Bioorganic & Medicinal Chemistry Letters 20 (2010) 5891–5894

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Identification and optimization of substituted 5-aminopyrazoles as potent and selective adenosine A1 receptor antagonists

Nils Griebenow [a,*], Lars Bärfacker [a], Heinrich Meier [a], Dirk Schneider [a], Nicole Teusch [b], Klemens Lustig [c], Raimund Kast [d], Peter Kolkhof [d]

[a] Bayer Schering Pharma AG, Global Drug Discovery, Medicinal Chemistry Wuppertal, Building 460, D-42096 Wuppertal, Germany
[b] Bayer Schering Pharma AG, Global Drug Discovery, Cell Biology, Building 456, D-42096 Wuppertal, Germany
[c] Bayer Schering Pharma AG, Global Drug Discovery, Research Pharmacokinetics, Building 468, D-42096 Wuppertal, Germany
[d] Bayer Schering Pharma AG, Global Drug Discovery, Cardiology Research, Building 500, D-42096 Wuppertal, Germany

ARTICLE INFO

Article history:
Received 10 July 2010
Revised 22 July 2010
Accepted 23 July 2010
Available online 1 August 2010

Keywords:
5-Aminopyrazole
Adenosin A_1 receptor antagonist
Heart failure
Diuresis
Natriuresis

ABSTRACT

Potent and selective adenosine A_1 receptor antagonists were disclosed. SAR and pharmacological profile of selected compounds were discussed.

Acute heart failure is one of the most common diseases in emergency medicine and associated with a poor prognosis. The acute decompensated heart failure registry (ADHERE) of 100,000 patients admitted with acute decompensated heart failure (ADHF) reveals that moderate and severe renal insufficiency, and even renal failure, are common in this population, and that normal renal function is rare.[1]

Diuretics are the mainstay of treatment for HF associated with volume overload. Diuretic therapy with loop diuretics can worsen renal function (e.g. reduction of glomerular filtration rate) in ADHF patients, and worsening renal function is associated with poorer outcomes.[2] Diuretic resistance in ADHF patients can also be considered as another indicator of poor prognosis in patients with chronic heart failure. New diuretic drugs which increase diuresis without worsening of renal function and, which overcome diuretic resistance in ADHF patients are urgently needed for patients to improve outcome and reduce re-hospitalization.

Adenosine, a purine nucleoside, is present in all cells and released under a variety of different physiologic and pathophysiologic circumstances to affect various physiological actions. The effects of adenosine are mediated by the stimulation of four distinct receptors A_1, A_{2a}, A_{2b}, and A_3.[3] Kidney A_1 receptors are expressed in afferent arterioles, the inner medullar collecting duct, the outer medullar collecting duct, the connecting tubule and cortical collecting duct, and Henle's loop (thin limb). It has been found that tissue distributions and signaling transduction pathways of the receptors show some species differences, but the main effects of kidney A_1 receptor activation are vasoconstriction of arterioles, inhibition of diuresis and inhibition of sodium transport. Inhibition of A_1 receptor activity by A_1 antagonists results in inhibition of vasoconstriction of renal arterioles by adenosine, increase of diuresis and natriuresis without worsening of glomerular filtration rate (GFR) and diuretic activity in Furosemide resistance. This has been proven in animal experiments and clinical studies with ADHF patients.[4] A_1 antagonists are, therefore, a promising drug class for the improvement of renal function in ADHF patients.

Our interest began with the identification of **1** as a moderate-potent and selective adenosine A_1 antagonist (Fig. 1). Amino-pyrazole **1** was discovered during the profiling and reevaluation of compounds from an internal diabetes project.[5] The development of adenosine A_1 receptor antagonists is an increasingly competitive field[6] and we were encouraged to find that 5-aminopyrazoles represented a novel chemotype.

However, inferior PK properties (see Table 3) and moderate potency prevented **1** from showing consistent in vivo activity in various diuresis models in rats.

* Corresponding author. Tel.: +49 202 36 5866; fax: +49 202 36 8149.
E-mail address: nils.griebenow@bayerhealthcare.com (N. Griebenow).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.095

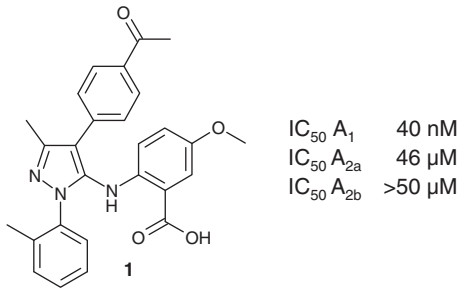


Figure 1. Initial lead structure from the reinvestigation of internal project compounds.

