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Original article

Tandem synthesis and *in vitro* antiplasmodial evaluation of new naphtho[2,1-*d*]thiazole derivatives

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ABSTRACT

A series of naphtho[2,1-*d*]thiazoles was prepared in good yields under microwave irradiation with an original protocol combining tandem direct arylation and intramolecular Knoevenagel reaction on 1,3-thiazole derivatives. Antiplasmodial evaluation of this series highlighted two hit compounds (compounds **11** and **13**) displaying promising *in vitro* activity on the multiresistant K1 *Plasmodium falciparum* strain. Structure-toxicity and structure–activity relationships are also discussed and reveal the importance of the R_1 and R_4 substituents of the naphthyl moiety for the biological profile of the series.

1. Introduction

Malaria is a devastating disease which in 2010 affected 216 million people worldwide and caused 655,000 deaths. This infection, transmitted via the bite of the female Anopheles mosquito, is caused by five species of protozoa parasites belonging to the *Plasmodium* genus, namely *malariae*, *vivax*, *ovale*, *knowlesi* and *falciparum*. *Plasmodium falciparum* is the most virulent [1], causing more than 95% of malaria-related morbidity and mortality. According to the WHO [2], significant and durable progress has been recorded for a few years, with the estimated incidence of malaria globally reduced by 17% since 2000. Moreover, malaria-specific mortality rates were also reduced by 26% between 2000 and 2010. These encouraging statistics are the combined result of vector control (insecticide-treated mosquito nets, indoor residual spraying), chemoprevention, diagnostic testing and malaria treatment. Nevertheless, the growing drug resistance of parasites around the world [3] remains a real and ever-present danger, attributable mainly to *P. falciparum*. Currently, the only fully effective class of antimalarial drug is artemisinin and its derivatives: artemisinin-based combination therapies (ACTs) have now been adopted as the first line of treatment in endemic areas. However, a *P. falciparum* resistance to artemisinin derivatives was identified and confirmed on the Cambodia-Thailand border in 2009 [4,5]. This emerging resistance could lead to a resurgence of more virulent levels of malaria unless a new chemical class of effective drugs is rapidly found. In this context, all innovative practices and encouraging results are being sponsored and shared [6,7], so as to accelerate the development and licensing of new antimalarial drugs.

1,3-thiazole nucleus is commonly found in the chemical structure of antiparasitic agents (nitazoxanide [8], aminitrozole [9], tenonitrozole). Among the chemical compounds already screened for inhibitors of *P. falciparum*, certain 1,3-thiazole derivatives have been reported to display promising antiplasmodial activity with new target hypothesis [6]. Recently, 1,3-thiazole derivatives combining good *in vitro* activity against *P. falciparum* with oral efficacy in a *Plasmodium berghei* mouse model have also been identified [10]. In continuation of our research program centered on

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the design and synthesis of novel bioactive compounds [11–14], a strong collaboration between our two research teams enabled us to synthesize and evaluate a group of new heterocyclic hit compounds displaying antiparasitic activities [15–19], which included newly-identified original antiplasmodial derivatives [20–24]. In this context, we decided to explore the antiplasmodial potential of new naphtho[2,1-*d*]thiazole derivatives which we synthesized using an original "one pot" methodology combining direct arylation and unexpected Knoevenagel intramolecular reaction on 1,3-thiazole derivatives substituted on the C-2 and C-4 positions.

