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# The design and synthesis of YC-1 analogues as probes for soluble guanylate cyclase

Kirk W. Hering,[a] Jennifer D. Artz,[b,c] William H. Pearson[d] and Michael A. Marletta[b,c,*]

[a]*Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA*
[b]*Department of Chemistry, University of California, Berkeley, CA 94720, USA*
[c]*Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA*
[d]*Berry & Associates, Inc., 2434 Bishop Circle East, Dexter, MI 48130, USA*

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**Abstract**—Soluble guanylate cyclase (sGC) is highly activated in the presence of both YC-1 (1-benzyl-3-(5′-hydroxymethyl-2′-furyl)-indazole) and CO. In this report, the design, synthesis, and activity (i.e., sGC activation) of photolabile analogues of 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1) are presented. Initial results with 6-azido-3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole led to the synthesis of a tritium-labeled analogue. When photoactivated, this analogue labeled the α-subunit of sGC.
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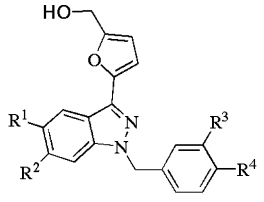
Soluble guanylate cyclase (sGC) catalyzes the conversion of GTP to cGMP and pyrophosphate. sGC is a heterodimeric hemoprotein that is activated several hundred-fold over the basal activity upon the binding of nitric oxide (NO). In the rat, sGC is comprised of an 80 kDa subunit and a 70 kDa subunit, and contains a heme protoporphyrin IX cofactor that serves as the binding site for NO. The cGMP formed in this reaction serves as a second messenger regulating several cellular events including smooth muscle relaxation,[1] platelet aggregation,[2] and neuronal communication.[1] sGC can also be activated 3- to 5-fold upon binding of carbon monoxide (CO) to the heme, or 8- to 12-fold in the presence of the benzylindazole derivative YC-1 [3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole], a known activator of sGC.[3] However, YC-1 and CO together act synergistically to activate the enzyme to a level similar to that observed with NO.[4,5] A variety of experimental approaches have been utilized in attempts to locate the YC-1 binding site within sGC including a site-directed mutagenesis study,[6] resonance Raman studies,[7,8] a photoaffinity labeling study,[9] and a deletion mutagenesis study,[10] to name a few. However, several of these reports have yielded conflicting results and thus the site of action of YC-1 has remained an open question. YC-1 has been shown to be efficacious in vivo, hence interest in this activator has remained high. We report here the synthesis of YC-1 and the photolabile YC-1 analogues **1**–**4** shown in Figure 1 that were designed as photoaffinity labels for sGC. Compound **2** was found to synergistically activate sGC in the presence of CO and was found to covalently bind to the α-subunit of sGC upon irradiation at 254 nm.

*Design and synthesis of azido YC-1 derivatives (compounds **1**–**4**).* Azides exhibit some undesirable properties for use in photoaffinity labeling studies in that they typically require irradiation at 254 nm, a wavelength that is




| compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| YC-1 | H | H | H | H |
| 1 | $N_3$ | H | H | H |
| 2 | H | $N_3$ | H | H |
| 3 | H | H | $N_3$ | H |
| 4 | H | H | H | $N_3$ |

**Figure 1.** Photolabile YC-1 analogues synthesized.

*Keywords*: Soluble guanylate cyclase; Nitric oxide; YC-1.
* Corresponding author. Tel.: +1 510 643 9325; fax: +1 510 643 9388; e-mail: marletta@berkeley.edu

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potentially damaging to hemoproteins, and they frequently exhibit a high degree of nonspecific binding.[11] However, azides are one of the smallest known photolabile moieties and the analogue design used here allowed for flexibility in placement around the YC-1 structure. This strategy should maximize the probability of mapping residues that make up the YC-1 binding pocket.

