

SUPPORTING INFORMATION

Hit Optimization of 5-Substituted-N-(piperidin-4-ylmethyl)-1H-indazole-3-carboxamides: Potent Glycogen Synthase Kinase-3 (GSK-3) Inhibitors with In Vivo Activity in Model of Mood Disorders

Guido Furlotti,^{,§} Maria Alessandra Alisi,[§] Nicola Cazzolla,[§] Patrizia Dragone,[§] Lucia Durando,[§] Gabriele Magarò,[§] Francesca Mancini,[§] Giorgia Mangano,[§] Rosella Ombrato,[§] Marco Vitiello,[§] Andrea Armirotti,[¢] Valeria Capurro,[¢] Massimiliano Lanfranco,[¢] Giuliana Ottonello,[¢] Maria Summa[¢] and Angelo Reggiani^{*,¢}*

Supporting Information include:

| | |
|---|----------|
| General method for the preparation of the amine intermediates 6o-q,u ; | pag. S1 |
| <i>In vivo</i> pharmacokinetic assessment methods; | pag. S2 |
| Enzymatic GSK-3 β assay full list of results; | pag. S2 |
| Cellular GSK-3 β assay full list of results; | pag. S4 |
| Kinase selectivity full lists of results for 14i | pag. S5 |
| Crystallization conditions and X-ray data collection | pag. S14 |

General method for the preparation of the amine intermediates 6o-q,u. To a stirred solution of *N*-[phenylmethylidene]-1-(piperidin-4-yl)methanamine (0.158 moles), prepared as described in WO2004/101548 in absolute ethanol (70 ml), the proper alkylhalide (0.237 moles) and potassium carbonate were added. The mixture was refluxed for 8 hours, cooled and concentrated evaporating the solvent under reduced pressure. The reaction mixture was diluted with 3N HCl and stirred at room temperature for 3 hours. The acid solution was washed with dichloromethane and made alkaline with NaOH. The aqueous phase was extracted with three portions of dichloromethane, which were reunited and dried over Na₂SO₄. The solvent was removed by evaporating under reduced pressure and the final product was purified with flash chromatography (SiO₂, CHCl₃/MeOH = 9/1).

1-{1-[4-(Trifluoromethyl)benzyl]piperidin-4-yl}methan-amine (6o). (78%) ¹H-NMR (300MHz, CDCl₃) δ = 8.11 (br. S., 2 H), 7.85 – 7.71 (m, 2 H), 7.41 – 7.28 (m, 2 H), 3.34 (s, 2 H), 2.81 (d, J = 7.3 Hz, 2 H), 2.58 (d, J = 11.8 Hz, 2 H), 1.99 (t, J = 11.6 Hz, 2 H), 1.69 – 1.50 (m, 1 H), 1.47 – 1.31 (m, 2 H), 1.27 – 1.06 (m, 2 H). [M+H⁺] exact mass found 289.1521 for C₁₄H₂₀F₃N₂O.

1-[1-(2,4-Dichlorobenzyl)piperidin-4-yl]methanamine (6p). (84%) ¹H-NMR (300MHz, CDCl₃) δ = 7.44 (d, J=8.3 Hz, 1H), 7.36 (d, J=2.3 Hz, 1H), 7.21 (dd, J=2.3, 8.3 Hz, 1H), 3.55 (s, 2H), 2.89 (d, J=11.9 Hz, 2H), 2.61 (d, J=5.9 Hz, 2H), 2.08 (t, J=11.4 Hz, 2H), 1.78 (s, 2H), 1.72 (d, J=11.2 Hz, 2H), 1.43 – 1.16 (m, 3H). [M+H⁺] exact mass found 273.0920 for C₁₃H₁₉Cl₂N₂.

