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THE SYNTHESIS AND EVALUATION OF BENZANNELATED-AZATOXINS: THE BENZAZATOXINS

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Abstract: The synthesis and evaluation of azatoxin congeners possessing annealed aromatic frameworks are described. The compounds were evaluated for their abilities to affect topoisomerase II inhibition through the stabilization of "cleavable complex" and for the inhibition of tubulin polymerization using purified bovine brain tubulin.

DNA topoisomerase II (topo II) is responsible for catalyzing the conformational and topological changes in DNA required for replication. Because of its critical role in maintaining cell viability and the discovery and development of agents that inhibit its functions, topo II has emerged as one of the foremost targets for the clinical treatment of cancer.[1-6] Our investigations of agents that inhibit topo II led to the postulate that many of these agents, which operate through both intercalative and non-intercalative associative interactions, possess a single pharmacophore (Figure 1).[7,8] This pharmacophore has been further corroborated and continues to be refined by ongoing research.[9-11] Additionally, some agents that inhibit the function of topo II also possess structural resemblance to inhibitors of tubulin polymerization. Azatoxin, for example, exhibits dual targeting of both topo II and tubulin (Figure 1).[7,8,11,12] This agent has been shown to exert its tubulin related activity by acting at the colchicine binding site on the tubulin α,β-dimer, which has been a target of prior studies in our laboratory summarized by Figure 2.[12-14] Agents that exhibit dual targeting in this regard may display cooperativity of these activities in the induction of cytotoxic responses. Therefore, enhancing our understanding of the structural prerequisites for the inhibition of topo II, the inhibition of tubulin polymerization, and dual inhibition may permit the rational design of agents which possess greater cytotoxic

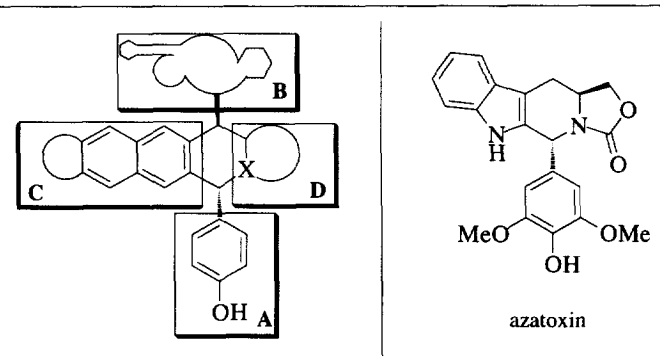

Figure 1. Proposed model pharmacophore for topo II inhibitors (left) and the structure of azatoxin (right).

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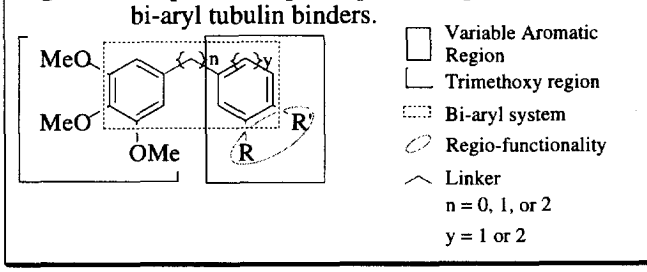
potential. As part of our research program directed at the development of agents that possess enhanced topo II inhibition activity and to further define pharmacophoric relationship between agents that exhibit topo II and tubulin polymerization inhibitory activities, we desired the synthesis and evaluation of agents structurally related to azatoxin which possess extended aromatic frameworks. This work, therefore, represents an effort to further define the intercalative domain of our pharmacophore (Figure 1, Domain C) for agents that inhibit topo II and to probe the structural limits of our composite pharmacophore for bi-aryl agents that inhibit the polymerization of tubulin (Figure 2). These studies have yielded additional insight into the structure-activity profiles of agents which exhibit cytotoxicity through these activities.

