9-Hydroxyellipticine and Derivatives as Chemotherapy Agents

Margaret M. Harding* and Annaleise R. Grummitt

School of Chemistry, The University of Sydney, N.S.W. 2006, Australia

Abstract: The hydroxy group in 9-hydroxyellipticines increases the apparent affinity for DNA, stabilisation of toposiomerase II-DNA cleavable complex, oxidation to reactive quinone-imine intermediates, phosphorylation of p53 suppressor proteins and cytotoxicity relative to the parent ellipticines. Recent studies have focused on the mechanism of inhibition of phosphorylation of the mutant type of p53 protein, structural characterisation of the drug-DNA complex, the synthesis of carbohydrate derivatives and calculations of physical parameters, including dipole moments, as potential screens to allow identification of new active derivatives. Derivatisation at the 2- and 9-positions has lead to significant improvements in the *in vivo* activity of the 9-hydroxyellipticine derivatives and has provided important insights into the mechanism of action of these compounds.

INTRODUCTION

Ellipticine **1** is a plant alkaloid, originally isolated in 1958 from the leaves of the plant *Ochrosia elliptica* of the *Apocynaceae* family [1]. This compound, as well as 9-methoxyellipticine **2**, shown in Fig. (**1a**), were identified as anticancer agents in 1967 [2], and later showed promising antitumor activity against a variety of tumor cell lines [3], with the lactate salt of 9-methoxyellipticine **2** being selected for clinical trials against acute myeloblastic leukaemia [4]. The hydrophobic structure and resulting limited aqueous solubility of these compounds, first observed by Goodwin and coworkers during the original isolation of these natural products [1], precluded clinical trials and initiated research to develop water-soluble derivatives of these alkaloids.

Over the past three decades, a range of derivatives of ellipticine **1**, as well as isomers of ellipticine and related heterocycles [5], have been studied in order to obtain compounds that retain the promising anticancer profiles of the natural products but have improved aqueous solubility. While incorporation of a 9-hydroxy substituent on the ellipticine core to give 9-hydroxyellipticine **4** (9OHE) [6,7] conferred somewhat improved aqueous solubility on the drug and a vastly improved biological profile, clinical trials were nevertheless terminated due to limiting aqueous solubility [8]. The introduction of charge into ellipticine via quaternization of the pyridinic nitrogen greatly enhanced the water solubility of 9OHE **4** and led to the development of ellipticinium acetate or Celiptium® **5**, which is the ellipticine derivative that has achieved most success to date in the clinic [8]. This compound was studied extensively in clinical trials [8,9] and shown to be a strong candidate for salvage treatment of metastatic breast cancer [10]. It also exhibited low haematological toxicity, lending it much promise as a component of combination therapy for the treatment of metastatic breast cancer. Celiptium **5** has since enjoyed exposure in the clinic, predominantly in combination with vinblastine, in the treatment of this form of cancer [5].

A large number of derivatives of the naturally occurring alkaloids ellipticine **1**, 9-methoxyelliptine **2**, and the related compound olivacine **3** (Fig. (**1a**)) have been reported. Recent developments in the mechanism of action of olivacine **3** have been summarized in a review [11], while the patent literature surrounding a range of heterocycles related to, and including, ellipticine **1** has also been reviewed [5]. Despite the close structural similarity between ellipticine **1** and olivacine **3**, which differ in the location of a single methyl group at position 11 or 1 respectively, these compounds differ with respect to cytotoxicity and metabolism. The subtle structural differences between the natural products **1**-**3**, and the disparate range of synthetic derivatives reported, have made comparisons of their properties and general conclusions regarding the mechanism of action of ellipticines difficult. This review will focus on a specific class of compounds related to 9OHE **4** which is derived from the natural product **2**. The properties and reactivity of 9OHE **4** will be reviewed, followed by structure-activity studies and a discussion of the mechanism of anticancer action of 9OHE **4** as well as 2-substituted, 9-substituted and 2,9-disubstituted derivatives of **4**.

PROPERTIES AND REACTIVITY OF 9-HYDROXYELLIPTICINE

9OHE **4** is classified as a nucleophile, due to both the pyridinic nitrogen at position 2 and the phenolic group at position 9, and also exhibits acid and base properties due to the presence of the phenolic and pyridinic nitrogen functional groups, respectively. These properties are significant as they have dictated the nature and variety of derivatives of **4** that have been reported and the extent to which these structural modifications have been pursued. These structural features also result in the formation of oxidation products not obtained with **1**-**3** that are highly relevant to the biological activity of 9OHE **4**, discussed in a later section.

*Address correspondence to this author at the School of Chemistry, University of Sydney, NSW 2006, Australia; Fax: +61 2 9351 6650; Email: harding@chem.usyd.edu.au

1389-5575/03 $41.00+.00

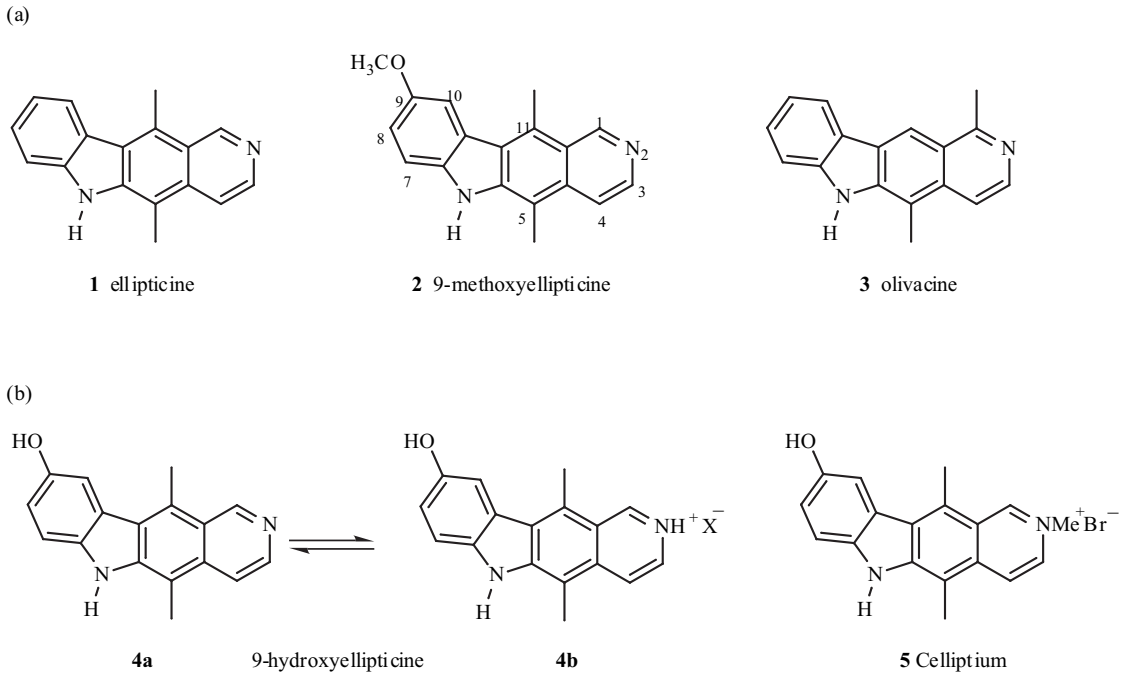


Fig. (1). Structures and numbering scheme of (a) natural products related to ellipticine and (b) 9-hydroxy ellipticines.

