Design, Synthesis, and Evaluation of Novel Thienopyrrolizinones as Antitubulin Agents

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Herein, we describe the structure−activity relationship study of a new 3-aryl-8*H*-thieno[2,3-*b*]pyrrolizin-8-one series of antitubulin agents. The pharmacological results from the National Cancer Institute in vitro human disease oriented tumor cell line screening allowed us to identify compound **1d** (NSC 676693) as a very efficient antitumoral drug in all cancer cell lines tested. This prompted us to define the structural requirements essential for this antiproliferative activity. Among all analogues synthesized in this study, compound **1o** was the most promising, being 10-fold more potent than compound **1d**. Its activity over a panel of nine tumoral cell lines was in the nanomolar range for all of the histological types tested, and surprisingly, the resistant KB-A1 cell line was also sensitive to this compound. Moreover, a flow cytometric study showed that L1210 cells treated by the most potent compounds were arrested in the G_2/M phases of the cell cycle with a significant percentage of cells having reinitiated a cycle of DNA synthesis without cell division. This interesting pharmacological profile, resulting from inhibition of tubulin polymerization, encouraged us to perform preliminary in vivo studies that led to a new prodrug chemical approach.

Introduction

Cancer today is still an important clinical problem with its prognosis remaining relatively poor for the majority of tumors. Surgery, radiotherapy, and chemotherapy all have an important role to play in the treatment of cancer, either alone or combined with each other to define more effective strategies. Moreover, we have gained remarkable biological knowledge about the exact steps necessary for cancer cells to grow, divide, and spread. This has opened the door for new prospects in chemotherapy to stop or reverse this proliferative process, especially using targeted approaches based on regulation of the cancer cell cycle like tubulin dynamic inhibition. Indeed, the mitotic spindle of eukaryotic cells is an attractive target for the development of compounds useful in anticancer therapy.[1−3] Microtubules show highly dynamic instability and play an essential role in mitosis.[4] Chemicals that attack microtubules through their major component, tubulin, disrupt or suppress both microtubule structure and normal functions by inhibition or promotion of microtubule assembly, resulting in cell arrest in mitosis.[5]

In previous chemical studies aimed at researching new microtubule polymerization inhibitors, we performed the synthesis of 3-substituted 8*H*-thieno[2,3-*b*]-pyrrolizin-8-ones **1a**−**d** (Figure 1).[6] To investigate the potential antitumor interest of this original chemical series, these products were submitted to the National Cancer Institute in vitro human disease oriented tumor cell line screening panel.[7] One of these compounds, **1d** (NSC 676693), expressed a cytotoxic activity in all cancer cell lines tested as shown in Table 1. These results obtained at submicromolar concentrations prompted the National Cancer Institute to perform hollow fiber and xenograft evaluations for **1d** and encouraged us to further investigate the structural parameters associated with its biological activities. Structure−activity relationship studies were started from compound **1d** to design and synthesize more potent analogues useful as anticancer agents and to define the minimal required structure to maintain the cytotoxic activity. So we successively explored the importance of cycle D (route 1), thiophene A (route 2), and pyrrolizine BC (route 3) (Figure 2).

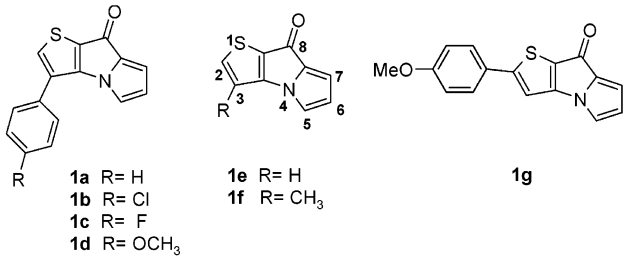

Figure 1.

Chemistry

Route 1. First, we decided to study the influence of an aromatic substitution with the synthesis of three compounds already described by our team: the unsubstituted thienopyrrolizinone **1e**,[8] the 3-methyl derivative **1f**,[9] and finally **1g**,[10] the isomer of **1d** (Figure 1). The

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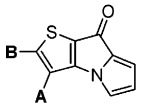
Table 1. Cytotoxicity of Compounds **1a**−**d** from the NCI 60 Tumoral Cell Lines Panel (log GI_{50} Effects in M[a])

cell type: cell line:	leukemia CCRF-CEM	NSCL[b] NCI-H522	colon SW-620	CNS[c] SF-295	melanoma M-14	ovarian OVCAR-3	renal XF-393	prostate PC-3	breast MDA-N
1a (NSC 683507)	−4.7	−4.9	−4.8	−4.7	−4.7	−4.8	−4.9	−4.9	−4.8
1b (NSC 683509)	−4.0	−5.5	−4.0	−4.0	−4.3	−4.0	−4.0	−4.0	−5.1
1c (NSC 683508)	−4.5	−4.8	−4.6	−4.6	−4.5	−4.3	−4.6	−4.5	−5.1
1d (NSC 676693)	−7.5	−7.8	−7.6	−7.4	−7.5	−7.4	−7.2	−7.4	−7.9

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition. [b] Non-small-cell lung. [c] Central nervous system.

Table 2. In Vitro Antiproliferative Activity and L1210 Cell Cycle Effects of Thienopyrrolizinone Analogues (Route 1)



compd	A	B	GI_{50} (μM)	effect on the cell cycle[a]
1d	$C_6H_4pOCH_3$	H	0.19	83% G2 + M + 8N at 0.5 μM
1e	H	H	85	NT[b]
1f	CH_3	H	>100	NT[b]
1g	H	$C_6H_4pOCH_3$	9.4	NT[b]
1h	$C_6H_4oOCH_3$	H	18.9	75% G_2 + M + 8N at 50 μM
1i	$C_6H_4mOCH_3$	H	5.2	94% G_2 + M + 8N at 25 μM
1j	$C_6H_3(m,p\text{di}OCH_3)$	H	11.7	86% G_2 + M + 8N at 25 μM
1k	$C_6H_2(o,m,p\text{tri}OCH_3)$	H	36.4	NT[b]
1l	$C_6H_3(m,pOCH_2O)$	H	4.2	88% G_2 + M + 8N at 10 μM
1m	$C_6H_3(m\text{OBn},pOCH_3)$	H	5.4	80% G_1 at 25μM
1n	$C_6H_3(mOCH_3,p\text{OBn})$	H	24.1	NT[b]
1o	$C_6H_3(m\text{OH},pOCH_3)$	H	0.015	86% G_2 + M + 8N at 0.5 μM
1p	$C_6H_3(mOCH_3,p\text{OH})$	H	8	86% G_2 + M + 8N at 25 μM
7a	$C_6H_4o\text{OH}$	H	3.7	72% G_1 at 50 μM
7b	$C_6H_4m\text{OH}$	H	29	NT[b]
7c	$C_6H_4p\text{OH}$	H	30.1	NT[b]
7d	$C_6H_3(m,p\text{diOH})$	H	0.28	86% G_2 + M + 8N at 0.5 μM
7e	$C_6H_2(o,m,p\text{triOH})$	H	12	NT[b]
8a	$C_6H_4pOC_2H_5$	H	0.43	88% G_2 + M + 8N at 1 μM
8b	$C_6H_4pOnC_3H_7$	H	28.1	NT[b]
8c	$C_6H_4pOiC_3H_7$	H	12.2	NT[b]
8d	$C_6H_4pOnC_4H_9$	H	29.6	NT[b]

[a] Percent of untreated cell in the phases of the cycle: 41% (G_1); 28% (S); 24% (G_2 + M); 1% (8N). [b] NT: not tested.

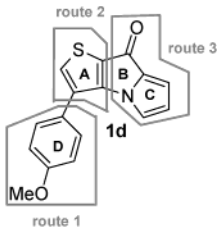


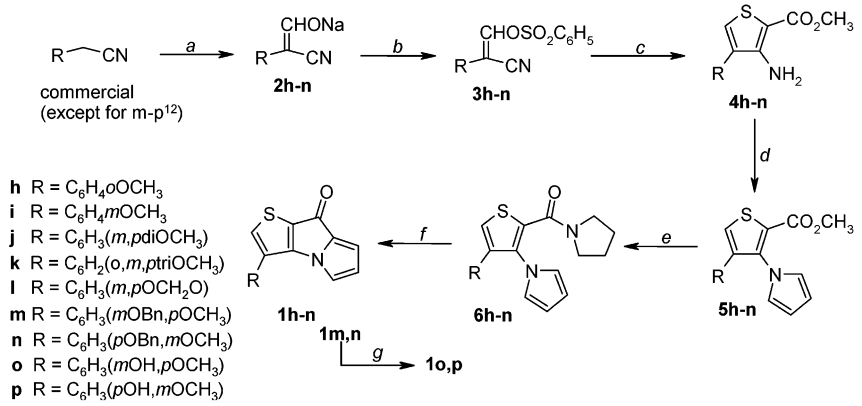
Figure 2.

in vitro antiproliferative activity of these three compounds **1e**−**g** on the L1210 cell line was 100-fold lower than that of compound **1a** (Table 2). This unsatisfactory result together with that obtained by the National Cancer Institute for the phenyl derivative **1a** and the *p*-chloro and *p*-fluorophenyl derivatives **1b**−**c** prompted us to synthesize a series of compounds where the thienopyrrolizinone moiety is substituted at position 3 by an alkoxyphenyl or a hydroxyphenyl group (derivatives **1h**−**p**, **7a**−**e**, **8a**−**d**, Schemes 1−3).[11] In a preliminary communication, we described a general route to prepare 3-substituted thienopyrrolizinones from suitable arylacetonitriles (Scheme 1). These nitriles precursors are commercially available excepted for those necessary for the synthesis of compounds **1m**−**p**, which were prepared from *O*-benzyl-protected isovanillin and vanillin.[12] Then treatment of arylacetonitriles with ethyl formate and sodium methoxide led to the corresponding hydroxyacrylonitrile sodium salts **2h**−**n** in 53−98% yields. These were then protected by a benzenesulfonyl group to give **3h**−**n**. Reaction of the latter compounds with methyl thioglycolate and sodium methoxide afforded methyl 3-amino-4-arylthiophen-2-carboxylates **4h**−**n** in good yields.[13−15] Treatment with dimethoxytetrahydrofuran (dimethoxyTHF) and 4-chloropyridine hydrochloride in dioxane gave the pyrrolylthiophene carboxylates **5h**−**n**, which led to the corresponding carboxamides **6h**−**n** in refluxing pyrrolidine. Cyclization of the latter compounds was performed by action of phosphorus oxychloride to give, via an intermediate iminium salt subsequently hydrolyzed in alkaline condition, the tricyclic ketones **1h**−**n** in 47−71% yields. Phenolic compounds **1o**−**p** were obtained from compounds **1m**−**n** in 53 and 58% yields, respectively, by cleavage of the *O*-benzyl protecting group using a 33% hydrobromic acid solution in glacial acetic acid (Scheme 1).

The methoxyphenyl derivatives **1d**,**h**−**k** were demethylated, affording the corresponding phenols **7a**−**e** (Scheme 2). The best yields were obtained using 1 equiv of boron tribromide for each methoxy group to cleave.[16] Then, to explore relationship between cytotoxic activity and steric parameters from alkoxy substituents, we synthesized a series of *p*-alkoxyphenyl derivatives from the **1d**-derived phenolic compound **7c**. This one readily reacted with various commercial alkyl bromides in the presence of sodium methoxide to yield the corresponding ethers **8a**−**d** in 63−77% yields (Scheme 3).

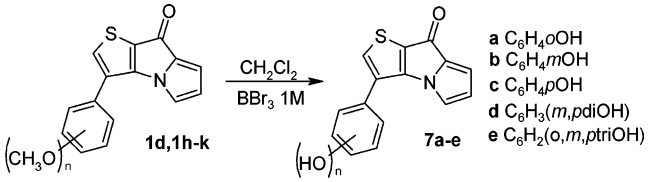
To continue our SAR study on compound **1d**, we decided to investigate the thieno[2,3-*b*]pyrrolizin-8-one skeleton and more precisely the role played by the

Scheme 1[a]

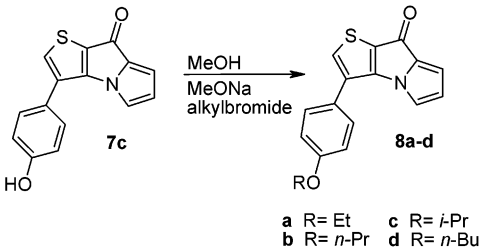


[a] Reaction conditions: (a) ethyl formate, MeONa, MeOH; (b) sulfonylbenzene chloride, DMF; (c) methyl thioglycolate, MeONa, MeOH; (d) dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (e) pyrrolidine; (f) (i) $POCl_3$, (ii) 10% NaOH; (g) 33% HBr in AcOH.

Scheme 2



Scheme 3



thiophene (route 2) and the pyrrolizinone rings (route 3) in global cytotoxic activity (Figure 2).

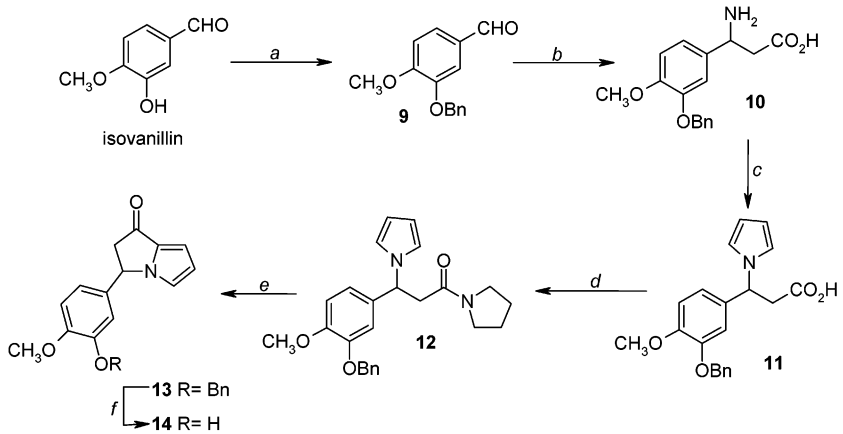
Route 2. Before synthesizing isosteric analogues of **1d**, we wanted to know what would be the activity of one analogue where cycle D was directly linked to the pyrrolizinone ring without fused thiophene.

So, considering the best cytotoxic compound **1o** ($IC_{50(L1210)} = 0.015\ \mu M$) obtained in route 1 (see Biological Results and Discussion part, Table 2), we synthesized the bicyclic pyrrolizinone analogue **14**. Its preparation was performed starting from the isovanillin, taking into account our previous work on the synthesis of such pyrrolizinones[17] and using the method of selective *O*-benzyl deprotection (Scheme 4).

Isovanillin was first protected by a benzyl group, providing the *O*-protected isovanillin **9** with excellent yield, which gave in a one-pot reaction the corresponding β-aminopropanoic acid **10** in 58% yield, using ammonium acetate and malonic acid in ethanol.[18,19] Compound **10** was then heated in acetic acid in the presence of 2,5-dimethoxyTHF to provide pyrrole **11**, which was converted into the corresponding amide **12** by treatment with ethyl chloroformate, triethylamine, and pyrrolidine. Intramolecular ring closure was performed using phosphorus oxychloride in toluene to yield a Vilsmeier salt, which was then hydrolyzed in alkaline conditions into pyrrolizinone **13**. Finally, deprotection of compounds **13** using a 33% hydrobromic acid solution in glacial acetic acid gave 3-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one **14** in 65% yield (Scheme 4).

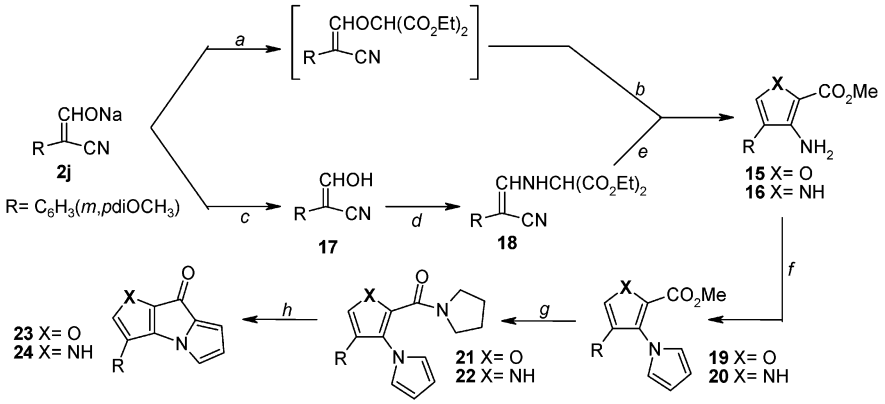
In the second part of route 2, a more exhaustive chemical study was run with the synthesis of bioisosteric analogues in the furane, pyrrole, and benzene series. The chemical strategy adopted for the heterocyclic ones (furane−pyrrole) is based on the general route described for thiophene series (Scheme 1). First, we needed to develop the original chemistry to synthesize

Scheme 4[a]



[a] Reaction conditions: (a) BnBr, K_2CO_3, MeOH; (b) malonic acid, ammonium acetate, EtOH; (c) 2,5-dimethoxyTHF, acetic acid; (d) (i) ethyl chloroformate, NEt_3, acetone, (ii) pyrrolidine; (e) (i) $POCl_3$, toluene, (ii) 5% NaOH; (f) 33% HBr in AcOH.

