# Inhibition of DNA Topoisomerase II by Azaelliptitoxins Functionalized in the Variable Substituent Domain

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A series of novel $C_{11}$-substituted derivatives of azaelliptitoxin (azatoxin) have been synthesized and tested for their inhibitory activity against human DNA topoisomerase II. Incorporation of a $C_{11}$ polyamine or amine resulted in an increase in the intercalation properties of the drug and a decrease of topoisomerase II activity. The structure−activity relationship (SAR) profile of the nonintercalating $C_{11}$ anilino azatoxin class follows the SAR of the (anilino)acridine family. 11-(4-Cyanoanilino)azatoxin (**14**) was found to be the most active analog in this series, exhibiting ∼10-fold higher activity than azatoxin **12** and etoposide.

## Introduction

Azaelliptitoxin (referred to as azatoxin) is a potent inhibitor of DNA topoisomerase II (topo II) and tubulin polymerization. This agent was rationally designed as a hybrid of etoposide and ellipticine.[1,2] As part of our continuing efforts aimed at the design of novel DNA topo II inhibitors, we have been investigating the individual contributions to activity from each of the three domains, hypothesized in a model pharmacophore of the azatoxin class.[1] Azatoxin and its analogs have been established to stabilize the ternary complex of drug, DNA, and DNA topo II, termed the "cleavable complex".[3,4]

We have previously reported the structure−activity relationship (SAR) of pendant group and indole-substituted azatoxin analogs.[5] The pendant group domain in the azatoxin and epipodophyllotoxin series has strict structural requirements for activity.[5,6] The variable substituent domain can be substituted by a variety of structurally diverse groups, with little SAR data available.[7,8] Since a significant increase in potency and selectivity, with regard to topo II versus tubulin,[9,10] has been observed in some 4-*epi*-functionalized podophyllotoxins, the incorporation of a substituent in the variable substituent domain of the azatoxin class was undertaken.

The structural requirements of the variable substituent domain in 4-*epi*-odophyllotoxin derivatives indicates that *O*-alkyl and *N*-alkyl chains of two or carbons with a terminal polar functionality enhances topo II activity.[11] In addition, *N*-aryl-substituted epipodophyllotoxins have been found to be potent topo II inhibitors.[12] As such, a representative group of substituted azatoxin analogs that fit the aforementioned structural requirements was synthesized and evaluated for their ability to promote protein-associated DNA strand breaks, a measure of cleavable complex formation. In addition, in an attempt to further define the structural demands for activity of the variable substituent domain, several $C_{11}$ (anilino)azatoxin analogs were synthesized and tested for topo II inhibition.

## Chemistry

Thirteen derivatives were synthesized and functionalized at the $C_{11}$ position of the azatoxin nucleus. These azatoxin analogs represent two classes of substituents in the variable substituent domain. The synthesis of one class, the $C_{11}$ alkoxy and aminoalkyl analogs, is depicted in Scheme 1. The synthesis of the N-aryl derivatives is outlined in Schemes 2 and 3.

Oxazolidinone **1,** generated from L-tryptophanol and diethyl carbonate with sodium ethoxide (98% yield), is the precursor for both classes (Scheme 1). Treatment of **1** with syringaldehyde dimethyl acetal and catalytic trifluoroacetic acid (15 mol %) gave azatoxin (91% yield). Functionalization of **1** at $C_{11}$ to yield **2,** as a 2:3 mixture of diastereomers, was accomplished by DDQ oxidation[13] of oxazolidinone **1** in THF/acetic acid at −78 °C (98%). Acetate **2** was unstable and was used as quickly as possible. Cyclization of **2** with syringaldehyde dimethyl acetal and catalytic *p*-TsOH (10 mol %) provided the 11-methoxyazatoxin **3** as a single diastereomer in 47% yield. Due to the insolubility of **3** in many solvent systems, the phenol was protected as the methyl carbonate, yielding **4**, which had a wider spectrum of solubility.

Acid-catalyzed exchange of the $C_{11}$ methoxy with water (**5**) or ethylene glycol (**7**) could be effected with catalytic *p*-TsOH in either a dioxane/water or dioxane/ethylene glycol mixture, respectively (Scheme 1). Deprotection of **5** was then accomplished with sodium methoxide in methanol. In order to functionalize the $C_{11}$ position with an amine, a variation of the literature procedure for the elaboration of the $C_4$ position of podophyllotoxin was developed (Scheme 1). The "standard method" of treatment of methoxyazatoxin **4** with HBr resulted in decomposition. A milder procedure involved treatment of alcohol **5** with acetyl chloride and TEA, followed by *N*,*N*-dimethylethlenediamine to yield **9** (54% yield). However, when anilines were used as nucleophiles, the yields were rather low. An alternative procedure utilizing $BF_3{\cdot}OEt_2$-catalyzed addition of the desired aniline was employed for synthesis of the anilino-substituted azatoxins. Treatment of **4** with 4-fluoroaniline, 4-nitroaniline, or 4-cyanoaniline and $BF_3{\cdot}OEt_2$ at 0 °C in $CH_2Cl_2$ afforded compounds **10a**−**c** in 50−60% yield (Scheme 2). Deprotection of **10a**−**c** proceeded smoothly with sodium methoxide in methanol to yield the arylamines **11, 13** and **14** (78%).

The methyl chloroformate protection group was replaced with the carbobenzyloxy group in the synthesis

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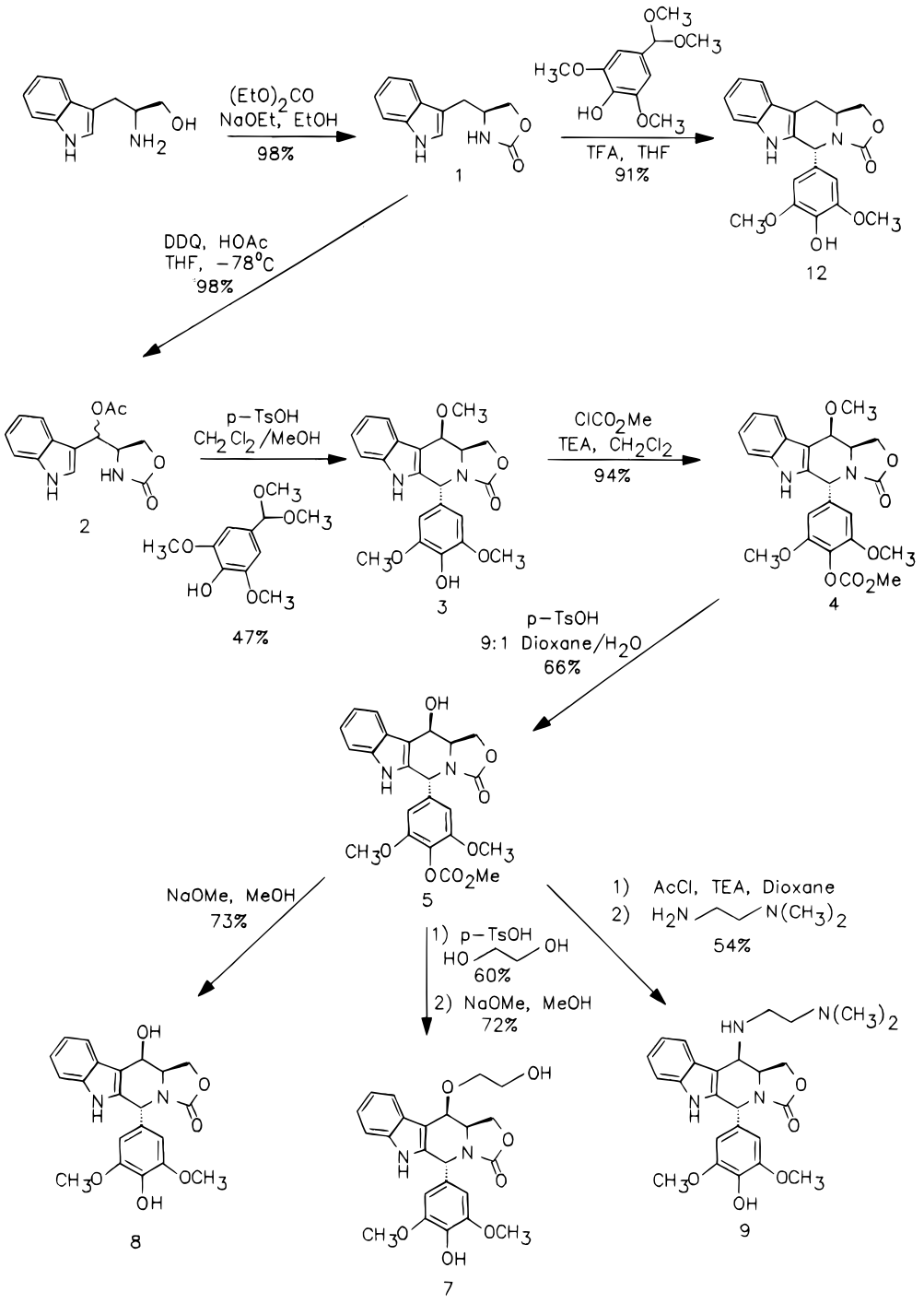
**Scheme 1**

