Direct Comparison of (N)-Methanocarba and Ribose-Containing 2-Arylalkynyladenosine Derivatives as A₃ Receptor Agonists

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ABSTRACT: A side-by-side pharmacological comparison of ribose and (N)-methanocarba (bicyclo[3.1.0]hexane) nucleosides as A₃AR agonists indicated that the bicyclic pseudoribose ring constraint provided higher affinity/selectivity at human and mouse A₃AR. The mean affinity enhancement for 5 pairs of 5'-methylamides was 11-fold at hA₃AR and 42-fold at mA₃AR. Novel C2-(5-fluorothien-2-ylethynyl) substitution enhanced affinity in the methanocarba but not ribose series, with highly hA₃AR-selective **16** (MRS7334) displaying K_i 280 pM and favorable pharmacokinetics and off-target activity profile. Molecular dynamics comparison of **16** and its corresponding riboside **8** suggested a qualitative entropic advantage of **16** in hA₃AR binding. The 5-F substitution tended to increase hA₃AR affinity (cf. 5-Cl) for methanocarba but not ribose derivatives. A representative methanocarba agonist **4** was shown to interact potently exclusively with A₃AR, among 240 GPCRs and 466 kinases. Thus, despite added synthetic difficulty, the (N)-methanocarba modification has distinct advantages for A₃AR agonists, which have translational potential for chronic disease treatment.

KEYWORDS: Adenosine receptor, G protein-coupled receptor, nucleosides, molecular dynamics, structure activity relationship

Agonists of the G_i-coupled A₃ adenosine receptor (A₃AR) are promising experimental therapeutic agents for the treatment of autoimmune inflammatory diseases, cancer, ischemia, pain, and other conditions.^{1–7} The A₃AR is expressed and demonstrated to act in the immune, nervous, and cardiovascular systems,^{7–9} and species differences in its pharmacology and physiological role have been described.^{10–12} For example, A₃AR agonists alone induce mast cell degranulation leading to hypothermia in rodents, but not in dog and human.¹⁰ Furthermore, >1200 human subjects treated with A₃AR agonist have reported no serious adverse effects.³ Phase 3 clinical trials of orally dosed agonist **1** (Chart 1) are in progress for rheumatoid arthritis and psoriasis. Its 2-chloro analogue **2** is in Phase 2 trials for hepatocellular carcinoma and nonalcoholic steatohepatitis (NASH).^{1,14} Furthermore, orally bioavailable agonists of unusually high human (h) A₃AR subtype selectivity (~10,000-fold) were shown in animal models to relieve or prevent the development of chronic neuropathic pain of various etiologies.^{7,15}

In probing the structure activity relationship (SAR) of adenosine derivatives as A₃AR agonists, we discovered that introduction of a (N)-methanocarba (bicyclo[3.1.0]hexyl) ring system in place of ribose leads to favorable affinity and selectivity.^{12,13} This rigid ribose substitution pre-establishes a ligand conformation that is highly compatible with the A₃AR binding requirements, possibly due to entropic reasons.^{16,17} As

demonstrated in both the ribose and methanocarba series, other modifications that are conducive to high A₃AR affinity are 5'-alkylamide and C2-ethynyl groups, as in 3–5.^{18–21} A 5'-methyl- or 5'-ethyl-amide group also tends to increase A₃AR agonist efficacy in functional assays. A C2-(5-chlorothien-2-ylethynyl) substitution was associated with a long duration and efficacy in chronic pain tests. For example, hypermodified analogues MRS5698 (**3**) and MRS5980 (**4**) were characterized as selective and highly efficacious A₃AR agonists in several species.¹⁵

In light of the extensive SAR reported for (N)-methanocarba derivatives as potent and selective A₃AR agonists,^{13,22–26} we reconsidered whether ribose derivatives similar to **4** and its congeners could serve as suitable alternatives. Ribosides are generally more readily synthesized than methanocarba nucleosides,^{15,27} which is a distinct advantage for pharmaceutical development. It is conceivable that with the combination of recently identified A₃AR-enhancing N⁶ and C2 position substitutions,^{23,25,28} it may not be necessary to incorporate

