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Semi-Rational Design of (N)-Methanocarba Nucleosides as Dual Acting A_1 and A_3Adenosine Receptor Agonists: Novel Prototypes for Cardioprotection

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Abstract

Ring-constrained adenosine analogues have been designed to act as dualagonists at tissue-protective A_1 and A_3 adenosine receptors (ARs). 9-Ribosides transformed into the ring-constrained (N)-methanocarba-2-chloro-5′-uronamides consistently lost affinity at A_1/A_{2A}ARs and gained at A_3AR. Among 9-riboside derivatives, only N^6-cyclopentyl and 7-norbornyl moieties were extrapolated for mixed A_1/A_3 selectivity and rat/human A_3AR equipotency. Consequently, 2 was balanced in affinity and potency at A_1/A_3ARs as envisioned and dramatically protected in an intact heart model of global ischemia and reperfusion.

There are four subtypes of adenosine receptors (ARs): A_1, A_{2A}, A_{2B}, and A_3, and their selective agonists are under development as therapeutic agents.[1–3] Activation of one or more of the ARs and receptor overexpression have been shown to have a cytoprotective role in ischemic models.[3–8] Specifically, activation of either A_1 or A_3ARs in cardiac myocytes in several species has been shown to mimic the cardioprotective effect of ischemic preconditioning.[9–11] The co-activation of A_1 or A_3ARs in the cardiac myocyte has been shown to be protective to a greater degree than activation of either subtype alone. *In vivo* experiments have also demonstrated the cardioprotective effects of A_1 and A_3ARs in certain species.[12] Activation of A_1 and A_3ARs leads to activation of PLC and PLD (phospholipase C and D), respectively, in cultured cardiac myocytes.[9] PLC and PLD converge on activation of protein kinase C (PKC), which mediates cardioprotection.[9,13] In the brain, activation of A_1 or chronic activation of A_3ARs has been shown to protect neurons against ischemia in a variety of models.[14,15] In a model of global ischemia in gerbils, the A_1 agonists CPA (N^6-cyclopentyladenosine) and ADAC (N^6-[4-[[[4-[[[(2-aminoethyl)amino]carbonyl]methyl]anilin]carbonyl]-methyl] phenyl]-adenosine) and the chronically administered A_3 agonist IB-MECA (1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuran uronamide) were cytoprotective at very low doses.

The concurrent activation of A_1 and A_3ARs has been carried out either by co- administering selective agonists for each subtype, or using novel conjugates of functionalized congeners of A_1 and A_3 agonists.[16] Some agonists of balanced potency have been reported,[17–19] however they are often partial agonists and of selectivities limited to a particular species. The careful design and covalent joining of these functionalized congeners provide binary conjugates that

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are balanced in their ability to activate A_1 and A_3ARs. However, due to the high molecular weights (in excess of 1000) and the presence of multiple hydrogen bond donors, these molecules do not satisfy the criteria proposed by Lipinski for prediction of oral bioactivity[20] and are of limited application *in vivo*.

We have taken a new approach, *i.e.* based on differential effects on ARs of the ring-constrained (N)-methanocarba ring system,[21] to design dual acting A_1/A_3AR agonists of relatively low molecular weight. The new agonists (1 – 3, Scheme 1) were synthesized subsequent to careful SAR analysis of a large number of adenosine derivatives at ARs with respect to both binding affinity and relative efficacy.[22-24] We have incorporated specific molecular features that provide a balance in potency at A_1 and A_3ARs and that also maintain full efficacy at the A_3AR.[21,22] In the present study, several derivatives have been shown in binding and functional assays to be dual acting at the two receptor subtypes. Furthermore, a potent anti–ischemic cardioprotective effect in an intact mouse heart model of global ischemia and reperfusion injury was demonstrated.[6,13] This mammalian heart model has been shown to express both AR subtypes.

