ChemPubSoc Europe

DOI: 10.1002/cmdc.201700151

CHEMMEDCHEM Full Papers

VIP *Very Important Paper*

Neladenoson Bialanate Hydrochloride: A Prodrug of a Partial Adenosine A_1 Receptor Agonist for the Chronic Treatment of Heart Diseases

Daniel Meibom,*[a] Barbara Albrecht-Küpper,*[b] Nicole Diedrichs+,[a] Walter Hübsch,[a] Raimund Kast,[b] Thomas Krämer,[a] Ursula Krenz,[a] Hans-Georg Lerchen,[a] Joachim Mittendorf,[a] Peter G. Nell+,[a] Frank Süssmeier,[a] Alexandros Vakalopoulos,[a] and Katja Zimmermann[c]

In memory of Jörg Keldenich

Adenosine is known to be released under a variety of physiological and pathophysiological conditions to facilitate the protection and regeneration of injured ischemic tissues. The activation of myocardial adenosine A_1 receptors (A_1Rs) has been shown to inhibit myocardial pathologies associated with ischemia and reperfusion injury, suggesting several options for new cardiovascular therapies. When full A_1R agonists are used, the desired protective and regenerative cardiovascular effects are usually overshadowed by unintended pharmacological effects such as induction of bradycardia, atrioventricular (AV) blocks, and sedation. These unwanted effects can be overcome by using partial A_1R agonists. Starting from previously reported capadenoson we evaluated options to tailor A_1R agonists to a specific partiality range, thereby optimizing the therapeutic window. This led to the identification of the potent and selective agonist neladenoson, which shows the desired partial response on the A_1R, resulting in cardioprotection without sedative effects or cardiac AV blocks. To circumvent solubility and formulation issues for neladenoson, a prodrug approach was pursued. The dipeptide ester neladenoson bialanate hydrochloride showed significantly improved solubility and exposure after oral administration. Neladenoson bialanate hydrochloride is currently being evaluated in clinical trials for the treatment of heart failure.

Introduction

Cardiovascular diseases are still the main cause of mortality worldwide, and they will be an increasing challenge in a growing elderly population. Although a high standard of care has been established for several cardiac indications including coronary artery disease and heart failure (HF), with a decreased ejection fraction,[1] there is still a high unmet medical need for new drugs that are capable of improving long-term prognosis and quality of life. Furthermore, despite medical advances in the treatment of HF over the last two decades, there are no approved treatment options for HF patients with preserved ejection fraction. Direct and selective activation of adenosine A_1 receptors (A_1Rs) is a new mode of action that is not currently addressed by other cardiovascular drugs. Activation of myocardial A_1Rs has been shown to inhibit a variety of myocardial pathologies associated with ischemia and reperfusion injury, including myocardial stunning, arrhythmogenesis, abnormalities of coronary vasomotor tone, ventricular dysfunction, acute myocardial infarction, apoptosis, and chronic HF,[2–4] suggesting a broad spectrum of therapeutic possibilities for the treatment of cardiac diseases.

Adenosine, a purine nucleoside, is present in all cells at tightly regulated concentrations. It is released under a variety of physiological and pathophysiological conditions to facilitate protection and regeneration of injured tissues. In the heart, adenosine-induced protective and regenerative effects are mediated predominantly by A_1R activation.[5] A_1Rs are expressed in cardiomyocytes, smooth muscle cells,[6] supraventricular tissue (atria), and ventricles.[7] They belong to the family of seven-transmembrane-spanning purinergic P1 receptors and couple to the inhibitory G_i protein, which decreases the concentration of the secondary messenger cyclic adenosine monophosphate (cAMP).[8,9] Furthermore, A_1Rs couple to phospholipase C, thereby influencing inositol triphosphate and Ca^{2+} release from internal stores. In addition, activation of A_1Rs is cardioprotective by modulating potassium channels such as mitochondrial K_{ATP} channels which improves and protects mitochondrial

[a] Dr. D. Meibom, Dr. N. Diedrichs,+ Dr. W. Hübsch, Dr. T. Krämer, U. Krenz, Dr. H.-G. Lerchen, Prof. Dr. J. Mittendorf, Dr. P. G. Nell,+ F. Süssmeier, Dr. A. Vakalopoulos
Medicinal Chemistry Wuppertal, Bayer AG, 42113 Wuppertal (Germany)
E-mail: daniel.meibom@bayer.com

[b] Dr. B. Albrecht-Küpper, Dr. R. Kast
Department of Cardiology Research Wuppertal, Bayer AG, 42113 Wuppertal (Germany)
E-mail: barbara.albrecht@bayer.com

[c] Dr. K. Zimmermann
Lead Discovery Wuppertal, Bayer AG, 42113 Wuppertal (Germany)

[+] Present address: Casebia Therapeutics, San Francisco, CA, 94158 (USA)

Supporting information for this article can be found under: https://doi.org/10.1002/cmdc.201700151.

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function in cardiomyocytes under hypoxia.[10] Effects on L-type calcium channels[2] are also known to be mediated by A_1Rs.

The main limitations of using full A_1R agonists in cardiovascular indications like HF are undesired cardiac effects, such as negative chronotropic and dromotropic effects leading to bradycardia and potentially to a higher degree, atrioventricular (AV) block as shown with the full A_1R agonists tecadenoson (**1**) and selodenoson (**2**) (Figure 1).[11]

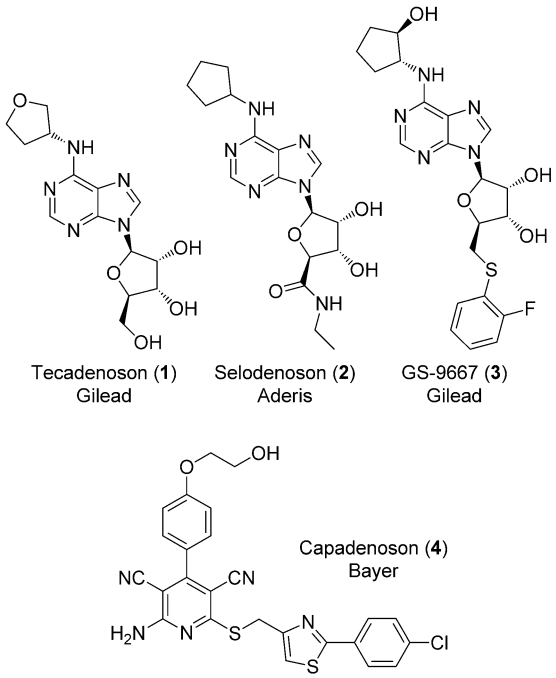

Figure 1. Full A_1R agonists: Tecadenoson (**1**) reached phase III clinical trials for the treatment of paroxysmal supraventricular tachycardia. Selodenoson (**2**) was evaluated in patients with atrial fibrillation. Partial A_1R agonists: GS-9667 (**3**) has been developed for type 2 diabetes, but development was terminated after phase I clinical trials. Capadenoson (**4**) was evaluated in phase II in patients with stable angina pectoris.

