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Orally available pyridinylpyrimidine derivatives as novel RANKL-induced osteoclastogenesis inhibitors

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ABSTRACT

An HTS campaign led to the identification of 4-pyrroldino-2-(pyridin-2-yl)pyrimidine compound **1** as an RANKL-induced osteoclastogenesis inhibitor. The compound **1** showed high clearance values in microsomes, however. Modification of the pyrrolidino group to a benzylamino group improved human microsomal stability with a slight loss of in vitro activity. Substitution at the *ortho* position of the benzyl group ameliorated in vitro activity, and further fluorination of the benzyl group improved microsomal stability in rodents. Representative members of this series, compounds **20** and **23,** exhibited efficacy in RANKL-induced osteopenic mice when administered orally at 0.3 mg/kg.

Osteoclasts are multinucleated cells derived from a monocyte lineage which express tartrate-resistant acid phosphatase (TRAP) on the cell surface as a differentiation marker, and play an essential role in bone resorption.[1] Osteoclasts and the bone forming cells, osteoblasts, cooperate in bone remodeling to maintain the mechanical competence of bone. Osteoblasts supply a critical factor, receptor activator of nuclear factor-κB (RANK) ligand (RANKL), which binds to its cellular acceptor, RANK, and triggers a signal transduction and subsequent gene expression. This in turn results in differentiation of the osteoclast precursor into TRAP-positive, multinucleated bone-resorptive cells.[2] Aging or diseases, including osteoporosis, bone metastasis and rheumatoid arthritis, cause excessive RANKL expression, which leads to abnormally increased bone resorption.[3,4]

Inhibition of osteoclastic activity is a primary therapeutic approach to bone loss.[1b] Bisphosphonates (BPs) are the major anti-resorptive therapy currently available.[5] These agents accumulate almost exclusively in skeletal sites and induce apoptosis in osteoclasts. The efficacy of BPs has been recognized, but gastrointestinal disorders are reported as common adverse effects. In addition, BP-related osteonecrosis of the jaw is a severe side effect which is difficult to treat.[6] Estrogen replacement therapy and selective estrogen receptor modulators (SERMs) also suppress bone resorption,[1b] but these therapies function only in the prevention and treatment of osteoporosis associated with estrogen deficiency in women, and estrogen replacement therapy increases the risk of cancer. These problems with current treatments emphasize the need for novel anti-resorptives.

The RANKL/RANK signaling pathway is a promising target for the development of effective osteoclastogenesis inhibitors. Denosumab, a humanized anti-RANKL antibody, is a new agent for the treatment of osteoporosis and other bone diseases.[4] In addition to biologics, which are administered parenterally, orally available small-molecule inhibitors might also be valuable in the treatment of bone loss, and several groups have in fact recently reported small-molecule inhibitors of RANKL-induced osteoclastogenesis.[7,8] Identification of potent small molecules of practical use has been challenging, however; efficacy through oral administration is not disclosed in most cases, and no clinical trial of small-molecule RANKL-induced osteoclastogenesis inhibitors has been reported.

Here, we report the modification of a high throughput screening (HTS) hit (**1**) which resulted in the discovery of a series of orally bioavailable anti-resorptive agents. An HTS campaign was performed using a RANKL-induced TRAP promoter-dependent reporter gene assay.[8] We successfully identified 4-pyrrolidino-2-(pyridin-2-yl)pyrimidine derivative **1** as an HTS hit with an IC_{50}

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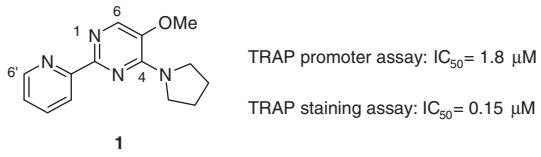


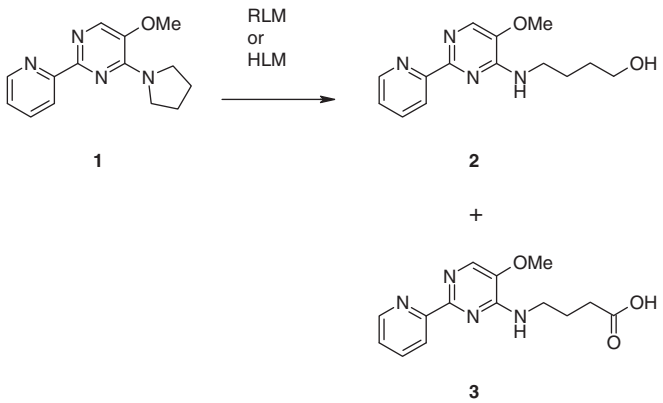
Figure 1. Structure and anti-osteoclastogenic activity of HTS hit **1**.

