2-(3-Aryl-3-oxopropen-1-yl)-9-*tert*-butyl-paullones: A New Antileishmanial Chemotype

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A screening program directed to find new agents against *Leishmania donovani*, the parasite causing visceral leishmaniasis, revealed that paullones attenuate the proliferation of axenic amastigotes. Because these structures were not active in a test system involving infected macrophages, a structure optimization campaign was carried out. Concomitant introduction of an unsaturated side chain into the 2-position and a *tert*-butyl substituent into the 9-position of the parent scaffold led to compounds inhibiting also parasites dwelling in macrophages. By inclusion of the so elaborated scaffold into a chalcone substructure, the toxicity against uninfected host cells was significantly reduced. For the synthesis of this new compound class, a novel modification of the Heck-type palladium-catalyzed C,C-cross coupling strategy was used, employing a ketone Mannich base as precursor for the alkene reactant. The so-prepared compounds exhibited improved antileishmanial activity both on axenic amastigotes ($GI_{50} < 1\ \mu M$) as well as on parasites in infected macrophages.

Introduction

Visceral leishmaniasis (VL[a]) is a disease caused by infection with human protozoan parasites belonging to the *Leishmania donovani* complex. These parasites exist in two developmental stages: the extracellular promastigote transmitted by the bite of the sandfly vector and the intracellular amastigote that is an obligate parasite of macrophages. VL occurs in tropical, subtropical, and temperate regions; however, approximately 90% of the cases occur in Bangladesh, Brazil, India, Nepal, and the Sudan. Symptoms of disease include hepatosplenomegaly, fever, anemia, immunosupression, hypergammaglobulinemia, and weight loss, and without early diagnosis and proper treatment, the disease is fatal. Unfortunately, treatment options for leishmaniasis are very limited. The main drugs in use today were introduced over 50 years ago, and all drug regimes have major drawbacks. First-line treatment based on pentavalent antimony (meglumine antimonate and sodium stibogluconate) show severe, unwanted side effects. Resistance to these compounds has emerged to such an extent in India that they can no longer be used in many regions. Similarly, use of second-line drugs, pentamidine and amphotericin B, is limited by toxicity. Liposomal amphotericin B is a highly effective option; however, these drug formulations are very expensive, limiting their use in most endemic regions. Miltefosine, a new drug recently registered for use in India is the first drug available for oral treatment of VL. However, due to reproductive toxicity, females of childbearing age cannot be treated without efficient contraception. Although current clinical trials with injectable paromomycin are showing encouraging results, an expanded catalogue of new drugs for these parasites is required to prevent the development of resistance. The WHO has designated leishmaniasis a "neglected and emerging disease", and the need for novel drugs against the parasites that cause them has been recognized. Considering the situation of the population in the developing countries threatened by the disease new drugs should be selective, nontoxic, inexpensive and orally available. Therefore, the search for innovative drugs based on new molecular scaffolds directed against novel biological targets should be highly prioritized.[1,2]

Extracellular promastigotes have been used frequently to screen compounds for activity against *Leishmania*; however, the two parasite stages show significant metabolic differences. Compounds that kill one stage, as in the case of the pentavalent antimony derivatives that are only active against the amastigote stage, may not be effective with the other stage and vice versa. In this context, we have developed two new assays suitable for medium to high throughput screening of compounds against amastigotes. Initial screening was carried out using a fluorescent viability microplate assay and *L. donovani* axenic amastigotes cultured under conditions, at 37 °C and acidic pH, which mimic the environment of tissue amastigotes. The second assay utilizes a human macrophage cell line (THP-1) infected with *L. donovani* stably transfected with the firefly luciferase gene. The effect of drugs on intracellular parasites survival can be measured rapidly and simply by adding an appropriate enzyme–substrate and measuring luminescence in a microplate reader. This assay can replace labor-intensive assays where infected macrophages are stained and the number of intracellular parasites and percentage of infected macrophages counted by light microscope. Finally, the toxicity of the compounds was determined on the human macrophage cell line using the Alamar Blue viability assay.

To identify new antileishmanial lead structures, we first screened a small in-house compound collection on *L.*

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[a]Abbreviations: λem, emission wavelength; λex, excitation wavelength; AxA, axenic amastigotes; calcd, calculated; cdc2, synonym of cyclin-dependent kinase 1; CDK, cyclin-dependent kinase; d, doublet; dec, decomposition; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; ED_{50}, effective dose 50%; EI, electron impact; EtOH, ethanol; GI_{50}, growth inhibition 50%; HPLC, high performance liquid chromatography; HRMS, high resolution mass spectrometry; *L.*, *Leishmania*; LmexCRK, *Leishmania mexicana* cdc2-related kinase; MO, Missouri; mp, melting point; ND, not determined; NMR, nuclear magnetic resonance; RA, retinoic acid; RPMI, Roswell Park Memorial Institute; s, singlet; SAR, structure−activity relationship; t, triplet; THP-1, human acute monocytic leukemia cell line; VL, visceral leishmaniasis; WHO, World Health Organization.

