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Anti-*Plasmodium* activity of imidazolium and triazolium salts

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ABSTRACT

We have previously reported that tetrazolium salts were both potent and specific inhibitors of *Plasmodium* replication, and that they appear to interact with a parasite component that is both essential and conserved. The use of tetrazolium salts in vivo is limited by the potential reduction of the tetrazolium ring to form an inactive, neutral acyclic formazan. To address this issue imidazolium and triazolium salts were synthesized and evaluated as *Plasmodium* inhibitors. Many of the imidazolium and triazolium salts were highly potent with active concentrations in the nanomolar range in *Plasmodium falciparum* cultures, and specific to *Plasmodium* with highly favorable therapeutic ratios. The results corroborate our hypothesis that an electron-deficient core is required so that the compound may thereby interact with a negatively charged moiety on the parasite merozoite; the side groups in the compound then form favorable interactions with adjacent parasite components and thereby determine both the potency and selectivity of the compound.

1. Introduction

Malaria, which is caused by infection with protozoan parasites of the genus *Plasmodium*, is a major global health problem and is responsible annually for the deaths of over a million people, mainly children in sub-Saharan Africa.[1] At present, prevention and treatment strategies are continuously hampered by the emergence of resistance in parasites to newly introduced drugs. Further, there are considerable costs involved in drug development, and the market for antimalarials is hard to value and is prone to logistical problems.[2] The development of effective malaria vaccines would change this situation,[3] however, in the absence of such vaccines, and because of the widespread resistance to currently used antimalarial drugs such as chloroquine, new chemotherapeutic agents are needed to help in the prevention and control of this disease.[4] Two major approaches have been employed in the search for new antimalarial drugs.[5] The more widely used approach is the development of chemical analogs of existing antimalarial agents.[6] An alternative approach is the identification of novel drug targets within the parasite, followed by the design of chemical entities acting on these targets.[7] Potential antimalarial chemotherapeutic targets have been classified into three main categories: inhibition of processes occurring in the digestive vacuole, inhibition of the enzymes involved in macromolecular and metabolite synthesis, and inhibition of membrane processing and signaling.[8] We are pursuing a novel strategy, namely, the prevention of malaria parasites from invading erythrocytes. The invasion of hepatocytes by *Plasmodium falciparum* depends on the interaction of parasite surface proteins with negatively charged carbohydrates, such as sialate residues or sulfated glycosaminoglycans (GAGs), on the surface of host cells. We observed[9] that anionic agents similar to such negatively charged carbohydrate structures also interfered with the entry of *P. falciparum* merozoites into human red blood cells, and, hence our 'first-generation' inhibitors consisted of short-chain aliphatic polysulfonates that were effective at blocking merozoite entry into erythrocytes. Our 'second-generation' inhibitors were based on sulfated cyclodextrins, and these provided a better definition of the structural features that inhibit the parasite/merozoite/host red-cell interaction.[10] During the course of an exploration of the relationship between structure and activity of the sulfated cyclodextrins, it was determined that the compounds interact with a specific component of the erythrocyte membrane, namely, the anion-transport protein AE1, causing a disruption to the invasion process. The observation that nitrotetrazolium blue chloride, a component of the parasite viability assays, formed stable complexes with the inhibitory sulfated cyclodextrins led us to the development of our 'third-generation' of anti-*Plasmodium* agents, which were based on tetrazolium salts. In an extensive study,[11] tetrazolium salts were found to be both potent and specific inhibitors of *Plasmodium* invasion, and were found to interact with a component of the *parasite* that is both essential and conserved. This parasite component, which we believe to be a sulfated or phosphorylated moiety such as in the phosphoinositol component of the Merozoite Surface Protein 1, is responsible for the recognition of

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erythrocytes by *Plasmodium* and allows the parasites to adhere to a receptor domain in the AE1 found on red cells and thereby initiate their invasion process.[12,13] In the presence of tetrazolium inhibitors, parasite entry into the erythrocyte is aborted and parasite cultures become unviable. The structure of one such 'third-generation' inhibitor, namely, tetrazolium red (commercially available), is shown in Figure 1. We hypothesize that the compound functions by attracting the parasite ligand by way of its electron-deficient tetrazolium ring and then forms favorable interactions involving its side groups. While the tetrazolium salts can be highly potent and selective for malaria parasites, a potential problem with this series of compounds is the possible reduction of the tetrazolium ring to form an inactive acyclic formazan (see Fig. 1); also, tetrazolium compounds may be susceptible to photochemical degradation. These issues of stability have been addressed in the present study by the replacement of tetrazolium salts by imidazolium or triazolium salts thereby allowing us to formulate the next generations of potential *Plasmodium* invasion inhibitors. A general depiction of the imidazolium and triazolium ring structures is shown in Figure 1. The anti-*Plasmodium* activities of a series of these cationic azolium salts were evaluated; it was observed that many of the compounds exhibited strong and specific inhibitory activity towards *P. falciparum* cultures, similar to that observed for tetrazolium salts. Indeed, these new imidazolium and triazolium containing compounds have provided drug candidates possessing greater potency and selectivity than the original tetrazolium compounds. Moreover, the results corroborate our hypothesis that an electron-deficient core is required for the compound's interaction with a negatively charged moiety on the parasite merozoite ligand and that the side groups in the compound then form favorable interactions with adjacent parasite components and thereby determine potency.

2. Results and discussion

2.1. Synthesis

The imidazolium compounds (**1**–**19**) and triazolium compounds (**21**–**30**) in Table 1 were synthesized by six main protocols depicted in Schemes 1–3. As a precursor to some imidazolium compounds, the 1-substituted imidazole, 1-(4-bromobenzyl)imidazole, was prepared by the alkylation of imidazole with 4-bromobenzyl bromide in KOH–DMSO following a reported similar procedure.[14] As shown in Scheme 1 (Protocol 1), most of the symmetrical 1,3-disubstituted imidazolium salts (including **1**, **3**, and **6–15**) were synthesized directly by the treatment of imidazole with an excess amount of alkyl halide {including substituted benzyl halides and 2-(bromomethyl)naphthalene} in toluene following a modification (omission of base) of a published procedure.[15,16] Alternate procedures for the formation of symmetrical 1,3-disubstituted imidazolium salts were also employed. Compounds **16** and **17** were prepared (Scheme 1, Protocol 2) by the dialkylation of imidazole with the appropriate α-bromoketone in DMF. Compound **19** was prepared (Scheme 1, Protocol 3) by the alkylation of 1-methylimidazole with iodomethane in 1-propanol following a related published procedure.[17] Also shown in Scheme 1 (Protocol 3), the unsymmetrical 1,3-disubstituted imidazolium salts **2**, **4**, **5**, and **18** were prepared by the alkylation of the appropriate 1-substituted imidazole with the appropriate alkyl or benzyl halide in toluene following a reported similar procedure.[18]

As shown in Scheme 3 and 1,4-disubstituted-[1,2,4]triazolium salts were prepared by two main protocols. Compound **27** was prepared (Protocol 5) by the alkylation of [1,2,4]triazole using benzyl bromide in K_2CO_3–THF, following reported similar procedures.[19,20] Similarly, compound **29** was prepared (Protocol 5) by the alkylation of [1,2,4]triazole using 4,2′-dibromoacetophenone in DMF. Using a different approach, compound **28** was prepared (Protocol 6) by the alkylation of **32** (free-base form) with benzyl bromide in 1-propanol.

The synthesis of tri- and tetra-substituted[1,2,4]triazoles involved the preparation of substituted triazole precursors. Thus, 3,5-diphenyl-[1,2,4]triazole was synthesized by the ring-forming condensation reaction of benzoic hydrazide with benzonitrile.[21] 1-Benzyl-3,5-diphenyl-[1,2,4]triazole and 1-(4-bromobenzyl)-3,5-diphenyl-[1,2,4]triazole were prepared by the alkylation of 3,5-diphenyl-[1,2,4]triazole with benzyl bromide and 4-bromobenzyl bromide, respectively, in K_2CO_3–DMF.[19] As shown in Scheme 2 (Protocol 4), the 1,3,4-trisubstituted-[1,2,4]triazolium salt **26** was synthesized by the methylation of 4-phenyl-1-(3-phenyl-[1,2,4]-triazol-1-yl)-butan-2-one in the 4-position using trimethyloxonium tetrafluoroborate in 1,2-dichloroethane following a modification of a reported similar procedure.[22] Similarly, the 1,3,4,5-tetrasubstituted-[1,2,4]triazolium salts (including **23**–**25**) were synthesized by the methylation of the appropriate 1,3,5-trisubstituted-[1,2,4]triazole in the 4-position using trimethyloxonium tetrafluoroborate in 1,2-dichloroethane, as shown in Scheme 2 (Protocol 4). The wide diversity of available starting materials (most notably substituted benzyl halides) allowed control over the nature and position of substituents when designing these imidazolium and triazolium compounds. This flexibility permitted a systematic exploration of structure–activity relationships of several imidazolium and triazolium salts (Table 1) with the goal of finding effective antimalarial candidates.