Hence, an exploratory program was initiated to rapidly investigate SAR around this novel chemotype as well as to identify an analogue with a PK profile suitable for preliminary validation studies in in vivo preclinical models. A number of analogs were synthesized according to the details in Scheme 1.

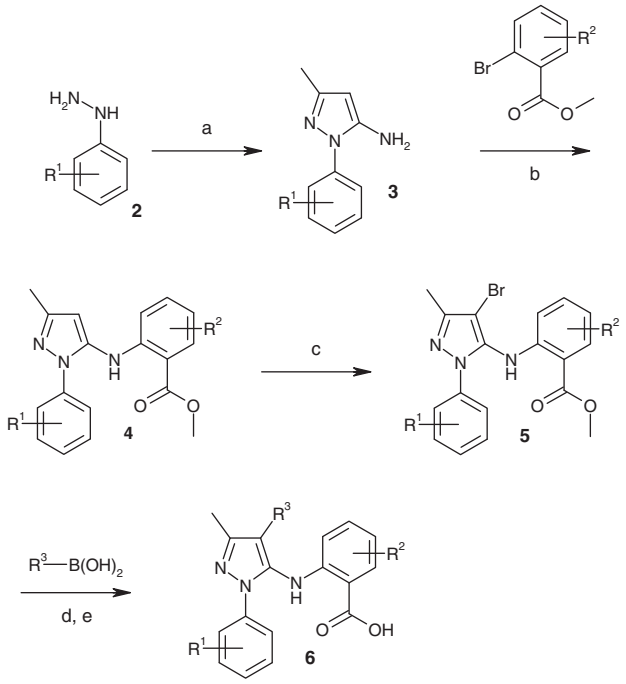
Compounds were build up in five linear steps. Initially, hydrazines **2** were condensed with 3-aminocrotononitrile to provide the 5-aminopyrazoles **3**. Subsequent Buchwald–Hartwig type cross-coupling of the 5-aminopyrazoles with methyl bromobenzoates yielded the *N*-aryl amino pyrazoles **4**. Bromination of **4** followed by Suzuki arylation with aryl boronic acids and consecutively saponification of the methyl esters provided the target compounds **6**.[7]

Early SAR exploration focused on aromatic variations at the C-4 of the pyrazole **5** which could be performed in a parallel library synthesis format utilizing Suzuki cross-coupling methodology. A list of selected compounds is shown in Table 1.

The SAR trends in Table 1 revealed that the combination of *meta*- and *para*-substitution on the phenyl resulted in more potent congeners compared to mono substituted phenyl rings (comparing **8** to **7**, **10** to **9**, and **12** to **11**).

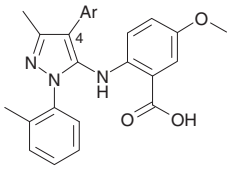
This is particularly true for alkoxy substituents (see compound **12**). Best potency resulted when the 3,4-substitution pattern is displayed by bicyclic ring systems, such as congeners **13** and **14**. Moreover, it was found that the 6–6 ring combination is more potent compared to the 6–5 ring system (**14** vs **13**). The quinoxaline **15** exhibited greatly improved A_1 potency. The downside of this type of analog is the moderate to high clearance and low bioavailability (see Table 3). It was found that **15** is rapidly metabolized at the *para*-methoxy group of the aminobenzoic acid (O-demethylation) but also at the quinoxaline (hydroxylation) and the carboxylic acid (glucuronidation). We had some concerns about the methoxy substituent, because quinone imine could be formed after demethylation and oxidation of the hydroxyaniline, which could cause adverse effects upon prolonged use.[8] As a result of these concerns we decided to proactively explore the feasibility to replace this substituent. Thus, replacement with chlorine, methyl, and hydrogen was investigated resulting in a slight loss of potency (see Table 2: **16**, **17**, and **18**).

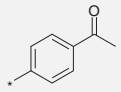
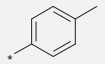
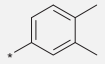
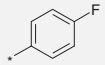
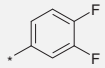
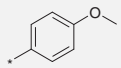
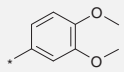
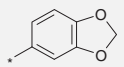
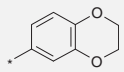
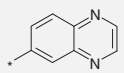
The SAR at the N-1 position of the pyrazole revealed that aromatic substituents, in particular *ortho*-substituted phenyl rings, are clearly favored over alkyl groups, including cyclohexyl. The ser-



Scheme 1. (a) 1 N HCl (aq), 3-aminocrotononitrile, reflux, 16 h; (b) BINAP, Cs_2CO_3, $Pd_2(dba)_3$, toluene, reflux, 16 h; (c) 1,3-dibromo-5,5-dimethylhydantoin, DCM, 0 °C then rt, 2 h; (d) $PdCl_2(dppf)$, toluene, dioxane, 2 N Na_2CO_3 (aq), 75 °C, 16 h; (e) NaOH, THF/MeOH/H_2O (v/v = 3:1:1), rt, 16 h (96%). R-groups are defined according to the examples in Table 1 and Table 2.