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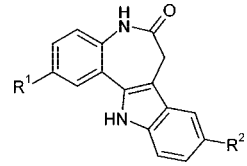
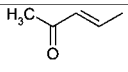
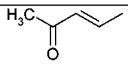
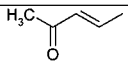
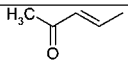
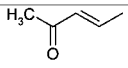
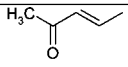
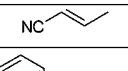
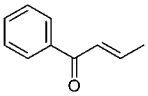
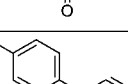
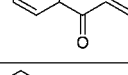
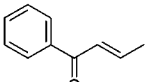
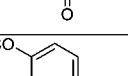
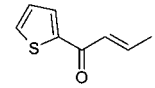
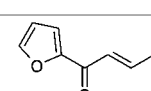
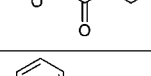
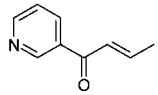
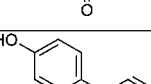
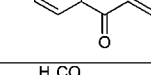
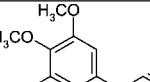
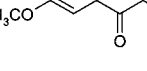
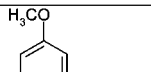
Table 1. Antileishmanial Activity and In Vitro Toxicity of Paullones **1**, **2**, **10**, **12**, and **15**[a]



	R^1	R^2	GI_{50} AxA* [μM]	% Inhibition AxA growth @ 15 μM	% Inhibition of parasite growth in infected Macrophages @ 5 μM	% Killing of Macrophages @ 5 μM
1	H-	$-CH_3$	15.7±0.1	25.5±2.3	0	0
2		$-CF_3$	3.1±0.1	88.8±4.2	0	57.5±2.5
10a		-H	3.4±0.2	98.7±2.0	11.4±11.4	0
10b		$-CH_3$	12.0±6.8	65.2±6.7	0	0
10c		-F	ND	45.4±5.2	0	0
10d		-CN	ND	9.8±2.4	0	48.0±3.0
10e		$-C(CH_3)_3$	2.8±0.2	99.0±0.2	56.2±22.4	62.0±3.0
10f		$-C(CH_3)_3$	3.6±0.2	63.8±8.0	47.3±7.8	0
12a		-H	5.0±0.0	99.6±0.2	0.0±0.0	0.0±0.0
12b		$-CH_3$	ND	17.4±10.3	0.0±0.0	0.0±0.0
12c		$-C(CH_3)_3$	1.1±0.1	99.0±0.4	61.8±25.7	0.0±0.0
12d		$-C(CH_3)_3$	1.2±0.0	97.5±1.1	71.7±19.7	0.0±0.0
12e		$-C(CH_3)_3$	1.2±0.3	98.3±0.4	76.8±13.5	0.0±0.0

	R^1	R^2	GI_{50} AxA* [μM]	% Inhibition AxA growth @ 15 μM	% Inhibition of parasite growth in infected Macrophages @ 5 μM	% Killing of Macrophages @ 5 μM
12f		$-C(CH_3)_3$	1.0±0.0	98.8±0.3	79.3±18.2	60.5±6.5
12g		$-C(CH_3)_3$	0.76±0.1	97.6±0.2	76.2±5.0	3.3±3.3
12h		$-C(CH_3)_3$	0.78±0.07	98.1±0.4	76.2±21.0	0.0±0.0
12i		$-C(CH_3)_3$	3.0	97.2±0.7	54.8±10.7	80.3±16.7
12j		$-C(CH_3)_3$	1.1	96.8±0.1	99.2±0.7	92.7±3.8
12k		$-C(CH_3)_3$	1.1	96.8±0.2	93.8±5.4	95.7±1.3
12l		$-C(CH_3)_3$	1.0±0.2	95.5±0.2	86.0±2.8	49.7±6.3
12m		$-C(CH_3)_3$	0.84±0.16	93.6±2.2	85.6±8.1	0.0±0.0
15		$-C(CH_3)_3$	0.86±0.54	79.6±3.9	72.0±17.4	9.0

[a] ND = not determined. Average ± standard error for values determined by duplicate or triplicate assays. Values reported without standard error were determined in singlet assays. * AxA = axenic amastigotes.

donovani axenic amastigotes. The structures were initially tested at a single concentration (50 μM). If >80% inhibition of parasite growth was observed, the compounds were examined at lower concentrations (30 and/or 15 μM). Interestingly, two compounds (**1** and **2**; Figure 1), belonging to the paullone structure class, strongly inhibited parasite growth at 50 μM (91.2 and 100%). Determination of the GI_{50} value showed that **2** was five times more potent compared to **1** (Table 1). On the other hand, paullone **2** showed a considerable cell-killing activity in a preliminary cellular toxicity assay for host cells, namely, THP-1 macrophages in vitro. Neither **1** or **2** were able to inhibit the growth of parasites in *Leishmania*-infected macrophages.

