In search for novel agents in therapy of tropical diseases and human immunodeficiency virus

Tim Goebel,[1] Daniela Ulmer,[1] Holger Projahn, [1] Melanie Glaser,[2] Alicia Pontre-Sucre,[2] Sabine Specht,[3] Salem Ramadan Sarite, [3] Achim Hoerauf,[3] Annette Kaiser,[4] Ilona Hauber,[5] Joachim Hauber,[5] Ulrike Holzgrabe[1*]

[1]Institute of Pharmacy and Food Chemistry, University of Wuerzburg,
Am Hubland, 97074 Wuerzburg, Germany

[2] Laboratory of Molecular Physiology, Universidad Central de Venezuela; at present: Institute for Molecular Infection Biology, University of Wuerzburg,
Roentgenring 11, 97070 Wuerzburg, Germany

[3]Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn,
Sigmund-Freud-Str. 29, 53105 Bonn, Germany

[4] FH-Bonn-Rhein-Sieg, Von-Liebig-Str. 20, 53359 Rheinbach, Germany

[5]Heinrich-Pette-Institute for Experimental Virology and Immunology,
Martinistr. 52, 20251 Hamburg, Germany

Received

Correspondence: Prof. Dr. Ulrike Holzgrabe,
Institut für Pharmazie und Lebensmittelchemie,
Am Hubland, D-97074 Würzburg, Germany
Phone 0931-888-5460; Fax 0931-888-5494
E-mail: holzgrab@pharmazie.uni-wuerzburg.de

Abstract

Malaria, sleeping sickness, Chagas' disease, and Aleppo boil are among the tropical diseases causing millions of infections and cases of deaths per year. In addition, AIDS is an emerging health problem, mainly in Africa and India. No or inefficient chemotherapy is available for all of these diseases. Since targeting the enzymes of the polyamine pathway may be a therapy option it was aimed to inhibit the deoxyhypusine hydroxylase (DOHH) which is an important step in the biosynthesis of the eukaryotic initiation factor 5A (eIF-5A). Previously, piperidone-type compounds have been shown to efficiently inhibit the DOHH likely by complexing the metal ion in the catalytic unit of the enzyme. In order to find new lead compounds for the development of drugs against the aforementioned diseases a library of piperidines was produced and tested against the corresponding microorganisms. The 3,5-diethyl piperidone 3,5-dicarboxylates **11** and **13** being substituted with 4-nitrophenyl rings in 2- and 6-position were found to be active against *Trypanosoma brucei brucei* and *Plasmodium falciparum* and to have a low cytotoxicity against macrophages. The corresponding monocarboxylates are only highly active against the *T. brucei brucei* The dichlorobenzyl ether of the piperidine oxime **53** showed the highest plasmodicidal activity. Moreover, the compounds **11** and **53** were also able to significantly inhibit replication of HIV-1. Thus, these compounds are promising leads for future drug development to combat tropical diseases and HIV-1 infections.

Keywords

Eukaryotic initiation factor 5A, eIF5A, hypusine, deoxyhypusine hydroxylase, malaria, trypanosomiasis, human immunodeficiency virus type 1, HIV-1, cytotoxicity

Introduction

The malaria parasite Plasmodium is a major cause of global human mortality with *Plasmodium falciparum* being the deadliest protozoan of 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 due to the emerging levels of resistance against plasmodicidal drugs and the lack of safe drugs against all forms of trypanosomiasis.[3] Even though facing 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) [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.[6,7,8] E.g. difluoromethylornithine (DFMO) 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.

Insert Scheme 1

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 by deoxyhypusine synthase (DHS) [EC 1.1.1.249] and by deoxyhypusine hydroxylase (DOHH) [EC1.14.99.29].[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 (Fig. 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} value of 8.2 μM. However, ciclopiroxolamine was ineffective *in vivo* in a rodent malaria model.

The plant amino acid L-mimosine (Fig. 1) can reversibly block mammalian cells at late G1-phase and leads to a notable reduction in the steady-state level of mature eIF-5A by means of DOHH inhibition.[16] These findings prompted the determination of the antiplasmodial effect resulting in an IC_{50} value 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.

Since the 2- and 4-piperidone skeleton of ciclopiroxolamine and mimosine are putatively complexing the essential catalytic metal ion (iron),[17] 4-oxo-piperidine-3-carboxylates (Fig. 1a), being perfect chelators of the metal either via the enolizable β-ketoester moiety (cf. acetylacetonate complexes of iron[18]) or via the three nitrogens, in position 1 and in the pyridines,[18,19] were recently tested for the inhibitory properties of DOHH in *P. falciparum* and found to be more active than ciclopiroxolamine and mimosine.[20] However, some of the piperidones lacked sufficient water solubility.

Insert Figure 1a

Insert Figure 1b

Moreover, the eIF-5A is an essential cofactor of the viral regulatory protein Rev which is important for HIV-1 replication.[21] 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[22,23,24,25] indicating that inhibition of hypusine (especially DHS and DOHH) is a preferred strategy for the development of anti-HIV drugs.

These findings proved the hypusine biosynthesis pathway to be a target for antimicrobial therapy[26] 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 Fig. 1b) is composed of a variety of 4-oxo-piperidine-3,5-dicarboxylates and 3-monocarboxylates carrying different substituents in 2- and 4-position 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. Applying this strategy it is 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-oxo-piperidine 3,5-dicarboxylates **1 – 18** (see Fig. 2) were achieved via a double Mannich condensation of one mole of a corresponding primary amine, two moles of pyridine-2-carbaldehyde and 3- or 4-nitrophenylcarbaldehyde, respectively, and dimethyl oxoglutarate in analogy to Merz and Haller,[27] Ashauer-Holzgrabe and Haller,[28] and Kuhl et al.[29] Here, it was 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 attachement of nitro groups to the aromatic ring in 2- and 6-positions (Tab. 1a). Especially the latter variation led to compounds of higher water solubility.

The analogous monocarboxylates **19 - 24** (Tab. 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 primary amines (cf. ref.[30]). The monocarboxylates were always isolated in the enol form. The corresponding dihydropyridine and tetrahydropyridine compounds **25 - 28** (see Fig. 2) can be achieved by means of an oxidation using cerium(IV)sulfate.[27,28,30]

Insert Figure 2

In order to obtain the spiropiperidine **33 – 41**, and **44, 45** and **47** the corresponding 4-oxo-piperidine-3,5-dicarboxylates **30a-d** have to be synthesized via a double Mannich condensation of ammonium bromide, two moles 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 was performed with concentrated hydrochloric acid to give the 2,6-alkyl substituted and non-substituted 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 finding of Siener et al.[31] N-Benzylation can be achieved by means of benzylbromide in acetonitrile in presence of an excess of potassium carbonate resulting in high yields of the piperidone **42**. Via a Strecker synthesis[32] the nitrile compounds **32a-f** and **43a,b** were synthesized in glacial acid using trimethylsilylcyanide, and aniline and benzylamine, respectively, 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 due to one axial alkyl group. Conversion of the compounds **32a-f** and **43a,b** with chlorosulfonylisocyanates and subsequent refluxing in 1 M hydrochloric acid leads 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 ^{o}C in analogy to Röver et al.[33] In contrast to Röver et al. the reduced compound **47** was forthwith isolated. Thus, the reduction step with $NaBH_4$ was dispensable.

Insert Figure 3

The N-alkylated oxime of the 4-piperidone **48** was synthesized starting from the piperidone which can be alkylated with phenylpropylbromide. Subsequently the ketone can be converted to the oxime by using hydroxylamine hydrochloride. **48** was alkylated with dichlorobenzylbromide to give the oxime ether **49**.

The synthesis of the corresponding 4-piperidinecarbaldehyde oximes started off from pyridine-4-carbaldehyde whose aldehyde function was firstly protected by means of ethylenglycol. The resulting acetal was N-alkylated with phenylpropylbromide 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 dichlorobenzylchloride to yield the oxime ether **53**.

Insert Figure 4

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* are summarized in Table 1 as well as the toxicity against macrophages after 48 h. Structure-activity relationships (SAR) will be qualitatively discussed chemical group by group.

Insert Table 1

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) felt in the higher micromolar range of concentration if the piperidones are armed with pyridines rings in 2- and 6-position (**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 are active against *T. brucei brucei* and **9** is additionally active against *P. falciparum*. In vitro effect of **9** on *P. falciparum* chloroquine sensitive NF-54 strains exhibits the percentage of parasitemia to be close to 0 after day 1 post treatment with 40 µM and after 2 day post treatment with 20 µM (see Fig. 5a). Additionally, the percentage of survival of C57BI/6 mice treated i.v. with **9** could be substantially prolonged (data not shown). However, the effective dosis for parasites and for the toxicity against macrophages are close together which makes **8** and **9** out of question as new lead compounds.

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 better soluble in water and buffer than the pyridine substituted analogues. Especially the diethyl dicarboxylates **11** ands **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 post treatment was close to 0 in case of **11** and the number of days of survival was increased (see Fig. 5b, c). The 3-nitrophenyl substituted compounds are only active against *T. brucei brucei*, but exhibit a higher toxicity.

Insert Figure 5

Since in a preliminary study the corresponding 3-monocarboxylates 4-piperidones **21** and **24** have shown high activity against the plasmodia after 48 h a series of compounds was synthesized having a varying substitution attached to the nitrogen. The activity of 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 amongst 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 hundred 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. The search for an independent synthesis pathway is therefore ongoing.

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[20] and was now found to be low for **28** after 48h. 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, a 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. Especially 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. **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. Due to 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.

Due to 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** are 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} value of 8.3 µM after 72 h, is able to kill the plasmodia completely (see Fig. 6). Thus, **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.

Insert Figure 6

Since the host cell eIF-5A, a co-factor 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 20% inhibition of virus replication was observed, the compound **11** was able to completely inhibit replication of HIV-1 *BaL* at a concentration of 5 µM and HIV-1 *NL4/3* at a concentration of 6 µM as 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 of compound **53,** virus replication was inhibited by 85% (Fig. 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.

Insert Figure 7

Conclusion

The evaluation of the library revealed several promising lead compounds for further drug development, i. e. the 3,5-diethyl piperidone 3,5-dicarboxylates **11** and **13** for drugs against *T. brucei brucei* and *P. falciparum* the corresponding monocarboxylates against the *T. brucei brucei* and the dichlorobenzyl ether of the piperidine oxime **53** against *P. falciparum*. Moreover, the compounds **11** and **53** appear to be promising leads for anti-HIV-1 drug development. Beside the good antiifective activity the main advantage of these compounds is the simple synthesis route and most of them were satisfyingly water soluble for biological evaluation. In the next step, it is not only necessary 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. Next, it has to be analyzed whether the growth inhibition of the microorganisms was caused by the interference with the polyamine pathway, especially the block of the DOHH. Corresponding work, e.g. the cloning of the DOHH for assay development, is in progress.

Acknowledgement

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

Supporting Information Available:

Microanalyses data are available free of charge via the Internet at http://pubs.acs.org.

Experimental

Material and Methods

Melting points were determined on a model B 540 Büchi or a Sanyo Gallenkamp melting point apparatus (Sanyo Gallenkamp, UK) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with a 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: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The center of the peaks of $CDCl_3$ and DMSO-d_6 was used as 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. Microanalyses (C, H, N) of the new compounds agreed with the theoretical value within ±0.4%.

The dimethyl 4-oxo-piperidine-3,5-dicarboxylates **1** and **2** were prepared according to ref. 29, the methyl 4-oxo-piperidine-3-monocarboxylates **20**, **21** and **24**, and the dihydro- and tetrahydropyridine compounds **25 - 28** were synthesized according to ref. 20.

Synthesis

General procedure for the synthesis of the 3,5-dialkyl 2,6-di-2-aryl-4-oxo-piperidine 3,5-dicarboxylates 1-18 and 30 a-d, modified after[34]

0.02 mol of the corresponding amine, 0.04 mol of pyridine-2-carboxaldehyde, and m- and p-nitrobenzaldehyde, respectively, were dissolved in 20 mL methanol and cooled to 0 °C. Over a course of about 1 h 0.02 mol of dimethyl oxoglutarate and diethyl oxoglutarate, respectively, were dropwise added. The solution was allowed to stand overnight at 5°C. The product was obtained by filtration of the precipitate formed. In the case no precipitate appeared, the solvent was removed *in vacuo* at 40 to 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 %.

3,5-Dimethyl (2*R*,6*S*)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (1) $C_{19}H_{19}N_3O_5$ (369.4 g/mol), yield: 33 % (67 %) ; **^{1}H NMR** (DMSO-d_6, δ = ppm, *J* = Hz) 2.94 (t, 1H, ^3J = *12.3*, N***H***); 3.53 (s, 6H, COOC***H*****$_3$**); 4.25 (d, 2H, $^3J_{aa}$ = *10.5*, C***H***C=O); 4.62 (dd, 2H, ^3J = *12.3*, $^3J_{aa}$ =*10.5*, C***H***NH); 7.32 (ddd, 2H, *J = 1.0*, *J = 4.8*, *J = 7.6*, ***H6'*** or ***H4'***); 7.45 (d, 2H, *J = 7.8*, ***H6'*** or ***H4'***); 7.78 (dt, 2H, *J = 7.6*, *J = 1.8*, ***H5'***); 8.50-8.56 (m, 2H, ***H3'***). **^{13}C NMR** (DMSO-d_6, δ = ppm) 51.6 (COO***C***H$_3$); 61.6 (***C***HC=O); 63.0 (***C***HNH); 123.2 (***C4'***/***C6'***); 137.0 (***C5'***); 148.9 (***C3'***); 158.0 (***C1'***); 168.6 (***C***OOCH$_3$); 202.1 (***C***=O). **IR** (cm^{-1}) 3296; 3021; 2954; 1739; 1701; 1432; 1335; 1213; 1110; 816; 776. mp. 175 °C (170-171 °C).[28]

3,5-Dimethyl (2*R*,6*S*)-1-allyl-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (2) $C_{22}H_{23}N_3O_5$ (409.5 g/mol), yield: 87 % ; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 3.00 (m, 2H, NC***H*****$_2$**); 3.59 (s, 3H, COOC***H*****$_3$**); 3.74 (s, 3H, COOC***H*****$_3$**); 4.18 (d, 1H, J = 10.3, C***H***C=O); 4.68 (d, 1H, *J = 10.3*, C***H***NH); 4.97 (s, 1H, NC***H***Cq); 5.19 (ddd, 2H, J = 17.3, J = 10.1, J = 1.2, =C***H***$_2$), 5.79 (m, 1H, J = 17.3, J = 10.1, NCH$_2$C***H***=); 7.07- 7.19 (m, 2H, ***H*****$_{aromat.}$**); 7.29 (m, 1H, ***H*****$_{aromat.}$**); 7.52- 7.71 (m, 3H, ***H*****$_{aromat.}$**); 8.43 (m, 1H, ***H*****$_{aromat.}$**); 8.60 (m, 1H, ***H*****$_{aromat.}$**); 12.52 (s, O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 44.32 (***C***HC=O); 50.53 (N***C***H$_2$); 51.76, 52.5 (COO***C***H$_3$); 59.43 (***C***HNCH$_2$); 60.38 (H$_2$CN***C***H); 97.78; 117.68 (=***C***H$_2$); 121.84; 122.20; 122.76; 123.06 (***C4'***/***C6'***); 135.86 (NCH$_2$***C***H=); 136.02; 136.12 (***C5'***); 148.13; 148.83 (***C3'***); 158.24 (***C1'***); 161.32 (***C1'***); 167.33 (***C***OOCH$_3$); 171.12, 172.05 (***C***OOCH$_3$). **IR** (cm^{-1}) 3040; 3000; 1720; 1650; 1430; 1360; 1250; 1005; 980; 825; 770. mp. 133 °C (134 °C).[28]

3,5-Dimethyl (2*R*,6*S*)-1-(2-hydroxyethyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (3) $C_{21}H_{23}N_3O_6$ (413.4 g/mol), yield: 55 % ; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 2.58-2.68 (m, 2H, CH$_2$C***H***$_2$OH); 3.35-3.43 (m, 1H, C***H***$_2$CH$_2$OH); 3.63 (s, 3H, CqCOOC***H*****$_3$**);

3.67-3.76 (m, 1H, C***H***$_2$CH$_2$OH); 3.74 (s, 3H, CHCOOC***H$_3$***); 4.26 (d, 1H, *J = 10.6*, CHC***H***COOCH$_3$); 4.46 (s, 1H, CH$_2$CH$_2$O***H***); 4.55 (d, 1H, J = 10.6, C***H***CHCOOCH$_3$) 5.03 (s,1H, CqC***H***); 7.08-7.14 (m, 2H, ***H3''*** and ***H5''***); 7.19-7.25 (m, 1H, ***H5'***); 7.33 (d, 1H, J = 7.8, ***H3'***); 7.55 (td, 1H, J = 7.7, J = 1.8, ***H4''***); 7.69 (td, 1H, J = 7.7, J = 1.8, ***H4'***); 8.40-8.47 (m, 1H, ***H6''***); 8.65-8.72 (m, 1H, ***H6'***). **^{13}C NMR** (CDCl$_3$, δ = ppm) 44.4 (CH***C***HCOOCH$_3$); 48.6 (CH$_2$***C***H$_2$OH); 52.0 (CqCOO***C***H$_3$); 52.7 (CHCOO***C***H$_3$); 59.3 (***C***HCHCOOCH$_3$); 60.2 (***C***H$_2$CH$_2$OH); 61.2 (Cq***C***H); 97.1 (***Cq***=C-OH); 121.8 (***C3'***); 122.4 (***C5'***); 1227, 124.1 (***C3''/ C5''***); 136.6 (***C4'/ C4''***); 148.4 (***C6''***); 149.8 (***C6'***); 157.5, 161.6 (***C2'/ C2''***); 169.6 (Cq=***C***OH); 171.3, 172.1 (***C***=O). **IR** (cm^{-1}) 3333; 2930; 1726; 1437. mp. 135 °C.

3,5-Dimethyl (2*R*,6*S*)-1-(2-hydroxypropyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (4) $C_{22}H_{25}N_3O_6$ (427.4 g/mol), yield: 39 % ; keto-enol ratio: 1:3. **^{1}H NMR** (CDCl$_3$, δ = ppm*, J* = Hz) 1.49 (qu, 2H, J = 6.8, CH$_2$C***H$_2$***CH$_2$OH, ketone); 1.59 (qu, 2H, *J = 6.7*, CH$_2$C***H$_2$***CH$_2$OH, enol); 2.23-2.35 (m, 2H, C***H$_2$***CH$_2$CH$_2$OH, ketone); 2.23-2.35, 2.42-2.49 (2m, each 1H, C***H$_2$***CH$_2$CH$_2$OH, enol); 3.15-3.30 (m 2H, CH$_2$CH$_2$C***H$_2$***OH, ketone); 3.15-3.30, 3.36-3.42 (2m, each 1H, CH$_2$CH$_2$C***H$_2$***OH, enol); 3.59 (2s, each 3H, COOC***H$_3$***, enol); 3.64 (s, 6H, COOC**H$_3$**, ketone); 4.10 (dd, 1H, *J = 0.8, J = 10.6*, C***H***COOCH$_3$, enol); 4.25 (d, J = 6.6, C***H***COOCH$_3$, ketone); 4.27-4.32 (m, 1H, CH$_2$O***H***, enol); 4.37 (d, 1H, J = 10.6, NC***H***CH, enol); 4.73 (d, 2H, J = 6.6, NC***H***CH, ketone); 4.93 (s, 1H, NC***H***Cq, enol); 7.22-7.36 (m, 2H, ***H3''*** and ***H5''***, enol, 1H, ***H5'***, enol, 2H, ***H5***, ketone); 7.49 (d, 2H, J = 7.8, ***H3***, ketone); 7.67 (d, 2H, J = 8.1, ***H3'***, enol); 7.73 (td, 1H, *J = 7.7*, J = 1.9, ***H4''***, enol); 7.82-7.89 (m, 2H, H4, ketone, 1H, ***H4'***, enol); 8.39-8.44 (m, 1H, ***H6''***, enol); 8.49-8.54 (m, 2H, H6, ketone, 1H, *H6'*, enol); 12.21(s, 1H, C=C-O***H***). **^{13}C NMR** (CDCl$_3$, δ = ppm) 30.6 (CH$_2$***C***H$_2$CH$_2$OH, ketone); 31.4 (CH$_2$***C***H$_2$CH$_2$OH, enol); 42.7 (***C***HC-OH, enol); 43.7 (N***C***H$_2$CH$_2$CH$_2$OH, enol); 44.6 (N***C***H$_2$CH$_2$CH$_2$OH, ketone); 51.6 51.7 (O***C***H$_3$, enol); 52.0 (O***C***H$_3$, ketone); 56.5 (***C***HC=O, ketone); 58.3 (CH$_2$CH$_2$***C***H$_2$OH, ketone); 58.7 (CH$_2$CH$_2$***C***H$_2$OH, enol); 59.3 (N***C***HCH, enol); 60.0 (N***C***HqC, enol); 63.2 (N***C***HCH, ketone); 98.0 (***Cq***=COH, enol); 122.2, 122.5, 122.7, 123.0 (***C3'/ C3'', C5'/ C5''***, enol); 123.0, 123.4 (***C3***, ***C5***, ketone); 136.6, 136.7 (***C4'/ C4''***, enol); 137.1 (***C4***, ketone); 148.0; 148.3 (***C6'/ C6''***, enol); 148.5 (***C6***, ketone); 157.4 (***C2***, ketone); 161.4, 157.9 (***C2'/ C2''***, enol); 166.2 (**C**OOCH$_3$, ketone); 169.0 (Cq=***C***-OH, enol); 170.6, 171.8 (**C**OOCH$_3$, enol); 198.6 (**C**=O, ketone); **IR** (cm^{-1}) 3524; 2957; 1736; 1438. mp. 136 °C.

