Bioorganic & Medicinal Chemistry Letters 26 (2016) 1525–1528

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

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ARTICLE INFO

Article history:
Received 13 December 2015
Revised 6 February 2016
Accepted 9 February 2016
Available online 10 February 2016

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

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http://dx.doi.org/10.1016/j.bmcl.2016.02.021
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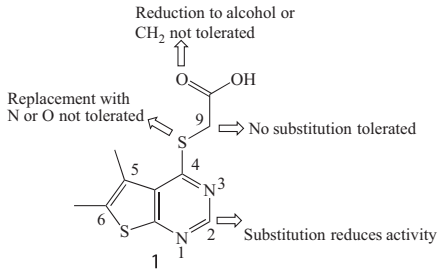


Figure 1. Preliminary SAR triage.

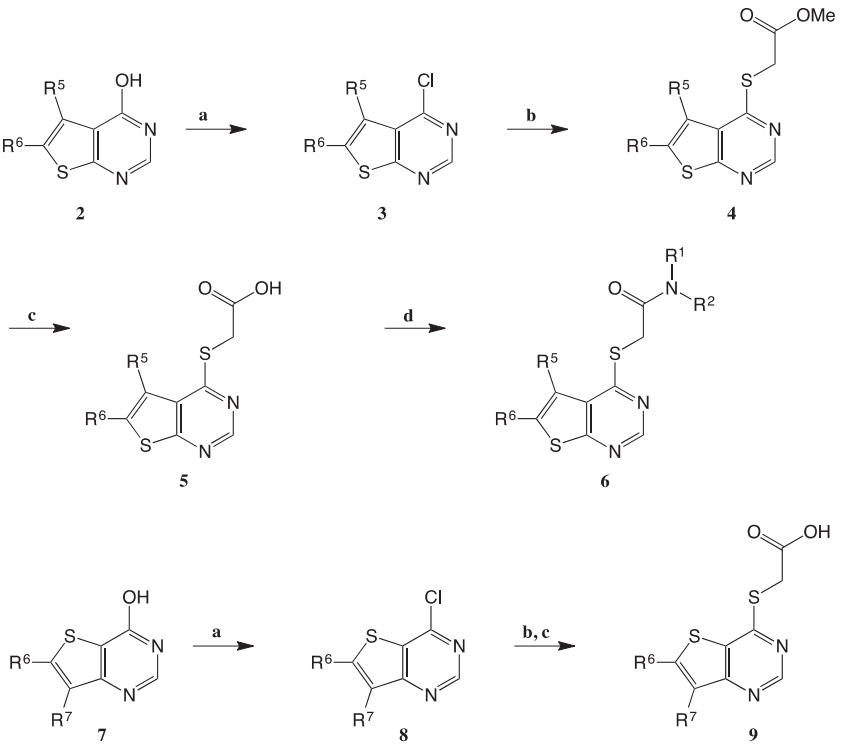
potency in cells. Once the OPTS assay potencies reached single-digit nanomolar range, we used mouse and human cellular assay EC_{50} values to further differentiate compounds, referred to as mEC_{50} and hEC_{50} respectively. Based on internal high throughput screening, we identified 2-((5,6-dimethylthieno[2,3-*d*]pyrimidin-4-yl)thio)acetic 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.