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Synthesis and Cytotoxic Activities of Analogues of Thuriferic Acid

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Abstract—Several analogues of thuriferic acid and derivatives, with the 3,4-methylenedioxyphenyl ring replaced by naphthalene, furan, thiophene and carbazole ring systems, have been prepared. The synthetic strategy is based on a conjugate addition–alkylation methodology followed by cationic cyclization in order to obtain the isopodophyllone analogues, which are transformed in the thuriferic acids. Their cytotoxic activities against several tumour cells lines are also described.

Thuriferic acid is a natural cyclolignan, isolated from *Juniperus thurifera* leaves,[1] whose structure (Fig. 1) and stereochemistry have been the subject of some debate and finally confirmed by means of an array of spectroscopic techniques and semisynthesis from podophyllotoxin.[2] The unambiguous establishment of the configuration of thuriferic acid followed its treatment with dry HCl and conversion of the terminal methylene group into a chloromethyl substituent. The carboxylic group and the α,β-unsaturated ketone, altogether with the *trans* relationship between the C7′ aryl group and the carboxylic group at C8′ are the most noticeable features of a fairly rigid compound. Despite the fact that the aforementioned structural characteristics are in overall disagreement with those usually cited in the structure–activity relationships for cytotoxic lignans:[3] presence of a *trans* lactone ring and a *cis* C7′–C8′ relationship (see podophyllotoxin in Figure 1 as a representative example), thuriferic acid displays a general cytotoxic effect with medium-low activity against the 51 cell lines of the NCI.[2]

Following our studies on the synthesis[4] and cytotoxic activities of non-natural lignans, such as heterolignans[5] (lignan analogues in which one or several carbon atoms of the basic lignanic skeleton have been replaced by heteroatoms), in this paper we have prepared and evaluated a series of thuriferic acid analogues. The benzodioxole system of the parent compound (A–B rings) has been substituted by different heterocyclic moieties, such as the furan, thiophene and carbazole ring systems, as well as the less polar naphthalene moiety, which has proven to be a good surrogate for the polyoxygenated phenyl rings in systems similar to the one now been considered.[6]

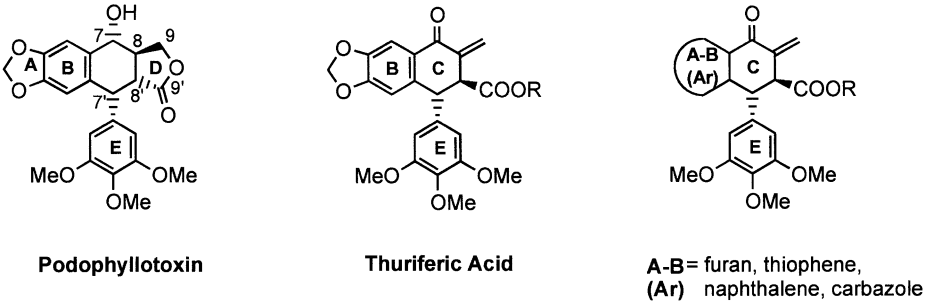

Figure 1. Structure of model lignans and synthesised compounds.

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The effects of these substitutions on the conformational preferences of the resulting compounds, as well as on their cytotoxic activities are discussed. The results show a totally unexplored structure–activity relationship (SAR) for the thuriferic acid derivatives, which might be of interest in the development of new antitumor agents.

Chemistry

Thuriferic acid itself can be prepared by basic treatment of either podophyllone or picropodophyllone.[2] The synthetic methodology we used for the preparation of the aryltetralones is based on the cationic cyclization of the conjugate addition–alkylation products of 5*H*-furan-2-one.[7] Such a general methodology has led us to the preparation of podophyllotoxin[8] as well as its thiophene, furan and naphthalene analogues.[4,9,10] In this paper we have adapted the synthetic sequence for preparing the desired thuriferic acid analogues from isopodo- and picropodophyllotoxone analogues (Fig. 2).

The synthetic methodology will be exemplified with the route to carbazole (Fig. 3) and naphthalene analogues of thuriferic acid, which will be described in more detail. The corresponding furan, thiophene, methylfuran, and methylthiophene analogues were synthesised in a similar way, as previously described.[10] The cytotoxic activities for all of them are here described for the first time.

The initial step implies a 'one pot' conjugate addition–alkylation sequence leading to the desired 2,3-bisaryl lactones.[7] The lithium anions of 2-substituted-1,3-dithianes (obtained by Lewis acid treatment of the corresponding aldehydes in the presence of 1,3-propanedithiol[11]), generated by means of *n*-BuLi in THF at −78 °C, are added to 5*H*-furan-2-one over a 3–4 h period, generating enolates which are alkylated with 3,4,5-trimethoxybenzaldehyde. The conjugate additions proceed with good yields,[12] but the alkylation steps require careful control of the temperature and time forcing the reaction to a maximum conversion (see Table 1). The reaction leads to racemic non-alkylated **1** and alkylated *trans*-lactones as mixtures of the two epimeric alcohols at C7′ (**2** and **3**). The **2:3** ratio depends on the nature of the 1,3-dithiane 2-substituent, the electrophile, and the actual reaction conditions in a non-straightforward way (see Table 1). The relative stereochemistry at C7′ was