3,5-Dimethyl (2*R*,6*S*)-1-[2-(2-hydroxy-ethoxy)-ethyl]-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (5): $C_{23}H_{27}N_3O_7$ (457.5 g/mol), yield: 63 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 2.44- 2.51 (m, 1H,); 2.67- 2.76 (m, 1H, NCH_2CH_2); 3.44- 3.58 (m, 3H, $NCH_2CH_2OCH_2$); 3.60 (s, 3H, $COOCH_3$); 3.75 (s, 3H, $COOCH_3$); 3.77- 3.80 (m, 2H,); 3.62- 3.69 (m, 1H,); 4.20 (d, 1H, J = 10.6, O=CCHCOH); 4.59 (d, 1H, J = 10.6, CHNCH); 5.41 (s, 1H, NCHCq); 7.08 (t, 1H, ***H4''***); 7.22- 7.29 (m, 2H, ***H6''/ H4'***); 7.45 (d, 1H, ***H6'***); 7.53 (dt, 1H, ***H5''***); 7.72 (t, 1H, ***H5'***); 8.42 (d, 1H, ***H3''***); 8.68 (d, 1H, **H3''**); 12.57 (s, OH). **^{13}C NMR** ($CDCl_3$, δ = ppm) 45.29 (O=CCHCOH); 48.63 (NCH_2CH_2); 53.37, 53.96 ($COOCH_3$); 60.97 (CHNCH); 62.74 ($NCH_2CH_2OCH_2CH_2OH$); 64.36 (NCHCq); 73.18 (NCH_2CH_2); 73.98 ($NCH_2CH_2OCH_2CH_2OH$); 98.83 (CqCOH); 123.82 (***C6'***); 123.87 (***C4'***); 123.94 (***C6''***); 125.20 (***C4''***); 137.67 (***C5'***); 138.38 (***C5''***); 149.57 (***C3''***); 150.61 (***C3'***); 159.61 (***C1'***); 162.15 (***C1''***); 170.17 (***Cq***OH); 173.02 , 173.58 ($COOCH_3$). **IR** (cm^{-1}) 3020; 3000; 2830; 1610; 1520; 1370; 1117; 821; 740. mp. 175°C.

3,5-Dimethyl (2*R*,6*S*)-1-(3-carboxypropyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (6): $C_{23}H_{25}N_3O_7$ (455.5 g/mol), yield: 70%; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.71- 1.83 (m, 1H, NCH_2CH_2), 1.89- 2.40 (m, 2H, $NCH_2CH_2CH_2$), 2.28- 2.47 (m, 2H, $NCH_2CH_2CH_2$), 2.51- 2.61 (m, 1H, NCH_2), 3.61 (s, 3H, $COOCH_3$); 3.74 (s, 3H, $COOCH_3$); 4.23 (d, 1H, J = 10.6, CHC=O); 4.52 (d, 1H, J = 10.6, CHNH); 5.06 (s, 1H, NCHCq); 7.08- 7.18 (m, 2H, $H_{aromat.}$); 7.28- 7.32 (t, 1H, $H_{aromat.}$); 7.50- 7.59 (m, 2H, $H_{aromat.}$); 7,78 (dt, 1H, $H_{aromat.}$); 8.42 (d, 1H, $H_{aromat.}$); 8.71 (d, 1H, $H_{aromat.}$); 12.54 (s, OH). **^{13}C NMR** ($CDCl_3$, δ = ppm) 23.70 (NCH_2CH_2); 32.59 ($NCH_2CH_2CH_2$); 43.96 (CHCqOH); 46.76 (NCH_2); 52.12, 52.71 ($COOCH_3$); 59.64 ($CHNCH_2$); 60.50 (H_2CNCH); 97.10 (NCH***Cq***); 122.73 (***C6''***); 122.97 (***C4'***); 123.31 (***C6'***); 124.02 (***C4''***); 136.59 (***C5''***); 137.74 (***C5'***); 148.33 (***C3''***); 148.75 (***C3'***); 157.79 (***C1''***); 160.26 ***C1'***); 168.98 (***Cq***OH); 171.50 , 172.04 ($COOCH_3$); 176.83 (COOH). **IR** (cm^{-1}) 3030; 2980; 1710; 1620; 1430; 1350; 1210; 950; 845; 700. mp. 169°C.

3,5-Dimethyl (2*R*,6*S*)-1-(3-carboxypentyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dicarboxylate (7): $C_{25}H_{29}N_3O_7$ (483.5 g/mol), yield: 43 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) keto-enol ratio: 1:5, **^{1}H NMR** ($CDCl_3$, δ = ppm, *J = Hz*) 0.99- 1.09 (m, 2H, C***H₂***, enol); 1.16- 1.34 (m, 2H, C***H₂***, enol); 1.36- 1.45 (m, 2H, C***H₂***, enol); 2.05- 2.10 (m, 2H, ***CH₂***, enol); 2.14- 2.21 (m, 2H, ***CH₂***, enol); 2.39- 2.45 (m, 2H, NCH_2CH_2, enol); 3.58 (s, 6H, $COOCH_3$, enol); 3.64 (s, 6H, $COOCH_3$, ketone); 4.11 (d, 1H, J = 10.5, CHCqOH, enol); 4.27 (d, 2H, J = 6.8, CHC=O, ketone); 4.36 (d, 1H, CHNCH, J = 10.5, ketone, CHNCH, enol); 4.71 (d, 2H, J = 6.8, NCHCH,

ketone); 4.89 (s, 1H, NC***H***Cq, enol); 7.20-7.35 (m, 2H, ***H6'/H6''***, enol, 2H, ***H4'/H4''***, enol, 2H, ***H4'***, ketone); 7.48 (d, 2H, ***H6'***, ketone); 7.67- 7.75 (m, 2H, ***H6'/H6''***, enol, 1H, ***H5'/H5''***, enol); 7.82-7.88 (m, 2H, H5', ketone, 2H, ***H5'/H5''***, enol); 8.42 (d, 1H, ***H3'/H3''***, enol); 8.51 (m, 2H, ***H3'/H3''***, ketone and enole). **^{13}C NMR** ($CDCl_3$, δ = ppm) 24.0 (***C***H_2, enol); 25.6 (***C***H_2, ketone); 25.7 (***C***H_2, enol); 26.3 (***C***H_2, ketone); 26.8 (***C***H_2, ketone); 30.5 (***C***H_2, ketone); 30.7 (***C***H_2, enol); 33.6 (***C***H_2, enol); 46.0 (***C***H_2, ***C5''***, enol); 51.8 (***C***H_2, ***C5''***, ketone); 51.9 (COO***C***H_3, enol); 52.2 (COO***C***H_3, ketone); 56.6 (***C***HC=O, ketone); 59.5 (N***C***HCH, enol); 60.3 (N***C***HCq, enol); 63.3 (N***C***HCH, ketone); 98.0 (***Cq***=C-OH, enol), 122.2, 122.6, 122.7, 123.1 (***C3'/ C3''*** and ***C5'/ C5''***, enol); 123.0, 123.4 (***C3*** and ***C5***, ketone); 136.6, 136.7 (***C4'/ C4''***, enol); 137.1 (***C4***, ketone); 148.0, 148.2 (***C6'/ C6''***, enol); 148.5 (***C6***, ketone); 157.4 (***C2***, ketone); 157.9, 161.6 (***C2'/ C2''***, enol); 166.0 (Cq=***C***-OH, enol); 169.0 (***C***$COOCH_3$, ketone); 170.6, 171.8 (***C***$COOCH_3$, enol); 174.4 (***C***OOH); 198.6 (***C***=O). **IR** (cm^{-1}) 3013; 2940; 2858; 1737; 1254. mp. 130 °C.

3,5-Dimethyl (2*R*,6*S*)-4-oxo-2,6-di(pyridine-2-yl)-1-(pyridine-2-yl-methyl)-piperidine 3,5-dicarboxylate (8): $C_{25}H_{24}N_4O_5$ (460.5 g/mol), yield: 47%; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) 3.51 (d, 1H, NC***H***$_2$); 3.53 (d, 1H, NC***H***$_2$); 3.61 (s, 6H, COOC***H*****$_3$**); 4.12 (d, 2H, $^3J_{aa}$ = 10.5, C***H***$COOCH_3$); 4.71 (d, 2H, $^3J_{aa}$ = 10.9, C***H***NC***H***); 7.17 (t, 2H, ***H5'/5"***); 7.26-7.31 (m, 2H, ***H6'/6"***); 7.66-8.10 (m, 2H, ***H3'/3"*** or ***H4'/4"***); 8.64 (t, 2H, ***H4'/4"*** *or* ***H3'/3"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 53.6 (COO***C***H_3); 55.8 (***C***H_2); 62.9 (***C***HC=O); 67.9 (N***C***H); 123.7, 123.9 (***C2'/2"/C4'/4"***); 130.3 (***C5'/5"***); 136.3 (***C6'/6"***); 145.3 (***C1'/1"***); 148.4 (***C3'/3"***); 170.1 (***C***$COOCH_3$); 192.4 (***C***=O). **IR** (cm^{-1}) 3020; 2980; 1710; 1640; 1500; 1405; 1310; 1119; 869; 727; 668. mp. 152-153 °C.

(2*R*,6*S*)-1-Benzyl-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3,5-dimethyl dicarboxylate (9): $C_{26}H_{25}N_3O_5$ (459.5 g/mol), yield: 65%; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) 3.53 (s, 2H, NC***H*****$_2$**); 3.62 (s, 3H, COOC***H*****$_3$**); 3.68 (s, 3H, COOC***H*****$_3$**); 3.94 (d, 2H, C***H***$COOCH_3$); 4.72 (d, 2H, NC***H***); 6.69-6.75 (m, 2H, C***H*****$_{Benzyl}$**); 6.95-6.99 (m, 3H, C***H*****$_{Benzyl}$**); 7.27 (t, 2H, ***H5'/5"***); 7.46-7.51 (m, 2H, ***H6'/6"***); 7.96-8.13 (m, 2H, ***H3'/3"*** or ***H4'/4"***); 8.36 (t, 2H, ***H4'/4"*** *or* ***H3'/3"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 53.2 (COO***C***H_3); 54.8 (***C***H_2); 64.6 (***C***HC=O); 69.7 (N***C***H); 122.7, 123.0 (***C2'/2"/C4'/4"***); 127.4, 128.3, 128.5 (***C***H_{Benzyl}); 134.3 (***C5'/5"***); 135.4 (***C6'/6"***); 138.6 (***Cq*****$_{Benzyl}$**); 143.1 (***C1'/1"***); 147.2 (***C3'/3"***); 169.7 (***C***$COOCH_3$); 193.9 (***C***=O). **IR** (cm^{-1}) 2980; 1728; 1657; 1531; 1342; 1146; 836; 791; 726. mp. 154 °C.

3,5-Diethyl (2*R*,6*S*)-1-allyl-4-hydroxy-2,6-bis-(4-nitrophenyl)-1,2,3,6-tetrahydropyridine 3,5- dicarboxylate (10): $C_{24}H_{23}N_3O_9$ (497.5 g/mol); yield: 39 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) 2.70-2.96 (NC***H*****$_2$**CH=CH_2); 3.54, 3.59 (2s, 3H, COOC***H*****$_3$**); 3.90 (dd, 2H, $^3J_{aa}$ = 9.9, ^3J = 2.0, C***H***$COOCH_3$); 4.42 (d, 2H, J = 9.6, NC***H*****$_2$**CH=CH_2); 4.86-4.95 (m, 2H, NC***H***Cq, ***H*****$_B$**); 5.23 (dd, 1H, $^3J_{AC}$ = 10.2, $^2J_{AB}$ = 1.4, ***H*****$_A$**); 5.71-5.86 (m, 1H, ***H*****$_C$**); 7.53-7.59 (m, 4H, ***H3'/3"***, ***H5'/H5"***); 8.15-8.24 (m, 4H, ***H6'/6"***, ***H2'/H2"***); 12.24 (O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 51.5 (N***C***H_2CH=CH_2); 51.9, 52.7 (COO***C***H_3); 55.3 (***C***HCOH); 59.7 (N***C***HCq); 62.6 (N***C***HCH); 101.4 (***Cq***=COH); 121.1 (NCH$_2$CH=***C***H_2); 123.4, 124.2 (***C6'/6"***, ***C2'/2"***); 129.6, 130.1 (***C5'/5"***, ***C3'/3"***); 130.2 (NCH$_2$***C***H=CH_2); 145.9, 151.0 (***C4'/4"***); 147.4, 148.2, (***C1'/1"***); 165.6 (Cq=***C***OH); 168.6, 170.5 (***C***=O). **IR** (cm^{-1}) 2953; 1733; 1665; 1518; 1439; 1346; 1243; 696. mp.154-155 °C.

3,5-Ethyl (2*R*,6*S*)-1-allyl-4-hydroxy-2,6-bis-(4-nitrophenyl)-1,2,3,6-tetrahydropyridine 3,5-dicarboxylate (11): $C_{26}H_{27}N_3O_9$, (525.5 g/mol) yield: 28 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.00-1.07 (m, 6H, $COOCH_2C$***H*****$_3$**); 2.81-2.98 (m, 2H, NC***H*****$_2$**); 3.95-4.07 (m, 5H, COOC***H*****$_2$**CH_3, C***H***C-OH); 4.44 (d, 2H, $^3J_{aa}$ = 9.9, NC***H***CH); 4.86-4.95 (m, 2H; NC***H***Cq, ***H*****$_B$**); 5.25 (dd, 1H, $^3J_{AC}$ = 12.2, $^2J_{AB}$ = 0.9, ***H*****$_A$**); 5.75-5.88 (m, 1H, ***H*****$_C$**); 7.57-7.67 (m, 4H, ***H3'/3"***, ***H5'/5"***); 8.17-8,25 (m, 4H, ***H2'/2"***, ***H6'/6"***); 12.38 (O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 14.0, 14.1 ($COOCH_2$***C***H_3); 51.4 (N***C***H_2); 55.1 (NCHC***H***); 61.4, 61.8 (COO***C***H_2CH_3); 62.2, 63.0 (N***C***H); 101.0 (***C***=COH); 123.3, 124.1 (***C2'/2"***, ***C6'/6"***); 123.9 (NCH***C***H=CH_2); 129.8 (NCHCH=***C***H_2); 130.0, 130.5 (***C3'/3"***, ***C5'/5"***); 147.5 (***C4'/4"***); 148.2 (***C1'/1"***); 165.9 (C=***C***OH); 168.1, 170.1 (***C***=O). **IR** (cm^{-1}) 2981; 2863; 1736; 1661; 1518; 1344; 1245; 700. mp.150-151 °C.

3,5-Dimethyl (2*R*,6*S*)-1-benzyl-4-hydroxy-2,6-bis-(4-nitrophenyl)-1,2,3,6-tetrahydro-pyridine 3,5-dicarboxylate (12):

$C_{28}H_{25}N_3O_9$ (547.6 g/mol), yield: 12 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 3.52, 3.66 (2d, each 1H, NC***H*****$_2$**); 3.55, 3.59 (2s, each 3H, COOC***H*****$_3$**); 3.95 (dd, 1H, $^3J_{aa}$ = 9.6, ^3J = 1.8, NCHC***H***); 4.49 (d, 1H, $^3J_{aa}$ = 9.6, NC***H***CH); 4.79 (d, 1H, ^4J = 1.5, NC***H***Cq); 6.78-6.83 (m, 2H, C***H*****$_{Benzyl}$**); 7.05-7.15 (m, 3H, C***H*****$_{Benzyl}$**); 7.42-7.52 (m, 4H, ***H3'/H3"***, ***H5'/H5"***); 8.06-8.11 (m, 4H, ***H2'/H2"***, ***H6'/H6"***); 12.27 (s, 1H, O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 52.0; 52.7 (COO***C***H_3); 54.5 (NCH***C***H); 56.5 (N***C***H_2); 61.6 (N***C***HCH); 64.7 (N***C***HCq); 101.3 (***C***=COH); 123.2, 123.8 (***C2'/C2"***, ***C6'/C6"***); 127.5, 128.4, 129.0 (***C***H_{Benzyl}); 129.7, 130.0 (***C3'/C3"***, ***C5'/C5"***); 137.0

(***Cq***$_{Benzyl}$); 146.4 (***C4'/C4"***); 151.3 (***C1'/C1"***); 165.8 (C=***C***OH); 168.5, 170.4 (***C***=O). **IR** (cm^{-1}) 2833; 1740; 1657; 1516; 1444; 1344; 1253; 731; 695. mp. 163-171 °C.

3,5-Diethyl (2*R*,6*S*)-1-benzyl-4-hydroxy-2,6-bis-(4-nitrophenyl)-1,2,3,6-tetrahydropyridine 3,5-dicarboxylate (13): $C_{30}H_{29}N_3O_9$ (575.7 g/mol), yield: 7 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.10-1.11 (m, 6H, $COOCH_2C\boldsymbol{H_3}$); 3.49-3.72 (2d, each 1H, ^2J = *14.9*, $NC\boldsymbol{H_2}$); 3.95-4.10 (m, 5H, $COOC\boldsymbol{H_2}CH_3$, NCHC***H***); 4.48 (d, 1H, $^3J_{aa}$ = 9.6, NC***H***CH); 4.86 (d, 1H, ^{4}J = 1.5, NC***H***Cq); 6.74-6.80 (m, 2H, $C\boldsymbol{H_{Benzyl}}$); 7.02-7.13 (m, 3H, $C\boldsymbol{H_{Benzyl}}$); 7.43-7.54 (m, 4H, ***H3'/3"***, ***H5'/5"***); 8.04-8.12 (m, 4H, ***H2'/2"***, ***H6'/6"***); 12.39 (O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 14.0, 14.1 ($CH_2\boldsymbol{C}H_3$); 54.7 (***C***HCOH); 56.5 ($N\boldsymbol{C}H_{2\ Benzyl}$); 61.4, 61.8 ($\boldsymbol{C}H_2CH_3$); 62.2 (N***C***HCH); 65.1 (N***C***HCq); 101.2 (***C***=COH); 123.2, 123.7 (***C2'/2"***, ***C6'/6"***); 127.4, 128.3, 128.8 ($\boldsymbol{C}H_{Benzyl}$); 130.0, 130.3 (***C3'/3"***, ***C5'/5"***); 137.0 ($\boldsymbol{Cq}_{Benzyl}$); 146.1, 151.2 (***C1'/1"***); 147.1, 148.0 (***C4'/4"***); 166.1 (C=***C***OH); 168.0, 170.1 (***C***=O). **IR** (cm^{-1}) 2988; 2857; 1740; 1655; 1516; 1345; 1243; 749; 698. mp. 181-184 °C.

3,5-Dimethyl (2*R*,6*S*)-1-(4-chlorbenzyl)-2,6-bis-(3-nitrophenyl)-4-oxo-piperidine 3,5-dicarboxylate (14): $C_{28}H_{24}N_3O_9Cl$ (582.0 g/mol), yield: 41 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 3.47 (s, 2H, $NC\boldsymbol{H_2}$); 3.55 (s, 3H, $COOC\boldsymbol{H_3}$); 3.58 (s, 3H, $COOC\boldsymbol{H_3}$); 4.02 (d, 2H, $^3J_{aa}$ = 10.8, C***H***$COOCH_3$); 4.59 (d, 2H, $^3J_{aa}$ = 10.8, NC***H***); 6.55-6.74 (m, 4H, $C\boldsymbol{H_{Benzyl}}$); 7.49 (t, 2H, J = 7.3, ***H5'/5"***); 7.78- 7.82 (m, 2H, ***H6'/6"***); 8.09- 8.13 (m, 2H, ***H4'/4"***); 8.35 (t, 2H, ***H2'/2"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 52.7 ($COO\boldsymbol{C}H_3$); 55.4 ($N\boldsymbol{C}H_2$); 63.9 (***C***HC=O); 68.1 (N***C***H); 113.9, 123.7, 123.9, 128.8, 129.9, 133.2, (***C2'/2"***, ***C4'/4"***, ***C5'/5"***, ***C6'/6"***, $\boldsymbol{C}H_{Benzyl}$); 139.3 ($\boldsymbol{Cq}_{Benzyl}$Cl); 140.8, 141.1, 148.3, 157.8 ($\boldsymbol{Cq}_{ar}$); 166.6 (***C***$OOCH_3$); 195.8 (***C***=O). **IR** (cm^{-1}) 3025; 2980; 1738; 1650; 1518; 1420; 1347; 1239; 880; 745; 697. mp. 144-146 °C.

