Bioorganic & Medicinal Chemistry Letters 27 (2017) 3408–3411

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of steroidal derivatives bearing a small ring as vitamin D receptor agonists

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ARTICLE INFO

Article history:
Received 11 May 2017
Revised 30 May 2017
Accepted 31 May 2017
Available online 1 June 2017

Keywords:
Steroids
Vitamin D receptor
Cyclopropanes
Cyclobutanes
[2+2] cycloaddition

ABSTRACT

A novel series of 3-ketolithocholic acid derivatives as well as estrone derivatives bearing a small ring for the conformational fixation of the side chain were synthesized by using a catalytic [2+2] cycloaddition and a ring-contraction rearrangement. The steroidal derivatives were evaluated for transcriptional activation of vitamin D receptor by luciferase reporter assays. Among them, two estrone derivatives showed a higher efficacy of the transactivation of vitamin D receptor than 3-ketolithocholic acid, and the small ring moieties were found to be important for the efficacy.

Calcitriol, which is the active form of vitamin D3, modulates a broad spectrum of biological functions such as bone homeostasis, immunity, cellular growth, and differentiation through binding to the vitamin D nuclear receptor (VDR) (Fig. 1).[1] The derivatives of calcitriol are effective in the treatment of osteoporosis and psoriasis.[2] However, their therapeutic use is limited because of their severe side effects such as hypercalciuria and hypercalcemia.[1c] It was found that lithocholic acid (LCA), which is a secondary bile acid, and its metabolite, 3-ketolithocholic acid (3-keto LCA), also bind to VDR and exhibit the agonistic activities although their structures fundamentally differ from that of calcitriol.[3] Lithocholic acid derivatives such as LCA acetate and LCA propionate act as selective VDR agonists with greater potency than LCA, and these derivatives can activate VDR without inducing hypercalcemia.[4] The structure of the complexes of the ligand-binding domain of VDR with LCA derivatives were solved by X-ray crystallography, which revealed that LCA and its derivatives bind to the same binding pocket as calcitriol, but in the opposite orientation.[5]

Conformational fixation by introduction of a structurally rigid moiety such as an unsaturated bond or a ring structure is a common strategy in drug development to increase biological activity and/or reduce side effects.[6] However, only a limited number of small ring carbocycles have been used to restrict a rotatable side chain because the practical synthetic methods are still lacking.[7] We recently developed a strategy for construction of a cyclobutane or a cyclopropane ring as a rigid structural unit on the D-ring of a steroidal backbone by using a stereoselective catalytic [2+2] cycloaddition and a stereospecific ring-contraction rearrangement.[8–10] We became interested in their biological activity as VDR agonists. In addition, we envisaged that we would apply the synthetic strategy to the synthesis of a new class of 3-keto LCA derivatives bearing a small ring for conformational regulation of the carbon side chain. Herein, we report synthesis of the 3-keto LCA derivatives and biological evaluation of the derivatives as well as estrone derivatives as VDR agonists.

Our synthesis of 3-keto LCA derivatives commenced from commercially available 4-androstene-3,17-dione, which was subjected to hydrogenation in 4-methylpydirine as a solvent and separation by recrystallization to give **1** in 74% yield as a single diastereomer (Scheme 1).[11] Selective protection of the less hindered A-ring carbonyl group, followed by treatment with *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf), delivered silyl enol ether **3**. $EtAlCl_2$-catalyzed [2+2] cycloaddition of **3** with hexafluoroisopropyl (HFIP) acrylate was carried out at different temperatures. When the reaction was performed at −78 °C, kinetic product *trans*-**4** was selectively obtained along with *cis*-**4** (*trans*:*cis* = 96:4) as a minor diastereomer. Reduction of the resulting diastereomeric

