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Design, synthesis and biological evaluation of nonsecosteroidal vitamin D_3 receptor ligands as anti-tumor agents

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ABSTRACT

1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, also known as calcitriol), the active form of vitamin D_3, is being increasingly recognized for cancer therapy. Our previous work showed that phenyl-pyrrolyl pentane analogs, which mimicked anti-proliferative activities against several cancer cell lines of the natural secosteroidal ligand 1,25-$(OH)_2D_3$. Here, in order to optimize the structural features and discover more potent derivative, a series of nonsecosteroidal vitamin D_3 receptor (VDR) ligands bearing acetylene bond linker was designed, synthesized and evaluated. Most of them showed moderate to good binding affinities and agonistic activities. Especially, compound **19f** displayed the most anti-proliferative activities against MCF-7 and PC-3 cells with the IC_{50} values of 1.80 and 5.35 μM, respectively, which was comparable to positive control 1,25-$(OH)_2D_3$. Moreover, compound **19f** exhibited reduced toxicity against human normal liver cell line (L02) compared with the parental compound **7**. Besides, the preliminary structure–activity relationships (SARs) were also analyzed.

The active form of vitamin D_3, the hormone 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, also known as calcitriol) participates in numerous biological processes.[1–3] In addition to its classical role in mineral homeostasis and bone mineralization,[4] this hormone regulates numerous cellular pathways that could have a role in determining cancer risk and prognosis.[5–9] Because of this "nonclassical" actions, 1,25$(OH)_2D_3$ has attracted considerable interest as a potential drug for the treatment of cancer disease. The effects of 1,25$(OH)_2D_3$ are mediated through the vitamin D_3 receptor (VDR) which is a ligand-dependent transcription factor belonging to the superfamily of nuclear hormone receptors.[10–14]

So far, more than 3000 secosteroid analogs have been synthesized,[15] and many of them exhibit efficient VDR activities and some analogs have been successfully used in the treatment of bone, mineral, and skin disorders (Fig. 1).[16,17] However, adverse effects, particularly hypercalcemia, limit the clinical application of secosteroid analogs in the management of cancer disease.[15] Recently, much attention has been directed towards nonsecosteroidal VDR ligands, which mimic various activities *in vitro* and *in vivo* of the natural ligand 1,25$(OH)_2D_3$ without direct structural relationship to 1,25 $(OH)_2D_3$, such as VDR binding and inhibition of proliferation of cancer cells.[18,19] Moreover, they have simpler structures compared with secosteroid analogs.[20–27] Above all, some nonsecosteroidal VDR ligands avoid hypercalcemia effect, such as LG190178 and its analogs. Therefore, the development of VDR ligands with nonsecosteroidal skeleton is required to create novel therapy for the VDR-related cancer disease.

We previously reported novel phenyl-pyrrolyl pentane skeleton as a nonsecosteroidal VDR ligand skeleton which had anti-proliferative effects against cancer cells and avoided hypercalcemia effect *in vivo*.[28,29] While the VDR binding affinities of all the synthesized compounds suffered a remarkable decrease as compared with 1,25 $(OH)_2D_3$, compound **7** had a moderate VDR binding affinity *in vitro*, which deserved further study. Here, we wish to report compound **7** as a lead for further optimization to explore the structure-activity relationships (SARs) and to discover more potent derivative with high VDR binding affinity and anti-proliferative effect against cancer cells. As shown in Fig. 2, first, we attempted to introduce acetylene bond linker to minimize the flexibility and to fix the direction of the side chain, and then designed 3-pentanolyl as the terminal hydrophobic group of the side chain based on previous SARs. Additionally, to explore the effects of substitutions of polarity and steric hindrance on the pyrrole ring, compounds **17c–i** and **18a–d** were designed. Second, phenyl-pentane group on C-4 position of pyrrole ring replaced C-5 position to investigate the influence of the substitution positions of the terminal hydrophilic group of the pyrrole ring to give compounds **19a**, **19f–j** and **20a–e**.

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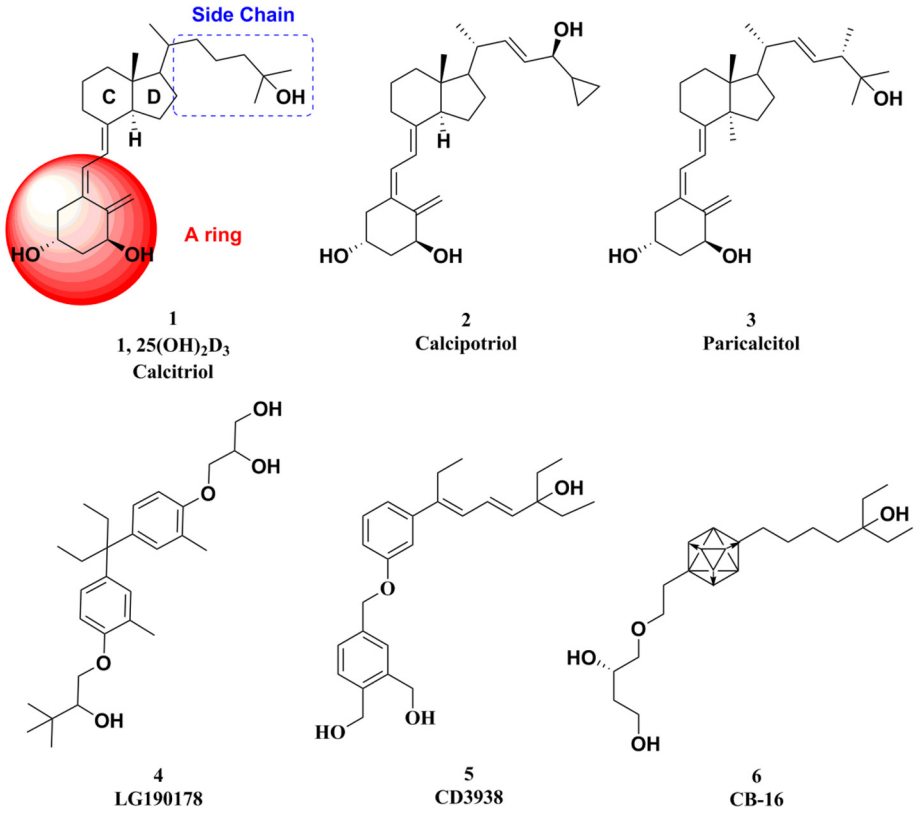


Fig. 1. Chemical structures of secosteroidal and nonsecosteroidal VDR ligands.

