CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Identification of Phenylphthalazinones as a New Class of Leishmania infantum Inhibitors

Authors: Maarten Sijm, Erik de Heuvel, An Matheeussen, Guy Caljon, Louis Maes, Geert-Jan Sterk, Iwan de Esch, and Rob Leurs

To be cited as: *ChemMedChem* 10.1002/cmdc.201900538

Link to VoR: http://dx.doi.org/10.1002/cmdc.201900538

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

10.1002/cmdc.201900538

Identification of Phenylphthalazinones as a New Class of *Leishmania infantum* Inhibitors

Maarten Sijm[1], Erik de Heuvel[1], An Matheeussen[2], Prof. Dr. Guy Caljon[2], Prof. Dr. Louis Maes[2], Dr. Geert-Jan Sterk[1], Prof. Dr. Iwan J.P. de Esch[1] and Prof. Dr. Rob Leurs[1].*

[1]Division of Medicinal Chemistry, Faculty of Sciences, Amsterdam Institute for Molecules, Medicines and Systems (AIMMS), Vrije Universiteit Amsterdam, De Boelelaan 1108 1081 HZ, Amsterdam, The Netherlands,

[2] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Universiteitsplein 1, 2610, Antwerpen, Belgium.

Abstract

Leishmaniasis is a neglected parasitic disease caused by over 20 different *Leishmania* species. Current treatments often rely on harsh regimes of pentavalent antimonials such as sodium stibogluconate, while more recent drugs suffer other shortcomings such as low stability and rapid emergence of treatment failure, amongst others. Furthermore, the effectiveness of drugs varies depending on the infecting *Leishmania* species, thus there is an urgent need for new and effective anti-leishmanial drugs. Screening of an in-house compound library identified the hexahydrophthalazinone NPD-2942 as a low micromolar hit with a pIC_{50} of 5.8 against *L. infantum* and a pIC_{50} of 4.6 for cytotoxicity against human MRC-5 fibroblasts. To derive Structure-Activity-Relationships, we modified the cyclohexyl ring of the hexahydrophthalazinone scaffold and 1,2,3-triazoles were attempted as replacement for the pyrazole ring, amongst other. Ultimately, the 2,3-pyrazole substituted hexahydrophthalazinone NPD-1289 was identified as the most potent analogue in this series with a pIC_{50} of 6.3, although some cytotoxicity towards MRC-5 cells (pIC_{50} = 5.1) was recorded as well. Replacement of the unsubstituted 2,3-pyrazole with 1,2,3-triazoles led to compounds with lower anti-leishmanial activity. The current scaffold is a valuable new starting point for optimization towards novel anti-leishmanial drugs.

Introduction

Leishmaniasis is a neglected parasitic disease which comes in several distinct clinical manifestations and is caused by over 20 different *Leishmania* species which are transmitted by phlebotomine sand flies.[1] Visceral leishmaniasis (VL) is caused by *L. donovani* and *L. infantum*, with 500.000 new infections annually resulting in 50000 deaths, although this number could be higher due to underreporting.[2] Estimations are that another 1.5 million people are suffering from cutaneous leishmaniasis (CL), which is caused by almost all other *Leishmania* species.[3]

For 70 years the standard treatment of leishmaniasis is with pentavalent antimonials meglumine antimonate (**1**) and sodium stibogluconate (**2**).[4] While quite effective outside the Indian subcontinent, the treatment regimens of 3 daily injections for 30 days, often accompanied with severe pains, are far from ideal.[3, 5] Furthermore, treatment is relatively expensive and often requires additional medical supervision as a result of side effects such as vomiting, cardiotoxicity and hepatotoxicity.[6] Due to different effectiveness of antimonials against the various *Leishmania* species and emerging drug resistance, amphotericin B (**3**) is currently recommended as first-line treatment.[2-3, 7] Some patients however suffer from infusion reactions like chills and fever, while serious toxicity has been reported as well.[8] As such, much research has been directed towards improved formulations of amphotericin B (**3**) to enhance drug delivery and reduce toxicity.[8]

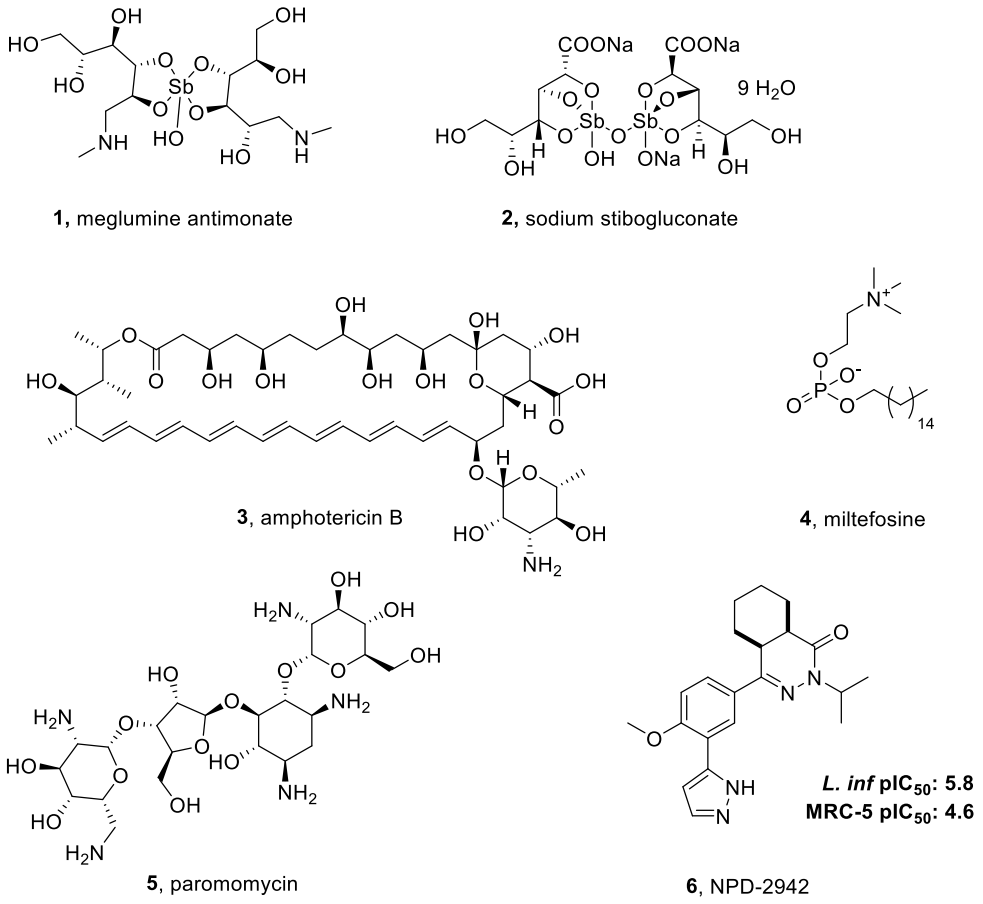


Figure 1 Pentavalent antimony drugs meglumine antiomonate (**1**), sodium stibogluconate (**2**), amphotericin B (**3**), miltefosine (**4**), paromomycin (**5**) and identified hit NPD-2942 (**6**).

