Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 24, pp. 2871-2876, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00437-4

STRUCTURE ACTIVITY OF TOPOISOMERASE I POISONS RELATED TO HOECHST 33342

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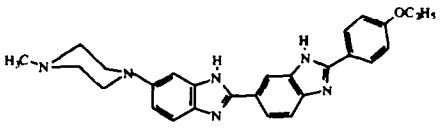
Abstract: A series of bisbenzimidazoles related to Hoechst 33342 were synthesized. Data on the relative activity of these bisbenzimidazoles as topoisomerase I poisons suggest that considerable flexibility exists in the location of the tertiary alkylamine moiety. With the exception of arylamine analogs, cytotoxicity was generally consistent with their relative potency as topoisomerase I poisons.

Introduction:

DNA topoisomerases represent a unique class of enzymes that alter the topological state of DNA by breaking and rejoining the phosphodiester backbone of DNA[1-3]. Topoisomerase inhibitors as a class of pharmacological agents have the potential to exhibit selective antibacterial, antifungal, antiprotozoal, anthelmintic, as well as antiviral activity. The broad spectrum of potent antineoplastic activity observed for camptothecin[4,5], a mammalian topoisomerase I inhibitor, has prompted further studies to identify other agents with similar pharmacological properties. It has recently been demonstrated that several minor groove-binding ligands (MGBLs) which exhibit antitumor activity can act as inhibitors of topoisomerase I [6,7]. Similar to camptothecin, these MGBLs have been shown to trap the reversible cleavable complex derived from DNA and topoisomerase I, resulting in a limited number of highly specific single-strand DNA breaks.

Preliminary studies have been performed with MGBLs such as distamycin, netropsin, berenil, 4',6-diamidino-2-phenylindole and 2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole. No clear correlation was observed between DNA binding affinity and topoisomerase I-mediated DNA cleavage. These data suggest that other factors, in addition to binding to the minor groove of DNA, contribute to the efficient trapping of the cleavable complex by specific MGBLs. The bisbenzimidazole derivative, 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, binds well to the minor groove of DNA with A + T specificity [7]. Several analogs of this bisbenzimidazole have been synthesized and examined for their potential to bind to DNA , with emphasis on their specificity to bind to various base pairs [8,9]. However, there has been no systematic study to examine those factors associated with this class of compounds which correlate with their potential to trap the cleavable DNA topoisomerase complex.

It has been recognized that the piperazine moiety, which exists positively charged at physiological pH, contributes to the DNA binding affinity of Hoechst 33342. In the present study a series of similarly substituted alkylamines related to Hoechst 33342 were synthesized, Figure 1, and evaluated for their potential to induce topoisomerase I-mediated DNA cleavage.



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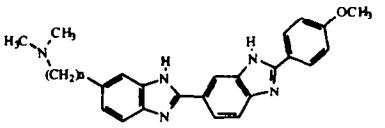
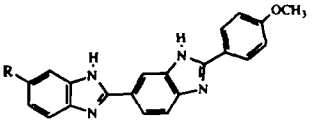
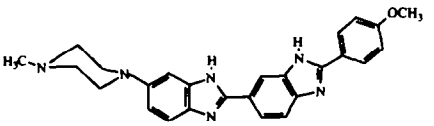


2; n = 3
3; n = 2
4, n = 1
5, n = 0



6, R = NO_2
7, R = NH_2



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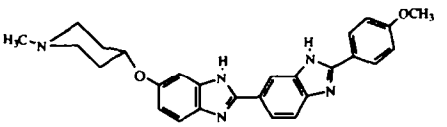

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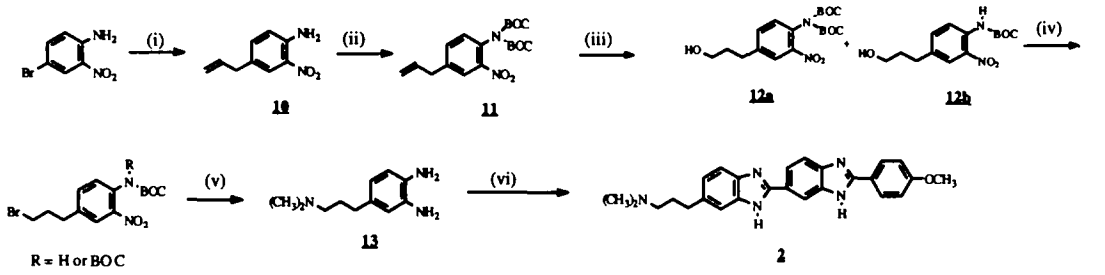
Chemistry:

