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The anti-malarial activity of bivalent imidazolium salts

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ABSTRACT

A series of compounds containing bivalent imidazolium rings and one triazolium analog were synthesized and evaluated for their ability to inhibit the replication of *Plasmodium falciparum* cultures. The activity and selectivity of the compounds for *P. falciparum* cultures were found to depend on the presence of electron-deficient rings that were spaced an appropriate distance apart. The activity of the compounds was not critically dependent on the nature of the linker between the electron-deficient rings, an observation that suggests that the rings were responsible for the primary interaction with the molecular target of the compounds in the parasite. The bivalent imidazolium and triazolium compounds disrupted the process whereby merozoites gain entry into erythrocytes, however, they did not appear to prevent merozoites from forming. The compounds were also found to be active in a murine *Plasmodium berghei* infection, a result consistent with the compounds specifically interacting with a parasite component that is required for replication and is conserved between two *Plasmodium* species.

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1. Introduction

The development and spread of drug resistance is a major concern in the current efforts to treat malaria. Therefore, there is a continuing need to develop new antimalarial compounds,[1] particularly those having a novel mechanism of action[2] based on novel chemical structures. Recent estimates suggest that as many as 3.2 billion people live in areas with a risk of malaria transmission, with the heaviest burden occurring in the low-resource regions of sub-Saharan Africa.[3] Antimalarial agents capable of widespread use in the developing world need to be inexpensive, effective, and safe even when used repeatedly by children and pregnant women. Unfortunately, many of the agents currently in use do not meet these criteria. We have previously observed that tetrazolium, triazolium, and imidazolium salts are preferentially toxic to *Plasmodium falciparum* cultures[4,5] because they appear to interfere with a step involved in the invasion of merozoites into erythrocytes. The structural motif that appears to make active agents both potent and selective towards *Plasmodium* cultures is comprised of an electron-deficient ring surrounded by hydrophobic side-groups. This motif was originally examined using tetrazolium-based structures,[4] however, compounds based on triazolium and imidazolium rings were found to be equally selective and potent[5] and appear to share the same mechanism of action. Triazolium and imidazolium structures are preferable in biological systems because they cannot be easily reduced to a neutral structure, as is the case with tetrazolium structures which yield a neutral formazan, and hence triazolium- and imidazolium-based compounds are amenable to evaluation of their antimalarial potential in a malaria animal model.

When triazolium- or imidazolium-containing compounds are added to *P. falciparum* cultures they inhibit the *Plasmodium* merozoite invasion process.[5] The initial events that allow a merozoite to recognize and tether itself to an erythrocyte are thought to be mediated by proteins present on the surface of the merozoite, particularly MSP-1. Merozoite Surface Proteins (MSPs) consist of a family of proteins with at least 10 members.[6] MSPs-1, -2, -4, -5, -8, and -10 are anchored to the merozoite membrane by a glycosylphosphatidylinositol (GPI)-lipid anchor, and the anchor also functions as a toxin that leads to host signalling through TLR molecules.[7] MSP-1 is a prime candidate for mediating the initial adhesion event[8] as MSP-1 is the most abundant protein on the surface of the merozoite.[9] MSP-1 is synthesized as a large (180–250 kDa) protein which then undergoes proteolytic processing until a 19 kDa fragment, referred to as MSP-1_{19}, is left on the GPI anchor. The role of MSP-1_{19} in merozoite invasion remains controversial, however, transfection studies have established that MSP-1 is required for merozoite survival[10] and therefore MSP-1_{19} is currently a candidate for merozoite invasion-blocking vaccines.[11] The exact mechanism by which tetrazolium, triazolium,

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and imidazolium salts impair merozoite invasion is unknown; however, their capacity to inhibit *P. falciparum* cultures was originally examined because the electron-deficient rings in tetrazolium salts have been observed to form tight complexes with sulfated or phosphorylated glycans,[4] some of which can also be potent inhibitors of the invasion process. Our current hypothesis is that tetrazolium, triazolium, and imidazolium compounds function by interfering with a phosphorylated moiety in a hydrophobic environment and that this target is either the parasite ligand itself, or is an essential component of the merozoite invasion process.[5] This description appears to fit the GPI anchor of the MSP proteins and therefore it is possible that tetrazolium, triazolium, and imidazolium salts inhibit merozoite invasion by interfering with the synthesis or function of the GPI anchor on MSP molecules. If GPI anchors, or other potential targets, are present in merozoites at a sufficiently high density, then bivalent compounds, that is, compounds containing two electron-deficient rings, should be able to interact with two target motifs simultaneously if the active centers, that is, the electron-deficient rings, are spaced sufficiently far apart. In the present work we have explored the effect of combining two such centers into a single molecule to produce bivalent compounds. Bivalency should have the beneficial effect of permitting the compound to interact with two target domains at once, leading to a higher avidity-interaction with the *Plasmodium* cultures. In this article we report the synthesis and evaluation of bivalent analogs of a triazolium salt and of imidazolium salts having alkyl spacers of varying lengths as well as varying types of spacers. The activity of the compounds was found to depend strongly on the length of the spacer, but less so on the nature of the spacer.

2. Results

2.1. Chemical synthesis

The synthesis of the bis-imidazolium salts is shown in Scheme 1, and involved the conversion of an α,ω-dihaloalkane into a bis-imidazole which, on treatment with an alkyl halide, afforded the corresponding bis-imidazolium salt. The non-commercially available dihaloalkanes were synthesized as follows. 1,13-Diiodotridecane and 1,18-diiodooctadecane were prepared by the method of Wang et al.[12] in two steps, namely, the reduction of the corresponding dicarboxylic acid using $LiAlH_4$ in THF, followed by treatment of the resulting diol with H_3PO_4–P_2O_5–KI. Similarly, 1,14-diiodotetradecane was prepared from 1,14-tetradecanediol by treatment with H_3PO_4–P_2O_5–KI. 1,15-Diiodopentadecane and 1,16-diiodohexadecane were prepared from pentadecanolide and hexadecanolide, respectively, by reduction of the cyclic ester using $LiAlH_4$ in THF,[13] followed by treatment of the resulting diol with H_3PO_4–P_2O_5–KI. The bis-1,2,4-triazole compound **38** was prepared by treatment of 1,12-dibromododecane with 1,2,4-triazole–NaOH–DMSO.

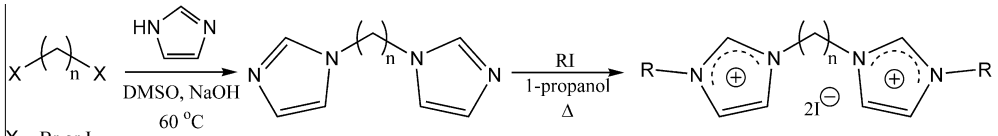
Compounds **27** and **29**, having phenyl-group linkers, were prepared from 1,4-dibromobenzene and 4,4′-dibromobiphenyl, respectively, by treatment with imidazole–K_2CO_3–$CuSO_4$.[14] Similarly, the benzimidazolium analog **35** was prepared from 4,4′-dibromobiphenyl by treatment with benzimidazole–K_2CO_3–$CuSO_4$. Compound **31** was prepared by the treatment of 4,4′-bis(chloromethyl)-1,1′-biphenyl with imidazole–NaOH–DMSO. Compound **36** was prepared by the dialkylation of hydroquinone using 1,3-dibromopropane, followed by treatment of the resulting dibromide with imidazole–NaOH–DMSO.

Alkylation of **27**, **29**, **31**, **36**, and **38** using iodomethane in 1-propanol gave the salts **28**, **30**, **32**, **37**, and **39**, respectively; the reaction of **11** with iodoethane or iodopropane afforded the corresponding salts **25** and **26**. Treatment of **29** with benzyl bromide in 1-propanol gave **33**, whereas treatment of **29** with 4-nitrobenzyl bromide in 1-propanol gave **34**.

2.2. Biological evaluation

2.2.1. Structure–activity relationships

Previous work[4,5] suggested that select tetrazolium, triazolium, and imidazolium compounds had anti-*Plasmodium* activity in cultures because they contained electron-deficient rings substituted by hydrophobic side groups. To determine if combining two electron-deficient centers in one molecule produced molecules that would selectively interact with *Plasmodium* cultures with a high avidity, a series of compounds that consisted of either imidazole or imidazolium rings linked by alkyl chains having 4, 6, 8, 10–16, 18, and 20 carbon atoms were synthesized (Table 1). The activity of compounds **1**–**24** in *P. falciparum* and mammalian (CHO) cell cultures was then determined (Table 1). Increasing the length of the alkyl chain in the bivalent (neutral) imidazole compounds led to a moderate decrease in the IC_{50} values observed in *P. falciparum* and CHO cultures (Fig. 1, top-left panel). Further, similar IC_{50} values were observed in both culture systems indicating that these compounds were not preferentially toxic to malaria parasites. In contrast, bivalent imidazolium compounds with spacers consisting



Bis-imidazoles

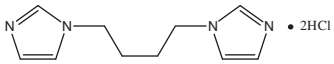
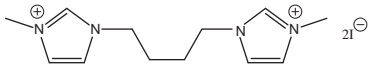
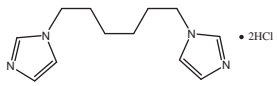
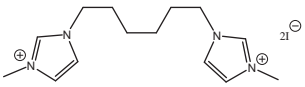
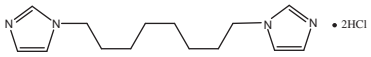
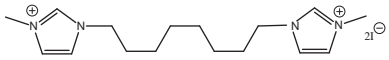
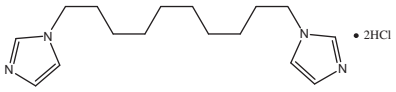
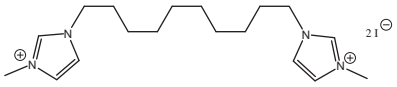
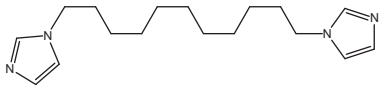
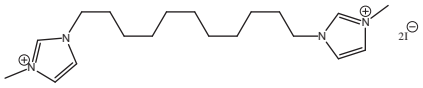
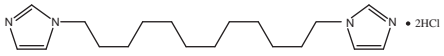
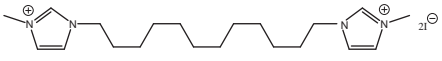
1 n = 4
3 n = 6
5 n = 8
7 n = 10
9 n = 11
11 n = 12
13 n = 13
15 n = 14
17 n = 15
19 n = 16
21 n = 18
23 n = 20

Bis-imidazolium salts

2 n = 4, R = CH_3
4 n = 6, R = CH_3
6 n = 8, R = CH_3
8 n = 10, R = CH_3
10 n = 11, R = CH_3
12 n = 12, R = CH_3
25 n = 12, R = CH_2CH_3
26 n = 12, R = $CH_2CH_2CH_3$
14 n = 13, R = CH_3
16 n = 14, R = CH_3
18 n = 15, R = CH_3
20 n = 16, R = CH_3
22 n = 18, R = CH_3
24 n = 20, R = CH_3

Scheme 1. Synthesis of selected bis-imidazoles and bis-imidazolium salts.

Table 1
Activities of compounds in *P. falciparum* and CHO cell cultures.

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO cells/ IC_{50} *P. falciparum*
1 · 2HCl		1066 ± 314	1900 ± 0	1.8
2		67 ± 22	122 ± 3	1.8
3 · 2HCl		90 ± 6	899 ± 80	10
4		2.6 ± 0.8	146 ± 14	56
5 · 2HCl		6 ± 2	140 ± 28	23
6		4.7 ± 0.9	452 ± 49	96
7 · 2HCl		9.2 ± 0.9	15 ± 11	1.6
8		0.082 ± 0.009	57 ± 5	695
9		1.9 ± 0.1	20 ± 5	11
10		0.0061 ± 0.0006	42 ± 3	6885
11 · 2HCl		4.1 ± 0.8	1.4 ± 0.2	0.3
12		0.006 ± 0.002	26 ± 5	4333

(*continued on next page*)

Table 1 (*continued*)

