Novel, Potent, and Selective Phosphodiesterase 5 Inhibitors: Synthesis and Biological Activities of a Series of 4-Aryl-1-isoquinolinone Derivatives

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A novel class of potent and selective phosphodiesterase 5 (PDE5) inhibitors, 4-aryl-1-isoquinolinone derivatives, which have been designed by the comparison of the structure of cGMP and a previously reported 1-arylnaphthalene lignan, was disclosed. Among these compounds, methyl 2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridinylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate dihydrochloride (**36a)** exhibited potent PDE5 inhibitory activity (IC_{50} = 1.0 nM) with high isozyme selectivities (IC_{50} ratio: PDE1/PDE5 = 1300, PDE2/PDE5 > 10 000, PDE3/PDE5 > 10 000, PDE4/PDE5 = 4700, PDE6/PDE5 = 28). Compound **36a** also showed the most potent relaxant effect on isolated rabbit corpus cavernosum (EC_{30} = 7.9 nM). Compound **63** (T-1032), the sulfate form of **36a**, was selected for further biological and pharmacological evaluation of erectile dysfunction.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes that catalyze the hydrolysis of cyclic nucleotides, cAMP and cGMP, to their respective 5′-nucleoside monophosphates by cleavage of the phosphodiester bond at the 3′-position. PDEs have been classified into at least 11 identified families to date,[1] which have been distinguished by their substrate specificities, mechanisms of regulation, and their sensitivities to various pharmacological agents. PDE5, cGMP-binding cGMP-specific PDE, is distributed in lung, kidney, spleen, endothelial cells, and smooth muscle cells, etc. and plays a key role in the regulation of the cellular level of cGMP (**1**, Figure 1).[2] Because cGMP mediates vascular functions, a PDE5 inhibitor that elevates cGMP level is an attractive means for the treatment of cardiovascular diseases, e.g., hypertension, angina, congestive heart failure, and erectile dysfunction.[3]

Many potent PDE5 inhibitors with a variety of scaffolds have been reported so far: Zaprinast-related inhibitors[4] exemplified by sildenafil, quinazoline derivatives,[5] phthalazine derivatives,[6] tetracyclic diketopiperazines,[7] indoles,[8] and pyrido[3,2,1-*jk*]carbazoles.[9] In connection with our continuous efforts in search of biologically active lignans,[10] we have recently disclosed a series of 1-arylnaphthalene lignans **2** as a new structural class of PDE5 inhibitors.[11] We have supposed that the superimposition of cGMP **1** and 1-arylnaphthalene **2** showed that the naphthalene ring in **2** significantly overlapped the purine nucleus in **1** and that the pendant phenyl group at the 1-position of **2** filled a space occupied by the cyclic phosphate group in **1**. Additionally, in the previous paper[11] we recognized the crucial effect of the amide carbonyl group at the 3-position of naphthalene derivatives **2** toward the PDE5 inhibitory activity. In comparing the structure of cGMP **1** with that of 1-arylnaphthalenes **2**, we envisaged that the spatial orientation of the carbonyl group shown in the box and the aromatic rings attached to the naphthalene were important for the PDE5 inhibition (Figure 1). To enhance potency as PDE5 inhibitors and to improve the isozyme selectivity (against PDE1, PDE2, PDE3, and PDE4), we designed 4-aryl-1(*2H*)-isoquinolinone derivatives **3** by moving the amide carbonyl group at the 3-position of **2** into the bicyclic ring system. In this paper, we report the structure−activity relationships (SARs) of 4-aryl-1(*2H*)-isoquinolinone derivatives **3** as PDE5 inhibitiors and the relaxant effects on isolated rabbit corpus cavernosum of selected compounds related to **3**.

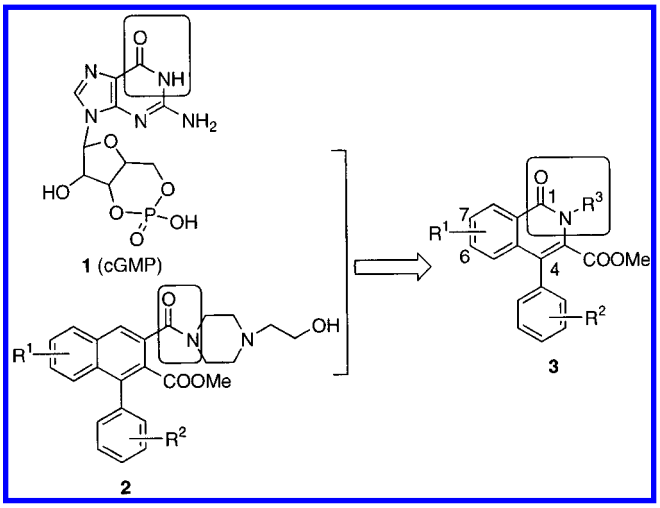

Figure 1. Compounds **1**−**3**.

Chemistry

The general synthetic method shown in Scheme 1 was used to prepare 1(*2H*)-isoquinolinones. Bromo acetals **4** were converted via keto acetals **5** to keto aldehydes **6**, which were oxidized to keto acids **9** (route 1). Alternatively, the oxidation of phthalides **8**, which were

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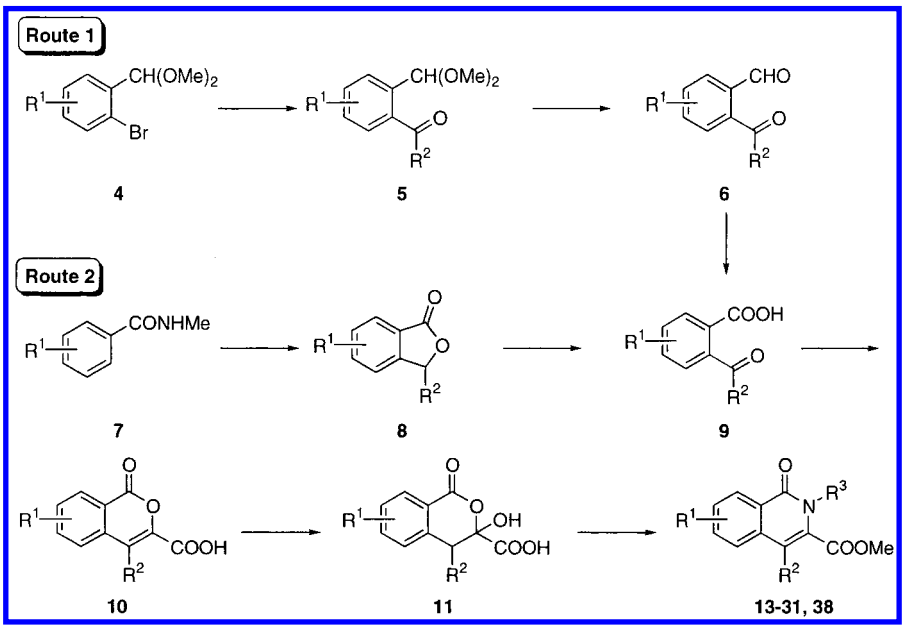
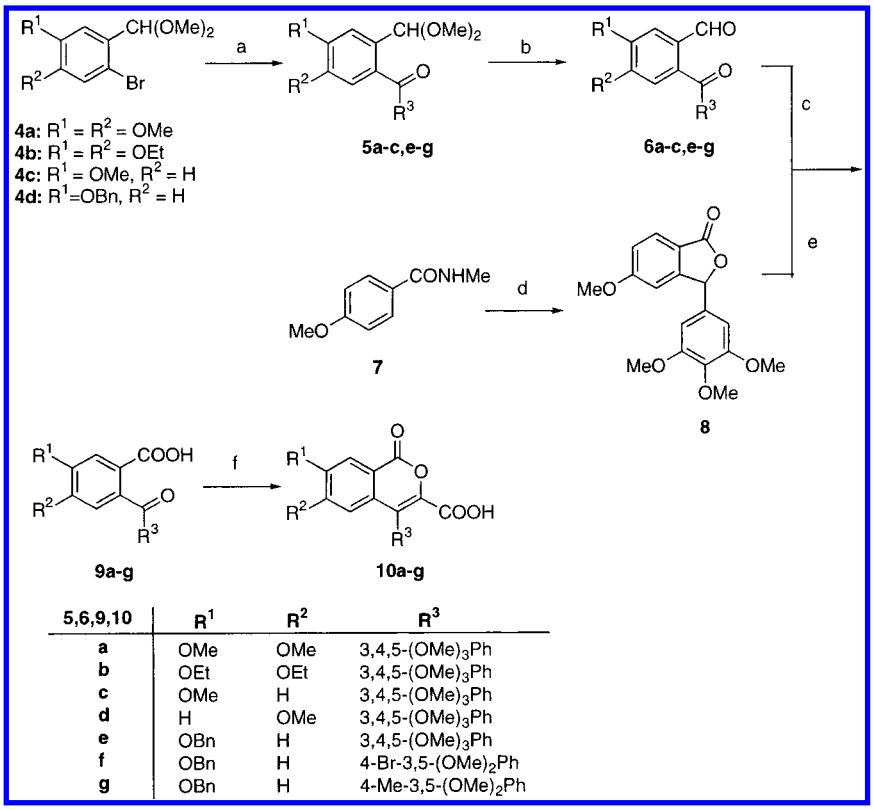
Scheme 1



Scheme 2[a]



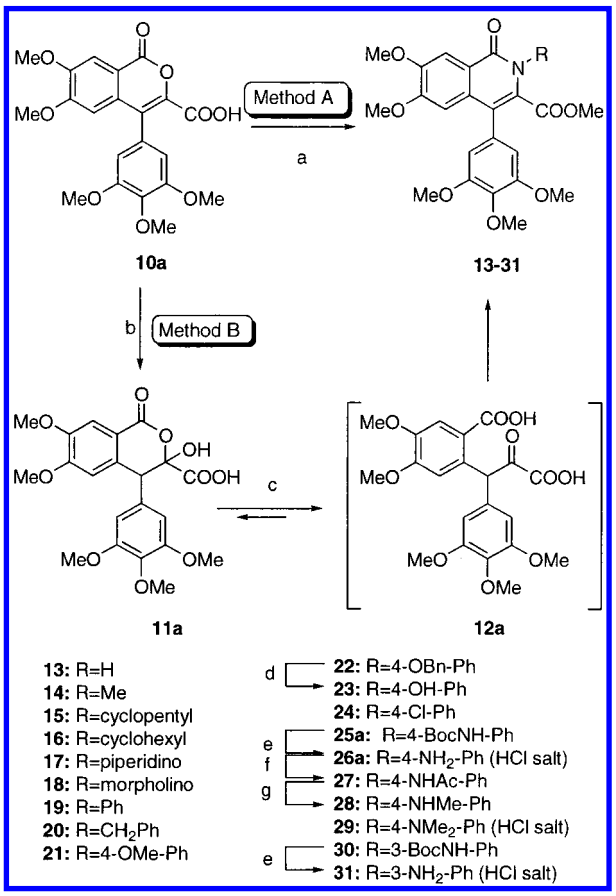
5,6,9,10	R^1	R^2	R^3
a	OMe	OMe	3,4,5-$(OMe)_3$Ph
b	OEt	OEt	3,4,5-$(OMe)_3$Ph
c	OMe	H	3,4,5-$(OMe)_3$Ph
d	H	OMe	3,4,5-$(OMe)_3$Ph
e	OBn	H	3,4,5-$(OMe)_3$Ph
f	OBn	H	4-Br-3,5-$(OMe)_2$Ph
g	OBn	H	4-Me-3,5-$(OMe)_2$Ph

[a] Reagents: (a) *n*-BuLi, R^3CONMe_2/THF, or (1) *n*-BuLi, R^3CHO/THF, (2) MnO_2/toluene; (b) 4 N HCl/AcOEt; (c) $NaClO_2$, resorcinol/acetate buffer (pH 3.8)−dioxane; (d) (1) *s*-BuLi, TMEDA, R^3CHO/THF, (2) concd HCl−dioxane; (e) $KMnO_4$, 15% aqueous KOH−pyridine; (f) (1) $BrCH(COOt\text{-}Bu)_2$, K_2CO_3/DMF, (2) 4 N HCl/AcOEt, (3) AcOH−dioxane.

transformed from benzamides **7**, also afforded keto acids **9** (route 2). Isocoumarins **10** were obtained from keto acids **9** under conditions modified from the general method[12] and converted to 1(*2H*)-isoquinolinones (vide infra).

The key intermediates, 4-arylisocoumarin-3-carboxylic acids **10**, were prepared as described in Scheme 2. Bromo acetals **4** were converted into keto acetals **5**, which were treated with aqueous HCl or TFA to give keto aldehydes **6**. Subsequent oxidation of **6** with $NaClO_2$ gave *o*-benzoylbenzoic acids **9a**−**c,e**−**g** in good yields. Alternatively, the oxidation of phthalide **8**, which was obtained from benzamide **7**, with $KMnO_4$ afforded **9d** in 95% yield. Alkylation of **9** with di-*tert*-butyl bromomalonate followed by the treatment with HCl/AcOEt and the subsequent reflux in AcOH−dioxane gave 4-arylisocoumarin-3-carboxylic acids **10** in a good yield. As shown in Scheme 3, 1(*2H*)-isoquinolinones were prepared according to the following methods, methods A and B. Treatment of **10a** with various

Scheme 3[a]

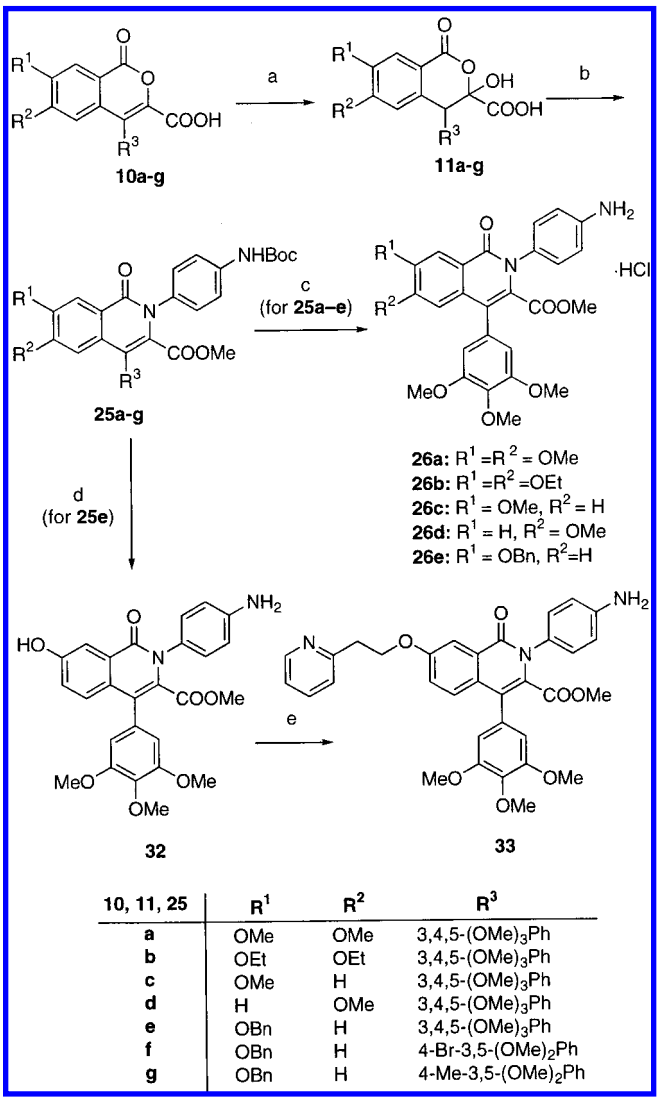


[a] Reagents: (a) (1) RNH_2/DMI or neat, (2) MeI, K_2CO_3/DMF; (b) NaOHaq/THF−MeOH; (c) (1) RNH_2, *i*-Pr_2NEt/DMI, (2) MeI, K_2CO_3/DMF; (d) H_2, Pd−C/DMF; (e) HCl/AcOEt−$CHCl_3$; (f) Ac_2O/CH_2Cl_2; (g) (1) MeI, K_2CO_3/DMF, (2) HCl_{aq}−MeOH.

alkylamines or hydrazines followed by the esterification directly gave isoquinolinones **13**−**20** and **24** (method A). However, in the case of less nucleophilic aromatic amines, this conversion resulted in relatively low yields even at higher temperature. Next we planned the reaction of aromatic amines with α-keto acid **12**, which was expected to be obtained by the hydrolysis of isocoumarins **10**.[13] Contrary to our expectation, the treatment of **10** with aqueous NaOH in THF−MeOH gave hydrated isocoumarins **11**; the structure was confirmed by X-ray analysis. The reaction of **11** with aromatic amines smoothly proceeded and gave the desired 1(*2H*)-isoquinolinones **21**, **22**, **25**, **29**, and **30** in modest to good yields (method B). We assume that this reaction proceeds via the condensation of amine with the highly reactive carbonyl group of α-keto acid **12** and subsequent ring closure.

