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The Role of the Iminium Bond in the Inhibition of Reverse Transcriptase by Quaternary Benzophenanthridines

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Abstract

The quaternary benzo[*c*]phenanthridines fagaronine, nitidine and *O*-methylfagaronine have been reviewed as potential antitumour and antiviral agents. Their mode of action has not been established, but their ability to bind with DNA by intercalation is believed to be involved.

Of the three synthetic analogues of *O*-methylfagaronine which we have synthesized, methoxidine and ethoxidine are active against HIV-1 reverse transcriptase (IC50 values 2·8 μM and 2·4 μM respectively) whereas hydroxidine is inactive. One of the prerequisites for the enzyme inhibitory activity of this class of molecule is the presence of an iminium group—it is well known that a positive charge on a polyaromatic nucleus facilitates intercalative binding with DNA. Through UV spectrophotometric and modelling studies, we have shown that the iminium bond plays a more fundamental role in enzyme inhibition through its susceptibility to nucleophilic attack—the inactive analogue hydroxidine has a non-electrophilic iminium bond.

Consequently, we have demonstrated that iminium bond electrophilicity is a parameter which needs to be considered in ternary complex formation with reverse transcriptase.

The pharmacological activity of the benzo[*c*]phenanthridines generally applies to conditions characterized by rapid cell growth (Simeon et al 1989). Sanguinarine (**1**) and chelerythrine (**2**) (Table 1) are active against fungal organisms (Hejtmankova et al 1984) and have significant antibacterial activity (Lenfield et al 1981); neither has antitumour activity (Caolo & Stermitz 1979). Fagaronine (**3**), nitidine (**4**) and *O*-methylfagaronine (**5**) have been reviewed as potential antitumour and antiviral agents, but do not have the biological activity of sanguinarine or chelerythrine (Messmer et al 1972; Zee-Cheng & Cheng 1975; Arisawa et al 1984; Comoe et al 1987, 1988). To date, the locus of activity of these analogues has not been established, but their ability to bind with DNA in what appears to be an intercalative mode is believed to be involved in their mechanism of action (Maiti et al 1982, 1984; Pezzuto et al 1983; Nandi & Maiti 1985; Casiano Torres & Baez 1986; Sen & Maiti 1994).

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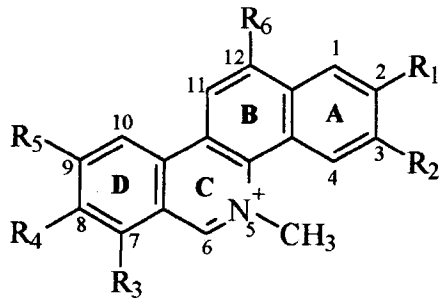
Since the 1970s, the activity of these compounds against a number of DNA-processing enzymes has been assessed (Sethi 1976, 1981, 1984, 1985a, b; Kakiuchi et al 1987; Barret & Sauvaire 1992). A correlation has been established between inhibitory activity against the RNA tumour viruses AMV and MuLV reverse transcriptase (RT) with activity against the P388 leukaemia cell line, because murine leukaemia P388 is an example of the conversion of healthy cells to tumour cells by infection with an oncogenic virus (Poiesz et al 1980). Sethi (1981, 1985a, b) showed that activities were dependent on the different substituent patterns around the benzo[*c*]phenanthridine nucleus, with maximum inhibition of viral DNA activity requiring 2,3,8,9-substituents and an iminium group. Stermitz et al (1975) observed that antitumour activity was confined to this substituent pattern, and that any variation resulted in loss of antitumour activity. RT inhibitory activity was in close agreement with this generalization because

Table 1. The structures of the quaternary benzo[*c*]phenanthridines.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
1		$-OCH_2O-$		$-OCH_2O-$	H	H
2		$-OCH_2O-$	CH_3O	CH_3O	H	H
3	OH	CH_3O	H	CH_3O	CH_3O	H
4		$-OCH_2O-$	H	CH_3O	CH_3O	H
5	CH_3O	CH_3O	H	CH_3O	CH_3O	H
6	CH_3O	CH_3O	CH_3O	OH	H	H
7	CH_3O	CH_3O	H	CH_3O	CH_3O	CH_3O
8	CH_3O	CH_3O	H	CH_3O	CH_3O	CH_2CH_3O
9	CH_3O	CH_3O	H	CH_3O	CH_3O	OH

chelerythrine and sanguinarine were only moderate inhibitors (Sethi 1985). Of significance was the observation that some of the benzo[*c*]phenanthridines formed a ternary complex with the RT and the nucleic acid to which it was bound (Sethi 1984). Fagaronine and nitidine when tested against RT from HIV-1 and HIV-2, showed comparable activity with AMV RT, and were the most potent of 156 pure natural products tested; chelerythrine was inactive (Tan et al 1992).

