Isomeric Kielcorins and Dihydroxyxanthones: Synthesis, Structure Elucidation, and Inhibitory Activities of Growth of Human Cancer Cell Lines and on the Proliferation of Human Lymphocytes *In Vitro*

by **Emília P. Sousa**[a][b], **Artur M. S. Silva**[c], **Madalena M. M. Pinto***[a], **Madalena M. Pedro**[a], **Fátima A. M. Cerqueira**[a], and **Maria S. J. Nascimento**[a]

[a]) Centro de Estudos de Química Orgânica, Fitoquímica e Farmacologia da Universidade do Porto, Faculdade de Farmácia, Rua Aníbal Cunha, 164, 4050-047, Porto, Portugal (fax: +351-222057358; e-mail: madalena@ff.up.pt)

[b]) Instituto Superior Ciências da Saúde-Norte, Rua Central da Gandra, 1317, 4580 Gandra, Portugal

[c]) Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

The synthesis, structure elucidation, and biological activities of five isomeric xanthonolignoids, (±)-*trans*-kielcorin C, (±)-*cis*-kielcorin C, (±)-*trans*-kielcorin D, (±)-*trans*-isokielcorin D, and (±)-*trans*-kielcorin E, are reported. The synthetic approach is based on the oxidative coupling of coniferyl alcohol with an appropriate xanthone. The influence of different oxidizing agents was studied, and the best results were obtained with potassium hexacyanoferrate(III). The structure elucidation was achieved by 2D-NMR techniques such as COSY, HETCOR, HSQC, and HMBC. Long-range C,H connectivities were used to establish the orientation of the substituents on the 1,4-dioxine rings, while NOE experiments were used to determine the configurations of these rings. These xanthonolignoids, as well as (±)-*trans*-kielcorin, (±)-*trans*-kielcorin B, (±)-*trans*-isokielcorin B, and the xanthonic building blocks 3,4-, 1,2-, and 2,3-dihydroxy-9*H*-xanthen-9-ones, and 2,3-dihydroxy-4-methoxy-9*H*-xanthen-9-one, were evaluated for their *in vitro* effect on the growth of three human cancer cell lines, MCF-7 (breast), TK-10 (renal), and UACC-62 (melanoma), and on the proliferation of human lymphocytes.

1. Introduction. – Natural kielcorins are xanthonolignoids isolated from several plants of Clusiaceae [1]. The biomimetic synthesis of kielcorin (**1**) [2] and kielcorin B (**2**) [3], as well as isokielcorin B (3) [3] (*Fig. 1*), by oxidative coupling of 3,4-dihydroxy-2-methoxy-9*H*-xanthen-9-one (**4**) and 2,3-dihydroxy-4-methoxy-9*H*-xanthen-9-one (**5**), respectively, with coniferyl alcohol (=4-(3-hydroxyprop-1-enyl)-2-methoxyphenol; **6**) was previously achieved (*Fig. 2*). These kielcorins, as well as their xanthonic building blocks, were assayed for their biological activities and exhibited interesting hepatoprotective activity against (*tert*-butyl)hydroperoxide-induced toxicity in isolated rat hepatocytes [4].

To obtain related bioactive compounds with a kielcorin framework, five constitutional isomers were designed. In this paper, the synthesis of *trans*- and *cis*-isomers of (±)-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-7*H*-1,4-dioxino-[2,3-*c*]xanthen-7-one, **7** and **8**, respectively, and the (±)-*trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-12*H*-1,4-dioxino[2,3-*a*]xanthen-12-one (**9**), as well as (±)-*trans*-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-12*H*-1,4-dioxino[2,3-*a*]xanthen-12-one (**10**), and (±)-*trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-11*H*-1,4-dioxino[2,3-*b*]xanthen-11-one (**11**) are reported (*Fig. 1*). The synthetic approach, based on a biomimetic pathway as a model,

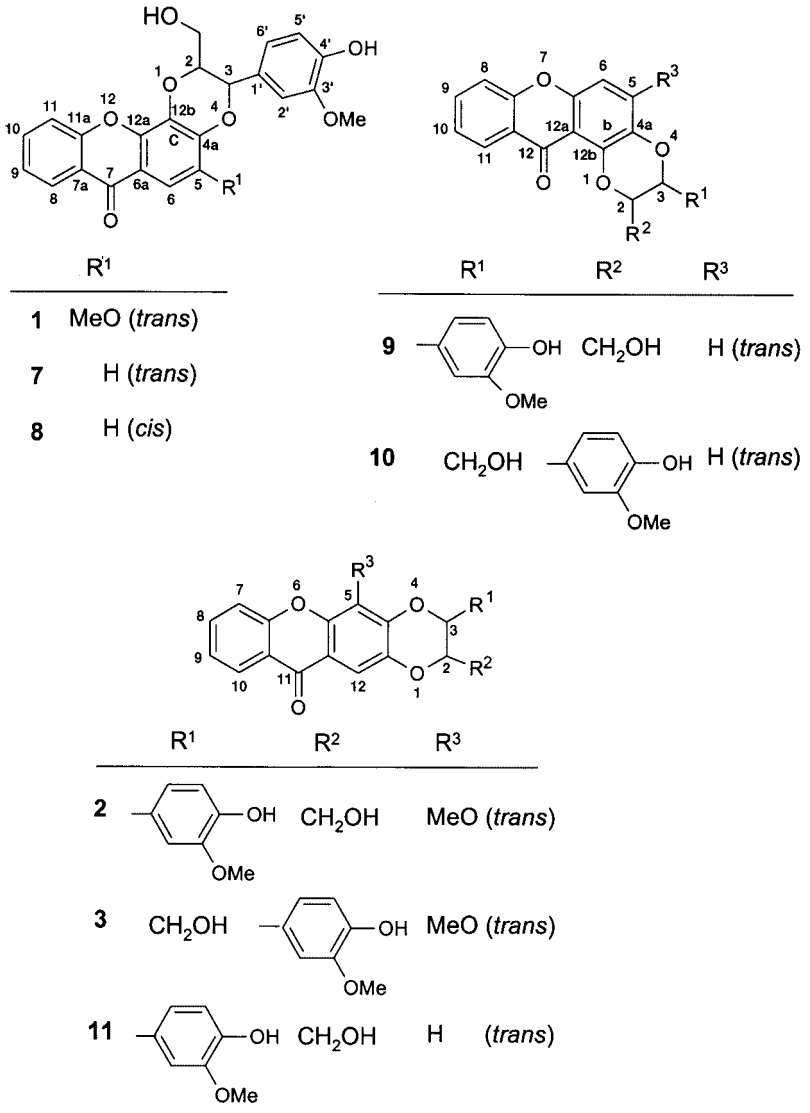
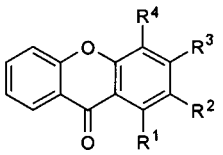
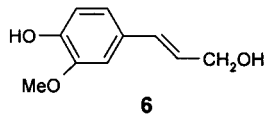


Fig. 1. *Structures of kielcorins* **1**–**3** *and* **7**–**11**

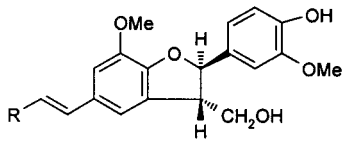
was by oxidative coupling of coniferyl alcohol (**6**) with an appropriate xanthone, 3,4-dihydroxy- (**12**), 1,2-dihydroxy- (**13**), and 2,3-dihydroxy-9*H*-xanthen-9-one (**14**), for the C, D, and E series of kielcorins, respectively (*Scheme 1*). Different oxidizing agents were used (*e.g.*, Ag_2O, Ag_2CO_3, and $K_3[Fe(CN)_6]$) to investigate the oxidative coupling reaction [5]. The total synthesis of demethoxykielcorin **7** has already been accomplished in low yield by a multi-step pathway [6]. Herein, the *cis*-isomer **8** was efficiently converted to the *trans*-isomer **7** [7].



