Identification of Diarylsulfone Sulfonamides as Secreted Frizzled Related Protein-1 (sFRP-1) Inhibitors

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Abstract: Inhibitor of secreted frizzled related protein-1 (sFRP-1) would be a novel potential osteogenic agent, since loss of sFRP-1 affects osteoblast proliferation, differentiation, and activity, resulting in improved bone mineral density, quality, and strength. We have identified small molecule diarylsulfone sulfonamide derivatives as sFRP-1 inhibitors. Structure−activity relationship generated for various regions of the scaffold was utilized to improve the biochemical profile, resulting in the identification of potent selective analogues, such as **16** with desirable pharmaceutical profile.

Wnts are secreted glycoproteins that mediate fundamental biological processes like embryogenesis, organogenesis, and tumorigenesis.[1] Many extracelluar and intracellular proteins control Wnt signaling. Among the extracelluar regulators are a variety of secreted proteins[2] that include Wnt inhibitory factor, secreted frizzled-related proteins (sFRPs),[3] Cerberus, and dickkopfs (DKKs).[a,4] Inhibitors like sFRPs that bind Wnts or frizzled receptors have the ability to blunt all Wnt-activated pathways, whereas DKKs only suppress the canonical pathway.

A sFRP was isolated from human osteoblast cells and identified as sFRP-1 (also known as SARP-2).[5] It is a 35 kDa protein consisting of 313 amino acids. This secreted protein contains two distinct structural domains, a netrin domain and a CRD domain, the latter of which is homologous to the frizzled receptor. sFRP-1 is thought to interact with Wnt via the CRD domain in a competitive manner with the frizzled receptor; thus, sFRP-1 is an antagonist of the Wnt signaling pathway. Overexpression of sFRP-1 in human osteoblasts in vitro accelerates programmed cell death, while deletion of sFRP-1 in mice leads to decreased osteoblast apoptosis and increased trabecular bone formation.[6] Loss of sFRP-1 also affects osteoblast proliferation, differentiation, and activity and improves bone mineral density, quality, and strength. Moreover, $sFRP^{-/-}$ animals do not have significant extra skeletal defects.[7] Thus, an inhibitor of sFRP-1 could have osteogenic potential, since it would prolong osteoblast life and allow these cells to produce more bone. Treatment with sFRP-1 inhibitor could increase bone mass and decrease the risk of osteoporotic fractures.

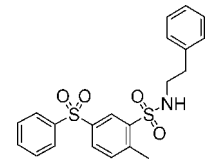

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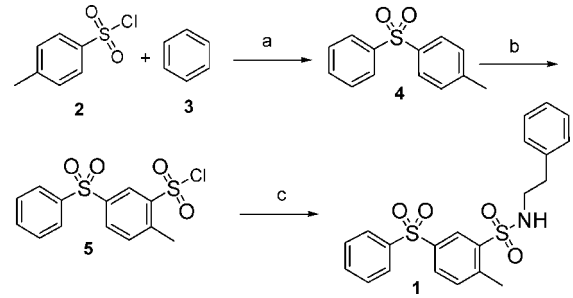
Figure 1. SFRP-1 inhibitor hit identified from HTS.

Scheme 1[a]



[a] Reagents: (a) $AlCl_3$, RT, overnight, 92%; (b) (1) $ClSO_3H$, neat, 50 °C, 16 h, 90%; (c) Phenethyl amine, Et_3N, CH_2Cl_2, 37 °C, 16 h, 82%.

A cell-based high-throughput screening (HTS) assay was developed in U2OS human osteosarcoma cells that were transfected with human sFRP-1, Wnt-3, and a TCF-luciferase reporter gene that measures activation of the canonical Wnt pathway.[8] One of the hits identified from this effort is the diphenylsulfone sulfonamide[9] **1** (Figure 1).

Compound **1** showed an EC_{50} of 3.9 μM in the cell based functional assay and was selective for sFRP-1 vs -2, -3, -4, and -5. It was active across species (human, mouse, and rat sFRP-1) and was active with Wnt-1 and Wnt-3. With fluorescence spectroscopy techniques, K_D of 0.35 μM was determined from the 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 a 1:1 ratio indicative of specific binding. The compound also suppressed osteocytic apoptosis induced by sFRP-1. The favorable biochemical profile exhibited by this hit prompted us to undertake further follow-up study. Herein, we describe our optimization efforts on this hit to obtain the structure−activity relationship and to improve pharmaceutical properties like aqueous solubility and microsomal stability.

