Eur. J. Med. Chem. 36 (2001) 561–568

PII: S0223-5234(01)01249-1/SCO

Short communication

Synthesis and biological evaluation of *O*-alkylated tropolones and related α-ketohydroxy derivatives as ribonucleotide reductase inhibitors

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Received 29 January 2001; revised 11 June 2001; accepted 12 June 2001

Abstract – A series of *O*-alkylated tropolones and related α-ketohydroxy compounds were evaluated for their biological activities and were shown to present an expected ribonucleotide reductase inhibition and cytotoxicity against some cancer cell lines but no antitubulin activity. Pharmacomodulation studies were realised to understand and enhance the observed activities. These original benzylic, heterocyclic and allylic compounds have been synthesised by a phase-transfer catalysed *O*-alkylation developed in our laboratories. © 2001 Éditions scientifiques et médicales Elsevier SAS

***O*-alkylated α-ketohydroxy compounds / phase-transfer *O*-alkylation / tropolones / anticancer / ribonucleotide reductase inhibitors**

1. Introduction

The ribonucleotide reductases play an important role in cell growth by supplying the 2′-deoxynucleotides required for DNA synthesis [1, 2]. Inhibition of these reductases interferes with the replication of genetic material required for cancer cell division or viral genome biosynthesis. Because of its importance in DNA synthesis, ribonucleotide reductase is a potential target for drug design. Few inhibitors, as hydroxyurea or tropolone derivatives [3–10], are known to block the cellular ribonucleotide reductase and are of great interest as inhibitors of human immunodeficiency virus-type 1 replication [3–10] or in cancer chemotherapy [11].

Yamato has shown that some tropolone derivatives could exhibit antitumoural activity probably related to iron chelation in the active site of ribonucleotide reductase [3–10]. These results prompted us to rationalise these observations by working on tropolone derivatives and related α-ketohydroxy compounds substituted by allylic, benzylic or heterocyclic moieties. The synthesis and the biological evaluation of these products are discussed here.

2. Chemistry

To explore the structure–activity relationships of various tropolone derivatives and α-ketohydroxy-related compounds, we decided to focus our work on alkylation reaction. Due to the tropolone moiety structure, we could imagine both *C*- or *O*-alkylation. Literature reports only one direct method to obtain *C*-alkylated tropolone analogues via palladium cross-coupling [12, 13] between a bromotropolone and a boronic acid derivative. A few other *C*-alkylated tropolones are described but resulted from a thermal Claisen-type rearrangement [14–17]. For *O*-alkylated tropolone synthesis [14–18], some methods are described, but all present drawbacks and a lack of generality.

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As part of our work on tropolone derivatives, we have recently described [19] a new general method for the synthesis of tropolone ethers. This method is a phase-transfer alkylation, as shown in *figure 1*, and had led us to synthesise various tropolone derivatives by reaction with benzyl, heterocyclic and allyl halides. This method was first applied to tropolone **3** and also extended to substituted tropolone **4** and α-ketohydroxy-related compounds like purpurogallin **5** and maltol **6**.

O-Alkylation of 4-isopropyl-tropolone **4** led to a 1:1 mixture of 4- and 6-isopropyl derivatives due to the possible isomerisation of the anion, as shown in *figure 2*. Only the 4-isopropyl derivatives **18** and **19** (*table II*) were obtained pure in sufficient quantities to be tested. Commercially available purpurogallin **5** was methylated by using dimethylsulphate to give a 1.5:1 mixture of tri- and tetramethylated products **20** and **20′**, as shown in *figure 3*. Maltol derivative **22** was converted in pyridinone analogue **24** by treatment with aqueous ammonia (*figure 4*). The alkylating agents are commercially available or have been easily prepared in our laboratory. The desired compounds were obtained in good to excellent yields and were exclusively *O*-alkylated. The tropolonyl esters **25**–**28** were prepared by classical condensation of tropolones **3** and **4** with the appropriate acyl chlorides in refluxing methylene chloride in the presence of anhydrous pyridine as described in Section 5 and *table IV*.

