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Original article

Synthesis of new troglitazone derivatives: Anti-proliferative activity in breast cancer cell lines and preliminary toxicological study

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ABSTRACT

Breast cancer is the most prevalent cancer in women. The development of resistances to therapeutic agents and the absence of targeted therapy for triple negative breast cancer motivate the search for alternative treatments. With this aim in mind, we synthesised new derivatives of troglitazone, a compound which was formerly used as an anti-diabetic agent and which exhibits anti-proliferative activity on various cancer cell lines. Among the compounds prepared, some displayed micromolar activity against hormone-dependent and hormone-independent breast cancer cells. Furthermore, the influence of the compounds on the viability of primary cultures of human hepatocytes was evaluated. This enabled us to obtain for the first time interesting structure–toxicity relationships in this family of compounds, resulting in **6b** and **8b**, which show good anti-proliferative activities and poor toxicity towards hepatocytes, compared to troglitazone.

1. Introduction

Breast cancer is the most prevalent cancer in women and represents the second highest leading cause of cancer death in this population after lung cancer. Approximately 75% of tumours express the oestrogen receptor ERα that allows for treatment with the anti-oestrogen tamoxifen. Additionally, human epidermal growth factor receptor 2 (HER2) expressing tumours may be treated with the monoclonal antibody trastuzumab [1]. Nevertheless, *de novo* or acquired resistance to these therapeutic agents often lead to treatment failure [2,3]. Therefore, there is an urgent need to develop alternative therapeutics. Moreover, there is no targeted therapy for the treatment of breast tumours known as triple negative: ERα-, PR- (progesterone receptor), HER2-. In this regard, ligands of peroxisome proliferator-activated receptor gamma (PPARγ) and especially thiazolidinediones (TZD) were envisioned as a therapeutic alternative towards advanced (or relapsing) breast cancer [4]. PPARγ is a nuclear receptor associated with glucose homeostasis. It can be activated by endogenous ligands such as 15d-PGJ(2) or synthetic compounds like TZD, including troglitazone, rosiglitazone, and pioglitazone [5]. Among these drugs, which were used in the treatment of type II diabetes, only pioglitazone is still therapeutically used in some countries; the others were withdrawn from the market because of side-effects such as Drug-Induced Liver Injury (DILI) [6]. Regarding breast cancer, several PPARγ ligands of the TZD family have been shown to possess anticancer properties *in vitro* and *in vivo* [7,8]. Among these, troglitazone (TGZ) seems to be particularly interesting and has even been tested in clinical trials for breast cancer before its withdrawal [9]. The anti-proliferative modes of action have been extensively studied, but are still not fully elucidated. Actually, increasing data suggest that its activity is mainly related to PPARγ-independent mechanisms [10–12]. Cyclin D1 ablation [13,14], inhibition of Bcl-xL/Bcl-2 functions [15], TGFβ signalling [16], ERα disruption [10], telomerase activity suppression [17], interaction with oestrogen-related receptor α and γ [18], energy restriction [19], and calcium and ERK-dependent expression of the early growth response gene 1 [20] are examples of TZD-induced PPARγ-independent events. A major aspect of this new therapeutic strategy lies in the fact that non-malignant cells seem to be resistant to these PPARγ-independent antitumour effects, which holds the translational potential of these agents [12].

New TGZ derivatives have already been prepared. In particular, the introduction of a double bond adjacent to the thiazolidinedione

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ring yielded Δ2-troglitazone (Δ2TGZ) which was devoid of PPARγ activity but exhibited an anti-proliferative ability similar to the one of TGZ [13,21]. Additional structural modulations were made on the Δ2TGZ template, especially by chromane phenol functionalisation, arising in low micromolar active molecules [22]. Nevertheless, a major drawback of TGZ derivatives lies in the fact that TGZ itself exhibits hepatotoxicity. The mechanisms underlying TGZ DILI are not fully understood. Yokoi [23] has recently reviewed the proposed mechanisms and suggested that the presence of the chromane moiety could be of importance in the induction of DILI since it can lead to toxic derivatives. Besides, Bharatam [24] recently reported a theoretical study to better understand the formation of these derivatives.

The goal of the present work was to synthesise new Δ2TGZ derivatives, aiming at increasing anti-proliferative activity towards breast cancer cell lines, and decreasing hepatotoxicity, as compared to TGZ.

2. Chemistry

Since phenolic functionalisation of Δ2TGZ seems to have a great influence on its biological activity, we envisioned the synthesis of many ester derivatives of this molecule. Thus, we first needed a straightforward access to Δ2TGZ. As mentioned by Halperin et al. [25], its synthesis is not trivial. Taking into account reported methods dealing with the synthesis of Δ2TGZ and derivatives [22,25–29], we started from commercial racemic Trolox® **1**, but intended to proceed without protection of the phenol of the chromane moiety with usual groups (*i.e.* Bn, TBDMS, MOM, MEM) whose introduction and removal proved to be troublesome. Therefore, after a simple esterification of the starting material [30,31] to give **2**, we smoothly introduced a *tert*-butyloxycarbonyl group to get compound **3a** (Scheme 1). After reduction to **4a**, triflate formation followed by substitution with *p*-hydroxybenzaldehyde gave **5a**. Then, condensation with 2,4-thiazolidinedione gave O-protected Δ2TGZ **6a** in 65% overall yield. In order to study the influence of the phenolic hydroxyl group on both activity and toxicity, we intended to remove this moiety. Thus, based on a method reported by Salvatore et al. [32] to get new tocopheryl derivatives, we prepared triflate **3b** in almost quantitative yield, followed by catalytic hydrogenation under basic conditions to get the deoxygenated compound **3c** in very good yield. The previously mentioned sequence afforded deoxygenated Δ2TGZ **6b** in 39% overall yield. Smooth removal of the phenolic protecting group of **6a** in acidic medium afforded Δ2TGZ **7** in 86% yield after chromatography, which enabled us to functionalise the molecule *via* an ester linkage.

It has been reported [33,34] that conjugation of molecules with biotin might increase their uptake in tumour cells since the latter often over-express biotin-specific receptors on their surface. Thus, we prepared the biotinoyl derivative **8a**, albeit in moderate yield. In order to facilitate recognition by the biotin receptor, we envisioned the introduction of a linker. Hence, Boc-protected aminocaprylic compound **8b** was obtained in good yield. Acidic deprotection afforded **8c** which was in turn linked to biotin to give **8d**. Furthermore, it has been reported that the succinoyl moiety linked to α-tocopherol induces antiadhesion properties on breast cancer cells [35]. We then synthesised the succinoyl derivative **8e**, along with non-acidic derivative **8f**.

In order to study the influence of the double bond of these molecules on the anti-proliferative activity and toxicity, we prepared saturated analogues. Thus, as reported by Ramachandran [28], 70 psi hydrogen afforded **9a** and **9b** in good yield from **6a** and **6b** respectively. Acidic treatment of **9a** gave TGZ **10** which was in turn converted into biotinoyl and succinoyl derivatives (**11** and **12** respectively).

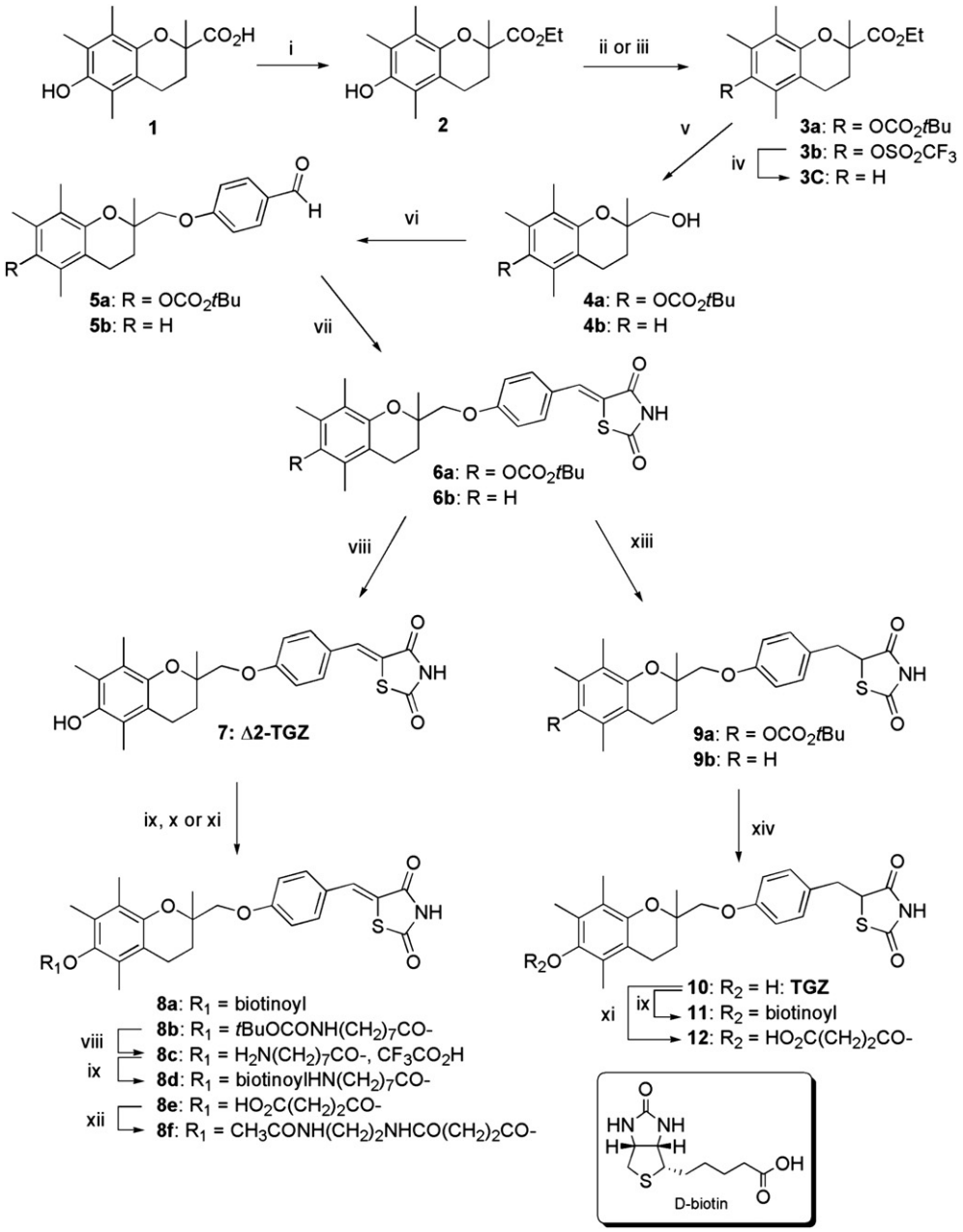
3. Biological studies and discussion

All of the TGZ derivatives were tested for their anti-proliferative activity against hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cell lines. Their hepatotoxicity was also evaluated by incubation with primary cultures of human hepatocytes. The results are summarised in Table 1.