Scheme 5[a]

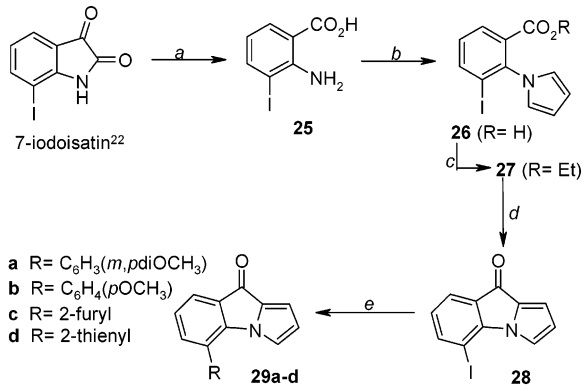


[a] Reaction conditions: (a) diethyl chloromalonate, DMF; (b) DBN, MeOH; (c) AcOH, H_2O; (d) AcONa, diethyl aminomalonate HCl, MeOH/H_2O; (e) MeONa, MeOH; (f) 2,5-dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (g) pyrrolidine (h) (i) $POCl_3$, (ii) 10% NaOH.

the key intermediates, i.e., methyl 3-amino-4-arylfuran-2-carboxylate **15** and methyl 3-amino-4-aryl-1*H*-pyrrol-2-carboxylate **16**, respectively. We describe herein only the synthesis of one compound for each series with 3,4-dimethoxyphenyl as the substituent because these syntheses are very difficult and are currently under investigation. The route outlined in Scheme 5 started with the hydroxyacrylonitrile sodium salt **2j**. Condensation of the latter with diethyl chloromalonate in *N*,*N*-dimethylformamide (DMF) provided an unstable vinyl ether intermediate that directly cyclized into **15** after removal of the solvent and in the presence of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in methanol.[20] The corresponding pyrrole **16** was obtained from **2j** following Elliot's procedure.[21] So compound **2j** under acidic conditions gave the corresponding enol **17**, which was then reacted with diethylaminomalonate hydrochloride to provide the enamine **18**. Ring closure was performed by treatment with sodium methoxide in methanol in 85% yield. At the end, the expected aminoesters **15** and **16** were submitted successively to the Clauson−Kaas reaction and condensation with pyrrolidine to yield the corresponding amides **21** and **22**, respectively. The latter compound was treated with phosphorus oxychloride, and a 10% aqueous sodium hydroxide solution gave the tricyclic pyrrolizinones **23** and **24**, respectively (Scheme 5).

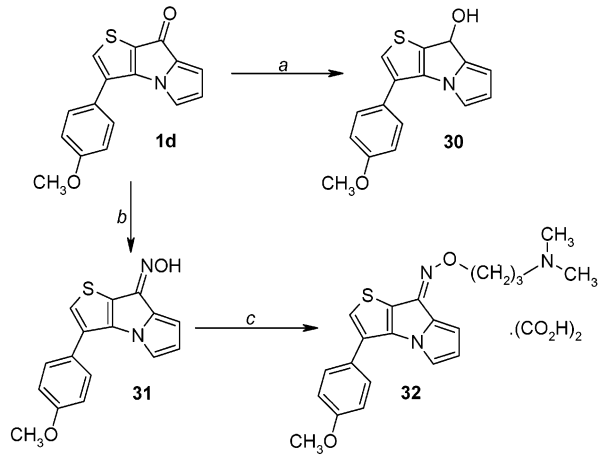
By analogy, the thiophene ring being considered as the first bioisoster of the benzene ring, this prompted us to prepare a series of 5-arylpyrroloindolones **29a**−**d** in which the phenyl ring was fused to pyrrolizinone instead of thiophene. Indeed, molecular modeling studies showed that the three-dimensional structures of these compounds were very close to those in the thiophene series as assessed by preliminary X-ray crystallographic data. So we synthesized the direct analogues **29** in which the aryl substituent is in the ortho position related to the pyrrole link and not in the meta. By analysis of possible approaches to obtain **29**, a convergent pathway starting from the easily accessible 7-iodoisatin and based on our previous studies[22] was followed. As shown in Scheme 6, oxidative cleavage of 7-iodoisatin was readily performed in an alkaline medium in the presence of hydrogen peroxide, giving the expected anthranilic acid **25** in 81% yield.[23] Introduction of a pyrrole ring was accomplished by reaction with 2,5-

Scheme 6[a]



[a] Reaction conditions: (a) 5% NaOH, 30% H_2O_2; (b) 2,5-dimethoxyTHF, 4-chloropyridine hydrochloride, dioxane; (c) $SOCl_2$, EtOH; (d) 1 M BBr_3, CH_2Cl_2; (e) $RB(OH)_2$, $NaHCO_3$, $Pd(PPh_3)_4$, DME/H_2O.

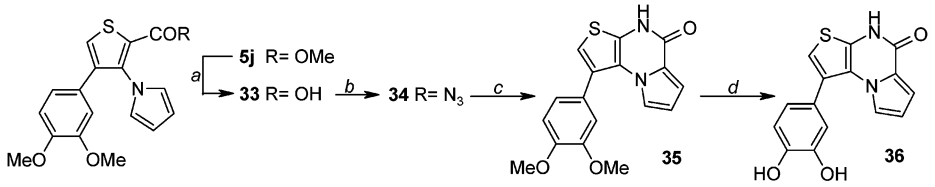
Scheme 7[a]



[a] Reaction conditions: (a) $NaBH_4$, MeOH; (b) $NH_2OH{\cdot}HCl$, pyridine; (c) (i) $Cl(CH_2)_3N(CH_3)_2$, K_2CO_3, HCl, acetone/H_2O, (ii) oxalic acid, isOH.

dimethoxyTHF. Initially it was thought that the intermediate acid **26** could be cyclized directly using Vilsmeier−Haack conditions (DMF/$POCl_3$), but all attempts failed. So we considered a subsequent esterification by treatment of **26** using thionyl chloride in ethanol. Treatment of the corresponding carboxylate **27** by boron tribromide in dichloromethane afforded 5-iodopyrroloin-

Scheme 8[a]



[a] Reaction conditions: (a) NaOH, EtOH; (b) NEt_3, NaN_3, acetone; (c) 1,2-dichlorobenzene; (d) 1 M BBr_3, CH_2Cl_2.

dolones **28** in 62% yield.[24] The last step was performed via a Suzuki cross coupling with commercial arylboronic acids providing 5-arylpyrroloindolones **29a**−**d** in 58−73% yields. So compounds **29a**−**d** were synthesized in five steps from 7-iodoisatin and with overall yields of 15−18% (Scheme 6).

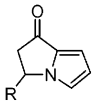
Route 3. In the third route aimed at studying the role played by the pyrrolizinone ring, the carbonyl group of **1d** was first reduced in alcohol **30** using sodium borohydride, and then condensation with hydroxylamine in pyridine gave the oxime **31** in 95% yield. The latter was subsequently substituted by a chloroamine chain under appropriate conditions to form the corresponding oxime ether **32** (Scheme 7). To determine the importance of the central ring size and the need for a planar tricyclic system, pyrrolothienopyrazinones **35** and **36** were prepared from ester **5j**. Saponification of **5j** leading to the corresponding acid **33** allowed formation of azide **34** in 70% yield. Treatment of **34** in hot *o*-dichlorobenzene provided the pyrazinone **35** by ring closure via a modified Curtius rearrangement.[25,26] Finally *O*-demethylation using boron tribromide gave the corresponding diphenol **36** (Scheme 8).

Biological Results and Discussion

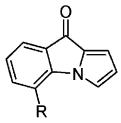
In vitro evaluation of the compounds' antitumor activity and particularly their effects on the cell cycle were studied on the L1210 cell line (Tables 2−4). Analysis of the results obtained for the derivatives resulting from our SAR study enables us to specify the structural requirements to identify a new lead compound and finally to begin understanding their mechanism of action.

First, thienopyrrolizinone **1d** (NSC 676693) confirmed the promising results provided by the National Cancer Institute because it expresses a GI_{50} of 0.19 μM on the L1210 cell line and this activity seemed closely dependent on the cycle in position 3. Indeed, absence of this cycle or its replacement by a small alkyl substituent totally abolished the activity (**1e**, $GI_{50} = 85$ μM; **1f**, $GI_{50} > 100$ μM). Similarly, **1g**, the position isomer of **1d**, was found to be 100 times less active ($GI_{50} = 9.4$ μM) (Table 2). Considering substitutions on the cycle in position 3, it appeared, as indicated in Table 2, that weak structural modifications were responsible for the large GI_{50} variation and that the hydroxy (**7a**−**d**) and methoxy (**1d**,**h**−**l**) series are not really comparable in term of SAR. Let us note here the activity of the diphenol **7d**, which expressed a GI_{50} of 0.28 μM, similar to those of **1d**. However, the substituent size seemed to be a feature closely related to the activity. Among the ethers **8a**−**d**, only the ethyl one **8a** ($GI_{50} = 0.43$ μM) kept an activity similar to that of **1d**, and any additional steric hindrance harmed the activity (**8b**−**d**, $GI_{50} > 10$ μM).

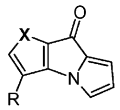
Table 3. In Vitro Antiproliferative Activity on L1210 Cells (Route 2)



compd	R	GI_{50} (μM)
14	C_6H_3(*m*OH,*p*OCH_3)	25

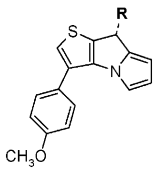


compd	R	GI_{50} (μM)
29a	C_6H_3(*m*,*p*diOCH_3)	72.2
29b	C_6H_4*p*OCH_3	3
29c	2-furyl	27.6
29d	2-thienyl	71.9

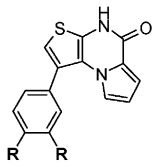


compd	X	R	GI_{50} (μM)
23	O	C_6H_3(*m*,*p*diOCH_3)	24.2
24	NH	C_6H_3(*m*,*p*diOCH_3)	11.8

Table 4. In Vitro Antiproliferative Activity on L1210 Cells (Route 3)



compd	R	GI_{50} (μM)
30	−OH	4.9
31	=NOH	34.3
32	=NO$(CH_2)_3$N$(CH_3)_2$ (oxalate)	3.9



compd	R	GI_{50} (μM)
35	OCH_3	>100
36	OH	8.8

Taking into account the fact that the two more active compounds are **1d** and **7d**, we considered the synthesis of "mixed" compounds bearing either a hydroxy or

Table 5. In Vitro Antiproliferative Activity of **1o** against a Panel of Nine Tumor Cell Lines

cell line:	L1210	B16	OVCAR3	A2780	DU145	A549	HT29	LS174T	KBA1	P388
1o GI_{50} (nM):	15	23	31	40	43	45	713	887	15	21

Chart 1. X-ray Crystal Structure for **1o**

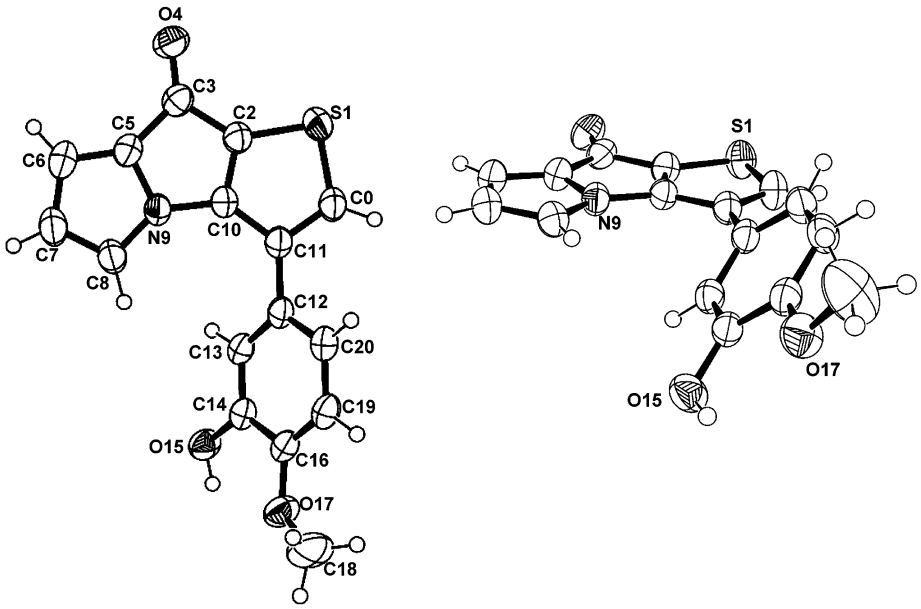
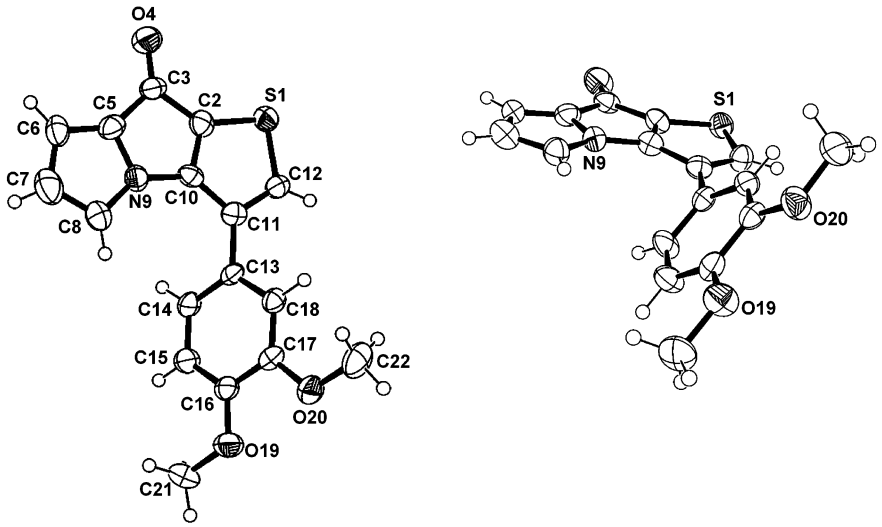


Chart 2. X-ray Crystal Structure for **1j**



methoxy substituent in the 3 and 4 positions. These vicinal methoxy and hydroxy groups on a phenyl ring appear to be a recurrent theme among antitubulin agents[1,5] and more particularly with combretastatin A-4 and analogues.[27] Our first attempts to prepare these analogues starting from the dimethoxy derivative **1j** failed to produce selective *O*-demethylation and gave only a mixture of monomethylated compounds. Similarly, mono-*O*-methylation of the dihydroxy derivative **7d** using various conditions also failed. This prompted us to prepare the two isomers **1o** and **1p** by unequivocal routes starting from vanillic and isovanillic derivatives, respectively (Scheme 1). Pharmacological evaluation of **1o**,**p** was beyond our expectations because one of the isomers, the isovanillic **1o** (GI_{50} = 0.015 μM), is about 1000-fold more active than the vanillic **1p** (GI_{50} = 8 μM) and is the best compound of the series, being 10-fold more potent than the parent 4-methoxy derivative **1d**. This result was confirmed over a panel of nine tumoral cell lines. Activities in the nanomolar range were measured for all of the histological types tested, and a surprising sensitivity for the multidrug resistant cell line KB-A1 (Table 5) was measured.

