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Original article

Novel nonsecosteroidal VDR agonists with phenyl-pyrrolyl pentane skeleton

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ABSTRACT

In order to find the vitamin D receptor (VDR) ligand whose VDR agonistic activity is separated from the calcemic activity sufficiently, novel nonsecosteroidal analogs with phenyl–pyrrolyl pentane skeleton were synthesized and evaluated for the VDR binding affinity, antiproliferative activity *in vitro* and serum calcium raising ability *in vivo* (tacalcitol used as control). Among them, several compounds showed varying degrees of VDR agonistic and growth inhibition activities of the tested cell lines. The most effective compound **2g** (EC_{50}: 1.06 nM) exhibited stronger VDR agonistic activity than tacalcitol (EC_{50}: 7.05 nM), inhibited the proliferations of HaCaT and MCF-7 cells with IC50 of 2.06 μM and 0.307 μM (tacalcitol: 2.07 μM and 0.057 μM) and showed no significant effect on serum calcium.

1. Introduction

1α,25-Dihydroxyvitamin D3 ($1,25(OH)_2D_3$, Fig. 1) is the biologically active form of vitamin D in human, which plays an important role in calcium homeostasis [1,2], cell differentiation and proliferation as well as immunomodulation [3,4]. The physiological effects of $1,25(OH)_2D_3$ are exerted by binding to vitamin D receptor (VDR) which belongs to the nuclear receptor superfamily [5]. Once binding with the ligand, VDR becomes active and forms a complex with co-factors, which consequently binds to vitamin D responsive element (VDRE) in the promoter moiety of the target genes leading to transactivation [6].

The secosteroidal VDR ligands (Fig. 1) have showed their therapeutic abilities in several human diseases such as psoriasis, osteoporosis and secondary hyperparathyroidism [7,8]. They have also exhibited efficacy in various cells and animal models of autoimmune diseases like arthritis, multiple sclerosis and inflammatory bowel disease as well as cancers of prostate, colon and breast [9]. However, the widely use of these classic VDR ligands as drugs is limited by their associated toxicity, namely hypercalcemia. Among these ligands, some decreased calcemic activity by structure modification, such as tacalcitol and calcipotriol (Fig. 1), which have been approved for topical treatments of mild to moderate psoriasis. But their pharmacological activities were separated from the calcemic activity insufficiently, so for the consideration of long-term therapy (especially oral) of diseases like cancers and severe psoriasis, the risk of hypercalcemia still existed [10]. Therefore, there is an unmet clinical need for exploring VDR agonists whose calcemic activity was effectively reduced.

In 1999, a series of nonsecosteroidal ligands of VDR (such as LG190155, Fig. 2) which mimic various activities of $1,25(OH)_2D_3$ *in vitro* were identified [11]. Although their VDR agonistic activities were weaker than $1,25(OH)_2D_3$, they showed no calcemic potential *in vivo*. These nonsecosteroidal VDR agonists provided opportunities for developing novel, oral medications to treat cancer, leukemia and psoriasis. In addition, the synthetic accessibility of these bis-phenyl derivatives made them attractive targets for drug discovery.

In order to find potent VDR agonists which rival $1,25(OH)_2D_3$ in biological activities but exhibit no calcemic activity like LG190155, we designed a series of phenyl-pyrrolyl pentane derivatives, using LG190155 as the leading compound. In this work, we found the potential of constructing the phenyl-pyrrolyl pentane skeleton, and introducing side chains which have abilities to form the hydrogen bond for the VDR agonists. First, we changed one benzene ring of the leading compound into the pyrrole ring to create a novel scaffold. Then we retained the 2-oxo-3,3-dimethylbutoxy chain at the 4-position of the benzene ring and introduced different amino acid esters to the 2-position of the pyrrole ring. At last, we adjusted the polarities of two side chains by reduction or hydrolysis reaction.

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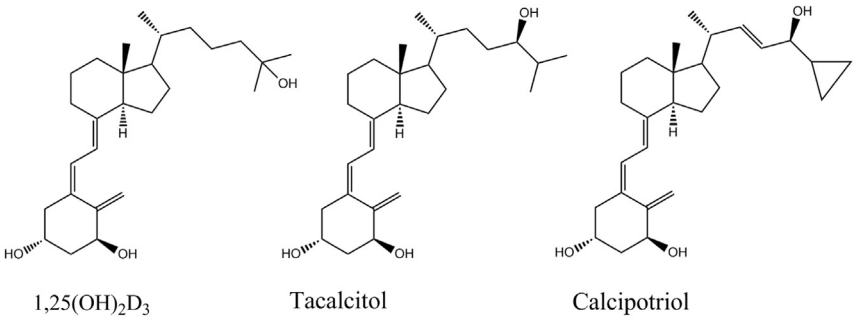


Fig. 1. The structures of secosteroidal VDR ligands.

Among the designed analogs, Compounds **2d**, **2e**, **2g** and **2h** demonstrated promising VDR binding affinities and antiproliferation activities against keratinocyte and tumor cells. Especially compound **2g** showed better VDR agonistic activity and the closely antiproliferation activities, compared with tacalcitol. Meanwhile, the calcium raising ability of **2g** was almost lost. Docking study was also carried out to understand the structure–activity relationships of these novel VDR agonists.

2. Results and discussion

2.1. Chemistry

The synthetic route was shown in Scheme 1. Compound **1**, esterificated by methanol, produced **2**. Then, protection of phenolic hydroxyl group of **2** with benzyl bromide created **3**, which, was given a further nucleophilic attack from ethylmagnesium bromide and **4** was generated. Coupling with ethylpyrrole-2-carboxylate, compound **4** produced the key intermediate **5** in the presence of boron fluoride ethyl ether. With different alkyl iodides, alkylating at 1-postion of pyrrole ring of **5** afforded **6**. After the deprotection of **6**, in the presence of K_2CO_3, the phenolic hydroxyl group was substituted by 1-chloropinacolone in the boiling acetone, and **8** was formed. Compound **8** was hydrolyzed by potassium hydroxide, and the exposed carboxyl group was acylated with different amino acid methyl ester hydrochlorides under the condition of EDCI/HOBT to form compounds **1a**–**1h**. In methanol, simultaneous reduction of both carbonyl group and methyl ester group of **1a**–**1h** was given by sodium borohydride, and compounds **2a**–**2h** could be formed. Hydrolyzed by lithium hydroxide, the methyl ester group of **1a**–**1h** produced compounds **3a**–**3h**. At last, reduced by sodium borohydride towards carbonyl group of **3a**–**3h**, compounds **4a**–**4h** were synthesized in methanol (Fig. 2).

2.2. Biological activities

2.2.1. In vitro HL-60 differentiation-inducing activity (VDR binding affinity)

It was examined that the HL-60 differentiation-inducing activity and the VDR binding affinity were relative, so the VDR binding

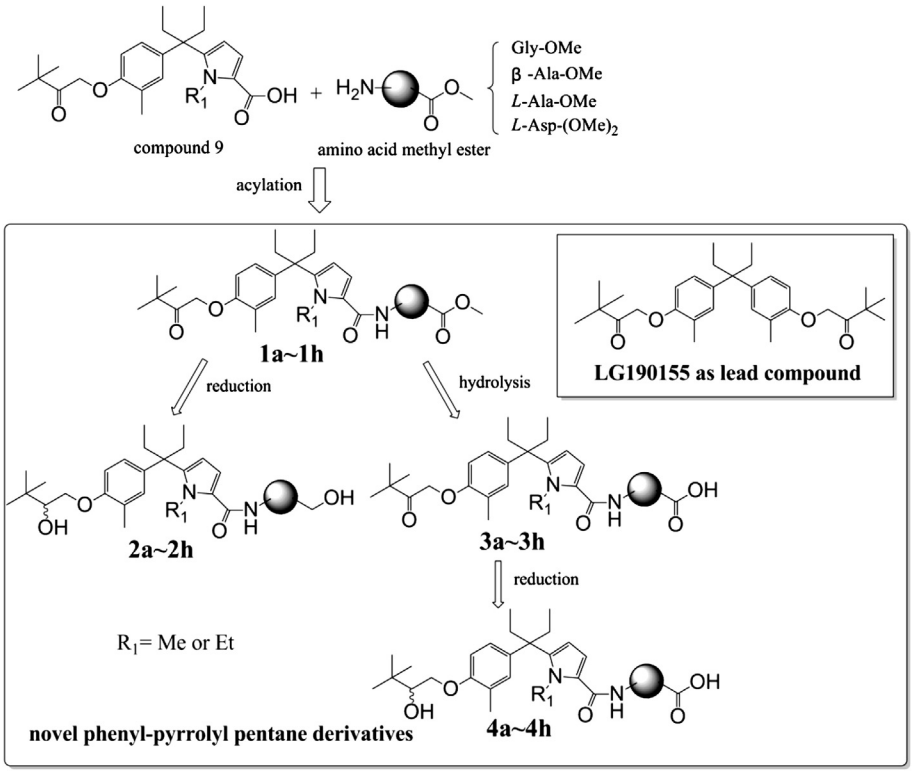
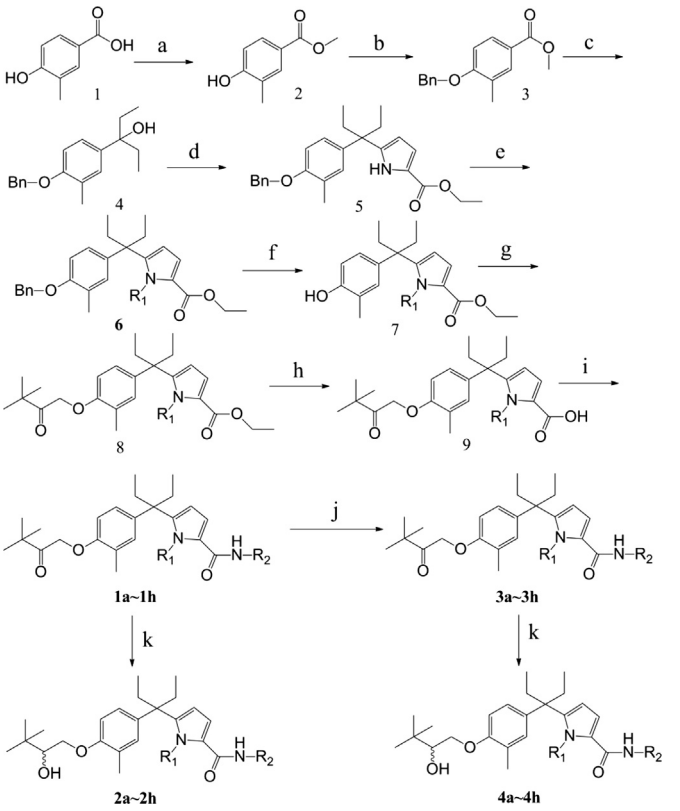


Fig. 2. The designed VDR agonists.



