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# Parallel Synthesis and Anti-Malarial Activity of a Sulfonamide Library

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**Abstract**—Solution-phase synthesis and evaluation of a library of 31 sulfonamides as inhibitors of a chloroquine-resistant strain of *Plasmodium falciparum* are described. The most potent compound displayed an activity 100-fold better than chloroquine. Experiments using a fluorescent sulfonamide derivative suggest that their site of action inside the parasite is different to that of chloroquine. 

## Introduction

The spread of multidrug-resistant *Plasmodium falciparum* has created an urgent need to develop new anti-malarial treatments, preferably drugs that are affordable to developing countries where malaria is prevalent.[1–3] Among standard therapies, chloroquine (CQ) is believed to exert its anti-malarial activity by inhibiting haemozoin formation in the food vacuole of the parasite.[4,5] Biochemical studies have indicated that CQ-resistant isolates accumulate less drug than their more sensitive counterparts. However opinion remains divided upon the mechanistic explanation for this reduction. Resistance to CQ may involve several mechanisms but its reversal by molecules such as verapamil, desipramine and chlorpromazine suggests that an enhanced CQ efflux by a multi-drug-resistant mechanism may be implicated.[6,7] Therefore, one possibility to overcome the resistance mechanism is to design hindered quinoline-based drugs that would not be recognized by the vacuolar efflux proteins.[8,9] In our ongoing efforts to design new series of 4-aminoquinoline derivatives active on resistant *P. falciparum* strains, we have synthesized bis-, tris- or tetra-quinolines using different spacers.[10] One of these derivatives, compound **1** (Fig. 1), based upon a piperazine linker, displayed a very good activity whatever the degree of CQ resistance of the *P. falciparum* strains tested[11] (e.g., on FcB1 strain, Table 1). Meanwhile experiments on the localization of a fluorescent sulfonamide analogue of **1**, dansyl compound **2**, (Fig. 1, Table 1) in infected red blood cells and using fluorescence microscopy, revealed an accumulation of the drug inside the parasite yet with the exception of the food vacuole (Fig. 2A and B). This result suggested that the mechanism of action of our compounds, differed from that of CQ.

This specific localization was lost by replacement of the piperazine moiety by a methylenic chain. Therefore, a library of 31 sulfonamide derivatives was designed to explore the structure–activity relationships of the piperazine series.

## Chemistry

### Synthesis

Amine **3** previously obtained by aromatic substitution of 4,7-dichloro-quinoline by 1,4-bis(3-aminopropyl)-piperazine (Scheme 1), was used as a precursor for all compounds. Sulfonamides were then obtained in parallel from compound **3** and commercially available sulfochlorides or sulfofluorides using the procedure given in Scheme 1, on a 20 μmol scale.

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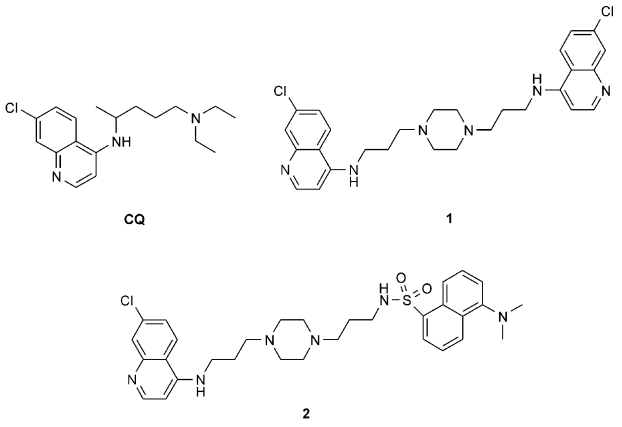



**Figure 1.** Chloroquine, bisquinoline **1** and its dansyl analogue **2**.

**Table 1.** Anti-malarial evaluation of compounds **1** and **2** on the CQ-resistant strain FcB1 of *P. falciparum*

| Compd | $IC_{50}$ (nM)[a] |
|---|---|
| Chloroquine | 126 (±26) |
| **1** | 112 (±17) |
| **2** | 23 (±9) |

[a]$IC_{50}$ values were obtained from triplicate experiments. Standard error is given in parentheses.

## Analytical control

Each crude product was tested for purity and identity using LC/MS. In all cases, the purity exceeded 80% and the mass spectrum was consistent with the anticipated product structure.