Regarding cell proliferation, it can first be noticed that the hormone-independent cell line (MDA-MB-231) is more sensitive to the synthesised molecules than the hormone-dependent cell line (MCF-7), with the exception of compound **8c**. These are interesting data that could be helpful for understanding the mechanism of action of TGZ derivatives in future studies. Besides, MDA-MB-231 cells display less variability than MCF-7 in their sensitivity to the various compounds: the IC_{50} values range from 3.2 to 5.0 μM for most of the active compounds (see **6a**, **8a**–d) in MDA-MB-231 whereas in the MCF-7 cell line they are ranging from 3.2 μM for the most active **8c** to 26.0 μM for the least active **8d**.

Dealing with structure–activity relationships, one can first notice that the double bond has an influence on the activity of the compounds. If we compare **6a** to **9a**, **6b** to **9b**, and **8a** to **11**, a slight positive effect is observed on both cell lines: unsaturated derivatives are more active than their saturated counterparts. Comparing Δ2TGZ to TGZ, a positive effect is also observed in MCF-7, but not in MDA-MB-231 cell line. Besides, phenol functionalisation with succinoyl residues (see **8e**, **8f**, **12**) appears deleterious to the anti-proliferative activity. This might be due to the increase of polarity that such substituents induce in the proximity of the chromane core. In the case of compound **8c**, the polar ammonium moiety is rather distant from the chromane, since it is linked to this heterocycle by seven apolar methylene groups, resulting in one of the most active molecules. Such methylene groups are also present in the case of biotinoyl derivatives **8a** and **8d** and in the case of Boc-linker intermediate **8b**, which are also active. Furthermore, strongly apolar Boc residue directly linked to the chromane afforded very active **6a**. A confirmation of the role of polarity came from compounds **6b** and **9b** in which the polar hydroxyl moiety was replaced by a hydrogen atom. Even if they are not the most active compounds, it resulted in a clear increase in potency. Furthermore, one can notice that the presence of the biotin residue (with or without a caprylic linker: see **8a** and **8d**) does not provide additive value compared to other substituents, unlike what we expected. Compounds **6a**, **8a**, and reference compounds **7** (Δ2TGZ) and TGZ were selected to exhibit proliferation curves for both cell lines, in Figs. 1 and 2.

In terms of hepatotoxicity assessment, it is important to take into account the pharmacokinetic parameters [23] and even more importantly, the potential tissue concentration in the liver, an organ potentially able to accumulate xenobiotics. As reported by Loi et al. [36], the maximum plasma concentration of TGZ in patients taking a dose of 600 mg/day reached 6.3 μM, while Sahi et al. [37] demonstrated that in rats the concentration in liver was 10–12 fold higher than that in the plasma. For this reason, in the present study, the cytotoxicity of TGZ and its derivatives were assessed using human hepatocytes at concentrations up to 100 μM, as reported by Yamamoto [38] for the evaluation of TGZ metabolites toxicity. Our study was conducted by measuring cell viability using the MTT assays on primary cultures of human hepatocytes and for clarity, we present the results as percentage of surviving cells after incubation with 100 μM of compound (Table 1). The study shows that, with the exception of non-active succinoyl derivatives, all Δ2TGZ derivatives are much less toxic than TGZ ones: compare Δ2TGZ (**7**) to TGZ, **6a** to **9a**, **6b** to **9b**, and **8a** to **11**. It has been reported that TGZ toxicity is at least partly due to glutathione depletion because of conjugation through linkage with the



Scheme 1. Reagents and conditions: i EtOH, cat. *p*-TSA, reflux, 95%; ii Boc_2O, cat. DMAP, 99%; iii Tf_2O, Pyr., 99%; iv TEA, H_2 70 psi, 10% Pd/C, 96%; v $LiAlH_4$, 91% (**4a**), 94% (**4b**); vi 1) Tf_2O, Pyr. 2) 4-hydroxybenzaldehyde, K_2CO_3 86% (**5a**), 84% (**5b**); vii 2,4-thiazolidinedione, piperidine, Δ, 88% (**6a**), 54% (**6b**); viii TFA, 86% (**7**), 94% (**8c**); ix D-biotin, TEA, IBCF, cat. DMAP, 35% (**8a** and **11**), 64% (**8d**); x Boc-aminocaprylic acid, TEA, IBCF, cat. DMAP, 75%; xi Succinic anhydride, cat. DMAP, 43% (**8e**), 63% (**12**); xii TEA, IBCF, *N*-acetylethylenediamine, 29%; xiii H_2 70 psi, 10% Pd/C, 99% (**9a**), 70% (**9b**); xiv HCl, quant.

thiazolidinedione ring [23,29,39]. The presence of a conjugated double bond might decrease the electrophilicity of the heterocycle, then disfavour conjugation with glutathione, and thus decrease the toxicity.

In addition, one can notice that Δ2TGZ derivatives bearing a lipophilic substituent on the chromane (see **6a** and **8b**) exhibit much less toxicity towards hepatocytes than others. Such residues might disfavour formation of reported toxic quinone-type metabolites [23,39]. From that point of view, compounds **6b** and **9b** devoid of hydroxyl group and thus unable to be transformed into that type of metabolite, show less toxicity towards hepatic cells than Δ2TGZ and TGZ respectively. Furthermore, the best viability value of the whole series is observed for **6b**. Fig. 3 shows the cytotoxicity profiles for TGZ, Δ2TGZ, **6b**, and **8b** which are similar for these compounds up to 50 μM but evidences that **6b** and **8b** are less cytotoxic, as compared to TGZ and Δ2TGZ at 100 μM.

4. Conclusion

A series of new TGZ and Δ2TGZ derivatives were prepared and tested for their anti-proliferative activity against hormone-dependent and hormone-independent breast cancer cell lines. These molecules were obtained *via* functionalisation or removal of the hydroxyl group of the chromane moiety. The most active molecules were Δ2TGZ derivatives, exhibiting low-micromolar range activity. Some of them also showed much less toxicity on primary cultured hepatocytes than parents TGZ and Δ2TGZ. This is a new step in the way to get more active and less toxic molecules to

Table 1
Anti-proliferative activity and hepatocyte viability related to prepared compounds and TGZ.

Compound	IC_{50}[a] ± SEM[b]		Hepatocyte viability[f] (%)
	MCF-7[c]	MDA-MB-231[d]	
6a[e]	7.7 ± 0.5	3.3 ± 0.1	75
6b	12.5 ± 0.9	10.8 ± 0.7	84
7[e] (**Δ2TGZ**)	29.7 ± 2.0	16.6 ± 1.0	63
8a[e]	11.2 ± 1.8	3.5 ± 0.1	66
8b[e]	13.0 ± 1.5	3.2 ± 0.3	79
8c[e]	3.2 ± 0.1	5.0 ± 0.7	57
8d	26.0 ± 5.7	3.7 ± 0.7	61
8e	>50	>25	54
8f	>50	>25	75
9a	11.2 ± 0.3	3.8 ± 0.4	47
9b	20.0 ± 1.0	12.3 ± 0.5	66
11	15.9 ± 0.8	6.5 ± 0.4	50
12	>50	>25	76
TGZ	35.4 ± 1.3	15.7 ± 0.1	52

[a] Concentration (μM) required to inhibit tumour cell proliferation by 50%.
[b] Standard Error of the Mean.
[c] Hormone-dependent breast cancer cell line.
[d] Hormone-independent breast cancer cell line.
[e] Anti-proliferative activity of these molecules on MCF-7 and MDA-MB-231 cell lines have already been reported [21].
[f] Percentage of surviving primary hepatocytes as compared to non-treated cells, after 90 min of incubation with compounds, at 100 μM.

treat breast cancer. Synthesis of new molecules aiming at reaching this goal is currently ongoing by our team.

5. Experimental protocols

Solvents and liquid reagents were purified and dried according to recommended procedures. Troglitazone used for anti-proliferative and hepatotoxicity assays was purchased from Sigma–Aldrich. TLC analyses were performed using standard procedures on Kieselgel 60 F254 plates (Merck). Compounds were visualised using UV light (254 nm) and a solution of cerium sulfate tetrahydrate and phosphomolybdic acid in 10% aqueous sulfuric acid as developing agent. Column chromatography was performed on Silica Gel SI 60 (63–200 μm) (Merck). Some chromatographic purifications were performed on Silica Gel 60H (5–40 μm) (Merck) using an Axxial® Modul Prep apparatus, working at 8 bars, with a 20 mm diameter column. FTIR spectra were recorded on a Perkin–Elmer spectrum 1000 apparatus on NaCl windows or KBr pellets. Melting points were determined with a Kofler bench and are uncorrected. ^{1}H and

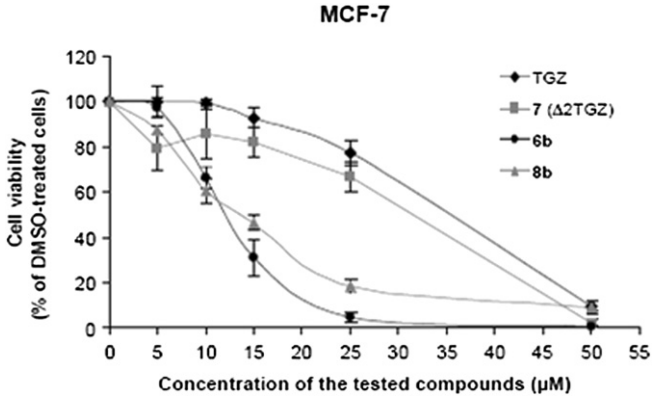


Fig. 1. Cytotoxicity of troglitazone (TGZ) and derived compounds **7** (Δ2TGZ), **6b** and **8b** in MCF-7 cells. Data are means +/− SEM of triplicate determinations. IC_{50} of **6b** and **8b** were significantly different from those measured for TGZ ($p < 0.05$) and Δ2TGZ ($p < 0.05$).