Scheme 1. Reagents and reaction conditions: (a) conc. H_2SO_4, CH_3OH, 70 °C, overnight; (b) BnBr, acetone, K_2CO_3, KI, reflux, 6 h; (c) C_2H_5MgBr, Et_2O, 25 °C, 2 h; (d) Ethylpyrrole-2-carboxylate, $BF_3{\cdot}Et_2O$, 25 °C, 1 h; (e) alkyl iodide, NaH, DMF, 0 °C–25 °C, 2 h; (f) Pd/C, $HCOONH_4$, CH_3OH, 25 °C, overnight; (g) 1-chloropinacolone, acetone, K_2CO_3, KI, reflux, 12 h; (h) KOH, H_2O, C_2H_5OH, 70 °C, overnight; (i) Amino acid methyl ester hydrochloride, EDCI, HOBt, Et_3N, DMF, 0 °C–25 °C, overnight; (j) $LiOH{\cdot}2H_2O$, THF, H_2O, 25 °C, overnight; (k) $NaBH_4$, CH_3OH, 0 °C–25 °C, 2 h–6 h.

affinity was measured in terms of the activity of differentiation of human promyelocytic leukemia cell line (HL-60) into macrophages, as had usually been done [12,13]. Tacalcitol was used as control for its strong VDR agonistic activity as $1,25(OH)_2D_3$ but weaker induction of hypercalcemia [14]. All the designed compounds were tested for the effect on the differentiation of HL-60 cells, as shown in Table 1. Among them, six compounds of **2a**–**2h** embodied varying degrees of VDR agonistic activities (EC_{50}: 5.64 μM–1.06 nM), with two side chains containing two or three hydroxyl groups. In particular, compound **2g** revealed the best VDR agonistic activity with EC_{50} at 1.06 nM, while the EC_{50} of tacalcitol was 7.05 nM. Four compounds of **4a**–**4h** revealed the VDR agonistic activities at the EC_{50} from 32.22 μM to 4.07 nM, and among them, compound **4g** showed the best differentiation-inducing activity with EC_{50} at 4.07 nM. There were also three compounds belonged to **1a**–**1h** and two compounds belonged to **3a**–**3h** showed moderate VDR agonistic activities whose structures had no hydroxyl group or carboxyl group in the side chains. (EC_{50}: 2.62 μM–23 nM).

According to the results, 75% compounds of **2a**–**2h** had the acceptability to an excellent VDR agonistic activities. It may due to the hydroxyl groups on the two side chains are able to mimic the function of hydroxyl groups in $1,25(OH)_2D_3$ [6] and form hydrogen bonds with amino acid residues of VDR. Although compound **4g** showed stronger agonistic activity than tacalcitol did, half compounds of **4a**–**4h** showed no significant agonistic activity, which suggested introducing carboxyl group into the side chain could reduce the VDR binding affinities of the compounds. It is presumed that the reduction of the binding affinity could be attributed to the increased polarity of these compounds. The least agonistic activity

Table 1
Structures and VDR binding affinities of the designed compounds.

Compd	R_1	R_2	VDR binding affinity[a] EC_{50}[b] (μM)
1a	Me	$-CH_2COOCH_3$	0.023
1b	Me	$-CH_2CH_2COOCH_3$	0.79
1c	Me	(*S*)-$CH(CH_3)COOCH_3$	—[c]
1d	Me	(*S*)-$CH(CH_2COOCH_3)COOCH_3$	—[c]
1e	Et	$-CH_2COOCH_3$	1.83
1f	Et	$-CH_2CH_2COOCH_3$	—[c]
1g	Et	(*S*)-$CH(CH_3)COOCH_3$	—[c]
1h	Et	(*S*)-$CH(CH_2COOCH_3)COOCH_3$	—[c]
2a	Me	$-CH_2CH_2OH$	—[c]
2b	Me	$-CH_2CH_2CH_2OH$	0.15
2c	Me	(*S*)-$CH(CH_3)CH_2OH$	0.57
2d	Me	(*S*)-$CH(CH_2CH_2OH)CH_2OH$	0.20
2e	Et	$-CH_2CH_2OH$	5.64
2f	Et	$-CH_2CH_2CH_2OH$	—[c]
2g	Et	(*S*)-$CH(CH_3)CH_2OH$	1.06E-03
2h	Et	(*S*)-$CH(CH_2CH_2OH)CH_2OH$	0.11
3a	Me	$-CH_2COOH$	—[c]
3b	Me	$-CH_2CH_2COOH$	—[c]
3c	Me	(*S*)-$CH(CH_3)COOH$	2.63
3d	Me	(*S*)-$CH(CH_2COOH)COOH$	0.41
3e	Et	$-CH_2COOH$	—[c]
3f	Et	$-CH_2CH_2COOH$	—[c]
3g	Et	(*S*)-$CH(CH_3)COOH$	—[c]
3h	Et	(*S*)-$CH(CH_2COOH)COOH$	—[c]
4a	Me	$-CH_2COOH$	4.95
4b	Me	$-CH_2CH_2COOH$	1.6
4c	Me	(*S*)-$CH(CH_3)COOH$	—[c]
4d	Me	(*S*)-$CH(CH_2COOH)COOH$	—[c]
4e	Et	$-CH_2COOH$	—[c]
4f	Et	$-CH_2CH_2COOH$	33.22
4g	Et	(*S*)-$CH(CH_3)COOH$	4.07E-03
4h	Et	(*S*)-$CH(CH_2COOH)COOH$	—[c]
Tacalcitol			7.05E-03

[a] VDR binding affinity was measured as HL-60 differentiation-inducing activity.
[b] Data shown are from triplicate experiments.
[c] Inactive at >50 μM.

showed in compounds of **1a**–**1h** and **3a**–**3h** could be explained by the weak hydrogen-bonding interactions between the compounds and amino acid residues of VDR when there were no conjunct hydroxyl groups or carboxyl groups in two side chains.

2.2.2. *In vitro cell cytotoxicity assay*

Cell cytotoxicity assay of the compounds exhibiting VDR binding affinity was evaluated against HaCaT and MCF-7 cells, using tacalcitol as the positive control. As shown in Table 2, compound **2g** which displayed the strongest VDR agonistic activity exhibited good antiproliferative activities against HaCaT and MCF-7 cells, with IC_{50} at 2.06 and 0.32 μM respectively. Compound **2d** only revealed the moderate VDR binding affinity though, showed the best antiproliferative effects, with each IC_{50} at 0.19 μM and 7.83 nM against HaCaT and MCF-7 cells, and this result indicated that it may work though the multiple molecular mechanisms.

Compounds **2d**, **2e**, **2g** and **2h** demonstrated favorable antiproliferative activities against both two cell lines. It was found that all the side chains of these four compounds have been substituted by hydroxyl groups. In this regard, it could be proved that the antiproliferative activity of the designed compounds is positively correlative with VDR binding affinity, and the hydroxyl groups in the side chains might be the pharmacophores of the phenylpyrrolyl pentane derivatives.

2.2.3. *In vivo calcemic activity assay*

Calcemic activity assay *in vivo* was measured to testify the effect of the designed nonsecosteroidal analogs on inducing hypercalcemic. The two representative compounds, **2g** and **2d**, were chosen for the test. The results were shown in Fig. 3.

Table 2
Cellular antiproliferative activities of the selected compounds.

Compd	Cell inhibition IC_{50}[a] (μM)		Compd	Cell inhibition IC_{50}[a] (μM)	
	HaCaT[c]	MCF-7		HaCaT[c]	MCF-7
1a	—[b]	—[b]	**2h**	1.25	0.30
1b	28.89	—[b]	**3c**	—[b]	—[b]
1e	—[b]	—[b]	**3d**	—[b]	0.16
2b	—[b]	16.35	**4a**	9.81	—[b]
2c	15.72	—[b]	**4b**	—[b]	—[b]
2d	0.19	7.83×10^{-3}	**4f**	—[b]	—[b]
2e	2.08	1.81	**4g**	—[b]	0.18
2g	2.06	0.32	Tacalcitol	2.07	0.057

[a] Data shown are from triplicate experiments.
[b] Inactive at >50 μM.
[c] HaCaT cell is an immortal human keratinocyte line commonly used as a model system for vitamin D_3 metabolism in human skin.

A significant increase in serum calcium (12.14 mg/dL compared with 8.76 mg/dL in control mice; $P < 0.05$) was noticed when mice were treated with 5 μg/kg tacalcitol each day. Compounds **2g** and **2d** had no effect on serum calcium at serial concentrations (8.09 and 8.91 mg/dL at 0.5 mg/kg/day, 8.30 and 9.29 mg/dL at 10 mg/kg/day, 9.08 and 8.94 mg/dL at 30 mg/kg/day). Meanwhile the two compounds showed a dramatic decrease in serum calcium, compared with tacalcitol ($P < 0.01$ for **2g** and $P < 0.05$ for **2d**).

2.3. Molecular docking study

Using Schrödinger Glide version 7.3 and MOE 2009, the docking study was carried out to understand the strong VDR agonistic activity of compound **2g**.

The two compounds **2c** and **2g** shared the same side chains were docked into the VDR ligand binding domain (VDR LBD, PDB ID: 2ZFX) and the resulting structures of the complexes were showed and compared in Fig. 4. The most suitable conformations of the ligands were selected based on the calculated docking scores by means of the bonding strength.

