Synthesis and Biological Evaluation of Novel Pyrazoles and Indazoles as Activators of the Nitric Oxide Receptor, Soluble Guanylate Cyclase

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Database searching and compound screening identified 1-benzyl-3-(3-dimethylaminopropyloxy)-indazole (benzydamine, **3**) as a potent activator of the nitric oxide receptor, soluble guanylate cyclase. A comprehensive structure−activity relationship study surrounding **3** clearly showed that the indazole C-3 dimethylaminopropyloxy substituent was critical for enzyme activity. However replacement of the indazole ring of **3** by appropriately substituted pyrazoles maintained enzyme activity. Compounds were evaluated for inhibition of platelet aggregation and showed a general lipophilicity requirement. Aryl-substituted pyrazoles **32**, **34**, and **43** demonstrated potent activation of soluble guanylate cyclase and potent inhibition of platelet aggregation. Pharmacokinetic studies in rats showed that compound **32** exhibits modest oral bioavailability (12%). Furthermore **32** has an excellent selectivity profile notably showing no significant inhibition of phosphodiesterases or nitric oxide synthases.

Introduction

Soluble guanylate cyclase (sGC, EC 4.6.1.2) catalyzes the conversion of guanosine 5′-triphosphate (GTP) to guanosine 3′,5′-cyclic monophosphate (cGMP) and is the only known receptor for the signaling molecule nitric oxide (NO).[1,2] NO may also participate in responses that are not mediated via sGC.[3] NO is a potent activator of sGC causing activation up to 600-fold greater than the basal level.[4] The NO-cGMP signaling pathway is important in many physiological processes including vasodilation, neurotransmission, and platelet aggregation.[5,6] Activators (or inhibitors) of sGC are therefore very desirable as both pharmacological tools to probe the NO-cGMP pathway and as therapeutic agents. sGC activators may be useful in a wide variety of conditions, and their potential in glaucoma has recently been highlighted.[7]

sGC exists as a heterodimeric heme protein with a total molecular mass of about 150 kDa, consisting of α and β subunits, both of which are required for catalytic activity.[8] Several isoforms of each subunit are known, with the $\alpha_1\beta_1$ heterodimer being the most studied.[2,9] The α and β subunits show considerable sequence homology with each other, particularly in the C-terminal catalytic domain, where they also share homology with adenylate cyclases and membrane-bound guanylate cyclases. sGC is widely expressed in human tissues with the $\alpha_1\beta_1$ isoform being predominant. The α_2 isoform shows a more restricted expression pattern with high levels in brain, placenta, spleen, and uterus only.[10] Tissue selectivity may derive from the differential desensitization[11] of the enzyme rather than from variation in isoform expression. sGC has been shown to bind one molecule of ferrous heme per heterodimer[4] in a five-coordinate complex with a histidine residue of the β subunit acting as the proximal ligand.[12] Spectral studies have demonstrated that NO forms a five-coordinate nitrosyl heme complex with sGC, breaking the weak Fe^{2+}−His bond and causing conformational changes leading to enzyme activation.[13,14] Carbon monoxide (CO) also shows limited activation of sGC.[14]

Presently there are few known potent modulators of sGC.[15] ODQ (**1**, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one) has been reported as an inhibitor of sGC.[16] The most studied class of sGC modulators are the NO donor compounds which activate sGC in a manner similar to endogenous NO by release of NO or an NO-related species.[6,17] The vasodilatory effects of NO donors have been exploited clinically for many years; however, it is only recently that their mode of action has been elucidated. NO donors can cause tolerance upon prolonged use, and problems associated with reactions of the compounds may arise, such as nitration of tyrosine residues.[6] Therefore there is an obvious need for novel activators of sGC that circumvent the problems associated with NO donors. YC-1 (**2**, 3-(5-hydroxymethyl-2-furyl)-1-benzylindazole) has been reported as an activator of sGC.[18] This compound does not act as an NO donor but activates purified sGC at high concentrations and shows synergistic activation in the presence of NO or CO.[19,20] The mechanism of activation is not yet fully understood. However YC-1 has recently been shown to also act as a nonspecific phosphodiesterase inhibitor[21]

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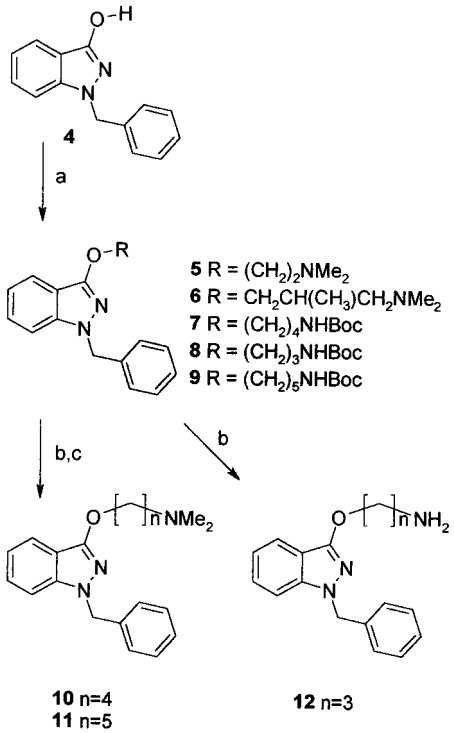
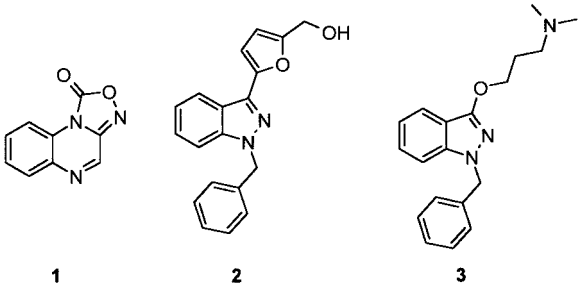
Scheme 1[a]



[a] Reagents: (a) R-Br or R-Cl, NaH, DMF, 100 °C; (b) with R = $(CH_2)_3NHBoc$, $(CH_2)_5NHBoc$: 50% TFA/CH_2Cl_2, with R = $(CH_2)_4NHBoc$: 1 M HCl, Et_2O; (c) HCHO, $NaCNBH_3$, AcOH, MeOH.

making interpretation of cell and tissue-based experiments difficult.

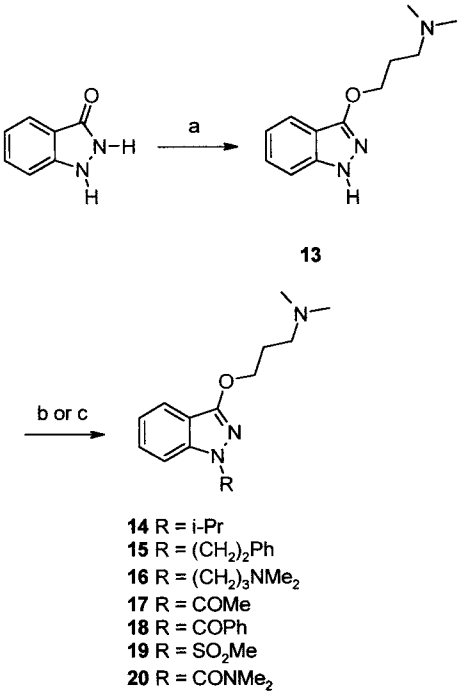


We have used the structure of compound **2** to generate 2-D substructural queries and performed 2-D substructural and 2-D similarity searches[22] of commercially available compound databases. From the subsequent compound screening it was found that benzydamine (**3**, 1-benzyl-3-(3-dimethylaminopropyloxy)indazole) is a more potent activator of sGC than YC-1 upon partially purified sGC. Benzydamine is a known antiinflammatory and analgesic; however, its mechanism of action is unknown.[23] We herein describe the synthesis and structure−activity relationships (SARs) of novel indazole and pyrazole activators of sGC based upon the benzydamine structure.

Chemistry

The synthesis of the benzydamine analogues which have been modified at the indazole C-3 substituent (Table 1) are depicted in Scheme 1. The starting material 1-benzyl-3-hydroxy-1*H*-indazole (**4**) was prepared by diazotization of *N*-benzylanthranilic acid fol-

Scheme 2[a]



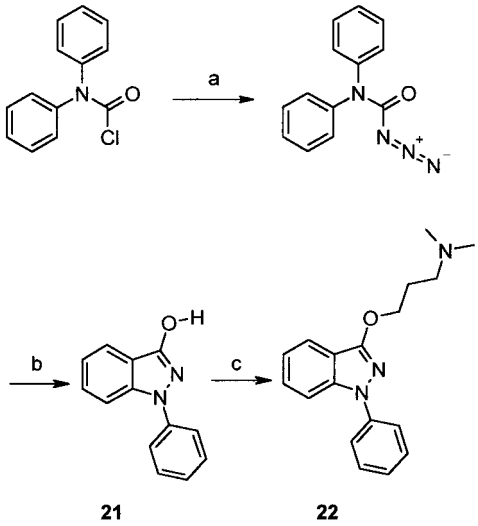
[a] Reagents: (a) $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene, 80 °C; (b) R-Cl or R-Br, *t*-BuOK, THF; (c) R-Cl, CH_2Cl_2.

lowed by sodium hydrosulfite reduction and subsequent cyclization as described previously.[23] Analogues **5** and **6** were synthesized from **4** via alkylation with the appropriate chloro-substituted alkyldimethylamino compound in the presence of sodium hydride. The first step in synthesizing analogues **10**−**12** was again alkylation of **4** using the appropriate BOC-protected bromoalkylamine. Removal of the BOC group of **8** was achieved using 50% TFA in dichloromethane to give the primary amine analogue **12**. Similarly deprotection of **7** and **9** followed by reductive amination of formaldehyde afforded analogues **10** and **11**, respectively. In this series of analogues where the hydroxyindazole **4** was alkylated, both *N*- and *O*-alkylations were possible. However IR spectroscopy proved that *O*-alkylation was the major product as there was no detection of a carbonyl stretch in the IR spectrum.

Analogues synthesized to investigate the SAR at the N-1 position of the indazole are shown in Table 2. All the analogues (with the exception of **22**) were synthesized from the intermediate **13** which was synthesized from 3-indazolinone and 3-dimethylamino-1-propanol via a Mitsunobu reaction using 1,1′-(azodicarbonyl)-dipiperidine (ADDP) and tributylphosphine as the redox system.[24] Compounds **14**−**16** were prepared from **13** by alkylation with the corresponding halide compound using potassium *tert*-butoxide as the base as shown in Scheme 2. Acylation of **13** afforded **17**−**20**. Analogue **22** was synthesized by alkylation of **21** with 3-dimethylaminopropyl chloride hydrochloride in anhydrous DMF using sodium hydride as base as shown in Scheme 3. Intermediate **21** was prepared by treatment of diphenylcarbamoyl chloride with sodium azide using standard procedures and subsequent conversion of the diphenylcarbamoyl azide to **21**.[25]

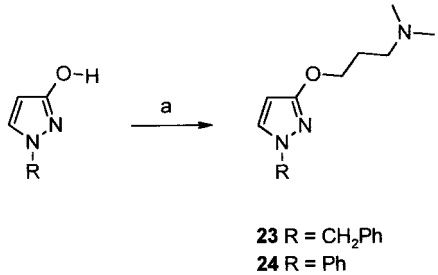
A series of novel pyrazole analogues were also synthesized. Table 3 outlines a wide variety of analogues

Scheme 3[a]



[a] Reagents: (a) NaN_3, acetone/H_2O; (b) xylene, △; (c) $Cl(CH_2)_3NMe_2$·HCl, NaH, DMF.

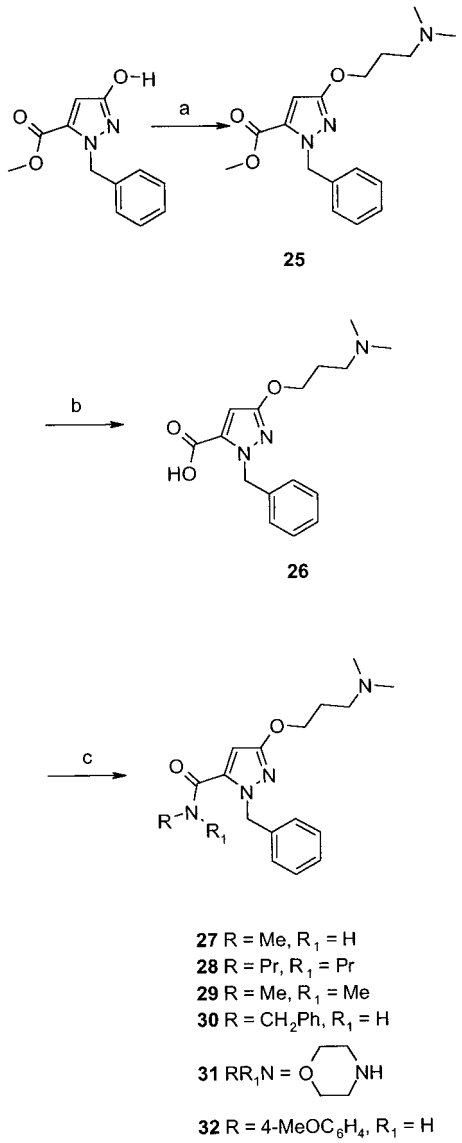
Scheme 4[a]



[a] Reagents: (a) with R = Ph: $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene, with R = CH_2Ph: $Cl(CH_2)_3NMe_2$·HCl, NaH, DMF.

all of which contain the 3-dimethylaminopropyloxy substituent at the pyrazole C-3 position but which vary at the N-1, C-4, and C-5 positions. Compound **23** was prepared via alkylation of 1-benzyl-3-hydroxy-1*H*-pyrazole[26] with the hydrochloride salt of 3-dimethylaminopropyl chloride using sodium hydride in DMF (Scheme 4). Analogue **24** was formed from the reaction of 3-hydroxy-1-phenyl-1*H*-pyrazole[27] with 3-dimethylamino-1-propanol under Mitsunobu conditions as described previously. The precursor to **26**, intermediate **25**, was prepared from 1-benzyl-3-hydroxy-5-methoxycarbonyl-1*H*-pyrazole[26] and 3-dimethylamino-1-propanol using the same Mitsunobu conditions as for compound **13**. The carboxylic acid **26** was subsequently prepared by saponification of **25** in alkaline solution and purification achieved using Dowex AG1 X8 (OH^- form) ion-exchange resin. Reaction of **26** in anhydrous DMF with the activating coupling reagent *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,-*N*′,*N*′-tetramethylammonium tetrafluoroborate (TBTU) and appropriate amine gave the amide derivatives **27**–**31** as shown in Scheme 5. For compound **32** *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was used as the coupling reagent to combat the decreased nucleophilic character of the amine. Pyrazole analogue **33** was prepared from 3-hydroxy-5-phenyl-1*H*-pyrazole[28] via reaction with 3-dimethylamino-1-propanol in the presence of the Mitsunobu reagent ADDP (Scheme 6). The tautomeric **33** gave access to the isomeric *N*-substituted analogues **34**