1-{1-[4-(Benzyloxy)benzyl]piperidin-4-yl}methan-amine (6q). (98%) ¹H-NMR (300MHz, DMSO-d₆) δ = 7.52 – 7.27 (m, 5H), 7.18 (d, J=8.5 Hz, 2H), 6.95 (d, J=8.8 Hz, 2H), 5.08 (s, 2H), 3.36 (s, 2H), 2.78 (d, J=11.4 Hz, 2H), 2.63 (d, J=7.0 Hz, 2H), 1.89 (s, 2H), 1.88 – 1.78 (m, 2H), 1.67 (d, J=12.0 Hz, 2H), 1.60 – 1.42 (m, 1H), 1.24 – 1.06 (m, 2H). [M+H⁺] exact mass found 311.2119 for C₂₀H₂₇N₂O.

1-{1-[2-(4-Methoxyphenyl)ethyl]piperidin-4-yl}-methanamine (6u). (82%) ¹H-NMR (300 MHz, DMSO-d₆). δ = 7.00 - 7.19 (m, 2H), 6.76 - 6.89 (m, 2H), 3.71 (s, 3H), 2.91 (d, J = 11.56 Hz, 2H), 2.55 - 2.72 (m, 4H), 2.37 - 2.47 (m, 2H), 1.90 (dt, J = 1.98, 11.56 Hz, 2H), 1.70 (d, J = 11.89 Hz, 2H), 1.52 (ddd, J = 3.96, 7.27, 10.90 Hz, 1H), 1.15 (dtd, J = 3.80, 12.01, 12.14 Hz, 2H). [M+H]⁺ exact mass found 249.1957 for C₁₄H₂₃N₂O.

***In Vivo* pharmacokinetic assessment.**

Compound administration and tissue preparation. Compounds were administered intraperitoneal to mice. Vehicle was: PEG400/Tween 80/Saline solution at 10/10/80% in volume respectively. Three animals per dose were treated. Animals were sacrificed and blood and brain samples were collected at time points after administration. Plasma was separated from blood by centrifugation for 15 minutes at 3500 rpm at 4°C, collected in a eppendorf tube and frozen (-80 °C). Control animals treated with vehicle only were also included in the experimental protocol. Brain samples were homogenized in RIPA buffer (150 mM NaCl, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate, 50 mM Tris, pH 8.0) and were then divided into two aliquots and kept at -80 °C until analysis. An aliquot was used for compound brain level evaluations. After 10 minutes centrifugation (3000 g for 10 minutes at 4°C), brain samples were prepared following the same procedure described for plasma samples. The second aliquot was kept for protein content evaluation by Bradford assay.

Samples preparation for UPLC-MS analysis. Samples (both plasma and brain homogenate) were thawed in an ice bath and after a short centrifugation transferred (50 µl) into a 96-deepwell plate and added with 150 µl of acetonitrile spiked with the internal standard to a final 200nM concentration. After agitation (3 minutes) the plate was centrifuged at 3000g for 10 minutes at 4°C. 80 µl of supernatant was then transferred in a 96-well plate and added with 80 µl of water. Standard compound was spiked in net solvent (PBS pH 7.4 added with 10% acetonitrile) to prepare a calibration curve over the 1 nM – 10 µM range. 3 quality controls samples were also prepared spiking the compound in blank rat plasma to final 20, 200 and 2000 nM concentrations. Calibrators and QCs were crashed with acetonitrile spiked with the I.S. as described for the plasma samples. Dosing solutions, previously diluted 2000 fold in the net solvent were also included in the samples and tested.

UPLC-MS/MS analysis. Compounds plasma levels were monitored on a Xevo TQ UPLC-MS/MS system (Waters, Milford USA), using the calibration curve and the internal standard (Warfarin). Chromatography was carried out on a Acquity BEH C18 column (2.1X50mm, 1,7µm particle size, Waters, Milford USA). Flow rate was 0.5ml/min. Eluents were A=water + Formic Acid 0.1% ; B= Acetonitrile + Formic Acid 0.1% . A linear gradient was applied from 5 to 100%B in 2min. 3µl of each sample were loaded on column. MS parameters and MRM transitions were carefully selected for each compound. Plasma levels data were analyzed using PKSolutions excel application (Summit Research Service, USA) to derive the most important pharmacokinetic parameters. Compound brain levels were normalized by total protein content.

Enzymatic GSK-3β assay results.

