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Synthesis and structure–activity relationships of 1,5-diazaanthraquinones as antitumour compounds

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Abstract—1,5-Diazaanthraquinone derivatives were synthesized employing single and double hetero Diels–Alder strategies. Their in vitro antitumour activity was assayed using three cell lines. Some of these compounds, specially those bearing methyl or ethyl groups at the C-3,7 positions or chloro at C-4 and methyl at C-7, showed IC_{50} values in the 10^{-8} M range for human lung carcinoma and human melanoma, which makes them attractive candidates for further development as anticancer agents.

Natural products containing a 9,10-anthracenedione substructure are an important class of antitumour compounds. They include the anthracyclines,[1] the pluramycins[2] and some of the enediyne antibiotics.[3] Isosteric substitution of one or more carbons of the benzene rings by nitrogen atoms should afford compounds with geometries similar to those of the parent compounds, but with increased affinity for DNA due to the presence of sites suitable for hydrogen bonding or ionic interactions. Also, the electron-withdrawing properties of the heterocyclic rings would facilitate the formation of DNA-damaging anion–radicals.[4] For these reasons, the preparation of azaanthraquinones as potential antitumour agents is an active field of research.[5]

The considerations outlined above would apply particularly well to azaanthraquinone natural products, such as the *Annonaceae* alkaloid cleistopholine.[6,7] The 1,5-diazaanthraquinone system seemed particularly interesting to us because of the presence of its imino derivatives as structural fragments of several marine natural products with potent antitumour activity. Compounds belonging to the pyridoacridine class of alkaloids,[8] such as ascididemin,[9] eilatin[10] and **1**, are representative examples (Fig. 1). The latter compound is a regioisomer of meridine that was initially synthesized in the course of our research into analogues of the pyridoacridine alkaloids,[11] but it has also been recently isolated from

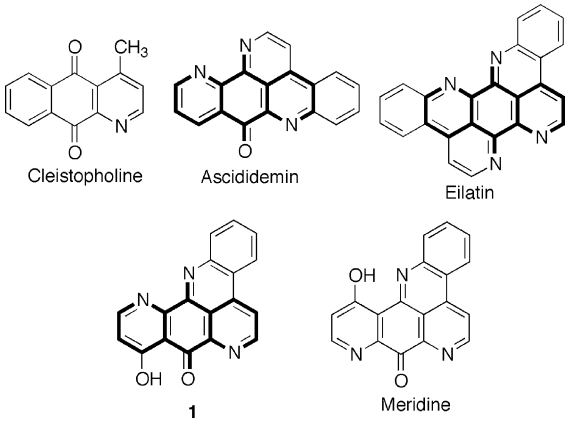

Figure 1. Structure of the natural azaanthraquinone cleistopholine and some marine natural products with 1,5-diazaquinonimine substructures.

Keywords: Antitumour compounds; Solid tumours; Heterocyclic quinones; Hetero Diels–Alder reactions.
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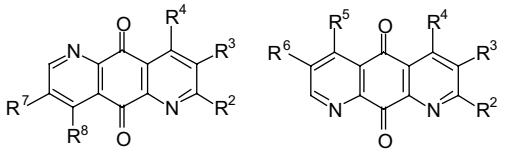


Figure 2. Structure of the compounds studied in this work.

