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Aza and diaza bioisosteric anthracene-9,10-diones as antitumor agents

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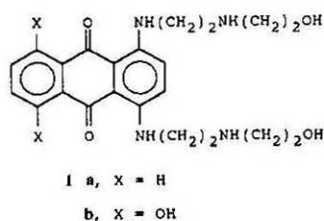
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Synthetic routes to aza and diaza bioisosteres related to the anthracene-9,10-dione, mitoxantrone, have been developed. The antitumor properties of these chemotypes are compared with those exhibited by the corresponding carbocyclic analogues. The sensitivity of the expressed cytotoxicities on the position(s) of the nitrogen atom(s) are discussed in terms of potential cellular targets. Several analogues show potential for clinical evaluations.

The anthracene-9,10-dione chemotypes amantantrone (**1a**) and mitoxantrone (**1b**) are clinically used in the treatment of leukemias, lymphomas and in combination therapy for advanced breast and ovarian cancer [1, 2]. The toxic side effects associated with the chemotherapeutic use of mitoxantrone (**1b**) are less pronounced than those exhibited by doxorubicin although myelotoxicity and cardiotoxicity are of clinical concern, especially in patients previously treated with doxorubicin [2].



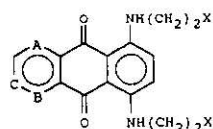
The need still exists for further studies of anthracene-9,10-dione congeners endowed with improved therapeutic efficacy, fewer toxic side effects, as well as effectiveness against drug resistant cell lines.

As in the anthracyclines, the cell killing events for the anthracene-9,10-dione are probably multimodal. Initial intercalation into DNA appears to be an important cellular event and this interaction may anchor the drugs at specific bases pair sites. This intercalated species can then disrupt DNA protein interactions, in particular, the interference of topoisomerase II [1, 3]. A few years ago our groups (University of Vermont and Boehringer Mannheim) launched a research effort to explore the effects of heteroatom substitution in the anthracene-9,10-dione chromophore on antitumor activity. The initial studies dealt with aza and diaza bioisosteres related to the carbocyclic models **1a** and **1b**. These chemotypes would retain the same spatial and planar characteristics for host molecular recognition such as DNA interaction and could be endowed with properties for increasing the affinity of the drug for DNA and/or effecting interactions with topoisomerase II [4]. In addition, in contrast to the carbocyclic models, these heterocyclic analogues would be

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much more readily reduced to anion-radicals and, therefore, be less prone to transfer an electron to molecular oxygen to produce undesirable radical species.

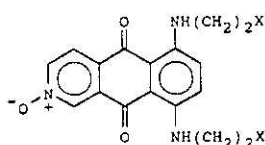
Towards these goals we have synthesized the azabioisosteres **2**, **3**, **4**, the N-oxides **5** and the diazabioisosteres **6**, **7**, **8**. The antitumor data for these analogues are presented and discussed.



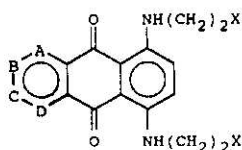
2 A = N, B = C = CH

3 C = N, A = B = CH

4 C = N, A = COH, B = CH



5



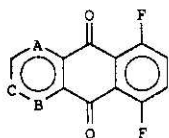
6 A = D = N, B = C = CH

7 B = D = N, A = C = CH

8 B = C = N, A = D = CH

SYNTHESIS

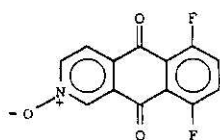
In general, the aza bioisosteres **2** [4, 5] and **3** [6] were synthesized by S_NAr displacements of the fluorides from **9** and **10**, respectively, by



9 A = N, B = C = CH

10 C = N, A = B = CH

11 C = N, A = COH, B = CH



12

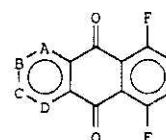
treatment with the appropriate aminoalkyl amine nucleophiles or aminoalkyl-N-t-butoxy-

carbonyl-amines (N-BOC-amines). The N-BOC analogues were then converted to the amine hydrochlorides by treatment with anhydrous hydrogen chloride.

The direct displacement of the fluorides from **11** to prepare analogues related to **4** was unsuccessful. This necessitated the conversion of **11** to the protected O-benzyl derivative, displacement of the fluorides with the appropriate amine nucleophile and removal of the benzyl groups by catalytic hydrogenation.

The analogues related to **5** were prepared by fluoride displacements from difluoro N-oxide **12** with the appropriate amino nucleophile [6].

The diazabioisosteres **6**, **7**, **8** were prepared in a similar pathway by displacements from **13**, **14**, **15**, respectively, with the appropriate amino nucleophiles [7].



13 A = D = N, B = C = CH

14 B = D = N, A = C = CH

15 B = C = N, A = D = CH

BIOLOGICAL RESULTS

A. Aza bioisosteres

(1) 6,9-Bis[(aminoalkyl)amino]benzo[g]quinoline-5,10-diones

The biological data [4] for some representative examples related to the 1-aza chemotype **2** are presented in Table 1.