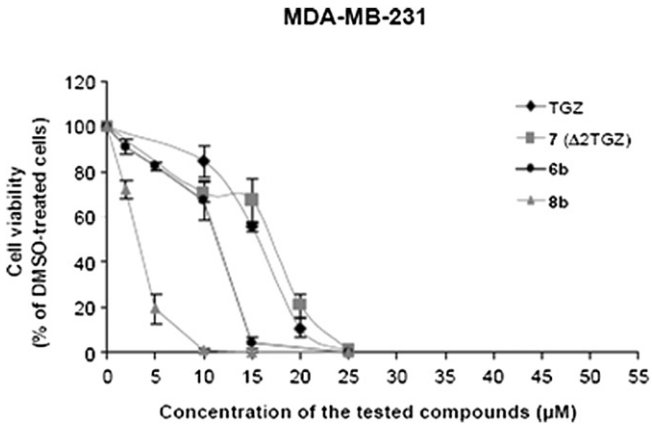


Fig. 2. Cytotoxicity of troglitazone (TGZ) and derived compounds **7** (Δ2TGZ), **6b** and **8b** in MDA-MB-231 cells. Data are means +/− SEM of triplicate determinations. IC_{50} of **6b** and **8b** were significantly different from those measured for TGZ ($p < 0.05$) and Δ2TGZ ($p < 0.05$).

^{13}C NMR spectra were mainly recorded on a Bruker spectrometer DPX250 (250 MHz and 62.9 MHz, respectively). A few spectra were recorded on a Bruker spectrometer DRX400 (400 MHz for ^{1}H and 100.6 MHz for ^{13}C). Chemical shifts (δ) are given in ppm relative to the solvent residual peak. The following abbreviations are used for multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, br = broad signal. The *J* values given refer to apparent multiplicities and do not represent the true coupling constants. Mass spectra were obtained on a VG-Platform Micromass-Waters (ESI+/quad). Elemental analyses were performed on a Thermofinnigan FlashEA 1112 apparatus.

5.1. Synthesis

5.1.1. (±) 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid ethyl ester (**2**)

To a solution of (±) 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid **1** (4.46 g, 18.82 mmol) in EtOH (175 mL) was added *p*-TSA (361 mg, 1.90 mmol), and the mixture was heated at reflux for 12 h. The solution was concentrated under vacuum, and the residue was dissolved in EtOAc (150 mL). The resulting solution

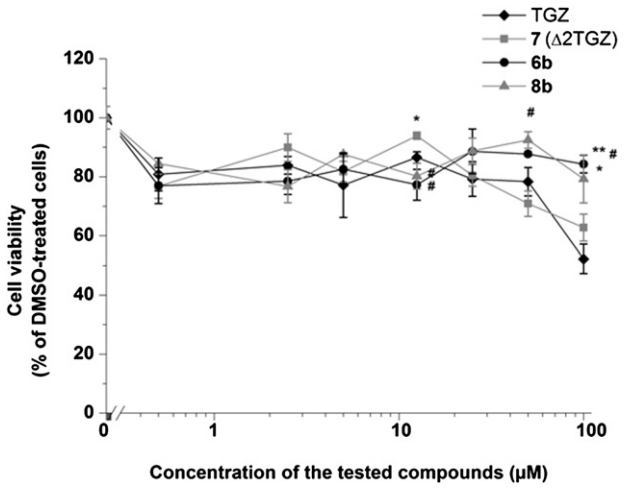


Fig. 3. Cytotoxicity of troglitazone (TGZ) and derived compounds **7** (Δ2TGZ), **6b** and **8b** in human hepatocytes. Data are means +/− SEM of triplicate determinations. *Statistical significantly different from TGZ ($^*p < 0.05$, $^{**}p < 0.01$). #Statistical significantly different from Δ2TGZ (#$p < 0.05$).

was washed with 5% aqueous $NaHCO_3$ solution (2 × 50 mL) and brine (2 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude product was dried to give 4.98 g of white crystals (17.90 mmol, 95% yield). M.p. 124 °C. IR (film) cm^{-1}: 3532, 2985, 2927, 1731, 1185, 1107. ^1H NMR ($CDCl_3$) δ: 1.18 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.60 (s, 3H, CH_3), 1.81–1.94 (m, 1H, chromane 3-H_aH_b), 2.06 (s, 3H, ArCH_3), 2.16 (s, 3H, ArCH_3), 2.19 (s, 3H, ArCH_3), 2.38–2.69 (m, 3H, chromane 3-H_aH_b and 4-H_2), 4.12 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 4.24 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ: 11.4 (ArCH_3), 11.9 (ArCH_3), 12.3 (ArCH_3), 14.2 (CH_3), 21.1 (CH_2), 25.5 (CH_3), 30.7 (CH_2), 61.1 (OCH_2), 77.0, 117.0, 118.5, 121.3, 122.7, 145.4, 145.8, 174.0 (C=O). ESI-MS (pos. mode): m/z = 205.42 $[M - CO_2Et]^+$, 301.30 $[M + Na]^+$, 317.27 $[M + K]^+$. Anal. Calcd for $C_{16}H_{22}O_4$ (278.3): C, 69.04; H, 7.97. Found: C, 68.70; H, 8.08.

5.1.2. (±) 6-tert-Butoxycarbonyloxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid ethyl ester (**3a**)

To a solution of **2** (4.72 g, 16.96 mmol) in CH_2Cl_2 (40 mL) were added di-*tert*-butyldicarbonate (4.08 g, 18.96 mmol) and 4,4-dimethylaminopyridine (207 mg, 1.69 mmol) under argon atmosphere. The solution was stirred at room temperature for 2 h and the solvent was evaporated. The residue was dissolved in EtOAc (100 mL), washed with a 1:1 solution of brine and 1 N aqueous HCl (60 mL), and a saturated aqueous solution of $NaHCO_3$ (2 × 40 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography (Hexane/EtOAc, 90:10) to give the titled compound (6.39 g, 16.88 mmol, 99% yield) as colourless liquid. IR (film) cm^{-1}: 2981, 2934, 1754, 1731, 1279, 1238, 1157, 1105. ^1H NMR ($CDCl_3$) δ: 1.17 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.54 (s, 9H, *t*-Bu), 1.59 (s, 3H, CH_3), 1.79–1.92 (m, 1H, chromane 3-H_aH_b), 1.99 (s, 3H, ArCH_3), 2.08 (s, 3H, ArCH_3), 2.16 (s, 3H, ArCH_3), 2.36–2.69 (m, 3H, chromane 3-H_aH_b and 4-H_2), 4.12 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ: 11.8 (ArCH_3 ×2), 12.7 (ArCH_3), 14.1 (CH_3), 20.8 (CH_2), 25.3 (CH_3), 27.7 (*t*-Bu), 30.2 (CH_2), 61.1 (OCH_2), 77.1, 82.7 (*t*-Bu), 117.1, 123.0, 125.1, 127.3, 141.6, 149.3, 152.2 (carbonate C=O), 173.6 (ester C=O). ESI-MS (pos. mode): m/z = 401.25 $[M + Na]^+$. Anal. Calcd for $C_{21}H_{30}O_6$ (378.5): C, 66.65; H, 7.99. Found: C, 66.24; H, 7.76.

5.1.3. (±) 2,5,7,8-Tetramethyl-6-trifluoromethanesulfonyloxy-chroman-2-carboxylic acid ethyl ester (**3b**)

To a stirred solution of **2** (500 mg, 1.80 mmol) in dry CH_2Cl_2 (10 mL) under argon was added pyridine (872 μL, 10.78 mmol) and the solution was cooled to 0 °C. Then, triflic anhydride (453 μL, 2.69 mmol) was added dropwise and the solution was stirred for 1 h at room temperature. The solution was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 30 mL), dried ($MgSO_4$) and concentrated to dryness. The liquid residue was purified by column chromatography (Hexane/EtOAc, 85:15) to give 735 mg (1.79 mmol, 99% yield) of colourless liquid. IR (film) cm^{-1}: 2922, 1457, 1417, 1403, 1228, 1208, 1140, 1111, 1034, 878. ^1H NMR ($CDCl_3$) δ: 1.20 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.66 (s, 3H, CH_3), 1.84–1.97 (m, 1H, chromane 3-H_aH_b), 2.19 (s, 3H, ArCH_3), 2.22 (s, 3H, ArCH_3), 2.27 (s, 3H, ArCH_3), 2.44–2.74 (m, 3H, chromane 3-H_aH_b and 4-H_2), 4.17 (dq, J_a = 7.1 Hz, J_b = 1.0 Hz, 2H, $COOCH_2CH_3$). ^{13}C NMR ($CDCl_3$) δ: 12.1, 13.3, 14.2 (2 peaks), 21.1, 25.4, 30.2, 61.4, 77.7, 118.4, 118.8 (q, J = 318 Hz, CF_3), 124.6, 126.8, 128.6, 140.5, 151.1, 173.2 (ester C=O). ESI-MS (pos. mode): m/z = 433.14 $[M + Na]^+$. Anal. Calcd for $C_{17}H_{21}O_6F_3S$ (410.4): C, 49.75; H, 5.16. Found: C, 49.87; H, 5.39.