In addition, the weak activity of **1p** (GI_{50} = 8 μM) is comparable with that observed for **1j** (GI_{50} = 11.7 μM), showing the harmful effect of the steric hindrance on the phenol function in meta position. This assumption is corroborated by the first X-ray crystallographic data obtained for compounds **1o** and **1j**. We can thus note very important differences in the relative positions of the phenyl cycle and the tricyclic system plans. The orientation observed in the best derivative **1o** (44.3°) (Chart 1) can be explained by the small steric hindrance of the phenol function. It seems that such an orientation

toward the interior of the medium plan of the molecule is essential for activity. This is confirmed by the fact that in the case of the dimethoxyphenyl **1j** analogue, which is 1000-fold less active than **1o**, we observed an orientation of the phenyl cycle of ±128° (Chart 2), which positions in all the cases the meta methoxy substituent toward the outside of the medium plan of the molecule. We currently try to crystallize the vanillic isomer **1p** because its X-ray crystallographic analysis would enable us to check this assumption.

Moreover, the results from routes 2 and 3 allowed us to refine the structural requirements of the family. Study of the influence of thiophene revealed that this cycle is essential for cytotoxicity, taking into account the result for pyrrolizinone **14** ($GI_{50} = 25\ \mu M$). Its substitution by a benzene in the pyrroloindole series **29** did not permit us to obtain activities similar to those in the thiophene series. For instance, we noted that the best compound **29b**, which expressed a GI_{50} of 3 μM, was more than 10 times less active than its thiophene analogue **1d** ($GI_{50} = 0.19\ \mu M$). On the other hand, if we consider the furan and pyrrole series, it appears that the nature of the heteroatom on the cycle has no influence on the activity observed because compounds **23** and **24** expressed a GI_{50} of 24.2 and 11.8 μM, respectively, while a GI_{50} of 11.7 μM was obtained for the thiophene analogue **1j** (Table 3). Last, the modifications made to the pyrrolizinone cycle, concerning the extension of the central cycle (**35** and **36** ($GI_{50} > 5\ \mu M$)) or the carbon 8 hybridization (**30**, $GI_{50} = 4.9\ \mu M$), and the nature of its substituents (**31**, **32**; $GI_{50} > 3\ \mu M$) led to a reduction or a loss of the initial activity of **1d**.

Finally, this SAR study indicated that our compounds must possess a tripentacyclic skeleton with a phenyl in position 3 bearing small substituents in the meta and para positions. Moreover, discovery of the new lead compound **1o** prompted us to carry out pharmacological investigations to elucidate the mechanism of action of the best compounds.

Preliminary flow cytometric studies (Table 2) showed that L1210 cells treated by the more potent compounds are arrested in the G_2/M phases of the cell cycle, with a significant percentage of cells having reinitiated a cycle of DNA synthesis without cell division (8N DNA content). Compound **1o** led to an accumulation of 86% of cells in G_2/M at 0.5 μM with 66% of cells having a DNA content greater than 4N chromosomes. This pharmacological profile is similar to that observed for the majority of the antimitotic agents and kinase inhibitors and led us to run the COMPARE program set up by the National Cancer Institute, which permits us to establish for any compound submitted to the human cancer cell lines screen a correlation (expressed by a Pearson correlation coefficient (PCC)) with the various references recorded in NCI database.[28,29] Any PCC value greater than 0.55 can be regarded as significant to assume a certain degree of similarity for the mechanism of action, independent of the structural considerations. Application of this program to **1d** (NSC 676693) confirmed the pharmacological effect observed on the cycle of L1210 leukemia cells because all PCC values greater than 0.55 are observed for products having tubulin as the biological target (e.g., paclitaxel, vinca alkaloids, maytansin, rhizoxin). These results prompted us to perform a tubulin polymerization inhibitory test on the best compound **1o** using deoxypodophyllotoxin as an internal reference ($IC_{50} = 2.4\ \mu M$). The result was beyond our expectations because **1o** inhibited microtubule polymerization with an IC_{50} of 2.9 μM. This capacity to interact with the mitotic spindle confirms the assumption of a powerful antimitotic activity, and our best compounds **1d**, **1o**, and **7d** were evaluated in an in vivo study, even if additional tubulin assays for compounds **7d** ($IC_{50} = 21\ \mu M$) and **1j** ($IC_{50} = 19\ \mu M$) did not shown any correlation between their effect on tubulin assembly and their cytotoxicity.

Table 6. Anticancer Activity of **1d** in the Hollow Fiber Assay[a]

compd	ip score	sc score[b]	total score	net cell kill
1d	16	0	16	yes

[a] A total of 12 ip and 12 sc cell lines were tested in triplicate at two dosage levels, and each cell line with a 50% or greater reduction in viable cell line was given a score of 2. [b] The ip and sc scores are the sums of all of the ip and sc scores. A compound with a combined ip + sc score ≥ 20, a sc score ≥ 8, or a net cell kill of one or more cell lines is considered as active in this assay.

Compound **1d** was first evaluated by the National Cancer Institute in an in vivo model where polyvinylidene fluoride hollow fibers containing various cancer cell cultures were implanted intraperitoneally (ip) and subcutaneously (sc) into mice. Compound **1d** was administered by the ip route,[30] and its effects on the reduction of tumoral cell mass were determined. The compound was tested against a panel of 12 human tumor cell lines as described previously.[31] It was solubilized in 10% DMSO in saline/Tween-80R and administered intraperitoneally once daily for a total of four doses at each of two dose levels. The day after the last dose, the fibers were collected and assessed for viable cell mass. A score of 2 was assigned each time the compound produced a 50% or greater reduction in viable cell mass compared to the vehicle-treated controls. The score for **1d** is summed in Table 6 for the intraperitoneal and subcutaneous fibers. Compound **1d** expressed a good ip score with, however, a total lack of subcutaneous activity (Table 6). Taking into account the ip administration, this profile indicates that the compound presented an insufficient bioavaibility due to a lack of solubility in physiological conditions. Nevertheless, evaluation of **1d** is currently continued for further in vivo testing in standard subcutaneous xenograft models (LOX IMVI, MDA-MB-231, SF-295, NCI-H23) because of its net cell kill effect found in the hollow fiber assay.

In parallel, the Institut de Recherche Servier undertook on compounds **1o** and **7d** with strong in vitro potentiality an evaluation of their in vivo activity in an ip P388 murine leukaemia model. In this test, the antitumor activity is expressed by the life span of mices (i.e., the average survival time of the treated mice (*T*) in comparison with the average survival time of mice controls (*C*)) measured following a single injection of the product by ip and iv routes. The result is significant when ratio *T*/*C* (%) is above 125% (Table 7). The same profile as that of **1d** was observed for **1o** and **7d** with a moderate ip activity at high concentrations but with no

Table 7. Anticancer Activity of **1o** and **7d** against an ip P388 Murine Model[a]

	ip (mg/kg)					iv (mg/kg)				
compd	25	50	100	200	400	25	50	100	200	400
7d *T/C* (%)	111	118	134	133	149	95	101	105	103	21
1o *T/C* (%)	108	116	121	117	121	102	104	102	103	19

[a] Mice were inoculated ip with 10^6 leukemic P388 cells, and the two compounds were tested with increasing concentrations (25−400 mg/kg) and administered the first day of the assay in a single injection. A compound is considered as active when the ratio *T/C* (%) is up to 125%.

iv activity. This confirmed the poor solubility of the compounds, and we are currently investigating prodrug approaches[27d,32] (esters of malic acid or retinoic acid and *N*-alkylcarbamate esters) in thiophene series (**1o**, **7d**) using the reactivity of the phenol function to obtain products with sufficient aqueous solubility and stability and high reconversion in vivo.

While additional kinetic in vivo studies are necessary to have a better understanding of the behavior of our compounds, these preliminary results are very encouraging. We have discovered a new antimitotic family based on the arylthienopyrrolizinone molecular skeleton with a growth inhibitory activity in the nanomolar range for the most promising compounds. The first results of this SAR study allowed us to specify structural requirements and prompted us to synthesize some new analogues to improve the in vivo results. In particular, we are currently developing an original prodrug chemistry on the best compounds **1o** and **7d**.

Experimental Section

Chemistry. Commercial reagents were used as received without additional purification. Melting points were determined on a Kofler melting point apparatus and are uncorrected. Elemental analyses for new compounds were within ±0.4% of theoretical values and were performed at the Institut de Recherche en Chimie Organique Fine (Rouen, France). IR spectra were recorded on a Genesis series FTIR infrared spectrometer using KBr pellets. The ^{1}H and ^{13}C NMR spectra were obtained on a JEOL Lambda 400 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and TMS as internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants are in hertz. Electron impact mass spectra (EIMS) were obtained using a JEOL JMS GCMate spectrometer. Reactions were monitored by thin-layer chromatography (TLC) using 0.2 mm Polygram Sil silica gel G/UV254 precoated plates with visualization by irradiation with a short-wavelength UV light. Silica gel flash chromatography was performed using 63−200 μm Kieselgel Merck 60 silica gel.

General Procedure for the Synthesis of the Thienopyrrolizinones 1h−n. A solution of the corresponding amide **6** (10 mmol) in phosphorus oxychloride (25 mL) was stirred at 70 °C for 2 h. After cooling, the reaction mixture was concentrated to give a red solid that was filtered, washed with Et_2O, and dried. Then, this intermediary iminium salt was added slowly to a 10% aqueous sodium hydroxide solution (70 mL) and the mixture was successively heated for 1 h to 50 °C and filtered when returned to room temperature to provide the crude product **1**, which was purified by column chromatography (chloroform).

3-(2-Methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1h). The thienopyrrolizinone **1h** was isolated as an orange powder in 58% yield: mp 142 °C; IR 1685 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.02 (s, 1H, H_2), 7.48 (m, 1H, $H_{4'}$), 7.41 (d, $J_{6',5'}$ = 7.5 Hz, 1H, $H_{6'}$), 7.18 (d, $J_{3',4'}$ = 8.2 Hz, 1H, $H_{3'}$), 7.07 (m, 1H, $H_{5'}$), 6.72 (d, $J_{7,6}$ = 3.8 Hz, 1H, H_7), 6.49 (d, $J_{5,6}$ = 2.6 Hz, 1H, H_5), 6.07 (m, 1H, H_6), 3.77 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 173.7, 155.8, 151.5, 135.4, 135.1, 130.1, 130.0, 125.5. 125.3, 120.8, 120.6, 120.5, 114.7, 112.6, 110.5, 54.7. Anal. ($C_{16}H_{11}NO_2S$) C, H, N.

3-(3-Methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1i). The thienopyrrolizinone **1i** was isolated as an orange powder in 71% yield: mp 150−152°C; IR 1680 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.13 (s, 1H, H_2), 7.43 (m, 1H, $H_{5'}$), 7.15 (m, 2H, $H_{2'}$ and $H_{6'}$), 7.03 (d, $J_{4',5'}$ = 8.0 Hz, 1H, $H_{4'}$), 6.89 (d, $J_{7,6}$ = 3.8 Hz, 1H, H_7), 6.75 (d, $J_{5,6}$ = 2.6 Hz, 1H, H_5), 6.11 (m, 1H, H_6), 3.80 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.5, 156.3, 150.2, 135.2, 134.8, 130.4, 129.3, 125.9, 125.1, 121.7, 120.7, 120.4, 115.3, 112.3, 111.3, 55.4. Anal. ($C_{16}H_{11}NO_2S$) C, H, N.

3-(3,4-Dimethoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1j). The thienopyrrolizinone **1j** was isolated as an orange powder in 53% yield: mp 190 °C; IR 1679 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.05 (s, 1H, H_2), 7.16 (s, 1H, $H_{2'}$), 7.09 (m, 2H, $H_{5'}$ and $H_{6'}$), 6.94 (m, 1H), 6.75 (m, 1H), 6.11 (m, 1H, H_6), 3.80 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 179.8, 150.8, 149.4, 149.2, 135.9, 134.1, 129.5, 124.9, 123.2, 120.6, 120.4, 115.6, 113.3, 111.3, 111.1, 56.1, 55.9. Anal. ($C_{17}H_{13}NO_3S$) C, H, N.

3-(3,4,5-Trimethoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1k). The thienopyrrolizinone **1k** was isolated as a yellow powder in 53% yield: mp 180−182 °C; IR 1680 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 1H, H_2), 7.02 (d, $J_{5,6}$ = 1.8 Hz, 1H, H_5), 6.88 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.75 (d, $J_{7,6}$ = 3.3 Hz, 1H, H_7), 6.11 (m, 1H, H_6), 3.82 (s, 6H, OCH_3), 3.71 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 173.1, 150.3, 151.4, 146.5, 135.3, 135.2, 133.9, 130.1, 125.9, 121.8, 115.7, 113.6, 106.7. Anal. ($C_{18}H_{15}NO_4S$) C, H, N.

3-(3,4-Methylenedioxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1l). The thienopyrrolizinone **1l** was isolated as an orange powder in 47% yield: mp 168−169 °C; IR 1691 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 1H, H_2), 7.24 (s, 1H, $H_{2'}$), 7.12 (m, 2H, $H_{5'}$ and $H_{6'}$), 6.96 (m, 1H, H_5), 6.82 (m, 1H, H_7), 6.18 (m, 3H, H_6 and CH_2); ^{13}C NMR ($CDCl_3$) δ 176.4, 152.5, 148.2, 148.0, 136.7, 134.9, 128.8, 126.5, 24.9, 121.8, 120.7, 115.8, 114.1, 112.5, 111.5, 87.9. Anal. ($C_{16}H_9NO_3S$) C, H, N.

3-(3-Benzyloxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1m). The thienopyrrolizinone **1m** was isolated as a yellow powder in 71% yield: mp 138 °C; IR 1680 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 6H, H_{arom}), 7.05 (dd, $J_{6',5'}$ = 8.2 Hz, $J_{6',2'}$ = 1.9 Hz, 1H, $H_{6'}$), 6.98 (d, $J_{2',6'}$ = 1.9 Hz, 1H, $H_{2'}$), 6.96 (d, $J_{5',6'}$ = 8.2 Hz, 1H, $H_{5'}$), 6.62 (dd, $J_{7,6}$ = 3.6 Hz, $J_{7,5}$ = 0.7 Hz, 1H, H_7), 6.45 (dd, $J_{5,6}$ = 2.6 Hz, $J_{5,7}$ = 0.7 Hz, 1H, H_5), 5.88 (dd, $J_{6,5}$ = 2.6 Hz, $J_{6,7}$ = 3.6 Hz, 1H, H_6), 5.21 (s, 2H, CH_2), 3.95 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.1, 150.7, 150.1, 148.1, 136.5, 135.8, 134.1, 129.4, 128.7, 128.0, 127.0, 126.9, 124.9, 121.1, 120.7, 115.5, 113.5, 113.3, 111.9, 70.8, 56.1. Anal. ($C_{23}H_{17}NO_3S$) C, H, N.

3-(4-Benzyloxy-3-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1n). The thienopyrrolizinone **1n** was isolated as an orange powder in 52% yield: mp 188−190 °C; IR 1672 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.39 (m, 6H, H_{arom}), 6.98 (m, 3H, $H_{2'}$, $H_{5'}$ and $H_{6'}$), 6.75 (d, $J_{5,6}$ = 2.2 Hz, 1H, H_5), 6.67 (d, $J_{7,6}$ = 3.4 Hz, 1H, H_7), 6.01 (dd, $J_{6,5}$ = 2.2 Hz, $J_{6,7}$ = 3.4 Hz, 1H, H_6), 5.21 (s, 2H, CH_2), 3.92 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.2, 150.9, 150.3, 148.3, 136.7, 136.1, 134.7, 129.9, 129.3, 128.1, 127.1, 126.5, 125.7, 121.3, 120.9, 116.2, 113.7, 113.3, 111.9, 70.8, 55.9. Anal. ($C_{23}H_{17}NO_3S$) C, H, N.

General Procedure for the Synthesis of the Thienopyrrolizinones 1o,p. A solution of the corresponding *O*-benzylthienopyrrolizinones **1m**,**n** (1.94 g, 5 mmol) in a 33% solution of hydrobromic acid in glacial acetic acid (20 mL) was stirred at room temperature for 1 h. After cooling, the reaction mixture was diluted with water (50 mL) and the resulting precipitate was extracted with ethyl acetate (3 × 30 mL). Then, the organic layers were combined, washed with a 10% aqueous $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to give an orange solid. This residue was purified by column chromatography, eluting by hexane/ethyl acetate (2:1) to provide **1o**,**p**.

