Synthesis and Structure−Activity Relationships of New Benzodioxinic Lactones as Potential Anticancer Drugs

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A set of disubstituted tetracyclic lactones has been synthesized and tested for potential antitumor activity. Several of them possess a noticeable cytotoxicity against L1210 and HT-29 colon cells *in vitro*. Relationships between chain nature and biological properties were sought. Lactones with a pentyl or hexyl substituent at C-11 are the most active ones. The introduction of a functional group at the side chain of C-11 modified the potency; carboxylic acid and primary amine decreased the cytotoxicity, whereas a cyano group increased the activity. An extensive structure−activity relationship study of these derivatives, including carbon homologues and bioisosteres has been performed. The synthesis and cytotoxicity of these compounds are discussed. Two lactones are recognized as potential lead compounds.

Introduction

Anticancer drugs have been classified according to the origin or the mechanism of action involved as antitumor antibiotic, antimetabolites, antitubulin agents, topoisomerase inhibitors, alkylating agents, and miscellaneous agents.[1] In recent years, difficulties in classifying new antitumoral compounds have arisen. Several of them exhibit multiple mechanisms of action in tumor cells and many demonstrate a wide range of biochemical and pharmacological properties. Major progress in chemotherapy requires new and useful antitumor agents to eradicate the entire range of cancer diseases in the world over. New compounds can be developed by a wide variety of approaches, ranging from empirical screening to rational design: (a) from natural products, (b) by screening of synthetic compounds prepared for other purposes, and (c) by semisynthesis or total synthesis using new biological concepts and structure−activity relationships of potentially interesting known compounds.[2]

It has been established that ellipticine, a drug isolated from *Ochrosia elliptica* (*Apocinaceae*), has powerful antiproliferative properties. Its chemical structure consists in a tetracyclic skeleton composed of pyridine fused with the carbazole system (subunits X and Y).[3] Ellipticine and related compounds exert their cytotoxic and antineoplastic effects by a multimodal mechanism of biological action (inhibition of DNA-topoisomerase II, DNA-intercalation, covalent binding to DNA, and redox generation of cytotoxic free radicals).[4] Several quaternary ellipticinium salts (datelliptium, pazellipticine, and others) were previously reported to possess high cytotoxic activity.[5] Datelliptium and pazellipticine bearing hydrophilic substituents on a pyridine ring which increase water solubility were pursued as targets for synthesis (Chart 1).[5]

These antitumor compounds are clinically effective, albeit they might cause serious adverse reactions. The toxicity of the great majority of the antitumor compounds, both alone and in combination with other antitumor agents, is a reason for their restricted deployment. These problems motivate the research of new compounds. There has been an enormous amount of effort directed toward the research of new anticancer agents, related or not, to known compounds and the elucidation of their structure−activity relationships.

The presence of polycyclic lactones in a wide variety of antineoplastic compounds of natural or synthetic origin[6] induced us to introduce this structural motif in a new research line. It is well documented that compounds possessing a butyrolactone fused to a polycyclic system, such as podophyllotoxin[7] or their semi-synthetic analogues etoposide and teniposide,[8] show an interesting cytotoxic and antitumor activity.

The relationships between structure and cytotoxicity from inhibition of topoisomerase II are not yet well established, and the research of further classes of new antitumor agents remains of high interest. We have previously studied 1,4-benzodioxino-isoquinolines designed and synthesized as related analogues to the ellipticine.[9] They are formally derived by substitution of the indole with a 1,4-benzodioxine subunit. This substitution leads to analogues that retain a noticeable *in vitro* cytotoxic activity. Structurally, they possess a basic nitrogen atom suitable for the DNA-intercalation. At a physiological pH, these compounds are ionized and it is expected as a limitation of their applicability. The present investigation was undertaken to determine the effect of introducing a nonbasic lactone instead of a pyridine. Therefore, we decided to further investigate a novel tetracyclic system consisting on an 1,4-benzodioxine skeleton fused with a benzofuranone, to form the new isobenzofuro[5,6-*b*][1,4]benzodioxines depicted in Chart 2. The aim of the present study was to establish new structure requirements for these new compounds and determine structural tolerances for cytotoxic activity in cancer cell cultures.

We focused our attention on the lactone due to its appealing antitumor activity for epipodophyllotoxins, such as etoposide, teniposide,[8] and other topoisomerase II inhibitors containing this moiety. The 1,4-benzodioxine, whose synthesis has been developed by us, was also chosen as a heterocyclic bioisostere

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Chart 1. Chemical Structures of Ellipticine, Datelliptium, and Etoposide
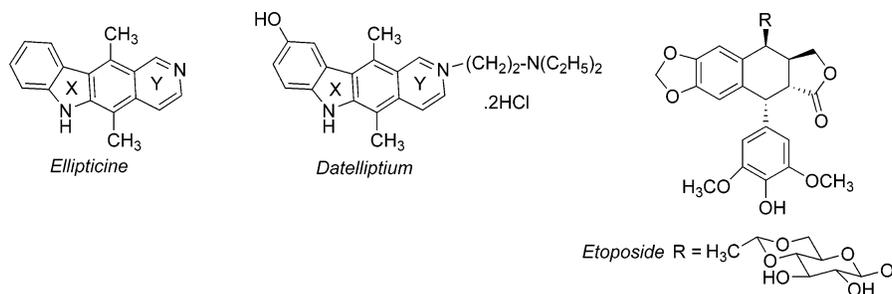
Chart 2. Design of Compounds **1−19** from Ellipticine
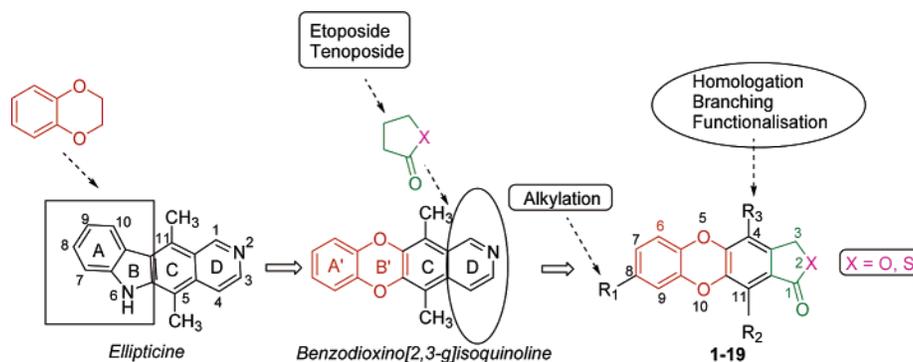
of indole.[9] Structure−activity relationship studies and theoretical determination of relevant parameters to their cytotoxic activity were very useful in the design and development of new compounds.

To our knowledge, the isobenzofuro[5,6-*b*][1,4]benzodioxine system has not previously been incorporated into an antitumor structure. Several modifications maintaining the same backbone were carried out, as those indicated in Chart 2: (a) modification of the R_2 and R_3 substituents, (b) modifications on the lactone ring, and (c) alkylation of the 1,4-benzodioxine ring.

Chemistry

Our initial task was to establish the most efficient method for performing the synthesis of the tetracyclic lactones (**1**, **2a−8a**, **2b−8b**) from the carboxylic acids **20** and **21**. The results are summarized in Schemes 1−3.

Treatment of 1,4-benzodioxin-6-yl carboxylic acids (**20** and **21**) with LDA and subsequent addition of the corresponding aldehyde allowed the preparation of the desired hydroxyacids (**22−34**). After the ring closure of the hydroxyacids (**22−34**) using PTSA (*p*-toluenesulfonic acid monohydrate) as catalyst in toluene at reflux led to the lactones (**35−47**) in very high yields.[10−11]

The intermediate lactones (**35−47**) were treated with alkyllithium followed by the addition of trimethylsilylchloride at −78 °C to give the furo-derivatives (**48−62**). These dienes were relatively stable at low temperatures but unstable at room temperature.[12]

Finally, the treatment of the furo[3,4-*b*][1,4]benzodioxines (**48−62**) with the lactone (**63**) or the thiolactone (**64**) in acidic media (Yb(OTf)$_3$, BF$_3$.Et$_2$O, SnCl$_4$, or TiCl$_4$) gives a mixture of tetracyclic lactones *a* and *b* in acceptable yields. Surprisingly, the expected Diels−Alder mixture of *exo-endo* adducts was not detected by reason of the facile cyclodehydration catalyzed by Lewis acid of the intermediate 1,4-epoxy-derivatives to the aromatic compounds. Aromatization of similar adducts by dehydration was generally effected by using catalytic amounts of acid.[13] Several Lewis acids were tested as catalysts in the

cycloaddition of the diene **48** and the 2(5*H*)-furanone at 90 °C. The results shown in the Table 1 indicate that Yb(OTf)$_3$ is the best catalyst for this cycloaddition reaction. When TiCl$_4$ as a typical Lewis acid was used in CH$_2$Cl$_2$, the reaction could be only poorly observed. Moreover, the reaction of cycloaddition with SiO$_2$ as catalyst did not lead to the desired product. On the other hand, it was found that BF$_3$·Et$_2$O catalyzed the Diels−Alder cycloaddition in lower yield than ytterbium(III) trifluoromethanesulfonate.

The one-pot formation of tetracyclic systems was investigated by using disubstituted dienes and lactone or thiolactone as dienophiles under the most suitable conditions at 90 °C in the presence of Lewis acid catalyst. It is noteworthy that a catalytic amount of Lewis acid is enough to give directly a mixture of two isomeric tetracyclic lactones (*a*, *b*). The diene **60** was obtained with difficulties in little yield, possibly due to the interaction of the heteroatom in the side chain with the reagents used for its preparation. The reaction between diene **60** and lactone **63** leads to the complex mixture of degradation compounds (dicarbonyl compound, diol, etc.) instead of the expected lactones.

