Potent, Orally Active Heterocycle-Based Combretastatin A-4 Analogues: Synthesis, Structure−Activity Relationship, Pharmacokinetics, and In Vivo Antitumor Activity Evaluation

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The synthesis and structure−activity relationship study of a series of compounds with heterocycles in place of the cis double bond in combretastatin A-4 (CA-4) are described. Substituted tosylmethyl isocyanides were found to be the key intermediates in construction of the heterocycles. Cytotoxicities of the heterocycle-based CA-4 analogues were evaluated against NCI-H460 and HCT-15 cancer cell lines. 3-Amino-4-methoxyphenyl and *N*-methyl-indol-5-yl were the best replacements for the 3-hydroxy-4-methoxyphenyl in CA-4. 4,5-Disubstituted imidazole was found to be the best for the replacement of the cis double bond in CA-4. Medicinal chemistry efforts led to the discovery of compounds **24h** and **25f** that were found to be 32 and 82% bioavailable, respectively, in rat. Evaluation of **24h** and **25f** against murine M5076 reticulum sarcoma in mice revealed that both compounds were orally efficacious with an increase in life span of 38.5 and 40.5%, respectively.

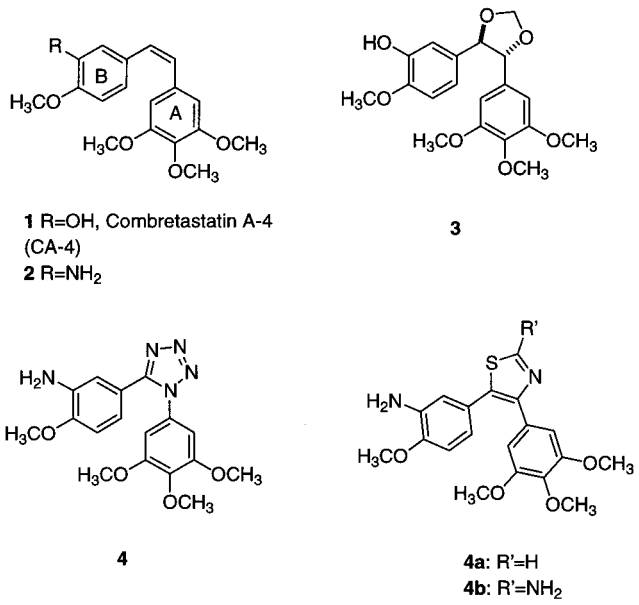
Introduction

Antimitotic agents, one of the major classes of cytotoxic drugs for cancer treatment, have commanded increasing attention recently, in part, due to the clinical success of taxol.[1] By interfering with the normal microtubule polymerization/depolymerization process, antimitotic agents cause mitotic arrest of eukaryotic cells. Microtubules are the dynamic pipelike protein fibers consisting of alternating α- and β-tubulins. The mitotic spindle, which is closely involved in cell replication, is made up of these microtubules.

There are at least three major binding regions on the microtubules for antimitotic agents. They are the taxane binding site, the vinca alkaloid binding site, and the colchicine binding site. Taxol, for example, stabilizes microtubules and prevents tubulin depolymerization, whereas vincristine interacts with tubulins to inhibit their polymerization. Although antimitotic drugs such as vincristine and paclitaxel have gained wide clinical use for the treatment of various cancers, they suffer from undesired side effects, difficulty in the dosing schedule, and lack of efficacy against multidrug resistance (MDR+) cancer cell lines. It is critical to discover novel antitumor agents with fewer side effects, improved pharmacokinetic properties, and better efficacy against MDR cancer cell lines.

Combretastatin A-4 (CA-4, **1**; Chart 1), a natural product isolated from the South African tree, *Combretum caffrum*, exhibits strong antitubulin activity by binding to the colchicine binding site of tubulin.[2] The IC_{50} of CA-4 against tubulin polymerization was found to range from 0.53 to 3.0 μM.[3] It exhibits potent cytotoxicity against a broad spectrum of human cancer lines including those that are MDR positive.[4] CA-4 is not a substrate of the MDR pump, a cellular pump which rapidly transports out foreign molecules, including many anticancer drugs. This is believed to be the major reason for its superior activity against MDR positive cancer cell lines.[5] The differential expression of various tubulin isotypes may contribute as well.[6]

Chart 1



CA-4 does not show in vivo efficacy against murine colon 26 adenocarinoma, in part, due to its poor pharmacokinetics resulting from its high lipophilicity and low aqueous solubility.[7] The cis double bond in CA-4 poses another liability. The double bond is prone to

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isomerize to the more thermally stable trans isomer, resulting in complete loss of cytotoxicity as shown in our lab as well as by other researchers.[8]

Because of its structural simplicity and potent cytotoxicity, CA-4 is a very attractive lead compound. Considerable effort has gone into modifying CA-4 to improve its in vivo efficacy. One example is the water-soluble disodium phosphate of CA-4.[8b,9] This prodrug showed potent antivascular activity. Ohsumi and co-workers reported that replacement of the phenolic hydroxyl group in CA-4 with an amino group (**2**) resulted in a marked increase in water solubility and in vivo antitumor activity against murine solid tumors when **2** was administered intraveously.[7] Shirai and co-workers have reported that the cis carbon−carbon double bond in CA-4 could be replaced by a dioxolane (**3**).[10] Ohsumi also demonstrated that a tetrazole (**4**) or a thiazole (**4a** or **b**) ring could replace the cis double bond to maintain potent cytotoxicity. All three compounds (**4** and **4a**,**b)** showed excellent antitumor activities against the colon 26 murine tumor when given intraveously.[11] Medarde and co-workers reported the synthesis and cytotoxic evaluation of indole-bridged combretastatin analogues.[12] Recently, Gwaltney and co-workers in our lab have shown that the cis double bond in CA-4 could also be replaced with a sulfonate to result in potent antitubulin agents.[13]

In our efforts to discover orally active antimitotic agents, we utilized 1,2-substituted five-membered aromatic heterocycles such as imidazole, oxazole, and pyrazole to mimic the cis double bond in CA-4. We were particularly interested in the imidazole ring. The basic nitrogen on the imidazole ring may lead to compounds with decreased lipophilicity that can be formulated into water-soluble salts to give improved physicochemical properties.

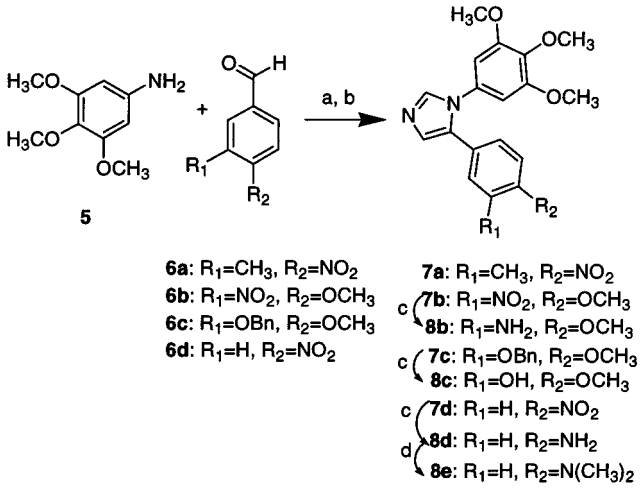
We now wish to report the syntheses and biological activities of novel five-membered heterocycle-based CA-4 derivatives. The cytotoxic effects of these compounds against two human cancer cell lines, HCT-15 (MDR+) and NCI-H460 (MDR−), and the inhibitory activity on tubulin polymerization were studied. The pharmacokinetics of selected compounds were evaluated in mouse, rat, dog, and monkey. The in vivo antitumor activities against murine M5076 reticulum sarcoma were evaluated in mouse.

Chemistry

The 1,5-disubstituted imidazoles **8b**−**e** were synthesized as outlined in Scheme 1. The enamines resulting from the reaction of 3,4,5-trimethoxyaniline **5** with a substituted benzaldehyde (**6a**−**d**) were treated with (*p*-tolylsulfonyl)methyl isocyanide (tosmic) in the presence of K_2CO_3 to afford the corresponding 1,5-disubstituted imidazoles **7a**−**d**.[14] Hydrogenation of **7b**−**d** gave the desired **8b**−**d**, respectively. The dimethylamino analogue **8e** was prepared by reductive amination of **8d**. Treatment of **7a** with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) followed by $TiCl_3$ gave the indole **9** (Scheme 2).[15] A one pot reduction and *N*-methylation resulted in the indoline **11**. *N*-Methylation of **9** with MeI and NaH in DMF gave **10**.

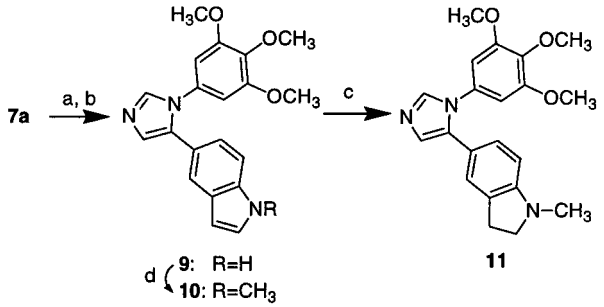
The syntheses of 1,2-disubstituted imidazoles **14** and **18** are described in Schemes 3 and 4, respectively. The

Scheme 1[a]



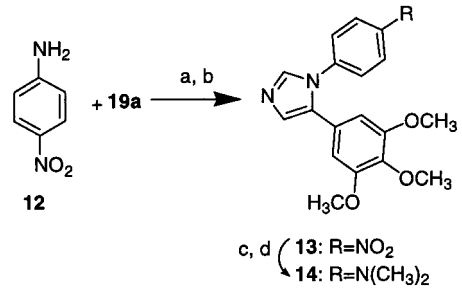
[a] Reagents and conditions: (a) EtOH, catalytic AcOH, reflux; (b) MeOH/DME (6:4), K_2CO_3, (*p*-tolysulfonyl)methyl isocyanide; (c) 5% Pd/C, EtOAc, H_2; (d) $NaB(CN)H_3$, $(CH_2O)n$, AcOH.

Scheme 2[a]



[a] Reagents and conditions: (a) DMF-DMA, DMF, reflux; (b) $TiCl_3$, MeOH, room temperature; (c) $NaB(CN)H_3$, $(CH_2O)n$, AcOH; (d) MeI, NaH, DMF, 0 °C.

Scheme 3[a]

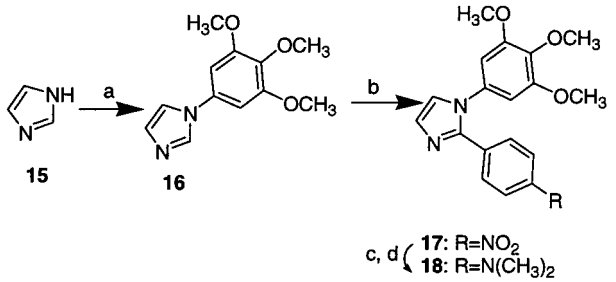


[a] Reagents and conditions: (a) EtOH, catalytic AcOH, reflux; **19**; (b) MeOH/DME (6:4), K_2CO_3, *(p*-tolysulfonyl)methyl isocyanide; (C) 5% Pd/C, H_2, EtOAc; (d) $NaB(CN)H_3$, $(CH_2O)n$, AcOH.

synthesis of **14** was conducted in a manner similar to that of **8e** (Scheme 3). Preparation of **18** begins with the reaction of imidazole and 1-bromo-3,4,5-trimethoxybenzene catalyzed by CuI and K_2CO_3 in DMF to give **16**. Copper-catalyzed reaction of **16** with 4-iodo-nitrobenzene gave **17**, which was followed by hydrogenation and *N*-methylation to afford **18**.[16] The structure of **17** was confirmed by X-ray crystallography.

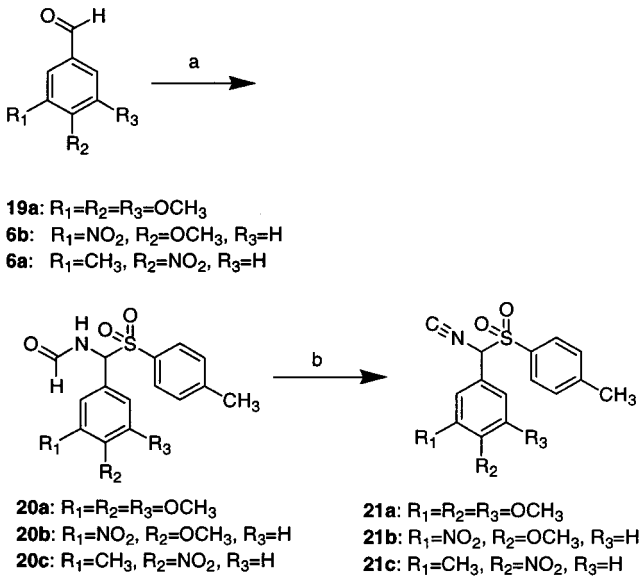
The construction of the 4,5-disubstituted imidazole and oxazole rings is based on the substituted tosmic reagents **21a**−**c** whose syntheses are shown in Scheme 5. Thus, substituted benzaldehydes **19a** and **6a**−**b** were allowed to react with *p*-toluenesulfinic acid and form-

Scheme 4[a]



[a] Reagents and conditions: (a) DMF, K_2CO_3, 1-bromo-3,4,5-trimethoxybenzene, CuI, 53%; (b) CuI, Cs_2CO_3, 4-iodo-nitrobenzene, DMF; (c) 5%Pd/C, H_2, EtOAc; (d) $NaB(CN)H_3$, $(CH_2O)n$, AcOH.