The preparation of 2-(4-aminophenyl)isoquinolinones **26a**−**e**, **33**, and **36a**−**e** is outlined in Schemes 4 and 5. As mentioned above, condensation of **11** with Boc-*p*-phenylenediamine followed by the esterification and the subsequent deprotection of the Boc group with HCl/AcOEt gave isoquinolinones **26** in good yields. The Mitsunobu reaction of **32**, which was obtained from **25e**, with 2-(2-hydroxyethyl)pyridine afforded **33** in 47% yield (Scheme 4). Hydrogenolysis of **25e**−**g** gave 7-hydroxy compounds **34a**−**c**, which were successively treated with picolyl chloride and HCl/AcOEt to afford 7-picolyloxy compounds **36a**−**e** (Scheme 5).

Scheme 4[a]



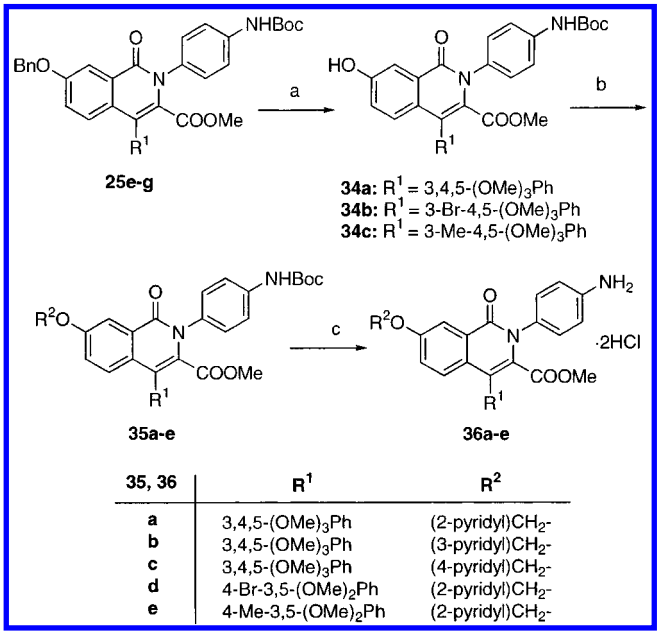
10, 11, 25	R^1	R^2	R^3
a	OMe	OMe	3,4,5-$(OMe)_3$Ph
b	OEt	OEt	3,4,5-$(OMe)_3$Ph
c	OMe	H	3,4,5-$(OMe)_3$Ph
d	H	OMe	3,4,5-$(OMe)_3$Ph
e	OBn	H	3,4,5-$(OMe)_3$Ph
f	OBn	H	4-Br-3,5-$(OMe)_2$Ph
g	OBn	H	4-Me-3,5-$(OMe)_2$Ph

[a] Reagents: (a) NaOHaq/THF−MeOH; (b) (1) Boc-*p*-phenylenediamine, *i*-Pr_2NEt/DMI, (2) MeI, K_2CO_3/DMF; (c) HCl/AcOEt−$CHCl_3$; (d) (1) H_2, Pd−C/THF−MeOH, (2) HCl/AcOEt−$CHCl_3$; (e) 2-(2-hydroxyethyl)pyridine, DEAD, PPh_3/THF.

The synthesis of hydroxyphenyl compounds **40** and **41** are summarized in Scheme 6. Isoquinolinone **39**, having an MOM protected phenolic hydroxy group on the phenyl ring at the 4-position, was prepared from **4d** and 3,4-dimethoxy-5-(methoxymethyloxy)benzaldehyde according to the analogous procedure for **35** described above. The Boc and MOM groups in **39** were removed by treatment with HCl/AcOEt to give **40** in 47% yield. The preparation of 4-(4-hydroxy-3,5-dimethoxy)phenyl compound **41** was directly accomplished by treatment of **36a** with concentrated HCl−dioxane under reflux conditions.

The modification of the ester group of **36a** is outlined in Scheme 7. The common intermediate **42** was obtained by the reaction of **11e** with Boc-*p*-phenylenediamine in good yield. The reaction of **42** with ethyl iodide or chloromethyl methyl ether provided the corresponding ethyl ester **43** or MOM ester **44**, respectively. Condensation of **42** with ammonia, methylamine, and dimethylamine with EDCI·HCl gave amide **45**, *N*-methylamide **46**, and *N*,*N*-dimethylamide **47** in good yields, respectively. Hydrogenolysis of **43**−**47** with Pd−C was fol-

Scheme 5[a]



35, 36	R^1	R^2
a	3,4,5-$(OMe)_3$Ph	(2-pyridyl)CH_2-
b	3,4,5-$(OMe)_3$Ph	(3-pyridyl)CH_2-
c	3,4,5-$(OMe)_3$Ph	(4-pyridyl)CH_2-
d	4-Br-3,5-$(OMe)_2$Ph	(2-pyridyl)CH_2-
e	4-Me-3,5-$(OMe)_2$Ph	(2-pyridyl)CH_2-

[a] Reagents: (a) H_2, Pd−C/THF−MeOH; (b) picolyl chloride, K_2CO_3/DMF; (c) HCl/AcOEt−$CHCl_3$.

lowed by the alkylation with 2-picolyl chloride to afford 7-(2-picolyloxy) derivatives **53**−**57**. Treatment of **53**−**57** with HCl/AcOEt or aqueous HCl gave ethyl ester **58**, carboxylic acid **59**, and amides **60**−**62** in good yields, respectively.

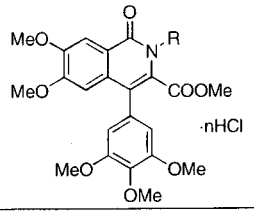
Biological Results and Discussion

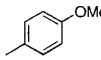
The compounds reported in this paper were first evaluated for the inhibition against three different forms of PDEs isolated from rat heart (PDE1), canine heart (PDE3), and canine lung (PDE5) (Tables 1−4). Compounds selected on the basis of the PDE5 inhibitory activity were next evaluated for relaxant effects on rabbit corpus cavernosum and investigated for the two additional different forms of PDEs isolated from bovine adrenal gland (PDE2) and canine lung (PDE4) (Table 5).

The effects of substituents at the 2-position of 1(*2H*)-isoquinolinones are listed in Table 1. We found that N−H compound **13** had a relatively selective PDE5 inhibitory activity (IC_{50} = 900 nM, selectivity for PDE5 against PDE1 and PDE3 is greater than 11). Introduction of a methyl group on the lactam resulted in improved potency against PDE1, PDE3, and PDE5 (**14**, IC_{50} = 2100 nM for PDE1, 8500 nM for PDE3, and 230 nM for PDE5). Compounds having cycloalkyl groups showed improved selectivity for PDE5 over PDE1 and PDE3 (compounds **15** and **16**); **16** was equipotent compared to **14**. Piperidino **17**, morpholino **18**, and phenyl **19** compounds exhibited potent PDE5 inhibitory activities (**17**, IC_{50} = 54 nM; **18**, IC_{50} = 26 nM; **19**, IC_{50} = 30 nM) with improved isozyme selectivities (**17**, PDE1/PDE5 and PDE3/PDE5 ratios greater than 185; **18**, PDE1/PDE5 and PDE3/PDE5 ratios greater than 385; **19**, PDE1/PDE5 and PDE3/PDE5 ratios greater than 333), while benzyl derivative **20** had a 13-fold lower potency than **19**. When comparing compounds **17** and **18**, we hypothesized that introduction of an oxygen atom on the 4-position of the six-membered ring at the 2-position might improve PDE5 inhibitory activity. Thus, we focused on the synthesis of 4-substituted phenyl compounds **21**−**29** and 3-substituted phenyl compound **31**. Introduction of the 4-methoxyphenyl group resulted in the marked loss of PDEs inhibitory activities (**21**, IC_{50} > 10 000 nM for PDE1, PDE3, and PDE5). Also, the introduction of the 4-hydroxyphenyl group and the 4-chlorophenyl group was detrimental (**23**, IC_{50} = 110 nM; **24** IC_{50} = 170 nM). On the other hand, 4-aminophenyl compound **26a** exhibited potent PDE5 inhibition (IC_{50} = 21 nM), although acetylamino **27**, methylamino **28**, and dimethylamino **29** compounds were inactive. Compound **31**, a regioisomer of **26a**, exhibited moderate PDE5 inhibitory activity (IC_{50} = 250 nM).

Next we investigated the effect of substituents on the 6- and 7-positions (R^2 and R^1, respectively) of **26a** shown in Table 2. Replacement of methoxy groups at the 6- and 7-positions of **26a** with ethoxy groups did not affect

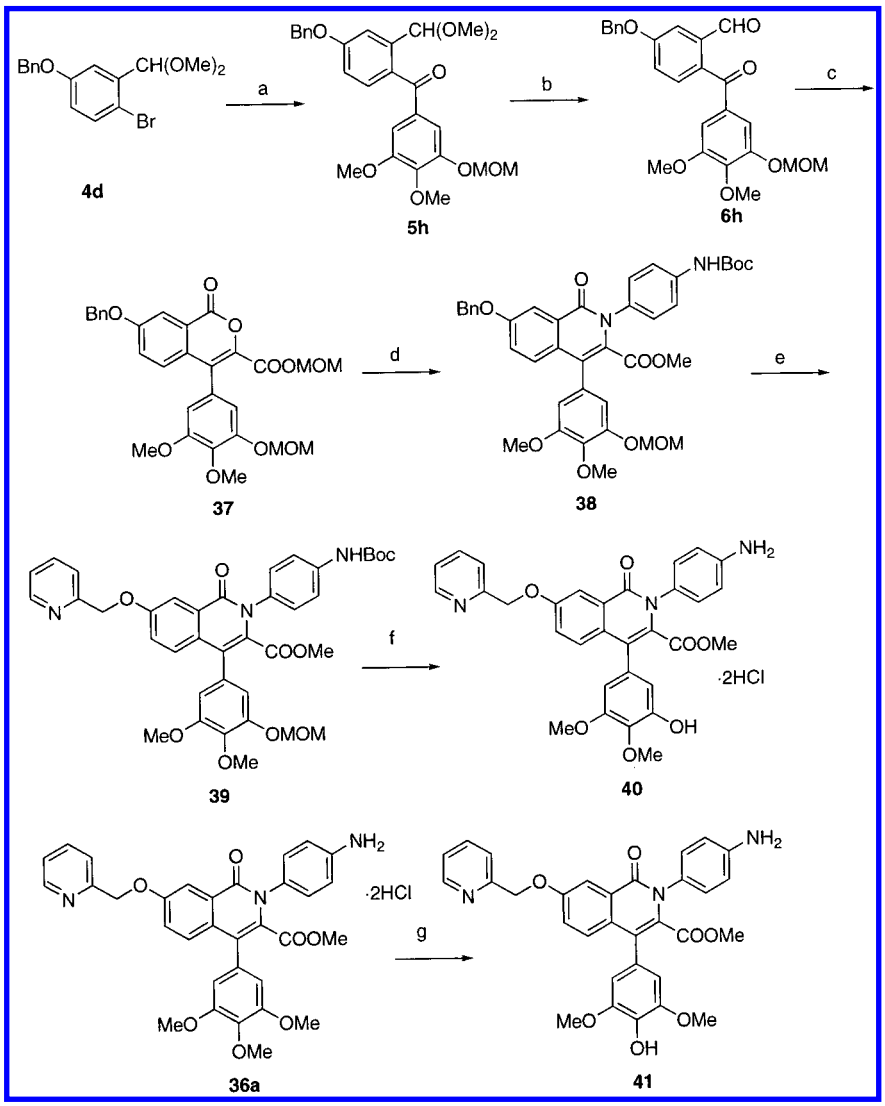
Table 1. Structures and PDE Inhibitions



Compd	R	n	PDE inhibition, IC_{50},[a] nM PDE1	PDE3	PDE5
13	H	0	>10000	>10000	900
14	Me	0	2100	8500	230
15		0	>10000	>10000	580
16		0	>10000	>10000	230
17		0	>10000	>10000	54
18		0	>10000	>10000	26
19		0	>10000	>10000	30
20		0	3100	>10000	380
21		0	>10000	>10000	>10000
23		0	>10000	>10000	110
24		0	>10000	>10000	170
26a		1	>10000	4400	21
27		0	>10000	>10000	>10000
28		0	>10000	>10000	>10000
29		1	>10000	>10000	>10000
31		1	>10000	>10000	250

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments, where the variation from the mean value is ±20% or less.

Scheme 6[a]



[a] Reagents: (a) *n*-BuLi, *N*,*N*-dimethyl-(3,4-dimethoxy-5-methoxymethoxy)benzamide/THF; (b) 2 N HCl_{aq}/THF; (c) (1) $NaClO_2$, resorcinol/acetate buffer (pH 3.8)−dioxane, (2) $BrCH(COOt\text{-}Bu)_2$, K_2CO_3/DMF, (3) 4 N HCl/AcOEt, (4) AcOH−dioxane, (5) MOMCl, *i*-Pr_2NEt/DMF; (d) (1) 2 N NaOHaq/THF−MeOH, (2) Boc-*p*-phenylenediamine/DMI, (3) MeI, K_2CO_3/DMF; (e) (1) H_2 (30 psi), Pd−C/THF−MeOH, (2) 2-picolyl chloride, K_2CO_3/DMF; (f) HCl/AcOEt−$CHCl_3$; (g) concd HCl−dioxane.