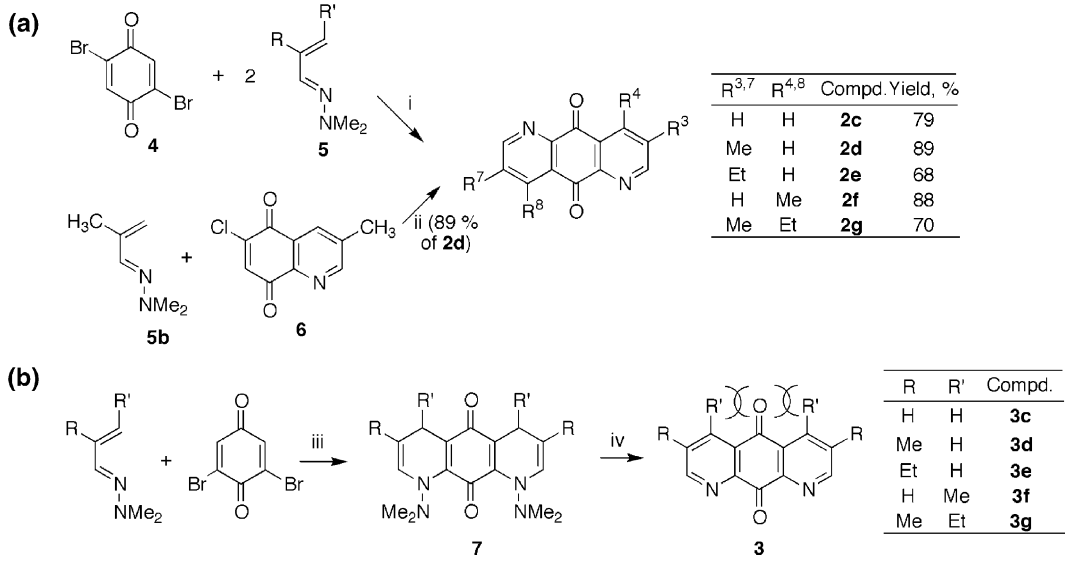
the sponge *Biemna fortis* (perhaps as an artifact) and shown to induce multipolar neuritogenesis at micromolar levels.[12] Because tumour cells often retain differentiation capabilities, substances able to inhibit cellular differentiation may exhibit selective cytotoxicity against tumour cells and are therefore considered as good anticancer candidates.

Derivatives of the 1,5-diazaanthraquinone system have received very little synthetic attention,[13] and their antitumour activity is almost unexplored.[14] We have previously studied compounds **2a** ($R^{2,3,7}=H$; $R^4=o$-$NO_2C_6H_4$; $R^8=OH$) and **2b** ($R^{2,3,7}=H$; $R^4=o$-$NO_2C_6H_4$; $R^8=Cl$), which we obtained as synthetic intermediates of **1**. Although compound **2a** lacked interest, **2b** showed a potent and selective activity for some solid tumours (human lung carcinoma and human melanoma), which warranted further investigation,[15] but unfortunately this compound showed a very short half-life in attempted in vivo studies. In an effort to improve the properties of **2b** and to systematically establish the potential usefulness of 1,5-diazaanthraquinones as a new class of antitumour agents, we describe here the preparation and in vitro antitumour activity of two series of derivatives of the 1,5-diazaanthracene-9,10-dione system, with general structure **2**, together with the comparison of some of these data with the ones obtained for their regioisomers derived from the 1,8-diazaanthracene-9,10-dione system (compounds **3**) (Fig. 2).

From the synthetic point of view, we reasoned that the use of a strategy based on double hetero Diels–Alder reactions[16] would allow a very fast and straightforward preparation of symmetrically substituted derivatives of the 1,5-diazaanthraquinone system. Although the double hetero Diels–Alder reactions of 2,6-dibromobenzoquinone have been used for the synthesis of 1,8-diazaanthraquinones,[17] a similar reaction of 2,5-dibromobenzoquinone was unknown.[18] To study the feasibility of this strategy, 2,5-dibromobenzoquinone **4** was prepared by oxidation of the corresponding hydroquinone with cerium ammonium nitrate.[19] Reaction of this quinone with 1-dimethylamino-1-azadienes **5**[20] proceeded almost instantly at room temperature through an efficient cascade of three reactions (Diels–Alder/HBr elimination/Me_2NH elimination) that gave 68–89% overall yields of the fully aromatic compounds **2**.[21] Alternatively, one of them (**2d**, $R^{3,7}=CH_3$, $R^{4,8}=H$) was prepared in 89% yield by cycloaddition of the known[13b] 3-methyl-6-chloroquinoline-5,8-quinone **6** to methacrolein dimethylhydrazone **5b**[21] followed by spontaneous aromatization (Scheme 1a). This finding paved the way for the synthesis of unsymmetrically substituted derivatives of the 1,5-diazaanthraquinone system by this route, which will be our next goal in this project.