Scheme 5[a]



[a] Reagents and conditions: (a) *p*-toluenesulfinic acid, $HCONH_2$, 10-camphorsulfonic acid, 60 °C; (b) $POCl_3$, DME, −10 °C.

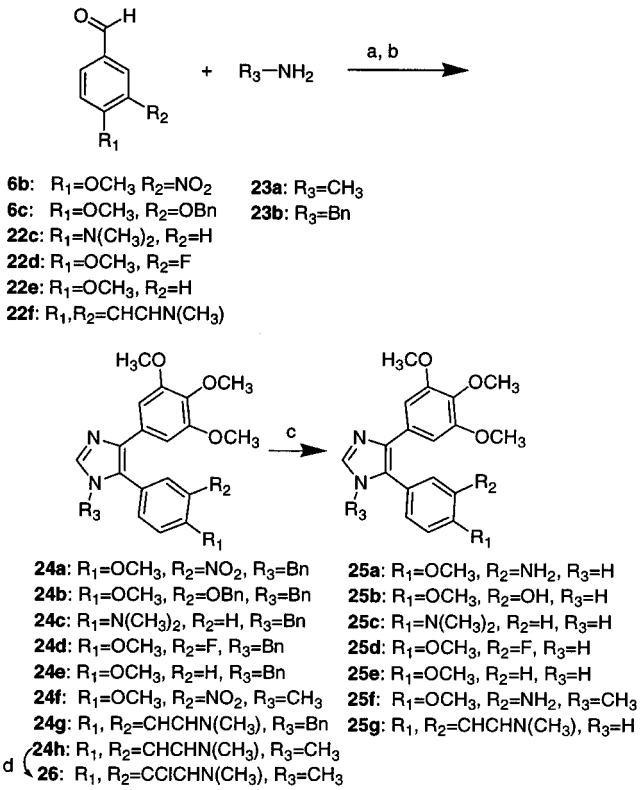
amide, catalyzed by 10-camphorsulfonic acid, to give **20a**−**c**. Dehydration with $POCl_3$ afforded the desired substituted tosmic reagents **21a**−**c**.

Substituted benzaldehydes **6b**−**c** and **22c**−**f**, when allowed to react with either benzylamine or methylamine to give the corresponding enamines, were subsequently treated with **21a** to afford **24a**−**h** (Scheme 6). Transfer hydrogenation of **24a**−**g** with ammonium formate and palladium on charcoal yielded the desired **25a**−**g**. Reaction of **24h** with *N*-chlorosuccinimide (NCS) in acetonitrile resulted in chlorination of the 3-position of the indole to give **26**.

When substituted benzaldehydes **6b**,**c**, **22c**, and **22f** reacted with tosmic reagent **21a**, 4,5-disubstituted oxazoles **27a**−**d** were obtained in excellent yields (Scheme 7). Transfer hydrogenation of **27a**,**b** with ammonium formate and palladium on charcoal afforded the desired **28a**,**b**. Oxazoles **30a**,**b** were prepared in a similar manner.

The preparation of *N*-methylimidazoles **32** and **33** is shown in Scheme 8. Reaction of 3,4,5-trimethoxybenzaldehyde (**19a**) with methylamine gave the corresponding enamine that was treated with either tosmic reagent **21b** or **21c** to generate *N*-methyl imidazole **31a**,**b**, respectively. *N*-Methyl-4-(*N*-methyl-indol-5-yl)-5-(3,4,5-

Scheme 6[a]



[a] Reagents and conditions: (a) EtOH, catalytic AcOH, reflux; (b) EtOH/DME (6:4), K_2CO_3, **21a**; (c) 5% Pd/C, $HCOONH_4$, MeOH, reflux; (d) NCS, CH_3CN, room temperature.

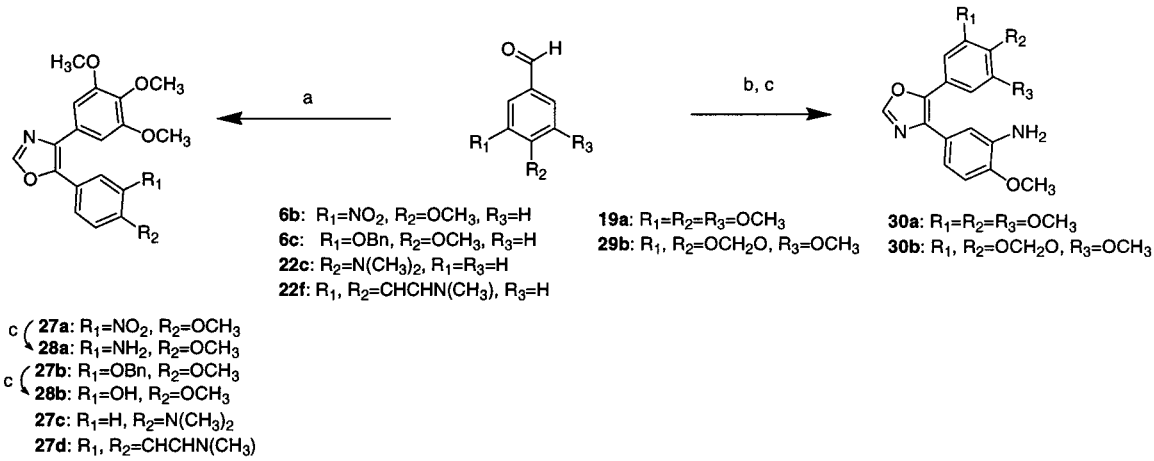
trimethoxyphenyl)imidazole **32** was prepared from **31b** in a manner similar to the synthesis of **10**. The nitro group of **31a** was reduced to give the desired amine **33**.

The preparation of **40a**,**b** is shown in Scheme 9. Indoline **34** was chlorinated in the 7-position with NCS. Hydrolysis of the *N*-acetyl followed by oxidation gave 5-bromo-7-chloro-indole **35**. Sequential treatment of **35** with KH and *t*-BuLi followed by the addition of DMF gave 7-chloro-5-formylindole **36a** in 83% overall yield.[17] Alternatively, the triflate of **37** was treated with 3 equiv of vinylmagnesium bromide[18] at −40 °C followed by carbonylation, which gave the methyl 7-fluoroindole-5-carboxylate. The ester was reduced to the hydroxymethyl and then oxidized to yield 7-fluoro-5-formylindole **36b**. The formation of **40a**,**b** from **36a**,**b** is carried out in a fashion similar to that described for **24h**.

The pyrazole-based CA-4 derivative **46** was prepared as shown in Scheme 10. Compound **42** was obtained from the reaction between 1-bromo-3,4,5-trimethoxybenezene and benzophenone hydrazone using the protocol developed by Buchwald's group.[19] Reaction between **42** and **44** afforded the desired 2,3-disubstituted pyrazole **45** along with the 1,3-disubstituted pyrazole (ratio 3:2, respectively). Reduction of the nitro group of **45** gave the desired compound **46**.

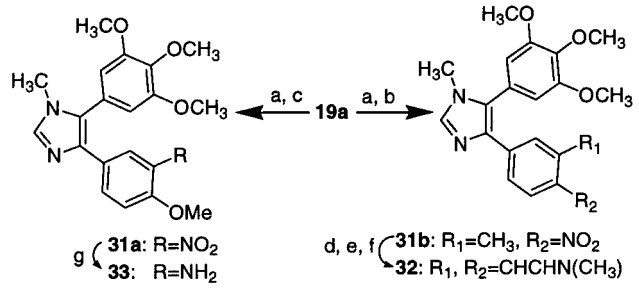
Additional heterocyclic analogues were prepared from the benzoins **48a**,**b** as described in Scheme 11. Substituted benzaldehydes **6c** and **22c** were treated with tetramethylsilane (TMS)−CN in the presence of ZnI_2 to give TMS cyanohydrins **47a**,**b**. Treatment of **47a**,**b** with LDA followed by the addition of **19a** gave **48a**,**b**, respectively. The benzoin **48a** was acetylated and then

Scheme 7[a]



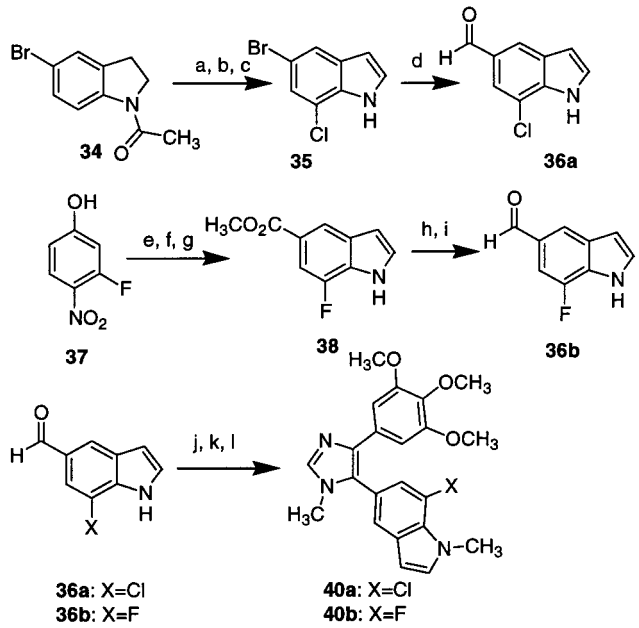
[a] Reagents and conditons: (a) EtOH/DME (6:4), K_2CO_3, **21a**; (b) EtOH/DME (6:4), K_2CO_3, **21b**; (c) 5% Pd/C, $HCOONH_4$, MeOH, reflux.

Scheme 8[a]



[a] Reagents and conditions: (a) MeOH, CH_3NH_2, reflux, catalytic AcOH; (b) EtOH/DME (6:4), K_2CO_3, **21c**; (c) K_2CO_3, EtOH/DME (6:4), **21b**; (c) DMF, DMF-DMA, (e) MeOH, $TiCl_3$; (f) NaH, MeI, DMF, 0 °C; (g) 5% Pd/C, H_2, EtOAc.

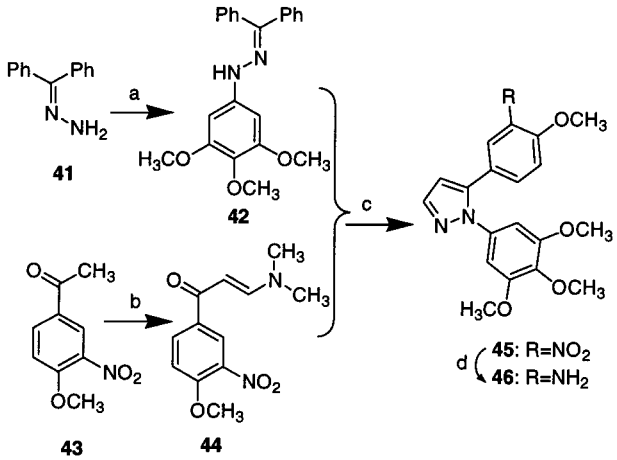
Scheme 9[a]



[a] Reagents and conditions: (a) NCS, CH_3CN, reflux; (b) LiOH, $MeOH/H_2O$; (c) O_2, salcomine, MeOH; (d) (1) KH, 0 °C, (2) *t*-BuLi, −78 °C, (3) DMF; (e) Et_3N, Tf_2O, CH_2Cl_2; (f) vinyl magnesium bromide, −40 °C, THF, 29%; (g) $Pd(dppf)Cl_2$, MeOH, Et_3N, CO; (h) DIBAL, toluene, −78 °C; (i) PCC, CH_2Cl_2, 0 °C; (j) EtOH, CH_3NH_2, AcOH, reflux; (k) EtOH/DME (6:4), K_2CO_3, **21a**; (l) CH_3I, NaH, DMF, 0 °C.

cyclized in the presence of NH_4OAc to form the oxazole ring. Hydrogenolysis resulted in the desired oxazole **49**.

Scheme 10[a]



[a] Reagents and conditions: (a) BINAP, NaO^{t}Bu, $PhCH_3$ $Pd(OAc)_2$, 1-bromo-3,4,5-trimethoxybenzene; (b) DMF, DMF-DMA, reflux; (c) EtOH, HCl(aq), reflux; (d) 5% Pd/C, H_2, EtOAc.

