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Structure-activity relationships for side chain oxysterol agonists of the hedgehog signaling pathway.

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hedgehog signaling pathway, oxysterols, GLI, liver X receptor

ABSTRACT: Oxysterols (OHCs) are byproducts of cholesterol oxidation that are known to activate the Hedeghog (Hh) signaling pathway. While OHCs that incorporate hydroxyl groups throughout the scaffold are known, those that act as agonists of Hh signaling primarily contain a single hydroxyl on the alkyl side chain. We sought to further explore how side chain hydroxylation patterns affect oxysterol-mediated Hh activation, by performing a structure-activity relationship study on a series of synthetic OHCs. The most active analogue, 23(*R*)-OHC (**35**) demonstrated potent activation of Hh signaling in two Hh-dependent cell lines (EC_{50} values 0.54 – 0.65 μM). In addition, OHC **35** was approximately three-fold selective for the Hh pathway compared to the liver X receptor, a nuclear receptor that is also activated by endogenous OHCs. Finally, **35** induced osteogenic differentiation and osteoblast formation in cultured cells, indicating functional agonism of the Hh pathway.

The Hedgehog (Hh) signaling pathway is a developmental signaling pathway that plays multiple roles during embryonic development, including directing neuronal cell growth and tissue patterning.[1] While the application of small molecule Hh pathway inhibitors as anti-cancer chemotherapeutics is more clearly defined,[2-3] modulation of the pathway also holds potential as a target for neurodegenerative disorders including Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and diabetic neuropathy.[1] Recent studies have also explored the osteoinductive effects associated with activation of the Hh signaling pathway. Stimulation of Hh signaling with several oxysterols (OHCs) resulted in significant osteoinductive effects in vitro and modest induction of bone formation in vivo.[4-7] De novo stimulation of bone formation is of significant clinical interest for a variety of situations, including spinal fusion surgery, bone fracture repair, and treatment of osteoporosis.[8] Taken together, these studies indicate that activators of Hh signaling are valid leads for further development as therapeutic agents for a variety of neuro- and osteodegenerative disorders.

OHCs are byproducts of cholesterol oxidation that exert numerous physiological effects through a variety of cellular receptors.[9] Several natural and synthetic OHCs have demonstrated the ability to activate Hh signaling in Hh-dependent cellular models, suggesting their potential as chemical probes for understanding endogenous mechanisms of Hh modulation and therapeutic agents for Hh-associated neurodegenerative disorders and orthopedic indications (Chart 1).[4-6,10] A major complicating factor for utilizing OHCs as selective agonists of the Hh signaling is their ability to modulate numerous signal

