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Epoxide-containing side chains enhance antiproliferative activity of paullones

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Abstract

The introduction of side chains bearing epoxide motifs into the molecular scaffold of kenpaullone and 9-trifluoromethylpaullone led to improved antiproliferative activity of the novel derivatives for human tumor cell lines. The syntheses were accomplished applying Stille coupling for the introduction of unsaturated side chains into the 2-position of the paullones and subsequently employing a hydrogen peroxide/nitrile mixture for the epoxidation of C,C-double bonds.

Keywords: Antiproliferative activity; Cyclin-dependent kinases; Epoxides; Paullones; Stille coupling

1. Introduction

The cyclin-dependent kinases (CDKs) are a group of serine/threonine kinases involved in the regulation of the cell cycle, neuronal functions, transcription and apoptosis [1]. Because in many human cancers hyperactivity of CDKs has been identified as one of the mechanisms underlying the pathological hyperproliferation [2] the inhibition of CDKs may offer a therapeutic option for cancer patients [3–6]. Consequently, a number of small molecules with CDK-inhibitory properties have been developed during the past decade [6–8]. At least four CDK inhibitors have been reported to be evaluated in clinical trials as anticancer drugs: flavopiridol, UCN-01, roscovitine (CYC202; seliciclib), and the aminothiazole BMS-387032 [9].

The paullones, a group of ATP-competitive 7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-ones, constitute a well established CDK inhibitor class [10,11]. Although many members of the paullone series provoke growth inhibition on cultivated tumor cells it has been pointed out that the antiproliferative activity is not paralleling the CDK-inhibitory properties of the paullones. This observation has been explained with additional targets of paullones, e.g. other kinases or completely unrelated proteins [12,13].

In order to increase the anticancer activity in the paullone series, derivatives with side chains bearing epoxide groups were designed and synthesized. The epoxide moieties were incorporated into the novel paullones to enable the formation of covalent bonds between the putative target proteins and the inhibitor, resulting in irreversible inhibition of the molecular target and consequently improved antiproliferative activity. The concept of irreversible enzyme inhibition by epoxides has been realized before by 1,2-epoxy-3-(*p*-nitrophenoxy)-propane (EPNP) [14], and by haloperidol-derived epoxides [15,16], which form covalent bonds to aspartyl side chains of the proteins. Compounds with epoxide groups were recently reported to exhibit antitumor activity in nude mice bearing prostate tumor xenografts [17]. The position for the epoxide-containing side chain attachment at the paullone scaffold was chosen considering the accommodation mode of the inhibitors in the ATP binding pocket of CDK1, in which the 2-position is directed towards the entrance gate of the cavity. Either a bromo- or a trifluoromethyl group was kept in the

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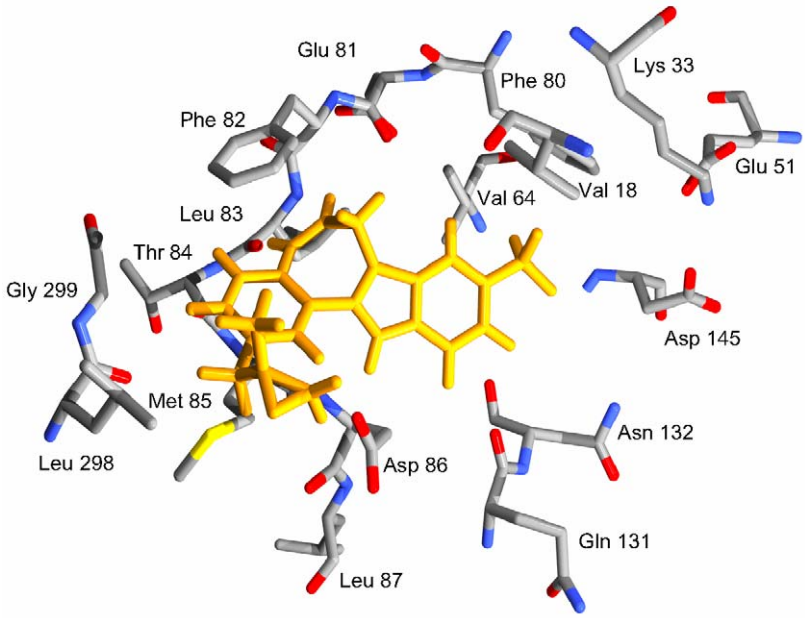


Fig. 1. Docking of epoxide **8** (dark yellow) in the ATP-binding-pocket of a CDK1/cyclin B homology model (FlexX module integrated in the SYBYL molecular modeling software; version 6.8; Tripos Associated Ltd., St. Louis, MO, USA). Distance between side chain carboxylate O of Asp 86 and epoxide-ring-carbon 4.13 Å. For sake of clarity, several amino acids on top (Ile 10, Gly 11, Glu 12) and at the bottom of the pocket (e.g. Leu 134) are not depicted.
Epoxide **8** docked into the ATP binding pocket of the CDK1/cyclin B complex.

