Antitumor *Psoropermum* Xanthones and *Sarcomelicope* Acridones: Privileged Structures Implied in DNA Alkylation[†]

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Fused isopropylfuran and dimethylpyran units are privileged structures present in numerous bioactive natural products exemplified, in the field of anticancer drugs, by the furanoxanthone psorospermin and the pyranoacridone acronycine. Psorospermin binds to the N-7 position of the guanine units in the presence of topoisomerase II. In contrast, acronycine derivatives such as *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine alkylate the 2-amino group of DNA guanine residues in the minor groove. Hybrid compounds associating the acridone or benzo[*b*]acridone chromophore of acronycine derivatives and the epoxyfuran alkylating unit present in psorospermin also display very potent antiproliferative activities, alkylating DNA guanine units at position N-7 in the major groove, as natural xanthones belonging to the psorospermin series.

Introduction

The crucial importance of secondary metabolites isolated from natural sources in the processes of drug discovery and development has been outlined in several recent reviews.[1−3] The role of defense played by natural products in the living organisms that synthesize them most probably explains their particular interest in the discovery of antifungal, antibacterial, antiparasitic, and anticancer drugs implying novel mechanisms of action. In those therapeutic fields, most new approved drugs are natural products, natural products derivatives, or synthetic mimics, which incorporate a pharmacophore present in a natural product.[4,5]

One of the striking characteristics of natural products is the relatively small number of biosynthetic pathways implied in their biosynthesis. Indeed, polyketide, shikimic, aliphatic amino acids, and isoprenic pathways and their various combinations account for the biosynthesis of most of the secondary metabolites isolated from microorganisms, animals, fungi, and higher plants.[6,7] In this way, natural products can be considered as resulting from a form of combinatorial or parallel chemistry developed by living organisms during the process of evolution.[8]

A fascinating example of diversification and parallel evolution is given by triterpenoids and steroids indispensable to the stabilization of biological membranes, and so to life. This role is played by a particular group of triterpenes, the hopanoids in archeobacteria, and by derivatives of ergosterol in fungi, cholesterol in animals, and phytosterols in higher plants.[9] The common feature in the synthesis of these compounds is the concerted cyclization of carbonium ions derived from squalene, whose biogenesis relies in turn on the reactivity of the active isoprene unit. Dimethyl allyl pyrophosphate, which is the starter unit of terpene metabolism, appears as a particularly reactive and efficient alkylating agent, with the phosphate being a good leaving group and the resulting carbonium ion being stabilized by charge delocalization. The high alkylating potency of dimethylallyl pyrophosphate also accounts for its implication in numerous mixed biogenetic pathways. Of particular interest in terms of molecular diversity is the alkylation at the oxygen atom or at the *ortho* position of natural phenolic substrates, which can generate, after subsequent cyclization, fused dimethylpyran or isopropylfuran systems, the latter often being simplified into the corresponding furan by oxidation followed by loss of acetone and water. The benzofuran and benzopyran structural motifs generated through this metabolic pathway are privileged structures present in numerous bioactive natural products.[10,11] The insecticidal rotenone (**1**)[12] and the photosensitizing furocoumarins bergapten (**2**) and xanthotoxin (**3**)[13] are relevant examples in the furan series. Similarly, the insecticidal precocenes I (**4**) and II (**5**),[14] the antiviral calanolide A (**6**),[15] and the cytotoxic kermadecins I (**7**) and II (**8**)[16] illustrate the biological interest of fused pyrans.

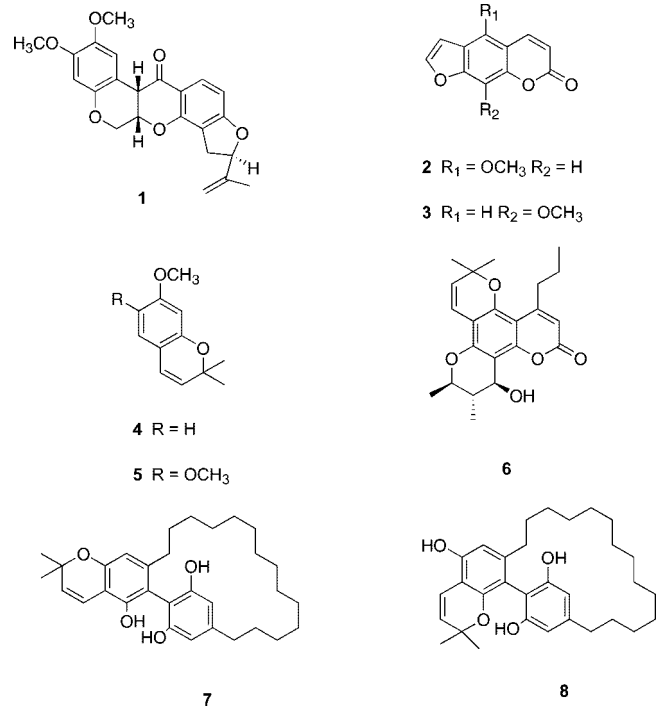

Figure 1. Selected examples of naturally occurring bioactive products including a benzofuran or benzopyran moiety: rotenone (**1**), bergapten (**2**), xanthotoxin (**3**), precocene I (**4**), precocene II (**5**), calanolide A (**6**), kermadecin I (**7**), and kermadecin II (**8**).

In the field of anticancer drugs, isopropylfuran and dimethylpyran units fused onto xanthone and acridone basic cores appear particularly interesting, with two natural products that were used as models for further chemical and biological development, the furanoxanthone psorospermin (**9**) isolated from *Psorospermum*

[†] Dedicated to Dr. David G. I. Kingston of Virginia Polytechnic Institute and State University for his pioneering work on bioactive natural products.

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Figure 2. Psorospermin (**9**) from *Psorospermum febrifugum* and acronycine (**10**) from *Sarcomelicope* species.

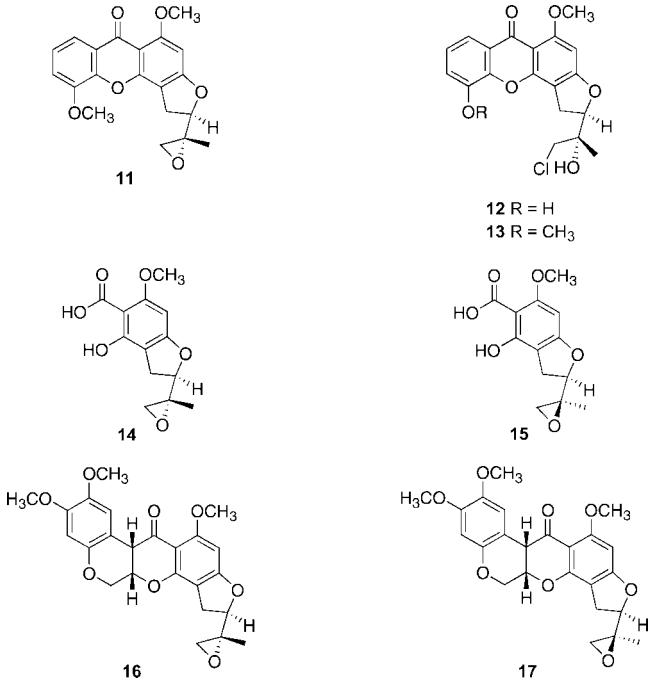


Figure 3. Psorospermin derivatives, epoxytubaic acids, and epoxyrotenones.

febrifugum Sprach.[17,18] and the pyranoacridone acronycine (**10**) present in several *Sarcomelicope* Engl. species.[19−21]

Psorospermin and Structural Analogues

The genus *Psorospermum* Sprach., usually included in the family Hypericaceae, tribe Vismieae, comprises some 40−50 species of trees and shrubs widespread in tropical Africa and Madagascar. It has been recently suggested to be congeneric with the relative genus *Harungana* Lamarck.[22] Several species, such as *Psorospermum guineense* Hochr. and *Psorospermum febrifugum*, are used locally in traditional medicine as febrifuges and purgatives as well as in the treatment of leprosy.[23,24] However, the interest of natural products chemists in the genus followed the isolation, through a bioguided fractionation of *Psorospermum febrifugum* root extract, of the cytotoxic and antitumor psorospermin (**9**), by Kupchan and co-workers in 1980.[17] The structure of psorospermin, an angular furanoxanthone bearing a reactive epoxide substituent, was established on the basis of its spectroscopic data and chemical correlations, but the absolute configuration could not be determined at that time. Observation of NOE correlations in the ^{1}H NMR spectrum gave evidence for the relative positions of the methoxyl and dihydrofuran ring and the angular fusion of this ring onto the xanthone basic core. Psorospermin gave the corresponding methyl ether **11** upon treatment with diazomethane. It was accompanied in the plant extracts by smaller amounts of psorospermin chlorohydrin (3′,4′-deoxy-4′-chloropsorospermin-3′-ol) (**12**), which was isolated as its methyl ether **13** after treatment of the relevant fractions with diazomethane. Chlorohydrin **13** was converted into 5-*O*-methylpsorospermin (**11**) by treatment with potassium *tert*-butoxide, whereas the reverse reaction was performed under various

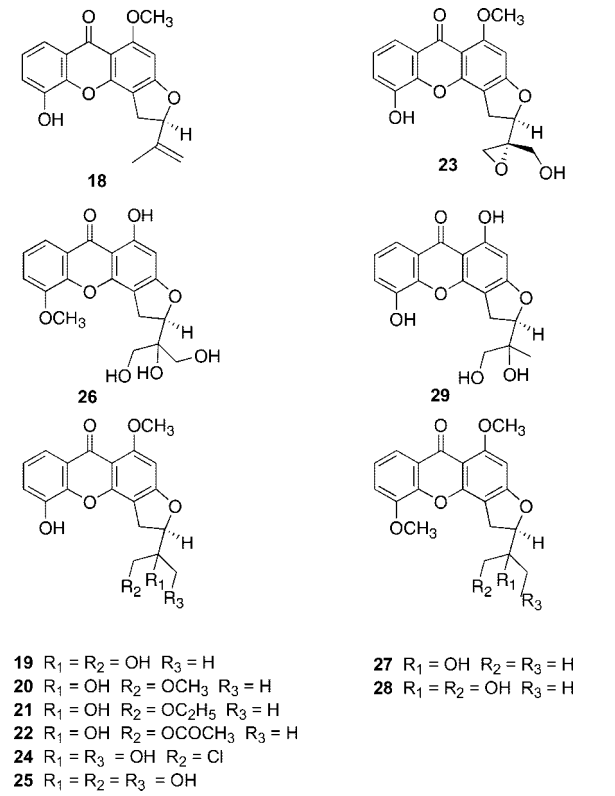


Figure 4. Furanoxanthones isolated from *Psorospermum febrifugum*.