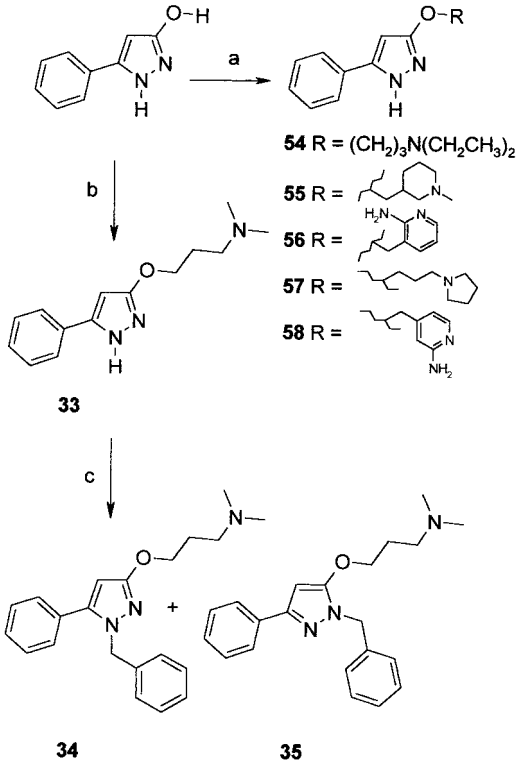
Scheme 5[a]



[a] Reagents: (a) $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene; (b) 2.5 M NaOH, MeOH; (c) RR_1NH, TBTU, DIPEA, except for **32**: 4-methoxyaniline, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, DIPEA.

and **35** in a ratio of 1:1.6, respectively, by reaction of **33** under Mitsunobu conditions with benzyl alcohol. The more reactive Mitsunobu reagent 1,1-azobisdimethylformamide was used in this case. The regiochemistry of **34** and **35** was determined on the basis of NOE experiments. For compound **34** an enhancement was observed between the benzylic protons and the *ortho* protons of the pyrazole C-5 substituted phenyl moiety. This enhancement was absent for **35**. The remaining pyrazole analogues were prepared by several different methods. In each case the 3-hydroxypyrazole was prepared initially and subsequently the C-3 substituent was introduced via Mitsunobu reaction with 3-dimethylamino-1-propanol using ADDP. The synthesis of pyrazoles can be achieved by reaction of alkynoates with a hydrazine.[29] The precursor to **43**, 1,5-diphenyl-3-hydroxy-1*H*-pyrazole (**36**), was prepared from phenylhydrazine and ethyl phenylpropiolate. Similarly the precursor to **44**, 3-hydroxy-5-trifluoromethyl-1*H*-pyrazole (**37**) was prepared from the reaction of ethyl 4,4,4-

Scheme 6[a]

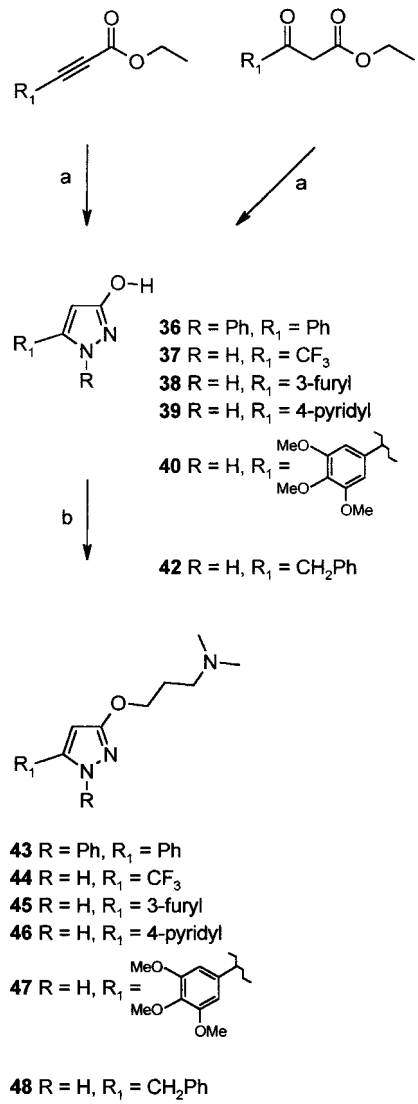


[a] Reagents: (a) ROH, ADDP, PBu_3, toluene; (b) $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene; (c) $PhCH_2OH$, 1,1-azobisdimethylformamide, PBu_3, toluene.

trifluoro-2-butynoate with hydrazine hydrate in ethanol (Scheme 7). Subsequent Mitsunobu reaction afforded the desired analogues **43** and **44**. Compounds **45**–**47** were all synthesized by a similar method as shown in Scheme 7. In each case the appropriate diketo ester **38**–**40** was reacted with hydrazine hydrate in ethanol to form the 3-hydroxypyrazole and converted to the desired product via Mitsunobu reaction. For compound **48** it was necessary to synthesize the diketo ester intermediate. This was accomplished as shown in Scheme 8 by reaction of phenylacetyl chloride with 2,2-dimethyl-1,3-dioxolane-4,6-dione (Meldrum's acid) in the presence of base followed by refluxing in ethanol to give ethyl 3-oxo-4-phenylbutyrate (**41**). Intermediate **41** was converted to the 3-hydroxypyrazole **42**, then to **48** as in previous examples. To form the C-4 substituted pyrazole **51**, methyl 3-dimethylamino-2-phenylacrylate (**49**) was prepared from the reaction of *tert*-butoxybis(dimethylamino)methane with methyl phenylacetate (Scheme 9). Subsequent reaction of **49** with hydrazine hydrate in refluxing ethanol gave 3-hydroxy-4-phenyl-1*H*-pyrazole (**50**). Treatment of **50** as previously described with 3-dimethylamino-1-propanol gave compound **51**.

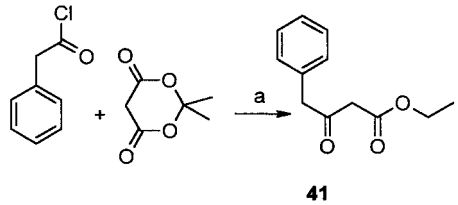
Table 4 depicts a series of pyrazole analogues that were synthesized to further investigate the pyrazole C-3 substituent. Compounds **54**–**58** were all synthesized from 3-hydroxy-5-phenyl-1*H*-pyrazole[28] under Mitsunobu conditions with the respective hydroxy compound using ADDP as shown in Scheme 6. The hydroxy precursors for **54** and **55** were available commercially. 3-Pyrrolidin-1-yl-1-propanol which was used to synthesize **57** was prepared from the alkylation of pyrrolidine with 3-chloro-1-propanol according to published procedures.[30] The precursor to **56**, 2-amino-3-pyridinemetha-

Scheme 7[a]



[a] Reagents: (a) with R = H: NH_2NH_2, EtOH, with R = Ph: $PhNHNH_2$, *t*-BuOK, THF; (b) $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene.

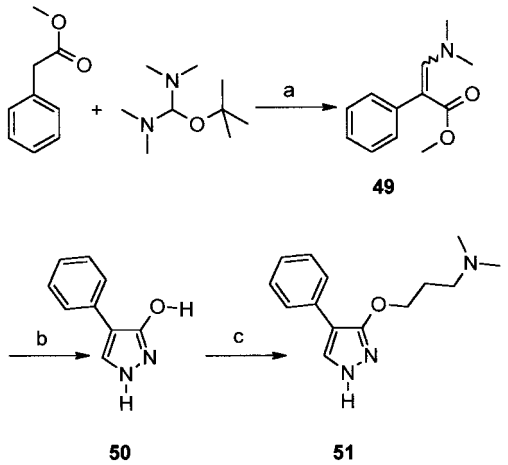
Scheme 8[a]



[a] Reagents: (a) Py, EtOH.

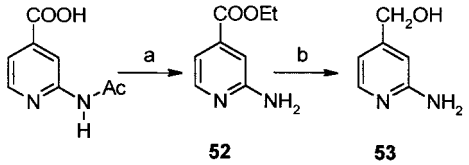
nol,[31] was prepared in two steps from 2-aminonicotinic acid. 2-Amino-4-pyridinemethanol (**53**), the reagent used in the synthesis of **58**, was synthesized in two steps from 2-acetylaminopyridine-4-carboxylic acid[32] as depicted in Scheme 10. Cleavage of the acetyl group and esterification of the carboxylate was achieved in one step using concentrated sulfuric acid in ethanol to give 2-amino-4-ethoxycarbonylpyridine (**52**). The final step involved the reduction of the ester **52** using lithium aluminum hydride to give **53**. The synthesis of **60** is shown in Scheme 11. First *N*1,*N*1-dimethyl-3-[(5-phenyl-1*H*-3-pyrazolyl)amino]propanamide (**59**) was prepared from

Scheme 9[a]



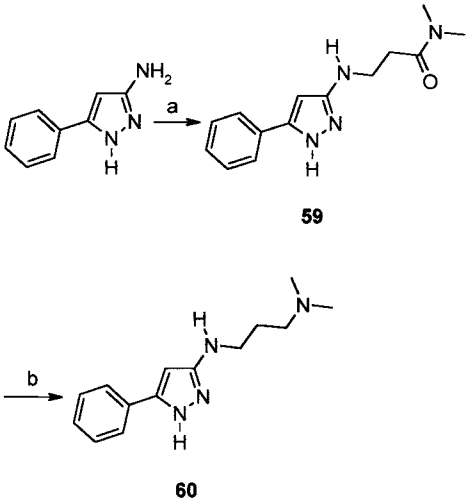
[a] Reagents: (a) THF, rt; (b) NH_2NH_2, EtOH; (c) $HO(CH_2)_3NMe_2$, ADDP, PBu_3, toluene.

Scheme 10[a]



[a] Reagents: (a) concd H_2SO_4, EtOH; (b) $LiAlH_4$, THF.

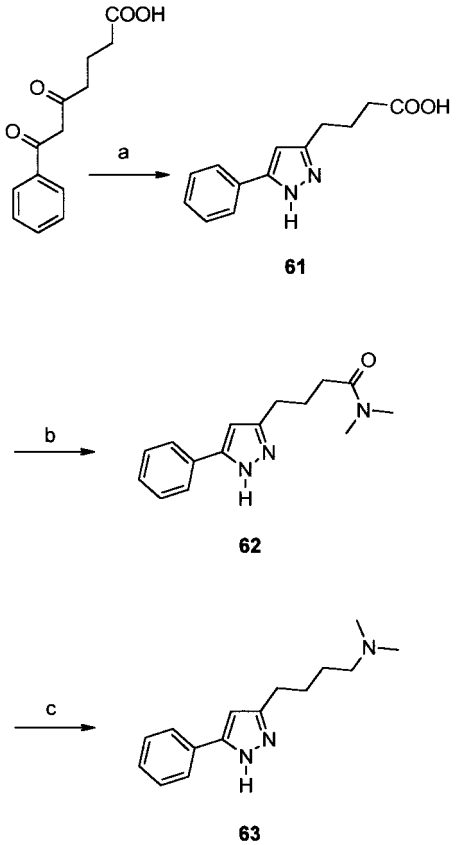
Scheme 11[a]



[a] Reagents: (a) *N*,*N*-dimethylacrylamide; (b) $LiAlH_4$ 1.0 M in THF, dioxane.

the Michael addition of commercially available 3-amino-5-phenyl-1*H*-pyrazole and *N*,*N*-dimethylacrylamide using previously described methodology.[33] Lithium aluminum hydride reduction of **59** in dioxane yielded **60**. The synthesis of **63** is depicted in Scheme 12. The diketone starting material, 7-phenyl-5,7-diketoheptanoic acid,[34] was prepared by known methods from acetophenone and glutaric anhydride. This was converted to the intermediate 4-(5-phenyl-1*H*-3-pyrazolyl)butanoic acid (**61**) by reaction with hydrazine hydrate in refluxing ethanol. Conversion of **61** to the acid chloride followed by quenching with dimethylamine gave *N*1,*N*1-dimethyl-4-(5-phenyl-1*H*-3-pyrazolyl)butanamide (**62**). The final step involved reduction of **62** with lithium aluminum hydride in dioxane to give **63**.

Scheme 12[a]



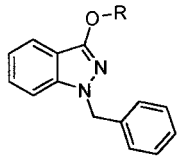
[a] Reagents: (a) NH_2NH_2, EtOH; (b) (i) $SOCl_2$, 60 °C, (ii) dimethylamine, Et_2O; (c) $LiAlH_4$, dioxane.

Results and Discussion

Activation of Soluble Guanylate Cyclase. All target compounds were assayed for sGC stimulation using baculovirus expressed and partially purified bovine sGC. cGMP levels were measured using a commercially available ELISA assay. sGC was submaximally stimulated with the NO donor compound DEA/NO (2-(*N*,*N*-diethylamino)diazenolate 2-oxide, 300 nM), and cGMP accumulation was evaluated for each compound at a single concentration of 1 μM. The cGMP produced by each compound was expressed first as a percentage of the DEA/NO response. Relative enzyme activities were calculated as the ratio between the percentage of the DEA/NO response for the compound and that for benzydamine (**3**), the lead compound. Dose−response curves in the presence of PAPA/NO ((*Z*)-1-[*N*-(3-ammoniopropyl)-*N*-(*n*-propyl)amino]diazen-1-ium-1,2-diolate, 30 nM) were produced for selected compounds and EC_{50} values calculated.