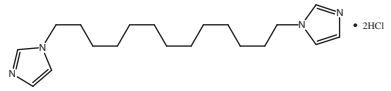
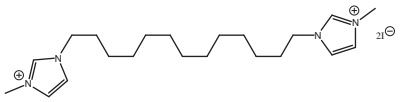
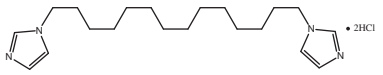
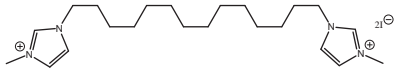
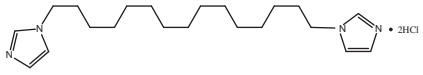
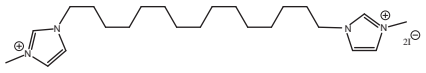
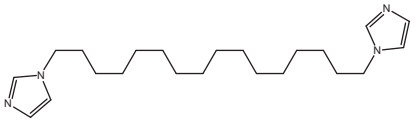
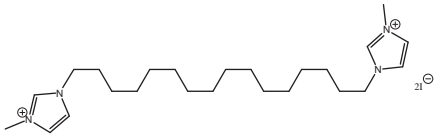
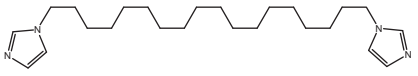
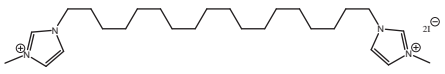
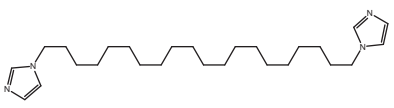
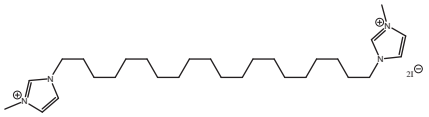
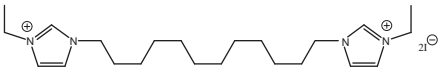
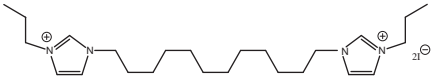
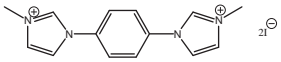
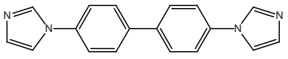
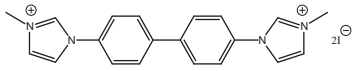
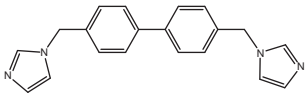
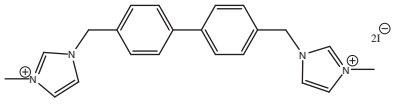
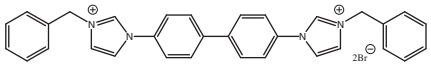
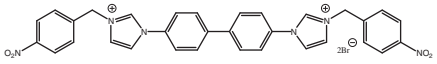
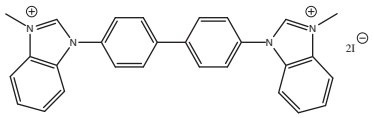
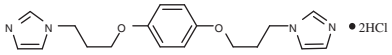
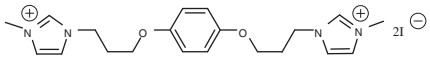
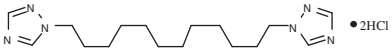
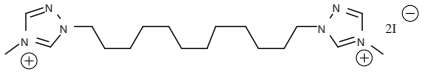
Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO cells/ IC_{50} *P. falciparum*
13 · 2HCl		0.56 ± 0.06	3.0 ± 0.3	5.4
14		0.007 ± 0.003	51 ± 11	7286
15 · 2HCl		1.6 ± 0.3	4.4 ± 0.7	2.8
16		0.0032 ± 0.0002	11 ± 2	3438
17 · 2HCl		0.93 ± 0.07	2.8 ± 0.4	3.0
18		0.002 ± 0.001	1.2 ± 0.2	600
19		0.65 ± 0.03	1.30 ± 0.09	2.0
20		0.0005 ± 0.0002	2.7 ± 0.1	5400
21		0.4 ± 0.1	1.2 ± 0.1	3.0
22		0.0009 ± 0.0002	1.4 ± 0.3	1556
23		2.5 ± 0.4	8.0 ± 0.6	3.2

Table 1 (*continued*)

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO cells/ IC_{50} *P. falciparum*
24		0.0021 ± 0.0005	1.4 ± 0.2	667
25		0.0120 ± 0.0005	34 ± 7	2833
26		0.0008 ± 0.0001	78 ± 14	97500
27		93 ± 4	1106 ± 127	12
28		11.2 ± 0.5	317 ± 64	28
29		3.1 ± 0.1	27 ± 7	9
30		0.009 ± 0.001	9 ± 2	1000
31		1.7 ± 0.1	65 ± 15	38
32		0.18 ± 0.04	125 ± 13	694
33		0.022 ± 0.003	12.1 ± 0.6	550
34		0.023 ± 0.003	14 ± 2	609

(*continued on next page*)

Table 1 (*continued*)

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO cells/ IC_{50} *P. falciparum*
35		0.07 ± 0.01	98 ± 24	1400
36 · 2HCl		4.9 ± 0.9	50 ± 4	10
37		0.045 ± 0.004	113 ± 12	2511
38 · 2HCl		19 ± 7	11.7 ± 0.5	0.6
39		0.008 ± 0.002	52 ± 9	6500

For each indicated structure the IC_{50} value was determined in both *P. falciparum* and CHO cell cultures. Values represent the mean of four independent determinations with the standard error of the mean indicated. Parallel experiments with chloroquine were included as a positive control.

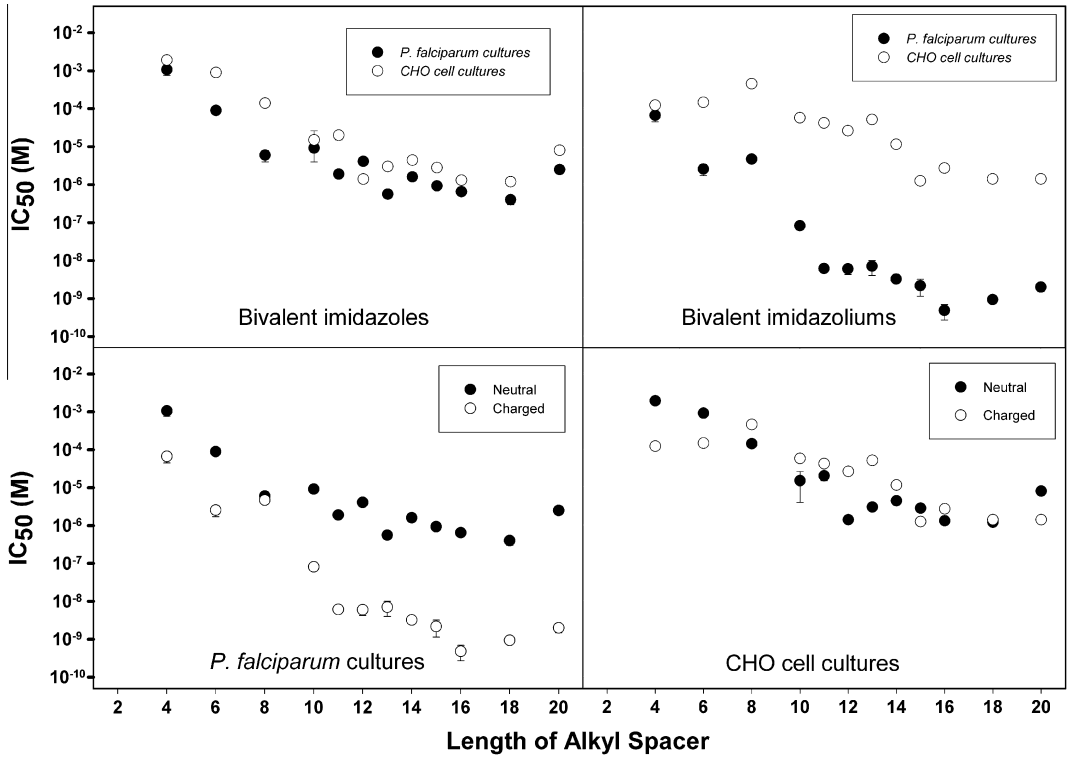


Figure 1. Effect of the alkyl spacer and charge on the activity of compounds in *P. falciparum* and CHO cell cultures. Compounds were synthesized either as bivalent imidazolium salts, or as neutral bivalent imidazole structures (see compounds **1**–**24** in Table 1). The compounds were then evaluated for activity in *P. falciparum* and CHO cell cultures as described in Section 4. When neutral imidazole compounds (top-left panel) were assayed, a moderate increase in activity was observed as the alkyl chain length increased, however, little, or no, selective toxicity was observed for *P. falciparum* cultures. When the experiment was repeated using similar bivalent imidazolium salts (top-right panel) it was observed that increasing the length of the alkyl chain between the imidazolium moieties resulted in a moderate increase in toxicity in CHO cultures throughout the range, however, a significant increase in activity is observed in *P. falciparum* cultures when an alkyl chain of more that 8 carbon atoms was present. When the data were plotted to allow a direct comparison of the effect of the imidazole and imidazolium compounds in *P. falciparum* cultures alone (bottom-left panel) it was observed that the presence of electron-deficient imidazolium rings was only a significant determinant of activity at alkyl spacer lengths greater than eight carbon atoms. Conversely, bivalent compounds containing either imidazole or imidazolium rings were found to have similar activities in CHO cell cultures (bottom-right panel).

of 4, 6, and 8 carbon atoms (compounds **2**, **4**, and **6**) had IC_{50} values in the micromolar range; the potency of the compounds in *P. falciparum* cultures increased dramatically as the length of the spacer was increased to 10 atoms and beyond. The activity of the bivalent imidazolium compounds in CHO cell cultures showed a general trend towards greater toxicity as the alkyl-spacer length increased, however, the effect was far less dramatic compared to that seen in *P. falciparum* cultures (Fig. 1, top-right panel). For example, the IC_{50} of compound **2** with a 4-carbon spacer was 67 ± 22 μM in *P. falciparum* cultures, whereas the IC_{50} of compound **24** with a 20-carbon spacer compound was 2.1 ± 0.5 nM; the corresponding values observed in CHO cell cultures for **2** and **24** were 122 ± 3 μM and 1.4 ± 0.2 μM, respectively. These results indicate that increasing the alkyl-chain length resulted in an 87-fold increase in potency in CHO cell cultures, and an ~32,000-fold increase in potency in *P. falciparum* cultures. The capacity of imidazolium compounds to be selectively toxic to *P. falciparum* cultures is also apparent when the IC_{50} values for imidazole and imidazolium compounds are plotted for *P. falciparum* cultures alone (Fig. 1, bottom-left panel) or CHO cell cultures alone (Fig. 1, bottom-right panel); these results are consistent with our hypothesis that imidazolium rings are interacting with a specific component of the parasite and that there is a minimum distance that is required between the imidazolium rings for them to interact with two target-domains. The imidazolium series has also been assayed using RAW cells (murine macrophage cells) and results similar to those seen for the CHO cells were observed (data not shown), an observation suggesting that the interaction is not a CHO-cell specific phenomenon. The effect of adding alkyl groups to the periphery of the imidazolium rings was also determined; thus, increasing the length of the alkyl substituents on a imidazolium compound having a 12-carbon spacer did result in increased potency and selectivity as was seen in the series **12**/**25** (methyl/ethyl) and **26** (propyl).

In order to determine if the nature of the spacer significantly affected the activity of a compound, bivalent imidazole- and imidazolium-containing compounds were synthesized having either a phenyl (**27**, **28**), a biphenyl (**29**, **30**), or a methylbiphenylmethyl (**31**, **32**) spacer. Consistent with our hypothesis of the necessity for the presence of a charged center, the uncharged compounds **27**, **29**, and **31** had poor activities in both culture systems. Compound **27** having a relatively short phenyl spacer did not display a high degree of potency or selectivity when assayed in CHO and *P. falciparum* cultures, however, the biphenyl imidazolium compound **30** was potent and selective (Fig. 2), as was **32**. In the case of **36**·**2HCl** and **37** which have a 1,4-dialkoxyphenyl spacer, the results obtained were consistent with the dependence of activity on charge, however, the presence of this particular spacer did not result in a loss of activity or selectivity. In summary, the activities of the compounds are more dependent on the length of the spacer than on its type.

To determine if the substituents on the heterocyclic rings significantly influenced the potency and selectivity of the compounds, a limited series of compounds, namely, **30** (methyl), **33** (benzyl), and **34** (4-nitrobenzyl), was examined. Relatively minor differences were seen in the IC_{50} values of these compounds in both CHO and *P. falciparum* cultures.

Interestingly, compound **35**, which contains benzimidazolium rings, was also both potent and selective.

In the present work an analog (**39**) containing two 1,2,4-triazolium rings was synthesized and evaluated (Fig. 3). Interestingly, it

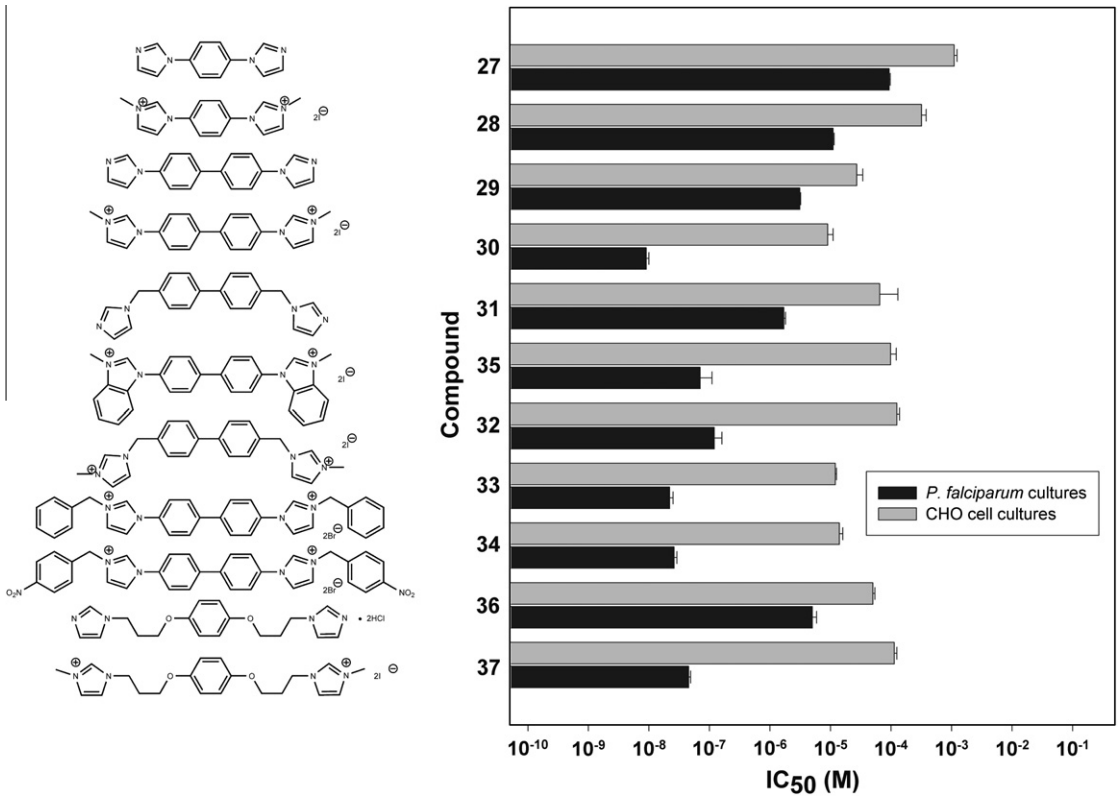


Figure 2. Effect of spacer chemistry and charge on the activity of the compound in *P. falciparum* and CHO cell cultures. Bivalent imidazole or imidazolium compounds with various spacers were synthesized. Compounds were then evaluated for activity in *P. falciparum* and CHO cell cultures as described in Section 4. Compounds with a phenyl spacer (**27** and **28**) were observed to have both low potency and selectivity for *P. falciparum* cultures. Increasing the spacer size to a biphenyl structure conferred activity and selectivity when the imidazolium rings were present (**30**, **35**, **33**, and **34**). Increasing the spacer length by two carbon atoms did not result in a significant change in activity (**31** and **32**). Adding phenyl groups (**35** and **33**) or 4-nitrophenyl groups (**34**) to the exterior of the imidazolium rings also did not significantly change the compounds' activities and the presence of a 1,4-dialkoxy moiety (**36**·**2HCl** and **37**) was equally well tolerated.

