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Rigidified A_3 Adenosine Receptor Agonists: 1-Deazaadenine Modification Maintains High in Vivo Efficacy

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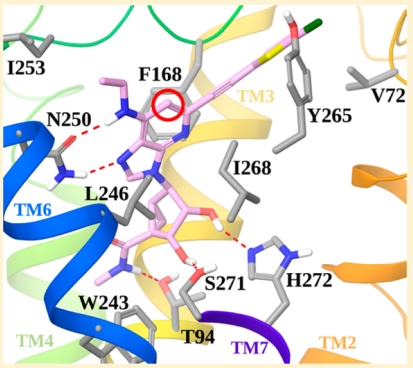
Supporting Information

ABSTRACT: Substitution of rigidified A_3 adenosine receptor (AR) agonists with a 2-((5-chlorothiophen-2-yl)ethynyl) or a 2-(4-(5-chlorothiophen-2-yl)-1*H*-1,2,3-triazol-1-yl) group provides prolonged protection in a model of chronic neuropathic pain. These agonists contain a bicyclo[3.1.0]hexane ((*N*)-methanocarba) ring system in place of ribose, which adopts a receptor-preferred conformation. N^6-Small alkyl derivatives were newly optimized for A_3AR affinity and the effects of a 1-deaza-adenine modification probed. 1-Deaza-N^6-ethyl alkyne **20** (MRS7144, K_i 1.7 nM) and 1-aza N^6-propyl alkyne **12** (MRS7154, K_i 1.1 nM) were highly efficacious in vivo. Thus, the presence of N1 is not required for nanomolar binding affinity or potent, long-lasting functional activity. Docking of 1-deaza compounds to a receptor homology model confirmed a similar binding mode as previously reported 1-aza derivatives. This is the first demonstration in nonribose adenosine analogues that the 1-deaza modification can maintain high A_3AR affinity, selectivity, and efficacy.

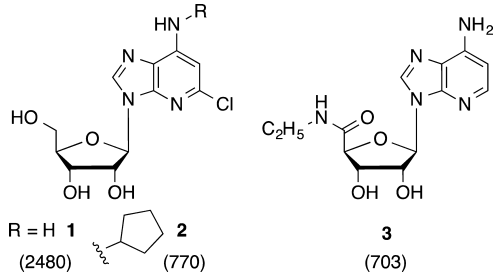


KEYWORDS: *G protein-coupled receptor, purines, chronic neuropathic pain, molecular modeling, adenosine receptor, crystallographic structure*

Agonists of the A_3 adenosine receptor (AR) have been shown to have anticancer and anti-inflammatory properties in vivo, and two nucleosides that are selective for this subtype have progressed to Phase III clinical trials for psoriasis and rheumatoid arthritis.[1−3] We recently developed a series of rigidified nucleoside full agonists that are thousands-fold selective for the A_3AR and display prolonged protection in models of chronic neuropathic pain.[4−6] The rigidity of these analogues, which seems to maintain a preferred conformation at the A_3AR, results from a bicyclic substitution of the ribose moiety, e.g., an appropriately positioned bicyclo[3.1.0]hexane ring system, termed North (*N*)-methanocarba. A combination with a rigid extension at the C2 position of the adenine in the form of a 2-(5-chlorothiophen-2-yl)ethynyl) or a 2-(4-(5-chlorothiophen-2-yl)-1*H*-1,2,3-triazol-1-yl) group further enhances A_3AR selectivity.[5,6] This modification suggested structural plasticity of the second transmembrane helix (TM2) of the A_3AR to accommodate the highly rigidified analogues.

Previous studies of modified ribonucleosides as AR ligands have demonstrated the importance of the adenine N3 and N7 positions; however, several 1-deaza-adenine analogues **1**−**3** of commonly used AR agonists were reported to display activity at various AR subtypes but with lower affinity (Chart 1).[7−9] Among these three analogues, the average loss of binding affinity at rat (r) ARs upon 1-deaza modification was: A_1, 12-fold; A_{2A}, 35-fold; A_3, 3.6-fold.[8,9] This suggested that the 1-deaza modification could be a means of enhancing A_3AR selectivity, possibly with a loss of affinity, but it had not been applied to nucleosides optimized for that AR subtype.