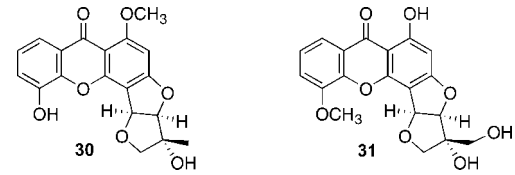


Figure 5. Difuranoxanthones isolated from *Psorospermum febrifugum*.

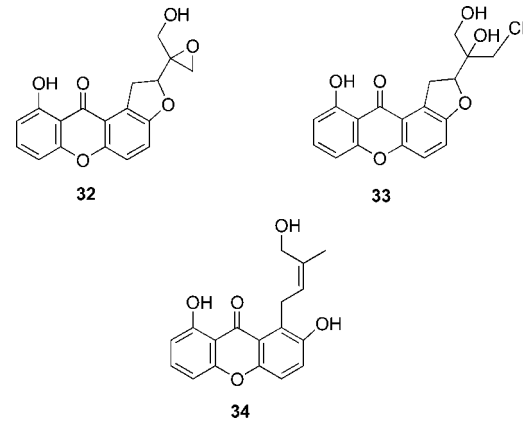


Figure 6. Furanoxanthones isolated from *Psorospermum molluscum*.

acidic conditions. Later on, a thorough study performed by the group of Cassady permitted the psorospermin absolute configuration to be determined[25−27] and several related bioactive furoxanthones[28,29] and difuroxanthones[30,31] to be isolated. These compounds were accompanied in the plant material by simple xanthones[31,32] and xantholignoids.[33] The (2′*R*,3′*R*) configuration of psorospermin was established by ORD, in comparison with the Cotton curves obtained for the epimeric (2′*R*,3′*R*) and (2′*R*,3′*S*) epoxytubaic acids (**14** and

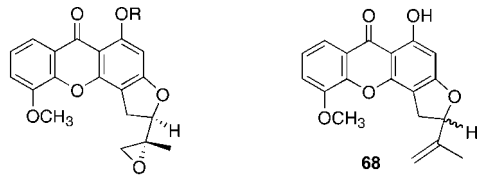


Figure 7. (±)-(2′*R**,3′*R**)-1-*O*-Demethyl-1-*O*-ethyl-5-*O*-methylpsorospermin (**66**), (±)-(2′*R**,3′*R**)-1-*O*-demethyl-1-*O*-isopropyl-5-*O*-methylpsorospermin (**67**), and 3′,4′-deoxyisopsorospermin (**68**).

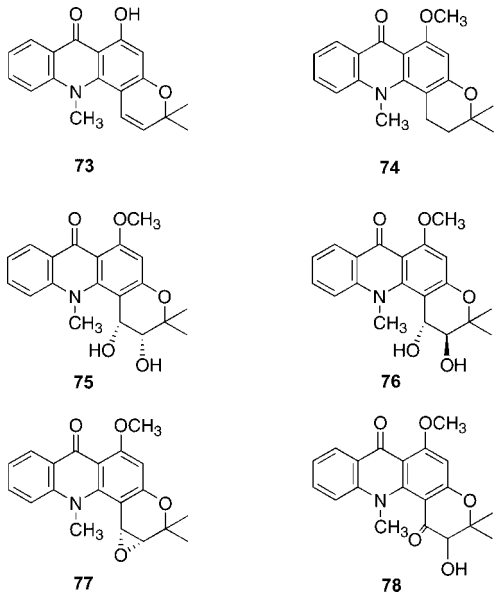


Figure 8. Noracronycine (**73**), 1,2-dihydroacronycine (**74**), *cis*-1,2-dihydroxy-1,2-dihydroacronycine (**75**), *trans*-1,2-dihydroxy-1,2-dihydroacronycine (**76**), acronycine epoxide (**77**), and 2-hydroxy-1-oxo-1,2-dihydroacronycine (**78**).

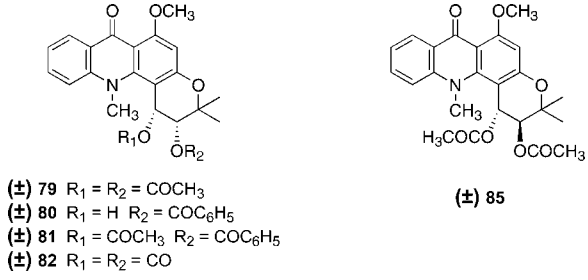
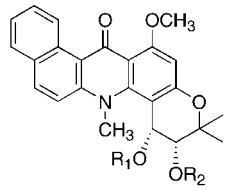


Figure 9. 1,2-Dihydroxy-1,2-dihydroacronycine diesters.

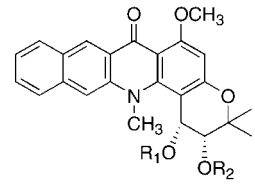
15) and (2′*R*,3′*R*) and (2′*R*,3′*S*) epoxyrotenones (**16** and **17**).[25] The stereochemistry of the latter epoxyrotenone **17**, prepared together with **16** by *meta*-chloroperbenzoic acid (MCPBA) epoxidation of natural rotenone (**1**) of known configuration, was assigned by X-ray crystallographic analysis.[25] Related furoxanthones isolated from *Psorospermum febrifugum* extracts included 3′,4′-deoxypsorospermin (**18**),[25] 3′,4′-deoxypsorospermin-3′,4′-diol (**19**),[25] 4′-*O*-methyl-3′,4′-deoxypsorospermin-3′,4′-diol (**20**),[29] 4′-*O*-ethyl-3′,4′-deoxypsorospermin-3′,4′-diol (**21**),[29] 4′-*O*-acetyl-3′,4′-deoxypsorospermin-3′,4′-diol (**22**),[31] 5′-hydroxypsorospermin (**23**),[29] 4′-chloro-3′,4′-deoxypsorospermin-3′,5′-diol (**24**),[29] 3′,4′-deoxypsorospermin-3′,4′,5′-triol (**25**),[31] 3′,4′-deoxyisopsorospermin-3′,4′,5′-triol (**26**),[29] 5-*O*-methyl-3′,4′-deoxypsorospermin-3′-ol (**27**),[25] 5-*O*-methyl-3′,4′-deoxypsorospermin-3′,4′-diol (**28**),[29] 1-*O*-demethyl-3′,4′-deoxypsorospermin-3′,4′-diol (**29**),[29] isolated as two different diastereoisomeric compounds, and two difuranoxanthones resulting from an additionnal cyclization of the prenyl substituent, psorofebrin (**30**)[30,31] and 5-hydroxyisopsorofebrin (**31**).[30,31] Other bioactive secondary metabolites isolated from *P. febrifugum* include various cytotoxic anthranoids with antimalarial and antileishmanial potential, such as anthrones, bianthrones, anthraquinones, and vismiones.[31,34−38] Compounds of these latter series, which are out of the scope of this review, have also been isolated from *Psorospermum tenuifolium* Kotschy,[39,40] *Psorospermum glaberrimum* Hochr.,[41−44] and *Psorospermum corymbiferum* Hochr.[44] Finally, a recent investigation of the madagascan species *Psorospermum molluscum* (Pers.) Hochr. by Kingston and co-workers permitted the isolation of psoroxanthin (**32**) and 3′,4′-deoxy-4′-chloropsoroxanthin-3′,5′-diol (**33**), which are the first representatives of a new series of furoxanthones structurally related to psorospermin, displaying promising cytotoxic activities.[45] They were accompanied by the open chain derivative, 8-(4′-hydroxyprenyl)-1,7-dihydroxyxanthone (**34**), and several simple xanthones.

The first synthesis of a psorospermin derivative was performed by the group of Cassady, in order to confirm the structure and relative stereochemistry of the natural product and to delineate some structure−activity relationships in the series.[27] Racemic (±)-(2′*R**,3′*S**)-5-*O*-methylpsorospermin (**35**) was obtained in 10 steps from 2,3-dimethoxybenzoic acid (**36**) and phloroglucinol (**37**) (Scheme 1). Condensation of **36** and **37** gave 1,3-dihydroxy-5-methoxyxanthone (**38**). Selective alkylation of the phenolic group at C-3, not chelated to the carbonyl group, with allyl bromide gave the ether **39**, which was methylated to **40**. Claisen rearrangement of the allyl ether **40** afforded 4-allyl-3-hydroxy-1,5-dimethoxyxanthone (**41**), which was benzylated to **42** and oxidized to aldehyde **43**. Formation of the *E*-olefin **44** was carried out by a Wittig reaction with (carbethoxyethylidene)triphenylphosphorane. Reduction of the carbomethoxy group gave the corresponding primary alcohol, **45**, which was converted to epoxide, **46**, by use of MCPBA. The primary alcohol **46** was activated as the corresponding mesylate, **47**. After deblocking of the benzyl group at C-3 an elegant zipper-type reaction performed on **48** gave the desired (±)-(2′*R**,3′*S**)-5-*O*-methylpsorospermin (**35**). The same zipper-type reaction, performed on an oxirane resulting from the osmium-catalyzed Sharpless asymmetric epoxidation of a *Z*-olefin, was used later to synthesize natural (2′*R*,3′*R*)-psorospermin (**9**) (Scheme 2).[46] Treatment of 4-allyl-3-hydroxy-1,5-dimethoxyxanthone (**41**) with boron tribromide gave the trihydroxyxanthone, **49**, which was converted to the bis-benzyl derivative, **50**. Methylation of the hydroxyl at C-1 afforded **51**. Johnson−Lemieux oxidation to aldehyde **52** was ensured by use of osmium tetroxide and sodium periodate. Condensation with ethyl (bis(2,2,2-trifluoroethoxy)phosphinyl)acetate under alkaline conditions at low temperature gave the required *Z*-olefin, **53**. DIBALH reduction of the carbomethoxy group afforded the allylic alcohol **54**. Sharpless asymmetric epoxydation of **54** gave **55** in good yield and 70% enantiomeric excess. Conversion to the corresponding mesylate **56** was followed by simultaneous zipper cyclization and debenzylation to (2′*R*,3′*R*)-psorospermin (**9**).

