Brief Articles

Synthesis of 1-Benzyl-3-(5′-hydroxymethyl-2′-furyl)indazole Analogues as Novel Antiplatelet Agents

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Received January 3, 2001

1-Benzyl-3-(5′-hydroxymethyl-2′-furyl)indazole (**28**, YC-1) was selected as the lead compound for systemic structural modification. After screening for antiplatelet activity, SARs of YC-1 analogues were established. Among these potent active derivatives, compounds **29**, **30**, **31**, **44**, and **45** functioned as potent activators of sGC and inhibitors of PDE5 with potency comparable to that of YC-1. In addition, compound **58** was found to be a selective and potent inhibitor of protease-activated receptor type 4 (PAR4)-dependent platelet activation.

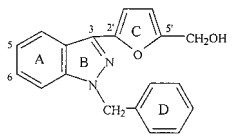
Introduction

In our previous work on the development of novel antiplatelet agents, we reported that 1-benzyl-3-(5′-hydroxymethyl-2′-furyl)indazole (**28**, YC-1) exhibited significant inhibitory effects against thrombin-, AA-, collagen-, and PAF-induced platelet aggregation.[1] Due to its antiplatelet activity of broad spectrum, we assumed that YC-1 interfered with platelet aggregation through a common pathway. Mechanism investigation revealed that the in vitro antiplatelet activity of YC-1 was associated with NO-independent activation of soluble guanylate cyclase (sGC),[1,2] although YC-1 and NO activated sGC in a synergistic manner.[3] In addition, YC-1 was similar to NO to enhance the response of sGC to CO.[3] Unlike NO, YC-1 activated sGC without involving heme but via binding to allosteric site of sGC which in turn decreased the dissociation rate of NO and CO.[3,4] Accordingly, YC-1 has been recognized as a new antiplatelet agent with unique mechanism of action.

Besides, YC-1 demonstrated NO-enhancing capability via sGC activation and PDE inhibition[5] and therefore may greatly elevate the cGMP level. These unique activities make YC-1 an ideal lead compound for new drug development. Furthermore, the ability of YC-1 to potentiate NO action may be of therapeutic potential, since it could reduce the dosage of nitrovasodilators and thus decrease the associated toxicity. Consequently, the pharmacological activities of YC-1 pertinent to its clinical efficacy were widely studied in recent years.

The structure−activity relationship (SAR) of YC-1 analogues has not been reported. As the inventors of YC-1, we feel obligated to report our extensive synthesis effort of YC-1 analogues to further the discovery of potential antiplatelet agents.

To examine whether the 5′-CH_2OH and aromatic ring of YC-1 may interact with the binding site of sGC, we prepared a series of YC-1 analogues with a fixed pyrazole (B) ring and with modifications made on peripheral 1-benzyl, 5′-CH_2OH, and 5,6-positions. In addition, the C ring was also replaced by benzene ring.



(28, YC-1)

Herein we report a SAR based on the antiplatelet activities of these analogues.

Results and Discussion

Chemistry. All key intermediates (**23**−**27**, **41**, **58**, **59**) were synthesized according to Scheme 1. The starting diaryl ketones (**7**−**11**, **52**, **53**) were treated with appropriate hydrazines (**12**, **38**) to yield the corresponding hydrazones (**13**−**17**, **39**, **54**, **55**) as mixtures of *E*- and *Z*-isomers[6] which were converted to indazoles (**23**−**27**, **41**, **58**, **59**) via the formation of azo intermediates (**18**−**22**, **40**, **56**, **57**).

The above hydrazones (**13**−**17**, **39**, **54**, **55**) 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 (**23**−**27**, **41**, **58**, **59**) via cyclization.

These indazoles (**23**−**27**, **41**, **58**, **59**) were either reduced with $Ca(BH_4)_2$ to afford the corresponding carbinol derivatives (**28**−**32**, **42**, **60**, **61**) or hydrolyzed

