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Synthesis and evaluation of vitamin D3 analogues with C-11 modifications as inhibitors of Hedgehog signaling

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ABSTRACT

Previous structure-activity relationship studies have provided potent and selective analogues of vitamin D3 as inhibitors of the Hedgehog (Hh) signaling pathway. These analogues contain both modified A- and *seco*-B ring motifs, and have been evaluated for anticancer therapeutic potential. To continue our studies on this scaffold, a new series of compounds were synthesized to explore additional interactions and spatial constraints. These compounds incorporate functional groups of varying size and hydrophobicity at the C-11 position. While large hydrophobic moieties (**9c**–**e**) resulted in significant loss of Hh inhibition, smaller or more flexible moieties (**9a**, **11**) maintain anti-Hh activity. These results call for additional and continued studies to identify the binding pocket to better understand these structure-activity relationships.

Dysregulation of the Hedgehog (Hh) signaling pathway is known to drive oncogenic transformation for a variety of cancers including basal cell carcinoma (BCC) and medulloblastoma (MB).[1] As a developmental pathway, Hh signaling plays a vital role in proper differentiation and growth of tissues during embryonic development. In adult tissues, Hh signaling is less active appearing to play a role in the maintenance of stem cell populations within the skin and central nervous system.[2] Constitutive activation of Hh signaling can arise from mutations in several components of the pathway that serve as signaling regulators. These include Smoothened (Smo), Patched (Ptch), or Suppressor of Fused (Sufu), all of which have been linked to the tumorigenesis and progression of both BCC and MB.[3,4] The FDA approval of two Smo inhibitors (Fig. 1), Vismodegib and Sonidegib for the treatment of locally advanced BCC, validated the therapeutic strategy of targeting Hh signaling for cancer treatment. Unfortunately, clinical relapse has already been observed following treatment with Vismodegib due to a variety of point mutations within its binding pocket on Smo.[5–7] With relapse and resistance[5–9] concerns for **1**, recent years have seen continued and further development of small molecule Hh inhibitors as anti-cancer treatment options.

Among the multiple scaffolds undergoing development, the endogenous *seco*-steroid, vitamin D3 (VD3, **3**) was identified as a non-selective Hh pathway inhibitor thought to act through direct binding to Smo.[10,11] As an endogenous small molecule, a primary concern for developing these compounds has been innate activation of the vitamin D receptor (VDR). Canonical VDR signaling is essential for numerous cellular functions and prolonged activation can lead to severe side-effects, such as hypercalcemia.[12] Even though VD3 does not bind to VDR directly, up-regulation of VDR target genes, such as *Cyp24a1*, has been observed in multiple studies suggesting that it is rapidly converted to a form that functionally activates VDR signaling. Following these observations, we have continued to explore structure-activity relationships (SAR) for Hh inhibition while attempting to minimize or eliminate VDR activation.

Our initial studies demonstrated that the northern region of VD3 (Grundmann's alcohol, CD-ring/side chain) is the pharmacophore of the scaffold.[13,14] With Grundmann's alcohol as a starting point, a series of esters that incorporated an aromatic A-ring mimic were synthesized and evaluated. This first series of analogues identified **4**, which demonstrated both improved potency and selectivity compared to VD3 in multiple Hh-dependent cell lines.[15] Further development of this scaffold through a series of analogues that modified the ester linker provided **5** as our most potent VD3 analogue prepared to date.[16] To continue our SAR studies, we decided to explore the area adjacent to the C-ring through the addition of various functionalities. The incorporation of these moieties would allow for probing of the binding pocket in this region for additional interactions and any spatial restraints for this region of the scaffold.