The first stage of exploring the SAR of benzydamine analogues was to modify the indazole C-3 substituent (Table 1) which afforded key information. Complete replacement of the dimethylamino functionality by nonbasic groups capable of hydrogen bonding such as hydroxy, methoxy, and carboxylate completely abolished enzyme activity (data not shown). Removal of a methylene unit (**5**) from the substituent decreased activity, but **10** with four methylene units only showed a slight decrease in REA. Indazole **11** with a further methylene unit gave a further drop in enzyme activation. The reduced activity of the methyl-substituted racemate **6**

Table 1. Activity Data for C-3 Substituted 1-Benzyl-1*H*-indazoles



compd	R	REA[a]	Platelet IC_{50} (μM)[b]
3	$(CH_2)_3NMe_2$	1.00	6.67 ± 0.15
5	$(CH_2)_2NMe_2$	0.33	
6	$CH_2CH(Me)CH_2NMe_2$	0.62	4.18 ± 1.11
10	$(CH_2)_4NMe_2$	0.79	7.62 ± 0.52
11	$(CH_2)_5NMe_2$	0.56	
12	$(CH_2)_3NH_2$	0.48	

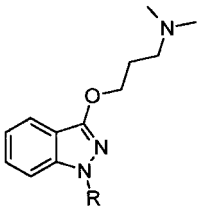
[a] Relative enzyme activity, enzyme activity relative to compound **3**. [b] Concentration required to inhibit collagen-induced platelet aggregation by 50%.

further indicates that the SAR surrounding the C-3 substituent is tight and that any bulk is not well-tolerated. Surprisingly primary amine **12** showed very little activity. Therefore the more lipophilic dimethylamino group is critical for enzyme activation.

To investigate the function of the indazole N-1 substituent (Table 2) a number of suitable substituents were selected as representatives of the groups most frequently attached to heteroaryl nitrogen atoms in the World Drug Index.[35] The phenyl-substituted compound **22** and benzoyl analogue **18** show comparable activity to the parent compound. However the phenylethyl-substituted **15** shows a decreased REA suggesting that the size increase is detrimental to activity. Conformational properties of these derivatives appear to be less important as both benzydamine and **15** have flexible substituents whereas the N-1 substituents of **18** and **22** will be conformationally restricted. The isopropyl and alkylamino derivatives **14** and **16** show decreased activity as do the acetyl- and dimethylamide-substituted compounds **17** and **20**. Surprisingly methylsulfonyl substitution (**19**) retained the activity of the parent, and unsubstituted **13** exhibited almost a 2-fold enhancement in relative enzyme activity over benzydamine. Therefore the N-1 unsubstituted indazole is favored for increased enzyme activity and substitutents containing an aryl ring also show good activation, but there does appear to be a steric requirement within the N-1 substituent binding site.

In an attempt to move into a novel series of compounds, replacement of the indazole ring by pyrazole was investigated (Table 3). Pyrazoles **23** and **24**, substituted at the N-1 position only, result in decreased enzyme activity. However the activity is regained upon the introduction of a phenyl ring substituted at the pyrazole C-4 or C-5 position. Indeed **34** and **43** show improved REA over benzydamine. The reduced activity of **35** suggests the C-3 exocyclic oxygen may play a crucial role as an electron-donating group via the +M

Table 2. Activity Data for N-1 Substituted 3-(3-Dimethylaminopropyloxy)-1*H*-indazoles



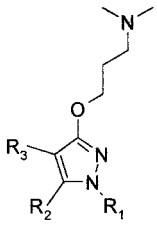
compd	R	REA[a]	Platelet IC_{50} (μM)[b]
3	CH_2Ph	1.00	6.67 ± 0.15
13	H	1.93	50% at 50 μM[c]
14	$CH(CH_3)_2$	0.66	
15	CH_2CH_2Ph	0.84	
16	$(CH_2)_3NMe_2$	0.79	
17	COMe	0.82	
18	COPh	1.04	8.57 ± 0.98
19	SO_2Me	1.07	21.6 ± 6.4
20	$CONMe_2$	0.78	
22	Ph	1.16	2.46 ± 0.33

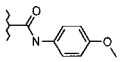
[a] Relative enzyme activity, enzyme activity relative to compound **3**. [b] Concentration required to inhibit collagen-induced platelet aggregation by 50%. [c] Inhibition at highest concentration tested.

effect. This renders the sp^2 hybridized nitrogen atom of the pyrazole ring more susceptible to donation of its lone pair of electrons in an activator−enzyme interaction. The N-1 unsubstituted, C-5 substituted pyrazoles **33** and **48** showed enhanced activity over benzydamine. When the aryl substituent is alternatively at the pyrazole C-4 position as in **51**, enzyme activity is also maintained. Replacement of the phenyl ring of **33** with 3-furyl (**45**) or 4-pyridyl (**46**) suggests that the electron-deficient pyridyl substituent was less favored. Trimethoxyphenyl analogue **47** also exhibited good enzyme activity. Further substitutions at the pyrazole C-5 position were also fruitful. Compounds **26**, **27**, and **44** all showed similar enzyme activation. Due to the ease of synthesis of **27** from **26** and the potential application to library synthesis, several amides were synthesized and evaluated. The amides were all synthesized as N-1 benzyl-substituted pyrazoles to increase lipophilicity and cell membrane penetration. Compounds **28**−**32** all showed enzyme activity similar to benzydamine.

Reduction of the pK_a of the dimethylamino function of the pyrazole series was studied. Aminopyridines have been proven to act as bioisosteres of guanidino groups for thrombin inhibitors.[36] The aminopyridyl pyrazoles **56** and **58** exhibited reduced enzyme activity in the case of sGC (Table 4), suggesting either the lowered pK_a or

Table 3. Activity Data for Substituted 3-(3-Dimethylaminopropyloxy)-1*H*-pyrazoles

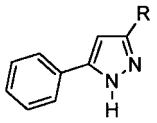


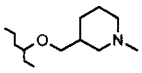
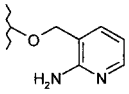
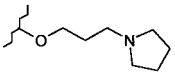
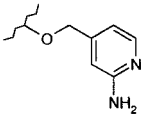
compd	R_1	R_2	R_3	REA[b]	Platelet IC_{50} (μM)[c]
3				1.00	6.67 ± 0.15
23	CH_2Ph	H	H	0.73	
24	Ph	H	H	0.71	3% at 100 μM[d]
26	CH_2Ph	CO_2H	H	1.01	
27	CH_2Ph	CONHMe	H	0.95	
28	CH_2Ph	$CON((CH_2)_2CH_3)_2$	H	1.22	35% at 100 μM[d]
29	CH_2Ph	$CONMe_2$	H	0.93	4% at 100 μM[d]
30	CH_2Ph	$CONHCH_2Ph$	H	1.22	25% at 100 μM[d]
31	CH_2Ph		H	0.84	90% at 100 μM[d]
32	CH_2Ph		H	1.10	2.84 ± 0.44
33	H	Ph	H	1.49	19% at 100 μM[d]
34	CH_2Ph	Ph	H	1.08	2.37 ± 0.42
35[a]				0.72	
43	Ph	Ph	H	1.28	2.84 ± 0.84
44	H	CF_3	H	1.13	8% at 100 μM[d]
45	H	3-Furyl	H	1.31	99.0 ± 8.9
46	H	4-Pyridyl	H	1.15	0% at 100 μM[d]
47	H		H	1.10	41.12 ± 3.37
48	H	CH_2Ph	H	1.54	49.2 ± 10.9
51	H	H	Ph	1.17	12.04 ± 1.98

[a] For the structure of compound **35**, see Scheme 6. [b] Relative enzyme activity, enzyme activity relative to compound **3**. [c] Concentration required to inhibit collagen-induced platelet aggregation by 50%. [d] Inhibition at highest concentration tested.

the additional steric bulk is unfavorable. The decrease in activity also observed for **54** and **55** indicates that steric bulk in the region of the pyrazole C-3 substituent is clearly not tolerated and is consistent with the SAR of the indazole series. When the dimethylamino group is constrained within a cyclic system as in **57**, a modest drop in activity occurs. Finally the role of the pyrazole exocyclic oxygen atom was probed. Replacement by amino (**60**) or methylene (**63**) resulted in decreased activity: this highlights the electronic and conformational importance of the oxygen atom providing a conjugated system with the pyrazole ring.

Table 4. Activity Data for Substituted 5-Phenyl-1*H*-pyrazoles



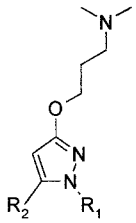
compd	R	REA[b]
3		1.00
54	$O(CH_2)_3N(CH_2CH_3)_2$	0.42
55		0.61
56		0.69
57[a]		0.85
58		0.53
60	$NH(CH_2)_3NMe_2$	0.66
63	$(CH_2)_4NMe_2$	0.68

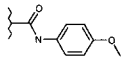
[a] Platelet aggregation IC_{50} 10.33 ± 2.44 μM for compound **57**. [b] Relative enzyme activity, enzyme activity relative to compound **3**.

Dose–response curves were carried out for selected compounds identified in the screening assay (Table 5). In general, evaluated EC_{50}'s mirror the trends observed in the screening assay. Comparison of the EC_{50}'s of YC-1 (**2**) with benzydamine and compounds **33**, **34**, **43**, and **48** clearly indicates the increase in potency that was achieved within the series. Furthermore the most active compound of the series **48** is almost 1 order of magnitude more potent against the enzyme than benzydamine. However compound **32** showed reduced enzyme potency upon comparison with benzydamine.

In summary, from our lead compound benzydamine, the dimethylamino function was proven critical for enzyme activity. The optimal length of the indazole C-3 substituent was identified as three methylene units, and substitution of this group is not tolerated. Phenyl, benzyl, and benzoyl are favored substituents at the indazole N-1 position, but larger groups appear to be subject to steric constraints. The unsubstituted analogue is particularly favored. The indazole moiety may be replaced by a pyrazole ring provided the pyrazole C-4 or C-5 positions are substituted. Groups with significant electron delocalization are favored at the C-5 position, in particular phenyl and benzyl. The pyrazole C-3 exocyclic oxygen is essential for activity and substitution of the dimethylaminopropyloxy group is unfavorable. Similarities are drawn between the indazole and pyrazole series, and it is speculated that the indazoles and

Table 5. Activation of sGC: EC_{50} Data for Selected Indazoles and Pyrazoles in the Presence of 30 nM PAPA/NO



compd	R_1	R_2	Activation of sGC EC_{50} (μM)
2			4.11 ± 3.16
3			1.02 ± 0.26
32	CH_2Ph		5.49 ± 2.34
33	H	Ph	0.72 ± 0.17
34	CH_2Ph	Ph	0.44 ± 0.18
43	Ph	Ph	0.58 ± 0.19
48	H	CH_2Ph	0.165 ± 0.074

pyrazoles are binding in a similar manner to the same site. This assumption allows a pharmacophore to be proposed consisting of the dimethylaminopropyl group and a hydrogen bond acceptor atom which is equivalent to the pyrazole or indazole N-2 nitrogen. However the enzyme-bound conformation of the flexible dimethylaminopropyl group cannot be predicted with any great certainty. The hydrogen bond acceptor is proposed on the basis of the reduced activity for compound **35**. This suggests that the conjugated system of the exocyclic oxygen and the pyrazole sp^2 hybridized nitrogen that is absent for **35** are important for enzyme activity and may participate in an activator–enzyme interaction. Therefore it is proposed that the electron-rich pyrazole or indazole N-2 nitrogen will accept a hydrogen bond in the activator–enzyme complex.

Inhibition of Platelet Aggregation. Compounds exhibiting significant potency in the enzyme assay were evaluated in a functional assay measuring the compounds ability to inhibit collagen-stimulated platelet aggregation (Tables 1–4). The most potent analogue **34** (IC_{50} = 2.37 μM) is almost 3-fold more potent than benzydamine. However compounds **33** and **48**, which both show good activity in the enzyme assay, show a decreased potency in the platelet assay upon comparison with benzydamine and **34**. This is attributed to poorer cell penetration, which is supported by a decrease in lipophilicity due to the lack of a hydrophobic substituent at the pyrazole N-1 position. A similar case is observed for the N-1 unsubstituted indazole **13**. Optimal inhibition of platelet aggregation appears to be achieved with a hydrophobic aryl group at the indazole and pyrazole N-1 position. The plot of $-\log[IC_{50}]$ versus calculated log P[37] and REA (Figure 1) clearly indicates there is a lipophilicity requirement for platelet activity. Compounds with an $IC_{50} \geq 100$ μM were capped with an assigned IC_{50} of 100 μM for the purposes of the plot only. From the plot it can be seen that a clogP greater than

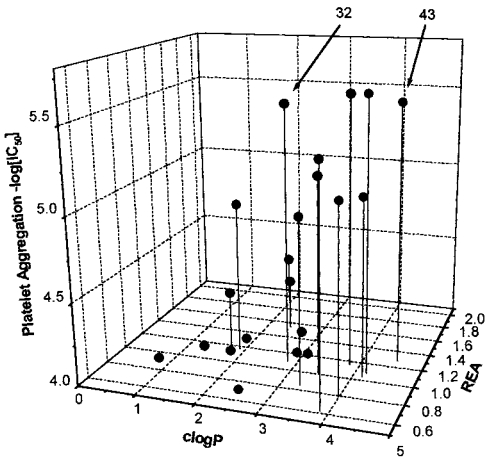


Figure 1. Plot of inhibition of collagen-induced platelet aggregation $-\log[IC_{50}]$ versus clogP and REA.

Table 6. Pharmacokinetic Parameters for sGC Activators

compd	C_{max} (ng/mL)	AUC (ng/h/mL)	t_{max} (h)	F (%)
22[a]	3.2	7.9	0.5	2
32[b]	6.4	61.1	2.7	12
34[a]	3.1	15.5	1.7	2
43[b]	1.4	3.3	0.5	<2

[a] Cassette dosing at 2 mg/kg po. [b] Dosed at 5 mg/kg po.

3.0 is generally required for potent inhibition of platelet aggregation. This observation can have important implications in library design of novel analogues. The clogP constraints from the plot can be used as a filter to ensure that synthesized compounds would have cell-based activity.

Selectivity and Pharmacokinetics. Compounds **22**, **32**, and **34** were evaluated for their selectivity against a number of enzymes including phosphodiesterases and adenylate cyclase at a single concentration of 10 μM (see Supporting Information). Compound **22** shows minimal inhibition of PDE II (23%), and compound **34** exhibits no significant activity at any of the enzymes studied. It is particularly noteworthy that **32** and **34** do not activate adenylate cyclase and unlike YC-1 (**2**) show no significant inhibition of any of the phosphodiesterases, particularly PDE V. Compound **32** was additionally assayed against the NOS isoforms, and minimal inhibition was observed with iNOS (25%) and nNOS (17%). Furthermore **32** shows no significant activity at any of the other enzymes studied.

