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Acidic triazoles as soluble guanylate cyclase stimulators

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ABSTRACT

A series of acidic triazoles with activity as soluble guanylate cyclase stimulators is described. Incorporation of the CF_3 triazole improved the overall physicochemical and drug-like properties of the molecule and is exemplified by compound **25**.

Soluble guanylate cyclase (sGC)[1] is a heterodimeric (α/β) heme-protein that converts guanosine-5′-triphosphate (GTP) to cyclic guanosine-3′5′-monophosphate (cGMP), 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 such as glycerol trinitrate or isosorbide dinitrate have been used as a treatment for angina pectoris. In vivo they are converted to NO which relaxes smooth muscles but develops tolerance on repeat dosing. Two mechanistic classes of direct stimulators of sGC have more recently been developed. One class of stimulators is NO-independent but heme-dependent, the other is both NO- and heme-independent.

The first reported direct stimulator of NO was YC-1,[2] followed by the discovery of more potent compounds such as BAY 41-8543 by Bayer[3] and their more recent clinical compound BAY 63-2521 (Fig. 1).[4] These compounds act synergistically with NO to potentiate stimulation of sGC and have been reported to bind to an allosteric binding site within the catalytic domain.

BAY 41-8543 and related compounds have a co-planar arrangement of the two heterocycles as demonstrated by X-ray analysis,[3] which seems a requisite for ligand function but could be detrimental to solubility. Neutral to weakly basic compounds can have a low volume of distribution which in turn could lead to short intrinsic half-life. Our medicinal chemistry strategy concentrated on keeping the potency and selectivity of these compounds while specifically addressing PK properties and solubility.

Changing the pendant heterocycles such as the furan in YC-1 and the diaminopyrimidine in BAY 41-8543 to heterocycles containing weakly acidic hydrogens should improve solubility at physiological pH while keeping the required functionality required for potency. However, initial reports on acidic heterocycles such as tetrazole showed they were not particularly active.[3] 1,2,4-Triazoles represented an attractive starting place because the pK_a of NH on the triazole can be modulated depending on the C_3 and C_5 substituents. Overall volume of distribution (V_d) of these slightly acidic compounds, however, is likely to be low (0.2–1.0 L/kg) so

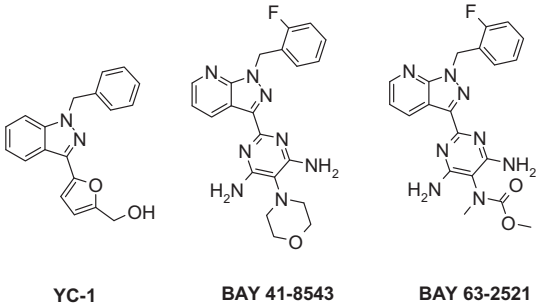

Figure 1. sGC stimulators YC-1, BAY41-8543, and BAY 63-2521.

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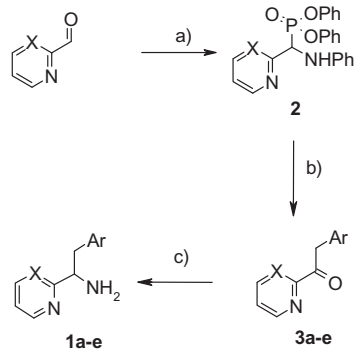
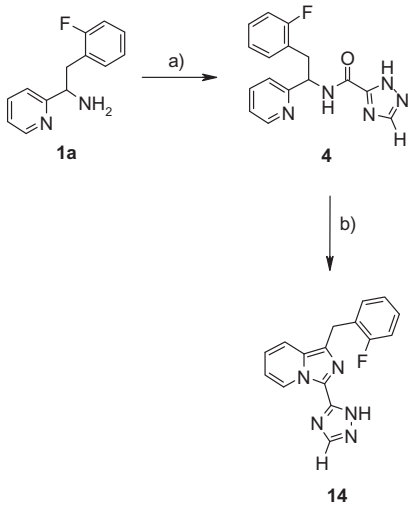


Scheme 1. Reagents and conditions: (a) PhO_2POH, $PhNH_2$, iPrOH, 50 °C; (b) (i) Cs_2CO_3, ArCHO, iPrOH, THF then (ii) HCl; (c) (i) $NH_2OH{\cdot}HCl$, NaOH, EtOH, H_2O, 75 °C; (ii) Zn, NH_4OAc, EtOH, H_2O, NH_4OH, 80 °C.