We chose to focus our C-ring modifications to the C-11 position primarily because synthetic routes to append functional groups at

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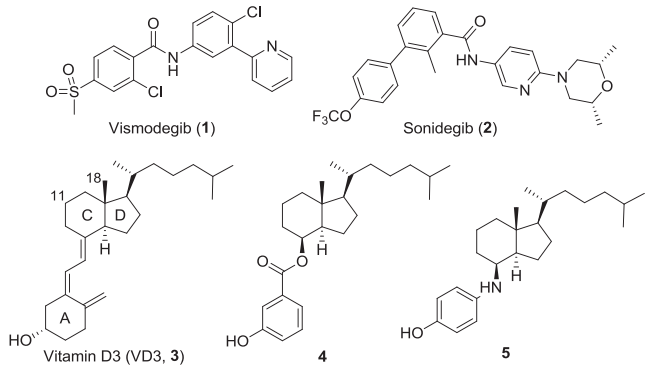
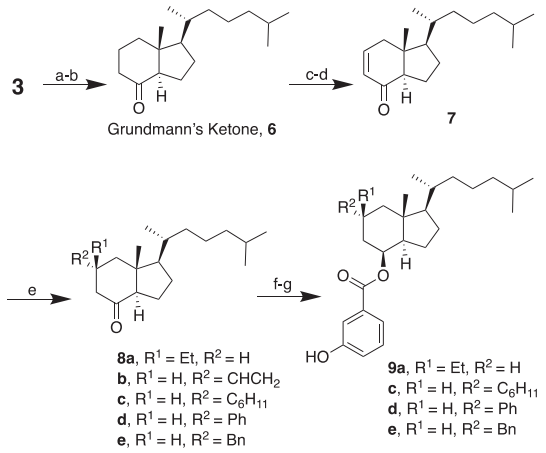


Fig. 1. Hedgehog (Hh) Pathway Inhibitors.

this position are well-characterized and straightforward. Utilizing standard procedures, Grundmann's ketone (**6**) can be obtained in high yield from VD3 in two steps (Scheme 1). α,β-unsaturation of the ketone was achieved through Saegusa-Ito oxidation of the silyl enol ether intermediate to give enone **7**. A slight modification to the published route to access **7**,[17] addition of *p*-benzoquinone as a co-oxidant to regenerate Pd (II), resulted in an increased yield. The desired alkyl functionalities were added to the C-11 position via 1,4-organocuprate addition. *In situ* formation of the organocuprate from the corresponding Grignard reagent provided a one-pot reaction to obtain the desired ketones (**8a**–**e**) in a stereoselective fashion. Standard sodium borohydride reduction followed by esterification and deprotection provided the desired esters (**9a,c**–**e**). The C-11 stereochemistry was determined by analysis of the ^{1}H NMR spectra in which the C-18 proton peak demonstrates a characteristic shift, similar to that previously reported.[17,18] Due to A1,3-strain in the C-ring, an interaction between the C-11 substituent and the C-18 methyl group would result in a downfield shift of the C-18 singlet, similar to what is observed when **6** is reduced to Grundmann's alcohol. Following the 1,4-addition to **7**, the C-18 methyl peak was observed at 0.6–0.8 ppm for **8b**–**8e**, indicating the α-orientation. For **8a**, an interaction with the C-18 methyl peak (1.1 ppm) was observed, indicating the β-orientation (Supplemental Information).

It is well-established that the reduction of **6** to Grundmann's alcohol with sodium borohydride is a highly stereoselective process that results in the β-alcohol due to the concavity and sterics

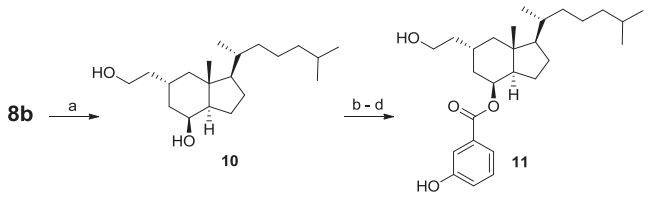


Scheme 1. Reagents and conditions: (a) i. O_3, pyridine, DCM:MeOH; ii. $NaBH_4$; 90% (b) PDC, DCM; 94% (c) i. LDA, THF; ii. TMSCl; (d) $Pd(OAc)_3$, *p*-benzoquinone, MeCN; 78% over two-steps; (e) R-MgBr, CuI, THF; 55–90% (f) $NaBH_4$, MeOH; (g) 3-OMOM-benzoic acid, DCC, DMAP, DCM; (h) (±)-CSA, MeOH; 17–32% over three-steps.