Furthermore, non-cardiac effects such as decreased neurotransmitter release in the central nervous system (CNS), resulting in sedation,[12,13] for example, are known to be triggered by full A_1R agonists. Therefore, partial A_1R agonists might be beneficial as a new therapeutic option in treating HF.[14] We previously reported that partial A_1R agonists modulate and trigger primarily favorable pharmacological responses for HF therapy, such as improvement of left ventricular (LV) function and cardioprotection without evoking the adverse effects of full A_1R agonists.[4] To date, the two partial A_1R agonists GS-9667 (**3**) and capadenoson (**4**) have reached clinical testing. GS-9667 (**3**) showed free fatty acid lowering effects in obese subjects while being devoid of hemodynamic properties.[15] However, the development of **3** was terminated due to unfavorable human pharmacokinetics data.[16] Capadenoson (**4**) belongs to the dicyanopyridine class of A_1R agonists, which was found in a high-throughput screen of our compound library. Hit optimization led to the identification of **4**, a potent and selective partial A_1R agonist with favorable DMPK properties.[2,3] In phase II clinical studies, capadenoson (**4**) improved total exercise time in patients with stable angina pectoris.[17] No induction of AV blocks was reported in this study. Furthermore, **4** showed beneficial effects, for example, on LV ejection fraction in a canine HF model without any effects on blood pressure or heart rate.[4] In a rodent model of acute myocardial infarction induced by temporary occlusion of the left anterior descending artery (LAD), treatment with capadenoson (**4**) decreased infarct size, presumably through pharmacological preconditioning.[2] The degree of agonism of **4**, however, turned out to be too high still, as central effects such as vertigo and dizziness have been observed in the above-mentioned clinical study.[17] Furthermore, the low solubility of capadenoson (**4**) hampered tablet development.[18] Taken together, safety and formulation aspects prompted us to start a follow-up project.

Results and Discussion

Addressing safety margins

CNS effects of adenosine including vertigo and sedation are mediated by the activation of A_1Rs in the brain. Full A_1R agonists such as tecadenoson (**1**) are capable of inducing prolonged sedation.[19] The central effects observed with the partial A_1R agonist capadenoson (**4**) were mild in nature and became visible in some cases at high plasma concentrations.[17] This led to the hypothesis that with a compound more partial than **4**, it might be possible to avoid unwanted A_1R-mediated pharmacological effects. More specifically: an A_1R agonist with the right degree of partiality could be capable of eliciting the desired pharmacological responses in the heart (e.g., cardioprotection) while being no longer agonistic enough to induce signaling in brain tissues. Limiting blood–brain barrier (BBB) permeation was not regarded as a valid strategy, as the BBB is often not intact in elderly heart disease patients.

The separation of desired from undesired pharmacological effects might be rationalized by different receptor reserves for A_1R-induced signaling cascades, as has been described for A_1Rs in guinea pig atrial myocytes.[20] Whereas a full A_1R agonist has high efficacy on A_1Rs and thereby elicits the full spectrum of physiological effects independent of the receptor reserve in different tissues, a partial A_1R agonist has lower efficacy and therefore only activates certain physiological responses, mostly with high receptor reserve. The decreased efficacy of a partial A_1R agonist is postulated to be due to its binding only to certain receptor activity states with compromised coupling to the G protein. This results in a selected activation of signaling cascades, as has been shown for the activation of A_{2a} receptors by partial A_{2a} receptor agonists.[21] The activation process can thus be viewed from the perspective of populations of key functional states, and the action of ligands on these conformational states through conformational selection.[21] The lower efficacy resulting in decreased signaling is therefore dependent on the conformational state addressed by the partial agonist and the specific receptor reserve involved in the induction of different physiological effects.

To quantify the partial agonistic activity on the A_1R, a GTP shift assay was used (see Supporting Information for details): K_i

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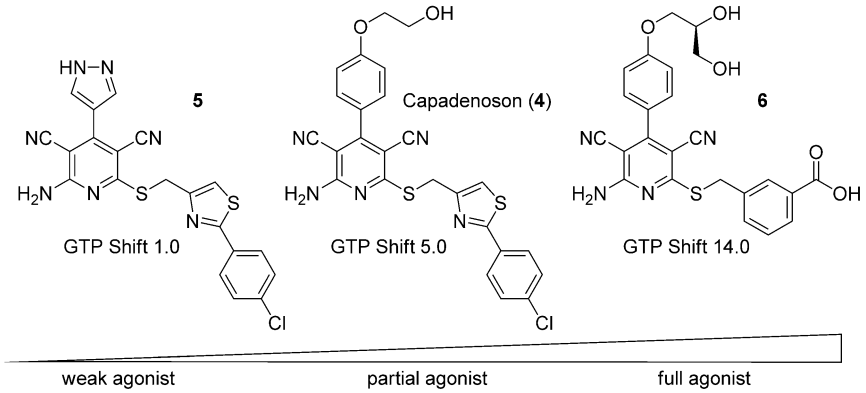

Figure 2. A GTP shift assay (Supporting Information) was used to categorize A_1R modulators as weak agonists, partial agonists, or full agonists. Rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP. Binding was determined after filtration and scintillation measurement. DPCPX (GTP shift 1.0) and tecadenoson (**1**, GTP shift 15.0) were used as controls.

measurements for A_1R modulators in the presence and absence of GTP were performed. The assay uses the fact that A_1R modulators have different affinities for the G-protein-coupled receptor (without GTP addition) or the uncoupled receptor (with GTP addition) depending on their partiality. Full A_1R agonists have high affinity for the G-protein-coupled receptor and low affinity for the uncoupled receptor, whereas weak agonists show high affinity in both cases. The factor between the K_i values from both measurements (K_{iGTP}/K_i) is the GTP shift, which is high for full agonists and intermediate for partial A_1R agonists (Figure 2). The degree of partiality of the agonists translates into different efficacies for signal induction by A_1R activation. Full agonists have high efficacy, whereas the efficacy is decreased with increasing partiality of the agonists.

