Bioorganic & Medicinal Chemistry Letters 18 (2008) 3522–3525

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis of diketopiperazine-based carboline homodimers and in vitro growth inhibition of human carcinomas

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ARTICLE INFO

Article history:
Received 18 March 2008
Received in revised form 3 May 2008
Accepted 6 May 2008
Available online 9 May 2008

Keywords:
Carboline
Diketopiperazine
Pictet–Spengler
Indole
Anti-cancer
Heterocycle
Tryptophan
Tryprostatin

ABSTRACT

Starting from D- or L-tryptophan, we have synthesized and characterized six compounds **2.29**–**2.31a** and **b** that belong to a class of nitrogen heterocycles: the carboline-based homodimers. Each individual homodimer features a 1,3-*trans* relationship on each side of the central diketopiperazine core, but differs in absolute stereochemistry and also in substitution on the 4′ and 4″ oxygens (–Bn, $-CH_3$, or –H). The in vitro cytotoxicity of the six compounds was evaluated by measuring the growth inhibition in NCI–H520 and PC-3 human carcinoma cells. Phenol **2.30a** inhibited cancer cell growth approximately three times better than its enantiomer **2.30b** and possessed a GI_{50} comparable to the clinically used agent etoposide in both cell lines. We have concluded that both the stereochemistry imparted by L-tryptophan and the presence of hydroxy substituents at the 4′ and 4″ positions are necessary to generate cytotoxic properties in the homodimer class. We are now employing **2.30a** as a new lead compound in our efforts to discover improved indole-based cancer chemotherapeutics.

Interest in the synthesis of novel indole-based heterocycles has increased in recent years due to the prevalence of indoles in biologically-active natural products such as the fumitremorgin class[1,2] and the significance of the indole moiety in clinical chemotherapeutics like ellipticine.[3,4] As Danishefsky,[5] Corey,[6] Boger,[7] Cook,[8] Joullié[9] and others[10] have independently demonstrated, indoles can be easily incorporated into a complex target molecule by starting from the amino acid tryptophan (Trp). While pursuing a program to synthesize β-carboline derivatives from D- or L-Trp–OMe using the Pictet–Spengler reaction,[11] we serendipitously discovered a new class of cytotoxic carboline analogs that are similar in structure to ellipticine,[3,12] azatoxin,[13] gypsetin,[5] the tryprostatins,[14] and other[15] heterocycles. This group of bivalent, nitrogen-rich heterocycles, which we identify as the carboline homodimer class, contains several characteristic structural features: (1) seven consecutive fused rings (2) a central diketopiperazine (DKP) core, and (3) indole rings that cap each end (Fig. 1). Although each homodimer retains a 1,3-*trans* relationship on each side of the central DKP ring, the six homodimers also differ by their absolute configuration at C_{7a}/C_{15a} and C_{14}/C_6, and by their substitution at the 4′ and 4″ phenol oxygen (Scheme 1).

We synthesized three enantiomeric pairs of the homodimers: **2.29a** and **b** (R = –Bn); **2.30a** and **b** (R = –H); or **2.31a** and **b**

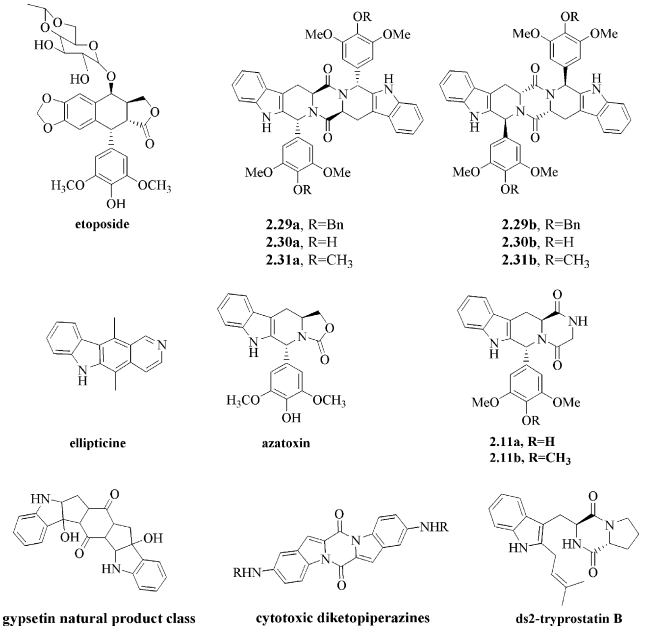

Figure 1. Structures of carboline homodimers, etoposide, and structurally-related nitrogen heterocycles.