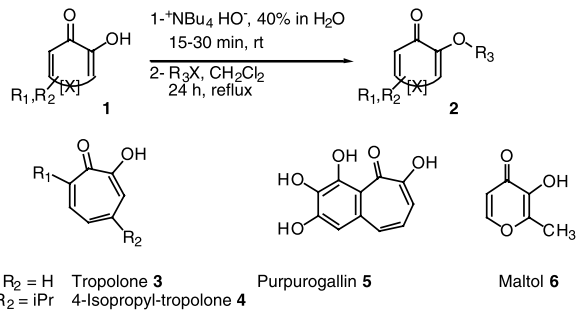


Figure 1. General method for *O*-alkylation of tropolones and related α-ketohydroxy derivatives, purpurogallin and maltol.

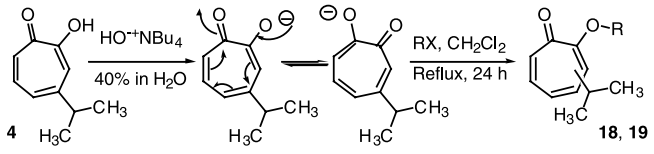


Figure 2. *O*-Alkylation of 4-isopropyltropolone.

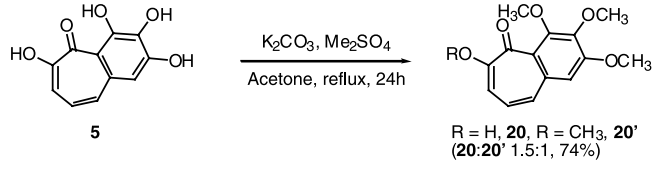


Figure 3. Preparation of trimethylpurpurogallin **20**.

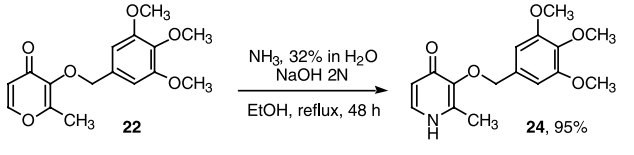


Figure 4. Conversion of *O*-alkylated maltol **22** in pyridinone analogue **24**.

3. Biological results and discussion

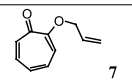
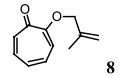
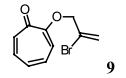
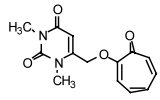
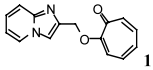
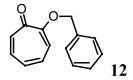
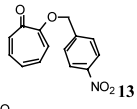
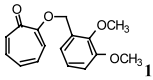
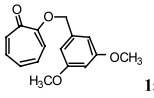
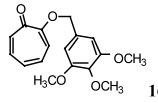
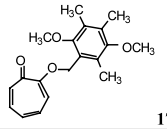
Biological evaluation of the allylic compounds (**7**–**9**) was first investigated in vitro on ribonucleotide reductase. As all products exhibit in vitro activity on ribonucleotide reductase comparable to that obtained with hydroxyurea as a reference compound, their cytotoxicity was evaluated on P388 cell lines as already described for Taxotere® [20]. Results were shown in *table I*.

As allylic derivatives (**7**–**9**) did not present any cytotoxicity on cancer cell lines, we turned our attention to the heterocyclic series (**10**, **11**). In the same way, they exhibit in vitro inhibition of ribonucleotide reductase but no cytotoxicity at all, as shown in *table I*.