More recently, topoisomerase enzymes have become a focus for the expression of antitumour activity and it has been shown (Fang et al 1993; Larsen et al 1993) that fagaronine and nitidine stabilized topoisomerase I in a ternary cleavable complex at low concentrations, whereas at higher concentrations they inhibited topoisomerase II, but did not stabilize the cleavable complex.

We have used UV spectrophotometric and molecular modelling studies to propose a mechanism for the inhibition of RT by the benzo[*c*]phenanthridines through the formation of these ternary benzo[*c*]phenanthridine–nucleic acid–enzyme complexes.

Materials and Methods

Chemistry

The quaternary benzo[*c*]phenanthridines hydroxidine, methoxidine and ethoxide were synthesized according to methods developed by Waigh and coworkers (Olugbade et al 1990; Mackay et al 1996, 1998). In summary, the 12-hydroxy-2,3,8,9-tetramethoxybenzo[*c*]phenanthridine (prepared by a four-step synthesis from veratraldehyde and veratrylamine) was treated with the appropriate di alkyl sulphate to produce the corresponding 12-alkoxy ethers, which were subsequently quaternized with methyl methanesulphonate to yield methoxidine and ethoxidine. Hydroxidine was prepared by initially treating the 12-hydroxy-2,3,8,9-tetramethoxybenzo[*c*]phenanthridine with benzyl bromide to yield the 12-benzyloxy analogue; this was quaternized with methyl methanesulphonate and the benzyl ether cleaved by reaction with glacial acetic acid and concentrated hydrochloric acid.

HIV RT Assay

HIV-1 RT (51/66K heterodimer) was expressed in *E. coli* (TG-1) from the recombinant plasmid pRT1 (Larder et al 1987). After a 1 M NaCl extraction procedure the RT was purified by chromatography on an RTMAb8 immunoabsorbent column (Tisdale et al 1988). RT was used to catalyse the incorporation of [^{3}H]thymidine 5′-monophosphate with rAdT (5 $\mu g\,mL^{-1}$ or 50 $\mu g\,mL^{-1}$, 3 : 1) used as template/primer. Reactions were incubated for 10 min at 37°C in the presence and absence of the three benzo[*c*]phenanthridines in a range of tenfold dilutions from 100 μM (dissolved in 1% DMSO reaction mixtures consisting of 50 mM Tris-HCl, pH 8·0, 10 mM dithiothreitol, 10 mM $MgCl_2$, 0·05% nonylphenoxy polyethoxy ethanol, 100 μM NaCl and 5 μM TTP, including 0·1 μM [^{3}H]TTP). At the

end of the incubation, reaction mixtures were spotted on to DE81 discs.

The discs were washed to remove unincorporated label and the activity determined by scintillation counting. IC50 values were calculated from the counts.

H9 DNA polymerase α assay

DNA polymerase α, purified from H9 cells (human lymphocytes) as described by Parker et al (1991), was used in assays to catalyse the incorporation of [^{3}H]deoxyAMP; activated calf thymus DNA (200 $\mu g\,mL^{-1}$) was used as template/primer. Reactions were incubated for 30 min at 37°C, in the presence and absence of the three benzo[*c*]phenanthridines, in a range of tenfold dilutions from 100 μM (dissolved in 5% DMSO reaction mixtures consisting of 1 mM dithiothreitol, 5 mM $MgCl_2$, 0·5 $mg\,mL^{-1}$ bovine serum albumin, 60 mM Tris-HCl pH 7·4, 100 μM deoxyCTP, 100 μM deoxyGTP, 100 μM thymidine triphosphate, 200 $\mu g\,mL^{-1}$ activated calf thymus DNA (Pharmacia), and 12 μM [^{3}H]deoxyATP). Reaction mixtures were spotted on to DE81 discs; these were then washed to remove unincorporated label and the activity was determined by scintillation counting. IC50 values were calculated from the counts.