9-position, where an electron-withdrawing substituent is required for CDK-inhibitory activity [11]. Based on these considerations, the paullone-derived epoxides **4**, **6**, **8**, **14**, and **15** were designed and docked into the ATP binding pocket of a CDK1/cyclin B homology model [18] using the FlexX module of the Sybyl software package [19]. The docking results revealed preferred ligand conformations placing the respective epoxide groups in close proximity to the Asp 86 carboxylate group as exemplified in Fig. 1 for epoxide **8**. Hence, the designed compounds fulfill the theoretical structural requirements for a covalent bond formation between protein and inhibitor resulting from a nucleophilic attack of the Asp 86 carboxylate towards the epoxide ring. In the present report the syntheses, the kinase inhibitory activities and the antiproliferative properties of the novel paullone-epoxides **4**, **6**, **8**, **14**, and **15** are described.

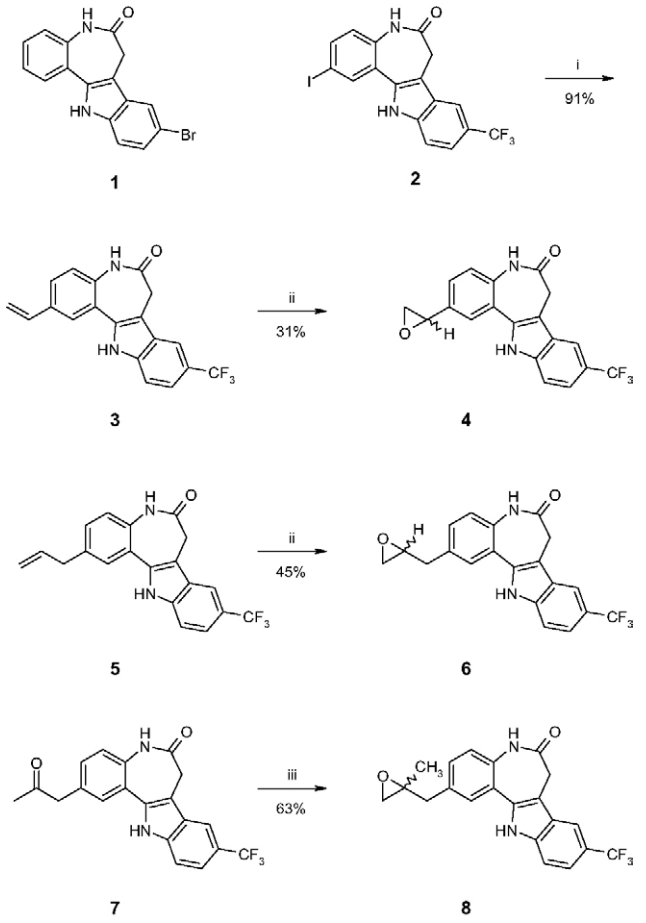
2. Chemistry

For the attachment of side chains at the 2-position of paullones the palladium-catalyzed Stille coupling procedure was employed. Hence, upon treatment of **2** with tributylvinyltin in the presence of 10 mol% $PdCl_2(PPh_3)_2$ in DMF the 2-vinyl-paullone **3** was obtained in a very good yield. In the following step, the terminal double bond of **3** turned out to be reluctant to *meta*-chloroperbenzoic acid as standard epoxidation reagent. In contrast, the use of hydrogen peroxide in the presence of acetonitrile [20] led to the desired paullone epoxide **4**, albeit in modest yield. An accordant epoxidation procedure starting with 2-allyl-9-trifluoromethylpaullone (**5**) [21] furnished the homologue **6**. For the synthesis of the epoxide **8**, Corey et al. [22] epoxidation method was used reacting the ketone **7** [21] with dimethyloxosulfonium methylide in dimethylsulfoxide (Scheme 1).

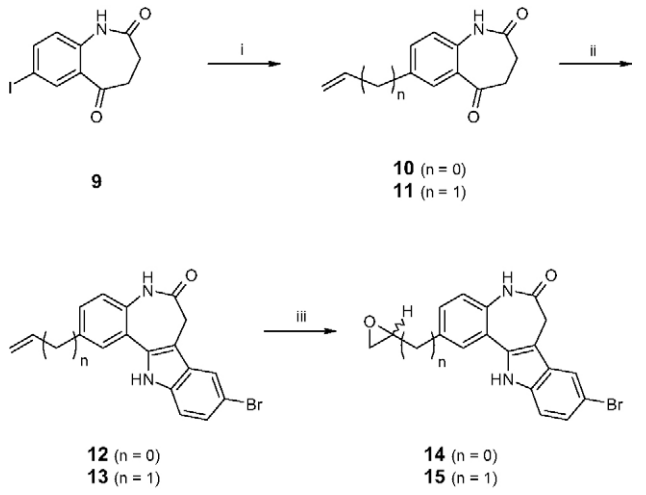
The synthesis of the 9-bromo analogues **14** and **15** is outlined in Scheme 2. In this reaction sequence the indole ring closure was carried out after the Stille coupling step in order to avoid an undesired oxidative addition of palladium to the carbon–bromo bond of the paullone scaffold. Thus, reaction of the lactam **9** [12] with tributylvinyltin catalyzed by $PdCl_2(PPh_3)_2$ furnished the vinyl derivative **10**, which subsequently was converted to the paullone ring system through a Fischer indolization reaction with 4-bromophenylhydrazine. Again, the following epoxidation with hydrogen peroxide/acetonitrile gave only low yields of the desired epoxide **14**. The corresponding homologue **15** was obtained by a similar procedure, in which allyltributyltin was employed in the Stille coupling instead of tributylvinyltin.