The paullones (7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-ones) are a class of protein kinase inhibitors acting predominantly on cyclin-dependent kinase 1 (CDK1), cyclin-dependent kinase 5 (CDK5), and glycogen synthase kinase-3.[3–6] Members of the paullone family have been used as biochemical tools in such diverse fields as Alzheimer's disease,[7,8] juvenile diabetes,[9] and development biology.[10] Distinct paullones like alsterpaullone **3** have been investigated as potential anticancer agents due to their growth inhibitory activity for cancer cell lines.[11]

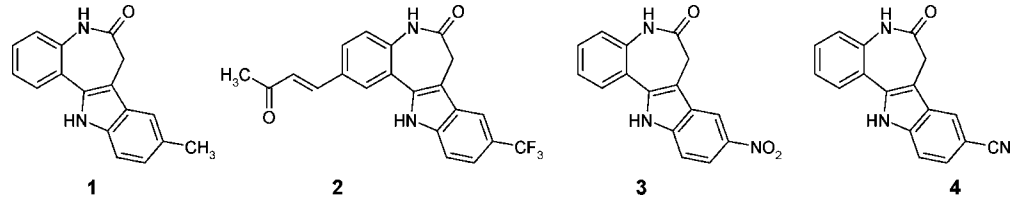
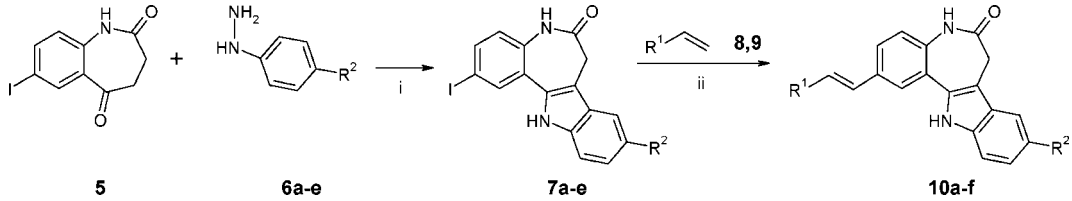


Figure 1. Structures of paullones **1**–**4**.

Scheme 1. Synthesis of Paullones **10a**−**f** with Unsaturated Side Chains in 2-Position[a]



[a] For designation of residues R^1 and R^2, refer to Table 1. Reagents and conditions: (i) 1. AcOH, 70 °C, 1 h; 2. AcOH, H_2SO_4, 70 °C, 1 h; (ii) $Pd(AcO)_2$, $P(Ph)_3$, DMF, triethylamine, N_2, 150 °C, 15 min–3 h.

The structure–activity relationships (SARs) in the paullone class of compounds have been extensively studied. It has been shown that an electron-withdrawing substituent in the 9-position is favorable for the CDK inhibitory activity.[12,13] According to this SAR, paullones **1** and **2** are 1 or 2 orders of magnitude inferior to **3** regarding inhibition of CDK1, respectively.[8] When alsterpaullone **3** was tested on axenic amastigotes it showed <80% inhibition at 50 μM and was excluded from further testing.

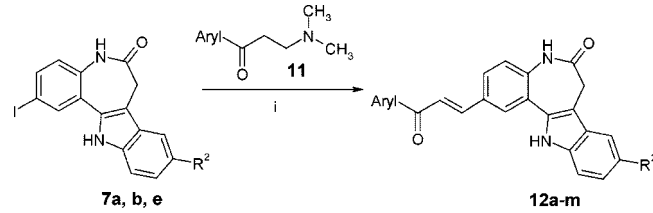
Scattered hints in the literature point to the fact that paullones have been considered as antileishmanial agents before. From the genome of the parasite *Leishmania major*, 11 cdc2 (= CDK1)-related kinases have been predicted, and the function of two putative cyclin-dependent kinases from *L. mexicana* (LmexCRK1 and LmexCRK3) have been investigated in detail. A screening program directed to find inhibitors of the LmexCRK3 revealed 42 active compounds, among them 9-cyanopaullone (**4**). Subsequent tests with **4** showed that the compound was able to inhibit the *L. donovani* infection of peritoneal mouse macrophages with an ED_{50} of 19.6 μM. The compound was not further pursued in this study because it exhibited toxicity for the host cells at 10 μM.[14]

Paullones bind to mitochondrial malate dehydrogenase when incubated with extracts of *Leishmania mexicana*, the parasite causing cutaneous leishmaniasis in South America. Indeed, alsterpaullone **3** inhibited the growth of in vitro cultivated *L. mexicana* promastigotes at 3 μM.[15] However, in our hands **3** proved to be inferior to the paullones **1** and **2** in the more relevant assay with axenic amastigotes, though differences in the efficacy of compounds between leishmanial species have been observed.