3-(3-Hydroxy-4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1o). The thienopyrrolizinone **1o** was isolated as an orange powder in 55% yield: mp 204 °C; IR 3427 (O−H), 1678 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36 (s, 1H, H_2), 7.01 (d, $J_{2',6'}$ = 1.7 Hz, 1H, $H_{2'}$), 6.92 (dd, $J_{6',5'}$ = 8.3 Hz, $J_{6',2'}$ = 1.7

Hz, 1H, $H_{6'}$), 6.86 (d, $J_{5',6'} = 8.3$ Hz, 1H, $H_{5'}$), 6.74 (d, $J_{5,6} = 2.4$ Hz, 1H, H_5), 6.59 (d, $J_{7,6} = 3.5$ Hz, 1H, H_7), 5.92 (m, 1H, H_6), 5.78 (br s, 1H, D_2O exchangeable, OH), 3.89 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.1, 150.8, 147.1, 146.1, 135.8, 134.1, 129.5, 126.9, 124.6, 121.0, 119.8, 115.7, 114.2, 113.2, 110.9, 56.1. Anal. ($C_{16}H_{11}NO_3S$) C, H, N.

3-(4-Hydroxy-3-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (1p). The thienopyrrolizinone **1p** was isolated as a yellow powder in 58% yield: mp 190−191 °C; IR 3396 (O−H), 1679 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 (s, 1H, H_2), 6.92 (m 3H, $H_{2'}$, $H_{5'}$, and $H_{6'}$), 6.68 (d, $J_{5,6} = 2.1$ Hz, 1H, H_5), 6.60 (d, $J_{7,6} = 3.7$ Hz, 1H, H_7), 5.93 (m, 1H, H_6), 5.77 (br s, 1H, D_2O exchangeable, OH), 3.86 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.1, 150.9, 146.8, 146.2, 135.9, 134.1, 129.7, 126.8, 124.4, 121.2, 120.7, 115.6, 114.9, 113.3, 110.5, 56.1. Anal. ($C_{16}H_{11}NO_3S$) C, H, N.

General Procedure for the Synthesis of 2-Aryl-3-hydroxyacrylonitriles Sodium Salts 2h−n. The corresponding arylacetonitrile (100 mmol) was added dropwise to a solution of ethyl formate (8.9 mL, 110 mmol) and sodium methoxide (5.9 g, 110 mmol) in methanol (150 mL). The mixture was refluxed for 2 h. The resulting precipitate was collected, washed with diethyl ether (2 × 50 mL), and dried under reduced pressure with P_2O_5 to give **2h−n**, which were used without further purification.

3-Hydroxy-2-(2-methoxyphenyl)acrylonitrile Sodium Salt (2h). 2h was obtained as a white powder in 85% yield: mp > 260 °C; IR 2180 (CN) cm^{-1}.

3-Hydroxy-2-(3-methoxyphenyl)acrylonitrile Sodium Salt (2i). 2i was obtained as a white powder in 73% yield: mp > 260 °C; IR 2176 (CN) cm^{-1}.

3-Hydroxy-2-(3,4-dimethoxyphenyl)acrylonitrile Sodium Salt (2j). 2j was obtained as a white powder in 83% yield: mp > 260 °C; IR 2166 (CN) cm^{-1}.

3-Hydroxy-2-(3,4,5-trimethoxyphenyl)acrylonitrile Sodium Salt (2k). 2k was obtained as a beige powder in 98% yield: mp > 260 °C; IR 2179 (CN) cm^{-1}.

3-Hydroxy-2-(3,4-methylenedioxyphenyl)acrylonitrile Sodium Salt (2l). 2l was obtained as a beige powder in 53% yield: mp > 260 °C; IR 2177 (CN) cm^{-1}.

3-Hydroxy-2-(3-benzyloxy-4-methoxyphenyl)acrylonitrile Sodium Salt (2m). 2m was obtained as a yellow powder in 83% yield: mp = 196 °C; IR 2172 (CN) cm^{-1}.

3-Hydroxy-2-(4-benzyloxy-3-methoxyphenyl)acrylonitrile Sodium Salt (2n). 2n was obtained as a yellow powder in 92% yield: mp = 204 °C; IR 2175 (CN) cm^{-1}.

General Procedure for the Synthesis of 2-Cyano-2-arylvinylbenzene Sulfonates 3h−n. A solution of the corresponding sodium salt **2** (100 mmol) in DMF (150 mL) was cooled with an ice bath to 0 °C before adding dropwise the benzene sulfonyl chloride (15.3 mL, 120 mmol). The reaction mixture was heated at 70 °C for 2 h and then diluted with water (200 mL) and stirred overnight to afford a precipitate. The latter was filtered, washed with water (3 × 100 mL), and dried over P_2O_5 under vacuum to give **3h−n**, which were used without further purification.

2-Cyano-2-(3-methoxyphenyl)vinylbenzene Sulfonate (3h). 3h was obtained as a beige powder in 32% yield: mp 78 °C; IR 2221 (CN), 1193 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.29 (m, 1H, H_{arom}), 8.10−7.75 (m, 5H, H_{arom}), 7.41 (m, 2H, H_{arom}), 7.07 (m, 2H, H_{arom}), 3.71 (m, 3H, OCH_3).

2-Cyano-2-(2-methoxyphenyl)Vinylbenzene Sulfonate (3i). 3i was obtained as a beige powder in 50% yield: mp 67−69 °C; IR 2222 (CN), 1215 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.03 (m, 1H, H_{arom}), 7.94−7.71 (m, 5H, H_{arom}), 7.32 (m, 3H, H_{arom}), 7.03 (m, 1H, H_{arom}), 3.67 (m, 3H, OCH_3).

2-Cyano-2-(3,4-dimethoxyphenyl)vinylbenzene Sulfonate (3j). 3j was obtained as a white powder (from diethyl ether) in 55% yield: mp 164 °C; IR 2233 (CN), 1196 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.01 (m, 2H, H_{arom}), 7.71 (m, 4H, H_{arom}), 6.99 (m, 1H, H_{arom}), 6.85 (m, 2H, H_{arom}), 3.89 (m, 6H, OCH_3).

2-Cyano-2-(3,4,5-trimethoxyphenyl)vinylbenzene Sulfonate (3k). 3k was obtained as a beige powder in 58% yield: mp 102 °C; IR 2226 (CN), 1193 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99 (m, 6H, H_{arom}), 6.75 (m, 2H, H_{arom}), 3.70 (m, 9H, OCH_3).

2-Cyano-2-(3,4-methylenedioxyphenyl)vinylbenzene Sulfonate (3l). 3l was obtained as a beige powder in 47% yield: mp 110°C; IR 2221 (CN), 1195 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.96 (m, 4H, H_{arom}), 7.01 (m, 5H, H_{arom}), 6.06 (m, 2H, CH_2).

2-Cyano-2-(3-benzyloxy-4-methoxyphenyl)vinylbenzene Sulfonate (3m). 3m was obtained as a beige powder (from methanol) in 50% yield: mp 136 °C; IR 2232 (CN), 1202 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.97 (m, 2H, H_{arom}), 7.75 (m, 1H, H_{arom}), 7.62 (m, 2H, H_{arom}), 7.46 (s, 1H, H_{vinyl}), 7.35 (m, 5H, H_{arom}), 6.95 (dd, $J_{6,5} = 8.4$ Hz, $J_{6,2} = 1.6$ Hz, 1H, H_6), 6.90 (d, $J_{2,6} = 1.6$ Hz, 1H, H_2), 6.85 (d, $J_{5,6} = 8.4$ Hz, 1H, H_5), 5.12 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3).

2-Cyano-2-(4-benzyloxy-3-methoxyphenyl)vinylbenzene Sulfonate (3n). 3n was obtained as a white powder (from methanol) in 52% yield: mp 104 °C; IR 2227 (CN), 1192 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98 (m, 2H, H_{arom}), 7.59 (m, 3H, H_{arom}), 7.38 (m, 6H, H_{arom}), 7.11 (m, 1H, H_{arom}), 6.86 (m, 2H, H_{arom}), 5.16 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3).

General Procedure for the Synthesis of Methyl 3-Amino-4-aryl-2-thiophene Carboxylates 4h−n. Sodium methylate was prepared from sodium (1.38 g, 60 mmol) in methanol (150 mL). After the mixture was cooled, methyl thioglycolate (2.16 mL, 24 mmol) and the sulfonate **3** (20 mmol) were slowly and successively added, and the reaction mixture was heated at 60 °C for 2 h. It was then concentrated and diluted with water (200 mL) and stirred overnight to afford a precipitate. The latter was filtered, washed with water (3 × 100 mL), dried over P_2O_5 under vacuum, and crystallized from ethanol to afford **4**.

Methyl 3-Amino-4-(2-methoxyphenyl)-2-thiophene Carboxylate (4h). 4h was obtained as a white powder in 58% yield: mp 110−111 °C; IR 3486 (N−H), 3371 (N−H), 1678 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.49 (s, 1H, $H_{thiophene}$), 7.38 (m, 1H, H_4), 7.19 (d, $J_{3,4} = 7.1$ Hz, 1H, H_3), 7.10 (d, $J_{6,5} = 8.2$ Hz, 1H, H_6), 7.02 (m, 1H, H_5), 6.01 (br s, 2H, D_2O exchangeable, NH_2), 3.75 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3). Anal. ($C_{13}H_{13}NO_3S$) C, H, N.

Methyl 3-Amino-4-(3-methoxyphenyl)-2-thiophene Carboxylate (4i). 4i was obtained as a white powder in 52% yield: mp 93 °C; IR 3453 (N−H), 3341 (N−H), 1685 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.67 (s, 1H, $H_{thiophene}$), 7.37 (m, 1H, H_4), 7.01 (m, 3H, H_{arom}), 6.29 (br s, 2H, D_2O exchangeable, NH_2), 3.79 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3). Anal. ($C_{13}H_{13}NO_3S$) C, H, N.

Methyl 3-Amino-4-(3,4-dimethoxyphenyl)-2-thiophene Carboxylate (4j). 4j was obtained as a white powder in 63% yield: mp 154 °C; IR 3426 (N−H), 3331 (N−H), 1667 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.58 (s, 1H, $H_{thiophene}$), 6.99 (m, 3H, H_{arom}), 6.25 (br s, 2H, D_2O exchangeable, NH_2), 3.79 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3). Anal. ($C_{14}H_{15}NO_4S$) C, H, N.

Methyl 3-Amino-4-(3,4,5-trimethoxyphenyl)-2-thiophene Carboxylate (4k). 4k was obtained as a white powder in 65% yield: mp 146 °C; IR 3468 (N−H), 3361 (N−H), 1675 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.65 (s, 1H, $H_{thiophene}$), 6.72 (s, 1H, H_{arom}), 6.70 (s, 1H, H_{arom}), 6.34 (br s, 2H, D_2O exchangeable, NH_2), 3.75 (s, 6H, OCH_3), 3.73 (m, 6H, OCH_3). Anal. ($C_{15}H_{17}NO_5S$) C, H, N.

Methyl 3-Amino-4-(3,4-methylenedioxyphenyl)-2-thiophene Carboxylate (4l). 4l was obtained as a beige powder in 48% yield: mp 168 °C; IR 3460 (N−H), 3345 (N−H), 1687 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.57 (s, 1H, $H_{thiophene}$), 6.95 (m, 3H, H_{arom}), 6.23 (br s, 2H, D_2O exchangeable, NH_2), 6.04 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3). Anal. ($C_{13}H_{11}NO_4S$) C, H, N.

Methyl 3-Amino-4-(3-benzyloxy-4-methoxyphenyl)-2-thiophene Carboxylate (4m). 4m was obtained as a white powder in 53% yield: mp 92 °C; IR 3464 (N−H), 3349 (N−H), 1677 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (m, 5H, H_{arom}), 7.10 (s, 1H, $H_{thiophene}$), 6.92 (m, 3H, H_2, H_5, and H_6), 5.42 (br s, 2H, D_2O exchangeable, NH_2), 5.18 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3),

3.82 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 164.9, 151.4, 149.4, 147.9, 136.6, 132.8, 128.5, 127.9, 127.8, 127.2, 126.7, 121.0, 113.9, 112.2, 100.8, 70.7, 55.9, 51.1. Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

Methyl 3-Amino-4-(4-benzyloxy-3-methoxyphenyl)-2-thiophene Carboxylate (4n). 4n was obtained as a white powder in 64% yield: mp 130−132 °C; IR 3420 (N−H), 3331 (N−H), 1676 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (m, 5H, H_{arom}), 7.18 (s, 1H, $H_{thiophene}$), 6.93 (m, 3H, H_2, H_5, and H_6), 5.61 (br s, 2H, D_2O exchangeable, NH_2), 5.19 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 174.5, 142.2, 141.5, 139.9, 138.5, 132.2, 128.9, 128.5, 127.3, 127.1, 120.3, 119.4, 114.1, 112.9, 111.7, 70.9, 56.1, 51.3. Anal. ($C_{20}H_{19}NO_4S$) C, H, N.

General Procedure for the Synthesis of Methyl 4-Aryl-3-(pyrrol-1-yl)-2-thiophene Carboxylates 5h−n. A solution of 2,5-dimethoxytetrahydrofuran (1.42 mL, 11 mmol) in dioxane (100 mL) was stirred for 15 min with 4-chloropyridine hydrochloride (1.65 g, 11 mmol). The methyl 3-amino-4-aryl-2-thiophene carboxylate **4** (10 mmol) was added, and the reaction mixture was refluxed for 3 h and filtered through a small pad of Celite. The solvent was evaporated to give a brown residue that was dissolved in methylene chloride (150 mL). The solution was washed with an 1 N aqueous hydrochloric acid solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a beige solid. An analytical sample was crystallized from diethyl ether.

Methyl 4-(2-Methoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5h). 5h was obtained as a white powder in 72% yield: mp 141−143 °C; IR 1723 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.88 (s, 1H, $H_{thiophene}$), 7.26 (dd, $J_{4,3} = 6.1$ Hz, $J_{4,5} = 7.1$ Hz, 1H, H_4), 6.95 (d, $J_{3,4} = 6.1$ Hz, 1H, H_3), 6.90 (d, $J_{6,5} = 8.3$ Hz, 1H, H_6), 6.84 (dd, $J_{5,6} = 8.3$ Hz, $J_{5,4} = 7.1$ Hz, 1H, H_5), 6.53 (m, 2H, $H_{\alpha pyrrole}$), 5.95 (m, 2H, $H_{\beta pyrrole}$), 3.69 (s, 3H, OCH_3), 3.48 (s, 3H, OCH_3). Anal. ($C_{17}H_{15}NO_3S$) C, H, N.

Methyl 4-(3-Methoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5i). 5i was obtained as a white powder in 84% yield: mp 74 °C; IR 1715 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.11 (s, 1H, $H_{thiophene}$), 7.17 (m, 1H, H_5), 6.82 (dd, $J_{6,5} = 8.3$ Hz, $J_{6,2} = 2.2$ Hz, 1H, H_6), 6.68 (m, 2H, $H_{\alpha pyrrole}$), 6.64 (m, 1H, H_4), 6.36 (d, $J_{2,6} = 2.2$ Hz, 1H, H_2), 6.12 (m, 2H, $H_{\beta pyrrole}$), 3.70 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3). Anal. ($C_{17}H_{15}NO_3S$) C, H, N.

Methyl 4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5j). 5j was obtained as a beige powder in 92% yield: mp 122−124 °C; IR 1714 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.04 (s, 1H, $H_{thiophene}$), 6.85 (m, 1H, H_{arom}), 6.68 (m, 3H, H_{arom} and $2H_{\alpha pyrrole}$), 6.23 (m, 1H, H_{arom}), 6.13 (m, 2H, $H_{\beta pyrrole}$), 3.70 (s, 6H, OCH_3), 3.49 (s, 3H, OCH_3). Anal. ($C_{18}H_{17}NO_4S$) C, H, N.

Methyl 4-(3,4,5-Trimethoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5k). 5k was obtained as a white powder in 77% yield: mp 110 °C; IR 1695 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.15 (s, 1H, $H_{thiophene}$), 6.70 (m, 2H, $H_{\alpha pyrrole}$), 6.21 (s, 2H, H_2 and H_6), 6.16 (m, 2H, $H_{\beta pyrrole}$), 3.69 (s, 3H, OCH_3), 3.60 (s, 9H, OCH_3). Anal. ($C_{19}H_{19}NO_5S$) C, H, N.

Methyl 4-(3,4-Methylenedioxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5l). 5l was obtained as a white powder in 93% yield: mp 116 °C; IR 1728 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.01 (s, 1H, $H_{thiophene}$), 6.80 (d, $J_{5,6} = 8.03$ Hz, 1H, H_5), 6.66 (m, 2H, $H_{\alpha pyrrole}$), 6.51 (dd, $J_{6,5} = 8.03$ Hz, $J_{6,2} = 1.4$ Hz, 1H, H_6), 6.35 (d, $J_{2,6} = 1.4$ Hz, 1H, H_2), 6.12 (m, 2H, $H_{\beta pyrrole}$), 5.96 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3). Anal. ($C_{17}H_{13}NO_4S$) C, H, N.

