Purine (N)-Methanocarba Nucleoside Derivatives Lacking an Exocyclic Amine as Selective A3 Adenosine Receptor Agonists

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Supporting Information

ABSTRACT: Purine (N)-methanocarba-5’-N-alkyluronamidobosi de A1 adenosine receptor (A₁AR) agonists lacking an exocyclic amine resulted from an unexpected reaction during a Sonogashira coupling and subsequent aminolysis. Because the initial C6-Me and C6-styryl derivatives had unexpectedly high A₁AR affinity, other rigid nucleoside analogues lacking an exocyclic amine were prepared. Of these, the C6-Me-(2-phenylethynyl) and C2-(5-chlorothienylethynyl) analogues were particularly potent, with human A₁AR Kᵢ values of 6 and 42 nM, respectively. Additionally, the C2-(5-chlorothienyl)-6-H analogue was potent and selective at A₁AR (MRS7220, Kᵢ 60 nM) and also completely reversed mouse sciatic nerve mechanoallodynia (in vivo, 3 μmol/kg, po). The lack of a C6 H-bond donor while maintaining A₁AR affinity and efficacy could be rationalized by homology modeling and docking of these hypermodified nucleosides. The modeling suggests that a suitable combination of stabilizing features can partially compensate for the lack of an exocyclic amine, an otherwise important contributor to recognition in the A₁AR binding site.

INTRODUCTION

There is an expanding effort to develop selective adenosine receptor (AR) agonists and antagonists for clinical use in inflammation, pain, ischemia, cancer, and other conditions.¹–³ Generally, insight into ligand affinity for the four ARs (A₁, A₂A, A₁B, and A₃) has come from screening, mutagenesis and structure-based studies,⁴–⁷ with the latter currently relying on homology with the known X-ray structures of ligand-bound A₂AARs.⁸–¹⁰ Structure-based optimization of known ligands and the discovery of novel chemotypes for the ARs have also been reported.¹¹ In general, these studies suggest that recognition of AR pharmacophores, especially as applied to adenosine derivatives, depends on a set of interactions with typically conserved amino acid residues. For example, the adenine ring engages in aromatic π−π stacking with a conserved Phe residue in EL2 (168 in the human (h) A₁AR), and the 5’-N-alkyluronamide of potent AR agonists such as I (nonselective) typically H-bonds with a conserved Thr/Ser 3.36 (using standard notation) in transmembrane helix (TM) 3. The N⁷ hydrogen, as H-bond donor, and N⁶, as H-bond acceptor, form a bidentate coordination with Asn (6.55). This latter interaction is the reason that 7-deaza adenosine derivatives are nearly inactive as AR agonists.¹²,¹³ The removal of H-bonding groups on the adenosine pharmacophore that interacts through these conserved recognition points often reduces agonist potency and/or efficacy across the four AR subtypes.

Although changes to the primary pharmacophore may disrupt binding completely, subtle changes in these conserved drug−receptor interactions can lead to subtype specificity. For example, substituting the ribose oxygen for sulfur reduced A₁ and increased A₂AAR affinity of 2-chloroadenosine.¹⁴ Conversely, replacing the flexible ribose conformation by a rigid Northern (N)-(methanocarba moiety decreased A₁ and increased A₂AAR affinity of 2-chloroadenosine.¹⁴ Similarly, N⁶-3-halobenzyl and 5’-N-methanuronamide moieties, along with combinations thereof, are particularly important for enhancing A₁AR selectivity.¹⁵ There are also derivatives lacking an exocyclic NH, such as C6-phenylpurine (nonriboside) derivatives that...
bind to the A1AR as antagonists and various purine-9-riboside derivatives that bind to the A2AR. For example, the N6-dimethyl 2 and inosine 3 analogues of the nonselective, potent agonist S'-N-ethylcarboxamidoadenosine maintain moderate A2AR binding affinity (Chart 1). Among nucleosides having a ring-constrained (N)-methanocarba (bicyclo[3.1.0]hexane) modification that maintains an A3AR-preferred conformation, several inosines, thioinosine derivatives show moderate binding affinity. Thus, compounds 2 and 6 lack a H-bond donor at the C6 position yet have μM A3AR binding affinity.

**RESULTS**

**Chemical Synthesis.** The opportunity to explore the structure–activity relationship (SAR) of C6-methylated and other C6-alkylated adenosine derivatives arose from a side reaction and an unanticipated fragmentation product that occurred during the attempted reaction of a 6-chloro-2-iodo intermediate 28. The attempted Sonogashira coupling of 28 was sought as an alternate route to synthesize C2-arylethynyl (N)-methanocarba nucleosides, e.g., 6-amino derivatives (Scheme 1), which we reported previously to be highly selective A3AR agonists. During the Sonogashira reaction of compound 28 with phenylacetylene, instead of the desired monosubstituted product 30, only disubstituted product 31a was obtained. To convert the 5'-ester group of compound 31a to an amide derivative, it was stirred with 40% methylamine at 70 °C.

Here, we considered whether other analogues lacking the H-bond donor at N6 behave as potent A3AR-selective agonists. Using structural modification of known A3AR agonists and assays for binding, function, and in vivo efficacy, we characterize such motifs. Also, we use molecular modeling based on an agonist-bound A3AR crystal structure to explore the binding of this redefined pharmacophore. The presence of other stabilizing interactions in these hypermodified analogues appears to compensate for the lack of an exocyclic NH. We show that this new variety of ligands not only enlarges the class of high affinity and specific A3AR ligands, which is highly desirable clinically but also gives new insight into the specificity attributes of the A3AR pharmacophore.