Table S. I. 1. IC₅₀ and 95% Confidence Intervals

| ID | IC ₅₀ (M) | 95% Confidence Intervals (M) |
|----|----------------------|------------------------------|
| 5 | 6.4E-07 | 4.06e-07 to 9.83e-07 |
| 9g | 1.7E-06 | 9.59e-07 to 2.91e-06 |

| ID | IC ₅₀ (M) | 95% Confidence Intervals (M) |
|-----|----------------------|------------------------------|
| 9a | 1.2E-06 | 9.04e-07 to 1.30e-06 |
| 9e | 8.7E-07 | 7.67e-07 to 1.23e-06 |
| 9p | 3.1E-07 | 1.11e-09 to 8.39e-05 |
| 9t | 3.5E-07 | 1.92e-07 to 6.73e-07 |
| 13i | 6.7E-08 | 5.75e-08 to 9.27e-08 |
| 9f | 1.0E-06 | 4.53e-07 to 2.56e-06 |
| 9u | 5.6E-07 | 4.02e-07 to 8.85e-07 |
| 9b | 6.7E-07 | 2.21e-07 to 1.44e-06 |
| 9m | 2.5E-07 | 2.31e-07 to 3.89e-07 |
| 13l | 3.8E-08 | 3.34e-08 to 4.90e-08 |
| 13m | 2.0E-08 | 1.84e-08 to 2.33e-08 |
| 13g | 1.5E-07 | 8.28e-08 to 2.54e-07 |
| 13q | 4.0E-08 | 2.76e-08 to 6.07e-08 |
| 9v | 5.1E-07 | 2.86e-07 to 8.20e-07 |
| 9c | 6.9E-07 | 4.90e-07 to 1.21e-06 |
| 14l | 3.1E-08 | 1.50e-08 to 6.76e-08 |
| 14m | 4.5E-08 | 3.83e-08 to 5.20e-08 |
| 14f | 2.0E-07 | 1.35e-07 to 3.08e-07 |
| 14g | 7.2E-08 | 3.34e-08 to 1.54e-07 |
| 14n | 2.6E-08 | 1.43e-08 to 4.79e-08 |
| 14q | 6.4E-08 | 4.37e-09 to 8.91e-07 |
| 14i | 1.8E-08 | 1.57e-08 to 2.11e-08 |
| 14k | 5.6E-07 | 8.16e-08 to 3.89e-06 |
| 14p | 1.4E-06 | 8.80e-08 to 1.55e-05 |
| 14o | 5.3E-08 | 3.07e-08 to 9.82e-08 |
| 14h | 5.8E-07 | 1.74e-07 to 1.87e-06 |
| 9y | 3.3E-07 | 9.91e-08 to 1.05e-06 |
| 15d | 2.6E-07 | 2.80e-08 to 2.45e-06 |
| 9x | 4.0E-07 | 3.02e-07 to 5.26e-07 |
| 15c | 2.1E-07 | 1.34e-07 to 3.25e-07 |
| 15m | 9.6E-09 | 4.05e-09 to 2.25e-08 |
| 15g | 1.7E-08 | 3.90e-09 to 7.14e-08 |
| 15i | 1.2E-08 | 4.36e-09 to 3.14e-08 |
| 15k | 3.0E-07 | 1.56e-07 to 6.18e-07 |
| 15o | 2.1E-08 | 1.48e-08 to 3.06e-08 |
| 15p | 3.6E-07 | 9.55e-08 to 1.31e-06 |
| 9o | 6.4E-07 | 1.08e-07 to 3.69e-06 |
| 9q | 3.5E-07 | 4.29e-08 to 1.73e-06 |
| 9n | 4.0E-07 | 2.71e-07 to 6.09e-07 |
| 17d | 5.3E-07 | 2.71e-07 to 1.10e-06 |
| 9ac | 1.3E-07 | 8.58e-08 to 2.01e-07 |
| 9aa | 2.1E-07 | 1.27e-07 to 3.42e-07 |
| 9af | 2.3E-07 | 1.69e-07 to 3.22e-07 |

