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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 1262–1278
Bioorganic & Medicinal Chemistry

Synthesis and antiplatelet activity of ethyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (YD-3) derivatives

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Received 30 August 2007; revised 19 October 2007; accepted 20 October 2007
Available online 25 October 2007

Abstract—Previously, ethyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (**YD-3**) was identified by us as the first non-peptide protease-activated receptor 4 (PAR4) antagonist. To continue on our development of novel anti-PAR4 agents, **YD-3** was used as a lead compound and a series of its derivatives were synthesized and evaluated for their selective anti-PAR4 activity. Through structure–activity relationship (SAR) study, we identified the important functional groups contributing to anti-PAR4 activity, and these functional groups were kept intact during subsequent structural modification. Several new compounds with anti-PAR4 activity comparable to **YD-3** were identified. Among them, ethyl 4-[1-(3-chlorobenzyl)-1*H*-indazol-3-yl]benzoate (**33**) showed the most potent inhibitory effect on PAR4-mediated platelet aggregation, ATP release, and P-selectin expression. On the other hand, ethyl 4-(1-phenyl-1*H*-indazol-3-yl)benzoate (**83**) exhibited dual inhibitory effects on PAR4 and thromboxane formation from arachidonic acid. The above findings can be used as guidelines for development of novel antiplatelet drug candidates.

1. Introduction

Thrombin-mediated platelet activation and aggregation are critical for arterial thrombosis in unstable angina and myocardial infarction.[1] Human platelet expresses two thrombin receptors PAR1, the principal receptor, and PAR4, which serves as an auxiliary factor to signal platelet activation.[2–4] It has been reported that pharmacological or genetic blockade of PAR1 or PAR4 inhibits arterial thrombosis in animal models.[5,6] Moreover, because PAR antagonists do not affect the ability of thrombin to cleave fibrinogen, they probably cause fewer bleeding complications than thrombin inhibitors. Therefore, PAR1 and PAR4 represent promising targets for development of new antithrombotic drugs.

In the previous study, we showed that ethyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (**YD-3**) represents the first selective non-peptide PAR4 antagonist.[7] **YD-3** selectively inhibited platelet aggregation caused by PAR4 agonist peptides at submicromolar concentrations ($IC_{50} \fallingdotseq 0.13\ \mu M$). Although **YD-3** alone hardly affected platelet aggregation induced by thrombin, it was able to act synergistically with PAR1 antagonists to inhibit thrombin-induced platelet aggregation and P-selectin expression.[8] In order to identify the key function groups for antiplatelet activity in **YD-3**, and to develop potent PAR4 antagonist, **YD-3** was selected as lead compound. A series of its derivatives were synthesized and evaluated for selective anti-PAR4 activity. Their structure–activity relationships (SAR) were established and utilized in subsequent structural modification. Compounds exhibiting superior and unique activities were further examined for mechanism of action to establish guidelines for further development of drug candidates.

Keywords: Protease-activated receptor 4 (PAR4) antagonist; Structure–activity relationships (SAR); **YD-3** derivatives.
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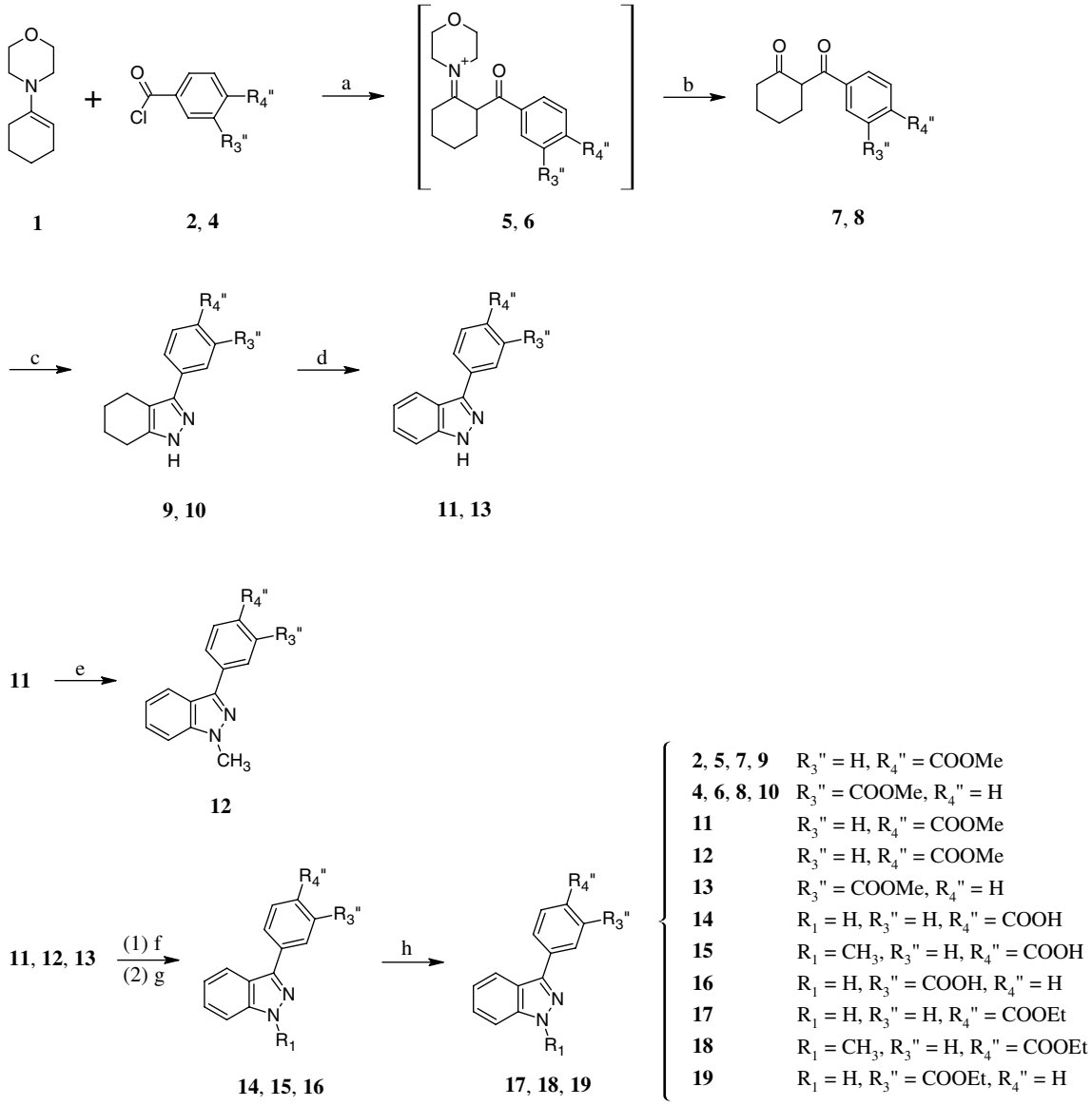
doi:10.1016/j.bmc.2007.10.070

2. Results and discussion

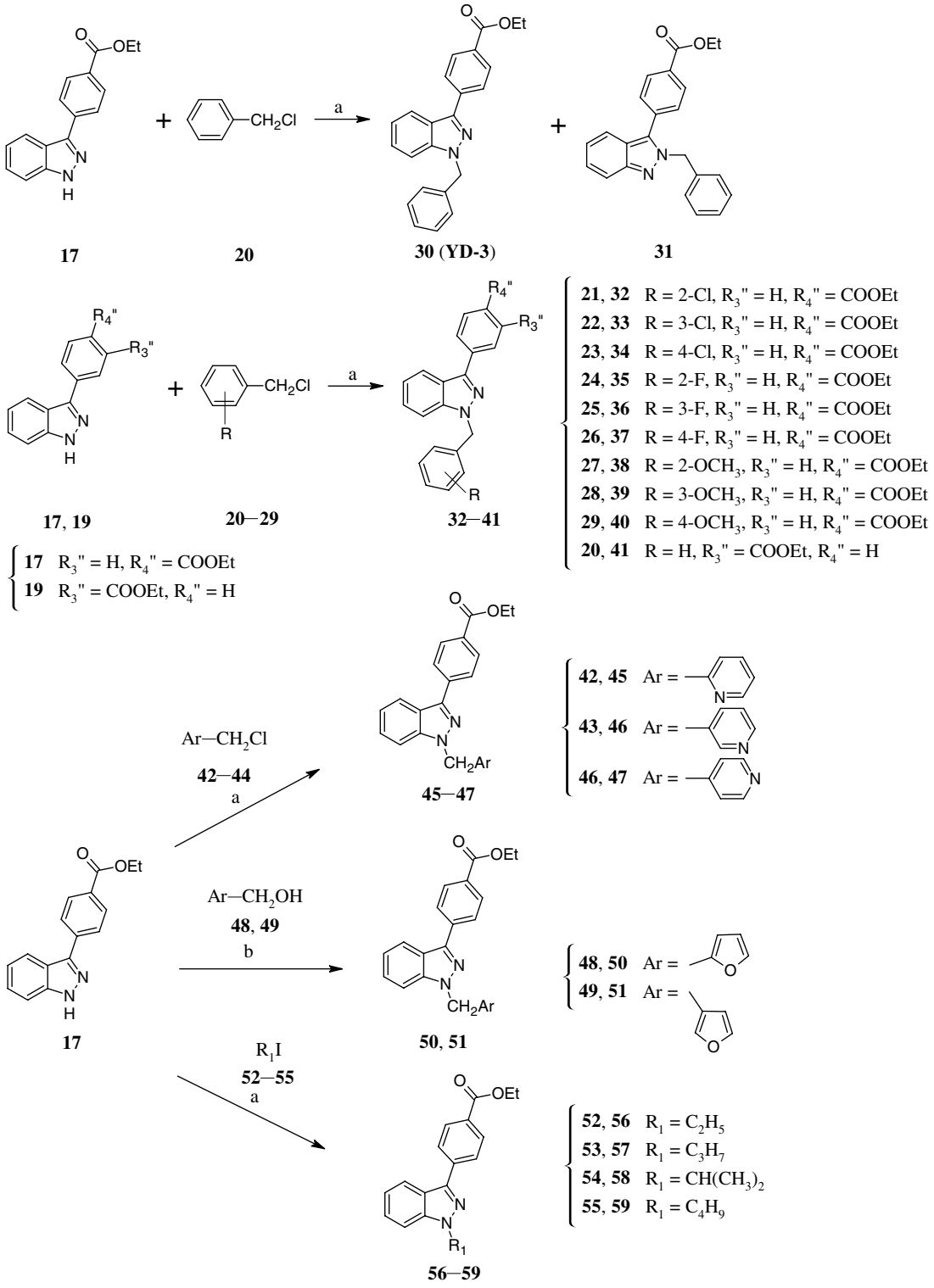
2.1. Chemistry

The key intermediates (**17**, **19**) for synthesis of target compounds were prepared according to Scheme 1.[9] First, 1-(*N*-morpholino)cyclohexene (**1**) was treated separately with two methoxy carbonylbenzoyl chlorides (**2**, **4**) in the presence of Et_3N, followed by acid treatment (20% HCl) under reflux, to yield the corresponding ketones (**7**, **8**). Condensation of compounds **7** and **8** with $NH_2NH_2 \cdot H_2O$ afforded the corresponding tetrahydroindazoles (**9**, **10**) that were then dehydrogenated over Pd/C, under elevated temperature, to produce the corresponding indazoles (**11**, **13**). Subsequent methylation of compound **11** with methyl iodide gave methyl 4-(1-methyl-1*H*-indazol-3-yl)benzoate (**12**). The above-synthesized compounds **11**–**13** were subjected to hydrolysis, followed by esterification with ethyl alcohol, to give the corresponding key intermediates (**17**, **19**) and target compound **18**.

Scheme 2 illustrates the synthetic method for compounds **30**–**41**, **45**–**47**, **50**, **51**, and **56**–**59**. First, the solution of compound **17** in EtOH was benzylated with benzyl chloride in the presence of EtONa to yield a mixture of compounds **30** (mp 79–81 °C, major product) and **31** (mp 112–113 °C, minor product). Mass spectra [*m*/*z*, 356 (M^+)] and elemental analysis data indicated that they are N^1- and N^2-benzyl regioisomers with the same molecular formula $C_{23}H_{20}N_2O_2$. Having the same characteristic IR, NMR, and Mass spectra as previously reported for **YD-3**,[10] compound **30** was identified as the N^1-benzyl isomer. On the other hand, the structure of compound **31** was confirmed by HMBC NMR spectra. As indicated by the HMBC correlation in Figure 1,



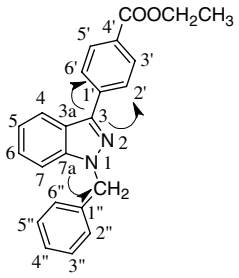
Scheme 1. Reagents and conditions: (a) Et_3N, $CHCl_3$, reflux, 3 h; (b) 20% HCl, reflux, 5 h; (c) 98% $NH_2NH_2 \cdot H_2O$, MeOH, reflux, 30 min; (d) *trans*-decalin, 10% Pd/C, reflux, 24 h; (e) methyl iodide, MeONa, MeOH, rt, 12 h; (f) MeOH, 10% NaOH, reflux, 1 h; (g) 10% HCl; (h) 99.5% EtOH, *p*-TSA, reflux, 2 h.



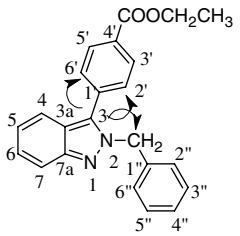
Scheme 2. Reagents and conditions: (a) EtONa, 99.5% EtOH, rt, 12 h; (b) 1,1′-(azodicarbonyl)dipiperidine, tri-*n*-butylphosphine, toluene, reflux, 16 h.

the ^3J-correlation from C_3-carbon to N-CH_2-, H-2′, and H-6′ observed in compound **31** differed from the ^3J-correlation seen in compound **30**.

