Pergamon

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Bioorganic & Medicinal Chemistry 11 (2003) 1475–1491

BIOORGANIC & MEDICINAL CHEMISTRY

Substituted Dibenzo[*c*,*h*]cinnolines: Topoisomerase I-Targeting Anticancer Agents

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Received 18 July 2002; accepted 24 October 2002

Abstract—Several substituted dibenzo[*c*,*h*]cinnolines were synthesized and evaluated for their potential to target topoisomerase I and for their relative cytotoxic activity. Select benzo[*i*]phenanthridines are capable of stabilizing the cleavable complex formed with topoisomerase I and DNA. This study was initiated to examine whether dibenzo[*c*,*h*]cinnolines, which are in essence aza analogues of benzo[*i*]phenanthridines, possess similar pharmacological properties. 2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine is one of the more potent benzo[*i*]phenanthridine derivatives in regard to topoisomerase I-targeting activity and cytotoxicity. The structure–activity relationship observed with these substituted dibenzo[*c*,*h*]cinnolines parallels that observed for benzo[*i*]phenanthridine derivatives. Compared to similarly substituted benzo[*i*]phenanthridines, the dibenzo[*c*,*h*]cinnoline analogues exhibit more potent topoisomerase I-targeting activity and cytotoxicity. The relative IC_{50} values obtained in assessing the cytotoxicity of 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline and 2,3-dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine in the human lymphoblastma cell line, RPMI8402, are 70 and 400 nM, respectively. In tumor cell lines selected for resistance to camptothecin and known to express mutant topoisomerase I, benzo[*i*]phenanthridine derivatives were not cross-resistant. In contrast, similarly substituted dibenzo[*c*,*h*]cinnolines with significant topoisomerase I-targeting activity did exhibit cross-resistance in these camptothecin-resistant cell lines. The cytotoxicity of these dibenzo[*c*,*h*]cinnolines was not diminished in cells overexpressing the efflux transporter, MDR1. These data indicate that substituted dibenzo[*c*,*h*]cinnolines can exhibit potent topoisomerase I-targeting activity and are capable of overcoming the multi-drug resistance associated with this efflux transporter.

Introduction

The topological state of DNA is regulated by DNA topoisomerases, which perform this function through the breaking and rejoining of DNA strands.[1–4] Studies have also demonstrated that these enzymes are involved in controlling template supercoiling during RNA transcription.[5,6] There are two major subtypes of topoisomerases based upon differences in their initial mechanisms. While the mechanism associated with topoisomerase I (TOP1) involves the formation of a single-strand DNA break, topoisomerase II (TOP2) functions by creating a double-strand DNA break. The antitumor activity of topoisomerase-targeting agents is associated with their ability to stabilize the enzyme-DNA cleavable complex. This drug-induced stabilization of the enzyme–DNA cleavable complex effectively converts these enzymes into cellular poisons.

Camptothecin and its structurally-related analogues are among the more extensively studied agents that target TOP1. Bi- and terbenzimidazoles,[7–10] certain benzo[*c*]-phenanthridine and protoberberine alkaloids and their synthetic analogues,[11–15] indolocarbazoles,[16] the fungal metabolites bulgarein[17] and saintopin,[18] and indenoisoquinolines,[19,20] phenazines,[21] and benzophenazines,[22] have been identified as TOP1-directed agents. Recently, several benzo[*i*]phenanthridines have been identified that exhibit potent activity as TOP1-targeting agents and significant cytotoxicity.[23,24] 2,3-Dimethoxy-8,9-methylenedioxybenzo[*i*]phenanthridine, **1** (Fig. 1), is

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PII: S0968-0896(02)00604-1

among the more active benzo[*i*]phenanthridine derivatives evaluated.

The benzo[*i*]phenanthridine derivatives **2a** and **2b** were previously selected for synthesis and pharmacological evaluation based upon their structural similarity to nitidine, **3**. Compound **1**, however, exhibited significantly greater cytotoxicity and TOP1-targeting activity than either **2a** or **2b**. Substituents on the A- and D-ring of benzo[*i*]phenanthridines can have a profound effect on pharmacological activity. It was assumed, therefore, that each of these benzophenanthridines, **1**–**3**, act by adopting an orientation wherein a similar molecular topology is achieved, but the relative position of the heteroatom differs. The presence of 2,3-dimethoxy substituents in ring-A, a 8,9-methylenedioxy moiety in ring-D, and a nitrogen heteroatom adjacent to the benzo-ring that possesses the methylenedioxy substituent appear to define key structural elements that are associated with the enhanced TOP1-targeting activity and cytotoxicity observed with **1** relative to other benzo[*i*]phenanthridine derivatives.[23,24]

The present study explores the structure activity relationships of substituted dibenzo[*c*,*h*]cinnolines. Dibenzo[*c*,*h*]cinnolines, which have incorporated into their structure two adjacent heteroatoms, are structurally similar to benzo[*c*]phenanthridines or benzo[*i*]phenanthridines The influence of substituents in both the A- and D-ring of dibenzo[*c*,*h*]cinnolines on both TOP1-targeting activity and cytotoxicity was investigated. Among the compounds selected for synthesis and pharmacological evaluation was 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline, **4**. To further evaluate the structure–activity relationships within this class of compounds, compounds **5**–**15** were synthesized and their pharmacological activities evaluated (Chart 1).

Chemistry

The synthetic method employed for the preparation of **5** is outlined in Scheme 1. Compound **16** was prepared from 6,7-dimethoxy-α-tetralone. Using conditions as employed in the Heck reaction, 6,7-dimethoxy-1,2-dihydronaphthalene **17** was coupled with *o*-iodoni-

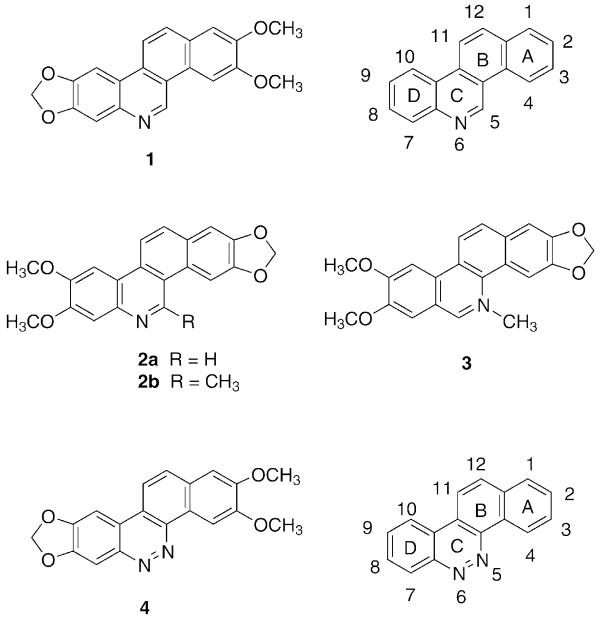
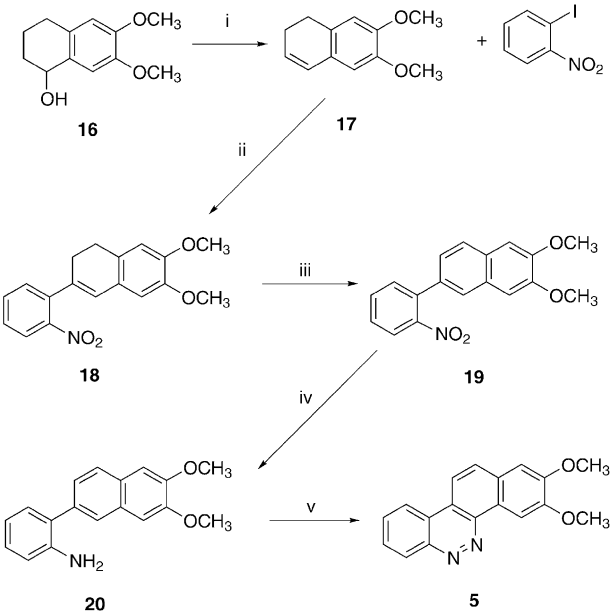
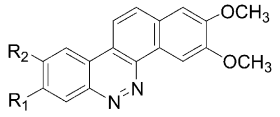


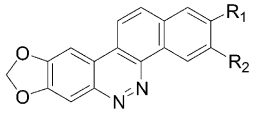
Figure 1. Structure of benzo[*i*]phenanthridines (**1**, **2a**,**b**), nitidine (**3**), 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline, **4**, and the numbering and ring designation of benzo[*i*]phenanthridines and dibenzo[*c*,*h*]cinnolines.



Scheme 1. Preparation of **5**: (i) H_3PO_4, DMF, 60 °C; (ii) $Pd(PPh_3)_2Cl_2$, $NaOCOCH_3$, DMA; (iii) DDQ, toluene, reflux; (iv) H_2, 10% Pd/C; (v) 48% HBr, $NaNO_2$, Cu powder.



5 R_1, R_2 = H
6 R_1, R_2 = OCH_3
7 R_1 = OCH_3; R_2 = H
8 R_1 = H; R_2 = OCH_3
9 R_1 = OCH_3; R_2 = OBn
10 OCH_3; R_2 = OH



4 R_1, R_2 = OCH_3
11 R_1 = OCH_3; R_2 = H
12 R_1 = H; R_2 = OCH_3
13 R_1 = H; R_2 = OBn
14 R_1 = H; R_2 = OH
15 R_1 = OCH_3; R_2 = CH_2OH

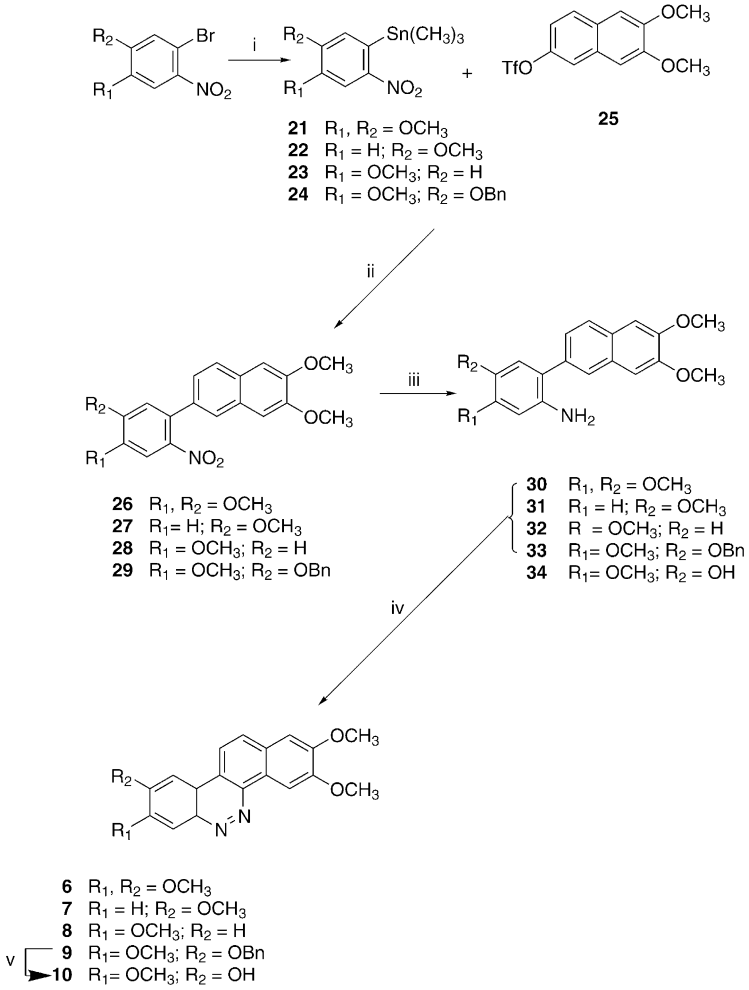
Chart 1.

trobenzene to provide **18** in low yield (30%).[25] Oxidation of **18** with DDQ provided the naphthyl derivative **19**, which was reduced to 2-(*o*-aminophenyl)-6,7-dimethoxynaphthalene, **20**. Diazotization of 6-(2-amino-4,5-dimethoxyphenyl)-2,3-dimethoxynaphthalene is known to give 2,3,8,9-tetramethoxydibenzo[*c*,*h*]cinnoline in approximately 20% yield.[26] Similarly, diazotization of **20** provided **5** in 18% yield. The instability of several of the intermediates, the low yields associated with several of the initial reactions, and the general inefficiency of this synthetic route prompted the development of an alternative synthetic strategy.

A substantially modified approach used for the preparation of the 2,3-dimethoxydibenzo[*c*,*h*]cinnolines **6**–**10** is outlined in Scheme 2. Stille coupling proved to be an effective method for preparing 2-(2-nitrophenyl)naphthalenes.[27] Using variously substituted *ortho*-nitroarylstannanes, Stille coupling of **21**–**24** with 6,7-dimethoxy - 2 - trifluoromethanesulfonyloxynaphthalene (25) provided the requisite 2-(2-nitrophenyl)naphthyl derivatives, **26**–**29**. These 2-(2-nitrophenyl)naphthyl derivatives were readily converted to their aniline derivatives, **30**–**34**, using Pd/C and H_2. Under these conditions, partial hydrogenolysis was observed with the benzyloxy analogue **29**, which resulted in the formation of a mixture of **33** and **34**. Diazotization of **30**–**33** provided the desired 2,3-dimethoxybenzo[*c*,*h*]cinnolines **6**–**9**. Treatment of **34** under similar conditions failed to provide **10**. Hydrogenolysis of **9**, however, did provide **10** in good yield.

