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PAPER

New synthetic approach to paullones and characterization of their SIRT1 inhibitory activity†

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A series of 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-ones (paullones) substituted at C9/C10 (Br) and C2 (Me, CF_3, CO_2Me) have been synthesized by a one-pot Suzuki–Miyaura cross-coupling of an *o*-aminoarylboronic acid and methyl 2-iodoindoleacetate followed by intramolecular amide formation. Other approaches to the paullone scaffold based on Pd-catalyzed C–H activation were unsuccessful. *In vitro* enzymatic assay with recombinant human SIRT-1 indicated a strong inhibitory profile for the series, in particular the analogue with a methoxycarbonyl group at C2 and a bromine at C9. These compounds are, in general, inducers of granulocyte differentiation of the U937 acute leukemia cell line and cause a marked increase in pre-G1 of the cell cycle.

Introduction

Sirtuins (Class III HDACs)[1] are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases that share homology with the yeast transcriptional repressor Sir2 (Silent Information Regulator 2) and regulate a variety of cellular functions such as conservation of the genome, longevity and metabolism of organisms ranging from bacteria to eukaryotes.[1–6] In contrast, other histone deacetylases (Class I and II HDACs) are Zn^{+2}-dependent hydrolases and do not share sequence similarities with the sirtuins.[7–9]

In mammals, the sirtuin family consists of seven members, SIRT1–7, which share a conserved catalytic domain of about 275 amino acids but differ in their cellular localization and their function.[1] SIRT1, SIRT2, SIRT3, SIRT5, SIRT6 and SIRT7 have activity as NAD^+-dependent deacetylases, while SIRT4 and SIRT6 show ADP-ribosyl transferase activity.[10] SIRT3 is also a mitochondrial protein with tumour suppressor function and is necessary to maintain mitochondrial integrity and metabolism during stress.[11]

Several crystal structures of SIRT enzymes – either uncomplexed or bound to NAD^+ analogs and the acetyl–lysine peptide substrates – have been reported, including the structures of human orthologs SIRT2,[12] SIRT3,[13] SIRT5[14] and SIRT6.[15] These systems contain a Rossmann fold domain for NAD^+ binding and a smaller domain composed of α-helical and Zn-binding modules. The NAD^+ binding site is usually divided into three binding pockets, the adenine ribose moiety in site **A**, the nicotinamide ribose moiety in site **B**, and the nicotinamide heterocycle in site **C**, which lies deep inside the pocket.[16] The nicotinamide part seems to be more flexible and the heterocycle has been found to occupy alternative locations in several of the crystal structures. The acetylated peptide binds in the major groove of sirtuins, and is located in a tunnel that leads to the NAD^+ binding site, close to residues (His and Asn) that are important for the catalytic activity. Binding of acetyl–lysine induces a strained conformation for NAD^+ (trapped in the crystal structure of a Michaelis complex) that buries the nicotinamide ring deep within the active site.[16–18] The precise details of the nicotinamide displacement step by the incoming nucleophile (the amide oxygen of the N^{ϵ}-acetyl–lysine group) to form an *O*-alkylamidate intermediate[17–19] by S_N1–,[20,21] or S_N2–type reactions are still subject to debate.[22]

The identification of small molecule modulators of sirtuins has provided additional valuable tools for understanding the roles of these enzymes in various biological systems.[6,17,23]

Mechanism-based small molecule SIRT inhibitors have been developed ranging from the simple N^{ϵ}-(thio)acetyl lysines (**1**,**2**),[24,25] to lysine-containing peptides (**3**–**6**) (Fig. 1). The key lysine residue of these peptides has been modified with the thioacetyl (**4**)[26] carbamoyl (**3**),[27] or N^{ϵ}-thiocarbamoyl (**6**),[28]

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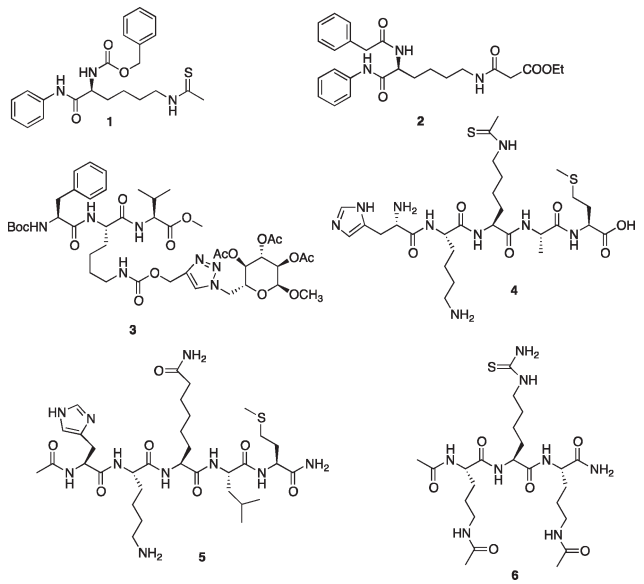


Fig. 1 Mechanism-based sirtuin inhibitors.

functional groups, and also L-2-amino-7-carboxamidoheptanoic acid has replaced lysine in tetrapeptide **5**.[29]

A fairly large collection of compounds derived from or inspired by natural product structures (**7–9**), such as **10**[30,31] and **11–17** have also been discovered by HTS or VLS protocols (Fig 2). For example, suramin (**11**), which has been co-crystallized with SIRT5,[14] and analogues are SIRT2-selective (IC_{50} = 93 nM for **11**),[32] the tenovins **12** are SIRT1/2 inhibitors,[33] whereas (*S*)-6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide, EX243 **13b**[34] is a nanomolar (IC_{50} = 120 nM) inhibitor (nicotinamide pocket binder) that shows about 23 times greater selectivity for SIRT1 than for SIRT2.[35] Nitrile AKG2 **14** was identified by docking a focused library into a model of human Sirt2[36] and it was proposed to occupy pocket C, thus competing for the nicotinamide part of NAD^+.[14–16] Kinase inhibitor Ro-318220 **15**, on the other hand, was considered to inhibit sirtuins by competing with the adenosine subpocket through the planar ring system of the maleimide group whereas the polar thioamidine functional group occupies a second binding site.[37] In the same campaign directed towards finding novel structures within those that target the adenosine binding pocket of enzymes or receptors, *N*-benzylkenpaullone **16b** and hydroxyamidinekenpaullone **17** were identified as SIRT inhibitors,[37] and were shown to compete for the NAD^+ binding site.

The 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-ones or paullones were first discovered during efforts directed towards finding inhibitors of cyclin–dependent kinases (CDKs, CDK-1, -2 and -5) based on the structure of flavopiridol, a semisynthetic flavonoid.[38,39] Together with the *in vitro* antiproliferative activity,[40] some members of the paullone family showed antitumour[39,41] and antileishmanial[42] activities. In addition, cazpaullone (9-cyano-1-aza-paullone) and analogues are inhibitors of glycogen synthase kinase-3 (GSK-3), an activity that might be relevant for the treatment of diabetes.[43] In fact, alsterpaullone (the C9-nitroderivative) acted in small screenings as a suppressor

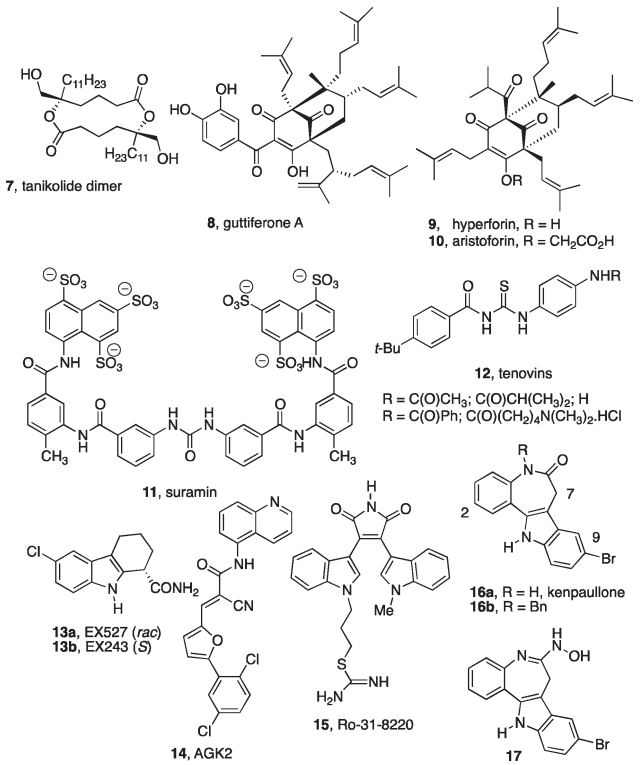
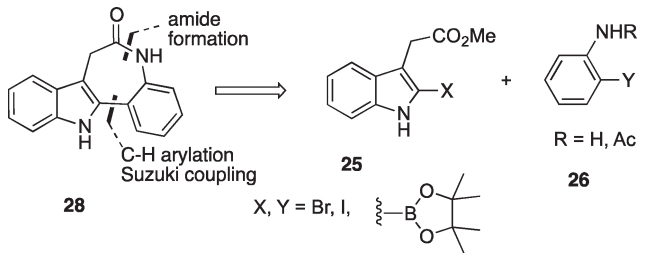


Fig. 2 Small-molecule sirtuin inhibitors.