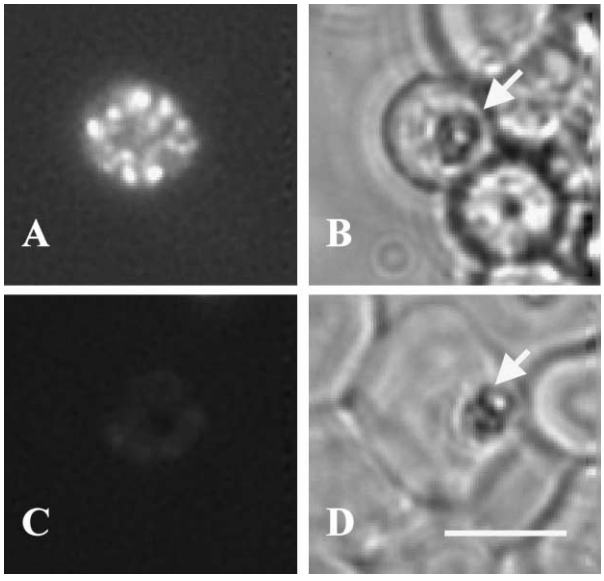



**Figure 2.** Localization of sulfonamide derivatives in *P. falciparum*-infected erythrocyte. Cells were incubated 40 min in the presence of 2 μM compound **2** (A, B) or 2 μM compound **2** and 50 μM compound **5** (C, D). Compound **2** fluorescence was concentrated in structures of parasite cytoplasm. No Food vacuole (arrow) and uninfected erythrocyte labelling were observed (A). Competition experiment with compound **5** completely displaces the fluorescence of compound **2**. B and D are corresponding phase images of A and C, respectively. Bar scale: 5 μm.

## Biological assays

### Anti-malarial activity and cytotoxicity

The sulfonamides were screened for their ability to inhibit parasite growth at 10 nM using a modified semi-automated micro-dilution technique.[12,13] Crude products displaying an inhibition percentage above 80% were selected for re-synthesis and further pharmacological characterization ($IC_{50}$) on fully purified and controlled samples. A few compounds displaying a lower inhibition percentage of parasite growth were also re-synthesized and their $IC_{50}$ evaluated as controls.

Cytotoxicity tests were performed on a human diploid embryonic lung cell line (MRC5) using the colorimetric MTT assay.[10]

### Fluorescence microscopy

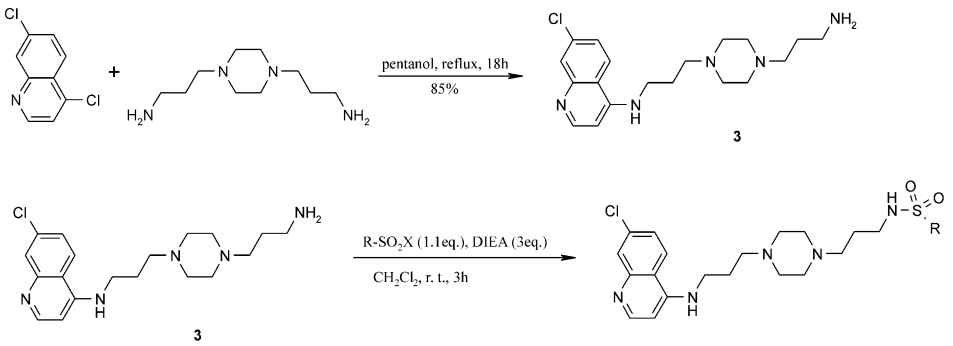
After incubation of infected erythrocytes with compounds in a culture medium, cells were washed three times and observed under a Nikon Eclipse TE 300 DV inverted microscope using UV emission filters. Image acquisition was performed with a back illuminated cooled detector (CCD EEV: NTE/CCD-1024-EB, Roper Scientific, France). Data acquisition and processing were performed with Metaview and Metamorph software (Universal Imaging Corporation, Roper Scientific, France).

## Results and Discussion

The introduction of a variety of sulfonamide fragments provided compounds with a broad range of inhibitory activities (Table 2). Those compounds displaying more than 80% inhibition of parasite growth contained exclusively aromatic sulfonamide templates. They exhibited low $IC_{50}$ values between 1.2 and 26.0 nM when compared with CQ ($IC_{50}$ = 126 nM). A naphthyl fragment revealed good inhibitory activity whereas aryl and thiophenyl moieties displayed activities substantially dependent upon substitution of the aromatic ring. The introduction of a vinyl (**19**) or methylene alkyl chain (**18**) between the sulfonamide moiety and the phenyl group led to a substantial loss in activity. Replacement of the aryl fragment with aliphatic groups as exemplified by **32**–**34**, generated relatively ineffective compounds.