**Scheme 1. Attempted Synthesis of 6-Chloro (N)-methanocarba Intermediate 30 as a Possible Precursor of Selective A3AR Agonists and the Redirected Route to A3AR Agonist Series Containing at the C6 Position Either a Substituted Styril 12–14 or a Methyl Group 15, 17 (Affinities in Table 1)**

<Diagram>
solution in methanol at room temperature overnight. It was interesting to observe that a hydroamination reaction had occurred at the C6-phenylacetylene group in addition to amidation to give compound 32a. We have never observed a similar hydroamination product when a phenylacetylene group is present at the C2 position. The structure of this product 32a was thoroughly characterized by various NMR studies (Figures S3–S5, Tables S1–S3, Supporting Information (SI)). Similar products were also observed during a reaction of compound 28 with 2-chloro-phenylacetylene and 4-t-butylphenylacetylene followed by amination to give the C2-aminostyryl derivatives 32b and 32c. To prove chemically that the hydroamination reaction occurred exclusively at the C6 phenylacetylene group, compound 34 was synthesized by a Sonogashira reaction of 2,6-dichloro derivative 33 with phenylacetylene (Scheme 2). Aminolysis of compound 34 with methylamine solution provided compound 11a. Attempted removal of the isopropylidene group of 32a with 10% TFA in methanol at 70 °C provided a hydrolyzed enol derivative 12 and the unexpected fragmented product 15. Enamines are known to be hydrolyzed to ketones under acidic conditions. Both products were extensively characterized by various NMR studies, and also a plausible mechanism for the formation of fragmented 6-Me product 15 is presented in Scheme S1 (SI).

We propose that under acidic conditions, methanol may attack the protonated keto tautomer equivalent of the enol group followed by a fragmentation that leaves a 6-Me group on the purine base. Similarly, hydrolysis of 32b–c and 11a provided enol derivatives 13, 14, and 11b. However, only a C6 functionalized product, e.g., 17, was observed in the hydrolysis reaction of 4-t-Bu-phenylethynyl derivative 32c. No fragmentation products were detected upon hydrolysis of 2-chlorophenylethynyl 32b and 2-chloro 11a derivatives.

C6-Me derivatives (16, 18, and 19) having different C2-arylethynyl groups were synthesized by an alternate route, which preinstalled a 6-Me group on the nucleobase (Scheme 3). The nucleobase intermediate 2-iodo-6-methyl purine 55 was prepared from a 9-protected 2-amino-6-methyl purine 52 as shown in Scheme S2 (SI). Similarly, C6-H derivatives were prepared from an intermediate 6-iodopurine (Scheme 4). However, it was observed that during attempted conversion of 5'-ester 39 to an amide, MeNH2 also replaced the iodo group at C2 position to give compound 40, which upon acid hydrolysis provided compound 23. To avoid this side reaction at the C2 position, a Sonogashira coupling was first performed on compound 39 with different arylalkynes to give 41a–c. Amidation of esters 41a and 41b with a methylamine solution followed by acid hydrolysis yielded C6-H derivatives 20 and 21, respectively. In contrast, the same reactions for the pyrazine derivative 41c yielded a hydroamination product 43, which upon acid hydrolysis gave the enol derivative 24.

For comparison, we also prepared one C6-methoxy (N)-methanocarba nucleoside 22, based on inosine, containing an extended C2 substituent. In the first route, we have synthesized a C6-OME derivative 44; however, the attempted conversion of the ester to an amide by treatment of 44 with methylamine solution gave a C6-NHMe substituted derivative 45 (Scheme 5). To avoid this side reaction, an alternate route featuring

Scheme 2. Application of the Redirected Route from Scheme 1 to the Preparation of C6-Substituted 2-Cl (N)-Methanocarba Derivative 11

Although many 2-Cl derivatives are potent A1AR agonists, this compound only weakly bound to the receptor (Table 1). (i) phenylacetylene, PdCl2(Ph3P)2, CuI, Et3N, DMF, rt; (ii) 40% MeNH2, MeOH, rt; (iii) 10% TFA, MeOH, 70 °C.

Scheme 3. Synthesis of C6-CH3 (N)-Methanocarba Derivatives 16, 18, and 19

Reagents and conditions: (i) 2-iodo-6-methyl purine 54, Ph3P, DIAD, THF, rt; (ii) 40% MeNH2, MeOH, rt; (iii) aryl alkynes, PdCl2(Ph3P)2, CuI, Et3N, DMF; (iv) 10% TFA, MeOH, H2O, 70 °C.
oxidation of a 5′-CH₂OH and MeNH₂ coupling was designed (Scheme 6). Compound 46 was converted to C6-OMe derivative 47, which upon TBDPS deprotection and PDC oxidation gave the acid derivative 49. Coupling of MeNH₂ with compound 49 in the presence of HATU gave the desired precursor 50, with no detectable C6-NHMe side products. Sonogashira coupling of compound 50 with 2-chloro-5-ethynylthiophene followed by acid hydrolysis afforded the C6-OMe derivative 22.

**Pharmacological Activity.** Table 1 lists the AR affinities for the various synthesized purine nucleoside analogues lacking a C6-exocyclic amino group and their related N⁶-substituted adenosine derivatives. Standard radioligand (25–27) binding assays were performed on human (h)A₁, A₂A, and A₃ ARs using reported methods, and IC₅₀ values were transformed to Kᵢ as described. The fortuitously synthesized initial C6-methyl derivative 15 had unexpectedly high binding at the hA₃AR (Kᵢ 6.01 nM). This motivated us to explore other accessible C6-alkyl or alkenyl derivatives containing either a 2-Cl (11b) or 2-arylethynyl (15–19) group. None of these compounds bound significantly to the hA₁ or A₂A ARs and were therefore selective for the A₃ AR. The 2-chloro analogue 11b bound only weakly to the hA₂AR (Kᵢ 1.14 μM). Many other 2-chloro nucleosides are known to have potent A₃AR agonist activity, which suggests that the presence of a rigid extension at the C2 position, e.g., the 6-styryl derivatives 12 and 13, enhances binding to the A₂AR in this series lacking an exocyclic NH.

