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Research paper

Design, synthesis and biological evaluation of non-secosteriodal vitamin D receptor ligand bearing double side chain for the treatment of chronic pancreatitis

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ABSTRACT

Chronic pancreatitis (CP) is a serious disease that characterized by the progressive replacement of functional pancreas tissue by fibrotic tissue. Vitamin D receptor (VDR) plays a critical role in the development of CP, since it inhibits excessive deposition of extracellular matrix (ECM) in activated pancreatic stellate cells (PSCs). Herein, a novel series of non-secosteriodal VDR ligands were designed and synthesized, and their VDR affinity and anti-fibrosis activity were evaluated. The identification of the potent compound **9c** was found over structural optimization, which inhibited ECM deposition and fibrotic gene expression in the western blot and qPCR assays, respectively. Further investigation revealed that compound **9c** inhibited pancreatic fibrosis *in vivo* without increase on serum calcium, which could cause hypercalcemia. These results provide novel insight in possible drug discovery for the treatment of CP using non-secosteroidal VDR modulators.

1. Introduction

Chronic pancreatitis (CP) is a seriously inflammatory disease of the pancreas. Repeated inflammatory events result in the replacement of exocrine and endocrine tissue by fibrotic tissue, leading to the loss of pancreatic function [1–3]. The advances in CP therapy were extremely slow, the treatment of CP has not improved appreciably in decades. Historically, treatment included medical management, drainage procedures, and total or subtotal pancreatectomy [4]. However, although medication such as antioxidants and pancreatic enzyme replacement therapy (PERT) have been employed to help relieve the pain, there is still no pharmacologic therapy that specifically targets the pancreas fibrosis, which leads to the frequently recalcitrant pain of CP [5].

In 1998, star-shaped cells in the pancreas, called pancreatic stellate cells (PSCs), were identified and characterized, which involved in many physiological processes like retinoids storing and wound healing [6,7]. Over a decade, there was accumulating evidence that activated PSCs played a key role in the development of pancreatic fibrosis in CP [8,9].

In healthy pancreas, PSCs are in a quiescent state, characterized by synthesizing low levels of extracellular matrix (ECM) component [10]. During pancreatic injury, PSCs are activated by fibrogenic factors, such as transforming growth factor β (TGF-β), and transdifferentiate to a myofibroblast-like cell [11]. Activated PSCs express the myofibroblast activation marker α-smooth muscle actin (α-SMA), acquire proliferative capacity, and synthesize abundant ECM proteins. Sustained PSC activation under the condition of chronic injury leading to fibrosis, which cause CP gradually [12–15].

In 2014, Evans et al. reported that Vitamin D receptor (VDR) agonist calcipotriol could inhibit the expression of α-SMA and ECM deposition *in vitro* and *in vivo* (Fig. 1) [16]. As a member of the nuclear receptor superfamily, VDR was involved in many physiological processes such as calcium homeostasis, cell differentiation and proliferation [17]. In addition, VDR could block the TGF-β associated signal, which is recognized as one of the most potent pro-fibrotic pathways [18]. Moreover, the finding of robust VDR expression in PSCs led us to consider VDR ligand as a possible modulator of pancreatitis through administering to PSCs [19].

To date, thousands of VDR ligands have been developed, which

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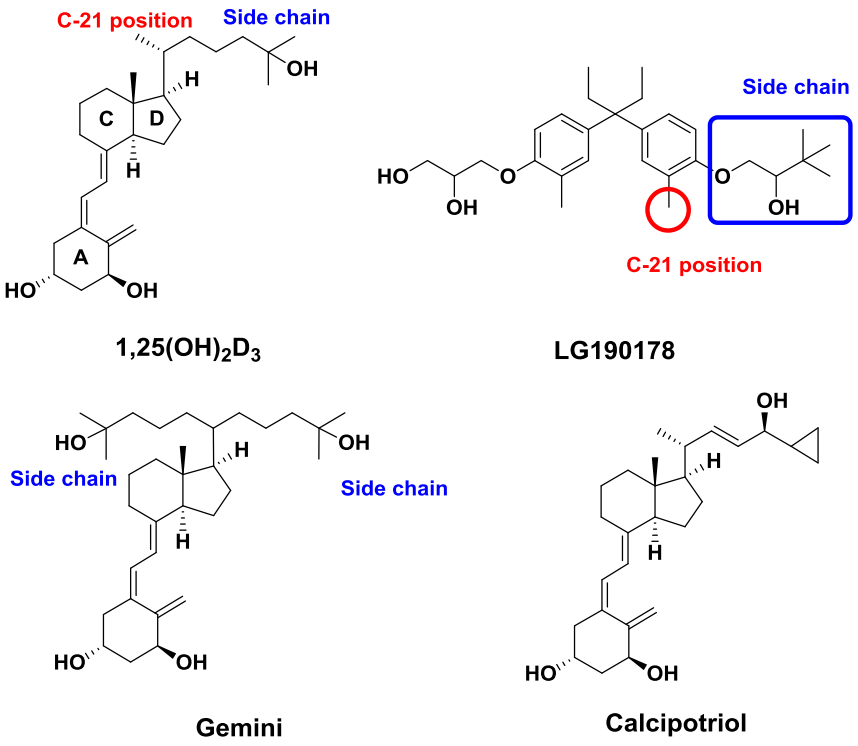


Fig. 1. Chemical structure of several VDR ligands.

can be classified into "secosteroid" and "non-secosteroid" based on their structure specificity. "Secosteroid" VDR ligands have a steroid backbone, such as 1α, 25-dihydroxyvitamin D_3 (1, $25(OH)_2$ D_3), calcipotriol and gemini (Fig. 1). And there is no steroid backbone in "non-secosteroid" ones, such as bisphenol derivative LG190178 (Fig. 1). Although all known VDR ligands are almost secosteroids, they can cause hypercalcemia, which could induce abdominal pain, kidney stones and cardiac arrest. Moreover, structural complexity of secosteroids results in synthetic inconvenience [20]. On the other hand, non-secosteroids was identified as compounds to avoid these disadvantages [21,22]. Non-secosteroidal VDR ligand mimics various activities of 1, $25(OH)_2$ D_3, an endogenous VDR agonist, *in vitro* and *in vivo*, including VDR binding, VDR-dependent transcriptional activation [23,24]. However, none study on non-secosteroidal vitamin D ligands for treating CP was reported until now. Therefore, it is noteworthy to discover non-secosteroidal vitamin D ligands for preventing the progression of CP.

In the present study, on the basis of the LG190178, we designed and synthesized non-secosteroidal VDR ligands bearing another side chain at the C-7 position, which correlate to the C-21 position of $1,25(OH)_2D_3$. As shown in Fig. 2, we attempted to introduce the 2- hydroxy-3,3-dimethylbutoxy chain at the C-7 position of the Y ring. Next, hydrophilic groups were added to the C-1' position of the X ring, which could form hydrogen bonds with amino acid residues of VDR. Herein, the syntheses and biological evaluations *in vitro* and *in vivo* of these non-secosteroidal VDR ligands are reported.

2. Results and discussion

2.1. Chemistry

The synthesis of VDR ligands was depicted in Scheme 1. Esterification of compound 1 by methanol gave compound **2**. Then, compound **3** was obtained by protection of phenolic hydroxyl group of **2** with p-toluene sulfonyl group, then a further nucleophilic attack using ethylmagnesium bromide and compound **4** was generated. Compound **4** coupling with 1-2-benzenediol, produced the key intermediate **5** in the presence of two phenolic hydroxyl group. The phenolic hydroxyl group was substituted by 1-chloropinacolone in the presence of K_2CO_3, and compound **6** was formed. Subsequently, reduction of compound **6** in the presence of sodium borohydride offered compound **7**, and then compound **7** was dissolved in methanol in the presence of magnesium to give the key intermediate **8**. Substitution of the phenolic hydroxyl group with corresponding halogen substituted alcohols provide the target compounds **9a-9c**. Compounds **10a-10e** were prepared in a single step by the treatment of compound **8** with different halogen substituted esters. By reduction or hydrolysis of the appropriate esters, the target compounds **11a-11d** and **12a-12e** were obtained, respectively. Finally, substitution of the phenolic hydroxyl group with corresponding sulfonyl chlorides lea to the target compounds **13a-13c**.

2.2. In vitro VDR binding affinity

In vitro receptor binding affinity of compounds for VDR was evaluated with VDR competitor assay and calcipotriol was used as the positive control. The target compounds was performed at a concentration of 1 μM and the binding affinity was displayed by a relative value based on calcipotriol being assigned as 100%. Structure-activity relationships (SARs) for these compounds are summarized in Table 1 and Table 2. We were encouraged that introduce another side chain at the C-7 position (**9a**) led to a moderate improvement in potency. This allowed for a variety of different analogues to be synthesized shown in Table 1. Replacing the A ring with sulfonic esters (analogues **6**–**7**, **13a-13c**) decreased the binding affinities. And we decided to substitution the A ring with hydrophilic group which contain hydrogen bond donor and hydrogen bond receptor. Consequently, we introduced carboxylic