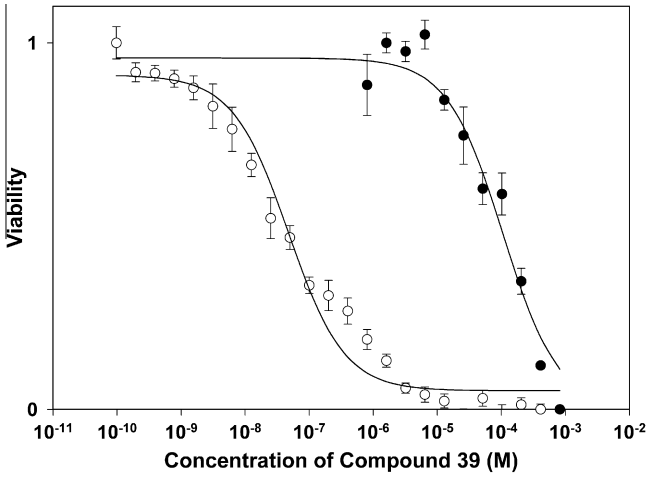


Figure 3. The effect of compound **39** on *P. falciparum* cultures and CHO cells. Compound **39** was serially diluted across the plate to produce twofold dilutions. A constant number of CHO cells or *P. falciparum* parasites were added to each well and the plate was allowed to incubate for 72 h before viability (vertical axis) was determined for each concentration of compound **39** (horizontal axis) using either a pLDH or MTT assay (see Methods). Each point represents the average value with standard error indicated. IC_{50} values were calculated using Sigma Plot and represent the mean of four determinations.

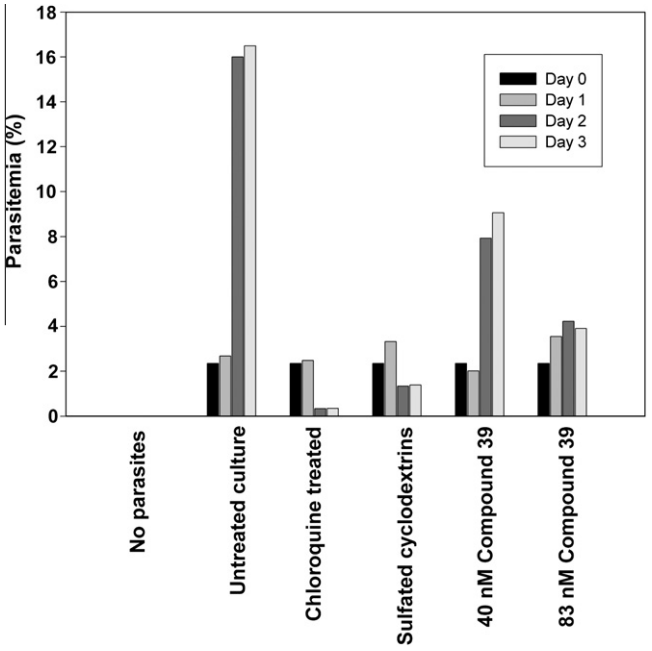


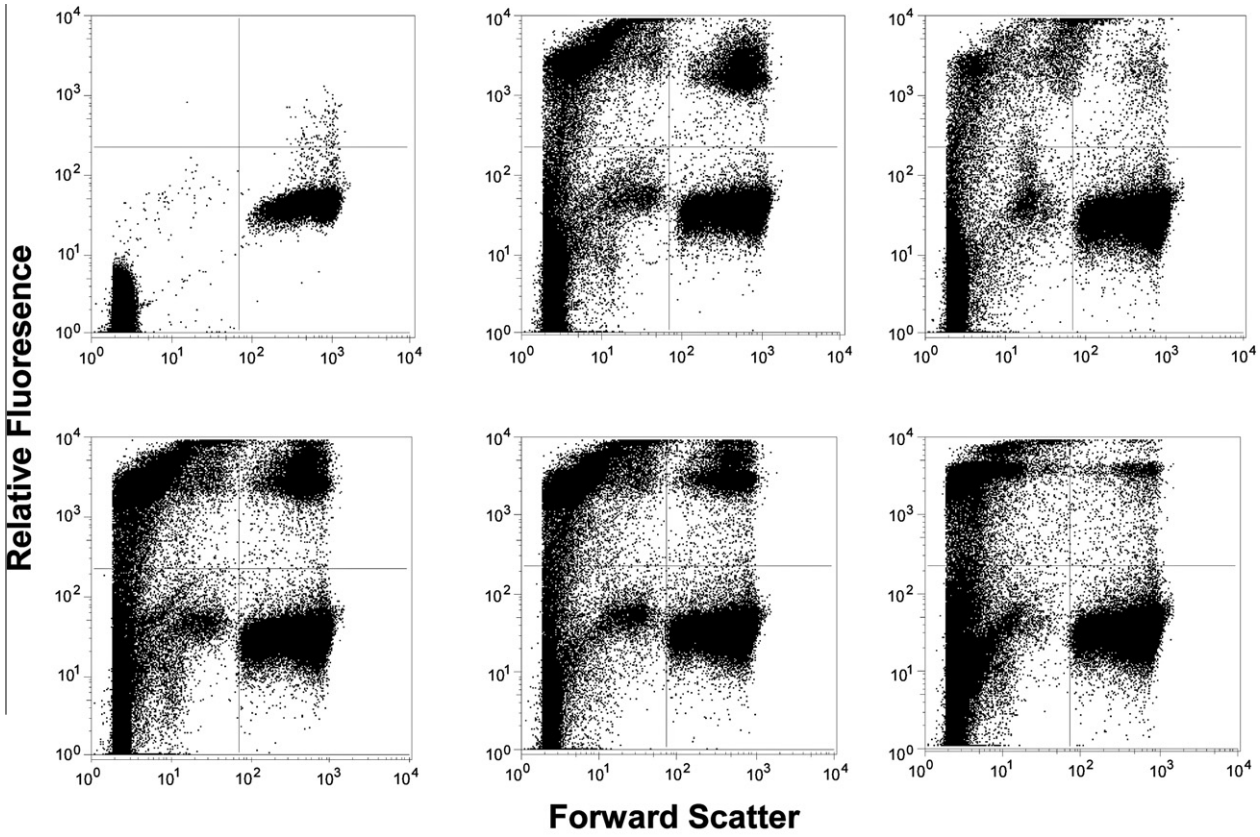
Figure 4. Determination of parasitemia of treated and untreated cultures. A Percoll gradient was used to synchronize the parasite cultures at the mature stage of development one day prior to the start of the parasite development assay. The cultures were then adjusted to a parasitemia of 2.4% and were treated for various periods of time with compounds (see *x*-axis) before the parasitemia present was determined by examining Giemsa-stained samples of the cultures. Untreated cultures showed a marked increase in parasitemia from Day 1 to Day 2, corresponding to the time of reinvasion, but no increase in parasitemia was observed between Day 0 to Day 1, or Day 2 to Day 3, corresponding to the time of intracellular maturation. Chloroquine-treated cultures produced results consistent with an intracellular action preventing maturation and a drop in parasitemia was observed between Days 1 and 2 with sulfated β-cyclodextrin (sodium salt)-treated cultures. When compound **39** was present at a concentration corresponding to five times its IC_{50} value the apparent parasitemia increased between Days 1 and 2, however, increasing the concentration to ten times the IC_{50} prevented an increase in parasitemia over time.

was found that **39** was a highly potent and selective compound with an IC_{50} value in *P. falciparum* cultures of 8 ± 2 nM and with an IC_{50} value in CHO cells of 52 ± 9 μM leading to a selectivity value of 6500. The observation that the uncharged analog (**38·2HCl**) of **39** was neither potent nor selective is consistent with our hypothesis of the presence of a charged species, however, the noteworthy values obtained with **39** warrant further exploration of 1,2,4-triazolium analogs, a study currently underway.

2.2.2. Effect of compounds on *P. falciparum* cultures

Previous work has indicated that negatively charged sulfated cyclodextrins interact with a receptor on the surface of an erythrocyte[15] while positively charged compounds that interact with these sulfated cyclodextrins might interact with a parasite component.[5] Examination of treated cultures suggests that bivalent imidazolium and triazolium compounds are active, at least in part, because they too inhibit merozoite invasion and their presence during the merozoite invasion event renders a culture unviable (Table 1). However, to exclude the possibility that these compounds are affecting intracellular parasite maturation, we determined the ability of parasites to go through their normal developmental stages in the presence of the bis-triazolium compound **39** by tracking the parasitemia of treated cultures over a period corresponding to two replication cycles, or 0–3 days (Fig. 4). Untreated synchronized culture parasites should be ring forms on Day 0, mature forms on Day 1, will be released to reinvade erythrocytes and become new ring forms on Day 2, and will develop into mature forms again on Day 3. As expected untreated parasite cultures displayed an increase in parasitemia only between Days 1 and 2, a period that coincides with the production and release of merozoites. Chloroquine prevents parasite maturation[16] and a drop in parasitemia was observed between Days 1 and 2. Cultures treated with sulfated β-cyclodextrin (sodium salt) showed a reduced parasitemia on Day 2, a result consistent with released merozoites not being able to associate with erythrocytes.[15] When compound **39** was present at a concentration corresponding to five times its IC_{50} value, the apparent parasitemia increased between Days 1 and 2, a result indicating that merozoite production was taking place; however, increasing the concentration to ten times the IC_{50} value prevented an increase in parasitemia over time, an observation suggesting that at this concentration the compound either prevented parasite association, as was seen with the sulfated cyclodextrin, or was toxic to intracellular parasites, as was seen with chloroquine.

We further examined the effect of compound **39** on parasite growth and development by analyzing treated cultures by flow cytometry (Fig. 5). Untreated cultures were observed to increase in parasitemia from 5% to 29% during this period, with a significant percentage of the DNA present not associated with the erythrocytes. The dissociation of DNA and erythrocytes suggests the presence of free merozoites. As intimated in Figure 4, treatment with chloroquine prevented the increase of parasitemia, however, treatment with putative invasion inhibitors resulted in a number of parasite particles (total bar heights in the graph in Fig. 5) that was equivalent to the control; this suggests that the production of DNA-containing particles, presumably merozoites, was normal but that the particles were less likely to be associated with erythrocytes. Treatment of the cultures with active compounds (Fig. 5) resulted in an increased number of parasite forms with a decreased size (Fig. 5, grey-bar section), however, treatment with sulfated β-cyclodextrin (sodium salt) produced the clearest indication that merozoite entry was not occurring. Further, when cultures treated with imidazolium or triazolium compounds were examined microscopically, it was observed that forms of the parasite appeared to adhere to the external surface of the erythrocytes (see picture in Fig. 5), a result suggesting that merozoites were still able to tether to erythrocytes, but that the entry process was inhibited. The