Methyl 4-(3-Benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5m). 5m was obtained as a white powder in 69% yield: mp 166 °C; IR 1725 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.43 (s, 1H, $H_{thiohene}$), 7.33 (m, 5H, H_{arom}), 6.81 (d, $J_{5,6} = 8.4$ Hz, 1H, H_5), 6.75 (dd, $J_{6,5} = 8.4$ Hz, $J_{6,2} = 2.1$ Hz, 1H, H_6), 6.62 (m, 2H, $H_{\alpha pyrrole}$), 6.28 (m, 2H, $H_{\beta pyrrole}$), 6.26 (d, $J_{2,6} = 2.1$ Hz, 1H, H_2), 4.84 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 160.9, 149.3, 147.9, 141.5, 140.5, 136.9, 128.4, 127.7, 127.2, 126.5, 126.3, 125.3, 122.4, 120.3, 112.0, 111.4, 109.3, 70.5, 55.8, 52.2. Anal. ($C_{24}H_{21}NO_4S$) C, H, N.

Methyl 4-(4-Benzyloxy-3-methoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylate (5n). 5n was obtained as a white powder in 76% yield: mp 124−126 °C; IR 1725 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.34 (m, 6H, H_{arom}), 6.80 (d, $J_{5,6} = 8$ Hz, 1H, H_5), 6.71 (d, $J_{6,5} = 8$ Hz, 1H, H_6), 6.63 (m, 2H, $H_{\alpha pyrrole}$), 6.25 (m, 2H, $H_{\beta pyrrole}$), 6.21 (s, 1H, H_2), 5.12 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 160.8, 149.2, 147.8, 141.3, 140.4, 136.8, 128.4, 127.8, 127.2, 126.6, 126.3, 125.9, 122.3, 119.7, 113.4, 109.9, 109.3, 70.7, 55.6, 52.1. Anal. ($C_{24}H_{21}NO_4S$) C, H, N.

General Procedure for the Synthesis of 4-Aryl-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamides 6h−n. A solution of methyl 4-aryl-3-(pyrrol-1-yl)-2-thenoate **5** (10 mmol) in pyrrolidine (40 mL) was refluxed for 2 h and 30 min. After the mixture was cooled and evaporated, the yellow oil was dissolved in chloroform (150 mL) and the solution was washed with an 1 N aqueous hydrochloric acid solution (2 × 150 mL), dried ($MgSO_4$), and evaporated to give, after cooling in an ice bath, a beige solid washed with petroleum ether (2 × 100 mL), filtered, and dried over P_2O_5 under vacuum to give **6h−n**.

4-(2-Methoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6h). 6h was obtained as a beige powder in 89% yield: mp 108 °C; IR 1627 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.66 (s, 1H, $H_{thiophene}$), 7.29 (m, 1H, H_4), 7.10 (d, $J_{6,5} = 6.8$ Hz, 1H, H_6), 6.92 (m, 2H, H_5 and H_3), 6.43 (m, 2H, $H_{\alpha pyrrole}$), 6.02 (m, 2H, $H_{\beta pyrrole}$), 3.80 (s, 3H, OCH_3), 3.30 (m, 2H, $H_{\alpha pyrrolidine}$), 2.57 (m, 2H, $H_{\alpha pyrrolidine}$), 1.64 (m, 2H, $H_{\beta pyrrolidine}$), 1.51 (m, 2H, $H_{\beta pyrrolidine}$). Anal. ($C_{20}H_{20}N_2O_2S$) C, H, N.

4-(3-Methoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6i). 6i was obtained as a beige powder in 82% yield: mp 91−94 °C; IR 1615 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.86 (s, 1H, $H_{thiophene}$), 7.19 (t, $J_{5,4} = 7.9$ Hz, $J_{5,6} = 7.9$ Hz, 1H, H_5), 6.82 (d, $J_{4,5} = 7.9$ Hz, 1H, H_4), 6.66 (dd, $J_{6,5} = 7.9$ Hz, $J_{6,2} = 2.2$ Hz, 1H, H_6), 6.57 (m, 2H, $H_{\alpha pyrrole}$), 6.44 (d, $J_{2,6} = 2.2$ Hz, 1H, H_2), 6.13 (m, 2H, $H_{\beta pyrrole}$), 3.59 (s, 3H, OCH_3), 3.34 (m, 2H, $H_{\alpha pyrrolidine}$), 2.78 (m, 2H, $H_{\alpha pyrrolidine}$), 1.68 (m, 2H, $H_{\beta pyrrolidine}$), 1.59 (m, 2H, $H_{\beta pyrrolidine}$). Anal. ($C_{20}H_{20}N_2O_2S$) C, H, N.

4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6j). 6j was obtained as a beige powder in 75% yield: mp 115 °C; IR 1633 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.79 (s, 1H, $H_{thiophene}$), 6.87 (d, $J_{5,6} = 8.5$ Hz, 1H, H_5), 6.69 (d, $J_{6,5} = 8.5$ Hz, 1H, H_6), 6.59 (m, 2H, $H_{\alpha pyrrole}$), 6.33 (s, 1H, H_2), 6.14 (m, 2H, $H_{\beta pyrrole}$), 3.71 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.34 (m, 2H, $H_{\alpha pyrrolidine}$), 2.80 (m, 2H, $H_{\alpha pyrrolidine}$), 1.68 (m, 2H, $H_{\beta pyrrolidine}$), 1.59 (m, 2H, $H_{\beta pyrrolidine}$). Anal. ($C_{21}H_{22}N_2O_3S$) C, H, N.

4-(3,4,5-Trimethoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6k). 6k was obtained as a beige powder in 83% yield: mp 68−72 °C; IR 1618 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.89 (s, 1H, $H_{thiophene}$), 6.62 (m, 2H, $H_{\alpha pyrrole}$), 6.25 (s, 2H, H_2 and H_6), 6.16 (m, 2H, $H_{\beta pyrrole}$), 3.60 (s, 9H, OCH_3), 3.32 (m, 2H, $H_{\alpha pyrrolidine}$), 2.83 (m, 2H, $H_{\alpha pyrrolidine}$), 1.68 (m, 2H, $H_{\beta pyrrolidine}$), 1.61 (m, 2H, $H_{\beta pyrrolidine}$). Anal. ($C_{22}H_{24}N_2O_4S$) C, H, N.

4-(3,4-Methylenedioxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6l). 6l was obtained as a beige powder in 71% yield: mp 124 °C; IR 1625 (C═O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.76 (s, 1H, $H_{thiophene}$), 6.82 (d, $J_{5,6} = 8.0$ Hz, 1H, H_5), 6.57 (m, 2H, $H_{\alpha pyrrole}$), 6.52 (d, $J_{6,5} = 8.0$ Hz, 1H, H_6), 6.44 (s, 1H, H_2), 6.14 (m, 2H, $H_{\beta pyrrole}$), 5.98 (s, 2H, CH_2O), 3.33 (m, 2H, $H_{\alpha pyrrolidine}$), 2.77 (m, 2H, $H_{\alpha pyrrolidine}$), 1.67 (m, 2H, $H_{\beta pyrrolidine}$), 1.59 (m, 2H, $H_{\beta pyrrolidine}$). Anal. ($C_{20}H_{18}N_2O_3S$) C, H, N.

4-(3-Benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6m). 6m was obtained as a beige powder in 77% yield: mp 120 °C; IR 1628 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (m, 6H, H_{arom}), 6.82 (d, $J_{5,6} = 8.3$ Hz, 1H, H_5), 6.75 (dd, $J_{6,5} = 8.3$ Hz, $J_{6,2} = 1.8$ Hz, 1H, H_6), 6.57 (m, 2H, $H_{\alpha pyrrole}$), 6.34 (d, $J_{2,6} = 1.8$ Hz, 1H, H_2), 6.18 (m, 2H, $H_{\beta pyrrole}$), 4.85 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.49 (m, 2H,

$H_{\alpha pyrrolidine}$), 2.75 (m, 2H, $H_{\alpha pyrrolidine}$), 1.76 (m, 2H, $H_{\beta pyrrolidine}$), 1.65 (m, 2H, $H_{\beta pyrrolidine}$); ^{13}C NMR ($CDCl_3$) δ 162.6, 149.1, 147.8, 137.6, 136.9, 134.8, 130.5, 128.4, 127.7, 127.2, 126.6, 123.1, 121.5, 120.5, 112.8, 111.4, 109.7, 70.5, 55.8, 47.4, 46.1, 25.6, 24.1. Anal. ($C_{27}H_{26}N_2O_3S$) C, H, N.

4-(4-Benzyloxy-3-methoxyphenyl)-3-(pyrrol-1-yl)thien-2-ylpyrrolidine Carboxamide (6n). 6n was obtained as a beige powder in 82% yield: mp 150−152 °C; IR 1626 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 6H, H_{arom}), 6.82 (d, $J_{5,6}$ = 8 Hz, 1H, H_5), 6.72 (dd, $J_{6,5}$ = 8 Hz, $J_{6,2}$ = 1.6 Hz, 1H, H_6), 6.57 (m, 2H, $H_{\alpha pyrrole}$), 6.29 (m, 1H, H_2), 6.16 (m, 2H, $H_{\beta pyrrole}$), 5.13 (s, 2H, CH_2), 3.61 (s, 3H, OCH_3), 3.50 (m, 2H, $H_{\alpha pyrrolidine}$), 2.76 (m, 2H, $H_{\alpha pyrrolidine}$), 1.78 (m, 2H, $H_{\beta pyrrolidine}$), 1.64 (m, 2H, $H_{\beta pyrrolidine}$); ^{13}C NMR ($CDCl_3$) δ 161.5, 149.2, 147.7, 137.6, 136.8, 134.9, 130.4, 128.5, 127.8, 127.2, 127.1, 123.1, 121.5, 119.9, 113.5, 110.8, 109.7, 70.8, 55.9, 47.4, 46.1, 25.6, 24.1. Anal. ($C_{27}H_{26}N_2O_3S$) C, H, N.

General Procedure for the Synthesis of 3-(Hydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-ones 7a−e. A solution of 3-(methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1** (3.5 mmol) in methylene chloride (30 mL) was stirred and cooled to 0 °C with an ice bath. Then, a 1 M boron tribromide solution in methylene chloride was added (3.5 mL, 3.5 mmol for each methyl ether bond to cleave) and the reaction mixture was stirred for 0.5 h to room temperature and diluted with cold water (100 mL). After 3 h, the orange solid was filtered, washed with methylene chloride (2 × 50 mL), dried over P_2O_5 under vacuum, and recrystallized from ethanol.

3-(2-Hydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (7a). 7a was obtained as an orange powder in 62% yield starting from 3-(2-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1h**: mp 192 °C; IR 3243 (O−H), 1660 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 10.08 (br s, 1H, D_2O exchangeable, OH), 7.99 (s, 1H, H_2), 7.31 (m, 2H, H_{arom}), 7.01 (m, 1H, H_{arom}), 6.91 (m, 1H, H_{arom}), 6.69 (m, 1H, H_{arom}), 6.60 (m, 1H, H_{arom}), 6.06 (m, 1H, H_6); ^{13}C NMR ($CDCl_3$) δ 175.3, 153.1, 150.3, 136.2, 131.5, 131.2, 129.4, 126.1, 124.0, 121.5, 119.3, 119.1, 115.7, 113.8, 112.2. Anal. ($C_{15}H_9NO_2S$) C, H, N.

3-(3-Hydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (7b). 7b was obtained as an orange powder in 77% yield starting from 3-(3-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1i**: mp > 260 °C; IR 3293 (O−H), 1661 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.78 (br s, 1H, D_2O exchangeable, OH), 8.08 (s, 1H, H_2), 7.31 (m, 1H, $H_{5'}$), 6.97 (m, 2H, H_{arom}), 6.90 (d, $J_{5,6}$ = 2.1 Hz, 1H, H_5), 6.85 (m, 1H, H_{arom}), 6.76 (d, $J_{7,6}$ = 3.6 Hz, 1H, H_7), 6.13 (m, 1H, H_6); ^{13}C NMR ($CDCl_3$) δ 175.1, 152.6, 151.4, 137.3, 132.3, 131.6, 129.6, 126.3, 124.7, 122.2, 118.8, 119.5, 116.3, 113.9, 113.2. Anal. ($C_{15}H_9NO_2S$) C, H, N.

3-(4-Hydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (7c). 7c was obtained as an orange powder in 88% yield starting from 3-(4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1d**: mp > 260 °C; IR 3165 (O−H), 1662 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.72 (br s, 1H, D_2O exchangeable, OH), 7.89 (s, 1H, H_2), 7.32 (d, J = 8.5 Hz, 2H, $H_{2'}$ and $H_{6'}$), 6.82 (d, J = 8.5 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.80 (d, $J_{5,6}$ = 2.4 Hz, 1H, H_5), 6.66 (d, $J_{7,6}$ = 3.4 Hz, 1H, H_7), 6.03 (m, 1H, H_6). Anal. ($C_{15}H_9NO_2S$) C, H, N.

3-(3,4-Dihydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (7d). 7d was obtained as an orange powder in 68% yield starting from 3-(3,4-dimethoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1j**: mp > 260 °C; IR 3491 (O−H), 1655 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.29 (br s, 2H, D_2O exchangeable, OH), 7.93 (s, 1H, H_2), 6.84 (m, 5H, H_{arom}), 6.11 (m, 1H, H_6); ^{13}C NMR (DMSO-d_6) δ 173.1, 150.4, 146.1, 145.7, 135.3, 135.2, 129.7, 125.9, 122.7, 121.6, 119.1, 116.1, 115.6, 115.1, 113.6. Anal. ($C_{15}H_9NO_3S$) C, H, N.

3-(3,4,5-Trihydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (7e). 7e was obtained as an orange powder in 83% yield starting from 3-(3,4,5-trimethoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1k**: mp > 260 °C; IR 3385 (O−H), 1667 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.23 (br s, 2H, D_2O exchangeable, $OH_{3'}$ and $OH_{5'}$), 8.49 (br s, 1H, D_2O exchangeable, $OH_{4'}$), 7.92 (s, 1H, H_2), 7.01 (m, 1H, H_5 or H_7), 6.75 (m, 1H, H_5 or H_7), 6.47 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.14 (m, 1H, H_6); ^{13}C NMR (DMSO-d_6) δ 173.2, 151.4, 150.3, 146.5, 135.3, 135.2, 133.9, 130.1, 125.9, 121.8, 115.7, 113.6, 106.7. Anal. ($C_{15}H_9NO_4S$) C, H, N.

General Procedure for the Synthesis of 3-(Alkoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-ones 8a−d. A solution of sodium (0.48 g, 2.1 mmol) in methanol (50 mL) was stirred and cooled to 0 °C before adding successively 3-(4-hydroxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **7c** (0.5 g, 2.1 mmol) and the corresponding alkyl bromide (2.1 mmol). The reaction mixture was refluxed for 3 h and concentrated under reduced pressure, and the beige residue was stirred in a 0.5 N aqueous sodium hydroxide solution (100 mL) for 15 min. The resulting precipitate was filtered, washed with water (2 × 50 mL), dried over P_2O_5 under vacuum, and recrystallized from ethanol/methanol (1:2).

3-(4-Ethoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (8a). 8a was obtained as an orange powder in 77% yield using ethyl bromide (0.15 mL, 2.1 mmol) as reagent: mp = 198−201 °C; IR 1687 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.02 (s, 1H, H_2), 7.50 (d, J = 8.6 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.03 (d, J = 8.6 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.86 (d, $J_{5,6}$ = 2.5 Hz, 1H, H_5), 6.75 (d, $J_{7,6}$ = 3.5 Hz, 1H, H_7), 6.03 (m, 1H, H_6), 4.08 (q, J = 6.9 Hz, 2H, CH_2), 1.35 (t, J = 6.9 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.2, 159.3, 150.9, 135.9, 133.9, 129.4, 129.1, 126.8, 124.4, 120.8, 115.6, 114.8, 113.2, 63.6, 14.7. Anal. ($C_{17}H_{13}NO_2S$) C, H, N.

