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Evaluation of Bis-Alkylamidoxime *O*-Alkylsulfonates as Orally Available Antimalarials

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The main threat to controlling malaria is the emerging multidrug resistance of *Plasmodium* sp. parasites. Bis-alkylamidines were developed as a potential new chemotherapy that targets plasmodial phospholipid metabolism. Unfortunately, these compounds are not orally available. To solve this absorption issue, we investigated a prodrug strategy based on sulfonate derivatives of alkylamidoximes. A total of 25 sulfonates were synthesized as prodrug candidates of one bis-*N*-alkylamidine and of six N-substituted bis-*C*-alkylamidines. Their antimalarial activities were evaluated in vitro against *P. falciparum* and in vivo against *P. vinckei* in mice to define structure–activity relationships. Small alkyl substituents on the sulfonate group of both C-alkyl- and N-alkylamidines led to the best oral antimalarial activities; alkylsulfonate derivatives are chemically transformed into the corresponding alkylamidines.

Introduction

Malaria is a significant cause of death and illness in children and adults, particularly in tropical countries. In 2010, there were an estimated 655 000 deaths by malaria and about 216 million cases.[1] Thanks to the Global Malaria Action Plan, 43 of the 99 countries with ongoing transmission documented decreases in the number of malaria cases by more than 50% in 2010 compared with 2000. However, mosquito resistance to insecticides and parasite resistance to antimalarial medicines still pose major threats to achieving global control of malaria. Indeed, resistance to antimalarial medicines has been documented for all classes of antimalarials,[2] including artemisinin derivatives.[3,4]

The emerging multidrug-resistant strains of *Plasmodium* parasites justify the development of new antimalarial chemotherapies. Therefore, Vial and co-workers studied phospholipid metabolism in *Plasmodium* and identified the effectors of de novo phosphatidylcholine biosynthesis as promising antimalarial targets.[5–8] Bis-thiazolium salts were further developed as choline analogues with potent antimalarial activity and lower toxicity (Figure 1).[9] This new strategy has been validated, and the lead compound **T3** [now named SAR97276 or Albitiazolium (INN)] has undergone the clinical trials for the parenteral treatment of severe malaria.[9–13] These new potent antimalarial agents contain permanent charges, which are the reason for their low oral bioavailability. Calas and co-workers thus developed bis-alkylamidine compounds[8,14] that are bioisosteres of bis-thiazolium salts and are potent antimalarial agents, acting as choline mimics. Indeed, their two cationic charges are due to the protonation of the alkylamidine functional group (base with pK_a ~12–14). The antimalarial potency of bis-alkylamidines against the human *P. falciparum* parasite correlates strongly with their high pK_a values, but their biscationic character also prevents oral absorption.[8,14] We have explored various approaches over the past few years to circumvent the physicochemical problems inherent with bis-alkylamidine compounds and to improve their oral bioavailability. We focused our work on the design of prodrug candidates, attempting to temporarily mask the positive charges of bis-alkylamidine derivatives.

Because the use of amidines is limited by their lack of oral bioavailability, Clement and co-workers studied pentamidine, an antiparasitic drug with a benzamidine moiety, and originally developed a prodrug strategy[15,16] based on the neutral benzamidoxime function.[17,18] Indeed, the cationic charge of benzamidines is masked by introducing an oxygen atom on the nitrogen atom of the amidine function. The resulting benzamidoximes are therefore less basic and remain unprotonated

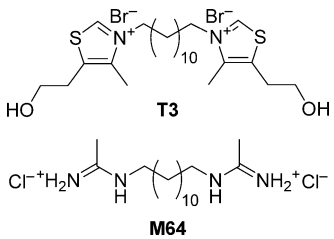

Figure 1. Lead compounds of bis-thiazolium salts and bis-*N*-alkylamidines.

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under physiological conditions. Clement's research group showed that reductases in the kidneys, liver, brain, lungs, and gastrointestinal tract are responsible for the rapid conversion of the inactive amidoximes to amidines.[19–21] The application of the amidoxime prodrug strategy to amidine-containing drugs led to bio-precursors with greater oral bioavailability than their parent drugs.[22–25] Consistent with these results, we previously reported that bis-alkylamidoxime derivatives have potent oral activities thanks to specific O-substituents.[26,27] The bis-methylsulfonate derivatives were able to temporarily mask the basic character and allowed oral delivery of the bis-alkylamidine drugs **M64** and **M34** with the highest antimalarial activities after oral administration (ED_{50} p.o. $< 50\ mg\,kg^{-1}$).

To the best of our knowledge, no other examples of *O*-alkylsulfonate amidoximes have been described as prodrug candidates. The bis-alkylamidoxime and corresponding methylsulfonate derivative were investigated as prodrug candidates of **M64**, the lead compound of the bis-*N*-alkylamidine series.[28] Pharmacokinetic studies in rat and in vitro metabolism by liver microsomes have shown that both prodrug candidates were converted into the active drug **M64**. However, transformation of the bis-methylsulfonate derivative was too rapid. Indeed, it is completely converted into **M64** after incubation for only 5 min.

The aim of this study was to generate new sulfonate derivatives as potential prodrugs in both the bis-*N*-alkylamidine and bis-*C*-alkylamidine series. We have shown that **M64** was the most potent drug in bis-*N*-alkylamidine series.[29] On the other hand, bis-*N*-substituents introduced on bis-*C*-alkylamidines could improve in vivo antimalarial potency relative to **M34**.[14,30,31] Indeed, the compounds shown in Figure 2[32] revealed IC_{50} values (drug concentration required to inhibit 50% parasite growth) lower than 15 nM and/or ED_{50} values after intraperitoneal (i.p.) administration lower than $10\ mg\,kg^{-1}$.[33] Consequently, we designed sulfonate prodrug candidates of the *N*-alkylamidine **M64** and of the six selected bis-*C*-alkylamidines **M38**, **M40**, **M42**, **M44**, **M45**, and **M46**, and defined structure–activity relationships governing their oral antimalarial activity (Figure 3).

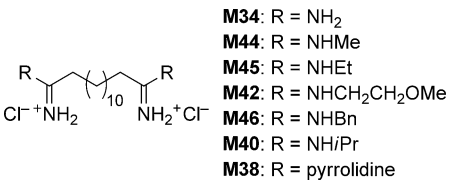


Figure 2. Modulation of the N-substituents of bis-*C*-alkylamidines.

Results and Discussion

Chemistry

The seven sulfonate prodrug candidates of bis-*N*-alkylamidine **M64** were prepared from bis-*N*-alkylamidoxime **8** (Scheme 1), which was obtained as previously described.[26] The bis-amidoxime **8** reacted with the suitable sulfonyl chloride reagents in the presence of pyridine to afford the targeted alkylsulfonates **1a**–**h**.[34] These could be prepared with satisfactory yields (60–80%), except for **1c**, presumably because 2-propanesulfonyl chloride is more hindered (39% yield).

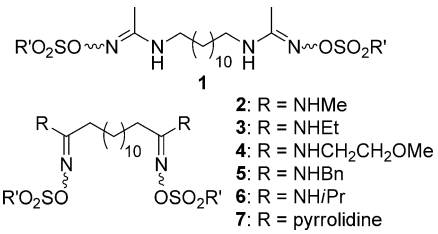
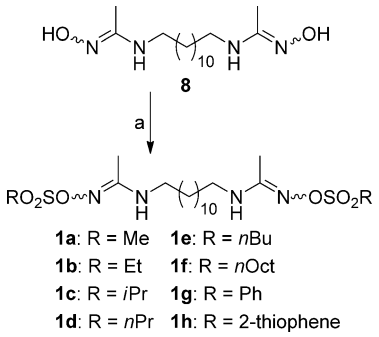


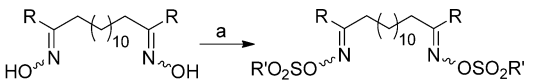
Figure 3. Targeted prodrug candidates of bis-*N*-alkylamidines and bis-*C*-alkylamidines.



Scheme 1. Synthesis of sulfonates in the bis-*N*-alkylamidine series. *Reagents and conditions:* a) sulfonyl chlorides, $CHCl_3$, pyridine, RT, 4 h. (yields **1a**–**c**, **e**–**h**: 60–80%; **1c**: 39%).

The targeted sulfonate prodrug candidates **2a**–**7c** of the six selected bis-*C*-alkylamidines **M38**, **M40**, **M42**, **M44**, **M45**, and **M46** were prepared from the corresponding N-substituted bis-*C*-alkylamidoximes **9a**–**f**, analogously to the previously described procedure (Scheme 2).[26,27] They were purified by precipitation in diethyl ether or by silica gel chromatography.

