Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Stimulation of cortical bone formation with thienopyrimidine based inhibitors of Notum Pectinacetylesterase

James E. Tarver Jr.[a,*], Praveen K. Pabba[a], Joseph Barbosa[a], Qiang Han[a], Michael W. Gardyan[a], Robert Brommage[b], Andrea Y. Thompson[b], James M. Schmidt[b], Alan G. E. Wilson[b], Wei He[a], Victoria K. Lombardo[a], Kenneth G. Carson[a]

[a] Lexicon Pharmaceuticals, 350 Carter Road, Princeton, NJ 08540, United States
[b] Lexicon Pharmaceuticals, 8800 Technology Forest Place, The Woodlands, TX 77381, United States

ARTICLE INFO

*Article history:*
Received 13 December 2015
Revised 6 February 2016
Accepted 9 February 2016
Available online xxxx

*Keywords:*
Notum Pectinacetylesterase
Osteoporosis
Thienopyrimidine
SAR
Femur cortical bone thickness

ABSTRACT

A group of small molecule thienopyrimidine inhibitors of Notum Pectinacetylesterase are described. We explored both 2-((5,6-thieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acids and 2-((6,7-thieno[3,2-*d*]pyrimidin-4-yl)thio)acetic acids. In both series, highly potent, orally active Notum Pectinacetylesterase inhibitors were identified.



Bone is a dynamic tissue continuously remodeled during life with bone mass depending on the coordinated activities of bone-forming osteoblasts and bone-resorbing osteoclasts. An imbalance in bone turnover between these anabolic and catabolic activities results in postmenopausal, aging-related and glucocorticoid-induced osteoporosis. Besides good nutrition, including adequate calcium and vitamin D intakes, current osteoporosis treatments include estrogens, bisphosphonates, selective estrogen receptor modulators (raloxifene and bazedoxifene) and the anti-RANKL antibody denosumab. These anti-resorptive agents all minimize additional bone loss by inhibiting osteoclastic bone resorption. Teriparatide, the amino-terminal fragment of parathyroid hormone given by daily subcutaneous injections, is the sole anabolic osteoporosis drug stimulating osteoblasts to produce new bone. Potential osteoporosis drugs under late stage clinical development include the anti-resorptive odanacatib inhibiting cathepsin K, the teriparatide analog abaloparatide, and anabolic anti-sclerostin antibodies. Because of the paucity of available anabolic drugs for osteoporosis treatment, there is an urgent need to develop orally-active small molecule therapies to treat this disease that are nontoxic, cost-effective, and easy to administer.[1–4]

The WNT signaling pathway, involving 19 secreted WNTs, 10 Frizzled membrane receptors, Lrp4/5/6 coreceptors, plus secreted Dickkopf (DKK) and Secreted Frizzled-Related Protein (SFRP) inhibitors, plays a key role transducing physical activity to new bone formation[5,6] and is a key pathway for drug development.[7,8] Lexicon's gene knockout mouse phenotyping campaign[9] identified the gene Notum Pectinacetylesterase as an important contributor to cortical bone mass and follow-up studies, subsequently confirmed by two independent laboratories,[10,11] demonstrated that NOTUM is a WNT-inactivating lipase that removes the palmitoleate essential for binding to Frizzled receptors. Inhibiting NOTUM stimulates bone formation on all endocortical (marrow-facing) bone surfaces.[12–14]

Herein we report a group of small molecules, namely thienopyrimidines, as potent inhibitors of Notum Pectinacetylesterase. $IC_{50}$ values were determined by incubation of conditioned media containing mouse or human NOTUM with trisodium 8-octanoyloxypyrene-l,3,6-trisulfonate (OPTS), a water-soluble enzyme substrate for fluorimetric assays of esterases and lipases. Compound $EC_{50}$ values were determined using a cell-based TCF/LEF CellSensor® assay as previously described.[12–14]

We used the OPTS assay as a primary screen and highly potent compounds in the OPTS assay were selected for additional profiling in mouse and human cellular assays. It was generally noticed that high potency in the OPTS assay ($IC_{50}$ <50 nM) was required to show

* Corresponding author. Tel.: +1 215 898 4193.
*E-mail address:* james.tarver2@gmail.com (J.E. Tarver).