3,5-Dimethyl (2*R*,6*S*)-1-(4-methoxybenzyl)-2,6-bis-(3-nitrophenyl)-4-oxo-piperidine 3,5-dicarboxylate (15): $C_{29}H_{27}N_3O_{10}$ (577.5 g/mol), yield: 29 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = H*z*) 3.49 (s, 2H, $NC\boldsymbol{H_2}$); 3.56 (s, 6H, $COOC\boldsymbol{H_3}$); 3.71 (s, 3H, $OC\boldsymbol{H_3}$); 4.01 (d, 2H, $^3J_{aa}$ = 11.1, C***H***C=O); 4.56 (d, 2H, $^3J_{aa}$ = 11.1, NC***H***); 6.57-6.62 (m, 4H, $C\boldsymbol{H_{Benzyl}}$); 7.52 (t, 2H, J = 7.6, ***H5'/5"***); 7.84 (m, 2H, ***H6'/6"***); 8.12 (m, 2H, ***H2'/2"*** or ***H4'/4"***); 8.36 (s, 2H, ***H4'/4"***or ***H2'/2"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 52.6 ($COO\boldsymbol{C}H_3$); 54.4 ($N\boldsymbol{C}H_2$); 55.4 ($O\boldsymbol{C}H_3$); 63.9 (***C***HC=O); 67.5 (N***C***H); 113.8, 123.8, 123.9 129.8, 129.9, 135.0, (***C2'/2"***, ***C4'/4"***, ***C5'/5"***, ***C6'/6"***, $\boldsymbol{C}H_{Benzyl}$); 137.3 ($\boldsymbol{Cq}_{Benzyl}CH_3$); 141.1, 141.4, 148.5, 158.8 ($\boldsymbol{Cq}_{ar}$); 166.6 (***C***$OOCH_3$); 195.6 (***C***=O). **IR** (cm^{-1}) 3536; 3088; 2957; 1732; 1524; 1436; 1352; 1248; 1172; 813; 741; 694. mp. 159-161 °C.

3,5-Dimethyl (2*R*,6*S*)-1-(4-methylbenzyl)-2,6-bis-(3-nitrophenyl)-4-oxo-piperidine 3,5-dicarboxylate (16): $C_{29}H_{27}N_3O_9$ (561.6 g/mol), yield: 41 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 2.19 (s, 3H, CqC***H*****$_3$**); 3.52 (s, 2H, NC***H*****$_2$**); 3.56 (s, 6H, COOC***H*****$_3$**); 4.07 (d, 2H, $^3J_{aa}$ = 11.1, C***H***C=O); 4.57 (d, 2H, $^3J_{aa}$ = 11.1, NC***H***); 6.57 (d, 2H, J = 7.8, ***H3/5*****$_{Benzyl}$**); 6.84 (d, 2H, J = 7.8, ***H2/6*****$_{Benzyl}$**); 7.50 (t, 2H, J = 7.1, ***H5'/5"***); 7.87 (m, 2H, ***H6'/6"***); 8.08-8.15 (m, 2H, ***H2'/2"*** or ***H4'/4"***); 8.34 (s, 2H, ***H4'/4"*** or ***H2'/2"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 20.9 (***C***H_3); 52.7 (O***C***H_3); 55.0 (N***C***H_2); 63.8 (***C***HC=O); 67.9 (N***C***H); 123.9, 124.0 (***C2'/2"/C4'/4"***); 128.4, 129.1 (***C***H_{Benzyl}); 129.9 (***C5'/5"***); 135.1 (CH_2***Cq*****$_{Benzyl}$**, ***C6'/6"***); 137.3 (***Cq*****$_{Benzyl}$**CH_3); 141.1 (***C1'/1"***); 148.5 (***C3'/3"***); 166.6 (***C***$OOCH_3$); 195.5 (***C***=O). **IR** (cm^{-1}) 2956; 1732; 1530; 1437; 1350; 1259; 1171; 810; 736; 695. mp. 170-172 °C.

3,5-Dimethyl (2*R*,6*S*)-1-benzyl-2,6-bis-(3-nitrophenyl)-4-oxo-piperidine 3,5-dicarboxylate (17): $C_{28}H_{25}N_3O_9$ (547.5 g/mol), yield: 15 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 3.52 (s, 2H, NC***H*****$_2$**); 3.57 (s, 6H, COOC***H*****$_3$**); 4.01 (d, 2H, $^3J_{aa}$ = 10.9, C***H***$COOCH_3$); 4.55 (d, 2H, $^3J_{aa}$ = 10.9, NC***H***); 6.65-6.72 (m, 2H, C***H*****$_{Benzyl}$**); 6.98-7.02 (m, 3H, C***H*****$_{Benzyl}$**); 7.47 (t, 2H, J = 8.0, ***H5'/5"***); 7.76-7.81 (m, 2H, ***H6'/6"***); 8.06-8.10 (m, 2H, ***H2'/2"*** or ***H4'/4"***); 8.34 (t, 2H, J = 1.9, ***H4'/4"*** *or* ***H2'/2"***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 52.6 (COO***C***H_3); 55.6 (***C***H_2); 63.9 (***C***HC=O); 68.3 (N***C***H); 123.8, 123.9 (***C2'/2"/C4'/4"***); 127.1, 128.2, 128.3 (***C***H_{Benzyl}); 129.8 (***C5'/5"***); 135.1 (***C6'/6"***); 137.0 (***Cq*****$_{Benzyl}$**); 141.3 (***C1'/1"***); 148.4 (***C3'/3"***); 166.6 (***C***$OOCH_3$); 195.7 (***C***=O). **IR** (cm^{-1}) 2953; 1748; 1528; 1441; 1348; 1242; 805; 741; 693. mp. 144-146 °C.

3,5-Dimethyl (2*R*,6*S*)-1-allyl-4-oxo-2,6-diphenylpiperidine 3,5-dicarboxylate (18): $C_{24}H_{25}NO_5$ (407.5 g/mol), yield: 81 %; keto-enol ratio: 4:3, **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz), 2.79-2.86 (m, 4H, NC***H*****$_2$**$CH=CH_2$, ketone, NC***H*****$_2$**$CH=CH_2$, enol); 3.53 (s, 6H, COOC***H*****$_3$**, ketone); 3.64, 3.68 (2s, each 3H, COOC***H*****$_3$**, enol); 3.81-3.90 (m, 2H, C***H***C=O, ketone); 3.94 (d, 1H, $^3J_{aa}$ = 10.1, C***H***C-OH, enol); 4.35-4.45 (m, 3H, NC***H***, ketone, NC***H***CH, enol); 4.59-4.66 (m, 1H, $NCH_2CH=C$***H*****$_2$**, ketone); 4.84 (s, 1H, NC***H***Cq, enol); 5.06-5.19 (m, 3H, $NCH_2CH=C$***H*****$_2$**, ketone, $NCH_2CH=C$***H*****$_2$**, enol); 5.67-5.87 (m, 2H, NCH_2C***H***$=CH_2$, ketone, NCH_2C***H***$=CH_2$, enol); 7.20-7.49 (m, 20H, C***H*****$_{ar}$**, ketone, C***H*****$_{ar}$**, enol); 12.45 (O***H***, enol). **^{13}C NMR** ($CDCl_3$, δ = ppm) 46.8 (C***H***C-OH, enol); 49.6, 50.9 (N***C***$H_2CH=CH_2$, ketone and enol); 52.0, 52.7 (COO***C***H_3, enol); 52.2 (COO***C***H_3, ketone); 57.8, 57.9 (N***C***H, ketone); 64.9, 66.3 (N***C***H, enol); 99.0 (***C***=C-OH, enol); 117.7 ($NCH_2CH=$***C***H_2, ketone); 120.5 ($NCH_2CH=$***C***H_2, enol); 127.2, 127.8, 128.1, 128.2, 128.4,

128.5, 128.5, 128.9, 129.2, 130.1 ($NCH_2\boldsymbol{C}H=CH_2$, enol and $\boldsymbol{C}H_{ar}$, ketone and enol); 136.8 ($NCH_2\boldsymbol{C}H=CH_2$, ketone); 138.8, 139.5, 141,8 ($\boldsymbol{Cq_{ar}}$, ketone and enol); 167.4 (C=$\boldsymbol{C}$-OH, enol and $\boldsymbol{C}OOCH_3$, ketone); 171.1, 172.3 ($\boldsymbol{C}$=O, enol); 197.5 ($\boldsymbol{C}$=O, ketone). **IR** (cm^{-1}) 2951; 2847; 1738; 1653; 1438; 1273; 1209; 764; 698. mp. 142 °C.

General procedure for the synthesis of the 3-methyl 2,6-di(pyridine-2-yl)-4-piperidone-3-monocarboxylates 19-24, modified after[35,36]

20 mmol of 2,4,6-trioxotetrahydropyrane were stirred in 30 mL methanol till dissolution and afterwards cooled to -20 °C. 20 mmol of the corresponding amine and 40 mmol pyridine-2-carboxaldehyde dissolved in 20 mL methanol, respectively, were added to the solution in parallel in a way that the reaction temperature does not exceed – 20 °C. After stirring 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 the ref.[35,36,37,38]

3-Methyl (2*R*,6*S*)-1-(benzyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3-carboxylate (19): $C_{24}H_{23}N_3O_3$ (401.5 g/mol), yield: 33% (53%)[36], mp. 155 °C (163-164).[38]

3-Methyl (2*R*,6*S*)-1-(4-methylbenzyl)-4-oxo-2,6-di(pyridine-2-yl)piperidine 3-carboxylate (20): $C_{25}H_{25}N_3O_3$ (415.5 g/mol), yield: 72% (41%)[36], mp. 145 °C (144).[38]

3-Methyl (2*R*,6*S*)-1-(4-chlorobenzyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3-carboxylate (21): $C_{24}H_{22}ClN_3O_3$ (435.9 g/mol), yield 68% (49%)[34], mp. 141 °C (142).[38]

3-Methyl (2*R*,6*S*)-1-(4-Methoxybenzyl)-4-oxo-piperidine-2,6-di(pyridine-2-yl) 3-carboxylate (22): $C_{25}H_{25}N_3O_4$ (431.5 g/mol), yield: 43% (46%)[34], mp. 140 °C (140).[38]

3-Methyl (2*R*,6*S*)-1-(3-methoxybenzyl)-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3-carboxylate (23): $C_{25}H_{25}N_3O_4$ (431.5 g/mol), yield: 56% (63%)[34], mp. 143 °C (144 °C).[38]

3-Methyl (2*R*,6*S*)-1-allyl-4-oxo-2,6-di(pyridine-2-yl)-piperidine 3-carboxylate (24): $C_{20}H_{21}N_3O_3$ (351.4 g/mol), yield: 44%, mp. 158°C.[38]

3,5-Dimethyl (2*R*,6*S*)-4,4-dihydroxy-2,6-dimethyl-piperidinium dicarboxylate hydrobromide (30a): $C_{11}H_{20}NO_6Br$ (342.2 g/mol), yield: 62 %; **^{1}H NMR** (DMSO-d_6, δ = ppm, J = Hz) 1.16 (d, 6H, J = 6.6, CHC$\boldsymbol{H_3}$); 2.81 (d, 2H, $^3J_{aa}$ = 11.6, C$\boldsymbol{H}$$COOCH_3$); 3.57 (m, 2H, C$\boldsymbol{H}$$CH_3$); 3.67 (s, 6H, COOC$\boldsymbol{H_3}$); 5.70, 6.43 (2s, each 1H, O$\boldsymbol{H}$); 8.83, 9.17 (2s, each 1H, N$\boldsymbol{H_2^+}$).

^{13}C NMR (DMSO-d_6, δ = ppm) 16.4 (CH***C***H_3); 50.4 (***C***HCH_3); 52.1 (COO***C***H_3); 55.9 (***C***$HCOOCH_3$); 92.3 (HO-**C**-OH); 168.7 (***C***=O). **IR** (cm^{-1}) 3489; 2943; 2888; 2780; 2736; 2477; 1744; 1719; 1383; 1221; 1024. mp. 178-182 °C.

3,5-Dimethyl (2*RS*,6*RS*)-4-hydroxy-2,6-diethyl-piperidine 3,5-dicarboxylate (30b): $C_{13}H_{21}NO_5$ (271.3 g/mol), yield: 97 % (oil); keto-enol ratio: 1:2. **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.84 (t, 3H, J = 7.6, $CHCH_2C$***H*****$_3$**, enol); 0.93 (t, 3H, J = 7.5, $CHCH_2C$***H*****$_3$**, enol); 0.94 (t, 3H, J = 7.3, $CHCH_2C$***H*****$_3$**, ketone); 0.96 (t, 3H, J = 7.3, $CHCH_2C$***H*****$_3$**, ketone); 1.24-1.66 (m, 7H, C***H*****$_2$$CH_3$, ketone, C***H*****$_2$$CH_3$, enol); 1.74-1.85 (m, 1H, C***H*****$_2$$CH_3$, enol); 2.61-2.68 (m, 1H, CHC***H***CH_2CH_3, enol); 2.84-2.89, 3.06-3.13 (2m, each 1H, C***H***CH_2CH_3, ketone); 3.22 (dd, 1H, $^3J_{ae}$ = 3.8, ^{3}J = 1.5, C***H***$COOCH_3$, enol); 3.43 (d, 1H, $^3J_{ae}$ = 3.3, C***H***$COOCH_3$, ketone); 3.58-3.64 (m, 2H, CHC***H***CH_2CH_3, enol, C***H***$COOCH_3$, ketone); 3.64, 3.68 (2s, each 3H, COOC***H*****$_3$**, ketone); 3.67, 3.72 (2s, each 3H, COOC***H*****$_3$**, enol); 11.9 (brs, 1H, O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 9.3 ($CHCH_2$***C***H_3, enol); 9.9 ($CHCH_2$***C***H_3, ketone); 11.0 ($CHCH_2$***C***H_3, enol and $CHCH_2$***C***H_3, ketone); 26.6, 27.9 (CH***C***H_2CH_3, enol); 26.8, 28.3 (CH***C***H_2CH_3, ketone); 48.3 (***C***$HCOOCH_3$, enol); 51.6, 52.2 (COO***C***H_3, enol); 52.0, 52.3 (COO***C***H_3, ketone); 53.5 (Cq***C***HCH_2CH_3, enol); 56.2 (CH***C***HCH_2CH_3, enol); 60.7, 61.5 (***C***$HCOOCH_3$, ketone); 60.9, 62.2 (***C***HCH_2CH_3, ketone); 103.4 (***Cq***=C-OH); 166.9 (Cq=***C***-OH, enol); 168.5, 169.8 (***C***$OOCH_3$, ketone); 170.8, 172.3 (***C***$OOCH_3$, enol); 200.5 (C=O, ketone).

3,5-Dimethyl (2*RS*,6*RS*)-4-hydroxy-2,6-dipropyl-piperidinium 3,5-dicarboxylate hydrobromide (30c): $C_{15}H_{26}NO_5Br$ (380.3 g/mol), yield: 50 %. **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.96 (t, 3H, J = 7.2, $CqCHCH_2CH_2C$***H*****$_3$**); 0.98 (t, 3H, J = 7.2, $CHCHCH_2CH_2C$***H*****$_3$**); 1.28-1.73 (m, 4H, CH_2C***H*****$_2$$CH_3$ and 1H, $CHCHC$***H*****$_2$$CH_2CH_3$); 1.93-2.19 (m, 2H, $CqCHC$***H*****$_2$$CH_2CH_3$); 2.45-2.57 (m, 1H, $CHCHC$***H*****$_2$$CH_2CH_3$); 3.42-3.52 (m, 1H, CHC***H***$CH_2CH_2CH_3$); 3.52-3.57 (d, 1H, $^3J_{ae}$ = 2.8, C***H***$COOCH_3$); 3.84 (s, 3H, $CqCOOC$***H*****$_3$**); 3.89 (s, 3H, $CHCOOC$***H*****$_3$**); 4.57-4.67 (m, 1H, CqC***H***$CH_2CH_2CH_3$); 7.86, 11.45 (2s, each 1H, N***H*****$_2^+$**); 12.23 (s, 1H, O***H***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 13.6, 14.0 (CH_2***C***H_3); 17.2, 18.8 (***C***H_2CH_3); 31.4, 34.8 (***C***$H_2CH_2CH_3$); 45.5 (***C***$HCOOCH_3$); 52.7 ($CqCOO$***C***H_3); 52.9 (Cq***C***HCH_2CH_3); 52.6 ($CHCOO$***C***H_3); 53.5 (CH***C***HCH_2CH_3); 98.9 (***Cq***=C-OH); 164.6 (Cq=***C***-OH); 169.1 (CH***C***$OOCH_3$); 169.8 (Cq***C***$OOCH_3$). **IR** (cm^{-1}) 3064; 2958, 2933; 2756; 2633; 1747; 1678; 1630; 1533; 1437. mp. 142-143 °C.

3,5-Dimethyl (2*RS*,6*RS*)-4-hydroxy -2,6-dibutyl-3,5-piperidinium dicarboxylate hydrobromide (30d): $C_{17}H_{30}NO_5Br$ (408.3 g/mol), yield: 24 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.91 (t, 6H, J = 7.2, $CHCH_2CH_2CH_3$); 1.19-1.63, 1.97-2.19 and 2.55-2.66 (3m, 12H, $CHCH_2CH_2CH_2CH_3$); 3.39-3.49 (m, 1H, $CHCHCH_2CH_2CH_2CH_3$); 3.54-3.58 (d, 1H, $^3J_{ae}$ = 3.3, $CHCOOCH_3$); 3.85 (s, 3H, $CqCOOCH_3$); 3.90 (s, 3H, $CHCOOCH_3$); 4.58-4.71 (m, 1H, $CqCHCH_2CH_2CH_3$); 6.52, 11.85 (2s, each 1H, NH_2^+); 12.27 (s, 1H, OH). **^{13}C NMR** ($CDCl_3$, δ = ppm) 13.8, 13.9 (CH_2CH_3); 22.1, 22.4 (CH_2CH_3); 25.4, 27.5 ($CH_2CH_2CH_3$); 29.2, 32.3 ($CH_2CH_2CH_2CH_3$); 44.8 ($CHCOOCH_3$); 52.8 ($CqCOOCH_3$); 52.9 ($CqCHCH_2CH_2CH_2CH_3$); 54.2 ($CHCOOCH_3$); 54.5 ($CHCHCH_2CH_2CH_2CH_3$); 98.5 (*Cq*=C-OH); 164.1 (Cq=*C*-OH); 170.1 ($CHCOOCH_3$); 170.3 ($CqCOOCH_3$). **IR** (cm^{-1}) 3080; 2957; 2929; 2864; 2638; 1748; 1666; 1630; 1534; 1440. mp. 144-146 °C.

(2*R*,6*S*)-2,6-Dimethyl-4-oxo-piperidinium chloride (31a): $C_7H_{12}NOCl$ (163.7 g/mol), yield: 95 %; **^{1}H NMR** (DMSO-d_6, δ = ppm, J = Hz) 1.36 (d, 6H, J = 6.3, $CHCH_3$); 2.44 (dd, 2H, ^{2}J = 15.4, $^3J_{ae}$ = 2. 8, CH_2); 2.62 (dd, 2H, ^{2}J = 15.4, $^3J_{aa}$ = 12.9, CH_2,); 3.61 (m, 2H, CH); 9.38, 9.46 (2s, each 1H, NH_2^+). **^{13}C NMR** (DMSO-d_6, δ = ppm) 18.5 ($CHCH_3$); 44.2 (CH_2); 50.8 (CH); 202.9 (C=O). **IR** (cm^{-1}) 2929; 2781; 2723; 2665; 2569; 1726; 1666; 1579; 1451; 1406. mp.: 227-229 °C.

(2*R*,6*S*)/(2*RS*,6*RS*)-2,6-Diethyl-4-oxo-piperidinium chloride (31b): $C_9H_{18}NOCl$ (191.7 g/mol), yield: 93 %; *cis-/trans* ratio: 5:1 **^{1}H NMR** (DMSO, δ = ppm, J = Hz) *cis*-isomer*:* 0.92 (t, 6H, CH_2CH_3, *J = 7.6*); 1.43-1.98 (m, 4H, CH_2CH_3); 2.45-2.63 (m, 4H, CH_2C=O); 3.54-3.57 (m, $CHCH_2CH_3$); 8.87-9.23 (m, 2H, NH_2^+), *trans*-isomer*:* 0.92 (t, 6H, J = 7.5, CH_2CH_3); 1.43-1.98 (m, 4H, CH_2CH_3); 2.45-2.63 (m, 2H, CH_2C=O); 2.72 (dd, 2H^{2}J = 15.3, $^3J_{ae}$ = 4.7, CH_2C=O,); 3.61-3.67 (m, $CHCH_2CH_3$); 9.23-9.32 (m, 2H, NH_2^+). **^{13}C NMR** (DMSO, δ = ppm) *cis*-isomer*:* 9.2 (CH_2CH_3); 25.4 (CH_2CH_3); 41.8 (CH_2C=O); 56.1 ($CHCH_2CH_3$); 202.9 (C=O), *trans*-isomer*:* 9.5 (CH_2CH_3); 24.3 (CH_2CH_3); 41.5 (CH_2C=O); 52.9 ($CHCH_2CH_3$); 203.5 (C=O). **IR** (cm^{-1}) 2929; 2781, 2723; 2665; 2569; 1726; 1666; 1579; 1451; 1406. mp. 181-183 °C.