It was found that the 2′-OH group and the oxygen atom beside the benzene ring of compound **2g** were able to form hydrogen bonds with the His 393 of VDR LBD. The 4-OH of compound **2g** interacted with Arg270 and Ser233 while 2-NH also formed a hydrogen bond with Ser233 of VDR LBD. Compound **2c** formed hydrogen-bonding interactions at 2′-OH with His 393 and 4-OH, as well as at Arg270 and Ser233. It is probable that the additional interactions with those amino acid residues of VDR LBD would make a stronger binding affinity. In other words, **2g** had a better VDR agonistic activity than **2c** did. These additional hydrogen-bonding interactions might be accredited to that a greater steric hindrance of ethyl group bonded to the 1-position of pyrrole ring is forcing the molecular conformation to be more curve than methyl group, which makes the near-end of the side chains closer to some amino acid residues of VDR LBD to form extra hydrogen bonds.

When the structure of the VDR LBD-**2g** complex overlapped with the VDR LBD-1,25$(OH)_2D_3$ complex, it could be found that, both **2g** and 1,25$(OH)_2D_3$ were embed in the same position of the binding pocket, as shown in Fig. 5. It was reported that the 25-OH group of 1,25$(OH)_2D_3$ could interact with His301 and His 393, and the 1-OH group could interact with Ser233 and Arg274 [15]. In comparison, the 2′-OH group of **2g** shared the same amino acid residue His 393 with 25-OH of 1,25$(OH)_2D_3$, and the 4-OH group of **2g** played the same role as 1-OH of 1,25$(OH)_2D_3$. No interactions with Tyr139 and Ser274 was observed in **2g**, while the 3-OH of 1,25$(OH)_2D_3$ formed hydrogen bonds with them was reported [15]. All of these demonstrated that compound **2g** worked similarly as the endogenous ligand.

3. Conclusions

In summary, a series of novel phenyl-pyrrolyl pentane derivatives were synthesized and first reported. Their VDR binding affinities were measured as the HL-60 differentiation-inducing activities *in vitro*. According to the results, the compounds containing groups which have abilities to form the hydrogen bond in the side chains exhibited good even excellent activities. Introducing hydroxyl groups to both side chains seemed to be the best choice to achieve the strongest activity. The results of cell cytotoxicity assay *in vitro* against HaCaT and MCF-7 cells demonstrated the conclusion indirectly as well. Compound **2g** revealed a remarkable VDR agonistic activity which even better than tacalcitol, with the EC_{50} value at 1.06 nM. Meanwhile, it had little effect on serum calcium. Simulated by the docking study, a summary of structure–activity relationships could be reached. Herein, these findings are suggesting the potential of constructing the hydrophobic phenyl-pyrrolyl pantene skeleton, and introducing side chains, about five atoms long, which are substituted by hydroxyl groups at the far-end for the vitamin D receptor agonists with extremely low calcemic mobilization.

4. Experiment

4.1. Regents and equipments

All commercially available starting materials, reagents and solvents were used without further purification unless otherwise stated. Melting points were determined and uncorrected employing an RY-1 apparatus from Tianjin Analytical Instrument Factory (China). High-resolution mass spectra (HRMS) were recorded on QSTAR XL Hybrid MS/MS mass spectrometer. ^1H NMR and ^{13}C NMR were recorded employing Bruker AV-300 or AV-500 instruments using $CDCl_3$ or DMSO-d_6. 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).

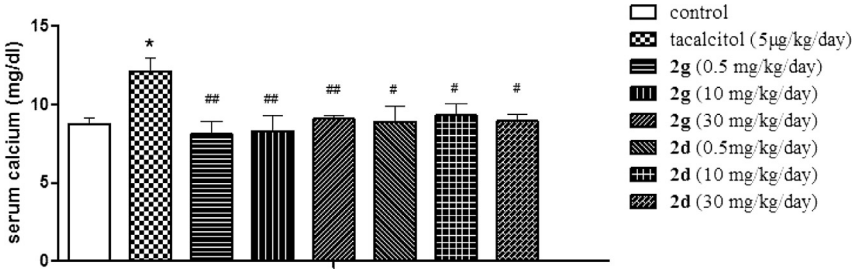


Fig. 3. *In vivo* calcemic effects of tacalcitol, compounds **2g** and **2d**.

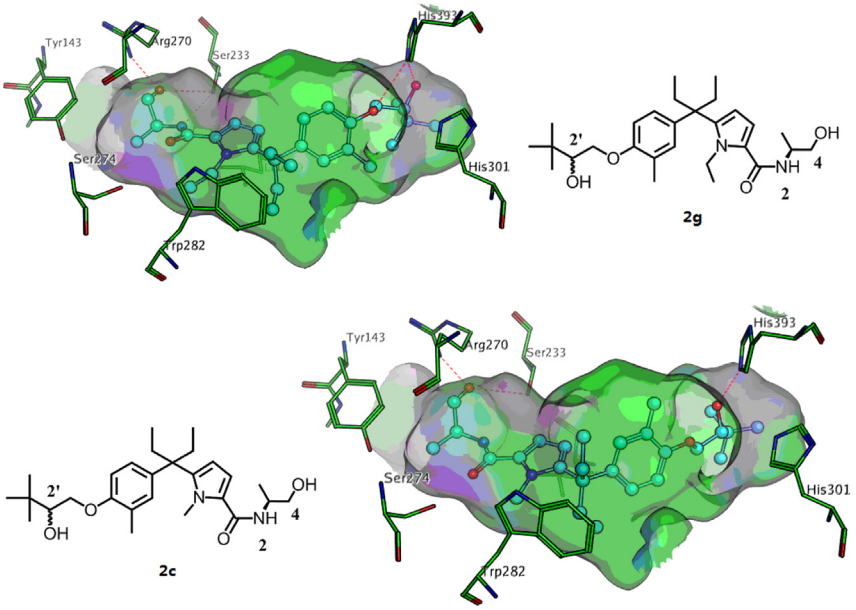


Fig. 4. Binding models of compound **2g** and **2c** docked into VDR ligand binding domain (VDR LBD).

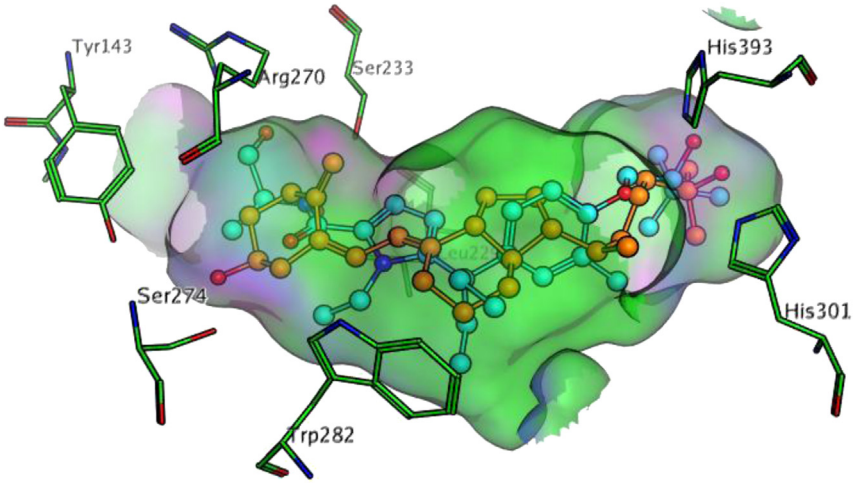


Fig. 5. Structure of the VDR LBD-2g complex overlapped with the VDR LBD-1,25(OH)$_2$D$_3$ complex.

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

To a solution of compound **1** (0.32 mol) in methanol (300 mL) was added conc. H_2SO_4 (45 mL). The reaction mixture was refluxed for 6 h and then cooled. The solution was adjusted to approx. pH 6 with 2 M NaOH and then poured into cold H_2O. The precipitate was filtered off and washed with cold water. The product was collected as a pink solid and dried (50.4 g, 92% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 6.98 (1H, d, J = 8.5 Hz), 6.50 (1H, d, J = 8.5 Hz), 5.40 (1H, bs), 3.88 (3H, s), 2.26 (3H, s).

4.3. Methyl-4-benzyloxy-3-methylbenzoate (**3**)

Benzyl bromide (112 mmol), K_2CO_3 (306 mmol) and KI (30.6 mmol) were added to a solution of compound **2** (102 mmol) in acetone. The mixture was refluxed for 6 h and then cooled. The precipitate was filtered off and the solution was evaporated. The residue was precipitated in petroleum ether to give compound **3** as pink crystal (25.3, 96% yield). Mp: 56–58 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46–7.31 (5H, m), 7.10 (1H, s), 7.04 (1H, d, J = 8.3 Hz), 6.69 (1H, d, J = 8.3 Hz), 5.04 (2H, s), 3.88 (3H, s), 2.24 (3H, s).

4.4. 3-(4-Benzyloxy-3-methylphenyl)pentan-3-ol (**4**)

To a solution of compound **3** (3.9 mmol) in ether (10 mL) was added ethyl Grignard reagent (15.6 mmol) dropwise at 0 °C. The reaction mixture was stirring at 30 °C for 2 h and then cooled. To the mixture was added an aqueous saturated solution of NH_4Cl and the two phases were separated. Aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give compound **4** as white oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49–7.28 (5H, m), 7.06 (1H, s), 7.03 (1H, d, J = 8.3 Hz), 6.79 (1H, d, J = 8.3 Hz), 5.04 (2H, s), 2.27 (3H, s), 1.77 (4H, q, J = 7.5 Hz), 0.97 (6H, t, J = 7.5 Hz).

4.5. Ethyl-5-(3-(4-benzyloxy-3-methylphenyl)pentan-3-yl)-1H-pyrrole-2-carboxylate (**5**)

$BF_3 \cdot Et_2O$ (1.3 mL) was added dropwise to a solution of compound **4** (4.6 mmol) and ethyl-1*H*-pyrrole-2-carboxylate (5.1 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was stirred for 1 h at 25 °C. Then the solution was added H_2O and organic phase was separated. The organic phases were washed

with brine and dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (12/1, v/v) to give compound **5** as yellow solid (1.35 g, 73% yield). Mp: 86–89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49–7.28 (5H, m), 7.03 (1H, s), 7.01 (1H, d, J = 8.5 Hz), 6.79 (1H, d, J = 8.5 Hz), 6.70 (1H, d, J = 2.0 Hz), 6.50 (1H, d, J = 2.0 Hz), 5.04 (2H, s), 4.30 (2H, q, J = 7.1 Hz), 2.24 (3H, s), 1.97 (4H, q, J = 7.3 Hz), 1.32 (3H, t, J = 7.1 Hz), 0.67 (6H, t, J = 7.3 Hz).