Dicyanopyridines were found to cover the full GTP shift range. To identify the optimal GTP shift range to achieve increased safety margins toward sedation on the one hand and cardioprotection on the other, we tested a variety of dicyanopyridines with a GTP shift lower than that of capadenoson (**4**, GTP shift 5.0) for effects on locomotion and infarct size reduction. Effects on locomotion were assessed in a mouse running wheel model (see Supporting Information for details), in which nighttime running distances of mice were used as a surrogate parameter for central sedative properties. As capadenoson decreased the running distance in this model and showed some hints for CNS effects in humans,[17] we concluded that results obtained in mice might translate to humans. Compounds with a GTP shift of 4.0 and lower did not decrease running distance significantly, as exemplified by the dicyanopyridine **7** (Figure 3).

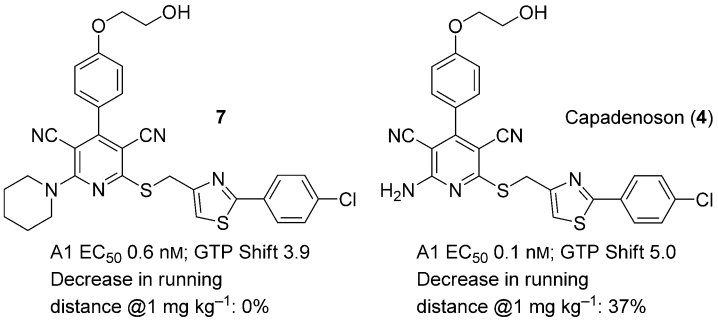

Figure 3. Effects on locomotion were assessed in a mouse running wheel model (Supporting Information). Compounds were dosed orally once daily by gavage, and running distances were analyzed over 8 h during the night activity phase. Potent partial A_1R agonists with similar PK properties (data not shown) and a GTP shift of 4.0 or lower did not decrease running distance significantly. Potency was measured by light emission of Chinese hamster ovary (CHO) cells expressing the human A_1R and a cAMP/luciferase reporter gene (Supporting Information). GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information).

Cardioprotective effects were analyzed in a rat model of acute myocardial infarction induced by occlusion of the LAD (see Supporting Information for details). Dicyanopyridines of similar potency were administered intravenously (i.v.) as bolus prior to occlusion of the LAD. After reperfusion for two hours, the infarcted area was quantified. It was found that compounds with a GTP shift of 3.0 or above are able to decrease infarct size, presumably through pharmacological preconditioning (Figure 4).

In an extension of our previous work[2,3] we started with a modification of the northern hydroxyethoxyphenyl substituent (Table 1) to identify compounds with suitable partiality (GTP shift 3.0–4.0). The pyridine derivative **11** was found to be a very potent and selective partial A_1R agonist, but with a GTP shift falling outside the desired range. Five-membered heterocycles, as represented by the hydroxyethyl-substituted pyrazole **12**, exhibited GTP shifts that were too low. Modification of the hydroxyethoxy side chain provided a variety of potent partial A_1R agonists (examples **9**, **13**–**17**). However, only the trifluoromethyl-substituted compound **9** and the dihydroxy derivative **13** displayed favorable GTP shifts between 3.0 and 4.0. Both compounds were deprioritized due to unfavorable PK characteristics based on a first assessment in rats (oral administration, data not shown; **9**) or insufficient selectivity versus the A_{2b} receptor (compound **13**).

We then turned our attention to the southeastern part of capadenoson (**4**, Table 2). Replacement of the thiazole moiety of

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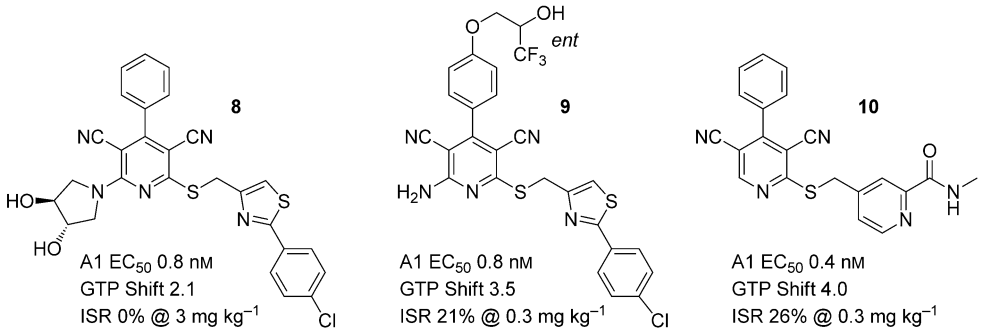
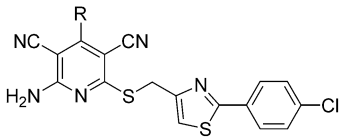


Figure 4. Cardioprotective properties of partial A_1R agonists were assessed in an acute myocardial infarction model in rats (Supporting Information). Compounds were dosed intravenously before ischemia by bolus injection, and infarct sizes were measured after reperfusion. Administration of potent dicyanopyridines with a GTP shift of 3.0 or above led to infarct size reduction (ISR). Potency was measured by light emission of CHO cells expressing the human A_1R and a cAMP/luciferase reporter gene (Supporting Information). GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information).

4 by an oxazole yielded compound **18** with a markedly decreased GTP shift of 1.6. Introduction of a 5-methyl group provided no improvement (**19**, Table 2). Variations on the phenyl ring, on the other hand, had a greater impact on the GTP shift, as represented by compounds **20** and **21**. However, partial agonism with a GTP shift in the desired range could not be ach-

Table 1. Exploration of northern SAR: potency, selectivity, and GTP shift.