The mixture of isomeric compounds proved difficult to separate by usual methods, but finally, after extensive experimentation, the mixture could be separated by meticulous column chromatography on silica gel. The structure of the isomer obtained was judged from ^{1}H NMR and ^{13}C NMR spectra. X-ray experiments of the lactone **4a** allowed us to unambiguously ascribe structure *a* to the minor constituent of the mixture and structure *b* to the main compound. Subsequent NMR studies agreed well with the X-ray structure (see Supporting Information).

To increase the number of structural changes, compounds **14**, **15**, **16**, and **19** were synthesized by transformation of the cyano-derivatives **12a** and **13a**. Thus, base hydrolysis of the cyano-derivatives (**12** and **13a**) with 2 N NaOH gave the corresponding carboxylic acids (**14** and **15**) in excellent yields. Based on molecular modeling, best activity was expected for the amine **16**. Treatment of the appropriate nitrile with H$_2$ in

Scheme 1. Synthesis of Dienes **48−62**

20. R_1= H
21. R_1= CH_3O

1) LDA
2) R_2CHO

22. R_1= H, R_2= CH_3
23. R_1= H, R_2= CH_2CH_3
24. R_1= H, R_2= $(CH_2)_2CH_3$
25. R_1= H, R_2= $(CH_2)_4CH_3$
26. R_1= H, R_2= $(CH_2)_5CH_3$
27. R_1= H, R_2= $(CH_2)_6CH_3$
28. R_1= H, R_2= $(CH_2)_7CH_3$
29. R_1= H, $R2$= $CH_2CH(CH_3)CH_2C(CH_3)_3$
30. R_1= H, R_2= $(CH_2)_4Br$
31. R_1= H, R_2= $CH_2OCH_2C_6H_5$
32. R_1= CH_3O, R_2= $(CH_2)_4CH_3$
33. R_1= H, R_2= $(CH_2)_4CN$
34. R_1= H, R_2= $(CH_2)_5CN$

PTSA
△

1) R_3Li, Me_3SiCl
2) NH_4Cl

35. R_1= H, R_2= CH_3
36. R_1= H, R_2= CH_2CH_3
37. R_1= H, R_2= $(CH_2)_2CH_3$
38. R_1= H, R_2= $(CH_2)_4CH_3$
39. R_1= H, R_2= $(CH_2)_5CH_3$
40. R_1= H, R_2= $(CH_2)_6CH_3$
41. R_1= H, R_2= $(CH_2)_7CH_3$
42. R_1= H, R_2= $CH_2CH(CH_3)CH_2C(CH_3)_3$
43. R_1= H, R_2= $(CH_2)_4Br$
44. R_1= H, R_2= $CH_2OCH_2C_6H_5$
45. R_1= CH_3O, R_2= $(CH_2)_4CH_3$
46. R_1= H, R_2= $(CH_2)_4CN$
47. R_1= H, R_2= $(CH_2)_5CN$

48. R_1= H, R_2= R_3= CH_3
49. R_1= H, R_2= CH_2CH_3, R_3= CH_3
50. R_1= H, R_2= $(CH_2)_2CH_3$, R_3= CH_3
51. R_1= H, R_2= CH_3, R_3= $(CH_2)_3CH_3$
52. R_1= H, R_2= $(CH_2)_4CH_3$, R_3= CH_3
53. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3
54. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= $(CH_2)_5CH_3$
55. R_1= H, R_2= $(CH_2)_6CH_3$, R_3= CH_3
56. R_1= H, R_2= $(CH_2)_7CH_3$, R_3= CH_3
57. R_1= H, R_2= $CH_2CH(CH_3)CH_2C(CH_3)_3$, R_3= CH_3
58. R_1= H, R_2= $(CH_2)_4CN$, R_3= CH_3
59. R_1= H, R_2= $(CH_2)_5CN$, R_3= CH_3
60. R_1= H, R_2= $CH_2OCH_2C_6H_5$, R_3= CH_3
61. R_1= CH_3O, R_2= $(CH_2)_4CH_3$, R_3= CH_3
62. R_1= H, R_2= $(CH_2)_4Br$, R_3= CH_3

methanol using PtO_2 as catalyst at room temperature afforded the corresponding amine in quantitative yields. The nucleophilic treatment of the bromide **18a** with methylamine hydrochloride in basic media afforded the corresponding amine **19** in acceptable yield (Scheme 3).

Results and Discussion

Structure−Activity Relationship. Many experimental studies indicate that the size, shape, and planarity of the ellipticine and derivatives are important factors for their mechanism of cytotoxicity and antitumor activity.[14] Furthermore, the acid-basic properties of ellipticine have been estimated. It is known that the pharmacological activities of drugs depend on their interaction. They are expected to be significantly different for the charged and uncharged drugs.[15]

A detailed similarity analysis, Figures 1 to 3, has shown a remarkable structural coincidence of 1-oxo-3(*H*)-isobenzofuro-[5,6-*b*][1,4]benzodioxine with the ellipticine nucleus. It is important to point out that only the behavior of the tetracyclic system was studied without considering the alkyl substituents.

This analysis was performed using fully optimized *ab initio* geometries, and electron density overlaps as a measure of similarity. Geometries were found to be almost planar. The density overlap alignment at the global maximum of the similarity function is presented in Figure 1. Figures 1 and 2c,d display the isocontour surface of the largest common pattern density, see Computational Methods for details and definitions. On the other hand, Figure 2a,b displays the electron density isocontours for the two molecules. The complete set of figures illustrates the extent of similarity, which embraces nearly the whole molecule but half of ring **A**. In the figures, the surfaces were plotted at the isocontour value of 0.03 and 0.03^2, in a.u., for the density and common pattern density, respectively, as being this value is an approximate equivalent for molecular accessible surface areas. Albeit being similar on an overall and structural analysis, the two electronic densities differ in some aspects. Differences on electronic rearrangements are manifest on the electrostatic potential maps. There are important differences in charge distribution among the studied compounds due to the presence of specific side groups. Figure 3 plots the density

Scheme 2. Synthesis of Tetracyclic Lactones by Diels−Alder Cycloaddition

48. R_1= H, R_2= CH_3, R_3= CH_3
49. R_1= H, R_2= CH_2CH_3, R_3= CH_3
50. R_1= H, R_2= $(CH_2)_2CH_3$, R_3= CH_3
51. R_1= H, R_2= CH_3, R_3= $(CH_2)_3CH_3$
52. R_1= H, R_2= $(CH_2)_4CH_3$, R_3= CH_3
53. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3
54. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= $(CH_2)_5CH_3$
55. R_1= H, R_2= $(CH_2)_6CH_3$, R_3= CH_3
56. R_1= H, R_2= $(CH_2)_7CH_3$, R_3= CH_3
57. R_1= H, R_2= $CH_2CH(CH_3)CH_2C(CH_3)_3$, R_3= CH_3
58. R_1= H, R_2= $(CH_2)_4CN$, R_3= CH_3
59. R_1= H, R_2= $(CH_2)_5CN$, R_3= CH_3
60. R_1= H, R_2= $CH_2OCH_2C_6H_5$, R_3= CH_3
61. R_1= CH_3O, R_2= $(CH_2)_4CH_3$, R_3= CH_3
62. R_1= H, R_2= $(CH_2)_4Br$, R_3= CH_3

63. Y= O
64. Y= S

1. R_1= H, R_2= CH_3, R_3= CH_3, Y= O
2a. R_1= H, R_2= CH_2CH_3, R_3= CH_3, Y= O
3a. R_1= H, R_2= $(CH_2)_2CH_3$, R_3= CH_3, Y= O
4a. R_1= H, R_2= $(CH_2)_3CH_3$, R_3= CH_3, Y= O
5a. R_1= H, R_2= $(CH_2)_4CH_3$, R_3= CH_3, Y= O
6a. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3, Y= O
7a. R_1= H, R_2= $(CH_2)_6CH_3$, R_3= CH_3, Y=O
8a. R_1= H, R_2= $(CH_2)_7CH_3$, R_3= CH_3, Y= O
9. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= $(CH_2)_5CH_3$, Y= O
10a. R_1= H, R_2= $CH_2CH(CH_3)CH_2C(CH_3)_3$, R_3= CH_3, Y= O
11a. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3, Y= S
12a. R_1= H, R_2= $(CH_2)_4CN$, R_3= CH_3, Y= O
13a. R_1= H, R_2= $(CH_2)_5CN$, R_3= CH_3, Y= O
17a. R_1= CH_3O, R_2= $(CH_2)_4CH_3$, R_3= CH_3, Y= O
18a. R_1= H, R_2= $(CH_2)_4Br$, R_3= CH_3, Y= O
19. R_1= H, R_2= $(CH_2)_4NHCH_3$, R_3= CH_3, Y= O

2b. R_1= H, R_2= CH_2CH_3, R_3= CH_3, Y= O
3b. R_1= H, R_2= $(CH_2)_2CH_3$, R_3= CH_3, Y= O
4b. R_1= H, R_2= $(CH_2)_3CH_3$, R_3= CH_3, Y= O
5b. R_1= H, R_2= $(CH_2)_4CH_3$, R_3= CH_3, Y= O
6b. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3, Y= O
7b. R_1= H, R_2= $(CH_2)_6CH_3$, R_3= CH_3, Y=O
8b. R_1= H, R_2= $(CH_2)_7CH_3$, R_3= CH_3, Y= O
10b. R_1= H, R_2= $CH_2CH(CH_3)CH_2C(CH_3)_3$, R_3= CH_3, Y= O
11b. R_1= H, R_2= $(CH_2)_5CH_3$, R_3= CH_3, Y= S
12b. R_1= H, R_2= $(CH_2)_4CN$, R_3= CH_3, Y= O
13b. R_1= H, R_2= $(CH_2)_5CN$, R_3= CH_3, Y= O
17b. R_1= CH_3O, R_2= $(CH_2)_4CH_3$, R_3= CH_3, Y= O
18b. R_1= H, R_2= $(CH_2)_4Br$, R_3= CH_3, Y= O

isocontours colored according to the potential values. As expected, the electronegative N and O atoms exhibit negative charge. The polarized $N^{\delta-} - H^{\delta+}$ bond is also reflected in this particular isosurface because the surface goes nearby the indole H.