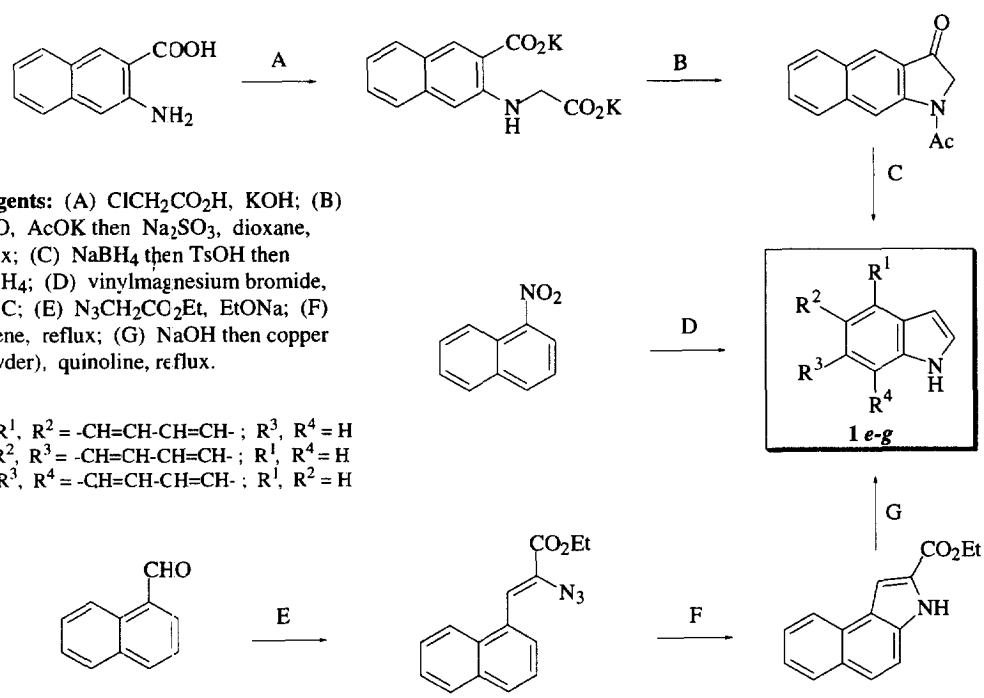
Figure 2. Proposed composite pharmacophore for bi-aryl tubulin binders.



Chemistry[15]

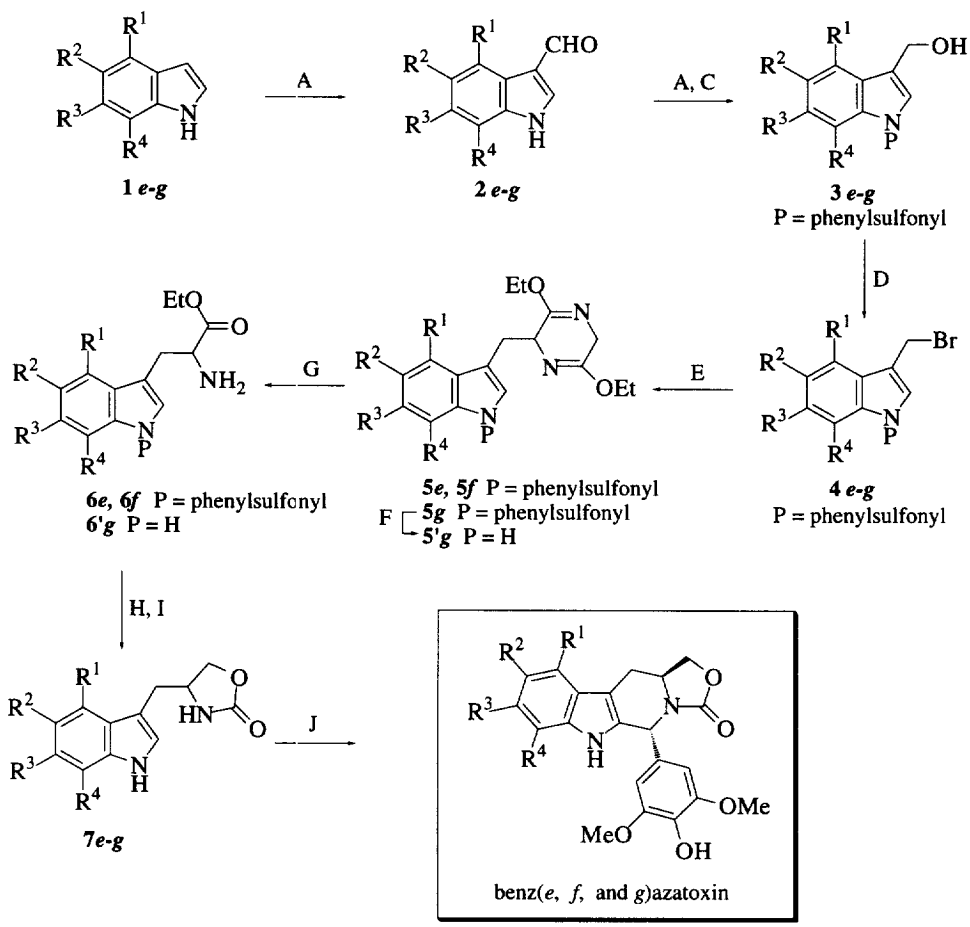
The requisite indolic core structures were prepared by the modification of existing methodology or by the utilization of literature preparations (Figure 3).[16-18] Thus, benz(*f*)indole was obtained in three steps from 2-amino-naphthoic acid. Benz(*e*)indole was obtained in five steps from 1-naphthaldehyde by utilizing Moody's azide method. Benz(*g*)indole was realized from 2-nitronaphthalene as reported by Bartoli *et al.*[18]

