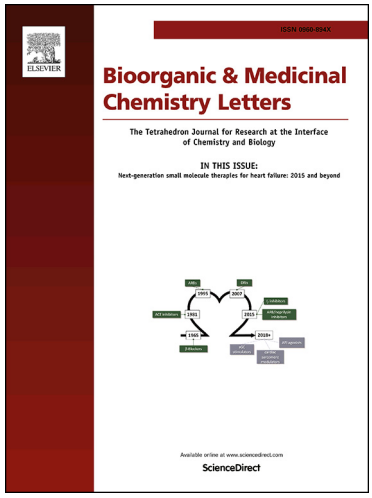
Accepted Manuscript



Design, synthesis and biological evaluation of deuterated Vismodegib for improving pharmacokinetic properties

Fangying Wang, Hongxia Jiang, Yufang Deng, Jiang Yu, Miao Zhan, Lifeng Zhao, Yuanwei Chen

PII: S0960-894X(18)30514-6
DOI: https://doi.org/10.1016/j.bmcl.2018.06.025
Reference: BMCL 25906

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 10 April 2018
Revised Date: 27 May 2018
Accepted Date: 13 June 2018

Please cite this article as: Wang, F., Jiang, H., Deng, Y., Yu, J., Zhan, M., Zhao, L., Chen, Y., Design, synthesis and biological evaluation of deuterated Vismodegib for improving pharmacokinetic properties, *Bioorganic & Medicinal Chemistry Letters* (2018), doi: https://doi.org/10.1016/j.bmcl.2018.06.025

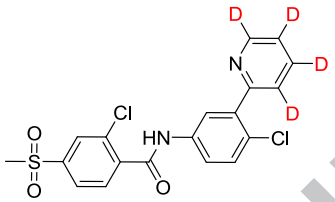
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
F ‘’ onts or abstract dimensions should not be changed or altered.

Design, synthesis and biological evaluation of deuterated Vismodegib for improving pharmacokinetic properties

Leave this area blank for abstract info.

Fangying Wang[a†] , Hongxia Jiang[a†] , Yufang Deng[a] , Jiang Yu[a] , Miao Zhan[d*] , Lifeng Zhao[c*] and Yuanwei Chen[a,b*]



SKLB-C2211

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Design, synthesis and biological evaluation of deuterated Vismodegib for improving pharmacokinetic properties

Fangying Wang[a†] , Hongxia Jiang[a†] , Yufang Deng[a] , Jiang Yu[a] , Miao Zhan[d*] , Lifeng Zhao[c*] and Yuanwei Chen[a,b*]

[a.] *State Key Laboratory of Biotherapy and Cancer Center/Collaborative Innovation Center of Biotherapy, West China Hospital, West China Medical School of Sichuan University, Chengdu 610041, China.*
[b.] *Hinova Pharmaceuticals Inc., Suite 402, Building B, #5 South KeYuan Road, Chengdu, 610041, China.*
[c.] *Chengdu University, Sichuan Industrial Institute of Antibiotics, Chengdu 610052, China*
[d.] *Frontier Institute of Science and Technology, Xi'an Jiaotong University, Xi'an, 710054, China*

ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
Vismodegib
Hedgehog signaling pathway
Basal cell carcinoma
Deuterated drug
Deuterium-hydrogen replacement
Pharmacokinetic properties

ABSTRACT

Vismodegib is an oral and high selective hedgehog (Hh) inhibitor used for the treatment of basal cell carcinoma (BCC). In this work, analogs of Vismodegib with deuterium-for-hydrogen replacement at certain metabolically active sites were prepared and found to have a better pharmacokinetic properties in mice. In particular, deuterated compound SKLB-C2211 obviously altered the blood circulation behavior compared to its prototype, which was demonstrated by significantly prolonged blood circulation half-life time ($t_{1/2}$) and increased $AUC_{0\rightarrow\infty}$. These results suggested SKLB-C2211 had the potential to be a long-acting inhibitor against Hh signaling pathway, and laid the foundation for the further research of its druggability.

∗ Corresponding author. Tel.: +86 186 0283 1330; fax: +86 2885058465; e-mail: ywchen@scu.edu.cn.

