Synthesis of Alkylamino Derivatives of 1,4,6,11-Tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione

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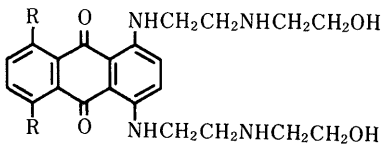
The synthesis of 7-[(3-*N*,*N*-dimethylamino)propylamino]-1,4,6,11-tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione derivatives is reported. The expected monoalkylamino substituted derivative (4a) was obtained from the 7-chloro-substituted compound (3a), whereas the 7,10-dichloro-substituted compound (3b) gave a mixture of the monoalkylamino derivative (4b) and the dialkylaminophthalimide (4c). The cytotoxic activity of 4a—c against HeLa cells was assayed. Compounds 4a and 4b showed a higher cytotoxicity than the starting adducts (3a and 3b).

Keywords ametantrone analogue; heteroanthracenedione; antitumor agent; diazapolycycle; tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione

Much effort has been devoted in the last decade to the preparation of synthetic anthracyclines,[1] because these compounds have been shown to be potent antitumor agents in the treatment of human cancers.[2] However, the usefulness of many anthracycline analogues synthesized so far is limited by high cardiotoxicity. It has been suggested that cardiotoxicity is related to the redox potential of the quinone moiety of anthracyclines.[3] Consequently, it seems to be interesting to prepare anthracyclinone mimetics containing a heteroaromatic ring, which acts as a bioisosteric replacement of the benzene ring in some drugs.[4]

On the other hand, the search for new synthetic compounds related to anthracyclinones has led to a structural class of antineoplastic agents called anthracenediones[5] [mitoxantrone (**1a**) and ametantrone (**1b**)]. These compounds exhibit excellent anticancer activities with diminished side-effects, and contain a planar aromatic part that may be inserted between the DNA nucleotide base pairs, and cationic side moieties which interact with the deoxyribophosphate backbone of DNA.[6] The presence of terminal nitrogen atoms at the side chains is crucial; they cannot be replaced by other heteroatoms.[7] However, two basic side-chains are not essential for activity; certain "one-armed" derivatives have shown significant activities. More than 600 tricyclic aromatic compounds with one or two side-chains have been synthesized as potential DNA intercalators[8] in a search for compounds with greater activity than **1a** or **1b**.

In the last few years, we have been engaged in the synthesis of diazatetracyclic systems as heterocyclic analogues of anthracyclinones.[9] In the present paper we wish to report the preparation of diazatricyclic compounds related to ametantrone.



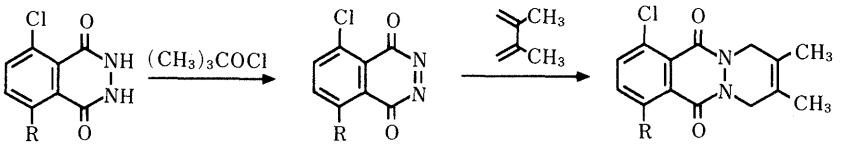
1a : R=H
1b : R=OH

The aromatic moiety has been prepared by a procedure previously developed by us,[10] which includes the oxidation of the mono- and dichloro substituted cyclic hydrazides **2a** and **2b** to the corresponding diazaquinones, and further cycloaddition of these with 2,3-dimethyl-1,3-butadiene to give **3a** and **3b**, respectively (Chart 1). In this way, one or two cationic side-chains could be introduced at the aromatic ring *via* nucleophilic substitution of the chlorine atoms.

In fact, treatment of the monochloro substituted adduct **3a** with an excess of dimethylpropylamine gave the monoalkylamino derivative **4a** in a 67% yield (Chart 2). However, the 7,10-dichloro substituted adduct **3b** afforded a mixture of the monoalkylamino derivative **4b** (70% yield) and a viscous oil that could not be crystallized and showed analytical and spectroscopic data consistent with the dialkylamino 6-chlorophthalimide derivative **4c** (29% yield).

