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RESEARCH ARTICLE

Semisynthesis of α-methyl-γ-lactones and *in vitro* evaluation of their activity on protein farnesyltransferase

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Abstract
The semisynthesis of xanthanolide derivatives is reported from xanthinin and 4-*epi*-isoxanthanol, two sesquiterpene lactones isolated from the crude chloroformic extract of the leaves of *Xanthium macrocarpum* DC. (Asteraceae) by liquid/liquid chromatography. *In vitro* evaluation of their protein farnesyltransferase (PFTase) inhibitory activity has been investigated. In contrast to other biological activities of xanthanolides, PFTase inhibition is not associated with the presence of the potentially toxic α-methylene-γ-lactone function.

Keywords: *Protein farnesyltransferase inhibitors; sesquiterpene lactone; xanthanolide; semisynthesis*

Introduction

Xanthium macrocarpum DC. (Asteraceae) is a plant originating from America and widely distributed in the floodplain zone of rivers. It was traditionally used in France for the treatment of several diseases such as catarrh, scrofula, and leprosy[1]. We earlier reported on the phytochemical study of its aerial parts that revealed the presence of several xanthanolides[2]. This subtype of sesquiterpene lactones is already known for its antimicrobial[3], antifungal[4], antimalarial[5], antileishmanial[6], cytotoxic[7,8], and anti-ulcerogenic activities[9]. A report also pointed out the potential of xanthanolides as protein farnesyltransferase inhibitors[10]. The protein farnesyltransferase (PFTase) is an enzyme responsible for the post-translational farnesylation of the oncogenic variants of Ras, and could be considered as a promising target for the treatment of cancer[11]. However, these xanthanolides (e.g. 8-*epi*-xanthatin, Figure 1) with PFTase inhibitory activity mainly differ in their stereochemistry from the xanthanolides (e.g. xanthatin **2**, Scheme 1) isolated from *Xanthium macrocarpum* DC. The 8-*epi*-xanthatin bears a 7,8-*cis* fused lactone ring with a high degree of conformational flexibility, whereas xanthatin has a 7,8-*trans* fused ring and is therefore probably much more rigid. Moreover, it was reported that, among helenanolide sesquiterpene lactones, minor differences in the stereochemistry significantly affect the bioactivity[12]. We therefore decided to evaluate the potential of 7,8-*trans* fused xanthanolides isolated from a natural source (**1–3**) and their synthetic derivatives (**4–7**) as PFTase inhibitors. The biological activities of sesquiterpene lactones are often explained by their ability to react *in vivo* as Michael acceptors with endogenous nucleophiles[13]. In fact, we recently reported that in contrast to **1** or **3**, reduced compounds such as **8** and **10** were devoid of any antifungal activity[4]. The alkylating potential of α-methylene-γ-lactone is also related to the toxicity of several plants rich in sesquiterpene lactones[14]. Moreover, only artemisinin, an α-methyl sesquiterpene lactone, is nowadays used in therapeutics[15]. As far as PFTase activity is concerned, there are only a few reported examples of monomeric[16] and dimeric[17] sesquiterpene lactones with such activity in the α-methyl-γ-lactone series. Therefore, with the aim to obtain potentially atoxic compounds, we decided to prepare several 7,8-*trans* fused derivatives in the α-methyl-γ-lactone series, starting from natural products **1–3**, and explore their ability to inhibit protein farnesyltransferase. Beforehand, it was necessary to purify compounds **1–3** present in the crude chloroformic extract from the leaves of *Xanthium macrocarpum* DC.

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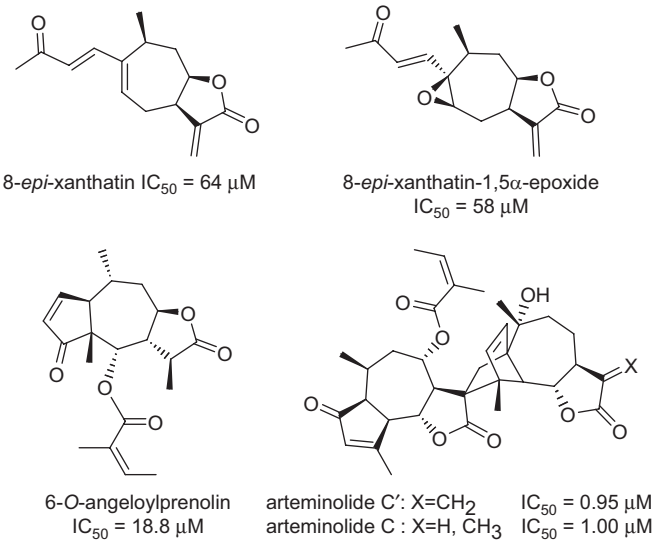


Figure 1. Xanthanolides exerting PFTase inhibitory activity.

by centrifugal partition chromatography (CPC), a liquid/liquid chromatography.

Materials and methods

General

All reagents and solvents were general purpose grade. The dried leaves from *Xanthium macrocarpum* were crushed with a Retsch Muhle crushing mill. The leaf powder was extracted in a Soxhlet apparatus (6 L solvent capacity). The purification of **1–3** was performed using a R 1000 rotor (980 mL column) on a bench scale FCPC (fast centrifugal partition chromatograph; Kromaton Technologies) with a KP 100 (100 mL/min, 100 bar) pump (Armen-Kromaton Technologies). Sample was injected with a loop (Kromaton Technologies; total bed volume 50 mL) and fractions were collected with a Gilson FC-204 collector. Column chromatographies were performed on silica gel 60 (Macherey-Nagel; 230–400 mesh) and preparative thin layer chromatography (TLC) with silica gel plates (Macherey-Nagel; SIL G/UV254, 0.25 mm). Eluates were monitored by TLC on silica 60 F254 (Macherey-Nagel; SIL G/UV254, 0.20 mm). The spots were detected under ultraviolet (UV) light at 254 nm or using sulfuric vanillin as revealing agent. Infrared (IR) spectra were determined on a Bruker Fourier transform (FT) IR Vector 22 spectrometer using neat liquid films. ^{1}H-nuclear magnetic resonance (NMR) spectra and ^{13}C-NMR spectra were recorded in $CDCl_3$ with, respectively, $CHCl_3$ at 7.26 ppm and 77.0 ppm as the internal standard on a Bruker Avance DRX 500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) machine or on a Jeol GSX 270 WB (^{1}H: 270 MHz; ^{13}C: 67.5 MHz) spectrometer. High-resolution electron ionization (EI) and electrospray ionization (ESI) mass spectroscopy analyses were done with a JMS-700 (Jeol) double focusing mass spectrometer with reversed geometry or an Orbitrap (Thermoelectron) machine in positive mode. Samples were solubilized with H_2O–MeOH (1:1) and injected with a syringe pump.