Compounds required for establishment of structure−activity relationship were prepared as shown in Scheme 1 starting from appropriately substituted phenylsulfonyl chlorides **2**. Friedel−Crafts sulfonylation of benzene **3** afforded the required diphenylsulfone intermediate **4**. The sulfone **4** underwent regioselective chlorosulfonylation to give the advanced intermediate sulfonyl chloride **5**. Reaction of **5** with various amines of interest afforded the final target sulfonamides. The reactions were carried out in parallel, and the products were purified by preparatory HPLC and characterized to ensure purity and integrity.

The hit to lead optimization was initiated by obtaining preliminary SAR for various regions of the molecule. As shown in Table 1, introducing a small substituent like methyl or fluoro to ring A of the diphenylsulfone scaffold was well tolerated (**6**

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[a] Abbreviations: sFRP-1, secreted frizzled related protein-1; DKKs, dickkopfs; SARP, secreted apoptosis related protein; CRD domain, cysteine-rich domain; HTS, high throughput screen.

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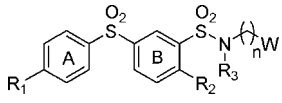
Table 1. sFRP-1 inhibitor activity of diphenylsulfone derivatives



Comp	R_1	R_2	R_3	n	W	Fold Induction[a]
1	H	Me	H	2	Phenyl	2.5[b]
6	Me	Me	H	2	Phenyl	2.2[b]
7	F	Me	H	2	Phenyl	2.7[b]
8	H	H	H	2	Phenyl	<1.0[2]
9	H	Et	H	2	Phenyl	3.3[b]
10	H	OMe	H	2	Phenyl	1.5[b]
11	Cl	Me	H	0	Phenyl	1.0[c]
12	H	Me	H	1	Phenyl	<1.0[c]
13	H	Me	H	3	Phenyl	1.3[b]
14	H	Me	Me	2	Phenyl	<1.0[b]
15	H	Et	H	C (Figure 2)	3.3[b]	
16	H	Et	H	A (Figure 2)	4.2[b]	
17	H	Et	H	G (Figure 2)	3.0[b]	

[a] Fold induction was determined by adding. [b] 15 μM of inhibitor (or). [c] 30 μM of inhibitor. The luciferase signal observed in absence of inhibitor was set at 1. Fold induction was measured in quadruplicate.

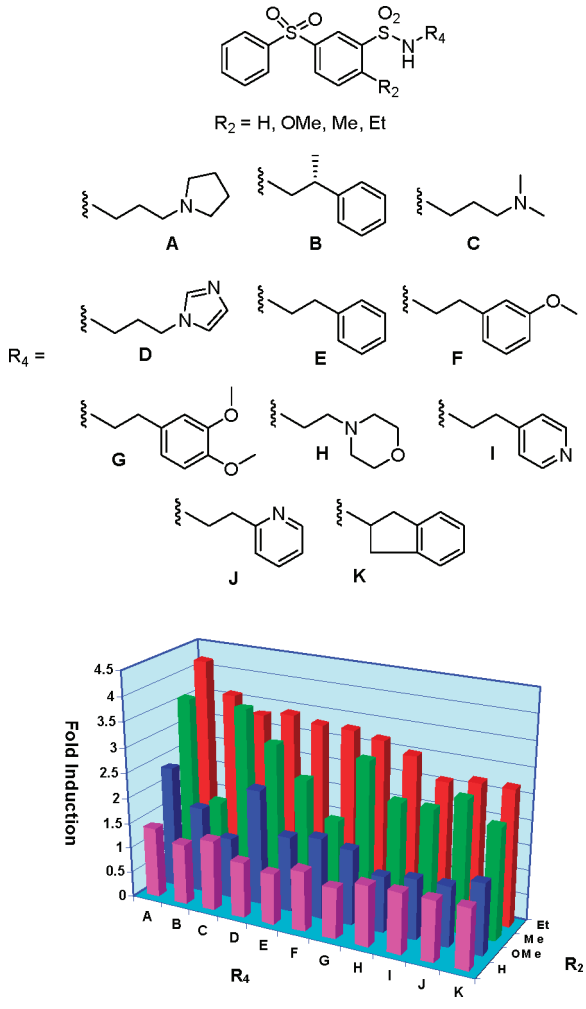


Figure 2. Structure activity relationship for R_2 and R_4 substituents of diphenyl sulfone sulfonamides. Fold Induction determined using 15 μM of the inhibitors when R_4 = Et, Me, OMe and 30 μM of the inhibitors when R_4 = H.

