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Short communication

Synthesis and antiprotozoal activity of original porphyrin precursors and derivatives

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ABSTRACT

Importance of heme in African trypanosomes, *Leishmania* sp. and *Plasmodium* sp. metabolisms justifies considering the potential of porphyrins and their precursors and derivatives as potential antiparasitic agents by interfering with heme metabolism. Consequently, twenty-four porphyrin precursors and derivatives were evaluated against *Leishmania donovani*, *Trypanosoma brucei* and *Plasmodium* sp. The best active compound against *Trypanosoma brucei brucei* was a new porphyrin derivative; compound **4i**, with a MEC value of 6.25 μM justifying further *in vivo* evaluation. Whereas these compounds were not active against intramacrophage amastigotes of *L. donovani*, another new porphyrin derivative, compound **4f** was active *in vitro* against *Plasmodium falciparum* at 20 nM and a slight delay of mice survival was observed on the *Plasmodium berghei*/Swiss mice model at 50 μmol/kg/day × 4. Pharmacomodulations should be further developed relying on a better knowledge on the porphyrin behaviour into the parasites comparatively to host cells.

1. Introduction

Malaria, leishmaniasis and human African trypanosomiasis are parasitic diseases responsible for high mortality and disability in tropical and sub-tropical areas [1]. The main limitations of classical drugs rely on their toxicity, and problems of drug resistance justifying the search for new compounds [2]. In order to get more specific drugs, a rational approach in the research of new antiparasitic compounds is to take advantage of different biochemical pathways (e.g.) between the parasite and its mammalian host.

Trypanosomatid protozoa such as *Leishmania* and *Trypanosoma* parasites require an exogenous source of heme for growth and transformation because of their inability to synthesize heme since they lack several key enzymes in the heme biosynthetic pathway [3]. Heme is a critical prosthetic group for proteins involved in metabolism and electron transport in these parasites. Leishmanias transform and grow only in medium containing either heme, usually supplied as hemin or protoporphyrin IX [4]. On one hand, compounds complexing heme, such as xanthones are toxic for *Leishmania* [5]. On the other hand, heme plays an important role in the cell host–parasite relationships on the *in vitro* intramacrophage *Leishmania* amastigote model [6]. These data suggest that derivatives of porphyrins could be efficient antileishmanial drugs by interfering with parasite heme metabolism.. Thus, dimethyl and diethyl carbaporphyrin ketals inhibit the growth of *Leishmania tarentolae* promastigotes *in vitro* [7].

Plasmodium parasites use host hemoglobin for their nutrition into erythrocytes, as a source of amino-acids, releasing the heme scaffold. Heme is highly toxic for *Plasmodium* parasites and heme detoxification is a critical step in the life cycle of the parasite, achieved by crystallization into physiologically insoluble hemozoin [8]. Porphyrins are interesting compounds to be tested against *Plasmodium* parasites because of their structural analogy with those of heme. Thus, any inhibition of heme detoxification is a rational strategy to fight the parasite.

Thus, all these data justify considering porphyrin derivatives as potential antiparasitic agents against *Trypanosoma*, *Leishmania* and *Plasmodium* parasites.

Consequently, this paper reports on the synthesis of dipyrrinato-metallates (**3a**–**d**), obtained from dipyrromethenes (**2a**–**e**), the latter being prepared from the known corresponding dipyrromethanes (**1a**–**e**). Porphyrins (**4a**–**k**) were also prepared through a known procedure and the synthesized products were evaluated for their antileishmanial, trypanocidal and antimalarial properties.

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2. Chemistry

Dipyrromethanes 1a–e were known and prepared through a published procedure from pyrrole and the desired aromatic aldehydes (Scheme 1) in the presence of trifluoroacetic acid [9,10] in excellent yields (62–89%).

Dipyrromethenes 2a–e were prepared, through a published method [11] from corresponding dipyrromethanes **1a–e** by DDQ mediated oxidation (Scheme 2) in good yields (53–95%).

Dipyrrinato-metallates 3a–d were prepared for the first time by mixing the corresponding dipyrromethenes **2c,d** and the iron (II) [12] or manganese (II) [13] salts (Scheme 3) in the presence of NEt_3 in a 1:1 mixture of MeOH : chloroform under reflux (39–98% yield) [14].

Porphyrins 4a–e, g and k were prepared as already reported by us [15]. Unreported porphyrin **4i** was prepared from dibromoporphyrin **4d** by a palladium-mediated cross coupling with pyrrolidinone in 11% yield as a secondary product besides major porphyrin **4j** [16]. Unknown porphyrin **4f** was prepared in 52% yield by saponification of porphyrin **4g** (Scheme 4).

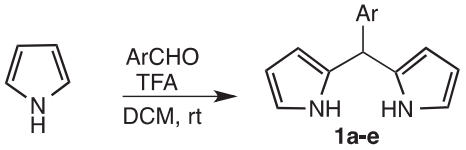
3. Biology

3.1. Antileishmanial evaluation

Promastigote forms of *Leishmania donovani* (MHOM/ET/67/HU3) were grown in M-199 medium supplemented with 40 mM HEPES, 100 μM adenosine, 0.5 mg/L haemin, 10% heat-inactivated foetal bovine serum (FBS) and 50 μg/mL gentamycin at 26 °C in a dark environment under an atmosphere of 5% CO_2. All the experiments were performed with parasites in their logarithmic phase of growth. Differentiation of promastigotes into axenic amastigotes was achieved by dilution of 1×10^6 promastigotes in 5 mL of axenic amastigote media (15 mM KCl; 8 mM glucose; 5 mM glutamine, 1 X M-199, 2.5% BBL_trypticase_peptone, 4 mM haemin, and 20% FBS). The pH was adjusted to pH 5.5. Axenic amastigotes were grown at 37 °C in 5% CO_2.