As the same results were obtained with the heterocyclic derivatives (**10**, **11**), we finally chose the benzylic series (**12**–**17**). As before, all tested compounds exhibit in vitro inhibition of ribonucleotide reductase. In that series, products **13**, **15** and **17** did not exhibit any cytotoxicity but compounds **12** and **16** showed some cytotoxicity. It was interesting to note that potency seemed to be related to the substituents of the benzylic moiety: electron-withdrawing substituent (**13**) did not lead to cytotoxicity, on the contrary no substituent (**12**) or well-oriented electron-donating substituent (**16**) improved the cytotoxicity, as shown in *table I*.

After modifications to enhance the observed cytotoxicity, we retained the 3,4,5-trimethoxybenzyl moiety and made some variations on the tropolone part.

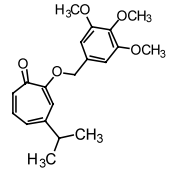
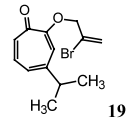
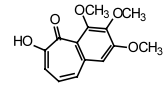
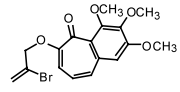
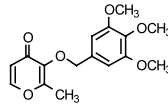
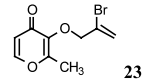
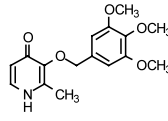
Table I. Biological activities of compounds **7**–**17** on ribonucleotide reductase and P388 cancer cell line.

Compounds	*RDR Inhibition*		*P388 cell growth Inhib.*	
	Conc. (mM)	% inhib.	Conc. (μg/ml)	% inhib.
 **7**	1	42	10	0
	0.1	34	1	0
 **8**	1	91	10	0
	0.1	77	1	0
 **9**	1	85	10	0
	0.1	37	1	0
 **10**	1	78	10	0
	0.1	25	1	0
 **11**	1	53	10	0
	0.1	12	1	0
 **12**			10	24
			1	16
	--	nd	0.1	21
			0.01	0
 **13**	--	nd	10	0
 **14**	1	82	10	0
	0.1	22	1	5
 **15**	1	83		
	0.1	50	10	0
	0.01	12	1	0
 **16**			IC_{50}	5 μg/ml
	1	79	10	99
	0.1	39	1	18
	0.01	29	0.1	11
 **17**	1	90	10	0
	0.1	50	1	0

First, we tried to change the binding properties by enhancing the steric hindrance of the substrate part. This was realised by adding an isopropyl substituent on the tropolone ring (**18**). On the contrary, the steric hindrance was decreased by fusing the two moieties and keeping the hydroxyl function free (**20**). Finally, we retained the 3,4,5-trimethoxybenzyl moiety and the tropolone ring was replaced by a maltol one (**22**) or a pyridinone one (**24**). Results are shown in *table II*. All modifications (**18**, **20**, **22**) resulted in a complete loss of cytotoxicity except in the case of the pyridinone derivative **24** which retains some cytotoxicity.

Finally, we decided to replace the ether link by an ester one (**25**–**28**) (*figure 5*). In those cases, cytotoxicity was retained only if an isopropyl substituent is present on the tropolone ring (**27**, **28**), as shown in *table III*.

Table II. Biological activities of 3,4,5-trimethoxybenzyl and 2-bromoallyl analogues **18**–**24** on ribonucleotide reductase and KB cancer cell line.

Compounds	*RDR Inhibition*		*KB cell growth Inhib.*	
	Conc. (mM)	% inhib.	Conc. (μg/ml)	% inhib.
 **18**	1	75	10	0
	0.1	18	1	0
 **19**	1	61	10	7
	0.1	9	1	5
	0.01	0	0.1	2
hydroxyurea	1	85	3.8	100
	0.1	57	0.76	26
			0.38	9
 **20**	1	98	10	71
	0.1	40	1	0
	0.01	7	0.1	0
 **21**	1	99	4	18
	0.1	26	0.9	5
	0.01	0	0.4	9
			0.09	3
hydroxyurea	1	94	3.8	61
	0.1	62		
 **22**	1	69	10	0
	0.1	21	1	0
 **23**	1	86	10	0
	0.1	29	1	0
hydroxyurea			3.8	95
	1	85	0.76	68
	0.1	57	0.38	28
 **24**	1	85	10	14
	0.1	25	1	15
	0.01	10	0.1	0