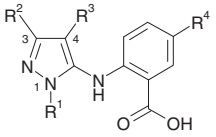
Table 1
SAR at the C-4 of the pyrazoles

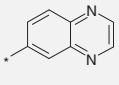
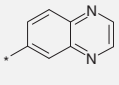
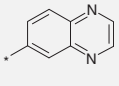
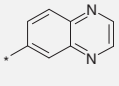
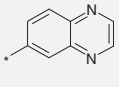
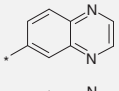
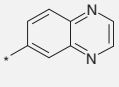
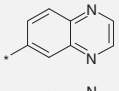
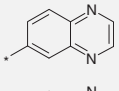
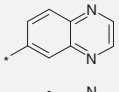
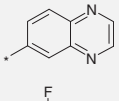
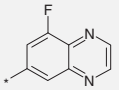


Compound	Ar	A_1 IC_{50}[a] [nM]
1		40
7		3.160
8		568
9		1.390
10		294
11		306
12		233
13		231
14		27
15		3.2

[a] IC_{50} data were determined in a cAMP functional assay, see Ref. 1; values are means of three experiments.

Table 2
Extended SAR of the pyrazoles



Compd	R^1	R^2	R^3	R^4	A_1 IC_{50}[a] [nM]	A_{2a} IC_{50}[a] [μM]	A_{2b} IC_{50}[a] [μM]	A_3 IC_{50}[a] [μM]
15		Me		OMe	3.2	>50	>50	13
16		Me		Cl	16	>50	>50	nd
17		Me		Me	5.1	>50	>50	nd
18		Me		H	8.0	>50	>50	nd
19		Me		OMe	2.8	5.5	1.1	nd
20		Me		OMe	435	>50	>50	nd
21		Me		OMe	7.3	>50	>50	nd
22		Me		OMe	6.4	>50	>50	nd
23		CF_3		H	9.0	>50	19	nd
24		Et		H	13	>50	>50	nd
25		H		H	277	>50	>50	nd
26		Me		H	0.6	>50	>50	>10

nd = not determined.
[a] IC_{50} data were determined in a cAMP functional assay, see Ref. 1; values are means of three experiments.

Table 3
Pharmacokinetic results for compounds **1**, **15** and **26** in Wistar rats[a]

Compound	CL_{blood}[b] [Lh^{-1} kg^{-1}]	$T_{1/2}$ [h]	F[c] [%]
1	4.00	1.5	20
15	2.62	0.4	20
26	0.57	1.8	97

[a] Intravenous administration of 1 mg kg^{-1} in 1% DMSO and 99% plasma; oral administration of 3 mg kg^{-1} in EtOH/PEG400/H_2O (1:4:5).
[b] Blood clearance.
[c] Oral bioavailability.

ies of methyl derivatives shows that mono-*ortho*-substitution is favored over disubstitution or the *meta*- and *para*-isomers (data not shown), but methyl can be replaced with almost no loss in potency by ethyl or chlorine (**22** and **21**). Additionally, selectivity over the A_{2a} and A_{2b} receptors is also mediated by the methyl substituent (compare **15** and **19**). Subsequently, SAR studies focused on variations at the C-3 position. Replacement of the methyl group by trifluoromethyl or ethyl was tolerated, however, introduction of a hydrogen at this position resulted in a significant loss in potency (see **25**). Finally, SAR investigations were carried out at the quinoxaline core. Fluorine-substitution at the C-5 position of the quinoxaline gave highly potent compounds (see **26**), compared with other substitution patterns (data not shown).

