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Original article

# Arylsulfonyl acridinyl derivatives acting on *Plasmodium falciparum*

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**Abstract**

Several arylacridinyl sulfones have been synthesized and their antimalarial action was tested on *Plasmodium falciparum*. PABA (*para*-aminobenzoic acid) has no antagonistic effect with these compounds as opposed to the observed effect with dapsone and sulfonamides previously studied. A possible relationship between the ability of cleavage of the S–9C acridinic bond and activity is suggested.


*Keywords:* Acridinic sulfone; *Plasmodium falciparum*; PABA

## 1. Introduction

Malaria remains a major health problem affecting 500 million people and causing 2.5–3 million deaths per year. The incidence of this disease is still increasing and basic research [1–3], novel drugs [4–11] are under investigation for needed new therapies.

Quinine which was the first natural drug used, has been replaced for a long time by mepacrine then chloroquine, but at present after decades of use, existence of resistant strains can no longer allow its use alone [12]. Artemisinin and related drugs, pyrimethamine–proguanil are currently administered. The target of chloroquine as well as that of mepacrine, a 9-aminoacridine derivative, is a ferriprotoporphyrine FPIX [13,14] located in the parasite vacuole. A drug–FPIX complex prevents the normal detoxification process leading to the parasite death. Artemisinin interferes with redox metabolism too [15]. Iron being essential to the parasite growth, numerous antimalarial drugs like deferiprone, desferrioxine [16], iron chelators [17,18] have been devised to deprive the parasites.

Antimetabolites, antifolic and antifolinic compounds often associated, act on the same biosynthesis pathway, namely the de novo folate biosynthesis, which is required for growth of malarial parasites. Antimicrobial agents, can act on several targets in this pathway (Scheme 1).

Dihydropteroate synthetase (DHPS) is the enzyme involved in the first step leading to 7,8-dihydropteroic acid, then dihydrofolate synthetase (DHFS) [19,20] produces dihydrofolic acid. Folylpolyglutamate synthetase (FGPS) catalyses polyglutamation of folate derivative [19] and the effective retention of folate and efficacy of antifolate drugs both depend upon the addition of polyglutamate tail by FGPS [21]. Dihydrofolate reductase (DHFR) effects reduction to tetrahydrofolate, which is the only active species. DHPS is targeted by sulfonamide and sulfone drugs [22] while antagonists like pyrimethamine or proguanil do the same with DHFR. It has been shown that inhibition of *para*-aminobenzoic acid (PABA) uptake might serve as a target for antimalarial chemotherapy [23] and that sulfones and sulfonamides inhibited DHPS in *Plasmodium berghei* [24,25] in *Plasmodium chabaudi* [26] and *Plasmodium falciparum* [22,27]. They are analogous to PABA and act as competitive inhibitors of DHPS [27].

*Abbreviations:* DHFS, dihydrofolate synthetase; DHPS, dihydropteroate synthetase; DMF-DMA, dimethylformamide dimethylacetal; FGPS, folylpolyglutamate synthetase; FPIX, ferriprotoporphyrine IX; *P*, *Plasmodium*; PABA, *para*-aminobenzoic acid; PPPK, 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase.

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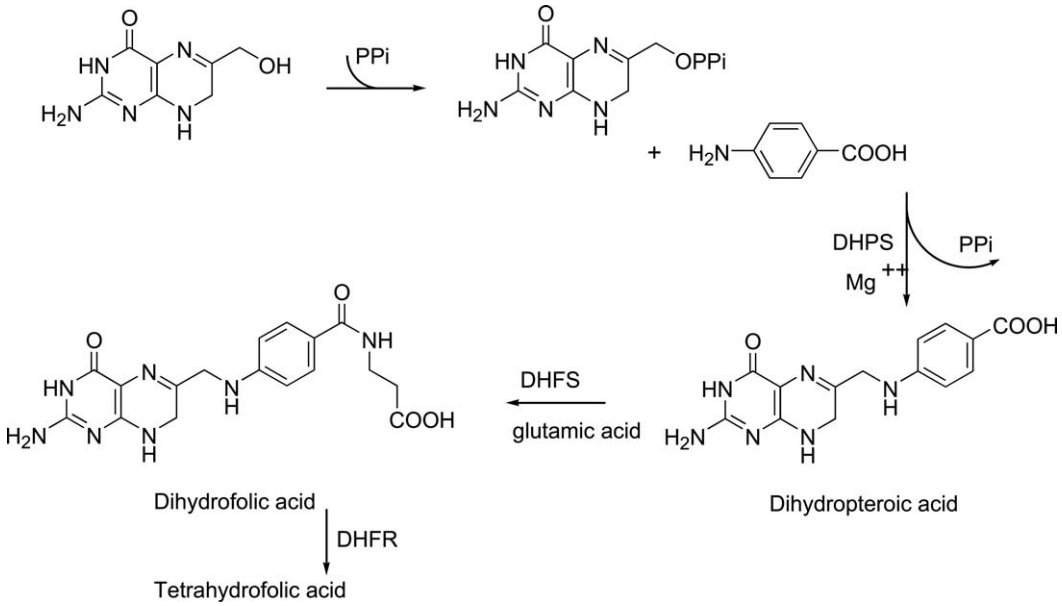



Scheme 1. Folate pathways.

We have tested nine compounds I for antimalarial activity (Table 1). They present at once acridinic ring and arylsulfone moiety (X = $SO_2$) (Scheme 2). Activities are compared to that of thioaryl derivative (I, X = S, R = 4′-$NH_2$, **10**).

## 2. Chemistry

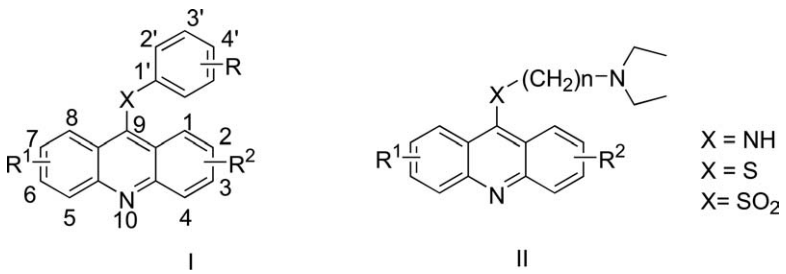
Acridinyl arylsulfones have been synthesized by action of the sodium salt of substituted benzene sulfinates on 9-chloroacridine derivatives (Scheme 3).