Plant material

The leaves of *Xanthium macrocarpum* DC. were collected at Saintes-Gemmes sur Loire (France) in September 2003. A voucher specimen (XM003) was deposited in the Department of Pharmacognosy, University of Angers, France.

Extraction

The dried and powdered leaves of *X. macrocarpum* DC. (9.0 kg) were extracted in several portions (~375 g) for 20 h with 6 L of $CHCl_3$ in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure gave 945 g of a crude chloroformic extract as a gum that was kept for 10 months before chromatography.

Isolation

Preparation of the two-phase solvent system

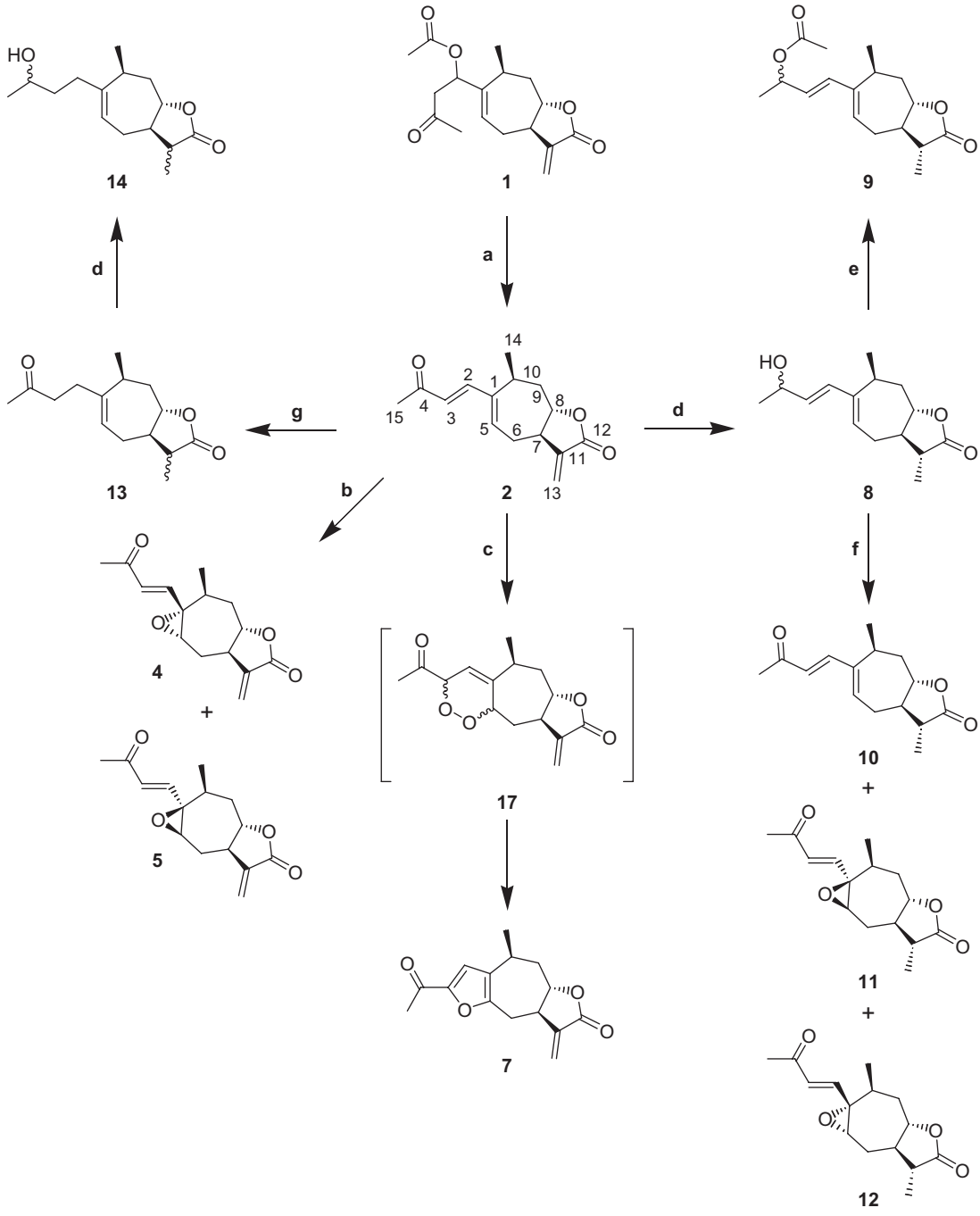
The same volumes of *n*-heptane, EtOAc, MeOH, and H_2O were mixed and thoroughly equilibrated in a separation funnel (4 L) at room temperature until two phases were discernible (30 min). The two phases were separated shortly before use.

Purification procedure on FCPC

The hydrostatic FCPC column was fully filled in ascending mode with the lower phase (stationary phase, 980 mL) using the KP 100 pump at a flow rate of 60 mL/min while the apparatus was run at 500 rpm. Then, 50 mL of sample (120 mg/mL) was injected while the apparatus was run at 1100 rpm. The sample was eluted with 30 mL of stationary phase at 20 mL/min, then the upper phase was pumped into the tail end of the inlet column at a flow rate of 10 mL/min during 5 min, and then at 20 mL/min. Some 390 mL of stationary phase eluted at the head end outlet. Next, 132 fractions of 15 mL were collected. After all desired compounds, monitored by TLC, were eluted, the elution was stopped. Fractions F14–18, F19–28, and F31–40 were respectively combined, concentrated under reduced pressure, and extracted with CH_2Cl_2. The dichloromethanic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The three fractions were recrystallized in *c*-hexane to give 385 mg (F14–18) of **1** (0.64% of dried leaves), 285 mg (F19–28) of **2** (0.47%), and 310 mg (F31–40) of **3** (0.51%). The final characterization of compounds **1–3** was carried out by ^{1}H-NMR, ^{13}C-NMR, and comparison with literature data.