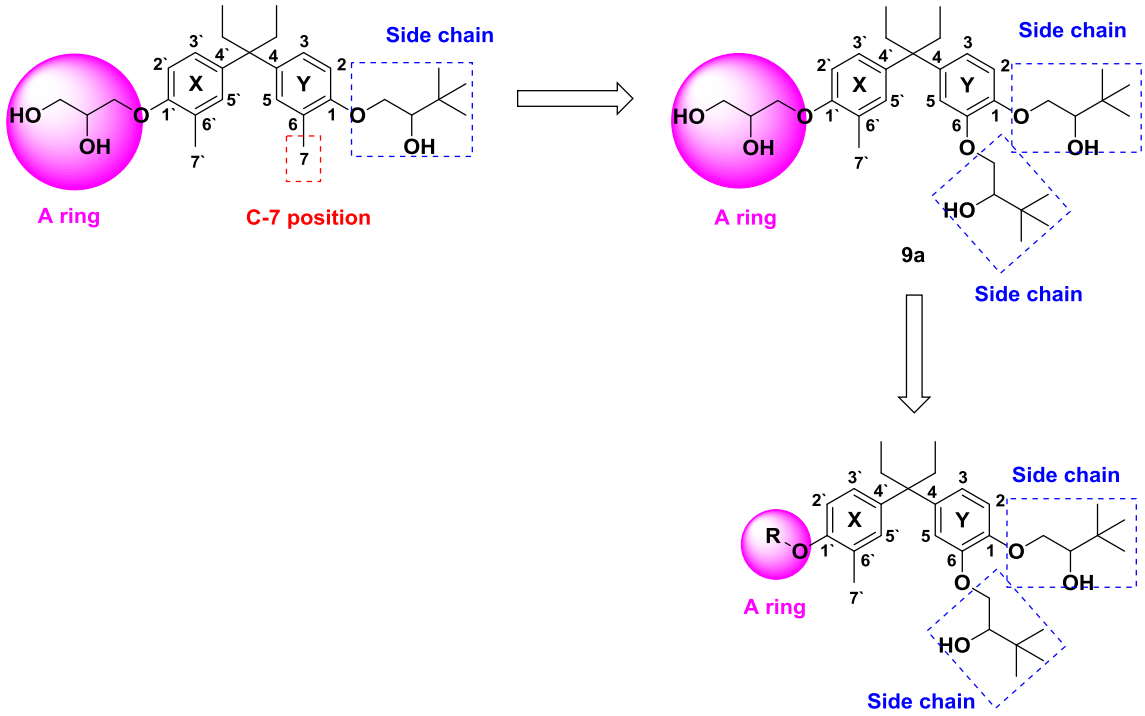


Fig. 2. Design concept of novel nonsecosteroidal VDR ligands.

acid with different chain length (analogues **12a-12e**), dramatically lost binding affinities completely, which suggests that carboxyl acid group may not preferred in the A ring. Gratifyingly, substitution the A ring with ethyl alcohol resulted in a compound with comparable potency (**9b**, Table 1). Given this result, we decided to extend the carbon chain length of **9b** to give compound **9c** and **11a-11d**. Among them, compound **9c** and **11a** showed significant binding affinities.

2.3. Transactivation

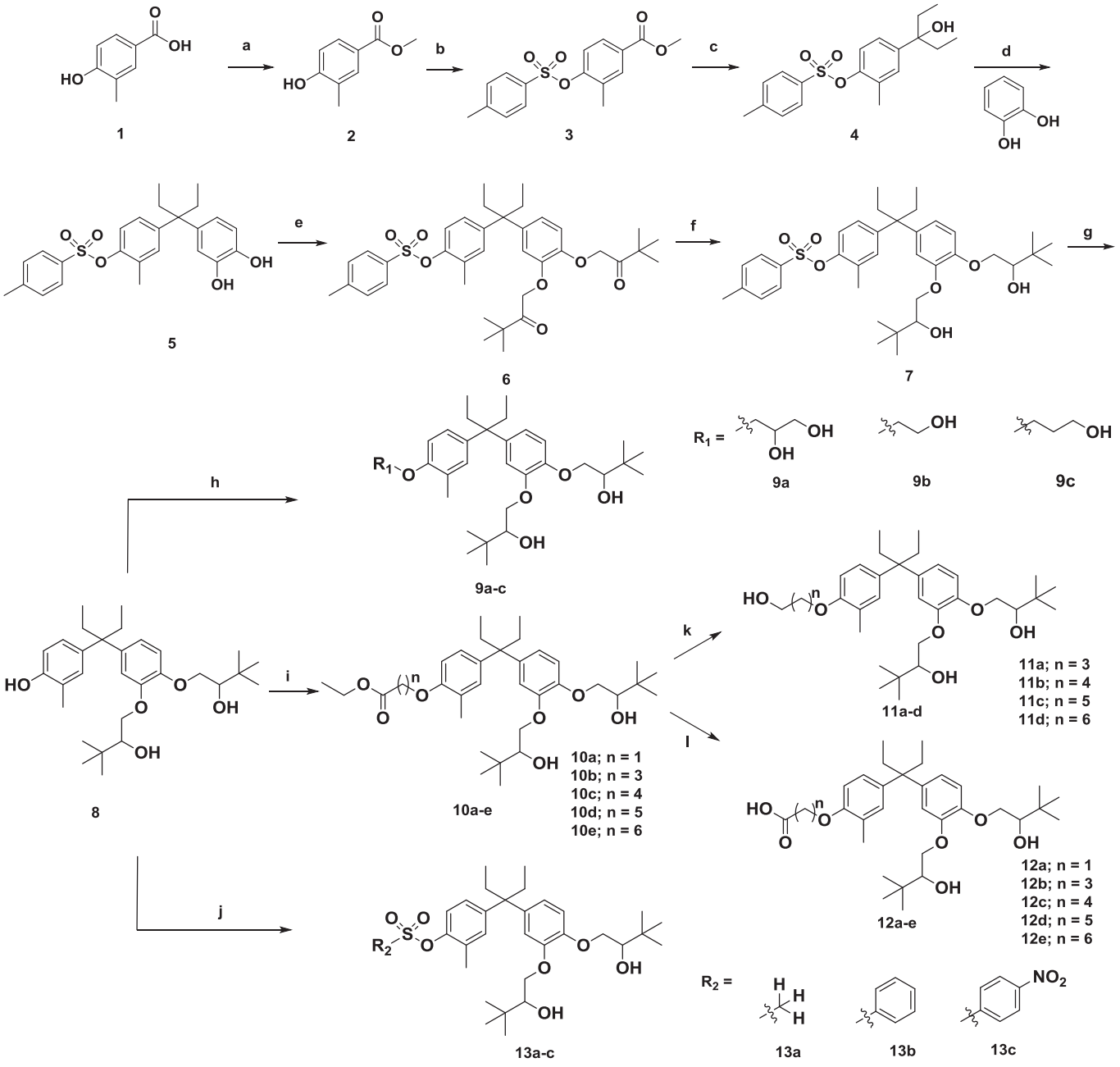
To estimate agonistic properties of the non-secosteroidal ligands bearing double side chain, a transactivation assay in HEK293 cells was performed using pGL4.27-SPP × 3-Luc reporter plasmid as the Vitamin D response element and luciferase reporter, pCMX- Renilla as the internal reference and to normalize the expression of luciferase, pCMX-VDR was to enhance the expression of VDR and pENTER-CMV-hRXRα expressing RXRα as the heterodimer partner of VDR. Compounds **9a**, **9c** and **11a** with excellent binding affinities at 1 μM were selected, LG190178 and calcipotriol were used as positive control and DMSO as the negative control. As shown in Fig. 3, all three compounds showed concentration-dependent transcriptional activity and acted as potent agonists. However, none of the compounds activated the reporter gene transcription better than positive controls.

2.4. In vitro anti-collagen I synthetic activities

CP characterized by the progressive replacement of functional pancreas tissue with ECM, and collagen I is recognized as the main component of ECM. Therefore, inhibiting the synthesis of collagen I is an effective anti-fibrotic strategy. To evaluate the anti-fibrotic effects of target compounds, LTC-14 cells (an immortalized rat PSC) were employed since they have been extensively characterized as a valuable cell-based model for studies of pancreatic fibrosis [25], DMSO was used as negative control, calcipotriol was used as positive control. And we defined anti-collagen I activity of the calcipotriol as 100%, so the relative anti-collagen I activity of testing compounds (%) = (OD $_{\text{Testing Compound}}$ - OD $_{\text{DMSO}}$)/(OD $_{\text{Calcipotriol}}$ - OD $_{\text{DMSO}}$) × 100% (Table 2). Compared with calcipotriol, six of the synthesized analogues (**7**, **9a-9c**, **11a-11b**) at the concentration of 0.5 μM, demonstrated more effective inhibitory potencies against collagen I synthesis, with values at the range of 128–189%. As similar as the SARs of binding affinities, compound **12a-12e** bearing terminal carboxylic acid group were completely inactive. In addition, compounds **9a-9c** and **11a-11b** bearing terminal alcohol group in the A ring displayed significant inhibitory activities. Among them, compound **9c** had the best inhibitory activity. And replacing the A ring with sulfonic esters only show moderately activity.

2.5. Effects on the levels of collagen I and α-SMA expression in LTC-14 cells

The effects of selected compounds **6**, **7**, **9a-9c**, **11a-11b** and **13c**, which displayed optimal potency on anti-collagen I synthetic activities were analyzed using Q-PCR and western blot, respectively. Collagen I and α-SMA are the markers of activated PSCs. As described above, CP is characterized by rapid accumulation of ECM proteins, mainly collagen I. In addition, the appearance of α-SMA-positive myofibroblasts is considered to be a key event in the progression of CP. So collagen I and α-SMA expression were selected to estimate the anti-fibrotic effects of selected compounds. As shown in Fig. 4A and B, compounds **6**, **9b**, **9c** and **11a** significantly reduced the expression of collagen I and α-SMA in TGFβ1-treated LTC-14 cells. Among them, compound **9c** displayed the best activity. Moreover, Q-PCR results (Fig. 4C and D) showed that compound **9c** possessed the best effective inhibitory potency against Col1α1 and α-SMA mRNA expression. Although LG190178 and calcipotriol showed better agonistic acitivity than **9c**, they displayed lower anti-fibrotic activity *in vitro*. Heterodimer partners of VDR, such as steroid receptor coactivator 1 and nuclear receptor corepressor 1, can intact with VDR and affect VDR biological



Scheme 1. Synthesis of target compounds. Reagents and conditions: (a) CH_3OH, conc. H_2SO_4, reflux, 12 h; (b) TsCl, K_2CO_3, acetone, reflux, 6 h; (c) C_2H_5MgBr, Et_2O, 30 °C, 5 h; (d) 1-2-Benzenediol, $BF_3 \cdot Et_2O$, DCM, 25 °C, 3 h; (e) 1-chloropinacolone, acetone, K_2CO_3, KI, reflux, 12 h; (f) $NaBH_4$, CH_3OH, 25 °C, 2 h; (g) NaOH, C_2H_5OH, 70 °C, 12 h; (h) halogen substituted alcohol, NaH, DMF, 70 °C, 12 h; (i) bromo ester, acetone, K_2CO_3, reflux, 12 h; (j) sulfonyl chloride, acetone, K_2CO_3, reflux, 6 h; (k) $LiAlH_4$, THF, 25 °C, 4 h; (l) NaOH, C_2H_5OH, H_2O, 70 °C, 24 h.

activity. In addition, there are other mechanisms in activation of PSC. Maybe this can explain the **9c** that has lower transcriptional activity can induce greater biological activity. And compound **9c** showed significant agonistic acitivity and the best anti-fibrotic activity *in vitro*, which is deserved to further evaluation.