It is interesting to note that the behaviour of quinone **4** towards 4-substituted 1-dimethylamino-1-azadienes was different to the one previously observed for 2,6-dibromobenzoquinone, which gave compounds **7** that required pyrolytic conditions to force their aromatization to **3**.[17] This difference can be attributed to steric compression between the carbonyl at C-10 and the substituents at the 4 and 5 positions of compounds **3**, which presumably hampers the dimethylamine elimination step, a problem that does not exist in their regioisomers **2** (Scheme 1b).

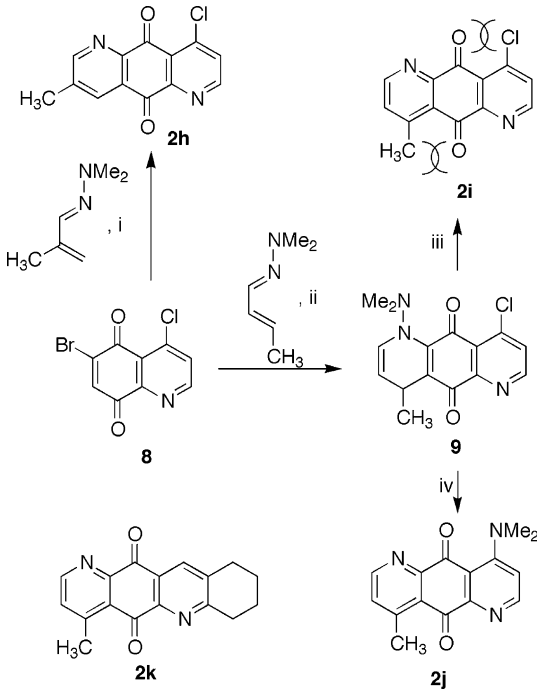
4-Chloro-1,5-diazaanthracene-9,10-dione derivatives with a closer relationship to **2b** were prepared as shown in



$R^{3,7}$	$R^{4,8}$	Compd.	Yield, %
H	H	**2c**	79
Me	H	**2d**	89
Et	H	**2e**	68
H	Me	**2f**	88
Me	Et	**2g**	70

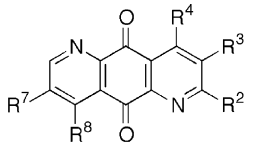
R	R'	Compd.
H	H	**3c**
Me	H	**3d**
Et	H	**3e**
H	Me	**3f**
Me	Et	**3g**

Scheme 1. Reagents and conditions: (i) $CHCl_3$, rt, 1 min; (ii) $(ClCH_2)_2$, 85 °C, 30 min; (iii) CH_2Cl_2 or $CHCl_3$, rt, 1 min; (iv) 110 °C, 0.1 torr, 2 h.



Scheme 2. Reagents and conditions: (i) CH_3CN–Et_2O, rt, 16 h; (ii) CH_3CN–Et_2O, rt, 22 h; (iii) 110 °C, 0.1 torr, 2 h; (iv) HCl 2 M, THF–H_2O, 80 °C, 30 min.

Table 1. Cytotoxic activity of compounds **2** and **3** (in brackets) dissolved in 1:9 DMSO/MeOH in several cultured cell lines[a] (IC_{50}, μM)



R^2	R^3	R^4	R^7	R^8	Cmpd.
H	H	*o*-NO_2Ph	H	OH	**2a**
H	H	*o*-NO_2Ph	H	Cl	**2b**
H	H	H	H	H	**2c**
H	Me	H	Me	H	**2d**
H	Et	H	Et	H	**2e**
H	H	Me	H	Me	**2f**
H	Me	Et	Me	Et	**2g**
H	H	Cl	Me	H	**2h**
H	H	Cl	H	Me	**2i**
H	H	NMe_2	H	Me	**2j**
$(CH_2)_4$		H	H	Me	**2k**