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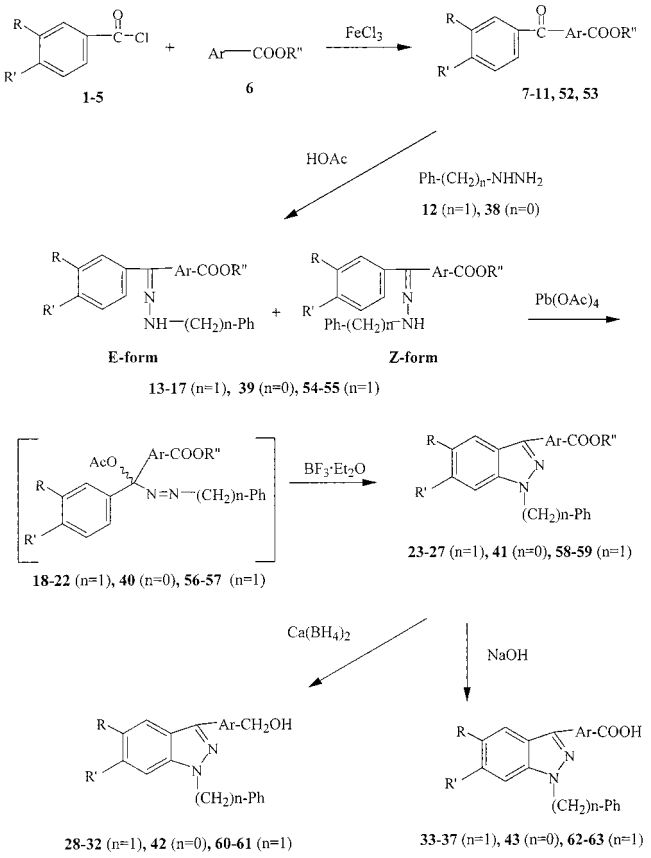
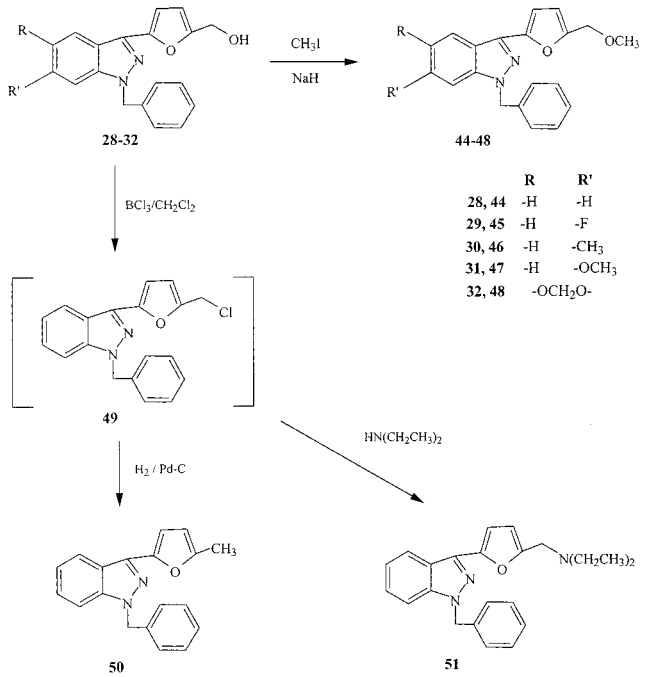
10.1021/jm010001h CCC: $20.00
Published on Web 09/28/2001

Scheme 1



Scheme 2



	R	R'
28, 44	-H	-H
29, 45	-H	-F
30, 46	-H	-CH_3
31, 47	-H	-OCH_3
32, 48	-OCH_2O-	

to give the corresponding carboxylic acid derivatives (**33**−**37**, **43**, **62**, **63**).

The -CH_2OH groups of compounds **28**−**32** were modified following Scheme 2 to give their corresponding methoxy methyl derivatives (**44**−**48**). Meanwhile, intermediate **49**, obtained by treating **28** with BCl_3, was either hydrogenated to yield 5′-methyl derivative (**50**) or treated with diethylamine to give its *N*,*N*-diethylaminomethyl derivative (**51**).

Antiplatelet Activity. The antiplatelet activities of 1,5,6-substituted 3-(5′-substituted-2′-furyl)indazoles (**23**−**51**) are summarized in Table 1. The lead compound (YC-1) was found to exhibit significant inhibitory effect against thrombin-, AA-, collagen-, and PAF-induced platelet aggregation. Replacing the 1-benzyl group with phenyl (**42**) improved antiaggregation potency toward thrombin, AA, and collagen inducers, whereas impaired the activity toward PAF-induced aggregation.

