeOH (0.1 mL) and stirred for 2d at room temperature. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH: NH_4OH = 5:1:0.1) to give the compound **8** (9 mg, 71%) as a colorless syrup. ^1H NMR (CD_3OD , 400 MHz) δ 8.12 (s, 1H), 7.33 (d, J = 4.0 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 5.08 (d, J = 6.8 Hz, 1H), 4.83 (s, 1H), 4.05 (d, J = 6.8 Hz, 1H), 3.60–3.49 (m,

2H), 3.19-3.09 (m, 3H), 2.19-2.16 (m, 1H), 1.94 (t, $J = 4.8$ Hz, 1H), 1.49-1.46 (m, 1H). HRMS calculated for $C_{21}H_{23}N_7O_3SCl$ ($M + H$)⁺: 488.1272; found 488.1273.

Methyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (9)—DCC (32 mg, 0.15 mmol), DMAP (2.5 mg, 0.02 mmol) and MeOH (10 μ L, 0.15 mmol) was added to a solution of compound **22** (23 mg, 0.05 mmol) in DMF (1 mL) and stirred at room temperature overnight. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH = 30:1) to afford the compound **9** (12 mg, 51%) as a colorless syrup. ¹H NMR (CD_3OD , 400 MHz) δ 8.03 (s, 1H), 7.33 (d, $J = 4.0$ Hz, 1H), 7.02 (d, $J = 4.0$ Hz, 1H), 5.22 (d, $J = 6.8$ Hz, 1H), 4.84 (s, 1H), 4.09 (d, $J = 6.8$ Hz, 1H), 3.79 (s, 3H), 3.13 (br s, 3H), 2.23-2.20 (m, 1H), 1.92 (t, $J = 5.2$ Hz, 1H), 1.66-1.62 (m, 1H). HRMS calculated for $C_{20}H_{19}N_5O_4SCl$ ($M + H$)⁺: 460.0846; found 460.0850.

Ethyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (10)—A solution of compound **33** (103 mg, 0.2 mmol) in methanol (3 mL) and 10% trifluoromethane sulfonic acid (3 mL) was heated at 70 °C for 4 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH = 30:1) to give the compound **10** (86 mg, 91%) as a colorless syrup. ¹H NMR (CD_3OD , 400 MHz) δ 8.08 (s, 1H), 7.35 (d, $J = 4.0$ Hz, 1H), 7.04 (d, $J = 4.0$ Hz, 1H), 5.21 (d, $J = 6.8$ Hz, 1H), 4.84 (s, 1H), 4.27-4.22 (m, 2H), 4.12 (d, $J = 6.4$ Hz, 1H), 3.14 (br s, 3H), 2.23-2.20 (m, 1H), 1.91 (t, $J = 5.2$ Hz, 1H), 1.67-1.63 (m, 1H), 1.29 (t, $J = 7.2$ Hz, 3H). HRMS calculated for $C_{21}H_{21}N_5O_4SCl$ ($M + H$)⁺: 474.1003; found 474.1007.

Methyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-bromothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (11)—MeONa (7.6 mg, 0.14 mmol) was added to a solution of compound **12** (25 mg, 0.048 mmol) in MeOH (3 mL) and stirred at room temperature overnight. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (hexane:ethylacetate = 1:1) to give the methyl ester derivative **11** (16 mg, 69%) as a colorless syrup. ¹H NMR (CD_3OD , 400 MHz) δ 8.02 (s, 1H), 7.28 (d, $J = 4.0$ Hz, 1H), 7.14 (d, $J = 4.0$ Hz, 1H), 5.22 (d, $J = 7.2$ Hz, 1H), 4.83 (s, 1H), 4.09 (d, $J = 7.2$ Hz, 1H), 3.78 (s, 3H), 3.13 (br s, 3H), 2.23-2.20 (m, 1H), 1.92 (t, $J = 5.2$ Hz, 1H), 1.65-1.61 (m, 1H). HRMS calculated for $C_{20}H_{19}N_5O_4SBr$ ($M + H$)⁺: 504.0341; found 504.0345.

Ethyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-bromothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (12)—Compound **12** (90%) was prepared from compound **34** following the same method as for compound **10**. ¹H NMR (CD_3OD , 400 MHz) δ 8.02 (s, 1H), 7.28 (d, $J = 4.0$ Hz, 1H), 7.14 (d, $J = 4.0$ Hz, 1H), 5.20 (d, $J = 6.6$ Hz, 1H), 4.84 (s, 1H), 4.27-4.22 (m, 2H), 4.13 (d, $J = 6.6$ Hz, 1H), 3.13 (br s, 3H), 2.23-2.20 (m, 1H), 1.92 (t, $J = 5.2$ Hz, 1H), 1.66-1.63 (m, 1H), 1.29 (t, $J = 7.2$ Hz, 3H). HRMS calculated for $C_{21}H_{21}N_5O_4SBr$ ($M + H$)⁺: 518.0498; found 518.0494.

Ethyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(6-((5-chlorothiophen-2-yl)ethynyl)-4-(methylamino)-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (13)—Compound **13** (71%) was

prepared from compound **50** following the same method as for compound **51**. ¹H NMR (CD₃OD, 400 MHz) δ 8.03 (s, 1H), 7.19 (d, *J*=4.0 Hz, 1H), 7.11 (s, 1H), 6.98 (d, *J*=4.0 Hz, 1H), 5.02 (d, *J*=7.6 Hz, 1H), 4.70 (s, 1H), 4.30-4.25 (m, 2H), 4.02 (d, *J*=6.4 Hz, 1H), 3.10 (s, 3H), 2.30-2.26 (m, 1H), 1.95 (t, *J*= 5.2 Hz, 1H), 1.74-1.71 (m, 1H), 1.33 (t, *J*= 7.2 Hz, 3H). HRMS calculated for C₂₂H₂₂N₄O₄SCl (M + H)⁺: 473.1050; found 473.1056.

***n*-Propyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (14)**—Compound **14** (53%) was prepared from compound **22** following the same method as

for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 8.01 (s, 1H), 7.33 (d, *J*= 4.0 Hz, 1H), 7.02 (d, *J*= 4.0 Hz, 1H), 5.21 (d, *J*= 6.8 Hz, 1H), 4.83 (s, 1H), 4.18-4.11 (m, 3H), 3.13 (br s, 3H), 2.24-2.20 (m, 1H), 1.92 (t, *J*= 5.2 Hz, 1H), 1.72-1.63 (m, 3H), 0.94 (t, *J*= 7.2 Hz, 1H). HRMS calculated for C₂₂H₂₃N₅O₄SCl (M + H)⁺: 488.1159; found 488.1162.

Isopropyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (15)—Compound **15** (52%) was prepared from compound **22** following the same method as

for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 7.99 (s, 1H), 7.33 (d, *J*= 4.0 Hz, 1H), 7.02 (d, *J*= 4.0 Hz, 1H), 5.20 (d, *J*= 6.4 Hz, 1H), 5.14-5.08 (m, 1H), 4.82 (s, 1H), 4.13 (d, *J*= 5.2 Hz, 1H), 3.13 (br s, 3H), 2.21-2.18 (m, 1H), 1.90 (t, *J*= 4.8 Hz, 1H), 1.66-1.62 (m, 1H), 1.27 (d, *J*= 8.0 Hz, 6H). HRMS calculated for C₂₂H₂₃N₅O₄SCl (M + H)⁺: 488.1159; found 488.1156.

Butyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (16)—Compound **16** (53%) was prepared from compound **22** following the same method as

for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 8.01 (s, 1H), 7.33 (d, *J*= 4.0 Hz, 1H), 7.02 (d, *J*= 4.0 Hz, 1H), 5.22 (d, *J*= 6.4 Hz, 1H), 4.84 (s, 1H), 4.24-4.17 (m, 2H), 4.12 (d, *J*= 6.4 Hz, 1H), 3.13 (br s, 3H), 2.23-2.19 (m, 1H), 1.91 (t, *J*= 5.2 Hz, 1H), 1.69-1.62 (m, 3H), 1.43-1.34 (m, 2H), 0.92 (t, *J*= 7.2 Hz, 3H). HRMS calculated for C₂₃H₂₅N₅O₄SCl (M + H)⁺: 502.1316; found 502.1308.

Isopentyl (1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (17)—Compound **17** (51%) was prepared from compound **22** following the same method as

for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 8.01 (s, 1H), 7.34 (d, *J*= 4.0 Hz, 1H), 7.02 (d, *J*= 4.0 Hz, 1H), 5.22 (d, *J*= 6.8 Hz, 1H), 4.83 (s, 1H), 4.29-4.21 (m, 2H), 4.12 (d, *J*= 6.4 Hz, 1H), 3.14 (br s, 3H), 2.22-2.18 (m, 1H), 1.91 (t, *J*= 5.2 Hz, 1H), 1.68-1.62 (m, 2H), 1.59-1.54 (m, 2H), 0.90 (d, *J*= 6.0 Hz, 6H). HRMS calculated for C₂₄H₂₇N₅O₄SCl (M + H)⁺: 516.1472; found 516.1467.