| ID | IC₅₀(M) | 95% Confidence Intervals (M) |
|-----------|---------------------------|-------------------------------------|
| 9ad | 9.5E-07 | 7.94e-07 to 1.16e-06 |
| 9ag | 1.4E-06 | 6.48e-08 to 1.74e-05 |
| 9ah | 2.1E-06 | 1.80e-06 to 2.48e-06 |
| 9r | 3.2E-07 | 2.29e-07 to 4.78e-07 |
| 9s | 2.7E-07 | 1.15e-07 to 6.54e-07 |
| 16d | 3.6E-07 | 1.40e-07 to 3.98e-07 |
| 17i | 2.7E-08 | 2.59e-08 to 2.87e-08 |
| 17n | 1.8E-08 | 1.55e-08 to 2.07e-08 |
| 9ab | 5.2E-08 | 5.19e-08 to 5.23e-08 |
| 9z | 7.0E-08 | 2.07e-08 to 2.19e-07 |
| 16i | 6.0E-09 | 5.10e-09 to 7.08e-09 |
| 9l | 5.8E-07 | 6.19e-08 to 8.39e-06 |
| 9i | 7.5E-07 | 7.84e-08 to 6.94e-06 |
| 9j | 1.5E-06 | 1.16e-06 to 1.99e-06 |
| 9h | 1.1E-06 | 2.63e-07 to 4.70e-06 |
| 9k | 5.3E-07 | 3.69e-07 to 7.96e-07 |
| 9ae | 4.9E-07 | 3.14e-07 to 6.54e-07 |
| 17e | 5.4E-07 | 2.69e-08 to 1.11e-05 |
| 17j | 4.0E-08 | 2.43e-08 to 6.55e-08 |
| 9d | 2.3E-07 | 5.02e-08 to 9.88e-07 |
| 9w | 3.6E-07 | 2.23e-08 to 5.65e-06 |

Cellular GSK-3 β assay results.

Table S. I. 2. IC₅₀ and 95% Confidence Intervals

| ID | IC₅₀ (M) | 95% Confidence Intervals (M) |
|-----------|----------------------------|-------------------------------------|
| 14o | 7.8E-07 | 6.29e-07 to 9.78e-07 |
| 9p | 1.4E-06 | 7.59e-07 to 2.55e-06 |
| 9af | 8.2E-07 | 3.86e-07 to 1.76e-06 |
| 9ac | 2.1E-06 | 1.44e-06 to 5.76e-06 |
| 9aa | 5.2E-06 | 3.51e-06 to 2.15e-05 |
| 14i | 3.5E-07 | 3.40e-07 to 4.63e-07 |
| 14n | 8.0E-07 | 7.02e-07 to 9.10e-07 |
| 16i | >100 | (Very wide) |
| 15o | 7.1E-05 | 1.31e-05 to 2.84e-04 |

Kinase selectivity results for 14i.

Table S. I. 3. Percent of inhibition of **14i** (@ 10 μ M) and standard deviation for a 216 kinases panel.