	R^1	R^2	R^3	R^4
4	H	MeO	OH	OH
5	H	OH	OH	MeO
12	H	H	OH	OH
13	OH	OH	H	H
14	H	OH	OH	H



6



21 R=CHO

22 R= CH_2OH

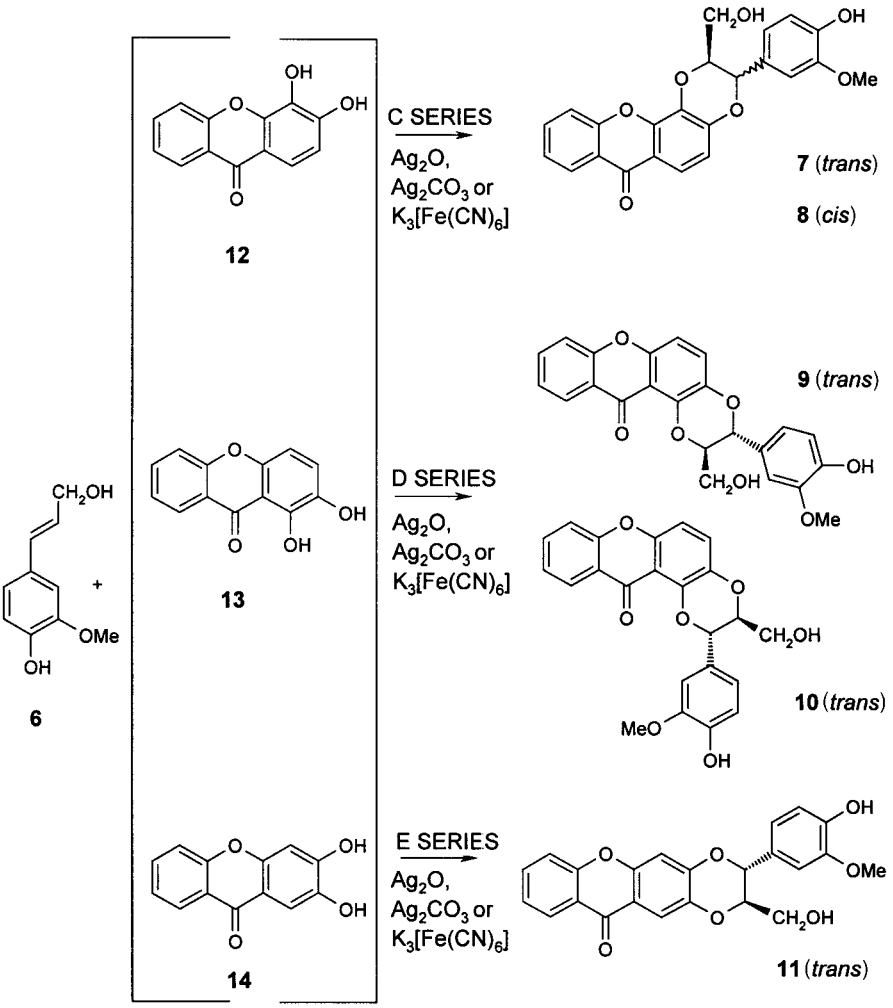
Fig. 2. *Structures of xanthones* **4**, **5** *and* **12**–**14**, *coniferyl alcohol* (**6**), *and the dimeric products* **21** *and* **22**

Few data are available for biological activities of kielcorins [4][8]. In this work, results of the effect of kielcorin derivatives **1**–**3** and **7**–**11**, and xanthones **5** and **12**–**14** on the growth of three human cancer cell lines MCF-7 (breast), TK-10 (renal), and UACC-62 (melanoma), as well as on the proliferation of human lymphocytes are described.

2. Results and Discussion. – 2.1. *Synthesis of Kielcorin Derivatives with Several Oxidizing Agents.* In a series of studies on the synthesis of bioactive lignans, flavonolignoids, and coumarinolignoids, several authors have investigated oxidative coupling reactions [5][9]. For simpler compounds, it was shown that the substituents on the catechol ring had a striking influence on the regioselectivity of the reaction; it was highly regioselective in cases where the catechol skeleton possessed electron-donating substituents and much less regioselective with catechols bearing electron-withdrawing substituents [9]. A free-radical coupling mechanism, with the first step being the intermolecular *O*-β coupling of two phenoxyl radicals, has been suggested [10]. Further studies on the synthesis of model compounds bearing a 3-aryl- or 2-aryl-1,4-benzodioxin skeleton achieved high regioselectivity by oxidative coupling in the presence of an Ag salt (oxide, carbonate) or $K_3[Fe(CN)_6]$, respectively [5a]. Based on these studies, an investigation was initiated to explore the regio- and stereoselectivity of the reaction with xanthones as one of the building blocks of benzodioxine rings that bear a C=O function as an electrophilic group and a heteroatom with electron-donating properties. It appeared reasonable to investigate the oxidative coupling in the presence of the same oxidizing agents (Ag_2O, Ag_2CO_3, and $K_3[Fe(CN)_6]$).

The synthesis of 1,2- and 2,3-dihydroxy-9*H*-xanthen-9-one, **13** and **14**, respectively (*Scheme 2*), involved as starting materials methyl 2-bromobenzoate (**15**) and 3,4-dimethoxyphenol (**16**); from the resulting methyl 2-(3,4-dimethoxyphenoxy)benzoate (**17**) [11], after hydrolysis and subsequent cyclization of 2-(3,4-dimethoxyphenoxy)-benzoic acid (**18**) with lithium diisopropylamide (LDA) [12] or with AcCl [13],

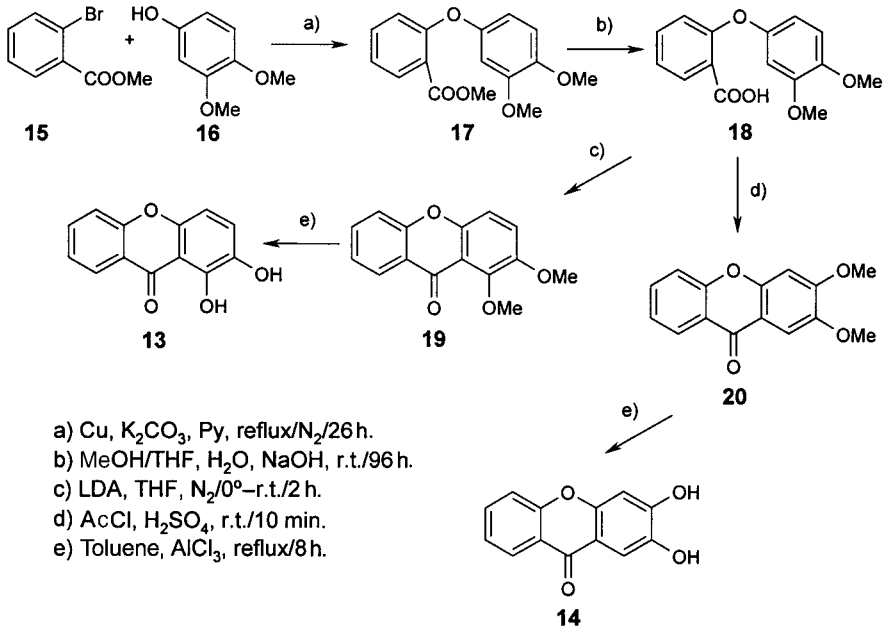
Scheme 1



compounds **19** and **20**, respectively, were obtained and demethylated to afford **13** and **14**, respectively. 3,4-Dihydroxy-9*H*-xanthen-9-one (**12**) was synthesized by a method described in [14]. For compounds **12**–**14**, and **20** are provided, for the first time, spectral data from INEPT, HETCOR, COSY, or HMBC experiments to clarify the structural elucidation of these compounds.

The oxidative coupling of the appropriate dihydroxyxanthones **12**–**14** and an equimolar amount of coniferyl alcohol (**6**) were carried out at room temperature, with 1.5 mol equiv. of Ag_2O or Ag_2CO_3, in toluene/Me_2CO, for 2–3 days. In the case of the other oxidizing agent, equimolar amounts of **12**–**14** and **6** were treated with 20 mol-equiv. of $K_3[Fe(CN)_6]$, at room temperature, in a mixture of Me_2CO/H_2O and an aqueous 4% AcONa solution (*Scheme 1*).

Scheme 2



The xanthonolignoid distribution in the crude products of the oxidative coupling reactions under the conditions indicated above is summarized in *Table 1*. The isomeric nature of the kielcorin derivatives obtained did not differ with the oxidizing agents: for the C series, (±)-*trans*-kielcorin C (**7**) and (±)-*cis*-kielcorin C (**8**) were obtained for all the oxidizing agents; the D series gave two isomeric xanthonolignoids, (±)-*trans*-kielcorin D (**9**) and (±)-*trans*-isokielcorin D (**10**); and the E series afforded (±)-*trans*-kielcorin E (**11**). The regioselectivity achieved with Ag salts or with $K_3[Fe(CN)_6]$ for simpler compounds was not verified for compounds **7**–**11**. In the C series, none of the oxidizing agents used were stereoselective, although, when Ag_2O and Ag_2CO_3 was used, a lower amount of the *cis*-isomer **8** was obtained. Two dimeric compounds, phenylcoumaran **21** and dehydrodiconiferyl alcohol **22**, were also found as by-products (*Fig. 2*) in all reactions [3]. By carrying out the oxidative coupling with $K_3[Fe(CN)_6]$, the quantities of **21** and **22** were markedly reduced, and a higher yield of the kielcorins was obtained.

The most efficient method involved the use of $K_3[Fe(CN)_6]$ as oxidizing agent in the oxidative coupling reaction (*Table 1*). Therefore, in the C series, the mixture of isomer **7** (*trans*) and **8** (*cis*) thus obtained was treated with anhydrous K_2CO_3 [7] to yield isomer **7**, exclusively (*Scheme 3*).