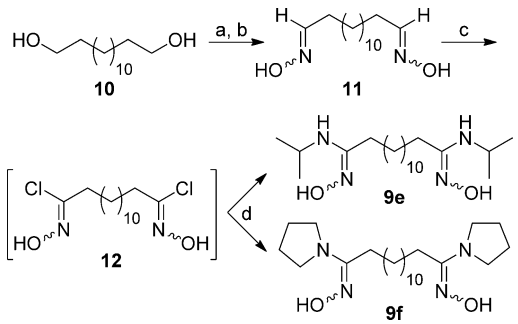
Two different routes were required to generate the N-substituted bis-*C*-alkylamidoximes **9a**–**f**. Indeed, the N-monosubstituted bis-*C*-alkylamidoximes **9a**–**d** could be prepared as previ-



	R	R' = Me	R' = Et	R' = *i*Pr
9a: R = NHMe	NHMe	**2a**	**2b**	**2c**
9b: R = NHEt	NHEt	**3a**	**3b**	**3c**
9c: R = $NHCH_2CH_2OMe$	$NHCH_2CH_2OMe$	**4a**	**4b**	**4c**
9d: R = NHBn	NHBn	**5a**	**5b**	**5c**
9e: R = NH*i*Pr	NH*i*Pr	**6a**	**6b**	**6c**
9f: R = pyrrolidine	pyrrolidine	**7a**	**7b**	**7c**

Scheme 2. Synthesis of *N*-alkylsulfonates in the bis-*C*-alkylamidine series. *Reagents and conditions:* a) alkylsulfonyl chlorides, pyridine, $CHCl_3$, RT, 4 h. (yields 41–72%).

ously described through N-alkylation of one oxadiazolone.[30] However, this strategy could afford neither the isopropyl compound **9e** in good yield nor the N,N-disubstituted compound **9f**. When we explored alternative access to N-substituted *C*-alkylamidoximes **9e**–**f**,[35] the hydroxyamination of bis-alkylthioamide derivatives did not lead to the expected compounds.[36,37] On the other hand, the second strategy based on the high reactivity of bis-hydroximinoyl chloride **12** was successful.[38] The *N*-isopropyl and *N*-pyrrolidinyl bis-*C*-alkylamidoximes **9e** and **9f** were generated in good yields in three steps by starting from the commercially available 1,14-tetradecanediol **10** and the corresponding amines (Scheme 3).[39]



Scheme 3. Synthesis of *C*-alkylamidoximes **9e** and **9f**. *Reagents and conditions:* a) IBX, DMSO, RT, 4 h; b) $NH_2OH{\cdot}HCl$, NEt_3, EtOH, RT, 16 h (74% over two steps); c) NCS, anhyd DMF, RT, 1 h; d) 2-propylamine or pyrrolidine, NEt_3, Et_2O, RT, 16 h (**9e**: 81%; **9f**: 82% over two steps).

The 1,14-tetradecanediol **10** was first oxidized by freshly prepared 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX) in DMSO.[40] The resulting bis-aldehyde was directly condensed with the hydroxylamine. The generated 1,14-tetradecanedioxime **11** reacted with *N*-chlorosuccinimide (NCS) to prepare the reactive hydroximinoyl chloride intermediate **12**.[41,42] This late 1,14-bis-(N^1,N^{14}-dihydroxyimidoyldichloride)tetradecane **12** was directly coupled with isopropylamine or pyrrolidine.[43] The desired bis-*C*-alkylamidoximes **9e** and **9f** were isolated as their respective hydrochloride salts owing to purification by reversed-phase chromatography. The pure neutral bis-amidoximes **9e** and **9f** were retrieved after neutralization. As previously observed for the amidines,[30] the *N*-methyl- and *N*-ethyl-bis-*C*-alkylamidoximes **9a** and **9b** could not be obtained by this strategy. All structures of the synthesized compounds were consistent with their ^1H and ^{13}C NMR, MS (ESI), and FTIR characterizations.

Optimization of the sulfonate prodrug strategy

Our main goal was to investigate more extensively the sulfonate prodrug strategy. Indeed, we previously reported that the bis-*O*-methylsulfonate derivative **1a** (**M64SMe**) constituted a new prodrug candidate with an ED_{50} p.o. value less than 50 mg kg^{-1}.[26] This was the first important step to improve the oral antimalarial activity of the bis-*N*-alkylamidines. This derivative **1a** was not sufficiently stable in biological media, as it is totally metabolized into the parent drug **M64** after 5 min at 37 °C.[28] This instability may be linked to 1) the the mode of introduction of the alkyl linker on the amidoxime function (on the carbon atom in the bis-*C*-alkylamidine series, or on the nitrogen atom in the bis-*N*-alkylamidine series), or 2) the methyl substituent of the sulfonate group. Here, the influence of this last parameter was studied by using the model of analogues of **1a**. An aromatic ring or an alkyl chain was introduced onto the sulfonate group in the bis-*N*-alkylamidine series, varying the length, steric bulk, lipophilicity, and electronic effects of these molecules. These modulations aimed at optimizing the stability and physicochemical properties, and at enhancing oral bioavailability and thus oral antimalarial activity. We focused on lipophilicity, one of the most important parameters. Calculated Clog *P* values are listed in the Table 1. As expected, the longer the alkyl chain, the higher the Clog *P* value; aromatic rings also led to enhanced Clog *P* values.

Table 1. In vitro and in vivo antimalarial activities of *O*-substituted sulfonate derivatives **1a**–**h** in *N*-alkylamidine series.

Compd	R′	Log *P*[a]	IC_{50} [nM][b]	ED_{50} [mg kg^{-1}][c] i.p.	p.o.
1a[d]	Me	3.97	12	4.7	42
1b	Et	5.03	12	7.2	60
1c	*i*Pr	5.73	49	5.8	90
1d	*n*Pr	6.09	14	>5[e]	>180
1e	*n*Bu	7.16	2.25	>5[e]	>180
1f	*n*Oct	11.41	61.5	>20	>180
1g	Ph	7.47	24.5	2.3	>180
1h	2-thiophene	6.67	41.7	>5[e]	>180

[a] Log *P* values were calculated using ACD/Log *P* DB, Advanced Chemistry Development Inc. [b] Toward *P. falciparum*; values are the mean of at least two independent experiments conducted in duplicate. [c] Antimalarial activities (*P. vinckei*) were determined after i.p. or p.o. administration once daily for four days to infected mice (three mice per dose). [d] Compound **1a** (**M64SMe**) was previously described.[26] [e] Toxicity appeared at higher doses.

The O-substituted sulfonates were evaluated for antimalarial activity in vitro against the human parasite *P. falciparum* and in vivo against *P. vinckei* in mice. As an initial part, the IC_{50} and ED_{50} values after i.p. or p.o. administration of the seven prodrug candidates in the bis-*N*-alkylamidine series are reported in Table 1. Results for **1a** are also listed as a reference.

The in vitro antimalarial activities were evaluated against a chloroquine-sensitive *P. falciparum* strain (Nigerian strain). The sulfonate derivatives **1a**–**h** constitute a homogeneous group, showing in vitro antimalarial activities in the low nanomolar range (IC_{50}: 2–62 nM). For four compounds, the antiplasmodial activity was moderate (**1c**: IC_{50} = 49 nM, **1f**: IC_{50} = 61.5 nM, **1g**: IC_{50} = 24.5 nM, **1h**: IC_{50} = 41.7 nM). We aimed at stabilizing the sulfonate moiety by using an aromatic group (**1g** and **1h**). The introduction of an aromatic ring as well as a heteroaromatic ring led to decreased antimalarial activity. With long (octyl) or hindered (isopropyl) alkyl chains, the antimalarial activity was also weaker than that of **1a**. The antimalarial potency may be hampered by the steric hindrance of the

O-substituent of the sulfonate. The other three substituted *O*-sulfonates exhibited potent in vitro antimalarial activities (**1b**: IC_{50} = 12 nM, **1d**: IC_{50} = 14 nM, **1e**: IC_{50} = 2.25 nM) similar to the one previously described (**1a** IC_{50} = 12 nM).[26,27] It appears that small O-substituents afford potent in vitro antiplasmodial activity.

The in vivo antimalarial activities were evaluated against the *P. vinckei petteri* strain (279BY) in female Swiss mice according to a modified version of the four-day suppressive test.[7] The mice (n = 3 per dose) were infected on day 0 and were treated once daily for four consecutive days (days 1–4 post-infection) by intraperitoneal (i.p.) or oral (p.o.) route (three appropriate doses). After i.p. administration, no antimalarial activity was detected at the tested doses with compounds sharing long aliphatic substituents at the sulfonate group (**1d**, **1e**, and **1f**), although **1d** and **1e** showed potent in vitro antimalarial activity. Doses of **1d** and **1e** higher than 5 mg kg^{-1} could not be used because toxicity appeared at higher doses; this precluded determination of whether higher doses could be active. For compound **1h** with the heteroaromatic ring, no ED_{50} i.p. could be determined, but at 5 mg kg^{-1}, compound **1h** decreased parasitemia by 43% relative to control, revealing significant i.p. antimalarial activity. Doses higher than 5 mg kg^{-1} could not be used because of toxicity issues. The presence of a long aliphatic substituent or a heteroaromatic ring greatly increases the toxicity of the compounds. Notably, the other three sulfonates **1b**, **1c**, and **1g** led to a total clearance of parasitemia, and ED_{50} values lower than 8 mg kg^{-1} were recorded, similar to **1a** and the parent drug **M64**.[44] Thus good in vivo antimalarial potencies are likely afforded by small alkyl or phenyl substituents on the sulfonate group, shared by compounds **1a**, **1b**, **1c**, and **1g**. However, **1c** and **1g** differ from **1a** and **1b**, as they they revealed potent in vivo antimalarial activities, while possessing moderate in vitro antiplasmodial activity.