| Assay ID | % Inhib. | S. D. |
|---------------------------|----------|-------|
| Abl kinase (h) | -7 | 8 |
| Ack (h) | 7 | 5 |
| ALK (h) | 78 | 13 |
| Akt1/PKBalpha (h) | -1 | 1 |
| Akt2/PKBBeta (h) | -2 | 1 |
| Akt3/PKBgamma (h) | 4 | 4 |
| ALK4 (h) (ACVR1B) | -1 | 2 |
| AMPKalpha (h) | 68 | 8 |
| Arg kinase (h) | -21 | 5 |
| ASK1 (h) | 50 | 1 |
| AurA/Aur2 kinase (h) | 10 | 1 |
| AurB/Aur1 kinase (h) | 0 | 6 |
| AurC/Aur3 kinase (h) | -1 | 3 |
| Axl kinase (h) | 33 | 2 |
| BMPRI1A (h) (ALK3) | 4 | 9 |
| Brk (h) | -3 | 1 |
| BRSK1 (h) | 50 | 5 |
| CaMK1alpha (h) | 40 | 4 |
| CaMK1delta (h) | 52 | 8 |
| CaMK2alpha (h) | 3 | 5 |
| CaMK4 (h) | 4 | 4 |
| CDC2/CDK1 (h) (cycB) | 80 | 4 |
| CDC7 /ASK (h) | 7 | 2 |
| CDK2 (h) (cycA) | 92 | 0 |
| CDK3 (h) (cycE1) | 55 | 0 |
| CDK4 (h) (cycD1) | 48 | 3 |
| CDK5 /p35 (h) | 77 | 2 |
| CDK6 (h) (cycD3) | 47 | 5 |
| CDK7 /MAT1 (h) (cycH) | 82 | 2 |
| CDK8 (h) (cycC) | -1 | 2 |
| CDK9 (h) (cycT1) | 91 | 0 |
| CHK1 (h) | -2 | 3 |
| CHK2 (h) | 2 | 6 |
| CK1alpha (h) | 6 | 2 |
| CK2 (h) (casein kinase 2) | 14 | 10 |
| c-kit kinase (h) | 7 | 2 |
| CLK1 (h) | 99 | 0 |
| CLK2 (h) | 83 | 2 |
| c-Met kinase (h) | 7 | 2 |
| COT kinase (h) (MAP3K8) | -9 | 0 |
| CRIK (h) | -4 | 0 |
| DAPK1 (h) | 47 | 4 |
| DAPK2 (h) | 17 | 3 |

| | | |
|---------------------------|-----|----|
| DCAMKL1 (h) | -51 | 3 |
| DCAMKL2 (h) | 24 | 8 |
| DDR2 kinase (h) | -8 | 4 |
| DLK1 (h) (MAP3K12) | 58 | 1 |
| DRAK1 (h) | 40 | 0 |
| DYRK1a (h) | 99 | 1 |
| DYRK2 (h) | 81 | 3 |
| DYRK4 (h) | -6 | 13 |
| EGFR kinase (h) | 1 | 1 |
| EphA1 kinase (h) | -4 | 10 |
| EphA2 kinase (h) | -25 | 5 |
| EphA3 kinase (h) | -12 | 3 |
| EphA4 kinase (h) | -4 | 3 |
| EphA5 kinase (h) | -28 | 6 |
| EphA7 kinase (h) | 8 | 2 |
| EphB1 kinase (h) | 30 | 16 |
| EphB2 kinase (h) | 2 | 2 |
| EphB3 kinase (h) | -12 | 11 |
| EphB4 kinase (h) | -1 | 10 |
| ERK1 (h) | 66 | 2 |
| ERK2 (h) (P42mapk) | 74 | 3 |
| ERK5 (h) (MAPK7) | 97 | 1 |
| FAK (h) | -10 | 3 |
| Fes kinase (h) | 20 | 7 |
| Fer kinase (h) | -49 | 12 |
| FGFR1 kinase (h) | 8 | 0 |
| FGFR2 kinase (h) | 5 | 1 |
| FGFR3 kinase (h) | 20 | 6 |
| FGFR4 kinase (h) | 2 | 6 |
| Fgr kinase (h) | -15 | 3 |
| FLT-1 kinase (h) (VEGFR1) | -11 | 4 |
| FLT-3 kinase (h) | 33 | 5 |
| FLT-4 kinase (h) (VEGFR3) | 33 | 10 |
| Fms/CSFR kinase (h) | -6 | 5 |
| FRK (h) | 22 | 3 |
| Fyn kinase (h) | -18 | 1 |
| GCK (h) (MAP4K2) | 40 | 5 |
| GRK2 (h) (ADRBK1) | 80 | 0 |
| GRK3 /BARK2 (h) (ADRBK2) | 8 | 3 |
| GSK3alpha (h) | 98 | 1 |
| GSK3beta (h) | 99 | 0 |
| HER2/ErbB2 kinase (h) | -1 | 2 |
| HGK (h) (MAP4K4) | 72 | 5 |
| HIPK2 (h) | 40 | 0 |
| HIPK4 (h) | 32 | 2 |