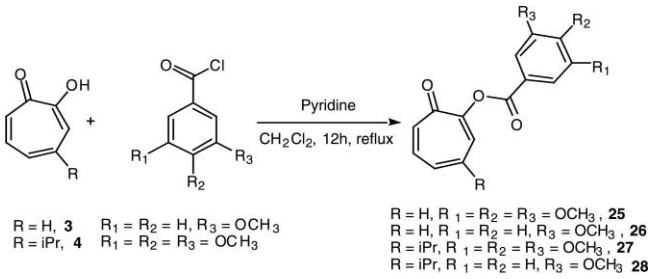
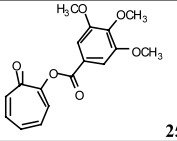
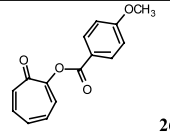
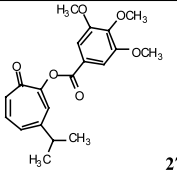
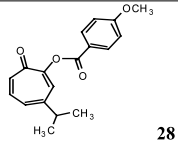


Figure 5. Preparation of ester derivatives **25**–**28**.

Table III. Biological activities of polymethoxybenzyl analogues in ester series **25**–**28** on ribonucleotide reductase and P388 cancer cell line.

Compounds	*Inhibition of RDR*		*P388 cell growth Inhib.*	
	Conc. (mM)	% inhib.	Conc. (μg/ml)	% inhib.
 **25**	--	nd	100	0
 **26**	--	nd	100	0
 **27**	1 0.1	71 27	10	95
 **28**	--	nd	100	100

Interestingly, when the same variations were made on compound **9**, which did not present any cytotoxicity, the introduction of an isopropyl group on the tropolone ring (**19**) or the fusion of a trimethoxyphenyl moiety (**21**) resulted in the apparition of poor cytotoxicity, not observed in the case of the maltol-related compound **23**.

In addition, compounds were tested for inhibition of tubulin polymerisation. Only compound **19** slightly inhibits tubulin polymerisation at high concentration (100 μg ml^{-1}). This activity disappears at lower concentration (10 μg ml^{-1}).

4. Conclusion

All tested compounds present an in vitro inhibition of the ribonucleotide reductase, but only few present cytotoxic properties on P388 or KB cell lines. In order to increase the potency observed for the best compound (**16**), we made some structural variations but, at this stage, we are not able to explain clearly which substituents are really involved in the interaction with the enzyme.

5. Experimental

5.1. Chemistry

Melting points were determined on a Buchi capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Centre de Microanalyses of the University of Aix-Marseille 3. Both ^{1}H- and ^{13}C-NMR spectra were determined on a Bruker AC 200 spectrometer. The ^{1}H-NMR shifts were reported in ppm downfield from Me_4Si and the ^{13}C-NMR shifts were referenced to the solvent peaks ($CDCl_3$; 76.9 ppm or DMSO-d_6; 39.6 ppm). Silicagel 60 (Merck, 0.063–0.200 mm, 70–230 mesh ASTM) was used for LC. TLC were performed on 5×10 cm aluminium plates coated with silicagel 60 F-254 (Merck) in an appropriate solvent. All commercial alkylating agents, purpurogallin **5**, and maltol **6** were purchased from Aldrich Chemical Co. Commercial products were of the highest purity available and were used as received. Substituted benzyl chlorides were prepared from the corresponding aldehydes by reduction with $NaBH_4$ and subsequent chlorination. Tropolone **3** [21], 4-isopropyl-tropolone **4** [21, 22], trimethylpurpurogallin **20** [23], 6-chloromethyl-1,3-dimethyl-1*H*,3*H*-pyrimidine-2,4-dione [24], and 2-chloromethylimidazo[1,2-*a*]pyridine [25] were prepared from known procedures.