Due to the high cost and low stability of amphotericin B (**3**), pentavalent antimonials are still commonly used in rural areas where a cold chain is absent. Other newly introduced drugs also have serious concerns. The phospholipid miltefosine (**4**) is the first oral drug available against leishmaniasis, initially showing high cure rates of 95% with only minor side effects.[9] However, more recent reports from the clinic indicate substantial levels of treatment failure.[10] The aminoglycoside paromomycin (**5**) shows cure rates up to 85% with a treatment regime for a 35 kg person costing less than 5 euro.[11] The largest downside of paromomycin is the relatively long treatment regime of injections for 21 days. Experimental resistance selection also showed a high

propensity for resistance development when used in monotherapy.[12] Combination treatments with sodium stibogluconate (**2**) improve the effectiveness for VL to cure rates of ~93%.[13]

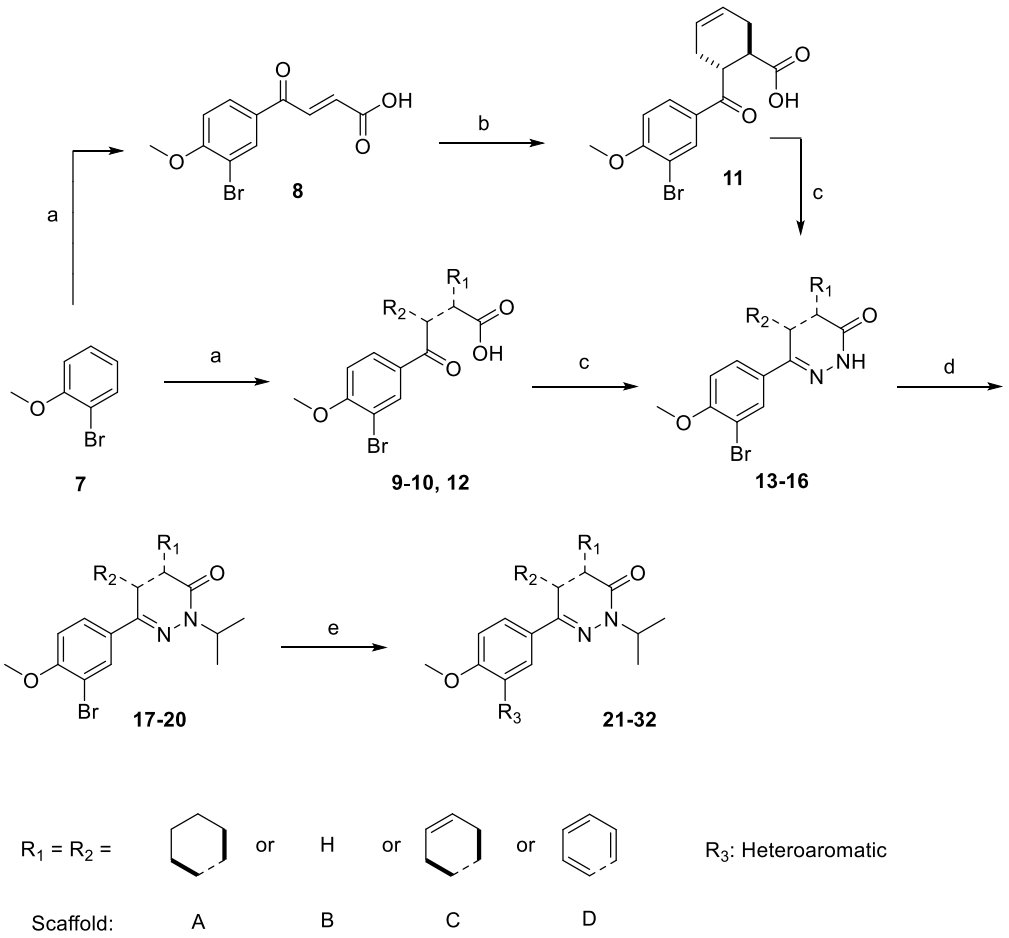
The last decades several drugs have been added to the toolbox against leishmaniasis, although each drug has its limitations.[14] Furthermore, there is a large interspecies difference of drug susceptibility and each clinical form of leishmaniasis has different requirements towards the pharmacokinetics of a drug. Hence, there remains an urgent need for new therapeutics.

To identify new hits against *Leishmania*, an in-house compound library was screened against *Leishmania infantum*. The majority of compounds in this library were previously obtained from other anti-parasitic programs. From this screening, hexahydrophthalazinone (**6**) was identified as a hit with a pIC_{50} of 5.8 against *L. infantum* and low toxicity against human MRC-5 cells. To investigate the Structure-Activity-Relationships (SAR) for this potential new chemical class of antiparasitic drugs, the cyclohexane part of the hexahydrophthalazinone, the phthalazinone-*N*-substituent and the aromatic substituent of the anisole ring were selected for modification.

Chemistry

First step of the synthesis of hexahydrophthalazinone analogues was the Friedel-Craft acylation of 2-bromoanisole with several anhydrides, yielding the corresponding keto-acids (**9**-**10**, **12**, scheme 1). The keto-acid precursor (**11**) for tetrahydrophthalazinone was prepared in two steps; first a Friedel-Craft acylation was done with maleic anhydride leading to trans-keto-acid **8**, which was followed by a Diels-Alder reaction with 1,3-butadiene resulting in trans-keto-acid **11**. Ring-closure of these keto-acids with hydrazine yielded the desired hexahydrophthalazinone (**13**), dihydropyridazinone (**14**), tetrahydrophthalazinone (**15**) and phthalazinone (**16**). An isopropyl moiety was installed on the unsubstituted phthalazinones and pyridazone using sodium hydride and isopropylbromide (**17**-**20**). In case of tetrahydrophthalazinone (**11**) the alkylation reaction with NaH resulted in the conversion of the trans-isomer to the cis-isomer as previously reported by de Heuvel *et al*.[15] After the alkylation the final aromatic substituents could be introduced via a Suzuki cross-coupling resulting in analogues substituted with various pyrazoles (**21**-**23**, **26**-**32**), 2-furan (**24**) and 3-tiophene (**25**).

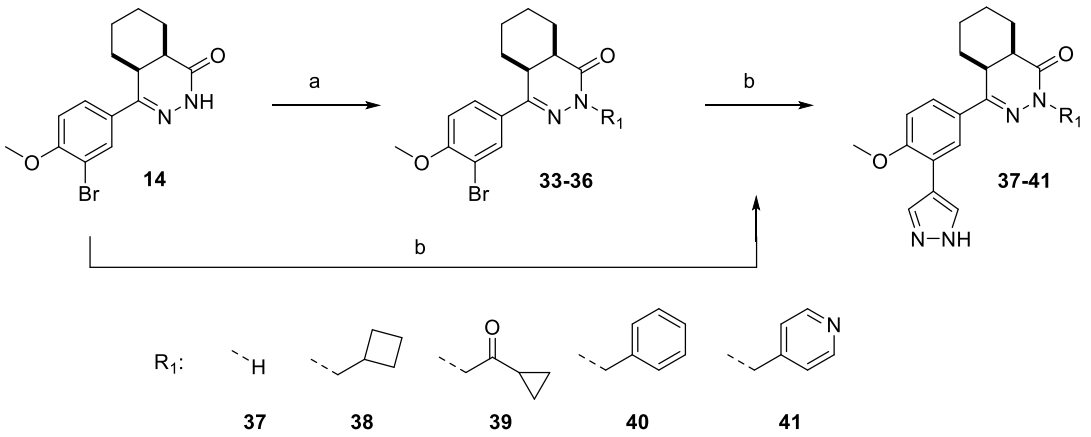
10.1002/cmdc.201900538



Scheme 1 Synthesis of various phthalazinones and pyridazinones. Reagents and conditions: a: $AlCl_3$, anhydride, DCM, rt, 16 h, 58-78 %, b: buta-1,3-diene, THF, 140 °C, 30 min, 10 bar, 97 %. c: $N_2H_4.H_2O$, EtOH, rt – 60 °C, 16 h, 20-89 %, d: isopropyl bromide, NaH, DMF, 50 °C, rt, 81-90 %, e: R_3-$B(OH)_2$ or R_3-pinacol, Na_2CO_3, $Pd(dppf)Cl_2$.DCM, H_2O:DME (1:3), 120 °C, 1 h, 8-26 %.

