# Novel Nonsecosteroidal Vitamin D Receptor Modulator Combined with Gemcitabine Enhances Pancreatic Cancer Therapy through Remodeling of the Tumor Microenvironment 

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#### Abstract

In a pancreatic tumor microenvironment, activated pancreatic stellate cells (PSCs) produce extracellular matrix (ECM) to form a barrier to drug penetration. Moreover, the interaction between cancer cells and activated PSCs promotes the tumor growth. Vitamin D receptor (VDR), as a key regulator to promote the recovery of PSCs to the resting state, is an attractive therapeutic target for pancreatic cancer. Herein, we reported the design and synthesis of 57 nonsecosteroidal VDR modulators based on the skeleton of phenyl-pyrrolyl pentane. Among them, compounds C4, I5, and I8 exhibited excellent VDR affinity and effective inhibition of the activation of PSCs, as well as potent suppression of the interaction between cancer cells and PSCs in vitro. In vivo, compound  I5 combined with gemcitabine achieved efficacious antitumor activity without causing hypercalcemia. In conclusion, the compounds designed in our study can remodel the tumor microenvironment and are expected to be candidates for the treatment of pancreatic cancer.


## INTRODUCTION

Due to difficulties in its early diagnosis, pancreatic cancer is one of the most lethal cancers. ${ }^{1,2}$ Unlike many other cancers, pancreatic cancer is characterized as a denser desmoplastic reaction, generating more than 50 percent occupation of the stroma in tumor tissue. ${ }^{3}$ Extracellular matrix (ECM), which is the main component of stroma and a barrier for chemotherapeutic drugs to penetrate into tumor tissue, has been implicated in the failure of many chemotherapeutic regimens. ${ }^{4,5}$

Pancreatic stellate cells (PSCs), located in periacinal or periductal regions of the exocrine pancreas, are important stromal cells. ${ }^{6}$ PSCs can be activated and acquire proliferative capacity in response to injury, inflammatory stimuli, and cancer. Activated PSCs synthesize abundant ECM proteins, leading to a physical barrier formation to resist chemotherapy drugs. ${ }^{8,9}$ Furthermore, the reciprocal relationship between PSCs and cancer cells has attracted increasing interest. For instance, pancreatic cancer cells secrete many kinds of fibrogenic and mitogenic factors that promote activation of PSCs, such as fibroblast growth factor (FGF), ${ }^{10}$ plateletderived growth factor (PDGF), ${ }^{11}$ periostin, and hepatocyte growth factor (HGF). ${ }^{12,13}$ Reciprocally, the cytokines produced by activated PSCs are very different from those of resting cells, ${ }^{14}$ such as insulin-like growth factor 1 (IGF-1) and
other factors to promote pancreatic cancer cell survival, proliferation, and migration. ${ }^{15,16}$ Therefore, a pharmacologic strategy to regulate the activation of PSCs would remodel the pancreatic tumor microenvironment through hindering ECM deposition and tumor-stroma crosstalk, resulting in enhanced clinical chemotherapy effect of pancreatic cancer.

Vitamin D receptor (VDR), as a ligand-dependent gene transcription factor, can interact with other coactivators and play a vital role in a variety of physiological processes like calcium homeostasis, cell proliferation, and differentiation. ${ }^{17}$ VDR agonist calcipotriol could inhibit the PSC activation and remodel the microenvironment of pancreatic cancer, while the side effects of hypercalcemia and shorter half-life greatly limit its clinical application. ${ }^{18-20}$ A series of threats to human health including abdominal pain, kidney stones, and an abnormal heart rhythm is attributed to hypercalcemia. ${ }^{21}$ Previous studies found that the hypercalcemia effect of calcipotriol was caused

[^0]


Figure 1. Design concept of the novel nonsecosteroidal compounds.
Table 1. Chemical Structures of Synthetized Novel Nonsecosteroidal VDR Agonists

|  |  |  |  |  |  |  |  |  |  |  |  | HN |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Compd. | $\mathbf{R}^{1}$ | R ${ }^{2}$ | n | X | Y | $\mathbf{R}^{3}$ | R ${ }^{4}$ | m | $\mathrm{R}^{5}$ | VDR binding <br> ability (\%) ${ }^{a}$ | Compd. | $\mathbf{R}^{1}$ |  |  | n |  |  | Y | $\mathbf{R}^{3}$ | R ${ }^{4}$ | m | $\mathrm{R}^{5}$ | VDR binding ability (\%) ${ }^{a}$ |
| C2 | Et | Et | 3 | N | C | Et | H | 2 |  | 38 | D13 | M |  | Me |  |  | C | N | H | Et | 3 |  | 24 |
| C3 | Et | Et | 4 | N | C | Et | H | 2 |  | 71 | D14 | Et |  | Et |  |  | C | N | H | Et | 3 |  | 46 |
| C4 | Me | Me | 2 | N | C | Et | H | 3 |  | 85 | D15 | n-Pr |  | n-P |  |  | C | N | H | Et | 3 |  | 64 |
| C5 | Me | Me | 4 | N | C | Et | H | 3 |  | 26 | D16 | M |  | H |  |  | C | N | H | Et | 3 |  | 78 |
| C6 | Me | Me | 5 | N | C | Et | H | 3 |  | 20 | D17 | Et |  | H |  |  | C | N | H | Et | 3 |  | 64 |
| C8 | Et | Et | 4 | N | C | Et | H | 3 |  | - ${ }^{\text {b }}$ | D18 | c-p |  | H |  |  | C | N | H | Et | 3 |  | 40 |
| C9 | Et | Et | 5 | N | C | Et | H | 3 |  | 33 | D19 | Ph |  | H |  |  | C | N | H | Et | 3 |  | 59 |
| C10 | $n-\mathrm{Pr}$ | $\mathrm{n}-\mathrm{Pr}$ | 2 | N | C | Et | H | 3 |  | 54 | H1 | M |  | Me |  |  | N | C | Et | H | 2 |  | 48 |
| C11 | $n-\mathrm{Pr}$ | $n-\mathrm{Pr}$ | 4 | N | C | Et | H | 3 |  | 64 | H2 | M |  | Me |  |  | N | C | Et | H | 2 |  | 36 |
| C12 | $n-\mathrm{Pr}$ | $n-\mathrm{Pr}$ | 5 | N | C | Et | H | 3 |  | - | H3 | M |  | Me |  |  | N | C | Et | H | 2 |  | 24 |
| C13 | Me | Me | 3 | N | C | Et | H | 3 |  | 33 | H4 | Me |  | Me |  |  | N | C | Et | H | 2 |  | 18 |
| C14 | Et | Et | 3 | N | C | Et | H | 3 |  | 23 | H5 | M |  | Me |  |  | N | C | Et | H | 2 |  | 68 |
| C15 | $n-\mathrm{Pr}$ | $\mathrm{n}-\mathrm{Pr}$ | 3 | N | C | Et | H | 3 |  | 61 | H6 | M |  | Me |  |  | N | C | Et | H | 1 |  | 59 |
| C16 | Me | H | 1 | N | C | Et | H | 3 |  | 30 | H7 | M |  | Me |  |  | N | C | Et | H | 1 |  | 77 |
| C17 | Et | H | 1 | N | C | Et | H | 3 |  | 28 | H8 | M |  | Me |  |  | N | C | Et | H | 0 |  | 60 |
| C18 | c-pr | H | 1 | N | C | Et | H | 3 |  | 39 | H9 | M |  | Me |  |  | N | C | Et | H | 3 |  | 63 |
| C19 | Ph | H | 1 | N | C | Et | H | 3 |  | 75 | H10 | M |  | Me |  |  | N | C | Et | H | 3 |  | 54 |
| D1 | Et | Et | 2 | C | N | H | Et | 2 |  | 80 | 11 | M |  | H |  |  | C | N | H | Et | 2 |  | 70 |
| D2 | Et | Et | 3 | C | N | H | Et | 2 |  | 31 | 12 | M |  | H |  |  | C | N | H | Et | 2 |  |  |
| D3 | Et | Et | 4 | C | N | H | Et | 2 |  | 41 | 13 | M |  | H |  |  | C | N | H | Et | 2 |  | - |
| D4 | Me | Me | 2 | C | N | H | Et | 3 |  | 67 | 14 | M |  | H |  |  | C | N | H | Et | 2 |  | 36 |
| D5 | Me | Me | 4 | C | N | H | Et | 3 |  | 33 | 15 | M |  | H |  |  | C | N | H | Et | 2 |  | 89 |
| D6 | Me | Me | 5 | C | N | H | Et | 3 |  | - | 16 | Me |  | H |  |  | C | N | H | Et | 1 |  | 60 |
| D7 | Et | Et | 2 | C | N | H | Et | 3 |  | 51 | 17 | Me |  | H |  |  | C | N | H | Et | 1 |  | 67 |
| D8 | Et | Et | 4 | C | N | H | Et | 3 |  | 27 | 18 | M |  | H |  |  | C | N | H | Et | 0 |  | 86 |
| D9 | Et | Et | 5 | C | N | H | Et | 3 |  | 32 | 19 | M |  | H |  |  | C | N | H | Et | 3 |  | 71 |
| D10 | n - Pr | n-Pr | 2 | C | N | H | Et | 3 |  | - | 110 | M |  | H |  |  | C | N | H | Et | 3 |  | 62 |
| D11 | n-Pr | n-Pr | 4 | C | N | H | Et | 3 |  | 44 | 111 | M |  | H |  | 1 | C | N | H | Et | 3 |  | 70 |

