

Short Note

3-Chlorokenpaullone

Oliver C. F. Orban, Ricarda S. Korn, Lisa Unger, Akim Yildiz and Conrad Kunick *

Institut für Medizinische und Pharmazeutische Chemie, Technische Universität Braunschweig, Beethovenstr. 55, 38106 Braunschweig, Germany

* Author to whom correspondence should be addressed; E-Mail: c.kunick@tu-braunschweig.de; Tel. +49-531-391-2754; Fax +49-531-391-2799.

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Abstract: 3-Chlorokenpaullone (9-bromo-3-chloro-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one) is a novel derivative of the protein kinase inhibitor kenpaullone. The title compound was synthesized by a Fischer indole reaction from 8-chloro-3,4-dihydro-1*H*-1-benzazepin-2,5-dione and 4-bromophenylhydrazine. It was characterized for structural identity by elemental analysis and spectroscopic methods (IR, NMR, EI-MS) and checked for purity by HPLC.

Keywords: kenpaullone; paullone; protein kinase inhibitor; Fischer indole synthesis

Kenpaullone (**1**; Figure 1) is the eponymous prototype of the paullones [1], a class of protein kinase inhibitors structurally based on the 7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one parent scaffold.

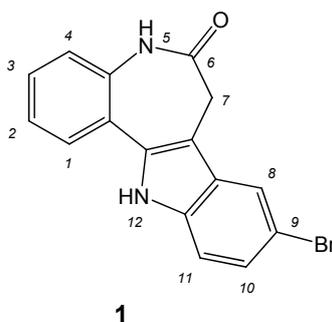
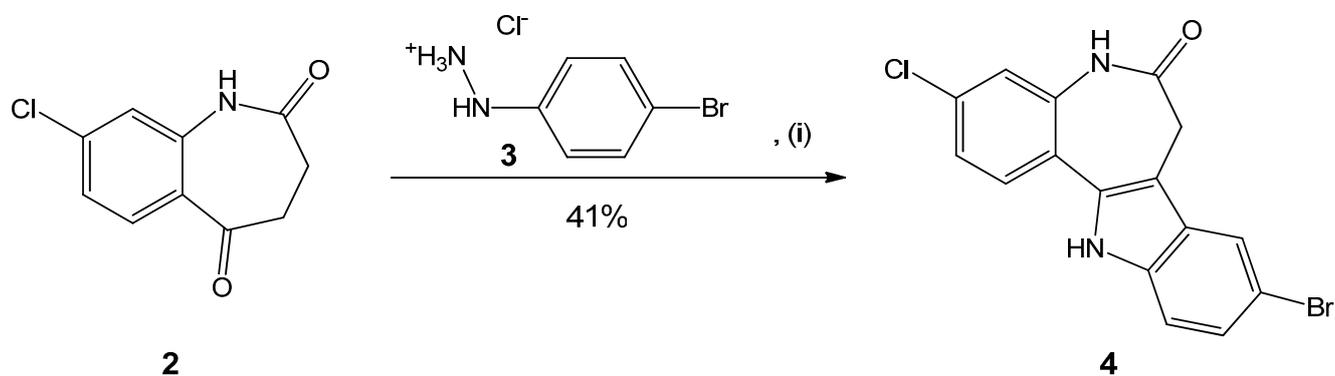


Figure 1. Structure of kenpaullone (**1**).