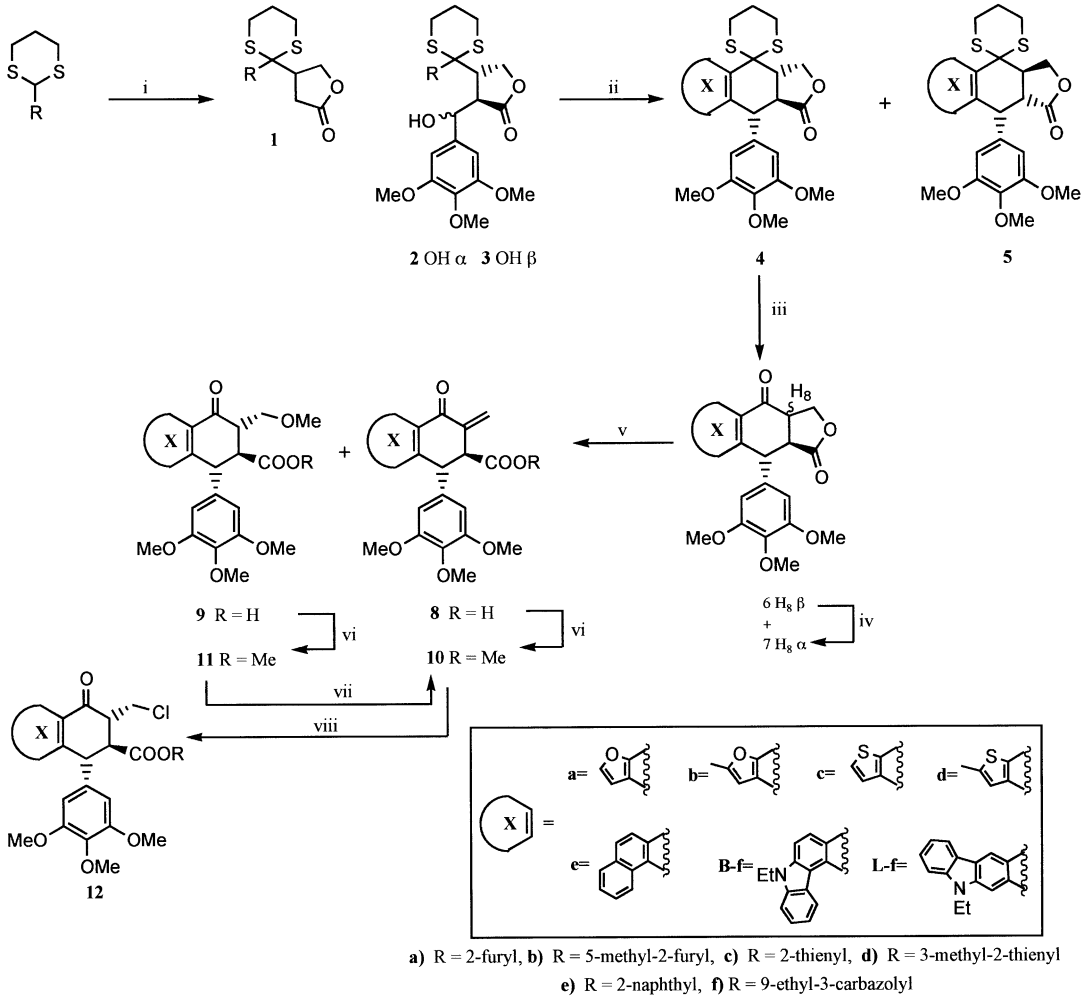


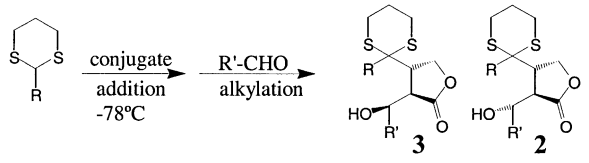
Figure 2. General synthesis of thuriferic acid analogues and their derivatives. Key: **(i)** (a) BuLi 1.6 M, THF, −78 °C, 45 min; (b) 5*H*-furan-2-one, THF, -78 °C, 3 h; (c) Ar–CHO, THF, TMEDA, −50 °C, 12 h. (ii) TFA, CH_2Cl_2, 24 h. (iii) HgO, $BF_3.Et_2O$, THF–H_2O (85:15), 0 °C–> rt, 24 h; (iv) EtOH/HOAc, reflux, 48 h. (v) 1% KOH/MeOH, rt, 1.5 h; then HCl 2 N. (vi) etheral CH_2N_2. (vii) *p*-TsOH, C_6H_6, reflux, 7 h. (viii) HCl dry stream, CH_2Cl_2, 2 h.

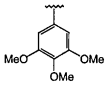
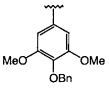
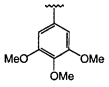
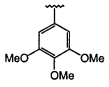
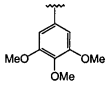
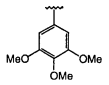
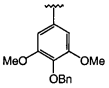
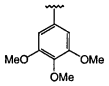
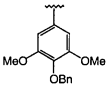
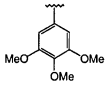
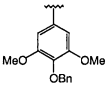
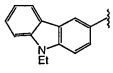
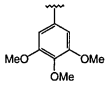
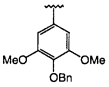
established by comparison with podorhizol and epipodorhizol,[13] based on the ^{1}H NMR chemical shifts of the C7′H (downfield shifted by the carboxyl group in the *threo* isomer **2** of podorhizol series) and the C7′H–C8′H *J*-coupling (larger in the epipodorhizol series **3**). The lack of stereoselectivity is not a problem, as both epimeric alcohols lead to the same compounds in subsequent steps and can be used without separation on the way to aryltetralin lactones.

The mixtures of alcohols, upon treatment with trifluoroacetic acid or tin tetrachloride ($SnCl_4$) in CH_2Cl_2 are transformed into cyclised products. For the furan, thiophene, methylfuran, and methylthiophene analogues only cyclisation products of the rings at C3 are observed.[4,10] For the carbazole series (Fig. 3), starting from the 2-(9-ethylcarbazol-3-yl)-1,3-dithiane, the cyclisation occurs mainly at position C4 of the carbazole (further referred to as the '*bent*' *B-series*), being the regioisomeric products of cyclisation at the C2 position (further referred to as the '*linear*' *L-series*) detected and identified as minor products. *'Bent'* and '*linear'* carbazole analogues, respectively, represent 41 and 13% of the crude (both are mixtures of the podophyllotoxin **5f** and isopodophyllotoxin **4f** relative stereochemistries, see below). For the naphthalene series, the cyclisation step proceeds exclusively on the more activated C1 position **4e**,**5e** (analogous to the carbazole '*bent' B-series*).

The relative stereochemistries of the cyclised products were easily established based upon the C7′H–C8′H and the C8′H–C8H coupling constants. All the lactones were C8–C8′*trans*; some series having exclusively a *trans* C7′–C8′ arrangement, some others being C7′–C8' *cis*/*trans* mixtures. The product ratios were determined by integration of the ^{1}H NMR signals of the well resolved C7′ protons (the aromatic resonances for the trimethoxyphenyl ring are in some cases unusually broadened, making them unsuitable for integration). The all-*trans* relative stereochemistry **4** (isopodophyllotoxin series) is preferentially obtained over the *trans–cis* stereochemistry of podophyllotoxin series **5**. The **4:5** ratio for the carbazole and naphthalene analogues is 3:1 to 2:1. For the thiophene, methylthiophene, furan and methylfuran series no podophyllotoxin-like **5** products were

Table 1.



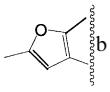
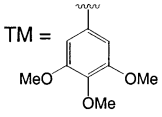
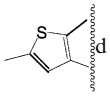
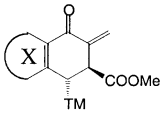
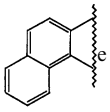
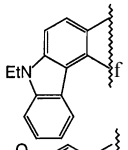
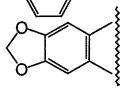
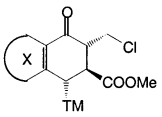
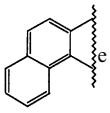
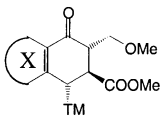
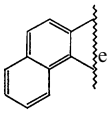
R	R′	T alk/TMEDA (equiv)	Overall yield % (erythro 3/threo 2)	R	R′	T alk/TMEDA (equiv)	Overall yield % (erythro 3/threo 2)
		−20 °C/1	57 (3/1)			−20 °C / 2	35 (5/1)
		−20 °C/1	71 (eryt)			−30 °C / 2	60 (5/1)
		−20 °C/1	62 (3/1)			−50 °C /2	77 (5/1)
		−20 °C /1	54 (eryt)			−50 °C / 2	90 (2/1)
		−20 °C/1	67 (2/1)			−50 °C / 2	77 (2/1)
		−20 °C/1	30 (eryt)			−50 °C /2	37 (2/1)
		−60 °C/1	28 (eryt)			−50 °C/2	66 (2/1)
		−20 °C/2	42 (eryt)			−50 °C /2	41 (2/1)
		−40 °C /2	34 (eryt)				

detected.[4,10] Such a stereochemical outcome is the main problem in the synthesis of podophyllotoxin analogues by this methodology.[14] However, on the way to thuriferic acid analogues both stereochemistries are converted to the same end product (see below) with the desired relative configurations, making this strategy much more efficient for this class of compounds.

The masked carbonyl group at position C7 is deprotected by means of $HgO/BF_3{\cdot}Et_2O$ catalyzed hydrolysis of the 1,3-dithiane ring producing *trans*-ketolactones **6**.[15] Under the reaction conditions the lactone ring is partially epimerised at position C8 to *cis*-ketolactones **7** (up to 50% depending upon the actual series and the reaction time). In order to improve the yield of the thuriferic acid stereochemistry, such epimerisation is further promoted by means of an acidic treatment with acetic acid, leading to total conversion. In the '*bent*' carbazoles (Fig. 3), under the reaction conditions, the *cis*-ketolactone is partially converted to the '*bent*-carbazolethuriferic' acid ethyl ester **B-10f** (R = Et).