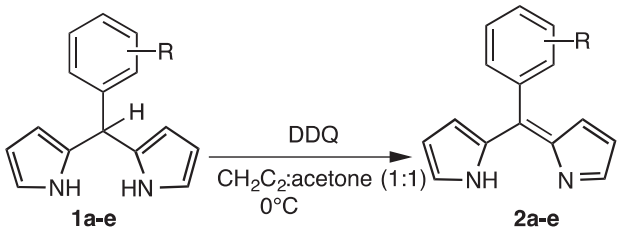
3.1.1. In vitro antileishmanial evaluation on promastigote forms

Promastigote forms from logarithmic phase culture were suspended to yield 10^6 cells/mL. Miltefosine was used as antileishmanial reference compound. Compounds to be evaluated and miltefosine were distributed in the plates by making a serial dilution. The final concentrations used were between 100 μM and 50 nM. Triplicates were used for each concentration. After a 3-day incubation period at 27 °C in the dark and under a 5% CO_2 atmosphere, the viability of the promastigotes was assessed using the tetrazolium-dye (MTT) colorimetric method, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent, a detergent solution (Triton X100, HCl) was added to lyse the cells and dissolve the coloured crystals. The absorbance at 570 nm, directly proportional to the number of viable cells, was measured using an ELISA plate reader. The results are expressed as the concentrations inhibiting parasite growth by 50% (IC_{50}) ± SD after a 3-day incubation period.



Entry	Aldehyde	TFA(equiv)	Yield(%)
1	PhCHO	0.1	**1a** : 89
2	2-Br-PhCHO	0.5	**1b** : 74
3	2,3,4,5,6-F_5-PhCHO	0.1	**1c** : 62
4	3,4,5-$(OMe)_3$-PhCHO	0.5	**1d** : 71
5	2,6-Cl_2-PhCHO	0.1	**1e** : 70

Scheme 1.



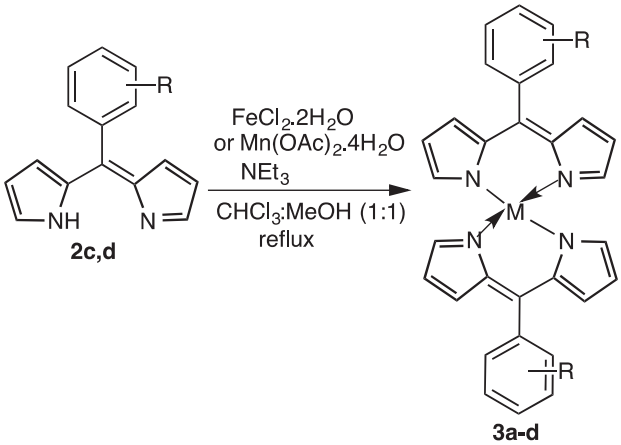
Entry	Dipyrromethane	Yield(%)
1	**1a** : R = H	**2a** : 68
2	**1b** : R = 2-Br	**2b** : 98
3	**1c** : R = 2,3,4,5,6-F_5	**2c** : 72
4	**1d** : R = 3,4,5-$(OMe)_3$	**2d** : 39
5	**1e** : R = 2,6-Cl_2	**2e** : 39

Scheme 2.

3.1.2. In vitro antileishmanial evaluation on intramacrophage amastigotes

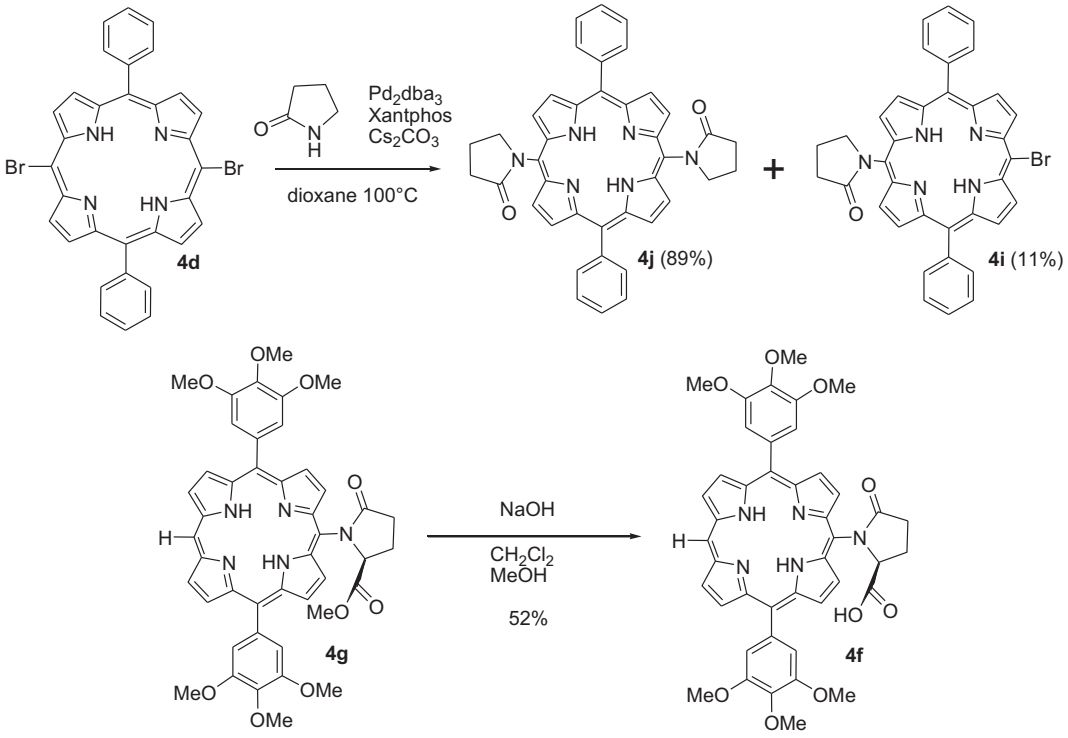
Concerning the amastigote *in vitro* model, the peritoneal macrophages of Swiss mice (female, 18–20 g, Janvier, France) were infected after a 24 h incubation period with promastigote forms of *L. donovani* in a stationary phase at a ratio of 10 parasites per macrophage, to obtain 87% of infected macrophages and 10 ± 3 amastigotes per macrophage. At 18 h after the promastigotes had entered macrophages, the free promastigotes were eliminated and intramacrophagic amastigotes were treated at various concentrations of the compounds.