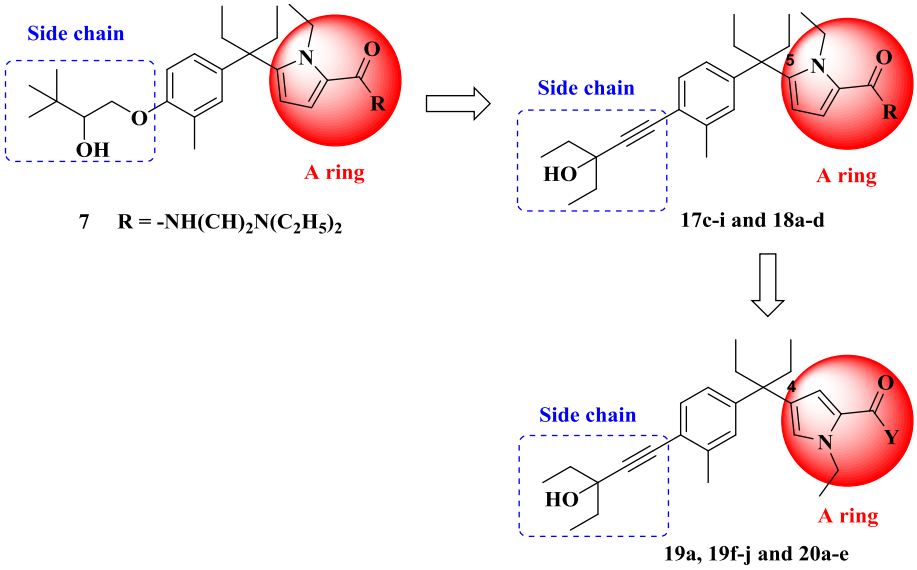
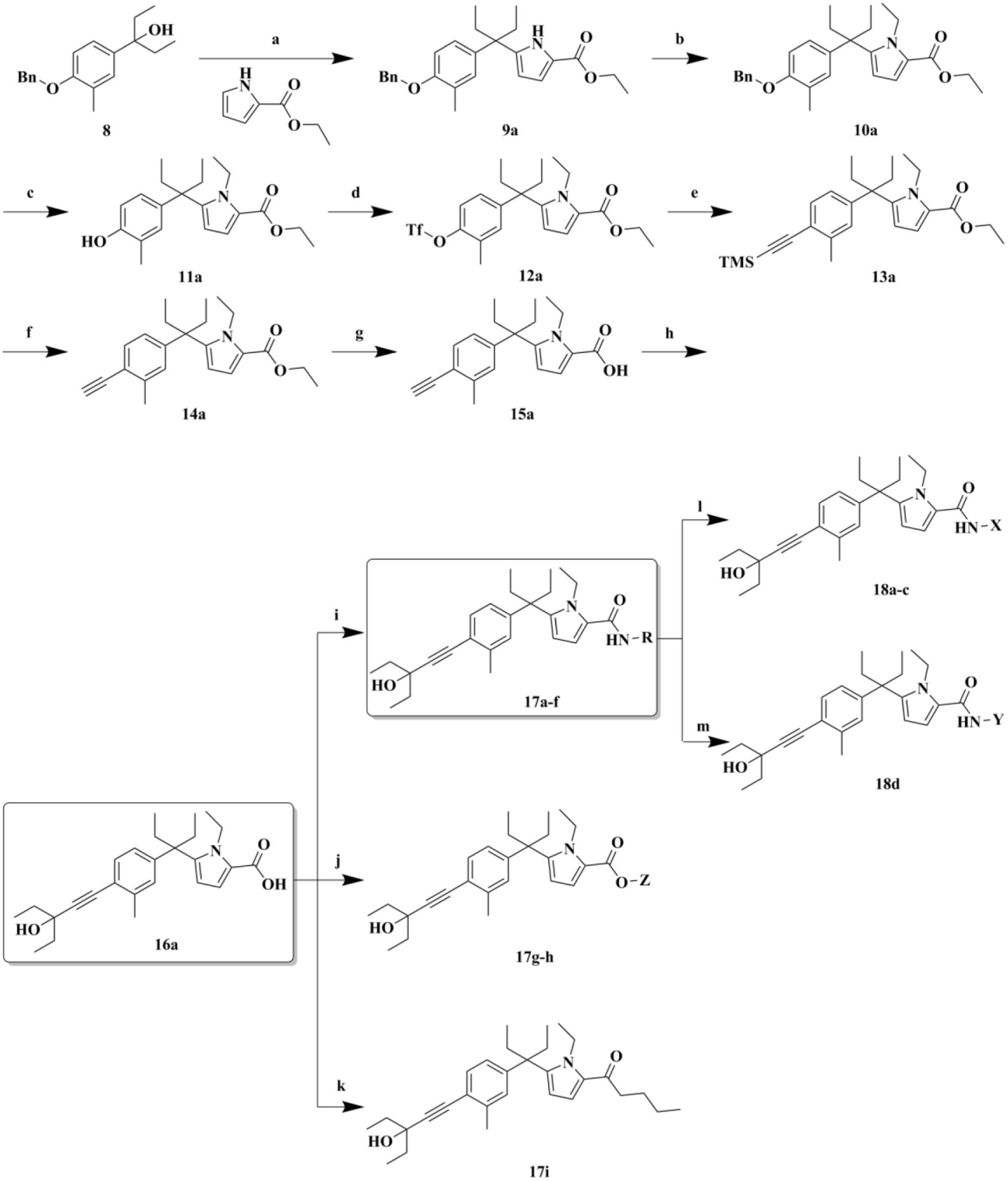


Fig. 2. Design of novel nonsecosteroidal vitamin D receptor ligands with phenyl-pyrrolyl pentane skeleton.