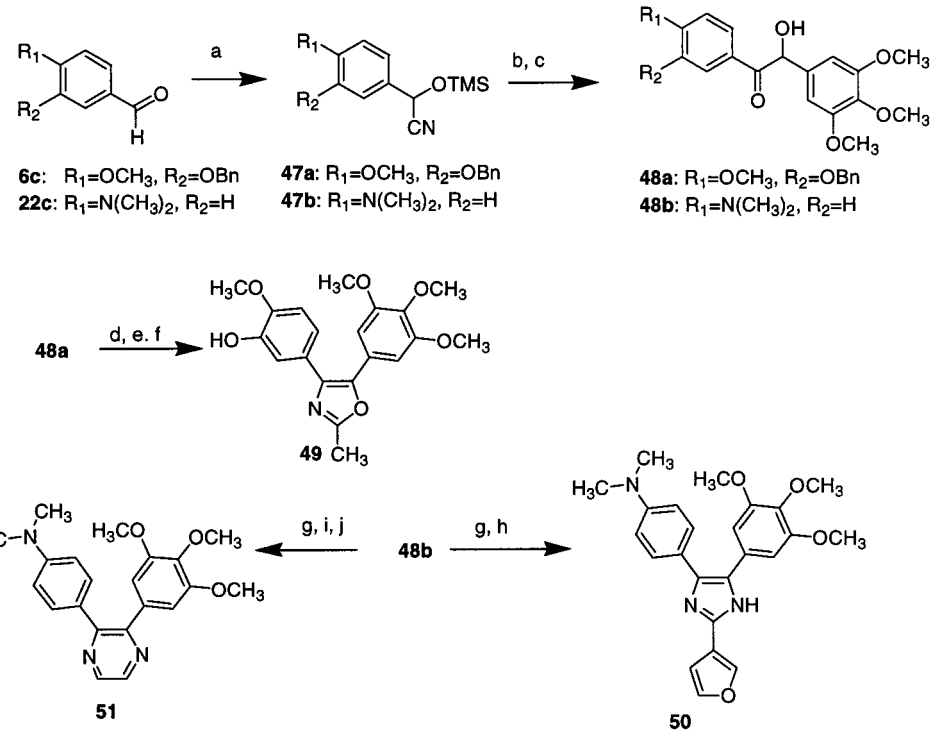
Oxidation of **48b** with $CuSO_4$ in aqueous pyridine followed by reaction with 3-furaldehyde in acetic acid gave the substituted imidazole **50**. Likewise, oxidation of **48b** with $CuSO_4$, followed by ethylenediamine in EtOH and then aromatization in the presence of elemental sulfur afforded the pyrazine **51**.

Results and Discussion

The results of the structure−activity relationship (SAR) study on the B-ring in which the cis double bond in CA-4 was replaced with the 1,5-imidazole ring are listed in Table 1. Both the hydroxyl group in **8c** and the amino group in **8b** showed similar antiproliferative potencies against NCI-H460 or HCT-15. It is interesting to note that **8e** with a 4-*N*,*N*-dimethylaminophenyl has cytotoxicities comparable to those of either **8b** or **8c**, whereas **8d**, with the unsubstituted aminophenyl, was completely inactive. While compounds **8b**, **8c**, and **8e** have comparable cytotoxicities, the antitubulin activity of **8e** is about three times less potent than that of either **8b** or **8c**.

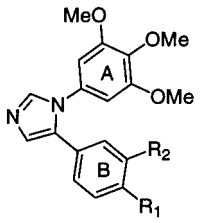
It is significant to note that the *N*-methyl-indol-5-yl (**10**) is a much better moiety than 3-amino-4-methoxyphenyl (**8b**) in this 1,5-disubstituted imidazole series. The *N*-methyl group in the indole ring of **10** plays an

Scheme 11[a]



[a] Reagents and conditions: (a) TMSCN, ZnI_2; (b) LDA, **19a**, THF, −78 °C; (c) HCl(aq); (d) Ac_2O, DMAP, CH_2Cl_2; (e) HOAc, NH_4OAc, reflux 6 h; (f) 5% Pd/C, 3:1 EtOH/EtOAc, H_2; (g) pyridine, $CuSO_4{\cdot}5H_2O$, H_2O, reflux; (h) 3-furancarboxyaldehyde, NH_4OAc, reflux; (i) ethylenediamine, EtOH, reflulx; (j) elemental sulfur, 140 °C, 30 min.

Table 1. SAR on the B-Ring Substituents



			IC$_{50}$		
no.	R_1	R_2	HCT-15 (nM)[a]	NCI-H460 (nM)[a]	antitubulin activity (μM)[b]
8b	OMe	NH_2	69	63	7.7
8c	OMe	OH	89	61	7.6
8d	NH_2	H	7260	8550	nd[c]
8e	$N(CH_3)_2$	H	36	79	23
9	−NHCHCH−		350	540	71
10	$-N(CH_3)CHCH-$		14	19	1.8
11	$-N(CH_3)CH_2CH_2-$		58	150	48
CA-4			1.7	3.0	1.2

[a] Drug concentration needed to inhibit the growth of cancer cells by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [b] Drug concentration needed to inhibit the tubulin polymerization by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [c] Not determined.

important role for both the antitubulin activity and the cytotoxicities for **10**. The des-methyl analogue **9** showed a 25−28-fold reduction in cytotoxicities and a 40-fold reduction in antitubulin activity when compared to the parent compound **10**. Unsaturation in the five-membered ring of the indole moiety is important. Reduction of the double bond to give the corresponding indoline **11** resulted in a marked loss in cytotoxicities (4−8-fold) and in antitubulin activity (~27-fold). However, the cytotoxicity values for **10** are 6−8 times less potent than those of CA-4, although both have comparable antitubulin activities.

Further investigations were carried out with several different five-membered ring heterocycles and different substitution patterns in place of the cis double bond of CA-4. A six-membered pyrazine derivative (**51**) was also prepared. The results are illustrated in Table 2. It is clear that the substitution pattern of the imidazole ring plays an important role for both the antitubulin and the antiproliferative activities (compare a series of compounds **8d**, **14**, **18**, and **25c**). Switching the positions of the two aryl groups (**8d** vs **14**) resulted in an approximately 7−9-fold loss in potency. Likewise, moving the 4-*N*,*N*-dimethylaminophenyl moiety from the 5-position of the imidazole ring in **8d** to the 2-position (**18**) resulted in an approximately 3-fold decrease in potency against both NCI-H460 and HCT-15.

The best substitution pattern for imidazole series is 4(5),5(4)-disubstituted imidazole as demonstrated by the compound **25c**. Although the antitubulin activity of **25c** is about three times weaker than that of CA-4, the antiproliferative activities against both NCI-H460 and HCT-15 are similar to those of CA-4. This discrepancy between cytotoxicity and antitubulin potency has been reported previously.[20] The possibility of an alternate mechanism was postulated.

Additional 4(5),5(4)-disubstituted imidazole analogues were examined (**25a**,**b**, **25d**,**e**, and **25g**). All of these analogues have potent cytotoxicities (Table 2) even for those that do not have either a 3-hydroxyl or an amino group in the B-ring (**25d**,**e**). Contrary to Ohsumi's finding, it appears that for the imidazole analogues, the

Table 2. SAR of Heterocycle-Based CA-4[a]

		IC$_{50}$			PK[d]				IC$_{50}$			PK[d]		
		HCT-15[b] (nM)	NCI-H460[b] (nM)	Anti-tubulin activity[c] (μM)	C_{max} (μg/ml)	AUC (PO) (μg•hr/ml)	F (%)		HCT-15[b] (nM)	NCI-H460[b] (nM)	Anti-tubulin activity[c] (μM)	C_{max} (μg/ml)	AUC (PO) (μg•hr/ml)	F(%)
8d		36	79	23		nd[e]		**27d**		7.4	12	0.79		nd	
14		333	589	130	<0.01	<0.01	0.01	**46**		28	33	nd		nd	
18		80	270	35		nd		**28a**		11	9.2	0.92	0.022	0.012	0.5
25a		8.1	8.5	0.68	<0.074	<0.218	45	**28b**		2.2	2.3	0.98		nd	
25b		10	11	0.73		nd		**30a**		7.2	11	nd	0.00	0.00	0.00
25c		4.4	2.0	3.7	0.0151	0.0162	1.3	**30b**		76	53	nd		nd	
25d		3.8	3.8	nd[b]		nd		**49**		15	35	nd		nd	
25e		7.8	7.2	2.8		nd		**50**		67	190	nd		nd	
25g		1.7	1.8	1.1	<0.01	0.0142	0.31	**51**		303	707	nd		nd	
27c		18	41	0.87		nd		**CA-4**		1.7	3.0	1.2			

[a] TMP, 3,4,5-trimethoxyphenyl; Ar_1, 4-*N*,*N*-dimethylaminophenyl; Ar_2, *N*-methyl-indol-5-yl; Ar_3, 3-amino-4-methoxyphenyl; Ar_4, 3-hydroxy-4-methoxyphenyl; Ar_5, 3-methoxy-4,5-methylenedioxyphenyl; Ar_6, 3-fluoro-4-methoxyphenyl; Ar_7, 4-methoxyphenyl. [b] Drug concentration needed to inhibit the growth of cancer cells by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [c] Drug concentration needed to inhibit the tubulin polymerization by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [d] Two animals were used for each dosing group (oral and iv, 5 mg/kg); the PK parameters reported here were the average of two animals. [e] Not determined.

presence of either an amino group or a hydroxyl group in the aryl 3-position is important to antitubulin activity (compare compounds **25a**,**b** and **25e**).[11]

For the oxazole-containing compounds **27c**,**d** and **28a**,**b**, the antitubulin activities of these are comparable to that of CA-4. However, the 3-hydroxy-4-methoxyphenyl (**28b**) appears to be better than either the 3-amino-4-methyoxyphenyl (**28a**) or the *N*-methyl-indol-5-yl (**27d**) for exerting antiproliferative activities, which is different from either 1,5- or 4,5-disubstituted imidazole series. The reduced cytotoxicities in **27c**,**d** and **28a** probably result from their poor permeabilities into cells. The substitution pattern in the oxazole series seems to have less effect on the antiproliferative activities than in the imidazole series. Switching the positions of the two aryls on the oxazoles (**28a** vs **30a**) resulted in little difference in potencies.

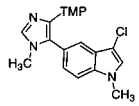
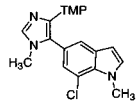
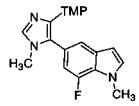
The 3,4,5-trimethoxyphenyl appears to be essential. A seemingly minor change such as replacing the 3,4,5-trimethoxyphenyl (**30a)** with the 3-methoxy-4,5-methylenedioxyphenyl (**30b**) caused approximately a 5−10-fold drop in cytotoxicity. This is in agreement with a previous report.[20]

Replacing the imidazole ring (**25a**) with a pyrazole ring (**46**) resulted in a modest decrease in antiproliferative activities. Substituting the imidazole (**25c**) with the six-membered pyrazine (**51**), however, gave a dramatic loss of cytotoxicities.

Introduction of substituents at the 2-position of the five-membered ring was not well-tolerated. A somewhat bulky furan substituent at the imidazole 2-position (**50**) caused a 15−95-fold drop in the antiproliferative potencies when compared to the unsubstituted **25c**. Even a relatively small methyl group (**49**) resulted in a 6−15-fold loss in the cytotoxicities (compare **49** with **28b**). The decrease in cytotoxicities was more pronounced when tested in the NCI-H460 assay than for the HCT-15 assay.

The pharmacokinetic properties in rat for selected imidazole and oxazole-containing CA-4 analogues were studied. The results are also summarized in Table 2. When possible, the compounds were converted into the corresponding water-soluble hydrochloride salts for the pharmacokinetic study. Despite their potent antitubulin activity and cytotoxicities, in general, the pharmacokinetic profiles for these compounds are disappointing. The pharmacokinetic profiles of these compounds are characterized by low C_{max} values, small values for the oral area under the plasma concentration curve (AUC), and poor bioavailability. The solubilities of the oxazole-containing CA-4 analogues were poor even with the presence of an amino group on the aryl group (**30a**).

Table 3. SAR of *N*-Methyl Imidazole-Based CA-4[a]

		IC$_{50}$			PK[d]		
		HCT-15 (nM)[b]	NCI-H460 (nM)[b]	Anti-tubulin binding (μM)[c]	C_{max} (μg/ml)	AUC (PO) (μg•hr/ml)	F (%)
25f		220	170	31	3.86	10.75	82.3
33		79	34	nd[e]	0.623	1.504	29.9
24h		20	32	2.0	0.169	0.461	35.7
32		27	51	nd	0.070	0.112	2.5
26		6.5	8.7	nd		nd	
40a		12	12	nd	0.13	0.22	10.2
40b		27	23	nd	0.209	0.34	12.3

[a] TMP, 3,4,5-trimethoxyphenyl; Ar_2, *N*-methyl-indol-5-yl; Ar_3, 3-amino-4-methoxyphenyl. [b] Drug concentration needed to inhibit the growth of cancer cells by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [c] Drug concentration needed to inhibit the tubulin polymerization by 50%. The compounds were assayed at least twice, and the IC$_{50}$ values reported here were the averages. [d] Two animals were used for each dosing group (oral and iv, 5 mg/kg); the PK parameters reported here were the average of two animals. [e] Not determined.

That is certainly one of the factors contributing to the poor pharmacokinetic profiles. Even though the imidazole-containing compounds had good aqueous solubility, they still suffered from poor pharmacokinetic properties.

It was found that the introduction of an *N*-methyl group on the imidazole ring dramatically improved the pharmacokinetics of the corresponding compounds. Although the presence of the *N*-methyl group in the imidazole ring increases lipophilicity, the resulting compounds are sufficiently soluble and maintain adequate levels of cytotoxicity. The results are summarized in Table 3. When examined in rat, **25f** showed a high C_{max}, a large oral AUC, and an outstanding bioavailability value. These pharmacokinetic properties are all much improved over those of **25a**. The same trend was observed between the *N*-methyl imidazole compound **24h** and the des-methyl imidazole **25g**, although to a lesser extent. It is worthwhile to note that the presence of the *N*-methyl group in compounds **25f** and **24h** does result in decreased cytotoxicities and antitubulin activity as compared to their parent compounds (**25a**,**g**, respectively).

The position of the *N*-methyl group on the imidazole has a profound influence on the pharmacokinetics and cytotoxicities of the imidazole-containing compounds. Comparison of the *N*-methyl regioisomers (**25f** vs **33**) shows a 3−5-fold improvement in the cytotoxicity of **33**. The pharmacokinetic properties, however, were markedly diminished for **33**. The same trend is observed when comparing the pharmacokinetics of the *N*-methyl isomers **24h** and **32**. The pharmacokinetic profile of imidazole **24h** is better than that of **32**. Compounds **24h** and **32**, however, showed little difference in antiproliferative activities.

