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THE BIOGENETIC, SYNTHETIC AND BIOCHEMICAL ASPECTS OF ELLIPTICINE, AN ANTITUMOR ALKALOID

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CONTENTS

1. INTRODUCTION 2389

2. BIOGENESES OF ELLIPTICINE AND RELATED ALKALOIDS 2390

3. SYNTHESES OF ELLIPTICINE AND ITS ANALOGUES 2393
3.1. Classification of the synthetic strategies 2393
3.1.1. B-Type syntheses 2393
3.1.2. C-Type syntheses 2394
3.1.3. D-Type syntheses 2399
3.1.4. B+C-Type syntheses 2400

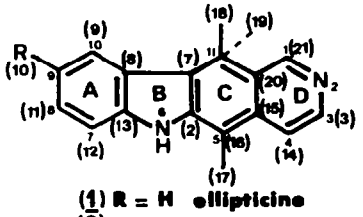
4. ANTITUMORAL ACTIVITY AND MECHANISM OF ACTION OF ELLIPTICINE 2401
4.1. Introduction 2401
4.2. Mechanism of action of ellipticine and derivatives 2403

5. CONCLUDING REMARKS 2406

REFERENCES AND NOTES 2407

1. INTRODUCTION

The plant alkaloid ellipticine 1 (5,11-dimethylpyrido [4,3-b] carbazole) was first isolated in 1959 from the leaves of Ochrosia elliptica Labill. (family Apocynaceae), a plant harvested in Florida (USDA plant introduction station)[1]. Subsequently ellipticine and its derivatives were isolated from various other species of genera Aspidosperma, Tabernaemontana and Strychnos[2-10].

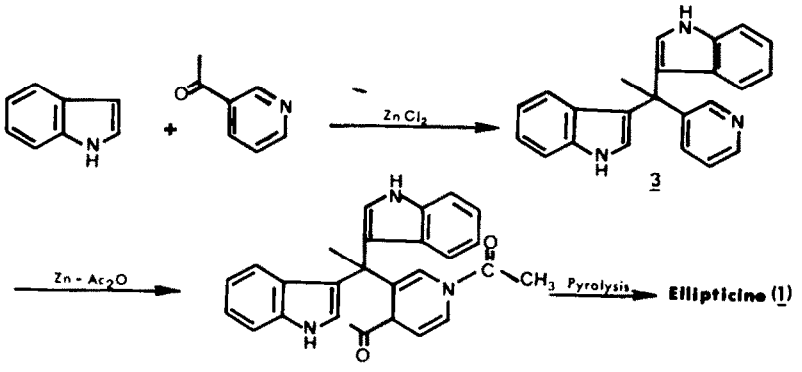


(1) R = H ellipticine
(2) R = OCH_3

Numbering in Parentheses is "biogenetic"

The structure of ellipticine was conclusively assigned by Woodward et al. in 1959 as a result of its first total synthesis[11]. They prepared ellipticine by the condensation of indole with

3-acetylpyridine to furnish 3 which on reduction with zinc and acetic anhydride, followed by pyrolysis yielded ellipticine, 1 in an overall yield of only 2% (Scheme 1).



Scheme 1

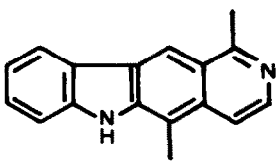
In 1967, a group of Australian scientists, Dalton et al.[12], showed that ellipticine, 1 and 9-methoxyellipticine, 2[13] (Svoboda et al. in 1968) are active against various experimental animal tumors, for example : Sarcoma-180, Adenocarcinoma 755 and Leukaemia L-1210.

The promising antitumoral activity of ellipticine and its analogues prompted chemists to discover a number of synthetic routes to the ellipticine nucleus and to synthesise a number of analogues for pharmacological evaluation. At the same time, biochemists and pharmacologists endeavoured to understand the fate of ellipticine in vivo. The simultaneous efforts of all these could furnish feed back information for obtaining better antitumoral agents. Since, to our knowledge, no report dealing with all the aspects of ellipticine and its analogues is available, we now present a critical view focusing on all these aspects of ellipticine and its analogues. We hope this will be useful to pertain central information regarding this group of compounds knowledge of which will be helpful to solve the existing problems in this field.

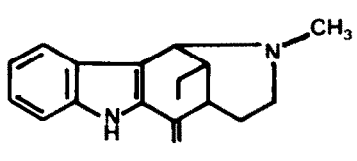
2. BIOGENESES OF ELLIPTICINE AND RELATED ALKALOIDS

To be able to both predict and prove the biogenesis of any alkaloid are the two fascinating and stimulating problems for understanding both the chemotaxonomy and chemistry of the secondary metabolites of plants. There are certain naturally occurring compounds for which several elegant and rational biogenetic pathways have been proposed. However, these hypotheses are still controversial in the absence of conclusive experimental support. Ellipticine and the related alkaloids fall in this category. Indeed, a number of elegant and rational pathways have been delineated for these alkaloids[11, 14-17]. However, because of the lack of experimental support[18,19], their biogenetic pathways still remain an interesting enigma to be solved.

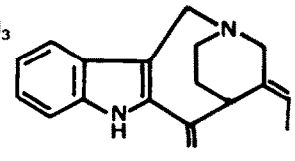
Ellipticine and its natural isomer olivacine 4 are usually present in the same plants alongwith uleine 5[20] and apparicine 6[21]. It is presumed, therefore, that these alkaloids have the same progenitor.



Olivacine (4)



Uleine (5)



Apparicine (6)

The biogenetic proposals for ellipticine and related alkaloids made by Woodward[11] and Boit[15] are hardly of historical importance and will not be discussed here.

The first detailed proposal on the biogenesis of ellipticine and related alkaloids was made by Wenkert in 1962[16]. He postulated a common precursor A (Figure 1), derived from glycosylidene-anthranilic acid, a tryptophan progenitor, and a seco-prephenateformaldehyde (SPF) unit or seco-loganine, as possible biogenetic intermediates for these alkaloids. Cyclisation of an SPF unit at C-19 (c), C-21 (b) with the indolic β-position followed by extrusion (via retro-aldolisation) of the β-glycosyl function in A could give rise to the Aspidosperma skeleta of ellipticine 1, olivacine 4 and uleine 5 (Figure 1).

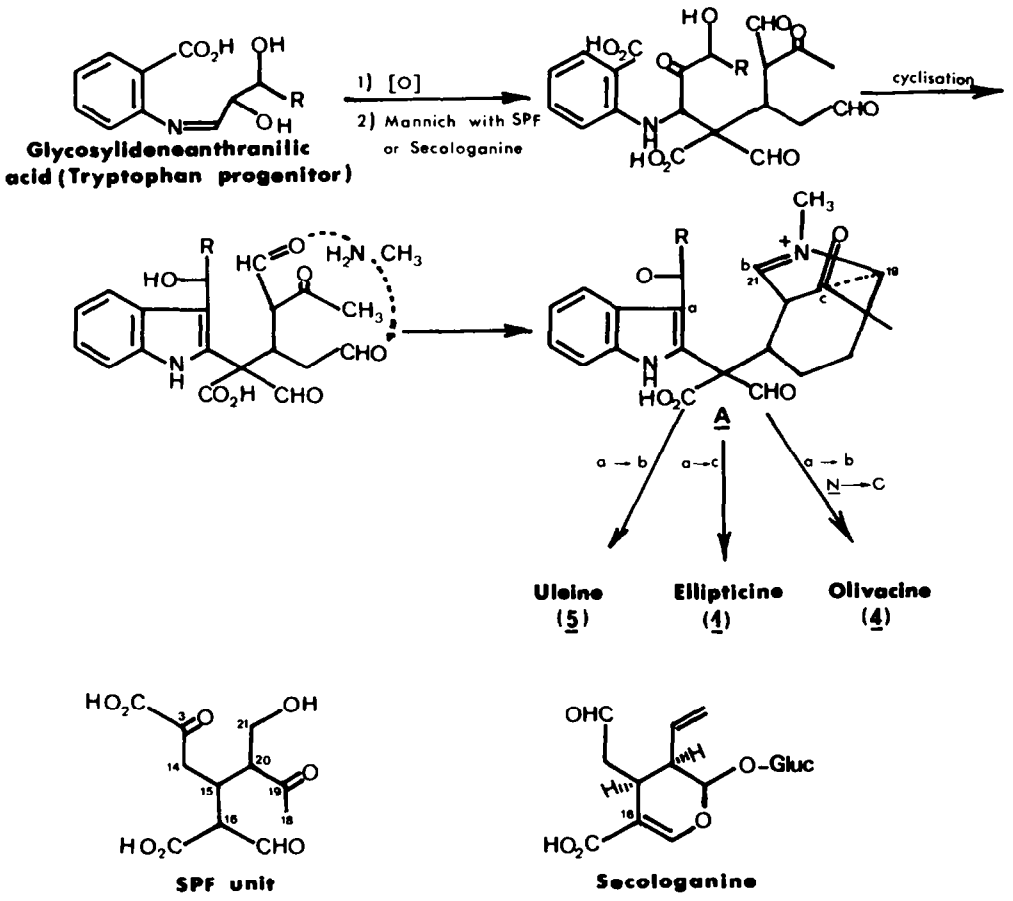


Figure 1

The most recent and widely accepted hypothesis, based upon a modified Polonovski reaction[22-25] was postulated by one of us (P.P.) and Janot[17]. We envisaged stemmadenine-N-oxide 7a as a possible precursor for these alkaloids which upon modified Polonovski fragmentation, followed by the corresponding transformation of the resultant product could lead to appericine 6, uleine 5, olivacine 4 and ellipticine 1 (Figure 2).