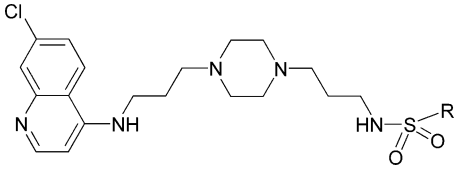
*para*-Substitution on the phenyl group led to higher activities (**11**, **12**, **21**, **22**, **24**) when compared with non-substituted phenyl group (**20**). Bulky substituents such as *tert*-butyl (**12**) and trifluoromethoxy (**11**) enhanced considerably the activities. In contrast, chloride (**21**), fluoride (**22**) or methyl (**24**) *para*-substitution on the phenyl group provided comparatively less effective compounds.

*ortho*-Substitution provided a loss in activity when compared with *para* (**22** and **23**) and *meta* (**25** and **26**). Poly-substitution on the phenyl group led to more effective inhibition than mono-substitution. Substitutions on the 2,5 positions (**27**, **29**, **30**) yielded less activity




**Scheme 1.** Synthesis of sulfonamide library.

**Table 2.** Variation of sulfonamide fragment



| Compd | R | % inhibition of parasite growth (10 nM) | $IC_{50}$ of parasite growth (nM)[a] | Cytotoxicity $IC_{50}$, μM[b] |
|---|---|---|---|---|
| | Chloroquine | | 126 (±26) | 50 |
| **4** | 2,3,4-Trichloro-phenyl | >80 | 9.73 (±3.1) | <3 |
| **5** | 3,5-Dichloro-phenyl | >80 | 18.7 (±0.9) | 1 |
| **6** | 4-Chloro-2,5-dimethyl-phenyl | >80 | 14.9 (±2.7) | 1 |
| **7** | 3-Chloro-2-methyl-phenyl | >80 | 16.7 (±2.0) | 1 |
| **8** | 2-Chloro-4-trifluoromethyl-phenyl | >80 | 15 (±0.5) | <3 |
| **9** | 2-Nitro-4-trifluoromethyl-phenyl | >80 | 26.0 (±6.9) | 2 |
| **10** | 3,5-di-Trifluoromethyl-phenyl | >80 | 10.2 (±0.4) | 3 |
| **11** | *p*-Trifluoromethoxyphenyl | >80 | 7.4 (±1.0) | 1 |
| **12** | *p*-Terbutyl-phenyl | >80 | 11.2 (±2.7) | 1 |
| **13** | Pentamethylphenyl | >80 | 16.9 (±1.2) | 1 |
| **14** | 2,5-Dichloro-4-bromo-thiophen-3-yl | >80 | 25.6 (±5.4) | 4 |
| **15** | 4,5-Dichloro-thiophen-2-yl | >80 | 16.6 (±0.3) | 4 |
| **16** | 4,5-Dibromo-thiophen-2-yl | >80 | 1.2 (±0.5) | <3 |
| **17** | 2-Naphtyl | >80 | 8.5 (±0.7) | <3 |
| **18** | Benzyl | 4 | | |
| **19** | *trans*-Phenylvinyl | 0 | | |
| **20** | Phenyl | 11 | 167 (±36) | 4 |
| **21** | *p*-Chloro-phenyl | 26 | | |
| **22** | *p*-Fluoro-phenyl | 20 | | |
| **23** | *o*-Fluoro-phenyl | 11 | | |
| **24** | *p*-Methyl-phenyl | 54 | | |
| **25** | *m*-Nitro-phenyl | 48 | | |
| **26** | *o*-Nitro-phenyl | 0 | | |
| **27** | 2-Methyl-5-nitro-phenyl | 56 | | |
| **28** | *m*-($CH_3CO$)-phenyl | 22 | | |
| **29** | 2-Chloro-5-trifluoromethyl-phenyl | 60 | | |
| **30** | 5-Bromo-2-methoxy-phenyl | 64 | | |
| **31** | Thiophen-2-yl | 20 | 69.0 (±0.7) | 6 |
| **32** | Methyl | 18 | | |
| **33** | Ethyl | 1 | | |
| **34** | Isopropyl | 25 | | |

[a]$IC_{50}$ values were obtained from triplicate experiments performed on the FcB1 strain. Standard error is given in parentheses.
[b]MRC-5 cells.