∗ Corresponding author. Tel.: +86 158 8241 1095; fax: +86 2885058465; e-mail: lifengzhao@scu.edu.cn; mzhan90@xjtu.edu.cn

†These authors contributed equally

Hedgehog (Hh) signaling pathway is highly conservative which plays a very important role in cell differentiation, development of embryo, organ formation, and keeping internal environment of mature organs.[1-5] This signaling pathway is mainly composed of Hh protein, two transmembrane receptors Patched (Ptc) and Smoothened (Smo), and downstream transcription factors (Gli family).[3] Under specific time or position-dependent stimuli, cells secrete the Hh family ligands (sonic, desert, and indian),[4] whose binding associates with Ptc on target cells. The role of Ptc, in the absence of Hh ligands, is to inhibit the activity of Smo, thus blocking Hh signal transduction. Binding of Hh proteins to Ptc relieves this inhibition and initiates activation of Smo. The increase in Smo activity leads to an augment in activated forms of Gli, which serves to regulate the expression of Hh target genes.[5,6,7] In adults the Hh pathway is mainly quiescent, activation of the Hh pathway has been implicated in the development of cancers in various organs, including brain, liver, prostate, mammary gland, lung and skin.[8,9,10,11] Basal cell carcinoma, the most common form of cancerous malignancy, has the closest association with Hh signaling pathway.[12]

Vismodegib (GDC-0449) is the first oral and high selective Hh signaling pathway targeting agent for the treatment of BCC,[14] and it is also undergoing clinical trials for metastatic colorectal cancer, small-cell lung cancer, advanced stomach cancer, pancreatic cancer, medulloblastoma and chondrosarcoma.[15] This drug acts as a cyclopamine-competitive antagonist of the Smo, Smo inhibition causes the transcription factors Gli1 and Gli2 to remain inactive, which prevents the expression of tumor mediating genes within the Hh pathway, which is pathogenetically relevant in more than 90% BCC[13,16]. Preclinical pharmacokinetic studies[17,18,19] shows that Vismodegib was metabolically stable in mouse, rat, dog, and human hepatocytes and had a more rapid turnover in monkey hepatocytes. The metabolites were identified by using various LC-MS/MS techniques.[19] The major metabolites and the metabolic pathways were illustrated in **Fig. 1**, three oxidative metabolites (**M1**, **M2** and **M3**)[17,18] were formed by oxidation of the pyridine or the middle benzene ring. Two glucuronide conjugates (**M4**, **M5**) and an oxidative sulfate metabolite (**M6**) were also produced subsequently. In addition, three major pyridine ring-opened metabolites (**M7**, **M8**, and **M9**)[19] have been found.

On the basis of this information, we considered that the blockade of metabolically active sites may slow down the metabolism rate. Replacing a hydrogen atom with fluorine has been extensively used to achieve this goal. Recently, the use of

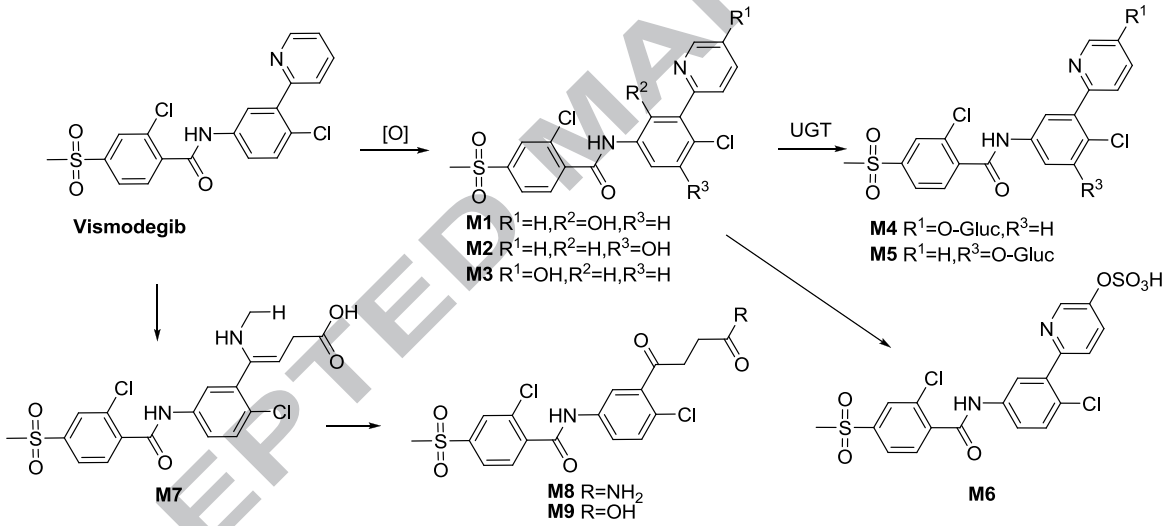


Fig 1. The structures of metabolites and major metabolic pathways of Vismodegib. UGT, UDP-glucuronosyltransferase; Gluc, glucuronic acid.