9OHE **4** is quite readily oxidized, with methanol solutions, in particular, of this compound observed to undergo significant degradation in the presence of oxygen [12]. The rate of this oxidation, its mechanism, and the products formed depend on the reaction conditions, the level of substitution at N2 and the solution pH [12-15]. When 9OHE **4** is quaternized as in **5** or protonated at N2 as in **4b**, electrochemical oxidation occurs via a single-electron process, while under basic conditions, the non-quaternized derivatives undergo an electrochemical oxidation involving two electrons [16]. Studies in the presence of chemical oxidants and using a number of techniques have confirmed the formation of a number of oxidation products including the quinone-imine **4d** (Fig. (**2**)) [12,15]. This quinone-imine is highly electrophilic and hence prone to attack by nucleophiles at the 10-position to give adducts of the general structure **4e** [3]. Independent electrochemical studies on Celiptium **5** have shown that, under basic conditions, the compound is readily oxidized to the di-*o*-quinone **5b**, presumably proceeding via the *p*-quinone-imine **5a** [12] (Fig. (**2**)).

The pK_a for protonation of 9OHE **4** is around 7.2 [16] and hence, under physiological conditions, 9OHE exists in

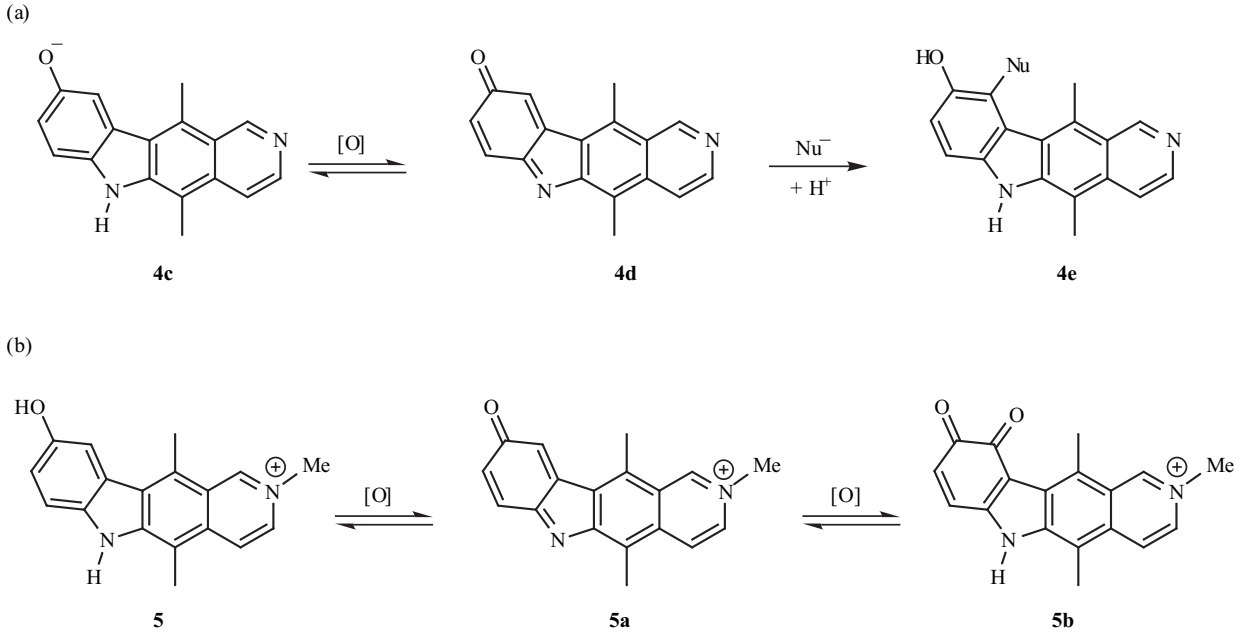


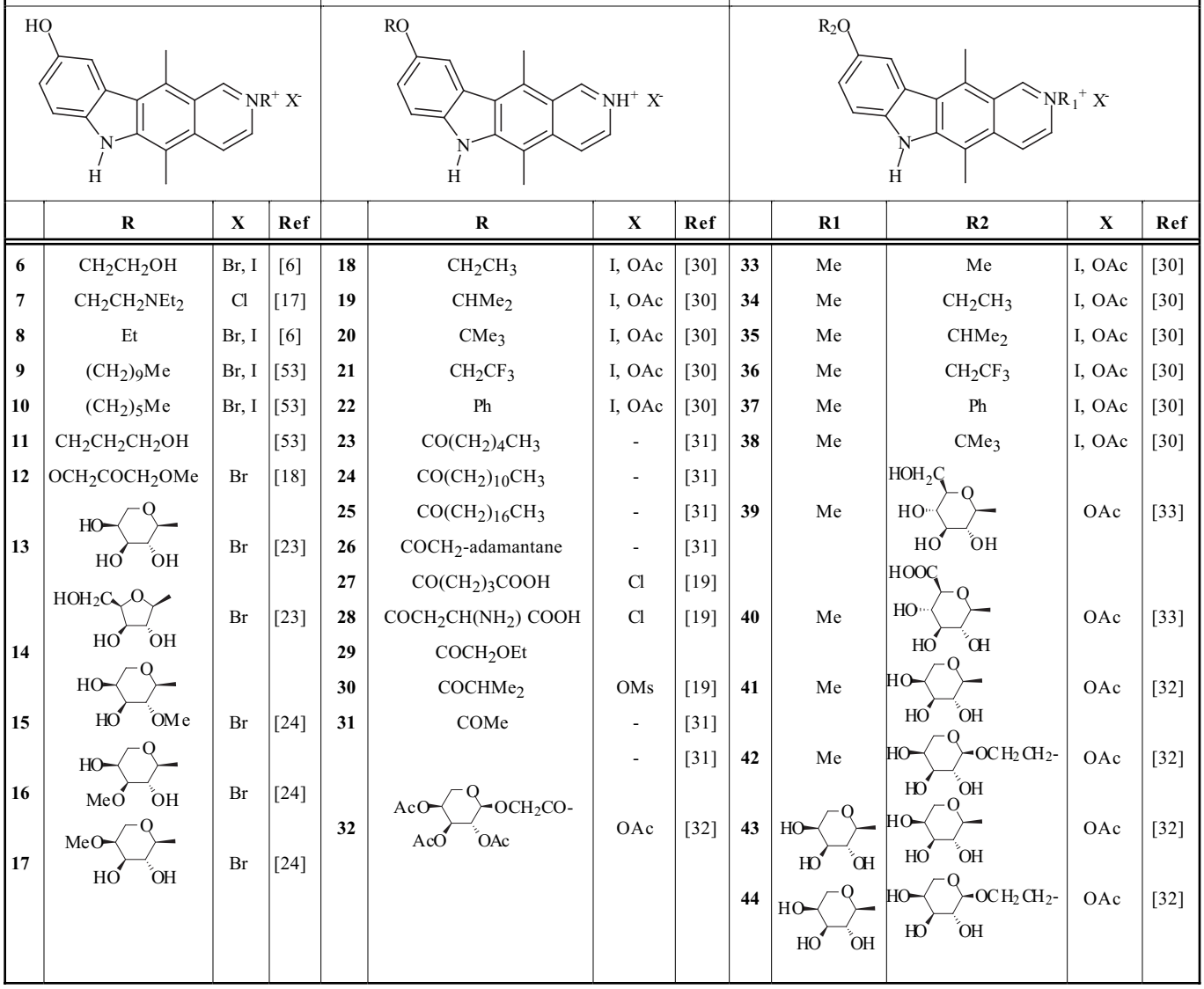
Fig. (2). Key intermediates formed on oxidation of 9OHE and Celiptium.

