In Search of Novel Agents for Therapy of Tropical Diseases and Human Immunodeficiency Virus

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Received June 27, 2007

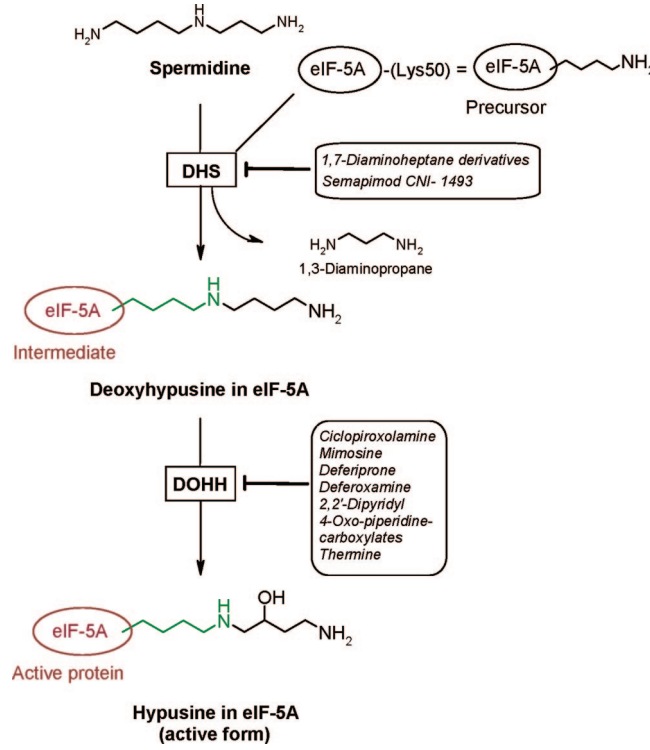
Malaria, sleeping sickness, Chagas' disease, Aleppo boil, and AIDS are among the tropical diseases causing millions of infections and cases of deaths per year because only inefficient chemotherapy is available. Since the targeting of the enzymes of the polyamine pathway may provide novel therapy options, we aimed to inhibit the deoxyhypusine hydroxylase, which is an important step in the biosynthesis of the eukaryotic initiation factor 5A. In order to identify new lead compounds, piperidines were produced and biologically evaluated. The 3,5-diethyl piperidone-3,5-dicarboxylates **11** and **13** substituted with 4-nitrophenyl rings in the 2 and 6 positions were found to be active against *Trypanosoma brucei brucei* and *Plasmodium falciparum* combined with low cytotoxicity against macrophages. The corresponding monocarboxylates are only highly active against the *T. brucei brucei.* The piperidine oximether **53** demonstrated the highest plasmodicidal activity. Moreover, compounds **11** and **53** were also able to inhibit replication of HIV-1.

Introduction

The malaria parasite *Plasmodium* is a major cause of global human mortality with *Plasmodium falciparum* being the deadliest protozoan to humans, causing one to two million deaths per year, mostly in subsaharian Africa.[1] Trypanosomatids such as *Trypanosoma brucei gambiense* and *T. b. rhodesiense* are the causative agents of African sleeping sickness, *T. cruzi* of South American Chagas' disease, and *Leishmania donovani, L. major*, and *L. tropica* of different forms of leishmaniasis, e.g., Aleppo boil. Worldwide, millions of people are infected by these parasites and tens of thousands of patients die every year.[2] The chemotherapy against all these diseases is very limited and unsatisfactory because of the emerging levels of resistance against plasmodicidal drugs and the lack of safe drugs against all forms of trypanosomiasis.[3] Even with the increasing number of infections with resistant and multiresistant pathogens, the major pharmaceutical companies are mostly neglecting this urgent medical problem and are frequently leaving the field of antiinfective drug discovery, especially in the case of tropical diseases.[4,5]

Thus, the purpose of this study was the search for novel agents active against plasmodia, trypanosomatids, and human immunodeficiency virus type 1 (HIV-1) by targeting the enzymes of the polyamine pathway (see Scheme 1). In fact, targeting of enzymes of this pathway like the ornithine decarboxylase (ODC[a]) [EC 4.1.1.17], adenosylmethionine decarboxylase (AdoMetDC) [4.1.1.50], and the spermidine synthase (SPDS) [EC 2.5.1.16] turned out to be valuable for both antiparasitic chemotherapy and prevention;[6–8] e.g., difluoromethylornithine,

Scheme 1. Polyamine Pathway and Available Inhibitors of the Enzymes[a]



[a] DHS, deoxyhypusine synthase; DOHH, deoxyhypusine hydroxylase.

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[a] Abbreviations: eIF5A, eukaryotic initiation factor 5A; DOHH, deoxyhypusine hydroxylase; DHS, deoxyhypusine synthase; ODC, ornithine decarboxylase; AdoMetDC, adenosylmethionine decarboxylase; CQS, chloroquine sensitive.

10.1021/jm070763y CCC: $40.75
Published on Web 12/27/2007

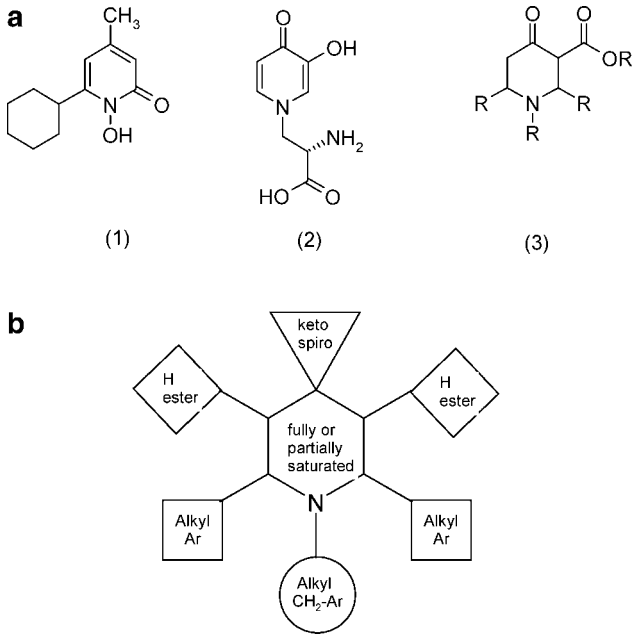

Figure 1. (a) Structural formulas of the DOHH inhibitors ciclopiroxolamine (1), L-mimosine (2), and 4-oxopiperidone 3-carboxylate (3). (b) Points of variation of the piperidine skeleton.

which is a specific inhibitor of ODC, blocks the erythrocytic schizogony of *P. falciparum* in culture, and recent results have shown that spermidine synthase from the malaria parasite can be inhibited by *trans*-4-methylcyclohexylamine with an inhibitory effect on cell proliferation.[9,10] In contrast to the enzyme of the parasite, the drug inhibits the mammalian enzyme without any antiproliferative effect.

The unusual amino acid hypusine (see Scheme 1) is a posttranslational modification of the eukaryotic initiation factor 5A (eIF-5A) and necessary for eIF-5A activity.[11] Hypusine is formed in two steps under catalysis of deoxyhypusine synthase (DHS) [EC 1.1.1.249] and by deoxyhypusine hydroxylase (DOHH) [EC 1.14.99.29].[12] DHS transfers an aminobutyl moiety from the triamine spermidine to a specific lysine residue in the eIF-5A precursor protein to give deoxyhypusine,[13] and subsequently DOHH hydroxylates this molecule, completing the hypusine biosynthesis.

Previous studies have shown that mature eIF-5A formation in plasmodia can be blocked by inhibition of DHS by means of 1,7-diaminoheptane in vitro.[14] Additionally, the eIF-5A formation can be prevented by inhibition of DOHH. Findings in rat testis have demonstrated that the DOHH can be inhibited by the antifungal drug ciclopiroxolamine (Figure 1). Ciclopiroxolamine showed antiangiogenetic effects in human vascular endothelial cells (HUVEC) and antiproliferative effects in the chick aortic arch sprouting assay.[15] Moreover, ciclopiroxolamine inhibits the in vitro proliferation of the chloroquine sensitive (CQS) NF54 *P. falciparum* strain with an IC_{50} of 8.2 μM. However, ciclopiroxolamine was ineffective in vivo in a rodent malaria model.[16]

The plant amino acid L-mimosine (Figure 1) can reversibly block mammalian cells at the late G1 phase and leads to a notable reduction in the steady-state level of mature eIF-5A by means of DOHH inhibition.[17] These findings prompted the determination of the antiplasmodial effect, resulting in an IC_{50} of 32 μM for the chloroquine susceptible (CQS) strain and 39 μM for the chloroquine resistant (CQR) strain. However, mimosine was toxic in a rodent malaria model.[16]

Complexing the essential catalytic metal ion (iron) was hypothesized to be the mechanism of inhibition by the DOHH by Park et al.,[18] because the hydroxamic acid moiety and the hydroxyl keton moiety of ciclopiroxolamine and mimosine, respectively, are putatively iron chelating subunits.[19] Following the iron complexing hypothesis, 4-oxopiperidine 3-carboxylates (Figure 1a), being chelators of the metal either via the enolizable β-ketoester moiety (cf. acetylacetonate complexes of iron[20]) or via the three nitrogens, in position 1 and in the pyridines[18,21] were recently tested for the inhibitory properties in *P. falciparum* and found to be more active than ciclopiroxolamine and mimosine in the same test.[16] However, this finding is an indirect proof-of-principle of the hypothesis only because the piperidones were not tested at the isolated enzyme. Some of the piperidones lacked sufficient water solubility.

Moreover, the eIF-5A is an essential cofactor of the viral regulatory protein Rev, which is important for HIV-1 replication.[22] Consequently, the direct inhibition of AdoMetDC, DHS, and DOHH with small molecules has been already shown to block the Rev activity and thus the virus replication,[23–26] indicating that inhibition of hypusine (especially DHS and DOHH) is a preferred strategy for the development of anti-HIV drugs.