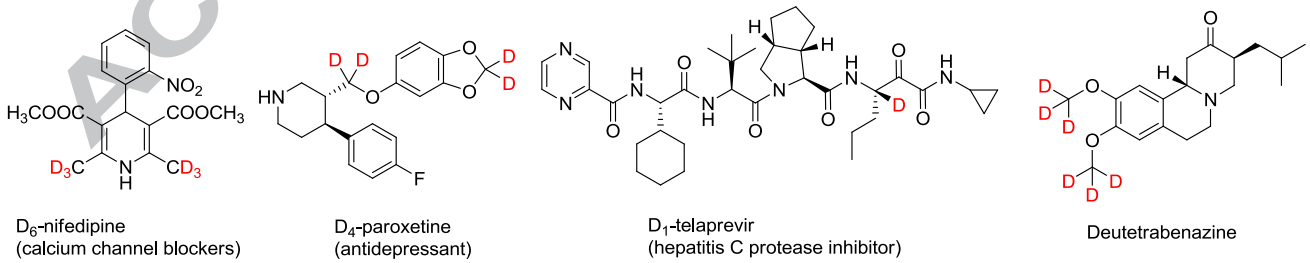


Fig 2. Recent examples of deuterated drug analogues. Deuterium used as a bioisostere of hydrogen to improve pharmacokinetic properties.

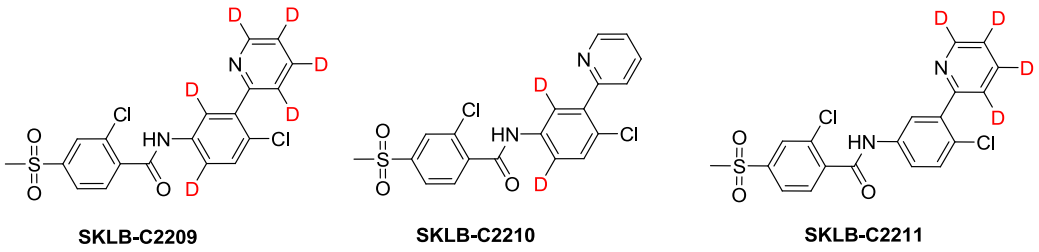
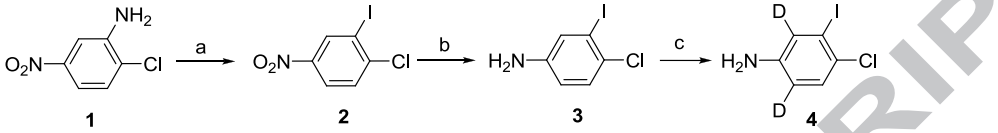
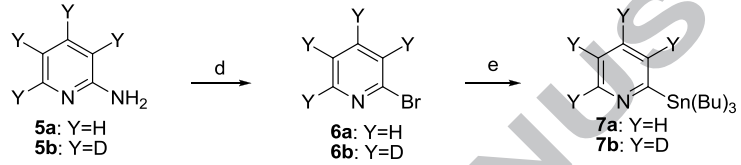


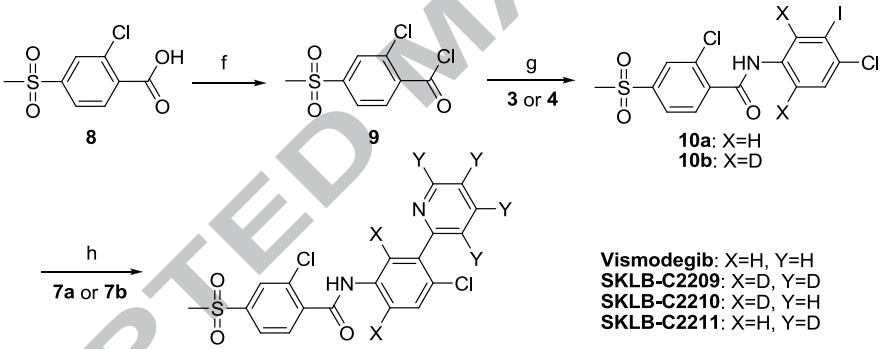
Fig 3. Chemical structure of deuterated analogues of Vismodegib.