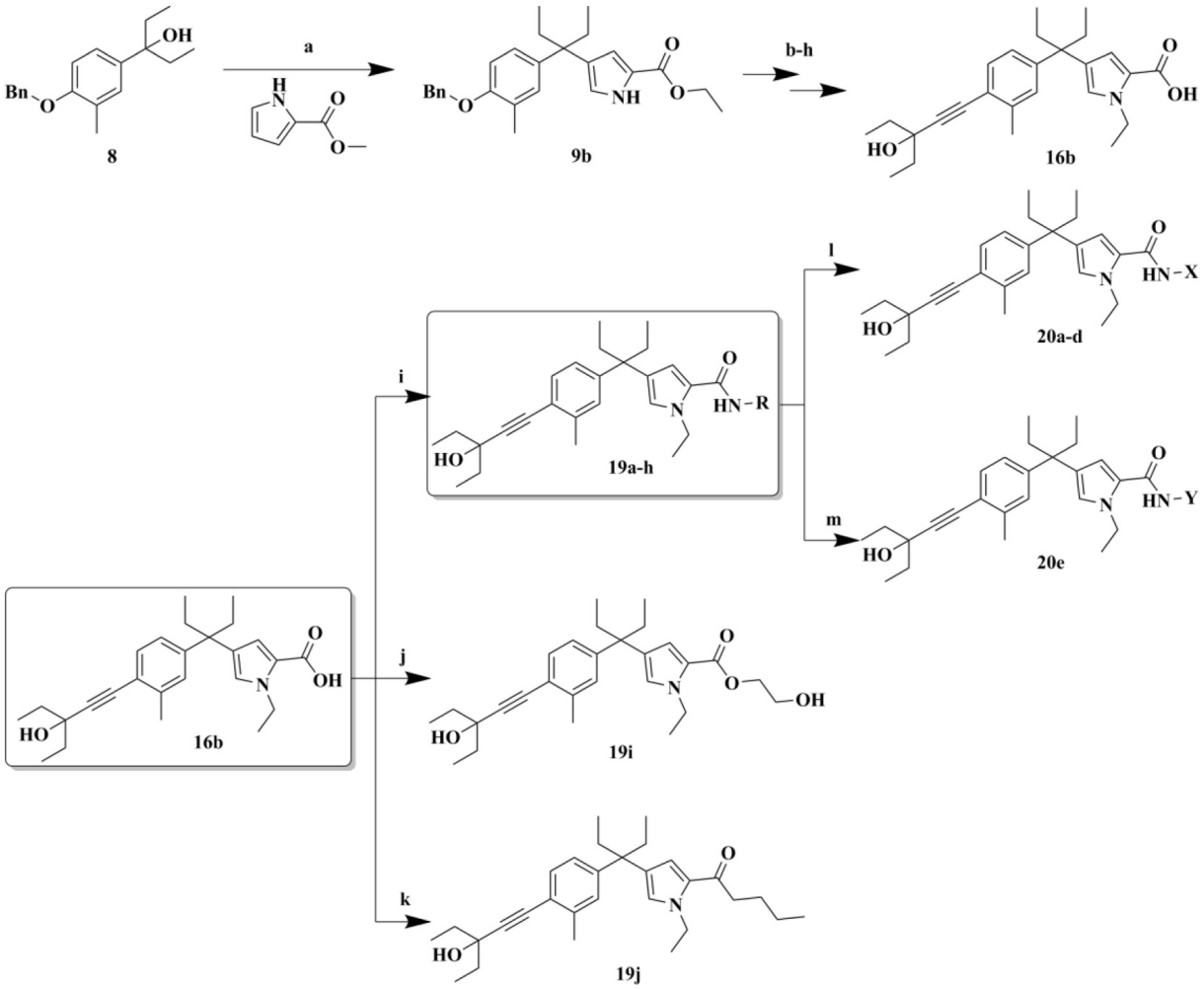
The synthetic pathway of target compounds **17c–i** and **18a–d** is outlined in Scheme 1. Key intermediate **8** was readily prepared using our previously reported approach,[25] and then it reacted with ethyl pyrrole-2-carboxylate in the presence of lewis acid $BF_3{\cdot}Et_2O$ at 0 °C to give intermediate **9a**, following the treatment with iodoethane in DMF to afford intermediate **10a**. The intermediate **11a** was obtained by reduction reaction of intermediate **10a** in the presence of $HCOONH_4$, which was acylated with trifluoromethanesulfonic anhydride to give **12a** in moderate yield. Intermediate **12a** subjected to sonogashira coupling reaction with trimethylsilylacetylene in the presence of palladium catalyst afforded **13a** and subsequent removal of trimethylsilyl group using tetrabutylammonium fluoride (TBAF) gave acetylene **14a**. Hydrolysis of intermediate **14a** by KOH produced the key intermediate **15a**, which was alkylated with *n*-butyl lithium and 3-pentanone at −78 °C to give key intermediate alcohol **16a**. Interestingly, followed by moving to room temperature for 2 h, alcohol **16a** transformed to compound **17i**, which were obtained by one step