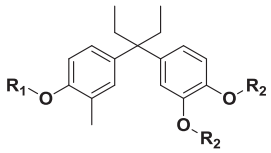
2.6. Preliminary pharmacokinetics study

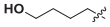
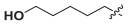
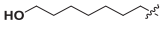
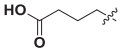
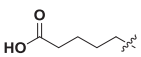
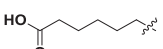
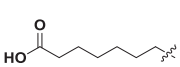
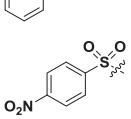
We determined pharmacokinetic properties of compound **9c** after intravenous injection (i.v.) and oral administration (p.o.) in C57BL/6J mice in order to guide future *in vivo* experiments. And the pharmacokinetic parameters were shown in Table 3. Compound **9c** exhibited moderate pharmacokinetics parameters with an oral bioavailability of 31%, a half-life value of 4.1 h and an AUC value of 20.6 mg/L × h after an oral dosing of 20 mg/kg.

2.7. In vivo anti-chronic pancreatitis efficacy of *9c*

The effects of compound **9c** on treating CP was evaluated in a caerulein induced CP model. The mice were administered compounds (0.5 mg/kg) orally five times per week. As shown in Fig. 5, the anti-fibrotic potential of compound **9c** was evaluated by histological examination. H&E staining supported that **9c** inhibited the progression of fibrosis because a much regular pancreatic duct

Table 1
The structures of all target compounds.



Compd	R_1	R_2
6		
7		
9a		
9b		
9c		
11a		
11b		
11c		
11d		
12a		
12b		
12c		
12d		
12e		
13a		
13b		
13c		

network was observed in pancreatitis tissue treated with **9c** compared with the placebo group. Compound **9c** significantly inhibited the accumulation of collagen in the mice pancreas treated with caerulein based on Masson's trichrome staining. Importantly, IHC staining clearly showed **9c** reduced α-SMA levels in the interstitial areas of pancreas tissues. Together, our data clearly demonstrate that compound **9c** could therapy CP *in vivo*.

2.8. Effects on serum calcium and body weight of chronic pancreatitis models

The level of serum calcium was measured to evaluate the safety profile of these compounds. As shown in Fig. 6A, a significant increase in serum calcium (0.37 μmol/mL compared with 0.25 μmol/mL in control mice) was noticed when mice were treated with 0.5 mg/kg calcipotriol. However, there was no significant change on serum calcium in mice when treated with compound **9c** in the same dose. And calcipotriol exhibited higher toxicity as indicated by the body weight loss compared with **9c** treatment, which did not affect mouse body weight (Fig. 6B).

2.9. Molecular docking study

Docking studies were carried out to understand the atomic level interaction of **9c** with VDR. Crystal structure of zVDR in complex with Gemini (PDB ID: 2HCD) was taken from protein databank (PDB). Compound **9c** was manually docked into the crystal structure of VDR using software Discovery Studio 3.0. Fig. 7A shows the superposition of the conformations of compound **9c** and the VDR ligand Gemini. As shown in Fig. 7B, hydroxyl group on the side chain of compound **9c** was able to form hydrogen binding interactions with the His333 and His423 of VDR LBD (VDR Ligand Binding Domain). The hydroxyl group in the A ring of **9c** formed hydrogen bonding interactions with Ser265 and Arg302.

3. Conclusions

In summary, we have described the design, synthesis, and biological evaluation of non-secosteroidal derivatives bearing double side chain as VDR ligands for preventing the progression of CP. The exploration of structure-activity relationship (SAR) led to the identification of highly agonistic and anti-collagen I synthetic activities derivative **9c**. Further investigations showed that compound **9c** demonstrated more efficient inhibitory activity against both collagen deposition and fibrotic gene expression in the western blot and Q-PCR assays, respectively. *In vivo* studies were performed in a cerulean induced CP model, demonstrating the anti-chronic pancreatitis efficacy of compound **9c**. In addition, compound **9c** not only can display better results for the reduction of CP than positive control calcipotriol but also had no significant change on serum calcium and body weight. These results provide novel insights into possible drug discovery for CP using non-secosteroidal VDR modulators.

4. Experiment section

4.1. General materials and methods

All material and reagents were purchased from commercial sources and, unless otherwise stated, were used without further purification. High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. ^{1}H NMR and ^{13}C NMR were recorded employing Bruker AV-300 or AV-500 instruments using $CDCl_3$. Chemical shifts were given as δ (ppm) units relative to the internal standard tetramethylsilane (TMS). Column chromatography separations were progressed on silica gel (200–300 mesh).

Table 2
The VDR binding affinities and anti-collagen I synthetic activities at 1 μM and 0.5 μM, respectively.

Compd	Relative VDR binding ability (%)[a]	Relative Anti-collagen I ability (%)[b]	Compd	Relative VDR binding ability (%)[a]	Relative Anti-collagen I ability (%)[b]
6	35	85	**12b**	14	–
7	43	137	**12c**	–	–
9a	78	147	**12d**	–	–
9b	38	128	**12e**	–	–
9c	76	172	**13a**	–	8
11a	71	189	**13b**	–	23
11b	67	133	**13c**	29	82
11c	52	74	**LG190178**	67	97
11d	5	-[d]	**1,25(OH)$_2$D$_3$**	100	NT[c]
12a	10	–	**Calcipotriol**	NT	100

[a] The values represent the mean of three independent experiments. 1,25(OH)$_2$D$_3$ is assigned as 100%.
[b] The values represent the mean of three independent experiments. Calcipotriol is assigned as 100%.
[c] NT means not tested.
[d] Means no activity.

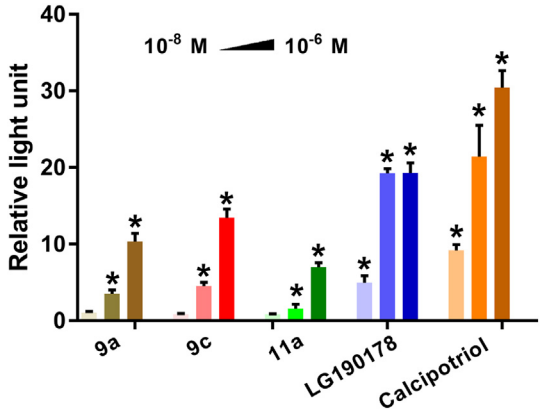


Fig. 3. Transcriptional activities of compounds were determined. HEK293 cells were cotransfected with TK-Spp × 3-LUC reporter plasmid, pCMX-Renilla, pENTER-CMV-hRXRα and pCMX–VDR. Eight hours after transfection, test compounds **(9a**, **9c** and **11a)** and positive control (LG190178 and calcipotriol) were added. Luciferase activity assay was performed 24 h later using the Dual-Luciferase Assay System. Firefly luciferase activity was normalized to the corresponding Renilla luciferase activity. Data are shown as mean ± SD. *$P < 0.05$ vs. DMSO. All the experiments were performed three times.

4.2. Methyl 4-hydroxy-3-methylbenzoate (**2**)

To a solution of compound **1** (100.0 g, 0.64 mol) in methanol (300 mL) was added conc. H_2SO_4 (100 mL). The reaction mixture was refluxed for 12 h and then cooled. The solution was poured into cold water (5 L). The precipitate was filtered off and washed with cold water. The product was obtained as a pink solid and dried (101.2 g, 93% yield). mp 67–78 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.60 (s, 1H), 7.55 (d, J = 8.28 Hz, 1H), 6.62 (d, 1H, J = 8.28 Hz), 3.68 (s, 1H), 2.06 (s, 1H). MS (TOF) m/z: 189.1 [M+Na]$^+$.

4.3. Methyl 3-methyl-4-[(4-methylbenzene-1-sulfonyl)oxy] benzoate (**3**)

To a stirred solution of **2** (50 g, 300.89 mmol) in acetone (200 ml) was added K_2CO_3 (49.90 g, 361.06 mmol) and tosyl chloride (68.83 g, 361.06 mmol) at 0 °C. The mixture was refluxed for 6 h and then cooled. The precipitate was filtered off, the solution was evaporated and water (150 mL) and EtOAc (300 mL) was added. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (4/1, v/v) to give compound **3** as white solid (90.0 g, 93% yield). mp 87–93 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.83 (s, 1H), 7.79 (d, J = 8.68 Hz, 1H), 7.72 (s, 1H), 7.69 (s, 1H). 7.23 (s, 1H), 7.29 (s, 1H), 7.08 (d, J = 8.68 Hz, 1H), 3.87 (s, 3H), 2.44 (s, 3H), 2.07 (s, 3H). MS (TOF) m/z: 343.1 [M+Na]$^+$.

4.4. 4-(3-Hydroxypentan-3-yl)-2-methylphenyl 4-methylbenzene-1-sulfonate (**4**)

To a stirred solution of **3** (20 g, 62.43 mmol) in ether (100 mL) was added EtMgBr (156.08 mmol) dropwise at 0 °C. The mixture was stirred at 30 °C for 5 h, water (50 mL) and EtOAc (200 mL) was carefully added to the mixture at 0 °C and organic phase was separated. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (10/1, v/v) to give compound **4** as colorless oil (17.2 g, 79% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.71 (s, 1H), 7.68 (s, 1H), 7.30 (s, 1H). 7.27 (s, 1H), 7.15 (d, J = 2.12 Hz, 1H), 7.08 (dd, J = 8.58 Hz, 2.12 Hz, 1H), 6.97 (d, J = 8.58 Hz, 1H), 2.43 (s, 3H), 2.02 (s, 3H), 1.77 (qd, J = 7.42 Hz, 3.45 Hz, 4H), 0.71 (t, J = 7.42 Hz, 6H). MS (TOF) m/z: 371.2 [M+Na]$^+$.