Oral administration of **1d**, **1e**, and **1f** sulfonates resulted in the detection of no antimalarial activity up to 180 mg kg^{-1}, as observed after i.p. administration. Surprisingly, compound **1g**, which exhibits the best antimalarial activity after i.p. administration, is not orally active up to 180 mg kg^{-1}. A slight oral effect could be observed with compound **1h** at 180 mg kg^{-1}: parasitemia was decreased by 39% relative to control. On the other hand, **1a**, **1b**, and **1c** revealed substantial antimalarial activity after oral administration, unlike **M64** alkylamidine, as a total clearance of parasitemia was observed with these three compounds. Thus, potent oral antimalarial activities are afforded only by derivatives with the smallest aliphatic substituents on the *O*-sulfonate group. These compounds possess the lowest Clog*P* values in the series (**1a**: Clog*P* = 3.97, **1b**: Clog*P* = 5.03, **1c**: Clog*P* = 5.73). They may be able to cross the gastrointestinal tract more efficiently than compounds with longer aliphatic and aromatic O-substituents (Clog*P* range: 6–11.5). The smaller the alkyl chain, the lower the Clog*P* value and consequently the better the oral activity. Regarding these results, methyl, ethyl, and isopropyl groups were selected as substituents on the sulfonate group applied to prodrug candidates in the bis-*C*-alkylamidine series.

Optimization of the alkylsulfonate prodrug in the bis-*C*-alkylamidine series

As observed for the *N*-alkylamidine series, N-substituted bis-*C*-alkylamidoximes did not reveal any significant antimalarial activity (i.p. or p.o.).[30,45] Indeed, specific O-substituents are likely needed to improve oral antimalarial activity. Thus, the alkylsulfonate derivatives were explored as prodrug candidates in the bis-*C*-alkylamidine series. According to the results observed for orally administered bis-*N*-alkylamidines, only small aliphatic *O*-alkylsulfonates (methyl, ethyl, and isopropyl compounds) were introduced on bis-*C*-alkylamidoximes to generate the 18 N-substituted alkylsulfonates **2a–c**, **3a–c**, **4a–c**, **5a–c**, **6a–c**, and **7a–c** as prodrug candidates of the six selected *C*-alkylamidines. The respective IC_{50} and ED_{50} values after i.p. or p.o. administration are listed in Table 2.

Table 2. In vitro and in vivo antimalarial activities of *O*-alkylsulfonate derivatives **2a–7c** in the *C*-alkylamidine series.

Compd	R	R′	Log *P*[a]	IC_{50} [nM][b]	ED_{50} [mg kg^{-1}][c] i.p.	p.o.
2a	NHMe	Me	3.97	12[d]	<2	44
2b	NHMe	Et	5.03	11[d]	3.05	47
2c	NHMe	*i*Pr	5.73	20[d]	4.1	110
3a	NHEt	Me	5.03	7.8[d]	<2	41
3b	NHEt	Et	6.09	7.5[d]	<2	93
3c	NHEt	*i*Pr	6.79	16[d]	<2	>180
4a	$NH(CH_2)_2OMe$	Me	3.99	23.5	>10	105
4b	$NH(CH_2)_2OMe$	Et	5.05	44	>10	>180
4c	$NH(CH_2)_2OMe$	*i*Pr	5.75	34	>10	>180
5a	NHBn	Me	7.52	15	2	130
5b	NHBn	Et	8.59	23.5	5.05	>180
5c	NHBn	*i*Pr	9.28	44.7	8.1	>180
6a	NH*i*Pr	Me	5.73	410	<2	35
6b	NH*i*Pr	Et	6.79	465	<2	73
6c	NH*i*Pr	*i*Pr	7.48	305	4	93
7a	Pyrrolidine	Me	4.3	18.5	4	>120
7b	Pyrrolidine	Et	5.37	19	2.2	>120
7c	Pyrrolidine	*i*Pr	6.06	25.5	>5[e]	>120

[a] Log*P* values were calculated using ACD/Log*P* DB, Advanced Chemistry Development Inc. [b] Toward *P. falciparum*; values are the mean of at least two independent experiments conducted in duplicate. [c] Antimalarial activities (*P. vinckei*) were determined after i.p. or p.o. administration once daily for four days to infected mice (three mice per dose). [d] Single value determined in duplicate. [e] Toxicity was observed at higher doses.

In contrast to the other compounds of this series, the in vitro antiplasmodial activities of sulfonates **6a–c** were weak, with IC_{50} values higher than 300 nM for all three (**6a**: IC_{50} = 410 nM, **6b**: IC_{50} = 465 nM, **6c**: IC_{50} = 305 nM). These *N*-isopropyl derivatives are prodrug candidates of **M40**. As observed for the sulfonate derivatives in the *N*-alkylamidine series, the other 15 compounds **2a–c**, **7a–c**, **4a–c**, **5a–c**, and **7a–c** form a homogeneous group with similar IC_{50} values in the low nanomolar range (IC_{50}: 7–34 nM). *O*-Isopropylsulfonate derivatives (group **c**) showed IC_{50} values slightly higher than those for the methyl and ethyl groups, except for **3c** and **4c**. The IC_{50} values of *O*-sulfonates **2a–c** and **3a–c** could be determined only once due to the degradation of sulfonates.

In vivo, after i.p. administration, all compounds in the bis-*C*-alkylamidine series showed substantial antimalarial activities, with ED_{50} i.p. values lower than 8 mg kg^{-1}, except **4a–c** (prodrugs of **M42**). These compounds revealed the weakest antimalarial effects. After i.p. administration of **4a**, **4b**, and **4c** at 10 mg kg^{-1}, parasitemia decreased by 23, 51, and 57% relative to control, respectively, and no ED_{50} value could be determined. The ED_{50} i.p. value of the *O*-isopropyl sulfonate derivative **7c** could not be determined because toxicity appeared at doses higher than 5 mg kg^{-1}. However, i.p. administration of **7c** at 5 mg kg^{-1} led to a 25% decrease in parasitemia relative to control. The other 14 alkylsulfonates **2a–c**, **3a–c**, **5a–c**, **6a–c**, and **7a–b** form a homogeneous group, with ED_{50} i.p. values lower than 5 mg kg^{-1}. Notably, the lower the Clog*P* value, the higher the activity of the alkylsulfonate prodrug candidates.

After oral administration of **4b**, **4c** (prodrugs of **M42**), **5b** and **5c** (prodrugs of **M42**) at 180 mg kg^{-1}, no antimalarial effect could be observed, and **4a** and **5a** revealed weak oral antimalarial activity, with ED_{50} p.o. values higher than 100 mg kg^{-1}. However, if these high (>100 mg kg^{-1}) ED_{50} p.o. values can be explained by high (>10 mg kg^{-1}) ED_{50} i.p. values for the prodrug candidates of **M42** (**4a**, **4b**, and **4c**), this is not the case for the prodrug candidates of **M46** (**5a**, **5b**, and **5c**), the ED_{50} i.p. values for which are ≤ 8 mg kg^{-1}. This apparent contradiction may be explained by the weaker antimalarial activity of the parent drug **M46** (ED_{50} i.p. $\gg 20$ mg kg^{-1}) and/or the higher Clog*P* values of the sulfonates **5b** and **5c**. *O*-Isopropyl sulfonates **2c**, **3c**, the **M38** prodrug candidates **7a**, **7b**, and **7c** were also weakly orally potent. Indeed, oral administration of **7a**, **7b**, and **7c** at 120 mg kg^{-1} decreased parasitemia in mice by 65, 66, and 76%, respectively, relative to control. With compounds **2c** and **3c** administered at 180 mg kg^{-1}, parasitemia was decreased by 76 and 47%, respectively. According to these results it seems that the prodrug candidates of at least **M46** (**5a–c**) and **M38** (**7a–c**), and the *O*-isopropyl sulfonates **2c** and **3c** possess weak oral bioavailability, although their Clog*P* values are not significantly higher than the others. The weak oral antimalarial activities of the prodrug candidates of **M38** (**7a**, **7b** and **7c**) are particularly disappointing, as the parent drug **M38** was the most potent *C*-alkylamidine.

Remarkably, potent oral antimalarial activities were observed for the other seven *O*-alkylsulfonates. A total clearance of parasitemia could be observed after oral administration of the O-methylated and ethylated sulfonates **2a**, **2b** (**M44** prodrugs), **3a**, and **3b** (**M45** prodrugs) at 180 mg kg^{-1}. Furthermore, parasitemia in mice was respectively decreased by 96, 95, and 91% with **6a**, **6b**, and **6c** (**M40** prodrugs).

Influence of lipophilicity and steric bulk on oral bioavailability and activity

To compare the results of the *O*-alkylsulfonates as prodrug candidates of **M64**, **M44**, **M45**, and **M40**, the ED_{50} p.o. values are presented in Figure 4. These derivatives are represented by different shades according to the three possible *O*-alkyl substituents (methyl in white, ethyl in grey, and isopropyl in black). Four groups of three *O*-alkylsulfonate prodrug candidates can be distinguished (**1a–c**, **2a–c**, **3a–c**, and **6a–c**) according to the corresponding parent drug **M64**, **M44**, **M45**, and **M40**. In each group, the weakest oral activities were obtained with the compounds bearing the *O*-isopropyl substituents, and the best results were attributed to the *O*-methyl derivatives. These observations led us to conclude that the oral antimalarial activity of the alkylsulfonates may depend on the bulkiness of the O-substituent and the consequent lipophilicity of the compounds. Indeed, the bulkier the O-substituent, the higher the Clog*P* value and the weaker the oral activity. Moreover, the oral antimalarial activities of the four methylsulfonates **1a**, **2a**, **3a**, and **6a** are in a similar range (ED_{50} p.o. between 35 and 44 mg kg^{-1}), suggesting that the way the alkyl linker is introduced at the amidoxime function is less important than the *O*-alkyl substituent on the sulfonate group. The best oral activity was observed with **6a** (ED_{50} p.o. $= 35$ mg kg^{-1}), while its antimalarial activity was moderate in vitro ($IC_{50} = 410$ nM) and potent in vivo.

