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Discovery and structure–activity relationship of thienopyridine derivatives as bone anabolic agents

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ABSTRACT

A cell-based assay was performed for the discovery of novel bone anabolic agents. Alkaline phosphatase (ALPase) activity of ST2 cells was utilized as an indicator of osteoblastic differentiation, and thienopyridine derivative **1** was identified as a hit compound. 3-Aminothieno[2,3-*b*]pyridine-2-carboxamide was confirmed to be a necessary core structure for the enhancement of ALPase activity, and then optimization of the C4-substituent on the thienopyridine ring was carried out. Introduction of cyclic amino groups to the C4-position of the thienopyridine ring improved the activity. Especially, *N*-phenyl-homopiperazine derivatives were found to be strong enhancers of ALPase among this new series. Furthermore, 3-amino-4-(4-phenyl-1,4-diazepan-1-yl)thieno[2,3-*b*]pyridine-2-carboxamide (**15k**) was orally administered to ovariectomized (OVX) rats over 6 weeks for evaluating the effects on areal bone mineral density (aBMD), and statistically significant improvements in aBMD were observed from the dosage of 10 mg/kg/day.

1. Introduction

Osteoporosis is a skeletal disorder characterized by low bone mass and deterioration of bone microarchitecture.[1] Under these pathological conditions, bones are more fragile and susceptible to fractures compared to healthy conditions.[2] It is estimated that over 200 million people worldwide have osteoporosis, and the number is growing with the increasing life expectancy.[3]

Bone is a dynamically remodeling tissue balanced by the coordinated actions of bone resorbing cells (osteoclasts) and bone forming cells (osteoblasts). In osteoporosis bone resorption exceeds bone formation resulting in bone loss. Agents for the treatment of osteoporosis are classified as either antiresorptive or anabolic.[4] Current therapeutic agents are mainly antiresorptive agents[5] such as bisphosphonates, calcitonins, estrogens, selective estrogen receptor modulators and the RANKL monoclonal antibody. On the other hand, recombinant human PTH is the only approved anabolic agent. PTH therapy has demonstrated that anabolic agents could increase bone mass to a greater degree than antiresorptive agents and also reduce fracture incidence.[6] However, the administration period of PTH is limited due to safety concerns.[6] Under these circumstances, the calcium-sensing receptor, the Wnt signaling pathway and β-adrenergic receptor have gained attention as molecular targets for developing bone anabolic agents.[7] However, mechanisms contributing to bone formation are complex and not yet completely understood. Therefore, we utilized a cell-based screening strategy instead of a target-based strategy. We believe that a cell-based screening, which detects differentiation of stromal cells to osteoblast cells, may provide compounds with a novel mechanism of action, and those compounds would lead to drug candidates.

For the screening, the stromal cell line ST2, derived from mouse bone marrow, was utilized, and alkaline phosphatase (ALPase) activity was detected as an indicator of differentiation to osteoblasts.[8] By a high-throughput screening of our corporate library, we identified 3-amino-4-(dimethylamino)thieno[2,3-*b*]pyridine-2-carboxamide (**1**)[9] as a hit compound with an EC_{200} = 0.17 μg/mL, a concentration to enhance ALPase activity to 200% of control (Fig. 1). Furthermore, hit compound **1** was evaluated with regard to calcification in rat bone marrow cells,[10] and found to induce the formation of mineralized nodules. These results indicated that

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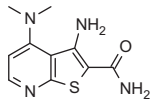

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Figure 1. Hit compound **1**.

compound **1** was a promising bone anabolic candidate and encouraged us to initiate chemical modification for potentiating the activity.

In this paper, we report the synthesis of thienopyridine derivatives and structure–activity relationships (SARs) with regard to enhancement of ALPase activity. In addition, the results of selected compounds **1** and **15k** evaluated in an in vivo animal osteoporosis model are described.

2. Chemistry

The synthetic routes for the compounds in Tables 1 and 2 are outlined in Schemes 1–3.

Scheme 1 describes the preparation of *N*-aryl-homopiperazine derivatives, which are employed as the C4-substituent of the thienopyridine ring. Appropriate halobenzenes **2** were coupled with *N*-protected homopiperazines **3** using transition metal catalysts,[11] or by nucleophilic aromatic substitution, and followed by cleavage of the protecting groups to give *N*-aryl-homopiperazines **5**. Benzamides **10a** and **10b** were prepared from 2,2,2,4′-tetrafluoroacetophenone (**6**).[12] Compound **6** was reacted with benzyl 1-homopiperazinecarboxylate to afford **7**. Compound **7** was treated with aqueous NaOH to give carboxylic acid **8**, and subsequent condensation with suitable amines, followed by deprotecting the benzyloxycarbonyl group furnished homopiperazines **10a** and **10b** in good yields.