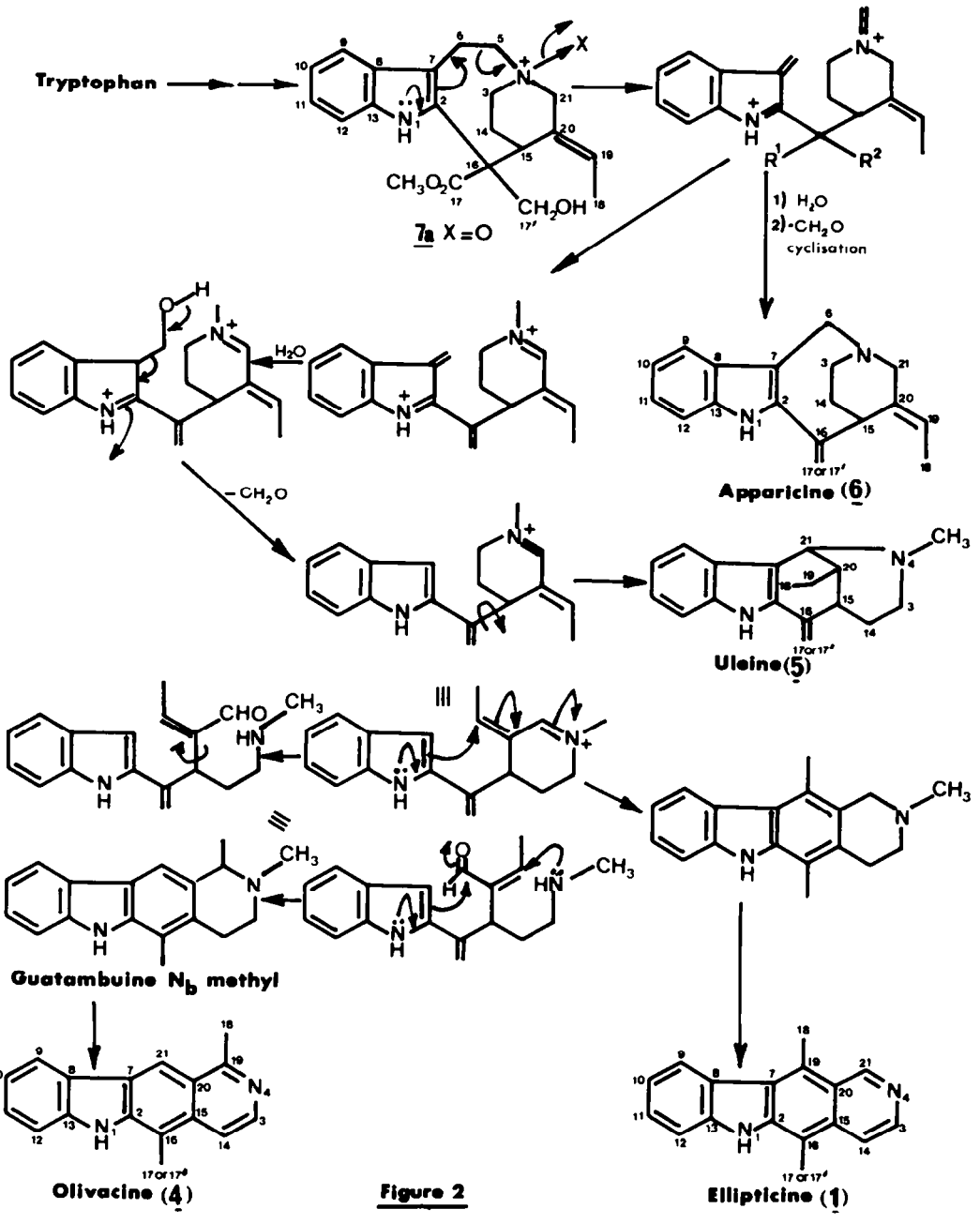


Figure 2

The biosynthetic pathway proposed by Wenkert[16] for ellipticine and related alkaloids, requires that the indolic part of these alkaloids originates from glycosylideneanthranilic acid and not from tryptophan. However, Potier and Janot[17] preferred tryptophan and stemmadenine as possible precursors for these alkaloids. Labelling experiments with both tryptophan and stemmadenine indicated a significant incorporation of both these compounds into apparicine. However, a very weak incorporation of tryptophan and stemmadenine was observed for uleine and none at all for both ellipticine and olivacine[18,19]. In the light of these observations, the Potier and Janot hypothesis seems more attractive at the present time, although further experimental support is still required to show its validity.

3. SYNTHESES OF ELLIPTICINE AND ITS ANALOGUES

Since ellipticine and its analogues possess potent antitumoral activity, a number of synthetic approaches have been developed for the synthesis of the ellipticine skeleton. Synthetic efforts made in this direction have been thoroughly reviewed up to the end of 1982[26,27]. We, therefore, describe the synthetic endeavours made in this direction from December 1982 to March 1985.

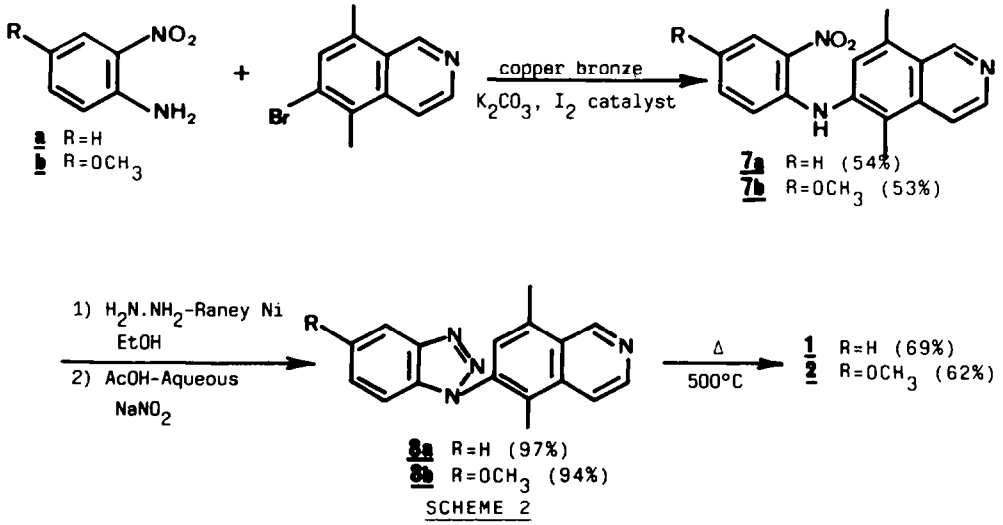
A review dealing with the syntheses of ellipticine has also recently appeared[28] which, however, does not cover the synthesis of the various ellipticine analogues. We, therefore, feel justified in including in our review a section on the recent synthetic strategies described towards ellipticine and its analogues.

3.1. Classification of the Synthetic Strategies

Sainsbury[26] has classified the synthetic approaches to ellipticine into three classes : B, C and D, based upon the last ring to be constructed. However, in a recent review[28], synthetic endeavours have been classified according to the last bond to be formed. We feel that the original classification is much simpler than the second one which becomes complicated if two rings are constructed simultaneously as described in a recent approach to ellipticine[29]. We, therefore, follow the original classification described by Sainsbury[26]. Since a new synthetic method[29], in which two rings (B + C), have been constructed simultaneously has been discovered, we, therefore, classified synthetic approaches to ellipticine into four classes B, C, D and B + C.

3.1.1. B-Type Syntheses

Recently Miller et al.[30] have reported a B-type synthesis using benzotriazoles, 8a & 8b as key intermediates for the construction of B-ring. Originally, this approach was employed by Bisagni et al.[31-34] for the synthesis of 9-azaellipticine. Thus Goldberg's coupling of substituted nitroanilines with 6-bromo-5,8-dimethylisoquinoline furnished the corresponding diarylamines 7a (54%) and 7b (53%) respectively. Reduction of the nitro group in 7a & 7b with hydrazine hydrate-Raney-Ni, followed by diazotisation gave benzotriazoles 8a (97%) and 8b (94%) respectively. Pyrolytic decomposition of 8a & 8b at 500°C yielded 1 (69%) and 2 (62%). However, a poor yield was obtained when 8a was heated (220°C) in polyphosphoric acid (16%) or photolysed in methanol (33%) (Scheme 2).