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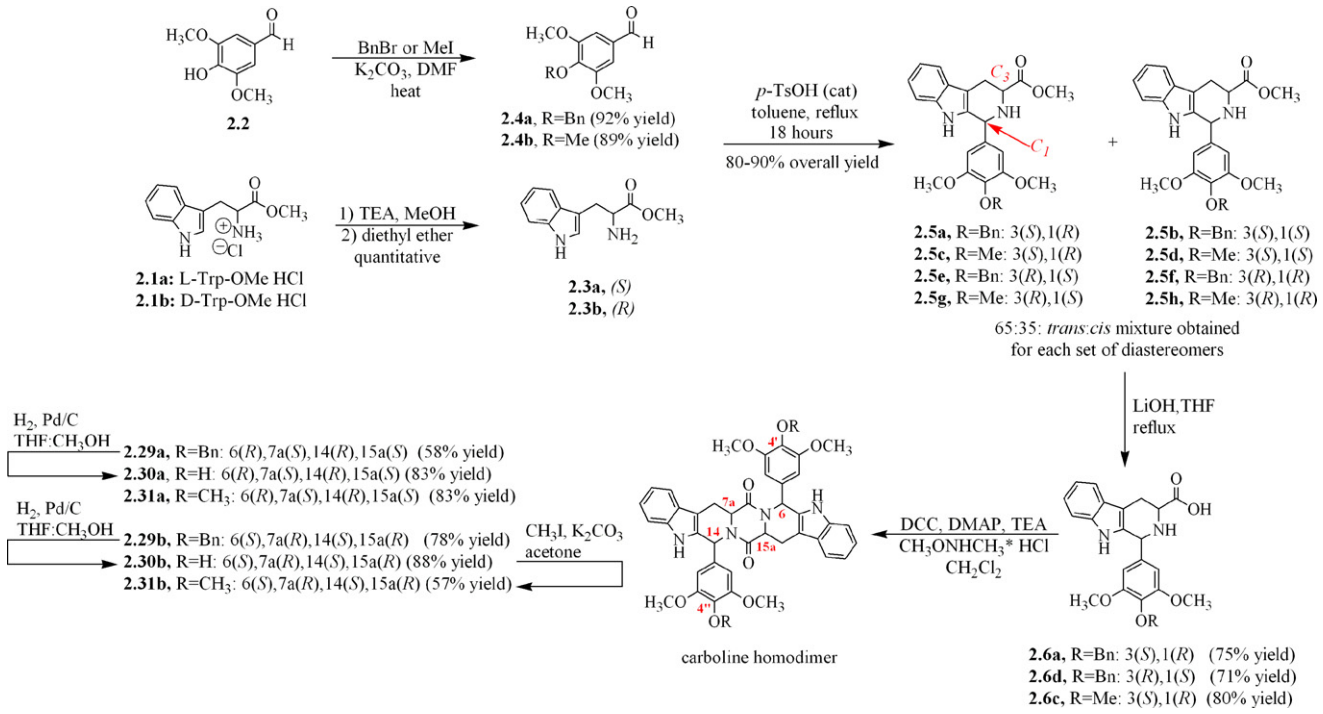
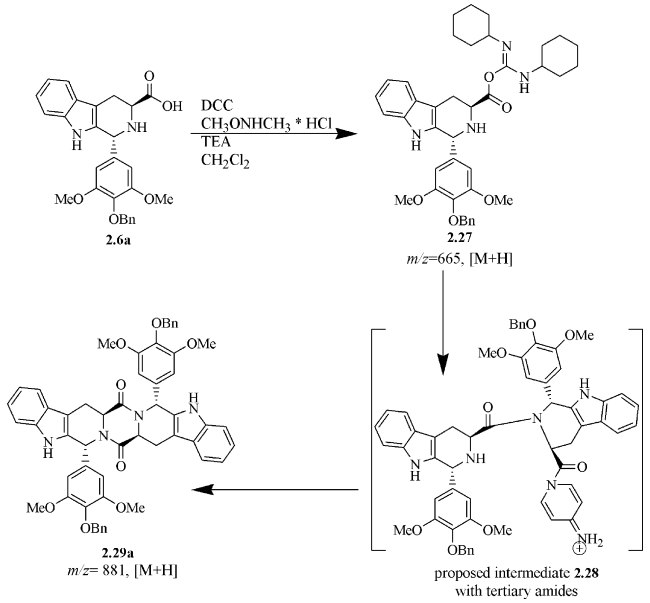
doi:10.1016/j.bmcl.2008.05.022



Scheme 1. Synthetic approach to synthesize carboline homodimers **2.29**–**2.31a** and **b**.