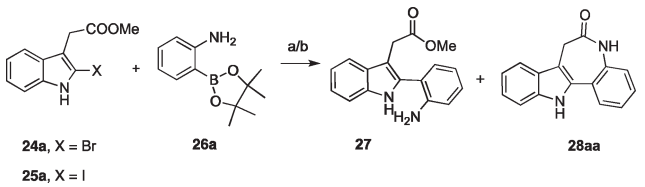
of cytokine-induced β-cell apoptosis, increasing their viability, and it therefore has potential for therapeutic intervention in type-1 diabetes.[44] Moreover, kenpullone (replacing Klf4), in combination with the transcription factors Oct4, Sox2 and C-Myc, can reprogramme MEFs into iPS cells and these colonies showed features of pluripotent ES cells.[45]

Given their interesting structure and biological activities, several groups have developed efficient syntheses to these compounds. These include functionalization of the 2-arylindole core structure built by traditional Fischer indole synthesis,[39,46] the cyclodehydration of an *N*-monosubstituted α-aminonitrile obtained by Strecker reaction of a protected 2-amino-benzaldehyde with ethyl 2-aminocinnamate,[47] the radical cyclization of iodoacetamides[48] and metal-catalyzed transformations. Among the latter approaches the Heck reaction,[49,50] oxidative coupling after rhodium(III)-catalyzed C–H functionalization of acetamides with alkynes,[51] the borylation–Suzuki coupling[52] and the Stille reaction[53] have all been employed inter- and/or intramolecularly with various degrees of success.

The presence of a haloindole fragment in both kenpaullones **16** and EX527/EX243 **13a,b** (Fig. 2) is suggestive of a similar pharmacophore in the interaction with SIRT enzymes. To our knowledge there have been no follow-up studies on the epigenetic activities of the paullones. In view of this we extended the previous work by Jung and co-workers[37] and set out to prepare novel analogues of the basic 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-one skeleton substituted at the C2-aryl ring and expanded the targets to the C9–Br (kenpaullones) and C10–Br analogues.



Scheme 1 Retrosynthetic analysis of the 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-one skeleton.



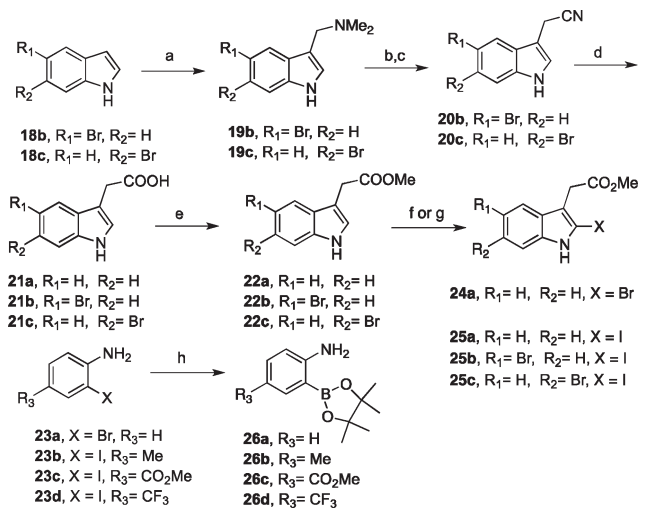
Scheme 3 a. $Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O, reflux, **27**, 14%; **28aa**, 29%. b. $Pd(OAc)_2$, dppf, CuCl, Cs_2CO_3, DMF; 100 °C for **24a**; **28aa**, 50%; 80 °C for **25a**; **28aa**, 84%.

Results and discussion

A rather straightforward retrosynthetic approach to the paullone skeleton (**28**) was considered first and this involved a direct C–H arylation[54–57] of the corresponding indole acetates with aniline derivatives followed by the intramolecular amide bond formation (Scheme 1). This method was deemed more convenient than the cross-coupling, since functionalization of the precursor(s) was not required or could be restricted to one of the components.

Methyl indoleacetate **22a**[58] was prepared from the commercial acid **21a** after *in situ* preparation of the acid chloride. The same step was used for the preparation of brominated analogues **22b**[59] and **22c**[60] from the acids **21b** and **21c**,[61] which in turn were obtained by hydrolysis of the corresponding nitriles **20b**[62] and **20c**.[61] These intermediates were produced from commercial 5- and 6-bromoindoles **18b** and **18c**, respectively, in a three-step sequence comprising the alkylation at C3 with Eschenmoser's salt, followed by *N*-methylation of **19b,c** and sodium cyanide-induced substitution of the non-isolated quaternary ammonium salts (Scheme 2).[60]

Neither of the protocols reported for the direct selective C2-arylation of indoles, a method pioneered by Ohta,[63] assayed using **22a** (and *N*-modified derivatives) and commercial 2-iodoaniline **23** as partners was successful. Among them, we tested the phosphine-free palladium-catalyzed arylation of unsubstituted (NH)-indoles described by Sames[64] and related methods that use co-catalysis by other metals, such as Fagnou's catalytic oxidative procedure with AgOAc;[65] Larrosa's phosphine-free $Pd(OAc)_2$-Ag_2O-mediated synthesis;[66] and Albericio-Lavilla's post-synthetic modification of tryptophans in peptides assisted by $Pd(OAc)_2$-$AgBF_4$.[67] Alternatively, Gaunt's reaction of *N*-acetylindoles with aryliodonium salts catalyzed by $Cu(OTf)_2$[68] also failed to provide the desired coupled product either under intramolecular or intermolecular fashion. In all cases, the control experiments afforded the reported C-2 substituted indoles. It is likely that the presence of a substituent at C3 of the indole moiety and *ortho* to the aniline unit makes the coupling sterically more difficult than in the unsubstituted cases reported to date.



Scheme 2 a. $Me_2N^+{=}CH_2I^-$, 19 : 1 CH_3CN/AcOH; b. MeI, EtOH, 25 °C; c. NaCN, DMF, H_2O, 70 °C; **20b**, 69%; **20c**, 58% for the three steps. d. KOH, H_2O, 100 °C, then HCl, 0 °C, **21b**, 97%; **21c**, 75%; e. $SOCl_2$, HOBt, MeOH, 0 to 25 °C; **22a**, 99%; **22b**, 99%; **22c**, 80%. f. NBS, $(PhCO)_2O$, CCl_4, 25 °C, 78%; g. AgOTf, I_2, THF, **25a**, 86%; **25b**, 79%; **25c**, 85%; h. $PdCl_2$(dppf), CH_2Cl_2, HB(pin), Et_3N, dioxane, 100 °C; **26a**, 84%; **26b**, 63%; **26c**, 58%; **26d**, 68%.

With **26a** in hand (commercial or prepared from the bromide **23a**), classical Suzuki coupling conditions ($Pd(PPh_3)_4$, $NaHCO_3$, DME/H_2O) were assayed with **25a**,[69] which afforded, after 8 h under reflux, a mixture of the primary coupling product **27** and the paullone skeleton **28aa** in 14 and 29% yield, respectively (Scheme 3). Longer reaction times led to deterioration of the products. The reaction of the coupling partners in water/dioxane with $Ba(OH)_2{\cdot}8H_2O$, $Pd(OAc)_2$, bis(cyclohexyl)biphenylphosphine at 100 °C[52] led to ester hydrolysis and incomplete conversions. Interestingly, when CuCl was added to the reaction mixture containing $Pd(OAc)_2$ and dppf as pre-catalyst in DMF, presumably to favour B-to-Cu transmetallation,[70] the paullone skeleton **28aa** was obtained in 84% yield. Both the Suzuki cross-coupling and the intramolecular formation of the amide took place in the same pot under the reaction conditions (Scheme 3). We also confirmed that the coupling of the C2-bromoindole **24a** required higher temperatures and afforded lower yields than the corresponding C2-iodoindole **25a** under otherwise identical conditions.