UV spectrophotometric studies

Determination of alkanolamine formation. The benzo[*c*]phenanthridines methoxidine (**7**), ethoxidine (**8**) and hydroxidine (**9**) were prepared as 10–15 mM solutions in doubly-distilled water. pH was measured with a Phillips PW9418 pH meter calibrated beforehand by use of pH buffer reference standards B-4895 (pH 10·0), B-4770 (pH 7·00) and B-5020 (pH 4·01) from Sigma. Absorbance measurements were taken over the range 330 to 450 nm (Uvikon 930 spectrophotometer) at pH 2·0, 8·0 and 12·0 (adjusted by addition of 2 M sodium hydroxide or hydrochloric acid solution to the drug solutions).

DNA binding studies. Concentrations of highly polymerized calf thymus DNA (type 1) from Sigma in 0·03 M Trizma (Sigma) and 0·018 M NaCl buffer, pH 7·4, were determined spectrophotometrically in terms of nucleotide phosphate calculated from an extinction coefficient of 6600 $M^{-1}\,cm^{-1}$ at a wavelength of 260 nm. Hydroxidine, methoxidine and ethoxidine were prepared as stock solutions in buffer and diluted (with buffer) to a final concentration within the range 30–45 μM. Spectrophotometric titrations were performed by adding different volumes of the calf thymus DNA solution to a solution (2 mL) of the drug in a quartz cuvette (3 mL) to vary the benzo[*c*]phenanthridine/DNA phosphate ratio until no further changes in absorbance were measured. Absorbance was measured over the range 330–450 nm (Uvikon 930 spectrophotometer). All measurements were made at 20°C and an equilibration time of 20 min was left after each addition of DNA. The measurements were repeated twice for each benzo[*c*]-phenanthridine assayed.

Molecular-modelling studies

Molecular orbital (MO) calculations for the benzo-[*c*]phenanthridines were performed using the MOPAC semi-empirical program with graphics visualization using Chem-X (October 1993 version; Chemical Design Ltd, Oxon, UK) and Insight II software (Version 95.0; MSI Technologies, San Diego, CA). Compounds were geometry-optimized using the AM1/MOPAC program, a charge of +1 was assigned to the quaternary nitrogen and −1 to the anionic oxygen of hydroxidine; electrostatic potentials, atom charges, bond-order matrices, $\sigma+\pi$ bonds and π-electron densities were calculated by the AM1/MOPAC semi-empirical method. Electrostatic potential maps of the benzo[*c*]phenanthridines using atom-based point charges determined by AM1 were visualized using the Chem-X graphics interface.

Results

HIV-1 RT inhibition

The enzyme inhibitory assays (Table 2) indicate that two of our synthetic analogues of *O*-methylfagaronine—the 12-alkoxy derivatives methoxidine (**7**) and ethoxidine (**8**)—are potent inhibitors of HIV-1 RT (cf. fagaronine; IC50 21·8 μM; Tan et al 1992). Hydroxidine (**9**) was inactive against both HIV-1 RT and human DNA polymerase α. Both methoxidine and ethoxidine showed some selectivity over human DNA polymerase α.

DNA binding studies

The UV spectra of the 12-alkoxy analogues titrated against DNA show an initial bathochromic and hypochromic change at high drug/DNA ratios, but

Table 2. HIV-1 reverse transcriptase inhibitory activity of the quaternary benzo[*c*]phenanthridines.

Benzo[*c*]phenanthridine	IC50 (μM) against HIV-1 reverse transcriptase	IC50 (μM) against DNA polymerase α
Hydroxidine	Inactive	Inactive
Methoxidine	2·8	31·0
Ethoxidine	2·4	34·0

below ratios of 1·00 (methoxidine) and 0·67 (ethoxidine) a further bathochromic shift occurs with a hyperchromic change, until no further absorption increases were observed below ratios of 0·10 and 0·12, for methoxidine and ethoxidine, respectively. The biphasic nature of the interaction (Figure 1), with no clear isosbestic points, indicates that more than one spectroscopically distinct bound form of the drug is present. These interactions are reminiscent of that of the related alkaloid, coralyne, with DNA (Wilson et al 1976), which is characterized by external association at high coralyne/DNA ratios and intercalation as the ratio decreases below 1·0. The interaction of hydroxidine with DNA also seems to involve a biphasic binding mechanism (Figure 2) with no further changes in absorption observed at a hydroxidine/DNA ratio of 0·11.