These findings suggested the hypusine biosynthesis pathway to be a target for antimicrobial therapy[27] and therefore prompted us to produce a target-oriented library of piperidine compounds being able to complex the catalytic metal ion of the DOHH and being adequately water-soluble. The library (see Figure 1b) is composed of a variety of 4-oxopiperidine 3,5-dicarboxylates and 3-monocarboxylates carrying different substituents in the 2 and 4 positions and on the nitrogen, of spiropiperidine compounds, and of piperidine oximes and related ethers. The library was tested for their in vitro and in vivo activity against *Plasmodium falciparum* (NF-54), *Trypanosoma brucei brucei*, *Leishmania major*, and bacteria, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Some representative compounds with high activity in plasmodia or trypanosomatids were also subjected to the inhibition assay of HIV-1 replication. Finally, most active compounds were tested for their capacity to inhibit the growth of the macrophage cell line J774.1. By application of this strategy, we aimed to find lead structures for the development of small compounds of selective activity against one of the aforementioned microorganisms and low cytotoxicity.

Chemistry

The 4-oxopiperidine 3,5-dicarboxylates **1**–**18** (see Figure 2) were achieved via a double Mannich condensation of 1 mol of a corresponding primary amine, 2 mol of pyridine-2-carbaldehyde and 3- or 4-nitrophenylcarbaldehyde, and dimethyl oxoglutarate in analogy to Merz and Haller,[28] Ashauer-Holzgrabe and Haller,[29] and Kuhl et al.[30] Here, we tried to enhance the water solubility of the dicarboxylates by introduction of polar substituents attached to the nitrogens, e.g., carboxylate and hydroxyl residues, and by attachment of nitro groups to the aromatic ring in the 2 and 6 positions (Table 1a). In particular, the latter variation led to compounds of higher water solubility. Moreover, the diesters are pretty stable; they can be hydrolyzed and subsequently decarboxylated only under strongly acidic conditions (cf. compound **31** below).

The analogous monocarboxylates **19**–**24** (Table 1b) can be obtained by conversion of 2,4,6-trioxotetrahydropyrane, formed by solvolytic cleavage with methanol, with freshly destilled pyridine-2-carbaldehyde and the correspondingly substituted

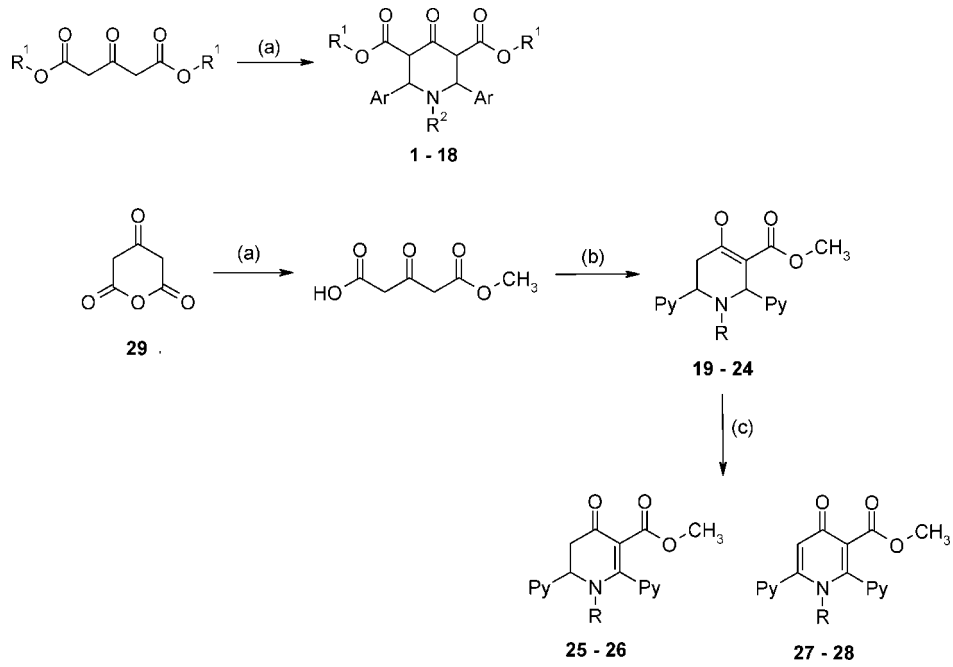

Figure 2. Synthesis pathway to 4-oxopiperidine 3,5-dicarboxylates **1**–**18** (reagents and conditions, (a) ArCHO, R^2NH_2, –5 °C; (b) $Ce(SO_4)_2$, 0–5 °C), monocarboxylates **19**–**24**, and dihydropyridine and tetrahydropyridine compounds **25**–**28** (reagents and conditions, (a) absolute MeOH, 25 °C; (b) 2 equiv of 2-pyridinealdehyde, RNH_2, –5 °C; (c) $Ce(SO_4)_2$, 0–5 °C).

primary amines (cf. ref 31). The monocarboxylates were always isolated in the enol form. The corresponding dihydropyridine and tetrahydropyridine compounds **25**−**28** (see Figure 2) can be achieved by means of oxidation using cerium(IV) sulfate.[28,29,31]

In order to obtain the spiropiperidines **33**–**41**, **44**, **45**, and **47** (see Figure 3), the corresponding 4-oxopiperidine 3,5-dicarboxylates **30a**−**d** have to be synthesized via a double Mannich condensation of ammonium bromide, 2 mol of the corresponding freshly destilled aliphatic aldehyde, and dimethyl oxoglutarate. The dicarboxylates were isolated as a mixture of keto–enol tautomers mostly occurring as trans isomers with regard to the alkyl groups. Ester hydrolysis and decarboxylation were performed with concentrated hydrochloric acid to give the 2,6-alkyl substituted and nonsubstituted 4-piperidones **31**, which are mainly isolated as cis isomers indicating a configurational change of the alkyl groups during the course of the reaction, which is in accordance with findings of Siener et al.[32] N-Benzylation can be achieved by means of benzyl bromide in acetonitrile in the presence of an excess of potassium carbonate, resulting in high yields of the piperidone **42**. Via a Strecker synthesis[33] the nitrile compounds **32a**−**f** and **43a**,**b** were synthesized in glacial acid using trimethylsilyl cyanide and using aniline and benzylamine as amine components. In order to avoid the hydrolysis of the amine, the reaction was stopped by addition of a concentrated ammonia solution. Only the cis isomer of all compounds was isolated because in the case of the trans isomer, the carbonyl group is difficult to attack because of one axial alkyl group. Conversion of the compounds **32a**−**f** and **43a**,**b** with chlorosulfonyl isocyanates and subsequent refluxing in 1 M hydrochloric acid lead directly to the spiro derivatives **33**–**36** and **44**. The nitrogen N3 in the imidazolidione skeleton can be selectively alkylated to give **37**–**41** and **45**. The spiro compound **47** was obtained via the amide **46** being formed by conversion with concentrated sulfuric acid and working-up with concentrated ammonia solution. Ring closure was achieved with formamide at 200 °C in analogy to Röver et al.[34] In contrast to Röver et al., the reduced compound **47** was forthwith isolated. Thus, the reduction step with $NaBH_4$ was dispensable.

Since oximes are known to complex iron ions, e.g., [2,2′]-furildioxime,[35] corresponding piperidone oximes were synthesized (see Figure 4). The N-alkylated oxime of the 4-piperidone **48** was synthesized starting from the piperidone, which can be alkylated with phenylpropyl bromide. Subsequently, the ketone can be converted to the oxime by using hydroxylamine hydrochloride. **48** was alkylated with dichlorobenzyl bromide to give the oxime ether **49**.

The synthesis of the corresponding 4-piperidinecarbaldehyde oximes started from pyridine-4-carbaldehyde whose aldehyde function was first protected by means of ethylene glycol. The resulting acetal was N-alkylated with phenylpropyl bromide in the microwave within 2 h to give compound **50**, which can be hydrogenated with PtO_2/H_2 to **51**. Acetal cleavage and subsequent oxime formation with hydroxylamine hydrochloride gave the piperidone oxime **52**, which was alkylated again with dichlorobenzyl chloride to yield the oxime ether **53**.

Results and Discussion

All piperidine compounds were subjected to the aforementioned microbiological assays. None of the compounds showed considerable activity against the bacteria *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and the fungi *C. albicans* (data not shown). Additionally, none of the compounds inhibited the formation of biofilm produced by *S. aureus* (data not shown). The inhibition activity against *P. falciparum*, *T. brucei brucei*, and *L. major* is summarized in Table 1 as well as the toxicity against macrophages after 48 h. Structure–activity relationships (SAR) will be qualitatively discussed by chemical group.

Whereas all piperidone dicarboxylates were not active against *L. major*, many of them show activity against *T. brucei brucei* and *P. falciparum*. For both parasites the activity (IC_{50} and ED_{50} values) is felt in the higher micromolar range of concentration if the piperidones are armed with pyridines rings in the 2 and 6 positions (**1**–**7**) and regardless of the substitution on the nitrogen. There are two exceptions, which are the compounds having a benzylic moiety (i.e., benzyl for **9** and pyridinylmethyl for **8**); both

Table 1. Antimicrobial Activity of All Piperidone Compounds against *P. falciparum* (NF-54), *T. brucei brucei*, and *L. major* and Cytotoxicity Measured in Macrophages (J774.1)[a]

(a) 4-Oxopiperidine 3,5-Dicarboxylates

					ED_{50} (μM)		
compd	Ar	R^1	R^2	IC_{50} (μM)*P. falciparum*	*T. brucei brucei*	*L. major*	macrophage after 48 h
1	2-pyridine	CH_3	H	na	28.09 ± 1.52	>100	>100
2	2-pyridine	CH_3	allyl	na	26.48 ± 7.64	>100	>100
3	2-pyridine	CH_3	2-hydroxyethyl	na	22.84 ± 5.57	>100	31.15 ± 0.14
4	2-pyridine	CH_3	3-hydroxypropyl	na	18.29 ± 2.59	>100	32.02 ± 0.63
5	2-pyridine	CH_3	2-(2-hydroxy-ethoxy)ethyl	na	24.14 ± 4.27	65.04 ± 3.60	38.48 ± 6.17
6	2-pyridine	CH_3	3-carboxypropyl	na	19.29 ± 2.28	>100	46.00 ± 5.92
7	2-pyridine	CH_3	7-carboxypentyl	na	23.98 ± 4.48	50.34 ± 8.26	33.83 ± 2.81
8	2-pyridine	CH_3	2-pyridinylmethyl	na	4.62 ± 1.39	>100	29.87 ± 2.32
9	2-pyridine	CH_3	benzyl	10.88 (72 h) 10.20 (24 h)	11.13 ± 1.91	55.99 ± 6.45	33.99 ± 1.56
10	4-NO_2phenyl	CH_3	allyl	na	2.41 ± 0.39	>100	50.29 ± 11.70
11	4-NO_2phenyl	C_2H_5	allyl	11.03 (72 h) + (m)	3.06 ± 0.55	nd	>100
12	4-NO_2phenyl	CH_3	benzyl	11.91 (72 h) + (m)	3.01 ± 0.27	>100	18.39 ± 2.93
13	4-NO_2phenyl	C_2H_5	benzyl	10.06 (72 h) + (m)	2.72 ± 0.51	nd	>100
14	3-NO_2phenyl	CH_3	4-chlorbenzyl	na	10.57 ± 2.83	>100	46.64 ± 9.86
15	3-NO_2phenyl	CH_3	4-methoxybenzyl	na	3.93 ± 1.10	>100	>100
16	3-NO_2phenyl	CH_3	4-methylbenzyl	na	2.66 ± 0.49	>100	49.78 ± 16.80
17	3-NO_2phenyl	CH_3	benzyl	na	2.49 ± 0.79	>100	>100
18	phenyl	CH_3	allyl	na	4.86 ± 0.63	nd	>100