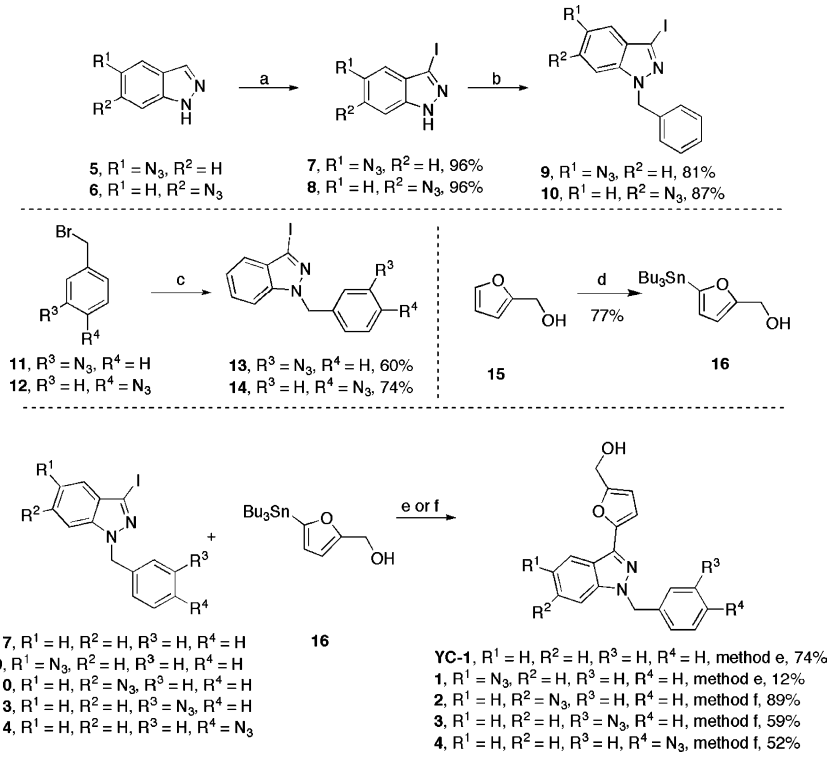
YC-1 and the YC-1 analogues **1**–**4** were synthesized as described in Scheme 1. The known compounds 5-azidoindazole (**5**) and 6-azidoindazole (**6**)[12] were treated with iodine in the presence of base by the method of Collot[13] to furnish the corresponding 3-iodoazidoindazoles **7** and **8** in excellent yields. Treatment of **7** and **8** with benzyl bromide, tetra-*n*-butylammonium iodide, and potassium *t*-butoxide furnished the Stille-coupling precursors **9** and **10** in 81% and 87% yields, respectively. Azides **13** and **14** were similarly prepared by reaction of 3-iodoindazole[13] with the known *m*- and *p*-azidobenzyl bromides[14] **11** and **12**, respectively. The furyl-coupling component **16** was easily obtained from furfuryl alcohol (**15**) by treatment with two equivalents of *n*-butyllithium, followed by quenching with tri-*n*-butyltin chloride. Reaction of stannane **16** with 1-benzyl-3-iodoindazole[13] (**17**) by the palladium-catalyzed cross-coupling method of Farina and Roth[15] furnished a 74% yield of YC-1. A similar reaction between stannane **16** and iodoindazole **9** resulted in only a 12% yield of the desired product **1**. This is likely due to a competing reaction of $Ph_3As$ with the azide in a manner analogous to the Staudinger reaction as reported by Cadogan and Gosney.[16] However, running the reaction at room temperature for a greater period of time allowed for selective palladium-catalyzed cross-coupling over arsinimine formation, and thus good yields of analogues **2**, **3**, and **4** were obtained by this route.

*Effect of YC-1 and novel azido YC-1 derivatives (compounds **1**–**4**) on sGC activity.* Analogues that compete at the YC-1 binding site would be expected to activate like YC-1 or act as competitive inhibitors of YC-1. Assays of analogues **1**–**4** at 100 μM with sGC in the presence and absence of CO gave the results shown in Table 1. Compounds **2** [6-azido-3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole] and **4** slightly activate sGC (3.4- and 4.7-fold, respectively) and act synergistically in the presence of CO, activating 29-fold and 20-fold, respectively. For comparison, the activation of sGC by

**Table 1.** Fold activation of sGC by YC-1 and compounds **1**–**4** (100 μM)[a]

| Compound | No CO | +CO |
|---|---|---|
| None | 1 | 3.9 ± 1.0 |
| YC-1 | 9.2 ± 0.1 | 139 ± 9 |
| **1** | 1.7 ± 0.2 | 4.4 ± 1.3 |
| **2** | 3.4 ± 0.1 | 29 ± 2.3 |
| **3** | 2.0 ± 0.2 | 7.1 ± 0.7 |
| **4** | 4.7 ± 0.1 | 20.3 ± 8.0 |

[a] Purified sGC was obtained from a baculovirus/SF9 expression system as described;[18] end point assays were performed with concentrations of DMSO of 4% v/v as described;[7] all errors were derived from the mean + range/2 for triplicate 2 min assays. Basal activity was 33 ± 3 nmol/min/mg ($n$ = 3).