5.1.1. Alkylation of tropolones and related compounds; general procedures [19]

5.1.1.1. Stoichiometric method

m The appropriate tropolone (6.07 mmol) was treated with Bu_4NOH (40% in water, 3.82 ml, 6.07 mmol) under an inert atmosphere at r.t. for 1 h. A solution of the appropriate alkylating agent (6.07 mmol) in CH_2Cl_2 (2.5 ml $mmol^{-1}$) was added slowly. The reaction was conducted at r.t. or at reflux depending on the alkylating

agent, and was monitored by TLC (CH_2Cl_2–EtOAc 7:3). After dilution with water (20 ml) and vigorous stirring for 10 min, the aqueous layer was extracted with CH_2Cl_2 (3×30 ml). The combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. Purification of the residue was done by chromatography on silicagel, eluting with 30% EtOAc in CH_2Cl_2.

5.1.1.2. Catalytic method

The appropriate tropolone (1 equiv., 8.20 mmol) was added to 25% aq. KOH (5 ml). After stirring at r.t., a yellow coloration was observed and H_2O was evaporated under 0.005 mbar at r.t. The yellow solid was recrystallised from acetone to give the potassium salt of tropolone in 95% yield. The appropriate alkylating agent (1 equiv.) was added slowly to the above potassium salt of tropolone (1 equiv.) followed by Bu_4NBr (0.1 equiv.) in H_2O (0.4 ml $mmol^{-1}$). The reaction was conducted at r.t. or reflux depending on the alkylating agent, and monitored by TLC (CH_2Cl_2–EtOAc 7:3). Workup and purification were identical as above.

*5.1.2. Conversion of maltol derivative **22** to the corresponding pyridinone analogue **24***

O-Alkylated maltol derivative **22** (2.01 g, 6.57 mmol) was treated with a mixture of EtOH (26.13 ml), NH_4OH (32% in H_2O, 56.28 ml) and NaOH 2 N (10.60 ml) under an inert atmosphere under reflux for 48 h. After cooling, the reaction mixture was concentrated under reduced pressure. The residue was then diluted with H_2O (10 ml) and pH 7 was reached by titration with a diluted hydrochloric solution. The aqueous layer was extracted with CH_2Cl_2 (3×30 ml) and the combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification of the residue done by chromatography on silicagel, eluting with 5% MeOH in CH_2Cl_2, followed by recrystallisation from acetone gave the desired pyridinone, 2-methyl-3-(3,4,5-trimethoxybenzyloxy)-1H-pyridin-4-one (**24**), as a white solid in 95% yield (1.90 g). m.p.: 187–188 °C . ^{1}H-NMR (DMSO-d_6) δ 2.10 (s, 3H), 3.64 (s, 3H), 3.74 (s, 6H), 4.96 (s, 2H), 6.85 [AB, $\Delta\nu = 1.3$: 6.14 (d, 1H, $J = 7.3$ Hz), 7.46 (d, 1H, $J = 7.0$ Hz)], 6.71 (s, 2H). ^{13}C-NMR (DMSO-d_6) δ 13.68, 55.78 (2x), 60.00, 71.89, 105.59 (2x), 115.99, 133.63, 134.56, 136.96, 139.06, 152.61 (2x), 161.00, 174.47.

Anal. Found: C, 62.93; H, 6.20; N, 4.60. Calc. for $C_{16}H_{19}NO_5$ (305.35): C, 62.94; H, 6.27; N, 4.59%.