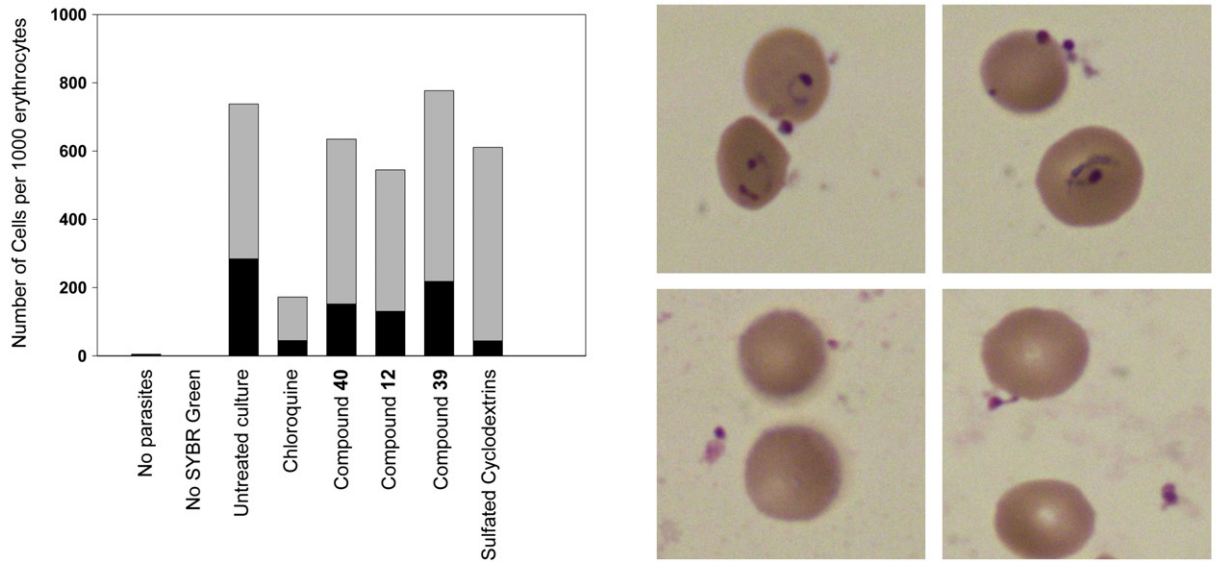


Figure 5. Flow cytometry analysis of treated cultures. Parasite cultures were synchronized as ring-stage cultures by sorbitol lysis. The cultures were then adjusted to a parasitemia of 5% and a hematocrit of 0.5% and were treated with various compounds for 48 h before 0.2 μL per mL of stock SYBR-Green solution was added and then the samples were subjected to flow cytometry. Each graph represents ~200,000 events and the *x*-axis represents forward scatter (size) while the *y*-axis indicates relative fluorescence. Diagrams represent data obtained from a culture stained with SYBR-Green that contained no parasites (top-left), an untreated parasite culture (top-middle), a parasite culture treated with 1 μM chloroquine (top-right), a culture treated with 20 μM compound **40** (bottom-left), a culture treated with 20 nM of compound **39** (bottom-middle), or a culture treated with 0.2 mg/mL sulfated β-cyclodextrin (sodium salt) (bottom-right). The data contained in the flow cytometry plots was quantitated by comparing the number of events observed in the lower-right quadrant (non-parasitized erythrocytes) compared to the upper-right quadrant (parasitized erythrocytes) and upper-left quadrant (small-sized material containing DNA) to the total number of erythrocytes present (upper- and lower-right quadrants). The sulfated β-cyclodextrin clearly prevented the association of parasite material with the erythrocytes in culture, however, the effect observed with compounds **12**, **40**, and **39** was less pronounced. Microscopic examination of the individual cultures suggested that in cultures treated with compounds **12**, **40**, and **39**, a significant number of parasite forms adhered to, but were not inside, the erythrocytes in the cultures (top-left photo, untreated; top-right photo, treated with compound **40**; bottom-left, treated with compound **39**; bottom-right photo, treated with the sulfated β-cyclodextrin).

maturation process ends in the production of 20–40 merozoites, followed by erythrocyte rupture and immediate reinvasion of the merozoites into new erythrocytes. It is interesting to note that the untreated culture contained a significant population of smaller DNA-containing elements which are presumably merozoites which have not completed the invasion process. This result is not

unexpected since the reinvasion efficiency of *P. falciparum* cultures is frequently well below the maximum possible, for example, a 5–10-fold increase is seen per cycle compared to the 20–40-fold increase in parasitemia that would be expected if the process were 100% successful (see Ref. 6). The presence of chloroquine prevented the appearance of an increased number of parasite forms containing DNA (bar graph, Fig. 5), and SYBR-Green I-staining populations (top-right panel, Fig. 5) are consistent with chloroquine treatment, since it would be expected to produce a diverse population of parasite forms and debris. In contrast, the presence of the other agents did not significantly affect the production of DNA-containing particles in the treated cultures, since the total height of the corresponding bars in Figure 5 are approximately equal to that of the control culture. The presence of moderate numbers of apparent ring-stage parasites detected by flow cytometry in cultures treated with **39** (as well as **12** or **40**) was surprising, since a result similar to that seen for the sulfated β-cyclodextrin (sodium salt)-treated sample was expected. However, examination of compound-treated cultures indicated that the parasite material appeared to be associated with the exterior of the erythrocytes; this suggests that merozoite production and release continue to occur, but that it is merozoite entry that is being aborted. The relative size and fluorescence of ring-stage infected erythrocytes and erythrocytes having adhered merozoites should be similar. The capacity of sulfated cyclodextrins to greatly reduce the number of parasites associated with red blood cells may reflect the fact that these molecules alter the surface charge of an erythrocyte.[15]

Other bis-cationic compounds have been studied for anti-*Plasmodium* activity, including bis-(quaternary ammonium) salts,[17–20] bis-pyridinium salts,[21] and bis-thiazolium salts.[22,23] In addition to inhibiting the formation of hemzoin inside the parasite food vacuole, many of these reported bis-cationic agents are considered mimics of choline, and target the membrane biogenesis of parasites in the erythrocytic stage by blocking the biosynthesis of phosphatidylcholine, the main component for the cytoplasmic membrane of the parasite. Although the possibility of reduced phosphatidylcholine production occurring inside the infected erythrocyte can not be eliminated in the case of our compounds, microscopic examination experiments suggest that the reported bis-imidazolium and bis-triazolium compounds appear to inhibit the process of merozoite invasion into the erythrocyte, and that the immobilization of the merozoite to the exterior surface of the erythrocyte is the main cause of parasite death. Thus, our compounds may act primarily by way of a mechanism of action similar to that seen using our tetrazolium salt compounds.[4] It is also possible that alteration of the merozoite surface proteins, GPI anchors, or other such merozoite features (while being constructed inside an infected erythrocyte), as a result of treatment with the bis-cationic compounds could lead to the inability of newly formed defected merozoites to reinvade other erythrocytes. These mechanistic aspects are currently under investigation in our laboratories.

It is recognized that the compounds in Table 1 may have surfactant properties (see, e.g., Refs. 24 and 25) and that the selective toxicity of the charged compounds in *P. falciparum* cultures may be attributable to surfactant effects. Surfactants can have the desirable property of interacting with, and crossing, biological membranes; however, this interaction depends on the relative contributions of the hydrophobic and hydrophilic components of the molecule. It should be noted that the environment found within *P. falciparum*-containing erythrocytes is known to differ from that of mammalian cells.[26,27] Three pieces of evidence suggest that the surfactant properties of the compounds do not explain the results found in Table 1. Firstly, erythrocytes parasitized with *Plasmodium* preferentially interact with surfactants having a higher hydrophilic content,[26] whereas the current compounds that are most active are more hydrophobic. Secondly, there appears to be a fair degree of latitude in the structure of the linker in active compounds, for example, in the series comprised of **12**, **30**, **32**, and **37**, and, therefore, there does not appear to be a direct relationship between hydrophobicity and activity, as seen also in the series comprised of **30**, **33**, and **34**. In the series comprised of **12**, **25**, and **26**, in which the only structural difference is the size of the alkyl substituents on the rings, there is a systematic decrease in toxicity in the CHO cell cultures. Thirdly, the IC_{50} values of the most active imidazolium compounds are sub-nanomolar, a result which is not consistent with a surfactant-like action.[28,29]

2.2.3. In vivo activity

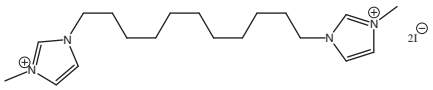
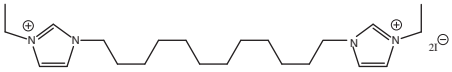
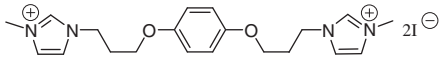
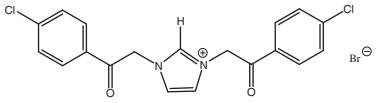
The observation that some of the compounds were selectively active in *P. falciparum* cultures compared to mammalian cell cultures suggested that these compounds might suppress a *Plasmodium* infection in a mouse model. Preliminary work using *Plasmodium berghei*-parasitized blood suggested that randomly selected compounds had similar, but not identical, activities in both *P. falciparum* cultures and *P. berghei* ex vivo assays (Table 2). *P. berghei* would be expected to differ from *P. falciparum* in at least two aspects: (1) *P. berghei* has a replicative cycle of 24 h versus the 48 h cycle seen in *P. falciparum*; and (2) *P. berghei* may vary from *P. falciparum* in its use of specific initial contact and tight-junction receptors. The similar activities of the compounds observed for both species (Table 2) suggests that a process common to both species is being inhibited by the compounds.

To determine if select compounds could positively influence the outcome of a murine malaria infection, mice were infected with 10^6 *P. berghei* parasites on Day 0 and their parasitemia was followed until parasites were observed in the peripheral circulation of the mice (Day 5, Fig. 6). The mice were then infused once daily for three days with: (1) a bivalent imidazolium salt having a 13-carbon spacer (compound **14**), (2) a bivalent triazolium salt compound having a 12-carbon spacer (compound **39**), (3) the equivalent amount of solvent (DMSO) in RPMI 1640, or (4) chloroquine, which functioned as a positive control. It was observed that the mice that were infused with **14** showed a less rapid development of parasitemia, however, mice infused with **39** showed both a delayed development of parasitemia and a lower final parasitemia, when compared to the RPMI 1640 control group (Fig. 6). Compound **39** was then subjected to a 4-day test[30] at two different doses, namely, 3.75 and 15 mg/kg, and it was observed that the parasitemia on the day following the final infusion was reduced by ~50% in the mice receiving the lower dose and by ~75% in the mice receiving the higher dose. Sulfated cyclodextrins, which bind to the corresponding receptor domain in the erythrocyte protein AE1, were also found to be effective in a mouse model, a result which is consistent with the hypothesis that *P. falciparum* and *P. berghei* share similar targets for both receptor inhibitors, for example, sulfated carbohydrates or the putative ligand inhibitors presented here. The results obtained suggest that complete suppression of a *Plasmodium* infection in vivo can be attained using compounds similar to those reported here.

3. Conclusions

Compounds containing two imidazolium rings (but not imidazole rings) show potent and selective activity in *P. falciparum* cultures and in a murine malaria model. A minimal spacing between the rings, for example, of more than eight carbon atoms, is necessary for both selectivity and potency, however, various spacers can be used to equal effect. Further, it is intimated that 1,2,4-triazolium rings can be substituted for imidazolium rings and the activity of the pharmacophore, both in vitro, and in vivo, is retained.

Table 2
Activities of compounds in *P. falciparum* and *P. berghei* cell cultures.

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) *P. berghei*
10		0.0061 ± 0.0006	0.05 ± 0.02
25		0.0120 ± 0.0005	0.12 ± 0.03
37		0.045 ± 0.004	0.046 ± 0.009
40		2.6 ± 0.1	4.2 ± 0.1

For each indicated structure the IC_{50} value was determined in both *P. falciparum* and *P. berghei* ex vivo cultures. Values represent the mean of four independent determinations with the standard error of the mean indicated. Compound **40** has been previously reported.[5]

4. Experimental

4.1. General

Flash column chromatography was performed on Silicycle silica gel (230–400 mesh, 60 Å). Analytical thin-layer chromatography was performed on glass-backed pre-coated Silica Gel 60 F254 plates (Silicycle), and the compounds were visualized either by UV illumination (254 nm), or by heating after spraying with phosphomolybdic acid in ethanol. Melting points were measured on a Mel-Temp II apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$, CD_3OD, or DMSO-d_6. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane.[31] The compounds synthesized were deemed >95% pure by ^1H NMR analysis. High-resolution ESI mass spectra were recorded on an Applied Biosystems/MDS Sciex QSTAR XL mass spectrometer with an Agilent HP1100 Cap-LC system. Samples were run in 50% aqueous MeOH at a flow rate of 6 μL/min. High-resolution EI mass spectra were recorded on a Waters/Micromass GC-TOF instrument.

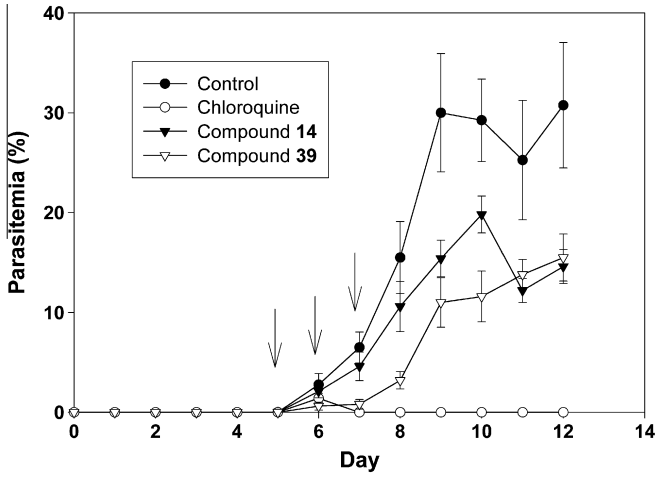


Figure 6. In vivo evaluation of compounds **14** and **39**. Groups of 5 female Balb/c mice were infected with 10^6 *P. berghei* parasites and their parasitemia was followed until parasites were observed in the peripheral circulation of the mice (Day 5). The mice were then infused once daily for three days (denoted by arrows) with: 1% (v/v) DMSO in RPMI-1640 (●); 60 μg per day of compound **14** (▼); 75 μg per day of compound **39** (open triangle); or 15 mg/kg per day of chloroquine (○, which functioned as a positive control). Parasitemia was determined by microscopic examination of Giemsa-stained peripheral blood films and the results are represented as mean with standard error shown using bars.

4.2. Materials

1,3-Dibromopropane, 1,4-dibromobutane, 1,6-dibromohexane, 1,8-dibromooctane, 1,10-dibromodecane, 4,4′-dibromobiphenyl, 1,4-dibromobenzene, 4,4′-bis(chloromethyl)-1,1′-biphenyl, 1,11-undecanedicarboxylic acid, and hydroquinone were obtained from Sigma–Aldrich. 1,11-Dibromoundecane and 1,14-tetradecanediol were obtained from Acros Organics. 1,12-Dibromododecane and 16-hexadecanolide were obtained from Alfa Aesar. 1,20-Dibromoeicosane was obtained from Karl Industries Inc. Octadecanedioic acid was obtained from TCI America. Sulfated β-cyclodextrin (sodium salt) was obtained from Sigma–Aldrich and contained 7–11 mol of sulfate residues per mol of β-cyclodextrin. All of the other chemicals were obtained from Sigma–Aldrich.