Semisynthesis

*Xanthatin-1,5-epoxides **4** and **5***[18] PCC (16.5 mg, 76 μmol) was added to a solution of xanthatin **2** (20 mg, 81 μmol) in dry CH_2Cl_2 (2 mL). The mixture was heated under reflux for 8 days. The precipitate was filtered on Celite®. CH_2Cl_2 was removed under reduced pressure and the residue was chromatographed over 5.4 g of silica gel (CH_2Cl_2–EtOAc, 9:1), yielding the xanthatin-1,5β-epoxide **4** (1 mg, 5% yield) and the xanthatin-1,5α-epoxide **5** (0.8 mg, 4% yield). **4**: ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.84 (1H, d, *J* = 15.6 Hz, H-2), 6.24 (1H, d, *J* = 15.6 Hz, H-3), 6.21 (1H, d, *J* = 2.5 Hz, H-13α), 5.49 (1H, d, *J* = 2.5 Hz, H-13β), 4.26 (1H, m, H-8), 2.98 (1H, t, *J* = 4.6 Hz, H-5), 2.85 (1H, m, H-7 and H-10), 2.67 (1H, m,



Scheme 1. Synthesis of compounds **2**, **4**, **5**, and **7–14**. Reagents: (a) SiO_2, RT, 2 days, 100%; (b) PCC, CH_2Cl_2, refluxing, 8 days (**4**, 5%; **5**, 4%); (c) dry air, tetraphenylporphine, CH_2Cl_2, hυ, 15°C, 5 h, 14%; (d) $NaBH_4$, MeOH, 0°C, 3 h (**8**, 95%; **14**, 95%); (e) AcCl, TEA, CH_2Cl_2, RT, 24 h, 12%; (f) DDQ, toluene, refluxing, 3h (**10**, 42%, **11**, 2%; **12**, 2%); (g) $NaBH_4$, $NiCl_2$, MeOH, 0°C, 3 h, 79%.

H-6α), 2.27 (3H, s, H-15), 2.01 (2H, m, H-6β and H-9α), 1.81 (1H, m, H-9β), 1.09 (3H, d, J = 7.1 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 190.4 (C-4), 169.2 (C-12), 144.9 (C-2), 138.8 (C-11), 128.9 (C-3), 118.5 (C-13), 80.1 (C-8), 63.6 (C-1), 63.1 (C-5), 42.9 (C-7), 34.4 (C-9), 31.1 (C-10), 28.3 (C-15), 26.3 (C-6), 16.4 (C-14); MS (ESI+) *m/z* calcd for $C_{15}H_{19}O_4$ 263.1278. Found 263.1278 $[M + H]^+$. **5**: ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.92 (1H, d, J = 15.6 Hz, H-2), 6.30 (1H, d, J = 15.6 Hz, H-3), 6.18 (1H, d, J = 3.2 Hz, H-13α), 5.45 (1H, d, J = 3.2 Hz, H-13β), 3.93 (1H, ddd, J = 2.5, 9.9, 12.4 Hz, H-8), 3.07 (1H, d, J = 5.3 Hz, H-5), 2.88 (1H, m, H-7), 2.70 (1H, m, H-6α), 2.62 (1H, m, H-10), 2.29 (3H, s, H-15), 2.14 (2H, m, H-6β and H-9α), 2.00 (1H, m, H-9β), 1.30 (3H, d, J = 7.4 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 190.4 (C-4), 169.2 (C-12), 144.8 (C-2), 138.8 (C-11), 128.9 (C-3), 118.5 (C-13), 79.9 (C-8), 63.6 (C-1), 63.1 (C-5), 42.9 (C-7), 34.2 (C-9), 31.1 (C-10), 28.2 (C-15), 26.3 (C-6), 16.4 (C-14).

Acetyl-4-epi-isoxanthanol ***6***[5] Triethylamine (370 µL, 2.6 mmol) and AcCl (190 µL, 2.6 mmol) were added in small portions during 4 days to a solution of 4-*epi*-isoxanthanol **3**

(105 mg, 0.3 mmol) in dry CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 5 days and then washed with H_2O (3 × 5 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed over 6 g of silica gel (CH_2Cl_2–EtOAc, 9:1), yielding the acetyl-4-*epi*-isoxanthanol **6** (12.3 mg, 12% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.15 (1H, d, *J* = 3.2 Hz, H-13α), 5.92 (1H, dd, *J* = 2.8 and 8.5 Hz, H-5), 5.44 (1H, d, *J* = 3.2 Hz, H-13β), 5.18 (1H, dd, *J* = 4.6 Hz, H-2), 4.90 (1H, m, H-4), 4.27 (1H, ddd, *J* = 2.8, 9.6, 12.4 Hz, H-8), 2.77 (1H, m, H-10), 2.55 (1H, m, H-6α), 2.44 (1H, m, H-7), 2.31 (2H, m, H-6β and H-9α), 2.04 (3H, s, H-17), 2.02 (3H, s, H-19), 1.82 (3H, m, H-3 and H-9β), 1.25 (3H, d, *J* = 6.0 Hz, H-15), 1.10 (3H, d, *J* = 7.1 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 170.5 (C-18), 170.0 (C-16), 169.7 (C-12), 145.6 (C-1), 139.3 (C-11), 126.3 (C-5), 118.5 (C-13), 82.1 (C-8), 67.2 (C-4), 67.1 (C-2), 47.9 (C-7), 39.9 (C-3), 36.8 (C-9), 29.6 (C-10), 25.3 (C-6), 21.2 (C-15), 21.1 (C19), 20.2 (C-17), 19.3 (14-C).