A different route was envisioned for the preparation of the four enantiomers of 5-*O*-methylpsorospermin required in the course of structure−activity relationship studies (Scheme 3).[47] Selective benzylation of the hydroxyl at C-3 of 1,3-dihydroxy-5-methoxyxanthone (**38**) gave **57**, which was further methylated to **58**. Deprotection of the benzyl group afforded the phenol **59**. Alkylation with 3-chloro-3-methyl-1-butyne gave the dimethylpropargyl ether **60**, which was reduced to the corresponding alkene **61** in the presence Lindlar's catalyst and quinoline. Wacker-type cyclization of the thermal Claisen rearrangement product **62** to isoprenyldihydrofuroxanthone **63** was followed by catalytic osmic oxidation to a diastereoisomeric mixture of the corresponding (±)-diols. The

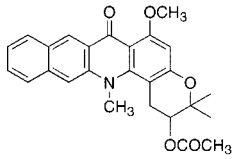


(±) 104 $R_1 = R_2 = H$
(±) 106 $R_1 = R_2 = COCH_3$
(±) 107 $R_1 = R_2 = COC_2H_5$
(±) 108 $R_1 = R_2 = COCH_2CH(CH_3)_2$
(±) 112 $R_1 = COCH_3$ $R_2 = COCH_2CH(CH_3)_2$
(±) 114 $R_1 = R_2 = CO$



(±) 105 $R_1 = R_2 = H$
(±) 109 $R_1 = R_2 = COCH_3$
(±) 110 $R_1 = R_2 = COC_2H_5$
(±) 111 $R_1 = R_2 = COCH_2CH(CH_3)_2$
(±) 113 $R_1 = COCH_3$ $R_2 = COCH_2CH(CH_3)_2$
(±) 115 $R_1 = R_2 = CO$

Figure 10. 1,2-Dihydroxy-1,2-dihydrobenzo[*a*]acronycine (±)-**104**, 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine (±)-**105**, and corresponding diesters and carbonates.



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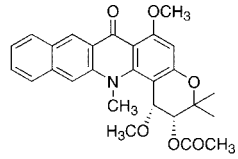
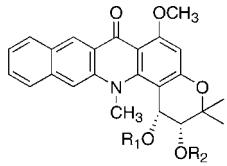


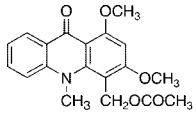
(±) 117

Figure 11. 2-Acetoxy-1,2-dihydrobenzo[*b*]acronycine and *cis*-2-acetoxy-1-methoxy-1,2-dihydrobenzo[*b*]acronycine.



(±) 118 $R_1 = H$ $R_2 = COCH_3$
(±) 119 $R_1 = H$ $R_2 = COCH_2CH(CH_3)_2$
(±) 120 $R_1 = H$ $R_2 = COC_3H_7$
(±) 121 $R_1 = H$ $R_2 = COCH_2CH_2CHCH_2$
(±) 122 $R_1 = COCH_3$ $R_2 = H$

Figure 12. 1,2-Dihydroxy-1,2-dihydrobenzo[*b*]acronycine monoesters.



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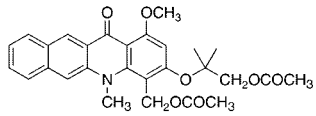

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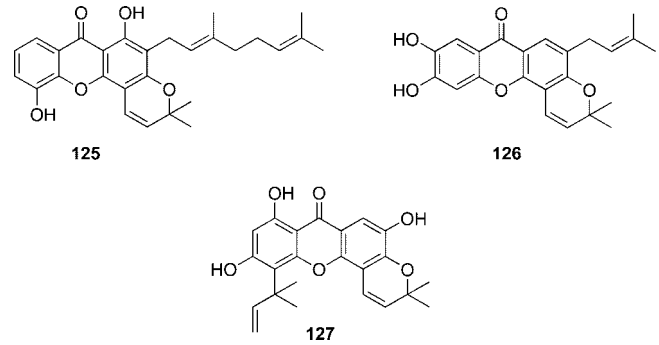
Figure 13. Analogues lacking the fused pyran ring but possessing an acetoxymethyl leaving group at position 4 in the acronycine and benzo[*b*]acronycine series.

diastereoisomers could be separated by column chromatography after conversion into the corresponding (±)-*tert*-butyldimethylsilyl ethers, (±)-**64** and (±)-**65**, prepared by treatment with *tert*-butyldimethylsilyl chloride in the presence of imidazole and 4-dimethylaminopyridine. Deprotection of (±)-**64** and (±)-**65** with tetrabutylammonium fluoride, followed by mesylation and alkaline treatment, provided the corresponding racemic epoxides, (±)-(2′*R**,3′*R**)-5-*O*-methylpsorospermin (**11**) and (±)-(2′*R**,3′*S**)-5-*O*-methylpsorospermin (**35**), respectively. Finally, resolution of the racemic mixtures using chiral column chromatography provided the four optically pure (2′*R*,3′*R*), (2′*S*,3′*S*), (2′*R*,3′*S*), and (2′*S*,3′*R*) isomers.

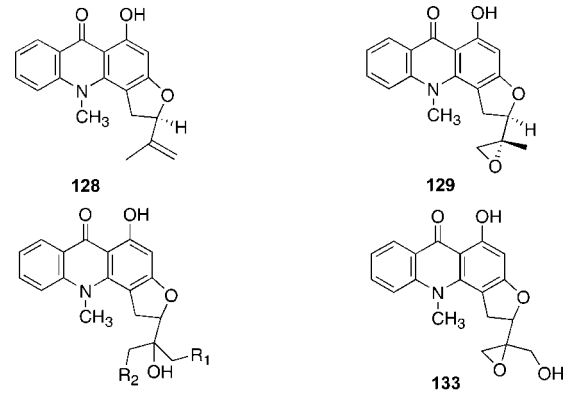
The cytotoxic and antitumor properties of psorospermin (**9**) were first reported by Kupchan, based on significant activity against the human epidermoid carcinoma cells 9KB in vitro (IC_{50} 0.29 μM) and the P388 mouse leukemia in vivo (T/C survival 158% at 8 mg/kg).[17] Further experiments showed that psorospermin was also significantly cytotoxic toward the human colon adenocarcinoma HT-29, human lung carcinoma A-549, and human breast adenocarcinoma MCF-7 cell lines.[18] Interestingly, structural analogues lacking an alkylating group at the 3′,4′ position, exemplified by 3′,4′-deoxypsorospermin-3′,4′-diol (**19**), 4′-*O*-acetyl-3′,4′-deoxypsorospermin-3′,4′-diol (**22**), and psorofebrin (**30**), displayed only marginal activity.[18] In contrast, 5′-hydroxypsorospermin (**23**), bearing a 3′,4′-epoxy group, exhibited both cytotoxic activity in vitro and antitumor properties in vivo.[29]

The mechanism of action of psorospermin (**9**) was first studied on simian virus 40 DNA replication in infected CV-1 monkey kidney cells.[48] With this model, psorospermin caused dose-dependent DNA strand breaks and protein-DNA cross-links, following reactions between primary amine groups of cellular proteins and the aldehyde groups at nonbasic sites. The density of the nonbasic sites generated on the DNA depended on the psorospermin dose.[48] Molecular details of the structure of the



125 **126** **127**

Figure 14. Selected examples of naturally occurring pyrano[3,2-*c*]xanthen-7-ones.



128 **129** **133**

130 $R_1 = Cl$ $R_2 = H$
131 $R_1 = OH$ $R_2 = H$
132 $R_1 = OCH_3$ $R_2 = H$
134 $R_1 = R_2 = OH$

Figure 15. Selected examples of naturally occurring furano[3,2-*c*]acridin-6-ones.

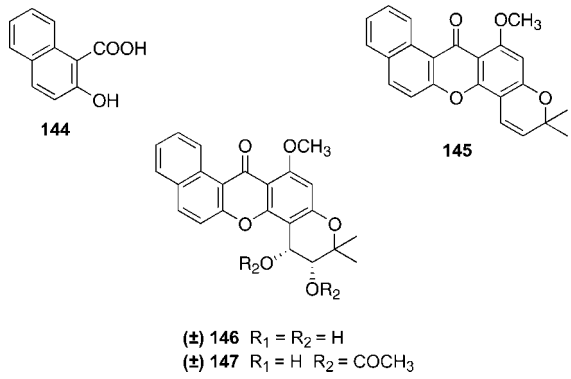


Figure 16. Isosters of benzo[*a*]acronycine in the benzo[*a*]pyrano[2,3-*i*]xanthen-7-one series.

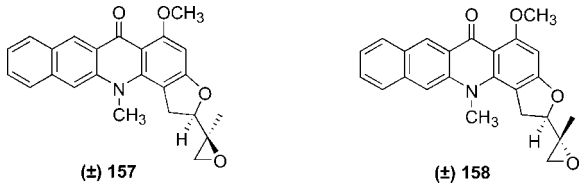


Figure 17. Psorospermin analogues in the benzo[*b*]furo[3,2-*h*]acridin-6-one series.

psorospermin-DNA complex were investigated using both high-field NMR and gel electrophoresis.[49] Psorospermin was shown to intercalate the DNA molecule, with the tricyclic xanthone chromophore in an orientation parallel to the adjacent base pairs. The reactive epoxide unit of psorospermine was placed in the major groove, permitting a selective electrophilic addition to N-7 of the DNA guanine units. The alkylation reaction was followed by the subsequent depurination of the adduct, which was the origin of the formation of the nonbasic sites on the DNA. Psorospermin showed alkylation selectivity, with a preference for the 5′GG sequences. DNA alkylation was greatly enhanced in the presence of topoisomerase II,[50] suggesting that the antitumor activity of psorospermin might be related to its specific interaction with the topoisomerase II-DNA complex.[51] This enhanced activity was dependent on pH, but independent of Mg^{2+} or ATP, indicating that topoisomerase II-mediated psorospermin alkylation occurred in the initial noncovalent binding step in the topoisomerase catalytic cycle. Position N-7 of the guanine residues was still the alkylation site in the presence of topoisomerase II, since the substitution of the target guanine by 7-deazaguanine prevented alkylation.[51]

In terms of structure−activity relationships, the presence of an alkylating group at the 3′,4′ position appears as an essential structural requirement to observe significant cytotoxic and antitumor activity. Indeed, only epoxides and, to a lesser extent, the corresponding chlorohydrins were found biologically active. The influence of the stereochemistry of the epoxyfuran moiety was investigated thoroughly, following the preparation of the four optically pure (2′*R*,3′*R*), (2′*S*,3′*S*), (2′*R*,3′*S*), and (2′*S*,3′*R*) stereoisomers of 5-*O*-methylpsorospermin.[47] In terms of cytotoxicity against 16 different cell lines of solid tumors, leukemias, and lymphomas, (2′*R*,3′*R*)-5-*O*-methylpsorospermin, having the same configuration as natural psorospermin, was the most active compound. Both the (2′*R*,3′*S*) and (2′*S*,3′*R*) isomers showed intermediate activity, whereas (2′*S*,3′*S*)-5-*O*-methylpsorospermin was the least active compound. The same order of potency was found in the topoisomerase II induced alkylation of DNA. The size of the alkoxy group at position 1 also plays an important role in the biological activity. Indeed, both (±)-(2′*R**,3′*R**)-1-*O*-demethyl-1-*O*-ethyl-5-*O*-methylpsorospermin (**66**) and (±)-(2′*R**,3′*R**)-1-*O*-demethyl-1-*O*-isopropyl-5-*O*-methylpsorospermin (**67**), prepared by selective demethylation of **63** to **68**, using boron trichloride, followed by alkylation with ethyl or isopropyl iodide and final epoxidation, were found to be 2 to 4 times less potent in cytotoxic activity against 11 cancer cell lines than (±)-(2′*R**,3′*R**)-5-*O*-methylpsorospermin (**35**).[47]