3-(4-*n*-Propoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (8b). 8b was obtained as an orange powder in 74% yield using *n*-propyl bromide (0.19 mL, 2.1 mmol) as reagent: mp = 172 °C; IR 1680 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.03 (s, 1H, H_2), 7.50 (d, J = 7.6 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.06 (d, J = 7.6 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.87 (d, $J_{5,6}$ = 2.3 Hz, 1H, H_5), 6.75 (d, $J_{7,6}$ = 3.4 Hz, 1H, H_7), 6.11 (m, 1H, H_6), 3.97 (m, 2H, OCH_2), 1.75 (m, 2H, CH_2), 0.98 (t, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.1, 159.5, 150.3, 135.7, 133.3, 129.1, 129.2, 127.1, 124.8, 121.5, 115.9, 114.9, 112.7, 62.4, 31.6, 13.7. Anal. ($C_{18}H_{15}NO_2S$) C, H, N.

3-(4-*i*-Propoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (8c). 8c was obtained as an orange powder in 63% yield using isopropyl bromide (0.19 mL, 2.1 mmol) as reagent: mp = 132−133 °C; IR 1687 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.02 (s, 1H, H_2), 7.48 (d, J = 8.2 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.04 (d, J = 8.2 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.88 (d, $J_{5,6}$ = 2.2 Hz, 1H, H_5), 6.75 (d, $J_{7,6}$ = 3.2 Hz, 1H, H_7), 6.11 (m, 1H, H_6), 3.67 (m, 1H, OCH), 1.28 (d, J = 5.9 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.2, 159.2, 150.8, 135.9, 133.6, 129.4, 129.2, 127.2, 125.0, 121.8, 116.3, 115.0, 112.8, 73.1, 19.6, 19.7. Anal. ($C_{18}H_{15}NO_2S$) C, H, N.

3-(4-*n*-Butoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one (8d). 8d was obtained as an orange powder in 75% yield using *n*-butyl bromide (0.23 mL, 2.1 mmol) as reagent: mp = 129 °C; IR 1679 (C═O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.02 (s, 1H, H_2), 7.50 (d, J = 8.2 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.06 (d, J = 8.2 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.87 (d, $J_{5,6}$ = 2.0 Hz, 1H, H_5), 6.75 (d, $J_{7,6}$ = 3.0 Hz, 1H, H_7), 6.10 (m, 1H, H_6), 4.01 (m, 2H, OCH_2), 1.71 (m, 2H, CH_2), 1.45 (m, 2H, CH_2), 0.93 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.1, 159.5, 150.8, 135.8, 133.8, 129.5, 129.1, 126.8, 124.3, 120.7, 115.5, 114.9, 113.1, 67.7, 31.2, 19.2, 13.8. Anal. ($C_{19}H_{17}NO_2S$) C, H, N.

3-Benzyloxy-4-methoxybenzaldehyde (9). Isovanillin (62.5 g, 410 mmol) was dissolved in methanol (250 mL) in the presence of potassium carbonate (68 g, 492 mmol) and benzyl bromide (58.5 mL, 492 mmol). The mixture was refluxed for 2 h and filtered, and the filtrate was evaporated under reduced pressure. The residue was taken up in chloroform (300 mL), and the solution was washed with water (2 × 200 mL), dried ($MgSO_4$), and evaporated to afford a white solid in 98% yield: mp = 68−70 °C; IR 1678 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.80 (s, 1H, CHO), 7.36 (m, 7H, H_{arom}), 6.97 (d, $J_{5,6}$ = 8.5 Hz, 1H, H_5), 5.17 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 190.7, 154.8, 148.5, 136.1, 129.9, 128.5, 128.1, 127.3, 126.8, 111.1, 110.6, 70.6, 56.1. Anal. ($C_{15}H_{14}O_3$) C, H, N.

3-Amino-3-(3-benzyloxy-4-methoxyphenyl)propanoic Acid (10). The aldehyde **9** (33 g, 136 mmol) was dissolved in ethanol (300 mL) with malonic acid (14.2 g, 136 mmol) and

ammonium acetate (21 g, 272 mmol). The reaction mixture was refluxed for 24 h until the appearance of a yellow precipitate. The latter was filtered, washed with hot ethanol (150 mL), and dried in a laboratory oven (60 °C): mp = 258 °C; IR 2103 (Zwitterion), 1676 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.42 (m, 5H, H_{arom}), 7.14 (m, 1H, H_{arom}), 6.93 (m, 2H, H_{arom}), 5.07 (s, 2H, CH_2), 4.18 (m, 1H, CH), 3.76 (s, 3H, OCH_3), 2.68 (m, 2H, CH_2). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

3-(3-Benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)propanoic Acid (11). A solution of 2,5-dimethoxytetrahydrofuran (2.37 mL, 18 mmol) in acetic acid (50 mL) was heated under stirring at 80 °C for 1 h and 30 min with the propanoic acid **10** (5 g, 17 mmol). The solvent was evaporated to give a brown residue. The latter was dissolved in methylene chloride (150 mL) and the solution was washed with a 1 N aqueous hydrochloric acid solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a beige solid. An analytical sample was recrystallized from diethyl ether to afford a white solid in 72% yield: mp = 92 °C; IR 3103 (O−H), 1699 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (m, 5H, H_{arom}), 6.71 (d, $J_{5',6'}$ = 8.3 Hz, 1H, $H_{5'}$), 6.63 (d, $J_{6',5'}$ = 8.3 Hz, 1H, $H_{6'}$), 6.55 (m, 3H, $H_{\alpha pyrrole}$ and $H_{2'}$), 6.04 (m, 2H, $H_{\beta pyrrole}$), 5.41 (m, 1H, CH), 4.95 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.05 (dd, $J_{2a,2b}$ = 16.1 Hz, $J_{2a,3}$ = 8.5 Hz, 1H, H_{2a}), 2.96 (dd, $J_{2b,2a}$ = 16.1 Hz, $J_{2b,3}$ = 6.6 Hz, 1H, H_{2b}); ^{13}C NMR ($CDCl_3$) δ 175.3, 149.3, 147.9, 136.6, 132.6, 128.4, 127.8, 127.4, 119.4, 118.9, 112.4, 111.6, 108.4, 70.9, 58.4, 55.8, 40.6. Anal. ($C_{21}H_{21}NO_4$) C, H, N.

3-(3-Benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)pyrrolidine Propionamide (12). 3-(3-Benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)propionic acid **11** (2.1 g, 6 mmol) was dissolved in anhydrous acetone (50 mL). After the mixture was cooled, TEA (0.836 mL, 6 mmol), ethyl chloroformate (0.576 mL, 6 mmol), and pyrrolidine (0.427 g, 6 mmol) were successively added slowly with a latent period of 15 min. The mixture was refluxed for 1 h and 30 min. After the mixture was cooled, the triethylammonium salt was filtered and the solvent was evaporated to afford a colorless oil in 62% yield: IR 1668 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18 (m, 5H, H_{arom}), 6.65 (m, 3H, $H_{2'}$, $H_{5'}$, and $H_{6'}$), 6.53 (m, 2H, $H_{\alpha pyrrole}$), 5.97 (m, 2H, $H_{\beta pyrrole}$), 5.56 (m, 1H, CH), 4.90 (s, 2H, CH_2), 3.66 (s, 3H, OCH_3), 3.20 (m, 2H, $H_{\alpha pyrrolidine}$), 2.94 (m, 2H, CH_2), 2.81 (m, 2H, $H_{\alpha pyrrolidine}$), 1.61 (m, 4H, $H_{\beta pyrrolidine}$). Anal. ($C_{25}H_{28}N_2O_3$) C, H, N.

3-(3-Benzyloxy-4-methoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one (13). A solution of 3-(3-benzyloxy-4-methoxyphenyl)-3-(pyrrol-1-yl)pyrrolidine propionamide **12** (1 g, 2.5 mmol) in toluene (50 mL) was cooled to 0 °C, and phosphorus oxychloride (0.46 mL, 5 mmol) was added slowly. The mixture was refluxed for 1 h, and the solvent was evaporated. The oily residue was taken up in water (50 mL), stirred overnight, and filtered. The aqueous layer was diluted with a 5% aqueous sodium hydroxide solution to pH 10, and the precipitate was dissolved in chloroform (70 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a white solid in 53% yield: IR 1681 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (m, 5H, H_{arom}), 6.73−6.38 (m, 6H, H_{arom}), 5.27 (m, 1H, CH), 4.93 (s, 2H, CH_2), 3.63 (s, 3H, OCH_3), 3.32 (m, 1H, H_{2a}), 2.73 (m, 1H, H_{2b}). Anal. ($C_{21}H_{19}NO_3$) C, H, N.

3-(3-Hydroxy-4-methoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one (14). A solution of the 3-(3-benzyloxy-4-methoxyphenyl)-2,3-dihydro-1*H*-pyrrolizin-1-one **13** (0.4 g, 1.2 mmol) in a 33% solution of hydrobromic acid in glacial acetic acid (10 mL) was stirred at room temperature for 1 h. After cooling, the reaction mixture was diluted with water (50 mL) and the resulting precipitate was extracted with ethyl acetate (3 × 20 mL). Then, the organic layers were combined, washed with an aqueous 10% $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to give a white solid purified by column chromatography, eluting by hexane/ethyl acetate (2:1) to provide **14** in 65% yield: mp = 186 °C; IR 3312 (O−H), 1683 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.81 (m, 3H, H_{arom}), 6.63 (m, 2H, H_{arom}), 6.52 (m, 1H, H_{arom}), 5.84 (br s, 1H, D_2O exchangeable, OH), 5.43 (m, 1H, CH), 3.89 (s, 3H, OCH_3), 3.49 (dd, $J_{2a,2b}$ = 18 Hz, $J_{2a,3}$ = 7.7 Hz, 1H, H_{2a}), 2.93 (dd, $J_{2b,2a}$ = 18 Hz, $J_{2b,3}$ = 2.6 Hz, 1H, H_{2b}); ^{13}C NMR ($CDCl_3$) δ 188.4, 146.7, 146.2, 133.6, 133.1, 122.5, 117.7, 117.3, 112.2, 110.9, 107.5, 57.8, 55.9, 49.6; EIMS *m*/*z* (relative intensity) 243 (M^+, 72), 150 (100), 135 (36), 94 (12), 77,8 (12). Anal. ($C_{14}H_{13}NO_3$) C, H, N.

Methyl 3-Amino-4-(3,4-dimethoxyphenyl)furan-2-carboxylate (15). To a solution of 3-hydroxy-2-(3,4-dimethoxyphenyl)acrylonitrile sodium salt **2j** (2 g, 8.8 mmol) in DMF (20 mL) was added diethyl chloromalonate (1.57 mL, 9.7 mmol). The reaction mixture was stirred for 5 h at room temperature, and the organic solvent was then removed under reduced pressure. The dark oil was extracted with methylene chloride (50 mL), and the organic layer was washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and evaporated to give an orange syrup. The orange syrup was dissolved in ethanol (50 mL) to which 1,5-diazabicyclo[4.3.0]non-5-ene (1.2 mL, 9.7 mmol) was added, and the mixture was stirred at room temperature overnight. The solution was concentrated, and an analytical sample was prepared by silica gel chromatography, eluting by ethyl acetate/cyclohexane (1:2). The orange powder was recrystallized from methanol to give a yellow solid in 25% yield: mp = 121−123 °C; IR 3464 (N−H), 3371 (N−H), 1688 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (s, 1H, H_{furan}), 6.94 (m, 3H, H_2, H_5, and H_6), 4.69 (br s, 2H, D_2O exchangeable, NH_2), 3.91 (s, 9H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 160.1, 149.1, 148.5, 141.7, 126.0, 122.2, 119.9, 119.6, 111.4, 110.5, 55.9, 55.1; EIMS *m*/*z* (relative intensity) 277 (M^+, 100), 262 (40), 203 (50), 174 (31), 146 (32). Anal. ($C_{14}H_{15}NO_5$) C, H, N.

Methyl 3-Amino-4-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2-carboxylate (16). To a solution of the enamine (3.85 g, 11 mmol) in methanol (80 mL) was added sodium methoxide (0.65 g, 12 mmol). The reaction mixture was refluxed for 45 min, and the dark solution obtained was then stirred for 2 h at room temperature until the precipitation of a beige solid. After cooling, the mixture was filtered, washed with cold methanol (2 × 50 mL), and dried over P_2O_5 under vacuum to afford a white solid in 85% yield: mp = 169 °C; IR 3463 (N−H), 3358 (N−H), 3275 (N−H), 1661 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.94 (m, 2H, H_5, H_{arom}), 6.86 (d, J = 8.2 Hz, 1H, H_{arom}), 6.77 (m, 1H, H_{arom}), 4.53 (br s, 3H, D_2O exchangeable, NH, NH_2), 3.84 (s, 9H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 162.3, 149.2, 147.6, 128.6, 128.5, 127.7, 126.7, 119.4, 114.6, 111.7, 110.7, 55.9, 55.8, 50.9; EIMS *m*/*z* (relative intensity) 276 (M^+, 64), 244 (100). Anal. ($C_{14}H_{16}N_2O_4$) C, H, N.

2-(3,4-Dimethoxyphenyl)-3-hydroxyacrylonitrile (17). A solution of 3-hydroxy-2-(3,4-dimethoxyphenyl)acrylonitrile sodium salt **2j** (10 g, 44 mmol) in water (100 mL) was acidified by glacial acetic acid to pH 4. The resulting precipitate was filtered, washed with water, and dried over P_2O_5 under vacuum to afford a white solid in 96% yield which was used without further purification: mp = 120−122 °C; IR 3194 (O−H), 2208 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.41 (s, 1H, H_3), 7.20 (m, 1H, H_{arom}), 6.88 (m, 2H, H_{arom}), 3.90 (s, 6H, OCH_3).

Diethyl ((2-Cyano-2-(3,4-dimethoxyphenyl)vinyl)amino)malonate (18). 2-(3,4-Dimethoxyphenyl)-3-hydroxyacrylonitrile **17** (8 g, 39 mmol) was dissolved in methanol/water (5:1), and diethyl aminomalonate hydrochloride (12.4 g, 59 mmol) and sodium acetate (10.6 g, 78 mmol) were added. The mixture was stirred for 48 h to room temperature, and the solvent was concentrated under reduced pressure. The aqueous solution was extracted with ethyl acetate (150 mL), and the organic layer was washed with water (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a yellow oil that crystallized from diethyl ether. The white solid was obtained in 35% yield: mp = 120 °C; IR 3194 (O−H), 2208 (CN) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.06 (d, $J_{H1,NH}$ = 12.9 Hz, 1H, H_1), 6.82 (m, 3H, H_{arom}), 5.72 (dd, $J_{NH,H1}$ = 12.9 Hz, $J_{NH,CH}$ = 8.3 Hz, 1H, D_2O exchangeable, NH), 4.70 (d, $J_{CH,NH}$ = 8.3 Hz, 1H, CH), 4.31 (m, 4H, CH_2), 3.88 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 1.32 (t, J = 7.1 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9, 149.2, 147.6, 144.5, 126.1, 117.8, 116.2, 111.6, 107.6, 84.5, 62.9, 62.3, 55.9, 55.8, 13.9. Anal. ($C_{18}H_{22}N_2O_6$) C, H, N.

Methyl 4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)furan-2-carboxylate (19). A solution of 2,5-dimethoxytetrahydrofuran (0.26 mL, 2 mmol) in dioxane (50 mL) was stirred for 15 min at room temperature with 4-chloropyridine hydrochlo-

ride (0.3 g, 2 mmol). Methyl 3-amino-4-(3,4-dimethoxyphenyl)-furan-2-carboxylate **15** (0.5 g, 1.8 mmol) was added to the reaction mixture, which was heated under reflux for 2 h and then filtered through a small pad of Celite. The filtrate was evaporated to give a beige residue. The beige residue was dissolved in methylene chloride (150 mL) and the solution was washed with an aqueous 1 N hydrochloric acid solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a beige solid in 81% yield: IR 1710 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.71 (s, 1H, H_{furan}), 6.79 (dd, $J_{6,5} = 8.1$ Hz, $J_{6,2} = 1.8$ Hz, 1H, H_6), 6.71 (m, 3H, H_5 and $2H_{\alpha pyrrole}$), 6.27 (m, 2H, $2H_{\beta pyrrole}$), 6.21 (d, $J_{2,6} = 1.8$ Hz, 1H, H_2), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3). Anal. ($C_{18}H_{17}NO_5$) C, H, N.