Next, the key intermediate **17** was subjected to alkylation with various substituted benzyl chlorides (**21**–**29**) to yield corresponding major products (**32**–**40**). Since the antiplatelet activity of the minor product **31** was found to be extremely weak, the minor byproducts obtained during synthesis of compounds **32**–**40** were neither purified nor tested for activities. Following the same synthetic method, the intermediate **19** was benzylated with benzyl chloride to afford the corresponding major product **41**. Similarly, intermediate **17** was alkylated



30 (YD-3)



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Compound 30: HMBC correlations		Compound 31: HMBC correlations	
^{1}H	^{13}C	^{1}H	^{13}C
H-4	C-6, C-7a	H-4	C-6, C-7a
H-5	C-3a, C-7	H-5	C-3a, C-7
H-6	C-4, C-7a	H-6	C-4, C-7a
H-7	C-3a, C-5	H-7	C-3a, C-5
H-2", 6"	-$\underline{C}H_2$-, C-6", 2", C-4"	H-2", 6"	-$\underline{C}H_2$-, C-6", 2", C-4"
H-3", 5"	C-1", C-5", 3", C-4"*	H-3", 5"	C-1", C-5", 3", C-4"*
H-2', 6'	C-3, C-4', C-6', 2'	H-2', 6'	C-3, C-4', C-6', 2'
H-3', 5'	4'-$\underline{C}OOCH_2CH_3$, C-1', C-5', 3'	H-3', 5'	4'-$\underline{C}OOCH_2CH_3$, C-1', C-5', 3'
N-C$\underline{H}_2$-	C-2", 6", C-7a	*N*-C$\underline{H}_2$-	C-3, C-2", 6"

*: $^{2}J_{CH}$ correlation; others are $^{3}J_{CH}$ correlation

Figure 1. HMBC correlations of compounds **30** and **31**.

with various picolyl chlorides (**42**–**44**) to obtain corresponding picolyl derivatives (**45**–**47**). Alternatively, the intermediate **17** was reacted with 2- or 3-hydroxymethyl furan (**48**, **49**) using Mitsunobu reaction to provide the corresponding furyl derivatives (**50**, **51**). Next, compound **17** was treated with various alkyl halides (**52**–**55**) to afford the corresponding major N^1-alkyl derivatives (**56**–**59**).

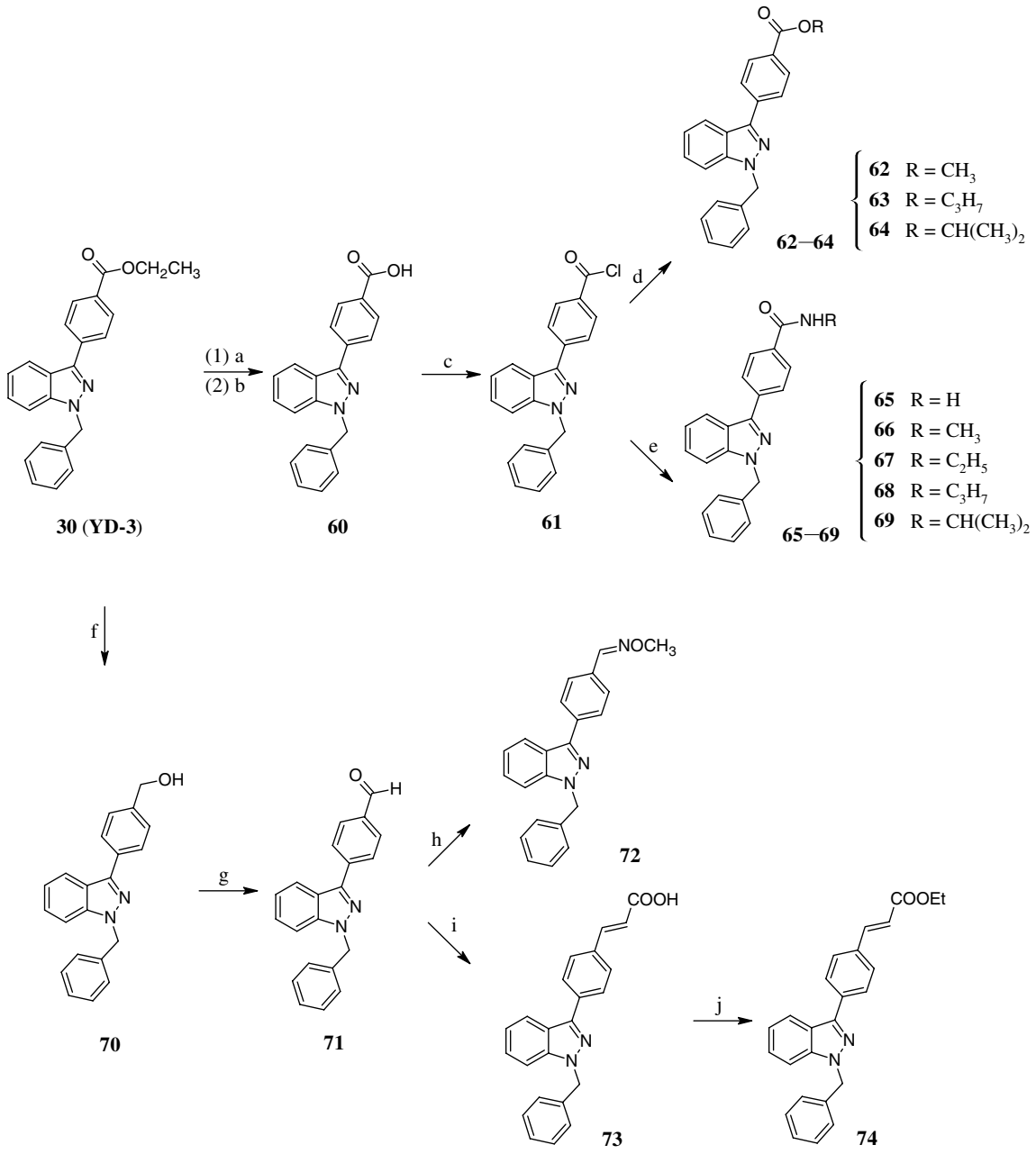
Starting from a different synthetic approach, shown in Scheme 3, compound **30** (**YD-3**) was first hydrolyzed, with 10% NaOH, into a carboxylic acid (**60**) which was converted into an acid chloride (**61**) by treatment with $SOCl_2$. The so-formed compound **61** was then either esterified with various alkyl alcohols to produce corresponding esters (**62**–**64**) or treated with various amines to give amides (**65**–**69**). Moreover, compound **30** was reduced with $Ca(BH_4)_2$ into a carbinol derivative (**70**) which was subsequently oxidized with MnO_2 into an aldehyde derivative (**71**). The so-formed aldehyde (**71**) was either converted into a Schiff base (**72**) by treatment with methylhydroxyamine, or was subjected to condensation with malonic acid to yield an acrylic acid derivative (**73**) which was subsequently esterified into compound **74**.

The 1-phenyl analog of **YD-3**, compound **83**, was prepared according to our previously reported method.[10] As described in Scheme 4, a 4-ethoxycarbonylphenyl phenyl ketone (**79**) was first prepared, which was then transformed into a phenylhydrazone (**82**) by reaction with phenylhydrazine. Following consecutive treatment with $Pb(OAc)_4$ and $BF_3 \cdot Et_2O$, compound **82** was converted into the target compound **83** by oxidative cyclization.

2.2. Antiplatelet activity

In washed human platelets, U46619 (2 μM, a stable thromboxane A_2 mimetic), collagen (10 μg/mL), thrombin (0.05 U/mL), SFLLRN (10 μM, a PAR1 agonist peptide), and GYPGKF (1.5 mM, a PAR4 agonist peptide) all elicited about 80–90% aggregation. The effect of test compounds on platelet aggregation was assessed and the results are summarized in Table 1. As indicated in Table 1, the lead compound **YD-3** demonstrated selective and potent inhibitory activity against GYPGKF-induced platelet aggregation with an IC_{50} value of 0.13 μM. However, it showed only weak inhibitory effect ($IC_{50} > 10$ μM) toward platelet aggregation induced by U46619, collagen, thrombin, and SFLLRN. Such results agree with the data in the literature,[7] and reconfirmed that **YD-3** is a selective and potent anti-PAR4 agent.

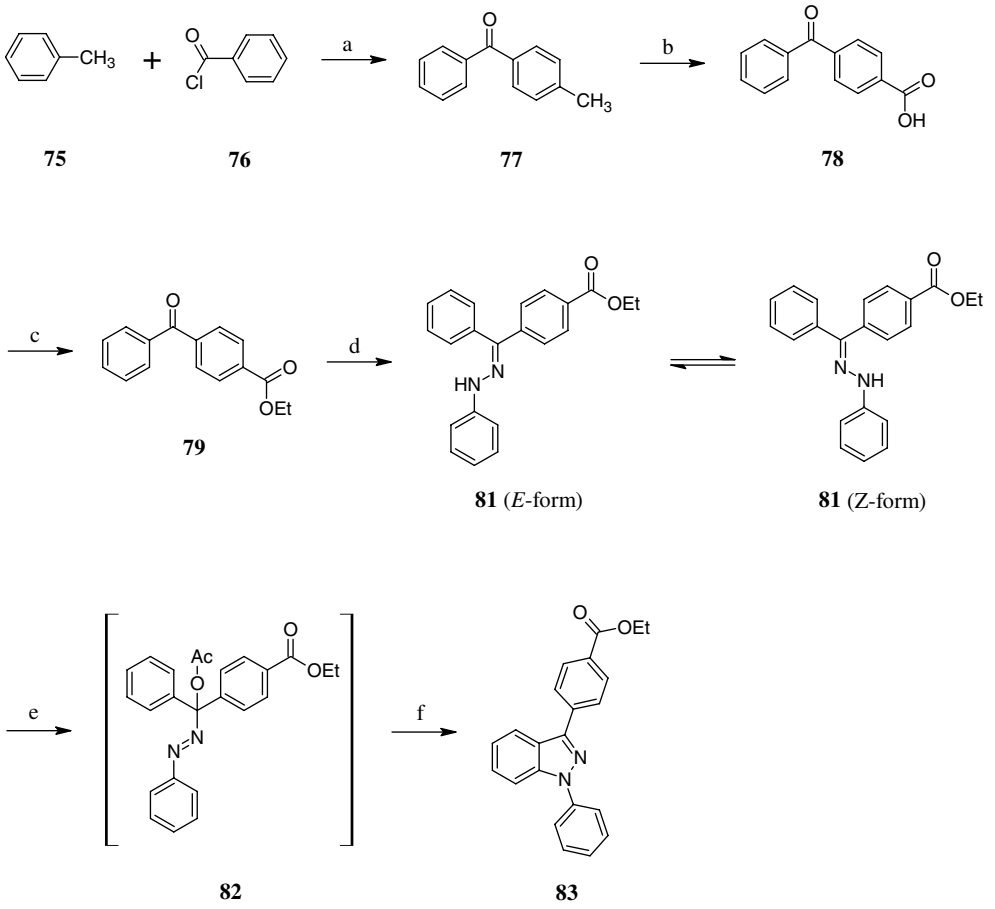
The replacement of the 4″-ethoxycarbonyl group of **YD-3** with a methoxycarbonyl group (compound **62**) retained its potency. Its IC_{50} toward GYPGKF-induced platelet aggregation raised from 0.13 to 0.56 μM, equivalent to the decrease of potency to about 1/4 that of **YD-3**. The attempt to replace the 4″-ethoxycarbonyl moiety of lead compound with different ester groups (**63**, **64**) raised the IC_{50} to greater than 10 μM toward platelet aggregation caused by all the tested inducers. This seemed to suggest that 4″-ethoxycarbonyl group is the optimized ester moiety for **YD-3**. The reposition of the



Scheme 3. Reagents and conditions: (a) MeOH, 10% NaOH, reflux, 1 h; (b) 10% HCl; (c) $SOCl_2$, CH_2Cl_2, reflux, 16 h; (d) substituted alcohol, reflux, 2 h; (e) substituted amine, rt, 30 min; (f) $Ca(BH_4)_2$, THF, reflux, 6 h; (g) MnO_2, acetone, rt, 20 h; (h) *O*-methylhydroxylamine, 99.5% EtOH, reflux, 3 h; (i) malonic acid, pyridine, 80 °C, 3 h; (j) 99.5% EtOH, *p*-TSA, reflux, 2 h.

ethoxycarbonyl group of **YD-3** from 4″- to 3″-position (**41**) drastically impaired its antiplatelet activity. Hydrolysis, or reduction, of the 4″-ethoxycarbonyl group of **YD-3** into carboxylic acid (**60**) and 4″-carbinol (**70**), respectively, also lowered the antiplatelet activity. Furthermore, the substitution of the 4″-ethoxycarbonyl group of **YD-3** with amide groups (**65**–**69**) also remarkably decreased the antiplatelet activity. Among them, compound **67** was the only one that retained selective but relatively weak anti-PAR 4 activity. Next, the substitution of the 4″-ethoxycarbonyl group of **YD-3** with 4″-CH=NOCH$_3$ (**72**) or –CH=CH–COOCH$_2$CH$_3$ (**74**) also decreased the antiplatelet activity.