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http://dx.doi.org/10.1016/j.bmcl.2017.05.089
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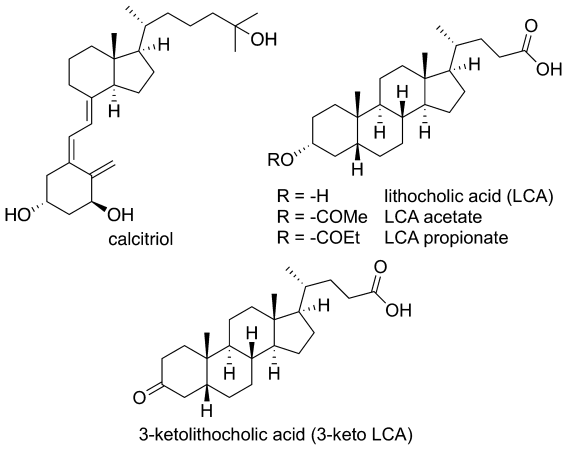
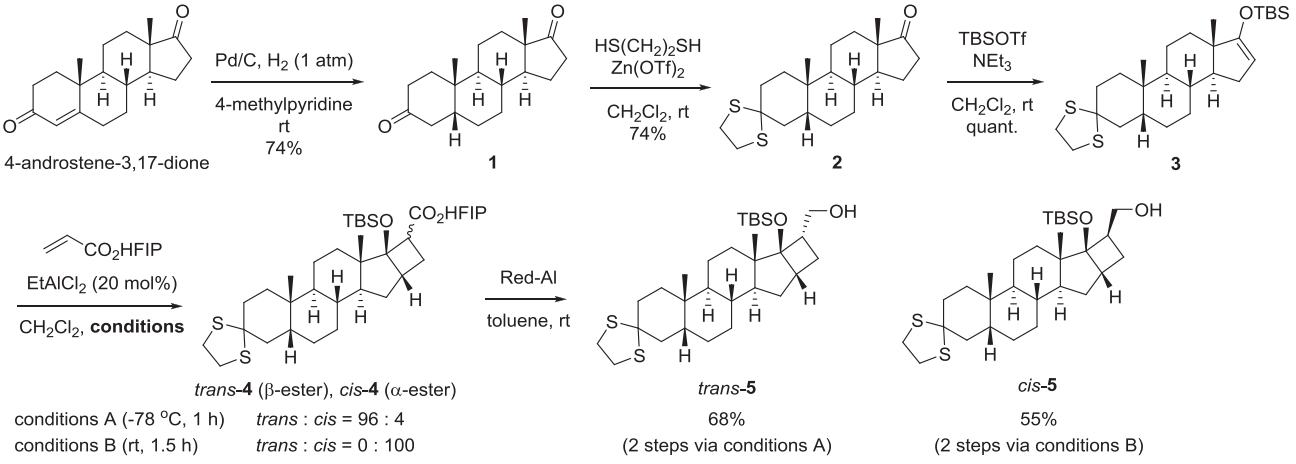


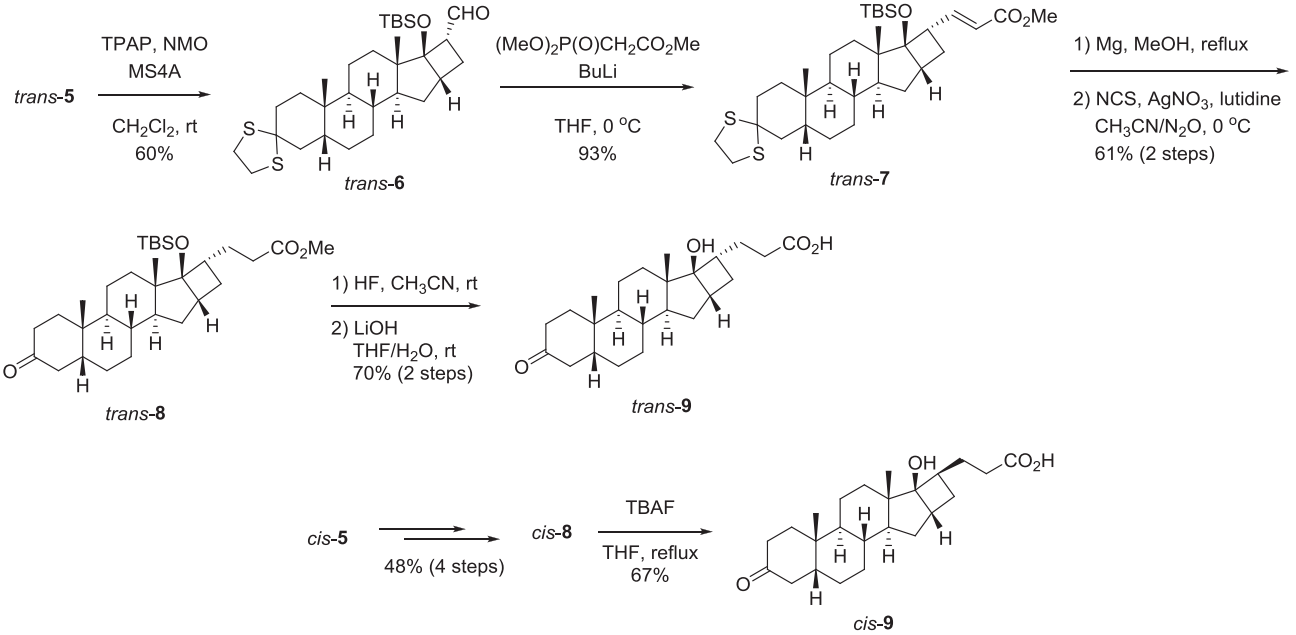
Fig. 1. Chemical structures of calcitriol, LCA, LCA acetate, LCA propionate, and 3-keto LCA.