Determination of alkanolamine formation

The formation of the alkanolamine form of the benzo[*c*]phenanthridines (Figure 3) at high pH is characterized by collapse of the λ_{max} at 400 nm; this is re-established when the pH is reduced, i.e. there is no absorbance by the alkanolamine form in the long-wave UV band (350–450 nm) (Simanek & Preininger 1977; Caolo & Stermitz 1979; Simanek 1985). These spectral changes are seen for methoxidine (data not shown) and ethoxidine (Figure 4), but not for hydroxidine (Figure 5). For hydroxidine, increasing the pH induces a bathochromic shift of the λ_{max} from 402 to 446 nm, characteristic of phenolic ionization; this again is reversed when the pH is reduced, a spectral change which is not associated with alkanolamine formation. We conclude that the iminium bond in hydroxidine is resistant to nucleophilic attack by the hydroxide ion, with ionization of the phenol group taking precedence over alkanolamine formation in the pH range covered in our study.

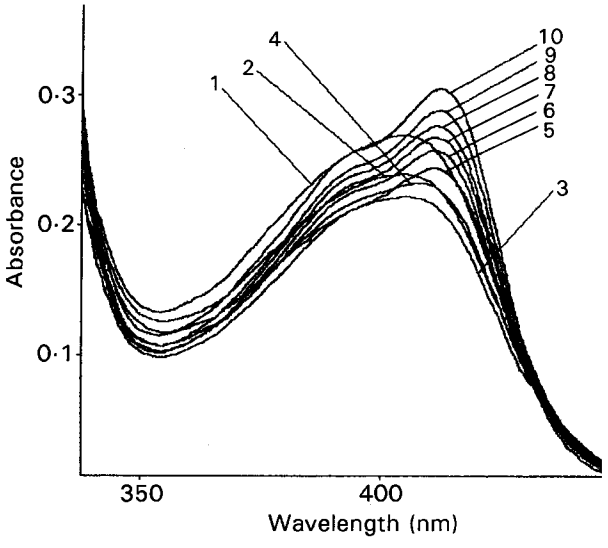


Figure 1. UV absorption spectra of 3·9 μM ethoxidine in 0·018 M NaCl and 0·03 M Tris buffer, pH 7·4, in the absence of DNA (curve 1) and after the addition of calf thymus DNA to yield benzo[*c*]phenanthridine/DNA (phosphate) ratios of 2·86 (curve 2), 1·43 (curve 3), 0·96 (curve 4), 0·58 (curve 5), 0·37 (curve 6), 0·33 (curve 7), 0·3 (curve 8), 0·22 (curve 9) or 0·19 (curve 10). For clarity not all titration curves are shown.

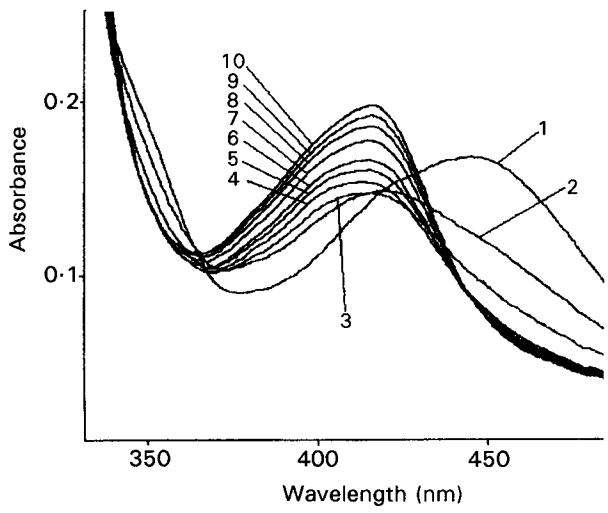


Figure 2. UV absorption spectra of 4·0 μM hydroxidine in 0·018 M NaCl and 0·03 M Tris buffer, pH 7·4, in the absence of DNA (curve 1) and after the addition of calf thymus DNA to yield benzo[*c*]phenanthridine/DNA (phosphate) ratios of 2·94 (curve 2), 1·47 (curve 3), 0·59 (curve 4), 0·43 (curve 5), 0·34 (curve 6), 0·30 (curve 7), 0·22 (curve 8), 0·18 (curve 9) or 0·14 (curve 10). For clarity not all titration curves are shown.

Molecular modelling studies

The electrostatic potential maps of the benzo[*c*]-phenanthridines indicate that the region of highest interaction energy with a unit positive charge is centred around the C-6 position of the iminium bond (>110 kcal mol^{-1}) (Figures 6 and 7) with the exception of hydroxidine. The lowest πz-electron densities for sanguinarine (**1**), chelerythrine (**2**), fagaronine (**3**), *O*-methylfagaronine (**5**) and ethoxidine (**8**) are observed at C-6 (Table 3). The electrostatic potential plot for the phenolate form of hydroxidine (**9**) (Figure 8) is indicative of a molecular electrostatic potential much lower than those of the other benzo[*c*]phenanthridines, tending to be predominantly negative, and a higher πz-electron density at C-6. It seems that the negative charge resident on the oxygen at the 12-position after ionization becomes distributed over the aromatic nucleus increasing the πz-electron density at the 6-position.