Methyl 4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-1*H*-pyrrole-2-carboxylate (20). A solution of 2,5-dimethoxytetrahydrofuran (1.48 mL, 11 mmol) and 4-chloropyridine hydrochloride (1.7 g, 11 mmol) in dioxane (50 mL) was stirred for 15 min at room temperature. Methyl 3-amino-4-(3,4-dimethoxyphenyl)-1*H*-pyrrole-2-carboxylate **16** (2.85 g, 10 mmol) was added to the reaction mixture, which was refluxed for 3 h and 30 min and filtered through a small pad of Celite. The filtrate was evaporated to give a beige residue. The latter was dissolved in methylene chloride (200 mL), and the solution was washed with a 1 N aqueous hydrochloric acid solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give a beige solid in 72% yield: mp = 158−161 °C; IR 3385 (N−H), 1704 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.68 (br s, 1H, D_2O exchangeable, NH), 7.09 (d, $J_{H5,NH} = 3.4$ Hz, 1H, H_5), 6.76 (s, 1H, $H_{2'}$), 6.74 (d, $J_{6',5'} = 2$ Hz, 1H, $H_{6'}$), 6.70 (m, 2H, $H_{\alpha pyrrole}$), 6.26 (m, 2H, $H_{\beta pyrrole}$), 6.21 (d, $J_{5',6'} = 2$ Hz, 1H, $H_{5'}$), 3.84 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 160.7, 148.7, 147.6, 127.7, 125.1, 123.1, 122.8, 118.9, 118.4, 117.0, 110.9, 109.3, 108.8, 55.6, 55.4, 51.6; EIMS *m*/*z* (relative intensity) 326 (M^+, 78), 294 (69), 136 (100), 83 (92). Anal. ($C_{18}H_{18}N_2O_4$) C, H, N.

4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)furan-2-pyrrolidine Carboxamide (21). A solution of methyl 4-(3,4-dimethoxyphenyl)-3-(pyrrol-1-yl)furan-2-carboxylate **19** (0.48 g, 1.5 mmol) in pyrrolidine (20 mL) was refluxed for 3 h. After the mixture was cooled and evaporated, the yellow oil was dissolved in methylene chloride (100 mL) and the solution was washed with a 1 N aqueous hydrochloric acid solution (2 × 100 mL), dried ($MgSO_4$), and evaporated to give **21** as a yellow oil in 63% yield: IR 1636 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.53 (s, 1H, H_{furan}), 6.70 (dd, $J_{6,5} = 8.3$ Hz, $J_{6,2} = 1.7$ Hz, 1H, H_6), 6.62 (d, $J_{5,6} = 8.3$ Hz, 1H, H_5), 6.61 (m, 2H, $H_{\alpha pyrrole}$), 6.18 (d, $J_{2,6} = 1.7$ Hz, 1H, H_2), 6.14 (m, 2H, $H_{\beta pyrrole}$), 3.75 (s, 6H, OCH_3), 3.47 (m, 2H, $H_{\alpha pyrrolidine}$), 3.12 (m, 2H, $H_{\alpha pyrrolidine}$), 1.72 (m, 4H, $H_{\beta pyrrolidine}$).

4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-1*H*-pyrrole-2-pyrrolidine Carboxamide (22). A solution of methyl 4-(3,4-dimethoxyphenyl)-3-(pyrrol-1-yl)-1*H*-pyrrole-2-carboxylate **20** (2.3 g, 7.1 mmol) in pyrrolidine (40 mL) was refluxed for 15 h. After the mixture was cooled and evaporated, the yellow oil was dissolved in methylene chloride (150 mL) and the solution was washed with water (3 × 100 mL), dried ($MgSO_4$), and evaporated to give a residue purified by column chromatography, eluting by ethyl acetate/cyclohexane (1:1) to afford a white powder in 55% yield: mp = 150−151 °C; IR 3223 (N−H), 1593 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.01 (br s, 1H, D_2O exchangeable, NH), 6.96 (d, $J_{H5,NH} = 2.7$ Hz, 1H, H_5), 6.69 (d, $J_{5,6} = 8.3$ Hz, 1H, H_5), 6.63 (d, $J_{6,5} = 8.3$ Hz, 1H, H_6), 6.55 (m, 2H, $H_{\alpha pyrrole}$), 6.17 (s, 1H, H_2), 6.12 (m, 2H, $H_{\beta pyrrole}$), 3.76 (s, 3H, OCH_3), 3.52 (m, 5H, OCH_3, $H_{\alpha pyrrolidine}$), 2.50 (m, 2H, $H_{\alpha pyrrolidine}$), 1.69 (m, 2H, $H_{\beta pyrrolidine}$), 1.53 (m, 2H, $H_{\beta pyrrolidine}$); ^{13}C NMR ($CDCl_3$) δ 161.7, 148.6, 147.4, 126.1, 123.5, 122.2, 120.8, 120.5, 118.9, 117.9, 110.9, 109.9, 109.5, 55.7, 55.3, 46.6, 46.4, 26.0, 23.8. Anal. ($C_{21}H_{23}N_3O_3$) C, H, N.

3-(3,4-Dimethoxyphenyl)-8*H*-furo[2,3-*b*]pyrrolizin-8-one (23). A solution of **21** (0.35 g, 0.96 mmol) in phosphorus oxychloride (15 mL) was refluxed for 6 h. After cooling, the reaction mixture was concentrated to give a red solid that was filtered, washed with Et_2O, and dried. Then, this intermediary iminium salt was added slowly to a 10% aqueous sodium hydroxide solution (70 mL) and the mixture was heated for 1 h to 50 °C. The precipitate was extracted by diethyl ether (100 mL), and the organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to give a brown residue purified by column chromatography, eluting by ethyl acetate/cyclohexane (1:2) to afford an orange powder in 38% yield: mp = 132−134 °C; IR 1684 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.51 (s, 1H, H_2), 6.98 (dd, $J_{6',5'} = 8.6$ Hz, $J_{6',2'} = 1.7$ Hz, 1H, $H_{6'}$), 6.89 (m, 2H, $H_{2'}$ and $H_{5'}$), 6.77 (d, $J_{5,6} = 2.5$ Hz, 1H, H_5), 6.59 (d, $J_{7,6} = 3.1$ Hz, 1H, H_7), 5.98 (m, 1H, H_6), 3.82 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 173.9, 150.8, 149.4, 149.2, 135.9, 134.1, 129.5, 126.8, 124.9, 120.6, 120.5, 115.6, 113.2, 111.4, 111.1, 56.0, 55.9; EIMS *m*/*z* (relative intensity) 295 (M^+, 51), 177 (81), 114 (100). Anal. ($C_{17}H_{13}NO_4$) C, H, N.

3-(3,4-Dimethoxyphenyl)-1,8-dihydropyrrolo[2,3-*b*]pyrrolizin-8-one (24). A solution of **22** (1.4 g, 3.8 mmol) in phosphorus oxychloride (60 mL) was heated at 70 °C for 5 h. After cooling, the reaction mixture was evaporated to give a red solid, which was filtered, washed with Et_2O, and dried. Then, this intermediary iminium salt was added slowly to a 10% aqueous sodium hydroxide solution (100 mL) and the mixture was heated for 3 h to 80 °C. The precipitate was extracted by diethyl ether (2 × 100 mL), and the organic layers were dried ($MgSO_4$) and evaporated under reduced pressure to give a brown residue purified by column chromatography, eluting by ethyl acetate to afford a red powder in 62% yield: mp = 194 °C; IR 3142 (N−H), 1655 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 11.18 (br s, 1H, D_2O exchangeable, NH), 6.93 (dd, $J_{6',5'} = 8.2$ Hz, $J_{6',2'} = 1.8$ Hz, 1H, $H_{6'}$), 6.91 (d, $J_{H2,NH} = 3$ Hz, 1H, H_2), 6.88 (d, $J_{2',6'} = 1.8$ Hz, 1H, $H_{2'}$), 6.84 (d, $J_{5',6'} = 8.2$ Hz, 1H, $H_{5'}$), 6.81 (d, $J_{5,6} = 2.2$ Hz, 1H, H_5), 6.47 (d, $J_{7,6} = 3.6$ Hz, 1H, H_7), 5.88 (dd, $J_{6,5} = 2.2$ Hz, $J_{6,7} = 3.6$ Hz, 1H, H_6), 3.84 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 171.4, 149.3, 148.4, 140.2, 136.2, 126.7, 125.1, 124.9, 121.7, 119.1, 114.6, 113.1, 112.2, 111.7, 110.1, 56.1. Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

2-Amino-3-iodobenzoic Acid (25). To a stirred suspension of 7-iodoisatin[22] (1 g, 3.7 mmol) in a 5% aqueous sodium hydroxide solution (15 mL) was added dropwise a 30% aqueous hydrogen peroxide solution (15 mL). The reaction mixture was stirred at 50 °C for 30 min and then allowed to reach room temperature. The filtered solution was acidified to pH 4 with an aqueous 1 N hydrochloric acid solution, and the white precipitate **25** was collected by filtration and dried over P_2O_5 under vacuum with 81% yield: mp = 182 °C; IR 3540 (O−H), 3230 (N−H), 3178 (N−H), 1665 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.96 (d, $J_{4,5} = 8$ Hz, 1H, H_4), 7.85 (d, $J_{6,5} = 7.6$ Hz, 1H, H_6), 6.44 (dd, $J_{5,4} = 8$ Hz, $J_{5,6} = 7.6$ Hz, 1H, H_5). Anal. ($C_7H_6NO_2I$) C, H, N.

3-Iodo-2-(pyrrol-1-yl)benzoic Acid (26). A solution of 2,5-dimethoxytetrahydrofuran (1.25 mL, 9.6 mmol) and 4-chloropyridine hydrochloride (1.44 g, 9.6 mmol) in dioxane (50 mL) was stirred for 15 min at room temperature. 2-Amino-3-iodobenzoic acid **25** (2.3 g, 8.7 mmol) was added to the reaction mixture, which was refluxed for 2 h and filtered through a small pad of Celite. The filtrate was evaporated to give a beige residue. The beige residue was dissolved in diethyl ether (100 mL), and the solution was washed with a 1 N aqueous hydrochloric acid solution (2 × 50 mL), dried ($MgSO_4$), and evaporated to give a white solid in 60% yield: mp = 170−172 °C; IR 3100 (O−H), 1681 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.17 (br s, 1H, D_2O exchangeable, OH), 8.01 (d, $J_{4,5} = 7.6$ Hz, 1H, H_4), 7.81 (d, $J_{6,5} = 7.6$ Hz, 1H, H_6), 7.12 (t, $J = 7.6$ Hz, 1H, H_5), 6.58 (m, 2H, $H_{\alpha pyrrole}$), 6.24 (m, 2H, $H_{\beta pyrrole}$). Anal. ($C_{11}H_8NO_2I$) C, H, N.

Ethyl 3-Iodo-2-(pyrrol-1-yl)benzoate (27). 3-Iodo-2-(pyrrol-1-yl)benzoic acid **26** (1.5 g, 4.8 mmol) was dissolved in ethanol (50 mL) in the presence of thionyl chloride (0.35 mL, 4.8 mmol), and the mixture was refluxed for 5 h. The solvent was evaporated under reduced pressure to afford an oil that slowly crystallized from petroleum ether as a beige solid in 83% yield: mp = 82−84 °C; IR 1672 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (d, $J_{4,5} = 8$ Hz, 1H, H_4), 7.84 (d, $J_{6,5} = 7.6$ Hz, 1H, H_6), 7.23 (dd, $J_{5,4} = 8$ Hz, $J_{5,6} = 7.6$ Hz, 1H, H_5), 7.10 (m,

2H, $H_{\alpha pyrrole}$), 6.36 (m, 2H, $H_{\beta pyrrole}$), 4.13 (q, J = 6.8 Hz, 2H, CH_2), 1.14 (t, J = 6.8 Hz, 3H, CH_3). Anal. ($C_{13}H_{12}NO_2I$) C, H, N.

5-Iodo-9*H*-pyrrolo[1,2-*a*]indol-9-one (28). A solution of ethyl 3-iodo-2-(pyrrol-1-yl)benzoate **27** (1.3 g, 3.8 mmol) in methylene chloride (50 mL) was cooled to 0 °C with an ice bath before adding a 1 M solution of boron tribromide in methylene chloride (4.2 mL, 4.2 mmol). The reaction mixture was stirred for 45 min to room temperature and diluted with cold water (100 mL). After 3 h, the organic layer was separated, washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The green residue was purified by column chromatography, eluting by ethyl acetate/hexane (1:4) to afford a yellow powder in 62% yield: mp = 138−140 °C; IR 1700 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.87 (dd, $J_{3,2}$ = 2.8 Hz, $J_{3,1}$ = 0.8 Hz, 1H, H_3), 7.58 (dd, $J_{8,7}$ = 8 Hz, $J_{8,6}$ = 1 Hz, 1H, H_8), 7.40 (dd, $J_{6,7}$ = 7.3 Hz, $J_{6,8}$ = 1 Hz, 1H, H_6), 6.72 (m, 2H, H_7 and H_1), 6.22 (dd, $J_{2,3}$ = 2.8 Hz, $J_{2,1}$ = 3.6 Hz, 1H, H_2); ^{13}C NMR ($CDCl_3$) δ 177, 145.2, 144.2, 132.3, 132.2, 126.5, 123.9, 121.6, 115.3, 114.7, 74.5. Anal. ($C_{11}H_6NOI$) C, H, N.

General Procedure for the Synthesis of 5-Aryl-9*H*-pyrrolo[1,2-*a*]indol-9-ones 29a−d. To a mixture of 5-iodo-9*H*-pyrrolo[1,2-*a*]indol-9-one **28** (0.3 g, 1 mmol) and $Pd(PPh_3)_4$ (0.035 g, 0.03 mmol) in DME (30 mL) was added successively the corresponding arylboronic acid (1.1 mmol) and sodium hydrogen carbonate (0.17 g, 2 mmol) in water (30 mL). The reaction mixture was refluxed with vigorous stirring under argon, and the rate of the reaction was followed by TLC. After the starting aryl halide **28** was consumed (8 h), the organic solvent was removed under reduced pressure. The residue was extracted with ethyl acetate (2 × 40 mL), and the organic layers were dried ($MgSO_4$) and evaporated. The orange solid was purified by column chromatography, eluting by ethyl acetate/hexane (1:4).

5-(3,4-Dimethoxyphenyl)-9*H*-pyrrolo[1,2-*a*]indol-9-one (29a). 29a was obtained as a yellow powder in 73% yield using 3,4-dimethoxyphenylboronic acid (0.2 g, 1.1 mmol) as reagent: mp = 198 °C; IR 1688 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (d, $J_{8,7}$ = 7.4 Hz, 1H, H_8), 7.31 (d, $J_{6,7}$ = 8.7 Hz, 1H, H_6), 7.16 (dd, $J_{7,6}$ = 8.7 Hz, $J_{7,8}$ = 7.4 Hz, 1H, H_7), 7.01 (m, 3H, $H_{2'}$, $H_{5'}$, and $H_{6'}$), 6.75 (d, $J_{1,2}$ = 4 Hz, 1H, H_1), 6.45 (d, $J_{3,2}$ = 2 Hz, 1H, H_3), 6.08 (dd, $J_{2,1}$ = 4 Hz, $J_{2,3}$ = 2 Hz, 1H, H_2), 3.97 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 179.0, 149.2, 148.9, 141.1, 136.1, 132.4, 130.7, 128.8, 127.1, 125.2, 123.1, 122.7, 121.2, 115.2, 113.8, 111.9, 111.2, 55.9, 55.8. Anal. ($C_{19}H_{15}NO_3$) C, H, N.

5-(4-Methoxyphenyl)-9*H*-pyrrolo[1,2-*a*]indol-9-one (29b). 29b was obtained as a yellow powder in 65% yield using 4-methoxyphenylboronic acid (0.17 g, 1.1 mmol) as reagent: mp = 145−147 °C; IR 1696 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.56 (d, $J_{8,7}$ = 7.3 Hz, 1H, H_8), 7.39 (d, J = 8.5 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.27 (d, $J_{6,7}$ = 7.8 Hz, 1H, H_6), 7.14 (dd, $J_{7,6}$ = 7.8 Hz, $J_{7,8}$ = 7.3 Hz, 1H, H_7), 7.02 (d, J = 8.5 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.75 (d, $J_{1,2}$ = 3.5 Hz, 1H, H_1), 6.45 (d, $J_{3,2}$ = 2.2 Hz, 1H, H_3), 6.07 (m, 1H, H_2), 3.89 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 179.5, 159.8, 141.3, 136.2, 132.5, 130.8, 130.1, 128.7, 127.1, 125.2, 123.1, 122.7, 115.2, 114.2, 113.8, 55.3. Anal. ($C_{18}H_{13}NO_2$) C, H, N.