Treatment with 1% methanolic KOH is needed to transform the *trans*-ketolactones **6** into the desired thuriferic acid analogues **8** in good yields (in some cases **9** is also produced by conjugate addition of methanol

Table 2. Cytotoxic potencies against several representative cell lines (lg IC_{50}M)

		P−388	A−549	HT−29	MEL−28
	 a	−6.5	−6.5	−6.5	−6.2
	 b	−6.2	−6.2	−6.2	−6.2
	 c	−6.6	−6.6	−6.6	−6.6
	 d	−6.6	−5.9	−5.9	−5.9
	 e	−6.6	−6.6	−6.6	−5.9
10	 f	−5.6	−5.6	−5.6	−5.6
		−6.6	−6.6	−6.6	−6.6
	(methyl thuriferate)				
	 a	−6.5	−6.2	−6.2	−6.2
	 c	−6.5	−6.2	−5.9	−5.9
12	 e	−6.7	−6.7	−6.7	−6.7
	 e	−5.7	−5.7	−5.7	−5.7
11					

under the basic conditions). Careful diazomethane treatments in dry ethyl ether render the methyl esters **10** and **11**. If the reaction is not carefully controlled and allowed to stand for long periods of time, **10** undergoes polar cycloaddition reactions of diazomethane onto the double bond to generate pyrazolines. For the naphthalene series, additional acidic treatment (*p*-TsOH in refluxing benzene) is necessary to regenerate the thuriferic acid analogue **10** from the product **11**. In order to ascertain the relative stereochemistry of the synthesised analogues, the methyl esters were treated with hydrochloric acid in dry CH_2Cl_2, rendering the corresponding chlorides **12**.

Biological Results

The cytotoxic activities against several representative tumor cell lines (P-388, HT-29, MEL-28 and A-549) were tested following a previously described protocol.[16] The results are summarised in Table 2. The IC_{50} for every compound tested against each cell line are in the micromolar range. The methyl thuriferates (**10**) display a potency similar to the chloro derivatives (**12**), as previously observed for thuriferic acid methyl ester and its chloro derivative. However, not every addition product to the double bond leads to active compounds, as the methoxy (**11**) and pyrazoline (not shown) derivatives of the naphthalene series are tenfold less potent. As a general trend, the furan and thiophene series are less potent than the naphthalene analogues (which in turn are almost equipotent to the natural series). The carbazoles are less potent than the naphthalenes.

Discussion

In previous papers, we have set the strategy for the synthesis of natural lignans and heteroanalogues based on a conjugate addition–alkylation reaction to assemble the basic lignan skeleton, followed by cyclisation towards the aryltetralin structures and epimerisation reactions to achieve the desired relative stereochemistries.[5,8] The replacement of the methylenedioxyphenyl ring of podophyllotoxin alters the synthetic intermediates and the final products, by modifying their stereochemical preferences and electronic properties and making them likely to have modified reactivity and/or biological properties.[4,5,9,10]

From a chemical point of view, the effects of the thiophene and furan substitutions with respect to the podophyllotoxin series can be summarised as a reduction of the solvent accessible surface of the A–B ring system and a more strained C–D ring system, probably due to the different bond angles imposed by the heteroaromatic moiety, which leads to a greater preference for the less strained picropodophyllin series **7**.[4,10]

For the naphthalene and carbazole series, the cyclisations onto the more reactive positions yield compounds in which the A–B ring systems form an angle with the newly formed C–D ring system ('*bent*' series). As a result, ring A comes closer to the pendant trimethoxyphenyl ring (ring E) than in the methylenedioxy-, furan-, and thiophene-'linear' series. This proximity reduces the conformational flexibility of the resulting molecules, as evidenced by comparison of their NMR spectral data

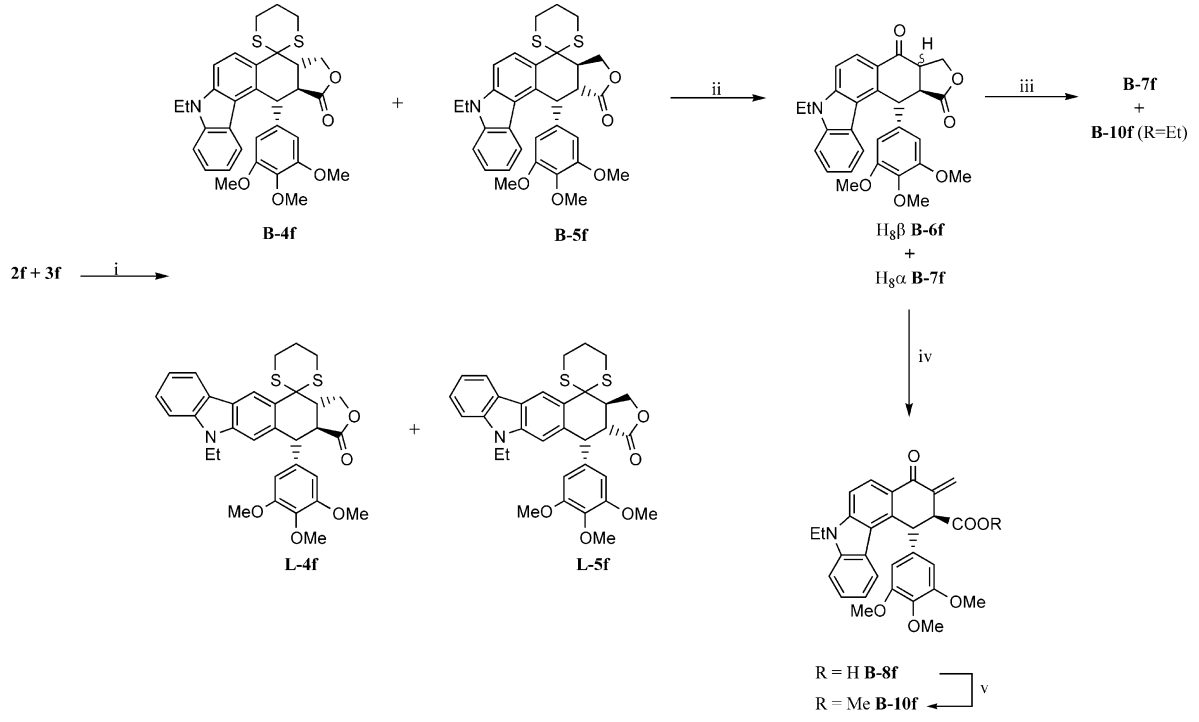


Figure 3. Synthesis of carbazole analogues. Key: (i) TFA, CH_2Cl_2, 24 h. (ii) HgO, $BF_3.Et_2O$, THF–H_2O (85:15), 0 °C–> rt, 24 h; (iii) EtOH/HOAc, reflux, 48 h. (iv) 1% KOH/MeOH, rt, 1.5 h; then HCl 2 N. (v) etheral CH_2N_2.