and **7**). However, in ring B, a substituent adjacent to the sulfonamide group was necessary and removal of the methyl group rendered **8** less active. Probably the conformational change brought about by a substituent in that postion is critical. Among the substitutents explored the ethyl analogue **9** was more active than the electron releasing methoxy analogue **10**. SAR for the linker region indicated that the sulfonamide NH is critical, since the N-methylated analogue **14** was found to be less active. Changing the phenethyl moiety to benzyl (**12**) or anilide (**11**) decreased the activity significantly. Extending the linker to phenylpropyl (**13**) was not advantageous as well.

However, the aromatic ring region of the phenethyl group was found to be very flexible, and a variety of groups were accommodated. Hence, this region was probed extensively. Basic heterocycles were introduced as water solubilizing groups in an effort to improve the pharmaceutical properties of the molecule. As seen in the Figure 2, the SAR preference observed for the R_2 substituents (i.e., Et > Me > OMe > H) using the phenethyl group was valid for the other R_4 modifications as well, using groups **A**−**K**. Observed SAR preference within the groups **A**−**K** was minimal; however, the tolerance of group **B** is noteworthy because it shows that substitution off the ethyl linker is accommodated. However, a ring constrained group at R_4 like **K** did not provide any improvement over the phenethyl moiety in the lead.

Some of the modifications carried out during this optimization process resulted in improved pharmaceutical properties like aqueous solubility and microsomal stability in analogues like **15** (R_2 = Et; R_4 = C) and **16** (R_2 = Et; R_4 = A) compared to the HTS hit **1** as shown in Table 2.

Table 2. Pharmaceutical Properties of sFRP-1 inhibitor diphenylsulfone derivatives

Properties	**1**	15	16	17
Cell-based TCF-reporter functional assay EC_{50} (μM)	3.88	1.27	9	6.97
Aq solubility @ pH7.4 (μg/mL)	1	26	>100	1
Rat microsomal stability ($t_{1/2}$, min)	5	12	16	1
In Vitro microsomal stability-Human ($t_{1/2}$, min)	-	26.7	-	-
Cyp 3A4 (% Inhibition @ 3 μM)	70	43	78	96
Cyp 2D6 (% Inhibition @ 3 μM)	12	9	25	11
Cyp 2C9 (% Inhibition @ 3 μM)	41	14	25	84

To further establish that this class of sFRP-1 inhibitors can indeed act as anabolic agents, **17** (R_2 = Et; R_4 = G) was evaluated in ex vivo mouse calvaria assay (Figure 3).[10] Treatment of calvaria with **17** at 5 μM resulted in a 50% increase in total bone area compared to the vehicle control. Significant increase in the number of osteoblasts was also observed.

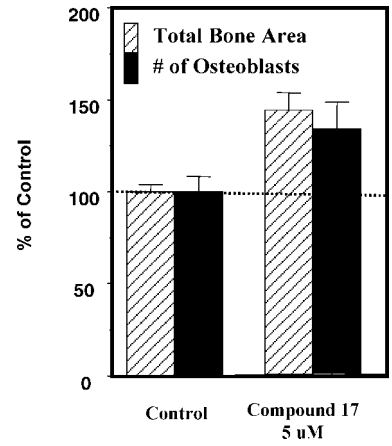


Figure 3. Evaluation of compound **17** in ex-vivo mouse calvaria assay at 5 μM. Each bar gives the Mean ± SE for 5 calvaria per treatment.

In summary, we have identified diphenylsulfone sulfonamide derivatives as sFRP-1 inhibitors. The hit to lead optimization afforded SAR for various regions of the molecule, resulting in inhibitors with improved physical properties. A representative compound from this class induced significant increase in total bone area in a murine calvarial organ culture assay.