Structural assignment of **4a,b** is unequivocal from analytical and spectroscopic data (Table I). The IR spectra of both compounds show the associated NH stretching vibration bands at 1630 and 1620 cm^{-1}, whereas the C=O stretching vibration is lowered by 20 cm^{-1} with respect to the values found for the starting adducts, in accordance with the formation of hydrogen bonding between carbonyl and amino groups, as observed by Greenhalgh and Hughes[11] for the reaction of leucoquinizarines with alkylenediamines.

In Table II are shown the main ^{1}H-NMR signals for **4a—c** and for the two starting adducts **3a, b**, which were used as authentic samples for comparison. It was observed that the chemical shifts corresponding to the dihydropyridazine ring were maintained, whereas significant changes were found for the aromatic moiety. Namely, the hydrogen



2a : R=H
2b : R=Cl

3a : R=H
3b : R=Cl

Chart 1

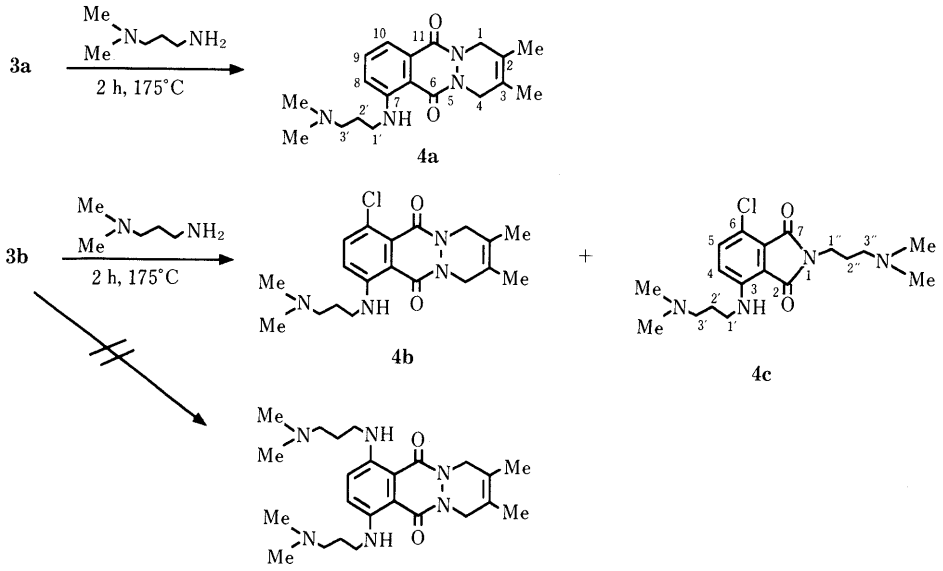


Chart 2

TABLE I. Physical and Spectroscopic Properties of the Alkylamino Substituted Derivatives

Compd.	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd (Found)				MS	IR (KBr, cm^{-1})
				C	H	N	Cl	*m/z*, M^+	
4a	82—83	67	$C_{19}H_{26}N_4O_2$	66.64 (66.72	7.65 7.88	16.36 16.19)		342 (50%)	3280 (NH), 1630 (C=O), 1270 (C–O)
4b	87—88	70	$C_{19}H_{25}ClN_4O_2 \cdot 1/2H_2O$	59.13 (59.24	6.79 6.63	14.51 14.86	9.18 9.43)	376 (16%)	3260 (NH), 1620 (C=O), 1260 (C–O)
4c	a)	29	$C_{18}H_{27}ClN_4O_2 \cdot H_2O$	56.17 (55.87	7.59 7.54	14.55 14.28	9.21 8.99)	368 (10%)	3380 (NH), 1760 (C=O), 1690 (C=O)

a) An oily material.

