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(1-(4-(Naphthalen-2-yl)pyrimidin-2-yl)piperidin-4-yl)methanamine: A Wingless β-Catenin Agonist That Increases Bone Formation Rate

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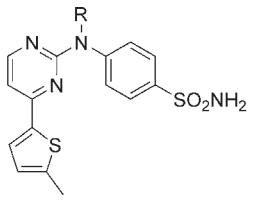
Abstract: A high-throughput screening campaign to discover small molecule leads for the treatment of bone disorders concluded with the discovery of a compound with a 2-aminopyrimidine template that targeted the Wnt β-catenin cellular messaging system. Hit-to-lead in vitro optimization for target activity and molecular properties led to the discovery of (1-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperidin-4-yl)methanamine (**5**, WAY-262611). Compound **5** has excellent pharmacokinetic properties and showed a dose dependent increase in the trabecular bone formation rate in ovariectomized rats following oral administration.

The canonical Wnt[a] β-catenin cellular messaging system controls several physiological events including bone homeostasis.[1] Bone forming osteoblasts express the proteins LRP5 and Fzd on the surface membrane. These behave as coreceptors for the soluble peptide agonist Wnt-3a. Once stimulated with Wnt-3a, internal concentrations of free β-catenin rise and enter the nucleus and recruit T-cell factor (TCF). Transcriptional events follow and result in the production of additional anabolic gene products. An additional soluble extracellular protein, Dkk-1, antagonizes this process by simultaneously binding to the cell surface receptors Kr2 and LRP5, effectively inhibiting Wnt-3a binding to LRP5. In addition, the Kr2/LRP5/Dkk-1 complex undergoes endocytosis to remove LRP5 from the cell membrane, thereby nullifying its function.[2]

Reports have described a familial gain-of-function mutation on the LRP5 receptor that results in bones of normal size and shape but with significantly increased density.[3] The mutation occurs at a single position on the LRP5 receptor, G171V, and results in a protein with substantially less affinity for Dkk-1 while affinity for Wnt-3a remains essentially unchanged.



1 R = H, TCF-luciferase EC_{50} = 7.0 μM, GSK-3β IC_{50} = 0.20 μM
2 R = CH_3, TCF-luciferase EC_{50} = 12.0 μM, GSK-3β IC_{50} = 63 μM

Figure 1. Structures, Dkk-1 mediated TCF-luciferase activity, and GSK-3β inhibition of **1** and **2**.

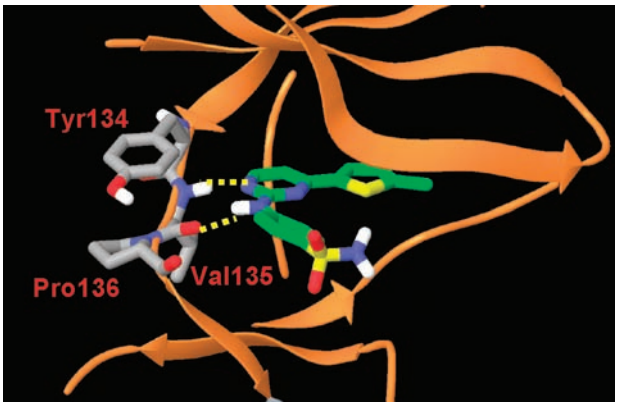


Figure 2. Docked structure of **1** in the active site of GSK-3β Yellow dotted lines indicate H-bond interactions with residues in the hinge region (see refs 9 and 10 for docking/structure details).

Hence, clinically relevant data indicate the important role of Dkk-1 in attenuating the Wnt β-catenin anabolic process and suggest that pharmacologic inhibition of Dkk-1 may lead to bones of greater strength for the treatment of osteoporosis and other diseases of low bone mass or quality.[4]

To identify small molecules that inhibit the action of Dkk-1, a high-throughput assay was conducted with the corporate collection using an osteosarcoma cell line transfected with Wnt-3a and Dkk-1. In addition, a TCF-luciferase response element and a renilla standard were transfected for light readouts of active compounds and control readout, respectively.[5] The 2-aminopyrimidine phenylsulfonamide **1** was identified as a weak agonist in this assay (EC_{50} = 7.0 μM). An *N*-methyl analogue, **2**, also potentiated the TCF-luciferase response in this assay at roughly the same potency (Figure 1). Resynthesized compounds **1** and **2** showed similar TCF-luciferase activity in HTS and benchtop formats.