*4.6. General procedure for the synthesis of ethyl-5-(3-(4-benzyloxy-3-methylphenyl) pentan-3-yl)-1-alkylpyrrole-2-carboxylate derivatives (**6a** and **6b**)*

*4.6.1. Ethyl-5-(3-(4-benzyloxy-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxylate (**6a**)*

To a solution of compound **5** (0.6 mmol) in DMF (5 mL), NaH (1.2 mmol) was added portionwise at 0 °C. After stirring for 0.5 h, methyl iodide (0.8 mmol) was added. The reaction mixture was stirred at 25 °C for 2 h and then H_2O (20 mL) was added dropwise followed by ethyl acetate (10 mL). The organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with H_2O, brine and then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (20/1, v/v) to give compound **6a** as yellow oil (205 mg, 82.4% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49–7.29 (5H, m), 7.03 (1H, s), 7.02 (1H, d, J = 8.4 Hz), 6.79 (1H, d, J = 8.4 Hz), 6.60 (1H, d, J = 1.9 Hz), 6.49 (1H, d, J = 1.9 Hz), 5.04 (2H, s), 4.25 (2H, q, J = 7.1 Hz), 3.86 (3H, s), 2.25 (3H, s), 1.94 (4H, q, J = 7.3 Hz), 1.30 (3H, t, J = 7.1 Hz), 0.67 (6H, t, J = 7.3 Hz).

*4.6.2. Ethyl-5-(3-(4-benzyloxy-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxylate (**6b**)*

Yellow oil, 480 mg, 66.9% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50–7.28 (5H, m), 7.04 (1H, s), 7.02 (1H, d, J = 8.6 Hz), 6.77 (1H, d, J = 8.6 Hz), 6.70 (1H, d, J = 2.0 Hz), 6.57 (1H, d, J = 2.0 Hz), 5.04 (2H, s), 4.32–4.20 (4H, m), 2.25 (3H, s), 1.95 (4H, q, J = 7.0 Hz), 1.35–1.26 (6H, m), 0.67 (6H, t, J = 7.0 Hz).

*4.7. General procedure for the synthesis of ethyl-5-(3-(4-hydroxy-3-methylphenylpentan-3-yl)-1-alkylpyrrole-2-carboxylate derivatives (**7a** and **7b**)*

*4.7.1. Ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxylate (**7a**)*

To a solution of compound **6a** (10.2 mmol) in methanol (20 mL), Pd/C (0.43 g) and ammonium formate (102 mmol) was added. The reaction mixture was stirred at 25 °C overnight. The precipitate was filtered off, H_2O (40 mL) and ethyl acetate (10 mL) was added to the solution. The organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine, then dried over anhydrous Na_2SO_4 and evaporated to give compound **7a** as white solid (3.3 g, 98% yield). Mp: 78–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.98 (1H, s), 6.96 (1H, d, J = 8.3 Hz), 6.70 (1H, d, J = 2.0 Hz), 6.69 (1H, d, J = 8.3 Hz), 6.49 (1H, d, J = 2.0 Hz), 4.25 (2H, q, J = 7.1 Hz), 3.85 (3H, s), 2.21 (3H, s), 1.95 (4H, q, J = 7.4 Hz), 1.31 (3H, t, J = 7.1 Hz), 0.67 (6H, t, J = 7.4 Hz).

*4.7.2. Ethyl-5-(3-(4-hydroxy-3-methylphenyl)pentan-3-yl)-1-ethypyrrole-2-carboxylate (**7b**)*

White solid, 4.2 g, 97% yield; Mp: 72–74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.92 (1H, s), 6.88 (1H, d, J = 8.6 Hz), 6.63 (1H, d, J = 2.1 Hz), 6.60 (1H, d, J = 8.6 Hz), 6.50 (1H, d, J = 2.1 Hz), 4.25–4.06 (4H, m), 2.28 (3H, s), 1.87 (4H, q, J = 7.4 Hz), 1.30–1.25 (6H, m), 0.60 (6H, t, J = 7.4 Hz).

*4.8. General procedure for the synthesis of ethyl-5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-alkylpyrrole-2-carbocxylate (**8a** and **8b**)*

*4.8.1. Ethyl-5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-methylpyrrole-2-carbocxylate (**8a**)*

1-Chloropinacolone (29 mmol), K_2CO_3 (57 mmol) and KI (3 mmol) were added to a solution of the compound **7a** (19 mmol) in acetone. The mixture was refluxed for 12 h and then cooled. The precipitate was filtered off and the solution was concentrated *in vacuo*. The residue was purified by column chromatography with petroleum ether/ethyl acetate (30/1, v/v) to give compound **8a** as yellow oil (6.9 g, 83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 6.97 (1H, d, J = 8.5 Hz), 6.68 (1H, d, J = 1.9 Hz), 6.50 (1H, d, J = 8.5 Hz), 6.49 (1H, d, J = 1.9 Hz), 4.84 (2H, s), 4.30 (2H, q, J = 7.1 Hz), 3.85 (3H, s), 2.26 (3H, s), 1.93 (4H, q, J = 7.3 Hz), 1.32 (3H, t, J = 7.1 Hz), 1.25 (9H, s), 0.65 (6H, t, J = 7.3 Hz).

*4.8.2. Ethyl-5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-ethylpyrrole-2-carbocxylate (**8b**)*

Yellow oil, 5.2 g, 86% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, s), 6.97 (1H, d, J = 8.5 Hz), 6.68 (1H, d, J = 2.0 Hz), 6.56 (1H, d, J = 2.0 Hz), 6.51 (1H, d, J = 8.5 Hz), 4.84 (2H, s), 4.31–4.10 (4H, m), 2.26 (3H, s), 1.93 (4H, q, J = 7.6 Hz), 1.46–1.30 (6H, m), 1.28 (9H, s), 0.66 (6H, t, J = 7.6 Hz).

*4.9. General procedure for the synthesis of 5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-alkylpyrrole-2-carboxylic acid (**9a** and **9b**)*

*4.9.1. 5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-methylpyrrole-2-carbocxylic acid (**9a**)*

Compound **8a** (4.2 mmol) was dissolved in ethanol (20 mL), treated with H_2O (10 mL) and KOH (20.1 mmol) and the reaction mixture was stirred at 80 °C for 5 h. The solution was diluted with H_2O (20 mL), the pH value was adjusted to about 3–4 using 1 M HCl. Then it was extracted with ethyl acetate. The aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (10/1, v/v) to give compound **9a** as yellow oil (1.5 g, 89% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.91 (1H, d, J = 8.2 Hz), 6.78 (1H, d, J = 1.9 Hz), 6.45 (1H, d, J = 1.9 Hz), 6.43 (1H, d, J = 8.2 Hz), 4.76 (2H, s), 3.78 (3H, s), 2.18 (3H, s), 1.86 (4H, q, J = 7.3 Hz), 1.24 (9H, s), 0.60 (6H, t, J = 7.3 Hz).

*4.9.2. 5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-ethylpyrrole-2-carbocxylic acid (**9b**)*

Yellow oil, 2.4 g, 86% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 6.97 (1H, d, J = 8.5 Hz), 6.85 (1H, d, J = 2.0 Hz), 6.60 (1H, d, J = 2.0 Hz), 6.51 (1H, d, J = 8.5 Hz), 4.84 (2H, s), 4.26 (2H, q, J = 7.1 Hz), 2.26 (3H, s), 1.93 (4H, q, J = 7.3 Hz), 1.41 (6H, t, J = 7.1 Hz), 1.28 (9H, s), 0.66 (6H, t, J = 7.3 Hz).

*4.10. General procedure for the synthesis of phenyl-pyrrolyl pentane derivatives (**1a**–**1h**, **2a**–**2h**, **3a**–**3h**, **4a**–**4h**)*

*4.10.1. Methyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) acetate (**1a**)*

To a solution of compound **9a** (0.75 mmol) in DMF (10 mL) was added Et_3N (3.3 mmol), followed by hydrochloride salt of glycine methyl ester (0.83 mmol), EDCI (0.91 mmol) and HOBT (0.91 mmol). The reaction mixture was stirred at 25 °C overnight and then poured into H_2O. The solution was extracted with ethyl acetate, and aqueous

phase was extracted with ethyl acetate. The combined organic phases were washed with H_2O, brine, then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (4/1, v/v) to give compound **1a** as white solid (0.29 g, 82% yield). Mp: 121–122 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{39}N_2O_5$ $(M + H)^+$, 471.2853; found, 471.2863; ^{1}H NMR (300 MHz, $CDCl_3$) δ: ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 7.01 (1H, s), 6.98 (1H, d, J = 8.5 Hz), 6.50 (1H, d, J = 8.5 Hz), 6.45 (1H, d, J = 1.8 Hz), 6.18 (1H, d, J = 1.8 Hz), 4.833 (2H, s), 3.88 (3H, s), 3.69 (3H, s), 3.59 (2H, t, J = 6.0 Hz), 2.60 (2H, t, J = 6.0 Hz), 2.26 (3H, s), 1.91 (4H, q, J = 7.3 Hz), 1.26 (9H, s), 0.65 (6H, t, J = 7.3 Hz); ^{13}C NMR (500 MHz, CDCl3) δ: 209.99, 170.75, 161.99, 154.09, 140.61, 131.23, 130.39, 126.69, 125.95, 125.71, 124.14, 111.89, 110.16, 69.57, 52.23, 44.82, 43.18, 40.94, 36.52, 30.20, 26.31, 16.59, 8.46.