Pentamidine and amphotericin B were used as reference compounds. Each experiment was performed in triplicate. The culture



Entry	Dipyrromethene	Metal	Yield(%)
1	**2d** : R = 3,4,5-$(OMe)_3$	M = Fe	**3a** : 68
2	**2d** : R = 3,4,5-$(OMe)_3$	M = Mn	**3b** : 98
3	**2c** : R = 2,3,4,5,6-F_5	M = Fe	**3c** : 72
4	**2c** : R = 2,3,4,5,6-F_5	M = Mn	**3d** : 39

Scheme 3.



Scheme 4.

medium was renewed 48 h later and a new culture medium containing the drug was added. The experiment was stopped at day 5, and the percentage of infected macrophages was evaluated microscopically after Giemsa staining. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, and expressed in μM ± SD.

3.2. In vitro trypanocidal evaluation

The drug incubation infectivity test (DIIT) was used for compound evaluation following a protocol previously described [17].

3.3. Antimalarial evaluation

3.3.1. In vitro antimalarial evaluation

Plasmodium falciparum 3D7 strain was maintained in O+ human erythrocytes in albumin RPMI supplemented medium under continuous culture using the candle-jar method [18]. The parasites were synchronized to the ring stage by repeated sorbitol treatment [19]. A 5%(v/v) erythrocytes suspension with 0.5% parasitemia (number of parasites per 100 red blood cell) was incubated with the compounds to be tested, previously dissolved in DMSO. Parasites were also incubated with culture medium (negative control) or with 4 μM chloroquine (positive control) in 96-well culture plates. After 44 h incubation at 37 °C, the plates were subjected to 3 freeze-thaw cycles to achieve complete hemolysis. The parasite growth was determined by the ELISA-Malaria antigentest (DiaMed, France), for the detection of *P. falciparum* lactate dehydrogenase (pLDH), as well as by microscopic examination under oil immersion of Giemsa-stained thin blood smears. Results were expressed as the percentage of reduction of parasite growth over the control receiving only the culture medium. Blood smears were prepared after 24 and 44 h by Giemsa staining. Parasitemia were determined by microscopic counting of at least 5000 erythrocytes under oil immersion.

3.3.2. In vivo antimalarial evaluation Plasmodium berghei

NK-173, a strain free of contamination with *Eperythrozoon coccoides* and sensitive to chloroquine, was used for *in vivo* antimalarial evaluation. This strain is known to induce high mortality in mice, providing a good model to estimate survival and antimalarial efficacy in reducing parasitemia, and is sensitive to all currently used antimalarial drugs.

Animal experiments were carried out according to the Principles of Laboratory Animal Care and legislation in force in France. Female outbred eperythrozoon-free Swiss albino mice weighing 18–20 g were obtained from Janvier, France. They were kept in a normal diurnal cycle and had free access to food and water throughout the experiments.

The four-day test was performed as described by Peters et al. [20]. An infective inoculum was prepared from a previously infected donor mouse with rising parasitemia (20%). On day 0 the mice were infected i.v. with 10^6 *P. berghei*-parasitized RBC in 0.2 ml of phosphate-buffered saline. They were randomly divided into groups of 10 and treated once daily, by the i.p. route with the compound for four consecutive days (days 0–3). The treatment was stopped when some toxicity was observed. Control groups received 100 μmoles chloroquine/kg. Thin blood smears were made from tail blood from untreated controls and from treated animals on days 4, 7, 8, 9, 10 and 14 after infection. Levels of parasitemia were measured in Giemsa-stained smears, and RBC numbers were determined on the same days. If mice were free from *Plasmodium* after at least 200 fields (magnification, ×1000) were checked, they were considered cured but were kept under observation for 60 days in case of relapse.

3.4. Cytotoxicity

The toxicity of the compounds was evaluated on mouse peritoneal macrophages, which were then used for the

intramacrophagic amastigotes *in vitro* model. Peritoneal macrophages were harvested from female Swiss mice (Janvier, France) 3 days after an intraperitoneal injection of 1.5 ml of sodium thioglycolate (Biomérieux, France) and were dispensed into eight-well chamber slides (LabTek Ltd.) at a concentration of 5×10^4/well (400_µl/well) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 25 mM Hepes, and 2 mM L-glutamine (Life Technologies, Cergy-Pontoise, France). Four hours after the macrophages were plated, they were washed to eliminate fibroblasts. The culture medium was renewed 48 h later and a new culture medium containing the drug was added. The experiment was stopped at day 5, and the viability of macrophages was checked using the tetrazolium-dye (MTT) colorimetric method. The cytotoxic concentrations 50% (CC_{50}) were determined by linear regression analysis, expressed in µM ± SD.

4. Results and discussion

4.1. Antileishmanial activity

Twenty-four compounds were first evaluated against the promastigote forms of *L. donovani*, as a primary screening and seven of them exhibited an IC_{50} value of less than 10 µM (Table 1). The most active compound was compound **2c** with an IC_{50} value at 3.6 µM, which belongs to the dipyrrin series. Eventhough tetrapyrroles and dipyrroles were reported to show cytotoxicity against tumour cells [21], it is the first time that antileishmanial properties are disclosed for such compounds. Among this series, 5-aryldipyrromethanes **1a**–**d** were inactive or poorly active whereas 5-aryldipyrromethenes **2a**–**e** were homogeneously active in the same concentration range (3.61–5.55 µM). Among dipyrrinato-metallates, compound **3b** with manganese exhibited a similar activity (IC_{50} = 4.52 µM) whereas analogs were poorly active or inactive. To the best of our knowledge, this is the first report of antileishmanial activity for such a complex. 5,15-diarylporphyrins **4a**–**k** were poorly active except new compound **4i** with an IC_{50} value of 6.37 µM. These results are quite disappointing in view of previous activities observed with tetraaryl porphyrins [7,22–24]. All compounds with IC_{50} values less than 10 µM were evaluated on *L. donovani* intramacrophage amastigotes and were found inactive at 20 µM, without toxicity for the cells at this concentration.