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Chart 1. Structures of Representative A₃AR Agonists, Including Ribosides (1, 2, and 5) and (N)-Methanocarba Derivatives (3 and 4)^a

^aBinding K_i values at the hA₃AR (nM): 1, 1.74; 2, 1.4; 3, 3.49; 4, 0.70; 5a, 0.44; 5b, 0.75.^{14,21,31}

an (N)-methanocarba ring in adenosine derivatives to achieve high selectivity. The original report of enhanced A₃AR affinity and selectivity of (N)-methanocarba adenosine compared to the riboside and the South (S)-methanocarba analogue was of limited structural scope.²² A direct side-by-side SAR pharmacological comparison of ribose and (N)-methanocarba nucleosides as A₃AR agonists is lacking, and here we have compared the A₃AR affinity in two species, h and mouse (m). Here, we extend the known SAR of C2-(5-chlorothien-2-ylethynyl) derivatives through multiple changes in both the (N)-methanocarba and 9-riboside series and characterize pharmacokinetic (PK) properties of selected analogues. The receptor interactions of several novel ligands were predicted using A₃AR homology modeling based on AR structures,^{29,30} ligand docking, and molecular dynamics (MD) simulations.

RESULTS

Chemical Synthesis. The novel nucleoside derivatives 8–10, 16–19, and 23 (Tables 1A and 1B) were prepared in order to explore the SAR at the A₃AR. Both 9-ribosides (6–10) and (N)-methanocarba 5'-methylamide derivatives (5, 11–19) were included. In Table 1A, the effects of N⁶-small alkyl (Me, Et, and Pr) and 1-deaza modifications of adenine in (N)-methanocarba and 9-riboside series were examined. Furthermore, a fluorine atom was inserted in the place of chlorine on the thienyl group present in 4. Also, the AR affinities of several corresponding 5'-ester derivatives (20–23) were compared (Table 1B). Synthetic routes were similar to those previously reported,^{23–25} although modification of the adenine moiety included novel C2 functionalization (Schemes S1–S3).

AR affinities expressed as an inhibition constant (K_i) were determined at several h and mA₃AR subtypes using standard radioligand binding assays.^{32,33} A comparison of corresponding changes made in the 9-riboside and (N)-methanocarba series indicated consistently greater A₃AR affinity in the (N)-methanocarba series. Some (N)-methanocarba F-thienyl analogues showed unprecedented hA₃AR selectivity; for

example, 16 had estimated ~40,000-fold selectivity compared to hA₁AR, while the corresponding riboside 8 was only 188-fold selective. Thus, the ratios of hA₃AR affinity enhancement for (N)-methanocarba compared to 9-riboside analogues were as follows: 2.1 (4 cf. 6); 5.7 (12 cf. 7); 8.2 (16 cf. 8); 7.5 (17 cf. 9); 30 (18 cf. 10). It is worth noting that the (N)-methanocarba modification also maintained high mA₃AR affinity better than in the riboside series. The mA₃AR affinity enhancement for the same set of (N)-methanocarba analogues compared to the corresponding 9-ribosides was (fold): 33 (4 cf. 6); 88 (12 cf. 7); 18 (16 cf. 8); 41 (17 cf. 9); 32 (18 cf. 10). Riboside 7 was equipotent in mA₁ and mA₃AR binding. Thus, we established that the (N)-methanocarba modification has an A₃AR affinity advantage over ribose substitution in this hypermodified series.