Compound	A-549	HT-29	MEL-28
2a	7.20	7.20	7.20
2b	0.03	0.27	0.03
2c (**3c**)	4.80 (4.80)	4.80 (5.70)	4.80 (4.80)
2d (**3d**)	0.04 (0.21)	0.42 (0.50)	0.04 (0.21)
2e (**3e**)	0.19 (0.45)	1.88 (1.88)	0.38 (0.79)
2f (**3f**)	0.50 (2.10)	1.00 (2.10)	1.00 (2.10)
2g (**3g**)	3.40 (8.50)	3.40 (8.50)	3.40 (8.50)
2h	0.03	0.04	0.03
2i	0.19	1.93	0.19
2j	0.37	0.93	0.37
2k	4.49	17.97	17.97
9	0.33	1.93	0.33
Adriamycin	0.01	0.09	0.03

[a] A-549: Human lung carcinoma (ATCC CCL-185); HT-29: Human colon carcinoma (ATCC HTB-38); MEL-28: Human melanoma (ATCC HTB-72).

Scheme 2. Treatment of quinone **8**[22] with 3-methyl-1-dimethylamino-1-azadiene gave directly the aromatized derivative **2h**. On the other hand, the use of 4-substituted 1-dimethylamino-1-azadienes such as crotonaldehyde dimethylhydrazone led to the 1-dimethylamino-1,4-dihydro derivative **9**, which was aromatized by elimination of dimethylamine under thermal conditions to give compound **2i**. As in the case of compounds **3**, the need to force the aromatization presumably arises from the steric compression due to the combined presence of the C-4 chlorine and C-8 methyl substituents in the planar compound **2i**. In a parallel experiment, treatment of **9** with dilute HCl led to its aromatization with concomitant displacement of the chlorine atom at C-4 by the liberated dimethylamine, affording compound **2j**. Finally, the antitumour activity of the previously described[23] compound **2k** was also measured in order to provide information about the influence of substituents at C-2 in the activity of 1,5-diazaanthraquinones.

Table 1 summarizes the cytotoxic activities of compounds **2**, compared with those of some of the regioisomeric 1,8-diazaanthraquinones **3**. Data obtained in the same assay for adriamycin, one of the most commonly used anti-cancer drugs worldwide and compounds **2a** and **2b**[11] are also included for reference purposes. The IC_{50} values contained in Table 1 were obtained using a literature procedure.[24]

Examination of the data in Table 1 leads to several pieces of information about structure–activity relationships. Although the unsubstituted compound **2c** showed poor activity, some of the di-, tri- and tetraalkyl derivatives studied had very potent activities, and were also selective for some solid tumours. Some of the most promising alkyl derivatives were compounds **2d** and **2e**, bearing small alkyl chains at C-3 and C-7 and specially the dimethyl derivative **2d**, which exhibited IC_{50} values in the 10^{-8} M range for human lung carcinoma and human melanoma. Compound **2f**, the analogue of **2d** bearing the same chains at C-4 and C-8 and also the tetrasubstituted compound **2g**, were less active. The very low activity found for **2k** proves that substituents at C-2 (and presumably at C-6) must be avoided.

Comparison of compounds **2c**–**g** with their regioisomers derived from 1,8-diazaanthraquinone (**3c**–**g**, data in brackets) shows that the latter are less active towards the lung and melanoma tumours.[25] The chloro-substituted compounds were also very interesting, with the 4-chloro-7-methyl derivative **2h** being more active, although less selective, than **2d**. The C-8 substituted analogue of **2h** (compound **2i**) was less active, in agreement with the conclusions obtained for the dialkyl series. A dimethylamino group at C-4, found in compound **2j**, is detrimental to activity. This observation, together with the low activity found for compound **2a**, bearing a hydroxy group at the equivalent C-8 position, suggests that electron-releasing substituents at C-4 and C-8 must be avoided. The 5-dimethylamino-5,8-dihydro compound **9** showed similar properties to its aromatic counterpart (compound **2j**).