Scheme 2. Reagents and conditions: (a) triazole CO_2H, water soluble carbodiimide, HOBT, DMF, NMM; (b) $POCl_3$, Et_3N, DCM.

modifications to the core template and the northern fragment to vary physicochemical properties to address clearance is also key for a good ADMET profile.

The imidazopyridines and related imidazopyrimidines **14–22** and **25–27** were constructed from the key amine intermediates **1a–e** (**1a** X = C—H and Ar = 2-F—Ph, **1b** X = N and Ar = 2-F—Ph, **1c** X = C—H and Ar = 5-pyrimidine, **1d** X = C—H and Ar = 2-methyl-5-pyrimidine, and **1e** X = C—F and Ar = 2-methyl-5-pyrimidine). These were synthesized *via* a modified Horner–Emmons condensation of an α-aminophosphonate ester **2** with an aldehyde followed by hydrolysis of the resultant enamine to give the intermediate ketones **3a–e**.[5] Simple oxime formation from the ketone followed by reduction with zinc and ammonium acetate gave amines **1a–e** (Scheme 1).

From amines **1a–e**, test compounds could be synthesized in either two ways. For compound **14**, **1a** was reacted with commercially available triazole carboxylic acid using water soluble carbodiimide and HOBT to give an intermediate amide **4** which was cyclized with $POCl_3$ to give the triazole (Scheme 2).

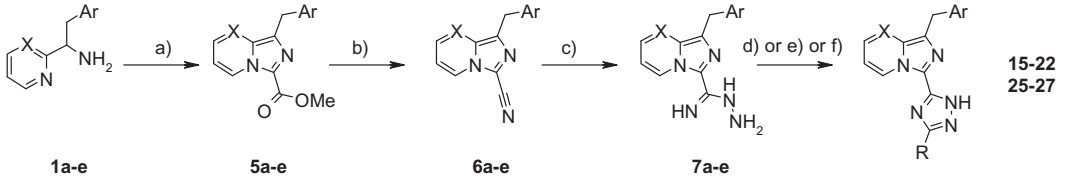
The other compounds were made using an alternative procedure (Scheme 3) as the substituted triazole carboxylic acids were not commercially available. The route involved some functional group manipulation but the chemistry was very straight forward. The first step involved addition of methyl oxalyl chloride to amines **1a–e** followed by cyclo-condensation with $POCl_3$ to give esters **5a–e**.[6] These esters were converted to the amides by aminolysis and dehydrated to nitriles **6a–e** using TFAA in pyridine.[7] The nitriles were reacted with hydrazine to give the versatile late stage amidrazone intermediates **7a–e**,[8] which were then cyclo-condensed with a number of carboxylic acids to give the substituted triazoles **15–19**, **22**, and **25–27**.

Methylsulfone analog **20** was synthesized by addition of 1,1′-thiocarbonyldiimidazole to the amidrazone **7a** followed by methylation with methyl iodide and finally oxidation with Oxone™. Amide **21** was synthesized by first reacting amidrazone **7a** with ethyl oxalylchloride followed by aminolysis of the resultant ester.