3. Biological evaluation and discussion

The in vitro antitumor activities of the novel compounds were evaluated in the Antitumor Drug Screen (ADS) of the National Cancer Institute (NCI). Details of this test system and the information that is encoded by the activity pattern over all cell lines have been published [23–26]. For every test compound, three parameters are determined for each of 60 human cancer cell lines: the GI_{50} value (GI_{50} = molar concentration of the compound that inhibits 50% net cell growth), the TGI value (TGI = molar concentration of the



Scheme 1. (i) Tributylvinyltin, 10 mol% $PdCl_2(PPh_3)_2$, DMF, 60 °C, N_2; (ii) H_2O_2 (35%), MeCN, K_2CO_3, MeOH, r.t. (iii) $((CH_3)_3SO)^+ I^-$, NaH, DMSO, N_2, 50 °C.



Scheme 2. (i) **10**: Tributylvinyltin, 5 mol% $PdCl_2(PPh_3)_2$, DMF, 60 °C, N_2, 92%; **11:** allyltributyltin, 10 mol% $PdCl_2(PPh_3)_2$, 20 mol% PPh_3, DMF, 95 °C, 2 h, N_2, 65%; (ii) 4-bromophenylhydrazine × HCl, NaOAc, AcOH, 70 °C, then H_2SO_4, 70 °C, 2 h (**12**: 52%; **13**: 62%); (iii) H_2O_2 (35%), MeCN, K_2CO_3, EtOH, r.t. (**14**: 38%; **15**: 26%).

compound leading to total inhibition of net cell growth) and the LC_{50} value (LC_{50} = molar concentration of the compound leading to 50% net cell death). Moreover, mean values are calculated, which indicate the antiproliferative activity averaged over all cell lines. For example, the mean graph midpoint $Log_{10}GI_{50}$ (MG_MID $log_{10}GI_{50}$) is the mean of all 60 $log_{10}GI_{50}$ values for all distinct cancer cell lines from the panel. Especially valuable are the selectivity patterns or "fingerprints" that originate from the selectivity of a compound on the 60 human cancer cell lines. These selectivity patterns are characteristic for individual molecular mechanisms underlying the growth inhibition. Consequently, compounds with identical mechanisms of action show a high pattern similarity, a phenomenon that can be used to discover new anticancer agents or novel molecular targets in cancer cells [27]. Kenpaullone (**1**), for example, the prototype of the paullone series, was identified using a similarity search in the NCI data base of selectivity patterns with the fingerprint of the CDK inhibitor flavopiridol as search template [10]. Although the paullones obviously display their antiproliferative activity not exclusively by CDK inhibition, a characteristic paullone-like pattern can be found for many members of the series in the testing data from the NCI ADS. For instance, the cell lines CCRF-CEM, SR (leukemia), HCT-116 (colon cancer), LOX IMVI (melanoma), and OVCAR-3 (ovarian cancer) are more sensitive to many paullones than the average cell line, whereas HOP-92 (lung cancer) and UACC-257 (melanoma) are less sensitive. The existence of such a typical paullone-like fingerprint gives strong evidence for a characteristic set of molecular targets of the paullones.

In Table 1, the growth inhibitory properties of the novel epoxide-substituted paullones are listed and compared with the results for kenpaullone. In the table, the mean values over all tested cell lines are given together with four of the mentioned indicator cell lines (HCT-116, LOX IMVI, UACC257, HOP-92). Of note, all five novel derivatives exhibited a stronger general antiproliferative activity. Three (entries **4**, **14**, **15**) out of the five new compounds outperformed kenpaullone (**1**) by roughly one order of magnitude. Most remarkably, all new derivatives showed the typical paullone-like pattern within the cell line panel, as exemplified in Table 1. Without exception, HCT-116 and LOX IMVI were inhibited by concentrations below the mean GI_{50} concentration, whereas UACC-257 and HOP-92 were either not inhibited or by concentrations above the mean GI_{50}. Apparently the enhanced antiproliferative activity of the paullone epoxides is not the result of an increased CDK1/cyclin B inhibitory potency, since the novel paullone epoxides exhibit IC_{50} values for this enzyme in the same order of magnitude as kenpaullone. Additional tests on the two closely related serine/threonine kinases CDK5/p25 and GSK-3β revealed that all novel compounds show the order of kinase inhibition typically found with paullones: GSK-3 β > CDK1/cyclin B > CDK5/p25.