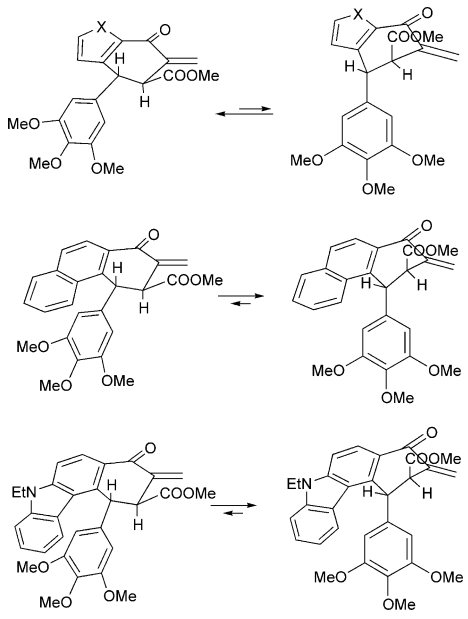


Figure 4. Conformational equilibrium of thiophene and furan, naphthalene and carbazole analogues of thuriferic acid.

with the '*linear*' ones (i.e., the resonances for aromatic protons of the trimethoxyphenyl ring in the ^{1}H NMR spectra appear line-broadened due to a slower motion of the otherwise freely rotating moiety). This closer spatial proximity of rings A and E must also be effective during the cyclisation reaction and affects its stereochemical outcome, leading to a higher proportion of compounds with a podophyllotoxin stereochemistry **5**. Such compounds are nearly unobserved in the '*linear*' series (furan, thiophene, methylenedioxyphenyl, etc.).[10]

The removal of the 1,3-dithiane protecting group proceeds similarly in every studied series, with moderate to good yields. Longer reaction times lead to the thermodynamically more stable epimerised products at C8 (**7**). This isomerisation, which has previously been used by us on the way to podophyllotoxin analogues,[8] is not essential in this synthetic route, as the stereochemical information is lost in the final products, carrying a double bond at that position.

The lactone opening reactions proceed in good yields to the desired products **8**. Their stereochemistries have been unambiguously established spectroscopically in the natural series and the thiophene and furan analogues, considering the ^{1}H NMR coupling constants of the thuriferic acids and the corresponding chloro-derivatives.[2,10] The differences in both series of compounds have been attributed to a higher contribution of an equatorial disposition of the pendant trimethoxyphenyl ring to the NMR data of the thuriferic acid analogues for the five-membered heteroaromatic rings (Fig. 4).[10] The more sterically demanding (A–B system larger and angled) naphthalene and carbazole rings, on the other hand impose and axial disposition to the aforementioned E ring, rendering the C7′ equatorially placed protons singlets in the ^{1}H NMR spectra. The whole series thus sample the conformational space, exhibiting distinct preferential conformations in solution.

The cytotoxic activities (Table 2) displayed by the synthesised compounds show that there are no stringent requirements for the nature of rings A and B of thuriferic acid and its derivatives. Replacement of the methylenedioxyphenyl ring by thiophene, furan, naphthalene or carbazole ring systems lead to compounds with comparable potencies, considering the substantial changes introduced in terms of stereochemical preferences (as previously discussed), hydrophobic accessible surface, steric and electronic properties. These results are contradicting with the described SAR for other lignan analogues, which usually are not tolerant to substitutions of the methylenedioxy ring.[3]

The cytotoxicity results, combined with the stereochemical preferences discussed above imply that the compounds are able to place their trimethoxyphenyl rings in a similar position, despite their intrinsical preferences. The higher potency of the naphthalene derivatives, combined with the fact that the equatorial arrangement for the E ring in such a system is disfavoured suggest a closer to axial disposition of the ring when bound to its target.

The furan and thiophene derivatives, with a preference for the equatorial disposition display a lower potency (the alternate conformation can be accessed at the expense of some energy loss, combined to the lower hydrophobic contact area). The carbazole ring, however, introduces a much bulkier substituent over the E ring, probably making unfavourable contacts or preventing the E ring from making favourable interactions with the target.

When compared to the stringent requirements for the A and B rings of podophyllotoxin analogues, including heteroanalogues such as those described in this paper, the tolerance described suggest a different mechanism of action or a different interaction with its target.[3] Such an observation is in agreement with the removal of the lactone ring, which is considered critical for the activity of most lignan analogues. Further experiments are under course in order to establish the action mechanism of these compounds.

Conclusions

Several new heterolignan analogues of thuriferic acid methyl ester have been synthesised and their cytotoxic activities against several tumor cell lines tested. The effects of the substitutions on the synthetic methodology are deep and also affect the structures of the compounds accessed. The unusual structural tolerance seen in the biological activity testing, as compared to other compounds of these series, could be explained by a different mechanism of action. These compounds could then be used as parent compounds for further defining and characterising the target. New compounds in which the trimethoxyphenyl ring (deemed essential for the usual mechanisms of the lignanic analogues) is replaced by other moieties in order to explore the diversity tolerated on ring E by such a target site, are currently being designed.

Experimental

General

Reagents were used as purchased without further purification. Solvents (THF) were dried and freshly distilled before use according to literature procedures. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040–0.063; Merck) chromatography. TLC was performed on precoated silica gel polyester plates (0.25 mm thickness) with fluorescent indicator UV 254 (Polychrom SI F_{254}). Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker WP 200-SY spectrometer at 200 MHz or on a Bruker SY spectrometer at 400 MHz. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard and coupling constants (*J* values) are in hertz. For FABMS analyses, a VG-TS250 apparatus (70 eV) was used.

N-Ethylcarbazole series

Conjugate addition–alkylation reaction:. To a solution of 2-(9-ethyl-3-carbazolyl)-1,3-dithiane (1.5 g, 4.8 mmols) in dry THF (48 mL) at −78 °C under argon was added *n*-BuLi (1.6 M in hexane) (3.3 mL, 5.3 mmols). After 45 min, a solution of 5*H*-furan-2-one (0.3 mL, 4.8 mmols) in dry THF (4.8 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 3 h and then allowed to warm to −50 °C. A solution of 3,4,5-trimethoxybenzaldehyde (1.4 g, 7.1 mmols) in dry THF (7.2 mL) and TMEDA (1.5 mL, 10 mmols) were successively added. The mixture was stirred for 12 h at −50ºC and then quenched by addition of NH_4Cl (concd solution), extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and evaporated in vacuo. Flash chromatography (hexane/ethyl acetate 70:30) afforded **1** (0.5 g, 26%), **2f** (0.5 g, 17%) and **3f** (0.7 g, 25%).

4-[2-(9-ethyl-3-carbazolyl)-1,3-dithian-2-yl]tetrahydrofuran-2-one (1). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47 (t, 3H, $J=7.3$), 1.90 (m, 2H), 2.48 (dd, 2H, $J=8.8$, 17.5), 2.70–3.01 (m, 4H), 3.20 (m, 1H), 4.20 (t, 1H, $J=8.2$), 4.44 (*c*, 2H, $J=7.3$), 4.50 (t, 1H, $J=8.1$), 7.45 (m, 4H), 8.06 (dd, 1H, $J=1.8$, 8.1), 8.14 (d, 1H, $J=8.0$), 8.68 (s, 1H); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 14.5 (CH_3), 25.5 (CH_2), 27.9 (CH_2), 27.9 (CH_2), 30.9 (CH_2), 38.3 (CH_2), 49.3 (CH), 62.1 (C), 69.3 (CH_2), 109.3 (C), 109.4 (CH), 119.8 (CH), 121.1 (CH), 121.5 (CH), 122.0 (CH), 123.0 (C), 124.0 (C), 126.7 (CH), 127.2 (CH), 130.0 (C), 139.8 (C), 141.1 (C), 176.6 (C=O); EIMS *m*/*z* (%) 397 (M^+, 16), 312 (100%).