5.1.4. (±) 2,5,7,8-Tetramethyl-chroman-2-carboxylic acid ethyl ester (**3c**)

In a Parr hydrogenator flask was first introduced 10% palladium on carbon (3.00 g), in order to avoid any ignition of the solvent. Please note that following reported procedure [32], Pd/C was purchased from Fisher/ACROS (catalogue #19503-0500). A solution of 4.96 g (12.09 mmol) of **3b** in THF (40 mL) was added to the flask, followed by MeOH (80 mL), and TEA (7.4 mL, 53.22 mmol). The suspension was shaken under 70 psi hydrogen pressure for 20 h at room temperature, then filtered on celite® and the resulting solution was concentrated. Column chromatography (Hexane/EtOAc, 90:10) afforded 3.03 g (11.55 mmol, 96% yield) of colourless liquid which upon storage at 4 °C gave low melting point (# 30 °C) white crystals. IR (film) cm^{-1}: 2981, 2935, 1753, 1732, 1462, 1312, 1202, 1178, 1137, 1108, 1023. ^1H NMR ($CDCl_3$) δ: 1.19 (t, J = 7.1 Hz, 3H, $COOCH_2CH_3$), 1.62 (s, 3H, CH_3), 1.82–1.95 (m, 1H, chromane 3-H_aH_b), 2.13 (s, 3H, ArCH_3), 2.16 (s, 3H, ArCH_3), 2.21 (s, 3H, ArCH_3), 2.38–2.69 (m, 3H, chromane 3-H_aH_b and 4-H_2), 4.14 (q, J = 7.1 Hz, 2H, $COOCH_2CH_3$), 6.58 (s, 1H, H_{arom}). ^{13}C NMR ($CDCl_3$) δ: 11.5, 14.2, 18.8, 19.8, 20.5, 25.4, 30.4, 61.2, 77.4, 116.6, 122.0, 123.4, 133.2, 134.9, 151.6, 173.8 (ester C=O). ESI-MS (pos. mode): m/z = 285.21 $[M + Na]^+$. Anal. Calcd for $C_{16}H_{22}O_3$ (262.4): C, 73.25; H, 8.45. Found: C, 73.63; H, 8.20.

5.1.5. (±) Carbonic acid tert-butyl ester 2-hydroxymethyl-2,5,7,8-tetramethyl-chroman-6-yl ester (**4a**)

To a suspension of $LiAlH_4$ (558 mg, 14.70 mmol) in anhydrous THF (15 mL) at 0 °C, was slowly added under argon, a solution of **3a** (6.34 g, 16.75 mmol) in anhydrous THF (40 mL). The suspension was stirred for 1 h (not more, to avoid reduction of the carbonate protecting group) at 0 °C, then, allowed to reach room temperature. The mixture was poured into a saturated aqueous solution of NH_4Cl (60 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (1 × 60 mL), brine (1 × 60 mL), dried ($MgSO_4$) and the solvent was evaporated. The crude product was purified by column chromatography (Hexane/EtOAc, 80:20) to give a colourless gum which was dissolved in hexane. Evaporation of the solvent afforded the titled compound (5.14 g, 15.28 mmol, 91% yield) as a white solid. M.p. 78–80 °C. IR (film) cm^{-1}: 3486, 2980, 2932, 1754, 1281, 1236, 1156, 1097. ^1H NMR ($CDCl_3$) δ: 1.22 (s, 3H, CH_3), 1.55 (s, 9H, *t*-Bu), 1.64–1.77 (m, 1H, chromane 3-H_aH_b), 1.87–2.02 (m, 1H, chromane 3-H_aH_b), 2.05 (s, 3H, ArCH_3), 2.09 (br s, 6H, 2× ArCH_3), 2.65 (m, 2H, chromane 4-H_2), 3.62 (m, 2H, CH_2OH). ^{13}C NMR ($CDCl_3$) δ: 12.0 (2× ArCH_3), 12.8 (ArCH_3), 20.2 (CH_2), 20.6 (CH_3), 27.5 (CH_2), 27.8 (*t*-Bu), 69.4 (OCH_2), 75.6, 82.9 (*t*-Bu), 117.4, 123.0, 125.5, 127.4, 141.3, 148.8, 152.3 (C=O). ESI-MS (pos. mode): m/z = 359.28 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{28}O_5$ (336.4): C, 67.83; H, 8.39. Found: C, 67.97; H, 8.19.

5.1.6. (±) (2,5,7,8-Tetramethyl-chroman-2-yl)methanol (**4b**)

To a suspension of $LiAlH_4$ (85 mg, 2.24 mmol) in anhydrous THF (5 mL) at 0 °C, was added dropwise under argon, a solution of **3c** (623 mg, 2.37 mmol) in anhydrous THF (10 mL). The suspension was stirred for 1 h at 0 °C, then $LiAlH_4$ (81 mg, 2.13 mmol) was anew added to the mixture. After 30 min the mixture was poured into a saturated aqueous solution of NH_4Cl (20 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (2 × 10 mL), dried ($MgSO_4$) and the solvent was evaporated. The crude product was purified by column chromatography (Hexane/EtOAc, 90:10) to give the titled compound (490 mg, 2.22 mmol, 94% yield) as a white solid. M.p. 81–82 °C. IR (film) cm^{-1}: 3367, 2931, 1458, 1310, 1106, 1049. ^1H NMR ($CDCl_3$) δ: 1.24 (s, 3H, CH_3), 1.68–1.78 (m, 1H, chromane 3-H_aH_b), 1.89 (t, J = 7.5 Hz, 1H, OH), 1.95–2.05 (m, 1H, chromane 3-H_aH_b), 2.08 (s, 3H, ArCH_3), 2.17 (s, 3H, ArCH_3), 2.21 (s, 3H, ArCH_3), 2.62–2.68 (m, 2H, chromane 4-H_2), 3.63 (m, 2H, CH_2OH), 6.89 (s, 1H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ: 11.5, 18.9, 19.7, 19.9, 20.7, 27.7, 69.6, 75.8, 116.9, 122.1, 123.0, 133.7, 135.0, 151.1. ESI-MS (pos. mode): m/z = 243.06 $[M + Na]^+$. Anal. Calcd for $C_{14}H_{20}O_2$ (220.3): C, 76.33; H, 9.15. Found: C, 76.52; H, 9.03.

*5.1.7. (±) Carbonic acid tert-butyl ester 2-(4-formylphenoxymethyl)-2,5,7,8-tetramethyl-chroman-6-yl ester (**5a**)*

Preparation of the triflate intermediate: to an ice-cooled solution of anhydrous pyridine (2.8 mL, 34.50 mmol) in dry CH_2Cl_2 (20 mL), was added dropwise trifluoromethanesulfonic anhydride (1.39 mL, 8.26 mmol) under argon. A solution of 4a (1.95 g, 5.80 mmol) in dry CH_2Cl_2 (20 mL) was added to the reaction mixture. The solution was stirred at 0 °C for 30 min and the solvent was evaporated. The excess of pyridine was co-evaporated with toluene and the residue so obtained was diluted in EtOAc (50 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ solution (2 × 30 mL), brine (2 × 30 mL), dried ($MgSO_4$) and the solvent was removed under vacuum to give a brown liquid residue.

Substitution with 4-hydroxybenzaldehyde: to a solution of the above triflate in anhydrous DMF (12 mL) were added 4-hydroxybenzaldehyde (709 mg, 5.81 mmol) and K_2CO_3 (1.61 g, 11.65 mmol). The reaction mixture was stirred under argon at room temperature for two days, and then water (30 mL) was added. The resulting solution was extracted with EtOAc (30 mL). The organic layer was washed with water (30 mL) dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by column chromatography (Hexane/EtOAc, 90:10) to give a colourless syrup which was dissolved in diethyl ether and concentrated under vacuum to give the titled compound as a white solid (2.20 g, 4.99 mmol, 86% yield over two steps). M.p. 124–125 °C. IR (film) cm^{-1}: 2980, 2934, 1752, 1693, 1601, 1278, 1237, 1157, 1096. ^1H NMR ($CDCl_3$) δ: 1.43 (s, 3H, CH_3), 1.55 (s, 9H, *t*-Bu), 1.80–1.95 (m, 1H, chromane 3-H_aH_b), 2.04 (s, 3H, ArCH_3), 2.06 (s, 3H, ArCH_3), 2.08 (s, 3H, ArCH_3), 2.11–2.19 (m, 1H, chromane 3-H_aH_b), 2.64 (m, 2H, chromane 4-H_2), 3.97, 4.08 (AB system, J = 9.3 Hz, 2H, CH_2O), 7.03 (d, J = 8.7 Hz, 2H, H_{arom}), 7.83 (d, J = 8.7 Hz, 2H, H_{arom}), 9.89 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$) δ: 11.9 ($ArCH_3$), 12.0 ($ArCH_3$), 12.8 ($ArCH_3$), 20.2 (CH_2), 22.8 (CH_3), 27.8 (*t*-Bu), 28.3 (CH_2), 72.8, 74.4, 82.9 (*t*-Bu), 115.1 (CH_{arom}), 117.2, 123.2, 125.5, 127.5, 130.2, 132.0 (CH_{arom}), 141.4, 148.8, 152.3 (carbonate C=O), 164.1, 190.9 (CHO). ESI-MS (pos. mode): m/z = 463.22 $[M + Na]^+$, 441.22 $[M + H]^+$ Anal. Calcd for $C_{26}H_{32}O_6$ (440.5): C, 70.89; H, 7.32. Found: C, 70.77; H, 7.29.

*5.1.8. (±) 4-(2,5,7,8-Tetramethyl-chroman-2-ylmethoxy)-benzaldehyde (**5b**)*

Preparation of the triflate intermediate: to an ice-cooled solution of anhydrous pyridine (930 μL, 11.50 mmol) in dry CH_2Cl_2 (10 mL), was added dropwise trifluoromethanesulfonic anhydride (480 μL, 2.85 mmol) under argon. A solution of **4b** (422 mg, 1.92 mmol) in dry CH_2Cl_2 (10 mL) was added to the reaction mixture. The solution was stirred at 0 °C for 20 min and the solvent was evaporated. The excess of pyridine was co-evaporated with toluene and the residue so obtained was dissolved in EtOAc (50 mL). The organic layer was washed with 5% aqueous $NaHCO_3$ solution (2 × 10 mL), brine (1 × 10 mL), dried ($MgSO_4$) and the solvent was removed under vacuum to give a brown oily residue.