A C2 modified, 5-chlorothienylethynyl substituent was associated with higher affinity in C6-modified analogues than most other aryl groups except an unsubstituted phenyl, as in 15. The 5-chlorothienylethynyl group in C6-Me (19) and C6-H (21) analogues produced Kᵢ values of 42 and 60 nM, respectively, and were both highly A₃AR selective, with hA₁ and A₂AARs Kᵢ values extrapolated to ≫10 μM. Other aryl groups, specifically substituted phenyl rings, did not achieve such high affinity. The rank order of decreasing hA₃AR affinity was: 15 > 19 > 16 > 17 ≳ 18. The least potent C6-Me analogues, compounds 17 (Kᵢ 305 nM, hA₃AR) and 18 (Kᵢ 343 nM) contained a bulky p-t-Bu-phenyl group or a pyrazine group, respectively. The disubstituted 6-styryl derivatives 12–14 were of intermediate affinity at the hA₃AR, ranging from Kᵢ ~ 80–500 nM. It is interesting that the 4-t-Bu group was highly detrimental to hA₃AR affinity in the case of C6-Me but not with a larger C6 substituent.
Selected nucleosides were tested in a binding assay at the mouse (m) A3AR expressed in HEK293 cells using reported methods. The $K_i$ values of 12 and 15 were $136 \pm 9$ and $158 \pm 10$ nM, respectively, which suggested that human vs mouse species differences are greater for the C6-methyl analogue than with a larger group at that position. Other analogues were weaker in binding at the mA3AR, with $K_i$ values: 19, $722 \pm 35$, and 21, $396 \pm 29$ nM.

Selected high affinity ligands (15, 19, and 21) were examined in a functional activity at hA3AR, e.g., the ability to inhibit production of cyclic AMP via the hA3AR expressed in CHO cells. All three compounds activated the hA3AR as full agonists with a similar rank order of potency as in the binding results; the EC$_{50}$ values (nM) were: 15, $3.16 \pm 0.72$; 19, $12.5 \pm 2.8$; 21, $26.9 \pm 8.4$ (Figure 1A). Compounds 12 and 15 were also tested in a functional assay at the mA3AR expressed in HEK293 cells, e.g., the ability to inhibit production of cyclic AMP. Figure 1B shows that these compounds were also full agonists for this receptor, with efficacy comparable to the reference compound 2-chloro-N$^6$-(3-iodobenzyl)-S$'$-N-methylcarboxamidoadenosine 55. Both 12 and 15 were quite potent in activating the mA3AR, with EC$_{50}$ values of 4.86 and 20.2 nM, respectively.

On the basis of the potent in vitro A3AR activity of these congeners, selected compounds were tested in vivo using previously reported methods for the ability to reduce chronic neuropathic pain following oral administration in the mouse chronic constriction injury (CCI) model (Figure 2). 2-(5-nitro-2-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate showed no significant binding inhibition at the diverse receptors, but at 10 μM it enhanced human dopamine transporter (hDAT) binding of [3H]methyl (1R,25,3S)-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate by roughly 200% (Figure S1, SI), similar to other (N)-methanocarba-adenosine derivatives. This unusual activity of this chemical series was shown by Janowsky et al. to correlate with an allosteric enhancement of the affinity of the tropane radioligand binding to DAT. Compound 19 showed only one such off-target interaction ($K_i$ at δ opioid receptor $5.8 \mu M$, 68% inhibition), and compound 21 showed no off-target interactions.

**Molecular Modeling.** Docking simulations were carried out to explore the environment of receptor-bound C6 substituted purine nucleosides. Selected compounds ($K_i < 100$ nM) were docked into the putative TM binding site of a previously reported homology model of the hA3AR, based on a hybrid A2AAR-β2 adrenergic receptor template. The docking poses were selected by taking into account optimal interaction geometries with the residues surrounding the binding site and by inspecting electrostatic and van der Waals contributions of computed residue interaction scores, denoted $I_{ele}$ and $I_{vdw}$ respectively. The “interaction score
maps" (ISMs) arising from the latter analysis (Figure S2 (SI)) identify a common binding mode for derivatives 15, 16, 19, and 21, involving residues located mainly in TM3, extracellular loop (EL) 2, TM6, and TM7. On the other side, the C6-styryl derivatives 12 and 14 interact with residues belonging to EL2, EL3, and TM7, thus implying that their placement in the binding site is shifted toward the extracellular side of the receptor (data not shown).

As an example of the binding mode exhibited by the majority of the considered nucleosides, Figure 3 shows the docking pose of compound 15 (Kᵢ = 6.0 nM). The ligand resides in the upper region of the TM bundle (see also SI, Video S1) with the C2 terminal cyclic group pointing toward the extracellular environment, and this mode features several interactions typical for AR agonists. The planar bicyclic core establishes an aromatic π-π stacking interaction with Phe168 (EL2), whereas the purine N7 engages the side chain of Asn250 (6.55) acting as H-bond donor. A tight hydrogen bond network with Thr94 (3.36), Ser271 (7.42), and His272 (7.43) anchors the methanocarba region of the compound in the binding pocket. In addition to these conserved recognition points, the ISMs (Figure S2B (SI)) report several other residues involved in favorable contacts with the ligand, including Leu91 (3.32), Ile92 (3.33), Val169 (EL2), Trp243 (6.48), Leu246 (6.51), and Ile268 (7.31).

### DISCUSSION

Previously, removal of the exocyclic NH of adenosine derivatives was not considered a feasible approach to the design of new, selective AR agonists. In an early, pioneering SAR paper by Bruns, purine-9-riboside 56 (nebularine) and 6-methylpurine-9-riboside (structures not shown) were described as weak AR agonists at 1 μM with only 15% and 6%, respectively, of the efficacy of 10 μM adenosine at a receptor in membranes prepared from CHO or HEK293 (hA₁AR and mA₃AR) cells stably expressing one of three hAR subtypes. The binding affinity for hA₁, hA₂A, and hA₃ARs was expressed as Kᵢ values (n = 3–4) measured using agonist radioligands [³H]N⁶-R-phenylisopropyladenosine 25, [³H]2-[2-carboxyethyl]phenyl-ethylamino]-S'-N-ethylcarboxamido-adenosine 26, or [¹²⁵I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyl-uronamide 27, respectively. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using adenosine-5'-N-methyluronamide 1 (10 μM at hARs, 100 μM at mAₓAR). The concentrations of radioligands and their Kᵢ values at the corresponding hARs in parentheses used to calculate Kᵢ values of competing ligands were all in nM: 25, 1.0 (1.5); 26, 10 (16.2); 27, 0.2 (1.22).