The above SAR results indicated that the 4″-ethoxycarbonyl group on **YD-3** is the major contributing functional group for its antiplatelet activity. Any modification of this moiety of **YD-3** lowered its antiplatelet activity. Therefore, during subsequent structural modification of the 1-benzyl group of **YD-3**, the 4″-ethoxycarbonyl group was kept intact. Next, the removal of the 1-benzyl group, or the aromatic moiety on the 1-benzyl group, from **YD-3** yielded compounds **17** and **18**, respectively, with selective but weaker anti-PAR4 activity. On the other hand, replacing the N^1-methyl group of compound **18** with N^1-ethyl (**56**), N^1-propyl (**57**), N^1-isopropyl (**58**), and N^1-butyl (**59**) groups all results in



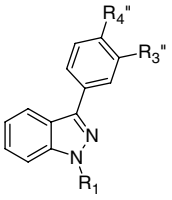
Scheme 4. Reagents and conditions: (a) $AlCl_3$, rt, 12 h; (b) HOAc, H_2SO_4, CrO_3, 100 °C, 4 h; (c) 99.5% EtOH, *p*-TSA, reflux, 2 h; (d) phenylhydrazine, HOAc, 99.5% EtOH, reflux, 12 h; (e) $Pb(OAc)_4$, CH_2Cl_2, 0–5 °C, 30 min; (f) $BF_3 \cdot Et_2O$, reflux, 1 h.

reduced activity. The removal of the $-CH_2-$ moiety from 1-benzyl group of **YD-3** led to a 1-phenyl derivative (**83**) with a shift in antiplatelet pattern from PAR4 specific inhibition to dual inhibition against both collagen- and GYPGKF-induced platelet aggregation. As comparison, the replacement of the 1-benzyl group with 1-picolyl group (**45**–**47**) or 1-furfuryl group (**50**, **51**) maintained the selective anti-PAR4 activity. The relocation of the 1-benzyl group to N^2-position resulted in 2-benzyl derivative (**31**) with considerable loss of antiplatelet activity. The above findings seem to suggest that the presence of benzyl group at the N^1-position of indazole ring is important for optimum activity. Consequently, during our subsequent structural modification, the benzyl group was fixed at the N^1-position while various substituents were introduced into it.

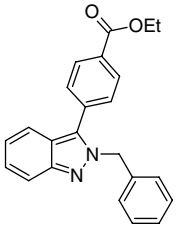
First, the substitution of chloro atom onto different sites of the 1-benzyl group led to 1-chlorobenzyl derivatives (**32**–**34**) with selective and potent anti-PAR4 activity. Among them, 1-(4-chlorobenzyl) derivative (**34**) was found to be equally potent as **YD-3**, whereas the 1-(3-chlorobenzyl) derivative (**33**), with an IC_{50} of 0.08 μM, is more potent than the leading **YD-3**. Similar substitution of fluoro atom onto the 1-benzyl group afforded 1-(fluorobenzyl) derivatives (**35**–**37**) with selective and potent anti-PAR4 activity. In particular, the activity of 1-(2-fluorobenzyl) derivative (**35**) was found to be comparable with **YD-3**. Finally, the introduction of methoxy group onto the 1-benzyl group of **YD-3** led to 1-(methoxybenzyl) derivatives (**38**–**40**) with slightly attenuated anti-PAR4 activity.

From the above SAR study, we have obtained the following important information: (1) Both the 4″-ethoxycarbonyl and 1-benzyl groups on **YD-3** were identified as the important functional groups contributing to the selective anti-PAR4 activity of **YD-3** derivatives studied. The relocation of either one of them led to significant loss in activity. (2) The substitution of the 4″-ethoxycarbonyl group on **YD-3** with other esters, amides, carboxylic acid, or carbinol moieties decreased its activity significantly. (3) The introduction of chloro, fluoro atom, or methoxy group onto the 1-benzyl group of **YD-3** maintained its selective and potent anti-PAR 4 activity, but led to considerable fluctuation in potency. (4) The replacement of the 1-benzyl group of **YD-3** with 1-alkyl, 1-picoyl, or 1-furfuryl group maintained the selective anti-PAR4 activity. (5) The removal of the 1-benzyl group, or the phenyl moiety on the 1-benzyl group, from **YD-3** led to selective but weaker anti-PAR4 activity. (6) The removal of the $-CH_2-$ moiety from the 1-benzyl group of **YD-3** afforded a 1-phenyl derivative with shift in antiplatelet pattern from PAR4 specific inhibition to dual inhibition against collagen- and GYPGKF-induced platelet aggregation.

Table 1. The inhibitory effects of **YD-3** and its derivatives on human platelet aggregation induced by U46619, collagen, thrombin, SFLLRN, and GYPGKF



17, **18**, **30**, **32**—**41**, **45**—**47**, **50**, **51**, **56**—**60**, **62**—**70**, **72**, **74**, **83**



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Compound	R_1	R_3''	R_4''	IC_{50} (μM)				
				U46619	Collagen	Thrombin	SFLLRN	GYPGKF
YD-3	Benzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.13 ± 0.02
62	Benzyl	H	$COOCH_3$	>10	>10	>10	>10	0.56 ± 0.03
63	Benzyl	H	$COOC_3H_7$	>10	>10	>10	>10	>10
64	Benzyl	H	$COOCH(CH_3)_2$	>10	>10	>10	>10	>10
41	Benzyl	$COOC_2H_5$	H	>10	>10	>10	>10	>10
60	Benzyl	H	COOH	>10	>10	>10	>10	>10
65	Benzyl	H	$CONH_2$	>10	>10	>10	>10	>10
66	Benzyl	H	$CONHCH_3$	>10	>10	>10	>10	>10
67	Benzyl	H	$CONHC_2H_5$	>10	>10	>10	>10	5.64 ± 0.40
68	Benzyl	H	$CONHC_3H_7$	>10	9.56 ± 0.51	>10	>10	>10
69	Benzyl	H	$CONHCH(CH_3)_2$	>10	>10	>10	>10	>10
70	Benzyl	H	CH_2OH	>10	>10	>10	>10	>10
72	Benzyl	H	$CH{=}NOCH_3$	>10	>10	>10	>10	>10
74	Benzyl	H	$CH{=}CHCOOC_2H_5$	>10	>10	>10	>10	>10
17	H	H	$COOC_2H_5$	>10	>10	>10	>10	5.65 ± 0.30
18	CH_3	H	$COOC_2H_5$	>10	>10	>10	>10	0.27 ± 0.02
56	C_2H_5	H	$COOC_2H_5$	>10	>10	>10	>10	0.36 ± 0.02
57	C_3H_7	H	$COOC_2H_5$	>10	>10	>10	>10	0.49 ± 0.02
58	$CH(CH_3)_2$	H	$COOC_2H_5$	>10	>10	>10	>10	0.65 ± 0.04
59	C_4H_9	H	$COOC_2H_5$	>10	>10	>10	>10	0.67 ± 0.01
83	Phenyl	H	$COOC_2H_5$	>10	4.81 ± 0.14	>10	>10	2.64 ± 0.29
45	2′-Picoyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.29 ± 0.03
46	3′-Picoyl	H	$COOC_2H_5$	>10	>10	>10	>10	5.55 ± 0.31
47	4′-Picoyl	H	$COOC_2H_5$	>10	>10	>10	>10	5.63 ± 0.17
50	2′-Furyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.34 ± 0.03
51	3′-Furyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.58 ± 0.03
31	—	—	—	>10	>10	>10	>10	>10
32	2′-Chlorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.23 ± 0.05
33	3′-Chlorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.08 ± 0.01
34	4′-Chlorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.12 ± 0.01
35	2′-Fluorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.11 ± 0.00
36	3′-Fluorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.27 ± 0.03
37	4′-Fluorobenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.19 ± 0.01
38	2′-Methoxybenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.47 ± 0.01
39	3′-Methoxybenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.26 ± 0.01
40	4′-Methoxybenzyl	H	$COOC_2H_5$	>10	>10	>10	>10	0.62 ± 0.03

Human platelets were incubated with tested sample or 0.3% DMSO at 37 °C for 3 min, then U46619 (2 μM), collagen (10 μg/mL), thrombin (0.05 U/mL), SFLLRN (10 μM), or GYPGKF (1.5 mM) was added to trigger the aggregation. Values are presented as means ± SE.
YD-3: positive control.

In addition to platelet aggregation, activated platelets also release the contents of their dense- (platelet agonists such as ADP and serotonin) and α-granules (P-selectin, fibrinogen, and growth factors), which are important for thrombosis, inflammation, and atherogenesis.[11] In the present study, the secretion of dense- and α-granule contents in platelets was evaluated by ATP release (which is co-released with ADP from dense granules) and P-selectin expression, respectively. **YD-3** and three representative derivatives (compounds **33**, **45**, and **83**) were

tested for their effect on platelet granule secretion. Compound **33** was chosen for its highest potency against PAR4, while compound **45** was selected for its ease of converting into an HCl salt which may expedite the dosage preparation process. Finally, compound **83** was selected due to its exhibition of different antiplatelet pattern. The inhibitory effect of **YD-3**, and compounds **33**, **45**, and **85** against PAR4-mediated ATP release is summarized in Table 2. As shown, the inhibitory effect of compound **33** at 0.1 and 0.2 μM concentration was slightly better than that of **YD-3**, meanwhile, complete inhibition of ATP release was observed in compound **45** at the concentration of 1 μM, and in compound **85** at 5 μM. In comparison, the inhibitory effect of the above compounds against PAR4-mediated P-selectin expression is shown in Table 3. As shown, the inhibitory effect of compound **33** at 0.05 and 0.1 μM concentration was also slightly better than that of **YD-3**. Similar to their potency in their inhibition of ATP release, complete inhibition of P-selectin expression was observed in compound **45** at the concentration of 1 μM, and in compound **83** at 5 μM.

Compound **83** inhibited both GYPGKF- and collagen-induced platelet aggregation, suggesting that in addition to anti-PAR4 effect, there is other effect of compound **83** in human platelets. It is known that platelet stimulation by collagen is largely dependent on the release of arachidonic acid (AA) and its subsequent conversion via cyclooxygenase to thromboxane A_2, and therefore can be prevented by cyclooxygenase inhibitors.[12] To examine the effect of compound **83** on thromboxane formation in human platelets, we used exogenous AA as a precursor, which circumvents receptor stimulation. As shown in Table 4, compound **83** (5 μM) inhibited AA-induced thromboxane formation by 98.5%. In parallel, platelet aggregation caused by AA was also abolished in the presence of compound **83** (data not shown). These results suggest that compound **83** is able to inhibit the metabolism of AA at the cyclooxygenase level. In contrast, **YD-3**, **33**, and **45**, even at the highest concentrations used, had no effect on AA-induced thromboxane formation and platelet aggregation, which indicated that they are all pure PAR4 antagonists. The dual inhibition of PAR4 and thromboxane formation by compound **83** may provide broader spectrum of antiplatelet activity and represents a lead for the development of new antiplatelet drugs.

Table 2. Effects of **YD-3**, **33**, **45**, and **83** on ATP release from activated platelets

		Released ATP ($ng/3 \times 10^8$ platelets)
Resting		1.3 ± 0.1
Control		100.2 ± 18.9
YD-3	0.2 μM	12.7 ± 2.4***
	0.1 μM	74.4 ± 14.7
33	0.2 μM	8.9 ± 3.6***
	0.1 μM	58.9 ± 15.4
45	1 μM	3.7 ± 0.3***
83	5 μM	1.8 ± 0.4***

Washed human platelets were incubated with DMSO (vehicle control) or test compounds at 37 °C for 3 min, then GYPGKF (1.5 mM) was added to trigger ATP release. Released ATP was measured by a luciferase/luciferin kit as described in Section 4. Results are presented as means ± SEM ($n = 3$). ***$P < 0.001$ as compared with the control.

Table 3. Effect of **YD-3**, **33**, **45**, and **83** on P-selectin expression

		% Positive P-selectin expression
Resting		1.7 ± 0.2
Control		88.7 ± 2.4
YD-3	0.1 μM	7.7 ± 0.8***
	0.05 μM	67.2 ± 5.1**
33	0.1 μM	5.2 ± 2.0***
	0.05 μM	39.3 ± 11.0**
45	1 μM	1.2 ± 0.6***
83	5 μM	1.6 ± 0.8***

Washed human platelets were pre-incubated with DMSO or test compounds for 3 min and thentreated with or without GYPGKF in the presence of FITC-conjugated anti-CD62P for 15 min at room temperature. The samples were then fixed and analyzed by flow cytometry. The levels of P-selectin expression were expressed as the percentages of cells positive for anti-CD62P. Results are presented as means ± SEM. ($n = 3$). ***$P < 0.001$, **$P < 0.01$ as compared with the control.

Table 4. Effects of **YD-3**, **33**, **45**, and **83** on the thromboxane B_2 (TxB_2) formation in human platelets stimulated with arachidonic acid

		TxB_2 ($ng/3 \times 10^8$ platelets)
Resting		0.52 ± 0.04
Control		1589.4 ± 266.1
YD-3	5 μM	1408.3 ± 266.2
33	5 μM	1368.4 ± 219.1
45	5 μM	1013.7 ± 301.4
83	5 μM	24.4 ± 4.4***

Washed human platelets were preincubated with DMSO or test compounds at 37 °C for 3 min and then arachidonic acid (150 μM) was added. Reactions were terminated by EDTA (2 mM) and indomethacin (50 μM) 5 min after the addition of arachidonic acid. Values are presented as means ± SEM ($n = 3$). ***$P < 0.001$ as compared with control.