(b) 4-Oxopiperidine 3-Monocarboxylates

			ED_{50} (μM)		
compd	R	IC_{50} (μM)*P. falciparum*	*T. brucei brucei*	*L. major*	macrophage after 48 h
19	benzyl	na	1.37 ± 0.02	25.97 ± 6.76	33.17 ± 0.69
20	4-methylbenzyl	nd	0.47 ± 0.17	83.83 ± 10.38	34.87 ± 0.45
21	4-chlorbenzyl	12.03 (72 h) 1.7 (48 h)	0.32 ± 0.09	87.93 ± 32.99	32.84 ± 0.49
22	4-methoxybenzyl	nd	0.43 ± 0.17	30	29.86 ± 0.33
23	3-methoxybenzyl	nd	>100	>100	>100
24	allyl	>40 1.4 (24 h)	1.08 ± 0.48	50	(35)
		4-Oxo-1,4,5,6-tetrahydropyridine 3-Monocarboxylates			
25	benzyl	4.7 (24 h)	>100	nd	nd
26	4-chlorbenzyl	18 (24 h)	nd	nd	nd
		4-Oxo-1,4-dihydropyridine 3-Monocarboxylates			
27	4-chlorbenzyl	9.4 (36 h)	>100	>100	>100
28	4-methylbenzyl	40 (48 h) 9.1 (24 h)	nd	nd	nd

(c) Spiropiperidines

					ED_{50} (μM)		
compd	R	R^1	R^2	IC_{50} (μM)*P. falciparum*	*T. brucei brucei*	*L. major*	macrophage after 48 h
33	CH_3	phenyl		na	>100	>100	>100
34	C_2H_5	benzyl		na	>100	>100	>100
35	C_3H_7	phenyl		na	>100	>100	36.02 ± 3.97
36	C_4H_9	phenyl		na	24.36 ± 14.10	>100	nd
37	CH_3	benzyl	2-hydroxyethyl	na	>100	>100	>100
38	C_2H_5	phenyl	benzyl	na	20.28 ± 3.55	nd	nd
39	CH_3	benzyl	4-nitrobenzyl	14.57 (72 h)	3.74 ± 0.77	>100	42.44 ± 14.49
40	C_2H_5	phenyl	2-phenylethyl	na	18.05 ± 5.34	41.10 ± 3.57	32.30 ± 2.52
41	C_2H_5	phenyl	3-phenylpropyl	na	13.44 ± 6.69	>100	>100
44				na	30.97 ± 2.18	>100	>100
45				na	25.37 ± 1.61	>100	>100
47				na	44.65 ± 15.50	nd	nd

(d) Oximes and Corresponding Ethers

		ED_{50} (μM)		
	IC_{50} (μM)*P. Falciparum*	*T. brucei brucei*	*L. major*	macrophage after 48 h
48	26.0	nd	nd	nd
49	31.0	>100	>100	>100
52	nd	>100	>100	>100
53	8.29 (72 h) 11.83 (24 h)	3.86 ± 1.95	30.72 ± 3.15	28.39 ± 5.92

[a] For comparison, pentamidine diisethionate ED_{50} (*T. brucei brucei*) = 0.0027 μM; suramine ED_{50} (*T. brucei brucei*) = 0.3 μM; eflornitine ED_{50} (*T. brucei brucei*) = 22.9 μM; mimosine IC_{50} (*P. falciparum* chloroquine-sensitive) = 32 μM, IC_{50} (*P. falciparum* chloroquine-resistent) = 39 μM, ED_{50} (*Trypanosoma brucei brucei*) > 100 μM; cyclopiroxolamine IC_{50} (*P. falciparum* chloroquine-sensitive) = 8.2 μM), ED_{50} (*T. brucei brucei*) = 0.62 ± 0.22 μM, ED_{50} (macrophages, 48 h) = 4.62 ± 0.97 μM; amphotericin B IC_{50} (*L. major*) = 5.07 ± 0.05 μM, IC_{50} (J774.1 macrophages) = 68.6 ± 16.48 μM. na = no activity. + (m) = activity in mice. nd = not determined.

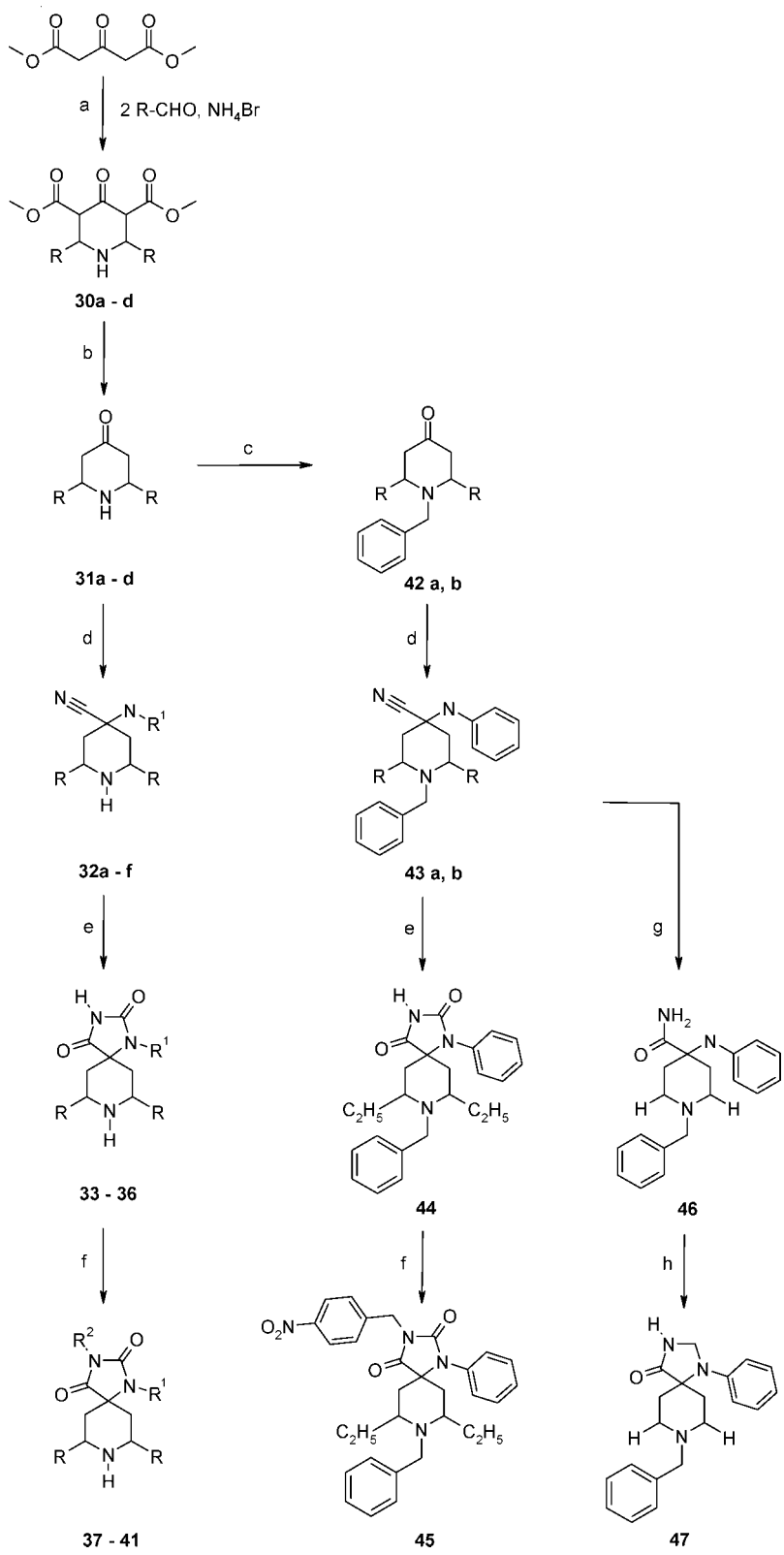

Figure 3. Synthesis pathway to the spiro compounds **33**–**41**, **44**, **45**, and **47**. Reagents and conditions: (a) MeOH, EtOH, MeOH/H_2O or THF, 0−25 °C; (b) 37% HCl, 70 °C; (c) RBr, K_2CO_3, acetonitrile, 60 °C; (d) glacial acetic acid, aniline or benzylamine, TMSCN, 25 °C; (e) (1) chlorosulfonyl isocyanate, absolute $CHCl_3$, 25 °C; (2) 1 M HCl, reflux, 90 °C; (f) R^2Br, K_2CO_3, acetonitrile, reflux, 70 °C; (g) (1) concentrated H_2SO_4, 25 °C; (2) concentrated NH_3, 0 °C; (h) formamide, 200 °C.

are active against *T. brucei brucei*, and **9** is additionally active against *P. falciparum*. The in vitro effect of **9** on *P. falciparum* chloroquine sensitive NF-54 strains results in the percentage of parasitemia to be close to 0 after day 1 posttreatment with 40 μM and after 2 day posttreatment with 20 μM (see Figure 5a). Additionally, the percentage of survival of C57BI/6 mice treated iv with **9** could be substantially prolonged (data not shown). However, the effective doses for parasites and for the toxicity against macrophages are close together, which makes **8** and **9** out of the question as new lead compounds.