4.10.2. Methyl-3-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoate (**1b**)

The same method as **1a** and the starting materials were **9a** and hydrochloride salt of β-alanine methyl ester. White solid, 0.25 g, 82% yield. Mp: 97–100 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_5$ $(M + H)^+$, 485.3010; found, 485.3016; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 7.01 (1H, s), 6.98 (1H, d, J = 8.5 Hz), 6.50 (1H, d, J = 8.5 Hz), 6.45 (1H, d, J = 1.8 Hz), 6.18 (1H, d, J = 1.8 Hz), 4.833 (2H, s), 3.88 (3H, s), 3.69 (3H, s), 3.59 (2H, t, J = 6.0 Hz), 2.60 (2H, t, J = 6.0 Hz), 2.26 (3H, s), 1.91 (4H, q, J = 7.3 Hz), 1.26 (9H, s), 0.65 (6H, t, J = 7.3 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 209.94, 173.06, 162.09, 154.11, 140.65, 131.05, 130.42, 126.36, 125.95, 125.70, 124.77, 111.15, 110.16, 69.61, 51.70, 44.82, 43.21, 36.52, 34.59, 34.12, 30.23, 26.33, 16.62, 8.49.

4.10.3. (S)-Methyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoate (**1c**)

The same method as **1a** and the starting materials were **9a** and hydrochloride salt of L-alanine methyl ester. White solid, 0.34 g, 89.7% yield. Mp: 92–93 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_5$ $(M + H)^+$, 485.3010; found, 485.3019; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.02 (1H, s), 6.98 (1H, d, J = 8.6 Hz), 6.51 (1H, d, J = 8.6 Hz), 6.48 (1H, d, J = 1.8 Hz), 6.28 (1H, d, J = 1.8 Hz), 4.84 (2H, s), 4.66 (1H, m), 3.87 (3H, s), 3.75 (3H, s), 2.26 (3H, s), 1.92 (4H, q, J = 6.5 Hz), 1.44 (3H, d, J = 7.2 Hz), 1.25 (9H, s), 0.65 (6H, t, J = 6.5 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 209.93, 173.85, 161.46, 154.09, 140.65, 131.15, 130.39, 126.64, 125.94, 125.72, 124.31, 111.60, 110.15, 69.57, 52.32, 47.73, 44.81, 43.18, 36.53, 30.17, 26.31, 18.53, 16.60, 8.46.

4.10.4. (S)-Dimethyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) succinate (**1d**)

The same method as **1a** and the starting materials were **9a** and hydrochloride salt of L-aspartic acid dimethyl ester. White solid, 0.60 g, 84% yield. Mp: 57–58 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{30}H_{43}N_2O_7$ $(M + H)^+$, 543.3065; found, 543.3078; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 6.98 (1H, d, J = 8.7 Hz), 6.68 (1H, d, J = 8.7 Hz), 6.47 (1H, d, J = 1.2 Hz), 6.32 (1H, d, J = 1.2 Hz), 4.84 (2H, s), 4.12 (1H, m), 3.86 (3H, s), 3.77 (3H, s), 3.69 (3H, s), 3.07 (1H, m), 2.91 (1H, m), 2.32 (3H, s), 1.92 (4H, q, J = 7.0 Hz), 1.26 (9H, s), 0.65 (6H, t, J = 7.0 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 209.99, 171.57, 161.52, 154.09, 140.58, 131.32, 130.36, 126.96, 125.96, 125.67, 123.98, 111.88, 110.21, 69.61, 52.73, 51.97, 48.25, 44.75, 43.19, 36.60, 36.41, 30.11, 26.32, 16.62, 8.46.

4.10.5. Methyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido)acetate (**1e**)

The same method as **1a** and the starting materials were **9b** and hydrochloride salt of glycine methyl ester. Colorless oil, 0.49 g, 80.5% yield. HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_5$ $(M + H)^+$, 485.3010; found, 485.3016; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 6.97 (1H, d, J = 8.5 Hz), 6.57 (1H, d, J = 1.4 Hz), 6.51 (1H, d, J = 8.5 Hz), 6.28 (1H, d, J = 1.4 Hz), 4.84 (2H, s), 4.32 (2H, q, J = 7.1 Hz), 4.10 (2H, d, J = 5.3 Hz), 3.76 (3H, s), 2.26 (3H, s), 1.92 (4H, q, J = 7.3 Hz), 1.36 (3H, t, J = 7.1 Hz), 1.26 (9H, s), 0.64 (6H, t, J = 7.3 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.02, 170.79, 161.83, 154.08, 140.62, 131.29, 130.43, 125.91, 125.78, 125.01, 123.33, 112.14, 110.14, 69.57, 52.25, 44.92, 43.72, 40.97, 34.21, 30.29, 26.33, 17.15, 16.64, 8.50.

4.10.6. Methyl-3-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido) propanoate (**1f**)

The same method as **1a** and the starting materials were **9b** and hydrochloride salt of β-alanine methyl ester. Colorless oil, 0.38 g, 87% yield. HRMS, ESI$^+$, *m/z*: Calcd for $C_{29}H_{43}N_2O_5$ $(M + H)^+$, 499.3166; found, 499.3175; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 7.01 (1H, s), 6.97 (1H, d, J = 8.5 Hz), 6.54 (1H, d, J = 1.9 Hz), 6.51 (1H, d, J = 8.5 Hz), 6.17 (1H, d, J = 1.9 Hz), 4.84 (2H, s), 4.31 (2H, q, J = 7.1 Hz), 3.68 (3H, s), 3.60 (2H, t, J = 6.1 Hz), 2.60 (2H, t, J = 6.1 Hz), 2.26 (3H, s), 1.90 (4H, q, J = 7.3 Hz), 1.36 (3H, t, J = 7.1 Hz), 1.26 (9H, s), 0.64 (6H, t, J = 7.3 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.00, 173.07, 161.91, 154.05, 140.65, 131.08, 130.43, 125.87, 125.75, 124.65, 123.94, 111.37, 110.11, 69.56, 51.70, 44.89, 43.66, 34.58, 34.11, 30.28, 26.32, 17.17, 16.62, 8.50.

4.10.7. (S)-Methyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido) propanoate (**1g**)

The same method as **1a** and the starting materials were **9b** and hydrochloride salt of L-alanine methyl ester. White solid, 0.51 g, 80% yield. Mp: 92–93 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{29}H_{43}N_2O_5$ $(M + H)^+$, 499.3166; found, 499.3175; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, s), 6.97 (1H, d, J = 8.7 Hz), 6.56 (1H, d, J = 1.5 Hz), 6.51 (1H, d, J = 8.7 Hz), 6.26 (1H, d, J = 1.5 Hz), 4.85 (2H, s), 4.66 (1H, m), 4.29 (2H, q, J = 6.9 Hz), 3.75 (1H, s), 2.27 (3H, s), 1.90 (4H, q, J = 7.2 Hz), 1.45 (3H, d, J = 7.2 Hz), 1.35 (3H, t, J = 6.9 Hz), 1.26 (9H, s), 0.64 (6H, t, J = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.06, 173.88, 161.27, 153.95, 140.60, 131.15, 130.36, 125.80, 125.71, 124.90, 123.38, 111.82, 109.98, 69.42, 52.33, 47.71, 44.78, 43.68, 35.20, 30.10, 26.27, 18.43, 17.14, 16.63, 8.44.

4.10.8. (S)-Dimethyl-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido) succinate (**1h**)

The same method as **1a** and the starting materials were **9b** and hydrochloride salt of L-aspartic acid dimethyl ester. Colorless oil, 0.52 g, 83% yield. HRMS, ESI$^+$, *m/z*: Calcd for $C_{31}H_{45}N_2O_7$ $(M + H)^+$, 557.3221; found, 557.3223; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, s), 6.98 (1H, d, J = 8.5 Hz), 6.66 (1H, d, J = 8.5 Hz), 6.54 (1H, d, J = 1.8 Hz), 6.31 (1H, d, J = 1.8 Hz), 4.95 (1H, m), 4.85 (2H, s), 4.28 (2H, q, J = 7.1 Hz), 3.77 (3H, s), 3.68 (3H, s), 3.01 (2H, m), 2.26 (3H, s), 1.92 (4H, q, J = 6.9 Hz), 1.35 (3H, t, J = 7.1 Hz), 1.26 (9H, s), 0.65 (6H, t, J = 6.9 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 209.97, 171.56, 161.37, 154.10, 140.57, 131.35, 130.41, 125.93, 125.76, 125.29, 123.22, 112.12, 110.22, 69.64, 52.71, 51.96, 48.31, 44.88, 43.76, 37.10, 36.43, 30.27, 26.33, 17.12, 16.63, 8.50.

4.10.9. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl) pentan-3-yl)-N-(2-hydroxyethyl)-1-methylpyrrole-2-carboxamide (**2a**)

To a solution of **1a** (0.23 mmol) in methanol (10 mL), $NaBH_4$ (2.3 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at 25 °C for 0.5 h and then added H_2O (10 mL). The

solution was extracted with ethyl acetate, and aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate (1/1, v/v) to give compound **2a** as white solid (0.10 mg, 96% yield). Mp: 98–100 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{26}H_{41}N_2O_4$ $(M + H)^+$, 445.3061; found, 445.3069; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.02 (1H, d, $J = 8.5$ Hz), 7.00 (1H, s), 6.70 (1H, d, $J = 8.5$ Hz), 6.49 (1H, d, $J = 1.9$ Hz), 6.21 (1H, d, $J = 1.9$ Hz), 4.09 (2H, m), 3.87 (3H, s), 3.72 (2H, m), 3.70 (1H, m), 3.46 (2H, m), 2.19 (3H, s), 1.91 (4H, q, $J = 7.5$ Hz), 1.01 (9H, s), 0.65 (6H, t, $J = 7.5$ Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 163.35, 154.46, 140.35, 131.28, 130.30, 126.28, 125.83, 125.52, 124.49, 111.59, 110.18, 69.30, 62.79, 44.82, 42.35, 36.58, 33.58, 30.14, 26.03, 16.59, 8.47.