Select 8,9-methylenedioxydibenzo[*c*,*h*]cinnolines were similarly prepared as illustrated in Scheme 3. Coupling of trimethyl-4,5-methylenedioxy-2-nitrophenylstannane, **35**, with 6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene (**25**), 2-bromo-6-methoxynaphthalene (**36**), or 7-methoxy-2-trifluoromethanesulfonyloxynaphthalene (**37**) provided 2-(2-nitrophenyl)naphthalenes **38**–**40**. Treatment of these 2-(2-nitrophenyl) naphthalenes with hydrogen in the presence of 10% Pd/C provided aniline derivatives **41**–**43**. Diazotization of **41**–**43** gave the 8,9-methylenedioxydibenzo[*c*,*h*]cinnolines, **4**, **11** and **12** as depicted in Scheme 3.

An alternative route for the preparation of **12**, **13** and **14** is outlined in Scheme 4. Nitration of **44**–**46** with 4 -methyl-4-nitro-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one provided **47**–**49** in 35-58% yield.[28] Using *N*-phenyltrifluoromethanesulfonimide[29] or trifluoromethanesulfonic anhydride, the triflates **50**–**52** were prepared and coupled with trimethyl-4,5-methylene-



Scheme 2. Preparation of dibenzo[*c*,*h*]cinnolines, **6**–**10**: (i) $(CH_3)_3SnSn(CH_3)_3$, $Pd(PPh_3)_4$, THF, reflux; (ii) $Pd(PPh_3)_4$, CuBr, THF; (iii) H_2, 10% Pd/C, EtOAc; (iv) $NaNO_2$, AcOH, concd HCl; (v) H_2, 10% Pd/C, EtOAc.

dioxy-2-nitrophenyl stannane (**35**) to give the dinitro intermediates, **53**–**55**. Reduction of **53** and **54** with LAH provided **12** and **13** in 75% and 42% yield, respectively. Preparation of **14** was accomplished by treatment of **55** using Raney Ni in the presence of hydrazine.[30]

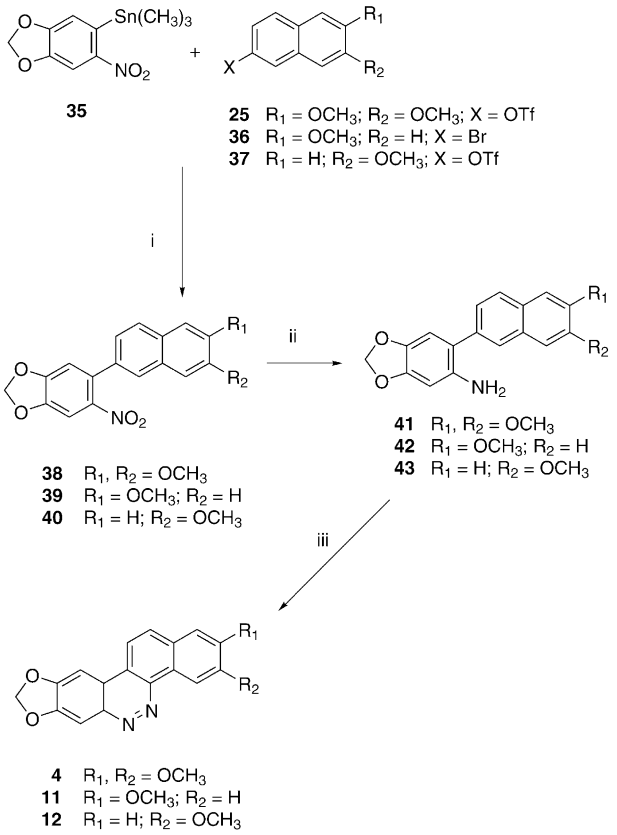
The phenolic dibenzo[*c*,*h*]cinnolines, **10** and **14**, represent derivatives that could be utilized to form new analogues or prodrugs that possess physico-chemical properties for improving solubility, formulation, and bioavailability. The synthesis of the 2-methoxy-3-hydroxymethylcinnoline **15** was also targeted in an effort to identify a dibenzo[*c*,*h*]cinnoline derivative suitable for prodrug development. The synthetic approach for the preparation of **15** is provided in Scheme 5. Coupling of the *ortho*-nitrostannane **35** with 7-bromo-3-methoxynaphthalene-2-carboxylic acid methyl ester gave **57** in 55% yield. Reduction of the NO_2 group of compound **57** with Ra–Ni and hydrazine hydrate provided **58**, which was converted to its hydroxymethyl derivative **59** with LAH. Diazotization of **59** gave the desired dibenzo[*c*,*h*]cinnoline, **15**.

Pharmacological evaluation

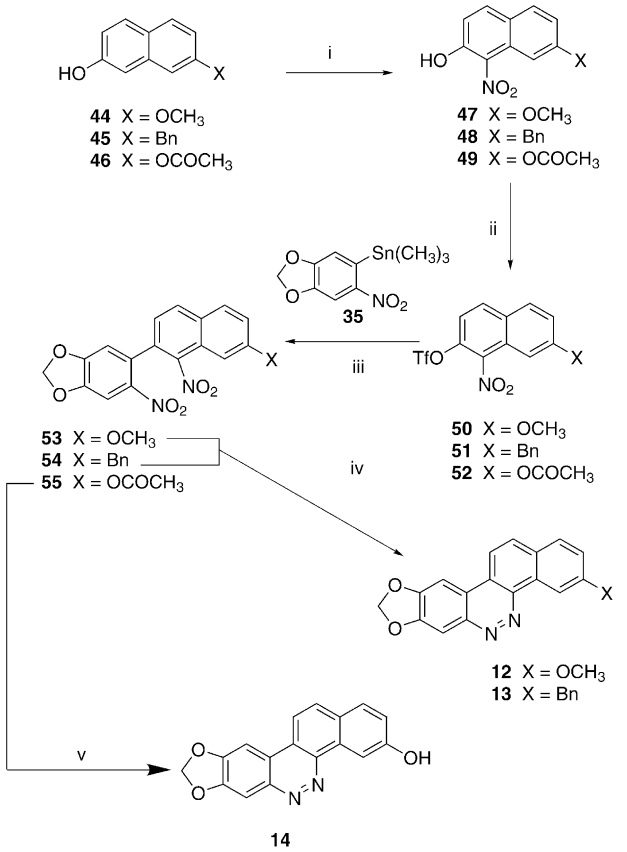
The relative topoisomerase-targeting activities (both TOP1 and TOP2) of **1**–**15** are listed in Table 1. Figures 2 and 3 are representative of the DNA cleavage induced by various substituted dibenzo[*c*,*h*]cinnolines in the presence of TOP1 and TOP2, respectively. The dibenzo[*c*,*h*]cinnolines were evaluated for cytotoxicity toward the human lymphoblast tumor cell line, RPMI8402 and its camptothecin-resistant variant, CPT-K5, U937 and its camptothecin-resistant variant U937/CR, as well as KB3-1 and its variant, KBV-1, which overexpresses the efflux transporter, P-glycoprotein (MDR1). These data are provided in Table 2. Table 3 summarizes the cytotoxicity observed with **1** and **3**–**7** against the human colon tumor cell line, HCT116, the human breast tumor cell line, ZR-75-1, the human cervical tumor cell line, HeLa, the human leukemia cell line, CEM and its TOP2 deficient variant, CEM/V-1.

Discussion

Previous studies demonstrated that the benzo[*i*]phenanthridine derivative **1** is an order of magnitude more potent in its TOP1-targeting activity than **2a** or **2b**, as determined by measuring cleavable complex formation in the presence of enzyme and DNA. A further enhancement in intrinsic TOP1-targeting activity was observed with the dibenzo[*c*,*h*]cinnoline analogue **4**, which is approximately twice as potent as **1**. The importance of the methylenedioxy substituent to the activity of these dibenzo[*c*,*h*]cinnolines as TOP1-targeting agents parallels previous observations noted in the structure–activity relationships observed with benzo[*i*]-



Scheme 3. Preparation of **4**, **11**–**12**: (i) $Pd(PPh_3)_4$, CuBr, THF; (ii) H_2, 10% Pd/C, EtOAc; (iii) $NaNO_2$, AcOH, concd HCl.



Scheme 4. Alternative synthetic route for the preparation of **12**–**14:** (i) $C_7H_3Br_4N_1O_3$, Et_2O; (ii) $(CF_3SO_2)_2O$ in pyridine or NaH, $(CF_3SO_2)_2NC_6H_5$, THF; (iii) $Pd(PPh_3)_4$, LiCl, CuBr, THF; (iv) LAH, THF; (v) $NH_2NH_2{\cdot}H_2O$, NaOH, EtOH.

phenanthridines. Removal of the methylenedioxy moiety, as in **5**, or its replacement by a methoxyl group, as in the case of **7** and **8**, resulted in a substantial loss of activity. Consistent with structure–activity data observed for benzo[*c*]phenanthridines and benzo[*i*]phenanthridines, replacement of the methylenedioxy moiety in Ring-D of **4** by two methoxyl groups, that is 2,3,8,9-teramethoxydibenzo[*c*,*h*]cinnoline **6**, a methoxyl and a benzyloxy group **9**, or a methoxyl and hydroxyl group **10**, results in the complete loss of TOP1-targeting activity. These data clearly substantiate the importance of the methylenedioxy moiety within the D-ring of these dibenzo[*c*,*h*]cinnolines for retention of potent TOP1-targeting activity.

The effect of altering substituents on the A-ring of these benzo[*c*,*h*]cinnolines was also examined. Relative to **4**, removal of the 3-methoxyl substituent, as in **11**, resulted in a loss of activity. In contrast, removal of the 2-methoxy substituent as in the case of **12**, resulted in a substantial reduction in TOP1-targeting activity. Nonetheless, at high concentrations of **12**, stabilization of cleavable complex formation was observed. Replacement of the methoxyl group of **12** with either a benzyloxy group or a hydroxyl moiety as in the case of **13** or **14** resulted in the loss of significant TOP1-targeting activity. Compound **15** was synthesized in an effort to develop a dibenzo[*c*,*h*]cinnoline derivative that retained TOP1-targeting activity, but possessed a hydroxyl group that could serve as a handle for forming either a derivative or prodrug with improved solubility. While **15** did retain activity as a TOP1-targeting agent, it was substantially less active than **4**.

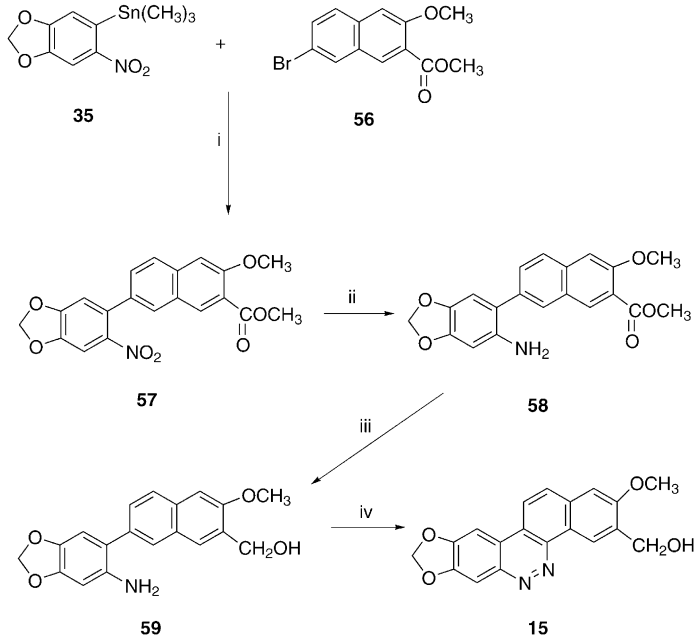
The cytotoxicity of **1**–**15** was evaluated in the human lymphoblast tumor cell line, RPMI8402 and its camptothecin-resistant variant, CPT-K5. In addition, cytotoxicity was also assessed in the leukemia cell line U937 and its camptothecin-resistant variant, U937/CR. Both of the cell lines are resistant to camptothecin and have a functional, but mutant TOP1 in which the cleavable complex formed between enzyme and DNA is not stabilized by camptothecin. The dibenzo[*c*,*h*]cinnoline derivative with the greatest potency as a TOP1-targeting agent **4** was the most cytotoxic analogue to RPMI8402 and U937 cells with IC_{50} values of 70 and 60 nM, respec-

Table 1. Topoisomerase-mediated DNA cleavage assays of dibenzo[*c*,*h*]cinnoline derivatives and related compounds

Compd	Topo I-mediated DNA cleavage[a]	Topo II-mediated DNA cleavage[b]
1	10	>1000
2	100	10
2b	100	100
3 (Nitidine)	10	1
4	5	500
5	100	>1000
6	>1000	500
7	100	>1000
8	500	>1000
9	>1000	>1000
10	>1000	>1000
11	5000	>1000
12	300	>1000
13	>1000	>1000
14	1000	>1000
15	100	>1000
CPT	1	>1000
VM-26	>1000	1

[a]Topoisomerase I cleavage values are reported as REC, relative effective concentration, that is concentrations relative to camptothecin (CPT), whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

[b]Topoisomerase II cleavage values are reported as REC, relative effective concentration, that is concentrations relative to VM-26, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase II.