All prepared lactones (compounds **1**−**19**) were assayed *in vitro* for the ability to inhibit cell proliferation L1210 (murine leukemia) and HT-29 (human colon carcinoma).[16] Also, the action over the phases of cell cycle was considered on the first cell line (L1210). The results expressed as IC_{50} values (mean of at least 4 determinations in triplicate obtained in independent experiments with variations of <5%) are listed in Table 2. The lactone **4a** was also evaluated against 60 tumor cell lines in the NCI (National Cancer Institute, MD).[17] The cytotoxic activity of these compounds was compared with the activity of ellipticine and adriamycin, both known antitumor compounds. Ellipticine and adriamycin were the chosen patterns for their structural similarity and for possessing bulky side chains, respectively.

Some of these compounds were found to possess significant activity at micromolar concentration. For analyzing structure−activity relationships, four structural components are considered: (a) the presence of substituents on ring **A**, (b) the nature of the ring **C** substituents, (c) position of the ring **C** substituents,

and (d) the nature of polycyclic lactone. First, with respect to the substituents on ring **A** (Figure 4), in general, the substitution of the H for a methoxy group presents a decreasing of activity (see Table 2, **17a** and **17b** versus **5a** and **5b**).

A parallel examination of compounds **1** and **2a**−**8a**, which have alkyl substituents on ring **C**, shows that the cytotoxic activity is best for compound **5a**, which possesses a pentyl chain on the C-11 and a methyl group on the C-4. The introduction of substituents with less than four C or more than six C at the C-11 position confers a decrease of cytotoxic activity. Compound **5a**, bearing a pentyl group on the C-11, showed an increase in the potency relative to the one bearing a propyl group substituent **3a**. The butyl homologue **4a** was less potent than **5a** and more potent than the new analogue compounds bearing a lower chain **3a**, **2a**, and **1**.

It is important to point out that the side chain at C-11 (the same side of the lactone carbonyl) confers greater activity for the inhibition of cell proliferation (**4a**, **5a**, and **6a** vs **4b**, **5b**, and **6b**). Thus, in general, compounds with the alkyl chain on the C-11 (series *a*) are more active than compounds with this chain on the C-4 (series *b*).

The lactones **5a** and **6a** showed similar cytotoxic activity,

Scheme 3. Preparation of Lactones **14−16** and **19**

Substrate	n	X	Conditions	Product	n	Y
12a	4	CN	1) NaOH 2N/Δ 2) HCl	**14**	4	COOH
13a	5	CN	1) NaOH 2N/Δ 2) HCl	**15**	5	COOH
12a	4	CN	H₂/PtO₂/HCl	**16**	5	NH₂·HCl
18a	4	Br	CH₃NH₂ / K₂CO₃	**19**	4	NHCH₃
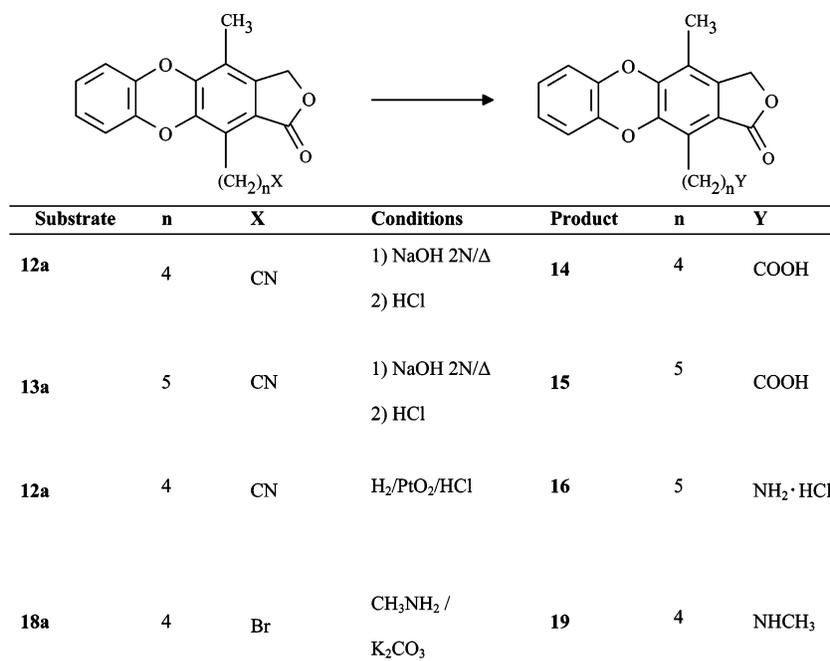
Table 1. Lewis Acids Tested as Catalyst in the Diels−Alder Reaction of **48**[a]

catalyst		SiO₂	MgBr₂[b]	BF₃·(CH₃CH₂)₂O[b]	SnCl₄[b]	TiCl₄[b]	Yb(CF₃SO₃)₃[b]
yield (%)	0*	0*	5*	40**	15**	16**	77***

[a] The symbol * designates a time of reaction = 12 h; ** designates a time of reaction = 10 min; and *** designates a time of reaction = 4 h. [b] Yields of isolated products.
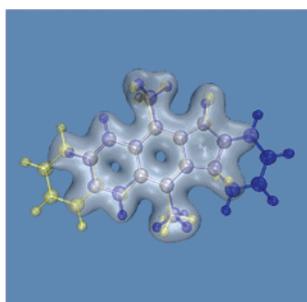
Figure 1. Electron density alignment of 4,11-dimethyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine and ellipticine, in yellow and blue, respectively. The largest $\rho_{CP}(\mathbf{r})$ isocontour surface is displayed at 0.001 $e^2\ a_0^{-6}$.

but **6a** was slightly more specific in disturbing cell cycle (G₂/M, 8N, 86%, 5 μM) than **5a**.

Particularly remarkable was the considerable cytotoxic activity of **4a** and **5a** on HT-29 human colon line (IC₅₀ = 5.8 × 10⁻⁷ M and IC₅₀ = 1.2 × 10⁻⁷ M, respectively) and also the high selectivity for the G₂ phase of the cell cycle (induced the accumulation of 67% of cells at the G₂/M phase by **4a** and 80% by **5a**).

On the other hand, the introduction of branched substituents at the alkyl chain on C-11 decreases the activity by more than 10 times (see **5a** vs **10a**).

The activity appears closely dependent on the number of methylenes in the side chain and on the nature of the substituents of this chain.

Until now, the C-4 and/or C-11 substituents had homologous alkyl chains, but it is interesting to study that the introduction of three distinct functions on the side chain is related to obtaining complementary results. It may be seen that **13a** exhibits greater
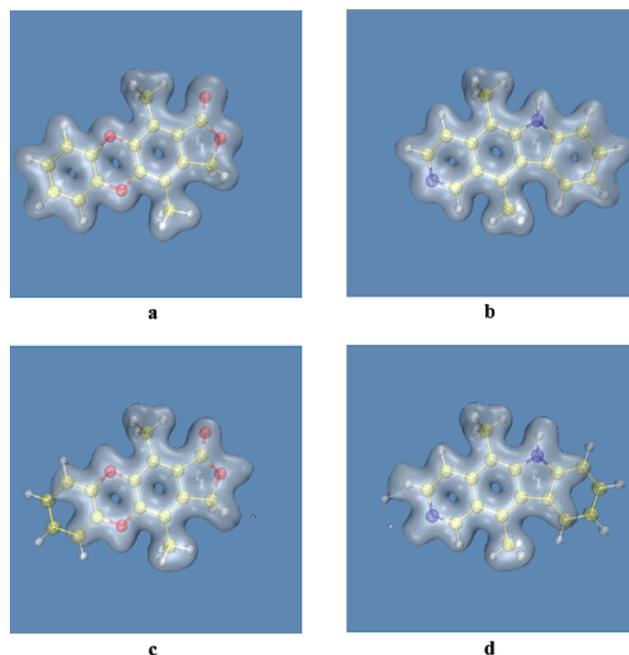
Figure 2. Electron density isocontour surfaces at 0.03 $e\ a_0^{-3}$, and ball and stick representation of (a) 4,11-dimethyl-1-oxo-3(*H*)-isobenzofuro-[5,6-*b*][1,4]benzodioxine and (b) ellipticine. The corresponding largest $\rho_{CP}(\mathbf{r})$ isocontour is displayed in (c) and (d), respectively, at 0.001 $e^2\ a_0^{-6}$.

activity according to the results of the nonfunctionalized chain compounds (3-fold more activity than **6a** and **5a** against leukemia L1210 and more activity than **6a** on HT-29 line cells). Whereas the introduction of the nitrile group on the side chain increases the activity (compare **13a** and **6a**), the introduction of some hydrophilic function as carboxylic acid (**15**) or primary
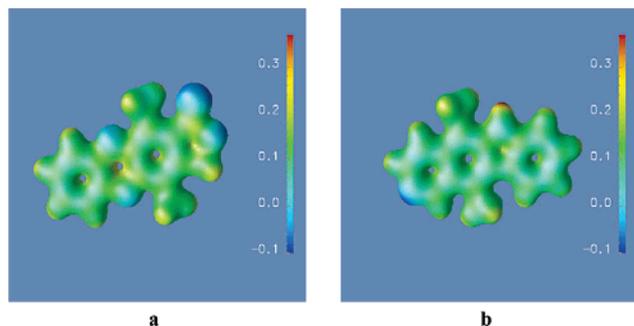
Figure 3. Electron density isocontour surface of (a) 4,11-dimethyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine and (b) ellipticine at 0.03 $e\ a_0^{-3}$, mapped with the electrostatic potential values.

amine on the C-11 chain (**16**) produces a 15-fold reduction of the activity on the colon HT-29 cell line and a 2-fold reduction on the leukemia L1210 cells, with respect to the unsubstituted compound **6a**.