The selection of N^6-, C2, and 5′-uronamide substituents in the target compounds has been based largely on our recent findings relating to AR affinity, selectivity, and relative efficacy for specific adenine-9-riboside derivatives as well as a series of adenine- (N)-methanocarba-5′-alkyl uronamide derivatives.[21,22] At the A_3AR, in contrast to the A_1AR, relative efficacy is easily diminished by substitution of the N^6 and C2 positions while preserving affinity. This reduction in efficacy is readily overcome by a flexible 5′-methyluronamide moiety.[22] The desired analogues would be balanced in high affinity at both human and rat A_1 and A_3ARs and would display full agonism. According to published SAR findings (correlated in Figure 1) the substitution of adenine-9-ribosides to obtain the corresponding 2-chloro-(N)-methanocarba-5′-alkyl uronamides produced consistent effects on AR affinity. Upon undergoing these modifications for various hydrophobic N^6-substituents there was roughly one order of magnitude loss of binding affinity at the A_1AR and slightly less loss at the A_{2A}AR. The effect of this transformation on binding affinity at the A_3AR resulted in either equal or greater affinity (up to 14-fold), due to the conformational preference of the A_3AR binding site.[21] Thus, we examined a large published series of seventy-four adenosine derivatives,[23,24] mono-substituted at N^6, for the ideal candidates predicted to have balanced binding affinity when adapted to the 2-Cl-(N)-methanocarba series. The following criteria were sought: 1) equipotency at rat and human A_3ARs; 2) roughly two orders of magnitude greater affinity at A_1ARs in comparison to A_3ARs; and 3) selectivity for A_1 and A_3ARs in comparison to both A_{2A} and A_{2B}ARs. Few N^6-substituents satisfied all criteria; for example, although the affinities at the rat and human A_1ARs were generally similar,[24] at the A_3ARs the species difference was as high as 1100-fold.[23] The most likely candidates identified were N^6- cycloalkyl groups of the A_1-selective agonists 4 – 6.

The synthetic route to three adenosine agonists 1 – 3 that were designed for highaffinity at the A_1 and A_3 adenosine receptors and low affinity at the A_2 receptors is shown in Scheme 1. The synthesis of the target cyclopentyl derivative 2 and the 7-norbornyl derivative 3, was according to the general route presented for 5′-uronamido-(N)-methanocarba derivatives.[21] The synthetic approach of Joshi et al.[25] has been followed to incorporate the 5′-uronamido-(N)-methanocarba ring system. The requisite 7-norbornylamine was prepared in three steps from 7-norbornyl bromide using a Curtius rearrangement. Accordingly, the 2,6-dichloro 5′-ester 7[21] was treated first with a cycloalkylamine, which substituted selectively at the 6-position. Subsequent treatment with a large excess of methylamine converted the ester group to the corresponding amide. The final step was acidic deprotection of the isopropylidene protecting group at the 2′,3′-hydroxyl groups. Both 2 and 3 contain the 2-chloro and 5′-uronamido-(N)-methanocarba substituents. The 2-Cl group in 2 was hydrogenolyzed to give 1 in good yield.

Binding and functional assays were carried out at human A_1, A_{2A} and A_3ARsexpressed in CHO (Chinese hamster ovary) cells. The results confirmed that 1 and 2were highly selective for A_1 and A_3ARs, and that the affinities were nearly balanced. However, 3 was considerably more potent in binding to the A_3 than to the A_1AR. For all three derivatives, the K_i values at the human A_{2A}AR were at least several hundredfold greater than at the A_1 or the A_3AR. Compound 2 was equipotent in binding to human and rat A_1ARs with K_i values of 18.3 and 17.4 nM, respectively. Also, the affinity of compound 2 was similar at human and rat A_3ARs with K_i values of 3.7 and 5.8 nM, respectively.

In functional assays consisting of measuring inhibition of forskolin-stimulatedproduction of 3′,5′-cyclic-adenosine monophosphate (cAMP) in intact transfected CHO cells, single concentration determinations (Table 1) indicated that full A_3AR agonism was maintained in compounds 1 – 3. Concentration response curves indicated that compound 2 was a dual acting full agonist with nearly equivalent functional potencies at human A_1 (EC_{50} = 8.2 nM) and A_3 (EC_{50} = 2.8 nM) ARs.

Compounds 1 – 3 were assayed for activation of the human A_{2B}AR stably expressed in CHO cells.[18] Each adenosine derivative was tested at a concentration of 10 μM. As for the ribosides 4 – 6, the EC_{50} values at the human A_{2B}AR of the (N)- methanocarba-5′-uronamide N^6-substituted nucleosides 1 – 3 were all >10 μM. Compound 2 also showed negligible effect in stimulation of adenylate cyclase at the murine A_{2A} or A_{2B}ARs endogenously expressed in PC12 (rat) and NIH/3T3 cells (mouse), respectively.