We next turned our attention to C–C bond formation between the indole and the aryl units using in particular a Suzuki–Miyaura cross-coupling reaction.[71] In order to select the best strategy to generate the organoboronic acid (or derivatives) partner, the reciprocal combination of functional groups on both coupling components (at the C2-position of the indole and at the *ortho*-position of the aniline) was investigated. Halogenation of the indole derivatives **22a–c** was straightforward using NBS and radical initiation for X = Br (**24a**)[72] and silver triflate-assisted iodination was successful for X = I (**25a–c**) using anhydrous AgOTf (Scheme 2).[73]

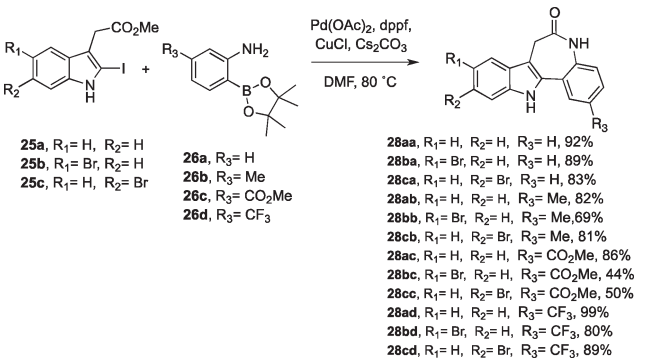
The preparation of the C2-indolyl pinacolboronate by Miyaura-type palladium-catalyzed borylation of *N*-benzyl-2-

bromoindoles[74] failed despite variations in the nature of the pre-catalysts and ligands, heating in the range 80–110 °C or under microwave irradiation or adding Et_3N. In most cases the dehalogenated substrate was obtained (for the alternative preparation of 2-borylindoles, see ref 75). We then turned our attention to the palladium-catalyzed borylation of *ortho*- iodoanilines.[52] Despite our efforts, we could not obtain the high yields reported for the reaction starting from *o*-iodoanilines and their acetate derivatives[52] using the same conditions [($Pd(OAc)_2$, bis(cyclohexyl) biphenylphosphine (1 : 4 ratio), Et_3N and HB(pin) in dioxane at 80 °C; a yield of 18% at most was obtained with *o*-iodoanaline, 25% if purified by reverse phase chromatography using MeOH/H_2O as eluent] or modified reaction conditions [using $Pd(OAc)_2$ and bis(cyclohexyl)biphenylphosphine or $PdCl_2(dppf)\cdot CH_2Cl_2$, pinacolborane and Et_3N in dioxane].[76] On the other hand, the borylation of *o*-iodoaniline **23a** using Buchwald's improved procedure[77] with $PdCl_2(CH_3CN)_2$, bis(cyclohexyl)biphenylphosphine, Et_3N and HB(pin) in dioxane at 110 °C was incomplete and afforded the product **26a** (Scheme 2) together with the dehalogenated aniline in a 53% combined yield. Moreover, boronate **26a** proved to be unstable to purification by column chromatography. Given this shortcoming, we decided to carry out the combined borylation–Suzuki coupling sequentially without isolation of the intermediate. To this end, treatment of 2-iodoaniline **23a** with $PdCl_2(CH_3CN)_2$, bis(cyclohexyl)biphenylphosphine (1 : 4 ratio), Et_3N and HB(pin) in dioxane at 110 °C,[77] followed by the appropriate work-up, afforded a residue that was immediately treated with methyl 2-iodoindoleacetate **25a** followed by the addition of $Pd(OAc)_2$, dppf, CuCl and Cs_2CO_3 in DMF and heating at 80 °C. The desired product was isolated in moderate yield (56%), and small amounts of the unreacted starting indole remained. Application of these conditions to methyl 5-bromo-2-iodoindoleacetate **25b** led to the desired product **28ba** in a disappointing 24% yield. Likewise, sequential borylation/Suzuki cross-coupling starting from substituted anilines **26b–c** led to the corresponding paullones **28ab** and **28ac** in moderate yields (31–33%).

When the borylation reaction was performed under the conditions reported by Hashimoto *et al.*[78] using $PdCl_2(dppf)\cdot CH_2Cl_2$, the yield of the reaction product **26a–d** was considerably improved (Scheme 2). Further optimization of the cross-coupling confirmed the requirement for 1 equivalent of CuCl, since the use of substoichiometric quantities led to longer reaction times and lower yields. Improved yields also resulted when the reaction time was shortened (2 h instead of 12 h), the work-up was carried out with ammonium chloride and the residue was purified by crystallization (Scheme 3). Applying these optimized conditions to the coupling of the fragments **25a–c** and **26a–d** led to the 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-ones **28xx** in good-to-excellent yields (Scheme 4). Of these compounds, **28aa** and **28ba** are known.[79] Starting from the brominated indoles chemoselective coupling at the C2-I position was exclusively obtained.

Biological evaluation

In vitro and *in vivo* tests were performed to evaluate the inhibitory activity of these novel paullones. The latter tests were carried out in the acute myeloid leukemia cell line U937 to assess the anti-proliferative potential and the alteration of cell cycle induced by the synthetic compounds. Compared to the vehicle-treated cells, the paullones produced some significant alterations on the cell cycle (Fig. 3A). In particular, the compounds at 50 μM caused an increase of the pre-G1 phase. Moreover, some of these compounds induced granulocytic



Scheme 4 General synthesis of 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-ones by one-pot Suzuki cross-coupling/amidation.

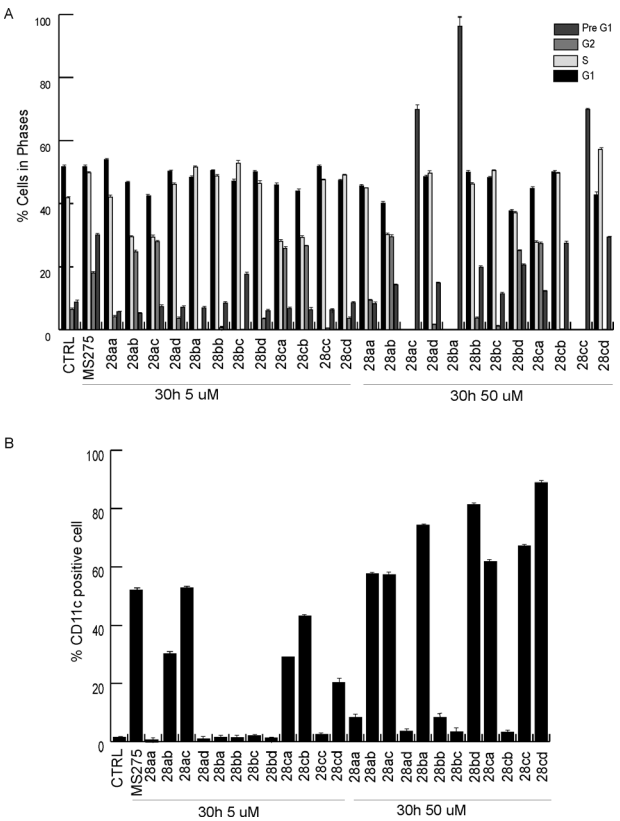


Fig. 3 Effects of paullones on cell cycle and granulocytic differentiation in the U937 acute myeloid leukemia cell line. A: Cytofluorimetric cell cycle analysis of U937 cells after treatment with the synthetic compounds at 5 μM and 50 μM for 30h. B: Cytofluorimetric differentiation analysis. CD11c expression after treatment with paullones at 5 μM and 50 μM for 30 h; MS-275 (5 μM) was used as positive control. The data represent the average value of independent triplicates.

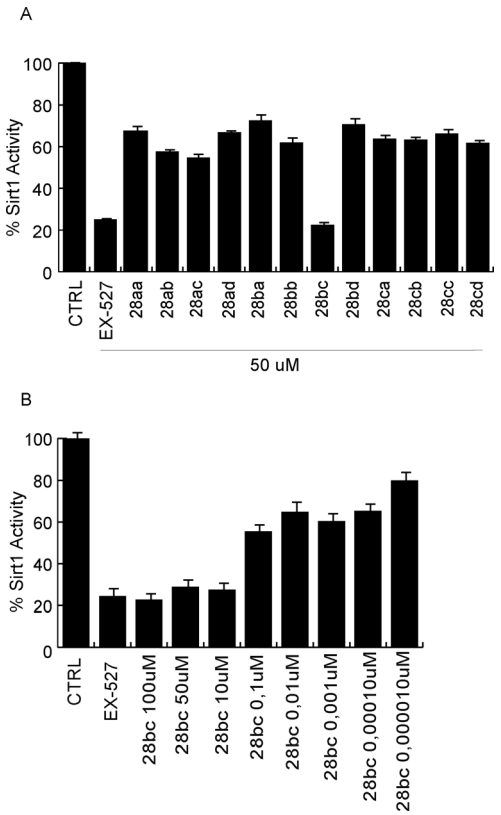


Fig. 4 *In vitro* SIRT1 activity analysis. The SIRT1 inhibitory activity of paullones was tested at 50 μM. The inhibition value was reported as percentage of residual activity in comparison to the control without compound incubation and to the known SIRT1 inhibitor EX-527 **13a**. The data represent the average value of independent triplicates.

differentiation of U937 cells after treatment at 5 μM and 50 μM for 30 h (Fig. 3B), as indicated by the increased expression of the granulocytic differentiation cell-surface marker CD11c using FACS analysis.