A pair wise correlation of the GI_{50} selectivity patterns found in the NCI screening yielded the Pearson correlation coefficients (PCCs) depicted in Table 2. These data con-

Table 1
Biological activity of paullones **1**, **4**, **6**, **8**, **14**, **15**: in vitro antiproliferative activity on cultivated human tumor cell lines[a], and kinase inhibitory activity on CDK1/cyclin B,[b] CDK5/p25,[b] and GSK3-β,[b]

Paullone	MG_MID[c]	HCT-116[d]	LOX IMVI[e]	UACC-257[e]	HOP-92[f]	IC_{50} (μM) CDK1/cyclin B	IC_{50} (μM) CDK5/p25	IC_{50} (μM) GSK-3β
1[g]	–4.37	–5.7	–5.3	> –4.0	> –4.0	0.40	0.85	0.023
4	–5.24	–5.70	–5.51	–4.25	–5.20	0.30	0.60	0.04
6[h]	–4.76	–5.57	–5.90	>–4.30	>–4.30	0.90	0.90	0.08
8	–4.88	–5.49	–5.40	–4.73	–4.64	0.40	1.6	0.17
14	–5.26	–5.46	–5.40	–4.66	–4.71	0.32	1.0	0.10
15	–5.40	–5.51	–5.91	–4.74	–4.60	0.50	1.2	0.14

[a] Data obtained from the NCI's in vitro ADS. Indicated are $\log_{10}$ values of molar concentrations for 50% growth inhibition. Two test runs were carried out, with 100 μM as the highest concentration tested, unless noted otherwise.
[b] See experimental part for details of kinase assays. Standard errors of the IC_{50} values are typically below 20%. In many cases, standard errors below 10% are found.
[c] MG_MID = mean graph midpoint = mean value for all tested cancer cell lines. If the indicated effect was not attainable for distinct cell lines within the used concentration interval, the highest tested concentration (= 100 μM) was used for the calculation.
[d] Colon cancer cell line. e Melanoma cell line.
[f] Non-small cell lung cancer cell line.
[g] Mean of four experiments for cancer cell growth inhibition tests.
[h] The highest concentration used for cancer cell growth inhibition tests with paullone **6** was 50 μM.

Table 2
PCCs[a] for pair wise correlation of the GI_{50} meangraphs of paullones **1**, **4**, **6**, **8**, **14**, **15**[b]

Paullone entry	**1**	**4**	**6**	**8**	**14**
15	0.527	0.534	0.475	0.661	0.542
14	0.401	0.377	0.361	0.506	
8	0.665	0.544	0.535		
6	0.388	0.514			
4	0.453				

[a] For an explanation of the COMPARE algorithm and the generation of the PCCs by pair wise comparison of selectivity patterns refer to [25].
[b] Data obtained by applying a matrix COMPARE analysis using the corresponding web accessible tool from the NCI DTP web site (http://dtp.nci.nih.gov/).

firmed the impression that the fingerprints of the novel paullones indeed display a high degree of similarity and are "paullone-like". For example, all correlations of the epoxide **8** with the pattern of other new derivatives gave PCCs > 0.5. Furthermore, a nice correlation (PCC = 0.665) was also found between **8** and the mother compound of the paullones, kenpaullone (**1**). Although a single high correlation between two isolated compounds might be coincidental, the concentration of high correlated patterns in this group of paullones is obviously significant and gives reason to suppose that the epoxide moiety is not just adding unspecific toxicity to the antiproliferative activity of the paullones but contributes to the characteristic antiproliferative mechanism.

Further investigations are required to determine the targets pivotal for the antiproliferative activity of the paullones. Once identified, it will be necessary to investigate whether the epoxide groups of the novel compounds actually form covalent bonds with the target proteins. Furthermore, the isolation of the optical isomers of the novel compounds will be of interest in order to investigate the influence of the configuration at the chiral center on the antiproliferative activity.

4. Experimental protocols

4.1. General remarks

Melting points (m.p.) were determined on an electric variable heater (Electrothermal 9100) and are not corrected. Elemental analyses were performed in the analytical department of the Institut für Pharmazie, Universität Hamburg. Infrared spectra were recorded using KBr pellets on a Philips PU 9712 spectrometer. Nuclear magnetic resonance spectra were recorded on a Bruker AMX 400 instrument, using tetramethylsilane as internal standard and dimethyl sulfoxide-d_6 as solvent unless stated otherwise. NMR signals are reported in ppm on a δ scale. High resolution FAB mass spectra (HMRS) were determined on a Finnigan MAT 311A instrument using 3-nitrobenzylic alcohol as matrix. Flash chromatography was performed using chromatography grade silica gel 60 (Merck). Thin-layer chromatography (TLC) was performed using fluorescent Polygram Sil G/UV_{254} silica gel plates. Spots were visualized under 254 nm UV illumination. Anhydrous solvents were prepared by distillation in the presence of an appropriate drying agent and were stored over 4 Å molecular sieves.