Based on the observations with **1** and **2** and the information from the literature a program for structure optimization was initialized. This program was directed to the development of paullone derivatives with improved potency for the protection of macrophages against *L. donovani* infection in vitro as well as minimized toxicity toward THP-1 macrophages. The structures designed and synthesized included the main feature of **2**, namely, the unsaturated side chain in position 2 of the paullone parent scaffold. Because in contrast to **2** and **4** the methyl derivative **1** lacked a toxic effect on macrophages we also included derivatives with electron-donating substituents in the 9-position in the series of new analogues.

Chemistry. An acid-catalyzed Fischer indole cyclization reaction with appropriately substituted phenyl hydrazines **6a**−**e** led from the cyclic ketone **5**[16] to the novel 2-iodo-substituted paullones **7a**−**e**. A Heck reaction with either methyl vinyl ketone **8** or acrylonitrile **9** catalyzed by a palladium acetate/triphenylphosphine system in DMF furnished the compounds **10a**−**f** as analogues of **2** (Scheme 1).

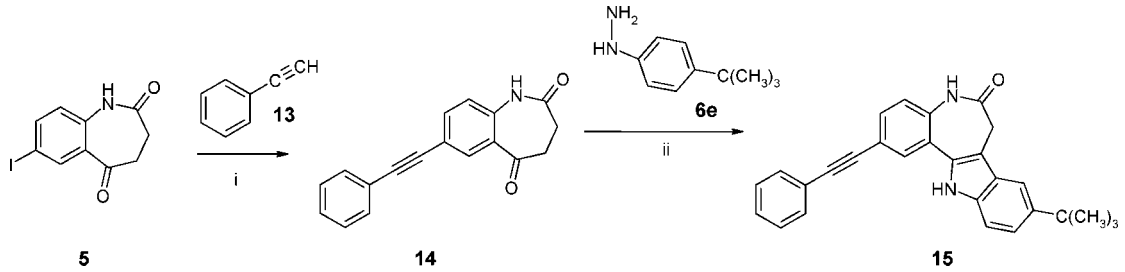
Scheme 2. Synthesis of 2-(3-Aryl-3-oxopropen-1-yl)paullones **12a**−**m**[a]



[a] For designation of residues aryl and R^2, refer to Table 1. Reagents and conditions: (i) either $Pd(AcO)_2$, DMF, triethylamine, $P(Ph)_3$, N_2, 150 °C, 30 min; or $Pd(AcO)_2$, DMF, triethylamine, carousel reactor station, 140 °C, 30 min.

For the preparation of the 2-(3-aryl-3-oxopropen-1-yl)paullones **12a**−**m** a conventional Heck reaction would have required the use of aryl vinyl ketones as reaction partners for **7**. However, aryl vinyl ketones are inconvenient to handle because they tend to polymerize at elevated temperatures.[17–19] Consistent with this finding, examples for the synthesis of 1,3-diarylpropenones by Heck reaction procedures employing aryl vinyl ketones are rare.[20–22] We therefore used the ketone Mannich bases **11** as precursors that under the typical conditions of the Heck reaction readily lose dimethylamine and release aryl vinyl ketones. Hence, the ketone Mannich bases **11** were heated with the 2-iodopaullones **7** in DMF in the presence of palladium acetate and triethylamine under nitrogen to furnish the expected 2-vinylpaullones **12a**−**m** (Scheme 2). The reaction could be transferred to a parallel synthesis procedure in 20 mL vials employing a parallel reactor station. The reaction worked well also in the absence of a phosphine ligand. To our knowledge, this is the first report on the use of Mannich bases in Heck reactions. The modest yields mentioned here (14–46%) are the result of compound loss during the workup procedures and still bear optimization potential. Because of the readily available starting materials, the simple protocol, and the short reaction times the Heck reaction with ketone Mannich bases and iodo arenes reported here favorably complements the well established

Scheme 3. Synthesis of 9-*tert*-Butyl-2-phenylethinylpaullone **15**[a]



[a] Reagents and conditions: (i) $Pd(AcO)_2$, DMF, triethylamine, N_2, 140 °C, 30 min; (ii) 1. AcOH, 70 °C, 1 h; 2. AcOH, H_2SO_4, 70 °C, 1 h.

Claisen−Schmidt synthesis for 1,3-diarylpropenones using aromatic aldehydes and acetophenone derivatives as starting materials.