*3,5-Oxyxanthatin **7*** Dried air was bubbled through the solution of xanthatin **2** (70 mg, 0.28 mmol) and tetraphenylporphine (3 mg, 0.004 mmol) as the photo-sensitizer in CH_2Cl_2 (30 mL). The reaction mixture was cooled to 15°C and irradiated with a halogen lamp (500 W) for 5 h. CH_2Cl_2 was removed under reduced pressure and the residue was purified on preparative TLC (EtOAc–CH_2Cl_2, 4:6) as the eluent, yielding the 3,5-oxyxanthatin (**7**) (10 mg, 14% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 7.04 (1H, s, H-2), 6.30 (1H, d, *J* = 3.2 Hz, H-13α), 5.59 (1H, d, *J* = 3.2 Hz, H-13β), 4.37 (1H, ddd, *J* = 2.5, 9.2, 11.7 Hz, H-8), 3.45 (1H, m, H-6α), 3.19 (1H, m, H-10), 2.89 (1H, m, H-7), 2.74 (1H, m, H-6β), 2.44 (3H, s, H-15), 2.10 (2H, m, H-9), 1.28 (3H, d, *J* = 7.2, H-14); ^{13}C NMR (67.5 MHz, $CDCl_3$): δ 186.1 (C-4), 169.1 (C-12), 152.3 (C-3), 150.1 (C-5), 138.6 (C-11), 128.0 (C-1), 120.3 (C-2), 120.2 (C-13), 80.5 (C-8), 44.9 (C-7), 38.5 (C-9), 31.9 (C-6), 29.7 (C-10), 22.6 (C-15), 14.8 (C-14); MS (ESI+) *m/z* calcd for $C_{15}H_{17}O_4$ 261.1121. Found 261.1121 $[M + H]^+$.

*2,3-(E)-Dehydro-4-epi-isoxanthanol **9*** Triethylamine (140 µL, 1 mmol) and AcCl (75 µL, 1 mmol) were added dropwise during 3 h to a solution of 4,11β,13-tetrahydroxanthatin **8** (58 mg, 230 µmol) in dry CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 24 h and was then washed with H_2O (3 × 5 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified on preparative TLC (CH_2Cl_2–EtOAc, 9:1), yielding the 2,3-(*E*)-dehydro-4-*epi*-isoxanthanol **9** as an unseparable diastereomeric mixture (8.2 mg, 12% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.12 (1H, d, *J* = 15.6 Hz, H-2), 5.82 (1H, dd, *J* = 2.8, 8.8 Hz, H-5), 5.61 (1H, m, H-3), 5.43 (1H, m, H-4), 4.29 (1H, ddd, *J* = 1.8, 9.9, 11.7 Hz, H-8), 3.01 (1H, m, H-10), 2.45 (1H, m, H-6α), 2.30 (3H, m, H-6β, H-7 and H-11), 2.05 (3H, s, H-17), 1.66 (2H, m, H-9), 1.35 (3H, d, *J* = 6.4 Hz, H-15), 1.23 (3H, d, *J* = 6.7 Hz, H-13), 15 (3H, d, *J* = 7.4 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 178.5 (C-12), 170.3 and 170.2 (C-16), 144.4 (C-1), 136.6 and 136.5 (C-2), 130.3 and 130.2 (C-3), 125.4 (C-5), 81.8 (C-8), 71.3 and 71.1 (C-4), 51.2 and 51.1 (C-7), 41.9 (C-11), 36.3 and 36.2 (C-9), 29.9 and 29.0 (C-10), 27.8 (C-6), 21.4 (C-15), 18.5 (C-14), 12.6 (C-13); MS (ESI+) *m/z* calcd for $C_{17}H_{25}O_4$ 293.1747. Found 293.1749 $[M + H]^+$.

*11β,13-Dihydroxanthatin **10** and 11β,13-dihydroxanthatin-1,5-epoxides **11** and **12*** DDQ (100 mg, 0.45 mmol) was added to a solution of the alcohol **8** (113 mg, 0.45 mmol) in toluene (10 mL). The mixture was heated under reflux for 3 h. The black precipitate was filtered on Celite®. Toluene was removed under reduced pressure and the residue was pre-purified on preparative TLC (CH_2Cl_2–EtOAc, 9:1). The interesting fractions were pooled and purified on preparative TLC (CH_2Cl_2–EtOAc, 7:3) to afford the 11β,13-dihydroxanthatin **10** (47.1 mg, 42% yield), the 11β,13-dihydroxanthatin-1,5α-epoxide **11** (2.8 mg, 2% yield), and the 11β,13-dihydroxanthatin-1,5β-epoxide **12** (2.1 mg, 2% yield). (**10**): see reference 5. (**11**): ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.90 (1H, d, *J* = 15.6 Hz, H-2), 6.29 (1H, d, *J* = 15.6 Hz, H-3), 3.94 (1H, ddd, *J* = 2.8, 6.7, 11.7 Hz, H-8), 3.03 (1H, d, *J* = 6.0 Hz, H-5), 2.59 (1H, m, H-10), 2.48 (1H, m, H-6α), 2.29 (1H, m, H-11), 2.28 (3H, s, H-15), 2.07 (1H, m, H-9α), 2.04 (1H, m, H-7), 1.91 (1H, m, H-9β), 1.86 (1H, m, H-6β), 1.13 (3H, d, *J* = 7.4 Hz, H-14), 1.08 (3H, d, *J* = 7.0 Hz, H-13); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 198.5 (C-4), 178.2 (C-12), 144.9 (C-2), 128.9 (C-3), 80.1 (C-8), 63.6 (C-1), 63.1 (C-5), 42.9 (C-7), 41.2 (C-11), 34.4 (C-9), 31.1 (C-10), 29.7 (C-15), 26.3 (C-6), 16.4 (C-14), 12.5 (C-13). (**12**): ^{1}H-NMR (270 MHz, $CDCl_3$): δ 6.78 (1H, d, *J* = 15.6 Hz, H-2), 6.23 (1H, d, *J* = 15.7 Hz, H-3), 4.17 (1H, ddd, *J* = 4.2, 9.9, 10.3 Hz, H-8), 2.95 (1H, t, *J* = 6.0 Hz, H-5), 2.75 (1H, m, H-10), 2.48 (1H, m, H-6α), 2.32 (1H, m, H-11), 2.27 (3H, s, H-15), 2.04 (1H, m, H-9α), 1.92 (1H, m, H-7), 1.88 (1H, m, H-9β), 1.78 (1H, m, H-6β), 2.00 (3H, d, *J* = 6.0 Hz, H-14), 1.88 (3H, d, *J* = 7.4 Hz, H-13); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 198.5 (C-4), 178.2 (C-12), 144.9 (C-2), 128.9 (C-3), 79.8 (C-8), 63.6 (C-1), 63.1 (C-5), 42.9 (C-7), 41.2 (C-11), 34.2 (C-9), 31.1 (C-10), 29.6 (C-15), 26.3 (C-6), 16.4 (C-14), 12.5 (C-13).