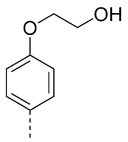
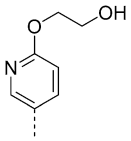
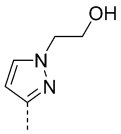
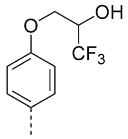
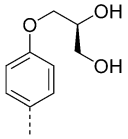
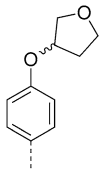
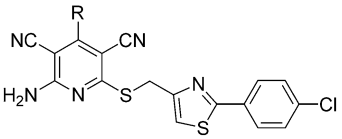
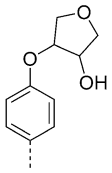
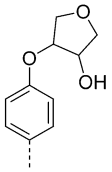
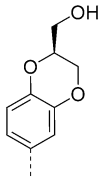
Compd	R	EC_{50} [nM][a] A_1	EC_{50} ratio[a] A_{2a}/A_1	A_{2b}/A_1	GTP shift[b]
4[c]		0.1	1800	900	5.0
11		0.04	30 000	10 000	5.2
12		1.9	1000	730	2.5
9 (*ent*)		0.8	7200	260	3.5
13		0.05	680	85	3.0
14 (*rac*)		0.3	4800	2400	1.5

Table 1. (Continued)



Compd	R	EC_{50} [nM][a] A_1	EC_{50} ratio[a] A_{2a}/A_1	A_{2b}/A_1	GTP shift[b]
15 (*ent1*,[d] *trans*)		0.3	3900	1500	2.0
16 (*ent2*,[d] *trans*)		0.7	3600	850	5.0
17		0.05	870	1500	6.2

[a] Potency was measured with CHO cells expressing either the human A_1, A_{2a}, or A_{2b} receptor and a cAMP/luciferase reporter gene (Supporting Information). A_1 cells were pre-stimulated with 1 μM forskolin. Cells were incubated with increasing doses of test compounds, and luciferase expression was measured by light emission. [b] GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information). [c] Capadenoson. [d] Enantiomer 1 was separated from enantiomer 2 by chiral chromatography; absolute stereochemistry was not elucidated.

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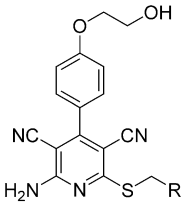
Table 2. Exploration of southeastern SAR: potency, selectivity, and GTP shift.



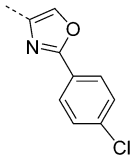
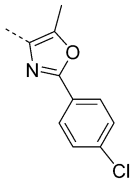
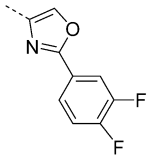
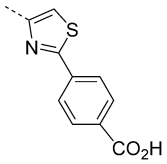
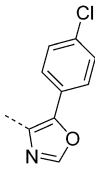
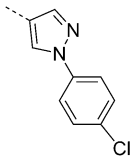
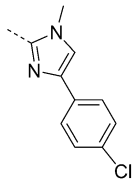
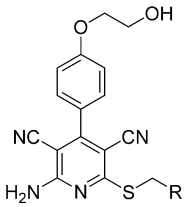
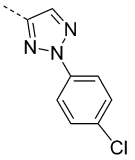
Compd	R	EC_{50} [nM][a] A_1	EC_{50} ratio[a] A_{2a}/A_1	A_{2b}/A_1	GTP shift[b]
18		0.1	410	180	1.6
19		0.3	2100	3200	2.0
20		0.3	2200	2600	4.8
21		0.02	610	140	10.0
22		35	10	15	3.6
23		1.0	870	800	4.2
24		0.4	2700	300	4.3

Table 2. (Continued)



Compd	R	EC_{50} [nM][a] A_1	EC_{50} ratio[a] A_{2a}/A_1	A_{2b}/A_1	GTP shift[b]
25		0.5	710	140	2.8

[a] Potency was measured with CHO cells expressing either the human A_1, A_{2a}, or A_{2b} receptor and a cAMP/luciferase reporter gene (Supporting Information). A_1 cells were pre-stimulated with 1 μM forskolin. Cells were incubated with increasing doses of test compounds, and luciferase expression was measured by light emission. [b] GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information).

ieved. Shifting the chlorophenyl substituent from the 2-position as in compound **18** to the 5-position of the oxazole resulted in significantly decreased A_1R agonistic potency (**22**, EC_{50} = 35 nM). Replacement of the thiazole of capadenoson **4** by nitrogen-containing heterocycles such as pyrazole **23**, imidazole **24**, and triazole **25** also provided no improvement with respect to potency and degree of partial agonism.

In contrast, evaluation of the southwestern region turned out to be more successful. As represented by the examples in Table 3, introduction of various substituents on the primary amino group of capadenoson (**4**) resulted in a decreased GTP shift while maintaining favorable potency and selectivity. Partial A_1R agonism within the desired range could be achieved with the glycine amide side chain (**27**) and especially with cyclic amino substituents (**7**, **28**–**31**), which was unexpected and is still not fully understood.[1] Amide **27** and piperidines **7** and **31** showed unfavorable PK properties in rats after oral administration (data not shown) and were not further pursued. The azetidine derivatives **28** and **29** had to be deprioritized due to instability of the four-membered rings under acidic conditions. The pyrrolidine **30** (neladenoson) showed the best overall profile in terms of potency, selectivity versus other adenosine receptors (including A_3, $EC_{50} > 3000$ nM), and partiality on the A_1R. Furthermore, no significant interference with 67 off-targets (MDS Pharma, Supporting Information), no CYP or hERG inhibition, and no in vitro mutagenicity were detected.

The in vivo PK profile of neladenoson (**30**) is characterized by low clearance and moderate to long half-life in rats, dogs, and monkeys (Table 4) after i.v. administration. Compared with capadenoson (**4**, Table 5), it showed an improved clearance and half-life, particularly in dogs. However, oral bioavailability values of neladenoson (**30**) were only in the low-to-moderate range due to low aqueous solubility (< 0.1 mg L^{-1}, crystalline

[1] *The molecular understanding of receptor activation by partial agonists is a topic of current research. A recent publication by Ye et al.[21] explains the mechanism of partial agonism on the A_{2a} receptor.*

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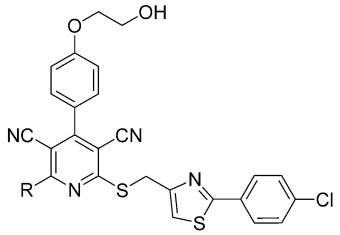
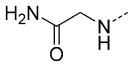
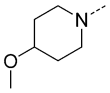
Table 3. Exploration of southwestern SAR: potency, selectivity, and GTP shift.



Compd	R	EC_{50} [nM][a] A_1	EC_{50} ratio[a] A_{2a}/A_1	A_{2b}/A_1	GTP shift[b]
26		0.1	460	360	4.3
27		0.9	790	900	3.6
28		0.3	1000	500	3.6
29		0.3	2000	8600	3.5
30[c]		0.1	6700	800	3.9
7		0.6	5000	5000	3.9
31		0.6	510	1200	3.2

[a] Potency was measured with CHO cells expressing either the human A_1, A_{2a}, or A_{2b} receptor and a cAMP/luciferase reporter gene (Supporting Information). A_1 cells were pre-stimulated with 1 μM forskolin. Cells were incubated with increasing doses of test compounds, and luciferase expression was measured by light emission. [b] GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information). [c] Neladenoson.

