

Short Note

(S)-3-(3-((7-Ethynyl-9H-pyrimido[4,5-*b*]indol-4-yl)amino)piperidin-1-yl)propanenitrile

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Abstract: The title compound (S)-3-(3-((7-ethynyl-9H-pyrimido[4,5-*b*]indol-4-yl)amino)piperidin-1-yl)propanenitrile (**2**) was synthesized in five steps, starting from 4-chloro-7-iodo-9H-pyrimido[4,5-*b*]indole (**3**), and was characterized by ¹H-NMR, ¹³C-NMR, MS and HPLC. Moreover, its structure was confirmed by single crystal X-ray diffraction. Pyrimido[4,5-*b*]indole **2** demonstrated an IC₅₀ value of 2.24 μM in a NanoBRET™ TE intracellular glycogen synthase kinase-3β assay.

Keywords: kinase inhibitor; glycogen synthase kinase 3β; tricyclic heterocycle; pyrimido[4,5-*b*]indole; single crystal diffraction

1. Introduction

Glycogen synthase kinase-3β (GSK-3β) is a highly multi-tasking serine/threonine kinase, which has been associated with various pathologic conditions including type II diabetes, cancer, cardiac hypertrophy and neurodegeneration [1]. Consequently, this disease-relevant enzyme is under ongoing investigation as a potential target for the development of novel drug candidates [2].

We recently reported on the optimization of pyrimido[4,5-*b*]indole-based inhibitors of GSK-3β, leading to the discovery of lead compound **1**, which displayed a micromolar IC₅₀ value in a NanoBRET™ target engagement (TE) intracellular GSK-3β assay (Promega) (Figure 1) [3,4]. To assess the contribution of the methyl group to the cellular target engagement potency of **1**, this substituent was removed, resulting in the title compound **2**.

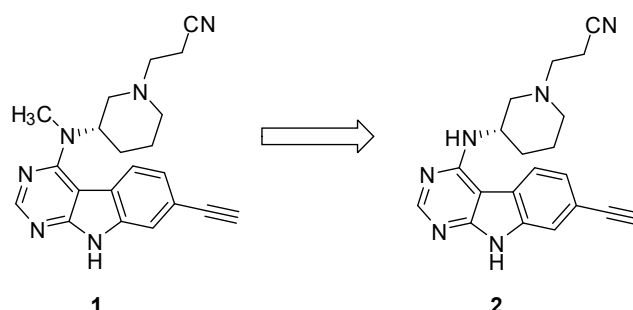


Figure 1. Structure of GSK-3β inhibitor **1** and the design of the title compound **2**.

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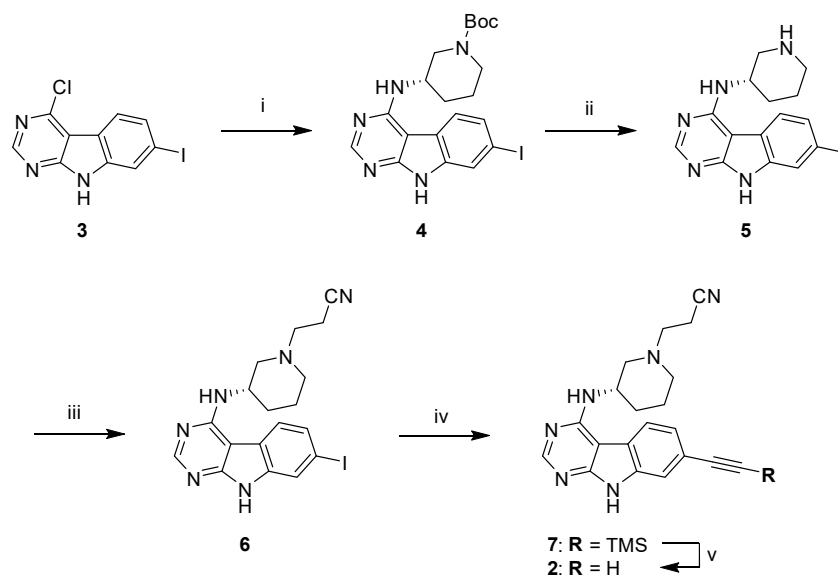


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2. Results and Discussion

2.1. Chemistry

The title compound **2** was prepared starting from the previously reported 4-chloro-7-iodo-9*H*-pyrimido[4,5-*b*]indole (**3**) [5], which was treated with an excess of commercially available *tert*-butyl (*S*)-3-aminopiperidine-1-carboxylate and DIPEA in *n*-BuOH [6] (Scheme 1). Substitution product **4** underwent Boc deprotection under acidic conditions, resulting in free piperidine **5**, which was reacted with acrylonitrile in MeOH to introduce the cyanoethyl substituent (**6**). The Sonogashira coupling of intermediate **6** with TMS-acetylene and the subsequent TMS-deprotection finally delivered the desired product **2**.