Figure 3. Synthesis of benz(*e-g*)indoles.



With the realization of the requisite indoles (**1** ***e-g***) accomplished, the synthesis of the benzazatoxins proceeded, in part, by the utilization of our previously described methods (Figure 4).[7,8,11] Accordingly, formylation via Vilsmeier-Haack conditions afforded aldehydes (**2** ***e-g***). Protection of the indolic nitrogen as the phenylsulfonyl derivative, followed by exposure to sodium borohydride afforded alcohols (**3** ***e-g***). Treatment with dibromotriphenylphosphorane afforded sensitive bromides (**4** ***e-g***) which were alkylated with lithio-2,5-

Figure 4. Synthesis of the benzazatoxins.



$e = R^1$, R^2 = -CH=CH-CH=CH- ; R^3, R^4 = H
$f = R^2$, R^3 = -CH=CH-CH=CH- ; R^1, R^4 = H
$g = R^3$, R^4 = -CH=CH-CH=CH- ; R^1, R^2 = H

Reagents: (A) $POCl_3$, DMF; (B) NaH, $PHSO_2Cl$, THF; (C) $NaBH_4$, THF; (D) Br_2PPh_3, CH_2Cl_2; (E) lithio-2,5-diethoxypyrazine; (F) sodium naphthalide; (G) 20% HCl; (H) $NaBH_4$, THF/H_2O; (I) NaOEt, HOEt, $(EtO)_2CO$; (J) syringaldehyde dimethylacetal.

diethoxypyrazine to afford (**5 *e-g***). Exposure to 10% HCl *in situ* afforded amino-esters (**6 *e*, 6 *f***); (**5 *g***) was further treated *in situ* with lithium naphthalide to afford (**5' *g***) prior to acidic hydrolysis to afford the free indole (**6'*g***). Reduction with sodium borohydride in aqueous ethanol followed by cyclization, and concomitant deprotection for (**6 *e*** and **6 *f***), with diethyl carbonate in ethanolic sodium ethoxide afforded oxazolidinones (**7 *e-g***). Modified Pictet-Spengler cyclization by reaction with syringaldehyde dimethylacetal afforded benz(*e, f,* and *g*)azatoxins.

Discussion

The data obtained for the inhibition of topo II, the inhibition of tubulin polymerization, and the growth inhibition (GI) of cultured cells are summarized in Table 1. While none of the benzazatoxins displayed marked topo II inhibition activity, these compounds were able to induce cytotoxic responses in cellular assays. This is in accord with prior studies in our laboratories[19,20] and with the work of Lee *et al.* in their investigations of agents that possess modified aromatic frameworks (Figure 5, upper) in the intercalative domain of our pharmacophore (Figure 1, Domain C).[9] The benzazatoxins, however, did display potent activity for the inhibition of tubulin polymerization. Benz(*e*)azatoxin and benz(*f*)azatoxin were found to possess nearly two fold greater activity than colchicine (Figure 5), a potent inhibitor of tubulin polymerization. Given the chirality requirements of ligands that bind to the colchicine binding site, the activity of the racemate is expected to represent one-half that of the optically pure active enantiomer.[21] Additionally, we have previously observed a fivefold increase in activity for the inhibition of tubulin polymerization upon conversion of the pendant dimethoxyphenol (Figure 1, Domain A)

Table 1. Summary of topo II activity, *in vitro* cellular assays, and tubulin polymerization inhibition.