Table 4. Pharmacokinetic profile of neladenoson (**30**) in selected species.[a]

Species	CL_b [L h^{-1} kg^{-1}][b]	V_{SS} [L kg^{-1}][c]	$t_{1/2}$ [h]	F [%][d]
Rat	1.1	8.0	6.3	37
Dog	0.2	1.9	9.6	51
Monkey	0.5	0.9	4.3	39

[a] Values were derived by i.v. (rat: bolus, dose 0.5 mg kg^{-1}; dog & monkey: 0.25 h infusion, dose 0.3 mg kg^{-1}) and oral (gavage, dose 1.0 mg kg^{-1}) administration of solutions in EtOH/PEG400/H_2O vehicles. [b] Blood clearance. [c] Volume of distribution at steady state. [d] Oral bioavailability.

material). The exposure was further markedly decreased when administered as a suspension of crystalline material in 0.5% Tylose ($F \approx 1\%$).

Addressing solubility and bioavailability

The low solubility and bioavailability of **30** in aqueous media prompted us to evaluate formulation techniques that make use of organic solvents like liquid-filled capsules and solid dispersions. However, these approaches were quickly dismissed, as the expected human dose was not soluble in acceptable capsule or tablet sizes. Several drugs are known, in which the introduction of prodrug moieties led to improved solubility and bioavailability.[22–25] We therefore turned our attention to investigating whether it would be beneficial to explore prodrug forms of neladenoson (**30**). A suitable candidate needed to have improved solubility and bioavailability from suspension and it had to be sufficiently stable for tablet development and to enable rapid liberation of the drug in vivo. The hydroxy group of **30** was selected as point of attachment for prodrug moieties.

Table 5. Pharmacokinetic profile of capadenoson (**4**) in selected species.[a]

Species	CL_b [L h^{-1} kg^{-1}][b]	V_{SS} [L kg^{-1}][c]	$t_{1/2}$ [h]	F [%][d]
Rat	1.4	10	8.4	57
Dog	1.2	2.3	2.4	21
Monkey	0.21	2.7	9.8	60

[a] Values were derived by i.v. and oral (gavage) administration. [b] Blood clearance. [c] Volume of distribution at steady state. [d] Oral bioavailability.

The first part of our prodrug screening cascade consisted of stability testing in aqueous solution at various pH values to identify prodrugs with good stability, particularly at pH ≤ 4, to ensure that parent drug liberation in the stomach at low pH does not take place. Esters of natural and unnatural amino acids,[25,26] which are protonated on the terminal amino group under acidic conditions, fulfilled this requirement (Figure 5).

The next filter in our prodrug screening cascade was the availability of crystalline prodrugs by stirring in various solvents. Crystalline material with defined hydrochloride stoichiometry was obtained from isopropanol as exemplified by the isoleucine derivative **34** (Figure 6).

Further criteria were bioavailability of **30** in rats after oral administration of suspensions of crystalline prodrugs and the seven-day stability of the solid prodrugs at 90 °C. Among different dipeptide esters, the bis-L-alanyl prodrug **35** (neladeno-

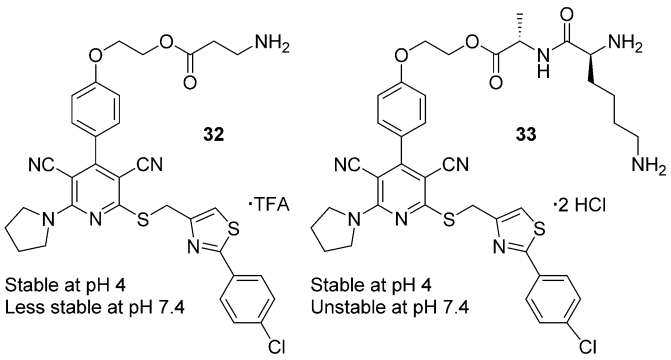


Figure 5. Stability of prodrugs in aqueous solution was monitored for 24 h. No ester cleavage and liberation of neladenoson (**30**) was detected at pH 4. At pH 7.4 slow conversion to **30** took place.

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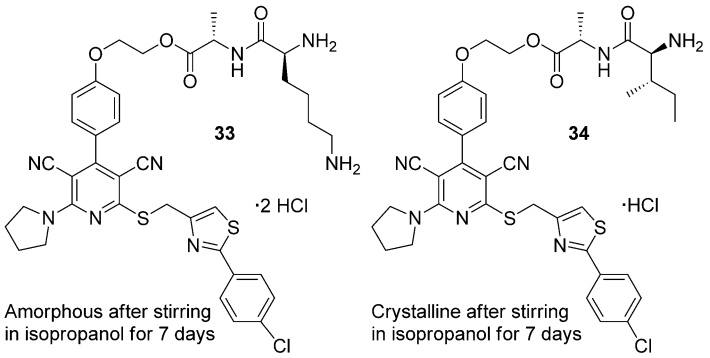
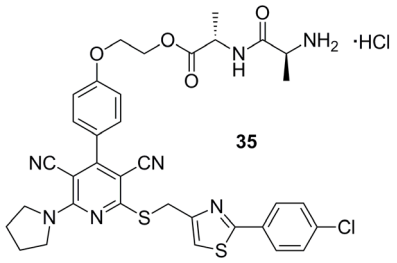


Figure 6. Crystallization experiments were performed with suspensions of prodrugs under a variety of conditions including a solvent screen. Stirring in isopropanol for seven days at room temperature gave good results.

son bialanate hydrochloride) showed the best overall profile with good solubility, rapid liberation of neladenoson (**30**) in vivo, improved oral exposure of **30**, and no degradation in the solid state. Compound **35** was deemed stable enough for tablet development and was selected as the development candidate (Table 6).

Table 6. Profile of neladenoson bialanate hydrochloride (**35**).