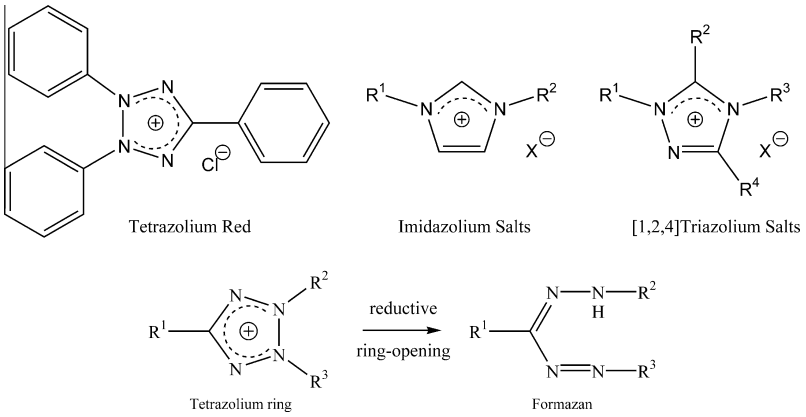


Figure 1. Tetrazolium red, generic structures of imidazolium and triazolium salts, and the reductive ring-opening reaction of tetrazolium salts to form acyclic formazans.

Table 1
Activities of imidazolium and [1,2,4]triazolium compounds in *P. falciparum* and CHO cell cultures[a]

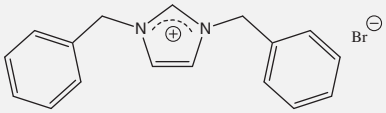
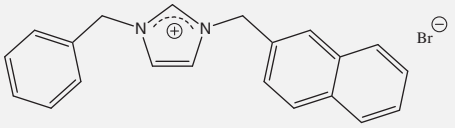
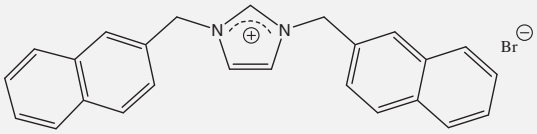
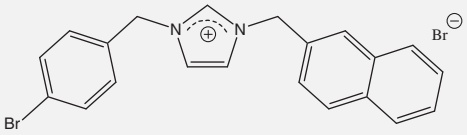
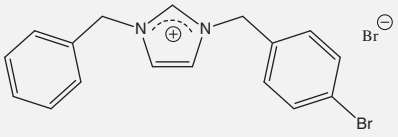
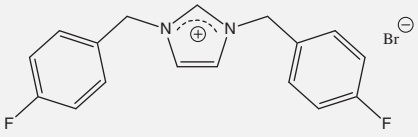
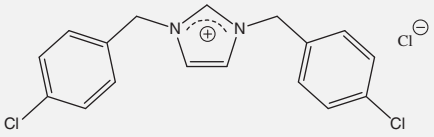
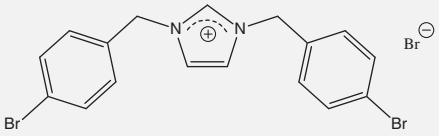
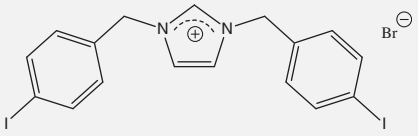
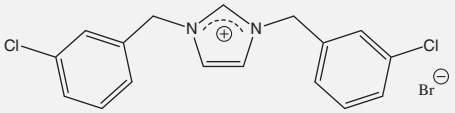
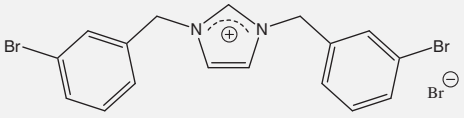
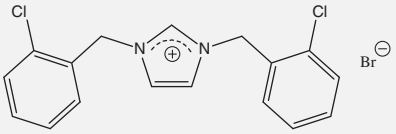
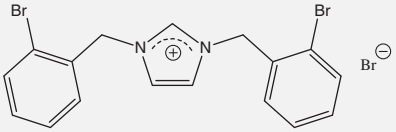
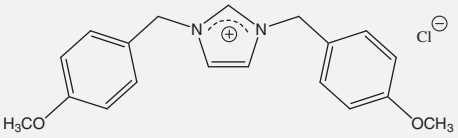
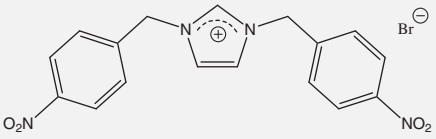
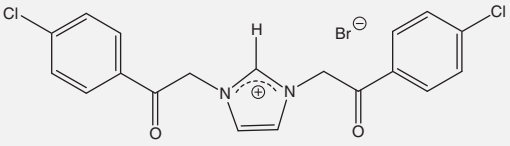
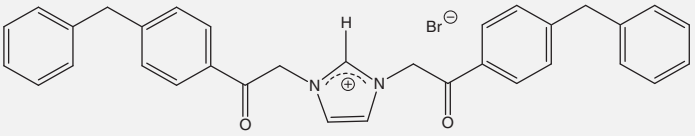
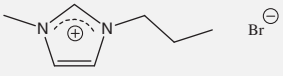
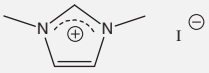
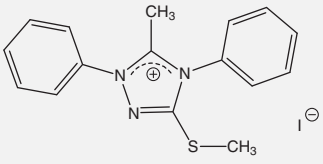
Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO/IC_{50} *P. falciparum*
Imidazolium compounds				
1		16 ± 1	191 ± 3	12
2		0.9 ± 0.2	108 ± 6	120
3		0.9 ± 0.4	27 ± 1	30
4		0.73 ± 0.03	27 ± 5	37
5		2.9 ± 0.9	484.5 ± 0.9	167
6		17 ± 3	56 ± 4	3
7		9 ± 2	105 ± 1	12
8		0.7 ± 0.1	171.9 ± 0.1	246
9		0.35 ± 0.06	33 ± 1	94
10		3.3 ± 0.4	334.2 ± 0.4	101
11		1.05 ± 0.06	132 ± 22	126

Table 1 (*continued*)

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO/IC_{50} *P. falciparum*
12		2.0 ± 0.2	110 ± 23	55
13		1.4 ± 0.6	125 ± 10	89
14		5 ± 1	30.6 ± 0.7	6
15		9 ± 2	227 ± 7	25
16		2.6 ± 0.1	53 ± 2	20
17		0.5 ± 0.1	4.3 ± 0.3	9
18		230 ± 83	998 ± 83	4
19		543 ± 66	894 ± 19	2
20		368	3675	10
Triazolium compounds				
21		3.1 ± 0.8	307 ± 37	99

(*continued on next page*)

Table 1 (*continued*)

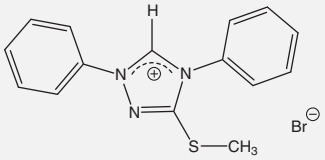
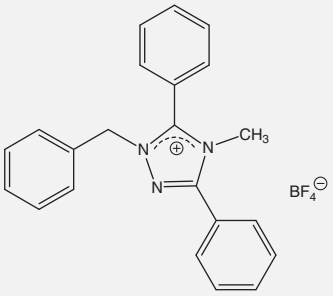
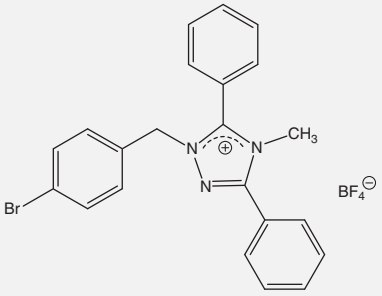
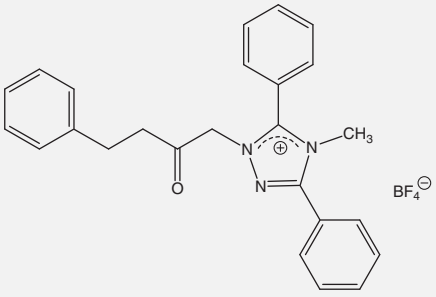
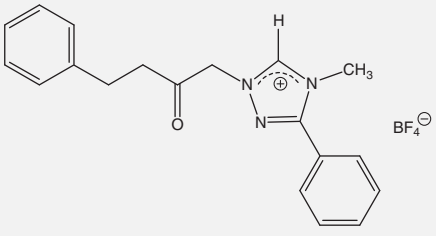
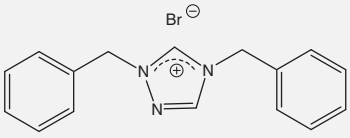
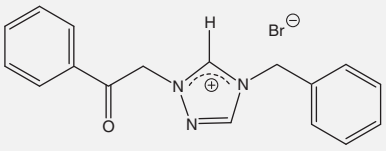
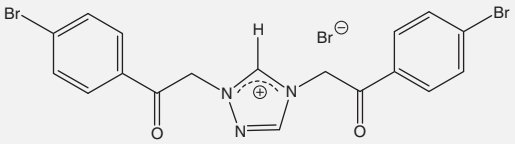
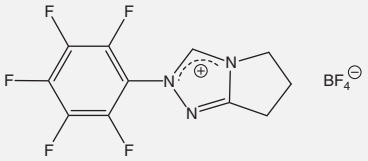
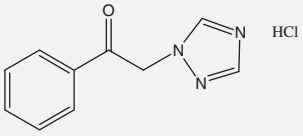
Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO/IC_{50} *P. falciparum*
22		69 ± 8	224 ± 21	3
23		7.8 ± 0.2	87 ± 8	11
24		1.68 ± 0.05	33 ± 2	20
25		0.10 ± 0.08	143 ± 14	1430
26		3.4 ± 0.2	237 ± 17	70
27		100 ± 8	74 ± 4	0.7