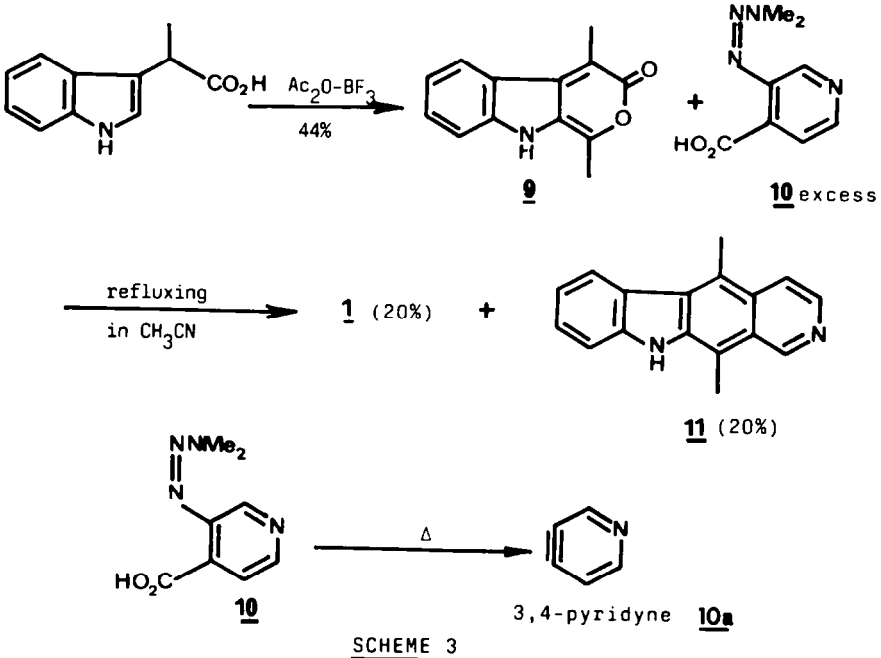


SCHEME 2

3.1.2. C-Type Syntheses

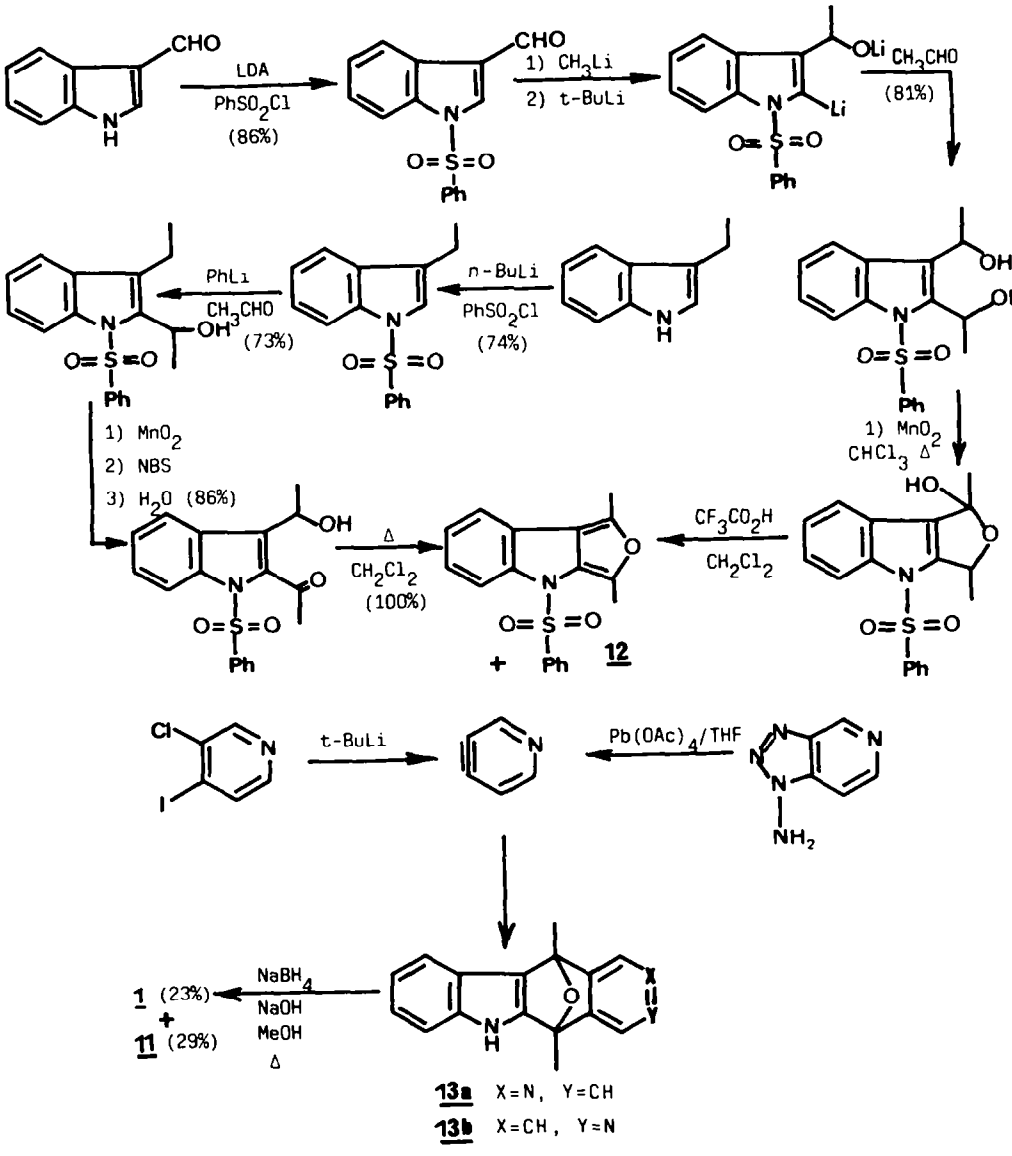
Recent synthetic reports in the literature directed to C-type syntheses not only include the extension and modification of the previous work but also describe a number of new elegant approaches to the ellipticine nucleus.

Moody et al.[35] have developed a new concise synthesis of ellipticine, by analogy with a Diels-Alder reaction of pyrano[3,4-b]indol-3-one with arynes[36]. The prerequisite diene 1,4-dimethylpyrano [3,4-b]indol-3-one 9 was prepared by the reaction of α-methylindole-3-acetic acid with Ac_2O in the presence of BF_3-Et_2O. A Diels-Alder reaction of 9 with 3-(3,3-dimethyl-triazen-1-yl)-pyridine-4-carboxylic acid 10, a precursor of 3,4-pyridyne 10a, gave ellipticine 1 (20%) together with an equal amount of isoellipticine 11 (Scheme 3).