Because of its favorable PK profile (Table 3: low clearance, high bioavailability) compound **26** was progressed further to pharmacological in vivo investigations in rats. The diuretic and natriuretic activity in vivo of the compound was investigated after oral as well as intravenous application in two independent experiments each.[9] Data from the first experiment after iv application of 0.3, 1.0, and 3.0 mg/kg of **26** revealed significant diuretic action at 0.3 mg/kg

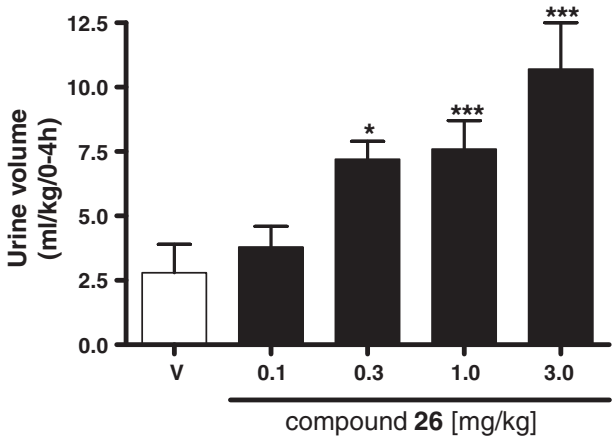


Figure 2. Diuretic activity of **26** in Wistar rats.

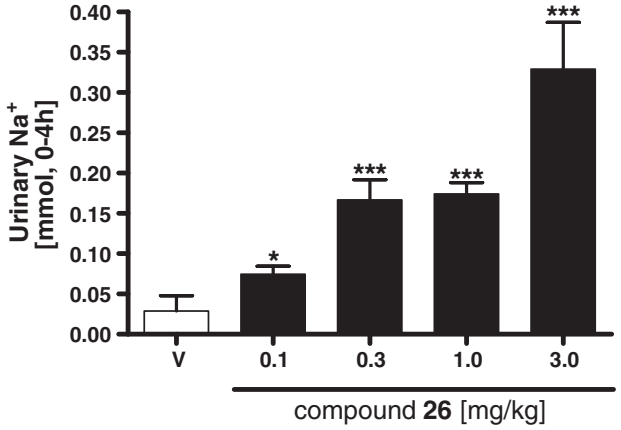


Figure 3. Natriuretic activity of **26** in Wistar rats.

(Fig. 2) as well as a significant increase in natriuresis at 0.1 mg/kg (Fig. 3).

In conclusion, we have identified a novel series of substituted 5-aminopyrazoles as potent and selective adenosine A_1 receptor antagonists. Lead optimization resulted in the identification of **26** with favorable PK properties suitable for in vivo studies. Lead **26** showed the expected profile of an A_1 receptor antagonist regarding diuresis and natriuresis. However, the recent failure of rolofylline (KW-3902) in the PROTECT trial[10] and the adjustment of tonapophylline (BG-9928) from phase III to phase II[11] have raised some concerns about the use of A_1 antagonists for the treatment of acute heart failure. Since these compounds are xanthine derivatives in contrast to our discovered class of 5-aminopyrazoles, it will be interesting to see whether non-xanthine-like A_1 antagonists will have a future in the treatment of acute heart failure.

Furthermore compound **26** may have a potentially superior safety profile compared to rolofylline and tonapophylline. On the one hand, rolofylline is able to cross the blood–brain barrier,[12] a fact that might be associated with pro-convulsive effects via inhibition of A_1 receptors in the central nervous system.[13] Whereas, compound **26** showed no pro-convulsive effects in relevant mouse models after iv application up to 30 mg/kg.[14] On the other hand, compound **26**, exhibited a significant higher selectivity versus the A_{2b} receptor compared with tonapophylline (A_{2b}/A_1 IC_{50} ratio: **26** >83.000, BG-9928 = 80, in-house data). One question in A_{2b} receptor antagonism is whether there are house-keeping pathways driven by A_{2b} receptor agonism whose disruption may lead to unexpected toxicities.[15]

Beyond acute heart failure, other potential targets of adenosine A_1 blockade include drug-induced renal injury (e.g., due to antibiotics [gentamicin], immunosuppressive agents [cyclosporine], or chemotherapy [cisplatin])[4] and acute kidney injury after cardiopulmonary bypass. Furthermore A_1 receptor antagonists may be useful in minimizing or preventing endotoxin-induced organ (e.g., lung) damage associated with sepsis as well as in the treatment of osteoporosis, prosthetic joint loosening.[16]

