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Dual acting antioxidant A_1 adenosine receptor agonists

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Abstract—Herein we report the synthesis and biological evaluation of some potent and selective A_1 adenosine receptor agonists, which incorporate a functionalised linker attached to an antioxidant moiety. N^6-(2,2,5,5-Tetramethylpyrrolidin-1-yloxyl-3-ylmethyl)-adenosine (VCP28, **2e**) proved to be an agonist with high affinity (K_i = 50 nM) and good selectivity ($A_3/A_1 \geqslant 400$) for the A_1 adenosine receptor. N^6-[4-[2-[1,1,3,3-Tetramethylisoindolin-2-yloxyl-5-amido]ethyl]phenyl]adenosine (VCP102, **5a**) has higher binding affinity (K_i = 7 nM), but lower selectivity ($A_3/A_1 = \sim 3$). All compounds bind weakly ($K_i > 1$ μM) to A_{2A} and A_{2B} receptors. The combination of A_1 agonist activity and antioxidant activity has the potential to produce cardioprotective effects.
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The cardioprotective effects of adenosine are well established and are the basis for a variety of therapeutic strategies. Adenosine is a key player in the phenomenon of 'preconditioning', a large number of studies in the early 1990s reported a process in which a prior instance of ischaemia protects the heart from future ischaemic events, and that adenosine re-uptake inhibitors could increase the level of protection.[1–3] More recently, the focus of attention has moved to the protectant effects of adenosine from reperfusion injury when adenosine is released (or administered) during and/or after a period of ischaemia.[4] The 'Acute Myocardial Infarction STudy of ADenosine (AMISTAD)' examined the effects of adenosine (administered to patients with thrombolytic therapy) within a few hours of myocardial infarction and reported a reduction in infarct size.[5,6] Cardioprotection has been shown in animal models to occur after activation of any of the four adenosine receptor subtypes, A_1, A_{2A}, A_{2B}, and A_3 receptors.[7–11] The greatest body of work has been performed on the A_1 receptor subtype, and a variety of mechanisms have been elucidated. Activation of the A_1 adenosine receptor results in inhibition of adenylyl cyclase and consequent decreases in cAMP as well as signal transduction events mediated by the release of βγ G protein subunits. The protective effects of A_1 adenosine receptor activation occur via several mechanisms, including activation of the protein kinase C/ERK pathway and opening of mitochondrial K^+ ATP channels.[12,13]

Antioxidants produce cardioprotection by several mechanisms including direct scavenging of the superoxide anion, the lynchpin radical in ischaemia-reperfusion injury. In addition, modulation of serum complement activity, as well as the reduction in the levels of C-reactive protein (CRP) and the membrane attack complex (MAC) in infarcted tissue, suggests a significant antiinflammatory component in the mechanism of antioxidants.[14] Exercise protects the heart in part by increasing the expression of antioxidant enzymes.[15] In addition, isomers of vitamin E have cardioprotective effects that appear to be due to inhibition of c-Src activation and proteasome stabilization.[16] Furthermore, some drugs currently in clinical use, such as anti-hypertensive agents including angio-

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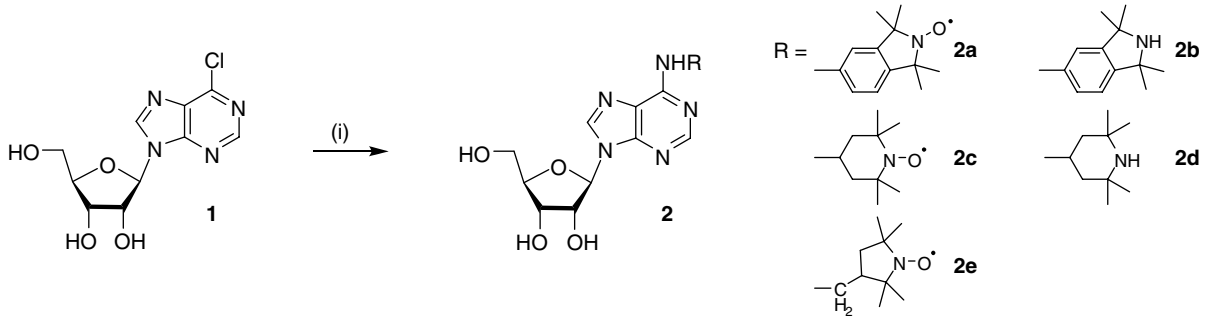
tensin-converting enzyme inhibitors and angiotensin receptor blockers and anti-hyperlipidemic reagents like statins, may also protect the heart via antioxidant action in addition to their primary pharmacological effect.[17,18]