In vitro assays with recombinant human sirtuin-1 revealed that paullones are potent inhibitors of SIRT1 (Fig. 4A). On average they reduce SIRT1 activity by up to 45%. For the most potent inhibitor of the series, **28bc**, an IC_{50} of 0.215 μM was determined (Fig. 4B), and therefore this compound surpasses the activity of *N*-benzylkenpaullone **16b** (Fig. 2, IC_{50} = 8 μM).

As the paullones have been reported as CDK inhibitors[38,39,43] we tested the synthesized compounds in an enzymatic assay with Cdc/Cdk1. As seen in Fig. 5, all the compounds were roughly equally active as inhibitors, with a potency similar to kenpaullone (**28ba**). Therefore compound **28bc** is more selective for SIRT1 than for Cdk1, and this finding suggests that additional studies, in combination with Molecular Modeling, are warranted to increase the selectivity profile of the series.

Conclusion

A novel synthesis of paullones substituted at C9/C10 (Br) and C2 (for the synthesis of other members with substituents at C9 and C2, see ref. 42) has been developed and is based on a one-pot Suzuki–Miyaura cross-coupling of an *o*-aminoarylboronic acid and a C2-iodoindoleacetic acid followed by intramolecular

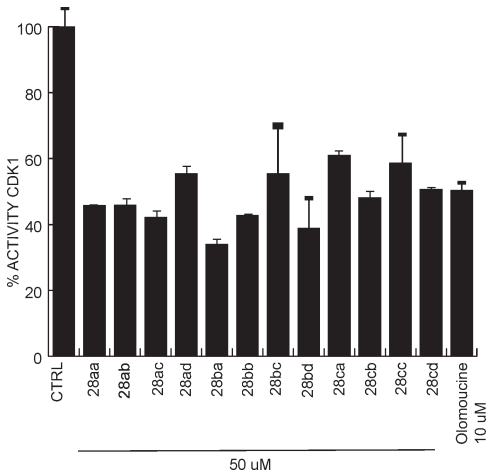


Fig. 5 *In vitro* cdc2 activity assay. The cdc2 inhibitory activity of compounds **28** was tested at 50 μM. The inhibition value was reported as percentage of residual activity in comparison to the control without compound incubation and to the known cdc2 inhibitor olomoucine at 10 μM concentration. The data represent the average value of independent triplicates.

amide formation to generate the 7,12-dihydroindolo[3,2-*d*][1]benzazepine-6(5*H*)-one skeleton.

SAR studies are only available for the CDK inhibition[39,80] and for antileishmanial activity[42] but not for SIRT inhibition or other epigenetic modulatory activities of the paullones. We tested the ability of the series of compounds to alter the cell cycle and induce differentiation of the acute myelogenous cell line U937 and carried out an *in vitro* enzymatic assay with recombinant human sirtuin-1. The analogue of kenpaullone with a methoxycarbonyl group at C2 is more than one order of magnitude more potent than *N*-benzylkenpaullone, thus showing that substituents at the anilide ring can indeed improve the SIRT1 inhibitory profile of this scaffold relative to other activities such as the inhibition of CDC2/Cdk1.

Experimental section

General procedures

Reagents and solvents were purchased as reagent-grade and used without further purification unless otherwise stated. Solvents were dried according to standard methods and distilled before use or dispensed from a Puresolv™ solvent purification system of Innovative Technology, Inc. All reactions were performed in oven-dried or flame-dried glassware under an inert atmosphere of Ar unless otherwise stated. Chromatography refers to flash chromatography (FC) on SiO_2 *60* (230–400 mesh) from Merck, head pressure of *ca.* 0.2 bar. TLC: UV_{254} SiO_2-coated plates from Merck, visualization by UV light (254 nm) or by spraying with a 15% ethanolic phosphomolybdic acid solution. NMR spectra were recorded in a Bruker AMX400 (400.13 MHz and 100.61 MHz for proton and carbon respectively) spectrometer at 298 K with residual solvent peaks as internal reference and the chemical shifts are reported in δ [ppm], coupling constants *J* are given in [Hz] and the multiplicities assigned with DEPT experiments and expressed as follows: s = singlet, d = doublet, t =

triplet, q = quartet, m = multiplet. For fluorinated compounds, a second multiplicity corresponding to the $^{13}C–^{19}F$ coupling is given in parenthesis together with the $J_{C–F}$ coupling constant. COSY, HMBC and HSQC methods were used to establish atom connectivities. Electrospray ionization (ESI) mass spectra were recorded on a Bruker APEX3 instrument. Infrared spectra (IR) were obtained on a JASCO FT/IR-4200 infrared spectrometer. Peaks are quoted in wave numbers (cm^{-1}) and their relative intensities are reported as follows: s = strong, m = medium, w = weak. Melting points were measured in a Stuart Scientific apparatus. Elemental analyses were carried out in a Fisons EA-1108 elemental analyzer. Irradiation with microwaves was performed using a CEM DISCOVER apparatus.

2-(5-Bromo-1*H*-indol-3-yl)acetic acid 21b. To a solution of 2-(5-bromo-1*H*-indol-3-yl)acetonitrile **20b** (0.86 g, 3.68 mmol) in MeOH (4.6 mL) was added a solution of KOH (1.67 g, 29.78 mmol) in water (14.9 mL) and the mixture was heated at 100 °C for 2 h. The reaction was diluted with water, cooled to 0 °C and treated with a 6 M aqueous solution of HCl until pH 1. The solid was filtered, washed twice with water and dried under high vacuum to give compound **21b** as a white solid (1.037 g, 97%). **m.p.**: 144–146 °C (methanol). **^{1}H-NMR** (400.13 MHz, CD_3OD): δ 7.67 (d, J = 1.8 Hz, 1H, ArH), 7.25 (d, J = 8.6 Hz, 1H, ArH), 7.19 (s, 1H, ArH), 7.17 (dd, J = 8.6, 1.8 Hz, 1H, ArH), 3.67 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, CD_3OD): δ 176.1 (s), 136.7 (s), 130.5 (s), 126.3 (d), 125.2 (d), 122.2 (d), 113.9 (d), 113.1 (s), 108.9 (s), 31.8 (t) ppm. **HRMS** (ESI^+): calcd. for $C_{10}H_8{}^{81}BrNNaO_2$ ($[M + Na]^+$), 277.9610; found, 277.9608. Calcd. for $C_{10}H_8{}^{79}BrNNaO_2$ ($[M + Na]^+$), 275.9631; found, 275.9629. **IR** (NaCl): ν 3500–3100 (br, OH), 3418 (s, NH), 3085 (w, C–H), 1703 (s, C═O), 1456 (m), 792 (m) cm^{-1}.

Methyl 2-(5-bromo-2-iodo-1*H*-indol-3-yl)acetate 25b. A solution of I_2 (236.7 mg, 0.93 mmol) in THF (5.5 mL) was added dropwise to a solution of methyl 2-(5-bromo-1*H*-indol-3-yl) acetate **22b** (250 mg, 0.93 mmol) and AgOTf (287.5 mg, 1.12 mmol) in THF (2.3 mL). Then, a second portion of AgOTf (23.9 mg, 0.09 mmol) was added and the mixture was stirred at ambient temperature for 30 min before the addition of an aqueous saturated solution of $Na_2S_2O_3$. The mixture was extracted with EtOAc (2x) and the combined organic layers were washed with brine, dried and the solvent was evaporated under vacuum to afford, after purification by column chromatography (silicagel, from 90 : 10 to 50 : 50 hexane/AcOEt), compound **25b** as a white solid (291 mg, 79%). **m.p.**: 115–118 °C (hexane/ethyl acetate). **^{1}H-NMR** (400.13 MHz, $CDCl_3$): δ 8.28 (br, 1H, NH), 7.64 (s, 1H, ArH), 7.2–7.1 (m, 1H, ArH), 7.1–7.0 (m, 1H, ArH), 3.71 (s, 3H, CH_3), 3.67 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, $CDCl_3$): δ 171.4 (s), 137.3 (s), 128.9 (s), 125.3 (d), 120.6 (d), 114.6 (s), 113.6 (s), 111.8 (d), 81.4 (s), 52.2 (q), 32.7 (t) ppm. **HRMS** (ESI^+): calcd. for $C_{11}H_9{}^{81}BrINNaO_2$ ($[M + Na]^+$), 417.8733; found 417.8730. Calcd. for $C_{11}H_9{}^{79}BrINNaO_2$ ($[M + Na]^+$), 415.8754; found 415.8750. **IR** (NaCl): ν 3326 (br, NH), 3000 (w, C–H), 2949 (w, C–H), 1723 (s, C═O), 1438 (m), 1334 (m), 795 (m) cm^{-1}.