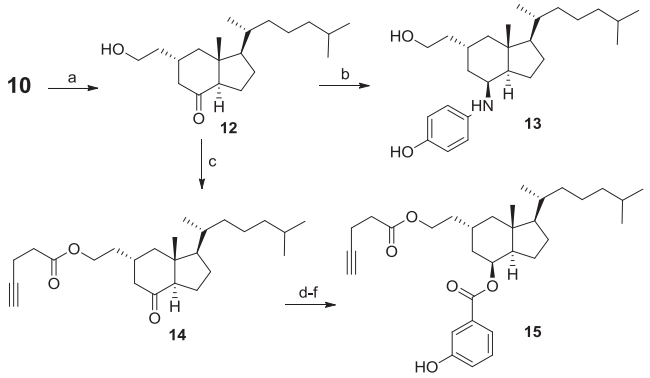
of the CD-ring system favoring backside hydride addition.[19] We believe that the cuprate addition works in a similar fashion and the steric bulk of the **8b**–**e** C-11 substituents results in α-addition to minimize the A1,3-strain. The smaller ethyl substituent, in comparison to the larger ring systems, decreased the favorability for the α -orientation by reducing the potential A1,3-strain between the C-11 substituent and the C-18 methyl groups, resulting in β-substitution.

In addition to incorporating hydrophobic functionalities, we also sought to append a hydroxyl moiety at C-11 to not only probe its effects on Hh inhibition, but also to provide a chemical handle for attaching a terminal alkyne. Analogues incorporating a terminal alkyne hold potential as probes to identify cellular proteins that bind the VD3 scaffold and mediate its anti-Hh effects. Hydroboration of the terminal olefin in **8b** with 9-borabicyclo(3.3.1)nonane (9-BBN) and reduction of the resulting aldehyde provided diol **10** (Scheme 2).[18] Selective protection of the primary alcohol at C-11, followed by esterification at the secondary alcohol and global deprotection provided analogue **11**. To incorporate the terminal alkyne, selective oxidation of the secondary alcohol in **10** with 2-iodoxybenzoic acid (IBX)[20] afforded ketone **12**, which allowed for esterification of the C-11 side chain hydroxyl (**14**, Scheme 3). Reduction of the ketone and coupling/deprotection with the phenolic 3-hydroxy A-ring yielded **15** in low yield. The low yield can be partially attributed to the deprotection step, which also partially cleaves the side chain ester. As noted above, our most potent VD3 analogue, **5**, incorporates a single amine linker between the CD-ring/side chain and the aromatic A-ring. Ketone **12** provided an opportunity to synthesize an amine linked analogue of **11** to see if the same SAR trend would be observed. Using a previously described reductive amination method,[21] analogue **13** was obtained from **12** as a single isomer.

Each of our synthesized analogues was evaluated in the C3H10T1/2 mouse embryonic fibroblast (MEF) cell line to deter-



Scheme 2. Reagents and conditions: (a) i. 9-BBN, THF; ii. NaOH, H_2O, H_2O_2, MeOH; 90% (b) TBSCl, imidazole, DCM; 88% (c) 3-OMOM-benzoic acid, DCC, DMAP, DCM; 55% (d) CSA, MeOH 65%.



Scheme 3. (a) IBX, tetrabutylammonium bromide, DCM:H_2O; 75% (b) 4-aminophenol, $NaBH(OAc)_3$, AcOH, DCE; 12% (c) 4-pentynoic acid, DCC, DMAP, DCM; 91% (d) $NaBH_4$, MeOH; 67% (e) 3-OMOM-benzoic acid, DCC, DMAP, DCM; 60% (f) CSA, MeOH; 15%.

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