Several 5-chlorothien-2-ylethynyl nucleosides 4 and 11–15, including 1-deaza analogues, were previously reported as potent A₃AR agonists.²³ Furthermore, the corresponding 5-bromo analogues were found to display similarly high A₃AR affinity. However, the corresponding 5-fluorothien-2-ylethynyl nucleosides 8–10 (ribosides) and 16–19 (methanocarba) were not previously reported. In each case, the ratios of hA₃AR affinity enhancement for 5-F compared to 5-Cl analogues were as follows: 2.5 (16 cf. 4); 1.0 (17 cf. 12); 3.3 (18 cf. 13); 0.61 (19 cf. 14). However, 18 and 13 were equipotent at mA₃AR. Thus, in some methanocarba derivatives there is an advantage at the hA₃AR for a 5-fluorothien-2-ylethynyl over 5-chlorothien-2-ylethynyl substitution at C2. However, the 5-F-thienyl substitution provided no affinity increase in the riboside series (8 cf. 6, 9 cf. 7). In general, 1-deaza analogues preserved hA₃AR affinity better in the methanocarba than the ribose series. Riboside 8 was of 12-fold lower affinity and distinctly lower hA₃AR selectivity than its 1-deaza analogue 10.

5'-Ester derivatives 20–23 displayed variable A₃AR affinity. These esters are expected to be partial A₃AR agonists; we reported previously that compound 20 was a low efficacy agonist at the hA₃AR in inhibition of cAMP formation (11.6 ±

Table 1A. Structures and Binding Affinities^a of A₃AR Ligands (5'-Methylamides) with Small N⁶-Alkyl Substitution, Including Reference Compounds 4, 6, 7, and 11–15

6 – 10		4, 11 – 19		
Compd.	R ² , R ³ , X	A ₁ AR % inhibition or K _i (nM) ^a	A _{2A} AR % inhibition or K _i (nM) ^a	A ₃ AR % inhibition or K _i (nM) ^a
Ribosides				
6 ^b	Me, Cl, N	58 ± 11% (h), 4170 ± 810 (m)	7 ± 4% (h)	1.55 ± 0.03 (h), 1170 ± 40 (m)
7 ^b	<i>n</i> -Pr, Cl, N	1160 ± 200 (h), 315 ± 2 (m)	49 ± 3% (h)	6.25 ± 1.55 (h), 597 ± 63 (m)
8	Me, F, N	432 ± 120 (h)	45 ± 2% (h)	2.30 ± 0.15 (h), 1510 ± 150 (m)
9	<i>n</i> -Pr, F, N	369 ± 59 (h)	2460 ± 710 (h)	8.51 ± 0.91 (h), 435 ± 12 (m)
10	Me, F, CH	47 ± 6% (h)	15 ± 1% (h)	26.6 ± 11.9 (h), 1200 ± 90 (m)
(N)-Methanocarba Derivatives				
4 ^c	Me, Cl, N	6 ± 1% (h), 38% (m) ^e	24 ± 13% (h), 7% (m) ^e	0.70 ± 0.11 (h), 36 ± 5 (m)
11 ^c	Et, Cl, N	28 ± 4% (h), 59 ± 3% (m)	12 ± 5% (h)	3.8 ± 1.5 (h), 27 ± 2 (m)
12 ^c	<i>n</i> -Pr, Cl, N	22 ± 5% (h), 2140 ± 260 (m)	34 ± 3% (h)	1.1 ± 0.3 (h), 6.8 ± 0.3 (m)
13 ^c	Me, Cl, CH	15 ± 7% (h)	8 ± 0% (h)	3.0 ± 0.8 (h), 31 ± 2 (m)
14 ^{c,d}	Et, Cl, CH	10 ± 4% (h), 43 ± 3% (m)	48 ± 1% (h)	1.7 ± 0.4 (h), 16 ± 3 (m)
15 ^c	<i>n</i> -Pr, Cl, CH	731 ± 22 (h), 270 ± 3 (m)	63 ± 0% (h)	38 ± 12 (h), 70.3 ± 12.0 (m)
16	Me, F, N	33 ± 4% (h), 3260 ± 250 (m)	4620 ± 360 (h), 5 ± 2% (m)	0.280 ± 0.102 (h), 85 ± 4 (m)
17	<i>n</i> -Pr, F, N	3980 ± 940 (h)	838 ± 17 (h)	1.13 ± 0.13 (h), 10.6 ± 1.3 (m)
18	Me, F, CH	20% (h) ^e , 51 ± 2% (m)	56 ± 4% (h), 6 ± 3% (m)	0.896 ± 0.172 (h), 37 ± 2 (m)
19	Et, F, CH	31% (h) ^e , 53 ± 34% (m)	61 ± 6% (h), 4 ± 2% (m)	2.78 ± 1.31 (h), 25 ± 2 (m)

