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Letters

1-Benzyl-3-(5′-hydroxymethyl-2′-furyl)-indazole (YC-1) Derivatives as Novel Inhibitors Against Sodium Nitroprusside-Induced Apoptosis

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Abstract: Antiapoptotic agents based on 1-benzyl-3-(5′-hydroxymethyl-2′-furyl)indazole (**22**, YC-1) derivatives were explored for effective treatment of sepsis and septic shock. We found that compound **22**, 1-benzyl-3-(5′-methoxymethyl-2′-furyl)indazole (**27**), and 1-phenyl-3-(5′-hydroxymethyl-2′-furyl)-indazole (**23**) were the most effective inhibitors of sodium nitroprusside-induced vascular smooth muscle cell apoptosis. These three compounds are proposed as potential therapeutic agents for the treatment of sepsis.

Introduction

Septic death is one of the most common causes of death in intensive care units[1] due to multiple organ failure (MOF) syndrome.[2,3] Several therapeutic approaches have recently been clinically included such as employing antioxidants, administering antiinflammatory agents, and inhibiting lipopolysaccharide (LPS)-induced nitric oxide (NO) synthesis.[4−6] However, the clinical outcome of these treatment strategies has been unsatisfactory.[7] More recently, inhibition of vascular smooth muscle cell (VSMC) apoptosis has been proposed for the treatment of sepsis. This is based on the theory that massive VSMC apoptosis, especially within vital organs, could induce MOF during septic attack.

NO and guanosine-3′,5′-cyclic monophosphate (cGMP) participate in multiple pathways in response to various stimuli including the fluctuation of vascular tone and cell growth.[8,9] The endothelium synthesizes micromolar concentrations of NO under normal physiological conditions. After severe physiological attack such as trauma and sepsis, however, the synthesis of exaggerated amounts of NO can occur.[10] When cells respond to bacterial products (such as LPS) or inflammatory cytokines [such as tumor necrosis factor (TNF-α)], they up-regulate inducible NO synthase (iNOS), which produces micromolar concentrations of NO.[11,12] Numerous lines of evidence demonstrate that NO is one of the key mediators of inflammation and such high concentrations of NO can contribute to the pathogenesis of vascular failure in sepsis.[13]

1-Benzyl-3-(5′-hydroxymethyl-2′-furyl)indazole (**22**, YC-1) was first synthesized in our laboratory as a novel NO-independent type of soluble guanylate cyclase (sGC) activator.[14,15] Compound **22** mimics many of the known functions of NO and NO donors. For instance, compound **22** inhibits platelet aggregation and adhesion and it also inhibits the contraction of vascular smooth muscles.[16−18] Moreover, compound **22** and NO activate sGC in a synergistic manner, and compound **22** may potentiate the action of exogenous and endogenous CO on sGC.[19]

In the present work, several derivatives of compound **22** were prepared according to our published procedure[15] and their effect on sodium nitroprusside (SNP)-induced apoptosis of cultured rat aortic smooth muscle cells was studied. The data indicate a high concentration of SNP (1 mmol/L)-induced profound cell apoptosis as determined by the MTT assay method and the TUNEL reaction technique.[9] Compound **22**, 1-benzyl-3-(5′-methoxymethyl-2′-furyl)indazole (**27**), and 1-phenyl-3-(5′-hydroxymethyl-2′-furyl)indazole (**23**) almost completely prevented VSMC apoptosis.

Results and Discussion

Chemistry. All key intermediates (**17**−**21**) were synthesized according to Scheme 1. The starting diaryl

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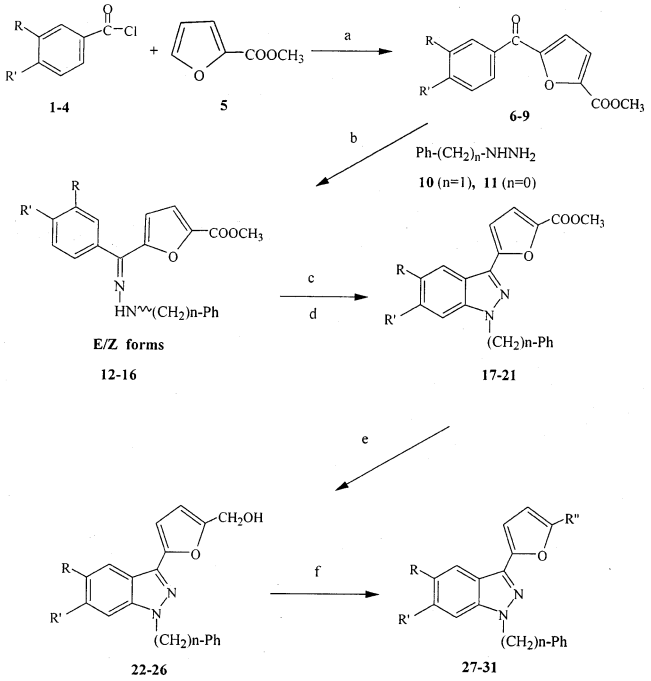
Scheme 1[a]