4.10.10. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl) pentan-3-yl)-N-(3-hydroxypropyl)-1-methylpyrrole-2-carboxamide (**2b**)

The same method as **2a** and the starting material was **1b**. White solid, 0.19 g, 98% yield. Mp: 80–81 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{43}N_2O_4$ $(M + H)^+$, 459.3217; found, 459.3223; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 7.00 (1H, s), 7.01 (1H, d, $J = 8.3$ Hz), 6.70 (1H, d, $J = 8.3$ Hz), 6.50 (1H, d, $J = 1.4$ Hz), 6.19 (1H, d, $J = 1.4$ Hz), 4.11 (2H, m), 3.88 (3H, s), 3.70 (1H, m), 3.63 (2H, m), 3.48 (2H, m), 2.19 (3H, s), 1.93 (4H, q, $J = 7.2$ Hz), 1.70 (2H, m), 1.00 (9H, s), 0.65 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 163.32, 154.41, 140.28, 131.20, 130.27, 126.43, 125.80, 125.45, 124.52, 111.25, 110.09, 69.23, 59.00, 44.77, 36.56, 35.59, 33.55, 32.59, 30.14, 26.01, 16.58, 8.45.

4.10.11. (S)-5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-N-(1-hydroxypropan-2-yl)-1-methylpyrrole-2-carboxamide (**2c**)

The same method as **2a** and the starting material was **1c**. White solid, 0.30 g, 95% yield. Mp: 88–89 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{43}N_2O_4$ $(M + H)^+$, 459.3217; found, 459.3223; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, s), 7.02 (1H, d, $J = 9.7$ Hz), 6.71 (1H, d, $J = 9.7$ Hz), 6.51 (1H, d, $J = 1.5$ Hz), 6.20 (1H, d, $J = 1.5$ Hz), 4.10 (2H, m), 3.86 (2H, m), 3.83 (1H, s), 3.69 (1H, m), 3.54 (1H, m), 2.19 (1H, s), 1.92 (4H, q, $J = 7.2$ Hz), 1.20 (3H, d, $J = 6.9$ Hz), 1.01 (9H, s), 0.65 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 162.92, 154.37, 140.34, 131.20, 130.28, 126.62, 125.77, 125.46, 124.47, 111.29, 109.94, 69.10, 67.71, 47.74, 44.71, 36.68, 33.53, 29.98, 26.02, 17.13, 16.64, 8.44.

4.10.12. (S)-N-(1,4-Dihydroxybutan-2-yl)-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamide (**2d**)

The same method as **2a** and the starting material was **1d**. White solid, 0.31 g, 90% yield. Mp: 80–82 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{45}N_2O_5$ $(M + H)^+$, 489.3323; found, 489.3316; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.94 (1H, d, $J = 8.6$ Hz), 6.93 (1H, s), 6.63 (1H, d, $J = 8.6$ Hz), 6.44 (1H, d, $J = 1.3$ Hz), 6.20 (1H, d, $J = 1.3$ Hz), 4.11 (1H, m), 4.02 (1H, m), 3.78 (3H, s), 3.75 (1H, m), 3.62 (2H, m), 3.59 (2H, m), 3.56 (1H, m), 2.11 (3H, s), 1.85 (4H, q, $J = 7.1$ Hz), 1.55 (2H, m), 0.93 (9H, s), 0.57 (6H, t, $J = 7.1$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 164.25, 153.27, 139.33, 130.39, 129.29, 125.85, 124.86, 124.52, 123.29, 110.71, 109.18, 68.29, 64.32, 57.78, 47.34, 43.80, 35.73, 33.51, 32.62, 29.10, 25.06, 15.63, 7.49.

4.10.13. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl) pentan-3-yl)-N-(2-hydroxyethyl)-1-ethylpyrrole-2-carboxamide (**2e**)

The same method as **2a** and the starting material was **1e**. White solid, 0.22 g, 97% yield. Mp: 81–82 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{43}N_2O_4$ $(M + H)^+$, 459.3217; found, 459.3217; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.03 (1H, d, $J = 8.3$ Hz), 7.00 (1H, s), 6.71 (1H, d, $J = 8.3$ Hz), 6.58 (1H, d, $J = 1.8$ Hz), 6.20 (1H, d, $J = 1.8$ Hz), 4.31 (2H, q, $J = 7.1$ Hz), 4.08 (1H, m), 3.86 (1H, m), 3.74 (1H, m), 3.70 (2H, m), 3.47 (2H, m), 2.04 (3H, s), 1.92 (4H, q, $J = 7.2$ Hz), 1.36 (3H, t, $J = 7.1$ Hz), 1.01 (9H, s), 0.64 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 163.21, 154.42, 140.35, 131.33, 130.30, 125.89, 125.44, 124.84, 123.67, 111.82, 110.13, 69.25, 62.85, 44.90, 43.72, 42.38, 33.56, 30.20, 26.03, 17.17, 16.61, 8.48.

4.10.14. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl) pentan-3-yl)-N-(3-hydroxypropyl)-1-ethylpyrrole-2-carboxamide (**2f**)

The same method as **2a** and the starting material was **1f**. White solid, 0.24 g, 93% yield. Mp: 104–106 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{45}N_2O_4$ $(M + H)^+$, 473.3374; found, 473.3379; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 7.01 (1H, s), 7.02 (1H, d, $J = 8.2$ Hz), 6.71 (1H, d, $J = 8.2$ Hz), 6.58 (1H, d, $J = 1.5$ Hz), 6.17 (1H, d, $J = 1.5$ Hz), 4.32 (2H, q, $J = 6.9$ Hz), 4.11 (1H, m), 3.86 (1H, m), 3.70 (1H, m), 3.63 (2H, t, $J = 5.6$ Hz), 3.47 (2H, t, $J = 5.7$ Hz), 2.20 (3H, s), 1.94 (4H, q, $J = 7.1$ Hz), 1.68 (2H, m), 1.36 (3H, t, $J = 6.9$ Hz), 1.00 (9H, s), 0.65 (6H, t, $J = 7.1$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 163.26, 154.44, 140.33, 131.29, 130.34, 125.93, 125.45, 124.83, 123.70, 111.58, 110.14, 69.27, 58.97, 44.94, 43.77, 35.56, 33.58, 32.72, 30.29, 26.05, 17.21, 16.63, 8.52.

4.10.15. 5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl) pentan-3-yl)-N-((S)-1-hydroxypropan-2-yl)-1-ethylpyrrole-2-carboxamide (**2g**)

The same method as **2a** and the starting material was **1g**. White solid, 0.28 g, 94% yield. Mp: 89–90 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{45}N_2O_4$ $(M + H)^+$, 473.3374; found, 499.3388; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.01 (1H, s), 7.02 (1H, d, $J = 8.1$ Hz), 6.72 (1H, d, $J = 8.1$ Hz), 6.58 (1H, d, $J = 1.5$ Hz), 6.18 (1H, d, $J = 1.5$ Hz), 4.31 (2H, q, $J = 7.1$ Hz), 4.11 (2H, m), 3.85 (1H, m), 3.70 (2H, m), 3.56 (1H, m), 2.20 (3H, s), 1.92 (4H, q, $J = 7.2$ Hz), 1.36 (3H, t, $J = 7.1$ Hz), 1.20 (3H, d, $J = 6.8$ Hz), 1.01 (9H, s), 0.65 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 162.79, 154.35, 140.33, 131.24, 130.28, 125.86, 125.40, 124.94, 123.69, 111.54, 109.95, 69.10, 67.77, 47.77, 44.82, 43.76, 33.52, 30.10, 26.01, 17.20, 17.12, 16.65, 8.46.

4.10.16. (S)-N-(1,4-Dihydroxybutan-2-yl)-5-(3-(4-(2-hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamide (**2h**)

The same method as **2a** and the starting material was **1h**. White solid, 0.17 g, 91% yield. Mp: 93–94 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{29}H_{47}N_2O_5$ $(M + H)^+$, 503.3479; found, 503.3483; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, d, $J = 8.9$ Hz), 7.00 (1H, s), 6.71 (1H, d, $J = 8.9$ Hz), 6.58 (1H, d, $J = 2.0$ Hz), 6.26 (1H, d, $J = 2.0$ Hz), 4.30 (2H, m), 4.07 (2H, q, $J = 7.0$ Hz), 3.85 (1H, m), 3.71 (2H, m), 3.67 (1H, m), 3.62 (2H, m), 2.19 (3H, s), 1.92 (4H, q, $J = 7.2$ Hz), 1.35 (3H, t, $J = 7.0$ Hz), 1.01 (9H, s), 0.65 (6H, t, $J = 7.2$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 163.10, 154.43, 140.32, 131.40, 130.29, 125.92, 125.46, 125.18, 123.45, 111.93, 110.16, 69.26, 65.39, 58.78, 48.26, 44.90, 43.83, 34.54, 33.59, 30.21, 26.04, 17.19, 16.62, 8.49.

4.10.17. 2-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-methylpyrrole-2-carboxamido)acetic acid (**3a**)

To a solution of **1a** (0.33 mmol) in the THF (5 mL) and H_2O (1 mL), $LiOH{\cdot}2H_2O$ (1.65 mmol) was added. The reaction mixture was stirred at 25 °C overnight and then H_2O (10 mL) was added followed by the pH value was adjusted to about 3–4. Then it was extracted with ethyl acetate, and the organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine, then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with dichloromethane/methanol (40/1, v/

v) to give compound **3a** as faint yellow solid (0.13 g, 83.9% yield). Mp: 104–105 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{26}H_{37}N_2O_5$ $(M + H)^+$, 457.2697; found, 457.2704; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.00 (1H, s), 6.95 (1H, d, J = 8.5 Hz), 6.50 (1H, d, J = 1.9 Hz), 6.49 (1H, d, J = 8.5 Hz), 6.32 (1H, d, J = 1.9 Hz), 4.83 (2H, s), 4.07 (2H, d, J = 5.3 Hz), 3.85 (3H, s), 2.24 (3H, s), 1.90 (4H, q, J = 7.3 Hz), 1.24 (9H, s), 0.64 (6H, t, J = 7.3 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 210.37, 172.99, 162.69, 154.07, 140.59, 131.46, 130.44, 127.15, 125.98, 125.73, 123.71, 112.58, 110.17, 69.53, 44.85, 43.20, 41.38, 36.63, 30.19, 26.33, 16.59, 8.47.

