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NO-Independent Stimulators of Soluble Guanylate Cyclase

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Abstract—SARs around a novel type of guanylate cyclase stimulator which act by a mechanism different from classical NO-donors are described. Several pyrazolopyridinylpyrimidines are shown to relax aortic rings and revealed a long-lasting blood pressure lowering effect in rats after oral application.

Introduction

Soluble guanylate cyclase (sGC)[1] is a heterodimeric (α/β) heme-protein that converts GTP to cGMP, which is an important messenger in signal transduction. Its natural stimulator is nitric oxide, which stimulates sGC via the formation of a nitrosyl-heme complex. Organic nitrates like glycerol trinitrate or isosorbide dinitrate have been used for decades as a treatment for angina pectoris. In vivo they are converted to NO which relaxes vascular smooth muscles. The major drawback of this therapy is the development of tolerance after repeated applications. Recently an indazole derivative, YC-1, has been described, which stimulates sGC directly via a distinct mechanism and sensitizes the enzyme towards its native activator NO.[2] We used YC-1 as the lead structure and selected the pyrazolopyridine Bay 41-2272 (**40**) as the most promising one out of a series of thousands of newly synthesized derivatives.

Chemistry[3]

Pd-catalyzed biaryl-coupling is the method of choice for the preparation of indazole-derived compounds. However this route gives low yields when applied to compounds possessing more *N*-heteroatoms. Therefore, in the case of pyrazolopyridinyl-pyrimidines we applied an alternative sequence, for which a representative example is shown in Scheme 1.

Pharmacology[4]

For biological characterization we measured IC_{50}s for the ability of the compounds to inhibit maximum constriction of phenylephrine-treated preconstricted (3×10^{-8} g/mL) rabbit aortic rings. In order to prove the mechanism of relaxation, the stimulatory effect on isolated recombinant sGC alone and in the presence of NO was measured. The in vivo efficacy was shown in anaesthetized rats after oral administration.[4]

Results and Discussion

We started the optimization process with the variation of the benzylic moiety of YC-1. At the methylene bridge no substitution like a carbonyl-oxygen or alkyl groups is tolerated. Omiting the methylene group with directly linked aromatics leads to inactive compounds. Table 1 reflects the influence of substituents at the aromatic part on the relaxation of preconstricted aortic rings. Fluorine proved to be preferable leading to compounds which show an increase in activity when changing the position of the substituent from 4 (**3**)→3 (**2**)→2 (**1**). Other halogens were less beneficial. The best activity was found with a 5-pyrimidinylmethyl residue (**10**); however because of its difficult synthetic accessibility and poor solubility we focused on the 2-fluorophenyl moiety for further optimization work.

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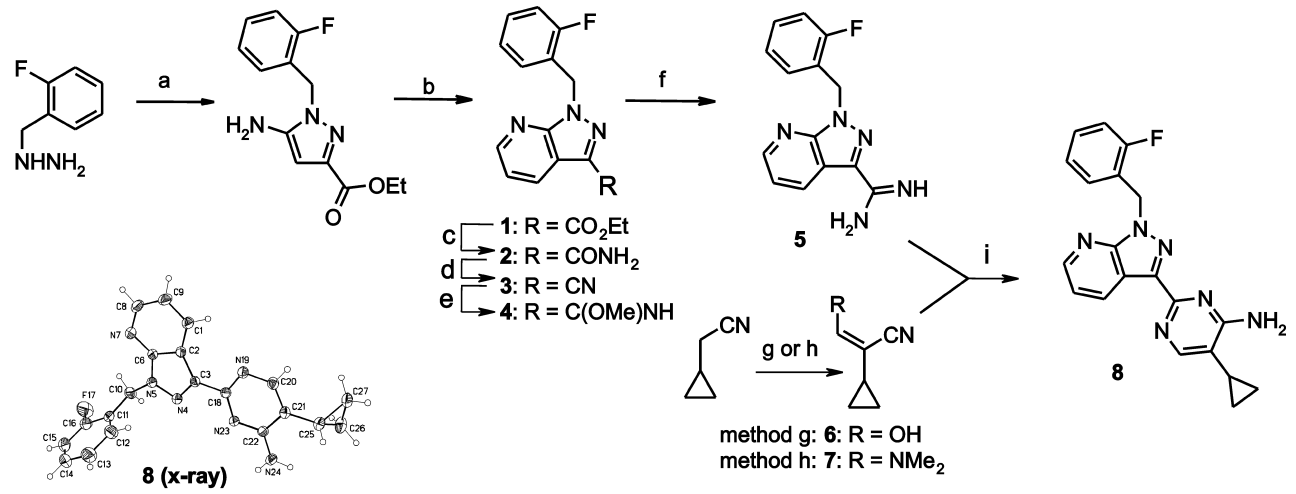
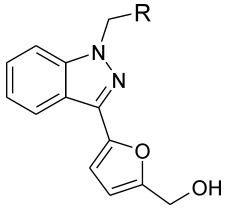


