Design, synthesis and biological evaluation of nonsecosteroidal vitamin D₃ receptor ligands as anti-tumor agents

Bin Wang, Meixi Hao, Can Zhang*

State Key Laboratory of Natural Medicines and Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of New Drug Discovery, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China

A R T I C L E   I N F O

Article info
Received 19 December 2016
Revised 17 January 2017
Accepted 27 January 2017
Available online 1 February 2017

Keywords:
Vitamin D₃
VDR ligand
Nonsecosteroidal
Phenyl-pyrrolyl pentane skeleton
Binding affinity
Agonistic activity
Anti-proliferative activity

A B S T R A C T

1α,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃, also known as calcitriol), the active form of vitamin D₃, 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)₂D₃. Here, in order to optimize the structural features and discover more potent derivative, a series of nonsecosteroidal vitamin D₃ 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 IC₅₀ values of 1.80 and 5.35 μM, respectively, which was comparable to positive control 1,25-(OH)₂D₃. Moreover, compound 19f exhibited reduced toxicity against human normal liver cell line (LO2) compared with the parental compound 7. Besides, the preliminary structure–activity relationships (SARs) were also analyzed.

© 2017 Elsevier Ltd. All rights reserved.

The active form of vitamin D₃, the hormone 1α,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃, also known as calcitriol) participates in numerous biological processes. In addition to its classical role in mineral homeostasis and bone mineralization, this hormone regulates numerous cellular pathways that could have a role in determining cancer risk and prognosis. Because of this “nonclassical” actions, 1,25(OH)₂D₃ has attracted considerable interest as a potential drug for the treatment of cancer disease. The effects of 1,25(OH)₂D₃ are mediated through the vitamin D₃ receptor (VDR) which is a ligand-dependent transcription factor belonging to the superfamily of nuclear hormone receptors.

So far, more than 3000 secosteroid analogs have been synthesized, and many of them exhibit efficient VDR activities and some analogs have been successfully used in the treatment of bone, mineral, and skin disorders. However, adverse effects, particularly hypercalcemia, limit the clinical application of secosteroid analogs in the management of cancer disease. 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)₂D₃ without direct structural relationship to 1,25(OH)₂D₃, such as VDR binding and inhibition of proliferation of cancer cells. Moreover, they have simpler structures compared with secosteroid analogs. 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. While the VDR binding affinities of all the synthesized compounds suffered a remarkable decrease as compared with 1,25(OH)₂D₃, 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.

* Corresponding author.
E-mail address: zhangcan@cpu.edu.cn (C. Zhang).

http://dx.doi.org/10.1016/j.bmcl.2017.01.084
0960-894X/© 2017 Elsevier Ltd. All rights reserved.
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, and then it reacted with ethyl pyrrole-2-carboxylate in the presence of lewis acid BF3\textsubscript{3}/\textsubscript{3}Et2O 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 HCOON\textsubscript{3}, 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
from carboxylic acid group to ketone group. By reaction of intermediate 16a with the corresponding amines or alcohols, target compounds 17c–h were obtained. Finaly, 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 1.** Synthesis of target compounds 17c–i and 18a–d. Reagents and conditions: (a) Ethyl 1H-pyrrole-2-carboxylate, BF₃·Et₂O, 0 °C, 1 h, 53%; (b) C₂H₅I, NaH, DMF, 0–25 °C, 2 h, 82%; (c) Pd/C, HCOONH₄, CH₃OH/ETOAc (10:1), 25 °C, 1 h, 98%; (d) Ts₂O, TEA, toluene, 0 °C, 2 h, 67%; (e) TMS acetylene, PdCl₂(dppe)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, RNH₂, 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) LiAlH₄, EtOAc, rt, 1 h, 86%.

---

**Scheme 2.**
reacting with ethyl pyrrole-2-carboxylate in the presence of lewis acid BF$_3$/Et$_2$O at 25 °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)$_2$D$_3$ 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)$_2$D$_3$. 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 was dramatically decreased compared with compound 18b, 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.

<table>
<thead>
<tr>
<th>Compd</th>
<th>X</th>
<th>Y</th>
<th>R¹</th>
<th>R²</th>
<th>R</th>
<th>Relative VDR Binding ability (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>17c</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17d</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>62.2 ± 3.5</td>
</tr>
<tr>
<td>17e</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>18.8 ± 1.8</td>
</tr>
<tr>
<td>17f</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>17g</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17h</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17i</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18a</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>55.4 ± 3.1</td>
</tr>
<tr>
<td>18b</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>18c</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>13.6 ± 1.5</td>
</tr>
<tr>
<td>18d</td>
<td>N</td>
<td>C</td>
<td>C₂H₅</td>
<td>H</td>
<td></td>
<td>59.7 ± 2.7</td>
</tr>
<tr>
<td>19a</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19f</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td>50.3 ± 3.1</td>
</tr>
<tr>
<td>19g</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td>55.3 ± 4.1</td>
</tr>
<tr>
<td>19h</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td>31.6 ± 1.7</td>
</tr>
<tr>
<td>19i</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td>29.7 ± 2.1</td>
</tr>
<tr>
<td>19j</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20a</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C₂H₅</td>
<td></td>
<td>14.6 ± 1.1</td>
</tr>
</tbody>
</table>
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)2D3 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