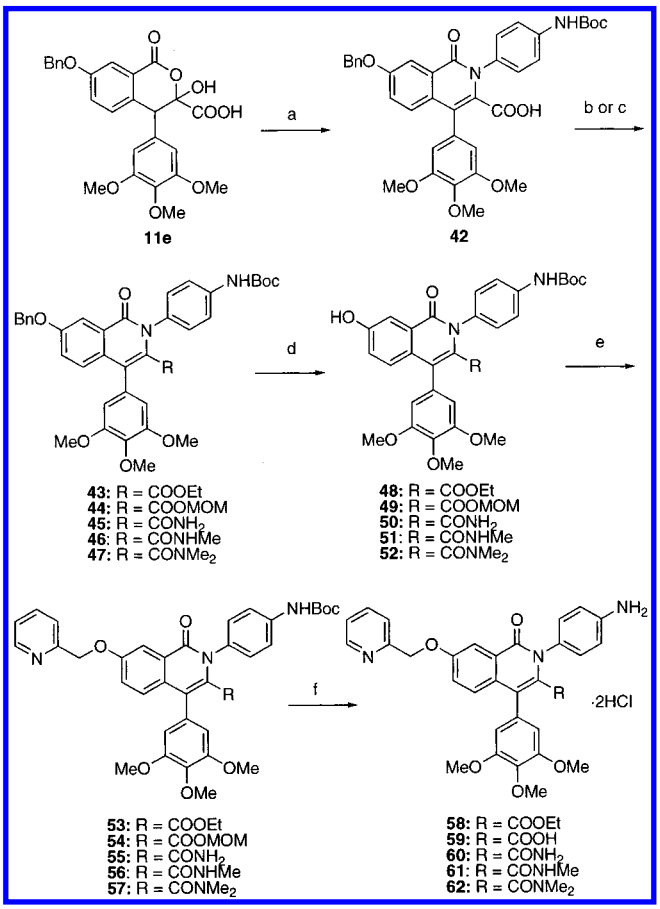
the PDE5 inhibitory activity. 7-Methoxy compound **26c** exhibited almost the same activity as **26a**, although 6-methoxy compound **26d** had lower activity than **26a** (**26c**, IC_{50} = 20 nM; **26d**, IC_{50} = 97 nM). This indicated that the presence of a substituent at the 7-position was crucial for PDE5 inhibition, while a substituent at the 6-position was unnecessary. The 7-benzyloxy compound **26e** exhibited an 18-fold higher activity than **26a**. To enhance the solubility of **26e** in water, we synthesized 7-picolyloxy compounds **36a**−**c**, which had potent PDE5 inhibitory activities (**36a**, IC_{50} = 1.0 nM; **36b**, IC_{50} = 1.1 nM; **36c**, IC_{50} = 3.1 nM). One carbon elongation of the substituent on the 7-position resulted in the loss of activity (**33** vs **36a**; **33**, IC_{50} = 5.0 nM).

As shown in Table 3, the methyl ester group at the 3-position was crucial for PDE5 inhibition. Ethyl ester **58** had an 18-fold lower activity than **36a** and exhibited poor selectivity for PDE5 over PDE1 (selectivity ratio = 31). Carboxylic acid **59**, amide **60**, methylamide **61**, and dimethylamide **62** showed only weak activities toward PDE5 (**59**, IC_{50} = 1900 nM; **60**, IC_{50} = 490 nM; **61**, IC_{50} = 220 nM; **62**, IC_{50} = 2100 nM).

Finally we investigated the modification of the 4-(3,4,5-trimethoxyphenyl) group of **36a** (Table 4). 4-Bromo and 4-methyl derivatives (**36d** and **36e**) possessed increased potencies compared with **36a** (**36a**, IC_{50} = 1.0 nM; **36d**, IC_{50} = 0.22 nM; **36e**, IC_{50} = 0.39 nM), while 3-hydroxy and 4-hydroxy derivatives (**40** and **41**) had lower activities (**40**, IC_{50} = 8.5 nM; **41**, IC_{50} = 14 nM).

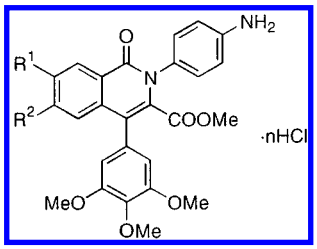
We selected six compounds (**26e**, **36a**−**e**) on the basis of PDE5 inhibitory activity and isozyme selectivities over PDE1 and PDE3 for further evaluation of two additional PDE (PDE2 and PDE4) inhibitory activities and relaxant effects on isolated rabbit corpus cavernosum, as shown in Table 5. These compounds showed modest to good selectivities for PDE5 against other isozymes. These compounds also possessed relaxant effects on rabbit corpus cavernosum, but the magnitude of these effects did not always correlate with PDE5 activities. For example, **36d** and **36e** exhibited lower relaxant effects than **36a** despite their high potencies for PDE5 inhibition (**36a**, EC_{30} = 7.9 nM; **36d**, EC_{30} = 44 nM; **36e**, EC_{30} = 37 nM). More lipophilic derivatives **26e**, **36d**, and **36e** (calculated log *D* at pH 7.4: **26e**, 4.90;

Scheme 7[a]



[a] Reagents: (a) Boc-*p*-phenylenediamine/DMI; (b) EtI, K_2CO_3 or MOMCl, *i*-Pr_2NEt/DMF; (c) NM_3 or $MeNH_2$ or Me_2NH, EDCl, HOBt/DMF; (d) H_2, Pd−C/THF−MeOH; (e) 2-picolyl chloride, K_2CO_3/DMF; (f) HCl/AcOEt−$CHCl_3$ or HCl_{aq}/THF.

Table 2. Structures and PDE Inhibitions

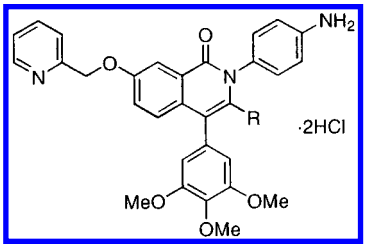


				PDE inhibition, IC_{50},[a] nM		
compd	R^1	*n*	R^2	PDE1	PDE3	PDE5
26a	MeO	1	MeO	>10000	4400	22
26b	EtO	1	EtO	>10000	>10000	27
26c	MeO	1	H	1000	>10000	20
26d	H	1	MeO	3900	>10000	97
26e	$PhCH_2O$	1	H	2100	>10000	1.2
36a	(2-pyridyl)CH_2O	2	H	1300	>10000	1.0
36b	(3-pyridyl)CH_2O	2	H	3900	>10000	1.1
36c	(4-pyridyl)CH_2O	2	H	9500	>10000	3.1
33	(2-pyridyl)$(CH_2)_2O$	0	H	>10000	>10000	5.0

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments, where the variation from the mean value is ±20% or less.

36d, 4.02; **36e**, 4.11) compared with picolyl derivatives **36a**−**c** (calculated log *D* at pH 7.4 = 3.41) showed less potency in this assay. These findings might be ascribed

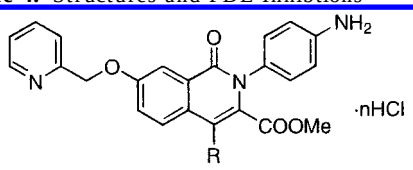
Table 3. Structures and PDE Inhibitions

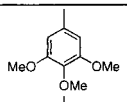
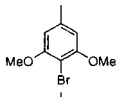
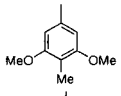
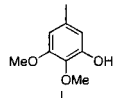
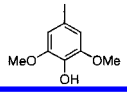


		PDE inhiition, IC_{50},[a] nM		
compd	R	PDE1	PDE3	PDE5
36a	COOMe	1300	>10000	1.0
58	COOEt	550	>10000	18
59	COOH	>10000	>10000	1900
60	$CONH_2$	>10000	>10000	490
61	CONHMe	>10000	>10000	220
62	$CONMe_2$	>10000	>10000	2100

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments, where the variation from the mean value is ±20% or less.

Table 4. Structures and PDE Inhibtions



			PDE inhibition, IC_{50},[a] nM		
Compd	R	n	PDE1	PDE3	PDE5
36a		2	1300	>10000	1.0
36d		2	460	8400	0.22
36e		2	1100	>10000	0.39
40		2	>10000	>10000	8.5
41		0	2600	>10000	14

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments, where the variation from the mean value is ±20% or less.

to the differences of physicochemical properties (cell permeability, water solubility) between these compounds.

36a was selected from the results of PDE5 inhibitory activity, PDE isozyme selectivity, and the relaxant effect on the isolated corpus cavernosum for further biological and pharmacological evaluation.

Because PDE6 is a cGMP-specific PDE found in the retina and because inhibition of PDE6 may cause visual disturbances, perception of bluish haze, or increased light sensitivity,[14] we examined the PDE6 inhibitory activity of **36a** to compare with that of sildenafil. **36a** (PDE6, IC_{50} = 28 nM; IC_{50} ratio PDE6/PDE5 = 28)

Table 5. PDE Inhibitions and Relaxant Effects on Isolated Rabbit Corpus Cavernosun

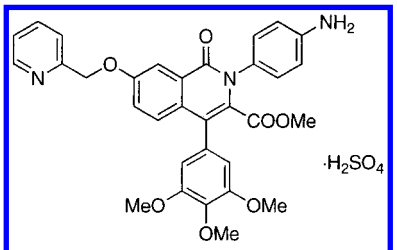
compd	PDE inhibition, IC_{50},[a] nM					relaxant effect EC_{30},[b] nM
	PDE1	PDE2	PDE3	PDE4	PDE5	
26e	2100	>10000	>10000	6100	1.2	130
36a	1300	>10000	>10000	4700	1.0	7.9
36b	3900	>10000	>10000	3600	1.1	9.5
36c	9500	>10000	>10000	7000	3.1	12
36d	460	2800	8400	750	0.22	44
36e	1100	3500	>10000	1700	0.39	37
sildenafil	300	>10000	>10000	8300	3.9	8.7

[a] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments, where the variation from the mean value is ±20% or less. [b] EC_{30} values were determined from the logarithmic concentration−inhibition curve. The value is given as the average of at least two experiments, where the variation from the mean value is ±30% or less.

showed improved selectivity against PDE6 than sildenafil (PDE6, IC_{50} = 29 nM; IC_{50} ratio, PDE6/PDE5 = 7.4).

Conclusion

Novel 1(*2H*)-isoquinolinone derivatives, designed by comparison of the structure of cGMP and that of previously reported 1-arylnaphthalene derivatives, were disclosed as a new structural class of potent and selective PDE5 inhibitors. Among them, **36a** showed the potent and selective PDE5 inhibitory activity (IC_{50} = 1.0 nM; IC_{50} ratio, PDE1/PDE5 = 1300, PDE2/PDE5 > 10 000, PDE3/PDE5 > 10 000, PDE4/PDE5 = 4700, PDE6/PDE5 = 28) and relaxant effects on isolated rabbit corpus cavernosum (EC_{30} = 7.9 nM). In our salt selection study of **36a**, three salts (dihydrochloride, mesylate, and sulfate) were crystallized and evaluated, but on the other hand, carboxylates (acetate, fumarate, citrate) were not crystallized. Dihydrochloride (**36a**) and mesylate were then eliminated because of poor crystallinity. Thus, compound **63** (T-1032), the sulfate form of



63: sulfate form of **36a** (**T-1032**)

36a, was selected for further biological and pharmacological evaluation of erectile dysfunction.

Experimental Section

General. Melting points were determined on a Büchi 535 capillary melting point apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400II analyzer. IR spectra were recorded on a Shimadzu IR-420 spectrophotometer. ^{1}H NMR spectra were obtained on a Bruker AC-200 instrument (200 MHz) with TMS as an internal standard. Mass spectra were recorded on a Hitachi M-2000A spectrometer or Finnigan MAT LC-Q. Column chromatography was performed with silica gel (E. Merck, 70−230 mesh) or NH−silica gel (Fuji silysia chemical, 200−350 mesh). *n*-Butyllithium was the 1.6 M solution in hexane, and *s*-butyllithium was the 1.3 M solution in cyclohexane supplied by Asia Lithium Co.

General Procedure for the Preparation of *o*-Benzoylbenzoic Acid Derivatives 9 (Scheme 1). Compounds **9a**−**c** and **9e** were essentially prepared according to the same procedure. The sequence (**5e** → **6e** → **9e**) is illustrated for **9e**, followed by analytical data for **9a**−**c**.

5-Benzyloxy-6-(3,4,5-trimethoxybenzoyl)benzaldehyde Dimethyl Acetal (5e). To a stirred solution of acetal **4d** (100 g, 0.297 mol) in THF (400 mL) was added *n*-BuLi (204 mL, 0.323 mol) at −78 °C under nitrogen atmosphere, and the mixture was stirred at the same temperature for 15 min. To this mixture was added dropwise *N*,*N*-dimethyl-3,4,5-trimethoxybenzamide (64.5 g, 0.27 mol) in THF (300 mL). The reaction mixture was warmed to −40 °C and stirred for 30 min. The mixture was poured into H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from *i*-Pr_2O gave **5e** (108 g, 81%), mp 230−231 °C. ^{1}H NMR ($CDCl_3$): δ 3.27 (s, 6H), 3.84 (s, 6H), 3.93 (s, 3H), 5.15 (s, 2H), 5.65 (s, 1H), 6.96 (dd, 1H, J = 8.5, 2.6 Hz), 7.05 (s, 2H), 7.25−7.52 (m, 7H). IR (KBr): 1654, 1582, 1128 cm^{-1}. MS (EI): *m*/*z* 452 (M^+), 437, 405, 269, 91 (base).

5-Benzyloxy-6-(3,4,5-trimethoxybenzoyl)benzaldehyde (6e). To a solution of **5e** (108 g, 239 mmol) in THF (600 mL) was added 2 N HCl (50 mL) at room temperature. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **6e** (90.8 g, 94%), mp 112−113 °C. ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 6H), 3.94 (s, 3H), 5.21 (s, 2H), 7.06 (s, 2H), 7.23 (dd, 1H, J = 8.5, 2.6 Hz), 7.30−7.55 (m, 5H), 7.55 (d, 1H, J = 8.5 Hz), 7.64 (d, 1H, J = 2.6 Hz), 10.07 (s, 1H). IR (KBr): 1689, 1623, 1565, 1230, 1130 cm^{-1}. MS (EI): *m*/*z* 406 (M^+), 375, 315, 287, 91 (base).

5-Benzyloxy-6-(3,4,5-trimethoxybenzoyl)benzoic Acid (9e). To a stirred solution of **6e** (76.5 g, 188 mmol) and resorcinol (24.8 g, 226 mmol) in a mixture of dioxane (450 mL) and 0.2 M acetate buffer (pH 3.8, 200 mL) was added $NaClO_2$ (20.4 g, 226 mmol) in H_2O (100 mL) at room temperature. The mixture was stirred at room temperature overnight. After evaporation of the organic solvent, the residue was acidified with 2 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **9e** (46.2 g, 58%), mp 161−162 °C. ^{1}H NMR ($CDCl_3$): δ 3.81 (s, 6H), 3.91 (s, 3H), 5.17 (s, 2H), 7.00 (s, 2H), 7.20 (dd, 1H, J = 8.5, 2.5 Hz), 7.30−7.51 (m, 6H), 7.64 (d, 1H, J = 2.5 Hz). IR (KBr): 2941, 1726, 1580, 1128 cm^{-1}. MS (SIMS): *m*/*z* 423 (M^+ + 1), 405, 91 (base).