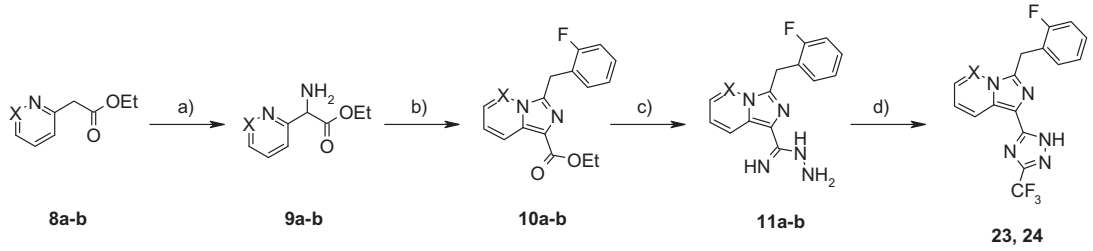
The reversed imidazopyridazine **23** (X = N) and imidazopyridine **24** (X = C—H) were synthesized according to Scheme 4. Ethyl 2-pyridylacetate and ethyl 3-pyridazinylacetate **8a–b** were each converted to the amino esters **9a–b** by nitration[9] followed by hydrogenation of the oxime to the amine in the presence of HCl due to stability of the free base. The amine was reacted with 2-fluorophenylacetic acid and water soluble carbodiimide then cyclo-condensed with $POCl_3$ to give esters **10a–b**. The ester was hydrolyzed to the acid, converted to the primary amide, dehydrated to the nitrile and finally converted to the amidazone **11a–b** in a similar sequence to that shown in Scheme 3. **11a–b** were then reacted with trifluoroacetic anhydride (TFAA) to give the CF_3 triazoles **23** and **24**.

Compounds **28** and **29** were synthesized from late stage nitrile intermediates **12a–b** previously described in the literature,[3] converted into the amidrazones **13a–b** through a similar sequence shown in Scheme 3 then finally reacted with TFAA to give the CF_3 triazoles (Scheme 5). The primary screen was a cell based enzyme assay and the more active compounds tested in isolated rat aorta (Table 1).[10,11]

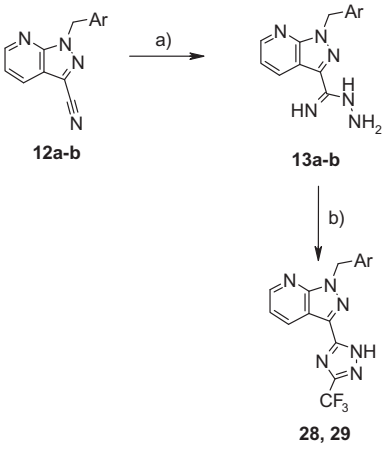
The pyrazolopyridine core was changed to an imidazopyridine in the first instance because it was novel, kept the key nitrogen required for activity based on previous SAR and was synthetically accessible. Simple alkyl substituents on the triazoles (compounds **14**–**19**) gave a range of potencies, while the more polar electron withdrawing groups such as the sulfone **20** and primary amide **21** being fairly weak. The CF_3 triazole **16** had the best balance of potency and physicochemical properties, with the pK_a of the NH around 6.5 and good potency in isolated rat aorta.



Scheme 3. Reagents and conditions: (a) (i) MeOCOCOCl, Hunig's base, DCM; (ii) $POCl_3$, 100 °C; (b) (i) NH_3, MeOH; (ii) TFAA, pyridine, THF; (c) $NH_2NH_2{\cdot}H_2O$, MeOH. For compounds **9**–**16**, **19**–**21**; (d) RCO_2H, 120 °C. For compound **17;** (e) (i) 1,1′-thiocarbonyldiimidazole, DBU, dioxane, 80 °C; (ii) MeI, K_2CO_3, DMF; (iii) Oxone™, MeOH, H_2O. For compound **18** (f) (i) EtOCOCOCl, Hunig's base, DCM; (ii) 7 M NH_3, MeOH, 100 °C.