The 2-aminopyrimidine motif is common to numerous kinase inhibitors.[6] Inhibition of the glycogen synthase kinase-3β (GSK-3β), an enzyme involved in β-catenin phosphorylation, would provide a false positive signal in the TCF-luciferase assay.[7] Therefore, compound evaluation in a GSK-3β enzyme assay was studied side by side with target evaluation.[8] Moderately potent enzyme inhibition was achieved with **1** (IC_{50} = 0.20 μM). To understand the key ligand features that are responsible for this activity, **1** was docked[9] in the active site of previously disclosed X-ray structure of GSK-3β.[10] As shown in Figure 2, the docked structure shows that ligand recognition is achieved by critical hydrogen bond interactions within the hinge region. The model also suggested

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[a] Abbreviations: Wnt, wingless; LRP5, low-density lipoprotein receptor-related protein 5; Fzd, frizzled; TCF, T-cell factor; Dkk-1, Dickkopf-1; Kr2, Kremen-2; GSK-3β, glycogen synthase kinase 3β; OVX, ovariectomized rat; BFR, bone formation rate; qd, once per day; hPTH, human parathyroid hormone; KO, knockout mouse; wt, wild type mouse.

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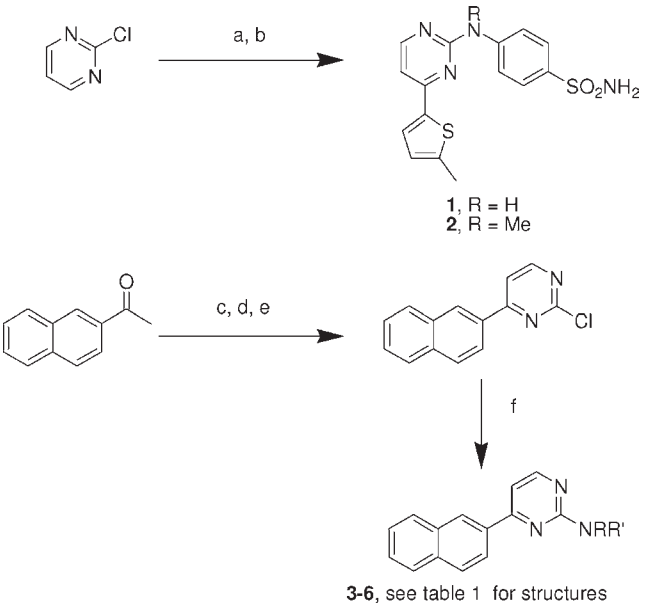
Scheme 1. Preparation of Compounds[a]



[a] Conditions: (a) 2-methylthiophene, *n*-BuLi, then DDQ (80%); (b) sulfanilamide or *N*′-methylsulfanilimide, toluenesulfonic acid, 1,4-dioxane, 95 °C (80−88%); (c) DMF−DMA, 85 °C (100%); (d) urea, NaOEt, EtOH, ref (54%); (e) $POCl_3$, ref (84%); (f) RR′NH, DMSO, 60 °C (69−70%).

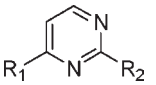
that alkylation of the 2-amino group to remove the H-bond donor would diminish enzyme activity. This was the case with **2** (see Figure 1). These experiments suggest that **2** potentiates the TCF response through Dkk-1 inhibition and not through a mechanism related to kinase inhibition. Hence, a structure−activity relationship (SAR) study centered on **2** was established.

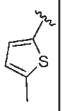
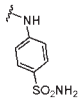
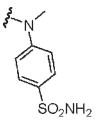
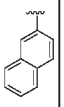
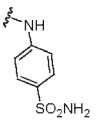
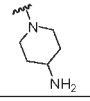
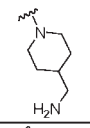
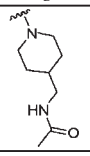
Compounds were synthesized according to the routes shown in Scheme 1. 4-(Thiophenyl)pyrimidines **1** and **2** were prepared by the method of Bursavich.[11] 4-(Naphthyl)pyrimidines **3**−**6** were obtained from the corresponding 2-naphthylmethyl ketone which was reacted with dimethylformamide dimethylacetal to provide the dimethylvinylogous amide.[12] Reaction of this product with urea under basic conditions gave the pyrimidinone sodium salt which was converted to the 2-chloropyrimidine with phosphorus oxychloride. Final products were obtained when the chloride was displaced with secondary amines.

