Bioorganic & Medicinal Chemistry Letters 19 (2009) 6337–6339

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification of iminooxothiazolidines as secreted frizzled related protein-1 inhibitors

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ARTICLE INFO

Article history:
Received 25 June 2009
Revised 21 September 2009
Accepted 22 September 2009
Available online 25 September 2009

Keyword:
sFRP1 inhibitor osteoporosis

ABSTRACT

Secreted frizzled related protein-1 (sFRP-1) inhibitors have the potential to be used for the treatment of osteoporosis or other bone related disorders, since the level of sFRP-1 affects osteoblast apoptosis and proliferation. From high throughput screening, we have identified a class of iminooxothiazolidines as sFRP-1 inhibitors. Structure–activity relationships were established for various regions of the scaffold along with the biochemical characterization of this class to probe selectivity, binding and ex vivo activity.

Wnts are a large family of growth factors that mediate fundamental biological processes like embryogenesis, organogenesis and tumorigenesis.[1–4] Recent research indicates that they also play an important role in bone formation, and the Wnt pathway components are being pursued as potential drug targets for osteoporosis and other metabolic bone diseases.[5–9] Secreted frizzled related proteins, sFRPs, are Wnt antagonists.[10,11] Overexpression of sFRP-1 in human osteoblasts in vitro accelerates apoptosis, and deletion of sFRP-1 in mice leads to osteoblast proliferation resulting in bone formation.[12,13] Moreover, sFRP-1 knockout animals do not have significant extra skeletal defects.[14] Thus, an sFRP-1 inhibitor has the potential to be a novel osteogenic agent, since it would prolong the life of osteoblasts and therefore allow these cells to produce more bone.

A cell-based high throughput screening (HTS) assay for sFRP-1 inhibitors was therefore developed. This assay involves transient transfection of U2OS human osteosarcoma cells with human sFRP-1, Wnt-3 and a TCF-luciferase reporter gene that measures activation of the canonical Wnt-pathway.[15,16] Iminooxothiazolidine **1** was identified as a hit from this screening effort (Fig. 1).[17]

Compound **1** showed an EC_{50} of 7.2 μM in the U2OS TCF-luciferase assay and was selective against sFRP-2 and -5. It was active across species (human, mouse and rat sFRP-1) and was active with Wnt-3. With fluorescence spectroscopic techniques a K_D of 0.72 μM was determined from changes in the endogenous tryptophan fluorescence of the protein upon inhibitor binding at the emission and excitation wavelengths of 340 and 295 nm, respectively. From these experiments the stoichiometry of binding was found to be 1:1, indicative of specific binding. The favorable biochemical profile displayed by this hit prompted us to conduct a follow up study around this scaffold. A structure–activity relationship was established for this chemotype and preliminary ex vivo data were obtained.

The compounds for exploration of SAR were synthesized by following synthetic routes analogous to those in Schemes 1–3. The iminooxothiazolidine derivatives were synthesized by a modified literature procedure.[18] Ring opening of maleic anhydride **3** with 2-amino-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester **2** resulted in the intermediate 2-(3-carboxy-acryloylamino)-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester **4**, which was further reacted with thiourea to give the 2-[2-(2-imino-4-oxo-thiazolidin-5-yl)-acetylamino]-4,5-dimethyl-thiophene-3-carboxylic acid ethyl ester **5** (Scheme 1). Following the same procedure and using different aminothiophenes or anilines with thiourea or

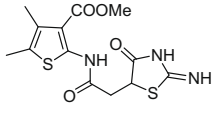

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Figure 1. High throughput screen hit identified.