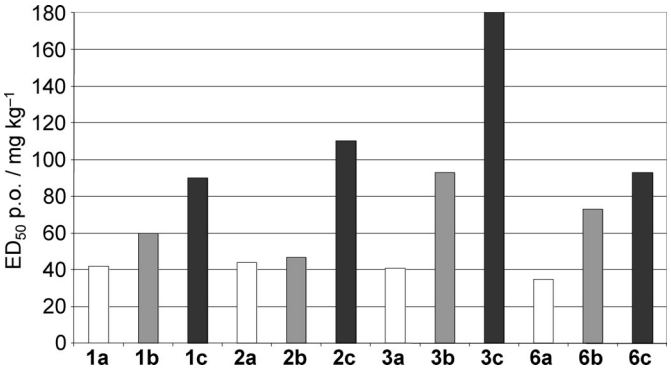


Figure 4. Oral antimalarial activities of *O*-methyl (□), ethyl (▩), and isopropyl (■) sulfonate prodrug candidates of **M64** and of N-substituted *C*-alkylamidines.

Transformation of the sulfonate prodrug candidates into the active amidines.

Compounds **1a**, **1b**, **1c**, **1g**, and **2a–c**, **3a–c**, **5a–c**, and **7a–b** all exert their antimalarial activity in the nanomolar range (in vitro) and with similar ED_{50} i.p. values (in vivo) as those of the corresponding bis-cationic bis-alkylamidines **M64**, **M38**, **M44**, **M45**, and **M46**.[33] The most likely explanation is that these prodrug candidates are efficiently converted in situ into the corresponding bis-alkylamidine, which is responsible for in vitro antiplasmodial activity.[27] Moreover, our assumption is consistent with the results obtained in Bressolle's group.[28] They observed that **1a** was rapidly transformed into **M64**. We assume that the desired transformation occurs in biological media for the seven analogues **1b–h** and analogously for the 18 sulfonates **2a–7c** in the bis-*C*-alkylamidine series. The observed IC_{50} value would reflect the rapidity of the desired conversion of the prodrug candidate into the corresponding drug in vitro, and the observed ED_{50} i.p. value would reflect the rate of transformation into the targeted drug in vivo.

Because no i.p. antimalarial activity could be detected for **1d**, **1e**, **1f**, **1h**, and **4a–c**, **7c**, it is likely that their conversion into the corresponding active drug is not efficient. They may be degraded into metabolites that differ from the targeted molecules **M64** or **M38**. Only small aliphatic or aromatic substituents on the *O*-sulfonate group likely permit the efficient transformation of the prodrug candidates into the active drug **M64**. Unfortunately, even with small substituents on the sulfonate group, **M42** was not efficiently produced from its prodrug candidates **4a–c**. The alkylsulfonate conversion may be substrate dependent and hampered by the methoxyethyl chain of **M42**.

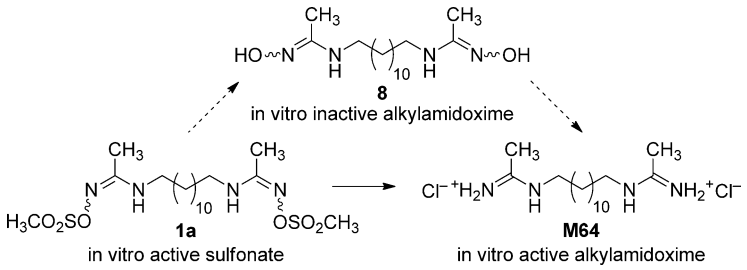


Figure 5. Possible conversion pathways of **M64SMe** (**1a**).

The three prodrug candidates **6a–c** of **M40** constitute a group of exceptions. They revealed potent i.p. and p.o. antimalarial activities while exhibiting very weak in vitro antiplasmodial activities ($IC_{50} > 300$ nM). One explanation for this apparent paradox is that the conversion of the prodrug candidates **6a–c** into the active **M40** occurs efficiently (the antimalarial activity observed in vivo may be credited to the parent drug **M40**), while this conversion occurs less rapidly in vitro. Compounds **6a–c** may be significantly more stable than the other prodrug candidates ($IC_{50} < 50$ nM).

The other key point to elucidate is how the sulfonate prodrug candidates are converted into the corresponding bis-alkylamidine drugs. Knowing that the sulfonates **1a–7c** are derivatives of the amidoxime **8**, **14a–d**, **12**, and **13**, we assumed at the beginning, the sulfonate derivatives **2a–7c** are prodrug candidates capable of being transformed into amidoxime **8**, **14a–d**, **12**, and **13**, and then reduced into bis-alkylamidines. However, the parent amidoximes **8**, **14a–d**, **12**, and **13** did not reveal any antimalarial activity in vitro or in vivo.[30,45] Thus, it is likely that the alkylsulfonates are not transformed into inactive amidoximes **8**, **14a–d**, **12**, and **13**, but directly into bis-alkylamidines **M64**, **M44**, **M45**, **M42**, **M46**, **M40**, and **M38**.

Because the desired conversion of the sulfonate prodrug candidates into the corresponding alkylamidine drug occurs in plasma used for in vitro evaluations, it is not likely that sulfonates are enzymatically converted. Indeed, the enzyme systems (cytochrome P_{450} reductases) that were predicted to transform amidoxime derivatives into the corresponding amidines are not present in the plasma. Moreover, to our knowledge, no enzyme system present in the plasma is capable of catalyzing the reduction of sulfonates into amidines. The most likely explanation is that sulfonates are chemically transformed into alkylamidines. These assumptions are consistent with the results of Bressolle's group[28] for **M64** in bis-*N*-alkylamidine series (Figure 5), which in analogy may also apply to bis-*C*-alkylamidines. The non-enzymatic transformation of the alkylsulfonate may consist of a chemical reduction possibly related to the reduction of amidoxime tosylates into *para*-toluenesulfonate salts of amidines, as reported by Le Berre et al.[46]

There are only few reports on prodrugs designed to rely exclusively on a non-enzymatic activation principle.[47] In this case, chemical stability issues have to be considered as a priority, especially when the prodrug candidates are supposed to be chemically transformed into active drugs.[48] The alkylsulfonate prodrug candidates of most of bis-alkylamidines (**M64**, **M38**, **M42**, **M44**, **M45**, and **M46**) are potent in vitro; their transformation into active bis-alkylamidines likely occurs very rapidly, except for those of **M40**, which revealed the weakest in vitro antiplasmodial potencies. The isopropyl group introduced on the nitrogen atom of the bis-*C*-alkylamidine led to the optimized antiplasmodial activity of **M40** while imparting the best stability of the alkylsulfonate prodrug candidates **6a–c** and an efficient conversion into active **M40**. Because the prodrug candidate **6a** is the most orally potent, this compound appears to be the most promising antimalarial agent based on bis-alkylamidine choline analogues, potentially working as an alkylsulfonate prodrug.

Conclusions

The main goal of the present study was to investigate the SAR of the sulfonate prodrug candidates in the bis-*N*-alkylamidine and bis-*C*-alkylamidine series. We wanted to improve the low stability of **1a** in biological media and the oral antimalarial activities. We first showed that a small alkyl substituent on the sulfonate group of prodrug candidates leads to the best oral antimalarial activities and that the alkylsulfonate prodrug strategy applies to *C*-alkylamidines as efficiently as it does to *N*-alkylamidines. The optimization of O-substituents on the selected N-substituted *C*-alkylamidoximes led to orally effective antimalarial alkylsulfonates in the *N*-alkylamidine series, as in *C*-alkylamidine series. According to these results, it appears that the alkyl substituent on the sulfonate moiety influences the lipophilicity of the derivatives and their diffusion across biological membranes, as well as the rate of conversion of the alkylsulfonate prodrug candidate, whereas the rate of conversion may be governed by the N-substituents in the bis-*C*-alkylamidine series. Compound **6a** revealed the most potent i.p. and p.o. potencies, as well as improved stability in biological media. We also came to the conclusion that alkylsulfonate derivatives are directly chemically transformed into the corresponding bis-alkylamidines, the mechanism for which remains unsolved.

Experimental Section

Drug inhibition of in vitro-cultured P. falciparum

A chloroquine-sensitive strain of *P. falciparum* (Nigerian strain)[6] was asexually cultured in human blood.[49] Compounds were dissolved in RPMI 1640 or DMSO (final concentration $<0.1\%$). Growths of *P. falciparum* cultures (0.6% initial parasitemia and 1.5% hematocrit) were measured in microtiter plates by [^{3}H]hypoxanthine incorporation after 48 h incubation with the compounds to determine the 50% inhibition concentration (IC_{50}), according to a modified Desjardins test.[6,50]

In vivo *studies*

All animal studies followed relevant laws and institutional guidelines. They were performed at the Centre d'Elevage et de Conditionnement Experimental des Modèles Animaux, Montpellier (France), under permission number A34370 (Centre National de la Recherche Scientifique). The in vivo antimalarial activities were evaluated against the *P. vinckei petteri* strain (279BY) in female Swiss mice according to a modified version of the four-day suppressive test.[7] The mice ($n=3$ per dose) were infected on day 0 and were treated once daily for four consecutive days (days 1–4 post-infection). Drugs and prodrugs were injected in 100 μL volumes of 0.9% NaCl or DMSO by intraperitoneal (i.p.) route, and in H_2O or DMSO by oral (p.o.) route (three appropriate doses). On day 5, parasitemia levels were monitored in Giemsa-stained blood smears and by flow cytometry on blood samples.[51]

Chemistry

General procedure A: synthesis of sulfonate derivatives: To a solution of amidoxime derivative (1 equiv) in $CHCl_3$ cooled at 0 °C was added pyridine (2.5 equiv). A solution of sulfonyl chloride derivative (2.5 equiv) in $CHCl_3$ was added to the medium. The reaction was stirred at room temperature for 4 h. After completion, the reaction was quenched with H_2O. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography twice with a step gradient of MeOH (0–1%) in CH_2Cl_2 to afford the desired sulfonate derivative.