Substitution with 4-hydroxybenzaldehyde: to a solution of the above triflate in anhydrous DMF (5 mL) were added 4-hydroxybenzaldehyde (236 mg, 1.93 mmol) and K_2CO_3 (528 mg, 3.82 mmol). The reaction mixture was stirred under argon at room temperature for two days, then water (10 mL) was added. The resulting solution was extracted with EtOAc (2 × 30 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. The crude product was purified by column chromatography (Hexane/EtOAc, 90:10) to give the titled compound as a colourless syrup (521 mg, 1.61 mmol, 84% yield over two steps). IR (film) cm^{-1}: 2933, 1693, 1600, 1577, 1310, 1246, 1157. ^1H NMR ($CDCl_3$) δ: 1.45 (s, 3H, CH_3), 1.86–1.97 (m, 1H, chromane 3-H_aH_b), 2.05 (s, 3H, ArCH_3), 2.08–2.17 (m, 1H, chromane 3-H_aH_b), 2.17 (s, 3H, ArCH_3), 2.20 (s, 3H, ArCH_3), 2.63 (m, 2H, chromane 4-H_2), 4.00, 4.09 (AB system, J = 9.3 Hz, 2H, CH_2O), 6.59 (s, 1H, H_{arom}), 7.03 (d, J = 8.8 Hz, 2H, H_{arom}), 7.83 (d, J = 8.8 Hz, 2H, H_{arom}), 9.89 (s, 1H, CHO). ^{13}C NMR ($CDCl_3$) δ 11.5, 18.9, 19.8, 19.9, 22.9, 28.5, 73.1, 74.6, 115.2, 116.7, 122.2, 123.0, 130.3, 132.1, 133.6, 135.2, 151.1, 164.2, 190.9 (CHO). ESI-MS: m/z = 347.14 $[M + Na]^+$. Anal. Calcd for $C_{21}H_{24}O_3$ (324.4): C, 77.75; H, 7.46. Found: C, 77.05; H, 6.98.

*5.1.9. (±) Carbonic acid tert-butyl ester 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester (**6a**)*

To a solution of **5a** (2.20 g, 4.99 mmol) in absolute EtOH (50 mL, which may be replaced by dry toluene), was added thiazolidine-2,4-dione (1.17 g, 9.99 mmol) and piperidine (247 μL, 2.50 mmol). The mixture was heated at reflux under argon for 12 h. The solution was concentrated under vacuum, and the residue was dissolved in EtOAc (100 mL). The resulting solution was washed with 5% aqueous $NaHCO_3$ solution (2 × 50 mL) and water (2 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated to give a yellow solid. The crude product was purified by column chromatography (Hexane/EtOAc, 80:20) to give the titled compound (2.37 g, 4.39 mmol, 88% yield) as a white solid. M.p. 195–196 °C. IR (film) cm^{-1}: 3189, 2981, 2933, 1747, 1705, 1595, 1510, 1245, 1154. ^1H NMR ($CDCl_3$) δ: 1.41 (s, 3H, CH_3), 1.56 (s, 9H, *t*-Bu), 1.81–1.93 (m, 1H, chromane 3-H_aH_b), 2.04 (s, 3H, ArCH_3), 2.05 (s, 3H, ArCH_3), 2.07 (s, 3H, ArCH_3), 2.08–2.16 (m, 1H, chromane 3-H_aH_b), 2.64 (br t, J = 6.7 Hz, 2H, chromane 4-H_2), 3.94, 4.01 (AB system, J = 9.3 Hz, 2H, CH_2O), 6.99 (d, J = 8.8 Hz, 2H, H_{arom}), 7.43 (d, J = 8.8 Hz, 2H, H_{arom}), 7.80 (s, 1H, ArCH=). ^{13}C NMR ($CDCl_3$) δ: 11.9 ($ArCH_3$), 12.0 ($ArCH_3$), 12.8 ($ArCH_3$), 20.2 (CH_2), 22.7 (CH_3), 27.8 (*t*-Bu), 28.4 (CH_2), 73.0, 74.5, 83.1 (*t*-Bu), 115.6 (CH_{arom}), 117.3, 119.6, 123.3, 125.6, 125.9, 127.7, 132.4 (CH_{arom}), 134.2 (=CH), 141.4, 148.8, 152.6 (carbonate C=O), 161.1, 167.0 (C=O), 167.5 (C=O). ESI-MS (pos. mode): m/z = 562.08 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{33}NO_7S$ (539.6): C, 64.54; H, 6.16; N, 2.60. Found: C, 64.38; H, 6.20; N, 2.65.

*5.1.10. (±) 5-[1-[4-(2,5,7,8-Tetramethyl-chroman-2-ylmethoxy)-phenyl]-meth-(Z)-ylidene]-thiazolidine-2,4-dione (**6b**)*

To a solution of **5b** (437 mg, 1.35 mmol) in absolute EtOH (13 mL), were added thiazolidine-2,4-dione (317 mg, 2.71 mmol) and piperidine (67 μL, 0.68 mmol). The mixture was heated at reflux under argon for 24 h. The solution was concentrated under vacuum, and the residue was dissolved in EtOAc (40 mL). The resulting solution was washed with 5% aqueous $NaHCO_3$ solution (2 × 10 mL) and brine (2 × 10 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography (Hexane/EtOAc, 80:20) to give the titled compound (308 mg, 0.73 mmol, 54% yield) as a yellow amorphous solid. M.p. 129–134 °C. IR (film) cm^{-1}: 3190, 3050, 2934, 1740, 1690, 1595, 1511, 1249, 1178, 1111, 1042, 827, 738. ^1H NMR ($CDCl_3$) δ: 1.44 (s, 3H, CH_3), 1.85–1.96 (m, 1H, chromane 3-H_aH_b), 2.05 (s, 3H, ArCH_3), 2.08–2.16 (m, 1H, chromane 3-H_aH_b), 2.16 (s, 3H, ArCH_3), 2.20 (s, 3H, ArCH_3), 2.63 (br t, J = 6.2 Hz, 2H, chromane 4-H_2), 3.98, 4.07 (AB system, J = 9.3 Hz, 2H, 2-CH_2O), 6.59 (s, 1H, H_{arom}), 7.02 (d, J = 9.0 Hz, 2H, H_{arom}), 7.44 (d, J = 9.0 Hz, 2H, H_{arom}), 7.81 (s, 1H, ArCH=), 8.28 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ 11.5, 18.9, 19.8, 19.9, 22.9, 28.5, 73.0, 74.6, 115.7, 116.8, 119.4, 122.2, 123.0, 125.9, 132.4, 133.6, 134.5, 135.2, 151.1, 161.3, 166.5 (C=O), 167.2 (C=O). ESI-MS (pos. mode): m/z = 446.17 $[M + Na]^+$. Anal. Calcd for $C_{24}H_{25}NO_4S$ (423.5): C, 68.06; H, 5.95; N, 3.31. Found: C, 67.82; H, 5.95; N, 3.34.

*5.1.11. (±) 5-[1-[4-(6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-ylmethoxy)phenyl]meth-(Z)-ylidene]thiazolidine-2,4-dione (**7**)*

To a solution of **6a** (601 mg, 1.11 mmol) in CH_2Cl_2 (15 mL), was added trifluoroacetic acid (5 mL). The mixture was stirred for 1 h

and the solvent was evaporated. The crude product was dissolved in EtOAc (50 mL) and washed with 5% aqueous $NaHCO_3$ solution (2 × 50 mL) and water (2 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude product was purified by column chromatography (Hexane/EtOAc, 80:20) and then recrystallised in MeOH to give the titled compound (422 mg, 0.95 mmol, 86% yield) as a yellow solid. M.p. 130–132 °C. IR (film) cm^{-1}: 3321, 3181, 2927, 1738, 1690, 1594, 1510, 1253, 1178. ^1H NMR ($CDCl_3$) δ: 1.42 (s, 3H, CH_3), 1.85–1.96 (m, 1H, chromane 3-H_aH_b), 2.05–2.20 (m, 1H, chromane 3-H_aH_b), 2.08 (s, 3H, ArCH_3), 2.11 (s, 3H, ArCH_3), 2.16 (s, 3H, ArCH_3), 2.65 (br t, J = 6.5 Hz, 2H, chromane 4-H_2), 3.96, 4.05 (AB system, J = 9.3 Hz, 2H, CH_2O), 4.31 (br s, 1H, OH), 7.02 (d, J = 8.7 Hz, 2H, H_{arom}), 7.44 (d, J = 8.7 Hz, 2H, H_{arom}), 7.81 (s, 1H, ArCH=), 8.75 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 11.5 ($ArCH_3$), 12.0 ($ArCH_3$), 12.4 ($ArCH_3$), 20.4 (CH_2), 22.7 (CH_3), 28.8 (CH_2), 72.9, 74.1, 115.7 (CH_{arom}), 117.3, 118.7, 119.3, 121.5, 122.9, 125.8, 132.4 (CH_{arom}), 134.5 (=CH), 145.0, 145.2, 161.3, 166.8 (C=O), 167.4 (C=O). ESI-MS (pos. mode): m/z = 440.15 $[M + H]^+$. Anal. Calcd for $C_{24}H_{25}NO_5S$,1/4H_2O (444.0): C, 64.92; H, 5.79; N, 3.16. Found: C, 64.84; H, 5.62; N, 3.35.