Parameter	Result
Stability in aqueous solution	stable 24 h at pH $\leq$ 5[a]
Solubility in aqueous medium	6 g L^{-1} at pH 4.5[b]
Prodrug concentrations in rat plasma	negligible[c]
Bioavailability of **30** from suspension of **35**	52%[d]
Stability as solid	stable 7 days at 90 °C[a]

[a] No degradation (ester cleavage) to neladenson (**30**) was detectable. [b] Measured from crystalline material. Solubility of neladenoson (**30**) at pH 4: $<$0.1 mg L^{-1}. [c] After oral administration of **35** to rats, **35** was only sporadically detected in the systemic circulation at single time points and at very low concentrations. [d] Calculation based on study with oral (gavage) administration of crystalline material of **35** as suspension (0.5% Tylose) to rats. Exposure of neladenoson (**30**) exceeded the one achievable by administration of a solution of **30**, whereas bioavailability of neladenson (**30**) from suspension of crystalline material of **30** accounted to only $\approx$1%.

Synthesis

Neladenoson bialanate hydrochloride (**35**) was synthesized in an eight-step sequence[27,28] starting from commercially available thioamide **36** and aldehyde **38** (Scheme 1). The first key step is the Hantzsch pyridine synthesis to afford capadenoson (**4**) in a one-pot/three-reaction sequence by reacting 2-cyanothioacetamide with two equivalents of the Knoevenagel product **39** and benzylic chloride **37**. The sequence likely starts by reacting one equivalent of **39** with 2-cyanothioacetamide, leading to a dihydropyridine intermediate which is oxidized in situ[29] to a pyridine by another equivalent of **39** in the second step. Finally, **37** alkylates the sulfur-substituted pyridine obtained in the previous reaction to give **4**. The pyrrolidine was introduced via Sandmeyer reaction and substitution of the chlorine atom. The dipeptide **43** was obtained via sequential coupling of two *tert*-butoxycarbonyl (Boc)-protected alanine moieties. Removal of the Boc group with 1 N HCl in the final step afforded the prodrug neladenoson bialanate hydrochloride (**35**).

In vivo pharmacodynamics studies

Capadenoson (**4**) and neladenoson (**30**) are both selective and potent A_1R agonists with EC_{50} values of 0.1 nM on human A_1Rs and a selectivity factor of 1800 and 6700 versus A_{2a}, 900 and 800 versus A_{2b}, respectively, and no significant activity on A_3 receptors.[2] The partial agonistic character of compounds **30** and **4** was analyzed relative to the full A_1R agonist 2-chloro-N^6-cyclopentyladenosine (CCPA) on human A_1Rs by a [^{35}S]GTP binding assay (Supporting Information). Neladenoson (**30**) and capadenoson (**4**) reached ~67 and 79% of the efficacy of CCPA, respectively (Figure 7).

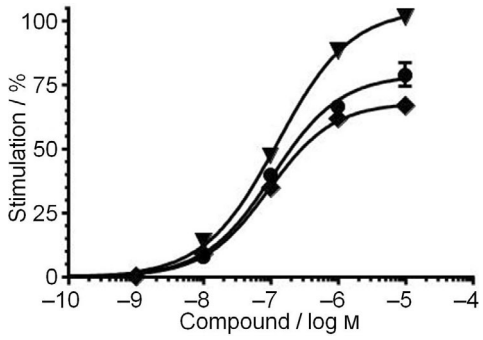


Figure 7. Degree of partiality of neladenoson (**30**, ◆) on A_1Rs compared with capadenoson (**4**, ●) and CCPA (▼). Partial character of A_1R agonists was assessed on human A_1Rs from frontal lobe in a [^{35}S]GTP binding assay (Supporting Information). Data are the mean ± SEM (n = 8).

Although the difference in the level of partiality between both compounds is only 12%, there were significant differences in their pharmacological effects. Capadenoson (**4**) showed a significant decrease of locomotion activity, as surrogate marker for sedative CNS effects, in a mouse running wheel model (Figure 8). The compound decreased the overnight running distance of mice in a dose-dependent manner starting from 0.3 mg kg^{-1} onward. In contrast, neladenoson (**30**) had no significant effect on the running distance up to doses of 3 mg kg^{-1}.

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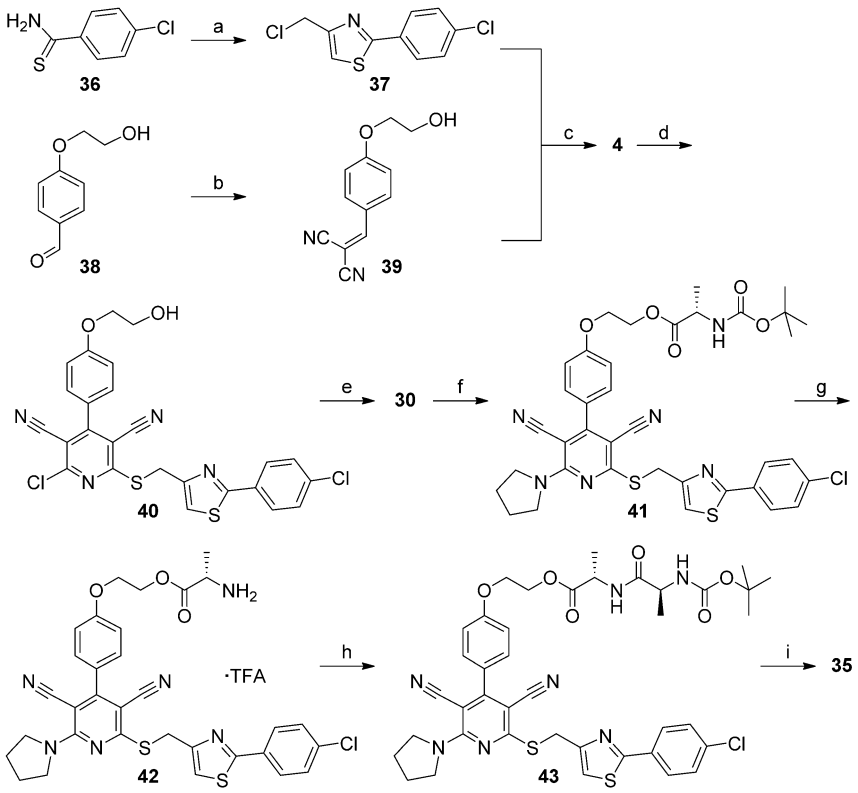

Scheme 1. Synthesis of neladenoson bialanate hydrochloride (**35**). *Reagents and conditions*: a) 1,3-dichloroacetone, 2-propanol, 55 °C, 7.5 h, 91 %; b) malononitrile, piperidine, 2-propanol, 80 °C, 16 h, 95 %; c) 2-cyanothioacetamide, tributylamine, MeOH, RT, 20 h, 68 %; d) isopentyl nitrite, $CuCl_2$, MeCN, 70 °C, 6 h, 62 %; e) pyrrolidine, THF, RT, 30 min, 81 %; f) Boc-L-alanine, EDC·HCl, HOBt·H_2O, *N*,*N*-diisopropylethylamine, DMF, RT, 1 day, 98 %; g) TFA, CH_2Cl_2, RT, 1 day, 98 %; h) Boc-L-alanine, EDC·HCl, HOBt·H_2O, *N*,*N*-diisopropylethylamine, DMF, RT, 3 h, 91 %; i) 1 N HCl in Et_2O, CH_2Cl_2, RT, 1 day, 91 %. EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; HOBt = hydroxybenzotriazole.