2. Results and discussion

2.1. Chemistry

Recently, we developed the application of the Suzuki-Miyaura cross-coupling reaction on thiazole series substituted on the C-2 and C-4 position [25]. We report herein the study of palladium-catalyzed direct arylation [26–33] on the thiazole ring C-5 position. This reaction was first described in 1992 by Ohta et al. [34]. In the literature, this procedure was described as being less efficient but complementary to the Suzuki-Miyaura cross-coupling reaction [35]. However, unexpectedly, when we tried for the first time to carry out this procedure on C-2 and C-4 substituted thiazole derivatives, we observed both the direct arylation of the compound and a simultaneous intramolecular Knoevenagel reaction. We then investigated the potential of this unexpected "one pot" strategy. Initially, the required starting material for the direct arylation is 2-methyl-4-(tosylmethyl)thiazole **1** which was prepared in 62% overall yield by sequential condensation between 1,3-dichloroacetone with thioacetamide [36], cyclization using $ZnCl_2$ in methanol [37], and condensation with *p*-toluenesulfinic acid sodium salt, in water and under microwave irradiation (Scheme 1).

Then, the reaction of 2-methyl-4-(tosylmethyl)thiazole **1** and 2-bromobenzaldehyde was carried out in order to synthesize 2-[2-methyl-4-(tosylmethyl)thiazol-5-yl]benzaldehyde as a product of direct arylation on C-5 position of the thiazole ring. Surprisingly, 2-methyl-4-tosylnaphtho[2,1-*d*]thiazole **4** was obtained, which was confirmed by X-ray structure analysis (Fig. 1).

The direct arylation of the thiazole nucleus on C-5 position by the bromide reagent forming the expected 2-[2-methyl-4-(tosylmethyl)thiazol-5-yl]benzaldehyde and the very favorable spatial position of the carbonyl substituent may allow a simultaneous intramolecular Knoevenagel [38] ring closure on the supposed methylene-activated intermediate (Scheme 2). Some examples of "one pot" strategies involving direct arylation have recently been published [39–42], but none describes a sequence with an intramolecular Knoevenagel reaction. The use of "one pot" organic reactions is currently attracting a great deal of attention, as they are performed without needing to isolate any intermediate product. In addition to their advantageous rate of synthesis, they offer the possibility of building up more complex compounds from simple components. These procedures are increasingly being applied to arylbromide reagents in non-heterocyclic series [43], to extend their reactivity. To verify the validity of this mechanism, we carried out the reaction of 2-methyl-4-(tosylmethyl)thiazole both with 3-bromobenzaldehyde and with 4-bromobenzaldehyde. No reaction occurred under any conditions. These initial results thus revealed the role of the carbonyl and the importance of its position in the mechanism of this reaction. Initially, we used the experimental conditions recommended by Primas et al. [35]. 1 equivalent (equiv) of 2-bromobenzaldehyde and 5 equiv of starting material **1** were dissolved in dimethylacetamide (DMA) and heated at 150 °C using $Pd(PPh_3)_4$ (0.05 equiv) as a catalyst and KOAc (3 equiv) as a base for 24 (Table 1, entry 1). The unexpected naphtho[2,1-*d*]thiazole **4** was then obtained with a 37% yield and no intermediate was isolated. In order to optimize the above method, we performed the reaction under microwave irradiation [44,45]: the desired product **4** was obtained with a better yield and a shorter reaction time (Table 1, entry 2). Then, we also tried to reduce the thiazole derivative amount (Table 1, entries 3–5). Finally, the best experimental conditions used 1.5 equiv of **1**, 3 equiv of KOAc, 0.05 equiv of $Pd(PPh_3)_4$ and microwave irradiation (300 W, 150 °C) (Table 1, entry 4).