The oral bioavailability of benzydamine in humans is claimed to be high,[38] though it is generally only used as a topical agent. Four compounds were examined for oral pharmacokinetics in rats (Table 6). In general the compounds were not particularly bioavailable though **32** did demonstrate 12% bioavailability. Further optimization of the compounds for oral bioavailability is likely to be required in order for them to be utilized as oral agents.

Conclusion

A series of novel analogues of benzydamine have been identified as potent activators of the NO receptor sGC. In our evaluation of sGC activators we chose an isolated enzyme system as our initial screen and inhibition of platelet aggregation as a well-characterized cellular system responsive to cGMP. We considered that an

intact cellular system would provide valuable confirmation of the activity as the isolated enzyme differs in some important aspects from the sGC present in intact cells. Thus the potency of NO is at least 10-fold greater in intact cells than against isolated enzyme. Further, inactivation of sGC in cells is far faster (seconds) than for purified enzyme.[11]

A comprehensive SAR study has been accomplished which demonstrates the severely restricted allowable modifications of the C-3 dimethylaminopropyloxy substituent. However substituted pyrazoles can replace the indazole ring of benzydamine maintaining the enzyme potency. More specifically aryl-substituted pyrazole analogues such as **33**, **43**, and **48** show enhanced potency over benzydamine, and a pharmacophore hypothesis has been proposed. Evaluation of compounds in the cell-based inhibition of platelet aggregation unveiled a general lipophilicity requirement for cell membrane penetration. Compounds **22**, **32**, **34**, and **43** were subsequently identified as sGC activators and potent inhibitors of platelet aggregation. Pharmacokinetic and selectivity studies have established that compound **32** (CFM1571) shows modest oral bioavailability (12%) and is selective over a number of related enzymes. In conclusion CFM1571 (**32**) has been identified as the first potent activator of sGC which is selective over phosphodiesterases and does not act as an NO donor. Further optimization of both pharmacokinetic parameters and potency is currently under investigation.

Experimental Section

General. All starting materials were either commercially available or reported previously in the literature unless noted. Solvents and reagents were used without further purification except THF which was dried over sodium. Reactions were monitored by TLC on precoated silica gel plates (Kieselgel 60 F_{254}, Merck). Purification was performed by flash chromatography using silica gel (particle size 40−63 μM, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 or Bruker AMX-400 spectrometer. Chemical shifts are reported as ppm (δ) relative to TMS as internal standard. Mass spectra were recorded on either a VG ZAB SE spectrometer (electron impact and FAB) or a Micromass Quattro electrospray LC-mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Microanalysis was carried out by the Analytical Services Section, Department of Chemistry, University College London.

1-Benzyl-3-(2-dimethylaminoethyloxy)-1*H*-indazole (5). To 1-benzyl-3-hydroxy-1*H*-indazole (**4**) (0.5 g, 2.23 mmol) in anhydrous DMF (20 mL) was added sodium hydride (60% dispersion in mineral oil) (0.11 g, 2.68 mmol) and the mixture was stirred under nitrogen at room temperature for 30 min. Simultaneously 2-dimethylaminoethyl chloride hydrochloride (0.35 g, 2.45 mmol) was stirred with sodium hydride (60% dispersion in mineral oil) (0.09 g, 2.23 mmol) in anhydrous DMF (10 mL) for 30 min. This mixture was then added to the flask containing the indazole and the reaction mixture was heated to 100 °C for 3 h. The reaction mixture was cooled to room temperature. Water (20 mL) was added and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using 0.88 ammonia/EtOH/EtOAc (1:5:94) to give **5** as a pale yellow oil (436 mg, 66%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, 1H, J = 7.7 Hz), 7.22−7.15 (m, 4H), 7.09−7.06 (m, 3H), 6.97−6.91 (m, 1H), 5.31 (s, 2H), 4.43 (t, 2H, J = 5.6 Hz), 2.74 (t, 2H, J = 5.6 Hz), 2.29 (s, 6H); MS (EI) m/z 296 $[MH]^+$. Anal. ($C_{18}H_{21}N_3O$) C, H, N.

1-Benzyl-3-(3-dimethylamino-2-methylpropyloxy)-1*H*-indazole (6). To 1-benzyl-3-hydroxy-1*H*-indazole (**4**) (1.0 g, 4.46 mmol) in anhydrous DMF (40 mL) was added sodium hydride (60% dispersion in mineral oil) (0.21 g, 5.35 mmol) and the mixture was stirred under nitrogen at room temperature for 30 min. Simultaneously 3-dimethylamino-2-methylpropyl chloride hydrochloride (0.85 g, 4.49 mmol) was stirred with sodium hydride (60% dispersion in mineral oil) (0.18 g, 4.46 mmol) in anhydrous DMF (10 mL) for 30 min. This mixture was then added to the flask containing the indazole and the reaction mixture was heated to 100 °C for 12 h then cooled to room temperature. Water (20 mL) was added and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **6** as an oil (650 mg, 45%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, 1H, J = 8.1 Hz), 7.23−7.17 (m, 4H), 7.10−7.07 (m, 3H), 6.98−6.95 (m, 1H), 5.31 (s, 2H), 4.37−4.27 (m, 1H), 4.19−4.10 (m, 1H), 2.42−2.33 (m, 1H), 2.20 (s, 6H), 1.04 (d, 3H, J = 6.3 Hz); MS (APCI$^+$) m/z 324 $[MH]^+$. Anal. ($C_{20}H_{25}N_3O$) C, H, N.

1-Benzyl-3-(4-*tert*-butoxycarbonylaminobutyloxy)-1*H*-indazole (7). To indazole **4** (0.5 g, 2.23 mmol) in anhydrous DMF (20 mL) was added sodium hydride (60% dispersion in mineral oil) (110 mg, 2.68 mmol) and the mixture was stirred under nitrogen at room temperature for 15 min. The reaction mixture was then heated to 100 °C while 4-bromobutylamine *tert*-butylcarbamate (620 mg, 2.45 mmol) in anhydrous DMF (10 mL) was added to the reaction mixture by syringe over a 30 min period. Once the reaction mixture reached 100 °C it was kept at this temperature for 2 h. The reaction mixture was then cooled to room temperature and stirred overnight. Water (20 mL) was added and the mixture was extracted with diethyl ether (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography using cyclohexane/EtOAc (70:30) to give **7** as a white solid (481 mg, 55%): mp 53−54 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 8.1 Hz), 7.34−7.24 (m, 5H), 7.17 (d, 2H, J = 8.5 Hz), 7.04 (t, 1H, J = 7.5 Hz), 5.40 (s, 2H), 4.68 (br s, 1H), 4.41 (t, 2H, J = 6.3 Hz), 3.23 (m, 2H), 1.90 (m, 2H), 1.71 (m, 2H), 1.46 (s, 9H); MS (EI) m/z 395 $[M]^+$. Anal. ($C_{23}H_{29}N_3O_3$) C, H, N.

1-Benzyl-3-(3-*tert*-butoxycarbonylaminopropyloxy)-1*H*-indazole (8). From **4** (0.5 g, 2.23 mmol) and 3-bromopropylamine *tert*-butylcarbamate (0.58 g, 2.45 mmol) using the same process as for compound **7**. The crude product was purified by flash chromatography using cyclohexane/EtOAc (70:30) to give **8** as a white solid (0.61 g, 72%) which was recrystallized from diethyl ether: mp 92−93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, 1H, J = 8.1 Hz), 7.36−7.22 (m, 7H), 7.05 (dt, 1H, J = 0.73, 7.4 Hz), 5.40 (s, 2H), 5.06 (br s, 1H), 4.48 (t, 2H, J = 6.1 Hz), 3.35 (q, 2H, J = 6.1 Hz), 2.05 (q, 2H, J = 6.3 Hz), 1.46 (s, 9H); MS (EI) m/z 381 $[M]^+$. Anal. ($C_{22}H_{27}N_3O_3$) C, H, N.

1-Benzyl-3-(5-*tert*-butoxycarbonylaminopentyloxy)-1*H*-indazole (9). From **4** (0.5 g, 2.23 mmol) and 5-bromopentylamine *tert*-butylcarbamate (0.65 g, 2.45 mmol) using the same process as for compound **7**. The crude product was purified by flash chromatography using cyclohexane/EtOAc (70:30) to give **9** as a clear oil (0.68 g, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, 1H, J = 8.1 Hz), 7.34−7.21 (m, 4H), 7.17 (br d, 3H, J = 8.1 Hz), 7.04 (m, 1H), 5.40 (s, 2H), 4.57 (br s, 1H), 4.39 (t, 2H, J = 6.6 Hz), 3.16 (m, 2H), 1.89 (t, 2H, J = 6.8 Hz), 1.56−1.55 (m, 4H), 1.46 (s, 9H); MS (EI) m/z 409 $[M]^+$. Anal. ($C_{24}H_{31}N_3O_3 \cdot 0.5H_2O$) C, H, N.

1-Benzyl-3-(4-dimethylaminobutyloxy)-1*H*-indazole (10). To **7** (200 mg, 0.51 mmol) in diethyl ether (10 mL) was added 1 M HCl in diethyl ether (1.0 mL, 1.0 mmol) and the reaction was stirred overnight at room temperature. Two further equivalents of 1 M HCl in diethyl ether (1.0 mL, 1.0 mmol) were added and the reaction mixture stirred for a further 48 h. A white solid precipitated out of solution which was filtered

and washed with diethyl ether. To the crude amine (72 mg, 0.22 mmol) in MeOH (5 mL) were added $NaCNBH_3$, 1 M in THF (0.32 mL, 0.32 mmol) and glacial acetic acid (0.2 mL). Formaldehyde (20 mg, 0.66 mmol) in MeOH (2 mL) was then added to the reaction and the mixture was stirred overnight at room temperature. The MeOH was removed under reduced pressure. Water (20 mL) was added to the residue which was subsequently washed with EtOAc (2 × 10 mL), then saturated with sodium carbonate and extracted again with EtOAc (3 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give **10** as a pale yellow oil (67 mg, 41%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, 1H, J = 8.1 Hz), 7.35−7.26 (m, 4H), 7.20−7.16 (m, 3H), 7.05 (m, 1H), 5.39 (s, 2H), 4.42 (t, 2H, J = 5.5 Hz), 2.85 (t, 2H, J = 7.5 Hz), 2.58 (s, 6H), 1.90 (m, 4H); MS (EI) m/z 322 [M − H]$^+$. Anal. ($C_{20}H_{25}N_3O{\cdot}2HCl{\cdot}0.5H_2O$) C, H, N.

1-Benzyl-3-(5-dimethylaminopentyloxy)-1*H*-indazole (11). From **9** (123 mg, 0.30 mmol) using the same process as for compound **10** except 50% TFA in CH_2Cl_2 at room temperature for 1 h was used in place of 1 M HCl in diethyl ether, to give **11** as a yellow oil (12 mg, 12%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (d, 1H, J = 8.1 Hz), 7.34−7.24 (m, 4H), 7.19−7.16 (m, 3H), 7.04 (t, 1H, J = 7.4 Hz), 5.40 (s, 2H), 4.39 (t, 2H, J = 6.3 Hz), 2.88−2.82 (m, 2H), 2.64 (s, 6H), 1.91−1.83 (m, 2H), 1.81−1.73 (m, 2H), 1.62−1.51 (m, 2H); MS (FAB) m/z 338 [MH]$^+$. Anal. ($C_{21}H_{27}N_3O{\cdot}0.5CF_3COOH{\cdot}0.5H_2O$) C, H, N.

1-Benzyl-3-(3-aminopropyloxy)-1*H*-indazole (12). Compound **8** (0.083 g, 0.22 mmol) was stirred in 50% TFA in CH_2Cl_2 (3 mL) at room temperature for 1 h. The solvent was removed under reduced pressure. The residue was taken up in EtOAc (20 mL) washed with saturated $NaHCO_3$ (30 mL) and saturated brine (30 mL) then concentrated under reduced pressure. The crude product was purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **12** as a colorless oil (46 mg, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, 1H, J = 8.3 Hz), 7.27−7.06 (m, 7H), 6.96 (t, 1H, J = 7.5 Hz), 5.27 (s, 2H), 4.83 (br s, 2H), 4.40 (t, 2H, J = 5.8 Hz), 3.00 (t, 2H, J = 6.6 Hz), 2.08 (quintet, 2H, J = 6.2 Hz); MS (EI) m/z 281 [M]$^+$. Anal. ($C_{17}H_{19}N_3O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-1*H*-indazole (13). To 3-indazolinone (10.0 g, 74.6 mmol), 3-dimethylamino-1-propanol (9.0 mL, 76.1 mmol), and tributylphosphine (18.5 mL, 75.0 mmol) in toluene (600 mL) was added 1,1′-(azodicarbonyl)-dipiperidine (19.0 g, 75.3 mmol) portionwise. The mixture was heated at 80 °C for 15 h. The cooled mixture was filtered and the residue was washed with EtOAc (200 mL). The combined filtrate was washed with 10% aqueous NaOH (2 × 200 mL) and water (200 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (2:20:78) to give **13** as an oil (5.40 g, 33%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (dt, 1H, J = 8.0, 0.9 Hz), 7.36 (ddd, 1H, J = 8.4, 6.8, 1.1 Hz), 7.30 (dt, 1H, J = 8.4, 0.9 Hz), 7.08 (ddd, 1H, J = 7.9, 6.7, 1.2 Hz), 4.47 (t, 2H, J = 6.4 Hz), 2.56 (br t, 2H, J = 7.5 Hz), 2.32 (s, 6H), 2.26−2.07 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 156.9, 142.3, 127.1, 119.6, 119.0, 112.1, 109.6, 67.0, 56.1, 45.0 (2C), 27.1; MS (FAB) m/z 220 [MH]$^+$. Anal. ($C_{12}H_{17}N_3O{\cdot}0.3H_2O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-1-(2-propyl)-1*H*-indazole (14). To a solution of indazole **13** (100 mg, 0.46 mmol) in THF (3.5 mL) were added successively *t*-BuOK (77 mg, 0.69 mmol) and 2-bromopropane (80 μL, 0.92 mmol). The reaction mixture was refluxed for 4 h, then allowed to cool and concentrated under reduced pressure. The crude residue was purified by flash chromatography using 5−10% MeOH/$CHCl_3$ to give **14** as an oil (72 mg, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dt, 1H, J = 8.0, 0.9 Hz), 7.34−7.25 (m, 2H), 7.01 (ddd, 1H, J = 7.9, 6.5, 1.2 Hz), 4.65 (dt, 1H, J = 13.3, 6.7 Hz), 4.44 (t, 2H, J = 6.5 Hz), 2.56−2.39 (m, 2H), 2.30 (s, 6H), 2.10−2.01 (m, 2H), 1.49 (d, 6H, J = 6.7 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.4, 140.4, 126.6, 120.0, 118.6, 112.7, 108.6, 67.2, 56.5, 49.7, 45.4 (2C), 27.5, 21.7 (2C); MS (FAB) m/z 278 [MH]$^+$. Anal. ($C_{15}H_{23}N_3O{\cdot}0.1H_2O$) C, H, N.