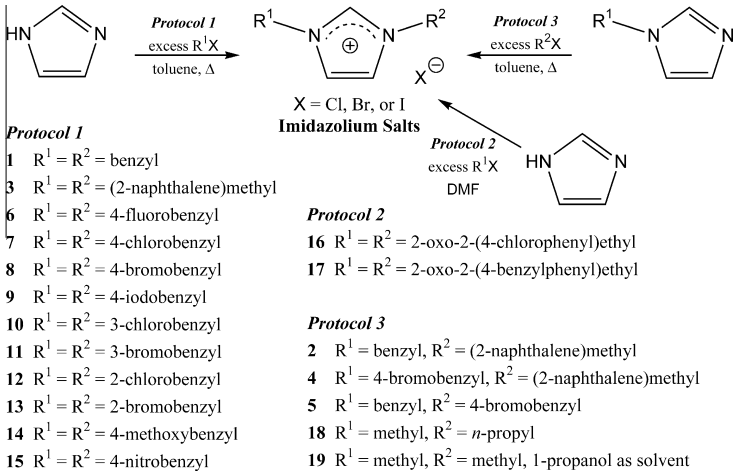
Table 1 (*continued*)

Compound	Structure	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) CHO cells	IC_{50} CHO/IC_{50} *P. falciparum*
28		73 ± 3	261 ± 3	4
29		10.9 ± 0.7	20.9 ± 0.5	2
30		89 ± 5	303 ± 15	3
31		2896	1273 ± 35	0.4
32		894	334 ± 11	0.4

[a] Each IC_{50} value represents the mean of four determinations with standard error indicated. The 'ratio of activities' given in the fifth column represents the IC_{50} value determined for CHO cells divided by the IC_{50} value determined for *P. falciparum* cultures. Values greater than unity indicate that the compound has greater potency in *P. falciparum* cultures. The strain line used was ItG. Parallel experiments were performed using chloroquine as a positive control. IC_{50} values of 10–50 nM were obtained indicating that the hematocrit, parasitemia, and incubation time used produced results that were comparable to those reported using other conditions.

2.2. Biological evaluation

The imidazolium and triazolium compounds in Table 1 were evaluated for anti-*Plasmodium* activity using *P. falciparum* cultures, whereas the general toxicity of these compounds was determined using Chinese hamster ovary (CHO) cell cultures. It should be noted that the *Plasmodium* strain employed is chloroquine-sensitive. The IC_{50} values are listed in Table 1; a selectivity index was calculated



Protocol 1

1 $R^1 = R^2$ = benzyl
3 $R^1 = R^2$ = (2-naphthalene)methyl
6 $R^1 = R^2$ = 4-fluorobenzyl
7 $R^1 = R^2$ = 4-chlorobenzyl
8 $R^1 = R^2$ = 4-bromobenzyl
9 $R^1 = R^2$ = 4-iodobenzyl
10 $R^1 = R^2$ = 3-chlorobenzyl
11 $R^1 = R^2$ = 3-bromobenzyl
12 $R^1 = R^2$ = 2-chlorobenzyl
13 $R^1 = R^2$ = 2-bromobenzyl
14 $R^1 = R^2$ = 4-methoxybenzyl
15 $R^1 = R^2$ = 4-nitrobenzyl

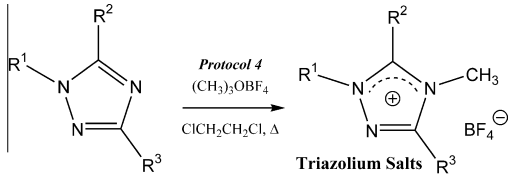
Protocol 2

16 $R^1 = R^2$ = 2-oxo-2-(4-chlorophenyl)ethyl
17 $R^1 = R^2$ = 2-oxo-2-(4-benzylphenyl)ethyl

Protocol 3

2 R^1 = benzyl, R^2 = (2-naphthalene)methyl
4 R^1 = 4-bromobenzyl, R^2 = (2-naphthalene)methyl
5 R^1 = benzyl, R^2 = 4-bromobenzyl
18 R^1 = methyl, R^2 = *n*-propyl
19 R^1 = methyl, R^2 = methyl, 1-propanol as solvent

Scheme 1. Synthesis of symmetrical and unsymmetrical imidazolium salts.



Protocol 4

23 R^1 = benzyl, R^2 = phenyl, R^3 = phenyl

24 R^1 = 4-bromobenzyl, R^2 = phenyl, R^3 = phenyl

25 R^1 = 2-oxo-4-phenylbutyl, R^2 = phenyl, R^3 = phenyl

26 R^1 = 2-oxo-4-phenylbutyl, R^2 = hydrogen, R^3 = phenyl

Scheme 2. Synthesis of 1,3,4-trisubstituted-[1,2,4]triazolium salts and 1,3,4,5-tetrasubstituted-[1,2,4]triazolium salts.

by dividing the IC_{50} values obtained in CHO cell cultures by the IC_{50} values obtained in *P. falciparum* cultures. The relative toxicity of these compounds was also compared graphically by plotting the IC_{50} values obtained in *P. falciparum* cultures against the values obtained in CHO cell cultures (Fig. 2). More than 90% of the compounds tested in Table 1 were found to be selectively toxic to *Plasmodium* as evidenced by the majority of data points in Figure 2 falling above the diagonal line that represents unity. This result is not unexpected since our synthetic design was based on the premise that triazolium and imidazolium components would interact with the parasite ligand, or some other parasite component, in a manner similar to that seen for the tetrazolium structures. The lack of a clear linear trend in this plot is also consistent with the compounds having a mechanism of action, for example, invasion inhibition, that is unique to *Plasmodium*, and, hence activity in the parasite cultures was poorly correlated with CHO cell toxicity. Microscopic examination of the inhibited assay wells containing individual imidazolium or triazolium salts confirmed the presence of a large number of extracellular merozoites and a lack of ring-stage parasites (see Fig. 3), a feature consistent with the hypothesis that inhibition of merozoite entry is also the possible primary mechanism of action of the triazolium and imidazolium generations of compounds. Interestingly, 19 of the 29 imidazolium and triazolium compounds exhibited modest selectivity (selectivity index >10) for *Plasmodium*. Furthermore, 11 of the 29 imidazolium and triazolium compounds exhibited enhanced selectivity with selectivity indexes >50, with one even approaching 1430 (compound **25**). Consistent with our hypothesis about the active motif, the uncharged triazoles **31** and **32** were not selective for *Plasmodium*. As regards anti-*Plasmodium* potency, 17 out of the 29 imidazolium and triazolium compounds tested exhibit IC_{50} values of less than 5 μM for *P. falciparum*, a result supportive of our hypothesis that they interact with a specific parasite component.