3. Conclusion

Through the SAR study of **YD-3** derivatives, we have identified the important functional groups contributing to the selective and potent anti-PAR4 activity. We also identified compound **33** as a more potent anti-PAR4 agent than **YD-3**. Furthermore, compound **83** was found to exhibit dual inhibitory effect on PAR4 and thromboxane formation. The above finding will provide guidelines for development of novel antiplatelet drug candidates.

4. Experimental

All of the solvents and reagents were obtained commercially and used without further purification. Reactions were monitored by thin-layer chromatography, using Merck plates with fluorescent indicator. Column chromatography was performed on silica gel.

Melting points were determined with a Yanaco MP-500D melting point apparatus and are uncorrected. IR spectra were recorded on Shimadzu IR-440 or Nicolet Impact 400 FT-IR spectrophotometers as KBr pellets. NMR spectra were obtained on a Bruker Avance DPX-200 FT-NMR spectrometer in DMSO-d_6 or $CDCl_3$. The following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; and br, broad. MS spectra were measured with an HP 5995 GC-MS instrument. The UV spectra were recorded on a Shimadzu UV-160A UV–vis recording spectrophotometer as methanolic solution. Elemental analyses (C, H, and N) were performed on a Perkin-Elmer 2400 Series II CHNS/O analyzer or Elementar vario EL III Heraeus CHNOS Rapid F002 and the results were within ±0.4% of the calculated values.

4.1. Methyl 4-(1*H*-indazol-3-yl)benzoate (11)

(A) Into a solution of 1-(*N*-morpholino)cyclohexene (**1**) (33.4 g, 0.2 mol) and triethylamine (28 mL) in $CHCl_3$ (100 mL) was added dropwise the solution of terephthalic acid monomethyl ester chloride (**2**) (19.9 g, 0.2 mol) in $CHCl_3$ (40 mL) at 45 °C. The reaction mixture was allowed to react for 3 h. Then 20% HCl (20 mL) was added, and the mixture was heated under reflux for 5 h and then allowed to stand at 25 ± 5 °C. The $CHCl_3$ layer was collected and washed with H_2O and dried over $MgSO_4$ and then evaporated. The residue was chromatographed (silica gel-$CHCl_3$/*n*-hexane = 1/2) to afford methyl 4-[(2-oxocyclohexyl)carbonyl]benzoate (**7**), yield 45.19 g, 86.5%; mp 91–92 °C.

(B) Compound **7** (15.8 g, 0.06 mol) was dissolved in MeOH (100 mL), then 3.4 mL of 98% $NH_2NH_2 \cdot H_2O$ was added dropwise at reflux. The mixture was allowed to react for 30 min and was then concentrated in vacuo until there is about 35 mL solution left. The residue was allowed to precipitate during cooling. The solid precipitate was washed with EtOH and dried to yield methyl 4-(4,5,6,7-tetrahydro-1*H*-indazol-3-yl)benzoate (**9**), yield 13.84 g, 90.1%; mp 184–187 °C.

(C) Into the solution of compound **9** (13.0 g, 0.05 mol) in *trans*-decahydronaphthalene (*trans*-decalin) was added 10% Pd/C (2.7 g), and the mixture was heated under reflux for 24 h and then concentrated to dry in vacuo in oil bath. The residue was chromatographed (silica gel-ethyl acetate/*n*-hexane = 1/3) to afford compound **11**, yield 10.42 g, 82.7%; mp 175–178 °C; MS (EI, 70 eV): *m*/*z* 252 (M^+); found: C, 71.40; H, 4.77; N, 11.11. $C_{15}H_{12}N_2O_2$ requires: C, 71.42; H, 4.79; N, 11.10; UV λ_{max} (log ε): 211 (4.3), 316 (4.1); IR (KBr): 1609 (C=N), 1697 (C=O), 3306 (–NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ3.88 (s, 3H), 7.20–7.28 (m, 1H), 7.34–7.46 (m, 1H), 7.62 (d, 1H, J = 8.2 Hz), 8.08 (d, 2H, J = 8.6 Hz), 8.13 (d, 1H, J = 9.6 Hz), 8.17 (d, 2H, J = 8.6 Hz), 13.48 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.36, 111.06, 120.35, 120.79, 121.78, 126.55, 126.86 (C × 2), 128.566, 130.05 (C × 2), 138.59, 141.89, 142.15, 166.30.

4.2. Methyl 4-(1-methyl-1*H*-indazol-3-yl)benzoate (12)

Into the solution of compound **11** (1.0 g, 0.004 mol) in absolute EtOH (50 mL) was added EtONa (0.9 g, 0.014 mol). The mixture was stirred at 25 ± 5 °C for 30 min. Methyl iodide (1.14 g, 0.008 mol) was added dropwise, and the mixture was stirred at 25 ± 5 °C for 12 h. The solid precipitate so formed was directly extracted several times with $CHCl_3$. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-$CHCl_3$) to afford compound **12**, yield 0.52 g, 48.9%; mp 114–116 °C; MS (EI, 70 eV): *m*/*z* 266 (M^+); found: C, 72.18; H, 5.30; N, 10.53. $C_{15}H_{12}N_2O_2$ requires: C, 72.16; H, 5.32; N, 10.52; UV λ_{max} (log ε): 211 (4.3), 318 (4.2); IR (KBr): 1609 (C=N), 1709 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.88 (s, 3H), 4.13 (s, 3H), 7.24–7.31 (m, 1H), 7.44–7.51 (m, 1H), 7.72 (d, 1H, J = 8.5 Hz), 8.05–8.09 (m, 2H), 8.11 (d, 1H, J = 5.7 Hz), 8.12–8.16 (m, 2H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 35.91, 52.37, 110.65, 120.96 (C × 2), 121.97, 126.62, 126.78 (C × 2), 128.57, 130.08 (C × 2), 138.10, 140.84, 141.53, 166.26.

4.3. Methyl 3-(1*H*-indazol-3-yl)benzoate (13)

(A) Into a solution of *mono*-methyl isophthalate (**3**) (10.0 g, 0.053 mol), thionyl chloride (6 mL, 0.082 mol), and dimethylformamide (DMF) (0.5 mL) in CH_2Cl_2 (100 mL) at reflux temperature to afford methyl 3-(cholrocarbonyl)benzoate (**4**).

(B) Into a solution of 1-(*N*-morpholino)cyclohexene (**1**) (10.0 g, 0.06 mol), triethylamine (60 mL), compound **4** (19.9 g, 0.2 mol), and $CHCl_3$ (150 mL) were allowed to react as in the preparation of compound **7** to afford methyl 3-[(2-oxocyclohexyl)carbonyl]benzoate (**8**).

(C) Into a solution of compound **8** (5.0 g, 0.0192 mol), 98% $NH_2NH_2 \cdot H_2O$ (3.4 mL), and MeOH (100 mL) were allowed to react as in the preparation of compound **9** to afford 3-(4,5,6,7-tetrahydro-1*H*-indazol-3-yl)benzoate (**10**), yield 4.5 g, 90.0%; mp 133–136 °C.

(D) Into a solution of compound **10** (4.0 g, 0.058 mol), *trans*-decalin (100 mL), and 10% Pd/C (5.0 g) were allowed to react as in the preparation of compound **11** to afford methyl 3-(1*H*-indazol-3-yl)benzoate (**13**), yield 9.85 g, 82.0%; mp 103–104 °C.

4.4. 4-(1*H*-Indazol-3-yl)benzoic acid (14)

Compound **11** (1.0 g, 0.004 mol) in MeOH (5 mL) was added 10% NaOH (20 mL). The mixture was stirred at 25 ± 5 °C for 30 min. The mixture was heated under reflux for 1 h and then cooled to 5 ± 1 °C. Then 10% HCl was added dropwise until white solid was no longer precipitated in ice-bath. The solid was washed several times with H_2O to afford compound **14**, yield 0.83 g, 87.2%; mp 231–234 °C; MS (EI, 70 eV): *m*/*z* 238 (M^+); found: C, 70.59; H, 4.24; N, 11.78. $C_{15}H_{12}N_2O_2$ requires: C, 70.58; H, 4.23; N, 11.76; UV λ_{max} (log ε): 211 (4.2), 317 (4.1); IR (KBr): 1616 (C=N), 1684 (C=O), 3014 (–OH), 3231 (–NH) cm^{-1}; ^1H NMR (200 MHz,

DMSO-d_6): δ 7.22–7.26 (m, 1H), 7.40–7.44 (m, 1H), 7.62 (d, 1H, J = 8.4 Hz), 8.07–8.16 (m, 5H), 13.42 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 111.30, 120.63, 121.08, 121.98, 126.78, 127.03 (C × 2), 130.06, 130.46 (C × 2), 138.44, 142.17, 142.58, 167.65.

4.5. 4-(1-Methyl-1*H*-indazol-3-yl)benzoic acid (15)

Compound **12** (1.0 g, 0.0038 mol) was allowed to react as in the preparation of compound **14** to afford compound **15**, yield 0.82 g, 85.6%; mp 234–237 °C; MS (EI, 70 eV): m/z 252 (M^+); found: C, 71.43; H, 4.78; N, 10.99. $C_{15}H_{12}N_2O_2$ requires: C, 71.42; H, 4.80; N, 11.03; UV λ_{max} (log ε): 211 (4.3), 319 (4.2); IR (KBr): 1609 (C═N), 1684 (C═O), 2983 (–OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.12 (s, 3H), 7.23–7.30 (m, 1H), 7.43–7.51 (m, 1H), 7.71 (d, 1H, J = 8.5 Hz), 8.04–8.15 (m, 5H), 12.97 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 35.88, 110.61, 120.99, 121.90, 126.54, 126.67 (C × 3), 129.79, 130.23 (C × 2), 137.71, 141.04, 141.51, 167.35.

4.6. 3-(1*H*-Indazol-3-yl)benzoic acid (16)

Compound **13** (1.0 g, 0.004 mol) was allowed to react as in the preparation of compound **14** to afford compound **16**, yield 0.8 g, 93.0%; mp 187–190 °C; MS (EI, 70 eV): m/z 238 (M^+); found: C, 70.60; H, 4.20; N, 11.79. $C_{15}H_{12}N_2O_2$ requires: C, 70.58; H, 4.23; N, 11.76; UV λ_{max} (log ε): 215 (4.5), 306 (4.7); IR (KBr): 1684 (C═O), 3449 (–OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 7.23 (t, 1H, J = 7.5 Hz), 7.42 (t, 1H, J = 7.5 Hz), 7.60–7.68 (m, 2H), 7.95–8.07 (m, 2H), 8.23 (d, 1H, J = 7.7 Hz), 8.57 (s, 1H), 13.42 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 111.05, 120.16, 120.53, 121.57, 126.46, 127.51, 128.59, 129.49, 130.89, 132.09, 134.26, 141.88, 142.47, 167.61.

4.7. Ethyl 4-(1*H*-indazol-3-yl)benzoate (17)

Compound **14** (1.0 g, 0.0042 mol) and toluene-4-sulfonic acid monohydrate (p-TSA) (0.5 g, 0.0026 mol) were dissolved in absolute EtOH (100 mL). The solution was heated under reflux for 2 h and then allowed to stand at 25 ± 5 °C. The solution was chromatographed (silica gel-ethyl acetate/n-hexane = 2/3) to afford compound **17**, yield 0.94 g, 84.1%; mp 133–135 °C; MS (EI, 70 eV): m/z 266 (M^+); found: C, 72.18; H, 5.29; N, 10.52. $C_{15}H_{12}N_2O_2$ requires: C, 72.16; H, 5.30; N, 10.52; UV λ_{max} (log ε): 211 (4.2), 317 (4.0); IR (KBr): 1609 (C═N), 1697 (C═O), 3306 (–NH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.0 Hz), 4.32 (q, 2H, J = 7.0 Hz), 7.20–7.27 (m,1H), 7.38–7.45 (m, 1H), 7.62 (d, 1H, J = 8.4 Hz), 8.08 (d, 2H, J = 8.4 Hz), 8.12 (d, 1H, J = 9.2 Hz), 8.16 (d, 2H, J = 8.4 Hz), 13.47 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 60.94, 111.05, 120.37, 120.78, 121.77, 126.53, 126.84 (C × 2), 128.86, 130.00 (C × 2), 138.53, 141.91, 142.21, 165.80.

4.8. Ethyl 4-(1-methyl-1*H*-indazol-3-yl)benzoate (18)

Compound **15** (1.0 g, 0.004 mol) was allowed to react as in the preparation of compound **17** to afford compound **18**, yield 0.73 g, 65.2%; mp 63–64 °C; MS (EI, 70 eV): m/z 280 (M^+); found: C, 72.87; H, 5.73; N, 9.98. $C_{15}H_{12}N_2O_2$ requires: C, 72.84; H, 5.75; N, 9.99; UV λ_{max} (log ε): 210 (4.3), 318 (4.2); IR (KBr): 1607 (C═N), 1707 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.34 (t, 3H, J = 7.0 Hz), 4.13 (s, 3H), 4.33 (q, 2H, J = 7.0 Hz), 7.24–7.31 (m, 1H), 7.44–7.52 (m, 1H), 7.73 (d, 1H, J = 8.0 Hz), 8.07 (d, 2H, J = 8.3 Hz), 8.15 (d, 3H, J = 8.3 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.44, 60.98, 110.67, 120.97 (C × 2), 121.98, 126.63, 126.77 (C × 3), 128.83, 130.05 (C × 2), 138.05, 140.86, 141.52, 165.76.