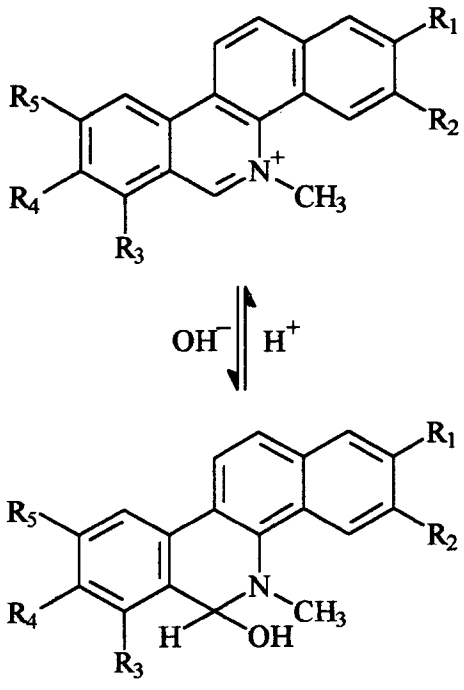


Figure 3. Formation of the alkanolamine form of the benzo-[*c*]phenanthridines.

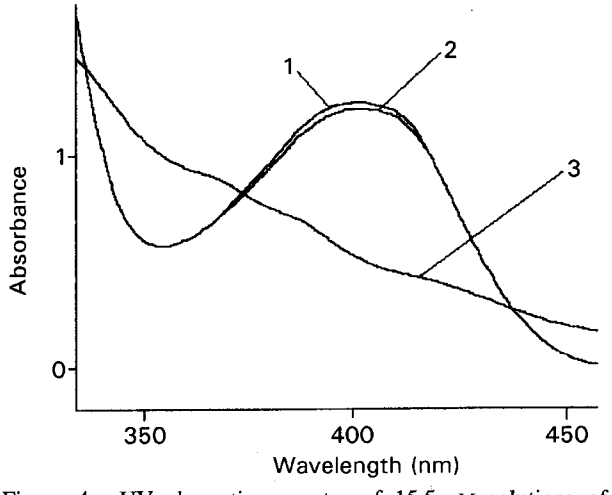


Figure 4. UV absorption spectra of 15·5 mM solutions of ethoxidine at pH 1·4 (curve 1), pH 8·0 (curve 2) and pH 11·4 (curve 3).

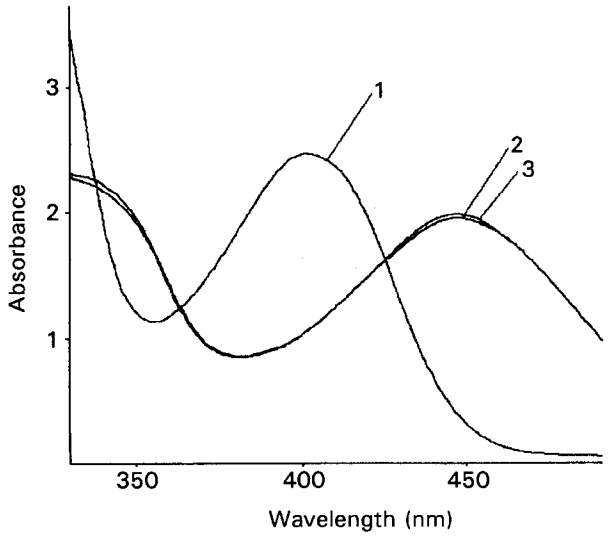


Figure 5. UV absorption spectra of 15 mM solutions of hydroxidine at pH 1·4 (curve 1), pH 8·0 (curve 2) and pH 11·4 (curve 3).