Our analysis of the results obtained in Table 1 revealed many specific structure–activity relationships. Our current hypothesis is that potency and the selective interaction of the compounds result from a combination of an electron-deficient ring and hydrophobic side groups. This hypothesis is illustrated by the

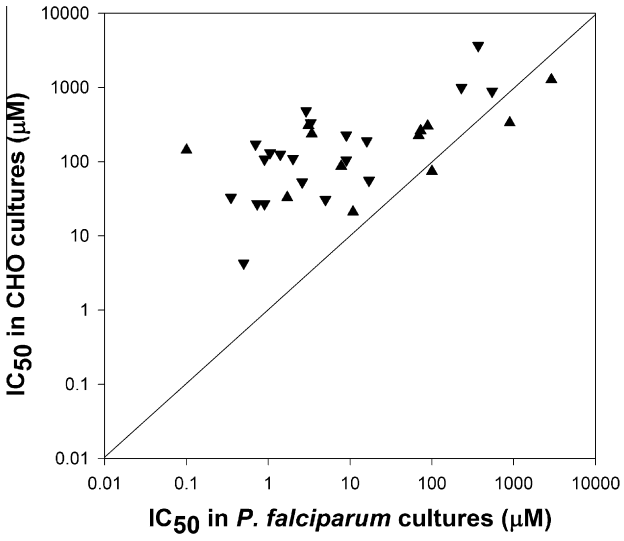
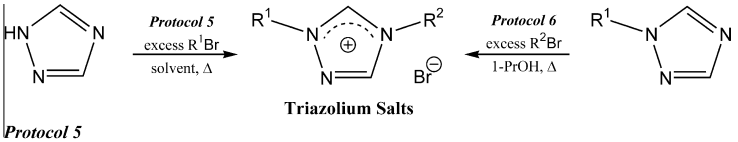


Figure 2. IC_{50} values for *P. falciparum* cultures were plotted against values obtained in CHO cell cultures. Points representing triazolium compounds (▲) and imidazolium compounds (▼) are depicted.

progressive changes seen in the series of compounds **20**, **19**, **18**, and **1**. Thus, there is a dramatic increase in both potency and selectivity in going from the uncharged compound **20** to the charged compound **1** having two benzyl moieties. In comparison to the parent dibenzylated imidazolium compound **1** (IC_{50} = 16 ± 1 μM, selectivity index of 12), the replacement of one benzene ring by a larger, more hydrophobic aromatic ring system such as that of naphthalene was found to increase antimalarial potency and selectivity. For example, compounds **2** and **3** both showed an 18-fold increase in potency when compared to compound **1**. Potency against *Plasmodium* is also increased by the incorporation of a halogen atom, apart from fluorine, in the phenyl substituents of these compounds. The presence of halogen atoms would have an electron-withdrawing effect on the imidazolium ring and thereby accentuate the electron deficiency of this component. Thus, the addition of halogens to the scaffold of **1** gave compounds **5** and **7**–**13**, all of which are significantly more potent against *P. falciparum* than **1**. For example, the addition of just one *p*-bromo substituent, gave compound **5** which exhibits an IC_{50} value of 2.9 ± 0.9 μM and a selectivity index of 167, whereas the addition of two *p*-bromo substituents gave compound **8** which exhibits an IC_{50} value of 0.7 ± 0.1 μM and a selectivity index of 246. The increased potency afforded by halogen incorporation was also noticed when comparing the results of imidazolium compounds **2** and **4**, and also the triazolium compounds **23** and **24**. Symmetrical imidazolium compounds having halogen atoms in the 4-position of the benzyl functionality showed a clear trend in potency against *P. falciparum* that was dependent on the size (and electronegativity) of the halogen. For example, the IC_{50} values (*P. falciparum*) for the directly comparable F, Cl, Br, I series of compounds (**6**, **7**, **8**, and **9**) were 17 ± 3,



Protocol 5

27 $R^1 = R^2$ = benzyl, K_2CO_3-THF as solvent

29 $R^1 = R^2$ = 2-oxo-2-(4-bromophenyl)ethyl, DMF as solvent

Protocol 6

28 R^1 = 2-oxo-2-phenylethyl, R^2 = benzyl

Scheme 3. Synthesis of 1,4-disubstituted-[1,2,4]triazolium salts.

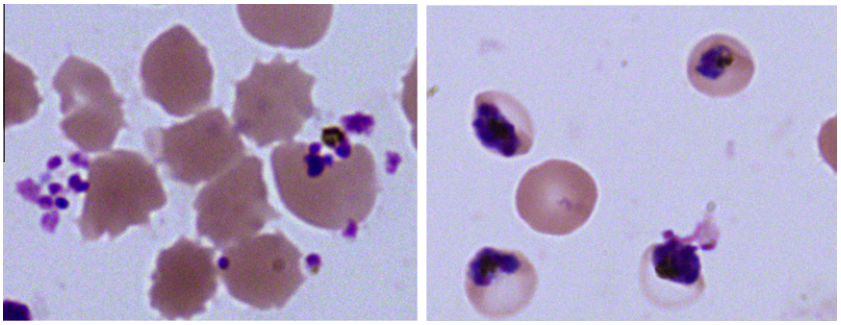


Figure 3. Photomicrograph of parasite cultures treated with 7 μM of compound **8** (left) or untreated (right).

9 ± 2, 0.7 ± 0.1, and 0.35 ± 0.06, respectively, the iodo compound **9** being the most potent; apart from the bromo and iodo compounds, a parallel trend was observed for the selectivity of these compounds for *Plasmodium*. As regards the effect of the positions of the halogen substituents on the aromatic rings, in the chlorine series (**12**, **10**, and **7**) the *o*-chloro compound **12** was the most potent and the *m*-chloro compound **10** was the most selective, whereas, for the bromine series (**13**, **11**, and **8**) the *p*-bromo compound **8** was the most potent and selective inhibitor. The presence of a *p*-methoxy group (as in **14**) or a *p*-nitro group (as in **15**) resulted in each case, in a lower IC_{50} value for *P. falciparum* when compared to that of **1**. Our hypothesis that an electron-deficient ring surrounded by hydrophobic side groups could form an active motif was also supported by the behavior of the triazolium compounds, as it was observed that, of the compounds studied herein, those having the more-substituted structures were the most-effective drug candidates. Thus, the tetrasubstituted triazolium compounds **21**, **23**, **24**, and **25** were more effective anti-*Plasmodium* agents than the trisubstituted triazolium compounds **22**, **26**, and **30**. Two direct comparisons were evident. The addition of a methyl substituent to the 5-position of **22** led to compound **21** which exhibited a 22-fold increase in potency and a 30-fold increase in selectivity towards *P. falciparum*. Similarly, the addition of a phenyl substituent to the 5-position of **26** led to compound **25** which exhibited a 34-fold increase in potency and a 21-fold increase in selectivity towards *P. falciparum*. Compound **25**, a 1,3,4,5-tetra-substituted triazolium tetrafluoroborate, displayed remarkable antimalarial properties, with an IC_{50} value for *Plasmodium* of 100 nM and a selectivity index of 1430 over CHO cells. To exclude the possibility that the tetrafluoroborate counterion influenced the viability assays, sodium tetrafluoroborate was also assayed as a control and IC_{50} values of 848 ± 135 μM for *P. falciparum* and 1991 ± 152 μM for CHO cells were observed, results indicating it is a well-tolerated counterion in both systems. The disubstituted triazolium compounds **27**, **28**, and **29** were active, but were found to be neither highly potent or selective agents against *P. falciparum*, suggesting that triazolium and imidazolium structures have similar, but not interchangeable, activities Thus, it was not surprising that the monosubstituted and unsubstituted triazoles **32** and **31**, respectively, were essentially inactive, particularly as they do not conform to our putative motif.

Although the comparisons are not direct, it appears that the presence of a β-keto functionality does increase potency, as can be seen by comparison of results for compounds **27**, **28**, and **29**, and also for compounds **7** and **16**. The electron-withdrawing properties of the keto functionality renders the central azolium ring system more electron-deficient, and this feature may contribute to the enhanced antimalarial activity. Indeed, some of our most-effective tetrazolium inhibitors[11] possessed strongly electron-withdrawing substituents such as fluorine.

In an effort to determine the effect that the cationic core structure has on activity, the directly comparable dibenzylated imidazolium and triazolium compounds **1** and **27**, respectively, were prepared. The imidazolium analog **1** was 6 times more potent and 17 times more selective for *Plasmodium*. A comparison between imidazolium compound **16** and triazolium compound **29** revealed similar results.

The precise nature of how these compounds affect the parasite cultures is not clear. Non-synchronous parasite cultures were used and the assay was allowed to incubate for 72 h. During this time parasites remained at the ring stage of development for 24 h prior to maturing to the point of merozoite production during the next 24 h. The parasitized cell then bursts releasing merozoites which will then invade new erythrocytes and will form ring-stage parasites. There were several observations that suggested that the compounds prevented merozoite entry instead of releasing rings from previously infected cells. Thus, for example, (1) external parasite forms were only seen after synchronized cultures had passed through the mature stage of development, (2) a much larger number of parasites were observed after 48 h than were in the original culture (suggesting replication had taken place), and (3) external parasites can be immunostained with an antibody against merozoite surface proteins (data not shown).

3. Conclusions

Several compounds that express excellent selectivity as inhibitors of *P. falciparum* relative to CHO cells have been synthesized. In particular, imidazolium salts **2**, **5**, and **8**–**11**, and triazolium salts **21** and **24**–**26** are noteworthy for their high potency and selectivity towards *Plasmodium*. The results obtained in *P. falciparum* and CHO cell assays support the hypothesis that potency in parasite cultures results from the presence of an electron-deficient ring attached to hydrophobic side groups. Tetrazolium, triazolium, and imidazolium salts all appear to provide candidates that show acceptable levels of activity in *P. falciparum* cultures coupled with selectivity for replicating parasites. These active compounds are unrelated to currently used antimalarial agents and further they have become very useful tools in elucidating the mechanistic aspects of how *Plasmodium* invades erythrocytes; their novel mechanism of action suggests that they could be developed for prophylactic and therapeutic applications. The increased stability of the current generation of compounds compared to the previously described tetrazolium series is a major step forward in the development of antimalarial therapeutics, since it will permit the evaluation of anti-invasion strategies in mouse models of malaria.