mixture **4** by sodium bis(2-methoxyethoxy)aluminumhydride (Red–Al), followed by separation of the products on silica gel column chromatography, gave *trans*-**5** as a pure diastereomer in 68% yield for two steps. The [2+2] cycloaddition at room temperature afforded thermodynamically more stable *cis*-**4** exclusively, which was reduced to *cis*-**5** with Red–Al.[12]

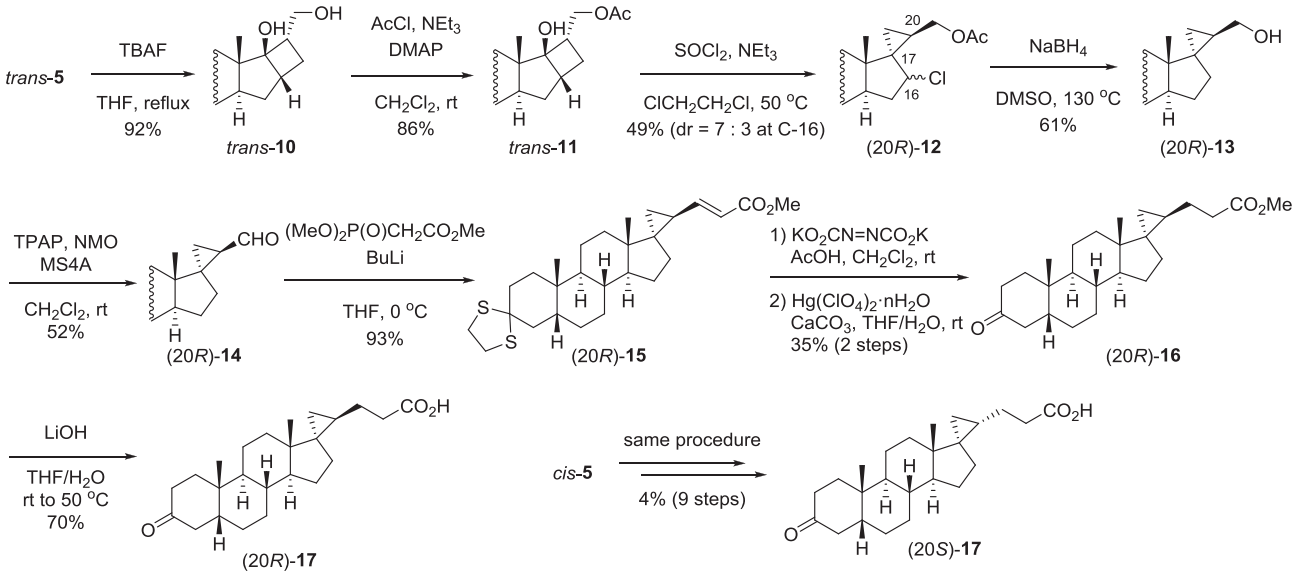
With the cyclobutane scaffolds constructed, we next installed a carbon side chain on the small ring (Scheme 2). Oxidation of *trans*-**5** by tetrapropylammonium perruthenate (TPAP), followed by Horner-Wadsworth-Emmons reaction, gave *trans*-**7**. Reduction of the resulting conjugated double bond by treatment with magnesium in methanol, followed by removal of the thioacetal with *N*-chlorosuccinimide (NCS), provided *trans*-**8**.[13,14] Removal of the TBS group using HF and saponification with LiOH furnished *trans*-**9**. Ester *cis*-**8** was also synthesized from *cis*-**5** by the same procedure. When *cis*-**8** was treated with tetrabutylammonium fluoride (TBAF) to remove the TBS group, simultaneous hydrolysis of the ester moiety was observed to afford *cis*-**9** in 67% yield.