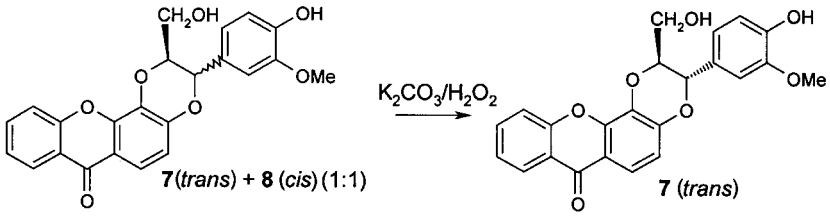
The position of the OH substituents had a striking effect on the nature of the kielcorin isomers obtained: when 3,4-dihydroxy-9*H*-xanthen-9-one (**12**) was used as a building block, a mixture of isomers **7** (*trans*) and **8** (*cis*) was formed; with 1,2-dihydroxy-9*H*-xanthen-9-one (**13**), the *trans*-isomers **9** and **10** were obtained, whereas 2,3-dihydroxy-9*H*-xanthen-9-one (**14**) gave only the *trans*-isomer **11**. The electron-

Table 1. *Distribution of Xanthonolignoids* **7**–**11** *in the Crude Reaction Products*

Compound	Yields [%]		
	Ag_2O	Ag_2CO_3	$K_3[Fe(CN)_6]$
7[a])	20	17	29
8[a])	3.5	3.0	11
9[b])	22	20	27
10[b])	0.7	0.4	0.8
11[c])	3.0	2.0	4.0

[a])[b])[c]) HPLC conditions: *C_{18}-Nucleosil* column, 25°, 1 ml/min. [a]) MeOH/1% AcOH in H_2O 6:4, 239 nm. [b]) MeCN/1% AcOH in H_2O 5:5, 239 nm. [c]) MeOH/1% AcOH in H_2O 7:3, 241 nm.

Scheme 3



withdrawing and electron-donating effects of the C=O group, and the heteroatom of these xanthones **12**–**14** could influence the electronic character of the radical formed on positions 3,4, 1,2, and 2,3 of the xanthone in different ways. The involvement of the olefinic C=C bound in the formation of an Ag complex can also be an explanation for this kind of oxidative coupling reaction and deserves further investigation.

2.2. *Structure Elucidation of Kielcorins.* The analysis of the ^{1}H- and COSY-NMR spectra of xanthonolignoids **7**–**11** allowed the assignment of the *ABCD* spin system corresponding to the resonances of H–C(8), H–C(9), H–C(10), and H–C(11) (in **7**–**10**) and H–C(7), H–C(8), H–C(9), and H–C(10) (in **11**). H–C(8) (H–C(11) in **9** and **10** and H–C(10) in **11**) (δ 8.04–8.20 ppm) is the more deshielded of these protons, due to the mesomeric and anisotropic deshielding effect of the C=O group, followed by H–C(10) in **7** and **8**, H–C(9) in **9** and **10**, and H–C(8) in **11** (δ 7.80–7.87 ppm), which only feels the mesomeric effect. The assignment of the corresponding ^{13}C resonances were made from the correlations found in the HETCOR or HSQC spectra, whereas those of the quaternary C-atoms of the unsubstituted ring were based on the connectivities found in the HMBC and on the one-dimensional selective INEPT experiments [15] of these compounds (H–C(8) → C(11a), C(7), C(10); H–C(11) and H–C(9) → C(7a)) (**a** and **b**; *Fig. 3*).

Due to the presence of electron-donating substituents on the 1,3,4-trisubstituted phenyl rings, originated from the coniferyl alcohol, their ^{1}H resonances were the more shielded ones (H–C(2′): 7.04–7.10 ppm; H–C(5′): 6.80–6.83 ppm; H–C(6′): 6.89–6.90 ppm). The corresponding protonated ^{13}C resonances were assigned based on the correlations found in the HETCOR or HSQC spectra, whereas those of the quaternary C-atoms were based on the connectivities found in their HMBC spectra (H–C(5′) and H–C(3) → C(1′); H–C(5′) → C(3′); H–C(2′) → C(4′) and C(3) (**a** and **b**; *Fig. 3*).

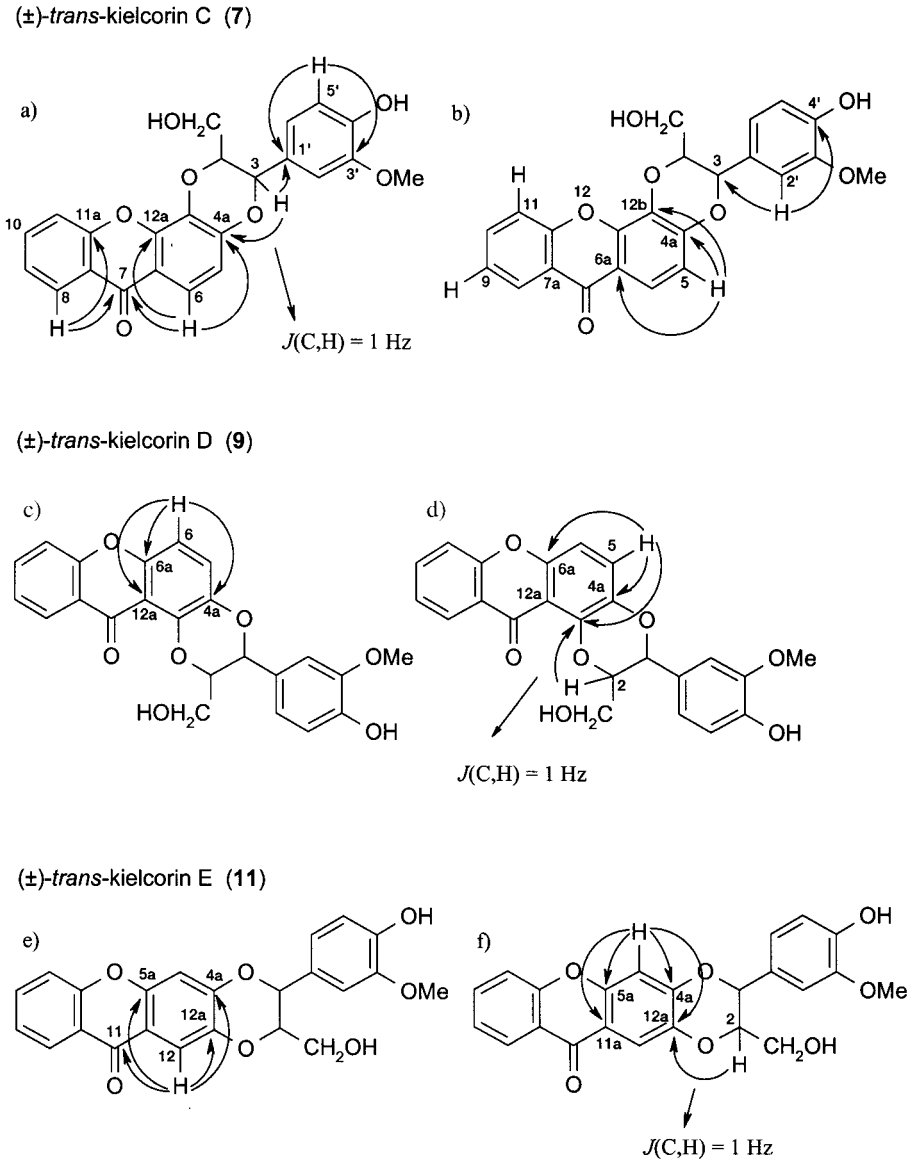


Fig. 3. *Main connectivities found in the HMBC and in the one-dimensional selective INEPT experiments of xanthonolignoids* **7**, **9**, *and* **11**

The major differences among the NMR spectra of the xanthonolignoids synthesized are due to the ^{1}H and ^{13}C resonances of the ring bearing the 'benzodioxane' moiety. In the case of (±)-*trans*-kielcorin C (**7**), H–C(5) and H–C(6) appear as two *doublets*, and the connectivities found in the HMBC allowed the assignment of the quaternary C-atoms C(4a), C(12a), C(12b), and C(6a) as depicted in *Fig. 3* (*a* and *b*). However, the

unequivocal identification of the structure of compound **7** as (±)-*trans*-kielcorin C was accomplished from the one-dimensional selective INEPT [15]. Upon H–C(3) irradiation, with a long-range C,H coupling constant optimized for 1 Hz [15b], enhancements on the C(4a) resonance was observed, allowing the establishment of the connection of the 1,4-dioxane moiety to the xanthone ring. The assignments of all of the ^{1}H and ^{13}C resonances of the substituted ring of the compounds **8**–**11**, and the connection of their 1,4-dioxane moiety were deduced by a similar procedure (*c*–*f*, *Fig. 3*).