Three halogenated indole analogues (**26** and **40a**,**b**) were prepared. Introduction of a chloride at the 3-position of indole (**26**) shows that the cytotoxicities were improved by about 3-fold. Incorporation of either a chloride (**40a**) or a fluoride (**40b**) at the 7-position of the indole had little effect on either the antiproliferative activities or the pharmacokinetics.

Additional pharmacokinetic studies of compounds **25f** and **24h** were carried out in dog, monkey, and mouse. The results are summarized in Table 4. Compound **25f** showed excellent pharmacokinetic profiles in all three

Table 4. Pharmacokinetic Studies in Dog, Monkey, and Mouse on Compounds **24h** and **25f**[a]

	24h			25f		
	C_{max} (mg/mL)	AUC (PO) (mg hr/mL)	F (%)	C_{max} (mg/mL)	AUC (PO) (mg hr/mL)	F (%)
dog	1.87	7.05	44.5	4.15	9.65	45.8
monkey	0.354	0.98	12.5	9.68	43.98	91.2
mouse	0.25	0.82	24.2	5.52	3.82	89.7

[a] Two animals were used for each dosing group (oral and iv, 5 mg/kg); the PK parameters reported here were the average of two animals.

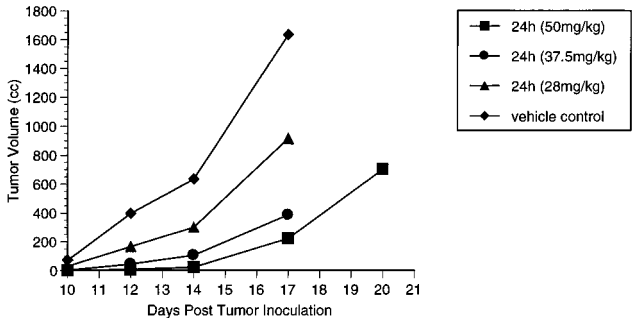


Figure 1. In vivo study of **24h** against murine M5076 reticulum sarcoma cell line.

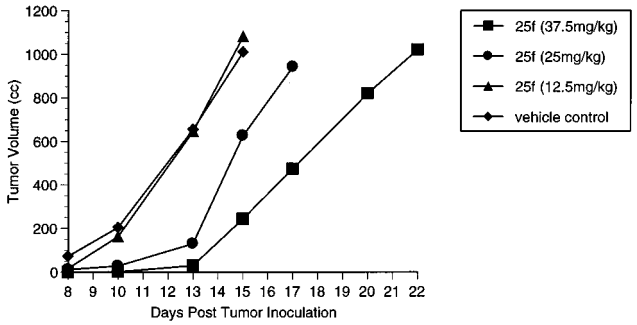


Figure 2. In vivo study of **25f** against murine M5076 reticulum sarcoma cell line.

species. Compound **24h** showed good absorption in dog. The pharmacokinetic behavior observed in monkey and mouse, however, was not as good.

Compounds **24h** and **25f** were selected for in vivo experiments using subcutaneous xenografts in nude mice against the murine M5076 reticulum sarcoma cell line, based on their favorable pharmacokinetic properties. Figures 1 and 2 show the results of the in vivo study on compound **24h** and **25f**, which were formulated into the corresponding hydrochloride salts. The maximum tolerated doses (MTD) for **24h** and **25f** were found to be 50 and 37.5 mg/kg/day, respectively. It is clear that at their MTD, both compounds **24h** and **25f** show a notable reduction in mean tumor mass for the animals dosed orally. The dose responses of **24h** and **25f** have also been observed. The increase in life span (ILS) in the animals studied for compounds **24h** and **25f** was found to be ca. 38.5 and 40.5%, respectively. It is interesting to note that although **24h** is about 6−10 times more potent than **25f** in antiproliferative activities, both compounds exhibit similar in vivo activity. This may be, in part, due to the better pharmacokinetic properties of **25f**. Figure 3 shows the results from the regression trial of **25f**. When given orally, compound **25f** (30 mg/kg/day) showed remarkable reduction in mean

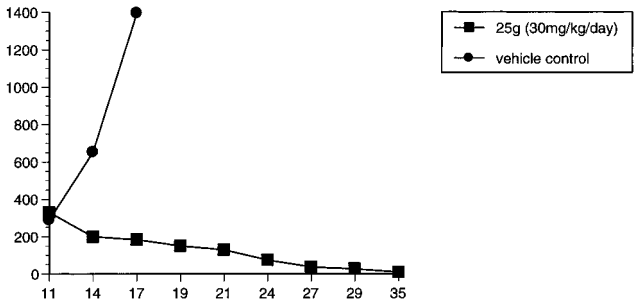


Figure 3. Regression trial of **25f** against murine M5076 reticulum sarcoma cell line.

tumor mass in the animals studied. Tumor mass in the untreated animals reached 1 g in approximately 15 days. Near the end of the regression trial (day 35), for the animals in the treatment groups, most of the solid tumors were not palpable. To our best knowledge, this is the first example of CA-4 analogues demonstrating oral in vivo antitumor activity against solid tumor.

Conclusion

We have demonstrated that the cis double bond in CA-4 can be replaced by a 1,2-disubstituted five-membered heterocycle such as imidazole, oxazole, or pyrazole. The 3-hydroxy-4-methoxyphenyl in CA-4 can be substituted by either an *N*-methyl-indol-5-yl or a 3-amino-4-methoxyphenyl. These compounds (e.g., **25a**,**d** and **28b**) have potent antitubulin and cytotoxic activity. Incorporation of an *N*-methyl group into the central imidazole ring leads to compounds **24h** and **25f** with much improved pharmacokinetic profiles, characterized by large oral AUC, longer half-life, and excellent bioavailability. The in vivo experiments with **24h** and **25f** in mice revealed that both have remarkable oral efficacy against the solid murine M5076 reticulum sarcoma cell line with ILS values of 38.5 and 40.5%, respectively. This is the first report of CA-4 analogues showing potent oral antitumor activity in vivo.

Experimental Section

All commercially available solvents and reagents were used without further treatment as received unless otherwise noted. DMF, DMA, methylene chloride, toluene, and THF were commercial anhydrous solvents from Aldrich. Fourier transform nuclear magnetic resonance (FT-NMR) spectra were obtained on Bruker 250 (62.5 MHz for ^{13}C), 300 (75 MHz for ^{13}C), or 400 MHz (100 MHz for ^{13}C) spectrometers. Elemental analyses were performed by Robertson Microlit Laboratories, Inc., of Madison, NJ. Thin-layer chromatography (TLC) was performed on Kiesegel 60 F254 plates (Merck) using reagent grade solvents. Flash chromatography was performed on Merck silica gel 60 (230−400 mesh) using reagent grade solvents. All reactions were performed under a nitrogen atmosphere. Small amounts of solvents were present in compounds **8d**, **25d**,**e**, and **49** detected by both NMR and elemental analyses.

General Procedure for the Formation of Substituted Isocyanides (21a−c). Toluenesulfinic Acid. A vigorously stirred solution of 4-methylbenzensulfinic acid sodium salt (150 g, 0.84 mol) in water (500 mL) and *tert*-butyl methyl ether (TMBE) (250 mL) was treated dropwise with concentrated HCl (75 mL). The resulting two layers were separated, and the aqueous layer was extracted with TMBE (100 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to near dryness. The resulting white solid was triturated with hexane (350 mL), filtered, and dried under vacuum to provide 96 g (73%) of the desired product.

***N*-[(Toluene-4-sulfonyl)-(3,4,5-trimethoxyphenyl)methyl]formamide (20a).** A solution of toluenesulfinic acid (22.3 g, 0.15 mol), **19a** (35.32 g, 0.18 mol), and camphorsulfonic acid (3.48 g, 1.5 mmol) in formamide (40 mL) was stirred vigorously at 65 °C for 16 h, cooled, and filtered. The resulting solid was washed with methanol and dried to provide 26.8 g (47%) of the desired compound.

3,4,5-Trimethoxyphenyl(tosyl)methyl Isocyanide (21a−c). The representative procedure for the preparation of substituted isocyanide **21a−c** is as follows. A solution of **20a** (13.8 g, 36.3 mmol) in 200 mL of 1,2-dimethoxyethane (DME) was cooled to −10 °C. To this solution was added $POCl_3$ (10.3 mL, 110 mmol) followed by dropwise addition of triethylamine (25.3 mL, 181.5 mmol) in DME (20 mL). The reaction mixture was stirred at −5 °C for 3 h, then poured into ice-cold H_2O (500 mL), and extracted with EtOAc (3 × 120 mL). The combined extracts were washed with 10% $NaHCO_3$ and brine, dried ($MgSO_4$), concentrated in vacuo to 20% of the original volume, and filtered to afford 7.5 g of the desired compound. The mother liquid was concentrated in vacuo, and the residue was purified by flash chromatography using 1:1EtOAc/hexane to provide an additional 1.6 g of the product (total 69%). ^{1}H NMR ($CDCl_3$): δ 7.65 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 6.49 (s, 2H), 5.53(s, 1H), 3.87 (s, 3H), 3.78 (s, 6H), 2.47(s, 3H). MS/ESI (+): 362 $(M + H)^+$, 377 $(M + NH_4)^+$. Anal. Calcd for $C_{18}H_{19}NO_5S$: C, 59.82; H, 5.30; N, 3.88. Found: C, 59.90; H, 5.37; N, 3.86.

General Procedure for the Preparation of 1,5-Disubstituted Imidazoles (8b−d). The representative procedure for the preparation of 1,5-disubstituted imidazoles (**8b−d**) is as follows.

(4-Nitrophenyl)-(3,4,5-trimethoxybenzylidene)amine. A mixture of 3,4,5-trimethoxyaniline **5** (1.83 g, 10 mmol), 4-nitrobenzaldehyde **6d** (1.51 g, 10 mmol), and 1 mL of acetic acid in 50 mL of ethanol was heated under reflux for 2 h. After it cooled to room temperature, the precipitation was collected by filtration to give 2.88 g (92%) of (4-nitrophenyl)-(3,4,5-trimethoxybenzylidene)amine as yellow crystals used directly for the next reaction without further purification.

5-(4-Nitrophenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (7d). A mixture of (4-nitrophenyl)-(3,4,5-trimethoxybenzylidene)amine (1.58 g, 5 mmol), tosylmethylisocyanide (1.47 g, 7.5 mmol), and potassium carbonate (1.38 g, 10 mmol) in 35 mL of methanol and 15 mL of DME was heated under reflux until the (4-nitrophenyl)-(3,4,5trimethoxybenzylidene)-amine disappeared monitored by TLC. After it was cooled to room temperature, the solution was concentrated in vacuo and partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 1.62 g (91%) of **7d** as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 8.15 (dd, J = 7.1, 2.0 Hz, 2H), 7.67 (s, 1H), 7.45 (s, 1H), 7.33 (dd, J = 8.8, 2.1 Hz, 2H), 6.42 (s, 2H), 3.90 (s, 3H), 3.76 (s, 6H).

5-(4-Aminophenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (8d). A mixture of **7d** (1.2 g, 3.4 mmol), 0.5 g of 5% palladium on carbon, and 100 mL of EtOAc was equipped with a balloon of hydrogen gas and stirred at room temperature. After uptake of H_2 was complete, the solution was filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography on silica gel eluting with 25:1 EtOAc/Methanol to give 1.01 g (92%) of **8d** as a white solid. ^{1}H ($CDCl_3$): δ 7.61 (d, J = 1.0 Hz, 1H), 7.23 (d, J = 1.0 Hz, 1H), 6.98 (d, J = 6.7 Hz, 1H), 6.90 (d, J = 6.7 Hz, 2H), 6.36 (s, 2H), 3.83 (s, 3H), 3.69−3.65 (m, 5H). ESI (+)/MS: 326 $(M + H)^+$. Anal. Calcd for $C_{18}H_{19}N_3O_3 \cdot 0.40H_2O$: C, 65.00; H, 6.00; N, 12.64. Found: C, 64.92; H, 6.23; N, 12.60.

5-(3-Amino-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (8b). Compound **8b** was prepared from **7b** by catalytic hydrogenation as described in the preparation of **8d**. Compound **7b** was prepared in a similar manner as described for the preparation of **7d**. ^{1}H NMR (DMSO-d_6): δ 7.87 (d, J = 1.1 Hz, 1H), 7.01 (d, J = 1.1 Hz, 1H), 6.72 (d, J = 8.1 Hz, 2H), 6.55 (s, 2H), 6.32 (dd, J = 8.1, 1.1 Hz, 2H), 3.73 (s, 3H), 3.65 (s, 6H). ESI (+)/MS: 356 $(M + H)^+$, 711 $(2M + H)^+$. Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.06; H, 5.92; N, 11.47.

5-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (8c). Compound **8c** was prepared from **7c** by catalytic hydrogenation as described in the preparation of **8d**. Compound **7c** was prepared in a similar manner as described for the preparation of **7d**. ^{1}H NMR (DMSO-d_6): δ 9.03 (s, 1H), 7.88 (d, J = 1.1 Hz, 1H), 7.09 (d, J = 0.7 Hz, 1H), 6.82 (d, J = 7.8 Hz, 2H), 6.55−6.63 (m, 4H), 3.74 (s, 3H), 3.68 (s, 6H), 3.66 (s, 6H). ESI (+)/MS: 357 $(M + H)^+$, 713 $(2M + H)^+$. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 64.10; H, 5.74; N, 7.84.