Since 2 was the most potent and still nearly matched in binding affinity and in function at the two AR subtypes known to be cardioprotective, this compound was chosen for further pharmacological studies in an intact mouse heart model of ischemia and reperfusion.[6,13] In this model, compound 2 at 30 nM exerted a potent anti-ischemic cardioprotective effect (Table 2). The mixed agonist was perfused until the induction of ischemia. The recovery of left ventricular developed pressure (LVPD), +dP/dt, -dP/dt and heart rate (HR) all improved significantly following treatment with the mixed agonist 2. The infarct size determined using computer morphometry[26] after staining with triphenyltetrazolium chloride (TTC) was significantly reduced in the group treated with 2 (Figure 2). The percent necrosis in the group treated with 2 was 15 ± 7% compared to 23 ± 8% in the vehicle-treated controls, n = 6. In the same model, the classical A_1AR agonist 5 at a higher concentration (100 nM) could also reduce myocardial infarct size. The percent necrosis following infusion of 5 was 15 ± 10%, n=15.

Thus, we have designed novel cardioprotective agents based on mechanistic andstructural considerations. The adenosine N^6-substituents, cyclopentyl and 7-norbornyl,were selected based on predictions made from the binding affinities of the corresponding adenosine derivatives,[22] and from the consistent effects on AR affinity of replacing the 9-riboside moiety with a 5′-uronamido-(N)-methanocarba-pseudoribose moiety in combination with the 2-Cl substituent. The sum of these effects on affinity at each of the three AR subtypes was generalized to design new N^6-cycloalkyl analogues having desired pharmacological properties. The results of Tchilibon et al.,[21] for substituted N^6-benzyl and N^6-phenylethyl derivatives suggested that in each case, in comparison to the corresponding adenine-9-riboside, the affinity at the human A_1AR decreased by at least one order of magnitude while the affinity at the human A_3AR tended to increase by typically one order of magnitude. In the case of N^6-cyclopentyl- and N^6-(7-norbornyl)-adenine 9-ribosides, 4 and 6, respectively,[22] the affinity of each was similar at rat and human A_3ARs, but the effects of such N^6-cycloakyl substitution had not yet been probed in the 5′-uronamido-(N)-methanocarba series. The affinity of both 9-ribosides at the human A_{2A} and A_{2B}ARs was weak, thus the corresponding 5′-uronamido-(N)-methanocarba derivatives were expected to be highly selective for A_1 and A_3ARs in comparison to A_{2A} and A_{2B}ARs. We have confirmed the anticipated selectivity for the N^6-

cyclopentyl derivatives 1 and 2. Also, based on the 9-ribosides a large species difference at the A_3AR common among N^6-substituted adenosine derivatives[24] was predicted to be absent in the new analogues. This prediction was confirmed in binding assays of all three newly-synthesized derivatives.

We have examined the mixed A_1/A_3 agonist 2 in an intact mouse heart model of ischemia and reperfusion injury,[6,13] in which either an A_1- or A_3-selective agonist acts as a potent cardioprotective agent. The initial findings validate the model for studying AR-dependent protection and illustrate the highly cardioprotective effect of 2. The role of cardiac A_3ARs is complex, with protective effects demonstrated in models of preconditioning, delayed cardioprotection,[13] and ischemia-reperfusion.[6,8] The activation of the A_3AR in the rat coronary circulation has been proposed to mediate vasodilation.[27] Also, potential side effects of adenosine agonists, such as hypotension and sedation, must be considered.[1] Therefore, additional pharmacological examination of 2 and similar mixed agonists will be needed.

In conclusion, we have used a semi-rational approach based on SAR analysis to focus on a small number (3) of candidate structures predicted to display the desired pharmacodynamic properties. Thus, a series of (N)-methanocarba nucleosides previously characterized as selective A_3AR agonists has now been adapted to mixed AR selectivity desired for cytoprotection in a variety of tissue systems. Indeed, two of the three compounds synthesized reached this goal as judged by functional analyses and/or *in vitro* binding. Further chemical optimization of the structure to enhance the affinity and potency of mixed A_1/A_3 agonists is now feasible. One of the candidates was already shown to be highly cardioprotective in the mouse. These compounds may serve as prototypical examples for more detailed pharmacological studies leading to the development of novel dual acting cardioprotective AR agonists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 2001;53:527–552. [PubMed: 11734617]
2. Yao L, Burbiel JC, Maass A, Müller CE. Adenosine receptor agonists: from basic medicinal chemistry to clinical development. Expert Opin Emerging Drugs 2003;8:537–576.
3. Liu GS, Richards SC, Olsson RA, Mullane K, Walsh RS, Downey JM. Evidence that the adenosine A3 receptor may mediate the protection afforded by preconditioning in the isolated rabbit heart. Cardiovasc Res 1994;28:1057–1061. [PubMed: 7954592]
4. Liang BT, Jacobson KA. A physiological role of the adenosine receptor:A_3 sustained cardioprotection. Proc Natl Acad Sci USA 1998;95:6995–6999. [PubMed: 9618527]
5. Tracey WR, Magee WP, Oleynek JJ, Hill RJ, Smith AH, Flynn DM, Knight DR. Novel N^6-substituted adenosine 5′-N-methyluronamides with high selectivity for human adenosine A_3 receptors reduce ischemic myocardial injury. AmJ Physiol Heart Circ Physiol 2003;285:H2780. [PubMed: 12919933]
6. Peart J, Flood A, Linden J, Matherne GP, Headrick JP. Adenosine-mediated cardioprotection in ischemic-reperfused mouse heart. J Cardiovasc Pharmacol 2002;39:117–129. [PubMed: 11743234]