value of 1.8 μM in the promoter assay (Fig. 1). Inhibitory activity against RANKL-induced osteoclastogenesis was confirmed with an IC_{50} of 0.15 μM in a TRAP staining assay in RAW264 cells, an immortalized mouse macrophage-like cell line possessing the ability to differentiate into osteoclasts.[9] However, compound **1** showed high clearance values in liver microsomes of mouse, rat and human (abbreviated as MLM, RLM and HLM, respectively) in vitro. We therefore conducted a preliminary search for the structure–activity relationships (SAR) of in vitro activity against osteoclastogenesis, and also investigated metabolic pathways in microsomes. The following results were obtained:

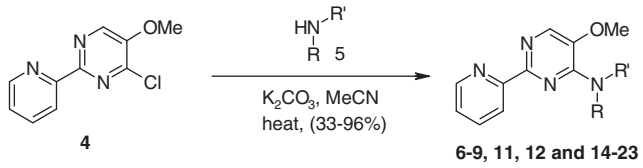
1. The 2-pyridinyl group on the pyrimidine ring was essential for inhibitory activity.
2. Substitution at either the 6-position of the pyrimidine ring or the 6′ position in the 2-pyridinyl group attenuated potency.
3. The pyrrolidine ring of **1** was identified as the main site of metabolism in both RLM or HLM in vitro by LC–MS/MS analysis (Scheme 1). One of the C–N bonds in the pyrrolidine ring was cleaved to generate two oxygenated metabolites **2** and **3**.[10]

On the basis of these preliminary results, we embarked on a modification of the pyrrolidine ring to improve both potency and metabolic stability.

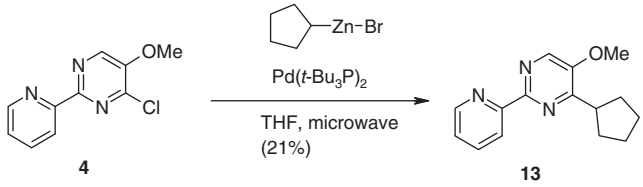
The general synthetic route for various 4-amino derivatives was efficient in only one step, as illustrated in Scheme 2. A commercially available chloropyrimidine **4** was reacted with amines **5** to afford the compounds **6–9, 11, 12** and **14–23** in moderate to high yields. The cyclopentyl compound **13** was prepared by Negishi coupling reaction of the chloropyrimidine **4** with cyclopentylzinc bromide under microwave irradiation as shown in Scheme 3.[11] The synthesized compounds were evaluated in vitro to investigate both their inhibitory activity against RANKL-induced osteoclastogenesis and microsomal stability, as shown in Table 1. The piperidine **6** and *N*-methyl-*N*-cyclopentylamine **9** showed equipotent activity with **1**, although clearance values in liver microsomes were increased. The azabicyclic compound **8** retained activity and stability, while conversion of the pyrrole group into azepane (**7**) decreased activity. These results suggested that replacement of the pyrrolidine



Scheme 1. Major metabolites of compound **1** in both RLM or HLM.

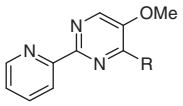


Scheme 2. Synthesis of compounds **6–9**, **11**, **12** and **14–23**.



Scheme 3. Synthesis of compound **13**.

Table 1
Potency in TRAP staining assay and microsomal stability of 4-amino/4-cyclopentyl pyrimidines[a]



Compd	R	TRAP staining[a] IC_{50} (M)	$Cl_{int,\ in\ vitro}$ (ml /min/kg)[b]		
			MLM	RLM	HLM
1	—N	0.15	>1000	987	458
6	—N	0.10	N.T.	>1000	657
7	—N	0.69	N.T.	N.T.	N.T.
8	—N	0.24	N.T.	734	512
9	—N(*c*-Pent)(Me)	0.18	N.T.	>1000	775
10	—N	>2	N.T.	N.T.	N.T.
11	—N	1.0	>1000	210	178
12	—N	1.4	>1000	395	310
13	*c*-Pent	1.8	N.T.	>1000	749
14	—NH—*c*-Pent	0.57	>1000	202	210
15	—NH—Ph	0.42	911	387	67

[a] Inhibitory activity of RANKL-induced osteoclastogenesis using RAW264 cells ($n \geqslant 3$).
[b] Average of two experiments. N.T.: Not tested.

ring with tertiary amino groups possessing simple aliphatic substituents might not improve metabolic stability sufficiently while retaining inhibitory activity. With the aim of blocking metabolism, introduction of electro-negative atoms and depletion of nitrogen were implemented. Disappointingly, embedding an oxygen atom in the ring as morpholine (**10**) resulted in the complete loss of activity. Fluorination of the pyrrolidine ring (**11**, **12**) and replacement of the nitrogen with a carbon (**13**) also reduced inhibitory activity, although the fluorination showed a tendency to improve