References and notes

1. Francis, G. C. *Clin. J. Med.* **2006**, *73*, S8.
2. Forman, D. E.; Butler, J.; Wang, Y.; Abraham, W. T.; O'Connor, C. M.; Gottlieb, S. S.; Loh, E.; Massie, B. M.; Rich, M. W.; Stevenson, L. W.; Young, J. B.; Krumholz, H. M. *J. Am. Coll. Cardiol.* **2004**, *43*, 61.
3. Fredholm, B. B.; Ijzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, J. *Pharmacol. Rev.* **2001**, *53*, 527.
4. Dohadwala, M. M.; Givertz, M. M. *Cardiovasc. Ther.* **2008**, *26*, 276.
5. Rudolph, J.; Cantin, L.-D.; Magnuson, S.; Bullock, W.; Bullion, A.-M.; Chen, L.; Chuang, C.-Y.; Liang, S.; Majumdar, D.; Ogutu, H.; Olague, A.; Qi, N.; Wickens, P. L. PCT Int. Appl., WO 2004050650, 2004.
6. (a) Hobbs, R. E. *Current Advances in Heart Disease*, Proceedings of the World Congress on Heart Disease, 14th International Academy of Cardiology Annual Scientific Sessions, Toronto, ON, Canada, July 26–29, 2008, 273–276.; (b) Doggrell, S. A. *Curr. Opin. Invest. Drugs* **2005**, *6*, 962.
7. (a) For experimental details and characterization of the compounds see: (a) Baerfacker, L.; Kast, R.; Griebenow, N.; Meier, H.; Kolkhof, P.; Albrecht-Kuepper, B.; Nitsche, A., Stasch, J.-P.; Schneider, D.; Teusch, N.; Rudolph, J.; Whelan, J.; Bullock, W.; Pleasic-Williams, S. PCT Int. Appl., WO 2010020363, 2010.; (b) Baerfacker, L.; Kast, R.; Griebenow, N.; Meier, H.; Kolkhof, P.; Albrecht-Kuepper, B.; Nitsche, A.; Stasch, J.-P.; Schneider, D.; Teusch, N. PCT Int. Appl., WO 2010020366, 2010.; (c) Baerfacker, L.; Kast, R.; Griebenow, N.; Meier, H.; Kolkhof, P.; Albrecht-Kuepper, B.; Nitsche, A.; Stasch, J.-P.; Schneider, D.; Teusch, N. Ger. Offen. DE 102008039083, 2010.; (d) Baerfacker, L.; Kast, R.; Griebenow, N.; Meier, H.; Kolkhof, P.; Albrecht-Kuepper, B.; Nitsche, A., Stasch, J.-P.; Schneider, D.; Teusch, N. Ger. Offen. DE 102008039082, 2010.
8. Chen, W.; Koenigs, L. L.; Thompson, S. J.; Peter, R. M.; Rettie, A. E.; Trager, W. F.; Nelson, S. D. *Chem. Res. Toxicol.* **1998**, *11*, 295.
9. After seven days of acclimatization, male Wistar rats (body mass 200–350 g, Charles River Breeding or Harlan) were placed in metabolic cages (Tecniplast Deutschland GmbH, Hohenpeißenberg, Germany) on water ad libitum. The test compounds were administered either orally or intravenously in 1 ml/kg vehicle solution (40% PEG400 and 60% Tris buffer) to 6–8 animals per dosage group. The urine of the animals was collected at the bottom of the cages in special collecting tubes. The usual duration of urine collection was 4 h. The urine samples were analyzed for volume as well as sodium and potassium on a flame photometer.
10. (a) Weatherley, B. D.; Cotter, G.; Dittrich, H. C.; DeLucca, P.; Mansoor, G. A.; Bloomfield, D. M.; Ponikowski, P.; O'Connor, C. M.; Metra, M.; Massie, B. M. *J. Card. Fail.* **2010**, *16*, 25; (b) Details on the design of PROTECT are available at http://www.clinicaltrials.gov, identifiers NCT00328692 and NCT00354458.
11. A global phase III trial began in August 2008; the trial was closed in December 2009. By March 2010, the drug was no longer listed on Biogen's pipeline and the program was presumed to have been discontinued.
12. Pronounced blood–brain-barrier penetration of KW-3902 has been found in rat (in house data).
13. Observations from clinical trials suggest that KW-3902 might lower the seizure threshold. See: (a) Givertz, M. M.; Massie, B. M.; Fields, T. K.; Pearson, L. L.; Dittrich, H. C. *J. Am. Coll. Cardiol.* **2007**, *50*, 1551; (b) Givertz, M. M. *Circ. Heart Fail.* **2009**, *2*, 519.
14. Inhouse data from MES and PTZ models. MES: maximal electroshock. PTZ: pentylenetetrazol.
15. Zablocki, J.; Elzein, E.; Kalla, R. *Expert Opin. Ther. Patents* **2006**, *16*, 1347.
16. Giorgi, I.; Nieri, P. *Expert Opin. Ther. Patents* **2008**, *18*, 677.