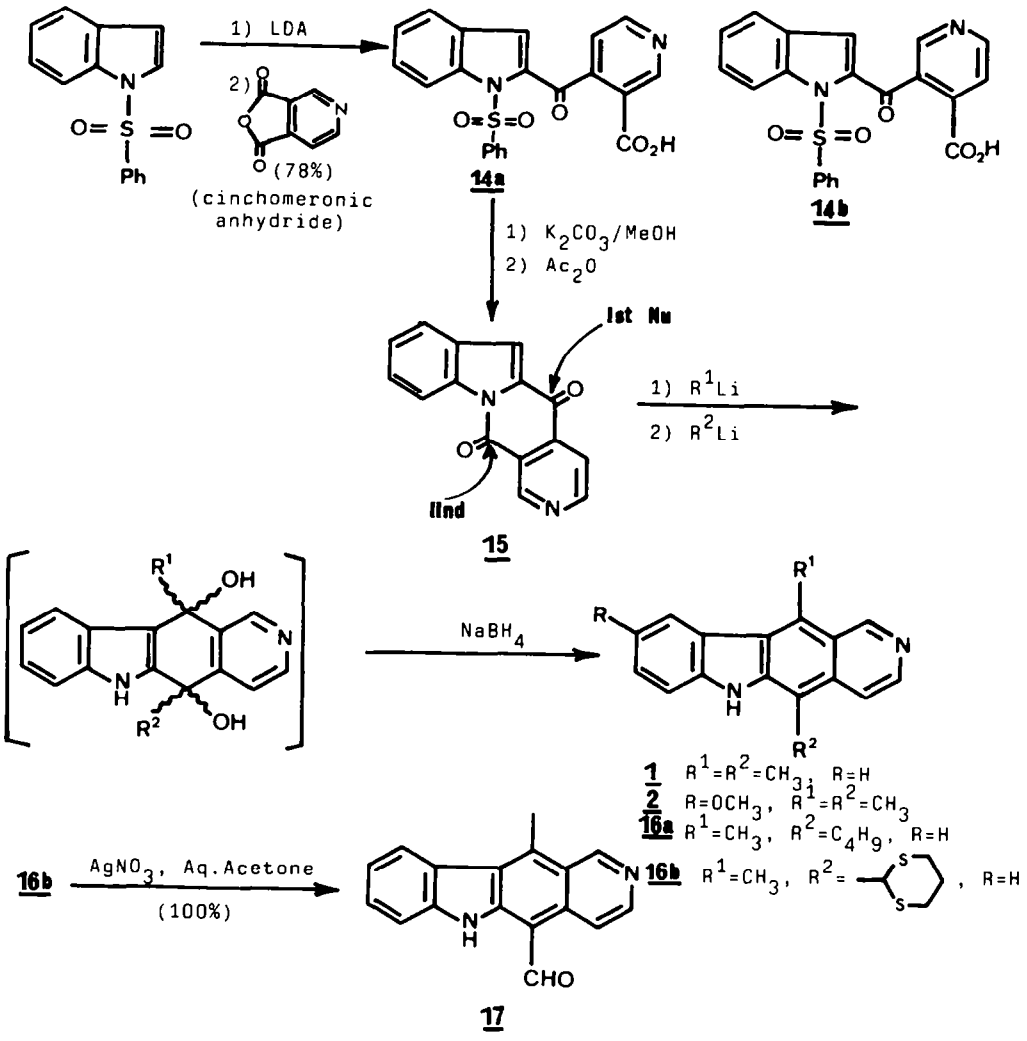
SCHEME 3

Another Diels-Alder approach for ellipticine and isoellipticine has been developed by Gribble et al.[37]. The key step of their synthesis involves the Diels-Alder reaction between 3,4-pyridyne and 1,3-dimethyl-4-(phenylsulphonyl)-4H-furo[3,4-b]indole 12 (Scheme 4) instead of 1,4-dimethylpyrano[3,4-b]indol-3-one, 9 (Scheme 3), followed by oxygen bridge extrusion of the resultant Diels-Alder products 13a & 13b to give a mixture of ellipticine 1 (23%) and isoellipticine, 11 (29%, Scheme 4). The key intermediate furoindole, 12 has been prepared by two routes starting from indole-3- carboxaldehyde and 3-ethylindole (Scheme 4). 3,4-Pyridyne is generated in situ either by lead tetraacetate oxidation of 1-aminotriazole[4,5-c]pyridine or from 3-chloro-4-iodopyridine and t-butyllithium. Oxygen bridge extrusion of the Diels-Alder products, 13a & 13b is achieved by treatment with $NaBH_4$-NaOH-MeOH to give an easily separable mixture of ellipticine, 1 (23%) and isoellipticine, 11 (29%).



SCHEME 4

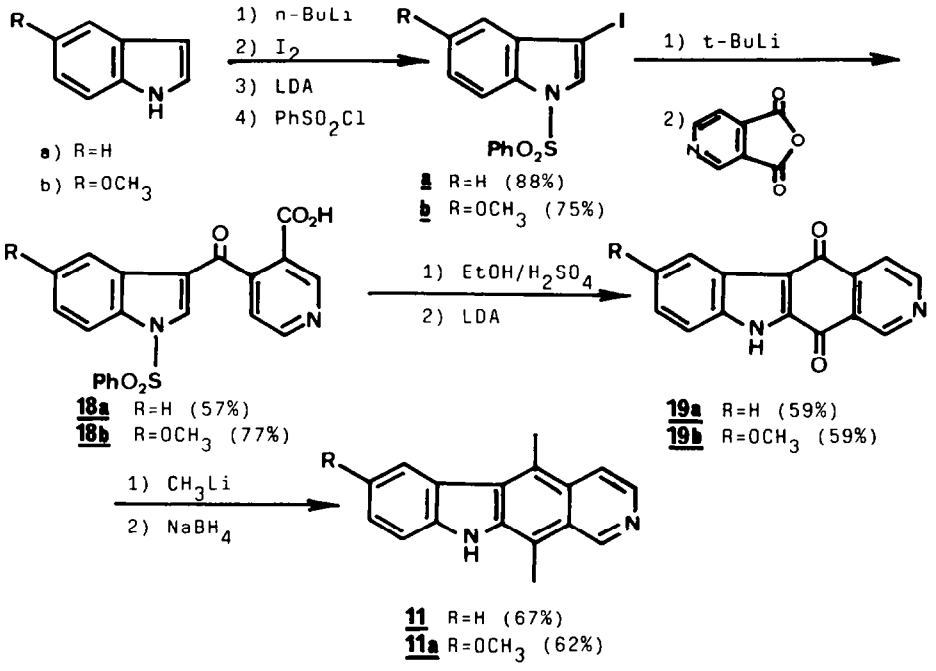
The same group has also developed another new elegant and high yielding synthesis of ellipticine[38]. Thus the reaction of the anion of 1-(phenylsulphonyl)indole, generated with LDA and 1-(phenylsulphonyl)indole at -100°C, with cinchomeronic anhydride gave a mixture of keto acids 14a & 14b (92:8, 78%). The major isomer 14a, on hydrolysis with K_2CO_3-MeOH, followed by treatment with hot Ac_2O furnished the keto lactam 15. This keto lactam, on treatment with methyllithium (2 eq) at -100°C, yielded a diastereomeric mixture of diol which on treatment with $NaBH_4$ gave ellipticine 1 in 54% overall yield from indole. When the same reaction sequences were extended to 5-methoxyindole, 9-methoxyellipticine 2 was produced in 47% overall yield (Scheme 5).



SCHEME 5

The above synthesis also allows the manipulation of the substitution pattern at positions 5 and 11 of ellipticine. The crucial step of this synthesis is the regiospecific and sequential addition of an alkyllithium to the keto lactam 15. Exploiting this observation, the same workers have also synthesised a Strychnos dinklages[39] alkaloid : 17-oxoellipticine 17[40] (scheme 5).

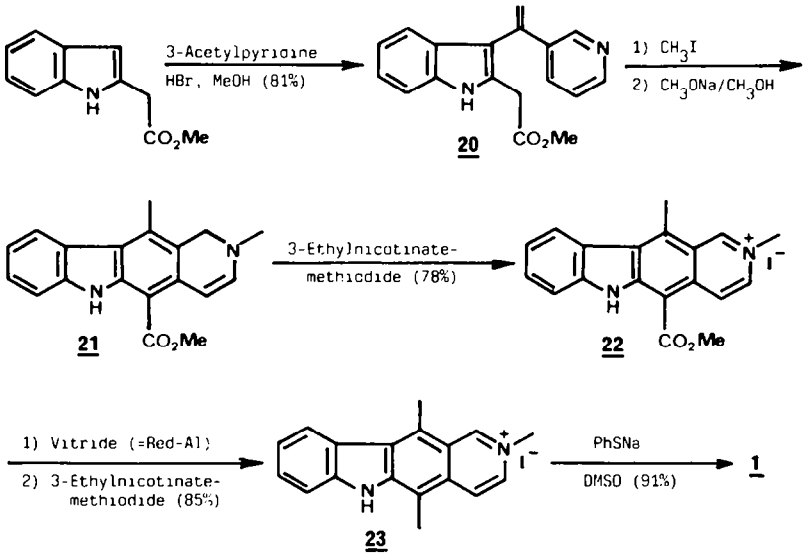
Gribble et al.[41] have also reported an elegant methodology for the synthesis of isoellipticine 11 and 7-methoxyisoellipticine 11a. This involves the regioselective acylation of 3-lithio-1-(phenylsulphonyl)indole with cinchomeronic anhydride to give the keto acids 18a (57%) and 18b (77%). A strong base mediated cyclisation of ethylester of 18a and 18b furnished the corresponding quinones 19a (59%) and 19b (59%) which on treatment with methyllithium, followed by $NaBH_4$ reduction afforded 11 and 11a in 20% and 21% overall yield from indole and 5-methoxyindole respectively (Scheme 6).