Scheme 1. Synthesis of intermediates **3** and **4**. Reagents and conditions: (a) HCl/H_2O, $NaNO_2$, KI, 0 ℃ to rt; (b) Fe, AcOH, EtOH, 60 ℃ to 70 ℃ to 23 ℃; (c) HCl, D_2O, microwave, 180 ℃.



Scheme 2. Synthesis of intermediates **7a** and **7b**. Reagents and conditions: (d) HBr, Br_2, $NaNO_2$, -2 ℃; (e) n-BuLi, THF, -78 ℃, chlorotributyltin, -78 ℃ to rt.



Scheme 3. Synthesis of Vismodegib analogs. Reagents and conditions: (f) $SOCl_2$, reflux; (g) DMAc, rt; (h) $Pd(PPh_3)_2Cl_2$, NMP, microwave, 130 ℃.

deuterium has also served a similar effect.[20,21,22] In fact, well-known applications using deuterium exist within every subdiscipline in pharmaceutical discovery and development.[23,24] Because of the isotope effect, deuterium-hydrogen replacement can have a major impact on improving pharmacokinetic properties (ADMET),[25,26] mainly were: (1) Reducing the metabolic rate, thus gaining a lower drug dose and improving the tolerance of drugs. (2) Decreasing the clearance rate and increasing the biological half-life of the drugs. These clear benefits result from a slower rate of C-D bond cleavage compared to C-H bond, making the deuterium-labeled drugs more resistant to chemical or enzymatic degradation.[27] As a result, many pharmacists devoted to the research of deuterium-hydrogen replacement at metabolically active sites expecting to achieve better drug candidates. Clinical trials of some known drugs have been initiated,[28,29,30] including D-venlafaxine (SD-254), D-atazanavir (CTP-518),[25] D_1-telaprevir, D_4-paroxetine, D_6-nifedipine.[22] On April 3rd, 2017, FDA approved the first deuterated drug-Deutetrabenazine.[31] (**Fig. 2**)

Here we wish to report the deuterium-for-hydrogen replacement at the metabolically active sites of Vismodegib to generate the corresponding deuterium-labeled drugs and the studies of their pharmacokinetic profiles. Therefore, we designed deuterated analogues of Vismodegib on the pyridine and/or the middle benzene ring, namely SKLB-C2209, SKLB-C2210, SKLB-C2211 (**Fig. 3**).

The designed deuterated analogues together with their intermediates (compounds **3**, **4**, **7**) were prepared according to **Schemes 1-3**.

The synthesis of key intermediates **3** and **4** commenced from commercially available 2-chloro-5-nitroaniline (**1**). As illustrated in **Scheme 1**, **1** was first transformed into **2** via a diazotizating iodination followed by a reduction reaction of nitro group using Iron to obtain **3**. Then **3** was efficiently deuterated at the ortho position of amino group in the presence of 1 equiv HCl in D_2O under microwave irradiation at 180 ℃, receiving compound **4** with nearly total deuterium incorporation.

As described in **Scheme 2**, intermediate **7a** was obtained by a two-step procedure. Bromo-substitution of 2-aminepyridine (**5a**) with HBr and Br_2 afforded compound **6a**, followed by nucleophilic substitution with chlorotributyltin to give compound **7a**. Corresponding deuterated compounds **5b**, **6b**, and **7b** were

Table 1. Pharmacokinetic parameters after oral compound administration to mice

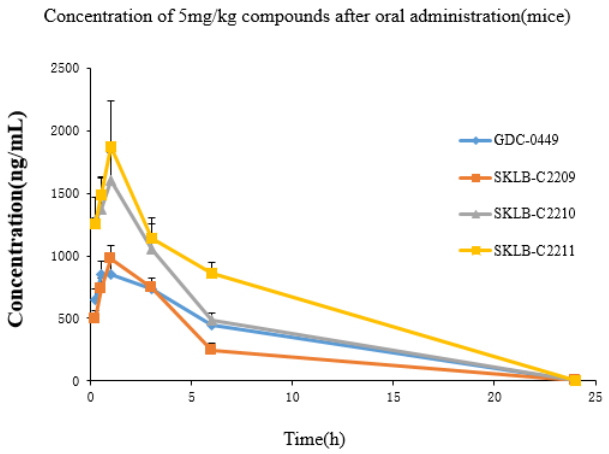
	GDC-0449	SKLB-C2209	SKLB-C2210	SKLB-C2211
Dose (mg/kg)	5	5	5	5
t_{max} (h)	1	1	1	1
$t_{1/2}$ (h)	5.617	2.556	2.82	8.547
C_{max} (ng/mL)	854	972.3	1606.7	1870
$AUC_{0\rightarrow t}$ (μg/L*h)	4049.8	6106.538	6284.838	6511.863
$AUC_{0\rightarrow\infty}$ (μg/L*h)	7657.678	6113.538	8290.712	17147.309