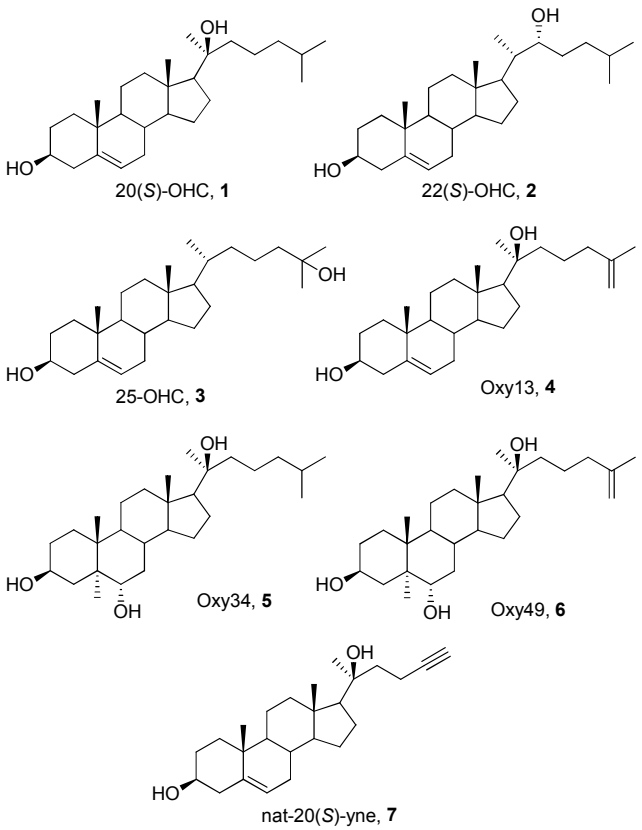


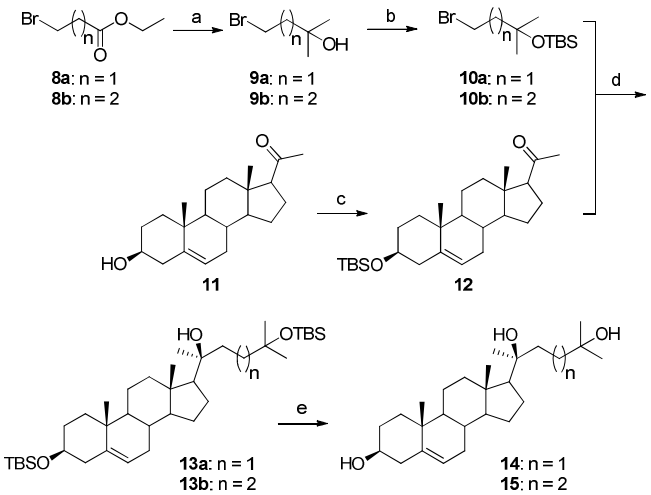
Chart 1. Natural (**1**-**3**) and synthetic (**4**-**7**) OHC Hh agonists.

ing pathways through a variety of cellular receptors. OHCs have been implicated as key regulators of lipid trafficking,[11] cholesterol homeostasis,[12] and leukocyte chemotaxis.[13] Of particular interest with respect to OHC modulation of the Hh pathway is the liver X receptor (LXR), a nuclear receptor that regulates genes involved in cholesterol homeostasis.[14] LXR was recently identified as a negative regulator of the Hh pathway and maintains affinity for many of the endogenous OHCs that activate Hh signaling;[15-17] therefore, in order to avoid competing effects on Hh signaling,

OHCs which act as specific agonists must be inactive against the LXR pathway. The enclosed manuscript details our initial efforts at identifying OHCs as selective Hh agonists and provides key SAR information for the future development of this class of compounds.

Preliminary SAR for naturally occurring and synthetic OHCs has demonstrated that a hydroxyl moiety on the side chain is essential for Hh activation (Chart 1). Oxysterols with a single hydroxyl moiety on the tetracyclic core scaffold, including 7β-OHC and 19-OHC, have proven ineffective at activating functional Hh signaling in vitro.[4-5] For this reason, our initial efforts to identify OHCs that act as selective Hh agonists focused on a systematic exploration of how varying the hydroxylation pattern on the side chain region of the scaffold effects Hh agonism. Predominantly, the synthesis of these OHCs followed established procedures with the minor modifications noted below.[18-26]

Scheme 1. Synthesis of 20,25-OHC analogues 14 and 15.[a]



[a]Reagents and conditions: (a) MeMgBr, -20°C, ether, 85-95%. (b) TBSOTf, 2,6-lutidine, CH_2Cl_2, 48-78%. (c) TBSCl, imidazole, DMF, quan (d) SmI_2, 4Å molecular sieves, HMPA, THF, 43-48%. (e) TBAF, THF, RT or 70°C, 42-55%.

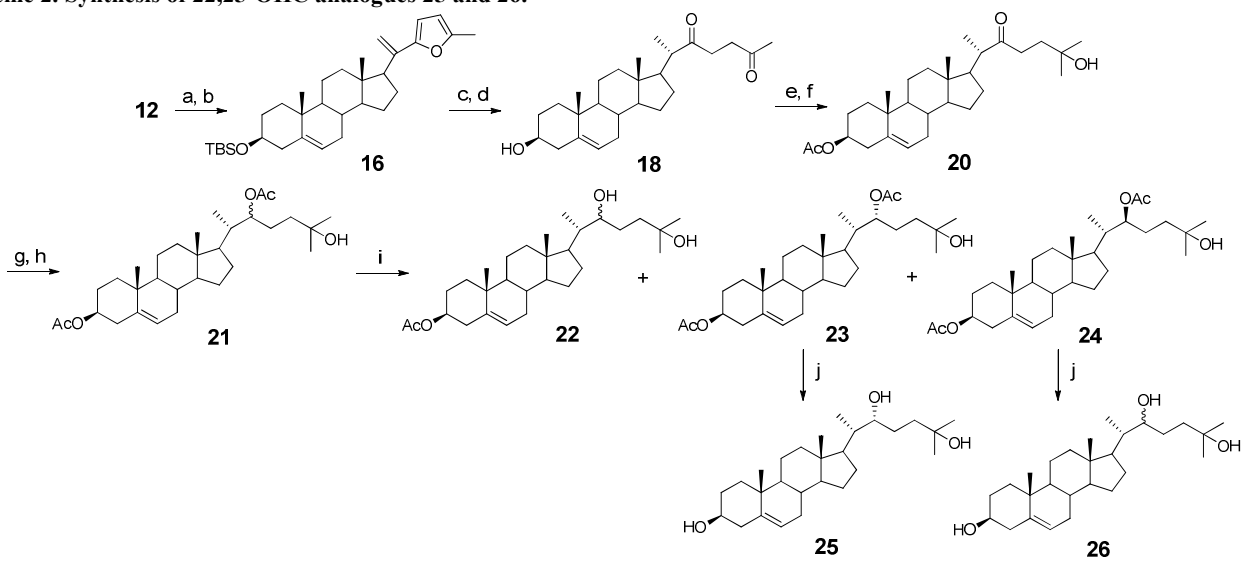
The hydroxylated side chains for the 20(*S*),25-OHC analogues were prepared as previously described[18-19] from ethyl 3-bromopropionate (**8a**) or ethyl 4-bromobutyrate (**8b**) by methyl Grignard addition to the ester and protection of the resulting tertiary alcohol as the TBS ether (Scheme 1). Bromoalkanes **10a** and **10b** were coupled directly to TBS-protected pregnenolone **12** in a stereoselective fashion via a samarium iodide-mediated Barbier reaction. It should be noted that freshly prepared samarium iodide was essential for the reaction to proceed. Global deprotection provided OHC analogues **14** and **15** in modest yields. Of note, deprotection of **13a** proceeded at room temperature: however, heat (70°C) was required for full deprotection of the side chain TBS ether of **13b**.

