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Original article

Development of 5-benzylpaullones and paullone-9-carboxylic acid alkyl esters as selective inhibitors of mitochondrial malate dehydrogenase (mMDH)

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ABSTRACT

A collection of paullones was tested for inhibitory activity against mitochondrial malate dehydrogenase (mMDH) as a biological target for antiproliferative activity. Based on the results of this screening, 5-benzylpaullones and paullone-9-carboxylic acid alkyl esters were developed as selective mMDH inhibitors. The new derivatives did not show noteworthy antiproliferative activity when tested on a panel of cancer cell lines, suggesting that mMDH inhibition is of minor relevance for the growth inhibition caused by paullones.

1. Introduction

The paullones are a family of ATP competitive and selective kinase inhibitors. The parent molecular scaffold of the paullones is based on the 7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one system, of which more than 280 derivatives have been described. Paullones substituted on distinct positions display manifold biological activities, e.g. inhibition of tumor cell growth [1], protection of insulin-producing pancreatic beta cells [2], and antiparasitic activity against *Leishmania* parasites [3]. The commercial available congener alsterpaullone is a widely used inhibitor of glycogen synthase kinase-3 (GSK-3) and cyclin-dependent kinases (CDKs) [4]. The potent antiproliferative acivity of alsterpaullone against human tumor cell lines appears to be due to perturbation of the mitochondrial membrane potential eventually leading to apoptosis [5]. Although the structure-activity relationships are well established for both CDK1 and GSK-3 inhibition by paullones [6], the structural requirements for the antitumor activity of paullones are still obscure. A recent publication indicated that an obvious correlation between kinase inhibition and tumor cell growth inhibition by paullones could not be shown [6]. Of note, some paullones with potent kinase inhibitory properties are devoid of noteworthy antiproliferative activity [6]. It was therefore speculated that additional biological targets besides GSK-3 and CDKs might, at least in part, be responsible for the antiproliferative activity of distinct paullones. In order to identify these additional targets, pull-down experiments have been carried out using paullones bound to solid matrices. These affinity investigations revealed that paullones bind mitochondrial malate dehydrogenase (mMDH) from various tissues, and subsequent enzymatic studies showed that alsterpaullone and kenpaullone inhibited mMDH in the low micromolar concentration range [7]. Although mMDH inhibition has been suggested as mechanistic rationale for the design of antineoplastic agents [8,9], these results were not sufficient to explain the relevance of the mMDH inhibition for the antiproliferative activity of paullones.

To discriminate between the influence of GSK-3/CDK inhibition and mMDH inhibition on growth inhibition by paullones, we initiated a program for the development of paullones with improved mMDH inhibitory activity but devoid of kinase inhibitory properties. Such mMDH-selective paullones were subsequently tested for antiproliferative activity against tumor cell lines.

In a first step, we determined the mMDH inhibition potential of a wide array of paullones. Initial tests were carried out at a 10 μM paullone concentration. The results of these tests are given in Table 1 together with the IC_{50} values for CDK1 and GSK-3. The results show on the one hand that the majority of the paullones exhibit only low inhibitory potency for mMDH and on the other hand that there is no obvious correlation between mMDH inhibition and GSK-3 or CDK1/cyclin B inhibition. **1j** (paullone-9-carboxylic acid methyl ester) and **2a** (5-benzylkenpaullone) showed a considerable mMDH inhibition

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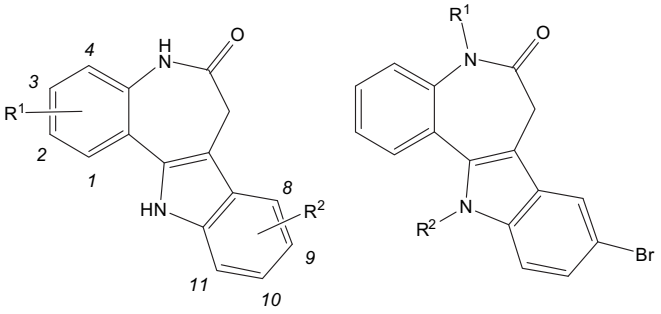
Table 1
mMDH inhibition (residual mMDH activity in the presence of 10 μm test compound) and kinase inhibition (IC_{50}) by paullones



1 **2**

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	R^1	R^2	residual mMDH activity @ 10 μM [%][a]	IC_{50} GSK-3 [μM]	IC_{50} CDK1/ Cyclin B [μM]
1a[b]	H	9-Br	74.5 ± 0.6	0.023	0.40
1b[b]	H	9-NO_2	54.2 ± 3.1	0.004	0.035
1c[b]	H	H	78.7 ± 4.0	0.62	7.0
1d[b]	H	9-Cl	73.4 ± 4.3	0.024	0.60
1e[b]	H	9-F	46.2 ± 5.4	0.080	1.6
1f[b]	H	9-CF_3	88.3 ± 0.9	0.030	0.40
1g[b]	H	9-CN	91.3 ± 5.5	0.010	0.024
1h[b]	H	9-CH_3	87.8 ± 7.4	0.13	2.0
1i	H	9-COOH	98.7 ± 0.6	30	47
1j	H	9-$COOCH_3$	60.9 ± 3.1	7.2	4.2
1k	H	9-SCH_3	76.6 ± 1.0	1.2	0.4
1l	H	9-$SOCH_3$	96.2 ± 1.3	13.0	0.28
1m[b]	2,3-di-OCH_3	9-CF_3	97.7 ± 0.9	0.075	0.28
1n	3-OCH_3	9-CF_3	83.9 ± 1.4	0.24	0.70
1o[b]	2,3-di-OCH_3	9-Br	56.3 ± 2.6	0.10	0.20
1p[c]	2-OCH_3	9-Br	58.7 ± 3.0	0.015	0.40
1q[c]	3-OH	9-Br	15.0 ± 8.0	0.018	0.60
1r[b]	4-OCH_3	9-Br	82.0 ± 0.1	16	250
1s[b]	4-OH	9-Br	25.9 ± 3.9	4.3	40
1t[b]	2,3-di-OCH_3	9-CN	89.2 ± 2.7	0.018	0.044
1u[b]	4-OCH_3	H	87.6 ± 3.1	140	430
1v[b]	H	11-Cl	65.3 ± 3.1	0.20	1.4
1w[b]	H	8,10-di-Cl	28.7 ± 3.0	5.0	2.5
2a[b]	Benzyl	H	8.6 ± 0.6	10	35
2b[b]	CH_3	H	81.9 ± 0.6	2.1	20
2c[b]	H	–$CH_2CH{=}CH_2$	74.1 ± 0.0	4.0	60
2d[b]	H	CH_3	98.0 ± 1.1	0.4	6.2

[a] mean ± SEM (=standard error of the mean).
[b] kinase inhibition data taken from [10].
[c] CDK1/cyclin B inhibition data taken from [11].

at 10 μM (39% and 91%, respectively) while exhibiting comparably poor kinase inhibition. We therefore synthesized a number of new 5-benzylpaullones **4a**–**k** and of paullone-9-carboxylic acid esters **5a**–**f** in order to discover compounds with enhanced mMDH inhibitory activity and selectivity.