Scheme 4. Reagents and conditions: (a) (i) $NaNO_2$, AcOH, H_2O, 0 °C to rt; (ii) 10%Pd/C, HCl, EtOH, H_2, 50 psi; (b) (i) 2F-$PhCH_2CO_2H$, water soluble carbodiimide, HOBT, Hunig's base, DCM; (ii) $POCl_3$, heat; (c) (i) $LiOH{\cdot}H_2O$, THF, H_2O; (ii) $(COCl)_2$, Et_3N, DCM, DMF; (iii) 880 NH_3, dioxane; (iv) $(CF_3SO_2)_2O$, DCM, Et_3N, 0 °C to rt; (v) $NH_2NH_2{\cdot}H_2O$, MeOH, rt; (d) TFAA, rt.

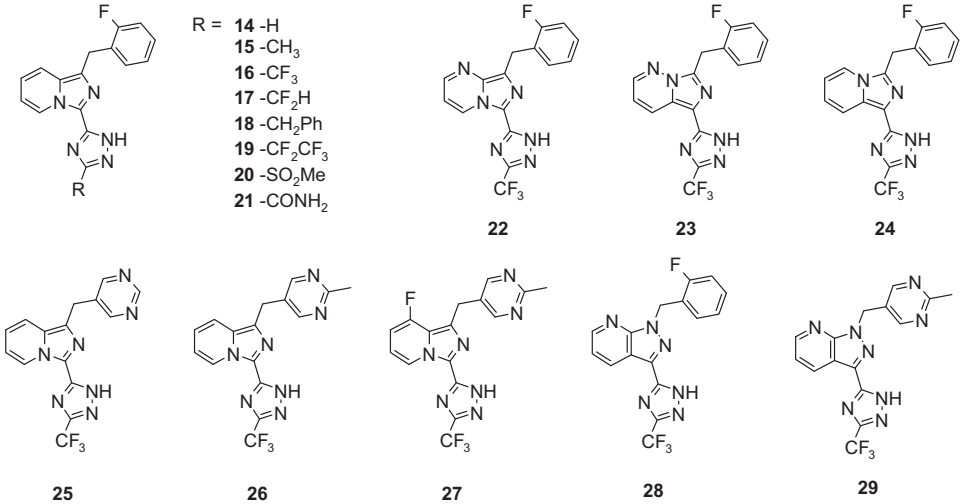


Scheme 5. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, MeOH, rt; (b) TFAA, rt.

The imidazopyridine core was modified by moving/adding nitrogens to the ring to give compounds **22**–**24**. Potency did improve for compound **23** but not for the other variants. Modification of the 2-fluorophenyl group to the pyrimidine **25** gave an improvement in terms of solubility and ADME properties without having a deleterious effect on potency presumably driven by the lower log*D*. The biggest problem with this compound was its potent CYP1A2 inhibition likely caused by its flat conformation and the accessible unflanked nitrogens on the pyrimidine ring. Sterically hindering the two nitrogens by placement of a methyl group between them (compound **26**) mitigated the CYP1A2 liability, however, the compound was less potent. Placing a fluorine on the pyridine ring in the 6-position to give compound **27** brought back the potency without greatly affecting the overall properties.

As comparators, compounds **28** and **29** were made using the pyrazolopyridine core with the CF_3 triazole attached. The 2-fluorophenyl group was replaced with the methylpyrimidine in compound **29**. The potency of these compounds were very good and

Table 1
Structures and data for compounds **14**–**29**



Compound	hsGC[a] EC_{10}, nM	hsGC[a] EC_{20}, nM	Rat aorta[b] IC_{50}, nM	Log $D_{7.4}$[c]	RRCK[d]	HLM Cl_{int}[e] μL/min/mg	Solubility[f]	CYP1A2 Inhibition at 3 μM[g]
YC-1	3360	–	–	3.7	6	212	14	69%
BAY 41-8543	44	11	11	3.1	ND	ND	<1	ND
14	2800	–	–	3.3	8	175	19	39%
15	393	–		3.3	1	124	58	23%
16	470	281	40	3.8	6	72	ND	57%
17	507	–	–	3.8	0.2	62	<0.3	49%
18	840	554	–	(4.9)	0.5	ND	<0.3	29%
19	1170	–	–	4.2	3	92	<0.3	31%
20	1340	–	–	1.2	ND	11	190	3%