Chart 1. Previous Examples of 1-Deaza Nucleosides as AR Ligands, with Binding K_i Values (nM) at the rA_3AR



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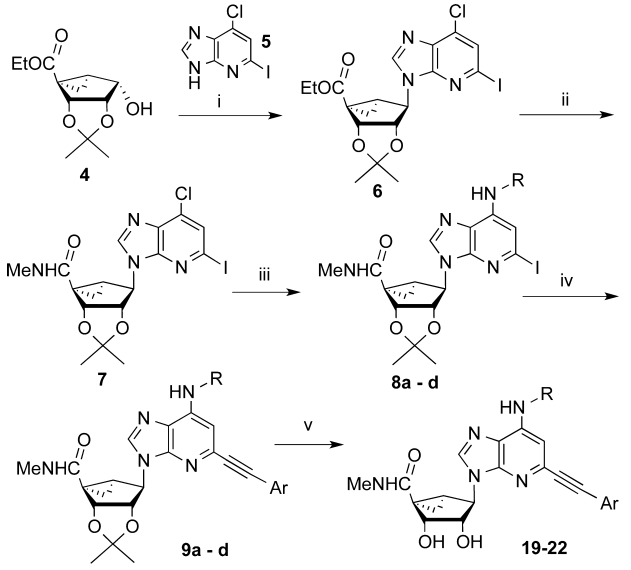
We first extended the structure−activity relationship (SAR) of the previous alkynyl and triazolyl series with analogues containing various N^6 groups synthesized by reported methods (Schemes S1 and S2, Supporting Information),[5,6] and then selected compounds were prepared in a 1-deaza form. The N^6 substituent was modified as small alkyl or cycloalkyl groups because the earlier studies emphasized N^6-methyl or N^6-(3-chlorobenzyl),[5,6] i.e., leaving a knowledge gap. The terminal aryl group was 5-chlorothiophen-2-yl, which was previously found to prolong the duration of the protective response in pain models.[5,6] The preparation of the 1-deaza-(*N*)-methanocarba derivatives containing an extended C2 alkynyl group is shown in Scheme 1.

Scheme 1. Synthesis of 1-Deaza-(*N*)-methanocarba-adenosine Alkynyl Analogues[a]



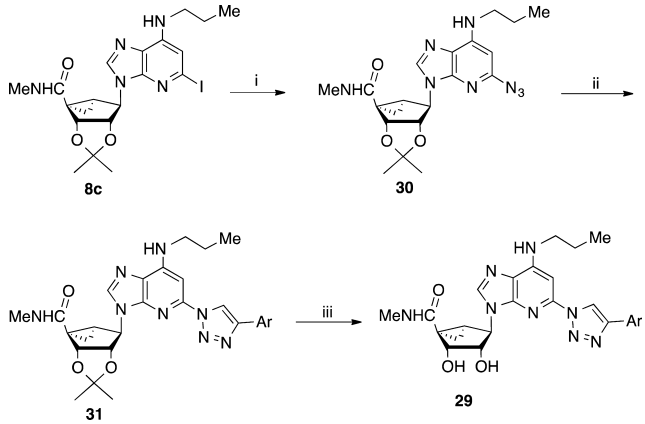
[a]Ar = 5-chlorothiophen-2-yl. N^6 groups of **8**, **9**: a, Me; b, Et; c, *n*-Pr; d, *c*-Pr. Reagents: (i) 1-deaza-6-chloro purine, Ph_3P, DIAD, THF, 51%; (ii) 40% $MeNH_2$, MeOH, rt, 72%; (iii) RNH_2, DIPEA, *i*-PrOH, 150 °C, microwave, 74-81%; (iv) Ar−C≡CH, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF, rt, 84-95%; (v) 10% TFA, MeOH, 70 °C, 86-91%.

A 2′,3′-isopropylidene [3.1.0]bicyclohexanol derivative **4**[5,15] was converted to nucleoside precursor **6** through a Mitsunobu reaction with 1-deaza-2-iodo-6-chloropurine **5** (Scheme S3, Supporting Information).[16,17] Ester aminolysis followed by nucleophilic substitution of the 6-chloro with an alkyl or cycloalkyl amine gave the N^6-substituted intermediates **8**. The C2 alkynyl group was installed by a Sonogashira reaction,[19] followed by isopropylidene deprotection in aqueous acid to provide 1-deaza nucleosides **19**−**22**. The 1-deaza C2-triazolyl derivative **29** was prepared by a modification of this route to include a click [3 + 2] cycloaddition step as shown in Scheme 2.