Attempts to improve primary assay potency and reduce potential metabolic activity led to replacement of the methylthiophene in **1** with a 2-naphthyl function (e.g., **3**). This structural change also led to enhanced GSK-3β inhibiton and substantially reduced solubility. It was assumed that replacement of the sulfanilamide functionality with a fully reduced cyclic structure appended with distal H-bond donors would retain TCF-luciferase activity, reduce GSK-3β activity via full alkylation of the exocyclic nitrogen, improve solubility by interfering with the π-stacking characteristics of the phenyl group, and maintain the cLogP between 3 and 4. The 4-aminopiperidine group satisfied these requirements and, in fact, led to an active molecule, **4**, with high solubility and low GSK-3β activity. Additional standard SAR studies showed the methylamine **5** to have the most potent activity in the primary assay, low kinase inhibition potential, and high solubility (Table 1).

In vitro pharmaceutical properties[13] of **5** indicated it would be an excellent candidate for in vivo pharmacokinetic evaluation in ovariectomized (OVX) rats (PAMPA membrane

Table 1. TCF-Luciferase, GSK-3β, and Solubility Data for Compounds **1**−**6**



No.	R_1	R_2	TCF-Luci EC_{50} (μM)[a, b, c]	GSK-3β IC_{50} (μM)[c,e]	Solubility at pH = 7.4 (μg/mL)[d]
1			7.8	0.20	8
2	"		9.8	65	15
3			1.0	0.31	< 0.1
4	"		1.0	> 50	> 100
5	"		0.63	>100	65
6	"		1.9	>10	1

[a] Efficacy for all six compounds ranged from 2.0 to 6.0 relative signal increase compared to DMSO alone. [b] Values represent the average of at least two experiments done in triplicate. [c] SD valuess are within ±25% of the EC_{50}/IC_{50}. [d] See ref 8 for assay details. [e] See ref 13 for assay details.

permeability, $P_e = 5.9 \times 10^{-6}$ cm/s; rat liver microsome stability, $t_{1/2} > 30$ min at 1.0 μM). Hence, **5** was administered to OVX rats (single dose iv and po). Pharmacokinetic parameters and plasma drug concentration−time curves are shown in Table 2 and Figure 3, respectively.

The PK profile indicated that **5** was a candidate for in vivo pharmacological evaluation with once a day dosing. Hence, OVX rats[14] were treated orally with **5** (po, vehicle = 0.5% methylcellulose/2% Tween-80, qd, 28 days) at four doses. Trabecular bone formation rate (BFR) in the tibia was established in all dose groups at the end of the in-life portion of the study. A clear dose response and activity as low as 0.3 mg/kg/day were observed (Figure 4).

To confirm activity via the Wnt pathway, the calvariae of wild type (wt) and Dkk-1 knockout (KO) mice were treated with **5** once a day for 7 days (DMSO solution, sc injection).[15] The KO animals were not expected to respond because of the inherent inability to inhibit a missing target protein, while wild

Table 2. Single Dose Pharmacokinetic Parameters for Compound **5** in OVX Rats

parameter	compound **5** po admin	compound **5** iv admin
vehicle	2% MC/0.5% T80[a]	DMSO
dose (mg/kg)	10	2.0
plasma $t_{1/2}$ (h)	5.6	8.2
T_{max} (h)	4.7	
C_{max} (ng/mL)	277	
$AUC_{0-\infty}$ (ng·h/mL)	3990	1029
F (%)	78	
Cl_p ((mL/min)/kg)		32
V_{ss} (L/kg)		22

[a] MC = methylcellulose. T80 = Tween-80.

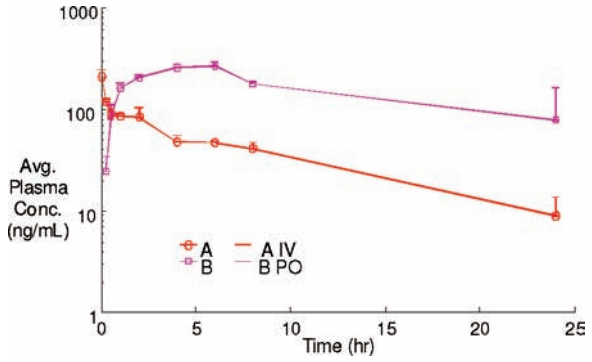


Figure 3. Plasma concentration as a function of time following a single dose of **5** in OVX rats.