As a further structure modification of **2**, the phenylethynyl derivative **15** was prepared (Scheme 3). Because the 2-iodopaullone **7e** gave unsatisfactory results in an attempted Sonogashira reaction with the phenyl acetylene **13**, the latter was reacted with 7-iodo-2,3,4,5-tetrahydro-1*H*-1-benzazepine-2,5-dione (**5**). The obtained cyclic ketone **14** was subsequently transformed to the paullone **15** by a Fischer indole ring-closure reaction.

Results and Discussion

The first series of analogues prepared from the hit compound **2** comprised 2-(2-oxobutenyl) derivatives **10a**−**e** differing in the 9-substituent. Testing in the axenic amastigote assay showed that structures with electron-withdrawing substituents, **10c** and **10d**, showed not more than 50% growth inhibition at 15 μM. Consistent with this finding, no inhibition of parasite growth in infected macrophages was found with either compound at 5 μM. In contrast, the derivatives **10a**, **b**, and **e** bearing a hydrogen or an electron-donating substituent exhibited a stronger (>50%) inhibition in the axenic amastigote assay. As a further structural modification, we prepared the compounds **12a**, **b**, and **c**, which are characterized by a 2-(3-aryl-3-oxopropenyl) residue. Of these derivatives, the 9-unsubstituted analogue **12a** and the 9-methyl-derivative **12b** proved to be inactive in the assay based on infected macrophages, though analogue **12a** showed good activity against the axenic amastigotes. In this assay, the structure with the 9-*tert*-butyl substituent **12c** exhibited the strongest potency, an observation that was also made with 9-*tert*-butyl-substituted derivatives **10e** and **10f** in the initial series of the analogues **10**. Therefore, the following compounds **12d**−**m** were designed as congeners of **12c** incorporating both the 2-(3-aryl-3-oxopropenyl) and the 9-*tert*-butyl substituent. Indeed, several of these structures demonstrated strong activity in the infected macrophages assay and a GI_{50} value in the axenic amastigote assay near or below 1 μM. Furthermore, **12d**, **12e**, **12g**, **12h**, and **12m** were devoid of noteworthy toxicity for the THP-1 macrophages. However, a small modification in the structure of **12m**, namely, the shift of the methoxy groups (derivatives **12k** and **12l**) or the addition of a third methoxy group (compound **12j**) led to increased toxicity in the THP-1 assay. The exchange of the 2-(3-aryl-3-oxopropenyl) substituent in **12a**−**m** for a phenylacetylene substituent resulted in compound **15** showing a comparable antileishmanial profile. This example illustrates that obviously there are still some more options for chemical modification at the 2-position of the parent scaffold that are worth exploring.

The most interesting derivatives reported here, **12d**, **12e**, **12g**, **12h**, and **12m** might be considered as paullones having a chalcone (= 1,3-diarylpropen-1-one) substructure. Chalcones

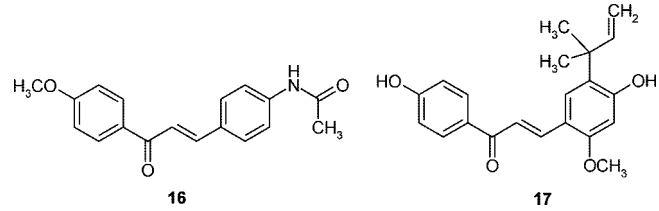


Figure 2. Structure of chalcone **16** as analogue of **12d** and structure of licochalcone A (**17**).

are well documented in the literature as antileishmanial agents.[23−29] In this context the question arises whether the paullone substructure is an indispensable prerequisite for the antileishmanial activity of **12**. To investigate this question, the chalcone **16**[30] was tested (Figure 2), which can be considered as a truncated analogue of **12d** lacking the indole element. With only 69.6 ± 2.0% inhibition in the axenic amastigote assay, **16** proved to be inferior to **12d**. We therefore assume that the paullone substructure in the compounds **12** is important for the antileishmanial activity.

The biological mechanism underlying the antileishmanial activity of 2-(3-aryl-3-oxopropenyl)-9-*tert*-butylpaullones **12** is not yet clear. As mentioned above, 2-unsubstituted paullones have been found to inhibit the cyclin-dependent kinase Lmex-CRK3 from *L. mexicana*.[14] Moreover, affinity experiments with immobilized paullones revealed that these moieties preferentially bind mitochondrial malate dehydrogenase from *L. mexicana* promastigote extracts.[15] On the other hand, chalcones have been shown to destroy the ultrastructure of *Leishmania* mitochondria and to inhibit the parasite respiration.[26,31] A variety of enzymes have been discussed as biological targets for chalcones in *Leishmania* parasites.[23] One of the most prominent antileishmanial chalcones of natural origin, licochalcone A (**17**), inhibited the fumarate reductase in permeabilized *L. major* promastigotes (IC_{50} = 1.2 μM) and in the crude mitochondria fraction of the parasite (IC_{50} = 14 μM).[32] For a further rational development of **12**, it will be important to identify the crucial biological target in *Leishmania*. Therefore, we intend to carry out affinity chromatography experiments with immobilized 2-(3-aryl-3-oxopropenyl)-9-*tert*-butyl-paullones **12** and parasite extracts. Proteins from the extracts binding to the immobilized pharmacophore moieties will be identified and analyzed for possible interactions with **12**. Moreover, animal studies are necessary to show that the class of compounds described here also exhibits antileishmanial activity in vivo.