Kenpaullone was initially identified as a potent inhibitor of cyclin-dependent kinases (CDKs) [2]. Later it was shown that kenpaullone also potently inhibits glycogen synthase kinase-3 (GSK-3), a putative target for drugs against diabetes and neurodegenerative diseases [3]. In recent stem cell research it was discovered that kenpaullone is able to replace the factor Klf4 in reprogramming murine fibroblasts to pluripotent stem cells [4]. Subtle molecular modifications at the kenpaullone structure have a formidable impact on its pharmacological profile [5,6]. For instance, replacement of C(1) for a nitrogen atom leads to 1-azakenpaullone, a selective GSK-3 inhibitor [7]. Further exchange of the bromo substituent for a nitrile group furnishes cazpaullone, a compound protecting pancreatic beta cells against glucolipotoxicity [8]. Other paullones were shown to exhibit antiparasitic activity against *Leishmania* [9] and *Trypanosoma* [10,11] parasites. While classical paullones inhibit Sirt1, a NAD⁺-dependent class of histone deacetylases [12], it was recently reported that 7-methylene-kenpaullone activates the enzyme [13]. In the course of our structure-based development of selective protein kinase inhibitors [14,15] we were interested to investigate 3-chlorokenpaullone (**4**). To our surprise, a survey of the literature revealed that **4** had not been reported before, neither as characterized compound nor as exemplified prophetic compound in patents. Recently, highly innovative metal-catalyzed paullone syntheses have been reported [12,13,16,17]. However, for the sake of simplicity we employed a standard acid-induced Fischer indolization procedure [18] for the preparation of **4**, starting from 8-chloro-3,4-dihydro-1*H*-1-benzazepine-2,5-dione (**2**) [19] and commercially available 4-bromophenylhydrazine hydrochloride (**3**) (Scheme 1).



Scheme 1. Synthesis of 3-chlorokenpaullone (**4**) by Fischer indole ring closure.

Reagents and conditions: (i) 1. AcOH, NaOAc, 70 °C, 1 h; 2. AcOH, H₂SO₄, 70 °C, 1 h.

Results and Discussion

For the preparation of **4**, the cyclic ketone **2** [19] was reacted with 4-bromophenyl hydrazine hydrochloride in glacial acetic acid at 70 °C in a two-step, one-pot method. In the presence of sodium acetate a phenyl hydrazone was formed, which was directly cyclized upon addition of concentrated sulfuric acid. Without the need for chromatographic purification, a moderated yield of analytical pure product was collected after crystallization from ethanol. Both isocratic and gradient HPLC methods indicated a degree of purity of the crystallized compound sufficient for biological studies (>95%). As expected, the IR spectrum displayed absorption maxima for the N-H (3223 cm⁻¹) and C=O (1647 cm⁻¹) stretching vibrations. In the ¹H-NMR spectrum, the methylene group appeared as a singlet at 3.56 ppm,

indicating a rapid inversion of the two mirror-imaged pseudoboat conformations of the azepine ring, leading to isochrony of the methylene protons. Besides the expected signals for the fourteen aromatic carbons, the signals of the methylene carbon (31.4 ppm) and carbonyl carbon (171.4 ppm) were unambiguously identified in the ^{13}C -NMR spectrum. In the EI mass spectrum, a $[\text{M}-29]^+$ signal was observed. This cation results from the loss of a CHO unit from the molecular ion and is characteristic for paullones [10].

Experimental

General

4-Bromophenylhydrazine hydrochloride **3** was purchased from Sigma Aldrich. The melting point was detected in an open-glass capillary on an electric variable heater (Electrothermal IA 9100). The infrared spectrum was recorded on a Thermo Nicolet FT-IR 200 spectrometer using KBr pellets. The ^{13}C -NMR and the ^1H -NMR spectra were recorded on a Bruker Avance AV II-600 spectrometer (Bruker Corporation, Billerica, MA, USA) (NMR Laboratories of the Chemical Institutes of the Technische Universität Braunschweig) in $\text{DMSO}-d_6$. Chemical shifts are presented in relation to TMS ($\delta = 0$ ppm). C nuclei were assigned based on results of a ^{13}C -DEPT135 experiment. HPLC was performed on a Merck Hitachi LaChrom Elite system (Hitachi High Technologies Inc., San Jose, CA, USA) (DAD detector: L-2450 (isocrat), UV detector: L-2400 (gradient); pump: L-2130; autosampler: L-2200; column: Merck LiChroCART 125-4, LiChrospher 100 RP-18 (5 μm); isocratic eluent: acetonitrile/water mixture 50:50; gradient elution: concentration acetonitril 0–2 min: 10%; 2–12 min: 10% \rightarrow 90% (linear) 12–20 min: 90%; elution rate: 1.000 mL/min; detection wavelength: 254 nm and 280 nm (isocrat.), 254 nm (gradient); overall run time: 15 min. (isocrat.); t_s = dead time; t_{ms} = total retention time). For mass spectrometry a MAT95XL spectrometer was used (ThermoFinnigan MAT, Bremen, Germany, Department of Mass Spectrometry of the Chemical Institutes of the Technische Universität Braunschweig). The elemental analysis was performed on a CE Instruments Flash EA® 1112 Elemental Analyzer. TLC: Polygram SIL G/UV₂₅₄, 0.2 mm thickness, (Macherey-Nagel).