In the case of (±)-*trans*-isokielcorin D (**10**), the one-dimensional selective INEPT did not allow the establishment of any connectivity between H–C(2) or H–C(3), and the C-atoms of the xanthone ring. However, a comparison of the ^{1}H and ^{13}C resonances on 1,4-dioxane ring with those of (±)-*trans*-kielcorin D (**9**) shows the downfield shifts of C(2) of **10** relative to C(3) of **9**. Comparable downfield shifts of C(2) of **9** relative to C(3) of **10** are also observed (*Fig. 4*). In both cases, the downfield shifts are clearly caused by the strong deshielded mesomeric effect exerted by the C=O group, through the O-substituent at C(12b) of both structures [3].

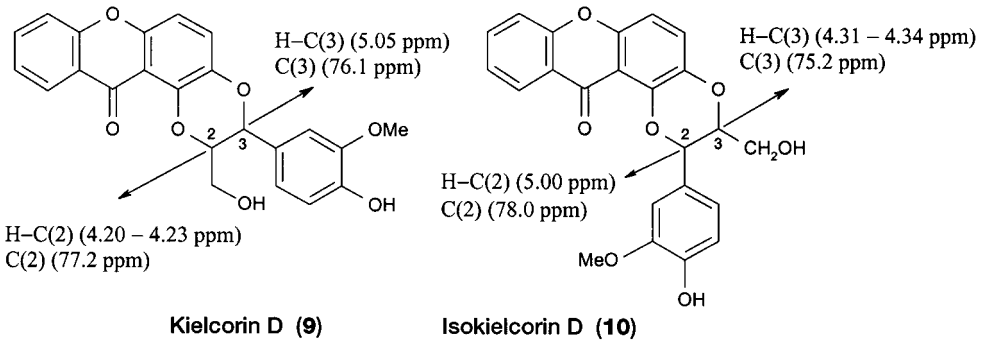


Fig. 4. *Comparison of the ^{1}H and ^{13}C resonances of the 1,4-dioxane of (±)*-trans-*kielcorin D* (**9**) and *(±)*-trans-*isokielcorin D* (**10**)

Taking into consideration the coupling constant values $^3J(2,3) = 7.6–8.1$ Hz in the ^{1}H-NMR spectra of xanthonolignoids **7**, **9**–**11**, and our previous report [3] allowed the establishment of a *trans*-configuration for these two H-atoms. The absence of a NOE effect between these two H-atoms supported this configurational assignment.

In the course of the synthesis of *trans*-kielcorin C (**7**), another compound was isolated with similar structural features. The main difference is the coupling constant value of $^3J(2,3) = 2.8$ Hz, which suggests a *cis*-configuration for these two H-atoms. This configuration was confirmed by the observed NOE enhancement (6%) in the H–C(3) resonance on irradiation of H–C(2), supporting their close proximity. This compound is named *cis*-isokielcorin C (**8**).

2.3. *Biological Activities. Table 2* summarizes the *in vitro* effects of (±)-*trans*-kielcorin (**1**), (±)-*trans*-kielcorin B (**2**), (±)-*trans*-isokielcorin B (**3**), (±)-*trans*-kielcorin C (**7**), (±)-*cis*-kielcorin C (**8**), (±)-*trans*-kielcorin D (**9**), (±)-*trans*-isokielcorin D (**10**), (±)-*trans*-kielcorin E (**11**) and of the four xanthonic building blocks 2,3-dihydroxy-4-methoxy-9*H*-xanthen-9-one (**5**), 3,4-, 1,2-, and 2,3-dihydroxy-

Table 2. *Effects of Xanthone derivatives* **1**–**3**, **5**, *and* **7**–**14** *on the Growth of Human Cancer Cell Lines and Proliferation of Human Lymphocytes*

Compound	GI_{50} [μM][a])[b])			IC_{50} [μM][a])[c])
	MCF-7 (Breast)	TK-10 (Renal)	UACC-62 (Melanoma)	Human lymphocytes
1	53.5 ± 8.0	72.1 ± 5.6	36.6 ± 2.4	> 100
5	37.2[d])	76.6 ± 7.0	19.8 ± 1.6	17.4 ± 2.7
2	62.5 ± 13.3	88.5 ± 8.6	80.8 ± 11.6	> 100
3	16.2 ± 3.4	30.9 ± 5.0	25.4 ± 1.3	41.9 ± 1.6
12	40.5 ± 1.5	59.2[d])	21.6 ± 2.6	12.2 ± 1.3
7	18.3 ± 0.7	25.9 ± 2.7	26.2 ± 1.3	36.2 ± 7.4
8	32.0 ± 2.4	45.2 ± 7.8	33.3 ± 2.1	48.6 ± 6.1
13	38.4 ± 2.7	65.8 ± 5.1	14.0 ± 0.3	73.3 ± 2.2
9	> 100	97.5 ± 10.5	> 100	> 100
10	82.8 ± 4.3	> 100	> 100	> 100
14	40.6 ± 1.3	61.4 ± 4.3	31.7 ± 3.7	31.2 ± 1.5
11	21.2 ± 8.2	23.1 ± 0.5	21.9 ± 3.9	> 100

[a]) Results are expressed as GI_{50} (concentrations of compounds that cause 50% inhibition of cancer cell growth) or IC_{50} (concentrations that cause 50% inhibition of lymphocytes proliferation), and show means ± SEM of 3–6 independent observations performed in duplicate. [b]) Doxorubicin was used as positive control in cancer cell lines growth (GI_{50} (MCF-7) = 42.8 ± 8.2 nM; GI_{50} (TK-10) = 548.0 ± 60.0 nM; GI_{50} (UACC-62) = 94.0 ± 9.4 nM). [c]) Cyclosporin A was used as positive control in lymphocyte proliferation (IC_{50} = 0.34 ± 0.04 μM). [d]) Data based on two independently run duplicate experiments.

9*H*-xanthen-9-ones **12**–**14** on the growth of the human cancer cell lines MCF-7, TK-10, UACC-62, and on the proliferation of human lymphocytes.

2.3.1. *Effect on the Growth of Human Cancer Cell Lines.* An inhibition of the growth of the human cancer cell lines was observed with almost all kielcorins and oxygenated xanthones studied. The growth inhibitory effect was, in general, moderate but was shown to be dose-dependent and due to growth arrest and not to cell death, as inferred from the sulforhodamine B (SRB) assay.

Considering the selectivity, the results in *Table 2* showed that 2,3-dihydroxy-4-methoxy-9*H*-xanthen-9-one (**5**), and 3,4- and 1,2-dihydroxy-9*H*-xanthen-9-ones (**12** and **13**, resp.) are significantly ($P < 0.05$) more active against UACC-62 than to MCF-7 and TK-10 cell lines.

The comparison between the effects of 3,4- and 2,3-dihydroxy-9*H*-xanthen-9-ones (**12** and **14**, resp.) with those of the (±)-*trans*-kielcorin derivatives **7** and **11**, respectively, revealed a significant ($P < 0.05$) increase of activity in MCF-7 and TK-10 cell lines. No significant difference was observed for UACC-62 ($P > 0.05$). On the contrary, compounds **2** and **9** were associated with a decrease and loss of growth-inhibitory activity, respectively, in all of the cancer cell lines when compared with their xanthonic building blocks **5** and **13**.

The isomerism of the tested kielcorins **7**–**11** seemed to influence the growth-inhibitory activity of the human cancer cell lines: the kielcorins of the C (**7** and **8**) and E (**11**) series were significantly more active ($P < 0.005$) than their D isomers (**9** and **10**). In the *cis*/*trans* pair, it is interesting to note that (±)-*trans*-kielcorin C (**7**) was shown to be a more potent inhibitor than its *cis*-isomer **8** ($P < 0.05$). Also, (±)-*trans*-isokielcorin

B (**3**) was associated with a significantly ($P < 0.05$) stronger effect compared with its constitutional isomer (±)-*trans*-kielcorin B (**2**).

It is also interesting to note that the absence of the MeO substituent in the xanthonic framework was associated with a significant ($P < 0.05$) increase of growth inhibitory activity as revealed by kielcorins **7** and **11**, relative to those of **1** and **2**, respectively.

2.3.2. *Effect of Kielcorins on Human Lymphocytes Proliferation*. Inhibition of human lymphocyte proliferation induced by phytohemagglutinin (PHA) was detected with all of the oxygenated xanthones **5** and **12**–**14**, and with (±)-*trans*-isokielcorin B (**3**) and the kielcorins in C series, **7** and **8**. The antiproliferative activity detected was dose-dependent and could not be attributed to a toxic effect on lymphocytes as demonstrated by the lymphocytoxicity assay (viability of exposed lymphocytes > 70%). (±)-*trans*-Kielcorin (**1**) and compounds in D and E series, **9**–**11**, showed no inhibitory effect even when tested at 100 μM.