7. Maddock HL, Mocanu MM, Yellon DM. Adenosine A_3 receptor activation protects the myocardium from reperfusion/reoxygenation injury. Am J Physiol Heart Circ Physiol 2002;283:H1307–H1313. [PubMed: 12234780]
8. Cross HR, Murphy E, Black RG, Auchampach J, Steenbergen C. Overexpression of A_3 adenosine receptors decreases heart rate, preserves energetics, and protects ischemic hearts. Am J Physiol Heart Circ Physiol 2002;283:H1562–1568. [PubMed: 12234810]
9. Parsons M, Young L, Lee JE, Jacobson KA, Liang BT. Distinct cardioprotective effects of adenosine mediated by differential coupling of receptor subtypes to phospholipases C and D. FASEB J 2000;14:1423–1431. [PubMed: 10877835]
10. Shneyvays V, Leshem D, Zinman T, Mamedova LK, Jacobson KA, Shainberg A. Role of adenosine A_1 and A_3 receptors in regulation of cardiomyocyte homeostasis after mitochondrial respiratory chain injury. Am J Physiol Heart Circ Physiol 2005;288:H2792–H2801. [PubMed: 15681707]
11. Carr CS, Hill RJ, Masamune H, Kennedy SP, Knight DR, Tracey WR, Yellon DM. Evidence for a role for both the adenosine A_1 and A_3 receptors in protection of isolated human atrial muscle against simulated ischaemia. Cardiovasc Res 1997;36:52– 59. [PubMed: 9415272]
12. Kodani E, Bolli R, Tang XL, Auchampach JA. Protection of IB-MECA against myocardial stunning in conscious rabbits is not mediated by the A_1 adenosine receptor. Basic Res Cardiol 2001;96:487–96. [PubMed: 11605996]
13. Zhao TC, Kukreja RC. Protein kinase C-delta mediates adenosine A_3 receptor-induced delayed cardioprotection in mouse. Am J Physiol Heart Circ Physiol 2003;285:H434–441. [PubMed: 12793983]
14. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int Rev Neurobiol 2005;63:191–270. [PubMed: 15797469]
15. von Lubitz DKJE, Lin RC, Bischofberger N, Beenhakker M, Boyd M, Lipartowska R, Jacobson KA. Protection against ischemic damage by adenosine amine congener, a potent and selective adenosine A_1 receptor agonist. Eur J Pharmacol 1999;369:313–317. [PubMed: 10225368]
16. Jacobson KA, Xie R, Young L, Chang L, Liang BT. A novel pharmacological approach to treating cardiac ischemia: binary conjugates of A_1 and A_3 adenosine receptor agonists. J Biol Chem 2000;275:30272–30279. [PubMed: 10887176]
17. Baraldi PG, Cacciari B, Pineda de las Infantas MJ, Romagnoli R, Spalluto G, Volpini R, Costanzi S, Vittori S, Cristalli G, Melman N, Park KS, Ji X-d, Jacobson KA. Synthesis and biological activity of a new series of N^6-arylcarbamoyl-, 2-(ar)alkynyl-N^6-arylcarbamoyl, and N^6-carboxamido-derivatives of adenosine-5′-*N*-ethyluronamide (NECA) as A_1 and A_3 adenosine receptor agonists. J Med Chem 1998;41:3174–3185. [PubMed: 9703463]
18. van Tilburg EW, van der Klein PAM, von Frijtag Drabbe Künzel J, de Groote M, Stannek C, Lorenzen A, IJzerman AP. 2,5′-Disubstituted adenosine derivatives: evaluation of selectivity and efficacy for the adenosine A_1,A_{2A}, and A_3 receptor. J Med Chem 2002;45:420–429. [PubMed: 11784146]
19. Cappellacci L, Franchetti P, Pasqualini M, Petrelli R, Vita P, Lavecchia A, Novellino E, Costa B, Martini C, Klotz KN, Grifantini M. Synthesis, biological evaluation, and molecular modeling of ribose-modified adenosine analogues as adenosine receptor agonists. J Med Chem 2005;48:1550–1562. [PubMed: 15743197]
20. Lipinski C, Hopkins A. Navigating chemical space for biology and medicine. Nature 2004;432:855–861. [PubMed: 15602551]
21. Tchilibon S, Joshi BV, Kim SK, Duong HT, Gao ZG, Jacobson KA. (N)- Methanocarba 2,N^6-disubstituted adenine nucleosides as highly potent and selective A_3 adenosine receptor agonists. J Med Chem 2005;48:1745–1758. [PubMed: 15771421]
22. Gao ZG, Kim SK, Biadatti T, Chen W, Lee K, Barak D, Kim SG, Johnson CR, Jacobson KA. Structural determinants of A_3 adenosine receptor activation: Nucleoside ligands at the agonist/antagonist boundary. J Med Chem 2002;45:4471–4484. [PubMed: 12238926]
23. Gao ZG, Blaustein J, Gross AS, Melman N, Jacobson KA. N^6-Substituted adenosine derivatives: Selectivity, efficacy, and species differences at A_3 adenosine receptors. Biochem Pharmacol 2003;65:1675–1684. [PubMed: 12754103]
24. Tchilibon S, Kim SK, Gao ZG, Harris BA, Blaustein J, Gross AS, Melman N, Jacobson KA. Exploring distal regions of the A_3 adenosine receptor binding site: Sterically-constrained N^6-(2-phenylethyl)