1,12-bis-(*N*,*N*′-Ethylsulfonyloxyacetamidinyl)dodecane 1 b: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (1 g, 3.18 mmol) and ethanesulfonyl chloride (0.6 mL, 6.37 mmol) affording **1 b** as a white powder (0.95 g, 60%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); mp: 56–57 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=5.25$ (m, 2H), 3.3 (q, $J=7.5$ Hz, 4H), 3.12 (q, $J=6.7$ Hz, 4H), 1.91 (s, 6H), 1.51 (m, 4H), 1.38 (m, 6H), 1.25 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.1$, 30.4, 29.4, 29.3, 29.1, 26.5, 14.4, 8 ppm; IR: $\tilde{\nu}=1630$, 2854, 2926, 3376 cm^{-1}; MS (ESI) *m/z* (%): 250 (18) $[(M+2H)/2]^+$, 499 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{43}N_4O_6S_2^+$: 499.2624, found: 499.2632.

1,12-bis-(*N*,*N*′-2-Propylsulfonyloxyacetamidinyl)dodecane 1 c: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (1 g, 3.18 mmol) and 2-propanesulfonyl chloride (0.7 mL, 6.37 mmol) affording **1 c** as a colorless oil (0.65 g, 39%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=5.25$ (m, 2H), 3.78 (m, 2H), 3.11 (q, $J=6.7$ Hz, 4H), 1.92 (s, 6H), 1.49 (m, 4H), 1.41 (m, 12H), 1.25 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.0$, 49.7, 30.5, 29.4, 29.3, 29.1, 26.5, 16.3, 14.4 ppm; IR: $\tilde{\nu}=1632$, 2854, 2926, 3381 cm^{-1}; MS (ESI) *m/z* (%): 1053.6 (11) $[2M+H]^+$, 264.2 (89) $[(M+2H)/2]^+$, 527.3 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_6S_2^+$: 527.2937, found: 527.2938.

1,12-bis-(*N*,*N*′-Propylsulfonyloxyacetamidinyl)dodecane 1 d: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (1 g, 3.18 mmol) and propanesulfonyl chloride (0.7 mL, 6.37 mmol) affording **1 d** as a white powder (1.3 g, 75%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); mp: 68–69 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=5.25$ (m, 2H), 3.29 (d, $J=7.8$ Hz, 4H), 3.10 (q, $J=6.7$ Hz, 4H), 1.91 (s, 6H), 1.87 (m, 4H), 1.38 (m, 4H), 1.26 (m, 16H), 1.06 ppm (t, $J=7.5$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.0$, 60.3, 50.1, 42.9, 30.4, 29.4, 29.3, 29.1, 26.5, 14.4, 14.1, 12.8 ppm; IR: $\tilde{\nu}=1643$, 2852, 2923, 3413 cm^{-1}; MS (ESI) *m/z* (%): 264.1 (35) $[(M+2H)/2]^{2+}$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_6S_2^+$: 527.2937, found: 527.2943.

1,12-bis-(*N*,*N*′-Butylsulfonyloxyacetamidinyl)dodecane 1 e: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (0.5 g, 1.6 mmol) and butanesulfonyl chloride (0.5 mg, 3.2 mmol) affording **1 e** as a colorless oil (0.9 g, 89%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=5.25$ (m, 2H), 3.30 (m, 4H), 3.10 (q, $J=6.7$ Hz, 4H), 1.91 (s, 6H), 1.81 (m, 4H), 1.49 (m, 8H), 1.26 (m, 16H), 0.93 ppm (t, $J=7.3$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.0$, 48.1, 42.9, 30.5, 29.4, 29.3, 29.1, 26.5, 25.2, 21.4, 14.4, 13.4 ppm; IR: $\tilde{\nu}=1644$, 2852, 2923, 3402 cm^{-1}; MS (ESI) *m/z* (%): 278.2 (17) $[(M+2H)/2]^{2+}$, 555.3 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{51}N_4O_6S_2^+$: 555.3250, found: 555.3242.

1,12-bis-(*N*,*N*′-Octylsulfonyloxyacetamidinyl)dodecane 1 f: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (0.5 g, 1.6 mmol) and octanesulfonyl chloride (0.62 mL, 3.2 mmol) affording **1 f** as a white powder (0.55 g, 70%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); mp: 82–83 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=5.24$ (m, 2H), 3.29 (t, $J=7.9$ Hz, 4H), 3.19 (q, $J=6.7$ Hz, 4H), 1.91 (s, 6H), 1.82 (m, 4H), 1.51 (m, 12H), 1.41 (m, 12H), 1.26 (m, 16H), 0.86 ppm (t, $J=6.5$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.0$, 60.3, 50.1, 42.9, 30.4, 29.4, 29.3, 29.1, 26.5, 14.4, 14.1, 12.8 ppm; IR: $\tilde{\nu}=1636$, 2849, 2926, 3388 cm^{-1}; MS (ESI) *m/z* (%): 334.2 (24) $[(M+2H)/2]^{2+}$, 667.3 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{32}H_{67}N_4O_6S_2^+$: 667.4502, found: 667.4508.

1,12-bis-(*N*,*N*′-Phenylsulfonyloxyacetamidinyl)dodecane 1 g: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (1 g, 3.18 mmol) and benzenesulfonyl chloride (0.8 mL, 6.37 mmol) affording **1 g** as a white powder (1.53 g, 80%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1); mp: 108–109 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.00$ (d, $J=3.6$ Hz, 4H), 7.62 (m, 4H), 7.52 (m, 4H), 5.25 (m, 2H), 3.22 (d, $J=6.7$ Hz, 4H), 3.10 (q, $J=6.4$ Hz, 4H), 1.91 (s, 6H), 1.51 (m, 4H), 1.26 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.1$, 136.1, 133.4, 42.8, 30.4, 29.4, 29.3, 29.1, 26.5, 14.4, ppm; IR: $\tilde{\nu}=1639$, 2854, 2931, 3388 cm^{-1}; MS (ESI) *m/z* (%): 298.1 (38) $[(M+2H)/2]^{2+}$, 595.1 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{28}H_{43}N_4O_6S_2^+$: 595.2624, found: 595.2618.

1,12-bis-(*N*,*N*′-2-Thiophenylsulfonyloxyacetamidinyl)dodecane 1 h: Using general procedure **A**, starting from 1,12-bis-(*N*,*N*′-hydroxyacetamidinyl)dodecane (**8**) (1 g, 3.18 mmol) and 2-thiophenesulfonyl chloride (1.18 g, 6.37 mmol) affording **1 h** as a white powder (1.3 g, 68%). $R_f=0.63$ (CH_2Cl_2/MeOH 9:1) mp: 85–86 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=7.79$ (m, 1H), 7.68 (m, 1H), 7.11 (m, 1H), 5.20 (m, 2H), 3.08 (q, $J=6.7$ Hz, 4H), 1.86 (s, 6H), 1.48 (m, 4H), 1.26 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=158.3$, 134.6, 133.5, 127.0, 42.9, 29.4, 29.3, 29.1, 26.8, 26.5, 14.3 ppm; IR: $\tilde{\nu}=1626$, 2852, 2922, 3402 cm^{-1}; MS (ESI) *m/z* (%): 304.1 (38) $[(M+$

2H)/2]$^{2+}$, 607.1 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{39}N_4O_6S_2^+$: 607.1752, found: 607.1745.

1,14-Tetradecanedial dioxime 11: A mixture of 1-hydroxy-1,2-benziodoxol-3-(1*H*)-one 1-oxide (IBX) (34.5 g, 123.3 mmol) in DMSO (200 mL) was cooled with an ice bath. 1,14-Tetradecanediol (9.5 g, 41,1 mmol) was added. The solution was stirred at room temperature for 5 h. Cold H_2O (500 mL) was added to the medium. The formed solid was filtered and washed with H_2O (3×50 mL). The solid was solubilized with EtOAc (500 mL). This organic layer was washed with brine (2×400 mL), dried over $MgSO_4$, filtered, and evaporated to give 1,14-tetradodecanedial as an oil becoming a white powder under high vacuum (8.7 g, crude). This was used directly in next step without further purification: R_f=0.50 (*c*Hex/EtOAc 7:3); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.76 (s, 2H), 2.39 (m, 4H), 1.61 (m, 4H), 1.24 ppm (m, 16H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=202.8, 43.8, 29.4, 29.3, 29.2, 29.0, 21.9 ppm; IR: $\tilde{\nu}$= 1710, 2848, 2912 cm^{-1}; MS (ESI) *m/z* (%): 227.2 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{14}H_{27}O_2$: 227.2011, found: 227.2015. Hydroxylamine hydrochloride (13.4 g, 192.8 mmol) was added to a solution of 1,14-tetradecanedial (8.7 g, 38.6 mmol) and anhydrous pyridine (21.6 mL, 268.6 mmol) in anhydrous EtOH (200 mL). The reaction was held at reflux overnight. After completion, the solvents were evaporated, and the solid obtained was washed with H_2O (3×50 mL), MeOH, and Et_2O to yield **11** as a white powder (7.7 g, 74% over two steps): R_f=0.64 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=10.66 (s, 2H, OH), 6.62 (t, *J*=5.4 Hz, 2H), 2.50 (m, 4H), 1.39 (m, 4H), 1.25 ppm (m, 16H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=153.3, 29.0, 28.9, 28.7, 28.5, 25.6, 24.5 ppm; MS (ESI) *m/z* (%): 257 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{14}H_{28}N_2O_2^+$: 257.2229, found: 257.2232.