Discussion

The quaternary benzo[*c*]phenanthridines occur as two pH-dependent forms (Caolo & Stermitz 1979)—at low pH the cationic species predominates whereas at high pH this species undergoes conversion to the alkanolamine form as a consequence of nucleophilic attack by a hydroxide anion at the electrophilic iminium bond at position 6 of the benzo[*c*]phenanthridine nucleus (Figure 3). The pK_{R+} of this equilibrium, which is analogous to the pK_a value for a Brønsted acid (denoting the pH at which the heterocyclic cation and alkanolamine are present at equal concentrations) is dependent on the nature and pattern of the oxygenated substituents around the nucleus, with the 7,8-substituted series having lower pK_{R+} values than the 8,9-substituted series (e.g. sanguinarine, 7·32; chelerythrine, 7·53; nitidine, 12·10) (Simanek 1985). The dichotomy in antitumour activity between the active 2,3,8,9-oxygenated and the inactive 2,3,7,8-oxygenated benzo[*c*]phenanthridines has been attributed to these differences in the cation–alkanolamine equilibria. The intercalative binding affinity of sanguinarine for DNA is pH-dependent (Sen & Maiti 1994), this being ascribed to the predominance of the cationic species at low pH (5·2) whereas at higher pH (10·5) the alkanolamine form predominates which, as a consequence of the loss of planarity in the tetracyclic system and the absence of a positive charge on the molecule, does not bind with DNA.

We believe there is a more substantial role for the iminium group in the inhibition of nucleic acid-binding enzymes than the provision of an electrostatic interaction for binding with DNA. We have shown that of the three analogues of *O*-methylfagaronine we have synthesized, methoxidine (**7**), ethoxidine (**8**) and hydroxidine (**9**), two inhibit HIV-1 RT with IC50 values of 2·8 μM (methoxidine) and 2·4 μM (ethoxidine) whereas hydroxidine is inactive. Our DNA binding studies indicate that all three compounds interact with DNA in a biphasic manner reminiscent of the interaction of the related alkaloid coralyne with DNA (Wilson et al 1976). Coralyne is also a potent inhibitor of MuLV and AMV RT (Sethi 1976, 1985a, b) and has a cation–alkanolamine equilibrium (Simanek

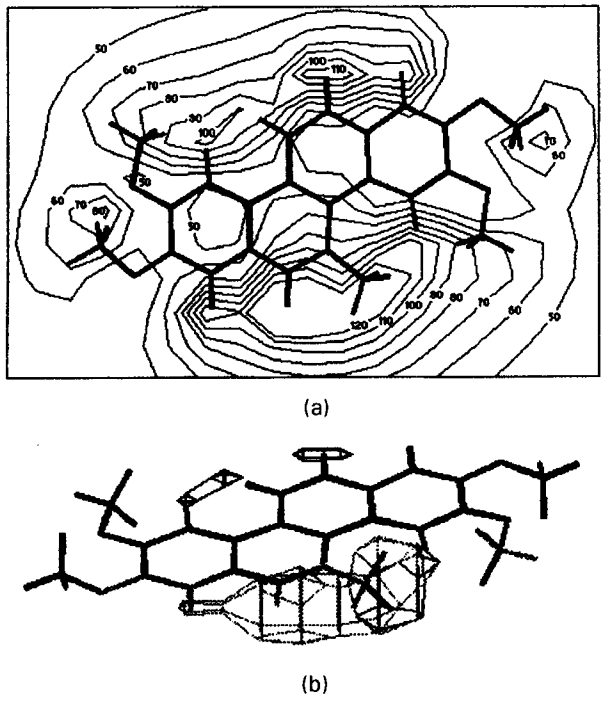


Figure 6. Molecular electrostatic potential map of *O*-methylfagaronine. (a) Electrostatic potential contours through the plane of the molecule with units expressed in kcal mol^{-1}. (b) Electrostatic potential grid showing regions greater than 110 kcal mol^{-1}, predominantly centred over the iminium bond.

