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Discovery of potent and bioavailable GSK-3β inhibitors

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ABSTRACT

Here we report on the discovery of a series of maleimides which have high potency and good selectivity for GSK-3β. The incorporation of polar groups afforded compounds with good bioavailability. The most potent compound **34** has an IC_{50} of 0.6 nM for GSK-3β, over 100-fold selectivity against a panel of other kinases, and shows efficacy in rat osteoporosis models. The X-ray structure of GSK-3β protein with **34** bound revealed the binding mode of the template and provided insights for future optimization opportunities.

In the US nearly 1 million fractures occur annually in people over the age of 65, mostly due to osteoporosis. The need to develop novel drugs that stimulate bone formation and thereby increase bone mass is an important research area for therapeutic intervention in the treatment of osteoporosis. Combined genetic and genomic studies revealed that a Wnt interacting protein (Frzb1) was a key element influencing peak bone density in humans as well as in mice.[1] Furthermore, glycogen synthase kinase-3β (GSK-3β), a serine/threonine protein kinase, has been identified as a regulatory enzyme in this pathway. Reported here is our effort to use small molecules to define the role of GSK-3β inhibition in the Wnt pathway and the resulting effect on bone density in a murine model of osteoporosis.

Human GSK-3 has two isozymes (α and β) sharing 95% homology around the catalytic domain. GSK-3 is a ubiquitous kinase that participates in a multitude of cellular processes, such as cell membrane-to-nucleus signaling, gene transcription, translation, cytoskeletal structuring and cell cycle progression and survival.[2,3] Therefore, there are potentially multiple therapeutic benefits by inhibiting GSK-3.

A number of chemical classes of GSK-3 inhibitors with varying potencies have been reported in the literature (Fig. 1).[4–8]

Our discovery effort to identify a potent class of GSK-3β inhibitors with oral bioavailability and in vivo efficacy was initiated from the directed screening of an in-house PKC inhibitor library containing bis-indolyl maleimides. Representative examples are shown in Figure 2.

The investigation was focused on the exploration of the SAR around the maleimide template with the aim of improving kinase selectivity, particularly against PKC, and improving in vivo bioavailability.

Compounds **8–26** were synthesized following the general route depicted in Scheme 1.

Acylation of *N*-methylindole **4** with oxalyl chloride was carried out in diethyl ether to give compound **5**, which was treated with

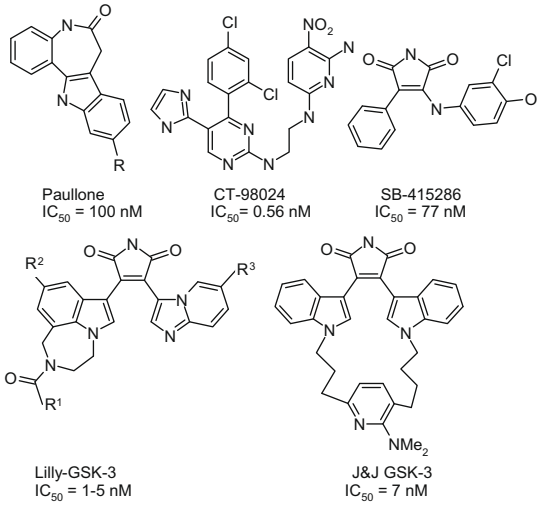

Figure 1. Different chemical classes of GSK-3 inhibitors.

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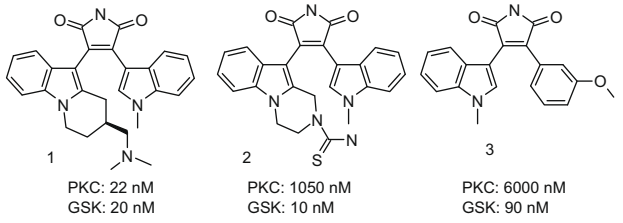
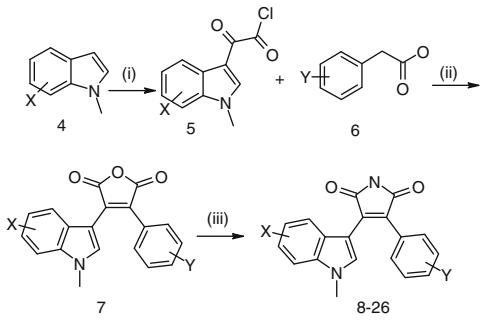


Figure 2. Examples of the screen hits from PKC inhibitor library.