2. Chemistry

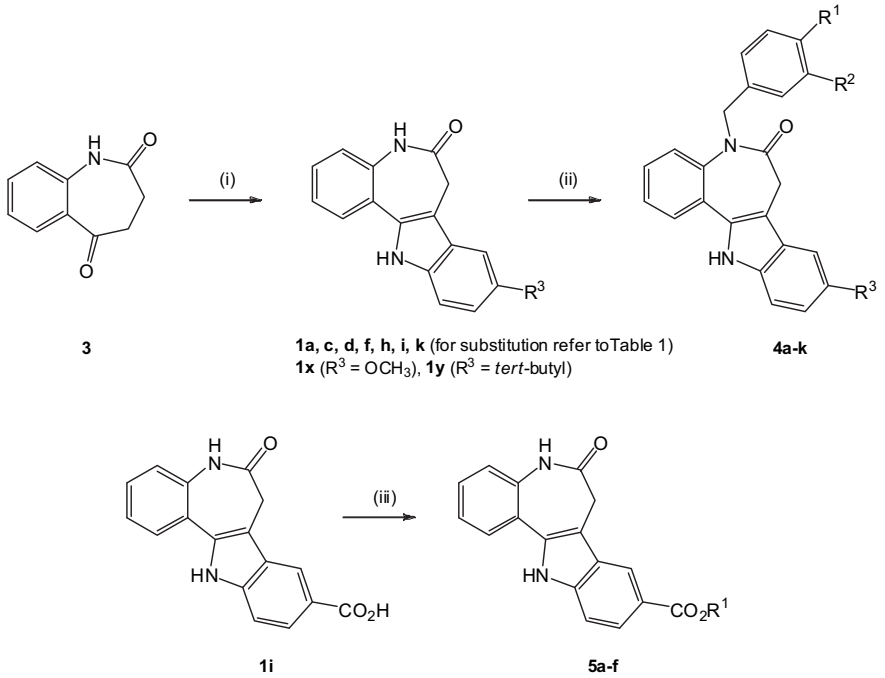
The synthesis of the novel 5-benzylpaullones **4** as analogues to **2a** and of the paullone-9-carboxylic acid esters **5** as analogues to **1j** is depicted in Scheme 1. The paullones **1** used as synthetic intermediates were prepared by a Fischer indole synthesis reacting 3,4-dihydro-1*H*-1-benzazepine-2,5-dione **3** [12] with appropriately substituted commercially available phenyl hydrazines in a mixture of acetic and sulphuric acid at 70 °C [11]. The 9-methylsulfinylpaullone **1l** was prepared by oxidation of the corresponding 9-methylthiopaullone **1k** by oxidation with m-chloroperbenzoic acid (not shown in Scheme 1). For the synthesis of 5-benzylpaullones **4a**–**k** as analogues of **2a**, the corresponding paullones **1** were selectively deprotonated by means of sodium hydride at the lactam nitrogen and the resulting anions alkylated with appropriately substituted benzyl bromides. To avoid additional substitution at the indole nitrogen the reaction was stopped before completion and the products were separated from unreacted starting materials **1** by column chromatography. The paullone-9-carboxylic acid **1i** was used as educt for the synthesis of the corresponding alkyl esters **5a**–**f** by a Mitsunobu reaction [13], employing the respective alcohols as nucleophiles in the presence of triphenylphosphane and di-*tert*-butyl azodicarboxylate in dry THF.

3. Biological evaluation and discussion

Results of the enzyme inhibition tests revealed that in compliance with our working hypothesis the new **2a**-related benzylpaullones **4a**–**k** were devoid of kinase inhibitory activity against GSK-3 and CDK1/cyclin B but showed improved mMDH inhibitory activity, e.g. derivative **4k** (IC_{50} = 0.77 μM). Similarly, the paullone-9-carboxylic esters **5a**–**f** exhibited poor kinase inhibitory activity. In this series, the octyl ester **5f** showed a noteworthy mMDH inhibition with an IC_{50} value of 2.7 μM (Table 2). In both compound families, a correlation was found between the mMDH inhibitory activity and the lipophilicity of the molecules expressed by the clogP values (Figs. 1 and 2, respectively). This observation suggests that hydrophobic interactions play a major role in the paullone-mMDH binding mode. Selected derivatives with a considerable degree of mMDH selectivity (**4b**, **4k**, **5d** and **5f**) were then tested for antiproliferative activity in the in vitro cell line screening project (IVCLSP) of the National Cancer Institute (NCI).

In the IVCLSP, the growth inhibitory potencies of test compounds are determined on 50–60 cancer cell lines derived from nine different tumor entities, representing cancers of the brain, lung, colon, ovary, breast, prostate, kidney, as well as leukaemia and melanoma. The screening system is a two step procedure. Initially, the test compounds are incubated in a 10 μM concentration with the cancer cells for two days. A sulforhodamine B assay is used for measurement of the surviving cells. If a compound shows considerable growth inhibitory activity and/or selectivity in the one-dose screening, a continuative test procedure is performed in which five concentrations between 10^{-8} to 10^{-4} M are employed and GI_{50} values (GI_{50} = concentration to inhibit cell growth by 50%) are calculated for the distinct cell lines. Furthermore, for each test compound an averaged GI_{50} value for all the cell lines in the screening panel is calculated defining the averaged antiproliferative activity. This parameter is designated meangraph midpoint (MG-MID).

Table 3 lists selected results of the IVCLSP for the mMDH-selective inhibitors **4b**, **4k**, **5d** and **5f**. For comparison, the table includes data of the 9-cyano-2,3-dimethoxy-paullone **1t** which inhibits the kinases CDK1/cyclin B and GSK-3, but is devoid of mMDH inhibitory activity (refer to Table 1). Since the mMDH inhibitors turned out to be weak antiproliferative agents, only the test data of cell growth at 10 μM inhibitor concentration are shown. Besides the averaged parameter for all cell lines of the panel, the data on four cell lines are included that are especially sensitive to characteristic kinase inhibitory paullones like **1t**, namely the colon cancer cell lines COLO205 and HCT 116 and the ovarian cancer cell lines IGROV1 and OVCAR3. As is shown in Table 3, **1t** (10 μM) completely suppresses the growth of COLO205 and of IGROV1 and provokes a net cell kill of the COLO205 and OVCAR3 cell lines. The GI_{50} values for these cell lines are in the low micromolar or submicromolar concentration range. In contrast, the four mMDH inhibitors **4b**, **4k**, **5d** and **5f** are, even in the high concentration of 10 μM, only modestly active or



Scheme 1. Synthesis of 5-benzylpaullones **4a–k** and paullone-9-carboxylic acid esters **5a–f** as analogues of **1j** and **2a**, respectively. Reagents and conditions: (i) 1. appropriately substituted phenylhydrazine, AcOH, 70 °C, 1–2 h; 2. H_2SO_4, 70 °C, 1 h; (ii) 1.NaH, dry THF; 2. appropriately substituted benzyl bromide, reflux; (iii) R^1OH, di-*tert*-butyl azodicarboxylate, triphenylphosphane, dry THF, 24 h.

completely inactive on the selected cell lines as well as on the other cell lines of the NCI panel.

4. Conclusion

A systematic investigation of 28 paullones failed to show a correlation between the mMDH and the kinase inhibitory activity within the compound family. Two series of novel paullones, namely 5-benzylpaullones **4** and paullone-9-carboxylic acid alkyl esters **5** were developed as selective inhibitors of mMDH versus kinases like CDK1/cyclin B and GSK-3. Within the two series, the mMDH inhibition was correlated to the lipophilicity of the compounds. In contrast to kinase inhibitory paullones like **1t** the mMDH inhibitors did not exhibit noteworthy antiproliferative activity on cancer cells. Hence, a contribution of the mMDH inhibition to the antiproliferative activity of paullones could not be proven. In contrast, the results suggest that mMDH inhibition is of minor relevance for the antiproliferative activity displayed by paullones.