4.5. 4-(3-(3,4-dihydroxyphenyl)pentan-3-yl)-2-methylphenyl 4-methylbenzenesulfonate (**5**)

$BF_3 \cdot Et_2O$ (2 mL) was added dropwise to a solution of compound **4** (1.5 g, 4.30 mmol) and catechol (0.56 g, 5.17 mmol) in dichloromethane (10 mL) at 0 °C. The mixture was stirred for 3 h at 25 °C, water (10 mL) and EtOAc (30 mL) was carefully added to the mixture at 0 °C and organic phase was separated. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (15/1, v/v) to give compound **5** as colorless oil (1.62 g, 85% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.72 (s, 1H), 7.69 (s, 1H), 7.31 (s, 1H). 7.28 (s, 1H), 6.91–6.90 (m, 1H), 6.89 (s, 1H), 6.74 (d, J = 7.86 Hz, 1H), 6.61–6.57 (m, 2H), 2.44 (s, 3H), 1.98 (s, 3H), 1.97 (q, J = 7.25 Hz, 4H), 0.57 (t, J = 7.25 Hz, 6H). MS (TOF) m/z: 463.2 [M+Na]$^+$.

4.6. 4-(3-(3,4-bis(3,3-dimethyl-2-oxobutoxy)phenyl)pentan-3-yl)-2-methylphenyl 4-methylbenzenesulfonate (**6**)

1-chloropinacolone (4.35 g, 32.35 mmol), K_2CO_3 (4.47 g, 32.35 mmol) and KI (0.21 g, 1.29 mmol) were added to a solution of the compound **5** (5.7 g, 12.94 mmol) in acetone. The mixture was refluxed for 12 h and then cooled. The precipitate was filtered off and the solution was evaporated. Water (30 mL) and EtOAc

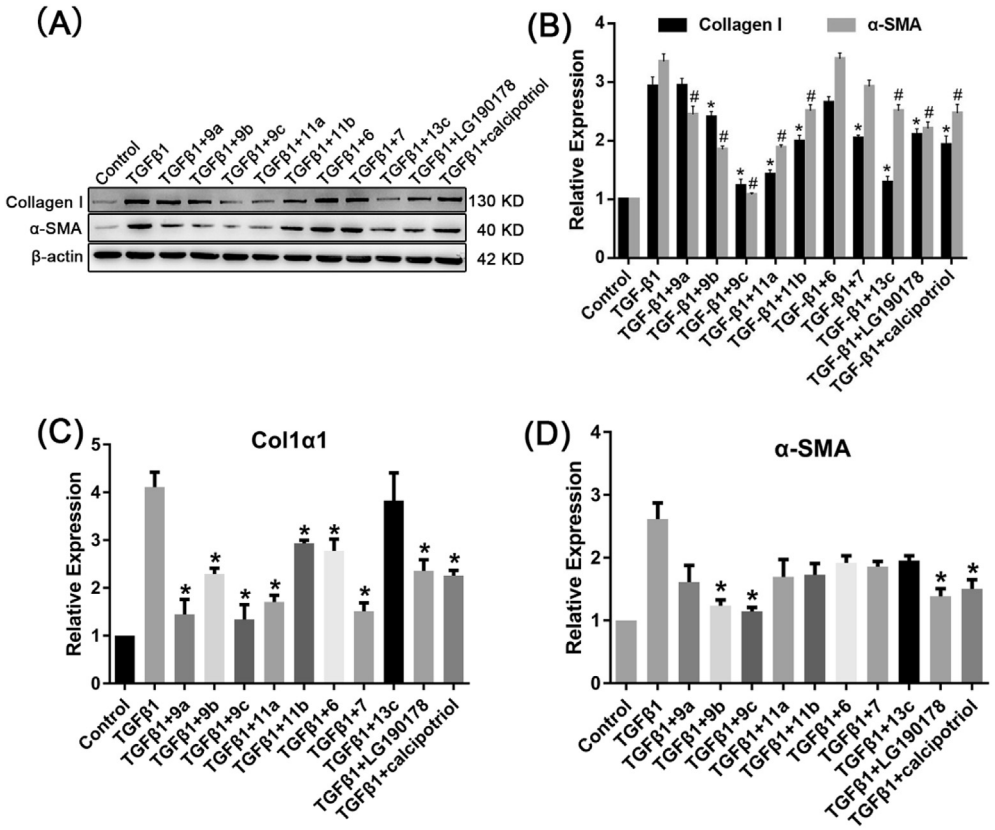


Fig. 4. Effects of representative compounds inhibited the expression levels of collagen I and α-SMA in LTC-14 cells. (A and B) LTC-14 cells were cultured with compounds (500 nM) for 24 h. Expression of collagen I and α-SMA on LTC-14 cells was examined by western blot. Representative gel electrophoresis bands are shown (A), and protein expression levels were quantified by densitometry and normalized to the expression of β-actin (B). Densitometry data are shown as mean ± SD. Collagen I: $^{*}P < 0.05$ vs. TGFβ1. α-SMA: #$P < 0.05$ vs. TGFβ1. (C and D) The expression levels of Col1α1 and α-SMA were measured by real-time PCR. Data are shown as mean ± SD. $^{*}P < 0.05$ vs. TGFβ1.

Table 3
Pharmacokinetics profiles for 9c.

route	$AUC_{0-\infty}$ (mg/L × h)	$T_{1/2}$ (h)	MRT (h)	CL (L/h)	F (%)
i.v.	32.56 ± 1.88	2.66 ± 0.15	4.65 ± 0.16	0.31 ± 0.02	–
p.o.	20.59 ± 0.89	4.12 ± 0.31	6.59 ± 0.22	1.21 ± 0.05	31.62

(100 mL) was added and the organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (25/1, v/v) to give compound **6** as white solid (7.01 g, 85% yield). mp 77–79 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.70 (s, 1H), 7.69 (s, 1H), 7.29 (s, 1H), 7.27 (s, 1H), 6.88 (s, 1H), 6.86 (s, 2H), 6.71–6.67 (m, 2H), 6.51 (s, 1H), 4.92 (s, 2H), 4.87 (s, 2H), 2.42 (s, 3H), 1.98 (s, 3H), 1.94 (q, J = 7.33 Hz, 4H), 1.20 (s, 9H), 1.11 (s, 9H), 0.54 (t, J = 7.33 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 147.65, 147.16, 146.52, 146.07, 145.12, 142.42, 133.26, 131.02, 130.30, 129.63, 128.28, 126.54, 126.41, 121.77, 121.26, 117.07, 114.89, 70.83, 70.80, 70.58, 70.56, 70.41, 49.20, 42.82, 29.29, 26.25, 26.16, 26.06, 21.59, 16.41, 8.28. ESI-HRMS calcd for $C_{37}H_{48}O_7S$ $[M+Na]^+$ 659.3121, found 659.3030.

*4.7. 4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl)pentan-3-yl)-2-methylphenyl 4-methylbenzenesulfonate (**7**)*

To a solution of **6** (1.3 g, 2.04 mmol) in methanol (10 mL), $NaBH_4$ (0.77 g, 20.4 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at 25 °C for 2 h and then added H_2O (10 mL). EtOAc (50 mL) was added and the organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (20/1, v/v) to give compound **7** as white solid (1.07 g, 81% yield). mp 74–77 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.72 (s, 1H), 7.71 (s, 1H), 7.30 (s, 1H), 7.28 (s, 1H), 6.92–6.91 (m, 3H), 6.82 (dd, J = 8.42 Hz, 3.75 Hz, 1H), 6.75 (dt, J = 8.42 Hz, 1.85 Hz, 1H), 6.70 (dd, J = 7.05 Hz, 2.0 Hz, 1H), 4.18–4.12 (m, 1H), 4.10–4.01 (m, 1H), 3.89–3.81 (m, 1H), 3.82–3.71 (m, 1H), 3.68–3.63 (m, 1H), 3.62–3.54 (m, 1H), 2.44 (s, 3H), 2.00 (s, 3H), 2.00 (q, J = 7.20 Hz, 4H), 0.98 (d, J = 1.25 Hz, 9H), 0.94 (d, J = 5.2 Hz, 9H), 0.59 (t, J = 7.20 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 147.29, 146.14, 145.12, 142.41, 142.35, 133.40, 131.04, 130.39, 129.65, 128.34, 126.55, 122.28, 122.18, 121.34, 117.39, 117.26, 114.52, 114.39, 77.39, 77.22, 76.95, 76.79, 49.25, 33.49, 33.45, 33.37, 33.34, 29.36, 29.30, 26.14, 26.10, 26.08, 26.02, 21.66, 16.51, 8.35. ESI-HRMS calcd for $C_{37}H_{48}O_7S$ $[M+Na]^+$ 663.3434, found 663.3336.

*4.8. 1,1'-((4-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**8**)*

To a stirred solution of **7** (2.2 g, 3.43 mmol) in a mixture of EtOH/H_2O 10:1 (40 mL) was added NaOH (1.37 g, 34.33 mmol) at 70 °C. After 24 h, the solution was evaporated, water (20 mL) and EtOAc (80 mL) was added. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (10/1, v/v) to give compound **8** as white solid (1.43 g, 86% yield). mp 63–68 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.87 (s, 1H), 6.83 (dd, J = 8.3 Hz, 2.15 Hz, 1H), 6.81–6.78 (m, 2H),