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**Table 1. Structures and Binding Affinities at Three ARs of Reference Compounds (7–10)²³,²⁶ and Newly Synthesized Nucleoside Derivatives (11–24)⁶**

| Compound | Structure | hA₁AR Kᵢ (nM) or % inhibition | hA₂AAR % inhibition | hA₃AR Kᵢ (nM) |
|----------|-----------|---------------------------------|---------------------|--------------|
| 7⁶       | ![Structure](image1) | 20% ± 3% | 27% ± 3% | 1.34 ± 0.30 |
| 8⁶       | ![Structure](image2) | 6% ± 4% | 41% ± 10% | 3.49 ± 1.84 |
| 9⁶       | ![Structure](image3) | 18% ± 8% | 14% ± 7% | 0.85 ± 0.22 |
| 10⁶      | ![Structure](image4) | 6% ± 1% | 24% ± 13% | 0.70 ± 0.11 |
| 11b      | ![Structure](image5) | 30% ± 12% | 24% ± 8% | 1140 ± 170 |
| 12       | ![Structure](image6) | 16% ± 5% | 16% ± 7% | 78.5 ± 19.8 |
| 13       | ![Structure](image7) | 28% ± 15% | 21% ± 1% | 515 ± 107 |
| 14       | ![Structure](image8) | 24% ± 9% | 19% ± 9% | 94.3 ± 30.9 |

| Compound | Structure | hA₁AR Kᵢ (nM) or % inhibition | hA₂AAR % inhibition | hA₃AR Kᵢ (nM) |
|----------|-----------|---------------------------------|---------------------|--------------|
| 15       | ![Structure](image9) | 15% ± 6% | 34% ± 7% | 6.01 ± 1.60 |
| 16       | ![Structure](image10) | 5430 ± 750 | 14% ± 2% | 98.5 ± 32.5 |
| 17       | ![Structure](image11) | 16% ± 10% | 15% ± 11% | 305 ± 39 |
| 18       | ![Structure](image12) | 15% ± 9% | 17% ± 7% | 343 ± 127 |
| 19       | ![Structure](image13) | <10% | 12% ± 5% | 42.2 ± 17.3 |
| 20       | ![Structure](image14) | 17% ± 9% | 12% ± 3% | 124 ± 23 |
| 21       | ![Structure](image15) | <10% | 18% ± 10% | 60 ± 19 |
| 22       | ![Structure](image16) | 796 ± 404 | 27% ± 8% | 684 ± 285 |
| 23       | ![Structure](image17) | 17% ± 1% | <10% | 604 ± 261 |
| 24       | ![Structure](image18) | 14% ± 2% | 20% ± 5% | 1480 ± 560 |
fibroblasts that was later identified as the human A2BAR. Compound 56 was 40-fold less potent than adenosine in activation of the canine coronary artery A2AAR, and the exocyclic NH was deemed essential for AR activation. Compound 56 is also a weak inhibitor of adenosine deaminase; however, we are not concerned about that off-target activity with respect to the potent A3AR agonists in this study because 5′-N-alkyluronamides have also been explored as anticancer and anti-infective agents through activities unrelated to ARs. We did not prepare the corresponding hypermodified 9-ribosides for direct comparison with (N)-methanocarba analogues in Table 1.

Many adenosine derivatives containing a monosubstituted N6 group, in combination with other substitutions, have been reported as potent A3AR agonists. We revisited these two previously rejected modifications of adenosine for AR agonists, C6-H and C6-Me, using highly optimized A3AR agonists as lead structures. In this compound series, the loss of the exocyclic NH still preserved moderate affinity and high selectivity for the A3AR. Moreover, these purine analogues maintained an ability to fully activate the Gi-coupled human

Figure 1. Functional agonism at the hA3AR (A) and the mA3AR (B) of nucleosides lacking an exocyclic NH, which remain selective A3AR ligands. (A) Compounds 15, 19, and 21 proved to be potent, full agonists at the hA3AR (% values relative to inhibition of forskolin-stimulated cyclic AMP accumulation by adenosine-5′-N-methyluronamide 1 at 10 μM). Compounds 12 (B) and 15 (C) in an essay of inhibition of forskolin-stimulated cyclic AMP accumulation with HEK293 cells expressing the mA3AR, as described. Concentration-effect curves with reference full agonist 2-chloro-N6-(3-iodobenzyl)-5′-N-methylcarboxamidoadenosine 55 are included. Data are the mean ± SEM, n = 4−7.

Figure 2. Time course of protection hind paw mechanoallodynia of the sciatic nerve in the CCI mouse model (po administration on day 7, 3 μmol/kg). The vehicle was 10% DMSO in 0.5% methylcellulose, which when administered alone had no effect on PWT. There was no effect on the contralateral paw. (A) CCI results (n = 3) for compounds 12 (●) and 15 (■). Data are the mean ± SEM. For comparison, compound 8 at the same dose provided 100% and 23.7 ± 10.8% protection against mechanoallodynia in the same model at 1 and 3 h, respectively. (B) CCI results (n = 2) for compounds 19 (●) and 21 (■).
and mouse A3ARs and to protect against neuropathic pain in the mouse, a characteristic A3AR effect.