5.1.3. Preparation of tropolonyl esters; general method (table IV)

To a solution of the appropriate tropolone (0.5 g, 4.10 mmol) in CH_2Cl_2 (10 ml) at r.t. under an inert atmosphere were added the appropriate acyl chloride (4.10 mmol) and anhydrous pyridine (1.5 ml, 18.65 mmol). After stirring under reflux for 12 h the solvents were removed under reduced pressure and the residue was diluted with CH_2Cl_2 (10 ml). The organic layer was washed with an aqueous saturated solution of Na_2CO_3, dried ($MgSO_4$) and concentrated under reduced pressure. The remaining oil was crystallised from EtOH to give the esters as white crystals [26].

5.2. Biology

5.2.1. Ribonucleotide Reductase test—principle of the test

RDR is one of the key enzymes for DNA replication and catalyses the reduction of ribonucleotides into deoxyribonucleotides. Enzymatic activity was correlated to the quantity of dCDP formed. After reaction, residual CDP and dCDP formed were, respectively, hydrolysed into cytidine (C) and deoxycytidine (dC). These two compounds were separated by chromatography and radioactive dC amounts were measured.

5.2.2. Reagents

All commercially available reagents were purchased from Sigma–Aldrich except the ^{14}C-labelled CDP purchased from CEA. Except buffers, all the reagents were prepared on ice and were stored as aliquots at −20 °C. Apyrase and alcaline phosphatase solutions were stored at −70 °C after preparation and/or utilisation. Borate buffer (50 mM, pH 9): a 19.06 g ml^{-1} solution of sodium tetraborate was prepared and pH 9 was reached by adding a 1 N solution of sodium hydroxide. Hepes buffer (27 mM, pH 7.4)–$MgCl_2$ (32.5 mM)–NaF (25.5 mM): a 1 l solution was prepared by adding Hepes (1 M, 27 ml), $MgCl_2$ (1 M, 32.5 ml) and NaF (1 M, 21.5 ml) and then pH was checked. Hepes buffer (20 mM, pH 7.4)–$MgCl_2$ (2 mM)–DTT (2 mM): a 1 l solution was prepared by adding Hepes (1 M, 20 ml), $MgCl_2$ (1 M, 2 ml) and DTT (1 M, 2 ml). Adenosine 5′-triphosphate (ATP, 13.5 mM): a 7.437 mg ml^{-1} solution was prepared with distilled water and neutralised carefully by adding 5 μl aliquots of a 1 N sodium hydroxide solution. DL–dithiothreitol (DTT 27 mM): a 4.16 mg ml^{-1} solution was prepared with distilled water. Cytidine 5′-diphosphate (CDP, 0.33 mM): a 0.133 mg ml^{-1}

solution was prepared with distilled water. 2′-Deoxycytidine: a 10 mg ml^{-1} solution was prepared with distilled water. Apyrase: a 3000 U ml^{-1} solution was prepared, corresponding to 666 μl of phosphate buffer (0.01 M, pH 7). Alcaline phosphatase: a 10 mg ml^{-1} solution was prepared from the commercially available form (3 U mg^{-1}) with phosphate buffer (0.01 M, pH 7). ^{14}C-labelled cytidine 5′-phosphate (50 μCi ml^{-1}): to the commercially available form (0.50 ml, 10% in EtOH, 0.054 mCi) was added 500 μl of distilled water.

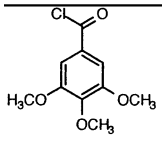
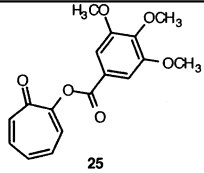
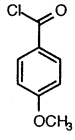
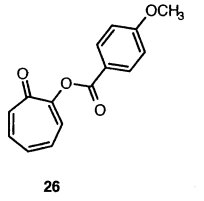
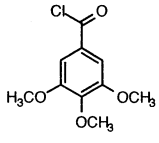
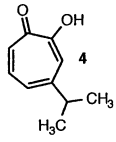
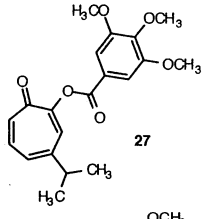
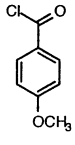
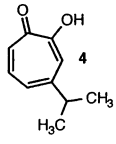
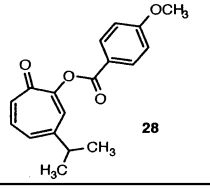
5.2.3. Enzyme preparation