(2) 6,9-Bis[(aminoalkyl)amino]benzo[g]isoquinoline-5,10-diones

The *in vitro* biological activities against L1210 murine leukemia and a human colon adenocarcinoma cell line sensitive (LoVo) and resistant to doxorubicin (LoVo/Dx) [6] for selected aza bioisosteres **3** are presented in Table 2.

Some *in vivo* data for analogue **3d** against P388 murine leukemia and a comparison with ametantrone (**1a**) are presented in Table 3.

The N-oxides **5a** [X = NH₂] and **5b** [X = N(CH₃)₂] exhibited ID₅₀ values (μg/mL) of 7.3 and 0.44, respectively, against the LoVo cell line and values of 62.6 and 2.6, respectively, against the LoVo/Dx cell line.

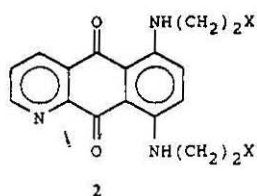
(3) 4-Hydroxy-6,9-bis[(aminoalkyl)amino]benzo[*g*]isoquinoline-5,10-diones

Only two congeners **4a** [$X = \text{NH}_2$] and **4b** [$X = \text{N}(\text{CH}_3)_2$] were evaluated in this series. The *in vitro* data against LoVo and LoVo/Dx along

with comparative data for **3d** and mitoxantrone (**1b**) are tabulated in Table 4.

Analogues **4b** and **3a** exhibited ID_{50} values ($\mu\text{g}/\text{mL}$) of 0.0015 and 0.006, respectively, in the L1210 murine leukemia screen.

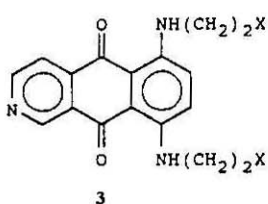
Table 1
Biological activities of analogues of **2**



Structure	X	L1210 Murine leukemia		
		In vitro ID ₅₀ ($\mu\text{g}/\text{mL}$)	In vivo	
			Dose (mg/kg)	T/C ^a (%)
2a	$\text{N}(\text{CH}_3)_2$	0.16	50, 25	130, 120
2b	$\text{CH}_2\text{N}(\text{CH}_3)_2$	1.7	50, 25	116, 106
2c	NH_2	1.5	20, 25	106, 114
2d	$\text{NH}(\text{CH}_2)_2\text{OH}$	5.8	25, 12.5	108, 116

^aMedian survival time of treated mice/ (median survival time of controls) $\times 100$.

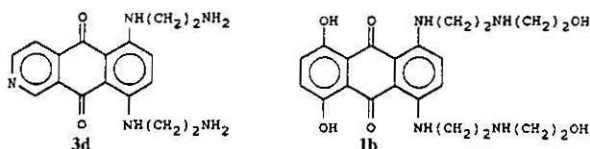
Table 2
In vitro cytotoxicities of analogues of **3**



Compounds	X	ID ₅₀ ($\mu\text{g}/\text{mL}$)		
		L1210	LoVo	LoVo/Dx
3a	$\text{N}(\text{CH}_3)_2$	0.006	0.08	0.13
3b	NHCH_2CH_3	0.01	– ^a	– ^a
3c	$\text{NH}(\text{CH}_2)_2\text{OH}$	0.06	1.3	99.5
3d(HCl)	NH_2	0.01	0.24	7.2
Ametantrone (1a)	–	0.03	0.4	34.7

^aNot determined.

Table 3
In vivo antitumor activities of **3d** and **1b** against P388 murine leukemia

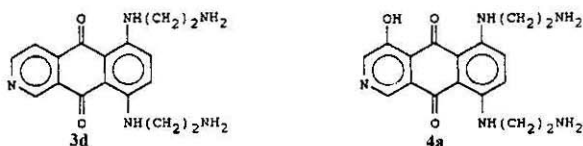


Compound	Dose ^a (mg/kg per day)	T/C ^b (%)
3d (maleate)	8	162
	12	170
	18	206
	22	250
	27	230
Mitoxantrone (1b)	2	172
	3	196
	4	175

^a 10^6 cells/mouse iv in CD2F1. Treatment iv on days 1, 4, 7 after tumor transplantation (day 0).

^bSee footnote a in Table 1.

Table 4
In vitro activities of **3d**, **4a** and mitoxantrone against LoVo and LoVo/Dx cells.

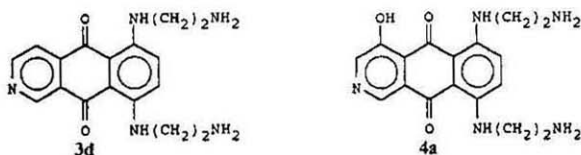


Compound	Dose ID ₅₀ ($\mu\text{g}/\text{mL}$)		
	LoVo	LoVo/Dx	RI ^a
3d (maleate)	0.24	7.2	30
4a (HCl)	0.1	0.7	7
Mitoxantrone (1b)	0.009	0.25	28

^aResistance index.

The *in vivo* data against P388 murine leukemia for **4a** and **3d** are listed in Table 5.