2-Cyclohexylethyl (1S,2R,3S,4R,5S)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (18)—Compound **18** (54%) was prepared from compound **22** following the same method as for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 8.02 (s, 1H), 7.34 (d, *J* = 4.0 Hz, 1H), 7.03 (d, *J* = 4.0 Hz, 1H), 5.24 (d, *J* = 7.6 Hz, 1H), 4.83 (s, 1H), 4.30-4.17 (m, 2H), 4.13 (d, *J* = 6.4 Hz, 1H), 3.13 (br s, 3H), 2.21-2.18 (m, 1H), 1.90 (t, *J* = 5.2 Hz, 1H), 1.70-1.61 (m, 6H), 1.58-1.53 (m, 2H), 1.32-1.26 (m, 2H), 1.19-1.15 (m, 2H), 0.94-0.85 (m, 2H). HRMS calculated for C₂₇H₃₁N₅O₄SCl (M + H)⁺: 556.1785; found 556.1786.

Benzyl (1S,2R,3S,4R,5S)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (19)—Compound **19** (59%) was prepared from compound **22** following the same method as for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 7.96 (s, 1H), 7.38-7.15 (m, 6H), 6.98 (d, *J* = 4.0 Hz, 1H), 5.24 (d, *J* = 4.8 Hz, 1H), 4.83 (s, 1H), 4.11 (d, *J* = 6.4 Hz, 1H), 3.57 (t, *J* = 6.8 Hz, 1H), 3.13 (br s, 3H), 2.68 (t, *J* = 8.0 Hz, 1H), 2.26-2.23 (m, 1H), 1.95 (t, *J* = 5.2 Hz, 1H), 1.70-1.66 (m, 1H). HRMS calculated for C₂₆H₂₃N₅O₄SCl (M + H)⁺: 536.1159; found 536.1153.

2-Phenylethyl (1S,2R,3S,4R,5S)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (20)—Compound **20** (58%) was prepared from compound **22** following the same method as for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 7.93 (s, 1H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.18-7.00 (m, 5H), 6.99 (d, *J* = 4.0 Hz, 1H), 5.19 (d, *J* = 6.8 Hz, 1H), 4.78 (s, 1H), 4.46-4.42 (m, 1H), 4.35-4.29 (m, 1H), 4.10 (d, *J* = 6.8 Hz, 1H), 3.14 (br s, 3H), 2.95 (t, *J* = 6.4 Hz, 2H), 2.07-2.03 (m, 1H), 1.86 (d, *J* = 5.2 Hz, 1H), 1.60-1.56 (m, 1H). HRMS calculated for C₂₇H₂₅N₅O₄SCl (M + H)⁺: 550.1316; found 550.1308.

3-Phenylpropyl (1S,2R,3S,4R,5S)-4-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (21)—Compound **21** (59%) was prepared from compound **22** following the same method as for compound **9**. ¹H NMR (CD₃OD, 400 MHz) δ 8.03 (s, 1H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.22-7.11 (m, 5H), 7.01 (d, *J* = 4.0 Hz, 1H), 5.25 (d, *J* = 6.4 Hz, 1H), 4.83 (s, 1H), 4.27-4.11 (m, 3H), 3.13 (br s, 3H), 2.67 (t, *J* = 7.6 Hz, 2H), 2.18-2.14 (m, 1H), 2.02-1.95 (m, 2H), 1.90 (t, *J* = 5.2 Hz, 1H), 1.65-1.61 (m, 1H). HRMS calculated for C₂₈H₂₇N₅O₄SCl (M + H)⁺: 564.1472; found 564.1463.

(1S,2R,3S,4R,5S)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylic acid (22)—2N NaOH solution (1.5 mL) was added to a solution of compound **10** (29.2 mg, 0.06 mmol) in MeOH (1.5 mL) and stirred for 1h at room temperature. Reaction mixture was neutralized with acetic acid and evaporated under vacuum. The residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH:TFA= 10:1:0.1) to give the compound **22** (22 mg, 82%) as colorless syrup. ¹H NMR (DMSO, 400 MHz) δ 8.12 (s, 1H), 7.98 (s, 1H), 7.48 (d, *J* = 4.0 Hz, 1H), 7.22 (d, *J* = 4.0 Hz, 1H), 4.92 (d, *J* = 6.0 Hz, 1H), 4.65 (s, 1H), 4.06-4.04

(m, 1H), 2.95 (br s, 3H), 2.03-2.00 (m, 1H), 1.69 (t, $J=4.8$ Hz, 1H), 1.48-1.44 (m, 1H). HRMS calculated for $C_{19}H_{17}N_5O_4SCl$ ($M + H$)⁺: 446.0690; found 446.0694.

(2S,3S,4R,5R)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydrofuran-2-carboxamide (27)—

Compound **27** (91%) was prepared from compound **44** following the same method as for compound **10**. ¹H NMR (DMSO, 400 MHz) δ 8.56 (d, $J=4.4$ Hz, 1H), 8.50 (s, 1H), 8.14 (d, $J=4.0$ Hz, 1H), 7.49 (d, $J=4.0$ Hz, 1H), 7.23 (d, $J=4.0$ Hz, 1H), 5.96 (d, $J=7.6$ Hz, 1H), 5.76 (d, $J=4.0$ Hz, 1H), 5.57 (d, $J=6.4$ Hz, 1H), 4.60-4.56 (m, 1H), 4.33 (s, 1H), 4.17-4.14 (m, 1H), 2.97 (br s, 3H), 2.78 (s, 3H). HRMS calculated for $C_{18}H_{18}N_6O_4SCl$ ($M + H$)⁺: 449.0799; found 449.0806.

(2S,3S,4R,5R)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(*n*-propylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydrofuran-2-carboxamide (28)—

Compound **28** (90%) was prepared from compound **45** following the same method as for compound **10**. ¹H NMR (CD₃OD, 400 MHz) δ 8.27 (s, 1H), 7.31 (d, $J=4.0$ Hz, 1H), 7.03 (d, $J=4.0$ Hz, 1H), 5.98 (d, $J=7.6$ Hz, 1H), 4.78-4.75 (m, 1H), 4.50 (s, 1H), 4.32 (d, $J=6.0$ Hz, 1H), 3.59 (br s, 3H), 1.77-1.71 (m, 2H), 1.05 (t, $J=7.6$ Hz, 3H). HRMS calculated for $C_{20}H_{22}N_6O_4SCl$ ($M + H$)⁺: 477.1112; found 477.1115.

Methyl (2S,3S,4R,5R)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-carboxylate (29)

—PdCl₂(PPh₃)₂ (1.77 mg, 0.002 mmol), CuI (1.0 mg, 0.001 mmol), 2-chloro-5-ethynylthiophene (10.8 mg, 0.075 mmol) and triethylamine (17 μ L, 0.12 mmol) was added to a solution of compound **43** (6 mg, 0.012 mmol) in anhydrous DMF (0.6 mL), and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was roughly purified on flash silica gel column chromatography (hexane:ethylacetate = 1:1) to give the compound **46**, which was subsequently stirred with 10% TFA (1 mL) and MeOH (1 mL) at 70 °C for 2 h. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH = 50:1) to afford the compound **29** (3.8 mg, 68%) as a colorless syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.58 (s, 1H), 7.31 (d, $J=4.0$ Hz, 1H), 7.02 (d, $J=4.0$ Hz, 1H), 6.20 (d, $J=4.8$ Hz, 1H), 4.64-4.60 (m, 2H), 4.48 (m, 1H), 3.84 (s, 3H), 3.14 (br s, 3H). HRMS calculated for $C_{18}H_{17}N_5O_5SCl$ ($M + H$)⁺: 450.0639; found 450.0645.

Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-((5-chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9H-purin-9-yl)-2,2-

dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (33)—PdCl₂(PPh₃)₂ (23.3 mg, 0.033 mmol), CuI (3.1 mg, 0.033 mmol), 2-chloro-5-ethynylthiophene (142 mg, 0.99 mmol) and triethylamine (0.23 mL, 1.66 mmol) was added to a solution of compound **32** (83 mg, 0.16 mmol) in anhydrous DMF (3 mL) and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethylacetate = 1:1) to give the compound **33** (71 mg, 83%) as a brownish glassy syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.11 (s, 1H), 7.37 (d, $J=4.0$ Hz, 1H), 7.03 (d, $J=4.0$ Hz, 1H), 5.89 (d, $J=6.8$ Hz,

1H), 5.02 (s, 1H), 4.82 (d, $J = 6.0$ Hz, 1H), 4.29-4.19 (m, 2H), 3.12 (br s, 3H), 2.31-2.27 (m, 1H), 1.71-1.67 (m, 1H), 1.57-1.54 (m, 4H), 1.29 (s, 3H), 1.23 (t, $J = 7.2$ Hz, 3H). HRMS calculated for $C_{24}H_{25}N_5O_4S$ (M + H)⁺: 514.1316; found 514.1318.

Ethyl (3a*R*,3b*S*,4a*S*,5*R*,5a*S*)-5-(2-((5-bromothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,2-

dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-*d*][1,3]dioxole-3b(3a*H*)-carboxylate (34)—Compound **34** (82%) was prepared from compound **32** following the same method as for compound **33**. ¹H NMR (CD₃OD, 400 MHz) δ 8.11 (s, 1H), 7.33 (d, $J = 4.0$ Hz, 1H), 7.15 (d, $J = 4.0$ Hz, 1H), 5.89 (d, $J = 7.2$ Hz, 1H), 5.02 (s, 1H), 4.83 (d, $J = 7.2$ Hz, 1H), 4.28-4.18 (m, 2H), 3.14 (br s, 3H), 2.31-2.27 (m, 1H), 1.71-1.67 (m, 1H), 1.57-1.54 (m, 4H), 1.29 (s, 3H), 1.23 (t, $J = 7.2$ Hz, 3H). HRMS calculated for $C_{24}H_{25}N_5O_4SBr$ (M + H)⁺: 558.0811; found 558.0814.