Methyl 2-(6-bromo-2-iodo-1*H*-indol-3-yl)acetate 25c. Following the general procedure described above for the iodination of indoles at C2, the reaction of methyl 2-(6-bromo-1*H*-indol-3-yl) acetate **22c** (250 mg, 0.93 mmol), AgOTf (287.5 mg, 1.12 mmol; 23.9 mg, 0.09 mmol) and I_2 (236.7 mg, 0.93 mmol) in THF (7.8 mL) afforded, after purification by column chromatography (silicagel, from 90 : 10 to 70 : 30 hexane/EtOAc), compound **25c** as a light brown solid (312.7 mg, 85%). **m.p.**: 135–137 °C (hexane/ethyl acetate). **^{1}H-NMR** (400.13 MHz, $CDCl_3$): δ 8.21 (br, 1H, NH), 7.38 (d, J = 1.8 Hz, 1H, H7), 7.37 (d, J = 8.4 Hz, 1H, H4), 7.19 (dd, J = 8.4, 1.6 Hz, 1H, ArH), 3.70 (s, 3H, CH_3), 3.69 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, $CDCl_3$): δ 171.4 (s), 139.3 (s), 126.2 (s), 123.5 (d), 119.3 (d), 116.2 (s), 115.3 (s), 113.3 (d), 80.4 (s), 52.2 (q), 32.8 (t) ppm. **HRMS** (ESI^+): calcd. for $C_{11}H_9{}^{81}BrINNaO_2$ ($[M + Na]^+$), 417.8739; found 417.8732. Calcd. for $C_{11}H_9{}^{79}BrINNaO_2$ ($[M + Na]^+$), 415.8754; found 415.8752. **IR** (NaCl): ν 3323 (br, NH), 3000 (w, C–H), 2949 (w, C–H), 1723 (s, C═O), 1437 (m), 1329 (m), 801 (m) cm^{-1}.

4-Methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) aniline 26b. General procedure for the borylation of anilines. To a solution of 2-iodo-4-methylaniline **23b** (1.0 g, 4.29 mmol) in 1,4-dioxane (11.0 mL) were added Et_3N (2.4 mL, 17.16 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (175.2 mg, 0.21 mmol) and pinacolborane (1.9 ml, 12.87 mmol). The reaction mixture was heated to 100 °C for 5 h. After cooling down to ambient temperature, the reaction mixture was filtered through Celite eluting with EtOAc. After removal of the solvent under vacuum, the residue was purified by column chromatography (silicagel, from 90 : 10 to 60 : 40 hexane/AcOEt), to afford compound **26b** as a white solid (628.4 mg, 63%). **m.p.**: 71–73 °C (hexane/ethyl acetate). **^{1}H-NMR** (400.13 MHz, CD_3OD): δ 7.34 (d, J = 1.6 Hz, 1H, ArH), 6.97 (dd, J = 8.2, 1.9 Hz, 1H, ArH), 6.55 (d, J = 8.2 Hz, 1H, ArH), 4.82 (s, 2H, NH_2), 2.15 (s, 3H, CH_3), 1.29 (s, 12H, 4×CH_3) ppm. **^{13}C-NMR** (100.61 MHz, CD_3OD): δ 152.8 (s), 137.6 (d), 134.5 (d), 126.8 (s), 116.6 (d), 112.5 (s), 84.6 (s, 2x), 25.3 (q, 4x), 20.6 (q) ppm. **HRMS** (ESI^+): calcd. for $C_{13}H_{21}BNO_2$ ($[M + H]^+$), 234.1662; found 234.1666. **IR** (NaCl): ν 3483 (m, NH), 3385 (m, NH), 2980 (m, C–H), 2928 (w, C–H), 1621 (s), 1496 (s), 1357 (s, B–O), 1146 (s) cm^{-1}.

Methyl 4-amino-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 26c. Following the general procedure described above for the borylation of anilines, the reaction of methyl 4-amino-3-iodobenzoate **23c** (277.1 mg, 1 mmol), Et_3N (557 µL, 4 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (40.8 mg, 0.05 mmol) and pinacolborane (435 µL, 3 mmol) in 1,4-dioxane (2.6 mL) afforded, after purification by column chromatography (silicagel, from 90 : 10 to 50 : 50 hexane/EtOAc), compound **26c** as a white solid (160.6 mg, 58%). **m.p.**: 167–169 °C (hexane/ethyl acetate). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 8.05 (d, J = 2.1 Hz, 1H, ArH), 7.71 (dd, J = 8.7, 2.2 Hz, 1H, ArH), 6.63 (d, J = 8.7 Hz, 1H, ArH), 6.24 (br, 1H, NH), 3.74 (s, 3H, CH_3), 1.30 (s, 12H, 4×CH_3) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 166.0 (s), 158.3 (s), 138.9 (d), 133.6 (d), 115.5 (d), 113.6 (s), 108.0 (s), 83.5 (s, 2x), 51.1 (q), 24.5 (q, 4x) ppm. **HRMS** (ESI^+): calcd. for $C_{14}H_{21}BNO_4$ ($[M + H]^+$), 278.1558; found 278.1554. **IR** (NaCl): ν 3481 (m, NH), 3374 (m, NH), 2981 (m, C–H), 1702 (s, C═O), 1608 (s), 1430 (m), 1371 (m, B–O), 1282 (s), 1246 (s), 1146 (s) cm^{-1}.

2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)aniline 26d. Following the general procedure described above for the borylation of anilines, the reaction of 2-iodo-4-(trifluoromethyl)aniline **23d** (1.0 g, 3.48 mmol), Et_3N (1.9 mL, 13.94 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (142.3 mg, 0.17 mmol) and pinacolborane (1.3 mL, 10.45 mmol) in 1,4-dioxane (8.9 mL) afforded, after purification by column chromatography (silicagel, from 90 : 10 to 20 : 80 hexane/EtOAc), compound **26d** as a white solid (676 mg, 68%). **m.p.**: 115–117 °C (hexane/ethyl acetate). **^{1}H-NMR** (400.13 MHz, CD_3OD): δ 7.69 (d, J = 1.7 Hz, 1H, ArH), 7.34 (dd, J = 8.7, 2.0 Hz, 1H, ArH), 6.66 (d, J = 8.6 Hz, 1H, ArH), 4.82 (br s, 2H, NH_2), 1.35 (s, 12H, 4×CH_3) ppm. **^{13}C-NMR** (100.61 MHz, CD_3OD): δ 158.8 (s), 134.9 (d) (q, $^3J_{C-F}$ = 3.8 Hz), 130.3 (d) (q, $^3J_{C-F}$ = 3.5 Hz), 126.7 (s) (q, $^1J_{C-F}$ = 269.2 Hz, CF_3), 118.4 (s) (q, $^2J_{C-F}$ = 32.3 Hz, C-CF_3), 115.3 (d), 110.1 (s), 85.3 (s, 2x), 25.2 (q, 4×CH_3) ppm. **HRMS** (ESI^+): calcd. for $C_{13}H_{18}BF_3NO_2$ ([M + H]$^+$), 288.1380; found 288.1383. **IR** (NaCl): ν 3473 (m, NH), 3378 (w, NH), 2981 (w, C–H), 1627 (m), 1374 (s, B–O), 1320 (s, B–O), 1149 (s), 1101 (s) cm^{-1}.