Scheme 5. Preparation of 3-hydroxymethyl-2-methoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline, **15**: (i) $Pd(PPh_3)_4$, CuBr, THF; (ii) Ra–Ni, NH_2NH_2, EtOH reflux; (iii) LAH, THF, rt; (iv) $NaNO_2$, AcOH, concd HCl.

tively. In both instances, significant cross-resistance (a >10-fold difference in cytotoxicity) was observed between these parent cell lines and their respective camptothecin-resistant variants, CPT-K5 and U937/CR. Compounds **5**, **7**, and **8**, in which the methylenedioxy group of **4** was removed or substituted by a single methoxyl group at either the 8- or 9-position, were substantially less cytotoxic in both the RPMI8402 and U937 tumor cell lines, with IC_{50} values ranging from 2.0 to 5.5 μM. In addition, the difference between the parent cell lines and the camptothecin-resistant variants was less pronounced than in the case of **4**. This correlates with their decreased potency as TOP1-targeting agents. Nitidine, **3**, is a TOP1 and TOP2-targeting agent. As it can exert its cytotoxic effect by targeting TOP2, it is not surprising that its cross-resistance to CPT-K5 is minimal. Those dibenzo[*c*,*h*]cinnoline derivatives that were devoid of TOP1-targeting activity (**6**, **9**, **10**, **11**, **13** and **14**) also had comparatively weak cytotoxic activities.

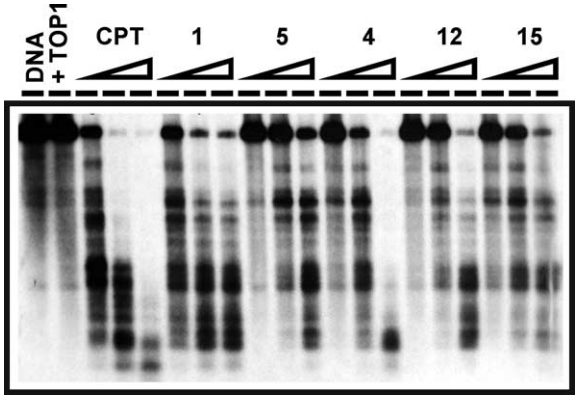


Figure 2. Stimulation of enzyme-mediated DNA cleavage by **1**, **4**, **5**, **12**, **15** and camptothecin (CPT) using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.1 to 10 μM for compound **1**, and from 0.01 to 1.0 μM for CPT and compounds **4**, **5**, **12**, and **15**.

These studies were extended to an evaluation of the relative cytotoxicity of **1**–**15** in KB3-1 tumor cell line and a variant, KBV-1, which overexpresses the efflux transporter, MDR-1 (P-glycoprotein). In these cell lines, the more potent TOP1-targeting dibenzo[*c*,*h*]cinnolines (**4**, **12** and **15**) have IC_{50} values that range from 50 to 640 nM in these cell lines. For each of the dibenzo[*c*,*h*]-cinnolines evaluated in this study, no significant difference in cytotoxicity (>10-fold) was observed between these two cell lines. Therefore, these substituted dibenzo[*c*,*h*]cinnolines are not substrates for this transporter and are capable of overcoming this form of multi-drug resistance.

The leukemia cell line CEM and its variants CEM/V1 and CEM/V2 are used to indicate whether certain cytotoxic agents are exerting their effect by targeting TOP2. A mutant, but functional TOP2 enzyme, is present in CEM/V1 and CEM/V2 and is responsible for their resistance to certain TOP2-targeting agents. Both VP-16 and VM-26 are known to target TOP2 and exhibit resistance to CEM/V1 and CEM/V2 relative to their parent cell line, CEM. As nitidine, **3**, targets both TOP1

Table 3. Relative cytotoxicities of substituted dibenzo[*c*,*h*]cinnolines

Compound	Cytotoxicity IC_{50} (μM)[a]					
	CEM	CEM/V1	CEM/V5	HeLa	HCT-116	ZR-75-1
1	0.1	0.4	0.2	0.2	0.3	0.2
3 (Nitidine)	0.16	1.0	0.8	0.07	1.9	0.6
4	0.03	0.04	0.06	0.04	0.04	0.03
5	1.5	4.1	6.2	4.7	2.8	3.2
6	>30	>30	>30	40	30	31
15	0.03	0.07	0.03	0.03	0.3	0.2
CPT[b]	0.003	0.004	0.005	0.004	0.004	0.004
VP-16[b]	0.3	9.4	34	0.3	0.5	0.3
VM-26[b]	0.04	2.1	24	0.05	0.15	0.05
DOX[b]	0.03	0.3	1.2	0.04	0.06	0.04
VBS[b]	0.001	0.003	0.001	0.001	0.002	0.001

[a]IC_{50} has been calculated after 4 days of continuous drug exposure.
[b]CPT is camptothecin; VP-16 is etoposide; VM-26 is teniposide; DOX is doxorubicin; VBS is vinblastine.

Table 2. Relative cytotoxicities of dibenzo[*c*,*h*]cinnolines

Compd	RPMI	CPT-K5	Cytotoxicity IC_{50} (μM)[a]			
			U937	U937/CR	KB3-1	KBV-1
1	0.38	0.36	0.13	0.11	0.31	0.24
2a	4.5	10.8	2.9	5	5.5	1.8
2b	7.6	9.7	3.0	5.3	12	19
3 (Nitidine)	0.4	4.0	0.8	3.5	0.06	2.5
4	0.07	5.5	0.06	7.5	0.05	0.12
5	5.5	10	3.8	20	6.4	3.5
6	>30	>30	>30	>30	27	29
7	2.0	5	2.0	4.0	3.2	2.5
8	4.0	28	3	7	3.3	2.5
9	39	61	67	58	28	75
10	>100	>100	>100	>100	>100	>100
11	41	41	26	30	57	200
12	0.45	100	0.33	>10	0.39	0.64
13	>10	>10	>10	>10	>10	>10
14	10	>10	7	>10	10	>10
15	0.32	0.38	0.065	0.1	0.21	0.17
CPT	0.005	61	0.006	0.65	0.01	0.03
VM-26	0.22	0.28	0.03	0.01	0.02	2.3

[a]IC_{50} has been calculated after 4 days of continuous drug exposure.

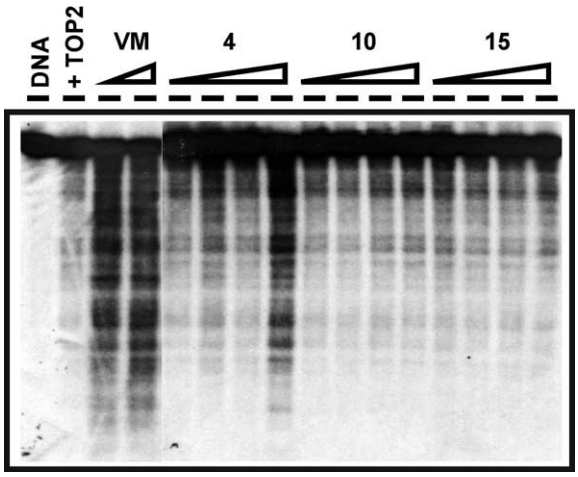


Figure 3. Stimulation of enzyme-mediated DNA cleavage by **4**, **10**, **15**, and VM-26 (VM) using human TOP2. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP2 and serially (10-fold each) diluted compound from 0.01 to 0.1 μM for VM and 0.1 to 100 for compounds **4**, **10** and **15**.

and TOP2, cross-resistance to these variant cell lines is minimal. While **4** did exhibit weak activity as a TOP2-targeting agent (≈0.5% of that of nitidine), both **4** and **15** did not exhibit any cross-resistance in these variant cell lines.

Compounds **1**–**6** and **15** were also evaluated together with camptothecin, VP-16 (etoposide), VM-26 (teniposide), doxorubicin, and vinblastine in several solid tumor cell lines. In the human tumor cell lines, HeLa (cervical), HCT-116 (colon), and breast (ZR-75-1), **4** exhibited significant cytotoxicity with IC_{50} values ranging from 30 to 40 nM. In the case of **15**, IC_{50} values for these cell lines ranged from 30 to 300 nM.

These data indicate that dibenzo[*c*,*h*]cinnolines can be developed that (1) target TOP1, (2) exhibit significant cytotoxic activity, and (3) are capable of overcoming MDR1-resistance. Compound **15** is a particularly interesting dibenzo[*c*,*h*]cinnoline as its hydroxymethyl moiety can serve as a synthetic handle for forming varied derivatives and prodrugs so as to alter its physicochemical properties. This could be of advantage in developing dibenzo[*c*,*h*]cinnolines to have properties that may improve solubility, facilitate formulation, and increase bioavailability.

Experimental

General

Column chromatography refers to flash chromatography conducted on Silitech 32–63 um, 60Å (ICN Biomedicals, Eschwege, Germany) using the eluting solvent systems as indicated. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were obtained on a Varian Gemini-200 Fourier Transform spectrometer. NMR spectra (200 MHz ^{1}H and 50 MHz ^{13}C) were recorded using deuterated chloroform as solvent, unless otherwise indicated. The chemical shifts were reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Melting points were determined with a Thomas-Hoover Unimelt capillary melting point apparatus. Infrared spectral data (IR) were obtained on a Perkin–Elmer 1600 Fourier transform spectrophotometer and are reported in cm^{-1}. Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry in the Department of Chemistry at Washington University, St. Louis, Mo. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone prior to use. Tetrakis(triphenylphosphine)palladium(0) was purchased from Aldrich Chemical Company (Milwaukee, WI) as bright yellow powder. 4-Methoxy-2-nitrobromobenzene, **36**, was purchased from Aldrich Chemical Company. The nitroarylstannanes, **21, 23**, and **35** were prepared as previously described.[27]

2,3-Dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (4). 6-(2-Amino-4,5-methylenedioxyphenyl)-2,3-dimethoxynaphthalene (**41**) (40 mg, 0.13 mmol) was dissolved in acetic acid (2 mL) and concentrated hydrochloric acid (0.3 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.09 g in 1.5 mL of water). The resulting diazonium solution was allowed to rise to room temperature slowly and left overnight. To the resulting red solution, which contained some precipitate, was added 50 mL of water. The mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with dilute sodium hydroxide solution, then with water and brine. The ethyl acetate extracts were dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 40:60 hexanes/ethyl acetate to give **4** (20 mg) in 48% yield; mp 292–294 °C; ^{1}H NMR δ 4.09 (3H, s), 4.22 (3H, s), 6.24 (2H, s), 7.31 (1H, s), 7.80 (1H, s), 7.95 (1H, s), 8.00 (1H, d, J = 9.2), 8.13 (1H, d, J = 8.9), 9.14 (1H, s); ^{13}C NMR δ 56.5, 56.9, 98.1, 102.9, 104.6, 107.5, 107.9, 117.1, 119.6, 120.6, 126.9, 128.5, 131.7, 141.9, 145.6, 150.2, 151.2, 152.1; HRMS (EI) calcd for $C_{19}H_{14}N_2O_4$ m/z: 334.0953; found: 334.0946.

2,3-Dimethoxydibenzo[*c*,*h*]cinnoline (5). 6-(2-Aminophenyl)-2,3-dimethoxynaphthalene (**20**) (70 mg, 0.25 mmol) was dissolved in 48% hydrobromic acid (4.25 mL), cooled in ice-salt bath, and treated dropwise with stirring with sodium nitrite (0.13 g) in water (2.2 mL). Stirring was continued for 0.5 h., and copper powder (0.5 g) was added to the cold solution with stirring. The mixture was allowed to rise slowly to room temperature and left overnight. The solid was filtered off and washed with hot chloroform. The chloroform solution was washed with dilute sodium hydroxide solution, water, dried (Na_2SO_4) and evaporated to give the crude product. Chromatography on silica gel using 50:50 hexanes/ethyl acetate afforded **5** (13 mg) in 18% yield; mp 201–203 °C; ^{1}H NMR δ 4.11 (3H, s), 4.24 (3H, s), 7.37 (1H, s), 7.89–7.94 (2H, m), 8.14 (1H, d, J = 8.9), 8.41 (1H, d, J = 8.8), 8.61–8.66 (1H, m), 8.75–8.80 (1H, m), 9.24 (1H, s); ^{13}C NMR δ 56.1, 56.4, 104.0, 107.3, 112.3,

116.5, 118.5, 121.7, 126.7, 128.6, 128.8, 131.1, 131.2, 131.9, 141.5, 146.3, 150.9, 151.0; HRMS (EI) calcd for $C_{18}H_{14}N_2O_2$ *m*/*z*: 290.1055; found: 290.1058.