${ }^{a}$ Relative VDR binding affinity $(\%)=\left(\mathrm{mP}_{\text {DMSO }}-\mathrm{mP}_{\text {Testing Compound }}\right) /\left(\mathrm{mP}_{\text {DMSO }}-\mathrm{mP}_{\text {Calcipotriol }}\right) \times 100 \%$. Calcipotriol is assigned as $100 \%$. $^{b}{ }^{-}$-, no activity.
by the secosteroid skeleton structure, which compose almost all of the reported VDR ligands. ${ }^{22-24}$

In order to discover new VDR agonists without hypercalcemia, our group have designed and characterized a series of nonsecosteroidal VDR agonists that bear a phenyl-pyrrolyl pentane scaffold. ${ }^{25-32}$ Among them, compounds $11 \mathbf{b}$ and 11d (Figure 1) showed satisfactory binding affinity and transcriptional activation. ${ }^{26}$ However, the structure of these compounds can further be optimized for better activity and application in more refractory indications. In the present study, 57 new compounds were designed and synthesized to further improve the efficacy and potency profile of nonsecosteroidal VDR agonists, and a variety of biological tests against pancreatic cancer were carried out. Among them, compounds C4, I5, and I8 exhibited excellent VDR affinity and could effectively inhibit the activation of PSCs. In vitro studies indicated that the ECM deposition in PSC spheres was decreased, and the interaction between cancer cells and PSCs was inhibited after the treatment. The combination of compound I5 with gemcitabine exerts strong antitumor effect in vivo without causing hypercalcemia.

## - RESULTS AND DISCUSSION

Novel Nonsecosteroidal Compounds Were Designed and Synthesized through Structure-Based Optimization. VDR agonists can be divided into steroidal VDR agonists and nonsecosteroidal VDR agonists according to whether they have the core skeleton of cyclopentane polyhephenanthrene. Steroidal VDR agonists with open-loop cyclopentane polyhydrophenanthrene core skeleton are collectively referred to as steroid VDR agonists. At present, several steroid VDR agonists have been put on the market and some are in the clinical research stage. Although the physiological activity of the improved steroids has been separated from the ability of increasing blood calcium to a certain extent, it still cannot meet the needs of clinical treatment due to the side effects of hypercalcemia. Therefore, many pharmaceutical chemists began to turn to the research and development of nonsecosteroidal VDR agonists, hoping to retain the VDR agonist activity and not combine with DBP, so as to completely avoid the side effects of hypercalcemia. LG190178 is one of the first synthesized nonsecosteroidal VDR agonist without inducing hypercalcemia, which mimics various physiological activities of $1,25(\mathrm{OH})_{2} \mathrm{D}_{3}$ in vitro and in vivo. ${ }^{33}$ However, compared with $1,25(\mathrm{OH})_{2} \mathrm{D}_{3}$, the binding affinity and transcriptional activity of LG190178 were modest. Therefore, to find more potential nonsecosteroidal VDR agonists, 225 nonsteroidal 1,25$(\mathrm{OH})_{2} \mathrm{D}_{3}$ analogues using LG190178 as the leading compound were designed, synthetized, and screened in our previous studies. ${ }^{25-32}$

At present, there are not many studies on the nonsecosteroidal VDR agonists. One of the main disadvantages of the nonsecosteroidal VDR agonists is that the activation activity is relatively poor, and the ability to stimulate VDR is much weaker than that of steroids. Therefore, our research group has been committed to the study of nonsecosteroidal VDR agonists with higher activity. After continuous research, the structure is also constantly optimized. Among these compounds, 11b and 11d (Figure 1) were identified as the potent agonists of VDR. To further improve the efficacy and potency profile of nonsecosteroidal VDR agonists, several series of chemically modified compounds based on 11b and 11d were generated (Figure 1 and Table 1). As shown in

Figure 1, first, for investigating the different side chain length and electronic property effects on VDR affinity and antitumor activity, the carbon chain length of the phenyl side chain was adjusted, and the side chain substituents were changed. Meanwhile, different fragments containing heteroatoms with different sizes and electronic properties were introduced into the pyrrolyl side chain to obtain compounds C4-C19 and $\mathbf{H 1}-\mathbf{H 1 0}$. For the sake of further investigation of the pyrrole ring substitution position effect on VDR affinity and antitumor activity, compounds D1-D19 and I1-I11 were synthesized. Overall, 57 new compounds bearing a phenyl-pyrrolyl pentane scaffold were designed and synthesized.

The intermediates $\mathbf{2 a} \mathbf{- 2 l}$ were generated by the Grignard reaction (Scheme 1).

Scheme 1. Synthesis of Intermediate 2a-21 ${ }^{a}$


The synthetic route of compounds C2-C6 and C8-C19 is shown in Scheme 2. In the presence of boron fluoride ethyl ether $\left(\mathrm{BF}_{3} \cdot \mathrm{Et}_{2} \mathrm{O}\right)$ at $0{ }^{\circ} \mathrm{C}$, intermediate 3 reacting with 1 H -pyrrole-2-carboxylate produced intermediate 4 . Intermediate 5 was produced following the treatment with iodoethane (EtI) in DMF. Then, reduction of intermediate 5 offered compound 6. Subsequently, hydrolyzed by NaOH , compound 7 was obtained. Following intermediate 7 reaction with different amines afford $8 \mathbf{a}$ ar $\mathbf{8 b}$. Then, in the presence of the corresponding bromine-containing fragment (2a-2l), 8a or $\mathbf{8 b}$ was dissolved in DMF to give target compounds C2-C6 and C8-C19.

The synthetic pathway of compounds D1-D19 is shown in Scheme 3. In the presence of $\mathrm{BF}_{3} \cdot \mathrm{Et}_{2} \mathrm{O}$ at $30^{\circ} \mathrm{C}$, intermediate 9 was obtained by reacting with ethyl pyrrole-2-carboxylate. Following treatment with EtI in DMF, intermediate 9 was to afford intermediate $\mathbf{1 0}$. Then, intermediate $\mathbf{1 0}$ was reduced to obtain intermediate 11. After hydrolysis by KOH, 11 was to give 12. Then, intermediates 13a or 13b was produced by the reaction of intermediate $\mathbf{1 2}$ with different amines. Target compounds D1-D19 were prepared by coupling intermediates $\mathbf{1 3 a}$ or $\mathbf{1 3 b}$ with $\mathbf{2 a} \mathbf{- 2 1}$.

The synthetic pathway of target compounds $\mathbf{H 1 - H 1 0}$ is summarized in Scheme 4. In the presence of 4-bromo-2-methylbutan-2-ol, intermediate 7 was dissolved in DMF to give 14. By reaction of $\mathbf{1 4}$ with different amines, target compounds H1-H10 were produced.

The synthetic pathway of compounds $\mathbf{I 1} \mathbf{- I 1 1}$ is shown in Scheme 5. In the presence of 1 -bromopropan- 2 -ol, intermediate 12 was dissolved in DMF to give 15. Target compounds I1-I11 were produced by reaction of intermediate 15 with different amines.

VDR Binding Affinity. To identify whether the synthesized phenyl-pyrrolyl pentane analogues act as VDR agonists, the VDR binding affinity of compounds was evaluated by VDR competitor assay. The relative binding affinity is listed in Table 1. As the results have shown, the volume of substituents was positively related to the activity, i.e., propyl > ethyl > methyl,

## Scheme 2. Synthesis of Compounds C2-C6 and C8-C19 ${ }^{\text {a }}$


${ }^{a}$ Reagents and conditions: (a) ethyl 1 H -pyrrole-2-carboxylate, $\mathrm{BF}_{3} \cdot \mathrm{Et}_{2} \mathrm{O},-20-0^{\circ} \mathrm{C}, 3 \mathrm{~h}$; (b) EtI, $\mathrm{NaH}, \mathrm{DMF}, 0-25^{\circ} \mathrm{C}, 5 \mathrm{~h}$; (c) $\mathrm{Pd} / \mathrm{C}, \mathrm{H} 2, \mathrm{MeOH}$, $25^{\circ} \mathrm{C}, 12 \mathrm{~h}$; (d) $\mathrm{NaOH}, \mathrm{EtOH} / \mathrm{H}_{2} \mathrm{O}(10: 1), 90^{\circ} \mathrm{C}, 24 \mathrm{~h}$; (e) EDCI, $\mathrm{HOBt}, \mathrm{TEA}, \mathrm{RNH}_{2}, \mathrm{DCM}$, rt, overnight; (f) $2 \mathrm{a}-21 \mathrm{NaH}, \mathrm{DMF}, 0-70{ }^{\circ} \mathrm{C}, 12$ h.