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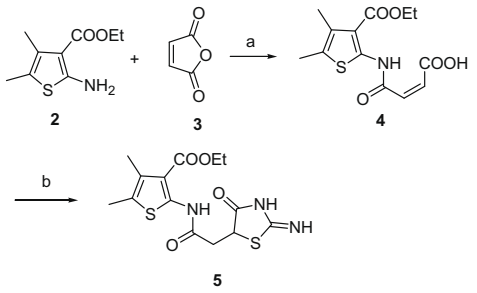
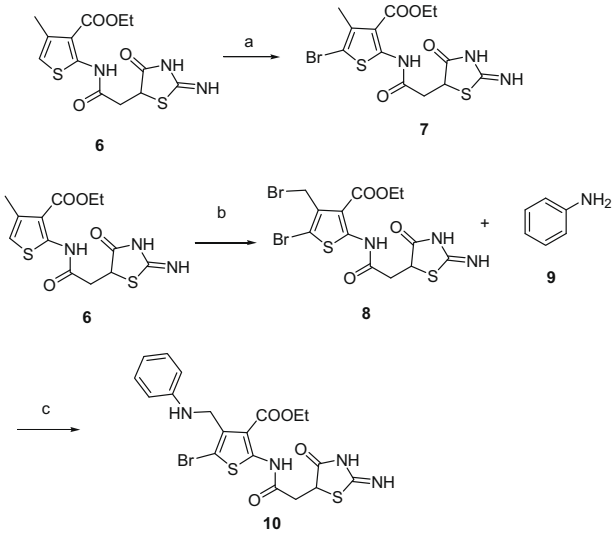
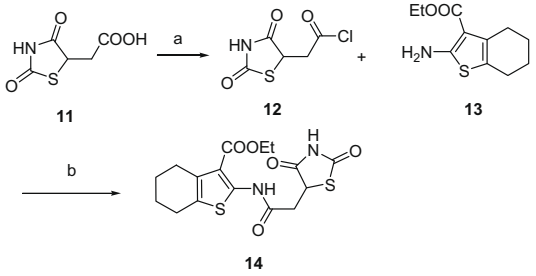
doi:10.1016/j.bmcl.2009.09.085



Scheme 1. Reagents and conditions: (a) Et_2O, rt 10 h, 72%; (b) HOBT, EDCI, thiourea, DMF, 60 °C, 12 h, 52%.



Scheme 2. Reagents and conditions: (a) NBS, chloroform, 0 °C 1 h, 67%; (b) bromine, chloroform, 6 h, 70%; (c) K_2CO_3, DMF, rt, 12 h, 25%.



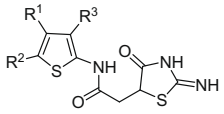
Scheme 3. Reagents and conditions: (a) oxalyl chloride, DMF (cat.), CH_2Cl_2 30 min; (b) pyridine, 1 h. 30%.

substituted thioureas, another series of iminooxothiazolidine analogs were synthesized for SAR studies. Compounds **7** and **8** were made by treating compound **6** with different brominating agents such as NBS or bromine. Compound **8** was further reacted with aniline to form the 5-bromo-2-[2-(2-imino-4-oxo-thiazolidin-5-yl)-acetylamino]-4-phenylamino methyl-thiophene-3-carboxylic acid ethyl ester **10** (Scheme 2). The 2,4-dioxo-thiazolidine **14** was synthesized from commercial acid **11** through a typical amide formation procedure (Scheme 3).

The hit to lead optimization was focused on exploring various regions of the hit molecule **1** to obtain preliminary SAR. As shown in Table 1, a methyl or ethyl ester at the 3-position of the thiophene scaffold provides the best activity of the functional groups surveyed. Replacing the ester with an amide (compound **24**) or nitrile (compound **25**) decreases the activity in the case of a bicyclic scaffold. From the analogs synthesized it was clear that a range of substituents like methyl (compounds **7** and **15**), phenyl (compound **20**) or anilinomethyl (compound **10**) were tolerated in the 4-position of the thiophene ring. However the 5-position showed less tolerance for steric bulk. The 5-phenyl analogs **18** and **19** were much less active compared to compounds with smaller substituents like methyl or ethyl at the 5-position (compounds **5** and **16**). However, a bromo group in this position, as in analogs **7** and **10**, was well accommodated indicating that the region was sensitive to the stereoelectronics of the substituent. Replacing the thiophene scaffold with cycloalkyl fused thiophenes (compounds **21**–**23**) was also tolerated.