(±)(3*R*,4*S*)-4-[2-(9-ethyl-3-carbazolyl)-1,3-dithian-2-yl]-3-[1*R*-(3,4,5-trimethoxyphenyl)hydroxymethyl]tetrahydrofuran-2-one (2f). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.87 (m, 2H), 2.55–2.58 (m, 2H), 2.70–2.81 (m, 2H), 1.46 (t, 3H, $J=7.3$), 2.92–2.99 (m, 1H), 3.09–3.13 (m,1H), 3.45 (brs, 6H), 3.78 (s, 3H), 4.34–4.46 (m, 2H), 4.40 (*c*, 2H, $J=7.3$), 4.96 (s, 1H), 6.14 (s, 2H), 7.10 (d, 1H, $J=8.2$), 7.25 (m, 2H), 7.50 (m, 2H), 8.08 (d, 1H, $J=8.0$), 8.65 (s, 1H); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 14.6 (CH_3), 25.3 (CH_2), 27.5 (CH_2), 27.8 (CH_2), 38.3 (CH_2), 48.5 (CH), 49.3 (CH), 55.7 (C), 56.3 (OCH_3), 56.3 (OCH_3), 61.5 (OCH_3), 70.5 (CH_2), 74.0 (CH), 102.6 (CH), 102.6 (CH), 108.6 (CH), 109.0 (CH), 109.0 (CH), 120.3 (CH), 121.5 (CH), 122.5 (C), 122.7 (C), 125.6 (C), 126.1 (CH), 128.8 (CH), 135.7 (C), 136.5 (C), 138.0 (C), 140.9 (C), 153.1 (C), 153.1 (C), 178.7 (C=O); EIMS *m*/*z* (%): 593 (M^+, 20).

(±)(3*R*,4*S*)-4-[2-(9-ethyl-3-carbazolyl)-1,3-dithian-2-yl)-3-[1*S*-(3,4,5-trimethoxyphenyl)hydroxymethyl]tetrahydrofuran-2-one (3f). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46 (t, 3H, $J=7.3$), 1.92 (m, 2H), 2.63–2.81 (m, 4H), 3.01 (m, 1H), 3.28 (m, 1H), 3.55 (s, 6H), 3.71 (t, 1H, $J=9.9$), 3.76 (s, 3H), 4.38 (*c*, 2H, $J=7.3$), 4.63 (d, 1H, $J=5.1$), 4.72 (dd, 1H, $J=2.9$, 9.9), 6.28 (s, 2H), 7.25 (t, 1H, $J=7.7$), 7.34 (d, 1H, $J=8.7$), 7.39 (d, 1H, $J=7.7$), 7.48 (t, 1H, $J=7.7$), 7.88 (dd, 1H, $J=1.8$, 8.7), 8.13 (d, 1H, $J=7.7$), 8.65 (d, 1H, $J=1.8$); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 13.8 (CH_3), 24.6 (CH_2), 26.8 (CH_2), 27.1 (CH_2), 37.6 (CH_2), 49.5 (CH), 51.2 (CH), 55.7 (OCH_3), 55.7 (OCH_3), 60.3 (OCH_3), 63.8 (C), 68.4 (CH_2), 73.9 (CH), 102.8 (CH), 102.8 (CH), 108.6 (CH), 108.9 (CH), 119.2 (CH), 120.4 (CH), 121.6 (CH), 122.5 (C), 123.2 (C), 126.3 (CH), 126.7 (CH), 128.9 (C), 135.4 (C), 137.3 (C), 139.0 (C), 140.4 (C), 152.9 (C), 152.9 (C), 176.3 (C=O); EIMS *m*/*z* (%): 593 (M^+, 20).

Cyclisation reaction

To a solution of **2f** and **3f** (1.2 g, 2.0 mmols) in CH_2Cl_2 (10.1 mL) at room temperature TFA (10.1 ml) was added dropwise. The reaction mixture was stirred for 24 h and then quenched with $NaHCO_3$ (concd solution), extracted with ethyl acetate, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. Crystallization of the crude product gave a 2:1 mixture (480 mg, 41%) of B-4f and B-5f as white crystals. Pure **B-4f** was isolated by crystallization. A 3:1 mixture (150 mg, 13%) of L-4f and L-5f was isolated from the mother liquors.

(±)(3a*S*,12*R*,12a*S*)-7-ethyl-4,4-trimethylenedithio-12-(3,4,5-trimethoxy phenyl)-3,3a,4,7,12,12a-hexahydroisobenzofuran[5,6-*c*]carbazol-1-one (B-4f). Mp 278–280 °C (hexane/ethyl acetate); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.46 (t, 3H, $J=7.3$), 2.17–2.27 (m, 2H), 2.79–3.13 (m, 4H), 3.35 (m, 1H), 3.51 (m, 1H), 3.64 (s, 6H), 3.70 (s, 3H), 4.35 (*c*, 2H, $J=7.3$), 4.53 (dd, 1H, $J=7.3$, 10.6), 4.72 (t, 1H, $J=7.3$), 5.23 (d, 1H, $J=8.8$), 6.49 (s, 2H), 7.03 (m, 1H), 7.34 (m, 2H), 7.42 (d, 1H, $J=8.8$), 8.03 (d, 1H, $J=7.8$), 8.43 (d, 1H, $J=8.8$); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 14.4 (CH_3), 24.4 (CH_2), 30.2 (CH_2), 31.0 (CH_2), 38.2 (CH_2), 43.9 (CH), 47.5 (CH), 54.0 (C), 56.6 (OCH_3), 56.6 (OCH_3), 57.3 (CH), 61.3 (OCH_3), 69.7 (CH_2), 106.4 (CH), 106.4 (CH), 108.3 (CH), 108.8 (CH), 119.5 (CH), 122.6 (C), 122.6 (C), 124.9 (C), 125.9 (CH), 127.1 (CH), 132.5 (C), 134.5 (C), 136.9 (C), 140.7 (C), 140.8 (C), 141.3 (C), 153.3 (C), 153.3 (C), 177.0 (C=O); EIMS *m*/*z* (%): 575 (M^+, 100).

(±)(3a*R*,12*R*,12a*R*)-7-ethyl-4,4-trimethylenedithio-12-(3,4,5-trimethoxy phenyl)-3,3a,4,7,12,12a-hexahydroisobenzofuran[5,6-*c*]carbazol-1-one (B-5f). ^{1}H NMR

(200 MHz, $CDCl_3$) δ 1.45 (t, 3H, $J=7.3$), 2.65–3.05 (m, 6H), 3.20 (m, 1H), 3.45 (m, 1H), 3.67 (s, 6H), 3.74 (s, 3H), 4.10–4.40 (m, 2H), 4.40 (*c*, 2H, $J=7.3$), 5.26 (d, 1H, $J=4.4$), 6.43 (s, 2H), 7.01 (m, 1H), 7.25–7.39 (m, 2H), 7.50 (d, 1H, $J=8.8$), 7.76 (d, 1H, $J=8.4$), 8.27 (d, 1H, $J=8.8$); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 13.7 (CH_3), 24.1 (CH_2), 27.1 (CH_2), 29.2 (CH_2), 37.5 (CH_2), 44.5 (CH), 45.2 (CH), 45.8 (CH), 56.1 (OCH_3),), 56.1 (OCH_3), 60.8 (OCH_3), 69.3 (CH_2), 69.9 (C), 105.4 (CH), 105.4 (CH), 108.0 (CH), 108.6 (CH), 119.3 (CH), 120.2 (CH), 120.8 (CH), 122.7 (C), 122.7 (C), 126.2 (CH), 131.0 (C), 134.0 (C), 139.8 (C), 140.8 (C), 141.7 (C), 141.8 (C), 152.8 (C), 152.8 (C), 178.0 (C=O); FABMS *m*/*z* (%): 575 (M^+, 75).