4.9. Ethyl 3-(1*H*-indazol-3-yl)benzoate (19)

Compound **16** (1.5 g, 0.007 mol) was allowed to react as in the preparation of compound **17** to afford compound **19**, yield 0.61 g, 36.0%; mp 112–113 °C; MS (EI, 70 eV): m/z 266 (M^+); found: C, 72.20; H, 5.31; N, 10.54. $C_{15}H_{12}N_2O_2$ requires: C, 72.16; H, 5.30; N, 10.52; UV λ_{max} (log ε): 215 (4.5), 306 (4.7); IR (KBr): 1719 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.34 (t, 3H, J = 7.0 Hz), 4.34 (q, 2H, J = 7.0 Hz), 7.24 (t, 1H, J = 7.5 Hz), 7.42 (t, 1H, J = 7.6 Hz), 7.60–7.71 (m, 2H), 7.95–8.08 (m, 2H), 8.28 (d, 1H, J = 7.8 Hz), 8.57 (s, 1H), 13.39 (br, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 61.15, 111.06, 120.15, 120.52, 121.64, 126.52, 127.42 (C × 2), 128.36, 129.71, 130.76, 131.38, 134.46, 141.88, 142.29, 165.88.

4.10. Ethyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (30; YD-3) and ethyl 4-(2-benzyl-2*H*-indazol-3-yl)benzoate (31)

Into the solution of compound **17** (4.0 g, 0.015 mol) in absolute EtOH (150 mL) was added EtONa (3.5 g, 0.052 mol). The mixture was stirred at 25 ± 5 °C for 30 min. Then benzyl chloride (**20**) (10.1 g, 0.08 mol) was added dropwise, and the mixture was stirred at 25 ± 5 °C for 12 h. The solid precipitate so formed was directly extracted several times with $CHCl_3$. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-$CHCl_3$) to afford compounds **30** (**YD-3**) and **31**. **YD-3**, yield 2.90 g, 54.3%; mp 79–81 °C; MS (EI, 70 eV): m/z 356 (M^+); found: C, 77.48; H, 5.68; N, 7.88. $C_{23}H_{20}N_2O_2$ requires: C, 77.51; H, 5.66; N, 7.86; UV λ_{max} (log ε): 211 (4.7), 320 (4.6); IR (KBr): 1611 (C═N), 1721 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H, J = 7.1 Hz), 5.75 (s, 2H), 7.22–7.30 (m, 6H), 7.44 (t, 1H, J = 7.0 Hz), 7.78 (d, 1H, J = 8.5 Hz,), 8.06–8.10 (m, 2H), 8.12 (d, 1H, J = 7.5 Hz), 8.15–8.18 (m, 2H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.40, 52.33, 60.97, 110.77, 121.14, 121.30, 122.14, 126.85, 126.95 (C × 2), 127.60 (C × 2), 127.83, 128.82 (C × 2), 129.03, 130.03 (C × 2), 137.41, 137.91, 141.23, 141.71, 165.75. Compound **31**, yield 0.47 g, 8.8%; mp 112–113 °C; MS (EI, 70 eV): m/z 356 (M^+); found: C, 77.49; H, 5.65; N, 7.87. $C_{23}H_{20}N_2O_2$ requires: C, 77.51; H, 5.66; N, 7.86; UV λ_{max} (log ε): 211 (4.7), 321 (4.3); IR (KBr): 1607 (C═N), 1708 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.31 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H,

J = 7.1 Hz), 5.70 (s, 2H), 6.98–7.04 (m, 2H), 7.09–7.14 (m, 1H), 7.19–7.24 (m, 3H), 7.27–7.34 (m,1H), 7.56 (d, 1H, J = 8.4 Hz), 7.69 (d, 3H, J = 8.3 Hz), 8.09 (d, 2H, J = 8.3 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.35, 54.39, 61.20, 117.51, 120.12, 121.19, 122.62, 126.52, 127.15 (C × 2), 127.84, 128.78 (C × 2), 129.84 (C × 2), 130.07 (C × 3), 133.75, 134.62, 137.04, 147.80, 165.48.

4.11. Preparation of ethyl 4-[1-(substituted benzyl)-1*H*-indazol-3-yl]benzoate (32–40)

4.11.1. Ethyl 4-[1-(2-chlorobenzyl)-1*H*-indazol-3-yl]benzoate (32). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 2-chlorobenzyl chloride (**21**) (24.3 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **32**, yield 3.20 g, 54.6%; mp 93–95 °C; MS (EI, 70 eV): m/z 390.5 (M^+); found: C, 70.70; H, 4.89; N, 7.15. $C_{23}H_{19}ClN_2O_2$ requires: C, 70.68; H, 4.90; N, 7.17; UV λ_{max} (log ε): 211 (4.7), 321 (4.6); IR (KBr): 1609 (C═N), 1711 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 7.1 Hz), 4.32 (q, 2H, J = 7.1 Hz), 5.82 (s, 2H), 6.87 (dd, 1H, J = 1.7, 7.6 Hz), 7.18–7.34 (m, 3H), 7.42–7.51 (m, 2H), 7.74 (d, 1H, J = 8.5 Hz), 8.04–8.18 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 49.96, 60.98, 110.65, 121.16 (C × 2), 122.27, 127.00 (C × 3), 127.74, 129.11, 129.38, 129.72 (C × 2), 130.02 (C × 2), 132.23, 134.74, 137.73, 141.62, 142.16, 165.72.

4.11.2. Ethyl 4-[1-(3-chlorobenzyl)-1*H*-indazol-3-yl]benzoate (33). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 3-chlorobenzyl chloride (**22**) (24.3 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **33**, yield 2.88 g, 49.2%; mp 76–77 °C; MS (EI, 70 eV): m/z 390.5 (M^+); found: C, 70.71; H, 4.89; N, 7.16. $C_{23}H_{19}ClN_2O_2$ requires: C, 70.68; H, 4.90; N, 7.17; UV λ_{max} (log ε): 211 (4.6), 320 (4.5); IR (KBr): 1609 (C═N), 1713 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.0 Hz), 4.33 (q, 2H, J = 7.0 Hz), 5.78 (s, 2H), 7.20–7.37 (m, 5H), 7.44–7.52 (m, 1H), 7.83 (d, 1H, J = 8.4 Hz), 8.06–8.17 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.42, 51.52, 61.01, 110.71, 121.25 (C × 2), 122.32, 126.27, 127.03 (C × 3), 127.45, 127.86, 129.12, 130.07 (C × 2), 130.83, 133.40, 137.75, 139.91, 141.28, 142.02, 165.74.

4.11.3. Ethyl 4-[1-(4-chlorobenzyl)-1*H*-indazol-3-yl]benzoate (34). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 4-chlorobenzyl chloride (**23**) (24.3 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **34**, yield 3.39 g, 57.9%; mp 89–91 °C; MS (EI, 70 eV): m/z 390.5 (M^+); found: C, 70.70; H, 4.88; N, 7.18. $C_{23}H_{19}ClN_2O_2$ requires: C, 70.68; H, 4.90; N, 7.17; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1611 (C═N), 1719 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H, J = 7.1 Hz), 5.76 (s, 2H), 7.24–7.39 (m, 5H), 7.43–7.50 (m, 1H), 7.80 (d, 1H, J = 8.4 Hz), 8.06–8.17 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.42, 51.51, 61.00, 110.72, 121.20, 121.28, 122.25, 126.99 (C × 3), 128.84 (C × 2), 129.08, 129.49 (C × 2), 130.04 (C × 2), 132.53, 136.43, 137.80, 141.22, 141.92, 165.74.

4.11.4. Ethyl 4-[1-(2-fluorobenzyl)-1*H*-indazol-3-yl]benzoate (35). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 2-fluorobenzyl chloride (**24**) (21.7 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **35**, yield 3.19 g, 56.9%; mp 94–96 °C; MS (EI, 70 eV): m/z 374 (M^+); $C_{23}H_{19}FN_2O_2$; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1612 (C═N), 1719 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H, J = 7.1 Hz), 5.80 (s, 2H), 7.11–7.32 (m, 5H), 7.44–7.52 (m, 1H), 7.80 (d, 1H, J = 8.5 Hz), 8.05–8.16 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 46.27, 60.99, 110.64, 115.74, 121.16 (C × 2), 122.23, 124.17, 124.93, 126.99 (C × 3), 129.10, 130.03, 130.18 (C × 2), 130.27, 137.77, 141.34, 141.99, 160.21, 165.74.

4.11.5. Ethyl 4-[1-(3-fluorobenzyl)-1*H*-indazol-3-yl]benzoate (36). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 3-fluorobenzyl chloride (**25**) (21.7 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **36**, yield 2.97 g, 53.0%; mp 86–87 °C; MS (EI, 70 eV): m/z 374 (M^+); $C_{23}H_{19}FN_2O_2$; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1611 (C═N), 1707 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H, J = 7.1 Hz), 5.78 (s, 2H), 7.07–7.14 (m, 3H), 7.24–7.36 (m, 2H), 7.43–7.51 (m, 1H), 7.82 (d, 1H, J = 8.5 Hz), 8.06–8.18 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 51.64, 60.99, 110.71, 114.33, 114.76, 121.21, 121.27, 122.27, 123.64, 127.02 (C × 3), 129.11, 130.05 (C × 2), 130.93, 137.78, 140.23, 141.29, 141.99, 162.37, 165.74.

4.11.6. Ethyl 4-[1-(4-fluorobenzyl)-1*H*-indazol-3-yl]benzoate (37). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 4-fluorobenzyl chloride (**26**) (21.7 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **37**, yield 3.13 g, 55.8%; mp 79–80 °C; MS (EI, 70 eV): m/z 374 (M^+); $C_{23}H_{19}FN_2O_2$; UV λ_{max} (log ε): 211 (4.6), 320 (4.4); IR (KBr): 1609 (C═N), 1711 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 7.1 Hz), 4.32 (q, 2H, J = 7.1 Hz), 5.74 (s, 2H), 7.08–7.22 (m, 2H), 7.26–7.39 (m, 3H), 7.41–7.49 (m, 1H), 7.80 (d, 1H, J = 8.5 Hz), 8.06–8.17 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.39, 51.52, 60.97, 110.72, 115.63 (C × 2), 121.16, 121.30, 122.18, 126.96 (C × 3), 129.06, 129.76 (C × 2), 130.02 (C × 2), 133.62, 137.85, 141.14, 141.84, 161.83, 165.74.

4.11.7. Ethyl 4-[1-(2-methoxybenzyl)-1*H*-indazol-3-yl]benzoate (38). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 2-methoxybenzyl chloride (**27**) (21 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **38**, yield 3.42 g, 56.9%; mp 80–81 °C; MS (EI, 70 eV): m/z 386 (M^+); found: C, 74.67; H, 5.72; N, 7.22. $C_{24}H_{22}N_2O_3$ requires: C, 74.59; H, 5.74; N, 7.25; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1611

(C=N), 1713 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.28 (t, 3H, J = 7.0 Hz), 3.77 (s, 3H), 4.28 (q, 2H, J = 7.0 Hz), 5.62 (s, 2H), 6.76–6.78 (m, 2H), 6.96 (d, 1H, J = 8.2 Hz), 7.18–7.25 (m, 2H), 7.36–7.40 (m, 1H), 7.65 (d, 1H, J = 8.3 Hz), 8.00–8.11 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.61, 47.59, 55.88, 61.19, 111.04, 111.37, 120.83, 121.28 (C × 2), 122.25, 125.32, 126.96, 127.11 (C × 2), 128.75, 129.16, 129.52, 130.23 (C × 2), 138.17, 141.70, 141.82, 156.96, 165.98.

4.11.8. Ethyl 4-[1-(3-methoxybenzyl)-1*H*-indazol-3-yl]benzoate (39). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 3-methoxybenzyl chloride (**28**) (21 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **39**, yield 2.87 g, 49.5%; mp 93–94 °C; MS (EI, 70 eV): m/z 386 (M^+); found: C, 74.54; H, 5.71; N, 7.23. $C_{24}H_{22}N_2O_3$ requires: C, 74.59; H, 5.74; N, 7.25; UV λ_{max} (log ε): 211 (4.7), 320 (4.4); IR (KBr): 1609 (C=N), 1713 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.29 (t, 3H, J = 7.1 Hz), 3.64 (s, 3H), 4.29 (q, 2H, J = 7.1 Hz), 5.68 (s, 2H), 6.74–6.85 (m, 3H), 7.12–7.23 (m, 2H), 7.40–7.43 (m, 1H), 7.75 (d, 1H, J = 8.4 Hz), 8.02–8.14 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.63, 52.44, 55.42, 61.21, 111.02, 113.07, 113.83, 119.86, 121.37, 121.48, 122.39, 127.17 (C × 3), 129.24, 130.27 (C × 3), 138.11, 139.16, 141.47, 141.91, 159.79, 165.98.

4.11.9. Ethyl 4-[1-(4-methoxybenzyl)-1*H*-indazol-3-yl]benzoate (40). Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and 4-methoxybenzyl chloride (**29**) (21 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **40**, yield 2.90 g, 50.1%; mp 89–90 °C; MS (EI, 70 eV): m/z 386 (M^+); found: C, 74.65; H, 5.72; N, 7.22. $C_{24}H_{22}N_2O_3$ requires: C, 74.59; H, 5.74; N, 7.25; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1609 (C=N), 1697 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.27 (t, 3H, J = 7.1 Hz), 3.61 (s, 3H), 4.27 (q, 2H, J = 7.1 Hz), 5.60 (s, 2H), 6.79 (d, 2H, J = 8.6 Hz), 7.16–7.21 (m, 1H), 7.23 (d, 2H, J = 8.6 Hz), 7.34–7.42 (m, 1H), 7.72 (d, 1H, J = 8.4 Hz), 8.02–8.13 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.58, 52.12, 55.39, 61.17, 110.98, 114.37 (C × 2), 121.31, 121.57, 122.26, 126.93, 127.10 (C × 2), 129.20, 129.33 (C × 2), 129.46, 130.23 (C × 2), 138.19, 141.21, 141.77, 159.21, 165.99.