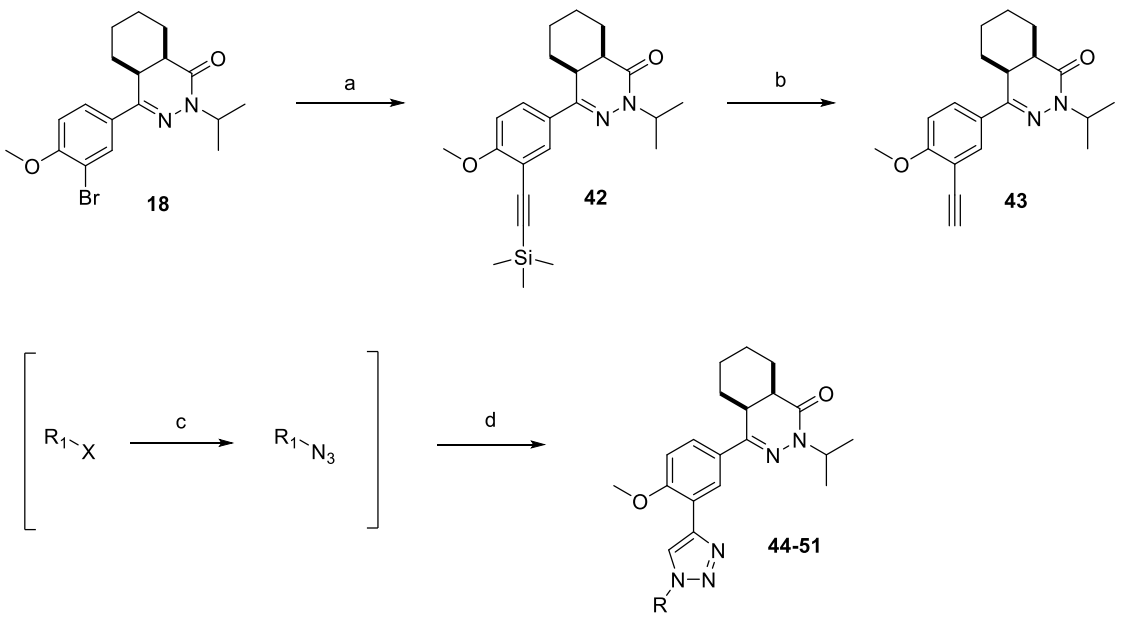
Further differentiation of the substituents on the hexahydrophthalazinone nitrogen (scheme 2), was done by deprotonation with sodium hydride followed by addition of the desired alkylhalides (**33**-**36**). On the alkylated intermediates the so far most promising 4-1*H*-pyrazolone was installed using a Suzuki cross-coupling (**37**-**41**).

10.1002/cmdc.201900538



Scheme 2 Synthesis of hexahydrophthalazinones. Reagents and conditions: a: alkyl bromide or chloride, NaH, DMF, 50 ºC, rt, 57-92 %, b: pyrazole-$B(OH)_2$, Na_2CO_3, $Pd(dppf)Cl_2$.DCM, H_2O:DME (1:3), 120 ºC, 1 h, 14-29 %.

Introduction of the 1,2,3-triazoles was done starting with isopropyl-hexahydrophthalazinone **18** (scheme 3), which was used in a Sonogashira reaction with trimetylsilylacetylene to yield **42**. The TMS protecting group (**42**) was removed with potassium hydroxide in MeOH, resulting in the acetylene substituted building-block (**43**). The desired azides were prepared *in situ* by refluxing sodium azide with the desired alkylhalide in MeOH:H_2O (8:1).[16] After 16 hours this mixture was cooled to room temperature and a portion of the azide solution was added to a mixture of the acetylene building block (**43**), $CuSO_4$ and *L*-ascorbate, yielding the desired 1,2,3-triazoles (**44**-**51**) after stirring overnight at room temperature.



Scheme 3 Synthesis of triazole substituted hexahydrophthalazinones. Reagents and conditions: a: a: $Pd(PPh_3)_4$, TEA, TMS-acetylene, 100 ºC, 1 h, 93 %, b: KOH, MeOH, rt, 86 %, 16 h, c: NaN_3, MeOH:H_2O (8:1), 80ºC, 16 h, d: R_1-N_3, $CuSO_4$.5 H_2O, *L*-ascorbate, MeOH: H_2O (8:1), rt, 16 h, 34-59 %.

Results and discussion

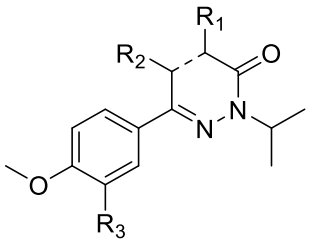
The first series of analogues synthesized around hit molecule NPD-2942 (**6**, figure 2) had modifications at two distinct positions. By modifying R_1 and R_2, the role of the hexyl ring of the hexahydrophthalazinone scaffold was investigated, while modifications on R_3 were introduced to investigate the SAR of the hetero-aromatic substituent (table 1). Introduction of a methyl group on the 1,2-pyrazole (**21**) reduced antiparasitic activity to 5.1 (pIC_{50}). Instead, installation of a 2,3-pyrazole ring, either unsubstituted (**22**) or methylated (**23**), improved activity compared to the original hit (**6**), with both compounds showing pIC_{50} values of 6.3. Cytotoxicity towards human MRC-5 cells was however higher for the methylated analogue (**23**, pIC_{50} = 5.6, SI = 5) than the

unsubstituted pyrazole (**22**, pIC_{50} = 5.1, SI = 16). Introduction of other heterocyclic rings, such as a 2-furan (**24**) and 3-thiophene ring (**25**) resulted in compounds with decreased activities (pIC_{50} values around 5.1). The removal of the cyclohexene ring (**26**-**27**) resulted in pIC_{50} values of 4.5, which is a decrease of 1.3 log units compared to original hit NPD-2942 (**6**). This shows the importance of having a cyclohexane ring on this position. Introduction of a cyclohexene ring instead of a cyclohexane, as in compounds **28**-**30**, resulted in a small increase in activity compared to the original hit (**6**) for the unsubstituted 1,2-pyrazole (**28**) with a pIC_{50} of 6.0. However, MRC-5 cytotoxicity of **28** also increased with 0.3 log unit showing 13 fold selectivity towards *L. inf*. The unsubstituted 2,3-pyrazole (**29**) had similar potency as the original hit (**6**) (pIC_{50} = 5.7) while the methylated analogue (**30**), was more potent with a pIC_{50} of 6.3. This increased potency of **30** was however accompanied with an increase of MRC-5 toxicity (pIC_{50} = 5.4). Replacing the cyclohexane ring with a phenyl moiety is tolerated, as 2,3-pyrazoles **31** and **32** showed pIC_{50} values of 5.6 and 6.0 respectively for the unsubstituted and methylated analogues. Both compounds, however, also showed some MRC-5 cytotoxicity with pIC_{50} values around 5.0.

After this first round of SAR, it was decided to continue with the unsubstituted 2,3-pyrazole on the R_3 position and a cyclohexene ring on R_1 and R_2. This compound, NPD-1289 (**22**), which has 16 fold selectivity over human cells with a pIC_{50} of 6.3 and a MRC-5 cytotoxicity of 5.1 (pIC_{50}), showed the best selectivity index in this series. The next position which was investigated for optimization was the substituent of the phthalazinone nitrogen (R_4, Table 2).

Table 1 Phenotypic activity of various *N*-isopropyl phthalazinones and dihydropyridazinone against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cell growth.