The following compounds were prepared similarly.

3-(3-Dimethylaminopropyloxy)-1-(2-phenylethyl)-1*H*-indazole (15). From **13** (127 mg, 0.50 mmol) and 1-bromo-2-phenylethane (0.2 mL, 1.4 mmol). Purified by flash chromatography using MeOH/$CHCl_3$ (3:97) to give **15** as an oil (51 mg, 32%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66 (dt, 1H, J = 8.0, 0.9 Hz), 7.31−7.14 (m, 6H), 7.07−6.99 (m, 2H), 4.47 (t, 2H, J = 6.5 Hz), 4.40 (br t, 2H, J = 7.5 Hz), 3.14 (br t, 2H, J = 7.5 Hz), 2.58 (br t, 2H, J = 7.6 Hz), 2.34 (s, 6H), 2.15−2.06 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.9, 141.4, 138.8, 128.8 (2C), 128.5 (2C), 127.1, 126.5 120.0, 118.8, 112.6, 108.5, 67.3, 56.5, 50.0, 45.5 (2C), 36.2, 27.5; MS (APCI$^+$) m/z 324 [MH]$^+$. Anal. ($C_{20}H_{25}N_3O{\cdot}0.5H_2O$) C, H, N.

1-(3-Dimethylaminopropyl)-3-(3-dimethylaminopropyloxy)-1*H*-indazole (16). From **13** (106 mg, 0.48 mmol) and 1-chloro-3-dimethylaminopropane hydrochloride (150 mg, 0.73 mmol of free amine). Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:10:89) to give **16** as an oil (117 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (dt, 1H, J = 7.1, 0.9 Hz), 7.31 (ddd, 1H, J = 8.5, 6.6, 1.1 Hz), 7.24 (dt, 1H, J = 8.5, 1.0 Hz), 6.98 (ddd, 1H, J = 7.8, 6.5, 1.1 Hz), 4.41 (t, 2H, J = 6.5 Hz), 4.20 (t, 2H, J = 6.8 Hz), 2.48 (br t, 2H, J = 7.5 Hz), 2.24 (s, 6H), 2.17 (s, 6H), 2.09−1.92 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 155.5, 141.3, 126.8, 119.8, 118.5, 112.2, 108.5, 67.1, 56.4, 56.3, 45.8, 45.4 (2C), 45.3 (2C), 27.6, 27.5; MS (FAB) m/z 305 [MH]$^+$. Anal. ($C_{17}H_{28}N_4O$) C, H, N.

1-Acetyl-3-(3-dimethylaminopropyloxy)-1*H*-indazole (17). To a solution of **13** (298 mg, 1.36 mmol) in dry CH_2Cl_2 (6 mL) was added acetic anhydride (0.30 mL, 3.10 mmol) and the mixture was stirred at room temperature for 48 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed successively with 1 M aqueous NaOH (5 mL) and water (5 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified by flash chromatography using MeOH/$CHCl_3$ (5:95) to give **17** as an oily solid (164 mg, 54%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39 (dt, 1H, J = 8.3, 1.0 Hz), 7.67 (dt, 1H, J = 7.9, 1.0 Hz), 7.55 (ddd, 1H, J = 8.3, 7.2, 1.2 Hz), 7.31 (ddd, 1H, J = 7.9, 7.3, 0.8 Hz), 4.50 (t, 2H, J = 6.5 Hz), 2.66 (s, 3H), 2.53 (br t, 2H, J = 7.4 Hz), 2.31 (s, 6H), 2.10−2.03 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 203.0, 159.1, 140.3, 130.1, 123.9, 119.6, 117.7, 115.7, 67.6, 56.2, 45.4 (2C), 27.1, 22.8; IR (film) 1706 (C=O) cm^{-1}; MS (FAB) m/z 262 [MH]$^+$. Anal. ($C_{14}H_{19}N_3O_2{\cdot}0.1H_2O$) C, H, N.

1-Benzoyl-3-(3-dimethylaminopropyloxy)-1*H*-indazole (18). To a solution of **13** (182 mg, 0.83 mmol) and pyridine (0.13 mL, 1.57 mmol) in CH_2Cl_2 (3 mL) at −30 °C was added benzoyl chloride (0.11 mL, 0.92 mmol) and the mixture was allowed to warm to room temperature over 6 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed successively with 1 M aqueous NaOH (5 mL) and water (5 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified by flash chromatography using a 0−3% gradient of MeOH/$CHCl_3$ to give **18** as an oil (112 mg, 42%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (br d, 1H, J = 8.4 Hz), 8.12 (dt, 2H, J = 7.0, 1.5 Hz), 7.72 (br d, 1H, J = 7.9 Hz), 7.64−7.46 (m, 4H), 7.37 (br t, 1H, J = 7.6 Hz), 4.45 (t, 2H, J = 6.5 Hz), 2.48 (br t, 2H, J = 7.1 Hz), 2.26 (s, 6H), 2.08−1.99 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 167.2, 159.5, 141.5, 133.7, 131.5, 130.7, 130.2, 129.3, 127.7, 127.6, 124.3, 119.6, 117.5, 116.2 67.5, 55.7, 44.7 (2C), 26.4; IR (film) 1676 (C=O) cm^{-1}; MS (FAB) m/z 324 [MH]$^+$. Anal. ($C_{19}H_{21}N_3O_2{\cdot}0.1H_2O$) C, H, N.

The following compounds were prepared similarly.

3-(3-Dimethylaminopropyloxy)-1-methylsulfonyl-1*H*-indazole (19). From **13** (100 mg, 0.46 mmol), pyridine (0.22 mL, 2.66 mmol), and methanesulfonyl chloride (85 μL, 1.1 mmol) in dry CH_2Cl_2 (3 mL) at room temperature for 20 h. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **19** as a white solid (64 mg, 47%): mp 35−36 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, 1H, J = 8.5 Hz), 7.72 (d, 1H, J = 8.0 Hz), 7.56 (t, 1H, J = 7.6 Hz), 7.36 (t, 1H, J = 7.6 Hz), 4.56 (t, 2H, J = 6.5 Hz), 3.07 (s, 3H), 2.53 (t, 2H, J = 7.3 Hz), 2.31 (s, 6H), 2.13−2.01 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 161.2, 142.6, 130.2, 124.1, 120.3, 117.5,

113.8, 67.9, 55.9, 45.1 (2C), 38.7, 26.8; MS (APCI$^+$) *m/z* 298 [MH]$^+$. Anal. ($C_{13}H_{19}N_3O_3S$) C, H, N.

1-Dimethylaminocarbonyl-3-(3-dimethylaminopropyloxy)-1*H*-indazole (20). From **13** (114 mg, 0.52 mmol), pyridine (72 μL, 0.89 mmol), and dimethylcarbamyl chloride (62 μL, 0.68 mmol) in dry CH_2Cl_2 (2 mL) at reflux for 15 h. Purified by flash chromatography using a 3−10% gradient of MeOH/$CHCl_3$ to give **20** as an oil (65 mg, 43%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (dt, 1H, J = 8.6, 0.9 Hz), 7.63 (dt, 1H, J = 8.0, 1.0 Hz), 7.46 (ddd, 1H, J = 8.4, 7.1, 1.2 Hz), 7.18 (ddd, 1H, J = 8.0, 7.1, 0.8 Hz), 4.45 (t, 2H, J = 6.5 Hz), 3.21 (s, 6H), 2.49 (br t, 2H, J = 7.1 Hz), 2.26 (s, 6H), 2.09−2.00 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 157.5, 154.3, 142.5, 129.2, 122.3, 119.5, 115.7, 114.9, 67.5, 56.3, 45.5 (2C), 38.8 (2C), 27.3; IR (film) 1680 (C=O) cm^{-1}; MS (APCI$^+$) *m/z* 291 [MH]$^+$. Anal. ($C_{15}H_{22}N_4O_2$) C, H, N.

3-Hydroxy-1-phenyl-1*H*-indazole (21). A solution of diphenylcarbamoyl chloride (10 g, 43.16 mmol) in acetone (20 mL) was added dropwise to a solution of NaN_3 (4.2 g, 64.61 mmol) in H_2O (14 mL) at 0 °C. The mixture was allowed to stir overnight at room temperature. The layers were then separated and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic material was dried ($MgSO_4$) and concentrated under reduced pressure to give the crude diphenylcarbamoyl azide as a solid (8.84 g, 86%). The material was used without further purification. A solution of diphenylcarbamoyl azide (4.0 g, 16.79 mmol) in xylenes (40 mL) was heated to reflux for 5 h. The solution was then cooled to 0 °C. The precipitate that formed was collected to give **21** as a white solid (1.21 g, 34%): mp 206−207 °C (lit.[25] mp 206−207 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.16 (br t, 1H, J = 7.5 Hz), 7.26 (br t, 1H, J = 7.4 Hz), 7.49 (m, 3H), 7.69 (br d, 2H, J = 7.4 Hz), 7.77 (m, 2H), 11.25 (br s, 1H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 156.64, 140.58, 139.55, 129.85 (2C), 128.73, 125.13, 120.97 (2C), 120.87, 120.67, 115.10, 110.68; MS (EI) *m/z* 210 [M]$^+$. Anal. ($C_{13}H_{10}N_2O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-1-phenyl-1*H*-indazole (22). Indazole **21** (0.50 g, 2.38 mmol) was added portionwise as a solid to a solution of NaH (60% dispersion in mineral oil, 0.10 g, 2.5 mmol) in DMF (20 mL) at room temperature. The mixture was stirred for 30 min, then a further portion of NaH (0.10 g, 2.5 mmol) was added and stirring was continued for another 15 min. 3-Dimethylaminopropyl chloride hydrochloride (0.41 g, 2.59 mmol) was then added and the mixture was heated to 100 °C for 2 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude residue was recrystallized (EtOH/H_2O) to give **22** as a solid (0.44 g, 63%): mp 63.5−64.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (m, 4H), 7.46 (m, 3H), 7.26 (m, 1H), 7.15 (m 1H), 4.54 (t, 2H, J = 6.5 Hz), 2.53 (t, 2H, J = 7.6 Hz), 2.30 (s, 6H), 2.10 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 158.57, 141.57, 140.99, 130.22 (2C), 129.03, 126.03, 122.45 (2C), 121.23, 121.14, 115.75, 110.99, 68.38, 57.37, 46.48 (2C), 28.47; MS (EI) *m/z* 295 [M]$^+$. Anal. ($C_{18}H_{21}N_3O$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazole (23). From 3-dimethylaminopropyl chloride hydrochloride (158 mg, 1.0 mmol) and 1-benzyl-3-hydroxy-1*H*-pyrazole[26] (174 mg, 1.0 mmol) using the same process as for compound **7** to give **23** (19 mg, 7%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 5H), 7.15 (d, 1H, J = 2 Hz), 5.67 (d, 2H, J = 2 Hz), 5.13 (s, 2H), 4.17 (t, 2H, J = 6 Hz), 2.46 (t, 2H, J = 8 Hz), 2.26 (s, 6H), 1.95 (m, 2H); MS (EI) *m/z* 260 [MH]$^+$. Anal. ($C_{15}H_{21}N_3O\cdot0.3H_2O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-1-phenyl-1*H*-pyrazole (24). From 3-hydroxy-1-phenyl-1*H*-pyrazole[27] (320 mg, 2.0 mmol) and 3-dimethylamino-1-propanol (206 mg, 2.0 mmol) using the same process as for compound **13**. The workup employed involved the addition of MeOH (20 mL) and Dowex 50W X8 (4 g) to the mixture, which was then swirled at room temperature for 1 h. The mixture was filtered and the resin washed with MeOH (50 mL). The resin was suspended in 0.88 ammonia/MeOH (15:85) (20 mL) and swirled for 30 min. Then filtered and washed with the same mixture (20 mL). The ammonia/MeOH washings were combined and concentrated under reduced pressure. The crude product was purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (2:10:88) to give **24** (100 mg, 20%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, 1H, J = 3 Hz), 7.61 (m, 2H), 7.41 (m, 3H), 7.20 (m, 1H), 5.90 (d, 1H, J = 3 Hz), 4.30 (t, 2H, J = 4.3 Hz), 2.48 (t, 2H, J = 8 Hz), 2.27 (s, 6H), 2.00 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 167.6, 140.2, 129.3, 127.6, 125.2, 117.8, 93.6, 67.6, 56.4, 45.5, 27.5; MS (EI) *m/z* 246 [MH]$^+$. Anal. ($C_{14}H_{19}N_3O\cdot0.1H_2O$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-methoxycarbonyl-1*H*-pyrazole (25). To 3-dimethylamino-1-propanol (5.61 mL, 47.4 mmol) in toluene (250 mL) were added 1-benzyl-3-hydroxy-5-methoxycarbonyl-1*H*-pyrazole[26] (11.0 g, 47.4 mmol) and tributylphosphine (9.6 g, 11.7 mL, 47.4 mmol) followed by 1,1′-(azodicarbonyl)dipiperidine (13.1 g, 52.1 mmol) portionwise. The mixture was heated to 100 °C for 2 h then cooled to room temperature and the reduced ADDP product was filtered off and washed with EtOAc (100 mL). The filtrate was extracted with 1 M HCl (2 × 100 mL) and the combined acidic extracts were washed with EtOAc (3 × 100 mL) and then basified with 0.88 ammonia solution (40 mL). This was extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give 16.2 g of a semisolid which still contained some reduced ADDP. The crude product was dissolved in THF (300 mL) and 20 equivalents of Dowex 50W X8 resin (200 g, 1 mol) was added. The mixture was stirred gently for 1 h then filtered and washed with methanol. The resin was then stirred in piperidine/methanol (5:95) for 3 h, filtered and the process repeated twice. The piperidine/methanol combined washes were concentrated under reduced pressure and the resulting residue was filtered through silica using MeOH/$CHCl_3$ (5:95) to give **25** as a solid (1.24 g, 39%): mp 141−142 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (m, 5H), 6.22 (s, 1H), 5.59 (s, 2H), 4.25 (t, 2H, J = 6 Hz), 3.84 (s, 3H), 3.15 (m, 2H), 2.76 (s, 6H), 2.34 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 137.1, 132.7, 128.5, 127.7, 127.5, 95.4, 66.0, 55.6, 54.4, 52.0, 43.0, 24.4; MS (EI) *m/z* 318 [MH]$^+$. Anal. ($C_{17}H_{23}N_3O_3$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazole-5-carboxylic Acid (26). To **25** (2.43 g, 7.66 mmol) in MeOH (24 mL) was added 2.5 M aqueous NaOH solution (4 mL, 10 mmol) and the reaction mixture stirred at room temperature overnight. The MeOH was removed under reduced pressure and water (10 mL) was added. The solution was added to an AG1 X8 (OH$^-$) form ion-exchange column and eluted with water (100 mL) and 0.1 M aqueous HCl. Fractions containing the product were concentrated under reduced pressure to give **26** as a solid (1.60 g, 69%): mp 169−174 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.29 (m, 3H), 7.12 (m, 2H), 6.32 (s, 1H), 5.56 (s, 2H), 4.14 (t, 2H, J = 6 Hz), 3.14 (m, 2H), 2.72 (s, 6H), 2.11 (m, 2H); ^{13}C NMR (67.8 MHz, D_2O) δ 168.5, 163.6, 143.9, 140.3, 131.2, 130.1, 129.3, 101.1, 95.5, 69.4, 57.7, 55.5, 45.2, 26.2; MS (FAB) *m/z* 304 [MH]$^+$. Anal. ($C_{16}H_{21}N_3O_3\cdot0.12HCl$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-methylaminocarbonyl-1*H*-pyrazole (27). To **26** (1 M solution in DMF, 0.5 mL, 0.5 mmol), 40% aqueous methylamine (0.5 mL) and DIPEA (2 M solution in DMF, 2 mL, 2 mmol) was added TBTU (1 M solution in DMF, 0.7 mL, 0.7 mmol) and the reaction mixture stirred at room temperature overnight. The DMF was removed under reduced pressure (~1 mmHg) and the residue taken up in saturated brine (20 mL), made basic with 0.88 ammonia and extracted with THF (3 × 20 mL). The THF extracts were dried (Na_2SO_4) and purified by flash chromatography using 0.88 ammonia/EtOH/$CHCl_3$ (2:14:84) to give **27** (26 mg, 16%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 5H), 5.93 (br s, 1H), 5.87 (s, 1H), 5.61 (s, 2H), 4.17 (t, 2H, J = 6 Hz), 2.92 (d, 3H, J = 5 Hz), 2.43 (m, 2H), 2.24 (s, 6H), 1.93 (m, 2H); MS (EI) *m/z* 317 [MH]$^+$. Anal. ($C_{17}H_{24}N_4O_2\cdot0.3CHCl_3$) C, H, N.