4.2. Syntheses

*4.2.1. 2-Ethenyl-9-trifluoromethyl-7,12-dihydroindolo[3,2-d] [1]benzazepin-6(5H)-one (**3**)*

To a solution of **2** (221 mg, 0.5 mmol) in DMF (6 ml) was added tributylvinyltin (190 mg, 0.62 mmol), $PdCl_2(PPh_3)_2$ (35 mg, 0.05 mmol) and 2,6-di-*tert.*-butyl-4-methylphenol (3 crystals) under a nitrogen atmosphere. The mixture was heated at 50–60 °C for 1 h, cooled to room temperature and poured into ice water. The precipitate was collected by suction filtration, washed with cyclohexane and purified by column chromatography (ethyl acetate/petrol ether = 40:60) to give 156 mg of a yellow solid (91%): m.p. > 330 °C (darken-

ing at 180 °C) (ethanol); IR 3200, 1640, 1300 cm^{-1}; ^1H NMR (400 MHz) 3.63 (s, 2 H), 5.33 (d, J = 11.7 Hz, 1 H), 5.92 (d, J = 17.3 Hz, 1 H), 6.80 (dd, J_1 = 17.6 Hz, J_2 = 10.8 Hz, 1 H), 7.25 (d, J = 8.6 Hz, 1 H), 7.46 (dd, J_1 = 8.6 Hz, J_2 = 1.5 Hz, 1 H), 7.54 (dd, J_1 = 8.5 Hz, J_2 = 2.0 Hz, 1 H), 7.62 (d, J = 8.5 Hz, 1 H), 7.86 (d, J = 2.0 Hz, 1 H), 8.14 (s, 1 H), 10.20 (s, 1 H), 12.11 (s, 1 H); ^{13}C NMR (100 MHz) 31.3, 108.4, 112.1, 114.3, 115.9 (q, J = 4.0 Hz), 118.3 (q, J = 2.0 Hz), 120.3 (q, J = 31 Hz), 122.1, 122.5, 125.5 (q, J = 270 Hz, CF_3), 124.7, 125.8, 126.2, 132.7, 134.5, 135.3, 135.6, 138.7, 171.2; Anal. $C_{19}H_{13}N_2OF_3$ (H,N) C. Calcd 66.67 C; found 66.19.

*4.2.2. 2-(Epoxyethyl)-9-trifluoromethyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4**)*

Prepared according to the method described for **6** (see below), starting with **3**, and yielding 31% of **4**; m.p. 309–310 °C (darkening at 280 °C); IR 3300, 1640, 1300, 1260, 1100 cm^{-1}, ^1H NMR (400 MHz) 2.97 (dd, J = 5.1/2.5 Hz, 1 H), 3.19 ("t", J = 5.0 Hz, 1 H), 3.62 (s, 2 H), 4.00–4.02 (m, 1 H), 7.26 (d, J = 8.1 Hz, 1 H), 7.36 (dd, J_1 = 8.6 Hz, J_2 = 1.5 Hz, 1 H), 7.47 (dd, J_1 = 8.6 Hz, J_2 = 1.5 Hz, 1 H), 7.62 (d, J = 8.1 Hz, 1 H), 7.68 (d, J = 1.5 Hz, 1 H), 8.20 (s, 1 H), 10.21 (s, 1 H), 12.12 (s, 1 H); ^{13}C NMR (125 MHz) 31.2, 50.2, 51.2, 108.5, 112.1, 115.9 (q, J = 5 Hz), 118.4 (q, J = 2 Hz), 120.0 (q, J = 30 Hz), 122.1, 122.4, 124.1, 125.5 (q, J = 273 Hz), 125.8, 126.1, 133.0, 134.4, 135.5, 138.7, 171.3; Anal. $C_{19}H_{13}N_2O_2F_3$ (H, N) C. Calcd 63.69 C; found 62.93; HRMS Calcd 358.0929; found 358.0947.

*4.2.3. 2-(2,3-Epoxypropyl)-9-trifluoromethyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**6**)*

To a suspension of **5** [21] (90 mg, 0.25 mmol) and K_2CO_3 (100 mg) in MeOH (10 ml) was added 1 ml of MeCN. H_2O_2 (35%, 5 ml) was added drop wise at r.t. over a period of 3 h. Ice water (50 ml) was added and a white solid precipitated, which was filtered off with suction, washed with water, and purified by column chromatography (ethyl acetate/petrol ether = 50:50) to recover 43 mg of **5** (48%) and to obtain 42 mg of **6** (45%): m.p. 295–296 °C (ethanol); IR 3400, 1660, 1500, 1300, 1260, 1100 cm^{-1}; ^1H NMR (400 MHz) 2.56–2.63 (m, 1 H), 2.77–2.80 (m, 1 H), 2.87 (d, J = 5.6 Hz, 2 H), 3.20–3.22 (m, 1 H), 3.60 (s, 2 H), 7.22 (d, J = 8.3 Hz, 1 H), 7.33 (d, J = 8.1 Hz, 1 H), 7.46 (d, J = 8.6 Hz, 1 H), 7.62 (d, J = 8.1 Hz, 1 H), 7.67 (s, 1 H), 8.14 (s, 1 H), 10.12 (s, 1 H), 12.26 (s, 1 H); ^{13}C NMR (100 MHz) 31.2, 37.5, 46.1, 51.8, 108.4, 112.0, 115.8 (q, J = 2 Hz), 118.0 (q, J = 31 Hz), 118.2 (q, J = 2 Hz), 122.1, 122.3, 125.5 (q, J = 270 Hz, CF_3), 125.8, 127.2, 129.2, 132.9, 134.2, 134.6, 138.7, 171.3; Anal. $C_{20}H_{15}N_2O_2F_3$ (C,H,N).