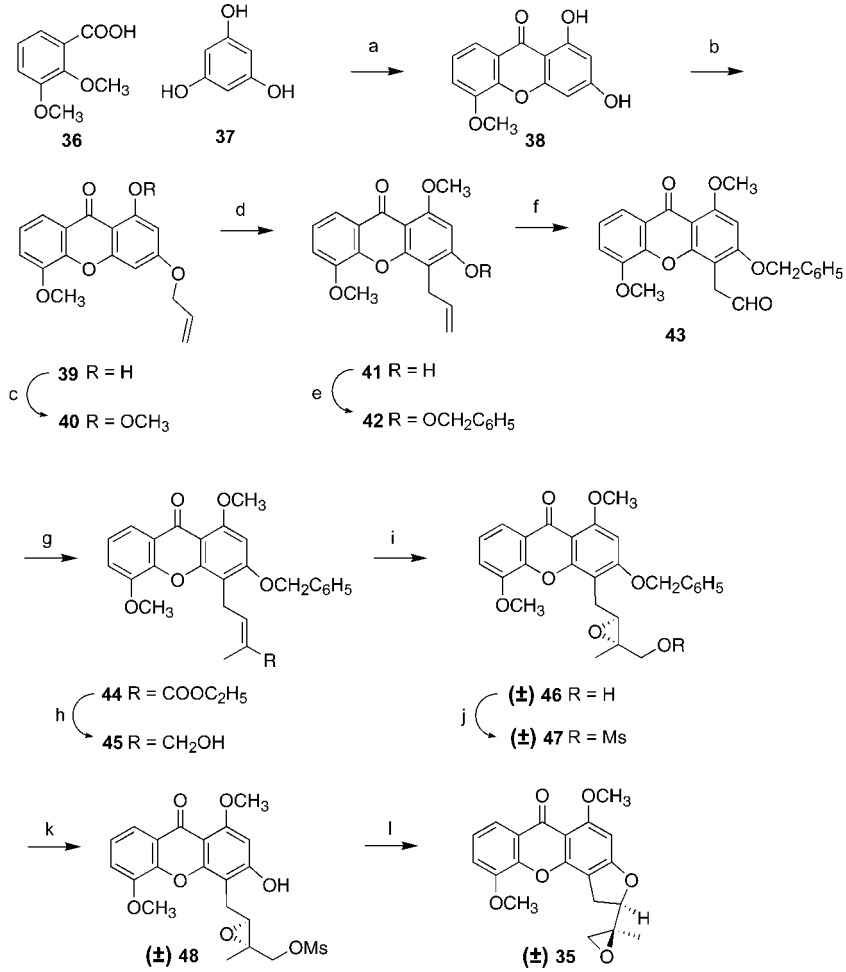
The difuranoxanthone 5-hydroxyisopsorofebrin (**31**)[30,31] has been used as a model for the design of conformationally restricted psorospermin analogues able to alkylate DNA (Scheme 4).[52] In the presence of catalytic amounts of selenium dioxide and acetic acid, allylic oxidation of furanoxanthone **68** gave **69**. Formation of the bisfuran heterocyclic system followed the biosynthesis postulated for natural psorofebrins, through the intermediacy of a quinone methide intermediate. Thus, oxidative cyclization of **69** afforded the *exo*-alkene **70**. Catalytic osmic dihydroxylation of **70** proved highly stereoselective, giving (±)-5-hydroxyisopsorofebrin (**31**) as a single diastereoisomer. Mesylation of the primary alcohol of (±)-(**31**), followed by intramolecular S_N2 reaction under alkaline conditions, gave the desired conformationally restricted epoxide, (±)-(**71**). Alternatively, treatment of the intermediate mesylate with TBAC gave the corresponding chlorohydrin, (±)-(**72**). The psorospermin analogue (±)-(**71**) was only marginally active when tested for DNA alkylation in the presence of topoisomerase II. In contrast, the chlorohydrin (±)-(**72**) retained the psorospermin-like DNA alkylation characteristics. Compared to 5-hydroxyisopsorofebrin, both compounds displayed increased cytotoxicity against a range of six human tumor cell lines, but remained far less potent than psorospermin (**9**).[52]

Acronycine and Structural Analogues

Acronycine (**10**) is a pyranoacridone alkaloid first isolated in 1948 from the stem bark of the Australian Rutaceous tree *Sarcomelicope simplicifolia* (Endl.) Hartley ssp. *simplicifolia* (= *Acronychia baueri* Schott),[19] and its structure was established in 1966 by Macdonald and Robertson.[20] Svoboda demonstrated in 1966, at the Eli Lilly Laboratories, that acronycine exhibited antitumor properties against a large panel of murine tumor models, including the S-180 and AKR sarcomas, X-5563 myeloma, S-115 carcinoma, and S-91 melanoma.[53,54] In contrast, it only showed borderline activity against murine leukemia models. Subsequently, phase I−II clinical evaluation of acronycine was performed in patients with refractory multiple myeloma.[55] Oral acronycine capsules produced one clear response in 16 patients. The remission was maintained for 72 weeks, using a daily dose of 300 mg/m^2. The limited success of that trial was probably related to the moderate potency of acronycine and to its very low water-solubility (ca. 1 mg per liter of water), which did not permit an efficient parenteral formulation of the drug. Despite this promising antitumor activity, the mechanism of action of acronycine at both the cellular and molecular levels has not yet been established clearly. In this context, none of the structural analogues prepared in the first 25 years following the description of the biological properties of acronycine presented a better profile than the parent compound.[21,56] However, these results permitted the delineation of some structural features indispensable for observing the pharmacological activity. The dimethylchromene unit corresponding to the C and D rings was shown to play an essential role. Indeed, the substituent at C-6 on the C-ring had a dramatic influence on the biological activity. Noracronycine (**73**), bearing a phenolic OH group instead of a methoxy group at C-6, did not possess any significant antitumor activity.[53,54] Similarly, the 1,2-double bond was also considered an important structural requirement to elicit antitumor activity, since 1,2-dihydroacronycine (**74**) was found to be inactive.[53]

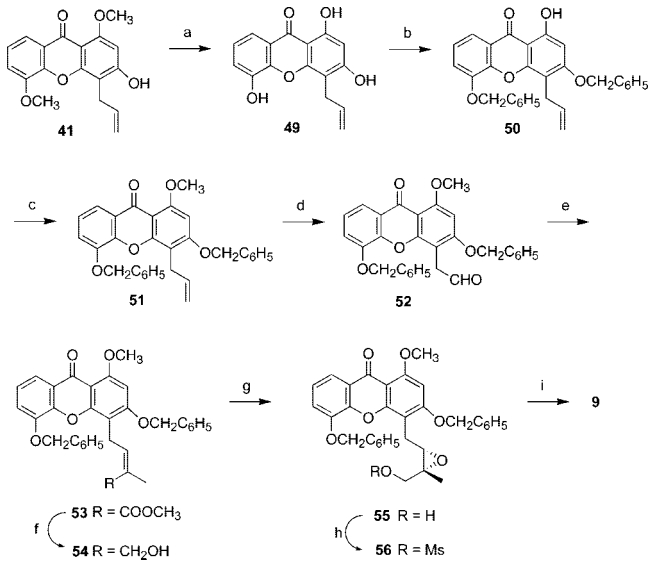
In the search for new acronycine derivatives, most of the species belonging to the genus *Sarcomelicope*, which includes some nine species distributed from eastern Australia to New Caledonia, Vanuatu, and the Fijian Islands,[57,58] were investigated for their alkaloid content,[59] leading to the isolation of several new acronycine derivatives oxidized at the pyran ring. Optically active *cis*-1,2-

Scheme 1. Synthesis of (±)-(2′*R**,3′*S**)-5-*O*-Methylpsorospermin (**35**)[27,a]



[a] Reagents: (a) $ZnCl_2$, $POCl_3$; (b) allyl bromide, K_2CO_3; (c) MeI, K_2CO_3; (d) 200 °C; (e) benzyl bromide, K_2CO_3; (f) OsO_4, $NaIO_4$; (g) $(Ph)_3P{=}C(CH_3)CO_2Et$; (h) $AlLiH_4$; (i) MCPBA; (j) MsCl, pyridine; (k) H_2, Pd/C; (l) *t*-BuOK.

Scheme 2. Synthesis of (−)-(2′*R*,3′*R*)-Psorospermin (**9**)[46,a]

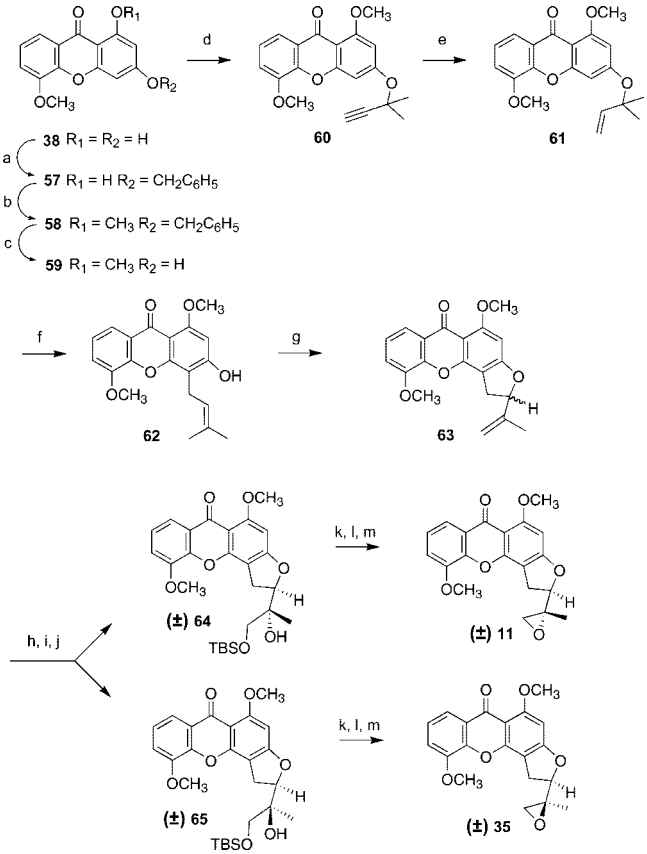


[a] Reagents: (a) BBr_3; (b) benzyl bromide, NaH; (c) MeI, NaH; (d) OsO_4, $NaIO_4$; (e) $(CF_3CH_2O)_2POCH(CH_3)COOCH_3$, KHMDS, 18-crown-6; (f) DIBALH; (g) *t*-BuOOH, (−)-DIPT, $Ti(i\text{-}OPr)_4$; (h) MsCl, Et_3N; (i) Raney Ni, K_2CO_3.