Table 5
Comparison of antitumor activities of **3d** and **4a** against P388 murine leukemia



Compound	Dose ^a (mg/kg per day)	T/C ^b (%)
3d (maleate)	8	162
	12	170
	18	206
	22	250
4a	0.6	158
	0.8	179
	1.3	216
	2	167
Mitoxantrone (1b)	2	122
	3	197

^aSee footnote a in Table 3.

^bSee footnote a in Table 1.

Diaza bioisosteres

(1) 6,9-Bis(aminoalkyl)amino]benzo[g]quinoline-5,10-diones

Three representative examples of congeners **6** [7] were evaluated against LoVo and LoVo/Dx. Congeners **6a** [X = N(CH₃)₂], **6b** [X = CH₂NH₂] and **6c** [X = NH₂, maleate salt] were found to be devoid of *in vitro* cytotoxicity against these cell lines. These analogues were also found to be inactive in the L1210 murine leukemia cell line with ID₅₀ values (μg/mL) of 4.2, > 10, and > 10, respectively. Chemotypes **6a** and **6b** were also inactive *in vivo* against P388 murine leukemia.

(2) 6,9-Bis[aminoalkyl)amino]benzo[g]quinazoline-5,10-diones

The synthesis of only one of these diaza bioisosteres **7** [7] was successful from the S_NAr

displacements of the fluorides in **14**. Competing side reactions involving pyrimidine ring opening thwarted this displacement process. Against L1210, S180 and S180/A10 cell lines, **7a** [X = N(CH₃)₂] exhibited ID₅₀ (μg/mL) values of 0.31, 3.5 and > 10, respectively. No *in vivo* studies were performed on this bioisostere.

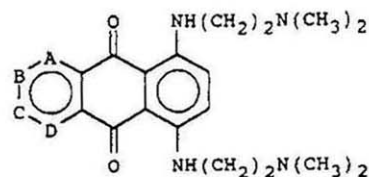
(3) 6,9-Bis[(aminoalkyl)amino]benzo[g]phthalazine-5,10-diones

The bioisosteres **8a** [X = N(CH₃)₂] and **8b** [X = NH₂] were screened in an L1210 assay and had ID₅₀ values (μg/mL) of 0.37 and 2.5, respectively. Both **8a** and **8b** were evaluated against LoVo and LoVo/Dx. Against LoVo **8a** and **8b** showed ID₅₀ values (μg/mL) of 0.86 and 2.8, respectively. In the LoVo/Dx screen **8a** and **8b** had ID₅₀ values of 1.84 and 2.2, respectively. These two compounds were devoid of antitumor activity *in vivo* against P388 murine leukemia.

Biological data comparisons of aza and diaza bioisosteres

Comparative data for the bioisosteres bearing the same side arms are presented in Table 6.

Table 6
Effect of the positioning of the N-atom(s) on the *in vitro* cytotoxicity of 5,8-bis-[2-(dimethylamino)ethyl]amino]aza- and diazaanthracene-9,10-diones against L1210 cells



Compound	A	B	C	D	ID ₅₀ (μg/mL)
-	CH	CH	CH	CH	0.08
2a	N	CH	CH	CH	0.16
3a	CH	N	CH	CH	0.0064
4b	COH	CH	N	CH	0.002
6a	N	CH	CH	N	4.2
7a	N	CH	N	CH	0.31
8a	CH	N	N	CH	0.37

DISCUSSION

An examination of the data presented in Tables 1 and 2 indicates that those analogues with the nitrogen at position 2 are more active than the comparative models with the same side arms but with the nitrogen atom at position 1. Compound **3d** (maleate salt) shows good *in vitro* activity against LoVo and LoVo/Dx (Table 3).

The N-oxide **5a** displayed little *in vitro* activity (LoVo) but significant *in vivo* activity against P388 murine leukemia. This is perhaps suggestive of a bioactivation by deoxygenation to yield **3d**.

The results of Tables 4 and 5 show good *in vitro* and *in vivo* activities for **3d** and **4a**. The presence of a hydroxy group at position 2 in the 2-aza series shows a modest increase in activity when compared with models divorced of this hydroxy substitution (Table 4).

The side arms in the 2-aza series for maximum activity are not identical to those present in ametantrone (**1a**) or mitoxantrone (**1b**).

The diaza analogues, in general, exhibit poor antitumoral activities in most of the screens in which they were evaluated (comparisons of data in Table 6).

Studies to rationalize the remarkable antitumor sensitivity to the position of the nitrogen atom have been commenced. In particular, the 1-aza analogue **2c** and the 2-aza analogue **3d** have been examined. Spectral shifts and gel mobilities have been evaluated in order to ascertain the relationship between DNA binding and cytotoxicity. The DNA affinity was 5–10 times higher for the 2-aza analogues. This cannot account for the differences in cytotoxicity. The cytotoxicity differences for the two series does not correlate with DNA damage or topoisomerase II interactions [8].

CONCLUSIONS

Aza bioisostere **3d** (maleate salt) (BBR 2778) is not cardiotoxic and at equiactive doses, less leukopenic than mitoxantrone (**1b**). It appears to be a favorable candidate for clinical evaluation.

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