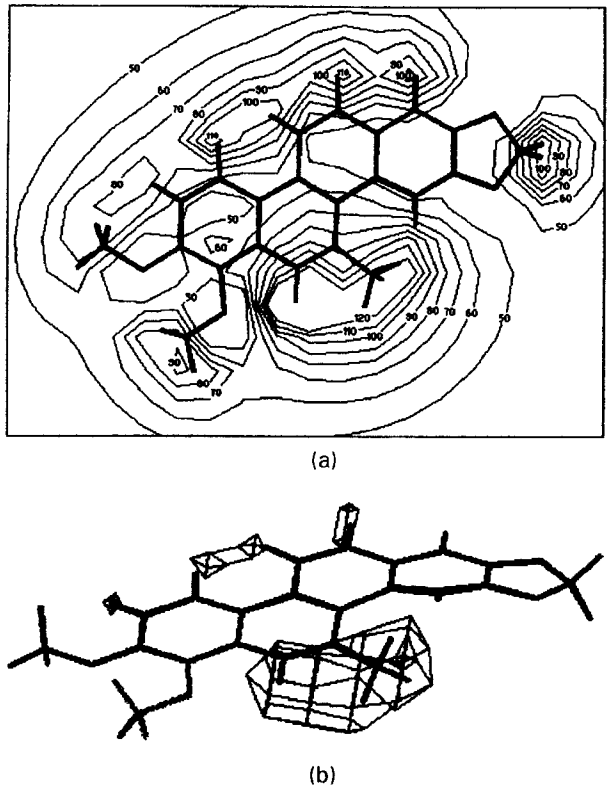


Figure 7. Molecular electrostatic potential map of chelerythrine. (a) Electrostatic potential contours through the plane of the molecule with units expressed in kcal mol^{-1}. (b) Electrostatic potential grid showing regions greater than 110 kcal mol^{-1}, predominantly centred over the iminium bond.

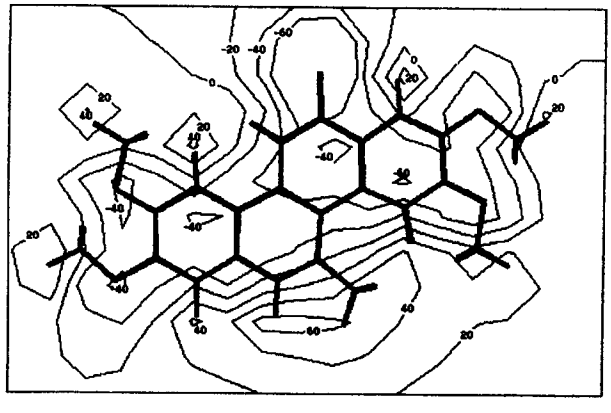
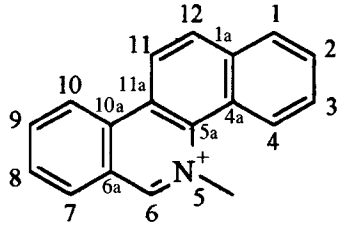


Figure 8. Molecular electrostatic potential contour map of the hydroxidine anion through the plane of the molecule with units expressed in kcal mol^{-1}.

& Preininger 1977). However, of the compounds prepared in this study, only the active 12-alkoxy benzo[*c*]phenanthridines are susceptible to nucleophilic attack at position 6 by the hydroxide anion. We believe that the inhibition of RT and the susceptibility of the iminium bond are linked, and to investigate the electrophilicity of the iminium bond further we have calculated the electrostatic potential maps and the πz-electron densities of the aromatic systems of a series of quaternary benzo[*c*]phenanthridines.

The molecular electrostatic potential is a much better indicator of the electrostatic properties of a molecule than atom-centred charges and can be calculated on a grid surrounding the molecule with contours connecting isopotential points, i.e. points at which the energy of interaction of the molecule with a unit positive charge is identical. Electrostatic potentials can therefore be viewed as a key factor in the control of the long-distance interactions during the approach of a nucleophile to the benzo[*c*]phenanthridine (Grassy et al 1985). Our electrostatic potential maps indicate that an approaching nucleophile would be attracted towards the region of highest interaction energy with a unit positive charge centred around the C-6 position of the iminium bond (>110 kcal mol^{-1}) (Figures 6 and 7) with the exception of hydroxidine. When these results are viewed in association with the calculated πz-electron densities of the aromatic systems, an indication of the site of electrophilicity within the benzo[*c*]phenanthridine can be deduced (Meunier et al 1988). Because the lowest πz-electron densities for sanguinarine (**1**), chelerythrine (**2**), fagaronine (**3**), *O*-methylfagaronine (**5**) and ethoxidine (**8**) are observed at C-6 (Table 3), we can

Table 3. πz-Electron densities of the quaternary benzo[*c*]phenanthridines.



