Novel Multidrug Resistance Reversal Agents

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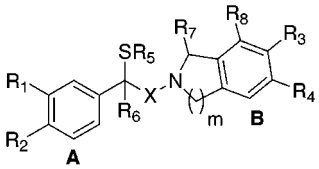
A series of 59 α-aryl-α-thioether-alkyl, -alkanenitrile, and -alkanecarboxylic acid methyl ester tetrahydroisoquinoline and isoindoline derivatives (**15a**−**48**) were synthesized and evaluated as multidrug resistance (MDR) reversal agents. The compounds were tested on S1-B1-20 human colon carcinoma cells selected for resistance to bisantrene. Both the cytotoxicity of the reversal agents and their ability to resensitize the cells to bisantrene were determined. All but two of these compounds (**15q**, **40**) were more effective MDR reversal agents in vitro than verapamil (VRP), a calcium channel antagonist which also has been shown to possess MDR modulating activity. Several showed good activity in this assay (IC_{50}'s < 0.5 μM), the most potent being isoindolines **44** (IC_{50} 0.26 μM) and **46** (IC_{50} 0.26 μM) and tetrahydroisoquinolines **47** (IC_{50} 0.29 μM) and **15m** (IC_{50} 0.30 μM). A number of compounds were evaluated in vivo against vincristine (VCR)-resistant murine P388 leukemia, as well as against human epidermoid carcinoma KB/8.5 implanted sc in athymic mice. The reversal agents which consistently showed the highest activity, together with low toxicity, were α-aryl-α-thiotolylalkanenitrile tetrahydroisoquinoline derivatives with electron-rich alkoxy substituents on the aromatic rings. Of the tested compounds, the most effective reversal agents for both tumor lines were **15h** (33% increased life span at 12.5 mg/kg, 0.2 mg/kg VCR versus VCR alone in the VCR-resistant P388 leukemia model and 59% relative tumor growth at 50 mg/kg, 8 mg/kg doxorubicin versus doxorubicin alone in the KB/8.5 model) and **39a** (48% increased life span at 50 mg/kg, 0.2 mg/kg VCR versus VCR alone in the VCR-resistant P388 leukemia model and 46% relative tumor growth at 25 mg/kg, 8 mg/kg doxorubicin versus doxorubicin alone in the KB/8.5 model). The mechanism of action of these compounds is believed to involve blocking the drug efflux pump, P-glycoprotein.

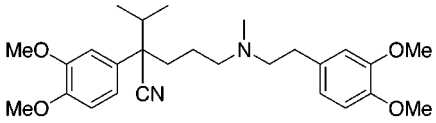
Introduction

Drug resistance is believed to be the single most important reason for the failure of cancer chemotherapy.[1] Certain tumors are unresponsive to chemotherapy from the outset, through inherent resistance. However, in many cases this occurs after a period of responsiveness to chemotherapy, through the process of acquired resistance. In tissue culture, several cellular mechanisms are responsible for mediating acquired resistance.[2] Tumor cells selected for resistance to one drug generally show cross-resistance to a number of functionally and structurally diverse hydrophobic drugs, including taxol, vinblastine, adriamycin, mitoxantrone, etoposide, and bisantrene. This is known as multidrug resistance (MDR).[3] Tumor cells which possess the MDR phenotype overexpress an energy-dependent drug efflux pump, P-glycoprotein (P-gp),[4] which lowers the intracellular concentration of cytotoxic agents by pumping them from the cell. While cells in culture can display drug resistance up to 1000-fold greater than a sensitive phenotype, drug-resistant tumors isolated from patients generally exhibit only a 2−10-fold increase in drug resistance. Thus, relatively low levels of P-gp in tumors are believed to contribute to the refractory behavior of tumors in clinical oncology.[5]

Studies have demonstrated that a variety of compounds are able to reverse MDR in vitro, thus restoring the levels of the anticancer agents within the cell to cytotoxic concentrations. These structurally diverse compounds are generally hydrophobic and include calcium channel antagonists such as verapamil (VRP),[6] dihydropyridines,[7,8] pyridine analogues,[9] vinca alkaloid analogues,[10] antiarrhythmic agents (quinidine),[11] immunosuppressants (cyclosporin A),[12] phenoxazines,[13] calmodulin antagonists (trifluoroperazine),[14] triazines (S-9788),[15] acridone carboxamides (GF-120918),[16] and pipecolinates (VX-710).[17] Several groups have synthesized analogues of dihydropyridines,[18] verapamil,[19,20] and cyclosporin A[21] to optimize their MDR reversal activity. Clinical trials with several of these compounds (most of which have been carried out with verapamil, cyclosporin A, and structurally related analogues) in conjunction with chemotherapeutic agents have demonstrated some benefit, although the effects have been modest and thus not universally accepted as useful.[22]

Chart 1





Verapamil

We used verapamil (Chart 1) as the structural basis for the program. Our intent was to identify compounds

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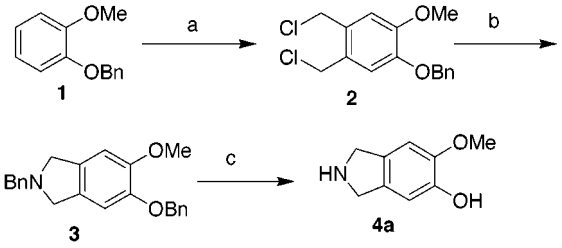
Scheme 1. Preparation of **4a** (Table 1)[a]



[a] Reagents and reaction conditions: (a) formaldehyde, ZnI_2, ether, HCl gas addition, 1.5 h, then 1.5 h, 25 °C; (b) $C_6H_5CH_2NH_2$, $(Bu)_4NCl$, toluene, aq NaOH, 25 °C, 48 h; (c) 35% $Pd(OH)_2/C$, H_2 (Parr, 50 lb/in.^{2}), EtOH, 21 h.

with greater potency and specificity for P-gp, but without the calcium channel-blocking activity of VRP. A similarity analysis of VRP to an in-house library of compounds revealed a number of related structures.[23] These compounds demonstrated good levels of in vitro biological activity, initiating a program to further modify their structures in order to optimize activity.

This paper describes our efforts to optimize the properties of these new compounds. Structurally, they are described by the general formula shown in Chart 1.

Chemistry

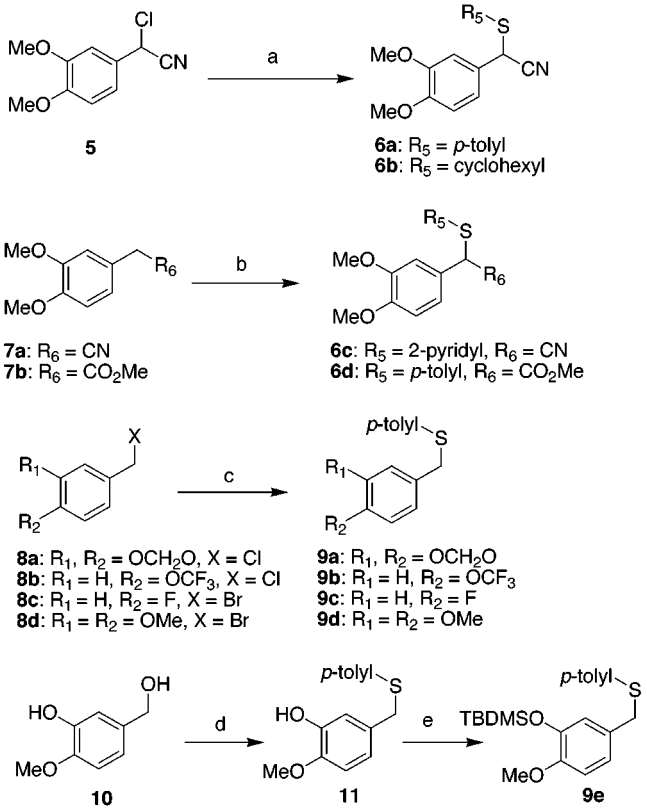
Tetrahydroisoquinolines which were not commercially available were prepared by literature methods (Table 1). The synthesis of isoindoline **4a** was achieved (Scheme 1) via an initial Friedel−Crafts acylation of **1** with paraformaldehyde, in the presence of HCl to produce dichloride **2**. Reaction of **2** with benzylamine and subsequent hydrogenation produced **4a**.

Scheme 2 outlines the synthesis of thioethers **6a**−**d** and **9a**−**e** (see Table 2). All were synthesized by reaction of a benzyl halide with *p*-thiocresol anion, except **6c,d**, which were produced by the reaction of the sodium anions of **7a**,**b** with 2-pyridyl and *p*-tolyl disulfide, respectively. The latter, shorter method subsequently became the preferred procedure for the large-scale preparation of **6a**.

Schemes 3−7 describe the syntheses of the target compounds listed in Tables 4 and 5. A significant number of the target compounds were constructed by thioether anion alkylation (methods A, A′) followed by N-alkylation (methods B, B′) as described in Scheme 3. Deprotonation of the nitrile- and ester-containing thioethers **6a**−**d** was readily achieved with sodium hydride, while *n*-butyllithium was required to produce the less stabilized anions of **9a**−**f**. Following reaction of these anions with an appropriate dihalide, the resulting intermediates **12a**−**k** and **13a**−**h** (Table 3) were N-alkylated by substituted isoindolines **4a**,**b** or tetrahydroisoquinolines **14a**−**i** to produce target compounds **15a**−**e′** (Table 4).

Compound **19**, possessing a more rigid alkynyl linker chain, was synthesized by a longer reaction sequence (method C, Scheme 4). Alkylation of **6a** with bromide **16** produced **17**. Following removal of the silyl protecting group, alcohol **18** was mesylated and N-alkylated by tetrahydroisoquinoline **14a** to provide the target compound **19.** The synthesis of **24** similarly required a somewhat lengthier reaction sequence (method D).

Scheme 2. Preparation of Intermediates **6a**−**d** and **9a**−**e** (Table 2)[a]



[a] Reagents and reaction conditions: (a) R_5SH, K_2CO_3, CH_3CN, 65 °C, 15 h; (b) $NaN(TMS)_2$, THF, 0 °C, 50 min, then R_5SSR_5, 5 °C, 40 min, 25 °C, 1.5 h; (c) *p*-thiocresol, $(Bu)_4NCl$, toluene, aq NaOH, 25 °C, 15 h, or *p*-thiocresol, NaH, DMF, 25 °C, 40 min; (d) HBr gas, toluene, 0 °C, 20 min, then *p*-thiocresol, $(Bu)_4NHSO_4$, toluene, aq NaOH, 25 °C, 15 h; (e) TBDMSCl, pyridine, reflux 3 h.

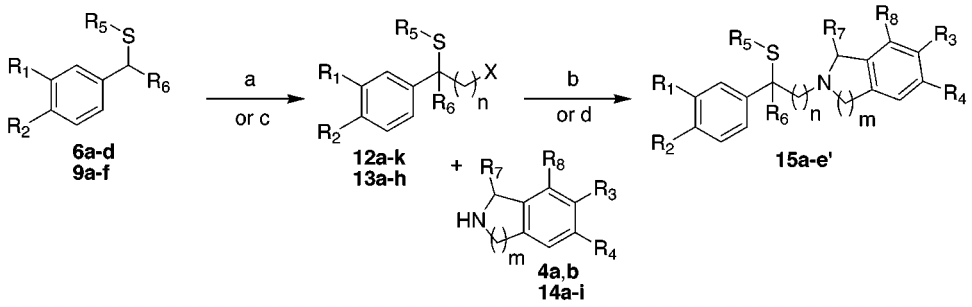
Following alkylation of **6a** with *m*-carboxybenzyl bromide, the benzoic acid residue was reduced to a benzyl alcohol, then converted to chloride **23**, and reacted with tetrahydroisoquinoline **14a** to provide target compound **24**.

Isoindolines **28a**−**c** were constructed as shown in Scheme 5 (method E). The alkyl chlorides **12e**,**h** were converted to the corresponding amines **26a**,**b** by reaction with potassium phthalimide and subsequent treatment with hydrazine. Reaction of the amines with *o*-bisbenzyl halides **27a**[24] and **27b**[25] produced the target compounds.

An alternative synthetic approach to compounds possessing various thioether substituents is outlined in Scheme 6. This reaction sequence was useful in cases where the required disulfides were not commercially available. Friedel−Crafts acylation of **29** with **30** followed by hydrolysis and coupling to tetrahydroisoquinoline **14a** produced intermediate **32**. Selective reduction of the ketone, followed by zinc(II) iodide-mediated alkanethiol displacement of benzyl alcohol **33a** provided compounds **34a**−**d** (method F).[26] This displacement reaction was particularly effective, as even the sterically congested *tert*-butyl thiol reacted readily. The final products **35a**−**d** were obtained by reduction of the amide linkages. Compound **35e** was obtained by treating ketone **32** with ethyl Grignard and then repeating the thiol displacement and reduction steps.

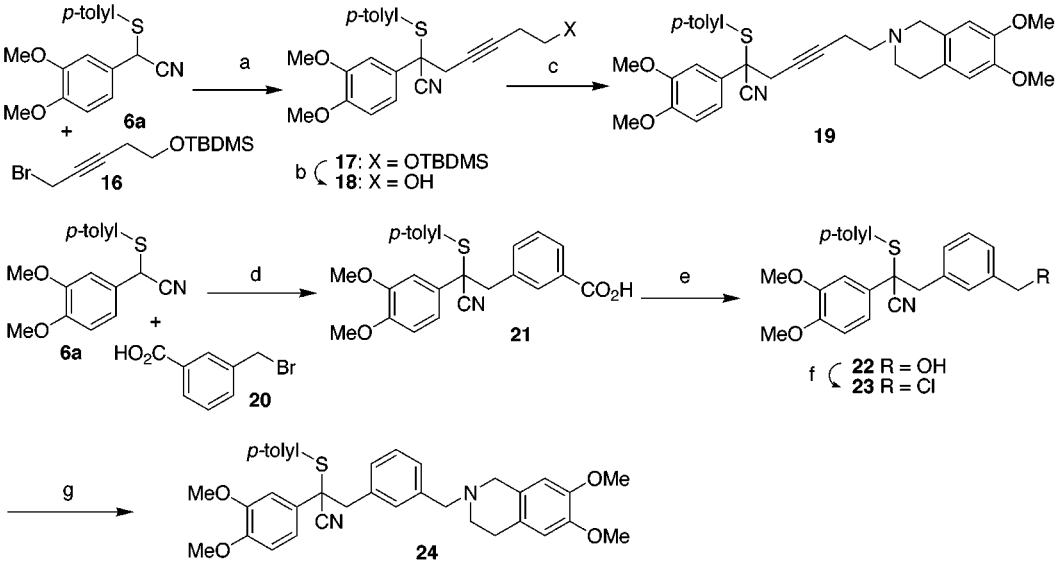
Scheme 7 outlines the chemistry utilized to attach

Scheme 3. Methods A and A′, Preparation of Intermediates **12a**−**k** and **13a**−**h** (Table 3), and Methods B and B′, Preparation of Target Compounds **15a**−**e′** (Tables 4 and 5)[a]



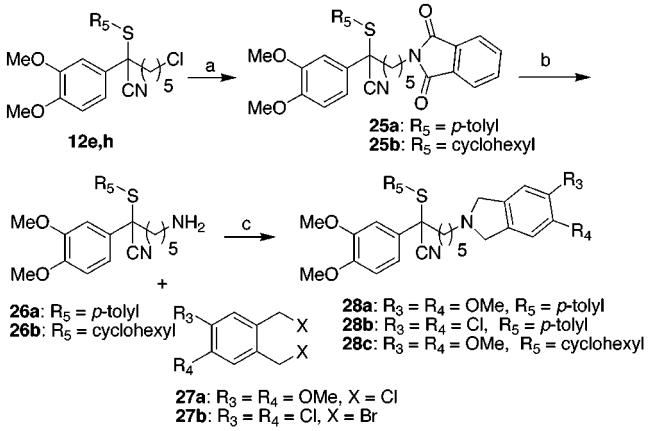
[a] Reagents and reaction conditions (X = Br or Cl): (a) NaH, DMF (or DMSO), 1.5 h, 25 °C, then $Br(CH_2)_nX$, 3 h (method A); (b) K_2CO_3, NaI, DMF (or 2-pyrrolidinone), 95 °C, 4−24 h (method B); (c) *n*-BuLi, THF, −78 °C, 30 min, then $Br(CH_2)_nX$, −78 to 25 °C (method A′); (d) $(i\text{-Pr})_2NEt$, NaI, CH_3CN, reflux 24−48 h (method B′).