prepared from the original material **5a**, which was deuterated using a Pd/C-H_2-D_2O system, details are described in Supporting Information.

Deuterium-labeled analogs SKLB-C2209, SKLB-C2210, and SKLB-C2211 were synthesized according to **Scheme 3**. Compound **8** was first transformed into **9** via reaction with thionyl chloride followed by an acylation reaction using compound **3** or **4**. The target compounds were obtained via Still Coupling reaction using **10a** / **10b** with **7a**/ **7b**.

The *in vivo* pharmacokinetic profile studies of Vismodegib and its deuterated analogues (SKLB-C2209, SKLB-C2210 and SKLB-C2211) in female CD-1 mouse were performed to evaluate the alteration of blood circulation behaviors by deuterium replacement at metabolically active sites of Vismodegib. (**Fig. 4**) Vismodegib and its analogs were suspended in vehicle (0.5% methyl cellulose with 0.2% Tween 80 in distilled water) and the suspension was administered orally to the mice within a day of preparation with dose of 5 mg/kg. Blood samples were taken at 0.25, 0.5, 1.0, 3.0, 6.0 and 24 h, and drug plasma concentration were quantified by LC-MS/MS. As shown in **Table 1**, deuterated compounds generally exhibited

Fig 4. Mean plasma concentration of 5mg/kg Vismodegib analogs after oral administration in mice (n=3/group)



blood circulation behavior compared to its prototype Vismodegib, and had the potential to be a long-acting inhibitor against Hh pathway with decreased dosage. These characters are important for clinical use because lowered dosage administration can significantly improve the compliance of patients and reduce side effects.

In conclusion, compound SKLB-C2211 with deuterium-for-hydrogen replacement in a metabolically active site significantly exhibited better pharmacokinetics profiles as compared to its prototype Vismodegib at the same dose and had a better plasma concentration which could prolong its half-life and improve the potency with a lower dosage. Thus, SKLB-C2211 may be a novel long-acting inhibitor of Hh signaling pathway.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No.81472418).

References and notes

1. Ruiz, i. Altaba. A.; Sánchez, P.; Dahmane, N. *Nat. Rev. Cancer* **2002**, *2*, 361.
2. Lum, L.; Beachy, P. A. *Science* **2004**, *304*, 1755.
3. Ruiz-Gómez, A.; Molnar, C.; Holguín, H.; Mayor, F.; de Celis, J. F. *Biochim. Biophys. Acta*. **2007**, *1768*, 901.
4. Rubin, L. L.; de Sauvage, F. J. *Nat. Rev. Drug Discov*. **2006**, *5*, 1026.
5. Varjosalo, M.; Taipale, J. *Genes. Dev*. **2008**, *22*, 2454.
6. Ma, G.; Xiao, Y.; He, L. *J. Genet. Genomics.* **2008**, *35*, 129.
7. Corbit, K. C.; Aanstad, P.; Singla, V.; Norman, A. R.; Stainier, D. Y.; Reiter, J. F. *Nature* **2005**, *437*, 1018.
8. Sahebjam, S.; Siu, L. L.; Razak, A. A. *Oncologist* **2012**, *17*, 1090.
9. Ma, HY.; Zhang, F.; Li, J.; Mo, M. L.; Cheng, Z.; Liu, L.; Zhou, H. M.; Sheng, Q. *Oncol. Lett.* **2011**, *2*, 1291.
10. Riobo, N. A. *Curr. Opin. Pharmacol*. **2012**, *12*, 736.
11. Pan, J. Y.; Zhou, S. H. *Pharmazie* **2012**, *67*, 475.
12. Wong, H.; Alicke, B.; West, K. A.; Pacheco, P.; La, H.; Januario, T.; Yauch, R.; de Sauvage, F.; Gould, S. E. *Clin. Cancer Res.* **2011**, *17*, 4682.
13. LoRusso, P. M.; Rudin, C. M.; Borad, M. J.; Vernillet, L.; Darbonne, W. C.; Mackey, H.; Dimartino, J. F.; de Sauvage, F.; Low, J. A.; VonHoff, D. D. *J. Clin. Oncol.* **2008**, *26*, 3516.
14. Geeraert, P.; Williams, J. S.; Brownell, I. *J. Drugs. Dermatol.* **2013**, *12*, 519.
15. Rudin, C. M.; Hann, C. L.; Laterra, J.; Yauch, R. L.; Callahan, C. A.; Fu, L.; Holcomb, T.; Stinson, J.; Gould, S. E.; Coleman, B.; LoRusso, P. M.; VonHoff, D. D.; de Sauvage, F. J.; Low, J. A. *N. Engl. J. Med.* **2009**, *361*, 1173.
16. Robarge, K. D.; Brunton, S. A.; Castanedo, G. M.; Cui, Y.; Dina, M. S.; Goldsmith, R.; Gould, S. E.; Guichert, O.; Gunzner, J. L.; Halladay, J.; Jia, W.; Khojasteh, C.; Koehler, M. F.; Kotkow,