5. Experimental protocols

5.1. Chemistry

5.1.1. General

Melting points (mp) were determined on an electric variable heater (Electrothermal IA 9100). Infrared spectra were recorded using KBr pellets on a Philips PU 9712 spectrometer or on a Thermo Nicolet FT-IR 200 spectrometer. Nuclear magnetic resonance spectra were recorded either on a Bruker AMX 400 instrument (Institute of Organic Chemistry, Universität Hamburg); on a Bruker Avance DRX-400 or a Bruker Avance II-600 (NMR laboratories of the Chemical Institutes of the Technische Universität Braunschweig) using DMSO-d_6 as solvent and tetramethylsilane as internal standard. NMR signals are reported in ppm on a δ scale. C, H, N analyses were performed with a CHN-O-Rapid, Heraeus or a EA 1108, Carlo Erba (Universität Hamburg) or with a CE Instruments FlashEA® 1112 Elemental Analyzer (Technische Universität Braunschweig). Analyses indicated by the symbols of elements were within ±0.4% of the theoretical values. Mass spectra were recorded on a VG 70-250S, VG Analytical, using a FAB xenon atom beam and PEG 300 or PEG 600 as matrices (Institute of Organic Chemistry, Universität Hamburg); or a Finnigan-MAT 95 instrument (department of mass spectrometry of the Chemical Institutes of the Technische Universität Braunschweig). The HPLC purity analyses were carried out using a Merck Hitachi LaChrom Elite system (pump: L-2130, DAD detector: L-2450; autosampler: L-2200; column: Merck LiChroCART 125-4, LiChrospher 100 RP-18 (5 μm); eluent: acetonitrile/water mixtures, elution rate 1.000 mL/min; detection wavelength: 254 nm and 280 nm; overall run time: 15 min.); t_M = hold-up time, t_N = net retention time.

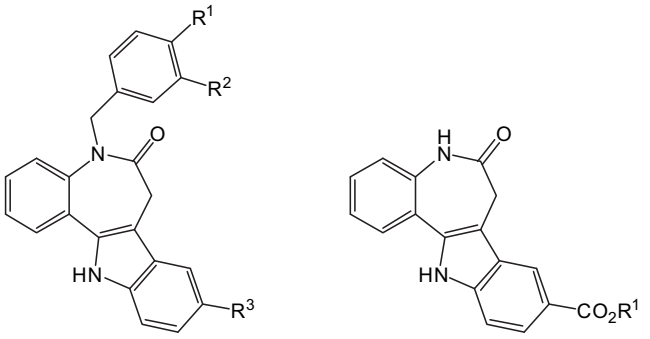
The following compounds were synthesized according to published procedures: **1a–h**, **1m**, **1o**, **1r–w**, **2a–d**, 9-methoxypaullone (**1x**) [14,15], **1p**, **1q** [11], **1i** [16], 9-*tert*-butylpaullone (**1y**) [16], **3** [12].

5.1.2. Preparation of paullones (7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-ones; (**1**)), general procedure

An appropriate 3,4-dihydro-1*H*-1-benzazepine-2,5-dione (1 mmol) and an appropriate substituted phenylhydrazine (1.50 mmol) [or the appropriate substituted phenylhydrazine hydrochloride (1.05 mmol) and sodium acetate (123 mg, 1.50 mmol)] are suspended in glacial acetic acid (10 mL) and stirred for 1 h at 70–80 °C. Subsequently, concentrated sulphuric acid (0.1 mL) is added, and stirring is continued at 70–80 °C for 1 h. After cooling to room temperature, the reaction mixture is poured into a 5% aqueous sodium acetate solution (30 mL). The resulting precipitate is filtered off with suction, washed with water, and crystallized from the given solvent.

*5.1.2.1. 9-(Methylthio)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**1k**).* Synthesis according to general procedure 5.1.2. Crystallization from ethanol afforded 60% beige powder; mp: >330 °C; IR (KBr): 3210 cm^{-1} (NH), 1640 cm^{-1} (C=O); ^1H NMR

Table 2
mMDH inhibition and kinase inhibition by 5-benzylpaullones **2a**, **4a–k** and paullone-9-carboxylic acid esters **1j**, **5a–f**



2a, 4a-k **1j, 5a-f**

.

Entry	Substitution pattern	IC_{50} mMDH [μM] ± SEM[a]	IC_{50} GSK-3 [μM]	IC_{50} CDK1-cyclin B [μM]
2a	R^1, R^2 = H; R^3 = Br	2.4 ± 0.4	10	35
4a	R^1, R^2 = H; R^3 = Cl	2.7 ± 0.05	>10	>10
4b	R^1, R^2 = H; R^3 = $C(CH_3)_3$	1.6 ± 0.1	>10	>10
4c	R^1, R^2 = H; R^3 = CF_3	4.2 ± 0.1	>10	>10
4d	R^1, R^2 = H; R^3 = CH_3	6.2 ± 1.2	>10	>10
4e	R^1, R^2 = H; R^3 = OCH_3	11.9 ± 0.4	>10	>10
4f	R^1 = CH_3, R^2 = H; R^3 = Br	1.6 ± 0.1	>10	>10
4g	R^1 = OCH_3, R^2 = H; R^3 = Br	1.8 ± 0.3	>10	>10
4h	R^1, R^2 = Cl; R^3 = Br	1.1 ± 0.1	>10	>10
4i	R^1 = CH_3, R^2, R^3 = H	10.5 ± 0.1	>10	>10
4j	R^1, R^2 = Cl; R^3 = H	7.9 ± 0.5	>10	>10
4k	R^1, R^2 = Cl; R^3 = $C(CH_3)_3$	0.77 ± 0.02	>10	>10
1j	R^1 = CH_3	13.5 ± 2.0	7.2	4.2
5a	R^1 = C_2H_5	20.0 ± 0.8	>100	25
5b	R^1 = n-C_3H_7	6.2 ± 0.2	700	13.0
5c	R^1 = n-C_4H_9	9.9 ± 0.3	>10	>10
5d	R^1 = n-C_5H_{11}	4.4 ± 0.6	600	25
5e	R^1 = n-C_6H_{13}	4.3 ± 0.2	>10	7.0
5f	R^1 = n-C_8H_{17}	2.7 ± 0.2	>1000	40

[a] mean of two tests. SEM = standard error of the mean.

(DMSO-d_6, 400 MHz): δ (ppm) = 2.51 (s, 3H, SCH_3), 3.51 (s, 2H, CH_2), 7.15 (dd, J = 8.4/1.5 Hz, 1H, ar H), 7.23–7.30 (m, 2H, ar H), 7.34–7.41 (m, 2H, ar H), 7.64 (d, J = 1.5 Hz, 1H, ar H), 7.73 (dd, J = 7.6/1.0 Hz, 1H, ar H), 10.10 (s, 1H, lactam NH), 11.63 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 17.2 (SCH_3), 31.4 (CH_2), 112.0, 117.3, 122.2, 123.1, 123.6, 126.8, 128.0 (tert. C), 107.0, 122.6, 127.0, 127.3, 133.2, 135.4, 135.9, 171.5 (quat. C); Anal. $C_{17}H_{14}N_2OS$ (C, H, N).

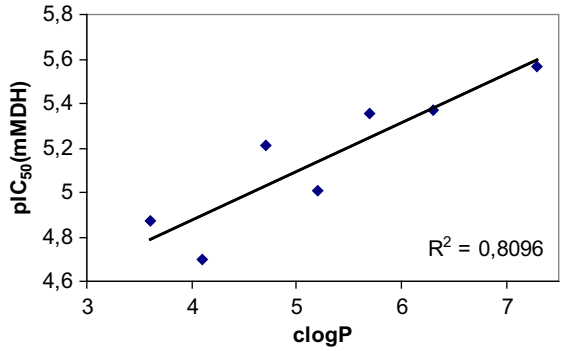


Fig. 1. Correlation between lipophilicity and mMDH inhibitory activity in the series of paullone-9-carboxylic acid alkyl esters **1j** and **5a–f**. The clogP values were calculated using ACD/Labs logP vers. 6.0.

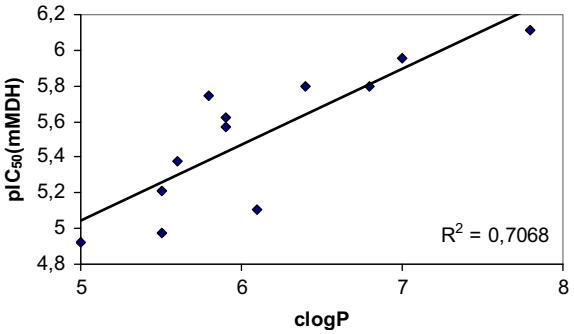


Fig. 2. Correlation between lipophilicity and mMDH inhibitory activity in the series of 5-benzylpaullones **2a** and **4a–k**. The clogP values were calculated using ACD/Labs logP vers. 6.0.