^aThe species is h (human) or m (mouse). Binding in membranes of HEK293 cells (all hARs and all mARs) stably expressing one of three hAR subtypes. The binding affinity for hA₁, A_{2A}, and A₃ARs was expressed as K_i values using agonist radioligands [³H]N⁶-R-phenylisopropyladenosine (55), [³H]2-[p-(2-carboxyethyl)phenyl-ethylamino]-5'-N-ethylcarboxamidoadenosine (56), or [¹²⁵I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide (57), respectively. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using 10 μM adenosine 5'-N-ethyluronamide (NECA, 58, hA₃AR) or N-(2-aminoethyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy]-acetamide (XAC, 59, hA₁AR, hA_{2A}AR). Values are expressed as the mean ± SEM (*n* = 3, unless noted). K_i values were calculated as reported.³³ Binding affinity for mA₁AR, A_{2A}AR, and A₃ARs (all expressed in HEK293 cells) was measured using agonist radioligands as described in the Supporting Information. Compound 4 (10 μM) was shown to be inactive at the hA_{2B}AR determined in a functional assay (Supporting Information). ^bReported in Tosh et al.^{26,34} ^cReported in Tosh et al.²³ ^dLow aqueous solubility observed for 14. ^eN = 1.

1.0% of a full agonist maximal effect at 10 μM).²⁶ The ester group of (N)-methanocarba analogues is relatively stable in vivo, likely due to steric hindrance.³⁴ Methanocarba ester 21 was the most potent hA₃AR ligand (K_i 5.38 nM) in this group but was weak at the mA₃AR, and 5-F derivative 23 was similar in hA₃AR affinity. Riboside 20 displayed nearly matched affinity at the two species, and it appears that high mA₃AR affinity of a 5'-ester requires a ribose rather than methanocarba ring system.

Forty-five off-target activities of all nucleoside derivatives were measured in radioligand binding assays by the NIMH Psychoactive Drug Screening Program (PDSP, Figure S1, Table S1, Supporting Information).³⁵ The data is represented as either a K_i value or a % inhibition at 10 μM, if <50%. Compounds 15 had considerable binding affinity at the 5-HT_{2B} serotonin receptor (K_i 760 nM), which occurs likely as an antagonist based on our previous study, which would potentially have antifibrotic activity.¹⁶ Most other off-target

Table 1B. Nucleoside 5'-Ester Derivatives 20–23^c

20	21 – 23			
Compd.	R ¹ , R ³ , X	A ₁ AR, % inhibition or K _i (nM) ^a	A _{2A} AR, % inhibition ^a	A ₃ AR, % inhibition or K _i (nM) ^a
Ribosides				
20 ^b	Me, Cl, N	46 ± 8% (h), 53 ± 1% (m)	8 ± 5% (h), 12 ± 4% (m)	11.5 ± 0.9 (h), 34 ± 2 (m)
(N)-Methanocarba Derivatives				
21 ^b	Me, Cl, N	24 ± 2% (h)	20 ± 1% (h)	5.38 ± 0.03 (h), 36 ± 1% (m)
22 ^b	Et, Cl, CH	17 ± 2% (h), 54 ± 1% (m)	18 ± 9% (h), 5 ± 1% (m)	29.4 ± 13.8 (h), 828 ± 51 (m)
23	Et, F, N	1670 ± 480 (h), 864 ± 67 (m)	27 ± 3% (h)	10.4 ± 4.1 (h) 625 ± 59 (m)