7,12-Dihydroindolo[3,2-*d*]benzazepin-6(5*H*)-one 28aa. General procedure for the synthesis of kenpaullone analogues. In a Schlenk tube, methyl 2-(2-iodo-1*H*-indol-3-yl)acetate **25a** (60 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26a** (62.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol), dppf (10.6 mg, 0.02 mmol) and anhydrous DMF (1.9 mL) were added. After the mixture was purged, the tube was closed and heated to 80 °C for 2 h. The reaction was cooled down, EtOAc and a saturated aqueous solution of NH_4Cl were added and the mixture was stirred for 30 min. After extracting with EtOAc, the combined organic layers were washed with H_2O, dried (Na_2SO_4) and the solvent was evaporated. The residue was recrystallized in EtOH and washed with hexane to afford compound **28aa** as a brown solid (43.6 mg, 92%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 11.58 (br, 1H, NH), 10.08 (br, 1H, NH), 7.75 (d, J = 6.6 Hz, 1H, ArH), 7.66 (d, J = 7.8 Hz, 1H, ArH), 7.44 (d, J = 8.1 Hz, 1H, ArH), 7.37 (td, J = 7.7, 1.4 Hz, 1H, ArH), 7.3–7.2 (m, 2H, ArH), 7.18 (t, J = 7.5 Hz, 1H, ArH), 7.08 (t, J = 7.5 Hz, 1H, ArH), 3.51 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.5 (s), 137.3 (s), 135.3 (s), 132.4 (s), 127.9 (d), 126.8 (d), 126.5 (s), 123.6 (d), 122.8 (s), 122.2 (d), 122.0 (d), 119.0 (d), 117.8 (d), 111.4 (d), 107.5 (s), 31.5 (t) ppm. **HRMS** (ESI^+): calcd. for $C_{16}H_{13}N_2O$ ([M + H]$^+$), 249.1022; found, 249.1022. **IR** (NaCl): ν 3220 (br, NH), 2922 (w, C–H), 2851 (w, C–H), 1641 (s, C═O), 1400 (w) cm^{-1}. **UV** (MeOH): λ_{max} 228, 314 nm. **Purity**: 90% (RPHPLC-ESI, Sunfire™ C18 5 µm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 16 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).[74]

9-Bromo-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28ba. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(5-bromo-2-iodo-1*H*-indol-3-yl)acetate **25b** (75.0 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26a** (62.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28ba** as a brown solid (55.4 mg, 89%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 11.81 (s, 1H, NH), 10.11 (s, 1H, NH), 7.91 (d, J = 1.5 Hz, 1H, ArH), 7.74 (d, J = 7.3 Hz, 1H, ArH), 7.5–7.4 (m, 2H, ArH), 7.3–7.2 (m, 3H, ArH), 3.52 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.4 (s), 136.0 (s), 135.6 (s), 133.9 (s), 128.4 (d), 128.2 (s), 126.9 (d), 124.4 (d), 123.6 (d), 122.3 (s), 122.2 (d), 120.3 (d), 113.3 (d), 111.6 (s), 107.1 (s), 31.3 (t) ppm. **MS** (EI): *m*/*z* (%) 328 (M^+, ^{81}Br, 100), 326 (M^+, ^{79}Br, 91), 299 (98), 297 (100), 218 (94). **HRMS** (EI): calcd. for $C_{16}H_{11}{}^{81}BrN_2O$ ([M]$^+$), 328.0034; found, 328.0047. Calcd. for $C_{16}H_{11}{}^{79}BrN_2O$ ([M]$^+$), 326.0055; found, 326.0056. **IR** (NaCl): ν 3217 (br, NH), 1642 (m, C═O), 1401 (w), 676 (w) cm^{-1}. **Purity**: 90% (RPHPLC-ESI, Sunfire™ C18 5 µm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 18 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).[74]

10-Bromo-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28ca. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(6-bromo-2-iodo-1*H*-indol-3-yl)acetate **25c** (75.0 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26a** (62.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28ca** as a brown solid (51.7 mg, 83%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.12 (s, 1H, NH), 7.73 (d, J = 7.2 Hz, 1H, ArH), 7.65 (d, J = 8.4 Hz, 1H, ArH), 7.59 (s, 1H, ArH), 7.4–7.3 (m, 1H, ArH), 7.3–7.2 (m, 2H, ArH), 7.20 (d, J = 8.2 Hz, 1H, ArH), 3.51 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.3 (s), 138.1 (s), 135.5 (s), 133.3 (s), 128.3 (d), 126.8 (d), 125.5 (s), 123.6 (d), 122.3 (s), 122.2 (d), 121.9 (d), 119.7 (d), 114.6 (s), 113.8 (d), 107.6 (s), 31.4 (t) ppm. **MS** (EI): *m*/*z* (%) 328 (M^+, ^{81}Br, 100), 326 (M^+, ^{79}Br, 68), 299 (91), 297 (93), 218 (81). **HRMS** (EI): calcd. for $C_{16}H_{11}{}^{81}BrN_2O$ ([M]$^+$), 328.0034; found, 328.0025. Calcd. for $C_{16}H_{11}{}^{79}BrN_2O$ ([M]$^+$), 326.0055; found, 326.0043. **IR** (NaCl): ν 3263 (br, NH), 2923 (w, C–H), 1656 (s, C═O), 1430 (w), 1162 (w) cm^{-1}. **Purity**: 96% (RPHPLC-ESI, Sunfire™ C18 5 µm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 18 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).[74]

2-Methyl-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28ab. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(2-iodo-1*H*-indol-3-yl)acetate **25a** (60 mg, 0.19 mmol), 4-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26b** (66.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28ab** as a brown solid (40.9 mg, 82%). **m.p.**: 229–232 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 11.54 (s, 1H, NH), 9.98 (s, 1H, NH), 7.64 (d, J = 7.7 Hz, 1H, ArH), 7.56 (s, 1H, ArH), 7.42 (d, J = 8.0 Hz, 1H, ArH), 7.2–7.1 (m, 3H, ArH), 7.06 (t, J = 7.4 Hz,

1H, ArH), 3.47 (s, 2H, CH_2), 2.38 (s, 3H, CH_3) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.3 (s), 137.3 (s), 133.1 (s), 132.6 (s), 132.5 (s), 128.6 (d), 126.9 (d), 126.4 (s), 122.6 (s), 122.1 (d), 121.9 (d), 119.0 (d), 117.8 (d), 111.3 (d), 107.3 (s), 31.5 (t), 20.4 (q) ppm. **HRMS** (ESI^+): calcd. for $C_{17}H_{15}N_2O$ ($[M + H]^+$), 263.1179; found, 263.1167. **IR** (NaCl): ν 3235 (br, NH), 2922 (w, C–H), 1646 (s, C═O), 1509 (w), 1408 (w) cm^{-1}. **Purity**: 98% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 16 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

9-Bromo-2-methyl-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28bb. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(5-bromo-2-iodo-1*H*-indol-3-yl)acetate **25b** (75.0 mg, 0.19 mmol), 4-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26b** (66.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28bb** as a brown solid (44.7 mg, 69%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.03 (s, 1H, NH), 7.90 (s, 1H, ArH), 7.56 (s, 1H, ArH), 7.39 (d, J = 8.5 Hz, 1H, ArH), 7.27 (dd, J = 8.6, 1.5 Hz, 1H, ArH), 7.22 (d, J = 7.7 Hz, 1H, ArH), 7.15 (d, J = 8.3 Hz, 1H, ArH), 3.49 (s, 2H, CH_2), 2.38 (s, 3H, CH_3) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.8 (s), 136.4 (s), 134.6 (s), 133.8 (s), 133.2 (s), 129.6 (d), 128.7 (s), 127.5 (d), 124.9 (d), 122.7 (d), 122.6 (s), 120.8 (d), 113.8 (d), 112.1 (s), 107.5 (s), 31.8 (t), 20.9 (q) ppm. **MS** (EI): *m*/*z* (%) 342 (M^+, ^{81}Br, 94), 340 (M^+, ^{79}Br, 97), 313 (98), 311 (100), 233 (22), 231 (36). **HRMS** (EI): calcd. for $C_{17}H_{13}{}^{81}BrN_2O$ ($[M]^+$), 342.0191; found, 342.0205. Calcd. for $C_{17}H_{13}{}^{79}BrN_2O$ ($[M]^+$), 340.0211; found, 340.0222. **IR** (NaCl): ν 3223 (br, NH), 2921 (w, C–H), 1646 (s, C═O), 1502 (w), 1302 (w), 973 (w) cm^{-1}. **Purity**: 95% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 19 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

10-Bromo-2-methyl-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28cb. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(6-bromo-2-iodo-1*H*-indol-3-yl)acetate **25c** (75.0 mg, 0.19 mmol), 4-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline **26b** (66.6 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28cb** as a brown solid (52.5 mg, 81%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.05 (s, 1H, NH), 7.64 (d, J = 8.3 Hz, 1H, ArH), 7.58 (s, 1H, ArH), 7.55 (s, 1H, ArH), 7.2–7.1 (m, 3H, ArH), 3.48 (s, 2H, CH_2), 2.38 (s, 3H, CH_3) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.1 (s), 138.0 (s), 133.4 (s), 133.2 (s), 132.6 (s), 128.9 (d), 126.8 (d), 125.4 (s), 122.1 (d), 122.0 (s), 121.8 (d), 119.6 (d), 114.4 (s), 113.7 (d), 107.4 (s), 31.3 (t), 20.2 (q) ppm. **MS** (EI): *m*/*z* (%) 342 (M^+, ^{81}Br, 98), 340 (M^+, ^{79}Br, 99), 313 (96), 311 (100), 233 (18), 231 (32). **HRMS** (EI): calculated for $C_{17}H_{13}{}^{81}BrN_2O$ ($[M]^+$), 342.0191; found, 342.0194. Calculated for $C_{17}H_{13}{}^{79}BrN_2O$ ($[M]^+$), 340.0211; found, 340.0224. **IR** (NaCl): ν 3207 (br, NH), 2923 (w, C–H), 1646 (s, C═O), 1409 (w), 1221 (w) cm^{-1}. **Purity**: 96% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 19 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