((3a*R*,4*R*,6*R*,6a*R*)-6-(6-Chloro-2-iodo-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methanol (35b)

—To a suspension of compound **35a** (1 g, 2.42 mmol) in acetone (50 mL), 2,2-dimethoxypropane (1.5 mL, 12.1 mmol) and *p*-TSA was added and stirred at room temperature for 5 h. After completion of starting material, the reaction mixture was neutralized by addition of solid NaHCO₃. Reaction mixture was filtered and the filtrate was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound **35b** (0.996 g, 91%) as a colorless syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.71 (s, 1H), 6.27 (d, $J = 2.8$ Hz, 1H), 5.33 (d, $J = 6.4$ Hz, 1H), 5.03 (d, $J = 6.4$ Hz, 1H), 4.41-4.38 (m, 1H), 3.38-3.71 (m, 2H), 1.62 (s, 3H), 1.41 (s, 3H). HRMS calculated for $C_{13}H_{15}N_4O_4ICl$ (M + H)⁺: 452.9827; found 452.9829.

((3a*R*,4*R*,6*R*,6a*R*)-6-(2-Iodo-6-(methylamino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-*d*][1,3]dioxol-4-yl)methanol (37)

—Methylamine hydrochloride (880 mg, 13 mmol) and triethylamine (3.6 mL, 26.1 mmol) was added to a solution of compound **35b** (1.18 g, 2.61 mmol) in methanol (25 mL) and stirred at room temperature overnight. The reaction mixture was evaporated under vacuum, and the residue was purified on flash column chromatography (hexane:ethyl acetate = 1:2) to give the desired product **37** (968 mg, 83%) as a colorless syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.13 (s, 1H), 6.09 (d, $J = 3.6$ Hz, 1H), 5.24 (d, $J = 6.0$ Hz, 1H), 5.02 (d, $J = 6.0$ Hz, 1H), 4.36-4.34 (m, 1H), 3.03 (br s, 3H), 1.62 (s, 3H), 1.39 (s, 3H). HRMS calculated for $C_{14}H_{19}N_5O_4I$ (M + H)⁺: 448.0482; found 448.0489.

((3a*R*,4*R*,6*R*,6a*R*)-6-(2-Iodo-6-(*n*-propylamino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-*d*][1,3]dioxol-4-yl)methanol (38)

—Compound **38** (85%) was prepared from compound **35b** following the same method as for compound **37**. ¹H NMR (CD₃OD, 400 MHz) δ 8.15 (s, 1H), 6.10 (d, $J = 3.2$ Hz, 1H), 5.24 (d, $J = 6.0$ Hz, 1H), 5.03 (d, $J = 6.0$ Hz, 1H), 4.36-4.34 (m, 1H), 3.79-3.71 (m, 2H), 3.52-3.49 (m, 2H), 1.72-1.62 (m, 2H), 1.62 (s, 3H), 1.40 (s, 3H), 1.01 (t, $J = 7.6$ Hz, 3H). HRMS calculated for $C_{16}H_{23}N_5O_4I$ (M + H)⁺: 476.0716; found 476.0734.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-Iodo-6-(methylamino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-*d*][1,3]dioxole-4-carboxylic acid (39)—PDC (530 mg, 1.4 mmol) was added to a solution of compound **37** (105 mg, 0.23 mmol) in dry DMF (2.5 mL) and heated at 40 °C overnight. After completion of starting material, water (10 mL) was added into the reaction mixture and extracted with ethyl acetate (3×10 mL). Combined organic layer was washed with brine (15 mL), dried, filtered, evaporated and the residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH = 35:1) to give the compound **39** (73 mg, 68%) as a colorless syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.08 (s, 1H), 6.29 (s, 1H), 5.66 (d, *J* = 6.0 Hz, 1H), 5.46 (d, *J* = 6.0 Hz, 1H), 4.75 (d, *J* = 1.6 Hz, 1H), 3.04 (br s, 3H), 1.58 (s, 3H), 1.43 (s, 3H). HRMS calculated for C₁₄H₁₇N₅O₅I (M + H)⁺: 462.0274; found 462.0265.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-Iodo-6-(*n*-propylamino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydrofuro [3,4-*d*][1,3]dioxole-4-carboxylic acid (40)—Compound **40** (67%) was prepared from compound **38** following the same method as for compound **39**. ¹H NMR (CD₃OD, 400 MHz) δ 8.10 (s, 1H), 6.29 (s, 1H), 5.65 (d, *J* = 6.0 Hz, 1H), 5.46 (d, *J* = 6.0 Hz, 1H), 4.76 (d, *J* = 1.6 Hz, 1H), 3.49 (br s, 2H), 1.71-1.65 (m 2H), 1.58 (s, 3H), 1.43 (s, 3H), 1.00 (d, *J* = 7.6 Hz, 3H). HRMS calculated for C₁₆H₂₁N₅O₅I (M + H)⁺: 490.0587; found 490.0583.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-Iodo-6-(methylamino)-9*H*-purin-9-yl)-*N*,2,2-trimethyltetrahydrofuro [3,4-*d*][1,3]dioxole-4-carboxamide (41)—MeNH₂ (48 μL, 2M solution in THF) and DIPEA (14 μL, 0.084 mmol) were added to a solution of compound **39** (30 mg, 0.065 mmol) and HATU (32 mg, 0.078 mmol) in dry DMF (1.3 mL). The reaction mixture was stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH = 35:1) to give the compound **41** (21 mg, 68%) as a colorless syrup. ¹H NMR (CD₃OD, 400 MHz) δ 8.05 (s, 1H), 6.27 (d, *J* = 1.2 Hz, 1H), 5.51 (d, *J* = 6.2 Hz, 1H), 5.35 (d, *J* = 6.2 Hz, 1H), 4.63 (d, *J* = 2.0 Hz, 1H), 3.05 (br s, 3H), 2.49 (s, 3H), 1.59 (s, 3H), 1.41 (s, 3H). HRMS calculated for C₁₅H₂₀N₆O₄I (M + H)⁺: 475.0591; found 475.0600.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-Iodo-6-(*n*-propylamino)-9*H*-purin-9-yl)-*N*,2,2-trimethyltetrahydrofuro [3,4-*d*][1,3]dioxole-4-carboxamide (42)—Compound **42** (69%) was prepared from compound **40** following the same method as for compound **41**. ¹H NMR (CD₃OD, 400 MHz) δ 8.05 (s, 1H), 6.27 (d, *J* = 1.6 Hz, 1H), 5.51 (d, *J* = 6.0 Hz, 1H), 5.35 (d, *J* = 6.0 Hz, 1H), 4.63 (d, *J* = 2.0 Hz, 1H), 3.49 (br s, 2H), 2.48 (s, 3H), 1.71-1.66 (m, 2H), 1.59 (s, 3H), 1.41 (s, 3H), 1.00 (t, *J* = 7.6 Hz, 3H). HRMS calculated for C₁₇H₂₄N₆O₄I (M + H)⁺: 503.0904; found 503.0912.

Methyl (3a*S*,4*S*,6*R*,6a*R*)-6-(2-Iodo-6-(*n*-propylamino)-9*H*-purin-9-yl)-2,2-dimethyltetrahydro furo[3,4-*d*][1,3]dioxole-4-carboxylate (43)—DCC (24 mg, 0.117 mmol), DMAP (2.38 mg, 0.019 mmol) and MeOH (10 μL, 0.19 mmol) was added to a solution of compound **39** (18 mg, 0.039 mmol) in DMF (0.8 mL) and stirred at room temperature overnight. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH = 35:1) to afford the compound **43** (9.6 mg,

52%) as a colorless syrup. $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 8.06 (s, 1H), 6.29 (s, 1H) 5.65 (d, $J=6.0$ Hz, 1H), 5.45 (d, $J=6.0$ Hz, 1H), 4.81 (s, 1H), 3.59 (s, 3H), 3.05 (br s, 3H), 1.57 (s, 3H), 1.43 (s, 3H). HRMS calculated for $\text{C}_{15}\text{H}_{19}\text{N}_5\text{O}_5\text{I}$ ($\text{M} + \text{H}$) $^+$: 476.0431; found 476.0433.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-*N*,2,2-trimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (44)

—Compound **44** (84%) was prepared from compound **41** following the same method as for compound **33**. $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 8.22 (s, 1H), 7.35 (d, $J=4.0$ Hz, 1H), 7.02 (d, $J=4.0$ Hz, 1H), 6.31 (d, $J=2.0$ Hz, 1H), 5.51 (d, $J=6.0$ Hz, 1H), 5.39 (d, $J=6.0$ Hz, 1H), 4.66 (d, $J=2.0$ Hz, 1H), 3.12 (br s, 3H), 2.52 (s, 3H), 1.62 (s, 3H), 1.42 (s, 3H). HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{N}_6\text{O}_4\text{SCl}$ ($\text{M} + \text{H}$) $^+$: 489.1112; found 489.1120.