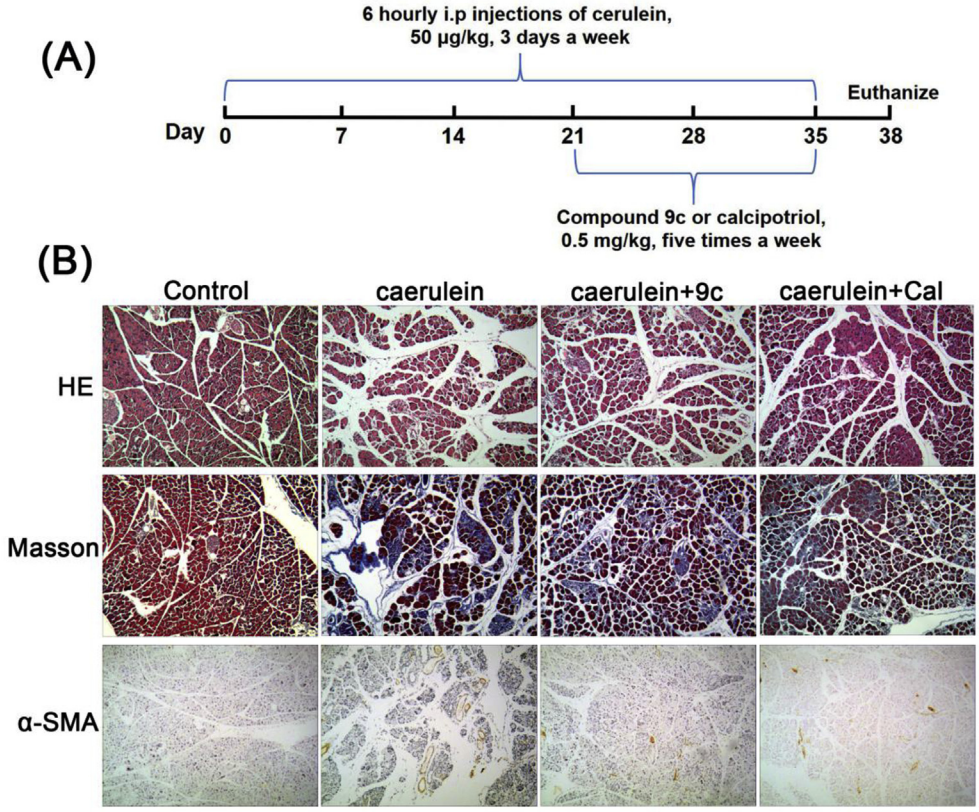


Fig. 5. (A) Treatment schedule for *in vivo* experiments. Mice were administered caerulein or PBS (control) via six hourly intraperitoneal (i.p.) injections per day, 3 days/week, for 5 weeks. Calcipotriol or compound 9c was given five times a week via oral gavage. Mice were sacrificed 3 days after the final caerulein injection. (B) Effects of **9c** inhibited the development of caerulein-induced pancreas lesions, collagen deposition and α-SMA overexpression. Pancreas lesions and collagen deposition were determined by H&E staining (× 100) and Masson's trichrome stain (× 100), respectively. α-SMA expression were determined by immunohistochemistry (× 100).

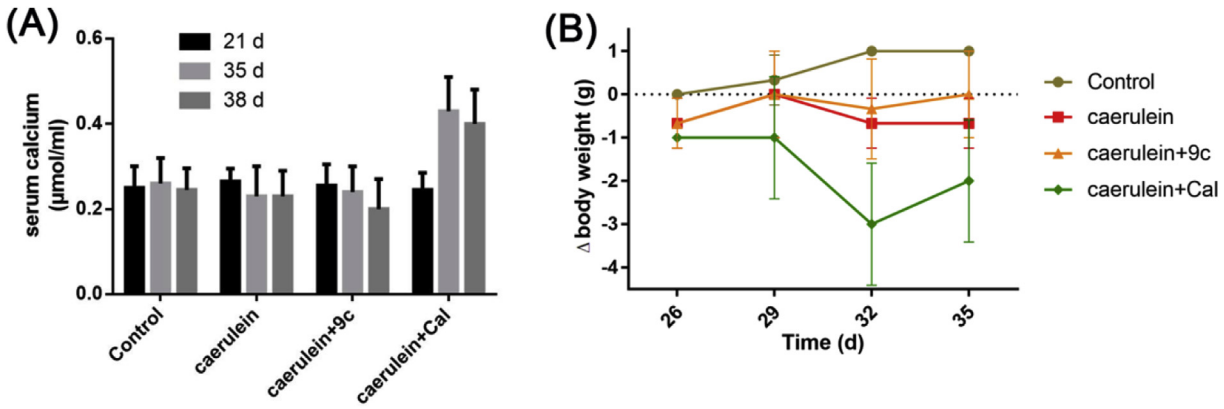


Fig. 6. (A) *In vivo* calcemic effects of compounds **9c** and calcipotriol was determined by calcium assay kit. (B) Changes in body weight of mice treated with vehicle, **9c** and calcipotriol were recorded on the indicated days.

6.75 (dd, $J = 7.0$ Hz, 1.65 Hz, 1H), 6.64 (d, $J = 8.3$ Hz, 1H), 4.17–4.12 (m, 1H), 4.10–4.03 (m, 1H), 3.89–3.81 (m, 1H), 3.81–3.72 (m, 1H), 3.68–3.64 (m, 1H), 3.61–3.53 (m, 1H), 2.18 (s, 3H), 2.00 (q, $J = 7.25$ Hz, 4H), 0.98 (s, 9H), 0.94 (d, $J = 4.80$ Hz, 9H), 0.59 (t, $J = 7.25$ Hz, 6H). MS (TOF) *m/z*: 509.3 $[M+Na]^+$.

4.9. General procedure 1 - synthesis of compounds ***9a-9c***

To a solution of compound **8** (0.2 g, 0.41 mmol) in DMF (50 mL), NaH (11.83 mg, 0.49 mmol) was added portionwise at 0 °C. After stirring for 0.5 h, appropriate halogen substituted alcohol (0.8 mmol) was added. The reaction mixture was stirred at 70 °C for 12 h and then H_2O (100 mL) was added dropwise followed by EtOAc (80 mL). The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

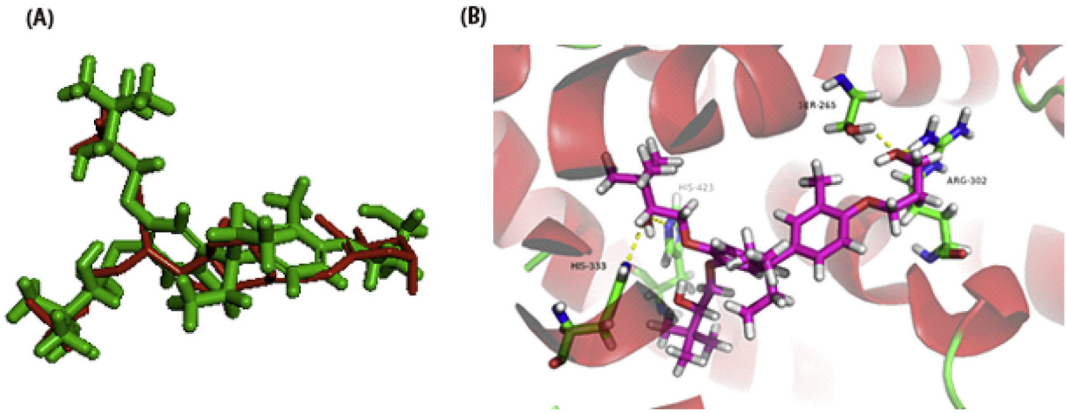


Fig. 7. (A) Superposition of compounds **9c** and Gemini. Compound **9c** is depicted in green and Gemini is depicted in red. (B) The binding mode between **9c** and VDR predicted by docking. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

*4.9.1. 3-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)propane-1,2-diol (**9a**)*

Petroleum ether/ethyl acetate (5/1). Colorless oil. 53% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.93 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 6.81–6.76 (m, 2H), 6.73 (dd, J = 8.0 Hz, J = 1.6 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 4.16–4.11 (m, 1H), 4.09–4.02 (m, 2H), 4.0 (d, J = 5.4 Hz, 2H), 3.86–3.84 (m, 1H), 3.83–3.81 (m, 1H), 3.74–3.72 (m, 1H), 3.67–3.65 (m, 1H), 3.57–3.51 (m, 1H), 2.14 (s, 3H), 2.04–1.98 (m, 4H), 0.97 (s, 9H), 0.94 (d, J = 6.4 Hz, 9H), 0.61 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.25, 147.95, 147.13, 143.08, 140.78, 130.56, 126.15, 125.56, 122.00, 116.97, 114.02, 110.24, 77.21, 77.18, 76.96, 76.68, 72.77, 72.42, 71.80, 71.55, 70.57, 69.13, 63.89, 48.84, 33.38, 29.42, 25.98, 16.44, 8.40. ESI-HRMS calcd for $C_{33}H_{52}O_7$ [M+Na] $^+$ 583.3713, found 583.3627.

*4.9.2. 1,1'-((4-(3-(4-(2-hydroxyethoxy)-3-methylphenyl)pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**9b**)*

Petroleum ether/ethyl acetate (5/1). Colorless oil. 43% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.94 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 6.89 (d, J = 2.2 Hz, 1H), 6.83–6.78 (m, 2H), 6.76 (dd, J = 7.0 Hz, 1.9 Hz, 1H), 6.70 (d, J = 8.4 Hz, 1H), 4.17–4.12 (m, 1H), 4.12–4.03 (m, 1H), 4.06 (t, J = 4.6 Hz, 2H), 3.95 (t, J = 4.5 Hz, 2H), 3.89–3.82 (m, 1H), 3.80–3.72 (m, 1H), 3.68–3.63 (m, 1H), 3.60–3.53 (m, 1H), 2.18 (s, 3H), 2.05–1.99 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 5.0 Hz, 9H), 0.61 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.43, 148.12, 147.25, 143.30, 140.69, 130.61, 126.16, 125.71, 122.29, 117.50, 114.47, 110.38, 77.32, 69.26, 61.69, 48.87, 33.45, 33.32, 29.47, 29.40, 26.14, 26.10, 26.07, 26.01, 16.47, 8.44. ESI-HRMS calcd for $C_{32}H_{50}O_6$ [M+Na] $^+$ 553.3607, found 553.3503.

*4.9.3. 1,1'-((4-(3-(4-(3-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**9c**)*

Petroleum ether/ethyl acetate (5/1). Colorless oil. 49% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.94 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 6.88 (d, J = 2.2 Hz, 1H), 6.82–6.78 (m, 2H), 6.75 (dd, J = 7.1 Hz, 1.9 Hz, 1H), 6.71 (d, J = 8.4 Hz, 1H), 4.17–4.11 (m, 1H), 4.09 (t, J = 5.8 Hz, 2H), 4.09–4.03 (m, 1H), 3.87 (t, J = 6.0 Hz, 2H), 3.87–3.84 (m, 1H), 3.82–3.72 (m, 1H), 3.68–3.63 (m, 1H), 3.60–3.52 (m, 1H), 2.15 (s, 3H), 2.07–2.05 (m, 2H), 2.04–1.98 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 5.1 Hz, 9H), 0.61 (t, J = 7.4 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.63, 147.98, 147.26, 143.28, 140.34, 130.49, 126.12, 125.45, 122.20, 117.39, 114.32, 109.93, 77.30, 77.21, 76.95, 76.70, 72.68, 71.75, 65.99, 60.95, 48.86, 33.36, 32.15, 29.48, 26.14, 26.10, 26.07, 26.01, 16.54, 8.44. ESI-HRMS calcd for $C_{33}H_{52}O_6$ [M+H] $^+$ 545.3764, found 545.3847.