*4.2.4. 2-(2-Methyl-2,3-epoxypropyl)-9-trifluoromethyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**8**)*

To dry DMSO (2 ml) in a 20 ml Schlenk tube was added NaH (60% in mineral oil, 6 mg, 0.15 mmol) under a N_2 atmosphere. Subsequently, trimethylsulfoxonium iodide (37 mg, 0.17 mmol, purchased from Aldrich) was added. The mixture was stirred at room temperature for 1 h, while H_2 evolved. Then **7** [21] (55 mg, 0.15 mmol) in dry DMSO (2 ml) was added drop wise and stirring was continued at 50 °C for 2 h. After cooling to room temperature, the mixture was poured into ice water (50 ml) and extracted with ethyl acetate (50 ml × 2). The combined organic layers were washed with water (10 ml × 2), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography (ethyl acetate/petrol ether = 50:50) furnishing 36 mg of **8** (63%): m.p. 270–271 °C (ethanol); IR 3320, 3200, 1670, 1510, 1310, 1270, 800 cm^{-1}; ^1H NMR (400 MHz) 1.25 (s, 3 H), 2.63, 2.71 (AB, J_{AB} = 4.6 Hz, 2 H), 2.85, 2.91 (AB, J_{AB} = 13.7 Hz, 2 H), 3.60 (s, 2 H), 7.20 (d, J = 8.1 Hz, 1 H), 7.29 (dd, J_1 = 8.6 Hz, J_2 = 1.5 Hz, 1 H), 7.46 (d, J = 8.1 Hz, 1 H), 7.61–7.64 (m, 2 H), 8.13 (s, 1 H), 10.11 (s, 1 H), 12.05 (s, 1 H); ^{13}C NMR (100 MHz) 20.6, 32.0, 41.7, 52.3, 56.8, 108.4, 112.0, 115.8, 118.2, 120.0 (q, J = 30 Hz), 121.9, 122.2, 125.5 (q, J = 270 Hz), 125.8, 127.7, 129.8, 132.8, 134.2, 134.6, 138.7, 171.3; HRMS ($C_{21}H_{17}N_2O_2F_3$). Calcd 386.1242; found 386.1273.

*4.2.5. 7-Ethenyl-1H-[1]benzazepine-2,5(3H,4H)-dione (**10**)*

To a solution of **9** [12] (260 mg, 0.86 mmol) in DMF (6 ml), tributylvinyltin (330 mg, 1.07 mmol), $PdCl_2(PPh_3)_2$ (30 mg, 0.04 mmol) and 2,6-di-*tert.*-butyl-4-methylphenol (six crystals) was added under nitrogen. The mixture was heated to 60 °C for 40 min, then cooled to room temperature and poured into ice water. The precipitate was collected by suction filtration, washed with cyclohexane and purified by column chromatography (ethyl acetate/petrol ether = 30:70) to give 160 mg yellow solid (92%): m.p. 170–171 °C (ethanol); IR 3300, 1650, 1490, 1400 cm^{-1}; ^1H NMR (400 MHz) 2.68 (m, 2 H), 2.91 (m, 2 H), 5.27 (d, J = 11.2 Hz, 1 H), 5.81 (d, J = 13.8 Hz, 1 H), 6.75 (dd, J_1 = 13.8 Hz, J_2 = 11.2 Hz, 1 H), 7.15 (d, J = 8.6 Hz, 1 H), 7.72 (dd, J_1 = 8.1 Hz, J_2 = 2.0 Hz, 1 H), 7.83 (d, J = 2.0 Hz, 1 H); ^{13}C NMR (100 MHz) 29.2, 38.0, 114.2, 122.0, 126.5, 128.2, 130.9, 132.2, 135.1, 138.7, 173.3, 195.0; Anal. $C_{12}H_{11}NO_2$ (H, N) C. Calcd 71.63 C; found 71.10.

*4.2.6. 7-(2-Propenyl)-1H-[1]benzazepine-2,5(3H,4H)-dione (**11**)*

To a mixture of **9** [12] (301 mg, 1 mmol), $PdCl_2(PPh_3)_2$ (70 mg, 0.1 mmol), PPh_3 (52 mg, 0.2 mmol) and 2,6-di-*tert.*-butyl-4-methylphenol (six crystals) in DMF (6 ml) was added allyltributyltin (342 mg, 1.03 mmol) under a nitrogen atmosphere. The corresponding mixture was stirred at r.t. for 5 min and heated to 95 °C for 1.5 h and then cooled to r.t. The mixture was poured into ice water. A solid precipitated, which was filtered with suction, washed with cyclohexane and water and purified by column chromatography (ethyl acetate/petrol ether = 30:70) to give 140 mg pale yellow solid (65%): m.p. 141–142 °C (ethanol); IR 3200, 2990, 1650, 1400 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 2.80 (m, 2 H), 3.01 (m, 2 H), 3.40 (d, J = 7.0 Hz, 2 H), 5.08–5.12 (m, 2 H), 5.89–5.98 (m, 1 H),

6.89 (d, J = 8.1 Hz, 1 H), 7.35 (dd, J_1 = 8.1 Hz, J_2 = 1.6 Hz, 1 H), 7.81 (d, J = 1.5 Hz, 1 H), 7.87 (brs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) 29.5, 38.9, 39.2, 116.7, 121.8, 127.3, 131.0, 134.9, 136.2, 136.4, 136.8, 174.6, 198.5. This material was directly used for the synthesis of **13**.