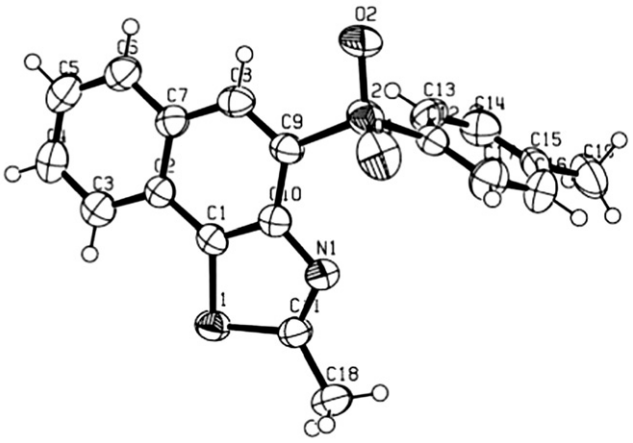
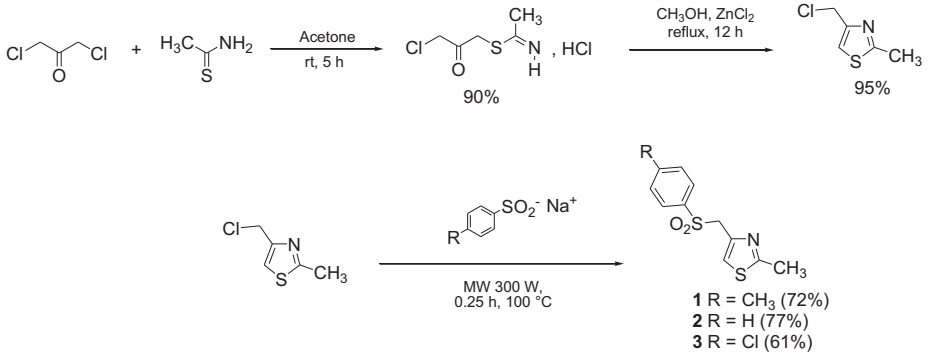
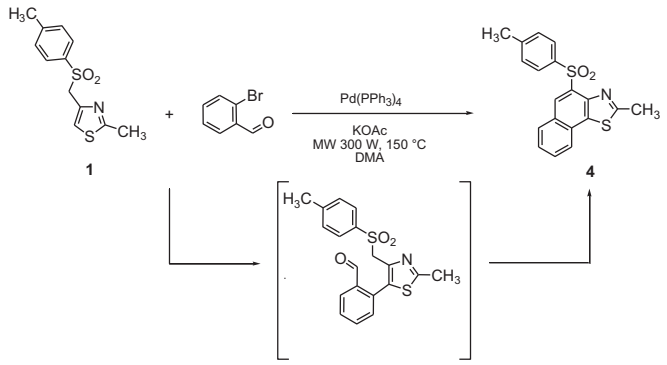


Fig. 1. ORTEP plot of 2-methyl-4-tosylnaphtho[2,1-*d*]thiazole **4**.

Based on these experimental results, we decided to diversify the chemical substituents on the naphtho[2,1-*d*]thiazole nucleus in



Scheme 1. Preparation of 4-arylsulfonylmethyl-1-methylthiazoles.



Scheme 2. Preparation of 2-methyl-4-tosylnaphtho[2,1-*d*]thiazole **4** by "one pot" direct arylation and intramolecular Knoevenagel reaction with 2-methyl-4-(tosylmethyl)thiazole **1**.

order to evaluate their influence on the antiplasmodial activity of the corresponding compounds. Then, we changed both the starting material and the brominated reagent (Table 2): the same procedure used to prepare **1** led to 2-methyl-4-(phenylsulfonylmethyl)thiazole **2** and 4-[(4-chlorophenylsulfonyl)methyl]-2-methylthiazole **3** from benzenesulfinic acid and *p*-chlorobenzenesulfinic acid sodium salts respectively, with 77% and 61% yields (Scheme 1). In addition, 2-bromobenzaldehyde was replaced by various substituted benzaldehyde and benzonitrile derivatives in order to pharmacomodulate the naphtho[2,1-*d*]thiazole scaffold. The productive "one pot" strategy was then extended to the new starting materials **2** and **3** (Table 2, entries 8–18), with a view to exploring the chemical and biological influence of the electron-withdrawing or -donating character of the substrates. No correlation could be established with chemical reaction yields. However, the nature of the halogen substituent on the benzaldehyde reagent had an influence on the synthesized product rate: the good yield obtained with 2-bromobenzaldehyde in optimal conditions (64% in 0.5 h) was not achieved with the other 2-halobenzaldehydes tested (from 35% to 42% with longer reaction times) (Table 2, entries 1 and 2). Moreover, this process was also successfully applied to 2-bromobenzonitriles (Table 2, entries 6, 7, 12, 13, 17 and 18) and led to the formation of some naphtho[2,1-*d*]thiazol-5-amines (compounds **8**, **9**, **14**, **15**, **19** and **20**) in good yields (from 44% to 70%). The structure of compound **8** was unambiguously confirmed by X-ray structure analysis (Fig. 2). The other structures were assigned by analogy and spectral comparison.