*4.10. General procedure 2 - synthesis of compounds **10a**-**10e***

Appropriate halogen substituted ester (1.38 mmol) and K_2CO_3 (0.32 g, 2.30 mmol) were added to a solution of the compound **8** (0.56 g, 1.15 mmol) in acetone. The mixture was refluxed for 12 h and then cooled. The precipitate was filtered off and the solution was evaporated. Water (30 mL) and EtOAc (100 mL) was added and the organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

*4.10.1. Methyl 2-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy) phenyl)pentan-3-yl)-2-methylphenoxy)acetate (**10a**)*

Petroleum ether/ethyl acetate (10/1). Colorless oil. 77% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.91–6.90 (m, 2H), 6.80 (q, J = 8.4 Hz, 2H), 6.76–6.75 (m, 1H), 6.58 (d, J = 9.2 Hz, 2H), 4.59 (s, 2H), 4.25 (q, J = 7.2 Hz, 2H), 4.16 (d, J = 9.4 Hz, 1H), 4.09 (d, J = 9.4 Hz, 1H), 3.80 (t, J = 9.3 Hz, 1H), 3.73 (t, J = 9.3 Hz, 1H), 3.67 (d, J = 9.4 Hz, 1H), 3.59 (d, J = 9.4 Hz, 1H), 2.22 (s, 3H), 2.01 (q, J = 7.3 Hz, 4H), 1.28 (t, J = 7.2, 3H), 0.98 (s, 9H), 0.94 (s, 9H), 0.59 (t, J = 7.3 Hz, 6H). MS (TOF) *m/z*: 572.37 [M+Na] $^+$.

*4.10.2. Methyl 4-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy) phenyl)pentan-3-yl)-2-methylphenoxy)butanoate (**10b**)*

Petroleum ether/ethyl acetate (10/1). Colorless oil. 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, J = 8.5 Hz, 2.3 Hz, 1H), 6.87 (s, 1H), 6.82–6.80 (m, 2H), 6.76 (dd, J = 6.9 Hz, 1.7 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.14 (m, 1H), 4.14 (q, J = 7.1 Hz, 2H), 4.11–4.03 (m, 1H), 3.97 (t, J = 6.0 Hz, 2H), 3.87 (t, J = 10.4 Hz, 1H), 3.83–3.72 (m, 1H), 3.65 (td, J = 10.4 Hz, 2.1 Hz, 1H), 3.60–3.53 (m, 1H), 2.52 (t, J = 7.4 Hz, 2H), 2.15 (s, 3H), 2.14–2.08 (m, 2H), 2.01 (q, J = 7.3 Hz, 4H), 1.25 (t, J = 7.1 Hz, 2H), 0.98 (s, 9H), 0.94 (d, J = 5.1 Hz, 9H), 0.61 (t, J = 7.3 Hz, 6H). MS (TOF) *m/z*: 623.4 [M+Na] $^+$.

*4.10.3. Methyl 5-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy) phenyl)pentan-3-yl)-2-methylphenoxy)pentanoate (**10c**)*

Petroleum ether/ethyl acetate (10/1). Colorless oil. 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (d, J = 8.4 Hz, 1H), 6.87 (s, 1H), 6.83–6.80 (m, 2H), 6.76 (d, J = 7.0 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 4.17–4.13 (m, 1H), 4.13 (q, J = 7.2 Hz, 2H), 4.10–4.03 (m, 1H), 3.94 (t, J = 5.4 Hz, 2H), 3.87 (t, J = 10.3 Hz, 1H), 3.82–3.72 (m, 1H), 3.65 (t, J = 10.3 Hz, 1H), 3.60–3.52 (m, 1H), 2.38 (t, J = 6.8 Hz, 2H), 2.15 (s, 3H), 2.03–1.97 (m, 4H), 1.85–1.82 (m, 4H), 1.25 (t, J = 7.2 Hz, 3H), 0.98 (s, 9H), 0.94 (d, J = 5.1 Hz, 9H), 0.61 (t, J = 7.3 Hz, 6H). MS (TOF) *m/z*: 637.5 [M+Na] $^+$.

4.10.4. Methyl 6-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy) phenyl)pentan-3-yl)-2-methylphenoxy)-2-methylhexanoate (**10d**)

Petroleum ether/ethyl acetate (15/1). Colorless oil. 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.91 (d, J = 8.5 Hz, 1H), 6.87 (s, 1H), 6.83–6.78 (m, 2H), 6.76 (d, J = 6.8 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.14 (m, 1H), 4.13 (q, J = 7.1 Hz, 2H), 4.10–4.03 (m, 1H), 3.92 (t, J = 6.2 Hz, 2H), 3.89–3.80 (m, 1H), 3.78–3.72 (m, 1H), 3.68–3.63 (m, 1H), 3.60–3.53 (m, 1H), 2.33 (t, J = 7.4 Hz, 2H), 2.15 (s, 3H), 2.05–1.97 (m, 4H), 1.83–1.77 (m, 2H), 1.74–1.68 (m, 2H), 1.55–1.49 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H), 0.98 (s, 9H), 0.94 (d, J = 4.6 Hz, 9H), 0.61 (t, J = 7.2 Hz, 6H). MS (TOF) m/z: 651.4 [M+Na] $^+$.

4.10.5. Methyl 7-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy) phenyl)pentan-3-yl)-2-methylphenoxy)heptanoate (**10e**)

Petroleum ether/ethyl acetate (15/1). Colorless oil. 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.91 (dd, J = 8.5 Hz, 2.2 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 6.82–6.78 (m, 2H), 6.76 (dd, J = 7.1 Hz, 1.7 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.14 (m, 1H), 4.12 (q, J = 7.2 Hz, 2H), 4.10–4.03 (m, 1H), 3.92 (t, J = 6.3 Hz, 2H), 3.89–3.84 (m, 1H), 3.84–3.72 (m, 1H), 3.68–3.63 (m, 1H), 3.60–3.52 (m, 1H), 2.30 (t, J = 7.4 Hz, 2H), 2.15 (s, 3H), 2.04–1.98 (m, 4H), 1.81–1.75 (m, 2H), 1.69–1.63 (m, 2H), 1.53–1.47 (m, 2H), 1.43–1.37 (m, 2H), 1.25 (t, J = 7.2 Hz, 3H), 0.98 (s, 9H), 0.94 (d, J = 5.7 Hz, 9H), 0.61 (t, J = 7.2 Hz, 6H). MS (TOF) m/z: 665.5 [M+Na] $^+$.

4.11. General procedure 3 - synthesis of compounds **11a-11d**

4.11.1. 1,1'-((4-(3-(4-(4-hydroxybutoxy)-3-methylphenyl)pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**11a**)

To a solution of appropriate ester **10b** (198.01 mg, 0.33 mmol) in THF (20 mL), $LiAlH_4$ (31.58 mg, 0.83 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at 25 °C for 4 h and then added H_2O (10 mL). EtOAc (50 mL) was added and the organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with dichloromethane/methanol (50/1). Colorless oil. 87% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, J = 8.4 Hz, 1.9 Hz, 1H), 6.87 (d, J = 1.9 Hz, 1H), 6.82–6.78 (m, 2H), 6.75 (d, J = 7.0 Hz, 1H), 6.68 (d, J = 8.4 Hz, 1H), 4.17–4.12 (m, 1H), 4.11–4.03 (m, 1H), 3.97 (t, J = 6.0 Hz, 2H), 3.89–3.82 (m, 1H), 3.80–3.74 (m, 1H), 3.72 (t, J = 6.3 Hz, 2H), 3.68–3.64 (m, 1H), 3.60–3.52 (m, 1H), 2.16 (s, 3H), 2.06–1.97 (m, 4H), 1.91–1.85 (m, 2H), 1.79–1.74 (m, 2H), 0.98 (s, 9H), 0.94 (d, J = 5.0 Hz, 9H), 0.61 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.78, 148.06, 147.18, 143.38, 140.03, 130.44, 126.03, 125.60, 122.27, 117.44, 114.40, 110.00, 77.26, 76.95, 76.68, 73.02, 71.99, 67.66, 62.60, 48.84, 33.44, 29.68, 26.13, 26.10, 26.06, 26.01, 25.99, 16.49, 8.44. ESI-HRMS calcd for $C_{34}H_{54}O_6$ [M+Na] $^+$ 581.3920, found 581.3826.

4.11.2. 1,1'-((4-(3-(4-((5-hydroxypentyl)oxy)-3-methylphenyl) pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**11b**)

Dichloromethane/methanol (50/1). Colorless oil. 66% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, J = 8.4 Hz, 2.1 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 6.83–6.78 (m, 2H), 6.76 (d, J = 1.8 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 4.16 (dd, J = 9.2 Hz, 2.2 Hz, 1H), 4.10 (dd, J = 9.5 Hz, 2.2 Hz, 1H), 3.94 (t, J = 6.3 Hz, 2H), 3.80 (t, J = 9.2 Hz, 1H), 3.73 (t, J = 9.5 Hz, 1H), 3.67 (t, J = 6.4 Hz, 2H), 3.67–3.65 (m, 1H), 3.59 (dd, J = 9.4 Hz, 2.2 Hz, 1H), 2.15 (s, 3H), 2.04–2.01 (m, 4H), 1.85–1.79 (m, 2H), 1.68–1.62 (m, 2H), 1.59–1.53 (m, 2H), 0.98 (s, 9H), 0.94 (s, 9H), 0.61 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.93, 148.09, 147.20, 143.47, 139.87, 130.40, 126.00, 125.65, 122.33, 117.56, 114.47, 109.96, 77.31, 73.10, 72.04, 67.67, 62.86, 48.84, 33.45, 33.33, 32.47, 29.23, 26.15, 26.08, 22.48, 16.49, 8.46. ESI-HRMS calcd for $C_{35}H_{56}O_6$ [M+Na] $^+$ 595.4077, found 595.3981.