Scheme 1. Construction of a cyclobutane ring on the steroidal D-ring.



Scheme 2. Synthesis of 3-keto LCA analogs bearing a cyclobutane ring.



Scheme 3. Synthesis of 3-keto LCA analogs bearing a spirocyclopropane ring.

Our attention turned toward synthesis of 3-keto LCA derivatives bearing a spirocyclic cyclopropane ring (Scheme 3). Cleavage of the TBS group of *trans*-**5**, followed by acetyl protection of the primary alcohol, gave *trans*-**11**. Ring-contraction rearrangement of *trans*-**11** was performed using thionyl chloride and triethylamine to give (20*R*)-**12** in 49% yield as a 7:3 mixture of diastereomers at the C-16 stereogenic center. Removal of the acetyl group and dechlorination were carried out in one-pot with $NaBH_4$ in DMSO at 130 °C to give alcohol (20*R*)-**13**. TPAP oxidation of (20*R*)-**13** and the successive olefination provided (20*R*)-**15**. The reduction of the resulting double bond was unsuccessful using magnesium and methanol, which resulted in a simultaneous ring opening of the cyclopropane ring. However, the chemoselective reduction was achieved by diimide reduction,[15] and removal of the thioacetal followed by hydrolysis afforded (20*R*)-**17**. The synthesis of (20*S*)-**17** was also accomplished from *cis*-**5** by the same procedure.

The synthesized LCA derivatives as well as estrone derivatives *trans*-**18**, *cis*-**18**, (20*R*)-**19** and (20*S*)-**19**, which were synthesized previously,[8b] were evaluated at various concentrations to determine transactivation of VDR by luciferase reporter assays (Fig. 2A). Among them, only estrone derivatives *trans*-**18** and (20*R*)-**19** showed a slightly higher efficacy, maximum effect, of the transcriptional activity of VDR than 3-keto LCA. The diastereomers *cis*-**18** and (20*S*)-**19** activated VDR less effectively than 3-keto LCA. Unfortunately, as seen in *trans*-**9**, *cis*-**9**, (20*R*)-**17** and (20*S*)-**17**, installation of the small ring onto the D-ring of 3-