5-(4-*N*,*N*-Dimethylaminophenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (8e). A mixture of **8d** (0.44 g, 1.35 mmol), paraformaldehyde (0.411 g, 13.5 mmol), and sodium cyanoborohydride (0.43 g, 6.75 mmol) in 30 mL of acetic acid was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc, washed with 10% NaOH and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with 100:1 EtOAc/methanol to give 0.33 g (63%) of **8e** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.65 (s, 1H), 7.14 (s, 1H), 7.03 (d, J = 8.8 Hz, 2H, 6.61 (d, J = 8.8 Hz, 2H), 6.41 (s, 2H), 3.87 (s, 3H), 3.72 (s, 6H), 2.94 (s, 6H). ESI (+)/MS: 354 $(M + H)^+$, 707 $(2M + H)^+$. Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.87; H, 6.60; N, 11.84.

5-(Indol-5-yl)-1-(3,4,5-trimethoxyphenyl)imidazole (9). A mixture of 1-(3,4,5-trimethoxyphenyl)-5-(4-nitro-3-methylphenyl)imidazole (0.369 g, 1 mmol), prepared in a similar manner as described for the preparation of **7d**, *N*,*N*-dimethylformamide dimethyl acetal (0.357 g, 3 mmol), and 3 mL of DMF was heated under reflux for 16 h. After the mixture was cooled to room temperature, the solvents were removed under reduced pressure to near dryness. The residue was then treated sequentially with 10 mL of methanol and 5 mL of 19 wt % $TiCl_3$ solution. The solution was stirred at room temperature for 20 min, then diluted with water, treated with 10% NaOH to pH > 8, and extracted with EtOAc. The extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography eluting with EtOAc to give 0.180 g (52%) of **9** as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ 11.16 (s, 1H), 7.93 (s, 1H), 7.45 (d, J = 0.7 Hz, 1H), 7.30−7.34 (m, 2H), 7.13 (s, H), 6.86 (d, J = 8.2 Hz, 1H), 3.65 (s, 3H), 3.58 (s, 6H). ESI (+)/MS: 350 $(M + H)^+$. Anal. Calcd for $C_{20}H_{19}N_3O_3$: C, 68.75; H, 5.48; N, 12.03. Found: C, 69.00; H, 5.77; N, 11.94.

5-(*N*-Methyl-indol-5-yl)-1-(3,4,5-trimethoxyphenyl)imidazole (10). 1-(3,4,5-trimethoxyphenyl)-5-(indol-5-yl)imidazole (0.28 g, 0.8 mmol) in 1 mL of anhydrous DMF was treated with 60% NaH in mineral oil (0.096 g, 4 mmol) at 0 °C. The solution was stirred for 2 h and then treated with methyl iodide (0.119 g, 0.84 mmol) in 1 mL of anhydrous DMF. The resulting solution was stirred for 1 h. The reaction mixture was partitioned between EtOAc and water. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 0.22 g (75%) of **10** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.50 (d, J = 1.4 Hz, 1H), 7.24 (s, 1H), 7.20 (d, J = 8.5 Hz, 1H), 7.05 (d, J = 3.1 Hz, 1H), 6.98 (dd, J = 8.5, 1.7 Hz, 1H), 6.42−6.43 (m, 3H), 3.89 (s, 3H), 3.78 (s, 3H), 3.66 (s, 3H). ESI (+)/MS: 364 $(M + H)^+$, 727 $(M + H)^+$. Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.14; H, 6.11; N, 11.20.

5-(*N*-Methyl-2,3-dihydro-indol-yl-5)-1-(3,4,5-trimethoxyphenyl)imidazole (11). A mixture of **9** (0.13 g, 0.37 mmol), paraformaldehyde (0.111 g, 3.7 mmol), and sodium cyanoborohydride (0.116 g, 1.85 mmol) in 10 mL of acetic acid was stirred at room temperature for 72 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc, and neutralized with 10% NaOH. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica

gel eluting with EtOAc to give 0.086 g (63%) of **11** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.75 (s, 1H), 7.15 (s, 1H), 6.85 (m, 2H), 6.05 (s, 2H), 6.36 (d, J = 8.1 Hz, 1H), 3.87 (s, 3H), 3.73 (s, 6H), 3.31 (t, J = 8.1 Hz, 2H), 2.88 (t, J = 8.1 Hz, 2H), 2.75 (s, 3H). ESI (+)/MS: 366 $(M + H)^+$. Anal. Calcd for $C_{21}H_{23}N_3O_3$: C, 69.02; H, 6.34; N, 11.50. Found: C, 68.82; H, 6.50; N, 11.25.

1-(4-Nitrophenyl)-5-(3,4,5-trimethoxyphenyl)imidazole (13). A mixture of (4-nitro-phenyl)-(3,4,5-trimethoxybenzylidene)amine (0.306 g, 0.9 mmol), prepared by condensation of **19a** and 4-nitroaniline in the presence of a catalytic amount of HOAc, tosylmethylisocyanide (0.264 g, 1.35 mmol), and potassium carbonate (0.250 g, 1.8 mmol) in 7 mL of methanol and 3 mL of DME was heated under reflux until the (4-nitro-phenyl)-(3,4,5-trimethoxybenzylidene)amine disappeared monitored by TLC. After the mixture was cooled to room temperature, the solution was concentrated in vacuo and partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 0.274 g (86%) of **13** as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 8.31 (dd, J = 6.8, 1.7 Hz, 2H), 7.84 (s, 1H), 7.39 (dd, J = 5.1, 1.7 Hz, 2H), 7.27 (d, J = 6.4 Hz, 1H), 6.32 (s, 2H), 3.86 (s, 3H), 3.68 (s, 6H).

1-(4-*N*,*N*-(Dimethylamino)-phenyl)-5-(3,4,5-trimethoxyphenyl)imidazole (14). Compound **14** was prepared from compound **13** in a similar manner as described for the preparation of **8e**. ^{1}H NMR ($CDCl_3$): δ 7.62 (d, J = 1.1 Hz, 1H), 7.25 (s, 1H), 7.07 (dd, J = 1.1, 9.1 Hz, 1H), 6.68 (dd, J = 1.1, 9.1 Hz, 2H), 6.37 (s, 2H), 3.83 (s, 3H), 3.66 (s, 6H), 2.93 (s, 6H). ESI (+)/MS: 354 $(M + H)^+$. Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.82; H, 6.54; N, 11.82.

1-(3,4,5-Trimethoxyphenyl)imidazole (16). A mixture of 1-bromo-3,4,5-trimethoxybenzene (1.23 g, 5 mmol), imidazole (0.403 g, 6 mmol), CuI (0.240 g, 1.25 mmol), and potassium carbonate (0.90 g, 6.5 mmol) in 5 mL of DMF was heated at 100 °C for 16 h. After the mixture was cooled to room temperature, the reaction mixture was diluted with EtOAc (120 mL), washed with water (3 × 50 mL) and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 0.62 g (53%) of **16**. ^{1}H NMR ($CDCl_3$): δ 7.9 (br, 1H), 7.25 (br, 2H), 6.59 (s, 2H), 3.92 (s, 6H), 3.88 (s, 6H). ESI (+)/MS: 235 $(M + H)^+$.

2-(4-Nitrophenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (17). A mixture of **16** (0.280 g, 1.2 mmol), 4-iodonitrobenzene (0.60 g, 2.4 mmol), Cs_2CO_3 (0.78 g, 2.4 mmol), CuI (0.46 g, 2.4 mmol), and PPh_3 (0.21 g, 0.8 mmol) in 10 mL of DMF was heated under N_2 at 140 °C for 20 h. After the mixture was cooled to room temperature, the reaction mixture was diluted with EtOAc (30 mL), washed with water (3 × 30 mL) and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 0.13 g (31%) of **17** as a pale white solid.

2-(4-*N*,*N*-Dimethylaminophenyl)-1-(3,4,5-trimethoxyphenyl)imidazole (18). Compound **18** was prepared from **17** in a similar manner as described for the preparation of **8e**. ^{1}H NMR ($CDCl_3$): δ 7.34 (dd, J = 9.2, 2.2 Hz, 2H), 7.20 (d, J = 1.1 Hz, 1H), 7.08 (d, J = 1.1 Hz, 1H), 6.60 (dd, J = 7.2, 1.8 Hz, 2H), 6.47 (s, 2H), 3.90 (s, 3H), 2.95 (s, 6H). ESI (+)/MS: 353 $(M + H)^+$. Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.86; H, 6.68; N, 11.75.

General Procedure for the Preparation of 4,5-Disubstituted Imidazoles (25a−e,g). The representative procedure for the preparation of 4,5-disubstituted imidazoles (**25a−e**,**g**) is as follows. A mixture of *N*-methyl-5-indolecarboxaldehyde (0.16 g, 1 mmol) and benzylamine (0.16 g, 1.5 mmol) in 15 mL of ethanol was treated with 0.5 mL of acetic acid. The solution was heated under reflux for 2 h. After the solution was cooled to room temperature, the solution was treated with 5 mL of DME, **21a** (0.49 g, 1.5 mmol), and potassium carbonate (0.552 g, 4 mmol). The solution was heated at reflux for 5 h. After it was cooled to room temperature, the solution was concentrated in vacuo and then partitioned between EtOAc and water. The organic layer was separated, washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc to give 0.42 g (93%) of 1-benzyl-4-(3,4,5-trimethoxyphenyl)-5-(*N*-methyl-indol-5-yl)imidazole (**24g**) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.66 (s, 1H), 7.54 (s, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.25−7.30 (m, 3H), 7.09 (m 2H), 7.00 (m, 2H), 6.82 (s, 2H), 6.45 (d, J = 3.3 Hz, 2H), 3.97 (s, 2H), 3.83 (s, 3H), 3.77 (s, 3H), 3.51 (s, 6H). ESI (+)/MS: 452 $(M + H)^+$.

A mixture of **24g** (0.42 g, 0.93 mmol), ammonium formate (0.586 g, 9.3 mmol), 5% palladium on carbon (0.2 g), and 30 mL of methanol was heated under reflux for 2 h. After it was cooled to room temperature, the solution was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with 25:1 CH_2Cl_2/methanol to give 0.303 g (93%) of 4(5)-(3,4,5-trimethoxyphenyl)-(4)5-(*N*-methyl-indol-5-yl)imidazole (**25g**) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.69 (m, 2H), 7.25 (m, 2H), 7.19 (m, 1H), 7.01 (d, J = 3.4 Hz, 1H), 6.81 (s, 2H), 6.39 (d, J = 3.4 Hz, 1H), 6.82 (s, 2H), 6.45 (d, J = 3.3 Hz, 2H), 3.77 (s, 3H), 3.74 (s, 3H), 3.56 (s, 6H). ESI (+)/MS: 366 $(M + H)^+$. Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.18; H, 5.53; N, 11.46.

4(5)-(3,4,5-Trimethoxyphenyl)-5(4)-(3-amino-4-methoxyphenyl)imidazole (25a). Compound **25a** was prepared in a similar manner as described for the preparation of **25g**. ^{1}H NMR (DMSO-d_6): δ 12.3 (br, 1H), 7.65 (m, 1H), 7.63−6.84 (m, 5H), 3.67−3.77 (m, 12H). APCI (+)/MS: 356 $(M + H)^+$. Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 63.94; H, 5.96; N, 11.47.

4(5)-(3,4,5-Trimethoxyphenyl)-5(4)-(3-hydroxy-4-methoxyphenyl)imidazole (25b). Compound **25b** was prepared in a similar way as described for the preparation of **25g**. ^{1}H NMR (HCl salt, MeOH-d_4): δ 8.99 (s, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.97−7.00 (m, 2H), 6.80 (s, 2H), 3.90 (s, 3H), 3.80 (s, 3H), 3.74 (s, 6H). ESI (+)/MS: 357 $(M + H)^+$. Anal. Calcd for $C_{19}H_{20}N_2O_5$·HCl: C, 58.09; H, 5.39; N, 7.13. Found: C, 58.31; H, 5.41; N, 7.05.

4(5)-(3,4,5-Trimethoxyphenyl)-5(4)-(4-*N*,*N*-dimethylaminophenyl)imidazole (25c). Compound **25c** was prepared in a similar manner as described for the preparation of **25g**. ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 1H), 7.35 (dd, J = 8.8, 2.0 Hz, 2H), 6.86 (s, 2H), 6.68 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H), 3.69 (s, 6H), 2.96 (s, 6H). APCI (+)/MS: 354 $(M + H)^+$. HRMS: Anal. Calcd for $C_{20}H_{23}N_3O_3$ *m*/*z*: 354.1818. Found: *m*/*z* 354.1826. Anal. Calcd for $C_{20}H_{23}N_3O_3$: C, 67.97; H, 6.56; N, 11.89. Found: C, 67.85; H, 6.39; N, 11.71.

4(5)-(3,4,5-Trimethoxyphenyl)-5(4)-(3-fluoro-4-methoxyphenyl)imidazole (25d). Compound **25d** was prepared in a similar manner as described for the preparation of **25g**. ^{1}H NMR ($CDCl_3$): δ 7.73 (s, 1H), 7.24−7.30 (m, 4H), 6.92 (m, 1H), 6.73 (s, 2H), 3.89 (s, 3H), 3.87 (s, 3H), 3.73 (s, 6H). DCI/NH_3 (+)/MS: 359 $(M + H)^+$. Anal. Calcd for $C_{19}H_{19}N_2O_4F$·0.4H_2O: C, 62.46; H, 5.60; N, 7.67. Found: C, 62.65; H, 5.79; N, 7.48.

