Bioorganic & Medicinal Chemistry Letters 20 (2010) 541–545

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and SAR of sulfonyl- and phosphoryl amidine compounds as anti-resorptive agents

Myung Yun Lee [a,d,†], Myung Hee Kim [b,†], Jinho Kim [c], Seok Hwan Kim [c], Bum Tae Kim [d], In Howa Jeong [a], Sukbok Chang [c], Seong Hwan Kim [b], Sung-Youn Chang [d,*]

[a] Department of Chemistry and Medical Chemistry, Yonsei University, Wonju 220-710, Republic of Korea
[b] Laboratory of Chemical Genomics, Pharmacology Research Center, Bio-Organic Science Division, Korea Research Institute of Chemical Technology, Sinseongno19, Yuseong-gu, Daejeon 305-600, Republic of Korea
[c] Department of Chemistry, Korea Advanced Institute of Science & Technology (KAIST), Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
[d] Medicinal Chemistry Research Center, Bio-Organic Science Division, Korea Research Institute of Chemical Technology, Sinseongno19, Yuseong-gu, Daejeon 305-600, Republic of Korea

ARTICLE INFO

Article history:
Received 23 September 2009
Revised 13 November 2009
Accepted 19 November 2009
Available online 26 November 2009

Keywords:
Sulfonyl amidine
Phosphoryl amidine
Osteoporosis
Osteoclast
Anti-resorptive agent

ABSTRACT

Sulfonyl amidines (**1**) and phosphoryl amidines (**2**), which were efficiently synthesized via a Cu-catalyzed one pot reaction, showed potent anti-bone resorptive activity in vitro. Structure activity relationship studies led to the identification of numerous osteoclast differentiation inhibitors.

A practical synthesis of amidines would be very helpful for medicinal chemists because amidines are found in many bioactive natural products[1] and identified as important pharmacophores.[2] Recently, efficient one-pot syntheses of amidines using Cu-catalyzed three component coupling reactions have been published.[3,4] The reaction was proposed to proceed via the formation of a ketenimine intermediate, which is generated in situ by the Cu-catalyzed cycloaddition of sulfonyl- or phosphoryl azides with terminal alkynes followed by the ring-cleavage of the resultant triazoles.[5] It is believed that the excellent reactivity of the ketenimine intermediate allows for the amazingly diverse reactions with pronucleophiles such as amines, alcohols, water, and imidazole. In the present study, we synthesized various sulfonyl- and phosphoryl amidines according to the reported procedure and evaluated their biological activity.

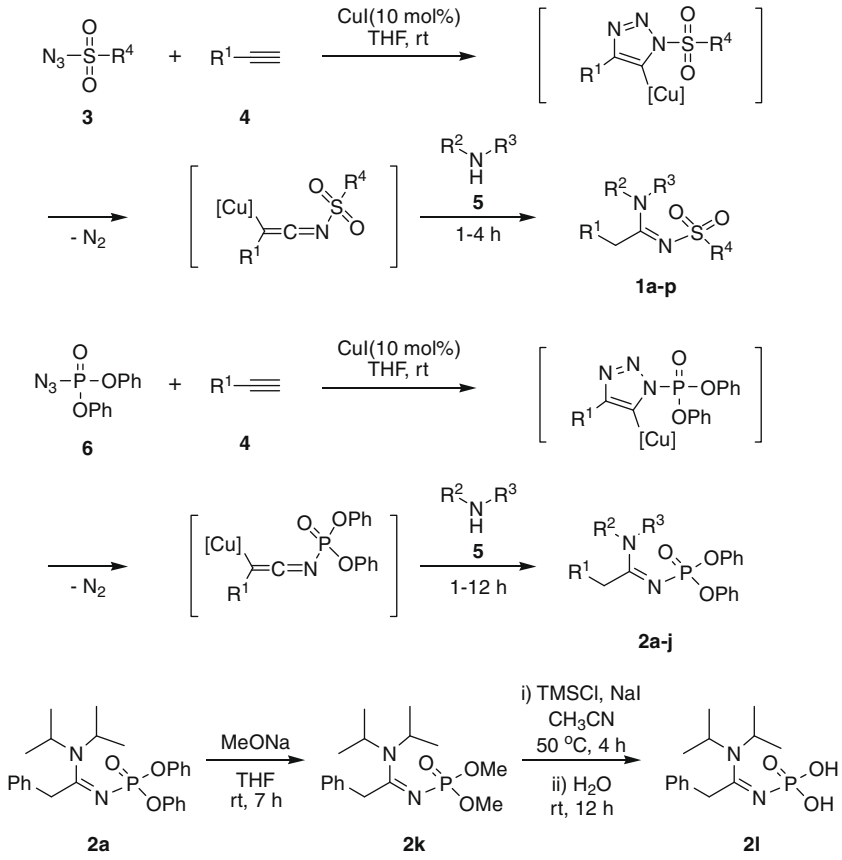
Sulfonyl amidines (**1**) were synthesized from alkyne, amine, and sulfonyl azide in the presence of CuI catalyst at rt in 66–99% yield.[6] In addition, phosphoryl amidines (**2**) were synthesized from phosphoryl azide instead of sulfonyl azide in 38–82% yield.[7] *N*-Dimethoxy phosphoryl amidine (**2k**), which had been previously obtained in relatively low yield with Cu-catalyzed one-pot reaction, was synthesized in 88% yield using substitution of the phenoxy group with methoxide.[4] Subsequent hydrolysis of the *N*-dimethoxy phosphoryl amidine (**2k**) with TMSCl in the presence of NaI gave the amidine containing phosphonic acid (**2l**) in 72% yield[8] (Scheme 1).