4.3. Procedures for the formation of α,ω-diiodoalkanes

4.3.1. 1,13-Diiodotridecane

This compound was prepared in two steps (94% overall yield) from 1,11-undecanedicarboxylic acid following the procedure for the formation of 1,18-diiodooctadecane given below and was obtained as a beige solid; mp 33–34 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 1.18–1.45 (m, 18H), 1.77–1.88 (m, 4H), 3.19 (t, J = 7.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.5, 28.7, 29.5, 29.6 (2C), 30.6, 33.7; HRMS (EI) $[M]^+$. Calcd for $C_{13}H_{26}I_2$: 436.0124. Found: 436.0121.

4.3.2. 1,14-Diiodotetradecane

Under an atmosphere of N_2, a 97% H_3PO_4 solution (8.7 equiv) was prepared by adding P_2O_5 (426 mg, 3.00 mmol, 1.4 equiv) to 85%

H_3PO_4 (0.86 mL, 1.22 g H_3PO_4, 12.41 mmol, 5.8 equiv). To this solution was added KI (2.06 g, 12.41 mmol, 5.8 equiv) and then 1,14-tetradecanediol (493 mg, 2.14 mmol, 1 equiv). The reaction mixture was stirred at ~115 °C for 4 h, cooled to rt, diluted with Et_2O (50 mL), and water (50 mL) was added. The organic layer was separated, and the aqueous phase extracted with an additional 50 mL of Et_2O. The combined organic extracts were washed with brine (30 mL), washed with a concentrated aqueous solution of $Na_2S_2O_3$ (10 mL), dried (Na_2SO_4), and then concentrated. High-vacuum drying gave 1,14-diiodotetradecane (842 mg, 1.87 mmol, 87%) as a white solid; mp 46–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.33 (m, 16H), 1.34–1.42 (m, 4H), 1.77–1.87 (m, 4H), 3.19 (t, J = 7.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.5, 28.7, 29.5, 29.6, 29.7, 30.7, 33.7; HRMS (EI) $[M]^+$. Calcd for $C_{14}H_{28}I_2$: 450.0281. Found: 450.0276.

4.3.3. 1,15-Diiodopentadecane

Under an atmosphere of N_2, a suspension of $LiAlH_4$ (228 mg, 6.01 mmol, 11.6 equiv) in dry THF (6 mL) was stirred at rt for 5 min. The mixture was cooled to 0 °C, and a solution of pentadecanolide (500 mg, 2.08 mmol, 1 equiv) in THF (6 mL) was added. The reaction mixture was brought to rt and then stirred at reflux temperature for 2 h. The mixture was cooled to 0 °C and then an aqueous solution of NaOH (2 M, 2 mL) was added. A white precipitate was formed and the mixture was stirred at reflux temperature for 0.5 h. The mixture was extracted with Et_2O (2 × 50 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried (Na_2SO_4), and concentrated. High-vacuum drying gave 1,15-pentadecanediol (482 mg, 1.97 mmol, 95%) as a white solid; mp 87–88 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.27–1.39 (m, 22H), 1.48–1.58 (m, 4H), 3.54 (t, J = 6.6 Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.0, 30.6, 30.8 (4C), 33.7, 63.0; HRMS (ESI) $[M+H]^+$. Calcd for $C_{15}H_{32}O_2$: 245.2481. Found: 245.2475.

Under an atmosphere of N_2, a 97% H_3PO_4 solution (8.7 equiv) was prepared by adding P_2O_5 (300 mg, 2.10 mmol, 1.4 equiv) to 85% H_3PO_4 (0.6 mL, 0.85 g H_3PO_4, 8.67 mmol, 5.8 equiv). To this solution was added KI (1.44 g, 8.70 mmol) and then 1,15-pentadecanediol (367 mg, 1.50 mmol, 1 equiv). The reaction mixture was stirred at ~115 °C for 4 h, cooled to rt, diluted with Et_2O (20 mL), and water (20 mL) was added. The organic layer was separated, and the aqueous phase extracted with an additional 50 mL of Et_2O. The combined organic extracts were washed with brine (20 mL), washed with a concentrated aqueous solution of $Na_2S_2O_3$ (10 mL), dried (Na_2SO_4), and then concentrated. High-vacuum drying gave 1,15-diiodopentadecane (683 mg, 1.47 mmol, 70%) as a white solid; mp 46–47 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19–1.45 (m, 22H), 1.77–1.87 (m, 4H), 3.19 (t, J = 7.2 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.5, 28.7, 29.5, 29.6, 29.7 (2C), 30.7, 33.7; HRMS (EI) $[M]^+$. Calcd for $C_{15}H_{30}I_2$: 464.0437. Found: 464.0436.

4.3.4. 1,16-Diiodohexadecane

This compound was prepared in two steps (84% overall yield) from 16-hexadecanolide following the procedure for the formation of 1,15-diiodopentadecane given above and was obtained as a white solid; mp 49–50 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18–1.45 (m, 24H), 1.75–1.89 (m, 4H), 3.19 (t, J = 7.1 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 7.5, 28.7, 29.6, 29.69, 29.74, 29.77, 30.7, 33.7; HRMS (EI) $[M]^+$. Calcd for $C_{16}H_{32}I_2$: 478.0594. Found: 478.0590.

4.3.5. 1,18-Diiodooctadecane

Under an atmosphere of N_2, octadecanedioic acid (2.00 g, 6.36 mmol, 1 equiv) was dissolved in freshly distilled THF (100 mL) and the solution was cooled to 0 °C; $LiAlH_4$ (228 mg, 6.01 mmol, 11.6 equiv) was added in small portions. The mixture was stirred at rt for 5 days then at reflux temperature for 5 h. The mixture was cooled to 0 °C and quenched with wet THF, then water, and finally an aqueous solution of NaOH (2 M, 2 mL) was added (pH ~10). The mixture was filtered through Celite and the filter cake washed with THF (100 mL) and Et_2O (100 mL). The organic filtrate was washed twice with brine, dried (Na_2SO_4), and then concentrated. High-vacuum drying gave 1,18-octadecanediol (1.40 g, 4.89 mmol, 77%) as a white solid; mp 93–94 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.27–1.38 (m, 28H), 1.48–1.57 (m, 4H), 3.54 (t, J = 6.6 Hz, 4H); ^{13}C NMR (100 MHz, CD_3OD): δ 26.9, 30.6, 30.7 (5C), 33.7, 63.0; HRMS (ESI) $[M+H]^+$. Calcd for $C_{18}H_{39}O_2$: 287.2950. Found: 287.2947.

Under an atmosphere of N_2, a 97% H_3PO_4 solution (8.7 equiv) was prepared by adding P_2O_5 (894 mg, 6.30 mmol, 1.4 equiv) to 85% H_3PO_4 (1.78 mL, 2.55 g H_3PO_4, 26.02 mmol, 5.8 equiv). To this solution was added KI (4.33 g, 26.10 mmol, 5.8 equiv) and then 1,18-octadecanediol (1.29 g, 4.50 mmol, 1 equiv). The reaction mixture was stirred at ~115 °C for 5 h, cooled to rt, diluted with Et_2O (20 mL), and water (20 mL) was added. The organic layer was separated, and the aqueous phase further extracted with Et_2O (3 × 100 mL). The combined organic extracts were washed with brine (20 mL), washed with a concentrated aqueous solution of $Na_2S_2O_3$ (10 mL), dried (Na_2SO_4), and then concentrated. High-vacuum drying gave 1,18-diiodooctadecane (2.27 g, 4.48 mmol, 99%) as a beige solid; mp 59–60 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.44 (m, 28H), 1.77–1.86 (m, 4H), 3.19 (t, J = 7.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 7.5, 28.7, 29.6, 29.70, 29.76, 29.80 (2C), 30.7, 33.7; HRMS (EI) $[M]^+$. Calcd for $C_{18}H_{36}I_2$: 506.0907. Found: 506.0921.

4.4. Representative procedure for the formation of bis-imidazole compounds, as outlined in Scheme 1

4.4.1. 1,4-Bis-(1*H*-imidazol-1-yl)butane dihydrochloride (1·2HCl)

Under an atmosphere of N_2, a mixture of imidazole (1.70 g, 25 mmol, 2 equiv) and sodium hydroxide (1.00 g, 25 mmol, 2 equiv) in dimethyl sulfoxide (5 mL) was stirred at 60 °C for 1.5 h. To this mixture was added carefully (very exothermic) 1,4-dibromobutane (2.70 g, 12.5 mmol, 1 equiv) and the mixture stirred at 60 °C for 2.5 h. The temperature was increased to ~100 °C and a stream of N_2 gas was blown over the mixture to remove dimethyl sulfoxide; the mixture solidified after 2 h. The mushy solid was dried under high vacuum leaving a beige solid which was extracted into benzene (3 × 75 mL) with excessive stirring and without the addition of water. The combined organic extracts were dried ($MgSO_4$), filtered, and the filtrate concentrated to a clear oil. {Alternatively, in cases where the alkane was longer than dodecane, the free base was isolated simply by cooling the DMSO-containing reaction mixture to 0 °C, diluting with water, collecting the solid precipitate by filtration, and washing the solid free base with water.} High-vacuum drying gave the crude free base as a white solid (1.22 g, 6.41 mmol, 51%). To a solution of the free base in warm 2-propanol (2 mL) was added a solution of 37% aqueous HCl (1.46 g, 14.8 mmol, 2.3 equiv) in 2-propanol (2 mL). The mixture was concentrated and dried under high vacuum. The residue was recrystallized from a mixture of methanol/ethanol/2-propanol. High-vacuum drying gave **1·2HCl** (881 mg, 3.35 mmol, 27%) as a white solid; mp >260 °C; R_f = 0.9 (MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 1.94–2.02 (m, 4H), 4.33–4.41 (m, 4H), 7.60 (s, 2H), 7.73 (s, 2H), 9.07 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 28.0, 49.8, 121.2, 123.3, 136.5; HRMS (ESI) $[M-H-2Cl]^+$. Calcd for $C_{10}H_{15}N_4$: 191.1297. Found: 191.1288.

4.5. Characterization of bis-imidazole compounds synthesized following the representative procedure (as given for compound 1)

4.5.1. 1,6-Bis-(1*H*-imidazol-1-yl)hexane dihydrochloride (3·2HCl)

This compound was prepared in one step (24% yield) from 1,6-dibromohexane and was obtained as a white solid (recrystallized

from a mixture of methanol/ethanol/2-propanol); mp 220–221 °C; $R_f = 0$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 1.38–1.49 (m, 4H), 1.88–2.00 (m, 4H), 4.30 (t, $J = 7.2$ Hz, 4H), 7.59 (s, 2H), 7.71 (s, 2H), 9.04 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 26.6, 30.9, 50.4, 121.1, 123.4, 136.3; HRMS (ESI) $[M\text{-}H\text{-}2Cl]^+$. Calcd for $C_{12}H_{19}N_4$: 219.1610. Found: 219.1599.

4.5.2. 1,8-Bis-(1*H*-imidazol-1-yl)octane dihydrochloride (5·2HCl)

This compound was prepared in one step (77% yield) from 1,8-dibromooctane and was obtained as a white solid (recrystallized from EtOH/2-propanol): mp 176–177 °C; $R_f = 0$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 1.30–1.46 (m, 8H), 1.84–1.98 (m, 4H), 4.28 (t, $J = 7.4$ Hz, 4H), 7.59 (app t, $J = 1.6$ Hz, 2H), 7.69 (app t, $J = 1.8$ Hz, 2H), 9.02 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.1, 29.8, 31.1, 50.6, 121.1, 123.3, 136.3; HRMS (ESI) $[M\text{-}H\text{-}2Cl]^+$. Calcd for $C_{14}H_{23}N_4$: 247.1923. Found: 247.1918.

4.5.3. 1,10-Bis-(1*H*-imidazol-1-yl)decane dihydrochloride (7·2HCl)

This compound was prepared in one step (84% yield) from 1,10-dibromodecane and was obtained as a white solid (recrystallized from EtOH/2-propanol); mp 143–144 °C; $R_f = 0$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 1.28–1.42 (m, 12H), 1.85–1.96 (m, 4H), 4.27 (t, $J = 7.4$ Hz, 4H), 7.59 (app t, $J = 1.6$ Hz, 2H), 7.69 (app t, $J = 1.6$ Hz, 2H), 9.02 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.3, 30.0, 30.4, 31.2, 50.6, 121.1, 123.3, 136.3; HRMS (ESI) $[M\text{-}H\text{-}2Cl]^+$. Calcd for $C_{16}H_{27}N_4$: 275.2236. Found: 275.2229.

4.5.4. 1,11-Bis-(1*H*-imidazol-1-yl)undecane (9)

This compound was prepared in one step (96% yield) from 1,11-dibromoundecane and was obtained as a golden oil (the solid free base isolated turned into an oil upon high-vacuum drying); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.15–1.34 (m, 14H), 1.68–1.80 (m, 4H), 3.90 (t, $J = 7.2$ Hz, 4H), 6.88 (s, 2H), 7.03 (s, 2H), 7.43 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 26.6, 29.1, 29.4 (2C), 31.1, 47.1, 118.9, 129.4, 137.1; HRMS (ESI) $[M]^+$. Calcd for $C_{17}H_{28}N_4$: 288.2314. Found: 288.2321.

4.5.5. 1,12-Bis-(1*H*-imidazol-1-yl)dodecane dihydrochloride (11·2HCl)

This compound was prepared in one step (89% yield) from 1,12-dibromododecane and was obtained as a white solid (recrystallized from 2-propanol/Et_2O); mp 164–165 °C; $R_f = 0$ (EtOAc); ^{1}H NMR (400 MHz, CD_3OD): δ 1.27–1.42 (m, 18H), 1.86–1.96 (m, 4H), 4.27 (t, $J = 7.4$ Hz, 4H), 7.58 (app t, $J = 1.6$ Hz, 2H), 7.69 (app t, $J = 1.6$ Hz, 2H), 9.01 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.3, 30.1, 30.5, 30.6, 31.2, 50.6, 121.1, 123.3, 136.3; HRMS (ESI) $[M\text{-}H\text{-}2Cl]^+$. Calcd for $C_{18}H_{31}N_4$: 303.2549. Found: 303.2535.