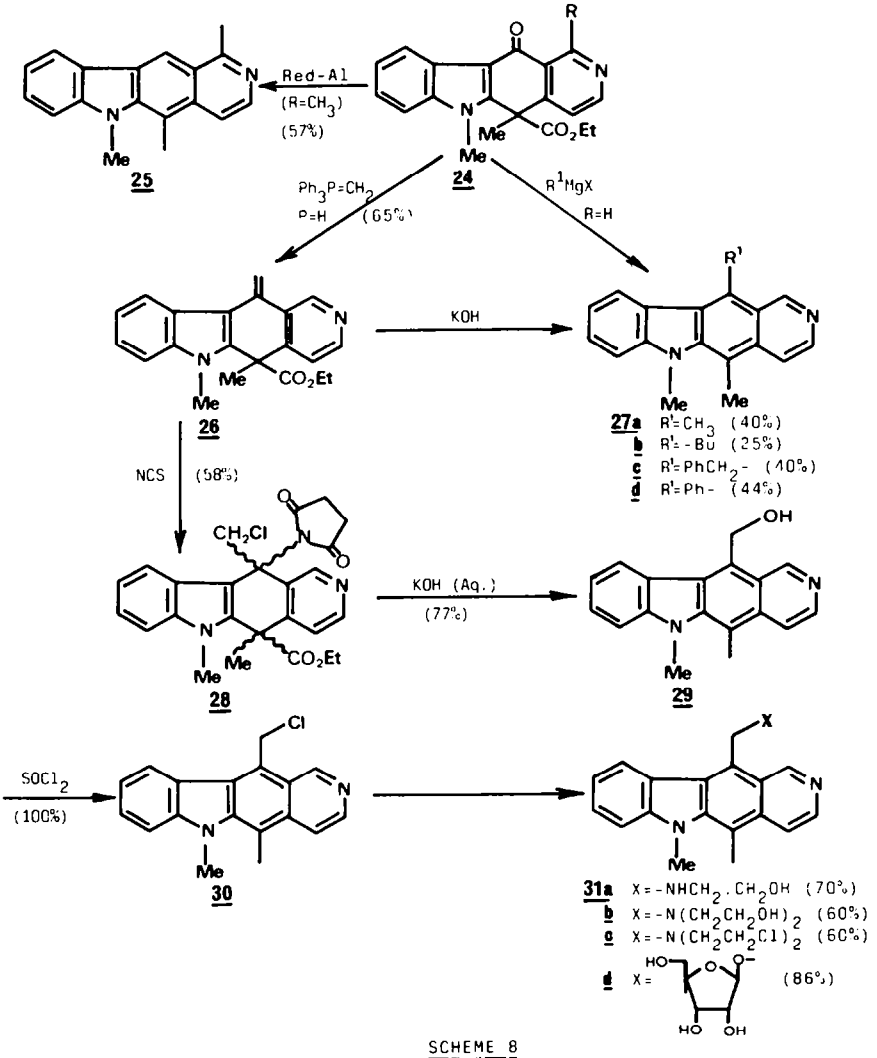
SCHEME 6

Recently Weller et al.[42] have reported a new synthesis of ellipticine, exploiting an intramolecular 1,4-addition of an ester anion to pyridinium salt (Scheme 7). Conceptually this approach is similar to the Bergmann approach[43,44] employed for the synthesis of ellipticine. A similar type of approach was previously used by Pandit et al.[45,46]. In their approach, the pyridinium ring was further activated by the carbonyl group at position 3. The condensation of 2-(carbomethoxymethyl)indole with 3-acetylpyridine under Bergmann conditions[43] gave the adduct 20 (81%). Quaternisation of the pyridinium nitrogen in 20 with methyl iodide, followed by treatment with sodium methoxide gave a labile dihydropyridine 21. Treatment of 21 with 3-ethylnicotinatemethiodide, an oxidising agent gave the quaternised salt 22 (78%). Reduction of 22 with vitride (=Red-Al) and oxidation of the resultant reduced product with 3-ethylnicotinatemethiodide gave N-2-methylellipticinium iodide, 23 (85%) which on nucleophilic demethylation with sodium thiophenoxide furnished ellipticine 1 (91%) (Scheme 7).

Pandit et al.[47] have recently published the details of their earlier work[45,46]. By exploiting the presence of an 11-keto group in the previously synthesised intermediate 24, they have prepared a large number of 6-methylellipticine derivatives, (27a-d & 31a-d) and 6-methylolivacine, 25. Reduction of the 11-keto in 24 with Red-Al gave 6-methylolivacine 25. However, the reaction of 24 with an excess of a Grignard reagent (CH_3MgI, BuMgI, $PhCH_2MgI$, PhMgI) afforded 6-methyl-11-substituted ellipticine derivatives 27a-d. 6-Methylellipticine 27a was also obtained by the reaction of 24 with methylenetriphenylphosphorane (Wittig reaction), followed by hydrolytic decarboxylation of the resulting intermediate 26. Furthermore, 26 on treatment with NCS, followed by hydrolytic decarboxylation furnished 29 which on reaction with thionyl chloride resulted in formation of the corresponding chloro derivative 30 in quantitative yield. The nucleophilic displacement of chlorine in 30 with various nucleophiles (substituted amines and sugar) furnished various 11-substituted-6-methylellipticine derivatives 31a-d (Scheme 8).



SCHEME 7

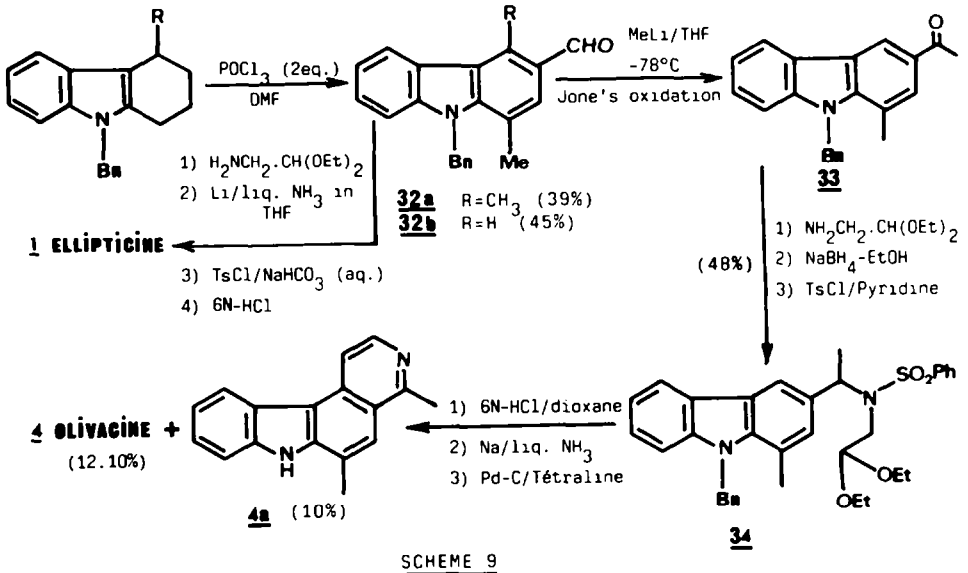


SCHEME 8

3.1.3. D-Type Syntheses

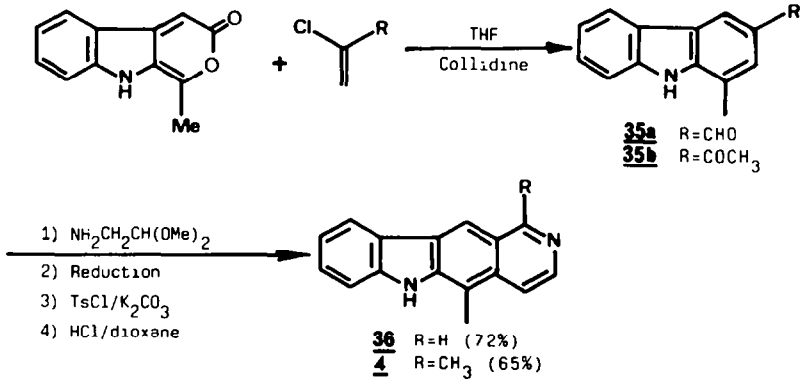
Only a couple of this class of new syntheses have recently been reported[50,51]. A number of new analogues have been synthesised for pharmacological evaluation, by exploiting the known intermediates prepared by this type of synthesis. The reaction sequences are essentially the same as described in the modified[48] Cranwell and Saxton[49] synthesis. Efforts are being made to obtain an appropriately substituted carbazoles in a moderate yield. The modified Cranwell and Saxton synthesis has now also been employed for the synthesis of olivacine.

Okuyama et al.[50] have synthesised 3-formylcarbazoles, 32a (39%) and 32b (45%), by carrying out a Vilsmeier-Haack reaction with substituted 1,2,3,4-tetrahydro-N-benzylcarbazoles. 32b, on treatment with methyllithium, followed by Jone's oxidation furnished 33. 32b & 33 were transformed[49] to ellipticine, 1 and olivacine, 4 in only a poor yield by employing the modified Cranwell & Saxton Synthesis[48] (Scheme 9).



