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

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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 reactive[13−16] 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 derivatives.[4,12,17,18] As reported by T. Nakanishi et al., NK109 (Figure 1) has interesting charge delocalization properties leading to higher stability.[4] At physiological pH, the phenolate anion on R_3 is in conjugation with the iminium moiety, preventing alpha addition of hydroxide[12] as well as improving resistance to biological reducing agents.[17]

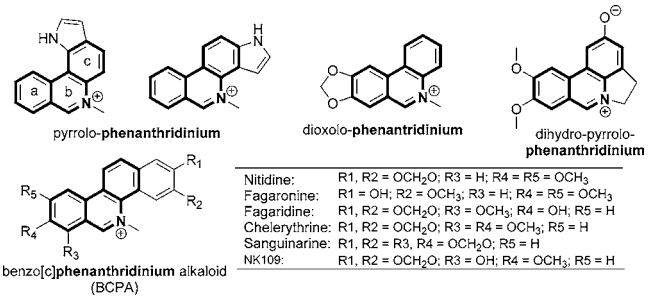
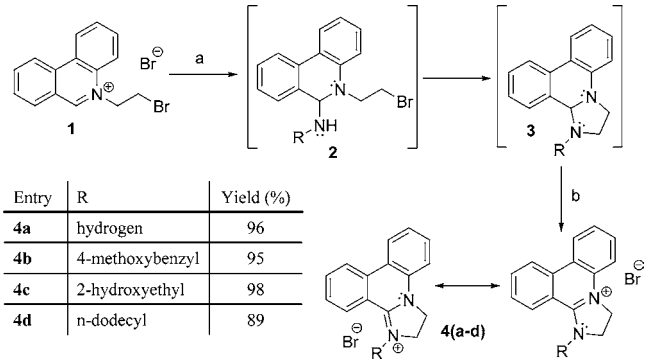


Figure 1. Some reported extensions around phenanthridinium core.

Scheme 1. [a] One-Pot Reaction Leading to DIP **4a**−**d**[14]



Entry	R	Yield (%)
4a	hydrogen	96
4b	4-methoxybenzyl	95
4c	2-hydroxyethyl	98
4d	n-dodecyl	89

[a] Reagent and conditions: (a) R-NH_2, Na_2CO_3, water/ethyl acetate, N_2, rt, 3H. (b) Aqueous wash, NBS, rt, 3 h in the dark.

Recently, we have exploited the reactivity of the phenanthridinium iminium moiety in an annelation reaction.[14] A primary amine reacts with 2-bromoethylphenanthridinium to yield a new phenanthridinium-ring-extended framework in one-pot: 2,3-dihydro-1*H*-imidazo[1,2-*f*]phenanthridinium (DIP) **4** (Scheme 1).

In an analogous manner to NK109, the dihydroimidazolium ring of DIP derivatives **4** (Scheme 1) should prevent reduction and pseudobase formation via delocalization of the positive charge. The resonance between the two nitrogen atoms of the DIP should decrease the reactivity of the CN double bond. Herein, we report the stability advantages of the DIP framework, along with preliminary in vitro cytotoxicity and DNA binding measurements.

The stability of the DIP framework has been demonstrated by two different experiments highlighting their resistance against both reducing agents and hydroxide addition. The resistance of the DIP framework toward reducing agent was investigated via a simple NMR phase/transfer experiment. In two different NMR tubes, the molecule **4b** and the reference 5-methyl-phenanthridinium bromide were dissolved in a $CDCl_3/D_2O$ biphasic solution. $NaCNBH_3$ (2 equiv) was added to make a 50 mM solution, and the NMR tubes were

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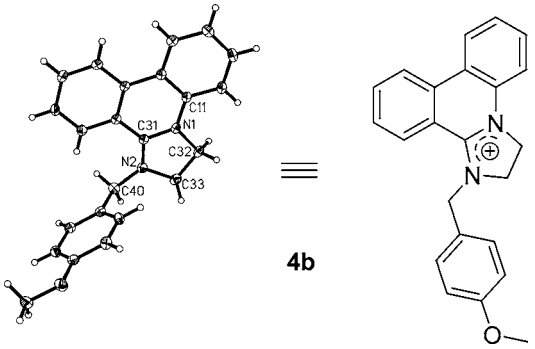

Figure 2. ORTEP representation of structure **4b**. Selected bond lengths showing delocalization of charge [Å]: N1−C31 1.340(3), N2−C31 1.333(3), N2−C33 1.472(3), N1−C32 1.475(3), N2−C40 1.457(3), N1−C11 1.389(3).

shaken. In the case of the reference molecule, a purple product instantaneously precipitates from the D_2O layer and shifts toward the $CDCl_3$ layer where 5-methyl-dihydro-phenanthridine was characterized by ^1H and ^{13}C 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.[17]

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.[20] 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 $pK(R_{OH})$, i.e: pH value at which 50% of the starting material is transformed into its pseudobase. Nevertheless, the apparent sensitivity of this reference to alkaline pH is in accordance with the $pK(R_{OH})$ of BCPAs sanguinarine, chelerythrine, and nitidine (Figure 1), reported to be 5.75, 6.67, and 9.76, respectively.[12,21] On the other hand, due to the introduction of an amidinium moiety, raising the electron density around the positive center, the DIP **4b** shows a much higher resistance to pseudobase formation, with a calculated $pK(R_{OH})$ of 11.4.

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**−**c** 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 IC_{50} value in the nanomolar range (Table 1).

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

compounds	IC_{50} (μM)[a] on cell line A2780	DNA binding constants, K (M^{-1}) × 10^4 on salmon testes DNA
carboplatin	5.22 ± 0.14	NA
chelerythrine	4.63 ± 0.52	46.6 ± 4.2
4a	1.56 ± 0.18	2.56 ± 0.08
4b	1.53 ± 0.09	2.89 ± 0.1
4c	11.7 ± 1.20	2.02 ± 0.06
4d	0.087 ± 0.01	12.9 ± 0.45

[a] IC_{50} is the concentration of drug necessary to kill 50% of the cells.

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.[22] 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[14] 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 druglike heterocycles.

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Supporting Information Available: More details for the reduction resistance experiment, $pK_a(R_{OH})$ 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

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