Several compounds related to Hoechst 33342 were prepared by coupling of the appropriately substituted phenylenediamine with 5-formyl-2-(4-methoxyphenyl)-1H-benzimidazole. For the preparation of **2** (Scheme 1), allyltributyltin was coupled with 4-bromo-2-nitroaniline in the presence of $Pd(PPh_3)_2Cl_2$ to provide a 96% yield of 4-allyl-2-nitroaniline, **10**. This aniline derivative was converted to its bis-BOC derivative, **11**, prior to hydroboration with BH_3·THF followed by treatment with H_2O_2 in the presence of NaOH to provide a 83% yield of a mixture of the bis-BOC propyl alcohol, **12a**, and the mono-BOC propyl alcohol, **12b**, in a ratio of approximately 3:1. Conversion of these primary alcohols to their bromide derivatives was accomplished with PPh_3 and CBr_4 in CH_2Cl_2. Reaction of these propyl bromides with dimethylamine in the presence of Hunig base provided a 96% yield of BOC-protected 4-(N,N-dimethylaminopropyl)-2-nitroanilines, which were deprotected with TFA in CH_2Cl_2 and reduced to the phenylenediamine, **13**, with H_2 and Pd/C in EtOAc.

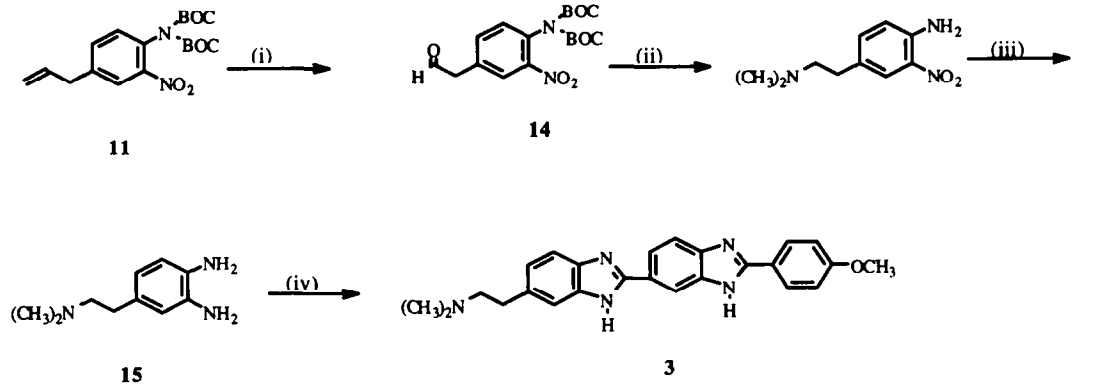
Scheme 1



(i): Allyltributyltin/$Pd(PPh_3)_2Cl_2$/PPh_3/DMF; (ii): $(BOC)_2O/Et_3N$/DMAP/CH_2Cl_2; (iii): a) BH_3·THF; b) $NaOH/H_2O_2$; (iv): PPh_3/CBr_4; (V): a) $(CH_3)_2NH$/Hunig Base, b) TFA/CH_2Cl_2; c) H_2/Pd-C/EtOAc; (vi): 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$.

The phenylenediamine required for the preparation of **3** (Scheme 2) was prepared by ozonolysis of **11** followed by treatment with methyl sulfide. Formation of the Schiff base of the resulting aldehyde was accomplished with N,N-dimethylamine. This Schiff base was reduced with $Na(CN)BH_3$ in MeOH to provide the N,N-dimethylamino derivative, which was treated with TFA in CH_2Cl_2 to remove the BOC groups and then reduced with Pd/C to yield the desired phenylenediamine, **15**.