In conclusion, 1,5-diazaanthraquinones are a very interesting class of antitumour compounds, particularly for some types of solid tumours. Further studies on their structure–activity relationships are in progress in our laboratories.

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References and notes

1. (a) Lown, J. W. *Chem. Soc. Rev.* **1993**, *22*, 165; (b) Gewirtz, D. A. *Biochem. Pharmacol.* **1999**, *57*, 727–741; (c) Monneret, C. *Eur. J. Med. Chem.* **2001**, *36*, 483–493.
2. (a) Abe, N.; Enoki, N.; Nakakita, Y.; Uchida, H.; Nakamura, T.; Munekata, M. *J. Antibiot.* **1993**, *46*, 1536–1549, and references cited therein; (b) Hansen, M.; Hurley, L. *J. Am. Chem. Soc.* **1995**, *117*, 2421–2429; (c) Lee, S. J.; Hurley, L. H. *J. Am. Chem. Soc.* **1999**, *121*, 8971–8977.
3. (a) Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715; (b) Jones, G. B.; Fouad, F. S. *Curr. Pharm. Des.* **2002**, *8*, 2415–2440.
4. (a) Kovacic, P.; Osuna, J. A. *Curr. Pharm. Des.* **2000**, *6*, 277–309; (b) Taatjes, D. J.; Koch, T. H. *Curr. Med. Chem.* **2001**, *8*, 15–29.
5. Krapcho, A. P.; Maresch, M. J.; Hacker, M. P.; Hazelhurst, L.; Menta, E.; Oliva, A.; Spinelli, S.; Beggiolin, G.; Giuliani, F. C.; Pezzoni, G.; Tignella, S. *Curr. Med. Chem.* **1995**, *2*, 803–824.
6. Total syntheses: (a) Bracher, F. *Liebigs Ann. Chem.* **1989**, 87–88; (b) Krapcho, A. P.; Ellis, M. *Arkivoc* **2000**, 43–50; (c) Pascual-Alfonso, E.; Avendaño, C.; Menéndez, J. C. *Synlett* **2000**, 205–208.
7. The *O*,*O*′-dimethyl derivative of cleistopholine hydroquinone, named annopholine, has also been isolated from natural sources (*Annona hayesii*): Rasamizafy, S.; Hocquemiller, R.; Cassels, B. K.; Cavé, A. *J. Nat. Prod.* **1987**, *4*, 759–761.
8. For reviews, see: (a) Álvarez, M.; Joule, J. A. *Heterocycles* **1992**, *34*, 2385–2405; (b) Molinski, T. F. *Chem. Rev.* **1993**, *93*, 1825–1838; (c) Ding, Q.; Chichak, K.; Lown, J. W. *Curr. Med. Chem.* **1999**, *6*, 1–27; (d) Delfourne, E.; Bastide, J. *Med. Res. Rev.* **2003**, *23*, 234–252.
9. Isolation and identification: Kobayashi, J.; Cheng, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177–1180; Total syntheses: (a) Bracher, F. *Heterocycles* **1989**, *29*, 2093–2095; (b) Moody, C. J.; Rees, C. W.; Thomas, R. *Tetrahedron* **1992**, *48*, 3589–3602; (c) Gellerman, G.; Rudi, A.; Kashman, Y. *Synthesis* **1994**, 239–241; (d) Lindsay, B. S.; Pearce, A. N.; Copp, B. R. *Synth. Commun.* **1997**, *27*, 2587–2592; (e) Álvarez, M.; Feliu, L.; Ajana, W.; Joule, J. A.; Fernández-Puentes, J. L. *Eur. J. Org. Chem.* **2000**, 849–855; (f) Cuerva, J. M.; Cárdenas, D. J.; Echavarren, A. M. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1360–1365.
10. Isolation: Rudi, A.; Benayahu, Y.; Goldberg, I.; Kashman, Y. *Tetrahedron Lett.* **1988**, *29*, 6655–6656; Total synthesis: Nakahara, S. H.; Kanaka, Y.; Kubo, A. *Heterocycles* **1993**, *36*, 1139–1193.