*5.1.2.2. 9-(Methylsulfinyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**1l**).* Under stirring, 9-(methylthio)-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**1k**) (150 mg, 0.50 mmol) was dissolved in acetone (60 mL) and cooled down to temperatures <0 °C. A solution of *meta*-chloroperbenzoic acid (141 mg of 70% *m*-CPBA, 0.57 mmol) in acetone (14 mL) was slowly added drop by drop to the dissolved educt, maintaining temperatures <0 °C and observing the progression of the reaction by TLC. After the completion of the reaction, the mixture was poured into water (300 mL). The resulting precipitate was filtered off with suction and washed with 7% aqueous sodium hydrogen carbonate solution. Crystallization from ethanol afforded 42% of a salmon colored powder, mp.: >330 °C; IR (KBr): 3200 cm^{-1} (NH), 1650 cm^{-1} (C=O), 1040 cm^{-1} (S=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 2.75 (s, 3H, $SOCH_3$), 3.57 (d, J = 2.6 Hz, 2H, CH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.7/7.5/1.5 Hz, 1H, ar H), 7.48 (dd, J = 8.5/1.7 Hz, 1H, ar H), 7.62 (d, J = 8.4 Hz, 1H, ar H), 7.77 (dd, J = 7.9/1.3 Hz, 1H, ar H), 8.04 (d, J = 1.2 Hz, 1H, ar H), 10.14 (s, 1H, lactam NH), 11.97 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.5 (CH_2), 43.6 ($SOCH_3$), 112.3, 114.3, 117.1, 122.2, 123.6, 126.9, 128.4 (tert. C), 108.0, 126.4, 134.3, 135.6, 136.3, 138.4, 171.4 (quat. C, one signal lacking due to peak overlapping); Anal. $C_{17}H_{14}N_2O_2S$ (C, H, N).

*5.1.2.3. 3-Methoxy-9-trifluormethyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**1n**).* Synthesis according to general procedure 5.1.2. Crystallization from ethanol afforded 25% yellow powder; mp. >330 °C; IR (KBr): 3230 cm^{-1} (NH), 1630 cm^{-1} (C=O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.59 (s, 2H, CH_2), 3.82 (s, 3H,

Table 3
Results of the in vitro cell line screening with five selected paullones **4b**, **4k**, **5d**, **5f**, and **1t**. Values indicate percentage of growth to untreated controls at 10 μM (**4b**, **4k**, **5d**, **5f**, **1t**) or GI_{50} values (**1t** [μM]).

	Colon cancer		Ovarian Cancer		Averaged parameter over all cell lines
	COLO 205[a]	HCT 116[a]	IGROV1[b]	OVCAR3[b]	
4b[c]	140%	91%	107%	125%	100%
4k[c]	98%	46%	74%	71%	67%
5d[d]	86%	69%	57%	79%	80%
5f[d]	94%	98%	92%	85%	88%
1t[c]	4%	−56%	2%	−34%	31%
1t[e]	0.47 μM	0.47 μM	0.93 μM	1.5 μM	5.6 μM[f]

[a] Colon cancer cell line.
[b] Ovarian cancer cell line.
[c] single one-dose test run @ 10 μM.
[d] averaged data from duplicate one-dose test runs @ 10 μM.
[e] GI_{50} values, averaged data from two 5-dose test runs.
[f] Meangraph midpoint (MG-MID).

OCH_3), 6.85 (d, 1H, 2.6 Hz, ar H), 6.95 (dd, 1H, 8.9/2.3 Hz, ar H), 7.43 (m, 1H, ar H), 7.59 (d, 1H, 8.6 Hz, ar H), 7.70 (d, 1H, 8.7 Hz, ar H), 8.09 (s, 1H, ar H), 10.01 (s, 1H, lactam NH), 11.98 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 55.3 (OCH_3), 31.2 (CH_2), 106.9, 110.3, 111.8, 115.4 (q, J_{CF} = 4 Hz), 117.8 (q, J_{CF} = 4 Hz), 128.1 (tert. C), 106.5, 115.1, 119.9, (q, J_{CF} = 32 Hz), 125.5 (q, J_{CF} = 271 Hz), 126.0, 134.9, 137.1, 138.5, 159.4, 171.1 (quat. C); Anal. $C_{18}H_{13}F_3N_2O_2$ (C, H, N).

*5.1.3. Preparation of 5-benzylpaullones **4a-4k**, general procedure*

A solution of the appropriate 7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one **1a**, **c**, **d**, **f**, **h**, **x**, **y** (1.0 mmol) in dried THF (15 mL) was refluxed under nitrogen atmosphere. Sodium hydride suspension in mineral oil (60%) (0.5 mmol) was added successively. As soon as gas formation was no longer observable, the appropriate benzyl bromide (0.5–1 mmol) was added to the mixture. After refluxing for the indicated reaction time, the reaction mixture was cooled to room temperature and water (50 mL) was added. The aqueous phase was extracted with dichloromethane (3 × 20 mL). The organic solvent was removed under reduced pressure and the obtained residue was purified by column chromatography and subsequent crystallization from ethanol/water (70:30).

*5.1.3.1. 5-Benzyl-9-chloro-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4a**).* Synthesis according to general procedure 5.1.3. (benzyl bromide 103 mg, 0.600 mmol, reaction time 1.5 h) yielded 27% white powder, mp.: 240 °C; IR (KBr): 3301 cm^{-1} (NH), 1646 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 3.16 (br s, 1H, azepine CH, superimposed by water peak), 4.03 (br s, 1H, azepine CH), 5.02 and 5.14 (2 br s, 2H, benzyl CH_2), 6.89–6.91 (m, 2H, ar H), 7.10–7.16 (m, 3H, ar H), 7.19 (dd, 1H, *J* = 8.7/2.1 Hz, ar H), 7.32–7.34 (m, 1H, ar H), 7.39–7.42 (m, 1H, ar H), 7.47 (d, 1H, *J* = 8.5 Hz, ar H), 7.58 (d, 1H, *J* = 8.3 Hz, ar H), 7.67 (dd, 1H, *J* = 7.9/1.5 Hz, ar H), 7.83 (d, 1H, *J* = 2.1 Hz, ar H), 11.95 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 30.0 (azepine CH_2), 50.6 (benzyl CH_2); 111.7, 116.2, 120.7, 122.9, 123.8, 124.8 (2C), 125.2, 125.9, 126.8 (2C), 127.0 (tert. C); 108.0, 122.4, 124.3, 125.6, 132.6, 134.3, 136.4, 137.8, 168.7 (quat. C); MS (EI): *m*/*z* = 372 $[M]^{+\bullet}$; HRMS ($C_{23}H_{17}ClN_2O$) calcd. 372.1029; found 372.1035; HPLC (ACN/H_2O; 60:40): 99.4% at 254 nm, 99.1% at 280 nm, t_N = 3.63 min, t_M = 1.02 min.