(R = –CH_3) as indicated in Scheme 1.[16] The key *trans*-1,3-disubstituted β-carboline synthons **2.5a**, **c**, **e**, and **g** were accessed via a Pictet–Spengler condensation in good yields,[17] and were subsequently saponified to their respective carboline-based carboxylic acids **2.6a**, **c**, and **d** using LiOH in THF.[11] *Trans*-carboline acid **2.6a** was then treated with *N*,*O*-dimethylhydroxylamine hydrochloride, DCC, and TEA in CH_2Cl_2 with the goal of synthesizing the Weinreb amide.[18] After this reaction was complete, the two products observed on thin-layer chromatography (TLC) were isolated using silica gel column chromatography in approximately a 1:1 ratio. Subsequent spectroscopic analysis revealed that the less polar product (R_f = 0.69; 9:1:$CHCl_3$/acetone) was in fact the DCC-based carboline intermediate **2.27** (Scheme 2), while the more polar spot (R_f = 0.58) was a 2,5-DKP-based dimer of our carboline core **2.29a**. Although 2,5-DKPs are well-documented products of α-amino acid coupling,[15a,16a,19] we were surprised and intrigued by our result; no DKP products were isolated when the reagent PyBop was used to couple **2.6a** with various amino acids during previous experiments.[11] Overall, and as detailed in Scheme 2, we think that **2.29a** resulted from attack of a second carboline molecule on the activated DCC intermediate **2.27** and that the ring closure was facilitated by populating accessible conformations of the two tertiary amides present in **2.28**.[20] Furthermore, to hasten the rate of our reaction and improve the homodimer yield we added *N*,*N*-dimethylaminopyridine (DMAP), a well known acylation catalyst. This simple change promoted complete conversion of starting material, improved our yield of sister compound **2.29b** to 78% and shortened our reaction time to 5 h.



Scheme 2. Proposed intermediates in the formation of the carboline homodimers.

With three pairs of homodimer enantiomers in hand, we next evaluated the compounds' abilities to inhibit the growth of lung (NCI–H520) and prostate (PC-3) human cancer cells as a measure of cytotoxicity. Two of the six dimers screened exhibited double-digit micromolar cytotoxicity (Tables 1 and 2). Phenol **2.30a** possessed a GI_{50} of 21.5 and 21.9 μM in lung and prostate lines, respectively, which was in the same range as the GI_{50} for etoposide and ds_2–Tps B (Fig. 1; Tables 1 and 2).[14c] Enantiomer **2.30b** was approximately three times less active in the same cell line (Table 2). Because phenol **2.30a** contains the same *trans* relationship and absolute stereochemistry as the azatoxin and etoposide cores, we were not surprised by its antiproliferative activity.

Table 1
In vitro GI_{50} data for homodimers **2.30a** and **b** against human carcinomas

Compound	NCI–H520[a]	PC-3[a]
Etoposide	13.5	12.5
Ds_2–Tps B	11.9	12.3
2.30a	21.5 (±1.1)	21.9 (±3.1)
2.30b	61.5 (±1.6)	55.3 (±1.0)

[a] GI_{50} is defined as the drug concentration required to inhibit 50% of cell growth.

Table 2
In vitro growth inhibition studies with carboline homodimers **2.29**–**2.31a** and **b** to measure cytotoxicity in human carcinoma lines

Compound	Percent cell survival[a]					
	NCI–H520			PC-3		
	10 μM[a]	50 μM[a]	100 μM[a]	10 μM[a]	50 μM[a]	100 μM[a]
Etoposide	78.6 (±3.3)	35.2 (±3.4)	21.3 (±2.7)	73.8 (±2.3)	41.2 (±4.2)	30.8 (±4.2)
2.29a	88.1 (±4.3)	81.3 (±3.2)	81.0 (±4.1)	98.2 (±5.4)	68.8 (±4.4)	54.5 (±0.6)
2.29b	>100	>100	84.5 (±3.4)	>100	92.7 (±2.3)	65.1 (±3.7)
2.30a	67.3 (±2.2)	16.5 (±1.6)	5.8 (±3.2)	80.7 (±2.4)	3.3 (±3.5)	1.6 (±4.8)
2.30b	88.7 (±4.4)	53.2 (±2.3)	26.8 (±1.7)	>100	43.6 (±3.7)	15.7 (±4.9)
2.31a	90.3 (±5.2)	90.8 (±4.6)	86.7 (±6.6)	93.0 (±3.2)	80.5 (±4.4)	65.0 (±2.5)
2.31b	99.8 (±1.3)	98.9 (±1.8)	98.9 (±1.9)	>100	>100	77.4 (±1.9)

Values are reported as the standard deviation of the mean.
[a] CellTiter 96 Aqueous nonradioactive cell proliferation assay (Promega) was used to determine growth inhibition. Percent inhibition values were calculated versus control wells and were completed in quadruplicate. Control wells contained 0.2% DMSO.