2,3,8,9-Tetramethoxydibenzo[*c*,*h*]cinnoline (6). 6-(2-Amino-4,5-dimethoxyphenyl)-2,3-dimethoxynaphthalene (**30**) (11 mg, 0.033 mmol) was dissolved in acetic acid (0.6 mL) and concentrated hydrochloric acid (0.06 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.026 g in 0.5 mL of water). The resulting diazonium solution was allowed to rise to room temperature slowly and left overnight. To the resulting red solution, which contained some precipitate, was added 30 mL of water. The mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with 1.0 N sodium hydroxide first, water and brine. The ethyl acetate extracts were dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 40:60 chloroform/ethyl acetate to give the **6** (5 mg) in 44% yield; mp >360 °C (lit[26] >360 °C); ^{1}H NMR δ 4.09 (3H, s), 4.18 (6H, s), 4.23 (3H, s), 7.31 (1H, s), 7.74 (1H, s), 8.00 (1H, s), 8.01 (1H, d, *J*=8.5), 8.20 (1H, d, *J*=8.9), 9.15 (1H, s); ^{13}C NMR δ 56.5, 56.9, 99.9, 104.5, 107.9, 109.5, 116.9, 118.5, 118.9, 127.1, 128.4, 131.6, 141.8, 144.6, 151.1, 151.2, 152.0, 153.9; HRMS (EI) calcd for $C_{20}H_{18}N_2O_4$ *m*/*z*: 350.1267; found: 350.1266.

2,3,8-Trimethoxydibenzo[*c*,*h*]cinnoline (7). 6-(2-Amino-4-methoxyphenyl)-2,3-dimethoxynaphthalene (**31**) (12 mg, 0.039 mmol) was dissolved in acetic acid (0.6 mL) and concentrated hydrochloric acid (0.06 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.026 g in 0.5 mL of water). The resulting diazonium solution was allowed to rise to room temperature slowly and left overnight. To the resulting red solution which contained some precipitate was added 30 mL of water and the mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with dilute sodium hydroxide solution, water and brine. The ethyl acetate extracts were dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 40:60 hexanes/ethyl acetate to give 7 (5 mg) in 40% yield; mp 244–246 °C; ^{1}H NMR δ 4.10 (6H, s), 4.22 (3H, s), 7.32 (1H, s), 7.54 (1H, dd, *J*=9.1, 2.6), 8.04–8.08 (2H, m), 8.28 (1H, d, *J*=8.9), 8.49 (1H, d, *J*=9.1), 9.16 (1H, s); ^{13}C NMR δ 56.3, 56.5, 56.9, 104.4, 107.9, 108.9, 116.9, 117.1, 119.5, 123.5, 124.5, 127.1, 128.4, 132.5, 141.8, 148.5, 151.1, 151.4, 160.5; HRMS (EI) calcd for $C_{19}H_{16}N_2O_3$ *m*/*z*: 320.1161; found: 320.0384.

2,3,9-Trimethoxydibenzo[*c*,*h*]cinnoline (8). 6-(2-Amino-5-methoxyphenyl)-2,3-dimethoxynaphthalene (**32**) (60 mg, 0.20 mmol) was dissolved in acetic acid (1.5 mL) and concentrated hydrochloric acid (0.3 mL). The solution was cooled in an ice bath and diazotized by dropwise addition of a solution of sodium nitrite (0.12 g in 1.2 mL of water). The resulting diazonium solution was allowed to rise to room temperature slowly and left overnight. To the resulting red solution, which contained some precipitate, was added 50 mL of water. This mixture was then extracted with ethyl acetate (40 mL×3). The organic layers were combined and washed with dilute sodium hydroxide solution, water and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 35:65 hexanes/ethyl acetate to give **8** (16 mg) in 26% yield; mp 215–217 °C; ^{1}H NMR δ 4.07 (3H, s), 4.09 (3H, s), 4.22 (1H, s), 7.29 (1H, s), 7.46 (1H, dd, *J*=9.1, 2.6), 7.72 (1H, d, *J*=2.5), 7.99 (1H, d, *J*=8.9), 8.20 (1H, d, *J*=9.0), 8.60 (1H, d, *J*=9.1), 9.17 (1H, s); ^{13}C NMR δ 56.3, 56.5, 56.9, 100.3, 104.6, 107.7, 117.0, 118.7, 121.3, 124.3, 127.0, 129.1, 131.4, 133.3, 141.8, 143.7, 151.2, 151.3, 162.0; HRMS (EI) calcd for $C_{19}H_{16}N_2O_3$ *m*/*z*: 320.1161; found: 320.1144.

9-Benzyloxy-2,3,8-trimethoxydibenzo[*c*,*h*]cinnoline (9). 6-(2 - Amino - 5 - benzyloxy - 4 - methoxyphenyl) - 2,3 - dimethoxynaphthalene **33** (35 mg, 0.084 mmol) was dissolved in acetic acid (0.65 mL) and concentrated hydrochloric acid (0.13 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.052 g in 0.52 mL of water). The reaction mixture was allowed to warm slowly to room temperature and left for 24 h. To the resulting red solution containing some precipitate was added 50 mL of water and the mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with dilute sodium hydroxide solution, water and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 20:80 hexanes/ethyl acetate to give **9** (24 mg) in 67% yield; mp 244–246 °C; ^{1}H NMR δ 4.07 (3H, s), 4.14 (3H, s), 4.21 (3H, s), 5.40 (2H, s), 7.25 (1H, s), 7.37–7.60 (5H, m), 7.91 (1H, d, *J*=9.0), 7.97 (1H, s), 8.01 (1H, d, *J*=9.0), 9.11 (1H, s); ^{13}C NMR δ 56.5, 56.9, 56.9, 71.6, 101.7, 104.4, 107.8, 109.6, 116.8, 118.2, 118.8, 127.0, 128.0, 128.3, 128.9, 129.3, 131.5, 136.3, 141.6, 144.5, 151.0, 151.1, 152.3, 152.9; HRMS (EI) calcd for $C_{26}H_{22}N_2O_4$ *m*/*z*: 426.1580; found: 426.1577.

9-Hydroxy-2,3,8-trimethoxydibenzo[*c*,*h*]cinnoline (10). 9-Benzyloxy-2,3,8-trimethoxydibenzo[*c,h*]cinnoline, **9**, (5 mg, 0.012 mmol) was hydrogenated overnight in ethyl acetate (25 mL) at 26 lb/inch^2 using 10% palladium on carbon (1.5 mg). The solution was passed through a Celite 545 bed and the sicciate was washed with ethyl acetate (10 mL×3). Concentration of the ethyl acetate solution in vacuo gave the crude product. Chromatography using a 50:45:5 mixture of hexanes/ethyl acetate/methanol as eluting solvent gave compound **10** (3 mg) in 76% yield; ^{1}H NMR (DMSO-d_6) δ 4.00 (3H, s), 4.10 (3H, s), 4.12 (3H, s), 7.66 (1H, s), 8.02 (2H, s), 8.21 (1H, d, *J*=8.4), 8.38 (1H, d, *J*=8.9), 8.96 (1H, s); ^{13}C NMR (DMSO-d_6) δ 55.9, 56.4, 103.1, 103.8, 108.5, 109.1, 117.4, 117.8, 118.0, 125.7, 128.1, 131.3, 140.4, 143.8, 150.5, 150.7, 151.3, 152.4; HRMS (EI) calcd for $C_{19}H_{16}N_2O_4$ *m*/*z*: 336.111; found: 336.1109.

2-Methoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (11). 6-(2-Amino-4,5-methylenedioxyphenyl)-2-methoxynaphthalene **42** (170 mg, 0.58 mmol) was dissolved in acetic

acid (4.5 mL) and concentrated hydrochloric acid (0.9 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.36 g in 3.6 mL of water). The resulting diazonium solution was allowed to warm slowly to room temperature and left for 1 day. To the resulting red solution containing some precipitate was added 50 mL water and the mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with dilute sodium hydroxide, water and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 50:50 hexanes/ethyl acetate to give **11** (20 mg) in 11% yield; mp 258–260 °C; ^{1}H NMR δ 4.01 (3H, s), 6.23 (2H, s), 7.30 (1H, J=2.6), 7.48 (1H, dd, J=9.1, 2.6), 7.75 (1H, s), 7.95 (1H, s), 8.00 (1H, d, J=9.2), 8.19 (1H, d, J=9.1), 9.62 (1H, d, J=9.2); ^{13}C NMR δ 56.0, 98.0, 102.9, 107.5, 108.3, 119.1, 119.6, 119.7, 120.6, 125.9, 126.6, 132.3, 134.6, 142.5, 145.9, 150.2, 152.1, 160.2; HRMS (EI) calcd for $C_{18}H_{12}N_2O_3$ m/z: 304.0848; found: 304.0843.

3-Methoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (12). Method 1. 7-(2-Amino-4,5-methylenedioxyphenyl)-2-methoxynaphthalene **43** (70 mg, 0.24 mmol) was dissolved in acetic acid (2.0 mL) and concentrated hydrochloric acid (0.4 mL). The solution was cooled in an ice bath and diazotized by the dropwise addition of a solution of sodium nitrite (0.16 g in 1.6 mL of water). The resulting diazonium solution was allowed to warm slowly to room temperature and left overnight. To the resulting red solution containing some precipitate was added 50 mL of water and the reaction mixture was extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with dilute sodium hydroxide, water and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The crude product was purified by column chromatography on silica gel using 55:45 hexanes/ethyl acetate to give 60 mg of **12** in 83% yield;

Method 2. Lithium aluminum hydride (46 mg, 1.2 mmol) was added to a stirred solution of compound **53** (74 mg, 0.2 mmol) in diethyl ether (10 mL) and benzene (10 mL). The mixture was stirred under reflux for 1 h. After cooling to room temperature, the excess lithium aluminum hydride was decomposed by sequential addition of 0.05 mL water, 0.05 mL 15% NaOH and 0.15 mL water, and the reaction mixture filtered through a Celite 545 bed. Evaporation of solvent in vacuo gave the crude product, which was purified by column chromatography using 50:50 hexanes/ethyl acetate mixture as eluting solvent. The yield was 46 mg (75%); mp 259–261 °C; ^{1}H NMR δ 4.12 (3H, s), 6.24 (2H, s), 7.37 (1H, dd, J=8.8, J=2.7), 7.80 (1H, s), 7.88 (1H, d, J=8.8), 7.96 (1H, s), 8.03 (1H, d, J=9.1), 8.09 (1H, d, J=9.0), 9.15 (1H, d, J=2.7); ^{13}C NMR δ 56.3, 98.3, 102.9, 104.0, 107.5, 116.4, 120.4, 120.5, 121.1, 128.0, 130.0, 132.5, 133.3, 142.0, 146.1, 150.5, 152.1, 160.3; HRMS (EI) calcd for $C_{18}H_{12}N_2O_3$ m/z: 304.0848; found: 304.0852.

3-Benzyloxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (13). Lithium aluminium hydride (37 mg, 1.0 mmol) was added to a stirred solution of compound **54** (111 mg, 0.25 mmol) in dry THF (20 mL). The mixture was stirred at room temperature for 1 h. Excess LAH was decomposed by sequential addition of 0.5 mL of water and 0.5 mL of 10% NaOH and the reaction mixture was filtered through Celite 545. The filtrate was concentrated in vacuo. The residue was extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and concentrated in vacuo to provide 40 mg (42%) of **13** as a solid: mp 246–247 °C; ^{1}H NMR (DMSO-d_6) δ 5.46 (2H, s), 6.40 (2H, s), 7.36–7.62 (6H, m), 8.02 (1H s,), 8.12 (1H, d, J=8), 8.26 (1H, d, J=8), 8.38 (1H, s), 8.54 (1H, d, J=8.2), 9.07 (1H, d, J=2.2); ^{13}C NMR (DMSO-d_6) δ 70.4, 99.3, 103.7, 105.3, 106.5, 117.9, 120.3, 121.1, 128.1, 128.5, 128.6, 129.2, 130.9, 132.7, 137.6, 141.4, 146.2, 151.1, 152.6, 159.1.

3-Hydroxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (14). Sodium hydroxide (50 mg) and 10% Pd/C (10 mg) were added to a solution of **55** (50 mg, 0.12 mmol) in EtOH (30 mL). The reaction mixture was heated to reflux and then hydrazine hydrate (0.2 mL) was added. The reaction mixture was refluxed for 1 h, allowed to cool to room temperature, and the catalyst removed by filtration through Celite 545. The filtrate was concentrated in vacuo. Water was added and pH adjusted to 7 using concd HCl. A yellow solid was filtered, washed with H_2O and allowed to dry. The solid was washed with ether, and then chloroform to remove impurities, to provide 18 mg of **14** (50% yield); mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 6.37 (2H, s), 7.26 (1H, dd, J=2.5, 9), 7.90 (1H, s), 8.19 (1H, d, J=2.5), 8.26 (1H, d, J=9), 8.32 (1H, s), 10.22 (1H, s); ^{13}C NMR (DMSO-d_6) δ 99.4, 101.0, 104.4, 106.6, 107.5, 116.5, 117.2, 120.5, 127.8, 129.8, 130.7, 134.2, 138.3, 151.7, 153.5, 158.1, 172.2.

3-Hydroxymethyl-2-methoxy-8,9-methylenedioxydibenzo-[*c*,*h*]cinnoline (15). Compound **59** (65 mg, 0.2 mmol) was dissolved in a mixture of acetic acid (3 mL) and concd HCl (1 mL). This mixture was cooled to 10 °C. A solution of $NaNO_2$ (100 mg) in 2 mL of water was then added dropwise. After the addition was complete, the reaction was allowed to stir at room temperature for 24 h. Water was added to reaction mixture. The reaction mixture was neutralized with NaOH to get a brown solid. The precipitate was filtered and washed with water and allowed to dry. The solid was then washed with ether and then chloroform to remove any impurities, to provide 40 mg (59.7%) of **59**; mp 219–220 °C; ^{1}H NMR (DMSO-d_6) δ 4.01 (3H, s), 4.79 (2H, s), 6.39 (2H, s), 7.63 (1H, s), 8.01 (1H, s), 8.26 (1H, d, J=8), 8.35 (1H, s), 8.64 (1H, d, J=8.2), 9.6 (1H, s); ^{13}C NMR (DMSO-d_6) δ 56.4, 59.3, 99.0, 103.7, 106.2, 107.3, 119.3, 120.2, 120.7, 122.2, 124.8, 132.6, 133.7, 134.6, 142.0, 145.5, 150.8,152.7, 157.5.