The correlation of activation of the A3AR with relief from chronic neuropathic pain has been established in various rodent models.3,28 Selected compounds evaluated in functional assays and in the CCI pain model in mice had A3AR selectively and activity comparable to nucleosides containing an exocyclic amine. Activation of the A1AR is also known to reduce neuropathic pain, 39 but except for two relatively weak analogues, 6-Me16 and 6-MeO22, these derivatives have no appreciable affinity for the A1AR. Therefore, we conclude that the antinociceptive activity of orally administered 12, 15, 19, and 21 was due to activation of the A3AR, with 21 completely reversing mechanoallodynia of the mouse sciatic nerve. With both peripheral and central mechanisms contributing to the antinociceptive effects, 3 a novel class of AR agonists that lacks the exocyclic amine (specifically an NH group) was considered an important recognition element for nucleosides binding to ARs in general, as it H-bonds to the conserved Asn (6.55). We have discovered that other structural features of the ligand can partially compensate for the lack of this important contributor and also increase A3AR specificity.

Figure 3. Hypothetical binding mode of C6-methyl (N)-methanocarba derivative 15 (orange carbon atoms, ball and stick representation), a potent and selective agonist, obtained after docking simulations at the hA3AR. Side chains of residues important for ligand recognition are reported as sticks (gray carbon atoms). H-bonds are pictured as green solid lines, whereas π−π stacking interactions as cyan dashed lines with the centroids of the aromatic rings displayed as cyan spheres. Nonpolar hydrogen atoms are omitted.

The C6-truncated or C6-methyl or styryl compounds prepared in this study display a wide range of A3AR affinities. Certain 6-methyl analogues were particularly potent, with $K_i$ values of 6 nM (15) to $\sim$50 nM (19, 21), and other 6-methyl analogues bound in the µM range. Unsubstituted phenyl and 5-chlorothienylethynyl groups at the C2 position appeared to promote higher A3AR affinity compared to other aryl groups. The loss of energetic stabilization provided by binding of an exocyclic NH of conventional A3AR agonists can be compensated by other groups at different locations on the nucleoside, such as the extended C2 substituent, the rigid bicyclic ring, or the 5′-methylamide. All of these groups contribute to A3AR affinity and selectivity and help to anchor

3256

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the ligand. For example, the C2 extended analogue 12 was 15-fold more potent at the hA3AR than the corresponding C2–Cl derivative 11b. The inspection of the ligand–receptor interactions suggests that the above-mentioned groups are engaged in hydrophobic contacts with several residues (mainly leucine side chains located in TM3 and TM7) surrounding the binding cavity. These ancillary interactions ensure an optimal orientation of the hydrogen-bonding groups toward the conserved recognition points. From the present set of ligands, we have no evidence that selective A2AR or A2AAR agonists lacking an exocyclic NH can be designed.

It was already observed that H-bonding groups located on the ribose moiety are more closely related to the activation of the A2AR, i.e., the “message” portion of the molecule, and the adenine constitutes the “address” portion of the nucleoside.41 The present findings reinforce that generalized division of function in that full agonism is observed in the absence of the NH.

**CONCLUSION**

In conclusion, this study is the first demonstration that removal of an H-bond donor group at C6 of purine nucleosides is still compatible with binding and activation of an AR subtype. This set of novel A2AR agonists arose from an unexpected series of reactions on the adenosine precursors that left a methyl or styryl group at the C6 position of adenine. After we discovered the biological utility of such truncated purine derivatives, we found synthetic approaches to enlarge the SAR beyond the accidental analogues. It is surprising that the adenine 6-NH group, which is traditionally considered essential for the biological utility of such truncated purine derivatives, we found synthetic approaches to enlarge the SAR beyond the accidental analogues. It is surprising that the adenine 6-NH group, which is traditionally considered essential for the recognition of nucleosides at the various ARs, is not universally essential. Importantly, these hypermodified nucleosides have lower polar surface area than the equivalent adenine analogues, which should be advantageous for bioavailability. Moreover, the C6-truncated and C6-C compounds are selective agonists of the A2AR that display considerable in vivo activity against chronic neuropathic pain in a mouse model.

**EXPERIMENTAL PROCEDURES**

**Materials and Instrumentation.** All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). Routine 1H NMR spectra were obtained at 298 K with a Bruker AVIII 400 MHz or AV 500 MHz spectrometer using CDCl3, CD3OD, and DMSO as solvents. Reported chemical shifts (δ, ppm) are referenced to tetramethylsilane (0.00) for CDCl3, methanol (3.30) for CD3OD, and water (3.30) for DMSO, unless otherwise noted. Conformation of the product structures was obtained by mass spectrometry and standard 1D and 2D NMR methods including COSY, TOCSY, HSQC, and HMBC. TLC analysis was carried out on glass sheets precoated with silica gel F254 (0.2 mm) from Aldrich. The purity of the final nucleoside derivatives was checked using a Hewlett–Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μm analytical column (30 mm × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). Mobile phase: linear gradient (PerkinElmer, Boston, MA). Interactions suggest that the above-mentioned groups are engaged in hydrophobic contacts with several residues (mainly leucine side chains located in TM3 and TM7) surrounding the binding cavity. These ancillary interactions ensure an optimal orientation of the hydrogen-bonding groups toward the conserved recognition points. From the present set of ligands, we have no evidence that selective A2AR or A2AAR agonists lacking an exocyclic NH can be designed.

It was already observed that H-bonding groups located on the ribose moiety are more closely related to the activation of the A2AR, i.e., the “message” portion of the molecule, and the adenine constitutes the “address” portion of the nucleoside.41 The present findings reinforce that generalized division of function in that full agonism is observed in the absence of the NH.