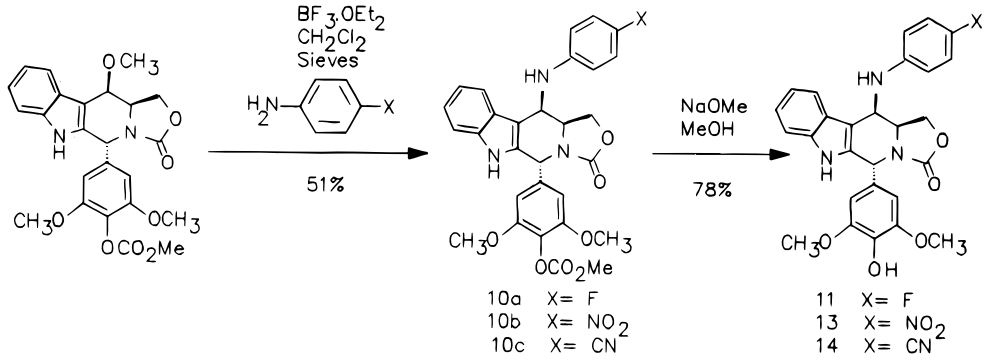



of 4′-hydroxy-substituted anilines, since basic or acidic deprotection often resulted in elimination of the aniline. The aniline precursors were synthesized through well-established procedures. Subsequent treatment of **15** with the aforementioned anilines and $BF_3{\cdot}OEt_2$ at 0 °C followed by hydrogenation over 10% Pd/C provided the compounds **26**−**31** (Scheme 3). However, oxidation of **30** occurred rapidly, resulting in the elimination of the aniline group.

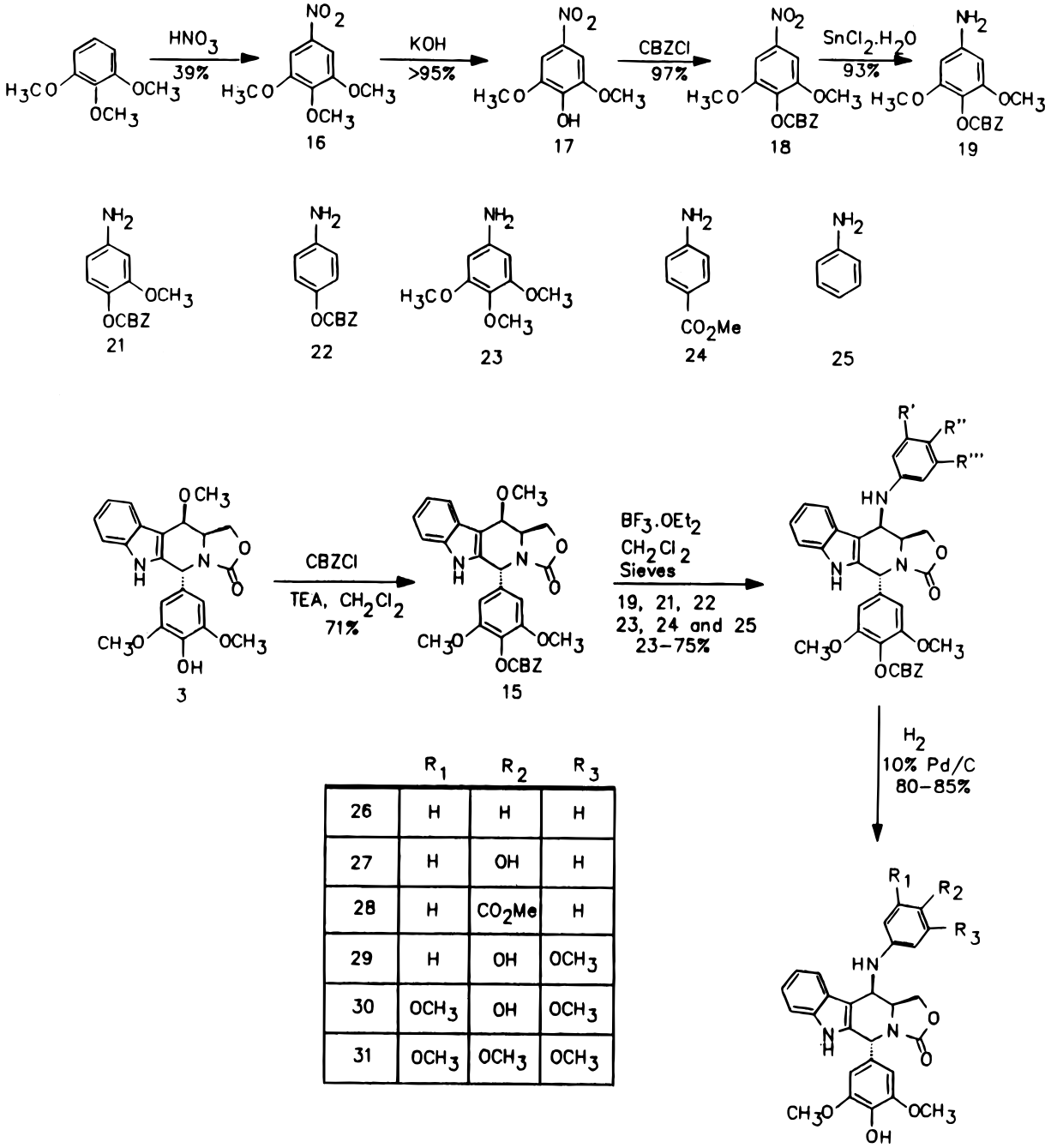
## Results and Discussion

Compounds **3**, **7**−**9**, **11**−**14**, and **26**−**31** were assayed for cleavable complex formation in the presence of linearized pUC18 DNA and topo II purified from human placenta, according to previously described techniques.[14] Table 1 provides a summary of the SAR data, and a representative cleavage gel is shown in Figure 1. The SAR data indicate that incorporation of a para-substituted anilino group in the variable substituent domain of azatoxin significantly increases the overall activity of the drug. Interestingly, an electron-withdrawing functionality in the paraposition of the aniline appears to show the highest increase in the potency of the agent. As illustrated by the di- and trisubstituted anilines, the SAR of the variable substituent domain of azatoxin follows the SAR of the (anilino)acridine family. The effects on *in vitro* topo II-mediated DNA cleavage activity of anilino-substituted in the (anilino)acridine series has demonstrated that (i) a para H-bonding substituent is not essential for activity and (ii) in