Scheme 1. Synthesis of target compounds **17c–i** and **18a–d**. Reagents and conditions: (a) Ethyl 1H-pyrrole-2-carboxylate, $BF_3{\cdot}Et_2O$, 0 °C, 1 h, 53%; (b) C_2H_5I, NaH, DMF, 0–25 °C, 2 h, 82%; (c) Pd/C, $HCOONH_4$, CH_3OH/EtOAc (10:1), 25 °C, 1 h, 98%; (d) Tf_2O, TEA, toluene, 0 °C, 2 h, 67%; (e) TMS acetylene, $PdCl_2(dppf)_2$, TEA, DMF, 100 °C, overnight, 63%; (f) TBAF, THF, rt, 1 h, 95%; (g) 2 mol/L KOH, EtOH, 80 °C, 6 h, 95%; (h) 3-pentanone, *n*-BuLi, THF, −78 to 0 °C, 2 h, 75%; (i) EDCI, HOBt, TEA, RNH_2, DCM, rt, overnight, 35–96%; (j) EDCI, DMAP, ROH, DCM, rt, overnight, 26–64%; (k) *n*-BuLi, THF, 25 °C, 2 h, 79%; (l) 2 mol/L KOH, EtOH, rt, 1 h, 83–94%; (m) $LiAlH_4$, EtOAc, rt, 1 h, 86%.

from carboxylic acid group to ketone group. By reaction of intermediate **16a** with the corresponding amines or alcohols, target compounds **17c–h** and intermediates **17a–b** were obtained. Finally, target compounds **18a–c** were obtained by hydrolysis reactions of intermediates **17a–c** in the presence of KOH. On the other hand, target compound **18d** was obtained by reduction reaction of compound **17c**.

The synthetic pathway of target compounds **19a**, **19f–j** and **20a–e** is outlined in Scheme 2. Intermediate **9b**, which was the regioselectivity isomer of intermediate **9a**, was produced by



Scheme 2. Synthesis of target compounds **19a**, **19f–j** and **20a–e**. Reagents and conditions: (a) Ethyl 1H-pyrrole-2-carboxylate, $BF_3{\cdot}Et_2O$, 25 °C, 1 h, 44%; (b) C_2H_5I, NaH, DMF, 0–25 °C, 2 h, 85%; (c) Pd/C, $HCOONH_4$, CH_3OH/EtOAc (10:1), 25 °C, 1 h, 97%; (d) Tf_2O, TEA, toluene, 0 °C, 2 h, 64%; (e) TMS acetylene, $PdCl_2(dppf)_2$, TEA, DMF, 100 °C, overnight, 53%; (f) TBAF, THF, rt, 1 h, 95%; (g) 2 mol/L KOH, EtOH, 80 °C, 6 h, 96%; (h) 3-pentanone, *n*-BuLi, THF, −78 to 0 °C, 2 h, 54%; (i) EDCI, HOBt, TEA, RNH_2, DCM, rt, overnight, 35–96%; (j) EDCI, DMAP, ROH, DCM, rt, overnight, 64%; (k) *n*-BuLi, THF, 25 °C, 2 h, 77%; (l) 2 mol/L KOH, EtOH, rt, 1 h, 83–94%; (m) $LiAlH_4$, EtOAc, rt, 1 h, 89%.

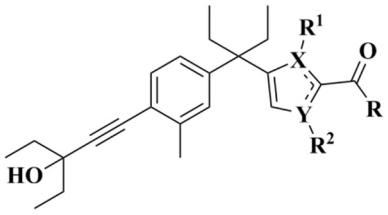
reacting with ethyl pyrrole-2-carboxylate in the presence of lewis acid $BF_3{\cdot}Et_2O$ at 20 °C instead of at 0 °C. By the same manner as described for the preparation of intermediates **10a–16a**, intermediates **10b–16b** were obtained. As described before, compound **19j** were obtained by one step from carboxylic acid group to ketone group. By reaction of intermediate **16b** with the corresponding amines or alcohols, target compounds **19a**, **19f–j** and intermediates **19b–e** were obtained. Finally, target compounds **20a–d** were obtained by hydrolysis reactions of intermediates **19a–d** in the presence of KOH. On the other hand, target compound **20e** was obtained by reduction reaction of compound **19e**.

The receptor binding affinity assay of synthesized compounds was initially performed at a concentration of 1 μM and the binding affinity was displayed by a relative value based on $1,25(OH)_2D_3$ being assigned as 100%. As shown in Table 1. the results displayed that although seven compounds (**17d**, **18a**, **18d**, and **19f–i**) demonstrated more effective binding affinities than the parental compound **7** (25.5%), with binding values at the range of 29.7–62.2%, none compound displayed equivalent binding affinity compared to $1,25(OH)_2D_3$. Additionally, five compounds (**17e**, **18c**, **20a**, **20c**, and **20e**) showed moderate binding affinities with the percentage of binding ranging from 13.6% to 20% and other compounds had no obvious binding affinities.

Our SARs analysis started with C-5 position of pyrrole ring bearing phenyl- pentane group and showed that compounds with the terminal hydrophilic groups in the A ring section, such as carboxylic acid (**18a**), hydroxyl (**18d**), and amino groups (**17d**) showed significant binding affinities. However, by one-atom extension of the hydrophilic groups of compounds **17d** and **18a**, **17e** and **18c** were synthesized and the binding affinities of them were dramatically decreased. Additionally, introducing a large carboxylic acid obtained compound **18b**, which displayed poor binding affinity, suggesting sterically hindered substitutes may not be accepted in VDR ligand binding domains. In order to verify the hypothesis, benzene and aromatic heterocyclic groups were introduced to synthesize compounds **17f** and **17h**. As expected, the low binding affinities of these compounds testify our hypothesis. Besides, when carboxylic acid group was protected by methyl group, the binding affinity of the resulting compound **17c** were dramatically decreased compared with compound **18c**, suggesting carboxylic acid group plays an important role in the VDR binding affinity. Furthermore, a similar binding affinities were observed when replacing ester group with hydrophobic groups, such as propargyl and *n*-butyl groups.

In order to explore the influence of substitution positions on the binding affinities, compounds **19a**, **19f–j** and **20a–e** were

Table 1
Structures and binding abilities of target compounds.