4.10.18. 3-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-methylpyrrole-2-carboxamido)propanoic acid (**3b**)

The same method as **3a** and the starting material was **1b**. Faint yellow solid, 0.22 g, 81% yield. Mp: 124–126 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{39}N_2O_5$ $(M + H)^+$, 471.2853; found, 471.2859; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 6.98 (1H, s), 6.93 (1H, d, J = 8.5 Hz), 6.45 (1H, d, J = 8.5 Hz), 6.21 (1H, d, J = 1.4 Hz), 6.04 (1H, d, J = 1.4 Hz), 4.81 (2H, s), 3.78 (3H, s), 3.45 (2H, t, J = 5.9 Hz), 2.41 (2H, t, J = 5.9 Hz), 2.20 (3H, s), 1.87 (4H, q, J = 7.2 Hz), 1.23 (9H, s), 0.60 (6H, t, J = 7.2 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 210.37, 176.05, 162.48, 154.03, 140.75, 131.02, 130.43, 126.37, 125.89, 125.73, 124.70, 111.59, 110.17, 69.51, 44.79, 43.17, 36.53, 35.44, 35.14, 30.14, 26.33, 16.58, 8.49.

4.10.19. (S)-2-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoic acid (**3c**)

The same method as **3a** and the starting material was **1c**. Faint yellow solid, 0.20 g, 84% yield. Mp: 148–150 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{39}N_2O_5$ $(M + H)^+$, 471.2853; found, 471.2860; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.90 (1H, d, J = 8.9 Hz), 6.42 (1H, d, J = 2.0 Hz), 6.21 (1H, d, J = 2.0 Hz), 6.20 (1H, d, J = 8.9 Hz), 4.78 (2H, s), 4.54 (1H, m), 3.79 (3H, s), 2.18 (3H, s), 1.85 (4H, q, J = 7.0 Hz), 1.42 (3H,d, J = 6.8 Hz), 1.18 (9H, s), 0.56 (6H, t, J = 7.0 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.34, 176.50, 162.26, 153.97, 140.55, 131.40, 130.37, 127.16, 125.90, 125.65, 123.68, 112.23, 109.99, 69.39, 48.15, 44.71, 43.16, 36.70, 30.01, 26.29, 17.86, 16.64, 8.43.

4.10.20. (S)-2-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) succinic acid (**3d**)

The same method as **3a** and the starting material was **1d**. Faint yellow solid, 0.15 g, 87% yield. Mp: 92–94 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{39}N_2O_7$ $(M + H)^+$, 515.2752; found, 515.2740; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.81 (1H, d, J = 2.3 Hz), 6.73 (1H, d, J = 2.3 Hz), 6.42 (1H, d, J = 8.5 Hz), 6.29 (1H, d, J = 8.5 Hz), 4.89 (1H, m), 4.78 (2H, s), 3.75 (3H, s), 2.98 (1H, m), 2.87 (1H, m), 2.16 (3H, s), 1.84 (4H, q, J = 7.2 Hz), 1.17 (9H, s), 0.56 (6H, t, J = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.76, 175.49, 174.89, 162.18, 153.97, 140.59, 131.57, 130.42, 127.54, 125.98, 125.74, 123.45, 112.79, 110.34, 69.59, 48.33, 44.78, 43.19, 36.80, 36.16, 30.16, 26.33, 16.58, 8.48.

4.10.21. 2-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-ethylpyrrole-2-carboxamido)acetic acid (**3e**)

The same method as **3a** and the starting material was **1e**. Faint yellow solid, 0.26 g, 82% yield. Mp: 104–106 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{39}N_2O_5$ $(M + H)^+$, 471.2853; found, 471.2853; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.92 (1H, s), 6.88 (1H, d, J = 8.5 Hz), 6.52 (1H, d, J = 1.9 Hz), 6.41 (1H, d, J = 8.5 Hz), 6.23 (1H, d, J = 1.9 Hz), 4.77 (2H, s), 4.21 (2H, q, J = 7.1 Hz), 4.00 (2H, m), 2.17 (3H, s), 1.83 (4H, q, J = 7.3 Hz), 1.27 (3H,t, J = 7.1 Hz), 1.17 (9H, s), 0.56 (6H, t, J = 7.3 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.42, 173.13, 162.48, 154.02, 140.59, 131.47, 130.44, 125.90, 125.78, 125.41, 122.89, 112.81, 110.13, 69.49, 44.91, 43.79, 43.18, 41.37, 30.26, 26.31, 17.09, 16.59, 8.47.

4.10.22. 3-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl) pentan-3-yl)-1-ethylpyrrole-2-carboxamido)propanoic acid (**3f**)

The same method as **3a** and the starting material was **1f**. Faint yellow solid, 0.24 g, 89% yield. Mp: 90–91 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_5$ $(M + H)^+$, 485.3010; found, 485.3010; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 6.93 (1H, s), 6.89 (1H, d, J = 8.5 Hz), 6.49 (1H, d, J = 1.9 Hz), 6.40 (1H, d, J = 8.5 Hz), 6.11 (1H, d, J = 1.9 Hz), 4.77 (2H, s), 4.23 (2H, q, J = 7.1 Hz), 3.60 (2H, t, J = 5.6 Hz), 2.53 (2H, t, J = 5.6 Hz), 2.16 (3H, s), 1.82 (4H, q, J = 7.1 Hz), 1.28 (3H, t, J = 7.1 Hz), 1.17 (9H, s), 0.56 (6H, t, J = 7.1 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.45, 176.21, 162.27, 154.00, 140.65, 131.19, 130.47, 125.86, 125.77, 124.86, 123.67, 111.89, 110.07, 69.46, 44.92, 43.70, 34.64, 34.37, 34.27, 30.27, 26.31, 17.14, 16.59, 8.50.

4.10.23. (S)-2-(5-(3-(4-(3,3-dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoic acid (**3g**)

The same method as **3a** and the starting material was **1g**. Faint yellow solid, 0.19 g, 83% yield. Mp: 114–117 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_5$ $(M + H)^+$, 485.3010; found, 485.3020; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.90 (1H, d, J = 8.4 Hz), 6.54 (1H, d, J = 1.9 Hz), 6.43 (1H, d, J = 8.4 Hz), 6.20 (1H, d, J = 1.9 Hz), 4.78 (2H, s), 4.53 (1H, m), 4.23 (2H, q, J = 7.1 Hz), 2.19 (3H, s), 1.84 (4H, q, J = 7.0 Hz), 1.43 (3H,d, J = 7.1 Hz), 1.29 (3H,t, J = 7.1 Hz), 1.19 (9H, s), 0.57 (6H, t, J = 7.0 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.25, 179.78, 162.04, 153.89, 140.67, 131.01, 130.35, 126.61, 125.76, 124.66, 123.64, 111.97, 110.16, 69.49, 50.52, 44.75, 43.51, 43.11, 30.23, 26.26, 17.92, 17.05, 16.61, 8.47.

4.10.24. (S)-2-(5-(3-(4-(3,3-Dimethyl-2-oxobutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido)succinic acid (**3h**)

The same method as **3a** and the starting material was **1h**. Faint yellow solid, 0.15 g, 81% yield. Mp: 252–255 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{29}H_{41}N_2O_7$ $(M + H)^+$, 529.2908; found, 529.2906; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.96 (1H, s), 6.95 (1H, d, J = 8.4 Hz), 6.47 (1H, d, J = 8.4 Hz), 6.52 (1H, d, J = 1.8 Hz), 6.30 (1H, d, J = 1.8 Hz), 4.85 (1H, m), 4.78 (2H, s), 4.24 (2H, q, J = 7.1 Hz), 2.95 (2H, m), 2.20 (3H, s), 1.92 (4H, q, J = 6.8 Hz), 1.35 (3H, t, J = 7.1 Hz), 1.26 (9H, s), 0.65 (6H, t, J = 6.8 Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 210.76, 175.37, 174.77, 162.02, 153.97, 140.60, 131.61, 130.46, 126.36, 125.95, 125.80, 122.69, 112.96, 110.33, 69.55, 48.37, 44.92, 43.85, 43.19, 36.09, 30.25, 25.52, 17.07, 16.56, 8.50.

4.10.25. 2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido)acetic acid (**4a**)

To a solution of **3a** (0.31 mmol) in methanol (10 mL), $NaBH_4$ (6.2 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at 25 °C for 0.5 h and then added H_2O (10 mL). The solution was extracted with ethyl acetate, then the organic phase was separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and then dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by column chromatography with dichloromethane/methanol (20/1, v/v) to give compound **4a** as faint yellow solid (0.12 g, 85% yield). Mp: 96–98 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{26}H_{39}N_2O_5$ $(M + H)^+$, 459.2853; found, 459.2858; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.92 (1H, d, J = 8.4 Hz), 6.63 (1H, d, J = 8.4 Hz), 6.37 (1H, d, J = 1.8 Hz), 6.25 (1H, d, J = 1.8 Hz), 4.01 (2H, m), 3.92 (2H, m), 3.81 (1H, m), 3.62 (3H, s), 2.10 (3H, s), 1.92 (4H, q, J = 6.8 Hz), 0.96 (9H, s), 0.57 (6H, t, J = 6.8 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 177.31, 163.04, 154.45, 140.46, 131.14, 130.13, 126.14, 125.69, 125.51, 124.68, 112.04, 110.38, 69.48, 67.67, 44.62, 43.97, 36.42, 33.63, 29.10, 26.06, 16.52, 8.45.

*4.10.26. 3-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoic acid (**4b**)*

The same method as **4a** and the starting material was **3b**. Faint yellow solid, 0.16 g, 83% yield. Mp: 158–160 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{41}N_2O_5$ $(M+H)^+$, 473.3010; found, 473.3014; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 6.92 (1H, s), 6.93 (1H, d, $J = 8.1$ Hz), 6.80 (1H, d, $J = 1.3$ Hz), 6.63 (1H, d, $J = 8.1$ Hz), 6.25 (1H, d, $J = 1.3$ Hz), 4.01 (2H, m), 3.81 (1H, m), 3.61 (3H, s), 3.27 (2H, t, $J = 5.6$ Hz), 2.21 (2H, t, $J = 5.6$ Hz), 2.09 (3H, s), 1.83 (4H, q, $J = 6.9$ Hz), 0.94 (9H, s), 0.57 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 179.66, 162.58, 154.47, 140.41, 131.10, 130.11, 125.91, 125.63, 125.52, 124.91, 111.56, 110.31, 69.50, 44.54, 37.53, 36.48, 36.39, 33.66, 29.75, 29.66, 26.06, 16.52, 8.43.