The cyano alkyl **12a** displayed less cytotoxicity than **5a**. The hexyl derivative **6a**, enhances the activity due to the addition of the cyano group (compare **6a** and **13a**). Comparison of the analogues **16** and **19** suggested that a secondary amine was more active than a primary amine.

Generally, these compounds were globally more potent on the colon HT-29 cell line than on the leukemia L1210 cells (15-fold in the case of **5a** and 4.5-fold in the case of **13a** and **19**). Compounds **4a**−**7a**, **12a**, **13a**, **16**, and **19** showed selectivity toward colon HT-29 cell lines, and only **5b**, **10a**, **11**, and **14** showed little selectivity toward leukaemia L1210 cell lines.

Particularly remarkable was the lack of activity obtained for the thiolactone **11a** (9- and 90-fold less activity than the lactone **6a** on L1210 and on colon HT-29 cell line, respectively).

Compound **9** has two long and flexible side chains at C-4 and C-11 of the tetracyclic lactone, which potentially could provide hindrance and make difficult the binding with the site of action. This fact could explain the inactivity of this compound.

On the other hand, the simplified lactone **35**, which bears important structural modifications on the proposed skeleton of this series, is inactive. The inactivity may be due to an insufficient structural similarity to the tetracyclic lactones, such as **5a**. The compounds **13a** and **5a** were the most promising, and **6a** was of particular interest because of its marked activity *in vitro* against leukemia L1210 and against colon HT-29 cell line. Compound **13a** exhibited potent activity against both tumor cell lines and, in particular, its cytotoxic effect against HT-29 ($IC_{50} = 0.12\ \mu M$) was greater than that of adriamycin ($IC_{50} = 0.48\ \mu M$) or ellipticine ($IC_{50} = 14.9\ \mu M$).

The favorable influence on cytotoxic activity of the presence of a lipophilic alkyl chain was not mentioned before in antitumor studies. Instead, the introduction of polar chains that facilitate the solubility and also the biological interactions are better known.

The compound **6a** induced the accumulation of 86% of L1210 cells in the G_2/M, 8 N phase.

In regard to the amino functionalized derivatives, the most active was the secondary amine **19** (5-fold more potent than the primary amine **16** on the L1210 cells and 10-fold more potent on HT-29 cells).

The bulk of the methoxy group could explain the fact that the substituted compounds (**17a** and **17b**) are inactive. In other works related to the ellipticine, compounds with a methoxy

group on the aromatic ring **A** showed more cytotoxicity than the unsubstituted parent.[18]

Some of these compounds (**4a**, **5a**, **5b**, **6a**, and **19**) could block the cell cycle progression of L1210 cells in the G_2/M phase in the same pattern as shown by adriamycin, while compounds **7a** and **13a** showed an affect on the 8 N phase.

In conclusion, a series of tetracyclic lactones were synthesized and evaluated *in vitro* against the leukemia L1210 cell line, colon HT-29 cell line, and also the cycle inhibition studied (Table 2). The presence of an alkyl chain at position C-11 appears as an important structural requirement to observe potent cytotoxic activity in this series. The decrease of cytotoxicity observed by lengthening and shortening the alkyl chain at the C-11 position of the tetracyclic system reveals that the optimal length was five C (see Figure 5). The presence of a cyano, carboxylic acid, or amino group on the side chain has a marked influence on the activity.

Considering substitutions on the side chain at the C-11, it appeared, as indicated in Table 2, that weak structural modifications were responsible for the activity variation and that this series and the ellipticine series are not comparable in terms of structure−activity relationships.[18]

Despite the interesting activity of lactones, the mechanism of their action at both cellular and molecular level has not yet been clearly established. Considering that the main accepted mode of action for ellipticine is based on the interaction with the enzyme topoisomerase II in terms of stabilization of the *cleavage complex*, which is formed between DNA and topoisomerase II during a particular stage of the cell cycle, **4a** and **13a** were examined for their ability to facilitate the formation of a *cleavage complex* as an indication of the inhibition of topoisomerase II.[19] These compounds did not show inhibition of topoisomerase II consistent with their cytotoxicity.

Among all of these compounds, only the compound **4a** (NSC: 707690-M/1) was selected by the NCI for testing *in vitro* against 60 cells lines of the NCI anticancer drug screen derived from eight cancer types (leukemia, non small cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer). The GI_{50} (GI_{50} values are the concentrations corresponding to 50% growth inhibition, and are averages of at least two determinations) and LC_{50} (a concentration at which 50% of the cells were killed in a 96 h assay) values obtained with selected cell lines are summarized in Table 3. For the most part, there are no differences between log GI_{50} or LC_{50} values for the cell lines tested, only a small CNS and colon selectivity at the levels for growth inhibition was displayed. However, the cytotoxicity remains at the micromolar level and confirms the results provided by the *Institut de Recherches Servier*.

Results from COMPARE searches often provide insight into the mechanism of action of new compounds.[20] COMPARE analysis of **4a** and standard anticancer agent database of the NCI anticancer drug screen using the determination of Pearson correlation coefficient found no significant correlation ($P > 100$) between the IC_{50} of the **4a** and the IC_{50} of known topoisomerase II inhibitors (9-hydroxy-2-methylellipticinium NSC, 264137; amsacrine NSC, 249992; etoposide NSC, 141540; adriamycin (doxorubicin) NSC, 123127; mitoxantrone NSC, 301739).[17]

The molecular target of these compounds is not known, and the study was continued by making additional biological studies. The capacity of compounds **4a** and **5a** to bind to DNA and to inhibit human DNA topoisomerases I and II[21] was investigated. However, no significant effect was observed compared with the reference compounds camptothecin or etoposide. These com-

Table 2. Cytotoxic Activity of **1−19**[a]

Compound	IC$_{50}$ L1210 (µM)	IC$_{50}$ HT-29 (µM)	Cycle (L1210)[b]	Compound	IC$_{50}$ L1210 (µM)	IC$_{50}$ HT-29 (µM)	Cycle (L1210)[b]
1	> 100	> 100	n.t.	10a	13.5	> 10	n.t.
2a	> 10	> 10	n.t.	10b	> 10 (70 %)	> 10	n.t.
2b	> 10	> 10	n.t.	11a	19	34.5	n.t.
3a	> 10 (111%)	> 10	n.t.	12a	77	24.1	n.t.
3b	> 10 (115%)	> 10	n.t.	13a	0.54	0.12	88% 8N 5µM
4a	2.4	0.58	G$_2$/M, 8N 67% 5µM	13b	> 10	> 10	n.t.
4b	> 100	17.7	n.t.	14	89	> 10	n.t.
5a	1.8	0.12	G$_2$/M, 8N 80% 5µM	15	> 10	> 10	n.t.
5b	7.2	9.2	G$_2$/M, 8N 35% 5µM	16	12.8	5.7	n.t.
6a	2.0	0.38	G$_2$/M, 8N 86% 5µM	17a	> 10 (78%)	> 10	n.t.
6b	> 10 (118.3 %)	18.2	n.t.	17b	> 10 (70 %)	> 10	n.t.
7a	3.9	1.9	70% 8N 20µM	19	2.4	0.54	G$_2$/M, 8N 86% 5µM
7b	> 10	21.8	n.t.	35	> 10	> 10	n.t.
8a	> 10	> 10	n.t.	adriamycin	0.029	0.48	G$_2$/M 86% 1 µM
8b	> 10	> 10	n.t.	Ellipticine	0.19	14.9	G$_2$ 50% 1 µM
9	> 10	> 10	n.t.		1.2	22.1	-

[a] Inhibition of L1210 and HT-29 cell proliferation measured by *in vitro* biological assay. IC$_{50}$ values are mean concentrations that inhibit growth by 50% (IC$_{50}$ values are the average of at least four determinations in triplicate obtained in independent experiments. Variation between replicates was less than 5%). [b] Percentage of L1210 cells arrested in cell cycle phase after 24 h of exposure to the indicated concentration of problem compound. n.t. = not tested (considered not interesting due to poor activity on L1210 cell lines).
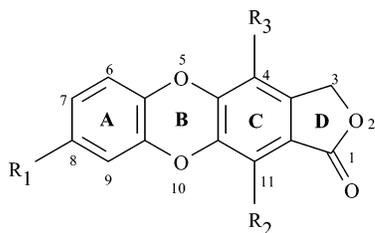
Figure 4. General structure of series *a* and *b* of lactones.
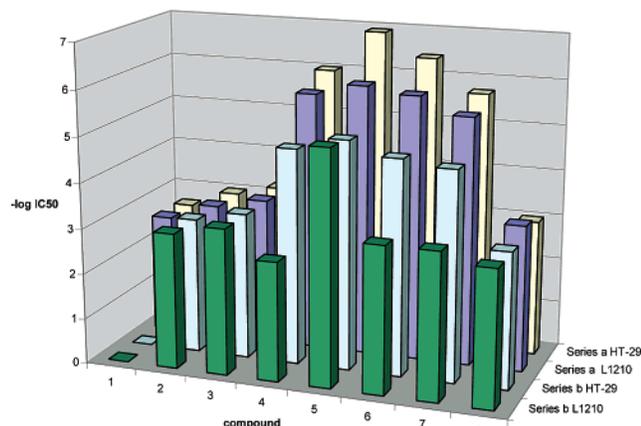
Figure 5. Plots $-\log_{10}(IC_{50})$ versus cancer type (L1210, and HT-29) for compounds **1**, **2a**−**8a**, and **2b**−**8b**.

pounds have no effect on the melting temperature of the DNA double helix, be it with calf thymus (42% GC) or with the polynucleotide poly(dAT)$_2$.