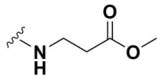
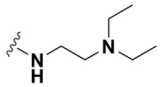
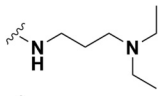
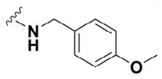
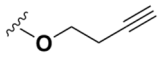
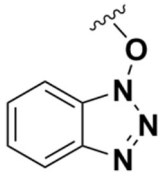
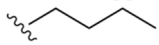
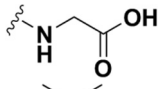
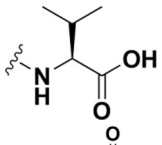
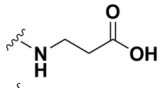
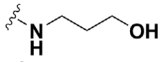
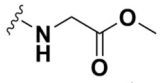
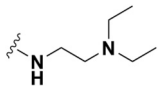
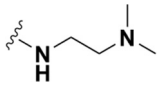
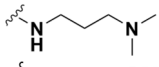
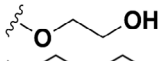
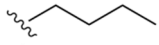
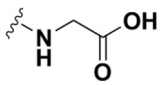
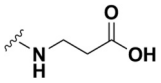
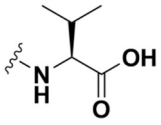
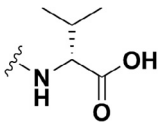
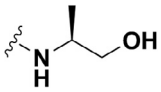
Compd	X	Y	R^1	R^2	R	Relative VDR Binding ability (%)[a]
17c	N	C	C_2H_5	H		–
17d	N	C	C_2H_5	H		62.2 ± 3.5
17e	N	C	C_2H_5	H		18.8 ± 1.8
17f	N	C	C_2H_5	H		4.0 ± 0.3
17g	N	C	C_2H_5	H		–
17h	N	C	C_2H_5	H		–
17i	N	C	C_2H_5	H		–
18a	N	C	C_2H_5	H		55.4 ± 3.1
18b	N	C	C_2H_5	H		5.9 ± 0.3
18c	N	C	C_2H_5	H		13.6 ± 1.5
18d	N	C	C_2H_5	H		59.7 ± 2.7
19a	C	N	H	C_2H_5		–
19f	C	N	H	C_2H_5		50.3 ± 3.1
19g	C	N	H	C_2H_5		55.3 ± 4.1
19h	C	N	H	C_2H_5		31.6 ± 1.7
19i	C	N	H	C_2H_5		29.7 ± 2.1
19j	C	N	H	C_2H_5		–
20a	C	N	H	C_2H_5		14.6 ± 1.1

Table 1 (*continued*)

Compd	X	Y	R^1	R^2	R	Relative VDR Binding ability (%)[a]
20b	C	N	H	C_2H_5		4.3 ± 0.1
20c	C	N	H	C_2H_5		20.0 ± 2.4
20d	C	N	H	C_2H_5		5.9 ± 0.3
20e	C	N	H	C_2H_5		19.8 ± 1.2
7	–	–	–	–	–	25.5 ± 1.4
$1,25(OH)_2D_3$	–	–	–	–	–	100.0 ± 2.3

[a] The values represent the mean ± SD of three independent experiments. $1,25(OH)_2D_3$ is assigned as 100%.

synthesized by removing the substitution on the C-5 position of pyrrole group to C-4 position. As shown in Table 1, similar SARs were observed. Although compound **19g** showed the most potent affinity among them, no better result was observed compared to compound **17d**. On the contrary, some compounds bearing same groups showed decreased binding affinity, such as compounds **20a**, **20b**, and **19f** as compared with **18a**, **18c**, and **17d**. The results suggest that the position of the substitute is important by affecting the conformation of the terminal hydrophilic group which is essential for binding, as in the case of the conformationally restricted A ring of secosteroid. To further explore the difference on the binding affinities between C-5 position of pyrrole group and C-4 position, we performed the docking analyses of compounds **18a** and **20a**, which had the same hydrophilic substitute, but displayed remarkable difference on binding affinities, based on crystallographic structure of $1,25(OH)_2D_3$ in complex with VDR (PDB code: 1DB1). As shown in Fig. 3C, docking analyses demonstrated that the side chain of both compounds present similar conformations. However, substitution of pyrrole group from C-5 position to C-4 position resulted in conformational change in the A ring section (Fig. 3A and B). In the docking, the introducing carboxylic acid of compound **18a** was beautifully adjusted in VDR and formed two hydrogen bonds with Ser237 and Arg274. Unfortunately, the change of substitution positions resulted in a longer distance