Scheme 1. Reaction conditions and yields: (i) $(COCl)_2$ in Et_2O at 0 °C; (ii) Et_3N in CH_2Cl_2 at 0 °C, 30%; (iii) NH_3 (aq) in DMF, 80–90%.

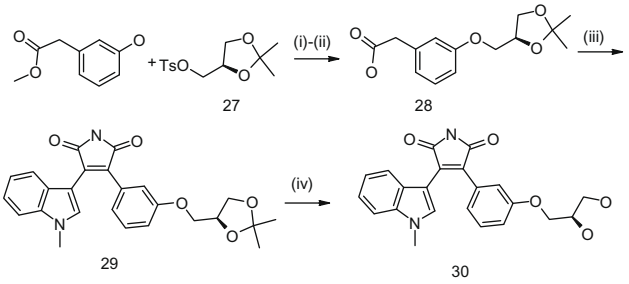
phenyl acetic acid **6** and triethylamine in CH_2Cl_2 at 0 °C to afford the corresponding furan-2,5-dione analogs **7**. A solution of **7** in DMF and aqueous ammonia was heated to 140 °C for 5 h to afford 80–90% of the maleimide analogs **8–26**.

Scheme 2 described the synthesis of compounds **30–32** from a commercially available chiral precursor.

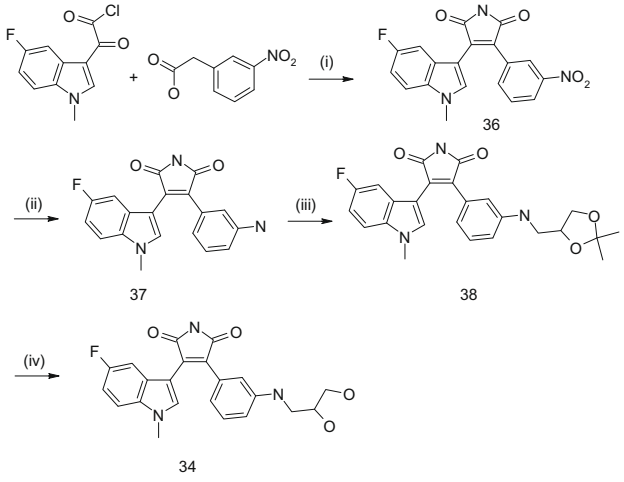
Compounds **33–35** were prepared via the route shown in Scheme 3, starting from 3-nitrophenylacetic acid. The reduction of the nitro group in **36**, followed by reductive amination and deprotection, afforded compound **34**.

The maleimide series identified from the screening exhibited dual inhibition against both PKC and GSK-3β. A close examination of the initial SAR of compounds **1–3** (Fig. 2) reveals two elements differentiate PKC from GSK. First, PKC potency requires two-indolyl rings attached to the maleimide whereas GSK potency could be maintained with one indolyl ring and one monocyclic ring such as phenyl. Second, PKC inhibition is favored by the presence of a basic nitrogen moiety whereas it is not essential for GSK activity. Based on these observations, the strategy we chose was to first confirm our hypothesis that GSK selectivity may be obtained by removing the second indole group, and then to optimize a mono-indolyl maleimide for potency (Table 1).

In general, a number of substitution patterns can be tolerated at the phenyl ring as exemplified in compounds **8–11** where 2-, 3-, 3,4-, and 2,4- substitutions are all potent for GSK-3β but 2,5-substitution (**12**) resulted in a less active compound. Thiophene **13** and pyridine **14** are tolerated by GSK-3β and have very good selectivity

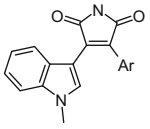


Scheme 2. Reaction conditions and yields: (i) NMP, K_2CO_3, 96 °C, 18 h, 68%; (ii) LiOH–H_2O (4 equiv) in MeOH/H_2O (10:1), >99%; (iii) Scheme 1 (i–iii); (iv) pTsOH (cat.), MeOH, 63%.



Scheme 3. Reaction conditions and yields: (i) Scheme 1 (ii–iii); (ii) $TiCl_3$ in acetone, rt, 18 h, 83%; (iii) 2,2-dimethyldioxolane-4-carboxaldehyde,[9] $Na(OAc)BH_3$, CH_2Cl_2, rt, 18 h, 25%; (iv) pTsOH (cat.), MeOH, 50 °C, 18 h, 66%.