Conclusion

We have developed a novel type of antileishmanial agents, namely, 2-(3-aryl-3-oxopropenyl)-9-*tert*-butyl-paullones **12**. Of note, several members of this compound class show activity both against axenic amastigotes and against parasites in host

macrophages without exhibiting toxicity for human host cells. Further studies are needed to elucidate the biological mechanism of the antileishmanial activity and for proof of the in vivo antiparasitic activity of these compounds.

Experimental Section

Parasite and Cell Culture. *L. donovani* (MHOM/SD/1962/1S-Cl2d) was used in all bioassays. Axenic amastigotes were grown at 37 °C in a 5% CO_2 incubator as described[33] in complete RPMI 1640 containing 20% fetal calf serum, pH 5.5. Stably transfected *L. donovani* promastigotes expressing the firefly luciferase gene (*Ld*:pSSU-int/LUC) were cultured in medium-199 adjusted to pH 6.8 and supplemented with L-glutamine (2 mM), adenosine (100 μM), folic acid (23 μM), 1× BME vitamin mix, 10% fetal calf serum, penicillin G (100 IU), streptomycin (100 μg/ml), and hygromycin B (100 μg/ml).

The human leukemia monocyte cell line (THP-1) was cultured in complete RPMI-1640 supplemented with antibiotics (100 IU penicillin G and 100 mg/ml streptomycin), 2 mM L-glutamine, and fetal calf serum (10% v/v).

Axenic Amastigote Viability Assay. Screening of the compounds for leishmanicidal activity was carried out using a alamarBlue (AbD Serotec, Oxford, U.K.) viability assay similar to that reported for leishmanial promastigotes.[34] Standardization and optimization of the assay for axenic amastigotes will be described elsewhere (Shimony and Jaffe, in preparation). Compounds to be assayed were diluted to twice the final concentration in the complete amastigote medium, containing 1% DMSO, and were aliquoted in triplicate (125 μL/well) into 96-well flat-bottom plates (Nunc, Roskilde, Denmark). Amastigotes (5.0×10^5 cells/mL; 125 μL/well) were added to each well and incubated for 24 h at 37 °C in a 5% CO_2 incubator. The alamarBlue viability indicator was added (25 μL/well) and the plates were incubated for an additional 24 h at which time the fluorescence (λex = 544 nm; λem = 590 nm) was measured in a microplate reader (Fluoroskan Ascent FL, Finland). Complete medium both with and without DMSO was used as negative controls (0% inhibition of amastigote growth). Amphotericin B (Sigma-Aldrich, St. Louis, MO), a drug used to treat VL, was included as a positive control on each plate and gave >90% inhibition of parasite growth at 1 μM.

Screening on Infected Macrophages. THP-1 cells in the log-phase of growth were differentiated by incubation for 3 days in complete RPMI-1640 containing 1 μM retinoic acid (RA, Sigma-Aldrich, St. Louis, MO).[35] Excess RA was removed by washing the cells three times with RPMI-1640 (250 × g, 10′, 4 °C), and the treated macrophages were suspended in complete RPMI-1640 and transferred to 75 mL tissue culture flasks (Costar Brand, NUNC, Denmark). Stationary-phase *Ld*:pSSU-int/LUC promastigotes were added to the treated macrophages (3:1 parasite/macrophage ratio) and incubated in a 5% CO_2 incubator for 16 h at 37 °C to allow for infection and differentiation of the *Leishmania* into intracellular amastigotes. Any remaining extracellular parasites were removed by centrifugation 4 – 5 times (210 × g, 8′, 4 °C). This was validated by phase microscopy. Infected THP-1 cells in complete RPMI-1640 were counted and aliquoted (1×10^5 cells in 50 μL/well) in triplicate into opaque 96-well flat bottom plates (Costar Brand, NUNC, Denmark). Drugs diluted in complete RPMI-1640 containing 1% DMSO (10 μM, 50 μL/well) were added to the infected cells. The cultures were incubated for 48 h (37 °C, 5% CO_2). Cells were lysed by the addition of Steady-Glo Luciferase Assay substrate (100 μL/well, Promega, MT, U.S.A.) to each well and the luminescence measured after 10 min using a microplate reader (Luminometer Mithras LB940, Berthold Technologies, Germany). Complete medium both with and without DMSO was used as negative controls (0% inhibition). Amphotericin B (Sigma-Aldrich, St. Louis, MO) was included as a positive control on each plate and gave >90% inhibition at 1 μM.