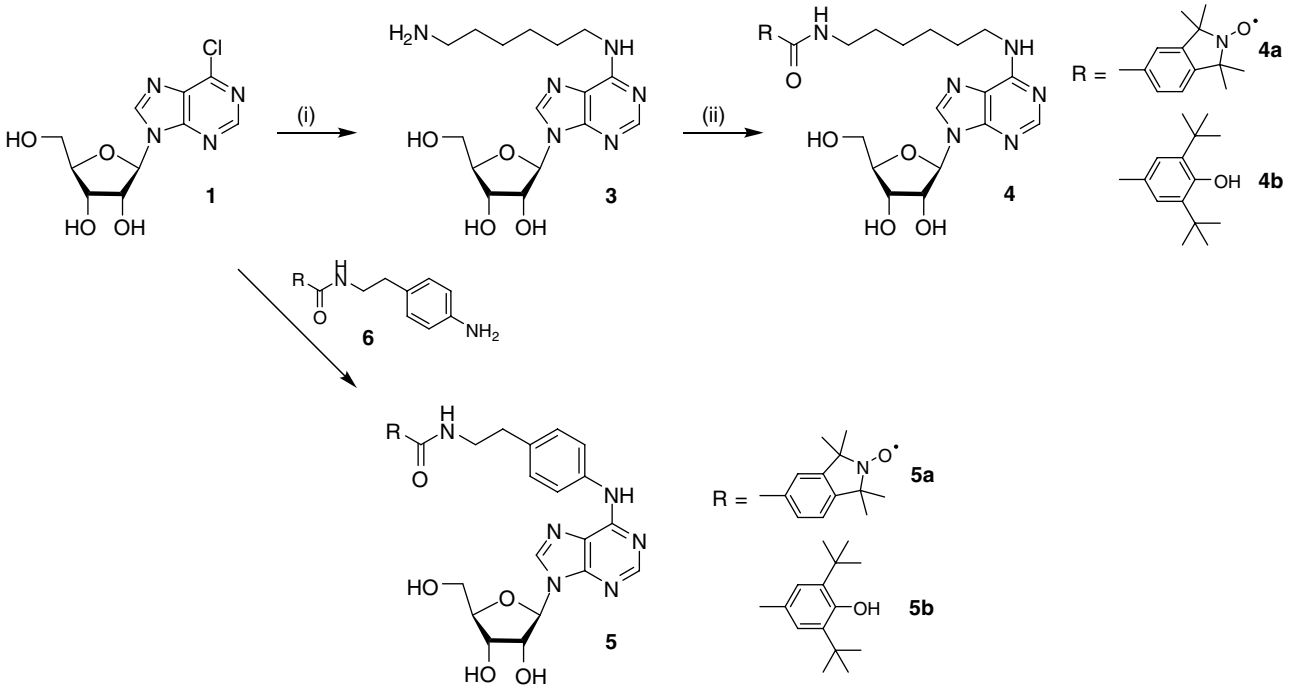
We now report the design, synthesis and preliminary pharmacological evaluation of dual acting antioxidant A_1 adenosine receptor agonists. More specifically, known antioxidant moieties have been incorporated in the design of new A_1AR agonists with the view to enhancing receptor affinity and selectivity in addition to providing antioxidant activity.