DNA sequence recognition was studied by DNase I footprinting.[22] No marked modification of the absorption spectra of the compounds was observed upon addition of DNA, and no specific binding site was detected in the DNase I footprinting assay, indicating also that these molecules do not appreciably bind to DNA.

In parallel, a plasmid supercoiled DNA (pKMp27 DNA) was used to study the relaxation and cleavage of DNA by purified topoisomerases I and II.[23] Here again, we found no effect of compounds **4a** and **5a** in these biochemical assays. Moreover, compounds **4a** and **13** were tested using another native supercoiled DNA (pYRG DNA), and the results were inconclusive (no DNA binding).[24] These compounds do not stimulate DNA cleavage by the enzymes and do not interfere with the poisoning effects of camptothecin on topoisomerase I or etoposide on topoisomerase II. The effect of no detectable binding to DNA, the absence of inhibition of either topoisomerase I or topoisomerase II, and the negative response to the DNA relaxation test confirm the results previously obtained from COMPARE analysis.

These compounds do not correlate with any known compound in the National Cancer Institute's data base, suggesting a unique mechanism of action.

The structure−activity relationships allowed refining the structural requirements of this series of dioxygenated compounds. Lactone appears essential for cytotoxicity, and its substitution by a thiolactone prevents us from obtaining the same activity. On the other hand, if we consider the side chain position and the nature of its substituents, it appears that the substituent on the C-11 position leads to an increase of the relative activity compared with C-4 substitution. Finally, this SAR study indicated that the best substituent must possess a linear alkyl chain of 5 C (**5a**) or a linear alkyl chain with a cyano group in position 5 (**13a**). These results strengthen our purpose of

performing new pharmacological investigations to elucidate the mechanism of action. These biological results corroborate the observation that a chemical modification of the structure framework may result in a profound impact in the biological activity.

In summary, several new synthetic tetracyclic lactones described in this paper have antitumor activity at the micromolar level. The cytotoxic activity is quantitatively comparable to the natural ellipticine, but they show a different mechanism of action. Likewise, the main structural features influencing the antitumor activity are different from the ellipticine series. Among the tested compounds, **4a**, **5a**, and **13a** are considered promising leads for further structural modifications, perhaps guided by the valuable information obtainable from detailed analysis of theoretical studies.

The results of this study, we believe, will provide useful information for the design of novel class of cytotoxic agents.

Experimental Section

Chemistry. Melting points were obtained on a MFB-595010M Gallenkamp apparatus in open capillary tubes and are uncorrected. IR spectra were obtained using a FTIR Perkin-Elmer 1600 infrared spectrophotometer. Only noteworthy IR absorptions are listed (v expressed in cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 (200 and 50.3 MHz, respectively) or Varian Gemini-300 (300 and 75.5 MHz) instrument using CDCl$_3$ as solvent with tetramethylsilane as internal standard or (CD$_3$)$_2$CO. Other ^{1}H NMR spectra and heterocorrelation ^{1}H−^{13}C (HMQC and HMBC) experiments were recorded on a Varian VXR-500 (500 MHz). Mass spectra were recorded on a Hewlett-Packard 5988-A. Column chromatography was performed with silica gel (E. Merck, 70−230 mesh). Reactions were monitored by TLC using 0.25 mm silica gel F-254 (E. Merck). Microanalysis was determined on a Carlo Erba−1106 analyzer. All reagents were of commercial quality or were purified before use. Organic solvents were of analytical grade or were purified by standard procedures. Commercial products were obtained from Sigma-Aldrich.

Preparation of Lactones (1−13a, 2b−13b, 17a−18a, 17b−18b) from Diels−Alder Cycloadditions of the Furans (48−62). General Procedure. 2(5*H*)-Furanone **63** (1.5 mmols) or 2(5*H*)-thiofuranone **64** (1.5 mmols) and a catalytic amount of ytterbium triflate were added to the corresponding diene (1 mmol) without solvent at room temperature.

The mixture was stirred in a hermetically closed flask on a preheated bath at 90−100 °C for 4 h. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate as eluent, allowed us to obtain two positional isomers that were accurately identified as *a* or *b*.

4,11-Dimethyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (1). Starting from the diene **48** (523 mg, 2.59 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **1** (535 mg, 1.99 mmol). The crude product was purified by silica gel column chromatography, using hexane/ethyl acetate 95/5 as eluent, giving the lactone as an oil in 77% of yield. Anal. (C$_{16}$H$_{12}$O$_4$) C, H.

11-Ethyl-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (2a). Starting from the diene **49** (621 mg, 2.87 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **2a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 95/5 as eluent, gave the lactone as a white solid in 32% of yield. When hexane/ethyl acetate 96/4 mixtures were used as eluent, the isomer **2b** was obtained in 44% yield. For **2a**: mp 205−208 °C (hexane/ethyl acetate). Anal. (C$_{17}$H$_{14}$O$_4$) C, H.

4-Ethyl-11-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (2b). Compound **2b** was obtained as a white solid, 44% yield; mp 185−188 °C (hexane /ethyl acetate). Anal. (C$_{17}$H$_{14}$O$_4$) C, H.

4-Methyl-11-propyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (3a). Starting from the diene **50** (681 mg, 2.96 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **3a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 96/4 as eluent, gave the lactone as a white solid in 33% yield. When hexane/ethyl acetate 97/3 mixtures were used as eluent, the isomer **3b** (378 mg, 1.27 mmol) was obtained as a white solid in 45% yield. For **3a**: mp 199−202 °C (hexane/ethyl acetate). Anal. ($C_{18}H_{16}O_4$) C, H.

11-Methyl-4-propyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (3b). Mp 191−194 °C (hexane /ethyl acetate). Anal. ($C_{18}H_{16}O_4$) C, H.

11-Butyl-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (4a). Starting from the diene **51** (695 mg, 2.84 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **4a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 97/3 as eluent, gave the lactone as a white solid in 31% of yield. When the same eluent was used, the isomer **4b** (380 mg, 1.2 mmol) was obtained in 43% yield; mp 122−125 °C (hexane/ethyl acetate). Anal. ($C_{19}H_{18}O_4$) C, H.

4-Butyl-11-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (4b). Mp 153−155 °C (hexane /ethyl acetate). Anal. ($C_{19}H_{18}O_4$) C, H.

4-Methyl-11-pentyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (5a). Starting from the diene **52** (598 mg, 2.32 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **5a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 98/2 as eluent, gave the lactone as a white solid in 28% yield (256 mg, 0.77 mmol). When hexane/ethyl acetate 99/1 was used as eluent, the isomer **5b** was obtained in 45% yield; mp 108−110 °C (hexane/ethyl acetate). Anal. ($C_{20}H_{20}O_4$) C, H.

11-Methyl-4-pentyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (5b). Mp 145−146 °C (hexane/ethyl acetate). Anal. ($C_{20}H_{20}O_4$) C, H.

11-Hexyl-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (6a). Starting from the diene **53** (603 mg, 2.21 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **6a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 98/2 as eluent, gave the lactone as a white solid in 36% of yield. When the same eluent was used, the isomer **6b** (292 mg, 0.86 mmol) was obtained in 39% yield; mp 131−133 °C (hexane/ethyl acetate). Anal. ($C_{21}H_{22}O_4$) C, H.

4-Hexyl-11-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (6b). Mp 149−150 °C (hexane/ ethyl acetate). Anal. ($C_{21}H_{22}O_4$) C, H.

11-Heptyl-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (7a). Starting from the diene **55** (627 mg, 2.19 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **7a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 98/2 as eluent, gave the lactone as a white solid in 29% of yield. When the same eluent was used, the isomer **7b** (302 mg, 0.86 mmol) was obtained as a white solid in 39% yield; mp 103−104 °C (hexane/ethyl acetate). Anal. ($C_{22}H_{24}O_4$) C, H.

4-Heptyl-11-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (7b). Mp 153−154 °C (hexane/ ethyl acetate). Anal. ($C_{22}H_{24}O_4$) C, H.

4-Methyl-11-octyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (8a). Starting from the diene **56** (598 mg, 1.99 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **8a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 98/2 as eluent, gave the lactone as a white solid in 27% of yield. When hexane/ethyl acetate 99/1 was used, the isomer **8b** (321 mg, 0.88 mmol) was obtained as a

white solid in 44% yield; mp 84−86 °C (hexane/ethyl acetate). Anal. ($C_{23}H_{26}O_4$) C, H.

