Supporting Information

Constituents of *Acacia nilotica* Delile with Novel Kinase Inhibitory Activity

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Isolation

The ethyl acetate extract (3 g), which was the most active against the protein kinases investigated, was subjected to silica gel column chromatography (40-63 µm; 50 × 3 cm) and eluted with a gradient of dichloromethane-methanol: 100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 80:20, 70:30, 60:40, 30:70, 10:90, and finally methanol (100%). Fractions of 50 mL were collected and monitored by TLC (ethyl acetate-chloroform 3:2, ethyl acetate-chloroform-methanol-water 15:8:4:1 and ethyl acetate-methanol-water 100:16.5:13.5). Fractions eluted with 5% methanol in dichloromethane (23-25), which showed a similar single spot on TLC using solvent system I and II, were pooled together to give compound 1 (11 mg). Fractions 27-29, also eluted with 5% methanol in dichloromethane, were subjected to gel filtration on Sephadex LH-20 in methanol to give compound 2 (13 mg).

Structural characterization

NMR spectra were recorded in CD$_3$OD on Bruker Avance 500 MHz and 125 MHz spectrophotometers for $^1$H and $^{13}$C-NMR, respectively, using TMS as the internal standard. UV spectra were recorded on a Pye-Unicam UV-Visible spectrophotometer. IR was performed on a Shimadzu spectrophotometer, while ES1-MS was recorded using an LCMS 1100 Agilent Finnigan LCQ DecaxP thermoquest. TLC was performed on a precoated TLC silica aluminum backed plate (0.2 mm) Silicycle, while pressurized column chromatography was carried out on silica gel G (200-400 mesh) Silicycle. MS analysis was performed employing an ESI-LTQ-orbitrap Discovery XL mass spectrometer (Thermo Scientific) connected to an Accela UHPLC.
system (Thermo Scientific). The UHPLC system was equipped with an autosampler, a vacuum degasser, a binary pump, and a temperature-controlled column. An ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 µm) reversed-phase column (Waters Corp.) was used for the analysis. The system was run in a binary gradient solvent mode consisting of 0.1% (v/v) formic acid/water (solvent A) and acetonitrile (solvent B). Sample analysis was carried out in both positive (ES+) and negative (ES-) ion modes. The flow rate was 0.4 mL/min. A gradient method of 32 min was used for metabolomic analysis as follows: 0 to 24 min: 95% A: 5% B; 24 to 28 min: 5% A: 95% B; 28-32 min: 95% A: 5% B.

**Protein kinase inhibitory studies**

The chloroform, ethyl acetate, and n-butanol fractions and purified compounds (1 and 2) were screened against a panel of diseased-related protein kinases. Kinase activity was assayed in appropriate buffer with either protein or peptide as the substrate in the presence of 15 µM \[\gamma^{33}\text{P}]\text{ATP} (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 µL following the assay described in [1]. Controls were performed with appropriate dilutions of dimethyl sulphoxide. Full-length kinases were used unless specified otherwise. Peptide substrates were obtained from Proteogenix.

**Buffers**

(A) 10 mM MgCl\(_2\), 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl, pH 7.5, 50 µg/mL heparin.
(B) 60 mM β-glycerophosphate, 30 mM p-nitrophenyl-phosphate, 25 mM MOPS (pH 7), 5 mM EGTA, 15 mM MgCl₂, 1 mM DTT, 0.1 mM sodium orthovanadate.

(D) 25 mM MOPS, pH 7.2, 12.5 mM β-glycerophosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT.

(H) MOPS 25 mM, pH 7.5, 10 mM MgCl₂.

(K) Tris 50 mM, pH 7.5, 20 mM MgCl₂, 2 mM MnCl₂.

(R) 1.67 mM MOPS, pH 7.2, 0.83 mM β-glycerophosphate, 1.33 mM MgCl₂, 0.83 mM MnCl₂, 0.33 mM EGTA, 0.13 mM EDTA, 16.67 μg/mL BSA, 0.017 mM DTT.

*HsCDK2/Cyclin A* (cyclin-dependent kinase 2, human; kindly provided by Dr. A. Echalier-Glazer, Leicester, UK) was assayed in buffer A (+0.15 mg/mL of BSA +0.23 mg/mL of DTT) with 0.8 μg/μL of histone H1 as the substrate.