dihydroxy-1,2-dihydroacronycine (**75**) and *trans*-1,2-dihydroxy-1,2-dihydroacronycine (**76**) were isolated from the bark of *Sarcomelicope glauca* Hartley and *Sarcomelicope dogniensis* Hartley.[60,61] Acronycine epoxide (**77**) was purified in minute amounts from *Sarcomelicope argyrophylla* Guill. and *Sarcomelicope simplicifolia* (Endl.) Hartley ssp. *neo-scotica* (P.S. Green) Hartley. This compound was very unstable, and its high reactivity led to speculation that it might be the active metabolite of acronycine in vivo, responsible for the alkylation of nucleophilic targets within the tumor cell.[62]

The instability of acronycine epoxide (**77**), particularly its rapid reaction with water to give the diols **75** and **76**, excluded its possible use as an anticancer agent. Consequently, 1,2-dihydroxy-1,2-dihydroacronycine diesters were prepared, in order to obtain new antitumor candidates with better stability than acronycine epoxide but similar reactivity at the benzylic 1-position toward nucleophilic agents.[63,64] The racemic *cis*-diol (±)-**75** was obtained by catalytic osmic oxidation of acronycine (**10**),[63] whereas the *trans* isomer (±)-**76** was prepared in two steps, by potassium permanganate oxidation of acronycine (**10**) to 2-hydroxy-1-oxo-1,2-dihydroacronycine (**78**), followed by sodium borohydride reduction.[64] Treatment of the *cis*-diol (±)-**75** with excess acetic anhydride gave the diacetate (±)-**79**. Monoesters at the less hindered 2-position, exemplified by benzoate (±)-**80**, were obtained under controlled conditions, when only 1 equiv of acylating agent was used. Further treatment of the monobenzoate (±)-**80** with excess acetic anhydride afforded the mixed ester (±)-**81**. Reaction of (±)-**75** with *N,N*′-carbonyldiimidazole gave the cyclic carbonate (±)-**82**.[63] In order to ensure that the reactivity of *cis*-1,2-dihydroxy-1,2-dihydroacro-

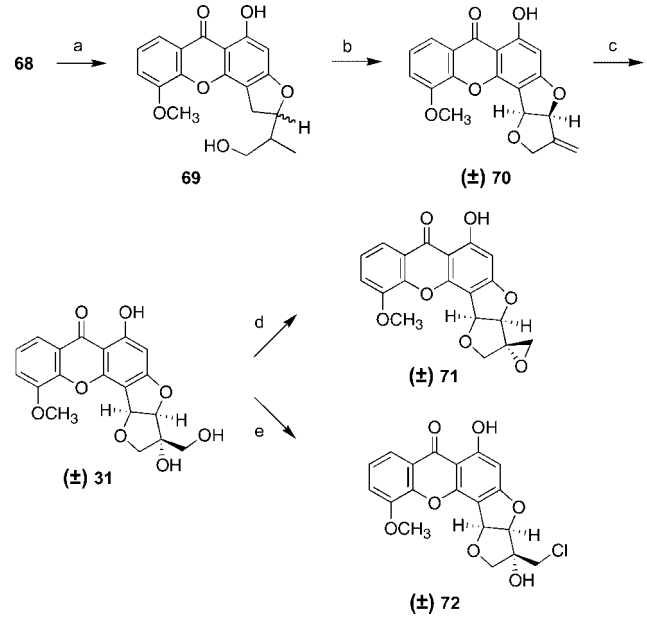
Scheme 3. Synthesis of All Four Enantiomers of 5-*O*-Methylpsorospermin[47,a]



[a] Reagents: (a) benzyl bromide, Cs_2CO_3; (b) MeI, Cs_2CO_3; (c) $FeCl_3$; (d) 3-chloro-3-methyl-1-butyne, KI, Cs_2CO_3; (e) H2, Pd/$CaCO_3$, quinoline; (f) 200 °C; (g) Pd[$(CH_3CN)_4(BF)_2$], benzoquinone; (h) OsO_4, NMO; (i) separation of diastereoisomers (column chromatography); (j) *tert*-butyldimethylsilyl chloride, imidazole, 4-dimethylaminopyridine; (k) TBAF; (l) MsCl, pyridine; (m) K_2CO_3, 18-crown-6.

nycine diesters toward nucleophilic agents was similar to that postulated for acronycine epoxide, the diacetate (±)-**79** was reacted with benzylmercaptan in acidic medium. As expected, the two isomeric *cis* and *trans* adducts (±)-**83** and (±)-**84**, with the sulfur atom linked to the benzylic position of the pyranyl ring, were obtained in almost quantitative yield (Scheme 5).[63] When tested for inhibition of L1210 cell proliferation in vitro, diesters (±)-**79** and (±)-**81** were found to be ca. 5-fold more potent than acronycine (**10**), while the most cytotoxic derivative, the cyclic carbonate (±)-**82**, was 75-fold more potent than acronycine in inhibiting L1210 cell proliferation. Two experimental models were used to test in vivo the *cis*-1,2-dihydroxy-1,2-dihydroacronycine diesters, the ip P388 leukemia and the sc colon 38 adenocarcinoma. The results are summarized in Table 1, in terms of percent T/C (survival or tumor volume) obtained at the optimal dosage. Against P388 leukemia, acronycine (**10**) was moderately active, while compounds (±)-**79**, (±)-**81**, and (±)-**82** were markedly active, at doses 4- to 16-fold lower. Against the colon 38 adenocarcinoma, compounds (±)-**81** and (±)-**82** were efficient, but the diacetate (±)-**79** was the most active, with all treated mice being tumor-free on day 23.[63] Acronycine was also active with this model, but less than (±)-**79**, and at a-16 fold higher dose.[65] In order to study the influence of the stereochemistry at C-1 and C-2 on the activity, the *trans*-diacetate, (±)-**85**, was prepared by acetylation of the *trans*-diol, (±)-**76**.[64] When evaluated in vivo against P388 leukemia, (±)-**85** was found to be more active than acronycine (**10**), but less active than the *cis*-diacetate (±)-**79**.[64] The influence of the configuration at C-1 and C-2 was explored only in the more active *cis* series.

Scheme 4. Synthesis of Conformationally Restricted Analogues of Prorospermin on the Model of 5-Hydroxyisopsorofebrin (**31**)[52,a]



[a] Reagents: (a) TBHP, SeO_2, AcOH; (b) $K_3Fe(CN)_6$, aq KOH; (c) OsO_4, NMO, 18-crown-6; (d) (i) MsCl, pyridine, (ii) K_2CO_3, 18-crown-6; (e) (i) MsCl, pyridine, (ii) TBAC.

Scheme 5. Reaction of Diacetate (±)-**79** with Benzylmercaptan

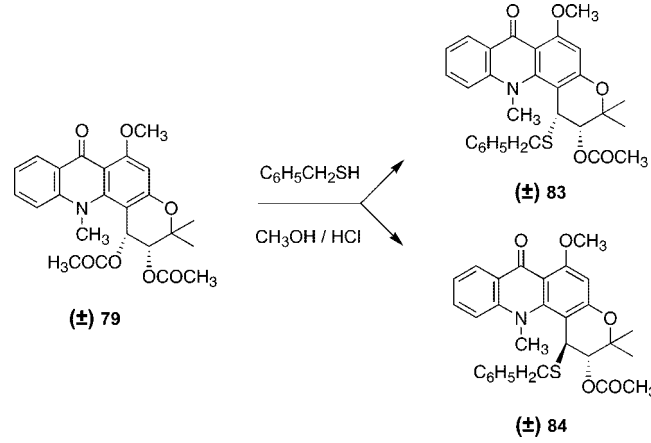
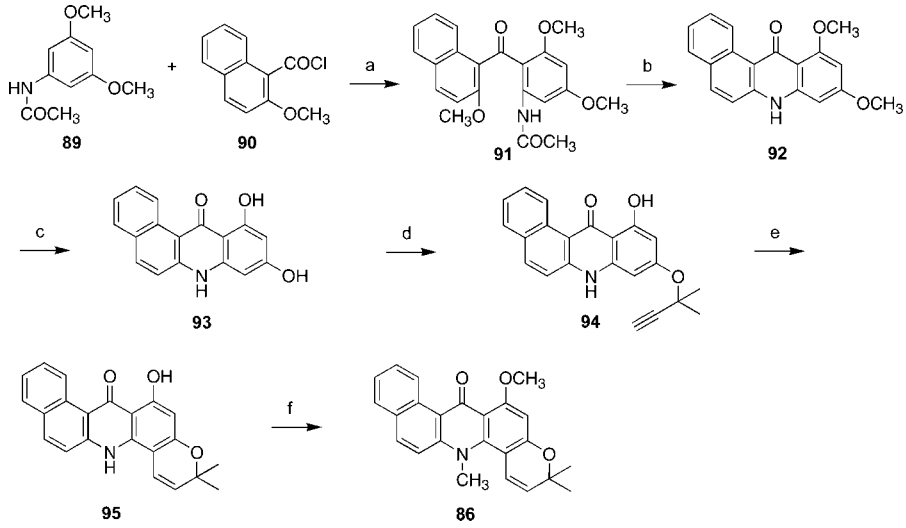


Table 1. Antitumor Activity (ip) of 1,2-Dihydroxy-1,2-dihydroacronycine Diesters in Comparison with Acronycine (**10**) at the Optimal Dosages[63,64]

	10	(±)-**79**	(±)-**81**	(±)-**82**	(±)-**85**
T/C	125	289	201	202	181
P388 (survival)	200 mg/kg	25 mg/kg	50 mg/kg	12.5 mg/kg	12.5 mg/kg
T/C	4	0	13	68	
C38 (tumor growth)	200 mg/kg	12.5 mg/kg	12.5 mg/kg	6.25 mg/kg	not tested

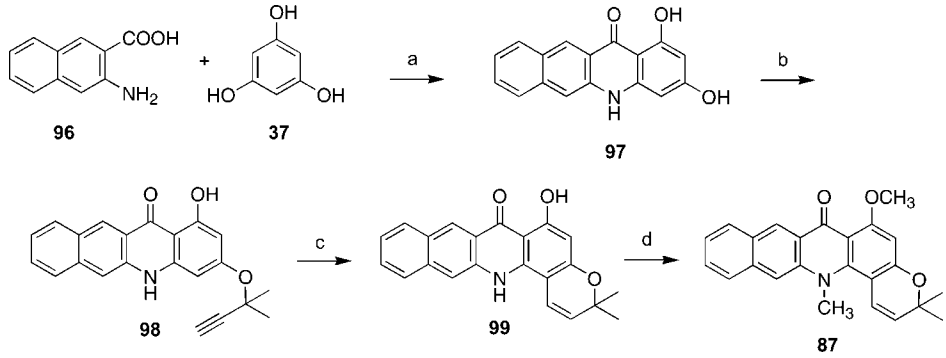
Sharpless osmium-catalyzed asymmetric dihydroxylation of acronycine (**10**) permitted the enantioselective synthesis of (1*R*,2*R*)-1,2-dihydroxy-1,2-dihydroacronycine and (1*S*,2*S*)-1,2-dihydroxy-1,2-dihydroacronycine.[66] Each enantiomer was purified using chiral high-performance liquid chromatography. The corresponding (1*R*,2*R*)-1,2-diacetoxy-1,2-dihydroacronycine and (1*S*,2*S*)-1,2-diacetoxy-1,2-dihydroacronycine were obtained subsequently by acetylation of the corresponding *cis*-diols. When tested in vitro on L1210 leukemia cells, no significant difference in cytotoxicity was observed between

Scheme 6. Synthesis of Benzo[*a*]acronycine (**86**)[71,a]



[a] Reagents: (a) $AlCl_3$; (b) NaH, DMF; (c) HBr, AcOH; (d) 3-chloro-3-methyl-1-butyne, KI, K_2CO_3; (e) DMF, 130 °C; (f) NaH, Me_2SO_4.

