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: amantantrone analogue; heteroanthracenedione; antitumor agent; diazopolycycle; tetrahydropyridazino[1,2-b]phthalazine-6,11-dione

Much effort has been devoted in the last decade to the preparation of synthetic antracyclines,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 antracyclines.3) Consequently, it seems to be interesting to prepare antracyclinone 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 antracyclines has led to a structural class of antineoplastic agents called anthraccenones5) [mitoxantrone (1a) and amantantrone (1b)]. These compounds exhibit excellent antineoplastic 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 intercalators8) 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 antracyclines.9) In the present paper we wish to report the preparation of diazatricyclic compounds related to amantantrone.

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 cyclodidion 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 1). 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 carboxyl and amino groups, as observed by Greenhalgh and Hughes11) for the reaction of leucoquinizarines with alkylenediamines.

In Table II are shown the main ¹H-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 dihydro pyridazino ring were maintained, whereas significant changes were found for the aromatic moiety. Namely, the hydrogen
Table I. Physical and Spectroscopic Properties of the Alkylamino Substituted Derivatives

<table>
<thead>
<tr>
<th>Compd.</th>
<th>mp (°C)</th>
<th>Yield (%)</th>
<th>Formula</th>
<th>Analysis (%)</th>
<th>MS</th>
<th>IR (KBr, cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>4a</td>
<td>82—83</td>
<td>67</td>
<td>C₁₄H₁₂N₂O₂</td>
<td>66.64 (66.72)</td>
<td>7.65 (7.88)</td>
<td>16.36 (16.19)</td>
</tr>
<tr>
<td>4b</td>
<td>87—88</td>
<td>70</td>
<td>C₁₄H₁₂ClN₄O₂·1/2H₂O</td>
<td>59.13 (59.24)</td>
<td>6.79 (6.63)</td>
<td>14.51 (14.86)</td>
</tr>
<tr>
<td>4c</td>
<td></td>
<td>29</td>
<td>C₁₄H₁₂N₂O₂·H₂O</td>
<td>56.17 (55.87)</td>
<td>7.59 (7.54)</td>
<td>14.55 (14.28)</td>
</tr>
</tbody>
</table>

Table II. ¹H-NMR Chemical Shifts for 3a, b and 4a—c (90 MHz, CDCl₃, δ scale, ppm)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Aromatic ring</th>
<th>Dihydropyrazine ring</th>
<th>CH₃ side-chains</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H-8</td>
<td>H-9, H-10</td>
<td>CH₃-C=</td>
<td>(CH₃)₂N(3') (CH₃)₂N(3')</td>
</tr>
<tr>
<td></td>
<td>N-CH₃-C=</td>
<td>CH₃-C=</td>
<td>1'</td>
<td>3'</td>
</tr>
<tr>
<td>3a</td>
<td>8.70—7.60</td>
<td>4.40</td>
<td>1.85</td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>7.67</td>
<td>4.40</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>4a</td>
<td>6.99</td>
<td>5.33</td>
<td>4.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(m)</td>
<td>(m)</td>
<td>(s)</td>
<td></td>
</tr>
<tr>
<td>4b</td>
<td>6.74</td>
<td>4.36</td>
<td>1.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d)</td>
<td>(m)</td>
<td>(s)</td>
<td></td>
</tr>
<tr>
<td>4c</td>
<td>6.80</td>
<td>7.30</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d)</td>
<td>(d)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Note: This signal corresponds to H-4 in 4c.

In 4c, disappearance of the signals of the dihydropyrazine 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 D₂O. The presence of the five-membered ring is confirmed in the IR spectrum by the two characteristic C=O imidic stretching vibration bands at 1760 and 1690 cm⁻¹. The shifting of the NH–C₄ stretching vibration band by 70 cm⁻¹ in 4c with respect to 4b is due to the atoms in ortho and para positions with respect to the alkylamino group are shielded by 0.70 (H₄) and 0.67 (H₁₂) ppm due to the increase in electron density caused by the substituent. The side-chain methyl z to the NH group appears as a multiplet (δ = 3.35 ppm) collapsing to a triplet on exchange with D₂O, whereas the NH signal is a triplet (δ = 8.89, 9.14 ppm, J = 4.5 Hz) that disappears in the presence of D₂O.
influence of the five-membered heterocyclic ring. In fact, in the \(^1\)H-NMR spectrum of 4c, the signal of the HN-C=O 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 chloride atom to give 4b, the electron donating effect of the amino group might result in an excess of negative charge at C10, 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.

The cytotoxic activity of compounds 3a, 4a, and 4a–c against HeLa cells 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 \(\mu\)g/ml). The bicyclic dialkylamino derivative 4c, with a diminished planarity with respect to 4b, had a larger IC\(_{50}\) value (40 \(\mu\)g/ml).

**Table III. Cytotoxic Activity of Starting Adducts and Alkylamino Substituted Derivatives**

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Aromatic ring substituents</th>
<th>IC(_{50}) ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>100</td>
</tr>
<tr>
<td>3b</td>
<td>Cl</td>
<td>100</td>
</tr>
<tr>
<td>4a</td>
<td>HN-(CH(_2)_3)-NMe(_2)</td>
<td>10</td>
</tr>
<tr>
<td>4b</td>
<td>HN-(CH(_2)_3)-NMe(_2)</td>
<td>10</td>
</tr>
<tr>
<td>4c</td>
<td>HN-(CH(_2)_3)-NMe(_2)</td>
<td>40</td>
</tr>
</tbody>
</table>

**Chart 3**

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-tetrahydropyridazine[1,2-b]thiazaline-6,11-dione (3a, b) and dimethylaminopropylamine (12 ml) was heated at 175°C for 2h. 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-tetrahydropyridazine[1,2-b]thiazaline-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-tetrahydropyridazine[1,2-b]thiazaline-6,11-dione (4b) and 6-Chloro-7-[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 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).

References