Determination of free brain concentrations of capadenoson (**4**) and neladenoson (**30**) showed equivalent exposures (data not shown). Therefore, the decreased effects on locomotion by **30** cannot be attributed to differences in the penetration of the BBB. It is more likely that differences on the A_1R reserve are responsible for the differentiated pharmacological effects depending on the level of partiality of the agonists. Based on our data, we assume that there is a low receptor reserve for the induction of sedative effects measured as decreased locomotor activity. The higher partiality of neladenoson (**30**) does not allow the induction of these effects. The findings are supported by clinical studies with neladenoson bialanate hydrochloride (**35**) in humans (HF patients and healthy subjects), in which no safety issue with respect to sedation was observed (manuscript in preparation).

Although neladenoson (**30**) had decreased effects on motor activity (sedation), it showed slightly higher cardioprotection measured as a decrease of infarct size (IS) relative to capadenoson (**4**), whereas both compounds are similar in their unbound exposure. When administered before induction of ischemia by left anterior descending artery (LAD) occlusion, **30** decreased IS from 28.7 ± 1.5 % to 19.8 ± 3.3 % at 0.03 mg kg^{-1} i.v. significantly in a rat model of acute myocardial infarction (Figure 9). In contrast, **4** decreased IS to 22.0 ± 3.4 % only at the higher dose of 0.1 mg kg^{-1}. The level of infarct size reduction by neladenoson (**30**) is similar to published data for full A_1R agonists such as CCPA.[30] Because capadenoson (**4**) showed beneficial effects in a canine model of HF with reduced ejection fraction,[4] benefits of neladenoson (**30**) on cardiac dysfunction were evaluated in a rodent HF model; chronic effects of **30** on heart function were measured in rats with permanent LAD occlusion. One week after LAD occlusion, treatment with 0.3 mg kg^{-1} **30** once daily was started over eight weeks followed by an invasive measurement of cardiac function by left ventricular catheterization (Supporting Information). Placebo rats showed a significant decrease in contractility and relaxation parameters and thereby systolic and diastolic function versus sham rats (dp/dt_{max} 5575 ± 479 versus 7501 ± 1031 mmHg s^{-1}; dp/dt_{min} 5399 ± 748 versus 7694 ± 1011 mmHg s^{-1}; tau 17.3 ± 3.1 versus 11.3 ± 1.5 ms) (Figure 10). Treatment with neladenoson (**30**) at 0.3 mg kg^{-1} p.o. once daily resulted in a significant improvement of contraction and relaxation parameters (dp/dt_{max} 7503 ± 765 mmHg s^{-1}; dp/dt_{min} 7385 ± 1298 mmHg s^{-1}; tau 14.4 ± 2.1 ms).

Conclusions

Starting from capadenoson (**4**) we evaluated options to identify partial A_1R agonists within a specific partiality range (GTP shift 3.0–4.0) to improve safety margins regarding CNS effects. The introduction of cyclic amines in the southwest region of the dicyanopyridine core provided compounds with suitable

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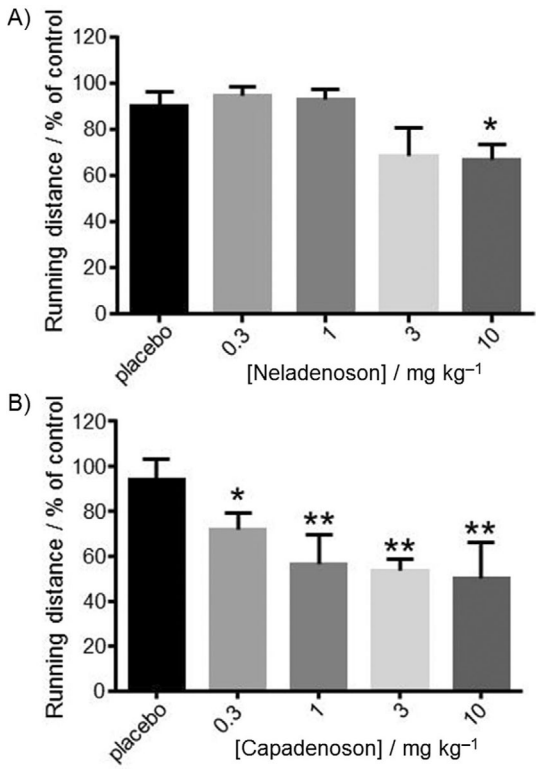

Figure 8. Effects of A) neladenoson (**30**) and B) capadenoson (**4**) on overnight running distance of mice in a running wheel. Running distance was automatically measured (Supporting Information). Both compounds were applied orally. Data are the mean ± SEM ($n = 8–10$); $^{*}p < 0.05$, $^{**}p < 0.01$ vs. placebo.

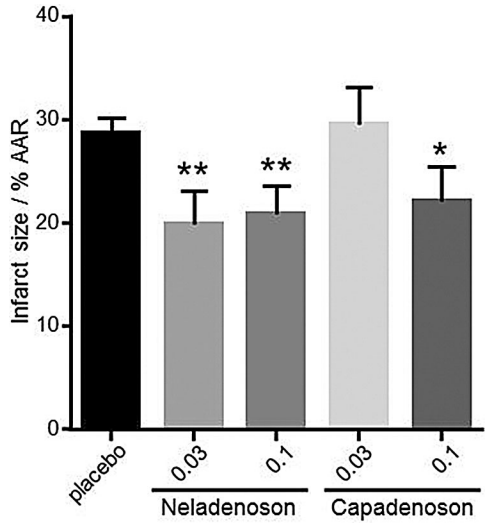

Figure 9. Cardioprotection of partial A_1R agonists in rodents. Cardioprotective properties of neladenoson (**30**) and capadenoson (**4**) by reduction of infarct size given as percentage of area at risk (AAR) were assessed in an acute myocardial infarction model in rats (Supporting Information). Compound **30** and **4** were dosed intravenously by bolus injection (0.03 and 0.1 mg kg^{-1}) before LAD occlusion and infarct sizes were measured after reperfusion. Data are the mean ± SEM ($n = 9–20$); $^{*}p < 0.05$, $^{**}p < 0.01$ vs. placebo.

