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Tricyclic indole and dihydroindole derivatives as new inhibitors of soluble guanylate cyclase

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ABSTRACT

The synthesis of new tricyclic fused indole and dihydroindole derivatives and preliminary results from their in vitro inhibitory activity against soluble guanylate cyclase (sGC) are presented.

Soluble guanylate cyclase (sGC) is an enzyme that catalyzes the conversion of guanosine 5′-triphospate (GTP) to the second messenger guanosine 3′,5′-cyclic monophosphate (cGMP). sGC carries a prosthetic heme groups that serves as a nitric oxide (NO) sensor, which activates sGC activity by up to 400-fold. Increased levels of cGMP have been shown to lead to smooth muscle relaxation, inhibition of platelet aggregation, anti-apoptotic and anti-inflammatory effects.[1] On the other hand, overactivation of the sGC/cGMP signaling pathway has been shown to occur in several pathological conditions such as during sepsis[2] and neurodegenerative disorders. Compounds that inhibit the activity of sGC could serve as potential therapeutic agents as well as chemical biology tools that will aid in unravelling the role of sGC-regulated pathways in vivo.

Several compounds have been described in the literature as inhibitors of the sGC. Among them, methylene blue[3] and LY83583[4] (Fig. 1) are not direct sGC inhibitors, but rather block cGMP formation by generating superoxide anion radicals that deactivate NO.[5] Despite their extensive use in plethora of research studies, the off-target effects of these compounds[6–8] limit their pharmacological value in the in vivo evaluation of the sGC activity. More specific inhibition of sGC has been obtained by other inhibitors like 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxaline-1-one (ODQ)[9] and its 8-bromo analogue NS2028.[10] ODQ acts as an oxidant of the heme group, as it has been revealed from Raman spectroscopy,[11] resulting in the desensitization of the sGC towards the activating action of NO. Although ODQ does not affect the activity of related cyclases (for example the particulate guanylate cyclase),[9] its binding affinity to the heme moiety may contribute to the unspecific interaction with other hemoproteins in vivo, leading to decreased enzyme selectivity and undesirable side effects.

In the context of the above mentioned data, it is evident that the development of new inhibitors with increased potency and selectivity against sCG remains a challenge. Additionally, sGC inhibitors might be useful therapeutically especially in disease states associated with hypotension.

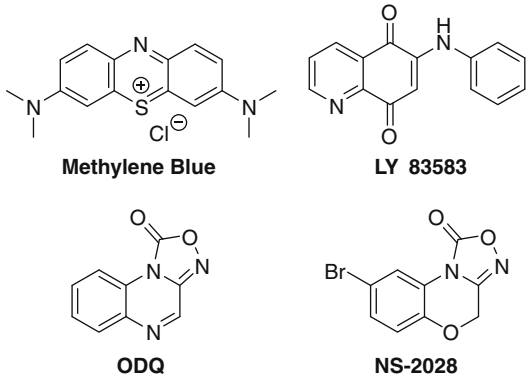

Figure 1. Inhibitors of soluble guanylate cyclase.

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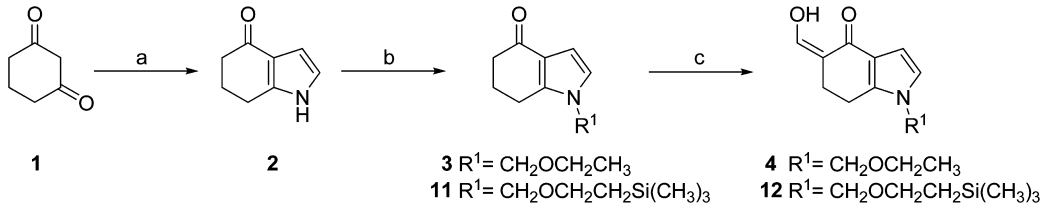
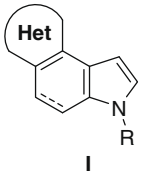


Scheme 1. Reagents and conditions: (a) glyoxal monoxime, Zn powder, AcOH, reflux, overnight, 20%; (b) *t*-BuOK, 18-crown-6, EOM-Cl or SEM-Cl, THF, 0 °C, 0.5 h, 87%; (c) HCOOEt, NaH, benzene, rt, overnight, 71–79%.