11. De la Fuente, J. A.; Martín, M. J.; Blanco, M. M.; Pascual-Alfonso, E.; Avendaño, C.; Menéndez, J. C. *Bioorg. Med. Chem.* **2001**, *9*, 1807–1814.
12. Aoki, S.; Wei, H.; Matsui, K.; Rachmat, R.; Kobayashi, M. *Bioorg. Med. Chem.* **2003**, *11*, 1969–1973.
13. (a) Tapia, R. A.; Quintanar, C.; Valderrama, J. A. *Heterocycles* **1996**, *43*, 447–461; (b) Brassard, P.; Lévesque, S. *Heterocycles* **1994**, *38*, 2205–2218; (c) Horiguchi, Y.; Toeda, A.; Tomoda, K.; Sano, T. *Heterocycles* **2000**, *53*, 315–322.
14. Nebois, P.; do Nascimento, S. C.; Boitard, M.; Bartoli, M. H.; Fillion, H. *Pharmazie* **1994**, *49*, 819–821.
15. The activity of compound **2b** was mistakenly attributed to its $R^3 = Br$ analogue in Ref. 11. The correct activity of **2b** is the one reported here.
16. For the use of α,β-unsaturated dimethylhydrazones as heterodienes, see: Serckx-Poncin, B.; Hesbain-Frisque, A.-M.; Ghosez, L. *Tetrahedron Lett.* **1982**, *23*, 3261–3264. For the use of bromoquinones in hetero Diels–Alder reactions, see, for instance, Ref. 6a.
17. Pérez, J. M.; López-Alvarado, P.; Avendaño, C.; Menéndez, J. C. *Tetrahedron* **2000**, *56*, 1561–1567.
18. For double hetero Diels–Alder reactions of compound **4** with carbodienes, see: Alonso, M. A.; López-Alvarado, P.; Avendaño, C.; Menéndez, J. C. *Tetrahedron* **2003**, *59*, 2821–2830.
19. López-Alvarado, P.; Avendaño, C.; Menéndez, J. C. *Synth. Commun.* **2002**, *32*, 3233–3239.
20. Waldner, A. *Helv. Chim. Acta* **1988**, *71*, 486–496.
21. Representative procedure: To a solution of quinone **4** (150 mg, 0.56 mmol) in $CHCl_3$ (15 mL) was added ethylacrolein dimethylhydrazone (142 mg, 1.12 mmol). The solution was stirred at room temperature for 1 min and evaporated. The residue was washed repeatedly with Et_2O, affording 102 mg (68%) of compound **2e**. Mp 218–220 °C. IR (KBr): 1683 (C=O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.93 (d, 2H, $J = 2.1$ Hz, H-2,6); 8.50 (d, 2H, $J = 2.1$ Hz, H-4,8); 2.85 (q, 4H, $J = 7.5$ Hz, $2CH_2$–CH_3), 1.35 (t, 6H, $J = 7.5$ Hz, $2CH_2$–CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 181.6 (C-9,10); 155.7 (C-2,6), 146.5 (C-9a,10a); 145.6 (C-3,7); 135.5 (C-4,8); 130.6 (C-4a,8a), 26.6 (CH_2–CH_3); 14.8 (CH_2–CH_3) ppm.
22. Kitahara, Y.; Tamura, F.; Nishimura, M.; Kubo, A. *Tetrahedron* **1998**, *54*, 8421–8432.
23. Blanco, M. M.; Avendaño, C.; Menéndez, J. C. *Tetrahedron* **1999**, *55*, 12637–12646.
24. Schroeder, A. C.; Hughes, R. G.; Bloch, A. *J. Med. Chem.* **1981**, *24*, 1078–1083.
25. Another difference between compounds **2** and **3** can be found in their behaviour towards the P-388 (mouse lymphoma) ATCC CCL-46 line. Thus, the two most relevant compounds **2d** and **2e** are about 50-fold more active for A-549 than for P-388, while this ratio was around 20 for **3d** and 8 for **3e**.