Table 1
Biological and physical data

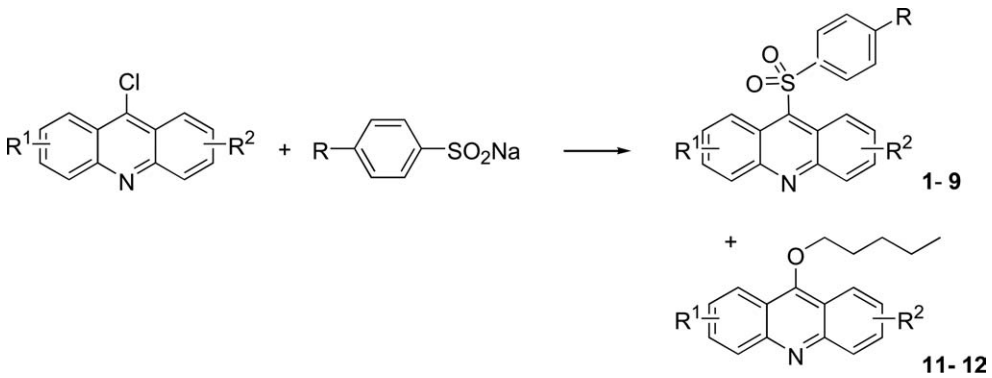
| Compound | $IC_{50}$ 3D7 | $IC_{50}$ W2 | $d$ [a] | $pK_{HB}$ |
|---|---|---|---|---|
| **1** | 35 | 31.3 | 1.690 | 1.09 |
| **2** | 6 | 11.4 | 1.723 | 1.33 |
| **3** | 15 | 16 | 1.710 | 1.26 |
| **4** | 14.3 | 20 | 1.717 | 1.37 |
| **5** | 31 | 22.8 | 1.710 | 1.24 |
| **6** | 17.2 | 17 | 1.711 | 1.27 |
| **7** | 4 | 6.5 | 1.716 | 1.30 |
| **8** | >500 | >500 | 1.705 | 1.47 |
| **9** | >99 | >99 | 1.700 | 1.60 |
| **10** | 33 | 77 | 1.700 | – |
| Dapsone | >100 | >100 | 1.670 | 1.54 |

[a] C9–S bond length in Å.

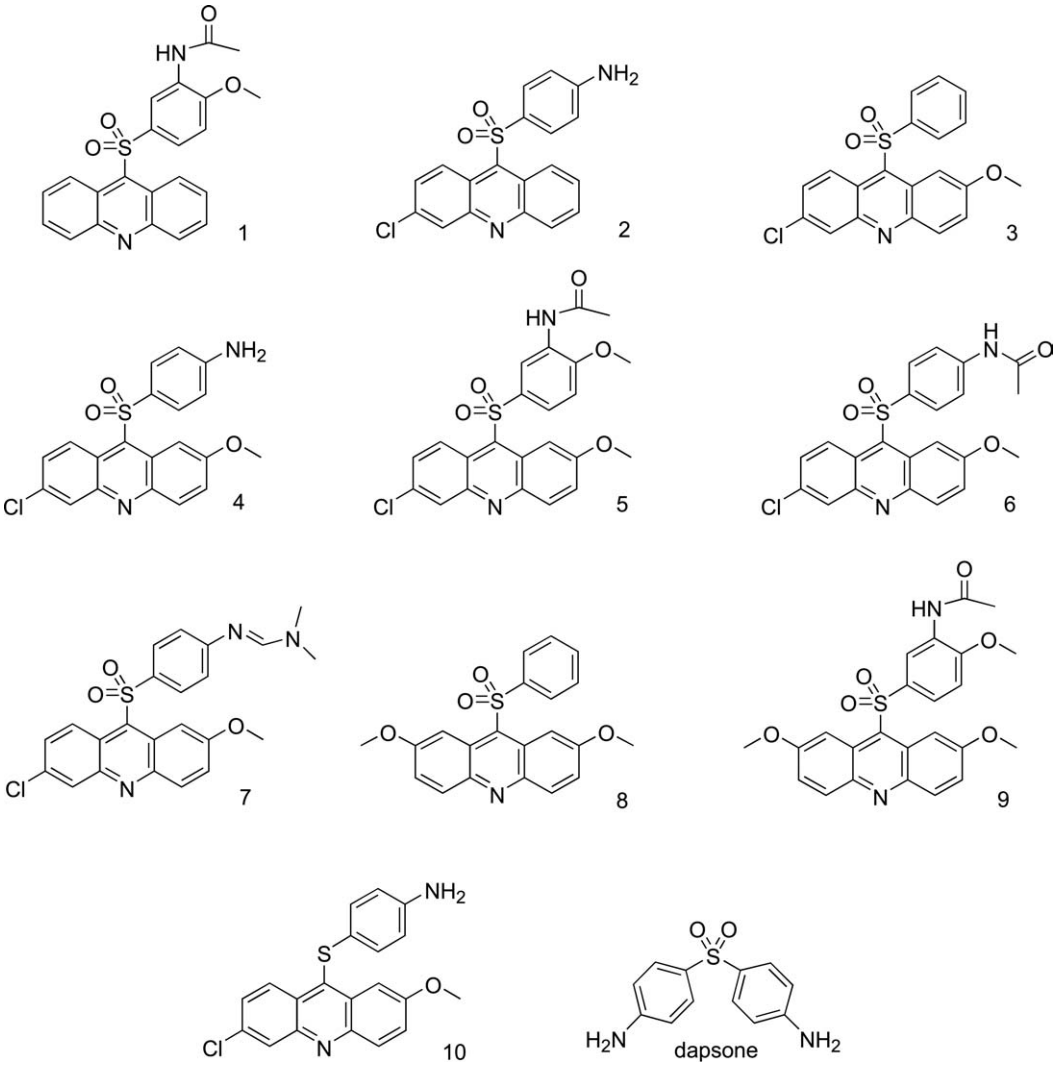
Sulfinic acids were obtained from commercial sources or prepared by known procedure, e.g. by reduction with sodium sulfite of the corresponding sulfonyl chloride [28]. Treatment of sulfone **4** by dimethylformamide dimethylacetal (DMF-DMA) leads to sulfone **7** (Scheme 4). In the general procedure, the sodium salt of a sulfinic acid and chloroacridine are heated in pentanol or pentanol–pyridine mixture, in the presence of copper and iodine in catalytic amount (Scheme 3). This procedure was chosen regarding the availability of substituted 9-chloroacridines and sulfinates. Direct oxidation of the corresponding arylacridinyl thioether was not efficient in our hands. Mild conditions used successfully with thioalkyl derivatives were not efficient with thioaryl compounds and stronger conditions were detrimental, leading to acridinones. Time and temperature conditions have been determined for each reaction. When benzene sulfinic acid was used, variations on time and temperature have been made as pentoxyacridine, a secondary product can be formed: when heating at 120 °C during 5 h, sulfone and chloroacridine were isolated in a 60:40 ratio, at 140 °C during 24 h pentoxyacridine was only isolated; at 120 °C during 7 h, the ratio sulfone/chloroacridine was 9:1 and pentoxy acridine was formed as a trace. When 9-chloro-2,7-dimethoxy acridine was used, heating at 120 °C during 11 h led to sulfone **8** without alkoxy




Scheme 2. Acridine derivatives.