| | | |
|-------------------------------|-----|----|
| IGF1R kinase (h) | 3 | 10 |
| IKKalpha (h) | 1 | 1 |
| IKKbeta (h) | 19 | 2 |
| IKKepsilon (h) (IKBKE) | 3 | 6 |
| IRAK1 (h) | 12 | 2 |
| IRAK4 (h) | -5 | 1 |
| IRK (h) (InsR) | 9 | 3 |
| IRR kinase (h) | -10 | 1 |
| ITK (h) | -4 | 3 |
| JAK1 (h) | 7 | 3 |
| JAK2 (h) | 23 | 1 |
| JAK3 (h) | 25 | 0 |
| JNK1 (h) | 82 | 2 |
| JNK2 (h) | 90 | 2 |
| JNK3 (h) | 72 | 5 |
| KDR kinase (h) (VEGFR2) | 49 | 0 |
| Lck kinase (h) | 11 | 3 |
| LIMK1 (h) | 3 | 1 |
| LTK (h) | 34 | 3 |
| Lyn A kinase (h) | -14 | 3 |
| Lyn B kinase (h) | 98 | 1 |
| MEK5 (h) (MAP2K5) | 5 | 0 |
| MEKK4 (h) (MAP3K4) | 14 | 1 |
| MAPKAPK2 (h) | 17 | 7 |
| MAPKAPK5 (h) (PRAK) | 79 | 1 |
| MARK1 (h) | 52 | 2 |
| MARK2 (h) | 66 | 4 |
| MARK3 (h) | 41 | 2 |
| MARK4 (h) | 74 | 2 |
| MEKK3 (h) (MAP3K3) | 9 | 6 |
| NIM1 kinase (h) (MGC42105) | 5 | 1 |
| MINK (h) | 0 | 2 |
| MKK6 (h) | -1 | 2 |
| MLK1 (h) | 8 | 7 |
| MNK1 (h) | 17 | 4 |
| MLK2 (h) (MAP3K10) | 23 | 6 |
| MNK2 (h) | 4 | 2 |
| MOS kinase (h) | -13 | 1 |
| MRCKalpha (h) | 5 | 6 |
| MSK2 (h) | -5 | 1 |
| MST1 kinase (STK4) (h) | 3 | 0 |
| MST2 kinase (h) | 3 | 3 |
| MST3 kinase (h) | 81 | 2 |
| MST4 kinase (h) | 75 | 0 |

| | | |
|-----------------------|-----|----|
| MusK (h) | 24 | 7 |
| MYT1 kinase (h) | 20 | 2 |
| NDR1 kinase (h) | 6 | 0 |
| NEK2 (h) | -4 | 4 |
| NEK4 (h) | -7 | 1 |
| NEK6 (h) | -40 | 9 |
| NEK7 (h) | -18 | 0 |
| NIK (h) | 11 | 3 |
| NuaK1 (h) (ARK5) | 31 | 4 |
| p38alpha kinase (h) | 1 | 1 |
| p38delta kinase (h) | 18 | 9 |
| p70S6K (h) | 14 | 1 |
| p70S6Kbeta (h) | -1 | 2 |
| PAK1 (h) | 3 | 4 |
| PAK2 (h) | 8 | 7 |
| PAK4 (h) | 32 | 6 |
| PASK (h) | 6 | 4 |
| PCTAIRE1 kinase (h) | 84 | 5 |
| PDGFRalpha kinase (h) | 26 | 7 |
| PDGFRbeta kinase (h) | 27 | 4 |
| PDK1 (h) | -8 | 2 |
| PEK (h) (EIF2AK3) | 11 | 0 |
| PhKgamma 1 (h) | 35 | 1 |
| PhKgamma 2 (h) | 0 | 1 |
| Pim1 kinase (h) | 17 | 3 |
| Pim2 kinase (h) | -6 | 3 |
| PKA (h) | -3 | 3 |
| PKCalpha (h) | 19 | 2 |
| PKCbeta 1 (h) | 28 | 3 |
| PKCbeta 2 (h) | 8 | 13 |
| PKCgamma (h) | -2 | 3 |
| PKCdelta (h) | -2 | 3 |
| PKCzeta (h) | 14 | 3 |
| PKCeta (h) | -9 | 3 |
| PKCtheta (h) | 22 | 1 |
| PKD2 (h) | 35 | 2 |
| PKD3 (h) | 90 | 3 |
| PKG1alpha (h) | 6 | 0 |
| PKG1beta (h) | 4 | 3 |
| PKG2 (h) | 5 | 1 |
| PKN1 (h) | 37 | 3 |
| PKN2 (h) | 45 | 4 |
| PKR (h) (EIF2AK2) | 1 | 0 |
| PLK1 (h) | 1 | 5 |
| PLK2 (h) | 9 | 1 |