2.2. In vitro antiplasmodial activity

To synthesize compounds of therapeutic interest, it was necessary to first determine whether these compounds were presenting a toxic profile on human cells, or if they could offer *in vitro* selective antiparasitic properties. Consequently, we performed cytotoxicity experiments, employing the MTT method [46] to determine the cytotoxic concentrations of 50% (CC_{50}) on the HepG2 cell line and using doxorubicin as a cytotoxic reference-compound (Table 3). HepG2 is a commonly used human-derived hepatocarcinoma cell line that has shown characteristics similar to those of primary hepatocytes. These cells express many of the hepatocyte-specific metabolic enzymes, thus enabling the cytotoxicity of tested product metabolites to be evaluated. The tests showed that a hydrogen atom as R_1 substituent conferred a lower overall cytotoxicity (11.3 μM ≤ CC_{50} ≤ 500 μM) than a methyl group (0.2 μM ≤ CC_{50} ≤ 250 μM) or a chlorine atom (0.5 μM ≤ CC_{50} ≤ 31.2 μM). Furthermore, whatever the R_1 substituent, compounds bearing an electron-donating group (CH_3, OCH_3) as R_4 substituent were non-cytotoxic (31.2 μM ≤ CC_{50} ≤ 250 μM) compared with doxorubicin used as reference drug (Table 3, entries 2, 4, 8, 10 and 15).

Then, the non-cytotoxic compounds were assessed for their *in vitro* antiplasmodial activity against a K1 multi-resistant strain of *P. falciparum* by using the SYBR Green I fluorescence-based method [47,48]. In order to identify compounds with significant potential, two reference drug compounds, chloroquine and doxycycline, were also tested in the same conditions (Table 3, entries 19 and 20). Their inhibitory concentrations 50% (IC_{50}) were then calculated, as well as their selectivity indexes (SI). Two hit compounds were identified, compounds **11** (4.8 μM) and **13** (2.7 μM), displaying interesting IC_{50} values intermediate between those of the reference drugs chloroquine (0.5 μM) and doxycycline (5 μM). These two hit compounds carried a hydrogen as R_1 substituent, which confirms that the nature of the R_1 substituent plays a key role in the cytotoxicity and the antiplasmodial activity of the naphtho[2,1-*d*]thiazole scaffold. The influence of other substituents could not be clearly established because of the limited number of compounds in the series tested. Nevertheless, from the results obtained, it seems that antiplasmodial activity could be greatly improved by adding an electron-donating group as R_4 substituent. Given the *in vitro* antiplasmodial activity and the cytotoxicity of the two hit compounds, their selectivity indexes (ratio CC_{50} (HepG2)/IC_{50} (K1)) were determined to identify their therapeutic potential. In particular, compound **13** (SI > 45) appeared to be an encouraging antiplasmodial compound, in comparison with doxycycline and chloroquine, which had selectivity indexes of 4 and 60 respectively.

Table 1
Reaction of **1** with 2-bromobenzaldehyde under different conditions.