*11,13-Dihydroxanthinosin **13*** $NaBH_4$ (13 mg, 0.343 mmol) was added in small portions during 15 min to a solution of nickel(II) chloride (48.4 mg, 0.375 mmol) and xanthatin **2** (50.3 mg, 0.204 mmol) in MeOH (6 mL) at 0°C. After 3 h, the black precipitate was filtered on Celite®, and the mixture was acidified to pH 4 with 10% HCl. MeOH was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic extract was successively washed with a saturated aqueous solution of $NaHCO_3$ (2 × 10 mL), 20% aqueous solution of NH_4Cl (2 × 10 mL), brine (2 × 10 mL), and H_2O (3 × 20 mL). The dichloromethanic extract was dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography over silica gel (CH_2Cl_2–EtOAc, 9:1) to afford the 11,13-dihydroxanthinosin **13** as an unseparable diastereomeric mixture (40.5 mg, 79% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.48 (1H, dd, *J* = 1.8 and 8.5 Hz, H-5), 4.26 (1H, ddd, *J* = 2.8, 9.6, 12.4 Hz, H-8), 2.53 (2H, m, H-3), 2.46 (1H, m, H-10), 2.27 (4H, m, H-2, H-6α, H-9α, H-11), 2.16 (3H, s, H-15), 1.99 (1H, m, H-6β), 1.60 (2H, m, H-7 and H-9β), 1.21 (3H, d, *J* = 6.7 Hz, H-13), 1.12 (3H, d, *J* = 7.1 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 208.0 (C-4), 178.6 (C-12), 146.2 (C-1), 122.2 (C-5), 81.2 (C-8), 51.6 (C-7), 42.6 (C-3), 41.8 (C-11), 36.5 (C-9), 34.5 (C-15), 34.1 (C-6), 30.0 (C-2), 27.1 (C-10), 18.2 (C-14), 12.6 (C-13); MS (ESI+) *m/z* calcd for $C_{15}H_{23}O_3$ 251.1642. Found 251.1642 $[M + H]^+$.

4,11,13-Tetrahydroxanthinosin ***14***[19] $NaBH_4$ (6 mg, 0.158 mmol) was added to a solution of 11,13-dihydroxanthinosin **13** (20 mg, 0.079 mmol) in MeOH (4 mL) at 0°C. After 3 h, the mixture was acidified to pH 4 with a 10% aqueous solution of HCl. MeOH was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic extract was successively washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), 20% aqueous NH_4Cl (2 × 10 mL), brine (2 × 10 mL), and H_2O (3 × 20 mL). The dichloromethanic extract was dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography over silica gel (CH_2Cl_2–EtOAc, 9:1) to afford the 4,11,13-tetrahydroxanthinosin **14** as an unseparable diastereomeric mixture (19 mg, 95% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.51 and 5.24 (1H, dd, H-5), 4.22 (1H, m, H-8), 3.8 (1H, m, H-4), 2.50 (1H, m, H-10), 2.22 (4H, m, H-3, H-6α and H-11), 2.02 (3H, m, H-2, H-6β and H-9α), 1.53 (2H, m, H-7 and H-9β), 1.19 (3H, d, *J* = 6.7 Hz, H-14), 1.17 (3H, d, *J* = 5.7 Hz, H-15), 1.12 (3H, d, *J* = 7.4 Hz, H-13); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 178.9 and 178.7 (C-12), 147.6, 147.3, 143.1 and 142.6 (C-1), 123.9, 123.7, 121.9 and 121.8 (C-5), 82.1 and 81.7 (C-8), 68.0, 67.8, 67.7 and 67.5 (C-4), 51.7, 51.6 and 48.8 (C-7), 42.4 and 42.3 (C-11), 39.2 and 39.1 (C-3), 38.0 and 37.2 (C-9), 31.2 and 31.1 (C-6), 29.9 and 29.8 (C-10), 27.4 and 27.2 (C-2), 23.7 and 23.0 (C-15), 18.2 and 17.8 (C-14), 12.9 and 12.5 (C-13); MS (ESI+) *m/z* calcd for $C_{15}H_{25}O_3$ 253.1798. Found 253.1798 $[M + H]^+$.

Acetyl-11β,13-dihydro-4-epi-isoxanthanol ***15***[20] $NaBH_4$ (20 mg, 0.172 mmol) was added in small portions to a solution of acetyl-4-*epi*-isoxanthanol **6** (30 mg, 0.086 mmol) in MeOH (4 mL) at 0°C. After 4 h, the mixture was acidified to pH 4 with 10% HCl. MeOH was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic extract was successively washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), 20% aqueous NH_4Cl (2 × 10 mL), brine (2 × 10 mL), and H_2O (3 × 20 mL). The dichloromethanic extract was dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography over silica gel (CH_2Cl_2–EtOAc, 9:1) to afford the acetyl-11β,13-dihydro-4-*epi*-isoxanthanol **15** (30 mg, 98% yield). ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.87 (1H, dd, *J* = 3.2, 9.2 Hz, H-5), 5.18 (1H, dd, *J* = 4.6, 9.6 Hz, H-2), 4.91 (1H, m, H-4), 4.29 (1H, ddd, *J* = 3.2, 9.6, 12.7 Hz, H-8), 2.74 (1H, m, H-10), 2.30 (3H, m, H-6α, H-9α and H-11), 2.04 (3H, s, H-17), 2.05 (3H, s, H-19), 1.82 (3H, m, H-3 and H-6β), 1.62 (2H, m, H-7 and H-9β), 1.22 (6H, m, H-13 and H-15), 1.11 (3H, d, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 178.3 (C-12), 170.4 (C-18), 170.0 (C-16), 145.2 (C-1), 126.8 (C-5), 81.9 (C-8), 76.5 (C-4), 67.1 (C-2), 51.2 (C-7), 41.6 (C-11), 39.8 (C-3), 36.3 (C-9), 29.6 (C-10), 26.4 (C-6), 22.7 (C-15), 21.2 (C-19), 20.3 (C-17), 19.1 (C-14), 12.5 (C-13); MS (ESI+) *m/z* calcd for $C_{21}H_{27}O_6Na$ 375.1784. Found 375.1793 $[M + Na]^+$.