We aimed to synthesize sGC inhibitors with potential in vitro and in vivo activity by rationally designing and synthesizing a series of new derivatives bearing the basic skeleton **I**. Principal structural feature of these new derivatives is an indole or a dihydroindole core fused with a five-membered heterocyclic ring resulting in the formation of a tricyclic skeleton. We considered this scaffold, either dihydrogenated or fully aromatized in the indole part, promising for the development of potential sCG inhibitors, as it presents molecular and structural similarity with already known inhibitors of sGC, like ODQ and NS-2028. Despite that the indole and the dihydroindole nucleus constitute essential structural components of many bioactive compounds, to the best of our knowledge similar derivatives have never been studied as inhibitors of sGC.

We herein describe the synthesis and preliminary results concerning the in vitro evaluation of these compounds.

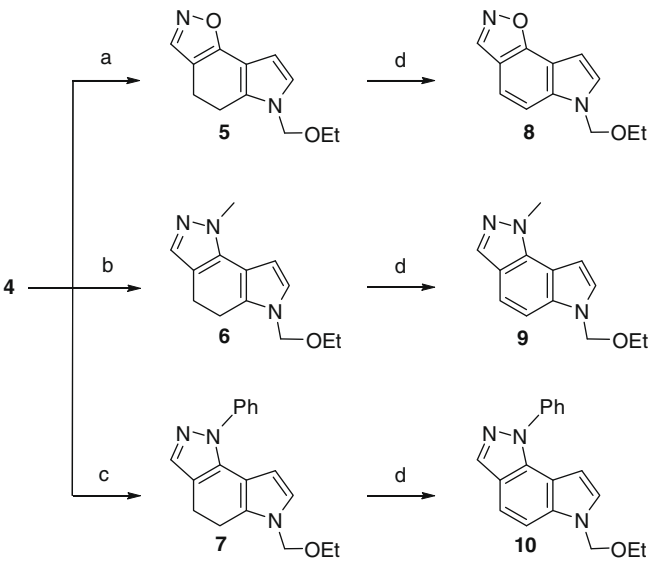


I

R = alkyl or H
Het = 5-membered heterocylic ring

Attempting to establish a simple and versatile synthetic methodology for the synthesis of the new tricyclic indole and dihydroindole derivatives, we used a 5-hydroxymethylene tetrahydroindolone, like **4** (Scheme 1) as a key intermediate molecule which would enable us to construct various fused tricyclic systems upon condensation with suitable reagents. Preparation of **4** was achieved from the commercially available cyclohexane-1,3-dione **1** following a three step procedure. Particularly, condensation of **1** with glyoxal-monoxime[12] in the presence of zinc powder in glacial acetic acid afforded the corresponding 4-keto-tetrahydroindole **2**.[13] Based on a previous successfully applied procedure for the protection of the pyrrole nitrogen of a 7-keto-tetrahydroindole derivative under phase transfer conditions,[14] treatment of **2** with chloromethyl ethyl ether (EOM-Cl) in the presence of potassium *tert*-butoxide (*t*-BuOK) and 18-crown-6 in tetrahydrofuran afforded the *N*-EOM protected tetrahydroindolone **3** in very good yields (87%). The latter was formylated with ethyl formate in the presence of sodium hydride affording the 5-hydroxymethylene derivative **4**.