Adenosine analogues with alkyl, cycloalkyl, bicycloalkyl, and aryl groups in the N^6-position are generally known to be potent and selective A_1AR agonists (in the absence of additional modifications).[19] In a number of cases, the presence of a heteroatom in the N^6-cycle further improved A_1AR activity. In our first series of target compounds a group with known antioxidant activity was incorporated directly in the N^6-position. These compounds were prepared via the reaction of 6-chloropurine riboside (**1**) with the appropriate amine in the presence of *N*,*N*-diisopropylethylamine (DIPEA). These reactions provided the desired target compounds **2a**–**e** in good to excellent yield, ranging from 71 to 89% (Scheme 1).

A second series of target compounds was prepared in which the antioxidant moiety was attached to the N^6-position via an alkyl linker. This initially involved the preparation of N^6-(6-aminohexyl)adenosine (**3**), followed by an EDCI mediated coupling to connect the antioxidant group (compounds **4a** and **4b**, Scheme 2). Alkyl aryl linkers were also targeted as the interaction of a phenyl group with the N^6-binding domain is known to enhance A_1AR affinity and selectivity.[19] In this approach the antioxidant moiety was first coupled to 4-(2-aminoethyl)aniline to afford the corresponding carboxamide of type **6**. The reaction of **6** with 6-chloropurine riboside afforded the desired N^6-substituted adenosines (**5a,b**) in good yield (Scheme 2).



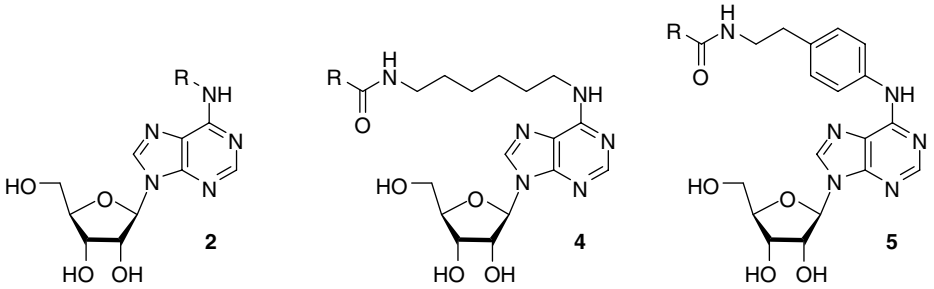
Scheme 1. Reagent and condition: (i) R-NH_2, N(*i*-Pr)$_2$Et, *t*-BuOH, 83 °C.

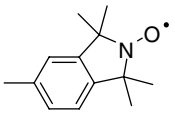
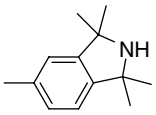
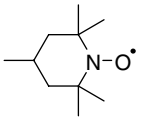
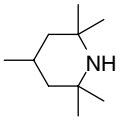
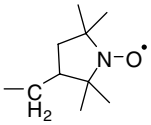
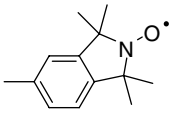
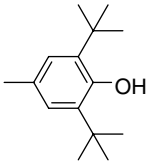
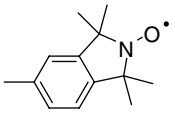
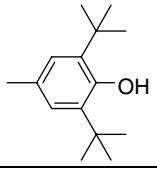


Scheme 2. Reagents and conditions: (i) $H_2N(CH_2)_6NH_2$, Et_3N, EtOH, 78 °C; (ii) R-COOH, EDCI, HOBt, N(*i*-Pr)$_2$Et, DMF, 25 °C.