Scheme 4. Method C, Preparation of Target Compound **19** (Table 5), and Method D, Preparation of Target Compound **24** (Table 5)[a]



[a] Reagents and reaction conditions: (a) NaH, THF, 1.5 h, 25 °C, then **16**, 3 h; (b) $(Bu)_4NF$, THF, 0 °C, then 25 °C, 1 h; (c) MsCl, pyridine, CH_2Cl_2, 25 °C, 5 h, then $(i\text{-Pr})_2NEt$, **14a**, CH_3CN, reflux 4 h; (d) *n*-BuLi, THF, −78 °C, 35 min, then **20**, −78 °C, 40 min, 25 °C, 15 min; (e) $BH_3{\cdot}SMe_2$, THF, 25 °C, 18 h; (f) $SOCl_2$, DMF, 70 °C, 17 h; (g) K_2CO_3, **14a**, DMF, 85 °C, 4 h.

Scheme 5. Method E, Preparation of Target Compounds **28a**−**c** (Table 4)[a]



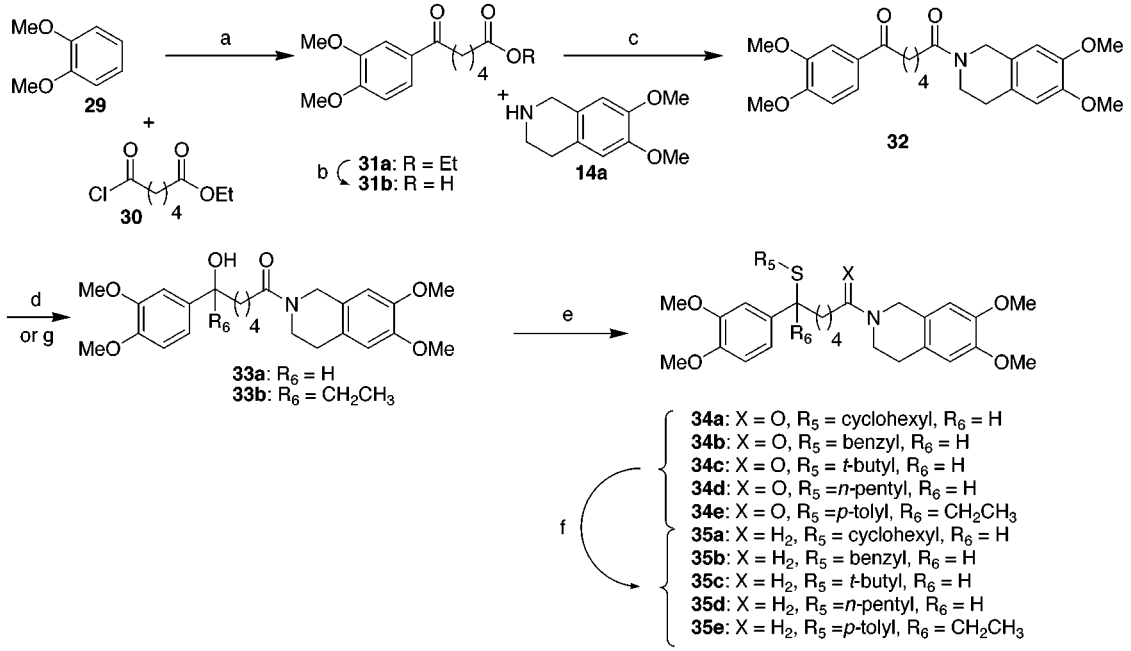
[a] Reagents and reaction conditions: (a) NaI, acetone, reflux 15 h, then potassium phthalimide, DMF, 100 °C for 2 h; (b) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux 1.5 h; (c) toluene, $(Bu)_4NCl$, aq NaOH, 25 °C, 48 h.

additional basic amine groups to the core structures. Because of the high lipophilicity of these compounds (clog *P* values are listed in Tables 4 and 5), they were poorly soluble in water. When protonated, the resulting salts were generally isolated as solids (many of the free bases were viscous gums) with greatly improved water solubility. After removing the silyl protecting group of **15w**, reaction of the resulting phenol **36** with chloroethyl tosylate produced chloride **37a**. Subsequent reaction of **37a** with imidazole provided the target compound **37b** (method G). Similarly, **15l,d′** and isoindoline **15b′** were converted to the ethylimidazole derivatives **39a**−**c** (method G′). Target compound **40** was obtained by the reaction of **15l** with chloroethyl dimethylamine under basic conditions (method H).

Results and Discussion

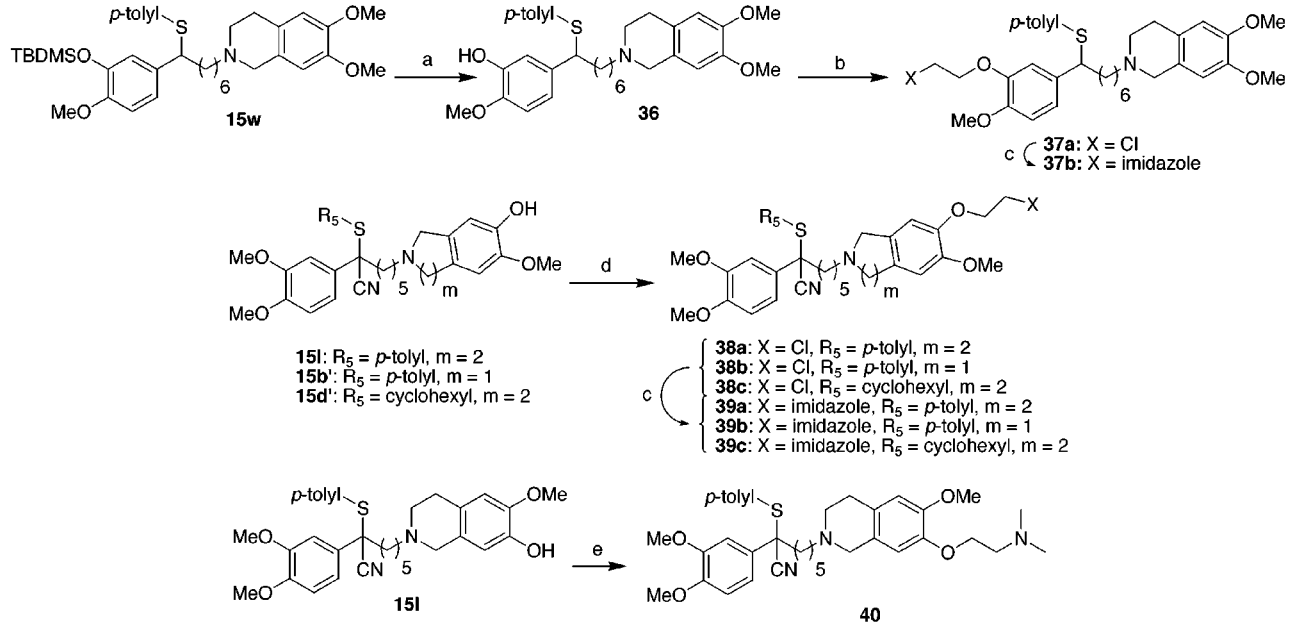
In Vitro Biological Results and Structure−**Activity Relationships (SAR).** Candidate agents were examined for their ability to restore sensitivity to antitumor drugs in tissue culture. To do this, a subclone of human colon carcinoma cells were selected for resistance to bisantrene (S1-B1-20). Such cells exhibit a profound overexpression of P-gp, are completely resistant to 20 μM bisantrene (100% survival), and are cross-resistant to drugs in the MDR phenotype (e.g., vinblas-

Scheme 6. Method F, Preparation of Compounds **35a**−**e** (Table 4)[a]



[a] Reagents and reaction conditions: (a) $AlCl_3$, CH_2Cl_2, 0 °C, 4 h, 25 °C, 15 h; (b) 10% NaOH/EtOH, 25 °C, 3 h; (c) $(EtO)_2P(O)CN$, DMF, 0 °C, then 25 °C, 15 h; (d) $NaBH_4$, MeOH, 25 °C, 15 h; (e) CH_2ClCH_2Cl, ZnI_2, R_5SH, 25 °C, 1 h; (f) $BH_3{\cdot}Me_2S$, THF, reflux 2 h; (g) EtMgBr, THF, 25 °C, 15 h.

Scheme 7. Method G, Preparation of Target Compounds **37a,b** (Table 4), Method G′, Preparation of Target Compounds **38a**−**c** and **39a**−**c** (Table 4), and Method H, Preparation of Target Compound **40** (Table 4)[a]



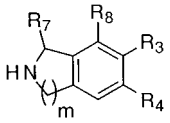
[a] Reagents and reaction conditions: (a) $(Bu)_4NF$, THF, 25 °C, 15 h; (b) $Cl(CH_2)_2OTs$, NaH, MEK, reflux 48 h; (c) imidazole, NaH, NaI, DMF, 25 °C, 48 h; (d) $Cl(CH_2)_2OTs$, 1 N NaOH, MEK, reflux 18 h; (e) NaH, DMF, 25 °C, 1 h, then $Cl(CH_2)_2N(CH_3)_2{\cdot}HCl$, KI, 25 °C, 18 h.

tine, doxorubicin, or paclitaxel).[27] Test agents (0.1−80 μM) were added to these cells alone or in combination with 20 μM bisantrene. After 3 days of continuous exposure, the number of cells that survived was determined. The difference score for each compound was determined as the difference between the number of cells that survived in the presence of the candidate agent alone minus those that survived in the presence of the candidate agent plus bisantrene. Minimum and maximum values in this assay are 0 and 100, respectively, with a value of 100 indicating the maximum possible reversal activity. The IC_{50}'s (difference scores) and LD_{50}'s (for reversal agents alone) are listed in Table 6.

Six regions of the target compounds were varied (Chart 1): (1) the substituents on ring A (R_1 and R_2); (2) the sulfide substituent (R_5); (3) the quaternary substituent (R_6); (4) the chain linker (X); (5) the ring size of the cyclic amine (m); and (6) the substituents on ring B (R_3, R_4 and R_7, R_8). On the basis of the in vitro results (Table 6), the following trends were observed:

1. In general, alkoxy substituents on the A ring

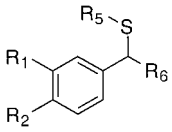
Table 1. Substituted Isoindoline (**4a,b**) and Tetrahydroisoquinoline (**14a**−**i**) Starting Materials



compd	R_3	R_4	R_7	R_8	*m*	yield (%)	formula[a]
4a	OH	OMe	H	H	1	87	$C_9H_{11}NO_2 \cdot 0.25H_2O \cdot 0.13EtOH$
4b	OMe	OMe	H	H	1	51	$C_{10}H_{13}NO_2$[37]
14a	OMe	OMe	H	H	2		*b*
14b	O-CH_2-O		H	H	2	90	*c*[38]
14c	H	OMe	H	H	2	69	$C_{10}H_{13}NO \cdot HCl$[39]
14d	OMe	OMe	H	OMe	2	69	$C_{12}H_{17}NO_3 \cdot HCl$[40]
14e	OH	OH	Me	H	2		*b*
14f	OMe	OMe	3,4-dimethoxybenzyl	H	2		*b*
14g	OBn	OMe	H	H	2	28	$C_{17}H_{19}NO_2 \cdot HCl$[41]
14h	H	H	H	H	2		*b*
14i	OH	OMe	H	H	2	65	*c*[42]

[a] Elemental analyses were within 0.4% of the theoretical values for the formulas given, unless otherwise stated. All compounds exhibited NMR spectra consistent with assigned structures. [b] Commercially available. [c] Synthesized by literature procedure; structure was confirmed by ^{1}H NMR.

Table 2. Benzyl Thioether Intermediates **6a**−**d** and **9a**−**f**



compd	R_1	R_2	R_5	R_6	yield (%)	formula[a]
6a	OMe	OMe	*p*-tolyl	CN	65	*c*[43]
6b	OMe	OMe	cyclohexyl	CN	55	$C_{16}H_{21}NO_2S$
6c	OMe	OMe	2-pyridyl	CN	64	$C_{15}H_{14}N_2O_2S$
6d	OMe	OMe	*p*-tolyl	CO_2Me	62	$C_{18}H_{20}O_4S$
9a	O-CH_2-O		*p*-tolyl	H	80	$C_{15}H_{14}O_2S$
9b	H	OCF_3	*p*-tolyl	H	81	$C_{15}H_{13}F_3OS$
9c	H	F	*p*-tolyl	H	86	$C_{14}H_{13}FS$
9d	OMe	OMe	*p*-tolyl	H	80	$C_{16}H_{18}O_2S$
9e	OTBDMS	OMe	*p*-tolyl	H	94	$C_{21}H_{30}O_2SSi$
9f	H	H	phenyl	H		*b*

[a−c] See corresponding footnotes for Table 1.

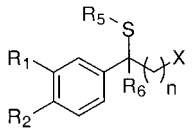
consistently provided the highest activity. With the exception of the particularly bulky *tert*-butyldimethylsilyloxy group, the P-gp binding site appeared to be remarkably insensitive to the nature of the alkoxy substituent at the R_1 position. This is illustrated by the almost identical activities of **36**, **15s**, and **37a**, which possess an R_1 hydroxy, methoxy, and chloroethoxy group, respectively. A compound possessing the ethoxyimidazole (**47**) at R_1 was a particularly active reversal agent. A cyclic benzodioxole compound **15x** was less active. It appears that the conformational rigidity and/or smaller size of the benzodioxole substituent reduces binding to its target site. Compounds possessing electron-withdrawing substituents such as fluoride (**15z**) and trifluoromethoxy (**15y**) were far less potent as MDR reversal agents. In summary, the greatest activity was achieved with hydrophobic electron-donating substituents.

2. Compounds possessing aromatic and aliphatic R_5 groups were synthesized. Of the aliphatic groups, cyclohexane provided the greatest amount of activity. With smaller pentyl (**35d**) and *tert*-butyl (**35c**) groups, the reversal agents were significantly less active, indicating too little contact was being made with the walls of an important binding pocket. Overall, the best R_5 group was the *p*-tolyl substituent. A benzyl derivative (**35b**) possessed poor MDR reversal properties. This indicated that the steric environment of P-gp binding site for the R_5 group is wide enough to accommodate the aromatic and cyclohexyl substituents very well but contains a narrow apex. Substitution of a pyridine residue (**15e′**) for a phenyl group (to provide greater water solubility) produced a surprisingly inactive compound. Evidently, a strongly hydrophobic R_5 group is required for optimal activity.

3. The quaternary substituent (R_6) which provided the greatest activity was a nitrile. Generally, similar compounds possessing hydrogens at R_6 were somewhat less potent. With a larger methyl ester group compound **15e** was significantly less active than the corresponding nitrile, **41**. Similarly, a reversal agent with an ethyl substituent (**35e**) possessed only marginal activity.

4. An optimal length of the linker section was important for activity, although a surprisingly large range was tolerated. When the linker section was X = $(CH_2)_n$, optimal potency was observed for $n = 5-8$. Shortening or lengthening the chain led to a decrease in activity. Rigidification of the linker chain by a phenyl ring (**24**) or an alkyne (**19**) led to a loss of activity. It appears that the rigidification prevented the compounds from binding in their most preferred conformations in the binding pocket, with unfavorable steric interactions by the bulky phenyl substituent of **24** possibly causing a larger decrease in binding.