Based on the methodology in Scheme 1, we prepared 64 sulfonyl amidine derivatives (**1**) and screened them in vitro for their anti-cancer, anti-obesity, bone forming, and anti-bone resorptive activities (Data are not shown). From the screening, amidine **1a** showed anti-resorptive activity with tartrate-resistant acid phosphatase (TRAP, a biomarker of osteoclastogenesis; IC_{50} value of 16.7 μM in RAW264.7 cells). In contrast, these same derivatives did not exhibit efficient anti-cancer, anti-obesity, and bone forming activities at a concentration of 10 μM.[9] These results suggested that amidine derivatives could be developed as selective anti-resorptive osteoporosis drugs.

Bone is constantly remodeled through osteoblast-mediated formation of bone matrix and osteoclast-mediated bone resorption in order to maintain skeletal strength and integrity. However, an imbalance in bone remodeling caused by increased bone resorption over bone formation leads to the reduction of bone mineral density that is a major cause of several bone disorders such as osteoporosis.[10] Since the loss of bone mass can increase the risk

* Corresponding author. Tel.: +82 42 860 7161; fax: +82 42 861 0307.
E-mail address: sychang@krict.re.kr (S.-Y. Chang).
† These authors contributed equally to this Letter.

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.11.104



Scheme 1. Syntheses of sulfonyl- and phosphoryl amidines.

of fractures, which can lead to serious problems including substantial skeletal deformity, pain, increased mortality and severe economic burden,[11] the prevention or treatment of loss of bone mass is an important means of improving the quality of life. Anti-resorptive agents have been considered as the therapeutic mainstay for osteoporosis, but there is a need for new anti-resorptive agents without the side effects such as bisphosphonate-related osteonecrosis of the jaw.[12] Therefore, in this report, amidines were investigated as potential alternative inhibitors of osteoclast differentiation.

To identify more efficient anti-resorptive amidine derivatives, a diverse range of sulfonyl- and phosphoryl amidine derivatives were synthesized as outlined in Scheme 1 and the structure-activity relationships (SAR) were investigated (Table 1 and Table 2). The activity was evaluated using a TRAP activity assay and the data are presented as % of control sample which received no added test compounds.[13] In cases of the most promising candidates, IC_{50} values were also calculated, with compounds tested over the concentration range of 0.3–30 μM.

The initial test compound **1a** containing *n*-butyl (R^1), diisopropylamino (NR^2R^3), and *p*-tolyl (R^4) groups showed potent anti-osteoclastogenic activity with 3% of control activity at 60 μM. To investigate the effect of the R^1 group on the activity, the diisopropylamino and *p*-tolyl groups were held fixed, and the R^1 group was varied. The bulkier *t*-butyl analogue (**1b**) showed similar activity at 60 μM, but introducing polar functionalities such as hydroxyl (**1c**) or chloro groups (**1d**) reversed the inhibitory activity. The phenyl derivative **1e** did not display a significant improvement in inhibition, regardless of the presence of the electron donating dimethylamino (**1f**) or electron withdrawing trifluoromethyl (**1g**) or nitro (**1h**) substituents at the *para* position. Moreover, pyridyl substitution (**1i**) resulted in an inactive analogue. Therefore, aliphatic chains without polar functionality appear to be the best substituents for R^1.