Scheme 7. Synthesis of Benzo[*b*]acronycine (**87**)[73,a]



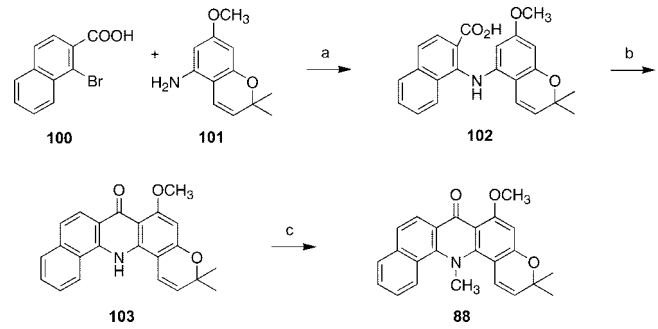
[a] Reagents: (a) *p*-TsOH; (b) 3-chloro-3-methyl-1-butyne, KI, K_2CO_3, DMF; (c) 130 °C; (d) NaH, Me_2SO_4.

the two (1*R*,2*R*)- and (1*S*,2*S*)-enantiomers and the (±)-(1*R**,2*R**)-*cis*-racemate.[66]

An investigation of the DNA-binding property of acronycine (**10**) suggested that the natural alkaloid should interact with DNA by some process able to stabilize the double helix against thermal denaturation.[67] Interaction with DNA is known to occur mainly for compounds with sufficiently large coplanar aromatic chromophores, as demonstrated in the case of numerous antitumor drugs currently used in the clinic, such as doxorubicin, camptothecine, or ellipticine derivatives. The assumption of a step involving DNA interaction in the mode of action of acronycine led to the development of structural analogues with an additional aromatic ring fused onto the natural alkaloid basic skeleton[68] in the three isomeric benzo[*a*]acronycine (**86**), benzo[*b*]acronycine (**87**), and benzo[*c*]acronycine (**88**) series (Schemes 6−8).

The general approach used to build the pentacyclic core of benzo[*a*]acronycine (**86**) (Scheme 6) was inspired by the biomimetic synthesis of acronycine developed by Lewis and co-workers, which involved base-catalyzed cyclization of an intermediate diphenylketone to construct the acridone skeleton.[69,70] Friedel−Crafts reaction of 3,5-dimethoxyacetanilide (**89**) with 2-methoxy-1-naphthoyl chloride (**90**) gave 2-methoxy-1-naphthyl-(6-acetamido-2,4-dimethoxy)phenylketone (**91**), which was cyclized to 9,11-dimethoxybenzo[*a*]acridin-12(7*H*)-one (**92**) by the use of sodium hydride in dimethylformamide. Demethylation of **92** with hydrogen bromide in acetic acid gave 9,11-dihydroxybenzo[*a*]acridin-12(7*H*)-one (**93**). Alkylation of **93** with 3-chloro-3-methyl-1-butyne gave the dimethylpropargyl ether **94**. Claisen rearrangement of **94** afforded 6-hydroxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*a*]pyrano[3,2-*h*]acridin-7-one (**95**), which was further methylated with dimethyl sulfate in the presence of sodium hydride to give the desired benzo[*a*]acronycine (**86**).[71]

Scheme 8. Synthesis of Benzo[*c*]acronycine (**88**)[77,a]



[a] Reagents: (a) $Cu(OAc)_2$, KOAc, Et_3N; (b) $(CF_3CO)_2O$; (c) 130 °C; (d) MeI, K_2CO_3.

The strategy employed to synthesize benzo[*b*]acronycine (**87**) (Scheme 7) was similar to that previously developed by Hlubucek and co-workers for the synthesis of acronycine.[72] Condensation of 3-amino-2-naphthalenecarboxylic acid (**96**) with phloroglucinol (**37**) furnished 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**97**). Treatment of **97** with 3-chloro-3-methyl-1-butyne gave 6-hydroxy-3,3-dimeth-

Table 2. Antitumor Activity (ip) of 1,2-Dihydroxy-1,2-dihydrobenzo[*b*]acronycine Diesters in Comparison with Acronycine (**10**) at the Optimal Dosages[73]

	10	(±)-**109**	(±)-**115**
T/C	125	327	213
P388 (survival)	200 mg/kg	12.5 mg/kg	12.5 mg/kg
T/C	4	4	18
C38 (tumor growth)	200 mg/kg	6.25 mg/kg	3.12 mg/kg

yl-3,14-dihydro-7*H*-benzo[*b*]pyrano[3,2-*h*]acridin-7-one (**99**), through Claisen rearrangement of the corresponding intermediate dimethylpropargyl ether **98**. Methylation of **99** with dimethyl sulfate gave benzo[*b*]acronycine (**87**).[73]

Construction of the pentacyclic basic core of benzo[*c*]acronycine (**88**) (Scheme 8) followed the strategy previously employed for the syntheses of acronycine,[74] 6-demethoxyacronycine,[75] and 6-demethoxybenzo[*b*]acronycine,[76] through Ullmann condensation of an aminochromene with an appropriate *ortho*-haloaromatic acid, followed by cyclization of the intermediate carboxylic diarylamine to the corresponding acridone under acidic conditions. Condensation of 1-bromonaphthalene-2-carboxylic acid (**100**)[77] with 7-methoxy-2,2-dimethyl-2*H*-1-benzopyran-5-ylamine (**101**)[78] gave the corresponding carboxylic diarylamine, **102**, on which cyclization to 6-methoxy-3,3-dimethyl-3,14-dihydro-7*H*-benzo[*c*]pyrano[3,2-*h*]acridin-7-one (**103**) was performed through the use of trifluoroacetic anhydride. Treatment of **103** with methyl iodide and potassium carbonate in acetone gave benzo[*c*]acronycine (**88**).[78]

When tested in vitro against L1210 cell proliferation, both benzo[*a*]acronycine (**86**) (IC_{50} 2.5 μM) and benzo[*b*]acronycine (**87**) (IC_{50} 1.9 μM) were found to be ca. 4−5-fold more potent than acronycine (**10**) (IC_{50} 10.4 μM), whereas benzo[*c*]acronycine (**88**) (IC_{50} 12.1 μM) displayed only marginal activity. In the most active benzo[*a*]acronycine and benzo[*b*]acronycine series, the *cis*-diols (±)-**104** and (±)-**105** were prepared by catalytic osmium oxidation of **86** and**87**, respectively.[71,73] Treatment of the diols with an excess of an appropriate acylating agent (acid anhydride or acyl halide) afforded the corresponding diesters, exemplified by **106**−**108** in the benzo[*a*]acronycine series and **109**−**111** in the benzo[*b*]acronycine series, respectively. Under controlled conditions, monoesters at the less hindered 2-position were obtained. A second acylation reaction performed on those monoesters gave access to mixed diesters, such as **112** and **113**. Treatment of diols (±)-**104** and (±)-**105** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux afforded the cyclic carbonates **114** and**115**, respectively.[71,73,79] When the 1,2-dihydroxy-1,2-dihydrobenzo[*a*] and -[*b*]acronycine diesters **106**−**115** were evaluated in vitro for cytotoxicity against the murine L1210 leukemia and the KB-3-1 human epidermoid carcinoma cell lines, all compounds were found to be significantly more potent (ca. 3−10-fold) for the solid tumor KB-3-1 cell line than for L-1210 leukemia. In most cases, diesters in the benzo-[*b*]acronycine series were more potent than their benzo[*a*]acronycine counterparts against the KB-3-1 cell line. Compared to acronycine (**10**), compounds **106**−**115** were markedly more potent, with the most cytotoxic derivative, cyclic carbonate **115** (IC_{50} 0.014 μM for L-1210, 0.005 for KB-3-1), being 1000-fold more potent than acronycine in inhibiting cell proliferation.

The most interesting compounds, diacetate (±)-**109** and cyclic carbonate (±)-**115**, both belonging to the benzo[*b*]acronycine series, were tested in vivo against two standard experimental models, the sensitive ip P388 leukemia and the more resistant sc colon 38 adenocarcinoma.[73] The results, in terms of percent T/C obtained at the dose giving the best therapeutic effect without toxicity, are summarized in Table 2. Against P388 leukemia, acronycine (**10**) was only marginally active, while compounds **109** and **115** were significantly active at doses 16-fold lower. Against the colon 38 adenocarcinoma, **109** and **115** were highly efficient, inhibiting by more than 80% the tumor growth. The diacetate (±)-**109** was the most active, since the tumor growth was inhibited by 96% at 6.25 mg/kg, and two mice out of seven were tumor-free on day 43.[73] Due to its favorable pharmacological profile, the most promising compound, diacetate (±)-**109**, was selected for further development under the code S23906-1. In order to define the efficacy of this compound toward human cancers, experiments involving aggressive models of human ovarian and lung carcinomas were conducted. Against the ovarian (IGROV-1 and NIH:OVCAR-3) and non-small cell lung tumors (NCI-H460 and A549), (±)-**109**, administered twice by the iv route at 1.56−6.25 mg/kg, increased the survival of tumor-bearing mice in a dose-dependent manner, being curative in the NIH:OVCAR-3 model, and was as efficient as paclitaxel, used as a reference compound. Against IGROV-1 and NCI-H460 tumors, (±)-**109** administered at 6.25 mg/kg induced T/C values of 193% and 162%, respectively.[80,81]