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 illumi-

nation (254 nm), or by heating after spraying with phosphomolybdic acid in ethanol. Melting points were taken on a Mel-Temp II apparatus and are uncorrected. ^{1}H 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.[23] 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.

4.2. Materials

2-(Bromomethyl)naphthalene, 3-chlorobenzyl bromide, and 2-chlorobenzyl bromide were obtained from Alfa Aesar® (Ward Hill, MA, USA). 5-Methyl-3-(methylthio)-1,4-diphenyl-1*H*-1,2,4-triazolium iodide (**21**) and 3-(methylthio)-1,4-diphenyl-1*H*-1,2,4-triazolium bromide (**22**) were obtained from Sigma–Aldrich® Canada, Ltd. (Oakville, ON, Canada). The rest of the chemical reagents were obtained from Sigma–Aldrich® Canada, Ltd. (Oakville, ON, Canada), and were used without further purification. The syntheses of 4′-benzyl-2-bromoacetophenone, 1-(3,5-diphenyl-[1,2,4]triazol-1-yl)-4-phenyl-butan-2-one, 4-phenyl-1-(3-phenyl-[1,2,4]triazol-1-yl)-butan-2-one, and **32** will be reported elsewhere.

4.3. Representative procedure for the formation of 1-substituted imidazole precursors

4.3.1. 1-(4-Bromobenzyl)imidazole

Imidazole (400 mg, 5.80 mmol, 1 equiv) was added to a mixture of DMSO (10 mL) and potassium hydroxide (394 mg, 7.05 mmol, 1.2 equiv) and the mixture was stirred for 30 min at 80 °C under an atmosphere of N_2. 4-Bromobenzyl bromide (1.76 g, 7.05 mmol, 1.2 equiv) was added and the mixture stirred for 2.5 h at 80 °C. The mixture was cooled to rt, water was added, and the mixture was extracted with ether (2 × 25 mL). The combined organic phase was washed with water, dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography on silica gel (30:1 v/v $CHCl_3$–CH_3OH as eluent, then CH_3OH) to give the product (680 mg, 2.87 mmol, 49%) as an orange oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.41 (s, 2H), 7.40 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.84 (s, 1H), 9.39 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 51.3, 119.8, 122.2, 122.9, 130.8, 132.0, 134.0, 136.4; HRMS (EI) $[M]^+$ Calcd for $C_{10}H_9N_2Br$: 235.9949. Found: 235.9940.

4.4. General procedure for the formation of symmetrical di-substituted imidazolium salts (Scheme 1)

Under an atmosphere of N_2, the benzyl or alkyl halide (11.01 mmol, 3 equiv) in toluene (5 mL) was added dropwise to a solution of imidazole (3.67 mmol, 1 equiv) in toluene (10 mL). The mixture was stirred for 30 min at rt and then for 72 h at 70 °C. After removal of the toluene, the crude product was washed with diethyl ether to remove the excess of benzyl or alkyl halide. Purification by flash column chromatography on silica gel (9:1 v/v CH_2Cl_2–CH_3OH) followed by recrystallization from 2-propanol afforded the corresponding symmetrical di-substituted imidazolium salt.

4.5. Characterization of the compounds synthesized following the general procedure for the formation of symmetrical di-substituted imidazolium salts

4.5.1. 1,3-Dibenzylimidazolium bromide (1)

Compound **1** was prepared using imidazole and benzyl bromide as starting materials to give the product (724 mg, 87%) as a clear oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.43 (s, 4H), 7.39–7.43 (m, 10H), 7.83 (d, J = 1.5 Hz, 2H), 9.41 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.1, 122.9, 128.3, 128.8, 129.0, 134.8, 136.3; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{17}N_2$: 249.1391. Found: 249.1388.

4.5.2. 1,3-Bis-(naphthalen-2-ylmethyl)imidazolium bromide (3)

Compound **3** was prepared using imidazole and 2-(bromomethyl)naphthalene as starting materials to give the product (754 mg, 48%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.61 (s, 4H), 7.55–7.58 (m, 7H), 7.90–7.97 (m, 7H), 8.00 (s, 2H), 9.50 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.3, 123.1, 125.7, 126.8, 127.6, 127.7, 127.9, 128.8, 132.2, 132.7, 132.8, 136.6; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{25}H_{21}N_2$: 349.1704. Found: 349.1700.

4.5.3. 1,3-Bis-(4-fluorobenzyl)imidazolium bromide (6)

Compound **6** was prepared using imidazole and 4-fluorobenzyl bromide as starting materials to give the product (737 mg, 55%) as an off-white oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.40 (s, 4H), 7.24–7.29 (m, 4H), 7.49–7.52 (m, 4H), 7.80 (d, J = 1.4 Hz, 2H), 9.36 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.4, 115.9, 116.1, 122.9, 131.0, 131.1, 136.2; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}F_2N_2$: 285.1203. Found: 285.1196.

4.5.4. 1,3-Bis-(4-chlorobenzyl)imidazolium chloride (7)

Compound **7** was prepared using imidazole and 4-chlorobenzyl chloride as starting materials to give the product (150 mg, 12%) as a white sticky solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.48 (s, 4H), 7.49–7.50 (m, 10H), 7.89 (d, J = 1.4 Hz, 2H), 9.68 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.1, 122.9, 129.0, 130.5, 133.5, 133.8, 136.6; HRMS (ESI) $[M-Cl]^+$ Calcd for $C_{17}H_{15}Cl_2N_2$: 317.0612. Found: 317.0628.

4.5.5. 1,3-Bis-(4-bromobenzyl)imidazolium bromide (8)

Compound **8** was prepared using imidazole and 4-bromobenzyl bromide as starting materials to give the product (263 mg, 47%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.43 (s, 4H), 7.40 (d, J = 8.5 Hz, 4H), 7.60 (d, J = 8.5 Hz, 4H), 7.85 (d, J = 1.6 Hz, 2H), 9.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.3, 122.2, 122.9, 130.7, 131.9, 134.0, 136.4; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}Br_2N_2$: 404.9601. Found: 404.9571.

4.5.6. 1,3-Bis-(4-iodobenzyl)imidazolium bromide (9)

Compound **9** was prepared using imidazole and 4-iodobenzyl bromide as starting materials to give the product (66 mg, 28%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.39 (s, 4H), 7.22 (d, J = 8.3 Hz, 4H), 7.79 (d, J = 8.3 Hz, 4H), 7.82 (d, J = 1.5 Hz, 2H), 9.38 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.4, 95.4, 122.9, 130.6, 134.3, 136.4, 137.7; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}I_2N_2$: 500.9324. Found: 500.9327.

4.5.7. 1,3-Bis-(3-chlorobenzyl)imidazolium bromide (10)

Compound **10** was prepared using imidazole and 3-chlorobenzyl bromide as starting materials to give the product (1.00 g, 68%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.45 (s, 4H), 7.38–7.43 (m, 2H), 7.45–7.48 (m, 4H), 7.55 (s, 2H), 7.85 (d, J = 1.4 Hz, 2H), 9.41 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.3, 122.9, 127.2, 128.4, 128.8, 130.9, 133.5, 136.6, 137.0; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}Cl_2N_2$: 317.0612. Found: 317.0601.

4.5.8. 1,3-Bis-(3-bromobenzyl)imidazolium bromide (11)

Compound **11** was prepared using imidazole and 3-bromobenzyl bromide as starting materials to give the product (581 mg, 32%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.43 (s, 4H), 7.38–7.45 (m, 4H), 7.60–7.62 (m, 2H), 7.69 (s, 2H), 7.85 (d, J = 1.4 Hz, 2H), 9.39 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.3, 122.1,

123.0, 127.6, 131.2, 131.2, 131.7, 136.7, 137.3; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}Br_2N_2$: 404.9601. Found: 404.961.

4.5.9. 1,3-Bis-(2-chlorobenzyl)imidazolium bromide (12)

Compound **12** was prepared using imidazole and 2-chlorobenzyl bromide as starting materials to give the product (660 mg, 45%) as a white solid; ^1H NMR (400 MHz, DMSO-d_6): δ 5.59 (s, 4H), 7.45–7.48 (m, 6H), 7.55–7.57 (m, 2H), 7.82 (d, J = 1.4 Hz, 2H), 9.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 50.2, 123.1, 128.0, 129.9, 130.9, 131.0, 131.9, 132.9, 137.4; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}Cl_2N_2$: 317.0612. Found: 317.0613.