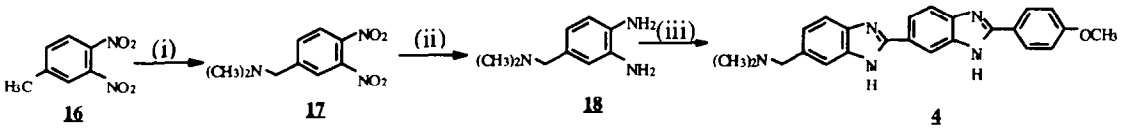
Scheme 2



(i): $O_3/(CH_3)_2S$; (ii): a) $(CH_3)_2NH$·HCl/$Na(CN)BH_3/CH_3OH$; b) TFA/CH_2Cl_2; (iii): H_2/Pd-C; iv): 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$.

The preparation of **4** (Scheme 3) was accomplished using 3,4-dinitrotoluene. Allylic bromination of this intermediate followed by reaction with dimethylamine gave **17** which was reduced to the corresponding phenylenediamine, **18**. Coupling of **18** with 5-formyl-2-(*p*-methoxyphenyl)benzimidazole resulted in the formation of **4**.

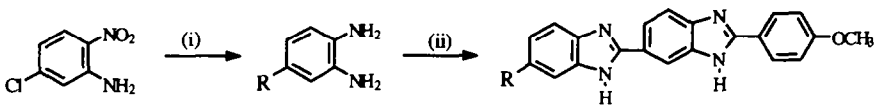
Scheme 3



(i): a) NBS/benzoyl peroxide/CCl_4/ hν, b) $(CH_3)_2NH$/Hunig base/acetone; (ii): H_2/Pd-C; (iii): 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$.

The formation of **5** and **8** was accomplished by synthesis of the requisite phenylenediamine using 4-chloro-2-nitroaniline as starting material (Scheme 4).

Scheme 4

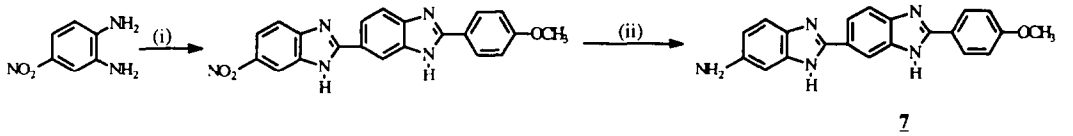


19, R = $N(CH_3)_2$ **5**, R = $N(CH_3)_2$
20, R = 4-Methylpiperazinyl **8**, R = 4-Methylpiperazinyl

(i): a) $(CH_3)_2NH$ or 1-methylpiperazine/K_2CO_3/DMF, b) H_2/Pd-C; (ii): 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$.

For the preparation of **7** and **9**, 4-nitrophenylenediamine and 4-(1-methylpiperidin-4-yloxy)phenylenediamine respectively were coupled with 5-formyl-2-(*p*-methoxyphenyl)benzimidazole (Schemes 5 and 6).

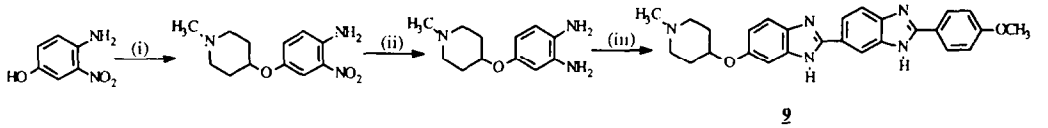
Scheme 5



7

(i): 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$; (ii): H_2/Pd-C.

Scheme 6



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(i): 1-methyl-4-hydroxypiperidine/DEAD/Ph_3P/THF; (ii): H_2/Pd-C; (iii) 5-formyl-2-(*p*-methoxyphenyl)benzimidazole/$PhNO_2$

Pharmacology:

Topoisomerase I-Mediated DNA Cleavage Assay: DNA topoisomerase I was purified from calf thymus gland by previously detailed procedures.[10] Plasmid YEPG was also purified by the alkali lysis methods followed by phenol deproteination and by CsCl/ethidium isopycnic centrifugation.[11] The end-labeling of the plasmid was accomplished as previously reported.[12] The cleavage assays were performed as reported previously.[13]

Evaluation of Cytotoxicity: The MTT-microtiter plate tetrazolium cytotoxicity assay (MTA) was used in this study to evaluate relative cytotoxicity.[14-16] CCRF CEM cells (30,000 cells/well) were seeded in 200 µl growth medium in Corning 96-well microtiter plates, and allowed to attach. Each dose of drug was evaluated in four replica plates. Each plate contained eight replicate control wells which were treated with DMSO only.