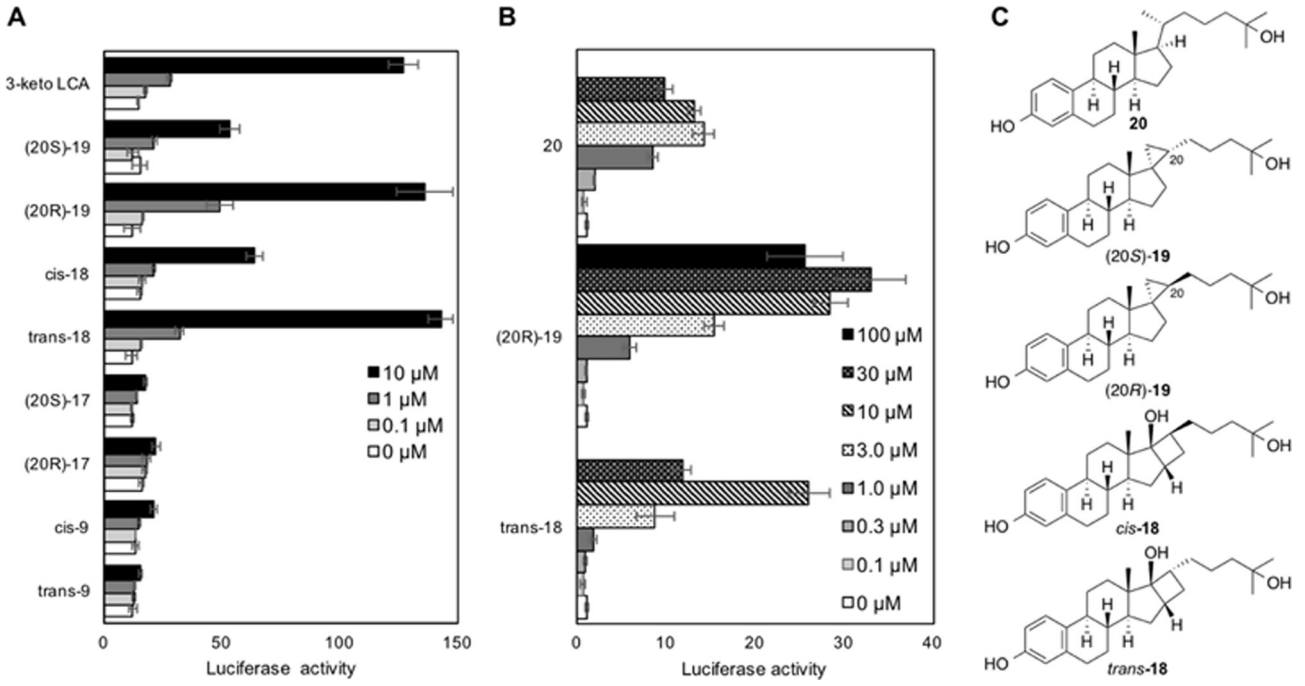


Fig. 2. (A) Transactivation of VDR by estrone derivatives or 3-keto LCA derivatives. (B) Comparison of the activity of estrone derivatives with or without a small ring. (C) Chemical structures of estrone derivatives.

keto LCA resulted in a significant loss of the activity. To examine the effect of the small rings of *trans*-**18** and (20*R*)-**19** on the transcriptional activity, estrone derivative **20** without a small ring was also evaluated (Fig. 2B). The reduction in the activity at higher concentrations results from cytotoxicity. Whereas **20** showed a higher potency (EC_{50} = 0.85 μM) than *trans*-**18** and (20*R*)-**19** (EC_{50} = 4.1 and 3.7 μM, respectively), *trans*-**18** and (20*R*)-**19** have a higher efficacy than **20**. We still struggle to understand how the structural difference affects the efficacy. However, a docking study on VDR with the synthetic ligands indicates that both of the oxygen atoms and the steroidal scaffold of *trans*-**18**, and those of (20*R*)-**19** occupy almost the same space in the binding pocket (see Supplementary data).[16] This increased efficacy of *trans*-**18** and (20*R*)-**19** may be attributed to cofactor interactions to induce recruitment of coactivators such as steroid receptor coactivator-1 (SRC-1) or dissociation of corepressors such as nuclear receptor corepressor (N-CoR).[17]

In summary, we have synthesized a new class of 3-keto LCA derivatives bearing a cyclobutane or a spirocyclopropane ring for conformational fixation of the carbon side chain. Our synthesis relies on a stereoselective catalytic [2+2] cycloaddition and a stereospecific ring-contraction rearrangement. The 3-keto LCA derivatives as well as estrone derivatives bearing a small ring were evaluated as VDR agonists in the luciferase reporter assays. Among them, estrone derivatives *trans*-**18** and (20*R*)-**19** showed a higher efficacy than 3-keto LCA, and the small ring moieties were found to be important for the efficacy.

Acknowledgments

This work was supported by MEXT KAKENHI Grant Number JP16H01147 in Middle Molecular Strategy, AMED Platform for Drug Discovery, Informatics, and Structural Life Science, and the Suzuken Memorial Foundation. N.A. acknowledges the Japan Society for the Promotion of the Science (JSPS) for the Research Fellowship for Young Scientists.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.05.089.

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