http://dx.doi.org/10.1016/j.bmcl.2016.02.021
0960-894X/

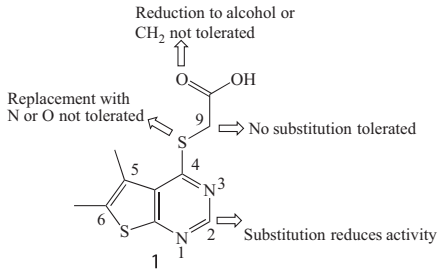



**Figure 1.** Preliminary SAR triage.

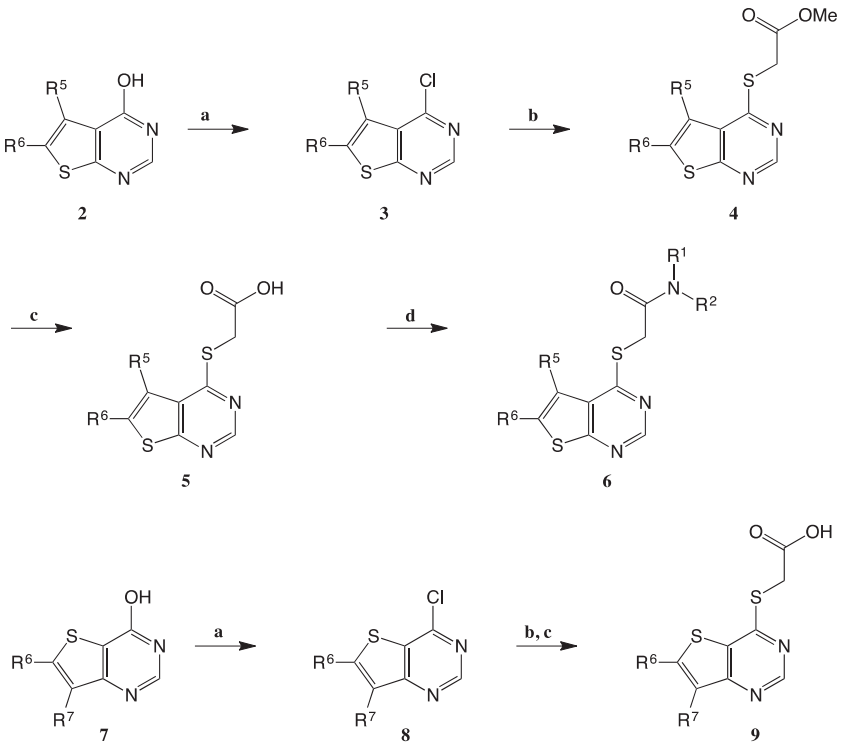
potency in cells. Once the OPTS assay potencies reached single-digit nanomolar range, we used mouse and human cellular assay $EC_{50}$ values to further differentiate compounds, referred to as $mEC_{50}$ and $hEC_{50}$ respectively. Based on internal high throughput screening, we identified 2-((5,6-dimethylthieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acid **1** as a small molecule lead (Fig. 1). Compound **1** had OPTS $IC_{50}$ of 2 nM, mouse $EC_{50}$ of 1020 nM and human $EC_{50}$ of 570 nM, which offered a good starting point for our SAR work. From the lead compound **1**, variations at different positions were explored (Fig. 1). The general observation was that replacement of the carboxylic acid with esters, heterocycles, sulfonamides and carboxamides was tolerated. No substitutions on the carbon at the 9-position were tolerated. Replacement of the thienopyrimidine ring with other heterocycles generally gave much lower potency.

The syntheses of our derivatives were carried out following the general synthetic methods shown in Scheme 1. The appropriate commercially available or prepared[12] thienopyrimidine-4-ols **2** were treated with phosphorus oxychloride to generate the 4-chlorothienopyrimidines **3**. Subsequent treatment with methyl 2-mercaptoacetate gave esters **4**. Saponification of the esters with aqueous base yielded carboxylic acids **5**, which could be further functionalized to amides, heterocycles, sulfonamides, carboxamides or ketoacids **6**. Similarly, commercially available or prepared[12] thienopyrimidine-4-ols **7** or other heterocyclic pyrimidines were converted to thioacetic acids **9** or other corresponding acids.