The synthesis of 22,25-OHC analogues also began with protected pregnenolone **12** (Scheme 2). Our synthetic route was slightly modified from the previously described procedure.[20-21] Coupling of in situ generated 2-lithio-5-methylfuran with **12** followed by gentle stirring in the presence of silica gel provided the furan **16**. Stereoselective catalytic hydrogenation of the side chain alkene and acid-mediated opening of the furan ring yielded the diketone **18**. Reprotection of the secondary hydroxyl as the acetate, followed by Grignard reaction of the distal ketone with methyl magnesium bromide afforded tertiary alcohol **20**. Sodium borohydride afforded tertiary alcohol **20**. Sodium borohydride reduction of the C-22 ketone and protection of the resulting hydroxyl group as the acetate afforded a mixture of diastereomers that could be separated via standard silica chromatography. Of note, this purification step also resulted in significant removal of the C-22 acetate to yield **22**; however, enough of the purified diacetylated analogues, **23** and **24**, were obtained. Interestingly, while global deprotection of **23** provided the final corresponding 22(*S*),25-OHC analogue **25**, deprotection of **24** resulted in a 1:1 mixture of the 22(*R*),25 and 22(*S*),25 OHC diastereomers that were not separable through standard chromatography conditions. Based on the previous results reported for 22(*R*)-OHC and our preliminary evaluation of dihydroxylated OHCs (vida infra, Table 1), further purification of **26** was not undertaken and it was evaluated as a 1:1 mixture.

The preparation of 23-OHC analogues began with conversion of the commercially available hyodeoxycholic acid to the corresponding carboxylic acid **27** via known procedures (Scheme 3).[22-24] Oxidative decarboxylation and exchange of protecting groups gave the THP protected alkene, **29**.[25-26] Hydroboration-oxidation provided the desired **30**, albeit in low yield. Oxidation of the side chain hydroxyl to the corresponding aldehyde followed by a Grignard reaction with isobutyl magnesium chloride afforded **32**:**33** as a mixture of diastereomers that were easily separable via standard silica gel chromatography. Removal of the 3-tetrahydropyran afforded the final 23-OHC analogues **34** and **35**. The absolute configuration of the hydroxyl moiety at C-23 was assigned for **34** and **35** based on TLC and NMR analysis as described previously.[25] Finally, removal of the protecting groups from the 3-hydroxyls of **30** and **20** provided the OHC analogues **36** and **37** (Scheme 4).

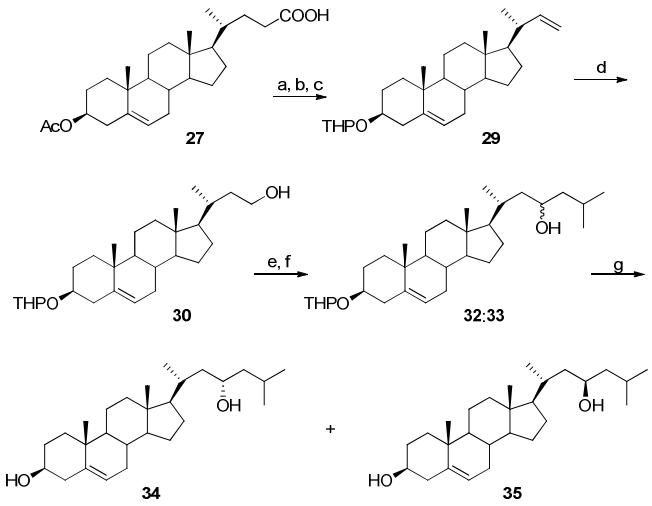
Initial evaluation of the OHC analogues focused on determining Hh and LXR agonist properties through up-regulation of known pathway target genes (Gli1 and ABCA1, respectively) in the Hh-dependent C3H10T1/2 cell line. For these studies, DMSO was used as a baseline control for both pathways (set at 1); values for OHC analogues are presented as fold induction of mRNA expression over DMSO control (Table 1). Compounds **1**-**3**, 7β-OHC, and 22(*R*)-OHC are the endogenous and naturally occurring OHCs that have been previously studied for Hh[4-5] and LXR[13-14] activation and were included in

