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Hit to lead studies on (hetero)arylpyrimidines—Agonists of the canonical Wnt-β-catenin cellular messaging system

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ABSTRACT

A series of (hetero)arylpyrimidines agonists of the Wnt-β-catenin cellular messaging system have been prepared. These compounds show activity in U2OS cells transfected with Wnt-3a, TCF-luciferase, Dkk-1 and tk-Renilla. Selected compounds show minimal GSK-3β inhibition indicating that the Wnt-β-catenin agonism activity most likely comes from interaction at Wnt-3a/Dkk-1. Two examples **1** and **25** show in vivo osteogenic activity in a mouse calvaria model. One example **1** is shown to activate non-phosphorylated β-catenin formation in bone.

Activation of the Wnt-β-catenin signaling pathway has been shown to play important roles in development, tissue regeneration, stem cell control, tumor progression and metastases.[1,2] This pathway has also been implicated in the regulation of bone homeostasis. The Wnt co-receptor LRP5 is central to this function where both gain- and loss-of-function mutations have been described in humans resulting in high bone mass or an osteoporosis pseudoglioma syndrome, respectively.[3–5] Murine models of both genetic conditions have been successfully generated.[6,7] Pharmacologic inhibition of either Dickkopf-1 (Dkk-1) or Sclerostin, two LRP5-binding negative regulators of Wnt-β-catenin signaling, results in increased bone mineral density in rodents.[8,9] These data provide additional evidence that agonists of Wnt-β-catenin activity could yield an osteogenic agent.

Signaling by the Wnt-β-catenin pathway has been extensively studied in many systems.[1,2,10] Briefly, secreted Wnt ligands bind to cells via Frizzled receptors and the LRP5 or LRP6 co-receptors. This ligand-receptor interaction activates the cytoplasmic protein Disheveled which in turn inactivates a protein complex comprising Axin, Adenomatous Polyposis Coli, and Glycogen Synthase Kinase-3β (GSK-3β). The resulting repression of GSK-3β activity leads to an accumulation of unphosphorylated β-catenin enabling it to translocate to the nucleus and form a transcriptional co-activator complex with T-cell factor/lymphoid enhancer-binding factor (TCF/LEF). Extracellularly, this mechanism can be antagonized by several secreted molecules including Dkk-1, Sclerostin and the Secreted Frizzled Related Proteins.

Both pharmacologic and genetic data support that the Wnt-β-catenin pathway is a key mediator of the normal adaptive response to mechanical loading in bone.[11–13] As a result, agents capable of mimicking the beneficial effects of Wnt-β-catenin activation on the skeleton would represent a novel approach for the treatment of osteoporosis and other bone disorders. In this Letter, we report on the hit to lead studies on a class of (hetero)arylpyrimidines: agonists of the Wnt-β-catenin pathway and their use as potential anabolic agents to increase bone mass starting from **1**, a (hetero)arylaminopyrimdine found from high-throughput screening.

Compounds **4** are generally prepared according to Scheme 1.[14] Thus 2-chloropyrimidine **2** when reacted with aryl/heteroaryl

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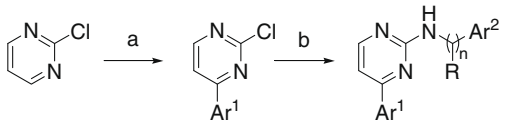
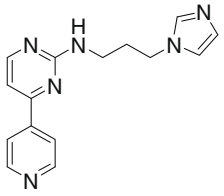


2 3 4

Scheme 1. Reagents and conditions: (a) (1) Ar^1Li, Et_2O, −78 to 0 °C; (2) DDQ, THF, 23 °C; (b) $H_2N(CHR)_nAr^2$, NMP, 90 °C or $H_2N(CHR)_nAr^2$, NaH, DMSO, 80 °C.

lithiums in Et_2O produces the corresponding 4-aryl/heteroaryl compounds.[15,16] Target compounds **4** are prepared by reacting **3** with amines in hot NMP or by first deprotonating the amine with NaH in DMSO and then heating with **3** in hot DMSO.