Table 3. Alkylated Benzyl Thioethers **12a**−**k** and **13a**−**h**



compd	R_1	R_2	R_5	R_6	*n*	X	method	yield (%)	formula[a]
12a	OMe	OMe	*p*-tolyl	CN	3	Cl	A	77	$C_{20}H_{22}ClNO_2S \cdot 0.07C_{20}H_{22}BrlNO_2S$[b]
12b	OMe	OMe	*p*-tolyl	CN	4	Cl	A	64	$C_{21}H_{24}ClNO_2S \cdot 0.01C_{21}H_{24}BrlNO_2S$[b]
12c	OMe	OMe	*p*-tolyl	CN	2	Cl	A	57	$C_{19}H_{20}ClNO_2S$
12d	OMe	OMe	*p*-tolyl	CN	11	Br	A	73	$C_{28}H_{38}BrNO_2S$
12e	OMe	OMe	*p*-tolyl	CN	5	Cl	A	77	$C_{22}H_{26}ClNO_2S \cdot 0.03C_{22}H_{26}BrlNO_2S$[b]
12f	OMe	OMe	*p*-tolyl	CN	8	Br	A	70	$C_{25}H_{32}BrNO_2S \cdot 0.11C_{17}H_{17}NO_2S$[b]
12g	OMe	OMe	*p*-tolyl	CN	6	Cl	A	82	$C_{23}H_{28}ClNO_2S$
12h	OMe	OMe	cyclohexyl	CN	5	Cl	A	76	$C_{21}H_{30}ClNO_2S \cdot 0.82C_{21}H_{30}BrlNO_2S$[b]
12i	OMe	OMe	*p*-tolyl	CN	5	Br	A	83	$C_{22}H_{26}BrNO_2S$
12j	OMe	OMe	2-pyridyl	CN	5	Br	A	82	$C_{20}H_{23}BrN_2O_2S$
12k	OMe	OMe	*p*-tolyl	CO_2Me	3	Cl	A	68	$C_{21}H_{25}ClO_4S$
13a	OMe	OMe	*p*-tolyl	H	3	Cl	A′	94	$C_{19}H_{23}ClO_2S \cdot 0.06CHCl_3$
13b	OMe	OMe	*p*-tolyl	H	5	Br	A′	51	$C_{21}H_{27}BrO_2S$
13c	OMe	OMe	*p*-tolyl	H	6	Br	A′	57	$C_{22}H_{29}BrO_2S \cdot 0.54C_{16}H_{18}NO_2S$[b]
13d	OTBDMS	OMe	*p*-tolyl	H	6	Br	A′	89	*d*
13e	O-CH_2-O		*p*-tolyl	H	5	Br	A′	67	$C_{20}H_{23}BrO_2S$
13f	H	OCF_3	*p*-tolyl	H	5	Br	A′	88	$C_{20}H_{22}BrF_3OS \cdot 0.33C_{15}H_{13}FO_3S$[c]
13g	H	F	*p*-tolyl	H	6	Br	A′	12	*d*
13h	H	H	phenyl	H	6	Br	A′	81	$C_{19}H_{23}BrS \cdot 0.03C_{13}H_{12}S$[c]

[a] See corresponding footnote for Table 1. [b] These compounds contained varying quantities of the chromatographically inseparable bromide analogues due to displacement of the chloride groups by Br^- during the course of reaction and/or reaction of the anion to some degree with the less reactive chloride portion of the bromochloroalkanes. [c] These compounds could not be chromatographically separated from starting materials. [d] Compound was synthesized and utilized in the subsequent step without purification.

5. Two series of cyclic compounds were examined: isoindolines and substituted tetrahydroisoquinolines (*m* = 1, 2). Several reversal agents possessing either heterocyclic system were very potent, although the oxidative instability of the isoindolines caused the measurements of activity to be more variable.

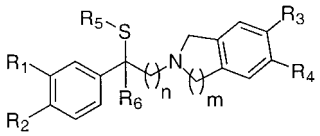
6. The B ring substituents (R_3 and R_4) which provided the highest activity were the electron-donating methoxy groups. A compound (**15v**) possessing an additional methoxy group at R_8 had similiar activity to the corresponding dimethoxy analogue (**15s**). The increased steric bulk provided by a chloroethoxy group at R_3 did not have a significant effect on activity (**38a** versus **15h**). However, compounds possessing ethoxyimidazole substituents at R_3 were somewhat less potent (for example, **48** versus **15h**). Significantly, when the imidazole was replaced by the less lipophilic dimethylamine group, the resulting compound **40** was essentially inactive. Benzodioxole compounds **15j**,**q** were poor reversal agents. Compounds with hydroxy substituents (**15l**, **42**) were far less active than the corresponding ethers. Also, electron-withdrawing substituents such as chlorides (**28b**) greatly reduced potency. Introduction of a bulky substituent at the benzylic position α to the nitrogen (R_7) on the tetrahydroisoquinoline moiety (**15t**) led to a diastereomeric mixture which possessed modest activity in comparison to the R_7 unsubstituted compound (**15h**). Apparently substituents at R_7 are tolerated but do not enhance the activity.

Practically all of the tested compounds showed greater activity than VRP (Table 6). Among the most potent were those that combined the optimal features of the above-mentioned regions of the reversal agents, such as the dimethoxytetrahydroisoquinolines **15h**,**i**,**m** (IC_{50}'s 0.37, 0.36, and 0.30 μM, respectively). These compounds were all α-dimethoxyphenyl,α-*p*-tolyl nitriles, differing only in the length of the linker chain. Compound **15o**, differing from **15h** only by having a hydrogen rather than a nitrile group at R_6, also showed good activity (IC_{50} 0.33 μM). Several similarly substituted isoindoline compounds were exceptionally active in vitro: **44** (IC_{50} 0.26 μM), cyclohexyl thioether **28c** (IC_{50} 0.35 μM), and its corresponding HCl salt **46** (IC_{50} 0.26 μM). Also, compounds possessing the water-solubilizing side chains (**39b**, **47**, **48**) were among the most active compounds (IC_{50}'s 0.50, 0.29, and 0.53 μM, respectively).

These compounds were up to 50-fold more potent than VRP in this assay. The onset of activity was observed at 0.2 μM for the more active compounds tested. At concentrations between 5 and 10 μM, the most potent MDR agents began to show signs of cytotoxicity, with LD_{50}'s at concentrations around 30-fold higher than the IC_{50}'s. Not only were most of the synthesized compounds far more potent than VRP, they were also more efficacious. This is graphically illustrated in Figure 1, where the activity of **15h** was compared to VRP. At doses of up to 2 μM, **15h** was nontoxic alone but killed cells in combination with bisantrene (at higher doses the compound killed tumor cells by itself, hence the biphasic dose−response), while VRP only began to show signs of activity at 5 μM. Furthermore, the maximal difference score (100) was observed with **15h**. Submaximal scores were attained with VRP because toxicity of the compound alone occurred before complete cell kill in the presence of bisantrene was achieved.

To determine whether the reversal agents increased the concentration of cytotoxic agents in the cells, accumulation of vinblastine was measured in S1-B1-20 cells (Figure 2). Compounds **15h** and **28a** were significantly more effective than VRP in their ability to restore vinblastine levels in the P-gp-expressing cells. The most potent reversal agent (**15h**) was approximately 5-fold more potent than verapamil in this assay. No effects were seen in cell lines that do not express P-gp.[28]

Table 4. Substituted Tetrahydroisoquinoline and Isoindoline α-Aryl-α-thioether-alkane, -alkanenitrile, and -alkanecarboxylic Acid Ester Derivatives

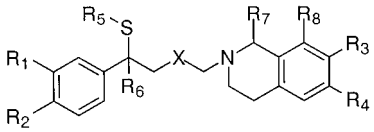


compd	R_1	R_2	R_3	R_4	R_5	R_6	*n*	*m*	method	yield (%)	log *P*[a]	formula[b,c]
15a	OMe	OMe	OMe	OMe	*p*-tolyl	CN	3	2	B	38	5.12	$C_{31}H_{36}N_2O_4S$
15c	OMe	OMe	OMe	OMe	*p*-tolyl	CN	4	2	B	42	5.65	$C_{32}H_{38}N_2O_4S{\cdot}CH_2Cl_2$
15d	OMe	OMe	OMe	OMe	*p*-tolyl	CN	2	2	B	20	4.59	$C_{30}H_{34}N_2O_4S{\cdot}0.4H_2O$
15e	OMe	OMe	OMe	OMe	*p*-tolyl	CO_2CH_3	3	2	B	20	5.54	$C_{32}H_{39}NO_6S$
15f	OMe	OMe	OMe	OMe	*p*-tolyl	CN	11	2	B	14	9.35	$C_{39}H_{52}N_2O_4S$
15g	OMe	OMe	H	H	*p*-tolyl	CN	5	2	B	40	6.72	$C_{31}H_{36}N_2O_2S$
15h	OMe	OMe	OMe	OMe	*p*-tolyl	CN	5	2	B	56	6.18	$C_{33}H_{40}N_2O_4S{\cdot}0.5H_2O$
15i	OMe	OMe	OMe	OMe	*p*-tolyl	CN	8	2	B	41	7.76	$C_{36}H_{46}N_2O_4S{\cdot}0.25C_6H_{14}$
15j	OMe	OMe	O-CH_2-O		*p*-tolyl	CN	5	2	B	61	6.08	$C_{32}H_{36}N_2O_4S{\cdot}2H_2O$
15k	OMe	OMe	H	OMe	*p*-tolyl	CN	5	2	B	46	6.64	$C_{32}H_{38}N_2O_3S$
15l	OMe	OMe	OH	OMe	*p*-tolyl	CN	5	2	B′	21	5.90	$C_{32}H_{38}N_2O_4S{\cdot}0.5H_2O$
15m	OMe	OMe	OMe	OMe	*p*-tolyl	CN	6	2	B	52	6.71	$C_{34}H_{42}N_2O_4S{\cdot}0.13H_2O$
15n	OMe	OMe	OMe	OMe	*p*-tolyl	H	3	2	B	12	6.36	$C_{30}H_{37}NO_4S{\cdot}0.25H_2O$
15o	OMe	OMe	OMe	OMe	*p*-tolyl	H	5	2	B	58	7.42	$C_{32}H_{41}NO_4S{\cdot}0.5H_2O$
15p	OMe	OMe	H	OMe	*p*-tolyl	H	5	2	B′	87	7.88	$C_{31}H_{39}NO_3S$
15q	OMe	OMe	O-CH_2-O		*p*-tolyl	H	5	2	B′	92	7.31	$C_{31}H_{37}NO_4S{\cdot}0.88H_2O$
15r	OMe	OMe	OMe	OMe	*p*-tolyl	H	6	1	B′	24	7.32	$C_{32}H_{41}NO_4S{\cdot}H_2O$
15s	OMe	OMe	OMe	OMe	*p*-tolyl	H	6	2	B′	38	7.95	$C_{33}H_{43}NO_4S{\cdot}H_2O$
15u	OMe	OMe	OMe	OMe	cyclohexyl	CN	5	2	B	65	6.28	$C_{32}H_{44}N_2O_4S$
15w	OTBDMS	OMe	OMe	OMe	*p*-tolyl	H	6	2	B′	82	11.68	$C_{38}H_{55}NO_4SSi$
15x	O-CH_2-O		OMe	OMe	*p*-tolyl	H	5	2	B′	84	7.31	$C_{31}H_{37}NO_4S$
15y	H	OCF_3	OMe	OMe	*p*-tolyl	H	5	2	B′	70	8.44	$C_{31}H_{36}F_3NO_3S$
15z	H	F	OMe	OMe	*p*-tolyl	H	6	2	B	73	8.63	$C_{31}H_{38}FNO_2S{\cdot}0.4EtOH$
15a′	OMe	OMe	OCH_2Ph	OMe	*p*-tolyl	CN	5	2	B	43	7.95	$C_{39}H_{44}N_2O_4S{\cdot}H_2O$
15b′	OMe	OMe	OH	OMe	*p*-tolyl	CN	5	1	B′	74	5.78	$C_{31}H_{36}N_2O_4S$
15d′	OMe	OMe	OH	OMe	cyclohexyl	CN	5	2	B′	79	6.00	$C_{31}H_{42}N_2O_4S$
15e′	OMe	OMe	OMe	OMe	pyridyl	CN	5	2	B′	58	4.72	$C_{31}H_{37}N_3O_4S$
28a	OMe	OMe	OMe	OMe	*p*-tolyl	CN	5	1	E	29	5.55	$C_{32}H_{38}N_2O_4S$
28b	OMe	OMe	Cl	Cl	*p*-tolyl	CN	5	1	E	95	7.40	$C_{30}H_{32}Cl_2N_2O_2S$[d]
28c	OMe	OMe	OMe	OMe	cyclohexyl	CN	5	1	E	28	5.65	$C_{31}H_{42}N_2O_4S$
35a	OMe	OMe	OMe	OMe	cyclohexyl	H	5	2	F	77	7.33	$C_{31}H_{45}NO_4S{\cdot}0.5EtOH$
35b	OMe	OMe	OMe	OMe	benzyl	H	5	2	F	83	6.63	$C_{32}H_{41}NO_4S$
35c	OMe	OMe	OMe	OMe	*t*-butyl	H	5	2	F	86	6.54	$C_{29}H_{43}NO_4S$
35d	OMe	OMe	OMe	OMe	*n*-pentyl	H	5	2	F	82	5.66	$C_{30}H_{43}NO_5S{\cdot}0.3EtOAc$
35e	OMe	OMe	OMe	OMe	*p*-tolyl	CH_2CH_3	5	2	F	81	8.34	$C_{34}H_{45}NO_4S{\cdot}0.5EtOH$
36	OH	OMe	OMe	OMe	*p*-tolyl	H	6	2	G	99	7.67	$C_{32}H_{41}NO_4S{\cdot}0.5H_2O$
37a	OCH_2CH_2Cl	OMe	OMe	OMe	*p*-tolyl	H	6	2	G	27	8.54	$C_{34}H_{44}ClNO_4S$
37b	OCH_2CH_2Im	OMe	OMe	OMe	*p*-tolyl	H	6	2	G	16	7.63	$C_{37}H_{47}N_3O_4S{\cdot}H_2O$
38a	OMe	OMe	OCH_2CH_2Cl	OMe	*p*-tolyl	CN	5	2	G′	42	6.77	$C_{34}H_{41}ClN_2O_4S$
38b	OMe	OMe	OCH_2CH_2Cl	OMe	*p*-tolyl	CN	5	1	G′	70	6.14	$C_{33}H_{39}ClN_2O_4S$
38c	OMe	OMe	OCH_2CH_2Cl	OMe	cyclohexyl	CN	5	2	G′	76	6.87	$C_{33}H_{45}ClN_2O_4S{\cdot}0.1CH_2Cl_2$
39a	OMe	OMe	OCH_2CH_2Im	OMe	*p*-tolyl	CN	5	2	G′	37	5.86	$C_{37}H_{46}N_4O_5S{\cdot}0.6EtOAc$
39b	OMe	OMe	OCH_2CH_2Im	OMe	*p*-tolyl	CN	5	1	G′	56	5.23	$C_{36}H_{42}N_4O_4S{\cdot}2HCl{\cdot}1.5H_2O$
39c	OMe	OMe	OCH_2CH_2Im	OMe	cyclohexyl	CN	5	2	G′	69	5.96	$C_{36}H_{48}N_4O_4S{\cdot}1.75HCl{\cdot}H_2O$
40	OMe	OMe	$OCH_2CH_2N(Me)_2$	OMe	*p*-tolyl	CN	5	2	H	21	6.10	$C_{36}H_{47}N_3O_4S{\cdot}0.6Et_2O$[e]
41	OMe	OMe	OMe	OMe	*p*-tolyl	CN	3	2	B	87	5.12	$C_{31}H_{36}N_2O_4S{\cdot}HCl$
43	OMe	OMe	OMe	OMe	*p*-tolyl	CN	5	2	B	87	6.18	$C_{30}H_{34}N_2O_4S{\cdot}HCl{\cdot}H_2O$
44	OMe	OMe	OMe	OMe	*p*-tolyl	CN	5	1	E	58	5.55	$C_{32}H_{38}N_2O_4S{\cdot}HCl{\cdot}0.75H_2O$
45	OMe	OMe	OMe	OMe	*p*-tolyl	CN	4	2	B	99	5.65	$C_{32}H_{38}N_2O_4S{\cdot}HCl$
46	OMe	OMe	OMe	OMe	cyclohexyl	CN	5	1	E	30	5.65	$C_{31}H_{42}N_2O_4S{\cdot}HCl$
47	OCH_2CH_2Im	OMe	OMe	OMe	*p*-tolyl	H	6	2	G	88	7.63	$C_{37}H_{47}N_3O_4S{\cdot}1.88HCl{\cdot}H_2O$
48	OMe	OMe	OCH_2CH_2Im	OMe	*p*-tolyl	CN	5	2	G′	90	5.86	$C_{37}H_{46}N_4O_5S{\cdot}2H_2O{\cdot}1.75HCl$

[a] Log *P* values were generated using MacClogP, version 1.03 (Biobyte Corp.), utilizing a fragment-based approach. [b] Elemental analyses were within 0.4% of the theoretical values for the formulas given, unless otherwise stated. All compounds exhibited NMR spectra consistent with assigned structures. [c] The majority of these compounds were isolated as thick oils or glassy, hygoscopic solids; thus a significant number of compounds contained solvents and/or water even after prolonged evacuation. In some cases compounds were dissolved in ethanol and then evacuated in order to remove halocarbon solvents prior to biological evaluation. [d] Cl: calcd, 12.76; found, 12.27. [e] C: calcd, 69.63; found, 70.66.

