Highly Stable Phenanthridinium Frameworks as a New Class of Tunable DNA Binding Agents with Cytotoxic Properties

Alexis D. C. Parenty, † Louise V. Smith, † Kevin M. Guthrie, † De-Liang Long, † Jane Plumb, † Robert Brown, † and Leroy Cronin*, †

Department of Chemistry, The University of Glasgow, Joseph Black Building, Glasgow, G12 8QQ, UK, and Cancer Research UK Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow, G6 1BD

Received April 7, 2005

Abstract: A new class of cytotoxic heteroaromatic cations is presented, based on the dihydro-imidazo-phenanthridinium framework (DIP), that have affinity for DNA and cytotoxicity toward cancerous cells. The DIP framework is particularly tunable due to the flexible synthetic methodology. Furthermore, the central moiety has proved to be very stable to hydrolysis and reduction compared to other phenanthridinium-based agents.

Nitrogen heteroaromatic cations are interesting compounds due to their reactivity and biological properties.1,2 In particular, the phenanthridinium moiety has given rise to a lot of attention because of its implication in the scaffold of a number of DNA intercalating agents with antitumor properties,3,4 DNA drug targeting applications,5,6 and DNA probes.7 With a view of increasing their biological properties, phenanthridinium derivatives have been subject to diverse ring extensions (Figure 1). Although annelation of rings a and c of the phenanthridinium framework have been extensively investigated,8–11 the heteroaromatic middle ring b has barely been explored.

The highly polar iminium moiety of the BCPAs (Figure 1) was proved to be essential to their antitumor properties.12 Nevertheless, the iminium moiety is highly reactive13–16 and therefore becomes an easy target for in vivo metabolism. Under physiological conditions, most of the phenanthridinium-based anticancer agents have the common drawback of being easily attacked by biological reducing agents such as NADH,17 as well as being subject to alpha addition of a hydroxide, forming pseudobases. Both transformations disturb the planarity of the framework and remove the positive charge necessary for the biological activity of the molecule.12 Moreover, the alkyl group on the quaternary nitrogen can be disconnected from the aromatic platform to give the corresponding neutral nonactive phenanthridine derivative.18 For these metabolic reasons, preclinical studies on the antileukemic compounds nitidine and fagaronine were abandoned due to their incompatibility with biological fluids.19

Therefore, a great deal of work has focused on trying to strengthen the scaffold of phenanthridinium deriva-

Scheme 1. a One-Pot Reaction Leading to DIP 4a–d

Figure 1. Some reported extensions around phenanthridinium core.

* Reagent and conditions: (a) R-NH2, Na2CO3, water/ethyl acetate, N2, rt, 3H. (b) Aqueous wash, NBS, rt, 3 h in the dark.
shaken. In the case of the reference molecule, a purple product instantaneously precipitates from the D₂O layer and shifts toward the CDCl₃ layer where 5-methyl-dihydro-phenanthridine was characterized by H and ¹³C NMR spectroscopy. No evidence of reduction was observed from compound 4b. A similar experiment was also undertaken with the biological reducing agent NADH leading to reduction of the reference within an 1 h, but leaving 4b unaffected, even after 24 h exposure time. Note that NADH is the coenzyme involved in the bioreduction of BCPAs.

The susceptibility of the control 5-methyl-phenanthridinium bromide and DIP 4b to undertake addition of hydroxide in aqueous medium was investigated by the method of Albert and Serjeant using UV spectrophotometry measurements. It was found that the reference 5-methyl-phenanthridinium bromide becomes affected around pH 8. The pseudo-base formation seems to be accompanied by an irreversible oxidation process, preventing the determination of an accurate pKₐₘₗₜ data for compound 4b.

Crystallographic analysis of 4b (Figure 2) shows the bonds N₂–C₃₁ and C₃₁–N₁ to be equidistant. This confirms the charge delocalization over the two nitrogen atoms, which is responsible for the higher stability of the DIP framework.

The demonstrated high stability of the DIP framework is expected to disfavor the cell metabolism processes that are involved in the case of BCPAs (Figure 1). In vitro cytotoxicity studies were undertaken with human ovarian tumor cell line A2780. A growth inhibition assay with 24 h drug exposure and a 3 day recovery period reveals that DIP derivatives 4a–d have cytotoxicity within the same order of magnitude as both chelerythrine and the clinically used antitumor agent carboplatin. However, compound 4d shows much higher cytotoxicity, with an IC₅₀ value in the nanomolar range (Table 1).

Like most of the BCPAs, the cytotoxicity of the DIP framework is likely to come from the intercalation of the aromatic platform between the DNA base pairs. To evaluate the DNA binding affinity of the DIP framework, ITC experiments were undertaken on a DNA solution. Binding constants in the region of 10⁻⁴–10⁻⁵ M⁻¹ were obtained (Table 1), which are comparable to other phenanthridinium-based DNA intercalating agents. Further studies concerning the mode of action of the DIPs are ongoing.

In summary, it has been shown that the fusion of a dihydro-imidazo moiety onto a phenanthridinium framework leads to important stability advantages. The general cytotoxicity associated with the phenanthridinium moiety is maintained in the resulting DIP framework and even dramatically improved in the case of molecule 4d. The DIP derivatives have also interesting DNA binding properties. The simplicity and generality of the annelation reaction could be used to strengthen the most labile BCPAs and should provide new horizons to some clinically abandoned phenanthridinium antitumor agents. Additionally, it could facilitate the development of new drug-like heterocycles.

Acknowledgment. This work was supported by the EPSRC and Cancer Research UK. We thank Prof A. Cooper for the DNA binding studies at the UK Center for Microcalorimetry funded by the BBSRC, and Dr. Jesus M. de la Fuente for fruitful discussions.

Supporting Information Available: More details for the reduction resistance experiment, pKₐₘₜ data, measurement, in vitro cytotoxicity assay, and DNA binding studies; synthetic procedures; analytical data. The cif file for compound 4b is also available. This material is available free of charge via the Internet at http://pubs.acs.org

Table 1. Cytotoxicity Activity and DNA Binding Affinity of DIP Derivatives 4a–d, along with Carboplatin and Chelerythrine for Comparison

<table>
<thead>
<tr>
<th>compounds</th>
<th>IC₅₀ (µM) on cell line A2780</th>
<th>DNA binding constants, K (M⁻¹) × 10⁴ on salmon testes DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>carboplatin</td>
<td>5.22 ± 0.14</td>
<td>NA</td>
</tr>
<tr>
<td>chelerythrine</td>
<td>4.63 ± 0.52</td>
<td>46.6 ± 4.2</td>
</tr>
<tr>
<td>4a</td>
<td>1.56 ± 0.18</td>
<td>2.56 ± 0.08</td>
</tr>
<tr>
<td>4b</td>
<td>1.53 ± 0.09</td>
<td>2.89 ± 0.1</td>
</tr>
<tr>
<td>4c</td>
<td>1.71 ± 1.20</td>
<td>2.02 ± 0.06</td>
</tr>
<tr>
<td>4d</td>
<td>0.987 ± 0.01</td>
<td>12.9 ± 0.45</td>
</tr>
</tbody>
</table>

* IC₅₀ is the concentration of drug necessary to kill 50% of the cells.

References


JM050320Z