TABLE II. ^{1}H-NMR Chemical Shifts for **3a**, **b** and **4a**—**c** (90 MHz, $CDCl_3$, δ scale, ppm)

Compd.	Aromatic ring		Dihydropyridazine ring		CH_2 side-chains						Other		
	H-8	H-9, H-10	N–CH_2–C=	CH_3–C=	1′	3′	2′	1″	3″	2″	NH	$(CH_3)_2$N(3′)	$(CH_3)_2$N(3″)
3a	8.70—7.60 (m)		4.40 (m)	1.85 (s)									
3b	7.67 (s)		4.40 (m)	1.77 (s)									
4a	6.99 (m)	7.53 (m)	4.43 (m)	1.83 (s)	3.35 (m)	2.43 (m)	1.93 (m)	—	—	—	8.89 (m)	2.26 (s)	—
4b	6.74 (d)	7.44 (d)	4.36 (m)	1.72 (s)	3.35 (m)	2.39 (m)	1.91 (m)	—	—	—	9.14 (m)	2.24 (s)	—
4c	6.80[a] (d)	7.30[b] (d)	—	—	3.32 (m)	2.40 (m)	1.83 (m)	3.65 (t)	2.40 (m)	1.83 (m)	6.73 (m)	2.20 (s)	2.23 (s)

a) This signal corresponds to H-4 in **4c**. b) This signal corresponds to H-5 in **4c**.

atoms in *ortho* and *para* positions with respect to the alkylamino group are shielded by 0.70 (H_8) and 0.67 (H_{10}) ppm due to the increase in electron density caused by the substituent. The side-chain methylene α to the NH group appears as a multiplet ($\delta = 3.35$ ppm) collapsing to a triplet on exchange with D_2O, whereas the NH signal is a triplet ($\delta = 8.89$, 9.14 ppm, $J = 4.5$ Hz) that disappears in the presence of D_2O.

In **4c**, disappearance of the signals of the dihydropyridazine ring and the presence of the two nitrogenated side-chains are observed in the NMR spectrum. The main difference between both side-chains corresponds to the methylene groups attached respectively to the N-2 and C-4 positions, which appear at 3.65 and 3.32 ppm. The first one is a triplet, whereas the last is a multiplet collapsing to a triplet with D_2O. The presence of the five-membered ring is confirmed in the IR spectrum by the two characteristic C=O imidic stretching vibration bands[12] at 1760 and 1690 cm^{-1}. The shifting of the HN–C_4 stretching vibration band by 70 cm^{-1} in **4c** with respect to **4b** is due to the

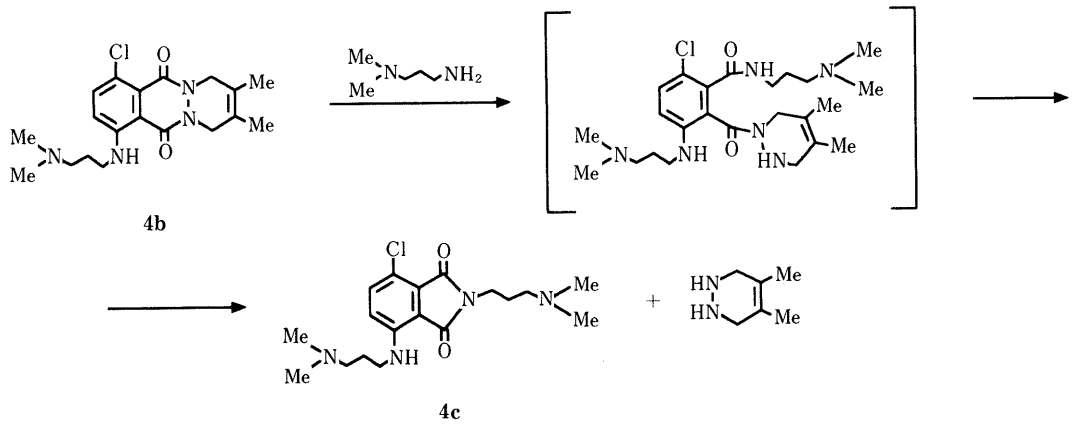


Chart 3

TABLE III. Cytotoxic Activity of Starting Adducts and Alkylamino Substituted Derivatives

Compd.	Aromatic ring substituents		IC_{50} (μg/ml)
3a	H	H	100
3b	Cl	Cl	100
4a	$HN-(CH_2)_3-NMe_2$	H	10
4b	$HN-(CH_2)_3-NMe_2$	Cl	10
4c	$HN-(CH_2)_3-NMe_2$	Cl	40

influence of the five-membered heterocyclic ring. In fact, in the ^{1}H-NMR spectrum of **4c**, the signal of the HN–C_4 proton is also highly shielded.