Every manipulation was done in an ice-bath. S180 tumour cell-lines were used. Tumours were washed and necrotic areas were cut. Then tumours were weighed (x g), roughly cut and added to (0.8 ml×x g) of Hepes buffer 20 mM pH 7.4–2 mM $MgCl_2$–2 mM DTT in a tube. The mixture was crushed (Ultraturrax) at least five times for 10 s at the higher speed with a break between each crushing to allow a good cooling. After centrifugation (Beckman Rotor 50Ti ultracentrifugation) of the obtained suspension (1 h 30 at 40 000 tr min^{-1}), the floating part was taken with a Pasteur pipette, avoiding taking the lipidic layer. The obtained suspension was dyalised at least for 1 h against 100× its volume of Hepes buffer 20 mM pH 7.4–2 mM $MgCl_2$–2 mM DTT in the cold room. The cellular extract was split (0.5 ml per tube), frozen at −70 °C and stored at −70 °C . Proteins were dosed by the Folin technique and concentration was supposed to be around 25 mg ml^{-1}.

5.2.4. Reaction

Each mixture was realised at 3 °C . In each 1.5 ml Eppendorf tube were added 10 μl Hepes buffer 27 mM–$MgCl_2$ 32.5 mM–NaF 21.5 mM, 10 μl of DTT 27 mM, 10 μl ATP 13.5 mM, 7.5 μl of CDP 0.33 mM, 2.5 μl of CDP* 50 μCi ml^{-1}, 2.5 μl of tested inhibitor (20 mM, 2 mM) and 10 μl enzyme preparation. After 30 min of incubation at 37 °C , the reaction was quenched by heating at 100 °C for 2–3 min. Incubation was

Table IV. *O*-Acylated products **25**–**28** prepared.

Acyl Halide	Substrate	Product	Yield (%)	mp (°C)	^{1}H NMR ($CDCl_3$ / TMS) δ(ppm), J (Hz)	Anal. C, H [26]
	 **3**	 **25**	79	129-130	3.91 (s, 6 H), 3.95 (s, 3 H), 7.12-7.27 (m, 5 H), 7.44 (s, 2 H)	$C_{17}H_{16}O_6$
	 **3**	 **26**	79	142-144	3.88 (s, 3 H), 6.96 (d, 2 H, J = 8.9), 7.11 (br m, 3 H), 7.26 (br m, 2 H), 8.11 (d, 2 H, J = 8.9)	$C_{15}H_{12}O_4$
	 **4**	 **27**	76	134-135	1.23 (d, 6 H, J = 6.8), 2.84 (sept, 1 H, J = 6.8), 3.92 (s, 6 H), 3.93 (s, 3 H), 7.03-7.26 (m, 4 H), 7.45 (s, 2 H)	$C_{20}H_{22}O_6$
	 **4**	 **28**	65	98-99	1.26 (d, 6 H, J = 6.8), 2.86 (sept, 1 H, J = 6.8), 3.89 (s, 3 H), 6.98 (d, 2 H, J = 8.8), 7.09-7.27 (br m, 4 H), 8.14 (d, 2 H, J = 8.8)	$C_{18}H_{18}O_4$

continued for 1 h at 37 °C after adding 5 μl apyrase (3000 U ml^{-1}). Another 1 h of incubation at 37 °C was done after adding 30 μl borate buffer (50 mM, pH 9.0), 5 μl cytidine (10 mg ml^{-1}), 5 μl deoxycytidine (10 mg ml^{-1}), 5 μl alcaline phosphatase (10 mg ml^{-1}). The reaction was quenched by heating at 100 °C for 10 min and tubes were centrifugated (Eppendorf bench centrifugation) for 2 min.