(3a*S*,4*S*,6*R*,6a*R*)-6-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-(*n*-propylamino)-9*H*-purin-9-yl)-*N*,2,2-trimethyltetrahydrofuro[3,4-*d*][1,3]dioxole-4-carboxamide (45)

—Compound **45** (82%) was prepared from compound **42** following the same method as for compound **33**. $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 8.22 (s, 1H), 7.35 (d, $J=4.0$ Hz, 1H), 7.02 (d, $J=4.0$ Hz, 1H), 6.31 (d, $J=2.0$ Hz, 1H), 5.50 (d, $J=6.0$ Hz, 1H), 5.38 (d, $J=6.0$ Hz, 1H), 4.66 (d, $J=2.0$ Hz, 1H), 3.57 (br s, 2H), 2.52 (s, 3H), 1.75-1.70 (m, 2H), 1.62 (s, 3H), 1.42 (s, 3H), 1.03 (d, $J=7.6$ Hz, 3H). HRMS calculated for $\text{C}_{23}\text{H}_{26}\text{N}_6\text{O}_4\text{SCl}$ ($\text{M} + \text{H}$) $^+$: 517.1425; found 517.1422.

Ethyl(3a*R*,3b*S*,4a*S*,5*R*,5a*S*)-5-(6-Bromo-4-chloro-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-*d*]

[1,3]dioxole-3b(3a*H*)-carboxylate (47)—DIAD (0.22 mL, 1.13 mmol) was added to a solution of triphenylphosphine (0.296 g, 1.13 mmol) and 3-deaza-2-bromo-6-chloro-purine (0.197 g, 0.845 mmol) in dry THF (4 mL) at 0 °C and after addition it was stirred at room temperature for 10 min. A solution of compound **36** (0.137 g, 0.566 mmol) in THF (2 mL) was added to the reaction mixture, and stirred overnight at room temperature. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate=2:1) to give the compound **47** (0.178 g, 69%) as a colorless foamy solid. $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 8.41 (s, 1H), 7.74 (s, 1H), 5.74 (d, $J=7.2$ Hz, 1H), 4.92 (s, 1H), 4.80 (d, $J=6.8$ Hz, 1H), 3.79-3.71 (m, 2H), 2.52-2.48 (m, 1H), 1.87-1.83 (m, 1), 1.66 (t, $J=5.2$ Hz, 1H), 1.54 (s, 3H), 1.34 (t, $J=7.2$ Hz, 1H), 1.29 (s, 3H). HRMS calculated for $\text{C}_{18}\text{H}_{20}\text{N}_3\text{O}_4\text{Br}$ ($\text{M} + \text{H}$) $^+$: 456.0326; found 456.0329.

Ethyl (3a*R*,3b*S*,4a*S*,5*R*,5a*S*)-5-(6-Bromo-4-(methylamino)-1*H*-imidazo[4,5-*c*]pyridin-1-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-*d*]

[1,3]dioxole-3b(3a*H*)-carboxylate (48)— $\text{MeNH}_2\cdot\text{HCl}$ (78.32 mg, 1.16 mmol) and DIPEA (0.4 mL, 2.32 mmol) was added to a solution of compound **47** (106 mg, 0.232 mmol) in isopropanol (2.5 mL) tube and heated at 125 °C under microwave condition in a sealed for 2.5 hrs. The reaction mixture was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethylacetate = 1:1) to give the compound **48** (80 mg, 76%) as a syrup. $^1\text{H NMR}$ (CD_3OD , 400 MHz) δ 7.98 (s, 1H), 6.83 (s, 1H), 5.70 (d, $J=7.2$ Hz, 1H), 4.81 (s, 1H), 4.70 (d, $J=6.8$ Hz, 1H), 4.33-4.25 (m, 2H),

3.06 (s, 3H), 2.43-2.39 (m, 1H), 1.85-1.81 (m, 1H), 1.59 (t, $J = 5.2$ Hz, 1H), 1.54 (s, 3H), 1.35 (t, $J = 7.2$ Hz, 3H), 1.28 (s, 3H). HRMS calculated for $C_{19}H_{24}N_4O_4Br$ ($M + H$)⁺: 451.0981; found 451.0977.

(3aR,3bS,4aS,5R,5aS)-5-(6-Bromo-4-(methylamino)-1H-imidazo[4,5-c]pyridin-1-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (49)—40% Methylamine solution (aqueous, 2.0 mL) was added to a solution of compound **48** (55 mg, 0.12 mmol) in methanol (3 mL) and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH=40:1) to give the compound **49** (37g, 71%) as a colorless syrup. ¹H NMR (CD_3OD , 400 MHz) δ 8.01 (s, 1H), 6.88 (s, 1H), 5.56 (d, $J = 7.2$ Hz, 1H), 4.95 (s, 1H), 4.65 (d, $J = 6.8$ Hz, 1H), 3.05 (s, 3H), 2.86 (s, 3H), 2.37-2.33 (m, 1H), 1.69-1.65 (m, 1H), 1.55 (s, 3H), 1.50 (d, $J = 5.2$ Hz, 1H), 1.28 (s, 3H). HRMS calculated for $C_{18}H_{23}N_5O_3Br$ ($M + H$)⁺: 436.0984; found 436.0987.

Ethyl (1S,2R,3S,4R,5S)-4-(6-Bromo-4-(methylamino)-1H-imidazo[4,5-c]pyridin-1-yl)-2,3-dihydroxybicyclo[3.1.0]hexane-1-carboxylate (50)—10% TFA (2 mL) was added to a solution of compound **48** (22 mg, 0.048 mmol) in MeOH (2 mL) and heated at 70 °C for 3 h. Solvent was evaporated and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH = 30:1) to give the compound **50** (18 mg, 91%) as a colorless syrup. ¹H NMR (CD_3OD , 400 MHz) δ 7.91 (s, 1H), 6.95 (s, 1H), 5.02 (d, $J = 6.8$ Hz, 1H), 4.63 (s, 1H), 4.30-4.24 (m, 2H), 4.02 (d, $J = 6.4$ Hz, 1H), 3.06 (s, 3H), 2.26-2.23 (m, 1H), 1.94 (t, $J = 5.2$ Hz, 1H), 1.73-1.69 (m, 1H), 1.33 (t, $J = 7.2$ Hz, 3H). HRMS calculated for $C_{16}H_{20}N_4O_4Br$ ($M + H$)⁺: 411.0668; found 411.0667.

(3aR,3bS,4aS,5R,5aS)-5-(6-((5-Chlorothiophen-2-yl)ethynyl)-4-(methylamino)-1H-imidazo[4,5-c]pyridin-1-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (51)— $PdCl_2(PPh_3)_2$ (10.3 mg, 0.014 mmol), CuI (1.4 mg, 0.007 mmol), 2-chloro-5-ethynylthiophene (63 mg, 0.44 mmol) and triethylamine (0.1 mL, 0.73 mmol) was added to a solution of compound **49** (32 mg, 0.073 mmol) in anhydrous DMF (1.2 mL) and was heated in a sealed tube at 130 °C under microwave condition. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2 :MeOH = 40:1) to give the compound **51** (23 mg, 63%) as a yellow syrup. ¹H NMR (CD_3OD , 400 MHz) δ 8.14 (s, 1H), 7.22 (d, $J = 4.0$ Hz, 1H), 7.03 (s, 1H), 6.98 (d, $J = 4.0$ Hz, 1H), 5.59 (d, $J = 7.2$ Hz, 1H), 5.01 (s, 1H), 4.69 (d, $J = 6.8$ Hz, 1H), 3.10 (s, 3H), 2.86 (s, 3H), 2.39-2.37 (m, 1H), 1.71-1.67 (m, 1H), 1.56 (s, 3H), 1.52 (t, $J = 5.2$ Hz, 1H), 1.28 (s, 3H). HRMS calculated for $C_{24}H_{25}N_5O_3SCl$ ($M + H$)⁺: 498.1367; found 498.1360.

Pharmacological procedures

Cell lines and radioligand binding—Initial screening at hDAT and other drug targets was performed by the PDSP. K_i determinations using 96-well plates and binding profiles in a broad screen of receptors and channels were generously provided by the National Institute of Mental Health's Psychoactive Drug Screening Program, Contract # HHSN-271-2008-00025-

C (NIMH PDSP). The NIMH PDSP is Directed by Bryan L. Roth MD, PhD at the University of North Carolina at Chapel Hill and Project Officer Jamie Driscoll at NIMH, Bethesda MD, USA. For experimental details please refer to the PDSP web site <http://pdsp.med.unc.edu/> and click on “Binding Assay” on the menu bar. The cell line used for preparing membranes for hDAT binding is HEK cells stably expressing hDAT, grown in DMEM medium containing 350 µg/mL geneticin and 10% fetal bovine serum. The buffer used for binding and washing was 10 mM HEPES, 135 mM NaCl, 5 mM KCl, 0.8 mM MgCl₂, 1 mM EGTA, pH 7.4, room temperature. The K_d of the radioligand **24** was determined to be 11.1±1.4 nM. The reference ligand **30** had a K_i value of 3.04±0.21 nM. The radioligands used by PDSP for NET and SERT binding were [³H]**52** and [³H]**53**, respectively.