Scheme 1. Synthesis of (*S*)-3-(3-((7-ethynyl-9*H*-pyrimido[4,5-*b*]indol-4-yl)amino)piperidin-1-yl)propanenitrile (**2**). Reagents and conditions: (i) *tert*-butyl (*S*)-3-aminopiperidine-1-carboxylate, DIPEA, *n*-BuOH, 120 °C; (ii) TFA, DCM, rt; (iii) acrylonitrile, MeOH, rt; (iv) TMS-acetylene, PdCl₂(PPh₃)₂, CuI, TEA, DMF, rt to 30 °C; (v) K₂CO₃, MeOH, rt.

Pyrimido[4,5-*b*]indole **2** was analyzed by ¹H-nuclear magnetic resonance spectroscopy (NMR), ¹³C-NMR, MS and high performance liquid chromatography (HPLC) (for details, see supplementary materials). Additionally, its chemical structure was confirmed by single crystal X-ray diffraction (Figure 2).

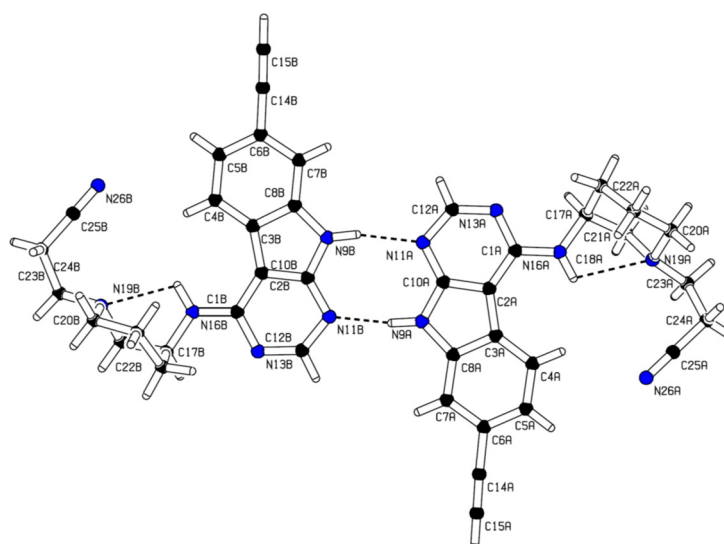


Figure 2. X-ray crystal structure of title compound **2**.

2.2. X-ray Structure

The three-dimensional network within the crystal packing of compound **2** features diverse inter- and intramolecular polar interactions (Figure 3). These include bidentate hydrogen bonds between the tricyclic scaffolds of adjacent molecules, as well as intramolecular interactions between the secondary aryl amine N-H group and the piperidine nitrogen.

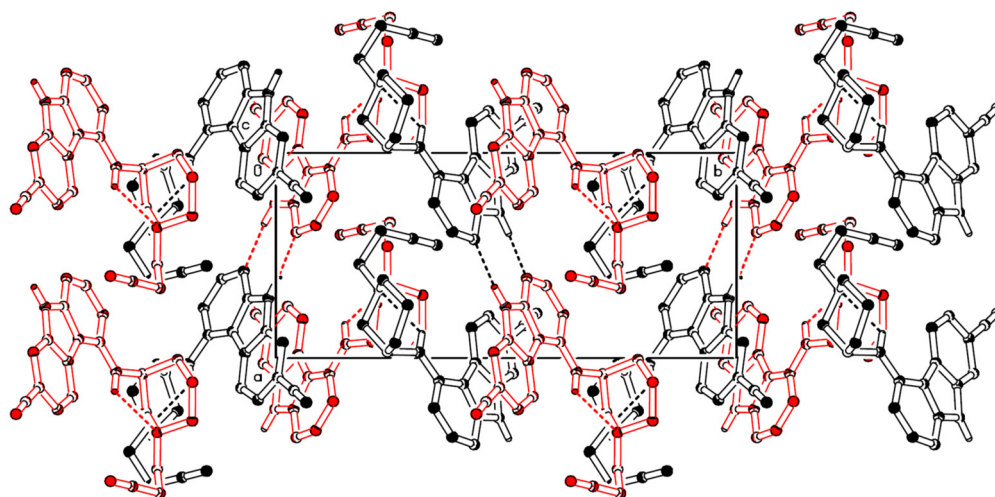


Figure 3. Partial packing diagram of title compound **2**. Hydrogen bonds are depicted as dashed lines.