4.5.10. 1,3-Bis-(2-bromobenzyl)imidazolium bromide (13)

Compound **13** was prepared using imidazole and 2-bromobenzyl bromide as starting materials to give the product (846 mg, 47%) as a beige solid; ^1H NMR (400 MHz, DMSO-d_6): δ 5.56 (s, 4H), 7.36–7.40 (m, 4H), 7.47–7.51 (m, 2H), 7.73 (d, J = 7.9 Hz, 2H), 7.81 (d, J = 1.4 Hz, 2H), 9.37 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.4, 123.1, 123.2, 128.5, 130.7, 131.1, 133.2, 133.5, 137.5; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}Br_2N_2$: 404.9601. Found: 404.9616.

4.5.11. 1,3-Bis-(4-methoxybenzyl)imidazolium chloride (14)

Compound **14** was prepared using imidazole and 4-methoxybenzyl chloride as starting materials to give the product (550 mg, 43%) as a white solid; ^1H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H), 5.32 (s, 4H), 6.96 (d, J = 8.6 Hz, 4H), 7.37 (d, J = 8.6 Hz, 4H), 7.58 (d, J = 1.5 Hz, 2H), 9.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.6, 55.2, 114.4, 122.6, 129.5, 130.2, 135.7, 159.6; HRMS (ESI) $[M-Cl]^+$ Calcd for $C_{19}H_{21}N_2O_2$: 309.1603. Found: 309.1617.

4.5.12. 1,3-Bis-(4-nitrobenzyl)imidazolium bromide (15)

Compound **15** was prepared using imidazole and 4-nitrobenzyl bromide as starting materials to give the product (731 mg, 47%) as a white solid; ^1H NMR (400 MHz, DMSO-d_6): δ 5.62 (s, 4H), 7.66 (d, J = 8.5 Hz, 4H), 7.9 (d, J = 0.9 Hz, 2H), 8.29 (d, J = 8.5 Hz, 4H), 9.45 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.2, 123.3, 124.1, 129.6, 137.3, 141.9, 147.7; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{15}N_4O_4$: 339.1093. Found: 339.110.

4.6. Alternate procedures for the formation of symmetrical disubstituted imidazolium salts (Scheme 1)

4.6.1. 1,3-Bis-[2-oxo-2-(4-chlorophenyl)ethyl]imidazolium bromide (16)

This compound was obtained as the minor product in a procedure designed for the synthesis of the monoalkylated analog. To a solution of 2-bromo-1-(4-chlorophenyl)ethanone (3.50 g, 15 mmol, 1 equiv) in DMF (15 mL) was added imidazole (3.06 g, 45 mmol, 3 equiv) under an atmosphere of N_2. The mixture was stirred at rt for 3 h, then slowly poured into water (250 mL), and stirring was continued for 0.5 h. The resulting white precipitate was removed by filtration, and the solid was washed with boiling toluene (100 mL) to remove the monoalkylated product. The toluene-insoluble material was dried under high vacuum and then recrystallized from 1:1 v/v MeOH–EtOH. The solid was removed by filtration and washed with EtOH. High-vacuum drying gave **16** (438 mg, 0.96 mmol, 13%) as a white solid; mp >260 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 6.17 (s, 4H), 7.74 (d, J = 8.4 Hz, 4H), 7.80 (s, 2H), 8.09 (d, J = 8.4 Hz, 4H), 9.09 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.6, 123.7, 129.2, 130.0, 132.5, 138.5, 139.3, 190.5; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{19}H_{15}Cl_2N_2O_2$: 373.0510. Found: 373.0488.

4.6.2. 1,3-Bis-[2-oxo-2-(4-benzylphenyl)ethyl]imidazolium bromide (17)

This compound was obtained as the minor product in a procedure designed for the synthesis of the monoalkylated analog. To a solution of 1-(4-benzylphenyl)-2-bromoethanone (1.21 g, 4.18 mmol, 1 equiv) in DMF (5 mL) was added imidazole (0.85 g, 12.49 mmol, 3 equiv) under an atmosphere of N_2. The mixture was stirred at rt for 3 h, then slowly poured into water (100 mL), and stirring was continued for 0.5 h. The resulting white precipitate was removed by filtration, and the solid was washed with boiling toluene (50 mL) to remove the monoalkylated product. The toluene-insoluble material was dried under high vacuum and then recrystallized from EtOH (5 mL). The solid was removed by filtration and washed with EtOH (1 mL). High-vacuum drying gave **17** (131 mg, 0.23 mmol, 11%) as a white solid; mp 215–217 °C; ^1H NMR (400 MHz, DMSO-d_6): δ 4.07 (s, 4H), 6.11 (s, 4H), 7.18–7.34 (m, 10H), 7.50 (d, J = 8.4 Hz, 4H), 7.76 (d, J = 1.2 Hz, 2H), 7.99 (d, J = 8.0 Hz, 4H), 9.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 41.0, 55.5, 123.7, 126.3, 128.5, 128.6, 128.8, 129.4, 131.8, 138.6, 140.4, 148.5, 190.8; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{33}H_{29}N_2O_2$: 485.2229. Found: 485.2219.

4.6.3. 1,3-Dimethylimidazolium iodide (19)

To a solution of 1-methylimidazole (202 mg, 2.46 mmol, 1 equiv) in 1-propanol (2 mL) at rt was added iodomethane (0.31 mL, 4.98 mmol, 2 equiv) under an atmosphere of N_2. The mixture was heated at 100 °C for 19 h, then cooled to 0 °C. Et_2O was added slowly with stirring resulting in a yellow precipitate that was removed by filtration and washed sequentially with Et_2O (3×) and also with EtOAc (5×). High-vacuum drying gave **19** (470 mg, 2.10 mmol, 85%) as a yellow solid; mp 78–80 °C; ^1H NMR (400 MHz, CD_3OD): δ 3.93 (s, 6H), 7.56 (s, 2H), 8.87 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 36.6, 124.8, 138.6; HRMS (ESI) $[M-I]^+$ Calcd for $C_5H_9N_2$: 97.0765. Found: 97.0765; HRMS (ESI) $[M-C_5H_9N_2]^-$ Calcd for I: 126.9045. Found: 126.9049.

4.7. General procedure for the formation of unsymmetrical disubstituted imidazolium salts (Scheme 1)

Under an atmosphere of N_2, the benzyl or alkyl halide (4.40 mmol, 1.2 equiv) in toluene (5 mL) was added dropwise to a solution of 1-benzylimidazole, 1-methylimidazole, or another 1-substituted imidazole (3.67 mmol, 1 equiv) in toluene (10 mL). The mixture was stirred for 30 min at rt and then for 72 h at 70 °C. After removal of the toluene, the crude product was washed with Et_2O to remove the excess of benzyl or alkyl bromide. Recrystallization from 2-propanol gave the corresponding unsymmetrical disubstituted imidazolium salt.

4.8. Characterization of the compounds synthesized following the general procedure for the formation of unsymmetrical disubstituted imidazolium salts

4.8.1. 1-Benzyl-3-(naphthalen-2-yl-methyl)imidazolium bromide (2)

Compound **2** was prepared using 1-benzylimidazole and 2-(bromomethyl)naphthalene as starting materials to give the product (46 mg, 32%) as a white solid; ^1H NMR (400 MHz, CD_3OD): δ 5.43 (s, 2H), 5.60 (s, 2H), 7.41–7.45 (m, 5H), 7.47 (dd, J = 2 Hz, 1H), 7.52–7.56 (m, 1H), 7.64–7.69 (m, 1H), 7.88–7.93 (m, 3H), 7.94 (s, 1H), 7.96 (s, 1H), 9.26 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 54.3, 54.4, 124.1, 124.2, 126.5, 128.0, 128.1, 128.9, 129.1, 129.4, 129.7, 130.4, 130.5, 132.4, 134.8, 134.9, 135.2, 137.7; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{21}H_{19}N_2$: 299.1548. Found: 299.1553.

4.8.2. 1-(4-Bromobenzyl)-3-(naphthalen-2-ylmethyl)imidazolium bromide (4)

Compound **4** was prepared using 1-(4-bromobenzyl)imidazole and 2-(bromomethyl)naphthalene as starting materials, recrystal-

lized from 2:1 v/v 2-propanol–Et_2O to give the product (312 mg, 64%) as a yellow-brown oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.43 (s, 2H), 5.60 (s, 2H), 7.39 (d, J = 8.5 Hz, 2H), 7.52–7.58 (m, 3H), 7.63 (d, J = 8.5 Hz, 2H), 7.84 (s, 1H), 7.88 (s, 1H), 7.91–7.99 (m, 4H), 9.43 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.3, 52.3, 122.1, 122.8, 123.0, 125.7, 126.7, 126.7, 127.6, 127.8, 128.8, 130.6, 131.9, 131.9, 132.7, 132.7, 134.0, 136.5; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{21}H_{18}BrN_2$: 377.0653. Found: 377.0641.