Results/Discussion:

The activity of these bisbenzimidazole derivatives as topoisomerase poisons and their cytotoxic activity in several human tumor cell lines relative to Hoechst 33342 are outlined in Table 1. These data reveal a major

Table 1. Pharmacological Activity of Substituted bisbenzimidazoles

Compound	Topoisomerase I-mediated DNA cleavage[a]	Cytotoxicity SK-Mel IC_{50} (µM)	Cytotoxicity PC3 IC_{50} (µM)	Cytotoxicity RPMI 8402 IC_{50} (µM)	Cytotoxicity CPT-K5 IC_{50} (µM)
1	1.0	1.6	0.71	0.005	0.5
2	0.5	10	28	1.2	7.1
3	0.2	8.5	43	0.61	6.1
4	0.4	10	52	0.38	6.3
5	0.001	26	4.9	0.13	0.65
6	0.001	n.a.	n.a.	0.65	3.9
7	0.01	6.2	19	1.7	14
8	1.0	1.1	0.84	<0.05	0.57
9	0.5	2.0	0.38	<0.04	0.44

[a]Values reported are the ratio of [Hoechst 33342] / [Drug] where [Hoechst 33342] and [Drug] are the concentrations that cause 50% cleavage of DNA in the presence of calf thymus topoisomerase I. Cleavage is calculated from the intensity of the strongest Hoechst specific band.

difference (10 to 500-fold) in the cytotoxic potency of these compounds toward lymphoblastoma (RPMI 8402) cells and either melanoma (SK-Mel) or prostatic carcinoma (PC3) cells. In the camptothecin resistant CPT-K5 cell line, compounds **1**, **8**, and **9** exhibited modest cross-resistance with IC_{50} values being 10 to 100 times higher. In contrast to these results, the differences in cytotoxicity between the parent cell line (RPMI 8402) and CPT-K5 for **2**, **3**, and **4**, were less generally than tenfold. While these compounds are active as topoisomerase I poisons, these data suggest that their mechanisms of inhibition may differ from camptothecin. In comparing the influence of increasing chain length on the relative potency of these derivatives as topoisomerase I poisons, **2** was more potent than either **3** or **4**. There were no major differences observed in the relative cytotoxicity between **2**, **3** or **4** in this study. It would appear that a wide-range of structural variation can be accommodated with retention of

activity as topoisomerase I poisons. Relative to compounds **1**, **8**, and **9**, these alkyl dimethylamines were approximately one-order of magnitude less cytotoxic. In view of their relatively weak topoisomerase I activity as observed *in vitro*, the arylamine derivatives, **5** and **7**, and the nitro derivative, **6**, were more cytotoxic than anticipated. Compounds **5**, **6**, and **7** did not exhibit major differences in cytotoxicity between RPMI 8402 and the camptothecin resistant subline, CPT-K5. This is consistent with the likelihood that mechanisms other than inhibition of topoisomerase I are associated with their cytotoxic activity.

These results indicate that the presence of a piperazine moiety is optimal for cytotoxic activity for this series of bisbenzimidazoles. The presence of a tertiary alkylamine linked by methylene groups at the 5-position in some of these bisbenzimidazoles appears to be related to increased potency as topoisomerase I poisons relative to either the 5-nitro analog, **6**, or the arylamine derivatives **5** and **7**.

Acknowledgment:

We are grateful to the Midwest Center for Mass Spectrometry at the University of Nebraska-Lincoln for providing mass spectral data and for the partial support of this facility by the National Science Foundation, Biology Division (Grant No. DIR9017262). We also wish to acknowledge Dr. Louis Barrows and Glenn Eldredge from the Department of Pharmacology and Toxicology, The University of Utah, Salt Lake City for performing the cytotoxicity studies in PC3 and SK-Mel cell lines. This study was supported by Grant CA 39662 from the National Cancer Institute (L.F.L.) and a fellowship grant from the Johnson & Johnson Discovery Research Fund (E.J.L.).

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(*Received in USA* 8 *September* 1994; *accepted* 9 *November* 1994)