4.2. Trypanocidal activity

The best active compound against *Trypanosoma brucei brucei* was new porphyrin derivative **4i**, with a MEC value of 6.25 µM (Table 1). This result has to be compared with other metallated porphyrins showing some activities against trypanosomes [25]. All 5-aryldipyrromethenes **2a**–**e** were active in a range from 12.5 to 50 µM and one dipyrrinato-metallate, compound **3b**, was active at 12.5 µM. However, these levels of activity did not justify an *in vivo* evaluation, except for compound **4i**.

4.3. Relationship between antileishmanial and trypanocidal activities

Except compound **3b**, which was active against *L. donovani* and not active against *T. brucei brucei*, these chemical series exhibited a strong positive correlation between antileishmanial and trypanocidal activities. This homogeneity to the drug susceptibility could suggest that a target common to leishmania and African trypanosomes could be affected.

4.4. Antimalarial activity

Only six porphyrin derivatives were evaluated *in vitro* against *P. falciparum* and new compound **4f** was the most active with an IC_{50} value of 20 nM (Table 1). This compound was poorly active against leishmania and African trypanosomes. Compound **4d** was also active but with an IC_{50} value of 200 nM and this activity was selective towards *Plasmodium*. To the best of our knowledge, this is the first report of antimalarial activity of a porphyrin derivative. The activity of compound **4f** justified an *in vivo* evaluation on the *P. berghei*/mice model. Compound **4f** was toxic at 130 µmol/kg with a slight delay of the animal death (Table 2). A dose regimen at 50 µmol/kg/day × 4 allowed a survival time of about 2 days comparatively to those of controls treated only with the excipient. Pyrroglutamic acid itself, a part of compound **4f**, was able to delay the animal death on about one day. Despite the dose regimen optimized to get the maximum antimalarial activity, discrimination between antimalarial activity and toxicity in animals could not be obtained. Thus, it was not possible to observe a dose for which an antimalarial activity was significant at Day 14 without toxicity.

5. Conclusion

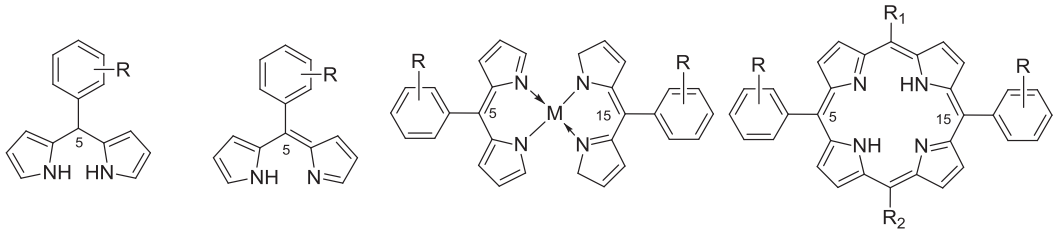
Among the four chemical series synthesized, three of them, 5-aryldipyrromethenes, dipyrrinato-metallates and 5,15-diarylporphyrins, can be considered as interesting leads for further pharmacomodulations because of their activity against three major parasites, leishmania, African trypanosomes and *Plasmodium*. Whereas these compounds were not active against intramacrophage amastigotes of *L. donovani*, one of them, new compound **4i** is worth of *in vivo* evaluation on the *T. brucei brucei*/Swiss mice model. More interestingly, new compound **4f** delayed the mice survival of *P. berghei* infected mice. The pharmacomodulations of these series will be optimized to get more active and less toxic analogues on the basis of a better knowledge of the porphyrin transport and action in *Plasmodium*, *Leishmania* and trypanosomes parasites.

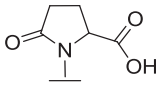
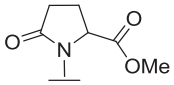
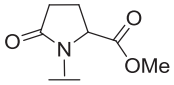
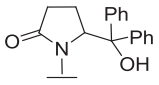
6. Experimental

6.1. General

^{1}H NMR spectra were recorded on 300 or 400 MHz in $CDCl_3$ and data are reported as follows: chemical shift in parts per million from tetramethylsilane as an internal standard, multiplicity (br = broad signal, s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, m = multiplet or overlap of non-equivalent resonances), integration. ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$ (unless otherwise specified) and data were reported as follows: chemical shift in parts per million from tetramethylsilane with the solvent as an internal indicator ($CDCl_3$ δ 77.0 ppm), multiplicity with respect to proton (deduced from JMOD experiments, br = broad signal, s = quaternary C, d = CH, t = CH_2, q = CH_3). Low Resolution Mass Spectra were recorded on an LC/MS equipment with ElectroSpray Ionisation method. Elemental analyses were recorded by our internal service. High-resolution mass spectra (HRMS) were performed by the service de masse (Gif-sur-Yvette IMAGIF – ICSN (Université Paris-Sud) on the spectrometer (LC) ESI/TOF (LCT, Waters) and MALDI-TOF (Voyager DE-STR, Applied Biosystems). UV–visible spectra were recorded on a Xenon source light wave diode spectrophotometer. DMF was distilled from CaH_2. Dichloromethane, THF, Et_2O, Toluene and were dried by filtration through activated molecular sieves. MeOH was dried by filtration through alumina. Other reagents were obtained from commercial suppliers and used as received.

Table 1
In vitro antileishmanial, antitrypanosomal, antimalarial and cytotoxic effects of porphyrins and their precursors and derivatives.