Scheme 2. Synthesis of 22,25-OHC analogues 25 and 26.[a]



[a]Reagents and conditions: (a) 2-methyllithio furan, THF, -78°C. (b) silica gel, CH_2Cl_2, 91% (two steps). (c) Pd/C 10%, H_2, benzene, 95%. (d) THF, H_2O, 10% H_2SO_4, AcOH. (e) Ac_2O, pyridine, 47% (two steps). (f) MeMgBr, THF, -78°C, 39%. (g) $NaBH_4$, MeOH, 0°C, 87%. (h) Ac_2O, pyridine. (i) silica gel, EtOAc/CH_2Cl_2. (j) KOH, MeOH, 80%.

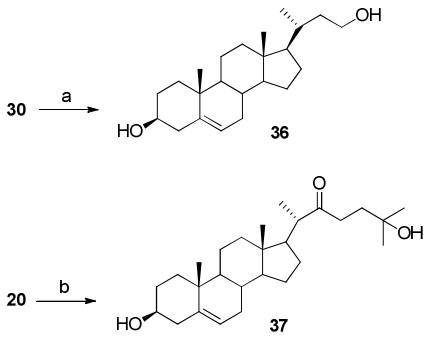
Scheme 3. Synthesis of 23-OHC analogues 34 and 35.[a]



[a]Reagents and conditions: (a) $Pb(OAC)_4$, $Cu(OAc)_2$, pyridine, benzene, reflux, 42%. (b) KOH, MeOH, 98%. (c) THP, *p*TSA, CH_2Cl_2, 53%. (d) BH_3-THF, THF, 3N NaOH, 30% H_2O_2, 0°C, 20%. (e) PCC, CH_2Cl_2, NaOAc, quantitative. (f) isobutyl magnesium chloride, THF, 0°C, 44%. (g) 2N HCl, MeOH, THF, 96-98%.

our studies for direct comparison with our synthetic OHCs. In addition, the commonly used standard for Hh activation is the combined administration of 20(*S*)- and 22(*S*)-OHC (**1** and **2**, 5 μM each) and this treatment was also included for compari son. Evaluation of these compounds has provided key structure-activity relationships (SAR) for side chain hydroxylated OHCs as agonists of the Hh pathway. First, while compounds that incorporate a C-25 hydroxyl (**14**-**15**, **25**-**26**, and **37**) exhibit a wide range of Hh activation, none of them are selective agonists of the Hh pathway. This is not surprising as previous studies have demonstrated potent LXR binding for oxysterols that incorporate hydroxyl moieties at C-24 and/or C-25.[17] Second, of the 23-OHCs (**34** and **35**) only the 23(*R*)-epimer, **35**, activated Hh signaling at a level equipotent to the **1**:**2** mixture and demonstrated greater than 10-fold selectivity for the Hh pathway. In the initial screen, 22(*S*)-OHC, **2**, demonstrated the greatest selectivity for Hh signaling; however, the absolute up-regulation of GLI1 was significantly reduced compared to **35** (52.5- and 87.1-fold, respectively). In addition, as 22(*S*)-OHC is an endogenous oxysterol with multiple known physiologic targets, we sought to further explore the activity of the synthetic OHC **35**. Finally, compound **37** exhibited the ability to activate LXR (22-fold induction and 3.6 fold selective for LXR). While these values represent modest LXR activation and selectivity, they are comparable to 22(*R*)-OHC, generally recognized as an LXR selective endogenous OHC. In addition, the overall up-regulation of ABCA1 demonstrated for **37** was significantly greater than a series of OHC-based LXR agonists recently reported.[27]

Scheme 4. Synthesis of OHC analogues 36 and 37.[a]



[a]Reagents and conditions: (a) 2N HCl, MeOH, THF, 86%. (b) KOH, MeOH, 53%.

Table 1. Initial In Vitro Evaluation of OHC analogues.

OHC	GLI1[a]	ABCA1[a]	Hh Selectivity[b]
DMSO	1.0 ± 0.1	1.0 ± 0.2	----
1	91.8 ± 5.8	13.4 ± 1.8	6.8

2	52.5 ± 1.0	2.3 ± 0.1	22.8
1:**2**	103.2 ± 3.4	17.4 ± 2.1	5.9
3	69.9 ± 2.9	9.8 ± 1.1	7.1
14	18.1 ± 0.8	10.3 ± 0.7	1.8
15	107.8 ± 0.9	26.3 ± 1.4	4.1
25	9.9 ± 0.2	2.9 ± 0.1	3.4
26	1.0 ± 0.03	0.9 ± 0.1	----
34	32.4 ± 0.1	3.6 ± 1.1	9.0
35	87.1 ± 0.8	4.9 ± 0.2	17.8
36	4.1 ± 0.8	0.4 ± 0.01	10.4
37	6.0 ± 0.3	21.7 ± 1.6	----
7β-OHC	1.6 ± 0.1	0.6 ± 0.1	2.6
22(*R*)-OHC	4.7 ± 0.4	24.1 ± 2.0	----

[a]Values are fold mRNA induction over DMSO control.
[b]Hh selectivity determined as Gli/ABCA1.