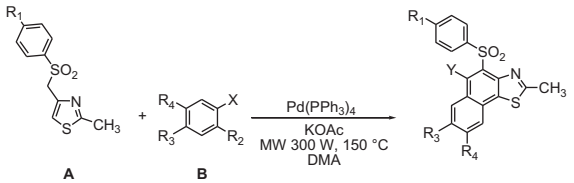
Entry	Compound 1	Microwave irradiation	Time (h)[a]	Yield 4 (%)
1	5 equiv	None	24	37
2	5 equiv	300 W	1.5	45
3	2.5 equiv	300 W	1	60
4	1.5 equiv	300 W	0.5	64
5	1 equiv	300 W	0.7	25

[a] All the reactions were performed using 1 equiv of 2-bromobenzaldehyde, 3 equiv of KOAc and 0.05 equiv of $Pd(PPh_3)_4$ in 5 mL of DMA. The temperature being ramped up from rt to 150 °C, where it was then held for a time *t*.

3. Conclusion

We have developed an original, rapid and efficient process to synthesize new naphtho[2,1-*d*]thiazole derivatives on C-2 and C-4 substituted thiazole series. These results constitute the first example of a "one pot" direct arylation and intramolecular Knoevenagel ring closure reaction on a heterocyclic substrate. Further investigations aimed at extending this method to other heterocycles are in progress. Among the synthesized compounds, we identified two hits (compounds **11** and **13**) displaying an encouraging *in vitro* antiplasmodial activity on the *K1* multi-resistant *P. falciparum* strain, compared with chloroquine and doxycycline used as reference drugs. Moreover, compound **13** (SI > 45) achieved a relatively high selectivity index score, compared with chloroquine and doxycycline (respectively SI60 and 4). Structure-toxicity and structure–activity relationships showed that the hydrogen atom as R_1 substituent plays a key role in cytotoxicity and antiplasmodial activity. The presence of an electron-donating group as R_4 substituent appeared to favor both the absence of cytotoxicity and increased antiplasmodial activity. The next step in our research program will be to examine the mechanism by which these compounds display their antiplasmodial activity.

Table 2
Microwave-mediated preparation of several naphtho[2,1-*d*]thiazoles.



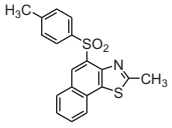
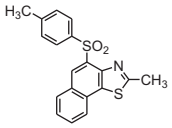
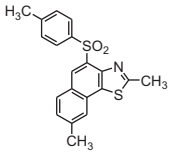
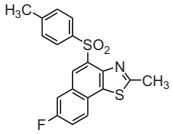
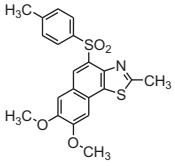
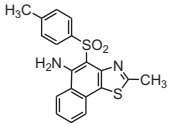
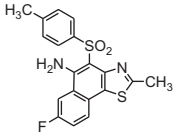
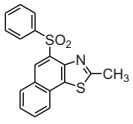
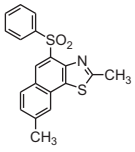
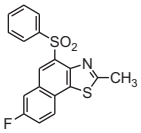
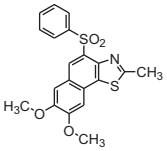
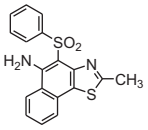
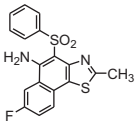
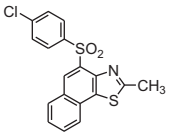
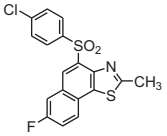
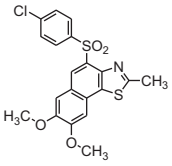
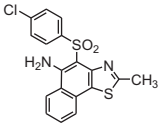
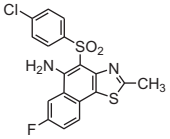
Entry	Reagent A	Reagent B	Product	Compound number	Reaction time (h)[a]	Yield (%)
1	$R_1 = CH_3$	X = Cl R_2 HO $R_3 = R_4 = H$		4	2	35
2	$R_1 = CH_3$	X = I R_2 = CHO $R_3 = R_4 = H$		4	3	42
3	$R_1 = CH_3$	X = Br R_2 = CHO R_3 = H $R_4 = CH_3$		5	2	87
4	$R_1 = CH_3$	X = Br R_2 = CHO R_3 = F R_4 = H		6	1	41
5	$R_1 = CH_3$	X = Br R_2 = CHO $R_3 = R_4 = OCH_3$		7	3	71
6	$R_1 = CH_3$	X = Br R_2 = CN $R_3 = R_4 = H$		8	3.5	59
7	$R_1 = CH_3$	X = Br R_2 = CN R_3 = F R_4 = H		9	1.5	70
8	R_1 = H	X = Br R_2 = CHO $R_3 = R_4 = H$		10	0.75	33
9	R_1 = H	X = Br R_2 = CHO R_3 = H $R_4 = CH_3$		11	1.5	72