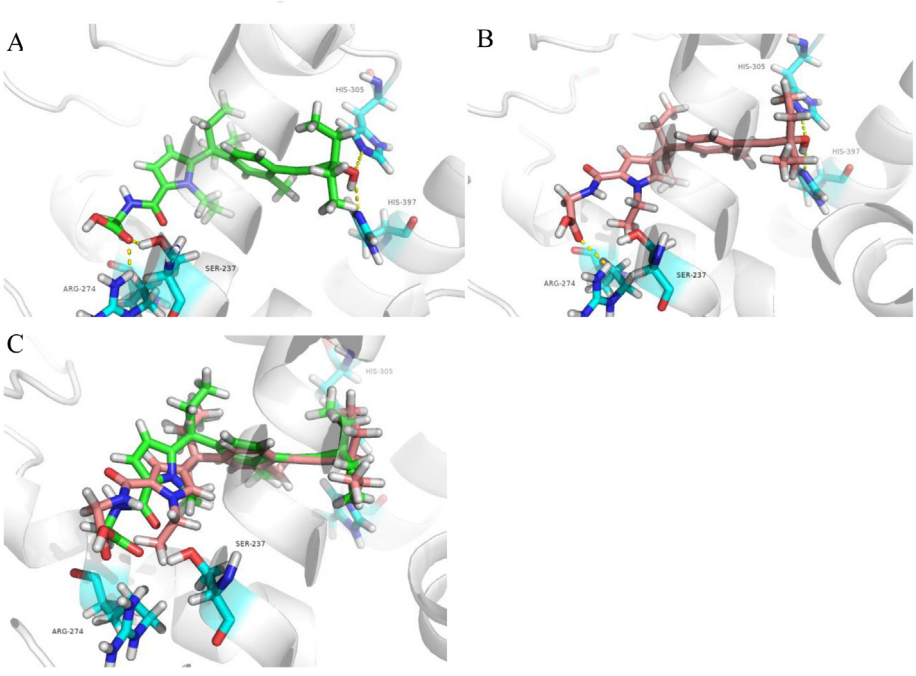


Fig. 3. (A) Docking structure of the complex VDR-compound **18a**. (B) Docking structure of the complex VDR-compound **20a**. (C) Superposition of compounds **18a** and **20a**. Compound **18a** is shown in stick representation with carbon and oxygen atoms in green and red, respectively. Compound **20a** is shown in stick representation with carbon and oxygen atoms in wheat and red, respectively. The hydrogen bonds formed are shown as yellow dashed lines.

Table 2
The binding affinities of selected compounds.

Compd	VDR binding (IC_{50}, nM)[a]	Compd	VDR binding (IC_{50}, nM)[a]
17d	73.21 ± 3.68	**19g**	51.31 ± 3.54
18a	85.66 ± 2.45	**7**	76.76 ± 5.12
18d	123.43 ± 2.32	$1,25(OH)_2D_3$	1.13 ± 0.11
19f	56.48 ± 2.17		

[a] IC_{50}: the concentration that causes 50% of cell proliferation inhibition. Data are expressed as mean ± SD from three independent experiments.

between the introduced carboxylic acid of compound **20a** and Ser237, so only one hydrogen bond with Arg274 was formed, which maybe the important reason of different binding affinities.

Subsequently, we further evaluated the VDR binding affinities (IC_{50}) of compounds **17d**, **18a**, **18d**, and **19f–g** with excellent inhibition at 1 μM. As shown in Table 2, introducing hydroxyl and carboxylic acid groups as the terminal hydrophilic groups showed decreased affinities compared with the parental compound **7**. However, compounds **17d**, **19f**, and **19g**, all of which were introduced amino groups instead displayed more potent than lead compound **7**, which deserved further investigations.

It is proved that vitamin D_3-agonistic activity is associated with HL-60 cell differentiation induction.[30,31] Therefore, the vitamin D_3-agonistic ability can be estimated as the potential to differentiate human promyelocytic leukemia cell line (HL-60) into macrophages. All synthesized compounds were tested for HL-60 cell differentiation using calcitriol as positive control, as shown in Table 3. Most compounds displayed good agonistic activities with EC_{50} values in the nanomole range. Among them, compounds **19f** and **20e** showed higher agonistic activities than positive control $1,25(OH)_2D_3$. As similar as the SARs of binding affinities, compounds with the terminal hydrophilic groups in the A ring section, such as carboxylic acid (**18a**) and anime groups (**17d**) also showed significant agonistic activities. By introducing a large carboxylic acid substitute, compound **18b** was synthesized and showed a slight decreased agonistic activity compared to compound **18a**, although a dramatically decreased binding affinity was observed. This discrepancy between agonistic activity and binding affinity could be explained by the interactions between the VDR ligand complex and other cofactors. For the transcriptional activation of VDR, it is required that the AF-2 transactivation motif of VDR interacts with several types of cofactor such as VDR interacting proteins (DRIPs).[32–35] Furthermore, introducing large sterically hindered substitutes, such as **17f** and **17h** displayed remarkable decreased agonistic activities. Additionally, when removing the substitution on the C-5 position of pyrrole group to C-4 position, most compounds with the same hydrophilic groups showed decreased agonistic activities. However, compound **19f** (EC_{50} = 6.98 nM) bearing N,N-diethyl-1,2-ethanediamine, which had less binding affinity than **17d**, showed the best agonistic activity. Unfortunately, no improvement in agonistic activities of all the synthesized compounds was observed compared with the parental compound **7** (EC_{50} = 6.54 nM). We estimate that acetylene bond introduced has weaker cell permeability than ether bond and results in the reduced agonistic activity.

Table 3
The HL-60 differentiation-inducing activities and anti-proliferative activities of synthesized compounds *in vitro*.

Compd	HL-60 differentiation -inducing activity(EC_{50}, nM)[a]	*In vitro* anti-proliferative activity (IC_{50}, μM)[b]	
		MCF-7	PC-3
17c	>50	>50	36.27 ± 0.76
17d	10.12 ± 0.86	3.77 ± 0.26	5.85 ± 0.37
17e	68.91 ± 2.29	17.80 ± 0.56	8.50 ± 0.63
17f	>50	>50	>50
17g	>50	>50	>50
17h	>50	>50	>50
17i	>50	>50	>50
18a	9.36 ± 0.86	5.14 ± 0.22	2.25 ± 0.14
18b	17.67 ± 1.15	13.00 ± 0.25	5.00 ± 0.28
18c	293.44 ± 5.68	>50	5.13 ± 0.35
18d	136.86 ± 7.43	5.95 ± 0.14	>50
19a	287.65 ± 10.59	26.46 ± 0.44	17.45 ± 0.66
19f	6.98 ± 0.36	1.80 ± 0.19	5.35 ± 0.31
19g	19.29 ± 0.86	13.44 ± 0.12	10.07 ± 0.47
19h	142.17 ± 7.13	20.17 ± 0.69	15.06 ± 0.50
19i	12.56 ± 0.59	1.88 ± 0.17	11.40 ± 0.21
19j	>50	>50	48.12 ± 0.22
20a	54.33 ± 4.33	17.54 ± 0.57	6.30 ± 0.44
20b	>50	30.55 ± 0.32	>50
20c	12.68 ± 0.76	12.60 ± 0.31	3.75 ± 0.14
20d	68.32 ± 4.13	18.02 ± 0.26	10.28 ± 0.33
20e	7.33 ± 0.28	1.91 ± 0.16	6.05 ± 0.40
7	6.54 ± 0.07	1.37 ± 0.02	2.8 ± 0.58
$1,25(OH)_2D_3$	8.12 ± 0.03	11.10 ± 0.26	16.20 ± 0.29

[a] EC_{50}: Vitamin D3-agonistic activity was estimated as HL-60 differentiation inducing ability. Data represent mean ± SD, n = 3, $^*P < 0.05$.