3,4-Dimethoxy-6-(3,4,5-trimethoxybenzoyl)benzoic Acid (9a). Yield 67% from **4a**; mp 182−184 °C. ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 6H), 3.92 (s, 3H), 3.92 (s, 3H), 4.00 (s, 3H), 6.83 (s, 1H), 7.00 (s, 2H), 7.55 (s, 1H). IR (KBr): 3400, 1678, 1584, 1125 cm^{-1}. MS (EI): *m*/*z* 376 (M^+, base), 301, 195.

3,4-Diethoxy-6-(3,4,5-trimethoxybenzoyl)benzoic Acid (9b). Yield 67% from **4b**; mp 194−196 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (t, 3H, J = 7.0 Hz), 1.51 (t, 3H, J = 7.0 Hz), 3.81 (s, 6H), 3.91 (s, 3H), 4.12 (q, 2H, J = 7.0 Hz), 4.20 (q, 2H, J = 7.0 Hz), 6.80 (s, 1H), 6.99 (s, 2H), 7.54 (s, 1H). MS (EI): 404 (M^+, base).

3-Methoxy-6-(3,4,5-trimethoxybenzoyl)benzoic Acid (9c). Yield 35% from **4c**; mp 197−199 °C. ^{1}H NMR ($CDCl_3$): δ 3.82 (s, 6H), 3.90 (s, 3H), 3.91 (s, 3H), 7.00 (s, 2H), 7.14 (dd, 1H, J = 8.5, 2.5 Hz), 7.36 (d, 1H, J = 8.5 Hz), 7.54 (d, 1H, J = 2.5 Hz), 7.68−8.18 (br s, 1H). IR (KBr): 2944, 1663, 1582 cm^{-1}. MS (EI): 346 (M^+, base).

5-Methoxy-3-(3,4,5-trimethoxyphenyl)phthalide (8). To a stirred mixture of 4-methoxy-*N*-methylbenzamide **7** (6.1 g, 36.9 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (11.1 mL, 73.8 mmol) in THF (100 mL) was added *s*-BuLi (62.5 mL, 81.3 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred at the same temperature for 30 min. To this mixture was added dropwise 3,4,5-trimethoxybenzaldehyde (9.04 g, 36.9 mmol) in 50 mL of THF. After being stirred at the same temperature for 30 min, the reaction mixture was poured into a mixture of H_2O and AcOEt. The

organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. A solution of the residue in concentrated HCl (5 mL)−dioxane (50 mL) was heated under reflux for 2 h. After evaporation of the organic solvent, the residue was diluted with H_2O and extracted with $CHCl_3$. The organic layer was washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from Et_2O to give **8** (8.42 g, 69%), mp 114−115 °C. ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 6H), 3.84 (s, 3H), 3.86 (s, 3H), 6.23 (s, 1H), 6.47 (s, 2H), 6.75 (d, 1H, J = 2.15 Hz), 7.07 (dd, 1H, J = 8.5, 2.1 Hz), 7.86 (d, 1H, J = 8.5 Hz). IR (KBr): 1756, 1596, 1127 cm^{-1}. MS (EI): 330 (M^+, base), 134.

4-Methoxy-2-(3,4,5-trimethoxybenzoyl)benzoic Acid (9d). To a stirred mixture of **8** (7.40 g, 22.4 mmol) in 25% aqueous KOH (90 mL) and pyridine (45 mL) was added powdered $KMnO_4$ (5.31 g, 33.6 mmol) portionwise at 50 °C, and the mixture was heated under reflux for 5 h. The mixture was filtered, and the residue was washed with H_2O. The filtrate was acidified with concentated HCl and extracted with AcOEt. The organic layer was washed successively with 10% HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from Et_2O to give **9d** (7.38 g, 95%), mp 207−209 °C. ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 6H), 3.74 (s, 3H), 3.85 (s, 3H), 6.92 (d, 1H, J = 2.6 Hz), 6.93 (s, 2H), 7.17 (dd, 1H, J = 8.7, 2.6 Hz), 7.95 (d, 1H, J = 8.7 Hz). IR (KBr): 2944, 1676 cm^{-1}. MS (EI): m/z 346 (M^+, base), 195.

5-Benzyloxy-6-(4-bromo-3,5-dimethoxybenzoyl)benzoic Acid (9f). To a stirred solution of acetal **4d** (20 g, 59.3 mmol) in THF (100 mL) was added *n*-BuLi (39 mL, 62.3 mmol) at −78 °C under nitrogen atmosphere, and the mixture was stirred at the same temperature for 30 min. To this mixture was added dropwise 3-bromo-4,5-dimethoxybenzaldehyde (14.5 g, 59.3 mmol) in THF (100 mL). After being stirred at the same temperature for 30 min, the mixture was poured into H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. A mixture of the residue, MnO_2 (50 g), and toluene (150 mL) was stirred at room temperature overnight. The mixture was filtered, and the residue was washed with $CHCl_3$. The filtrate was concentrated under reduced pressure. Crystallization of the residue from *i*-Pr_2O gave **5f** (17.7 g, 60%) as crude material. To a stirred solution of **5f** in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (0.5 mL) and H_2O (0.5 mL) at room temperature, and the mixture was stirred for 2 h. The reaction mixture was washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a mixture of the residue, resorcinol (5.8 g, 52.7 mmol) in a mixture of dioxane (150 mL), and 0.2 M acetate buffer (pH 3.8, 80 mL) was added $NaClO_2$ (4.76 g, 52.7 mmol) in H_2O (50 mL) at room temperature. The mixture was stirred at room temperature for 30 min. The residue was acidified with 2 N HCl and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from *i*-Pr_2O gave **9f** (10.6 g, 38% from **4d**), mp 179−180 °C. ^{1}H NMR ($CDCl_3$): δ 3.85 (s, 6H), 5.18 (s, 2H), 6.92 (s, 2H), 7.22 (dd, 1H, J = 8.5, 2.6 Hz) 7.31−7.53 (m, 6H), 7.63 (d, 1H, J = 2.5 Hz). IR (KBr): 2943, 1670, 1580, 1234 cm^{-1}. MS (SIMS): m/z 471/473 (M^+ + 1), 91 (base).

5-Benzyloxy-6-(4-methyl-3,5-dimethoxybenzoyl)benzoic Acid (9g). 9g was prepared from **4d** and 3,5-dimethoxy-4-methylbenzaldehyde as described for **9f**. Yield 36%; mp 173−175 °C. ^{1}H NMR ($CDCl_3$): δ 2.12 (s, 3H), 3.77 (s, 6H), 5.17 (s, 2H), 6.91 (s, 2H), 7.20 (dd, 1H, J = 8.5, 2.5 Hz), 7.30−7.50 (m, 6H), 7.63 (d, 1H, J = 2.5 Hz). IR (KBr): 3004, 1698, 1584, 1318, 1141 cm^{-1}. MS (EI): m/z 406 (M^+), 91 (base).

General Procedure for the Preparation of 4-Aryliso-coumarin-3-carboxylic Acids 10 (Scheme 1). Compounds **10a**−**g** were essentially prepared according to the same procedure. The procedure is described for **10a**, followed by analytical data for **10b**−**g**.

6,7-Dimethoxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (10a). A mixture of **9a** (29 g, 77 mmol), K_2CO_3 (23.4 g, 169 mmol), and di-*tert*-butyl bromomalonate (26.0 g, 84.7 mmol) in DMF (250 mL) was stirred at room temperature overnight. The mixture was diluted with AcOEt and washed with H_2O. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To the residue was added 4 N HCl/AcOEt (250 mL), and the mixture was stirred at room temperature overnight and concentrated under reduced pressure. To the residue were added AcOH (100 mL) and dioxane (500 mL), and the mixture was heated under reflux for 6 h. The reaction mixture was cooled to room temperature and the crystals precipitated were collected by filtration and washed with Et_2O to give **10a** (12.8 g, 53%), mp >250 °C. ^{1}H NMR ($CDCl_3$): δ 3.68 (s, 3H), 3.74 (s, 3H), 3.75 (s, 6H), 3.94 (s, 3H), 6.58 (s, 1H), 6.66 (s, 2H), 7.65 (s, 1H), 13.42 (br s, 1H). IR (KBr): 3000, 1733, 1586, 1254 cm^{-1}. MS (SIMS): m/z 417 (M^+), 46 (base).

6,7-Diethoxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (10b). Yield 55%; mp >250 °C. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 0.94 (t, 3H, J = 7.0 Hz), 1.06 (t, 3H, J = 6.9 Hz), 3.39 (s, 6H), 3.45 (s, 3H), 3.49 (q, 2H, J = 7.0 Hz), 3.78 (q, 2H, J = 6.9 Hz), 6.08 (s, 1H), 6.09 (s, 2H), 7.22 (s, 1H). MS (EI): m/z 444 (M^+, base), 371.

7-Methoxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (10c). Yield 62%; mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H), 3.74 (s, 6H), 3.92 (s, 3H), 6.63 (s, 2H), 7.10 (d, 1H, J = 8.9 Hz), 7.47 (dd, 1H, J = 8.9, 2.7 Hz), 7.69 (d, 1H, J = 2.7 Hz). IR (KBr): 2944, 1735 cm^{-1}. MS (EI): m/z 386 (M^+, base), 340, 313.

6-Methoxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (10d). Yield 67%; mp 219−221 °C. ^{1}H NMR (DMSO-d_6): δ 3.74 (s, 3H), 3.75 (s, 6H), 3.78 (s, 3H), 6.52 (d, 1H, J = 2.5 Hz), 6.65 (s, 2H), 7.34 (dd, 1H, J = 8.8, 2.5 Hz), 8.24 (d, 1H, J = 8.8 Hz). IR (KBr): 2946, 1738, 1598 cm^{-1}. MS (EI): m/z 386 (M^+), 330 (base).

6-Benzyloxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (10e). Yield 87%; 236−238 °C. ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 6H), 3.94 (s, 3H), 5.20 (s, 2H), 6.46 (s, 2H), 7.15 (d, 1H, J = 8.9 Hz), 7.35−7.52 (m, 6H), 7.91 (d, 1H, J = 2.6 Hz). IR (KBr): 2942, 1728 cm^{-1}. MS (SIMS): m/z 463 (M^+ + 1), 91 (base).

6-Benzyloxy-4-(4-bromo-3,5-dimethoxyphenyl)-3-isocoumarincarboxylic Acid (10f). Yield 59%; mp >250 °C. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 3.87 (s, 6H), 5.20 (s, 2H), 6.49 (s, 2H), 7.10 (d, 1H, J = 8.9 Hz), 7.28−7.52 (m, 6H), 7.89 (d, 1H, J = 2.7 Hz). IR (KBr): 3088, 1739 cm^{-1}. MS (SIMS): m/z 511/513 (M^+ + 1), 91 (base).

6-Benzyloxy-4-(4-methyl-3,5-dimethoxyphenyl)-3-isocoumarincarboxylic Acid (10g). Yield 86%; mp 245−246 °C. ^{1}H NMR (DMSO-d_6): δ 2.06 (s, 3H), 3.73 (s, 6H), 5.30 (s, 2H), 6.57 (s, 2H), 7.10 (d, 1H, J = 8.9 Hz), 7.30−7.58 (m, 6H), 7.78 (d, 1H, J = 2.7 Hz)). IR (KBr): 2951, 1736, 1583 cm^{-1}. MS (SIMS): m/z 447 (M^+ + 1), 91 (base).

3,4-Dihydro-3-hydroxy-6,7-dimethoxyoxy-4-(3,4,5-trimethoxyphenyl) 3-Isocoumarincarboxylic Acid (11a). To a stirred suspension of **10a** (8.0 g, 19.2 mmol) in THF (160 mL) and MeOH (40 mL) was added dropwise 2 N aqueous NaOH (38.4 mL) at 0 °C, and the mixture was stirred at room temperature for 2 h. To the reaction mixture were added 2 N aqueous HCl (40 mL) and $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from *i*-Pr_2O gave **11a** (6.0 g, 72%), mp 207−208 °C dec. ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H), 3.80 (s, 6H), 3.87 (s, 3H), 3.95 (s, 3H), 4.91 (s, 1H), 6.43 (s, 1H), 6.60 (s, 2H), 7.61 (s, 1H). IR (KBr): 3287, 1723, 1601 cm^{-1}. MS (SIMS): m/z 435 (M^+ + 1), 417, 361, 344, 313, 193 (base).

7-Benzyloxy-3,4-dihydro-3-hydroxy-4-(3,4,5-trimethoxyphenyl)-3-isocoumarincarboxylic Acid (11e). Yield 97%; mp 105−106 °C. ^{1}H NMR ($CDCl_3$): δ 3.78(s, 6H), 3.87 (s, 3H), 4.90 (s, 1H), 5.12 (s, 2H), 6.61 (s, 2H), 6.91 (d, 1H, J = 8.6 Hz), 7.16 (dd, 1H, J = 8.6, 2.7 Hz), 7.30−7.52 (m, 5H), 7.76

(d, 1H, J = 2.7 Hz). IR (KBr): 2942, 1738 cm^{-1}. MS (SIMS): m/z 481 ($M^+ + 1$), 463, 91 (base).

Methyl 1,2-Dihydro-6,7-dimethoxy-2-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (13). A solution of **10a** (15.2 g, 36.5 mmol) in 5 N NH_3−MeOH (350 mL) was stirred at room temperature overnight. After evaporation of the solvent, to the residue was added 4 N HCl/AcOEt (150 mL), and the mixture was stirred at room temperature overnight and concentrated under reduced pressure. The crystals precipitated were collected by filtration and washed with Et_2O to give 1,2-dihydro-6,7-dimethoxy-2-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylic acid (13.8 g, 91%). To a suspension of the acid in MeOH (150 mL) was added H_2SO_4 (30 mL) at room temperature, and the mixture was refluxed for 8 h. The mixture was poured into H_2O and extracted with $CHCl_3$. The organic layer was washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was crystallized from Et_2O to give **13** (12.1 g, 85%), mp 204−207 °C. ^{1}H NMR ($CDCl_3$): δ 3.70 (s, 3H), 3.74 (s, 3H), 3.84 (s, 6H), 3.96 (s, 3H), 4.05 (s, 3H), 6.47 (s, 2H), 6.64 (s, 1H), 7.89 (s, 1H), 9.32 (s, 1H). IR (KBr): 1739, 1657 cm^{-1}. MS (EI): m/z 429 (M^+, base). Anal. ($C_{22}H_{23}NO_8 \cdot 0.5H_2O$) C, H, N.