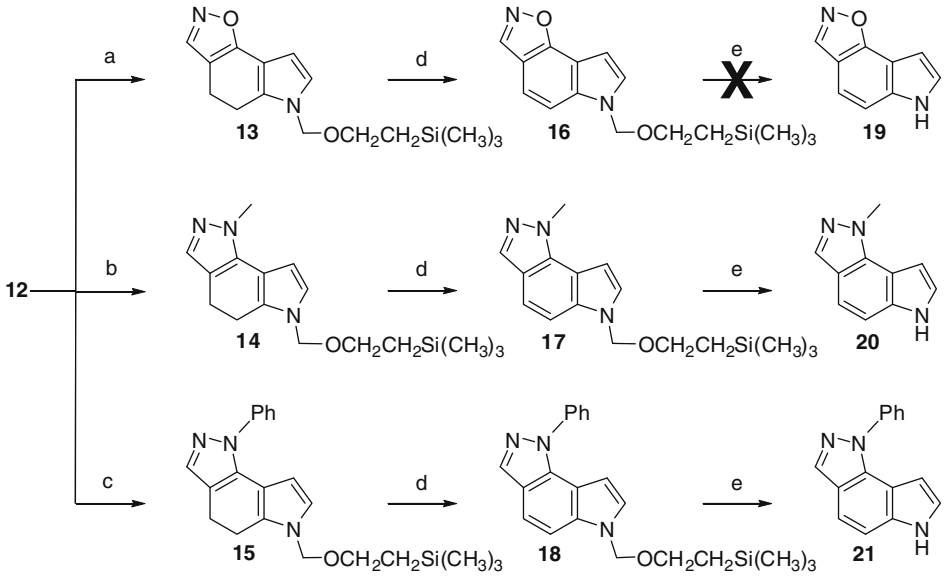
Condensation of **4** with hydroxylamine hydrochloride under mild conditions led to the formation of the 4,5-dihydro-isoxazolo[5,4-*e*]indole **5** (Scheme 2) the structure of which has been confirmed.[15,16] The 4,5-dihydro-pyrrolo[2,3-*g*]indazole derivatives **6** and **7** were obtained upon condensation of **4** with methylhydrazine and phenylhydrazine, respectively. Confirmation of the structures is consistent with literature data[16,17] and additionally is supported by the ^{1}H NMR signal for 8-H which is observed to



Scheme 2. Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, EtOH, 0 °C, 1 h, 75%; (b) CH_3NHNH_2, EtOH, 0 °C, 1 h, 72%; (c) $PhNHNH_2$, EtOH, 0 °C, 1 h, 46%; (d) DDQ, 1,4-dioxane, 1 h, rt, 58–87%.

shift relatively upfield (5.83 δ) in compound **7** compared to that of compound **6** (6.32 δ) as a consequence of its interference with the shielding cone of phenyl ring. Subsequently, the derivatives **5**, **6** and **7** were dehydrogenated on treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxane to the corresponding fused tricyclic indole derivatives **8**, **9** and **10**. In an effort to gain access to the *N*-unprotected derivatives and investigate their possible inhibitory activity against sGC, we decided to remove the EOM protective group. We chose the derivative **10** as model compound for initial trial experiments. Extensive attempts to cleave the EOM group using HCl in dioxane, or HCl in EtOH under various conditions proved unsuccessful resulting either in recovery or in decomposition of the starting material **10**.

The above-mentioned results prompted us to exchange the EOM moiety with an alternative alkyloxymethyl one which would be susceptible to hydrolysis under milder conditions. We considered the employment of the 2-(trimethylsilyl)ethoxymethyl (SEM) group for the *N*-pyrrole protection of the 4-keto-tetrahydroindole **2.** Treatment of **2** with SEM-Cl under the phase transfer conditions described above afforded the *N*-SEM protected tetrahydroindolone **11** in excellent yields (87%) (Scheme 1). Subsequently, base-catalyzed formylation gave the 5-hydroxymethylene derivative **12**. Upon condensation of the latter with appropriate reagents the corresponding tricyclic fused dihydroindole derivatives **13**–**15** (Scheme 3) were obtained while dehydrogenation with DDQ afforded the indole derivatives **16**–**18**. Cleavage of the SEM protective group was accomplished upon treatment with $BF_3{\cdot}Et_2O$ in dichloromethane which generated the corresponding *N*-hydroxymethyl-intermediates. The crude mixture of them was further