The mechanism of action of S23906-1 (**109**) at both cellular and molecular levels was studied, due to its promising antitumor activity. At the cellular level, (±)-**109** induced an irreversible S-phase blockade of the cell cycle and efficiently triggered apoptosis in several cancer cell types.[68,73,82−84] It also caused irreversible inhibition of DNA synthesis. A short treatment with (±)-**109** was sufficient to induce an increase of the cyclin E level in HT-29 cells, which were arrested in the S-phase and subsequently underwent apoptosis.[82] At the molecular level, (±)-**109** and other 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters were shown to bind covalently with both purified DNA fragments and genomic DNA extracted from treated tumor cells.[76,85] The base selectivity for alkylation was determined by the use of synthetic duplex oligonucleotides containing a unique set of guanine-cytosine (G·C), adenine-thymine (A·T), or inosine-cytosine (I·C) base pairs. Adducts were characterized by polyacrylamide gel shift retardation and/or a fluorescence assay. These experiments demonstrated that 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters readily alkylated G·C base pairs, but did not alkylate A·T and I·C base pairs. The I·C base pair differs from the G·C base pair only by the lack of the NH_2 group at position 2 of guanine, which is exposed in the minor groove of DNA. Therefore, 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters should be considered as specific alkylating agents of the exocyclic NH_2 group of the guanine units. Study of the structure−activity relationships indicated a strong correlation between DNA alkylation by the various 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters and their respective cytotoxic potential. Additional evidence for guanine alkylation was obtained by mass spectrometry of the adducts obtained using short 7-base pair hairpin oligonucleotides with selected diesters. The adducts of highest molecular weight observed corresponded to the addition of one molecule of drug and to the loss of one acyloxy group when the oligonucleotide contained one guanine unit, and to the addition of three molecules of drug and to the loss of three acyloxy groups when the oligonucleotide contained three guanine units. Involvement of the ester group at position 1 of the drug in the alkylation of DNA guanine units was deduced from the structure−activity relationships in the benzo[*b*]acronycine series. Indeed, all 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters that were tested exhibited both DNA alkylating and cytotoxic properties. In contrast, structural analogues without an ester leaving group at position 1, such as 2-acetoxy-1,2-dihydrobenzo[*b*]acronycine (**116**) or *cis*-2-acetoxy-1-methoxy-1,2-dihydrobenzo[*b*]acronycine (**117**), were inert toward DNA alkylation and devoid of significant cytotoxic properties.[76,84] The covalent binding to DNA of 1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine diesters such as **109** was shown to induce a marked destabilization of the double helix, with the formation of single-stranded DNA. Therefore, alkylation of guanines in DNA by dihydrobenzo[*b*]acronycine derivatives leads to a local denaturation of DNA, a consequence strikingly different from those observed with other antitumor drugs, including the N-2 guanine alkylating agent ecteinascidin 743, which stabilize duplex DNA.[86,87]

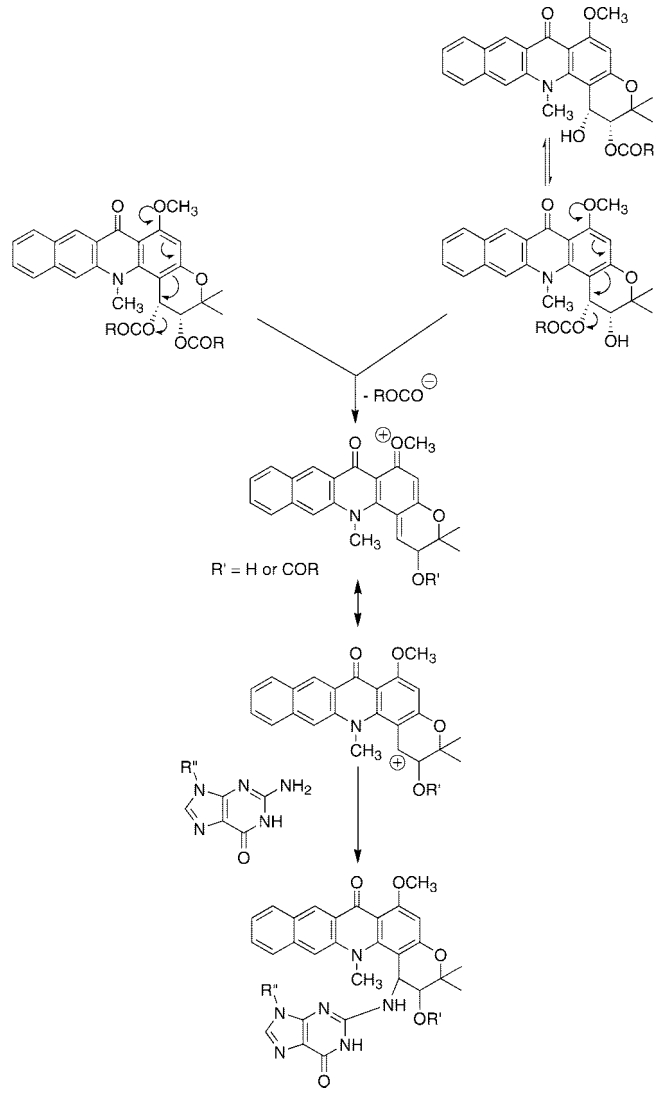
In the course of studying the structure−activity relationships, *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine monoesters at position 2, exemplified by acetate **118**, isovalerate **119**, butanoate **120**, and pentenoate **121**, showed cytotoxic activities within the same order of magnitude as the corresponding diesters, despite the lack of an ester leaving group at position 1.[76] Also, in vivo antitumor activities similar to that of *cis*-diacetate **109** were observed with monoesters **118**−**121**. Gel retardation experiments performed on monoesters **118**−**121** demonstrated that these compounds were able to link covalently to DNA. Mass spectrometric analysis revealed that the molecular weight of the adducts obtained with 7-base pair hairpin oligonucleotides differed by one acyl group per alkylated guanine residue from those of the heaviest adducts obtained with the corresponding diesters. These results suggested strongly that *cis*-monoesters at position 2 can lead spontaneously to the isomeric more reactive *cis*-monoesters at position 1, by a transesterification process. Indeed, this reaction could explain the similar reactivity observed in both series and the difference of molecular mass in the adducts obtained with DNA. Experimental evidence for spontaneous transesterification was obtained through NMR study of *cis*-monoacetate **118**. When kept at 20 °C in DMSO containing 15% D_2O, the ^1H NMR data of **118** evolved, revealing an equilibrium between the 2-monoacetate **118** and the 1-monoacetate **122**. A stable 80:20 equilibrium between **118** and **122** was obtained within 48 h at room temperature and did not evolve further when the duration of the experiment was increased.[76,88] The mechanism of action at the molecular level of *cis*-1,2-dihydroxy-1,2-dihydrobenzo[*b*]acronycine esters is outlined in Scheme 9.

In terms of structure−activity relationships, the presence of the fused dimethylpyran privileged structure appears to be an indispensable structural requirement to observe significant cytotoxic activity in both the acronycine and benzo[*b*]acronycine series. Indeed, simplified analogues lacking the fused pyran ring, but possessing an acetoxymethyl leaving group at position 4, as exemplified by **123** and **124**, displayed only marginal antiproliferative activity compared to the parent compounds.[89]

Hybrid Compounds: Pyranoxanthones and Epoxyfuroacridones

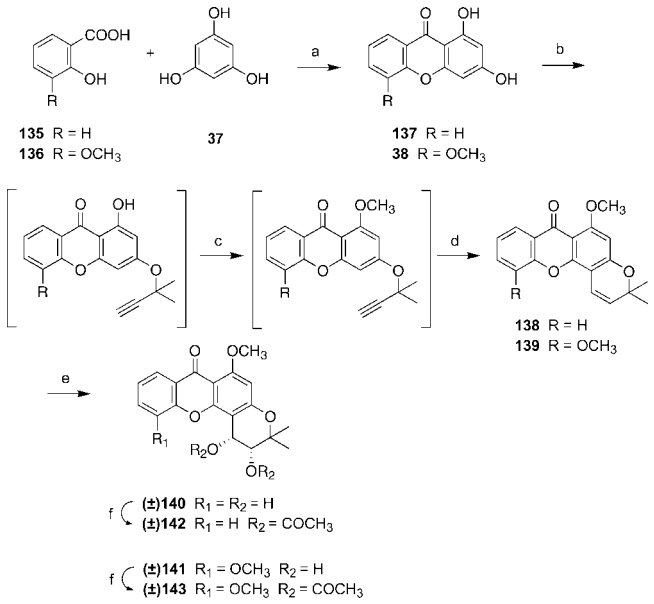
In the search for novel antitumor candidates, it was considered of interest to prepare hybrid compounds between the psorospermin (**9**) and the acronycine (**10**) series. The isosteric replacement of the acridone nitrogen atom of acronycine and benzoacronycine by an oxygen on one hand and of the xanthone oxygen of psorospermin by a nitrogen on the other hand was envisaged. It should be emphasized that both the pyrano[3,2-*c*]xanthen-7-one and furano[3,2-*c*]acridin-6-one basic cores constitute the backbone of natural products. Most naturally occurring pyrano[3,2-*c*]xanthones, exemplified by formoxanthone B (**125**) isolated from *Cratoxylum formosum* (Jack) Benth. & Hook. f. (Guttiferae),[90] bear prenyl substituents on the basic chromophore. Some of them have been reported to display cytotoxic activity. Cudratricusxanthone (**126**) isolated from the roots of *Cudrania tricuspidata* (Carr.) Bur. (Moraceae) exhibited significant antiproliferative activity against several human digestive apparatus tumor cell lines (HCT-116, SMMC-7721, and SGC-7901), with IC_{50} values within the 4−10 μM range.[91] The angular pyranoxanthone **127**, which was isolated from the same source, exhibited marginal cytotoxic activity against several human cancer cell lines.[92] In the furano[3,2-*c*]acridone series, several alkaloids, which were first isolated from *Ruta graveolens* L. (Rutaceae), possess substituents on the fused furanic system identical with those encountered in furanoxanthones isolated from *Psorospermum febrifugum*.[93] Rutacridone (**128**)[94] and its epoxide (**129**),[95] for which the absolute (2′*R*) and (2′*R*,3′*R*) (psorospermin numbering) configurations were recently established using a combination of CD and NMR techniques,[96] include the same furanoid moieties as 3′,4′-deoxypsorospermin (**18**) and psorospermin (**9**), respectively. Other furano[3,2-*c*]acridones isolated from *Ruta graveolens* include the chlorohydrin isogravacridonchlorine (**130**),[97] the diol gravacridondiol (**131**),[98] the ether gravacridondiol monomethyl ether (**132**),[98] the epoxy alcohol hydroxyrutacridone epoxide (**133**),[99] the triol gravacridontriol (**134**),[100] and several related esters and glycosides. When tested for their cytotoxic activity against three human cancer cell lines (HeLa, MCF-7, and A431), alkaloids **128**, **131**, and **132**, although devoid of epoxide or chlorohydrin alkylating groups, were significantly active, with IC_{50} values within the 3−20 μM range, whereas triol **134** displayed only marginal activity.[101]