*5.1.3.2. 5-Benzyl-9-tert-butyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4b**).* Synthesis according to general procedure 5.1.3. (benzyl bromide 173 mg, 1.00 mmol, reaction time 8 h) yielded 24% beige powder, mp.: 211–212 °C; IR (KBr): 3233 cm^{-1} (NH), 1637 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.37 (s, 9H, 3x CH_3), 3.15 (br s, 1H, azepine CH), 4.00 (br s, 1H, azepine CH), 5.09 (br s, 2H, benzyl CH_2), 6.89–6.91 (m, 2H, ar H), 7.08–7.16 (m, 3H, ar H), 7.28–7.32 (m, 2H, ar H), 7.35–7.41 (m, 2H, ar H), 7.59 (dd, 1H, *J* = 8.2/1.1 Hz, ar H), 7.65–7.66 (m, 2H, ar H), 11.57 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 30.7 (azepine CH_2); 31.8 (3C) (CH_3); 34.4 ($C(CH_3)_3$); 51.9 (benzyl CH_2); 111.2, 113.6, 120.6, 125.1, 125.8, 126.4 (2C), 126.6, 127.1, 127.7, 128.2 (2C) (tert. C); 110.0, 124.2, 126.5, 132.4, 135.6, 137.9, 138.8, 141.6, 170.3 (quat. C); MS (EI): *m*/*z* (%) = 394 $[M]^{+\bullet}$; HRMS ($C_{27}H_{26}N_2O$) calcd. 394.2045; found. 394.2047; HPLC (ACN/H_2O; 60:40): 99.8% at 254 nm, 99.4% at 280 nm, t_N = 6.79 min, t_M = 1.02 min.

*5.1.3.3. 5-Benzyl-9-trifluoromethyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4c**).* Synthesis according to general procedure 5.1.3. (benzyl bromide 103 mg, 0.600 mmol, reaction time 2 h) yielded 16% orange crystals; mp.: 224 °C; IR (KBr): 3305 cm^{-1} (NH), 1648 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 3.21 (br s, 1H, azepine CH, superimposed by water peak), 4.18 (br s, 1H, azepine CH), 5.02 and 5.17 (2 br s, 2H, benzyl CH_2), 6.90–6.91 (m, 2H, ar H), 7.10–7.17 (m, 3H, ar H), 7.35 (dt, 1H, *J* = 7.5/1.1 Hz, ar H), 7.42–7.45 (m, 1H, ar H), 7.49 (dd, 1H, *J* = 8.7/1.7 Hz, ar H), 7.61 (dd, 1H, *J* = 8.4/1.0 Hz, ar H), 7.65 (d, 1H, *J* = 8.5 Hz, ar H), 7.70 (dd, 1H, *J* = 7.8/1.6 Hz, ar H), 8.22 (s, 1H, ar H), 12.25 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 31.3 (azepine CH_2), 52.0 (benzyl CH_2); 112.3, 116.2 (q, $^3J_{C,F}$ = 3.9 Hz, C–C–CF_3), 118.4 (q, $^3J_{C,F}$ = 3.3 Hz, C–C–CF_3), 124.3, 125.2, 126.2 (2C), 126.6, 127.3, 128.2 (2C), 128.6 (tert. C); 110.6, 120.0 (q, $^2J_{C,F}$ = 31.2 Hz, C–CF_3), 125.4 (q, $^1J_{C,F}$ = 271.3 Hz, CF_3), 125.5, 134.5, 137.7, 138.6, 139.2, 170.1 (1 quaternary C atom not detected under application of 512 Scans) (quat. C); Anal. $C_{24}H_{17}F_3N_2O$ (C,H,N); HPLC (ACN/H_2O; 60:40): 99.9% at 254 nm, 100.0% at 280 nm, t_N = 4.62 min, t_M = 1.02 min.

*5.1.3.4. 5-Benzyl-9-methyl-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4d**).* Synthesis according to general procedure 5.1.3. (benzyl bromide 173 mg, 1.00 mmol, reaction time 3 h) yielded 36% orange crystals; mp.: 214 °C; IR (KBr): 3322 cm^{-1} (NH), 1650 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 2.42 (s, 3H, CH_3), 3.14 (br s, 1H, azepine CH, superimposed by water peak), 3.93 (br s, 1H, azepine CH), 5.02 and 5.13 (2 br s, 2H, benzyl CH_2), 6.90–6.91 (m, 2H, ar H), 7.02 (dd, 1H, *J* = 8.3/1.7 Hz, ar H), 7.10–7.16 (m, 3H, ar H), 7.31 (dt, 1H, *J* = 7.5/1.2 Hz, ar H), 7.35–7.37 (m, 2H, ar H), 7.49 (d, 1H, *J* = 0.6 Hz, ar H), 7.57 (dd, 1H, *J* = 8.3/1.1 Hz, ar H), 7.66 (dd, 1H, *J* = 7.7/1.7 Hz, ar H), 11.58 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 21.1 (CH_3); 31.6 (azepine CH_2), 52.0 (benzyl CH_2); 111.3, 117.7, 123.8, 124.1, 125.1, 126.2 (2C), 126.5, 127.1, 127.8, 128.1 (2C) (tert. C); 109.2, 126.1, 126.3, 127.7, 132.4, 135.7, 137.8, 138.9, 170.1 (quat. C); Anal. ($C_{24}H_{20}N_2O$) (C,H,N); HPLC (ACN/H_2O; 60:40): 99.2% at 254 nm, 99.0% at 280 nm, t_N = 3.62 min, t_M = 1.02 min.

*5.1.3.5. 5-Benzyl-9-methoxy-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4e**).* Synthesis according to general procedure 5.1.3. (benzyl bromide 86 mg, 0.50 mmol, reaction time 3 h) yielded 8% yellow crystals; mp.: 222 °C (Lit. [17] 213–215 °C); IR (KBr): 3429 cm^{-1} (NH), 1635 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 3.15 (br s, 1H, azepine CH, superimposed by water peak), 3.81 (s, 3H, OCH_3), 3.99 (br s, 1H, azepine CH), 5.03 and 5.14 (2 br s, 2H, benzyl CH_2), 6.83 (dd, 1H, *J* = 8.7/2.5 Hz, ar H), 6.91–6.92 (m, 2H, ar H), 7.10–7.17 (m, 3H, ar H), 7.23 (d, 1H, *J* = 2.5 Hz, ar H), 7.31 (dt, 1H, *J* = 7.5/1.2 Hz, ar H), 7.34–7.37 (m, 2H, ar H), 7.57 (dd, 1H, *J* = 8.3/1.1 Hz, ar H), 7.64 (dd, 1H, *J* = 7.7/1.7 Hz, ar H), 11.56 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 30.2 (azepine CH_2), 50.7 (benzyl CH_2); 53.9 (OCH_3); 98.2, 111.0, 111.4, 122.8, 123.7, 124.9 (2C), 125.2, 125.7, 126.4, 126.8 (2C) (tert. C); 108.3, 124.9, 125.0, 131.1, 131.5, 136.5, 137.5, 152.2, 168.9 (quat. C); HRMS ($C_{24}H_{20}N_2O_2$) calcd. 368.1525; found 368.1529; HPLC (ACN/H_2O; 60:40): 96.3% at 254 nm, 96.6% at 280 nm, t_N = 2.08 min, t_M = 1.02 min.

*5.1.3.6. 9-Bromo-5-(4-methylbenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4f**).* Synthesis according to general procedure 5.1.3. (4-methylbenzyl bromide 93 mg, 0.50 mmol, reaction time 2.5 h) yielded 28% off-white powder; mp.: 274 °C; IR (KBr): 3318 cm^{-1} (NH), 1639 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 2.17 (s, 3H, CH_3), 3.14 (br s, 1H, azepine CH, superimposed by water peak), 4.01 (br s, 1H, azepine CH), 4.98 and 5.09 (2 br s, 2H, benzyl CH_2), 6.76–6.78 (m, 2H, ar H), 6.92–6.94 (m, 2H, ar H), 7.29–7.34 (m, 2H, ar H), 7.38–7.44 (m, 2H, ar H), 7.59 (dd, 1H, *J* = 8.1/1.0 Hz, ar H), 7.66 (dd, 1H, *J* = 7.7/1.6 Hz, ar H), 7.96 (d, 1H, *J* = 2.0 Hz, ar H), 11.93 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 20.4 (CH_3); 31.3 (azepine CH_2), 51.7 (benzyl CH_2); 113.5, 120.6, 124.3, 124.6, 125.2, 126.3 (2C), 127.2, 128.3, 128.7 (2C) (tert. C); 109.3, 111.7, 125.7, 127.7, 133.9, 134.6, 135.6, 135.9, 139.1, 170.0 (quat. C); HRMS ($C_{24}H_{19}BrN_2O$) calcd. 430.0681; found 430.0676; HPLC (ACN/H_2O; 70:30): 97.2% at 254 nm, 97.1% at 280 nm, t_N = 2.48 min, t_M = 1.03 min.