4(5)-(3,4,5-Trimethoxyphenyl)-5(4)-(4-methoxyphenyl)-imidazole (25e). Compound **25e** was prepared in a similar manner as described for the preparation of **25g**. ^{1}H NMR ($CDCl_3$): δ 7.73 (s, 1H), 7.44 (dd, J = 7.1, 2 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.79 (s, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 3.70 (s, 6H). DCI/NH_3 (+)/MS: 341 $(M + H)^+$. Anal. Calcd for $C_{19}H_{20}N_2O_4$·0.20H_2O: C, 66.35; H, 5.98; N, 8.14. Found: C, 66.61; H, 5.71; N, 7.97.

General Procedure for the Formation of *N*-Methyl-4,5-disubstituted Imidazoles. The representative procedure for the formation of *N*-methyl-4,5-disubstituted imidazoles is as follows. A mixture of *N*-methyl-5-indolecarboxaldehyde (0.16 g, 1 mmol) and 2.0 M methylamine in methanol (2.5 mL, 5 mmol) in 15 mL of ethanol was treated with 0.3 mL of acetic acid. Then, the solution was heated under reflux for 2 h. After it was cooled to room temperature, the solution was treated with 5 mL of DME, **21a** (0.49 g, 1.5 mmol), and potassium carbonate (0.552 g, 4 mmol). The solution was heated at reflux

for 6 h. After it was cooled to room temperature, the reaction mixture was concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel eluting with EtOAc to afford 0.341 g (90%) of 1-methyl-4-(3,4,5-trimethoxyphenyl)-5-(*N*-methyl-indol-5-yl)imidazole (**24h**) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.63 (s, 1H), 7.60 (s, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.20 (dd, J = 7.8, 1.3 Hz, 1H), 7.12 (d, J = 3.1 Hz, 1H), 6.81 (s, 2H), 6.51 (d, J = 3.1 Hz, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 3.53 (s, 6H), 3.48 (s, 3H). ESI (+)/MS: 378 (M + H)$^+$. Anal. Calcd for $C_{22}H_{23}N_3O_3$·HCl: C, 63.84; H, 5.84; N, 10.15. Found: C, 63.61; H, 5.96; N, 10.10.

1-Methyl-4-(3,4,5-trimethoxyphenyl)-5-(3-amino-4-methoxyphenyl)imidazole (25f). Compound **24f**, 1-methyl-4-(3,4,5-trimethoxyphenyl)-5-(3-nitro-4-methoxyphenyl)imidazole, was prepared in a similar way to that of **24h**. ^{1}H NMR ($CDCl_3$): δ 7.89 (d, J = 1.7 Hz, 1H), 7.62 (s, 1H), 7.52 (dd, J = 8.8, 2 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 6.72 (s, 2H), 4.02 (s, 3H), 3.82 (s, 3H), 3.69 (s, 6H), 3.54 (s, 3H). ESI (+)/MS: 400 (M + H)$^+$.

A mixture of **24f** (11.0 g, 27 mmol), $SnCl_2$·$2H_2O$ (12.43 g, 55.0 mmol), and 100 mL of concentrated HCl in 300 mL of EtOH was refluxed for 6 h. The solvent was removed under reduced pressure. The residue was diluted with 1 L of water, neutralized with 50% NaOH, and extracted with EtOAc. The combined organic layers were then washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was recryststallized from MeCN to afford pure 6.8 g (68%) of **25f**. ^{1}H NMR (HCl salt, in MeOH-d_4): δ 9.14 (s, 1H), 7.68−7.56 (m, 4H), 6.65 (s, 2H), 4.07 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H). ESI (+)/MS: 370 (M + H)$^+$, 392 (M + Na)$^+$. Anal. Calcd for $C_{20}H_{23}N_3O_4$·HCl: C, 59.18; H, 5.96; N, 10.35. Found: C, 58.85; H, 5.81; N, 10.23.

1-Methyl-4-(3,4,5-trimethoxyphenyl)-5-(*N*-methyl-3-chloro-indol-5-yl)imidazole (26). A mixture of **24h** (0.377 g, 1 mmol) and NCS (0.16 g, 1.2 mmol) in 10 mL of acetonitrile was stirred for 20 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel eluting with 100:3 EtOAc/methanol to give 0.240 g (58%) of **26** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.66 (m, 1H), 7.59 (s, 1H), 7.40 (dd, J = 8.5, 0.7 Hz, 1H), 7.22 (dd, J = 8.5, 1.7 Hz, 1H), 7.11 (s, 1H), 6.81 (s, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 3.56 (s, 6H), 3.48 (s, 3H). ESI (+)/MS: 378 (M + H)$^+$. Anal. Calcd for $C_{22}H_{22}N_3O_3Cl$: C, 64.15; H, 5.38; N, 10.20. Found: C, 64.28; H, 5.52; N, 10.06.

General Procedure for the Formation of 4,5-Disubstituted Oxazoles. The representative procedure for the formation of 4,5-disubstituted oxazoles is as follows. A mixture of **21a** (0.43 g, 1.2 mmol), 3-benzyloxy-4-methoxybenzaldehyde **6c** (0.242 g, 1 mmol), and potassium carbonate (0.1.34 g, 2.4 mmol) in 10 mL of methanol and 3 mL of DME was refluxed for 2 h. After it was cooled to room temperature, the solution was concentrated in vacuo and diluted with EtOAc. The solution was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with 1:1 EtOAc/hexane to give 0.40 g (89%) of 4-(3,4,5-trimethoxyphenyl)-5-(3-benzyloxy-4-methoxyphenyl)oxazole (**27b**) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.89 (s, 1H), 7.18−7.34 (m, 6H), 6.92−6.89 (m, 4H), 5.06 (s, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.79 (s, 6H). ESI (+)/MS: 448 (M + H)$^+$.

A mixture of **27b** (0.28 g, 0.63 mmol), ammonium formate (0.400 g, 6.3 mmol), 5% palladium on carbon (0.1 g), and 30 mL of methanol was heated under reflux for 2 h. After it was cooled to room temperature, the solution was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by flash chromatography on silica gel eluting with 3:2 EtOAc/hexane to give 0.202 g (90%) of 4-(3,4,5-trimethoxyphenyl)-5-(3-hydroxy-4-methoxyphenyl)oxazole (**28b**) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.91 (s, 1H), 7.26 (d, J = 2.3 Hz, 1H), 7.16 (dd, J = 8.1, 1.9 Hz, 1H), 6.94 (s, 2H), 6.86 (d, J = 8.5 Hz, 1H), 5.5 (br, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.809 (s, 6H). ESI (+)/MS: 358 (M + H)$^+$. Anal. Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.93; H, 5.37; N, 3.88.

4-(3,4,5-Trimethoxyphenyl)-5-(4-*N*,*N*-dimethylaminophenyl)oxazole (27c). Compound **27c** was prepared in a similar manner as described for the preparation of **27b**. ^{1}H NMR ($CDCl_3$): δ 7.88 (s, 1H), 7.53 (d, J = 8.9 Hz, 2H), 6.95 (s, 2H), 6.72 (d, J = 8.8 Hz, 2H), 3.83 (s, 3H), 3.80 (s, 6H), 3.01 (s, 6H). ESI (+)/MS: 355 (M + H)$^+$. Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.83; H, 6.32; N, 7.84.

4-(3,4,5-Trimethoxyphenyl)-5-(*N*-methyl-indol-5-yl)oxazole (27d). Compound **27d** was prepared in a similar manner as described for the preparation of **27b**. ^{1}H NMR ($CDCl_3$): δ 7.95 (m, 2H), 7.50 (dd, J = 8.4, 1.4 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 7.10 (d, J = 3.4 Hz, 1H), 7.00 (s, 2H), 6.51 (d, J = 2.9 Hz, 1H), 3.88 (s, 3H), 3.83 (s, 3H), 3.74 (s, 6H). ESI (+)/MS: 357 (M + H)$^+$, 379 (M + Na)$^+$. Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.14; H, 5.55; N, 7.61.

4-(3,4,5-Trimethoxyphenyl)-5-(3-amino-4-methoxyphenyl)oxazole (28a). Compound **28a** was prepared in a similar manner as described for the preparation of **28b**. ^{1}H NMR (DMSO-d_6): δ 8.40 (s, 1H), 6.95−6.97 (m, 3H), 6.88 (d, J = 8.5 Hz, 1H), 6.80 (dd, J = 8.2, 2.1 Hz, 1H), 4.98 (s, 2H), 6.51 (d, J = 2.9 Hz, 1H), 3.80 (s, 3H), 3.70 (s, 6H), 3.69 (s, 3H). ESI (+)/MS: 357 (M + H)$^+$, 379 (M + Na)$^+$. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 63.95; H, 5.55; N, 7.82.

4-(3-Amino-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)oxazole (30a). Compound **30a** was prepared in a similar manner as described for the preparation of **28b**. ^{1}H NMR ($CDCl_3$): δ 7.90 (s, 1H), 7.16 (d, J = 1.0 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 6.88 (s, 2H), 6.81 (d, J = 8.5 Hz, 1H), 3.88 (s, 6H), 3.79 (s, 6H). ESI (+)/MS: 357 (M + H)$^+$, 379 (M + Na)$^+$. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, C, 63.97; H, 5.53; N, 7.80.

4-(3-Amino-4-methoxyphenyl)-5-(3-methoxy-4,5-methylenedioxyphenyl)oxazole (30b). Compound **30b** was prepared in a similar manner as described for the preparation of **28b**. ^{1}H NMR ($CDCl_3$): δ 7.86 (s, 1H), 7.01−7.05 (m, 2H), 6.85 (d, J = 1.0 Hz, 1H), 6.77−6.81 (m, 2H), 6.00 (s, 2H), 3.88 (s, 3H), 3.82 (s, 3H). ESI (+)/MS: 341 (M + H)$^+$. Anal. Calcd for $C_{18}H_{16}N_2O_5$: C, 63.54; H, 4.74; N, 8.23. Found: C, 63.43; H, 4.49; N, 8.15.

1-Methyl-4-(4-methoxy-3-nitrophenyl)-5-(3,4,5-trimethoxyphenyl)imidazole (31a). Compound **31a** was prepared in a similar manner as described for the preparation of **24h**. ^{1}H NMR ($CDCl_3$): δ 8.05 (d, J = 2 Hz, 1H,), 7.72 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 1.1 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.54 (s, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 3.83 (s, 6H), 3.54 (s, 3H). ESI (+)/MS: 400 (M + H)$^+$.

1-Methyl-4-(4-nitro-3-methylphenyl)-5-(3,4,5-trimethoxyphenyl)imidazole (31b). Compound **31b** was prepared from **19a**, tosmic agent **21c**, and methylamine in a similar manner as described for the preparation of **24h**. ^{1}H NMR ($CDCl_3$): δ 7.84 (d, J = 8.9 Hz, 1H), 7.71 (s, 1H), 7.63 (s, 1H), 7.32 (d, J = 8.9 Hz, 1H), 6.54 (s, 2H), 3.96 (s, 3H), 3.83 (s, 6H), 3.53 (s, 3H). ESI (+)/MS: 384 (M + H)$^+$.

1-Methyl-4-(*N*-methyl-indol-5-yl)-5-(3,4,5-trimethoxyphenyl)imidazole (32). Compound **32** was prepared from **31b** in a similar manner as described for the preparation of compound **10**. ^{1}H NMR ($CDCl_3$): δ 7.85 (d, J = 1.0 Hz, 1H), 7.57 (s, 1H), 7.43 (dd, J = 8.8, 1.6 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 6.98 (d, J = 3.1 Hz, 1H), 6.58 (s, 2H), 6.38 (dd, J = 3.1, 0.3 Hz, 1H), 3.94 (s, 3H), 3.78 (s, 3H), 3.75 (s, 6H), 3.52 (s, 3H). ESI (+)/MS: 378 (M + H)$^+$. Anal. Calcd for $C_{22}H_{23}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C, 69.71; H, 6.30; N, 11.10.

1-Methyl-4-(3-amino-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)imidazole (33). Compound **33** was prepared from **31a** in a similar manner as described for the preparation of **25f**. ^{1}H NMR (HCl salt, in MeOH-d_4): δ 9.14 (1H, s), 7.63 (dd, j = 8.4, 2.2 Hz, 1H), 7.53 (d, J = 1.9 Hz, 1H), 7.44 (d, J = 8.8 Hz, 1H), 6.65 (s, 2H), 4.07 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H), 3.68 (s, 6H). APCI (+)/MS: 370 (M + H)$^+$. Anal. Calcd for $C_{20}H_{23}N_3O_4$·1.1HCl: C, 58.66; H, 5.93; N, 10.26. Found: C, 58.51; H, 5.95; N, 10.25.

5-Bromo-7-chloroindole (35). 1-Acetyl-5-bromo-7-chloroindoline. A mixture of 1-acetyl-5-bromoindoline (10.0 g, 42 mmol) and NCS (6.11 g, 46 mmol) in 300 mL of acetonitrile was heated under reflux for 24 h. After it was cooled to room temperature, the solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel eluting with 1:1 EtOAc/hexane to give 4.23 g (53%) of 1-acetyl-5-bromo-7-chloroindoline as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.38 (m, 1H), 7.27 (m, 1H), 4.18 (t, J = 7.7 Hz, 2H), 3.04 (t, J = 7.7 Hz, 2H), 2.76 (s, 3H). ESI (+)/MS: 275 $(M + H)^+$.