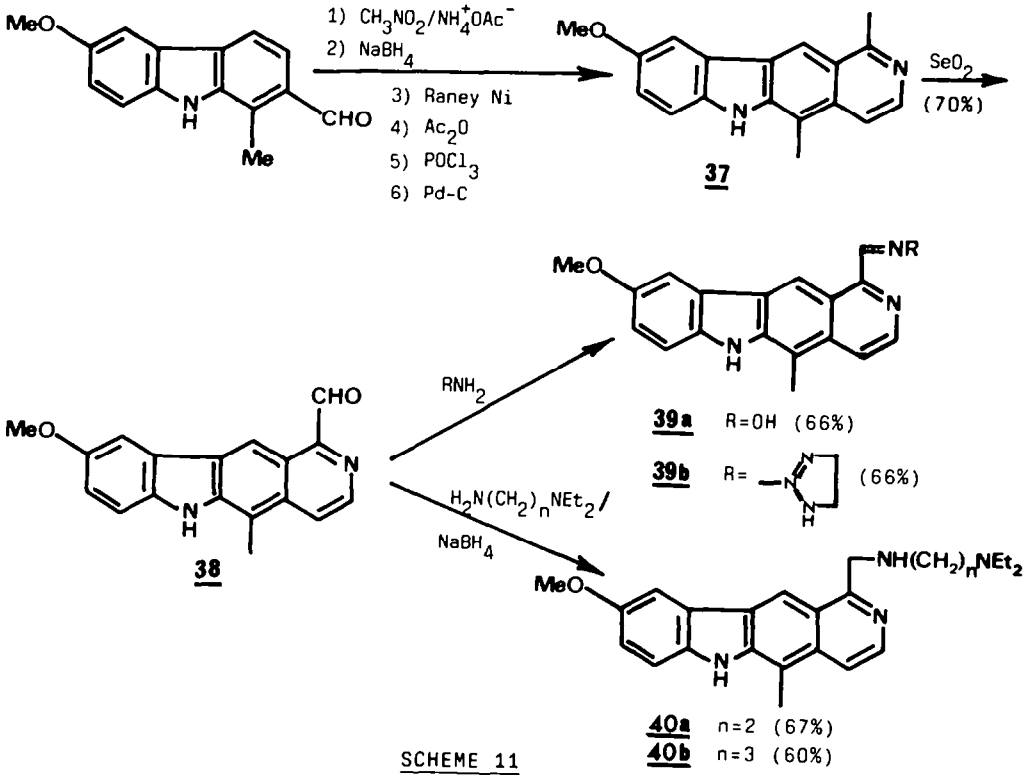
SCHEME 9

Narsimhan et al.[51] have developed a method to synthesise 3-formyl- and 3-acetyl-carbazoles using the cycloaddition reaction of 1-methylpyrano[3,4-b]indol-3-one with appropriately substituted haloalkenes[36] in a high yield. The resulting carbazoles 35a and 35b have been converted to olivacine 4 and 11-desmethylellipticine 36, following the modified Cranwell and Saxton synthesis (Scheme 10). It is noteworthy that the yields of the cyclised products depend on the amound of conc. HCl used.



SCHEME 10

Bisagni et al.[52] have synthesised a number of 1-aminomethylpyrido[4,3-b]carbazole derivatives for pharmacological evaluation, starting from 9-methoxyolivacine 37. Thus selenium dioxide oxidation of 37 gave 1-formyl-11-desmethylellipticine 38 (70%) which upon reductive amination (amine/$NaBH_4$) afforded 1-aminomethyl-N-(diethylaminoalkyl)-11-desmethylellipticine 40a (67%) and 40b (60%) (Scheme 11). However 38 on reaction with amines gave Schiff's bases 39a (66%) and 39b (66%). The starting material 9-methoxyolivacine, 37 has been synthesised from 1-methyl-2-formyl-6-methoxycarbazole[53] in six steps, following the classical procedures[54-57].

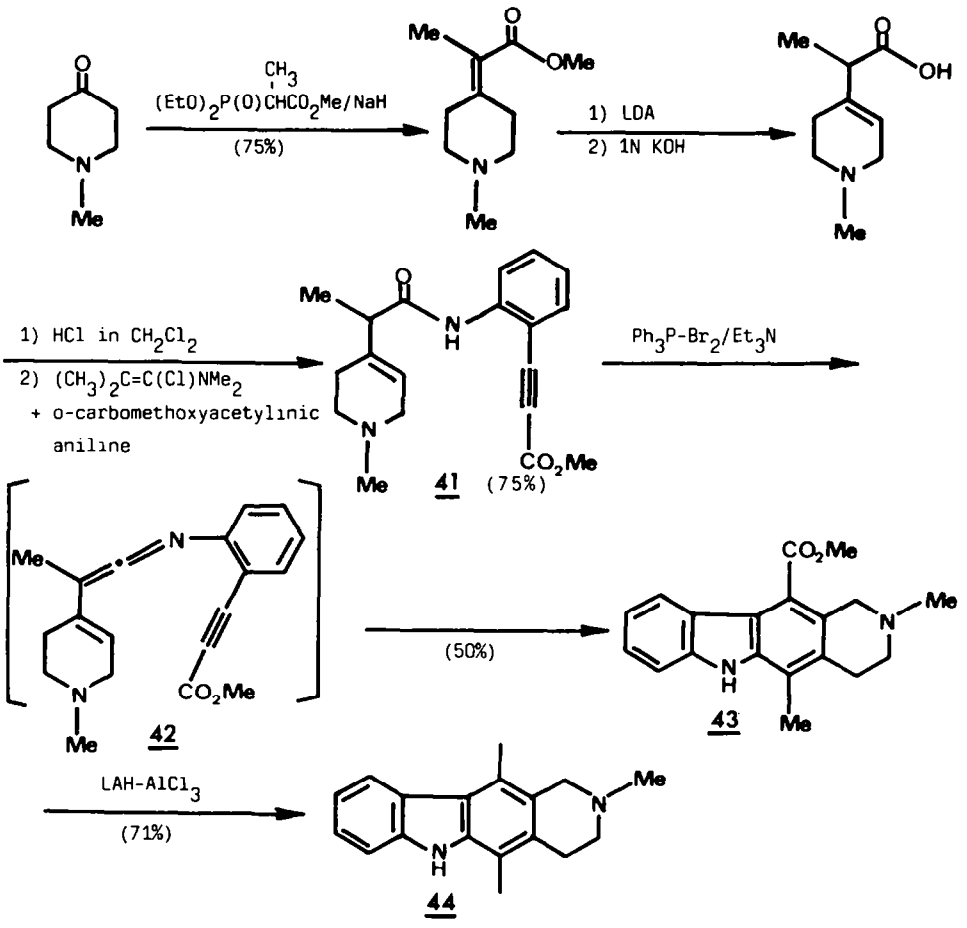


SCHEME 11

Pharmacologically all these compounds exhibit weak cytotoxic and antitumoral activity.

3.1.4. B + C-Type Syntheses

A new elegant synthetic approach to ellipticine has recently been published by Differding and Ghosez[29], employing an intramolecular Diels-Alder cycloaddition of an acetylenic vinylketenimine as a key step to construct the B + C ring of the ellipticine simultaneously. The prerequisite intermediate 41 has been synthesised from N-methylpiperid-4-one in 42% over all yield. On refluxing 41 with Ph_3P-Br_2 and Et_3N, the vinylketenimine 42 is generated which undergoes an intramolecular cycloaddition to furnish 43 (50%). The mixed hydride reduction (LAH + $AlCl_3$) of 43 readily furnished N-2-methyltetrahydroellipticine 44 (71%) which could be transformed to ellipticine as reported earlier[4,55,58] (Scheme 12).



SCHEME 12

A number of unknown ellipticine derivatives which have been prepared during the mechanistic studies of ellipticine in vitro, have been discussed in Section 4.2.

4. ANTITUMORAL ACTIVITY AND MECHANISM OF ACTION OF ELLIPTICINE

4.1. Introduction

Ellipticine and its derivatives have been shown to inhibit the growth of various experimental tumors for example S-180, adenocarcinoma-755, Leukaemia L-1210 and myeloblastic leukaemia[12,13,59-61]. Biometabolic studies of ellipticine indicate that ellipticine is oxidised in vivo to 9-hydroxyellipticine (9-HE, 45, major) and 7-hydroxyellipticine (7-HE, 46, minor) in the presence of cytochrome-P 450 mixed oxygenase[62-67]. 9-HE is fourty times more active than ellipticine on Leukaemia L-1210 although 7-HE is six times less active than ellipticine. The quaternisation of the pyridinium nitrogen of 9-HE has furnished a highly active compound, 9-hydroxy-N-2-methylellipticinium acetate (9-HNME$^+$, NSC 264137, 47)[68] which has recently been used for example in the treatment of osteolytic metastases of breast cancers[69,70].

The antitumoral activity of 9-HE 45 and 9-HNME$^+$ 47, tested against various experimental tumors in mice and rats by the pharmacological group of the European Organization for Research and Treatment of Cancer (EORTC) is given in Table 1[71]. The activity of various ellipticine derivatives has been presented in Table 2[72].