(2*R*,6*S*)-/ (2*RS*,6*RS*)-2,6-Dipropyl-4-oxo-piperidinium chloride (31c): $C_{11}H_{22}NOCl$ (219.8 g/mol), yield: 92 %; *cis-/trans* ratio: 4:1 **^{1}H NMR** (DMSO, δ = ppm, J = Hz), *cis*-isomer*:* 0.88 (t, 6H, J = 7.3, CH_2CH_3); 1.21-1.90 (m, 8H, $CH_2CH_2CH_3$); 2.42-2.52 (m, 2H, CH_2C=O); 2.61 (dd, 2H, ^{2}J = 15.3, $^3J_{aa}$ = 12.8, CH_2C=O); 3.54-3.65 (m, $CHCH_2CH_2CH_3$); 8.88-9.35 (m, 2H, NH_2^+), *trans*-isomer*:* 0.88 (t, 6H, J = 7.3, CH_2CH_3); 1.21-1.90 (m, 4H, $CH_2CH_2CH_3$); 2.42-2.52 (m, 2H,

C***H₂***C=O); 2.72 (dd, 2H, ^{2}J = 15.4, $^3J_{ae}$ = 4.8, C***H₂***C=O); 3.61-3.78 (m, C***H***CH_2CH_3); 9.35-9.48 (m, 2H, N***H₂***$^+$). **^{13}C NMR** (DMSO, δ = ppm) *cis*-isomer*:* 13.5 (CH_2***C***H_3); 17.5 (***C***H_2CH_3); 34.3 (***C***$H_2CH_2CH_3$); 42.2 (***C***H_2C=O); 54.4 (***C***HCH_2CH_3); 202.8 (***C***=O), *trans*-isomer*:* 13.6 (CH_2***C***H_3); 17.9 (***C***H_2CH_3); 33.2 (***C***$H_2CH_2CH_3$); 41.9 (***C***H_2C=O); 51.4 (***C***HCH_2CH_3); 203.5 (***C***=O). **IR** (cm^{-1}) 2929; 2791; 1726; 1596; 1408. mp. 194 °C.

(2*R*,6*S*)-/ (2*RS*,6*RS*)-2,6-Dibutyl-4-oxo-piperidinium chloride (31d): $C_{13}H_{26}NOCl$ (247.9 g/mol), yield: 90 %; *cis-/trans* ratio: 2:1 **^{1}H NMR** (DMSO-d_6, δ = ppm, , J = Hz) *cis*-isomer*:* 0.88 (t, 6H, J = 6.8, CH_2C***H₃***); 1.19-1.97 (m, 12H, C***H₂***C***H₂***C***H₂***CH_3); 2.44-2.52 (m, 2H, C***H₂***C=O); 2.59 (dd, 2H, ^{2}J = 15.3, $^3J_{aa}$ = 12.8, C***H₂***C=O); 3.47-3.61 (m, C***H***$CH_2CH_2CH_2CH_3$); 8.92-9.39 (m, 2H, N***H₂***$^+$), *trans*-isomer*:* 0.87 (t, 6H, J = 6.7, CH_2C***H₃***); 1.19-1.97 (m, 12H, C***H₂***C***H₂***C***H₂***CH_3); 2.44-2.52 (m, 2H, C***H₂***C=O); 2.71 (dd, 2H, ^{2}J = 15.3, $^3J_{ae}$ = 4.7, C**H₂**C=O); 3.65-3.72 (m, C***H***$CH_2CH_2CH_2CH_3$); 9.39-9.50 (m, 2H, N***H₂***$^+$). **^{13}C NMR** (DMSO-d_6, δ = ppm) *cis*-isomer: 13.6 (CH_2***C***H_3); 21.7, 26.1, 32.0 (***C***H_2***C***H_2***C***H_2CH_3); 42.3 (***C***H_2C=O); 54.7 (***C***$HCH_2CH_2CH_2CH_3$); 202.8 (***C***=O), *trans*-isomer*:* 13.6 (CH_2***C***H_3); 21.7, 26.5, 30.8 (***C***H_2***C***H_2***C***H_2CH_3); 41.9 (***C***H_2C=O); 51.5 (***C***$HCH_2CH_2CH_2CH_3$); 203.5 (***C***=O). **IR** (cm^{-1}) 2928; 2780, 2723; 1726; 1664; 1579; 1448; 1406. mp. 212 °C.

General procedure for the synthesis of the carbonitrile compounds 32a-f and 43

To 25 mmol corresponding piperidone dissolved in 80 mL glacial acid 25 mmol of the corresponding amine and 3.1 mL (25 mmol) trimethylsilylcyanide were added. The solution was stirred for 12 h at 25 ^{o}C. After completion of the reaction the solution was alkalized with concentrated ammonia (pH 11) keeping the temperature under -5 ^{o}C. The solution was 3 times extracted with 100 mL chloroform, the organic layer was dried over sodium sulfate and the solvent removed *in vacuo*. The obtained residue was either crystallized with diethyl ether or *tert.*-butylethyl ether (**32a, c-e**), or a column chromatography with ethyl acetate on basic aluminum oxide was performed (Rf 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-phenylamino-piperidine-4-carbonitrile (32a): $C_{14}H_{19}N_3$ (229.3 g/mol), yield: 71 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) 1.16 (d, 6H, *J = 6.3*, C***H₃***); 1.27 (dd, 2H, ^{2}J = 12.6, $^3J_{aa}$ = 11.6, C***H₂***); 1.57 (brs, N***H***); 2.36 (d, 2H*, ^2J = 12.6*, C***H₂***); 3.08-3.18 (m, 2H, C***H***); 3.62 (s, 1H, N***H***); 6.89-6.97 (t, 3H, J = 8.1, C***H***$_{ar}$); 7.22-7.29 (m, 2H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ

= ppm) 22.0 (***C***CH_3); 44.2 (***C***H_2); 48.6 (***C***H); 54.7 (***C***-CN); 121.1 (***C***N); 118.3, 121.2, 129.4 (***C***H_{ar}); 143.4 (***Cq$_{ar}$***). **IR** (cm^{-1}) 3368; 3304; 2965; 2928; 2874; 2228; 1602; 1499; 1317; 1163; 752; 695. mp. 126 – 128 ^oC.

(2*R*,6*S*)-4-Benzylamino-2,6-dimethylpiperidine-4-carbonitrile (32b): $C_{15}H_{21}N_3$ (243.4 g/mol), yield: 68 % (oil); ratio of rotamers: 4:1. **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) isomer A: 1.13 (t, 6H, *J = 6.1*, CHC***H$_3$***); 1.22 (dd, 2H, ^{2}J = 12.6, $^3J_{aa}$ = 11.6, C***H$_2$***-Cq); 2.02 (d, 2H, *^2J = 12.6*, C***H$_2$***-Cq); 3.01-3.11 (m, 2H, C***H***); 3.91 (s, 2H, NHC***H$_2$***); 7.24-7.38 (m, 5H, C***H$_{ar}$***), isomer B: 1.04 (t, 6H, *J = 6.3,* CH_2C***H$_3$***); 1.53 (dd, 2H, ^{2}J = 13.9, $^3J_{aa}$ = 10.9, C***H$_2$***-Cq); 1.94 (d, 2H, *^2J = 13.9*, C***H$_2$***-Cq); 3.15-3.25 (m, 2H, C***H***); 3.85 (s, 2H, NHC***H$_2$***); 7.24-7.38 (m, 5H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) isomer A: 22.1 (***C***H_3); 43.6 (***C***H_2-Cq); 48.4 (NH***C***H_2); 48.6 (***C***H); 57.3 (***Cq***); 122.0 (***C***N); 127.5, 128.4, 128.6 (***C***H_{ar}); 139.2 (***Cq***$_{ar}$), isomer B: 22.1 (***C***H_3); 42.1 (***C***H_2-Cq); 48.8 (NH***C***H_2); 45.5 (***C***H); 54.6 (***Cq***); 122.3 (***C***N); 127.5, 128.4, 128.6 (***C***H_{ar}); 139.3 (***Cq***$_{ar}$). **IR** (cm^{-1}) 3311; 2962; 2927; 2841; 2218; 1454; 1376; 1318; 1159; 739; 701. mp. 126-128 °C.

(2*R*,6*S*)-2,6-Diethyl-4-phenylamino-piperidine-4-carbonitrile (32c): $C_{16}H_{23}N_3$ (257.4 g/mol), yield: 52 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.98 (t, 6H, J = 7.6, C***H$_3$***); 1.51-1.71 (m, 6H, C***H$_2$***CH_3, Cq-C***H$_2$***); 2.44-2.50 (d, 2H, *^2J = 13.1,* Cq-C***H$_2$***); 2.95-3.06 (m, 2H, C***H***); 6.91-6.98 (m, 3H, C***H$_{ar}$***); 7.23-7.28 (m, 2H, C***H$_{ar}$***). **^{13}C NMR** ($CDCl_3$, δ = ppm) 10.2 (***C***H_3); 27.7 (***C***H_2); 40.7 (***C***H_2); 54.2 (***C***H); 55.2 (***Cq***); 120.7 (***C***N); 118.2, 121.3, 129.5 (***C***H_{ar}); 143.4 (***Cq$_{ar}$***). **IR** (cm^{-1}) 3258; 2971; 2940; 2225; 1603; 1557; 1497; 1404; 1319; 1258; 748; 695. mp. 142-143 °C.

(2*R*,6*S*)-4-Benzylamino-2,6-diethylpiperidine-4-carbonitrile (32d): $C_{17}H_{25}N_3$ (271.4 g/mol), yield: 47 %; ratio of rotamers: 5:1. **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) isomer A: 0.96 (t, 6H, *J = 7.5*, CH_2C***H$_3$***); 1.20 (dd, 2H, ^{2}J = 12.9, $^3J_{aa}$ = 11.6, C***H$_2$***-Cq); 1.34-1.58 (m, 4H, C***H$_2$***CH_3); 2.09 (d, 2H, *^2J = 12.9,* C***H$_2$***Cq); 2.78-2.87 (m, 2H, C***H***); 3.94 (s, 2H, C***H$_2$***NH); 7.26-7.39 (m, 5H, C***H***$_{ar}$), isomer B: 0.88 (t, 6H, J = 7.5, CH_2C***H$_3$***); 1.63 (dd, 2H, *^2J = 13.6, $^3J_{aa}$ = 11.6*, C***H$_2$***-Cq); 1.34-1.58 (m, 4H, C***H$_2$***CH_3); 2.04 (d, 2H, ^{2}J = 13.6, C***H$_2$*** Cq); 2.97-3.03 (m, 2H, C***H***); 3.87 (s, 2H, NHC***H$_2$***); 7.26-7.39 (m, 5H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) isomer A: 10.3 (***C***H_3); 29.1 (***C***H_2CH_3); 41.9 (***C***H_2 Cq); 48.6 (NH***C***H_2); 54.7 (***C***H); 57.4 (***Cq***); 122.0 (***C***N); 127.6, 128.5, 128.7 (***C***H_{ar}); 139.2 (***Cq***$_{ar}$), isomer B: 10.2 (***C***H_3); 28.8 (***C***H_2CH_3); 40.0 (***C***H_2 Cq); 49.0 (NH***C***H_2); 51.6 (***C***H); 54.5 (***Cq***); 122.3 (***C***N); 127.7, 128.6, 128.7 (***C***H_{ar}); 139.3 (***Cq***$_{ar}$). **IR** (cm^{-1}) 3304; 3237; 2963; 2923; 2877; 2218; 1558; 1456; 13.81; 1329; 1142; 1093; 740; 699. mp. 109-111 °C.

(2*R*,6*S*)-4-Phenylamino-2,6-dipropylpiperidine-4-carbonitrile (32e): $C_{18}H_{27}N_3$ (285.4 g/mol), yield: 27 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = *Hz*) 0.89 (t, 6H, *J = 6.9*, C***H*****$_3$**); 1.16 (dd, 2H, ^{2}J = 12.5, $^3J_{aa}$ = 12.0, CqC***H*****$_2$**); 1.30-1.42 (m, 8H, C***H*****$_2$**C***H*****$_2$**CH_3); 2.34 (d, 2H, ^{2}J = 12.5, CqC***H*****$_2$**); 2.70-2.79 (m, 2H, C***H***); 6.72 (t, 1H, J = 7.3, C***H***$_{ar}$); 6.86 (d, 2H, J = 7.8, C***H***$_{ar}$); 7.16 (2t, each 1H, J = 7.7, J = 7.6, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) 14.1 (***C***H_3); 18.4, 37.8 (***C***H_2***C***H_2CH_3); 41.9 (Cq***C***H_2); 52.3 (***C***H); 53.6 (***Cq***); 121.2 (***C***N); 115.7, 118.3, 128.8 (***C***H_{ar}); 144.8 (***Cq***$_{ar}$). **IR** (cm^{-1}) 3371; 2958; 2928; 2870; 2230; 1603; 1500; 1321; 1256; 1156; 751; 693. mp. 94 °C.

(2R,6S)-2,6-Dibutyl-4-phenylamino-piperidine-4-carbonitrile (32f): $C_{20}H_{31}N_3$ (313.5 g/mol), yield: 21 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.91 (t, 6H, J = 6.9, C***H*****$_3$**); 1.27-1.41, 1.56-1.91 (2m, 14H, C***H*****$_2$**C***H*****$_2$**C***H*****$_2$**CH_3, Cq-C***H*****$_2$** axial); 2.48-2.55 (d, 2H, *^2J = 12.9*, Cq-C***H*****$_2$** äquatorial); 3.11-3.22 (m, 2H, C***H***); 6.92-6.99 (m, 3H, C***H***$_{ar}$); 7.24-7.29 (m, 2H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) 14.1 (***C***H_3); 22.6 (***C***H_2); 27.9 (***C***H_2); 33.8 (***C***H_2); 40.6 (***C***H_2); 54.0 (***C***H); 54.2 (***Cq***); 120.4 (***C***N); 118.5, 121.6, 129.6 (***C***H_{ar}); 143.1 (***Cq***$_{ar}$). **IR** (cm^{-1}) 3370; 2958; 2928; 2870; 2230; 1603; 1500; 1321; 1257; 1156; 751; 694. mp. 79 °C.

General procedure for the synthesis of the spiro compounds 33-36 and 44

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

(2*R*,6*S*)-7,9-Dimethyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (33): $C_{15}H_{19}N_3O_2$ (273.3 g/mol), yield: 52 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.05 (d, 6H, J = 6.3, C***H*****$_3$**); 1.33 (dd, 2H, ^{2}J = 12.5, $^3J_{aa}$ = 12.3, C***H*****$_2$** Cq); 1.87 (d, 2H, *^2J = 12.5*, C***H*****$_2$** Cq); 3.45-3.58 (m, 2H, C***H***); 7.15-7.19 (m, 2H, C***H*****$_{ar}$**); 7.41-7.47 (m, 3H, C***H*****$_{ar}$**). **^{13}C NMR** ($CDCl_3$, δ = ppm) 22.3 (***C***H_3); 39.5

(Cq***C***H_2); 46.6 (***C***H); 65.7 ***(Cq)***; 129.4, 129.8, 130.9 (***C***H_{ar}); 132.6 (***Cq$_{ar}$***); 154.9 (***C***=O); 176.2 (Cq-***C***=O). **IR** (cm^{-1}) 2978; 2942; 2716; 1716; 1410; 1386; 1152; 706; 629. mp. 256 °C.

(2*R*,6*S*)-1-Benzyl-7,9-diethyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (34): $C_{18}H_{25}N_3O_2$ (315.4 g/mol), yield: 84 %; ratio of rotamers: 5:1 **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) rotamere A: 0.86 (t, 6H, J = 7.5, CH_2C***H$_3$***); 1.13-1.78 (m, 4H, C***H$_2$***CH_3, 4H, C***H$_2$***-Cq); 3.17-3.29 (m, 2H, C***H***); 4.51 (s, 2H,); 7.20-7.36 (m, 5H, C***H***$_{ar}$), rotamere B: 0.64 (t, 6H, J = 7.5, CH_2C***H$_3$***); 1.13-1.78 (m, 4H, C***H$_2$***CH_3, 4H, C***H$_2$***-Cq); 2.51-2.62 (m, 2H, C***H***); 4.87 (s, 2H, NC***H$_{2\ Benzyl}$***); 7.20-7.36 (m, 5H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) rotamere A: 10.2 (***C***H_3); 29.5 (***C***H_2CH_3); 37.4 (***C***H_2-Cq); 42.0 (NH-***C***H_2); 52.4 (***C***H); 64.5 (***Cq***); 127.6, 127.6, 128.7 (***C***H_{ar}); 138.0 (***Cq***$_{ar}$); 155.0 (Cq-***C***=O); 179.8 (***C***=O), rotamere B: 9.9 (***C***H_3); 30.1 (***C***H_2CH_3); 38.8 (***C***H_2-Cq); 45.5 (NH-***C***H_2); 53.9 (***C***H); 65.9 (***Cq***); 129.9, 127.6, 128.8 (***C***H_{ar}); 137.8 (***Cq***$_{ar}$); 156.9 (Cq-***C***=O); 177.0 (***C***=O). **IR** (cm^{-1})3291; 2963; 2925; 2876; 1709; 1413; 1131; 703; 623. mp. 284-286 °C

(2*R*,6*S*)-1-Phenyl-7,9-dipropyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (35): $C_{19}H_{27}N_3O_2$ (329.5 g/mol), yield: 66 %; **^{1}H NMR** (DMSO, δ = ppm, J = Hz) 0.84 (t, 6H, J = 7.1, C***H$_3$***); 1.17-1.32 (m, 10H, C***H$_2$***C***H$_2$***CH_3, CqC***H$_2$***); 1.78-1.89 (m, 2H, CqC***H$_2$***); 3.21-3.33 (m, 2H, C***H***); 6.96-7.14 (m, 2H, C***H$_{ar}$***); 7.33-7.54 (m, 3H, C***H$_{ar}$***). **^{13}C NMR** (DMSO, δ = ppm) 14.3 (***C***H_3), 19.1 (CH_2***C***H_2CH_3); 38.6 (***C***$H_2CH_2CH_3$); 39.2 (Cq***C***H_2); 50.7 (***C***H); 65.9 (***Cq***); 129.3, 129.8, 130.9 (***C***H_{ar}); 132.6 (***Cq$_{ar}$***); 154.7 (Cq***C***=O); 176.1 (N***C***=ON). **IR** (cm^{-1}) 3270; 2961; 2924; 2871; 1709; 1387; 1152; 700; 625. mp. 216-218 °C.

(2*R*,6*S*)-7,9-Dibutyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (36): $C_{21}H_{31}N_3O_2$ (357.5 g/mol), yield: 42 %; **^{1}H NMR** (DMSO, δ = ppm, J = Hz) 0.87 (t, 6H, J = 6.8, CH_2C***H$_3$***); 1.18-1.40 and 1.74-1.86 (2m, 12H, C***H$_2$***C***H$_2$***C***H$_2$***CH_3); 1.54 (dd, 2H, ^{2}J = 13.9, $^3J_{aa}$ = 12.4, C***H$_2$***-Cq); 2.41 (d, 2H*, ^2J = 13.9,* C***H$_2$***-Cq); 3.53-3.65 (m, 2H, C***H***); 7.30-7.36 (m, 2H, C***H***$_{ar}$); 7.42-7.53 (m, 2H, C***H***$_{ar}$). **^{13}C NMR** (DMSO, δ = ppm) 13.7 (***C***H_3); 21.7 (CH_2CH_2***C***H_2CH_3); 26.5 (CH_2***C***$H_2CH_2CH_3$); 31.9 (***C***$H_2CH_2CH_2CH_3$); 33.1 (***C***H_2-Cq); 51.5 (***C***H); 62.0 (***Cq***); 128.7, 129.4, 131.0 (***C***H_{ar}); 133.0 (***Cq***$_{ar}$); 154.7 (Cq-***C***=O); 176.1 (N***C***=ON). **IR** (cm^{-1}) 3502; 3387; 2957; 2932; 2862; 2726; 1715; 1397; 1150; 705; 625. mp. 305 °C.

(2*R*,6*S*)-8-Benzyl-7,9-diethyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (44): $C_{24}H_{29}N_3O_2$ (391.5 g/mol), yield: 59 %; **^{1}H NMR** (DMSO, δ = ppm, J = Hz) 0.70 (t, 6H, J = 7.3, CH_2C***H$_3$***); 0.95-1.07 (m, 2H, C***H$_2$***CH_3); 1.31-1.46 (m, 2H, C***H$_2$***-Cq axial, 2H, C***H$_2$***CH_3); 1.68 (d, 2H, ^{2}J = 12.6, C***H$_2$***-Cq äquatorial); 3.32-3.42 (m, 2H, NC***H$_2$***, 2H, C***H***); 7.09 (t, 1H, J = 7.2,

C***H***$_{ar}$); 7.20 (t, 2H, J = 7.5, C***H***$_{ar}$); 7.26 (t, 4H, J = 8.0, C***H***$_{ar}$); 7.35 (t, 1H, J = 7.3, C***H***$_{ar}$); 7.45 (t, 2H, J = 7.6, C***H***$_{ar}$). **^{13}C NMR** (DMSO, δ = ppm) 10.9 (***C***H$_3$); 27.3 (***C***H$_2$CH$_3$); 32.6 (***C***H$_2$-Cq); 47.7 (N***C***H$_2$); 59.1 (***C***H); 63.9 (***Cq***); 125.7, 127.1, 127.7, 128.9, 130.1, (***C***H$_{ar}$); 136.0, 143.4 (***Cq***$_{ar}$); 157.7 (***C***=O); 183.5 (Cq-***C***=O). **IR** (cm^{-1}) 2963; 2930; 2865; 1734; 1603; 1494; 1367; 1135; 727; 696. mp. 294-295 °C.