*4.2.7. 9-Bromo-2-ethenyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**12**)*

To a slurry of **10** (165 mg, 0.82 mmol) in acetic acid (10 ml) was added sodium acetate (80 mg, 0.97 mmol) and 4-bromophenylhydrazine hydrochloride (217 mg, 0.97 mmol). The mixture was stirred at 70 °C for 1 h and then cooled to room temperature. Concentrated sulfuric acid (0.2 ml) was added and stirring was continued for 2 h at 70 °C. After cooling to room temperature, the mixture was poured into 5% aqueous sodium acetate solution (20 ml). A yellow solid precipitated, which was filtered off with suction and purified by recrystallization from ethanol/toluene to give 150 mg of yellow crystals (52%): m.p. > 330 °C (darkening at 200 °C); IR 3300, 1660, 1390, 1300, 820 cm^{-1}; ^{1}H NMR (400 MHz) 3.53 (s, 2 H), 5.32 (d, J = 10.6 Hz, 1 H), 5.90 (d, J = 17.3 Hz, 1 H), 6.79 (dd, J_1 = 17.3 Hz, J_2 = 11.2 Hz, 1 H), 7.23 (d, J = 8.6 Hz, 1 H), 7.28 (d, J = 8.6 Hz, 1 H), 7.40 (d, J = 8.6 Hz, 1 H), 7.52 (d, J = 7.6 Hz, 1 H), 7.83 (s, 1 H), 7.92 (s, 1 H), 10.16 (s, 1 H), 11.84 (s, 1 H); ^{13}C NMR (100 MHz) 31.3, 107.1, 111.6, 113.3, 114.1, 120.4, 122.2, 122.4, 124.5, 124.7, 126.0, 128.2, 132.6, 133.8, 135.1, 135.6, 135.9, 171.2; HRMS ($C_{18}H_{13}BrN_2O$). Calcd 352.0211; found 352.0209.

*4.2.8. 9-Bromo-2-(2-propenyl)-7,12-dihydroindolo[3,2-d]-[1]benzazepin-6(5H)-one (**13**)*

Prepared according to the method described for **12**, starting with **11** and yielding 62% of **13**; m.p. 308–309 °C (darkening at 260 °C); IR 3190, 1640, 1410, 1300, 790 cm^{-1}; ^{1}H NMR (400 MHz) 3.43 (d, J = 6.6 Hz, 2 H), 3.54 (s, 2 H), 5.09–5.18 (m, 2 H), 5.98–6.06 (m, 1 H), 7.17–7.23 (m, 2 H), 7.27 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1 H), 7.39 (d, J = 8.6 Hz, 1 H), 7.56 (s, 1 H), 7.90 (d, J = 1.5 Hz, 1 H), 10.84 (s, 1 H), 11.79 (s, 1 H); ^{13}C NMR (100 MHz) 31.0, 38.8, 107.1, 111.6, 113.3, 116.0, 120.3, 122.3, 122.4, 124.4, 126.6, 128.2, 128.5, 133.8, 133.9, 135.1, 135.9, 137.3, 171.3; HRMS ($C_{19}H_{15}BrN_2O$). Calcd 366.0368; found 366.0358.

*4.2.9. 9-Bromo-2-epoxyethyl-7,12-dihydroindolo[3,2-d]-[1]benzazepin-6(5H)-one (**14**)*

To a suspension of **12** (80 mg, 0.22 mmol) and K_2CO_3 (40 mg) in ethanol (20 ml) was added MeCN (2 ml). H_2O_2 (35%, 5 ml) was added dropwise at room temperature over 3 h. Upon addition of ice water (50 ml) a pale yellow solid precipitated, which was filtered off with suction, washed with water, and purified by flash chromatography (ethyl acetate/petrol ether = 50:50) recovering 40 mg of **12** (50%) and yielding 32 mg of **14** (38%), m.p. 320–321 °C (darkening at 260 °C); IR 3300, 1640, 1300, 830 cm^{-1}; ^{1}H NMR (400 MHz) 2.95 (dd, J = 5.6/2.5 Hz, 1 H), 3.17 ("t", J = 4.6 Hz, 1 H), 3.49, 3.53 (AB, J_{AB} = 14.7 Hz, 2 H), 3.99–4.01 (m, 1 H), 7.24 (d, J = 8.6 Hz, 1 H), 7.28 (dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz, 1 H), 7.33 (dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz, 1 H), 7.40 (d, J = 8.6 Hz, 1 H), 7.64 (d, J = 2.0 Hz, 1 H), 7.91 (d, J = 2.0 Hz, 1 H), 10.15 (s, 1 H), 11.84 (s, 1 H); ^{13}C NMR (100 MHz) 31.3, 50.2, 51.2, 107.3, 111.6, 113.3, 120.4, 122.3, 124.1, 124.6, 125.9, 128.2, 133.0, 133.7, 135.4, 136.0, 171.3; HRMS ($C_{18}H_{13}BrN_2O_2$). Calcd 368.0160; found 368.0168.