6,7-Dimethoxy-1-hydroxytetralin (16). A solution of sodium borohydride (1 g, 26 mmol) in 10 mL of methanol (caution: in forming this solution, heat was generated and a significant amount of H_2 gas was generated) was added dropwise to a stirred solution of 6,7-dimethoxy-1-tetralone (1 g, 4.9 mmol) in 20 mL

methanol. This mixture was stirred for 10 min, at which time the reaction was complete as indicated by thin layer chromatography. After the solvent was evaporated, the residue was filtered through a very short column using hexanes/ethyl acetate (70:30) as eluting solvent. The eluent was concentrated in vacuo to give 1.0 g of 1-hydroxy-6,7-dimethoxytetralin **16** in 100% yield; mp 169–171 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–1.69 (2H, m), 1.83–1.89 (2H, m), 2.59–2.63 (2H, m), 3.71 (3H, s), 3.72 (3H, s), 4.46–4.51 (1H, m), 6.62 (1H, s), 6.95 (1H, s); ^{13}C NMR (DMSO-d_6) δ 19.5, 28.8, 32.7, 55.7, 66.3, 66.4, 111.7, 112.1, 128.7, 132.5, 147.1, 147.9.

1,2-Dihydro-6,7-dimethoxynaphthalene (17). 6,7-Dimethoxy-1-hydroxytetralin, **16**, (0.60 g, 2.9 mmol) was dissolved in 15 mL of *N*,*N*-dimethylformamide. To this solution was added orthophosphoric acid 0.25 mL (3.7 mmol). The reaction mixture was stirred at 60 °C for 20 min until the reaction was complete as indicated by thin layer chromatography. Ethyl acetate (50 mL) was added and the reaction mixture was washed with water (60 mL×3). The organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified by column chromatography using a 80:20 mixture of hexanes/ethyl acetate. The first eluting compound was the desired product **17**. This reaction provided 0.55 g (100% yield) of **17**; mp 35.5–36 °C; ^{1}H NMR δ 2.24–2.32 (2H, m), 2.72 (2H, t, J=8.3), 3.86 (3H, s), 3.87 (3H, s), 5.92 (1H, dt, J=9.6, 4.8), 6.38 (1H, d, J=9.6), 6.60 (1H, s), 6.66 (1H, s); ^{13}C NMR δ 23.7, 27.7, 56.5, 110.4, 112.0, 126.9, 127.4, 127.7, 128.4, 147.8, 148.1.

6-(2-Nitrophenyl)-7,8-dihydro-2,3-dimethoxynaphthalene (18). $Pd(PPh_3)_2Cl_2$ (840 mg, 1.2 mmol) and sodium acetate (200 mg, 2.4 mmol) were added to a solution of 1,2-dihydro-6,7-dimethoxynaphthalene, **17**, (700 mg, 3.7 mmol) and 1-iodo-2-nitrobenzene (925 mg, 3.7 mmol) in *N*,*N*-dimethylacetamide (50 mL). The mixture was stirred under nitrogen at 140 °C overnight, and then concentrated in vacuo. Ethyl acetate (60 mL) was added to the residue and washed with distilled water (50 mL). The organic layer was separated and filtered through a Celite 545. The organic layer was then washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed to give **18** (330 mg) in 29% yield; mp 139–141 °C; ^{1}H NMR δ 2.51 (2H, t, J=8.1), 2.92 (2H, t, J=8.1), 3.87 (3H, s), 3.90 (3H, s), 6.45 (1H, s), 6.67 (1H, s), 6.73 (1H, s), 7.38–7.45 (2H, m), 7.54–7.62 (1H, m), 7.88–7.93 (1H, m); ^{13}C NMR δ 28.5, 28.5, 56.6, 111.0, 111.7, 124.9, 127.0, 127.1, 128.1, 128.3, 131.3, 133.3, 135.7, 138.7, 148.0, 148.9; HRMS (EI) calcd for $C_{18}H_{17}NO_4$ m/z: 311.1157; found: 311.1145.

6-(2-Nitrophenyl)-2,3-dimethoxynaphthalene (19). 6-(2-Nitrophenyl)-7,8-dihydro-2,3-dimethoxynaphthalene (**18**) (100 mg, 0.32 mmol) was refluxed overnight in toluene (20 mL) with DDQ (109 mg, 0.48 mmol). The reaction mixture was allowed to cool to room temperature and was then filtered through a Celite 545 bed. The filtrate was evaporated to dryness to give the crude product and chromatographed on silica gel using 80:20 hexanes/ethyl acetate to afford **19** (90 mg) in 91% yield; mp 139–141 °C; ^{1}H NMR δ 4.00 (3H, s), 4.01 (3H, s), 7.12 (1H, s), 7.14 (1H, s), 7.27 (1H, dd, J=8.4, 1.7), 7.46–7.62 (3H, m), 7.66 (1H, s), 7.72 (1H, d, J=8.4), 7.86 (1H, d, J=8.1); ^{13}C NMR δ 56.4, 106.6, 107.1, 124.5, 124.6, 125.9, 127.3, 128.4, 129.3, 129.6, 132.7, 133.6, 137.0, 149.9, 150.5, 150.6; HRMS (EI) calcd for $C_{18}H_{15}NO_4$ m/z: 309.1001; found: 309.0999.

6-(2-Aminophenyl)-2,3-dimethoxynaphthalene (20). 6-(2-Nitrophenyl)-2,3-dimethoxynaphthalene (**19**) (70 mg, 0.23 mmol) in ethyl acetate (45 mL) was hydrogenated overnight at 40–45 lb/inch^2 using 10% palladium on carbon (20 mg). The solution was passed through a Celite 545 bed and the catalyst was washed with ethyl acetate (3×10 mL). Concentration in vacuo gave **20** (60 mg) in 99% yield; mp 145–147 °C; ^{1}H NMR δ 4.02 (3H, s), 4.04 (3H, s), 6.82 (1H, d, J=8.0), 6.85–6.93 (1H, m), 7.16 (1H, s), 7.18 (1H, s), 7.20–7.26 (2H, m), 7.47 (1H, dd, J=8.3, J=1.6), 7.78 (1H, d, J=8.8), 7.80 (1H, s); ^{13}C NMR δ 56.4, 106.7, 106.9, 116.1, 119.2, 126.1, 126.9, 127.3, 128.3, 128.7, 128.9, 129.9, 131.2, 135.8, 144.3, 150.2, 150.3; HRMS (EI) calcd for $C_{18}H_{17}NO_2$ m/z: 279.1259; found: 279.1267.

Trimethyl(5-methoxy-2-nitrophenyl)stannane (22). A mixture of hexamethylditin (2.0 g, 6.13 mmol), 3-methoxy-6-nitrobromobenzene (0.70 g, 3.0 mmol) and $Pd(PPh_3)_4$ (100 mg) in anhydrous THF (20 mL) was heated to reflux under nitrogen until thin layer chromatography no longer showed the presence of starting material. After cooling to room temperature, THF was evaporated and methylene chloride was added to the residue. To this mixture, aqueous potassium fluoride (7.0 M, 1.5 mL) was added dropwise with vigorous stirring. The mixture was passed through a Celite 545 bed and the filtrate washed with brine. The methylene chloride layer was dried (Na_2SO_4), filtered and the solution concentrated in vacuo. The residue was chromatographed using a 125:2 mixture of hexanes/ethyl acetate to give **22** (200 mg) in 21% yield; ^{1}H NMR δ 0.34 (9H, s), 3.91 (3H, s), 6.92 (1H, dd, J=9.1, 2.7), 7.13 (1H, d, J=2.8), 8.33 (1H, d, J=9.1); ^{13}C NMR δ-7.1, 56.2, 114.2, 122.4, 127.0, 143.6, 146.8, 164.1.

3-Methoxy-6-nitrobromobenzene. Nitric acid (70%, 5 mL) was placed in a 25 mL round-bottomed flask. Concentrated sulphuric acid (4 mL) was then added dropwise with stirring. The mixture was kept cool during the addition by immersing the flask in an ice bath. 3-Methoxybromobenzene (4 g, 21.5 mmol) was then introduced dropwise. The reaction mixture was then heated to 50 °C and stirred for 5 h. After cooling, the mixture was poured into 100 mL of cold water and extracted with ethyl acetate (30 mL×3). The organic layers were combined and washed with water (50 mL×4) and brine. The ethyl acetate layer was dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using 95:5 hexanes/ethyl acetate. The first compound that eluted from the column was 3-methoxy-4-nitrobromobenzene (1.2 g) in 24% yield; ^{1}H NMR δ 3.96 (3H, s), 7.17 (1H, dd, J=8.6, 1.9), 7.24 (1H, d, J=1.9), 7.75 (1H, d, J=8.6); ^{13}C NMR δ 57.3, 117.6, 124.0,

127.4, 129.1, 142.3, 154.0. The second compound eluting from the column was the desired 3-methoxy-6-nitrobromobenzene (1.5 g, 30% yield); mp 43–45 °C; ^{1}H NMR δ 3.89 (3H, s), 6.91 (1H, dd, J = 9.1, 2.7), 7.21 (1H, d, J = 2.7), 7.98 (1H, d, J = 9.1); ^{13}C NMR δ 56.7, 114.0, 117.3, 120.6, 128.5, 163.2.

Trimethyl(3-benzyloxy-4-methoxy-6-nitrophenyl)stannane (24). A mixture of hexamethylditin (2 g, 6.13 mmol), 3-benzyloxy-4-methoxy-6-nitro-bromobenzene (1.4 g, 4.14 mmol) and $Pd(PPh_3)_4$ (200 mg) in anhydrous THF (40 mL) was heated to reflux under nitrogen for 2 days. After cooling to room temperature, THF was evaporated and methylene chloride was added to the residue. To this mixture, aqueous potassium fluoride (7.0 M, 1.5 mL) was added dropwise with vigorous stirring. The mixture was passed through a Celite 545 bed and the filtrate washed with brine. The methylene chloride layer was dried (Na_2SO_4), filtered and evaporated in vacuo. The residue was chromatographed using a 90:10 mixture of hexanes/ethyl acetate to give **24** (1.16 g) in 66% yield; mp 81–83 °C; ^{1}H NMR δ 0.27 (9H, s), 3.97 (3H, s), 5.29 (2H, s), 7.04 (1H, s), 7.36–7.44 (5H, m), 7.91 (1H, s); ^{13}C NMR δ −7.2, 56.8, 71.6, 108.0, 119.6, 127.8, 128.9, 129.3, 132.6, 136.4, 146.9, 150.2, 153.3; HRMS (EI) calcd for $C_{17}H_{21}NO_4Sn$ m/z: 408.0258; found: 408.0243.

3-Benzyloxy-4-methoxy-6-nitrobromobenzene. 3-Benzyloxy-1-bromo-4-methoxybenzene (1 g, 3.4 mmol) was dissolved in 50 mL acetic acid in a 100 mL round-bottomed flask and cooled to 0 °C using an ice bath. 2.5 mL of nitric acid (70%) in 6 mL of acetic acid was added dropwise. The reaction mixture was allowed to slowly rise to room temperature. After 3 h no starting material was detected by thin layer chromatography. Evaporation of acetic acid gave the crude product, which was filtered through a short silica gel column using a 80:20 mixture of hexanes/ethyl acetate to give 3-benzyloxy-4-methoxy-6-nitrobromobenzene (1.15 g) in quantitative yield; mp 134–135 °C; ^{1}H NMR δ 3.93 (3H, s), 5.19 (2H. s), 7.17 (1H, s), 7.38–7.45 (5H, m), 7.57 (1H, s); ^{13}C NMR δ 57.0, 72.0, 107.7, 109.7, 118.7, 128., 129.1, 129.4, 135.5, 142.4, 149.2, 152.4; HRMS (EI) calcd for $C_{14}H_{12}NO_4Br$ m/z: 336.9950; found: 336.9941.

3-Benzyloxy-1-bromo-4-methoxybenzene. A solution of 5-bromo-2-methoxyphenol (2.0 g, 10 mmol) and α-bromotoluene (2.6 g, 15.3 mmol) in CH_3CN (30 mL) and acetone (25 mL) was treated with potassium carbonate (2.1 g, 15.2 mmol). The resulting mixture was heated to reflux under nitrogen for 18 h. After cooling to room temperature, the reaction mixture was filtered through a Celite 545 bed. The acetone was removed in vacuo and 50 mL ethyl acetate was added to the residue. The ethyl acetate solution was washed with water, brine, dried (Na_2SO_4), and then evaporated in vacuo. The residue was chromatographed using a 90:10 mixture of hexanes/ethyl acetate to give 3-benzyloxy-1-bromo-4-methoxybenzene (2.77 g) in 96% yield; mp 70–71 °C; ^{1}H NMR δ 3.86 (3H, s), 5.12 (2H. s), 7.77 (1H, J = 9.2), 7.04–7.08 (2H, m), 7.33–7.48 (5H, m); ^{13}C NMR δ 56.7, 71.7, 113.0, 113.5, 117.7, 124.5, 127.9, 128.6, 129.1, 136.9, 149.5, 149.5; HRMS (EI) calcd for $C_{14}H_{13}O_2Br$ m/z: 292.0099; found: 292.0085.