Methyl 1,2-Dihydro-6,7-dimethoxy-2-methyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (14). Yield 77%; mp 170−171 °C. ^{1}H NMR ($CDCl_3$): δ 3.61 (s, 3H), 3.62 (s, 3H), 3.78 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.03 (s, 3H), 6.57 (s, 2H), 6.72 (s, 1H), 7.89 (s, 1H). IR (KBr): 1738, 1649, 1510, 1229 cm^{-1}. MS (EI): m/z 443 (M^+, base). Anal. ($C_{23}H_{25}NO_8 \cdot 0.5H_2O$) C, H, N.

Methyl 2-Cyclopentyl-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (15). A mixture of **10a** (1.5 g, 3.6 mmol) and cyclopentylamine (5 g, 58.7 mmol) was refluxed for 3 h. The mixture was diluted with $CHCl_3$ and washed with 1 N HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To the residue was added 4 N HCl/AcOEt (150 mL), and the mixture was stirred at room temperature overnight and concentrated under reduced pressure. To a mixture of the residue, K_2CO_3 (995 mg, 7.2 mmol), and DMF (30 mL) was added MeI (0.5 mL, 8.0 mmol) at room temperature, and the mixture was stirred for 30 min. The reaction mixture was diluted with AcOEt and washed with H_2O. The organic layer was washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/AcOEt = 10:1) gave **15** (1.25 g, 70%), mp 132−134 °C. ^{1}H NMR ($CDCl_3$): δ 1.62−1.76 (m, 2H), 1.84−2.29 (m, 4H), 2.40−2.65 (m, 2H), 3.61 (s, 3H), 3.77 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.01 (s, 3H), 4.06−4.23 (m, 1H), 6.58 (s, 2H), 6.65 (s, 1H), 7.86 (s, 1H). IR (KBr): 1738, 1655, 1509, 1221 cm^{-1}. MS (EI): m/z 497 (M^+), 429 (base). Anal. ($C_{27}H_{31}NO_8 \cdot 0.4H_2O$) C, H, N.

Methyl 2-Cyclohexyl-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (16). Yield 24%; mp 165−167 °C. ^{1}H NMR ($CDCl_3$): δ 1.15−1.40 (m, 4H), 1.60−1.75 (m, 1H), 1.78−2.00 (m, 4H), 2.58−2.90 (m, 2H), 3.61 (s, 3H), 3.77 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.01 (s, 3H), 6.56 (s, 2H), 6.64 (s, 1H), 7.87 (s, 1H). IR (KBr): 3422, 2937, 1736, 1657, 1209, 1122 cm^{-1}. MS (EI): m/z 511 (M^+), 429 (base). Anal. ($C_{28}H_{33}NO_8 \cdot 0.2H_2O$) C, H, N.

Methyl 1,2-Dihydro-6,7-dimethoxy-1-oxo-2-piperidino-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (17). A mixture of **10a** (416 mg, 1.0 mmol) and 1-aminopiperidine (0.22 mL, 2.0 mmol) in DMI (3 mL) was stirred at 100 °C overnight. The mixture was diluted with AcOEt and washed with saturated aqueous $NaHCO_3$. The aqueous layer was acidified with 2 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (152 mg, 1.1 mmol), and DMF (5 mL) was added MeI (75 μL, 1.2 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 10:1) gave **17** (100 mg, 20%), mp 125−128 °C. ^{1}H NMR ($CDCl_3$): δ 1.25−1.85 (m, 6H), 3.10−3.23 (m, 2H), 3.72 (s, 3H), 3.75−4.05 (m, 2H), 3.78 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.01 (s, 3H), 6.62 (s, 2H), 6.75 (s, 1H), 7.85 (s, 1H). IR (KBr): 2937, 1745, 1655 cm^{-1}. MS (EI): m/z 512 (M^+), 429 (base). Anal. ($C_{27}H_{32}N_2O_8 \cdot 0.6H_2O$) C, H, N.

Methyl 1,2-Dihydro-6,7-dimethoxy-2-morpholino-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (18). 18 was prepared from **10a** and 4-aminomorpholine as described for **17**. Yield 69%; mp 219−220 °C. ^{1}H NMR ($CDCl_3$): δ 3.00 (d, 2H, J = 9.4 Hz), 3.40−3.62 (m, 2H), 3.73 (s, 3H), 3.79 (s, 3H), 3.85 (s, 6H), 3.94 (s, 3H), 4.02 (s, 3H), 3.87−4.05 (m, 2H), 4.29 (dt, 2H, J = 11, 3.0 Hz), 6.61 (s, 2H), 6.75 (s, 1H), 7.85 (s, 1H). IR (KBr): 1743, 1659 cm^{-1}. MS (EI): m/z 514 (M^+), 429 (base). Anal. ($C_{26}H_{30}N_2O_9 \cdot 0.2H_2O$) C, H, N.

Methyl 1,2-Dihydro-6,7-dimethoxy-1-oxo-2-phenyl-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (19). A mixture of **10a** (1.2 g, 2.88 mmol) and aniline (5 mL) was heated at 130 °C for 8 h. After it was cooled to room temperature, the mixture was poured into 2 N HCl and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (796 mg, 5.76 mmol), and DMF (25 mL) was added MeI (613 mg, 4.32 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$) gave **18** (840 mg, 58%), mp 214−216 °C. ^{1}H NMR ($CDCl_3$): δ 3.20 (s, 3H), 3.81 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.02 (s, 3H), 6.63 (s, 2H), 6.80 (s, 1H), 7.32−7.58 (m, 5H), 7.91 (s, 1H). IR (KBr): 1774, 1661 cm^{-1}. MS (EI): m/z 505 (M^+, base). Anal. ($C_{28}H_{27}NO_8 \cdot 0.5H_2O$) C, H, N.

Methyl 2-Benzyl-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (20). A mixture of **10a** (1.5 g, 3.6 mmol), benzylamine (1.18 mL, 10.8 mmol), and DMI (5 mL) was heated at 120 °C for 20 h. After it was cooled to room temperature, the mixture was diluted with $CHCl_3$ and washed with brine. The organic layer was washed successively with 2 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (995 mg, 7.2 mmol), and DMF (30 mL) was added MeI (1.02 g, 7.2 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 20:1) gave **20** (1.49 g, 80%), mp 182−183 °C. ^{1}H NMR ($CDCl_3$): δ 3.29 (s, 3H), 3.79 (s, 3H), 3.83 (s, 6H), 3.91 (s, 3H), 4.04 (s, 3H), 5.44 (s, 2H), 6.55 (s, 2H), 6.75 (s, 1H), 7.13−7.40 (m, 5H), 7.95 (s, 1H). IR (KBr): 1727, 1647, 1509, 1231 cm^{-1}. MS (EI): m/z 519 (M^+, base), 428. Anal. ($C_{29}H_{29}NO_8 \cdot 0.5H_2O$) C, H, N.

Methyl 1,2-Dihydro-6,7-dimethoxy-2-(4-methoxyphenyl)-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (21). A mixture of **11a** (500 mg, 1.15 mmol), *p*-anisidine (425 mg, 3.45 mmol), and DMI (5 mL) was heated at 110 °C overnight. The mixture was diluted with saturated aqueous $NaHCO_3$ and washed with AcOEt. The aqueous layer was acidified with 2 N HCl and extracted with AcOEt. The organic layer was washed successively with 2 N HCl and brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (191 mg, 1.38 mmol), and DMF (5 mL) was added MeI (196 mg, 1.38 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 20:1) gave **21** (406 mg, 66%), mp 210−211 °C. ^{1}H NMR ($CDCl_3$): δ 3.25 (s, 3H), 3.80 (s, 3H), 3.85 (s, 9H), 3.92 (s, 3H), 4.02 (s, 3H), 6.63 (s, 2H), 6.79 (s, 1H), 6.91−7.03 (m, 2H), 7.22−7.35 (m, 2H), 7.90 (s, 1H). IR (KBr): 1740, 1658,

1510, 1249 cm^{-1}. MS (EI): *m/z* 535 (M^+, base), 525. Anal. ($C_{29}H_{29}NO_9 \cdot 0.2H_2O$) C, H, N.

Methyl 2-(4-Benzyloxyphenyl)-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (22). 22 was prepared from **11a** and (4-benzyloxy)-aniline as described for **21**. Yield 46%; mp 231−233 °C. ^1H NMR ($CDCl_3$): δ 3.23 (s, 3H), 3.81 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.02 (s, 3H), 5.10 (s, 2H), 6.63 (s, 2H), 6.79 (s, 1H), 6.99−7.13 (m, 2H), 7.21−7.52 (m, 7H), 7.90 (s, 1H). IR (KBr): 1740, 1652, 1508, 1241 cm^{-1}. MS (SIMS): *m/z* 612 ($M^+ + 1$, base).

Methyl 1,2-Dihydro-2-(4-hydroxyphenyl)-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (23). A mixture of **22** (300 mg, 0.49 mmol), 10% palladium−carbon (54% H_2O) (50 mg), and DMF (50 mL) was stirred under hydrogen at room temperature overnight. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 5:1) gave **23** (244 mg, 95%), mp 183−185 °C. ^1H NMR ($CDCl_3$): δ 3.24 (s, 3H), 3.82 (s, 3H), 3.85 (s, 6H), 3.92 (s, 3H), 4.04 (s, 3H), 6.62 (s, 2H), 6.71 (d, 2H, J = 8.7 Hz), 6.81 (s, 1H), 7.11 (d, 2H, J = 8.7 Hz), 7.93 (s, 1H). IR (KBr): 3434, 1744, 1653, 1510, 1233 cm^{-1}. MS (SIMS): *m/z* 522 ($M^+ + 1$, base). Anal. ($C_{28}H_{27}NO_9 \cdot 1H_2O$) C, H, N.

Methyl 2-(4-Chlorophenyl)-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (24). 24 was prepared from **10a** and 4-chloroaniline as described for **19**. Yield 39%; mp 194−195 °C. ^1H NMR ($CDCl_3$): δ 3.25 (s, 3H), 3.81 (s, 3H), 3.85 (s, 6H), 3.93 (s, 3H), 4.02 (s, 3H), 6.61 (s, 2H), 6.79 (s, 1H), 7.30−7.54 (m, 4H), 7.89 (s, 1H). IR (KBr): 1744, 1660, 1510, 1216 cm^{-1}. MS (EI): *m/z* 539/541 (M^+, base). Anal. ($C_{28}H_{26}ClNO_8 \cdot 0.5H_2O$) C, H, N.

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (25a). A mixture of **11a** (500 mg, 1.15 mmol), Boc-*p*-phenylenediamine (287 mg, 1.38 mmol), *i*-Pr_2NEt (481μL, 2.76 mmol), and DMI (5 mL) was heated at 110 °C for 8 h. The mixture was diluted with AcOEt and washed with saturated aqueous $NaHCO_3$. The aqueous layer was acidified with citric acid and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (191 mg, 1.38 mmol), and DMF (5 mL) was added MeI (86 μL, 1.38 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 10:1) gave **25a** (350 mg, 49%), mp 240−243 °C. ^1H NMR (DMSO-d_6): δ 1.49 (s, 9H), 3.18 (s, 3H), 3.72 (s, 6H), 3.76 (s, 6H), 3.90 (s, 3H), 6.66 (s, 2H), 6.84 (s, 1H), 7.20 (d, 2H, J = 8.8 Hz), 7.55 (d, 2H, J = 8.8 Hz), 7.70 (s, 1H), 9.60 (s, 1H). IR (KBr): 2952, 1739, 1509, 1238 cm^{-1}. MS (ESI): *m/z* 621 ($M^+ + 1$, base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-6,7-diethoxy-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (25b). 25b was prepared from **11b** and Boc-*p*-phenylenediamine as described for **25a**. Yield 45%; mp 204−206 °C. ^1H NMR ($CDCl_3$): δ 1.43 (t, 3H, J = 7.0 Hz), 1.51 (t, 3H, J = 7.0 Hz), 1.52 (s, 9H), 3.24 (s, 3H), 3.84 (s, 6H), 3.92 (s, 3H), 3.98 (q, 2H, J = 7.0 Hz), 4.24 (q, 2H, J = 7.0 Hz), 6.61 (s, 2H), 6.62 (s, 1H), 6.75 (s, 1H), 7.28 (d, 2H, J = 8.4 Hz), 7.46 (d, 2H, J = 8.7 Hz), 7.88 (s, 1H). IR (KBr): 2981, 1723, 1508, 1234 cm^{-1}. MS (SIMS): *m/z* 649 ($M^+ + 1$), 593, 57 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-methoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (25c). 25c was prepared from **11c** and Boc-*p*-phenylenediamine as described for **25a**. Yield 43%; mp 230−232 °C dec. ^1H NMR ($CDCl_3$): δ 1.52 (s, 9H), 3.25 (s, 3H), 3.84 (s, 6H), 3.91 (s, 3H), 3.94 (s, 3H), 6.59 (s, 2H), 6.63 (s, 1H), 7.20−7.41 (m, 4H), 7.42−7.56 (m, 2H), 7.93 (d, 1H, J = 2.6 Hz). IR (KBr): 1738, 1590, 1161 cm^{-1}. MS (SIMS): *m/z* 591 ($M^+ + 1$), 535 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-6-methoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (25d). 25d was prepared from **11d** and Boc-*p*-phenylenediamine as described for **25a**. Yield 26%; mp 233−235 °C dec. ^1H NMR ($CDCl_3$): δ 1.52 (s, 9H), 3.25 (s, 3H), 3.80 (s, 3H), 3.84 (s, 6H), 3.92 (s, 3H), 6.61 (s, 2H), 6.68 (s, 1H), 6.79 (d, 1H, J = 2.4 Hz), 7.13 (dd, 1H, J = 8.9, 2.4 Hz), 7.28 (d, 2H, J = 8.8 Hz), 7.46 (d, 2H, J = 8.8 Hz), 8.45 (d, 1H, J = 8.9 Hz). IR (KBr): 1739, 1654 cm^{-1}. MS (EI): *m/z* 590 (M^+), 516 (base).

Methyl 7-Benzyloxy-2-[(4-*tert*.-butoxycarbonylamino)-phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (25e). 25e was prepared from **11e** and Boc-*p*-phenylenediamine as described for **25a**. Yield 82%; mp 148−149 °C. ^1H NMR ($CDCl_3$): δ 1.52 (s, 9H), 3.24 (s, 3H), 3.83 (s, 6H), 3.91 (s, 3H), 5.21 (s, 2H), 6.59 (s, 2H), 6.63 (s, 1H), 7.25−7.56 (m, 11H), 8.02 (d, 1H, J = 2.0 Hz). IR (KBr): 2938, 1731, 1644, 1591 cm^{-1}. MS (SIMS): *m/z* 667 ($M^+ + 1$), 611, 519, 91 (base).

Methyl 7-Benzyloxy-4-(4-bromo-3,5-dimethoxyphenyl)-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-3-isoquinolinecarboxylate (25f). 25f was prepared from **11f** and Boc-*p*-phenylenediamine as described for **25a**. Yield 72%; mp 143−145 °C dec. ^1H NMR ($CDCl_3$): δ 1.53 (s, 9H), 3.24 (s, 3H), 3.88 (s, 6H), 5.20 (s, 2H), 6.59 (s, 2H), 6.62 (s, 1H), 7.25−7.55 (m, 11H), 8.01−8.03 (m, 1H). IR (KBr): 2984, 1731, 1644 cm^{-1}. MS (SIMS): *m/z* 715/717 ($M^+ + 1$), 659/661, 91 (base).