K.; La, H.; Lalonde, R. L.; Lau, K.; Lee, L.; Marshall, D.; Marsters, J. C. Jr.; Murray, L. J.; Qian, C.; Rubin, L. L.; Salphati, L.; Stanley, M. S.; Stibbard, J. H.; Sutherlin, D. P.; Ubhayaker, S.; Wang, S.; Wong, S.; Xie, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5576.
17. Wong, H.; Chen, J. Z.; Chou, B.; Halladay, J. S.; Kenny, J. R.; La, H.; Marsters, J. C. Jr.; Plise, E.; Rudewicz, P. J.; Robarge, K.; Shin, Y.; Wong, S.; Zhang, C.; Khojasteh, S. C. *Xenobiotica* **2009**, *39*, 850.
18. Wong, H.; Theil, F. P.; Cui, Y.; Marsters, J. C. Jr.; Khojasteh, S. C.; Vernillet, L.; La, H.; Song, X.; Wang, H.; Morinello, E. J.; Deng, Y.; Hop, C. E. *Drug. Metab. Dispos.* **2010**, *38*, 1029.
19. Yue, Q.; Chen, Y. H.; Mulder, T.; Deese, A.; Takahashi, R.; Rudewicz, P. J.; Reynolds, M.; Solon, E.; Hop, C. E.; Wong, H.; Khojasteh, S. C. *Drug. Metab. Dispos.* **2011**, *39*, 952.
20. Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529.
21. Zhu, Y.; Zhou, J.; Jiao, B. *ACS. Med. Chem. Lett.* **2013**, *4*, 349.
22. Gant, T. G. *J. Med. Chem.* **2014**, *57*, 3595.
23. Xu, G.; Lv, B.; Roberge, J. Y.; Xu, B.; Du, J.; Dong, J.; Chen, Y.; Peng, K.; Zhang, L.; Tang, X.; Feng, Y.; Xu, M.; Fu, W.; Zhang, W.; Zhu, L.; Deng, Z.; Sheng, Z.; Welihinda, A.; Sun, X. *J. Med. Chem.* **2014**, *57*, 1236.
24. Sanderson, K. *Nature* **2009**, *458*, 269.
25. Harbeson, S. L.; Tung, R. D. *Annu. Rep. Med. Chem.* **2011**, *46*, 403.
26. Katsnelson, A. *Nat. Med.* **2013**, *19*, 656.
27. Butear, K. C. *J. High. Tech. Law*. **2010**, *10*.
28. Yarnell, A. *Chem. Eng. News.* **2009**, *87*, 36.
29. Frank, S.; Moritz, H.; Rossen, K.; Rudolf-Giesbert, A. *Arzneim. Forsch.* **2006**, *56*, 295.
30. Szostak, M.; Spain, M.; Procter, D. J. *Org. Lett.* **2014**, *16*, 5052.
31. Heo, Y. A.; Scott. L. J. *Drugs*. **2017**, *77*, 1857.

Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

Click here to remove instruction text...

Highlights:

- SKLB-C2211 has prolonged blood circulation half-life time (t1/2) and increased AUC.
- Three deuterated analogues produced greater $AUC_{0\rightarrow t}$ than Vismodegib.
- SKLB-C2211 is potential to be a long-acting inhibitor against Hh signaling pathway.