In Vivo Biological Results. Indications of good potency and/or therapeutic index were important criteria in choosing compounds for in vivo testing. Compounds were evaluated against vincristine-resistant murine leukemia P388/VCR cells in CDF1 mice using a variety of treatment schedules, comparing treatments with VCR alone (Table 7). The drugs were introduced by an ip route. As mentioned previously, many of the reversal agents were poorly soluble in water. After considerable experimentation, cremophor/ethanol solution or propylene glycol/ethanol were chosen as vehicles for in vivo administration (it was possible to avoid the use of these solvent systems with water-soluble analogues containing additional salt-forming functional groups). Administration of the reversal agents together with VCR produced significant increases in life span compared to treatment with VCR alone. The largest increases in lifespan were observed with compounds **15h**

Table 5. Substituted Alkyl Chain (X) and Highly Substituted (R_3, R_4, R_7, R_8) Tetrahydroisoquinolinealkane and -alkanenitrile Derivatives



compd	R_1,R_2	R_3,R_4	R_5	R_6	R_7	R_8	X	method	yield (%)	log P^a	formula[b,c]
15b	OMe	OH	*p*-tolyl	CN	CH_3	H	CH_2	B	18	4.92	$C_{30}H_{34}N_2O_4S{\cdot}0.5H_2O$
15t	OMe	OMe	*p*-tolyl	CN	3,4-dimethoxybenzyl	H	$(CH_2)_3$	B′	61	7.57	$C_{42}H_{50}N_2O_6S$
15v	OMe	OMe	*p*-tolyl	H	H	OMe	$(CH_2)_4$	B′	76	7.38	$C_{34}H_{45}NO_5S{\cdot}0.25H_2O$
15c′	H	OMe	phenyl	H	H	H	$(CH_2)_4$	B′	36	7.99	$C_{30}H_{37}NO_2S$
19	OMe	OMe	*p*-tolyl	CN	H	H	$C{\equiv}CCH_2$	C	30	5.52	$C_{33}H_{36}N_2O_4S{\cdot}0.1H_2O$
24	OMe	OMe	*p*-tolyl	CN	H	H	*m*-phenyl	D	63	5.91	$C_{36}H_{38}N_2O_4S{\cdot}0.7EtOH$
42	OMe	OH	*p*-tolyl	CN	CH_3	H	CH_2	B	73	4.92	$C_{30}H_{34}N_2O_4S{\cdot}HCl{\cdot}0.75H_2O$[d]

[a–c] See corresponding footnotes for Table 4. [d] Cl: calcd, 6.24; found, 5.80.

(33% at 12.5 mg/kg, 0.2 mg/kg VCR), **15c′** (46% at 25 mg/kg, 0.2 mg/kg VCR), **28a** (41% at 25 mg/kg, 0.2 mg/kg VCR), and the water-soluble analogue **39a** (48% at 50 mg/kg, 0.2 mg/kg VCR). Of these compounds, the activity of **15c′** was anomalous. In vitro, **15c′** was a relatively poor reversal agent, as might be expected for a compound lacking the appropriate substituents on ring A. It is therefore possible that this highly lipophilic compound (clog *P* 7.99) was acting via non-P-gp mechanisms, altering the pharmacokinetics of VCR uptake into the cells.[29] Dosed at 100 mg/kg in the absence of VCR, both **15a**,**h** showed no observable signs of toxicity to the animals, as well as no antitumor effects. Similarly, the cremophor/ethanol solution or propylene glycol/ethanol vehicles alone were not toxic to the animals.

Further in vivo evaluation of the reversal agents was carried out on human epidermoid carcinoma KB/8.5 implanted sc (subcutaneous) in athymic mice (Table 8). These mice were treated with a single iv dose of doxorubicin (DOX), bracketed by two subcutaneous doses of placebo or reversal agent. Relative tumor growth was then determined for each group and compared with the group treated with DOX alone.

Many of the reversal agents showed some level of activity if administered at high enough doses (100−200 mg/kg for the less active compounds). The compounds of interest were those that showed significant activity ($p \leq 0.01$) at 50 mg/kg or less. The most active isoindoline compound in this test was **28a**. When administered at 50 mg/kg, together with 8 mg/kg doxorubicin, tumor growth was 65% of that observed in mice treated with DOX alone (44% of the tumor growth observed in the placebo-treated mice). However, these compounds showed significant toxicity at doses > 100 mg/kg, with weight loss, convulsions, and several deaths observed. Because of this toxicity, and the chemical instability of the isoindoline analogues in general (several of the more active isoindolines possessing electron-donating substituents were rapidly air-oxidized), these compounds were not considered to be useful as potential drug candidates.

Several tetrahydroisoquinolines possessed promising activity as reversal agents in this model. When compounds **15a**,**f**,**h** were administered at 50 mg/kg, together with 8 mg/kg DOX, tumor growth in these mice was 63%, 61%, and 59%, respectively, of that observed in mice treated with DOX alone (25%, 24%, and 41% of the tumor growth observed in the placebo-treated mice). No signs of toxicity were observed for these compounds at the high dose of 200 mg/kg. The water-soluble analogue **39a** was the most active compound in this assay. When administered at 25 mg/kg, together with 8 mg/kg DOX, tumor growth was 46% of that observed in mice treated with DOX alone (31% tumor growth as compared to the placebo-treated mice).

In choosing compounds for further evaluation as potential drug candidates, the two reversal agents which consistently displayed activity in vitro and in both animal models were **15h** and **39a**. Because the synthesis of **15h** could be achieved in three steps from commercially available starting materials, this reversal agent was produced on a large scale for further testing. An account describing the detailed biological evaluation of **15h** has been published.[30] One particularly important finding was that **15h** did not alter the pharmacokinetic disposition of DOX in mice at optimum efficacious doses. Thus, when **15h** was administered with escalating doses of DOX, significantly less toxicity was observed in mice as compared to those similarly treated with DOX and an optimal dose of the reversal agent cyclosporin A. Cyclosporin A is known to alter the pharmacokinetic disposition of anticancer drugs in animals.[31] Consistent with this fact, cyclosporin A was shown to enhance the tumoricidal activity of DOX when tested on mice implanted with a drug-sensitive P-gp-negative KB cell line, while **15h** had no effect.[30]

Conclusion

A series of compounds have been synthesized and evaluated as MDR reversal agents. With few exceptions, these compounds are far more effective than verapamil. The in vitro data demonstrates activity at lower doses for these compounds, as well as a more efficacious response based on the difference scores obtained in the assay. In vivo, several reversal agents restore the activity of vincristine in mice bearing vincristine-resistant murine tumors. At the proper doses, the reversal agents are not cytotoxic and do not possess anticancer activity in the absence of a chemotherapeutic drug. These compounds also potentiate the action of doxorubicin to reduce the tumor size in “nude” mice bearing the doxorubicin-resistant human epidermoid carcinoma KB/8.5.

Of this series, compounds **15h** and **39a** (and the corresponding HCl salt **48**) are particularly effective

Table 6. In Vitro Activity of Compounds on the S1-B1-20 Cell Line

compd	IC_{50}[a] (μM)	LD_{50}[b] (μM)
VRP	17.27	*c*
15d	2.30	33.67
15e	1.47	32.76
15f	1.74	10.77
15g	2.56	24.65
15h	0.37	13.57
15i	0.36	11.62
15j	3.09	30.03
15k	1.92	15.72
15l	3.11	24.85
15m	0.30	13.52
15n	1.23	25.82
15o	0.33	16.85
15p	3.09	22.21
15q	IA	24.95
15r	1.84	22.06
15s	0.90	13.20
15t	1.06	78.24
15u	0.46	13.95
15v	0.70	13.98
15w	10.68	49.11
15x	0.50	15.91
15y	2.07	16.91
15z	2.70	26.99
15a′	0.85	40.00
15c′	3.82	24.61
15e′	2.12	50.82
19	0.97	36.00
24	2.19	23.33
28a	0.81	22.81
28b	10.49	*c*
28c	0.35	14.96
35a	0.73	16.06
35b	1.98	15.13
35c	3.58	76.75
35d	0.50	16.33
35e	1.23	11.76
36	0.84	13.13
37a	0.96	10.34
37b	0.69	5.52
38a	0.37	16.80
39a	0.69	14.68
39b	0.50	8.37
39c	0.96	8.39
40	IA	6.68
41	0.46	23.99
42	4.70	*c*
44	0.26	11.36
45	0.62	41.77
46	0.26	11.83
47	0.29	5.64
48	0.53	11.61

[a] Concentration at which (percent survival of cells treated with test agent alone) − (percent survival of cells treated with test agent and 20 μM bisantrene) = 50. [b] Concentration at which 50% of cells treated with test agent alone survived. [c] Determinations could not be made due to the limiting solubilities of the reversal agents. See Experimental Section for details.

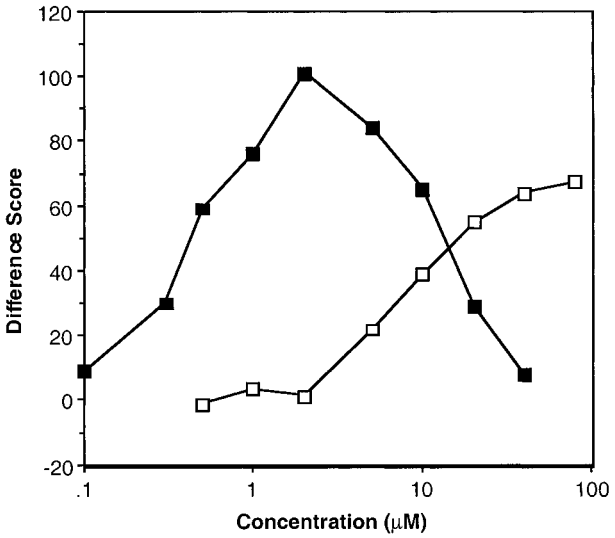


Figure 1. Comparison of reversal activity of **15h** (■) and verapamil (□) in S1-B1-20 MDR cells. Difference score = (percent survival of cells treated with test agent alone) − (percent survival of cells treated with test agent and 20 μM bisantrene). See Experimental Section for details.

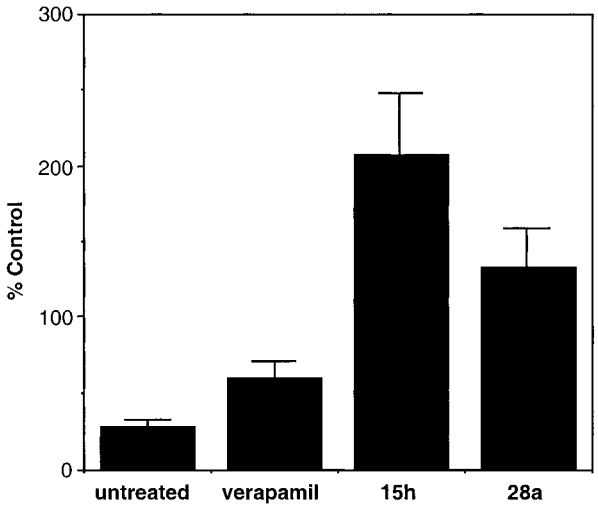


Figure 2. Effect of reversal agents on vinblastine accumulation in S1-B1-20 cells. One million cells were grown for 2 days in drug-free medium. After preincubation in serum-free media (60 min), cells were incubated in the absence or presence of 20 μM test agent (90 min), followed by continued incubation with the addition of 1 μM [^{3}H]vinblastine (90 min). The experiment was terminated by rapidly washing cells in ice-cold phosphate-buffered saline, briefly trypsinizing the cells, and determining the cell number as well as the radioactivity in the cell suspension by liquid scintillation counting. Data is expressed as percentage of drug-sensitive S1 cells. Values are means ± SE; n = 3−5 independent experiments.

modulators of MDR resulting from the overexpression of the *MDR-1* gene. In addition to the results presented in this work, other studies have provided strong evidence that the effects of **15h** are due to its interaction with P-gp:[30] Reversal agent **15h** selectively restores the cytotoxic activity of hydrophobic anticancer agents such as vinblastine, bisantrene, colchicine, and doxorubicin in resistant cells, by increasing drug accumulation in such cells. Sensitivity to water-soluble drugs such as cisplatin is not altered by **15h**. No resensitization is found in cells lacking P-gp. These results suggest that the reversal agents inhibit the P-gp-mediated efflux of cytotoxic agents, probably by blocking the ability of P-gp to interact with antitumor drugs. Given the hydrophobic nature of the P-gp substrates, it seems likely that the hydrophobic reversal agents also bind to P-gp. Consistent with this, the binding of the photoaffinity probe [^{125}I]iodoarylazidoprazosin (which is believed to be a reporter for the vinblastine binding site) to P-gp is inhibited by **15h** and verapamil. However, further studies described in this work show that unlike verapamil (and cyclosporin A), **15h** is not effluxed from cells by P-gp, which suggests that it binds more strongly to or at a distinct site of the binding pocket. It appears likely that the superior reversal activity of **15h** versus

Table 7. Antitumor Activity of Vincristine Alone and in Combination[a] with an Optimal Dose of MDR Reversal Agents against Vincristine-Resistant Murine Leukemia P388/VCR

compd	VCR[b]	% $ILS_{placebo}$[c]	% ILS_{VCR}[d]	compd dose (mg/kg)	*t*-test[e]
15a[f]	51	73	15	50	<0.01
15d	67	89	13	12.5	<0.01
15e	13	25	11	50	<0.01
15f	24	42	14	25	<0.01
15g	14	27	11	25	<0.01
15h	37	83	33	12.5	<0.01
15i	24	34	8	6.3	0.03
15j	24	38	16	25	<0.01
15k	26	56	24	12.5	<0.01
15m	37	68	23	12.5	<0.01
15o	21	37	13	6.3	0.02
15c′	15	68	46	25	<0.01
28a	15	63	41	25	<0.01
39a	15	71	48	50	<0.01
PG/ET/Sal[g]		−1		0	
Crm/ET/Sal[h]		1		0	
15a[i]		−3		100	
15h[i]		−4		100	

[a] MDR reversal agents plus 0.2 mg/kg vincristine were administered simultaneously (both ip) once daily on days 1−7, where day 1 is 1 day after tumor implantation. [b] Percent increase in mean life span when 0.2 mg/kg vincristine was administered ip once daily on days 1−7, relative to placebo control treatment. [c] Percent increase in mean life span, relative to placebo control treatment. [d] Percent increase in mean life span, relative to treatment with an equivalent dose of vincristine alone. [e] Student *t*-test analysis of mean survival time, comparing treatment with vincristine plus MDR reversal agents to treatment with vincristine alone. [f] **15a** was administered ip 1 h before each daily 0.4 mg/kg ip dose of vincristine on days 1−3. [g] Propylene glycol/ethanol/saline vehicle administered at a dose equivalent to a 100 mg/kg/day dose of MDR reversal agent, together with a 0.5 mg/kg ip dose of vincristine once daily on days 1−7. [h] Cremophor EL/ethanol/saline vehicle administered at a dose equivalent to a 100 mg/kg/day dose of MDR reversal agent, together with a 0.5 mg/kg ip dose of vincristine once daily on days 1−7. [i] MDR reversal compounds at a dose of 100 mg/kg in Cremophor/ethanol/saline vehicle administered ip without vincristine, once daily on days 1−7.