Table 2 (*continued*)

Entry	Reagent A	Reagent B	Product	Compound number	Reaction time (h)[a]	Yield (%)
10	$R_1 = H$	$X = Br$ $R_2 = CHO$ $R_3 = F$ $R_4 = H$		12	2	45
11	$R_1 = H$	$X = Br$ $R_2 = CHO$ $R_3 = R_4 = OCH_3$		13	4	58
12	$R_1 = H$	$X = Br$ $R_2 = CN$ $R_3 = R_4 = H$		14	4	48
13	$R_1 = H$	$X = Br$ $R_2 = CN$ $R_3 = F$ $R_4 = H$		15	1.75	46
14	$R_1 = Cl$	$X = Br$ $R_2 = CHO$ $R_3 = R_4 = H$		16	1	60
15	$R_1 = Cl$	$X = Br$ $R_2 = CHO$ $R_3 = F$ $R_4 = H$		17	1	72
16	$R_1 = Cl$	$X = Br$ $R_2 = CHO$ $R_3 = R_4 = OCH_3$		18	4	54
17	$R_1 = Cl$	$X = Br$ $R_2 = CN$ $R_3 = R_4 = H$		19	2	44
18	$R_1 = Cl$	$X = Br$ $R_2 = CN$ $R_3 = F$ $R_4 = H$		20	2.25	52

[a] All the reactions were performed using 1.5 equiv of reagent A, 1 equiv of halogenated reagent B, 3 quiv of KOAc and 0.05 equiv of $Pd(PPh_3)_4$ in 5 mL of DMA. An initial microwave irradiation of 300 W was used, the temperature being ramped up from rt to 150 °C, where it was then held for a time *t*.

4. Experimental section

Yields refer to purified products and are not optimized. Melting points were determined on a Büchi melting point B-540 apparatus and are uncorrected. Both ^1H- and ^{13}C NMR spectra were determined on a Bruker Avance 200 spectrometer (operating at 200 MHz for ^1H and 50 MHz for ^{13}C).^1H and ^{13}C NMR shifts (δ) were reported in parts per million (ppm) with respect to $CDCl_3$ 7.26 ppm for ^1H and 77.0 ppm for ^{13}C and DMSO-d_6 2.50 for ^1H and 39.7 ppm for ^{13}C. Multiplicities are represented by s (singulet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are in Hertz (Hz). Elemental analyses were carried out at the Spectropole,