[b] IC_{50}: the concentration that causes 50% of cell proliferation inhibition. Data are expressed as mean ± SD from three independent experiments.

To evaluate the anti-proliferative activities of synthesized compounds, human breast cancer cell line (MCF-7)[36,37] and human prostate cancer cell line (PC-3),[38] which over express VDR were selected as cell models to test the anti-proliferative effects by the standard MTT assay, with $1,25(OH)_2D_3$ as positive control. As shown in Table 3, the results displayed that most of the synthesized compounds showed moderate to good activities with IC_{50} values in the micromole range and in some cases better than that of $1,25(OH)_2D_3$, while compounds **17h–i**, and **17k** were proved to be poor activities against both cell lines. Interestingly, compounds **17d**, **18a**, **18d**, **19f**, **19i** and **20e** showed higher anti-proliferative activities than $1,25(OH)_2D_3$ against MCF-7 cells. In addition, thirteen compounds exhibited remarkable anti-proliferative activities with the IC_{50} values ranging from 2.2 to 15.1 μM, which were comparable to that of $1,25(OH)_2D_3$ (IC_{50} = 17.2 μM) on PC-3 cells. Notably, compounds **17d**, **18a**, **19f**, **19i** and **20e** displayed more effective anti-proliferative activities against both cell lines compared with $1,25(OH)_2D_3$, although only compounds **19f** and **20e** showed better agonistic activities than $1,25(OH)_2D_3$, which suggests that they may work though the multiple molecular mechanisms. This primary screening results revealed that phenylpyrrolyl pentane derivatives with acetylene bond linker exhibited strong anti-proliferative activities. As similar as the SARs of the binding affinity and agonistic activity, compounds bearing hydrophilic groups, such as **17d**, **18a**, **19f**, and **19i**, showed better agonistic activities than that of hydrophobic groups, such as **17c**, **17i** and **19j**, which suggested that it is necessary to introduce hydrophilic groups into the A ring section. Compound **18d** bearing hydroxyl group displayed poor anti-proliferative activity against PC-3 cells, although it showed significant binding affinity and moderate agonistic activity, which suggests that chemical modification of compound **18d** may induce AF2 conformations and cofactor interactions distinct from those of natural ligands and can result in cell type-selective modulation of target gene expression.[39] Besides, compound **18b** also showed a slight decreased anti-proliferative activity compared to compound **18a** alike that of agonistic activities. Furthermore, compound **17f–i** bearing sterically hindered or hydrophobic substitutes also exhibited poor anti-proliferative activities against both cell lines as similar as the binding affinities and agonistic activities. Additionally, as similar as the SARs of agonistic activities, most synthesized compounds exhibited decreased anti-proliferative activities compared with that of the same A ring section, such as **18a**, **18b** and **18c–20a**, **20c** and **20b**, respectively. In this regard, it could be proved that the

Table 4
The anti-proliferative activities of selected compounds and $1,25(OH)_2D_3$ over L02 normal cell line and selectivities for both cancer cells.

Compd	*In vitro* anti-proliferative activities (IC_{50}, μM)[a]			Selectivities	
	L02	MCF-7	PC-3	L02/MCF-7	L02/PC-3
17d	20.52 ± 0.12	3.77 ± 0.26	5.85 ± 0.37	5.4	3.5
19f	42.14 ± 0.46	1.80 ± 0.19	5.35 ± 0.31	23.4	7.9
20e	35.43 ± 0.34	1.91 ± 0.16	6.05 ± 0.40	18.5	5.9
7	21.24 ± 0.32	1.37 ± 0.02	2.8 ± 0.58	15.5	7.6
$1,25(OH)_2D_3$	>50	11.10 ± 0.26	16.20 ± 0.29	>4.5	>3.1

[a] IC_{50}: the concentration that causes 50% of cell proliferation inhibition. Data are expressed as mean ± SD from three independent experiments.

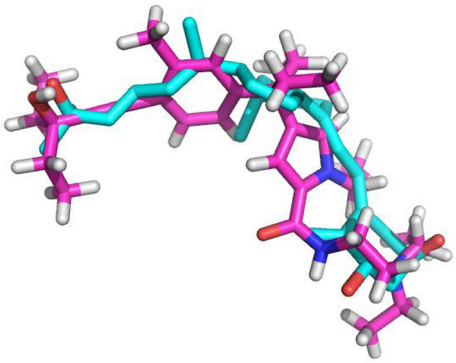


Fig. 4. Superposition of compounds **19f** and $1\alpha,25\text{-}(OH)_2\text{-}D_3$. Compound **19f** is depicted in magenta and $1\alpha,25\text{-}(OH)_2\text{-}D_3$ is depicted in cyan.

anti-proliferative activities of synthesized compounds are positively correlative with VDR agonistic activities. Moreover, compound **19f** also showed the most anti-proliferative activities against MCF-7 and PC-3 cells with the IC_{50} values of 1.80 and 5.35 μM, respectively, which was comparable to positive control $1,25\text{-}(OH)_2D_3$.