4.8.3. 1-Benzyl-3-(4-bromobenzyl)imidazolium bromide (5)

Compound **5** was prepared using 1-benzylimidazole and 4-bromobenzyl bromide as starting materials to give the product (330 mg, 51%) as a beige solid; ^{1}H NMR (400 MHz, CD_3OD): δ 5.44 (s, 2H), 5.45 (s, 2H), 7.38–7.436 (m, 4H), 7.63–7.65 (m, 4H), 7.86 (d, J = 1.6 Hz, 2H), 9.47 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 51.2, 52.0, 122.2, 122.8, 122.9, 128.4, 128.8, 129.0, 130.7, 131.9, 134.1, 134.7, 136.3; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{16}BrN_2$: 327.0496. Found: 327.0513.

4.8.4. 3-Methyl-1-propylimidazolium bromide (18)

Compound **18** was prepared using 1-methylimidazole and 1-bromopropane as starting materials to give the product (519 mg, 83%) as an orange oil; ^{1}H NMR (400 MHz, CD_3OD): δ 0.96 (t, J = 7.4 Hz, 3H), 1.88 (m, 2H), 3.96 (s, 3H), 4.19 (t, J = 7.2 Hz, 2H), 7.60 (s, 1H), 7.67 (s, 1H), 9.02 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 10.9, 24.5, 36.6, 52.3, 123.7, 125.0, 137.9; HRMS (ESI) $[M-Br]^+$ Calcd for $C_9H_{13}N_2$: 125.1078. Found: 125.1084.

4.9. Representative procedure for the formation of 3,5-disubstituted [1,2,4]triazoles

4.9.1. 3,5-Diphenyl-[1,2,4]triazole

Under an atmosphere of N_2, a mixture of benzoic hydrazide (4.00 g, 29.30 mmol, 1 equiv) and benzonitrile (39.60 g, 384.00 mmol, 13.1 equiv) was stirred at reflux temperature for 14 h in a round-bottom flask equipped with a Dean–Stark apparatus to remove water. The mixture was cooled to rt, and the resulting precipitate collected by filtration and washed with 2-propanol. Recrystallization from 2-propanol gave the product (3.77 g, 58%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.41–7.59 (m, 6H), 8.07–8.10 (m, 2H), 8.12 (1s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 125.9, 126.1, 128.7, 129.1, 130.2, 131.3; HRMS (EI) $[M]^+$ Calcd for $C_{14}H_{11}N_3$: 221.0953. Found: 221.0948.

4.10. Representative procedures for the formation of 1,3,5-trisubstituted [1,2,4]triazoles

4.10.1. 1-Benzyl-3,5-diphenyl-[1,2,4]triazole

Under an atmosphere of N_2, a mixture of 3,5-diphenyl-[1,2,4]triazole (250 mg, 1.13 mmol) and potassium carbonate (471 mg, 3.39 mmol) in DMF (5 mL) was stirred at 75 °C for 1 h. A solution of benzyl bromide (386 mg, 2.26 mmol) in DMF (3 mL) was added, and the mixture heated at reflux temperature with stirring for 48 h; the progress of the reaction was monitored by TLC. The mixture was cooled to rt, diluted with EtOAc (25 mL), and the organic layer washed sequentially with water (2 × 25 mL) and a saturated aqueous solution of $NaHCO_3$ (2 × 25 mL), dried (Na_2SO_4), and concentrated to a beige solid. Recrystallization from hexanes gave the product (282 mg, 3.2 mmol, 80%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41–7.59 (m, 6H), 8.07–8.10 (m, 2H), 8.12 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 52.4, 120.9, 125.8, 126.8, 127.6, 127.8, 128.5, 128.8, 129.0, 129.3, 130.3, 130.7, 136.3, 155.6, 160.3; HRMS (EI) $[M]^+$ Calcd for $C_{21}H_{17}N_3$: 311.1422. Found: 311.1426.

4.10.2. 1-(4-Bromobenzyl)-3,5-diphenyl-[1,2,4]triazole

Under an atmosphere of N_2, a mixture of 3,5-diphenyl-[1,2,4]triazole (500 mg, 2.26 mmol) and potassium carbonate (942 mg, 6.78 mmol) in DMF (10 mL) was stirred at 75 °C for 1 h. A solution of 4-bromobenzyl bromide (1.41 g, 5.65 mmol) in DMF (3 mL) was added, and the mixture was stirred at reflux temperature for 48 h; the progress of the reaction was monitored by TLC. The mixture was cooled to rt, diluted with EtOAc (25 mL), and the organic layer washed sequentially with water (2 × 25 mL) and a saturated aqueous solution of $NaHCO_3$ (2 × 25 mL), dried (Na_2SO_4), and concentrated to an orange oil. Purification by flash column chromatography on silica gel ($CHCl_3$ as eluent to remove impurities, followed by elution of the product with MeOH) gave the product (563 mg, 64%) as an orange oil; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.55 (s, 2H), 7.11 (d, J = 8.3 Hz, 2H), 7.44–7.56 (m, 8H), 7.7–7.72 (m, 2H), 8.05 (s, 1H), 8.072–8.074 (d, J = 1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 51.79, 120.92, 125.85, 127.48, 128.52, 128.75, 128.98, 129.1, 129.31, 130.34, 130.64, 131.66, 135.7, 155.6, 160.42; HRMS (EI) $[M]^+$ Calcd for $C_{21}H_{16}N_3Br$: 389.0528. Found: 389.0536.

4.11. Representative procedures for the formation of 1,3,4,5-tetrasubstituted-[1,2,4]triazolium salts (Scheme 2)

4.11.1. 1-Benzyl-4-methyl-3,5-diphenyl-[1,2,4]triazolium tetrafluoroborate (23)

To a solution of 1-benzyl-3,5-diphenyl-[1,2,4]triazole (200 mg, 0.64 mmol) in 1,2-dichlorethane (2 mL) was added a solution of trimethyloxonium tetrafluoroborate (104 mg, 0.70 mmol) in 1,2-dichlorethane (1 mL) under an atmosphere of N_2. The mixture was heated at 65 °C with stirring for 5 h; the progress of the reaction was monitored by TLC (9:1 v/v CH_2Cl_2–CH_3OH as eluent). The mixture was concentrated to a clear oil that was purified by flash column chromatography on silica gel ($CHCl_3$ as eluent to remove impurities, followed by elution of the product with MeOH). High-vacuum drying gave **23** (175 mg, 66%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66 (s, 3H), 5.55 (s, 2H), 7.26–7.28 (m, 2H), 7.36–7.38 (m, 3H), 7.69–7.87 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.6, 54.1, 119.3, 123.1, 128.1, 128.7, 128.8, 129.4, 129.5, 129.8, 130.2, 132.2, 133.3, 133.5, 152.3, 154.0; HRMS (ESI) $[M-BF_4]^+$ Calcd for $C_{22}H_{20}N_3$: 326.1657. Found: 326.1663.

4.11.2. 1-(4-Bromobenzyl)-4-methyl-3,5-diphenyl-[1,2,4]triazolium tetrafluoroborate (24)

To a solution of 1-(4-bromobenzyl)-3,5-diphenyl-[1,2,4]triazole (300 mg, 0.77 mmol) in 1,2-dichlorethane (5 mL) was added a solution of trimethyloxonium tetrafluoroborate (136 mg, 0.92 mmol) in 1,2-dichlorethane (2 mL) under an atmosphere of N_2. The mixture was heated at 65 °C with stirring for 10 h; the progress of the reaction was monitored by TLC (9:1 v/v CH_2Cl_2–CH_3OH as eluent). The mixture was concentrated to a clear oil. Purification by flash column chromatography on silica gel ($CHCl_3$ as eluent to remove impurities, followed by elution of the product with MeOH) gave **24** (184 mg, 49%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.66 (s, 3H), 5.54 (s, 2H), 7.24 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.1 Hz, 3H), 7.71–7.84 (m, 8H), 7.86 (d, J = 0.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 34.58, 53.35, 119.21, 121.99, 123.03, 129.35, 129.46, 129.84, 130.19, 130.43, 131.64, 132.27, 132.65, 133.51, 152.42, 154.08; HRMS (ESI) $[M-BF_4]^+$ Calcd for $C_{22}H_{19}BrN_3$: 404.0762. Found: 404.0771.