5.1.12. (±) 5-((3aS,4S,6aR)-2-Oxo-hexahydro-thieno[3,4-d] imidazol-4-yl)-pentanoic acid 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester (**8a**)

To a solution of D(+)-biotin (46 mg, 0.19 mmol) in DMF (4 mL) under an argon atmosphere were added CH_2Cl_2 (2 mL) and triethylamine (26 μL, 0.19 mmol). The solution was cooled to 0 °C and isobutyl chloroformate (24 μL, 0.19 mmol) was added. The mixture was stirred 45 min at this temperature, then **7** (76 mg, 0.17 mmol) and DMAP (2 mg, 0.017 mmol) dissolved in CH_2Cl_2 (6 mL) were added. The solution was stirred at room temperature for 12 h. The CH_2Cl_2 was evaporated and the liquid residue was diluted with EtOAc (30 mL). The solution was washed with 5% aqueous $NaHCO_3$ solution (2 × 20 mL), 5% aqueous citric acid solution (2 × 20 mL), water (2 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. Column chromatography (CH_2Cl_2/MeOH, 97:3) using Axxial® Modul Prep apparatus (see above) afforded a colourless residue which was suspended in a 1:1 mixture of water and CH_3CN (5 mL) and freeze dried to give 40 mg (0.06 mmol, 35% yield) of white powder. M.p. 196 °C. IR (KBr) cm^{-1}: 3422, 2928, 1736, 1702, 1598, 1509, 1250, 1160. ^1H NMR (DMSO-d_6, 400 MHz) δ: 1.28 (s, 3H, CH_3), 1.35–1.70 (m, 6H, biotin CH_2), 1.75–2.00 (m, 2H, chromane 3-CH_2), 1.86 (s, 6H, ArCH_3), 1.91 (s, 3H, ArCH_3), 2.50–2.65 (m, 5H, CH_2CO + chromane 4-CH_2 + CH_aH_bS), 2.78 (A part of an ABX system, J = 5.1, 12.4 Hz, 1H, CH_aH_bS), 3.07 (m, 1H, CHS), 4.08 (m, 3H, OCH_2 + CHNH), 4.26 (m, 1H, CHNH), 6.32 (s, 1H, NH), 6.41 (s, 1H, NH), 7.10 (d, J = 8.8 Hz, 2H, H_{arom}), 7.48 (d, J = 8.8 Hz, 2H, H_{arom}), 7.69 (s, 1H, ArCH=), 12.45 (br s, 1H, thiazolidinedione NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 11.7 ($ArCH_3$), 12.0 ($ArCH_3$), 12.8 ($ArCH_3$), 19.4 (CH_2), 21.5 (CH_3), 24.6 (biotin CH_2), 27.6 (CH_2), 28.1 (biot CH_2), 28.2 (biot CH_2), 33.0 (CH_2), 40.2 (CH_2S), 55.4 (CHS), 59.2 (CHNH), 61.1 (CHNH), 72.6 (OCH_2), 74.5 ($OCCH_3$), 115.7 (CH_{arom}), 117.4, 120.4, 122.0, 125.0, 125.7, 126.5, 131.8 (=CH), 132.0 (CH_{arom}), 140.6, 148.2, 160.5, 162.7, 167.5, 168.0, 171.6. ESI-MS (pos. mode): m/z = 666.21 $[M + H]^+$, 688.19 $[M + Na]^+$. Anal. Calcd for $C_{34}H_{39}N_3O_7S_2$, 1/2H_2O (674.8): C, 60.51; H, 5.97; N, 6.23. Found: C, 60.75; H, 6.21; N, 6.21.

5.1.13. (±) 8-tert-Butoxycarbonylamino-octanoic acid 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester (**8b**)

To an argon-flushed solution of 8-(*tert*-butoxycarbonylamino) caprylic acid (532 mg, 2.05 mmol, prepared as previously reported [40]) in CH_2Cl_2 (40 mL) was added TEA (286 μL, 2.05 mmol) and the mixture was cooled with an ice bath. Then, isobutyl chloroformate (266 μL, 2.05 mmol) was added, the solution was stirred for 1 h at the same temperature, and a solution of **7** (751 mg, 1.71 mmol) and DMAP (25 mg, 0.21 mmol) in CH_2Cl_2 (40 mL) was added. The reaction mixture was stirred for 12 h at room temperature, then washed with 5% aqueous $NaHCO_3$ solution (2 × 40 mL), 5% aqueous citric acid solution (2 × 40 mL), brine (2 × 40 mL), dried ($MgSO_4$) and concentrated to dryness. Column chromatography (CH_2Cl_2/EtOAc, 95:5) using Axxial® Modul Prep apparatus (see above) afforded 873 mg (1.28 mmol, 75% yield) of light yellow amorphous low melting point solid. IR (film) cm^{-1}: 3375, 3181, 2932, 1747, 1705, 1597, 1511, 1250, 1178. ^1H NMR ($CDCl_3$) δ: 1.42 (s, 3H, CH_3), 1.45 (s, 9H, *t*-Bu), 1.32–1.55 (m, 8H, caprylic CH_2× 4), 1.80 (m, 2H, caprylic CH_2), 1.92 (m, 1H, chromane 3-H_aH_b), 1.97 (s, 3H, ArCH_3), 2.01 (s, 3H, ArCH_3), 2.05 (s, 3H, ArCH_3), 2.10 (m, 1H, chromane 3-H_aH_b), 2.62 (m, 4H, chromane 4-CH_2 + caprylic CH_2), 3.12 (br q, J = 6.4 Hz, 2H, caprylic CH_2), 3.99 (m, 2H, CH_2O), 4.53 (br s, 1H, carbamate NH), 6.99 (d, J = 8.7 Hz, 2H, H_{arom}), 7.43 (d, J = 8.7 Hz, 2H, H_{arom}), 7.79 (s, 1H, ArCH=), 8.87 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 12.0, 12.3, 13.1, 20.3, 22.8, 25.2, 26.8, 28.5, 28.6, 29.1, 29.4, 30.2, 34.2, 40.8, 73.1, 74.5, 77.4, 79.3, 115.7, 117.4, 119.7, 123.3, 125.3, 126.0, 127.4, 132.3, 134.1, 141.2, 148.8, 156.2, 161.1, 166.8, 167.3, 172.6. ESI-MS (pos. mode): m/z = 703.27 $[M + Na]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_8S$ (680.8): C, 65.27; H, 7.11; N, 4.12. Found: C, 64.99; H, 6.89; N, 4.14.

5.1.14. (±) 8-Amino-octanoic acid 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester (**8c**)

To a solution of **8b** (306 mg, 0.45 mmol) in CH_2Cl_2 (15 mL) was added trifluoroacetic acid (5 mL) and the mixture was stirred at room temperature for 45 min. The solution was concentrated to dryness. The residue was dissolved in CH_2Cl_2 (10 mL) and Et_2O (10 mL) was added to the solution. The suspension was concentrated to dryness and carefully dried to give 290 mg (0.42 mmol, 94% yield) of light yellow solid. M.p. 114 °C. IR (KBr) cm^{-1}: 3423, 2930, 1745, 1686, 1598, 1252, 1204, 1179, 1147. ^1H NMR (DMSO-d_6) δ: 1.33 (br s, 9H, CH_3 + caprylic CH_2× 3), 1.52 (m, 2H, caprylic CH_2), 1.66 (m, 2H, caprylic CH_2), 1.85 (m, 1H, chromane 3-H_aH_b), 1.91 (s, 6H, 2× ArCH_3), 1.96 (s, 3H, ArCH_3), 2.03 (m, 1H, chromane 3-H_aH_b), 2.63 (m, 4H, chromane 4-CH_2 + caprylic CH_2), 2.78 (m, 2H, caprylic CH_2), 4.12 (s, 2H, OCH_2), 7.15 (d, J = 8.9 Hz, 2H, H_{arom}), 7.54 (d, J = 8.9 Hz, 2H, H_{arom}), 7.68 (br s, 3H, NH_3^+), 7.75 (s, 1H, ArCH=), 12.54 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 11.6, 11.9, 12.7, 19.4, 21.5, 24.4, 25.7, 27.0, 27.6, 28.2, 28.3, 30.7, 33.1, 72.6, 74.5, 115.7, 117.4, 120.4, 122.0, 125.0, 125.7, 126.5, 131.8, 132.0, 140.6, 148.2, 160.5, 167.4, 167.9, 171.6. ESI-MS (pos. mode): m/z = 581.28 $[M]^+$. Anal. Calcd for $C_{34}H_{41}N_2O_8SF_3$, 1/2H_2O (703.8): C, 58.02; H, 6.02; N, 3.98. Found: C, 57.79; H, 5.91; N, 3.91.

5.1.15. (±) 8-[5-((3aR,6R,6aS)-2-Oxo-hexahydro-thieno[2,3-d] imidazol-6-yl)-pentanoylamino]-octanoic acid 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester(**8d**)

To an argon-flushed solution of biotin (35 mg, 0.14 mmol) in dry DMF (4 mL) were added dry CH_2Cl_2 (1 mL) and TEA (50 μL, 0.36 mmol). The solution was cooled in an ice bath, isobutyl chloroformate (19 μL, 0.14 mmol) was added, and the solution was stirred for 45 min. Then a solution of **8c** (100 mg, 0.14 mmol) in DMF (2 mL) was added and the mixture was stirred for 12 h. The solution was diluted with EtOAc (50 mL) and washed with 5% aqueous $NaHCO_3$ solution (2 × 30 mL), 5% aqueous citric acid solution (2 × 30 mL), brine (2 × 30 mL), dried ($MgSO_4$) and concentrated to dryness. Column chromatography (CH_2Cl_2/MeOH, 97:3 to 90:10) using Axxial® Modul Prep apparatus (see above) afforded 72 mg (0.089 mmol, 64% yield) of white powder. M.p. 147 °C. IR (KBr) cm^{-1}: 3422, 2927, 1741, 1699, 1597, 1510, 1250, 1151. ^1H NMR (DMSO-d_6,

400 MHz) δ: 1.12–1.42 (m, 15H, CH_3 + 6× CH_2), 1.50 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 1.80–2.02 (m, 2H, chromane 3-CH_2), 1.91 (s, 6H, 2× ArCH_3), 1.96 (s, 3H, ArCH_3), 2.04 (m, 2H, caprylic CH_2), 2.40–2.70 (m, 5H, chromane 4-CH_2 + CH_2CONH + C$H_a$$H_b$S), 2.80 (dd, J = 4.7, 12.8 Hz, 1H, $CH_a$$H_b$S), 3.01 (m, 2H, caprylic CH_2), 3.08 (m, 1H, CHS), 4.11 (m, 3H, OCH_2 + CHNH), 4.29 (m, 1H, CHNH), 6.36 (s, 1H, NH), 6.43 (s, 1H, NH), 7.15 (d, J = 8.4 Hz, 2H, H_{arom}), 7.54 (d, J = 8.4 Hz, 2H, H_{arom}), 7.74 (m, 2H, ArCH═ + CONH), 12.50 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 12.5 (CH_3), 12.8 (CH_3), 13.6 (CH_3), 20.3 (CH_2), 22.4 (CH_3), 25.3 (CH_2), 26.2 (CH_2), 27.1 (CH_2), 28.5 (CH_2), 28.9 (CH_2), 29.1 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 30.0 (CH_2), 34.0 (CH_2), 36.1 (CH_2), 39.2 (CH_2), 55.8 (CH_2), 56.3 (CH), 60.0 (CH), 61.9 (CH), 73.5, 75.4, 116.5 (CH_{arom}), 118.2, 121.6, 122.8, 125.8, 126.7, 127.4, 132.2 (ArCH═), 132.8 (CH_{arom}), 141.4, 149.0, 161.2, 163.6, 168.7, 169.0, 172.5, 172.6. ESI-MS (pos. mode): m/z = 829.33 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{54}N_4O_8S_2$, 1/2H_2O (816.0): C, 61.81; H, 6.79; N, 6.87. Found: C, 61.72; H, 6.80; N, 6.68.