The following compounds were prepared similarly.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-dipropylaminocarbonyl-1*H*-pyrazole (28). From **26** (102 mg, 0.33 mmol) and dipropylamine (33 mg, 0.33 mmol). The reaction mixture

was heated to 110 °C and the following workup was employed. The crude reaction mixture was cooled and water (1 mL) was added followed by MeOH (5 mL) and Dowex 50W X8 ion-exchange resin (3 g). The mixture was stirred for 30 min, filtered and washed with MeOH/H_2O (90:10) (10 mL). The product was eluted from the resin with MeOH/0.88 ammonia (70:30) (10 mL). Fractions containing product were concentrated under reduced pressure and purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (2:10:88) to give **28** as an oil (39.5 mg, 31%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (m, 5H), 5.71 (s, 1H), 5.28 (s, 2H), 4.19 (t, 2H, J = 6 Hz), 3.31 (t, 2H, J = 7 Hz), 3.03 (t, 2H, J = 8 Hz), 2.45 (t, 2H, J = 8 Hz), 2.26 (s, 6H), 1.95 (m, 2H), 1.52 (m, 2H), 1.34 (m, 2H), 0.89 (t, 3H, J = 7 Hz), 0.72 (t, 3H, J = 7 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 162.1, 161.6, 137.4, 137.1, 128.4, 127.7, 127.6, 91.0, 67.7, 56.3, 53.8, 50.3, 46.7, 45.5, 27.5, 21.8, 20.4, 11.4, 10.9; MS (EI) m/z 387 [MH]$^+$. Anal. ($C_{22}H_{34}N_4O_2$·0.2H_2O) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-(dimethylaminocarbonyl)-1*H*-pyrazole (29). From **26** (306 mg, 1.0 mmol) and dimethylamine (45 mg, 1 mmol) using NMP as solvent at 100 °C. Purification by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **29** as an oil (125 mg, 38%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 5H), 5.74 (s, 1H), 5.29 (s, 2H), 4.18 (t, 2H, J = 6.4 Hz), 2.96 (s, 3H), 2.76 (s, 3H), 2.45 (t, 2H, J = 8 Hz), 2.25 (s, 6H), 1.94 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 161.5, 137.4, 136.5, 128.4, 127.8, 127.6, 91.8, 67.6, 56.3, 53.9, 45.5, 38.7, 35.0, 27.5; MS (EI) m/z 331 [MH]$^+$. Anal. ($C_{18}H_{26}N_4O_2$·0.2H_2O) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-benzylaminocarbonyl-1*H*-pyrazole (30). From **26** (306 mg, 1 mmol) and benzylamine (107 mg, 1 mmol) using NMP as solvent at 100 °C. Purification by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **30** as a solid (125 mg, 32%): mp 78−80 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 10H), 6.19 (m, 1H), 5.90 (s, 1H), 5.63 (s, 2H), 4.54 (d, 2H, J = 6 Hz), 4.17 (t, 2H, J = 6 Hz), 2.42 (t, 2H, J = 7 Hz), 2.23 (s, 6H), 1.92 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 137.7, 137.6, 128.8, 128.4, 127.7, 127.5, 91.1, 67.7, 56.3, 54.1, 45.4, 43.5, 27.4; MS (EI) m/z 393 [MH]$^+$. Anal. ($C_{23}H_{28}N_4O_2$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-(*N*-morpholinocarbonyl)-1*H*-pyrazole (31). From **26** (102 mg, 0.33 mmol) and morpholine (29 mg, 0.33 mmol). The workup followed that described for **28** to give **31** as a solid (53 mg, 43%): mp 75−76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.19 (m, 5H), 5.70 (s, 1H), 5.32 (s, 2H), 4.21 (t, 2H, J = 6 Hz), 3.59 (m, 4H), 3.27 (m, 2H), 3.17 (m, 2H), 2.46 (t, 2H, J = 8 Hz), 2.27 (s, 6H), 1.96 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 160.9, 137.5, 128.6, 127.9, 127.8, 91.9, 67.7, 66.4, 56.3, 53.9, 45.5, 27.5; MS (EI) m/z 373 [MH]$^+$. Anal. ($C_{20}H_{28}N_4O_3$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-(4-methoxyphenylaminocarbonyl)-1*H*-pyrazole (32). To **26** (1.51 g, 5.0 mmol), 4-methoxyaniline (0.61 g, 5.0 mmol) and DIPEA (1.29 g, 1.74 mL, 10 mmol) in THF (20 mL) was added *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (1.90 g, 5 mmol) and the reaction was heated to 100 °C for 1 h. The reaction mixture was cooled, poured onto aqueous 1 M NaOH (100 mL) and extracted with EtOAc (3 × 50 mL). The extracts were dried (Na_2SO_4) and the product purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (2:10:88) to give **32** (1.4 g, 69%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.69 (br s, 1H), 7.75 (d, 2H, J = 9.0 Hz), 7.43 (m, 4H), 7.31 (d, 1H, J = 6.8 Hz), 7.05 (d, 2H, J = 9.0 Hz), 6.80 (s, 1H), 5.73 (s, 2H), 4.30 (t, 2H, J = 6.0 Hz), 3.88 (s, 3H), 3.32 (m, 2H), 2.90 (s, 3H), 2.88 (s, 3H), 2.27 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 160.9, 157.7, 156.2, 138.4, 136.7, 131.6, 128.8, 127.7, 127.5, 122.6, 114.1, 93.1, 66.5, 66.5, 55.6, 54.1, 53.6, 42.3, 24.2; MS (FAB) m/z 409 [MH]$^+$. Anal. ($C_{10}H_{17}N_3O_3$·0.1H_2O) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-phenyl-1*H*-pyrazole (33). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (1.6 g, 10 mmol) as the starting material using the same process as for compound **13**. Purification by flash chromatography using 0.88 ammonia/EtOH/EtOAc (2:10:88) to give **33** as a solid (1.2 g, 49%): mp 70−72 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (m, 2H), 7.42−7.26 (m, 3H), 5.92 (s, 1H), 4.13 (t, 2H, J = 6 Hz), 2.41 (t, 2H, J = 7 Hz), 2.21 (s, 6H), 1.90 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 130.2, 128.9, 128.4, 125.4, 87.3, 67.8, 56.3, 45.4, 27.5; MS (FAB) m/z 246 [MH]$^+$. Anal. ($C_{14}H_{19}N_3O$) C, H, N.

1-Benzyl-3-(3-dimethylaminopropyloxy)-5-phenyl-1*H*-pyrazole (34) and 1-Benzyl-5-(3-dimethylaminopropyloxy)-3-phenyl-1*H*-pyrazole (35). Benzyl alcohol (132 mg, 0.126 mL, 1.22 mmol), tributylphosphine (246 mg, 0.3 mL, 1.22 mmol) and **33** (300 mg, 1.22 mmol) were dissolved in toluene (10 mL) and 1,1-azobisdimethylformamide (211 mg, 1.22 mmol) added and the reaction mixture was stirred overnight. Further equivalents of benzyl alcohol, tributylphosphine and 1,1-azobisdimethylformamide were added and the reaction mixture stirred for 4 h, then MeOH (20 mL) and Dowex 50W X8 (4 g) were added and the mixture swirled at room temperature for 1 h. The mixture was filtered and the resin washed with MeOH (50 mL). The resin was suspended in 0.88 ammonia/MeOH (15:85) (20 mL) and swirled for 30 min then filtered and washed with the same mixture (20 mL). The ammonia/MeOH washings were combined and concentrated under reduced pressure. The products were purified by flash chromatography using MeOH/$CHCl_3$ (8:92) to give **34** as a solid (90 mg, 22%): mp 60−61 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42−7.20 (m, 8H), 7.08 (m, 2H), 5.80 (s, 1H), 5.18 (s, 2H), 4.22 (t, 2H, J = 7 Hz), 2.48 (t, 2H, J = 8 Hz), 2.26 (s, 6H), 1.97 (m, 2H); MS (EI) m/z 336 [MH]$^+$. Anal. ($C_{21}H_{25}N_3O$) C, H, N. Also obtained was **35** as an oil (146 mg, 36%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, 2H, J = 7 Hz), 7.43−7.21 (m, 8H), 5.88 (s, 1H), 5.22 (s, 2H), 4.14 (t, 2H, J = 6 Hz), 2.34 (t, 2H, J = 6 Hz), 2.22 (s, 6H), 1.93 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 155.1, 149.6, 137.3, 134.0, 128.5, 128.5, 127.5, 127.4, 127.3, 125.3, 82.5, 70.0, 55.9, 50.9, 45.4, 27.2; MS (EI) m/z 336 [MH]$^+$. Anal. ($C_{21}H_{25}N_3O$·0.5H_2O) C, H; N: calcd, 12.20; found, 12.75.

1,5-Diphenyl-3-hydroxy-1*H*-pyrazole (36). A solution of *t*-BuOK (1.0 M in THF, 41 mL, 41 mmol) was added to a mixture of phenylhydrazine (2.0 mL, 20.33 mmol) in THF (350 mL) at room temperature. To this was added ethyl phenylpropiolate (3.40 mL, 20.59 mmol) and the mixture stirred at room temperature overnight, then heated to reflux for 4 h. The solution was allowed to cool to room temperature and the solvent was removed under reduced pressure. The residue was dissolved in water and extracted with CH_2Cl_2 (2 × 100 mL). The aqueous layer was acidified with concentrated HCl and then the solid was collected by filtration. The crude solid was recrystallized (cyclohexane/CH_2Cl_2) to give **36** as a white solid (1.75 g, 36%): mp 251−253 °C; ^{1}H NMR (DMSO-d_6) δ 7.18−6.96 (m, 10H), 5.75 (s, 1H); MS (EI) m/z 236 [M]$^+$.

3-Hydroxy-5-trifluoromethyl-1*H*-pyrazole (37). A solution of ethyl 4,4,4-trifluoro-2-butynoate (0.50 g, 3.01 mmol) and hydrazine hydrate (0.15 mL, 3.09 mmol) in EtOH (5 mL) was stirred at room temperature for 15 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using MeOH/$CHCl_3$ (15:85) to give **37** as a solid (263 mg, 57%): mp 205−208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.84 (br, 1H), 11.25 (br, 1H), 5.67 (s, 1H); MS (EI) m/z 152 [M$^+$].

The following compounds were prepared similarly.

3-Hydroxy-5-(3-furyl)-1*H*-pyrazole (38). From ethyl 3-(3-furyl)-3-oxopropanoate (0.50 g, 2.75 mmol). Precipitation of the reaction mixture afforded **38** as a white solid (0.21 g, 50%): mp >200 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.80 (br, 1H), 9.49 (br, 1H), 7.99 (s, 1H), 7.70 (s, 1H), 6.79 (s, 1H), 5.67 (s, 1H); MS (FAB) m/z 151[MH]$^+$.