Receptor binding assays: The receptor binding affinity of the initial series of compounds bearing a substituent known to confer antioxidant activity in the N^6-position is described in Table 1. All of the N^6-nitroxide compounds (**2a**, **2c**, and **2e**) had sub-micromolar K_i values at the A_1AR and good selectivity versus $A_{2A}AR$ and

Table 1. Receptor affinity of the target compounds



No.	R	A_1 K_i (μM)	$A_{2A}K_i$ (μM)	$A_{2B}K_i$ (μM)	A_3 K_i (μM)
2a		0.10 ± 0.03	>10	>10	0.79 ± 0.56
2b		0.29 ± 0.03	>10	>10	0.28 ± 0.17
2c		0.05 ± 0.01	>10	>10	8.56 ± 3.20
2d		1.58 ± 0.45	>10	>10	25.0 ± 6.40
2e		0.05 ± 0.01	>10	>10	21.2 ± 8.30
4a		0.015 ± 0.0003	>10	>10	0.089 ± 0.031
4b		0.040 ± 0.0003	>10	>10	0.014 ± 0.048
5a		0.007 ± 0.001	>10	1.45 ± 0.08	0.023 ± 0.01
5b		0.032 ± 0.006	>10	8.58 ± 0.90	0.084 ± 0.001

the A_{2B}AR. Affinity for the A_3AR ranged from very modest in the case of compound **2a** to high for compound **2e**. Interactions between a nitroxide moiety and the N^6-binding domain of adenosine receptors have not been explored to date. In order to probe the effects of this group on A_1AR affinity and selectivity, analogues in which the nitroxide was replaced by the corresponding secondary amine were prepared and evaluated (Table 1, compounds **2b** and **2d**). A comparison of **2a** with **2b** and **2c** with **2d** suggests that the presence of a stable free radical in these positions is not deleterious for interaction with the A_1 adenosine receptor.

Although it is well known that the N^6-binding domain can accommodate a wide range of aryl and cycloalkyl groups, the incorporation of very bulky tetramethylisoindolin-2-yloxyl and tetramethylpiperidin-1-yloxyl groups in this position afforded agonists with modest affinity for the A_1AR. In order to improve receptor affinity, the antioxidant moiety was attached via an alkylamino linker. Linkers of this type have previously proven to be effective in connecting A_1 adenosine receptor agonists with fluorescent markers and also for the preparation of bivalent and bifunctional targets.[20–22]

A functionalised linker was employed in order to achieve further improvements in A_1AR affinity. This linker incorporated an N^6-phenyl group to allow interaction with the hydrophobic region of the N^6-binding domain, attached to 4-alkyl amino functionality which provided a handle for the attachment of the antioxidant group.

Cardiomyocyte model of ischaemia: Cultured H9C2 embryonic rat atrial cardiomyocytes were exposed to conditions used previously to mimic in vivo ischaemia. This type of ischaemic model produces necrotic and apoptotic cell death,[20] which results in membrane dysfunction and allows entry of propidium iodide.[23] Under simulated ischaemia conditions, approximately 40% of all cells stained positively for propidium iodide