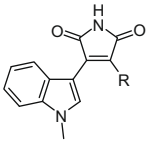
Table 1
Enzymatic activity of monoindolyl maleimides[a]



Compound	Ar	GSK3β/PKCα IC_{50}, nM[a]
8	2-Cl-Ph	3.6/760
9	3-NH2-Ph	1.8/1900
10	2,4-Di-OMe-Ph	2.4/5500
11	3,4-Di-OMe-Ph	6.6/6600
12	2,5-Di-OMe-Ph	22/380
13	2-Thiophene	22/8900
14	3-Pyridyl	42/>30,000
15	4-Ph-Ph	730/NA

[a] Compounds were characterized by mass spectral, ^1H NMR, elemental analysis and mp. IC_{50} values are an average of multiple determinations ($n \geqslant 2$). Assay conditions are described in Ref. 10; N.A. = no data obtained.

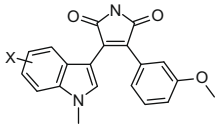
Table 2
Enzyme activity of phenyl analogs with linkers



Compound	R	GSK3β IC_{50}[a] (nM)
16	–SPh	2000
17	–NHPh	350
18	–OPh	2800
19	–N(BOC)Ph	3100
20	–N-morpholine	8200

[a] Compounds were characterized by mass spectral, ^1H NMR, elemental analysis and mp. IC_{50} values are an average of multiple determinations ($n \geqslant 2$). Assay conditions are described in Ref. 10; N.A. = no data obtained.

Table 3
Enzyme activity of substituted indolyl analogs



Compound	X	GSK3b/PKCa IC_{50}[a] (nM)
21	H	90/6000
22	2-Me	220/NA
23	6-F	31/4200
24	5-Cl	6.4/1400
25	6-Me	35/2900
26	4-OMe	1300/NA

[a] Compounds were characterized by mass spectral, ^{1}H NMR, elemental analysis and mp. IC_{50} values are an average of multiple determinations ($n \geqslant 2$). Assay conditions are described in Ref. 10; N.A. = no data obtained.

against PKC whereas biphenyl **15** has reduced activity. Compounds **16–19** demonstrate that placing a linker between the maleimide and phenyl rings results in less active inhibitors (Table 2). The N-linked morpholine analog **20** is also weak.

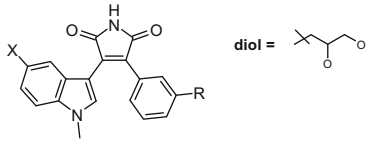
Our focus then shifted to substitution of the indole ring. Substitution at position 5 or 6 enhanced the potency with the effect being more pronounced at position 5 whereas the 2-Me and 4-OMe analogs were less active (Table 3).

Having attained the desired potency and selectivity, the next challenge was to achieve good bioavailability. In the course of evaluating this series, compound **21** was found to be extremely insoluble in addition to being extensively metabolized. We attributed both problems to the highly lipophilic nature of the series and we envisaged that reduction of the lipophilicity by incorporating a polar functionality would improve both the solubility and the metabolic stability. Compounds **30–35** were synthesized based on this rationale.

Incorporation of a number of polar or ionizable groups at the 3-position of the phenyl ring afforded compounds that maintained GSK-3β potency and PKC selectivity (Table 4). More gratifying was that solubility and metabolic stability were improved as compared to compound **21** (Table 5).

Shown in Figures 3 and 4 is the X-ray structure of GSK-3β with compound **34** occupying the ATP binding pocket.[11] The structure revealed several key interactions by the compound with GSK-3β.

Table 4
Enzyme activity of substituted phenyl analogs with polar groups



Compound	X	R	GSK3β/PKCα IC_{50}[a] (nM)
30	H	O-diol (R)	3.2/1000
31	H	O-diol (S)	8.2/1400
32	F	O-diol (R/S)	1.8/2200
33	H	$NH(CH_2)_3OH$	1/740
34	F	NH-diol (R/S)	0.6/400
35	Cl	NH-diol (R/S)	0.4/260

[a] Compounds were characterized by mass spectral, ^{1}H NMR, elemental analysis and mp. IC_{50} values are an average of multiple determinations ($n \geqslant 2$). Assay conditions are described in Ref. 10; N.A. = no data obtained.