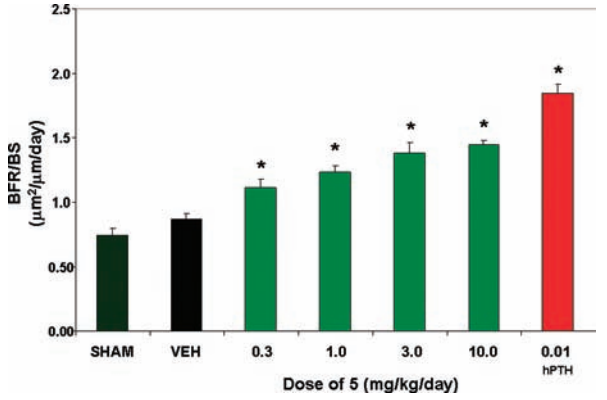


Figure 4. Increase in trabecular bone formation rate (BFR) in the tibia of OVX rats following once a day oral administration of **5** for 28 days (vehicle = 0.5% methylcellulose/2% Tween-80). hPTH (=human parathyroid hormone) (positive control) was administered sc.

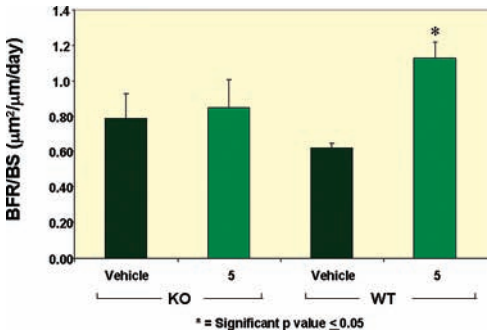


Figure 5. BFR in the calvaria of wild type (WT) mice compared to Dkk-1 knockout (KO) mice after 7 day treatment with **5**: (*) $p < 0.05$.

type animals with fully expressed Dkk-1 were expected to show a pharmacological response. Calvariae from wt mice treated with **5** showed statistically increased BFR, while similarly treated KO animals were no different from control (Figure 5). This indicates that **5** is acting via the Wnt β-catenin pathway and most likely through inhibition of Dkk-1.

In conclusion, 2-aminopyrimidines that potentiate the Wnt β-catenin cellular signaling pathway were discovered via high-throughput screening. Lead optimization led to a compound with enhanced in vitro activity. Compound **5** was shown to possess excellent pharmaceutical and PK properties, and the compound was able to enhance the bone formation rate in OVX rats following oral adminstration.

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

References

(1) (a) Logan, C. Y.; Nusse, R. The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* **2004**, *20*, 781–810. (b) Moon, R. T.; Kohn, A. D.; De Ferrari, G. V.; Kaykas, A. Wnt and β-catenin signaling: diseases and therapies. *Nat. Rev. Genet.* **2004**, *5*, 689–699.

(2) (a) Shahnazari, M.; Yao, W.; Corr, M.; Lane, N. E. Targeting the Wnt signaling pathway to augment bone formation. *Curr. Osteoporosis Rep.* **2008**, *6*, 142–148. (b) Kubota, T.; Michigami, T.; Ozono, K. Wnt signaling in bone metabolism. *J. Bone Miner. Metab.* **2009**, *27*, 265–271. (c) Mao, B.; Wu, W.; Dividson, G.; Marhold, J.; Li, M.; Mechler, B. M.; Delius, H.; Hoppe, D.; Stannek, P.; Walter, C.; Glinka, A.; Niehrs, C. Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signaling. *Nature* **2002**, *417*, 664–667.

(3) (a) Boyden, L. M.; Mao, J.; Belsky, J.; Mitzner, L.; Farhi, A.; Mitnick, M. A.; Wu, D.; Insogna, K.; Lifton, R. P. High bone density due to a mutation in LDL receptor related protein 5. *N. Engl. J. Med.* **2002**, *346*, 1513–1521. (b) Little, R. D.; Carulli, J. P.; Del Mastro, R. G.; Dupuis, J.; Osborne, M.; Folz, C.; Manning, S. P.; Swain, P. M.; Zhoa, S.-C.; Eustace, B.; Lappe, M. M.; Spitzer, L.; Zweier, S.; Braunschweiger, K. A mutation in the LDL receptor related protein 5 results in the autosomal dominant high-bone-mass trait. *Am. J. Hum. Genet.* **2002**, *70*, 11–19. (c) Johnson, M. L. The high bone mass family: the role of Wnt/Lrp5 signalling in the regulation of bone mass. *J. Musculoskeletal Neuronal Interact.* **2004**, *4*, 135–138.