adenosine derivatives as potent ligands. Bioorg Med Chem 2004;12:2021–2034. [PubMed: 15080906]

25. Joshi BV, Moon HR, Fettinger JC, Marquez VE, Jacobson KA. A new synthetic route to (N)-methanocarba nucleosides designed as A_3 adenosine receptor agonists. J Org Chem 2005;70:439–447. [PubMed: 15651784]
26. Ichinose F, Bloch KD, Wu JC, Hataishi R, Aretz HT, Picard MH, Scherrer-Crosbie M. Pressure overload-induced LV hypertrophy and dysfunction in mice are exacerbated by congenital NOS3 deficiency. Am J Physiol Heart Circ Physiology 2004;286:H1070–H1075.
27. Hinschen AK, Rose′Meyer RB, Headrick JP. Adenosine receptor subtypes mediating coronary vasodilation in rat hearts. J Cardiovasc Pharmacol 2003;41:73–80. [PubMed: 12500024]
28. Klotz KN, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, Lohse MJ. Comparative pharmacology of human adenosine receptor subtypes –characterization of stably transfected receptors in CHO cells. Naunyn-Schmiedeberg′s Arch Pharmacol 1998;357:1–9.
29. van Galen PJM, van Bergen AH, Gallo-Rodriguez C, Melman N, Olah ME, IJzerman AP, Stiles GL, Jacobson KA. A binding site model and structure-activity relationships for the rat A_3 adenosine receptor. Mol Pharmacol 1994;45:1101–1111. [PubMed: 8022403]