2.3. Biological Evaluation

Pyrimido[4,5-*b*]indole **2** was evaluated in a NanoBRET™ TE intracellular GSK-3β assay [4], where it displayed a two-fold higher IC₅₀ value compared to parent compound **1** (Table 1). These data indicate that the removal of the methyl group at the piperidinyl amino function resulted in reduced cellular target engagement potency.

Table 1. Evaluation of pyrimido[4,5-*b*]indoles **1** and **2** in a NanoBRET™ TE intracellular GSK-3β assay.

| Compound | IC ₅₀ (μM ± SEM) ^a |
|----------|--|
| 1 | 1.18 ± 0.03 |
| 2 | 2.24 ± 0.17 |

^a IC₅₀ values are mean values from two independent experiments under identical conditions ± SEM.

3. Materials and Methods

3.1. General

All reagents and solvents were of commercial quality and utilized without further purification. The chromatographic retention times of all the reported compounds and the purity of the title compound **2** were determined on an Agilent 1100 Series HPLC system, equipped with an ultraviolet diode array detector (detection at 254 and 230 nm) from Agilent Technologies (Santa Clara, CA, USA). The chromatographic separation was carried out on a XBridge™ C18 column (150 × 4.6 mm, 5 μm) from Waters (Milford, MA, USA). The injection volume was 5 μL and the flow was 1.5 mL/min, using the following gradient: 0.01 M KH₂PO₄, pH 2.3 (mobile phase A), MeOH (mobile phase B), 40% B to 85% B in 8 min; 85% B for 5 min; 85% B to 40% B in 2 min; stop time 16 min. Column chromatography was performed on Geduran Si₆₀ 40–63 μm silica from Merck (Darmstadt, Germany) or commercial 50 μm silica columns from Interchim (Montluçon, France), using an Interchim PuriFlash XS520Plus automated flash chromatography system. NMR spectra were

measured on an Avance 300 MHz or an Avance 400 MHz NMR spectrometer from Bruker (Billerica, MA, USA). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane. All spectra were calibrated against the (residual proton) peak of the deuterated solvent. Mass spectrometry was carried out on an Agilent 6540 UHD Accurate-Mass Q-TOF liquid chromatography coupled electrospray ionization mass spectrometer (LC-ESI-MS) from Agilent Technologies (Santa Clara, CA, USA), at the analytical department of the University of Regensburg. X-ray diffraction data were collected on a STOE IPDS 2T diffractometer (STOE & Cie, Darmstadt, Germany) using monochromated Mo K α radiation (0.71073 Å).

3.2. Chemistry

3.2.1. *tert*-Butyl (S)-3-((7-iodo-9H-pyrimido[4,5-*b*]indol-4-yl)amino)piperidine-1-carboxylate (4)

4-Chloro-7-iodo-9H-pyrimido[4,5-*b*]indole (3) (170.0 mg, 0.52 mmol), *tert*-butyl (S)-3-aminopiperidine-1-carboxylate (206.6 mg, 1.03 mmol) and DIPEA (200.0 mg, 1.55 mmol) were stirred in *n*-BuOH at 120 °C for 24 h. After cooling down to room temperature (rt), the mixture was diluted with EtOAc (40 mL) and washed with brine (3 × 20 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, DCM-MeOH gradient elution from 97.5–2.5 to 93.5–6.5) to obtain 193 mg of the title compound as a brown solid (76% yield). ¹H-NMR (400 MHz, DMSO-*d*₆) δ 12.00 (s, 1H), 8.38 (s, 1H), 8.14 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 1.2 Hz, 1H), 7.55 (dd, *J* = 8.3, 1.3 Hz, 1H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.39–4.24 (m, 1H), 4.13–3.90 (m, 1H), 3.87–3.72 (m, 1H), 3.19–2.80 (m, 2H), 2.04–1.93 (m, 1H), 1.88–1.70 (m, 2H), 1.50–1.25 (m, 10H); ESI-MS (*m/z*) 494.1 [M+H]⁺; HPLC *t*_r = 9.515 min.