11β,13-Dihydro-4-epi-isoxanthanol ***16***[20] $NaBH_4$ (38 mg, 1 mmol) was added in small portions to a solution of 4-*epi*-isoxanthanol **3** (80 mg, 0.261 mmol) in MeOH (5 mL) at 0°C. After 6 h, the mixture was acidified to pH 4 with 10% HCl. MeOH was removed under reduced pressure and the aqueous solution was extracted with CH_2Cl_2 (3 × 10 mL). The organic extract was successively washed with saturated aqueous $NaHCO_3$ (2 × 10 mL), 20% aqueous NH_4Cl (2 × 10 mL), brine (2 × 10 mL), and H_2O (3 × 20 mL). The dichloromethanic extract was dried with Na_2SO_4, concentrated under reduced pressure, and purified by column chromatography over silica gel (CH_2Cl_2–EtOAc, 7:3) to afford the 11β,13-dihydro-4-*epi*-isoxanthanol **16** (30 mg, 0.097 mmol) in 37% yield. ^{1}H-NMR (270 MHz, $CDCl_3$): δ 5.87 (1H, dd, *J* = 2.8, 8.8 Hz, H-5), 5.35 (1H, dd, *J* = 3.2, 9.5 Hz, H-2), 4.29 (1H, ddd, *J* = 2.5, 9.6, 12.0 Hz, H-8), 3.71 (1H, m, H-4), 2.75 (1H, m, H-10), 2.30 (3H, m, H-6α, H-9α and H-11), 2.09 (3H, s, H-17), 1.81 (3H, m, H-3 and H-6β), 1.58 (2H, m, H-7 and H-9α), 1.22 (6H, m, H-13 and H-15), 1.15 (3H, d, *J* = 7.1 Hz, H-14); ^{13}C-NMR (67.5 MHz, $CDCl_3$): δ 171.2 (C-12), 169.8 (C-16), 145.8 (C-1), 126.2 (C-5), 82.1 (C-8), 77.2 (C-2), 63.9 (C-4), 51.3 (C-7), 48.3 (C-3), 43.4 (C-11), 38.8 (C-9), 29.9 (C-6), 25.3 (C-15), 21.2 (C-10), 19.8 (C-17), 19.3 (C-14), 12.5 (C-13); MS (ESI+) *m/z* calcd for $C_{17}H_{26}O_5Na$ 333.1678. Found 333.1678 $[M + Na]^+$.

Fluorescence-based PFTase inhibition assay

Assays were realized on 96-well plates, prepared with Biomek NKMC and Biomek 3000 from Beckman Coulter and read on a Wallac Victor fluorimeter from PerkinElmer. Per well, 20 µL of farnesyl pyrophosphate (10 µM) was added to 180 µL of a solution containing 2 µL of varied concentrations of tested compounds (dissolved in methanol) and 178 µL of a solution composed by 0.1 mL of partially purified recombinant yeast FTase (2.2mg/mL) and 7.0 mL of Dansyl-GCVLS peptide (in the following buffer: 5.8 mM DTT, 12 mM $MgCl_2$, 12 µM $ZnCl_2$, and 0.09% (w/v) CHAPS, 53 mM Tris/HCl, pH 7.5). Then the fluorescence development was recorded for 15 min (0.7 s per well, 20 repeats) at 30°C with an excitation filter at 340 nm and an emission filter at 486 nm.

Results and discussion

Purification

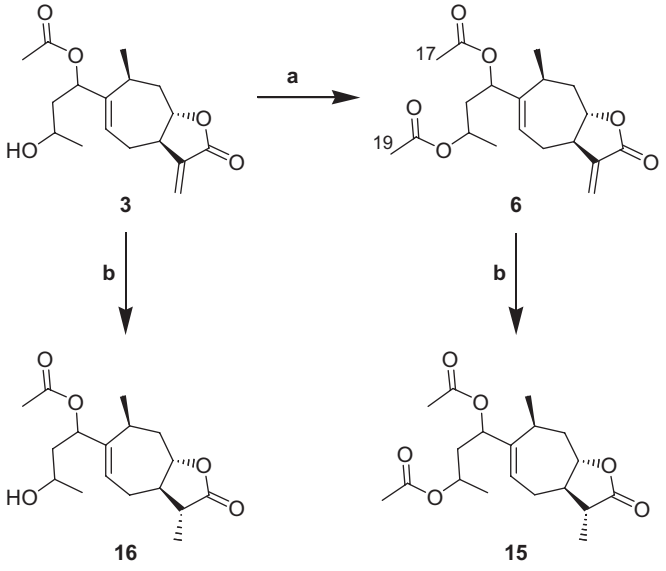
The liquid/liquid chromatography, CPC, is a method of purification which presents some advantages compared to the traditional solid/liquid chromatography. Indeed, the absence of a solid support avoids irreversible adsorption (no sample loss) or artifact formation. A reduction of solvent consumption can also be expected and the scaling up from analytical to preparative scale is easy and reliable. The centrifugal partition chromatography of the crude chloroformic extract of the leaves of *X. macrocarpum* DC. was optimized with a 1 L bench scale FCPC®[21] and the compounds **1–3** were obtained in, respectively, 0.64%, 0.47%, and 0.51% yield.

Chemistry

Synthesis in the α-methylene-γ-lactone series mainly focused on modification of the side chain realized by oxidation of the dienonic part of xanthatin **2** (Scheme 1) and led to a new tricyclic furan **7** and two epoxides **4** and **5** already isolated from *Xanthium strumarium* L.[18].