Table 8. In Vivo Evaluation of MDR Reversal Agents (dosed at 12.5−200 mg/kg) against Human Epidermoid Carcinoma KB/8.5 Implanted sc in Athymic 'Nude' Mice

	optimal tumor growth inhibition			
compd	DOX[a]	% TGI[b]	compd dose (mg/kg)	*t*-test
15a	39	63	50	<0.01
15f	39	61	50	<0.01
15h	69	59	50	<0.01
15o	58	68[c]	100	0.01
15q	100	57	200	0.04
15r	75	60	100	<0.01
15s	45	64	100	0.03
15t	64	58	200	<0.01
15u	67	69[c]	100	0.09
15v	64	54	100	<0.01
15x	100	39	200	<0.01
15y	100	51	100	0.02
15a′	100	71	200	0.10
15c′	59	65	100	<0.01
28a	67	65[d]	50	<0.01
28b	57	94	200	0.34
35a	67	57	100	<0.01
35b	77	68	100	0.05
35c	67	53	200	<0.01
35e	77	72	100	0.06
36	75	54	200	<0.01
37b	72	67	100	<0.01
38a	75	64	100	<0.01
39a	67	46	25	<0.01
47	80	56	100	<0.01

[a] Relative tumor growth as a percentage of saline control on day 14 when treated with DOX (8 mg/kg) alone. [b] Percent tumor growth inhibition (TGI) obtained for the combination of DOX (8 mg/kg) together with MDR reversal agent (at the specified dose), versus DOX (8 mg/kg) alone on day 14. p (*t*-test) values < 0.05 are statistically significant. See Experimental Section for details. [c] Toxicity was observed at a dose of 200 mg/kg MDR reversal agent, together with 8 mg/kg DOX: 2/5 dead/total. [d] Toxicity was observed at a dose of 200 mg/kg MDR reversal agent, together with 8 mg/kg DOX: 4/5 dead/total.

verapamil is at least in part due to the fact that it is accumulated in greater concentrations, particularly in cells overexpressing P-gp.

Despite the structural similarity of the compounds to VRP, they possess significantly less calcium channel activity. When **15h** was assayed for calcium channel antagonistic activity in rat thoracic aortic strips, it was found to be 70-fold less potent than racemic verapamil.[30] Additional tests (results not shown) for other representative compounds showed the same level of activity. Thus, the structural differences are sufficient to provide a significantly different activity profile. Consequently, the calcium channel-blocking effect (hypotension) is not expected to be a side effect of these compounds.

As in the case of verapamil, most of the active compounds possess a chiral quaternary center. While some studies have shown that both enantiomers of verapamil exhibit equal activity as MDR reversal agents,[32,33] the *R*-enantiomer is approximately 10-fold less active as a calcium channel blocker than the *S*-enantiomer.[34] Although an enantioselective synthesis of verapamil has been carried out,[35] the structural differences of our lead compounds have necessitated a different synthetic approach toward producing the pure enantiomers to study their relative activities. The results will be reported in due course.

While no clear trend was observed correlating the lipophilicity of the reversal agents with activity, the most active compounds overall were relatively lipophilic (clog $P > 5.5$). Altering the steric bulk of certain substituents (the R_5 and R_6 groups) appeared to strongly affect binding to P-gp, while relatively large changes down the long axis (the linker chain length n and the size of the alkoxy substituents R_1-R_4) seemed to be well-accommodated. The presence of certain functional groups was critical for activity; for example, electron-donating alkoxy substituents on the aromatic rings provide far greater activity than halogens in the same positions. Thus, despite the fact that P-gp acts as an efflux pump for a variety of structurally unrelated hydrophobic substrates, it displays a significant sensitivity to structural changes within a particular series of compounds.

Experimental Section

Biological Procedures. 1. In Vitro Testing. Cells: A clonal cell line, S1, derived from human colon carcinoma cells, LS 174 T, was subjected to increasing concentrations of bisantrene (CL 216,942; from Lederle Laboratories, Pearl River, NY) over a period of 12−16 weeks. The resistant cell line was designated S1-B1-20.[27]

Screening assay for reversal agents: Cells, prepared by trypsinization, were plated at 20 000 cells/well in media with and without bisantrene (20 μM). Then, test agents at varying concentrations were added to the cells. Test agents were solubilized in 100% DMSO. The final DMSO concentration in the seeded plates did not exceed 0.1%. Plates were incubated for 3 days at 37 °C in a moisturized 7% CO_2 atmosphere. Cell survival was estimated by the sulforhodamine B assay.[36] Dye

content/well was read at 540 nm in a microtiter reader. Values were electronically processed, percent cell survival compared to untreated (without bisantrene) cells was computed, and the results were expressed as the difference between these values. The difference scores of those two experiments were determined at concentrations from 0.1 to 80 μM (typically at 0.1, 0.5, 1, 5, 10, 20, 40, and 80 mM concentrations). Two to four sets of measurements were made for each compound, and the results were plotted. The maximum variation of these data points was ±15%. A best fit equation was then applied (with $R^2 \geq 0.990$), and the IC_{50}'s were determined. The LD_{50}'s were determined similarly by utilizing the percent cell survival data from experiments in which test agent alone was used.

2. In Vivo Testing. Evaluation of MDR-1 reversal agents against the vincristine-resistant murine leukemia P388/VCR implanted in mice: The vincristine-resistant murine leukemia P388/VCR was propagated by intraperitoneal (ip) injection of 1×10^6 tumor cells into syngeneic DBA/2 mice (Charles River Laboratories, Inc.). Tumor cell ascites was harvested 5−7 days later. For drug testing, groups of 10−15 CDF1 mice (*DBA/2* × *Balb/c F1* hybrids; Charles River Laboratories, Inc.) were injected ip with 0.5 mL of diluted ascitic fluid containing 1×10^6 viable P388/VCR tumor cells. All mice were of one sex, weighing a minimum of 18 g, and all within a 3-g weight range per test. Drugs were administered once daily, by ip, starting 1 day after tumor implantation and using a variety of treatment schedules. Mice were weighed periodically, survival was recorded for up to 30 days post-tumor implantation, and mean survival times were calculated for all groups of mice. Statistical analyses of mean survival times were carried out using the Student *t*-test. Positive MDR-1 drug resistance reversal activity was evidenced by a statistically significant ($p < 0.05$) increase in mean survival time for groups of mice treated with vincristine plus MDR-1 compound, compared to groups of mice treated with an equivalent dose of vincristine alone.

Evaluation of MDR-1 reversal agents against the human epidermoid carcinoma KB/8.5 implanted in athymic "nude" mice: The MDR human epidermoid carcinoma KB/8.5 was propagated in RPMI 1640 medium supplemented with 5% fetal calf serum, gentamicin (50 μg/mL), ITS culture supplement (Collaborative Medical Products, Bedford, MA), and colchicine (10 ng/mL) to maintain drug resistance. Colchicine was then removed from the medium, for two in vitro passages, prior to subcutaneous (sc) implant of 8×10^6 KB/8.5 tumor cells into athymic "nude" mice (Harlan Sprague−Dawley, Inc., Indianapolis, IN). Approximately 7−10 days post-tumor implant, the sc tumors attained a mass of 100−300 mg. At this time (day 0 of the test period) mice were allocated to treatment groups of 5−10 mice/group so that the groups had mean tumor masses as comparable as possible. Drug treatment was initiated 1 day later, according to the following treatment schedule: doxorubicin was administered as a single intravenous dose (8 mg/kg), and either placebo or MDR-1 reversal agent was administered in two sc doses (12.5−200 mg/kg/dose) given 2 h before and 2 h after the single iv dose of doxorubicin. The tumor mass of each mouse and the mean tumor mass of each treatment group were determined on days +14 and +21 of the test period. For each treatment group, the relative tumor growth (RTG) was calculated as follows:

$$\text{RTG} = \text{mean tumor mass on day } +14 \text{ or day } +21$$

Statistical analysis of log RTG was carried out using the Student *t*-test. Transformation of the data from absolute mean tumor mass (mg of tumor mass) to log RTG prior to statistical analysis has two beneficial effects: (1) it corrects the data for differences in mean tumor mass of the different treatment groups at the start of the test (day 0); and (2) the log transformation of the data more adequately reflects the exponential growth pattern of sc implanted tumors and makes the use of the Student *t*-test more appropriate. Positive drug resistance reversal activity is indicated by a statistically significant ($p < 0.05$) reduction in RTG for groups treated with doxorubicin plus MDR-1 reversal compound, compared to treatment with an equivalent dose of doxorubicin alone.

Chemistry. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Fast atom bombardment (FAB) mass spectra were determined on a VG-ZAB SE mass spectrometer. Electron impact (EI) and chemical ionization (CI) mass spectra were determined on a Finnigan MAT-90 mass spectrometer. IR spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. ^{1}H NMR spectra were determined at 300 MHz using a Nicolet QE-300 WB spectrometer; chemical shifts (δ) given are in parts per million relative to tetramethylsilane. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within 0.4% of the theoretical value. Unless otherwise noted all reagents and solvents obtained from commercial suppliers were used without further purification.

1,2-Bis(chloromethyl)-4-methoxy-5-(phenylmethoxy)-benzene (2). Into a mixture of 12.0 g (56.0 mmol) of 1-methoxy-2-(phenylmethoxy)benzene **(1)**, 18.0 mL (240.0 mmol) of 37% formaldehyde, 2.0 g (6.27 mmol) of zinc iodide, and 200 mL of ether was bubbled HCl gas at a rate such that a gentle reflux was maintained. The addition of HCl gas was stopped after 1.5 h, and the solution was stirred for an additional 1.5 h. The solution was poured into ice water and extracted with ether. The organic layer was washed with brine, dried with anhydrous Na_2SO_4, and filtered and the filtrate was evaporated to yield 15.0 g of an oil. This oil was taken up in ether and passed through a short pad of magnesol with 400 mL of ether. The volatiles were removed, and the residual oil was purified by flash chomatography (4:96 ethyl acetate/hexane). This afforded 1.36 g (8%) of **2** as a white fluffy solid, mp 114−116 °C: ^{1}H NMR ($CDCl_3$) δ 7.46−7.31 (m, 5H), 6.91 (s, 1H), 6.90 (s, 1H), 5.15 (s, 2H), 4.69 (s, 1H), 4.46 (s, 1H), 3.88 (s, 3H). Anal. Calcd for $C_{16}H_{16}Cl_2O_2 \cdot 0.18C_{15}H_{13}ClO_2$: C, H, Cl.

2,3-Dihydro-5-methoxy-6-(phenylmethoxy)-2-(phenylmethyl)-1*H*-isoindole (3). To a stirred solution of 2.5 mL (25.0 mmol) of 10 N NaOH, 0.61 mL (0.6 g, 5.6 mmol) of benzylamine, and 0.1 g (0.36 mmol) of *n*-tetrabutylammonium chloride was added a solution of 1.0 g (3.2 mmol) of **2** in 10 mL of toluene under argon, and the resulting mixture was stirred vigorously for 3 days. The mixture was poured into water, and the two layers were separated. Following extraction of the aqueous layer with ethyl acetate, the combined organic layers were washed with brine. The crude product was dried with anhydrous Na_2SO_4 and evaporated to yield an oil, which was purified by flash chromatography on silica gel eluting with a gradient of 0.2−1% CH_3OH/CH_2Cl_2 to yield 0.74 g (67%) of **3**, as a beige solid, mp 94−96 °C: ^{1}H NMR ($CDCl_3$) δ 7.43−7.24 (m, 10H), 6.75 (s, 1H), 6.72 (s, 1H), 5.10 (s, 2H), 3.88 (s, 4H), 3.85 (s, 3H), 3.82 (s, 2H); MS (FAB) *m*/*z* 346 ($M + H^+$). Anal. ($C_{23}H_{23}NO_2 \cdot 0.03CH_2Cl_2$) C, H, N.

2,3-Dihydro-6-methoxy-1*H*-isoindol-5-ol (4a). A 0.5-g (1.45-mmol) portion of **3** was warmed in 35 mL of ethanol until dissolution. After cooling to room temperature, 0.13 g of 35% palladium hydroxide on carbon was added. The resulting mixture was shaken on a Parr apparatus at 50 psi of hydrogen for 21 h. The solution was filtered through a pad of Celite, and the pad was washed with ethanol. Evaporation of the volatiles afforded 0.209 g (87%) of **4a** as a tan wax: ^{1}H NMR ($CDCl_3$) δ 6.80 (s, 1H), 6.72 (1H), 4.10 (d, 4H), 3.80 (s, 2H). Anal. ($C_9H_{11}NO_2 \cdot 0.25H_2O \cdot 0.125EtOH$) C, H, N.

Preparation of Compounds 6a,b (Table 2). Representative Example: 3,4-Dimethoxy-α-[(4-methylphenyl)thio]-benzeneacetonitrile (6a). A mixture of 4.12 g (19.5 mmol) of α-chloro-3,4-dimethoxybenzeneacetonitrile **(5)**,[44] 2.48 g (20.0 mmol) of *p*-thiocresol, 2.76 g (18.0 mmol) of K_2CO_3, and 100 mL of CH_3CN was stirred at 65 °C for 12 h. After the salts were filtered, the reaction mixture was concentrated under vacuum, leaving a golden oil. The oil was dissolved in a minimum amount of ethyl acetate and diluted with 3 volumes of hexane. On standing, 3.81 g (65%) of **6a** as light-yellow crystals were obtained: ^{1}H NMR ($CDCl_3$) δ 7.37 (d, 2H, $J = 8$ Hz), 7.15 (d, 2H, $J = 8$ Hz), 6.82 (m, 3H), 4.87 (s, 1H), 3.88 (s,

3H), 3.82 (s, 3H), 2.36 (s, 3H); MS (EI) 299 (M^+). Anal. ($C_{17}H_{17}NO_2S$) C, H, N.

Preparation of Compounds 6c,d (Table 2). Representative Example: (3,4-Dimethoxyphenyl)(2-pyridylthio)-acetonitrile (6c). To a 5 °C solution of 2.66 g (15.0 mmol) of (3,4-dimethoxyphenyl)acetonitrile **(7a)** in 40 mL of THF was added dropwise a solution of 30.0 mL (30.0 mmol) of a 1.0 M solution of sodium bis(trimethylsilyl)amide in THF over a period of 20 min. The resulting mixture was stirred at 5 °C for 50 min and then added dropwise via cannula to a solution of 3.33 g (15.0 mmol) of 2,2′-dipyridyl disulfide in 10.0 mL of THF. After stirring at 5 °C for 40 min and then at room temperature for 1.5 h, the mixture was poured into ice water. Following extraction with ethyl acetate, the organic layer was washed with brine, dried, and concentrated in vacuo to yield an oil which solidified on standing. The solid was recrystallized from ethyl acetate/hexane to yield 2.75 g (64%) of **6c**, as an orange solid, mp 108−110 °C: ^{1}H NMR ($CDCl_3$) δ 8.56−8.54 (m, 1H), 7.62−7.56 (m, 1H), 7.24−7.11 (m, 3H), 7.05 (d, 1H, J = 2 Hz), 6.87 (d, 1H, J = 8.3 Hz), 6.06 (s, 1H), 3.90 (s, 3H), 3.89 (s, 3H). Anal. ($C_{15}H_{14}N_2SO_2$) C, H, N, S.