**Scheme 2**



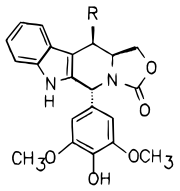

**Scheme 3**

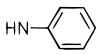
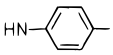
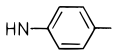
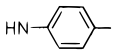
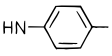
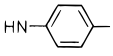
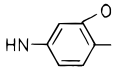
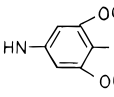
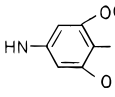



| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| 26 | H | H | H |
| 27 | H | OH | H |
| 28 | H | $CO_2Me$ | H |
| 29 | H | OH | $OCH_3$ |
| 30 | $OCH_3$ | OH | $OCH_3$ |
| 31 | $OCH_3$ | $OCH_3$ | $OCH_3$ |

*p*-hydroxyanilines, ortho substitution depresses activity. Thus, (*p*-hydroxyanilino)acridine > (*o*-methoxyhydroxyanilino)acridine > (*o*,*o*′-dimethoxyhydroxyanilino)acridine. In addition, the results are consistent with previous studies which found that analogously substituted podophyllotoxins exhibited a closely related SAR for aniline substitution at the $C_4$ position of podophyllotoxins. These results support our hypothesis that the

**Table 1.** Biological Evaluation of Azatoxin Substituted in the Variable Substituent Domain



| Compound | R | Activity* |
|---|---|---|
| 12 | H | 1 |
| 3 | $OCH_3$ | inactive |
| 8 | OH | 0.25 |
| 7 | $OCH_2CH_2OH$ | 0.25 |
| 9 | $NHCH_2CH_2N(CH_3)_2$ | 0.5 |
| 26 |  | 3 |
| 27 |  | 1 |
| 28 |  | 2 |
| 11 |  | 4 |
| 13 |  | 6 |
| 14 |  | 9 |
| 29 |  | inactive |
| 30 |  | not tested |
| 31 |  | inactive |

* Relative DNA cleavage was determined by densitometric analysis of the protein-associated DNA cleavage assays and quantified through comparison with the previously reported drug-induced cleavage of several of the azatoxin derivatives.[3] The cleavage activities are expressed relative to azatoxin and were reproducible within 30% of the reported activities.

anilino group of the aminoacridine family resides in the variable substituent domain of the model pharmacophore.

Of all the compounds tested, compound **14** is the most active analog in this series exhibiting ~10-fold higher activity than azatoxin **12** and etoposide. The (nitroanilino)azatoxin **13** was shown to be sensitive to P-glycoprotein-mediated cell efflux as observed in multiple drug resistance (MDR).[15] The (fluoroanilino)azatoxin derivative **11** was not found to be sensitive to the multidrug transporter and was selected for preclinical evaluation of its antitumor activity.

In addition to the potential increase in potency of these functionalized azatoxin analogs, the $C_{11}$ aniline-substituted azatoxins exhibit a profound increase in topo II selectivity. Azatoxin **12** was designed to be a dual

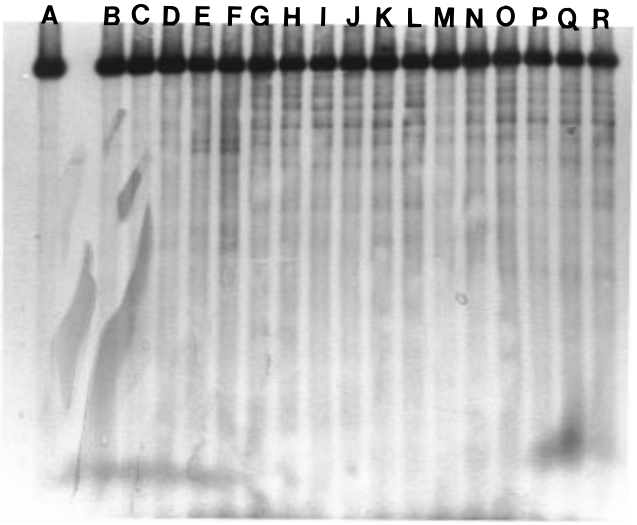



**Figure 1.** DNA cleavable complex assay with azatoxin analogs: lane A, DNA only; lane B, DNA +topo II; lane C, 1% DMSO; lanes D−F, 5, 10, 50 $\mu$M azatoxin; lanes G−I, 5, 10, 50 $\mu$M (4-fluoroanilino)azatoxin (**11**); lanes J−L, 5, 10, 50 $\mu$M anilinoazatoxin (**26**); lanes M−O, 5, 10, 50 $\mu$M (4-hydroxyanilino)azatoxin (**27**); lanes P−R, 5, 10, 50 $\mu$M (4-(carboxymethyl)anilino)azatoxin (**28**).

inhibitor of tubulin polymerization and topo II.[10] Based on the similarity in structure and the protein-associated DNA cleavage patterns of 4′-demethyl-4-deoxypodophyllotoxin (DDP) and azatoxin **12**, it has been postulated that tubulin might have a comparable drug binding site.[3] The association with the tubulin binding site can be eliminated when the drug is substituted in the variable substituent domain of our model pharmacophore. The enhancement of topo II selectivity has been previously illustrated by the transformation of the dual inhibitor, DDP, into the selective topo II inhibitor, etoposide, by substitution in the variable substituent region. In the case of azatoxin, the incorporation of a substituent in the variable substituent domain similarly eliminates the inhibition of tubulin polymerization and results in selective topo II inhibition.[3] Our data indicate that the variable substituent domain contributes to both drug selectivity, by eliminating the association with tubulin binding sites, and drug potency at the topo II target site.

In contrast to the increase in potency and selectivity resulting from the substitution of para-substituted anilines in the variable substituent domain, the other substituents depressed topo II activity. Although 4′-demethyl-epipodophyllotoxin has been reported to be equipotent to etoposide, the analogously substituted azatoxin **8** is considerably less active than **12**. However, the reduced activity of the glycol ether **7** is comparable to that observed in the epipodophyllotoxin series.[16]

The difference in cleavage patterns, between azatoxin and the anilino-substituted azatoxins, observed in Figure 1, is thought to be the result of the subtly different orientations of the drug within the enzyme−DNA complex. Azatoxin **12** and the anilino-substituted azatoxin analogs (**11**, **13**, **14**, **26**−**28**) are nonintercalative topo II inhibitors which exert their cytotoxicity through the stabilization of the cleavable complex in the catalytic cycle of topo II. The association of the drug with the DNA−enzyme complex can occur through several "modes" of interaction, illustrated by the diversity of DNA cleavage patterns.[17, 18] Each cleavage site presum-

ably reflects on destinct drug−DNA−enzyme complex with a distinct, but similar, drug-induced DNA conformation. As illustrated by our model of the pharmacophore for topo II active agents, we postulate that the variable substituent domain of azatoxin **12** is located in the minor groove of the DNA. The additional binding energy derived from placement of a group in this structurally accesible locus contributes to the stability of the drug in the DNA−enzyme complex. We hypothesize that the aniline in the variable substituent domain of azatoxin enables a drug-induced DNA conformation with which the enzyme binds more tightly, thereby increasing the stabilization of the DNA−enzyme cleavable complex and resulting in a profound increase of potency.