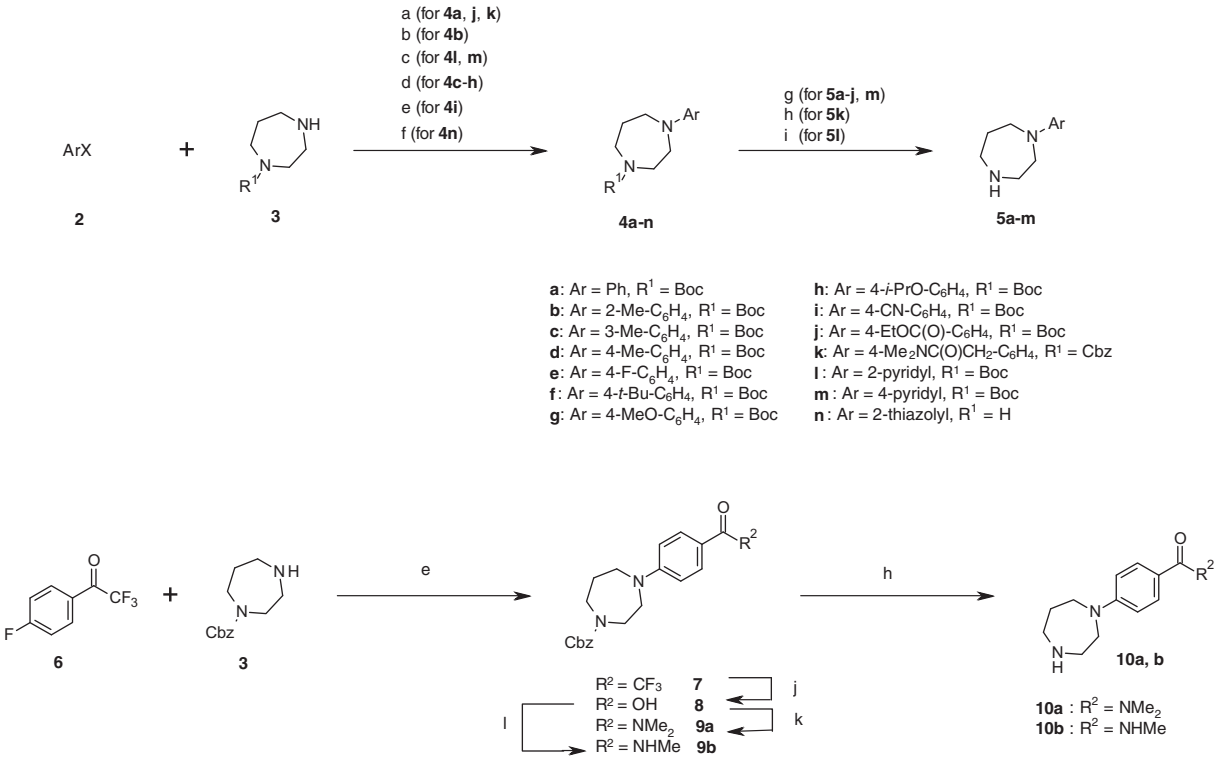
The synthesis of thienopyridine derivatives is shown in Scheme 2. Compound **11**[13] was treated with suitable amines to give enaminothioamides **12**. Condensation of **12** with *N*,*N*-dimethylformamide dimethyl acetal (DMF–DMA), and subsequent refluxing in aqueous NaOH afforded thiopyridones **14** via the intermediates **13**.[9] Cyclization to the thiopyridone ring was also performed in DMF or DMA under heating conditions without a base. Eventually, the resulting thiopyridones **14** were reacted with 2-chloroacetamide in the presence of bases to furnish thienopyridines **1** and **15**. Since the major product was 4-(dimethylamino)thiopyridone **14a** when 3-morpholinobutenethioamide **12g** was reacted with DMF–DMA, 4-(dimethoxymethyl)morpholine was used instead of DMF–DMA in the preparation of morpholine derivative **15g**.

The preparation of compounds **15aa**, **15ab** and **15ac** is shown in Scheme 3. Deprotection of the *tert*-butoxycarbonyl group of **15h** provided **15aa**. Saponification of ester **15t** furnished carboxylic acid **15ab**. Carboxamide **15ac** was prepared by the hydration of nitrile **15s**.

3. Results and discussion

The effects of the synthesized compounds on cellular ALPase activity, an indicator of differentiation to osteoblasts, were evaluated using the stromal cell line ST2, derived from mouse bone marrow. The results are shown in Tables 1 and 2, and the potency is expressed as EC_{200}, concentrations to enhance ALPase activity to 200% of control.

Initial chemical efforts were focused on the modification of the thienopyridine core of hit compound **1**. Replacement of the ring



Scheme 1. Reagents and conditions: (a) $Pd_2(dba)_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, NaO*t*-Bu, *t*-BuOH, 1,4-dioxane, 100 °C; (b) $Pd(OAc)_2$, 2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane, NaO*t*-Bu, toluene, 80 °C; (c) $Pd_2(dba)_3$, 1,3-bis-(2,6-diisopropylphenyl)-imidazolium chloride, KO*t*-Bu, 1,4-dioxane, 90 °C; (d) CuI, K_2CO_3, ethylene glycol, *i*-PrOH, 80 °C; (e) Et_3N, DMSO, 100 °C; (f) *n*-BuOH, reflux; (g) HCl, MeOH, 1,4-dioxane, rt; (h) H_2, Pd/C, EtOH, rt; (i) TFA, CH_2Cl_2, rt; (j) aq NaOH, DMF, 80 °C, 93%; (k) CDI, Me_2NH, THF, rt, 100%; (l) CDI, $MeNH_2$, THF, rt, 100%.