4.5.6. 1,13-Bis-(1*H*-imidazol-1-yl)tridecane dihydrochloride (13·2HCl)

This compound was prepared in one step (88% yield) from 1,13-diiodotridecane and was obtained as an orange oil; ^{1}H NMR (400 MHz, CD_3OD): δ 1.25–1.42 (m, 18H), 1.85–1.95 (m, 4H), 4.27 (t, $J = 7.4$ Hz, 4H), 7.59 (s, 2H), 7.69 (s, 2H), 9.02 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.3, 30.1, 30.5, 30.6, 30.7, 31.2, 50.6, 121.1, 123.3, 136.3; HRMS (ESI) $[M\text{-}H\text{-}2Cl]^+$. Calcd for $C_{19}H_{33}N_4$: 317.2699. Found: 317.2696.

4.5.7. 1,14-Bis-(1*H*-imidazol-1-yl)tetradecane dihydrochloride (15·2HCl)

This compound was prepared in one step (99% yield) from 1,14-diiodotetradecane and was obtained as a white solid (not recrystallized); mp 142–143 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.20–1.44 (m, 20H), 1.85–1.98 (m, 4H), 4.20–4.35 (m, 4H), 7.58 (s, 2H), 7.68 (s, 2H), 8.99 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 27.4, 30.1, 30.6, 30.7, 30.8, 31.3, 50.7, 121.2, 123.4, 136.4; HRMS (ESI) $[M-2Cl]^{2+}$. Calcd for $C_{20}H_{36}N_4$: 166.1464. Found: 166.1463.

4.5.8. 1,15-Bis-(1*H*-imidazol-1-yl)pentadecane dihydrochloride (17·2HCl)

This compound was prepared in one step (100% yield) from 1,15-diiodopentadecane and was obtained as a yellow solid (not recrystallized); mp 98–99 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 1.24–1.43 (m, 22H), 1.85–1.98 (m, 4H), 4.20–4.35 (m, 4H), 7.57 (s, 2H), 7.67 (s, 2H), 8.98 (s, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 18.3, 27.4, 30.2, 30.6, 30.7, 30.8, 31.3, 50.9, 121.2, 123.5, 136.6; HRMS (ESI) $[M\text{-}2Cl]^{2+}$. Calcd for $C_{21}H_{38}N_4$: 173.1542. Found: 173.1544.

4.5.9. 1,16-Bis-(1*H*-imidazol-1-yl)hexadecane (19)

This compound was prepared in one step (98% yield) from 1,16-diiodohexadecane and was obtained as a yellow solid (not recrystallized); mp 46–48 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20–1.44 (m, 24H), 1.68–1.82 (m, 4H), 3.90 (t, $J = 6.8$ Hz, 4H), 6.88 (s, 2H), 7.03 (s, 2H), 7.44 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.6, 29.2, 29.5, 29.6, 29.7 (2C), 31.2, 47.1, 118.9, 129.4, 137.2; HRMS (ESI) $[M+H]^+$. Calcd for $C_{22}H_{39}N_4$: 359.3174. Found: 359.3196.

4.5.10. 1,18-Bis-(1*H*-imidazol-1-yl)octadecane (21)

This compound was prepared in one step (93% yield) from 1,18-diiodooctadecane and was obtained as a beige solid (not recrystallized); mp 56–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08–1.40 (m, 28H), 1.68–1.80 (m, 4H), 3.91 (t, $J = 7.0$ Hz, 4H), 6.89 (s, 2H), 7.04 (s, 2H), 7.44 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.7, 29.2, 29.5, 29.6, 29.7 (2C), 29.8, 31.2, 47.2, 118.9, 129.5, 137.2; HRMS (EI) $[M+H]^+$. Calcd for $C_{24}H_{43}N_4$: 387.3487. Found: 387.3499.

4.5.11. 1,20-Bis-(1*H*-imidazol-1-yl)eicosane (23)

This compound was prepared in one step (68% yield) from 1,20-dibromoeicosane and was obtained as an off-white solid (isolated as the free base, not recrystallized); mp 58–59 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.18–1.36 (m, 32H), 1.70–1.80 (m, 4H), 3.91 (t, $J = 7.0$ Hz, 4H), 6.89 (s, 2H), 7.04 (s, 2H), 7.45 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.7, 29.2, 29.55, 29.64, 29.7, 29.77, 29.81 (2C), 31.2, 47.2, 118.9, 129.5, 137.2; HRMS (EI) $[M]^+$. Calcd for $C_{26}H_{46}N_4$: 414.3722. Found: 414.3748.

4.6. Representative procedure for the formation of bis-imidazolium compounds, as outlined in Scheme 1

4.6.1. 1,4-Bis-(3-methyl-1*H*-imidazolium-1-yl)butane diiodide (2)

The dihydrochloride salt **1·2HCl** (400 mg, 1.52 mmol) was dissolved in water (2 mL) and the solution basified with an excess of Na_2CO_3 (400 mg, 3.77 mmol). The mixture was extracted with EtOAc (3×), and the combined organic extracts were dried (Na_2SO_4), concentrated, and dried under high vacuum leaving the free base **1** (167 mg, 0.88 mmol, 58%). The free base **1** (167 mg, 0.88 mmol, 1 equiv) was dissolved in 1-propanol (2 mL) at rt and to this solution was added iodomethane (0.22 mL, 3.52 mmol, 4 equiv). The mixture was heated at reflux temperature for 24 h, then cooled to 0 °C. The resulting oil was washed with Et_2O (3 × 10 mL, decanting off the Et_2O layer each time), then concentrated at 60 °C and dried under high vacuum leaving a beige solid (395 mg, 0.83 mmol, 94%). The solid was recrystallized from boiling 2-propanol (4 mL), and washed with Et_2O. High-vacuum drying gave **2** (383 mg, 0.81 mmol, 92%) as a white solid; mp 103–104 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.70–1.86 (m, 4H), 3.85 (s, 6H), 4.15–4.26 (m, 4H), 7.71 (s, 2H),

7.76 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.1, 36.0, 48.1, 122.3, 123.7, 136.6; HRMS (ESI) $[M\text{-}2I]^{2+}$. Calcd for $C_{12}H_{20}N_4$: 110.0838. Found: 110.0840.

4.7. Characterization of the bis-imidazolium compounds synthesized following the representative procedure shown for 2, unless otherwise stated

4.7.1. 1,6-Bis-(3-methyl-1*H*-imidazolium-1-yl)hexane diiodide (4)

This compound was prepared in one step (95% yield) from free base **3** and was obtained as a white solid (recrystallized from 2-propanol/EtOH); mp 156–157 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.20–1.32 (m, 4H), 1.71–1.83 (m, 4H), 3.85 (s, 6H), 4.16 (t, J = 7.0 Hz, 4H), 7.70 (s, 2H), 7.77 (s, 2H), 9.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.9, 29.2, 35.9, 48.7, 122.3, 123.6, 136.5; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{14}H_{24}N_4$: 124.0994. Found: 124.0998.

4.7.2. 1,8-Bis-(3-methyl-1*H*-imidazolium-1-yl)octane diiodide (6)

This compound was prepared in one step (88% yield) from free base **5** and was obtained as a white–orange solid (recrystallized from 2-propanol/Et_2O); mp 115–116 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16–1.32 (m, 8H), 1.71–1.82 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.2 Hz, 4H), 7.70 (s, 2H), 7.76 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.4, 28.2, 29.4, 35.9, 48.8, 122.3, 123.6, 136.4; HRMS (ESI) $[M\text{-}I]^+$. Calcd for $C_{16}H_{28}N_4I$: 403.1358. Found: 403.1368.

4.7.3. 1,10-Bis-(3-methyl-1*H*-imidazolium-1-yl)decane diiodide (8)

This compound was prepared in one step (76% yield) from free base **7** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16–1.32 (m, 12H), 1.71–1.81 (m, 4H), 3.85 (s, 6H), 4.14 (t, J = 7.2 Hz, 4H), 7.69 (s, 2H), 7.76 (s, 2H), 9.10 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.7, 29.4, 35.8, 48.8, 122.3, 123.6, 136.4; HRMS (ESI) $[M-I]^+$. Calcd for $C_{18}H_{32}N_4I$: 431.1671. Found: 431.1658.

4.7.4. 1,11-Bis-(3-methyl-1*H*-imidazolium-1-yl)undecane diiodide (10)

This compound was prepared in one step (96% yield) from free base **9** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12–1.34 (m, 14H), 1.68–1.84 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.2 Hz, 4H), 7.71 (s, 2H), 7.78 (s, 2H), 9.13 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.8, 28.9, 29.4, 35.8, 48.7, 122.2, 123.6, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{19}H_{34}N_4$: 159.1386. Found: 159.1380.

4.7.5. 1,12-Bis-(3-methyl-1*H*-imidazolium-1-yl)dodecane diiodide (12)

This compound was prepared in one step (97% yield) from free base **11** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15–1.32 (m, 16H), 1.70–1.82 (m, 4H), 3.85 (s, 6H), 4.14 (t, J = 7.2 Hz, 4H), 7.69 (s, 2H), 7.76 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.8, 28.9, 29.4, 35.8, 48.8, 122.3, 123.6, 136.4; HRMS (ESI) $[M\text{-}I]^+$. Calcd for $C_{20}H_{36}N_4I$: 459.1984. Found: 459.1977.

4.7.6. 1,13-Bis-(3-methyl-1*H*-imidazolium-1-yl)tridecane diiodide (14)

This compound was prepared in one step (96% yield) from free base **13** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.16–1.32 (m, 18H), 1.71–1.82 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.2 Hz, 4H), 7.71 (s, 2H), 7.77 (s, 2H), 9.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.9, 29.0 (2C), 29.4, 35.8, 48.8, 122.2, 123.6, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{21}H_{38}N_4$: 173.1542. Found: 173.1544.

4.7.7. 1,14-Bis-(3-methyl-1*H*-imidazolium-1-yl)tetradecane diiodide (16)

This compound was prepared in one step (95% yield) from free base **15** and was obtained as a amber oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15–1.35 (m, 20H), 1.70–1.85 (m, 4H), 3.85 (s, 6H), 4.10–4.20 (m, 4H), 7.71 (s, 2H), 7.77 (s, 2H), 9.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.9, 29.0, 29.1, 29.4, 35.8, 48.8, 122.3, 123.6, 136.5; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{22}H_{40}N_4$: 180.1621. Found: 180.1622.

4.7.8. 1,15-Bis-(3-methyl-1*H*-imidazolium-1-yl)pentadecane diiodide (18)

This compound was prepared in one step (80% yield) from free base **17** (using instead 2-propanol as reaction solvent) and was obtained as an amber oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15–1.35 (m, 22H), 1.70–1.85 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.2 Hz, 4H), 7.70 (s, 2H), 7.77 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.4, 28.3, 28.8, 28.9, 29.0, 29.3, 35.7, 48.7, 122.2, 123.5, 136.4; HRMS (ESI) $[M\text{-}2I\text{-}H]^{2+}$. Calcd for $C_{23}H_{41}N_4$: 373.3325. Found: 373.3326.

4.7.9. 1,16-Bis-(3-methyl-1*H*-imidazolium-1-yl)hexadecane diiodide (20)

This compound was prepared in one step (90% yield) from free base **19** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10–1.40 (m, 24H), 1.70–1.85 (m, 4H), 3.85 (s, 6H), 4.14 (t, J = 6.8 Hz, 4H), 7.70 (s, 2H), 7.76 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.8, 29.0, 29.05, 29.09, 29.4, 35.8, 48.8, 122.2, 123.6, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{24}H_{44}N_4$: 194.1777. Found: 194.1783.

4.7.10. 1,18-Bis-(3-methyl-1*H*-imidazolium-1-yl)octadecane diiodide (22)

This compound was prepared in one step (90% yield) from free base **21** and was obtained as a brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.10–1.40 (m, 28H), 1.70–1.85 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.0 Hz, 4H), 7.70 (s, 2H), 7.77 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.8, 28.96, 29.03, 29.07 (2C), 29.3, 35.8, 48.8, 122.2, 123.6, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{26}H_{48}N_4$: 208.1934. Found: 208.1946.

4.7.11. 1,20-Bis-(3-methyl-1*H*-imidazolium-1-yl)eicosane diiodide (24)

This compound was prepared in one step (70% yield) from free base **23** and was obtained as a yellow solid (recrystallized from 2-propanol/Et_2O); mp 78–80 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18–1.30 (m, 32H), 1.72–1.82 (m, 4H), 3.85 (s, 6H), 4.15 (t, J = 7.2 Hz, 4H), 7.70 (s, 2H), 7.77 (s, 2H), 9.11 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.5, 28.4, 28.8, 28.9, 29.1 (4C), 29.3, 35.8, 48.7, 122.2, 123.6, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{28}H_{52}N_4$: 222.2090. Found: 222.2089.