5-Bromo-2-methoxyphenol. To a solution of 5-bromo-2-methoxybenzaldehyde (2.4 g, 11.2 mmol) in 50 mL CH_2Cl_2, *m*-chloroperoxybenzoic acid (70–75%, 7.0 g, containing 28.4 mmol *m*-CPBA) was added and the mixture was stirred at ambient temperature for 2 days. The reaction was quenched with aqueous saturated $NaHCO_3$ solution and extracted with ethyl acetate (50 mL×3). The organic extract was dried (Na_2SO_4) and filtered through silica gel. Evaporation of the solvent gave the product (2.1 g) in 92% yield; mp 62–64 °C; ^{1}H NMR δ 3.87 (3H, s), 6.71 (1H, d, J = 8.6), 6.97 (1H, dd, J_1 = 8.6, J_2 = 2.4), 7.07 (1H, d, J = 2.4); ^{13}C NMR δ 56.6, 112.4, 113.8, 118.3, 123.3, 146.4, 147.0.

6,7-Dimethoxy-2-trifluoromethanesulfonyloxynaphthalene (25). 3,4-Dihydro-6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene (200 mg, 0.59 mmol) was refluxed overnight in toluene (30 mL) with DDQ (166 mg, 0.73 mmol). This reaction mixture was allowed to cool to room temperature and then filtered through Celite 545. The filtrate was concentrated in vacuo to give the crude product. Chromatography on silica gel using a 80:20 hexanes/ethyl acetate afforded **25** (190 mg) in 95% yield; ^{1}H NMR δ 4.00 (6H, s), 7.10 (1H, s), 7.12 (1H, s), 7.21 (1H, dd, J = 8.9, J = 2.5), 7.58 (1H, d, J = 2.5), 7.71 (1H, d, J = 8.9); ^{13}C NMR δ 56.4, 106.6, 106.6, 109.7, 116.1, 117.9, 118.2, 122.5, 128.9, 129.0, 129.8, 146.6, 150.9, 151.3; HRMS (EI) calcd for $C_{13}H_{11}O_5F_3S$ m/z: 336.0279; found: 336.0288.

3,4-Dihydro-6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene. A solution of 6,7-dimethoxy-2-tetralone (250 mg, 1.2 mmol) in THF (5 mL) was added to a suspension of sodium hydride (60 wt% in mineral oil, 75 mg, 1.9 mmol) in THF (10 mL) cooled by ice bath. This mixture was stirred for 0.5 h. A solution of *N*-phenyltrifluoromethanesulfonimide (500 mg, 1.4 mmol) in THF (5 mL) was then added, and the reaction stirred at 0 °C for 9 h. After concentration in vacuo, the residue was chromatographed using 80:20 hexanes/ethyl acetate to give 3,4-dihydro-6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene (300 mg) in 73% yield as a pale yellow liquid at room temperature; ^{1}H NMR δ 2.66 (2H, t, J = 8.5), 3.00 (2H, t, J = 8.4), 3.86 (3H, s), 3.88 (3H, s), 6.40 (1H, s), 6.62 (1H, s), 6.68 (1H, s); ^{13}C NMR δ 27.1, 28.9, 56.5, 111.3, 111.7, 115.9, 118.7, 122.3, 124.0, 126.0, 148.2, 148.9, 149.3; HRMS (EI) calcd for $C_{13}H_{13}O_5F_3S$ m/z: 338.0436; found: 338.0453.

6-(4,5-Dimethoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene (26). Tetrakis(triphenylphosphine)palladium (0) (80 mg) and cuprous bromide (16 mg) were added to a solution of 6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene **25** (220 mg, 0.66 mmol) and trimethyl(nitroaryl)stannane **21** (220 mg, 0.64 mmol) in THF (25 mL) at room temperature and stirred for 0.5 h. The mixture was then refluxed under N_2 overnight. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was

washed with water. The organic layer was separated and passed through a Celite 545 bed to remove suspended particles. The organic layer was then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 60:40 mixture of hexanes/ethyl acetate to give **26** (120 mg) in 54% yield; mp 190–192 °C (lit[26] 189–190); ^{1}H NMR δ 3.95 (3H, s), 3.99 (6H, s), 4.01 (3H, s), 6.86 (1H, s), 7.12 (1H, s), 7.14 (1H, s), 7.23 (1H, dd, J = 8.4, 1.8), 7.56 (1H, s), 7.61 (1H, d, J = 1.7), 7.70 (1H, d, J = 8.4); ^{13}C NMR δ 56.4, 56.9, 106.7, 107.0, 108.3, 114.4, 124.9, 125.7, 127.0, 129.0, 129.5, 132.2, 134.6, 141.6, 148.4, 150.4, 152.7.

6-(5-Methoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene (27). Tetrakis(triphenylphosphine)palladium (0) (80 mg) and cuprous bromide (6 mg) were added to a solution of 6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene **25** (200 mg, 0.60 mmol) and trimethyl(nitroaryl)stannane **22** (200 mg, 0.64 mmol) in THF (25 mL) at room temperature and the mixture was stirred for 0.5 h. The mixture was then refluxed under N_2 overnight. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was washed with water. The organic layer was separated and passed through a Celite 545 bed to remove suspended particles. The organic layer was then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 75:25 mixture of hexanes/ethyl acetate to give a mixture of two compounds with similar R_f values. This mixture was used for the next step without further purification.

6-(4-Methoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene (28). Tetrakis(triphenylphosphine)palladium (0) (60 mg) and cuprous bromide (10 mg) were added to a solution of 6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene 25 (150 mg, 0.45 mmol) and trimethyl(nitroaryl)stannane **23** (140 mg, 0.45 mmol) in THF (20 mL) at room temperature and the mixture was stirred for 0.5 h. The mixture was then refluxed under N_2 for 36 h. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was washed with water. The organic layer was separated and passed through a Celite 545 bed to remove suspended particles. The organic layer was then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 70:30 mixture of hexanes/ethyl acetate to give **28** (60 mg) in 43% yield; ^{1}H NMR δ 3.92 (3H, s), 4.01 (3H, s), 4.02 (3H, s), 7.12–7.26 (4H, m), 7.39–7.46 (2H, m), 7.61 (1H, d, J = 1.7), 7.71 (1H, d, J = 8.4); ^{13}C NMR δ 56.4, 106.6, 107.0, 109.5, 119.2, 124.8, 125.9, 127.2, 129.0, 129.4, 129.6, 133.5, 150.3, 150.4, 159.5; HRMS (EI) calcd for $C_{19}H_{17}NO_5$ m/z: 339.1107; found: 339.1108.

6-(5-Benzyloxy-4-methoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene (29). Tetrakis(triphenylphosphine)palladium (0) (200 mg) and cuprous bromide (20 mg) were added to a solution of 6,7-dimethoxy-2-trifluoromethanesulfonyloxynaphthalene **25** (500 mg, 1.49 mmol) and trimethyl(nitroaryl)stannane **24** (950 mg, 2.25 mmol) in THF (40 mL) at room temperature and the mixture was stirred for 0.5 h. The mixture was then refluxed under N_2 for 2 days. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was washed with water. The organic layer was separated and passed through a Celite 545 bed to remove suspended particles. The organic layer was then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 70:30 mixture of hexanes/ethyl acetate to give **29** (230 mg) in 35% yield; mp 151–153 °C; ^{1}H NMR δ 3.98 (3H, s), 3.99 (3H, s), 4.01 (3H, s), 5.20 (2H. s), 6.94 (1H, s), 7.10 (1H, s), 7.14 (1H, s), 7.18 (1H, dd, J = 8.4, 1.8), 7.36–7.43 (5H, m), 7.54 (1H, d, J = 1.5), 7.56 (1H, s), 7.68 (1H, d, J = 8.3); ^{13}C NMR δ 56.4, 57.0, 71.7, 106.7, 107.0, 108.7, 116.4, 124.9, 125.8, 127.0, 128.0, 128.9, 129.0, 129.2, 129.5, 131.8, 134.5, 136.2, 141.9, 149.0, 150.4, 151.9; HRMS (EI) calcd for $C_{26}H_{23}NO_6$ m/z: 445.1525; found: 445.1355.

6-(2-Amino-4,5-dimethoxyphenyl)-2,3-dimethoxynaphthalene (30). 6-(4,5-Dimethoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene **26** (12 mg, 0.033 mmol) was hydrogenated overnight in ethyl acetate (20 mL) at 40∼45 lb/inch^2 using 10% palladium on carbon (10 mg) as catalyst. The reaction solution was passed through a Celite 545 bed and the catalyst was washed with ethyl acetate (10 mL×3). Concentration of the solution in vacuo gave **30** (11 mg) in 100% yield; mp 200–202 °C (lit[26] 201–202 °C); ^{1}H NMR δ 3.84 (3H, s), 3.89 (3H, s), 4.01 (3H, s), 4.02 (3H, s), 6.41 (1H, s), 6.80 (1H, s), 7.14 (1H, s), 7.15 (1H, s), 7.43 (1H, dd, J = 8.4, J = 1.6), 7.75 (1H, d, J = 1.5), 7.75 (1H, d, J = 8.4); ^{13}C NMR δ 56.4, 57.2, 101.3, 106.6, 106.8, 115.2, 119.9, 126.2, 126.8, 127.3, 128.5, 129.9, 135.7, 138.0, 142.7, 149.8, 150.1, 150.3.

6-(2-Amino-5-methoxyphenyl)-2,3-dimethoxynaphthalene (31). Crude 6-(5-methoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene **27** (100 mg, approximately 90%) was hydrogenated overnight in ethyl acetate (40 mL) at 40∼45 lb/inch^2 using 10% palladium on carbon (30 mg) as catalyst. The reaction solution was passed through a Celite 545 bed and the catalyst was washed with ethyl acetate (10 mL×3). Concentration in vacuo gave the crude product. The residue was chromatographed using a 50:50 mixture of hexanes/ethyl acetate to give **31** (66 mg); mp 158–160 °C; ^{1}H NMR δ 3.57 (2H, s), 3.79 (3H, s), 4.01 (3H, s), 4.03 (3H, s), 6.77–6.84 (3H, m), 7.15 (1H, s), 7.16 (1H, s), 7.45 (1H, dd, J = 8.3, 1.8), 7.75–7.79 (2H, m); ^{13}C NMR δ 56.3, 56.4, 106.6, 106.9, 114.8, 116.4, 117.4, 126.0, 126.8, 127.3, 128.7, 129.4, 129.8, 135.8, 137.9, 150.2, 150.3, 153.6; HRMS (EI) calcd for $C_{19}H_{19}NO_3$ m/z: 309.1365; found: 309.1375.

6-(2-Amino-4-methoxyphenyl)-2,3-dimethoxynaphthalene (32). 6-(4-Methoxy-2-nitrophenyl)-2,3-dimethoxynaphthalene **28** (18 mg, 0.053 mmol) was hydrogenated overnight in ethyl acetate (20 mL) at 40∼45 lb/inch^2 using 10% palladium on carbon (10 mg) as catalyst. The reaction solution was filtered through Celite 545 and the sicciate was washed with ethyl acetate (10 mL×3). Concentration in vacuo gave the crude product. The residue was chromatographed using a 60:40 mixture of hexanes/ethyl acetate to give **32** (14 mg) in 85% yield; ^{1}H NMR δ 3.83 (3H, s), 4.01 (3H, s), 4.03 (3H, s), 6.37

(1H, d, $J=2.5$), 6.45 (1H, dd, $J=8.3$, 2.4), 7.12–7.16 (3H, m), 7.42 (1H, dd, $J=8.4$, 1.7), 7.72–7.77 (2H, m); ^{13}C NMR δ 55.7, 56.4, 101.6, 104.8, 106.6, 106.8, 121.5, 126.4, 126.8, 127.2, 128.5, 129.9, 132.0, 135.6, 145.3, 150.0, 150.3, 160.5; HRMS (EI) calcd for $C_{19}H_{19}NO_3$ m/z: 309.1365; found: 309.1355.

6-(2-Amino-5-benzyloxy-4-methoxyphenyl)-2,3-dimethoxynaphthalene (33) and 6-(2-amino-5-hydroxy-4-methoxyphenyl)-2,3-dimethoxynaphthalene (34). Compound **29** (50 mg, 0.112 mmol) was hydrogenated in ethyl acetate (40 mL) at 30 lb/inch^2 using 10% palladium on carbon (15 mg) as catalyst for 16 h. The solution was passed through a Celite 545 bed and the catalyst was washed with ethyl acetate (10 mL×3). Concentration of the ethyl acetate solution in vacuo gave the crude product. Column chromatography was performed using a 35:65 mixture of hexanes/ethyl acetate as eluting solvent to give compounds **33** and **34**.