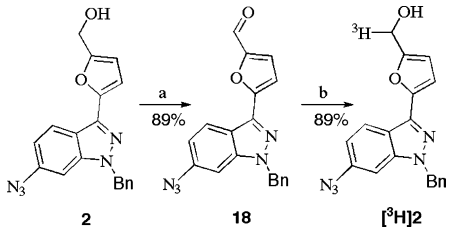

**Scheme 1.** Synthesis of YC-1 and azide-containing YC-1 analogues. Reagents and conditions: (a) $I_2$, KOH, DMF; (b) BnBr, KOt-Bu, $Bu_4NI$, THF; (c) 3-iodoindazole, KOt-Bu, $Bu_4NI$, THF; (d) (i) 2 equiv BuLi, THF, −78 °C then −20 °C, 2 h; (ii) $Bu_3SnCl$, THF, −78 °C then rt, 16 h; (e) $Pd_2(dba)_3$, $AsPh_3$, DMF, 1 h, 100 °C; (f) $Pd_2(dba)_3$, $AsPh_3$, DMF, 3 days, rt.

YC-1 was 9.2-fold in the absence of CO and 139-fold in the presence of CO. Compound **2** was chosen for further studies based on the observed activity and the novel placement of the photolabile moiety on the YC-1 skeleton.

In order to determine the potency of **2**, the effect of 50 μM **2** on YC-1 activation of sGC–CO was determined (Fig. 2). These data were fit to a standard four-parameter logistic equation (sigmoid) with variable slope and the calculated $EC_{50}$ values of sGC–CO activation by YC-1 in the absence and presence of **2** were determined to be $14.5 \pm 4.4$ μM and $21.9 \pm 8.7$ μM, respectively. The results show that **2** is either a very weak inhibitor of YC-1 action or consistent with it acting at a distinct site (see below).

Koesling and coworkers report that the addition of YC-1 to a solution of sGC–CO in the presence of GTP causes the Soret absorption peak to shift from 424 nm to 420 nm.[17] Examination of the sGC–CO heme Soret with **2** showed that it did not act like YC-1, in that **2** did not shift the heme Soret from 424 nm for sGC–CO in the presence or absence of GTP (data not shown).

*Photoaffinity labeling studies using compound [$^3$H]2.* The synergistic activation of sGC in the presence of CO and **2** provided a compelling case for further study, therefore, photoaffinity labeling studies were pursued. Tritium labeled **2** was prepared as shown in Scheme 2. The alcohol **2** was oxidized to the aldehyde **18** by treatment with $MnO_2$. Preliminary studies showed that reaction of **18** with $NaBH_4$ resulted in reduction of both the aldehyde and the azide moieties. However, it was found that treatment with $CeCl_3$ and $NaBH_4$ allowed for selective reduction of the aldehyde in the presence of the azide. Thus, reaction of aldehyde **18** with [$^3$H]$NaBH_4$ and $CeCl_3$ gave an excellent yield of [$^3$H]**2** with a specific activity of 533.3 Ci/mol.




**Scheme 2.** Synthesis of tritium-labeled **2**. Reagents and conditions: (a) $MnO_2$, $CH_2Cl_2$, rt, 20 h, 89%; (b) [$^3$H]$NaBH_4$, $CeCl_3$, MeOH/$CH_2Cl_2$, rt, 5 min, 89%.

sGC and [$^3$H]**2** were irradiated at 254 nm and 20 °C in the presence and absence of either a 10-fold excess of unlabeled **2** or a 10-fold excess of YC-1, under argon with the lamp 1 cm above the solution for 10 min. Samples were then separated by SDS–PAGE, fixed in EN$^3$HANCE Autoradiography Enhancer, dried, and exposed to X-ray film for 7 days. The results of this study, shown in Figure 3, indicate that [$^3$H]**2** bound primarily to the α-subunit of sGC, similar to the result reported by Stasch et al. in a related photoaffinity study of sGC with the YC-1-like compound BAY 51-9491.[9] In addition, these data indicated that unlabeled **2** almost completely competes out the binding of [$^3$H]**2**, suggesting a specific site of interaction. However, these results also show that YC-1 has little or no competition with [$^3$H]**2**, giving further evidence that **2** could bind in an alternate site on sGC than