Removal of 1-benzyl group of YC-1 to yield compound **I**[7] dramatically reduced antiplatelet activity, suggesting that the presence of an aromatic ring at 1-position is essential for potent antiplatelet activities of broad spectrum.

We then explored the effects of altering R_5 and R_6 substituents. Placing an F atom at the 6-position yielded **29** which significantly enhanced the inhibition toward AA-, collagen-, and PAF-induced aggregation, but reduced the inhibition against thrombin-induced aggregation. Substitution of 6-position with CH_3 (**30**) and OCH_3 (**31**) did not alter the activity. Finally, bridging the 5,6-positions with -OCH_2O- (**32**) significantly diminished the activity.

Then, 5′-CH_2OH was converted to -CH_2OCH_3 (**44**) and resulted in greatly increased activity. Likewise, the ether **45** significantly enhanced the inhibition toward thrombin-induced aggregation, whereas it reduced the inhibition against AA-, collagen-, and PAF-induced aggregation. Similar conversion of **30** to **46** resulted in only a minor change in activity, while conversion of **31** and **32** into **47** and **48** led to reduced activity.

In another approach, amination of the 5′-CH_2OH group provided **51** and considerably improved the inhibition activity toward PAF- and thrombin-induced aggregation but was inactive toward AA- and collagen-induced aggregation. Meanwhile, elimination of the OH group provided **50**, showing markedly increased inhibition, especially toward thrombin- and PAF-induced aggregation.

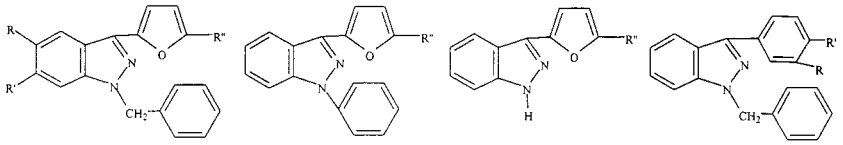
Subsequently, the 5′-CH_2OH group was converted into -$COOCH_3$ to give **23**, which demonstrated weak inhibition against AA- and collagen-induced aggregation and no inhibition toward thrombin- and PAF-induced aggregation at a concentration as high as 300 μM. The pattern of preferential inhibition of **23** toward platelet aggregation differed widely from that of YC-1. Like **23**, esters **24**−**27** were less potent than their corresponding 5′-CH_2OH counterparts **29**−**32** and had a different pattern of preferential inhibition from YC-1.

A marked decrease in inhibition potency toward thrombin- and PAF-induced aggregation for N-phenyl compounds was observed when 5′-CH_2OH (**42**) was converted into -$COOCH_3$ (**41**).

The 5′-COOH derivative **33** was found less potent than YC-1. Again, as expected, the 5′-COOH derivatives **34**−**37** were also less potent than their 5′-CH_2OH counterparts (**29**−**32**).

Replacing the 3-furan ring of YC-1, **23** and **33** with a benzene ring yielded **58**−**63**, which were generally weaker than their 3-furyl counterparts (Table 1). However, compound **58** displayed unexpectedly great inhibition against thrombin-induced aggregation (IC_{50} = 29.3 μM) but very little inhibition toward AA-, collagen-, and PAF-induced aggregation. Such highly selective anti-

Table 1. Inhibitory Effect of Compounds **23**−**37**, **41**−**48**, **50**−**51**, **58**−**63**, and **I** on Platelet Aggregation Induced by Thrombin, AA, Collagen, and PAF[a]