4.12. Ethyl 3-(1-benyl-1*H*-indazol-3-yl)benzoate (41)

Compound **19** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and benzyl chloride (**20**) (10.1 g, 0.08 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **41**, yield 3.01 g, 52.0%; mp 54–55 °C; MS (EI, 70 eV): m/z 356 (M^+); found: C, 77.55; H, 5.68; N, 7.89. $C_{23}H_{20}N_2O_2$ requires: C, 77.51; H, 5.66; N, 7.86; UV λ_{max} (log ε): 217 (4.6), 311 (4.7); IR (KBr): 1603 (C=N), 1718 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.34 (t, 3H, J = 7.1 Hz), 4.36 (q, 2H, J = 7.1 Hz), 5.75 (s, 2H), 7.23–7.35 (m, 6H), 7.45 (m, 1H), 7.66 (m, 1H), 7.78 (d, 1H, J = 8.4 Hz), 7.98 (d, 1H, J = 7.8 Hz), 8.07 (d, 1H, J = 8.02 Hz), 8.25 (d, 1H, 7.8 Hz), 8.55 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 52.23, 61.16, 110.76, 120.89, 121.08, 122.02, 126.86, 127.30, 127.55 (C × 2), 127.80, 128.56, 128.83 (C × 2), 129.76, 130.83, 131.50, 133.89, 137.53, 141.22, 141.84, 165.83.

4.13. Ethyl 4-[1-(pyridin-2-ylmethyl)-1*H*-indazol-3-yl]benzoate (45)

Compound **17** (4.0 g, 0.015 mol), EtONa (4.2 g, 0.062 mol), and 2-picolyl chloride hydrochloride (**42**) (4.92 g, 0.03 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **45**, yield 1.4 g, 26.3%; mp 80–82 °C; MS (EI, 70 eV): m/z 357 (M^+); found: C, 73.99; H, 5.34; N, 11.73. $C_{22}H_{19}N_3O_2$ requires: C, 73.93; H, 5.36; N, 11.76; UV λ_{max} (log ε): 211 (4.6), 320 (4.5); IR (KBr): 1611 (C=N), 1709 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.32 (t, 3H, J = 7.1 Hz), 4.32 (q, 2H, J = 7.1 Hz), 5.84 (s, 2H), 7.10 (d, 1H, J = 7.8 Hz), 7.24–7.31 (m, 2H), 7.41–7.45 (m, 1H), 7.67–7.76 (m, 2H), 8.07 (d, 2H, J = 8.5 Hz), 8.14 (d, 3H, J = 8.5 Hz), 8.49 (d, 1H, J = 4.7 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.41, 54.21, 61.00, 110.92, 121.09, 121.23, 121.82, 122.19, 123.09, 126.96 (C × 3), 129.05, 130.03 (C × 2), 137.39, 137.85, 141.71, 141.90, 149.53, 156.67, 165.77.

4.14. Ethyl 4-[1-(pyridin-3-ylmethyl)-1*H*-indazol-3-yl]benzoate (46)

Compound **17** (4.0 g, 0.015 mol), EtONa (4.2 g, 0.062 mol), and 3-picolyl chloride hydrochloride (**43**) (4.92 g, 0.03 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **46**, yield 0.24 g, 4.4%; MS (EI, 70 eV): m/z 357 (M^+); found: C, 73.88; H, 5.34; N, 11.80. $C_{22}H_{19}N_3O_2$ requires: C, 73.93; H, 5.36; N, 11.76; UV λ_{max} (log ε): 211 (4.6), 320 (4.4); IR (KBr): 1610 (C=N), 1709 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.29 (t, 3H, J = 7.0 Hz), 4.29 (q, 2H, J = 7.1 Hz), 5.78 (s, 2H), 7.25–7.32 (m, 2H), 7.40–7.45 (m, 1H), 7.59–7.61 (m, 1H), 7.85 (d, 1H, J = 8.2 Hz), 8.02–8.13 (m, 5H), 8.43 (d, 1H, J = 4.7 Hz), 8.59 (d, 1H, J = 2.2 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.64, 50.01, 61.23, 110.92, 121.36, 121.50, 122.54, 124.21, 127.26 (C × 3), 129.33, 130.27 (C × 2), 133.23, 135.74, 137.96, 141.46, 142.33, 149.26, 149.44, 165.96.

4.15. Ethyl 4-[1-(pyridin-4-ylmethyl)-1*H*-indazol-3-yl]benzoate (47)

Compound **17** (4.0 g, 0.015 mol), EtONa (4.2 g, 0.062 mol), and 4-picolyl chloride hydrochloride (**44**) (9.84 g, 0.06 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **47**, yield 0.89 g, 16.6%; mp 90–92 °C; MS (EI, 70 eV): m/z 357 (M^+); found: C, 73.98; H, 5.33; N, 11.74. $C_{22}H_{19}N_3O_2$ requires: C, 73.93; H, 5.36; N, 11.76; UV λ_{max} (log ε): 211 (4.7), 320 (4.4); IR (KBr): 1611 (C=N), 1708 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.31 (t, 3H, J = 7.1 Hz), 4.31 (q, 2H,

J = 7.1 Hz), 5.78 (s, 2H), 7.27–7.33 (m, 2H), 7.42–7.46 (m, 1H), 7.64 (d, 1H, J = 7.9 Hz), 7.75 (d, 1H, J = 8.5 Hz), 8.05 (d, 2H, J = 8.3 Hz), 8.12 (d, 3H, J = 8.3 Hz), 8.44 (dd, 1H, J = 1.6, 4.8 Hz), 8.58 (d, 1H, J = 2.2 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.63, 50.01, 61.22, 110.90, 121.51, 121.63, 122.52, 124.20, 127.25 (C × 3), 129.34, 130.26 (C × 2), 133.22, 135.73, 137.97, 141.46, 142.34, 149.26, 149.34, 165.96.

4.16. Ethyl 4-[1-(2-furylmethyl)-1*H*-indazol-3-yl]benzoate (50)

Compound **17** (4.0 g, 0.015 mol), tri-*n*-butylphosphine (7.74 mL, 0.03 mol), 1,1′-(azodicarbonyl)dipiperidine (7.5 g, 0.03 mol), and 2-furfuryl alcohol (**48**) (2.63 g, 0.03 mol) were dissolved in anhydrous toluene (50 mL). The mixture was heated under reflux for 16 h and then allowed to stand at 25 ± 5 °C. The solid precipitate so formed was directly extracted several times with diethyl ether. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-$CHCl_3$) to afford compound **50**, yield 2.83 g, 54.6%; mp 80–82 °C; MS (EI, 70 eV): m/z 346 (M^+); found: C, 72.84; H, 5.25; N, 8.11. $C_{21}H_{18}N_2O_3$ requires: C, 72.82; H, 5.24 N, 8.09; UV λ_{max} (log ε): 210 (4.3), 320 (4.2); IR (KBr): 1612 (C═N), 1717 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.33 (t, 3H, J = 7.1 Hz), 4.33 (q, 2H, J = 7.1 Hz), 5.76 (s, 2H), 6.40 (dd, 1H, J = 1.9, 3.2 Hz), 6.55 (d, 1H, J = 3.0 Hz), 7.24–7.31 (m, 1H), 7.44–7.51 (m, 1H), 7.56–7.57 (m, 1H), 7.84 (d, 1H, J = 8.5 Hz), 8.05–8.16 (m, 5H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.40, 45.67, 60.98, 109.26, 110.85 (C × 2), 121.07, 121.29, 122.21, 126.85, 126.96 (C × 2), 129.07, 130.03 (C × 2), 137.80, 141.09, 141.70, 143.38, 150.07, 165.75.

4.17. Ethyl 4-[1-(3-furylmethyl)-1*H*-indazol-3-yl]benzoate (51)

Compound **17** (4.0 g, 0.015 mol) and 3-furfuryl alcohol (**49**) (2.63 g, 0.03 mol) were allowed to react as in the preparation of compound **50** to afford compound **51**, yield 2.49 g, 48.0%; mp 56–57 °C; MS (EI, 70 eV): m/z 346 (M^+); found: C, 72.87; H, 5.22; N, 8.06. $C_{21}H_{18}N_2O_3$ requires: C, 72.82; H, 5.24 N, 8.09; UV λ_{max} (log ε): 210 (4.6), 320 (4.3); IR (KBr): 1611 (C═N), 1717 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.30 (t, 3H, J = 7.0 Hz), 4.34 (q, 2H, J = 7.0 Hz), 5.55 (s, 2H), 6.37 (d, 1H, J = 0.8 Hz), 7.23–7.27 (m, 1H), 7.43–7.44 (m, 1H), 7.53–7.54 (m, 1H), 7.75–7.76 (m, 1H), 7.81 (d, 1H, J = 8.5 Hz), 8.05 (d, 2H, J = 8.3 Hz), 8.09 (d, 1H, J = 8.1 Hz), 8.12 (d, 2H, J = 8.3 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.65, 43.93, 61.21, 110.89, 111.00, 121.33, 121.48, 121.56, 122.35, 126.98, 127.19 (C × 2), 129.21, 130.25 (C × 2), 138.16, 141.10, 141.34, 141.73, 144.24, 165.98

4.18. Ethyl 4-(1-ethyl-1*H*-indazol-3-yl)benzoate (56)

Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and ethyl iodide (**52**) (12.1 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **56**, yield 3.02 g, 68.4%; MS (EI, 70 eV): m/z 294 (M^+); found: C, 73.49; H, 6.14; N, 9.49. $C_{18}H_{18}N_2O_2$ requires: C, 73.45; H, 6.16; N, 9.52; UV λ_{max} (log ε): 211 (4.4), 318 (4.2); IR (KBr): 1611 (C═N), 1699 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.34 (t, 3H, J = 7.2 Hz), 1.45 (t, 3H, J = 7.2 Hz), 4.32 (q, 2H, J = 7.2 Hz), 4.54 (q, 2H, J = 7.2 Hz), 7.25–7.29 (m, 1H), 7.45–7.48 (m, 1H), 7.75 (d, 1H, J = 8.4 Hz), 8.08 (d, 2H, J = 8.4 Hz), 8.12–8.15 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.69, 15.35, 43.89, 61.24, 110.79, 121.32 (C × 2), 122.25, 126.83, 127.08 (C × 2), 129.11, 130.29 (C × 2), 138.39, 140.90, 141.30, 166.06.

4.19. Ethyl 4-(1-propyl-1*H*-indazol-3-yl)benzoate (57)

Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and propyl iodide (**53**) (14.5 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **57**, yield 2.69 g, 58.3%; MS (EI, 70 eV): m/z 308 (M^+); found: C, 74.06; H, 6.57; N, 9.05. $C_{19}H_{20}N_2O_2$ requires: C, 74.00; H, 6.54; N, 9.08; UV λ_{max} (log ε): 210 (4.3), 320 (4.3); IR (KBr): 1609 (C═N), 1702 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.86 (t, 3H, J = 7.4 Hz), 1.34 (t, 3H, J = 7.1 Hz), 1.87–1.92 (m, 2H), 4.36 (q, 2H, J = 7.1 Hz), 4.45 (t, 2H, J = 6.9 Hz), 7.25–7.29 (m, 1H), 7.44–7.48 (m, 1H), 7.76 (d, 1H, J = 8.5 Hz), 8.08 (d, 2H, J = 8.4 Hz), 8.12–8.15 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 11.80, 14.85, 23.51, 50.55, 61.39, 111.05, 121.34, 121.45, 122.36, 126.99, 127.26 (C × 2), 129.28, 130.45 (C × 2), 138.56, 141.47, 141.70, 166.21.

4.20. Ethyl 4-(1-isopropyl-1*H*-indazol-3-yl)benzoate (58)

Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and isopropyl iodide (**54**) (14.5 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **58**, yield 1.65 g, 35.7%; MS (EI, 70 eV): m/z 308 (M^+); found: C, 74.05; H, 6.52; N, 9.01. $C_{19}H_{20}N_2O_2$ requires: C, 74.00; H, 6.54; N, 9.08; UV λ_{max} (log ε): 210 (4.3), 319 (4.2); IR (KBr): 1609 (C═N), 1704 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ1.34 (t, 3H, J = 7.1 Hz), 1.54 (d, 6H, J = 6.6 Hz), 4.34 (q, 2H, J = 7.1 Hz), 5.06–5.09 (m, 1H), 7.25–7.28 (m, 1H), 7.43–7.47 (m, 1H), 7.79 (d, 1H, J = 8.5 Hz), 8.08 (d, 2H, J = 8.3 Hz), 8.11–8.15 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.87, 22.67 (C × 2), 50.38, 61.39, 110.97, 121.45, 121.51, 122.43, 126.82, 127.28 (C × 2), 129.24, 130.44 (C × 2), 138.72, 140.70, 141.32, 166.24.