General procedure for the alkylation in position 3 of the spiro compounds 37-41 and 45

1.75 mmol of compound **33-36** and **44**, respectively, were dissolved in 20 mL acetonitrile, 0.62 g (4.5 mmol) potassium carbonate and subsequently 2 mmol of the corresponding alkyl or aromatic bromide added. The solution was refluxed for 16 h and then cooled to 25 °C and filtrated. 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-hydroxy-ethyl)-7,9-dimethyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (37): $C_{18}H_{25}N_3O_3$ (331.4 g/mol), yield: 48 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.06 (d, 6H, J = 6.3, C***H*****$_3$**); 1.48 (dd, 2H, ^{2}J = 13.3, $^3J_{aa}$ = 11.0, CqC***H*****$_2$**); 1.59 (dd, 2H, ^{2}J = 13.3, $^3J_{ae}$ = 1.9, CqC***H*****$_2$**); 3.46-3.56 (m, 2H, C***H***CH$_3$); 3.74-3.78 (m, 2H, NCH$_2$C***H*****$_2$**OH); 3.80-3.84 (m, 2H, NC***H*****$_2$**CH$_2$OH); 4.53 (s, 2H, NC***H*****$_2$**); 7.25-7.32 (m, 5H, C***H*****$_{ar}$**). **^{13}C NMR** ($CDCl_3$, δ = ppm) 22.2 (***C***H$_3$); 38.8 (Cq***C***H$_2$); 41.9 (CH$_2$***C***H$_2$OH); 42.4 (N***C***H$_2$); 46.7 (N***C***H); 61.2 (***C***H$_2$CH$_2$OH); 63.5 (***Cq***); 127.5, 127.7, 128.8 (***C***H$_{ar}$); 156.6 (N***C***=ON); 176.4 (Cq***C***=O). **IR** (cm^{-1}) 3302; 2963; 2934; 2853; 1695; 1447; 1048; 700. mp. 195-196 °C.

(2*R*,6*S*)-3-Benzyl-7,9-diethyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (38): $C_{24}H_{29}N_3O_2$ (391.5 g/mol), yield: 55 %; ratio of rotamers: 6:1. **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) rotamer A: 0.90 (t, 6H, J = 7.5, CH$_2$C***H*****$_3$**); 1.20-1.45 (m, 6H, C***H*****$_2$**-Cq axial, C***H*****$_2$**CH$_3$); 1.82 (d, 2H, ^{2}J = 12.6, C***H*****$_2$**-Cq äquatorial); 3.25-3.35 (m, 2H, C***H***); 4.73 (s, 2H, C***H*****$_{2\ Benzyl}$**); 7.12-7.48 (m, 10H, C***H***$_{ar}$), rotamer B: 0.81 (t, 6H, J = 7.5, CH$_2$C***H*****$_3$**); 1.20-1.45 (4H, C***H*****$_2$**CH$_3$); 1.73-1.79 (m, 2H, C***H*****$_2$**-Cq axial); 1.97 (d, 2H, *^2J = 13.9,* C***H*****$_2$**-Cq äquatorial); 2.22-2.31 (m, 2H, C***H***); 4.73 (s, 2H, C***H*****$_{2\ Benzyl}$**); 7.12-7.48 (m, 10H, C***H***$_{ar}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) rotamer A: 10.3 (***C***H$_3$); 29.4 (***C***H$_2$CH$_3$); 38.0 (***C***H$_2$-Cq); 42.4 (***C***H$_2$ Benzyl); 52.7 (***C***H); 64.3 (***Cq***); 127.9; 128.7; 128.8; 129.1, 129.7, 130.8 (***C***H$_{ar}$); 132.9 (N-***Cq***$_{ar}$); 136.3 (CH$_2$-***Cq***$_{ar}$); 155.0 (N***C***=ON); 175.3 (Cq-***C***=O), rotamer B: 10.1 (***C***H$_3$); 30.0 (***C***H$_2$CH$_3$); 39.3 (***C***H$_2$-Cq); 43.0 (***C***H$_2$ Benzyl); 52.9 (***C***H); 65.6 (***Cq***); 127.3; 128.0; 128.6; 129.4, 129.6, 131.0 (***C***H$_{ar}$); 136.2 (N-***Cq***$_{ar}$); 136.5 (CH$_2$-***Cq***$_{ar}$); 155.5

(N***C***=ON); 175.5 (Cq-***C***=O). **IR** (cm^{-1}) 2962; 2933; 1704; 1434; 1412; 1152; 743; 697. mp. 74-75 °C.

(2*R*,6*S*)-1-Benzyl-7,9-dimethyl-3-(4-nitro-benzyl)-1,3,8-triaza-spiro[4.5]decane-2,4-dione (39): $C_{23}H_{26}N_4O_4$ (422.5 g/mol), yield: 71 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 1.11 (d, 6H, J = 6.3, C***H*****$_3$**); 1.45 (d, 2H, ^{2}J = 12.4, C***H*****$_2$** Cq äquatorial); 1.80 (dd, 2H, ^{2}J = 12.4, $^3J_{aa}$ = 12.9, C***H*****$_2$**Cq); 3.52-3.62 (m, 2H, C***H***); 4.50 (s, 2H, C***H*****$_{2\ Benzyl}$**); 4.70 (s, 2H, C***H*****$_{2\ 4\text{-}Nitrobenzyl}$**); 7.17-7.31 (m, 5H, C***H*****$_{Benzyl}$**); 7.46-7.51 (m, 2H, C***H*****$_{4\text{-}Nitrobenzyl}$**); 8.11-8.17 (m, 2H, C***H*****$_{4\text{-}Nitrobenzyl}$**). **^{13}C NMR** ($CDCl_3$, δ = ppm) 20.6 (***C***H_3); 37.1 (CH***C***H_2); 41.7 (***C***$H_{2\ 4\text{-}Nitrobenzyl}$); 42.6 (***C***$H_{2\ Benzyl}$); 47.5 (***C***H); 62.7 (***Cq***); 124.2, 127.9, 127.9, 128.9, 129.5 (***C***H_{ar}); 137.6 (CH_2-***Cq*****$_{Benzyl}$**); 143.1 (CH_2-***Cq*****$_{4\text{-}Nitrobenzyl}$**); 147.9 (***Cq***-NO_2); 155.3 (N***C***=ON); 175.1 (Cq-***C***=O). **IR** (cm^{-1}) 2974; 2934; 1708; 1575; 1518; 1439; 1413; 1343; 703; 646. mp. 161-162 °C.

(2*R*,6*S*)-7,9-Diethyl-3-phenethyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (40): $C_{25}H_{31}N_3O_2$ (405.5 g/mol), yield: 62 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.87 (t, 6H, *J = 7.6*, CH_2C***H*****$_3$**); 1.15-1.32 (m, 6H, C***H*****$_2$**-Cq axial, C***H*****$_2$**CH_3); 1.59 (d, 2H, *^2J = 12.4,* C***H*****$_2$**-Cq äquatorial); 3.03 (t, 2H, NCH_2C***H*****$_2$**); 3.09-3.18 (m, 2H, C***H***); 3.85 (t, 2H, NC***H*****$_2$**CH_2); 4.73 (s, 1H, N***H***); 7.05-7.44 (m, 10H, C***H*****$_{ar}$**). **^{13}C NMR** ($CDCl_3$, δ = ppm) 10.4 (***C***H_3); 29.8 (***C***H_2CH_3); 33.7 (NCH_2***C***H_2); 38.2 (***C***H_2-Cq); 39.3 (N***C***H_2CH_2); 52.4 (***C***H); 64.2 (***Cq***); 126.8; 128.5; 129.0; 129.3, 129.7, 130.8 (***C***H_{ar}); 133.0 (N-***Cq*****$_{ar}$**); 138.0 (CH_2-***Cq*****$_{ar}$**); 155.1 (N***C***=ON); 175.5 (Cq-***C***=O). **IR** (cm^{-1}) 2934; 2857; 1700; 1443; 1415; 1128; 739; 699; 623. mp. 146-147 °C.

(2*R*,6*S*)-7,9-Diethyl-1-phenyl-3-(3-phenyl-propyl)-1,3,8-triaza-spiro[4.5]decane-2,4-dione (41): $C_{26}H_{33}N_3O_2$ (419.6 g/mol), yield: 63 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.91 (t, 6H, J = 7.5, CH_2C***H*****$_3$**); 1.24-1.38 (m, 6H, C***H*****$_2$**-Cq axial, C***H*****$_2$**CH_3); 1.75 (d, 2H, *^2J = 12.1,* C***H*****$_2$**-Cq äquatorial); 2.05 (quin, 2H, J = 7.5, NCH_2C***H*****$_2$**CH_2); 2.69 (t, 2H, J = 7.7, NCH_2CH_2C***H*****$_2$**); 3.21-3.31 (m, 2H, C***H***); 3.64 (t, 2H, J = 7.3, NC***H*****$_2$**CH_2CH_2); 7.12-7.46 (m, 10H, C***H*****$_{ar}$**). **^{13}C NMR** ($CDCl_3$, δ = ppm) 10.4 (***C***H_3); 29.5 (NCH_2***C***H_2CH_2); 29.8 (***C***H_2CH_3); 33.3 (NCH_2CH_2***C***H_2); 38.4 (***C***H_2-Cq); 38.7 (N***C***$H_2CH_2CH_2$); 52.5 (***C***H); 64.3 (***Cq***); 126.2, 128.5, 128.6, 129.1, 129.7, 130.9 (***C***H_{ar}); 133.1 (N-***Cq*****$_{ar}$**); 141.3 (CH_2-***Cq*****$_{ar}$**); 155.3 (N***C***=ON); 175.9 (Cq-***C***=O). **IR** (cm^{-1}) 2958; 2914; 1704; 1450; 1416; 741; 695; 645. mp. 118-120 °C

1-Benzyl-4-piperidone (42a): $C_{12}H_{15}NO$ (189.3 g/mol), yield: 86 %

42a is commercially available but was synthesized from 4-oxo-piperidone-HCl: 20 mmol of 4-oxo-piperidine-HCl and 6.92g (50 mmol) K_2CO_3 were dissolved in 50 mL acetonitrile and 1.8 ml (24 mmol) benzyl bromide added. After heating for 16 h the solution was cooled to 25 °C and filtrated. To isolate the product the remaining solid was suspended in acetonitrile and filtrated again. The filtrates were combined and the solvent evaporated *in vacuo* to obtain the oily product that can be used for the following synthetic steps without further purification.

(2*R*,6*S*)/(2*RS*,6*RS*)-1-Benzyl-2,6-diethyl-4-piperidone (42b): $C_{16}H_{23}NO$ (245.4 g/mol), yield: 91 % (oil); *cis*-*trans*-ratio: 2:1 **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) *cis*-isomer: 0.97 (t, 6H, J = 7.5, $CH_2C\boldsymbol{H_3}$); 1.36-1.66 (m, 4H, $C\boldsymbol{H_2}CH_3$); 2.09 (dd, 2H, ^2J = 13.9, $^3J_{aa}$ = 11.9, $C\boldsymbol{H_2}C$=O); 2.40 (dd, 2H, ^2J = 13.9, $^3J_{ae}$ = 1.8, $C\boldsymbol{H_2}C$=O); 2.72-2.82 (m, 2H, $C\boldsymbol{H}CH_2CH_3$); 3.08 (s, 2H, $NC\boldsymbol{H_2}$); 7.24-7.50 (m, 5H, $C\boldsymbol{H_{ar}}$), *trans*-isomer: 0.94 (t, 6H, J = 7.3, $CH_2C\boldsymbol{H_3}$); 1.36-1.66 (m, 4H, $C\boldsymbol{H_2}CH_3$); 2.20 (ddd, 2H, $^3J_{ae}$ = 1.4, $^3J_{aa}$ = 6.7, ^2J = 13.9, $C\boldsymbol{H_2}C$=O); 2.49 (ddd, 2H, $^3J_{ae}$ = 1.4, $^3J_{ee}$ = 4.7, ^2J = 13.9, $C\boldsymbol{H_2}C$=O); 3.02-3.13 (m, 2H $C\boldsymbol{H}CH_2CH_3$); 3.64, 4.02 (2d, 2H, J = 13.9, $NC\boldsymbol{H_2}$); 7.24-7.50 (m, 5H, $C\boldsymbol{H_{ar}}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) *cis*-isomer: 10.0 ($\boldsymbol{C}H_3$); 29.6 ($\boldsymbol{C}H_2CH_3$); 47.9 ($\boldsymbol{C}H_2$); 57.9 ($\boldsymbol{C}H_2$); 64.6 ($\boldsymbol{C}HCH_2CH_3$); 126.8, 128.2, 128.3, 129.1, 130.5, 133.3 ($\boldsymbol{C}H_{ar}$ *cis* and *trans*); 139.9 ($\boldsymbol{Cq_{ar}}$); 209.7 ($\boldsymbol{C}$=O), *trans*-isomer: 10.7 ($\boldsymbol{C}H_3$); 25.7 ($\boldsymbol{C}H_2CH_3$); 42.6 ($\boldsymbol{C}H_2$); 57.5 ($\boldsymbol{C}H_2$); 63.2 ($\boldsymbol{C}HCH_2CH_3$); 126.8, 128.2, 128.3, 129.1, 130.5, 133.3 ($\boldsymbol{C}H_{ar}$ *trans* and *cis*); 141.4 $\boldsymbol{Cq_{ar}}$); 210.2 ($\boldsymbol{C}$=O). **IR** (cm^{-1}) 3028; 2963; 2874; 2801; 1707; 1453; 731; 698.

1-Benzyl-4-phenylamino-piperidine-4-carbonitrile (43a): $C_{19}H_{21}N_3$ (291.4 g/mol), yield: 62 %, mp. 146-148 °C, for spectroscopic data see ref. [39]

(2*R*,6*S*)-1-Benzyl-2,6-diethyl-4-phenylamino-piperidine-4-carbonitrile (43b): $C_{23}H_{29}N_3$ (347.5 g/mol), yield: 59 %; **^{1}H NMR** (DMSO-d_6, δ = ppm, J = Hz) 0.79 ($C\boldsymbol{H_3}$); 1.26-1.39 (m, 2H, $C\boldsymbol{H_2}CH_3$); 1.48-1.60 (m, 4H, $C\boldsymbol{H_2}CH_3$, $CqC\boldsymbol{H_2}$ axial); 2.31 (d, 2H, ^2J = 13.4, $CqC\boldsymbol{H_2}$ äquatorial); 2.70-2.80 ($C\boldsymbol{H}$); 3.70 (s, 2H, $NC\boldsymbol{H_2}$); 6.04 (s, 1H, $N\boldsymbol{H}$); 6.75 (t, 1H, J = 7.3, $C\boldsymbol{H_{ar}}$); 6.88 (d, 2H, *J = 7.8*, $C\boldsymbol{H_{ar}}$); 7.14-7.23 (m, 4H, $C\boldsymbol{H_{ar}}$); 7.28 (t, 2H, *J = 7.6*, $C\boldsymbol{H_{ar}}$); 7.38 (d, 2H, *J = 7.6*, $C\boldsymbol{H_{ar}}$). **^{13}C-NMR** (DMSO-d_6, δ = ppm) 10.4 ($\boldsymbol{C}H_3$); 26.5 ($\boldsymbol{C}H_2CH_3$); 37.3 ($Cq\boldsymbol{C}H_2$); 50.2 ($N\boldsymbol{C}H_2$); 53.2 ($\boldsymbol{Cq}$); 60.8 ($N\boldsymbol{C}H$); 121.1 ($\boldsymbol{C}N$); 125.8, 118.5, 126.0, 127.2, 127.9, 129.0 ($\boldsymbol{C}H_{ar}$); 142.3, 144.7 ($\boldsymbol{Cq_{ar}}$). **IR** (cm^{-1}) 3375; 2966; 2934; 2877; 2229; 1601; 1499; 1317; 759; 730; 696. mp. 123-125 °C.

(2*R*,6*S*)-8-Benzyl-7,9-diethyl-3-(4-nitro-benzyl)-1-phenyl-1,3,8-triaza-spiro[4.5]decane-2,4-dione (45): $C_{31}H_{34}N_4O_4$ (526.6 g/mol), yield: 46 %; **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz) 0.76 (t, 6H, J = 7.3, $CH_2C\boldsymbol{H_3}$); 1.04-1.17 (m, 2H, $C\boldsymbol{H_2}CH_3$); 1.56-1.73 (m, 6H, $C\boldsymbol{H_2}CH_3$, $C\boldsymbol{H_2}$-Cq); 3.29-3.40 (m, 2H, C***H***); 3.44 (s, 2H, CHNC$\boldsymbol{H_{2\ Benzyl}}$); 4.81 (s, 2H, O=CNC$\boldsymbol{H_{2\ 4\text{-}Nitrobenzyl}}$); 7.10-7.15 (m, 1H, $\boldsymbol{H4_{Anilin}}$); 7.17-7.53 (m, 9H, $\boldsymbol{H2/3/5/6_{Anilin}}$, $C\boldsymbol{H}_{Benzyl}$); 7.56-7.62 (m, 2H, $\boldsymbol{H2/6\ _{4\text{-}Nitrobenzyl}}$); 8.17-8.22 (m, 2H, $\boldsymbol{H3/5\ _{4\text{-}Nitrobenzyl}}$). **^{13}C NMR** ($CDCl_3$, δ = ppm) 11.0 ($\boldsymbol{C}H_3$); 27.8 ($\boldsymbol{C}H_2CH_3$); 32.9 ($\boldsymbol{C}H_2$-Cq); 41.7 (O=CN$\boldsymbol{C}H_2$); 48.9 (CHN$\boldsymbol{C}H_2$); 59.6 (***C***H); 64.7 (***Cq***); 124.1, 126.2; 127.3, 128.0, 129.3; 129.6, 129.9, 130.6 ($\boldsymbol{C}H_{ar}$); 132.9 (N-$\boldsymbol{Cq_{Anilin}}$); 142.8 (CH_2-$\boldsymbol{Cq}_{ar}$); 143.4 ($\boldsymbol{Cq}_{ar}$); 147.8 (***Cq***-NO_2); 154.7 (N-***C***=ON); 175.2 (Cq-***C***=O). **IR** (cm^{-1}) 2987; 2932; 2866; 1703; 1520; 1432; 1341; 742; 700. mp. 185-187 °C

1-Benzyl-4-phenylamino-piperidine-4-carboxylic acid amide (46): 7.5 mmol of the corresponding carbonitrile compound **32** was stirred in 60 mL of concentrated sulphuric acid for about 12 h at room temperature. During neutralization of the solution with concentrated ammonia the temperature is not allowed to exceed 0 ^oC. 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.[40]

8-Benzyl-1-phenyl-1,3,8-triaza-spiro[4.5]decane-4-one (47):

10 mmol of 46 were dissolved in 20 mL formamide and refluxed for 2 h. The solution is allowed to cool to room temperature, poured into ice water, the aqueous solution 3 times extracted with chloroform and the organic layer dried over sodium sulphate. After evaporation of the solvent **47** was crystallized with acetone/methanol. $C_{20}H_{23}N_3O$ (321.4 g/mol), yield: 68 %; **^{1}H NMR** (DMSO-d_6, δ = ppm, J = Hz) 1.57 (d, 2H, J = 13.6, CqC$\boldsymbol{H_2}$); 2.52-2.63 (m, 2H, CqC$\boldsymbol{H_2}$); 2.67-2.78 (m, 4H, NC$\boldsymbol{H_2}CH_2$); 3.52 (s, 2H, NC$\boldsymbol{H_{2\ Benzyl}}$); 4.57 (s, 2H, NC$\boldsymbol{H_2}$N); 6.76 (t, 2H, J = 7.8, C$\boldsymbol{H_{ar}}$); 6.87 (d, 1H, J = 7.1, C$\boldsymbol{H_{ar}}$); 7.21-7.39 (m, 7H, C$\boldsymbol{H_{ar}}$); 8.60 (s, 1H, N***H***). **^{13}C NMR** (DMSO-d_6, δ = ppm) 28.4 (Cq$\boldsymbol{C}H_2$); 49.2 (N$\boldsymbol{C}H_2CH_2$); 58.2 (N$\boldsymbol{C}H_2$N); 58.7 (N$\boldsymbol{C}H_{2\ Benzyl}$); 62.1 (***Cq***); 114.3, 117.7, 126.8, 128.2, 128.7, 129.0 ($\boldsymbol{C}H_{ar}$); 138.7, 143.3 ($\boldsymbol{Cq}_{ar}$); 176.2 (***C***=ONH). **IR** (cm^{-1}) 3180; 3108; 3067; 2960; 2926; 2818; 1703; 1599; 1373; 1312; 1098; 1030; 796; 735; 689. mp. 234-237 °C.