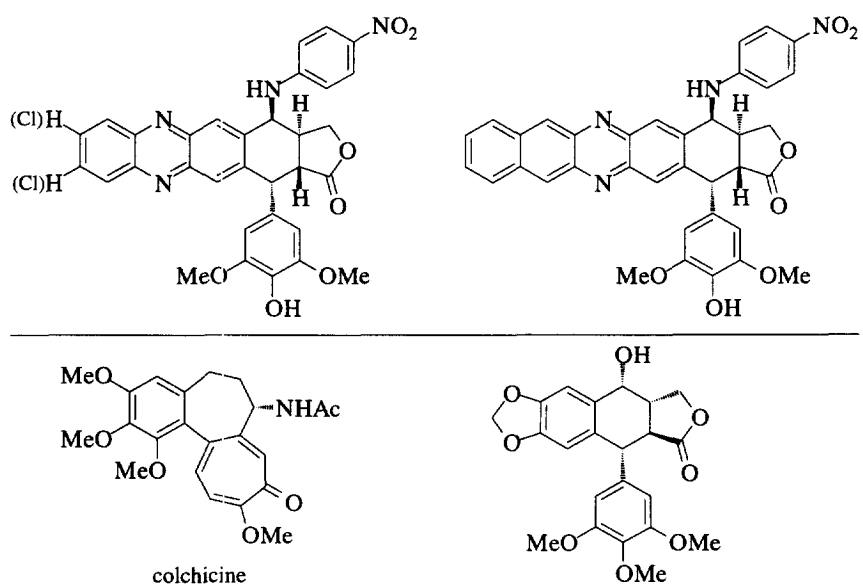
Compound	topo II Activity[a]	GI_{50}[b] MCF-7	PC-3	NCI-H520	Inhib. of Tubulin Poly. (IC_{50})[c]
colchicine	inactive	< 0.05 μM	< 0.05 μM	< 0.05 μM	11.8 μM
benz(e)azatoxin	inactive	2 μM	2 μM	0.8 μM	5.6 μM
benz(f)azatoxin	inactive	5 μM	10 μM	4 μM	6.7 μM
benz(g)azatoxin	inactive	>50 μM	>50 μM	>50 μM	51 μM
azatoxin	active	0.25 μM	0.2 μM	0.12 μM	20.3 μM

[a]As measured by the methods described ref 12. [b]GI_{50} values represent the concentration required to inhibit culture growth to 50% of control cultures. [c]IC_{50} values for three data points were obtained and averaged. Conditions were as follows: Purified bovine brain tubulin (120 μL, 4 mg/mL), PME (240 μL, 1 mM $MgSO_4$, 2 mM EGTA, 100 mM PIPES, pH 6.9), Benzazatoxin (32 μL, DMSO), and GTP (8 μL, 50 mM) were allowed to polymerize for 10 min. Absorbencies were recorded with a Varian DMS 90 UV-VIS spectrophotometer at 351 nm in a temperature controlled curvet holder at 30 °C.

to the corresponding trimethoxyaryl derivatives.[19,20] Given this, trimethoxy-benzazatoxin adducts may possess even greater activity for the inhibition of tubulin polymerization. Because of this activity increase for the inhibition of tubulin polymerization in the azatoxin series upon incorporation of a trimethoxyaryl into the pendant domain, we have postulated that this domain (Figure 1, Domain A) possesses structural correspondence with the trimethoxyaryl substituents found in many bi-aryl tubulin toxins. This modification, however, has also been shown to eliminate topo II inhibitory activity and thus precludes the use of trimethoxyaryl pendant domains in the design of agents which exhibit dual inhibition. Benz(*g*)azatoxin showed minimal activity for the inhibition of tubulin polymerization, which concurs with data obtained from cellular assays.

The extended aromatic domain of the benzazatoxins and other related agents[10] (Figure 5, upper) appears to have a markedly deleterious effect on the inhibition of topo II activity. These agents therefore may represent a spatial threshold in the aromatic framework to achieving topo II inhibitory activity. For the inhibition of tubulin polymerization, the benzazatoxins yield additional insight into the spacial tolerance of the colchicine binding site. Many bi-aryl inhibitors of tubulin polymerization possess aromatic frameworks in the "*Variable Aromatic Region*" (Figure 2) that are limited to one ring (colchicine, Figure 5) or two rings (podophyllotoxin, Figure 5). The benzazatoxins which possess potent activity for the inhibition of tubulin polymerization extend the "*Variable Aromatic Region*" (Figure 2) to include aromatic frameworks which possess three contiguous rings. Given the

Figure 5. Compounds structurally related to benz(*f*)azatoxin (upper). Colchicine and podophyllotoxin (lower).



increase in activity over the parent, azatoxin, for the inhibition of tubulin polymerization, the benzazatoxins provide a new avenue of exploration for the development for agents which possess enhanced tubulin mediated antimitotic activity. The benzazatoxins, however, provide limited insight into the structural prerequisites for dual inhibitor design.