5.1.16. (±) Succinic acid mono-(2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl) ester (**8e**)

To a solution of **7** (82 mg, 0.18 mmol) in CH_2Cl_2 (10 mL) were added succinic anhydride (37 mg, 0.37 mmol) and DMAP (2 mg, 0.018 mmol), and the mixture was refluxed for 12 h. Succinic anhydride (18 mg, 0.18 mmol) was anew added and the mixture was refluxed for another 12 h. The solvent was evaporated, the residue was dissolved in EtOAc (20 mL) and the solution was washed with 5% aqueous citric acid solution (3 × 10 mL), water (3 × 10 mL), dried ($MgSO_4$) and concentrated to dryness. Column chromatography (CH_2Cl_2/MeOH, 98:2 then 96:4) using Axxial® Modul Prep apparatus (see above) afforded a residue which was suspended in a 1:1 mixture of water and CH_3CN (5 mL) to give after freeze drying 43 mg (0.08 mmol, 43% yield) of light yellow powder. M.p. 131–136 °C. IR (KBr) cm^{-1}: 3433, 2926, 1736, 1686, 1598, 1510, 1253, 1151. ^{1}H NMR (DMSO-d_6) δ: 1.34 (s, 3H, CH_3), 1.92 (s, 6 H, ArCH_3× 2), 1.96 (s, 3H, ArCH_3), 1.78–2.11 (m, 2H, chromane 3-CH_2), 2.61 (m, 4H, CH_2), 2.83 (m, 2H, CH_2), 4.12 (s, 2H, OCH_2), 7.15 (d, J = 8.9 Hz, 2H, H_{arom}), 7.54 (d, J = 8.9 Hz, 2H, H_{arom}), 7.73 (s, 1H, ArCH═), 12.29 (s, 1H, NH), 12.47 (br s, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ: 11.6, 11.8, 12.6, 19.4, 21.5, 27.6, 28.4, 28.7, 72.6, 74.5, 115.7, 117.3, 120.6, 121.9, 125.1, 125.7, 126.7, 131.6, 132.0, 140.6, 148.1, 160.4, 167.6, 168.0, 170.9, 173.3. ESI-MS (pos. mode): m/z = 562.20 $[M + Na]^+$. Anal. Calcd for $C_{28}H_{29}NO_8S$, 1/2H_2O (548.6): C, 61.30; H, 5.51; N, 2.55. Found: C, 60.98; H, 5.40; N, 2.58.

5.1.17. (±) N-(2-Acetylamino-ethyl)-succinamic acid 2-{4-[2,4-dioxo-thiazolidin-(5Z)-ylidenemethyl]-phenoxymethyl}-2,5,7,8-tetramethyl-chroman-6-yl ester (**8f**)

To an argon-flushed solution of **8e** (101 mg, 0.18 mmol) in CH_2Cl_2 (6 mL) was added TEA (28 μL, 0.20 mmol). The solution was cooled with an ice bath, then isobutyl chloroformate (26 μL, 0.20 mmol) was added. The mixture was stirred at this temperature for 45 min, then it was added to a solution of *N*-acetylethylenediamine (23 mg, 0.20 mmol) in MeOH (10 mL), and the mixture was stirred at room temperature for 12 h. The solvents were evaporated. Column chromatography (CH_2Cl_2/MeOH, 98:2 then 96:4) using Axxial® Modul Prep apparatus (see above) afforded a residue which was suspended in a 1:1 mixture of water and CH_3CN (5 mL) to give after freeze drying 34 mg (0.054 mmol, 29% yield) of white powder. M.p. 150–154 °C. IR (KBr) cm^{-1}: 3432, 2930, 1743, 1698, 1648, 1598, 1511, 1251, 1153. ^{1}H NMR (DMSO-d_6) δ: 1.33 (s, 3H, CH_3), 1.78 (s, 3H, CH_3CO), 1.87 (m, 1H, chromane 3-$H_a$$H_b$), 1.91 (s, 6H, 2× ArC$H_3$), 1.96 (s, 3H, ArC$H_3$), 2.01 (m, 1H, chromane 3-$H_a$$H_b$), 2.46 (t, J = 6.7 Hz, 2H, succinic CH_2), 2.63 (br t, 2H, chromane 4-CH_2), 2.82 (t, J = 6.7 Hz, 2H, succinic CH_2), 3.07 (m, 4H, ethylenediamine CH_2), 4.12 (s, 2H, OCH_2), 7.15 (d, J = 8.6 Hz, 2H, H_{arom}), 7.54 (d, J = 8.6 Hz, 2H, H_{arom}), 7.82 (br t, 1H, NH), 7.94 (br t, 1H, NH), 12.49 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 11.6, 11.9, 12.7, 19.4, 21.5, 22.6, 27.6, 28.7, 29.8, 38.3, 38.4, 72.6, 74.5, 115.7, 117.3, 120.7, 121.8, 125.1, 125.7, 126.7, 131.6, 132.0, 140.6, 148.1, 160.4, 167.7, 168.1, 169.3, 170.7, 171.1. ESI-MS (pos. mode): m/z = 646.39 $[M + Na]^+$. Anal. Calcd for $C_{32}H_{37}N_3O_8S$, 1/2H_2O (632.7): C, 60.74; H, 6.05; N, 6.64. Found: C, 60.83; H, 5.92; N, 6.50.

5.1.18. (±) Carbonic acid tert-butyl ester 2-[4-(2,4-dioxo-thiazolidin-5-ylmethyl)-phenoxymethyl]-2,5,7,8-tetramethyl-chroman-6-yl ester (**9a**)

In a Parr hydrogenator flask was first introduced 10% palladium on carbon (400 mg), in order to avoid any ignition of the solvent. A solution of **6a** (500 mg, 0.93 mmol) in dioxane (40 mL) was then added and the suspension was shaken under 70 psi hydrogen pressure for 20 h at room temperature, then filtered on celite® and the resulting solution was concentrated. Thorough drying of the residue afforded 499 mg (0.92 mmol, 99% yield) of amorphous solid. M.p. 143 °C. IR (film) cm^{-1}: 3218, 2928, 1755, 1704, 1514, 1239, 1156. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 3H, CH_3), 1.55 (s, 9H, *t*-Bu), 1.89 (m, 1H, chromane 3-$H_a$$H_b$), 2.04 (s, 3H, ArC$H_3$), 2.07 (s, 6H, 2× ArC$H_3$), 2.14 (m, 1H, chromane 3-$H_a$$H_b$), 2.63 (t, J = 6.3 Hz, 2H, chromane 4-CH_2), 3.09 (dd, A part of an ABX system, J = 9.5, 14.1 Hz, 1H, PhCH_2), 3.45 (dd, B part of an ABX system, J = 3.8, 14.1 Hz, 1H, PhCH_2), 3.86,3.97 (AB system, J = 9.2 Hz, 2H, CH_2O), 4.48 (dd, X part of an ABX system, J = 3.8 Hz, 9.5 Hz, 1H, CH), 6.87 (d, J = 8.6 Hz, 2H, H_{arom}), 7.13 (d, J = 8.6 Hz, 2H, H_{arom}), 7.26 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 11.9, 12.0, 12.8, 20.3, 23.0, 27.9, 28.5, 37.9, 53.8, 72.8, 74.7, 82.9, 115.2, 117.5, 123.2, 125.5, 127.5, 128.0, 130.4, 141.4, 149.0, 152.5, 158.7, 170.1, 173.9. ESI-MS (pos. mode): m/z = 564.29 $[M + Na]^+$. Anal. Calcd for $C_{29}H_{35}NO_7S$ (541.6): C, 64.30; H, 6.51; N, 2.59. Found: C, 64.49; H, 6.96; N, 2.44.

5.1.19. (±) 5-[4-(2,5,7,8-Tetramethyl-chroman-2-ylmethoxy)-benzyl]-thiazolidine-2,4-dione (**9b**)

In a Parr hydrogenator flask was first introduced 10% palladium on carbon (102 mg), in order to avoid any ignition of the solvent. A solution of **6b** (98 mg, 0.23 mmol) in dioxane (20 mL) was then added and the suspension was shaken under 70 psi hydrogen pressure for 20 h at room temperature, then filtered on celite® and the resulting solution was concentrated. The crude product was purified by column chromatography (Hexane/EtOAc, 80:20) to give a colourless syrup which was dissolved in diethyl ether and concentrated under vacuum to give 67 mg of white solid (0.16 mmol, 70% yield). Low melting point solid. IR (film) cm^{-1}: 3212, 2924, 1755, 1699, 1512, 1310, 1243, 1159. ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 3H, CH_3), 1.88 (m, 1H, chromane 3-$H_a$$H_b$), 2.06 (s, 3H, ArC$H_3$), 2.13 (m, 1H, chromane 3-$H_a$$H_b$), 2.16 (s, 3H, ArC$H_3$), 2.20 (s, 3H, ArC$H_3$), 2.62 (br t, J = 7.0 Hz, 2H, chromane 4-H_2), 3.11 (dd, A part of an ABX system, J = 9.3, 14.1 Hz, 1H, PhCH_2), 3.45 (dd, B part of an ABX system, J = 3.8, 14.1 Hz, 2H, PhCH_2), 3.88, 3.98 (AB system, J = 9.1 Hz, 2H, CH_2O), 4.50 (dd, X part of an ABX system, J = 3.8, 9.3 Hz, 1H, CH), 6.57 (s, 1H, H_{arom}), 6.87 (d, J = 8.7 Hz, 2H, H_{arom}), 7.13 (d, J = 8.7 Hz, 2H, H_{arom}), 7.74 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 11.5, 18.9, 19.8, 19.9, 23.1, 28.5, 37.9, 53.8, 72.8, 74.7, 115.2, 116.9, 122.2, 122.9, 127.9, 130.4, 133.6, 135.0, 151.2, 158.8, 170.0, 173.7. ESI-MS (pos. mode): m/z = 448.22 $[M + Na]^+$. Anal. Calcd for $C_{24}H_{27}NO_4S$ (425.5): C, 67.74; H, 6.40; N, 3.29. Found: C, 67.53; H, 6.28; N, 3.37.