11-Methyl-4-octyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (8b). Mp 137−138 °C (hexane/ ethyl acetate). Anal. ($C_{23}H_{26}O_4$) C, H.

4,11-Dihexyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (9). Starting from the diene **54** (721 mg, 2.11 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **9**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 99/1 as eluent, gave the lactone as a white solid in 73% of yield; mp 115−117 °C (hexane/ethyl acetate). Anal. ($C_{26}H_{32}O_4$) C, H.

4-Methyl-11-(2,4,4-trimethylpentyl)-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (10a). Starting from the diene **57** (659 mg, 2.01 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **10a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 98/2 as eluent, gave the lactone as a white solid in 29% of yield. When hexane/ethyl acetate 99/1 was used, the isomer **10b** (356 mg, 0.90 mmol) was obtained as a white solid in 45% yield; mp 182−183 °C (hexane/ethyl acetate). Anal. ($C_{23}H_{26}O_4$) C, H.

11-Methyl-4-(2,4,4-trimethylpentyl)-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (10b). Mp 161−162 °C (hexane/ethyl acetate). Anal. ($C_{23}H_{26}O_4$) C, H.

11-Hexyl-4-methyl-1-oxo-3(*H*)-isobenzothiofeno[5,6-*b*][1,4]-benzodioxine (11a). Starting from the diene **53** (700 mg, 2.57 mmol) and the thiolactone **64**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **11a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 80/20 as eluent, gave the lactone as a white solid in 36% of yield. When hexane/ethyl acetate 70/30 was used, the isomer **11b** (382 mg, 1.06 mmol) was obtained as a white solid in 42% yield; mp 126−129 °C (hexane/ ethyl acetate). Anal. ($C_{21}H_{22}O_3S$) C, H.

4-Hexyl-11-methyl-1-oxo-3(*H*)-isobenzothiofeno[5,6-*b*][1,4]-benzodioxine (11b). Mp 131−133 °C (hexane/ethyl acetate). Anal. ($C_{21}H_{22}O_3S$) C, H.

11-(4-Cyanobutyl)-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*]-[1,4]benzodioxine (12a). Starting from the diene **58** (458 mg, 1.70 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **12a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 99/1 as eluent, gave the lactone as a white solid in 30% of yield. When hexane/ethyl acetate 98/2 was used, the isomer **12b** (245 mg, 0.73 mmol) was obtained as a white solid in 43% yield; mp 169−171 °C (hexane/ ethyl acetate). Anal. ($C_{20}H_{17}NO_4$) C, H, N.

4-(4-Cyanobutyl)-11-methy-1-oxo-3(*H*)-isobenzofuro[5,6-*b*]-[1,4]benzodioxine (12b). Mp 178−181 °C (hexane/ethyl acetate). Anal. ($C_{20}H_{17}NO_4$) C, H, N.

11-(5-Cyanopentyl)-4-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*]-[1,4]benzodioxine (13a). Starting from the diene **59** (597 mg, 2.11 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **13a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 99/1 as eluent, gave the lactone as a white solid in 36% of yield. When hexane/ethyl acetate 98/2 was used, the isomer **13b** (318 mg, 0.92 mmol) was obtained as a white solid in 43% yield; mp 110−113 °C (hexane/ ethyl acetate). Anal. ($C_{21}H_{19}NO_4$) C, H, N.

4-(5-Cyanopentyl)-11-methyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*]-[1,4]benzodioxine (13b). Mp 137−139 °C (hexane/ethyl acetate). Anal. ($C_{21}H_{19}NO_4$) C, H, N.

4-Methyl-8-methoxy-11-pentyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine (17a). Starting from the diene **61** (527 mg, 1.83 mmol) and the lactone **63**, and following the general procedure described above for the preparation of lactones, we obtained the lactone **17a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 99/1 as eluent, gave

the lactone as a white solid in 34% of yield. When hexane/ethyl acetate 98/2 was used, the isomer **17b** (304 mg, 0.82 mmol) was obtained as a white solid in 45% yield; mp 137−139 °C (hexane/ethyl acetate). Anal. ($C_{21}H_{22}O_5$) C, H.

11-Methyl-7-methoxy-4-pentyl-1-oxo-3(H)-isobenzofuro[5,6-b][1,4]benzodioxine (17b). Mp 117−119 °C (hexane/ethyl acetate). Anal. ($C_{21}H_{22}O_5$) C, H.

4-Methyl-11-(4-bromobutyl)-1-oxo-3(H)-isobenzofuro[5,6-b]-[1,4]benzodioxine (18a). Starting from the diene **62** (602 mg, 1.86 mmol) and the lactone **63**, following the general procedure described above for the preparation of lactones, we obtained the tetracyclic compound **18a**. Purification of the crude product by silica gel column chromatography, using hexane/ethyl acetate 90/10 as eluent, gave the lactone as a white solid in 29% of yield. The isomer **18b** (308 mg, 0.78 mmol) was obtained as a white solid in 43% yield using hexane/ethyl acetate 88/12 as eluent; mp 146−148 °C (hexane/dichloromethane). Anal. ($C_{19}H_{17}BrO_4$) C, H.

11-Methyl-4-(4-bromobutyl)-1-oxo-3(H)-isobenzofuro[5,6-b]-[1,4]benzodioxine (18b). Mp 160−162 °C (hexane/dichloromethane). Anal. ($C_{19}H_{17}BrO_4$) C, H.

11-(4-Carboxybutyl)-4-methyl-1-oxo-3(H)-isobenzofuro[5,6-b][1,4]benzodioxine (14). A solution containing the corresponding lactone (**12a**; 50 mg, 0.15 mmol) in 2 N NaOH (40 mL) and methanol (5 mL) was refluxed for 2 h. After 1 N HCl was slowly added until pH = 1, and the suspension was filtered. The residue was dried to give the lactone **14** as a white solid (48 mg, 0.14 mmol) in a 91% yield; mp 197−200 °C (H_2O). Anal. ($C_{20}H_{18}O_6$) C, H.

11-(5-Carboxypentyl)-4-methyl-1-oxo-3(H)-isobenzofuro[5,6-b][1,4]benzodioxine (15). A solution containing the corresponding lactone (**13a**; 60 mg, 0.17 mmol) in 2 N NaOH (40 mL) and methanol (5 mL) was refluxed for 2 h. HCl (1 N) was slowly added until pH = 1, and the suspension was filtered. The residue was dried to give the lactone **15** as a white solid (57 mg, 0.16 mmol) in a 96% yield; mp 182−185 °C (H_2O). Anal. ($C_{21}H_{20}O_6$) C, H.

11-(5-(Aminopentyl)-4-methyl-1-oxo-3(H)-isobenzofuro[5,6-b][1,4]benzodioxine Hydrochloride (16). To a solution containing the corresponding lactone (**12a**; 15 mg, 0.045 mmol) in methanol (15 mL) was added PtO_2 (5 mg) and two drops of 1 N HCl. The suspension was stirred at room temperature under hydrogen atmosphere for 12 h. Then the suspension was filtered, and the solvent was removed under vacuum. The residue obtained was diluted with water and extracted with dichloromethane. The organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the reduced compound **16** (15 mg, 0.04 mmol) as a white solid in 88% yield. Anal. ($C_{20}H_{22}ClNO_4 \cdot H_2O$) C, H, N.

11-(5-(Aminomethyl)pentyl-4-methyl-1-oxo-3(H)-isobenzofuro-[5,6-b][1,4]benzodioxine (19). To a solution of the lactone **18a** (20 mg, 0.05 mmol) in DMF (3 mL) were added K_2CO_3 (22 mg, 0.15 mmol) and methylamine hydrochloride (5 mg, 0.08 mmol). The resulting mixture was stirred seven days at room temperature. Every two days, the same amount of K_2CO_3 and methylamine hydrochloride was added. The reaction mixture was diluted with water (50 mL) and was extracted with ether (3 × 12 mL). The organic layer was dried over Na_2SO_4, filtered off, and the solvent removed under vacuum. The residue was purified by silica gel column chromatography using hexane/ethyl acetate as eluent, giving the desired amine in 57% yield; mp 114−116 °C (methanol). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

General Procedure for the Preparation of Hydroxyacids (22−34). *ortho*-Lithiation of Carboxylic Acids (20−21). To a solution of the carboxylic acid (1 mmol) in THF cooled at −78 °C was added dropwise LDA (2.2 mmol; 2 M solution in THF/heptane), and this mixture was stirred. After 3.5 h, the electrophile (1.2 mmol) was added dropwise. The resulting mixture was stirred for 12 h, and then the reaction mixture was allowed to warm to room temperature. A solution of 0.5 N HCl was added until the pH of the mixture was acidic. Then the mixture was stirred at room temperature for 5 min. Finally the reaction mixture was diluted with water and extracted with ether (4 × 15 mL). The organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced

pressure. The hydroxyacid was obtained as a white solid that was further transformed into the corresponding lactone.

3-(1-Hydroxyethyl)-1,4-benzodioxin-2-carboxylic Acid (22). Following the general procedure applied to the carboxylic acid **20** (1 g, 5.60 mmol), the crude residue was purified to furnish the hydroxyacid (1.19 g, 5.36 mmol) in a 96% of yield; mp 210−212 °C (methanol). Anal. ($C_{11}H_{10}O_5$) C, H.

3-(1-Hydroxypropyl)-1,4-benzodioxin-2-carboxylic Acid (23). Following the general procedure applied to the carboxylic acid **20** (0.8 g, 4.20 mmol) to furnish the hydroxyacid **23** (0.9 g, 3.86 mmol) in a 92% yield; mp 151−153 °C (methanol). Anal. ($C_{12}H_{12}O_5$) C, H.