equilibrium between the protonated form **4b**, and the free base **4a**. The acidity of the 9-hydroxy group is comparable to related phenols ($pK_a \sim 10$). The proton on N6 is more acidic than the corresponding proton on indole, and the positive charge in quaternized ellipticines such as **5** facilitates removal of the proton in a manner similar to the formation of anhydronium bases reported for related plant alkaloids [1]. The ionisation state of 9OHE **4** has a profound effect on solubility and also reactivity, which has hampered the preparation of analogues functionalized at the 9-position. Thus, while **4a** is soluble in water, alcohols, DMF and $MeNO_2$, the salt **4b** is restricted to only water, alcohols and DMF. Both the 2- and 9-positions of 9OHE **4** have, however, been functionalized with electrophilic reagents. Due to the competing oxidative degradation pathways that are promoted under basic conditions (Fig. (**2a**)), as well as solubility constraints, the pre-formation of phenolate salts has generally been avoided. Fig. (**3**) summarizes key 2- and 9-substituted derivatives of ellipticine, as well as 2,9-disubstituted derivatives that have been reported.

2-SUBSTITUTED DERIVATIVES

Quaternary salts of 9OHE **4** formed by alkylation at N2 have been widely studied due to the significant benefits that quaternization confers on the aqueous solubility of the ellipticines. Three classes of quaternary salts have been reported which are illustrated by the *N*-alkyl derivatives **6**-**11**, the *N*-oxide alkyl derivative **12** and the *N*-glycosides **13**-**17**.

In addition to conferring improved aqueous solubility, the nature of the *N*-alkyl substituent in simple alkyl derivatives is important in determining the biological activity of the ellipticine derivative. Thus, within the group **6**-**11**, datelliptium **7**, which contains a (dialkylamino)alkyl sidechain, was identified for clinical evaluation. Datelliptium **7** showed a similar biological profile to Celiptium **5**, but its enhanced lipophilicity resulted in greater cytotoxicity, possibly due to an increase in diffusion across the cellular membrane and a more favourable *in vivo* biodistribution [17]. Datelliptium **7**, as with Celiptium **5**,



	R	X	Ref
6	CH_2CH_2OH	Br, I	[6]
7	$CH_2CH_2NEt_2$	Cl	[17]
8	Et	Br, I	[6]
9	$(CH_2)_9Me$	Br, I	[53]
10	$(CH_2)_5Me$	Br, I	[53]
11	$CH_2CH_2CH_2OH$		[53]
12	OCH_2COCH_2OMe	Br	[18]
13		Br	[23]
14		Br	[23]
15		Br	[24]
16		Br	[24]
17		Br	[24]

	R	X	Ref
18	CH_2CH_3	I, OAc	[30]
19	$CHMe_2$	I, OAc	[30]
20	CMe_3	I, OAc	[30]
21	CH_2CF_3	I, OAc	[30]
22	Ph	I, OAc	[30]
23	$CO(CH_2)_4CH_3$	-	[31]
24	$CO(CH_2)_{10}CH_3$	-	[31]
25	$CO(CH_2)_{16}CH_3$	-	[31]
26	$COCH_2$-adamantane	-	[31]
27	$CO(CH_2)_3COOH$	Cl	[19]
28	$COCH_2CH(NH_2)$ COOH	Cl	[19]
29	$COCH_2OEt$		
30	$COCHMe_2$	OMs	[19]
31	COMe	-	[31]
32		OAc	[32]

	R1	R2	X	Ref
33	Me	Me	I, OAc	[30]
34	Me	CH_2CH_3	I, OAc	[30]
35	Me	$CHMe_2$	I, OAc	[30]
36	Me	CH_2CF_3	I, OAc	[30]
37	Me	Ph	I, OAc	[30]
38	Me	CMe_3	I, OAc	[30]
39	Me		OAc	[33]
40	Me		OAc	[33]
41	Me		OAc	[32]
42	Me		OAc	[32]
43			OAc	[32]
44			OAc	[32]

Fig. (3). Summary of key derivatives of 9-hydroxyellipticine.

was active in salvage treatment for metastatic breast cancer [5].

The second class of quaternary salts of 9OHE **4** are derivatives of 9-hydroxyellipticine 2-*N*-oxide, which were designed to enhance the drug's water solubility [18]. The *N*-oxide **12** showed potent activity against P388 leukaemia, colon 26 and Lewis lung carcinoma and good water solubility, but pharmacological evaluation showed that these derivatives were highly unstable in water and were readily hydrolyzed to the parent compound 9OHE **4** [19].

Given the importance of carbohydrates in many naturally occurring DNA-binding antitumor antibiotics [20], as well as the significant improvements in aqueous solubility conferred by carbohydrates [21], Honda *et al* have reported the synthesis and biological activity of 49 *N*-glycosides of 9OHE **4** against a range of cell lines [22,23]. The stereochemistry and ring size (furanose and pyranose) of the sugars was systematically varied and, on the basis of preliminary investigations, 5 glycosides were selected as promising candidates for further clinical evaluation against L1210 leukaemia, P388 leukaemia, B16 melanoma, colon 38 carcinoma, Ehrlich ascites carcinoma and sarcoma 180 [23]. The arabinosyl **13** and xylosyl **14** derivatives outperformed ellipticine **1**, 9OHE **4** and Celiptium **5** in these tests and, due to their high activity, were entered into clinical trials. Clinical trials of the arabinosyl glycoside **13** (ellipravin), were discontinued as a result of high liver toxicity [5], while the progress of the xylosyl derivative **14** through clinical trials has not been reported. Further structure-activity studies on ellipravin **13** focused on the importance of the three hydroxy groups in the arabinose sugar for conferring biological activity. Masking each of these three hydroxy groups as methyl ethers (derivatives **15-17**) resulted in drastic reduction in the drug's activity against L1210 leukemia in mice, demonstrating the importance of the fully hydroxylated sugars in **13** for activity [24].

Recent theoretical calculations on substituted ellipticines, including 9OHE **4** and Celiptium **5**, have revealed a simple correlation between the dipole moment of the drug and antitumor activity [25]. Low dipole moments of a range of ellipticines were correlated with high cytotoxicity. On this basis, the authors proposed that modification of the dipole of ellipticines provides a method to improve and design new antitumor ellipticine derivatives, as well as to select promising molecules from untested groups [25].