Comparing the antiproliferative activity of the dihydroxy-9*H*-xanthen-9-ones, it was concluded that the position of the *ortho*-dihydroxy groups influence the suppresser effect of these compounds (*Table 1*). Positions 3,4- were associated with a significantly ($P < 0.01$) stronger antiproliferative effect when compared with the 1,2- and 2,3-positions. Kielcorins **3**, **7**, and **8** exhibited a significantly ($P < 0.05$) less-potent suppresser effect than that of their xanthonic building blocks **5** and **12**.

3. Conclusions. – In summary, the synthesis of five new structures based on models of natural kielcorins is reported. For this kind of compound, the most efficient method in the oxidative coupling reaction involves the use of $K_3[Fe(CN)_6]$ as oxidizing-agent. Long-range C,H connectivities and NOE experiments permit unambiguous establishment of the structures of the different isomers. The compounds of the C and E series are the most active for the inhibition of the growth activity. For the inhibition of human lymphocyte proliferation, generally, the kielcorins are less potent than their xanthonic building blocks. The differences in the isomerism and in the nature of the substituents in the xanthonolignoid framework will allow us to evaluate the structure features, and further modifications are underway to improve the activity of these compounds.

The authors thank *FCT* (I & D No. 226/94 and No. 62/94), *POCTI* (QCA III), and *FEDER* for financial support, and to *PRAXIS XXI* and *FCT* for the Ph.D. grants to *M. M. P.* (SFRH/BD/1456/2000), *F. A. M. C.* (PRAXIS XXI/BD/21801/99), and to *E. P. S.* (PRAXIS XXI/BD/15663/98). We are indebted to the *National Cancer Institute*, Bethesda, MD, USA, for the generous provision of the cancer cell lines and to Dr. *G. Eaton*, Department of Chemistry, University of Leicester, UK, for HR-MS measurements.

Experimental Part

1. *General*. Column chromatography (CC): *Merck* silica gel *60* (0.50–0.20 mm). Flash chromatography (FC): *Merck* silica gel *60* (0.040–0.063 mm). Anal. and prep. TLC: *Merck* silica gel *60* (GF_{254}). The reactions were monitored by TLC ($CHCl_3$/MeOH) and interrupted, when one of the reagents was no longer detectable by TLC. Ag_2CO_3, AcONa, and $K_3[Fe(CN)_6]$ were supplied by *Merck* (D-Darmstadt). Coniferyl alcohol and Ag_2O were obtained from *Sigma* (D-Steunheim). Lithium diisopropylamide (LDA) soln. was purchased from *Fluka* (D-Neu-Ulm). Structure elucidations of phenylcoumaran (**21**) and dehydrodiconiferyl alcohol (**22**; *Fig. 2*) were accomplished by comparison with authentic samples. Reversed-phase (RP) HPLC: *Jasco 880-PU*, equipped with a *Jasco 875-UV* detector, and a *CSW 1.7* integrator; *Nucleosil-C_{18}* column (250 × 4.6 mm, 5 μm; *Macherey-*

Nagel, D-Düren) equipped with a guard pre-column (*Macherey-Nagel*); linear gradient for compounds **7** and **8** with MeOH/1% AcOH in H_2O 6:4, for compounds **9** and **10** with MeCN/1% AcOH in H_2O 5:5, and for compound **11** with MeOH/1% AcOH in H_2O 7:3; at 25°; flow rate of 1 ml/min; sample injection volume 20 µl; detection at 239 nm for kielcorins **7**–**10** and 241 nm for kielcorin **11**; t_R in min. M.p. *Kofler* microscope; uncorrected. The optical rotation was zero for compounds **7**–**11** and was obtained on a *Polartronic Universal* polarimeter. IR Spectra: *Perkin-Elmer 257* spectrophotometer; KBr microplates; in cm^{-1}. UV Spectra: *Varian CARY 1E* spectrophotometer; λ_{max} in nm. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AC-200, DRX-300*, or *DRX-500* spectrometer; at r.t., $CDCl_3$ or (D_6)DMSO soln.; δ in ppm relative to Me_4Si as an internal reference; coupling constants J in Hz. ^{1}H-NMR assignments were made by means of 2D COSY experiments, while ^{13}C-NMR assignments were made by 1D selective INEPT and 2D HETCOR, HSQC, and HMBC experiments (long-range C,H coupling constants were optimized to 7 and 1 Hz). NOE Experiments were conducted by means of NOE difference technique, with an irradiation time of 2 s and a relaxation delay of 4 s. EI-MS Spectra: *Hitachi Perkin-Elmer RMU-6M* spectrometer, in m/z. HR-MS Spectra: *VG AutoSpec-Q* spectromer; recorded as EI (electronic impact) mode.

Fetal bovine serum and *RPMI-1640* medium were obtained from *Gibco BRL*. Cyclosporin A, DMF, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DMSO, doxorubicin hydrochloride, gentamicin, L-glutamine, *Histopaque-1077*, phytohemagglutinin (PHA), sodium lauryl sulfate (SDS), and sulforhodamine B (SRB) were purchased from *Sigma*. *National Cancer Institute* (Bethesda, MD, USA) kindly provided the cancer cell lines. Stock solns. of compounds were prepared in DMSO and stored at −20° providing uniform samples for retesting. These frozen concentrates were then diluted to the desired final concentrations with the appropriate diluents immediately prior the biological assays. Final concentrations of DMSO showed no interference with the biological activities tested. Unpaired Student's *t*-tests were used. Differences with P values below 0.01 or 0.05 were considered statistically significant. Whenever $GI_{50} > 100$ µM, values of 100 µM were considered to evaluate the statistical significance of differences.

2. *Synthesis of Kielcorins* **7**–**11**. *General Procedure for the Oxidative Coupling Reaction of Dihydroxy-9H-xanthen-9-ones* **12**–**14** *and Coniferyl Alcohol* (**6**). *Method A: with* a) Ag_2O *and* b) Ag_2CO_3. The appropriate dihydroxyxanthones **12**–**14** (615 mg, 2.7 mmol) and **6** (500 mg, 2.7 mmol) were taken up in toluene/Me_2CO 1:1 (50 ml) and stirred in the presence of 3.1 mmol of the oxidizing agent (*a*) 815 mg of Ag_2O or *b*) 860 mg of Ag_2CO_3), at r.t., in the dark for 2–3 days. The suspension was filtered, the filtrate evaporated, and the crude product was analyzed by HPLC. The crude reaction products were purified by CC with a mixture of hexane/$CHCl_3$/MeOH (several proportions). The isolation of the components was then carried out by prep. TLC ($CHCl_3$/MeOH 95:5). HPLC: t_R (% yield Ag_2O, Ag_2CO_3): 17.2 (20, 17) **7**, 20.2 (3.5, 3.0) **8**, 4.9 (22, 20) **9**, 8.4 (0.7, 0.4) **10**, 12.8 (3.0, 2.0) **11**.

Method B: with $K_3[Fe(CN)_6]$. To a stirred soln. of the appropriate dihydroxyxanthones **12**–**14** (315 mg, 1.4 mmol) and **6** (250 mg, 1.4 mmol) in Me_2CO/H_2O 1:1 (60 ml), AcONa (0.9 g, 11 mmol, in 25 ml of H_2O) and then $K_3[Fe(CN)_6]$ soln. (1.0 g, 30 mmol in 25 ml of H_2O) were added at r.t. After the reaction, the mixture was slightly acidified with 10% HCl and diluted with H_2O. The product was extracted with CH_2Cl_2; the extract was washed with H_2O, dried, and the solvent was evaporated to dryness. The crude product was analyzed by HPLC. The isolation of the components, was then carried out by prep. TLC ($CHCl_3$/MeOH 95:5). HPLC: t_R (% yield): 17.2 (29) **7**, 20.2 (11) **8**, 4.9 (27) **9**, 8.4 (0.8) **10**, 12.8 (4.0) **11**.

*Conversion of (±)-*cis-*Kielcorin C* (**8**) *to (±)-*trans-*Kielcorin C* (**7**). To the mixture of **7** and **8** obtained with $K_3[Fe(CN)_6]$ as described above (409.6 mg), H_2O (200 ml) was added, and the suspension was alkalinized with K_2CO_3. The suspension was stirred at r.t. for 1 h, until conversion of *cis*-isomer **8** to the *trans*-isomer **7** was complete. The mixture was extracted with AcOEt, washed with H_2O, dried (Na_2SO_4), filtered, and concentrated in vacuum to reveal a white solid (407.2 mg, 99%).