The nucleosides were assayed by radioligand binding at three human (h) AR subtypes by standard methods (Table 1).[5] Only two compounds, **12** and **20**, were evaluated and found to be inactive at the $hA_{2B}AR$; it was shown previously that (*N*)-methanocarba adenosine derivatives have greatly reduced $hA_{2B}AR$ affinity.[5] Reference alkyne **10** and triazoles **23**, **24**, **27**, and **28** were included for comparison.[5,6]

We first optimized affinity in the 1-aza series and then proceeded to introduce the 1-deaza modification. Except for **21**, the nucleoside alkyne derivatives only weakly inhibited binding (<50% at 10 μM) at the hA_1AR and $hA_{2A}AR$, and many bound at

Scheme 2. Synthesis of a 1-Deaza-(*N*)-methanocarba-2-triazolyladenosine Analogue 29[a]



[a]Ar = 5-chlorothiophen-2-yl. Reagents: (i) NaN_3, sodium ascorbate, $CuSO_4.5H_2O$, L-proline, Na_2CO_3, ^{t}BuOH-H_2O, 100 °C, microwave, 1.5 h, 92%; (ii) Ar−C≡CH, sodium ascorbate, $CuSO_4·5H_2O$, TBTA, ^{t}BuOH-H_2O, rt, 89%; (iii) 10%TFA-MeOH, 70 °C, 78%.

the hA_3AR with nearly nanomolar affinity. N^6-Cyclopropyl derivative **15** displayed close to 50% inhibition at the hA_1AR and $hA_{2A}AR$. In the 1-aza ethynyl series **10**−**18**, homologation to N^6-ethyl **11** diminished A_3AR affinity, but the N^6-propyl analogue **12** was more potent with a K_i value of 1.1 nM at hA_3AR. The effects on affinity of branching of the N^6 group at the α-carbon were mixed (cf. **15**, **16**), and a cycloalkyl group on the β-carbon (**17**, **18**) lowered the hA_3AR affinity. In the 1-deaza ethynyl series **19**−**22**, homologation to N^6-ethyl **20** increased A_3AR affinity to a K_i value of 1.7 nM, but the N^6-propyl analogue **21** was less selective and 23-fold less potent than **20**. Direct comparison of N^6-ethyl analogues **11** and **20** indicated slightly higher affinity with a 1-deaza modification. Compounds **19** and **20** were completely inactive in binding to the A_1AR and $A_{2A}AR$. In the 1-aza triazolyl series **23**−**28**, homologation beyond N^6-ethyl diminished A_3AR affinity and somewhat increased the micromolar affinity at A_1AR. Thus, the N^6-methyl triazole analogue **23** was favored in this in vitro A_3AR assay. Like the corresponding alkyne **21**, the 1-deaza N^6-propyl triazolyl analogue **29** displayed only moderate A_3AR affinity. Analogues **21** and **25**−**28** displayed significant micromolar A_1AR affinity; **26** displayed an A_3AR K_i value of 21 nM (only 33-fold A_3AR-selective). The A_3AR selectivities of the highest affinity nucleosides in the present study are much greater than 2000-fold.

Selected alkyne derivatives were assayed in binding to the mouse (m) and canine (c) A_3ARs in membranes of A_3AR-expressing HEK293 cells.[5] The affinities (nM; m, c; mean ± SEM, n = 3) were: **10**, (36 ± 5,[5] 8.5 ± 0.7); **11**, (27 ± 2, 1.5 ± 0.2); **12**, (6.8 ± 0.3, 5.8 ± 0.2); **19** (31 ± 2, 75 ± 7); **20** (16 ± 3, 49 ± 4). Thus, **12** was consistently potent in binding at h and mA_3ARs. Off-target activity at various receptors (Psychoactive Drug Screening Program,[20] Supporting Information) indicated only an occasional interaction in the micromolar range. Analogue **12** showed none, and **20** showed only one such interaction (K_i at $5HT_{2B}R$ = 2.5 μM).