4.8. Other synthetic procedures

4.8.1. 1,12-Bis-(3-ethyl-1*H*-imidazolium-1-yl)dodecane diiodide (25)

The free base **11** (205 mg, 0.68 mmol, 1 equiv) was dissolved in 1-propanol (2 mL) at rt and to this solution was added iodoethane (0.49 mL, 955 mg, 6.12 mmol, 9 equiv). The mixture was stirred at reflux temperature (~110 °C) for 24 h, another portion of iodoethane (0.25 mL) was added, and the mixture stirred at ~110 °C for

3 h. The mixture was cooled to 0 °C, and Et_2O was added. The resulting insoluble oil was washed with Et_2O (3 × 10 mL, Et_2O layer removed using a pipette each time), then dried under high vacuum leaving an amber oil (323 mg, 0.53 mmol, 78%). The oil (R_f = 0, EtOAc) was purified using preparatory-scale thin-layer chromatography: the plate was loaded using MeOH, dried, and then eluted twice using 95:5 (v/v) EtOAc–MeOH, and then twice using 90:10 (v/v) EtOAc–MeOH; the bottom of the plate was excised and extracted using MeOH, the extract was filtered, and the filtrate concentrated. High-vacuum drying gave **25** (189 mg, 0.31 mmol, 46%) as an amber oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12–1.35 (m, 16H), 1.42 (t, J = 7.2 Hz, 6H), 1.73–1.87 (m, 4H), 4.10–4.28 (m, 8H), 7.79 (s, 2H), 7.81 (s, 2H), 9.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.0, 25.5, 28.4, 28.8, 28.9, 29.3, 44.2, 48.8, 122.1, 122.4, 135.6; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{22}H_{40}N_4$: 180.1627. Found: 180.1623.

4.8.2. 1,12-Bis-(3-propyl-1*H*-imidazolium-1-yl)dodecane diiodide (26)

The free base **11** (265 mg, 0.88 mmol, 1 equiv) was dissolved in 1-propanol (7 mL) at rt and to this solution was added 1-iodopropane (854 mg, 5.26 mmol, 6 equiv). The mixture was stirred at reflux temperature for 42 h and then concentrated. To the residue were added MeOH (5 mL) and Et_2O (20 mL) and the mixture concentrated, and then dried under high vacuum leaving an impure solid (525 mg). The solid (R_f ~0, 9:1 EtOAc–MeOH) was purified using flash column chromatography (colored impurities eluted first using 9:1 (v/v) EtOAc–MeOH, and then the product using 1:1 (v/v) EtOAc–MeOH). High-vacuum drying gave **26** (40 mg, 0.06 mmol, 7%) as a beige solid; mp 121–123 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (t, J = 7.4 Hz, 6H), 1.16–1.32 (m, 16H), 1.74–1.86 (m, 8H), 4.10–4.18 (m, 8H), 7.79 (s, 2H), 7.80 (s, 2H), 9.18 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.4, 22.7, 25.5, 28.3, 28.8, 28.9, 29.3, 48.9, 50.3, 122.4 (2C), 135.9; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{24}H_{44}N_4$: 194.1777. Found: 194.1774.

4.8.3. 1,4-Bis-(1*H*-imidazol-1-yl)benzene (27)

Under an atmosphere of N_2, a mixture of 1,4-dibromobenzene (2.50 g, 10.60 mmol, 1 equiv), imidazole (3.03 g, 44.52 mmol, 4.2 equiv), K_2CO_3 (4.69 g, 33.93 mmol, 3.2 equiv), and $CuSO_4$ (34 mg, 0.21 mmol, 0.02 equiv) was stirred at ~180 °C for 24 h. The mixture was cooled to rt, and diluted with water (100 mL). The resulting solid was collected by filtration and washed with water (100 mL). The brown solid obtained was dissolved in hot EtOH (200 mL), the solution filtered, and the filtrate concentrated. High-vacuum drying gave **27** (1.87 g, 8.90 mmol, 84%) as a white solid; mp 208–209 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23 (s, 2H), 7.30 (s, 2H), 7.52 (s, 4H), 7.87 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 118.3, 123.0, 131.1, 135.7, 136.6; HRMS (EI) $[M]^+$. Calcd for $C_{12}H_{10}N_4$: 210.0905. Found: 210.0900.

4.8.4. 1,4-Bis-(3-methyl-1*H*-imidazolium-1-yl)benzene diiodide (28)

Under an atmosphere of N_2, a mixture of 1,4-bis-(1*H*-imidazol-1-yl)benzene (**27**) (200 mg, 0.95 mmol, 1 equiv), 1-propanol (3 mL), and iodomethane (0.57 mL, 1.30 g, 9.50 mmol, 10 equiv) was stirred at rt for 1 h, then stirred at ~85 °C for 24 h. The mixture was cooled to rt and diluted with Et_2O (30 mL). The resulting white precipitate was collected by filtration and washed with Et_2O (20 mL). High-vacuum drying gave **28** (335 mg, 0.68 mmol, 72%) as a white solid; mp 324–325 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.98 (s, 6H), 8.01 (s, 2H), 8.11 (s, 4H), 8.40 (s, 2H), 9.91 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.4, 120.9, 123.4, 124.6, 135.2, 136.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{14}H_{16}N_4$: 120.0681. Found: 120.0680.

4.8.5. 4,4′-Bis-(1*H*-imidazol-1-yl)biphenyl (29)

Under an atmosphere of N_2, a mixture of 4,4′-dibromobiphenyl (5.00 g, 16.03 mmol, 1 equiv), imidazole (4.58 g, 67.33 mmol, 4.2 equiv), K_2CO_3 (6.98 g, 50.50 mmol, 3.2 equiv), and $CuSO_4$ (51.08 mg, 0.32 mmol, 0.02 equiv) was stirred at ~180 °C for 24 h. The mixture was cooled to rt, water (200 mL) was added, and the solid collected by filtration. The solid was dissolved in warm EtOH (200 mL), the solution filtered, and the filtrate concentrated and dried under high vacuum. The resulting off-white solid was purified using flash column chromatography on silica gel (silica plug was made using MeOH, washed with EtOAc, and eluted with MeOH). High-vacuum drying gave **29** (0.87 g, 3.04 mmol, 19%) as an off-white solid; mp 288–289 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.14 (s, 2H), 7.78 (d, J = 8.4 Hz, 4H), 7.83 (s, 2H), 7.88 (d, J = 8.8 Hz, 4H), 8.35 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 117.9, 120.7, 128.0, 130.0, 135.5, 136.3, 137.3; HRMS (EI) $[M]^+$. Calcd for $C_{18}H_{14}N_4$: 286.1218. Found: 286.1219.

4.8.6. 4,4′-Bis-(3-methyl-1*H*-imidazolium-1-yl)biphenyl diiodide (30)

Under an atmosphere of N_2, a mixture of 4,4′-bis-(1*H*-imidazol-1-yl)biphenyl (**29**) (200 mg, 0.70 mmol, 1 equiv), 1-propanol (3 mL), and iodomethane (0.44 mL, 994 mg, 7.00 mmol, 10 equiv) was stirred at ~85 °C for 6 h. The solution was stirred at rt for 24 h, then diluted with Et_2O. The solid was collected by filtration and washed with Et_2O. High-vacuum drying gave **30** (365 mg, 0.64 mmol, 91%) as a yellow solid; mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.98 (s, 6H), 7.93 (d, J = 8.4 Hz, 4H), 8.00 (s, 2H), 8.11 (d, J = 8.0 Hz, 4H), 8.39 (s, 2H), 9.87 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 36.3, 120.9, 122.3, 124.5, 128.5, 134.5, 136.0, 139.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{20}H_{20}N_4$: 158.0838. Found: 158.0857.

4.8.7. 4,4′-Bis-(1*H*-imidazol-1-yl-methyl)biphenyl (31)

Under an atmosphere of N_2, a mixture of imidazole (569 mg, 8.36 mmol, 2.1 equiv) and sodium hydroxide (334 mg, 8.36 mmol, 2.1 equiv) in dimethyl sulfoxide (3 mL) was stirred at 70–80 °C for 1 h. The mixture was cooled to rt and a solution of 4,4′-bis(chloromethyl)-1,1′-biphenyl (1.00 g, 3.98 mmol, 1 equiv) in dimethyl sulfoxide (5 mL) was slowly added. The mixture was stirred at 70–80 °C for 24 h, then cooled to rt and diluted with water (300 mL). The resulting white precipitate was collected by filtration, washed with water (100 mL), dissolved in MeOH (10 mL), and the solution was concentrated. High-vacuum drying gave **31** (981 mg, 3.12 mmol, 78%) as an off-white solid; mp 161–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.17 (s, 4H); 6.94 (s, 2H), 7.12 (s, 2H), 7.23 (d, J = 8.0 Hz, 4H), 7.55 (d, J = 7.6 Hz, 4H), 7.60 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 50.6, 119.4, 127.7, 127.9, 130.1, 135.7, 137.6, 140.5; HRMS (EI) $[M]^+$. Calcd for $C_{20}H_{18}N_4$: 314.1531. Found: 314.1519.

4.8.8. 4,4′-Bis-(3-methyl-1*H*-imidazolium-1-yl-methyl)biphenyl diiodide (32)

Under an atmosphere of N_2, a mixture of 4,4′-bis-(1*H*-imidazol-1-yl-methyl)biphenyl (**31**, 200 mg, 0.64 mmol, 1 equiv), 1-propanol (3 mL), and iodomethane (0.40 mL, 908 mg, 6.40 mmol, 10 equiv) was stirred at ~85 °C for 24 h, then stirred at rt for 24 h. The resulting yellow precipitate was collected by filtration and washed with Et_2O (50 mL). High-vacuum drying gave **32** (346 mg, 0.58 mmol, 91%) as yellow solid; mp 215–216 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.87 (s, 6H), 5.48 (s, 4H), 7.53 (d, J = 8.0 Hz, 4H), 7.73 (d, J = 6.8 Hz, 4H), 7.74 (s, 2H), 7.84 (s, 2H), 9.26 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 35.9, 51.5, 122.3, 124.0, 127.2, 129.0, 134.3, 136.7, 139.7; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{22}H_{24}N_4$: 172.0994. Found: 172.0992.

4.8.9. 4,4′-Bis-(3-benzyl-1*H*-imidazolium-1-yl)biphenyl dibromide (33)

Under an atmosphere of N_2, 4,4′-bis-(1*H*-imidazol-1-yl)biphenyl (**29**, 200 mg, 0.70 mmol, 1 equiv) was suspended in 1-propanol (3 mL) and to this mixture was added benzyl bromide (0.33 mL, 479 mg, 2.80 mmol, 4 equiv). The mixture was stirred at ~85 °C for 24 h, and then slowly cooled to 0 °C, to give a white solid precipitate. The solid was collected by filtration and washed with cold 1-propanol and then with Et_2O. The white solid obtained was recrystallized from hot MeOH. High-vacuum drying gave **33** (176 mg, 0.28 mmol, 40%) as a white solid; mp 308–309 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.56 (s, 4H), 7.41–7.50 (m, 6H), 7.98 (d, J = 8.4 Hz, 4H), 7.98 (d, J = 8.4 Hz, 4H), 8.11 (d, J = 8.8 Hz, 6H), 8.45 (s, 2H), 10.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.4, 121.6, 122.4, 123.3, 128.4, 128.6, 128.9, 129.0, 134.5, 134.5, 135.6, 139.4; HRMS (ESI) $[M-2Br]^{2+}$. Calcd for $C_{32}H_{28}N_4$: 234.1151. Found: 234.1148.

4.8.10. 4,4′-Bis-[3-(4-nitrobenzyl)-1*H*-imidazolium-1-yl]biphenyl dibromide (34)

Under an atmosphere of N_2, 4,4′-bis-(1*H*-imidazol-1-yl)biphenyl (**29**) (200 mg, 0.70 mmol, 1 equiv) was suspended in 1-propanol (6 mL) and to this mixture was added 4-nitrobenzyl bromide (907 mg, 4.20 mmol, 6 equiv). The mixture was stirred at ~110 °C for 72 h, and then slowly cooled to 0 °C, to give a solid precipitate. The solid was collected by filtration and washed with cold 1-propanol and then with Et_2O. The yellow solid obtained was recrystallized from hot MeOH (20 mL). High-vacuum drying gave **34** (127 mg, 0.18 mmol, 26%) as a yellow solid; mp 302–303 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.74 (s, 4H), 7.83 (d, J = 8.4 Hz, 4H), 7.98 (d, J = 8.4 Hz, 4H), 8.12 (d, J = 7.2 Hz, 6H), 8.31 (d, J = 8.4 Hz, 4H), 8.49 (s, 2H), 10.20 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.5, 121.7, 122.4, 123.4, 124.0, 128.4, 129.8, 134.5, 136.2, 139.5, 141.6, 147.7; HRMS (ESI) $[M\text{-}2Br]^{2+}$. Calcd for $C_{32}H_{26}N_6O_4$: 279.1002. Found: 279.1002.

4.8.11. 4,4′-Bis-(3-methyl-1*H*-benzimidazolium-1-yl)biphenyl diiodide (35)

Under an atmosphere of N_2, a mixture of 4,4′-dibromobiphenyl (1.00 g, 3.20 mmol, 1 equiv), benzimidazole (1.89 g, 16.00 mmol, 5 equiv), K_2CO_3 (1.37 g, 9.92 mmol, 3.1 equiv), and $CuSO_4$ (16 mg, 0.10 mmol, 0.03 equiv) was stirred at ~180 °C for 24 h. The mixture was cooled to rt, water (200 mL) was added, and the solid collected by filtration. The solid was dissolved in hot MeOH (200 mL), the solution filtered, and the filtrate concentrated and dried under high vacuum. The resulting grey solid was washed with hot water (3 × 50 mL) and then recrystallized from hot EtOAc (30 mL). High-vacuum drying gave 4,4′-bis-(1*H*-benzimidazol-1-yl)biphenyl (91 mg, 0.24 mmol, 8%) as a brown solid; mp 258–259 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30–7.42 (m, 4H), 7.72 (d, J = 7.6 Hz, 2H), 7.81 (d, J = 7.6 Hz, 2H), 7.85 (d, J = 8.4 Hz, 4H), 8.04 (d, J = 8.4 Hz, 4H), 8.66 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 110.8, 120.1, 122.6, 123.6, 124.2, 128.4, 135.6, 138.3; HRMS (ESI) $[M+H]^+$. Calcd for $C_{26}H_{19}N_4$: 387.1609. Found: 387.1607.