10.1002/cmdc.201900538

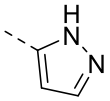
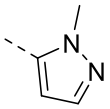
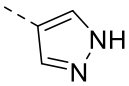
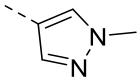
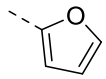
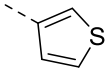
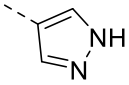
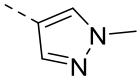
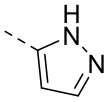
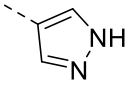
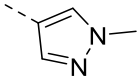
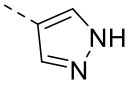
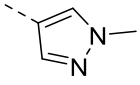


A

B

C

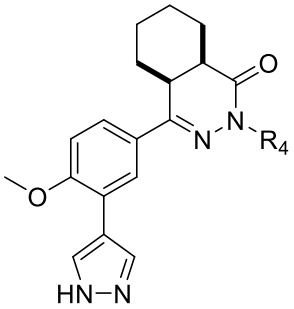
D

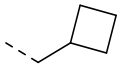
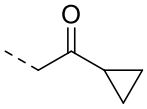
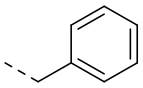
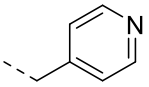
Cmpnd	R^1/R^2	R^3	*L. inf* (pIC_{50}) [a]	MRC-5 (pIC_{50}) [a]	Selectivity Index (SI)[b]
6 NPD-2942	A		5.8	4.6	16
21	A		5.1	4.6	3
22	A		6.3	5.1	16
23	A		6.3	5.6	5
24	A		5.1	4.5	4
25	A		5.2	< 4.2	> 10
26	B		4.5	4.5	1
27	B		4.5	4.5	1
28	C		6.0	4.9	13
29	C		5.7	4.6	13
30	C		6.3	5.4	8
31	D		5.6	5.2	3
32	D		6.0	5.0	10

[a] all reported values are within a standard deviation of ± 0.2 [b] the SI was calculated by dividing the activity (IC_{50}) against *L. inf* by IC_{50} measured against MRC-5.

Removal of the isopropyl moiety led to compound **37**, which was considered inactive (pIC_{50} = 4.2) against *L. infantum* but did show substantial cytotoxicity towards MRC-5 cells (pIC_{50} = 5.5). Installation of a cyclobutylmethyl instead of the isopropyl moiety of **22** resulted in analogue **38.** This molecule shows low micromolar activity against *L. infantum* (pIC_{50} = 5.9), but is accompanied with substantial cytotoxicity (pIC_{50} = 5.6). The three cyclopropyl-2-oxoethyl **39**, benzyl **40** and 4-pyridinylmethyl **41** analogues all showed even higher cytotoxicity for MRC-5 cells compared to their activities against *L. infantum*. Based on these findings, further modifications of the R_4 position were discontinued for now.

Table 2 Phenotypic activity of hexahydrophthalazinones with various *N*-substituents against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cells.



Cmpnd	R^4	*L. inf* (pIC_{50}) [a]	MRC-5 (pIC_{50}) [a]	Selectivity Index (SI)[b]
37		4.2	5.5	0.05
38		5.9	5.6	2
39		4.4	5.0	0.25
40		5.4	5.6	0.63
41		5.4	6.1	0.20

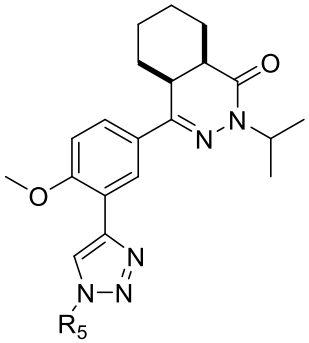
[a] all reported values are within a standard deviation of ±0.2 [b] the SI was calculated by dividing the activity (IC_{50}) against *L. inf* by IC_{50} measured against MRC-5.

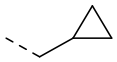
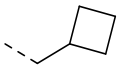
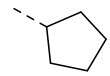
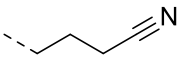
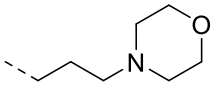
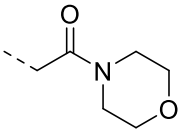
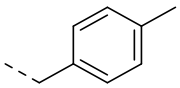
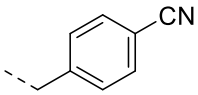
Next, analogues were prepared with modifications on the heteroaromatic ring on R_5 (Table 3), aiming for lower toxicity. Moving away from the pyrazoles, instead 1,2,3-triazoles were introduced by utilizing click reactions on acetylene substituted hexahydrophthalazinone **43**. Having small aliphatic substituents on the 1,2,3-triazole (**44**-**47**) resulted in four compounds with activities around 5.7 (pIC_{50}), however large differences in MRC-5 toxicity were observed. While methylcyclopropyl **44** and butyronitrile (**47**) showed some cytotoxicity against MRC-5 cells (pIC_{50} = 5.0 and 4.7), methylcyclobutyl (**45**), cyclopentyl (**46**) showed virtually no cytotoxicity with pIC_{50} values of 4.3 and <4.2 respectively, resulting in high SI's of 25 and >31. Attempts to introduce

some polar side chains, by installing morpholine moieties (**48**, **49**), resulted in compounds with moderate activities against *L. infantum* with pIC_{50} values of 5.1 and 4.7 respectively, while showing cytotoxicity towards MRC-5 cells at similar concentrations. Installing aromatic moieties, such as benzyl derivatives **50** and **51**, resulted in two compounds with activities around 5.4 (pIC_{50}) and no observable toxicity against MRC-5 cells ($pIC_{50} < 4.2$). While 1,2,3-triazoles showed substantially lower cytotoxicity than their pyrazole counterparts, their antiparasitic activities are also lower. The cyclopentyl substituted triazole, NPD-3189 (**46**) does however show some promise with a pIC_{50} of 5.7 and no toxicity.

10.1002/cmdc.201900538

Table 3 Phenotypic activity against intracellular amastigotes of *Leishmania infantum* and against MRC-5 cell growth by 1,2,3-triazole substituted hexahydrophthalazinones



Cmpnd	R^5	*L. inf* (pIC_{50}) [a]	MRC-5 (pIC_{50}) [a]	Selectivity Index (SI)[b]
44		5.7	5.0	5
45		5.7	4.3	25
46		5.7	< 4.2	> 31
47		5.6	4.7	8
48		5.1	4.7	3
49		4.5	4.5	1
50		5.2	< 4.2	> 10
51		5.5	< 4.2	> 20

[a] all reported values are within a standard deviation of ±0.2 [b] the SI was calculated by dividing the activity (IC_{50}) against *L. inf* by IC_{50} measured against MRC-5.

Conclusion

Starting from the screening hit NPD-2942 (**6**) a series of close analogues has been prepared to investigate SAR around the hexahydrophthalazinone scaffold. Variations of the core scaffold shows that removal of the cyclohexane moiety leads to compounds with decreased activity (pIC_{50}s around 4.5), modification of the cyclohexane to a cyclohexene or phenyl moiety is tolerated, although this generally does not improve potency. Introduction of several heteroaromatic rings on R_3 revealed that an unsubstituted 2,3-pyrazole on the hexahydrophthalazinone scaffold (**22**, NPD-1289) is the most promising analogue of this series with a pIC_{50} value of 6.3 and a 16-fold selectivity over toxicity for MRC-5 cells. Close analogue **30** shows a similar potency, however is less promising with a relative high cytotoxicity (pIC_{50} value = 5.4, SI = 8). Modifications of the isopropyl moiety (**37**-**41**) led to compounds with high cytotoxicity towards MRC-5 cells and this position was not further investigated. Replacing the unsubstituted 2,3-pyrazole with 1,2,3-triazoles (**44**-**51**) yielded a set of compounds with lower activity against *L. infantum*, although higher selectivity over MRC-5 cells was achieved with methylcyclobutyl **45** (SI = 25) and cyclopentyl **46** (SI >31). Overall, the current series shows the promise of the hexahydrophthalazinone scaffold. This new chemical class shows submicromolar antileishmanial activity and is a valuable starting point for further anti-leishmanial hit-to-lead optimization.