5-(Fur-2-yl)-9*H*-pyrrolo[1,2-*a*]indol-9-one (29c). 29c was obtained as a yellow powder in 71% yield using fur-2-ylboronic acid (0.12 g, 1.1 mmol) as reagent: mp = 121 °C; IR 1692 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.52 (m, 1H, $H_{5'}$), 7.47 (d, $J_{8,7}$ = 7.2 Hz, 1H, H_8), 7.38 (d, $J_{6,7}$ = 7.8 Hz, 1H, H_6), 7.07 (dd, $J_{7,6}$ = 7.8 Hz, $J_{7,8}$ = 7.2 Hz, 1H, H_7), 7.02 (m, 1H, H_{arom}), 6.74 (m, 1H, H_{arom}), 6.58 (m, 1H, H_{arom}), 6.51 (m, 1H, H_{arom}), 6.13 (m, 1H, H_2); ^{13}C NMR ($CDCl_3$) δ 179.1, 149.4, 142.6, 140.5, 134.5, 132.5, 131.6, 125.3, 124.1, 123.8, 116.4, 115.6, 114.3, 111.9, 109.6. Anal. ($C_{15}H_9NO_2$) C, H, N.

5-(Thien-2-yl)-9*H*-pyrrolo[1,2-*a*]indol-9-one (29d). 29d was obtained as a yellow powder in 58% yield using thien-2-ylboronic acid (0.14 g, 1.1 mmol) as reagent: mp = 112 °C; IR 1690 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (m, 1H, $H_{5'}$), 7.49 (d, $J_{8,7}$ = 7.1 Hz, 1H, H_8), 7.37 (d, $J_{6,7}$ = 8.2 Hz, 1H, H_6), 7.26 (m, 2H, $H_{3'}$ and $H_{4'}$), 7.17 (dd, $J_{7,6}$ = 8.2 Hz, $J_{7,8}$ = 7.1 Hz, 1H, H_7), 6.77 (d, $J_{1,2}$ = 3.2 Hz, 1H, H_1), 6.55 (d, $J_{3,2}$ = 2.4 Hz, 1H, H_3), 6.12 (m, 1H, H_2); ^{13}C NMR ($CDCl_3$) δ 179.1, 157.2, 142.1, 138.1, 132.4, 130.9, 127.8, 127.5, 127.1, 125.1, 124.2, 122.6, 119.8, 115.5, 114.1. Anal. ($C_{15}H_9NOS$) C, H, N.

3-(4-Methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-ol (30). 3-(4-Methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1d** (0.5 g, 1.78 mmol) was dissolved in tetrahydrofuran (30 mL). After the mixture was cooled to 0 °C with an ice bath, lithium aluminum hydride (0.17 g, 4.44 mmol) was slowly added to the mixture, which was stirred for 2 h at 0 °C before the reaction was quenched with crushed ice. The solvent was concentrated under reduced pressure, and the resulting suspension was extracted with diethyl ether (2 × 50 mL). The organic layers were collected, washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated to give a yellow residue purified by column chromatography, eluting by ethyl acetate/cyclohexane (1:2) to give **30** as a gray powder with 78% yield: mp = 124 °C; IR 3386 (O−H) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.35 (d, J = 7.9 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.19 (d, J = 7.9 Hz, 2H, $H_{3'}$ and $H_{5'}$), 7.09 (s, 1H, $H_{thiophene}$), 6.70 (m, 1H, H_5), 6.26 (m, 1H, H_7), 6.04 (m, 2H, 1H D_2O exchangeable, H_6 and OH), 5.62 (s, 1H, H_8), 3.84 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 142.8, 142.0, 137.9, 132.8, 130.8, 129.5, 128.1, 126.6, 113.5, 110.9, 106.6, 67.0, 55.5. Anal. ($C_{16}H_{13}NO_2S$) C, H, N.

8-Hydroxyimino-3-(4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizine (31). 3-(4-Methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizin-8-one **1d** (1 g, 3.6 mmol) was dissolved in pyridine (10 mL), and hydroxylamine hydrochloride (0.55 g, 7.9 mmol) was slowly added to the reaction mixture, which was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the residue was taken up in ethyl acetate (100 mL). The solution was washed with a 1 N aqueous hydrochloric acid solution (2 × 100 mL) and the organic layer was dried ($MgSO_4$) and evaporated to give a yellow residue purified by column chromatography, eluting by ethyl acetate/cyclohexane (1:4). **31** was obtained as a yellow powder with 95% yield in a mixture of *Z* and *E* isomers (30:70) not separated: mp = 191−194 °C; IR 3225 (O−H), 1607 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6), *Z* isomer, δ 12.07 (br s, 1H, D_2O exchangeable, OH), 7.65 (s, 1H, H_2), 7.56 (d, J = 8.3 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.12 (d, J = 8.3 Hz, 2H, $H_{3'}$ and $H_{6'}$), 6.93 (m, 1H, H_5), 6.72 (m, 1H, H_7), 6.22 (m, 1H, H_6), 3.85 (s, 3H, OCH_3); ^1H NMR (DMSO-d_6), *E* isomer, δ 12.11 (br s, 1H, D_2O exchangeable, OH), 7.79 (s, 1H, H_2), 7.56 (d, J = 8.3 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.12 (d, J = 8.3 Hz, 2H, $H_{3'}$ and $H_{6'}$), 6.93 (m, 1H, H_5), 6.55 (m, 1H, H_7), 6.18 (m, 1H, H_6), 3.85 (s, 3H, OCH_3). Anal. ($C_{16}H_{12}N_2O_2S$) C, H, N.

8-(*N*,*N*-Dimethylaminopropyloxyimino)-3-(4-methoxyphenyl)-8*H*-thieno[2,3-*b*]pyrrolizine Monooxalate (32). To a solution of the oxime **31** (0.5 g, 1.7 mmol) in anhydrous acetone (50 mL) were added potassium carbonate (0.47 g, 3.4 mmol), 3-chloro-*N*,*N*-dimethylpropylamine hydrochloride (0.3 g, 1.9 mmol), and water (1 mL). The reaction mixture was refluxed for 48 h and concentrated under reduced pressure. The residue was taken up in diethyl ether (100 mL), and the solution was washed with water (3 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a yellow oil. The yellow oil was dissolved in propan-2-ol, and oxalic acid (0.15 g, 1.7 mmol) was added to the suspension, which was refluxed for 10 min. After the mixture was cooled, the precipitate was filtered and dried with anhydrous diethyl ether (3 × 50 mL) and then in a laboratory oven (40 °C) to afford yellow plates with 45% yield in a mixture of *Z* and *E* isomers (30:70) not separated: mp = 107 °C; IR 3417 (O−H), 1719 (C=O), 1612 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6), *Z* isomer, δ 7.68 (s, 1H, H_2), 7.51 (d, J = 8.2 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.08 (d, J = 8.2 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.91 (m, 1H, H_5), 6.74 (d, $J_{7,6}$ = 3.4 Hz, 1H, H_7), 6.16 (m, 1H, H_6), 4.32 (m, 4H, D_2O exchangeable, 2 × OH and OCH_2), 3.80 (s, 3H, OCH_3), 3.12 (m, 2H, NCH_2), 2.74 (s, 6H, $N(CH_3)_2$), 2.10 (m, 2H, CH_2); ^1H NMR (DMSO-d_6), *E* isomer, δ 7.83 (s, 1H, H_2), 7.51 (d, J = 8.2 Hz, 2H, $H_{2'}$ and $H_{6'}$), 7.08 (d, J = 8.2 Hz, 2H, $H_{3'}$ and $H_{5'}$), 6.91 (m, 1H, H_5), 6.56 (d, $J_{7,6}$ = 3.4 Hz, 1H, H_7), 6.14 (m, 1H, H_6), 4.32 (m, 4H, D_2O exchangeable, 2 × OH and OCH_2), 3.80 (s, 3H, OCH_3), 3.12 (m, 2H,

NCH_2), 2.74 (s, 6H, $N(CH_3)_2$), 2.10 (m, 2H, CH_2). Anal. ($C_{23}H_{25}N_3O_6S$) C, H, N.

4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Carboxylic Acid (33). An aqueous 2.5% sodium hydroxide solution (40 mL) was added dropwise to a stirred solution of methyl 4-(3,4-dimethoxyphenyl)-3-(pyrrol-1-yl)-2-thenoate **5j** (5 g, 14.6 mmol) in ethanol (50 mL). After 10 h at room temperature, water (50 mL) was added and the reaction mixture was acidified with 12 N hydrochloric acid to pH 3. The precipitate was extracted with ethyl acetate (50 mL); the organic layer was washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give a white solid with 95% yield: mp = 148−151 °C; IR 3100 (O−H), 1659 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.07 (br s, 1H, D_2O exchangeable, OH), 7.97 (s, 1H, $H_{thiophene}$), 6.85 (m, 1H, H_{arom}), 6.68 (m, 3H, H_{arom} and $2H_{\alpha pyrrole}$), 6.22 (m, 1H, H_{arom}), 6.11 (m, 2H, $H_{\beta pyrrole}$), 3.70 (s, 3H, OCH_3), 3.49 (s, 3H, OCH_3). Anal. ($C_{17}H_{15}NO_4S$) C, H, N.

4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene Azide (34). 4-(3,4-Dimethoxyphenyl)-3-(pyrrol-1-yl)-2-thiophene carboxylic acid **33** (1.5 g, 4.6 mmol) was dissolved in anhydrous acetone (40 mL). The solution was cooled to 0 °C with an ice bath, and TEA (0.7 mL, 5 mmol), ethyl chloroformate (0.5 mL, 5 mmol), and sodium azide (0.33 g, 5 mmol) were added successively with a latent period of 15 min. After 2 h of stirring at room temperature, the reaction mixture was filtered and diluted with water (100 mL) and the resulting precipitate was extracted with ethyl acetate (2 × 50 mL). The organic layers were collected, washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated to give a pink solid with 70% yield, which was used without further purification: mp < 100 °C; IR 2139 (N_3), 1803 (C=O) cm^{-1}.

1-(3,4-Dimethoxyphenyl)-4,5-dihydropyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazin-5-one (35). The azide **34** (1.14 g, 3.2 mmol) was added to hot 1,2-dichlorobenzene (15 mL, 180 °C). After 15 min, the mixture was cooled at room temperature until the appearance of a precipitate. The precipitate was filtered and recrystallized from methanol to give a white powder with 76% yield: mp = 242 °C; IR 3108 (N−H), 1642 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.56 (br s, 1H, D_2O exchangeable, NH), 7.59 (s, 1H, H_2), 7.36 (m, 1H, H_8), 7.03 (m, 3H, H_{arom}), 6.81 (m, 1H, H_6), 6.39 (m, 1H, H_7), 3.84 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); EIMS *m*/*z* (relative intensity) 326 (M^+, 100), 311 (27), 283 (27), 251 (18), 163 (19), 91 (14). Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N.

1-(3,4-Dihydroxyphenyl)-4,5-dihydropyrrolo[1,2-*a*]-thieno[2,3-*e*]pyrazin-5-one (36). A solution of 1-(3,4-dimethoxyphenyl)-4,5-dihydropyrrolo[1,2-*a*]thieno[2,3-*e*]pyrazin-5-one **35** (0.25 g, 0.76 mmol) in methylene chloride (20 mL) was stirred and cooled to 0 °C with an ice bath. Then, a 1 M solution of boron tribromide in methylene chloride was added (1.5 mL, 1.5 mmol) to the reaction mixture, which was stirred for 0.5 h to room temperature before being diluted with cold water (100 mL). After 3 h, the organic layer was extracted, washed with water (2 × 50 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The solid residue was crystallized from methanol: mp > 260 °C; IR 3231 (N−H), 1651 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.43 (br s, 1H, D_2O exchangeable, NH), 9.24 (br s, 2H, D_2O exchangeable, OH), 6.95 (s, 1H, H_2), 6.85 (m, 2H, H_{arom}), 6.76 (m, 2H, H_{arom}), 6.68 (m, 1H, H_{arom}), 6.42 (m, 1H, H_7); EIMS *m*/*z* (relative intensity) 298 (M^+, 100), 251 (14), 140 (15), 112 (10). Anal. ($C_{15}H_{10}N_2O_3S$) C, H, N.

Pharmacology. Standard Proliferation Assay. The principle of this assay and its application to anticancer drug screening have been the subject of publications.[33] Adherent cells were trypsinized and seeded in 96-well microplates at the indicated densities, previously determined to maintain control cells in the exponential phase of growth for the duration of the experiment and to obtain a linear relationship between the optical density and the number of viable cells.[34] The plates were incubated with the tested compounds for four doubling times, the maximum duration being 7 days. At the end of this period, an amount of 15 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, MO) was added to each well and the plates were incubated for 4 h at 37 °C. The medium was aspirated, and the formazan was solubilized by 100 μL of DMSO. The optical density (OD) was read at 540 nm with a plate reader (Multiskan MCC, Labsystem) connected to a computer. The percentage of growth was calculated for each well: % growth = [(OD treated cells)/(OD control cells)] × 100. The percentages of growth of the tri- or hexaplicate were then averaged and plotted as a function of the log of the concentration. The GI_{50} (concentration reducing by 50% of the OD) was calculated by a linear regression performed on the linear zone of the curve.

Cell Cycle Analysis. L1210 cells (2.5 × 10^5/mL) were incubated for 21 h (approximately two doubling times) with various concentrations of cytotoxic drugs. Cells were then fixed by 70% ethanol, washed twice with PBS, and incubated for 30 min in PBS containing 100 μg/mL RNAse, 25 μg/mL PI. For each sample, 10 000 cells were analyzed by flow cytometry. Results are expressed as the percentage of cells accumulated in the G2 + M + 8N phase of the cell cycle.

Tubulin Binding Assay. Calf brain tubulin was purified according to the method of Shelanski,[35] by three cycles of assembly−disassembly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 2 mM EGTA, and 1 mM GTP, pH 6.6 (the concentration of tubulin was about 2−3 mg/mL). Tubulin assembly was monitored and recorded continuously by turbidimetry at 400 nM in a UV spectrophotometer, equipped with a thermostated cell at 37 °C.[36] We determined for drugs the IC_{50} values for their concentrations that decreased by 50% the maximum assembly rate of the tubulin without drug. The IC_{50} values were compared to the IC_{50} of deoxypodophyllotoxin, measured the same day under the same conditions.

In Vivo Evaluation of Antitumor Activity. Female B6D2F1 (C57B1/6 x DBA2) mice were used in murine tumor models. All mice were purchased from Iffa Credo (Lyon, France). They were aged 4−6 weeks and weighed 20−22 g at the start of the experiment. These were conducted in accordance with the protocols published by the National Cancer Institute (NCI) and European Organization for Research and Treatment of Cancer (EORTC) members.[37,38] All tumors used for experiments were provided by the Division of Cancer Treatment, Tumor Repository, NCI. Mice were inoculated ip with 10^6 leukemic P388 cells, and drugs are administrated in an ip or iv single injection with increasing concentrations (25−400 mg/kg) the first day of the experiment. The evaluation of antitumor activity was represented vs BCNU as an internal reference by the life span of mices. The median survival time (MST) of the treated group (*T*) was compared with that of the control group (*C*), and the results were expressed as *T*/*C* (%). *T*/*C* (%) = [(MST of treated group)/(MST of control group)] × 100.

X-ray Crystallography. Suitable crystals of the title compounds were obtained by slow evaporation from methylene chloride/methanol (v/v) at room temperature, and they were mounted on a glass fiber.

Diffraction data were collected on an Enraf-Nonius CAD4 diffractometer with Mo Kα radiation (λ = 0.710 73 Å) at room temperature. Data were measured using $\theta-2\theta$ scan. The data treatment, polarization and decay corrections, was carried out with JANA98 program.[39] The crystal structure was solved by direct methods using the SHELX97 package.[40] All non-hydrogen atoms were refined anisotropically. For **1o**, all H atoms were determined via difference Fourier maps and refined with isotropic atomic displacement parameters, with the exception of H atoms of the methoxy group for which the H atoms were fixed. For **1j**, the majority of H atoms were fixed.

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Supporting Information Available: X-ray crystallographic files for **1j** and **1o** including crystal data and structure refinement, atomic coordinates, and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, and hydrogen coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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