^aThe species is h (human) or m (mouse). Binding in membranes of HEK293 cells (all hARs and all mARs) stably expressing one of three hAR subtypes. The binding affinity for hA₁, A_{2A}, and A₃ARs was expressed as K_i values using agonist radioligands [³H]N⁶-R-phenylisopropyladenosine (55), [³H]2-[p-(2-carboxyethyl)phenyl-ethylamino]-5'-N-ethylcarboxamidoadenosine (56), or [¹²⁵I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide (57), respectively. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using 10 μM adenosine 5'-N-ethyluronamide (NECA, 58, hA₃AR) or N-(2-aminoethyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8-yl)-phenoxy]-acetamide (XAC, 59, hA₁AR, hA_{2A}AR). Values are expressed as the mean ± SEM (n = 3, unless noted). K_i values were calculated as reported.³³ Binding affinity for mA₁AR, A_{2A}AR, and A₃ARs (all expressed in HEK293 cells) was measured using agonist radioligands as described in the Supporting Information. Compound 4 (10 μM) was shown to be inactive at the hA_{2B}AR determined in a functional assay (Supporting Information).^b Reported in Tosh et al.^{26,34} ^cCompounds 20–22 were previously reported and their expanded characterization is included here for comparison to 23. R¹ = Et, unless noted.

Figure 1. Docking pose of compound 16 (A) and 8 (B) at hA₃AR (coordinate files in Supporting Information). The receptor is rendered by a gray ribbon and residues within 4 Å from the ligand by gray sticks. Compound 16 is in green sticks and compound 8 in orange. Cyan lines indicate H-bonds between ligand and A₃AR.

activities detected were in the micromolar range. Several compounds bound to the β₃-adrenergic (12–14, 18, 19), κ-opioid, or σ receptors or to the translocator protein (TSPO). In a separate assay, compound 4 (10 μM) was tested, as a representative (N)-methanocarba selective A₃AR agonist, in the GPCRMax and Kinome screens (DiscoverX), and no other GPCR hits or any potent kinase hits emerged (Table S4 and Figure S8, Supporting Information).

However, some analogues (4, 6, 8, 11, 12, 16, 17, 20, 21, and 23) at 10 μM displayed a strong negative percent value for human dopamine transporter (DAT) inhibition, indicating an allosteric interaction with DAT to enhance radioligand binding, as described.²⁶ The 1-deaza modification reduced interaction with DAT, as shown for the 1-deazaadenine

derivative 18 compared to its adenine congener 16. However, one 1-deaza analogue, 15, was clearly inhibitory in DAT binding with a K_i value of 3.94 μM. Thus, the effect of N⁶-alkyl homologation of 4 and 11, both of which enhanced DAT radioligand binding, combined with the 1-deaza modification in 15, qualitatively altered the pharmacological effect on DAT.

In vitro ADME-tox characterization and in vivo PK of five nucleosides 4, 11, 14, 16, and 17 in the rat were determined (Figure S2, Table S2). High plasma and microsomal stability and low HEP-G2 cell toxicity and CYP inhibition were demonstrated. The compounds' oral bioavailability was demonstrated to be in the range of 14%–99%F (Supporting Information). The complex PK profile of 16, in comparison to the data for 4, 12, and 14, was suggestive of enterohepatic

recirculation.³⁶ In addition to the initial high concentration of **16** following oral administration, a second concentration peak was observed at ~8 h.