Scheme 9. Mechanism of Action of S-23906 and Related Compounds



Xanthone counterparts of acronycine and 1,2-hydroxy-1,2-dihydroacronycine dieters, bearing no sustituent or a methoxy group at C-11, were prepared from the corresponding 1,3-dihydroxyxanthen-9-one (**137**) and 1,3-dihydroxy-5-methoxyxanthen-9-one (**38**), obtained by Ulmann condensation of phloroglucinol (**37**) with 2-hydroxybenzoic acid (**135**) or 2-hydroxy-3-methoxybenzoic acid (**136**), respectively (Scheme 10).[102] Conversion of **137** and **38** into the corresponding dimethylpropargyl ethers, followed by methylation and Claisen rearrangement, afforded **138** and **139**, accompanied by their linear regioisomers. *cis*-Dihydroxylation of the pyran double bond by catalytic osmic oxidation gave the racemic diols (±)-**140** and (±)-**141**, which were converted into the corresponding acetates (±)-**142** and (±)-**143**.[102] A similar strategy starting from 2-hydroxy-1-naphthalenecarboxylic acid (**144**) permitted the preparation of the isostere of benzo[*a*]acronycine, 6-meth-

Scheme 10. Synthesis of Pyrano[3,2-*c*]xanthone Counterparts of Acronycine[97,a]

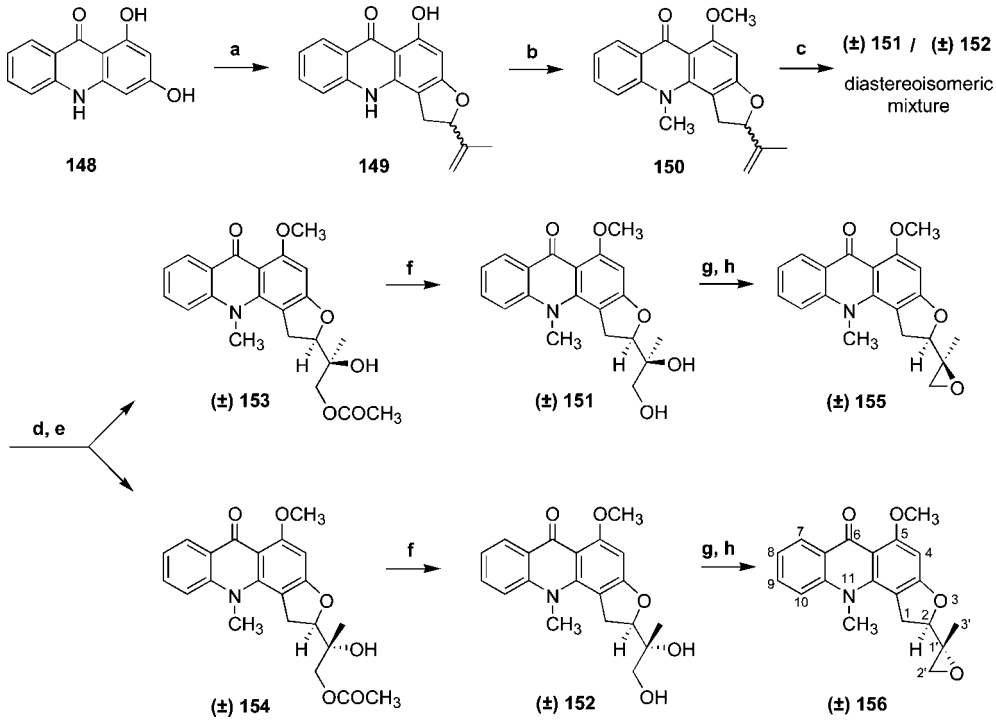


[a] Reagents: (a) $ZnCl_2$, $POCl_3$; (b) 3-chloro-3-methyl-1-butyne, CuI, NaI, K_2CO_3, DMF; (c) NaH, Me_2SO_4, THF; (d) 210 °C; (e) OsO_4, NMO; (f) Ac_2O, pyridine.

oxy-3,3-dimethyl-3*H*,7*H*-benzo[*a*]pyrano[2,3-*i*]xanthen-7-one (**145**), and the corresponding *cis*-diol **146** and diacetate **147**.[103] Surprisingly, diesters **142** and **143** were found to be inactive when tested against the proliferation of L1210 leukemia cells, whereas unsaturated compounds **138**, **139**, and **145** and diester **147** were only marginally active.[102,103]

Analogues of psorospermin (**9**) in the furo[2,3-*c*]acridin-6-one and benzo[*b*]furo[3,2-*h*]acridin-6-one series were prepared with the aim of determining the respective influence of the acridone or xanthone basic core on one hand and the fused pyran or furan unit on the other hand on cytotoxic properties and effects on DNA alkylation.[104] In both series, construction of the fused 2-isopropenyl-2,3-dihydrofuran system was envisioned through treatment of a phenol precursor with (*E*)-1,4-dibromobut-2-ene ("dibromoisoprene") in alkaline medium,[105] since this method had previously given satisfactory results in the synthesis of the related natural alkaloid rutacridone.[106−108] Reaction of 1,3-dihydroxyacridone (**148**)[72] with (*E*)-1,4-dibromo-2-methylbut-2-ene gave (±)-2-isopropenyl-5-hydroxy-1,2-dihydro-11*H*-furo[2,3-*c*]acridin-6-one (**149**), which was methylated to **150**. Catalytic osmium tetroxide oxidation of **150** led to a diasteroisomeric mixture of racemic (2*R**,1′*S**) (**151**) and (2*R**,1′*R**) (**152**) diols, which could be conveniently separated by OPLC on a preparative scale after conversion into the corresponding monoacetates **153** and **154**. After regeneration of the diols, mesylation followed by alkaline treatment gave the desired (2*R**,1′*S**) and (2*R**,1′*R**) epoxides, **155** and **156** (Scheme 11).[104] A similar reaction sequence, starting from 1,3-dihydroxybenz[*b*]acridin-12(5*H*)-one (**97**), gave an efficient entry to the corresponding (2*R**,1′*S**) and (2*R**,1′*R**) epoxides, **157** and **158**. When evaluated for their cytotoxicity against the murine leukemia cell line L1210 and the human epidermoid carcinoma cell line KB-3-1, a dramatic difference of potency was observed between the two diastereoisomeric epoxides in the furo[2,3-*c*]acridin-6-one series. Indeed, the (2*R**,1′*S**) isomer **155** exhibited cytotoxic properties with micromolar IC_{50} values against both cell lines, whereas the (2*R**,1′*R**) epoxide **156** was found to be nearly 100-fold more potent in inhibiting cell proliferation.[104] These results were consistent with those obtained for O^5-methylpsorospermin, whose (2′*R**,3′*R**) diasteroisomeric pair, containing the naturally occurring (2′*R*,3′*R*) epoxyfuroxanthone, was much more active than the isomeric (2′*S**,3′*R**) pair when tested against a wide range of tumor cells.[47] In the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series, both epoxides **157** and **158** exhibited IC_{50} values in the 10−100 nM range. The (2*R**,1′*S**) isomer was the more potent, but the

Scheme 11. Synthesis of Furo[2,3-*c*]acridin-6-one Analogues of Psorospermin[104,a]



[a] Reagents: (a) (*E*)-1,4-dibromobut-2-ene, K_2CO_3; (b) Me_2SO_4, NaH, DMF; (c), OsO_4, NMO; (d) Ac_2O, pyridine; (e) separation of diastereoisomers (OPLC); (f) K_2CO_3, MeOH; (g) MsCl, pyridine; (h) K_2CO_3, 18-crown-6.

difference between the cytotoxic activities of the two diastereoisomers was much less important than that observed in the 11*H*-furo[2,3-*c*]acridin-6-one series.[104] The most potent epoxide in the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series, **158**, was shown, using gel shift assays, to form covalent complexes with DNA and to classically stabilize the DNA helix upon binding. Compound **158** induced DNA cleavage at guanine positions. Experiments conducted with synthetic oligonucleotides containing a single type of base pairs showed that bonding essentially occurred with G·C and, to a weaker extent, with A·T and I·C base pairs. Further insight in the base recognition was established by incubating **158** with hairpin oligonucleotides containing either guanines or 7-deaza-guanines as targets. Strong alkylation of DNA containing guanines but not 7-deaza-guanines gave evidence that the N-7 position of guanines in the major groove was the target of **158**.

Conclusions

The epoxyfuranoxanthone psorospermin (**9**) isolated from the African plant *Psorospermum febrifugum* apprears as a promising agent, active against drug-resistant leukemia lines and AIDS-related lymphoma. Its mechanism of action implies interaction with DNA, through its exocyclic oxirane group, which binds covalently to the N-7 position of the guanine units in the major groove. This activity is dramatically enhanced in the presence of topoisomerase II, since in the absence of this enzyme alkylation is both weak and unspecific. Structure−activity relationship studies have emphasized the importance of the natural (2′*R*,3′*R*) stereochemistry to observe optimum DNA alkylation and antitumor activity.

The pyranoacridone acronycine (**10**), isolated from the Australian and New-Caledonian shrub *Sarcomelicope simplicifolia*, exhibits antitumor properties against a large panel of solid tumors, but only marginal activity against leukemia. The moderate potency and extremely low water solubility of this drug severely hampered the subsequent clinical trials. A hypothesis of bioactivation of the 1,2-double bond of acronycine into the corresponding oxirane in vivo led to the development of potent structural analogues, exemplified by *cis*-1,2-diacetoxy-1,2-dihydrobenzo[*b*]acronycine (**109**), which recently underwent phase I clinical trials under the code S23906-1. Its mechanism of action implies alkylation of the 2-amino group of DNA guanine residues in the minor groove by the carbocation resulting from the elimination of the ester leaving group at position 1 of the drug, followed by a marked destabilization of the double helix, with the formation of single-stranded DNA.

Hybrid compounds associating the acridone or benzo[*b*]acridone chromophore of acronycine derivatives and the epoxyfuran alkylating unit present in psorospermin also displayed very potent antiproliferative activities. Interstingly, these compounds alkylate DNA guanine units at position N-7 in the major groove. From a structure−activity viewpoint, the ester-pyran or epoxy-furan pharmacophore appears to play a crucial role in the site of guanine DNA alkylation. Epoxides in the 13*H*-benzo[*b*]furo[3,2-*h*]acridin-6-one series alkylate DNA at the same guanine N-7 position as natural xanthones belonging to the psorospermin series.

These results emphasize the application to natural products chemistry of the concept of privileged structures initially developed by Evans for synthetic benzodiazepines and benzazepines.[109] Indeed, both isopropylfuran and dimethylpyran fused systems present in natural secondary metabolites are rigid units, able to orient substituents in a defined three-dimensional space.

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