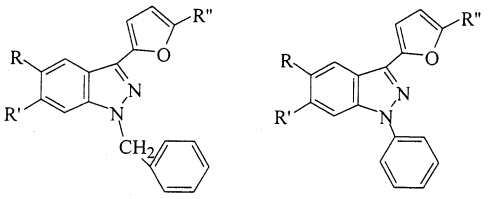
[a] Reagents: (a) $FeCl_3$, CH_2Cl_2, reflux. (b) HOAc, MeOH, reflux. (c) $Pb(OAc)_4$, CH_2Cl_2, 30 °C. (d) $BF_3{\cdot}Et_2O$, reflux. (e) $Ca(BH_4)_2$, THF, reflux. (f) Compound **22** reacts with NaH and MeI in THF in a ice bath to afford **27**; **22** reacts with BCl_3 in CH_2Cl_2 to afford **28**; **28** reacts with $MeNH_2$ or Et_2NH to afford **29** or **30**; **28** reacts with H_2/Pd−C in THF to afford **31**.

ketones (**6**−**9**) were treated with appropriate hydrazines (**10** and **11**) to yield the corresponding hydrazones (**12**−**16**) as mixtures of *E*- and *Z*-isomers. These hydrazones (**12**−**16**) were then treated with $Pb(OAc)_4$ in CH_2Cl_2 at low temperature. Subsequently, $BF_3{\cdot}Et_2O$ was added, and the mixture was heated to yield the desired indazoles (**17**−**21**) via cyclization. The indazoles (**17**−**21**) were then reduced with $Ca(BH_4)_2$ to afford the corresponding carbinol derivatives (**22**−**26**).

The $-CH_2OH$ group of compound **22** was modified to give its corresponding methoxy methyl derivative (**27**). Meanwhile, intermediate **28**, obtained by treating **22** with BCl_3, was either treated with alkylamine to give its alkylaminomethyl derivatives (**29** and **30**) or hydrogenated to yield the 5′-methyl derivative (**31**).

Antiapoptotic Effect. As shown in Table 1, at a concentration of 30 μM, compound **22** almost completely reversed the SNP-induced apoptosis of VSMCs. Replacement of the 1-benzyl group with a phenyl group resulted in compound **23** with similar activity as compound **22**. Introduction of F (**24**) or OCH_3 (**25**) groups into the 6-position of compound **22** resulted in slightly reduced potency. Replacement of the 5- and 6-substituents of compound **22** with a bridging $-OCH_2O-$ group provided compound **26** with even lower activity. On the other hand, converting the $-CH_2OH$ group of compound **22** into $-CH_2OCH_3$ (**27**) retained the activity of compound **22**. Subsequent conversion of compound **27** to $-CH_2NHCH_3$ (**29**) resulted in significantly reduced activity, and conversion to $-CH_2N(CH_2CH_3)_2$ (**30**) impaired its activity further. Conversion of the $-CH_2OH$ group of compound **22** into $-CH_3$ (**31**) produced a cytotoxic effect in VSMC and caused considerable reduction in antiapoptotic activity. Finally, conversion of the $-CH_2OH$ group of compound **22** into $-COOCH_3$ (**17**) slightly reduced its activity to the level of compound **26**.

Table 1. Antiapoptotic Effect of Various Benzylindazole Compounds on the Sodium Nitroprusside-Mediated Apoptosis of VSMCs[a]



17, 22, 24-27, 29-31 **23**

compd	R	R′	R″	cell survival (% of control)
SNP				41.3 ± 3.7
22	−H	−H	$-CH_2OH$	94.7 ± 7.6**
23	−H	−H	$-CH_2OH$	93.9 ± 10.4**
24	−H	−F	$-CH_2OH$	83.3 ± 1.5**
25	−H	$-OCH_3$	$-CH_2OH$	84.5 ± 10.3**
26	$-OCH_2O-$		$-CH_2OH$	75.8 ± 1.5**
27	−H	−H	$-CH_2OCH_3$	93.2 ± 9.1**
29	−H	−H	$-CH_2NHCH_3$	57.2 ± 7.6
30	−H	−H	$-CH_2N(CH_2CH_3)_2$	39.7 ± 8.3
31	−H	−H	$-CH_3$	31.1 ± 7.6
17	−H	−H	$-COOCH_3$	78.0 ± 10.6*

[a] Effects of SNP and benzylindazole derivatives on the survival of rat aortic smooth muscle cells. Cells were pretreated with benzylindazole derivatives (30 μM) for 30 min. Then, vehicle or SNP (1 mM) was added for another 24 h. After the incubation period, cell viability was assayed by the MTT assay and TUNEL techniques. Data are expressed as mean ± SEM of 5−7 determinations (each in triplicate). * $p < 0.01$ and ** $p < 0.001$ as compared with SNP alone.

After screening the above derivatives for their inhibitory effect on SNP-induced apoptosis, we concluded that compound **22** together with compounds **27** and **23** were the most effective inhibitors of SNP-induced apoptosis.

In a preliminary in vivo study, the intraperitoneal application of LPS (60 mg/kg) caused death in all mice within 24 h ($n = 9$). However, oral administration of compound **22** (10 mg/kg) at 6 h after LPS application markedly decreased the septic death rate (66.7%) in mice. A complete report of our in vivo study will be published elsewhere. The present work demonstrated remarkable therapeutic potential for compound **22** derivatives in the treatment of sepsis and suggested that these drugs may be developed for clinical use soon.

Supporting Information Available: Synthetic methods, yields, and complete physical and spectral data for compounds **6**−**31**. Experimental details for cell culture, cytotoxicity assay, and statistical analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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