Based on the promising initial results for **35**, we sought to further explore its ability to selectively activate Hh signaling in a dose-dependent fashion in C3H10T1/2 and M2-10B4 cells, a multipotent murine bone stromal cell line that responds to Hh agonists with characteristic pathway up-regulation (Figure 2). Compound **35** activated Hh signaling in both cells lines in a dose-dependent manner as measured by up-regulation of GLI (Figure 1A and 1B) and PTCH (Figure 1C) expression. Similar EC_{50} values were obtained in both cell lines for Hh activation (0.54 - 0.65 μM), demonstrating that pathway agonism was not cell type dependent (Table 2). By contrast EC_{50} values for LXR activation and ABCA1 up-regulation were three-fold higher (EC_{50}s = 1.53 and 1.54, Figure 1D) in both cell lines, further demonstrating the selectivity for the Hh pathway. Of note, while the absolute up-regulation of GLI in C3H10T1/2 cells was approximately 5-fold greater than in M2-10B4s, these absolute values correspond well with values previously reported for complete Hh activation.[4-5]

Table 2. EC_{50} Values for Pathway Regulation by OHC 35.

Target Gene	C3H10T1/2	M2-10B4
GLI1	0.54 ± 0.1[a]	0.57 ± 0.1
PTCH	0.65 ± 0.06	0.58 ± 0.1
ABCA1	1.53 ± 0.2	1.54 ± 0.1

[a]All values are in μM.

To verify that the up-regulation of GLI and PTCH were the results of Hh pathway activation we evaluated the ability of cyclopamine (Cyc), an Hh-specific inhibitor, to attenuate this response (Figure 2). Treatment of M2-10B4 cells with **35** (5 μM) resulted in a robust up-regulation of the Hh pathway target genes GLI1 and PTCH that correlated well with the results demonstrated for the standard **1**:**2** OHC cocktail utilized for pathway activation. Pretreatment with Cyc (5 μM) resulted in complete attenuation of GLI1 and PTCH overexpression, verifying these results are due to Hh pathway activation. Of