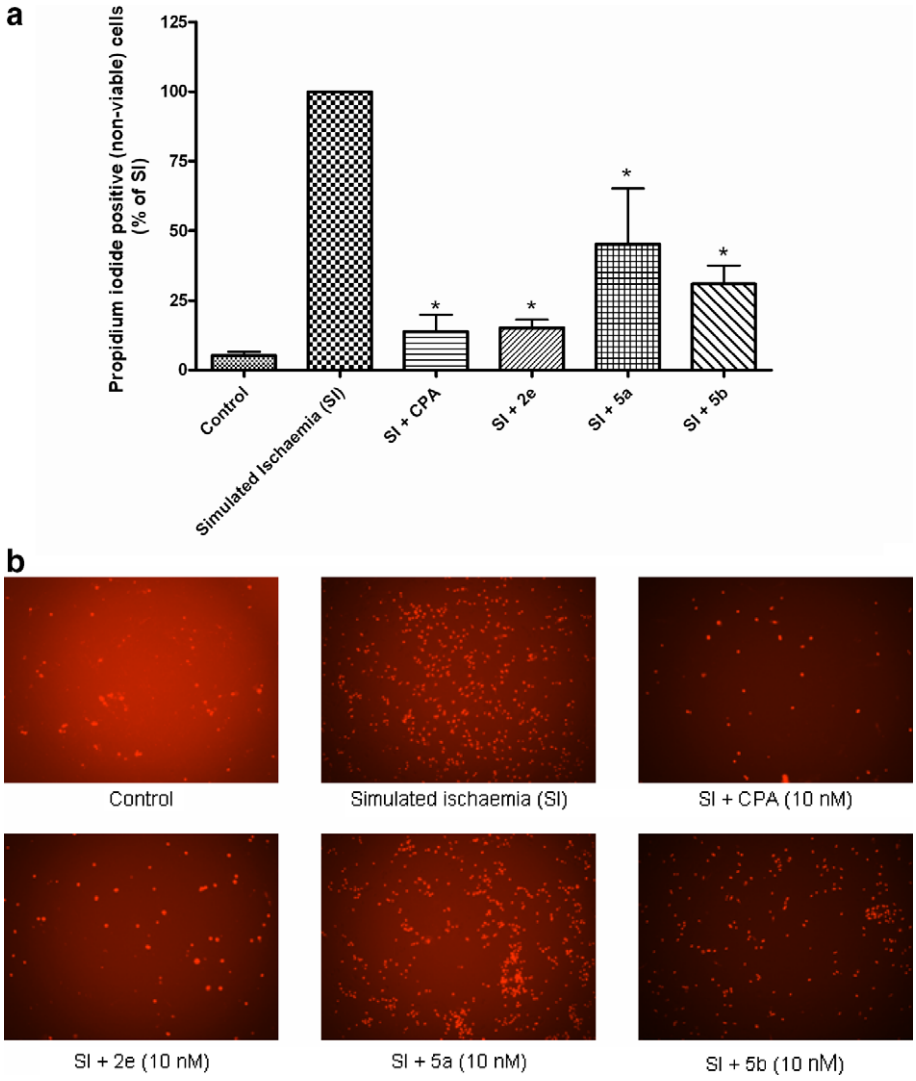


Figure 1. (a) The effects of adenosine receptor agonists on a cell model of ischaemia. Cells were grown in either normal medium (control) or hypoxic simulated ischaemia medium (all other treatments) for 12 h. Propidium iodide exclusion was then used to determine the number of viable cells, and cell death calculated for each treatment, with the simulated ischaemia treatment normalised to 100% (mean ± SEM, $n = 3$). (b) Representative images from cells incubated as described in Figure 1a.

(Fig. 1—the PI-positive cell number in the simulated ischaemia treatment group was normalised to 100%). When cells were incubated in the simulated ischaemia conditions in the presence of N^6-cyclopentyladenosine (CPA, 10 nM), the number of PI-positive cells was reduced by 86.18 ± 6.19%, to a level similar to that seen in cells exposed to normal oxygenated media (control). The series of adenosine receptor agonists tested in this assay all demonstrated cardioprotective properties at the same concentration (10 nM). Compound **2e** (84.84 ± 3.0% reduction in dead cell number) demonstrated similar efficacy to CPA at this concentration, whilst compounds **5a** (54.74 ± 19.89% reduction in dead cell number) and **5b** (73.24 ± 7.8% reduction in dead cell number) were less efficacious.

These protective effects of CPA and analogues **2e**, **5a**, and **5b** were all significantly reduced in the presence of the A_1AR antagonist 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX), over the range of agonist concentrations of 10–1000 nM (ANOVA, $P < 0.05$).

These data indicate that the adenosine receptor analogues evaluated have significant cardioprotective effects at low nanomolar concentrations. The protective effects of the analogues were reduced but not abolished in the presence of the A_1AR antagonist DPCPX, raising the possibility that there was some benefit derived from the antioxidant attachments. We are currently investigating this potential cardioprotective mechanism further.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.035.

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