3.2.2. (S)-7-Iodo-N-(piperidin-3-yl)-9H-pyrimido[4,5-*b*]indol-4-amine (5)

tert-Butyl (S)-3-((7-iodo-9H-pyrimido[4,5-*b*]indol-4-yl)amino)piperidine-1-carboxylate (4) (185.0 mg, 0.375 mmol) was stirred in a mixture of dry DCM (5 mL) and trifluoroacetic acid (1 mL) at rt for 1.5 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (SiO₂, DCM-(2N NH₃ in MeOH) 9-1) to obtain 126 mg of the title compound as a light beige solid (86% yield). ¹H-NMR (300 MHz, MeOD) δ 8.33 (s, 1H), 7.96 (d, *J* = 8.3 Hz, 1H), 7.86 (d, *J* = 1.4 Hz, 1H), 7.59 (dd, *J* = 8.3, 1.5 Hz, 1H), 4.51–4.38 (m, 1H), 3.31–3.24 (m, 1H), 3.02–2.93 (m, 1H), 2.77–2.60 (m, 2H), 2.16–2.06 (m, 1H), 1.89–1.60 (m, 3H); ESI-MS (*m/z*) 394.1 [M+H]⁺; HPLC *t*_r = 4.847 min.

3.2.3. (S)-3-(3-((7-Iodo-9H-pyrimido[4,5-*b*]indol-4-yl)amino)piperidin-1-yl)propanenitrile (6)

(S)-7-Iodo-N-(piperidin-3-yl)-9H-pyrimido[4,5-*b*]indol-4-amine (5), TEA (64.0 mg, 0.631 mmol) and acrylonitrile (20.1 mg, 0.378 mmol) were stirred in MeOH of HPLC grade (15 mL) at rt for 16 h. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography (SiO₂, DCM-(EtOAc-MeOH 95-5) gradient elution from 3–1 to 1–4) to obtain 113 mg of the title compound as a light yellow solid (80% yield). ¹H-NMR (300 MHz, DMSO-*d*₆) δ 11.99 (s, 1H), 8.37 (s, 1H), 8.10 (d, *J* = 8.3 Hz, 1H), 7.77 (d, *J* = 1.4 Hz, 1H), 7.52 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.64 (d, *J* = 8.2 Hz, 1H), 4.57–4.43 (m, 1H), 2.93–2.82 (m, 1H), 2.77–2.58 (m, 5H), 2.41–2.32 (m, 1H), 2.28–2.18 (m, 1H), 1.86–1.47 (m, 4H); ESI-MS (*m/z*) 447.1 [M+H]⁺; HPLC *t*_r = 5.098 min.

3.2.4. (S)-3-(3-((7-((Trimethylsilyl)ethynyl)-9H-pyrimido[4,5-b]indol-4-yl)amino)piperidin-1-yl)propanenitrile (7)

(S)-3-(3-((7-Iodo-9H-pyrimido[4,5-b]indol-4-yl)amino)piperidin-1-yl)propanenitrile (6) (50.0 mg, 0.112 mmol) and CuI (3.6 mg, 0.019 mmol) were mixed with dry DMF in a reaction tube. The mixture was degassed by purging with an argon balloon. TEA (34.0 mg, 0.336 mmol), TMS-acetylene (33.0 mg, 0.336 mmol) and PdCl₂(PPh₃)₂ were added under argon atmosphere and the reaction tube was sealed. The mixture was stirred at rt for 40 min and then warmed to 30 °C by a water bath for another 40 min. The mixture was then diluted with EtOAc (40 mL) and washed with brine (3 × 20 mL), dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, DCM-MeOH 95-5) to obtain 41 mg of the title compound as a beige solid (88% yield). ESI-MS (*m/z*) 417.2 [M+H]⁺; HPLC *t_r* = 8.180 min.

3.2.5. (S)-3-(3-((7-Ethynyl-9H-pyrimido[4,5-b]indol-4-yl)amino)piperidin-1-yl)propanenitrile (2)