Scheme 1. Reagents and conditions: (a) NC-CH=C(ONa)CO_2Et/TFA, dioxane, reflux, 8 h; (b) Me_2NCH=CH-CHO/TFA/dioxane, reflux, 3 days, 50% for steps (a) and (b); (c) NH_3/MeOH, rt, 2 days, quant.; (d) TFAA/pyridine, pyridine, rt, 8 h, quant.; (e) MeONa/MeOH, rt, 2 h, quant.; (f) 1. NH_4Cl/HAc, MeOH, reflux, 8 h; 2. Na_2CO_3/H_2O, rt, 76.4%; (g) 1. HCO_2Et, *t*-BuOK, THF, rt, 3 h; 2. HCl→pH 4; (h) $[Me_2N]_2$CHO*t*-Bu, neat, 100 °C, 46 h, 83.5%; (i) **7**, neat, 100 °C, 12 h, 23.4%; or **6**, toluene, reflux, 8 h, 31.8%.

Concerning the pyrazol-type central moiety (see Table 2), among all the core heterocycles investigated the pyrazolo[3,4-*b*]pyridin ring system (**12**) seems to be the optimum with respect to in vitro effects, whereas indazoles usually are somewhat weaker.

As shown in Table 3, several heterocycles can replace the furyl moiety. Besides other five-membered ring systems like oxazoles (**20**–**22**), we synthesized a 2-pyridyl derivative (**25**) which had moderate activity. However, after replacing it with a 2-pyrimidinyl-residue (**26**), which displays rotational symmetry with respect to the heteroatoms, a marked increase in potency could be observed. In contrast to the furyl system no further increase in activity was achieved by introduction of a hydroxymethyl group (**27**). However, a 4-amino group significantly enhanced the activity (**28**).

Table 1. Variation of the *N*-substituent

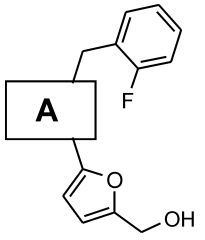


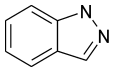
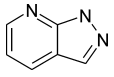
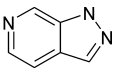
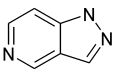
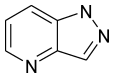
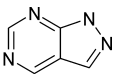
Compounds	R	Relaxation of preconstricted aortic rings IC_{50}, μM[a]
YC-1	Ph	10
1	2-F-Ph	4.9
2	3-F-Ph	10
3	4-F-Ph	19
4	2,6-F_2-Ph	15
5	2-CN-Ph	8.7
6	3-CN-Ph	26
7	2-(5-Chlorothiophene)	7.2
8	2-Pyridyl	16
9	3-Pyridyl	9.6
10	5-Pyrimidinyl	3.2

[a]Values are standardized to YC-1 = 10 μM.