Scheme 3. Synthesis of Target Compounds D1-D19 ${ }^{a}$


${ }^{a}$ Reagents and conditions: (a) ethyl 1 H -pyrrole-2-carboxylate, $\mathrm{BF}_{3} \cdot \mathrm{Et}_{2} \mathrm{O}, 30^{\circ} \mathrm{C}, 3 \mathrm{~h}$; (b) EtI, NaH, DMF, $0-25{ }^{\circ} \mathrm{C}, 5 \mathrm{~h}$; (c) $\mathrm{Pd} / \mathrm{C}, \mathrm{H} 2, \mathrm{MeOH}, 25$ ${ }^{\circ} \mathrm{C}, 12 \mathrm{~h}$; (d) NaOH, EtOH/ $\mathrm{H}_{2} \mathrm{O}(10: 1)$, $90^{\circ} \mathrm{C}$, 24 h ; (e) EDCI, HOBt, TEA, RNH 2 , DCM, rt, overnight; (f) 2a-2l, NaH, DMF, 0-70 ${ }^{\circ} \mathrm{C}, 12 \mathrm{~h}$.

## Scheme 4. Synthesis of Compounds H1-H10 ${ }^{a}$



[^1]Scheme 5. Synthesis of Compounds I1-I11 ${ }^{a}$

${ }^{a}$ Reagents and conditions: (a) 1-bromopropan-2-ol, $\mathrm{K}_{2} \mathrm{CO}_{3}$, acetone, reflux, 12 h ; (b) EDCI, HOBt, TEA, RNH , DCM, rt, overnight.
while the activity was partially decreased when $R^{1}$ or $R^{2}$ is $H$, indicating that the side substituents might not be enough to provide a steric effect, which was adverse to the activity. However, when the side substituents were phenyl, the activity was still excellent (C19). When the end substituent is disubstituted methyl or ethyl, the activity order of the carbon chain length was $3>6>5>4$. When the end substituent was di-n-propyl, the activity order of carbon chain length was $4>5$ $>3>6$.

In the second round of design, the substitution position of the pyrrole ring in the core framework from C5 to C 4 was changed to generate compounds D1-D19. The results showed that the volume effect of end substituents on the activity was basically the same as that of compounds C2-C19, but the effect of the carbon chain length on the activity was slightly different. When the end was substituted by dimethyl or diethyl, the effect of " n " on the activity was $4>6>5>3$. However, in this round of design, the most active molecule appeared in the compound D16 with the length of carbon chain of 1 and the end substituted by H and methyl. The two optimized molecules C4 and D16 from the first and second rounds were taken as the starting point to carry out the third round of structural transformation, and the right hydrophilic end segment without modifying side chain structure was optimized. In the optimization of $\mathbf{C 4}$, we found the significantly decreased activity of shortening one carbon atom of the right carbon chain, i.e., when " $m$ " was 2 (H2). No matter the simplification of the end N atom substituent to methyl (H1), cyclization (H3), or the introduction of the second heteroatom (H4H5), could not restore the VDR affinity, and the above effects also appeared when " $m$ " was shortened to 1 , indicating that the optimal length of the carbon atom on the right side of the core skeleton was 3 concerning the extraction of pyrrole C5. The activity would be reduced with the length reduction of the carbon atom and no more active molecule could be found for the optimization of C4. The optimization starting from D16 showed that compounds I5 and I8 had better activity. It was worth mentioning that the right carbon chain length of these two molecules was 2 , which was the preferred length for the pyrrole C4 substituted skeleton.

Phenyl-pyrrolyl Pentane Analogues Inhibited the Activation of PSCs. The immortalized rat PSC cell line, LTC-14 cells were selected for the experiment. ${ }^{34}$ To avoid the influence in activation of PSCs due to cytotoxicity, the toxicity of all compounds to LTC-14 cells were tested by the MTT assay. as shown in Table S1, the compounds showed no significant toxicity to LTC-14 cells, and the IC50 values of all compounds to LTC-14 cells were greater than $10 \mu \mathrm{M}$. Therefore, $0.5 \mu \mathrm{M}$ was selected for the subsequent activity screening test.

Collagen I and $\alpha$-smooth muscle actin ( $\alpha$-SMA) are over expressed when PSCs are activated. ${ }^{35}$ Therefore, the expression level of $\alpha$-SMA and collagen I can be used as an
important indicator of PSC activation. In order to evaluate the effect of target compounds on the suppression of PSC activation, LTC-14 cells were treated with different compounds in the presence of $0.5 \mathrm{ng} / \mathrm{mL}$ TGF- $\beta 1$ for 24 h . Acta2 and Colla1 are mRNA of $\alpha$-SMA and collagen I, respectively. The expression levels of Colla1 and Acta2 in LTC-14 cells were detected by Q-PCR. Meanwhile, the expression of collagen I protein was determined by western blot. As shown in Figure 2, most of the compounds showed the ability to suppress these gene expressions in varying degrees. Considering the results of activity, the effect of the side chain, the end hydroxyl side substituents $\mathrm{R}^{1}$ and $\mathrm{R}^{2}$, on the activity was propyl > ethyl > methyl, indicating that larger substituents had better activity. In addition, when $R^{1}$ was phenyl and $R^{2}$ was $H$, the compounds had better activity. The length of the side chain carbon atom also had an obvious influence on the activity. When the core framework pyrrole ring was substituted at the C5 position, the influence of the carbon chain length on the activity was: $3>6>5>4$. When the core framework pyrrole ring was substituted at the C 4 position, the influence of the length of the carbon chain on the activity was: $4>6>5>3$. The selected compounds C4 and D16 had better ability to inhibit the activation of PSCs. When optimizing the right hydrophilic segment without modification of the left side chain structure, it was found that the structure optimization starting from C4 did not get better active molecules, while the optimization starting from D16 produced many better active compounds, which suggested that the activity of C4 substitution with the core skeleton pyrrole was higher than that of C5 substitution.

Compounds Inhibited the Oncogenic Factor Expression of PSCs. In the microenvironment of pancreatic tumor, the interaction between cancer cells and activated PSCs promotes tumor growth and metastasis. ${ }^{36}$ Cancer cells can promote PSCs to secrete many cytokines, such as periosteal osteoblast specific factor 2 (POSTN), PDGF, FGF and HGF, which in turn enhance the cancer cell proliferation, migration, and invasion ${ }^{37}$. In pancreatic cancer, PSCs play the role of "accomplice", and thus inhibiting the interaction of cancer cells and PSCs may reshape the pancreatic tumor microenvironment and improve the chemotherapy effect.

After comprehensive consideration of the affinity and ability to inhibit activation of PSCs, compounds C4, C15, D11, H2, H3, H5, I3, I5, and I8 were selected for the follow-up activity study, calcipotriol as the positive control. LTC-14 cells were treated with the conditioned media (CM) of rat pancreatic cancer cell DSL-6A/C1 in the presence of different compounds $(0.5 \mu \mathrm{M})$ for 24 h . The expression of POSTN and PDGF-A protein in LTC-14 cells was determined by western blot, and the mRNA expression of Pdgfa, Pdgfb, Fgf, Hgf, and Periodin was measured by Q-PCR. As shown in Figure 3A-C, compounds C4, D11, H5, I5, and I8 can significantly inhibit the expression of PDGF-A and POSTN protein in


Figure 2. The expression levels of Col1a1, Acta2, and collagen I in LTC-14 cells after different compound treatment. (A and B) The expression of Colla1 and Acta2 were determined by Q-PCR. Data are shown as mean $\pm$ SD. ${ }^{*} P<0.05$; $* * P<0.01$; ***P $<0.001$ compared to TGF- $\beta 1$ alone. (C) The expression level of collagen I protein on LTC-14 cells was determined by western blot, protein expression levels were quantified by densitometry. Data are shown as mean $\pm \mathrm{SD} .{ }^{*} P<0.05 ; * * P<0.01$; ${ }^{* * * P}<\mathbf{0 . 0 0 1}$ compared to TGF- $\beta 1$ alone.

LTC-14 cells after CM treatment. In addition, the mRNA expression of Pdgfa, Pdgfb, Fgf, Hgf, and Periodin in LTC-14 cells, which is related to PSC activation, was inhibited in varying degrees after CM treatment (Figure 3D-H). Therefore, these preferred compounds can inhibit the oncogenic factor expression of PSCs that are promoted by pancreatic cancer cells.