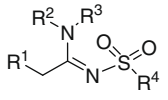
Interestingly, substitution of the NR^2R^3 group of inactive derivative **1d** resulted in improved inhibitory activity. Substitution of the diisopropylamino NR^2R^3 group with pyrrolidine (**1j**), piperidine (**1k**), and cis-2,6-dimethylpiperidine (**1l**) gave an improvement in activity (88%, 57%, and 5% TRAP activity of control at 10 μM, respectively) as compared to **1d** itself (105% of control at 60 μM). Refinement of amidine compound **1l** with *n*-butyl R^1 group (**1m**) instead of a 3-chloropropyl group resulted in similar activities (TRAP IC_{50} values of 1.8 and 3.3 μM for **1l** and **1m**, respectively). Changing the *p*-tolyl R^4 group of sulfonyl amidine **1e** into *p*-nitrophenyl (**1n**), 2-pyridyl (**1o**), and methyl (**1p**) groups was not effective. Thus, in the sulfonyl amidine series, **1l** and **1m** were the most potent inhibitors of osteoclast differentiation with IC_{50} values of <5 μM.

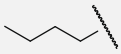
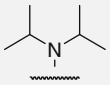
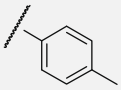
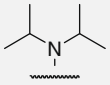
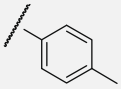
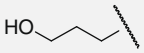
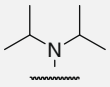
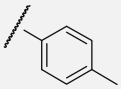
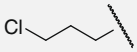
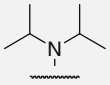
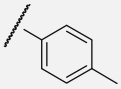
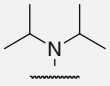
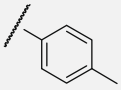
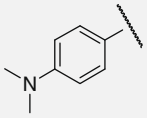
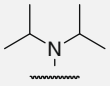
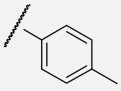
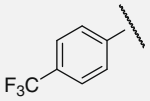
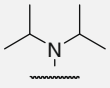
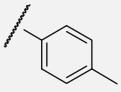
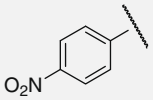
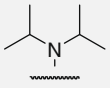
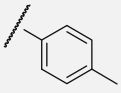
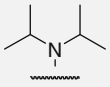
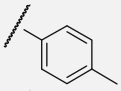
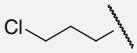
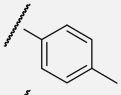
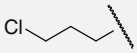
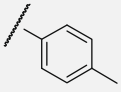
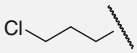
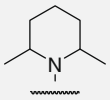
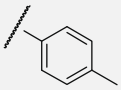
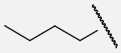
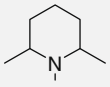
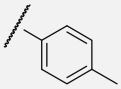
In addition to sulfonyl amidines, phosphoryl amidine derivatives (**2a**–**2l**) were synthesized according to Scheme 1 and SAR of the series were also investigated (Table 2).

In general, the phosphoryl amidines were more potent than the sulfonyl amidines, showing anti-resorptive activity at 10 μM whereas most of the sulfonyl amidines required concentrations as high as 60 μM to exhibit a similar activity (Table 1).

The phosphoryl amidine compound **2a** containing phenyl R^1, diisopropylamino NR^2R^3, and phenyl R^5 showed potent activity at 10 μM, with activity ~8-fold more potent than the control.[14] With the exception of **2b**, modification of the R^1 group of phosphoryl amidine **2a**, while holding the diisopropylamino NR^2R^3 and the phenyl R^5 groups constant, resulted in similar activity against osteoclast differentiation. Introducing the electron-donating

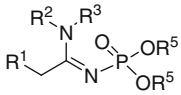
Table 1
SAR studies of sulfonyl amidines