6-(2-Amino-5-benzyloxy-4-methoxyphenyl)-2,3-dimethoxynaphthalene (33). The higher R_f material on thin layer chromatography proved to be compound **33**. The yield was 34 mg (73%); ^{1}H NMR δ 3.90 (3H, s), 4.01 (3H, s), 4.02 (3H, s), 5.08 (2H, s), 6.42 (1H, s), 6.87 (1H, s), 7.12 (1H, s), 7.15 (1H, s), 7.33–7.48 (6H, m), 7.71–7.75 (2H, m); ^{13}C NMR δ 56.4, 56.4, 56.5, 73.1, 101.5, 106.6, 106.8, 119.1, 120.0, 126.2, 126.8, 127.3, 128.2, 128.2, 128.5, 128.9, 129.9, 135.6, 138.2, 138.9, 141.7, 150.1, 150.3, 150.9; HRMS (EI) calcd for $C_{26}H_{25}NO_4$ m/z: 415.1784; found: 415.1775.

6-(2-Amino-5-hydroxy-4-methoxyphenyl)-2,3-dimethoxynaphthalene (34). The lower R_f material on thin layer chromatography proved to be compound **34**. The yield was 6 mg (16%); IR (KBr) 3432, 2937, 2364, 1625, 1508, 1459, 1252, 1232 cm^{-1}; ^{1}H NMR δ 3.88 (3H, s), 4.01 (3H, s), 4.02 (3H, s), 6.40 (1H, s), 6.85 (1H, s), 7.12 (1H, s), 7.15 (1H, s), 7.41 (1H, dd, $J=8.4$, 1.7); 7.73 (1H, d, $J=1.5$), 7.74 (1H, d, $J=8.3$); ^{13}C NMR δ 56.4, 100.5, 106.6, 106.8, 116.9, 121.1, 126.3, 126.8, 127.3, 128.5, 129.9, 135.5, 137.2, 139.1, 147.1, 150.1, 150.3.

7-Methoxy-2-trifluoromethanesulfonyloxynaphthalene (37). A solution of 7-methoxy-2-naphthol (0.75 g, 4.3 mmol) in THF (10 mL) was added to a suspension of sodium hydride (60 wt%, 205 mg, 5.1 mmol) in THF (10 mL) cooled by ice bath and stirred for 1.5 h. A solution of *N*-phenyltrifluoromethanesulfonimide (1.55 g, 4.34 mmol) in THF (10 mL) was then added, and the reaction mixture was stirred for 9 h. After evaporation of the solvent in vacuo, the residue was mixed with silica gel (4 g) and then chromatographed using 500:18 hexanes/ethyl acetate to give **37** (1.19 g) in 90% yield; mp 34 °C (lit[31] 34 °C); ^{1}H NMR 3.93 (3H, s), 7.13–7.25 (3H, m), 7.65 (1H, d, $J=2.5$), 7.77 (1H, d, $J=9.1$), 7.83 (1H, d, $J=8.8$); ^{13}C NMR δ 55.9, 106.3, 116.1, 117.5, 118.5, 120.8, 122.5, 128.3, 129.9, 130.7, 135.4, 148.3, 159.4; HRMS (EI) calcd for $C_{12}H_9SO_4F_3$ m/z: 306.0174; found: 306.0176.

6-(4,5-Methylenedioxy-2-nitrophenyl)-2,3-dimethoxynaphthalene (38). Tetrakis(triphenylphosphine)palladium(0) (126 mg) and cuprous bromide (21 mg) were added to a solution of **25** (500 mg, 1.5 mmol) and the trimethyl(nitroaryl)stannane **35**, (0.6 g, 1.83 mmol) in THF (30 mL) at room temperature and stirred for 0.5 h. The mixture was then refluxed under N_2 overnight. After cooling, THF was evaporated and ethyl acetate was added to the residue. The solution was washed with water. The organic layer was filtered through Celite 545 and then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 70:30 mixture of hexanes/ethyl acetate to give **38** in 42% yield; ^{1}H NMR δ 3.92 (3H, s), 6.19 (2H, s), 6.76 (1H, s), 7.12 (1H, d, $J=2.5$), 7.18 (1H, d, $J=8.3$), 7.28 (1H, dd, $J=9.0$, 2.3), 7.70 (1H, s), 7.85 (1H, d, $J=9.2$), 7.93 (1H, d, $J=8.4$); ^{13}C NMR δ 56.08, 100.59, 103.93, 106.31, 110.70, 121.54, 124.07, 126.47, 128.71, 129.55, 130.33, 130.99, 131.23, 142.83, 148.92, 152.07, 160.68.

6-(4,5-Methylenedioxy-2-nitrophenyl)-2-methoxynaphthalene (39). Tetrakis(triphenylphosphine)palladium(0) (120 mg) and cuprous bromide (20 mg) were added to a solution of 2-bromo-6-methoxynaphthalene (0.3 g, 1.27 mmol) and trimethyl(3,4-methylenedioxy-6-nitrophenyl)stannane **35** (0.45 g, 1.37 mmol) in THF (30 mL) at room temperature and the mixture stirred for 0.5 h. The mixture was then refluxed under N_2 for 16 h. After cooling, THF was evaporated and 50 mL ethyl acetate was added to the residue. The solution was washed with water, filtered through Celite 545, then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 80:20 mixture of hexanes/ethyl acetate to give the desired product **39** (0.29 g) in 71% yield; mp 165–167 °C; ^{1}H NMR δ 3.94 (3H, s), 6.14 (2H, s), 6.88 (1H, s), 7.16–7.21 (2H, m), 7.31 (1H, dd, $J=8.5$, 1.9), 7.47 (1H, s), 7.67–7.77 (3H, m); ^{13}C NMR δ 55.9, 103.5, 105.9, 106.2, 111.7, 119.9, 126.9, 127.0, 127.6, 129.2, 130.1, 133.8, 133.9, 134.5, 143.4, 147.5, 151.5, 158.6; HRMS (EI) calcd for $C_{18}H_{13}NO_5$ m/z: 323.0794; found: 323.0788.

7-(4,5-Methylenedioxy-2-nitrophenyl)-3-methoxynaphthalene (40). Tetrakis(triphenylphosphine)palladium(0) (120 mg) and cuprous bromide (20 mg) were added to a solution of 7-methoxy-2-trifluoromethanesulfonyloxynaphthalene **37** (336 mg, 1.1 mmol) and trimethyl(nitroaryl)stannane **35** (300 mg, 0.92 mmol) in THF (30 mL) at room temperature and the mixture was stirred for 0.5 h. The mixture was then refluxed under N_2 overnight. After cooling, THF was evaporated in vacuo and ethyl acetate (30 mL) was added to the residue. The solution was washed with water. The organic layer was filtered through Celite 545 bed, then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 80:20 mixture of hexanes/ethyl acetate to give **40** (100 mg) in 34% yield; ^{1}H NMR δ 3.92 (3H, s), 6.15 (2H, s), 6.88 (1H, s), 7.13–7.23 (3H, m), 7.48 (1H, s), 7.64 (1H, s), 7.74–7.81 (2H, m); ^{13}C NMR δ 55.8, 103.5, 105.9, 106.5, 111.6, 119.8, 124.1, 126.0, 128.5, 128.7, 129.8, 133.9, 135.0, 136.6, 143.4, 147.6, 151.5, 158.6; HRMS (EI) calcd for $C_{18}H_{13}NO_5$ m/z: 323.0794; found: 323.0787.

6-(2-Amino-4,5-methylenedioxyphenyl)-2,3-dimethoxynaphthalene (41). 6-(4,5-Methylenedioxy-2-nitrophe-

nyl)-2,3-dimethoxynaphthalene (38) (25 mg) was hydrogenated overnight in ethyl acetate (40 mL) at 40–45 lb/inch^2 under palladium catalysis (10 wt % on activated carbon, 20 mg). The solution was passed through a Celite 545 bed and the catalyst was washed with ethyl acetate (3×10 mL). Concentration in vacuo gave the crude product. Chromatography using 60:40 hexanes/ethyl acetate gave **41** (15 mg) in 66% yield; ^{1}H NMR δ 4.01 (3H, s), 4.02 (3H, s), 5.91 (2H, s), 6.40 (1H, s), 6.73 (1H, s), 7.13 (1H, s), 7.15 (1H, s), 7.39 (1H, dd, J=8.2, 1.8), 7.72 (1H, s), 7.74 (1H, d, J=8.5); ^{13}C NMR δ 56.4, 98.3, 101.2, 106.6, 106.8, 110.7, 120.5, 126.3, 127.0, 127.3, 128.5, 129.9, 135.7, 138.9, 141.1, 148.0, 150.1, 150.3; HRMS calcd for $C_{19}H_{17}NO_4$: 323.1157; found: 323.1170.

6-(2-Amino-4,5-methylenedioxyphenyl)-2-methoxynaphthalene (42). 6-(4,5-Methylenedioxy-2-nitrophenyl)-2-methoxynaphthalene **39** (260 mg, 0.81 mmol) was hydrogenated overnight in ethyl acetate (35 mL) at 40–45 lb/inch^2 using 10% palladium on carbon (70 mg) as catalyst. The reaction solution was filtered through Celite 545 and the sicciate was washed with ethyl acetate (10 mL×3). The filtrate was concentrated and the residue chromatographed using a 3:1 mixture of hexanes/ethyl acetate to give **42** (180 mg) in 76% yield; mp 130–132 °C; ^{1}H NMR δ 3.56 (2H, s), 3.95 (3H, s), 5.92 (2H, s), 6.40 (1H, s), 6.75 (1H, s), 7.17–7.22 (2H, m), 7.51 (1H, dd, J=8.5, 1.6), 7.73–7.82 (3H, m); ^{13}C NMR δ 55.8, 98.3, 101.3, 106.1, 110.7, 119.7, 120.3, 127.8, 128.3, 128.6, 129.6, 129.9, 134.0, 135.1, 139.0, 141.2, 148.1, 158.3; HRMS (EI) calcd for $C_{18}H_{15}NO_3$ m/z: 293.1052; found: 293.1051.

7-(2-Amino-4,5-methylenedioxyphenyl)-2-methoxynaphthalene (43). 7-(4,5-Methylenedioxy-2-nitrophenyl)-2-methoxynaphthalene **40** (100 mg, 0.31 mmol) was hydrogenated overnight in ethyl acetate (35 mL) at 40–45 lb/inch^2 using 10% palladium on carbon (30 mg) as catalyst. The reaction solution was filtered through Celite 545 and the sicciate washed with ethyl acetate (10 mL×3). The filtrate was concentrated in vacuo and the residue chromatographed using a 75:25 mixture of hexanes/ethyl acetate to give **43** (75 mg) in 83% yield; ^{1}H NMR δ 3.64 (2H, s), 3.94 (3H, s), 5.92 (2H, s), 6.40 (1H, s), 6.76 (1H, s), 7.15–7.20 (2H, m), 7.40 (1H, dd, J=8.3, 1.7), 7.75–7.85 (3H, m); ^{13}C NMR δ 55.8, 98.4, 101.3, 106.3, 110.7, 119.4, 120.3, 125.8, 127.4, 128.3, 128.7, 129.7, 135.3, 138.0, 139.0, 141.2, 148.2, 158.5; HRMS (EI) calcd for $C_{18}H_{15}NO_3$ m/z: 293.1052; found: 293.1052.

2-Hydroxy-7-methoxy-1-nitronaphthalene (47). 7-Methoxy-2-naphthol (871 mg, 5 mmol) was dissolved in 40 mL of dry ether. To this solution was added 4-methyl-4-nitro-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one[26] (2.33 g, 5 mmol) as a solid. The color of the solution slowly became red, and eventually dark red with some dark precipitate adhering to the inside surface of the flask. The reaction continued for 2.5 h at room temperature. Evaporation of the solvent gave the crude product. To the residue was added 20 mL of methanol/water (80/20). The reaction mixture was filtered and the sicciate was washed with methanol/water (80/20). The filtrate was then evaporated under vacuum and chromatographed using a 90:10 mixture of hexanes and ethyl acetate as the eluting solvent to yield 380 mg of **47** (35%); mp 130–131 °C (lit[26] 130 °C); ^{1}H NMR δ 3.96 (3H, s), 7.04 (1H, d, J=9.0), 7.10 (1H, dd, J=9.0, 2.6), 7.67 (1H, d, J=8.9), 7.87 (1H, d, J=8 .9), 8.37 (1H, d, J=2.5); ^{13}C NMR δ 56.0, 104.3, 116.8, 117.3, 124.2, 129.4, 131.5, 139.6, 160.4, 162.8.

2-Hydroxy-7-benzyloxy-1-nitronaphthalene (48). 7-Benzyloxy-2-naphthol[32] (2.5 g, 10.0 mmol) was dissolved in 100 mL of dry ether. To this solution was added 4-methyl-4-nitro-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one[26] (4.69 g, 10 mmol) as a solid. The color of the solution became red and eventually dark brown. The reaction was continued for 3 h at room temperature. The reaction mixture was concentrated in vacuo. The residue was chromatographed using a 85:15 mixture of hexanes and ethyl acetate to provide 1.6 g (55%) of **48**; mp 177–178 °C; ^{1}H NMR δ 5.22 (2H, s), 7.10 (d, 1H, J=8), 7.21 (1H, dd, J=4, 10.2), 7.38–7.56 (5H, m), 7.74 (1H, d, J=8.0), 7.93 (1H, d, J=8.2), 8.56 (1H, s,), 12.42 (1H, s).