4.11.3. 1,1'-((4-(3-(4-((6-hydroxyhexyl)oxy)-3-methylphenyl) pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**11c**)

Dichloromethane/methanol (70/1). Colorless oil. 86% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.90 (dd, J = 8.5 Hz, 2.1 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 6.83–6.78 (m, 2H), 6.75 (dd, J = 7.0 Hz, 1.6 Hz, 1H), 6.67 (d, J = 8.4 Hz, 1H), 4.17–4.12 (m, 1H), 4.11–4.03 (m, 1H), 3.93 (t, J = 6.3 Hz, 2H), 3.89–3.80 (m, 1H), 3.80–3.71 (m, 1H), 3.69–3.63 (m, 1H), 3.65 (t, J = 6.5 Hz, 2H), 3.60–3.52 (m, 1H), 2.15 (s, 3H), 2.04–1.98 (m, 4H), 1.82–1.77 (m, 2H), 1.63–1.58 (m, 2H), 1.54–1.48 (m, 2H), 1.47–1.42 (m, 2H), 0.98 (s, 9H), 0.94 (d, J = 4.8 Hz, 9H), 0.61 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.97, 148.09, 147.20, 143.48, 139.82, 130.39, 126.00, 125.65, 122.32, 117.57, 114.47, 109.96, 77.31, 73.09, 72.04, 67.72, 62.91, 48.84, 33.45, 33.32, 32.72, 29.50, 29.42, 26.15, 26.11, 26.08, 26.03, 25.55, 16.47, 8.46. ESI-HRMS calcd for $C_{36}H_{58}O_6$ [M+Na] $^+$ 609.4233, found 609.4138.

4.11.4. 1,1'-((4-(3-(4-((7-hydroxyheptyl)oxy)-3-methylphenyl) pentan-3-yl)-1,2-phenylene)bis(oxy))bis(3,3-dimethylbutan-2-ol) (**11d**)

Dichloromethane/methanol (60/1). Colorless oil. 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.91 (dd, J = 8.5 Hz, 2.0 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 6.82–6.78 (m, 2H), 6.75 (d, J = 7.0 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.11 (m, 1H), 4.11–4.03 (m, 1H), 3.92 (t, J = 6.4 Hz, 2H), 3.89–3.80 (m, 1H), 3.80–3.71 (m, 1H), 3.67–3.62 (m, 1H), 3.64 (t, J = 6.6 Hz, 2H), 3.60–3.52 (m, 1H), 2.15 (s, 3H), 2.03–1.98 (m, 4H), 1.81–1.75 (m, 2H), 1.59–1.56 (m, 2H), 1.50–1.47 (m, 2H), 1.40–1.38 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 4.9 Hz, 9H), 0.61 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 154.99, 148.07, 147.18, 143.47, 139.78, 130.37, 125.98, 125.65, 122.29, 117.52, 114.43, 109.94, 77.21, 76.69, 73.06, 72.02, 67.80, 62.96, 48.83, 33.44, 32.70, 29.50, 29.38, 29.19, 26.17, 26.14, 26.11, 26.07, 26.01, 25.70, 16.47, 8.46. ESI-HRMS calcd for $C_{37}H_{60}O_6$ [M+Na] $^+$ 623.4390, found 623.4287.

4.12. General procedure 4 - synthesis of compounds **12a-12e**

4.12.1. 2-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)acetic acid (**12a**)

To a stirred solution of appropriate ester **10a** (2.2 g, 3.43 mmol) in a mixture of EtOH/H_2O 10:1 (40 mL) was added NaOH (1.37 g, 34.33 mmol) at 70 °C. After 24 h, the solution was evaporated, water (20 mL) and EtOAc (80 mL) was added. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography with dichloromethane/methanol (50/1). Colorless oil. 86% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.93–6.91 (m, 2H), 6.81 (dd, J = 8.4 Hz, 3.2 Hz, 1H), 6.78 (dd, J = 8.4 Hz, 1.8 Hz, 1H), 6.73 (dd, J = 7.3 Hz, 1.8 Hz, 1H), 6.61 (d, J = 8.3 Hz, 1H), 4.63 (s, 2H), 4.17–4.12 (m, 1H), 4.10–4.02 (m, 1H), 3.89–3.81 (m, 1H), 3.81–3.72 (m, 1H), 3.69–3.65 (m, 1H), 3.61–3.53 (m, 1H), 2.22 (s, 3H), 2.04–1.98 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 5.7 Hz, 9H), 0.60 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 172.58, 153.59, 148.14, 147.27, 143.14, 141.75, 130.90, 126.19, 126.13, 122.28, 117.46, 114.47, 110.56, 77.43, 77.33, 72.99, 71.91, 65.36, 48.93, 33.36, 29.44, 26.14, 26.11, 26.07, 26.02, 16.43, 8.43. ESI-HRMS calcd for $C_{32}H_{48}O_7$ [M+Na] $^+$ 567.3400, found 567.3299.

4.12.2. 4-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)butanoic acid (**12b**)

Dichloromethane/methanol (50/1). Colorless oil. 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, J = 8.5 Hz, 1.8 Hz, 1H), 6.88 (s, 1H), 6.82–6.78 (m, 2H), 6.75 (dd, J = 6.8 Hz, 1.8 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.12 (m, 1H), 4.09–4.03 (m, 1H), 3.98 (t,

J = 6.0 Hz, 2H), 3.89–3.83 (m, 1H), 3.83–3.72 (m, 1H), 3.68–3.65 (m, 1H), 3.61–3.53 (m, 1H), 2.58 (t, J = 7.3 Hz, 2H), 2.15 (s, 3H), 2.13–2.11 (m, 2H), 2.04–1.98 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 5.5 Hz, 9H), 0.60 (t, J = 7.2 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 178.32, 154.60, 148.08, 147.19, 143.37, 140.17, 130.48, 126.03, 125.66, 122.29, 117.46, 114.45, 109.88, 77.33, 66.43, 48.85, 33.33, 30.61, 29.42, 26.14, 26.11, 26.07, 26.01, 24.63, 16.42, 8.45. ESI-HRMS calcd for $C_{34}H_{52}O_7$ $[M+Na]^+$ 595.3713, found 595.3611.

4.12.3. 5-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)pentanoic acid (**12c**)

Dichloromethane/methanol (50/1). Colorless oil. 77% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (d, J = 8.5 Hz, 1H), 6.87 (s, 1H), 6.83–6.78 (m, 2H), 6.75 (d, J = 7.0 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.12 (m, 1H), 4.11–4.03 (m, 1H), 3.95 (t, J = 5.3 Hz, 2H), 3.89–3.83 (m, 1H), 3.83–3.72 (m, 1H), 3.68–3.64 (m, 1H), 3.60–3.53 (m, 1H), 2.44 (t, J = 6.6 Hz, 2H), 2.15 (s, 3H), 2.03–1.98 (m, 4H), 1.87–1.83 (m, 4H), 0.98 (s, 9H), 0.94 (d, J = 5.3 Hz, 9H), 0.60 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 178.26, 154.79, 147.20, 143.39, 140.00, 130.44, 126.02, 125.67, 122.21, 117.49, 114.46, 114.33, 109.88, 77.35, 67.19, 48.85, 33.50, 33.34, 29.50, 29.42, 28.77, 26.15, 26.11, 26.07, 26.02, 21.58, 16.49, 8.46. ESI-HRMS calcd for $C_{35}H_{54}O_7$ $[M+Na]^+$ 609.3870, found 609.3796.

4.12.4. 6-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)hexanoic acid (**12d**)

Dichloromethane/methanol (40/1). Colorless oil. 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.92 (dd, J = 8.5 Hz, 2.3 Hz, 1H), 6.87 (d, J = 1.7 Hz, 1H), 6.82–6.78 (m, 2H), 6.75 (dd, J = 6.5 Hz, 1.7 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.12 (m, 1H), 4.11–4.03 (m, 1H), 3.93 (t, J = 6.3 Hz, 2H), 3.89–3.79 (m, 1H), 3.79–3.72 (m, 1H), 3.68–3.64 (m, 1H), 3.60–3.53 (m, 1H), 2.38 (t, J = 7.5 Hz, 2H), 2.15 (s, 3H), 2.04–1.98 (m, 4H), 1.82–1.78 (m, 2H), 1.75–1.69 (m, 2H), 1.57–1.53 (m, 2H), 0.98 (s, 9H), 0.94 (d, J = 5.0 Hz, 9H), 0.60 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 178.32, 154.89, 148.08, 147.19, 143.46, 139.90, 130.41, 126.01, 125.66, 122.30, 117.51, 114.45, 109.95, 77.34, 73.02, 71.99, 67.49, 48.85, 33.77, 33.34, 29.10, 26.14, 26.07, 26.02, 25.76, 24.47, 16.46, 8.46. ESI-HRMS calcd for $C_{36}H_{56}O_7$ $[M+Na]^+$ 623.4026, found 623.3934.

4.12.5. 7-(4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenoxy)heptanoic acid (**12e**)

Dichloromethane/methanol (50/1). Colorless oil. 89% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 6.91 (dd, J = 8.5 Hz, 2.2 Hz, 1H), 6.87 (d, J = 1.7 Hz, 1H), 6.83–6.78 (m, 2H), 6.75 (dd, J = 6.8 Hz, 1.7 Hz, 1H), 6.67 (d, J = 8.5 Hz, 1H), 4.17–4.12 (m, 1H), 4.11–4.03 (m, 1H), 3.92 (t, J = 6.4 Hz, 2H), 3.87–3.83 (m, 1H), 3.81–3.72 (m, 1H), 3.68–3.64 (m, 1H), 3.60–3.53 (m, 1H), 2.38 (t, J = 7.5 Hz, 2H), 2.15 (s, 3H), 2.04–1.99 (m, 4H), 1.81–1.76 (m, 2H), 1.70–1.64 (m, 2H), 1.53–1.47 (m, 2H), 1.45–1.39 (m, 2H), 0.98 (s, 9H), 0.94 (d, J = 5.1 Hz, 9H), 0.60 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 178.44, 154.94, 147.97, 147.19, 143.48, 139.83, 130.39, 126.00, 125.66, 122.21, 117.50, 114.46, 109.96, 77.34, 67.67, 48.84, 33.75, 33.34, 29.69, 29.50, 29.43, 29.24, 28.82, 26.15, 26.11, 26.08, 26.02, 25.86, 24.63, 16.47, 8.47. ESI-HRMS calcd for $C_{36}H_{56}O_7$ $[M+Na]^+$ 637.4183, found 637.4089.