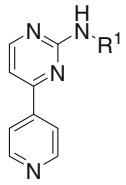
1

Wnt-3a/Dkk-1/TCF-Luci EC_{50}: 4.2 μM (2.9 FI @ 20 μM)
GSK-3β IC_{50}: 51 μM

Compounds were assayed using U2OS cells transfected with Wnt-3a, TCF-luciferase, Dkk-1 and tk-Renilla as a signal normalizer compared to control U2OS cells transfected with Wnt-3a, TCF-Luciferase and tk-Renilla.[17] The signal from the TCF-luciferase is reduced in the presence of the inhibitor Dkk-1. Compounds that modulate Dkk-1 activity or act downstream of the Wnt-3a/Dkk-1 complex (i.e., GSK-3β inhibition) cause an increase in the luciferase signal. For all compounds in this manuscript, EC_{50}s were determined as well as the fold-induction response at 20 μM compared to the control U2OS cells. A [^{32}P]-GSK-3β was used to assay compounds for Wnt/β-catenin activity downstream (of, from) Wnt-3a/Dkk-1.

Compound **1** shows a robust TCF-Luciferase response at 20 μM as well as a μM-EC_{50} (**1**: TCF (20 μM): 2.9; EC_{50}: 4.2 μM) (Table 1). Since arylamino-pyrimidines are well-known kinase inhibitors,[18] **1** was assayed for GSK-3β inhibition but little kinase inhibition was seen (IC_{50}: 51 μM). Modulation of the R^1 substituent to ethylpyridines (**5** and **6**) produces compounds of comparable efficacy/potency to **1**. The replacement of R^1 with a propyl(dimethylpyrazole) group

Table 1
Wnt-β-catenin activities of compounds **1**, **5**–**9**



1, 5-9

Compd	R^1	TCF[a] (20 μM)	EC_{50}[b] (μM)
1	*N*-(3-(1*H*-Imidazol-1-yl)propane)	2.9	4.2
5	*N*-(2-(Pyridin-4-yl)ethane)	4.3	6.8
6	*N*-(2-(Pyridin-3-yl)ethane)	2.8	4.6
7	*N*-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)propyl)	2.1	29.6
8	*N*-(2-(1*H*-Indol-3-yl)ethane)	7.5	2.7
9	*N*-(*S*)-3-(1*H*-Indol-3-yl)-2-propan-1-ol amine	2.2	FTC[c]

[a] U2OS: Wnt-3a/Dkk-1/TCF-Luci fold induction at 20 μM. Values are the average of 2 or 3 runs. Error ±20%.
[b] U2OS: Wnt-3a/Dkk-1/TCF-Luci EC_{50}. Values are the average of 2 or 3 runs. Error ±20%.
[c] FTC: EC_{50} determination failed to converge.

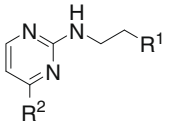
produces a less potent compound (**7**) while the ethylindole derivative (**8**) gives a 2.5-fold more efficacious analog of **1** (**8**: TCF (20 μM): 7.5; EC_{50}: 2.7 μM). The more complex **9** containing a propanolindole substituent possesses similar efficacy to **1** but the EC_{50} fails to converge.

Table 2 outlines the effects of varying the 4-pyrimidine position on compounds similar to **1**. Changing R^2 from a pyridine-4-yl to a pyridine-3-yl results in a slight increase in efficacy (**10**: TCF (20 μM): 4.7) while a 3-NO_2-phenyl group gives a much less potent compound (**11**: EC_{50}: 46.3 μM). Compound **13** where R^1 = 3-(1*H*-indole) shows μM-potency with large fold-induction (**13**: TCF (20 μM): 10.0; EC_{50}: 4.1 μM).

Of the 4-(1*H*-imidazole) indole compounds **15**–**17**, the 2-(benzo[*b*]thiophene) analog **16** possesses the best combination of potency and efficacy (EC_{50}: 3.3 μM, TCF (20 μM): 3.7). Several phenethyl analogs were also prepared, but they showed poor Wnt-β-catenin agonism.