Compounds Inhibited Tumor Cell Proliferation that Were Induced by PSCs and Decreased the ECM Deposition in PSC Spheres. PSCs secrete many factors to increase the cancer cell proliferation ${ }^{38}$. In order to detect the inhibition ability of selected compounds on cancer cell proliferation that were induced by PSCs, cancer cells were treated with culture medium supernatant of PSCs (PSC-CM) in the presence of the compound (PSC-CM + compound) for


Figure 3. Compounds inhibit the oncogenic factors expression of PSCs. (A) The expression of PDGF-A and POSTN was determined by western blot. (B, C) The expression of PDGF-A and POSTN proteins was quantified by densitometry and normalized to $\beta$-actin. (D-H) The expression of Pdgfa, Pdgfb, Fgf 2, Hgf, and Periodin in LTC-14 cells after CM treatment was measured by Q-PCR. Three repeats were performed. Data are shown



Figure 4. Compounds inhibit the promotion effect of PSCs on cancer cells. (A-C) Cancer cells were treated with a PSC culture medium (PSC$\mathrm{CM})$ in the presence of the compound $(0.5 \mu \mathrm{M})$ for 24 h . Otherwise, the culture supernatant of compound-pretreated PSCs for 24 h was used to culture tumor cells (compound treated PSC-CM). The cancer cell proliferation was determined by MTT assay. Data are shown as mean $\pm$ SD. ${ }^{* * P}$ $<0.01$; ${ }^{* * * P}<0.001$ versus PSC-CM. (D, E) LTC-14 cells were used for 3D culture in vitro to form PSC spheres. After the PSC spheres were treated with compounds, doxorubicin (DOX) with red fluorescence was added. The content of DOX in PSC spheres was detected by a laser confocal microscope. Three repeats were performed. Data are shown as mean $\pm$ SD. $* P<0.05$; **P $<0.01$ versus control.

24 h . Otherwise, the culture supernatant of compound pretreated PSCs for 24 h was used to culture tumor cells
(compound treated PSC-CM). The cancer cell proliferation was determined by MTT assay. In order to prevent the residual


Figure 5. C4, I5, and I8 are VDR agonists. (A) Chemical structure of selected compounds C4, I5, and I8. (B) VDR binding affinity of compounds C4, I5, and I8. The affinity was determined by fluorescence polarization competition assay ( $n=3$ ). (C) Compound transcriptional activities were determined in HEK293 cells. TK-Spp×3-LUC reporter plasmid, pCMX-Renilla, pENTER-CMV-hRXR $\alpha$, and pCMX-VDR were cotransfected into HEK293 cells. Test compounds and calcipotriol were added after 8 h . After 24 h , luciferase activity was performed using the Dual-Luciferase Assay System. (D) Predicted binding model of C4, I5, and I8. Using the discovery studio, VDR-LBD (PDB code: 2ZFX) was applied for the molecular docking analysis.
compounds in PSC-CM from directly inhibiting proliferation of cancer cells, the cytotoxicity of the compounds to DSL-6A/ C 1 were tested. All the selected compounds had no obvious toxicity to DSL-6A/C1 at the concentration of $0.5 \mu \mathrm{M}$ (Figure S1). As shown in Figure 4A-C, the proliferation of DSL-6A/ C1 cells increased significantly after PSC-CM treatment compared with the control. When the selected compounds were added, the proliferation of cancer cells had no significant change compared with PSC-CM alone. Meanwhile, it is noteworthy that the cancer cell proliferation was significantly decreased after the compound-treated-PSC-CM treatment, which indicated that the compound could cause PSCs to lose inhibitory effect on cancer cell proliferation.

Previous studies have shown that the activated PSCs can secrete a large amount of collagen, resulting in excessive deposition of ECM in the tumor microenvironment and providing a physical barrier for cancer cells. In order to investigate whether compounds can reduce ECM deposition after inhibiting PSC activation, LTC-14 cells were used for 3D culture in vitro to form PSC spheres. After the PSC spheres were treated with different compounds, doxorubicin (DOX) with red fluorescence was added as the model drug. The content of DOX in PSC spheres was detected by a laser confocal microscope, which reflected whether the compounds could increase the permeation rate of drug via ECM barrier. As shown in Figure 4D,E, red fluorescence was only observed on the surface of PSC spheres in the control group after administration of DOX, while DOX could enter PSC spheres in varying degrees after administration of calcipotriol, C4, I5, and I8, indicating that the dense of ECM secreted by PSCs was inhibited and the permeation rate of drug via physical barrier
was increased by the compounds acting as permeation enhancers.

Compounds C4, 15, and 18 Showed VDR Agonist Activities. The structures of compounds C4, I5, and I8 were shown in Figure 5A. To evaluate the VDR binding affinities of the compounds, various concentrations of compounds were tested for the VDR binding ability. Compounds $\mathrm{C} 4\left(\mathrm{IC}_{50}=\right.$ $92.51 \mathrm{nM})$, $\mathrm{I} 5\left(\mathrm{IC}_{50}=57.19 \mathrm{nM}\right)$, and $\mathbf{I 8}\left(\mathrm{IC}_{50}=81.59 \mathrm{nM}\right)$ showed excellent VDR binding affinity (Figure 5B). Affinity is the degree of interaction between ligand and VDR protein. The premise of activating VDR is that the ligand must have a certain affinity with VDR protein. Therefore, affinity can be used to characterize the matching degree of ligand and protein binding, which can be used as a standard to judge whether the design of the nonsteroidal structure is reasonable and whether it can simulate the binding of natural steroid ligand and protein receptor. Meanwhile, there are many other mechanisms involved in the interaction of VDR, including the ability to interact with many other cofactors. The ability to activate VDR can be evaluated in many aspects, including the binding activity, transcriptional activity, and related biological activity.

To further evaluate the agonistic properties of target compounds, transactivation assay was performed in HEK293 cells. Compounds C4, I5, and I8 were tested, and calcipotriol was applied as positive control. pGL4.27-SPP $\times 3$-Luc reporter plasmid acts as the VDRE activated reporter, and pRL-TK normalizes the expression of luciferase as the internal reference. pENTER-CMV-hVDR was applied to increase the VDR expression, and pENTER-CMV-hRXR $\alpha$ expressing $\operatorname{RXR} \alpha$ acts as the VDR heterodimer partner. As shown in Figure 5C, compounds C4, I5, and I8 showed a concentration-


Figure 6. C4, I5, and I8 plus gemcitabine shows anti-pancreatic cancer efficacy in vivo. (A) Schematic illustrating the treatment of tumor-bearing mice. DSL-6A/C1-luci and LTC-14-GFP cells were co-inoculated into the nude mice pancreas to construct the orthotopic xenotransplantation model of pancreatic cancer. Fourteen days after tumor grafting, mice were randomly divided into groups: vehicle, gemcitabine, or gemcitabine and compound combination. Gemcitabine was administered once every 3 days and compounds were administered daily ( $n=5$ per group). After 12 days administration, mice were photographed by in vivo imaging and tumors were harvested. (B) Tumor growth in mice was examined by an IVIS optical imaging system. Representative animal images are shown. (C) Structures of tumor tissue were determined by H\&E staining ( $\times 100$ ), collagen deposition was examined by Masson's trichrome stain ( $\times 100$ ). (D) The expression of $\alpha$-SMA (red) in the PSCs (green) of tumor sections was tested by immunofluorescence. Scale bars: $10 \mu \mathrm{~m}$.
dependent transcriptional activity, indicating that the compounds were potent agonists.

To further elucidate the interaction mode between these compounds and VDR at atomic level, C4, I5, and I8 were selected for molecular docking with VDR ligand binding domain (rat VDR-LBD). The results of molecular docking showed that the compound I8 pyrrole ring interacted with the indole ring of TRP 282 of VDR protein (PDB: 2ZFX). The hydroxyl group interacted with the imidazole ring of HIS 301 and HIS 393 at the same time, and the hydroxyl group of SER 233 at the other end formed a hydrogen bond force and formed a water molecule-mediated hydrogen bond force with the ARG 270 guanidine group. Compound I5 was obtained by replacing the propylene glycol side chain of $\mathbf{I 8}$ with methylpiperazine, which could also form $\pi-\pi$ interaction with TRP 282, but methylpiperazine could not interact with HIS 301 and HIS 393. Moreover, due to the steric hindrance of methylpiperazine, the hydroxyl side chain only formed hydrogen bonding force with the hydroxyl group of SER 233. When the side chain of methylpiperazine was replaced by the diethylamino group to obtain $\mathbf{C 4}$, due to the further increase of steric hindrance, the torsion of the pyrrole ring resulted in the destruction of the formation of $\pi-\pi$ interaction, resulting in the formation of only the end hydroxyl group with SER 233 and ARG 270.

C4, I5, and 18 plus Gemcitabine Showed AntiPancreatic Cancer Effect. Based on in vitro results, we selected the compounds C4, I5, and I8 for the pharmacodynamic test in vivo. DSL-6A/C1-luci and LTC-14-GFP cells were co-inoculated into the nude mice pancreas to construct
the orthotopic xenotransplantation model of pancreatic cancer. Fourteen days after tumor grafting, mice were randomly divided into six groups: vehicle, gemcitabine (Gem), Gem + calcipotriol, Gem +C 4 , Gem + I5, and Gem + I8. Once every 3 days, gemcitabine ( $100 \mathrm{mg} / \mathrm{kg}$ ) was administered by intraperitoneal injection. Meanwhile, compounds (C4, I5, and I8) were administered at $500 \mu \mathrm{~g} / \mathrm{kg}$ and calcipotriol was administered at $60 \mu \mathrm{~g} / \mathrm{kg}$. After 12 days of administration, mice were photographed by in vivo imaging and tumors were harvested (Figure 6A). As shown in Figure 6B and Figures S2 and S3, the tumor did not decrease significantly after gemcitabine administration alone. The tumor volume was significantly reduced after treatment with gemcitabine combined with the compounds. In particular, compound I5 combined with gemcitabine showed the best antitumor activity.