Table 5
Solubility and Rat oral PK

Compound	Solubility (μg/ml)[a]	Dose (mpk)	Cmax (μg/ml)	AUC (μg h/ml)
21	<0.5	10[b]	BQL[c]	BQL[c]
32	2.8	10	0.40	1.06
34	104	10	0.11	0.35
35	42	25	0.11	0.10

[a] Aqueous solubility: pH 6.6.
[b] The route of administration was IP in mice.
[c] BQL: Below Quantification Limit of 4 ng/ml.

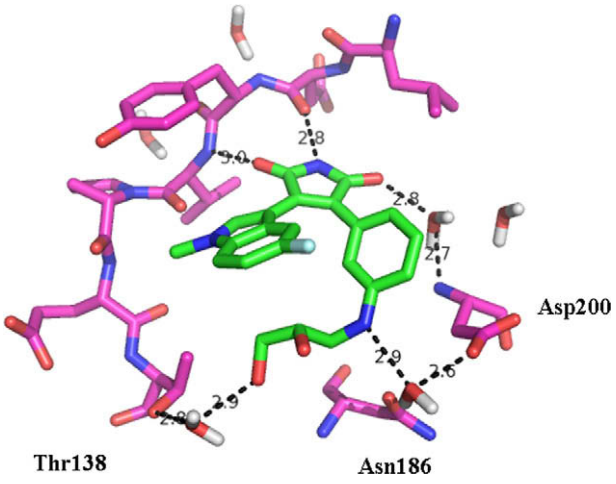


Figure 3. X-ray of **34** bound to GSK-3β.

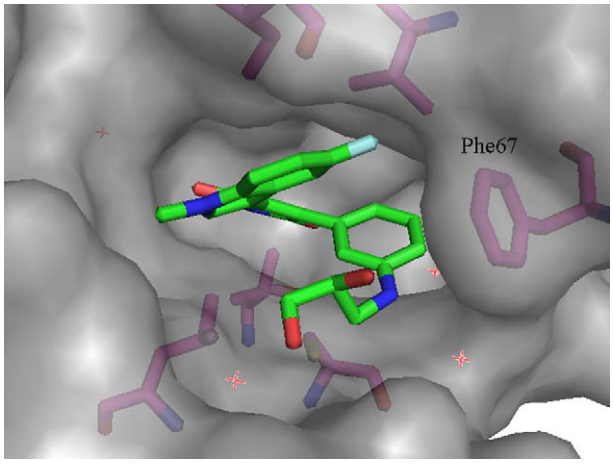


Figure 4. Surface representation of **34** bound to GSK-3β.

With regard to polar interactions, the maleimide NH acts as a H-bond donor interacting with Asp133 carbonyl group and one of the carbonyl groups from the maleimide acts as a H-bond acceptor to interact with Val135 NH. Together, they form the hinge interaction as seen in most kinase inhibitors. The other carbonyl group from the maleimide interacts with Asp200 through water. The side chain shows additional polar interactions: the NH with Asn186 via water and the terminal OH with Thr138 via water. As shown in Figure 4, the indole and phenyl rings stack on top of each other and occupy the hydrophobic part of ATP pocket. In addition, the phenyl

Table 6
Kinase selectivity profile of compound **34**

Kinase	K_d[a] (μM)	Ratio over GSK3β	Kinase	K_d[a] (μM)	Ratio over GSK3β
GSK3β	0.000053	1	CDK9	0.41	>2000
GSK3α	0.0015	28	ERK5	5.6	>2000
CLK2	0.010	180	TTK	6.7	>2000
PCTK1	0.012	230	CDK5	6.8	>2000
CLK1	0.016	300	PRKR	14	>2000
ERK8	0.032	600	AURKC	23	>2000
CDK7	0.041	770	PLK1	23	>2000
PCTK2	0.069	1300	AURKA	40	>2000
CDK3	0.080	1500	ERK3	40	>2000
DYRK1B	0.087	1600	AURKB	40	>2000
CLK4	0.11	>2000	PLK3	40	>2000
PCTK3	0.11	>2000	CDK11	40	>2000
CDK2	0.15	>2000	ERK1	40	>2000
STK16	0.23	>2000	ERK2	40	>2000
CLK3	0.31	>2000	ERK4	40	>2000
PLK4	0.32	>2000	CDK8	40	>2000