Hypothetical A₃AR binding modes of riboside **8** and corresponding methanocarba **16** were compared. These nucleosides were docked to the orthosteric binding site of a previously generated hA₃AR homology model,²⁸ and then the complexes were subjected to molecular dynamics (MD) simulations to assess their stability over time. In each case, the MD simulation having the best average interaction energy (sum of electrostatic and van der Waals) among three replicates was selected for comparison. Compounds **16** and **8** (Figure 1A and B, respectively) docked in similar poses, which were comparable with the binding mode experimentally observed for AR agonists bound to A₁ and A_{2A}ARs.^{29,30,37} The adenine moiety maintained a π - π interaction with conserved Phe168 (EL2) and a bidentate hydrogen bond with Asn250 (6.55), through the endocyclic N7 nitrogen and the exocyclic N⁶H. The ribose and (N)-methanocarba moieties of compounds **8** and **16**, respectively, faced the bottom of the orthosteric pocket and were engaged in a triplet of H-bonds involving the following: Ser271 (7.42), His272 (7.43), and Thr94 (3.36) with, respectively, the 3'-OH and 2'-OH groups and the 5'-methylamide NH group. The F-thienyl-alkynyl substituent at C2 faced TM2, in an accommodation that was enabled by the outward displacement of transmembrane helix 2 (TM2), which was modeled after an antagonist-bound A₁AR template.^{28,37} The compounds were surrounded within 4 Å by Tyr15 (1.35), Ser73 (2.65), Leu90 (3.32), Leu91 (3.33), Thr94 (3.36), His95 (3.37), Gln167 (EL2), Phe168 (EL2), Val169 (EL2), Met174 (5.35), Met177 (5.38), Trp243 (6.48), Leu246 (6.51), Ile249 (6.54), Asn250 (6.55), Ile253 (6.58), Leu264 (7.35), Tyr265 (7.36), Ile268 (7.39), Ser271 (7.42), and His272 (7.43), and most of these ligand contacts persisted during the MD simulations (Figure S3C for **16** and Figure S4C for **8**). H-bonding with Asn250 (6.55) and Thr94 (3.36) was maintained during >85% and >40% of the simulation frames in all the replicates, respectively. Ser271 (7.42) and His272 (7.43) remained in contact with the ligand, but they interact through a hydrogen bond to a reduced extent (Table S3, Figures S3D and S4D for the selected replicates). The initial poses were geometrically stable during the MD simulations, with average ligand RMSD over the replicates of 2.03 Å (**16**) or 1.76 Å (**8**). In the selected replicates, the RMSD of **16** fluctuated with average RMSD 2.03 Å (Table S3) for the whole simulation (Figure S3A), while the final MD pose of compound **8** stabilized with RMSD ~1.5 Å compared to its initial pose (Figure S4A). A comparison of the interaction energy profile of compounds **16** and **8** (Figure S3B and S4B, respectively, for the selected replicates) showed a similar electrostatic component for the two ligands, with an average value over the three replicates of -25.76 kcal/mol (**16**) and -25.75 kcal/mol (**8**) (Table S3). A slightly favorable van der Waals contribution appeared for **16**, with an average value of -43.92 kcal/mol over the three replicates, compared to -38.69 kcal/mol for **8** (Table S3). This was not surprising, given the additive character of van der Waals interactions and the introduction of multiple atoms in bicyclic **16** to replace the riboside O4'. In fact, focusing on the selected replicates, we computed the van der Waals contribution of O4' of **8** (Figure S5B) and of the two carbon atoms and attached hydrogens replacing O4' in the (N)-methanocarba ring of **16** (Figure

S5A): the average van der Waals contribution of these atoms was -0.90 kcal/mol (**8**) and -3.21 kcal/mol (**16**).