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Supporting Information Available: Experimental details and spectral data for the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Wodarz, A.; Nusse, R. Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **1998**, *14*, 59–88. (b) Miller, J. R. The Wnts. *Genome Biol.* **2002**, *3*, 3001. (c) Moon, R. T.; Kohn, A. D.; De Ferrari, G. V.; Kaykas, A. WNT and beta-catenin signalling: diseases and therapies. *Nat. Rev. Genet.* **2004**, *5*, 691–701. (d) Logan, C. Y.; Nusse, R. The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* **2004**, *20*, 781–810.

(2) Kawano, Y.; Kypta, R. Secreted antagonists of the Wnt signalling pathway. *J. Cell Sci.* **2003**, *116* (13), 2627–2634.

(3) Melkonyan, H. S.; Chang, W. C.; Shapiro, J. P.; Mahadevappa, M.; Fitzpatrick, P. A.; Kiefer, M. C.; Tomei, L. D.; Umansky, S. R. SARPs: a family of secreted apoptosis-related proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13636–13641.

(4) (a) Morvan, F.; Boulukos, K.; Clement-Lacroix, P.; Roman-Roman, S.; Suc-Royer, I.; Vayssiere, B. Deletion of a single allele of the Dkk1 gene leads to an increase in bone formation and bone mass. *J. Bone Miner. Res.* **2006**, *21*, 934–945. (b) Li, X.; Ominsky, M. S.; Niu, Q.-T.; Sun, N.; Daugherty, B.; D'Agostin, D. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. *J. Bone Miner. Res.* **2008**, *23*, 860–869.

(5) (a) Rattner, A.; Hsieh, J. C.; Smallwood, P. M.; Gilbert, D. J.; Copeland, N. G.; Jenkins, N. A.; Nathans, J. A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2859–2863. (b) Chong, J. M.; Uren, A.; Rubin, J. S.; Speicher, D. W. Disulfide bond assignments of secreted frizzled-related protein-1 provide insights about frizzled homology and netrin modules. *J. Biol. Chem.* **2002**, *277*, 5134–5144.

(6) (a) 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. The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. *Mol. Endocrinol.* **2004**, *18*, 1222–1237. (b) 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. The Wnt antagonist secreted frizzled-related protein-1 controls osteoblast and osteocyte apoptosis. *J. Cell. Biochem.* **2005**, *96*, 1212–1230.

(7) 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. Secreted frizzled-related protein-1 regulates Wnt signaling for BMP2 induced chondrocyte differentiation. *J. Cell. Physiol.* **2006**, *208*, 87–96.

(8) (a) Bhat, R. A.; Stauffer, B.; Komm, B. S.; Bodine, P. V. Regulated expression of sFRP-1 protein by the GeneSwitch system. *Protein Expression Purif.* **2004**, *37*, 327–335. (b) Bhat, R. A.; Stauffer, B.; Komm, B. S.; Bodine, P. V. N. Structure−function analysis of secreted frizzled-related protein-1 for its Wnt antagonist function. *J. Cell. Biochem.* **2007**, *103*, 1519–1528.

(9) Gopalsamy, A.; Moore, W. J.; Kern, J. C.; Molinari, A. J.; Shi, M.; Welmaker, G. S.; Wilson, M. A.; Krishnamurthy, G.; Commons, T. J.; Webb, M. B.; Woodworth, R. P. Preparation of Diarylsulfone Sulfonamides and Their Use as Secreted Frizzled Related Protein-1 Modulators for Bone Disorders Such as Osteoporosis. U.S. 2006/0276464, 2006.

(10) (a) Mundy, G.; Garrett, R.; Harris, S.; Chan, J.; Chen, D.; Rossini, G.; Boyce, B.; Zhao, M.; Gutierrez, G. Stimulation of bone formation in vitro and in rodents by statins. *Science* **1999**, *286*, 1946–1949. (b) Traianedes, K.; Dallas, M. R.; Garrett, I. R.; Mundy, G. R.; Bonewald, L. F. 5-Lipoxygenase metabolites inhibit bone formation in vitro. *Endocrinology* **1998**, *139*, 3178–3184.

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