Methyl 6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*]benzazepin-2-carboxylate 28ac. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(2-iodo-1*H*-indol-3-yl)acetate **25a** (60 mg, 0.19 mmol), methyl 4-amino-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate **26c** (79.1 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28ac** as a brown solid (50.0 mg, 86%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.45 (s, 1H, NH), 8.39 (s, 1H, ArH), 7.92 (d, J = 7.8 Hz, 1H, ArH), 7.68 (d, J = 7.5 Hz, 1H, ArH), 7.46 (d, J = 7.5 Hz, 1H, ArH), 7.36 (d, J = 8.1 Hz, 1H, ArH), 7.2–7.1 (m, 1H, ArH), 7.1–7.0 (m, 1H, ArH), 3.90 (s, 3H, CH_3), 3.58 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.3 (s), 165.6 (s), 139.1 (s), 137.6 (s), 131.4 (s), 128.3 (d), 128.2 (d), 126.3 (s), 124.4 (s), 122.5 (s), 122.4 (d), 122.1 (d), 119.1 (d), 118.0 (d), 111.5 (d), 107.9 (s), 52.0 (q), 31.6 (t) ppm. **MS** (EI): *m*/*z* (%) 306 (M^+, 75), 305 (45), 277 (100), 218 (30). **HRMS** (EI): calcd. for $C_{18}H_{14}N_2O_3$ ($[M]^+$), 306.1004; found, 306.1006. **IR** (NaCl): ν 3313 (br, NH), 2923 (w, C–H), 1707 (s, C═O), 1671 (s, C═O), 1435 (m), 1257 (m) cm^{-1}. **Purity**: 83% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 17 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

Methyl 9-bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*]benzazepin-2-carboxylate 28bc. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(5-bromo-2-iodo-1*H*-indol-3-yl)acetate **25b** (75.0 mg, 0.19 mmol), methyl 4-amino-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate **26c** (79.1 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28bc** as a brown solid (32.6 mg, 44%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 12.00 (s, 1H, NH), 10.46 (s, 1H, NH), 8.38 (s, 1H, ArH), 7.94 (ap. s, 2H, ArH), 7.41 (d, J = 8.6 Hz, 1H, ArH), 7.36 (d, J = 8.5 Hz, 1H, ArH), 7.30 (d, J = 8.8 Hz, 1H, ArH), 3.90 (s, 3H, CH_3), 3.59 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.2 (s), 165.5 (s), 139.4 (s), 136.2 (s), 132.9 (s), 128.8 (d), 128.3 (d), 128.1 (s), 124.8 (d), 124.5 (s), 122.3 (d), 122.0 (s), 120.5 (d), 113.5 (d), 111.8 (s), 107.5 (s), 52.1 (q), 31.4 (t) ppm. **MS** (EI): *m*/*z* (%) 386 (M^+, ^{81}Br, 97), 384 (M^+, ^{79}Br, 97), 358 (20), 357 (96), 356 (22), 355 (100). **HRMS** (EI): calcd. for $C_{18}H_{13}{}^{81}BrN_2O_3$ ($[M]^+$), 386.0089; found, 386.0075. Calcd. for $C_{18}H_{13}{}^{79}BrN_2O_3$ ($[M]^+$), 384.0110; found, 384.0122. **IR** (NaCl): ν 3312 (br, NH), 2922 (s, C–H), 2851 (m, C–H), 1706 (s, C═O), 1670 (s, C═O), 1434 (m), 1256 (m) cm^{-1}. **Purity**:

96% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 18 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

Methyl 10-bromo-6-oxo-5,6,7,12-tetrahydroindolo[3,2-*d*]benzazepin-2-carboxylate 28cc. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(6-bromo-2-iodo-1*H*-indol-3-yl)acetate **25c** (75.0 mg, 0.19 mmol), methyl 4-amino-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate **26c** (79.1 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28cc** as a brown solid (36.4 mg, 50%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 8.37 (s, 1H, ArH), 7.95 (d, J = 8.1 Hz, 1H, ArH), 7.68 (d, J = 8.2 Hz, 1H, ArH), 7.61 (s, 1H, ArH), 7.37 (d, J = 8.5 Hz, 1H, ArH), 7.22 (d, J = 8.6 Hz, 1H, ArH), 3.91 (s, 3H, CH_3), 3.59 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.2 (s), 165.5 (s), 139.2 (s), 138.3 (s), 132.3 (s), 128.7 (d), 128.2 (d), 125.4 (s), 124.5 (s), 122.2 (d), 122.1 (d), 122.0 (s), 119.9 (d), 115.0 (s), 113.9 (d), 108.0 (s), 52.1 (q), 31.5 (t) ppm. **MS** (EI): *m*/*z* (%) 386 (M^+, ^{81}Br, 100), 384 (M^+, ^{79}Br, 100), 358 (20), 357 (88), 356 (22), 355 (90). **HRMS** (EI): calcd. for $C_{18}H_{13}{}^{81}BrN_2O_3$ ($[M]^+$), 386.0089; found, 386.0084. Calcd. for $C_{18}H_{13}{}^{79}BrN_2O_3$ ($[M]^+$), 384.0110; found, 384.0112. **IR** (NaCl): ν 3301 (br, NH), 2924 (w, C–H), 2852 (w, C–H), 1703 (m, C═O), 1670 (s, C═O), 1434 (w), 1287 (w), 1257 (w) cm^{-1}. **Purity**: 80% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 18 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

2-(Trifluoromethyl)-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28ad. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(2-iodo-1*H*-indol-3-yl)acetate **25a** (60 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)aniline **26d** (82.0 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28ad** as a brown solid (59.5 mg, 99%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 8.10 (s, 1H, ArH), 7.8–7.7 (m, 2H, ArH), 7.5–7.4 (m, 2H, ArH), 7.3–7.2 (m, 1H, ArH), 7.11 (t, J = 7.5 Hz, 1H, ArH), 3.60 (s, 2H CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.5 (s), 138.3 (s), 137.6 (s), 131.0 (s), 126.3 (s), 124.3 (d) (d, $^3J_{C-F}$ = 3.3 Hz), 124.2 (s) (q, $^1J_{C-F}$ = 274.3 Hz, CF_3), 123.9 (d) (d, $^3J_{C-F}$ = 3.6 Hz), 123.7 (s), 122.9 (s), 122.8 (d), 122.7 (d), 119.3 (d), 118.2 (d), 111.5 (d), 108.5 (s), 31.6 (t) ppm. **MS** (EI): *m*/*z* (%) 316 (M^+, 58), 315 (35), 288 (18), 287 (100), 286 (7). **HRMS** (EI): calcd. for $C_{17}H_{11}F_3N_2O$ ($[M]^+$), 316.0823; found, 316.0826. **IR** (NaCl): ν 3253 (br, NH), 2923 (s, C–H), 2854 (m, C–H), 1654 (s, C═O), 1322 (m), 1127 (m) cm^{-1}.

9-Bromo-2-(trifluoromethyl)-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28bd. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(5-bromo-2-iodo-1*H*-indol-3-yl)acetate **25b** (75.0 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)aniline **26d** (82.0 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28bd** as a brown solid (59.8 mg, 80%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.50 (s, 1H, NH), 8.10 (s, 1H, ArH), 7.98 (d, J = 1.4 Hz, 1H, ArH), 7.75 (dd, J = 8.4, 1.4 Hz, 1H, ArH), 7.44 (dd, J = 8.5, 2.4 Hz, 2H, ArH), 7.32 (dd, J = 8.6, 1.6 Hz, 1H, ArH), 3.62 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.3 (s), 138.6 (s), 136.2 (s), 132.5 (s), 128.0 (s), 125.0 (d), 124.7 (d) (d, $^3J_{C-F}$ = 3.3 Hz), 124.1 (d) (d, $^3J_{C-F}$ = 3.8 Hz), 124.1 (s) (q, $^1J_{C-F}$ = 271.8 Hz, CF_3), 123.7 (s), 122.8 (d), 122.3 (s), 120.6 (d), 113.5 (d), 111.8 (s), 108.0 (s), 31.3 (t) ppm. **MS** (EI): *m*/*z* (%) 396 (M^+, ^{81}Br, 89), 394 (M^+, ^{79}Br, 100), 393 (43), 368 (17), 367 (98), 366 (21), 365 (96). **HRMS** (EI): calcd. for $C_{17}H_{10}{}^{81}BrF_3N_2O$ ($[M]^+$), 395.9908; found, 395.9894. Calcd. for $C_{17}H_{10}{}^{79}BrF_3N_2O$ ($[M]^+$), 393.9929; found, 393.9919. **IR** (NaCl): ν 3293 (br, NH), 2925 (m, C–H), 1659 (s, C═O), 1317 (m), 1124 (m) cm^{-1}. **Purity**: 84% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 20 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