Scheme 3. Sulfone synthetic pathways.




Scheme 4. Arylsulfones and aryl thio acridine studied.

derivative. When using *p*-aminobenzenesulfinic acid, the best conditions were 120 °C during 3–3:30 h.

## 3. Results and discussion

The studied compounds I (Scheme 4) have been synthesized to be tested for their effect on *P. falciparum*, as antimalarial activity of these aryl-heteroarylsulfones can be expected due to their analogy with dapsone and related derivatives [29] or that with acridinic derivatives (mepacrine or anilinoacridines) [30].

Activities against *P. falciparum* 3D7 and W2 strains (chloroquinosusceptible and resistant strains, respectively) have been measured. $IC_{50}$ values are included from 4 upto >500

μM for 3D7 and from 6.5 upto >500 μM for W2 strain (Table 1). Compounds are diversely substituted on the acridinic ring in 3-chloro, 2-methoxy-6-chloro and 2,7-dimethoxyacridine. The aryl part is a phenyl ring unsubstituted (**3, 8**) or branched with an amino substituent (or an acetamido one which can be cleaved in amino) (**1, 2, 4, 5, 6, 9**) and an amidino group (**7**). The thio derivative **10** [31] has been tested to be compared with the corresponding sulfone **4**. To date, acridinic compounds known for their antimalarial activity belong either to the 9-aminoalkylamino series analogous to the mepacrine series (II, X = NH) or, more recently, to the anilino derivatives (I, X = NH) [30,32].

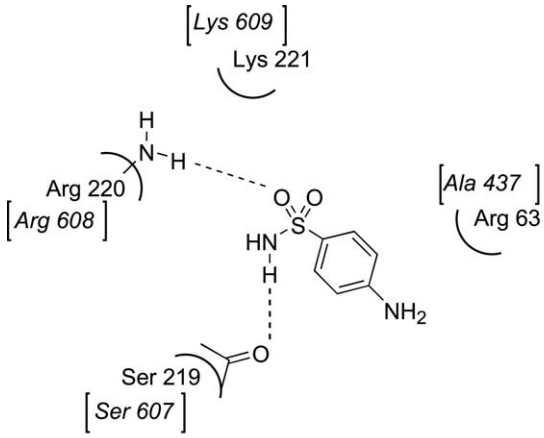
9-Anilinoacridines [32] have been shown to inhibit parasite DNA topoisomerase II activity in vitro but it seems there are no correlation between DNA binding and antimalarial activity [33]. For compounds I, when X = S instead of X = $SO_2$, decrease in activity is observed. If we compare $IC_{50}$ values for **4** (X = $SO_2$, R = $NH_2$) and **10** (X = S, R = $NH_2$), they varies from 14.3 to 33 μM with 3D7 strain and from 20 to 77 μM with W2 strain. Hence, the fact that sulfone group plays an essential role is emphasized. Sulfone **3** in which R = H, also displays an activity, although replacement of one aryl $NH_2$ group by an hydrogen atom in dapsone derivatives resulted in total loss of activity [34].

Dapsone used extensively for the treatment of leprosy has been known since 1941 to also possess antimalarial activity [35].

Sulfones and sulfonamides known to inhibit DHPS, interacts with identical binding sites [36]. These drugs analogous to PABA act as competitive inhibitors of DHPS [27] and the gene encoding *P. falciparum* enzyme DHPS has been cloned [20]. The Pf DHPS protein was found to be joined to a region homologous to the PPPK enzyme of other organisms, suggesting that this enzyme is bifunctional in *P. falciparum*.

Crystal structure of *Escherichia coli* DHPS has been reported [37] and binding sites for sulfanilamide have been located (Scheme 5). Sulfanilamide is sandwiched between the main chain of Arg 220 and on the one side, the side chain of Lys 221 and the side chain of Arg 63 on the other side. Amino groups from the sulfonamide function gives hydrogen bond to the carbonyl of Ser 219 whilst one sulfonamide oxygen accepts hydrogen bond from the guanidinium of Arg 220 [37]. For Pf DHPS a correspondence for aminoacids has been established [20] in which Ala 437, Ser 607, Arg 608, Lys 609 replace, respectively, Arg 63, Ser 219, Arg 220, Lys 221 of *E. coli* DHPS. Metabolism of sulfadoxine by DHPS in *P. falciparum* is reduced in sulfadoxine-resistant parasites suggesting that the S436F, A437G, A581G, A613S mutations in this enzyme are involved in the mechanism.

Extracts of *E. coli* use as substrate either PABA to yield dihydropteroic acid as product or *p*-aminobenzoyl glutamic acid to yield dihydrofolic acid as product. However, PABA is at least a 10 times more effective substrate than *p*-aminobenzoyl glutamic acid [38]. Sulfonamides become incorporated into sulfa-pterin adducts that are metabolically inert and diffuse from cell [39,40]. Study of the dapsone




Modelling of Sulfanilamide in DHPS *E.coli* [37]
with the corresponding *P.f* aminoacids in brackets [20]

Scheme 5. Modeling of sulfanilamide in DHPS.

action on *E. coli* [41] showed that an analog of dihydropteroic acid is formed as well. Besides studies on dapsone and related compounds [29], phosphanilic acid [42], sulfanilic acid, *p*-aminosalicylic acid [23] have been tested for their PABA inhibition. It is suggested a second mode of action for phosphanilic acid, other than inhibition of DHPS. This mode of action also appeared to be shared by PABA. Sulfanilic acid displays a high inhibitory action on *E. coli* in cell free systems (IC = 5 μM) [43] but a total inactivity in whole cell cultures. Nevertheless, in contrast to sulfanilamide, this compound has been shown to be a more effective inhibitor of DHPS and its weak in vitro activity is due to hydrophobicity. Portage transport has circumvent the poor drug permeation [44,45]. According to several studies on sulfones and sulfonamides, the necessary presence of an anilino group has been established but besides the interactions with DHPS, these compounds interacts with DHFS on identical binding sites and a decisive factor is a negative charge at the oxygen atoms of the $SO_2$ group which is favored in case of sulfones by electron-releasing substituents branched on the aromatic ring and by electron-attracting groups in case of sulfonamides [46].