(±)(3a*S*,12*R*,12a*S*)-10-ethyl-4,4-trimethylenedithio-12-(3,4,5-trimethoxy phenyl)-1,3,3a,4,12,12a-hexahydroisobenzofuran-[5,6-*b*]carbazol-3-one (L-4f). ^1H NMR (200 MHz, $CDCl_3$) δ 1.46 (t, 3H, $J=7.3$), 2.00–3.27 (m, 6H), 3.31 (m, 1H), 3.75 (s, 6H), 3.84 (s, 3H), 3.58–3.84 (m, 1H), 4.37 (*c*, 2H, $J=7.3$), 4.37 (m, 1H), 4.56 (d, 1H, $J=6.9$), 4.75 (dd, 1H, $J=4.0$, 10.6), 6.52 (s, 2H), 7.24 (m, 1H), 7.34 (m, 1H), 7.45 (m, 1H), 8.12 (d, 1H, $J=7.7$), 8.16 (d, 1H, $J=7.7$), 8.79 (s, 1H).

(±)(3a*R*,12*R*,12a*R*)-10-ethyl-4,4-trimethylenedithio-12-(3,4,5-trimethoxyphenyl)-1,3,3a,4,12,12a-hexahydroisobenzofuran-[5,6-*b*] carbazol-3-one (L-5f). ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (m, 3H), 2.00–3.27 (m, 6H), 3.40–3.87 (m, 2H), 3.64 (s, 3H), 3.75 (s, 6H), 3.90–4.13 (m, 2H), 4.12 (m, 2H), 5.26 (d, 1H, $J=4.4$), 6.92 (s, 2H), 7.20–7.48 (m, 1H), 7.34 (s, 1H), 7.48 (m, 1H), 8.01 (d, 1H, $J=8.8$), 8.26 (d, 1H, $J=8.8$), 8.67 (s, 1H).

Deprotection reaction

A solution of **B-4f** and **B-5f** (0.48 g, 0.83 mmols) in 25 mL of THF/H_2O (85:15) was added to a suspension of HgO (390 mg, 1.9 mmols) in THF/H_2O (85:15) at 0 °C BF_3OEt_2 (0.23 mL, 1.9 mmols). The reaction mixture was stirred for 24 h at room temperature, then CH_2Cl_2 (20 mL) was added and the precipitate filtered. The organic layer was washed with brine, dried over Na_2SO_4 and the solvent evaporated in vacuo. The crude product was purified by crystallization in hexane/ethyl acetate affording a mixture (2:1) of **B-6f** and **B-7f** (379 g, 79%), which were then separated by chromatography (hexane/ethyl acetate 8:2).

(±)(3a*S*,12*R*,12a*S*)-7-ethyl-12-(3,4,5-trimethoxyphenyl)-3a,7,12,12a-tetrahydro-3*H*-isobenzofuran[5,6-*c*]carbazol-1,4-dione (B-6f). Mp 242–244 °C (hexane/ethyl acetate); ^1H NMR (200 MHz, $CDCl_3$) δ 1.45 (t, 3H, $J=6.9$), 3.12 (dd, 1H, $J=9.8$, 16.4), 3.45 (m, 1H), 3.63 (s, 6H), 3.70 (s, 3H), 4.40 (*c*, 2H, $J=6.9$), 4.50 (t, 1H, $J=8.8$), 4.62 (m, 1H), 5.32 (d, 1H, $J=9.8$), 6.49 (brs, 2H), 7.08 (m, 1H), 7.39 (m, 2H), 7.48 (d, 1H, $J=9.0$), 7.92 (d, 1H, $J=9.6$), 8.21 (d, 1H, $J=9.0$); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 13.7 (CH_3), 37.8 (CH_2), 44.8 (CH), 48.3 (CH), 48.7 (CH), 56.1 (OCH_3), 56.1 (OCH_3), 60.7 (OCH_3), 66.3 (CH_2), 106.6 (CH), 106.6 (CH), 108.2 (CH), 108.7 (CH), 120.0 (C), 120.0 (C), 121.9 (C), 124.5 (CH), 124.6 (CH), 126.2 (CH), 126.8 (C), 138.3 (C), 138.3 (C), 140.5 (C), 140.5 (C), 143.7 (C), 152.8 (C), 152.8 (C), 174.1 (C=O), 194.0 (C=O); EIMS *m*/*z* (%): 485 (M^+, 60).

(±)(3a*R*,12R,12a*S*)-7-ethyl-12-(3,4,5-trimethoxyphenyl)-3a,7,12,12a-tetrahydro-3*H*-isobenzofuran[5,6-*c*]carbazol-1,4-dione (B-7f). ^1H NMR (200 MHz, $CDCl_3$) δ 1.46 (t, 3H, $J=7.7$), 3.36 (dd, 1H, $J=6.2$, 8.0), 3.53 (d, 1H, $J=8.0$), 3.69 (s, 6H), 3.74 (s, 3H), 4.41 (dd, 1H, $J=6.2$, 9.0), 4.85 (d, 1H, $J=9.0$), 5.67 (s, 1H), 6.41 (s, 2H), 7.21 (m, 1H), 7.46 (m, 3H), 8.11 (d, 1H, $J=8.0$), 8.31 (d, 1H, $J=8.8$); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 13.9 (CH_3), 37.8 (CH_2), 40.8 (CH), 43.7 (CH), 47.9 (CH), 56.3 (OCH_3), 56.3 (OCH_3), 60.8 (OCH_3), 71.0 (CH_2), 105.0 (CH), 105.0 (CH), 109.0 (CH), 109.3 (CH), 120.7 (C), 120.7 (C), 122.6 (C), 123.4 (CH), 124.8 (C), 125.8 (CH), 126.3 (CH), 137.2 (C), 137.2 (C), 138.5 (C), 140.7 (C), 143.9 (C), 153.8 (C), 153.8 (C), 175.9 (C=O), 194.9 (C=O); FABMS *m*/*z* (%): 486 (M^+, 5).

Acidic epimerisation

To a solution of **B-6f** (250 mg, 0.5 mmols) in ethanol (45 mL) was added glacial acetic acid (35 mL, 0.6 mmols). The reaction mixture was stirred at reflux for 48 h and then allowed to cool to room temperature and quenched with a concd solution of $NaHCO_3$, extracted with ethyl acetate, washed with brine, dried over Na_2SO_4 and the organic solvent evaporated in vacuo. Flash chromatography of the crude product afforded **B-7f** (92 mg, 38%) and **B-10f** (R = Et)(120 mg, 47%).

B-10f (R = Et). ^1H NMR (200 MHz, $CDCl_3$) δ 0.98 (t, 3H, $J=7.1$), 1.45 (t, 3H, $J=7.3$), 3.69 (s, 3H), 3.75 (s, 3H), 3.96 (*c*, 2H, $J=7.1$), 4.04 (s, 1H), 4.40 (*c*, 2H, $J=7.3$), 5.33 (s, 1H), 5.73 (s, 1H), 6.37 (s, 3H), 7.20 (m, 1H), 7.24–7.50 (m, 2H), 7.46 (d, 1H, $J=8.8$), 8.09 (d, 1H, $J=8.0$), 8.40 (d, 1H, $J=8.8$); ^{13}C NMR (50.13 MHz, $CDCl_3$) 13.8 (CH_3), 13.8 (CH_3), 37.8 (CH_2), 46.1 (CH), 56.1 (OCH_3), 56.1 (OCH_3), 56.9 (CH), 60.7 (OCH_3), 61.3 (CH_2), 105.7 (CH), 105.7 (CH), 108.5 (CH), 108.9 (CH), 120.2 (C), 120.3 (C), 122.6 (CH), 125.3 (C), 125.3 (C), 125.7 (CH_2), 125.9 (CH), 126.0 (CH), 136.1 (C), 136.9 (C), 138.3 (C), 138.5 (C), 140.6 (CH), 143.4 (C), 153.3 (C), 153.3 (C), 171.8 (CO), 185.6 (C=O). FABMS *m*/*z* (%) 514 (M^+, 15), 150 (100).