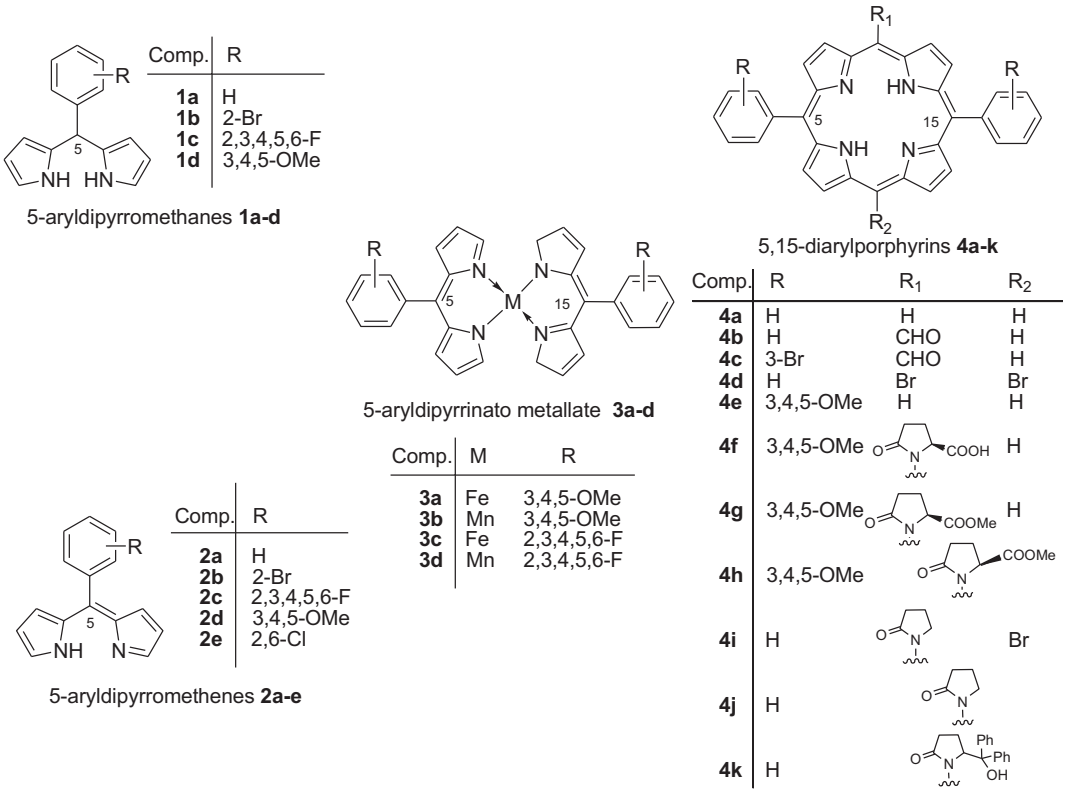
	R	R_1	R_2	Compound	*L. donovani* promastigote $IC_{50} \pm SD$ (μM)	*T. brucei brucei* MEC (μM)	*P. falciparum* $IC_{50} \pm SD$ (μM)	Cytotoxicity $CC_{50} \pm SD$ (μM)
DIPYRRINS	H	–	–	**1a**	≥100	>100	–	>100
	2-Br	–	–	**1b**	44.74 ± 2.80	>100	–	>100
	2,3,4,5,6-F_5	–	–	**1c**	44.1 ± 3.67	>100	–	>100
	3,4,5-$(OMe)_3$	–	–	**1d**	≥100	100	–	>100
	H	–	–	**2a**	5.23 ± 0.41	25	–	>100
	2-Br	–	–	**2b**	5.38 ± 0.47	50	–	>100
	2,3,4,5,6-F_5	–	–	**2c**	3.61 ± 0.43	12.5	–	>100
	3,4,5-$(OMe)_3$	–	–	**2d**	4.71 ± 0.23	25	–	>100
	2,6-Cl_2	–	–	**2e**	5.55 ± 0.28	12.5	–	>100
	3,4,5-$(OMe)_3$		–	**3a (Fe)**	56.79 ± 3.84	12.5	–	>100
	3,4,5-$(OMe)_3$	–	–	**3b (Mn)**	4.52 ± 0.33	>100	–	>100
	2,3,4,5,6-F_5	–	–	**3c (Fe)**	≥ 100	>100	–	>100
	2,3,4,5,6-F_5	–	–	**3d (Mn)**	58.77 ± 6.15[b]	100	–	>100
Porphyrins	H	H	H	**4a**	≥100	>100	>20	>100
	H	CHO	H	**4b**	≥100	>100	>20	>100
	3-Br	CHO	H	**4c**	≥100	>100	>20	>100
	H	Br	Br	**4d**	≥100	>100	0.2	>100
	3,4,5-$(OMe)_3$	H	H	**4e**	≥100	>100	>20	>100
	3,4,5-$(OMe)_3$		H	**4f**	57.23 ± 3.13	25	0.02	>100
	3,4,5-$(OMe)_3$		H	**4g**	≥100	–	–	>100
	3,4,5-$(OMe)_3$			**4h**	≥100	100	–	>100
	H		Br	**4i**	6.37 ± 0.42	6.25	–	>100
	H			**4j**	≥100	>100	–	>100
	H			**4k**	53.01 ± 4.40	50	–	>100
REF	Pentamidine			–	3.10 ± 0.21	5	–	26.3 ± 2.1
	Chloroquine			–	–	–	15–25	–
	Miltefosine			–	4.26 ± 0.40	–		15.8 ± 0.9

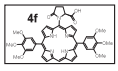
Analytical thin layer chromatography (TLC) was performed on silica gel plates visualized either with a UV lamp (254 nm), or by using solutions of *p*-anisaldehyde/sulfuric acid/acetic acid in EtOH or $KMnO_4/K_2CO_3$ in water followed by heating. Flash chromatographies were performed on silica gel (230–400 mesh). All the reactions were carried out under N_2 atmosphere unless specified. Only spectroscopic data of new compounds are described. It is worth noting that NMR analyses of dipyrromethene complexes **3a**–**e** were not satisfying because of the presence of Fe (II) or Mn(II), thus HRMS analyses were crucial for their structural determinations.

6.2. General protocol for the synthesis of dipyrromethenes ***2a***–***e***

To 5-aryldipyrromethane (1 g, 3.20 mol) in a 9:1/CH_2Cl_2:acetone mixture was added at 0 °C a solution of DDQ (799 mg, 3.52 mol, 1.1

Table 2
In vivo antimalarial activity of compound **4f** on the *P. berghei*/Swiss mice model.