3-Hydroxy-5-(4-pyridyl)-1*H*-pyrazole (39). From ethyl isonicotinoylacetate (1.0 g, 5.18 mmol). Precipitation of the reaction mixture and recrystallization (EtOH/H_2O) afforded **39** (0.73 g, 88%): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 12.44 (br, 1H), 9.81 (br, 1H), 8.58 (br s, 2H), 7.64 (d, 2H, J = 5.5 Hz), 6.14 (br s, 1H); MS (EI) m/z 161 [M]$^+$.

3-Hydroxy-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole (40). From ethyl 3,4,5-trimethoxybenzoylacetate (2.0 g, 7.09 mmol). Precipitation of the reaction mixture afforded **40** as a white

solid (1.47 mg, 78%): mp 230−232 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.02 (s, 2H), 5.96 (s, 1H), 3.86 (s, 6H), 3.71 (s, 3H); MS (EI) *m/z* 250 [M]$^+$.

Ethyl 3-Oxo-4-phenylbutyrate (41). Phenylacetyl chloride (5.0 mL, 37.81 mmol) was added dropwise to a solution of 2,2-dimethyl-1,3-dioxolane-4,6-dione (Meldrum's acid; 5.45 g, 37.81 mmol) and pyridine (6.2 mL) in CH_2Cl_2 (70 mL) at 0 °C. The solution was stirred for a further 30 min at 0 °C, then allowed to warm slowly to room temperature overnight. The reaction mixture was then washed with 10% aqueous HCl (2 × 50 mL) and H_2O (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude residue was dissolved in EtOH (100 mL) and heated to reflux for 4 h. The mixture was allowed to cool to room temperature and then concentrated under reduced pressure. The dark, oily residue was purified by flash chromatography using EtOAc/cyclohexane (20:80) followed by Kugelrohr distillation (oven temperature 130 °C/ca. 1 mmHg) to give **41** as a pale yellow oil (5.68 g, 73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.18 (q, 2H, J = 7.0 Hz), 3.84 (s, 2H), 3.46 (s, 2H), 1.27 (t, 3H, J = 7.0 Hz); MS (EI) *m/z* [M]$^+$ 206.

3-Hydroxy-5-benzyl-1*H*-pyrazole (42). From ethyl 3-oxo-4-phenylbutyrate **(41)** (2.0 g, 9.70 mmol) using the same process as for compound **37**. Precipitation of the reaction mixture afforded **42** as a white solid (1.26 g, 75%): mp 196−197 °C; ^{1}H NMR (DMSO-d_6) δ 11.39 (br s, 1H), 9.34 (br s, 1H), 7.32−7.17 (m, 5H), 5.21 (s, 1H), 3.78 (s, 2H); MS (EI) *m/z* 174 [M]$^+$.

1,5-Diphenyl-3-(3-dimethylaminopropyloxy)-1*H*-pyrazole (43). From **36** (0.20 g, 0.85 mmol) using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/EtOH/EtOAc (1:5:94) to give **43** as an oil (0.39 g, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.2−7.1 (m, 10H), 5.9 (s, 1H), 4.2 (t, 2H, J = 6.4 Hz), 2.4 (t, 2H, J = 7.5 Hz), 2.15 (s, 6H), 1.85−1.81 (m, 2H); MS (EI) *m/z* 321 [M]$^+$. Anal. ($C_{20}H_{23}N_3O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-trifluoromethyl-1*H*-pyrazole (44). From **37** using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) followed by recrystallization (hexane/CH_2Cl_2) to give **44** as a solid (0.16 g, 51%): mp 85.5−86.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.86 (s, 1H), 4.24 (t, 2H, J = 5.9 Hz), 2.66 (t, 2H, J = 6.8 Hz), 2.44 (s, 6H), 2.06 (m, 2H); MS (EI) *m/z* 237 [M]$^+$. Anal. ($C_9H_{14}N_3OF_3$) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-(3-furyl)-1*H*-pyrazole (45). From **38** (0.18 g, 1.20 mmol) using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:10:89) to give **45** as a solid (0.11 g, 39%) which was recrystallized (hexane/CH_2Cl_2): mp 100−101 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.49 (s, 1H), 6.59 (s, 1H), 5.86 (s, 1H), 4.22 (t, 2H, J = 6.3 Hz), 2.47 (t, 2H, J = 7.4 Hz), 2.27 (s, 6H), 1.97 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 143.95, 139.74, 117.06, 108.99, 87.57, 68.33, 56.64, 45.81 (2C), 27.84; MS (EI) *m/z* 235 [M]$^+$. Anal. ($C_{12}H_{17}N_3O_2$) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-(4-pyridyl)-1*H*-pyrazole (46). From **39** (0.40 g, 2.48 mmol) using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:10:89) to give **46** as a solid (0.39 g, 64%) which was recrystallized (hexane/CH_2Cl_2): mp 103−105 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, 2H, J = 6.1 Hz), 7.66 (d, 2H, J = 6.1 Hz), 6.18 (s, 1H), 4.36 (t, 2H, J = 6.3 Hz), 2.65 (t, 2H, J = 7.2 Hz), 2.44 (s, 6H), 2.13 (t, 2H, J = 6.8 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 164.00, 163.20, 151.07 (2C), 139.53, 120.48 (2C), 87.98, 69.33, 56.87, 46.20 (2C), 28.19; MS (EI) *m/z* 246 [M]$^+$. Anal. ($C_{13}H_{18}N_4O$) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrazole (47). From **40** (500 mg, 1.90 mmol) using the same process as for **13**. Purified by flash chromatography using 0.88 ammonia/EtOH/EtOAc (1:10:89) to give **47** as an oil (507 mg, 79%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.71 (s, 2H), 5.83 (s, 1H), 4.15 (t, 2H, J = 6.3 Hz), 3.83 (s, 6H), 3.80 (s, 3H), 2.46 (t, 2H, J = 7.4 Hz), 2.25 (s, 6H), 1.92 (quintet, 2H, J = 6.9 Hz); MS (EI) *m/z* 335 [M]$^+$. Anal. ($C_{17}H_{25}N_3O_4$·1.1H_2O) C, H, N.

3-(3-Dimethylaminopropyloxy)-5-benzyl-1*H*-pyrazole (48). From **42** (0.50 g, 2.87 mmol) using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:10:89) followed by recrystallization (hexane) to give **48** as a solid (393 mg, 53%): mp 52−54 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51−7.36 (m, 5H), 5.68 (s, 1H), 4.29 (t, 2H, J = 6.4 Hz), 4.08 (s, 2H), 2.59 (t, 2H, J = 7.5 Hz), 2.40 (s, 6H), 2.08 (quintet, 2H, J = 7.0 Hz); MS (EI) *m/z* 259 [M]$^+$. Anal. ($C_{15}H_{21}N_3O$·0.65H_2O) C, H; N: calcd, 15.50; found, 15.03.

Methyl 3-Dimethylamino-2-phenylacrylate (49). To a solution of methyl phenylacetate (2.0 mL, 13.9 mmol) in THF (30 mL) was added *tert*-butoxybis(dimethylamino)methane (3.2 mL, 15.5 mmol). The resultant solution was stirred at room temperature for 15 h and then the solvent was removed under reduced pressure. The residual oil was purified by flash chromatography EtOAc/cyclohexane (20:80) followed by Kugelrohr distillation to give **49** as an oil (1.70 g 59%): bp 150 °C/∼1 mmHg (oven); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (s, 1H), 7.36−7.20 (m, 5H), 3.64 (s, 3H), 2.68 (s, 6H); MS (FAB) *m/z* 206 [MH]$^+$ 205.

3-Hydroxy-4-phenyl-1*H*-pyrazole (50). A solution of **49** (1.0 g, 4.87 mmol) and hydrazine hydrate (0.26 mL, 5.36 mmol) in EtOH (20 mL) was heated to reflux for 5 h. The cooled solution was concentrated under reduced pressure and the solid was recrystallized (MeOH) to give **50** (0.67 g, 86%): mp 221.5−223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.76 (br, 1H), 10.12 (br, 1H), 7.90 (s, 1H), 7.67 (d, 2H, J = 7.4 Hz), 7.29 (t, 2H, J = 7.7 Hz), 7.09 (t, 1H, J = 7.4 Hz); MS (EI) *m/z* 160 [M]$^+$.

3-(3-Dimethylaminopropyloxy)-4-phenyl-1*H*-pyrazole (51). From **50** (0.40 g, 2.50 mmol) using the same process as for **13**. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:10:89) to give **51** as a solid (0.48 g, 78%) which was recrystallized (hexane/CH_2Cl_2): mp 98.5−100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68−7.65 (m, 3H), 7.36 (t, 2H, J = 7.9 Hz), 7.21 (t, 1H, J = 7.4 Hz), 4.36 (t, 2H, J = 6.3 Hz), 2.52 (t, 2H, J = 7.5 Hz), 2.29 (s, 6H), 2.04 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 160.75, 132.61, 128.91, 127.89, 126.22, 126.05, 106.93, 67.72, 56.80, 45.80 (2C), 27.91; MS (EI) *m/z* 245 [M]$^+$. Anal. ($C_{14}H_{19}N_3O$) C, H, N.

2-Amino-4-ethoxycarbonylpyridine (52). 2-Acetylaminopyridine-4-carboxylic acid[32] (2.0 g, 11.1 mmol) and concentrated H_2SO_4 (3 mL) in dry EtOH (20 mL) was refluxed for 6 h. The cooled mixture was basified with aqueous ammonia solution and extracted with EtOAc (3 × 100 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. The crude material was purified by flash chromatography using a 0−2% gradient of MeOH/$CHCl_3$ to give **52** as a white solid (1.40 g, 75%): mp 85−86 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (d, 1H, J = 5.3 Hz), 7.16 (br d, 1H, J = 1.5 Hz), 7.09 (dd, 1H, J = 5.3, 1.5 Hz), 4.40 (q, 2H, J = 7.1 Hz), 1.42 (t, 3H, J = 7.1 Hz); ^{13}C NMR (75.5 MHz, CD_3OD) δ 166.7, 161.6, 148.9, 140.9, 112.4, 110.0, 62.6, 14.4; MS FAB *m/z* 167 [MH]$^+$.

2-Amino-4-pyridinemethanol (53). To a solution of **52** (1.1 g, 6.62 mmol) in dry THF (20 mL) at 0 °C was added a 1 M solution of $LiAlH_4$ in THF (8.6 mL, 8.6 mmol). The mixture was stirred at room temperature for 2 h and an excess of MeOH was added. The mixture was concentrated under reduced pressure and the crude material purified by flash chromatography using a 5−10% gradient of MeOH/$CHCl_3$ to give **53** as a white solid (721 mg, 90%): mp 43−44 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (d, 1H, J = 5.5 Hz), 6.63 (s, 1H), 6.60 (d, 1H, J = 5.5 Hz), 4.54 (s, 2H); ^{13}C NMR (75.5 MHz, CD_3OD) δ 160.6, 154.4, 147.4, 111.9, 107.1, 63.4; MS (APCI$^+$) *m/z* 125 [MH]$^+$. Anal. ($C_6H_8N_2O$) C, H, N.

3-(3-Diethylaminopropyloxy)-5-phenyl-1*H*-pyrazole (54). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (0.25 g, 1.56 mmol) and 3-diethylamino-1-propanol (0.205 g, 1.56 mmol) using the same process as for compound **13** except the reaction was carried out in THF at 70 °C for 3.5 h and a Dowex resin workup

was employed. Purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) to give **54** (0.12 g, 30%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.47 (m, 2H), 7.40−7.27 (m, 3H), 5.91 (s, 1H), 4.17 (t, 2H, J = 6.3 Hz), 2.61−2.49 (m, 6H), 1.95−1.86 (m, 2H), 0.10 (t, 6H, J = 7.2 Hz); MS (EI) m/z 273 $[M]^+$. Anal. ($C_{16}H_{23}N_3O{\cdot}1H_2O$) C, H, N.

3-(1-Methyl-3-piperidinyl)methyloxy-5-phenyl-1*H*-pyrazole (55). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (250 mg, 1.56 mmol) and 1-methyl-3-hydroxymethylpiperidine (202 mg, 1.56 mmol) using the same process as for compound **13** except the reaction was carried out in THF at 70 °C for 3.5 h and a Dowex resin workup was employed. The crude compound was purified by flash chromatography using 0.88 ammonia/MeOH/$CHCl_3$ (1:5:94) followed by recrystallization (cyclohexane/EtOAc) to give **55** (138 mg, 33%): mp 110−111 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, 2H, J = 7.0 Hz), 7.45−7.33 (m, 3H), 5.96 (s, 1H), 4.07 (dd, 1H, J = 5.9, 9.9 Hz), 4.00 (dd, 1H, J = 7.4, 9.6 Hz), 2.98 (d, 1H, J = 11.0 Hz), 2.79 (d, 1H, J = 11.0 Hz), 2.28 (s, 3H), 2.14 (m, 1H), 1.94 (td, 1H, J = 11.0, 2.9 Hz), 1.84−1.58 (m, 4H), 1.07 (m, 1H); MS (EI) m/z 273 $[M + 2H]^+$. Anal. ($C_{16}H_{21}N_3O$) C, H, N.

5-Phenyl-3-[(2-aminopyridin-3-yl)methyloxy]-1*H*-pyrazole (56). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (550 mg, 3.44 mmol) and 2-amino-3-pyridinemethanol[31] (640 mg, 5.2 mmol) using the same process as for **13** except the reaction was carried out in dry THF at 80 °C for 15 h. The crude material was purified by flash chromatography using a 0−5% gradient of MeOH/$CHCl_3$ followed by recrystallization (CH_2Cl_2/cyclohexane) to give **56** as a solid (64 mg, 7%): mp 151−152 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (dd, 1H, J = 5.2, 1.8 Hz), 7.57−7.52 (m, 3H), 7.36−7.23 (m, 3H), 6.60 (dd, 1H, J = 7.3, 5.2 Hz), 6.07 (s, 1H), 5.04 (s, 2H); MS (APCI$^+$) m/z 267 $[MH]^+$. Anal. ($C_{15}H_{14}N_4O$) C, H, N.