Lactone opening reaction

Compound **B-7f** (250 mg, 0.5 mmols) was treated with a solution of KOH in MeOH (1%) for 1.5 h and then quenched by addition of HCl (2 N), extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and evaporated in vacuo affording **B-8f** (225 mg, 93%).

(±)(1*R*,2*S*)-7-ethyl-3-methylen-1-(3,4,5-trimethoxyphenyl)-4-oxo-1,2,3,4-tetrahydrobenzo[*c*]carbazole-2-carboxylic acid (B-8f). ^1H NMR (200 MHz, $CDCl_3$) δ 1.44 (t, 3H, $J=6.9$), 3.65 (s, 6H), 3.72 (s, 3H), 3.98 (m, 1H), 4.36 (*c*, 2H, $J=6.9$), 5.29 (s, 1H), 5.65 (s, 1H), 6.31 (s, 2H), 6.34 (s, 1H), 7.12–7.21 (m, 1H), 7.41–7.48 (m, 2H), 7.47 (d, 1H, $J=8.0$), 7.98 (d, 1H, $J=8.0$), 8.38 (d,0 1H, $J=8.8$);^{13}C NMR (50.13 MHz, $CDCl_3$) *δ*: 14.0 (CH_3),

37.8 (CH_2), 45.7 (CH), 56.2 (OCH_3), 56.2 (OCH_3), 56.5 (CH), 60.7 (OCH_3), 105.5 (CH), 105.5 (CH), 108.8 (CH), 109.1 (CH), 120.3 (C), 120.4 (C), 122.5 (CH), 123.1 (C), 125.1 (C), 126.1 (CH), 126.3 (CH), 126.9 (CH_2), 136.1 (C), 137.0 (C), 137.8 (C), 138.1 (C), 140.6 (CH), 143.6 (C), 153.3 (C), 153.3 (C), 176.7 (C=O), 185.4 (C=O).

Esterification reaction

Compound **B-8f** (225 mg, 0.46 mmols) was treated with a saturated solution of CH_2N_2 in ether for 5 min. The solvent was then evaporated and the crude product was purified by chromatography (hexane/ethyl acetate 1:1) affording **B-10f (R = Me)** (206 mg, 90%).

(±)(1*R*,2*S*)-7-ethyl-3-methylen-1-(3,4,5-trimethoxyphenyl)-4-oxo-1,2,3,4-tetrahydrobenzo[*c*]carbazole-2-methylcarboxylate (B-10f; R = Me). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47 (t, 3H, J = 6.9), 3.61 (s, 3H), 3.69 (s, 6H), 3.79 (s, 3H), 3.99 (s, 1H), 4.42 (*c*, 2H, J = 6.9), 5.54 (s, 1H), 6.36 (brs, 2H), 6.59 (brs, 2H), 7.10 (m, 2H), 7.44 (m, 2H), 7.52 (d, 1H, J = 8.6), 8.44 (d, 1H, J = 8,6); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 13.8 (CH_3), 37.8 (CH_2), 45.4 (CH), 52.6 (OCH_3), 56.1 (OCH_3), 56.1 (OCH_3), 59.6 (CH), 60.9 (OCH_3), 105.7 (CH), 105.7 (CH), 108.9 (CH), 109.0 (CH), 120.4 (C), 120.6 (C), 122.1 (CH), 123.1 (C), 125.0 (C), 126.0 (CH_2), 126.1 (CH), 126.7 (CH), 135.9 (C), 136.8 (C), 137.3 (C), 137.3 (C), 140.5 (CH), 143.4 (C), 153.5 (C), 153.5 (C), 173.1 (C=O), 194.7 (C=O); EIMS m/z (%): 499 (5), 391 (15).

Naphthalene series

Lactone opening reaction. Compound **6e** (280 mg, 0.7 mmols) was treated with a solution of KOH in MeOH (1%) for 1.5 h and then quenched by addition of HCl (2 N), extracted with ethyl acetate, washed with brine, dried over $MgSO_4$ and evaporated in vacuo affording a mixture (2:1) of **8e** and **9e** (250 mg).

Esterification reaction

A mixture of **8e** and **9e** (200 mg) was treated with a saturated solution of CH_2N_2 in ether for 5 min. The solvent was then evaporated and the crude product was purified by chromatography (hexane/ethyl acetate 1:1) affording **10e** (60 mg, 28%) and **11e** (140 mg, 64%).

(±)(3*S*, 4*R*)-2-methylen-1-oxo-4-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydrophenantrene-3-methylcarboxylate (10e). Mp 218 °C (MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.68 (s, 6H), 3.75 (s, 3H), 4.04 (s, 1H), 5.41 (s, 1H), 5.58 (s, 1H), 6.27 (s, 2H), 6.43 (s, 1H), 7.50 (t, 1H, J = 8.4), 7.87 (t, 1H, J = 8.4), 7.89 (d, 1H, J = 8.4), 8.02 (d, 1H, J = 8.4), 8.28 (d, 1H, J = 8.4); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 44.8 (CH), 52.6 (OCH_3), 55.9 (CH), 56.0 (OCH_3), 56.0 (OCH_3), 60.7 (OCH_3), 105.1 (CH), 105.1 (CH), 122.9 (CH), 125.4 (CH), 126.7 (CH), 126.8 (CH_2), 127.2 (CH), 128.5 (CH), 128.7 (CH), 130.5 (C), 131.0 (C), 136.3 (C), 136.7 (C), 136.9 (C), 137.6 (C), 140.0 (C), 153.3 (C), 153.3 (C), 172.1 (C=O), 186.1 (C=O); FABMS m/z (%): 433 (M^+, 100).

(±)(2*S*, 3*S*, 4*R*)-2-methoxymethyl-1-oxo-4-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydrophenanthrene-3-methylcarboxylate (11e). ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.18 (ddd, 1H, J = 4.1, 4.7, 8.3), 3.29 (s, 3H), 3.57 (s, 3H), 3.67 (s, 6H), 3.73 (dd, 1H, J = 8.3, 9.9), 3.75 (dd, 1H, J = 4.7, 8.2), 3.80 (s, 3H), 4.12 (dd, 1H, J = 5.4, 9.9), 5.38 (d, 1H, J = 8.8), 6.28 (s, 2H), 7.47 (t, 1H, J = 8.6), 7.54 (t, 1H, J = 8.6), 7.80 (d, 1H, J = 8.6), 7.83 (d, 1H, J = 8.6), 7.90 (d, 1H, J = 8.6), 8.24 (d, 1H, J = 8.6); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 42.6 (CH), 44.0 (CH), 49.3 (CH), 51.5 (OCH_3), 55.4 (OCH_3), 55.4 (OCH_3), 58.1 (OCH_3), 60.0 (OCH_3), 69.1 (CH_2), 104.8 (CH), 104.8 (CH), 121.4 (CH), 124.8 (CH), 126.4 (CH), 127.5 (CH), 127.9 (CH), 128.1 (CH), 129.8 (C), 130.3 (C), 135.5 (C), 136.0 (C), 136.4 (C), 137.3 (C), 152.8 (C), 152.8(C), 172.0 (C=O), 195.1 (C=O); FABMS m/z (%) 465 (M^+, 30).