It should be notes that structual restrictions on the $C_{11}$ substituent are apparent. For example, incorporation of a polyamine or amine into the nucleus of a topo II inhibitor structure normally results in an analog with increased activity, although the azatoxin polyamine derivative **9** is less active than azatoxin **12**. Interestingly, the cleavage intensity of **9** does not increase from 50 to 100 mM, but appears to decrease slightly. This result suggests the same autoinhibitory phenomenon that is observed with intercalators. Compound **9** exhibits limited intercalative ability in an ethidum bromide displacement assay.[19] The resulting concentration−response curve reveals that at 50 mM of **9**, 15% displacement occurs, while displacement does not significantly increase at 100 mM. This asymptotic behavior is typical of weaker intercalative agents. Furthermore, **9** is the first epipodophyllotoxin-like topo II inhibitor to exhibit any "classical" intercalative behavior. This result represents a significant observation in that it exemplifies the continuous spectrum of intercalation and topo II activity suggested by the composite pharmacophore model.

The incorporation of several other substituents in the variable substituent domain of azatoxin, such as several glycosyl units and additional *N*-arylamino units, are under investigation, and their biological results will be published in the near future.

## Experimental Section

All melting points were taken on a Thomas-Hoover UNIMELT melting point apparatus and are uncorrected. $^1$H NMR spectra were obtained using a General Electric QE300 spectrometer at 300 MHz. All chemical shifts are recorded in ppm. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and in house on a Perkin-Elmer PE 2400 C, H, N analyzer. Analytical thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates and were visualized with a phosphomolybdic acid/ethanol solution.

**4(*S*)-(1*H*-Indol-3-ylmethyl)-2-oxazolidinone (1).** Diethyl carbonate (8.83 g, 74.8 mmol) and L-tryptophanol (12.94 g, 68.02 mmol) in ethanol (100 mL) was added to a solution of sodium (1.56 g, 68.1 mmol) in absolute ethanol (150 mL). The solution was heated at reflux for 5 h and cooled to room temperature, and the solvent was removed under reduced pressure. Saturated aqueous $NH_3Cl$ (100 mL) and $CH_2Cl_2$ (200 mL) were added, and the organic layer was separated. The aqueous layer was washed with $CH_2Cl_2$ (2 × 100 mL), and the organic fractions were combined and dried over $Na_2SO_4$. The solvent was removed under reduced pressure. Recrystallization from MeOH/$H_2O$ yielded a white solid (11.66 g, 79%): mp 155 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.17 (sb,1H), 7.57 (d, $J$ = 7.94 Hz, 1H), 7.40 (d, $J$ = 8.06 Hz, 1H), 7.19 (m, 2H), 7.08 (d, $J$ = 2.2 Hz, 1H), 5.22 (sb, 1H), 4.50 (m, 1H), 4.21 (m, 2H), 3.04 (m, 2H); $[\alpha]^{24}_D$ +8.4° (*c* 9.4, MeOH). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

**Preparation of Syringaldehyde Dimethyl Acetal.** Syringaldehyde (1 g) was dissolved in trimethyl orthoformate (7 mL), and a catalytic amount of *p*-TsOH (40 mg) was added. The reaction was followed to completion by TLC. The solvent was removed under reduced pressure. The remaining oil was dissolved in $CHCl_3$ and filtered through a plug of silica. The solvent was again removed under reduced pressure. The remaining oil was stored in a desicator until use.

**(5*R*,11*aS*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (12).** TFA (0.15 equiv) was added to a solution of oxazolidinone **1** (0.522 mmol) in 3 mL of THF and syringaldehyde dimethyl acetal (1.5 equiv). The solution was refluxed for 5 h, cooled to room temperature, quenched with saturated aqueous $NaHCO_3$, and extracted with ethyl acetate. The organic layer was separated, dried over $Na_2SO_4$, and filtered. The product was purified by flash chromatography (12% acetone in $CHCl_3$, $R_f$ = 0.28), producing a white solid in 91% yield: mp dec slow 175 °C; $[\alpha]^{22}_D$ −139.6° (*c* 1.0, $CHCl_3$); $^1$H NMR (300 MHz, $CD_3CN$) $\delta$ 7.94 (sb, 1H), 7.51 (d, $J$ = 7.91 Hz, 1H), 7.30 (d, $J$ = 7.57 Hz, 1H), 7.09 (m, 2H), 6.59 (s, 2H), 6.27 (s, 1H), 5.88 (d, $J$ = 1.7 Hz, 1H), 4.54 (dd app t, $J$ = 8.3 Hz, 1H), 4.33 (m, 1H), 4.21 (dd, $J$ = 8.5, 4.7 Hz, 1H), 3.75 (s, 6H), 3.16, (dd, $J$ = 15, 4.6 Hz, 1H), 2.76 (ddd, $J$ = 15, 10.38 Hz, 1.73 Hz, $J$ = 1.73 Hz, 1H). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-methoxy-1*H*,6*H*-oxazolo[3′,4′:1,6]-pyrido[3,4-*b*]indol-3-one (3).** Glacial acetic acid (30 mL) was added dropwise to a solution of oxazolidinone **1** (1.02 g, 4.72 mmol) in THF (30 mL) at −78 °C. The cooling bath was removed. When dissolution of the acetic acid occurred, the reaction flask was reemerged in the cooling bath, and immediately a solution of DDQ (1.17 g, 5.2 mmol) in THF (30 mL) was added over 5 min. The solution was allowed to stir at −78 °C for 30 min. The cooling bath was removed, and the reaction mixture was allowed to stir at room temperature for 24 h. Toluene (60 mL) was added, and the solvent was removed under reduced pressure. The remaining brown oil was redissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ (5 × 75 mL). The organic layer was dried over $Na_2SO_4$ and filtered quickly through a plug of florisil. The solvent was removed under reduced pressure to yield the acetate **2** (1.28 g, 98%) as a mixture of diastereomers.

*p*-TsOH (0.80 mg, 0.47 mmol) was added to a solution of syringaldehyde dimethyl acetal (1.60 g, 7.01 mmol) in anhydrous $CH_2Cl_2$/MeOH (9:1 mixture, 8 mL) and was allowed to stir for 5 min. The solution was cooled to 0 °C, and a solution of acetate **2** (1.28 g, 4.67 mmol) in 9:1 $CH_2Cl_2$/MeOH (8 mL) was added in small portions. The precipitate which was formed after 4 h was collected by filtration in a scintered glass funnel and washed with cold $CH_2Cl_2$. The remaining solid was dried in a vacuum desicator to yield the methoxyazatoxin analog **3** (0.91g, 47%); $[\alpha]^{22}_D$ = −134 (*c* 0.9, DMSO); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.50 (sb, 1H), 8.42 (sb, 1H), 7.65 (d, $J$ = 7.28 Hz, 1H), 7.31 (d, $J$ = 7.71 Hz, 1H), 7.06 (m, 2H), 6.48 (s, 2H), 5.85 (s, 1H), 4.63 (d, $J$ = 1.8 Hz, 1H), 4.43 (m, 3H), 3.64 (s, 6H), 3.28 (s, 3H); $^{13}$C NMR (DMSO-$d_6$) 157.5, 148.9, 137.3, 136.8, 135.0, 130.7, 127.6, 122.5, 120.4, 119.2, 112.5, 108.5, 106.6, 68.0, 65.1, 57.2, 57.0, 55.4, 54.2. Anal. ($C_{22}H_{22}N_2O_6$) C, H, N.