In conclusion, this study is the first demonstration that removal of an H-bond donor group at C6 of purine nucleosides is still compatible with binding and activation of an AR subtype. This set of novel A2AR agonists arose from an unexpected series of reactions on the adenosine precursors that left a methyl or styryl group at the C6 position of adenine. After we discovered the biological utility of such truncated purine derivatives, we found synthetic approaches to enlarge the SAR beyond the accidental analogues. It is surprising that the adenine 6-NH group, which is traditionally considered essential for the recognition of nucleosides at the various ARs, is not universally essential. Importantly, these hypermodified nucleosides have lower polar surface area than the equivalent adenine analogues, which should be advantageous for bioavailability. Moreover, the C6-truncated and C6-C compounds are selective agonists of the A2AR that display considerable in vivo activity against chronic neuropathic pain in a mouse model.
methyl product was identified in this reaction. 1H NMR (CD3OD, 400 MHz) (8.43, s, 1H), (7.79, d, 6.0, 1H), (7.67, d, 6.8, 1H), (7.60, d, 7.2, 1H), (7.53–7.49, m, 2H), (7.48–7.42, m, 3H), (6.47, s, 1H), (5.13, d, 5.6, 1H), (5.00, s, 1H), (4.13, d, 6.4, 1H), (2.84, s, 3H), (2.18–2.14, m, 1H), (1.88, t, 5.2, 1H), (1.44–1.40, m, 1H). HRMS calculated for C26H30N5O3 (M + H)+, 460.2349; found, 460.2341.

15.2R,35,4R,5S)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (14). A solution of compound 32c (24 mg, 0.035 mmol) in methanol (2.5 mL) and 10% trifluorothianesulfonic acid (2.5 mL) was heated at 70 °C overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH2Cl2:MeOH = 3:1) to give the compound 14 (12 mg, 55%) as a colorless syrup. Column was further eluted with (CH2Cl2:MeOH = 1:1) to give the C6-methyl compound 17 (2.8 mg, 17%) as colorless syrup.

**Compound 17.** 1H NMR (CD3OD, 400 MHz) (8.51, s, 1H), (7.62, d, 8.4, 1H), (7.52, d, 8.4, 1H), (5.15, d, 6.4, 1H), (5.00, s, 1H), (4.11, d, 6.8, 1H), (2.86, s, 3H), (2.83, s, 3H), (2.16–2.13, m, 1H), (1.88, t, 5.2, 1H), (1.38, s, 18H). HRMS calculated for C24H25N5O6 (M + H)+, 620.3237; found, 620.3232.

**Compound 18.** 1H NMR (CD3OD, 400 MHz) (8.52, s, 1H), (7.64–7.61, m, 1H), (7.55–7.50, m, 1H), (7.43–7.36, m, 1H), (5.14, d, 6.8, 1H), (5.00, s, 1H), (4.11, d, 5.6, 1H), (2.84, s, 3H), (2.83, s, 3H), (2.16–2.12, m, 1H), (1.88, t, 5.2, 1H), (1.43–1.39, m, 1H). HRMS calculated for C26H25N5O5 (M + H)+, 440.1534; found, 440.1530.

**Compound 19.** 1H NMR (CD3OD, 400 MHz) (8.52, s, 1H), (7.64, s, 1H), (7.50, d, 6.8, 1H), (5.01, s, 1H), (4.13, d, 5.6, 1H), (2.86, s, 3H), (2.85, s, 3H), (2.16–2.12, m, 1H), (1.86, t, 5.2, 1H), (1.44–1.40, m, 1H). HRMS calculated for C27H27N5O5 (M + H)+, 466.1628; found, 466.1621.

**Compound 20.** 1H NMR (CD3OD, 400 MHz) (8.52, s, 1H), (7.64, d, 4.0, 1H), (7.05, d, 4.0, 1H), (5.12, d, 6.4, 1H), (4.99, s, 1H), (4.10, d, 5.6, 1H), (2.88, s, 3H), (2.81, s, 3H), (2.16–2.12, m, 1H), (1.87, t, 5.2, 1H), (1.43–1.39, m, 1H). HRMS calculated for C25H22N5O5Cl (M + H)+, 444.0897; found, 444.0899.

**Compound 21.** 1H NMR (CD3OD, 400 MHz) (8.52, s, 1H), (7.36, d, 4.0, 1H), (7.05, d, 4.0, 1H), (5.12, d, 6.4, 1H), (4.99, s, 1H), (4.10, d, 5.6, 1H), (2.88, s, 3H), (2.16–2.14, m, 1H), (1.87, t, 5.2, 1H), (1.43–1.40, m, 1H). HRMS calculated for C27H27N5O5 (M + H)+, 466.1387; found, 466.1385.
(3aR,3bS,4aS,5R,5aS)-N,2-Trimethyl-5-(6-((Z)-2-(methylamino)vinyl)-2-(phenylethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxamide (32a). Methylamine solution (40%, 0.5 mL) was added to a solution of compound 31a (97 mg, 0.173 mmol) in methanol (6 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH₂Cl₂:MeOH = 40:1) to give the compound 32a (67.4 mg, 68%) as a yellowish syrup. NMR (CDCl₃, δ): 3.97 (3H, s, 9H), 1.54 (3H, s, 9H), 1.48 (3H, s, 9H), 1.29 (3H, s, 9H), 1.19 (3H, s, 9H), 0.98 (3H, t, 7.2 Hz, 3H), 0.84 (3H, t, 7.2 Hz, 3H), 0.79 (3H, s, 9H). HRMS calculated for C₂₄H₂₇N₃O₂ (M + H)+, 394.2340; found, 394.2340.

Journal of Medicinal Chemistry

1.32, s, 3H), (1.18, d, 7.2, 3H). HRMS calculated for C₁₁H₁₄N₂O (M + H)+, 167.1012; found, 167.1010.

(3aR,3bS,4aS,5R,5aS)-N,2-Trimethyl-5-(6-((Z)-2-(methylamino)vinyl)-2-(phenylethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxamide (32b). Compound 32b (71%) was prepared from compound 31b following the same method for compound 32a. δ: 3.97 (3H, s, 9H), 1.54 (3H, s, 9H), 1.48 (3H, s, 9H), 1.29 (3H, s, 9H), 1.19 (3H, s, 9H), 0.98 (3H, t, 7.2 Hz, 3H), 0.84 (3H, t, 7.2 Hz, 3H), 0.79 (3H, s, 9H). HRMS calculated for C₂₄H₂₇N₃O₂ (M + H)+, 394.2340; found, 394.2340.