The detailed comparison of binding enhancement at human DAT, NET and SERT were performed as described.^{3,48} Membranes from human embryonic kidney (HEK)-293 cells stably expressing the recombinant hDAT (HEK-hDAT), hSERT (HEK-hSERT), or hNET (HEK-hNET) were used. Transporter binding assays were performed in Krebs-HEPES assay buffer (25 mM HEPES, 122 mM NaCl, 5 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1 µM pargyline, 100 µM tropolone, 2 mg glucose/mL, 0.2 mg ascorbic acid/mL, pH 7.4). Membranes were preincubated at room temperature for 10 min with the test compound before radioligand (either [¹²⁵I]**23**, 40-80 pM final concentration, or [³H]**26**) was added. Concentrations of [³H]**26** were 1-3 nM for DAT and NET and 10-13 nM for SERT. The assay mixture was then incubated radioligand for 90 min at room temperature in the dark before filtration. Nonspecific binding was defined using 5 µM mazindol (HEK-hDAT and HEK-NET) or 5 µM imipramine (HEK-hSERT). In a typical hDAT binding experiment, the basal specific binding was ~2900 cpm, and the maximal stimulated specific binding was 7000-8000 cpm.

The full length coding region of the mouse DA transporter (mDAT) cDNA in PCMV6 vector (OriGene, Rockville, MD) was prepared using a Qiagen (Chatsworth, CA) miniprep kit following transformation of One-Shot Top 10 chemically competent Escherichia coli (Invitrogen, Carlsbad, CA). HEK-293 cells were transfected with 10 µg mDAT cDNA using Lipofectamine 2000 (Invitrogen). Cells were plated in DMEM supplemented with 10% Fetal Clone I (HyClone, South Logan, UT), then selected for G418 resistance (600 µg/ml G418). Forty-eight surviving colonies were chosen and plated in the above media with 300 µg/ml G418, 10 units/ml penicillin and 10 µg/ml streptomycin (HyClone). Clonal lines that had high levels of specific [³H]DA (20 nM) uptake, defined as the difference between uptake in the absence and presence of mazindol (5 µM), were tested using [¹²⁵I]**23** saturation binding assays (0.05 nM –16.6 nM) and competitive [¹²⁵I]**23** (40 pM) binding against mazindol and cocaine (1 nM-10 µM). The HEK-mDAT line used for the binding and uptake assays herein had a K_d value in [¹²⁵I]**23** binding of 3.9 ± 1.8 nM and a B_{max} value of 2410 ± 630 fmol/mg protein. In the binding enhancement experiments, the fixed concentration of [¹²⁵I]**23** was 45-50 pM. In a typical mDAT binding experiment, the basal specific binding was 750-900 cpm, and the maximal stimulated specific binding was 7000-8000 cpm.

A₃AR affinity was measured in two species as described using [¹²⁵I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide as radioligand.^{2,18,19} Protein was determined as reported.⁴⁹

In all the binding experiments, EC₅₀ values of K_i values were calculated using GraphPad Prism software (San Diego, CA). Values are expressed as mean ± SEM.

Uptake assays—Assays of uptake of [³H]-neurotransmitters (DA, norepinephrine, or 5HT; each 20 nM final concentration) in the same transporter-expressing HEK cell lines as above, used as intact and detached cells, were performed as described.³ The assay used Krebs-HEPES assay buffer and was incubated for 10 min at 25 °C before termination by filtration. Values are expressed as mean ± SEM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AR	adenosine receptor
CCI	chronic constriction injury
DA	dopamine
DAT	dopamine transporter (SLC6A3)
DCC	dicyclohexylcarbodiimide
DHTB	dihydrotrabenzine
DMAP	4-(dimethylamino)pyridine
DMF	dimethylformamide
DIPEA	diisopropylethylamine
DIAD	diisopropyl azodicarboxylate
GPCR	G protein-coupled receptor
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluorophosphate
HEK	human embryonic kidney 293

HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRMS	high resolution mass spectroscopy
5HT	serotonin
mazindol	(±)-5-(4-chlorophenyl)-3,5-dihydro-2 <i>H</i> -imidazo[2,1- <i>a</i>]isoindol-5-ol
METH	methamphetamine
NET	norepinephrine transporter (SLC6A2)
nisoxetine	(<i>R,S</i>)-3-(2-methoxyphenoxy)- <i>N</i> -methyl-3-phenylpropan-1-amine
PDC	pyridinium dichromate
PDSP	Psychoactive Drug Screening Program
SERT	serotonin transporter (SLC6A4)
TSPO	translocator protein
p-TSA	p-toluenesulfonic acid
VMAT2	vesicular monoamine transporter 2 (SLC18A2)

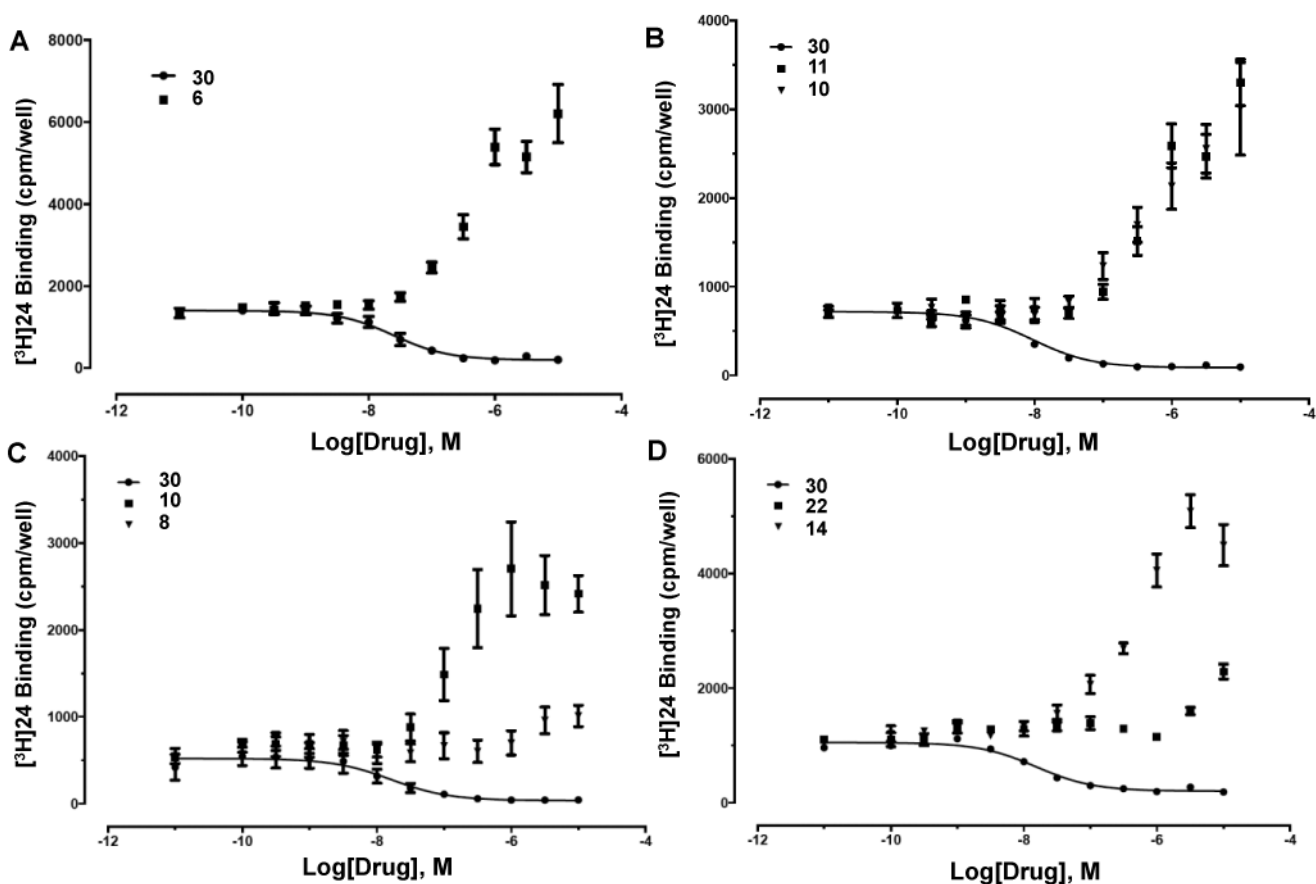


Figure 1.

Drug-induced enhancement of [^3H]24 binding to hDAT (PDSP) by (N)-methanocarba analogues (representative of 2 - 5 determinations): (A) 3-deaza-5'-methylamide **6**; (B) 5'-ethyl ester **10** and 2-(5-bromothiénylethynyl)-5'-methyl ester **11**; (C) 2-(5-chlorothiénylethynyl)-5'-*N*-(2-aminoethyl)-carboxamide **8** and its 5'-ethyl ester analogue **10**; (D) 5'-*n*-propyl ester **14** and 5'-carboxylic acid **22**. Comparison with binding inhibition by 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3 phenylpropyl)-piperazine (GBR12909, **30**) is shown.