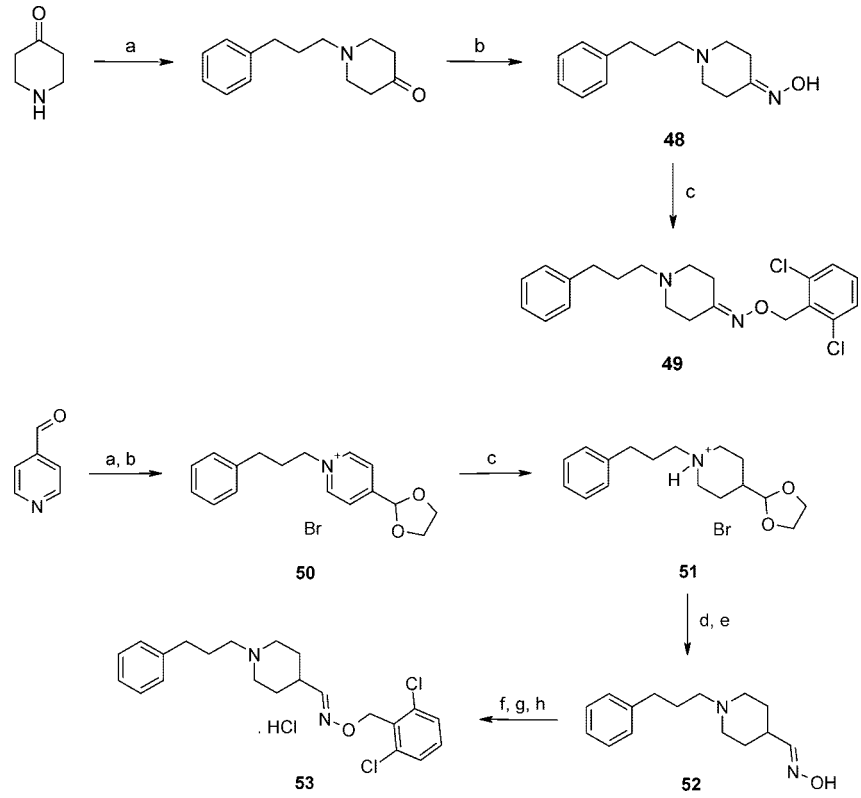

Figure 4. Synthesis pathway to oximes **48** and **52** and their corresponding ethers **49** and **53**. Reagents and conditions for **48** and **49**: (a) $Ph(CH_2)_3Br$, K_2CO_3, CH_3CN, 60 °C; (b) K_2CO_3, NH_2OH. HCl, absolute EtOH; (c) 2,6-dichlorobenzylchloride, KO^{t}Bu, 55 °C. Reagents and conditions for **52** and **53**: (a) ethylene glycol, pTosOH, toluene; (b) $Ph(CH_2)_3Br$, 2 h, 90 °C, microwave; (c) PtO_2, H_2, 60 °C, 20 bar, microwave; (d) H_2SO_4, microwave; (e) $NH_2OH-HCl$; (f) KOtBu; (g) 2,6-dichlorobenzyl chloride; (h) MeOH/HCl.

The replacement of the pyridine rings with 4-nitrophenyl and with some restriction with 3-nitrophenyl rings enhances the activity against *T. brucei brucei* and *P. falciparum* considerably in connection with low cytotoxicity against macrophages. In addition, these compounds are much more soluble in water and buffer than the pyridine substituted analogues. In particular, the diethyl dicarboxylates **11** and **13** rather than the corresponding dimethyl dicarboxylates **10** and **12** show activity against both microorganisms in the lower micromolar concentration range with concomitant very low cytotoxicity and can thus be regarded as lead compounds for further improvement. This is supported by the facts that the percentage of *P. falciparum* parasitemia of C57BL/6 mice 4 days after treatment was close to 0 in the case of **11** and the number of days of survival was increased (see parts b and c of Figure 5). The 3-nitrophenyl substituted compounds are only active against *T. brucei brucei* but exhibit a higher toxicity.

Since in a preliminary study the corresponding 3-monocarboxylates 4-piperidones **21** and **24** have shown high activity against the plasmodia after 48 h, compounds were synthesized having varying substituents attached to the nitrogen. The aforementioned compounds showed a weaker activity after 72 h and did not completely kill the plasmodia in vivo. All other monocarboxylates did not show any activity against plasmodia and demonstrated a low activity against leishmania. However, the monocarboxylates are among the compounds with the highest activity against *T. brucei brucei*. With exception of the N-3-methoxybenzyl substituted compound **23**, the trypanocidal activity was in the lower and submicromolar range of concentration, more or less independent from substitution of the benzyl substituent. Since the cytotoxicity in macrophages was found to be 100 times higher than the trypanocidal activity, these compounds can be regarded as leads for the development of trypanocidal drugs. Unfortunately, using the classical synthesis pathway, we were not yet able to produce the 2-and 6-(*p*-nitrophenyl) substituted analogues whose corresponding dicarboxylates showed higher activity and water solubility in comparison to the pyridine substituted ones. Thus, these compounds are being currently produced via an independent synthesis pathway.

Since mimosine and ciclopiroxolamine can be regarded as dihydropyridine derivatives, structurally corresponding compounds were considered in our library. The antiplasmodial activity of the dihydro- and tetrahydropyridine monocarboxylates **25**–**28** was previously reported[16] and was now found to be low for **28** after 48 h. The trypanocidal activity of all compounds **25**–**28** is rather low. Since these compounds are difficult to isolate from oxidation of the corresponding piperidone, we did not embark on this strategy. The lack of activity may be due to the fact that the β-ketocarboxylate is not enolizable because of double bonds next to this moiety. Thus, complexation of the DOHH via the metal ion is difficult.

A similar reason can be given for the missing antiplasmodial activity of all spiro compounds. There is only one exception, compound **39** having a *p*-nitrobenzyl substituent attached to the imidazolidione ring. The antiplasmodial activity is in the same range of concentration as that found for the most promising compound **11**. However, the therapeutic distance to cytotoxicity of the compound is small. In contrast, some of the spiro compounds show an interestingly high trypanocidal activity being in the same concentration range as eflornitine. In particular, a phenylalkyl substitution in position N3 of the imidazolidione ring seems to be advantageous. With increasing length of the alkyl spacer, the inhibitory activity increases (cf.

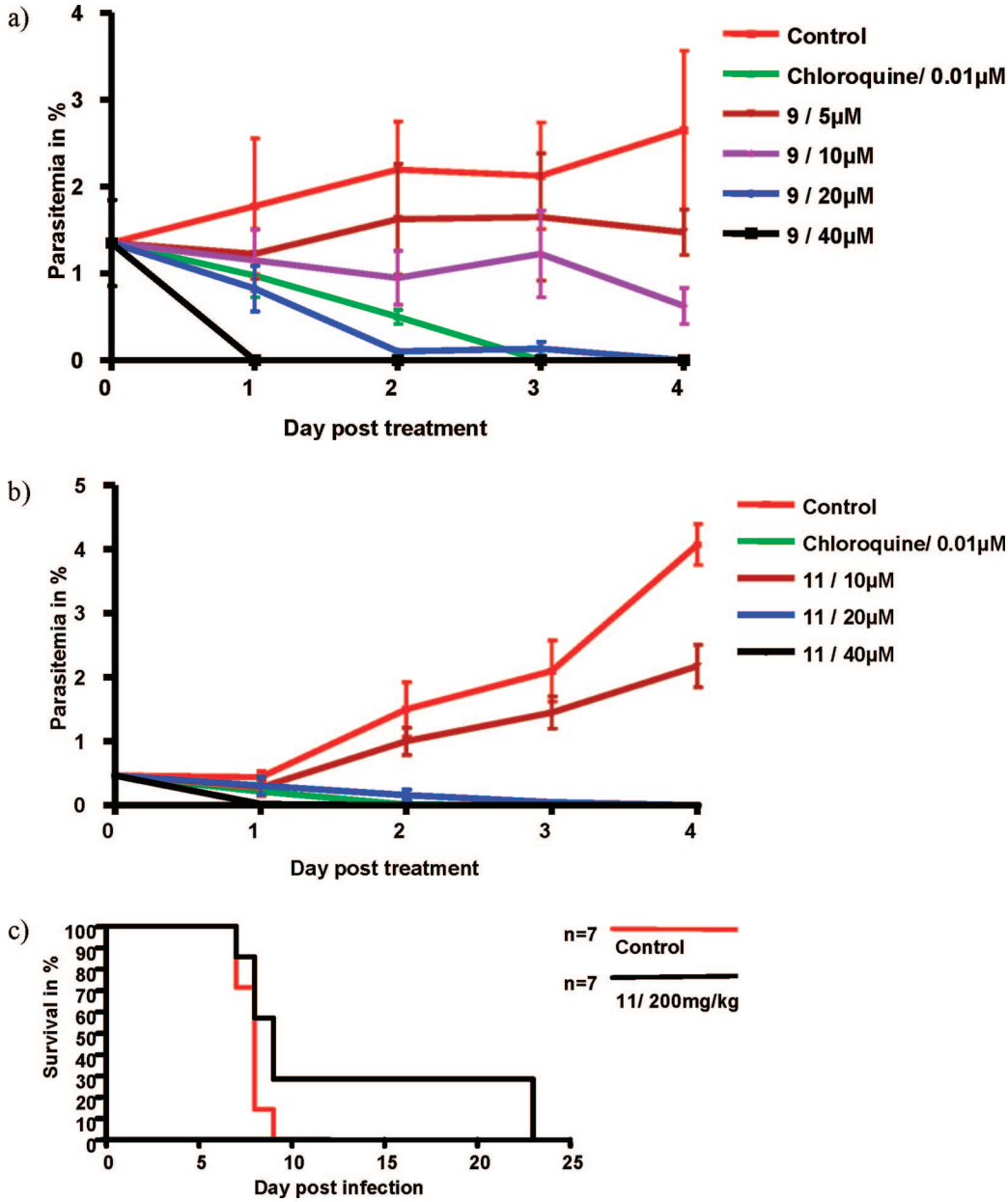

Figure 5. (a) In vitro parasitemia of **9** on Pf/NF-54 strains. (b) In vitro parasitemia of C57BL/6 mice treated iv with **11**. (c) Survival of C57BL/6 mice treated orally with **11**.