Toxicity Assay. Effect of the compounds on human cells was assessed using the alamarBlue viability assay. Drugs to be tested were diluted in the complete medium containing 1% DMSO (10 μM) and aliquoted in triplicate (125 μL/well) into 96-well flat-bottom plates. THP-1 macrophages in complete RPMI-1640 were added (8×10^5 cells/ml, 125 μL/well) to the plates and incubated for 48 h (37 °C, 5% CO_2). The viability indicator alamarBlue (25 μL) was added, the plates were incubated for an additional 3 h, and the fluorescence was read as described above. Complete medium both with and without DMSO was used as negative controls (0% inhibition).

Synthetic Chemistry. Melting points: IA 9100 instrument (Barnstedt Electrothermal), not corrected. Infrared spectra: KBr pellets. Thermo FT-IR 200 (Thermo Nicolet). NMR: Bruker Avance DRX-400, solvent [d_6]-DMSO, internal standard trimethylsilane, signals in ppm (δ scale). Mass spectrometry: Finnigan-MAT 90 instrument. Reverse-phase HPLC: Merck/Hitachi LaChrome Elite system and LiChroCART 125-4, LiChrospher 100 RP-18 (5 μM) column, eluent acetonitrile/water mixtures. Elemental analyses: CE Instruments FlashEA 1112 Elemental Analyzer (Thermo Quest). Results obtained were within ± 0.4%, unless indicated otherwise. Thin-layer chromatography: Polygram Sil G/UV_{254} silica gel plates (Macherey-Nagel); 254 nm UV illumination. Parallel synthesis: Carousel 12 Place Reaction Station (Radley Discovery Technologies). Compounds **5**[16] and **7a**[8] were prepared according to published procedures. The ketone Mannich base hydrochlorides were prepared according to a standard procedure.[36] Details for the synthesis of compounds **7c–e**, **10b−f**, **12b−m**, **14**, and **15** can be found in the Supporting Information.

General Procedure A for Preparation of Paullone Derivatives 7b−e by Acid-Catalyzed Fischer Indole Reaction. A mixture of 7-iodo-3,4-dihydro-1*H*-benzazepine-2,5-dione (**5**)[16] (452 mg, 1.5 mmol), an appropriate phenylhydrazine [2.0 mmol; or the appropriate phenylhydrazine hydrochloride (2.0 mmol) and sodium acetate (164 mg, 2.0 mmol)] in glacial acetic acid (15 mL) is stirred at 70 °C for 1 h. Concentrated sulfuric acid (0.1 mL) is added and stirring is continued for 1 h. After cooling to room temperature, the mixture is poured into 5% aqueous sodium acetate solution (20 mL). A precipitate is formed that is filtered off with suction and purified by crystallization from the given solvent.

2-Iodo-9-methyl-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (7b). Preparation according to general procedure A from 7-iodo-3,4-dihydro-1*H*-benzazepine-2,5-dione (**5**)[16] and 4-methylphenylhydrazine hydrochloride yielded 67% of an orange solid, mp 312–323 °C (dec; EtOH); IR 3199 (NH), 1648 (*C*═O); ^{1}H NMR 2.41 (s, 3 H), 3.48 (s, 2 H), 7.00–7.05 (m, 2 H), 7.32 (d, 1 H, J = 8.3 Hz), 7.44 (s, 1 H), 7.67 (dd, 1 H, J = 2.0/8.6 Hz), 8.06 (d, 1 H, J = 2.0 Hz), 10.14 (s, 1 H), 11.51 (s, 1 H); ^{13}C NMR 21.2 (prim C), 31.6 (sec C), 111.2, 117.6, 124.2, 124.3, 134.7, 136.0 (tert C), 87.6, 107.8, 125.2, 126.6, 127.8, 131.0, 135.0, 136.0, 171.3 (quat C); Anal. ($C_{17}H_{13}IN_2O$) C, H, N.

General Procedure B for the Preparation of 3-Oxo-1-butenyl Substituted Compounds (10a−e). The 9-substituted 2-iodo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**7a−e**; 0.50 mmol), methyl vinyl ketone (0.4 mL, 5 mmol), palladium acetate (23 mg, 0.10 mmol), triphenylphosphine (26 mg, 0.10 mmol), and triethylamine (1 mL) were suspended in DMF (10 mL) and stirred at 150 °C under nitrogen atmosphere. The mixture was filtered after 15 min. After addition of silica gel (1.5 g), the mixture was dried in vacuo. The remaining silica gel/reaction product mixture was added onto a silica gel pad in a glass frit and was then eluted with ethyl acetate (150 mL). After evaporation of the solvent, the remaining solid was purified by crystallization from ethanol.