9-Bromo-3-chloro-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4**)

8-Chloro-3,4-dihydro-1H-1-benzazepin-2,5-dione (**2**, 211 mg, 1.01 mmol) [19] and 4-bromophenylhydrazine hydrochloride (**3**, 298 mg, 1.33 mmol) were suspended in glacial acetic acid (10.0 mL) and anhydrous sodium acetate (113 mg, 1.38 mmol) was added. The mixture was stirred for 1 h at 70 °C. Concentrated sulfuric acid (100 μL) was added and stirring was continued for 1.25 h at 70 °C. After the mixture was cooled down to room temperature, 5% aqueous sodium acetate solution (20.0 mL) was added. The mixture was stored overnight at 6 °C. An orange precipitate formed which was filtered off, washed with water and crystallized from ethanol to yield a yellow powder (151 mg, 41.8%).

M.p.: 247–249 °C (dec.).

MS (EI, rel. intensity) m/z : 362 ($[\text{M}]^+$, 100), 333 ($[\text{M}-29 (-\text{CHO})]^+$, 62).

IR (KBr) (cm^{-1}): 3223 (N-H), 1647 (C=O).

$^1\text{H-NMR}$ (600.1 MHz, $\text{DMSO-}d_6$): δ (ppm) = 11.87 (s, 1H, NH-indole), 10.23 (s, 1H, NH-lactam), 7.93 (d, $J = 1.9$ Hz, 1H, ArH), 7.74 (d, $J = 8.5$ Hz, 1H, ArH), 7.40 (d, $J = 8.4$ Hz, 1H, ArH), 7.37 (dd, $J = 8.4, 2.1$ Hz, 1H, ArH), 7.31 (d, $J = 2.2$ Hz, 1H, ArH), 7.29 (dd, $J = 8.6, 1.9$ Hz, 1H, ArH), 3.56 (s, 2H, $-\text{CH}_2$).

$^{13}\text{C-NMR}$ (151 MHz, $\text{DMSO-}d_6$): δ (ppm) = 31.35 (CH_2); 113.47, 120.59, 121.24, 123.54, 124.84, 128.24 (CH); 107.43, 111.82, 121.57, 128.61, 132.43, 133.01, 136.13, 136.78, 171.40 (C).

HPLC (AUC %): 98.74% at 254 nm; 98.00% at 280 nm, $t_{\text{ms}} = 5.88$ min, t_{s} (DMSO) = 1.06 min (isocrat); 95.43% at 254 nm, $t_{\text{ms}} = 12.02$ min (gradient).

TLC (petroleum ether/ethyl acetate 50:50): $R_f = 0.73$.

Anal. calculated for $\text{C}_{16}\text{H}_{10}\text{BrClN}_2\text{O}$ (361.62): C, 53.14; H, 2.79; N, 7.75. Found: C, 53.21; H, 2.77; N, 7.51.

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Author Contributions

Lisa Unger, Akim Yildiz: Experimental synthetic work, HPLC, writing of manuscript; Oliver C. F. Orban, Ricarda S. Korn: Experimental synthetic work, literature search, HPLC, NMR and MS interpretation; Conrad Kunick: Synthesis planning, literature search, writing of manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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