| | | |
|------------------------|-----|----|
| PLK4 (h) | 44 | 9 |
| PRKX (h) | 4 | 4 |
| PYK2 (h) | 13 | 0 |
| B-Raf kinase (h) | 2 | 10 |
| RAF-1 kinase (h) | 19 | 3 |
| Ret kinase (h) | -5 | 8 |
| RIPK2 (h) | 18 | 3 |
| ROCK1 (h) | -2 | 0 |
| ROCK2 (h) | 7 | 6 |
| Ron kinase (h) | -13 | 5 |
| RSK1 (h) | -20 | 0 |
| RSK2 (h) | 4 | 2 |
| RSK3 (h) | 1 | 2 |
| SGK1 (h) | 7 | 4 |
| SGK3 (h) | -1 | 2 |
| SIK (h) | 19 | 6 |
| Src kinase (h) | -9 | 6 |
| STK33 (h) | 30 | 1 |
| Syk (h) | 1 | 3 |
| TAK1-TAB1 (h) (MAP3K7) | 37 | 3 |
| TAOK2 (TAO1) (h) | 79 | 1 |
| TBK1 (h) | 8 | 6 |
| TIE2 kinase (h) | -21 | 28 |
| Tnk1 (h) | 20 | 5 |
| TRKA (h) | 14 | 2 |
| TRKB (h) | 38 | 6 |
| TRKC (h) | -1 | 25 |
| TSSK1 (h) | 8 | 5 |
| TTK (h) | 14 | 5 |
| TXK (h) | 18 | 9 |
| Tyk2 (h) (JTK1) | 13 | 5 |
| Tyro3 /Sky kinase (h) | 3 | 3 |
| ULK1 (h) | 11 | 1 |
| Wee1 kinase (h) | 9 | 2 |
| WNK2 (h) | 6 | 0 |
| WNK3 (h) | 0 | 0 |
| WNK4 (h) | 9 | 0 |
| Yes kinase (h) | 1 | 3 |
| ZAP70 kinase (h) | -14 | 1 |

Table S. I. 4. IC₅₀ values for the selected kinases.

| Assay ID | IC ₅₀ (M) | 95% Confidence Intervals (M) |
|-----------------------|----------------------|------------------------------|
| ALK (h) | 2.0E-05 | 1.77e-05 to 2.15e-05 |
| AMPKalpha (h) | 6.3E-06 | 4.05e-06 to 1.21e-05 |
| BRSK1 (h) | 9.1E-06 | 5.56e-06 to 3.75e-05 |
| CaMK1delta (h) | 4.4E-06 | 2.68e-06 to 6.11e-06 |
| CDC2/CDK1 (h) (cycB) | 6.1E-06 | 3.98e-06 to 7.49e-06 |
| CDK2 (h) (cycA) | 3.5E-06 | 2.37e-06 to 4.96e-06 |
| CDK3 (h) (cycE1) | 4.8E-06 | 3.51e-06 to 1.11e-05 |
| CDK5 /p35 (h) | 3.4E-06 | 2.61e-06 to 6.73e-06 |
| CDK7 /MAT1 (h) (cycH) | 1.1E-05 | 9.28e-06 to 1.81e-05 |
| CDK9 (h) (cycT1) | 1.3E-06 | 9.08e-07 to 1.68e-06 |
| CLK1 (h) | 7.1E-08 | 6.90e-08 to 1.07e-07 |
| CLK2 (h) | 9.5E-07 | 5.92e-07 to 1.66e-06 |
| DLK1 (h) (MAP3K12) | 1.1E-05 | 6.04e-06 to 2.02e-05 |
| DYRK1a (h) | 4.0E-08 | 3.00e-08 to 6.82e-08 |
| DYRK2 (h) | 5.3E-07 | 4.24e-07 to 5.71e-07 |
| ERK1 (h) | 1.2E-05 | 6.49e-06 to 2.75e-05 |
| ERK2 (h) (P42mapk) | 6.0E-06 | 3.63e-06 to 1.38e-05 |
| ERK5 (h) (MAPK7) | 2.2E-07 | 2.06e-07 to 3.45e-07 |
| GRK2 (h) (ADRBK1) | 5.3E-07 | 2.83e-07 to 5.64e-07 |
| GSK3alpha (h) | 4.0E-08 | 3.31e-08 to 5.12e-08 |
| HGK (h) (MAP4K4) | 4.4E-06 | 1.84e-06 to 7.18e-06 |
| JNK1 (h) | 3.2E-06 | 1.72e-06 to 8.79e-06 |
| JNK2 (h) | 1.2E-06 | 8.41e-07 to 1.53e-06 |
| JNK3 (h) | 5.8E-06 | 5.17e-06 to 6.28e-06 |
| Lyn B kinase (h) | 1.7E-07 | 1.26e-07 to 3.31e-07 |
| MAPKAPK5 (h) (PRAK) | 1.8E-06 | 1.08e-06 to 7.91e-06 |
| MARK1 (h) | 1.3E-05 | 6.60e-06 to 2.65e-05 |