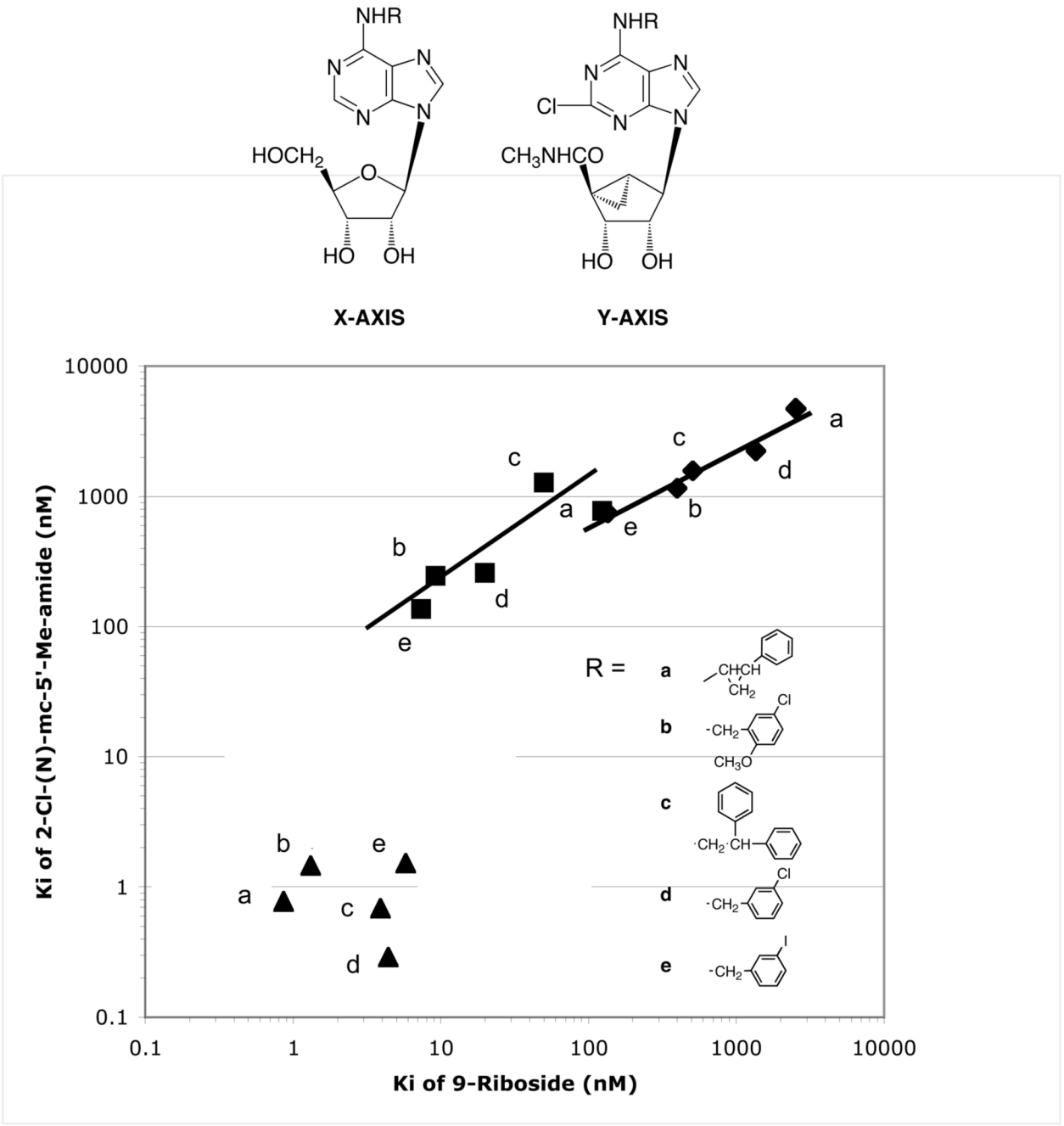


Figure 1.

Correlation of K_i values at human A_1 (■), A_{2A} (◆), and A_3 (▲) receptors of adenosine derivatives in two structural series were compared. The two series compared are: mono-substituted adenosine (9-riboside) derivatives, and tri-subsituted 2-chloro-(N)-methanocarba derivatives. In each case, pairs of compounds in which both members have the same N^6-sustitution (as indicated) are correlated. The five (N)-methanocarba analogues depicted in this graph contained N^6-benzyl and phenylethyl-type groups, however the general effects on affinity at each of the three adenosine receptor subtypes were generalized to design new N^6-cycloalkyl analogues having desired pharmacological properties. For the N^6 substitutions shown, there was consistent loss of affinity at A_1 and A_{2A}ARs and the effect at the A_3AR

ranged from no change to a 14-fold gain of affinity. The (N)-methanocarba compounds shown here were all selective for the A_3 receptor, while the desired property in the new analogues was balanced affinity at A_1 and A_3 receptors.