(3aR,3bS,4aS,5R,5aS)-5-(6-((Z)-2-(2-Chlorophenyl)-2-(methylamino)vinyl)-2-(phenylethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxamide (32c). Compound 32c (70%) was prepared from compound 31c following the same method for compound 32a. δ: 3.97 (3H, s, 9H), 1.54 (3H, s, 9H), 1.48 (3H, s, 9H), 1.29 (3H, s, 9H), 1.19 (3H, s, 9H), 0.98 (3H, t, 7.2 Hz, 3H), 0.84 (3H, t, 7.2 Hz, 3H), 0.79 (3H, s, 9H). HRMS calculated for C₂₄H₂₇N₃O₂ (M + H)+, 394.2340; found, 394.2340.

Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-Chloro-6-(phenylethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxylate (34). Dicl (PPh₃)₂ (138.9 mg, 0.20 mmol), Cul (19 mg, 0.10 mmol), phenylacetylene (64 μL, 0.58 mmol) and triethylamine (0.13 mL, 0.80 mmol) was added to a solution of compound 33 (40.6 mg, 0.098 mmol) in anhydrous DMF (1.5 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound 34 (40.4 mg, 86%) as a colorless glassy syrup. δ: 7.86 (3H, t, 7.5 Hz, 3H), 7.80 (d, 7.5 Hz, 3H), 7.73 (d, 7.5 Hz, 3H), 7.72 (d, 7.5 Hz, 3H), 7.55 (s, 1H), 6.81 (s, 1H), 5.89 (s, 1H), 5.14 (s, 1H), 4.90 (s, 1H), 4.36 (7.2 Hz, 1H). HRMS calculated for C₂₅H₂₅N₃O₂ (M + H)+, 338.1844; found, 338.1844.

(3aR,3bS,5R,5aS)-5-(6-((Z)-2-(pyrazin-2-yl(ethyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxamide (35). Compound 35 (89%) was prepared from compound 34 following the same method for compound 35a. δ: 7.97 (3H, t, 7.5 Hz, 3H), 7.88 (d, 7.5 Hz, 3H), 7.82 (d, 7.5 Hz, 3H), 7.72 (d, 7.5 Hz, 3H), 7.36 (s, 1H), 6.95 (s, 1H), 5.93 (s, 1H), 5.14 (s, 1H). HRMS calculated for C₂₆H₂₈N₄O₂Cl (M + H)+, 494.2107; found, 494.2107.

Ethyl (3aR,3bS,5R,5aS)-5-(2-Iodo-6-methyl-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]dioxole-3(3aH)-carboxylate (36). DIAD (0.23 mL, 1.2 mmol) was added to a solution of triphenylphosphine (0.326 g, 1.242 mmol) and 2-iodo-6-methylpyrine (0.234 g, 0.9 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 35 (0.145 g, 0.6 mmol) in THF (2 mL) was added to the reaction mixture and stirred overnight at room temperature. Solvent was evaporated, and after the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound 36 (0.243 g, 84%) as a colorless foamy solid. δ: 7.97 (3H, t, 7.5 Hz, 3H), 7.88 (d, 7.5 Hz, 3H), 7.85 (d, 7.5 Hz, 3H), 7.82 (d, 7.5 Hz, 3H), 7.72 (d, 7.5 Hz, 3H), 7.36 (s, 1H), 6.95 (s, 1H), 5.93 (s, 1H). HRMS calculated for C₂₅H₂₅N₃O₂Cl (M + H)+, 494.2107; found, 494.2107.
h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH\_2\_Cl\_2:MeOH = 30:1) to give the compound 40 (22.4 mg, 64%) as a colorless syrup. \(^2\)H NMR (CD\_OD, 400 MHz) (8.56, s, 1H), (8.05, s, 1H), (7.80, d, 7.2 Hz, 1H), (4.96–4.94, m, 2H), (2.96, s, 3H), (2.77, s, 3H), (2.25–2.22, m, 2H), (1.55, s, 3H), (1.52–1.48, m, 1H), (1.42, t, 1.2 Hz, 3H), (1.31, s, 3H). HRMS calculated for C\(_{25}\)H\(_{23}\)N\(_4\)O\(_4\)F\(_2\) (M + H\(^+\)) = 481.1687; found, 481.1689.

(1.56, s, 3H), (1.30, s, 3H), (1.18, t, 6.8 Hz, 3H). HRMS calculated for C\(_35\)H\(_30\)N\(_5\)O\(_4\)F\(_2\) (M + H\(^+\)) = 541.1875; found, 541.1873.

\(\text{Methyl (3aR,3bS,4aS,5R,5aS)-5-(2-lodo-6-methoxy-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]-dioxole-3(3H)-carboxylic Acid} \) (49). PDC (567 mg, 1.5 mmol) was added to a solution of compound 48 (115 mg, 0.25 mmol) in dry DMP (2 mL) and the mixture heated at 80°C overnight. After completion of starting material, water (10 mL) was added into the reaction mixture and extracted with ethyl acetate (3 x 10 mL). Combined organic layer was washed with brine (15 mL), dried, filtered, and evaporated, and the residue was purified on flash silica gel column chromatography (CH\_2\_Cl\_2:MeOH = 20:1) to give the compound 49 (115 mg, 91%) as a solid. \(^3\)H NMR (CD\_OD, 400 MHz) (9.10, s, 1H), (8.60, s, 1H), (7.75–7.70, m, 1H), (7.61–7.58, m, 1H), (7.43–7.37, m, 1H), (5.87, d, 7.2 Hz, 1H), (5.16, s, 1H), (4.94, d, 6.4 Hz, 1H), (2.78, s, 3H), (2.26–2.22, m, 2H), (1.60–1.57, m, 1H), (1.49, t, 5.2 Hz, 1H), (1.32, s, 3H). HRMS calculated for C\(_{22}\)H\(_{21}\)N\(_5\)O\(_3\)I (M + H\(^+\)) = 528.0789; found, 528.0795.