A number of other groups including porphyrins [26] and steroids [27] have been attached to 9OHE **4** via N2 (structures not shown). Estradiol and (*E*)-clomiphene have been attached to 9OHE **4** and Celiptium **5** as specific vectors for the targeting of breast tissue [27]. However, competition experiments with estradiol on the hormone-dependent human MCF-7 breast cancer cell line only showed activity in ellipticine conjugates lacking the 9-hydroxy substituent.

9-SUBSTITUTED DERIVATIVES

9-Substituted derivatives of 9OHE **4** fall into two general classes: ethers (eg., **18-22**) and esters (eg., **23-32**). The ethers may be considered analogues of the natural product 9-methoxyellipticine **2** [28], which is highly cytotoxic to malignant cultured cells [2], and induces remissions from myeloblastic acute leukaemia in humans [4]. 9-Methoxyellipticine **2** has been proposed to undergo *O*-demethylation *in vivo* to generate 9OHE **4** as the active species [29].

The *O*-alkyl derivatives **18-22** were evaluated for *in vitro* activity against the National Cancer Institute human tumor cell line panels [30]. A range of activity was seen with different tumor cell lines, but all derivatives were non-specific for central nervous system (CNS) tumors and analogues with bulkier substituents at C9 retained activity.

Forty ester derivatives of 9OHE **4**, including **27-31,** were prepared in order to assess the feasibility of designing water-soluble pro-drugs of 9OHE **4** that have improved pharmacokinetics and tissue distribution [19]. The ester sidechains incorporated alkyl, amino acid, amide and acid functional groups. Screening of these derivatives against P388 leukaemia, colon 26, Lewis lung carcinoma and B16 melanoma in mice identified the glutarate ester **27** as the most potent compound. The stability of the glutarate ester **27** in blood plasma was measured, and 9OHE **4** was identified as the metabolite released with a half life of only a few minutes. Thus, ester derivates of 9OHE most probably act as pro-drugs to release 9OHE **4** *in vivo*, as discussed further in a later section.

In an independent study [31], a range of long chain esters including **23-26** were tested for activity against the solid form of Sarcoma 180 and Ehrlich carcinoma in mice. While a number of the short chain esters, including **23**, showed significant antitumor activity, none of the derivatives showed superior activity to 9-methoxyellipticine **2** and neither of the long chain esters **24** and **25** exhibited any antitumor activity.

Given the promising results obtained with *N*-glycosides **13-17**, including the development of ellipravin **13**, a logical extension of this work is the attachment of carbohydrates to the 9-hydroxy position of 9OHE. However, selectivity for the 9-position is difficult to attain in the direct glycosidation of 9OHE **4** due to the competing nucleophilicity of the pyridinic N2. This difficulty was overcome by incorporating an ester linkage between the sugar and the 9-hydroxy group to give derivative **32**, which utilizes a short glycolate linker [32]. However, the glycolate ester linkage was very susceptible to hydrolysis and the compound is thus unsuitable for biological evaluation.

2,9-DISUBSTITUTED DERIVATIVES

2,9-Disubstituted derivatives of 9OHE **4** have been prepared from Celiptium **5** (R1 = Me) and ellipravin **13** (R1 = arabinosyl) (Fig. (**3**)). Derivatives **33-38** are related to the 9-substituted ellipticine ethers **18-22**. The bismethyl derivative **33** has been shown to exhibit significant selective cytotoxicity against a subpanel of human CNS tumors when compared with a broader panel of human cells *in vitro* [30]. This selectivity also occurs with Celiptium **4** but only at

low drug concentrations. In contrast to the unquaternized compounds **18**-**22**, the corresponding quaternized derivatives **34**-**38** displayed CNS tumor selectivity, the extent of which was reduced by increasing the steric bulk of the 9-alkoxy group. The CNS selectivity of the 9-phenoxyderivative **37** was less pronounced than for the other alkyl derivatives which was attributed to the fact that a diaryl ether would not be expected to be cleaved *in vivo*. Thus, the 9-phenoxy salt **37** was proposed as a new lead structure for the development of new CNS selective drugs in this class [30]. The increased selectivity of the trifluoroethoxy ether **36** compared to the ethoxy ether **34** suggest that electronic differences may also be important in CNS selectivity [30].

In contrast to *N*-glycosidation of 9OHE **4**, there is only a single report of *O*-glycosidation of Celiptium **5** to give glycosides **39** and **40**. The 9-*O*-glucoside **39** and the 9-*O*-glucuronide **40** were reported to be stable to hydrolysis in the pH range 1-13 [33]. Glucose was chosen as the carbohydrate portion due to the high activity of β-glucuronidase in neoplastic cells and the absence of the *O*-glucuronide of 9OHE in studies on the metabolism of 9OHE **4**. However, the compounds were found to be 10 and 200 times, respectively, less active than Celiptium **5** against L1210 leukaemia [33]. Glycosides **39** and **40** were designed to function as pro-drugs which would release Celiptium **5** in the presence of a glycosidase enzyme specific for the carbohydrates present in these derivatives. As the glycoside linkages were reported to be stable, the reduced activity was explained by poor penetration of the glycosides into the cells and a lack of enzymatic hydrolysis to release **5** *in vivo*.

Our group have synthesized a range of *O*-glycosides of Celiptium, including derivatives **41** and **42** [32]. In contrast to the rationale of Dugue *et al* [33], carbohydrates were chosen as DNA recognition motifs that may enhance or modulate the interaction of 9OHE **4** with DNA and/or DNA/topoisomerase II cleavable complexes. Derivatives **43** and **44** are the analogous carbohydrate derivatives synthesized from ellipravin **13** [32]. The arabinosyl derivatives **41** and **43** formally contain a glycosidic bond to the 9-hydroxy position, while **42** and **44** contain an ether linker for attachment of the arabinose sugar. Due to the vastly different stabilities of these linkers, in particular the high stability of the ether functionality, it is expected that these compounds will undergo different reactions *in vivo*. Biological testing of these derivatives has yet to be reported.

Finally, ditercalinium (structure not shown), in which two molecules of 9-methoxyellipticine were linked by a substituted aminocyclohexane linker, was designed to function as a DNA bisintercalator, and showed high activity against human cell lines *in vitro* [34]. Accumulation of the drug in the mitochondria of the liver and the related high levels of liver toxicity resulted in withdrawal of the drug from clinical trials [35].

DNA-BINDING PROPERTIES

While the strong interaction of ellipticine **1** with DNA via intercalation is well-established [36], peripheral substituents on the ring, including the 9-hydroxy substituent perturbate the electronic distribution in the heterocycle [25,37], and influence the binding constants and binding orientation of ellipticine derivatives with DNA. There is a 13-fold increase in the association constant of 9OHE **4** with DNA ($K_{assoc} = 2\times10^6\ M^{-1}$), compared to that of ellipticine **1** [38].