The most potent 1-deaza compound **20** was shown to be a full agonist with EC_{50} of 1.5 ± 0.3 nM (cf. 38 ± 16 nM for **44**) in hA_3AR-induced inhibition of the production of cyclic AMP in membranes of hA_3AR-expressing CHO cells (Supporting Information).[5]

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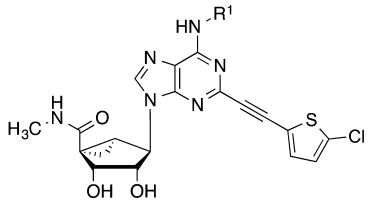
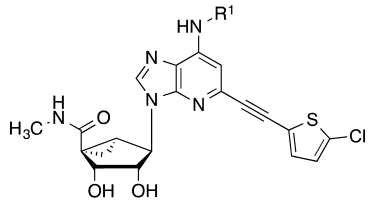
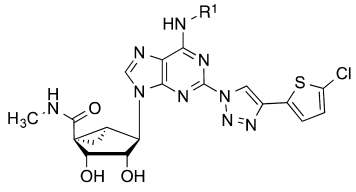
Table 1. Structures and Binding Affinities[a] of A_3AR Agonists, Including Reference Compounds 10, 23, 24, 27, and 28



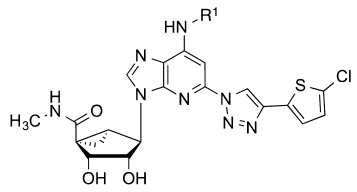
10 – 18



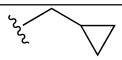
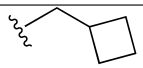
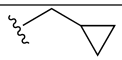
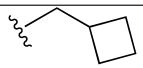
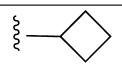
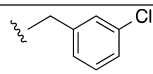
19 – 22



23 – 28



29

Compd.	R^1	A_1AR % inhibition or K_i (nM)[a]	A_{2A}AR % inhibition[a]	A_3AR % inhibition or K_i (nM)[a]
10[b]	Me	*6 ± 1%*	*24 ± 13%*	0.70 ± 0.11
11	Et	*28 ± 4%*	*12 ± 5%*	3.8 ± 1.5
12	*n*-Pr	*22 ± 5%*	*34 ± 3%*	1.1 ± 0.3
13	*n*-Bu	*6 ± 2%*	*0%*	2.9 ± 0.8
14	*i*-Pr	*26 ± 1%*	*17%*	2.9 ± 1.1
15	*c*-Pr	*47 ± 1%*	*44%*	1.0 ± 0.0
16	*c*-Bu	*39 ± 3%*	*34 ± 3%*	4.5 ± 2.0
17		*24 ± 4%*	*3 ± 3%*	7.9 ± 2.7
18		*34 ± 3%*	*5 ± 5%*	15 ± 5
19	Me	*15 ± 7%*	*8 ± 0%*	3.0 ± 0.8
20	Et	*10 ± 4%*	*0%*	1.7 ± 0.4
21	*n*-Pr	2440[d]	*11%*	38 ± 12
22	*c*-Pr	*26 ± 5%*	*11%*	10 ± 5
23[b]	Me	*27 ± 8%*	*34 ± 5%*	0.73 ± 0.10
24[b]	Et	*15 ± 11%*	*32 ± 5%*	1.2 ± 0.3
25		1350[d]	*44%*	9.4 ± 6.7
26[c]		710 ± 360	*42%*	21 ± 7
27[b]		*77%*	*43%*	7.1 ± 5.8
28[b]		*65%*	*32%*	10 ± 5
29	*n*-Pr	*44 ± 4%*	*4%*	110 ± 40

[a]Binding in membranes of CHO or HEK293 (A_{2A} only) cells stably expressing one of three hAR subtypes. The binding affinity for hA_1, A_{2A}, and A_3ARs was expressed as K_i values using agonists [^{3}H]N^6-*R*-phenylisopropyladenosine **41**, [^{3}H]2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamidoadenosine **42**, or [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide **43**, respectively. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using adenosine 5′-*N*-ethyluronamide **44** (10 μM). Values are expressed as the mean ± SEM (n = 3, unless noted). K_i values were calculated as reported.[14] Compounds **12** and **20** were shown to be inactive at the hA_{2B}AR (Supporting Information). [b]Data from Tosh et al.[5,6] [c]Low aqueous solubility observed for **26**. [d]N = 1.