Experimental section biology

Leishmania infantum cellular assay. L. infantum MHOM/MA(BE)/67 amastigotes were collected from the spleen of an infected donor hamster and used to infect primary peritoneal mouse macrophages. To determine *in vitro* antileishmanial activity, 3×10^4 macrophages were seeded in each well of a 96-well plate. After 2 d of outgrowth, 5×10^5 amastigotes/well were added and incubated for 2 h at 37 °C. Solutions with or without test compound were subsequently added, and the plates were further incubated for 5 d at 37 °C and 5% CO_2 . Parasite burdens (mean number of amastigotes/macrophage) were microscopically assessed on 500 cells after Giemsa staining of the test plates and expressed as a percentage of the blank controls without test compound. In the case of observed toxicity to the macrophages, the lowest concentration was recorded at which the toxicity was observed, and this was used as a qualitative phenotypic assessment.[17] As a positive control Miltefosine was used.

MRC-5 cytotoxicity cellular assay. MRC-5 SV2 cells, originally from a human diploid lung cell line, were cultivated in MEM, supplemented with L-glutamine (20 mM), 16.5 mM sodium hydrogen carbonate and 5% FCS. For the assay, 10^4 cells/well were seeded onto the test plates containing the pre-diluted sample and incubated at 37°C and 5% CO_2 for 72 h. Cell viability was assessed fluorimetrically 4 h after addition of 10 µg/mL resazurin (excitation 550 nm, emission 590 nm). The results are expressed as percentage reduction in cell viability compared to untreated controls.[17] Tamoxifen was used as a positive control in this experiment.

Experimental section chemistry

Chemicals and reagents were obtained from commercial suppliers and were used without further purification. Anhydrous DMF, THF and DCM were obtained by passing them through an activated alumina column prior to use. Microwave reactions were executed using a Biotage® Initiator microwave system. ^{1}H

NMR spectra were recorded on a Bruker Avance 250 (250 MHz), Bruker Avance 400 (400 MHz), Bruker Avance 500 (500 MHz) or Bruker 600 Avance (600 MHz) spectrometer. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = double doublet, t = triplet, dt = double triplet, q = quartet, p = pentet, h = heptet, bs = broad singlet, m = multiplet), and coupling constants (Hz). Chemical shifts are reported in ppm with the natural abundance of deuterium in the solvent as the internal reference ($CDCl_3$: δ 7.26, $(CD_3)_2SO$: δ 2.50). ^{13}C NMR spectra were recorded on a Bruker Avance 500 (126 MHz) or Bruker Avance 600 (150 MHz). Chemical shifts are reported in ppm with the solvent resonance resulting from incomplete deuteration as the internal reference ($CDCl_3$: δ 77.16 or $(CD_3)_2SO$: δ 39.52). Systematic names for molecules according to IUPAC rules were generated using the Chemdraw AutoName program. LC-MS data was gathered using a Shimadzu HPLC/MS workstation with a LC-20AD pump system, SPD-M20A diode array detection, and a LCMS-2010 EV mass spectrometer. The column used is an Xbridge C_{18} 5 μm column (100 mm × 4.6 mm). Solvents used were the following: solvent B = ACN, 0.1% formic Acid; solvent A = water, 0.1% formic acid. The analysis was conducted using a flow rate of 1.0 mL/min, start 5% B, linear gradient to 90% B in 4.5 min, then 1.5 min at 90% B, linear gradient to 5% B in 0.5 min and then 1.5 min at 5% B, total run time of 8 min. All reported compounds have purities >95%, measured at 254 nm, unless otherwise mentioned. All HRMS spectra were recorded on a Bruker microTOF mass spectrometer using ESI in positive-ion mode. Column purifications were either carried out automatically using Biotage equipment or manually, using 60-200 mesh silica. TLC analyses were performed with Merck F254 alumina silica plates using UV visualization. All reactions were done under N_2 atmosphere, unless specifically mentioned.

Experimental data

(*E*)-4-(3-Bromo-4-methoxyphenyl)-4-oxobut-2-enoic acid (**8**)

To an ice-cooled mixture of 1-bromo-2-methoxybenzene (**6**)(65 mL, 0.52 mol) and furan-2,5-dione (77 g, 0.78 mol) in DCM (465 mL) was added $AlCl_3$ (84 g, 0.63 mol). The reaction mixture was stirred at rt overnight. The orange suspension was quenched in 3 M aq. HCl (1.5 L) and extracted using EtOAc (4 × 1 L). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to obtain a dark yellow solid. Trituration with Et_2O provided the product as a light yellow solid (97.5 g, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (d, *J* = 2.2 Hz, 1H), 7.69 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.53 (d, *J* = 15.4 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 1H), 6.51 (d, *J* = 15.4 Hz, 1H), 3.69 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 187.2, 167.1, 166.7, 160.2, 133.8, 133.0, 131.3, 130.6, 112.9, 111.8, 57.3. LC-MS (ESI) *m/z* found: 285 $[M+H]^+$; retention time: 4.09 minutes. HRMS (ESI): *m/z*: $[M + H]^+$ calcd. for $C_{11}H_{10}BrO_4$ 284.9757, found 284.9763.

(1R,2S)-2-(3-bromo-4-methoxybenzoyl)cyclohexanecarboxylic acid (**9**)

cis-hexahydroisobenzofuran-1,3-dione (17.3 g, 112 mmol) was added to a round bottom flask, followed by 1-bromo-2-methoxybenzene (**6**) (20 g, 107 mmol) and DCM (250 ml). The mixture was stirred and cooled with an ice-bath. Aluminum trichloride (17.1 g, 128 mmol) was added in small portions over approximately 5 min, after which the ice-bath was removed and the mixture stirred for 16 h, by then the mixture was a deep red solution. The mixture was quenched in a mixture of ice and concentrated hydrochloric acid (3:1) after which a white solid precipitated. Solids were filtered off, washed with water (3x 50 mL) and dried in vacuo to yield 24 g (63 mmol, 58%) of the title compound. ^{1}H-NMR: (500 MHz, DMSO-*d*6) δ 12.05 (s, 1H), 8.04 (d, 8.6 Hz, 1H), 7.94 (d, 8.6 Hz, 1H), 7.21 (d, *J* = 8.6 Hz, 1H), 3.93 (s, 3H), 3.39 (s, 1H), 2.73 – 2.58 (m, 1H), 2.03 (q, *J* = 9.5 Hz, 1H), 1.93 – 1.78 (m, 2H), 1.78 – 1.67 (m, 1H), 1.67 – 1.56 (m, 1H), 1.44 – 1.23 (m, 2H), 1.24 – 1.10 (m, 1H). LC-MS (ESI) *m/z* found: 341 $[M+H]^+$; retention time: 4.43 minutes, purity: 85%. HRMS-ESI $[M+H]^+$ calculated for $C_{15}H_{18}BrO_4$: 341.0383, found 341.0392.

trans-6-(3-Bromo-4-methoxybenzoyl)cyclohex-3-ene-1-carboxylic acid (**11**)

A mixture of keto-acid **8** (70.0 g, 221 mmol) and buta-1,3-diene in THF (~ 13% w/w, 150 mL, 300 mmol) was divided over 8 microwave vials. Each vial was stirred under microwave irradiation at 140 °C for 30 min (the internal pressure reached ~ 10 bar). The reaction mixtures were pooled and concentrated *in vacuo* to obtain the crude product. Trituration with toluene provided the product as a white solid (72.4 g, 97%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.30 (s, 1H), 8.16 (s, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 5.79 – 5.66 (m, 2H), 3.95 (s, 3H), 3.83 – 3.72 (m, 1H), 2.92 – 2.75 (m, 1H), 2.54 – 2.10 (m, 3H), 1.93 – 1.79 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ 200.8, 176.4, 159.61 133.4, 130.6, 130.3, 125.8, 125.7, 112.8, 111.6, 57.2, 42.2, 41.6, 29.4, 28.4. LC-MS (ESI) *m/z* found: 339 $[M+H]^+$; retention time: 4.53 minutes. HRMS (ESI): *m/z*: $[M + H]^+$ calcd. for $C_{15}H_{16}BrO_4$ 339.0226, found 339.0213.