Our SAR work began by exploring point substitutions at each position. A logical step was to see if improvements could be made to the 5,6-di-methyl substitution. Most of these compounds showed significant losses in potency. As stated above, 2-((5,6-dimethylthieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acid **1** had an OPTS $IC_{50}$ of 2 nM . The demethylated analog, compound **10**, showed a reduction in potency, OPTS $IC_{50}$ to 2.8 μM. Mono-methyl compounds substituted at the 5- or 6-position, **11** and **12**, had $IC_{50}$'s of 92 nM and 152 nM respectively, demonstrating a preference for bis-substitution on the thiophene ring. Expanding dialkyl substitution by incorporating a ring in compound **16** led to a noticeable loss of activity but suggested that larger substitutions in this region of the molecule could be tolerated. Replacing the thiophene with a thiazole led to dramatic loss of activity, compound **13** exhibited a micromolar $IC_{50}$. Phenyl, pyrrole and pyrazole, compounds **15**, **17**, and **18**, also proved poor surrogates for the thiophene, registering significant losses in potency. Transposing the thiophene ring in compound **14**, however, had similar activity to its constitutional isomers, compounds **11** and **12**.

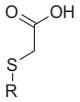
A survey of the carboxyl terminus based on converting acid **1** to esters, amides or other heterocycles is summarized in Table 2. These modifications generally led to modest losses in potency. In the case of the amide derivatives, we found that they exhibited poor metabolic stability and pharmacokinetic issues. In contrast, we observed that the parent carboxylic acids generally had good ADME profiles, and so we chose to focus our efforts on thioacetic acids.

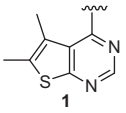
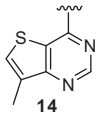
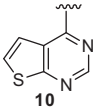
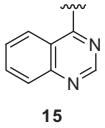
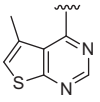
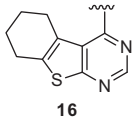
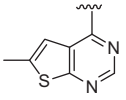
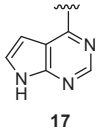
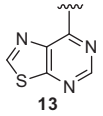
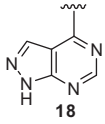
Further SAR of 2-((5,6-thieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acids is summarized in Table 3. The 5-ethyl-6-methyl compound **29** had a $hEC_{50}$ of 22 μM. But shifting the positions, 5-methyl-6-ethyl compound **30** made a major impact on potency, with a $hEC_{50}$ of 835 nM. Retaining the 5-methyl and changing the 6-position to bromo (**31**) further improved $hEC_{50}$ potency to 144 nM,




**Scheme 1.** Reagents and conditions: (a) $POCl_3$; (b) $HSCH_2CO_2Me$, $Et_3N$; (c) NaOH, $THF/H_2O$; (d) HATU, $R^1NHR^2$.

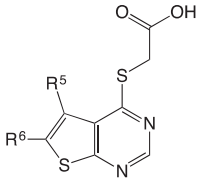
**Table 1**
SAR on thieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acid ring for Notum Pectinacetylesterase



| Compounds | OPTS $IC_{50}$ (μM) | Compounds | OPTS $IC_{50}$ (μM) |
|---|---|---|---|
|  **1** | 0.002 |  **14** | 0.193 |
|  **10** | 2.8 |  **15** | 1.2 |
|  **11** | 0.092 |  **16** | 0.620 |
|  **12** | 0.152 |  **17** | 15 |
|  **13** | 15 |  **18** | 15 |

while 5-bromo-6-methyl isomer **32** had a $hEC_{50}$ of 208 nM. Compound **32** suggested that electronics were important for increasing activity compared with the hydrophobic nature of bromine, as both

**Table 3**
SAR of 2-((5,6-thieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acids for Notum Pectinacetylesterase

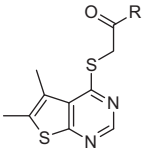


| Compounds | $R^5$ | $R^6$ | $mEC_{50}$ (nM) | $hEC_{50}$ (nM) |
|---|---|---|---|---|
| **1** | H | H | 1020 | 570 |
| **29** | Et | Me | 65,394 | 21,928 |
| **30** | Me | Et | 2637 | 835 |
| **31** | Me | Br | 485 | 144 |
| **32** | Br | Me | 375 | 208 |
| **33** | Me | Cl | 2561 | 885 |
| **34** | Cl | Me | 409 | 107 |
| **35** | Cl | Et | 1442 | 393 |
| **36** | Cl | *i*-Pr | 828 | 477 |
| **37** | Cl | *c*-Pr | 1006 | 250 |
| **38** | Cl | Pr | 342 | 88 |

these analogs were more potent than the dialkyl congeners. 5-Methyl-6-chloro compound **33** decreased the potency to 885 nM, while 5-chloro-6-methyl compound **34** improved $hEC_{50}$ potency to 107 nM. We decided next to fix the 5-chloro while varying the 6-alkyl group. This effort led to a 6-ethyl and 6-isopropyl compounds **35** and **36** that increased potency to 393 nM and 477 nM respectively. However, cyclopropane **37** slightly improved potency to 250 nM and *n*-propyl compound **38** continued the trend with $hEC_{50}$ of 88 nM.