TABLE 1

Antitumoral activity of 9-hydroxyellipticine (9-HE) and 9-hydroxy-N-2-methylellipticinium acetate (9-HNME$^+$) against various experimental tumors,[71] reported by EORTC Screening and Pharmacological group

Experimental animal	Experimental tumor	9-HE	9-NHME$^+$
Mice	Leukaemia L 1210	+	++
	Leukaemia P 388	+	++
	Lewis lung Carcinoma	±	±
	Myeloma (ADJ-PCGA)	+	
	Osteosarcoma		-
	Ependymoblastoma		++
	Melanoma B 16	+	+
Rat	Yoshida lymphosarcoma (in vitro)		++
	Gardner lymphosarcoma OG		++
	Squamous Cell Carcinoma		+
	EHTH Myeloid Leukaemia		

TABLE 2

Comparative effect of ellipticine derivatives on L 1210 cells in vitro and in vivo and DNA binding affinity[72]

Compound	ID$_{50}$[a]		ILS(%) in term of LD$_o$[b]			LD$_o$[c]	DNA
	ng/ml	µM	1	1/2	1/5	(mg/kg)	Affinity[d]
E	242	0.99	68	40	12	50	1.5×10^5
9-HE	3.9	0.015	53	58	28	50	2×10^6
2-NME$^+$	437	1.68	18	22	17	13	2.3×10^5
9-H-6-NME	6.0	0.022	-	-	-	-	1.2×10^7
9-H-2-NEtE$^+$	9.6	0.033	57 (47%)[e]	88 (32%)	119 (15%)	50	1.0×10^6
9-HNME$^+$	13.8	0.05	62	53	28	5	1.3×10^6
9-H-2,6-N,N-DME$^+$	11.9	0.041	78	75	55	15	4.0×10^6
9-NH$_2$-E	138	0.53	18	0	-	150	1.2×10^6
9-OCH$_3$-E	164	0.60	70	-	-	70	1.0×10^5
9-FE	1035	3.94	f	-	-	>250	6.4×10^5
9-Br-E	4115	12.7	inactive.....			>500	4.0×10^5
Actinomycin D	1.24	.001	45	-	36	0.87	2.3×10^6

a Dose which reduces the cell growth by 50% after 48h as compared to control.
b ILS (increase in life span) over controls (10^5 cells/mouse IP ; single injection after 24h after cell grafting).
c LD$_o$ = highest nonlethal dose (IP treatment).
d Measurement carried out at 25°C in 0.1 M NaCl-0.1 M tris-HCl (Ph = 7.4).
e Percentage of cured mice which survive longer than 45 days.
f = not tested due to insufficient quantity of the compound.
Abbreviation : DM = dimethyl, E = ellipticine, E$^+$ = ellipticinium, H = Hydroxy, NM = N-methyl.

4.2. Mechanism of Action of Ellipticine and Derivatives

The mechanism of action of ellipticine in vivo and its derivatives is still not well understood. However, a tremendous amount of work has been carried out to solve this enigma. Ellipticine and its derivatives bind to double stranded DNA with an affinity coefficient of 10^{-5} to 10^{-6} M[73], destroy the kinoplastic DNA[74], base pairing and denature the DNA[75]. Ellipticine also induces DNA strand breaks and stable DNA protein complexes[76]. Kohn and Waring et al.[77] have established that ellipticine binds to DNA by intercalation which has been further confirmed by NMR studies[78]. Furthermore Jain et al.[79] have cocrystallized ellipticine with 5-iodocytidylyl (3'-5') guanosine and have solved the three dimensional structure of the complex by X-ray analysis.

The high affinity of ellipticine and its derivatives to DNA and its intercalation between DNA base pairs have long been considered the two main factors responsible for their antitumoral activity. However, there are certain ellipticine derivatives for example 9-aminoellipticine (9-NH_2E, 48) and 9-fluoroellipticine (9-FE, 49)[72,73], which have nearly the same DNA binding constants but are less active or inactive (Table 2). This observation led one to think that ellipticine and its analogues act in vivo through more than one mechanism. The strong cytotoxic activity of 9-HE, a biometabolite of ellipticine, suggests that ellipticine is biometabolised to 9-HE prior to interact with DNAs.

The antitumoral activity of ellipticine 1 or, in general pyridocarbazoles could be explained on the basis of four hypotheses. The first two are related to DNA. However, the last two designate RNAs and protein molecules as possible targets for 9-HE. These hypotheses are illustrated in Figure 3.

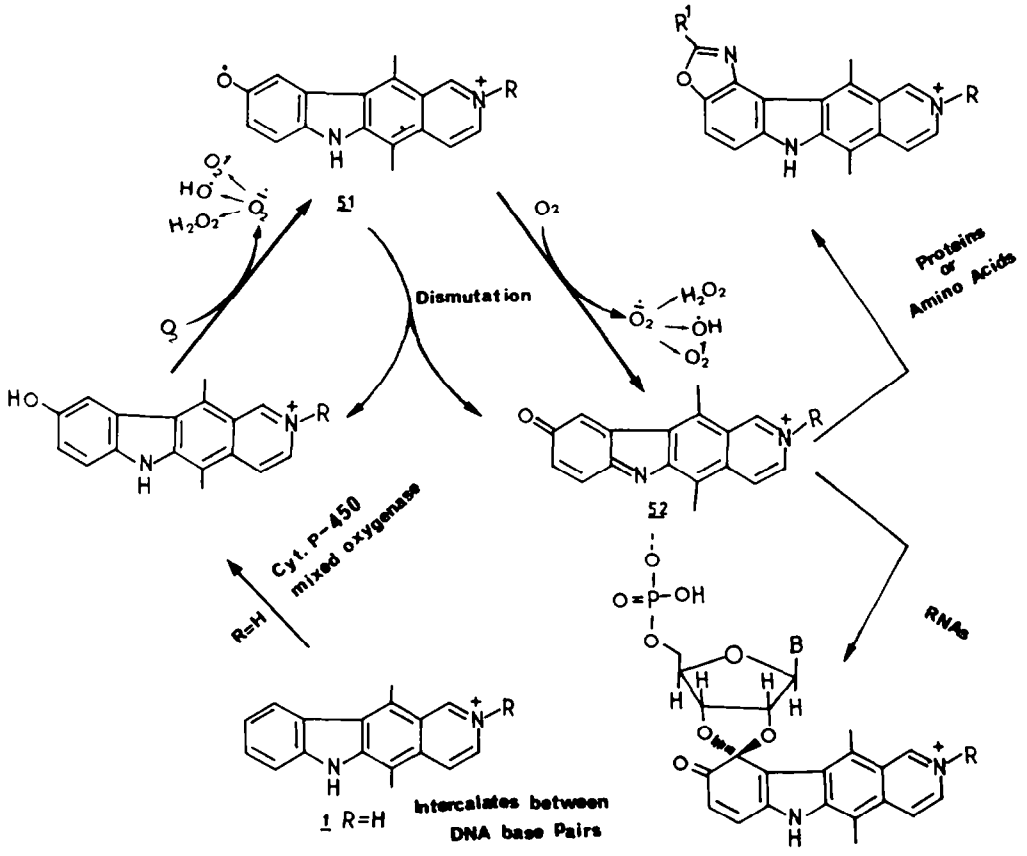


Figure 3

In the first hypothesis, the enhancement of cytotoxic activity of ellipticine on hydroxylation at the 9-position could be attributed to the high affinity of the 9-HE for DNA, since the presence of the 9-hydroxy group in 9-HE could stabilize the intercalating complex by hydrogen bonding with the phosphate groups or bases present in the DNA[71].

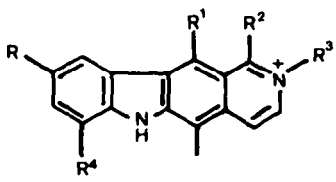
In the second hypothesis, the damage of the protein bound DNA by 9-HE and 9-HNME$^+$ has been explained by the formation of phenoxy radicals 51. Since 9-HE and 9-HNME$^+$ gave quinone-imines 52 upon peroxidase oxidation (peroxidase/H_2O_2) by undergoing a two electron oxidation[80]. The generation of these quinone-imines could be conceived therefore through the intermediacy of phenoxy radicals 51 which have been considered responsible for DNA strand breaks, resulting an irreversible damage to the DNA[81]. The cytotoxic activity of a number of benzanthraquinones : adriamycin, daunorubicin, carminomycin, aclacinomycin A and the N-heterocyclic quinones : streptonigrin and mitomycin has also been explained on the basis of the formation of these transitory phenoxy radicals[82-86]. The formation of the phenoxy radicals 51 in the case of 9-HE 45 and 9-HNME$^+$ 47 has been shown by in vitro experiments in the presence of NaOH/DMSO or peroxidase-H_2O_2[80,87]. However, we believe that these phenoxy radicals are not directly responsible for DNA strand breaks, since, phenoxy radicals are very stable[88] and, therefore, can not attack DNA.

The in vivo formation of these phenoxy radicals could be explained by the reduction of molecular oxygen to superoxide ions. It is, therefore, quite likely that instead of these phenoxy radicals 51, superoxide ions could be a responsible factor for DNA strand breaks. The reaction of superoxide ions with water could furnish highly reactive hydroxyl radicals (OH) which could damage DNA and may also initiate lipid peroxidation and subsequently result in the cell damage[89] (Figure 3).

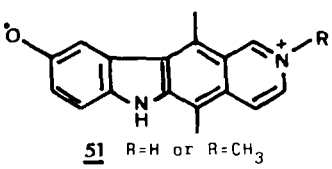
The thus generated phenoxy radicals 51 could further undergo one electron transfer oxidation to give the electrophilic quinone-imines 52 either by the further reduction of molecular oxygen to superoxide ion or by dismutation to quinone-imines and to parent 9-hydroxypyridocarbazoles (Figure 3). This type of quinone-imines could covalently bind to biomolecules (such as proteins and nucleic acids). This is also analogous to the quinonemethide intermediates proposed by Moore in his "bioreductive alkylation model" for various anticancer agents[90]. Indirect evidence has also been obtained for the formation of quinone-imines in vivo. Since the 10-S-glutathione conjugates of 9-HNME$^+$ and its positional isomer 9-hydroxy-N-2-methylolivacinium acetate (9-HNMO$^+$, 50) have been isolated as excretory metabolites of rats treated with 9-HNME$^+$ and 9-HNMO$^+$ respectively[91].