General procedure B: synthesis of amidoxime derivatives: To a solution of 1,14-tetradecanedial dioxime (**11**) (1 equiv) in anhydrous DMF at 0 °C under N_2 atmosphere was added dropwise a solution of *N*-chlorosuccinimide (2.4 equiv) in anhydrous DMF. The reaction was stirred at room temperature for 1 h and then quenched with cold H_2O. Extractions with Et_2O were carried out, then the combined organic layers were washed with brine. After separation, the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford 1,14-bis-(N^1,N^{14}-dihydroxyimidoyldichloride)tetradecane (**12**) as a white powder (90%, crude). This crude was directly used in the next step without further purification: R_f=0.22 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, MeOD): δ= 2.46 (t, *J*=7.3 Hz, 4H), 1.63 (m, 4H), 1.30 ppm (m, 16H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=162.8, 36.5, 29.3, 29.2, 28.9, 28.3, 26.1 ppm; IR: $\tilde{\nu}$=1635, 2848, 2916, 3273 cm^{-1}. To a solution of **12** (1 equiv) in anhydrous Et_2O at 0 °C was simultaneously slowly added Et_3N (5 equiv) and 2-iodopropylamine (4 equiv). The reaction was stirred at room temperature overnight. After completion, the solvents were removed under reduced pressure. The residue was taken up in H_2O, and CH_2Cl_2 extractions were carried out. After separation of the layers, the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The remaining residue was taken up in MeOH. An aqueous solution of 37% HCl was added. The solution was stirred at room temperature for 15 min, and then the solvents were evaporated. The crude product was subjected to reversed-phase column chromatography with a step gradient of MeOH (0–30%) in H_2O pH 3. The isolated HCl salt was taken up in MeOH (50 mL) and neutralized by the addition of a solution of MeONa (5 M) in MeOH until pH>7/pH<8. The solvents were then evaporated to afford the desired amidoxime derivative.

1,12-bis-(*N*-Isopropyl-*N*′-hydroxyamidinyl)dodecane 9e: Using general procedure **B**, starting from 1,14-tetradecanedial dioxime (**11**) (7.7 g, 30.0 mmol) and 2-iodopropylamine (9.5 mL, 110.0 mmol) affording **9e** as a beige powder (8.9 g, 81% over two steps). R_f=0.30 (CH_2Cl_2/MeOH 9:1); mp: 78–79 °C; ^{1}H NMR (300 MHz, MeOD): δ=3.64 (hept, *J*=4.8 Hz, 2H), 2.18 (t, *J*=7.6 Hz, 4H), 1.54 (m, 4H), 1.31 (m, 16H), 1.18 ppm (m, 12H); ^{13}C NMR (75 MHz, MeOD): δ=157.5, 44.9, 30.7, 30.6, 30.4, 30.3, 29.5, 28.8, 24.7 ppm; IR: $\tilde{\nu}$=1662, 2851, 2917, 3143, 3418 cm^{-1}; MS (ESI) *m/z* (%): 741 (12) $[2M+H]^+$, 371 (85) $[M+H]^+$, 186 (100) $[(M+2H)/2]^{2+}$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{20}H_{43}N_4O_2^+$: 371.3386, found: 371.3394.

1,12-bis-(*N*-Pyrrolidinyl-*N*′-hydroxyamidinyl)dodecane 9f: Using general procedure **B**, starting from 1,14-tetradecanedial dioxime (**11**) (3.15 g, 9.7 mmol) and pyrrolidine (3.2 mL, 38.70 mmol) affording **9f** as a beige powder (3.36 g, 82% over two steps). R_f=0.46 (CH_2Cl_2/MeOH 9:1); mp: 125–126 °C; ^{1}H NMR (300 MHz, MeOD): δ= 3.24 (m, 8H), 2.51 (t, *J*=7.9 Hz, 4H), 1.88 (m, 8H), 1.57 (m, 4H), 1.33 ppm (m, 16H); ^{13}C NMR (75 MHz, MeOD): δ=163.7, 47.7, 30.8, 30.7, 30.6, 30.4, 27.3, 27.2, 26.0 ppm; IR: $\tilde{\nu}$=1627, 2853, 2923, 3258 cm^{-1}; MS (ESI) *m/z* (%): 789 (6) $[2M+H]^+$, 395 (41) $[M+H]^+$, 198 (100) $[(M+2H)/2]^{2+}$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{43}N_4O_2^+$: 395.3386, found: 395.3395.

1,12-bis-(*N*-Methanesulfonyloxy-*N*′-methylamidinyl)dodecane 2a: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-methylamidinyl)dodecane (**9a**) (0.53 g,1.7 mmol) and methanesulfonyl chloride (0.33 mL, 4.2 mmol) affording **2a** as a white powder (0.32 g, 41%). R_f=0.78 (CH_2Cl_2/MeOH 9:1); mp: 90–91 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.22 (m, 2H), 3.11 (s, 6H), 2.87 (d, *J*= 5.2 Hz, 6H), 2.24 (t, *J*=7.8 Hz, 4H), 1.59 (m, 4H), 1.27–1.36 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.8, 36.0, 29.6, 29.5, 29.3, 28.1, 26.2 ppm; IR: $\tilde{\nu}$=1633, 2854, 2927, 3401 cm^{-1}; MS (ESI) *m/z* (%): 236 (7) $[(M+2H)/2]^+$, 941 (41) $[2M+H]^+$, 471 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{18}H_{39}N_4O_6S_2^+$: 471.2311, found: 471.2326.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-methylamidinyl)dodecane 2b: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-methylamidinyl)dodecane (**9a**) (0.5 g, 1.6 mmol) and ethanesulfonyl chloride (0.38 mL, 4.0 mmol) affording **2b** as a colorless oil (0.459 g, 58%). R_f=0.78 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.23 (m, 2H), 3.33 (q, *J*=7.4 Hz, 4H), 2.87 (d, *J*=5.2 Hz, 6H), 2.24 (t, *J*=7.7 Hz, 4H), 1.59 (m, 4H), 1.39 (t, *J*=7.4 Hz, 6H), 1.26–1.42 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.6, 43.1, 29.6, 29.5, 29.2, 28.0, 26.2, 8.1 ppm; IR: $\tilde{\nu}$=1632, 2854, 2926, 3401 cm^{-1}; MS (ESI) *m/z* (%): 250 (12) $[(M+2H)/2]^+$, 997 (56) $[2M+H]^+$, 499 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{20}H_{43}N_4O_6S_2^+$: 499.2624, found: 499.2633.

1,12-bis-(*N*-Isopropanesulfonyloxy-*N*′-methylamidinyl)dodecane 2c: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-methylamidinyl)dodecane (**9a**) (0.5 g, 1.6 mmol) and 2-propanesulfonyl chloride (0.45 mL, 4.0 mmol) affording **2c** as a colorless oil (0.392 g, 50%). R_f=0.78 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.24 (m, 2H), 3.77 (hept, *J*=6.9 Hz, 2H), 2.87 (d, *J*= 5.1 Hz, 6H), 2.23 (t, *J*=7.7 Hz, 4H), 1.58 (m, 4H), 1.42 (t, *J*=6.9 Hz, 6H), 1.26–1.43 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.6, 50.0, 29.7, 29.6, 29.3, 28.1, 26.3, 16.6 ppm; IR: $\tilde{\nu}$=1632, 2854, 2926, 3399 cm^{-1}; MS (ESI) *m/z* (%): 1053 (7) $[2M+H]^+$, 264 (22) $[(M+2H)/2]^+$, 527 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_6S_2^+$: 527.2937, found: 527.2947.

1,12-bis-(*N*-Ethyl-*N*′-methanesulfonyloxyamidinyl)dodecane 3a: Using general procedure **A**, starting from 1,12-bis-(*N*-ethyl-*N*′-hy-

droxyamidinyl)dodecane (**9b**) (0.6 g, 1.8 mmol) and methanesulfonyl chloride (0.34 mL, 4.4 mmol) affording **3a** as a white powder (0.539 g, 62%). R_f=0.78 (CH_2Cl_2/MeOH 9:1); mp: 42–43 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.10 (m, 2H), 3.20 (m 4H), 3.12 (s, 6H), 2.24 (t, J=7.7 Hz, 4H), 1.59 (m, 4H), 1.27 (m, 16H), 1.20 ppm (t, J=7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.9, 37.5, 36.1, 29.6, 29.3, 19.7, 28.4, 26.5, 16.0 ppm; IR: $\tilde{\nu}$=1615, 2853, 2924 3290 cm^{-1}; MS (ESI) *m/z* (%): 250 (14) $[(M+2H)/2]^+$, 997 (17) $[2M+H]^+$, 499 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{20}H_{43}N_4O_6S_2^+$: 499.2624, found: 499.2637.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-ethylamidinyl)dodecane 3b: Using general procedure **A**, starting from 1,12-bis-(*N*-ethyl-*N*′-hydroxyamidinyl)dodecane (**9b**) (0.6 g, 1.8 mmol) and ethanesulfonyl chloride (0.42 mL, 4.4 mmol) affording **3b** as a white powder (0.665 g, 72%). R_f=0.86 (CH_2Cl_2/MeOH 9:1); mp: 25–26 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.10 (m, 2H), 3.35 (q, J=7.4 Hz, 4H), 3.20 (m, 4H), 2.20 (t, J=7.7 Hz, 4H), 1.55 (m, 4H), 1.39 (t, J=7.4 Hz, 6H), 1.26–1.40 (m, 16H), 1.21 ppm (t, J=7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.8, 43.1, 37.5, 29.7, 29.6, 29.3, 28.4, 26.5, 16.0, 8.2 ppm; IR: $\tilde{\nu}$=1628, 2854, 2926, 3384 cm^{-1}; MS (ESI) *m/z* (%): 264 (15) $[(M+2H)/2]^+$, 1053 (25) $[2M+H]^+$, 527 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_6S_2^+$: 527.2937, found: 527.2950.