In the reaction of **3b** with dimethylpropylamine, the 7,10-dialkylamino derivative was not synthesized. After the substitution of the first chlorine atom to give **4b**, the electron donating effect of the amino group might result in an excess of negative charge at C_{10}, and this must be the reason for the lack of reactivity of **4b** for further nucleophilic attack of the amine.

The transformation of **4b** to **4c** under the strongly basic conditions of the reaction can be explained in terms of the nucleophilic attack of a second molecule of the amine on one of the amide bonds, to give an intermediate that undergoes a further cyclization including simultaneous elimination of the tetrahydropyridazine ring (Chart 3). The presence of chloro or amino substituents *ortho* to the carbonyl groups is known to favor the formation of the *N*-substituted derivatives of phthalimide from acyclic amides.[10]

The cytotoxic activity of compounds **3a, b** and **4a—c** against HeLa cells[13] was tested, and the results obtained are shown in Table III. It was observed that the introduction of a dimethylaminopropylamino group in the side-chain significantly decreases IC_{50} values, since the starting adducts **3a** and **3b** were inactive, while **4a** and **4b** showed a rather high activity (10 μg/ml). The bicyclic dialkylamino derivative **4c**, with a diminished planarity with respect to **4a** and **4b**, had a larger IC_{50} value (40 μg/ml).

Experimental

Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 257 spectrometer. ^{1}H-NMR spectra were recorded on a Varian XL-30 spectrometer, using tetramethylsilane as an internal standard. Analytical TLC was performed on aluminium sheets coated with a 0.2 mm layer of Silica gel 60 F_{254} (Merck). Flash chromatography was performed on a column using Silica gel G60 (Merck). Compounds **3a** and **3b** were obtained following the procedure reported by Lora-Tamayo *et al.*[10]

General Procedure A mixture of 7-chloro or 7,10-dichloro-2,3-dimethyl-1,4,6,11-tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione (**3a, b**) and dimethylaminopropylamine (12 ml) was heated at 175°C for 2 h. The excess of dimethylaminopropylamine was removed *in vacuo* and the residue was dissolved in chloroform (25 ml). The resulting solution was treated with 5% aqueous sodium hydroxide solution (50 ml). The organic layer was then dried over magnesium sulfate and removed *in vacuo*. The residue was purified as specified below for each case.

Synthesis of 7-[(3-*N*,*N*-Dimethylamino)propylamino]-2,3-dimethyl-1,-4,6,11-tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione (4a) Following the general procedure, 0.33 g (1.2 mmol) of **3a** gave a residue, which was recrystallized from hexane to give 0.28 g (67%) of analytically pure **4a** (see Tables I and II for the data).

Synthesis of 10-Chloro-7-[(3-*N*,*N*-dimethylamino)propylamino]-2,3-dimethyl-1,4,6,11-tetrahydropyridazino[1,2-*b*]phthalazine-6,11-dione (4b) and 6-Chloro-3-[(3-*N*,*N*-dimethylamino)propylamino]-*N*-(3-*N*,*N*-dimethylamino)propylphthalimide (4c) Following the general procedure, 0.30 g (0.96 mmol) of **3b** afforded an oil, which was chromatographed over a silica gel column [400 g, 200—400 mesh, eluent: ethyl acetate/ethanol/25% aqueous ammonium hydroxide (v/v 15/5/1)]. The fractions were monitored by analytical TLC and the appropriate fractions were combined to give two major compounds of Rf=0.66, 0.48. The removal of the solvents from the fraction of Rf=0.66 afforded 0.25 g of a solid corresponding to **4b** (70% yield). The fraction of Rf=0.48 gave 0.10 g of an oil corresponding to **4c** (29% yield).

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