*(±)-(2*R*,3*R*)- and (±)-(2*S*,3*S*)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-7H-1,4-dioxino[2,3-c]xanthen-7-one* (*(±)-*trans-*kielcorin C*; **7**). M.p. 243–247° (Me_2CO). UV (EtOH): 309, 283, 239, 204. IR (KBr): 3398, 1642, 1605, 1562, 1449, 1338, 1264, 1025, 861. ^{1}H-NMR (300.13 MHz, (D_6)DMSO): 9.25 (*s*, HO–C(4′)); 8.20 (*dd*, J = 7.7, 1.6, H–C(8)); 7.87 (*ddd*, J = 8.3,7.4, 1.6, H–C(10)); 7.70 (*d*, J = 8.8, H–C(6)); 7.69 (*d*, J = 8.3, H–C(11)); 7.49 (*ddd*, J = 7.7, 7.4, 0.7, H–C(9)); 7.07 (*d*, J = 8.8, H–C(5)); 7.07 (*d*, J = 1.7, H–C(2′)); 6.92 (*dd*, J = 8.1, 1.7, H–C(6′)); 6.83 (*d*, J = 8.1, H–C(5′)); 5.15 (*d*, J = 7.9, H–C(3)); 5.13 (*t*, J = 6.1, CH_2OH); 4.36–4.40 (*m*, H–C(2)); 3.79 (*s*, MeO); 3.71–3.76 (*m*, CH_2OH); 3.40–3.50 (*m*, CH_2OH). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 175.1 (C(7)); 155.4 (C(11a)); 148.8 (C(4a)); 147.7 (C(3′)); 147.2 (C(4′)); 145.9 (C(12a)); 135.2 (C(10)); 131.8 (C(12b)); 126.7 (C(1′)); 126.0 (C(8)); 124.5 (C(9)); 121.0 (C(7a)); 120.7 (C(6′)); 118.1 (C(11)); 117.5 (C(6)); 115.7 (C(6a)); 115.4 (C(5′)); 113.9 (C(5)); 111.8 (C(2′)); 78.0 (C(2)); 76.5 (C(3)); 59.9 (CH_2OH); 55.7 (MeO). EI-MS: 406 (6, M^+), 356 (26), 341 (22), 327 (17), 306 (18), 292 (12), 281

(100), 261 (38), 253 (5), 228 (13), 215 (8), 180 (10), 165 (8), 141 (10), 115 (18), 105 (9), 91 (17), 77 (18), 63 (10), 55 (13). Anal. calc. for $C_{23}H_{19}O_7$: 407.1131; found: 407.1130.

(±)-(2S,3R*)- and (±)-(2R*,3S*)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-7H-1,4-dioxino[2,3-c]xanthen-7-one* (*(±)*-cis-*Kielcorin C*; **8**). M.p. 200–202° (Me_2CO). UV (EtOH): 308, 283, 239, 204. IR (KBr): 3420, 1644, 1607, 1511, 1454, 1379, 1267, 1100, 860. ^{1}H-NMR (300.13 MHz, (D_6)DMSO): 9.09 (*s*, HO–C(4′)); 8.21 (*dd*, *J* = 7.6, 1.6, H–C(8)); 7.83 (*ddd*, *J* = 8.0, 7.5, 1.6, H–C(10)); 7.75 (*d*, *J* = 8.7, H–C(6)); 7.67 (*d*, *J* = 8.0, H–C(11)); 7.46 (*dd*, *J* = 7.6, 7.5, H–C(9)); 7.05 (*d*, *J* = 8.8, H–C(5)); 6.99 (br. *s*, H–C(2′)); 6.95–6.74 (*m*, H–C(5′), and H–C(6′)); 5.46 (*d*, *J* = 2.8, H–C(3)); 4.98 (*t*, *J* = 5.4, CH_2OH); 4.63–4.68 (*m*, H–C(2)); 3.83 (*s*, MeO); 3.42–3.61 (*m*, CH_2OH). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 175.5 (C(7)); 155.9 (C(11a)); 148.7 (C(4a)); 147.8 (C(3′)); 147.1 (C(4′)); 146.7 (C(12a)); 135.2 (C(10)); 131.1 (C(12b)); 126.7 (C(1′)); 126.4 (C(8)); 124.6 (C(9)); 121.5 (C(7a)); 119.5 (C(6′)); 118.4 (C(11)); 118.2 (C(6)); 116.4 (C(6a)); 115.9 (C(5′)); 114.1 (C(5)); 111.4 (C(2′)); 77.8 (C(2)); 76.4 (C(3)); 58.6 (CH_2OH); 56.0 (MeO). EI-MS: 406 (19, M^+), 281 (1.8), 246 (24), 184 (14), 153 (100), 149 (8), 137 (17), 111 (1.3), 93 (60), 83 (22), 72 (8), 65 (38), 59 (14), 57 (41). Anal. calc. for $C_{23}H_{19}O_7$: 407.1131; found: 407.1131.

(±)-(2R,3R*)- and (±)-(2S*,3S*)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxyphenyl)-12H-1,4-dioxino[2,3-a]xanthen-12-one* (*(±)*-trans-*Kielcorin D*; **9**). M.p. 258–260° (Me_2CO). UV (EtOH): 372, 278, 259, 239, 237, 212. IR (KBr): 3498, 1635, 1602, 1309, 1270, 1228, 1047, 762. ^{1}H-NMR (300.13 MHz, (D_6)DMSO): 9.20 (*s*, HO–C(4′)); 8.04 (*dd*, *J* = 7.9, 1.6, H–C(11)); 7.80 (*ddd*, *J* = 8.1, 7.8, 1.6, H–C(9)); 7.56 (*d*, *J* = 8.1, H–C(8)); 7.45 (*d*, *J* = 9.1, H–C(5)); 7.40 (*dd*, *J* = 7.9, 7.8, H–C(10)); 7.14 (*d*, *J* = 9.1, H–C(6)); 7.10 (*d*, *J* = 1.7, H–C(2′)); 6.92 (*dd*, *J* = 8.1, 1.7, H–C(6′)); 6.83 (*d*, *J* = 8.1, H–C(5′)); 5.05 (*d*, *J* = 7.6, H–C(3)); 5.05 (*t*, *J* = 7.6, CH_2OH); 4.20–4.23 (*m*, H–C(2)); 3.78 (*s*, MeO); 3.57–3.62 (*m*, CH_2OH); 3.41 (under H_2O signal, CH_2OH). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 174.9 (C(12)); 154.6 (C(7a)); 150.9 (C(6a)); 147.6 (C(3′)); 147.0 (C(4′)); 143.1 (C(12b)); 138.9 (C(4a)); 134.8 (C(9)); 127.1 (C(1′)); 125.9 (C(11)); 124.0 (C(10)); 123.7 (C(5)); 121.7 (C(11a)); 120.5 (C(6′)); 117.6 (C(8)); 115.3 (C(5′)); 111.92, 111.86 (C(12a), C(2′)); 109.5 (C(6)); 77.2 (C(2)); 76.1 (C(3)); 60.0 (CH_2OH); 55.7 (MeO). EI-MS: 406 (6, M^+), 369 (2), 327 (7), 285 (6), 228 (14), 184 (22), 153 (100), 137 (10), 125 (13), 110 (9), 93 (61), 81 (9), 65 (35). Anal. calc. for $C_{23}H_{19}O_7$: 407.1131; found: 407.1130.

(±)-(2R,3R*)- and (±)-(2S*,3S)-2,3-Dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-12H-1,4-dioxino[2,3-a]xanthen-12-one* (*(±)*-trans-*Isokielcorin D*; **10**). M.p. 136–139° (Me_2CO). UV (EtOH): 374, 280, 260, 240, 209. IR (KBr): 3436, 1626, 1520, 1459, 1383, 1319, 1269, 1110, 1060, 1028, 809. ^{1}H-NMR (500.13 MHz, (D_6)DMSO): 9.20 (*s*, HO–C(4′)); 8.12 (*dd*, *J* = 7.8, 1.4, H–C(11)); 7.81 (*ddd*, *J* = 8.1, 7.4, 1.4, H–C(9)); 7.57 (*d*, *J* = 8.1, H–C(8)); 7.43 (*d*, *J* = 9.1, H–C(5)); 7.43 (*dd*, *J* = 7.8, 7.4, H–C(10)); 7.12 (*d*, *J* = 9.1, H–C(6)); 7.04 (*d*, *J* = 1.4, H–C(2′)); 6.89 (*d*, *J* = 8.1, 1.4, H–C(6′)); 6.80 (*d*, *J* = 8.1, H–C(5′)); 5.00 (*d*, *J* = 7.8, H–C(2)); 4.99 (*t*, *J* = 7.6, CH_2OH); 4.31–4.34 (*m*, H–C(3)); 3.77 (*s*, MeO); 3.71–3.73 (*m*, CH_2OH); 3.41 (under H_2O signal, CH_2OH). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 174.6 (C(12)); 154.5 (C(7a)); 150.0 (C(6a)); 147.7 (C(3′)); 147.3 (C(4′)); 143.2 (C(12b)); 139.2 (C(4a)); 135.0 (C(9)); 127.3 (C(1′)); 126.2 (C(11)); 123.8 (C(5)); 123.7 (C(10)); 122.3 (C(11a)); 120.8 (C(6′)); 117.7 (C(8)); 115.1 (C(5′)); 111.7 (C(12a), C(2′)); 107.9 (C(6)); 78.0 (C(3)); 75.2 (C(2)); 59.4 (CH_2OH); 55.4 (MeO). EI-MS: 406 (50, M^+), 239 (7), 228 (100), 199 (25), 180 (73), 170 (11), 153 (18), 137 (64), 124 (44), 103 (5), 91 (16), 77 (14), 65 (10). Anal. calc. for $C_{23}H_{19}O_7$: 407.1131; found: 407.1131.