5-Bromo-7-chloroindoline. A mixture of 1-acetyl-5-bromo-7-chloroindoline (4.13 g, 15 mmol) and LiOH (1.44 g, 60 mmol) in 50 mL of MeOH and 50 mL of water was heated under reflux for 12 h. After it was cooled to room temperature, the solvent was removed in vacuo and the residue was purified by flash chromatography eluting with 1:1 EtOAc/hexane to give 3.45 g (99%) of 5-bromo-7-chloroindoline as a white solid. ^{1}H ($CDCl_3$): 7.13 (d, J = 1.7 Hz, 1H), 7.07 (d, J = 1.7 Hz, 1H), 3.97 (br, 1H), 3.62 (t, J = 8.5 Hz, 2H), 3.10 (t, J = 8.5 Hz, 2H). ESI (+)/MS: 233 $(M + H)^+$.

5-Bromo-7-chloroindole (35). A mixture of 5-bromo-7-chloroindoline (3.45 g, 14.8 mmol) and salcomine (0.48 g, 1.48 mmol) in MeOH (600 mL) was stirred overnight while a stream of O_2 was bubbled though the solution. The MeOH was removed, and the residue was purified by flash chromatography eluting with 1:9 EtOAc/hexane to give 3.28 g (96%) of **35** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.75 (br, 1H), 7.67 (dd, J = 1.3, 0.7 Hz, 1H), 7.32 (d, J = 1.7 Hz, 1H), 7.26 (m, 1H), 6.65 (dd, J = 3.1, 2.0 Hz, 1H). ESI (+)/MS: 231 $(M + H)^+$.

5-Formyl-7-chloroindole (36a). A solution of **35** (0.922 g, 4.0 mmol) in 5 mL of anhydrous ether was added to a suspension of 35% KH in mineral oil (0.512 g, 4.5 mmol) in 20 mL of anhydrous ether dropwise at 0 °C. The resulting solution was stirred for 15 min at 0 °C. The solution was cooled to −78 °C and treated with precooled (−78 °C) 1.7 M *t*-BuLi in pentane (5.0 mL, 8.5 mmol). The resulting solution was stirred at −78 °C for 30 min. The solution was treated with DMF (0.585 g, 8 mmol) in 5 mL of anhydrous ether. The resulting solution was stirred at −78 °C for 1 h and then slowly warmed to room temperature. The reaction was treated with 10 mL of 10% HCl(aq) partitioned between EtOAc and water. The organic layer was separated, and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 1:4 EtOAc/hexane to give 0.60 g (83%) of **36a** as a white solid. ^{1}H NMR ($CDCl_3$): δ 10.00 (s, 1H), 8.60 (br, 1H), 8.08 (s, 1H), 7.79 (d, J = 1.3 Hz, 1H), 7.37 (dd, J = 3.2, 2.4 Hz, 1H), 6.77 (dd, J = 3.3, 2.3 Hz, 1H). ESI (+)/MS: 180 $(M + H)^+$.

Trifluoromethanesulfonic Acid 3-Fluoro-4-nitrophenyl Ester. A mixture of 3-fluoro-4-nitrophenol (0.628 g, 4 mmol) and triflic anhydride (2.27 g, 8 mmol) in 30 mL of anhydrous CH_2Cl_2 was treated dropwise with triethylamine (1.62 g, 16 mmol) at 0 °C. The solution was stirred at room temperature for 3 h. The reaction mixture was poured into water, and the layers were separated. The aqueous layer was extracted with additional CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 1:4 EtOAc/hexane to give 1.10 g (98%) of trifluoromethanesulfonic acid 3-fluoro-4-nitrophenyl ester as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.23 (m, 1H), 7.30 (m, 2H). ESI (+)/MS: 290 $(M + H)^+$.

Trifluoromethanesulfonic Acid 7-Fluoroindol-5-yl Ester. Trifluoromethanesulfonic acid 3-fluoro-4-nitrophenyl ester (1.10 g, 3.8 mmol) was dissolved in 50 mL of THF and cooled to −40 °C. The solution was treated dropwise with 1.0 M vinylmagnesium bromide in THF (11.4 mL, 11.4 mmol) at −40 °C. The solution was stirred at −40 °C for 2 h and quenched with aqueous NH_4Cl. The mixture was partitioned between EtOAc and water. The organic layer was separated, and the aqueous layer was extracted with additional EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 15:85 EtOAc/hexane to give 0.307 g (29%) of trifluoromethanesulfonic acid 7-fluoro-indol-5-yl ester as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.50 (br, 1H), 7.36 (m, 2H), 6.92 (dd, J = 10.5, 2.3 Hz, 1H), 6.66 (m, 1H). ESI (+)/MS: 284 $(M + H)^+$.

5-Carbomethoxy-7-fluoroindole (38). A mixture of trifluoromethanesulfonic acid 7-fluoro-indol-5-yl ester (1.19 g, 4.2 mmol), Pd(dppf)Cl_2 (0.11 g, 0.15 mmol), triethylamine (0.85 g, 8.4 mmol), and 40 mL of MeOH in an autoclave was charged with carbon monoxide to 450 psi. The autoclave was heated at 120 °C for 20 h. After it was cooled to room temperature, the reaction mixture was filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with 15:85 EtOAc/hexane to give 0.63 g (84%) of 5-carbomethoxy-7-fluoro-1H-indole as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.55 (br, 1H), 8.22 (s, 1H), 7.60 (dd, J = 11.7, 1.1 Hz, 1H), 7.31 (m, 1H), 6.69 (dd, J = 5.5, 3.3 Hz, 1H), 3.94 (s, 3H). ESI (+)/MS: 194 $(M + H)^+$.

5-Hydroxymethyl-7-fluoroindole. Compound **38** (0.393 g, 2.03 mmol) was dissolved in 10 mL of anhydrous toluene, cooled to −78 °C, and treated with 1.5 M DIBAL in toluene (2.73 mL, 4.1 mmol) at −78 °C. The solution was stirred at −78 °C for 2 h and then warmed to room temperature gradually. The solution was poured in 100 mL of 10% HCl solution and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 3:7 EtOAc/hexane to give 0.300 g (89%) of 5-hydroxymethyl-7-fluoroindole as a white solid. ^{1}H NMR ($CDCl_3$): δ 8.33 (br, 1H), 8.39 (m, 1H), 7.24 (m, 1H), 6.95 (dd, J = 12.3, 1.3 Hz, 1H), 6.56 (m, 1H), 4.74 (d, J = 4.1 Hz, 2H), 1.63 (m, 1H). ESI (+)/MS: 166 $(M + H)^+$.

5-Formyl-7-fluoroindole (36b). A mixture of 5-hydroxymethyl-7-fluoroindole (0.30 g, 1.82 mmol) and PCC (0.77 g, 3.64 mmol) in 50 mL of CH_2Cl_2 was stirred at 0 °C for 2 h. The reaction mixture was diluted with ether and filtered through a pad of Celite. The filtrate was concentrated, and the residue was purified by flash chromatography eluting with 3:7 EtOAc/hexane to give 0.240 g (81%) of **36b** as a white solid. ^{1}H ($CDCl_3$): 10.0 (s, 1H), 8.76 (br, 1H), 7.97 (s, 1H), 7.47 (dd, J = 11.0, 1.2 H, 1H), 7.35 (m, 1H), 6.76 (m, 1H). ESI (+)/MS: 164 $(M + H)^+$.

***N*-Methyl-4-(3,4,5-trimethoxyphenyl)-5-(*N*-methyl-7-chloro-indole-5-yl)imidazole (40a).** Compound **40a** was prepared from **36a** in a similar manner as described for the preparation of **24h**. ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H), 7.50 (d, J = 1.5 Hz, 1H), 7.16 (s, 1H), 7.06 (d, J = 3.0 Hz, 1H), 6.82 (s, 2H), 6.50 (d, J = 3.0 Hz, 1H), 4.19 (s, 3H), 3.80 (s, 3H), 3.59 (s, 6H), 3.49 (s, 3H). ESI (+)/MS: 419 $(M + H)^+$. Anal. Calcd for $C_{22}H_{22}N_3O_3Cl$: C, 64.15; H, 5.38; N, 10.20. Found: C, 64.11; H, 5.36; N, 10.13.

***N*-Methyl-4-(3,4,5-trimethoxyphenyl)-5-(*N*-methyl-7-fluoro-indol-5-yl)imidazole (40b).** Compound **40b** was prepared from **36b** in a similar manner as described for the preparation of **24h**. ^{1}H NMR ($CDCl_3$): δ 7.57 (s, 1H), 7.38 (d, J = 1.5 Hz, 1H), 7.07 d, J = 2.9 Hz, 1H), 6.85 (dd, J = 12.9, 1.1 Hz Hz, 1H), 6.82 (s, 2H), 6.50 (m, 1H), 4.04 (d, J = 1.5 Hz, 3H), 3.79 (s, 3H), 3.68 (s, 6H), 3.47 (s, 3H). ESI (+)/MS: 396 $(M + H)^+$. Anal. Calcd for $C_{22}H_{22}N_3O_3F$: C, 66.82; H, 5.61; N, 10.63. Found: C, 66.75; H, 5.74; N, 10.38.

***N*-Benzhydrylidene-*N'*-(3,4,5-trimethoxyphenyl)hydrazine (42).** A mixture of benzophenone hydrazone (0.614 g, 3 mmol), (±)-BINAP (0.056 g, 0.09 mmol), and Pd(AcO)$_2$ (0.013 g, 0.06 mmol) was suspended in 5 mL of toluene. The mixture was heated at 100 °C for 5 min then and cooled to room temperature. To this red solution, 3,4,5-trimethoxybromobenzene (0.667 g, 2.7 mmol) and sodium *t*-butoxide (0.366 g, 3.8 mmol) were added. The flask was purged with N_2 and then reheated to 100 °C for 16 h. After it was cooled to room temperature, the reaction mixture was filtered through a pad of silica gel and washed with EtOAc. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography eluting with 3:7 EtOAc/hexane to give 0.840 g

(77%) of **42** as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.53−7.62 (m, 5H), 7.29−7.39 (m, 5H), 6.34 (s, 2H), 3.86 (s, 6H), 3.79 (s, 3H). ESI (+)/MS: 363 $(M + H)^+$.

3-Dimethylamino-1-(3-nitro-4-methoxyphenyl)propenone (44). A mixture of 3-nitro-4-methoxyacetophenone **43** (0.390 g, 2 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (0.60 g, 5 mmol) in 5 mL of DMF was heated under reflux for 16 h. The solvents were removed under reduced pressure to afford 0.50 g (98%) of **44** as a yellow solid. ^{1}H NMR ($CDCl_3$): δ 8.38 (d, J = 2.4 Hz, 1H), 8.17 (dd, J = 8.8, 2.3 Hz, 1H), 7.84 (d, J = 12.2 Hz, 1H), 7.12, (d, J = 8.5 Hz, 1H), 5.67 (d, J = 12.2 Hz, 1H), 4.01 (s, 3H), 3.18 (s, 3H), 2.96 (s, 3H). ESI (+)/MS: 251 $(M + H)^+$.

5-(3-Nitro-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)pyrazole (45). Compound **42** (0.73 g, 2 mmol) in 10 mL of ethanol was treated with 10 mL of 6 N HCl(aq). The solution was refluxed until TLC indicated that all of the compound was consumed. The solution was treated with **44** (0.5 g, 2 mmol) and 30 mL of ethanol. The solution was heated under reflux for 16 h. After it was cooled to room temperature, the reaction mixture was concentrated in vacuo, diluted with EtOAc, washed with 2 N Na_2CO_3(aq) and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography eluting with 1:1 EtOAc/hexane to give 0.358 g (48%) of **45** as a pale yellow solid. ^{1}H ($CDCl_3$): δ 7.86 (d, J = 2 Hz, 1H), 7.72 (d, J = 2.1 Hz, 1H), 7.34 (dd, J = 8.8, 2.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.55 (d, J = 1.7 Hz, 1H), 6.52 (s, 2H), 3.97 (s, 3H), 3.87 (s, 3H), 3.74 (s, 6H). ESI (+)/MS: 386 $(M + H)^+$.

5-(3-Amino-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)pyrazole (46). Compound **46** was prepared from **45** in a manner similar to that of **33**. ^{1}H NMR (DMSO-d_6): δ 7.66 (d, J = 1.5 Hz, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.58 (d, J = 1.8, Hz, 1H), 6.57 (s, 2H), 6.43 (d, J = 1.4 Hz, 1H), 6.40 (dd, J = 8.5, 1.9 Hz, 1H), 4.83 (s, 2H), 3.75 (s, 3H), 3.67 (s, 3H), 3.61 (s, 6H). ESI (+)/MS: 356 $(M + H)^+$. Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.82. Found: C, 64.18; H, 6.30; N, 11.77.