Upon further examination of the homodimer growth inhibition data in Tables 1 and 2, it is also striking to note that *only* the phenol-based enantiomers possessed growth inhibitory properties; placing an ether at the 4′ and 4″ O generates homodimers devoid of activity. Interestingly, our findings are in contrast to Ganesan et al.'s research on phenol **2.11a** and its methyl ether **2.11b**; the methyl ether possessed a notably lower GI_{50} than the corresponding phenol (Fig. 1).[2a] Our lab has also independently synthesized **2.11a** did not find that it possessed significant anti-proliferative properties against human carcinoma lines.[11] This suggests that homodimer **2.30a** (seven consecutive rings fused by a central DKP) may function via a different mechanism than glycine-based DKP ether **2.11b** (4 fused rings and a terminal DKP). Although mechanism was not explored here, we wonder if **2.30a** could be targeting topoisomerase II (like azatoxin and etoposide), tubulin, or interacting with DNA via groove binding or intercalation. Intercalation, like that found in the ellipticines,[21] is possible due to the presence of the aromatic indole moiety, and the aromatic pendant groups on C_6 and C_{14}, respectively. Molecular modeling of homodimer **2.30a** clearly shows the planarity of the terminal indole, but also suggests that the molecule can adopt a cup-like conformation that is controlled by the flexible DKP/carboline core (Fig. 2). Whatever the discrete molecular target or mechanism, enantiospecificity with growth inhibition data in a series of related compounds can be associated with a preferred binding conformation and/or site.

We were also particularly interested in the carboline homodimers when considering the concept of bivalency in drug design.

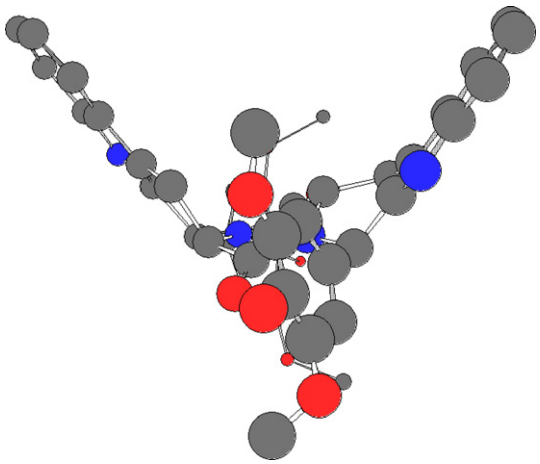


Figure 2. One representative low energy cup-like conformation of homodimer **2.30a** achieved after completing an energy minimization (Chem3D, MM2 Force Field).

Carlier et al. have recently shown that dimerization of a biologically inactive precursor to the natural product huperzine A produces a drug with twice its potency.[22] From Carlier's and others[23] research, it has become clear that bivalency is a useful derivatization and diversification technique for a synthetic agent or natural product in drug discovery. Remarkable enhancements of dimer biological activity over the individual monomer units have been observed, but it is important to note that the effectiveness of this technique depends entirely upon the nature of the drug target's binding site(s). Although most of the current bivalency literature involves use of a tether to connect two identical halves, our application of the concept to the aforementioned symmetrical fused-ring homodimers is also relevant. To our knowledge, this concept has not yet been formally extended to this class of cytotoxic indole-based homodimers.

In summary, we serendipitously discovered a new class of cytotoxic carboline analogs that are similar in structure to azatoxin and the fumitremorgin class of natural products while pursuing a program to synthesize carboline derivatives from D- or L-Trp. This group of bivalent, nitrogen-rich heterocycles, the carboline homodimer class, contains seven consecutive fused rings and is characterized by a central DKP core and two terminal indoles that cap each end of the molecule. We synthesized carboline homodimers **2.29a** and **b**–**2.31a**and **b** (Fig. 1) and evaluated their growth inhibitory properties in PC-3 and NCI–H520 cell lines as a measure of cytotoxicity. The enantiomeric pair of phenols **2.30a** and **b** possessed GI_{50}'s in both lung and prostate cell lines in the micromolar range. Interestingly enantiomer **2.30a** was twice as cytotoxic as **2.30b** and possessed a GI_{50} comparable to that of etoposide and ds2–Tps B[14c] in the cell lines investigated (Fig. 1). Considering these data, we conclude that the stereochemistry around the DKP ring junction is coupled to growth inhibition. Additionally, our data suggest that the presence of the 4-hydroxy-3,5-dimethoxybenzene pendant group was essential to elicit an inhibitory response. Overall for the homodimer class, –H $\gg$ –Me $\approx$ –Bn at the 4′ and 4″-positions, respectively. Because of **2.30a**'s structural similarity to etoposide and axatoxin, further biological testing is intended to determine whether **2.30a** may target topoisomerase II, tubulin, or interact with DNA. Overall, we remain interested in structurally diverse indole-based heterocycles like the carboline homodimers. and continue to explore the relationship between structure, activity, and mechanism in the carboline homodimer series and related compounds.

Acknowledgment

Dr. A.M. Deveau gratefully acknowledges the support of the University of New England for a University Faculty Mini-Grant.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.05.022.

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