38, **40**, and **41**). The activity can be enhanced by a nitrobenzyl substitution resulting in **39**, a compound with the highest activity within this series and a therapeutic index of 10, which has to be improved. Further SARs are difficult to derive. Because of the multistep synthesis of these compounds and the fact that activity is often connected with cytotoxicity, the spiro compounds were discarded from the list of potential lead compounds even though some active compounds were found.

Because of the hypothesis that compounds complexing metal ions are able to inhibit the DOHH and to show antiplasmodial activity, oximes with piperidone moiety (**48** and **52**) were synthesized in addition to the corresponding dichlorobenzyl ethers (**49** and **53**). Interestingly, neither the oximes **48** and **52** nor the oxime ether **49** is active against trypanosomes and plasmodia, respectively. In contrast, the oxime ether of the piperidine aldehyde **53** is active against plasmodia, trypanosomes, and leishmania; however, the activity against leishmania is low. In addition, **53**, showing an IC_{50} of 8.3 μM after 72 h, is able to kill the plasmodia completely (see Figure 6). Thus,

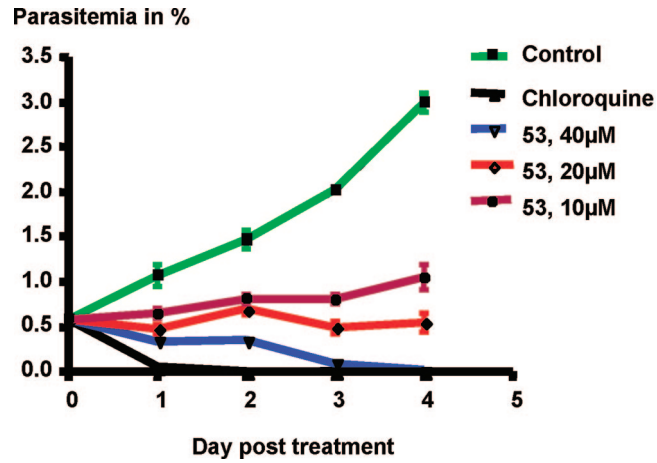

Figure 6. Parasitemia of C57BL/6 mice treated iv with **53**.

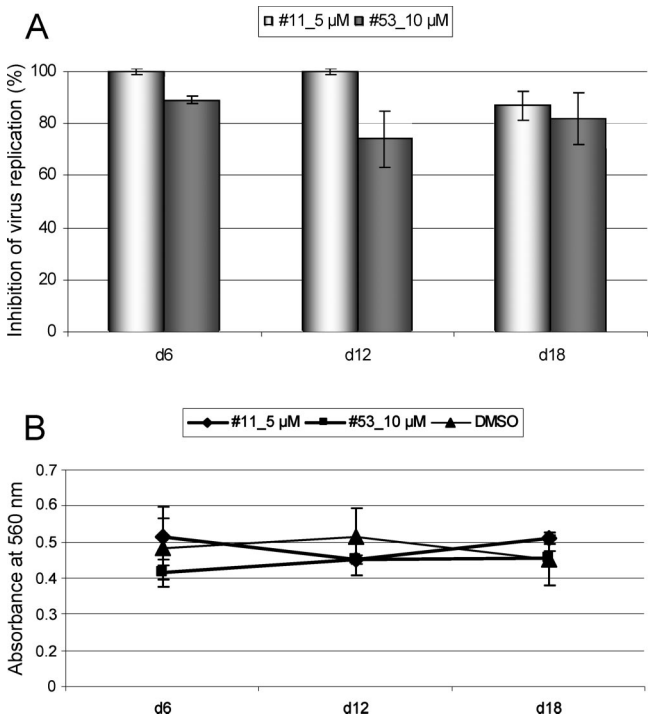

Figure 7. Inhibition of HIV-1 BaL replication by piperidones. HIV-1 BaL-infected PM1 cells were cultured in the presence of the indicated concentrations of **11**, **53**, or DMSO as solvent control. Culture medium was changed at days 6 and 12 postinfection, and the cells were split 1:1. $p24^{Gag}$ antigen levels and cell viabilities were determined at days 6, 12, and 18. (A) The percentage of inhibition of virus replication in the drug-treated cell culture, compared to the untreated controls, is shown. (B) Cell viabilities from the same cultures were determined by alamarBlue assay (Serotec).

53 is the most active compound against plasmodia in the entire library, but it is also cytotoxic, having a therapeutic index of about 10. However, **53** will be a new lead compound for further development. Since **53** is not able to form a complex with metal ions, the mode of action has to be elucidated in the future.

Since the host cell eIF-5A, a cofactor of the HIV-1 protein Rev, is involved in the virus replication, the inhibition of DOHH and DHS will reduce the multiplication rate of the virus. Consequently, the most active compounds **11**–**13** and **53** were tested against the R5-tropic HIV-1 strain BaL and/or against the X4-tropic HIV-1 strain NL4/3 in the human T cell line PM1. In contrast to the compounds **12** and **13**, where only 20% inhibition of virus replication was observed, the compound **11** was able to completely inhibit replication of HIV-1 BaL at 5 μM and HIV-1 NL4/3 at 6 μM compared to the respective control cultures (which were treated with the drug solvent DMSO). When HIV-1 BaL infected PM1 cells were exposed to a concentration of 10 μM **53**, virus replication was inhibited by 85% (Figure 7). Finally, the parallel analysis of cellular metabolic activities revealed that these antiviral effects were not caused by deleterious effects of the respective drugs on the host cell.

Conclusion

The evaluation of the library revealed several promising lead compounds for further drug development, i.e., the 3,5-diethylpiperidone 3,5-dicarboxylates **11** and **13** for drugs against *T. brucei brucei* and *P. falciparum,* and the corresponding monocarboxylates against the *T. brucei brucei* and the dichlorobenzyl ether of the piperidine oxime **53** antiproliferative activity against *P. falciparum*. Moreover, the compounds **11** and **53** appear to be promising leads for anti-HIV-1 drug development. Besides the good antiifective activity, the main advantage of these compounds is their simple synthesis route. Most of the compounds were satisfyingly water-soluble for biological evaluation. In the next step, it is necessary not only to increase the antimicrobial activity but also to reduce the cytotoxicity, especially of compound **53**, by variation of the substitution pattern.

However, the small molecules were only tested against entire organisms so far. Thus, the mechanism of action is not fully understood. Whereas the piperidone 3,5-dicarboxylate **11** is in principle able to complex the iron ion via the enolizable β-ketoester moiety, which is also typical for many HIV integrase inhibitors,[36] or the terpyridine skeleton, the piperidine oximether **53** cannot chelate an iron. Since the pK_A value of the piperidine nitrogen was determined to be approximately 7,[37] **53** will be partially protonated at physiological conditions and therefore able to interact with the negatively charged amino acids in the active site of the DOHH.[38] Taken together, it has to be analyzed whether the growth inhibition of the microorganisms and inhibition of the replication of HIV-1 were caused by the interference with the polyamine pathway, especially the block of the DOHH. Respective work, e.g., the cloning of the DOHH for assay development, is in progress.

Experimental Section

Material and Methods. Melting points were determined on a model B 540 Büchi or a Sanyo Gallenkamp melting point apparatus (Sanyo Gallenkamp, U.K.) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker AV 400 spectrometer (^{1}H, 400.132 MHz; ^{13}C, 100.613 MHz). Chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. Abbreviations for data quoted are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The centers of the peaks of $CDCl_3$ and DMSO-d_6 were used as an internal reference. FT-IR spectra were recorded on a Bio-Rad PharmalyzIR equipped with an ATR unit. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microanalysis results (C, H, N) of the new compounds agreed with the theoretical values within $\pm$0.4%.

The dimethyl-4-oxopiperidine 3,5-dicarboxylates **1** and **2** were prepared according to ref 30, and the methyl 4-oxopiperidine 3-monocarboxylates **20**, **21**, and **24** and the dihydro- and tetrahydropyridine compounds **25**−**28** were synthesized according to ref 16.

Synthesis. General Procedure for the Synthesis of the 3,5-Dialkyl-2,6-di-2-aryl-4-oxopiperidine 3,5-Dicarboxylates 1–18 and 30a−d, Modified after Reference 39. The corresponding amine (0.02 mol), pyridine-2-carboxaldehyde (0.04 mol), and *m*- and *p*-nitrobenzaldehyde were dissolved in methanol (20 mL) and cooled to 0 °C. Over the course of about 1 h, dimethyl oxoglutarate and diethyl oxoglutarate (0.02 mol) were added dropwise. The solution was allowed to stand overnight at 5 °C. The product was obtained by filtration of the precipitate formed. In the case where no precipitate appeared, the solvent was removed in vacuo at 40−50 °C and the remaining oil dissolved in methanol/diethyl ether or treated with diethyl ether. The obtained crystals could be washed with a mixture of methanol/diethyl ether and recrystallized from methanol. The piperidones could be isolated in yields ranging from 28% to 81%.

Dimethyl (2*R*,6*S*)-4-oxo-2,6-di(pyridin-2-yl)piperidine-3,5-dicarboxylate (1): yield 33% (67%); mp 175 °C (170–171 °C).[29]

Dimethyl (2*R*,6*S*)-1-allyl-4-oxo-2,6-di(pyridin-2-yl)piperidine-3,5-dicarboxylate (2): yield 87%; mp 133 °C (134 °C).[29]

Dimethyl (2*R*,6*S*)-1-(2-hydroxyethyl)-4-oxo-2,6-di(pyridin-2-yl)piperidine-3,5-dicarboxylate (3): yield 55%; mp 135 °C. Anal. ($C_{21}H_{23}N_3O_6$) C, H, N.

Dimethyl (2*R*,6*S*)-1-(2-hydroxypropyl)-4-oxo-2,6-di(pyridin-2-yl)piperidine 3,5-dicarboxylate (4): yield 39%; keto−enol ratio, 1:3; mp 136 °C. Anal. ($C_{22}H_{25}N_3O_6$) C, H, N.

Dimethyl (2*R*,6*S*)-1-[2-(2-hydroxyethoxy)ethyl]-4-oxo-2,6-di(pyridin-2-yl)piperidine-3,5-dicarboxylate (5): yield 63%; mp 175 °C. Anal. ($C_{23}H_{27}N_3O_7$) C, H, N.