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Potent in vivo efficacy of the A_3AR-selective nucleosides was demonstrated in a mouse chronic constriction injury (CCI) model of neuropathic pain,[10] as described in our previous studies.[5,6] The compounds were administered by oral gavage at the point of peak pain (day 7) to assess bioavailability and duration of action (Table 2). Unlike many of the compounds we reported earlier that lost most of the protection by 3 h post-administration, in this phenotypic screen most of the compounds examined maintained at least 80% of full protection at the 3 h time-point. Homologation of the N^6 group to ethyl in **11** in the alkynyl 1-aza series prolonged the duration of action in vivo with only a small increase in the ED_{50} value to 0.43 mg/kg (Figure S1, Supporting Information). By comparison, the reference agonist **10** displayed an ED_{50} value of 0.55 mg/kg.[4] For comparison with an established treatment of neuropathic pain, the ED_{50} of gabapentin (i.p.) at maximal reversal (1 h) in the CCI model was 140 μmol/kg,[18] i.e., much less potent than **10** and **11** (footnote b, Table 1). N^6-Propyl analogue **12** also displayed a long duration of action in vivo. Other small N^6 groups in **13**−**15** resulted in a reduction of peak protective effect (Figure 1). However, the N^6-cyclobutyl analogue **16** displayed 95% protection at 3 h. Comparable high efficacies were observed in the 1-deaza series, indicating that the lack of N1 does not detract from in vivo activity. The most potent 1-deaza analogue in A_3AR binding **20** achieved 93% and 84% reduction of chronic neuropathic pain at 1 and 3 h, respectively. The full time course for the in vivo action of 1-deaza N^6 ethyl analogue **20** indicated a very long duration of action of at least 5 h (Figure 1). The cLog P values were in a favorable range for drug-like molecules, e.g., 3.49 and 2.71 for N^6-ethyl analogues **20** and **11**, respectively (Supporting Information).[11]

The observation that nanomolar A_3AR affinity could be maintained in the 1-deaza series was explored structurally through molecular modeling using a hybrid homology model[5,6] of the receptor (methodological details have been previously reported).[12] Figure 2 shows the docking pose of compound **20** at the hA_3AR model.

Table 2. Activity of Orally Administered AR Agonists (3 μmol/kg) in CCI Model of Neuropathic Pain in the Mouse[a]

compd	max. effect E_{max} (% ± SEM)[a]	effect at 3 h (% ± SEM)
10[b]	93 ± 5	46 ± 9
11[b]	98 ± 2	87 ± 6
12	95 ± 2	93 ± 4
13	68 ± 11	24 ± 6
14	88 ± 6	39 ± 9
15	76 ± 1	36 ± 7
16	97 ± 3	95 ± 5
17[c]	94 ± 11	85 ± 18
18	98 ± 2	90 ± 2
19	91 ± 6	80 ± 8
20	93 ± 4	84 ± 7
27[d]	91 ± 6	85 ± 9
28[d]	95 ± 3	91 ± 5

[a]Effect shown for ipsilateral hind paw; there is no effect on the contralateral side. Time of peak protection (corresponding to E_{max}) was 1 h for each compound in Table 2. n = 4, unless noted. [b]ED_{50} values at peak effect were 0.34 mg/kg (0.73 μmol/kg; n = 5)[4] for **10** and 0.43 mg/kg (0.9 μmol/kg; n = 5) for **11** (Supporting Information). [c]n = 3. [d]Data from Tosh et al.[5,6]

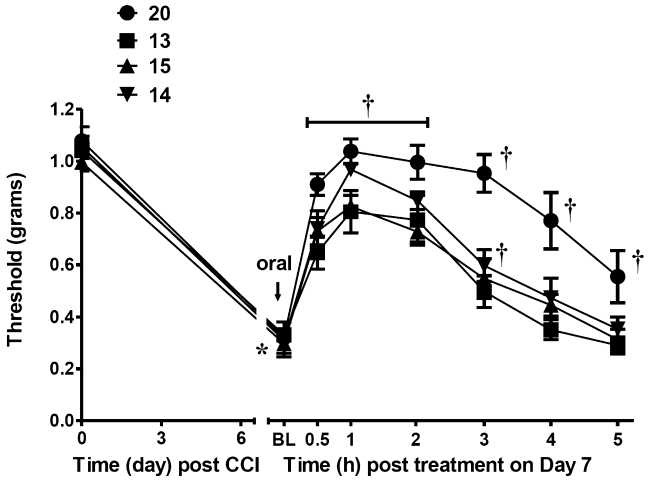

Figure 1. Time course of protection hind paw mechanoallodynia of the sciatic nerve in the CCI mouse model (p.o. administration on day 7, 3 μmol/kg, p.o.; n = 4). Data are the mean ± SEM analyzed by two-tailed, two-way ANOVA with Bonferroni comparisons: *P < 0.05 vs D0; †P < 0.05 vs D7. Additional plots are shown in the Supporting Information.