10-Bromo-2-(trifluoromethyl)-7,12-dihydroindolo[3,2-*d*]benzazepin-6-(5*H*)-one 28cd. Following the general procedure described above for the synthesis of kenpaullone analogues, the reaction of methyl 2-(6-bromo-2-iodo-1*H*-indol-3-yl)acetate **25c** (75.0 mg, 0.19 mmol), 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)aniline **26d** (82.0 mg, 0.29 mmol), Cs_2CO_3 (248.2 mg, 0.76 mmol), CuCl (18.8 mg, 0.19 mmol), $Pd(OAc)_2$ (2.1 mg, 0.01 mmol) and dppf (10.6 mg, 0.02 mmol) in DMF (1.9 mL) afforded, after purification by recrystallization, compound **28cd** as a brown solid (67.0 mg, 89%). **m.p.**: >300 °C (ethanol/hexane). **^{1}H-NMR** (400.13 MHz, DMSO-d_6): δ 10.50 (s, 1H, NH), 8.09 (s, 1H, ArH), 7.72 (d, J = 8.5 Hz, 1H, ArH), 7.69 (d, J = 8.3 Hz, 1H, ArH), 7.63 (s, 1H, ArH), 7.45 (d, J = 8.3 Hz, 1H, ArH), 7.23 (d, J = 8.5 Hz, 1H, ArH), 3.61 (s, 2H, CH_2) ppm. **^{13}C-NMR** (100.61 MHz, DMSO-d_6): δ 171.3 (s), 138.5 (s), 138.3 (s), 131.9 (s), 125.4 (s), 124.7 (d) (d, $^3J_{C-F}$ = 3.1 Hz), 124.0 (d) (d, $^3J_{C-F}$ = 4.0 Hz), 124.2 (s) (q, $^1J_{C-F}$ = 266.5 Hz, CF_3), 123.7 (s), 122.8 (d), 122.3 (s), 122.2 (d), 120.0 (d), 115.3 (s), 114.0 (d), 108.6 (s), 31.4 (t) ppm. **MS** (EI): *m*/*z* (%) 396 (M^+, ^{81}Br, 91), 394 (M^+, ^{79}Br, 100), 393 (42), 368 (17), 367 (95), 366 (18), 365 (95). **HRMS** (EI): calcd. for $C_{17}H_{10}{}^{81}BrF_3N_2O$ ($[M]^+$), 395.9908; found, 395.9893. Calcd. for $C_{17}H_{10}{}^{79}BrF_3N_2O$ ($[M]^+$), 393.9929; found, 393.9915. **IR** (NaCl): ν 3276 (br, NH), 2925 (w, C–H), 1658 (s, C═O), 1322 (s), 1123 (s) cm^{-1}. **Purity**: 92% (RPHPLC-ESI, Sunfire™ C18 5 μm, 46 × 250 mm, gradient A/B, 0 : 100 to 100 : 0, 30 min, 1 mL min^{-1}, t_R = 20 min, A: CH_3CN/HCOOH 99 : 1, B: H_2O/HCOOH 99 : 1).

Compounds

MS-275 (Bayer-Schering AG), EX-527 (Alexis-Biochemicals) and paullones were dissolved in DMSO (Sigma-Aldrich) and used at 5 μM or 50 μM.

Cell lines

U937 (human leukemic monocyte lymphoma cell line-ATCC) were grown in RPMI 1640 medium (Euroclone) supplemented with 10% heat-inactivated FBS (Euroclone), 1% glutamin (Lonza), 1% penicillin/streptomycin (Euroclone) and 0.1% gentamycin (Lonza), at 37 °C in air and 5% CO_2.

Cell cycle analysis

2.5×10^5 U937 cells were collected by centrifugation after 30 h stimulation with reference compounds or paullones at 5 μM or 50 μM. The cells were resuspended in 500 μL of hypotonic buffer (0.1% NP-40, 0.1% sodium citrate, 50 μg ml^{-1} PI, RNAse A) and incubated in the dark for 30 min. The analysis was performed by FACS-Calibur (Becton Dickinson) using the Cell Quest Pro software (Becton Dickinson) and ModFit LT version 3 software (Verity). The experiment was performed in triplicate.

Granulocytic differentiation analysis

2.5×10^5 U937 cells were collected by centrifugation after 30 h stimulation with reference compound MS-275 at 5 μM concentration or paullones at 5 μM and 50 μM. The cells were washed with PBS and incubated in the dark at 4 °C for 30 min with 10 μL of PE-conjugated anti-CD11c surface antigen antibody or with 10 μL of PE-conjugated IgG, in order to define the background signal. At the end of the incubation the samples were washed again and resuspended in 500 μL of PBS containing 0.25 $\mu g\ mL^{-1}$ PI. The analysis was performed by FACS-Calibur (Becton Dickinson) using the Cell Quest Pro software (Becton Dickinson). The experiment was performed in triplicate and PI positive cells were excluded from the analysis.

SIRT1 fluorimetric assay

SIRT1 assays were performed in the presence of 3 mM Sirt assay buffer (#KI-286: 50 mM Tris-HCl pH8, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, BSA 1 mg mL^{-1}). For these experiments, SIRT1 enzyme at concentration 0.2U μL^{-1} (#BML-SE239) was added to the reaction mix 2X solution by diluting the Fluor the Lys (substrate KI-177, 50 mM stock solution) and NAD^+ (#KI-282, 10 mM stock solution) to 500 μM and 1 mM, respectively, in SIRT1 assay buffer. After a pre-incubation of 15 min at 37 °C a 50 μM solution of the specific inhibitor was added. The reaction was carried out for 90 min at 37 °C with gentle shaking. Then, 50 μL/well of Developer solution were added to reaction mix (39 μL tripsin buffer - 50 mM Tris-HCl pH8, 100 mM NaCl; 3 μL nicotinamide - diluted at 50 mM in assay buffer from 120 mM stock solution dissolved in DMSO; 8 μL Trypsin-6 mg mL^{-1}) and incubated for 30 min at 37 °C with gentle shaking. The fluorescence was quantified with a TECAN INFINITY 200 station (TECAN) at 360 nm excitation and 460 nm emission.

In the IC_{50} analysis, compound **28bc** was used at different scalar concentration from 100 μM to 0.00001 μM (Fig. 4B).

Cdc2 kinase activity assay

Cdc2 kinase activity assay was performed using the MESACUP cdc2 kinase Assay Kit (Medical and Biological Laboratories Co, Ltd.). 20×10^6 U937 cells were collected by centrifugation after 16 h stimulation with colcemid at a concentration of 0.02 μg mL^{-1}. U937 were lysed by adding lysis Buffer (50 mM Tris HCl pH 7.5, 0.5 M NaCl, 5 mM EDTA, 2 mM EGTA, 0.01% TritonX, 50 mM β-mercaptoethanol, 25 mM β-glycerophosphate, 1 mM sodium orthovanadate, 1 mM PMSF, 0.05 mg mL^{-1} leupeptin) and by sonication for 25 s at 400W cm^{-2}. Cell extract was separated by centrifugation at $10^5 \times g$ for 1 h at 4 °C. U937 samples were preincubated with the paullones or reference compound olomoucine (Promega), at 50 μM and 10 μM concentration respectively, for 2 h at 37 °C in a specific phosphorylation reaction mixture (1X cdc2 Reaction Buffer, 1mM ATP). Then the samples were incubated with Cdc2 kinase substrate, biotinylated MV peptide, at 30 °C for 30 min. Following supplier's instruction, the final reaction mixtures were transferred to microwell strip coated with Monoclonal Antibody (4A4) for ELISA cdc2 kinase activity detection. The activity of cdc2 was measured by horseradish peroxidase substrate emission at 492 nm with a microplate reader (TECAN Infinite M200).

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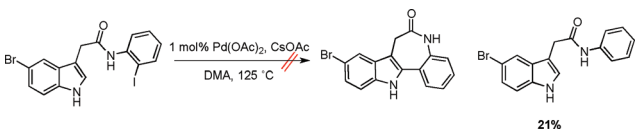

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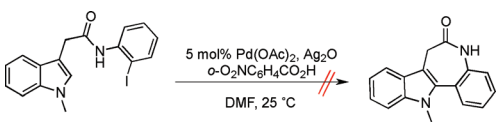

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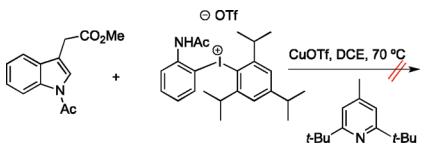

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