Although many early modifications of the heterocyclic core structure were not fruitful (as in Table 1), one of the most tolerated core changes was the transposition of the thiophene ring, such as compound **14**. Our next approach was to explore the change in

**Table 2**
SAR of the carboxyl group for thieno[2,3-*d*]pyrimidin-4-yl)thio)acetic acid



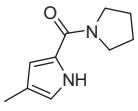
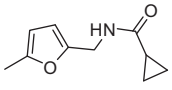
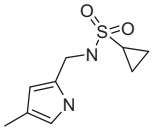
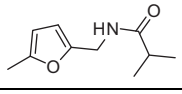
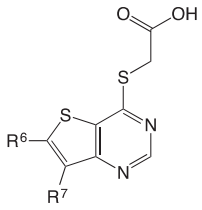
| Compounds | R | OPTS $IC_{50}$ (nM) | $hEC_{50}$ (nM) | Compounds | R | OPTS $IC_{50}$ (nM) | $hEC_{50}$ (nM) |
|---|---|---|---|---|---|---|---|
| **1** | –OH | 2 | 570 | | | | |
| **19** | –NHCN | 2 | 400 | **25** |  | 10 | 280 |
| **20** | –NHOH | 2 | 556 | | | | |
| **21** | –OMe | 2 | 445 | **26** |  | 5 | 249 |
| **22** | –NHMe | 27 | 789 | **27** |  | 2 | 168 |
| **23** | $–NH_2$ | 2 | 857 | | | | |
| **24** | $–NHSO_2Me$ | 11 | 1462 | **28** |  | 6 | 211 |

**Table 4**
SAR of 2-((6,7-thieno[3,2-*d*]pyrimidin-4-yl)thio)acetic acids for Notum Pectinacetylesterase



| Compounds | $R^6$ | $R^7$ | $mEC_{50}$ (nM) | $hEC_{50}$ (nM) |
|---|---|---|---|---|
| **39** | Me | Cl | 5508 | 1965 |
| **40** | Cl | Me | 1444 | 435 |
| **41** | Br | Me | 1360 | 417 |
| **42** | Me | Me | 4468 | 881 |
| **43** | Me | *c*-Pr | 408 | 115 |
| **44** | Cl | *c*-Pr | 55 | 21 |
| **45** | $CF_3$ | *c*-Pr | 43 | 12 |

**Table 5**
Mouse pharmacokinetic data for compounds **34** and **44**

| Compounds | $C_{max}$ (μM) at 10 mg/kg po | AUC (μM * h) at 10 mg/kg po | Clearance (mL/min/kg) | $V_d$ (L/kg) | %*F* |
|---|---|---|---|---|---|
| **34** | 157 | 515 | 1.22 | 0.16 | 95 |
| **44** | 129 | 1533 | 0.49 | 0.13 | 65 |

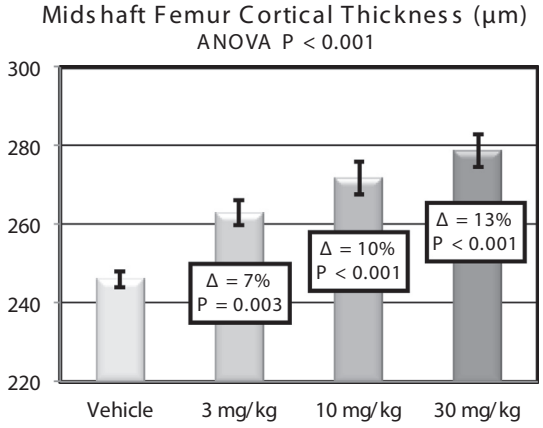



**Figure 2.** Change in cortical bone thickness after treatment for 25 days.

thiophene orientation to give 2-((6,7-thieno[3,2-*d*]pyrimidin-4-yl) thio)acetic acids. In this manner, we hoped to preserve the geometry of the core and the molecular arrangement of substituents but somewhat alter the electronics of the system. The SAR is summarized in Table 4. With $R_6$ = Me, $R_7$ = Cl, compound **39** had a $hEC_{50}$ of close to 2 μM. By exchanging the $R_6$ and $R_7$ substituents, the potency of **40** improved to 435 nM. Fixing $R_7$ as the methyl and changing $R_6$ to bromo gave $hEC_{50}$ of 417 nM, similar to chloro compound **40**, and changing $R_6$ to methyl **42** reduced the potency to 881 nM. When we selected $R_6$ = Me and introduced cyclopropyl at $R_7$ in compound **43**, we obtained a major boost in potency to 115 nM. Keeping $R_7$ as cyclopropyl, and modifying $R_6$ substitution produced compound **44** ($R_6$ = Cl), $hEC_{50}$ of 21 nM, and compound **45** ($R_6$ = $CF_3$), $hEC_{50}$ of 12 nM.