(4aS,8aR)-4-(3-bromo-4-methoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**13**)

Keto-acid **10** (13.0g, 38.1 mmol) was charged to a round bottom flask followed by ethanol (150 mL). This suspension was stirred and hydrazine hydrate (50-60% (20 mL, 381 mmol) was added in portions. The mixture was heated at 60ºC for 16 h after which it was allowed the cool to rt while stirring. White precipitation was visible and 50 mL of water was added, increasing precipitation. Solids were filtered off and dried *in vacuo* to yield 11.4 gram (33.8 mmol, 89%) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, DMSO-*d6*) δ 10.89 (s, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.76 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.15 (d, *J* = 8.8 Hz, 1H), 3.88 (s, 3H), 3.29 – 3.20 (m, 1H), 2.71 (s, 1H), 2.28 (d, *J* = 12.5 Hz, 1H), 1.67 (d, *J* = 12.6 Hz, 1H), 1.56 – 1.47 (m, 2H), 1.45 – 1.33 (m, 2H), 1.30 – 1.16 (m, 2H). LC-MS (ESI) *m/z* found: 337 $[M+H]^+$; retention time: 4.41 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{15}H_{18}BrN_2O_2$: 337.0546, found 337.0536.

(4aS,8aR)-4-(3-bromo-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**17**)

Phthalazinone **13** (5.0 g, 14.8 mmol) was dissolved in DMF (100 mL) and sodium hydride (60% in mineral oil) (652 mg, 16.3 mmol) was added. The mixture was stirred for 15 min after which 2-bromopropane (1.67 mL, 17.8 mmol) was added and the mixture was stirred for 16 h at 60ºC. The reaction was quenched pouring the mixture in water (250 mL) and the aqueous layer was extracted with MTBE (250 mL). The organic layer was washed with sat. aqueous $NaHCO_3$ (2x 250 mL) and brine (250 mL) after which the organic layer was dried over Na_2SO_4. The solids were filtered off and volatiles were evaporated *in vacuo* after which the remaining crude was purified over SiO_2 using a gradient of 10% EtOAc in heptane towards 50% EtOAc in heptane yielding 4.6g (12.1 mmol, 82%) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.69 (d, *J* = 8.6 Hz, 1H), 6.90 (d, 1H), 5.03 (hept, *J* = 6.4 Hz, 1H), 3.03 – 2.93 (m, 1H), 2.63 (s, 1H), 2.60 – 2.47 (m, 1H), 1.84 – 1.72 (m, 1H), 1.68 – 1.56 (m, 2H), 1.40 – 1.30 (m, 3H), 1.29 (d, *J* = 6.6 Hz, 3H), 1.21 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ 166.45, 156.68, 151.24, 130.75, 129.19, 126.15, 112.06, 111.47, 56.36, 46.40, 36.69, 35.48, 26.90, 25.67, 24.28, 23.98, 20.65, 20.18. LC-MS (ESI) *m/z* found: 379 $[M+H]^+$; retention time: 5.51 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{18}H_{24}BrN_2O_2$: 379.1016, found 379.1009.

2-isopropyl-4-(4-methoxy-3-(1H-pyrazol-4-yl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**22**)

Phthalazinone **17** (100 mg, 0.26 mmol) and (1H-pyrazol-4-yl)boronic acid (35 mg, 0.32 mmol) were added to a microwave tube with a stirring bean, subsequently DME (3 ml) and 1M sodium carbonate (0.9 mL, 0.9 mmol) were added and the mixture was degassed for 5 minutes with N_2. After addition of $PdCl_2$(dppf).CH_2Cl_2 (20 mg, 0.026 mmol) the mixture was degassed for another 2 min and the vessel was sealed and heated for 1 h at 120ºC. The mixture was diluted with EtOAc (25 mL), filtered over Celite and the organic layer was washed with water (2x 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, solids were filtered off and the residue was concentrated in vacuo. Remaining crude was purified over SiO_2 using a gradient of 50% heptanes in EtOAc towards 5% MeOH in EtOAc to yield the 18 mg (0.05 mmol, 19%) of title compound as an off-white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 8.08 (s, 2H), 8.04 (d, *J* = 2.3 Hz, 1H), 7.64 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.99 (d, *J* = 8.6 Hz, 1H), 5.08 (hept, *J* = 6.6 Hz, 1H), 3.95 (s, 3H), 3.13 – 3.03 (m, 1H), 2.72 – 2.65 (m, 1H), 2.61 – 2.52 (m, 1H), 1.85 – 1.76 (m, 1H), 1.73 – 1.60 (m, 2H), 1.49 – 1.35 (m, 4H), 1.33 (d, *J* = 6.6 Hz, 3H), 1.25 (d, *J* = 6.6 Hz, 3H). ^{13}C-NMR: (126 MHz,

$CDCl_3$) δ 166.60, 156.95, 152.67, 133.16, 127.93, 125.30, 125.22, 121.55, 117.70, 110.93, 55.65, 46.40, 36.76, 35.61, 25.76, 24.44, 24.06, 22.06, 20.69, 20.24. LC-MS (ESI) *m/z* found: 367 $[M+H]^+$; retention time: 4.55 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{21}H_{27}N_4O_2$: 367.2129, found 367.2116.

(4aS,8aR)-4-(3-bromo-4-methoxyphenyl)-2-(cyclobutylmethyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**33**)

Phthalazinone **13** (450 mg, 1.33 mmol) was dissolved in DMF (10 mL) and sodium hydride (60% in mineral oil) (59 mg, 1.5 mmol) was added. The mixture was stirred for 15 min after which (bromomethyl)cyclobutane (0.18 mL, 1.6 mmol) was added and the mixture was stirred for another 16 h. The reaction was quenched pouring the mixture in water (50 mL) and the aqueous layer was extracted with MTBE (50 mL). The organic layer was washed with sat. aqueous $NaHCO_3$ (2x 50 mL) and brine (50 mL) after which the organic layer was dried over Na_2SO_4. The solids were filtered off and volatiles were evaporated *in vacuo* after which the remaining crude was purified over SiO_2 using a gradient of 10% EtOAc in heptane towards 70% EtOAc in heptane yielding 430 mg (1.06 mmol, 79%) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 2.1 Hz, 1H), 7.66 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 1H), 4.13 (dd, *J* = 13.2, 7.7 Hz, 1H), 3.93 (s, 3H), 3.65 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.05 – 2.95 (m, 1H), 2.75 (h, *J* = 7.8 Hz, 1H), 2.70 – 2.64 (m, 1H), 2.59 – 2.49 (m, 1H), 2.07 – 1.97 (m, 2H), 1.93 – 1.76 (m, 5H), 1.69 – 1.59 (m, 3H), 1.45 – 1.23 (m, 5H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ 167.17, 156.74, 151.27, 130.83, 128.89, 126.20, 112.11, 111.47, 56.37, 53.30, 36.40, 35.96, 26.12, 26.09, 25.71, 24.40, 23.95, 22.00, 18.46. LC-MS (ESI) *m/z* found: 405 $[M+H]^+$; retention time: 5.91 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{20}H_{26}BrN_2O_2$: 405.1172, found 405.1165.