Preparation of Compounds 9a−c (Table 2). Representative Example: 5-[[(4-Methylphenyl)thio]methyl]-1,3-benzodioxole (9a). A mixture of 28.5 g (230 mmol) of *p*-thiocresol, 250 mL of water, and 8.8 g (220 mmol) of NaOH was warmed on a steam bath and then stirred vigorously while cooling to room temperature. Next, 250 mL of toluene, 39.71 g (116.4 mmol) of 4-(methylenedioxy)benzyl chloride **(8a)** in 30 mL of CH_2Cl_2, and 3.29 g (11.0 mmol) of tetrabutylammonium chloride were added to the reaction. The mixture was stirred at room temperature for 1 h; then 4.4 g (11.0 mmol) of NaOH was added. After stirring for 12 h, the layers were separated and the organic layer was washed with first with 1 N NaOH and then with brine. The organic layer was dried with Na_2SO_4 and concentrated under vacuum to a brown oil. Flash chromatography on silica gel, using a hexane/$CHCl_3$ and then a $CH_3OH/CHCl_3$ gradient, gave 24.01 g (80%) of **9a** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.20 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.79 (s, 1H), 6.68 (s, 2H), 5.92 (s, 2H), 3.98 (s, 2H), 2.30 (s, 3H); MS (EI) 259 (M^+). Anal. ($C_{15}H_{14}O_2S$) C, H, S.

1,2-Dimethoxy-4-[[(4-methylphenyl)thio]methyl]benzene (9d). To a solution of 0.745 g (6.00 mmol) of *p*-thiocresol in 5 mL of DMF was added 0.24 g (6.0 mmol) of 60% sodium hydride in oil. After the effervescence had stopped, 1.33 g (5.76 mmol.) of 3,4-dimethoxybenzyl bromide[45] **(8d)** was added. The reaction was left to stand for 12 h and then evaporated under vacuum and the residue partitioned between $CHCl_3$ and water. The organic layer was dried (sodium sulfate) and evaporated again. Chromatography (silica gel with gradient elution progressing from hexane/$CHCl_3$ to $CH_3OH/CHCl_3$) gave 1.35 g of crystalline solid, mp 49−51 °C. Recrystallization from hexane, with filtration of the hot solution through diatomaceous silica, gave 1.29 g (82%) of white needles, mp 50−52 °C: ^{1}H NMR ($CDCl_3$) δ 7.21 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.76 (m, 3H), 4.02 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 2.30 (s, 3H); MS (EI) 274 (M^+). Anal. ($C_{16}H_{18}O_2S$) C, H, S.

2-Methoxy-5-[[(4-methylphenyl)thio]methyl]phenol (11). A mixture of 53.34 g (430 mmol) of *p*-thiocresol, 1 L of water, 24.08 g (430 mmol) of KOH, 100 mL of toluene, and 2.54 g (7.5 mmol) of tetrabutylammonium hydrogen sulfate was stirred for 1 h. Meanwhile, a suspension of 22.10 g (143 mmol) of 3-hydroxy-4-methoxybenzyl alcohol **(10)** in 720 mL of toluene was cooled and stirred in an ice bath while hydrogen bromide was bubbled in at a rapid rate for 30 min. The clear reddish supernatant, containing 3-hydroxy-4-methoxybenzyl bromide, was decanted from a small quantity of gum into the thiocresol solution. The 3-hydroxy-4-methoxybenzyl bromide formation was repeated twice more until a total of 54.97 g (356.7 mmol) of **17** had been consumed. After the reaction was stirred for 12 h, the layers were separated, and the organic layer was washed with dilute HCl. After drying with Na_2SO_4, the solvent was evaporated under vacuum. On standing, the residue crystallized. Chromatography on silica gel with a gradient elution progressing from hexane to $CHCl_3$ followed by recrystallization from 2-propanol afforded 70.52 g (76% yield) of **11** as white crystals, mp 62−63.5 °C: ^{1}H NMR ($CDCl_3$) δ 7.22 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.88 (s, 1H), 6.74 (s, 2H), 5.56 (s, 1H), 3.99 (s, 2H), 3.85 (s, 3H), 2.30 (s, 3H); MS (CI) 278 (M^+). Anal. ($C_{15}H_{16}O_2S$) C, H, S.

(1,1-Dimethylethyl)[2-methoxy-5-[[(4-methylphenyl)-thio]methyl]phenoxy]dimethylsilane (9e). A mixture of 11.1 g (42.6 mmol) of **11**, 7.46 g (49.5 mmol) of *tert*-butyldimethylsilyl chloride, and 0.3 g (2.45 mmol) of 4-(dimethylamino)pyridine was heated under reflux in 40 mL of pyridine. After cooling, the mixture was poured into water and extracted with 3 × 75 mL of ether. The ether extract was washed with brine, dried with anhydrous sodium sulfate, and filtered and the filtrate was evaporated to yield a brown oil. The crude residue was distilled at 161−165 °C/0.03 mmHg, affording 15.06 g (94% yield) of **9e** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.20 (d, 2H, J = 8 Hz), 7.05 (d, 2H, J = 8 Hz), 6.75 (m, 3H), 3.96 (s, 2H), 3.77 (s, 3H), 2.30 (s, 3H), 0.97 (s, 9H), 0.11 (s, 6H); MS (CI) 392 (M^+). Anal. ($C_{21}H_{30}O_2SSi$) C, H, S.

Preparation of Compounds 12a−k (Table 3): Method A. Representative Example: α-(4-Chlorobutyl)-3,4-dimethoxy-α-[(4-methylphenyl)thio]benzeneacetonitrile (12b). To a solution of 3.79 g (12.7 mmol) of **13a** in 40 mL of DMSO under an argon atmosphere was added 0.516 g (12.9 mmol) of 60% sodium hydride in oil with stirring. After the mixture stirred for 1.5 h, 1.50 mL (13.0 mmol) of 1-bromo-4-chlorobutane was added and the reaction mixture was further stirred for 5 h. The reaction mixture was poured into 200 mL of ice water and twice extracted with ether, and the combined organic extracts were dried with $MgSO_4$. The ethereal solution was filtered and concentrated in vacuo to give 3.18 g (64%) of **12b** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.25 (d, 2H, J = 8 Hz), 7.07 (d, 2H, J = 8 Hz), 6.95 (d, 1H, J = 8 Hz), 6.89 (s, 1H), 6.78 (d, 1H, J = 8 Hz), 3.88 (s, 3H), 3.84 (s, 3H), 3.48 (t, 2H, J = 6 Hz), 2.32 (s, 3H), 2.22 (m, 2H), 1.80 (m, 2H), 1.68 (m, 1H), 1.45 (m, 1H); MS (CI) m/z 407 ($M + NH_4^+$). Anal. Calcd for $C_{21}H_{24}ClNO_2S \cdot 0.01C_{21}H_{24}BrNO_2S$: C, H, N, S, Cl, Br.

Preparation of Compounds 13a−h (Table 3): Method A′. Representative Example: 4-[4-Chloro-1-[(4-methylphenyl)thio]butyl]-1,2-dimethoxybenzene (13a). To a solution of 13.72 g (50.0 mmol) of **9d** and a crystal of phenanthroline in 250 mL of dry THF under nitrogen at −78 °C was added 21.0 mL (52.5 mmol) of 2.5 M *n*-butyllithium in hexane via syringe. After 10 min, the brown solution was warmed to 0 °C for 1 h, then it was recooled to −78 °C, and 8.02 mL (75.0 mmol) of 1-bromo-3-chloropropane was added via syringe. The reaction was warmed to room temperature and left for for 12 h. To the light-yellow reaction was added 10 mL of ethyl acetate, and the resulting solution was concentrated in vacuo. The residue was partitioned between ethyl acetate and water and the organic phase dried (Na_2SO_4) and concentrated under vacuum to a yellow oil. Purification by chromatography on silica gel with gradient elution progressing from hexane/$CHCl_3$ to $CHCl_3/CH_3OH$ afforded 16.47 g (94% yield) of **13a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.15 (d, 2H, J = 8 Hz), 7.02 (d, 2H, J = 8 Hz), 6.72 (m, 3H), 4.01 (m, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.49 (t, 2H, J = 7 Hz), 2.29 (s, 3H), 2.05 (m, 2H), 1.82 (m, 2H); MS (CI) m/z 227, 229 ($M + H^+ - C_7H_7S$). Anal. ($C_{19}H_{23}ClO_2S \cdot 0.055CHCl_3$) C, H, Cl, S.

Preparation of Compounds 15a−e′ (Tables 4−6): Method B. Representative Example: α-(3,4-Dimethoxyphenyl)-3,4-dihydro-6,7-dimethoxy-α-[(4-methylphenyl)-thio]-2(1*H*)-isoquinolineheptanenitrile (15h). To a solution of 4.65 g (11.5 mmol) of **19e** in 60 mL of DMF, under argon, were added 4.24 g (30.7 mmol) of K_2CO_3, 0.511 g (3.1 mmol) of KI, and 4.25 g (18.5 mmol) of **14a**. The reaction mixture was then heated to 95 °C for 4 h and cooled to ambient temperature. After 12 h, the solvent was evaporated and the residue was partitioned between ethyl ether and water. The ether layer was dried with $MgSO_4$, filtered, and evaporated to give a brown oil. Silica gel chromatography (ethyl acetate) afforded 3.67 g (56%) of **15h**, as a beige gum: ^{1}H NMR ($CDCl_3$)

δ 7.24 (d, 2H, J = 8 Hz), 7.07 (d, 2H, J = 8 Hz), 6.95 (d, 1H, J = 8 Hz), 6.77 (d, 1H, J = 8 Hz), 6.58 (s, 1H), 6.51 (s, 1H), 3.88 (s, 3H), 3.84 (s, 9H), 3.51 (br s, 2H), 2.80 (t, 2H, J = 6 Hz), 2.68 (t, 2H, J = 6 Hz), 2.44 (t, 2H, J = 7 Hz), 2.32 (s, 3H), 2.20 (t, 2H, J = 7 Hz), 1.55 (m, 3H), 1.36 (m, 3H); HR FAB (MS) calcd for $C_{33}H_{41}N_2O_4S$ (M + H) 561.2287, found 561.2287. Anal. Calcd for $C_{33}H_{40}N_2O_4S \cdot 0.5H_2O$: C, H, N, S.

Method B′. Representative Example: 2-[6-(3,4-Dimethoxyphenyl)-6-[(4-methylphenyl)thio]hexyl]-1,2,3,4-tetrahydro-6-methoxyisoquinoline (15p). To a solution of 4.23 g (10 mmol) of **13b** in 40 mL of CH_3CN were added 3.88 g (20 mmol) of **14c**, 1.74 mL (10 mmol) of diisopropylethylamine, and 0.01 g (0.067 mmol) of NaI. The solution was heated at reflux for 43 h and cooled to room temperature. The reaction mixture was partitioned between $CHCl_3$ and saturated K_2CO_3 and the organic layer dried with Na_2SO_4. Following evaporation in vacuo, the residue was chromatographed on silica gel using a gradient elution of hexane/$CHCl_3$ to $CHCl_3$/CH_3OH. The residue from the chromatography was dissolved in ether and passed through diatomaceous silica. This afforded 4.4 g (87% yield) of **15p** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.14 (d, 2H, J = 8 Hz), 7.01 (d, 2H, J = 8 Hz), 6.72 (m, 6H), 4.00 (m, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 3.52 (s, 2H), 2.86 (m, 2H), 2.66 (m, 2H), 2.43 (m, 2H), 2.28 (s, 3H), 1.90 (m, 2H), 1.54 (m, 2H), 1.33 (m, 4H); MS (FAB) m/z 506 (M + H$^+$). Anal. ($C_{31}H_{39}NO_3S$) C, H, N, S.

[(5-Bromo-3-pentynyl)oxy](1,1-dimethylethyl)dimethylsilane (16). A solution of 0.20 g (0.93 mmol) of 5-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-pentyn-1-ol[46] in 6 mL of CH_2Cl_2 under argon was cooled to −23 °C. To this were added 0.183 g (1.03 mmol) of *N*-bromosuccinimide and 0.294 g (1.12 mmol) of triphenylphosphine. After stirring at −23 °C for 1 h, the reaction was quenched with 30 mL of saturated $NaHCO_3$. An additional 50 mL of ether was added to the mixture, and the layers were separated. The organic layer was washed with 30 mL of brine, dried with $MgSO_4$, and concentrated in vacuo. Following purification by silica gel chromatography (98:2 hexane/ethyl acetate), 0.12 g (46%) of **16** was obtained as a clear oil: ^{1}H NMR ($CDCl_3$) δ 3.91 (t, 2H, J = 3 Hz), 3.72 (t, 2H, J = 7 Hz), 2.49−2.43 (m, 2H), 0.90 (s, 9H), 0.08 (s, 6H); MS (CI) m/z 277. Anal. ($C_{11}H_{21}BrOSi \cdot 0.1C_6H_{14}$) C, H, Br.

Method C. (a) α-[5-[[(1,1-Dimethylethyl)dimethylsilyl]-oxy]-2-pentynyl]-3,4-dimethoxy-α-[(4-methylphenyl)thio]-benzeneacetonitrile (17). To a solution of 0.108 g (0.36 mmol) of **6a** in 3 mL of anhydrous THF under an argon atmosphere was added 0.017 g (0.43 mmol) of 60% sodium hydride in mineral oil. After the mixture stirred for 1 h, 0.10 g (0.36 mmol) of **16** was added to the reaction mixture. The reaction was stirred for 1 h and then poured into 10 mL of water. The aqueous layer was extracted with 3 × 30 mL of ether. The combined ether layers were dried with $MgSO_4$, and solvent was removed in vacuo. Silica gel chromatography (5:1 hexane/ethyl acetate) provided 0.14 g (78%) of **17** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.38 (d, 2H, J = 8 Hz), 7.12 (d, 2H, J = 8 Hz), 7.08 (dd, 1H, J = 8 Hz, J = 2 Hz), 7.02 (d, 1H, J = 2 Hz), 6.81 (d, 1H, J = 8 Hz), 3.89 (s, 3H), 3.87 (s, 3H), 3.59 (t, 2H, J = 7 Hz), 3.02 (t, 2H, J = 2 Hz), 2.34 (s, 2H), 0.86 (s, 9H), 0.02 (s, 6H); HR MS (FAB) calcd for $C_{28}H_{37}NO_3SSiNa$ (M + Na) 518.2161, found 518.2166. Anal. ($C_{28}H_{37}NO_3SSi$) C, H, N, S.

(b) α-(5-Hydroxy-2-pentynyl)-3,4-dimethoxy-α-[(4-methylphenyl)thio]benzeneacetonitrile (18). A 0.289-g (0.58 mmol) portion of **17** was dissolved in 5 mL of THF and cooled to 0 °C. To this was added 0.90 mL (0.90 mmol) of 1.0 M tetrabutylammonium fluoride (in THF) while stirring. The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction was quenched with 5 mL of saturated aqueous $NaHCO_3$ solution and 5 mL of water. Following extraction of the aqueous phase with 3 × 10 mL of ether, the combined organic layers were washed with 10 mL of brine and dried with $MgSO_4$. The solvent was evaporated in vacuo. Silica gel chromatography (1:1 hexane/ethyl acetate) afforded 0.184 g (83%) of **18** as a clear oil: ^{1}H NMR (CDCl3) δ 7.37 (d, 2H, J = 8 Hz), 7.12 (d, 2H, J = 8 Hz), 7.08 (dd, 1H, J = 8 Hz, J = 2 Hz), 7.01 (d, 1H, J = 2 Hz), 6.82 (d, 1H, J = 8 Hz), 3.89 (s, 3H), 3.87 (s, 3H), 3.61 (t, 2H, J = 6 Hz), 3.04 (t, 2H, J = 2 Hz), 2.34 (s, 2H), 1.83 (br s, 1H); HR MS (EI) calcd for $C_{22}H_{23}NO_3S$ 381.1398, found 381.1386. Anal. ($C_{22}H_{23}NO_3S$) C, H, N, S.