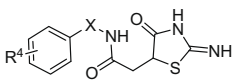
Scaffold hopping was attempted next using substituted phenyl (compounds **26–30**) and benzyl derivatives (compound **31**) in the place of the thiophene, as shown in Table 2. Overall this effort did not improve the activity for the series. Variations on the iminooxothiazolidine region were also explored and the results are shown in Table 3. Thus, the unsubstituted iminooxothiazolidine was found to be critical for activity. Replacing it with a 2,4-dioxo-thiazolidine (**14**) or alkylation of the iminooxothiazolidine nitrogen (**32–34**) decreases activity significantly, irrespective of the scaffold employed.

Table 1
sFRP-1 inhibitor activity of iminooxothiazolidine derivatives



Compd	R^1	R^2	R^3	Fold induction (@15 μM)
1	Me	Me	COOMe	1.9
5	Me	Me	COOEt	2.3
7	Me	Br	COOEt	2.6
15	Me	H	COOEt	2.0
16	H	Et	COOEt	1.7
17	H	H	COOMe	1.2
18	H	Ph	COOMe	0.9
19	Me	Ph	COOEt	0.8
20	Ph	Me	COOEt	1.8
10	CH_2NHPh	Br	COOEt	2.5
21	$-CH_2CH_2CH_2-$		COOEt	1.5
22	$-CH_2(CH_2)_3CH_2-$		COOEt	1.9
23	$-CH_2(CH_2)_2CH_2-$		COOEt	1.6
24	$CH_2(CH_2)_2CH_2-$		$CONH_2$	1.1
25	$CH_2(CH_2)_2CH_2-$		CN	1.2

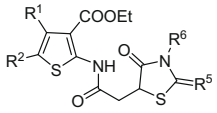
Table 2
SAR for central ring scaffold replacement



Compd	R^4	X	Fold induction (@30 μM)
26	3-Cl	Bond	1.7
27	3-COOEt	Bond	1.5
28	2-COOEt	Bond	1.1[a]
29	H	Bond	1.1
30	2,-3 DiCl	Bond	1.0
31	3-Cl	CH_2	1.0

[a] Fold induction was determined at 15 μM inhibitor concentration.

Table 3
SAR for iminooxothiazolidine replacement



Compd	R^1	R^2	R^5	R^6	Fold induction (@15 μM)
5	Me	Me	NH	H	2.3
32	Me	Me	NMe	H	1.2
33	Me	Me	NEt	H	1.2
34	Me	Me	NMe	Me	1.2
23	$-CH_2(CH_2)_2CH_2-$		NH	H	1.6
14	$CH_2(CH_2)_2CH_2-$		O	H	1.0

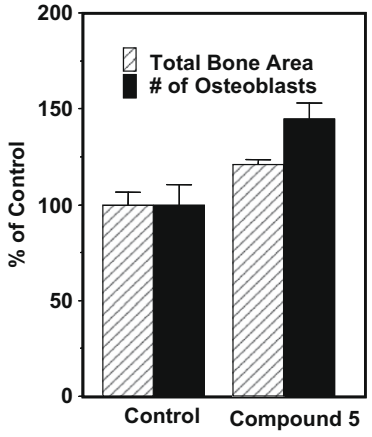


Figure 2. Evaluation of compound **5** in ex vivo mouse calvaria assay at 15 μM. Each bar gives the mean ± SE for 5 calvaria per treatment.

Further characterization of compound **5** in an ex vivo calvarial bone formation assay showed that the compound increased total bone area and the number of osteoblasts as shown in Figure 2.[19]

In summary, a series of iminooxothiazolidines identified by high throughput screening was investigated to understand the SAR requirements of the scaffold. These compounds represent a novel sFRP-1 inhibitor scaffold with an interesting in vitro and ex vivo profile.

Acknowledgements

The authors thank Dr. John Ellingboe for his support of this work. We also thank Discovery Analytical Chemistry group for analytical data.