GTP shift values. The potent and selective A_1R agonist neladenoson (**30**) had the preferred level of partiality and a favorable

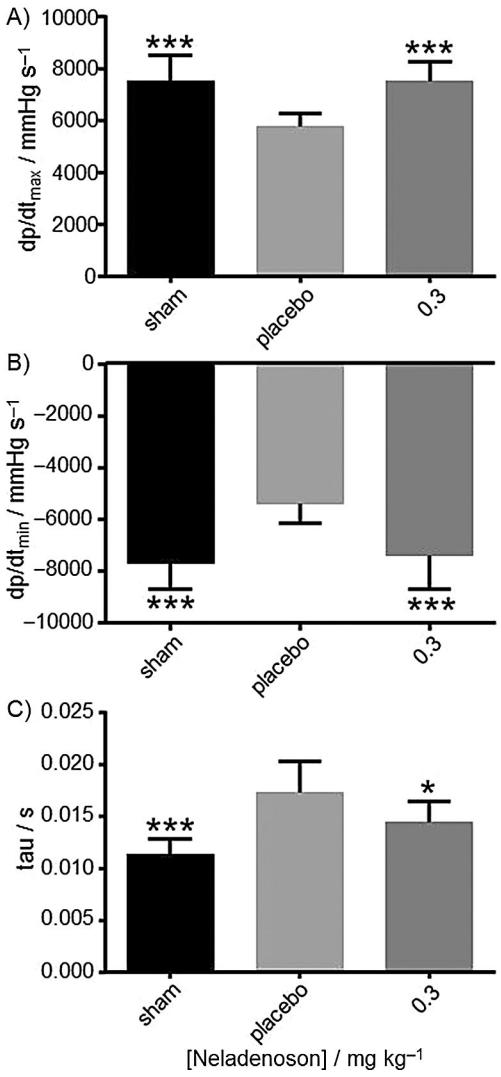

Figure 10. Effects of neladenoson (**30**) on heart function in rats treated with sham (control), placebo, and **30** (0.3 mg kg^{-1} p.o.) in a rat HF model of permanent LAD occlusion (Supporting Information). Improvement in contractility [A) dp/dt_{max}] and relaxation [B) dp/dt_{min}; C) tau] were observed for neladenoson (**30**) after eight weeks treatment. Data are the mean ± SEM ($n = 9–14$); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. placebo.

PK profile after i.v. administration. However, low solubility led to low oral exposure and potential tablet development issues. We therefore embarked on a prodrug approach to identify a candidate with good solubility and bioavailability. Stability was tailored to allow efficient liberation of the drug in vivo while showing no degradation in the solid state. The hydrochloride form of the dipeptide ester neladenoson bialanate (**35**) fulfilled all desired criteria (Table 7).

Neladenoson (**30**) preserved the high potency and cardioprotective effects observed with capadenoson (**4**), while having significantly reduced central effects. In phase I and phase IIa studies the neladenoson prodrug **35** was well tolerated and showed no safety issues with respect to sedation or AV block induction (manuscript in preparation), which makes the compound suitable for chronic treatment of patients with cardiac diseases such as heart failure (HF). Neladenoson bialanate

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Table 7. Comparison of capadenoson (**4**), neladenoson (**30**), and neladenoson bialanate hydrochloride (**35**).

Compd	A_1 EC_{50} [nM][a]	GTP shift[b]	Aq. sol. [mg L^{-1}]	F [%][c]
4	0.1	5.0	<0.1[d]	≈ 1
30	0.1	3.9	<0.1[d]	≈ 1
35	–	–	6000[e]	52

[a] Potency was measured with CHO cells expressing the human A_1R and a cAMP/luciferase reporter gene (Supporting Information). A_1 cells were pre-stimulated with 1 μM forskolin. Cells were incubated with increasing doses of test compounds, and luciferase expression was measured by light emission. [b] GTP shift: rat brain membranes expressing the A_1R were incubated with [^{3}H]DPCPX as radioligand and A_1R modulator at various concentrations in the presence and absence of GTP (Supporting Information). [c] Oral bioavailability in rats from suspension in 0.5% Tylose. [d] Measured at pH 4. [e] Measured at pH 4.5.

hydrochloride (**35**) is currently under evaluation in clinical trials for the treatment of HF. Phase IIb studies are planned for 2017.

Experimental Section

All experimental data are shown in the Supporting Information.

Acknowledgements

We thank Heike Gielen-Haertwig as well as Stephan Vettel for continuous support of this project and Stefanie Vogt for valuable technical assistance with the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Keywords: adenosine A_1 receptor · biological activity · dicyanopyridines · medicinal chemistry · partial agonists · prodrugs

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Manuscript received: March 7, 2017
Revised: April 12, 2017
Accepted Article published: April 12, 2017
Final Article published: ■■ ■■, 0000

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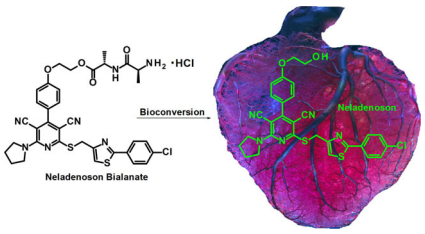
Good gets even better: Partial adenosine A_1 receptor (A_1R) activation is associated with a positive impact on heart failure without the detriments of full A_1R agonism. Starting from capadenoson, we describe the identification of neladenoson bialanate hydrochloride, which has a good pharmacokinetic and safety profile. Neladenoson bialanate hydrochloride is currently being assessed in clinical studies for the treatment of heart failure.



D. Meibom, B. Albrecht-Küpper,* N. Diedrichs, W. Hübsch, R. Kast, T. Krämer, U. Krenz, H.-G. Lerchen, J. Mittendorf, P. G. Nell, F. Süssmeier, A. Vakalopoulos, K. Zimmermann*

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Neladenoson Bialanate Hydrochloride: A Prodrug of a Partial Adenosine A_1 Receptor Agonist for the Chronic Treatment of Heart Diseases

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