(c) α-[5-(3,4-Dihydro-6,7-dimethoxy-2(1*H*)-isoquinolinyl)-2-pentynyl]-3,4-dimethoxy-α-[(4-methylphenyl)thio]-benzeneacetonitrile (19). To a solution containing 0.18 g (0.47 mmol) of **18** in 5 mL of CH_2Cl_2 was added 0.060 g (0.52 mmol) of mesyl chloride. The solution was cooled to 0 °C, and 0.1 mL (0.57 mmol) of diisopropylethylamine was added. The mixture was warmed to room temperature and stirred for 5 h. The solvent was removed with a stream of argon; then 5 mL of acetonitrile, 0.25 mL (2.61 mmol) of diisopropylethylamine, and 0.087 g (1.41 mmol) of **14a** were added. After heating to reflux under argon for 4 h, the reaction mixture was cooled and evaporated and the residue partitioned between ethyl acetate and water. The organic layer was washed with saturated brine, dried, and concentrated in vacuo. Silica gel chromatography (CH_2Cl_2/CH_3OH (95:5)) afforded 0.065 g (25%) of **19** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.38 (d, 2H, J = 8 Hz), 7.12 (d, 2H, J = 8 Hz), 7.09 (dd, 1H, J = 8 Hz, J = 2 Hz), 7.02 (d, 1H, J = 2 Hz), 6.79 (d, 1H, J = 8 Hz), 6.56 (s, 1H), 6.50 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.55 (s, 2H), 3.03 (t, 2H, J = 2 Hz), 2.78 (t, 2H, J = 5 Hz), 2.70 (d, 2H, J = 5 Hz) 2.62 (d, 2H, J = 7 Hz) 2.34 (s, 2H); HR MS (EI) calcd for $C_{26}H_{29}N_2O_4$ (M − C_7H_7S) 433.2127, found 433.2120. Anal. ($C_{33}H_{36}N_2O_4S \cdot 0.1H_2O$) C, H, N, S.

Method D. (a) 3-[2-Cyano-2-(3,4-dimethoxyphenyl)-2-[(4-methylphenyl)thio]ethyl]benzoic Acid (21). To a −78 °C solution of 1.86 g (6.2 mmol) of **13a** in 15 mL of THF was added dropwise a solution of 2.9 mL (6.34 mmol) of *n*-butyllithium (2.2 M) in hexane. This solution was stirred at −78 °C for 35 min. A solution of 0.67 g (3.1 mmol) of *m*-carboxybenzyl bromide (**20**) in 5 mL of anhydrous THF was added dropwise; the mixture was stirred at −78 °C for 40 min and then at room temperature for 15 min. The mixture was quenched with water and extracted with ethyl acetate. The extract was washed with brine, dried with $MgSO_4$, and evaporated in vacuo to yield a yellow oil. Flash chromatography (95:5 CH_2Cl_2/CH_3OH) afforded 1.15 g (86%) of **21**, as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 7.95 (d, 1H, J = 7.5 Hz), 7.73 (s, 1H), 7.37 (d, 2H, J = 8 Hz), 7.36−7.2 (m, 2H), 7.1 (d, 2H, J = 8 Hz), 6.98−6.92 (m, 2H), 6.75 (d, 1H, J = 8 Hz), 3.87 (s, 3H), 3.83 (s, 3H), 3.49 (s, 2H), 2.34 (s, 3H). Anal. ($C_{25}H_{23}NSO_4 \cdot 0.3H_2O$) C, H, N, S.

(b) α-(3,4-Dimethoxyphenyl)-3-(hydroxymethyl)-α-[(4-methylphenyl)thio]benzenepropanenitrile (22). A mixture of 1.0 g (2.3 mmol) of **21** and 0.7 mL (7.0 mmol) of borane−methyl sulfide complex (10 M) in 50 mL of THF was stirred at room temperature for 18 h. The mixture was quenched with CH_3OH, treated with HCl gas for 5 min, and stirred, and the solvent was evaporated to yield a white foam. After the foam was dissolved in water, the solution was made basic with 5 N sodium hydroxide and extracted with ethyl acetate. The extract was washed with brine, dried with $MgSO_4$, filtered, and evaporated in vacuo to yield a yellow oil. This was purified by flash chromatography (98:2 CH_2Cl_2/CH_3OH) to afford 0.55 g (52%) of **22** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.35 (d, 1H, J = 2 Hz), 7.34 (d, 1H, J = 2 Hz), 7.22−7.08 (m, 4H), 6.95−6.87 (m, 4H), 6.74 (d, 1H, J = 8 Hz), 4.57 (s, 2H), 3.87 (s, 3H), 3.82 (s, 3H), 3.42 (s, 2H), 2.33 (s, 3H). Anal. ($C_{25}H_{25}NO_3S$) C, H, N, S.

(c) 3-(Chloromethyl)-α-(3,4-dimethoxyphenyl)-α-[(4-methylphenyl)thio]benzenepropanenitrile (23). A mixture of 0.1 mL (1.37 mmol) of thionyl chloride and 0.12 mL (1.42 mmol) of DMF was stirred at room temperature for 30 min. To this was added a solution of 0.34 g (0.81 mmol) of **22** in 1.0 mL of DMF. The resulting mixture was heated at 70 °C for 17 h, cooled to room temperature, and poured into water. Following extraction with ether, the ether extract was washed with brine, dried with $MgSO_4$, filtered, and evaporated in vacuo to yield an oil. This was purified by flash chromatography (9:1 hexane/ethyl acetate) to afford 0.27 g (76%) of **23** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.38 (d, 2H, J = 8 Hz),

7.29−7.09 (m, 4H), 6.96−6.91 (m, 4H), 6.73 (d, 1H, J = 8 Hz), 4.49−4.44 (m, 2H), 3.90 (s, 3H), 3.8 (s, 3H), 3.42 (s, 2H), 2.33 (s, 3H). Anal. ($C_{25}H_{24}ClNO_2S$) C, H, N, Cl, S.

(d) 3-(3,4-Dihydro-6,7-dimethoxy-2(1*H*)-isoquinolyl)-α-(3,4-dimethoxyphenyl)-α-[(4-methylphenyl)thio]benzenepropanenitrile (24). A mixture of 0.21 g (0.47 mmol) of **32**, 0.091 g (0.47 mmol) of **14a**, and 0.3 g (2.16 mmol) of anhydrous K_2CO_3 in 2.0 mL of DMF was heated at 85 °C for 4 h, cooled, poured into water, and extracted with ethyl acetate. The extract was washed with brine and dried with $MgSO_4$. The filtrate was evaporated in vacuo to yield an oil, which was purified by flash chromatography (98:2 CH_2Cl_2/CH_3OH) and then precipitated from cold EtOH to afford 0.18 g (63%) of **24** as a yellow glassy solid: ^{1}H NMR ($CDCl_3$) δ 7.35−6.88 (m, 10H), 6.70 (d, 1H, J = 8.5 Hz), 6.58 (s, 1H), 6.48 (s, 1H), 3.86−3.79 (m, 12H), 3.58 (s, 2H), 3.50 (s, 2H), 3.43 (s, 2H), 2.78−2.71 (m, 2H), 2.67−2.55 (m, 2H), 2.32 (s, 3H); MS (FAB) 595 (M + H$^+$). Anal. ($C_{36}H_{38}N_2O_4S$·0.7EtOH) C, H, N, S.

Preparation of Compounds 28a−c (Tables 4 and 5): Method E. Representative Example (28a): (a) α-(3,4-Dimethoxyphenyl)-1,3-dihydro-α-[(4-methylphenyl)thio]-1,3-dioxo-2*H*-isoindole-2-heptanenitrile (25a). A mixture of 4.50 g (11.1 mmol) of **12e**, 48 g (320 mmol) of NaI, and 155 mL of acetone was heated at reflux for 12 h, protected from light. The volatiles were evaporated in vacuo, and 500 mL of ether was added to the residue. This was filtered and evaporated. Silica gel chromatography (hexane/ethyl acetate, 2:1) afforded 6.24 g of α-(5-iodopentyl)-3,4-dimethoxy-α-[(4-methylphenyl)thio]benzeneacetonitrile as a clear oil.

A mixture of 6.12 g (11.1 mmol) of this intermediate, 2.22 g (12.0 mmol) of potassium phthalimide, and 50 mL of DMF was heated on a steam bath for 2 h. After evaporation of solvent, the residue was partitioned between ether and water, with agitation. The organic layer was washed with 1 N NaOH, followed by aqueous K_2CO_3. The solution was dried with Na_2SO_4, and evaporated in vacuo. Chromatography on silica gel, with gradient elution progressing from hexane/$CHCl_3$ to $CHCl_3$/CH_3OH, afforded 4.77 g (83% yield) of **25a** as a fluorescent oil: ^{1}H NMR ($CDCl_3$) δ 7.84 (m, 2H), 7.71 (m, 2H), 7.23 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.88 (m, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.63 (t, 2H, J = 7 Hz), 1.65 (m, 2H), 1.34 (m, 4H,); MS (FAB) m/z 515 (M + H$^+$). Anal. ($C_{30}H_{30}N_2O_4S$·0.375H_2O) C, H, N, S.

(b) α-(5-Aminopentyl)-3,4-dimethoxy-α-[(4-methylphenyl)thio]benzeneacetonitrile (26a). A mixture of 4.72 g (9.17 mmol) of **25a**, 100 mL of ethanol, and 7.0 mL (225.0 mmol) of hydrazine hydrate was heated at reflux. After 30 min, a heavy precipitate formed. After 1.3 h, the reaction was cooled to room temperature and concentrated in vacuo. The residue was warmed on a steam bath with 2 N HCl for 10 min. Diatomaceous silica and $CHCl_3$ were added; the mixture was shaken thoroughly and then filtered through more diatomaceous silica. The organic phase was washed first with aqueous ammonia and then with brine. After drying with Na_2SO_4, the filtrate was concentrated in vacuo to provide 3.57 g (99.8%) of **26a** as a yellow oil: ^{1}H NMR ($CDCl_3$ + TFA) δ 7.21 (d, 2H, J = 8 Hz), 7.09 (d, 2H, J = 8 Hz), 6.88 (m, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.10 (m, 2H), 2.33 (s, 3H), 2.23 (m, 2H), 1.70 (m, 2H), 1.43 (m, 4H); MS (CI) m/z 385 (M + H$^+$). Anal. ($C_{22}H_{28}N_2O_2S$·0.58H_2O) C, H, N, S.

(c) α-(3,4-Dimethoxyphenyl)-1,3-dihydro-5,6-dimethoxy-α-[(4-methylphenyl) thio]-2*H*-isoindole-2-heptanenitrile (28a). To a solution of 3.03 g (7.88 mmol) of **26a**, 1.85 g (7.88 mmol) of **27a**,[24] 20 mL of toluene, and 0.22 g (0.79 mmol) of tetrabutylammonium chloride was added 2.0 g (50 mmol) of NaOH in 40 mL of water. The mixture was stirred for 48 h. After the pH of the aqueous layer was adjusted to 9 with solid $NaHCO_3$, the layers were separated and the aqueous layer was further extracted with $CHCl_3$. The combined organic extracts were dried with $NaSO_4$, passed through a pad of hydrous magnesium silicate, and concentrated in vacuo to a brown gum. This gum was purified by HPLC using a reverse-phase C-18 radial-pak column (6 cm × 30 cm) with a buffered solvent system (150 mL of ammonium hydroxide in water was adjusted to a pH of 4.00 with acetic acid and then diluted to 7.875 L with water, 5700 mL of acetonitrile, and 1425 mL of CH_3OH). Concentration in vacuo afforded 0.85 g of **28a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.25 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.96 (d, 1H, J = 8 Hz), 6.90 (s, 1H), 6.77 (d, 1H, J = 8 Hz), 6.73 (s, 2H), 3.85 (m, 16H), 2.67 (t, 2H, J = 6 Hz), 2.31 (s, 3H), 2.20 (t, 2H, J = 6 Hz), 1.55 (m, 3H), 1.40 (m, 3H); HR MS (EI) calcd for $C_{32}H_{38}N_2O_4S$ 546.2553, found 546.2569. Anal. ($C_{32}H_{38}N_2O_4S$) C, H, N, S.

Preparation of Compounds 35a−e (Table 4): Method F. Representative Example (35a): (a) Ethyl 6-(3,4-Dimethoxy-(*E*)-oxobenzene)hexanoate (31a). To a 0 °C solution of 1.52 g (11.0 mmol) of veratrole (**29**) in 15 mL of CH_2Cl_2 was added 2.66 g (20.0 mmol) of anhydrous aluminum chloride in portions so as to maintain the temperature at 0 °C. A solution of 1.93 g (10.0 mmol) of adipic acid chloride monoethyl ester **(30)** in 10 mL of CH_2Cl_2 was added dropwise. The resulting mixture was stirred at 0 °C for 4 h and warmed to room temperature for 12 h. The crude product was poured into a mixture of ice/concentrated HCl. The aqueous layer was further extracted with CH_2Cl_2. The combined organic layers were washed with brine and dried ($MgSO_4$). The filtrate was evaporated to an oil, which was purified by silica gel chromatography (hexane/ethyl acetate, 4:1) to afford 2.76 g (94%) of **31a** as a white solid, mp 50−52 °C: ^{1}H NMR ($CDCl_3$) δ 7.58 (dd, 1H, J = 2 Hz, J = 8 Hz), 7.53 (d, 1H, J = 2 Hz), 6.90 (d, 1H, J = 8 Hz), 4.13 (q, 2H, J = 7 Hz), 3.95 (s, 3H), 3.94 (s, 3H), 2.96 (t, 2H, J = 7 Hz), 2.36 (t, 2H, J = 7 Hz), 1.78−1.72 (m, 4H), 1.26 (t, 3H, J = 7 Hz). Anal. ($C_{16}H_{22}O_5$) C, H.

(b) 6-(3,4-Dimethoxy-(*E*)-oxobenzene)hexanoic Acid (31b). A mixture of 2.5 g (8.5 mmol) of **31a** and 16 mL of 10% ethanolic NaOH was stirred at room temperature for 3 h. The mixture was diluted with water, and the ethanol was evaporated in vacuo. Concentrated HCl was added to acidify the solution. The solid obtained was collected by filtration, washed with water, and dried in vacuo to afford 2.2 g (97%) of **31b**, as a beige solid, mp 123−125 °C: ^{1}H NMR ($CDCl_3$) δ 7.58 (dd, 1H, J = 2 Hz, J = 8.5 Hz), 7.53 (d, 1H, J = 2 Hz), 6.89 (d, 1H, J = 8.5 Hz), 3.95 (s, 3H), 3.94 (s, 3H), 2.97 (t, 2H, J = 7 Hz), 2.43 (t, 2H, J = 7 Hz), 1.81−1.74 (m, 4H). Anal. ($C_{14}H_{18}O_5$·0.1H_2O) C, H.

(c) 2-[6-(3,4-Dimethoxyphenyl)-1,6-dioxohexyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (32). To a suspension of 1.73 g (7.5 mmol) of **14a** in 20 mL of DMF was added 4.2 mL (3.05 g, 30.13 mmol) of triethylamine. The solution was stirred for 5 min and then cooled with an ice bath. To this solution were added 2.0 g (7.5 mmol) of **31b** followed by 1.5 g (9.2 mmol) of diethyl cyanophosphonate. The mixture was stirred at room temperature for 12 h. The crude product mixture was poured into water, extracted with ethyl acetate, washed with 2 N HCl, a saturated solution of $NaHCO_3$, and brine, and dried ($MgSO_4$). Following evaporation of the solvent, 3.15 g (95%) of **32** was obtained as a white solid, mp 101−103 °C: ^{1}H NMR ($CDCl_3$) δ 7.60 (dd, 1H, J = 8 Hz, J = 2 Hz), 7.53 (br s, 1H), 6.90−6.86 (m, 1H), 6.63−6.60 (m, 2H), 4.65 (s, 1H), 4.56 (s, 1H), 3.95−3.93 (m, 6H), 3.87−3.85 (m, 6H), 3.83 (t, 1H, J = 6 Hz), 3.67 (t, 1H, J = 6 Hz), 2.99 (t, 2H, J = 7 Hz), 2.82 (t, 1H, J = 6 Hz), 2.76 (t, 1H, J = 6 Hz), 2.47 (t, 2H, J = 7 Hz), 1.88−1.76 (m, 4H). Anal. ($C_{25}H_{31}NO_6$·0.2H_2O) C, H, N.