As shown in Table 5, when dosed orally to mice at 10 mg/kg, compounds **34** and **44** were both able to achieve high $C_{max}$ and AUC while having low clearance and volume of distribution. Compound **34** was advanced into in vivo mouse models of bone growth and was found to increase cortical bone thickness.[12]

The in vivo effect of 2-((6-chloro-7-cyclopropylthieno[3,2-*d*] pyrimidin-4-yl)thio)acetic acid **44** was determined by treating F1 male hybrid (129xC57) mice for 25 days with the compound, starting at 8.7 weeks of age. The compound was administered by daily oral gavage (vehicle = 0.1% Tween 80 in water). Four groups of mice ($N$ = 13) were used: control, 3 mg/kg compound, 10 mg/kg compound, and 30 mg/kg compound. Midshaft femur cortical thickness was measured by microCT (Scanco μCT40). As shown in Figure 2, an increase in cortical bone thickness was observed at all doses compared to control: 7% ($p$ = 0.003) at 3 mg/kg; 10% ($p$ <0.001) at 10 mg/kg; and 13% ($p$ <0.001) at 30 mg/kg.

In summary, we have developed novel inhibitors of Notum Pectinacetylesterase showing in vivo efficacy in significantly increasing midshaft femur cortical bone thickness in mice and rats.

## References and notes

1. Allen, J. G.; Fotsch, C.; Babij, P. *J. Med. Chem.* **2010**, *53*, 4332.
2. Guo, H.; Shao, H.; Yang, Z.; Xue, S.; Li, X.; Liu, Z.; He, X.; Jiang, J.; Zhang, Y.; Si, S.; Li, Z. *J. Med. Chem.* **2010**, *53*, 1819.
3. Makras, P.; Delaroudis, S.; Anastasilakis, A. D. *Metabolism* **2015**, *64*, 1199.
4. Cairoli, E.; Zhukouskaya, V. V.; Eller-Vainicher, C.; Chiodini, I. *J. Endocrinol. Invest.* **2015**, *38*, 303.
5. Baron, R.; Kneissel, M. *Nat. Med.* **2013**, *19*, 179.
6. Maupin, K. A.; Droscha, C. J.; Williams, B. O. *Bone Res.* **2013**, *1*, 27.
7. Kahn, M. *Nat. Rev. Drug Disc.* **2014**, *13*, 513.
8. Brommage, R. *J. Cell. Biochem.* **2015**, *116*, 2139.
9. Brommage, R.; Liu, J.; Hansen, G. M.; Kirkpatrick, L. L.; Potter, D. G.; Sands, A. T.; Zambrowicz, B.; Powell, D. R.; Vogel, P. *Bone Res.* **2014**, *2*, 14034.
10. Kakugawa, S.; Langton, P. F.; Zebisch, M.; Howell, S. A.; Chang, T. H.; Liu, Y.; Feizi, T.; Bineva, G.; O'Reilly, N.; Snijders, A. P.; Jones, E. Y.; Vincent, J. P. *Nature* **2015**, *519*, 187.
11. Zhang, X.; Cheong, S. M.; Amado, N. G.; Reis, A. H.; MacDonald, B. T.; Zebisch, M.; Jones, E. Y.; Abreu, J. G.; He, X. *Dev. Cell* **2015**, *32*, 719.
12. Barbosa, J.; Carson, K. G.; Gardyan, M. W.; He, W.; Lombardo, V.; Pabba, P.; Tarver, J. Jr., U.S. Patent 65, 200, **2012**.
13. Brommage, R.; Feng, X.; Hong, S.; Landes, G.; Liu, J.; Potter, D. G.; Powell, D. R. U.S. Patent 302,346, **2013**.
14. Han, Q.; Pabba, P. K.; Barbosa, J.; Mabon, R.; Healy, J. P.; Gardyan, M. W.; Terranova, K. M.; Brommage, R.; Thompson, A. Y.; Schmidt, J. M.; Wilson, A. G. E.; Xu, X.; Tarver, J. E., Jr.; Carson, K. G. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1184.