1-(3-Phenylpropyl)-4-piperidine oxime (48):

13.60 g (100.0 mmol) 4-oxo-piperidine monohydrate hydrochloride, 19.90 g (100.0 mmol) 1-bromo-3-phenylpropane and 69.10 g (500.0 mmol) potassium carbonate were mixed in 400 mL acetonitrile and stirred at 60 °C for 18 h. The solid was filtered off, washed with acetonitrile and the solvent was evaporated. The residue was diluted with 100 mL of dichloromethane and 50 mL sat. sodium hydrogencarbonate solution and the layers were separated. The aqueous layer was extracted twice with 50 mL dichloromethane 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 200 mL abs. ethanol and a solution of 1-(3-phenylpropyl)-4-piperidinone (16.95 g 78.0 mmol) in 100 mL abs ethanol was added and refluxed for 1 h. The mixture was allowed to cool to 25 °C, filtrated and washed with ethanol. The solvent was evaporated and a yellow solid was achieved. Yield: 13.84 (59%). $C_{14}H_{20}N_2O$ (232.3 g/mol); **^{1}H NMR** (d_4-MeOH, δ = ppm, J = Hz): 7.14 – 7.18 (5 H, m, Ph-H), 3.21 – 3.14 (4 H, m, Ph-C***H*****$_2$**-CH_2-C***H*****$_2$**-N), 2.99 (2 H, t, J = 4.6, 2-H_{eq}, 6-H_{eq}), 2.77 - 2.64 (2 H, m, 2-H_{ax}, 6-H_{ax}), 2.60 - 2.49 (4 H, m, 3-H, 5-H), 2.03 – 1.95 (2 H, m, Ph-CH_2-C***H*****$_2$**-CH_2-N). **^{13}C NMR** (d_4-MeOH, δ= ppm): 151.3 (–C=N), 141.6 (C-1 arom.), 129.7, 129.5 (C-2, C-2, C-2, C-6 arom.), 127.5 (C-4 arom.), 57.6 (Ph-CH_2-CH_2-***C***H_2-N), 53.7 and 52.5 (C-2, C-6, rotameres), 33.7 (Ph-***C***H_2-CH_2-CH_2-N), 29.3 (Ph-CH_2-***C***H_2-CH_2-N), 27.4 and 22.5 (C-3, C-5, rotameres), **IR** (ATR, cm^{-1}): 3147 (OH), 3074 (=C–H), 2946 (–CH_2), 1656 (–C=N), 1600 (–C=C- arom.), 954 (N-O), 763, 704 (out of plane). mp. 182.5 °C.

1-(2-Phenylpropyl)—4-oxo-piperidine O-(2,6-dichloro-benzyl)-oxime (49):

1.22 g (6.25 mmol) 2,6-dichlorobenzylchloride, 1.20 g (5.0 mmol) 1-(3-phenylpropyl)piperidin-4-oxime and 0.71 g (6.25 mmol) potassium-*tert*-butoxide were dissolved in dry 100 mL THF and stirred for 24 h at 55°C in the dark under Ar. The solvent was evaporated and the residue was diluted with 100 mL dichloromethane and 50 mL 2.0 M NaOH. The layers were separated and aqueous layer extracted with dichloromethane (2 x 80 mL). The combined organic layers were dried and evaporated to achieve a colorless solid. Yield: 0.68 g (28%). $C_{21}H_{24}N_2OCl_2$ (391.3 g/mol); **^{1}H NMR** ($CDCl_3$, δ = ppm): 7.13 – 7.04 (8 H, m, aromatic); 5.23 (2 H, s, O-CH_2), 2.58 – 2.42 (8 H, m, 2-H, 3-H, 5-H, 6-H); 2.41 – 2.31 (4 H, m, Ph-C***H*****$_2$**-CH_2-C***H*****$_2$**-N); 1.82 – 1.77 (2 H,

m, Ph-CH_2-C***H*****$_2$**-CH_2-N). **^{13}C NMR** ($CDCl_3$, δ = ppm): 152.1 (–C=N), 136.1 (C-1''), 133.4 (C-1'), 130.2 (C-2'', C-6''), 128.5, 128.4, 128.2 (C-2', C-3' C-5', C-6', C-3'', C-5''), 125.8, 125.6 (C-4', C-4''), 57.5 (CH_2-O), 53.4 (Ph-CH_2-CH_2-***C***H_2-N), 52.4 (Ph-***C***H_2-CH_2-CH_2-N), 40.7 (C-2, C-6), 33.6 (C-3, C-5), 31.1 (Ph-***C***H_2-CH_2-CH_2-N). **IR** (ATR, cm^{-1}): 3026 (=C–H); 2945 and 2812 (–CH_2), 1660 (–C=N), 1563 (–C=C- arom.), 739, 705, 700, 642 (out of plane). mp. 147 °C.

4-(1,3-Dioxolan-2-yl)-*N*-(3-phenylpropyl)pyridinium bromide (50):

1.93 g (18.0 mmol) 4-pyridine-carbaldehyde, 2.26 g (36.0 mmol) ethylenglykole and 3.80 g (20.0 mmol) toluene-4-sulfonic acid were dissolved in 100 mL toluene and refluxed for 4 h to remove the water out of the reaction mixture. The solution was allowed to cool to 25 °C and the solvent was evaporated. The residue was diluted with 2.0 M NaOH (50 mL) and 50 mL dichloromethane. The layers were separated and the aqueous layer was extracted with dichloromethane (2 x 50 mL) and the combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated to achieve 4-(1,3-dioxolane-2yl)-pyridine as colorless oil, which was used in the next step without further purification. This 2.07 g (13.6 mmol) intermediate and 4.06 g (20.4 mmol) bromo-3-phenylpropane were dissolved in 20 mL toluene. The solution was filled into a sealed 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 and the oily residue was dissolved in 20 mL acetone and diethyl ether was added until the solution becomes 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 %) colorless crystals. $C_{17}H_{20}NO_2Br$ (350.3 g/mol); **^{1}H NMR** ($CDCl_3$, δ = ppm): 9.45 (2 H, br, 2-H, 6-H pyridine), 7.94 (2 H, br, 3-H, 5-H pyridine), 7.21 – 7.06 (m, 5 H, Ph-H), 5.88 (s, 1H, O–CH–O), 5.01 (br, 2 H, Ph-CH_2-CH_2-C***H*****$_2$**-N), 4.04 – 3.94 (m, 4 H, O–$(CH_2)_2$–O), 2.74 (t, 2 H, J = 6.6, Ph-C***H*****$_2$**-CH_2-CH_2-N), 2.36 – 2.29 (m, 2 H, Ph-CH_2-C***H*****$_2$**-CH_2-N^+), **^{13}C NMR** ($CDCl_3$, δ= ppm): 156.8 (pyridine C-4), 145.5 (pyridine C-2, C-6), 139.5 (phenyl C-1), 128.7 und 128.4 (phenyl C-2, C-3, C-5, C-6), 126.5 (pyridine C-3, C-5), 125.5 (phenyl C-4), 100.0 (O–CH–O), 65.9 (O–$(CH_2)_2$–O), 61.4 (Ph-CH_2-CH_2-***C***H_2-N), 33.1 (Ph-***C***H_2-CH_2-CH_2-N), 32.2 (Ph-CH_2-***C***H_2-CH_2-N). **IR** (ATR, cm^{-1}: 3026 (=C–H); 2976 and 2897 (–CH); 1708 (–C=C- aromatic); 1096 (C-O), mp. 78.1 °C.

(1,3-Dioxolane-2-yl)-*N*-(3-phenylpropyl)piperidine hydrobromide (51)

1.84 g (8.18 mmol) 4-(1,3-dioxolane-2-yl)-1-(3-phenylpropyl)pyridinium bromide **50** and 40 mg PtO_2 were dissolved in 100 mL methanol 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 the get a colorless solid. Yield 1.84 g (98%). $C_{17}H_{26}O_2N_1Br$ (355.1 g/mol); **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz): 11.29 (1 H, br, NH^+), 7.30 – 7.16 (5 H, m, arom.), 4.79 and 4.64 (1 H, d, rotameres, J = 5.6, O-CH-O), 4.08 – 3.99 (4 H, m, O-CH_2-CH_2-O), 3.70 – 3.60 (2 H, m, Ph-CH_2-CH_2-C***H*****$_2$**-N), 3.07 – 2.71 (4 H, m, 2-H, 6-H), 2.68 – 2.16 (7 H, m, 3-H, 4-H, 5-H, Ph-C***H*****$_2$**-CH_2-CH_2-N), 1.76 – 1.69 (2 H, m, Ph-CH_2-C***H*****$_2$**-CH_2-N). **^{13}C NMR** ($CDCl_3$, δ = ppm): 139.4 (phenyl C-1), 128.8, 128.4 (phenyl, C-2, C-3, C-5, C-6), 126.7 (phenyl C-4), 105.3 (O–CH–O), 66.0 (O–$(CH_2)_2$–O), 57.3 (Ph-CH_2-CH_2-***C***H_2-N), 52.6 (C-2, C-6), 38.7 (C-4), 32.9 (Ph-***C***H_2-CH_2-CH_2-N), 24.9 (Ph-CH_2-***C***H_2-***C***H_2-N), 23.9 (C-3, C-5). **IR** (ATR, cm^{-1}):3029 (=C–H), 2926 and 2885 (–CH_2), 1600 (–C=C- arom.), 1045 cm^{-1} (C-O). mp. 132 °C (dec.)

1-1-(3-Phenyl-propyl)-piperidine-4-carbaldehyde oxime (52)

The acetal **51** (10.0 mmol, 3.55 g) was dissolved in 30 mL water and 0.2 mL sulphuric acid (98 %) were added. The mixture was heated up in the microwave within 4 min to 110 °C. The temperature was hold for 30 min and the solution was allowed to cool to 25 °C. 30 mL of 1.0 M Na_2HPO_4 and 6.65 g (100.0 mmol) hydroxylamine hydrochloride were added and the sodium hydroxide solution was added until a pH of 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 x 100 mL). The combined organic layers were dried, filtered and evaporated. The oily residue was dissolved in diethyl ether, filtered and the filtrate was evaporated. Recrystallization from cyclohexane gave a colorless solid. Yield: 1.90 g (55 %), $C_{15}H_{22}N_2O$ (246.4 g/mol); **^{1}H NMR** ($CDCl_3$, δ = ppm, J = Hz): 9.11 (1 H, br, OH), 7.64 – 7.10 (6 H, m, 5 H arom., HC=N), 2.91 – 2. 88 (2 H, m, 2-H_{eq}, 6-H_{eq}), 2.55 (2 H, t, J = 7.8, Ph-CH_2-CH_2-C***H*****$_2$**-N), 2.34 – 2.30 (2 H, m, 2-H_{ax}, 6-H_{ax}), 2.14 – 1.53 (9 H, m, 3-H, 4-H, 5-H, Ph-C***H*****$_2$**-CH_2-C***H*****$_2$**-N). **^{13}C NMR** ($CDCl_3$, δ = ppm): 154.1 (HC=N), 141.9 (C-1, arom.), 128.4, 128.3 (C-2, C-3, C-5, C-6 arom.), 125.6 (C-4 arom.), 58.3 (C-2, C-6), 53.0, Ph-CH_2-CH_2-***C***H_2-N), 33.7 (C-4), 29.0 (C-3, C-

5), 28.4, 28.2 (Ph-***C***H_2-***C***H_2-CH_2-N). **IR** (ATR, cm^{-1}): 3050 – 2750 (OH, br), 3021 (=CH), 2937, 2827 (-CH), 1652 (C=N), 1601, 1495 (C=C, arom.), 942 (N-O), 740 and 700 cm^{-1} (out of plane), mp. 111 °C.

4-[(2,6-Dichloro-benzyloxyimino)-methyl]-1-(3-phenyl-propyl)-piperidinium hydrochloride (53)

The oxime **52** (0.7 g, 2.84 mmol) was dissolved in 40 mL abs. MeOH 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 50 mL dry acetonitrile and 0.56 g (2.84 mmol) 2,6-dichlorobenzylchloride was added. The solution was refluxed for 2 h and the solvent was removed *in vacuo*. Column chromatography (silica gel, eluent: EtAc/EtOH 1:1 + 0.7 % triethylamine) gave a colorless oil. This oil dissolved in 10 mL diethyl ether and 1 mL 1.0 M hydrochloric acid in methanol was added. After 12 h at 4 °C a colorless solid was isolated. Yield: 0.51 g (40 %). $C_{22}H_{27}Cl_3N_2O$ (412.5 g/mol); **^{1}H NMR** (d_6-DMSO, δ ppm, J = Hz): 10.28 (1 H, br, NH^+), 7.50 – 7.18 (9 H, HC=N, 8 H arom.), 5.23 (2 H, s, CH_2-O), 3.58 – 3.38 (2 H, m, 2-H_{eq}, 6-H_{eq}), 3.00 – 2.80 (4 H, m, Ph-CH_2-CH_2-C***H*****$_2$**-N, 2-H_{ax}, 6-H_{ax}), 2.61 (2 H, t, J = 7.6, Ph-C***H*****$_2$**-CH_2-CH_2-N), 2.41 – 2.37 (1 H, m, 4-H), 2.00 – 1.68 (6 H, m, 3-H, 5-H, Ph-CH_2-C***H*****$_2$**-CH_2-N). **^{13}C NMR** (d_6-DMSO, δ = ppm): 153.0 (C=N), 140.5 (C-1'), 136.1 (C-2'', C-6''), 132.0 (C-1''), 131.2 (C-4''), 128.6, 128.4, 128.2 (C-2', C-3', C-5', C-6', C-3'', C-5''), 126.1 (C-4'), 69.4 (CH_2-O), 55.6 (Ph-CH_2-CH_2-***C***H_2-N), 50.9 (C-2, C-6), 33.8 (C-4), 32.0 (Ph-***C***H_2-CH_2-CH_2-N), 26.0 (C-3, C-5), 24.8 (Ph-CH_2-***C***H_2-CH_2-N^+). **IR** (ATR, cm^{-1}): 2910 (-CH), 2512 (NH^+) 1715 (C=N), 1599 and 1563 (C=C, arom.), 1021 (C-O), 931 (N-O), 777, 767, 753 and 698 cm^{-1} (out of plane), Mp.: 198 °C (dec).

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) according to a protocol from ref. [41],[42], in a gaseous phase of 90 % N_2, 5% CO_2 and 5% O_2. Parasites were cultured in human erythrocytes (blood group A^+) in RPM1640 medium (Sigma) supplemented with 25 mM HEPES, 20 mM sodium hydrogen carbonate, and 10 % heat inactivated human A+

plasma at 10 % (v/v) hematocrit. 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, growth medium was changed once a day for four days and the total inhibitor treatment lasted 96 hours. Inhibitors were used in concentrations of 1 to 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 10^{-8} M chloroquine 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 hours were computed into plots with linear regression analysis from y axis (inhibition %) to x (axis) (inhibitor concentration µM) according to [43].

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 3 mice. Parasites were passaged ones to three new BALB/c mice, and 2 days later mosquitoes were allowed to a blood meal on these mice. 21 days after the mosquito infection, salivary glands of the mosquitoes were dissected to isolate the sporozoites. A dose of 500 sporozoites in 200 µl of phosphate buffer saline (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 i.p. 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 (1000x).

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

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[44,45]

Parasite culture: Trypomastigote forms of *T. brucei brucei* laboratory strain TC 221 were cultured in Baltz medium according to standard conditions.[46]

In vitro cytotoxicity assays: The test compounds were dissolved in DMSO or 0.1 M NaOH. A defined number of parasites (10^4 trypanosomes per mL) 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 in 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 wave length of 630 nm, using the Alamar Blue®.

Macrophage Assay according to[47]

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 200 µl of complete RPMI medium 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 20 µl of Alamar Blue, 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[48]

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), 2 mM L-glutamine (Biochrom, Berlin, Germany), 10 mM HEPES buffer pH 7.2 (Invitrogen), 100 µg mL^{-1} penicillin, 160 µg mL^{-1} gentamicin, 7.5% $NaHCO_3$ and 5×10^{-5} M 2-mercaptoethanol (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 20 µl of Alamar Blue (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) 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 20 µl of Alamar Blue, the plates were incubated again and the OD measured 24, 48 and 72 h later as described. Amphotericin B (Sigma-Aldrich) was used as 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 the drug concentrations that inhibit 50% cell growth or cell survival (IC_{50}) *via* linear interpolation.[45]

HIV-1 Infection Experiments:

HIV-1 infection experiments using the T-cell tropic (X4) strain NL4/3, 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 x 10^7 cells were resuspended in 500 µl culture medium without drugs and incubated at 37 °C for 3 h with 100 ng of HIV-1 viral stocks. 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 x 10^5 / mL) of infected cells were further cultured in 24-well plates (triplicates) in the presence of the drugs (dissolved in DMSO) at various concentrations, or in medium with DMSO as control for the calculation of the inhibition of virus replication. Culture medium was changed and cells were splited twice a week post-infection. 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.

Table 1: Antimicrobial activity of all piperidone compounds against *P. falciparum* (NF-54), *T. brucei brucei* and *L. major* as well cytotoxicity measured in macrophages (J774.1), 7d. 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 b.b.*) > 100 µM; Ciclopiroxolamine 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

a) 4-Oxo-piperidine 3,5-dicarboxylates

No	**Ar**	**R^1**	**R^2**	**IC_{50} [µM] *P. falciparum***	**ED_{50} [µM] *T. brucei brucei***	**ED_{50} [µM] *L. major***	**ED_{50} [µM] macrophage after 48h**
1	2-Pyridine	CH_3	H	n.a.	28.09 ± 1.52	> 100	> 100
2	2-Pyridine	CH_3	Allyl	n.a.	26.48 ± 7.64	> 100	> 100
3	2-Pyridine	CH_3	2-Hydroxyethyl	n.a.	22.84 ± 5.57	> 100	31.15 ± 0.14
4	2-Pyridine	CH_3	3-Hydroxypropyl	n.a.	18.29 ± 2.59	> 100	32.02 ± 0.63
5	2-Pyridine	CH_3	2-(2-Hydroxy-ethoxy)ethyl	n.a.	24.14 ± 4.27	65.04 ± 3.60	38.48 ± 6.17
6	2-Pyridine	CH_3	3-Carboxypropyl	n.a.	19.29 ± 2.28	> 100	46.00 ± 5.92
7	2-Pyridine	CH_3	7-Carboxypentyl	n.a.	23.98 ± 4.48	50.34 ± 8.26	33.83 ± 2.81
8	2-Pyridine	CH_3	2-Pyridinylmethyl	n.a.	4.62 ± 1.39	> 100	29.87 ± 2.32
9	2-Pyridine	CH_3	Benzyl	10.88 (72h) 10.20 (24h)	11.13 ± 1.91	55.99 ± 6.45	33.99 ± 1.56
10	4-NO_2phenyl	CH_3	Allyl	n.a.	2.41 ± 0.39	> 100	50.29 ± 11.70
11	4-NO_2phenyl	C_2H_5	Allyl	11.03 (72h) + (m)	3.06 ± 0.55	n.d.	> 100
12	4-NO_2phenyl	CH_3	Benzyl	11.91 (72h) + (m)	3.01 ± 0.27	> 100	18.39 ± 2.93
13	4-NO_2phenyl	C_2H_5	Benzyl	10.06 (72h) + (m)	2.72 ± 0.51	n.d.	> 100
14	3-NO_2phenyl	CH_3	4-Chlorbenzyl	n.a.	10.57 ± 2.83	>100	46.64 ± 9.86
15	3-NO_2phenyl	CH_3	4-Methoxybenzyl	n.a.	3.93 ± 1.10	>100	> 100
16	3-NO_2phenyl	CH_3	4-Methylbenzyl	n.a.	2.66 ± 0.49	>100	49.78 ± 16.80
17	3-NO_2phenyl	CH_3	Benzyl	n.a.	2.49± 0.79	>100	> 100
18	Benzyl	CH_3	Allyl	n.a	4.86 ± 0.63	n.d.	> 100

b) 4-Oxo-piperidine 3-monocarboxylates

No	R	IC_{50} [µM] *P. falciparum*	ED_{50} [µM] *T. brucei brucei*	ED_{50} [µM] *L. major*	ED_{50} [µM] macrophage after 48h
19	Benzyl	n.a.	1.37 ± 0.02	25.97 ± 6.76	33.17 ± 0.69
20	4-Methylbenzyl	n.d.	0.47 ± 0.17	83.83 ± 10.38	34.87 ± 0.45
21	4-Chlorbenzyl	12.03(72h) 1.7 (48h)	0.32 ± 0.09	87.93 ± 32.99	32.84 ± 0.49
22	4-Methoxybenzyl	n.d.	0.43 ± 0.17	30	29.86 ± 0.33
23	3-Methoxybenzyl	n.d.	> 100	> 100	> 100
24	Allyl	> 40 1.4 (24h)	1.08 ± 0.48	50	(35)
4-Oxo-1,4,5,6-tetrahydropyridine 3-monocarboxylates					
25	Benzyl	4.7 (24h)	> 100	n.d.	n.d.
26	4-Chlorbenzyl	18 (24h)	n.d.	n.d.	n.d.
4-Oxo-1,4-dihydropyridine 3-monocarboxylates					
27	4-Chlorbenzyl	9.4 (36h)	> 100	> 100	> 100
28	4-Methylbenzyl	40 (48h) 9.1 (24h)	n.d.	n.d.	n.d.