(4) (a) Chan, A.; van Bezooijen, R. L.; Lowik, C. W. G. M. A new paradigm in the treatment of osteoporosis: Wnt pathway proteins and their antagonists. *Curr. Opin. Invest. Drugs* **2007**, *8*, 293–298. (b) Niida, A.; Hiroko, T.; Kasai, M.; Furukawa, Y.; Nakamura, Y.; Suzuki, Y.; Sugano, S.; Akiyama, T. Dkk-1, a negative regulator of Wnt signaling, is a target of the β-catenin/TCF pathway. *Oncogene* **2004**, 1–7. (c) Baron, R.; Rawadi, G. Targeting the Wnt/β-catenin pathway to regulate bone formation in the adult skeleton. *Endocrinology* **2007**, *148*, 2635–2643.

(5) Bhat, B. M.; Allen, K. M.; Liu, W.; Graham, J.; Morales, A.; Anisowicz, A.; Lam, H.-S.; McCauley, C.; Coleburn, V.; Cain, M.; Fortier, E.; Bhat, R. A.; Bex, F. J.; Yaworsky, P. J. Structure-based mutation analysis shows the importance of LRP5 β-propeller 1 in modulating Dkk 1-mediated inhibition of Wnt signaling. *Gene* **2007**, *391*, 103–112.

(6) Ring, D. B.; Johnson, K. W.; Henriksen, E. J.; Nuss, J. M.; Goff, D.; Kinnick, T. R.; Ma, S. T.; Reeder, J. W.; Samuels, I; Slabiak, T.; Wagman, A. S.; Wernette-Hammond, M.-E.; Harrison, S. D. Selective glycogen synthase kinase inhibitors potentiate insulin activation of glucose transport utilization in vitro and in vivo. *Diabetes* **2003**, *52*, 588–595.

(7) Lui, J.; Wu, X.; Mitchell, B.; Kintner, C.; Ding, S.; Schultz, P. G. A small-molecule agonist of the wnt signaling pathway. *Angew. Chem., Int. Ed.* **2005**, *44*, 1987–1990.

(8) The GSK-3β kinase assay was performed essentially as described by the manufacturer (Upstate Biotechnology/Millipore Corporation, Billerica, MA).

(9) McMartin, C.; Bohacek, R. QXP: powerful, rapid computer algorithms for structure-based drug design. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 333–344. Docking details are provided in Supporting Information .
(10) Bertrand, J.; Thieffine, S.; Vulpetti, A.; Cristiani, C.; Valsasina, B.; Knapp, S.; Kalisz, H.; Flocco, M. Structural characterization of the GSK-3β active site using selective and non-selective ATP-mimetic inhibitors. *J. Mol. Biol.* **2003**, *333*, 393–347.
(11) Bursavich, M. G.; Lombardi, S.; Gilbert, A. M. Expedient parallel synthesis of 2-amino-4-heteroarylpyrimidines. *Org. Lett.* **2005**, *7*, 4113–4116.
(12) Ioachim, E.; Medlycott, E. A.; Polson, M. I. J.; Hanan, G. S. Synthesis of a novel series of 6,6′-disubstituted-4,4′-bipyrimidines by radical anion coupling: new π-accepting ligands for coordination chemistry. *Eur. J. Org. Chem.* **2005**, 3775–3780.
(13) Di, L.; Kerns, E. H. Application of pharmaceutical profiling assays for optimization of drug-like properties. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 495–504.
(14) Kharode, Y. P.; Sharp, M.C.; Bodine, P.V.N. Utility of the Ovariectomized Rat as a Model for Human Osteoporosis in Drug Discovery. In *Osteoporosis: Methods and Protocols*; Westendorf, J. J., Ed.; Methods in Molecular Biology, Vol. 455; Humana Press: Totowa, NJ, 2008; Chapter 8, pp 111−124.
(15) Robinson, J. A.; Chatterjee-Kishore, M.; Yaworsky, P. J.; Cullen, D. M.; Zhao, W.; Li, C.; Kharode, Y.; Sauter, L.; Babij, P.; Brown, E. L.; Hill, A. A.; Akhter, M. P.; Johnson, M. L.; Recker, R. R.; Komm, B. S.; Bex, F. J. Wnt/β-catenin is a normal physiological response to mechanical loading in bone. *J. Biol. Chem.* **2006**, *281*, 31720–31728.