3-(1-Hydroxybutyl)-1,4-benzodioxin-2-carboxylic Acid (24). Following the general procedure applied to the acid **20** (0.78 g, 4.40 mmol) furnished the hydroxyacid **24** (1.04 g, 4.16 mmol) in a 95% yield; mp 152−154 °C (methanol). Anal. ($C_{13}H_{14}O_5$) C, H.

3-(1-Hydroxyhexyl)-1,4-benzodioxin-2-carboxylic Acid (25). Following the general procedure applied to the acid **20** (0.95 g, 5.33 mmol) furnished the hydroxyacid **25** (1.23 g, 4.42 mmol) in 83% yield; mp 154−157 °C (methanol). Anal. ($C_{15}H_{18}O_5$) C, H.

3-(1-Hydroxyheptyl)-1,4-benzodioxin-2-carboxylic Acid (26). Following the general procedure applied to the acid **20** (1.01 g, 5.67 mmol) furnished the hydroxyacid **26** (1.35 g, 4.62 mmol) in 82% yield; mp 154−155 °C (methanol). Anal. ($C_{16}H_{20}O_5$) C, H.

3-(1-Hydroxyoctyl)-1,4-benzodioxin-2-carboxylic Acid (27). Following the general procedure applied to the acid **20** (1.31 g, 7.35 mmol) furnished the hydroxyacid **27** (1.78 g, 5.81 mmol) in 79% yield; mp 150−152 °C (methanol). Anal. ($C_{17}H_{22}O_5$) C, H.

3-(1-Hydroxynonyl)-1,4-benzodioxin-2-carboxylic acid (28). Following the general procedure applied to the acid **20** (0.98 g, 5.50 mmol) furnished the hydroxyacid **28** (1.43 g, 4.46 mmol) in 81% yield. Mp 148−150 °C (methanol). Anal. ($C_{18}H_{24}O_5$) C, H.

3-(3,5,5-Trimethyl-1-hydroxyhexyl)-1,4-benzodioxin-2-carboxylic Acid (29). Following the general procedure applied to the acid **20** (0.68 g, 3.82 mmol) furnished the hydroxyacid **29** (1.11 g, 3.19 mmol) in 83% yield; mp 168−170 °C (methanol). Anal. ($C_{18}H_{24}O_5$) C, H.

3-(5-Bromo-1-hydroxypentyl)-1,4-benzodioxin-2-carboxylic Acid (30). Following the general procedure applied to the acid **20** (0.87 g, 4.90 mmol) furnished the hydroxyacid **30** (1.49 g, 4.32 mmol) in 89% yield; mp 160−162 °C (methanol). Anal. ($C_{14}H_{15}BrO_5$) C, H.

3-(2-Benzyloxy-1-hydroxyethyl)-1,4-benzodioxin-2-carboxylic Acid (31). Following the general procedure applied to the acid **20** (0.97 g, 5.45 mmol) furnished the hydroxyacid **31** (1.59 g, 4.84 mmol) in 89% yield; mp 145−147 °C (methanol). Anal. ($C_{18}H_{16}O_6$) C, H.

3-(1-Hydroxyhexyl)-6-methoxy-1,4-benzodioxin-2-carboxylic Acid (32). Following the general procedure applied to the acid **21** (0.97 g, 4.65 mmol) furnished the hydroxyacid **32** as colorless oil (1.19 g, 3.86 mmol) in 83% yield. Anal. ($C_{16}H_{20}O_6$) C, H.

3-(5-Cyano-1-hydroxypentyl)-1,4-benzodioxin-2-carboxylic Acid (33). Following the general procedure applied to the acid **20** (0.72 g, 4.04 mmol) furnished the hydroxyacid **33** (0.88 g, 3.10 mmol) in 76% yield; mp 158−160 °C (methanol). Anal. ($C_{15}H_{15}NO_5$) C, H, N.

3-(6-Cyano-1-hydroxyhexyl)-1,4-benzodioxin-2-carboxylic Acid (34). Following the general procedure applied to the acid **30** (0.53 g, 2.96 mmol) furnished the hydroxyacid **34** (0.67 g, 2.21 mmol) in 75% yield; mp 162−163 °C (methanol). Anal. ($C_{16}H_{17}NO_5$) C, H, N.

Preparation of Lactones (35−47). Lactonization of Hydroxyacids (22−34). General Procedure. To a solution of the carboxylic acid (1 mmol) in dry toluene (25 mL) under an argon atmosphere were added PTSA (catalytic amount) and molecular sieves of 4 Å (100 mg), and the mixture was refluxed for 12 h. Then the suspension was filtered and washed with ethyl acetate. The crude product was extracted with ether and washed with 1 N NaOH until

basic pH. The obtained residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate mixtures.

3-Methyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (35). Starting from the hydroxyacid **22** (0.95 g, 4.28 mmol) and following the general procedure, the lactone **35** (0.78 g, 3.82 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 89% yield; mp 125−127 °C (hexane/ethyl acetate). Anal. ($C_{11}H_8O_4$) C, H.

3-Ethyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (36). Starting from the hydroxyacid **23** (0.7 g, 3.02 mmol) and following the general procedure, the lactone **36** (0.55 g, 2.57 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 85% yield. Anal. ($C_{12}H_{10}O_4$) C, H.

3-Propyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (37). Starting from the hydroxyacid **24** (1.1 g, 4.40 mmol) and following the general procedure, the lactone **37** (0.88 g, 3.79 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 86% yield; mp 80−81 °C (hexane/ethyl acetate). Anal. ($C_{13}H_{12}O_4$) C, H.

3-Pentyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (38). Starting from the hydroxyacid **25** (0.63 g, 2.26 mmol) and following the general procedure, the lactone **38** (0.51 g, 1.96 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 87% yield; mp 92−93 °C (hexane/ethyl acetate). Anal. ($C_{15}H_{16}O_4$) C, H.

3-Hexyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (39). Starting from the hydroxyacid **26** (0.74 g, 2.52 mmol) and following the general procedure, the lactone **39** (0.63 g, 2.30 mmol) was obtained as a white solid. The crude product was purified on a silica gel column, eluting with hexane/ethyl acetate (8/2), to furnish the desired lactone in 91% yield; mp 82−83 °C (hexane/ethyl acetate). Anal. ($C_{16}H_{18}O_4$) C, H.

3-Heptyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (40). Starting from the hydroxyacid **27** (0.99 g, 3.22 mmol) and following the general procedure, the lactone **40** (0.79 g, 2.74 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (8/2), to furnish the desired lactone in 85% yield; mp 84−85 °C (hexane/ethyl acetate). Anal. ($C_{17}H_{20}O_4$) C, H.

3-Octyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]⁴benzodioxine (41). Starting from the hydroxyacid **28** (1.13 g, 3.53 mmol) and following the general procedure, the lactone **41** (0.88 g, 2.91 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (8/2), to furnish the desired lactone in 82% yield; mp 82−83 °C (hexane/ethyl acetate). Anal. ($C_{18}H_{22}O_4$) C, H.

3-(2,4,4-Trimethylpentyl)-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (42). Starting from the hydroxyacid **29** (1.11 g, 3.19 mmol) and following the general procedure, the lactone **42** (0.88 g, 2.66 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (8/2), to furnish the desired lactone in 84% yield; mp 69−70 °C (hexane/ethyl acetate). Anal. ($C_{18}H_{22}O_4$) C, H.

3-(4-Bromobutyl)-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (43). Starting from the hydroxyacid **30** (0.52 g, 1.52 mmol) and following the general procedure, the lactone **43** (0.41 g, 1.26 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 83% yield; mp 82−84 °C (hexane/ethyl acetate). Anal. ($C_{14}H_{13}BrO_4$) C, H.

3-(Benzyloxymethyl)-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (44). Starting from the hydroxyacid **31** (0.82 g, 2.49 mmol) and following the general procedure, the lactone **44** (0.61 g, 1.97 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 79% yield; mp 95−97 °C (hexane/ethyl acetate). Anal. ($C_{18}H_{14}O_5$) C, H.

6-Methoxy-3-pentyl-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (45). Starting from the hydroxyacid **32** (0.92 g, 2.97 mmol) and following the general procedure, the lactone **45** (0.77 g, 2.65 mmol) was obtained as a yellow oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 89% yield. Anal. ($C_{16}H_{18}O_5$) C, H.

3-(4-Cyanobutyl)-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (46). Method a. Starting from the hydroxyacid **33** (0.43 g, 1.48 mmol) and following the general procedure, the lactone **46** (0.32 g, 1.20 mmol) was obtained as a white solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 81% yield; mp 103−105 °C (hexane/ethyl acetate). Anal. ($C_{15}H_{13}NO_4$) C, H.

Method b. To a solution of the corresponding lactone (110 mg, 0.34 mmol) in DMF (3 mL) was added HMPA (1 mL) and KCN (37 mg, 0.58 mmol). The obtained mixture was stirred at room temperature for 12 h. Then the solvent was removed and the crude of reaction was purified by silica gel column chromatography to give catechol (32 mg, 0.29 mmol).

3-(5-Cyanopentyl)-1-oxo-3(*H*)-furo[3,4-*b*][1,4]benzodioxine (47). Starting from the hydroxyacid **34** (0.94 g, 3.10 mmol) and following the general procedure, the lactone **47** (0.77 g, 2.70 mmol) was obtained as a yellow solid. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (7/3), to furnish the desired lactone in 87% yield; mp 117−118 °C (hexane/ethyl acetate). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

Synthesis of Dienes (48−60). General Procedure. The lactone (1 mmol) was dissolved in dry THF (4 mL) under argon, and the solution was then cooled to −78 °C. A solution of trimethylsilyl chloride (6 mmol) in THF and the corresponding alkyllithium (3 mmol) were added with stirring over 5 min, and stirring was continued until the mixture reached room temperature. A solution of NH₄Cl (5 mL) was added and the organic layer was separated. The combined organic solutions were dried (Na₂SO₄), and the solvent was removed to give the crude product. The product was purified by column chromatography (silica gel, hexane/ethyl acetate).