In order to determine the possible analogy of the biological action of sulfones **1–7** and dapsone or sulfonamides, we have studied the PABA association to sulfone **3**. Antagonistic effect of PABA on the antimalarial activity of sulfonamide derivatives has been recognized in *P. lophurae* infection in ducks [47] as well in chickens infected with *P. gallinaceum* [48] in which PABA blocks the action of sulfamides but does not affect action of quinine or atebrin. Antimalarial activity on FCR3 strain of *P. falciparum* of sulfonamides and dapsone is blocked by 100 μM of PABA [22].

In the present study, an amount of PABA from 0 upto 100 mM, was added to sulfone **3**. Effect has been studied on 3D7 and W2 strains (Table 2). PABA alone displayed a very low antimalarial activity from 0% to 15% (3D7) and from 0%

Table 2
Percentage of parasites g.i. for the association PABA and sulfone **3** (concentration in μM)

| PABA | 0 μM | 1 μM | 10 μM | 50 μM | 100 μM | 500 μM | 1 mM | 100 mM |
|---|---|---|---|---|---|---|---|---|
| ***3** on 3D7* | | | | | | | | |
| 0 | 0 | 0 | 0 | 1 | 5 | 8 | 7 | 15 |
| 15 | 40 | 39 | 41 | 45 | 46 | 50 | 55 | 64 |
| 30 | 51 | 50 | 52 | 55 | 59 | 61 | 64 | 73 |
| 75 | 75 | 74 | 76 | 78 | 77 | 78 | 80 | 84 |
| ***3** on W2* | | | | | | | | |
| 0 | 0 | 0 | 0.5 | 3 | 4 | 4.5 | 6.5 | 6.5 |
| 15 | 52.5 | 54 | 55.5 | 60 | 61 | 64.5 | 67 | 74.5 |
| 30 | 61.5 | 62 | 62 | 66.5 | 67 | 71.5 | 73 | 81.5 |
| 75 | 82.5 | 83 | 82 | 82 | 83.5 | 85 | 85.5 | 89.5 |

to 6.5% (W2), when its concentration varies from 0 to 100 mM. A quite additive effect and a slight synergistic effect were observed for the highest concentrations of PABA that is 500 μM upto 100 mM.

In Fig. 1, we present the percentage of parasites growth inhibition (g.i.) (yellow) for various mixtures of sulfone **3** (blue) and PABA (pink) (at concentrations, *c*, of 0 upto 100 μM).

It is clear that activity is related to the amount of sulfones and not linked to the presence of PABA. These results indicate a definite difference in the action of PABA and the studied sulfones compared to the antagonistic effect observed previously with sulfonamides and dapsone.

The observed antimalarial activities of compounds I are in the same range that activity of sulfanilic acid is in cell free system. These values were expected although the acridine ring size is larger than that of usual rings encountered with the other studied sulfones. It has been established that analogues of antifolates with 4-aminobenzoyl group replaced by the 4-amino-1-naphthoyl one, indicated ample spatial accommodation for the naphthalene ring and even larger groups when describing the binding of methotrexate to human DHFR [49].

The sulfonyl oxygen atoms of sulfones are good hydrogen bond acceptors (HBA) [50]. The hydrogen bond basicity of a solute can be obtained from the equilibrium constant $K_c$ of the reaction of association of 4-fluorophenol with the base in carbon tetrachloride.

Strength of the different HBA groups can be ranked according to the p$K_{HB}$ scale (p$K_{HB}$ = log $K_c$). This p$K_{HB}$ scale pionnered by Taft et al. [51] has now been developed for more than 1000 solutes containing a large variety of accepting groups [52]. In the case of the acridinic sulfones it is not possible to get direct experimental values of the basicity of the sulfonic group since the phenol will primarily associates on the much more basic acridinic nitrogen atom. We have thus estimated the hydrogen bond basicities of the $SO_2$ oxygens of dapsone and compounds **1–9** using AM1 semi empirical calculations of the enthalpies $\Delta H°$ of the association reaction (Eq. 1)

$$4-FC_6H_4OH + R^1PhSO_2R^2 \rightleftharpoons 4-FC_6H_4\ OH\cdots SO_2R^2\ PhR^1 \quad \Delta H° \tag{1}$$

Following Le Questel et al. [53], the calculated enthalpies of this reaction can predict correctly the hydrogen bond basicity of homogeneous family of bases in spite of the fact that AM1 underestimates the association reaction enthalpy. A calibration with experimental data of hydrogen bond basicity is, therefore, necessary for each new family of base. In our case, these reference calculations can be performed with the p$K_{HB}$ values of five phenylsulfones studied by Chardin et al. [50] yielding Eq. (2) where *r* is the correlation coefficient, *n* the number of points and *s* the standard error on the estimate

$$\text{p}\ K_{HB} = -0.699\Delta H° - 3.595 \tag{2}$$

$$r = 0.992, \quad n = 5, \quad s = 0.09$$

The results obtained for the five reference compounds are gathered in Tables 1 and 3 for the acridinic sulfones **1–9**.

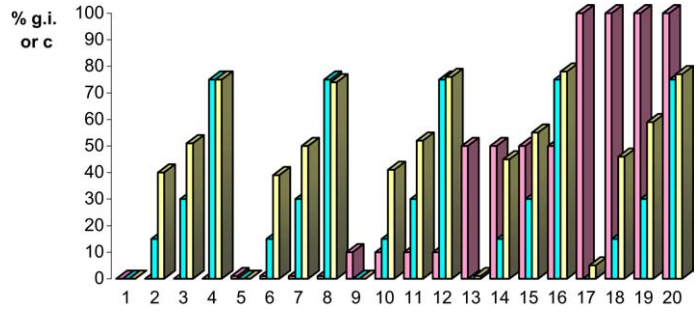



Fig. 1. Percentage of activity on 3D7 (yellow) of sulfone **3** (blue) and PABA (pink).

Table 3
Experimental and calculated hydrogen-bond basicities of arylsulfonyl bases

| Compound | $\Delta H°$ [a] (kcal mol$^{-1}$) | p$K_{HB}$ experimental [50] | p$K_{HB}$ calculated |
|---|---|---|---|
| $PhSO_2Ph$ | –6.95 | 1.21 | 1.27 |
| $PhSO_2NMe_2$ | –6.86 | 1.19 | 1.20 |
| $PhSO_2N{=}CHNMe_2$ | –7.54 | 1.81 | 1.68 |
| $pMePhSO_2N{=}SMe_2$ | –8.29 | 2.14 | 2.20 |
| $pMePhSO_2N^-N^+Me_3$ | –8.86 | 2.61 | 2.61 |

[a] $\Delta H°$ (reaction 1) = $\Delta H°_f$(4-$FC_6H_4$ … $OHSO_2R_2PhR_1$) – $\Delta H°_f$($SO_2R_2PhR_1$) – $\Delta H°_f$(4-$FC_6H_4OH$).