Conclusion

In summary, the synthesis of congeners of azatoxin which possess extended aromatic frameworks has been disclosed. These agents, which provide valuable insight into the design of agents which inhibit topo II and tubulin polymerization, represent an effort to increase the potency of the parent, azatoxin. While none of the benzazatoxins displays topo II inhibitory activity, benz(*e*)azatoxin and benz(*f*)azatoxin displayed activity greater than that of colchicine for the inhibition of tubulin polymerization. Results from our continued study of these novel inhibitors of tubulin polymerization and our further investigations into dual inhibitor design will be reported in future correspondence.

References and Notes

1. Menta, E.; Palumbo, M. *Expert Opinion on Therapeutic Patents* **1997**, *7*, 1401.
2. Hoffman, L.; Moller, P.; Pedersenbjergaard, J.; Waage, A.; Pedersen, M.; Hirsch, F. R. *Annals of Oncology* **1995**, *6*, 781.
3. Sinha, B. K. *Drugs* **1995**, *49*, 11.
4. Corbett, A. H.; Osheroff, N. *Chem. Res. Toxicol.* **1993**, *6*, 585.
5. Pommier, Y. *Cancer Chemother. Pharmacol.* **1993**, *32*, 103.
6. Cummings, J.; Smyth, J. F. *Annals of Oncology* **1993**, *4*, 533.
7. Leteurtre, F.; Maddalengoitia, J.; Orr, A.; Guzi, T.; Lehnert, E.; Macdonald, T.; Pommier, Y. *Cancer Res.* **1992**, *52*, 4478.
8. Leteurtre, F.; Sackett, D. L.; Madalengoitia, J.; Kohlhagen, G.; Macdonald, T. L.; Hamel, E.; Paull, K. D.; Pommier, Y. *Biochem. Pharmacol.* **1995**, *19*, 1283.
9. Cho, S. J.; Kashiwada, Y.; Bastow, K, F.; Cheng, Y. -C.; Lee, K. -H. *J. Med. Chem.* **1996**, *39*, 1396.
10. Cho, S. J.; Tropsha, A.; Suffness, M.; Cheng, Y. -C.; Lee, K. -H. *J. Med. Chem.* **1996**, *39*, 1383.
11. Tepe, J. J.; Madalengoitia, J.; Slunt, K. M.; Werbovetz, K. W.; Spoors, P. G.; Macdonald, T. L. *J. Med. Chem.* **1996**, *39*, 2188.
12. Solary, E.; Leteurtre, F.; Paull, K. D.; Scudiero, D.; Hamel, E.; Pommier, Y. *Biochem. Pharmacol.* **1993**, *45*, 2449.
13. Andres, C. J.; Bernardo, J. E.; Yan, Q.; Hastie, S. B.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 565.
14. Miller, T. A.; Bulman, A. L.; Thompson, C. D.; Macdonald, T. L. *Bioorg. Med. Chem. Lett.*, submitted.
15. All compounds reported gave spectral data in accord with assigned structures.
16. Cook, J. M.; Allen, M. S.; Hamaker, L. K.; LaLoggia, A. J. *Syn. Comm.* **1992**, *22*, 2077.
17. Etienne, A.; Staehelin, A. *Bull. Soc. Chim.* **1954**, 748.
18. Bartoli, G.; Palmieri, G.; Bosco, M.; Dalpozzo, R. *Tetrahedron Lett.*, **1989**, *30*, 2129.
19. Unpublished Results.
20. Madalengoitia, J. S.; Werbovetz, K. A.; Lehnert, E. K.; Tepe, J. J.; Macdonald, T. L. *J. Med. Chem.* submitted.
21. Brossi, A.; Yeh, H. J. C.; Chrzanoska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quin, F.; Suffness, M.; Silverton, J. *J. Med. Res. Rev.* **1988**, *8*, 77.