There are no X-ray crystal structures or NMR structures of 9OHE **4** or any of its derivatives with short oligonucleotides, and hence the molecular level detail of the exact binding orientation of 9OHE **4** with respect to the DNA bases and the effect of binding on the DNA conformation are unknown. While early CD studies of the interaction of Celiptium **5** with DNA predicted that the chromophore was oriented perpendicular to the helix axis [39], the exact orientation could not be determined. Two recent studies have resolved this issue and provided some important details on the nature of the drug-DNA complex. Theoretical studies on the interaction of 9OHE **4** with the oligonucleotide, $d(ATATATATATAT)_2$, have shown that the presence of the 9-hydroxy group results in the chromophore being orientated perpendicular to the long axis of the DNA base pairs [40]. Furthermore, the orientation of the chromophore with respect to the DNA base pairs is specific in positioning the pyridinic nitrogen N2 into the major groove and the 9-hydroxy group in the minor groove where it participates in hydrogen bonding with water molecules (Fig. (**4**)). This proposed orientation was confirmed by spectroscopic studies on the interaction of 9OHE **4** with calf thymus DNA, poly$[d(AT)_2]$ and poly$[d(GC)_2]$ using circular and linear dichroism, resonance light scattering and molecular dynamics [41].

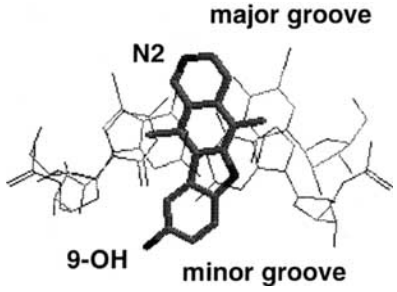


Fig. (4). Computer generated view of the binding orientation of 9OHE with DNA.

Very few studies on the interaction of the 9- or 2-substituted derivatives of 9OHE **4** with DNA have been reported. The nature of these substituents is clearly important as it may influence the electronic distribution in the chromophore and hence dictate the stacking interactions of the drug with the DNA base pairs. Celiptium **5** intercalates [28] with an increased binding constant for DNA due to quaternization at N2 [13]. The introduction of the lipophilic (diethylamino)ethyl group present in datelliptium **7**, does not affect the association constant of the drug with DNA or its ability to intercalate with DNA. However, the DNA unwinding angle associated with binding of the drug is decreased [17]. An affinity constant of ellipticines for

DNA of 10^5 M^{-1} or greater has been proposed to be necessary, but not sufficient, for antitumor activity [38].

Molecular modelling studies on ellipravin **13** have predicted that the *N*-glycosides of 9-hydroxy- and 9-methoxyellipticine prefer to intercalate perpendicular to the DNA base pairs with the sugars oriented in the major groove [24]. This binding mode is in agreement with that observed with 9OHE **4** shown in Fig. (**4**). However there is no experimental data to support this prediction, and the difference in energy between the two possible binding modes that position the arabinose sugar in either the major groove or the minor groove is small [24].

The carbohydrate containing 2,9-disubstituted derivatives **43** and **44** have been designed as potential DNA-threaders [32]. The binding of 9OHE **4** with DNA is reminiscent of the naturally occurring anthracyclines, including anthracycline DNA-threaders, which bind to DNA by intercalation [42]. The anthracyclines and 9OHE **4** both contain four fused aromatic rings which result in the long axis of the drug orienting perpendicular to the DNA base pairs. As shown in Fig. (**4**), this orientation allows the attachment of minor and major groove binding motifs via the phenol and pyridinic nitrogen.

INHIBITION OF TOPOISOMERASE ENZYMES

There is strong evidence that the cytotoxicity of the ellipticines is related, at least in part, to their ability to induce DNA strand breaks by stabilisation of the DNA-topoisomerase II cleavable complex. Despite much interest in this topoisomerase II poison activity, few comprehensive studies have been conducted to clearly delineate the structure-activity relationships involved in this activity. 9OHE **4** exhibits topoisomerase II poison activity, through the formation of ternary enzyme-drug-DNA cleavable complexes [38]. The 9-hydroxy group of 9OHE **4** is a key feature required for this activity, with formation of a quinone-imine, such as **4d** (Fig. (**2b**)), proposed to be an essential characteristic of general topoisomerase II poisons [38]. The 9-hydroxy group in **4** plays a crucial role in the stabilisation of the cleavable complex, with derivatives such as ellipticine **1** and 9-methoxyellipticine **2**, that lack the 9-hydroxy substituent, or ellipticines containing a hydroxy group at other positions on the ring, inducing much lower levels of DNA cleavage [13]. Compared to the free base ellipticines, quaternization of the nitrogen (Celiptium **5** and datelliptium **7**) does not significantly change the cleavage potency, and it has been proposed that the degree of DNA unwinding upon binding of the drug may be a determinant in the topoisomerase II-DNA-drug complex stability [13].

The topoisomerase II-mediated DNA cleavage activity induced by Celiptium **5** and datelliptium **7** in a human small cell lung carcinoma has been compared with the established topoisomerase II inhibitor 4'-(9-acridinylamino)methanesulfonate (*m*-AMSA). While datelliptium **7** and Celiptium **5** both induced double and single strand breaks, the amount of damage was reduced compared with *m*-AMSA. The limited cleavage activity is due to a secondary inhibition of the cleavable complex formation by intercalation of the drug with DNA [44]. Datelliptium **7** presented a weaker secondary inhibition effect on the DNA cleavage reaction than did Celiptium, which was suggested to result from a greater affinity of datelliptium for DNA [43].

The above data suggest that derivatives of 9OHE **4** in Fig. (**3**) that lack the critical phenol functional group, or are unable to be metabolized to generate this species *in vivo*, will be unable to stabilize the DNA-topoisomerase II cleavable complex in the same way as 9OHE **4**. The interaction of the 9-OH group with the DNA-enzyme complex, as well as DNA-intercalation are key requirements for topoisomerase II poison activity [13], whilst substituents at the 2-position influence the inhibition pathway to a much lower extent. [13].

INHIBITION OF PHOSPHORYLATION OF MUTANT PROTEIN P53

The p53 tumor suppressor gene is mutated in >50% of human tumors [45], with the majority of clinical agents for cancer treatment more active against the wild-type than the mutant protein [46]. The phosphorylation of mutant p53 is selectively inhibited by 9OHE **4** [5]. Recently, 9OHE **4** has been shown to influence the drug sensitivity of human pancreatic cancer cells displaying p53 mutations to different anticancer agents, with the cells showing increased sensitivity to some drugs, such as cis-platin, and reduced sensitivity to others, such as 5-fluorouracil. Whilst this mechanism is not clearly understood, it is thought to be related to the restoration of the wild-type p53 activity, and may involve cellular mechanisms such as growth arrest induced by overexpression of p21 in response to this p53 activity [47]. Independent studies have proposed that 9OHE **4** may cause arrest in the G1 phase and induction of G1 phase-restricted apoptosis by this restoration of wild-type p53 function [48].

A comprehensive cluster analysis of 112 ellipticine analogues by the National Cancer Institute investigated the CNS selectivity of these compounds and their relative activities against mutant and wild-type p53 [46]. The 2-substituted quaternized ellipticines, including Celiptium **5** and datelliptium **7**, showed selectivity for cells of CNS origin and were also more potent on average against p53 mutant cells than against the p53 wild-type cells. This was not the case for the non-quaternized derivatives, such as ellipticine **1** and 9-methoxyellipticine **2**.