Methyl 7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)-phenyl]-4-(3,5-dimethoxy-4-methylphenyl)-1,2-dihydro-1-oxo-3-isoquinolinecarboxylate (25g). 25g was prepared from **11g** and Boc-*p*-phenylenediamine as described for **25a**. Yield 62%; mp 132−134 °C. ^1H NMR ($CDCl_3$): δ 1.52 (s, 9H), 2.13 (s, 3H), 3.23 (s, 3H), 3.79 (s, 6H), 5.20 (s, 2H), 6.54 (s, 2H), 6.64 (s, 1H), 7.25−7.60 (m, 11H), 8.02 (d, 1H, J = 2.4 Hz). IR (KBr): 2950, 1732, 1644 cm^{-1}. MS (SIMS): *m/z* 651 ($M^+ + 1$), 595, 503, 91 (base).

Methyl 2-(4-Aminophenyl)-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (26a). A mixture of **25a** (300 mg, 0.48 mmol), 4 N HCl/AcOEt (10 mL), and $CHCl_3$ (10 mL) was stirred at room temperature overnight. The crystals precipitated were collected by filtration and washed with Et_2O to give **26a** (256 mg, 95%), mp 203−205 °C. ^1H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.73 (s, 6H), 3.76 (s, 6H), 3.91 (s, 3H), 6.66 (s, 2H), 6.85 (s, 1H), 7.28 (d, 2H, J = 8.8 Hz), 7.35 (d, 2H, J = 8.8 Hz), 7.70 (s, 1H). MS (EI): *m/z* 520 (M^+ − HCl, base). Anal. ($C_{28}H_{28}N_2O_8 \cdot 1HCl \cdot 0.4H_2O$) C, H, N.

Methyl 2-(4-Aminophenyl)-6,7-diethoxy-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (26b). 26b was prepared from **25b** as described for **26a**. Yield 95%; mp 222−225 °C dec. ^1H NMR (DMSO-d_6): δ 1.32 (t, 3H, J = 6.9 Hz), 1.39 (t, 3H, J = 6.9 Hz), 3.18 (s, 3H), 3.73 (s, 3H), 3.75 (s, 6H), 3.96 (q, 2H, J = 7.0 Hz), 4.17 (q, 2H, J = 7.0 Hz), 6.65 (s, 2H), 6.81 (s, 1H), 7.24 (d, 2H, J = 8.8 Hz), 7.32 (d, 2H, J = 8.8 Hz), 7.69 (s, 1H). IR (KBr): 2940, 1733, 1584, 1507, 1236 cm^{-1}. MS (EI): *m/z* 548 (M^+ − HCl). Anal. ($C_{30}H_{32}N_2O_8 \cdot 1HCl \cdot 0.5H_2O$) C, H, N.

Methyl 2-(4-Aminophenyl)-1,2-dihydro-7-methoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (26c). 26c was prepared from **25c** as described for **26a**. Yield 94%; mp 213-218 °C dec. ^1H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.72 (s, 3H), 3.75 (s, 6H), 3.90 (s, 3H), 6.62 (s, 2H), 7.15 (d, 2H, J = 8.5 Hz), 7.28 (d, 2H, J = 8.5 Hz), 7.25−7.50 (m, 2H), 7.74 (d, 1H, J = 2.4 Hz). IR (KBr): 3427, 2941, 1737, 1650, 1506 cm^{-1}. MS (SIMS): *m/z* 491 (M^+ − HCl). Anal. ($C_{27}H_{26}N_2O_7 \cdot 1HCl \cdot 0.6H_2O$) C, H, N.

Methyl 2-(4-Aminophenyl)-1,2-dihydro-6-methoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (26d). 26d was prepared from **25d** as described for **26a**. Yield 88%; mp 222−225 °C. ^1H NMR (DMSO-d_6): δ 3.19 (s, 3H), 3.73 (s, 3H), 3.75 (s, 6H), 3.78 (s, 3H), 6.65 (s, 2H), 6.77 (d, 1H, J = 2.4 Hz), 7.06 (d, 2H, J = 8.6 Hz), 7.15−7.38 (m, 3H), 8.27 (d, 1H, J = 8.9 Hz). IR (KBr):

3426, 2943, 1738, 1583, 1237 cm^{-1}. MS (APCI): *m/z* 491 (M^+ − HCl). Anal. ($C_{27}H_{26}N_2O_7$·1HCl·0.5H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-7-benzyloxy-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (26e). 26e was prepared from **25e** as described for **26a**. Yield 88%; mp 207−208 °C dec. ^1H NMR ($CDCl_3$): δ 3.23 (s, 3H), 3.80 (s, 6H), 3.90 (s, 3H), 5.17 (s, 2H), 6.56 (s, 2H), 7.28−7.54 (m, 10H), 7.70−8.00 (m, 2H). IR (KBr): 1738, 1647, 1506, 1126 cm^{-1}. MS (SIMS): *m/z* 567 ($M^+ + 1$ − HCl), 475, 91 (base). Anal. ($C_{33}H_{30}N_2O_7$·1HCl·0.3H_2O) C, H, N.

Methyl 2-[(4-Acetylamino)phenyl]-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (27). To a stirred solution of the free base of **26a** (312 mg, 0.60 mmol) in CH_2Cl_2 (2 mL) was added acetic anhydride (85 μL, 0.90 mmol), and the reaction mixture was stirred at room temperature for 3 h. The mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **27** (270 mg, 80%), mp 235−237 °C. ^1H NMR ($CDCl_3$): δ 2.12 (s, 3H), 3.23 (s, 3H), 3.82 (s, 3H), 3.85 (s, 6H), 3.92 (s, 3H), 4.03 (s, 3H), 6.62 (s, 2H), 6.81 (s, 1H), 7.24 (d, 2H, J = 8.6 Hz), 7.52 (d, 2H, J = 8.6 Hz), 7.90 (s, 1H), 8.27 (s, 1H). IR (KBr): 1740, 1649 cm^{-1}. MS (EI): *m/z* 562 (M^+, base). Anal. ($C_{30}H_{30}N_2O_9$·0.6H_2O) C, H, N.

Methyl 6,7-Dimethoxy-2-[(4-methylamino)phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (28). To a stirred solution of **27** (260 mg, 0.47 mmol) in THF (3 mL) was added NaH (60% dispersion in mineral oil, 28 mg, 0.71 mmol), and the reaction mixture was stirred at room temperature for 30 min. To the mixture was added MeI (59 μL, 0.94 mmol), and the mixture was stirred for 5 h. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. A solution of the residue in MeOH (5 mL)−2 N HCl (5 mL) was heated under reflux overnight. After evaporation of the organic solvent, the residue was diluted with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **28** (170 mg, 68%), mp 239−241 °C. ^1H NMR ($CDCl_3$): δ 2.87 (s, 3H), 3.27 (s, 3H), 3.81 (s, 3H), 3.84 (s, 6H), 3.92 (s, 3H), 4.01 (s, 3H), 6.63 (s, 2H), 6.65−6.85 (m, 3H), 7.19 (d, 2H, J = 8.6 Hz), 7.90 (s, 1H). IR (KBr): 1741, 1653 cm^{-1}. MS (EI): *m/z* 534 (M^+, base). Anal. ($C_{29}H_{30}N_2O_8$·0.4H_2O) C, H, N.

Methyl 2-[(4-Dimethylamino)phenyl]-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (29). 29 was prepared from **11a** and (4-dimethylamino)aniline as described for **21** and isolated as the HCl salt. Yield 17%; mp 226−229 °C. ^1H NMR (DMSO-d_6): δ 2.98 (s, 6H), 3.19 (s, 3H), 3.62−3.83 (m, 9H), 3.90 (s, 3H), 3.91 (s, 3H), 6.66 (s, 2H), 6.80−6.94 (m, 3H), 7.14 (d, 2H, J = 8.8 Hz), 7.70 (s, 1H). IR (KBr): 1741, 1663, 1509, 1216 cm^{-1}. MS (EI): *m/z* 548 (M^+ − HCl, base). Anal. ($C_{30}H_{32}N_2O_8$·1HCl·0.3H_2O) C, H, N.

Methyl 2-(3-Aminophenyl)-1,2-dihydro-6,7-dimethoxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Hydrochloride (31). 31 was prepared from **11a** and Boc-*m*-phenylenediamine as described for **26a**. Yield 24%; mp 238−240 °C dec. ^1H NMR (DMSO-d_6): δ 3.20 (s, 3H), 3.73 (s, 6H), 3.76 (s, 6H), 3.91 (s, 3H), 6.67 (s, 2H), 6.85 (s, 1H), 7.02−7.18 (m, 2H), 7.12−7.26 (m, 1H), 7.46 (dd, 1H, J = 7.9, 7.8 Hz), 7.71 (s, 1H). IR (KBr): 3426, 2948, 1738, 1584, 1510, 1227, 1123 cm^{-1}. MS (APCI): *m/z* 521 ($M^+ + 1$ − HCl, base).

Methyl 2-(4-Aminophenyl)-1,2-dihydro-7-hydroxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (32). To a solution of **34a** (4.84 g, 8.4 mmol) in $CHCl_3$ (20 mL) and MeOH (5 mL) was added 4 N HCl/AcOEt (30 mL), and the mixture was stirred at room temperature for 30 min. The mixture was neutralized with 2 N aqueous NaOH and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **32** (3.87 g, 97%). ^1H NMR (DMSO-d_6): δ 3.19 (s, 3H), 3.72 (s, 3H), 3.74 (s, 6H), 5.35 (s, 2H), 6.58 (d, 2H, J = 8.7 Hz), 6.60 (s, 2H), 6.91 (d, 2H, J = 8.6 Hz), 7.18−7.30 (m, 2H), 7.66 (d, 1H, J = 1.6 Hz), 10.22 (s, 1H). IR (KBr): 3380, 1736, 1647, 1588, 1126 cm^{-1}. MS (SIMS): *m/z* 477 ($M^+ + 1$, base).

Methyl 2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-[2-(2-pyridyl)ethyloxy]-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (33). To a stirred solution of **32** (300 mg, 0.63 mmol) and PPh_3 (249 mg, 0.95 mmol) in THF (10 mL) was added DEAD (150 μL, 0.95 mmol), and the reaction mixture was stirred at room temperature for 2 h. After the reaction mixture was concentrated under reduced pressure, the residue was purified by NH−SiO_2 gel chromatography (AcOEt) to give **33** (171 mg, 47%), mp 182−183 °C. ^1H NMR ($CDCl_3$): δ 3.26 (s, 3H), 3.78 (t, 2H, J = 6.4 Hz), 3.83 (s, 6H), 3.91 (s, 3H), 4.53 (t, 2H, J = 6.4 Hz), 6.58 (s, 2H), 6.63−6.80 (m, 2H), 7.05−7.41 (m, 8H), 7.64−7.80 (m, 1H), 7.95 (d, 1H, J = 2.6 Hz), 8.57 (d, 1H, J = 4.2 Hz). IR (KBr): 3426, 1732, 1656, 1514, 1290, 1125 cm^{-1}. MS (SIMS): *m/z* 582 ($M^+ + 1$), 477, 106 (base). Anal. ($C_{33}H_{31}N_3O_7$·0.1H_2O) C, H, N.

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (34a). A mixture of **25e** (260 mg, 0.39 mmol), 10% palladium−carbon (54% H_2O) (50 mg), THF (30 mL), and MeOH (15 mL) was stirred under hydrogen at room temperature overnight. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **34a** (225 mg, 87%), mp 230−231 °C. ^1H NMR ($CDCl_3$): δ 1.53 (s, 9H), 3.24 (s, 3H), 3.83 (s, 6H), 3.91 (s, 3H), 6.59 (s, 2H), 6.65 (s, 1H), 7.15−7.42 (m, 4H), 7.51 (d, 2H, J = 8.8 Hz), 7.91 (br s, 1H), 8.46 (d, 1H, J = 2.5 Hz). IR (KBr): 3307, 1728, 1692, 1655 cm^{-1}. MS (SIMS): *m/z* 577 ($M^+ + 1$), 521 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (35a). A mixture of **34a** (300 mg, 0.52 mmol), 2-picolyl chloride hydrochloride (94 mg, 0.57 mmol), K_2CO_3 (158 mg, 1.14 mmol), and DMF (5 mL) was stirred at 40 °C overnight. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Crystallization of the residue from Et_2O gave **35a** (250 mg, 72%), mp 136−138 °C. ^1H NMR ($CDCl_3$): δ 1.53 (s, 9H), 3.24 (s, 3H), 3.84 (s, 6H), 3.91 (s, 3H), 5.35 (s, 2H), 6.59 (s, 2H), 6.61 (s, 1H), 7.20−7.62 (m, 8H), 7.75 (dt, 1H, J = 7.7, 1.7 Hz), 8.03 (m, 1H), 8.59−8.69 (m, 1H). IR (KBr): 2940, 1729, 1592 cm^{-1}. MS (SIMS): *m/z* 608 ($M^+ + 1$), 612, 520, 57 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-7-(3-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (35b). 35b was prepared from **34a** and 3-picolyl chloride hydrochloride as described for **35a**. Yield 89%; mp 144−145 °C. ^1H NMR ($CDCl_3$): δ 1.53 (s, 9H), 3.25 (s, 3H), 3.84 (s, 6H), 3.91 (s, 3H), 5.24 (s, 2H), 6.59 (s, 2H), 6.66 (s, 1H), 7.25−7.56 (m, 7H), 7.80−7.91 (m, 1H), 8.01 (d, 1H, J = 2.5 Hz), 8.61 (dd, 1H, J = 4.9, 1.6 Hz), 8.73 (d, 1H, J = 1.7 Hz). IR (KBr): 2940, 1732, 1590 cm^{-1}. MS (SIMS): *m/z* 668 ($M^+ + 1$), 612, 520, 92, 57 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-7-(4-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (35c). 35c was prepared from **34a** and 4-picolyl chloride hydrochloride as described for **35a**. Yield 92%; mp 150−151 °C. ^1H NMR ($CDCl_3$): δ 1.52 (s, 9H), 3.25 (s, 3H), 3.84 (s, 6H), 3.92 (s, 3H), 5.25 (s, 2H), 6.59 (s, 2H), 6.66 (s, 1H), 7.25−7.57 (m, 8H), 7.96 (d, 1H, J = 2.3 Hz), 8.64 (d, 2H, J = 5.7 Hz). IR (KBr): 2939, 1730, 1590, 1510 cm^{-1}. MS (SIMS): *m/z* 668 ($M^+ + 1$), 612, 520, 475, 57 (base).