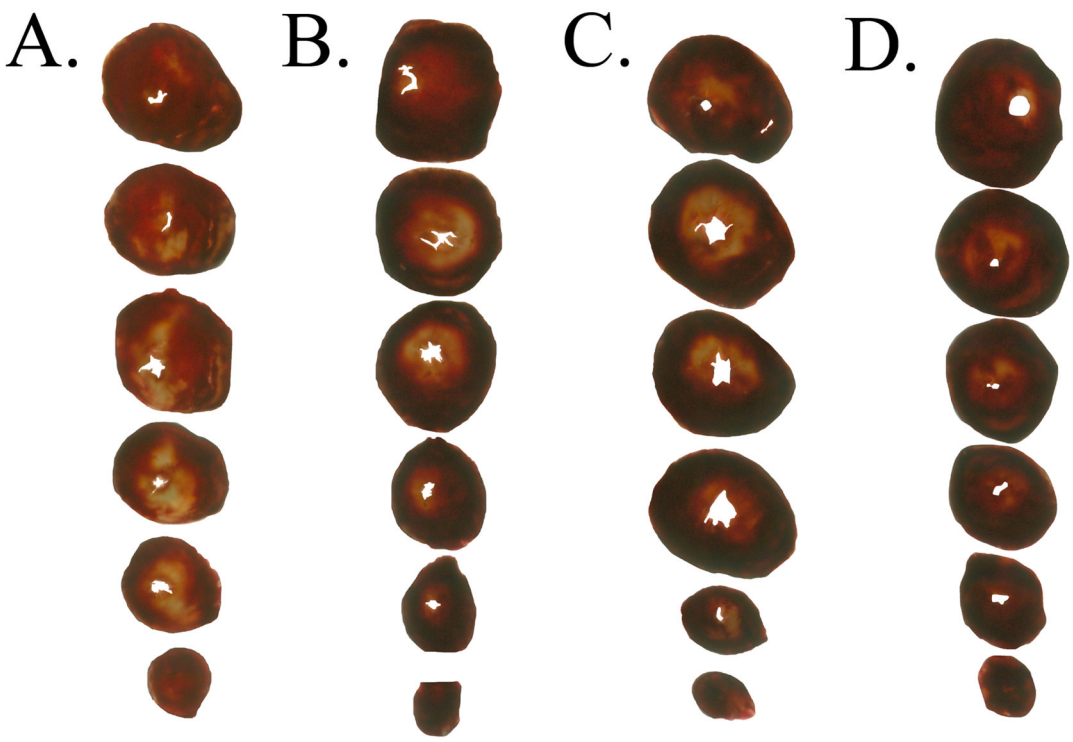
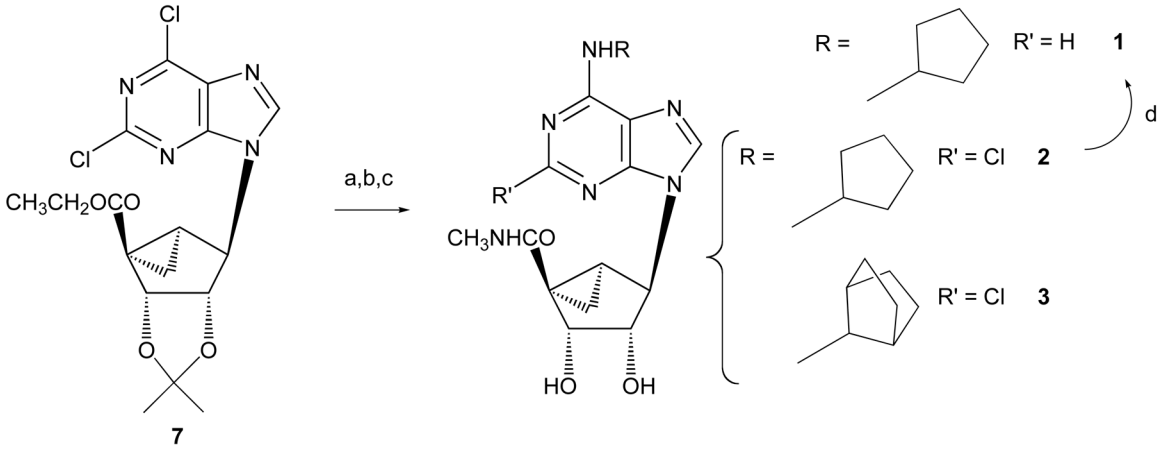


Figure 2.

Anti-ischemic infarct-reducing effects of adenosine receptor agonists. Murine hearts were excised and subjected to normothermic global ischemia and reperfusion with or without (A) adenosine receptor agonists as described in Methods. The adenosine receptor agonists were: (B) A_3 agonist Cl-IB-MECA, 30 nM; (C) A_1 agonist **5**, 100 nM; (D) Mixed A_1/A_3 agonist **2**, 30 nM. Agonists were infused for five min till the induction of ischemia. The heart was stained with TTC after 35 min of ischemia and 120 min of reperfusion and the infarcted areas were visualized as TTC-negative (pale, white). The infarct was quantified by morphometry and normalized to the whole heart as % necrosis. Data were representative of 6 (adenosine receptor agonist-treated) and 16 (DMSO/vehicle-treated) mice. A vehicle control not subjected to ischemia showed no pale or TTC-negative area.

