Bioorganic & Medicinal Chemistry Letters 26 (2016) 1184–1187

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

4*H*-Thieno[3,2-*c*]chromene based inhibitors of Notum Pectinacetylesterase

Qiang Han [a], Praveen K. Pabba [a], Joseph Barbosa [a], Ross Mabon [a], Jason P. Healy [a], Michael W. Gardyan [a], Kristen M. Terranova [a], Robert Brommage [b], Andrea Y. Thompson [b], James M. Schmidt [b], Alan G. E. Wilson [b], Xiaolian Xu [a], James E. Tarver Jr. [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 12 January 2016
Accepted 14 January 2016
Available online 18 January 2016

Keywords:
Notum Pectinacetylesterase
Osteroporosis
Thienochromene
SAR
Femur cortical bone thickness

ABSTRACT

A group of small molecule thienochromenes inhibitors of Notum Pectinacetylesterase are described. We developed SAR on three series based on carbon, oxygen and sulfur replacement of the 5-position. In each series, highly potent Notum Pectinacetylesterase inhibitors were identified.

Bone is a living, dynamic tissue, that is, continuously remodeled during the adult life of all mammals. Bone mass depends on the coordinated activities of bone-forming osteoblasts and bone-resorbing osteoclasts. Bone turnover reflects a balance between these anabolic and catabolic cellular functions and ensures that the mature skeleton can repair itself when damaged and sustain its endocrine function by release of minerals such as calcium and phosphorous into the circulation. Modest loss of bone mineral density (BMD), referred to as osteopenia, and severe loss of bone known as osteoporosis represent the early and late manifestations of alterations in bone turnover that result in bone disease.[1–3]

The current standard of care for the treatment of osteoporosis utilizes the bisphosphonate class of oral, small molecule antiresorptives. Raloxifene™, calcium, and vitamin D supplements are also typically used in osteoporosis treatment.[4] More recently, the FDA approved denosumab™ as treatment of postmenopausal osteoporosis. Longer duration of bisphosphonate therapy is associated with a higher risk of atypical femur fractures.[5] Combination therapy with teriparatide and denosumab appears to increase bone mineral density to a greater extent than either therapy alone in postmenopausal women at high risk for fracture.[5,6] There are several novel therapies under investigation for the treatment of osteoporosis, which are in various stages of development.[5,6] While antiresorptive agents prevent bone loss, anabolic agents are capable of increasing bone mass, improving bone quality and increasing bone strength.[4] In the United States, teriparatide is the only FDA-approved anabolic agent.[5,6] Because of the paucity of available anabolic agents for osteoporosis treatment, there is an urgent need to develop small molecule drugs to treat this disease that are non-toxic, cost-effective, and easy to administer.[1–3]

Although the development of pharmacological agents that stimulate bone formation is less advanced compared to antiresorptive therapies, several pathways are known to facilitate osteoblast function. These pathways include bone morphogenic proteins, transforming growth factor β, parathyroid hormone, insulin-like growth factor, fibroblast growth factor, and wingless-type MMTV integration site (WNT) signaling.[3] One of these pathways to our interest is the WNT pathway, which is implicated in a variety of developmental and regenerative processes.[7,8] The WNT family is comprised of 19 secreted proteins that regulate many aspects of cell growth, differentiation, function and death, including osteoblastogenesis and adipogenesis. Recent analysis of gene expression data has led to the identification of new targets of WNT signaling.[9] One such target is Notum Pectinacetylesterase, as NOTUM is a WNT-inactivating lipase that removes the palmitoleate essential for binding to Frizzled receptors[10,12] and inhibition of NOTUM stimulates endocortical bone formation.[13]

* 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.2016.01.038
0960-894X/

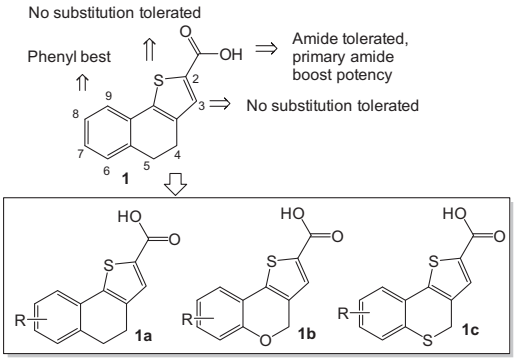


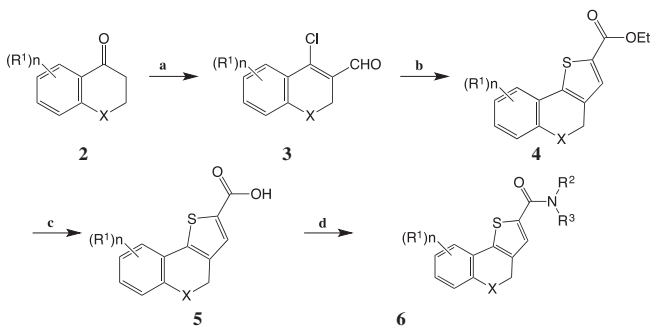
Figure 1. Lead compounds and lessons from hit-to-lead.

Herein we report a group of small molecules, namely thienochromenes, as potent inhibitors of Notum Pectinacetylesterase. Compound EC_{50} values were determined using a cell-based TCF/LEF CellSensor® assay having a β-lactamase reporter gene under control of the β-catenin/LEF/TCF response element where β-lactamase activity was measured using a FRET-based substrate (GeneBLAzer®, Invitrogen) technology.[11–13] Based on high throughput screening, we identified 4,5-dihydronaphtho[1,2-*b*]thiophene-2-carboxylic acid **1** as an interesting small molecule lead. Compound **1** had a mouse EC_{50} of 1060 nM and human EC_{50} of 361 nM, which offered a reasonable starting point for potency optimization. Structural modifications of lead compound **1** were explored with focus on the carboxyl terminus, cyclic core, and substitution on the aromatic rings.