(±)-(2R,3S*)- and (±)-(2S*,3R)-2,3-Dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-11H-1,4-dioxino[2,3-b]xanthen-11-one* (*(±)*-trans-*Kielcorin E*; **11**). M.p. 247–249° (Me_2CO). UV (EtOH): 353, 307, 277, 241. IR (KBr): 3498, 1635, 1602, 1309, 1270, 1228, 1047, 762. ^{1}H-NMR (300.13 MHz, (D_6)DMSO): 9.25 (*s*, HO–C(4′)); 8.17 (*dd*, *J* = 7.7, 1.2, H–C(10)); 7.84 (*ddd*, *J* = 8.0, 7.6, 1.2, H–C(8)); 7.62 (*d*, *J* = 8.0, H–C(7)); 7.61 (*s*, H–C(12)); 7.46 (*ddd*, *J* = 7.7, 7.5, H–C(9)); 7.23 (*s*, H–C(5)); 7.06 (*d*, *J* = 1.3, H–C(2′)); 6.90 (*dd*, *J* = 8.1, 1.3, H–C(6′)); 6.82 (*d*, *J* = 8.1, H–C(5′)); 5.13 (*d*, *J* = 8.1, H–C(3)); 5.06 (br. *s*, CH_2OH); 4.33–4.35 (*m*, H–C(2)); 3.78 (*s*, MeO); 3.58 (br. *d*, *J* = 11.4, CH_2OH): 3.38 (under H_2O signal, CH_2OH). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 174.8 (C(11)); 155.7 (C(6a)); 151.1 (C(4a)); 150.6 (C(5a)); 147.7 (C(3′)); 147.3 (C(4′)); 141.3 (C(12a)); 135.0 (C(8)); 126.5 (C(1′)); 125.8 (C(10)); 124.1 (C(9)); 120.8 (C(6′)); 120.6 (C(10a)); 118.0 (C(7)); 115.4 (C(5′), C(11a)); 111.9 (C(2′)); 111.2 (C(12)); 104.8 (C(5)); 77.8 (C(2)); 77.0 (C(3)); 60.0 (CH_2OH); 55.7 (MeO). EI-MS: 406 (0.02, M^+), 369 (0.8), 327 (6), 310 (0.8), 285 (6), 267 (0.6), 260 (1.4), 246 (1), 215 (2), 208 (0.6), 184 (21), 153 (100), 137 (3), 125 (12), 110 (9), 93 (62), 81 (8), 65 (35). Anal. calc. for $C_{23}H_{19}O_7$: 407.1131; found: 407.1131.

3. *Synthesis of Xanthones* **12**–**14**. The following compounds were obtained according to the procedures cited below.

3,4-Dihydroxy-9H-xanthen-9-one (**12**) [14]. M.p. >330° $CHCl_3$ ([16]: 240–241° (EtOH/H_2O); [17]: 238–240° (MeOH)). UV, IR, and EI-MS: in accord with those in [17]. The analysis of the ^{1}H, ^{13}C, COSY, HETCOR,

and HMBC spectra of **12** permits us to correct the literature [17] assignment of the ^{1}H resonances of H–C(6) and H–C(5) to 7.81 (*ddd*, *J* = 8.6, 6.9, 1.7, H–C(6)) and 7.62 (*dd*, *J* = 8.6, and 0.9, H–C(5)).

*1,2-Dihydroxy-9*H*-xanthen-9-one* (**13**) [14][18]. M.p. 163–165° (CH_2Cl_2/hexane); [19]: 166–167° (EtOH/H_2O)). UV (EtOH): 261, 243, 232, 203 (33300), 28800, 14200, 11000); UV (EtOH + NaOH): 311, 236, 222 (76000, 23900, 17000). UV (EtOH + $AlCl_3$): 305, 283, 266, 204 (33000, 22900, 8700). IR (KBr): 3467, 1606, 1475, 1365, 1288, 1045, 748. ^{1}H-NMR (300.13 MHz, (D_6)DMSO): [20] 12.45 (*s*, HO–C(1)); 9.42 (*s*, HO–C(2)); 8.16 (*dd*, *J* = 7.9, 1.6, H–C(8)); 7.81 (*ddd*, *J* = 8.4, 7.0, 1.6, H–C(6)); 7.60 (*dd*, *J* = 8.4, 0.8, H–C(5)); 7.46 (*ddd*, *J* = 7.9, 7.0, 0.8, H–C(7)); 7.32 (*d*, *J* = 9.0, H–C(4)); 6.96 (*d*, *J* = 9.0, H–C(3)). ^{13}C-NMR (75.47 MHz, (D_6)DMSO): 182.3 (C(9)); 155.9 (C(4b)); 148.3 (C(4a)); 147.6 (C(1)); 140.2 (C(2)); 136.4 (C(6)); 125.4 (C(8)); 124.5 (C(3)); 124.2 (C(7)); 119.3 (C(8a)); 118.0 (C(5)); 108.8 (C(9a)); 106.2 (C(4)). EI-MS: 230 (1, $[M+2]^+$), 229 (1, $[M+1]$), 228 (70, M^+), 199 (13), 126 (5), 115 (4), 78 (95), 63 (100).

*2,3-Dihydroxy-9*H*-xanthen-9-one* (**14**) [14][17][21]. M.p. >330° (Me_2CO/hexane) ([14]: >350° (AcOEt); [17]: 293–295° ($CHCl_3$/MeOH)). UV, IR and EI-MS: in accord with those in [17]. The unequivocal assignments of ^{13}C resonances, mainly on the basis of 1D selective INEPT and 2D HETCOR experiments, allowed us to correct some ^{1}H and ^{13}C resonances of **14** [17], namely the assignment of the ^{1}H resonances of H–C(6) to 7.78 (*ddd*, *J* = 8.4, 6.8, 1.7) and H–C(5) to 7.58 (*d*, *J* = 8.4) and the ^{13}C resonances of C(2) (144.1), C(3) (151.3), and C(4a) (154.2).

Methyl 2-Bromobenzoate (**15**) [22], *Methyl 2-(3,4-Dimethoxyphenoxy)benzoate* (**17**) [11–13], *2-(3,4-Dimethoxyphenoxy)benzoic Acid* (**18**) [11][12], *and 1,2-Dimethoxy-9*H*-xanthen-9-one* (**19**) [12][24]: Data identical to those reported in [12][25].

*2,3-Dimethoxy-9*H*-xanthen-9-one* (**20**) [13][25]. M.p. 170–171° (CH_2Cl_2/pentane) ([26]: 164–165° (Me_2CO); [17]: 157–158° ($CHCl_3$); [27]: 155–159° (CH_2Cl_2/heptane); [28]: 165–167° ($CHCl_3$/petroleum ether)). IR and EI-MS: in accord with those in [17]. The unequivocal assignments of ^{13}C resonances, mainly on the basis of 1D selective INEPT and 2D HETCOR experiments, allowed us to correct some ^{1}H and ^{13}C resonances of **20** [29]. ^{1}H-NMR (200.13 MHz, (D_6)DMSO): 8.16 (*dd*, *J* = 7.9, 1.5, H–C(8)); 7.82 (*ddd*, *J* = 8.4, 7.4, 1.5, H–C(6)); 7.60 (*d*, *J* = 8.4, H–C(5)); 7.49 (*s*, H–C(1)); 7.44 (*ddd*, *J* = 7.9, 7.4, 0.9, H–C(7)); 7.20 (*s*, H–C(4)); 3.94 (*s*, MeO–C(3)); 3.87 (*s*, MeO–C(2)). ^{13}C-NMR (50.03 MHz, (D_6)DMSO): 174.8 (C(9)); 155.7 (C(4b)); 155.6 (C(4a)); 152.0 (C(3)); 146.7 (C(2)); 134.8 (C(6)); 125.9 (C(8)); 124.3 (C(7)); 120.9 (C(8a)); 118.0 (C(5)); 114.0 (C(9a)); 104.7 (C(1)); 100.5 (C(4)); 56.6 (*Me*O–C(3)); 55.9 (*Me*O–C(2)).