Scheme 3. Reagents and conditions: (a) $H_2NOH{\cdot}HCl$, EtOH, 0 °C, 1 h, 80%; (b) CH_3NHNH_2, EtOH, 0 °C, 1 h, 63%; (c) $PhNHNH_2$, EtOH, 0 °C, 1 h, 40%; (d) DDQ, 1,4-dioxane, 1 h, rt, 63–75%; (e) (i) $BF_3{\cdot}Et_2O$, DCM, 0 °C to rt, 1 h; (ii) Triton B, acetonitrile, 0 °C, 4 h, 26–37%.

treated with Triton B in acetonitrile effecting the loss of formaldehyde and affording the *N*-unprotected products **20**–**21** in moderate yields (26–37%) over two steps. Unfortunately, **16** proved extremely labile under deprotection conditions leading to decomposition upon treatment with $BF_3{\cdot}Et_2O$.

To study whether the synthesized compounds are effective sGC inhibitors, RASMCs were pretreated for 30 min with two different concentrations (1 and 100 μM) of each of the tested compound and then exposed to the nitric oxide donor SNP (10 μM). RASMC treated with SNP alone exhibited a substantial increase in cGMP levels (Fig. 2). The response to SNP was reduced in cells exposed to the newly synthesized compounds to varying degrees. Pretreatment with compound **5** only inhibited SNP-induced cGMP formation at the highest concentration tested. Compound **6** was effective in attenuating NO-induced cGMP formation and was more potent than **5** as it inhibited cGMP at a lower concentration; it should be noted that higher concentrations of this compound were not as effective in inhibiting cGMP formation. Derivative **7** reduced SNP-stimulated cGMP accumulation in a concentration-dependent manner that was maximal at 100 μM.

To test the ability of compound **8** (which had shown activity in preliminary screening tests) throughout a broader concentration range, RASMCs were pretreated for 30 min with 0.1, 1, 10 or 100 μM of compound **8** and then exposed to the nitric oxide donor SNP (10 μM). The response to SNP was reduced in a concentration-dependent manner in cells exposed to the derivative **8** (Fig. 3). Both compounds **7** and **8** were more efficacious than compounds **5** and **6**, as pretreatment with **7** and **8** led to the greatest amount of inhibition of cGMP formation.

To study the selectivity of the tested compounds on sGC, we examined the effect of one of the synthesized compounds (**8**) that was most effective on sGC-stimulated cGMP formation. Exposure of RASMC to ANF (1 μM) increased their cGMP content through activation of pGC; as expected although ANF-induced cGMP levels were lower than what was observed for SNP (Fig. 4). The response to ANF was unaffected by the addition of 100 μM of **8** (Fig. 4). It

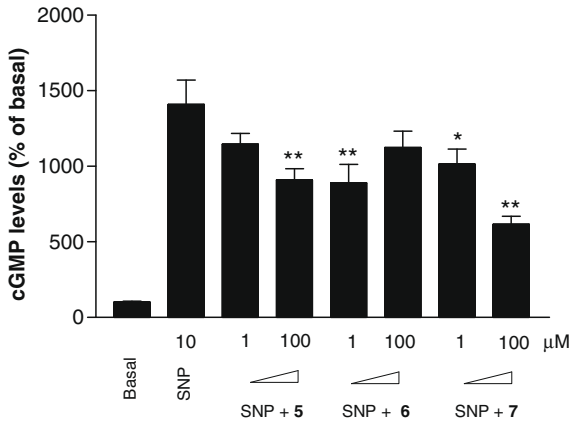


Figure 2. Effect of newly synthesized compounds on SNP-induced cGMP production. Confluent RASMCs were incubated with the tested compounds (1 and 100 μM) for 30 min. Cells were washed twice with HBSS and then treated with basal or SNP (10 μM) in the presence of IBMX (1 mM) for 15 min. cGMP was extracted and measured as described in supplementary data. Data are means ± SEM; $n = 8$ wells. $^{**}P < 0.01$, $^{*}P < 0.05$ compared to SNP.