Compound	Dose		Route of administration	Reduction parasitemia at Day 4 (%)	Quantal survival by days[a]					Toxicity: number of dead mice [day of death post-infection]	% Surviving mice at Day 30
	(! moles/kg/day × *n*)	mg/kg/day × *n*			Day 7	Day 8	Day 9	Day 10	Day 14		
	130 × 2	100 × 2	i.p.	60	5/10	5/10	5/10	4/10	0/10	3[Day 2]; 2[Day 4]	0
	100 × 1	76.9 × 1	i.p.	40	10/10	6/10	5/10	0/10	0/10	No toxicity	0
	50 × 1	38.5 × 1	i.p.	0	10/10	5/10	0/10	0/10	0/10	No toxicity	0
	50 × 4	38.5 × 4	i.p.		10/10	10/10	7/10	3/10	0/10	No toxicity	0
Pyrroglutamic acid	774 × 4	100 × 4	i.p.	/	10/10	10/10	5/10	0/10	0/10	No toxicity	0
Chloroquine	100 × 4	51.5 × 4	i.p.	100	10/10	10/10	10/10	10/10	10/10	No toxicity	100
Excipient	/	/	i.p.	0	10/10	6/10	0/10	0/10	0/10	No toxicity	0

[a] The numerators represent the number of surviving mice each day relative to the total number of mice in each group.

equiv.) in acetone (8 mL). Additional acetone (5 mL) was added and the reaction mixture was stirred at room temperature for 45 min. After concentration, the residue was stirred 10 min in cold $CHCl_3$/DCM (1:1), filtered, and washed with cold $CHCl_3$. The filtered was concentrated and the crude material was then purified over neutral alumina DCM/AcOEt +1%NEt_3 to yield the corresponding dipyrromethene.

*6.2.1. 5-Phenyldipyrromethene (**2a**)*

Reaction from 5-phenyldipyrromethane **1a** (50 mg, 0.225 mmol) and DDQ (56.2 mg, 0.47 mmol, 1.1 equiv.) following the general protocol afforded dipyrromethene **2a** (26 mg, 53%). ^1H ($CDCl_3$, 300 MHz) δ 6.40 (m, 2H, CH), 6.60 (m, 2H, CH), 7.47 (m, 5H, Ph-H), 7.65 (s, 2H, CH), 11.5 (brs, 1H, NH). ^{13}C ($CDCl_3$, 75 MHz) δ 143.6 (CH), 142.0 (CH), 140.9 (Cq), 137.3 (Cq), 130.8 (CH), 128.8 (CH), 127.6 (CH), 117.6 (CH). LRMS-ESI: *m*/*z* 221 $[M + H]^+$. HRMS-ESI: *m*/*z* calculated for $[M + H]^+$ $C_{15}H_{13}N_2$ 221.1079, obtained 221.1070.

*6.2.2. 5-(2-Bromophenyl)dipyrromethene (**2b**)*

Reaction from 5-(2-bromophenyl)dipyrromethane **1b** (50.0 mg, 0.166 mmol) and DDQ (41.5 mg, 0.183 mmol, 1.1 equiv) following the general protocol afforded dipyrromethene **2b** (47.3 mg, 95%). ^1H ($CDCl_3$, 300 MHz) δ 6.38 (m, 4H, CH), 7.32 (m, 1H), 7.40 (m, 2H), 7.64 (s, 2H), 7.68 (d, J = 7.8 Hz, 1H), 11.8 (brs, 1H, NH). ^{13}C ($CDCl_3$, 75 MHz) δ 144.1 (2CH), 140.5 (C), 139.3 (C), 137.9 (C), 132.6 (CH), 131.6 (CH), 129.9 (CH), 127.9 (2CH), 126.5 (CH), 123.6 (C–Br), 117.9 (2CH). LRMS-ESI: *m*/*z* 299 $[M + H]^+$ (^{79}Br). HRMS-ESI: *m*/*z* calculated for $[M + H] + 299.0184$, obtained 299.0185. R_f 0.49 (CH_2Cl_2).

*6.2.3. 5-Pentafluorophenyldipyrromethene (**2c**)*

Reaction from 5-(pentafluorophenyl)dipyrromethane **1c** (983 mg, 3.02 mmol) and DDQ (760 mg, 3.32 mmol, 1.1 equiv.) following the general protocol afforded dipyrromethene **2c** (638.9 mg, 68%). ^1H ($CDCl_3$, 400 MHz) δ 6.42 (dd, J = 4.2, 1.2 Hz, 2H), 6.48 (d, J = 4.2 Hz, 2H), 7.66 (s, 2H), 12.3 (brs, 1H, NH). ^{13}C ($CDCl_3$, 100 MHz) δ 145.4 (2CH), 143.5 (Cq), 140.4 (Cq), 139.5 (dm,

J = 190 Hz, C–F), 138.6 (m, Cq), 136.2 (m, Cq), 126.9 (2CH), 123.9 (Cq), 118.9 (2CH). ^{19}F ($CDCl_3$, 188 MHz) δ −161.41 (dd, J = 20.9, 14.8 Hz, 2F), −152.83 (tt, J = 20.9, 1.7 Hz, 2F), −138.76 (m, 1H). $[M + H]^+$. HRMS-ESI: m/z calculated for $[M + H]^+$ 311.0608, obtained 311.0608. R_f 0.63 (CH_2Cl_2).

*6.2.4. 5-(3,4,5-Trimethoxyphenyl)dipyrromethene (**2d**)*

Reaction from 5-(3,4,5-methoxyphenyl)dipyrromethane **1d** (1 g, 3.20 mmol) and DDQ (799 mg, 3.52 mmol) following the general protocol afforded dipyrromethene **2d** (690.8 mg, 69%). ^{1}H ($CDCl_3$, 300 MHz) δ 3.86 (s, 6H, 2OCH_3), 3.94 (s, 3H, OCH_3), 6.41 (d, J = 4.1 Hz, 2H), 6.71 (d, J = 4.1 Hz, 2H), 6.75 (s, 2H), 7.65 (s, 2H), 12.6 (brs, 1H, NH). ^{13}C ($CDCl_3$, 75 MHz) δ 152.3 (Cq), 143.6 (CH), 141.7 (Cq), 140.7 (Cq), 138.5 (Cq), 132.6 (Cq), 128.6 (CH), 117.5 (CH), 108.4 (CH), 60.9 (4OCH_3), 56.2 (3OCH_3). LRMS-APCI: m/z 310 $[M + H]^+$. HRMS-ESI: m/z calculated for $[M + H]^+$ $C_{18}H_{18}N_2O_3$ 311.1396, obtained 311.1396. R_f 0.62 (CH_2Cl_2).