(*continued on next page*)

Table 1 (*continued*)

Compound	hsGC[a] EC_{10}, nM	hsGC[a] EC_{20}, nM	Rat aorta[b] IC_{50}, nM	Log $D_{7.4}$[c]	RRCK[d]	HLM Cl_{int}[e] μL/min/mg	Solubility[f]	CYP1A2 Inhibition at 3 μM[g]
21	2810	–	–	2.8	13	<8	2	16%
22	724	221	–	2.5	28	17	32	17%
23	224	–	–	(2.2)	ND	ND	ND	ND
24	658	1070	–	3.9	17	20	ND	66%
25	210	80	60	1.8	19	<8	195	82%
26	366	245	–	2.1	27	<8	17	5%
27	–	73	–	2.0	23	<8	ND	15%
28	60	22	33	3.2	20	<11	6	20%
29	–	58	–	(1.3)	(27)	<7	59	<5%

ND, not determined.
[a] See Ref. 10 for details.
[b] See Ref. 11 for details.
[c] Log *D* measured in octanol:pH 7.4 buffer (clog *D* value given in parenthesis).
[d] RRCK assessment of passive permeability PappAB 10^{-6} cm/s (value in parenthesis are from PAMPA screen).
[e] Cl_{int} is the intrinsic metabolic clearance in microsomes in μL/min/mg of microsomal protein.
[f] Pseudo-thermodynamic solubility assay at pH 6.5.
[g] Cytochrome P450 CYP1A2 % inhibition.

Table 2
Selected data and rat pharmacokinetics of **25** and **28** dosed at 2 mg/kg iv and po

	16	**28**
Log $D_{7.4}$	1.8	
HLM, Cl_i μL/min/mg	<7	
RLM, Cl_i μL/min/mg	<16	
hERG activity	>10 μM	
Cerep/Bioprint™	>10 μM	
Rat PK	Cl 9.9 ml/min/kg[a] V_d 3.7 L/kg[b] $T_{1/2}$ 4.1h[c] *F* (%) 61%[d]	Cl 8.0 mL/min/kg V_d 3.1 L/kg $T_{1/2}$ 4.7 h *F* (%) 66%

[a] In vivo clearance after iv dosing.
[b] Volume of distribution at steady state after iv dosing.
[c] Half-life after iv dosing.
[d] Bioavailability after oral dosing.

compound **29** looks attractive from a physicochemical perspective as it had good HLM stability, good solubility with no CYP1A2 activity.

As compound **25** had an attractive profile in terms of potency in rat aorta and good solubility, it was investigated further. It was found to be selective (all IC_{50} values >10 μM) in wide-ligand profiling over a range of >70 targets including PDE's (CEREP, Bioprint™, http://www.cerep.fr and Dundee Kinase). The pharmacokinetics of **25** and **28** were also determined in rat (Table 2).

Compound **25** was progressed to in vivo studies and shown to be efficacious in the conscious SHR model of hypertension.[12]

In summary, optimization of a series of triazole containing sGC stimulators is described. A combination of the acidic CF_3 triazole group coupled with a pyrimidine gave compounds having sGC activity with a good pharmacokinetic profile.

Acknowledgments

We also thank Nicola Lindsay for ADME assessment of all compounds and Mark Lewis for running rat PK.