We next investigated the SAR for various substituents at the aminopyrimidine (Table 4). A wide diversity of substituents is tolerated in the 5-pyrimidine position without considerably affecting the activity. For alkyl residues, there seems to be an optimum at 2–5 carbon

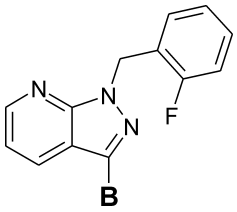
Table 2. Variation of the pyrazole type core heterocycle

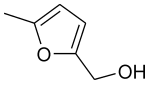
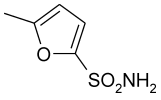
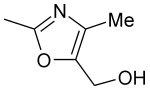
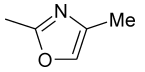
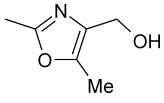
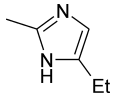
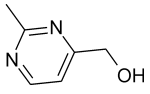
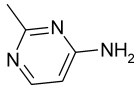


Compounds	Heterocycle A	Relaxation of preconstricted aortic rings IC_{50}, μM[a]
11		4.9
12		1.7
13		6.932
14		9.8[b]
15		20.4 (27%)[c]
16		9.4[d]

[a]Values are standardized to YC-1 = 10 μM.
[b]In combination with 5-hydroxymethyl-4-methyl-1,3-oxazol-2-yl instead of 5-hydroxymethyl-2-furyl (cf. compound **20** in Table 3).
[c]Percent of inhibition.
[d]In combination with benzyl instead of 2-fluorobenzyl.

Table 3. Variation of the attached heterocycle



Compounds	B	Relaxation of preconstricted aortic rings IC_{50}, μM[a]
17		1.7
18		9.3
19		8.0
20		0.9
21		1.2
22		1.5
23		3.2
24		0.6
25		4.8
26		1.25
27		1.3
28		0.27
29		1.8
30		8.6

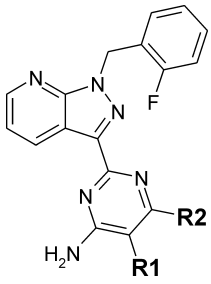
[a]Values are standardized to YC-1 = 10 μM.

chain length. With increasing steric demand and lipophilicity, the activity decreases; the introduction of polar residues generally leads to more potent compounds as compared to alkyl chains with similar steric demand.

We selected **40** for further investigations. The X-ray crystallographic analysis of **40** revealed a coplanar arrangement of the biaryl system (Scheme 1). Since this is a prerequisite for ligand function, we reasoned that these molecules might display metal-complexing properties. Indeed we were able to prepare a silver complex by adding $AgNO_3$ to **40** in acetonitrile. The X-ray crystal structure is shown in Fig. 1. The relevance of this phenomenon for the biological mechanism is still unclear. However, substituting the core heterocycle by an indole which lacks the ligand nitrogen leads to inactive compounds.

Compound **40** concentration-dependently stimulated isolated sGC from 0.1 nM–100 μM (Fig. 2). When given

Table 4. Variation of the pyrimidine substituents



Compounds	R1	R2	Relaxation of preconstricted aortic rings IC_{50} (nM)[a]
31	H	H	265
32	Me	H	675
33	Et	H	272
34	*n*-Propyl	H	252
35	*i*-Propyl	H	207
36	*n*-Butyl	H	218
37	*t*-Butyl	H	709
38	*n*-Pentyl	H	200
39	*n*-Hexyl	H	1041
40	Cyclopropyl	H	304
41	Cyclobutyl	H	633
42	Cyclopentyl	H	904
43	1-Cyclopentenyl	H	836
44	Cyclohexyl	H	561
45	CN	H	391
46	2-Cyanoethyl	H	412
47	5-Cyanopentyl	H	588
48	6-Cyanoheptyl	H	1294
49	OMe	H	343
50	SMe	H	243
51	SO_2Me	H	261
52	SO_2propyl	H	378
53	SO_2*i*-propyl	H	517
54	$PO(OEt)_2$	H	414
55	*N*-Morpholino	H	306
56	F	H	478
57	CH_2CF_3	H	508
58	*N*-Thiomorpholino	H	650
59	CH_2NH_2	H	1400
60	H	Me	536
61	Cyclopropyl	NH_2	233

[a]Values are standardized to YC-1 = 10 μM.