4-[(2*R*,6*S*)-3,5-bis(methoxycarbonyl)-4-oxo-2,6-di(pyridin-2-yl)piperidin-1-yl]butanoic acid (6): yield 70%; mp 169 °C. Anal. ($C_{23}H_{25}N_3O_7$) C, H, N.

8-[(2*R*,6*S*)-3,5-bis(methoxycarbonyl)-4-oxo-2,6-di(pyridin-2-yl)piperidin-1-yl]octanoic acid (7): yield 43%; mp 130 °C. Anal. ($C_{25}H_{29}N_3O_7$) C, H, N.

Dimethyl (2*R*,6*S*)-4-oxo-2,6-di(pyridine-2-yl)-1-(pyridine-2-ylmethyl)-piperidine-3,5-dicarboxylate (8): yield 47%; mp 152–153 °C. Anal. ($C_{25}H_{24}N_4O_5$) C, H, N.

Dimethyl (2*R*,6*S*)-1-benzyl-4-oxo-2,6-di(pyridin-2-yl)piperidine-3,5-dicarboxylate (9): yield 65%; mp 154 °C. Anal. ($C_{26}H_{25}N_3O_5$) C, H, N.

Dimethyl (2*R*,6*S*)-1-allyl-4-hydroxy-2,6-bis(4-nitrophenyl)-1,2,3,6-tetrahydropyridine-3,5-dicarboxylate (10): yield 39%; mp 154–155 °C. Anal. ($C_{24}H_{23}N_3O_9$) C, H, N.

Diethyl (2*R*,6*S*)-1-allyl-4-hydroxy-2,6-bis(4-nitrophenyl)-1,2,3,6-tetrahydropyridine-3,5-dicarboxylate (11): yield 28%; mp 150–151 °C. Anal. ($C_{26}H_{27}N_3O_9$) C, H, N.

Dimethyl (2*R*,6*S*)-1-benzyl-4-hydroxy-2,6-bis(4-nitrophenyl)-1,2,3,6-tetrahydropyridine-3,5-dicarboxylate (12): yield 12%; mp 163–171 °C. Anal. ($C_{28}H_{25}N_3O_9$) C, H, N.

Diethyl (2*R*,6*S*)-1-benzyl-4-hydroxy-2,6-bis(4-nitrophenyl)-1,2,3,6-tetrahydropyridine-3,5-dicarboxylate (13): yield 7%; mp 181–184 °C. Anal. ($C_{30}H_{29}N_3O_9$) C, H, N.

Dimethyl (2*R*,6*S*)-1-(4-chlorobenzyl)-2,6-bis(3-nitrophenyl)-4-oxopiperidine-3,5-dicarboxylate (14): yield 41%; mp 144–146 °C. Anal. ($C_{28}H_{24}N_3O_9Cl$) C, H, N.

Dimethyl (2*R*,6*S*)-1-(4-methoxybenzyl)-2,6-bis(3-nitrophenyl)-4-oxopiperidine-3,5-dicarboxylate (15): yield 29%; mp 159–161 °C. Anal. ($C_{29}H_{27}N_3O_{10}$) C, H, N.

Dimethyl (2*R*,6*S*)-1-(4-methylbenzyl)-2,6-bis(3-nitrophenyl)-4-oxopiperidine 3,5-dicarboxylate (16): yield 41%; mp 170–172 °C. Anal. ($C_{29}H_{27}N_3O_9$) C, H, N.

Dimethyl (2*R*,6*S*)-1-benzyl-2,6-bis(3-nitrophenyl)-4-oxopiperidine-3,5-dicarboxylate (17): yield 15%; mp 144–146 °C. Anal. ($C_{28}H_{25}N_3O_9$) C, H, N.

Dimethyl (2*R*,6*S*)-1-allyl-4-oxo-2,6-diphenylpiperidine-3,5-dicarboxylate (18): yield 81%; keto–enol ratio, 4:3; mp 142 °C. Anal. ($C_{24}H_{25}NO_5$) C, H, N.

General Procedure for the Synthesis of the 3-Methyl 2,6-Di(pyridine-2-yl)-4-piperidone-3-monocarboxylates 19–24, Modified after References 40 and 41. 2,4,6-Trioxotetrahydropyrane (20 mmol) were stirred in methanol (30 mL) till dissolution and afterward cooled to −20 °C. The corresponding amine (20 mmol) and pyridine-2-carboxaldehyde (40 mmol), each dissolved in methanol (20 mL), were added to the solution in parallel such that the reaction temperature did not exceed −20 °C. After the mixture was stirred for 2 h at −10 °C, the solvent was removed in vacuo at room temperature. The obtained residue was covered with a small amount of methanol, and the product crystallized at 4 °C. The analytical and spectroscopic data of **19**–**24** are in accordance with refs 40–43.

Methyl (2′*R*,6′*S*)-1′-benzyl-4′-hydroxy-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (19): yield 33% (53%);[41] mp 155 °C (163–164 °C).[43]

Methyl (2′*R*,6′*S*)-4′-hydroxy-1′-(4-methylbenzyl)-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (20): yield 72% (41%);[41] mp 145 °C (144 °C).[43]

Methyl (2′*R*,6′*S*)-1′-(4-chlorobenzyl)-4′-hydroxy-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (21): yield 68% (49%);[34] mp 141 °C (142 °C).[43]

Methyl (2′*R*,6′*S*)-4′-hydroxy-1′-(4-methoxybenzyl)-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (22): yield 43% (46%);[34] mp 140 °C (140 °C).[43]

Methyl (2′*R*,6′*S*)-4′-hydroxy-1′-(3-methoxybenzyl)-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (23): yield 56% (63%);[34] mp 143 °C (144 °C).[43]

Methyl (2′*R*,6′*S*)-1′-allyl-4′-hydroxy-1′,2′,5′,6′-tetrahydro-2,2′;6′,2″-terpyridine-3′-carboxylate (24): yield 44%; mp 158 °C.[43]

Dimethyl (2*R*,6*S*)-4,4-dihydroxy-2,6-dimethyl-piperidinium-3,5-dicarboxylate hydrobromide (30a): yield 62%; mp 178–182 °C.

Dimethyl (2*RS*,6*RS*)-4-hydroxy-2,6-diethylpiperidine-3,5-dicarboxylate (30b): yield 97% (oil); keto–enol ratio, 1:2.

Dimethyl (2*RS*,6*RS*)-4-hydroxy-2,6-dipropylpiperidinium-3,5-dicarboxylate hydrobromide (30c): yield 50%; mp 142–143 °C.

Dimethyl (2*RS*,6*RS*)-4-hydroxy-2,6-dibutyl-3,5-piperidinium-dicarboxylate hydrobromide (30d): yield 24%; mp 144–146 °C.

(2*R*,6*S*)-2,6-Dimethyl-4-oxopiperidinium chloride (31a): yield 95%; mp 227–229 °C.

(2*R*,6*S*)/(2*RS*,6*RS*)-2,6-Diethyl-4-oxopiperidinium chloride (31b): yield 93%; cis/trans ratio, 5:1; mp 181–183 °C.

(2*R*,6*S*)/(2*RS*,6*RS*)-2,6-Dipropyl-4-oxopiperidinium chloride (31c): yield 92%; cis-/trans ratio, 4:1; mp 194 °C.

(2*R*,6*S*)/(2*RS*,6*RS*)-2,6-Dibutyl-4-oxopiperidinium chloride (31d): yield 90%; cis/trans ratio, 2:1; mp 212 °C.

General Procedure for the Synthesis of the Carbonitrile Compounds 32a−f and 43. To the corresponding piperidone (25 mmol) dissolved in glacial acid (80 mL) the corresponding amine (25 mmol) and trimethylsilyl cyanide (3.1 mL, 25 mmol) were added. The solution was stirred for 12 h at 25 °C. After completion of the reaction, the solution was alkalized with concentrated ammonia (pH 11), keeping the temperature under −5 °C. The solution was extracted 3 times with chloroform (100 mL). The organic layer was dried over sodium sulfate, and the solvent was removed in vacuo. The obtained residue was crystallized with diethyl ether or *tert*-butyl ethyl ether (**32a**,**c**−**e**), or a column chromatography with ethyl acetate on basic aluminum oxide was performed (R_f between 0.5 and 0.9, **32f**). **32b** was forwarded to the next reaction as an oil.

(2*R*,6*S*)-2,6-Dimethyl-4-phenylaminopiperidine-4-carbonitrile (32a): yield 71%; mp 126–128 °C.

(2*R*,6*S*)-4-Benzylamino-2,6-dimethylpiperidine-4-carbonitrile (32b): yield 68% (oil); ratio of rotamers, 4:1; mp 126–128 °C.

(2*R*,6*S*)-2,6-Diethyl-4-phenylaminopiperidine-4-carbonitrile (32c): yield 52%; mp 142–143 °C.

(2*R*,6*S*)-4-Benzylamino-2,6-diethylpiperidine-4-carbonitrile (32d): yield 47%; ratio of rotamers, 5:1; mp 109–111 °C.

(2*R*,6*S*)-4-Phenylamino-2,6-dipropylpiperidine-4-carbonitrile (32e): yield 27%; mp 94 °C.

(2*R*,6*S*)-2,6-Dibutyl-4-phenylaminopiperidine-4-carbonitrile (32f): yield 21%; mp 79 °C.

General Procedure for the Synthesis of the Spiro Compounds 33–36 and 44. The corresponding carbonitrile compounds (2.5 mmol) were suspended in nonaqueous chloroform (20 mL). Chlorosulfonyl isocyanate (0.22 mL, 2.5 mmol) was added to achieve **33**–**36**, and chlorosulfonyl isocyanate (0.44 mL, 5 mmol) was added to obtain **44**. The solution was stirred for 2 h at room temperature and afterward evaporated to dryness. To the residue hydrochloric acid (50 mL, 1 M) was added, and the obtained solution was refluxed for 2 h. After neutralization with NaOH (5 M) under intensive cooling, the product crystallized directly or the pH had to be adjusted to pH 9–10 and the solution extracted 5 times with dichloromethane (50 mL). The combined organic phases were dried over sodium sulfate. The solvent was reduced in vacuo, and the obtained crystals were collected and washed with diethyl ether or ethyl acetate. If necessary, column chromatography on silica gel was performed with chloroform/methanol = 25/1, R_f = 0.6–0.9.

(2*R*,6*S*)-7,9-Dimethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (33): yield 52%; mp 256 °C. Anal. ($C_{15}H_{19}N_3O_2$) C, H, N.