H\&E-staining of tumor sections further confirmed that the density of pancreatic tumor tissue decreased with the combination of I5 and gemcitabine. According to Masson staining results, the collagen deposition in the group of I5 combined with gemcitabine was significantly inhibited, and the inhibition activity was better than that of the positive control (Figure 6C). The results in Figure 6D and Figure S4 show that after treatment with gemcitabine + calcipotriol, gemcitabine + C4, gemcitabine + I5, and gemcitabine + I8, the $\alpha$-SMA protein expression of LTC-14 cells decreased in varying degrees, indicating that the activation of PSCs in the tumor microenvironment was inhibited, and compound I5 combined with gemcitabine showed the best efficacy. In addition, the combination treatment of compounds and gemcitabine can
prolong the survival time of tumor-bearing mice in a certain degree (Figure S5). More importantly, the compounds do not cause hypercalcemia in mice (Figure S6). These results indicated that the compounds designed in our study can provide new effective candidate strategies for the treatment of pancreatic cancer.

## - CONCLUSIONS

In summary, 57 new compounds were designed and synthesized in our study on the basis of previous works. Among them, compounds C4, I5, and $\mathbf{I 8}$ exhibited excellent VDR affinity and effective inhibition of the activated PSCs. In vitro studies indicated that after the compound treatment, the ECM deposition in PSC spheres was decreased and the interaction between pancreatic cancer cells and PSCs was inhibited. Meanwhile, in the antitumor study in vivo, compound I5 combined with gemcitabine had a strong antitumor effect without causing hypercalcemia in mice. In light of the presented findings, we concluded that these novel nonsecosteroidal VDR modulators may have potent application in combined treatment of pancreatic cancer.

## EXPERIMENTAL SECTION

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 a QSTAR XL Hybrid MS/MS mass spectrometer. ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR were recorded employing Bruker AV-300 or AV-500 instruments using $\mathrm{CDCl}_{3}$. Chemical shifts were given as $\delta(\mathrm{ppm})$ units relative to the internal standard tetramethylsilane (TMS). Column chromatography separations were progressed on a silica gel ( $200-300$ mesh). Purity of all final compounds was $\geq 95 \%$ as determined by HPLC.
Synthesis of the New Compounds. General Procedure 1: Synthesis of Compounds $2 a-21$. To a stirred solution of 1 (55 $\mathrm{mmol})$ in ether $(100 \mathrm{~mL})$ was added $\mathrm{RMgBr}(138 \mathrm{mmol})$ dropwise at $0^{\circ} \mathrm{C}$. The mixture was stirred at $30^{\circ} \mathrm{C}$ for 6 h , water ( 50 mL ) and EtOAc ( 200 mL ) was carefully added to the mixture at $0^{\circ} \mathrm{C}$, and the sorganic phase was separated. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with an appropriate mixture as indicated in each case.

4-Bromo-2-methylbutan-2-ol (2a). Petroleum ether/ethyl acetate (100/1). Colorless oil. $42 \%$ yield. ${ }^{1}$

5-Bromo-2-methylpentan-2-ol (2b). Petroleum ether/ethyl acetate (100/1). Colorless oil. $38 \%$ yield.

6-Bromo-2-methylhexan-2-ol (2c). Petroleum ether/ethyl acetate ( $80 / 1$ ). Colorless oil. $53 \%$ yield.

7-Bromo-2-methylheptan-2-ol (2d). Petroleum ether/ethyl acetate ( $70 / 1$ ). Colorless oil. $62 \%$ yield.

1-Bromo-3-ethylpentan-3-ol (2e). Petroleum ether/ethyl acetate (30/1). Colorless oil. $35 \%$ yield.

6-Bromo-3-ethylhexan-3-ol (2f). Petroleum ether/ethyl acetate (40/1). Colorless oil. $40 \%$ yield.

7-Bromo-3-ethylheptan-3-ol (2g). Petroleum ether/ethyl acetate (50/1). Colorless oil. $32 \%$ yield.

8-Bromo-3-ethyloctan-3-ol (2h). Petroleum ether/ethyl acetate (100/1). Colorless oil. $20 \%$ yield.

4-(2-Bromoethyl)heptan-4-ol (2i). Petroleum ether/ethyl acetate ( $100 / 1$ ). Colorless oil. $22 \%$ yield.

1-Bromo-4-propylheptan-4-ol (2j). Petroleum ether/ethyl acetate (50/1). Colorless oil. $52 \%$ yield.

8-Bromo-4-propyloctan-4-ol (2k). Petroleum ether/ethyl acetate (50/1). Colorless oil. $41 \%$ yield.

9-Bromo-4-propyInonan-4-ol (2l). Petroleum ether/ethyl acetate (70/1). Colorless oil. $37 \%$ yield.

Ethyl-5-(3-(4-(Benzyloxy)-3-methylphenyl)pentan-3-yl)-1H-pyr-role-2-carboxylate (4). $\mathrm{BF}_{3} \cdot \mathrm{Et}_{2} \mathrm{O}(5 \mathrm{~mL})$ was added dropwise to a solution of compound $3(2.0 \mathrm{~g}, 7.03 \mathrm{mmol})$ and ethyl 1 H -pyrrole-2carboxylate $(1.17 \mathrm{~g}, 8.44 \mathrm{mmol})$ in dichloromethane $(20 \mathrm{~mL})$ at -20 ${ }^{\circ} \mathrm{C}$. The mixture was stirred for 3 h at $0^{\circ} \mathrm{C}$, water ( 10 mL ) and EtOAc ( 30 mL ) was carefully added to the mixture at $0^{\circ} \mathrm{C}$, and the organic phase was separated. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate ( $100 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound 4 as colorless oil ( $1.73 \mathrm{~g}, 61 \%$ yield).
Ethyl-5-(3-(4-(Benzyloxy)-3-methylphenyl)pentan-3-yl)-1-ethyl-1H-pyrrole-2-carboxylate (5). To a solution of compound 4 ( 0.87 g , $2.15 \mathrm{mmol})$ in DMF $(15 \mathrm{~mL})$ was added $\mathrm{NaH}(0.10 \mathrm{mg}, 4.29 \mathrm{mmol})$ at $0^{\circ} \mathrm{C}$ and stirred for 1 h . Ethyl bromide was added to the mixture at $0^{\circ} \mathrm{C}$. The reaction mixture was stirred for 5 h at room temperature, water $(10 \mathrm{~mL})$ and EtOAc $(30 \mathrm{~mL})$ was added and the organic phase was separated. The organic phase were washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with petroleum ether/ethyl acetate ( $80 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound $\mathbf{5}$ as oil ( $0.97 \mathrm{~g}, 85 \%$ yield).

Ethyl-1-ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1 H -pyrrole-2-carboxylate (6). To a solution of $5(2.00 \mathrm{~g}, 4.61 \mathrm{mmol})$ in methanol $(30 \mathrm{~mL})$, $\mathrm{Pd} / \mathrm{C}(0.2 \mathrm{~g})$ was added. The reaction mixture was stirred under a $\mathrm{H}_{2}$ atmosphere at for 12 h . The $\mathrm{Pd} / \mathrm{C}$ was filtered off, and then the residue was purified by column chromatography with petroleum ether/ethyl acetate ( $20 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound $\mathbf{6}$ as oil ( $1.33 \mathrm{~g}, 84 \%$ yield).

1-Ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyr-role-2-carboxylic acid (7). To a stirred solution of $6(0.30 \mathrm{~g}, 0.87$ $\mathrm{mmol})$ in a mixture of $\mathrm{EtOH} / \mathrm{H}_{2} \mathrm{O}$ 10:1 $(40 \mathrm{~mL})$ was added NaOH $(0.35 \mathrm{~g}, 8.73 \mathrm{mmol})$ at $90^{\circ} \mathrm{C}$. After 24 h , the solution was evaporated, and water ( 20 mL ) and EtOAc ( 80 mL ) was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with dichloromethane/methanol ( $50 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound 7 as oil ( $0.21 \mathrm{~g}, 76 \%$ yield).

N-(2-(Diethylamino)ethyl)-1-ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (8a). To a solution of compound $7(100.93 \mathrm{mg}, 0.32 \mathrm{mmol})$ in dichloromethane ( 50 mL ), 3-(ethyliminomethylideneamino)- $\mathrm{N}, \mathrm{N}$-dimethylpropan-1amine (hydrochloride) ( $74.38 \mathrm{mg}, 0.48 \mathrm{mmol}$ ), 1-hydroxybenzotriazole ( $64.74 \mathrm{mg}, 0.48 \mathrm{mmol}$ ), and triethylamine $(48.49 \mathrm{mg}, 0.48$ mmol ) were added at $0^{\circ} \mathrm{C}$. After stirring for $0.5 \mathrm{~h}, \mathrm{~N}, \mathrm{~N}$-diethylethane1,2 -diamine ( $55.78 \mathrm{mg}, 0.48 \mathrm{mmol}$ ) was added. The reaction mixture was stirred at room temperature for 12 h and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with dichloromethane/methanol ( $30 / \mathrm{l}, \mathrm{v} / \mathrm{v}$ ) to give compound 8 a as oil $(0.11 \mathrm{~g}, 83 \%$ yield).