REDOX CHEMISTRY

While interaction with DNA is a feature common to both ellipticine **1** and 9OHE **4**, the hydroxy group in 9OHE **4** facilitates a number of redox pathways that are important in its mechanism of action. As discussed in the previous sections, the quinone-imine **4d** (Fig. (**2**)) is strongly electrophilic and particularly prone to attack by biological nucleophiles including glutathione, amino acids and nucleic acids [3]. Thus, 9OHE **4** may be considered to act as a pro-alkylating agent. The high reactivity of ribonucleosides and

ribonucleotides with 9OHE **4** under oxidative conditions to give 10-substituted derivatives of the general structure **4e** [3,49] suggests that these reactions can occur *in vivo*, and is supported by the covalent binding of Celiptium **5** to nucleic acids in L1210 leukaemia cells [50].

The oxidation processes (Fig. (**2**)) require the presence of an hydroxy (or similar) group at C-9, in a position para to the indole nitrogen, to allow the generation of the quinone-imines **4d** and **5a**. Such oxidation processes occurring *in vivo* are often coupled to the generation of superoxide radicals as well as radical intermediates of the compound itself, which are able to react with DNA to produce strand breaks, resulting in apoptosis of the cell [3,38].

The importance of these pathways in the overall antitumor activity of 9OHE **4** is highlighted in studies on the isostructural 9-amino and 9-fluoro derivatives of ellipticine. These compounds retain a high affinity for DNA but have little or no *in vivo* activity [3], presumably due, at least in part, to the disabling of the redox pathways that are accessible to 9OHE **4**.

METABOLISM

As discussed earlier, derivatives of 9-hydroxyellipticine-*N*-oxide (eg, **12**) are unstable in water, being readily converted to 9OHE **4**. Similarly 9-acyloxy derivatives including **23**-**32**, while more stable *in vitro*, are presumed to be hydrolyzed *in vivo* to generate 9OHE **4** as the active species. Nevertheless, the 9-acyloxy pro-drugs allow a convenient means for modifying the lipophilicity and water solubility of 9OHE **4** and hence affect the pharmacokinetics and tissue distribution of the drug [19]. Thus, while all esters are believed to be hydrolyzed *in vivo*, the nature of the ester sidechain does influence the antitumor profile of the drug. Studies into hydrolysis of the glutarate derivative **27** in phosphate buffer at body temperature gave a half life of 2.6 h, but in mouse plasma or blood, hydrolysis occurred in minutes to produce 9OHE **4** [19].

The phenol functional group in 9OHE **4** is essential for the redox chemistry outlined in Fig. (**2**) that leads to the formation of adducts with biological nucleophiles. Stable derivatives, including the 9-substituted ethers **18**-**22**, that lack the phenol functional group but retain antitumor activity may, therefore, operate via different mechanisms. However, metabolism of 9-methoxyellipticine **2** to produce 9OHE **4**, and conversion of the quaternized 9-methoxy salt **33** to Celiptium **5**, may occur *in vivo* as well as other mechanisms that do not require conversion to 9OHE **4** (Fig. (**5**)). Deprotonation to form an azaquinone methide **33a** is possible, but the significance or existence of such a species remains to be demonstrated [30]. Oxidative *O*-demethylation of **2** is catalyzed by cytochrome P450 and by hydrogen

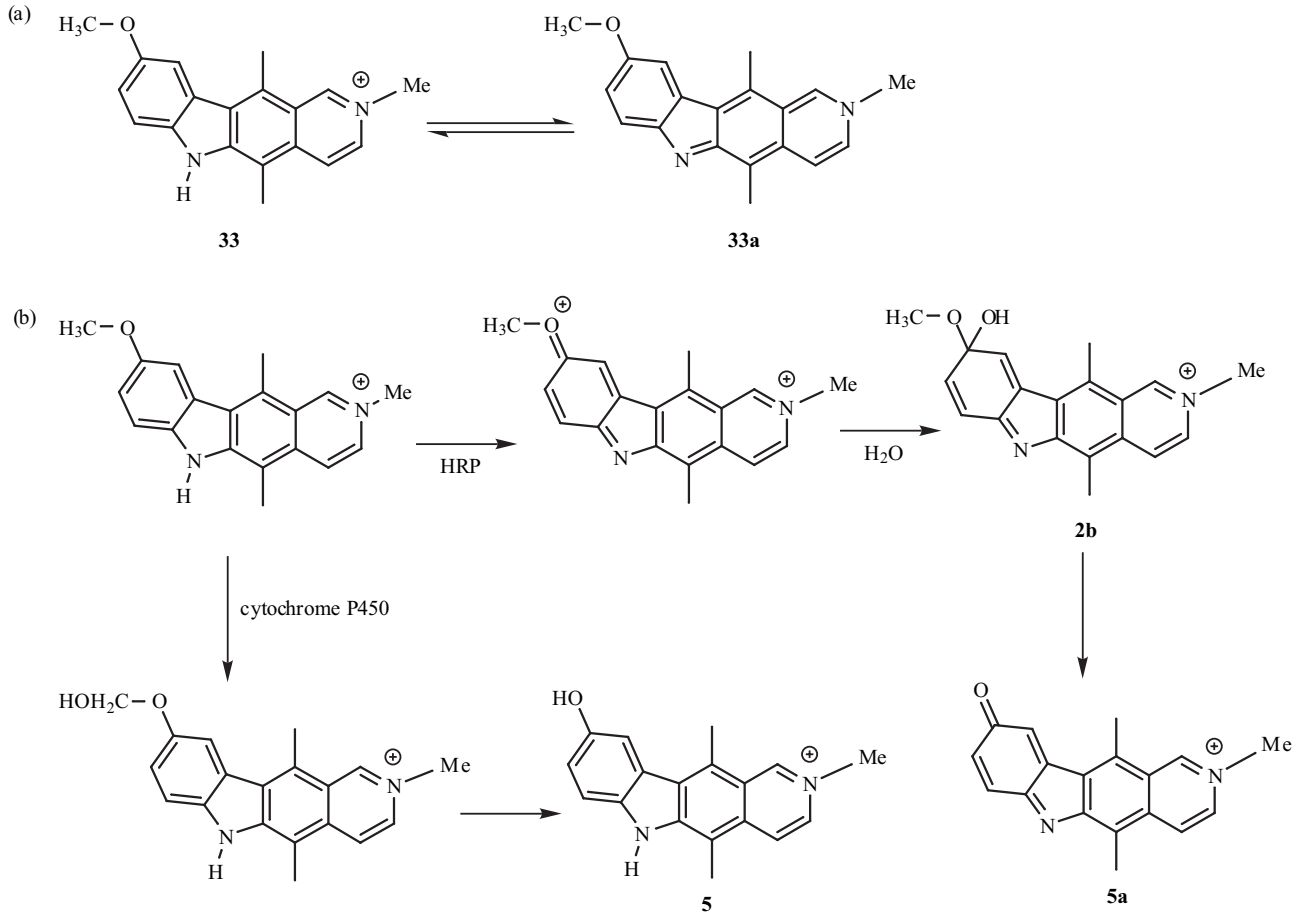


Fig. (5). Proposed pathways for *O*-demethylation of **33** to generate (a) an azaquinone methide and (b) oxidation by horseradish peroxidase or cytochrome P450 to generate 9OHE or the quinone-imine.

peroxide and horse radish peroxidase (HRP) via two distinct pathways shown in Fig. (**5b**) [29,30]. In the case of cytochrome P450, the mechanism proceeds via an oxene in which the reactive species is singlet oxygen bound to heme iron in the enzyme active site. The HRP-catalyzed reaction also occurs via a series of electron transfer reactions to give a hemiacetal **2b** which is then converted to the quinone-imine **5a** [51].