4. *Cell-Growth Assay.* The effects of compounds on the growth of human cancer cell lines were evaluated according to the procedure adopted in the NCI's *in vitro* anticancer drug screening that uses the SRB assay to assess growth inhibition [30]. This colorimetric assay estimates cell number indirectly by staining cellular protein with the protein-binding dye SRB. Three human cancer cell lines were used, MCF-7 (breast cancer), TK-10 (renal cancer), UACC-62 (melanoma). Cells were routinely maintained as adherent cell cultures in *RPMI-1640* medium containing 5% heat-inactivated fetal bovine serum, 2 mM glutamine, and 50 μg/ml gentamicin at 37° in a humidified air incubator containing 5% CO_2. For the SRB assay, each cell line was plated at an appropriate density (1.5×10^5 cells/ml to MCF-7 and TK-10, 1.0×10^5 cells/ml to UACC-62) in 96-well plates and allowed to attach overnight. Cells were then exposed for 48 h to serial concentrations of compounds and to the positive control doxorubicin. Following this incubation period, the adherent cells were fixed *in situ*, and washed and dyed with SRB. The bound stain was solubilized, and the absorbance was measured at 492 nm in a microplate reader. Growth inhibition of 50% (GI_{50}) as well as toxicity of compounds was determined as described in [31]. Toxicity of xanthone derivatives was inferred from the SRB assay by comparing, after 48 h, the absorbance of the wells with xanthone derivative-treated cells with the absorbance of the wells containing untreated cells that were fixed at time zero (time at which compounds were added). Lower absorbances after 48 h of treatment indicate occurrence of cell death instead of growth arrest.

5. *Lymphocyte Assays.* The effects of compounds on the mitogenic response of human lymphocytes to PHA (10 μg/ml) were evaluated with a modified colorimetric MTT assay [32]. This assay was previously described by us [33]. Mononuclear cells were isolated from heparinized peripheral blood of healthy volunteers by *Histopaque-1077* density centrifugation. The toxicity of compounds against human lymphocytes was evaluated by an assay based on the ability of viable cells to reduce the colourless tetrazolium salt MTT to a colored formazan product. Briefly, in flat-bottom 96-well plates, $2-3 \times 10^6$ mononuclear cells/ml in *RPMI-1640* medium containing 10% heat-inactivated foetal bovine serum, 2 mM glutamine, and 50 μg/ml of gentamicin were exposed for 24 h to the various concentrations of each compound. Following this incubation period, the MTT soln. (1 mg/ml) was added. After incubation for 4 h, the MTT formazan products were solubilized with SDS/DMF soln. (20% SDS in a 50% soln. of DMF, pH 4.7) overnight at 37°. Absorbance (OD 550 nm) of the colored soln. was measured with a plate reader. Lymphocytoxicity, determined in terms of the percentage of

viable cells, was present when the viability of the exposed cells was less than 70% of the nonexposed control cells.

REFERENCES

[1] O. R. Gottlieb, A. A. L. Mesquita, E. M. Silva, M. T. Melo, *Phytochemistry* **1969**, *8*, 665.
[2] M. M. M. Pinto, A. A. L. Mesquita, O. R. Gottlieb, *Phytochemistry* **1987**, *26*, 2045.
[3] E. G. R. Fernandes, M. M. M. Pinto, A. M. S. Silva, J. A. S. Cavaleiro, O. R. Gottlieb, *Heterocycles* **1999**, *51*, 821.
[4] E. R. Fernandes, F. D. Carvalho, F. G. Remião, M. L. Bastos, M. M. Pinto, O. R. Gottlieb, *Pharm. Res.* **1995**, *12*, 1756.
[5] a) S. Antus, E. Baitz-Gáes, R. Bauer, A. Gottsegen, O. Seligmann, H. Wagner, *Liebigs Ann. Chem*. **1989**, 1147; b) S. Maeda, H. Masuda, T. Tokoroyama, *Chem. Pharm. Bull*. **1995**, *43*, 84; c) M. Massacret, C. Goux, P. Lhoste, D. Sinou, *Tetrahedron Lett*. **1994**, *5*, 6093.
[6] R. A. Vishwakarma, R. S. Kapil, S. P. Popli, *Indian J. Chem.*, *Sect. B* **1986**, *25*, 1021.
[7] a) X. She, X. Jing, X. Pan, A. S. C. Chan, T. Yang, *Tetrahedron Lett*. **1999**, *40*, 4570; b) M. F. Hibert, M. W. Gittos, D. N. Middlemiss, A. K. Mir, J. R. Fozard, *J. Med. Chem*. **1988**, *31*, 1087.
[8] a) M. Abou-Shoer, A. Habib, C. Chang, J. M. Cassady, *Phytochemistry* **1989**, *28*, 2483; b) H. Tosa, M. Iinuma, T. Tanaka, H. Nozaki, S. Ikeda, K. Tsutsui, K. Tsutsui, M. Yamada, S. Fujimori, *Chem. Pharm. Bull*. **1997**, *45*, 418.
[9] L. Merlini, A. Zanarotti, A. Pelter, M. P. Rochefort, R. Hänsel, *J. Chem. Soc., Perkin Trans. 1* **1980**, *3*, 775.
[10] L. Merlini, A. Zanarotti, *Tetrahedron Lett*. **1975**, 3621.
[11] W. T. Jackson, R. J. Boyd, L. L. Froelich, D. M. Gapinski, B. E. Mallett, J. S. Sawyer, *J. Med. Chem*. **1993**, *36*, 1726.
[12] L. Gales, M. E. Sousa, M. M. M. Pinto, A. Kijjoa, A. M. Damas, *Acta Crystallogr., Sect. C* **2001**, *57*, 1319.
[13] R. A. Finnegan, P. L. Bachman, *J. Pharm. Sci*. **1965**, *54*, 633.
[14] O. R. Gottlieb, A. A. L. Mesquita, G. G. Oliveira, M. T. Melo, *Phytochemistry* **1970**, *9*, 2537.
[15] a) A. Bax, *J. Magn. Reson.* **1984**, *57*, 314; b) G. Venkatraman, L. J. Harrison, K. Y. Sim, *Tetrahedron Lett*. **1996**, *37*, 2643; c) L.-J. Lin, G. A. Cordell, *J. Chem. Res. (M)* **1988**, 3052; d) G. A. Cordell, A. D. Kinghorn, *Tetrahedron* **1988**, *47*, 3521.
[16] P. K. Grover, G. D. Shah, R. C. Shah, *J. Chem. Soc*. **1955**, 3982.
[17] C. N. Lin, S. S. Liou, F. N. Ko, C. M. Teng, *J. Pharm. Sci*. **1993**, *82*, 11.
[18] B. Vermes, O. Seligmann, H. Wagner, *Helv. Chim. Acta* **1985**, *68*, 2359.
[19] J. S. H. Davies, F. Lamb, H. Suschitzky, *J. Chem. Soc*. **1958**, 1790.
[20] a) D. Barraglough, H. D. Locksley, F. Scheinmann, M. T. Magalhães, *J. Chem. Soc. B* **1970**, 603; b) P. Arends, P. Helboe, *Acta Chem. Scand.* **1972**, *26*, 4181.
[21] O. R. Gottlieb, A. A. L. Mesquita, G. G. Oliveira, M. T. Melo, *Anais da Academia Brasileira de Ciências* **1970**, *42*, 117.
[22] a) A. Ault, 'Techniques and Experiments for Organic Chemistry', 5th edn., Allyn and Bacon, Inc., Newton, Massachusetts, 1987, p. 392; b) B. S. Furniss, A. J. Hannaford, P. W. G. Smith, A. R. Tatchell, 'Vogel's Textbook of Practical Organic Chemistry', Longman Scientific and Technical, Harlow, England, 1989, p. 1077.
[23] A. Moroz, M. S. Shvartsberg, *Russ. Chem. Rev*. **1974**, *43*, 679.
[24] a) O. B. Familoni, I. Ionica, J. F. Bower, V. Snieckus, *Synlett* **1997**, 1081; b) B. Bennetau, J. Mortier, J. Moyround, J. Guesnet, *J. Chem. Soc., Perkin Trans. 1* **1995**, 1265.
[25] C. Morel, D. Séraphin, J.-M. Oger, M. Litaudon, T. Sévenet, P. Richomme, J. Bruneton, *Phytochemistry* **2000**, *53*, 1471.
[26] C. H. Hassal, J. R. Lewis, *J. Chem. Soc*. **1961**, *2*, 2312.
[27] F. D. Monache, M. M. Mac-Quhae, G. D. Monache, G. B. M. Betollo, R. A. Lima, *Phytochemistry* **1983**, *22*, 227.
[28] A. A. L. Gunatilaka, A. M. Y. J. Silva, S. Sotheeswaran, *Phytochemistry* **1982**, *21*, 1751.
[29] A. J. Quillinan, F. Scheinmann, *J. Chem. Soc., Perkin Trans. 1* **1973**, *2*, 1329.
[30] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107.

[31] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757.
[32] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55.
[33] M. J. Gonzalez, M. S. J. Nascimento, H. M. Cidade, M. M. M. Pinto, A. Kijjoa, C. Anantachoke, A. M. S. Silva, W. Herz, *Planta Med*. **1999**, *65*, 368.

Received June 22, 2002