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (8b). To a solution of compound $7(100.93 \mathrm{mg}, 0.32 \mathrm{mmol})$ in dichloromethane $(50 \mathrm{~mL})$, 3-(ethyliminomethylideneamino)- $\mathrm{N}, \mathrm{N}$-dimethylpropan-1amine (hydrochloride) ( $74.38 \mathrm{mg}, 0.48 \mathrm{mmol}$ ), 1-hydroxybenzotriazole ( $64.74 \mathrm{mg}, 0.48 \mathrm{mmol}$ ), and triethylamine ( $48.49 \mathrm{mg}, 0.48$ mmol ) were added at $0{ }^{\circ} \mathrm{C}$. After stirring for $0.5 \mathrm{~h}, \mathrm{~N}, \mathrm{~N}$ -dimethylpropan-1,3-diamine ( $62.46 \mathrm{mg}, 0.48 \mathrm{mmol}$ ) was added. The reaction mixture was stirred at room temperature for 12 h , and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with dichloromethane/methanol ( $30 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound $\mathbf{8 b}$ as oil $(0.11 \mathrm{~g}$, $83 \%$ yield).

General Procedure 2: Synthesis of Compounds C2 and C3. To a solution of compound 8a ( $200 \mathrm{mg}, 0.48 \mathrm{mmol}$ ) in DMF ( 50 mL ), $\mathrm{NaH}(18.28 \mathrm{mg}, 0.72 \mathrm{mmol})$ was added portionwise at $0{ }^{\circ} \mathrm{C}$. After stirring for $0.5 \mathrm{~h}, 2 \mathrm{f}$ or $2 \mathrm{~g}(0.72 \mathrm{mmol})$ was added. The reaction mixture was stirred at $70{ }^{\circ} \mathrm{C}$ for 12 h and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added dropwise followed by EtOAc ( 80 mL ). The organic phase was
separated and washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

N-(2-(Diethylamino) ethyl)-1-ethyl-5-(3-(4-((4-ethyl-4-hydroxyhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C2). Dichloromethane/methanol (100/1). Oil. 46\% yield.

N-(2-(Diethylamino) ethyl)-1-ethyl-5-(3-(4-((5-ethyl-5-hydroxyheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2carboxamide (C3). Dichloromethane/methanol (50/1). Oil. 52\% yield.

General Procedure 3; Synthesis of Compounds C4-C6 and C8C19. To a solution of compound $\mathbf{8 b}(205 \mathrm{mg}, 0.48 \mathrm{mmol})$ in DMF $(50 \mathrm{~mL}), \mathrm{NaH}(18.28 \mathrm{mg}, 0.72 \mathrm{mmol})$ was added portionwise at 0 ${ }^{\circ} \mathrm{C}$. After stirring for 0.5 h , one of $\mathbf{2 a} \mathbf{- 2 1}(0.72 \mathrm{mmol})$ was added. The reaction mixture was stirred at $70{ }^{\circ} \mathrm{C}$ for 12 h , and then $\mathrm{H}_{2} \mathrm{O}(100$ mL ) was added dropwise followed by EtOAc ( 80 mL ). The organic phase was separated, washed with brine, dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with the appropriate mixture as indicated in each case.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-(3-hydroxy-3-meth-ylbutoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C4). Dichloromethane/methanol (70/1). Oil. $67 \%$ yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((5-hydroxy-5-methylhexyl)oxy)-3-methylphenyl) pentan-3-yl)-1 H-pyrrole-2-carboxamide (C5). Dichloromethane/methanol (60/1). Oil. 44\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((6-hydroxy-6-methylheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C6). Dichloromethane/methanol (70/1). Oil. 48\% yield.
$N$-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((5-ethyl-5-hydroxyheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2carboxamide (C8). Dichloromethane/methanol (70/1). Oil. 71\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-5-(3-(4-((6-ethyl-6-hydroxyoctyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C9). Dichloromethane/methanol (100/1). Oil. 56\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((3-hydroxy-3-propylhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C10). Dichloromethane/methanol (100/1). Oil. 41\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((5-hydroxy-5-propyloctyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C11). Dichloromethane/methanol (70/1). Oil. 40\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((6-hydroxy-6-propylnonyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C12). Dichloromethane/methanol (70/1). Oil. 39\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-((4-hydroxy-4-methylpentyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C13). Dichloromethane/methanol (100/1). Oil. 53\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-5-(3-(4-((4-ethyl-4-hydroxyhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C14). Dichloromethane/methanol (70/1). Oil. 42\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-5-(3-(4-((4-hydroxy-4-propylheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C15). Dichloromethane/methanol (100/1). Oil. 48\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C16). Dichloromethane/methanol (70/1). Oil. 54\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-(2-hydroxybutoxy)-3-methylphenyl) pentan-3-yl)-1H-pyrrole-2-carboxamide (C17). Dichloromethane/methanol (70/1). Oil. 43\% yield.

5-(3-(4-(2-Cyclopropyl-2-hydroxyethoxy)-3-methylphenyl)-pentan-3-yl)-N-(3-(diethylamino)propyl)-1-ethyl-1H-pyrrole-2-carboxamide (C18). Dichloromethane/methanol (70/1). Oil. 41\% yield. N-(3-(Diethylamino)propyl)-1-ethyl-5-(3-(4-(2-hydroxy-2-phenyl-ethoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (C19). Dichloromethane/methanol (70/1). Oil. 33\% yield.

Ethy-4-(3-(4-(benzyloxy)-3-methylphenyl)pentan-3-yl)-1H-pyr-role-2-carboxylate (9). By the same manner as described for the preparation of 4, intermediate 9 was prepared at $30^{\circ} \mathrm{C}$ and purified
by silica gel chromatography with petroleum ether/ethyl acetate (50/ 1, v/v). Yield: $40 \%$.

Ethyl-4-(3-(4-(benzyloxy)-3-methylphenyl)pentan-3-yl)-1-ethyl-1H-pyrrole-2-carboxylate (10). By the same manner as described for the preparation of 5 , intermediate $\mathbf{1 0}$ was prepared from intermediate 9 and purified by silica gel chromatography with petroleum ether/ ethyl acetate ( $50 / 1$, v/v). Yield: $76 \%$.

Ethyl-1-ethyl-4-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxylate (11). By the same manner as described for the preparation of $\mathbf{6}$, intermediate $\mathbf{1 1}$ was prepared from intermediate $\mathbf{1 0}$ and purified by silica gel chromatography with petroleum ether/ethyl acetate (20/1, v/v). Yield: $73 \%$.

1-Ethyl-4-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyr-role-2-carboxylic acid (12). By the same manner as described for the preparation of 7 , intermediate 12 was prepared from intermediate 11 and purified by silica gel chromatography with dichloromethane/ methanol (70/1, v/v). Yield: $68 \%$.

N-(2-(Diethylamino)ethyl)-1-ethyl-4-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (13a). By the same manner as described for the preparation of $\mathbf{8 a}$, the intermediate 13a was prepared from the intermediate 12 and purified by silica gel chromatography with dichloromethane/methanol (30/1, v/v). Yield: $84 \%$.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (13b). By the same manner as described for the preparation of $\mathbf{8 b}$, the intermediate $\mathbf{1 3 b}$ was prepared from the intermediate 12 and purified by silica gel chromatography with dichloromethane/methanol (60/1, v/v). Yield: $90 \%$.

General Procedure 4: Synthesis of Compounds D1-D3. To a solution of compound 13a ( $200 \mathrm{mg}, 0.48 \mathrm{mmol}$ ) in DMF ( 50 mL ), $\mathrm{NaH}(18.28 \mathrm{mg}, 0.72 \mathrm{mmol})$ was added portionwise at $0^{\circ} \mathrm{C}$. After stirring for $0.5 \mathrm{~h}, 2 \mathrm{f}$ or $2 \mathrm{~g}(0.72 \mathrm{mmol})$ was added. The reaction mixture was stirred at $70{ }^{\circ} \mathrm{C}$ for 12 h , and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added dropwise followed by EtOAc $(80 \mathrm{~mL})$. The organic phase was separated and washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.
$N$-(2-(Diethylamino)ethyl)-1-ethyl-4-(3-(4-((3-ethyl-3-hydroxypentyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2carboxamide (D1). Dichloromethane/methanol (50/1). Oil. 45\% yield.

N-(2-(Diethylamino) ethyl)-1-ethyl-4-(3-(4-((4-ethyl-4-hydroxyhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D2). Dichloromethane/methanol (70/1). Oil. 46\% yield.

N-(2-(Diethylamino) ethyl)-1-ethyl-4-(3-(4-((5-ethyl-5-hydroxyheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2carboxamide (D3). Dichloromethane/methanol (100/1). Oil. 53\% yield.