c) Spiropiperidines

No	R	R^1	R^2	IC_{50} [µM] *P. falciparum*	ED_{50} [µM] *T. brucei brucei*	ED_{50} [µM] *L. major*	ED_{50} [µM] macrophage after 48h
33	CH_3	Phenyl		n.a.	> 100	>100	>100
34	C_2H_5	Benzyl		n.a.	> 100	>100	>100
35	C_3H_7	Phenyl		n.a.	> 100	>100	36.02 ± 3.97
36	C_4H_9	Phenyl		n.a.	24.36 ± 14.10	>100	n.d.
37	CH_3	Benzyl	2-Hydroxyethyl	n.a.	> 100	>100	> 100
38	C_2H_5	Phenyl	Benzyl	n.a.	20.28 ± 3.55	n.d.	n.d.
39	CH_3	Benzyl	4-Nitrobenzyl	14.57(72h)	3.74 ± 0.77	>100	42.44±14.49
40	C_2H_5	Phenyl	2-Phenylethyl	n.a.	18.05 ± 5.34	41.10 ± 3.57	32.30 ± 2.52
41	C_2H_5	Phenyl	3-Phenylpropyl	n.a.	13.44 ± 6.69	>100	>100
44				n.a.	30.97 ± 2.18	>100	>100
45				n.a.	25.37 ± 1.61	>100	> 100
47				n.a.	44.65 ± 15.50	n.d.	n.d.

d) Oximes and corresponding ethers

	IC_{50} [µM] ***P. falciparum***	**ED_{50} [µM]** ***T. brucei brucei***	**ED_{50} [µM]** ***L. major***	**ED_{50} [µM] macrophage after 48h**
48	26.0	n.d.	n.d.	n.d.
49	31.0	> 100	>100	>100
52	n.d.	> 100	>100	>100
53	8.29 (72h) 11.83 (24h)	3.86 $\pm$ 1.95	30.72 $\pm$ 3.15	28.39 $\pm$ 5.92

n.a. = no activity; + (m) = activity in mice; n.d. not determined

Scheme 1[a] Polyamine pathway and available inhibitors of the enzymes

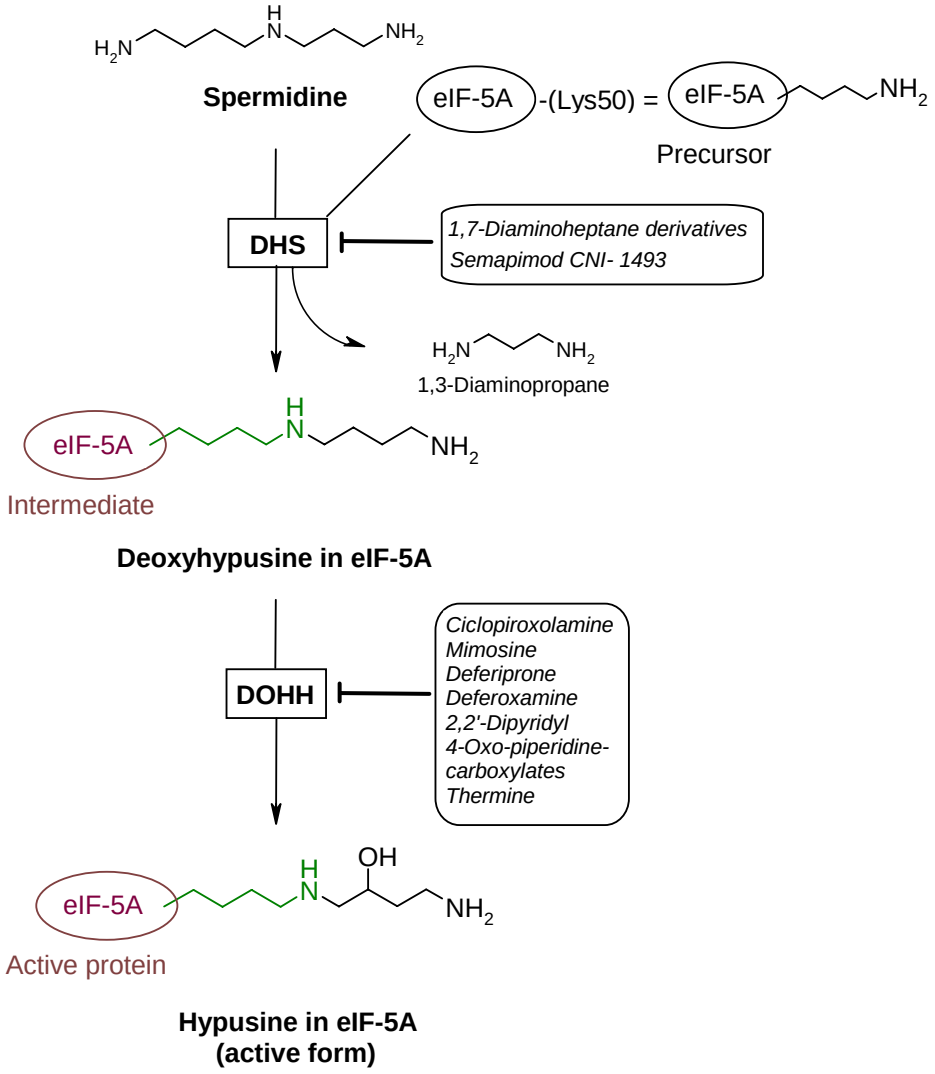
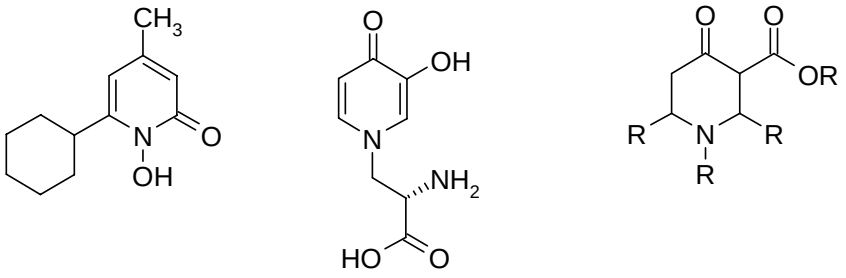


Figure 1a



(1) (2) (3)

Figure 1b

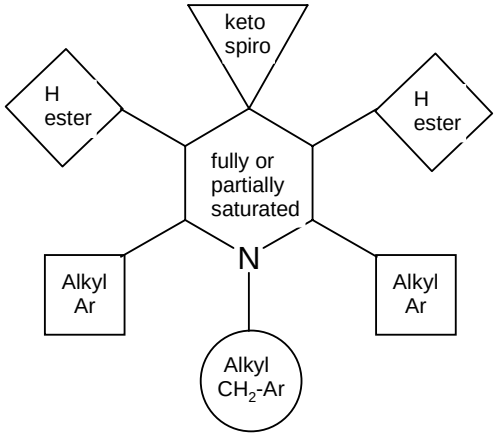


Figure 2.

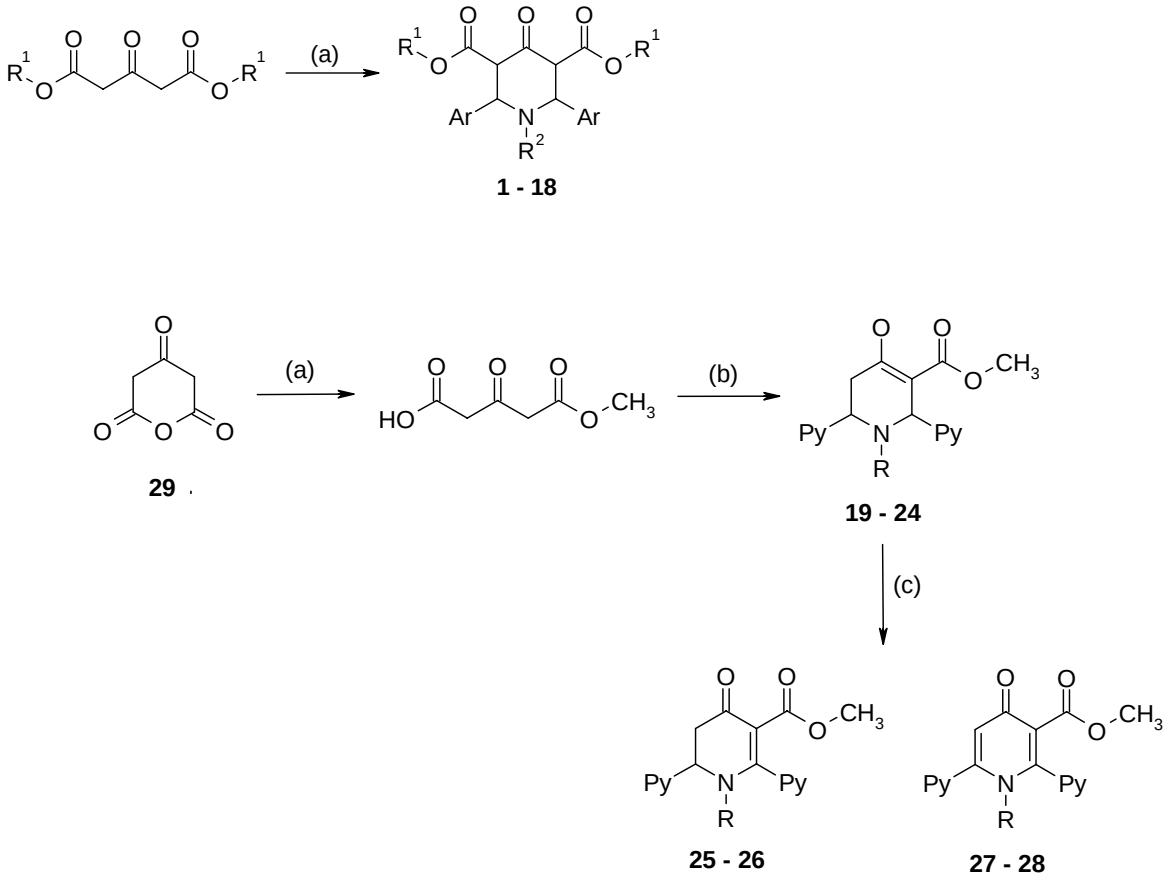


Figure 3

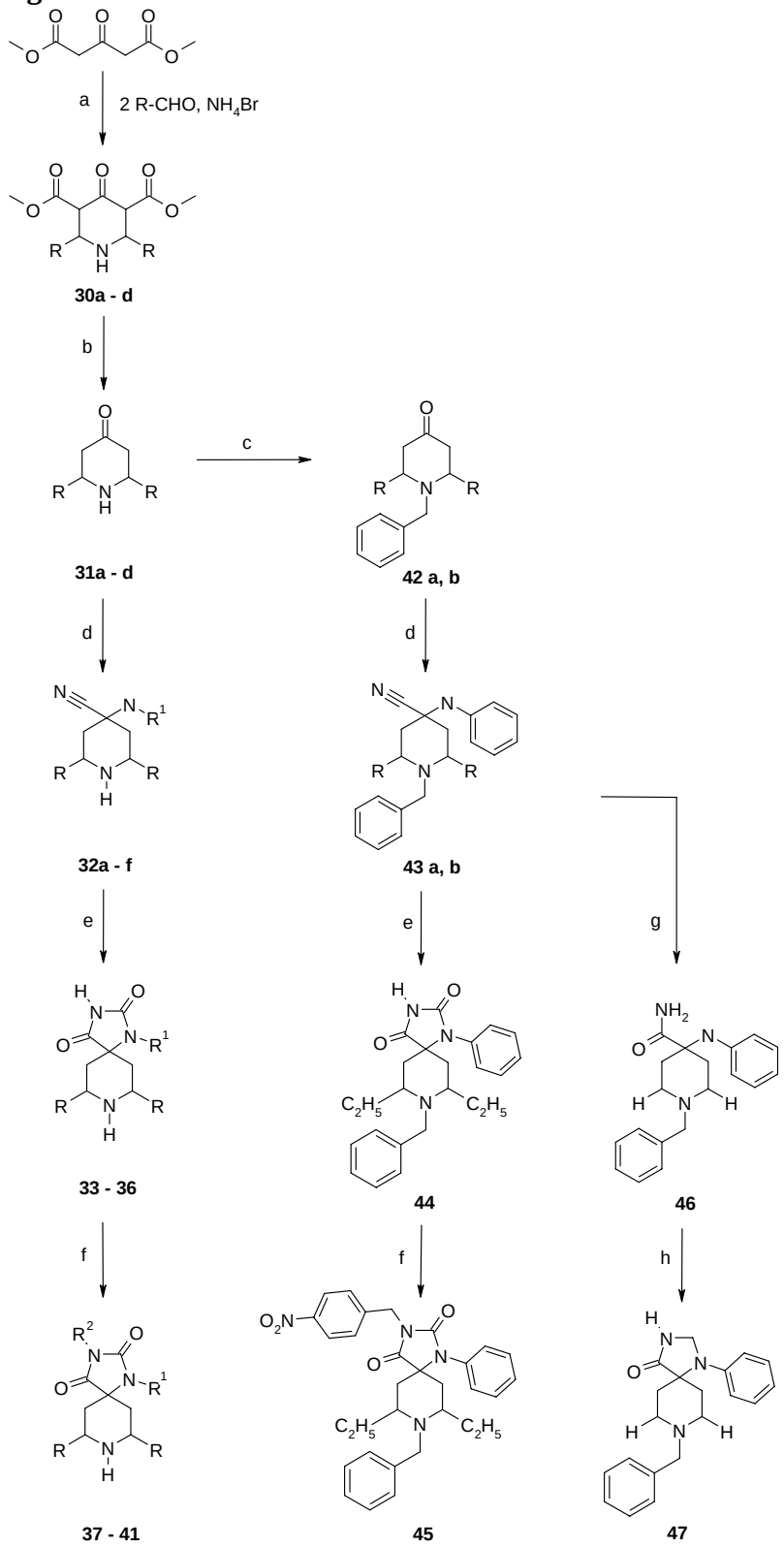
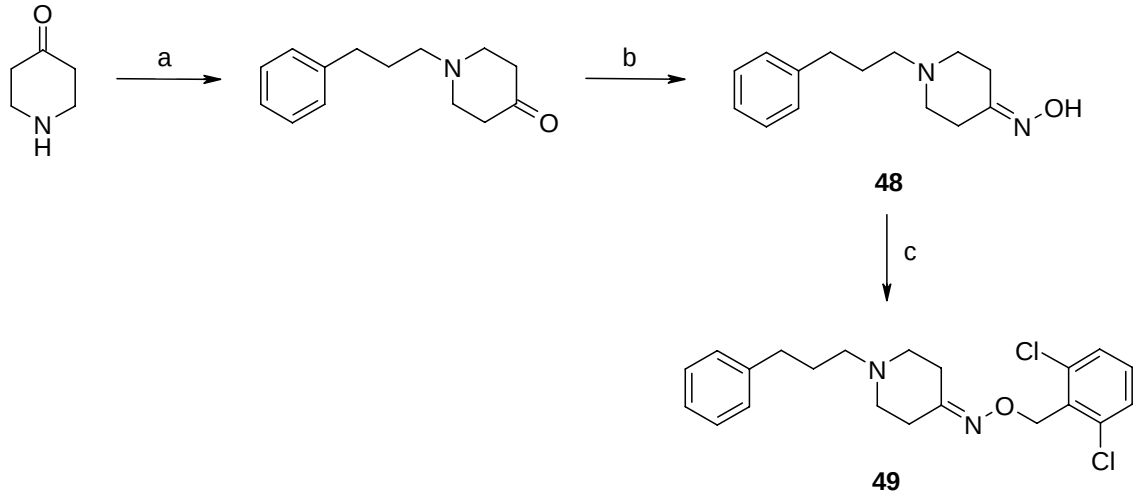

a
2 R-CHO, NH4Br
30a - d
b
31a - d
c
42 a, b
d
32a - f
43 a, b
e
33 - 36
44
g
46
f
37 - 41
45
h
47

Figure 4



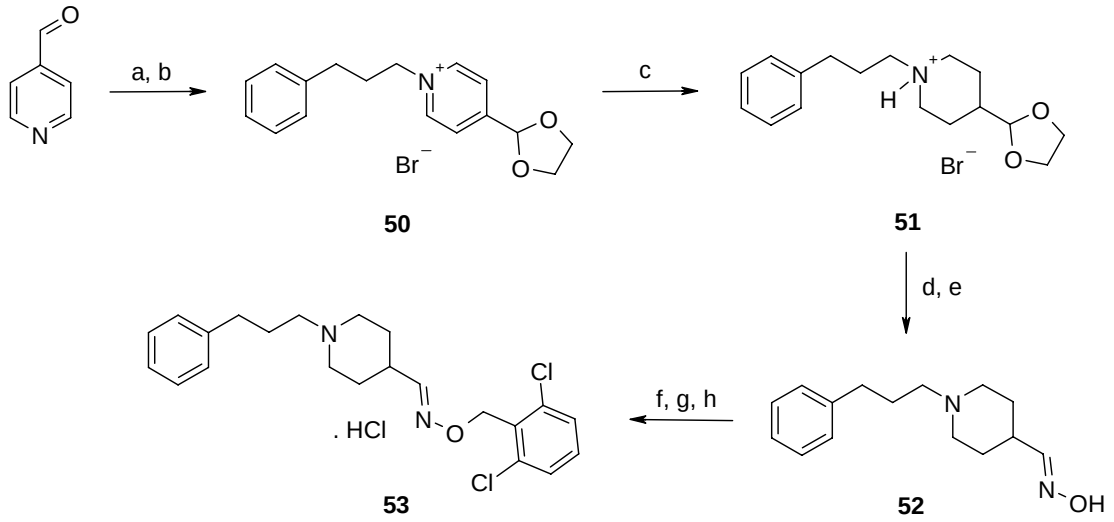
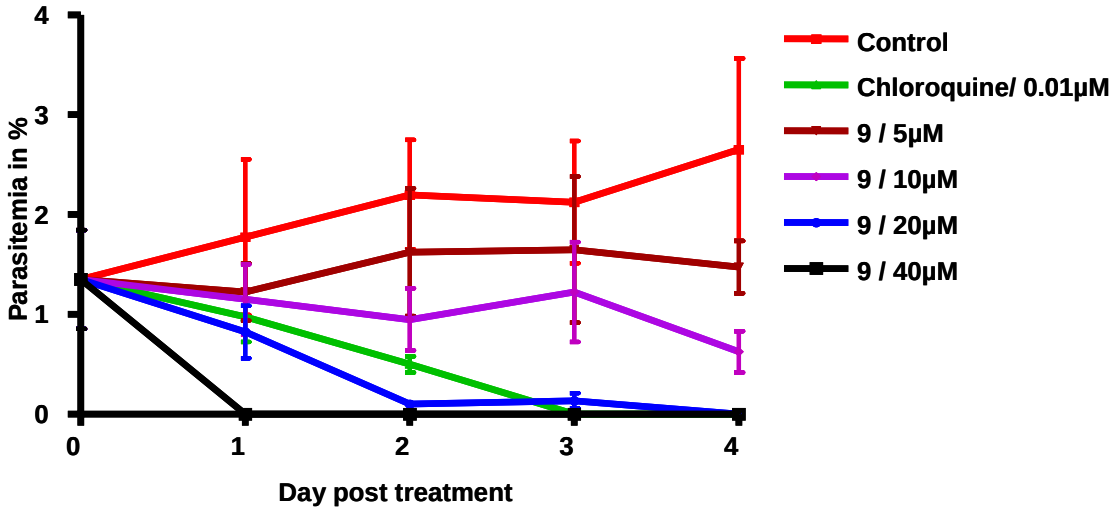
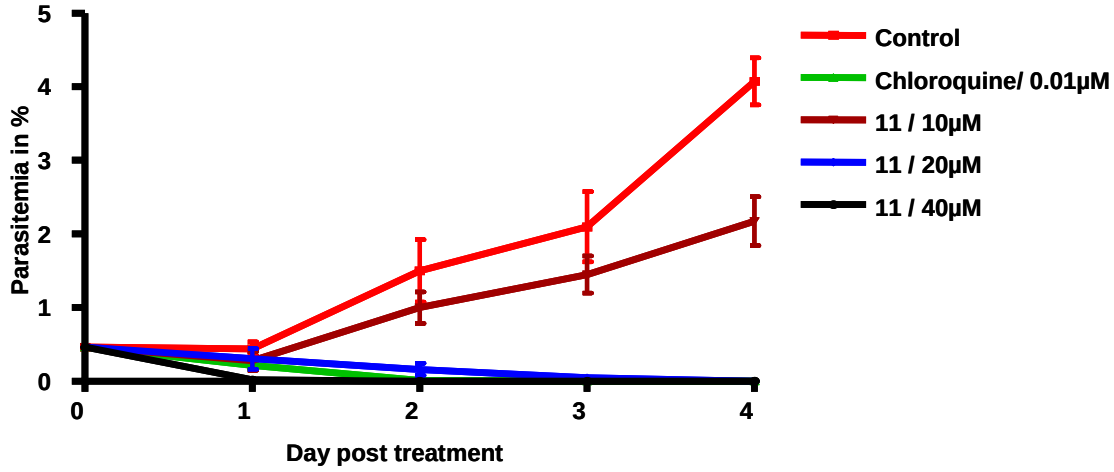