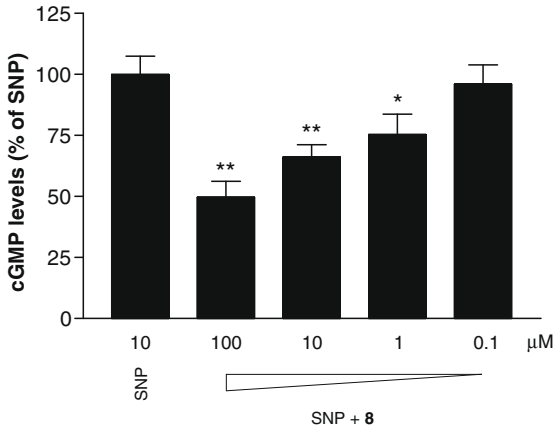


Figure 3. Effect of compound **8** on SNP-induced cGMP production. Confluent RASMCs were incubated with the tested compound (0.1, 1, 10 and 100 μM) for 30 min. Cells were washed twice with HBSS and then treated with vehicle or SNP (10 μM) in the presence of IBMX (1 mM) for 15 min. cGMP was extracted and measured as described in supplementary data. Data are means ± SEM; $n = 8$–16 wells. $^{**}P < 0.01$, $^{*}P < 0.05$ compared to SNP.

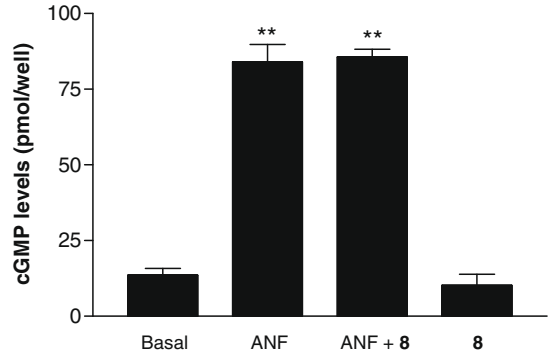


Figure 4. Effect of compound **8** on pGC. Confluent RASMCs were incubated with **8** (100 μM) for 30 min. Cells were washed twice with HBSS and then treated with basal or ANF (1 μM) in the presence of IBMX (1 mM) for 15 min. cGMP was extracted and measured as described in supplementary data. Data are means ± SEM; $n = 4$ wells. $^{**}P < 0.01$ compared to Basal.

should be noted that **8** did not alter baseline cGMP levels in smooth muscle cells, indicating that this compound does not block basal sGC activity. This finding suggests that the mechanism of action of compound **8** might be similar to ODQ, (i.e., heme oxidation), that only blocks NO-stimulated sGC activity.

In the context of the above mentioned data it is obvious that both tricyclic fused 6,7-dihydroindole and indole rings are promising core structures for novel sGC inhibitors. Preliminary in vitro evaluation indicates that derivatives bearing five-membered heterocycles fused with a 6,7-dihydroindole or an indole ring (compounds **5**–**8**) exhibit significant inhibitory potency. Among them, the derivatives **7** and **8** bearing a fused phenylpyrrazole and an isoxazole ring, respectively, showed superior and equipotent, indicating that the pattern and the substitution of the fused heterocyclic ring may be varied to some extent. This is in agreement to previous reports, which have shown that structurally diverse classes of compounds such as organic phosphates,[18] purine and pyrimidine nucleotides,[19] pyridopyrimidine derivatives,[20] halogenated volatile anesthetics,[21] various metalloporphyrins[22,23] and the related biliverdin IX,[24] poly-hydroxylated benzylidenemalononitrile derivatives (tyrphostins)[25] and L-ascorbic acid[26] inhibit sGC. On the other hand, comparing the isoxazolo derivatives **5** and **8** it is concluded that the fully aromatic character of the tricyclic core of **8** contributes substantially to the enhancement of anti-sGC activity.

In conclusion, following a simple and versatile synthetic methodology we synthesized a series of new tricyclic indole and dihydroindole derivatives and we evaluated distinct members as sGC inhibitors. Preliminary results from in vitro studies demonstrate the potential of these compounds as candidate sCG inhibitors. Specifically, the isoxazolo[5,4-*e*]indole derivative **8** represents a promising hit compound of the series with inhibitory activity in the micromolar range and selectivity for sGC. Further structural optimization studies and pharmacological investigations are in progress.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.06.047.

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