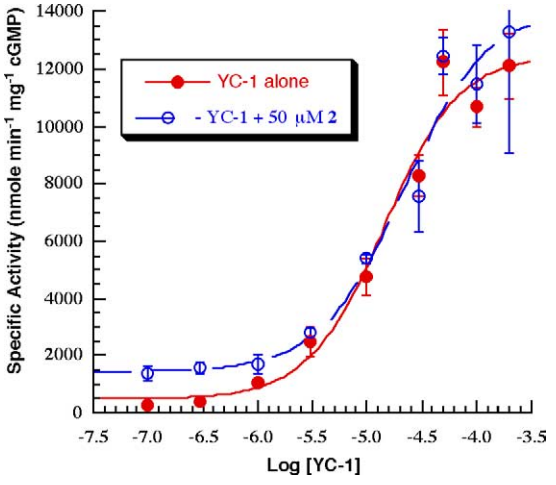



**Figure 2.** Activation of sGC by a range of YC-1 concentrations in the presence and absence of 50 μM **2**. Purified sGC was obtained from a baculovirus/SF9 expression system as described;[18] end point assays were performed with concentrations of DMSO of 4% v/v as described;[7] all errors were derived from the mean + range/2 for triplicate 2 min. assays. The data were fit with a standard four-parameter logistic equation (sigmoid), and the calculated $EC_{50}$ values of sGC–CO activation by YC-1 in the absence and presence of **2** were determined to be $14.5 \pm 4.4$ and $21.9 \pm 8.7$ μM, respectively.

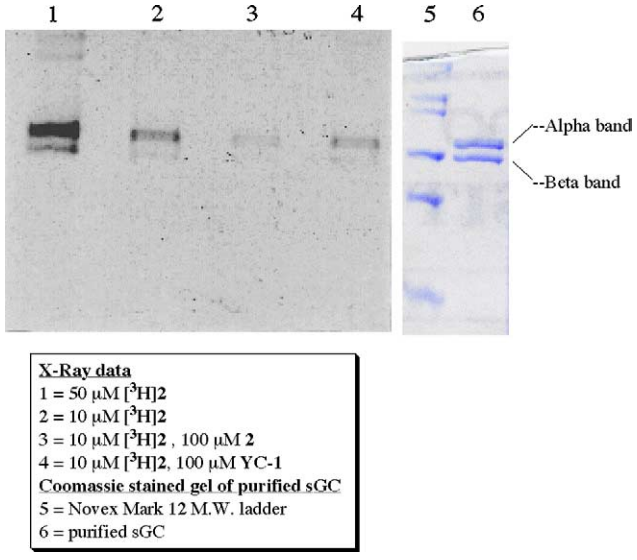



**Figure 3.** Autoradiograph of [$^3$H]**2** (50 μM, 26.7 mCi, lane 1; 10 μM, 5.3 mCi, lanes 2–4) labeled sGC (25 μg) in the presence and absence of 100 μM **2** (lane 3) and 100 μM YC-1 (lane 4) and Coomassie stained gel of Novex Mark 12 M.W. ladder (lane 5) and purified sGC (lane 6) after separation by SDS–PAGE.

YC-1. The latter result is particularly important since it shows that there are distinct sites on sGC for small molecule activators that could be exploited in drug design.

In conclusion, a number of photolabile compounds structurally related to YC-1 were synthesized and their activation of sGC was determined. Although, none of the synthesized analogues were as effective as YC-1 in terms of activity toward sGC–CO, compound **2** was identified as the best activator of sGC–CO (29-fold) among those tested. Competition binding experiments with **2** and YC-1 indicated that **2** was a relatively weak binder or was binding to a different site than YC-1. In order to further examine the interaction between **2** and sGC, the tritium-labeled analogue [$^3$**H**]**2** was prepared and utilized in a photoaffinity labeling experiment. It was found that this analogue was covalently bound primarily to the α-subunit of sGC. However, the photoaffinity labeling experiment failed to show that this compound was competitive with YC-1. These data support a conclusion that the sGC-activating YC-1 derivative **2** may be binding in a distinct pocket and further suggest the existence of novel activators of sGC distinct from YC-1. Currently, further studies are under way to specify the [$^3$**H**]**2** binding site in the α-subunit of sGC and to further characterize its mechanism of action.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.10.093.

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