General Procedure 5: Synthesis of Compounds D4-D19. To a solution of compound $\mathbf{1 3 b}(205 \mathrm{mg}, 0.48 \mathrm{mmol})$ in DMF ( 50 mL ), $\mathrm{NaH}(18.28 \mathrm{mg}, 0.72 \mathrm{mmol})$ was added portionwise at $0{ }^{\circ} \mathrm{C}$. After stirring for 0.5 h , one of $\mathbf{2 a} \mathbf{- 2 1}(0.72 \mathrm{mmol})$ was added. The reaction mixture was stirred at $70{ }^{\circ} \mathrm{C}$ for 12 h and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added dropwise followed by EtOAc $(80 \mathrm{~mL})$. Organic phase was separated and washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-(3-hydroxy-3-meth-ylbutoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D4). Dichloromethane/methanol (100/1). Oil. 35\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-4-(3-(4-((5-hydroxy-5-methylhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D5). Dichloromethane/methanol (100/1). Oil. $45 \%$ yield.

N-(3-(Diethylamino) propyl)-1-ethyl-4-(3-(4-((6-hydroxy-6-methylheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D6). Dichloromethane/methanol (100/1). Oil. 62\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((3-ethyl-3-hydroxypentyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-
carboxamide (D7). Dichloromethane/methanol (100/1). Oil. 42\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((5-ethyl-5-hydroxyheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2carboxamide (D8). Dichloromethane/methanol (100/1). Oil. 66\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-4-(3-(4-((6-ethyl-6-hydroxyoctyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D9). Dichloromethane/methanol (100/1). Oil. $43 \%$ yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((3-hydroxy-3-propylhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D10). Dichloromethane/methanol (100/1). Oil. 49\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((5-hydroxy-5-propyloctyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D11). Dichloromethane/methanol (100/1). Oil. 34\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((6-hydroxy-6-propylnonyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D12). Dichloromethane/methanol (100/1). Oil. 62\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((4-hydroxy-4-methylpentyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D13). Dichloromethane/methanol (100/1). Oil. 50\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((4-ethyl-4-hydroxyhexyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D14). Dichloromethane/methanol (100/1). Oil. 61\% yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-((4-hydroxy-4-propylheptyl)oxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D15). Dichloromethane/methanol (100/1). Oil. 53\% yield.

N-(3-(Diethylamino) propyl)-1-ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D16). Dichloromethane/methanol ( $50 / 1$ ). Oil. $71 \%$ yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-(2-hydroxybutoxy)-3-methylphenyl) pentan-3-yl)-1H-pyrrole-2-carboxamide (D17). Dichloromethane/methanol (50/1). Oil. $58 \%$ yield.

4-(3-(4-(2-Cyclopropyl-2-hydroxyethoxy)-3-methylphenyl)-pentan-3-yl)-N-(3-(diethylamino)propyl)-1-ethyl-1H-pyrrole-2-carboxamide (D18). Dichloromethane/methanol ( $50 / 1$ ). Oil. $43 \%$ yield.

N-(3-(Diethylamino)propyl)-1-ethyl-4-(3-(4-(2-hydroxy-2-phenyl-ethoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (D19). Dichloromethane/methanol (50/1). Oil. $51 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-1H-pyrrole-2-carboxylic acid (14). To a solution of compound $7(200 \mathrm{mg}, 0.63 \mathrm{mmol})$ in DMF ( 50 mL ) was added NaH ( $22.84 \mathrm{mg}, 0.95 \mathrm{mmol}$ ) at $0{ }^{\circ} \mathrm{C}$ and stirred for 1 h .4 -Bromo-2-methylbutan- 2 -ol ( $157 \mathrm{mg}, 0.95 \mathrm{mmol}$ ) was added to the mixture at 0 ${ }^{\circ} \mathrm{C}$. The mixture reacted for 12 h at $70^{\circ} \mathrm{C}$, water $(10 \mathrm{~mL})$ and EtOAc $(30 \mathrm{~mL})$ was added, and the organic phase was separated. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with dichloromethane/methanol (100/1, v/ v) to give compound 14 as oil ( $0.18 \mathrm{~g}, 71 \%$ yield).

General Procedure 6: Synthesis of Compounds H1-H10. To a solution of compound $14(200 \mathrm{mg}, 0.50 \mathrm{mmol})$ in dichloromethane ( 50 mL ), 3-(ethyliminomethylideneamino)- $\mathrm{N}, \mathrm{N}$-dimethylpropan-1amine (hydrochloride) ( $116.2 \mathrm{mg}, 0.75 \mathrm{mmol}$ ), 1-hydroxybenzotriazole ( $101.0 \mathrm{mg}, 0.75 \mathrm{mmol}$ ), and triethylamine ( $75.6 \mathrm{mg}, 0.75$ mmol ) was added at $0{ }^{\circ} \mathrm{C}$. After stirring for 0.5 h , the corresponding amine fragment $(0.75 \mathrm{mmol})$ was added. The reaction mixture was stirred at room temperature for 12 h , and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with the appropriate mixture as indicated in each case.

N-(2-(Dimethylamino)ethyl)-1-ethyl-5-(3-(4-(3-hydroxy-3-meth-ylbutoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (H1). Dichloromethane/methanol (70/1). Oil. $83 \%$ yield.

N-(2-(Diethylamino)ethyl)-1-ethyl-5-(3-(4-(3-hydroxy-3-methyl-butoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (H2). Dichloromethane/methanol (100/1). Oil. $73 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(2-(piperidin-1-yl)ethyl)-1H-pyrrole-2-carboxamide (H3). Dichloromethane/methanol (50/1). Oil. $66 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(2-morpholinoethyl)-1H-pyrrole-2-carboxamide (H4). Dichloromethane/methanol (100/1). Oil. 71\% yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(2-(4-methylpiperazin-1-yl)ethyl)-1H-pyrrole-2-carboxamide (H5). Dichloromethane/methanol (40/1). Oil. $80 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-((tetrahydrofuran-2-yl)methyl)-1H-pyrrole-2-carboxamide (H6). Dichloromethane/methanol (50/1). Oil. $61 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrole-2carboxamide (H7). Dichloromethane/methanol (40/1). Oil. 51\% yield.

N-(2,3-Dihydroxypropyl)-1-ethyl-5-(3-(4-(3-hydroxy-3-methylbu-toxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (H8). Dichloromethane/methanol (70/1). Oil. $90 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(3-morpholinopropyl)-1H-pyrrole-2-carboxamide (H9). Dichloromethane/methanol ( $50 / 1$ ). Oil. $53 \%$ yield.

1-Ethyl-5-(3-(4-(3-hydroxy-3-methylbutoxy)-3-methylphenyl)-pentan-3-yl)-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-2carboxamide (H10). Dichloromethane/methanol (40/1). Oil. 88\% yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxylic acid (15). To a stirred solution of 12 $(200 \mathrm{mg}, 0.63 \mathrm{mmol})$ in acetone $(50 \mathrm{~mL})$ was added $\mathrm{K}_{2} \mathrm{CO}_{3}(131.3$ $\mathrm{mg}, 0.95 \mathrm{mmol})$ and 1 -bromopropan-2-ol $(131.1 \mathrm{mg}, 0.95 \mathrm{mmol})$ at 0 ${ }^{\circ} \mathrm{C}$. The mixture was refluxed for 6 h and then cooled. The precipitate was filtered off, the solution was evaporated, and water ( 100 mL ) and EtOAc ( 80 mL ) was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by column chromatography with dichloromethane/methanol ( $100 / 1, \mathrm{v} / \mathrm{v}$ ) to give compound 15 as oil ( 0.25 g , $71 \%$ yield).

General Procedure 7: Synthesis of Compounds 11-111. To a solution of compound $15(186.6 \mathrm{mg}, 0.50 \mathrm{mmol})$ in dichloromethane $(50 \mathrm{~mL}), 3$-(ethyliminomethylideneamino)- $\mathrm{N}, \mathrm{N}$-dimethylpropan-1amine (hydrochloride) ( $116.2 \mathrm{mg}, 0.75 \mathrm{mmol}$ ), 1-hydroxybenzotriazole ( $101.0 \mathrm{mg}, 0.75 \mathrm{mmol}$ ), and triethylamine ( $75.6 \mathrm{mg}, 0.75$ mmol ) was added at $0^{\circ} \mathrm{C}$. After stirring for 0.5 h , the corresponding amine fragment $(0.75 \mathrm{mmol})$ was added. The reaction mixture was stirred at room temperature for 12 h , and then $\mathrm{H}_{2} \mathrm{O}(100 \mathrm{~mL})$ was added. The organic phase was washed with brine and dried over anhydrous $\mathrm{Na}_{2} \mathrm{SO}_{4}$, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case.
N-(2-(Dimethylamino)ethyl)-1-ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (I1). Dichloromethane/methanol (40/1). Oil. 70\% yield.

N-(2-(Diethylamino)ethyl)-1-ethyl-4-(3-(4-(2-hydroxypropoxy)-3methylphenyl) pentan-3-yl)-1H-pyrrole-2-carboxamide (12). Dichloromethane/methanol (40/1). Oil. $60 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-(2-(piperidin-1-yl)ethyl)-1H-pyrrole-2-carboxamide (I3). Dichloromethane/methanol (30/1). Oil. 73\% yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-(2-morpholinoethyl)-1H-pyrrole-2-carboxamide (14). Dichloromethane/methanol (40/1). Oil. $82 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-(2-(4-methylpiperazin-1-yl)ethyl)-1H-pyrrole-2-carboxamide (I5). Dichloromethane/methanol (30/1). Oil. $85 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-((tetrahydrofuran-2-yl)methyl)-1H-pyrrole-2-carboxamide (I6). Dichloromethane/methanol (50/1). Oil. $84 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-((tetrahydro-2H-pyran-4-yl)methyl)-1H-pyrrole-2-carboxamide (I7). Dichloromethane/methanol (40/1). Oil. $88 \%$ yield.