*6.2.5. 5-(2,6-Dichlorophenyl)dipyrromethene (**2e**)*

Reaction from 5-(2,6-dichlorophenyl)dipyrromethane **1e** (500 mg, 1.71 mmol) and DDQ (428 mg, 1.89 mmol, 1.1 equiv.) following the general protocol afforded dipyrromethene **2e** (73.4 mg, 15%). ^{1}H ($CDCl_3$, 300 MHz) δ 6.37 (m, 4H, CH), 7.34 (dd, J = 9.0, 6.9 Hz, 1H), 7.40 (d, J = 7.0 Hz, 1H), 7.45 (d, J = 1.49 Hz, 1H), 7.64 (s, 2H), 11.8 (brs, 1H, NH). ^{13}C ($CDCl_3$, 75 MHz) δ 144.4 (CH), 139.9 (CH), 135.6 (C), 134.9 (C–Cl), 134.8 (C–Cl), 130.1 (C), 127.9 (CH), 126.7 (CH), 118.3 (CH). LRMS-ESI: m/z 290 $[M + H]^+$. HRMS-ESI: m/z calculated for $[M + H]^+$ 289.0299, obtained 289.0290. R_f 0.67 (CH_2Cl_2).

*6.3. General protocol for the synthesis of dipyrromethenes complexes **3a**–**e***

To a solution of dipyrromethene **2a**–**e** in 1:1/$CHCl_3$:MeOH was added Et_3N (1 equiv) followed by the metal salt, e.g. $FeCl_2 \cdot 2H_2O$ or $Mn(OAc)_2$ (10 equiv). The reaction mixture was then heated to reflux and controlled by TLC until complete conversion. The reaction mixture was then diluted with CH_2Cl_2, washed with brine and deionized water, dried over sodium sulfate, filtered, and concentrated under vacuum. The residue was purified over neutral alumina (CH_2Cl_2: Et_3N, 99:1) to furnish complexes (39–98%)

*6.3.1. Bis-(5-(3,4,5-methoxyphenyl)dipyrrinato)iron (II) complex (**3a**)*

Reaction from 5-(3,4,5-methoxyphenyl)dipyrromethene **2d** (345 mg, 1.11 mmol) and $FeCl_2 \cdot H_2O$ (10 equiv.) following the general protocol afforded bis-(5-(3,4,5-methoxy-phenyl)dipyrrinato) iron (II) complex **3a** (255.8 mg, 68%). ^{1}H ($CDCl_3$, 300 MHz) δ 10.46 (s, 4H), 4.90 (s, 12H), 4.66 (s, 6H). LRMS-APCI: m/z 674.4 $[M]^+$. HRMS-APCI: m/z calculated for $[M + H]^+$. $C_{36}H_{35}FeN_4O_6$ 674.1828, obtained 674.1815. R_f 0.26 (Cyclohexane/AcOEt: 7/3).

*6.3.2. Bis-(5-(3,4,5-methoxyphenyl)dipyrrinato)manganese (II) complex (**3b**)*

Reaction from 5-(3,4,5-methoxyphenyl)dipyrromethene **2d** (345 mg, 1.11 mmol) and $Mn(OAc)_2$ (10 equiv.) following the general protocol afforded bis-(5-(3,4,5-methoxy-phenyl)dipyrrinato)manganese (II) complex **3b** (395.1 mg, 98%). ^{1}H ($CDCl_3$, 300 MHz) δ 7.46 (brs, 4H), 3.91 (s, 6H), 3.81 (s, 12H). LRMS-APCI: m/z 674.2 $[M + H]^+$. HRMS-APCI: m/z calculated for $[M + H]^+$. $C_{36}H_{35}MnN_4O_6$ 674.1932, obtained 674.1921. R_f 0.17 (Cyclohexane/AcOEt: 7/3).

*6.3.3. Bis-(5-pentafluorophenyl)dipyrrinato)iron (II) complex (**3c**)*

Reaction from 5-(3,4,5-methoxyphenyl)dipyrromethane **2c** (388.9 mg, 1.25 mmol) and $FeCl_2 \cdot H_2O$ (10 equiv.) following the general protocol afforded bis-(5-pentafluoro-phenyl)dipyrrinato) iron (II) complex **3c** (302.1 mg, 72%). ^{19}F ($CDCl_3$, 188 MHz) δ −135.77 (brd, J = 11.3 Hz, 4F), −151.91 (t, J = 20.7 Hz, 2F), −160.23 (brt, J = 18.8 Hz, 4F). LRMS-APCI: m/z 674.2 $[M]^+$. HRMS-APCI: m/z calculated for $[M + H]^+$. $C_{30}H_{13}F_{10}FeN_4$ 674.0267, obtained 674.0267. R_f 0.87 (Cyclohexane/AcOEt:7/3).

*6.3.4. Bis-(5-pentafluorophenyl)dipyrrinato)manganese (II) complex (**3d**)*

Reaction from 5-(3,4,5-methoxyphenyl)dipyrromethane **2c** (200 mg, 0.65 mmol) and $Mn(OAc)_2$ (10 equiv.) bis-(5-pentafluorophenyl)dipyrrinato)manganese (II) complex **3d** (84 mg, 39%). ^{19}F ($CDCl_3$, 188 MHz) δ −142.32 (brs, 4F), −153.45 (t, J = 20.7 Hz, 2F), −161.60 (brs, 4F). LRMS-APCI: m/z 673.0 $[M + H]^+$. HRMS-APCI: m/z calculated for $[M]^+$. (^{55}Mn) $C_{30}H_{12}F_{10}MnN_4$ 673.0283, obtained 673.0277. R_f 0.7 (CH_2Cl_2).