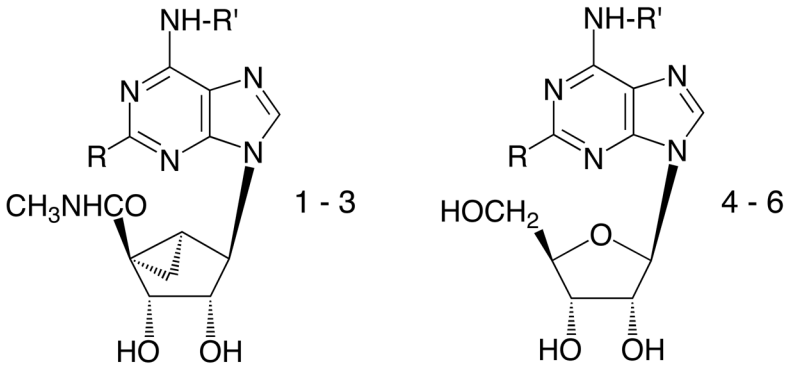


Scheme 1.

Reagents: a) RNH_2, MeOH, triethylamine; b) CH_3NH_2, MeOH; c) TFA, H_2O, MeOH, 70°C; d) 10% Pd/C/ H_2, MeOH.

Table 1

Potency of adenosine derivatives at human A_1, A_{2A}, and A_3ARs and the rat A_3AR [a] and maximal agonist effects at human A_3ARs expressed in CHO cells.[a]



No.	N^6-R′	C2-R	K_i (hA_1AR) nM[a]	K_i ($hA_{2A}AR$) nM[a]	K_i (hA_3AR) nM[a]	% Activation (hA_3AR)[b] at 10 μM	K_i (rA_3AR)
1[c]	CP	H	34.1 ± 6.1	6420 ± 630	13.1 ± 5.1	93 ± 7	10.2 ± 2.1
2[c]	CP	Cl	18.3 ± 6.3[g]	3250 ± 300	3.7 ± 0.9	101 ± 10	5.8 ± 1.6
3[c]	NB	Cl	190 ± 37	>10,000	14.6 ± 3.2	92 ± 6	9.6 ± 2.7
4[d]	CP	H	0.45±0.04[b]	462	240±36	72±12	97±4
5[d]	CP	Cl	0.83[e]	2270[e]	38 ± 6	0	237±71[f]
6[d]	NB	H	0.48±0.01	>10,000	229±76	112±25	103±1

[a] All AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human or rat ARs. Percent activation of the human A3AR was determined at 10 μM. Binding at human A_1 and A_{2A}ARs in this study was carried out as described in Methods using [^{3}H]R-PIA or [^{3}H]CGS 21680 as a radioligand. Values from the present study are expressed as mean ± s.e.m., n = 3–5.

[b] Percent activity at 10 μM, relative to 10 μM Cl-IB-MECA (A_3).

[c] **1,** MRS3706; **2**, MRS3630; **3,** MRS3638.

[d] Data from Gao et al., unless noted.[22,23]

[e] Data from Klotz et al.[28]

[f] Data from van Galen et al.[29]

[g] K_i at rat A_1 AR is 17.4 ± 2.7 nM.

ND not determined. CP, cyclopentyl; NB, 7-norbornyl.

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Table 2

Recovery of left ventricular function in a mouse heart model of ischemia/reperfusion.[a]

Parameter	Vehicle[b]	Compound 5[c]	Significance
LVDP[d]	8.5 ± 5.3	26.0 ± 4.8	t=7.1, P < 0.0001
+dP/dt[d]	6.3 ± 3.9	21.1 ± 4.9	t=7.4, P < 0.0001
-dP/dt[d]	8.2 ± 3.7	24.6 ± 7	t=7.22, P < 0.0001
HR[d]	37.1 ± 32.6	93.8 ± 19.3	t=4.1, P < 0.0005
% necrosis	23.4 ± 7.8	15.0 ± 6.9	t=2.32, P[e]=0.029

[a] Values were obtained after 35 global ischemia followed by reperfusion.

[b] DMSO, n = 16.

[c] A concentration of 30 nM 2 (initially dissolved in DMSO) was used, n = 6. During buffer perfusion of heart via the side port, the spontaneous heart rate dropped by 24 ± 8.2% (SEM) due to a decrease in the perfusion pressure. Perfusion of buffer containing 30 nM 2 was associated with a larger decrease in the heart rate of 57 ± 6.7%, likely because of a negative inotropic effect of 2.

[d] % of baseline prior to ischemia/reperfusion.

[e] Two-tailed.