To follow-up compound **16** which shows μM potency and moderate efficacy in the Wnt-3a/Dkk-1/TCF-Luci assay, 2-(naphthyl)-2-(benzo[*b*]thiophene)-derivatives were prepared according to the

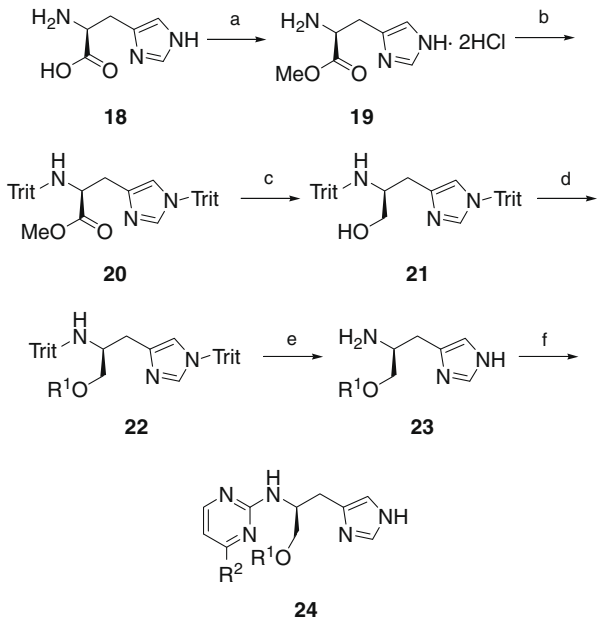
Table 2
Wnt-β-catenin activities of compounds **1**, **10**–**11**



1, 10-17

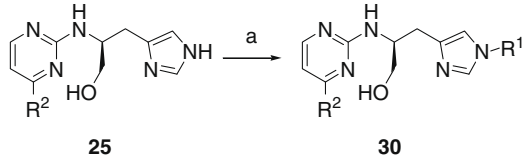
Compd	R^1	R^2	TCF[a] (20 μM)	EC_{50}[b] (μM)
1	CH_2-1*H*-Imidazole	4-(Pyridin-4-yl)	2.9	4.2
10	CH_2-1*H*-Imidazole	4-(Pyridin-3-yl)	4.7	8.8
11	CH_2-1*H*-Imidazole	4-(3-Nitrophenyl)	3.6	46.3
12	4-Pyridine	4-(Pyridin-3-yl)	3.4	20.2
13	3-(1*H*-Indole)	4-(Pyridin-3-yl)	10.0	4.1
14	3-(2-Methyl-1*H*-indol-5-ol)	4-(pyridin-3-yl)	5.1	12.2
15	4-(1*H*-Imidazole)	4-(Pyridin-3-yl)	3.1	28.8
16	4-(1*H*-Imidazole)	2-(Benzo[*b*]thiophene)	3.7	3.3
17	4-(1*H*-Imidazole)	2-(Naphthyl)	1.1	12.1

[a] U2OS: Wnt-3a/Dkk-1/TCF-Luci fold induction at 20 μM. Values are the average of 2 or 3 runs. Error ±20%.
[b] U2OS: Wnt-3a/Dkk-1/TCF-Luci EC_{50}. Values are the average of 2 or 3 runs. Error ±20%.



Scheme 2. Reagents and conditions: (a) $SOCl_2$, MeOH, 60 °C; (b) Trit–Cl, TEA, MeCN, 23 °C; (c) $LiAlH_4$. THF 0 to 23 °C; (d) NaH, R^1X, DMF, 50 °C; (e) HCl, THF, reflux; (f) Scheme 1, step b.

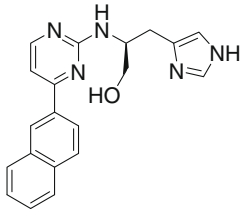
chemistry outlined in Schemes 2 and 3. Histidine **18** may be converted to methyl ester **19** using standard conditions. After being bis(tritylated) to produce **20**, reduction with $LiAlH_4$ produces the primary alcohol **21**. Alkylation with various alkyl and aryl bromides produces **22**. Deprotection with HCl yields the derivatized histidines **23** which were incorporated into products **4** using the procedure of Scheme 1, Step b. Analogs where the imidazole nitrogen is substituted are prepared by reacting compounds like **25** with NaH followed by the addition of an alkylating agent to produce **30**.



Scheme 3. Reagents and conditions: (a) NaH, R^1X, THF/DMSO, 23 °C.