5-Phenyl-3-[(3-pyrrolidin-1-yl)-propyloxy]-1*H*-pyrazole (57). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (500 mg, 3.13 mmol) and 3-pyrrolidin-1-yl-propan-1-ol[30] (400 mg, 3.13 mmol) using the same process as for compound **13**. Purified by flash chromatography using 0.88 ammonia/EtOH/EtOAc (1:5:94) followed by recrystallization (cyclohexane/EtOAc) to give **57** as a solid (440 mg, 52%): mp 109−110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, 2H, J = 7.2 Hz), 7.46−7.37 (m, 3H), 5.98 (s, 1H), 4.26 (t, 2H, J = 6.4 Hz), 2.66 (t, 2H, J = 7.6 Hz), 2.57 (br s, 4H), 2.09−2.00 (m, 2H), 1.81 (t, 4H, J = 3.6 Hz); MS (EI) m/z 271 $[M]^+$. Anal. ($C_{16}H_{21}N_3O$) C, H, N.

5-Phenyl-3-[(2-aminopyridin-4-yl)methyloxy]-1*H*-pyrazole (58). From 3-hydroxy-5-phenyl-1*H*-pyrazole[28] (300 mg, 1.88 mmol) and **53** (303 mg, 2.44 mmol) using the same process as for compound **13** except the reaction was carried out in dry THF at 80 °C for 15 h. Purified by flash chromatography using a 2−4% gradient of MeOH/$CHCl_3$ to give **58** as a solid (261 mg, 52%): mp 134−135 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (d, 1H, J = 5.3 Hz), 7.56 (br d, 2H, J = 6.8 Hz), 7.48−7.39 (m, 3H), 6.72 (br d, 1H, J = 5.6 Hz), 6.63 (s, 1H), 6.05 (s, 1H), 5.21 (s, 2H); MS (APCI$^+$) m/z 267 $[MH]^+$. Anal. ($C_{15}H_{14}N_4O$) C, H, N.

***N*1,*N*1-Dimethyl-3-[(5-phenyl-1*H*-3-pyrazolyl)amino]-propanamide (59).** A mixture of 3-amino-5-phenyl-1*H*-pyrazole (159 mg, 1 mmol) and dimethylacrylamide (120 mg, 1.2 mmol) was heated at 80 °C for 2 h then allowed to cool. Purification was by flash chromatography using CH_2Cl_2/MeOH (95:5), to give **59** (175 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80−7.70 (m, 2H), 7.50−7.30 (m, 3H), 5.75 (s, 1H), 4.70 (br s, 2H), 4.18 (m, 2H), 3.00 (s, 3H), 2.95 (s, 3H), 2.95 (m, 2H); MS (EI) m/z 258 $[M]^+$.

3-(3-Dimethylaminopropylamino)-5-phenyl-1*H*-pyrazole Dihydrochloride (60). Lithium aluminum hydride powder (40 mg, 1.05 mmol) was added to a solution of **59** (150 mg, 0.58 mmol) in dry dioxane (15 mL) and the mixture heated at 80 °C for 6 h. After cooling, water (0.5 mL) was added followed by 1.0 M NaOH solution (0.5 mL). The solution was refluxed for 1 h then filtered and the precipitated salts washed with more dioxane. The combined filtrates were concentrated under reduced pressure and the crude product was purified by flash chromatography using 0.88 ammonia/CH_2Cl_2/MeOH (1:95:5) to give **60** as a clear oil (80 mg, 57%) which was converted to its hydrochloride salt with HCl (1.0 M in diethyl ether): mp 225 °C (HCl salt); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 8 Hz), 7.40−7.20 (m, 3H), 5.75 (s, 1H), 4.80 (br s, 2H), 4.10 (m, 2H), 2.20 (s, 6H), 2.15 (m, 2H), 2.05 (m, 2H); MS (APCI$^+$) m/z 245 $[MH]^+$. Anal. ($C_{14}H_{20}N_4{\cdot}2HCl{\cdot}1H_2O$) C, H, N.

4-(5-Phenyl-1*H*-3-pyrazolyl)butanoic Acid (61). 7-Phenyl-5,7-diketoheptanoic acid[34] (2 g, 8.5 mmol) and hydrazine hydrate (2 mL) were refluxed in EtOH (20 mL). After 2 h the cooled solution was concentrated under reduced pressure to give **61** as a crude oil (1.8 g, 92%) which solidified on standing and was used without further purification or characterization: MS (APCI$^-$) m/z 229 $[M - H]^+$.

***N*1,*N*1-Dimethyl-4-(5-phenyl-1*H*-3-pyrazolyl)butanamide (62).** Crude compound **61** (1.5 g, 6.5 mmol) was treated with thionyl chloride (5 mL) at 60 °C for 3 h then cooled and concentrated under reduced pressure. The resulting dark oil was suspended in diethyl ether and dimethylamine (2.0 M in THF, 5 mL) and the mixture was stirred overnight. The solution was concentrated under reduced pressure and purified by flash chromatography using CH_2Cl_2/MeOH (95:5), to give **62** as a solid (450 mg, 20%): mp 148 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 8 Hz), 7.45−7.25 (m, 3H), 6.40 (s, 1H), 2.95 (s, 6H), 2.80 (t, 3H, J = 6 Hz), 2.45 (m, 2H), 2.05 (m, 2H); MS (APCI$^+$) m/z 258 $[MH]^+$.

3-(4-Dimethylaminobutyl)-5-phenyl-1*H*-pyrazole (63). Compound **62** (150 mg, 0.58 mmol) was added to dioxane (20 mL) and $LiAlH_4$ (1.0 M in THF, 2 mL) was added. The resulting solution was heated at 80 °C for 6 h then cooled. Dioxane moistened with water (2 mL) was added, followed by 1 M NaOH (1 mL) and the mixture heated at 80 °C for 45 min then cooled and filtered. The filtrate was concentrated under reduced pressure, toluene added and evaporation repeated. Purification was by flash chromatography using 0.88 ammonia/CH_2Cl_2/MeOH (1:89:10) to give **63** as a solid (120 mg, 82%): mp 91−93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 8 Hz), 7.45−7.25 (m, 3H), 6.35 (s, 1H), 2.70 (t, 2H, J = 7 Hz), 2.20 (s, 6H), 1.70 (m, 2H), 1.55 (m, 2H); MS (FAB) m/z 244 $[MH]^+$. Anal. ($C_{15}H_{21}N_3{\cdot}0.25H_2O$) C, H, N.

sGC Enzyme. Recombinant bovine sGC was obtained from Sf21 cells infected with baculovirus carrying sGC cDNA. Original clones containing bovine sGC α_1 and β_1 cDNA were a kind gift from Doris Koesling (Germany). The cDNA for both subunits was subcloned into p2Bac, a dual promoter baculovirus vector (Invitrogen). Sf21 cells (1×10^8) were seeded on 4-$\times$ 600-cm^2 plates in 60 mL of complete TC 100 medium. Cells were allowed to attach overnight and then medium removed. 40 mL of TC 100 medium, 6 mL of high-titer virus (>10^8 plaque-forming units/mL) and 2.5 μg/mL hemin were added and the cells were incubated at 27 °C for 48 h. The cells were collected by centrifugation at 300*g* for 5 min. The cells were washed three times in cold PBS and then resuspended in 10 mL of buffer A (25 mM TEA pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.2 mM benzamidine, 5 mM DTT). After sonication, the particulate fraction was pelleted by centrifugation at 15000*g* for 10 min. The soluble fraction was then applied to a 1 mL Mono-Q column (Amersham-Pharmacia) that had been equilibrated with buffer A. A 40-mL linear gradient of 100 mM to 1 M NaCl in buffer A was used to elute sGC. The 1-mL collected fractions were assayed for sGC activity using a cyclic GMP ^{3}H assay system (Amersham-Pharmacia). The peak fractions of activity were pooled, made 50% with glycerol and stored at −80 °C for subsequent use.

sGC Enzyme Assay. Test compounds (10 mM) were dissolved in DMSO and diluted with Tris buffer (40 mM trizma hydrochlorate, 5 mM magnesium chloride hexahydrate, pH 7.4, 1 M NaOH). Into each well 10 μL of the test compound at 20$\times$ the final concentration was added with 100 μL of reaction buffer (120 μg/mL recombinant sGC, 1.1 mg/mL IBMX (Sigma), 2.6 mg/mL GTP (Sigma) in Tris buffer). The reaction was started with the addition of 90 μL of 667 nM DEA/NO (RBI) to give a reaction DEA/NO concentration of 300 nM. The plates were then incubated at room temperature for 10 min. To

determine the amount of cGMP produced the Biotrak cGMP enzyme immunoassay (EIA) system from Amersham was used according to manufacturer's instructions. Briefly the assay was based upon the competition between unlabeled cGMP and a fixed quantity of peroxidase labeled cGMP for a limited amount of cGMP specific antibody. The peroxidase ligand that was bound to the antibody was immobilized on precoated microtiter wells and the amount of labeled cGMP was determined using a one pot stabilized substrate. The concentration of unlabeled cGMP in a sample was subsequently determined by interpolation from a standard curve. The standard curve was prepared in duplicate and each test compound was evaluated in duplicate with at least $n = 2$. The measured cGMP for each compound was expressed as a percentage of the DEA/NO response, and relative enzyme activities (REA) were calculated as a ratio between the percentage DEA/NO response for the compound and that for benzydamine (**3**).

Dose−response curves were carried out using an identical assay except the recombinant sGC was stimulated by the addition of 30 nM of PAPA/NO (Alexis Biochemicals). EC_{50}'s were calculated by nonlinear regression and reported as means ± standard error of the mean of at least 3 independent experiments. Data analysis was performed with Origin software (Microcal Software).

Platelet Aggregation Assay. Platelets were prepared as described previously.[39] Briefly, fresh human blood was collected into tubes as a 1:9 solution of sodium citrate solution (3.15%):blood and centrifuged immediately at 260*g* for 20 min to separate the red cells from the platelet-rich plasma (PRP). The PRP was decanted and PGI_2 (0.3 μg/mL, ICN Pharmaceuticals) in Tris buffer (0.05 M, pH 9) was added. The PRP was then centrifuged at 180*g* for 10 min to sediment the remaining red and white cells. The resulting PRP was decanted into a new tube, PGI_2 (0.15 μg/mL) in Tris buffer was added and centrifuged at 950*g* for 10 min to sediment the platelets. The resultant platelet-poor plasma (PPP) was discarded and the platelet pellet was resuspended in an equal volume of Tyrodes buffer without calcium (140 mM NaCl, 3 mM KCl, 12 mM $NaHCO_3$, 0.4 mM $NaH_2PO_4 \cdot H_2O$, 2 mM $MgCl_2 \cdot 6H_2O$, 0.1% glucose, pH 7.4 with 0.05 M Hepes) by gently pipetting up and down. The suspension was centrifuged at 870*g* for 10 min at 4 °C. The supernatant was discarded and the platelet pellet was resuspended in an equal volume of Tyrodes buffer as before. The platelets were counted (using a Coulter Counter model T540) and normalized to 250 000 cells/μL using Tyrodes buffer. The resultant suspension was placed on ice for approximately 1 h until use.

Test compounds (10 mM) were dissolved in DMSO and subsequent dilutions made in Tyrodes buffer. The final assay concentration of DMSO did not exceed 0.1% (which is without effect on platelet reactivity). Platelet aggregation was monitored using either a Chrono-Log model 560-CA dual channel or a 570-4S four-channel aggregometer (Chrono-Log Corp., Havertown, PA). Aggregation was analyzed by using 0.5-mL aliquots of the platelet suspension at 37 °C using percent (%) light transmittance. For each sample a baseline reading was established for a 3-min period, followed by addition of test compound or buffer. An EC_{50} dose of collagen (collagenreagent Horm, Nycomed Arzneimittel GmbH, Munchen) was added 1 min later and the response measured 3 min after addition of collagen. The amplitude of each aggregatory response, normalized to the collagen control, was used to plot dose−response curves. The concentration of drug that inhibited collagen-induced platelet aggregation by 50% (IC_{50}) was calculated from the dose−response curves and is presented as means ± standard error of the mean of at least 2 independent experiments. Data analysis was performed with Origin software (Microcal Software).

Selectivity Studies. All selectivity assays were determined using published procedures by CEREP (France). Briefly, compounds were evaluated in each assay at 10 μM in duplicate. For each experiment a reference compound was evaluated at a minimum of 7 concentrations to obtain an inhibitor or stimulation curve for experimental validation. Reference compounds and procedures were as follows: COX_1, diclofenac;[40] COX_2, NS398;[40] PDE I, 8-methoxy-3-isobutyl-1-methylxanthine;[41] PDE II, *erythro*-9-(2-hydroxy-3-nonyl)adenine;[42] PDE III, milrinone;[43] PDE IV, rolipram;[42] PDE V, dipyridamole;[43] adenylate cyclase (stimulated), SQ 22,536;[44] adenylate cyclase (basal), forskolin;[44] iNOS, L-NMMA;[45] eNOS, L-NMMA;[46] nNOS, L-NMMA.[47]

Pharmacokinetics Studies. Pharmacokinetics studies were carried out by TNO BIBRA (U.K.). Pharmacokinetics parameters were determined in male Sprague−Dawley rats. Compounds **32** and **43** were dosed at a level of 5 mg/kg prepared in a PBS solution. For the animals dosed iv blood samples were taken before dosing and at 0.25, 0.5, 1, 1.5, 2, 4, 6, and 8 h after dosing. For the animals dosed po blood samples were taken before dosing and at 0.5, 1, 2, 3, 4, 6, 10 and 24 h after dosing. Compounds **22** and **34** were dosed at a level of 2 mg/kg prepared in a PBS solution. For the animals dosed iv blood samples were taken before dosing and at 5, 15 and 30 min and 1, 1.5, 2, 4, 6, and 8 h after dosing. For the animals dosed po blood samples were taken before dosing and at 15 min and 0.5, 1, 2, 3, 4, 6 and 10 h after dosing.

Serum was separated by centrifugation and stored at −20 °C prior to analysis. For analysis, compounds were extracted by liquid−liquid extraction with diethyl ether. Chromatography was performed on a 3.9- × 150-mm Nova-Pak C18 column (Waters Chromatography) using a mobile phase of acetonitrile/methanol/0.25% ammonia solution 50:45:5. Mass spectrometry was performed on a Micromass Quattro-LC QLC 9025 quadrupole instrument in the positive ion electrospray (ES+) mode.

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Supporting Information Available: Elemental analysis, calculated log *P* values, and activity summary for compounds **22**, **32**, and **34** against various enzymes. This material is available free of charge via the Internet at http://pubs.acs.org.

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