References and notes

1. Wodarz, A.; Nusse, R. *Annu. Rev. Cell Dev. Biol.* **1998**, *14*, 59.
2. Miller, J. R. *Genome Biol.* **2002**, *3*, 3001.
3. Moon, R. T.; Kohn, A. D.; De Ferrari, G. V.; Kaykas, A. *Nat. Rev. Genet.* **2004**, *5*, 691.
4. Logan, C. Y.; Nusse, R. *Annu. Rev. Cell Dev. Biol.* **2004**, *20*, 781.
5. Westendorf, J. J.; Kahler, R. A.; Schroeder, T. M. *Gene* **2004**, *341*, 19.
6. Rawadi, G.; Roman-Roman, S. *Expert Opin. Ther. Targets* **2005**, *9*, 1063.
7. Krishnan, V.; Bryant, H. U.; Macdougald, O. A. *J. Clin. Invest.* **2006**, *16*, 1202.
8. Gopalsamy, A.; Shi, M.; Stauffer, B.; Bahat, R.; Billiard, J.; Ponce-de-Leon, H.; Seestaller-Wehr, L.; Fukayama, S.; Mangine, A.; Moran, R.; Krishnamurthy, G.; Bodine, P. *J. Med. Chem.* **2008**, *51*, 7670.
9. Moore, W. J.; Kern, J. C.; Bhat, R.; Commons, T. J.; Fukayama, S.; Goljer, I.; Krishnamurthy, G.; Magolda, R. L.; Nogle, L.; Pitts, K.; Stauffer, B.; Trybulski, E. J.; Welmaker, G. S.; Wilson, M.; Bodine, P. V. N. *J. Med. Chem.* **2009**, *52*, 105.
10. Rattner, A.; Hsieh, J. C.; Smallwood, P. M.; Gilbert, D. J.; Copeland, N. G.; Jenkins, N. A.; Nathans, J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2859.
11. Chong, J. M.; Uren, A.; Rubin, J. S.; Speicher, D. W. *J. Biol. Chem.* **2002**, *277*, 5134.
12. Bodine, P. V.; Zhao, W.; Kharode, Y. P.; Bex, F. J.; Lambert, A. J.; Goad, M. B.; Gaur, T.; Stein, G. S.; Lian, J. B.; Komm, B. S. *Mol. Endocrinol.* **2004**, *18*, 1222.
13. Bodine, P. V. N.; Billiard, J.; Moran, R. A.; Ponce-de-Leon, H.; McLarney, S.; Mangine, A.; Scrimo, M. J.; Bhat, R. A.; Stauffer, B.; Green, J.; Stein, G. S.; Lian, J. B.; Komm, B. S. *J. Cell Biochem.* **2005**, *96*, 1212.
14. Gaur, T.; Rich, L.; Lengner, C. J.; Hussain, S.; Trevant, B.; Ayers, D.; Stein, J. L.; Bodine, P. V. N.; Komm, B. S.; Stein, G. S.; Lian, J. B. *J. Cell Phys.* **2006**, *208*, 87.
15. Bhat, R. A.; Stauffer, B.; Komm, B. S.; Bodine, P. V. N. *Protein Expr. Purif.* **2004**, *37*, 327.
16. Bhat, R. A.; Stauffer, B.; Komm, B. S.; Bodine, P. V. N. *J. Cell Biochem.* **2007**, *103*, 1519.
17. Gopalsamy, A.; Shi, M. (Wyeth, John, and Brother Ltd, USA). PCT Int. Appl. WO 2006124887.
18. Balasubramaniyan, V.; Balasubramaniyan, P.; Wani, M. J. *Indian J. Chem., Sec. B: Org. Chem. Incld. Med. Chem.* **1990**, *29B*, 1092.
19. Bodine, P.; Stauffer, B.; Ponce-de-Leon, H.; Bhat, R.; Mangine, A.; Seestaller-Wehr, L.; Moran, R.; Billiard, J.; Fukayama, S.; Komm, B.; Pitts, K.; Krishnamurthy, G.; Gopalsamy, A.; Shi, M.; Kern, J.; Commons, T.; Woodworth, R.; Wilson, M.; Welmaker, G.; Trybulski, E.; Moore, W. *Bone* **2009**, *44*, 1063.