O-Substituted derivatives in Table 3 show moderate Wnt-3a/Dkk-1/TCF-Luci efficacy at 20 μM, but most of the compounds were not as potent as and efficacious as **16**. This was true for alkyl, aryl and alkynyl derivatives **25**–**29**. The exception was hydroxyl analog **25**. Analogs of **16** where the imidazole nitrogen is alkylated show similar to greater efficacy compared to the O-substituted analogs as a number of substituents are tolerated (**31**, **32**: benzyl, **33**: propynyl, **34**: acetamide, **35**, **36**: 3-propanitrile), but comparatively weaker potency to **25** (see Table 4).

Example **25** represents a compound with good properties of a chemical lead based on **1**. It has robust Wnt-3a/Dkk-1/TCF-Luci efficacy @ 20 μM (FI 4.9), good potency (EC_{50}: 6.8 μM) and an acceptable ligand efficiency. The calculated physical properties are in line for a molecule with good drug-like properties. Good aqueous solubility, moderate PAMPA permeability and good rat liver microsome stability is also seen.

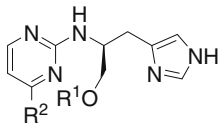


25

Wnt-3a/Dkk-1/TCF-Luci fold induction at 20 μM: 4.9
Wnt-3a/Dkk-1/TCF-Luci EC_{50}: 6.8 μM
LE: 0.27
LELP: 8.9
MW: 345.40
clogP: 2.4
TPSA: 87
Aqueous Solubility: 65 μg/mL
PAMPA: 0.13 x 10^{-6} cm/s
RLM $t_{1/2} \geq 80$ min

In order to establish the osteogenic activity of this series, **1** was evaluated in vivo via local subcutaneous injection over the right side of the calvaria of wild type C57BL/6 mice.[11] As a positive control, an inhibitor of GSK-3β (GSKi), 3-(3-chloro-4-hydroxyphenylamino)-4-(2-nitrophenyl)-1*H*-pyrole-2,5-dione),[19] was administered at 1 mg/kg/day as previously described.[11] Vehicle alone was used as the negative control. Using standard dynamic histomorphometry measurements, the mineral apposition rate of new bone formation was

Table 3
Wnt-β-catenin activities of compounds **25**–**29**

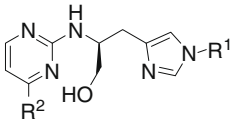


25-29

Compd	R^1	R^2	TCF[a] (20 μM)	EC_{50}[b] (μM)
25	H	2-(Naphthyl)	4.9	6.8
26	Ethyl	2-(Naphthyl)	1.9	14.4
27	Methylenecyclohexyl	2-(Naphthyl)	2.2	20.8
28	(2-Fluoro-3-(trifluoromethyl)benzyl	2-(Naphthyl)	1.6	10.9
29	Prop-2-ynyl	2-(Benzo[*b*]thiophene)	3.5	FTC

[a] Values are the average of 2 or 3 runs. Error ±20%.
[b] FTC: EC_{50} failed to converge.

Table 4
Wnt-β-catenin activities of compounds **31**–**38**



31-36

Compd	R^1	R^2	TCF[a] (20 μM)	EC_{50}[b] (μM)
31	3,5-Difluorobenzyl	2-(Benzo[*b*]thiophene)	1.7	16.0
32	3,5-Difluorobenzyl	2-(Naphthyl)	2.0	FTC
33	Prop-2-ynyl	2-(Benzo[*b*]thiophene)	3.0	14.6
34	2-Acetamide	2-(Naphthyl)	3.9	FTC
35	3-Propanitrile	2-(Benzo[*b*]thiophene)	4.1	FTC
36	3-Propanitrile	2-(Naphthyl)	5.5	FTC

[a] Values are the average of 2 or 3 runs. Error ±20%.
[b] FTC: EC_{50} determination failed to converge.