2-Hydroxy-7-acetoxy-1-nitronaphthalene (49). 7-Acetoxy-2-naphthol[33] (1.01 gm, 5.0 mmol) was dissolved in 100 mL of dry ether. To this solution was added 4-methyl-4-nitro-2,3,5,6-tetrabromo-2,5-cyclohexadien-1-one[26] (2.35 g, 5.0 mmol) as a solid. The color of the solution became red and eventually dark brown. The reaction was continued for 3 h at room temperature. The reaction mixture was concentrated in vacuo and the crude material was purified by flash chromatography eluting with hexanes/ethyl acetate (85:15), to provide 715 mg (58%) of **49**; ^{1}H NMR δ 2.41 (3H, s), 7.21 (1H, d, J=4), 7.35 (1H, d, J=4), 7.83 (1H, d, J=8.0), 8.02 (1H, d, J=8.2), 8.76 (1H, s), 12.38 (1H, s).

7-Methoxy-1-nitro-2-trifluoromethanesulfonyloxynaphthalene (50). A solution of compound **49** (380 mg, 2.24 mmol) in THF (15 mL) was added to a suspension of sodium hydride (60 wt% in mineral oil, 90 mg, 2.25 mmol) in THF (10 mL) at 0 °C and the mixture stirred for 0.5 h. A solution of *N*-phenyltrifluoromethanesulfonimide (800 mg, 2.24 mmol) in THF (10 mL) was then added, and the reaction stirred at 0 °C for 8 h. After concentration in vacuo, the residue was chromatographed using 85:15 hexanes/ethyl acetate to give **50** (526 mg) containing approximately 10% *N*-phenyltrifluoromethanesulfonamide.

7- Benzyloxy-1-nitro-2-trifluoromethanesulfonyloxynaphthalene (51). To a cold solution of **48** (147 mg, 0.5 mmol) in dry pyridine (4 mL), trifluoromethanesulfonic anhydride (0.08 mL, 0.5 mmol) was added dropwise at 0 °C and stirring was continued for 30 min at 0 °C. Then the ice bath was removed and the reaction was stirred at room temperature overnight. The reaction mixture was poured on ice-water. A light yellow solid separated which was filtered, washed with water and dried to provide 155 mg of **51** (70% yield).

7-Acetoxy-1-nitrotrifluoromethanesulfonyloxynaphthalene (52). To a cold solution of **49** (705 mg, 2.85 mmol) in dry pyridine (5 mL), trifluoromethanesulfonic anhy-

dride (0.48 mL, 2.85 mmol) was added dropwise at 0 °C with stirring. After being allowed to stir for 30 min at 0 °C, the mixture was allowed to warm to room temperature. The reaction mixture was poured on ice-water. A light yellow solid separated which was filtered washed with water and dried to provide 975 mg (90% yield) of **52**; mp 108–109 °C; ^{1}H NMR δ 2.42 (3H, s), 7.48–7.68 (3H, m), 8.12 (1H, d, J = 12), 8.24 (1H, d, J = 12).

6-(4,5-Methylenedioxy-2-nitrophenyl)-2,3-dimethoxy-5-nitronaphthalene (53). Tetrakis(triphenylphosphine)-palladium(0) (100 mg) and cuprous bromide (20 mg) were added to a solution of 7-methoxy-1-nitro-2-trifluoromethanesulfonyloxynaphthalene **50** (366 mg, 1.04 mmol) and trimethyl(nitroaryl)stannane **35** (500 mg, 1.52 mmol) in THF (30 mL) at room temperature and the mixture was stirred for 0.5 h. The mixture was then refluxed under N_2 overnight. After cooling, THF was evaporated and ethyl acetate (30 mL) was added to the residue. The solution was washed with water. The organic layer was filtered through Celite 545 bed and then washed with brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed using a 70:30 mixture of hexanes/ethyl acetate to give **53** (160 mg) in 42% yield; mp 187–189 °C; IR (KBr) 2925, 1628, 1526, 1487, 1364, 1332, 1265, 1230 cm^{-1}; ^{1}H NMR δ 3.92 (3H, s), 6.19 (2H, s), 6.76 (1H, s), 7.12 (1H, d, J = 2.5), 7.18 (1H, d, J = 8.3), 7.28 (1H, dd, J = 9.0, 2.3), 7.70 (1H, s), 7.85 (1H, d, J = 9.2), 7.93 (1H, d, J = 8.4); ^{13}C NMR δ 56.1, 100.6, 103.9, 106.3, 110.7, 121.5, 124.1, 126.5, 128.7, 129.6, 130.3, 131.0, 131.2, 142.8, 148.9, 152.1, 160.7.

2-(4,5-Methylenedioxy-2-nitrophenyl)-7-methoxy-1-nitronaphthalene (54). Tetrakis(triphenylphosphine)-palladium(0) (90 mg), lithium chloride (240 mg) and cuprous bromide (60 mg) were added to a solution of **51** (886 mg, 2.0 mmol) and trimethyl(nitroaryl)stannane **35** (804 mg, 2.4 mmol) in THF (200 mL) and the reaction mixture was heated to reflux for 24 h under N_2. After cooling, THF was evaporated and chloroform (100 mL) was added to the residue. The solution was washed with water. The organic layer was separated, filtered through Celite 545 and then washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed using a mixture of hexanes/ethyl acetate (85:15) to provide 520 mg (58.5%) of **54**; mp 151–152 °C; ^{1}H NMR δ 5.2 (2H, s), 6.19 (2H, t, J = 2.2), 6.77 (1H, s), 7.21 (1H, dd, J = 1.4, 8.4), 7.34 (1H, s), 7.36 (6H, m), 7.65 (1H, s), 7.88 (1H, d, J = 9.2), 7.95 (1H, d, J = 8.4); ^{13}C NMR δ 70.5, 101.7, 103.5, 105.9, 110.3, 121.3, 123.9, 126.1, 127.9, 128.3, 128.4, 128.0, 129.3, 130.0, 130.7, 130.8, 136.1, 142.5, 145.2, 148.5, 151.7, 159.4.

2-(4,5-Methylenedioxy-2-nitrophenyl)-7-acetoxy-1-nitronaphthalene (55). Tetrakis(triphenylphosphine)-palladium(0) (23 mg), lithium chloride (126 mg) and cuprous bromide (15 mg) were added to a solution of **52** (379 mg, 1.0 mmol) and trimethyl(nitroaryl)stannane **35** (402 mg, 2.4 mmol) in THF (200 mL) and reaction mixture was heated to reflux for 72 h under N_2. After cooling, THF was evaporated and chloroform (100 mL) was added to the residue. The solution was washed with water. The organic layer was separated and filtered through Celite 545. The organic layer was then washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was chromatographed using a mixture of hexanes/ethyl acetate (80:15) to provide 130 mg (40% yield) of **55**; mp 166–167 °C; ^{1}H NMR δ 2.38 (3H, s), 6.19 (2H, dd, J = 1.5, 2.5), 6.75 (1H, s), 7.33 (1H, s), 7.37 (1H, s), 7.45 (1H, dd, J = 2.5, 11.5), 7.72 (1H, s), 8.02 (t, 1H, J = 9); ^{13}C NMR δ 21.1, 103.4, 103.6, 105.7, 105.9, 109.6, 110.2, 113.8, 123.7, 125.3, 126.2, 127.8, 129.9, 130.9, 131.4, 148.7, 151.1, 151.8, 169.2.

7-Bromo-3-methoxynaphthalene 2-carboxylic acid methyl ester[32] (56). A mixture of 6-bromo-3-hydroxy-2-naphthoic acid (5.0 g, 18.0 mmol), anhydrous K_2CO_3 (12.9 gm) and dimethyl sulfate (4.5 g, 41 mmol) in dry acetone (150 mL) were refluxed for 4 h. The reaction mixture was cooled and water (5 mL) was added. The reaction mixture was then stirred for 2 h to allow for hydrolysis of any remaining dimethyl sulfate. The reaction mixture was then filtered and the filtrate concentrated in vacuo. The residue was dissolved in $CHCl_3$ and washed several times with water, was dried, (Na_2SO_4) and the solvent removed in vacuo to afford 4.6 g (83.6%) of (**56**) as a solid; mp 166–167 °C.

2-Acetoxy-6-(4,5-methylenedioxy-2-nitrophenyl)-3-methoxynaphthalene (57). Tetrakis(triphenylphosphine)-palladium(0) (280 mg) and cuprous bromide (150 mg) were added to a solution of **56** (2.95 g, 10 mmol) and the trimethyl(nitroaryl)stannane **35** (3.55 g, 10.6 mmol) in THF (200 mL). The reaction mixture was heated to reflux for 96 h under N_2. After allowing the reaction mixture to cool, THF was removed in vacuo. The residue was dissolved in 150 mL of chloroform. The solution was washed with water. The organic layer was separated and filtered through Celite 545. The organic layer was then washed with brine, dried (Na_2SO_4) and concentrated in vacuo. The residue was chromatographed using hexanes/ethyl acetate (85:15) to give 2.1 g of **57** (55.3% yield); mp 186–187 °C; ^{1}H NMR δ 3.97 (3H, s), 4.03 (3H, s), 6.17 (2H, s), 6.87 (1H, s), 7.25 (1H, d, J = 4), 7.39 (1H, dd, J = 2, 8), 7.50 (1H, s), 7.73 (1H, d, J = 4), 7.78 (1H, s), 8.30 (1H, s); ^{13}C NMR δ 52.3, 56.2, 103.1, 105.6, 106.9, 111.2, 122.6, 126.8, 127.5, 128.6, 132.9, 134.3, 135.5, 143.0, 147.4, 151.2, 156.4, 166.6.

2-Acetoxy-6-(2-Amino-4,5-methylenedioxyphenyl)-3-methoxynaphthalene (58). To a refluxing solution of **57** (760 mg, 2.0 mmol) and Ra–Ni (200 mg) in ethanol (50 mL), hydrazine hydrate (2.5 mL) was added dropwise. After the addition was complete, the reaction mixture was heated to reflux for 2 h. The mixture was filtered through Celite 545. The filtrate was concentrated in vacuo to provide 700 mg (99% yield) of solid; mp 192–193 °C; ^{1}H NMR δ 3.98 (3H, s), 4.04 (3H, s), 5.94 (2H, s), 6.40 (2H, s), 6.78 (1H, s), 7.28 (1H, d, J = 2), 7.61 (1H, dd, J = 2, 8), 7.80 (1H, s), 7.86 (1H, d, J = 4), 8.32 (1H, s).

6-(2-Amino-4,5-methylenedioxyphenyl)-2-hydroxymethyl-3-methoxynaphthalene (59). To a solution of **58** (702 mg, 2.0 mmol) in dry THF (60 mL), LAH (115 mg, 3.0 mmol) was added in portions with stirring at room temperature under N_2. The reaction was stirred at room temperature for 2 h. Excess LAH was decomposed with

a few drops of 10% NaOH solution. The reaction mixture was filtered through Celite 545 and the sicciate was washed with THF. The filtrate was concentrated in vacuo and dried (Na_2SO_4) to provide 470 mg (73% yield) of **58** as a light yellow solid; mp 143–144 °C; ^{1}H NMR δ 4.00 (3H, s), 4.83 (2H, s), 5.92 (2H, s), 6.40 (1H, s), 6.73 (1H, s), 7.15 (1H, s), 7.50 (1H, dd, J = 1.4, 8.2), 7.75–7.81 (3H, m); ^{13}C NMR δ 55.5, 62.1, 98.1, 100.9, 105.1, 110.3, 120.1, 127.1, 127.6, 127.9, 128.1, 129.0, 131.2, 133.1, 135.0, 138.2, 140.9, 147.7, 156.2.

Topoisomerase-mediated DNA cleavage assays. Human topoisomerase I was expressed in *Escherichia coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[13] Recombinant human topoisomerase IIα was isolated and purified as previously described.[34] Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[35] The end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[36] The cleavage assays were performed as previously reported.[8] The drug and the DNA in presence of topoisomerase I was incubated for 30 min at 37 °C. The reactions were terminated by the addition of 5 μL of 5% SDS and 1 mg/mL protein kinase K with an additional 1 h of incubation at 37 °C. Samples were then alkali denatured by the addition of NaOH, EDTA, sucrose, and bromophenol blue to final concentrations of 75 mM, 2.5%, and 0.05 mg/mL, respectively, prior to loading onto a neutral agarose gel. After development of the gels, typically 24-h exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, i.e., concentrations relative to camptothecin, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I. Topoisomerase II-mediated DNA cleavage values are reported as REC, Relative Effective Concentration, wherein potency was based upon the relative amount of drug needed to induce approximately 10% DNA fragmentation. REC values for topoisomerse II-mediated cleavage are reported relative to VM-26, whose value is arbitrarily set as 1.0.

Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[37–39] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[40] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). For determination of IC_{50}, cells were exposed continuously for 4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

Acknowledgements

We are grateful to Washington University Resource for Biomedical and Bio-Organic Mass Spectrometry for providing mass spectral data and for the partial support of this facility by the National Institutes of Health (Grant No. P41RR0954). This study was supported by AVAX Technologies, Inc, (E.J.L.) and Grant CA39662 (L.F.L.) and Grant CA077433 (L.F.L.) from the National Cancer Institute.

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