N-(2,3-Dihydroxypropyl)-1-ethyl-4-(3-(4-(2-hydroxypropoxy)-3methylphenyl) pentan-3-yl)-1H-pyrrole-2-carboxamide (I8). Dichloromethane/methanol (70/1). Oil. $67 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-(3-morpholinopropyl)-1H-pyrrole-2-carboxamide (I9). Dichloromethane/methanol (40/1). Oil. $79 \%$ yield.

1-Ethyl-4-(3-(4-(2-hydroxypropoxy)-3-methylphenyl)pentan-3-yl)-N-(3-(4-methylpiperazin-1-yl)propyl)-1H-pyrrole-2-carboxamide (I10). Dichloromethane/methanol (40/1). Oil. 81\% yield.

N-(3-(bis(2-Hydroxyethyl)amino)propyl)-1-ethyl-4-(3-(4-(2-hy-droxypropoxy)-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxamide (I11). Dichloromethane/methanol (30/1). Oil. 66\% yield.

VDR Binding Assay. The VDR binding affinity of nonsecosteriodal VDR ligands was measured by a PolarScreen VDR Competitor Assay following the procedure previously described. All compounds were tested for their binding affinity at $1 \mu \mathrm{M}$ in triplicates. Fluorescence polarization was measured on an Ultra384 microplate reader (Biotek) using a 535 nm excitation filter ( 25 nm bandwidth) and a 590 nm emission filter ( 20 nm bandwidth).

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 $\times 3$-LUC reporter plasmid, 20 ng of pCMXRenilla, 30 ng of $\mathrm{pENTER}-\mathrm{CMV}-\mathrm{hRXR} \alpha$, and 100 ng of $\mathrm{pCMX}-\mathrm{VDR}$ for each well using a Lipofectamine2000 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.

MTT Assay. The effects of the compounds on the proliferation of the DSL-6A/C1 cells were evaluated by MTT (3-(4,5-dimethylth-iazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; Sigma) assay. Briefly, after the PSC cells (LTC-14) were treated with compounds for 24 h , the medium supernatant of PSC (PSC-CM) was collected and added into the pancreatic cancer cells DSL-6A/C1. After incubation with $10 \mu \mathrm{~L}$ of MTT $(5 \mathrm{mg} / \mathrm{mL})$ for 4 h , absorbance of the soluble MTT product was measured at 570 nm . The antiproliferation assay was performed in triplicate.

Anti-PSC Activity Assay. 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. Approximate 1 $\times 10^{5}$ cells, suspended in the medium, were plated into each well of a 24-well plate and grown at $37^{\circ} \mathrm{C}$ in a humidified atmosphere with $5 \%$ $\mathrm{CO}_{2}$ for 24 h . The following day, tested compounds $(0.5 \mu \mathrm{M})$ and TGF- $\beta 1(5 \mathrm{ng} / \mathrm{mL})$ were added to the culture medium and incubated for 24 h . RNA extracts were derived from LTC-14 cells for Q-PCR assay and proteins were purified for western blot.

RNA Extraction and Quantitative Real-Time Polymerasechain Reaction (Q-PCR). cDNA was generated from RNA extracts derived from cultured LTC-14 cells and pancreas tissues using a reverse transcription kit (Vazyme). $\beta$-actin (rat) was used as an internal control. Q-PCR was performed using the SYBR Green Master Mix (Yeasen Biotech Co., Ltd.) using a StepOnePlus RealTime PCR System (Applied Biosystems).

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: rabbit anti- $\alpha$-SMA, rabbit anticollagen I, rabbit anti- PDGF-A, rabbit anti-POSTN, and rabbit anti-$\beta$-actin (Absin, Shanghai, China). Horseradish peroxidase-conjugated goat anti-rabbit/mouse IgG (Boster, Wuhan, China) was used as the secondary antibody. Immunoreactive bands were visualized with a Tanon 5200 Multi Chemiluminescence Imaging System and analyzed with Image J .

Antitumor Effect of Compound In Vivo. Female BALB/c nude mice ( $18-22 \mathrm{~g}$ ) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. All animals were treated according to the guidelines for the care and use of laboratory animals approved by the Animal Science Ethics Committee of China Pharmaceutical University. Mice were randomly divided into six groups (five mice in each group). All mice were maintained under standard conditions with free access to water and laboratory rodent food. LTC-14-GFP ( $1 \times 10^{6}$ cells per mouse) and DSL-6A/C1 Luci $\left(1 \times 10^{6}\right.$ cells per mouse) were co-inoculated in situ on the pancreas of nude mice, and the administration was started 7 days after tumor grafting. The compound was dissolved in ethanol/polyoxyethylene castor oil/normal saline ( $1: 1: 18$ ) mixed solvent, and the mice were intraperitoneally injected with normal saline, gemcitabine, gemcitabine + calcipotriol $(60 \mu \mathrm{~g} / \mathrm{kg})$, gemcitabine $+\mathbf{C} 4(500 \mu \mathrm{~g} / \mathrm{kg})$, gemcitabine $+\mathrm{I} 5(500 \mu \mathrm{~g} / \mathrm{kg})$, and gemcitabine $+\mathrm{I} 8(500 \mu \mathrm{~g} / \mathrm{kg})$, once every 3 days. The dosage of gemcitabine was $100 \mathrm{mg} / \mathrm{kg}$ in each group. Tumor images were taken on the Maestro in vivo imaging system (PerkinElmer) during the 12 day treatment study. Mice were sacrificed 3 days after the final administration. Mouse pancreas and serum were obtained for histopathology, collagen assay, biochemical, and molecular analyses.

Immunofluorescence Analysis. Identification of the $\alpha$-SMA by immunofluorescence microscopy was carried out as follows: The tumor sections were fixed with $4 \%$ paraformaldehyde for 10 min , followed by $0.5 \%$ Triton X-100 to perforate cell membranes at room temperature. After careful washing with PBS three times, the samples were blocked at $37{ }^{\circ} \mathrm{C}$ for 30 min with $5 \%$ BSA. The tumor sections were incubated with primary antibodies to $\alpha$-SMA overnight at $4{ }^{\circ} \mathrm{C}$. After careful washing with PBS, tumor sections were incubated with the fluorescent secondary antibody Alexa Fluor ${ }^{\text {TM }} 594$ donkey antirabbit $\operatorname{IgG}(\mathrm{H}+\mathrm{L})$ in a $37{ }^{\circ} \mathrm{C}$ incubator for 1 h . The clusters were washed twice with PBS and stained with DAPI for 15 min at room temperature. After washing twice more, the samples were observed using a laser confocal scanning microscope (LSM880, Carl Zeiss Jena).

Statistical Analysis. Statistical analyses were performed using GraphPad Prism 8.0. All plots show mean $\pm$ SD from at least three independent experiments. Student's t-test was applied for comparisons between two groups, one-way ANOVA with Dunnett's post hoc test was used for comparisons of multiple groups, and a log-rank (Mantel-Cox) test was used to analyze the statistical significance of difference for survival analysis. Statistical significance was set at $* P<$ 0.05 , $* * P<0.01$ and $* * * P<0.001$.

## ASSOCIATED CONTENT

## si Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01197.
(Table S1) Cell toxicity of all target compounds against LTC-14; (Figure S 1 ) cell toxicity of representative compounds against DSL-6A/C1 cells; (Figure S2) relative BLI intensities of mice that received different treatments; (Figure S3) inhibition rate of tumor growth after tumor-bearing mice received different treatments; (Figure S4) relative fluorescence intensities of $\alpha$-SMA after mice received different treatments; (Figure S5) survival percentages of mice that received different treatments; (Figure S6) in vivo hypercalcemic effects in the orthotopic transplantation pancreatic cancer model; NMR spectra information; HPLC traces; and ${ }^{1} \mathrm{H}$ NMR and ${ }^{13} \mathrm{C}$ NMR spectra (PDF)

Molecular Formula Strings (CSV)

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## Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

ECM, extracellular matrix; LBP, ligand binding pocket; VDR, vitamin D receptor; PSC, pancreatic stellate cell; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; HGF, periostin and hepatocyte growth factor; IGF-1, insulinlike growth factor 1; TGF, transforming growth factor; $\alpha$-SMA, $\alpha$-smooth muscle actin; POSTN, periosteal osteoblast specific factor 2; CM, conditioned media; DOX, doxorubicin; RXR $\alpha$, retinoid X receptor alpha

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[^1]:    ${ }^{a}$ Reagents and conditions: (a) 4-bromo-2-methylbutan-2-ol, NaH, DMF, 0-70 ${ }^{\circ} \mathrm{C}$, 12 h ; (b) EDCI, HOBt, TEA, RNH 2 , DCM, rt, overnight.