*6.4. Synthesis of porphyrins (**4i**) and (**4j**)*

To a round bottom flask, containing a magnetic bar, solid reagents were weighed : 5-bromo-10,15-diphenylporphyrin **4d** (31 mg, 0.05 mmol, 1 equiv.), Pd_2dba_3 (1.6 mg, 0.0018 mmol, 10 mol %), Xantphos (4.1 mg, 0.0071 mmol, 40 mol%) and Cs_2CO_3 (23.2 mg, 0.071 mmol, 2 eq.) and the flask was purged with nitrogen. A solution of pyrrolidinone (30.8 μl, 0.40 mmol, 8 equiv.) in Dioxane was added and the flask was purged again with nitrogen. The reaction mixture was stirred at 100 °C for 24 h. The flask tube was cooled down to room temperature and then concentrated. The crude reaction mixture was then purified on a silica gel column (solid deposit) and purified affording the title porphyrin **4j** (28 mg, 89%) along mono coupled porphyrin **4i** (4 mg, 11%).

*6.4.1. 5,15-Di-(N-(2′-Pyrrolidinone-yl)-10,20-diphenylporphyrin (**4j**) [16]*

^{1}H ($CDCl_3$, 300 MHz) δ −2.87 (s, 2H), 2.83 (m, 4H), 3.24 (m, 4H), 4.73 (td, 4H, J = 6.7 Hz, J = 14.3 Hz), 7.79 (m, 6H, Ar), 8.17 (m, 4H, Ar), 8.90 (d, 4H, J = 4.9 Hz), 9.13 (d, 4H, J = 4.7 Hz). ^{13}C ($CDCl_3$, 75 MHz) δ 178.3 (N–C=O), 141.2 (Cq), 134.6 (CH), 127.8 (CH), 41.9 (CH_2), 32.0 (CH_2), 20.2 (CH_2). UV–visible (nm, ε, c 0.06 mM, CH_2Cl_2): 421 (36,860), 513 (7805), 547 (2623), 590 (2670), 646 (1696). LRMS-APCI: m/z 629 $[M + H]^+$. HRMS-MALDI: m/z calculated for $[M + H]^+$ $C_{40}H_{33}N_6O_2$ 629.2659, obtained 629.2629. IR (cm^{-1}): 2037, 2023, 1397, 1277, 750. R_f 0.27 (AcOEt).

*6.4.2. 5-Bromo-15-(N-(2′-pyrrolidinone-yl)-10,20-diphenylporphyrin (**4i**)*

^{1}H ($CDCl_3$, 400 MHz) δ −2.78 (s, 2H, 2NH), 2.82 (quint, J = 7.5 Hz, 2H), 3.23 (t, J = 7.9 Hz, 2H), 4.71 (t, J = 7.1 Hz, 2H), 7.78 (m, 6H, Ar), 8.17 (m, 4H, Ar), 8.86 (m, 4H, β-H), 9.10 (d, J = 4.5 Hz, 2H, β-H), 9.65 (d, J = 4.5 Hz, β-H). ^{13}C ($CDCl_3$, 100 MHz) δ 178.1 (N–C=O), 141.3 (Cq), 134.7 (CH), 134.6 (CH), 134.5 (CH), 128.1 (CH), 126.9 (CH), 126.8 (CH), 121.1 (Cq), 114.6 (Cq), 58.4 (CH_2), 32.0 (CH_2), 20.2 (CH_2). LRMS-APCI: m/z 624 $[M + H]^+$. HRMS-MALDI: m/z calculated for $[M + H]^+$ $C_{36}H_{27}N_5OBr$ 624.1393, obtained 624.1395. UV–visible (nm, ε 0.1 mM, $CHCl_3$): 421 (19,923), 517 (2070), 551 (1017), 593 (892), 652 (732). R_f 0.56 (AcOEt).

*6.5. 5,15-Di-(3,4,5-trimethoxyphenyl)-10-(5-oxopyrrolidine-2(S)-carboxylate)) porphyrin (**4f**)*

Methyl ester **4g** (133 mg, 0.17 mmol, 1 equiv.) was dissolved in a CH_2Cl_2/MeOH (1:1) mixture and NaOH 3M (1 ml) was added. The mixture was stirred at room temperature for 4 h then the reaction was neutralized with a solution of 10% HCl until pH 4–5 was reached. The solution was extracted with dichloromethane several

times and the different organic phases were combined, washed with distilled water and the solvent was removed on rotavapor affording carboxylic acid porphyrin **4f** after column chromatography purification on silica gel (68.7 mg, 52%). ^{1}H ($CDCl_3$, 300 MHz) δ −2.55 (bs, 2H, NH), 2.41–2.65 (m, 2H, CH_2), 2.66–2.96 (m, 2H,CH_2), 3.79 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.92 (s, 3H, OMe), 3.94 (s, 3H, OMe), 4.01 (s, 3H, OMe), 4.17 (s, 3H, OMe), 4.92 (m, 1H, CH–N), 7.32 (s, 2H, Ar), 7.38 (s, 2H, Ar), 8.48 (s, 1H), 8.90 (s, 2H), 9.02 (dd, J = 4.3 Hz, J = 13.5 Hz, 2H), 9.23 (s, 1H), 9.81 (s,1H), 10.06 (s, 1H), 10.25 (s, 1H), 12.43 (s, 1H, C(O)OH). ^{13}C ($CDCl_3$, 75 MHz) δ 177.9 (N–C═O), 151.5 (Cq), 137.8 (Cq), 136.5 (Cq), 134.0 (CH), 132.9 (CH), 132.3 (CH), 131.3 (CH), 130.4 (CH), 128.4 (CH), 125.8 (CH), 125.8 (CH), 120.1 (Cq), 112.7 (CH), 106.4 (CH), 61.2 (OCH_3), 61.1 (OCH_3), 56.3(OCH_3), 53.4 (CH_2). UV–visible (nm, ε, c 0.05 mM, MeOH): 419 (44,013), 508 (9449), 542 (2848), 583 (3406), 641 (1520). LRMS-APCI$^-$: m/z 768 [M − H]$^-$. HRMS-MALDI: m/z calculated for [M + H]$^+$ $C_{43}H_{40}N_5O_9$ 770.2821, obtained 770.2850. IR (cm^{-1}): 3300, 2366, 1581, 1408, 1126. R_f 0.17 (CH_2Cl_2/MeOH : 8/2).

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Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.06.002.

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