1,3-Dimethylfuro[3,4-*b*][1,4]benzodioxine (48). Starting from the lactone **35** (631 mg, 3.09 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **48** (531 mg, 2.63 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 85% yield. Anal. ($C_{12}H_{10}O_3$) C, H.

1-Ethyl-3-methylfuro[3,4-*b*][1,4]benzodioxine (49). Starting from the lactone **36** (501 mg, 2.34 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **49** (440 mg, 2.06 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 87% yield. Anal. ($C_{13}H_{12}O_3$) C, H.

1-Methyl-3-propylfuro[3,4-*b*][1,4]benzodioxine (50). Starting from the lactone **37** (750 mg, 3.23 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **50** (625 mg, 2.71 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 84% yield. Anal. ($C_{14}H_{14}O_3$) C, H.

1-Butyl-3-methylfuro[3,4-*b*][1,4]benzodioxine (51). Starting from the lactone **35** (523 mg, 2.56 mmol) and following the general procedure using butyllithium (1.6 M in hexane), the furan **51** (425 mg, 1.74 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 68% yield. Anal. ($C_{15}H_{16}O_3$) C, H.

1-Methyl-3-pentylfuro[3,4-*b*][1,4]benzodioxine (52). Starting from the lactone **38** (541 mg, 2.08 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **52** (457 mg, 1.77 mmol) was obtained as an oil. The crude product was

purified on silica gel column, eluting with hexane/ethyl acetate (99/1), to furnish the desired furan in 85% yield. Anal. ($C_{16}H_{18}O_3$) C, H.

1-Hexyl-3-methylfuro[3,4-*b*][1,4]benzodioxine (53). Starting from the lactone **39** (480 mg, 1.75 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **53** (396 mg, 1.45 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (99/1), to furnish the desired furan in 83% yield. Anal. ($C_{17}H_{20}O_3$) C, H.

1,3-Dihexylfuro[3,4-*b*][1,4]benzodioxine (54). Starting from the lactone **39** (637 mg, 2.32 mmol) and following the general procedure using hexyllithium (2.5 M in hexane), the furan **54** (453 mg, 1.32 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (99/1), to furnish the desired furan in 87% yield. Anal. ($C_{22}H_{30}O_3$) C, H.

1-Heptyl-3-methylfuro[3,4-*b*][1,4]benzodioxine (55). Starting from the lactone **40** (523 mg, 1.81 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **55** (435 mg, 1.52 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (99/1), to furnish the desired furan in 84% yield. Anal. ($C_{18}H_{22}O_3$) C, H.

1-Methyl-3-octylfuro[3,4-*b*][1,4]benzodioxine (56). Starting from the lactone **41** (527 mg, 1.74 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **56** (444 mg, 1.49 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (99/1), to furnish the desired furan in 85% yield. Anal. ($C_{19}H_{24}O_3$) C, H.

3-Methyl-1-(2,4,4-trimethylpentyl)furo[3,4-*b*][1,4]benzodioxine (57). Starting from the lactone **42** (625 mg, 1.89 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **57** (503 mg, 1.53 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 87% yield. Anal. ($C_{19}H_{24}O_3$) C, H.

1-(4-Cyanobutyl)-3-methylfuro[3,4-*b*][1,4]benzodioxine (58). Starting from the lactone **46** (440 mg, 1.62 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **58** (353 mg, 1.31 mmol) was obtained as an oil. The crude product was purified on silica gel column chromatography, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 81% yield. Anal. ($C_{16}H_{15}NO_3$) C, H, N.

1-(5-Cyanopentyl)-3-methylfuro[3,4-*b*][1,4]benzodioxine (59). Starting from the lactone **47** (520 mg, 1.82 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **59** (407 mg, 1.44 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 79% yield. Anal. ($C_{17}H_{17}NO_3$) C, H, N.

1-(Benzyloxymethyl)-3-methylfuro[3,4-*b*][1,4]benzodioxine (60). Starting from the lactone **44** (535 mg, 1.72 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **60** (58 mg, 0.19 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 11% yield. Anal. ($C_{19}H_{16}O_4$) C, H.

1-Methyl-6-methoxy-3-pentylfuro[3,4-*b*][1,4]benzodioxine (61). Starting from the lactone **45** (823 mg, 2.83 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **61** (677 mg, 2.34 mmol) was obtained as an oil. The crude mixture was purified on silica gel column, eluting with hexane/ethyl acetate (98/2), to furnish the desired furan in 83% yield. Anal. ($C_{17}H_{20}O_4$) C, H.

1-Methyl-3-(4-bromobutyl)furo[3,4-*b*][1,4]benzodioxine (62). Starting from the lactone **43** (530 mg, 1.63 mmol) and following the general procedure using methyllithium (1.6 M in ether), the furan **62** (441 mg, 1.52 mmol) was obtained as an oil. The crude product was purified on silica gel column, eluting with hexane/

ethyl acetate (99/1), to furnish the desired furan in 84% yield. Anal. ($C_{15}H_{15}BrO_3$) C, H.

Biological Experimental

Biological Materials. Cell Culture and Cytotoxicity. L1210 leukemia and HT-29 cells were grown in nutrient medium RPMI 1640 or DMEM (Dulbecco's modified Eagle's medium, Sigma, St. Louis, MO), respectively, supplemented with 2 mM L-glutamine, 200 IU/mL penicillin, 50 μg/mL streptomycin, and 20% heat inactivated horse serum. They were incubated in a 5% CO_2 atmosphere at 37 °C. For the experiments, the drugs were dissolved in dimethyl sulfoxide (0.5% final) and added to the cells in exponential phase of growth at an initial concentration of 0.8×10^5 cells/mL. The cells were counted in quadruplicate after 48 h for L1210 cells and 96 h for HT-29 cells with Coultronics Coulter Counter, and results were expressed as the drug concentration that inhibited cell growth by 50% as compared to the controls (IC_{50}). The IC_{50} values were calculated from regression lines obtained from the probit of the percent cell growth inhibition plotted as a function of the logarithm of the dose. This experiment was performed four independents times in triplicate for every compound.

Inhibition of Cellular Proliferation and Cell Cycle Effects. L1210 leukemia cells were grown in nutrient medium RPMI 1640 supplemented with 2 mM L-glutamine, 200 IU/mL penicilium, 50 μg/mL streptomycin, and 20% heat inactivated horse serum. They were incubated in a 5% CO_2 atmosphere at 37 °C for 21 h with several drug concentrations. Cells were fixed by ethanol (70% v/v), then washed, and incubated with PBS containing 100 μM RNAse and 25 μM/mL propidium iodide for 30 min at room temperature. For each concentration, 10-4 cells were analyzed on an Epics XL flow cytomer (Modele Beckman Coulter, French). Results were expressed as a percentage of cells accumulated in each phase of the cell cycle and are indicated.

Effect on Topoisomerase I and II. The complete methodology to measure topoisomerase I and topoisomerase II inhibition by relaxation assay has been previously described.[21]

DNA Interaction. Sequence recognition was determined by DNase I footprinting using a protocol previously described in detail.[22]

Formation of Cleavage Complex. Topoisomerase II Inhibition. Topoisomerase II inhibition activity by **4a** and **13a** was measured by quantization of the formation of the cleavage complex on pYRG plasmid DNA by human topoisomerase II (purified from placenta).[19] The cleavage complex product, linear DNA, was quantized (relative to ellipticine) by densitometric analyses.[24]

Computational Methods

The molecular geometries of ellipticine and 4,11-dimethyl-1-oxo-3(*H*)-isobenzofuro[5,6-*b*][1,4]benzodioxine were optimized at the B3LYP/6-31G(d) level of theory. The B3LYP/6-31G(d) electron densities at the optimized geometries were fitted to spherical atomic shell approximation (ASA) expansions[25] and were used to perform the molecular alignment[26] of the two structures. Once aligned, the electron densities were computed at the finer level B3LYP/6-311G (d,p) to analyze and visualize the charge distributions and electrostatic potentials. The computation of the molecular geometries, electron densities, and electrostatic potentials was carried out using the Gaussian 03[27] suite of programs. The electron density alignment was produced with ASASim,[26] and the visualization figures were prepared with VMD[28] and OpenDX.[29]

Figures were arranged to display electron density isocontours at the aligned positions. A novel visualization technique for the analysis of similarity, that is, isocontour plots of the product of densities, was used. Briefly, the common pattern density $\rho_{CP}(\mathbf{r}) = \rho_A(\mathbf{r})\rho_B(\mathbf{r})$ on an information or similarity theoretic setting gives the simultaneous probability distribution for the two entities $\rho_A(\mathbf{r})$ and $\rho_B(\mathbf{r})$. If A and B were two equal and aligned molecules, $\rho_{CP}(\mathbf{r})$ would possess exactly the same set of isocontour surfaces as $\rho_A(\mathbf{r})$, with the values shifted and given by the product $\rho_A(\mathbf{r})\rho_A(\mathbf{r})$. In general, at the global maximum arrangement of the similarity

function, $\rho_{CP}(\mathbf{r})$ displays the largest common pattern (LCP) for the two molecules A and B.

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