*4.2.10. 9-Bromo-2-(2,3-epoxypropyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**15**)*

Prepared from **13** according to the method described for **14**. Flash chromatography was used to recover 50% of **13** and to yield 26% of **15**; m.p. 308–310 °C (darkening at 260 °C); IR 3200, 1640, 1400, 1300, 1220, 830 cm^{-1}; ^{1}H NMR (400 MHz) 2.61 (dd, J = 4.6/2.5 Hz, 1 H), 2.78 ("t", J = 4.1 Hz, 1 H), 2.86 (d, J = 5.6 Hz, 2 H), 3.17–3.21 (m, 1 H), 3.49 (s, 2 H), 7.19 (d, J = 8.1 Hz, 1 H), 7.27 (dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz, 1 H), 7.30 (dd, J_1 = 8.1 Hz, J_2 = 2.0 Hz, 1 H), 7.40 (d, J = 8.6 Hz, 1 H), 7.63 (s, 1 H), 7.90 (s, 1 H), 10.07 (s, 1 H), 11.80 (s, 1 H); ^{13}C NMR (100 MHz) 31.3, 37.5, 46.1, 51.8, 107.1, 111.6, 113.3, 120.3, 122.2, 122.3, 124.4, 127.1, 128.0, 129.0, 132.9, 133.9, 134.1, 135.9, 171.3. Anal. $C_{19}H_{15}BrN_2O_2$ (N) C. Calcd 59.55 H. Calcd 3.95 C; found 59.03 H; found 4.44; HRMS Calcd 382.0317; found 382.0322.

4.3. Kinase assays

4.3.1. Reagents

Homogenization buffer: 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM Mops (pH 7.2), 15 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM sodium vanadate, 1 mM NaF, 1 mM phenylphosphate, 10 μg leupeptin ml^{-1}, 10 μg aprotinin ml^{-1}, 10 μg soybean trypsin inhibitor ml^{-1}, and 100 μg benzamidine. Buffer A: 10 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, 25 mM Tris–HCl pH 7.5, 50 μg heparin ml^{-1}. Buffer C: homogenization buffer but 5 mM EGTA, no NaF and no protease inhibitors. Kinase activities were assayed in duplicates in buffer A or C at 30 °C, at a final ATP concentration of 15 μM. The order of mixing the reagents was: buffers, substrate, enzyme, and inhibitor. There was no preincubation at 30 °C. Addition of radio-labeled ATP was considered as time 0 of the incubation period. Assays were run under conditions where less than 5% of the radio-labeled phosphate was incorporated. Blank values were subtracted and activities calculated as pmol of phosphate incorporated for a 10-min incubation. The activities are usually expressed in percentage of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO.

4.3.2. CDK1/cyclin B

CDK1/cyclin B was extracted in homogenization buffer from M phase starfish (*Marthasterias glacialis*) oocytes and purified by affinity chromatography on p9^{CKShs1}-Sepharose beads, from which it was eluted by free p9^{CKShs1} as previ-

ously described [28]. The kinase activity was assayed in buffer C, with 1 mg histone H1 per ml, in the presence of 15 μM [γ-^{32}P]ATP (3000 Ci mmol^{-1}; 1 mCi ml^{-1}) in a final volume of 30 μl. After a 10-min incubation at 30 °C, 25 μl aliquots of supernatant were spotted onto P81 phosphocellulose papers and treated as described above.

4.3.3. CDK5/p25

CDK5/p25 was reconstituted by mixing equal amounts of recombinant mammalian CDK5 and p25 expressed in *E. coli* as glutathione S-transferase (GST) fusion proteins and purified by affinity chromatography on glutathione-agarose (vectors kindly provided by J.H. Wang, Department of Medical Chemistry, University of Calgary, Alberta, Canada) (p25 is a truncated version of the p35, the 35-kDa CDK5 activator). Its activity was assayed in buffer C as described for CDK1/cyclin B.

4.3.4. GSK-3β

GSK-3β was expressed in and purified from insect sf9 cells. [29] It was assayed, following a 1/100 dilution in 1 mg BSA per ml 10 mM dithiothreitol, with 5 μl 40 μM GS-1 peptide as a substrate, in buffer A, in the presence of 15 μM [γ-^{32}P]ATP (3000 Ci mmol^{-1}; 1 mCi ml^{-1}) in a final volume of 30 μl. After 30 min incubation at 30 °C, 25 μl aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper, and, 20 s later, the filters were washed five times (for at least 5 min each time) in a solution of 10 ml phosphoric acid per l of water. The wet filters were counted in the presence of 1 ml ACS (Amersham) scintillation fluid.

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