Under an atmosphere of N_2, a mixture of 4,4′-bis-(1*H*-benzimidazol-1-yl)biphenyl (59 mg, 0.15 mmol, 1 equiv), 1-propanol (2 mL), and iodomethane (0.09 mL, 213 mg, 1.50 mmol, 10 equiv) was stirred at ~85 °C for 24 h and then stirred at rt for 24 h. Et_2O (50 mL) was added, and the resulting solid precipitate was collected by filtration, washed with Et_2O, and dried under high vacuum to obtain a brown solid. The solid was recrystallized from hot MeOH (7 mL) with a few drops of Et_2O. High-vacuum drying gave **35** (20 mg, 0.03 mmol, 20%) as orange solid; mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.21 (s, 6H), 7.70–8.05 (m, 10H), 8.15–8.25 (m, 6H), 10.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 33.6, 114.0, 125.8, 128.9, 130.9, 131.9, 133.1, 140.3, 143.2; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{28}H_{24}N_4$: 208.0994. Found: 208.0992.

4.8.12. 1,4-Bis[3-(1*H*-imidazol-1-yl)propoxy]benzene dihydrochloride (36·2HCl)

Under an atmosphere of N_2, a mixture of hydroquinone (2.75 g, 25 mmol, 1 equiv), 1,3-dibromopropane (20.2 g, 100 mmol, 2 equiv), and anhydrous K_2CO_3 (10.35 g, 75 mmol, 6 equiv) in acetone (75 mL) was stirred at reflux temperature for 24 h. The solid was removed by filtration and discarded, and the filtrate was concentrated. The semi-solid residue was diluted with hexanes (125 mL), and the resulting solid was removed by filtration and discarded. The filtrate was set aside overnight, and the crystals that formed were collected by filtration (2.05 g). The filtrate was concentrated to a yellow oil that was diluted with 2-propanol (10 mL) and refrigerated overnight to afford a second crop (1.28 g) of crystals. The combined crystalline material was recrystallized from ethanol (10 mL). High-vacuum drying gave 1,4-bis (3-bromopropyloxy)benzene (2.88 g, 65%) as a yellow solid; mp 72–73 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.25–2.34 (m, 4H), 3.60 (t, J = 6.6 Hz, 4H), 4.06 (t, J = 5.8 Hz, 4H), 6.84 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.2, 32.6, 66.2, 115.7, 153.2; HRMS (EI) $[M]^+$. Calcd for $C_{12}H_{16}Br_2O_2$: 349.9512. Found 349.9521.

Under an atmosphere of N_2, a mixture of imidazole (1224 mg, 18 mmol, 6 equiv) and NaOH (720 mg, 18 mmol, 6 equiv) in DMSO (8 mL) was stirred at 70–80 °C for 1 h, then 1,4-bis(3-bromopropyloxy)benzene (1056 mg, 3 mmol, 1 equiv) was added, and the mixture stirred at 70–80 °C overnight. The mixture was partitioned between water (120 mL) and ethyl acetate (30 mL), the organic phase separated, and the aqueous phase was further extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with water (50 mL) and then with brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated to a brown solid (784 mg, 80%). A portion of this solid free base (228 mg, 0.70 mmol, 1 equiv) was dissolved in methanol (3 mL) and to this solution was added 37% aqueous HCl (300 mg, 3.05 mmol, 2.2 equiv). The mixture was concentrated and the residue recrystallized from 2-propanol. High-vacuum drying gave **36·2HCl** (146 mg, 0.37 mmol, 53% from free base, 42% overall) as a hygroscopic tan solid; mp 203–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.20–2.31 (m, 4H), 3.92 (t, J = 6.0 Hz, 4H), 4.38 (t, J = 6.8 Hz, 4H), 6.81 (s, 4H), 7.69 (t, J = 1.0 Hz, 2H), 7.84 (t, J = 1.0 Hz, 2H), 9.26 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.1, 46.1, 65.1, 115.3, 119.7, 122.1, 135.3, 152.4; HRMS (ESI) $[M-2Cl]^{2+}$. Calcd for $C_{18}H_{24}N_4O_2$: 164.0944. Found 164.0953.

4.8.13. 1,4-Bis[3-(3-methyl-1*H*-imidazolium-1-yl)propoxy]-benzene diiodide (37)

Under an atmosphere of N_2 atmosphere, the free base **36** (458 mg, 1.40 mmol, 1 equiv) was dissolved in 1-propanol (4 mL) at rt and to this solution was added iodomethane (1.99 g, 14.00 mmol, 10 equiv). The mixture was stirred at reflux temperature overnight, and then cooled to rt. The dark-brown solution was diluted with diethyl ether (25 mL), and the solution was stirred for 2 h resulting in an insoluble oil. The supernatant was removed using a pipette, and the oil washed with diethyl ether (2 × 25 mL). The brown oil was dissolved in hot ethanol (10 mL), diluted with ethyl acetate (10 mL), and stirred vigorously overnight. The solid that formed was collected by filtration. High-vacuum drying gave **37** (510 mg, 60%) as a brown solid; mp 127–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.14–2.29 (m, 4H), 3.85 (s, 6H), 3.95 (t, J = 6.0 Hz, 4H), 4.34 (t, J = 6.8 Hz, 4H), 6.84 (s, 4H), 7.72 (t, J = 1.6 Hz, 2H), 7.81 (t, J = 1.6 Hz, 2H), 9.17 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 29.1, 35.8, 46.4, 64.9, 115.4, 122.4, 123.5, 136.7, 152.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{20}H_{28}N_4O_2$: 178.1100. Found 178.1100.

4.8.14. 1,12-Bis(1*H*-1,2,4-triazol-1-yl)dodecane dihydrochloride (38·2HCl)

Under an atmosphere of N_2, a mixture of 1,2,4-triazole (1035 mg, 15.00 mmol, 2.5 equiv) and NaOH (600 mg, 15.00 mmol, 2.5 equiv) in DMSO (4 mL) was stirred at 70–80 °C for 1 h, then 1,12-dibromododecane (1.97 g, 6.00 mmol, 1 equiv) was added, and the mixture stirred at 70–80 °C overnight. The mixture was diluted with water (50 mL), and the solid that formed was collected by filtration and air-dried to give the crude bis-triazole product (1.50 g, 4.93 mmol, 82%). The solid was dissolved in ethanol (10 mL), and then a 37% aqueous solution of HCl (2 g, 20.30 mmol, 2.1 equiv) in ethanol (2 mL) was added. The precipitate that formed was collected by filtration and recrystallized from ethanol. High-vacuum drying gave **38·2HCl** (1.45 g, 64%) as a white solid; mp 192–193 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.21 (br s, 16H), 1.72–1.83 (m, 4H), 4.20 (t, J = 7.0 Hz, 4H), 8.24 (s, 2H), 8.91 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.6, 28.3, 28.8, 28.9, 49.6, 142.8, 148.0; HRMS (ESI) $[M-2Cl]^{2+}$. Calcd for $C_{16}H_{30}N_6$: 153.1261. Found 153.1265.

4.8.15. 1,12-Bis(4-methyl-1*H*-1,2,4-triazolium-1-yl)dodecane diiodide (39)

A sample of **38·2HCl** (530 mg, 1.40 mmol) was suspended in water (30 mL) and the mixture basified with an aqueous solution of NaOH (2 M). The mixture was extracted with ethyl acetate (30 mL). The organic phase was washed successively with water (30 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. High-vacuum drying gave the free base **38** (417 mg, 1.37 mmol, 98%) as a white solid. Under an atmosphere of N_2, the free base **38** (417 mg, 1.37 mmol, 1 equiv) was dissolved in warm 1-propanol (7 mL) and the solution was treated with iodomethane (1.95 g, 13.72 mmol, 10 equiv). The mixture was stirred at reflux temperature overnight, and then it was cooled to rt and diluted with diethyl ether (20 mL). The solid that formed was collected by filtration, washed with diethyl ether, and then recrystallized from methanol. High-vacuum drying gave **39** (492 mg, 61%) as an off-white solid; mp 203–204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.18–1.34 (m, 16H), 1.77–1.89 (m, 4H), 3.89 (s, 6H), 4.35 (t, J = 7.0 Hz, 4H), 9.13 (s, 2H), 10.05 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 25.4, 28.1, 28.3, 28.8, 28.9, 34.1, 51.4, 142.9, 145.4; HRMS (ESI) $[M-2I]^{2+}$. Calcd for $C_{18}H_{34}N_6$: 167.1417. Found 167.1419.

4.9. Determination of anti-*Plasmodium* activity

P. falciparum cultures were grown in O+ blood obtained by venipuncture of volunteers. Cultures of the laboratory line ItG were maintained by the method of Trager and Jensen[32] using RPMI-1640 supplemented with 10% human serum and 50 μM hypoxanthine (RPMI-A). The effects of the test compounds on the viability of *P. falciparum* cultures were evaluated using a lactate dehydrogenase (LDH) enzyme assay specific to the enzyme found in *P. falciparum* (pLDH).[33,34] Briefly, compounds to be tested were dissolved in DMSO to afford a solution having a concentration of 10 mg/mL. Fifty microliters of RPMI-A were added to every well in a 96-well plate, then 5 μL of the 10 mg/mL compound solution mixed with 45 μL of RPMI-A were added to the first well, and then serially diluted across the plate to produce a compound gradient with two-fold dilutions. Fifty microliters of parasite culture (2% hematocrit, 2% parasitemia) were added to each well and the plates were then incubated at 37 °C in 95% N_2, 3% CO_2, and 2% O_2 for 72 h. The contents of the wells were then re-suspended and 15-μL samples were removed and added to 100 μL of pLDH enzyme assay mixture in the corresponding well of a second 96-well plate.[33] After 1 h the absorbance of the wells at 595 nm was determined using a ThermoMax microplate reader (Molecular Devices, Sunnyvale, CA). The IC_{50} values of individual compounds were determined using a non-linear regression analysis of the data[35] using the computer program SigmaPlot (Jandel Scientific). The IC_{50} values represent the mean ± SEM, n = 4. Parallel experiments using chloroquine were included as a positive control. The determination of the activities of compounds in *P. berghei* cultures presented in Table 2 was performed using the SYBR-Green method[35] as previously described.[4] *P. berghei* was obtained by cardiac puncture of an infected mouse (parasitemia of 5%); the blood was washed 3 times in RPMI and was then plated at a 2% hematocrit in a gradient of compounds as above. After 24 h the contents of the well were agitated to effect merozoite release[36] and the plate was incubated for a further 24 h before SYBR-Green I was added and the relative fluorescence was determined using a Fluostar Optima plate reader (BMG Labtech, Offenburg, Germany).

4.10. Mammalian strains and culture

The effect of the compounds on CHO cell cultures was determined by using the MTT assay[37] as described previously.[5] CHO cells (ATCC®, Manassas, VA, USA) were grown in RPMI-1640 supplemented with 10% fetal calf serum (Sigma–Aldrich® Canada, Ltd, Oakville, ON, Canada), 25 mM HEPES, and gentimicin (referred to as RPMI-10). Cells were seeded in 96-well plates and grown to 50% confluency in 100 μL of RPMI-10 per well prior to the addition of either DMSO alone, or a 10 mg/mL solution of a test compound in DMSO. Compound gradients were prepared by adding 90 μL of RPMI-10 mixed with 10 μL of compound solution to the first well in the series, mixing, transferring 100 μL to the next well, and repeating until the next-to-last well was reached. After 48 h, the viability of the cells was determined by discarding the media in the wells and adding 100 μL of 10 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich® Canada, Ltd, Oakville, ON, Canada) in RPMI-10, incubating the plates for a further 30 min, and then removing the media and adding 100 μL of DMSO and reading the absorbance at 650 nm (see Ref. 37). The IC_{50} values of individual compounds were determined using a non-linear regression analysis of the sigmoidal dose-response curves using the computer program SigmaPlot (Jandel Scientific®, San Rafael, CA, USA).

4.11. Examination of treated parasite cultures using light microscopy

A Percoll gradient[38] was used to synchronize 3D7 parasite cultures at the mature stage of development one day prior to the start of the parasite development assay. The cultures were then adjusted to have a hematocrit of 1% and a parasitemia of 2%. Parasites were then treated with either 40 nM or 83 nM of compound **39** and sampled every 24 h over a period of 4 days. Controls included uninfected RBCs, untreated cultures, treatment with sulfated β-cyclodextrin (sodium salt, 7–11 mol of sulfate residues per mol of β-cyclodextrin), and treatment with chloroquine.

4.12. Flow cytometry analysis

Parasite cultures were synchronized as ring-stage cultures by sorbitol lysis.[38] The cultures were then adjusted to a parasitemia of 5% and a hematocrit of 0.5% and were treated with various compounds for 48 h before an equal volume of RPMI-1640 containing 0.2 μL per mL of stock SYBR-Green solution was added, and the mixture was allowed to incubate for 1 h in the dark. The samples were subjected to flow cytometry using a FACS Calibur Flow Cytometer in a level II BSC. Data from the sample were collected and analysed using the FlowJo software program.

4.13. In vivo evaluation of compounds

Female 6–8 week-old Balb/c mice (Charles River) were infected with 10^6 *P. berghei* parasites on Day 0 and their parasitemia was followed by producing Giemsa-stained peripheral blood films until parasites were observed in the peripheral circulation of the mice. The mice were then infused once daily for three days with either 0.5 mL of RPMI-1640 or RPMI-1640 containing compound. The health of the animals was monitored closely and the parasitemia of the mice was determined by examining Giemsa-stained peripheral blood films. Experimental groups contained five mice each and had unrestricted access to food and water during the experiment.

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