**General Procedure A for Methyl Formate Protection of Phenols.** Methyl chloroformate (3.69 mL, 47.7 mmol) was added dropwise to a suspension of methoxy azatoxin **3** (1.96 g, 4.77 mmol) in $CH_2Cl_2$ (10 mL) and TEA (6.7 mL, 47.7 mmol) at 0 °C. The solution was diluted with $CH_2Cl_2$ after 2 h and washed with water. The organic layer was dried over $Na_2SO_4$ and filtered, and the solvent was removed under reduced pressure. The product was purified by flash chromatography (eluting with acetone−chloroform).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbomethoxyoxyphenyl)-11-methoxy-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (4).** Methoxyazatoxin analog **4** was prepared as described in procedure A. The product was purified by flash chromatography eluting with 8% acetone

in $CHCl_3$ to yield the product as a white solid (2.09 g, 94%). An analytical sample was obtained by recrystallization from ethyl acetate: mp dec 219−220 °C; $[\alpha]^{22}_D = -83.5$ (*c* 1.25, $CHCl_3$); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.26 (sb, 1H), 7.71 (d, $J$ = 6.72 Hz, 1H), 7.37 (d, $J$ = 6.37 Hz, 1H), 7.28−7.21 (m, 2H), 6.51 (s, 2H), 6.08 (s, 1H), 4.75 (dd, $J$ = 8.32 Hz, $J$ = 3.82 Hz, 1H), 4.67 (d, $J$ = 2.29 Hz, 1H), 4.45 (t, $J$ = 8.71 Hz, 1H), 4.20 (dt, $J$ = 8.83 Hz, 1H), 3.89 (s, 3H), 3.72 (s, 6H), 3.4 (s, 3H). Anal. ($C_{24}H_{24}N_2O_8$) C, H.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbomethoxyoxyphenyl)-11-hydroxy-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (5).** *p*-TsOH (130 mg, 0.70 mmol) was added to a solution of the methoxyazatoxin analog **4** (1.88 g, 7.00 mmol) in a dioxane/water solution (9:1 mixture, 30 mL). The solution was followed to completion by TLC and diluted with $CHCl_3$. The resulting mixture was washed with saturated aqueous $NaHCO_3$, dried over $Na_2SO_4$ and filtered. The solvent was removed under reduced pressure. The product was purified by flash chromatography eluting with 20% acetone in $CHCl_3$, yielding the hydroxyazatoxin analog **5** as white solid (1.21 g, 66%) and the epimer as the minor product (0.16 g, 13%). An analytical sample was obtained by recrystallization from toluene: mp dec 205−207 °C; $^1$H NMR (300 MHz, $CDCl_3$) 8.09 (sb, 1H), 7.75 (d, $J$ = 7.17 Hz, 1H), 7.38 (d, $J$ = 7.29 Hz, 1H), 7.32−7.22 (m, 2H), 6.53 (s, 2H), 6.14 (s, 1H), 5.01 (sb, 1H), 4.84 (dd, $J$ = 8.39 Hz, $J$ = 4.56 Hz, 1H), 4.49 (t, $J$ = 8.8 Hz, 1H), 4.22−4.16 (m, 1H), 3.91 (s, 3H), 3.37 (s, 6H). Anal. ($C_{23}H_{22}N_2O_8$) C, H.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-hydroxy-1*H*,6*H*-oxazolo[3′,4′:1,6]-pyrido[3,4-*b*]indol-3-one (8).** A solution of the hydroxyazatoxin analog **5** (64.5 mg, 141 mmol) in absolute ethanol was added to a solution of sodium methoxide (0.51 mmol) in absolute methanol (1 mL). The solution was allowed to stir at room temperature for 3 h. The reaction mixture was carefully acidified to pH 7 by the addition of 1% HCl, and stirring was continued for an additional 20 min. A precipitate formed, which was collected by filtration in a scintered glass funnel and dried in a vacuum desiccator to yield the product **8** as a white solid (41 mg, 73%): $[\alpha]^{22}_D$ −126.7° (*c* 0.3, DMSO); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.9 (sb, 1H), 8.42 (sb, 1H), 7.54 (d, $J$ = 7.29 Hz, 1H), 7.28 (d, $J$ = 7.78 Hz, 1H), 7.08−7.00 (m, 2H), 6.47 (s, 2H), 5.80 (s, 1H), 5.26 (d, $J$ = 6.7 Hz, 1H), 4.79 (dd, $J$ = 6.43 Hz, $J$ = 2.25 Hz, 1H), 4.55−4.51 (m, 1H), 4.37 (t, $J$ = 8.37 Hz, 1H), 4.29−4.27 (m, 1H), 3.65 (s, 6H). Anal. ($C_{21}H_{20}N_2O_6$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-hydroxy-1*H*,6*H*-oxazolo[3′,4′:1,6]-pyrido[3,4-*b*]indol-3-one (7).** *p*-TsOH (10 mol %) was added to a solution of the hydroxyazatoxin analog **5** (0.2 g, mmol) in dioxane/ethylene glycol (9:1 mixture, 6 mL), and the reaction mixture was followed to completion by TLC. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ and $CH_2Cl_2$. The organic layer was separated, dried over $Na_2SO_4$, and filtered. The solvent was removed under reduced pressure. Purification by flash chromatography yielded the product **7** as a white solid (60%): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.55 (sb, 1H), 7.66 (d, $J$ = 7.19 Hz, 1H), 7.32 (d, $J$ = 8.00 Hz, 1H), 7.11−7.02 (m, 2H), 6.63 (s, 2H), 5.94 (s, 1H), 4.81 (s, 1H), 4.64 (d, $J$ = 4.78 Hz, 1H), 4.58 (t, $J$ = 3.64 Hz, 1H), 4.55−4.48 (m, 2H), 3.76 (s, 3H), 3.70 (s, 6H), 3.66−3.59 (m, 1H), 3.51−3.43 (m, 3H).

Deprotected as in **8** (72%): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.02 (sb, 1H), 8.43 (sb, 1H), 7.65 (d, $J$ = 7.26 Hz, 1H), 7.30 (d, $J$ = 7.9 Hz, 1H), 7.12−7.03 (m, 2H), 6.46 (s, 2H), 5.83 (s, 1H), 4.78 (s, 1H), 4.61 (d, $J$ = 3.84 Hz, 1H), 4.56 (t, $J$ = 5.15 Hz, 1H), 4.49−4.42 (m, 2H), 3.65 (s, 6H), 3.64−3.59 (m, 1H), 4.57−4.42 (m, 3H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-((2-*N*,*N*-dimethylamino)ethyl)-amino)-1*H*,6*H*-oxazolo[3′4′:1,6]pyrido[3,4-*b*]indol-3-one (9).** TEA (640 mmol) and acetyl chloride (535 mmol) were added to a solution of **5** (97 mg, 214 mmol) in anhydrous dioxane (2 mL) at 0 °C. After 15 min of stirring, the solvent was removed under reduced pressure, and $BaCO_3$ (1.1 mmol), *N*,*N*-dimethylethylenediamine (1.1 mmol), dioxane (3 mL) were added. The reaction mixture was heated at 50 °C, and the reaction was followed to completion by TLC. The solvent was removed under reduced pressure, and the remaining solid was purified by flash chromatography, eluting with 8% TEA in $CH_2Cl_2$ ($R_f$ = 0.35) to afford the product **9** as a white solid (54 mg, 54%): $[\alpha]^{22}_D$ −145° (*c* 0.5, DMSO); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.41 (sb, 1H), 7.59 (d, $J$ = 7.34 Hz, 1H), 7.27 (d, $J$ = 7.77 Hz, 1H), 7.07−6.97 (m, 2H), 6.47 (s, 2H), 5.78 (s, 1H), 4.63 (dd, $J$ = 7.31 Hz, $J$ = 4.23 Hz, 1H), 4.42−4.31 (m, 2H), 3.97 (d, $J$ = 2.11 Hz, 1H), 3.65 (s, 6H), 2.74−2.7 (m, 1H), 2.63−2.60 (m, 1H), 2.26−2.17 (m, 2H), 1.96 (s, 6H). Anal. ($C_{25}H_{30}N_4O_5$) C, H, N.