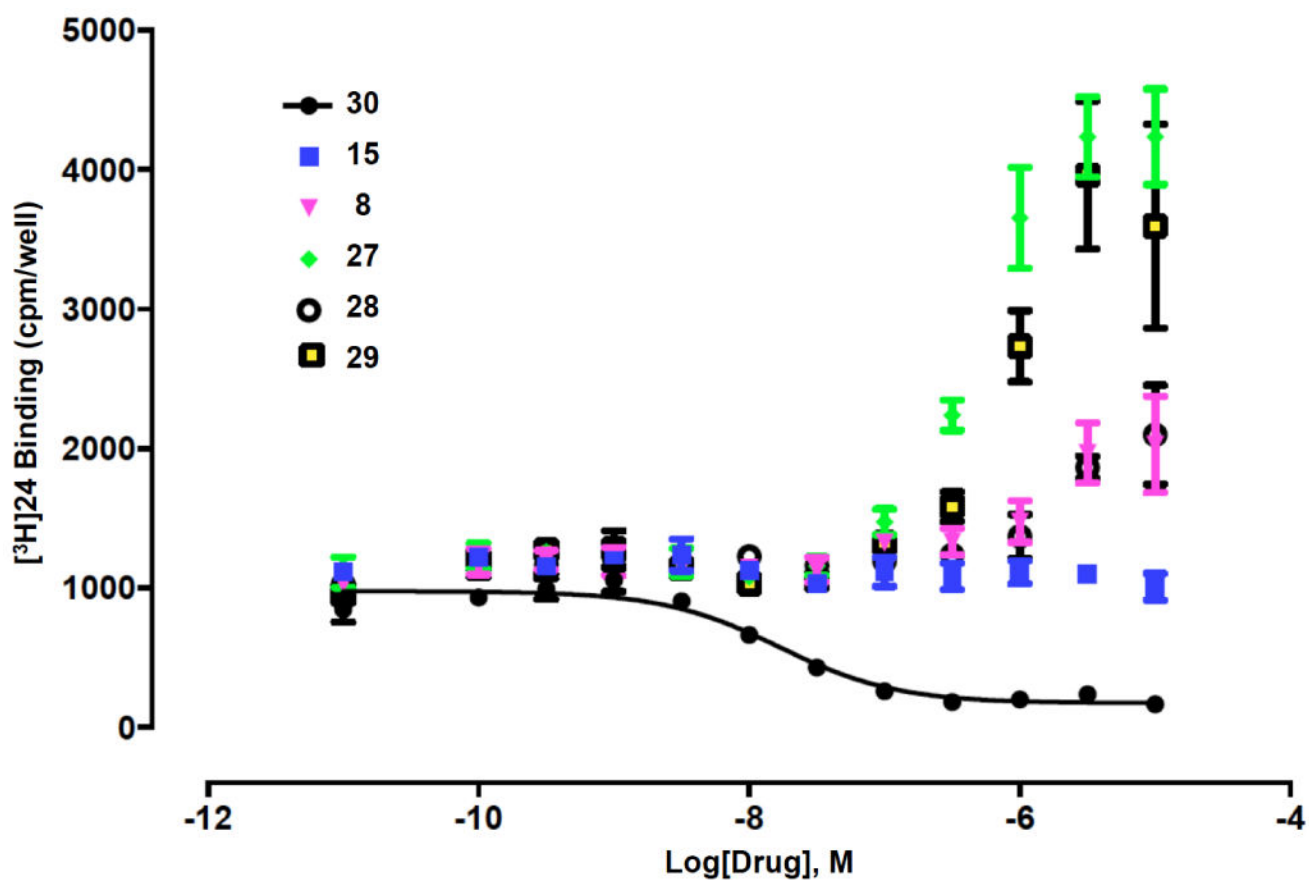


Figure 2.
(A) Drug-induced enhancement of [³H]24 binding to hDAT (PDSP) by ribosides **27 - 29** (representative of 4 determinations) in comparison to weak enhancers (N)-methanocarpa 5'-N-(2-aminoethyl)-carboxamide **8** and 5'-isopropyl ester **15**. Comparison with binding inhibition by **30** is shown.

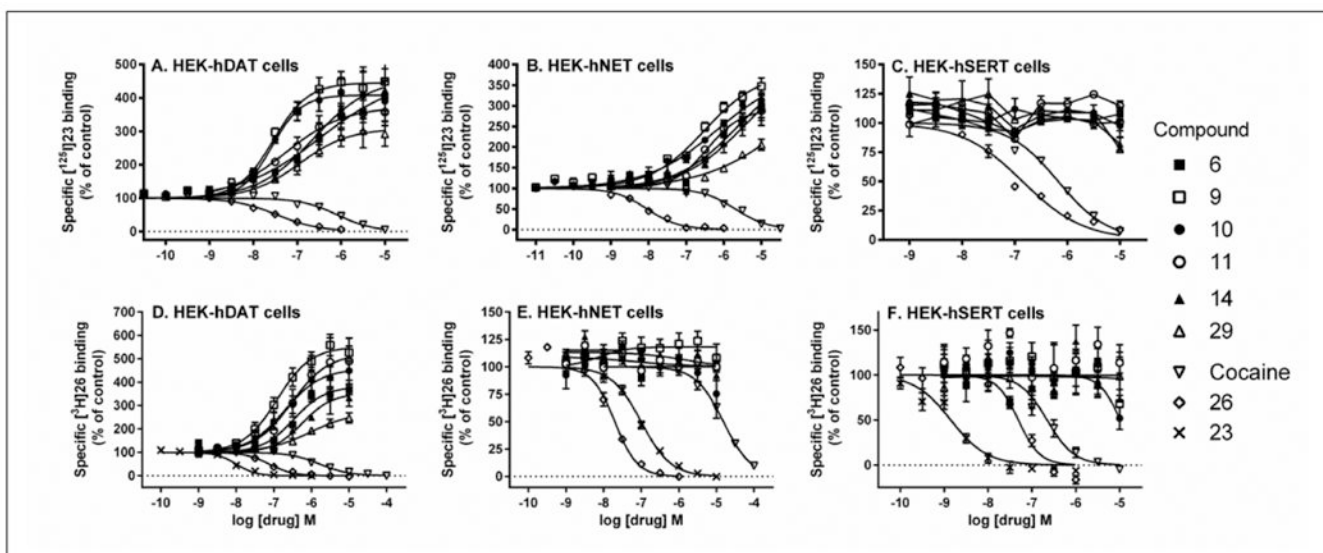
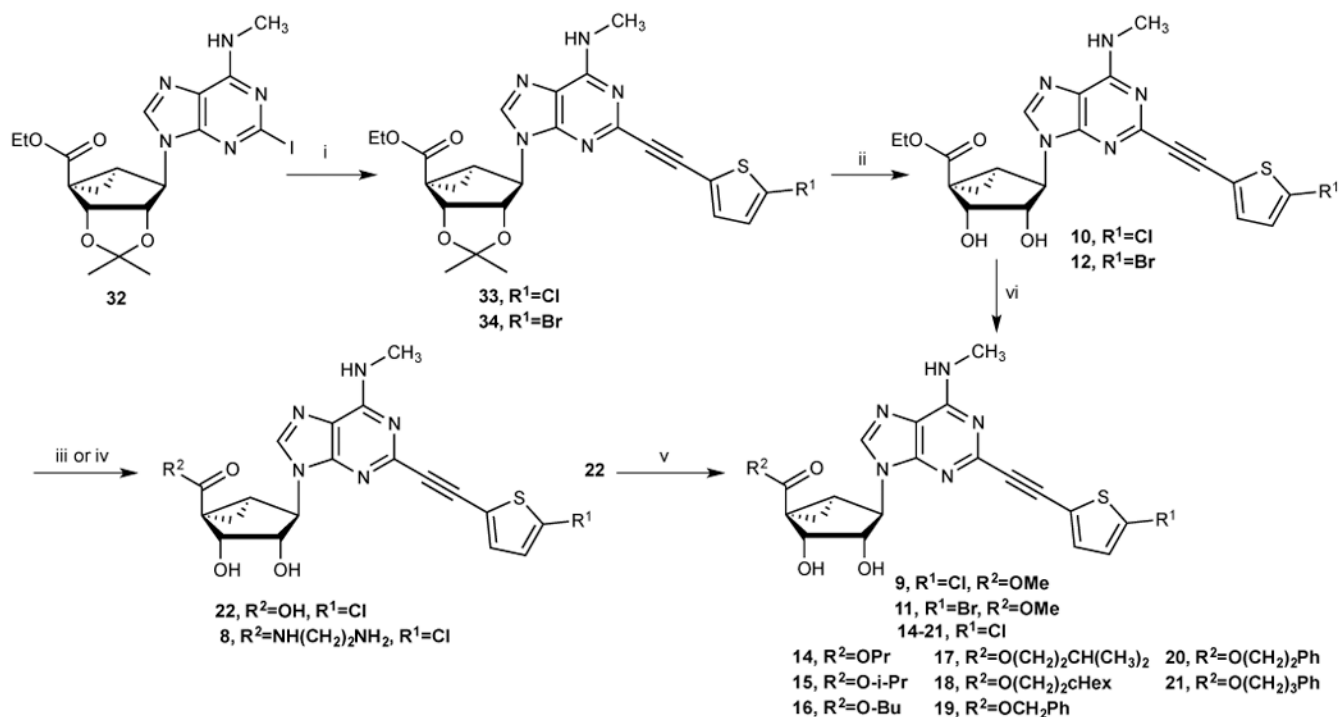
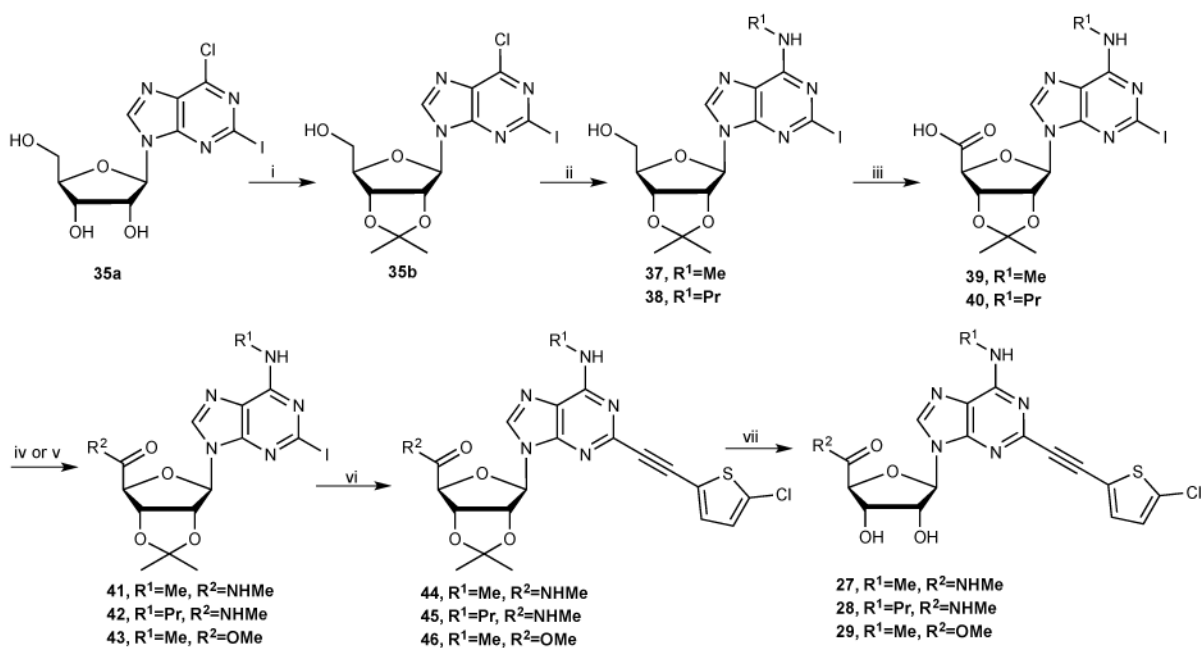