The quinone-imines 52 are strongly electrophilic and undergoe an addition of various nucleophiles such as pyridine[92], amino acids[93], α-substituted primary amines[94], sulphydryl derivatives[92], methanol, ethylene glycol, ribo-nucleosides and -nucleotides[95-98] to give 53 - 60 respectively. The structures of amino acids 61[99,106], methanol 62[92] and ribonucleosides 63[87,100] adducts of 9-HNME$^+$ were incorrectly assigned by Meunier et al. and have been recently revised by us to 54[93], 57 and 59[95,96] respectively.

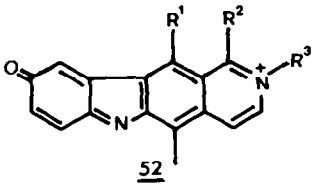
During the reaction of ribo-nucleosides and -nucleotides with 9-HNME$^+$ and 9-HNMO$^+$ under oxidative conditions, the formation of the regio- and stereo-selective ketalic linkage by the both 2' and 3'-hydroxyl groups of the ribose ring at the 10-position of 9-HNME$^+$ and 9-HNMO$^+$ has been observed[95-98]. However, the reaction of 2'-deoxyadenosine with 9-HNME$^+$ under oxidative conditions resulted exclusively an orthoquinone 64[96]. In the light of these observations we have proposed that the antitumoral activity of this class of compounds could also be explained on the basis of alkylation at the terminal end of t-RNA to stop the formation of aminoacyl t-RNA, end of poly-A-tail of the m-RNA or "cap" present at the 5'-end of m-RNA (whereas the free 2' and



45 R=OH, $R^1=CH_3$, $R^2=R^3=R^4=H$
46 R^4=OH, $R^1=CH_3$, $R=R^2=R^3=H$
47 R=OH, $R^1=R^3=CH_3$, $R^2=R^4=H$
48 R=NH_2, $R^1=CH_3$, $R^2=R^3=R^4=H$
49 R=F, $R^1=CH_3$, $R^2=R^3=R^4=H$
50 R=OH, $R^2=R^3=CH_3$, $R^1=R^4=H$



51 R=H or R=CH_3



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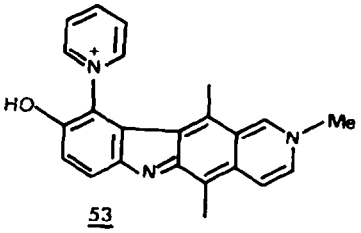

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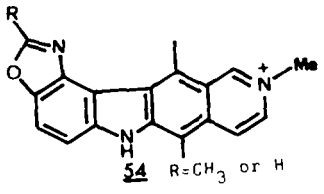
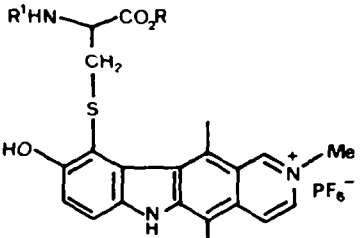
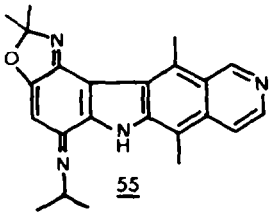


54 R=CH_3 or H



56 R=H, R^1=H, R=H, $R^1=OCOCH_3$
R=CH_3, R^1=H



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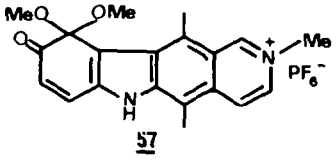

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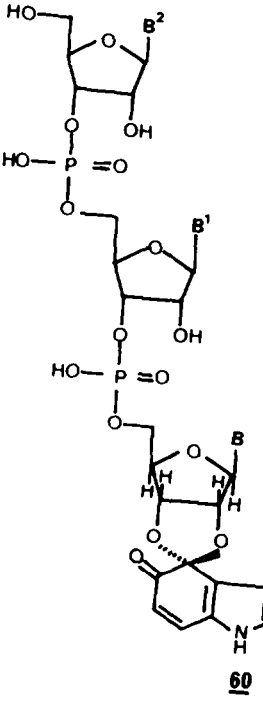

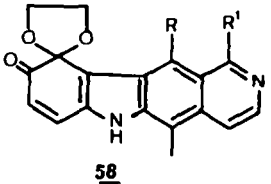

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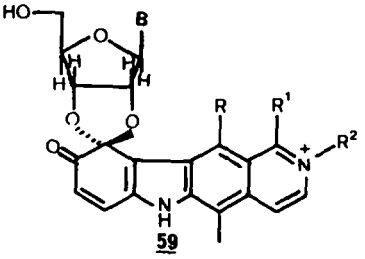

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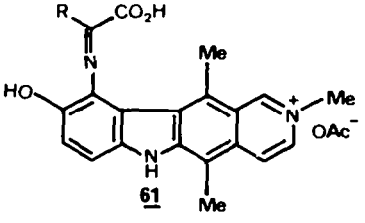

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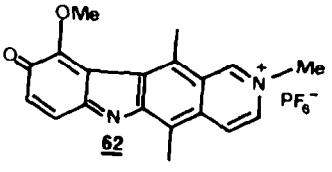

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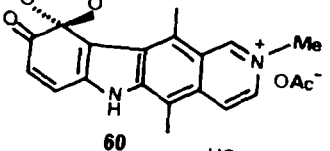

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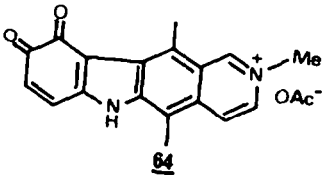

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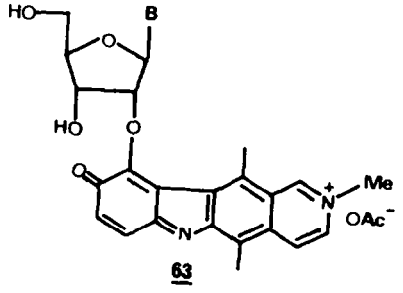

63

3'-hydroxyl groups are present together) and could consequently inhibit the biosynthesis of proteins[95-97]. Blockage of the synthesis of polyribonucleotides and polypeptide chains at the elongation step by olivacine 4[101] and inhibition of RNA synthesis by ellipticine[102-105] could be the consequence of this type of reaction and could further support this hypothesis.

In the light of the reaction of 9-hydroxypyridocarbazoles with aminoacids and α-substituted primary amines under oxidative conditions, it could be hypothesised that the thus generated quinone-imines could also be a target for protein molecules (such as enzymes). The reaction of 9-HE with α-substituted primary amines under oxidative conditions[94] indicates that this type of compound could act as a biselectrophile. If this type of reaction occurs in vivo, then the generated quinone-imines could cross link to protein molecules (whereas the free amino group and other nucleophile such as (-SH) are present in the same molecule). Inhibition of ribonucleic acid polymerase activity by ellipticine could possibly be the result of this type of reaction[105]. Furthermore, it has also been demonstrated by in vitro experiments that bovine serum albumin (BSA) and other proteins bind to 9-HNME$^+$ irreversibly under bioxidative conditions[106] and could be responsible for some anaphyllactic side effects seldomly observed during cancer patients treatment by Celliptium-®(9-HNME$^+$).

5. CONCLUDING REMARKS

In conclusion this report has pointed out the following facts :

1. The biosynthetic pathway of ellipticine and related alkaloids is still not substantiated by experiment. However, the Potier and Janot biosynthetic pathway seems to be involved in plants, since both tryptophan and stemmadenine are incorporated in to apparicine.

2. A number of elegant synthetic routes are available now, not only for the synthesis of simple pyridocarbazole alkaloids but also allowing one to vary the substitution pattern at most positions of the ellipticine nucleus. However, very little attention has been focused on the systematic pharmacological evaluation of pyridocarbazole derivatives for establishing clear structural activity relationship. It is, therefore, necessary to exploit and to explore this missing link of the pyridocarbazole story in order to establish the pharmacophore essential for their antitumoral activity.

3. The in vivo mechanism of action of pyridocarbazole derivatives has still to be established. However, from the available information, it may be concluded that pyridocarbazole derivatives interact in vivo by more than one mechanism. In the light of the reaction of ribo-nucleosides and -nucleotides with 9-hydroxypyridocarbazole derivatives under oxidative conditions, it has been proposed that this type of compounds besides "classical" intercalation into DNA, could also react with RNAs by forming stable covalent bonds and can cause irreversible damage to cells. Furthermore, reaction of isopropylamine with 9-hydroxypyridocarbazole derivatives has illustrated that an oxidised form of 9-hydroxypyridocarbazole could also act as a biselectrophile. This type of compound, therefore, could cross link to biomolecules (such as proteins-enzymes) and consequently could inhibit their action.

It is quite probable that, in the near future, the continuous interest of the scientific community working in this field will overcome the existing problems and possibly lead to new more active anticancer drugs in the pyridocarbazole series.

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