(4aS,8aR)-2-(cyclobutylmethyl)-4-(4-methoxy-3-(1H-pyrazol-4-yl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**38**)

Phthalazinone **33** (150 mg, 0.37 mmol) and (1H-pyrazol-4-yl)boronic acid (62 mg, 0.56 mmol) were added to a microwave tube with a stirring bean, subsequently DME (4 ml) and 1M sodium carbonate (2 mL, 2.0 mmol) were added and the mixture was degassed for 5 min with N_2. After addition of $PdCl_2$(dppf).CH_2Cl_2 (30 mg, 0.037 mmol) the mixture was degassed for another 2 minutes and the vessel was sealed and heated for 1 h at 120°C. The mixture was diluted with EtOAc (25 mL), filtered over Celite and the organic layer was washed with water (2x 25 mL) and brine (25 mL). The organic layer was dried over Na_2SO_4, solids were filtered off and the residue was concentrated in vacuo. Remaining crude was purified over SiO_2 using a gradient of 50% heptanes in EtOAc towards 5% MeOH in EtOAc to yield 37 mg (0.09 mmol, 25%) of the title compound as an off-white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 8.09 (s, 2H), 8.00 (d, *J* = 1.8 Hz, 1H), 7.59 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 1H), 4.17 (dd, *J* = 13.2, 7.6 Hz, 1H), 3.94 (s, 3H), 3.68 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.14 – 3.03 (m, 1H), 2.82 – 2.68 (m, 2H), 2.61 – 2.51 (m, 1H), 2.12 – 1.97 (m, 2H), 1.94 – 1.76 (m, 5H), 1.73 – 1.59 (m, 2H), 1.50 – 1.29 (m, 4H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ 167.35, 157.02, 152.87, 133.38, 127.59, 125.35, 125.22, 121.77, 117.58, 110.94, 105.69, 55.63, 53.25, 36.47, 36.12, 34.86, 26.20, 25.78, 24.55, 24.02, 22.08, 18.52. LC-MS (ESI) *m/z* found: 393 $[M+H]^+$; retention time: 4.83 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{23}H_{28}N_4O_2$: 393.2285, found 393.2276.

(4aS,8aR)-2-isopropyl-4-(4-methoxy-3-((trimethylsilyl)ethynyl)phenyl)-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**42**)

Phthalazinone **13** (2.0 g, 5.3 mmol) was added to a microwave tube and TEA (12 ml) was added. The mixture was degassed with N_2 for 5 min after which Tetrakis (0.61 g, 0.53 mmol) and CuI (0.1 g, 0.53 mmol) were added. The tube was heated in the microwave at 100 °C for 1 h, after which the mixture was allowed to cool to rt, diluted with MTBE (25 mL) and filtered over Celite. The organic layer was washed with saturated aqueous $NaHCO_3$ (2x 25 mL) and brine (25 mL), after which it was dried over Na_2SO_4 and solids were filtered off. The remaining black crude was concentrated *in vacuo* and purified over SiO_2 using a gradient of heptane towards 25% EtOAc in heptane yielding 1.95 g (4.9 mmol, 93%) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.83 – 7.76 (m, 2H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.05 (hept, *J* = 6.6 Hz, 1H), 3.92 (s, 3H), 3.07 – 2.99 (m, 1H), 2.63 (s, 1H), 2.55 (d, *J* = 9.8 Hz, 1H), 1.83 – 1.75 (m, 1H), 1.68 – 1.60 (m, 2H), 1.58 (s, 2H), 1.41 – 1.32 (m, 2H), 1.31 (d, *J* = 6.6 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H), 0.29 (s, 8H). LC-MS (ESI) *m*/*z* found: 397 $[M+H]^+$; retention time: 6.09 minutes.

(4aS,8aR)-4-(3-ethynyl-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**43**)

Phthalazinone **42** (2.0 g, 5.0 mmol) was charged to a round bottom flask, followed by MeOH (50 mL) and 2.5M aqueous potassium hydroxide. The mixture was stirred overnight at rt for 16 h, after which the aqueous layer was extracted with EtOAc (50 mL). The organic layer was washed with brine (50 mL) and dried over Na_2SO_4 after which the solids were filtered off. Volatiles were removed *in vacuo*, after which the remaining crude was purified over SiO2 using a gradient of 10% EtOAc in heptanes towards 70% EtOAc in heptanes yielding 1.4 g (5.0 mmol, 86%) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 2.3 Hz, 1H), 7.81 (dd, *J* = 8.8, 2.3 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 5.05 (hept, *J* = 6.6 Hz, 1H), 3.95 (s, 3H), 3.35 (s, 1H), 3.07 – 2.97 (m, 1H), 2.67 – 2.62 (m, 1H), 2.59 – 2.50 (m, 1H), 1.84 – 1.76 (m, 1H), 1.68 – 1.60 (m, 2H), 1.47 – 1.32 (m, 4H), 1.30 (d, *J* = 6.6 Hz, 3H), 1.23 (d, *J* = 6.7 Hz, 3H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ 166.47, 161.36, 151.60, 131.59, 128.01, 127.84, 111.30, 110.66, 81.51, 79.74, 56.11, 46.36, 36.70, 35.47, 25.69, 24.28, 24.01, 22.00, 20.65, 20.18. LC-MS (ESI) *m*/*z* found: 325 $[M+H]^+$; retention time: 5.12 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{20}H_{25}N_2O_2$: 325.1911, found 325.1904.

(4aS,8aR)-4-(3-(1-(cyclopropylmethyl)-1H-1,2,3-triazol-4-yl)-4-methoxyphenyl)-2-isopropyl-4a,5,6,7,8,8a-hexahydrophthalazin-1(2H)-one (**44**)

(azidomethyl)cyclopropane was freshly prepared by refluxing (bromomethyl)cyclopropane (0.49 g, 3.6 mmol) and sodium azide (0.47 g, 7,2 mmol) in 4 mL MeOH and 0.5 mL H_2O for 16 h. To a tube 1 mL of the azide solution (0.8 M) was added followed by alkyne **43** (50 mg, 0.15 mmol), (+)-sodium L-ascorbate (3 mg, 0.015 mmol) and $CuSO_4$.5 H_2O (4 mg, 0.015 mmol), after which the tube was sealed. The reaction was stirred overnight at room temperature after which the reaction was quenched in 1M aqueous Na_2CO_3 (25 mL) and extracted with EtOAc (2x 25 mL). The organic layer was washed with 1M aqueous Na_2CO_3 (2x 25 mL) and brine (25 mL), dried over Na_2SO_4 and filtered off. The remaining residue was concentrated *in vacuo* and purified over

SiO_2 using a gradient of 70% heptanes in EtOAc towards 30% heptanes in EtOAc yielding 27 mg (0.06 mmol, 43 %) of the title compound as a white solid. ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 8.66 (d, *J* = 2.3 Hz, 1H), 8.13 (s, 1H), 7.92 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.03 (d, *J* = 8.8 Hz, 1H), 5.06 (hept, *J* = 6.7 Hz, 1H), 4.28 (d, *J* = 7.2 Hz, 2H), 3.99 (s, 3H), 3.28 – 3.18 (m, 1H), 2.70 – 2.64 (m, 1H), 2.59 – 2.50 (m, 1H), 1.81 – 1.57 (m, 5H), 1.49 – 1.33 (m, 5H), 1.32 (d, *J* = 6.6 Hz, 3H), 1.25 (d, *J* = 6.7 Hz, 3H), 0.75 – 0.68 (m, 2H), 0.52 – 0.44 (m, 2H). ^{13}C-NMR: (126 MHz, $CDCl_3$) δ 166.70, 156.47, 152.97, 142.69, 138.93, 133.38, 128.32, 126.80, 125.27, 122.79, 121.77, 119.38, 111.22, 55.70, 54.95, 46.34, 36.80, 35.38, 25.64, 24.27, 24.01, 22.07, 20.66, 20.21, 11.31, 4.24. LC-MS (ESI) *m/z* found: 422 $[M+H]^+$; retention time: 5.12 minutes. HRMS-ESI $[M+H]^+$ calculated for $C_{24}H_{31}N_5O_2$: 422.2551, found 422.2543.

Corresponding author

Rob Leurs, r.leurs@vu.nl

Author contributions

M.S. and E. d. H. contributed to the synthesis of the molecules, A.M., G.C. and L.M. were involved with the parasitological screenings, M.S., L.M., G.S and R.L. were involved in the design of the molecules and experiments. L.M, G.C., G.S, I.J.P de E and R.L obtained the necessary funding. M.S., G.S. and R.L. contributed to the writing of this manuscript. All authors have read and checked the manuscript.

Funding Sources

The PDE4NPD project was funded by the European Union under the FP-7-Health program, project ID: 602666.