**3,4,5-Trimethoxynitrobenzene (16).** Fuming nitric acid (3.5 mL) was added to a solution of trimethoxybenzene (60 mmol) in 20 mL of glacial acetic acid at 0 °C. The solution was stirred for 1 h, after which it was poured over ice. The beige/brown precipitate was filtered off by suction, yielding a orange/yellow solid. The product was purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.8), yielding trimethoxynitrobenzene as a beige/yellow solid (39% yield): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.52 (s, 2H), 3.88 (s, 9H).

**3,5-Dimethoxy-4-hydroxynitrobenzene (17).** A solution of 3,4,5-trimethoxynitrobenzene (0.034 mol) in 20% KOH/$H_2O$ (100 mL) was refluxed for 2 days. The pH was adjusted to pH ∼3 with a 10% HCl solution. The solution was extracted with $CHCl_3$ (3 × 25 mL), and the organic layers were combined, dried over $Na_2SO_4$, and filtered. The solvent was removed under reduced pressure. The product was purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.5), yielding the product **17** as a yellow solid (600 mg, 9% yield). The remaining 91% was starting material which was demethylated by repeating the procedure (∼10% yield per repeat): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.57 (s, 2H), 6.12 (sb, 1H), 4.01 (s, 6H).

**General Procedure B for Carbobenzoxy Protection of Nitrophenols.** CBZCl (19.6 mmol) was added to a solution of the nitrophenol (7.18 mmol) and TEA (20.0 mmol) in 22 mL $CH_2Cl_2$ at 0 °C. The solution was stirred for 2 h. The solution was diluted with of $CH_2Cl_2$ and extracted with $H_2O$ (3 × 10 mL). The organic layer was separated, dried over $Na_2SO_4$, and filtered. The solvent was removed under reduced pressure. The product was purified by flash chromatography (acetone−chloroform).

**4-(Carbobenzoxyoxy)benzene.** This nitrophenol analog was prepared as described in procedure B (95% yield) and purified by flash chromatography eluting with 10% acetone in chloroform ($R_f$ = 0.45): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.27 (d, $J$ = 9.3 Hz, 2H), 7.24 (m, 7H), 5.38 (s, 2H).

**3-Methoxy-4-(carbobenzoxyoxy)nitrobenzene.** This nitrophenol analog was prepared as described in procedure B (96%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.58): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85 (m, 2H), 7.42 (m, 5H), 7.27 (d, $J$ = 13.9 Hz, 1H), 5.29 (s, 2H), 3.87 (s, 3H).

**3,5-Dimethoxy-4-(carbobenzoxyoxy)nitrobenzene (18).** Nitrophenol analog **18** was prepared as described in procedure B (89%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.7): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.52 (s, 2H), 7.39 (m, 5H), 5.31 (s, 2H), 3.84 (s, 6H).

**General Procedure C for Preparation of 4-(Carbobenzoxyoxy)anilines.** $SnCl_2 \cdot H_2O$ and $H_2O$ (0.6 mL) were added to a solution of the 4-(carbobenzoxyoxy)nitrobenzene (3.34 mmol) in ethanol (30 mL). The solution was refluxed until completed by TLC (∼30 min). The solution was cooled to room temperature and poured onto ice. The pH was neutralized to pH 7−8 with $NaHCO_3$ powder, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The organic layers were combined and washed with a brine solution (3 × 25 mL). The organic layer was separated, dried over $Na_2SO_4$, and filtered. The solvent as removed under reduced pressure. The product was purified by flash chromatography (acetone−chloroform).

**4-(Carbobenzoxyoxy)aniline (22).** Aniline analog **22** was prepared as described in procedure C (93%) and purified by flash chromatography eluting with 5% acetone in chloroform

($R_f$ = 0.48): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.48 (m, 7H), 6.96 (d, $J$ = 8.8 Hz, 2H), 6.63 (d, $J$ = 8.8 Hz, 2H), 5.22 (s, 2H), 3.63 (sb, 2H).

**3-Methoxy-4-(carbobenzoxyoxy)aniline (21).** Aniline analog **21** was prepared as described in procedure C (97%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.40): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.40 (m, 5H), 6.88 (d, $J$ = 8.4 Hz, 1H), 6.29 (d, $J$ = 2.4 Hz, 1H), 6.20 (dd, $J$ = 8.5 Hz, 2.59 Hz, 1H), 5.23 (s, 2H), 3.75 (s, 3H), 3.62 (sb, 2H).

**3,5-Methoxy-4-(carbobenzoxyoxy)aniline (19).** Aniline analog **19** was prepared as described in procedure C (93%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.30): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.40 (m, 5H), 5.92 (s, 2H), 5.25 (s, 2H), 3.78 (s, 6H), 3.63 (sb, 2H).