The general observations in substitutions of the carboxylic acid were that secondary and tertiary amides were tolerated, while primary amides generally showed significant increases in potency. Position 1 would not tolerate replacement of the sulfur atom and no substitution for the sp^2-carbon on the 3-position was tolerated, requiring a thiophene for activity. Replacement of the phenyl ring with hetero-aromatics generally gave much lower potency (data not shown).

The synthesis of our derivatives was carried out following the general synthetic methods shown in Scheme 1. The appropriate commercially available or synthesized[13] ketones **2** were treated with phosphorus oxychloride in *N*,*N*-dimethylformamide under Vilsmeier–Haack conditions to generate the α,β-unsaturated β-chloroaldehydes **3**.[14–16] Subsequent treatment with ethyl 2-mercaptoacetate and sodium ethoxide effected cyclization to generate substituted thiophenes **4**. Saponification of the esters with aqueous base yielded a range of carboxylic acids **5**, which could be further functionalized to amides **6**.[13]

Conversion of acids **5** to amides **6** was generally tolerated, and primary amides stood out with the biggest improvement in



Scheme 1. Reagents and conditions: (a) $POCl_3$, DMF; (b) $HSCH_2CO_2Et$, NaOEt; (c) NaOH, THF/H_2O; (d) HATU, R^2NHR^3.

potency. We chose the primary amide derivatives to explore substitution on the central core, allowing us the best chance to observe subtle potency trends. Some representative analogs are summarized in Table 1. It is worth noting that while we were more concerned about modulation of activity at the human enzyme, potency at the mouse homolog was still a consideration since initial in vivo studies were planned in murine models. We anticipated some metabolic stability challenges, particularly at positions 4 and 5 of the dihydronaphtho[1,2-*b*]thiophene chemotype due to high oxidation potential of those carbons and chose to aggressively explore modifications at these points of the molecule. Addition of a methyl group at the 4-position (**8**) led to a slight impairment of potency, while 5-methyl substitution (**9**) had no impact. Compound **10** further clarified that steric modifications to the 5-position were well tolerated. Expansion of the cyclic core to a seven-membered ring (e.g., **11**, **12**) retained potency. ADME profiling of the amide derivatives revealed poor metabolic stability and fast clearance. However, the excellent potency of most of the primary amides did not translate to related carboxylic acids. For example, the related carboxylic acids of **11** and **12** were 100-fold less potent. In contrast, we observed that carboxylic acids generally had more acceptable ADME profiles, so we decided to focus our optimization efforts on carboxylic acids. Eventually we focused our efforts on the six-membered ring systems without the 4,5-substitutions.

Based on the preliminary SAR, our synthetic efforts mainly converged on three subseries **1a**, **1b**, and **1c** (Fig. 1) based on carbon, oxygen and sulfur at the 5-position. The three subtypes demonstrated that the phenyl ring could tolerate of a range of electron rich substituents. We wanted to probe the effect of a more electron deficient system on activity and identify functionality that could improve solubility and metabolic stability. The SAR of 4,5-dihydronaphtho[1,2-*b*]thiophene-2-carboxylic acids is summarized in Table 2. A preliminary survey of substituent position modifications on the phenyl ring afforded the highly potent 6-cyano compound (**13**). The combination of 6-cyano and 7-position substitutions demonstrated reasonable potency gains; 7-methoxy compound (**14**) improved hEC_{50} to 36 nM while 7-fluoro (**15**) and 7-methyl (**16**) compounds gave 3- to 4-fold improvements in hEC_{50}, respectively. Di-substituted compounds with halide substitutions at the 6-position provided some of the most potent compounds, in particular dichloro- and difluoro-combinations. 6,7-Difluoro substitution (**23**) gave a hEC_{50} of 17 nM, 6-chloro-7-fluoro compound **17** gave a hEC_{50} of 46 nM, whereas shifting the fluoro group to the 8-position (**18**) improved hEC_{50} to 13 nM. Subsequent replacement of the 8-F with 8-Cl (**19**) gave a hEC_{50} of 20 nM, but moving the chloro group to the 7-position (**20**) gave hEC_{50} of 12 nM. However, the most potent analogs arose from modulating the 8-position substitution of 6-fluoro compounds and 6-fluoro-8-methyl substitution (**24**) produced a hEC_{50} of 9 nM. The best potency was achieved with 6-F, 8-Me and 9-F tri-substitution (**25**), which had single-digit nanomolar mEC_{50} and hEC_{50}.

The SAR of 4*H*-thieno[3,2-*c*]chromene-2-carboxylic acids is summarized in Table 3. The unsubstituted 4*H*-thieno[3,2-*c*]chromene-2-carboxylic acid **26** had hEC_{50} of 1.36 μM, about three-fold less active than carbocyclic compound **1**. We postulated that the apparent decrease in activity might be due to the increased electron density in the phenyl ring and attempted to modulate the electronics by probing substitution using a small electron withdrawing group such as fluorine, a strategy which had proven effective in the dihydronaphtho-thiophene series. Preliminary SAR showed the 9-fluoro substitution (**27**) gave a two-fold potency increase to hEC_{50} of 706 nM, while 8-fluoro substitution (**28**) further improved the potency to hEC_{50} of 175 nM. Changing the halogen to chlorine at the 8-position (**29**) slightly increased the potency to 133 nM. Subsequent efforts showed that substitution on the 9-position generally reduced potency, so our main focus was on