(2*R*,6*S*)-1-Benzyl-7,9-diethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (34): yield 84%; ratio of rotamers, 5:1; mp 284–286 °C. Anal. ($C_{18}H_{25}N_3O_2$) C, H, N.

(2*R*,6*S*)-1-Phenyl-7,9-dipropyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (35): yield 66%; mp 216–218 °C. Anal. ($C_{19}H_{27}N_3O_2$) C, H, N.

2*R*,6*S*)-7,9-Dibutyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (36): yield 42%; mp 305 °C. Anal. ($C_{21}H_{31}N_3O_2$) C, H, N.

(2*R*,6*S*)-8-Benzyl-7,9-diethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (44): yield 59%; mp 294–295 °C. Anal. ($C_{24}H_{29}N_3O_2$) C, H, N.

General Procedure for the Alkylation in Position 3 of the Spiro Compounds 37–41 and 45. Compounds **33**–**36** and **44** (1.75 mmol) were each dissolved in acetonitrile (20 mL), and potassium carbonate (0.62 g, 4.5 mmol) and the corresponding alkyl or aromatic bromide (2 mmol) were added. The solution was refluxed for 16 h and then cooled to 25 °C and filtered. The solvent was partially removed in vacuo and the precipitate collected and washed with diethyl ether or ethyl acetate.

(2*R*,6*S*)-1-Benzyl-3-(2-hydroxyethyl)-7,9-dimethyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (37): yield 48%; mp 195–196 °C. Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

(2*R*,6*S*)-3-Benzyl-7,9-diethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (38): yield 55%; ratio of rotamers, 6:1; mp 74–75 °C. Anal. ($C_{24}H_{29}N_3O_2$) C, H, N.

(2*R*,6*S*)-1-Benzyl-7,9-dimethyl-3-(4-nitrobenzyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (39): yield 71%; mp 161–162 °C. Anal. ($C_{23}H_{26}N_4O_4$) C, H, N.

(2*R*,6*S*)-7,9-Diethyl-3-phenethyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (40): yield 62%; mp 146–147 °C. Anal. ($C_{25}H_{31}N_3O_2$) C, H, N.

(2*R*,6*S*)-7,9-Diethyl-1-phenyl-3-(3-phenylpropyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione (41): yield 63%; mp 118–120 °C. Anal. ($C_{26}H_{33}N_3O_2$) C, H, N.

1-Benzyl-4-piperidone (42a). 42a is commercially available but was synthesized from 4-oxopiperidone−HCl. 4-Oxopiperidine−HCl (20 mmol) and K_2CO_3(6.92 g, 50 mmol) were dissolved in acetonitrile (50 mL), and benzyl bromide (1.8 mL, 24 mmol) was added. After being heated for 16 h, the solution was cooled to 25 °C and filtered. To isolate the product, the remaining solid was suspended in acetonitrile and filtered again. The filtrates were combined and the solvent was evaporated in vacuo to obtain the oily product that can be used for the following synthetic steps without further purification. Yield 86%.

(2*R*,6*S*)/(2*RS*,6*RS*)-1-Benzyl-2,6-diethyl-4-piperidone (42b): yield 91% (oil); cis/trans ratio, 2:1.

1-Benzyl-4-phenylaminopiperidine-4-carbonitrile (43a): yield 62%; mp 146–148 °C. For spectroscopic data, see ref 44.

(2*R*,6*S*)-1-Benzyl-2,6-diethyl-4-phenylaminopiperidine-4-carbonitrile (43b): yield 59%; mp 123–125 °C.

(2*R*,6*S*)-8-Benzyl-7,9-diethyl-3-(4-nitrobenzyl)-1-phenyl-1,3,8-triazaspiro[4.5]decane-2,4-dione (45): yield 46%; mp 185–187 °C. Anal. ($C_{31}H_{34}N_4O_4$) C, H, N.

1-Benzyl-4-phenylaminopiperidine-4-carboxylic Acid Amide (46). The corresponding carbonitrile compound **32** (7.5 mmol) was stirred in concentrated sulfuric acid (60 mL) for about 12 h at room temperature. During neutralization of the solution with concentrated ammonia, the temperature is not allowed to exceed 0 °C. The resulting precipitate was collected, efficiently washed with water, and dried in vacuo: $C_{19}H_{23}N_3O$ (309.4 g/mol), yield 96%; mp 188–189 °C. For spectroscopic data, see ref 45.

8-Benzyl-1-phenyl-1,3,8-triazaspiro[4.5]decane-4-one (47). 46 (10 mmol) was dissolved in formamide (20 mL) and refluxed for 2 h. The solution was allowed to cool to room temperature and was then poured into ice–water. The aqueous solution was extracted 3 times with chloroform, and the organic layer was dried over sodium sulfate. After evaporation of the solvent, **47** was crystallized with acetone/methanol. Yield 68%; mp 234–237 °C. Anal. ($C_{20}H_{23}N_3O$) C, H, N.

1-(3-Phenylpropyl)-4-piperidine Oxime (48). 4-Oxopiperidine monohydrate hydrochloride (13.60 g, 100.0 mmol), 1-bromo-3-phenylpropane (19.90 g, 100.0 mmol), and potassium carbonate (69.10 g, 500.0 mmol) were mixed in acetonitrile (400 mL) and stirred at 60 °C for 18 h. The solid was filtered off and washed with acetonitrile, and the solvent was evaporated. The residue was diluted with dichloromethane (100 mL) and saturated sodium hydrogen carbonate solution (50 mL), and the layers were separated. The aqueous layer was extracted twice with dichloromethane (50 mL), and the combined organic layers were dried and evaporated. The pale-yellow solid was used for the next step without further purification.

Potassium carbonate (10.84 g, 156.0 mmol) and hydroxylamine hydrochloride (21.60 g, 156.0 mmol) were dissolved with absolute ethanol (200 mL). A solution of 1-(3-phenylpropyl)-4-piperidinone (16.95 g, 78.0 mmol) in absolute ethanol (100 mL) was added, and the mixture was refluxed for 1 h. The mixture was allowed to cool to 25 °C, filtered, and washed with ethanol. The solvent was evaporated, and a yellow solid was achieved. Yield 13.84 (59%); mp 182.5 °C. Anal. ($C_{14}H_{20}N_2O$) C, H, N.

1-(2-Phenylpropyl)-4-oxopiperidine *O*-(2,6-Dichlorobenzyl)oxime (49). 2,6-Dichlorobenzyl chloride (1.22 g, 6.25 mmol), 1-(3-phenylpropyl)piperidin-4-oxime (1.20 g, 5.0 mmol), and potassium *tert*-butoxide (0.71 g, 6.25 mmol) were dissolved in dry THF (100 mL) and stirred for 24 h at 55 °C in the dark under Ar. The solvent was evaporated, and the residue was diluted with dichloromethane (100 mL) and NaOH (50 mL, 2.0 M). The layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 80 mL). The combined organic layers were dried and evaporated to achieve a colorless solid. Yield 0.68 g (28%); mp 147 °C. Anal. ($C_{21}H_{24}N_2OCl_2$) C, H, N.

4-(1,3-Dioxolan-2-yl)-*N*-(3-phenylpropyl)pyridinium Bromide (50). 4-Pyridinecarbaldehyde (1.93 g, 18.0 mmol), ethylene glycol (2.26 g, 36.0 mmol), and toluene-4-sulfonic acid (3.80 g, 20.0 mmol) were dissolved in toluene (100 mL) and refluxed for 4 h to remove the water from the reaction mixture. The solution was allowed to cool to 25 °C, and the solvent was evaporated. The residue was diluted with NaOH (50 mL, 2.0 M) and dichloromethane (50 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and evaporated to achieve 4-(1,3-dioxolane-2-yl)pyridine as a colorless oil, which was used in the next step without further purification. This intermediate (2.07 g, 13.6 mmol) and bromo-3-phenylpropane (4.06 g, 20.4 mmol) were dissolved in toluene (20 mL). The solution was sealed in a quartz glass vessel equipped with a Weflon plate (diameter 1.7 cm, thickness 0.3 cm) and was heated in the microwave oven at 110 °C for 2 h (5 min for 25–110 °C). After that time the solvent was evaporated, the oily residue was dissolved in acetone (20 mL), and diethyl ether was added until the solution became turbid. The solution was cooled to −20 °C for 24 h. The crystals were filtered off, washed with diethyl ether, and dried. Yield, 1.78 g (37%) of colorless crystals; mp 78.1 °C.

(1,3-Dioxolane-2-yl)-*N*-(3-phenylpropyl)piperidine Hydrobromide (51). 4-(1,3-Dioxolane-2-yl)-1-(3-phenylpropyl)pyridinium bromide **50** (1.84 g, 8.18 mmol) and PtO_2 (40 mg) were dissolved in methanol (100 mL) and hydrogenated in a microwave hydrogenation reactor at 60 °C and 20 bar for 1.5 h. The catalyst was filtered off, and the solvent was evaporated. The residue was dissolved in acetone and crystallized with diethyl ether at 4 °C to give a colorless solid. Yield 1.84 g (98%); mp 132 °C (dec).

1-[1-(3-Phenylpropyl)piperidine]-4-carbaldehyde Oxime (52). Acetal **51** (10.0 mmol, 3.55 g) was dissolved in water (30 mL), and sulfuric acid (0.2 mL, 98%) was added. The mixture was heated in a microwave within 4 min to 110 °C. The temperature was held for 30 min, and the solution was allowed to cool to 25 °C. Na_2HPO_4 (30 mL, 1.0 M) and hydroxylamine hydrochloride

(6.65 g, 100.0 mmol) were added, and the sodium hydroxide solution was added until pH 8.5 was achieved. The mixture was stirred at 25 °C for 18 h, and the solution was alkalized with potassium carbonate and extracted with dichloromethane (3 × 100 mL). The combined organic layers were dried, filtered, and evaporated. The oily residue was dissolved in diethyl ether and filtered, and the filtrate was evaporated. Recrystallization from cyclohexane gave a colorless solid. Yield 1.90 g (55%); mp 111 °C. Anal. ($C_{15}H_{22}N_2O$) C, H, N.