5.2.5. Chromatographies

Chromatographies were done on 20×20 plastic support coated with a cellulose sheet with fluorescence indicator (Sclucher and Schuell). Spots of 5 μl were spaced at 1.5 cm and dried before elution with a mobile phase composed of ammonium acetate 5 M pH 9.0 (10 ml), saturated sodium tetraborate (40 ml), EtOH (90 ml) and EDTA 0.5 M (500 μl). Time separation was ca. 4 h.

5.2.6. Results

Radioactivity was measured using a Berthold analyser.

5.2.7. Colchicine test—principle of the test

Tubulin, a heterodimeric protein, polymerises to form microtubules. This assembly can be inhibited by colchicine type compounds and some natural substances. In order to evaluate the potency of potential drugs, tubulin polymerisation is induced in the presence of the drug. The amount of microtubules formed is then monitored by measuring the turbidity of the solution.

5.2.8. Reagents

EGTA and $MgCl_2$ were purchased from Sigma. Mes was purchased from Calbiochem. Bidistilled glycerol was purchased from Prolabo, and lithium bisalt of GTP was purchased from Boehringer Mannheim.

5.2.9. Preparation of pure tubulin

Brain pig tubulin was prepared from three polymerisation–depolymerisation cycles followed by P11 phosphocellulose chromatography (Whatman). Tubulin was eluted and concentrated by ultrafiltration and adjusted to the following data [(Mes: 0.05 M); (pH 6.8); ($MgCl_2$: 0.25 mM); (EGTA: 0.5 mM); (Glycerol: 3.4 M); (GTP: 0.2 mM)] and stored at −80 °C at a concentration ca. 5–10 mg ml^{-1}.

5.2.10. Microtubule assembly assay

Tubulin (10 μl, 1 mg ml^{-1}) was equilibrated in assembly buffer [(Mes: 50 mM); (pH 6.8); ($MgCl_2$: 0.25 mM); (EGTA: 0.5 mM); ($MgCl_2$: 6 mM); (glycerol: 3.4 mM); (GTP: 1 mM)]. The reaction was started by a temperature variation between 6 and 37 °C in a thermostat cell with a 1 cm optical distance, and was monitored the turbidity measurement at 400 nm (Uvikon 931 Spectrophotometer). When a potential inhibitor (100 μg ml^{-1}) was tested, it was added to tubulin solution just before the temperature progression.

Acknowledgements

Financial support by the CNRS and Rhône-Poulenc Rorer (Aventis Pharma) through a Grant (BDI for I.T.T.) is gratefully acknowledged.

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[26] All the compounds tested gave satisfactory elemental analyses. Elemental analyses for the new esters: 3,4,5-trimethoxybenzoic acid 7-oxocyclohepta-1,3,5-trienyl ester (**25**): Anal. Found: C, 64.52; H, 5.14. Calc. for $C_{17}H_{16}O_6$ (316.31): C, 64.55; H, 5.10. 4-Methoxybenzoic 7-oxocyclohepta-1,3,5-trienyl ester (**26**): Anal. Found: C, 70.32; H, 4.70. Calc. for $C_{15}H_{12}O_4$ (256.26): C, 70.31; H, 4.72. 3,4,5-Trimethoxy benzoic acid 3-isopropyl-7-oxocyclohepta-1,3,5-trienyl ester (**27**): Anal. Found: C, 66.99; H, 6.17. Calc. for $C_{20}H_{22}O_6$ (358.39): C, 67.03; H, 6.19. 4-Methoxybenzoic 3-isopropyl-7-oxocyclohepta-1,3,5-trienyl ester (**28**): Anal. Found C, 72.44; H, 6.05. Calc. for $C_{18}H_{18}O_4$ (298.34): C, 72.47; H, 6.08.