Thus, a multigram-scale, solvent-free, and quantitative β-elimination of **1** was realized in 2 days on silica to yield the dienone **2**[22]. To achieve the 1,5-oxidized analogs of **2**, we initially planed to use a classical oxidant such as magnesium monoperoxyphthalate[23]. However, under this condition, no evolution was observed with the deactivated dienone **2**. We then attempted synthesis of the 1,5-oxidized analogs of **2** by a photooxygenation process in the presence of a photosensitizer, a method already applied to other dienones[24]. Instead of the desired epoxide, the furan **7** was obtained, probably via the postulated endoperoxide **17**[25]. A chromium epoxidation of deactivated alkenes (unsaturated acids) was recently reported[26]. Therefore, the PCC oxidation of **2** was attempted to finally, slowly (8 days) lead to the desired epoxides **4** and **5**, albeit in low yield. Then, to evaluate the influence of the side chain substituents on the PFTase inhibitory activity, the 4-hydroxyl group of 4-*epi*-isoxanthanol **3** was acetylated to give **6** (Scheme 2)[27]. In contrast to the xanthanolides in the α-methylene-γ-lactone series, α-methyl-γ-lactone xanthanolides are not so frequently isolated from natural sources. Moreover, their total synthesis is only realized in modest overall yield due to the difficulty in obtaining the fused rings and their asymmetric centers[28]. We therefore thought that preparation of α-methyl-γ-lactone xanthanolides would be easier through semisynthesis from the α-methylene-γ-lactones **1** and **3** isolated from *X. macrocarpum* DC. Indeed, a small library of α-methyl-γ-lactones (**8–16**) was synthesized. Among them, **9**, **11**, and **12** are original compounds whereas **13–16** were already isolated from *Stevia isomeca* Grashoff[19], *Iva dealbata* A. Gray[20], and *Dittrichia graveolens* (L.) Greuter[29], but never prepared by semisynthesis.

Reacting with sodium borohydride, only one of the three carbon–carbon double bonds of **2** was reduced, as well as the ketonic carbonyl group, to yield **8**. It should be noted that using this achiral reducing agent, the α-methylene-γ-lactone reduction appeared as stereoselective, as already noted in the case of arteannuin B[30]. Under the same reduction condition, **3** behaved similarly, yielding the stereomer **16** (Scheme 2). On the other hand, such diastereoselectivity was not observed when the $NaBH_4$ reduction of **2** was realized in the presence of nickel chloride. Moreover, with this reagent, a second reduction affected the C2–C3 double bond instead of the ketonic carbonyl, yielding the non-conjugated ketone **13**. Therefore, synthesis of alcohol **14** necessitated an extra reductive step ($NaBH_4$). Non-hydroxylic derivatives of **8** were also prepared either by its acetylation to **9** or by its oxidation (DDQ). Under those conditions, the expected dihydroxanthatine **10** was also accompanied by the minor epoxides **11** and **12**. A higher structural diversity in the α-methyl-γ-lactone series could be obtained with the synthesis of reduced derivatives of **3** (Scheme 2). Besides the abovementioned alcohol **16**, the synthesis of the diacetate **15** was also realized starting from **3**.



Scheme 2. Synthesis of compounds **6**, **15**, and **16**. Reagents: (a) AcCl, TEA, RT, 12%; (b) $NaBH_4$, RT (**16**, 37%; **15**, 98%).

Farnesyl transferase inhibition

In a first step, the IC_{50} values of compounds **1–16** were evaluated toward recombinant yeast PFTase (Table 1)[31]. Compounds **1–16** could be sorted into two categories: (i) α-methylene-γ-lactone series (compounds **1–7**) and (ii) α-methyl-γ-lactone series (compounds **8–16**). Comparison of the PFTase activities of the 7,8-*trans* lactones **2** (IC_{50} = 100 μM) and **5** (IC_{50} = 100 μM) with the literature data for their corresponding *cis* fused isomers (Figure 1), respectively, 8-*epi*-xanthatin (IC_{50} = 64 μM) and 8-*epi*-xanthatin-1,5α-epoxide (IC_{50} = 58 μM), indicated that stereochemistry at C7 and C8 had only a minor impact on the level of activity. Therefore, the influence of the modification of the western part of the xanthanolide skeleton was evaluated in the α-methylene-γ-lactone series and then confirmed in the α-methyl-γ-lactone series. Analysis of group (i) revealed that the compounds bearing a hydroxyl group at the 4-position of the side chain were inactive. The IC_{50} of alcohol **3** was above 1000 μM. The disfavorable effect of a hydrogen bond donor was also confirmed by comparison of the PFTase inhibitory activity of

Table 1. Effects of compounds **1–16** on PFTase.

Series	Compound	IC_{50} (μM)
α-Methylene-γ-lactone	**3**	>1000
	1	150
	2	100
	4	500
	5	100
	7	>1000
	6	40
α-Methyl-γ-lactone	**8**	>1000
	14	>1000
	16	633
	10	210
	11	90
	12	66
	13	240
	9	900
	15	40

the 4-hydroxyl derivative **3** with the corresponding 4-oxo derivative **1** (IC_{50} = 150 μM). Moreover, except for **7**, the 4-oxo derivatives **2**, **4**, and **5** were more active than **3**. The lack of activity of the tricyclic compound **7** (IC_{50} >1000 μM) indicated the disfavorable effect of the backbone-rigidification supported by the furanic ring, in contrast to its deoxy precursor **2** (IC_{50} = 100 μM). Moreover, the 1,5-oxidized analogs of **2**, the tricyclic epoxides **4** and **5**, were also more active than the furanic derivative **7**. However, the orientation of the ketonic side chain proved to be an important factor, since isomer **5** (IC_{50} = 100 μM) was more potent than **4** (IC_{50} = 500 μM). The favorable parameters for PFTase inhibitory activity were then confirmed in the α-methyl-γ-lactone series. Indeed, the 4-hydroxyl derivatives **8**, **14**, and **16** were less active (IC_{50} >500 μM) than the 4-oxo derivatives (**10–13**) (IC_{50} < 240 μM). Among them, the two oxiranes **11** (IC_{50} = 90 μM) and **12** (IC_{50} = 66 μM) were the most potent with the same level of activity. Again, the 4-acetyl derivatives **9** and **15** were more active than the corresponding alcohols, respectively, **8** and **16**. Therefore, as in the case of the dimeric arteminolides (Figure 1) and contrary to what is observed for numerous other biological activities, we clearly demonstrated that the unsaturated α-methylene-γ-lactone was not required for the inhibition of PFTase. Moreover, reduction of the α-methylene-γ-lactone does not affect the inhibitory activity as exemplified by compounds **6** and **15**, which exhibited the same inhibitory activity (IC_{50} = 40 μM).