The *O*-alkyl ethers **18**-**22** and **33**-**38** were designed to inhibit the cytochrome P450 mediated metabolic conversion of 9-methoxyellipticine to 9OHE **4** [30]. While this pathway is established for the methyl ether derivative **33,** steric effects are known to retard the rate of cytochrome P450 mediated *O*-dealkylation. Hence the size and electronic characteristics of the alkyl groups were varied in the series **18**-**22** (and in the series **33**-**37**) to investigate the importance of these characteristics in the dealkylation process. The reduced selectivity of the diaryl ether **37** for CNS tumors was proposed to be related to the fact that this group would not be cleaved *in vivo* via mechanisms including those shown in Fig. (**5b**) that require an α-hydrogen for C-H insertion or hydrogen atom abstraction. The trifluoroethoxy ether **36** may be more resistant to dealkylation than the non-fluorinated ethers, but systematic studies in this area, or alternate strategies to avoid *O*-dealkylation, have not been carried out.

Cytochrome P450 has also been indirectly implicated in the metabolism of ellipticine **1**. Ellipticine was shown to be metabolized *in vivo* to 9OHE **4**, and this process was enhanced in patients receiving barbiturates, which are known to be potent cytochrome P450 inducers [3].

CONCLUSIONS

The presence of a hydroxy group at C9 of ellipticine confers significant changes in the properties of 9OHE **4** compared with the parent compound ellipticine **1**. This structural modification leads to increased antitumor activity, increased apparent affinity for DNA, increased inhibition of topoisomerase II and inhibition of the phosphorylation of mutant p53. The drug retains its ability to intercalate with DNA, with current evidence suggesting a unique orientation that positions the OH group in the minor groove and the pyridinic nitrogen in the major groove of DNA. The weakly acidic phenol functional group also promotes the formation of highly reactive intermediates, including the quinone-imine **4d**, that are susceptible to attack by a range of biological nucleophiles.

Substitution at N2 by a methyl or a dialkylaminoalkyl substituent to give a quaternized derivative results in an increase in antitumor activity and improved aqueous solubility. This does not, however, affect the capacity of the compound to intercalate with DNA. This structural alteration also enhances the selectivity of the drug for mutant p53, and confers selectivity for tumor cells of CNS origin. Similarly, glycosidation at N2 yields more potent compounds in terms of their antitumor profiles.

Several opportunities exist for the development of new derivatives of 9OHE that may operate via different mechanisms. The establishment of the binding orientation of 9OHE **4** with DNA allows the rational design of derivatives in which major and/or minor groove binding motifs are tethered to the ellipticine core. The tethering of sequence specific substituents to 9OHE **4** provides the opportunity to deliver ellipticine to a specific DNA sequence, an approach that has been successfully used recently with the anthracycline, daunomycin [52].

Current evidence suggests that the nature of substitution at N2 may be used to modulate lipophilicity and biodistribution without significant alteration to the interaction of the drug with DNA and topoisomerase II. The remarkable activity of datelliptium compared with other alkyl groups and the high activity of ellipravin **13** compared with 48 other closely related glycosides highlights the fact that further structural modification at N2 to enhance the drug's interaction with DNA and/or topoisomerases may produce interesting novel derivatives of 9OHE.

Most 9-substituted derivatives reported to date have functioned as pro-drugs that deliver 9OHE **4** *in vivo*. The preparation of derivatives at the 9-position via stable functional groups that are not metabolized *in vivo* may lead to novel ellipticine derivatives that retain the ability to interact with DNA and stabilize the DNA-topoisomerase II cleavable complex, but are unable to undergo redox reactions generating reactive intermediates in a manner similar to 9OHE **4**. The 9-phenoxyellipticinium acetate salt **37** has been identified as a lead compound which could be modified to increase the potency and selectivity of ellipticines for CNS tumors by structure-activity studies involving the aryl ring.

ACKNOWLEDGEMENTS

Financial assistance from the Sydney University Cancer Research Fund (M. M. H.) and the award of a Sydney University Postgraduate Scholarship (A.R.G.) are gratefully acknowledged.

REFERENCES

[1] Goodwin, W.; Smith, A. F.; Horning, E. C. *J. Am. Chem. Soc.,* **1959**, *81*, 1903.

[2] Dalton, L. K.; Demerac, S.; Elmes, B. C.; Loder, J. W.; Swan, J. M.; Teitei, T. *Aust. J. Chem.,* **1967**, *20*, 2715.

[3] Potier, P. *Chem. Soc. Rev.,* **1992**, 113.

[4] Mathe, G.; Hayat, M.; de Vassal, F.; Schwarzenberg, L.; Schneider, M.; Schlumberger, J. R.; Jasmin, C.; Rosenfeld, C. *Rev. Eur. Etud. Clin. Biol.,* **1970**, *15*, 541.

[5] Ohashi, M.; Oki, T. *Exp. Opin. Ther. Patents,* **1996**, *6*, 1285.

[6] Le Pecq, J. B.; Paoletti, C.; Dat-Xuong, N. In *Patent US4045565*; Agence Nationale de Valorisation de la Recherche: France, **1974**.

[7] Le Pecq, J.-B.; Dat-Xuong, N.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. USA,* **1974**, *71*, 5078.

[8] Paoletti, C.; Le Pecq, J.-B.; Dat-Xuong, N.; Juret, P.; Garnier, H.; Amiel, J.-L.; Rouesse, J. *Recent Res. Cancer Res.,* **1980**, *74*, 107.

[9] Juret, P.; Tanguy, A.; Girard, A.; Le Talaer, J. Y.; Abbatucci, J. S.; Dat-Xuong, N.; Le Pecq, J. B.; Paoletti, C. *Eur. J. Cancer,* **1978**, *14*, 205.

[10] Rouëssé, J.; Spielmann, M.; Turpin, F.; Le Chevalier, T.; Azab, M.; Mondésir, J. M. *Eur. J. Cancer,* **1993**, *29A*, 856.

[11] Pierré, A.; Atassi, G.; Devissaguet, M.; Bisagni, E. *Drugs of the Future,* **1997**, *22*, 53.

[12] Formisyn, P.; Pautet, F.; Tran Minh, C.; Bourgois, J. *J. Pharmaceut. Biomed. Anal.,* **1992**, *10*, 427.