1-(4-Dimethylaminophenyl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)ethanone (48b). A mixture of 4-*N*,*N*-dimethylaminobenzaldehyde (7.0 g; 46.9 mmol) and trimethylsilyl cyanide (6.3 mL; 4.7 g: 47.2 mmol) in 100 mL of CH_2Cl_2 was treated with ZnI_2 (50 mg) and stirred at room temperature for 2 h. The reaction was diluted with additional CH_2Cl_2 and washed with water (2×) and brine (1×), dried (Na_2SO_4), and evaporated. The reaction yielded the cyanohydrin **47b** (11.1 g; 95%) as a brown oil that solidified upon standing. The cyanohydrin (10.0 g; 40.3 mmol) in 50 mL of THF was cooled to −78 °C and treated with LDA (48 mL of a 1.05 M solution in THF; 50 mmol). The mixture was stirred for 20 min at −78 °C. Compound **19a** (8.3 g; 42.3 mmol) in 20 mL of THF was added, and the cold bath was removed. The reaction was stirred at room temperature for 1 h. The mixture was acidified with 50 mL of 10% HCl(aq) and stirred for 3 h. The reaction was diluted with water and extracted into EtOAc (3×). The combined extracts were washed with water (3×) and brine (2×), dried ($MgSO_4$), and evaporated. The product was recrystallized from EtOAc/hexane to yield the benzoin product **48b** (6.5 g; 47%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (d, J = 12 Hz, 2H), 6.73 (s, 2H), 6.68 (d, J = 12 Hz, 2H), 5.87 (d, J = 10 Hz, 1H), 5.68 (d, J = 10 Hz, 1H), 3.72 (s, 6H), 3.60 (s, 3H), 2.99 (s, 6H). MS (DCI/NH_3): *m*/*e* 346 $(M + H)^+$.

1-(3-Benzyloxy-4-methoxyphenyl)-2-hydroxy-2-(3,4,5-trimethoxyphenyl)ethanone (48a). The benzoin **48a** was prepared in the same manner as described for the preparation of **48b** using 3-benzyloxy-4-methoxybenzaldehyde instead of 4-*N*,*N*-dimethylaminobenzaldehyde. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.77 (dd, J = 12 Hz, 3 Hz, 1H), 7.60 (d, J = 3 Hz, 1H), 7.32−7.55 (m, 5H), 7.04 (d, J = 12 Hz, 1H), 6.75 (s, 2H), 5.99 (d, J = 10 Hz, 1H), 5.89 (d, J = 10 Hz, 1H), 5.15 (d, J = 12 Hz, 1H), 5.08 (d, J = 12 Hz, 1H), 3.83 (s, 3H), 3.71 (s, 6H), 3.60 (s, 3H). MS (DCI/NH_3): *m*/*e* 439 $(M + H)^+$, *m*/*e* 456 $(M + NH_4)^+$.

2-Methyl-4-(3-hydroxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)oxazole (49). The benzoin **48a** (300 mg; 0.68 mmol), acetic anhydride (216 mg; 2.12 mmol), and DMAP (5 mg) were combined in 5 mL of CH_2Cl_2 and stirred at room temperature for 4 h. The reaction was diluted with EtOAc and washed with water (2×) and brine (1×), dried over $MgSO_4$, and evaporated to give acetic acid 2-(3-benzyloxy-4-methoxyphenyl)-2-oxo-1-(3,4,5-trimethoxyphenyl)ethyl ester (325 mg, 99%) as an off-white solid that was used with no further purification. ^{1}H NMR (300 MHz, DMSO−d_6): δ 7.79 (dd, J = 12 Hz, 3 Hz, 1H), 7.61 (d, J = 3 Hz, 1H), 7.32−7.53 (m, 5H), 7.07 (d, J = 12 Hz, 1H), 6.85 (s, 2H), 5.17 (d, J = 12 Hz, 1H), 5.05 (d, J = 12 Hz, 1H), 3.82 (s, 3H), 3.73 (s, 6H), 3.61 (s, 3H).

Acetic acid 2-(3-benzyloxy-4-methoxyphenyl)-2-oxo-1-(3,4,5-trimethoxyphenyl)ethyl ester (300 mg; 0.62 mmol) and NH_4-OAc (670 mg; 6.24 mmol) were combined in 5 mL of glacial acetic acid and heated at reflux for 6 h. The cooled reaction mixture was poured into 2 N NaOH(aq) and extracted into EtOAc (2×). The combined extracts were washed with water (2×) and brine (1×), dried ($MgSO_4$), and evaporated. The product was isolated by flash chromatography on silica gel eluting with 30% EtOAc/hexane to yield 2-methyl-4-(3-benzyloxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)oxazole **(**215 mg, 74%) as an off-white, fluffy solid. ^{1}H NMR (300 MHz, DMSO−d_6): δ 7.32−7.48 (m, 5H), 7.17−7.24 (m, 2H), 7.03 (d, J = 12 Hz, 1H), 6.81 (s, 2H), 4.98 (s, 2H), 3.79 (s, 3H), 3.71 (s, 6H), 3.69 (s, 3H), 3.65 (s, 3H). MS (DCI/NH_3): *m*/*e* 462 $(M + H)^+$.

2-Methyl-4-(3-benzyloxy-4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)oxazole (200 mg; 0.43 mmol) and 10% Pd/C (40 mg) in 5 mL of 3:1 EtOH:EtOAc were stirred for 2 h under an atmosphere of H_2(g) provided via a balloon. The mixture was filtered through Celite and rinsed with EtOH. Evaporation of the solvent yielded the product as a white solid (143 mg; 89%).^{1}H NMR (300 MHz, DMSO-d_6): δ 9.50 (br s, 1H), 7.05−7.10 (m 2H), 6.90 (d, J = 12 Hz, 2H), 6.80 (s, 2H), 3.8 (s, 3H), 3.70 (s, 9H), 2.47 (s, 3H). MS (DCI/NH_3): *m*/*e* 372 $(M + H)^+$. Anal. Calcd for $C_{20}H_{21}NO{\cdot}0.6CH_3OH$: C, 63.34; H, 6.04; N, 3.58. Found: C, 63.14; H, 5.81; N, 3.24; mp 70−73 °C.

4(5)-(4-*N*,*N*-Dimethylaminophenyl)-2-(3-furyl)-5(4)-(3,4,5-trimethoxyphenyl)imidazole (50). Benzoin **48b** (3.0 g; 8.7 mmol) was dissolved in 15 mL of pyridine and treated with a solution of $CuSO_4{\cdot}5H_2O$ (5.4 g; 21.7 mmol in 5 mL water). The mixture was heated at reflux for 2 h. The mixture was diluted with water and extracted into EtOAc. The extracts were rinsed with brine (2×), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography on silica gel eluting with 35% EtOAc/hexane to yield 1-(4-dimethylaminophenyl)-2-(3,4,5-trimethoxyphenyl)ethane-1,2-dione (2.5 g; 84%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, J = 12 Hz, 2H), 7.76 (s, 2H), 6.67 (d, J = 12 Hz, 2H), 3.93 (s, 3H), 3.88 (s, 6H), 3.11 (s, 6H). MS (DCI/NH_3): *m*/*e* 344 $(M + H)^+$, *m*/*e* 361 $(M + NH_4)^+$.

1-(4-Dimethylaminophenyl)-2-(3,4,5-trimethoxyphenyl)-ethane-1,2-dione (343 mg; 1.0 mmol), 3-furan carboxyaldehyde (2.0 g; 2.1 mmol), and NH_4OAc (2.1 g; 20 mmol) in 10 mL of HOAc were heated at reflux for 4 h. The reaction mixture was poured into water and neutralized with solid K_2CO_3. The mixture was extracted into EtOAc (3×). The combined extracts were rinsed with saturated $NaHCO_3$(aq) and then filtered through Celite. The filtrate was rinsed with brine, dried (Na_2-SO_4), and evaporated. The residue was purified by flash chromatography on silica gel eluting with 45% EtOAc/hexane. The material was recrystallized from EtOAc to yield 140 mg (33%) of the product as a white solid. The product was a 2:1 mixture of imidazole tautomers (as determined by ^{1}H NMR). Major tautomer: ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.29 (br s, 1H), 8.18−8.20 (m, 1H), 7.74−7.78 (m, 1H), 7.32 (d, J = 8 Hz, 2H), 6.98−7.00 (m, 1H), 6.86 (s, 2H), 6.78 (d, J = 8 Hz, 2H), 3.71 (s, 3H), 3.63 (s, 6H), 2.92 (s, 6H). Minor tautomer: ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.23 (br s, 1H), 8.18−8.20 (m, 1H), 7.74−7.78 (m, 1H), 7.41 (d, J = 8 Hz, 2H), 6.98−7.00 (m, 1H), 6.81 (s, 2H), 6.71 (d, J = 8 Hz, 2H), 3.71 (s, 3H), 3.66 (s, 6H), 2.89 (s, 6H). MS (DCI/NH_3): *m*/*e* 420 $(M + H)^+$. Anal. Calcd for $C_{24}H_{25}N_3O_4$: C, 68.72; H, 6.01; N, 10.02. Found: C, 68.66; H, 6.15; N, 10.01.

2-(4-*N*,*N*-Dimethylaminophenyl)-3-(3,4,5-trimethoxyphenyl)pyrazine (51). 1-(4-Dimethylaminophenyl)-2-(3,4,5-trimethoxyphenyl)ethane-1,2-dione (225 mg; 0.65 mmol) in 10 mL of EtOH was treated with ethylenediamine (72 mg; 1.20 mmol), heated at reflux for 12 h, and then stirred for 2 days at room temperature. Additional ethylenediamine was added (22 mg; 0.37 mmol), and the reaction was heated at reflux overnight. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2. The solution was washed with water (1×) and brine (2×), dried (Na_2SO_4), and evaporated. The resulting material was treated with elemental sulfur (40 mg; 1.22 mmol) and heated at 140 °C for 30 min. The product was isolated by flash chromatography on silica gel eluting with 15% EtOAc/CH_2Cl_2 to yield **51** (95 mg; 42%) as a sticky yellow foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (br s, 1H), 8.46 (br s, 1H), 7.37 (br d, 2H), 6.76 (s, 2H), 6.64 (br d, 2H), 3.86 (s, 3H), 3.72 (s, 6H), 2.97 (s, 6H). MS (DCI/NH_3): *m/e* 366 $(M + H)^+$. Anal. Calcd for $C_{21}H_{23}N_3O_3$: C, 69.02; H, 6.34; N, 11.50. Found: C, 68.97; H, 6.37; N, 11.40.

Cell Proliferation Assay. An in vitro antitumor assay was conducted in a 96 well microtiter format utilizing HCT-15 (human colon adenocarcinoma, MDR positive) and NCI-H460 (human lung large cell carcinoma, MDR negative) cancer lines based on the sulforhodamine protocol established by the NCI.[22] Each compound was added to the cultured cells, with final concentrations in the range of 10^{-11} to 10^{-4} M. Two controls were performed within each microtiter plate: a solvent (DMSO) control without drug to yield a 0% inhibition level and trichloracetic acid (TCA)-treated wells to yield a 100% inhibition level. The cells were then grown in culture (37 °C, 5% CO_2 atmosphere) for 48 h. The cells were fixed by the addition of TCA and then stained with sulforhodamine. After the cells were washed, the adherent dye was solubilized by the addition of Tris buffer, and the absorbance of the solutions was measured with a Molecular Devices SpectraMax 340 plate reader. The IC_{50} values were determined by a nonlinear regression analysis using Microsoft Excel. Compounds were tested at least twice independently, and the IC_{50} values reported here are the geometric mean of these measurements.

Microtubule Polymerization Assay. A microtubule polymerization assay was conducted in Corning-Costar Half-Area (A/2) 96 well plates according to the established protocol of Gaskin et al.[23] Microtubule proteins were purified from bovine brain by the procedure of Shelanski et al.,[24] or purified tubulin in glycerol (T237) was purchased from Cytoskeleton. Drug or solvent control (5 μL) was first added to each well. The tubulin and buffer components were prechilled to 4 °C, and finally, GTP (guanosine 5′-triphosphate) was added. The tubulin−buffer−GTP solution was mixed thoroughly, and then, 95 μL was added to each test well containing drug or solvent. The plate reader was prewarmed to 37 °C, and polymerization began within 2−3 min as the temperature of the solutions rose. The light scattering of the microtubule solutions was followed at 340 nm for 30 min. The degree of polymerization was determined from the AUC for the polymerization profiles, and the IC_{50} values were determined by a nonlinear regression analysis using Microsoft Excel. Compounds were tested at least twice independently, and the IC_{50} values reported here are the geometric means of these measurements.

In Vivo Antitumor Activity. Approximately 7 week old C57BL/6 inbred mice (10/study group) were inoculated subcutaneously (SC) with 0.5 mL of a 1:10 brei of the murine M5076 reticulum sarcoma cells on study day 0. Therapy was initiated on day 1 for all study drugs and lasted 5 days. The compounds **24h** and **25f** formulated as their hydrochloride salts were given once orally at doses of 50, 37.5, and 28 mg/kg/day and 37.5, 25, 12.5 and mg/kg/day, respectively. Vehicles for **24h** and **25f** were administrated as the untreated control. In the regression trial, animals were treated with compound **25f** orally on day 11 after inoculation of the murine M5076 reticulum sarcoma cells. The therapy consisted of continuous once daily dosing of **25f** at 30 mg/kg/day. The average tumor mass was approximately 300 mg when treatment was started. The mice were monitored daily for clinical signs. Tumors were measured 3 times per week. Animals were euthanized when moribund or when tumors exceeded 1 g. Tumor mass was calculated from the formula mass $= (L \times W^2)/2$. The day of termination was recorded for each individual and was used to calculate percent ILS $[(T \text{ (treated)} - C \text{ (control)}/C] \times 100\%$ where $[T - C]$ = mean days delay to 1 g.

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Supporting Information Available: The X-ray structure report of compound **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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