(d) 2-[6-(3,4-Dimethoxyphenyl)-6-hydroxy-1-oxohexyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (33a). A mixture of 1.4 g (3.1 mmol) of **32** and 0.28 g (7.4 mmol) of sodium borohydride in 25 mL of CH_3OH was stirred at room temperature for 12 h. The mixture was poured into water and extracted with ethyl acetate. The ethyl acetate extract was washed with brine and dried (Na_2SO_4). Evaporation of the filtrate afforded 1.4 g of **33a** as a colorless oil, which was used in the subsequent step without further purification.

(e) 2-[6-(Cyclohexylthio)-6-(3,4-dimethoxyphenyl)-1-oxohexyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (34a). To a solution of 1.0 g (2.2 mmol) of crude **33a** in 10 mL of 1,2-dichloroethane were added 0.72 g (2.2 mmol) of zinc iodide followed by the addition of 0.30 g (2.6 mmol) of

cyclohexyl mercaptan.[26] The resulting mixture was stirred at room temperature for 1 h, poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with NaOH and brine. After drying ($MgSO_4$), the solvent was evaporated in vacuo to afford 1.06 g (89%) of **34a**, as a glassy solid: ^{1}H NMR ($CDCl_3$) δ 6.88 (d, 1H, J = 1.5 Hz), 6.79−6.78 (m, 2H), 6.63−6.56 (m, 2H), 4.65 (s, 1H), 4.56 (s, 1H), 3.90−3.85 (m, 12H), 3.75 (m, 2H), 3.62 (t, 1H, J = 5.9 Hz), 2.85−2.70 (m, 2H), 2.34 (t, 3H, J = 7.6), 2.00−1.20 (m, 16H). Anal. ($C_{31}H_{43}NO_5S$) C, H, N, S.

(f) 2-[6-(Cyclohexylthio)-6-(3,4-dimethoxyphenyl)hexyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (35a). A mixture of 0.95 g (1.75 mmol) of **34a** and 0.35 mL (3.5 mmol) of borane−methyl sulfide complex (10 M) in 10 mL of THF was heated at reflux for 2 h, cooled, and quenched with CH_3OH. Volatiles were evaporated in vacuo, and the residue was taken up in 10 mL of ethanol. To this was added 8 mL of 1 N NaOH, and the resulting mixture was heated at reflux for 2 h. The solution was cooled, diluted with water, and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and filtered. Evaporation of the filtrate yielded an oil, which was purified by flash chromatography (2% CH_3OH/CH_2Cl_2) to afford 0.71 g (77%) of **35a** as a light-yellow oil: ^{1}H NMR ($CDCl_3$) δ 6.90 (s, 1H), 6.82 (m, 2H), 6.58 (s, 1H), 6.51 (s, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 3.51 (s, 2H), 2.80 (t, 2H, J = 6.5 Hz), 2.68 (t, 2H, J = 6.5 Hz), 2.44 (t, 2H, J = 8.5 Hz), 2.33 (m, 1H), 2.20−1.00 (m, 19H). Anal. ($C_{31}H_{45}O_4NS\cdot 0.5EtOH$) C, H, N, S.

Intermediate for Synthesis of 35e: (g) 2-[6-(3,4-Dimethoxyphenyl)-6-hydroxy-1-oxooctyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (33b). To a solution of 3.0 g (6.8 mmol) of **32** in 20 mL of anhydrous THF was added 15.0 mL (15.0 mmol) of ethylmagnesium bromide (1 M in THF). The resulting mixture was stirred at room temperature for 12 h. After pouring the mixture into ice and saturated ammonium chloride, the aqueous layer was extracted with ether. The extract was washed with brine, dried ($MgSO_4$), and evaporated to afford 3.0 g (94%) of **33b** as a colorless oil, which was subsequently used without further purification: ^{1}H NMR ($CDCl_3$) δ 7.00−6.55 (m, 5H), 4.63 (s, 1H), 4.49 (s, 1H), 3.89−3.85 (m, 12H), 3.80 (t, 1H, J = 6 Hz), 3.60 (t, 1H, J = 6 Hz), 2.76 (m, 2H), 2.32 (m, 2H), 1.83−1.70 (m, 3H), 1.69−0.80 (m, 5H), 0.76 (t, 3H, J = 7.5 Hz).

5-[7-(3,4-Dihydro-6,7-dimethoxy-2(1*H*)-isoquinolinyl)-1-[(4-methylphenyl)thio]heptyl]-2-methoxyphenol (36). Compound **36** was prepared by the procedure of compound **18**. A 10.28-g (15.8 mmol) portion of **15x** was reacted with 49 mL (49.0 mmol) of 1.0 M tetrabutylammonium fluoride/THF in 150 mL of THF. The crude product was dissolved in ether and passed through a short hydrous magnesium silicate pad to afford, after solvent removal, 8.75 g (99.5%) of **36** as a brown oil: ^{1}H NMR ($CDCl_3$) δ 7.16 (d, 2H, J = 8 Hz), 7.01 (d, 2H, J = 8 Hz), 6.85 (d, 1H, J = 2 Hz), 6.70 (m, 2H), 6.58 (s, 1H), 6.51 (s, 1H), 5.6 (br s, 1H), 3.96 (m, 1H), 3.83 (m, 9H), 3.52 (s, 2H), 2.81 (m, 2H), 2.70 (m, 2H), 2.44 (m, 2H), 2.28 (s, 3H), 1.87 (m, 2H), 1.55 (m, 2H), 1.28 (m, 6H); MS (CI) m/z 536 (M + H$^+$). Anal. ($C_{32}H_{41}NO_4S\cdot 0.5H_2O$) C, H, N, S.

Method G. (a) 2-[7-[3-(2-Chloroethoxy)-4-methoxyphenyl]-7-[(4-methylphenyl)thio]heptyl]-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (37a). To a stirred solution of 5.57 g (1.02 mmol) of **36** and 3.05 g (13.0 mmol) of 2-chloroethyl tosylate in 50 mL of 2-butanone was added 0.44 g (11.0 mmol) of 60% sodium hydride in oil. The solution was heated at reflux for 48 h and then cooled to room temperature. The crude product mixture was partitioned between $CHCl_3$ and brine and the organic layer dried (Na_2SO_4). Following evaporation of the solvent in vacuo, the residue was chromatographed on silica gel with a gradient elution going from hexane/$CHCl_3$ to $CHCl_3$/CH_3OH. The product was dissolved in ether and passed through a short hydrous magnesium silicate pad. The solvent was evaporated in vacuo to afford 1.6 g (27% yield) of **37a** as a light-tan gum: ^{1}H NMR ($CDCl_3$) δ 7.11 (d, 2H, J = 8 Hz), 7.03 (d, 2H, J = 8 Hz), 6.77 (m, 2H), 6.71 (m, 1H), 6.58 (s, 1H), 6.51 (s, 1H), 4.17 (m, 2H), 3.97 (m, 1H), 3.83 (m, 9H), 3.75 (t, 2H, J = 7 Hz), 3.54 (s, 2H), 2.83 (m, 2H), 2.71 (m, 2H), 2.47 (m, 2H), 2.28 (s, 3H), 1.90 (m, 2H), 1.55 (m, 2H), 1.30 (br s, 6H); MS (CI) m/z 598 (M + H$^+$). Anal. ($C_{34}H_{44}ClNO_4S$) C, H, Cl, N, S.

(b) 1,2,3,4-Tetrahydro-2-[7-[3-[2-(1*H*-imidazol-1-yl)ethoxy]-4-methoxyphenyl]-7-[(4-methylphenyl)thio]heptyl]-6,7-dimethoxyisoquinoline (37b). To 0.76 g (11.0 mmol) of imidazole in 5 mL of dry DMF was added 0.18 g (4.5 mmol) of 60% sodium hydride in oil. When the effervescence subsided, 0.26 g (1.7 mmol) of NaI was added, followed by 1.12 g (1.87 mmol) **37a** in 7 mL of DMF. After stirring for 48 h, the solution was concentrated in vacuo and the residue partitioned between ethyl acetate and water. The organic layer was extracted with 1 N HCl, the acid layer basified with aqueous ammonia, and the resulting oil extracted into ethyl acetate. After drying with Na_2SO_4, the extract was concentrated in vacuo to 0.53 g of a tan oil. Flash chromatography on silica gel (gradient elution: hexane to $CHCl_3$ to CH_3OH) gave a tan oil which was taken up in ethyl acetate and passed through a pad of hydrous magnesium silicate. This afforded 0.194 g (16% yield) of **37b** as a yellow gum: ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1H), 7.12 (s, 1H), 7.08 (d, 2H, J = 8), 7.07 (s, 1H), 6.99 (d, 2H, J = 8), 6.76 (m, 2H), 6.64 (m, 1H), 6.59 (s, 1H), 6.51 (s, 1H), 4.3 (m, 2H), 4.17 (m, 2H), 3.95 (m, 1H), 3.83 (m, 9H), 3.53 (s, 2H), 2.81 (m, 2H), 2.71 (m, 2H), 2.45 (m, 2H), 2.26 (s, 3H), 1.88 (m, 2H), 1.55 (m, 2H), 1.29 (m, 6H); MS (CI) m/z 630 (M + H$^+$). Anal. ($C_{37}H_{47}N_3O_4S\cdot H_2O$) C, H, N, S.

Preparation of Compounds 38a−c and 39a−c (Table 4): Method G′. Representative Example: (a) 7-(2-Chloroethoxy)-α-(3,4-dimethoxyphenyl)-3,4-dihydro-6-methoxy-α-[(4-methylphenyl)thio]-2(1*H*)-isoquinolineheptanenitrile (38a). A mixture of 0.93 g (1.70 mmol) of **15l**, 10 mL of 1 N NaOH, 0.92 mL (5.1 mmol) of 2-chloroethyl *p*-toluenesulfonate, and 25 mL of 2-butanone was heated at reflux for 18 h under argon. The mixture was cooled and concentrated in vacuo, and the residue was partitioned between ethyl acetate and water. After the organic layer was washed with brine and dried ($MgSO_4$), concentration in vacuo afforded an oil. Flash chromatography (ethyl acetate) afforded 0.433 g (76%) of **38a** as a colorless gum: ^{1}H NMR ($CDCl_3$) δ 7.24 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.95 (d, 1H, J = 8 Hz), 6.89 (s, 1H), 6.77 (d, 1H, J = 8 Hz), 6.61 (s, 1H), 6.57 (s, 1H), 4.24 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.81 (m, 8H), 3.49 (s, 2H), 2.79 (t, 2H, J = 6 Hz), 2.67 (t, 2H, J = 6 Hz), 2.44 (t, 2H, J = 8 Hz), 2.32 (s, 3H), 2.19 (t, 2H, J = 8 Hz), 1.54 (m, 3H), 1.31 (m, 3H); EI (HRMS) calcd for $C_{34}H_{41}N_2O_4SCl$ 608.2476, found 608.2483. Anal. ($C_{34}H_{41}ClN_2O_4S$) C, H, Cl, N, S.

(b) α-(3,4-Dimethoxyphenyl)-3,4-dihydro-7-[2-(1*H*-imidazol-1-yl)ethoxy]-6-methoxy-α-[(4-methylphenyl)thio]-2-(1*H*)-isoquinolineheptanenitrile (39a). Compound **39a** was prepared by the procedure of compound **37b**. A 0.366-g (0.60 mmol) portion of **38a** was reacted with 0.062 g (0.91 mmol) of imidazole and 0.037 g (0.91 mmol) of 60% sodium hydride in oil in 16 mL of DMF. Flash chromatography (98:2 CH_2Cl_2/CH_3OH), followed by precipitation of a polar impurity from ether/ethyl acetate, afforded 0.144 g (37%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.63 (s, 1H), 7.23 (d, 2H), 7.08 (m, 4H), 6.95 (d, 1H, J = 8 Hz), 6.89 (s, 1H), 6.79 (d, 1H, J = 8 Hz), 6.60 (s, 1H), 6.43 (s, 1H), 4.32 (t, 2H, J = 5 Hz), 4.20 (t, 2H, J = 5 Hz), 3.88 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.46 (s, 2H), 2.80 (t, 2H, J = 6 Hz), 2.68 (t, 2H, J = 6 Hz), 2.43 (t, 2H, J = 7 Hz), 2.32 (s, 3H), 2.19 (t, 2H, J = 7 Hz), 1.54 (m, 3H), 1.35 (m, 3H). Anal. ($C_{37}H_{44}N_4O_4S\cdot 0.6EtOAc$) C, H, N, S.

Method H. α-(3,4-Dimethoxyphenyl)-7-[2-(dimethylamino)ethoxy]-3,4-dihydro-6-methoxy-α-[(4-methylphenyl)thio]-2(1*H*)-isoquinolineheptanenitrile (40). To a solution of 0.583 g (1.07 mmol) of **15l** in 20 mL of DMF was added 0.051 g (1.3 mmol) of 60% sodium hydride in oil, and the solution was stirred for 1 h. To this solution were added 3.50 mg (3.2 mmol) of freshly prepared 2-(dimethylamino)ethyl chloride and 0.06 g (0.36 mmol) of KI. The solution was stirred at room temperature for 18 h, and the volatiles were evaporated at in vacuo. The residue was dissolved in a minimum amount of

ethyl acetate, and 5 volumes of ether was added. After the solution was passed through a short pad of magnesol, the volatiles were evaporated in vacuo. Silica gel chromatography (gradient of ether/ethyl acetate to ethyl acetate/CH_3OH) afforded 0.140 g (21%) of **40** as a clear glass: ^{1}H NMR ($CDCl_3$) δ 7.24 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 6.97 (d, 1H, J = 8 Hz), 6.89 (s, 1H), 6.77 (d, 1H, J = 8 Hz), 6.58 (s, 1H), 6.54 (s, 1H), 4.06 (t, 2H, J = 6 Hz), 3.88 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.49 (s, 2H), 2.77 (m, 4H), 2.66 (t, 2H, J = 6 Hz), 2.42 (t, 2H, J = 7 Hz), 2.34 (s, 6H), 2.32 (s, 3H), 2.19 (t, 2H, J = 7 Hz), 1.54 (m, 3H), 1.31 (m, 3H). Anal. ($C_{36}H_{47}N_3O_4S\cdot 0.6Et_2O$) C, H, N, S.

Preparation of Hydrochloride Salts (41−48). Representative Example: α-(3,4-Dimethoxyphenyl)-3,4-dihydro-7-[2-(1*H*-imidazol-1-yl)ethoxy]-6-methoxy-α-[(4-methylphenyl)thio]-2(1*H*)-isoquinolineheptanenitrile Hydrochloride (48). To a solution of 0.8 g (1.25 mmol) of **39a** in 100 mL of ether and 5 mL of ethanol was added 0.83 mL (3.74 mmol) of 4.5 M HCl in ethanol. The solution was stirred for 20 min. A white solid precipitate was collected, washed three times with small portions of ether, and dried in vacuo to afford 0.84 g (90%) of **48** as a white solid, mp 70 °C dec. ^{1}H NMR (DMSO-d_6) δ 9.06 (br s, 1H), 7.76 (m, 1H), 7.63 (m, 1H), 7.22 (d, 2H, J = 8 Hz), 7.17 (d, 2H, J = 8 Hz), 6.97−6.86 (m, 3H), 6.83 (s, 1H), 6.80 (s, 1H), 4.58 (t, 2H, J = 5 Hz), 4.31−4.02 (m, 4H), 3.75 (m, 9H), 3.45−3.32 (m, 4H), 3.06 (t, 2H, J = 6 Hz), 2.41 (t, 1H, J = 7 Hz), 2.30 (s, 3H), 2.16 (t, 1H, J = 7 Hz), 1.76 (m, 2H), 1.37 (m, 3H), 1.21 (m, 1H). Anal. ($C_{37}H_{44}N_4O_4S\cdot 1.75HCl\cdot 2.0H_2O$) C, H, N, S, Cl.

Supporting Information Available: Analytical data for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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