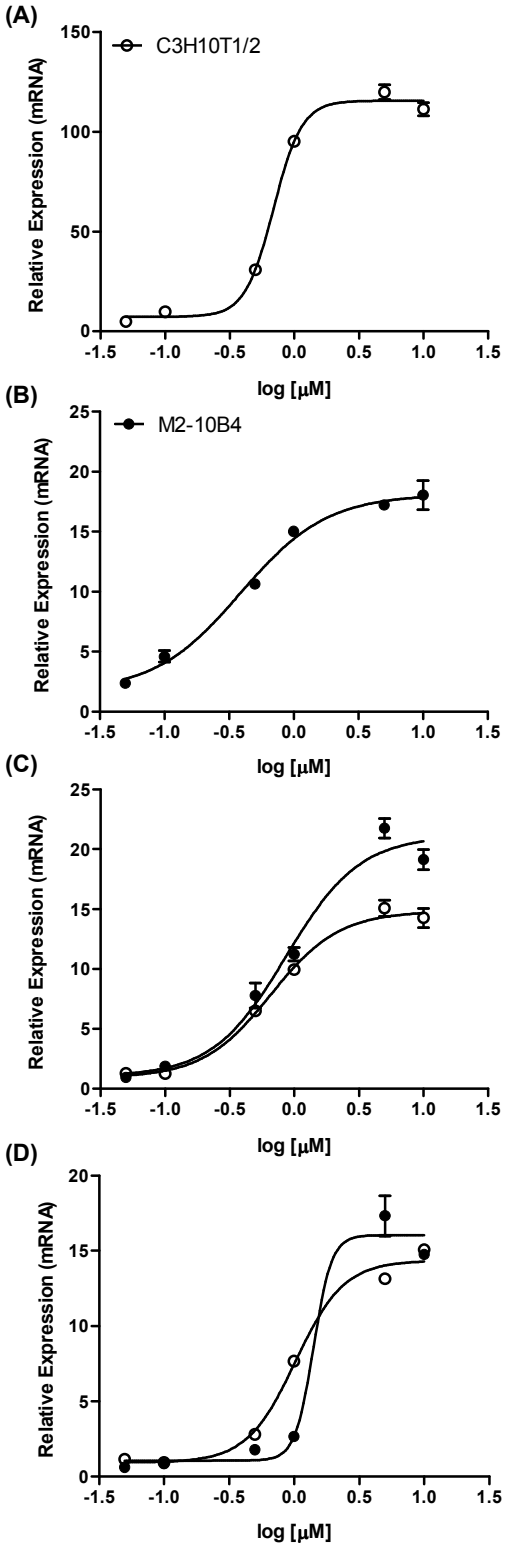


Figure 1. Hh and LXR activation in C3H10T1/2 and M2-10B4 cells by **35**. The relative expression of GLI1 (C3H10T1/2, A and M2-10B4, B), PTCH (C), and ABCA1 (D) were determined relative to DMSO control. Open circles represent data in C3H10T1/2 while closed circles represent data from M2-10B4 cells.

note, pretreatment with **35** or **1**:**2** prior to Cyc addition demonstrated similar results (data not shown).

Activation of the Hh pathway in M2-10B4 cells promotes osteogenic differentiation and results in mature osteoblastic cells; therefore, our next series of experiments sought to measure early and late transcriptional markers of osteogenic differentiation to verify that OHC **35** functionally activates Hh signaling.[8-9] Treatment of M2-10B4 cells with **35** (5 μM) resulted in a modest (5-fold) up-regulation of the early stage osteogenic marker Osterix (Osx) at 24 hrs and a robust up-regulation (>30-fold) at 48 hr (Figure 3). Enhanced expression of Osx was maintained up to 96 hr after initial addition of **35**. The formation of mature osteoblasts was assessed by evaluating time-dependent induction of mRNA expression for alkaline phosphatase (ALP). Similar to Osx, **35** induced modest up-regulation of ALP at 24 hr with a more robust overexpression seen at 48 and 96 hr post treatment. Taken together, these data indicate that activation of the Hh pathway by **35** results in osteogenic differentiation and osteoblast formation in cultured M2-10B4 cells.

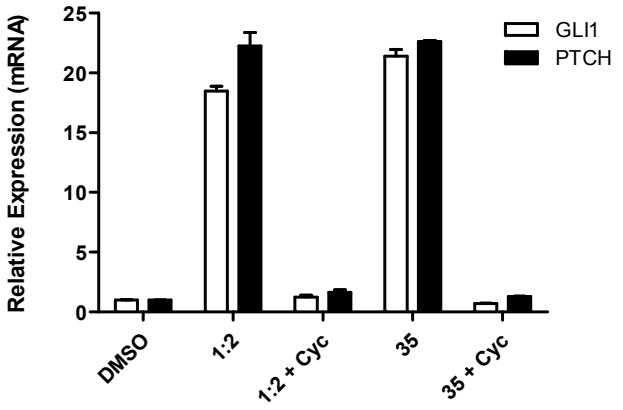


Figure 2. Activation of the Hh signaling pathway. The relative expression of GLI1 and PTCH were determined in M2-10B4 cells following treatment with the standard Hh activating OHCs (**1**:**2**) or **35** and the Hh-specific inhibitor Cyc. Cells were pretreated with Cyc (5 μM) for 2 hr and then treated with OHC (5 μM). mRNA expression was determined following 24 hr incubation. Data are from a representative experiment performed in triplicate.

In summary, through the synthesis and evaluation of a series of side chain OHC analogues we identified key SAR for this class of compounds relative to their ability to selectively activate the Hh signaling pathway. The most active of these compounds, 23(*R*)-hydroxycholesterol, **35**, is a potent activator of the Hh pathway (EC_{50} values = 0.54–0.65 μM) and demonstrates approximately 3-fold selectivity relative to LXR. The most active synthetic OHC reported, **7**, induced Hh signaling with an EC_{50} value of approximately 390 nM; however, no selectivity parameters for this OHC analogue have been disclosed.[28] OHC **35** In addition, as measured by the up-regulation of early and late stage markers of osteogenic differentiation, **35** induced functional activation of Hh signaling. Finally, while not our initial intent, we have identified a synthetic OHC, **37**, that potently and selectivity up-regulates LXR and can serve as a valuable chemical tool to further probe LXR activated signaling.