1,12-bis-(*N*-Ethyl-*N*′-2-propanesulfonyloxyamidinyl)dodecane 3c: Using general procedure **A**, starting from 1,12-bis-(*N*-ethyl-*N*′-hydroxyamidinyl)dodecane (**9b**) (0.6 g, 1.8 mmol) and 2-propanesulfonyl chloride (0.49 mL, 4.4 mmol) affording **3c** as a colorless oil (0.588 g, 61%). R_f=0.86 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.10 (m, 2H), 3.77 (hept, J=6.9 Hz, 2H), 3.19 (m, 4H), 2.21 (t, J=7.7 Hz, 4H), 1.59 (m, 4H), 1.42 (d, J=6.9 Hz, 6H), 1.25–1.36 (m, 16H), 1.20 ppm (t, J=7.2 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.8, 50.0, 37.5, 29.7, 29.6, 29.3, 28.4, 26.6, 16.5, 16.0, ppm; IR: $\tilde{\nu}$=1628, 2854, 2926, 3383 cm^{-1}; MS (ESI) *m/z* (%): 1109 (13) $[2M+H]^+$, 278 (15) $[(M+2H)/2]^+$, 555 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{51}N_4O_6S_2^+$: 555.3250, found: 555.3250.

1,12-bis-(*N*-Methanesulfonyloxy-*N*′-2-methoxyethylamidinyl)dodecane 4a: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-2-methoxyethylamidinyl)dodecane (**9c**) (0.5 g, 1.2 mmol) and methanesulfonyl chloride (0.24 mL, 3.1 mmol) affording **4a** as a white powder (0.34 g, 49%). R_f=0.76 (CH_2Cl_2/MeOH 9:1); mp: 77–78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.44 (m, 2H), 3.46 (t, J=5.1 Hz, 4H), 3.37 (s, 6H), 3.32 (m, 6H), 3.12 (s, 6H), 2.25 (t, J=7.8 Hz, 4H), 1.58 (m, 4H), 1.26–1.42 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.7, 71.6, 59.2, 42.6, 36.1, 29.7, 29.6, 29.3, 28.5, 26.5 ppm; IR: $\tilde{\nu}$=1629, 2854, 2926, 3387 cm^{-1}; MS (ESI) *m/z* (%): 280 (25) $[(M+2H)/2]^+$, 559 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_8S_2^+$: 559.2835, found: 559.2844.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-2-methoxyethylamidinyl)dodecane 4b: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-2-methoxyethylamidinyl)dodecane (**9c**) (0.5 g, 1.2 mmol) and ethanesulfonyl chloride (0.30 mL, 3.1 mmol) affording **4b** as a colorless oil (0.38 g, 52%). R_f=0.76 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.46 (m, 2H), 3.46 (t, J=5.2 Hz, 4H), 3.37 (s, 6H), 3.32 (m, 8H), 2.25 (t, J=7.8 Hz, 4H), 1.57 (m, 4H), 1.40 (t, J=7.4 Hz, 6H), 1.26–1.42 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.6, 71.6, 59.1, 43.1, 42.5, 29.6, 29.5, 29.2, 28.5, 26.4, 8.2 ppm; IR: $\tilde{\nu}$=1628, 2854, 2925, 3388 cm^{-1}; MS (ESI) *m/z* (%): 294 (56) $[(M+2H)/2]^+$, 587 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{51}N_4O_8S_2^+$: 587.3148, found: 587.3154.

1,12-bis-(*N*-2-Methoxyethyl-*N*′-2-propanesulfonyloxyamidinyl)dodecane 4c: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-2-methoxyethylamidinyl)dodecane (**9c**) (1.0 g, 2.5 mmol) and 2-propanesulfonyl chloride (0.7 mL, 6.2 mmol) affording **4c** as a colorless oil (1.0 g, 67%). R_f=0.76 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.45 (m, 2H), 3.77 (hept, J=6.9 Hz, 2H), 3.46 (t, J=5.2 Hz, 4H), 3.36 (s, 6H), 3.32 (m, 4H), 2.24 (t, J=7.7 Hz, 4H), 1.57 (m, 4H), 1.43 (d, J=6.9 Hz, 6H), 1.26–1.39 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.3, 71.7, 59.0, 49.8, 42.4, 29.5, 29.4, 29.1, 28.3, 26.3, 16.4 ppm; IR: $\tilde{\nu}$=1628, 2854, 2926, 3421 cm^{-1}; MS (ESI) *m/z* (%): 308 (30) $[(M+2H)/2]^+$, 615 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{26}H_{55}N_4O_8S_2^+$: 615.3461, found: 615.3457.

1,12-bis-(*N*-Benzyl-*N*′-methanesulfonyloxyamidinyl)dodecane 5a: Using general procedure **A**, starting from 1,12-bis-(*N*-benzyl-*N*′-hydroxyamidinyl)dodecane (**9d**) (0.7 g, 1.5 mmol) and methanesulfonyl chloride (0.29 mL, 3.8 mmol) affording **5a** as a colorless oil (0.471 g, 50%). R_f=0.83 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.24–7.39 (m, 10H), 5.59 (m, 2H), 4.36 (d, J=6.2 Hz, 4H), 3.13 (s, 6H), 2.27 (t, J=7.7 Hz, 4H), 1.60 (m, 4H), 1.24–1.36 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.1, 137.7, 129.1, 128.1, 127.0, 46.5, 36.1, 29.6, 29.5, 29.3, 28.4, 26.5 ppm; IR: $\tilde{\nu}$=1627, 2854, 2926, 3397 cm^{-1}; MS (ESI) *m/z* (%): 312 (11) $[(M+2H)/2]^{2+}$, 529 (35) $[(M\text{-}CH_3SO_3)+H]^{2+}$, 623 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{30}H_{47}N_4O_6S_2^+$: 623.2937, found: 623.2944.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-benzylamidinyl)dodecane 5b: Using general procedure **A**, starting from 1,12-bis-(*N*-benzyl-*N*′-hydroxyamidinyl)dodecane (**9d**) (0.7 g, 1.5 mmol) and ethanesulfonyl chloride (0.36 mL, 3.8 mmol) affording **5b** as a colorless oil (0.696 g, 71%). R_f=0.83 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.24–7.40 (m, 10H), 5.58 (m, 2H), 4.36 (d, J=6.2 Hz, 4H), 3.36 (q, J=7.4 Hz, 4H), 2.27 (t, J=7.7 Hz, 4H), 1.60 (m, 4H), 1.4 (t, J=7.4 Hz, 6H), 1.24–1.43 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.8, 137.4, 128.9, 127.9, 126.8, 46.3, 43.0, 29.4, 29.3, 29.1, 28.2, 26.3, 8.0 ppm; IR: $\tilde{\nu}$=1626, 2854, 2926, 3393 cm^{-1}; MS (ESI) *m/z* (%): 326 (13) $[(M+2H)/2]^+$, 651 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{32}H_{51}N_4O_6S_2^+$: 651.3250, found: 651.3251.

1,12-bis-(*N*-2-Propanesulfonyloxy-*N*′-benzylamidinyl)dodecane 5c: Using general procedure **A**, starting from 1,12-bis-(*N*-benzyl-*N*′-hydroxyamidinyl)dodecane (**9d**) (0.7 g, 1.5 mmol) and 2-propanesulfonyl chloride (0.42 mL, 3.8 mmol) affording **5c** as a white powder (0.55 g, 55%). R_f=0.83 (CH_2Cl_2/MeOH 9:1); mp: 95–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.24–7.34 (m, 10H), 5.59 (m, 2H), 4.36 (d, J=6.1 Hz, 4H), 3.80 (hept, J=6.9 Hz, 4H), 2.26 (t, J=7.6 Hz, 4H), 1.60 (m, 4H), 1.40 (d, J=6.9 Hz, 6H), 1.24–1.44 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.9, 137.7, 129.2, 128.1, 127.0, 50.1, 46.5, 29.7, 29.6, 29.3, 28.5, 26.6, 16.6 ppm; IR: $\tilde{\nu}$=1629, 2849, 2923, 3390 cm^{-1}; MS (ESI) *m/z* (%): 340 (13) $[(M+2H)/2]^+$, 679 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{34}H_{55}N_4O_6S_2^+$: 679.3563, found: 679.3558.