[a] Binding constant (K_d) Determination: Quantitative affinity measurement (11-point curve) using Ambit's competition binding assay.[12]

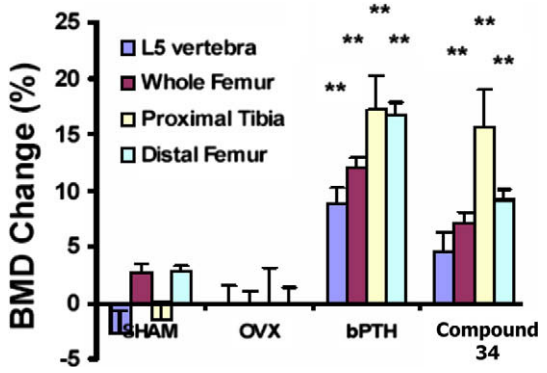


Figure 5. Compound **34**'s anabolic effect in rat (OVX) model.[13]

ring from the inhibitor also has an edge/face interaction with Phe67.

Compound **34** was screened against 317 kinases at Ambit Biosciences. In this panel, compound **34** was found to inhibit only 36 kinases with >90% inhibition at 10 μM. The K_ds of these 36 kinases were then determined and the results are shown in Table 6. Overall, compound **34** shows good selectivity over other kinases.

Combined with its reasonable oral exposure and desired potency against GSK-3β, **34** was chosen for in vivo evaluation in our rat osteoporosis model. It was administrated at 30 mpk daily for 3 weeks to ovariectomized (Ovx) rats to assess its ability to increase the bone mineral density (BMD).[13] Compound **34** showed a pronounced anabolic effect at multiple locations (Fig. 5) and the results are comparable to the effect of known anabolic hormone, bovine PTH.

Starting from a focused screen of a PKC inhibitor library, we have discovered a novel series of monoindolyl maleimides by dialing out PKC activity and improving GSK-3β activity. The chemistry effort has resulted in compound **34**, an orally active, potent and selective GSK-3β inhibitor. In vivo studies with **34** have demonstrated that GSK-3β plays a role in influencing bone density in a murine model of osteoporosis.

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10. The GSK-3β inhibition assay was described in Gong, L.; Peltz, G. Grupe, A. U.S. Patent 6,479,490, 2002. In detail, it was performed in 50 μl reactions in a 96 well polypropylene plate, each reaction containing 20 mM magnesium chloride, 40 μM ATP, 2 mM DTT, 88.5 μM biotinylated and phosphorylated CREB-peptide substrate (biotin-KRREILSRRPS(PO_4)YR-OH, and [γ-^{33}P] ATP (1 μCi). 15 μl of the test compounds in DMSO (various concentrations) were added and the reaction mixture was incubated at 30 °C for 1 h. The reaction was stopped by transferring 25 μl of the reaction mixture to a phosphocellulose plate containing 130 μl of 1.85% phosphoric acid. The free radionucleotides in the membrane were washed off under vacuum with 1.85% phosphoric acid (five times). After the last wash, the plate was transferred to an adaptor plate and 50 μl of scintillation cocktail (Microscint-20, Packard, cat. #20-133) were added to each well and the amount of radioactivity was counted in a top counter.
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13. OVX model was described in Day-Lollini, P. A.; Gong, L. US20030176484, 2003. In detail, three month old rats were ovariectomized (OVX) and administered either bovine parathyroid hormone (AminoAcids$^{1\text{-}34}$) (bPTH) or compound **34** once a day by mouth starting at least 4 weeks post-ovariectomy and continuing until final sacrifice after 3 weeks of daily treatment. Control groups, both sham (rats that were not ovariectomized) and OVX, received vehicle only. Bovine parathyroid hormone, bPTH, was tested at 40 mg/kg (optimal dose) was an internal positive control for anabolic activity. The bone mineral density of the right femur was determined using the High Resolution Software on a QDR-4500W Bone Densitometer™ (Hologic, Waltham, Mass.). The animals were scanned by placing them on a plexiglass block in a supine position such that the right leg was perpendicular to the main body and the tibia was perpendicular to the femur. The increase in the bone density was expressed as % change at the end of 3 week experiment.