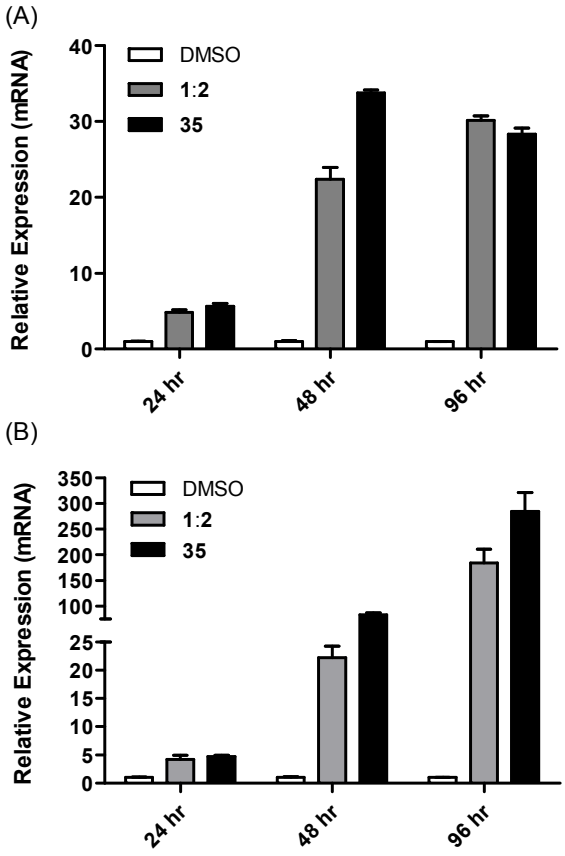


Figure 3. Induction of osteogenic differentiation markers. The relative expression of osterix (A) and alkaline phosphatase (B) were determined relative to DMSO control in M2-10B4 cells. Cells were treated with either **1**:**2** (5 μM each) or **35** (5 μM) for the indicated time period. Data are from a representative experiment performed in triplicate.

ASSOCIATED CONTENT

Supporting Information. Synthetic methods, biological assay protocols, spectroscopic data, and HPLC purity analysis. This material is available free of charge via the internet at http://pubs.acs.org.

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Author Contributions

A.C. synthesized and characterized the oxysterols in the manuscript. A.M.D. performed all the biological analysis and HPLC characterization. All authors contributed to the preparation of the manuscript. □These authors contributed equally to this work.

ABBREVIATIONS

Hh, Hedgehog; OHC, oxysterol; GLI, glioma-associated oncogene; PTCH, patched; LXR, liver X receptor;

REFERENCES

1. Dellovade, T.; Romer, J.T.; Curran, T.; Rubin, L.L. The hedgehog pathway and neurological disorders. *Annu. Rev. Neurosci.* **2006**, *29*, 539-563.