*5.1.3.7. 9-Bromo-5-(4-methoxybenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4g**).* Synthesis according to general procedure 5.1.3. (4-methoxybenzyl bromide 101 mg, 0.500 mmol, reaction time 4 h) with a reaction time of 4 h yielded 28% white powder; mp.: 236 °C; IR (KBr): 3319 cm^{-1} (NH), 1638 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 3.11 (br s, 1H, azepine CH, superimposed by water peak), 3.64 (s, 3H, OCH_3), 4.02 (br s, 1H, azepine CH), 4.91 and 5.12 (2 br s, 2H, benzyl CH_2), 6.68–6.70 (m, 2H, ar H), 6.79–6.82 (m, 2H, ar H), 7.30 (dd, 1H, J = 8.7/1.9 Hz, ar H), 7.32–7.34 (m, 1H, ar H), 7.39–7.43 (m, 2H, ar H), 7.62 (dd, 1H, J = 8.3/0.8 Hz, ar H), 7.65 (dd, 1H, J = 7.7/1.7 Hz, ar H), 7.97 (d, 1H, J = 1.5 Hz, ar H), 11.94 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 31.3 (azepine CH_2), 51.2 (benzyl CH_2); 54.8 (OCH_3); 113.5 (2C), 113.5, 120.6, 124.4, 124.6, 125.1, 127.2, 127.6 (2C), 128.2 (tert. C); 109.3, 111.6, 125.8, 129.5, 133.8, 135.8, 139.0, 157.8, 170.0 (quat. C); HRMS ($C_{24}H_{19}BrN_2O_2$) calcd. 446.0630; found 446.0633; HPLC (ACN/H_2O; 60:40): 97.3% at 254 nm, 96.6% at 280 nm, t_N = 4.19 min, t_M = 1.02 min.

*5.1.3.8. 9-Bromo-5-(3,4-dichlorobenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4h**).* Synthesis according to general procedure 5.1.3. (3,4-dichlorobenzyl bromide 120 mg, 0.500 mmol, reaction time 5.5 h) yielded 25% beige powder, mp.: 251 °C; IR (KBr): 3166 cm^{-1} (NH), 1636 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 3.18 (br s, 1H, azepine CH, superimposed by water peak), 4.04 (br s, 1H, azepine CH), 4.97 and 5.17 (2 br s, 2H, benzyl CH_2), 6.90 (dd, 1H, J = 8.3/2.1 Hz, ar H), 7.01 (d, 1H, J = 1.9 Hz, ar H), 7.31 (dd, 1H, J = 8.5/2.0 Hz, ar H), 7.35–7.38 (m, 1H, ar H), 7.42–7.45 (m, 3H, ar H), 7.57 (d, 1H, J = 8.3 Hz, ar H), 7.69 (dd, 1H, J = 7.7/1.5 Hz, ar H), 7.99 (d, 1H, J = 1.9 Hz, ar H), 12.00 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 31.2 (azepine CH_2), 51.0 (benzyl CH_2); 113.6, 120.7, 124.4, 124.7, 125.5, 126.7, 127.3, 128.1, 128.5, 130.3 (tert. C); 109.2, 111.7, 125.8, 127.6, 130.8, 133.7, 136.0, 138.8, 139.0, 170.2 (quat. C, 1 quaternary C atom not detected due to peak overlapping); HRMS ($C_{23}H_{15}BrCl_2N_2O$) calcd. 483.9745; found 483.9747; HPLC (ACN/H_2O; 70:30): 99.3% at 254 nm, 98.7% at 280 nm, t_N = 3.60 min, t_M = 1.03 min.

*5.1.3.9. 5-(4-Methylbenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4i**).* Synthesis according to general procedure 5.1.3. (4-methylbenzyl bromide 93 mg, 0.50 mmol, reaction time 3.5 h) yielded 26% yellow crystals; mp.: 228–229 °C; IR (KBr): 3229 cm^{-1} (NH), 1640 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 2.17 (s, 3H, CH_3), 3.14 (br s, 1H, azepine CH), 3.97 (br s, 1H, azepine CH), 4.96 and 5.12 (2 br s, 2H, benzyl CH_2), 6.78–6.79 (m, 2H, ar H), 6.93–6.95 (m, 2H, ar H), 7.09–7.11 (m, 1H, ar H), 7.18–7.21 (m, 1H, ar H), 7.30–7.33 (m, 1H, ar H), 7.36–7.38 (m, 1H, ar H), 7.46 (dd, 1H, J = 8.1/0.8 Hz, ar H), 7.58 (dd, 1H, J = 8.3/0.8 Hz, ar H), 7.67 (dd, 1H, J = 7.7/1.7 Hz, ar H), 7.71 (d, 1H, J = 7.7 Hz, ar H) 11.72 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 19.4 (CH_3); 30.5 (azepine CH_2), 50.5 (benzyl CH_2); 110.5, 117.1, 118.0, 121.1, 123.1, 124.0, 125.2 (2C), 126.0, 126.7, 127.6 (2C) (tert. C); 108.6, 124.8, 125.1, 131.2, 133.6, 134.5, 136.2, 137.8, 169.0 (quat. C); HRMS ($C_{24}H_{20}N_2O$): calcd. 352.1575; found 352.1565; HPLC (ACN/H_2O; 65:35): 98.6% at 254 nm, 98.7% at 280 nm, t_N = 2.28 min, t_M = 1.02 min.

*5.1.3.10. 5-(3,4-Dichlorobenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4j**).* Synthesis according to general procedure 5.1.3. (3,4-dichlorobenzyl bromide 120 mg, 0.500 mmol, reaction time 4 h) yielded 32% yellow crystals; mp.: 232–233 °C; IR (KBr): 3252 cm^{-1} (NH), 1634 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.21 (br s, 1H, azepine CH, superimposed by water peak), 3.96 (br s, 1H, azepine CH), 5.07 (br s, 2H, benzyl CH_2), 6.90 (dd, 1H, J = 8.3/2.3 Hz, ar H), 7.03 (d, 1H, J = 2.0 Hz, ar H), 7.08–7.12 (m, 1H, ar H), 7.18–7.22 (m, 1H, ar H), 7.33–7.43 (m, 3H, ar H), 7.46–7.49 (m, 1H, ar H), 7.57 (dd, 1H, J = 8.1/1.3 Hz, ar H), 7.68–7.73 (m, 2H, ar H), 11.75 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 31.4 (azepine CH_2), 51.0 (benzyl CH_2); 111.6, 118.2, 119.1, 122.3, 124.3, 125.4, 126.7, 127.1, 128.0, 128.2, 130.3 (tert. C); 109.6, 125.8, 126.2, 129.0, 130.8, 132.1, 137.3, 138.5, 139.1, 170.3 (quat. C); Anal. ($C_{23}H_{16}Cl_2N_2O$) (C,H,N); HPLC (ACN/H_2O; 60:40): 99.7% at 254 nm, 99.8% at 280 nm, t_N = 4.89 min, t_M = 1.02 min.