4.21. Ethyl 4-(1-butyl-1*H*-indazol-3-yl)benzoate (59)

Compound **17** (4.0 g, 0.015 mol), EtONa (3.5 g, 0.052 mol), and butyl iodide (**55**) (17.0 mL, 0.15 mol) were allowed to react as in the preparation of compound **30** (**YD-3**) to afford compound **59**, yield 2.42 g, 50.2%; MS (EI, 70 eV): m/z 322 (M^+); found: C, 74.55; H, 6.90; N, 8.65. $C_{20}H_{22}N_2O_2$ requires: C, 74.51; H, 6.88; N, 8.69; UV λ_{max} (log ε): 210 (4.4), 320 (4.2); IR (KBr): 1607 (C═N), 1701 (C═O) cm^{-1}; ^{1}H NMR

(200 MHz, DMSO-d_6): δ 0.89 (t, 3H, J = 7.3 Hz), 1.26–1.31 (m, 2H), 1.34 (t, 3H, J = 7.0 Hz), 1.82–1.89 (m, 2H), 4.35 (q, 2H, J = 7.0 Hz), 4.48 (t, 2H, J = 6.9 Hz), 7.25–7.29 (m, 1H), 7.44–7.48 (m, 1H), 7.76 (d, 1H, J = 8.5 Hz), 8.09 (d, 2H, J = 8.2 Hz), 8.12–8.15 (m, 3H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.17, 14.86, 20.12, 32.17, 48.75, 61.39, 111.01, 121.37, 121.46, 122.36, 126.99, 127.25 (C × 2), 129.28, 130.45 (C × 2), 138.56, 141.45, 141.58, 166.21.

4.22. 4-(1-Benzyl-1*H*-indazol-3-yl)benzoic acid (60)

Compound **30** (**YD-3**) (1.0 g, 0.0028 mol) was allowed to react as in the preparation of compound **14** to afford compound **60**, yield 0.83 g, 89.8%; mp 203–206 °C; MS (EI, 70 eV): m/z 328 (M^+); found: C, 76.84; H, 4.90; N, 8.50. $C_{21}H_{16}N_2O_2$ requires: C, 76.81; H, 4.91; N, 8.53; UV λ_{max} (log ε): 211 (4.6), 320 (4.5); IR (KBr): 1572 (C═N), 1624 (C═O), 3441 (–OH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.74 (s, 2H), 7.23–7.30 (m, 6H), 7.45 (t, 1H, J = 6.9 Hz), 7.79 (d, 1H, J = 8.4 Hz), 8.05–8.08 (m, 2H), 8.10 (d, 1H, J = 3.8 Hz), 8.11–8.16 (m, 2H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.28, 110.76, 121.26 (C × 2), 122.11, 126.86 (C × 3), 127.62 (C × 2), 127.84, 128.84 (C × 3), 130.24 (C × 2), 137.44 (C × 2), 141.20, 141.86, 167.39.

4.23. Methyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (62)

Into a solution of compound **60** (1.0 g, 0.003 mol) in CH_2Cl_2 (50 mL) was added thionyl chloride (5 mL, 0.068 mol). The mixture was reacted under reflux for 16 h and then allowed to stand at 25 ± 5 °C. The mixture was concentrated to dry in vacuo in water bath to afford 4-(1-benzyl-1*H*-indazol-3-yl)benzoyl chloride (**61**), then MeOH (15 mL) was added into the residue. The solution was reacted under reflux for 2 h and then allowed to stand at 25 ± 5 °C. The solution was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-$CHCl_3$) to afford compound **62**, yield 0.56 g, 53.3%; mp 95–97 °C; MS (EI, 70 eV): m/z 328 (M^+); found: C, 77.20; H, 5.32; N, 8.19. $C_{22}H_{18}N_2O_2$ requires: C, 77.17; H, 5.30; N, 8.18; UV λ_{max} (log ε): 211 (4.6), 320 (4.5); IR (KBr): 1609 (C═N), 1705 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$-d_1): δ 3.97 (s, 3H), 5.69 (s, 2H), 7.22–7.41 (m, 8H), 8.04–8.12 (m, 2H), 8.15 (d, 1H, J = 9.9 Hz), 8.18–8.22 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$-d_1): δ 51.91, 53.02, 109.64, 120.98, 121.42, 121.90, 126.36, 126.92 (C × 4), 127.61, 128.54 (C × 2), 128.94, 129.88 (C × 2), 136.36, 137.96, 140.92, 142.68, 166.79.

4.24. Propyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (63)

Compound **60** (1.0 g, 0.003 mol) and *n*-propanol (15 mL) were allowed to react as in the preparation of compound **62** to afford compound **63**, yield 0.47 g, 43.3%; mp 57–58 °C; MS (EI, 70 eV): m/z 370 (M^+); found: C, 77.84; H, 5.98; N, 7.58. $C_{24}H_{22}N_2O_2$ requires: C, 77.81; H, 5.99; N, 7.56; UV λ_{max} (log ε): 211 (4.3), 321 (4.3); IR (KBr): 1611 (C═N), 1709 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.07 (t, 3H,J = 7.5 Hz), 1.79–1.89 (m, 2H), 4.33 (t, 2H,J = 6.6 Hz), 5.69 (s, 2H), 7.23–7.40 (m, 8H), 8.03–8.08 (m, 2H), 8.14 (d, 1H, J = 10.9 Hz), 8.18–8.21 (m, 2 H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 10.31, 21.93, 53.02, 66.34, 109.63, 120.98, 121.34, 121.92, 126.33, 126.92 (C × 4), 127.59, 128.53 (C × 2), 129.33, 129.83 (C × 2), 136.36, 137.84, 140.92, 142.74, 166.36.

4.25. Isopropyl 4-(1-benzyl-1*H*-indazol-3-yl)benzoate (64)

Compound **60** (1.0 g, 0.003 mol) and isopropanol (15 mL) were allowed to react as in the preparation of compound **62** to afford compound **64**, yield 0.51 g, 46.7%; mp 75–77 °C; MS (EI, 70 eV): m/z 370 (M^+); found: C, 77.84; H, 6.01; N, 7.55. $C_{24}H_{22}N_2O_2$ requires: C, 77.81; H, 5.99; N, 7.56; UV λ_{max} (log ε): 211 (4.7), 320 (4.6); IR (KBr): 1605 (C═N), 1713 (C═O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42 (d, 6H, J = 6.2 Hz), 5.24–5.37 (m, 1H), 5.69 (s, 2H), 7.20–7.39 (m, 8H), 8.03–8.07 (m, 2H), 8.12 (d, 1H, J = 10.5 Hz), 8.17–8.20 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.81 (C × 2), 53.00, 68.19, 109.66, 121.00, 121.42, 121.95, 126.35, 126.87 (C × 2), 126.97 (C × 2), 127.61, 128.55 (C × 2), 129.77, 129.83 (C × 2), 136.41, 137.78, 140.95, 142.76, 165.81.

4.26. 4-(1-Benzyl-1*H*-indazol-3-yl)benzamide (65)

Compound **60** (1.0 g, 0.003 mol) and $NH_{3(g)}$ in toluene (30 mL) were reacted for 30 min at 25 ± 5 °C. The mixture was directly extracted several times with ethyl acetate. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-ethyl acetate/$CHCl_3$ = 2/1) to afford compound **65**, yield 0.37 g, 36.7%; mp 182–185 °C; MS (EI, 70 eV): m/z 327 (M^+); found: C, 77.07; H, 5.24; N, 12.87. $C_{21}H_{17}N_3O$ requires: C, 77.04; H, 5.23; N, 12.84; UV λ_{max} (log ε): 211 (4.3), 317 (4.2); IR (KBr): 1558 (C═N), 1636 (C═O), 3099 (-NH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 5.74 (s, 2H), 7.26–7.31 (m, 5H), 7.41–7.45 (m, 2H), 7.79 (d, 1H, J = 8.5 Hz), 7.99–8.05 (m, 2H), 8.07 (d, 1H, J = 7.7 Hz), 8.09–8.16 (m, 2H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.23, 110.69, 121.24 (C × 2), 121.98, 126.62 (C × 2), 126.82, 127.61 (C × 2), 127.82, 128.42 (C × 2), 128.84 (C × 2), 133.54, 136.05, 137.51, 141.17, 142.08, 167.78.

4.27. 4-(1-Benzyl-1*H*-indazol-3-yl)-*N*-methylbenzamide (66)

Compound **60** (1.0 g, 0.003 mol) and methylamine$_{(g)}$ in toluene (30 mL) were allowed to react as in the preparation of compound **65** to afford compound **66**, yield 0.42 g, 40.0%; mp 187–189 °C; MS (EI, 70 eV): m/z 341 (M^+); found: C, 77.44; H, 5.62; N, 12.33. $C_{22}H_{19}N_3O$ requires: C, 77.40; H, 5.61; N, 12.31; UV λ_{max} (log ε): 211 (4.7), 317 (4.5); IR (KBr): 1552 (C═N), 1636 (C═O), 3018 (-NH) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.80 (d, 3H, J = 4.3 Hz), 5.74 (s, 2H), 7.22–7.29 (m, 6H), 7.45 (t, 1H, J = 7.0 Hz), 7.78 (d, 1H, J = 8.3 Hz), 7.95–7.98 (m, 2H), 8.02 (d, 1H, J = 12.7 Hz), 8.11–8.15 (m, 2H), 8.52 (q, 1H, J = 4.4 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 26.52, 52.23, 110.69, 121.23 (C × 2), 121.97, 126.68 (C × 2),

126.81, 127.60 (C × 2), 127.82, 127.98 (C × 2), 128.84 (C × 2), 133.76, 135.86, 137.51, 141.17, 142.06, 166.49.

4.28. 4-(1-Benzyl-1*H*-indazol-3-yl)-*N*-ethylbenzamide (67)

Compound **60** (1.0 g, 0.003 mol) and ethylamine$_{(g)}$ in toluene (30 mL) were allowed to react as in the preparation of compound **65** to afford compound **67**, yield 0.47 g, 43.3%; mp 161–164 °C; MS (EI, 70 eV): *m*/*z* 355 (M^+); found: C, 77.69; H, 5.98; N, 11.79. $C_{23}H_{21}N_3O$ requires: C, 77.72; H, 5.96; N, 11.82; UV λ_{max} (log ε): 211 (4.3), 317 (4.1); IR (KBr): 1547 (C═N), 1631 (C═O), 3125 (-NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.14 (t, 3H, J = 7.2 Hz), 3.23–3.43 (m, 2H), 5.74 (s, 2H), 7.19–7.32 (m, 6H), 7.45 (t, 1H, J = 7.3 Hz), 7.78 (d, 1H, J = 8.5 Hz), 7.96–8.00 (m, 2H), 8.03 (d, 1H, J = 10.1 Hz), 8.09–8.15 (m, 2H), 8.55 (t, 1H, J = 5.4 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 15.05, 34.29, 52.22, 110.68, 121.23 (C × 2), 121.95, 126.63 (C × 2), 126.80, 127.58 (C × 2), 127.80, 128.03 (C × 2), 128.83 (C × 2), 133.94, 135.83, 137.51, 141.18, 142.09, 165.76.

4.29. 4-(1-Benzyl-1*H*-indazol-3-yl)-*N*-propylbenzamide (68)

Compound **60** (1.0 g, 0.003 mol) and propylamine (15 mL) in toluene (30 mL) were allowed to react as in the preparation of compound **65** to afford compound **68**, yield 0.45 g, 40.1%; mp 135–137 °C; MS (EI, 70 eV): *m*/*z* 369 (M^+); found: C, 78.04; H, 6.28; N, 11.35. $C_{24}H_{23}N_3O$ requires: C, 78.02; H, 6.27; N, 11.37; UV λ_{max} (log ε): 211 (4.5), 317 (4.4); IR (KBr): 1543 (C═N), 1628 (C═O), 3152 (–NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.90 (t, 3H, J = 7.3 Hz), 1.49–1.60 (m, 2H), 3.19–3.29 (m, 2H), 5.74 (s, 2H), 7.21–7.32 (m, 6H), 7.45 (t, 1H, J = 8.0 Hz), 7.78 (d, 1H, J = 8.4 Hz), 7.96–8.00 (m, 2H), 8.02 (d, 1H, J = 9.9 Hz), 8.09–8.15 (m, 2H), 8.55 (t, 1H, J = 5.5 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 11.72, 22.65, 41.24, 52.22, 110.70, 121.23 (C × 2), 121.97, 126.64 (C × 2), 126.82, 127.59 (C × 2), 127.82, 128.07 (C × 2), 128.84 (C × 2), 133.98, 135.82, 137.52, 141.18, 142.10, 165.97.

4.30. 4-(1-Benzyl-1*H*-indazol-3-yl)-*N*-isopropylbenzamide (69)

Compound **60** (1.0 g, 0.003 mol) and isopropylamine (15 mL) in toluene (30 mL) were allowed to react as in the preparation of compound **65** to afford compound **69**, yield 0.56 g, 49.9%; mp 169–171 °C; MS (EI, 70 eV): *m*/*z* 369 (M^+); found: C, 78.06; H, 6.26; N, 11.35. $C_{24}H_{23}N_3O$ requires: C, 78.02; H, 6.27; N, 11.37; UV λ_{max} (log ε): 211 (4.4), 317 (4.3); IR (KBr): 1628 (C═N), 1723 (C═O), 3157 (-NH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.30 (d, 6H, J = 6.5 Hz), 4.29–4.39 (m, 1H), 5.67 (s, 2H), 6.07 (d, 1H, J = 7.5 Hz), 7.19–7.35 (m, 6H), 7.37 (d, 2H, J = 3.7 Hz), 7.90 (d, 2H, J = 8.3 Hz), 8.02 (d, 1H, J = 8.5 Hz), 8.08 (d, 2H, J = 8.3 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 22.67 (C × 2), 41.74, 52.96, 109.60, 120.96, 121.32, 121.87, 126.33, 126.92 (C × 3), 127.12 (C × 4), 127.58, 128.52 (C × 2), 133.72, 136.41, 140.91, 142.78, 166.17.