2. Yang, L.; Xie, G.; Fan, Q.; Xie, J. Activation of the hedgehog-signaling pathway in human cancer and the clinical implications. *Oncogene* **2010**, *29*, 469-481.
3. Peukert, S. and Miller-Moslin, K. Small-molecule inhibitors of the hedgehog signaling pathway as cancer therapeutics. *ChemMedChem* **2010**, *5*, 500-512.
4. Corcoran, R.B.; Scott, M.P. Oxysterols stimulate sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc. Natl. Acad. Sci.* **2006**, *103*, 8408-8413.
5. Dwyer, J.R.; Sever, N.; Carlson, M.; Nelson, S.F.; Beachy, P.A.; Parhami, F. Oxysterols and novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells. *J. Bio. Chem.* **2007**, *282*, 8959-8968.
6. Kha, H.T.; Basseri, B.; Shouhed, D.; Richardson, J.; Tetradis, S.; Hahn, T.J.; Parhami, F. Oxysterols regulate differentiation of mesenchymal stem cells: pro-bone and anti-fat. *J. Bone Miner. Res.* **2004**, *19*, 830-840.
7. Aghaloo, T. L.; Amantea, C. M.; Cowan, C. M.; Richardson, J. A.; Wu, B.M. Parhami, F.; Tetradis, S. Oxysterols enhance osteoblast differentiation in vitro and bone healing in vivo. *J. Orthop. Res.* **2007**, *25*, 1488-1497.
8. Giannoudis, P. V.; Dinopoulos, H.; Tsiridis, E. Bone substitutes: an update. *Injury* **2005**, *36*, S20-S27.
9. Olkkonen, V.A.; Beaslas, O.; Nissila, E. Oxysterols and their cellular effectors. *Biomolecules* **2012**, *2*, 76-103.
10. Johnson, J.S.; Meliton, V.; Kim, W.K.; Lee, K.-B.; Wang, J.C.; Nguyen, K.; Yoo, D.; Jung, M.E.; Atti, E.; Tetradis, S.; Pereira, R.C.; Magyar, C.; Nargizyan, T.; Hahn, T.J.; Farouz, F.; Thies, S.; Parhami, F. Novel oxysterols have pro-osteogenic and anti-adipogenic effects in vitro and induce spinal fusion in vivo. *J. Cell. Biochem.* **2011**, *112*, 1673-1684.
11. LeBlanc, M. A. and McMaster, C. R. Lipid binding requirements for oxysterol-binding protein Kes1 inhibition of autophagy and endosome-trans-Glogi trafficking pathways. *J. Biol. Chem*. **2010**, *285*, 33875-33884.
12. Peet, D.J.; Janowski, B.A.; Mangelsdorf, D.J. The LXRs: a new class of oxysterol receptors. *Curr. Opin. Genet. Dev.* **2008**, *8*, 571-575.
13. Liu, C.; Yang, X. V.; Wu, J.; Kuei, C.; Mani, N. S.; Zhang, L.; Yu, J.; Sutton, S. W.; Qin, N.; Banie, H.; Karlsson, L.; Sun, S.; Lovenberg, T. W. Oxysterols direct B-cell migration through EBI2. *Nature* **2011**, *475*, 519-523.
14. Peet, D.J.; Janowski, B.A.; Mangelsdorf, D.J. The LXRs: a new class of oxysterol receptors. *Curr. Opin. Genet. Dev.* **2008**, *8*, 571-575.
15. Kim, W.-K.; Meliton, V.; Park, K.W.; Hong, C.H.; Tontonoz, P.; Niewiadomski, P.; Waschek, J.A.; Tetradis, S.; Parhami, F. Negative regulation of hedgehog signaling by liver X receptors. *Mol. Endocrinol.* **2009**, *23*, 1532-1543.
16. Janowski, B.A.; Willy, P.J.; Devi, T.R.; Falck, J.R.; Mangelsdorf, D.J. An oxysterol signaling pathway mediated by the nuclear receptor LXRα. *Nature* **1996**, *383*, 728-731.
17. Janowski, B.A.; Grogan, M.J.; Jones, S.A.; Wisely, G.B.; Kliewer, S.A.; Corey, E.J.; Mangelsdorf, D.J. Structural requirements of ligands for the oxysterol liver X receptors LXRα and LXRβ. *Proc. Natl. Acad. Sci.* **1999**, *96*, 266-271.
18. Baggiolini, E.G.; Baggiolini, B.J.; Hennessy, B.M.; Shiuey, S-J.: Truitt, G.A. Uskokovic, M.R.; Vitamin D_3 analogs, U.S. Patent 5,087,619, **1992**.
19. DeLuca, H.F.; Bulli, P.T.; Plum, L.A.; Clagett-Dame, M. 2-Methylene-(20E)-20(22)-dehydro-19-nor-vitamin D analogs. U.S. Patent Application 2010/009940A1, **2010**.
20. Kametani, T.; Katoh, T.; Tsubuki, M.; Honda, T. A facile preparation of ecdysone side chain by utilizing furan derivative. *Chemistry Letters*, **1985**, 485-488.
21. Kametani, T.; Tsubuki, M.; Nemoto, H. A novel synthetic approach to the exdysone side-chain via furan derivatives. *JCS Perkin I,* **1981**, 3077-3082.
22. Jones, S.R.; Kinney, W.A.; Zhang, X; Jones, L.M.; Selinsky, B.S. The synthesis and characterization of analogs of the antimicrobial compound squalamine: 6β-hydroxy-3-aminosterols synthesized from hyodeoxycholic acid. *Steroids*, **1996**, *61*, 565-571.
23. Bharucha, K.R.; Buckley, G.C.; Cross, C.K.; Rubin, L.J.; Ziegler, P. The conversion of hyodesoxycholic acid to progesterone. *Can. J. Chem.,* **1956**, *34*, 982-990.
24. Shen, Y.; Wen,J; A new route for synthesizing cholesterol analogs with fluorocarbon side chains and their liquid-crystalline aliphatic esters. *J. Fluor. Chem.* **2002**, *113*, 13-15.
25. Hirano, Y.; Eguchi, T; Ishiguor, M.; Ikekawa, N. 23,25-Dihydroxycholesterols. *Chem. Pharm. Bull.* **1983**, *31*, 394-400.
26. Honda, T.; Katoh, M.; Yamane, S-I. Stereoselective synthesis of petrosterol and a formal synthesis of aragusterols, *JCS Perkin I,* **1996**, 2291-2296.
27. Parhami, F.; Jung, M.E.; Nguyen, K.T.; Matsui, W.; Yoo, D. Meliton, V. Oxysterols that activate liver X receptor signaling and inhibit hedgehog signaling. *WO* 2011103175. **2011**.
28. Nachtergaele, S.; Mydock, L.K.; Krishnan, K.; Rammohan, J.; Schlesinger, P.H.; Covey, D.F.; Rohatgi, R. Oxysterols are allosteric activators of the oncoprotein smoothened. *Nat. Chem. Biol.* **2012**, *8*, 211-220.

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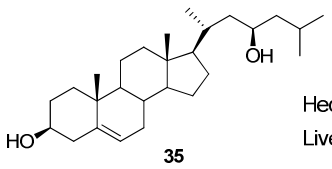

35

Hedgehog Pathway; EC_{50} = 0.54 - 0.65 μM

Liver X Receptor; EC_{50} = 1.54 μM