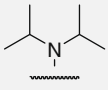
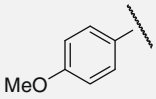
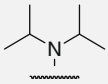
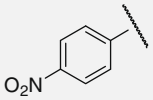
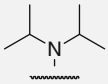
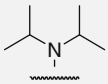
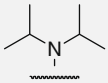
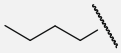
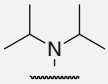
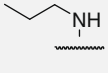
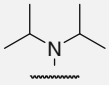
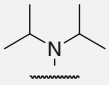


Compds	R^1	R^2R^3N	R^4	Trap activity[a] (% of control)
1a				3
1b				10
1c				59
1d				105
1e				98
1f				114
1g				105
1h				105
1i				107
1j				88[b]
1k				57[b]
1l				5[b]
1m				0
1n				124
1o				84
1p				98

[a] TRAP activity at 60 μM of amidine compounds except **1j**, **1k**, and **1l**.
[b] TRAP activity at 10 μM of amidine compounds.

Table 2
SAR studies of phosphoryl amidines



Compds	R^1	NR^2R^3	R^5	Trap activity[a] (% of control)	IC_{50} (μM)
2a			Ph	13	2.0
2b			Ph	67	21.2
2c			Ph	14	2.6
2d			Ph	14	nd
2e			Ph	0.4	2.4
2f			Ph	10	7.3
2g			Ph	24	4.2
2h			Ph	51	10.6
2i			Ph	27	4.8
2j			Ph	28	6.5
2k			Me	90	nd
2l			H	107	nd

[a] TRAP activity at 10 μM of amidine compounds.

methoxy group at the *para*-position of the phenyl R^1 group (**2b**) modestly inhibited the activity (67% of control), but substitution of the methoxy group by the electron withdrawing NO_2 group (**2c**) restored the inhibitory activity. The phosphoryl amidine derivatives containing a heteroaromatic ring such as pyridyl (**2d**), a cycloalkane such as cyclohexyl (**2e**), and an alkyl chain such as *n*-butyl (**2f**) groups afforded potent activities comparable to **2a**.

The phenyl R^1 and R^5 groups were then held constant, allowing the SAR investigation for optimization of the NR^2R^3. Substitution of diisopropylamine with piperidine (**2g**), *t*-butylamine (**2i**) and *n*-propylamine (**2j**) was successfully carried out, yielding several potent inhibitors with decreased anti-resorptive activity to ~25% of control values, but replacement by a morpholine (**2h**) at this position resulted in a twofold decrease in activity to 51% of control.

While keeping phenyl (R^1) and diisopropylamino (NR^2R^3) groups constant, substitution of the diphenyl phosphonate (R^5 = Ph) group with either dimethyl phosphonate (**2k**) or phosphonic acid (**2l**) resulted in an inactive inhibitor implying that the diphenyl phosphonate functionality (R^5 = Ph) is essential for activity. From the phosphoryl amidine series, 7 compounds showed potent activity with IC_{50} values of <10 μM.

In conclusion, a novel series of sulfonyl- and phosphoryl amidines were synthesized efficiently via a Cu-catalyzed one pot reaction. SAR studies revealed that phosphoryl amidines are more potent than sulfonyl amidines, and the diphenyl phosphonate functionality of phosphoryl amidines is critical for anti-resorptive activity. Target protein identification with an affinity probe and in vivo experiments with ovariectomized mice are currently underway.

Acknowledgments

This work was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health, Welfare & Family Affairs, Republic of Korea (A084242) and Korea Research Foundation Grant (KRF-2005-015-C00248). This research was also supported by the KOSEF (R01-2007-000-10618-0) and the KRICT (SI-0905).