However, in our case, the antimalarial activity does not seem to be dependent on the presence and strength of those HBA groups; for example, **8** and **9** display the highest p$K_{HB}$ values and no antimalarial activity. So, it can be wondered if the studied sulfones actually work after cleavage of the sulfur acridine (S–9C) bond leading to the corresponding sulfinic acid and acridinone. In other acridinic derivatives, the X–9C bond (X = NH, $NCH_3$) [54] is known to easily undergo hydrolysis and in our hands, with alkyl derivatives, sulfones were less stable than the corresponding thioethers. Hydrolysis of sulfones in oxidative medium to recover acridinone was a side and easy reaction (unpublished results). As a decrease in activity is observed from the 2-methoxy-6-chloro to the 2,7-dimethoxy (**3** and **8**) and from the 3-chloro to the 2-methoxy-6-chloro derivatives (**2–4**), it seemed likely to consider a possible relationship between activity and the S–9C bond cleavage. With this aim, bond length $d$ and the p$K_a$ value $p$ of the sulfinic part were selected as variables. $p$ values are for benzenesulfinic acid, 1.26; for 4-aminobenzenesulfinic acid, 0.80; for 4-acetamidobenzenesulfinic acid, 1.34; for 3-acetamido-4-methoxybenzenesulfinic acid, 1.24; for 4-(dimethylaminomethyleneamino)-benzenesulfinic acid, 1.46. They were calculated with the Pallas 2.0 program [55].

At first, only compounds **3–7** which have the same heterocyclic moiety, i.e. a 2-methoxy-7-chloro substituted acridine ring, have been considered.

Multiple linear regression (MLR) calculation was performed with the Regression VI.1a, Medicinal Chemistry Regression Machine program (1987) [56,57]. MLR relationship was identified between $IC_{50}$ and $d$ and $p$, in case of the resistant W2 strain.

$$IC_{50} = -1429.77\ (\pm 522.39)\ d - 22.67\ (\pm 7.07) p + 2493.12\ (\pm 898.30)$$

$$n = 5;\ s = 3.23;\ r = 0.929;\ F = 6.28;\ F(2,2,0.01) = 99.$$

Equation shows that this two variable model described 86% of results. The larger is the $d$ value, the easier the cleavage of S–C bond and the higher the activity of the compound as it entails decrease in $IC_{50}$ values.

Consecutively, 3-chloro derivative **2** was added. Then, the relation explains 88% of results. In a same way, **1**, which is a nonsubstituted derivative, was added and 86% of results are still explained. These results with seven compounds show that parameters used account for differences in substitution of the acridine ring.

However, with 2,7-dimethoxy derivatives **8** and **9**, the relation leads to theoretical activities of 20.67 and 24.97 μM instead of the experimental ones that are more than 500 and 100 μM. This suggests that a steric hindrance parameter would have to be included in the relation with the aim to account for the presence of the second methoxy substituent which experimentally is known to prevent nucleophilic attack on the C9 acridinic ring.

By the way, it can be noted that when d parameter is only used, the regression explains 66% of the results. This clearly demonstrates that ionization of the sulfinic acid groups improves the activity.

In addition, in case of the chloroquinosusceptible strain 3D7, MLR, with the same compounds, explains 71% of results.

Acridinone moiety results also from the bond cleavage. Acridinones are known to be devoid of biological in vivo activity due to their low solubility [58]. Actually, it has been observed that compounds from the mepacrine series can be cleaved into aliphatic diamines and acridinones under well-defined conditions. Cleavage products have always been found to be inactive [59,60].

With respect to this, activities of 2-methoxy-7-chloroacridinone (A), sodium salt of 4-methoxy-3-acetamido benzene sulfinic acid (B) and an equimolar mixture of A and B have been measured (Table 4). They were compared to those of sulfones **3–7** which have the same ring. Results gathered in Table 4, which were selected as a matter of example, do not allow to infer a link between activities of sulfone **5** and those of separate components. Consequently, relation between antimalarial activity of the sulfone under evaluation and the C–S9 bond breaking seems to be well documented but do not allow to clarify the mode of action.

Sulfones were also tested as chloroquinoresistance reversal agents, but they do not present any activity except antagonistic effect detected in some cases when added to chloroquine.

Table 4
Antimalarial activity of A, B, A + B and **5**

| $IC_{50}$ (in μM) | A | B | A + B (1/1) | **5** | CQ |
|---|---|---|---|---|---|
| 3D7 | 18.6 | >100 | 88.2 | 31 | 0.02 |
| W2 | 12.5 | >100 | 63.3 | 22.8 | 0.95 |

## 4. Conclusion

Arylacridinyl sulfones **1–7** display an antimalarial activity on *P. falciparum*. This action was suspected because of structural analogy with dapsone and sulfonamides. At first, we present evidences that activities are not dependent on the presence of PABA unlike dapsone and sulfonamides, which have been found to inhibit PABA uptake. Second, even if sulfonyl group is important for the activity, there is no correlation with either charge density on oxygen atoms or $pK_{HB}$ values. Finally a good relationship is established between activity and cleavage of the S–9C bond and to a lesser extent with $pK_a$ of the benzene sulfinic acids formed after the cleavage. So, it must be concluded that acridine ring confers a particular activity to arylsulfones.

## 5. Experimental protocols

### *5.1. Chemistry*

Liquid chromatography was performed on silica gel 60 (230–400 mesh) and TLC on silica gel 60 $F_{254}$. Melting points (m.p.) were determined on a Büchi apparatus and are given uncorrected. $^1$H and $^{13}$C NMR spectra were recorded on Brüker spectrometer (300 MHz) with TMS as internal reference; chemical shifts $\delta$ are given in the ppm scale with $J$ values in Hz. Elemental analysis are within ±0.4% of the theoretical values.