Treatment with *p*-TsOH

Compound **11e** (140 mg, 0.3 mmols) was treated with *p*-TsOH (excess) in benzene and stirred at reflux for 7 h. The reaction mixture was quenched with a concd solution of $NaHCO_3$, extracted with ethyl acetate, washed with brine, dried over Na_2SO_4 and the organic solvent evaporated in vacuo affording compound **10e** (140 mg, 100%).

Chlorination reaction

Compound **10e** (200 mg, 0.5 mmols) in CH_2Cl_2 (15 mL) was treated with dry HCl gas for 2 h. The organic solvent was evaporated in vacuo and the crude product purified in MeOH, affording **12e** (180 mg, 80%).

(±)(2*S*, 3*S*, 4*R*)-2-chloromethyl-1-oxo-1-(3,4,5-trimethoxyphenyl)-1,2,3,4-tetrahydrophenantrene-3-methylcarboxylate (12e). Mp 194 °C (MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.18 (dd, 1H, J = 5.0, 8.8), 3.59 (s, 3H), 3.61 (s, 6H), 3.82 (s, 3H), 3.90 (m, 2H), 4.42 (dd, 1H, J = 5.0, 11.7), 5.46 (s, 1H), 6.25 (s, 2H), 7.47 (t, 1H, J = 8.4), 7.58 (t, 1H, J = 8.4), 7.82 (d, 1H, J = 8.4), 7.88 (d, 1H, J = 8.4), 7.90 (d, 1H, J = 8.4), 8.20 (d, 1H, J = 8.4); ^{13}C NMR (50.13 MHz, $CDCl_3$) δ 43.2 (CH_2), 45.3 (CH), 46.3 (CH), 50.4 (CH), 53.1 (OCH_3), 56.8 (OCH_3), 56.8 (OCH_3), 61.4 (OCH_3), 106.1 (CH), 106.1 (CH), 122.9 (CH), 126.2 (CH), 127.9 (CH), 129.2 (CH), 129.5 (CH), 129.5 (CH), 130.9 (C), 130.9 (C), 131.7 (C), 136.7 (C), 136.9 (C), 138.9 (C), 154.3 (C), 154.3 (C), 173.0 (C=O), 195.2 (C=O); FABMS m/z (%) 469 (M^+, 10).

Antitumor assays[17]

P-388 cells (suspension culture) were seeded into 16 mm wells at 1×10^4 cells/well in 1 mL aliquot of medium MEM 10FCS containing the indicate (Table 2) concentration of sample. The remaining cell lines: A-549, HT-29 and MEL-28 (monolayer cultures) were seeded into 16 mm wells at 2×10^4 cells /well in 1 mL aliquots of MEM 10FCS. The day after the inoculum, media were replaced by 1 mL aliquots of MEM 10FCS containing the different (Table 2) concentrations of sample. In both cases a separate set of cultures without sample was

counted daily to ensure that the cells remained in exponential growth. Cells were incubated at 37 °C in a 10% CO_2 humid atmosphere. All determinations were carried out in duplicate. After three days of incubation, cells were counted and the IC_{50} for each sample was determined.

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References and Notes

1. San Feliciano, A.; López, J. L.; Medarde, M.; Miguel del Corral, J. M.; de Pascual Teresa, B.; Puebla, P. *Tetrahedron* **1988**, *44*, 7255.
2. (a) López-Pérez, J. L.; Olmo, E.; de Pascual-Teresa, B.; Merino, M.; Martín, S.; San Feliciano, A. *Tetrahedron* **1995**, *51*, 6343. (b) López-Pérez, J. L.; Olmo, E.; de Pascual-Teresa, B.; Merino, M.; San Feliciano, A. *Tetrahedron* **1996**, *52*, 4903.
3. (a) Ter Haar, E.; Rosenkranz, H. S.; Hand, E.; Day, B. W. *Bioorg. Med. Chem.* **1996**, *4*, 1659. (b) Tepe, J. J.; Madalengoitia, J. S.; Slunt, K. M.; Werbovetz, K. W.; Spoors, P. G.; Macdonald, T. L. *J. Med. Chem.* **1996**, *39*, 2188. (c) Cho, S. J.; Kashiwada, Y.; Bastow, K. F.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1396.
4. Medarde, M.; Ramos, A. C.; Caballero, E.; López, J. L.; Peláez, R.; San Feliciano, A. *Tetrahedron Lett.* **1998**, *39*, 2001.
5. Ramos, A. C.; Peláez, R.; Medarde, M. *Heterocycles* **1999**, *51*, 1443.
6. (a) Maya, A. B.; del Rey, B.; Peláez, R.; Caballero, E.; Bararoain, I.; Andreu, J. M.; Medarde, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2549. (b) López-Pérez, J. L.; Olmo, E.; de Pascual-Teresa, B.; Merino, M.; Martín, S.; San Feliciano, A. *Tetrahedron* **1995**, *51*, 6343.
7. Ziegler, F. E.; Schwartz, J. A. *J. Org. Chem.* **1978**, *43*, 985.
8. Medarde, M.; Ramos, A. C.; Caballero, E.; López, J. L.; Peláez-Lamamié, R.; San Feliciano, A. *Tetrahedron Lett.* **1996**, *37*, 2663.
9. (a) Medarde, M.; Peláez Lamamié de Clairac, R.; Tomé, F.; López, J. L.; San Feliciano, A. *Arch. Pharm. (Weinheim)* **1994**, *327*, 91. (b) Medarde, M.; Peláez Lamamié de Clairac, R.; Tomé, F.; López, J. L.; San Feliciano, A.; Grávalos, D. G. *Arch. Pharm. (Weinheim)* **1995**, *328*, 403.
10. Ramos, A. C.; Peláez, R.; López, J. L.; Caballero, E.; Medarde, M.; San Feliciano, A. *Tetrahedron* **2001**, *57*, 3963.
11. Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, John Wiley & Sons: New York; **1991**, p. 198–207.
12. Medarde, M.; Ramos, A. C.; Peláez, R.; Caballero, E.; San Feliciano, A. *J. Chem. Soc., Perkin Trans.* **1994**, *1*, 45.
13. Khun, M.; von Wartburg, A. *Helv. Chim. Acta* **1967**, 1546.
14. (a) González, A. G.; de la Rosa, R.; Trujillo, J. M. *Tetrahedron* **1986**, *42*, 3899. (b) Boissin, P.; Dhal, R.; Brown, E. *Tetrahedron* **1992**, *48*, 687. (c) Ogiku, T.; Yoshida, S.; Ohmizu, H.; Iwasaki, T. *J. Org. Chem.* **1995**, *60*, 4585. (d) Pelter, A.; Ward, R. S.; Pritchard, M. C. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1615. (e) Tomioka, K.; Ishiguro, T.; Koga, K. *Chem. Pharm. Bull.* **1985**, *33*, 4333. (f) Brown, E.; Daugan, A. *Tetrahedron* **1986**, *45*, 141.
15. Vedejs, E.; Fuchs, P. J. *J. Org. Chem.* **1971**, *36*, 366.
16. Medarde, M.; Peláez-Lamamié de Clairac, R.; López, J. L.; Grávalos, D.G[a]; San Feliciano, A. *Arch. Pharm. (Weinheim)* **1995**, *328*, 640.
17. Bergeron, R. J.; Cavanaugh, P. F., Jr.; Kline, S. J.; Hughes, R. G., Jr.; Elliott, G. T.; Porter, C. W. *Bioch. Bioph. Res. Commun.* **1984**, *3*, 121 .