1,12-bis-(*N*-Methanesulfonyloxy-*N*′-2-propylamidinyl)dodecane 6a: Using general procedure **A**, starting from 1,12-bis(*N*-hydroxy-*N*′-2-propylamidinyl)dodecane (**9e**) (0.5 g, 1.35 mmol) and methanesulfonyl chloride (0.261 mL, 3.37 mmol) affording **6a** as a colorless oil (0.37 g, 52%). R_f=0.85 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ=4.95 (m, 2H), 3.59 (m, 2H), 3.08 (s, 6H), 2.2 (t, J=6.4 Hz 4H), 1.56 (m, 4H), 1.23 (m, 16H), 1.16 ppm (d, J=6.5 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=159.8, 44.5, 35.8, 29.4, 29.3, 29.1, 28.9, 28.3, 26.9, 24.5 ppm; IR: $\tilde{\nu}$=1637, 2855, 2927, 3275 cm^{-1}; MS (ESI) *m/z* (%): 264 (7) $[(M+2H)/2]^+$, 527 (91) $[M+$

H]$^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{47}N_4O_6S_2^+$: 527.2937, found: 527.2945.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-2-propylamidinyl)dodecane 6b: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-2-propylamidinyl)dodecane (**9e**) (0.5 g, 1.4 mmol) and ethanesulfonyl chloride (0.32 mL, 3.4 mmol) affording **6b** as a colorless oil (0.46 g, 61%). R_f=0.86 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.01 (m, 2H), 3.61 (m, 2H), 3.34 (q, J=7.4 Hz, 4H), 2.23 (t, J=7.7 Hz, 4H), 1.58 (m, 4H), 1.26–1.41 (m, 22H), 1.20 ppm (d, J=6.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.0, 44.7, 43.1, 24.5, 29.7, 29.6, 29.3, 28.5, 27.0, 8.2 ppm; IR: $\tilde{\nu}$=1637, 2854, 2926, 3279 cm^{-1}; MS (ESI) *m/z* (%): 224.2 (17) $[([M-CH_3CH_2SO_3]+2H)/2]^{2+}$, 339.4 (20) $[(M-2CH_3CH_2SO_3)+H]^+$, 555.4 (20) $[M+H]^+$, 170.2 (85) $[([M-2CH_3CH_2SO_3]+2H)/2]^{2+}$, 447.4 (100) $[(M-CH_3CH_2SO_3)+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{51}N_4O_6S_2^+$: 555.3250, found: 555.3277.

1,12-bis-(*N*-2-Propanesulfonyloxy-*N*′-2-propylamidinyl)dodecane 6c: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-2-propylamidinyl)dodecane (**9e**) (0.2 g, 0.5 mmol) and 2-propanesulfonyl chloride (0.15 mL, 1.4 mmol) affording **6c** as a colorless oil (0.15 g, 48%). R_f=0.86 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.00 (m, 2H), 3.75 (hept, J=6.8 Hz, 2H), 3.59 (m, 4H), 2.20 (t, J=7.7 Hz, 4H), 1.55 (m, 4H), 1.20–1.40 ppm (m, 28H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=159.7, 49.7, 44.5, 29.5, 29.4, 29.1, 28.3, 26.0, 24.3, 16.3 ppm; IR: $\tilde{\nu}$=1637, 2854, 2926, 3279 cm^{-1}; MS (ESI) *m/z* (%): 292.2 (16) $[(M+2H)/2]^{2+}$, 461.4 (71) $[(M-(CH_3)_2CHSO_3)+H]^+$, 583.4 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{26}H_{55}N_4O_6S_2^+$: 583.3563, found: 583.3542.

1,12-bis-(*N*-Methanesulfonyloxy-*N*′-pyrrolidinylamidinyl)dodecane 7a: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-pyrrolidinylamidinyl)dodecane (**9f**) (0.3 g, 0.76 mmol) and methanesulfonyl chloride (0.15 mL, 1.90 mmol) affording **7a** as a colorless oil (0.2 g, 47%). R_f=0.87 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.40 (m, 2H), 3.09 (m, 8H), 2.48 (t, J=8.7 Hz, 4H), 1.89 (m, 8H), 1.80 (m, 4H), 1.41 (m, 12H), 1.16 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.7, 46.9, 35.7, 29.7, 29.6, 29.5, 29.2, 26.3, 25.2 ppm; IR: $\tilde{\nu}$=1627, 2854, 2926, 3279 cm^{-1}; MS (ESI) *m/z* (%): 551.3 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{24}H_{47}N_4O_6S_2^+$: 551.2932, found: 551.2943.

1,12-bis-(*N*-Ethanesulfonyloxy-*N*′-pyrrolidinylamidinyl)dodecane 7b: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-pyrrolidinylamidinyl)dodecane (**9f**) (0.3 g, 0.76 mmol) and ethanesulfonyl chloride (0.18 mL, 1.90 mmol) affording **7b** as a colorless oil (0.242 g, 55%). R_f=0.74 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (300 MHz, $CDCl_3$): δ=5.10 (m, 2H), 3.40 (m, 2H), 3.09 (m, 8H), 2.48 (t, J=8.7 Hz, 4H), 1.89 (m, 8H), 1.87 (m, 4H), 1.39 (m, 12H), 1.25 ppm (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.4, 46.7, 45.7, 24.7–29.7 ppm; IR: $\tilde{\nu}$=1627, 2854, 2926, 3279 cm^{-1}; MS (ESI) *m/z* (%): 579.3 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{26}H_{51}N_4O_6S_2^+$: 579.3245, found: 579.3259.

1,12-bis-(*N*-2-Propanesulfonyloxy-*N*′-pyrrolidinylamidinyl)dodecane 7c: Using general procedure **A**, starting from 1,12-bis-(*N*-hydroxy-*N*′-pyrrolidinylamidinyl)dodecane (**9f**) (0.3 g, 0.76 mmol) and 2-propanesulfonyl chloride (0.21 mL, 1.9 mmol) affording **7c** as a colorless oil (0.2 g, 42%). R_f=0.74 (CH_2Cl_2/MeOH 95:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.79 (m, 2H), 3.29 (m, 8H), 2.48 (t, J=8.7 Hz, 4H), 1.89 (m, 8H), 1.52 (m, 4H), 1.41 (d, J=6.9 Hz, 12H), 1.25 ppm (m, 16H), ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.5, 49.6, 46.9, 16.6–29.8 ppm; IR: $\tilde{\nu}$=1637, 2855, 2927, 3279 cm^{-1}; MS (ESI) *m/z* (%): 607.9 (100) $[M+H]^+$; HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{28}H_{55}N_4O_6S_2^+$: 607.8890, found: 607.8896.

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Keywords: antimalarial activity · phospholipids · structure-activity relationships · parasitemia · prodrugs

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[32] **M40**: ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.24$ (d, $J = 6.5$ Hz, 12H), 1.20–1.45 (m, 16H), 1.65 (m, 4H), 2.14 (q, $J = 7.9$ Hz, 4H), 3.78 ppm (hept, $J = 6.5$ Hz, 4H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 20.0$, 27.2, 28.4, 28.8, 29.1, 29.2, 32.8, 44.5, 166.8 ppm; MS (ESI) *m/z* (%): 339 (100) $[M+H]^+$. **M40**: ^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.24$ (d, $J = 6.5$ Hz, 12H), 1.20–1.45 (m, 16H), 1.65 (m, 4H), 2.14 (q, $J = 7.9$ Hz, 4H), 3.78 ppm (hept, $J = 6.5$ Hz, 4H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 20.0$, 27.2, 28.4, 28.8, 29.1, 29.2, 32.8, 44.5, 166.8 ppm; MS (ESI) *m/z* (%): 339 (100) $[M+H]^+$.
[33] **M34**: $IC_{50} = 0.3$ nM; **M44**: $IC_{50} = 14.4$ nM; **M45**: $IC_{50} = 9.4$ nM, ED_{50} i.p. = 6.3 mg kg^{-1}; **M42**: $IC_{50} = 9.2$ nM, ED_{50} i.p. = 8 mg kg^{-1}; **M46**: $IC_{50} = 4.6$ nM; **M40**: $IC_{50} = 31$ nM, ED_{50} i.p. = 4.1 mg kg^{-1}; **M38**: $IC_{50} = 0.75$ nM, ED_{50} i.p. = 1.3 mg kg^{-1}.
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[44] **M64**: $IC_{50} = 9.3$ nM, ED_{50} i.p. = 3.1 mg kg^{-1}, ED_{50} p.o. > 200 mg kg^{-1}.
[45] **M40AH**: $IC_{50} = 5850$ nM, ED_{50} i.p. > 10 mg kg^{-1}, ED_{50} p.o. > 90 mg kg^{-1}. **M38AH**: $IC_{50} = 67.5$ nM, ED_{50} i.p. = 5 mg kg^{-1}, ED_{50} p.o. > 90 mg kg^{-1}.
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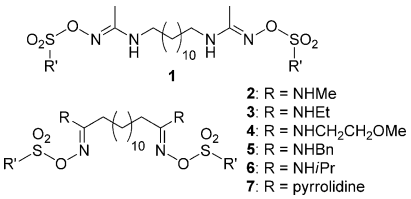
Evaluation of Bis-Alkylamidoxime *O*-Alkylsulfonates as Orally Available Antimalarials



Improved bioavailability: Bis-alkylamidines were originally developed as potential new antimalarial agents that target phospholipid metabolism, but these compounds are not orally bioavailable. To solve this issue, 25 sulfonates were investigated as prodrug candidates. Their antimalarial activities were evaluated in vitro and in vivo to define structure–activity relationships.