#### *5.1.1. 9-(3′-Acetamido-4′-methoxy-phenylsulfonyl)-acridine (**1**)*

9-Chloroacridine (1 g, 4.7 mmol), sodium 3-acetamido-4-methoxy-benzenesulfinate (from commercial sulfinic acid 1.1 g and NaOH, 0.24 g, 5 mmol) are heated in the same conditions used for **2** during 7 h. Solvents are removed under vacuum. Product is isolated by chromatography (ether/$CHCl_3$/$CH_3OH$, 200:10:0–5). Yield: 37%. M.p.: 199–200 °C. $^1$H NMR (DMSO-$d_6$): 11.76 (s, 1H), 8.35 (s, 1H), 8.24 (dd, $J$ = 8.1 Hz, $J$ = 1.5 Hz, 2H); 7.74 (ddd, $J$ = 8.4 Hz, $J$ = 6.9 Hz, $J$ = 1.5 Hz, 2H); 7.56 (d, $J$ = 8.1 Hz, 2H); 7.37 (dd, $J$ = 8.5 Hz, $J$ = 2.2 Hz, 1H); 7.27 (ddd, $J$ = 8.0 Hz, $J$ = 7.0 Hz, $J$ = 1.1 Hz, 2H); 7.20 (d, $J$ = 8.5 Hz, 1H); 3.90 (s, 3H); 2.13 (s, 3H). $^{13}$C NMR (DMSO-$d_6$): 177.26 (s); 169.37 (s); 141.37 (s); 140.65 (s); 133.92 (d); 128.37 (s); 126.48 (d); 121.46 (d); 120.97 (d); 117.81 (d); 111.49 (d); 56.55 (q); 23.39 (q).

#### *5.1.2. 3-Chloro-9-(4′-aminophenylsulfonyl)-acridine (**2**)*

3,9-Dichloro-acridine (1.24 g, 5 mmol), 4-aminophenyl-sulfinic acid (2.6 g, 10 mmol), $K_2CO_3$ (2.7 g, 20 mmol), pentanol (20 ml), pyridine (10 ml), Cu (cat.), iodine (cat.), were heated under stirring at 120 °C during 3 h. Solvents are removed under vacuum and solid is extracted three times with hot acetone. Product is isolated by chromatography on silica gel and eluted with ether. Yield: 32%. M.p.: 303–305 °C. $^1$H NMR ($CDCl_3$): 9.46 (m, 2H); 8.26 (d, $J$ = 2.4 Hz, 1H); 8.23 (d, $J$ = 9.3 Hz, 1H); 7.85–7.69 (m, 2H); 7.76 (d, $J$ = 8.8 Hz, 2H); 7.62 (dd, $J$ = 9.8 Hz, $J$ = 2.3 Hz, 1H); 6.58 (d, $J$ = 8.7 Hz, 2H); 4.17 (br.s, 2H). $^{13}$C NMR ($CDCl_3$): 151.35 (s); 150.00 (s); 149.35 (s); 141.48 (s); 136.05 (s); 130.69 (d); 130.37 (d); 130.11 (s); 129.61 (d); 128.98 (d); 128.89 (d); 128.78 (d); 126.60 (d); 125.11 (d); 122.73 (s); 121.18 (s); 114.07 (d).

#### *5.1.3. 3-Chloro-9-pentoxy-acridine (**11**)*

M.p.: 62–63 °C (acetonitrile). $^1$H NMR ($CDCl_3$): 8.26–8.14 (m, 4H); 7.76 (t, $J$ = 7.2 Hz, 1H); 7.51 (d, $J$ = 6.8 Hz, 1H); 7.43 (d, $J$ = 9.2 Hz, $J$ = 2.0 Hz, 1H); 4.31 (t, $J$ = 6.6 Hz, 2H); 2.03 (q, $J$ = 6.7 Hz, 2H); 1.83–1.40 (m, 4H); 0.98 (t, $J$ = 7.0 Hz, 3H). $^{13}$C NMR ($CDCl_3$): 161.41 (s); 151.08 (s); 150.27 (s); 136.35 (s); 130.79 (d); 129.50 (d); 128.00 (d); 126.21 (d); 125.20 (d); 124.05 (d); 122.53 (d); 120.39 (s); 118.77 (s); 77.57 (t); 30.21 (t); 28.05 (t); 22.46 (t); 13.97 (q).

#### *5.1.4. 2-Methoxy-6-chloro-9-phenylsulfonyl-acridine (**3**)*

2-Methoxy-6,9-dichloro-acridine (1.2 g, 4.3 mmol), sodium benzenesulfinate (1.3 g, 7.9 mmol), $K_2CO_3$ (1.25 g, 12.7 mmol), pentanol (20 ml), Cu (cat.), were heated under stirring at 120 °C during 7 h. Solvents are removed under vacuum; solid is extracted with chloroform and organic phase washed with $H_2O$ and dried on $MgSO_4$. Product is isolated by chromatography on silica gel and eluted with ether/pentane 2:98 then 10:90 and ether. Yield: 38%. M.p.: 239–240 °C. $^1$H NMR ($CDCl_3$): 9.33 (d, $J$ = 9.7 Hz, 1H); 8.56 (d, $J$ = 2.6 Hz, 1H); 8.25 (d, $J$ = 2.2 Hz, 1H); 8.12 (d, $J$ = 9.4 Hz, 1H); 7.92 (br, d, $J$ = 8.0 Hz, 2H); 7.60 (dd, $J$ = 9.8 Hz, $J$ = 2.3 Hz, 2H); 7.48 (m, 3H); 4.01 (s, 3H). $^{13}$C NMR ($CDCl_3$): 159.76 (s); 147.62 (s); 147.36 (s); 142.68 (s); 135.88 (s); 134.86 (s); 133.71 (d); 132.45 (d); 130.06 (d); 129.38 (d); 129.16 (d); 126.27 (d); 126.22 (d); 125.81 (d); 125.00 (s); 121.90 (s); 100.55 (d); 55.93 (q).

#### *5.1.5. 2-Methoxy-6-chloro-9-pentoxy-acridine (**12**)*

2-Methoxy-6,9-dichloro-acridine (1.2 g, 4.3 mmol), sodium benzene sulfinate (1.3 g, 7.9 mmol), $K_2CO_3$ (1.25 g, 12.7 mmol), pentanol (20 ml), Cu (cat.), were heated under stirring at 140 °C during 24 h. Solvents are removed under vacuum; solid is extracted with chloroform and organic phase is washed with $H_2O$ and dried on $MgSO_4$. The product is isolated by chromatography on silica gel and eluted with ether/pentane 5:5 then ether. Yield: 53%. M.p.: 75.8–76.5 °C. $^1$H NMR ($CDCl_3$): 8.16 (s, 1H); 8.13 (d, $J$ = 7.7 Hz, 1H); 8.05 (d, $J$ = 9.4 Hz, 1H); 7.44 (dd, $J$ = 9.8 Hz, $J$ = 2.8 Hz, 1H); 7.41 (dd, $J$ = 9.1 Hz, $J$ = 2.0 Hz, 1H); 7.35 (d, $J$ = 2.8 Hz, 1H); 4.24 (t, $J$ = 6.6 Hz, 2H); 3.97 (s, 3H); 2.01 (quint, $J$ = 6.6 Hz, 2H); 1.65 (m, 2H); 1.46 (sext, $J$ = 7.1 Hz, 2H); 0.98 (t, $J$ = 7.1 Hz, 3H). $^{13}$C NMR ($CDCl_3$): 159.32 (s); 157.07 (s); 148.76 (s); 148.42 (s); 135.22 (s); 131.30 (d); 128.21 (d); 126.54 (d); 126.03 (d); 123.69 (d); 121.30 (s); 119.14 (s); 97.80 (d); 76.70 (t); 55.56 (q); 30.39 (t); 28.39 (t); 22.63 (t); 14.13 (q).