Figure 3.

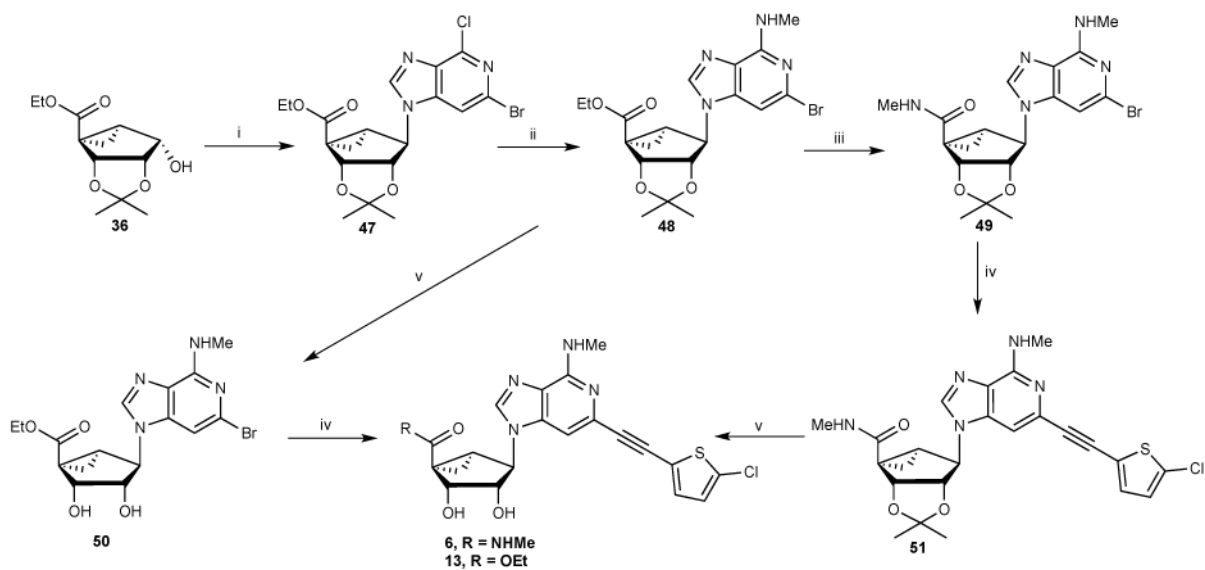
Drug-induced enhancement or inhibition of [¹²⁵I]23 binding and [³H]26 binding to HEK-hDAT, HEK-hNET and HEK-hSERT cell membranes.

**Scheme 1.**

Synthesis of (N)-methanocarpa 5'-esters **9-21**, carboxylate **22**, and extended amide **8** derivatives. Reagents and Conditions (% yields): (i) 2-Chloro or 2-bromo-5-ethynylthiophene, Pd(Ph₃P)₂Cl₂, CuI, Et₃N, DMF, rt, 82-83%; (ii) 10% TFA, MeOH, 70 °C, 90-91%; (iii) for **22**: 1N NaOH, MeOH, rt, 82%; (iv) for **8**: ethylenediamine, MeOH, rt, 71%; (v) for **14 - 21**: ROH, DCC, DMAP, DMF, rt, 51-59%; (vi) for **9, 11**: MeONa, MeOH, rt, 69%.

**Scheme 2.**

Synthesis of 5'-ester and 5'-methylamides **27-29** in the riboside series. Reagents and Conditions (% yields): (i) 2,2-dimethoxypropane, acetone, *p*-TSA, rt, 91%; (ii) RNH₂, Et₃N, MeOH, rt, 83-85%; (iii) PDC, DMF, 40 °C, 67-68%; (iv) for **41**, **42**: RNH₂, HATU, DIPEA, DMF, rt, 68-69%; (v) for **43**: MeOH, DCC, DMAP, DMF, rt, 52%; (vi) 2-chloro-5-ethynylthiophene, Pd(Ph₃P)₂Cl₂, CuI, Et₃N, DMF, rt, 82-84%; (vii) 10% TFA, MeOH, 70 °C, 90-91%.

**Scheme 3.**

Synthesis of 3-deaza-(N)-methanocarbanucleosides **6** and **13**. Reagent and Conditions (% yields): (i) 3-deaza-2-bromo-6-chloropurine, Ph_3P , DIAD, THF, 69%; (ii) $\text{MeNH}_2 \cdot \text{HCl}$, DIPEA, isopropanol, 125 °C, microwave, 76%; (iii) 40% MeNH_2 , MeOH, rt; (iv) 5-chloro-2-ethynylthiophene, $\text{PdCl}_2(\text{Ph}_3\text{P})_2$, CuI, DMF, 130 °C, μwave , 63%; (v) 10% TFA, MeOH, 70 °C, 90-91%.

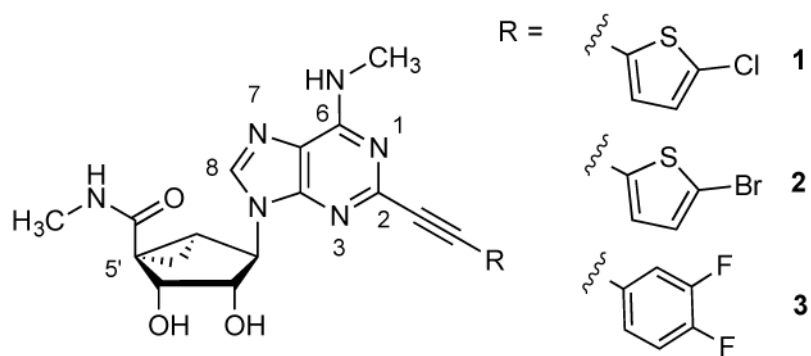
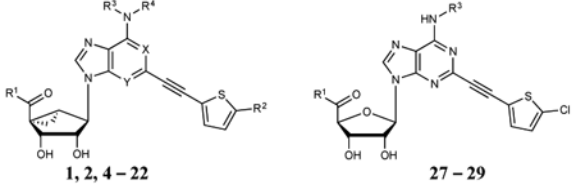


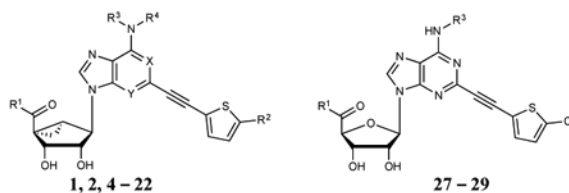
Chart 1.
Structures of potent A₃AR agonists that displayed activity at hDAT.

Table 1

Structures, initial screening results at hDAT^{a,b} (³H]24, unless noted) and binding affinity at A₃AR^d of (N)-methanocarba adenosine derivatives (**1-22**) and ribose derivatives (**27 - 29**). R² = Cl; R³ = CH₃; R⁴ = H; and X and Y = N, unless noted.