[13] Fossé, P.; René, B.; Charra, M.; Paoletti, C.; Saucier, J.-M. *Mol. Pharmacol.,* **1992**, *42*, 590.

[14] Meunier, G.; De Montauzon, D.; Bernadou, J.; Grassy, G.; Bonnafous, M.; Cros, S.; Meunier, B. *Mol. Pharmacol.,* **1988**, *33*, 93.

[15] Bernard, S.; Schwaller, M. A.; Moiroux, J.; Bazzaoui, E. A.; Lévi, G.; Aubard, J. *J. Raman Spectros.,* **1996**, *27*, 539.

[16] Moiroux, J.; Armbruster, A. M. *J. Electroanal. Chem. Interfac. Electrochem.,* **1984**, *165*, 231.

[17] Auclair, C.; Pierre, A.; Voisin, E.; Pepin, E.; Cros, S.; Colas, C.; Saucier, J.-M.; Verschuere, B.; Gros, P.; Paoletti, C. *Cancer Res.,* **1987**, *47*, 6254.

[18] Harada, N.; Kawaguchi, T.; Inoue, I.; Oda, K.; Ohashi, M.; Hashiyama, T.; Tsujihara, K. *Chem. Pharm. Bull.,* **1997**, *45*, 134.

[19] Harada, N.; Ozaki, K.; Oda, K.; Nakanishi, N.; Ohashi, M.; Hashiyama, T.; Tsujihara, K. *Chem. Pharm. Bull.,* **1997**, *45*, 1156.

[20] Kahne, D. *Chem. Biol. ,* **1995**, *2*, 7.

[21] Palomino, E. *Adv. Drug Deliv. Review,* **1994**, *13*, 311.

[22] Honda, T.; Inoue, M.; Kato, M.; Shima, K.; Shimamoto, T. *Chem. Pharm. Bull.,* **1987**, *35*, 3975.

[23] Honda, T.; Kato, M.; Inoue, M.; Shimamoto, T.; Shima, K.; Nakanishi, T.; Yoshida, T.; Noguchi, T. *J. Med. Chem.,* **1988**, *31*, 1295.

[24] Shimamoto, T.; Imajo, S.; Honda, T.; Yoshimura, S.; Ishiguro, M. *Bioorg. Med. Chem. Lett.,* **1996**, *6*, 1331.

[25] Barone, P. M. V. B.; Dantas, S. O.; Galvao, D. S. *J. Mol. Struct. (Theochem.),* **1999**, *465*, 219.

[26] Etemad-Moghadam, G.; Ding, L.; Tadj, F.; Meunier, B. *Tetrahedron,* **1989**, *45*, 2641.

[27] Delbarre, A.; Oberlin, R.; Rocques, B. P.; Borgna, J.-L.; Rochefort, H.; Le Pecq, J.-B.; Jacquemin-Sablon, A. *J. Med. Chem.,* **1985**, *28*, 752.

[28] Festy, B.; Poisson, J.; Paoletti, C. *FEBS Letters,* **1971**, *17*, 321.

[29] Meunier, G.; Meunier, B. *J. Biol. Chem.,* **1985**, *260*, 10576.

[30] Anderson, W. K.; Gopalsamy, A.; Reddy, P. S. *J. Med. Chem.,* **1994**, *37*, 1955.

[31] Guthrie, R. W.; Brossi, A.; Mennona, F. A.; Mullin, J. G.; Kierstead, R. W.; Grunberg, E. *J. Med. Chem.,* **1975**, *18*, 755.

[32] Grummitt, A. R.; Harding, M. M. **2003**, *Eur. J. Org. Chem.,* in press.

[33] Dugue, B.; Meunier, B.; Paoletti, C. *Eur. J. Med. Chem. - Chim. Ther.,* **1983**, *18*, 551.

[34] Leon, P.; Garbay-Jaureguiberry, C.; Barsi, M. C.; Le Pecq, J. B.; Roques, B. P. *J. Med. Chem.,* **1987**, *30*, 2074.

[35] Fellouus, R.; Coulaud, D.; El Abed, I. *Cancer Res.,* **1988**, *48*, 6542.

[36] Jain, S. C.; Bhandary, K. K.; Sobell, H. M. *J. Mol. Biol.,* **1979**, *135*, 813.

[37] Carvalho, A. C. M.; Laks, B. *J. Mol. Struct. (Theochem.),* **2001**, *539*, 273.

[38] Auclair, C. *Arch. Biochem. Biophys.,* **1987**, *259*, 1.

[39] Monnot, M.; Mauffret, O.; Lescot, E.; Fermandjian, S. *Eur. J. Biochem.,* **1992**, *204*, 1035.

[40] Elcock, A. H.; Rodger, A.; Richards, W. G. *Biopolymers,* **1996**, *39*, 309.

[41] Ismail, M. A.; Sanders, K. J.; Fennell, G. C.; Latham, H. C.; Wormell, P.; Rodger, A. *Biopolymers,* **1998**, *46*, 127.

[42] Roche, C. J. T.; Thomson, J. A.; Crothers, D.M. *Biochemisry*, **1994**, *33*, 926.

[43] Multon, E.; Riou, J.-F.; LeFevre, D.; Ahomadegbe, J.-C.; Riou, G. *Biochem. Pharmacol.,* **1989**, *38*, 2077.

[44] Tewey, K. M.; Chen, G. L.; Nelson, E. M.; Liu, L. F. *J. Biol. Chem.,* **1984**, *259*, 9182.

[45] Harris, C. C. *Carcinogenesis,* **1996**, *17*, 1187.

[46] Shi, L. M.; Myers, T. G.; Fan, Y.; O'Connor, P. M.; Paull, K. D.; Friend, S. H.; Weinstein, J. N. *Mol. Pharmacol.,* **1998**, *53*, 241.

[47] Mizumoto, K.; sato, N.; Kusumoto, M.; Niiyama, H.; Maehara, N.; Nishio, S.; Li, Z.; Ogawa, T.; Tanaka, M. *Cancer Letts.,* **2000**, *149*, 85.

[48] Sugikawa, E.; Hosoi, T.; Naoko, Y.; Gamanuma, M.; Nakanishi, N.; Ohashi, M. *Anticancer Res.,* **1999**, *19*, 3099.

[49] Kansal, V. K.; Potier, P. *Tetrahedron,* **1986**, *42*, 2389.

[50] Dugue, B. C.; Auclair, C.; Paoletti, C.; Meunier, B. *Cancer Res.,* **1986**, *46*, 3828.

[51] Bernadou, J.; Bonnafous, M.; Labat, G.; Loiseau, P.; Meunier, B. *Drug Metab. Disp.,* **1991**, *19*, 360.

[52] Garbesi, A.; Bonazzi, S.; Zanella, S.; Capobianco, M. L.; Giannini, G.; Arcamone, F. *Nucl. Acids Res.,* **1997**, *25*, 2121.

[53] Nguyen, D. X.; Adeline, M. T.; Lecointe, P.; Janot, M. M. *Hebd. Seances Acad. Sci. Ser. C,* **1975**, *281*, 623.