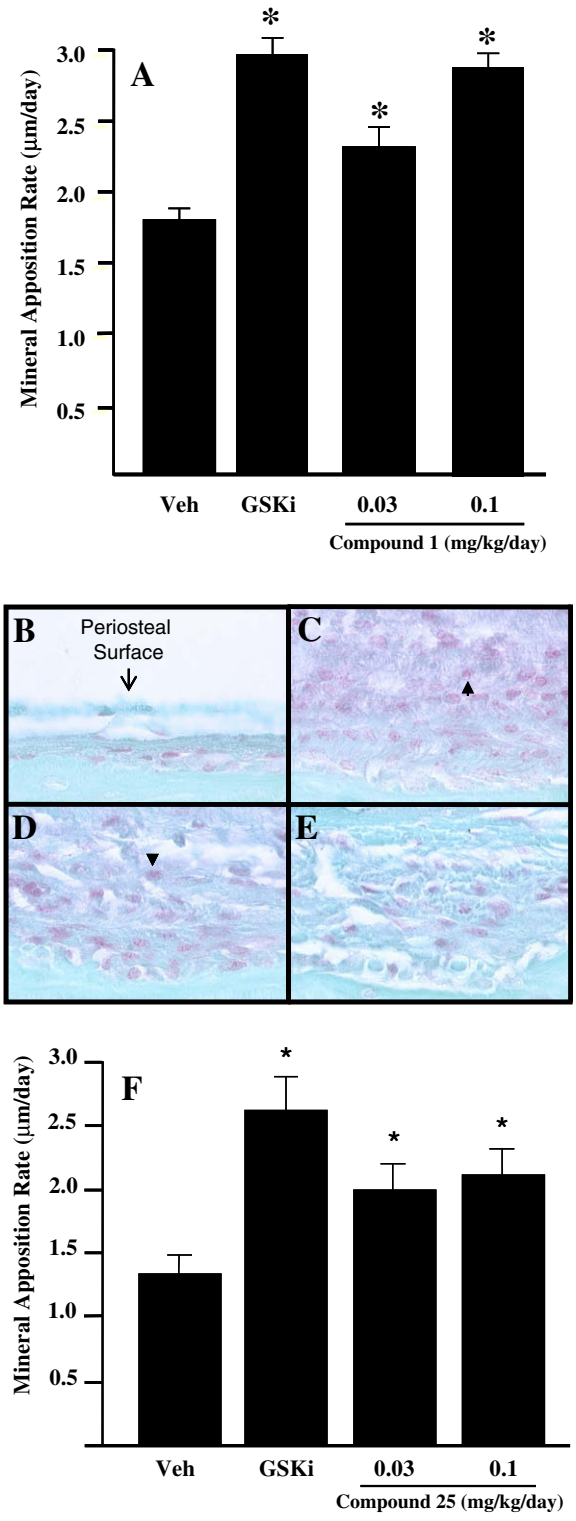


Figure 1. Compounds **1** and **25** are osteogenic in vivo. (A) Quantitative dynamic histomorphometry was performed on murine calvaria following 7 days of **1** treatment. Mineral apposition rates were calculated and are presented as an index of anabolic osteoblast activity. Veh, vehicle; GSKi, Glycogen synthase kinase-3β inhibitor;[19] * p <0.01 versus vehicle. (B–D) Immunohistochemical detection of non-phosphorylated β-catenin is enhanced in compound-treated calvaria suggesting that Wnt-β-catenin signaling is activated. Positive cells are identified by the pink staining as indicated by the arrowheads. Vehicle (B), GSKi (C), 0.3 mg/kg/day **1** (D), 1.0 mg/kg/day **1** (E). (F) Compound **25** is osteogenic in the same assay showing significantly elevated mineral apposition rates versus vehicle.

calculated exactly as previously described.[11] All doses of **1** showed a significant increase in anabolic bone activity over vehicle with levels equivalent to that attained by the GSKi positive control in all but the lowest tested dose of **1** (Fig. 1A). Mechanistically, this anabolic bone activity could be ascribed to activated Wnt-β-catenin signaling as visualized by the immunohistochemical detection of non-phosphorylated β-catenin in the osteoblastic cells lining the periosteal surface of the calvaria (Fig. 1B–E). Furthermore, using the same in vivo model, **25** was also shown to be an anabolic bone agent (Fig. 1F). Together these data support that **1** and **25** are both Wnt-β-catenin pathway agonists and efficacious osteogenic agents in vivo.

In conclusion, we have disclosed a series of (hetero)arylpyrimidines that act as agonists of the Wnt-β-catenin pathway. While we currently don't know their exact mechanism of action, these compounds don't function via inhibition of GSK-3β. Moreover, examples of this equity show osteogenic in vivo activity in mouse calvaria as well as immunohistochemical data indicating that Wnt-β-catenin activity is activated in bone.

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