The larger difference between compounds **16** and **8** was their ring conformational variation, which was (N)-constrained in **16** (Figure S6). Three MD simulations of **8** in the free (unbound) state were collected, and the riboside ring puckering parameters²² were analyzed in comparison to those in the bound state. When unbound, **8** explored a wide range of ring puckers (Figures S7D-F), spanning from (N) to (S) regions of the pseudorotational wheel, with several conformations in the West portion of the graph. Compound **8** was initially docked to hA₃AR in an (N) conformation, comparable to A_{2A}AR- and A₁AR-bound adenosine derivatives in reported structures.^{29,30,37} The time course of compound **8** puckering showed two predominant conformational clusters when hA₃AR-bound (Figures S7A-C), concentrated in the pure (N) and (S) portions of the graph. The transition of **8** from free to the bound state reduced conformational scatter, i.e. conformational space explored by the ligand, with a consequent possible qualitative entropic disadvantage. From the MD trajectory analysis, both the (N) and (S) puckers appeared compatible with receptor binding, so the (N) conformation constraint through the (N)-methanocarba ring of **16** may provide an entropic advantage over **8** to lower the difference between the conformational variation displayed by the ligand in the bound vs unbound states. This was consistent with the higher affinities of the corresponding (N)-methanocarba nucleosides.

DISCUSSION

Adenosine derivatives in the ribose or ribose 5'-alkylamide series, containing 2-phenylalkynyl or 2-benzylalkynyl combined with N⁶-small alkyl substitution (or unsubstituted), e.g. **5**, were previously characterized as potent A₃AR agonists.^{18,19,31} On the other hand, a long synthetic sequence is required to prepare (N)-methanocarba agonists, for example, 16 steps from D-ribose to obtain highly selective agonist **3**.³ Therefore, we re-examined adenine 9-riboside derivatives, containing combinations of more recently introduced A₃AR-favorable functionalities, as potentially useful A₃AR agonists. We recently reconsidered the SAR distinction between ribose and (N)-methanocarba nucleosides as A₁AR agonists, and ribosides were shown to be superior at that subtype.³⁸ We now extend a similar side-by-side nucleoside agonist comparison to the A₃AR, and we reached the opposite conclusion, i.e. the (N)-methanocarba agonists are consistently of higher A₃AR affinity and selectivity.

In conclusion, the (N)-methanocarba (bicyclo[3.1.0]-hexane) nucleosides displayed higher affinity/selectivity at two A₃AR species homologues than their equivalent ribosides. The mean affinity enhancement for 5 pairs was 11 ± 5 fold at hA₃AR and 42 ± 12-fold at mA₃AR. Novel C2-(5-fluorothiophenylethynyl) substitution enhanced affinity in the methanocarba but not ribose series, with compound **16** displaying K_i 280 pM at hA₃AR and favorable PK and off-target activity profile. MD simulation of A₃AR binding of **16** and riboside **8** showed a close overlap in interactions with conserved polar H-bonding and nonpolar π - π stacking amino acid residues, but a qualitative entropic advantage of the rigid bicyclic ring of **16** compared to **8** was suggested. The 5-F-thienyl substitution compared to 5-Cl-thienyl tended to increase affinity at the hA₃AR in the methanocarba but not ribose series. A representative methanocarba agonist **4** was

shown to interact potently exclusively with the A₃AR, among 167 known and 73 orphan GPCRs and 466 kinases. Thus, despite the added synthetic difficulty of this series, the (N)-methanocarba nucleosides have distinct advantages for achieving high A₃AR selectivity and their potential use in treating chronic conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmmedchemlett.9b00637>.

Chemical synthesis schemes S1–S3 and associated procedures and spectra; PDSP and DiscoverX screening results; in vitro ADME-tox data for **4**, **11**, **14**, **16**, and **17** and in vivo PK in rat; and molecular modeling methods and results (PDF)

Video of MD simulation of hA₃AR-bound **8** (AVI)

Video of MD simulation of hA₃AR-bound **16** (AVI)

Docking pose coordinates of receptor-bound **8** (PDB)

Docking pose coordinates of receptor-bound **16** (PDB)

Molecular formula strings (XLSX)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS:

AR, adenosine receptor; HEK293, human embryonic kidney 293; DAT, dopamine transporter; GPCR, G protein-coupled receptor; MD, molecular dynamics; PDSP, NIMH Psychoactive Drug Screening Program; PK, pharmacokinetics; RMSD, root-mean-square deviation; SAR, structure–activity relationship; TM, transmembrane helical domain; TSPO, translocator protein

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