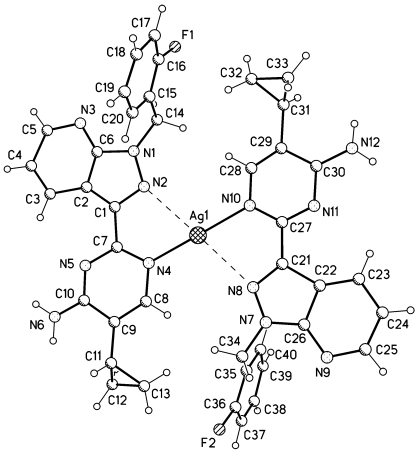


Figure 1. Single crystal X-ray structure of the silver (Ag(I)) complex of **40** (NO_3^--counterion not shown).

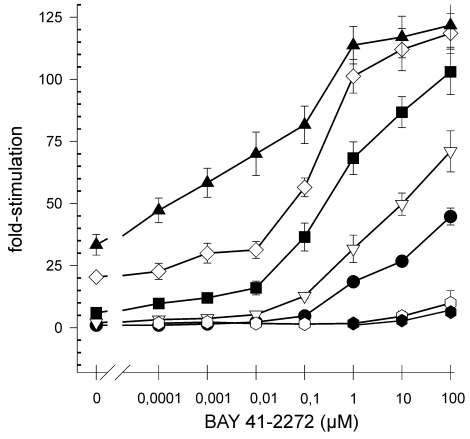


Figure 2. Stimulation of purified sGC by **40** (0.0001–100 μM) in the absence (-●-) and presence of SIN-1 (-▽- 0.01, -■- 0.1, -◇- 1, and -▲- 10 μM) and ODQ (-⬢- 10 μM). Stimulation of -⬡- heme-free sGC. The specific activity of sGC is expressed as *x*-fold stimulation versus basal activity (basal activity in the presence of Mg^{2+} 152 nmol/mg/min). The data presented represent means ± SEM, from four independent experiments performed in duplicate.

in combination, **40** and the NO donor SIN-1 revealed a strong potentiating effect over a wide range of concentrations. As shown in Fig. 2, **40** does not activate the heme-free enzyme. The sGC inhibitor ODQ completely inhibited the effect of **40** at intact sGC. Investigations concerning the molecular mechanism of action are published elsewhere.[5] After oral administration to anaesthetized rats it dose-dependently produced a long-lasting decrease in blood pressure (Fig. 3).

Our data demonstrate SARs of NO-independent sGC stimulators which may offer a novel approach for treatment of cardiovascular diseases.

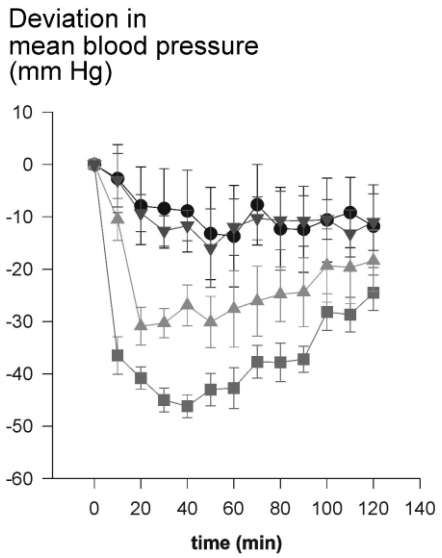


Figure 3. Effect of **40** on blood pressure of anesthetized rats: -●- Control ($n=6$); -▼- **40**: 0.3 mg/kg po ($n=4$); -▲- **40**: 1.0 mg/kg po ($n=5$); -■- **40**: 3.0 mg/kg po ($n=4$).

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3. (a) All final compounds were characterized by NMR and mass spectrometry. Crystallographic data for **40** and the Ag complex of **40** have been deposited at the Cambridge Crystallographic Data Centre (**40**: CCDC 157014; **40**-complex: CCDC 157015). For experimental details see Straub, A.; Feurer, A.; Alonso-Alija, C.; Stasch, J.-P.; Perzborn, E.; Hütter, J.; Dembowsky, K.; Stahl, E. WO 2,000,006,568, 2000. *Chem. Abstr.* **2000**, *132*, 122629. (b) Gordon, D. W. *Synlett* **1998**, 1065. (c) Collot, V.; Dallemagne, P.; Bovy, P. R.; Rault, S. *Tetrahedron* **1999**, *55*, 6917.

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