*5.1.3.11. 9-tert-Butyl-5-(3,4-dichlorobenzyl)-7,12-dihydroindolo[3,2-d][1]benzazepin-6(5H)-one (**4k**).* Synthesis according to general procedure 5.1.3 (3,4-dichlorobenzyl bromide 120 mg, 0.500 mmol, reaction time 3 h) yielded 16% yellow powder; mp.: 221–222 °C; IR (KBr): 3241 cm^{-1} (NH), 1637 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 1.37 (s, 9H, 3x CH_3), 3.15 (br s, 1H, azepine CH), 4.01 (br s, 1H, azepine CH), 4.97 and 5.21 (2 br s, 2H, benzyl CH_2), 6.89 (dd, 1H, J = 8.3/2.1 Hz, ar H), 7.05 (d, 1H, J = 1.9 Hz, ar H), 7.30 (dd, 1H, J = 8.7/1.9 Hz, ar H), 7.34 (dt, 1H, J = 7.5/1.0 Hz, ar H), 7.37–7.42 (m, 3H, ar H), 7.57 (dd, 1H, J = 8.2/0.8 Hz, ar H), 7.65–7.68 (m, 2H, ar H), 11.62 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ (ppm) = 30.8 (azepine CH_2), 31.1 (3C) (CH_3); 33.7 ($C(CH_3)_3$); 50.2 (benzyl CH_2); 110.6, 113.0, 120.1, 123.7, 124.8, 126.1, 126.5, 127.2, 127.7, 129.7 (tert. C); 109.3, 125.0, 125.9, 128.5, 130.2, 131.6, 135.0, 137.7, 138.5, 141.0, 169.8 (quat. C); HRMS ($C_{27}H_{24}Cl_2N_2O$) calcd. 462.1266; found 462.1255; HPLC (ACN/H_2O; 70:30): 99.2% at 254 nm, 98.6% at 280 nm, t_N = 5.84 min, t_M = 1.02 min.

*5.1.4. Preparation of paullone-9-carboxylic esters **1j** and **5a–f**, general procedure*

To a suspension of 6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*][1]benzazepine-9-carboxylic acid (**1i**) (0.5 mmol) in freshly dried THF (3.5 mL), 2.5 equiv. of triphenylphosphane (1.25 mmol) and 2.5 equiv. of an appropriate anhydrous alcohol (1.25 mmol) are added. The reaction mixture is stirred in an ice bath for 30 min before adding 2.5 equiv. of di-*tert*-butyl azodicarboxylate (1.25 mmol), and stirring is continued for another 30 min. The ice bath is removed, and the mixture is allowed to warm up to room temperature. The reaction mixture is then stirred for a total of 24 h, adding 1 mL of the appropriate alcohol after 20 h. The solid is filtered off with suction and crystallized from methanol unless stated otherwise.

*5.1.4.1. Methyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**1j**).* Preparation according to general procedure 5.1.4. yielded 23% white powder; mp.: >330 °C; IR (KBr): 3370 cm^{-1} (NH), 3200 cm^{-1} (NH), 1690 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 3.56 (s, 2H, CH_2), 3.87 (s, 3H, ester–OCH_3), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.6/7.6/1.5 Hz, 1H, ar H), 7.52 (d, J = 8.4 Hz, 1H, ar H), 7.77 (dd, J = 7.8/1.4 Hz, 1H, ar H), 7.81 (dd, J = 8.7/1.5 Hz, 1H, ar H), 8.35 (d, J = 1.3 Hz, 1H, ar H), 10.14 (s, 1H, lactam NH), 12.03 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 31.4 (CH_2), 51.6 (ester–OCH_3), 111.4, 120.4, 122.3, 122.9, 123.6, 126.9, 128.4 (tert. C), 108.6, 120.6, 122.1, 126.0, 134.2, 135.6, 139.8, 167.0, 171.2 (quat. C); Anal $C_{18}H_{14}N_2O_3$ (H, N); C calcd. 70.58; found 70.06.

*5.1.4.2. Ethyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5a**).* Preparation according to general procedure 5.1.4. yielded 29% white powder after crystallization from ethanol; mp.: >330 °C; IR (KBr): 3380 cm^{-1} (NH), 3200 cm^{-1} (NH), 1690 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.37 (t, J = 7.1 Hz, 3H, CH_3), 3.56 (s, 2H, azepine CH_2), 4.34 (q, J = 7.1 Hz, 2H, ester OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.8/7.6/1.4 Hz, 1H, ar H), 7.52 (d, J = 8.6 Hz, 1H, ar H), 7.77 (dd, J = 7.7/1.1 Hz, 1H, ar H), 7.82 (dd, J = 8.6/1.5 Hz, 1H, ar H), 8.34 (d, J = 1.3 Hz, 1H, ar H), 10.15 (s, 1H, lactam NH), 12.02 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 14.2 (CH_3), 31.4 (azepine CH_2),

60.1 (ester OCH_2), 111.3, 120.3, 122.3, 122.9, 123.6, 126.9, 128.4 (tert. C), 108.6, 120.9, 122.2, 126.0, 134.2, 135.6, 139.8, 166.5, 171.2 (quat. C); Anal. $C_{19}H_{16}N_2O_3$ (C,H,N).

*5.1.4.3. Propyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5b**).* Preparation according to general procedure 5.1.4. yielded 28% white powder, mp.: 285 °C (dec.); IR (KBr): 3360 cm^{-1} (NH), 3200 cm^{-1} (NH), 1690 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 1.01 (t, J = 7.4 Hz, 3H, CH_3), 1.77 (sext., J = ~7.1 Hz, 2H, ester CH_2), 3.56 (s, 2H, azepine CH_2), 4.25 (t, J = 6.7 Hz, 2H, OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.8/7.6/1.4 Hz, 1H, ar H), 7.52 (d, J = 8.4 Hz, 1H, ar H), 7.77 (dd, J = 7.9/1.3 Hz, 1H, ar H), 7.82 (dd, J = 8.5/1.7 Hz, 1H, ar H), 8.34 (d, J = 1.3 Hz, 1H, ar H), 10.15 (s, 1H, lactam NH), 12.01 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 10.4 (CH_3), 21.7 (CH_2), 31.4 (azepine CH_2), 65.6 (OCH_2), 111.4, 120.2, 122.3, 122.9, 123.7, 126.9, 128.4 (tert. C), 108.5, 120.9, 122.2, 126.0, 134.2, 135.6, 139.8, 166.6, 171.3 (quat. C); Anal. $C_{20}H_{18}N_2O_3$ (C, H, N)

*5.1.4.4. Butyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5c**).* Preparation according to general procedure 5.1.4. yielded 30% white powder; mp: >330 °C (dec. starting at 285 °C); IR (KBr): 3370 cm^{-1} (NH), 3200 cm^{-1} (NH), 1690 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 0.97 (t, J = 7.4 Hz, 3H, CH_3), 1.47 (sext., J = ~7.4 Hz, 2H, CH_2), 1.74 (quint., J = ~7.1 Hz, 2H, CH_2), 3.56 (s, 2H, azepine CH_2), 4.30 (t, J = 6.6 Hz, 2H, OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.6/7.6/1.5 Hz, 1H, ar H), 7.52 (d, J = 8.4 Hz, 1H, ar H), 7.77 (dd, J = 7.9/1.3 Hz, 1H, ar H), 7.81 (dd, J = 8.5/1.7 Hz, 1H, ar H), 8.33 (d, J = 1.3 Hz, 1H, ar H), 10.15 (s, 1H, lactam NH), 12.01 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 14.0 (CH_3), 19.2, 30.8 (ester CH_2), 31.9 (azepine CH_2), 64.3 (ester OCH_2), 111.8, 120.7, 122.7, 123.4, 124.1, 127.3, 128.9 (tert. C), 109.0, 121.4, 122.6, 126.5, 134.7, 136.1, 140.3, 167.0, 171.7 (quat. C); Anal. $C_{21}H_{20}N_2O_3$ (C, H, N).