No.	R ¹ , and other changes as noted	hDAT Binding Enhancement, unless noted ^a , % of control (n)	hDAT Binding Inhibition, unless noted ^b , % of control	A ₃ AR Binding K _i , nM (species), or % at 10 μM ^d
1 ^d	NHCH ₃	556%	NE	0.70±0.11 (h), 36.1±4.7 (m)
2 ^d	NHCH ₃ , R ² = Br	235%	NE	0.44±0.12 (h), 43.7±2.1 (m)
3 ^d	NHCH ₃ ^e	322%	NE	1.65±0.08 (h), 86±6 (m)
4 ^d	NHCH ₃ , R ⁴ = CH ₃	NE	25%	23.5±10.6 (h), 597±63 (m)
5a ^d	NHCH ₃ , X = CH	c	c	2.34±0.20 (h), 31.3±1.9 (m)
5b	NHCH ₃ , X = CH, R ³ = CH ₂ CH ₃	c	c	1.69 ± 0.44 (h), 16.2±3.0 (m)
6	NHCH ₃ , Y = CH	334±35% (5)	NE	9.33 (h), 38±1% (m)
7 ^d	NHCH ₃ , R ³ = (CH ₂) ₂ CH ₃	159%	NE	1.11 ± 0.34 (h), 6.79±0.28 (m)
8	NH(CH ₂) ₂ NH ₂	136±15% (5)	NE	156±8 (h), 0% (m)
9	OCH ₃	352±50% (4)	NE	5.38±0.03 (h), 36±1% (m)
10	OCH ₂ CH ₃	539±106% (4)	NE	14.5±2.3 (h), 45±5% (m)
11	OCH ₃ , R ² = Br	370±91% (5)	NE	8.56±0.10 (h), 57±1% (m)
12	OCH ₂ CH ₃ , R ² = Br	430±86% (3)	NE	6.42±0.35 (h), 1800±90 (m)
13	OCH ₂ CH ₃ , Y = CH	354%	NE	169±24 (h), 18±4% (m)
14	O(CH ₂) ₂ CH ₃	426±14% (2)	NE	5.78±1.45 (h), 2810±150 (m)
15	OCH(CH ₃) ₂	NE	36%	42.9±22.8 (h), 30±1% (m)
16	O(CH ₂) ₃ CH ₃	285±35% (2)	NE	17.5±1.6 (h), 54±1% (m)
17	O(CH ₂) ₂ -CH(CH ₃) ₂	196 ±58%	NE	24.4±2.8 (h), 32±2% (m)
18	O(CH ₂) ₂ -cHex	c	c	770±75 (h), 5±1% (m)
19	OCH ₂ -Ph	c	c	7.31±0.29 (h), 48±2% (m)
20	O(CH ₂) ₂ -Ph	c	c	262±44 (h), 3±2% (m)
21	O(CH ₂) ₃ -Ph	c	c	255±30 (h), 9±1% (m)
22	OH	50±32% (4)	NE	684±195 (h), 0% (m)
27	NHCH ₃	321±49% (4)	NE	1.55±0.03 (h), 1170±40 (m)



No.	R ¹ , and other changes as noted	hDAT Binding Enhancement, unless noted ^a , % of control (n)	hDAT Binding Inhibition, unless noted ^b , % of control	A ₃ AR Binding K _i , nM (species), or % at 10 μM ^d
28	NHCH ₃ , R ³ = (CH ₂) ₂ CH ₃	84±35% (4)	NE	6.25±1.55 (h), 597±63 (m)
29	OCH ₃	220±54% (4)	NE	11.5±0.9 (h), 34±2 (m)

^aValues indicate enhancement at 10 μM of [³H]**24** binding at hDAT by PDSP, i.e. a negative percent inhibition in that screen is shown as a positive % here. Full curves for selected compounds appear in Figures 1 and 2 and in Supporting Information. Nonspecific binding was determined using 10 μM **30**. Values are expressed as the mean ± SEM.

^bValues indicate inhibition at 10 μM of [³H]**24** binding at hDAT by PDSP. Full curves for selected compounds appear in the Supporting information. Nonspecific binding was determined using 10 μM **30**. Values are expressed as the mean ± SEM.

^cFound to have negligible or no modulation of [³H]**24** binding at hDAT by PDSP, i.e. % inhibition at 10 μM was between -20% and 20% (see Figures 1 and 2).

^dData for hA₃AR and mA₃AR affinity (n = 2 – 3) of **1 – 5** and **7** are from Tosh et al.^{2,18} and PDSP data (except for **5b**) are from Janowsky et al.³ The radioligand used for A₃AR in both species was [¹²⁵I]Λ⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide **25**. Values are expressed as the mean ± SEM. Nonspecific binding was determined using 10 μM adenosine-5'-N-ethyluronamide.

^eStructure is in Chart 1.

NE – no effect.

Table 2

Affinities, potencies and efficacies of adenosine derivatives for hDAT, hNET and hSERT (n = 3-6).

No.	hDAT			hNET			hSERT		
	[¹²⁵ I]23 binding, EC ₅₀ ^a or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]DA uptake, IC ₅₀ (nM)	[¹²⁵ I]23 binding, EC ₅₀ or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]NE uptake, IC ₅₀ (nM)	[¹²⁵ I]23 binding, EC ₅₀ or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]5-HT uptake, IC ₅₀ (nM) ^c
1^b	35.1 ± 8.4 ^a 550 ± 110%	131 ± 31 ^a 403 ± 33%	253 ± 92	1180 ± 360 ^a 386 ± 73%	>10 μM NE	>10 μM	>10 μM NE	>10 μM NE	>10 μM
2^b	9.1 ± 1.7 ^a 217 ± 24%	870 ± 130 ^a 369 ± 31%	229 ± 30	670 ± 200 ^a 285 ± 22%	>10 μM NE	6110 ± 570	>10 μM NE	>10 μM NE	>10 μM
3^b	70 ± 26 ^a 690 ± 180%	128 ± 18 ^a 438 ± 41%	92 ± 16	1760 ± 640 ^a 371 ± 67%	>10 μM NE	>10 μM	>10 μM NE	>10 μM NE	>10 μM
6	239 ± 79 ^a 437 ± 60%	550 ± 180 ^a 405 ± 18%	163 ± 42	740 ± 190 ^a 318 ± 22%	>10 μM NE	5060 ± 360	>10 μM NE	>10 μM NE	>10 μM
7^b	1120 ± 220 ^a 268 ± 32%	ND	>8700	900 ± 320 ^a 159 ± 1%	ND	ND	ND	ND	ND
9	35 ± 13 ^a 458 ± 71%	124 ± 21 ^a 540 ± 33%	127 ± 40	310 ± 110 ^a 375 ± 24%	>10 μM NE	3380 ± 290	>10 μM NE	>10 μM NE	>10 μM
10	34 ± 13 ^a 434 ± 64%	267 ± 80 ^a 455 ± 39%	107 ± 14	490 ± 120 ^a 365 ± 11%	>8,400 NE	3980 ± 330	>10 μM NE	>10 μM NE	>10 μM
11	53.5 ± 9.8 ^a 374 ± 50%	308 ± 20 ^a 520 ± 100%	246 ± 10	400 ± 130 ^a 293 ± 32%	>10 μM NE	3560 ± 500	>10 μM NE	>10 μM NE	>10 μM
14	294 ± 82 ^a 461 ± 67%	535 ± 29 ^a 347 ± 60%	1145 ± 52	980 ± 270 ^a 302 ± 24%	>10 μM NE	7900 ± 1400	>10 μM NE	>10 μM NE	>10 μM
29	127 ± 32 ^a 312 ± 45%	860 ± 180 ^a 269 ± 28%	396 ± 83	1520 ± 180 ^a 227 ± 18%	>10 μM NE	2690 ± 220	>10 μM NE	>10 μM NE	>10 μM
Cocaine	720 ± 140	1270 ± 530	274 ± 31	2410 ± 590	10,700 ±	221 ± 13	657 ± 56	229 ± 56	277 ± 24

No.	hDAT			hNET			hSERT		
	[¹²⁵ I]23 binding, EC ₅₀ ^a or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]DA uptake, IC ₅₀ (nM)	[¹²⁵ I]23 binding, EC ₅₀ or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]NE uptake, IC ₅₀ (nM)	[¹²⁵ I]23 binding, EC ₅₀ or K _i (nM), % control	[³ H]26 binding, EC ₅₀ or K _i (nM), % control	[³ H]5-HT uptake, IC ₅₀ (nM) ^c
23	NE	NE	ND	NE	1100	ND	NE	1.27 ± 0.29	ND
	ND	11.0 ± 2.9	ND	ND	70.0 ± 8.8	ND	ND		
26	34.6 ± 5.7	74.2 ± 8.4	13.0 ± 0.6	11.3 ± 3.5	13.5 ± 1.5	0.92 ± 0.10	136 ± 15	45 ± 10	54 ± 11
	NE	NE		NE	NE		NE		

^aThe EC₅₀ value for drugs that enhanced radioligand binding.

^bData from Janowsky et al.³

K_i values are provided for drugs that inhibited binding.

NE, No enhancement of binding.

ND, Not determined.

Table 3

Affinities, potencies and efficacies of adenosine derivatives for mDAT expressed in HEK-293 cells.

No.	[¹²⁵ I]23 binding, EC ₅₀ ± SEM (nM)	Maximal % control [¹²⁵ I]23 binding	[³ H]DA uptake, IC ₅₀ ± SEM (nM)
1	2230 ± 430	1150 ± 60%	2290 ± 470
2	1100 ± 290	810 ± 110%	3260 ± 830
6	2760 ± 310	1000 ± 0%	4300 ± 1000
9	810 ± 270	1440 ± 190%	940 ± 130
10	1070 ± 320	1560 ± 200%	1170 ± 150
	[¹²⁵ I]23 binding K _i ± SEM (nM)		
Cocaine	850 ± 170	NA	200 ± 24
26	404 ± 88	NA	13.0 ± 2.6

NA, not applicable.