| Assay ID | IC ₅₀ (M) | 95% Confidence Intervals (M) |
|---------------------|----------------------|------------------------------|
| MARK2 (h) | 3.7E-06 | 1.10e-06 to 6.42e-06 |
| MARK4 (h) | 4.5E-06 | 3.12e-06 to 5.75e-06 |
| MST3 kinase (h) | 6.0E-06 | 3.33e-06 to 1.46e-05 |
| MST4 kinase (h) | 1.1E-05 | 7.18e-06 to 2.29e-05 |
| PCTAIRE1 kinase (h) | 9.4E-07 | 6.81e-07 to 2.45e-06 |
| PKD3 (h) | 2.1E-06 | 9.25e-07 to 7.22e-06 |
| TAOK2 (TAO1) (h) | 5.6E-06 | 2.76e-06 to 8.91e-06 |

Crystallization conditions and X-ray data collection.

Crystals were obtained using hanging drop vapour diffusion set-ups. 1 µl of human purified GSK-3β protein solution (9.6 mg/ml in 50 mM MES, 175 mM NaCl, 10 mM DTT, 7 mM MgCl₂, 5 % glycerol, pH 6.5, incubated with 5-fold molar excess of compound **5** for 1h) was mixed with 1 µl of reservoir solution (10 % (w/v) PEG 4000, 10 % (v/v) 2-propanol, 0.1 M HEPES/NaOH, pH 7.5) and equilibrated over 0.4 ml of reservoir solution. Small crystals appeared overnight and grew to full size within a few days. Data were collected from one crystal by ID14.4 beamline at the ESRF synchrotron radiation source (Grenoble, France). A complete data set could be collected to a resolution of 2.5 Å but because of the high mosaicity leading to a strongly increasing Rmerge during the measurement, the completeness of the data included in refinement was reduced to 80 %. However, the overall Rmeas remained very high with a value of 0.19. Nevertheless, the quality of the data set was sufficient for structure determination. Several rounds of alternating manual rebuilding and refinement with REFMAC5 resulted in the final model. The published structure of GSK-3β with the PDB accession code 3F88 was used as a starting model for molecular replacement [see: Saitoh, M.; Kunitomo, J.; Kimura, E.; Hayase, Y.; Kobayashi, H.; Uchiyama, N.; Kawamoto, T.; Tanaka, T.; Mol, C. D.; Dougan, D. R.; Textor, G. S.; Snell, G. P.; Itoh, F., Design, synthesis and structure-activity relationships of 1,3,4-oxadiazole derivatives as novel inhibitors of glycogen synthase kinase-3beta. *Bioorganic & Medicinal Chemistry* **2009**, 17 (5), 2017-29]