*5.1.6. 2-Methoxy-6-chloro-9-(4′-amino-phenylsulfonyl)-acridine (**4**)*

Procedure is the same to that described with **2**. Yield: 58%. M.p.: 230 °C (from toluene). $^1$H NMR (DMSO-$d_6$): 9.41 (d, $J$ = 9.8 Hz, 1H); 8.52 (d, $J$ = 2.4 Hz, 1H); 8.19 (d, $J$ = 2.1 Hz, 1H); 8.06 (d, $J$ = 9.5 Hz, 1H); 7.79 (dd, $J$ = 9.8 Hz, $J$ = 2.3 Hz, 1H); 7.71 (d, $J$ = 8.7 Hz, 2H); 7.57 (dd, $J$ = 9.5 Hz, $J$ = 2.5 Hz, 1H); 6.60 (d, $J$ = 8.7 Hz, 2H); 6.32 (br.s, 2H); 3.99 (s, 3H). $^{13}$C NMR (DMSO-$d_6$): 158.84 (s); 154.24 (s); 146.89 (s); 146.74 (s); 138.44 (s); 133.73 (s); 132.08 (d); 129.39 (d); 128.88 (d); 128.37 (d); 126.41 (d); 125.89 (d); 125.61 (s); 123.47 (s); 120.70 (s); 113.09 (d); 100.84 (d); 55.89 (q).

*5.1.7. 2-Methoxy-6-chloro-9-(3′-acetamido-4′-methoxy-phenyl-sulfonyl)-acridine (**5**)*

A mixture of 2-methoxy-6,9-dichloroacridine (1.3 g, 5 mmol), sodium 3-acetamido-4-methoxy-benzenesulfinate (from 1.30 g of the corresponding sulfinic acid and NaOH 0.23 g), $K_2CO_3$ (1 g), pentanol (30 ml), and pyridine (10 ml), Cu (cat.) was heated at 120 °C during 5 h. Solvents are removed under vacuo. After addition of hot water a solid is isolated, washed with a small amount of chloroform and methanol. Yield: 35%. M.p.: 217–218 °C (methanol–water). $^1$H NMR (DMSO-$d_6$): 9.43 (br.s, 1H); 9.38 (d, $J$ = 9.8 Hz, 1H); 8.97 (d, $J$ = 2.1 Hz, 1H); 8.50 (d, $J$ = 2.5 Hz, 1H); 8.20 (d, $J$ = 2.3 Hz, 1H); 8.05 (d, $J$ = 9.4 Hz, 1H); 7.82 (m, 2H); 7.57 (dd, $J$ = 9.4 Hz, $J$ = 2.5 Hz, 1H); 7.20 (d, $J$ = 8.8 Hz, 1H); 4.03 (s, 3H); 3.86 (s, 3H); 2.07 (s, 3H). $^{13}$C NMR (DMSO-$d_6$): 169.58 (s); 159.34 (s); 152.97 (s); 146.98 (s); 146.72 (s); 136.90 (s); 133.91 (s); 132.52 (s); 132.28 (d); 129.88 (d); 128.61 (d); 126.30 (d); 126.07 (d); 123.86 (s); 123.11 (d); 120.93 (s); 118.10 (d); 111.72 (d); 100.54 (d); 56.45 (q); 55.91 (q); 24.05 (q).

*5.1.8. 2-Methoxy-6-chloro-9-(4′-acetamido-phenylsulfonyl)-acridine (**6**)*

Procedure is the same to that described with **2**.

Product is isolated by chromatography on silica gel eluted with ether/chloroform mixture 90:10, then 1–5% methanol. Yield: 53%. M.p.: 245 °C (pyridine–water). $^1$H NMR ($C_5D_5N$): 11.28 (s, 1H); 9.74 (d, $J$ = 9.7 Hz, 1H); 8.91 (d, $J$ = 2.0 Hz, 1H); 8.38–8.17 (m, 4H); 8.08 (d, $J$ = 8.8 Hz, 2H); 7.69 (dd, $J$ = 9.8 Hz, $J$ = 2.2 Hz, 1H); 7.52 (dd, $J$ = 9.4 Hz, $J$ = 2.3 Hz, 1H); 3.92 (s, 3H); 2.08 (s, 3H). $^{13}$C NMR ($C_5D_5N$): 169.62 (s); 160.03 (s); 148.00 (s); 147.74 (s); 145.57 (s); 137.51 (s); 136.29 (s); 134.65 (s); 132.99 (d); 129.98 (d); 129.61 (d); 128.42 (d); 126.73 (d); 126.30 (d); 125.03 (s); 122.00 (s); 119.60 (d); 101.29 (d); 55.89 (q); 24.29 (q).

*5.1.9. 2-Methoxy-6-chloro-9-[(4′-*N,N*-dimethyl amino-methyleneamino)-phenylsulfonyl)]-acridine (**7**)*

2-Methoxy-7-chloro-9-(4′-aminophenylsulfonyl)-acridine (0.39 g, 1 mmol) and DMF-DMA (10 g) were heated at 110 °C during 24 h. The product recovered after evaporation under vacuo was chromatographed on silica gel, with ether/methylene chloride/methanol as eluents 200:10:5. Yield: 38%. M.p.: 226–227 °C. $^1$H NMR ($CD_2Cl_2$): 9.40 (d, $J$ = 10.4 Hz, 1H); 8.57 (d, $J$ = 2.6 Hz, 1H); 8.19 (d, $J$ = 2.4 Hz, 1H); 8.06 (d, $J$ = 9.2 Hz, 1H); 7.80 (d, $J$ = 9.0 Hz, 2H); 7.61 (dd, $J$ = 10 Hz, $J$ = 2.4 Hz, 1H); 7.48 (br.s, 1H); 7.46 (dd, $J$ = 9.4 Hz, $J$ = 2.8 Hz, 1H); 6.91 (d, $J$ = 9.0 Hz, 2H); 4.02 (s, 3H); 2.98 (s, 3H); 2.95 (s, 3H). $^{13}$C NMR ($CD_2Cl_2$): 159.46 (s); 157.49 (s); 153.84 (d); 147.66 (s); 147.46 (s); 134.59 (s); 134.43 (s); 133.29 (d); 129.43 (d); 128.94 (d); 127.82 (d); 126.15 (d); 125.82 (d); 121.26 (d); 100.88 (d); 55.87 (q); 40.14 (q).