23-37, 44-48, 50, 51 41-43 I 58-63

				IC_{50} (μM)[b]			
compd	R	R′	R″	thrombin	AA	collagen	PAF
28 (YC-1)	-H	-H	$-CH_2OH$	173.0	54.3	53.8	87.3
42	-	-	$-CH_2OH$	158.2	17.9	13.7	145.4
I	-	-	$-CH_2OH$	>270	100.9	>270	>270
29	-H	-F	$-CH_2OH$	217.4	21.7	25.2	53.4
30	-H	$-CH_3$	$-CH_2OH$	146.9	49.4	45.9	72.0
31	-H	$-OCH_3$	$-CH_2OH$	168.3	49.7	46.7	89.2
32	$-OCH_2O-$		$-CH_2OH$	238.8	96.6	104.6	163.8
50	-H	-H	$-CH_3$	39.4	47.3	27.5	39.6
44	-H	-H	$-CH_2OCH_3$	143.4	8.2	6.6	25.5
45	-H	-F	$-CH_2OCH_3$	115.8	45.9	64.1	108.7
46	-H	$-CH_3$	$-CH_2OCH_3$	125.9	32.4	59.3	76.3
47	-H	$-OCH_3$	$-CH_2OCH_3$	255.7	108.2	114.8	176.6
48	$-OCH_2O-$		$-CH_2OCH_3$	>270	101.6	113.0	195.6
51	-H	-H	$-CH_2NEt_2$	101.9	55.7	50.4	55.9
23	-H	-H	$-COOCH_3$	>270	81.1	161.5	>270
41	-	-	$-COOCH_3$	>270	10.6	18.2	>270
24	-H	-F	$-COOCH_3$	>270	94.0	101.1	148.6
25	-H	$-CH_3$	$-COOCH_3$	>270	64.2	78.3	>270
26	-H	$-OCH_3$	$-COOCH_3$	>270	92.3	156.9	>270
27	$-OCH_2O-$		$-COOCH_3$	259.8	136.2	168.4	253.5
33	-H	-H	-COOH	>270	83.7	75.5	>270
43	-	-	-COOH	>270	>270	>270	>270
34	-H	-F	-COOH	>270	119.1	179.5	>270
35	-H	$-CH_3$	-COOH	>270	150.3	182.5	>270
36	-H	$-OCH_3$	-COOH	>270	150.0	139.7	>270
37	$-OCH_2O-$		-COOH	>270	148.1	150.3	>270
58	-H	$-COOC_2H_5$	-	29.3	184.8	212.4	164.0
59	$-COOC_2H_5$	-H	-	>270	124.7	143.8	>270
60	-H	$-CH_2OH$	-	192.7	100.9	122.6	142.9
61	$-CH_2OH$	-H	-	>270	51.3	98.1	173.6
62	-H	-COOH	-	>270	129.6	156.1	178.7
63	-COOH	-H	-	>270	155.5	148.2	235.4
aspirin				>270	20.0	>270	>270

[a] Platelets were incubated with test compound at 37 °C for 1 min, then thrombin (0.1 unit/mL), AA (100 μM), collagen (10 μg/mL), or PAF (2 ng/mL) was added to trigger the aggregation. Values are expressed as mean ± SE from three to six separations. Aspirin acts as a positive control. [b] The accuracy of IC_{50} values are within ±10%.

platelet activity prompted us to further investigate its mechanism of action.

Thus far, from the antiplatelet activity of these YC-1 analogues, the following SARs can be established.

YC-1 analogues with a benzyl or phenyl group at the 1-position all exhibited potent antiplatelet activity of broad spectrum. Replacing 5′-CH_2OH with $-CH_2OCH_3$, $-CH_2N(CH_2CH_3)_2$, or $-CH_3$ maintained the potent activity. In contrast, replacing the 5′- CH_2OH with $-COOCH_3$ or -COOH resulted in a markedly decreased activity of narrower spectrum.

Placing an F atom at the 6-position strengthened the inhibition toward AA-, collagen-, and PAF-induced aggregation. Conversely, the introduction of a 6-CH_3 or 6-OCH_3 caused no noticeable effect, while 5,6-OCH_2O- profoundly reduced the activity.

Replacing the furan ring of YC-1, **23**, and **33** with a benzene ring was generally deleterious to the antiplatelet activity.

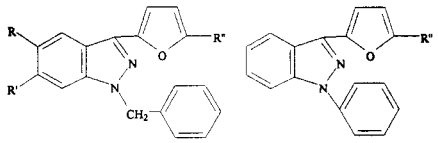
On the basis of the results of our antiplatelet screening, nine indazole derivatives (**29**−**31**, **42**, **44**−**46**, **50**, and **51**) were identified with significant antiplatelet activity of broad spectrum as YC-1. Therefore, these nine promising compounds were further tested for the activation of sGC and inhibition of PDE5.

Activation of sGC and Inhibition of PDE5. The sGC activation data of the above nine compounds are summarized in Table 2. Compounds **29**, **30**, **31**, **44**, and **45** all exhibited potent activities comparable to YC-1. Furthermore, their inhibition activity toward PDE5 was stronger than that of YC-1. Among them, compounds **31** and **45** demonstrated 10-fold more potency than YC-1. However, the potency for sGC activation and PDE5 inhibition did not parallel their antiplatelet activities. The reason for such discrepancy might be explainable by that a cGMP-independent mechanism is involved in the antiplatelet activity.