2-[(1*E*)-3-Oxo-1-butenyl]-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (10a). Preparation following general procedure B from 2-iodo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**7a**)[8] yielded 54% of an orange solid, mp >330 °C; IR 3306 (NH), 3192 (NH), 1671 (*C*═O); ^{1}H NMR 2.37 (s, 3

H), 3.57 (s, 2H), 6.88 (d, 1 H, J = 16.3 Hz), 7.01 – 7.11 (m, 1H), 7.18–7.22 (m, 1 H), 7.29 (d, 1 H, J = 8.5 Hz), 7.46 (d, 1 H, J = 8.1 Hz), 7.65–7.71 (m, 3 H), 8.11 (d, 1 H, J = 1.8 Hz), 10.30 (s, 1 H), 11.63 (s, 1 H); ^{13}C NMR 21.2 (prim C), 31.7 (sec C), 111.4, 118.1, 119.2, 122.4, 122.6, 126.9, 128.1, 129.7, 142.5 (tert C), 107.6, 122.9, 126.5, 126.8, 131.9, 137.0, 137.5, 171.3, 198.0 (quat C); ($C_{20}H_{16}N_2O_2$) HRMS (EI) (m/z) calcd for [M^+], 316.12119; found, 316.12039.

General Procedure C for the Preparation of 2-[(1*E*)-3-Aryl-3-oxo-1-propenyl]-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-ones 12 by Heck-Type Reaction with Ketone Mannich Bases. A mixture of a 9-substituted 2-iodo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (0.50 mmol; **7a**, **b**, or **e**), a ketone Mannich base hydrochloride (0.55 mmol), palladium(II)acetate (11 mg, 0.050 mmol), triphenylphosphine (13 mg, 0.050 mmol), triethylamine (2 mL), and DMF (10 mL) is stirred at 150 °C under nitrogen. After filtration, silica gel (1.5 g) is added to the filtrate and the solvent is evaporated. The remaining silica gel/reaction product mixture is added onto a silica gel pad in a glass frit and is then eluted with ethyl acetate (200 mL). After evaporation of the solvent the remaining solid is purified by crystallization from ethanol. For the synthesis of the derivatives **12a**−**c** and **12g**−**m**, the procedure was adapted to the use of a parallel synthesis reactor. In this case, the reaction was carried out without addition of triphenylphosphine in 20 mL vials with 2 mL DMF as solvent. The vessel reactor block temperature was set to 140 °C. The work-up procedure was carried out as described above.

2-[(1*E*)-3-Oxo-3-phenyl-1-propenyl]-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (12a). Preparation following general procedure C from 2-iodo-7,12-dihydroindolo[3,2-*d*][1]benzazepin-6(5*H*)-one (**7a**)[8] and *N*,*N*-dimethyl-3-oxo-3-phenyl-1-propanaminium chloride[37] yielded 40% of a yellow solid, mp 256 °C (dec); IR 3309 (NH), 3222 (NH), 1652 (*C*=O); ^{1}H NMR 3.59 (s, 2 H), 7.10 (ddd, 1 H, J = 9.0/7.97 Hz), 7.21 (ddd, 1 H, J = 8.0/1.1 Hz), 7.33 (d, 2 H, J = 8.5 Hz), 7.49 (d, 2 H, J = 8.1 Hz), 7.59 – 7.63 (m, 2 H), 7.68 – 7.72 (m, 2 H), 7.81 (d, 2 H, J = 15.6 Hz), 7.91 (dd, 1 H, J = 8.6/2.0 Hz), 7.98 (d, 1 H), 8.16 – 8.18 (m, 2 H), 8.25 (d, 1H, J = 1.9 Hz,), 10.34 (s, 1 H), 11.63 (s, 1 H); ^{13}C NMR 31.6 (sec C), 111.4, 118.0, 119.1, 121.4, 122.3, 122.4, 127.6, 128.3, 128.4 (2 C), 128.8 (2 C), 133.0, 143.3 (tert C), 107.5, 122.8, 126.4, 129.8, 131.8, 137.1, 137.4, 137.5, 171.2, 189.0 (quat C); ($C_{25}H_{18}N_2O_2$) HRMS (EI; m/z, M^+) calcd, 378.13681; found, 378.13611.

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Supporting Information Available: Details for the synthesis of compounds **7c–e**, **10b**−**f**, **12b**−**m**, **14**, and **15**, spectroscopic data, HPLC purity data, and data of elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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