(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methoxy-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]-dioxole-3(3H)-carboxamide (43). Methyl amine solution (40%, 0.5 mL) was added to a solution of compound 41c (21 mg, 0.044 mmol) in methanol (2 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound 41 (11 mg, 25%) as a colorless syrup. \(^3\)H NMR (CD\_OD, 400 MHz) (8.21, s, 1H), (5.85, d, 6.8 Hz, 1H), (5.04, s, 1H), (4.88, d, 6.8 Hz, 1H), (4.15, s, 3H), (3.85, s, 3H), (2.31–2.27, m, 1H), (1.68–1.65, m, 1H), (1.57–1.51, m, 4H), (1.29, s, 3H). HRMS calculated for C\(_{25}\)H\(_{23}\)N\(_4\)O\(_4\)F\(_2\) (M + H\(^+\)) = 463.2206; found, 463.2208.

(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-(methylamino)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]-dioxole-3(3H)-carboxamide (41b). Combined 41b (8%) was prepared from compound 39 following the same method for compound 38a. \(^4\)H NMR (CD\_OD, 400 MHz) (8.20, s, 1H), (5.85, d, 6.8 Hz, 1H), (5.04, s, 1H), (4.88, d, 6.8 Hz, 1H), (4.15, s, 3H), (3.85, s, 3H), (2.31–2.27, m, 1H), (1.68–1.65, m, 1H), (1.57–1.51, m, 4H), (1.29, s, 3H). HRMS calculated for C\(_{25}\)H\(_{23}\)N\(_4\)O\(_4\)F\(_2\) (M + H\(^+\)) = 481.1687; found, 481.1689.

(3aR,3bS,4aS,5R,5aS)-5-(2-(5-Chlorothiophen-2-yl)ethylthio)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopenta[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxylic Acid (44). Sodium methoxide (77.8 mg, 1.44 mmol) was added to a solution of compound 46 (202 mg, 0.28 mmol) in methanol (5 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound 47 (192 mg, 96%) as a colorless syrup. \(^3\)H NMR (CD\_OD, 400 MHz) (7.95, s, 1H), (5.72, d, 7.2 Hz, 1H), (4.93, s, 1H), (4.85, d, 6.8 Hz, 1H), (4.15, s, 3H), (3.85, d, 7.2 Hz, 1H), (2.45–4.14, m, 2H), (2.41–2.37, m, 1H), (1.76–1.73, m, 1H), (1.61, t, 5.6 Hz, 1H), (1.56, s, 3H), (1.30, s, 3H), (1.18, t, 6.8 Hz, 3H). HRMS calculated for C\(_{22}\)H\(_{20}\)N\(_5\)O\(_3\)Cl (M + H\(^+\)) = 458.1042; found, 458.1042.
compound 49 (46 mg, 0.09 mmol) and HATU (48.15 mg, 0.12 mmol) in dry DMF (1.5 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 = 60:1) to give the compound 50 (31 mg, 67%) as a colorless powder. 1H NMR (CD3OD, 400 MHz) (7.05, d 4.0, 1H), (5.82, d 6.4, 1H), (4.90, s, 1H), (4.87, d 6.8, 1H), (4.73, s, 1H), (2.73, s, 1H). HRMS calculated for C23H23N5O4SCl (M + H)+, 500.1159; found, 500.1150.

Institutes of Health guidelines on laboratory animal welfare and the International Association for the Study of Pain and the National Institutes of Health guidelines on laboratory animal welfare, and the residue was washed with saturated sodium bisulphate solution followed by brine, dried, filtered, and evaporated under vacuum, and the residue was purified on ash silica gel column chromatography (CH2Cl2:MeOH = 40:1) to give the compound 51 (24 mg, 77%) as a yellowish syrup. 1H NMR (CD3OD, 400 MHz) (8.37, s, 1H), (7.42, d 8.8, 2H), (6.91, d 8.8, 2H), (4.87, s, 2H), (3.78, s, 3H), (2.73, s, 3H). HRMS calculated for C14H14N4OI (M + H)+, 460.0846; found, 460.0852.

In the studies described herein, male Sprague Dawley rats (350–450 g) were used. All in vivo experiments were performed by methods described and in accordance with the animal care and use guidelines of the American Association for the Accreditation of Laboratory Animal Care. The authors declare no competing financial interest.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01998.

NMR analysis and mass spectra of selected synthesized compounds, results of PDSP screening, and supplementary chemical schemes (PDF)

Molecular formula strings (CSV)

Video related to Figure 3 (AVI)

3D coordinates of the modeled hA3AR complex with 15 (PDB)

3D coordinates of the modeled hA3AR complex with 21 (PDB)

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Notes

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ABBREVIATIONS USED

AR, adenosine receptor; cAMP, adenosine 3',5'-cyclic monophosphate; CCI, chronic constriction injury; CHO, Chinese hamster ovary; DIPPEA, disopropylethylamine; DMEM, Dulbecco’s Modified Eagle Medium; DMF, N,N-dimethylformamide; EL, extracellular loop; GPCR, G protein-coupled receptor; HATU, hexafluorophosphate; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; HEK, human embryonic kidney; HMBC, heteronuclear multiple bond correlation; HRMS, high resolution mass spectroscopy; IS$_{diss}$, per residue interaction score, electrostatic; IS$_{piv}$, per residue interaction score, van der Waals; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; PDC, pyridinium dichromate; DPSO, Psychopharmacology Drug Screening Program; PWT, paw withdrawal threshold; RMS, root-mean-square; SAR, structure–affinity relationship; TBAP, tetrabutylammonium dihydrogen phosphate; TB DPS, tert-butyldiphenylsilyl; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TM, transmembrane helix; tPSA, total polar surface area; MW, molecular weight

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