References and notes

1. Greenhill, J. V.; Lue, P. *Prog. Med. Chem.* **1993**, *30*, 203.
2. Boyd, G. V. In *Chemistry of Amidines and Imidates*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1991; Vol. 2, Chapter 8.3,.
3. (a) Bae, I.; Han, H.; Chang, S. *J. Am. Chem. Soc.* **2005**, *127*, 2038; (b) Cho, S. H.; Yoo, E. J.; Bae, I.; Chang, S. *J. Am. Chem. Soc.* **2005**, *127*, 16046; (c) Yoo, E. J.; Bae, I.; Cho, S. H.; Han, H.; Chang, S. *Org. Lett.* **2006**, *8*, 1347; (d) Chang, S.; Lee, M.; Jung, D. Y.; Yoo, E. J.; Cho, S. H.; Han, S. K. *J. Am. Chem. Soc.* **2006**, *128*, 12366; (e) Cho, S. H.; Chang, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 1897; (f) Cho, S. H.; Hwang, S. J.; Chang, S. *Org. Synth.* **2008**, *85*, 131; (g) Kim, J. Y.; Kim, S. H.; Chang, S. *Tetrahedron Lett.* **2008**, *49*, 1745; (h) Cho, S. H.; Chang, S. *Angew. Chem., Int. Ed.* **2008**, *47*, 2836; (i) Yoo, E. J.; Chang, S. *Org. Lett.* **2008**, *10*, 1163; (j) Yoo, E. J.; Park, S. H.; Chang, S. *Org. Lett.* **2009**, *12*, 1155.
4. Kim, S. H.; Jung, D. Y.; Chang, S. *J. Org. Chem.* **2007**, *72*, 9769.
5. (a) Cassidy, M. P.; Raushel, J.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 3154; (b) Yoo, E. J.; Ahlquist, M.; Kim, S. H.; Bae, I.; Fokin, V. V.; Sharpless, K. B.; Chang, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 1730; (c) Yoo, E. J.; Ahlquist, M.; Bae, I.; Fokin, V. V.; Sharpless, K. B.; Chang, S. *J. Org. Chem.* **2008**, *73*, 5520.
6. *General procedure for the preparation of sulfonyl amidines* (**1**): To a stirred mixture of alkyne (0.5 mmol, 1.0 equiv), azide (0.6 mmol, 1.2 equiv), and CuI (0.05 mmol, 10 mol %) in THF (1 mL) was slowly added amine nucleophile (0.6 mmol, 1.2 equiv) at room temperature under an N_2 atmosphere. After the reaction was completed, which was monitored with TLC, the reaction mixture was diluted by adding CH_2Cl_2 (2 mL) and aqueous NH_4Cl solution (3 mL). The mixture was stirred for an additional 30 min and two layers were separated. After the aqueous layer was extracted with CH_2Cl_2 (3 mL × 3), the combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatograph with an appropriate eluting solvent system.
7. *General procedure for the preparation of phosphoryl amidines* (**2**): For the synthesis of phosphoryl amidines(**2**), amine nucleophile (0.75 mmol, 1.5 equiv) was added to the mixture of azide (0.5 mmol, 1.0 equiv), alkyne (1 mmol, 2.0 equiv), and CuI(0.05 mmol, 10 mol %) in THF (1 mL) at room temperature under N_2 atmosphere. After the reaction was completed, the same procedure was followed as for the sulfonyl amidines.
8. Morita, T.; Okamoto, Y.; Sakurai, H. *Tetrahedron Lett.* **1978**, *28*, 2523.
9. Those activities were evaluated in breast cancer cells by cell viability assay, adipocytes derived 3T3-L1 cells by oil red S stained fat level, and MC3T3-E1 subclone 4 cells by measuring the deposited calcium level, respectively.
10. Boyle, W. J.; Simonet, W. S.; Lacey, D. L. *Nature* **2003**, *423*, 337.
11. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy. JAMA **2001**, *285*, 785.
12. Dannemann, C.; Gratz, K. W.; Riener, M. O.; Zwahlen, R. A. *Bone* **2007**, *40*, 828.
13. TRAP activity assay was performed as the following; mouse monocyte RAW264.7 cells purchased from the American Type Culture Collection (VA, USA) were suspended in α-MEM with 10% FBS and 100 ng/ml of receptor activator of nuclear factor-κB ligand, and plated in 96-well plates at a density of 1×10^3 cells/well. After 24 h, amidine compounds (**1** or **2**) were treated into cells. On differentiation day 4, cells were fixed with 10% formalin for 10 min and 95% ethanol for 1 min, and then 100 μl of citrate buffer (50 mM, pH 4.6) containing 10 mM of sodium tartrate and 5 mM of *p*-nitrophenylphosphate (Sigma) was added to the fixed cells-containing wells. After incubation for 1 h, the enzyme reaction mixtures in the wells were transferred into new plates containing an equal volume of 0.1 N NaOH. Absorbance was measured at 410 nm with a Wallac EnVision HTS microplate reader (PerkinElmer, Finland). The experiment was performed in triplicate.
14. No cytotoxicity of **1l** or **2a** was observed up to 10 μM (data not shown).