As further evaluation for the selective cytotoxicities of the promising compounds **17d**, **19f**, and **20e**, they were tested over human normal liver cell line (L02) using MTT assay. As illustrated in Table 4, all compounds displayed moderate cytotoxicities against L02 cells. Notably, compound **19f** (IC_{50} = 42.14 μM) had remarkable decreased cytotoxicity compared with the parental compound **7** (IC_{50} = 21.24 μM) and showed better selectivities for both cancer cells (L02/MCF-7 = 23.4, L02/PC-3 = 7.9). Finally, an investigation on the binding affinities and anti-proliferative activities for cancer and normal cells of these derivatives showed that compound **19f** exhibited desirable results.

To confirm the detailed interactions of the most promising compound **19f**, molecular docking study was conducted based on crystallographic structure of $1,25(OH)_2D_3$ in complex with VDR (PDB code: 1DB1). Compound **19f** was manually docked into the crystal structure of VDR using software Discovery Studio 3.0. Fig. 4 shows the superposition of the conformations of compound **19f** and the

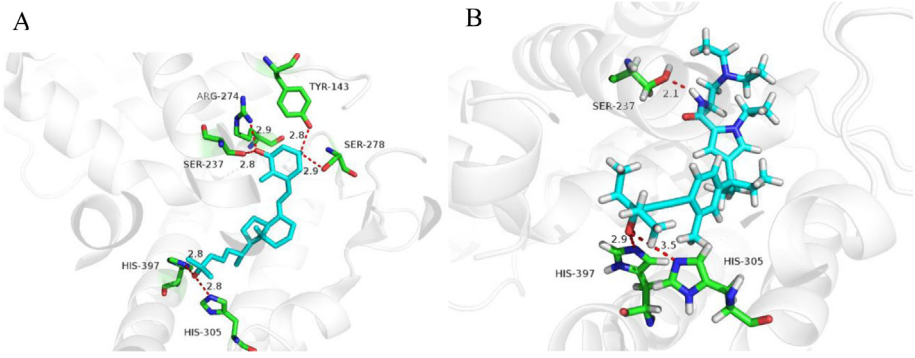


Fig. 5. (A) Structure of the complex VDR-$1\alpha,25\text{-}(OH)_2\text{-}D_3$. (B) Docking structure of the complex VDR-compound **19f**. The ligands are shown in stick representation with carbon and oxygen atoms in cyan and red, respectively. The hydrogen bonds formed are shown as red dashed lines.

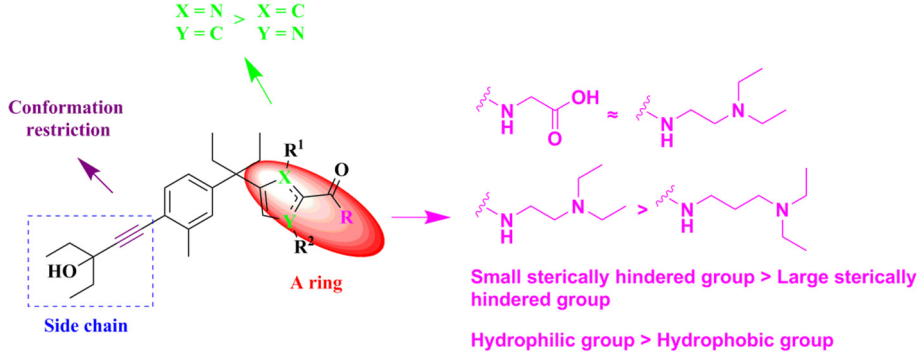


Fig. 6. Schematic diagram of structure-activity relationships.

natural ligand 1,25$(OH)_2D_3$. Docking analyses demonstrated that the side chain and A ring part of compound **19f** present similar conformations to those observed in the presence of 1,25$(OH)_2D_3$. As shown in Fig. 5, the hydroxyl group in the side chain was able to form the same hydrogen-bonding interactions with His 305 and His 397 as the hVDR LBD bound to 1α,25-$(OH)_2$-D_3 complex. However, the A ring part of compound **19f** form hydrogen-bonding interaction only with Ser 237 by amide bond, while 1α,25-$(OH)_2$-D_3 binded with Ser 237, Arg 274, Tyr 143, and Ser 278. In addition, the N,N-diethyl ethyl amine group introduced made longer A ring part than that of 1α,25-$(OH)_2$-D_3. These factors might play important roles in reducing the binding affinity of compound **19f** to VDR (see Fig. 6).

According to the above results (Tables 1 and 3), we can draw some conclusions: (1) introducing the hydrophilic moieties at the R group is important to improve the VDR binding affinities and anti-proliferative activities, and introduction of the hydrophobic segments may lead to a remarkable decrease or even loss of binding affinities and anti-proliferative activities. (2) Introduction of large groups such as benzene ring cannot be tolerated, nearly leading to a loss of affinities and activities. (3) C-5 position of pyrrole ring bearing phenyl-pentane group is not absolutely required but may increase the binding affinity.

In summary, we synthesized and evaluated a series of novel phenyl-pyrrolyl pentane derivatives with acetylene bond linker as VDR ligands. Structural optimization of the parental compound **7** led to the synthesis of 22 derivatives. Seven analogs (**17d**, **18a**, **18d**, and **19f–i**) demonstrated more effective binding affinities than the parental compound **7**. Moreover, compound **19f** not only showed excellent agonistic activity to VDR but also displayed more anti-proliferative effect against MCF-7 and PC-3 cells with the IC_{50} values of 1.80 and 5.35 μM, respectively. Besides, compound **19f** exhibited reduced toxicity against human normal liver cell line (L02) compared with the parental compound **7**. In conclusion, on the basis of the abilities of these compounds, introducing acetylene bond linker for phenyl-pyrrolyl pentane derivatives may be a strategy for the discovery of new drugs for the treatment of cancer diseases.

Based on the preliminary investigation results, our efforts are now focused on the modification and understanding the mode of action of these novel molecules. It is expected that the biological results described and further modification studies will expedite the development of new chemotherapeutic agents for the clinical intervention of cancer disease.

Acknowledgments

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.01.084.

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