Fig. 5

a)


Parasitemia in %
Day post treatment
Control
Chloroquine/ 0.01µM
9 / 5µM
9 / 10µM
9 / 20µM
9 / 40µM

b)


Parasitemia in %
Day post treatment
Control
Chloroquine/ 0.01µM
11 / 10µM
11 / 20µM
11 / 40µM

c)

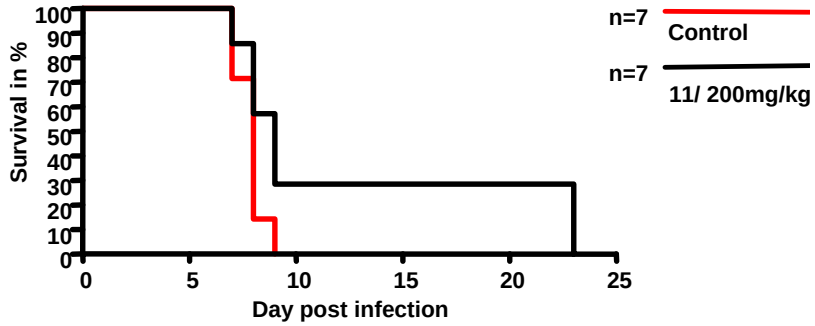

Survival in %
Day post infection
n=7
Control
n=7
11/ 200mg/kg

Fig. 6

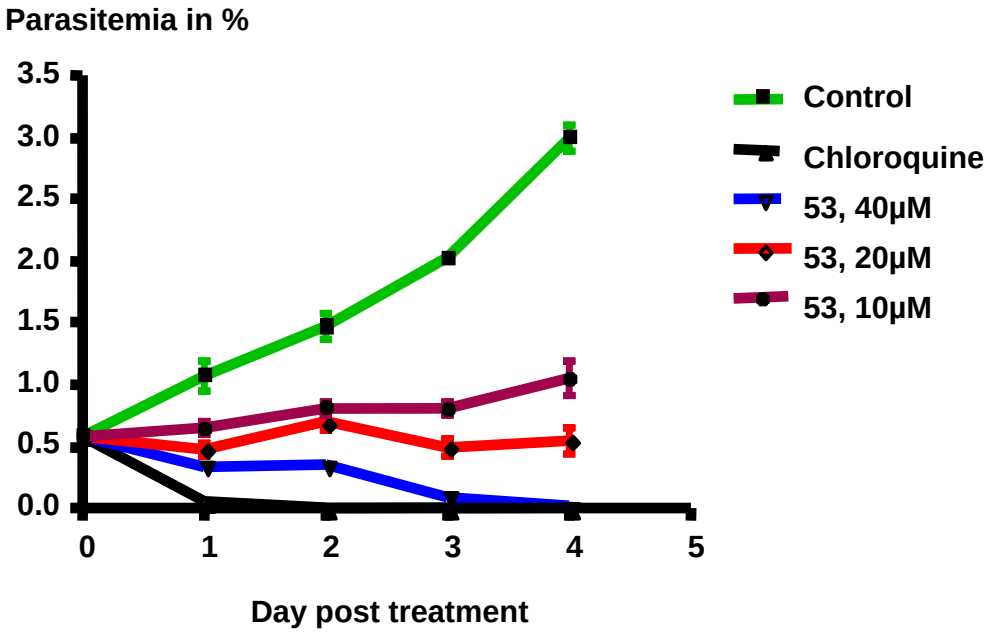

Parasitemia in %
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
Day post treatment
Control
Chloroquine
53, 40µM
53, 20µM
53, 10µM

Figure 7:

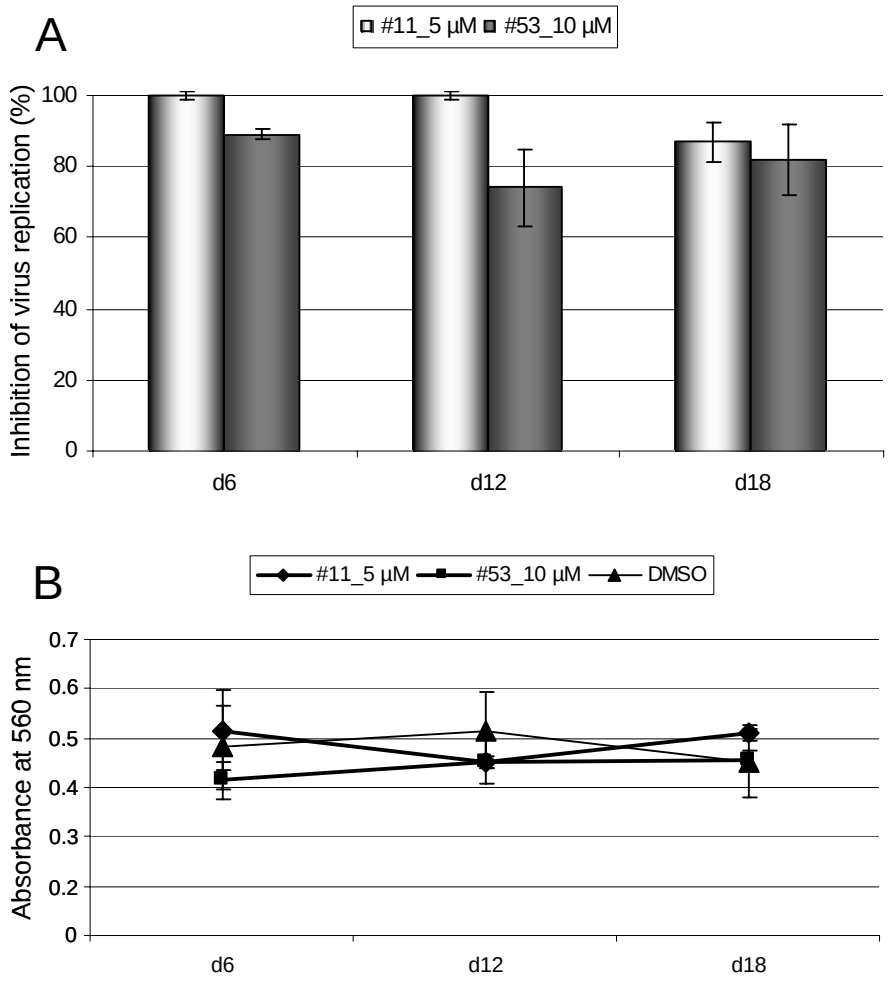

A
#11_5 µM
#53_10 µM
Inhibition of virus replication (%)
100
80
60
40
20
0
d6
d12
d18
B
#11_5 µM
#53_10 µM
DMSO
Absorbance at 560 nm
0.7
0.6
0.5
0.4
0.3
0.2
0
d6
d12
d18

Figure Legends:

Scheme 1: Polyamine pathway and available inhibitors of the enzymes. DHS = Deoxyhypusine synthase; DOHH = Deoxyhypusine hydroxylase

Figure 1a: Structural formulae of the DOHH inhibitors ciclopiroxolamine (1), L-mimosine (2), and 4-oxo-piperidone 3-carboxylate (3)

Figure 1b: Points of variation of the piperidine skeleton

Figure 2: Synthesis pathway to 4-oxo-piperidine 3,5-dicarboxylates **1 – 18 (**Reagents: (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: (a) MeOH abs. ,25 °C; (b) 2 eq 2-pyridine aldehyde, R-NH_2, -5 °C; (c) $Ce(SO_4)_2$, 0 – 5 °C)

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

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, EtOH abs.; (c) 2,6- dichlorobenzylchloride, KOtBu, 55 °C. Reagents and conditions for **52** and **53**: (a) ethylene glycole, pTosOH, toluene; (b) $Ph(CH_2)_3Br$, 2h, 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- dichlorobenzylchloride; (h) MeOH / HCl

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

Figure 6: Parasitemia of C57BL/6 mice treated i.v. with **53**

Figure 7: Inhibition of HIV-1 BaL replication by piperidones. HIV-1 BaL-infected PM1 cells were cultured in presence of the indicated concentrations of the compounds **11**, **53** or DMSO as solvent control. Culture medium was changed at day 6 and 12 post-

infection and the cells were split 1:1. $p24^{Gag}$ antigen levels and cell viabilities were determined at day 6, 12 and 18. (A) The percentage of inhibition of virus replication in the drug-treated cell culture, as compared to the untreated controls is shown. (B) Cell viabilities from the same cultures were determined by alamarBlue™ assay (Serotec).

References

[1] Greenwood, B.M.; Bojang, K.; Whitty, C.J.M.; Targett, G.A.T. Malaria. *Lancet* **2005**, *365*, 1487-1498.

[2] http://www.who.int/tdr/diseases/tryp/; http://www.who.int/tdr/diseases/chagas/diseaseinfo.htm; http://www.who.int/leishmaniasis/en/

[3] D´Silva, C. Human African trypanosomiasis: future propects for chemotherapy. *Drugs Fut*. **2007**, *32*, 149-160.

[4] Projan, S.J. Why is big Pharma getting out of antibacterial drug discovery. *Curr. Op. Microbiol*. **2003**, *6*, 427-430.

[5] Nwaka, S.; Hudson, A. Innovative lead discovery strategies for tropical diseases. *Nat. Drug Discov.* **2006**, *5*, 941-955.

[6] Müller, S.; Da'dara A.; Lürsen K.; Wrenger C.; Das Gupta, R.; Madhubala, R.; Walter, R. D. In the human malaria parasite *Plasmodium falciparum*, polyamines are synthesized by a bifunctional ornithine decarboxylase, S-adenosylmethionine decarboxylase. *J. Biol. Chem*. **2000***, 275*, 8097-8102.

[7] Haider, N.; Eschbach, M. L; Dias Sde, S.; Gilberger, T. W.; Walter; R. D.; Luersen, K. The spermidine synthase of the malaria parasite *Plasmodium falciparum*: Molecular and biomchemical characterization of the polyamine synthesis enzyme. *Mol. Biochem. Parasitol*. **2005***, 142*, 224-236.

[8] Casero Jr, R.A.; Marton, L.J. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. *Nature Rev. Drug Discov*. **2007**, *7*, 373-390

[9] Das Gupta, R.; Krause-Ihle, T.; Bergmann, B.; Muller, I.B.; Khomutov, A.R.; Müller, S.; Walter, R,D,; Luersen, K. 3-Aminooxy-1-aminopropane and derivatives have an antiproliferative effect on cultured *Plasmodium falciparum* by decreasing intracellular polyamine concentrations. *Antimicrob. Agents Chemother*. **2005**, *49*, 2857-2864.

[10] Kaiser, A.; Gottwald, A.; Wiersch, C.; Lindenthal, B.; Maier, W.; Seitz, H.M. Effect of drugs inhibiting spermidine biosynthesis and metabolism on the in vitro development of *Plasmodium falciparum*. *Parasitol. Res*. **2001**, *87*, 963-972.

[11] Park, M.H.; Wolff, E.C., Folk, J.E. Hypusine: Its post-translational formation in eukaryotic initiation factor 5A and its potential in cellular regulation. *Biofactors* **1993**, *4*, 95-104.

[12] Abbruzzese, A.; Park, M.H.; Folk, J.E. Deoxyhypusine hydroxylase from rat testis. Partial purification and characterization. *J. Biol. Chem*. **1988**, *261*, 3085-3089.

[13] Park, M.H.; Wolff, E.C. Cell-free synthesis of deoxyhypusine. Separation of protein substrate and enzyme and identification of 1,3-diaminopropane as a product of spermidine cleavage. *J. Biol. Chem*. **1988**, *263*, 15264-15269.

[14] Njuguna, J.T.; Nassar, M.; Hoerauf, A.; Kaiser, A.E. Cloning, expression and functional activity of deoxyhypusine synthase from *Plasmodium vivax*. *BMC Microbiol*. **2006**, *6,* 91

[15] Clement, P.M.; Hanauske-Abel, H.M.; Wolff, E,C,; Kleinmann, H.K.; Park, M.H. The antifungal drug ciclopirox inhibits deoxyhypusine and proline hydroxylation, endothelial cell growth and angiogensis in vitro. *Int. J. Cancer* **2002**, *100*, 491-498.

[16] Dong, Z.; Arnold, R.; Yang, Y.; Park, M.H.; Hrncirova, P.; Mechref, Y.; Novotny, M.V.; Zhang, J.T. Modulation of differentiation-related gene-1 expression by cell cycle blocker mimosine, revealed by proteomic analysis. *Mol. Cell. Proteomics* **2005**, *4*, 993- 1001.

[17] Park, M.H. The post-translational synthesis of a polyamine-derived amino acid, hypusine, in the eukaryotic translation initiation factor 5A (eIF-5A). *J. Biochem*. **2006**, *139*, 1-9.

[18] Cotton, F.A, Wilkinson, G. *Advanced Inorganic Chemsitry*, 5th Ed. Wiley, New York **1988**, p.710

[19] Haller, R. Metallchelate pyridyl-(2)-substituierter Piperidone und Piperidinole. *Arch. Pharm*. **1968**, *301*, 741-749.

[20] Saeftel, M.; Sarite, R.S.; Njuguna, T.; Holzgrabe, U.; Ulmer, D.; Hoerauf, A.; Kaiser, A. Piperidones with activity against *Plasmodium falciparum*. *Parasitol. Res*. **2006**, *99*, 281-286.

[21] Bevec, D.; Jaksche, H.; Oft, M.; Wohl, T.; Himmelspach, M.; Pacher, A.; Schebesta, M.; Koettnitz, K.; Dobrovnik, M.; Csonga, R.; Lottspeich, F.; Hauber, J. Inhibition of HIV-1 replication in lymphocytes by mutants of the Rev cofactor eIF-5A. *Science* **1996**, *271,* 1858-1860.

[22] Schäfer, B.; Hauber, I.; Bunk, A.; Heukeshoven, J.; Düsedau, A.; Bevec, D.; Hauber, J. Inhibition of multi-resistant HIV-1 by interference with cellular S-adenosylmethionine decarboxylase activity. *J. Inf. Dis*. **2006**, *194*, 740-750.

[23] Hauber, I.; Bevec, D.; Heukeshoven, J.; Krätzer, F.; Horn, F.; Choidas A.; Harrer, T.; Hauber, J. Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy. *J. Clin. Invest*. **2005**, *115*, 76-85.

[24] Hart, R.A.; Billaud, J.N.; Choi, S.J.; Phillips, T.R. Effects of 1,8-diaminooctane on the FIV Rev regulatory system. *Virology* **2002**, *304*, 97-104.

[25] Andrus, L.; Szabo, P.; Grady, R. W.; Hanauske, A.-R.; Huima-Byron, T.; Slowinska, B.; Zagulska, S.; Hanauske-Abel, H. M. Antiretroviral effects of deoxyhypusyl hydroxylase inhibitors. *Biochem. Pharmacol*. **1998**, *55*, 1807-1818.

[26] Kaiser, A.; Ulmer, D.; Goebel, T.; Holzgrabe, U.; Saeftel, M.; Hoerauf, A. Inhibition of hypusine biosynthesis in plasmodium: a possible, new startegy in prevention and therapy of malaria. *Mini-Rev. Med. Chem*. **2006**, *6*, 1231-1241.

[27] Merz, K.W.; Haller, R. Synthesen mit Pyridin- und Chinolinaldehyden. *Pharm. Acta Helv.* **1963**, *38*, 442-456.

[28] Ashauer-Holzgrabe, U.; Haller, R. Zur Umsetzung von N-benzylsubstituierten Piperidoncarbonsäuren mit Cer(IV)sulfat. *Arch. Pharm. (Weinheim)* **1986**, *319*, 1079-1083.

[29] Kuhl, U.; Englberger, W.; Haurand, M.; Holzgrabe, U. Diazabicyclo[3.3.1]nonanone-type ligands for the opioid receptors. *Arch. Pharm. Pharm. Chem*. **2000**, *333*, 226-230.

[30] Holzgrabe, U.; Piening, B.; Kohlmorgen, R.; Stoll, E. Synthese verschieden substituierter 5-Oxo-2,6-methano-2-benzazocine. *Arch. Pharm*. *(Weinheim)* **1988**, *321*, 917-920.

[31] Siener, T.; Holzgrabe, U.; Drosihn, S.; Brandt W. Conformational and configurational behaviour of κ-agonistic 3,7-diazabicyclo[3.3.1]nonan-9-ones - synthesis, nuclear magnetic resonance studies and semiempirical PM3 calculations. *J. Chem. Soc. Perkin 2*, **1999**, 1827-1834.

[32] Smith, M.B.; March, J. *March´s advanced organic chemistry*, 5th ed., Wiley-Interscience Publ., New York **2001**, p. 1240.

[33] Röver, S.; Adam, G.; Cesura, A.M.; Galley, G.; Jenck, F.; Monsma, F.J.; Wichmann, J.; Dautzenberg, F.M. High-affinity, non-peptide agonists for the ORL1 (Orphanin FQ/Nociceptin) receptor. *J. Med. Chem*. **2000**, *43*, 1329-1338.

[34] Holzgrabe, U.; Erciyas, E. Synthese und Stereochemie potentiell stark analgetischer 2,4-m-diarylsubstituierter 3,7-Diazabicyclo[3.3.1]nonan-9-on-1,5-diester. *Arch. Pharm. (Weinheim)* **1992**, *325*, 657-663.

[35] Holzgrabe, U.; Piening, B.; Hesse, K.-F.; Höltje, H.-D.; Worch, M. Stereochemistry of 2,6-dipyridine substituted N-benzyl-4-piperidone mono- and dicarboxylates and the corresponding reduction products. *Z. Naturforsch*. **1988**, *44b*, 565-574.

[36] Haller, R.; Kohlmorgen, R. Synthese und Struktur substituierter N-Benzyl-piperidoncarbonsäureester. *Arch. Pharm*. *(Weinheim)* **1976**, *309*, 206-214.

[37] Haller, R. Zur Synthese 2,6-disubstituierter Piperidonmonocarbonsäureester. *Arch. Pharm*. **1965**, *298*, 787-794.

[38] Holzgrabe, U.; Piening, B.; Kohlmorgen, R.; Stoll, E. Cer(IV)oxidationen von ß-Aminoketonen. 5. Mitt. Synthese verschieden substituierter 5-Oxo-2,6-methano-2-benzazocine. *Arch. Pharm*. *(Weinheim)* **1988**, *321*, 917-920.

[39] van Parys, M.; Vandewalle, M. The Synthesis of 1,8-Diazaspiro[4,4]decanes. *Bull Soc Chim Belg* **1981**, *90*, 749-756

[40] Taber, D.F.; Rahimizadeh, M. Amide to ester conversion: A practical route to the carfentanil class of analgetics. *J. Org. Chem.* **1992**, *57*, 4037-4038.

[41] Trager, W.; Williams, J. Extracellular (axenic) development *in vitro* of the erythrocytic cycle of *Plasmodium falciparum*. *Proc. Nat. Acad. Sci*. **1992**, *89*, 5351-5355.

[42] Moloney, M.B.; Pawluk, A.R.; Ackland, N.R. *Plasmodium falciparum* growth in deep culture. *Trans. R. Soc. Trop. Med. Hyg*. **1990**, *84*, 516-518.

[43] Singh, S.; Puri, S.K.; Singh, S.K.; Srivastava, R.; Das Gupta, R.C.; Pandey, V.C. Characterization of simian malarial parasite (*Plasmodium knowlesi*)-induced putrescine transport in rhesus monkey erythrocytes. A novel putrescine conjugate arrests *in vitro* growth of simian malarial parasite (*Plasmodium knowlesi*) and cures multidrug resistant murine malaria (*Plasmodium yoelii*) infection *in vivo*. *J. Biol. Chem*. **1997**, *272*, 13506-13511.

[44] Räz, B.; Iten, M.; Grether-Bühler, Y.; Kaminsky, R.; Brun, R. The Alamar Blue assay to determine drug sensitivity of African trypanosomes (*T. brucei rhodesiense* and *T. brucei gambiense*) in vitro. *Acta Trop*. **1997**, *68*, 139-147.

[45] Huber, W.; Koella, J.C. A comparison of three methods of estimating EC_{50} in studies of drug resistance of malaria parasites. *Acta Trop*. **1993**, *55*, 257-261.

[46] Baltz, T.; Baltz, D.; Giroud, C.; Crocket, J. Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense. EMBO J*. **1985**, *4,* 1273-1277.

[47] Ahmed, S.A.; Gogal, R.M.; Walsh, J.E. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [^{3}H]thymidine incorporation assay. *J. Immunol. Methods* **1994**, *170*, 211-224.

[48] Ponte-Sucre, A.; Vicik, R.; Schultheis, M.; Schirmeister, T.; Moll, H. Aziridine-2,3-dicarboxylates: Peptidomimetic cysteine protease inhibitors with antileishmanial activity *Antimicrob. Agents Chemother*. **2006**, *50*, 2439-2447.