References and notes

1. Hobbs, A. J.; Stasch, J.-P. In *Nitric Oxide*; Ignarro, L. J., Ed., 2nd ed.; Elsevier: New York, 2010; p 301.
2. Ko, F. N.; Wu, C. C.; Kuo, S. C.; Lee, F. Y.; Teng, C. M. *Blood* **1994**, *84*, 4226.
3. Straub, A.; Stasch, J.-P.; Alonso-Alija, C.; Benet-Buchholz, J.; Ducke, B.; Feurer, A.; Fuerstner, C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 781.
4. Mittendorf, J.; Weigard, S.; Alsono-Alija, C.; Bischoff, E.; Feurer, A.; Gerisch, M.; Kern, A.; Knorr, A.; Lang, D.; Muenter, K.; Radtke, M.; Schirok, H.; Schlemer, K.-H.; Stahl, E.; Straub, A.; Wunder, F.; Stasch, J.-P. *ChemMedChem* **2009**, *4*, 853.
5. Journet, M.; Cai, D.; Larsen, R. D.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 1717.
6. Ford, N. F.; Browne, L. J.; Campbell, T.; Gemenden, C.; Goldstein, R.; Gude, C.; Wasley, J. W. F. *J. Med. Chem.* **1985**, *28*, 164.
7. Subhas Bose, D.; Jayalaskshmi, B. *Synthesis* **1999**, *1*, 64.
8. Neilson, D. G.; Roger, R.; Heatlie, J. W. M.; Newlands, L. R. *Chem. Rev.* **1970**, *70*, 151.
9. Van, Zyl G.; DeVries, D. L.; Decker, R. H.; Niles, E. T. *J. Org. Chem.* **1961**, *26*, 3373.
10. (a) An LC–MS enzyme assay method was used to measure production of the cyclic nucleotide, cGMP from the activation of sGC in the presence of a nitric oxide (NO) donor, SIN-1 (Biotium Inc., California). The assay evaluated the ability of human sGC to catalyze the conversion of GTP to cGMP, which was subsequently detected and quantified by LC–MS. This assay determined an EC_{10} value (the concentration of chemical causing a 10% activation relative to a standard compound) for each compound. (b) A FP assay was used to measure the activation of human soluble guanylate cyclase (sGC) in vitro using purified enzyme in the presence of the NO donor, SIN-1. The sGC enzyme converts GTP to cGMP, which binds a cGMP specific antibody included in the reactions. This assay determined an EC_{20} value (the concentration of chemical causing a 20% activation relative to a standard compound) for each compound.
11. Compounds elicited a relaxation of aortic rings by enhancing the cGMP signal evoked by a stable exogenous NO-donor, DETA-NO. An EC_{50}, with 95% confidence intervals, for compound-evoked relaxation was calculated as an index of potency. Male Sprague–Dawley rats (250–350 g) were asphyxiated by CO_2 gas and their thoracic aorta carefully excised and placed in Krebs buffer. The aortas were then carefully dissected free of connective tissue and divided into eight sections, each 3–4 mm in length. Aortic rings were suspended between parallel stainless steel wires in a water jacketed (37 °C), 15 mL tissue bath under a resting tension of 1 gram. Tension was measured using isometric tension transducers and recorded using Ponemah tissue platform system. Each preparation was allowed to equilibrate for at least 60 min prior to drug testing. During this time, the tissues were also incubated with 200 μM L-NMMA, and the incubation media changed every 15–20 min (L-NMMA was added after each wash to maintain the final concentration at 200 μM in each tissue bath). Following the equilibration period, baseline tensions were recorded for each tissue. The vasoconstrictor response to phenylepherine (1 μM) was assessed and when the response to phenylepherine reached a maximum, vascular reactivity was subsequently assessed by a challenge of acetylcholine (1 μM). Following another washout period, a second baseline value was recorded, the vasoconstrictor noradrenaline (25 nM) was added to each bath and the tissues incubated for a time period (~15 min) to achieve a stable tone. An exogenous NO drive was supplied using the stable NO-donor, DETA-NO. The concentration of DETA-NO was titrated (cumulatively in half-log increments) to achieve approximately 5–15% relaxation of the noradrenaline-evoked preconstriction. Cumulative concentration–response curves were constructed in a single ring, typically using 5 doses/ring and allowing 15 min between each addition.
12. This will be published on in the near future.