*5.1.4.5. Pentyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5d**).* Preparation according to general procedure 5.1.4. yielded 15% white powder; mp.: 293 °C (dec.); IR (KBr): 3370 cm^{-1} (NH), 3200 cm^{-1} (NH), 1690 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 0.92 (t, J = 7.1 Hz, 3H, CH_3), 1.33–1.47 (m, 4H, CH_2–CH_2), 1.76 (quint., J = ~7.0 Hz, 2H, CH_2), 3.56 (s, 2H, azepine CH_2), 4.29 (t, J = 6.6 Hz, 2H, OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.8/7.6/1.4 Hz, 1H, ar H), 7.52 (d, J = 8.4 Hz, 1H, ar H), 7.77 (dd, J = 7.6/1.3 Hz, 1H, ar H), 7.81 (dd, J = 8.5/1.6 Hz, 1H, ar H), 8.33 (d, J = 1.5 Hz, 1H, ar H), 10.16 (s, 1H, lactam NH), 12.03 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 13.8 (CH_3), 21.7, 27.7, 27.9 (CH_2), 31.4 (azepine CH_2), 64.1 (OCH_2), 111.4, 120.2, 122.3, 122.9, 123.7, 126.9, 128.4 (tert. C), 108.5, 120.9, 122.2, 126.0, 134.2, 135.6, 139.8, 166.6, 171.3 (quat. C); Anal $C_{22}H_{22}N_2O_3$ (H, N); C calcd. 72.91; found 72.04. HRMS–FAB (m/z): $[M + H]^+$ calcd. 363.1709, found 363.1702.

*5.1.4.6. Hexyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5e**).* Preparation according to general procedure 5.1.4. yielded 28% white powder; mp.: 280 °C (dec.); IR (KBr): 3370 cm^{-1} (NH), 3200 cm^{-1} (NH), 1680 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 0.89 (t, J = 7.0 Hz, 3H, CH_3), 1.28–1.49 (m, 6H, CH_2–CH_2–CH_2), 1.76 (quint., J = ~7.1 Hz, 2H, CH_2), 3.56 (s, 2H, azepine CH_2), 4.29 (t, J = 6.6 Hz, 2H, OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.6/7.6/1.5 Hz, 1H, ar H), 7.52 (d, J = 8.7 Hz, 1H, ar H), 7.77 (dd, J = 7.9/1.3 Hz, 1H, ar H), 7.81 (dd, J = 8.6/1.5 Hz, 1H, ar H), 8.33 (d, J = 1.5 Hz, 1H, ar H), 10.16 (s, 1H, lactam NH), 12.03 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 14.3 (CH_3), 22.4, 25.6, 28.6, 31.3 (CH_2), 31.9 (azepine CH_2), 64.6 (OCH_2), 111.8, 120.7, 122.7, 123.4, 124.1, 127.3, 128.9 (tert. C), 109.0, 121.4, 122.6, 126.5, 134.7, 136.1, 140.3, 167.0, 171.7 (quat. C); Anal ($C_{23}H_{24}N_2O_3$) (C, H, N).

*5.1.4.7. Octyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-d][1]benzazepine-9-carboxylate (**5f**).* Preparation according to general procedure 5.1.4. yielded 33% white powder; mp.: 292 °C (dec.); IR (KBr): 3360 cm^{-1} (NH), 3200 cm^{-1} (NH), 1680 cm^{-1} (C═O), 1670 cm^{-1} (C═O); ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) = 0.86 (t, J = 6.7 Hz, 3H, CH_3), 1.21–1.48 (m, 10H, CH_2), 1.75 (quint., J = ~7.0 Hz, 2H, CH_2), 3.55 (s, 2H, azepine CH_2), 4.28 (t, J = 6.6 Hz, 2H, OCH_2), 7.25–7.33 (m, 2H, ar H), 7.41 (dt, J = 7.8/7.6/1.5 Hz, 1H, ar H), 7.52 (d, J = 8.6 Hz, 1H, ar H), 7.77 (dd, J = 7.6/1.0 Hz, 1H, ar H), 7.81 (dd, J = 8.6/1.5 Hz, 1H, ar H), 8.32 (d, J = 1.3 Hz, 1H, ar H), 10.15 (s, 1H, lactam NH), 12.02 (s, 1H, indole NH); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ (ppm) = 13.8 (CH_3), 22.0, 25.5, 28.2, 28.5, 28.6, 31.1 (CH_2), 31.4 (azepine CH_2), 64.1 (OCH_2), 111.4, 120.2, 122.3, 122.9, 123.6, 126.9, 128.4 (tert. C), 108.5, 120.9, 122.2, 126.0, 134.2, 135.6, 139.8, 166.5, 171.2 (quat. C); Anal. $C_{25}H_{28}N_2O_3$ (H, N); C calcd. 74.23; found 73.65; HRMS–FAB (m/z): $[M + H]^+$ calcd. 405.2178, found 405.2175.

5.2. Biological assays

5.2.1. mMDH inhibition assay

5.2.1.1. Reagents. Trizma buffer, pH 8.5: Trizma®-base (Sigma T-1503) (12.11 g, 100.0 mmol) is dissolved in aqua bidest. The pH is adjusted to 8.5 with HCl (10%).

L-malate, 0.5 M: L-malic acid disodium salt (Sigma, M 9138) (1.78 g, 10.0 mmol) is dissolved in Trizma buffer to yield 20.0 mL.

NAD, 16 mM: NAD (Fluka 43410) (10.614 mg, 16.000 μmol) is dissolved in Trizma buffer (1000 μL).

mMDH solution 0.5%: mMDH (5 μL, Sigma M 2634, solution in 50% glycerol) is dissolved in Trizma buffer pH 8.5 to yield 1000 μL. The enzyme solution is prepared freshly and kept at 0 °C.

Solutions of test compounds, 10 mM: test compound (1.000 mg) is dissolved in a measured volume of DMSO to yield a 10 mM stock solution. The stock solution is dissolved in DMSO to yield solutions of 0.050, 0.100, 0.250, 0.500, 1000, 2500 mM. All solutions except the enzyme solution are maintained and handled at 25 °C.

5.2.1.2. Assay conditions. mMDH catalyzes the conversion of L-malate to oxaloacetate, which is coupled to the formation of the reduced cofactor $NADH/H^+$. In the applied spectral photometric assay, absorbance at 340 nm caused by $NADH/H^+$ was used as a read-out. For calculation of enzyme activity, a time interval of 2.5 min, during which a linear absorbance increase was observed, was monitored. The mMDH assays were run in a dual-trace spectrometer Specord® 200 (Analytik Jena AG) equipped with a constant temperature holder maintained at 25 °C. Assays were performed in disposable 1.5 mL polymethylmethacrylate cuvettes (Sarstedt AG, Nümbrecht, Germany) of 1 cm width. For the assay, Trizma buffer (790 μL), enzyme solution (3–10 μL, depending on activity), and inhibitor solution (10 μL) were incubated in a cuvette for 3 min. The reaction was started by addition of NAD (50 μL of 16 mM solution) and L-malate (150 μL of 0.5 M solution). The absorbance increase at 340 nm was monitored over 2.5 min against a blank solution (same mixture of reagents without enzyme solution). The slope rate $\Delta A_{340}/\Delta t$ is a measure for the reaction velocity. The mMDH activity is expressed as ratio of the velocities of the inhibited and the uninhibited reaction. For determination of the velocity of the uninhibited reaction the assay was performed with 10 μL DMSO instead of the inhibitor solution. Dose response-curves were constructed by drawing the residual enzyme activities against the final inhibitor concentrations. IC_{50} values were calculated from the dose response-curves using GraphPad Prism 4.00 (Graphpad Software, San Diego, CA, USA, 2003). Assays were performed in duplicate.

5.2.2. Kinase inhibition assays

Kinase inhibition assays were performed according to the methodology developed by the "Protein Phosphorylation & Human Disease" Group of the Station Biologique, Roscoff, France [18]

5.2.3. In vitro cell line screening

Screening compounds are tested against 60 human tumor cell lines initially at 10^{-4} M test compound concentration. After 48 h of continuous drug exposure, a sulforhodamine B protein assay is used to determine cell viability or growth. The percentage of growth is calculated on the basis of the optical density of the treated cultures compared to untreated control cell lines. In case of a noteworthy antiproliferative activity and selectivity, the tests are repeated at five concentrations (10^{-8} M to 10^{-4} M) and dose response-curves are calculated for each cell line. Details concerning the performance of this screening have been published [19,20].

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