*HsCDK9/Cyclin T* (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer A (+0.15 mg/mL of BSA +0.23 mg/mL of DDT) with 0.27 μg/μL of peptide YSPTSPYSPTSPSYSPTSPSKKKK as the substrate.

*HsCDK5/p25* (human, recombinant, expressed in bacteria) was assayed in buffer B, with 0.8 μg/μL of histone H1 as the substrate.
HsPIM1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed in buffer B, 0.8 µg/µL of histone H1 (Sigma #H5505) as the substrate.

HsHaspin-kd (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed in buffer H with 0.007 µg/µL of histone H3 (1-21) peptide ARTKQTARKSTGGKAPRKQLA as the substrate.

HsRIPK3 (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed in buffer R with 0.1 µg/µL of MBP as the substrate.

HsAuroraB (human, recombinant, expressed by baculovirus in Sf9 insects cells, Signal Chem, product #A31-10G) was assayed in buffer D with 0.2 µg/µL of MBP as the substrate.

SscGSK-3α,β (glycogen synthase kinase 3, porcine brain, native, affinity purified) was assayed in buffer A (+0.15 mg/mL of BSA +0.23 mg/mL of DTT) with 0.010 µg/µL of GS-1 peptide, a GSK-3 selective substrate (YRRAAVPPPSLSRHSSPHQSpEDEEE, “Sp” stands for phosphorylated serine).

SscCK1 δ/ε (casein kinase 1 δ/ε, porcine brain, native, affinity purified) was assayed in buffer B with 0.022 µg/µL of peptide RRKHAAILGSpAYSITA as the CK1-specific substrate).
RnDYRK1A-kd (*Rattus norvegicus*, amino acids 1 to 499 including the kinase domain, recombinant, expressed in bacteria, DNA vector provided by Dr. W. Becker, Aachen, Germany) was assayed in buffer A (+0.5 mg/mL of BSA +0.23 mg/mL of DTT) with 0.033 µg/µL of peptide KKISGRLSPIMTEQ as the substrate.

*Mm*CLK1 (from *Mus musculus*, recombinant, expressed in bacteria) was assayed in buffer A (+0.15 mg/mL of BSA + 0.23 mg/mL of DTT) with 0.027 µg/µL of peptide GRSRSRSRSRSR as the substrate.

*Pf*GSK-3 (from *Plasmodium falciparum*, recombinant, expressed in bacteria) was assayed in buffer A (+0.15 mg/mL of BSA +0.23 mg/mL of DTT) with 0.010 µg/µL of GS-1 peptide, a GSK-3 selective substrate (YRRAAVPPPSLISRHSPPHQSpEDEEE, “Sp” stands for phosphorylated serine).

*Ld*TLK (tousled-like kinase, from *Leishmania donovani*, recombinant, expressed in bacteria) was assayed in buffer K with 0.6 µg/µL of casein dephosphorylated from bovine milk (Sigma #C4032) as the substrate.

*Lm*CK1 (from *Leishmania major*, recombinant, expressed in bacteria was assayed in buffer B (adjusted at pH 8) with 0.028 µg/µL of peptide RRKHAAIGSpAYSITA as the CK1-specific substrate.
To validate the kinase assay, model inhibitors were used for each tested enzyme.

GSK’872 (#530389, purity ≥ 98%, Calbiochem) for HsRIPK3.

Barasertib (AZD1152-HQPA, #S1147, purity 97.31%, Selleckchem) for HsAuroraB.

Staurosporine from Streptomyces sp. (#S5921, purity ≥ 95%, Sigma-Aldrich) for SscCK1, LmCK1 and LdTLK.

Indirubin-3’-oxime (#I0404, purity ≥ 98%, Sigma-Aldrich) for SscGSK-3α,β, HsPIM1, CDKs, RnDYRK1A-kd, and MmCLK1.

CHR-6494 (#SML0648, purity ≥ 98%, Sigma-Aldrich) for HsHaspin-kd.

3-Amino-4-arylthieno[2,3-b]pyridine derivative called “5m” for PfGSK-3 [2].

Supplemental references