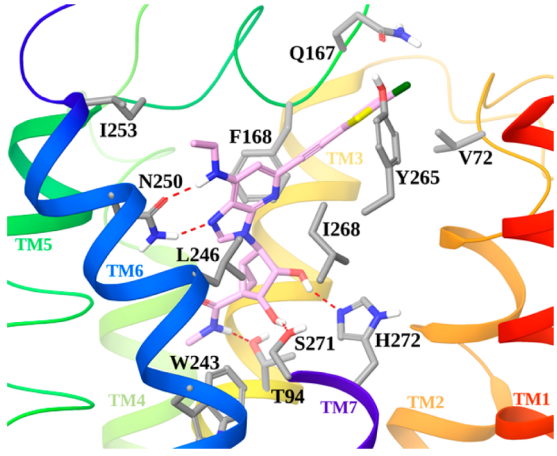

Figure 2. Docking pose of compound **20** (pink carbon sticks) at the hA_3AR model. Side chains of residues important for ligand recognition are shown in sticks (gray carbons), and H-bonding interactions are indicated by red, dashed lines. Nonpolar hydrogen atoms are not displayed. The view of TM7 is partially omitted.

As with the 1-aza analogues,[5,6] conserved H-bonds for adenosine derivatives were maintained between the 3′- and 2′-hydroxyl groups of docked **20** and Ser271 (7.42) and His272 (7.43), respectively. As expected, the 5′-*N*-methyluronamide of **20** formed a H-bond with the side chain of Thr94 (3.36). The necessity of having an N7 reflected its association as H-bond acceptor with Asn250 (6.55); the same residue accepts a hydrogen bond from the 6-amino group. As with the 1-aza analogues, the adenine ring formed a π−π stacking with Phe168 (EL2) and strong hydrophobic interactions with Leu246 (6.51) and Ile268 (7.39). The C2 group of **20** was accommodated in an exofacial interface region generated by the outward movement of TM2 in the hybrid A_3AR model, as previously proposed for derivatives bearing rigid and extended C2 substituents.[12,13] Thus, the major conserved recognition points for A_3AR agonists were preserved in the 1-deaza analogues, and as expected, the N1 of adenosine is not required for binding. Several different N^6 substitutions can be tolerated in this series, slightly modulating the affinity depending on their accommodation in a region

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delimited by TM6 and EL2 and exposed toward the extracellular environment. Consistent with our previous report,[6] there was a correspondence in A_3AR affinity between 1-aza alkynes and 1-aza triazoles. We have no structural explanation for the lack of correlation of A_3AR affinity between 1-aza and 1-deaza variants of the alkynes containing N^6-Pr or *c*-Pr substituents.

In conclusion, we have found that the 1-deaza modification may promote affinity and selectivity in (*N*)-methanocarba nucleosides that are optimized for activation of the A_3AR subtype, but not consistently in all cases. A_3AR docking suggests no major difference in the binding mode of the 1-aza and 1-deaza nucleosides. The preferred N^6 substituents, i.e., small alkyl groups, provided high A_3AR affinity and selectivity and maintained the in vivo efficacy. 1-Deaza N^6-ethyl **20** and 1-aza N^6-propyl **12** analogues were particularly potent in vitro and in vivo and displayed a long duration of action in reducing chronic neuropathic pain.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, physicochemical properties, mass spectra, NMR and mass spectra, HPLC, biological assay procedures and results, and off-target activity. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00150.

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Author Contributions

All authors contributed to this manuscript and have given approval to its final version.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

AR, adenosine receptor; CHO, Chinese hamster ovary; EL, extracellular loop; GPCR, G protein-coupled receptor; HEK, human embryonic kidney; TM, transmembrane helix

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