**General Procedure D for the Substitution of Anilines in the Variable Substituent Domain.** 4-Carbobenzoxyoxy-protected aniline (0.366 mmol) was added to a solution of **25** (0.183 mmol) in anhydrous $CH_2Cl_2$ (3 mL) over 4A molecular sieves in an argon-charged flame-dried 10 mL two-neck round bottom flask. The solution was stirred at room temperature, and $BF_3{\cdot}OEt_2$ (5−10 mol % $BF_3{\cdot}OEt_2$, 1−2 $\mu$L) was added. The reaction was judged to completion by TLC after 4 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL) and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layers were combined, dried over $Na_2SO_4$, and filtered. The solvent was removed under reduced pressure. The product was purified by flash chromatography (acetone−chloroform).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-(4-(carbobenzoxyoxy)-anilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one.** The protected ((carbobenzoxyoxy)anilino)azatoxin was prepared as described in procedure D (23%) and purified by flash chromatography eluting 5% acetone in chloroform ($R_f$ = 0.32); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.20 (s, 1H), 7.47−7.20 (m, 13H), 7.11 (t, $J$ = 7.61 Hz, 1H), 7.07 (d, $J$ = 8.80 Hz, 2H), 6.66 (d, $J$ = 8.89 Hz, 2H), 6.54 (s, 2H), 6.06 (s, 1H), 5.27 (s, 4H), 4.86 (dd, $J$ = 7.80, 2.74 Hz, 1H), 4.48 (m, 3H), 3.82 (db, 1H), 3.69 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-(3-methoxy-4-(carbobenzoxyoxy)anilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one.** The protected (methoxy(carbobenzoxyoxy)-anilino)azatoxin was prepared as described in procedure D (33%) and purified by flash chromatography eluting with 20% acetone in chloroform ($R_f$ = 0.55): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.38 (s, 1H), 7.46−7.22 (m, 14H), 7.10 (t, $J$ = 7.55 Hz, 1H), 7.01 (d, $J$ = 8.23 Hz, 1H), 6.52 (s, 2H), 6.22 (s, 1H), 6.08 (s, 1H), 5.28 (s, 2H), 5.26 (s, 2H), 4.86 (dd, $J$ = 7.8, 2.74 Hz, 1H), 4.49−4.44 (m, 3H), 3.85 (db, 1H), 3.73 (s, 3H), 3.66 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-(3,5-dimethoxy-4-(carbobenzoxyoxy)anilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido-[3,4-*b*]indol-3-one.** The protected (dimethoxy(carbobenzoxy-oxy)anilino)azatoxin was prepared as described in procedure D (23%) and purified by flash chromatography eluting with 15% acetone in chloroform ($R_f$ = 0.65): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.27 (s, 1H), 7.46−7.26 (m, 13H), 7.13 (t, $J$ = 7.54 Hz, 1H), 6.53 (s, 2H), 6.11 (s, 1H), 5.90 (s, 2H), 5.29 (s, 2H), 5.27 (s, 2H), 4.87 (dd, $J$ = 7.8, 2.74 Hz, 1H), 4.58−4.43 (m, 3H), 3.85 (db, 1H), 3.77 (s, 6H), 3.67 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-anilino-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one.** The protected (anilino)-azatoxin was prepared as described in procedure D (75%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.35): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.27 (s, 1H), 7.43−7.19 (m, 10H), 7.07 (t, $J$ = 7.41 Hz, 1H), 6.81 (t, $J$ = 7.18 Hz, 1H), 6.67 (d, $J$ = 8.23 Hz, 2H), 6.55 (s, 2H), 6.13 (s, 1H), 5.27 (s, 2H), 4.91 (dd, $J$ = 7.8, 2.75 Hz, 1H), 4.81 (db, 1H), 4.52−4.43 (m, 3H), 3.69 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-(3,4,5-trimethoxyanilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one.** The protected (trimethoxyanilino)azatoxin was prepared as described in procedure D (60%) and purified by 15% acetone in chloroform ($R_f$ = 0.6): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.42 (sb, 1H), 7.41−7.18 (m, 7H), 7.05 (t, $J$ = 7.5 Hz, 1H), 6.90 (t, $J$ = 7.3 Hz, 1H), 6.48 (s, 2H), 6.07 (s, 2H), 5.82 (s, 1H), 5.21 (s, 2H), 4.97 (dd, $J$ = 7.7, 2.69 Hz, 1H), 4.52 (m, 1H), 4.45 (t, $J$ = 8.5 Hz, 1H), 4.18 (dd, $J$ = 6.4, 4.87 Hz, 1H), 3.66 (s, 6H), 3.63 (s, 6H), 3.52 (s, 3H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbobenzoxyoxy)phenyl)-11-(4-aminobenzoxy)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one.** The protected (aminobenzoxy)azatoxin was prepared as described in procedure D (55%) and purified by flash chromatography eluting with 10% acetone in chloroform ($R_f$ = 0.55): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.49 (sb, 1H), 7.69 (d, $J$ = 8.43 Hz, 2H), 7.38−7.20 (m, 6H), 7.15 (d, $J$ = 7.82 Hz, 1H), 7.04 (t, $J$ = 7.5 Hz, 1H), 6.87 (m, 2H), 6.68 (d, $J$ = 8.82 Hz, 1H), 6.49 (s, 2H), 5.87 (s, 1H), 5.16 (s, 2H), 5.12 (dd, $J$ = 8.7, 2.74 Hz, 1H), 4.55 (m, 1H), 4.45 (t, $J$ = 8.7 Hz, 1H), 4.03 (dd, $J$ = 8.2 Hz, 5.42 Hz, 1H), 3.72 (s, 3H), 3.66 (s, 6H).

**General Procedure E for the Deprotection of 4-(Carbobenzoxyoxy)benzenes.** A suspension of the 4-carbobenzoxyoxy-protected phenol (0.013 mmol) in ethanol (5 mL) containing 10% Pd/C (20 mg) was stirred under a $H_2$ atmosphere (balloon pressure) at room temperature overnight. The solution was filtered through a plug of Celite. The solvent was evaporated off under reduced pressure, and the product was purified by flash chromatography (acetone−chloroform).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(4-hydroxyanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (27).** The (hydroxyanilino)-azatoxin analog **27** was prepared as described in procedure E (80%) and purified by flash chromatography eluting with 15% acetone in chloroform ($R_f$ = 0.42): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.02 (sb, 1H), 7.40−7.18 (m, 4H), 7.09 (t, $J$ = 7.4 Hz, 1H), 6.78 (d, $J$ = 8.82 Hz, 2H), 6.59 (d, $J$ = 8.87 Hz, 2H), 6.54 (s, 2H), 6.61 (s, 1H), 5.56 (s, 1H), 4.74 (dd, $J$ = 7.8, 2.72 Hz, 1H), 4.58 (m, 1H), 4.42 (m, 2H), 3.80 (s, 6H), 3.44 (db, 1H). Anal. ($C_{26}H_{27}N_3O_6$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(3-methoxy-4-hydroxyanilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (29).** The (methoxyhydroxyanilino)azatoxin analog **29** was prepared as described in procedure E (81%) and purified by flash chromatography eluting with 20% acetone in chloroform ($R_f$ = 0.32): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.06 (sb, 1H), 7.35−7.20 (m, 3H), 7.11 (t, $J$ = 7.4 Hz, 1H), 6.85 (d, $J$ = 8.36 Hz, 1H), 6.53 (s, 2H), 6.25 (dd, $J$ = 8.4, 2.45 Hz, 1H), 6.20 (d, $J$ = 2.41 Hz, 1H), 6.11 (s, 1H), 5.56 (sb, 1H), 5.13 (sb, 1H), 4.80 (dd, $J$ = 7.5, 2.74 Hz, 1H), 4.59 (m, 1H), 4.43 (m, 2H), 3.81 (s, 3H), 3.79 (s, 6H), 3.49 (db, 1H). Anal. ($C_{27}H_{29}N_3O_7$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(3,5-dimethoxy-4-hydroxyanilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indole-3-one (30).** The (dimethoxyhydroxyanilino)azatoxin analog **30** was prepared as described in procedure E and purified by flash chromatography eluting with 20% acetone in chloroform ($R_f$ = 0.23): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.99 (sb, 1H), 7.36−7.15 (m, 3H), 6.52 (s, 2H), 6.11 (s, 2H), 6.07 (s, 1H), 5.54 (sb, 1H), 5.23 (sb, 1H), 4.71 (dd, $J$ = 7.5, 2.73 Hz, 1H), 4.67 (d, $J$ = 1.7 Hz, 1H), 4.43 (t, $J$ = 8.4 Hz, 1H), 4.22 (m, 1H), 3.66 (s, 6H), 3.58 (s, 6H), 3.52 (db, $J$ = 7.2 Hz, 1H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-phydroxyhenyl)-11-(3,4,5-trimethoxyanilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (31).** The (trimethoxyanilino)azatoxin analog **31** was prepared as described in procedure E (78%) and purified by flash chromatography eluting with 20% acetone in chloroform ($R_f$ = 0.42): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.42 (sb, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.19 (d, $J$ = 7.6 Hz, 1H), 7.05 (t, $J$ = 7.2 Hz, 1H), 6.90 (t, $J$ = 7.4 Hz, 1H), 6.48 (s, 2H), 6.07 (s, 2H), 5.82 (s, 1H), 5.50 (d, $J$ = 8.41 Hz, 1H), 4.97 (dd, $J$ = 7.7, 2.69 Hz, 1H), 4.52 (m, 1H), 4.45 (t, $J$ = 8.6 Hz, 1H), 4.18 (dd, $J$ = 7.5, 4.87 Hz, 1H), 3.66 (s, 6H), 3.64 (s, 6H), 3.52 (s, 3H). Anal. ($C_{30}H_{33}N_3O_8$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-anilino-1*H*,6*H*-oxazolo[3′,4′:1,6]-pyrido[3,4-*b*]indole-3-one (26).** The anilinoazatoxin analog