than 3,5 (**5**, **10**), 3,2 (**7**) and 2,4 (**8**, **9**). The bis trifluoromethyl compound (**10**) was more active than the dichloro analogue (**5**). The nitro substituent in **9** proved less active than the chloride (**8**). An increase in phenyl substitution (**4**, **6** and **13**) provided highly potent inhibitors of parasite growth. The thiophenyl group displayed characteristics similar to the phenyl group. Substitution of the thiophenyl ring in **15** and **16** enhanced considerably the inhibitory activity when compared with non-substituted thiophenyl ring **31**. Substitution of a bromine atom provided an increase in activity when compared with chlorine atom as revealed by compounds **15** and **16**.

Taken together, these biological results show that antimalarial activity is related to the presence of terminal

aromatic rings and is enhanced by bulky hydrophobic substituents. Rigidity and electronic delocalisation of the terminal fragment promote activity, which is in accord with the higher potency of phenylic sulfonamides when compared with benzylic or phenyl vinylic compounds. As the parasitical target domain is not known we can only speculate about the existence of hydrophobic interactions between the aromatic sulfonamides and their target. It is likely that differences in the uptake of the compounds also contribute to the variation in anti-malarial activity.[14] Competition assays conducted upon some aromatic sulfonamides (**5**, **12** and **15**) revealed that these compounds displaced the fluorescence of the dansyl compound **2** from its cytosolic localization as shown in Figure 2C and D for compound **5**. In contrast, no displacement of fluorescence was observed with an excess of CQ. These results strongly suggest that all compounds have a similar site of action which might be saturated, located inside the parasitic cytosol.

The average cytotoxicity of our compounds upon MRC-5 cells (human diploid embryonic lung cell line) extended from 1 to 6 μM (Table 2). This range provided a number of favourable selectivity indices (ratio of cytotoxicity $IC_{50}$ to $IC_{50}$ of parasite growth). If they retain their activity in vivo, their use in combined therapy with chloroquine could be successful.

## Conclusion

Parallel synthesis of a library of sulfonamide compounds enabled us to easily achieve a first study of structure–activity relationships of this novel series of potential anti-malarial compounds. The potent anti-malarial activity displayed by some of these compounds and their site of action being different to that of CQ, as suggested by fluorescence assays, deserves a further and wider investigation along with the preparation of a larger, more diverse library.

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## References and Notes

1. Van Est, H. G.; Skamene, G. E.; Schurr, E. *Clin. Invest. Med.* **1993**, *16*, 285.
2. White, N. J. *J. Antimicrob. Chemother.* **1992**, *30*, 571.
3. WHO, 1999. *Report of Infectious Diseases*; World Health Organization: Geneva.
4. Dorn, A.; Stoffel, R.; Matile, H.; Bubendorf, A.; Ridley, R. G. *Nature* **1995**, *374*, 269.
5. Dorn, A.; Vippagunta, S. R.; Matile, H.; Jaquet, C.; Vennerstrom, J. L.; Ridley, R. G. *Biochem. Pharmacol.* **1998**, *55*, 727.
6. Krogstad, D. J.; Gluzman, I. Y.; Kyle, D. E.; Oduola, A. M.; Martin, S. K.; Milhous, W. K.; Schlesinger, P. H. *Science* **1987**, *238*, 1283.
7. Reed, M. B.; Saliba, K. J.; Caruana, S. R.; Kirk, K.; Cowman, A. F. *Nature* **2000**, *403*, 906.
8. Vennerstrom, J. L.; Ellis, W. Y.; Ager, A. L.; Andersen, S. L.; Gerena, L.; Milhous, W. K. *J. Med. Chem.* **1992**, *35*, 2129.
9. Raynes, K.; Galatis, D.; Cowman, A. F.; Tilley, L.; Deady, L. W. *J. Med. Chem.* **1995**, *38*, 204.
10. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemière, P.; Debreu, M. A.; Davioud-Charvet, E.; Sergheraert, C. *J. Med. Chem.* **2000**, *43*, 2646.
11. Unpublished results.
12. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents. Chemother.* **1979**, *16*, 710.
13. Delarue, S.; Girault, S.; Maes, L.; Debreu-Fontaine, M.-A.; Labaïed, M.; Grelleir, P.; Sergheraert, C. *J. Med. Chem.* **2001**, *44*, 2827.
14. Experiments on log D in progress.