Methyl 2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Dihydrochloride (36a). 36a was prepared from **35a** as described for **26a**. Yield 98%; mp 207−208 °C dec. ^1H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.73 (s, 3H), 3.75 (s, 6H), 5.52 (s, 2H), 6.63 (s, 2H), 7.35−7.50 (m, 5H), 7.58 (dd, 1H, J =

9.0, 2.8 Hz), 7.63−7.75 (m, 1H), 7.77−7.92 (m, 2H), 8.22 (dt, 1H, J = 7.8, 1.6 Hz), 8.77 (d, 1H, J = 4.5 Hz). IR (KBr): 3418, 1735, 1584 cm^{-1}. MS (EI): m/z 567 (M$^+$ − 2HCl, base), 475. Anal. ($C_{32}H_{29}N_3O_7$·2HCl·2H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-1,2-hydro-1-oxo-7-(3-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Dihydrochloride (36b). 36b was prepared from **35b** as described for **26a**. Yield 44%; mp 207−209 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.73 (s, 3H), 3.75 (s, 6H), 5.49 (s, 2H), 6.63 (s, 2H), 7.20−7.49 (m, 5H), 7.56 (dd, 1H, J = 9.0, 2.7 Hz), 7.89 (d, 1H, J = 2.7 Hz), 7.99 (dd, 1H, J = 8.0, 5.6 Hz), 8.55 (d, 1H, J = 8.1 Hz), 8.82−8.90 (m, 1H), 8.99 (m, 1H). IR (KBr): 3406, 1734, 1657 cm^{-1}. MS (EI): m/z 567 (M$^+$ − 2HCl, base), 490, 475. Anal. ($C_{32}H_{29}N_3O_7$·2HCl·2.4H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(4-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Dihydrochloride (36c). 36c was prepared from **35c** as described for **26a**. Yield 95%; mp 245−248 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.19 (s, 3H), 3.73 (s, 3H), 3.75 (s, 6H), 5.64 (s, 2H), 6.63 (s, 2H), 7.15 (d, 2H, J = 8.6 Hz), 7.28 (d, 2H, J = 8.6 Hz), 7.42 (d, 1H, J = 9.0 Hz), 7.58 (dd, 1H, J = 9.0, 2.8 Hz), 7.85 (d, 1H, J = 2.7 Hz), 8.07 (d, 2H, J = 6.6 Hz), 8.91 (d, 2H, J = 6.6 Hz). IR (KBr): 3426, 2839, 1730, 1650, 1508 cm^{-1}. MS (EI): m/z 567 (M$^+$ − 2HCl), 475, 107 (base). Anal. ($C_{32}H_{29}N_3O_7$·2HCl·0.4H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-4-[(4-bromo-3,5-dimethoxy)phenyl)]-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-3-isoquinolinecarboxylate Dihydrochloride (36d). Yield 51% from **25f**; mp 194−197 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.19 (s, 3H), 3.81 (s, 6H), 5.44 (s, 2H), 6.70 (s, 2H), 7.16 (d, 2H, J = 8.7 Hz), 7.29 (d, 2H, J = 8.7 Hz), 7.38 (d, 1H, J = 8.9 Hz), 7.48−7.62 (m, 2H), 7.70 (d, 1H, J = 7.8 Hz), 7.84 (d, 1H, J = 2.7 Hz), 8.04 (dt, 1H, J = 7.7, 1.7 Hz), 8.68 (d, 1H, J = 4.3 Hz). IR (KBr): 3417, 2839, 1735, 1660 cm^{-1}. MS (SIMS): m/z 616/618 (M$^+$ + 1 − 2HCl, base). Anal. ($C_{31}H_{26}BrN_3O_6$·2HCl·1.4H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-4-[(3,5-dimethoxy-4-methyl)phenyl]-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-1(2*H*)-isoquinolinone-3-carboxylate Dihydrochloride (36e). Yield 51% from **25g**; mp 203−206 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.05 (s, 3H), 3.17 (s, 3H), 3.74 (s, 6H), 5.51 (s, 2H), 6.58 (s, 2H), 7.30−7.48 (m, 5H), 7.56 (dd, 1H, J = 9.0, 2.8 Hz), 7.61−7.72 (m, 1H), 7.77−7.90 (m, 2H), 8.13−8.25 (m, 1H), 8.75 (d, 1H, J = 4.4 Hz). IR (KBr): 3427, 2840, 1736, 1656 cm^{-1}. MS (SIMS): m/z 552 (M$^+$ + 1 − 2HCl, base), 460.

3-Benzyloxy−6-[(4,5-dimethoxy-3-methoxymethoxy)-benzoyl]benzaldehyde Dimethyl Acetal (5h). 5h was prepared from **4d** and *N*,*N*-dimethyl-4,5-dimethoxy-3-(methoxymethoxy)benzamide as described for **5e**. Yield 83%; powder. ^{1}H NMR ($CDCl_3$): δ 3.25 (s, 6H), 3.46 (s, 3H), 3.87 (s, 3H), 3.94 (s, 3H), 5.15 (s, 2H), 5.17 (s, 2H), 5.68 (s, 1H), 6.95 (dd, 1H, J = 8.5, 2.6 Hz), 7.12−7.55 (m, 9H). MS (EI): m/z 482 (M$^+$), 467, 451, 435, 403, 281, 269, 91 (base).

Methoxymethyl 7-Benzyloxy-4-[(4,5-dimethoxy-3-methoxymethoxy)benzoyl]-3-isocoumarincarboxylate (37). To a solution of **5h** (9.60 g, 19.9 mmol) in THF (80 mL) was added 2 N HCl (20 mL) at room temperature. The mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and washed with brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/hexane/AcOEt = 5:5:1) gave **6h** (5.66 g, 65%). To a stirred solution of **6h** (5.66 g, 13.0 mmol) and resorcinol (2.14 g, 19.5 mmol) in a mixture of dioxane (60 mL) and 0.2 M acetate buffer (pH 3.8, 50 mL) was added $NaClO_2$ (1.76 g, 19.5 mmol) in H_2O (20 mL) at room temperature. The mixture was stirred at room temperature overnight. After evaporation of the organic solvent, the residue was acidified with 2 N HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a solution of the residue in DMF (30 mL) was added K_2CO_3 (5.37 g, 38.9 mmol) and di-*tert*-butyl bromomalonate (5.75 g, 19.5 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. A mixture of the residue and 4 N HCl/AcOEt was stirred at room temperature overnight and concentrated under reduced pressure. To the concentrate were added AcOH (30 mL) and dioxane (50 mL), and the mixture was heated under reflux for 4 h and concentrated under reduced pressure. To a mixture of the residue and DMF (50 mL) were added chloromethyl methyl ether (4.0 mL, 52.7 mmol) and *i*-Pr_2NEt (9.3 mL, 53.4 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/hexane/AcOEt = 5:5:1) gave **37** (1.82 g, 17% from **5h**), mp 131−133 °C. ^{1}H NMR ($CDCl_3$): δ 3.23 (s, 3H), 3.49 (s, 3H), 3.84 (s, 3H), 3.94 (s, 3H), 5.20 (s, 4H), 5.26, 5.27 (ABq, 2H, J = 5.9 Hz), 6.53 (d, 1H, J = 1.8 Hz), 6.72 (d, 1H, J = 1.8 Hz), 7.16 (d, 1H, J = 8.9 Hz), 7.32−7.52 (m, 6H), 7.92 (d, 1H, J = 2.7 Hz). IR (KBr): 1736, 1605 cm^{-1}. MS (EI): m/z 536 (M$^+$), 91 (base).

Methyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-7-benzyloxy-4-[(4,5-dimethoxy-3-methoxymethyl)phenyl]-1,2-dihydro-1-oxo-3-isoquinolinecarboxylate (38). To a stirred solution of **37** (1.82 g, 3.39 mmol) in THF (15 mL) and MeOH (5 mL) was added dropwise 2 N aqueous NaOH (3.39 mL) at 0 °C. The mixture was stirred at room temperature for 0.5 h, and 2 N HCl (3.39 mL) was added. After evaporation of the organic solvent, the residue was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. A mixture of the residue, Boc-*p*-phenylenediamine (2.12 g, 10.2 mmol), and DMI (10 mL) was heated at 100 °C for 2 h. The mixture was diluted with AcOEt and washed with saturated aqueous $NaHCO_3$. The aqueous layer was acidified with aqueous saturated citric acid and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred mixture of the residue, K_2CO_3 (514 mg, 3.72 mmol), and DMF (10 mL) was added MeI (250 μL, 4.4 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was diluted with AcOEt and washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/hexane/AcOEt = 5:5:2) gave **38** (540 mg, 23%), mp 142−144 °C. ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 3.25 (s, 3H), 3.49 (s, 3H), 3.84 (s, 3H), 3.93 (s, 3H), 5.19 (s, 2H), 5.20 (s, 2H), 6.61 (s, 1H), 6.64 (d, 1H, J = 1.9 Hz), 6.80 (d, 1H, J = 1.9 Hz), 7.25−7.52 (m, 11H), 8.02 (d, 1H, J = 2.4 Hz). IR (KBr): 1728, 1645, 1499, 1159 cm^{-1}. MS (SIMS): m/z 697 (M$^+$ + 1, base), 641, 91.

Methyl 2-(4-Aminophenyl)-1,2-dihydro-4-[3-hydroxy-4,5-dimethoxy]phenyl]-1-oxo-7-(2-pyridylmethoxy)-3-isoquinolinecarboxylate Dihydrochloride (40). A mixture of **38** (540 mg, 0.78 mmol), 10% palladium−carbon (54% H_2O) (200 mg), THF (5 mL), and MeOH (3 mL) was stirred under hydrogen (30 psi) at room temperature for 2 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The mixture of the residue, K_2CO_3 (235 mg, 1.7 mmol), 2-picolyl chloride hydrochloride (140 mg, 0.85 mmol), and DMF (5 mL) was stirred at 50 °C overnight. The mixture was poured into H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a stirred solution of the obtained residue in $CHCl_3$ (5 mL) and MeOH (10 mL) was added 4 N HCl/AcOEt (2 mL), and the mixture was stirred at 40 °C for 3 h and concentrated under reduced pressure. Crystallization of the residue from AcOEt gave **40** (230 mg, 47%), mp 196−199 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.73 (s, 6H), 5.49 (s, 2H), 6.42 (d, 1H, J = 1.9 Hz), 6.46 (d, 1H, J = 1.9 Hz), 7.25−7.48 (m, 5H), 7.51−7.70 (m, 2H), 7.75−7.89 (m, 2H), 8.16 (m, 1H), 8.74 (m, 1H). IR (KBr): 3406, 1733, 1653 cm^{-1}. MS (SIMS): m/z 554 (M$^+$ + 1 − 2HCl, base).

Methyl 2-(4-Aminophenyl)-1,2-dihydro-4-[(4-hydroxy-3,5-dimethoxy)phenyl]-1-oxo-7-(2-pyridylmethoxy)-3-isoquinolinecarboxylate (41). A mixture of **36a** (1.92 g, 3.0 mmol), concentrated HCl (30 mL), and dioxane (30 mL) was heated under reflux overnight. The reaction mixture was neutralized with 2 N aqueous NaOH and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by silica gel chromatography ($CHCl_3$/acetone = 2:1) gave **41** (920 mg, 55%), mp 202−205 °C. ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 3H), 3.87 (s, 6H), 5.39 (s, 2H), 6.60 (s, 2H), 6.74 (d, 2H, J = 8.6 Hz), 7.14 (d, 2H, J = 8.6 Hz), 7.25−7.45 (m, 3H), 7.52−7.64 (m, 1H), 7.80 (m, 1H), 8.03 (m, 1H), 8.48−8.70 (m, 1H). MS (EIMS): m/z 554 (M^+ + 1, base).

7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylic Acid (42). A mixture of **11e** (1.5 g, 3.12 mmol), Boc-*p*-phenylenediamine (780 mg, 3.74 mmol), *i*-Pr_2-NEt (1.3 mL, 7.49 mmol), and DMI (10 mL) was heated at 100 °C overnight. The mixture was diluted with AcOEt and washed with aqueous saturated citric acid. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crystallization of the residue from *i*-Pr_2O gave **42** (1.51 g, 74%), mp 151−153 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 3.72 (s, 3H), 3.74 (s, 6H), 5.26 (s, 2H), 6.66 (s, 2H), 7.17−7.62 (m, 11H), 7.80 (d, 1H, J = 2.6 Hz), 9.57 (s, 1H), 13.28 (br s, 1H). IR (KBr): 3258, 1723, 1645, 1235, 1159 cm^{-1}. MS (ESI): m/z 653 (M^+ + 1).

Ethyl 7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)-phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (43). 43 was prepared from **11e** and Boc-*p*-phenylenediamine as described for **25a**, using EtI instead of MeI. Yield 61%; mp 183−185 °C. ^{1}H NMR ($CDCl_3$): δ 0.79 (t, 3H, J = 7.1 Hz), 1.53 (s, 9H), 3.71 (q, 2H, J = 7.1 Hz), 3.83 (s, 6H), 3.90 (s, 3H), 5.20 (s, 2H), 6.60 (s, 3H), 7.26−7.57 (m, 11H), 8.02 (d, 1H, J = 1.6 Hz). IR (KBr): 1732, 1668, 1161 cm^{-1}. MS (ESI): m/z 681 (M^+ + 1).

Methoxymethyl 7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (44). 44 was prepared from **11e** and Boc-*p*-phenylenediamine as described for **25a**, using chloromethyl methyl ether instead of MeI. Yield 62%; mp 151−154 °C. ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 2.99 (s, 3H), 3.84 (s, 6), 3.89 (s, 3H), 4.77 (s, 2H), 5.21 (s, 2H), 6.63 (s, 3H), 7.32−7.58 (m, 11H), 8.03 (d, 1H, J = 1.7 Hz). IR (KBr): 1722, 1668, 1516, 1162 cm^{-1}. MS (SIMS): m/z 697 (M^+ + 1), 641, 91 (base).

7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (45). To a mixture of **42** (500 mg, 0.77 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) (176 mg, 0.92 mmol), and 1-hydroxybenzotriazole hydrate (HOBt·H_2O) (129 mmol, 0.84 mmol) in DMF (5 mL) was added 28% aqueous NH_3 (0.5 mL), and the mixture was stirred at room temperature for 3 h. The mixture was poured into H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crystallization of the residue from AcOEt gave **45** (373 mg, 75%), mp 242−246 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 3.72 (s, 3H), 3.74 (s, 6H), 5.25 (s, 2H), 6.69 (s, 2H), 7.15−7.58 (m, 12H), 7.75 (s, 1H), 7.79 (d, 1H, J = 2.7 Hz), 9.52 (s, 1H). IR (KBr): 3438, 1646, 1500, 1237 cm^{-1}. MS (SIMS): m/z 652 (M^+ + 1), 596, 91 (base).

7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-*N*-methyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (46). 46 was prepared from **42** and methylamine as described for **45**. Yield 76%; mp 206−208 °C. ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 2.34 (d, 3H, J = 3.5 Hz), 3.84 (s, 6H), 3.91 (s, 3H), 5.19 (s, 2H), 5.35 (q, 1H, J = 3.5 Hz), 6.62 (s, 3H), 7.12−7.53 (m, 11H), 8.01 (d, 1H, J = 2.5 Hz). IR (KBr): 3484, 1647, 1238 cm^{-1}. MS (SIMS): m/z 666 (M^+ + 1), 610, 518, 91 (base).