Conclusion

Starting from the lead compound, we retained the pyrazole (B) ring as a nucleus and systematically modified the peripheral 1-benzyl group, 5,6-substituents, and 5′-CH_2OH, as well as varied the A and C rings, to obtain several series of YC-1 analogues. After screen-

Table 2. Activation of sGC and Inhibition of PDE5 of **28**−**31**, **42**, **44**−**46**, **50**−**51**[a]



28-31, 44-46, 50-51 **42**

compd	R	R′	R″	sGC (pmol cGMP/ min/mg protein)	PDE5 (IC_{50}, μM)
control				8.0 ± 3.4	
28 (YC-1)	-H	-H	$-CH_2OH$	44.6 ± 6.3[c]	31.9
29	-H	-F	$-CH_2OH$	37.2 ± 11.7[a]	14.0
30	-H	$-CH_3$	$-CH_2OH$	35.1 ± 9.9[a]	8.4
31	-H	$-OCH_3$	$-CH_2OH$	39.9 ± 7.1[b]	3.5
44	-H	-H	$-CH_2OCH_3$	32.0 ± 12.8[b]	13.8
45	-H	-F	$-CH_2OCH_3$	41.8 ± 11.2[c]	3.9
46	-H	$-CH_3$	$-CH_2OCH_3$	22.1 ± 8.7	9.3
42			$-CH_2OH$	27.8 ± 7.0	36.3
50	-H	-H	$-CH_3$	30.9 ± 3.8	22.3
51	-H	-H	$-CH_2N(CH_2CH_3)_2$	17.9 ± 1.1	63.0
IBMX				ND	63.0

[a] sGC activity in the supernatant fraction of platelet homogenate was determined in the presence of [α-^{32}P]GTP and 82 μM of test compound at 37 °C for 10 min. Values are presented as pmol cGMP/min/mg protein. PDE activity fraction was determined in the presence of [^{3}H]cGMP and various concentrations of test compound at 37 °C for 10 min. Values are presented as the concentration (μM) by 50% inhibition of PDE5 (IC_{50}). IBMX (3-isobutyl-1-methylxanthine) acts as a positive control. ND: not determined. *P < 0.05. **P < 0.01. ***P < 0.001.

ing for antiplatelet activity, SARs of these YC-1 analogues were established in this study.

Five indazole compounds (**29**, **30**, **31**, **45**, and **46**) were identified as promising antiplatelet candidates. Together with YC-1, these compounds are currently under pharmacological investigations.

Another important contribution of our work is the discovery of compound **58**, which demonstrated highly selective and potent inhibition toward thrombin-induced aggregation. Mechanism study revealed that compound **58** selectively inhibited platelet aggregation and phosphoinositide breakdown induced by thrombin without affecting the proteolytic activity of thrombin. On the other hand, compound **58** inhibited thrombin-induced platelet activation only in the impairment of protease-activated receptor type 1 (PAR1)[8−10] in human platelets. Previously,[11] we suggested that **58** could interfere with platelet activation elicited by thrombin through the blockade of a non-PAR1 thrombin receptor. Recently, after detailed study, we learned that compound **58** inhibited the aggregation of washed human platelets stimulated by the protease-activated receptor type 4 (PAR4)[12] agonist peptide GYPGKF (IC_{50} 0.10 ± 0.01 μM). These recent results indicate that compound **58** is a selective and potent inhibitor of PAR4-dependent platelet activation. To our knowledge, compound **58** is currently the only PAR4 antagonist available, and it could act as a probe to investigate the functional role of PAR4-mediated signaling in platelet and other cells. Therefore, compound **58** can serve as a lead compound for antiplatelet agents. Details about the action mechanism of **58** will be published elsewhere.

Experimental Section

Evaluation of Antiplatelet Aggregation Activity. Antiplatelet aggregation activity was determined as previously described.[13,14]

Measurement of Guanylate Cyclase Activity. Guanylate cyclase activity was determined as previously described.[15]

Measurement of Phosphodiesterase Activity. Phosphodiesterase activity was determined as previously described.[16]

Supporting Information Available: Synthetic methods, yields, and complete physical and spectral data for compounds **7**−**65**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM010001H