4.11.3. 4-Methyl-1-(2-oxo-4-phenylbutyl)-3,5-diphenyl-[1,2,4] triazolium tetrafluoroborate (25)

To a solution of 1-(3,5-diphenyl-[1,2,4]triazol-1-yl)-4-phenylbutan-2-one (107 mg, 0.29 mmol, 1 equiv) in 1,2-dichloroethane (2 mL) was added trimethyloxonium tetrafluoroborate (46 mg,

0.31 mmol, 1.07 equiv) in 1,2-dichloroethane (1 mL) under an atmosphere of N_2. The mixture was heated at 65 °C with stirring for 3.5 h, and then concentrated to a golden oil. High-vacuum drying left a foamy white solid which was purified by flash chromatography on silica gel ($CHCl_3$ as eluent to remove impurities, followed by elution of the product with EtOAc) gave **25** (49 mg, 0.10 mmol, 34%) as a clear oil; $R_f = 0$ ($CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 2.82–2.93 (m, 4H), 3.76 (s, 3H), 5.42 (s, 2H), 7.08–7.25 (m, 5H), 7.60–7.95 (m, 10H); ^{13}C NMR (100 MHz, CD_3OD): δ 30.0, 35.3, 42.2, 60.4, 120.3, 124.4, 127.3, 129.3, 129.5, 130.5, 130.7, 131.0, 131.2, 133.5, 135.0, 141.5, 155.3, 156.4, 201.8; ^{19}F NMR (376 MHz, CD_3OD): δ −156; ^{1}H–^{1}H NOESY: no observable NOE between protons at 5.42 ppm and the protons at 3.76 ppm (confirming methylation at the 4-position); HRMS (ESI) $[M-BF_4]^+$ Calcd for $C_{25}H_{24}N_3O$: 382.1919. Found: 382.1903. HRMS (ESI) $[M-C_{25}H_{24}N_3O]^-$ Calcd for BF_4: 87.0029. Found: 87.0031.

4.12. Representative procedure for the formation of 1,3,4-trisubstituted [1,2,4]triazolium salts (Scheme 2)

4.12.1. 4-Methyl-1-(2-oxo-4-phenylbutyl)-3-phenyl-[1,2,4]triazolium tetrafluoroborate (26)

Compound **26** was prepared using the procedure for the formation of 1,3,4,5-tetrasubstituted-[1,2,4]triazolium salts above to give a white solid in 40% yield from 4-phenyl-1-(3-phenyl-[1,2,4]triazol-1-yl)-butan-2-one and trimethyloxonium tetrafluoroborate; mp ~50 °C; $R_f = 0.05$ ($CHCl_3$); ^{1}H NMR (400 MHz, CD_3OD): δ 2.92–3.05 (m, 4H), 4.01 (s, 3H), 5.53 (s, 2H), 7.15–7.20 (m, 1H), 7.21–7.30 (m, 4H), 7.60–7.73 (m, 3H), 7.78–7.84 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ 30.0, 35.3, 42.3, 60.8, 123.9, 127.3, 129.4, 129.6, 130.5 (3C), 133.5, 141.8, 156.1, 201.2; ^{1}H–^{1}H NOESY: no observable NOE between protons at 4.01 ppm and the protons at 5.53 ppm (confirming methylation at the 4-position); HRMS (ESI) $[M-BF_4]^+$ Calcd for $C_{19}H_{20}N_3O$: 306.1606. Found: 306.1603.

4.13. Representative procedures for the formation of 1,4-disubstituted-[1,2,4]triazolium salts (Scheme 3)

4.13.1. 1,4-Dibenzyl-[1,2,4]triazolium bromide (27)

Under a atmosphere of N_2, a mixture of [1,2,4]triazole (500 mg, 14.40 mmol) and potassium carbonate (426 mg, 3.09 mmol) in THF (10 mL) was stirred at rt for 10 min. Benzyl bromide (5.00 g, 28.80 mmol) was added dropwise and the mixture stirred at reflux temperature for 48 h. The mixture was cooled to rt, the white precipitate was removed by filtration, and the filtrate was concentrated to a yellow oil. The oil was treated with dichloromethane and the resulting precipitate was removed by filtration. Recrystallization from 2-propanol gave **27** (530 mg, 35%) as a white solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.52 (s, 2H), 5.61 (s, 2H), 7.40–7.51 (m, 10H), 9.33 (s, 1H), 10.32 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 50.65, 54.89, 128.82, 128.87, 128.91, 128.96, 129.09, 133.13, 133.5, 142.74, 145.0; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{16}H_{16}N_3$: 250.1344. Found: 250.1341.

4.13.2. 4-Benzyl-1-(2-oxo-2-phenylethyl)-[1,2,4]triazolium bromide (28)

A solution of the hydrochloride salt **32** (132 mg, 0.59 mmol) in water (~5 mL) was basified using an excess of K_2CO_3 (~132 mg, 0.96 mmol). The mixture was extracted with EtOAc (3×), and the combined organic extracts were dried (Na_2SO_4), concentrated, and dried under high vacuum to afford the free base (112 mg, 0.59 mmol, 100%). To a solution of the free base (112 mg, 0.59 mmol, 1 equiv) in 1-propanol (2 mL) at rt was added benzyl bromide (0.1 mL, 0.84 mmol, 1.4 equiv). The mixture was heated at reflux temperature for 4 h, then cooled to 0 °C. Et_2O was added, and the resulting precipitate was removed by filtration, washed with Et_2O (10×), and dried under high vacuum. Purification by flash column chromatography on silica gel (9:1 v/v $CHCl_3$–MeOH as eluent) gave **28** (24 mg, 0.07 mmol, 12%) as a beige solid; mp 166–170 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.66 (s, 2H), 6.31 (s, 2H), 7.40–7.55 (m, 5H), 7.63 (app t, J = 7.8 Hz, 2H), 7.77 (app t, J = 7.4 Hz, 1H), 8.00–8.10 (m, 2H), 9.45 (s, 1H), 10.22 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 50.9, 58.4, 128.5, 128.8, 129.3, 129.4 (2C), 133.5, 133.7, 134.9, 144.4, 144.9, 190.6; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{17}H_{16}N_3O$: 278.1293. Found: 278.1283.

4.13.3. 1,4-Bis-[2-oxo-2-(4-bromophenyl)ethyl]-[1,2,4]triazolium bromide (29)

This compound was obtained as the minor product in a procedure designed for the synthesis of the monoalkylated analog. To a solution of 2-bromo-1-(4-bromophenyl)ethanone (1.12 g, 4 mmol, 1 equiv) in DMF (6 mL) was added [1,2,4]triazole (828 mg, 12 mmol, 3 equiv) under an atmosphere of N_2. The mixture was stirred at rt for 3 h, then water was added. The resulting white precipitate was removed by filtration, and the solid was washed with boiling benzene (50 mL) to remove the monoalkylated product. The benzene insoluble material was dried under high vacuum and then purified by flash column chromatography on silica gel (9:1 v/v EtOAc–MeOH as eluent). The yellow solid obtained was washed once with MeOH to remove the yellow color. Recrystallization from MeOH gave **29** (100 mg, 0.18 mmol, 9%) as a white solid; mp 244–245 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.20 (s, 2H), 6.40 (s, 2H), 7.80–7.92 (m, 4H), 7.95–8.08 (m, 4H), 9.24 (s, 1H), 10.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 54.3, 58.5, 129.0, 129.1, 130.3, 130.4, 132.3, 132.4 (2C), 132.6, 145.4, 145.9, 189.8, 189.9; HRMS (ESI) $[M-Br]^+$ Calcd for $C_{18}H_{14}Br_2N_3O_2$: 461.9452. Found: 461.9440.

4.14. Determination of anti-*Plasmodium* activity

P. falciparum cultures were grown in O+ blood obtained by venipuncture of volunteers. Cultures of the laboratory line 3D7 were maintained by the method of Trager and Jensen[24] using RPMI-1640 supplemented with 10% human serum (a kind gift obtained under ethical consent from the Chemo Day Care Department of the Princess Margaret Hospital, Toronto, Canada) and 50 μM hypoxanthine (referred to as RPMI-10Pf). The effects of the test compounds on the viability of *P. falciparum* cultures were determined using a Lactate Dehydrogenase (LDH) enzyme assay specific for the enzyme found in *P. falciparum* (pLDH).[25,26] Briefly, compounds to be tested were dissolved in DMSO to afford a solution having a concentration of 10 mg/mL. Twofold serial dilutions were then produced in 50 μL of RPMI-10Pf in a 96-well plate and then 50 μL 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 using a multi-channel pipettor and a 15-μL sample was removed from each well and was added to 100 μL of pLDH enzyme assay mixture. After 1 h the absorbance of the wells at 595 nm was determined using a Thermo-Max microplate reader (Molecular Devices, Sunnyvale, CA, USA). The IC_{50} values of individual compounds were determined using a non-linear regression analysis of the data[27] using the computer program SigmaPlot (Jandel Scientific®, San Rafael, CA, USA). The IC_{50} values represent the mean ± standard error calculated from four independent determinations. To verify if the poor viability of the cultures was related to inhibited merozoite invasion, samples were taken from treated wells and the presence of extracellular merozoites was confirmed by microscopy. We have frequently used SYBR-Green 1 assays to determine parasite viability, and the values obtained were virtually identical to those obtained from the LDH assay. The LDH method was employed

because some compounds caused interference with the SYBR-Green 1 assay.

4.15. Mammalian strains and culture

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. 28). 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).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.05.020.

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