4.13. General procedure 5 - synthesis of compounds **13a**-**13c**

To a stirred solution of **8** (0.2 g, 0.41 mmol) in acetone (30 ml) was added K_2CO_3 (113.59 mg, 0.82 mmol) and appropriate sulfochlorides (0.62 mmol) at 0 °C. The mixture was refluxed for 6 h and then cooled. The precipitate was filtered off, the solution was evaporated and water (10 mL) and EtOAc (60 mL) was added. The organic phase were washed with brine and dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

4.13.1. 4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenyl methanesulfonate (**13a**)

Petroleum ether/ethyl acetate (50/1). Colorless oil. 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.16 (d, J = 8.52 Hz, 1H), 7.04 (d, J = 8.75 Hz, 1H), 7.01 (d, J = 8.75 Hz, 1H), 6.85–6.75 (m, 2H), 6.65–6.58 (m, 1H), 4.74–4.71 (m, 1H), 4.64–4.61 (m, 1H), 4.26–4.14 (m, 2H), 4.10–3.97 (m, 2H), 3.17 (s, 3H), 2.30 (s, 3H), 2.03 (q, J = 7.33 Hz, 4H), 1.07 (s, 9H), 1.01–0.99 (m, 9H), 0.59 (t, J = 7.33 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 147.45, 147.34, 146.18, 145.65, 142.59, 131.31, 130.23, 126.88, 121.83, 121.21, 115.61, 113.60, 88.90, 88.59, 69.26, 68.94, 49.37, 39.06, 38.10, 34.17, 29.68, 29.29, 26.31, 26.27, 26.24, 26.12, 16.87, 8.41. ESI-HRMS calcd for $C_{31}H_{48}O_7S$ $[M+Na]^+$ 587.3121, found 587.3017.

4.13.2. 4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenyl benzenesulfonate (**13b**)

Petroleum ether/ethyl acetate (65/1). Colorless oil. 74% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.85 (d, J = 1.2 Hz, 1H), 7.83 (d, J = 1.2 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.50 (t, J = 7.5 Hz, 2H), 6.91 (s, 3H), 6.82 (dd, J = 8.5 Hz, 3.6 Hz, 1H), 6.74 (dt, J = 8.5 Hz, 2.1 Hz, 1H), 6.69 (dd, J = 7.2 Hz, 2.1 Hz, 1H), 4.17–4.11 (m, 1H), 4.09–4.01 (m, 1H), 3.89–3.82 (m, 1H), 3.80–3.70 (m, 1H), 3.68–3.63 (m, 1H), 3.61–3.53 (m, 1H), 2.00 (q, J = 7.3 Hz, 4H), 1.99 (s, 3H), 0.98 (s, 9H), 0.94 (d, J = 4.9 Hz, 9H), 0.59 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 148.30, 147.52, 147.43, 146.10, 142.37, 142.32, 136.34, 134.05, 131.09, 130.37, 129.06, 128.33, 126.61, 122.28, 122.18, 121.33, 117.40, 114.54, 77.40, 77.23, 73.15, 72.00, 49.27, 33.47, 29.36, 26.15, 26.11, 26.09, 26.03, 16.48, 8.36. ESI-HRMS calcd for $C_{36}H_{50}O_7S$ $[M+Na]^+$ 649.3277, found 649.3193.

4.13.3. 4-(3-(3,4-bis(2-hydroxy-3,3-dimethylbutoxy)phenyl) pentan-3-yl)-2-methylphenyl 4-nitrobenzenesulfonate (**13c**)

Petroleum ether/ethyl acetate (65/1). Colorless oil. 82% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 8.37 (s, 1H), 8.35 (s, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 6.96–6.94 (m, 2H), 6.90–6.89 (m, 1H), 6.82 (dd, J = 8.4 Hz, 3.6 Hz, 1H), 6.73 (dt, J = 8.4 Hz, J = 2.1 Hz, 1H), 6.70 (dd, J = 6.6 Hz, 2.1 Hz, 1H), 4.18–4.12 (m, 1H), 4.11–4.02 (m, 1H), 3.89–3.82 (m, 1H), 3.80–3.71 (m, 1H), 3.68–3.64 (m, 1H), 3.62–3.54 (m, 1H), 2.04 (s, 3H), 2.00 (q, J = 7.3 Hz, 4H), 0.98 (s, 9H), 0.94 (d, J = 4.4 Hz, 9H), 0.59 (t, J = 7.3 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 148.23, 147.67, 145.81, 142.16, 142.12, 141.97, 131.46, 130.15, 129.74, 126.91, 124.28, 122.32, 122.23, 121.02, 117.50, 117.39, 114.58, 114.48, 77.48, 49.32, 33.49, 33.38, 29.71, 29.31, 29.25, 26.17, 26.13, 26.11, 26.05, 16.62, 8.38. ESI-HRMS calcd for $C_{36}H_{49}NO_9S$ $[M+Na]^+$ 694.3128, found 649.3037.

4.14. VDR binding assay

The VDR binding affinity of non-secosteriodal VDR ligands were measured by PolarScreen VDR Competitor Assay following the procedure previously described [26]. All compounds were tested for their binding affinity at 1 μM in triplicates. Fluorescence polarization was measured on an Ultra384 microplate reader (Biotek) using a 535 nm excitation filter (25 nm bandwidth) and 590 nm emission filter (20 nm bandwidth).

4.15. Transcription assay

Luciferase activity assay was performed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI) according to the manufacturer's instructions. HEK293 cells of 85%–

90% confluence were seeded in 48-well plates. Transfections of 140 ng of TK-Spp × 3-LUC reporter plasmid, 20 ng of pCMX- Renilla, 30 ng of pENTER-CMV-hRXRα and 100 ng of pCMX-VDR for each well using Lipofectamine®2000 Reagent (Invitrogen). Eight hours after transfection, test compounds were added. Luciferase activity assay was performed 24 h later using the Dual-Luciferase Assay System. Firefly luciferase activity was normalized to the corresponding Renilla luciferase activity. All the experiments were performed three times.

4.16. Anti-collagen I synthetic activity assay

Anti-collagen I synthetic activity assay was performed using the Rat COL1 (Collagen Type I) ELISA Kit (Elabscience, Wuhan). Rat PSC line LTC-14, provided by Prof. Gisela Sparmann (Department of Gastroenterology, University of Rostock, Germany) was maintained in Iscove's Modified Dulbecco's medium (IMDM, Gibco) supplement with 10% fetal bovine serum (FBS) and 1% Penicillin-Streotomycin [27]. Approximate 1×10^5 cells, suspended in medium, were plated into each well of a 24-well plate and grown at 37 °C in humidified atmosphere with 5% CO_2 for 24 h. The following day tested compounds (0.5 μM) and TGF-β1 (5 ng/mL) were added to the culture medium and incubated for 24 h. Centrifuge supernate for 20 min to remove insoluble impurity and cell debris at 1000 × g at 2–8 °C. Collect the clear supernate and carry out the assay immediately. Then follow the description of the Kit. Finally, the optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Rat COL1.

4.17. The caerulein-induced mouse CP model

Male C57BL/6J mice (8 weeks old) were purchased from the Medical School of Yangzhou University (Yangzhou, China). All mice were maintained under standard conditions with free access to water and laboratory rodent food. CP was induced by repetitive caerulein injections. In brief, mice were given six hourly intraperitoneal (i.p.) injections of 50 μg/kg body weight caerulein 3 days per week, for a total of 5 weeks. Control mice received vehicle (DMSO in corn oil) instead. For the evaluation of the effect of Vitamin D analogues on caerulein-induced mouse pancreatic fibrosis, 3 weeks after the first dose of caerulein, calcipotriol or compound **9c** (0.5 mg/kg body weight) was administered by oral gavage five times a week. Mice were sacrificed 3 days after the final caerulein injection. Mouse pancreas and serum were obtained for histopathology, collagen assay, biochemical, and molecular analyses.

4.18. RNA extraction and quantitative real-time polymerase chain reaction (Q-PCR)

cDNA was generated from RNA extracts derived from cultured LTC-14 cells and pancreas tissues using a reverse transcription kit (Transgen, Beijing, China). β-actin (rat) was used as an internal control. Q-PCR was performed using the SYBR Green Master Mix (Vazyme) using a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Primer pairs of mRNA used for rat are as shown in Table S1.

4.19. Western blot

Proteins were purified from LTC-14 cells. Proteins were separated using 10% SDS-polyacrylamide gel electrophoresis and were electrophoretically transferred to polyvinylidene fluoride (PVDF) membranes using standard procedures. The following primary antibodies were employed: rat anti-α-SMA (Boster, Wuhan, China), rabbit anti-collagenI(Boster, Wuhan, China), and rat anti-β-actin (Boster, Wuhan, China). Horseradish peroxidase-conjugated goat anti-rabbit/rat IgG (Boster, Wuhan, China) was used as the secondary antibody. Immunoreactive protein bands were detected using an Odyssey Scanning System (LI-COR Inc).

4.20. Pharmacokinetics study

Compounds **9c** was dissolved in ethanol/EL/saline (1:1:18). C57BL/6J mice were injected compound **9c** 10 mg/kg intravenously and 20 mg/kg orally (n = 4). Blood plasma samples were collected at 0 h, 0.083 h, 0.167 h, 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h after administration of compound **9c**, and then immediately centrifuged (12000 rpm, 10 min) to obtain plasma samples. The concentration of **9c** in plasma was measure by HPLC. The pharmacokinetic parameters were calculated using Kinetica 4.4 software.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.ejmech.2018.01.073. These data include MOL files and InChiKeys of the most important compounds described in this article.

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