Atom	Benzo[*c*]phenanthridine π z-electron density of compound					
	1	**2**	**3**	**5**	**8**	**9**
1	1·0171	1·0195	1·0311	1·0311	1·0185	0·9291
2	0·9748	0·9764	0·9469	0·9469	0·9744	1·0226
3	0·9437	0·9493	0·9551	0·9551	0·9218	0·9713
4	1·0501	1·0504	1·0671	1·0671	1·0495	1·0575
4a	1·0172	1·0208	1·0367	1·0367	1·0082	1·0031
5a	1·0658	1·0582	1·0376	1·0375	1·0842	1·1660
5	1·4108	1·4237	1·4307	1·4307	1·4129	1·3672
6	0·8219	0·8115	0·8596	0·8596	0·8783	1·0349
6a	1·1305	1·1318	1·0901	1·0901	1·0805	1·0404
7	0·9696	0·9409	1·0222	1·0222	1·0251	1·0265
8	1·0154	1·0239	0·9665	0·9665	0·9632	0·9858
9	0·9306	0·9567	0·9024	0·9024	0·9187	0·9832
10	1·0081	1·0185	1·1077	1·1077	1·0991	0·9882
10a	0·9655	0·9567	0·9235	0·9235	0·9452	0·9773
11a	0·9924	0·9984	1·0081	1·0081	0·9676	0·9729
11	1·0137	1·0111	1·0208	1·0207	1·1487	1·1614
12	0·9302	0·9378	0·9359	0·9359	0·8798	0·7677
12a	0·9921	0·9915	0·9884	0·9884	1·0359	1·0259

*Calculated as the phenolate anion

conclude that nucleophilic attack will be directed towards this site in these compounds. The 2,3,7,8-substituted benzo[*c*]phenanthridines have the lowest πz-electron densities at C-6; this is in accord with their greater susceptibility to attack by the hydroxide anion (Caolo & Stermitz 1979; Simanek 1985; Dostal & Potacek 1990). The electrostatic potential plot for the phenolate form of hydroxidine (**9**) (Figure 8) shows the molecular electrostatic potential to be much lower than those of the other benzo[*c*]phenanthridines; it also shows that the πz-electron density at C-6 is higher, indicating that it has a non-electrophilic iminium bond resistant to nucleophilic attack by the hydroxide ion, as shown by our spectroscopic studies. The electron density at C-12 is low, but in the absence of a corresponding high positive potential ($-60\,kcal\,mol^{-1}$), nucleophilic attack at this position is unlikely. Our conclusion therefore, is that RT inhibitory activity is linked to the presence of an electrophilic iminium bond in the quaternary benzo[*c*]phenanthridines.

Other workers have shown that the iminium bond can be attacked by carbon, nitrogen and sulphur nucleophiles (Dostal & Potacek 1990) and have suggested a role for in-vivo hydration, alkylation or addition at position 6 by certain biological species (Zee-Cheng & Cheng 1975). Studies with AMV and MuLV RT indicate that the inhibitory action of fagaronine and *O*-methylfagaronine is associated with the formation of a labile enzyme–nucleic acid–alkaloid ternary complex (Sethi 1984). The model for inhibition which we propose is that on complexing with the nucleic acid substrate the quaternary benzo[*c*]phenanthridine is bound in such a way that it undergoes nucleophilic attack by a residue within the enzyme (such as cysteine, serine, threonine or tyrosine) when it binds to the substrate-benzo[*c*]phenanthridine complex. The resulting ternary complex is stabilized by a labile covalent bond between the enzyme and the benzo[*c*]phenanthridine which, in turn, is bound to the nucleic acid substrate and enzyme inhibition is effected. Hydroxidine, with a non-electrophilic iminium bond, would be unable to undergo this complexation process and therefore enzyme inhibition would not occur.

Electrostatic potential plots and πz-electron densities for sanguinarine and chelerythrine also revealed electrophilicity at the iminium bond. The weak RT inhibitory activity associated with these alkaloids might be a consequence of the assays being performed at pH 7·0, where a significant percentage of the compounds would exist as the alkanolamine form, thus reducing their affinity for the template-primers (Sen & Maiti 1994).

The absence of inhibitory activity of fagaronine, *O*-methylfagaronine and nitidine with nucleic acid substrates containing only G and C (even though binding is evident) (Sethi 1981, 1984, 1985a, b; Kakiuchi et al 1987) suggests that iminium bond electrophilicity is not the only factor governing enzyme inhibition. Enzyme inhibition might be influenced by the orientation of the benzo[*c*]phenanthridine when bound between the base pairs of the nucleic acid substrate; this, in turn, might be a function of base-pair composition. If inhibition requires the formation of a covalent bond with a residue in the enzyme, then the relative position of the iminium bond will be important in terms of proximity to attack. The macromolecular structure of the enzyme bound with a particular nucleic acid substrate might exert considerable influence on the geometry of the bound benzo[*c*]phenanthridine, and modelling studies are currently in progress to investigate these complexes. We must also consider the electronic effects of different neighbouring base-pairs on the electronic charge distribution of the bound drugs, particularly considering the proposed role for the iminium bond, for which we are also developing appropriate models.

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