4-[(2,6-Dichlorobenzyloxyimino)methyl]-1-(3-phenylpropyl)piperidinium Hydrochloride (53). The oxime **52** (0.7 g, 2.84 mmol) was dissolved in absolute MeOH (40 mL), and potassium *tert*-butoxide was added. The solution was stirred at 25 °C for 3 h, and the solvent was removed in vacuo.The residue was suspended in dry acetonitrile (50 mL), and 2,6-dichlorobenzyl chloride (0.56 g, 2.84 mmol) was added. The solution was refluxed for 2 h, and the solvent was removed in vacuo. Column chromatography (silica gel, eluent 1:1 EtAc/EtOH + 0.7% triethylamine) gave a colorless oil. This oil was dissolved in diethyl ether (10 mL), and 1.0 M hydrochloric acid (1 mL) in methanol was added. After 12 h at 4 °C, a colorless solid was isolated. Yield 0.51 g (40%); mp 198 °C (dec). Anal. ($C_{22}H_{27}Cl_3N_2O$) C, H, N.

Plasmodium Assays. Culturing of *P. falciparum* Chloroquine Sensitive NF54 and Chloroquine Resistant R Strains. *P. falciparum* isolate NF54 and R strain were maintained in small Petri dishes (5 cm) in a gaseous phase of 90% N_2, 5% CO_2, and 5% O_2, according to the protocol from refs 46 and 47. Parasites were cultured in human erythrocytes (blood group A^+) in RPM1640 medium (Sigma) supplemented with HEPES (25 mM), sodium hydrogen carbonate (20 mM), and 10% heat inactivated human A^+ plasma at hematocrit (10% v/v). The parasitemia of infected erythrocytes was determined by light microscopy and estimated by Giemsa stained smears. Parasitemias detected in the cultures were scored visually with a 100-fold oil immersion objective, counting at least 1000 infected erythrocytes to determine the parasitemia.

Inhibitor Experiments by Monitoring Multiplication and Growth of Plasmodia. Cultures were adjusted to a parasitemia of 1.5%. Aliquots were diluted 1:10-fold in RPMI medium, dispensed into 12-well microculture trays, and incubated at 37 °C in a candle jar. Thereafter, the growth medium was changed once a day for 4 days and the total inhibitor treatment lasted 96 h. Inhibitors were used in concentrations of 1−10 μM and dissolved in either DSMO or RPMI medium before they were added to the media. Parasitemias and stage distribution were estimated as triplicates daily from Giemsa stained smears by counting 1000 erythrocytes. Control experiments were performed with chloroquine (10^{-8} M) and the appropriate *P. falciparum* strain without drug treatment.

Evaluation of Inhibitor Experiments by Determination of the IC_{50} Values. Data obtained from the inhibitor-dependent concentration growth curve of 96 h were plotted as percent inhibition vs inhibitor concentration (μM) and analyzed with linear regression according to ref 48.

Infection with *Plasmodium berghei.* The *P. berghei* ANKA strain was used in all experiments. *P. berghei* ANKA was maintained by periodic passages through the mosquito vector *Anopheles stephensi*. *A. stephensi* mosquitoes were bred at the animal facility of the Institute for Medical Microbiology, Immunoloy and Parasitology, University of Bonn. Stock blood-stage parasites stored in liquid nitrogen were used to infect three mice. Parasites were passaged to three new BALB/c mice, and 2 days later mosquitoes were allowed to a blood meal on these mice. Twenty-one days after the mosquito infection, salivary glands of the mosquitoes were dissected to isolate the sporozoites. A dose of 500 sporozoites in of phosphate buffer saline (200 μL) (PBS) was administered intravenously to two new naive mice, and parasitized erythrocytes were taken from these mice at a parasitemia of 20% to generate frozen aliquots. For infection with parasitized erythrocytes, mice were infected with the identical frozen aliquots of parasite. The parasitized blood was diluted in PBS and injected ip in concentrations of 50 000 *P. berghei* infected erythrocytes per mice. The percentage of the parasitemia was calculated by examining Giemsa stained smears under a microscope with an oil immersion lens (1000×).

Treatment of Mice. The treatment of *P. berghei* infected BALB/c mice was started with the 4-piperidone compounds ip when parasitemia in the mice had reached 5%. Five mice were used in each group during the experiments. DOHH inhibitors were dissolved in a concentration of 300 mg/kg in PBS (200 μL) with DMSO (10% v/v). As a negative control, mice were treated with PBS with DMSO (10% v/v) only. As a positive control, four mice were treated with 25 mg/kg chloroquine dissolved in sterile PBS for four days ip.

Detection of Parasites. Blood samples were collected daily (from the beginning of the *P. berghei* infection) from experimental mice via the tail vein. Giemsa stained (Merck, Darmstadt, Germany) thin blood smears were prepared, and *P. berghei* parasitemia was quantified as the percentage of infected red blood cells (iRBCs) per 500 RBCs per slide.

Trypanosome Assay According to References 49 and 50. Parasite Culture. Trypomastigote forms of *T. brucei brucei* laboratory strain TC 221 were cultured in Baltz medium according to standard conditions.[51]

In Vitro Cytotoxicity Assays. The test compounds were dissolved in DMSO or NaOH (0.1 M). A defined number of parasites (10^4 trypanosomes per milliliter) were exposed in test chambers of 96-well plates to various concentrations of the test substances in a final volume of 200 μL. Positive (trypanosomes in culture medium) and negative controls (test substance without trypanosomes) were run with each plate.

The plates were then incubated at 37 °C in an atmosphere of 5% CO_2 for a total time period of 72 h. A reading was done at 48 h. The effect of test substances was quantified as ED_{50} values by linear interpolation[18] of three different measurements. The activity of the test substances was measured by light absorption in an MR 700 microplate reader at a wavelength of 550 nm with a reference wavelength of 630 nm, using Alamar Blue.

Macrophage Assay According to Reference 52. The macrophage cell line J774.1 was maintained in complete Click RPMI medium. For the experimental procedures, cells were detached from the flasks with a rubber police, washed twice with PBS, and suspended at 2×10^6 cells mL^{-1} in complete Click RPMI medium.

J774.1 macrophages were plated in complete RPMI medium (200 μL) without phenol red in 96-well plates in the absence or presence of various concentrations of the compounds and incubated for 24 h at 37 °C, 5% CO_2, 95% humidity. Following the addition of of Alamar Blue (20 μL), the plates were further incubated at similar conditions. The plates were then read 24 and 48 h later. Control experiments to examine the effect of cell density, incubation time, and DMSO concentration were performed. Absorbance in the absence of compounds was set as 100% of growth control.

Leishmania Assay According to Reference 53. Parasites. The cloned virulent *L. major* isolate MHOM/IL/81/FE/BNI was maintained by passage in BALB/c mice. Promastigotes were grown in blood agar cultures at 26 °C, 5% CO_2, 95% humidity. For the experiments described here, promastigotes were washed twice with phosphate buffered saline (PBS) and suspended at 1×10^8 cells mL^{-1} in Click RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS) (PAA Laboratories, Linz, Austria), L-glutamine (2 mM) (Biochrom, Berlin, Germany), HEPES buffer pH 7.2 (10 mM) (Invitrogen), penicillin (100 μg mL^{-1}), gentamicin (160 μg mL^{-1}), 7.5% $NaHCO_3$, and 2-mercaptoethanol (5×10^{-5}M) (Sigma-Aldrich) (complete medium).

Cells and Cell Lines. The macrophage cell line J774.1 was maintained in complete medium. For the experimental procedures, cells were detached from the flasks with a police scraper, washed twice with PBS, and suspended at 2×10^6 cells mL^{-1} in complete medium.

Analysis of in Vitro Antiproliferative Activity. Promastigotes were seeded into 96-well plates in complete medium without phenol red (200 μL) in the absence or presence of increasing concentrations

of the compounds. They were then incubated for 24 h at 26 °C, 5% CO_2, 95% humidity. Following the addition of Alamar Blue (20 μL) (Trinova Biochem, Giessen, Germany), the plates were incubated again and the optical densities (OD) measured 24 and 48 h later with a MultiAscent ELISA reader (Thermo Electon Corporation, Dreieich, Germany) using a test wavelength of 540 nm and a reference wavelength of 630 nm. Absorbance in the absence of compounds was set as 100% of growth. J774.1 macrophages, peritoneal macrophages, and BMDC were cultured in complete medium without phenol red, and fibroblasts in DMEM (200 μL) were cultured in the absence or presence of increasing concentrations of the compounds for 24 h at 37 °C, 5% CO_2, 95% humidity. Following the addition of Alamar Blue (20 μL), the plates were incubated again, and the OD was measured 24, 48, and 72 h later as described. Amphotericin B (Sigma-Aldrich) was used as a reference compound and positive control.

Statistical Analysis. Data on antiproliferative activity (from at least two experiments) were analyzed with Ascent Software and Microsoft Excel. OD values at 48 h were used to calculate, via linear interpolation, the drug concentrations that inhibit 50% cell growth or cell survival (IC_{50}).[50]

HIV-1 Infection Experiments. HIV-1 infection experiments using the T-cell tropic (X4) strain NL4/3 and the macrophage tropic (R5) strain BaL were routinely performed using PM1 cells (virus laboratory strains and cells were obtained from the NIH AIDS Research and Reference Reagent Program). Cells were cultured in RPMI medium containing 10% fetal calf serum (Pansystems GmbH) and antibiotics (penicillin and streptomycin). For HIV-1 infection, 5×10^7 cells were resuspended in culture medium (500 μL) without drugs and incubated at 37 °C for 3 h with HIV-1 viral stocks (100 ng). After infection, cells were washed twice with PBS without Ca^{2+} and Mg^{2+} to avoid false positive p24 antigen determination. Cells were resuspended, and identical aliquots (5×10^5/mL) of infected cells were further cultured in 24-well plates (triplicates) in the presence of the substances **11**–**13** and **53** (dissolved in DMSO) at various concentrations or in medium with DMSO as a control for the calculation of the inhibition of virus replication. Culture medium was changed, and cells were split twice a week postinfection. Viability of the cells (as measured by Alamar Blue) and p24 antigen levels (as measured by ELISA, Innogenetics N.V.) were determined at different time points.

Acknowledgment. This work was financially supported by grants from the Deutsche Forschungsgemeinschaft to U.H., A.H., and J.H. and by the SFB 630 with respect to the testing of the compounds against *T. brucei brucei*, *L. major* and cytotoxicity.

Supporting Information Available: Microanalysis and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM070763Y