*4.10.27. (S)-2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) propanoic acid (**4c**)*

The same method as **4a** and the starting material was **3c**. Faint yellow solid, 0.18 g, 87% yield. Mp: 92–93 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{41}N_2O_5$ $(M+H)^+$, 473.3010; found, 473.3016; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.93 (1H, s), 6.64 (1H, d, $J = 8.2$ Hz), 6.44 (1H, d, $J = 1.8$ Hz), 6.22 (1H, d, $J = 1.8$ Hz), 6.16 (1H, d, $J = 8.2$ Hz), 4.55 (1H, m), 4.04 (1H, m), 3.80 (3H, s), 3.77 (1H, m), 3.65 (1H, m), 2.10 (3H, s), 1.85 (4H, q, $J = 6.8$ Hz), 1.41 (3H, d, $J = 7.0$ Hz), 0.94 (9H, s), 0.58 (6H, t, $J = 6.8$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 176.53, 162.15, 154.36, 140.24, 131.45, 130.27, 127.13, 125.76, 125.50, 123.75, 112.09, 109.98, 69.03, 48.08, 44.72, 36.73, 33.53, 30.04, 26.02, 20.71, 17.99, 16.63, 8.44.

*4.10.28. (S)-2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-methylpyrrole-2-carboxamido) succinic acid (**4d**)*

The same method as **4a** and the starting material was **3d**. Faint yellow solid, 0.16 g, 82% yield. Mp: 223–225 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{41}N_2O_7$ $(M+H)^+$, 517.2908; found, 517.2903; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.00 (1H, d, $J = 7.9$ Hz), 6.97 (1H, s), 6.79 (1H, d, $J = 7.9$ Hz), 6.67 (1H, d, $J = 2.2$ Hz), 6.38 (1H, d, $J = 2.2$ Hz), 4.81 (1H, m), 4.29 (1H, m), 4.02 (1H, m), 3.77 (3H, s), 3.45 (1H, m), 2.50 (1H, m), 2.39 (1H, m), 2.12 (3H, s), 1.89 (4H, q, $J = 6.9$ Hz), 0.92 (9H, s), 0.59 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 179.45, 175.24, 160.35, 154.45, 139.44, 130.15, 129.31, 125.59, 125.44, 124.87, 124.58, 110.94, 110.22, 75.92, 69.85, 44.06, 40.06, 38.95, 36.07, 33.93, 29.29, 26.06, 16.49, 8.41.

*4.10.29. 2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido)acetic acid (**4e**)*

The same method as **4a** and the starting material was **3e**. Faint yellow solid, 0.18 g, 84% yield. Mp: 160–162 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{27}H_{41}N_2O_5$ $(M+H)^+$, 473.3010; found, 473.3017; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, d, $J = 8.4$ Hz), 6.94 (1H, s), 6.53 (1H, d, $J = 8.4$ Hz), 6.38 (1H, d, $J = 1.3$ Hz), 6.33 (1H, d, $J = 1.3$ Hz), 4.13 (2H, m), 4.01 (2H, m), 3.82 (2H, q, $J = 6.9$ Hz), 3.63 (1H, m), 2.11 (3H, s), 1.85 (4H, q, $J = 6.3$ Hz), 1.16 (3H, t, $J = 6.9$ Hz), 0.96 (9H, s), 0.57 (6H, t, $J = 6.3$ Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 177.14, 162.71, 154.47, 140.48, 131.28, 130.19, 125.79, 125.51, 124.36, 123.93, 112.26, 110.42, 69.52, 44.77, 43.51, 33.65, 30.06, 26.08, 17.05, 16.53, 8.49.

*4.10.30. 3-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido) propanoic acid (**4f**)*

The same method as **4a** and the starting material was **3f**. Faint yellow solid, 0.17 g, 85% yield. Mp: 163–166 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{43}N_2O_5$ $(M+H)^+$, 487.3166; found, 487.3175; ^{1}H NMR ($CDCl_3$, 300 Hz) δ: 6.94 (1H, s), 6.95 (1H, d, $J = 8.4$ Hz), 6.65 (1H, d, $J = 8.4$ Hz), 6.40 (1H, d, $J = 1.9$ Hz) 6.20 (1H, d, $J = 1.9$ Hz), 4.12 (2H, q, $J = 6.9$ Hz), 4.02 (1H, m), 3.86 (1H, m), 3.65 (1H, m), 3.29 (2H, t, $J = 5.5$ Hz), 2.22 (2H, t, $J = 5.5$ Hz), 2.11 (3H, s), 1.85 (4H, q, $J = 6.6$ Hz), 1.19 (3H, t, $J = 6.9$ Hz), 0.96 (9H, s), 0.58 (6H, t, $J = 6.6$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 179.30, 162.31, 154.48, 140.47, 131.14, 130.21, 125.79, 125.50, 124.23, 111.62, 110.34, 69.51, 44.75, 43.51, 39.70, 37.08, 36.11, 33.66, 30.00, 26.07, 17.11, 16.56, 14.17, 8.47.

*4.10.31. (S)-2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido) propanoic acid (**4g**)*

The same method as **4a** and the starting material was **3g**. Faint yellow solid, 0.23 g, 88% yield. Mp: 110–111 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{28}H_{43}N_2O_5$ $(M+H)^+$, 487.3166; found, 487.3179; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.02 (1H, d, $J = 8.1$ Hz), 7.01 (1H, s), 6.72 (1H, d, $J = 8.1$ Hz), 6.61 (1H, d, $J = 1.3$ Hz), 6.27 (1H, d, $J = 1.3$ Hz), 4.61 (1H, m), 4.31 (2H, m), 4.09 (2H, q, $J = 7.2$ Hz), 3.73 (1H, m), 2.20 (3H, s), 1.92 (4H, q, $J = 6.9$ Hz), 1.49 (3H, d, $J = 6.9$ Hz), 1.36 (3H, t, $J = 7.2$ Hz), 1.01 (9H, s), 0.65 (6H, t, $J = 6.9$ Hz); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 180.16, 162.11, 154.43, 140.41, 131.24, 130.23, 125.81, 125.46, 124.44, 123.99, 112.04, 110.34, 69.50, 65.54, 50.83, 44.82, 43.50, 33.65, 30.12, 26.07, 18.13, 17.09, 16.57, 8.51.

*4.10.32. (S)-2-(5-(3-(4-(2-Hydroxy-3,3-dimethylbutoxy)-3-methylphenyl)pentan-3-yl)-1-ethylpyrrole-2-carboxamido)succinic acid (**4h**)*

The same method as **4a** and the starting material was **3g**. Faint yellow solid, 0.24 g, 82% yield. Mp: 280–284 °C; HRMS, ESI$^+$, *m/z*: Calcd for $C_{29}H_{43}N_2O_7$ $(M+H)^+$, 531.3065; found, 531.3064; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.96 (1H, s), 6.96 (1H, d, $J = 8.5$ Hz), 6.79 (1H, d, $J = 8.5$ Hz), 6.71 (1H, d, $J = 1.8$ Hz), 6.36 (1H, d, $J = 1.8$ Hz), 4.75 (1H, m), 4.25 (2H, m), 3.76 (2H, q, $J = 6.3$ Hz), 3.45 (1H, m), 2.50 (2H, m), 2.12 (3H, s), 1.90 (4H, q, $J = 6.0$ Hz), 1.22 (3H, t, $J = 6.3$ Hz), 0.92 (9H, s), 0.58 (6H, t, $J = 6.0$ Hz); ^{13}C NMR (300 MHz, DMSO-d_6) δ: 177.68, 173.25, 160.20, 154.43, 139.41, 130.20, 129.30, 125.49, 124.51, 124.09, 124.03, 111.15, 110.22, 75.91, 69.83, 44.14, 42.75, 40.33, 38.66, 33.92, 29.34, 26.05, 17.26, 16.48, 8.42.

4.11. Measurement of VDR binding affinity by HL-60 differentiation-inducting assay

Differentiation of HL-60 cells was quantified in terms of morphology using nitroblue tetrazolium (NBT) reduction assay [12]. Target cell line which was grown well was diluted to 1×10^4 cells/mL. Then 200 μL of the obtained cell suspension was added to each well of 96-well culture plates and the tested compounds at pre-set concentration in DMSO were added. The HL-60 cells were incubated for 96 h at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. After incubation, the nitroblue tetrazolium (NBT) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was added. Then the mixture was incubated at 37 °C for 25 min, and cells were collected by centrifugation. Each sample was added 150 μL DMSO and the value of absorbance was measured at 570 nm then the EC_{50} value was calculated. The compounds whose efficacies were larger than 85% were tested of the second screening. And the final EC_{50} value was calculated for each tested compound.

4.12. Measurement of antiproliferation activities by MTT assay

The cytotoxicity was evaluated by MTT assay using HaCaT cells and MCF-7 cells. First, target cells which were grown well were diluted to 1×10^4 cells/mL. Then 200 μL of the obtained cell suspension was added to each well of 96-well culture plates and the tested compounds at pre-set concentration in DMSO were added.

The target cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2/air without medium change. After 48 h incubation, MTT (5 mg/mL) and DMEM culture medium (200 μl) was added to each well and reacted for 4 h. The medium was replaced by 150 μL DMSO to solubilize the purple formazan crystals produced. The absorbance at 570 nm of each well was measured on an ELISA plate reader. And the IC_{50} value was calculated by a dose of Logarithmic linear regression analysis. The compounds whose efficacies were larger than 85% were tested of the second screening. And the final IC_{50} value was calculated for each tested compound.

4.13. In vivo calcemic activity assay

ICR mice, aged 10 weeks, of clean grade, weighing 18–22 g, were provided by Jiangsu University and fed with a vitamin D-replete diet (0.2% calcium, 1% phosphate, and 2000 units vitamin D/kg) for 7 days. The hypercalcemic effect of the analogs was tested by daily s.c. injections of serial dilutions of tacalcitol or analogs for 7 consecutive days. Serum calcium was measured as calcemic parameter using a commercially available kit (Shanghai ShiFeng Biological) [16].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.09.015.

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