Acknowledgements

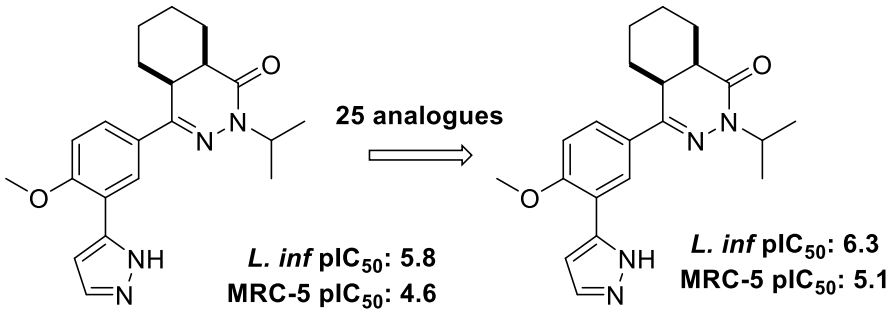
Authors would like to thank H. Custers and A. van der Stolpe for their technical assistance.

List of abbreviations

ACN: acetonitrile, bs: broad singlet, CL: cutaneous leishmaniasis, cLogP: calculated logarithm of the partition-coefficient, d: doublet, DCM: dichloromethane, dd: double doublet, DME:

dimethoxyethane, dt: double triplet, DMF: dimethylformamide, dppf: 1,1′-Ferrocenediyl-bis(diphenylphosphine), ESI: electron spray ionization, EtOAc: ethylacetate, EtOH: ethanol, h: heptet, h: hour, HPLC: high pressure liquid chromatography, HRMS: high resolution mass spectroscopy, Hz: hertz, *J*: coupling constant, *L. inf.*: *Leishmania infantum*, LC-MS: liquid chromatography- mass spectroscopy, LDA: lithium di-isopropyl amide, m: multiplet, MeOH: methanol, MHz: megahertz, MRC-5: medical research council cell strain 5, MS: mass spectroscopy, MTBE: methyl tert-butyl ether, nm = nanometer, NMR: nuclear magnetic resonance, p = pentet, pIC_{50} = inverse logarithm of the concentration at which 50% is inhibited, ppm = parts per million, q = quartet, s: singlet, SAR: structure activity relationship, SEM: standard error of the mean, SI: selectivity index, t: triplet, THF: tetrahydrofuran, VL: visceral leishmaniasis.

Table of contents



6, NPD-2942 **22**, NPD-1289

Leishmaniasis is caused by protozoan parasites of the *Leishmania* family and targets millions of people worldwide. Currently used drugs have several limitations and/or side effects. As such the need for new molecules in the drug discovery pipeline is high. Phenylphthalazinone NPD-2942 was identified as a hit and 25 close analogues were prepared to investigate SAR. Several promising analogues were identified which are useful starting points for further optimization.

References

[1] WHO, WHO fact sheet on Leishmaniasis. https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis, Vol. 2018, **2018**.

[2] F. Chappuis, S. Sundar, A. Hailu, H. Ghalib, S. Rijal, R. W. Peeling, J. Alvar, M. Boelaert, *Nat Rev Micro* **2007**, *5*(11), 873-882.

[3] R. Reithinger, J.-C. Dujardin, H. Louzir, C. Pirmez, B. Alexander, S. Brooker, *The Lancet Infectious Diseases* **2007**, *7*(9), 581-596.

[4] a) E. Moore, D. O' Flaherty, H. Heuvelmans, J. Seaman, H. Veeken, S. d. Wit, R. N. Davidson, *Bulletin of the World Health Organization* **2001**, *79*, 388-393; b) WHO, *World Health Organisation Technical Report Series* **2012**, *949*.

[5] B. L. Herwaldt, J. D. Berman, *The American Journal of Tropical Medicine and Hygiene* **1992**, *46*(3), 296-306.

[6] F. Frézard, C. Demicheli, R. Ribeiro, *Molecules* **2009**, *14*(7), 2317.

[7] S. L. Croft, S. Sundar, A. H. Fairlamb, *Clinical Microbiology Reviews* **2006**, *19*(1), 111-126.

[8] S. Sundar, J. Chakravarty, *Journal of Global Infectious Diseases* **2010**, *2*(2), 159-166.

[9] S. K. Bhattacharya, P. K. Sinha, S. Sundar, C. P. Thakur, T. K. Jha, K. Pandey, V. R. Das, N. Kumar, C. Lal, N. Verma, V. P. Singh, A. Ranjan, R. B. Verma, G. Anders, H. Sindermann, N. K. Ganguly, *The Journal of Infectious Diseases* **2007**, *196*(4), 591-598.

[10] a) V. Bhandari, A. Kulshrestha, D. K. Deep, O. Stark, V. K. Prajapati, V. Ramesh, S. Sundar, G. Schonian, J. C. Dujardin, P. Salotra, *PLOS Neglected Tropical Diseases* **2012**, *6*(5), e1657; b) K. Pandey, S. Pun, B. Pandey, *Indian Journal of Medical Microbiology* **2012**, *30*(2), 227-229; c) R. Obonaga, O. L. Fernández, L. Valderrama, L. C. Rubiano, M. D. M. Castro, M. C. Barrera, M. A. Gomez, N. Gore Saravia, *Antimicrobial agents and chemotherapy* **2014**, *58*(1), 144-152.

[11] a) R. N. Davidson, M. den Boer, K. Ritmeijer, *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2009**, *103*(7), 653-660; b) A. Ben Salah , N. Ben Messaoud , E. Guedri , A. Zaatour , N. Ben Alaya , J. Bettaieb , A. Gharbi , N. Belhadj Hamida , A. Boukthir , S. Chlif , K. Abdelhamid , Z. El Ahmadi , H. Louzir , M. Mokni , G. Morizot , P. Buffet , P. L. Smith , K. M. Kopydlowski , M. Kreishman-Deitrick , K. S. Smith , C. J. Nielsen , D. R. Ullman , J. A. Norwood , G. D. Thorne , W. F. McCarthy , R. C. Adams , R. M. Rice , D. Tang , J. Berman , J. Ransom , A. J. Magill , M. Grogl *New England Journal of Medicine* **2013**, *368*(6), 524-532.

[12] S. Hendrickx, A. Mondelaers, E. Eberhardt, P. Delputte, P. Cos, L. Maes, *Antimicrobial Agents and Chemotherapy* **2015**, *59*(8), 4714.

[13] C. P. Thakur, T. P. Kanyok, A. K. Pandey, G. P. Sinha, A. E. Zaniewski, H. H. Houlihan, P. Olliaro, *Transactions of the Royal Society of Tropical Medicine and Hygiene* **2000**, *94*(4), 429-431.

[14] H. Goto, J. A. L. Lindoso, *Expert Review of Anti-infective Therapy* **2010**, *8*(4), 419-433.

[15] E. de Heuvel, A. K. Singh, E. Edink, T. van der Meer, M. van der Woude, P. Sadek, M. P. Krell-Jørgensen, T. van den Bergh, J. Veerman, G. Caljon, T. D. Kalejaiye, M. Wijtmans, L. Maes, H. P. de Koning, G. Jan Sterk, M. Siderius, I. J. P. de Esch, D. G. Brown, R. Leurs, *Bioorganic & Medicinal Chemistry* **2019**, *27*(18), 15.

[16] S. Nijmeijer, H. Engelhardt, S. Schultes, A. C. van de Stolpe, V. Lusink, C. de Graaf, M. Wijtmans, E. E. J. Haaksma, I. J. P. de Esch, K. Stachurski, H. F. Vischer, R. Leurs, *British Journal of Pharmacology* **2013**, *170*(1), 12.

[17] A. R. Blaazer, K. M. Orrling, A. Shanmugham, C. Jansen, L. Maes, E. Edink, G. J. Sterk, M. Siderius, P. England, D. Bailey, I. J. P. de Esch, R. Leurs, *Journal of Biomolecular Screening* **2014**, *20*(1), 10.