In order to clarify the possible mode of action of this class of inhibitors, compounds **12** and **15**, with the most significant PFTase inhibitory activity in the α-methyl-γ-lactone series, were selected for further investigation. Thus, **12** and **15** were subjected to kinetic studies, in a fluorescence-based assay. Analysis of the Lineweaver–Burk plots (Figures 2 and 3) clearly shows that compounds **12** and **15** are non-competitive inhibitors toward both peptide and farnesyl diphosphate (FPP). These results show that these compounds bind neither to the peptide nor to the FPP binding site. However, it has been shown that before leaving the enzyme, the farnesylated peptide binds to an exit groove that might be the binding site of the studied compounds[32]. A weak cytotoxic activity was also revealed against human colon tumor cell line HCT 116, with 11.4 and 7.4% for **12** and 12.3 and 7.3% for **15** at, respectively, 50 μM and 10 μM.

Conclusion

Sixteen xanthatin derivatives were investigated as potent inhibitors of protein farnesyltransferase. The results showed that, like the previously reported 7,8-*cis* fused

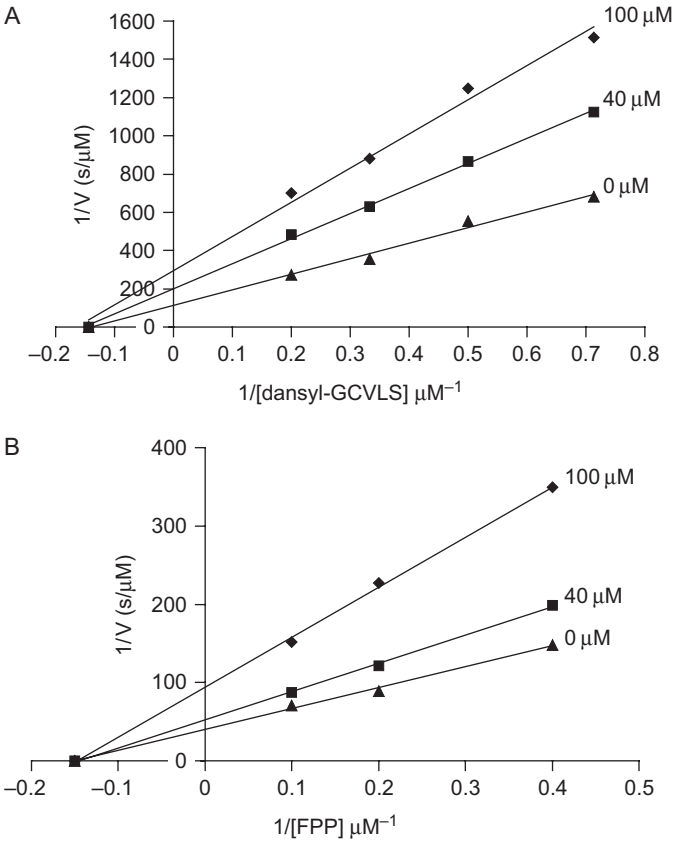


Figure 2. Lineweaver–Burk plots for the inhibition of PFTase isolated from yeast by compound **15** in the presence of dansyl-GCVLS peptide and FPP as substrates. Concentrations of inhibitor **15** were 0, 40, and 100 μM with excess FPP (A), or with excess dansyl-GCVLS (B).

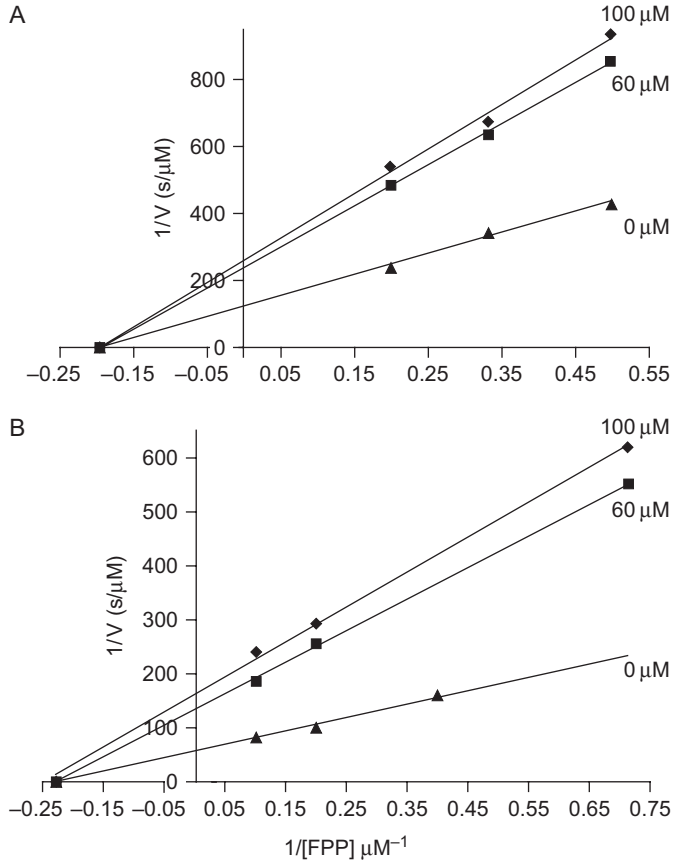


Figure 3. Lineweaver–Burk plots for the inhibition of PFTase isolated from yeast by compound **12** in the presence of dansyl-GCVLS peptide and FPP as substrates. Concentrations of inhibitor **12** were 0, 60, and 100 μM with excess FPP (A), or with excess dansyl-GCVLS (B).

xanthanolides, 7,8-*trans* derivatives also inhibit the PFTase. Six compounds showed inhibitory activity with IC_{50} values between 40 and 100 μM in both the α-methylene-γ-lactone (**2**, **5**, and **6**), and the α-methyl-γ-lactone series (**11**, **12**, and **15**). Moreover, the structure–activity relationships of the western part of the xanthanolide core revealed several features favorable to PFTase inhibitory activity such as the lack of a polar function or the presence of a 1,5-epoxide. Kinetic experiments illustrated that the two selected α-methyl-γ-lactone derivatives are non-competitive inhibitors of the peptide or FPP. This study clearly indicated that the α-methylene-γ-lactone group, already known to be responsible for side effects such as allerginicity, is not required to insure PFTase inhibitory activity. Although they have to be confirmed by cytotoxic assays, these results suggest that α-methyl-γ-lactone could represent a new lead for the development of non-competitive PFTase inhibitors, which are seldom studied.

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Declaration of interest

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