5.1.20. (±) 5-((3aS,4S,6aR)-2-Oxo-hexahydro-thieno[3,4-d]imidazol-4-yl)-pentanoic acid 2-[4-(2,4-dioxo-thiazolidin-5-ylmethyl)-phenoxymethyl]-2,5,7,8-tetramethyl-chroman-6-yl ester (**11**)

Synthesis of troglitazone **10**: A suspension of **9a** (100 mg, 0.18 mmol) in CH_3CN (10 mL) and 3 M HCl (5 mL) was refluxed for

45 min. The mixture of solvents was evaporated, the residue was dissolved in EtOAc (20 mL) and the solution was washed with water (3 × 20 mL), dried ($MgSO_4$) and concentrated to dryness to give a colourless residue.

Coupling with biotin: To a solution of D(+)-biotin (50 mg, 0.20 mmol) in DMF (4 mL) were added CH_2Cl_2 (2 mL) and TEA (28 μL, 0.20 mmol). The solution was cooled with an ice bath, isobutyl chloroformate (26 μL, 0.20 mmol) was added under an argon atmosphere, then the mixture was stirred for 45 min, and a solution of **10** and DMAP (2 mg, 0.02 mmol) in CH_2Cl_2 (6 mL) was added. Stirring was continued at room temperature for 12 h, and CH_2Cl_2 was evaporated. The residue was diluted with EtOAc (30 mL) and the solution was washed with 5% aqueous $NaHCO_3$ solution (2 × 20 mL), 5% aqueous citric acid solution (2 × 20 mL), water (2 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. Column chromatography (CH_2Cl_2/MeOH, 97:3) using Axxial® Modul Prep apparatus (see above) afforded a residue which was suspended in a 1:1 mixture of water and CH_3CN (5 mL) to give after freeze drying 43 mg (0.064 mmol, 35% yield) of white powder. M.p. 133 °C. IR (KBr) cm^{-1}: 3423, 2926, 1752, 1702, 1511, 1459, 1244, 1160. ^1H NMR (DMSO-d_6) δ: 1.32 (s, 3H, CH_3), 1.46 (m, 2H, biotin CH_2), 1.70 (m, 2H, biotin CH_2), 1.86 (m, 1H, chromane 3-H_aH_b), 1.91 (s, 6H, 2× ArCH_3), 1.98 (s, 6H, ArCH_3), 2.03 (m, 1H, 3-H_aH_b), 2.62 (m, 5H, CH_2CO + chromane 4-CH_2 + CH_aH_bS), 2.84 (A part of an ABX system, J = 5.0, 12.4 Hz, 1H, CH_aH_bS), 3.06 (dd, A part of an ABX system, J = 8.9, 14.3 Hz, 1H, PhCH_2), 3.13 (m, 1H, biotin CHS), 3.29 (m, 1H, PhCH_2, overlapped with H_2O from solvent), 3.99 (m, 2H, OCH_2), 4.16 (m, 1H, C*H*NH), 4.31 (m, 1H, C*H*NH), 4.86 (dd, X part of an ABX system, J = 4.4, 8.9 Hz, 1H, $PhCH_2CH$), 6.35 (br s, 1H, biotin NH), 6.43 (br s, 1H, biotin NH), 6.92 (d, J = 8.5 Hz, 2H, H_{arom}), 7.14 (d, J = 8.5 Hz, 2H, H_{arom}), 11.98 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 11.6, 11.9, 12.7, 19.4, 21.7, 24.6, 27.7, 28.0, 28.1, 32.9, 36.2, 39.8, 53.0, 55.3, 59.2, 61.1, 72.4, 74.5, 114.7, 117.4, 122.0, 124.9, 126.5, 128.8, 130.3, 140.5, 148.2, 157.8, 162.7, 171.5, 171.6, 175.7. ESI-MS (pos. mode): m/z = 690.23 $[M + Na]^+$. Anal. Calcd for $C_{34}H_{41}N_3O_7S_2$ (667.8): C, 61.14; H, 6.19; N, 6.29. Found: C, 60.87; H, 6.52; N, 6.31.

5.1.21. (±) Succinic acid mono-{2-[4-(2,4-dioxo-thiazolidin-5-ylmethyl)-phenoxymethyl]-2,5,7,8-tetramethyl-chroman-6-yl} ester (**12**)

To a solution of **10** (82 mg, 0.19 mmol, obtained from **9a** as reported above) in CH_2Cl_2 (14 mL) were added succinic anhydride (37 mg, 0.37 mmol) and DMAP (2 mg, 0.019 mmol). The solution was refluxed for 2 days. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (30 mL). The solution was washed with 5% aqueous citric acid solution (2 × 20 mL), water (2 × 20 mL), dried ($MgSO_4$) and concentrated to dryness. The solid residue was suspended in CH_2Cl_2 (5 mL), sonicated, filtered and washed with CH_2Cl_2. Careful drying afforded 63 mg (0.12 mmol, 63% yield) of white powder. M.p. 204 °C. IR (KBr) cm^{-1}: 3418, 3189, 2926, 1743, 1680, 1515, 1246, 1142. ^1H NMR (DMSO-d_6) δ: 1.32 (s, 3H, CH_3), 1.85 (m, 1H, chromane 3-H_aH_b), 1.91 (s, 6H, 2× ArCH_3), 1.97 (s, 6H, ArCH_3), 2.00 (m, 1H, 3-H_aH_b), 2.61 (m, 4H, succinic CH_2CH_2), 2.83 (m, 2H, chromane 4-CH_2), 3.05 (dd, A part of an ABX system, J = 9.0, 14.1 Hz, 1H, CH_2CH), 3.29 (dd, B part of an ABX system, J = 4.3, 9.0 Hz, 1H, CH_2CH), 3.98 (m, 2H, OCH_2), 4.86 (dd, X part of an ABX system, J = 4.3, 9.0 Hz, 1H, CH_2CH), 6.92 (d, J = 8.4 Hz, 2H, H_{arom}), 7.14 (d, J = 8.4 Hz, 2H, H_{arom}), 12.04 (br s, 1H), 12.29 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ: 11.7, 11.9, 12.7, 19.5, 21.7, 27.7, 28.5, 28.7, 36.3, 53.1, 72.4, 74.6, 114.7, 117.4, 122.0, 125.1, 126.7, 128.9, 130.3, 140.5, 148.2, 157.8, 171.0, 171.8, 173.4, 175.9. ESI-MS (pos. mode): m/z = 564.05 $[M + Na]^+$. Anal. Calcd for $C_{28}H_{31}NO_8S$, 1/2 CH_2Cl_2 (584.1): C, 58.60; H, 5.52; N, 2.40. Found: C, 58.81; H, 5.43; N, 2.45.

5.2. Anti-proliferative activity measurement

5.2.1. Cell culture and reagents

MCF-7 and MDA-MB231 human breast cancer cell lines were obtained from American Type Culture Collection (Rockville, MD). Both cell lines were grown at 37 °C, according to American Type Culture Collection recommendations, in phenol red Dulbecco's modified Eagle medium (DMEM, Invitrogen, Cergy Pontoise, France) for MCF-7 and in L-15 medium (Invitrogen) for MDA-MB-231. These media were supplemented with 10% fetal calf serum (Sigma–Aldrich, Lyon, France) and 2 mM L-glutamine (Invitrogen).

5.2.2. Cell proliferation assay

Cells were seeded in 6-well plates at the density of 8.10^4 cells/well in 2 mL of medium supplemented with 10% FCS and 2 mM L-glutamine. After 24 h of cell attachment, the medium was replaced by fresh medium supplemented with 1% FCS and 2 mM L-glutamine. Cell proliferation was studied after 24 h of treatment. Control wells received 0.1% DMSO. At the end of the treatment, cells were washed with PBS, trypsinized and counted with the CellTiter-GloTM Luminescent Cell Viability Assay (Promega, Charbonnieres, France). Each treatment was performed in triplicate. For the different compounds, the concentration leading to a decrease of 50% in the number of viable cells (IC50) was measured.

5.3. Hepatotoxicity evaluation

5.3.1. Cell culture and reagents

Cryopreserved human hepatocytes were provided by Kaly-cell (Illkirch, France). They were distributed in 96-well plates at the density of 0.1 × 106 cells/well in 50 μl of DMEM (Gibco, Invitrogen, UK) supplemented with gentamycin (50 mg/L, Sigma Aldrich, France), insulin (4 mg/L, Sigma Aldrich, France) and dexamethasone (10 μM, Sigma Aldrich, France) under a CO_2/air (5%/95%) humidified atmosphere at 37 °C. After 30 min of equilibration, under stirring at 900 rpm, 50 μL of test compounds or DMSO (0.4%) were added. Compounds were tested at 7 concentrations (0.5, 2.5, 5, 12.5, 25, 50 and 100 μM) in triplicate.

5.3.2. Cytotoxicity assay (MTT assay)

After 90 min of treatment, MTT (10 μL/well at 10 mg/mL, Sigma Aldrich, France) was added and incubated during 30 min at 37 °C. Plates were centrifuged, MTT was removed and 100 μL DMSO was distributed per well. The absorbance was read at 595 nm by microplate spectrophotometry. Cell viability was expressed as percentage over controls (DMSO).

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