*5.1.10. 2,7-Dimethoxy-9-(phenylsulfonyl)-acridine (**8**)*

Procedure is the same to that described with **3** (heating at 120 °C during 11 h). Yield: 41%. M.p.: 252.5–253.6 °C. $^1$H NMR ($CDCl_3$): 8.55 (d, $J$ = 2.6 Hz, 2H); 8.10 (d, $J$ = 9.4 Hz, 2H); 7.92 (d, $J$ = 9.4 Hz, 2H); 7.57–7.36 (m, 5H); 3.98 (s, 6H). $^{13}$C NMR ($CDCl_3$): 159.57 (s); 144.45 (s); 142.98 (s); 133.32 (d); 132.36 (d); 129.09 (d); 125.99 (d); 125.30 (s); 123.80 (d); 100.45 (d); 55.69 (q).

*5.1.11. 2,7-Dimethoxy-9-(3′-acetamido-4′-methoxy-phenyl sulfonyl)-acridine (**9**)*

Procedure is the same to that described with **5** (heating at 120 °C during 20 h). Chromatography with ether/$CHCl_3$/$CH_3OH$, 200:20:5–10. Yield: 19%. M.p.: 222.5–223.5 °C. $^1$H NMR ($CDCl_3$): 9.26 (d, $J$ = 2.2 Hz, 1H); 8.69 (d, $J$ = 2.6 Hz, 2H); 8.05 (d, $J$ = 9.4 Hz, 2H); 7.75 (br.s, 1H); 7.66 (dd, $J$ = 8.7 Hz, $J$ = 2.3 Hz, 1H); 7.37 (dd, $J$ = 9.4 Hz, $J$ = 2.6 Hz, 2H); 6.83 (d, $J$ = 8.7 Hz, 1H); 4.07 (s, 6H), 3.83 (s, 3H), 2.14 (s, 3H). $^{13}$C NMR ($CDCl_3$): 168.12 (s); 159.59 (s); 150.92 (s); 144.61 (s); 135.01 (s); 133.59 (s); 132.20 (d); 128.39 (s); 125.14 (s); 123.82 (d); 122.39 (d); 117.25 (d); 109.76 (d); 100.80 (d); 56.21 (q); 55.90 (q); 24.87 (q).

## 5.2. Biology

*5.2.1. Strains of* P. falciparum

The chloroquine resistant clone W2 from Indochina and the chloroquine susceptible 3D7 from Africa, were maintained in culture. When required for drug assays, cultures were synchronized by sorbitol lysis [61].

*5.2.2. Drugs*

PABA was obtained from Sigma Chemical (St. Louis, MO, USA). Stock solutions of sulfones were prepared in sterile distilled water and DMSO (1%). Twofold serial dilutions were prepared in DMSO (1%) and RPMI 1640 medium (Invitrogen, United Kingdom) buffered with 25 mM HEPES and 25 mM $NaHCO_3$. Final concentrations distributed for the evaluation of drug were ranged from 0.15 to 500 μM. Stock solution of PABA was prepared in methanol (1%) and distilled water. Twofold serial dilutions were prepared in RPMI 1640 medium buffered with 25 mM HEPES and 25 mM $NaHCO_3$. Final concentrations distributed for the evaluation of the interaction of PABA and sulfone **3** were ranged from 1 μM to 100 mM.

*5.2.3. In vitro assay*

With the aim to determine intrinsic activity, 25 µl per well of sulfones derivatives and 175 µl per well of parasitized erythrocytes suspension (final parasitemia and hematocrit, 0.5% and 1.5%) was distributed in 96-well plates for the in vitro isotopic microtests.

To assess interaction between PABA and sulfone compounds, 25 µl of sulfone **3**, 25 µl of PABA and 150 µl of parasitized red blood cells suspension (final parasitemia and hematocrit, 0.5% and 1.5%) were distributed in each well. Parasite growth was assessed by adding 1 µCi of $^3$H-hypoxanthine with a specific activity 14.1 Ci mmol$^{-1}$ (NEN Products, Dreiech, Germany) to each well at $t_0$. Then, plates were incubated for 48 h at 37 °C in 10% $O_2$, 5% $CO_2$, 85% $N_2$ atmosphere, at 95% humidity. Immediately after incubation, plates were frozen then thawed to lyse erythrocytes. The contents of each well were collected on standard filter microplates (Unifilter™ GF/B, Perkin–Elmer, USA) and washed using a cell harvester (FilterMate™ Cell Harvester, Perkin–Elmer). Filter microplates were dried and 25 µl of scintillation cocktail (Microscint™ O, Perkin–Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured using a scintillation counter (Top Count™, Perkin–Elmer).

The 50% inhibitory concentration ($IC_{50}$), i.e. the drug concentration corresponding to 50% of the uptake of $^3$H-hypoxanthine by the parasites in drug-free control wells, was determined by non-linear regression analysis of log-dose/response curves. Data were analyzed after logarithmic transformation and expressed as the geometric mean $IC_{50}$ and 95% confidence intervals (95% CI) were calculated. Combinations of PABA and sulfone **3** were expressed in % of parasite g.i.

To assess interaction between chloroquine and arylsulfonyl acridinyl compounds, 25 µl of chloroquine, 25 µl of subinhibitory fixed concentrations of drugs tested and 150 µl of the suspension of parasitized erythrocytes were distributed in each well. Isobolograms were constructed by plotting a pair of fractional $IC_{50}$ for each combination of chloroquine and the different compounds and for both parasite strains. The different sulfone derivatives fractional $IC_{50}$ was calculated by dividing their fixed concentrations by the $IC_{50}$ of tested drugs alone and plotted on the horizontal axis. The corresponding chloroquine fractional $IC_{50}$ was calculated by dividing the $IC_{50}$ of chloroquine combined with fixed concentrations of sulfone derivatives and plotted on the vertical axis. Points lying above the straight diagonal line (corresponding to the points where there is no interaction between the drugs) are antagonistic, points below the straight diagonal line are considered to be synergistic [62].

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