**26** was prepared as described in procedure E (63%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.19): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.27 (s, 1H), 7.34−7.19 (m, 4H), 7.10 (t, $J$ = 7.3 Hz, 1H), 6.81 (t, $J$ = 7.2 Hz, 1H), 6.68 (d, $J$ = 8.66 Hz, 2H), 6.52 (s, 2H), 6.11 (s, 1H), 5.57 (sb, 1H), 4.92 (dd, $J$ = 7.6, 2.52 Hz, 1H), 4.52−4.42 (m, 3H), 3.81 (db, 1H), 3.77 (s, 6H). Anal. ($C_{26}H_{27}N_3O_5$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(4-aminobenzoxy)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (33).** The (aminobenzoxy)-azatoxin analog **33** was prepared as described in procedure E (82%) and purified by flash chromatography eluting with 5% acetone in chloroform ($R_f$ = 0.19): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.49 (s, 1H), 7.69 (d, $J$ = 8.43 Hz, 2H), 7.30 (d, $J$ = 8.11 Hz, 1H), 7.15 (d, $J$ = 7.82 Hz, 1H), 7.04 (t, $J$ = 7.3 Hz, 1H), 6.87 (m, 2H), 6.68 (d, $J$ = 8.82 Hz, 1H), 6.49 (s, 2H), 5, 87 (s, 1H), 5.12 (dd, $J$ = 8.74, 2.74 Hz, 1H), 4.55 (m, 1H), 4.45 (t, $J$ = 8.7 Hz, 1H), 4.03 (dd, $J$ = 8.2, 5.42 Hz, 1H), 3.72 (s, 3H), 3.66 (s, 6H). Anal. ($C_{28}H_{29}N_3O_7$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbomethoxyoxy))-11-(4-fluoroanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (10a).** The protected (fluoroanilino)azatoxin analog **10a** was prepared as described in procedure A and purified by flash chromatography eluting with 6% acetone in chloroform ($R_f$ = 0.30): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.31 (d, $J$ = 8.05 Hz, 1H), 7.08 (m, 2H), 6.96 (m, 3H), 6.81 (m, 2H), 6.65 (s, 2H), 5.94 (s, 1H), 5.66 (d, $J$ = 8.31 Hz, 1H), 4.87 (dd, $J$ = 2.81, $J$ = 8.04 Hz, 1H), 4.62 (m, 1H), 4.49 (t, $J$ = 8.5 Hz, 1H), 4,18 (dd, $J$ = 5.27, $J$ = 8.04 Hz, 1H), 3.77 (s, 3H), 3.71 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbomethoxyoxy))-11-(4-nitroanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (10b).** The protected (nitroanilino)azatoxin analog **10b** was prepared as described in procedure A and purified by flash chromatography eluting with 10% acetone in chloroform ($R_f$ = 0.42): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.76 (s, 1H), 8.14 (d, $J$ = 9.17 Hz, 2H), 7.35 (m, 2H), 7.23 (t, $J$ = 7.78 Hz, 1H), 7.14 (t, $J$ = 7.27 Hz, 1H), 6.76 (d, $J$ = 9.11 Hz, 2H), 6.46 (s, 2H), 6.06 (s, 1H), 5.17 (m, 2H), 4.47 (m, 2H), 4.34 (m, 1H), 3.86 (s, 3H), 3.63 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-(carbomethoxyoxy)-11-(4-cyanoanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido [3,4-*b*]indol-3-one (10c).** The protected (cyanoanilino)azatoxin analog **10c** was prepared as described in procedure A and purified by flash chromatography eluting with 6% acetone in $CH_2Cl_2$ ($R_f$ = 0.35): $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.39 (s, 1H), 7.52 (d, $J$ = 8.59 Hz, 2H), 7.35−7.26 (m, 3H), 7.14 (t, $J$ = 7.58 Hz, 1H), 6.73 (d $J$ = 8.73 Hz, 2H), 6.51 (s, 2H), 6.11 (s, 1H), 5.02 (dd, $J$ = 8.50, 2.03 Hz, 1H), 4.46−4.36 (m, 3H), 3.89 (s, 3H), 3.70 (s, 6H).

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(4-fluoroanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (11).** The (fluoroanilino)-azatoxin **10a** (16.5 mg, 30 mmol) was added to a solution of sodium methoxide (90 mmol) in methanol (1 mL). The reaction was followed to completion by TLC. Saturated aqueous $NH_4Cl$ (200 mL) was added followed by $CH_2Cl_2$. The organic layer was separated, dried over $Na_2SO_4$, and filtered. The solvent was removed under reduced pressure. The product was purified by flash chromatography eluting with 20% acetone in $CHCl_3$ ($R_f$ = 0.36) to yield the product **11** as a white solid (10.8 mg, 73%) as a white solid: mp slow dec 150 °C; $[\alpha]^{22}_D$ −139° (*c* 0.3, DMSO); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.48 (s, 1H), 7.28 (d, $J$ = 8.0 Hz, 1H), 7.09−7.04 (m, 2H), 7.01-6.86 (m, 2H), 6.78 (dd, $J$ = 8.92 Hz, $J$ = 4.55 Hz, 2H), 6.49 (s, 2H), 5.86 (s, 1H), 5.65 (d, $J$ = 8.26 Hz, 2H), 4.85 (dd, $J$ = 8.14 Hz, $J$ = 2.95 Hz, 1H), 4.53−4.49 (m, 1H), 4.41 (t, $J$ = 4.95 Hz, 1H), 4.12 (dd, $J$ = 8.07 Hz, $J$ = 5.41 Hz, 1H), 3.66 (s, 6H). Anal. ($C_{26}H_{26}N_3O_5F$) C, H. N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(4-nitroanilino)-1*H*,6*H*-oxazolo[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (13).** The (nitroanilino)azatoxin analog was deprotected as in **11**. Purification by flash chromatography eluting with 12% acetone in $CH_2Cl_2$ ($R_f$ = 0.35) yielded the product **13** as a white solid (64%): mp dec 215 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.06 (s, 1H), 7.49 (d, $J$ = 8.47 Hz, 1H), 7.31 (d, $J$ = 8.06, 1H), 7.15 (d, $J$ = 7.86 Hz, 1H), 7.07 (dd apparent t, $J$ = 7.32 Hz, 1H), 6.95−6.86 (m, 3H), 6.65 (s, 2H), 5.96 (s, 1H), 5.15 (dd, $J$ = 8.98 Hz, 2.92 Hz, 1H), 4.68−4.64 (m, 1H), 4.50 (t, $J$ = 8.68, 1H), 4.02 (dd, $J$ = 8.52, 5.15 Hz, 1H), 3.71 (s, 6H). Anal. ($C_{26}H_{26}N_4O_7$) C, H, N.

**(5*R*,11*R*,11a*S*)-4,5,11,11a-Tetrahydro-5-(3,5-dimethoxy-4-hydroxyphenyl)-11-(4-cyanoanilino)-1*H*,6*H*-oxazolo-[3′,4′:1,6]pyrido[3,4-*b*]indol-3-one (14).** The (cyanoanilino)-azatoxin was deprotected as in **11**. Purification by flash chromatography eluting with 15% acetone in $CH_2Cl_2$ ($R_f$ = 0.29) yielded the product **14** as a white solid (71%): mp dec 190−194 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.10 (s, 1H), 8.49 (s, 1H),7.48 (d, $J$ = 8.32 Hz, 2H), 7.30 (d, $J$ = 8.03 Hz, 1H), 7.14 (d, $J$ = 7.84 Hz, 1H), 7.05 (dd apparent t, $J$ = 7.33 Hz, 1H), 6.94−6.83 (m, 3H), 6.49 (s, 2H), 5.88 (s, 1H), 5.15 (dd, $J$ = 8.59, 2.40 Hz, 1H), 4.56−4.52 (m, 1H), 3.98 (dd, $J$ = 7.99, 5.70 Hz, 1H), 3.66 (s, 6H). Anal. ($C_{27}H_{27}N_4O_5$) C, H, N.

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