4.31. [4-(1-Benzyl-1*H*-indazol-3-yl)phenyl]methanol (70)

Anhydrous calcium chloride ($CaCl_2$, granular, 2.0 g, 0.018 mol) was added into the solution of sodium borohydride ($NaBH_4$, 2.0 g, 0.052 mol) in anhydrous THF (100 mL). The mixture was stirred at 25 ± 5 °C for 4 h. Then compound **30** (**YD-3**) (1.0 g, 0.0028 mol) was added. The above mixture was heated under reflux for 6 h and then allowed to stand at 25 ± 5 °C. The reaction mixture was then poured into the ice-water (300 mL). The mixture was extracted several times with CH_2Cl_2. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-ethyl acetate/*n*-hexane = 2/3) to afford compound **70**, yield 0.7 g, 79.7%; mp 102–104 °C; MS (EI, 70 eV): *m*/*z* 314 (M^+); found: C, 80.27; H, 5.79; N, 8.89. $C_{21}H_{18}N_2O$ requires: C, 80.23; H, 5.77; N, 8.91; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1614 (C═N), 3418 (–OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.57 (d, 2H, J = 5.7 Hz), 5.27 (t, 1H, J = 5.7 Hz), 5.71 (s, 2H), 7.18–7.30 (m, 6H), 7.38–7.45 (m, 1H), 7.47 (d, 2H, J = 8.2 Hz), 7.75 (d, 1H, J = 8.5 Hz), 7.80 (d, 2H, J = 8.2 Hz), 8.07 (d, 1H, J = 8.2 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.11, 62.94, 110.50, 121.24 (C × 2), 121.55, 126.64, 126.84 (C × 2), 127.18 (C × 2), 127.59 (C × 2), 127.76, 128.80 (C × 2), 131.86, 137.68, 141.12, 142.51, 142.99.

4.32. 4-(1-Benzyl-1*H*-indazol-3-yl)benzaldehyde (71)

Into a solution of compound **70** (1.0 g, 0.0032 mol) in acetone (30 mL), manganese(IV) oxide (0.55 g, 0.0064 mol) was added, and the mixture was stirred for 20 h at 25 ± 5 °C. The reaction mixture was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-ethyl acetate/*n*-hexane = 1/3) to afford compound **71**, yield 0.8 g, 80.1%; mp 91–92 °C; MS (EI, 70 eV): *m*/*z* 312 (M^+); found: C, 80.83; H, 5.13; N, 8.93. $C_{21}H_{16}N_2O$ requires: C, 80.75; H, 5.16; N, 8.97; UV λ_{max} (log ε): 211 (4.7), 320 (4.5); IR (KBr): 1603 (C═N), 1692 (C═O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 5.71 (s, 2H), 7.19–7.26 (m, 6H), 7.37–7.41 (m, 1H), 7.74 (d, 1H, J = 8.5 Hz), 7.98 (d, 2H, J = 8.3 Hz), 8.10 (d, 1H, J = 8.2 Hz), 8.18 (d, 2H, J = 8.3 Hz), 10.00 (s, 1H, H-23); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.60, 111.02, 121.40, 121.58, 122.52, 127.15, 127.52 (C × 2), 127.85 (C × 2), 128.09, 129.06 (C × 2), 130.65 (C × 2), 135.60, 137.54, 139.32, 141.45, 141.83, 193.05.

4.33. 4-(1-Benzyl-1*H*-indazol-3-yl)benzaldehyde *O*-methyloxime (72)

Into a solution of compound **71** (1.0 g, 0.0032 mol) in 99.5% EtOH (50 mL), *O*-methylhydroxylamine (1.2 mL, 0.014 mol) was added. The mixture was heated at reflux for 3 h, and then allowed to stand at 25 ± 5 °C. The reaction mixture was extracted

several times with CH_2Cl_2. The combined extract was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-ethyl acetate/*n*-hexane = 1/8) to afford compound **72**, yield 0.78 g, 71.5%; mp 65–67 °C; MS (EI, 70 eV): *m*/*z* 341 (M^+); found: C, 77.44; H, 5.59; N, 12.34. $C_{22}H_{19}N_3O$ requires: C, 77.40; H, 5.61; N, 12.31; UV λ_{max} (log ε): 210 (4.7), 320 (4.4); IR (KBr): 1611 (C=N), 2940 (–CH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.92 (s, 3H), 5.73 (s, 2H), 7.21–7.30 (m, 6H), 7.35–7.43 (m, 1H), 7.75 (d, 2H, J = 8.3 Hz), 7.76 (d, 1H, J = 8.4 Hz), 8.05 (d, 2H, J = 8.3 Hz), 8.11 (d, 1H, J = 8.2 Hz), 8.29 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.24, 61.90, 110.66, 121.22 (C × 2), 121.91, 126.80, 127.24 (C × 2), 127.59 (C × 3), 127.67 (C × 2), 128.83 (C × 2), 131.41, 134.82, 137.53, 141.19, 142.20, 148.67.

4.34. (2*E*)-3-[4-(1-Benzyl-1*H*-indazol-3-yl)phenyl]acrylic acid (73)

Into a solution of compound **71** (1.0 g, 0.0032 mol) in toluene (30 mL), malonic acid (2.5 g, 0.024) and piperidine (3.5 g, 0.041 mol) were added. The mixture was heated at 80 °C for 3 h and then allowed to stand at 25 ± 5 °C. The reaction mixture was concentrated in vacuo for solvent removal. The residue was chromatographed (silica gel-CH_2Cl_2/EtOH = 16/1) to afford compound **73**, yield 0.98 g, 78.7%; mp 186–189 °C; MS (EI, 70 eV): *m*/*z* 354 (M^+); found: C, 78.02; H, 5.11; N, 7.87. $C_{23}H_{18}N_2O_2$ requires: C, 77.95; H, 5.12; N, 7.90; UV λ_{max} (log ε): 211 (4.6), 321 (4.4); IR (KBr): 1605 (C=C), 1630 (C=N), 1674 (C=O), 3030 (–OH) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 5.69 (s, 2H), 6.54 (d, 1H, J = 16.0 Hz), 7.18–7.27 (m, 6H), 7.37–7.41 (m, 1H), 7.61 (d, 1H, J = 16.0 Hz), 7.74 (d, 1H, J = 9.3 Hz), 7.78 (d, 2H, J = 8.4 Hz), 8.00 (d, 2H, J = 8.3 Hz), 8.08 (d, 1H, J = 8.2 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 52.46, 110.91, 119.67, 121.46 (C × 2), 122.21, 127.05, 127.50 (C × 2), 127.85 (C × 2), 128.06, 129.06 (C × 2), 129.31 (C × 2), 134.05, 135.30, 137.71, 141.38, 142.37, 143.89, 168.08.

4.35. Ethyl (2*E*)-3-[4-(1-benzyl-1*H*-indazol-3-yl)phenyl]acrylate (74)

Compound **73** (1.0 g, 0.0028 mol) was allowed to react as in the preparation of compound **17** to afford compound **74**, yield 0.8 g, 75.0%; mp 87–89 °C; MS (EI, 70 eV): *m*/*z* 382 (M^+); found: C, 78.57; H, 5.77; N, 7.30. $C_{25}H_{22}N_2O_2$ requires: C, 78.51; H, 5.80 N, 7.32; UV λ_{max} (log ε): 211 (4.7), 321 (4.4); IR (KBr): 1605 (C=C), 1630 (C=N), 1705 (C=O) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.22 (t, 3H, J = 7.0 Hz), 4.15 (q, 2H, J = 6.9 Hz), 5.69 (s, 2H), 6.63 (d, 1H, J = 16.1 Hz), 7.17–7.27 (m, 6H), 7.36–7.44 (m, 1H), 7.67 (d, 1H, J = 16.2 Hz), 7.73 (d, 1H, J = 9.0 Hz), 7.81 (d, 2H, J = 8.3 Hz), 8.00 (d, 2H, J = 8.1 Hz), 8.07 (d, 1H, J = 7.7 Hz); ^{13}C NMR (50 MHz, DMSO-d_6): δ 14.65, 52.46, 60.53, 110.90, 118.52, 121.45 (C × 2), 122.20, 127.04, 127.48 (C × 2), 127.85 (C × 2), 128.06, 129.05 (C × 2), 129.45 (C × 2), 133.82, 135.52, 137.68, 141.38, 142.32, 144.35, 166.71.

4.36. Ethyl 4-(1-phenyl-1*H*-indazol-3-yl)benzoate (83)

(A) Into a solution of aluminum chloride (85.0 g, 0.64 mol) in toluene (**75**) (90 mL) was added dropwise the benzoyl chloride (**76**) (50 mL, 0.43 mol) in ice-bath. The mixture was heated at 100 °C for 2 h and then allowed to stand at 25 ± 5 °C. The mixture was concentrated to dry in vacuo in water bath to afford 4-methylbenzophenone (**77**), yield 60.21 g, 70.8%; mp 54–56 °C.

(B) Into a solution of compound **77** (25.0 g, 0.127 mol) in glacial acetic acid (130 mL), chromium oxide (40.0 g, 0.4 mol) and H_2SO_4 (25 mL) were added. The mixture was heated at 100 °C for 2 h and then allowed to stand at 25 ± 5 °C to afford 4-benzoylbenzoic acid (**78**), yield 21.74 g, 75.6%; mp 197–200 °C.

(C) Compound **78** (20.0 g, 0.0884 mol) was allowed to react as in the preparation of compound **17** to afford ethyl 4-benzoylbenzoate (**79**), yield 15.96 g, 71.0%; mp 47–49 °C.

(D) Into a solution of compound **79** (10.0 g, 0.039 mol) in absolute EtOH (100 mL), phenylhydrazine (**80**) (8.4 g, 0.078 mol) and glacial acetic acid (1 mL) were added. The mixture was heated under reflux for 24 h and then allowed to stand at 25 ± 5 °C to afford ethyl 4-[(phenylhydrazono)(phenyl)methyl]benzoate (**81**), yield 8.8 g, 65.6%; mp 135–137 °C.

(E) Into a solution of lead tetraacetate (20.0 g) in CH_2Cl_2 (100 mL) was added the solution of compound **81** (10.0 g, 0.029 mol) in CH_2Cl_2 (100 mL). The mixture was reacted for 30 min in the ice-bath. Then boron trifluoride etherate (200 mL) was added dropwise and the mixture was heated under reflux for 30 min. The reaction mixture was poured into the ice-bath to afford compound **83**, yield 5.34 g, 53.8%; mp 85–87 °C; MS (EI, 70 eV): *m*/*z* 342 (M^+); found: C, 77.22; H, 5.32; N, 8.15. $C_{22}H_{18}N_2O_2$ requires: C, 77.17; H, 5.30 N, 8.18; UV λ_{max} (log ε): 242 (4.6), 303 (4.2); IR (KBr): 1598 (C=N), 1723 (C=O) cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.45 (t, 3H, J = 7.1 Hz), 4.44 (q, 2H, J = 7.1 Hz), 7.26–7.51 (m, 3H), 7.57 (d, 2H, J = 8.1 Hz), 7.76–7.83 (m, 3H), 8.10 (d, 1H, J = 8.10 Hz), 8.17 (d, 2H, J = 8.72 Hz), 8.21 (d, 2H, J = 8.16 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.15, 60.91, 110.66, 121.13, 122.16, 122.93 (C × 3), 126.80, 127.05, 127.16 (C × 2), 129.30 (C × 2), 129.70, 129.85 (C × 2), 137.41, 137.68, 140.25, 144.63, 166.26.

4.37. Preparation of washed human platelets and measurement of platelet aggregation

Human blood anticoagulated with acid citrate dextrose was obtained from healthy human volunteers and the platelet suspension was then prepared according to the procedures described previously.[13] Platelets were finally suspended in Tyrode's solution containing Ca^{2+} (2 mM) at a concentration of 3×10^8 platelets/mL. Platelet

aggregation was measured turbidimetrically with a light-transmission aggregometer. The extent of platelet aggregation was measured as the maximal increase of light transmission within 5 min after the addition of stimulators.

4.38. ATP release assay

ATP release from platelet dense granule was measured as described previously.[13] Washed human platelets were pre-incubated with DMSO or test compounds for 3 min and thentreated with GYPGKF for additional 5 min. The reactions were stopped with EDTA (5 mM), and the samples were immediatelycentrifuged at 4 °C. ATP was measured in the supernatantsby the addition of a luciferase/luciferin reagent in a microplate luminometer.

4.39. Measurement of P-selectin expression

P-selectin expression in platelets was measured as described previously.[14] Washed human platelets were pre-incubated with DMSO or test compounds for 3 min and then treated with or without GYPGKF in the presence of FITC-conjugated anti-CD62P for 15 min at room temperature. The samples were then fixed at 4 °C with 1% paraformaldehyde. Flow cytometric analysis was performed on a flow cytometer. Platelets were identified by logarithmic signal amplification for forward and side scatter. The levels of P-selectin expression were expressed as the percentages of cells positive for anti-CD62P.

4.40. Measurement of thromboxane B_2 formation

Because thromboxane A_2 is very unstable and rapidly converted to more stable metabolite thromboxane B_2, we thus measured the latter instead of thromboxane A_2. After challenge of platelets with arachidonic acid for 5 min, EDTA (2 mM) and indomethacin (50 μM) were added. The platelet suspensions were centrifuged for 3 min at 13,000 rpm, and the thromboxane B_2 in the supernatants was assayed using enzyme immunoassay kits according to the procedure described by the manufacturer.

Acknowledgments

The investigation was supported by research grants to S.C. Kuo and L.J. Huang from the National Science Council of the Republic of China (NSC 93-2320-B-039-003) and China Medical University (CMU 93-PC-06) in Taiwan.

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