<table>
<thead>
<tr>
<th>Compd</th>
<th>X</th>
<th>Y</th>
<th>R1</th>
<th>R2</th>
<th>R</th>
<th>Relative VDR Binding ability (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>20b</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C3H5</td>
<td></td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>20c</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C3H5</td>
<td></td>
<td>20.0 ± 2.4</td>
</tr>
<tr>
<td>20d</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C3H5</td>
<td></td>
<td>5.9 ± 0.3</td>
</tr>
<tr>
<td>20e</td>
<td>C</td>
<td>N</td>
<td>H</td>
<td>C3H5</td>
<td></td>
<td>19.8 ± 1.2</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.5 ± 1.4</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.0 ± 2.3</td>
</tr>
</tbody>
</table>

a The values represent the mean ± SD of three independent experiments. 1,25(OH)2D3 is assigned as 100%.

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.
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\textsubscript{50}) of compounds 17d, 18a, 18d, and 19f–g with excellent inhibition at 1 \mu M. 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\textsubscript{3}-agonistic activity is associated with HL-60 cell differentiation induction.\textsuperscript{30,31} Therefore, the vitamin D\textsubscript{3}-agonistic activity was estimated as HL-60 differentiation inducing ability. Data represent mean ± SD, n = 3, *P < 0.05.

To evaluate the anti-proliferative activities of synthesized compounds, human breast cancer cell line (MCF-7)\textsuperscript{36,37} and human prostate cancer cell line (PC-3),\textsuperscript{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)\textsubscript{2}D\textsubscript{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\textsubscript{50} values in the micromole range and in some cases better than that of 1,25(OH)\textsubscript{2}D\textsubscript{3} while compounds 17b–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)\textsubscript{2}D\textsubscript{3} against MCF-7 cells. In addition, thirteen compounds exhibited remarkable anti-proliferative activities with the IC\textsubscript{50} values ranging from 2.2 to 15.1 \mu M, which were comparable to that of 1,25(OH)\textsubscript{2}D\textsubscript{3} (IC\textsubscript{50} = 17.2 \mu M) on PC-3 cells. Notably, compounds 17d, 18a, 19f, 19i and 20e showed more effective anti-proliferative activities against both cell lines compared with 1,25(OH)\textsubscript{2}D\textsubscript{3}, although only compounds 19f and 20e showed better agonistic activities than 1,25(OH)\textsubscript{2}D\textsubscript{3}, which suggested 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.\textsuperscript{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 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 (DIRPs).\textsuperscript{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\textsubscript{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\textsubscript{50} = 6.54 nM). We estimate that acetylene bond introduced has weaker cell permeability than ether bond and results in the reduced agonistic activity.
The 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 IC50 values of 1.80 and 5.35 μM, respectively, which was comparable to positive control 1,25-(OH)2D3.

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 (IC50 = 42.14 μM) had remarkable decreased cytotoxicity compared with the parental compound 7 (IC50 = 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)2D3 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

Table 4
The anti-proliferative activities of selected compounds and 1,25(OH)2D3 over L02 normal cell line and selectivities for both cancer cells.

<table>
<thead>
<tr>
<th>Compd</th>
<th>In vitro anti-proliferative activities (IC50, μM)</th>
<th>Selectivities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L02</td>
<td>MCF-7</td>
</tr>
<tr>
<td>17d</td>
<td>20.52 ± 0.12</td>
<td>3.77 ± 0.26</td>
</tr>
<tr>
<td>19f</td>
<td>42.14 ± 0.46</td>
<td>1.80 ± 0.19</td>
</tr>
<tr>
<td>20e</td>
<td>35.43 ± 0.34</td>
<td>1.91 ± 0.16</td>
</tr>
<tr>
<td>7</td>
<td>21.24 ± 0.32</td>
<td>1.37 ± 0.02</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>&gt;50</td>
<td>11.10 ± 0.26</td>
</tr>
</tbody>
</table>

*IC50: 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 1α,25-(OH)2-D3. Compound 19f is depicted in magenta and 1α,25-(OH)2-D3 is depicted in cyan.

Fig. 5. (A) Structure of the complex VDR-1α,25-(OH)2-D3. (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)_2D_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)_2D_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)_2D_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-4) 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 IC50 values of 1.80 and 5.35 μM, respectively. Besides, compound 19f exhibited reduced toxicity against human normal liver cell line (LO2) 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

This work was supported by the National Natural Science Foundation of China (81273468, 81473153), National Basic Research Program of China (2015CB755500), Natural Science Foundation of Jiangsu Province of China (BK20140672), and 111 Project from the Ministry of Education of China and the State Administration of Foreign Expert Affairs of China (No. 111-2-07).

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.