(S)-3-(3-((7-((Trimethylsilyl)ethynyl)-9H-pyrimido[4,5-b]indol-4-yl)amino)piperidin-1-yl)propanenitrile (7) (35.0 mg, 0.084 mmol) was suspended in MeOH of HPLC grade (20 mL) and the mixture was shortly warmed to 50 °C to improve the solubility of the reactant. K₂CO₃ (15.1 mg, 0.109 mmol) was added and the mixture was stirred at rt for 5 h, and then concentrated under reduced pressure. EtOAc (40 mL) was added to the residue, as well as small amounts of MeOH to improve the solubility. The mixture was washed with brine (3 × 20 mL), dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO₂, DCM-MeOH gradient elution from 97–3 to 93–7) to obtain 19 mg of the title compound as a beige solid (66% yield). ¹H-NMR (400 MHz, DMSO-*d*₆) δ 12.01 (s, 1H), 8.37 (s, 1H), 8.26 (d, *J* = 8.2 Hz, 1H), 7.52 (d, *J* = 1.0 Hz, 1H), 7.31 (dd, *J* = 8.1, 1.4 Hz, 1H), 6.69 (d, *J* = 8.0 Hz, 1H), 4.56–4.46 (m, 1H), 4.17 (s, 1H), 2.95–2.83 (m, 1H), 2.77–2.57 (m, 5H), 2.45–2.34 (m, 1H), 2.31–2.19 (m, 1H), 1.85–1.64 (m, 3H), 1.61–1.50 (m, 1H); ¹³C-NMR (101 MHz, DMSO-*d*₆) δ 156.04, 155.96, 155.4, 135.8, 123.7, 121.4, 120.2, 119.9, 117.2, 114.2, 95.4, 84.5, 80.1, 57.6, 52.9, 52.4, 46.5, 29.1, 23.3, 15.1; ESI-HRMS (*m/z*) calculated 345.1822 [M+H]⁺, found 345.1825 [M+H]⁺; HPLC *t_r* = 3.127 min. Single crystals suitable for X-ray diffraction were obtained by slow evaporation of a solution of compound 2 in CHCl₃/MeOH at rt. Crystal data for C₂₀H₂₀N₆ (*M_r* = 344.42 g mol^{−1}): monoclinic space group P2₁ (4), *a* = 8.2564 (3) Å, *b* = 17.6500 (7) Å, *c* = 12.8922 (4) Å, *V* = 1788.16 (11) Å³, *Z* = 4, *T* = 120 (2) K, μ(MoKα) = 8.825 mm^{−1}, *D_{calc}* = 1.279 Mg m^{−3}, 16354 reflections measured (2.31° ≤ 2θ ≤ 28.35°), 8489 unique (*R_{int}* = 0.0381) which were used in all calculations. CCDC 2190577 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (accessed on 30 August 2022).

3.3. Biological Evaluation

The NanoBRET™ TE intracellular GSK-3β assay was performed as previously described [4], with minor modifications in the preparation of the HEK293T cells. One day prior to the experiment, cells were treated with trypsin and centrifuged (210 g, 5 min). Afterwards, cells were resuspended in Leibovitz' L-15 medium supplemented with 5% FCS and 10 mM HEPES and were adjusted to a density of 300.000 cells per mL. Meanwhile, the transfection reagent was prepared. For a 96-well plate, 4 μg of transfection carrier DNA (Promega, Fitchburg, WI, USA) and 0.45 μg of NanoLuc®-GSK3B Fusion vector (Promega) were diluted in 450 μL of L-15 and 13.5 μL of X-tremeGENE™ HP (Roche Diagnostics, Mannheim, Germany) were added. The DNA complex was allowed to form by incubating it for 20 min at rt. Subsequently, the lipid DNA complex was added to 9 mL of the cell suspension. Then, 80 μL of this cell suspension were added to each well of a white 96-well plate (Brand, Wertheim, Germany). The plate was incubated at 37 °C for 24 h (no additional CO₂).

4. Conclusions

The synthesis of (S)-3-(3-((7-ethynyl-9H-pyrimido[4,5-b]indol-4-yl)amino)piperidin-1-yl)propanenitrile (**2**) was achieved by a five-step route, starting from 4-chloro-7-iodo-9H-pyrimido[4,5-b]indole (**3**). The analytical characterization of compound **2** comprised ¹H-NMR, ¹³C-NMR, MS, HPLC and single crystal X-ray diffraction. The novel compound demonstrated an IC₅₀ value of 2.24 μM in a NanoBRET™ TE intracellular GSK-3β assay, indicating a minor contribution of the methyl group present in parent compound **1** to the cellular target engagement potencies of these congeners.

Supplementary Materials: Figures S1–S5: Analytical characterization of title compound **2**, including ¹H-NMR, ¹³C-NMR, high-resolution ESI-MS and HPLC; Figures S6–S8: ¹H-NMR spectra of intermediates **4–6**; Figures S9–S12: Mass spectra of intermediates **4–7**.

Author Contributions: S.A., N.P., D.S. and P.K. conceived and designed the experiments; S.A. performed synthesis; N.P. carried out the biological assay; S.A., N.P., D.S. and P.K. analyzed the data; S.A. and P.K. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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