7-Benzyloxy-2-[(4-*tert*-butoxycarbonylamino)phenyl]-1,2-dihydro-*N*,*N*-dimethyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (47). 47 was prepared from **42** and dimethylamine as described for **45**. Yield 76%; mp 171−172 °C. ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 2.42 (s, 3H), 2.74 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.91 (s, 3H), 5.20 (s, 2H), 6.54 (d, 1H, J = 1.8 Hz), 6.62 (s, 1H), 6.77 (d, 1H, J = 1.8 Hz), 7.05−7.54 (m, 10H), 7.70 (d, 1H, J = 8.0 Hz), 8.02 (d, 1H, J = 2.6 Hz). IR (KBr): 3484, 1725, 1646, 1497, 1238 cm^{-1}. MS (SIMS): m/z 680 (M^+ + 1, base), 624, 579, 532, 91.

Ethyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (48). 48 was prepared from **43** as described for **34a**. Yield 90%; mp 222−223 °C dec. ^{1}H NMR ($CDCl_3$): δ 0.78 (t, 3H, J = 7.1 Hz), 1.53 (s, 9H), 3.70 (q, 2H, J = 7.1 Hz), 3.83 (s, 6H), 3.90 (s, 3H), 6.60 (s, 2H), 6.62 (s, 1H), 7.12−7.42 (m, 4H), 7.42−7.60 (m, 2H), 8.42 (d, 1H, J = 2.5 Hz). IR (KBr): 3357, 1729, 1647, 1505, 1232 cm^{-1}. MS (SIMS): m/z 591 (M^+ + 1, base), 535.

Methoxymethyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (49). 49 was prepared from **44** as described for **34a**. Yield 89%; powder. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 2.90 (s, 3H), 3.70 (s, 3H), 3.74 (s, 6H), 4.74 (s, 2H), 6.64 (s, 2H), 7.15−7.32 (m, 4H), 7.54 (d, 2H, J = 8.8 Hz), 7.66 (s, 1H), 9.57 (s, 1H), 10.29 (s, 1H). IR (KBr): 3319, 1732, 1648, 1506, 1161 cm^{-1}. MS (SIMS): m/z 607 (M^+ + 1, base), 551, 489.

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (50). 50 was prepared from **45** as described for **34a**. Yield 83%; mp >250 °C. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 3.71 (s, 3H), 3.74 (s, 6H), 6.67 (s, 2H), 7.08−7.31 (m, 5H), 7.49 (d, 2H, J = 8.8 Hz), 7.63 (d, 1H, J = 2.4 Hz), 7.72 (s, 1H), 9.52 (s, 1H), 10.10 (s, 1H). IR (KBr): 3384, 1675, 1644, 1239 cm^{-1}. MS (SIMS): m/z 562 (M^+ + 1, base), 506, 489.

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-*N*-methyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (51). 51 was prepared from **46** as described for **34a**. Yield 97%; mp 223−225 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 2.12 (d, 3H, J = 4.6 Hz), 3.71 (s, 3H), 3.73 (s, 6H), 6.64 (s, 2H), 7.11−7.25 (m, 4H), 7.49 (d, 2H, J = 8.8 Hz), 7.63 (s, 1H), 8.21 (q, 1H, J = 4.6 Hz), 9.52 (s, 1H), 10.13 (s, 1H). IR (KBr): 3355, 1643, 1239 cm^{-1}. MS (SIMS): m/z 576 (M^+ + 1), 520 (base), 489.

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-7-hydroxy-*N*,*N*-dimethyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (52). 52 was prepared from **47** as described for **34a**. Yield 81%; mp 217−220 °C dec. ^{1}H NMR (DMSO-d_6): 1.49 (s, 9H),2.28 (s, 3H), 2.74 (s, 3H), 3.71 (s, 6H), 3.76 (s, 3H), 6.59 (m, 1H), 6.65 (m, 1H), 7.10−7.32 (m, 4H), 7.40−7.70 (m, 3H), 9.54 (s, 1H), 10.14 (s, 1H). IR (KBr): 3361, 1640 cm^{-1}. MS (SIMS): m/z 590 (M^+ + 1, base), 534, 489.

Ethyl 2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate (53). 53 was prepared from **48** as described for **35a**. Yield 86%; mp 210−212 °C dec. ^{1}H NMR ($CDCl_3$): δ 0.78 (t, 3H, J = 7.1 Hz), 1.52 (s, 9H), 3.71 (q, 2H, J = 7.1 Hz), 3.84 (s, 6H), 3.90 (s, 3H), 5.35 (s, 2H), 6.60 (s, 2H), 6.62 (s, 1H), 7.21−7.62 (m, 8H), 7.76 (dt, 1H, J = 7.7, 1.7 Hz), 8.03 (s, 1H), 8.63 (d, 1H, J = 4.2 Hz). IR (KBr): 3329, 2975, 1727, 1667 cm^{-1}. MS (SIMS): m/z 682 (M^+ + 1, base), 628, 534.

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (55). 55 was prepared from **50** as described for **35a**. Yield 63%; mp 236−238 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 3.72 (s, 3H), 3.74 (s, 6H), 5.32 (s, 2H), 6.69 (s, 2H), 7.15−7.52 (m, 9H), 7.69−7.82 (m, 2H), 7.84 (dt, 1H, J = 7.7, 1.8 Hz), 8.59 (d, 1H, J = 4.1 Hz), 9.53 (s, 1H). IR (KBr): 1680, 1650, 1508, 1238 cm^{-1}. MS (SIMS): m/z 653 (M^+ + 1, base), 597.

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-*N*-methyl-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (56). 56 was prepared from **51** as described for **35a**. Yield 89%; mp 203−206

°C. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 2.13 (d, 3H, J = 4.6 Hz), 3.71 (s, 3H), 3.74 (s, 6H), 5.32 (s, 2H), 6.65 (s, 2H), 7.18 (d, 2H, J = 8.8 Hz), 7.28 (d, 1H, J = 8.9 Hz), 7.30−7.42 (m, 1H), 7.42−7.60 (m, 4H), 7.78 (d, 1H, J = 2.7 Hz), 7.85 (dt, 1H, J = 7.7, 1.8 Hz), 8.25 (q, 1H, J = 4.6 Hz), 8.56−8.62 (m, 1H), 9.53 (s, 1H). IR (KBr): 3381, 1722, 1660, 1240 cm^{-1}. MS (SIMS): m/z 667 (M^+ + 1), 611, 518, 57 (base).

2-[(4-*tert*-Butoxycarbonylamino)phenyl]-1,2-dihydro-*N*,*N*-dimethyl-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide (57). 57 was prepared from **52** as described for **35a**. Yield 63%; mp 159−161 °C. ^{1}H NMR (DMSO-d_6): δ 1.49 (s, 9H), 2.28 (s, 3H), 2.74 (s, 3H), 3.71 (s, 3H), 3.72 (s, 3H), 3.76 (s, 3H), 5.33 (s, 2H), 6.60 (d, 1H, J = 1.6 Hz), 6.66 (d, 1H, J = 1.6 Hz), 7.10−7.27 (m, 2H), 7.27−7.62 (m, 6H), 7.78 (d, 1H, J = 2.7 Hz), 7.84 (dt, 1H, J = 7.7, 1.8 Hz), 8.56−8.62 (m, 1H), 9.55 (s, 1H). IR (KBr): 3409, 2935, 1724, 1649 cm^{-1}. MS (SIMS): m/z 681 (M^+ + 1), 625, 532, 92, 72 (base), 57.

Ethyl 2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Dihydrochloride (58). 58 was prepared from **53** as described for **26a**. Yield 89%; mp 206−209 °C. ^{1}H NMR (DMSO-d_6): δ 0.67 (t, 3H, J = 7.1 Hz), 3.62 (q, 2H, J = 7.1 Hz), 3.71 (s, 3H), 3.75 (s, 6H), 5.49 (s, 2H), 6.63 (s, 2H), 7.28−7.46 (m, 5H), 7.50−7.69 (m, 2H), 7.73−7.89 (m, 2H), 8.15 (dt, 1H, J = 7.7, 1.6 Hz), 8.73 (d, 1H, J = 4.4 Hz). IR (KBr): 3414, 2834, 1722, 1582, 1507 cm^{-1}. MS (SIMS): m/z 582 (M^+ + 1 − 2HCl, base). Anal. ($C_{33}H_{31}N_3O_7$·2HCl·2H_2O) C, H, N.

2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylic Acid Dihydrochloride (59). A mixture of **49** (177 mg, 0.29 mmol), 2-picolyl chloride hydrochloride (50 mg, 0.31 mmol), K_2CO_3 (85 mg, 0.61 mmol), and DMF (5 mL) was stirred at 50 °C overnight. The mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. To a solution of the residue in THF (5 mL) was added 6 N aqueous HCl, and the mixture was stirred at room temperature overnight. After the mixture was concentrated under reduced pressure, crystallization of the residue from AcOEt gave **59** (162 mg, 89%) as a powder. ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H), 3.75 (s, 6H), 5.54 (s, 2H), 6.69 (s, 2H), 7.30−7.98 (m, 9H), 8.18−8.37 (m, 1H), 8.79 (d, 1H, J = 4.4 Hz). IR (KBr): 3426, 1731, 1644 cm^{-1}. MS (SIMS): m/z 554 (M^+ + 1 − 2HCl, base). Anal. ($C_{31}H_{27}N_3O_7$·2HCl·3H_2O) C, H, N.

2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide Dihydrochloride (60). 60 was prepared from **55** as described for **26a**. Yield 80%; mp 230−232 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.72 (s, 3H), 3.74 (s, 6H), 5.46 (s, 2H), 6.70 (s, 2H), 7.20−7.70 (m, 8H), 7.71−7.82 (m, 2H), 7.89 (s, 1H), 8.14 (dt, 1H, J = 7.7, 1.6 Hz), 8.73 (d, 1H, J = 4.4 Hz). IR (KBr): 3416, 1643, 1509 cm^{-1}. MS (SIMS): m/z 553 (M^+ + 1 − 2HCl, base). Anal. ($C_{31}H_{28}N_4O_6$·2HCl·6H_2O) C, H, N.

2-(4-Aminophenyl)-1,2-dihydro-*N*-methyl-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide Dihydrochloride (61). 61 was prepared from **56** as described for **26a**. Yield 86%; powder. ^{1}H NMR (DMSO-d_6): δ 2.13 (d, 3H, J = 4.5 Hz), 3.72 (s, 3H), 3.74 (s, 6H), 5.52 (s, 2H), 6.69 (s, 2H), 7.33 (d, 1H, J = 9.0 Hz), 7.40−7.61 (m, 5H), 7.72 (t, 1H, J = 6.4 Hz), 7.83 (d, 1H, J = 2.7 Hz), 7.88 (d, 1H, J = 7.9 Hz), 8.26 (dt, 1H, J = 7.7, 1.6 Hz), 8.45−8.58 (m, 1H), 8.78 (d, 1H, J = 4.6 Hz). IR (KBr): 3427, 1646 cm^{-1}. MS (SIMS): m/z 567 (M^+ + 1 − 2HCl, base). Anal. ($C_{32}H_{30}N_4O_6$·2HCl·6H_2O) C, H, N.

2-(4-Aminophenyl)-1,2-dihydro-*N*,*N*-dimethyl-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxamide Dihydrochloride (62). 62 was prepared from **57** as described for **26a**. Yield 98%; mp 195−200 °C. ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H), 2.76 (s, 3H), 3.71 (s, 3H), 3.72 (s, 3H), 3.77 (s, 3H), 5.45 (s, 2H), 6.60 (d, 1H, J = 1.7 Hz), 6.68 (d, 1H, J = 1.7 Hz), 7.25−7.45 (m, 5H), 7.45−7.65 (m, 2H), 7.74 (d, 1H, J = 7.9 Hz), 7.81 (d, 1H, J = 2.7 Hz), 8.10 (dt, 1H, J = 7.7, 1.6 Hz), 8.71 (d, 1H, J = 4.3 Hz). IR (KBr): 3432, 1634 cm^{-1}. MS (SIMS): m/z 581 (M^+ + 1 − 2HCl), 72 (base). Anal. ($C_{33}H_{32}N_4O_6$·2HCl·4H_2O) C, H, N.

Methyl 2-(4-Aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinolinecarboxylate Sulfate (63). To a stirred solution of the free base of **36a** (30.1 g, 53 mmol) in EtOH (870 mL) was added 2 N aqueous H_2SO_4 (52.9 mL, 106 mmol) at 80 °C. The mixture was stirred at room temperature overnight, and the crystals precipitated were collected by filtration and washed with Et_2O to give **63** (31.4 g, 89%), mp 221−223 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.18 (s, 3H), 3.72 (s, 3H), 3.75 (s, 6H), 5.44 (s, 2H), 6.63 (s, 2H), 7.12 (d, 2H, J = 8.7 Hz), 7.28 (d, 2H, J = 8.7 Hz), 7.40 (d, 1H, J = 8.9 Hz), 7.51−7.65 (m, 2H), 7.71 (d, 1H, J = 7.8 Hz), 7.83 (d, 1H, J = 2.7 Hz), 8.06 (dt, 1H, J = 7.7, 1.7 Hz), 8.69 (d, 1H, J = 4.3 Hz). IR (KBr): 2839, 1742, 1660 cm^{-1}. MS (SIMS): m/z 568 (M^+ + 1 − H_2SO_4, base).

Assay of Phosphodiesterase Activity. PDE activity was determined by a modification of the method of Thompson et al.[15] The assay buffer contained 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 0.33 mg/mL of BSA (Sigma), unlabeled cGMP or cAMP, and 12.5 nM [^{3}H]cGMP or 4.88 nM [^{3}H]cAMP. The reaction was started by mixing the substrate into 500 μL of assay buffer, and tubes were incubated at 37 °C for 30 min. After boiling for 1.5 min, the mixtures were added to 100 μL of 1 mg/mL solution of *Crotalus atrox* snake venom and incubated at 37 °C for 30 min. The reaction was stopped by the addition of 500 μL of methanol, and the resultant solutions were applied to a Dowex (1 × 8-400) column (volume 0.25 mL). Aqueous scintillation fluid was added to each eluate, and the radioactivity was measured.

Relaxant Effect in the Isolated Rabbit Corpus Cavernosum. The initial resting isometric tension of each tissue strip was adjusted to 1.5 g by gradual incremental stretching in 10 mL of organ bath chambers containing physiological salt solution (PSS) at 37 ± 0.5 °C, continuously aerated with 95% O_2 and 5% CO_2. The contractile response to high-KCl (120 mM) PSS was checked twice. Phenylephrine (PE) (5×10^{-6} M) was added into each organ bath chamber in order to obtain a tonic contraction. After the PE contractile response was stabilized, test compound (10^{-10}−10^{-6} M) or vehicle was added to the preparation at an interval of 30 min. Papaverine hydrochloride was added into each organ bath chamber (final concentration, 10^{-4} M) to confirm the maximal relaxation of the tissue strips at the end of experiment. The composition of PSS was as follows (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.5, KH_2PO_4 1.2, $NaHCO_3$ 25.0, dextrose 11.0, EDTA−2Na 0.023 (pH 7.3 or 7.4).

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