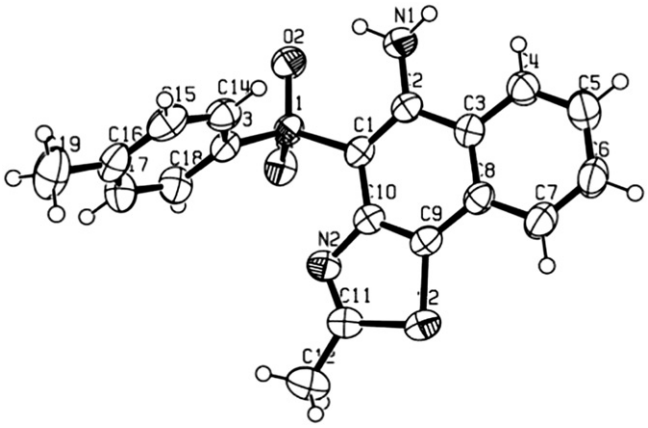


Fig. 2. X-ray structure of 2-methyl-4-tosylnaphtho[2,1-*d*]thiazol-5-amine **8**.

Faculté des Sciences et Techniques de Saint-Jérome. The following adsorbent was used for column chromatography: silica gel 60 (Merck, 70–230 mesh). Thin-layer chromatography (TLC) was performed with Merck 60F-254 silica gel (0.25 mm layer thickness) in an appropriate solvent.

4.1. 2-Methyl-4-(tosylmethyl)thiazole (**1**)

To a mixture of 4-chloromethyl-2-methylthiazole (19 g, 128.7 mmol) in water (20 mL) was added *p*-toluenesulfinic acid sodium salt (3 equiv). The reaction mixture was irradiated in a microwave oven (ETHOS Synth Lab station) and reaction was carried out under microwave irradiation at 150 °C at 300 W for 0.25 h. A precipitate appeared and was filtered after cooling, then washed with water (3 × 20 mL). The precipitate was purified by chromatographic column, eluting with EtOAc/chloroform (5/5). Recrystallization from isopropanol (*i*-PrOH) gave the compound **1** as a brown solid. mp 174 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.41 (s, 3 H), 2.59 (s, 3 H), 4.48 (s, 2 H), 7.13 (s, 1 H), 7.27 (d, $J = 8.2$ Hz, 2 H), 7.60 (d, $J = 8.2$ Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.9 (CH_3), 21.6 (CH_3), 58.2 (CH_2), 119.7 (CH), 128.5 (2 CH), 129.6 (2 CH), 135.5 (C), 142.9 (C), 144.7 (C), 165.9 (C). Anal. Calcd for $C_{12}H_{13}NO_2S_2$: C, 53.91; H, 4.90; N, 5.24. Found: C, 54.13; H, 5.01; N, 5.23.

Table 3
Human cell toxicity and antiplasmodial activity of the studied compounds.

Entry	Compound	Human cell toxicity HepG2 CC_{50}[a]	Antiplasmodial activity K1 IC_{50}[a]	Selectivity index (SI)[b]
1	4	2.2 (±0.37)	–	–
2	5	>250[e]	>25[f]	ND[g]
3	6	3.1(±1.33)	–	–
4	7	>125[e]	>25[f]	ND[g]
5	8	0.2 (±0.01)	–	–
6	9	0.2 (±0.03)	–	–
7	10	11.3 (±3.17)	>25[f]	< 0.5
8	11	>125[e]	4.8 (±0.04)	> 26
9	12	26.0 (±0.81)	>25[f]	< 1
10	13	>125[e]	2.7 (±0.11)	> 45
11	14	>500[e]	>25[f]	ND[g]
12	15	>500[e]	>25[f]	ND[g]
13	16	6.0 (±2.12)	–	–
14	17	3.4 (±0.22)	–	–
15	18	>31.2[e]	>25[f]	ND[g]
16	19	1.0 (±0.02)	–	–
17	20	0.5 (±0.16)	–	–
18	Doxorubicin[c]	0.2	–	–
19	Doxycycline[d]	20	5	4
20	Chloroquine[d]	30	0.5	60

[a] Mean of three independent experiments.
[b] Selectivity index was calculated according to the following formula : SI K1 = CC_{50} (HepG2)/IC_{50} (K1).
[c] Doxorubicin was used as reference drug compound for human cell toxicity.
[d] Doxycycline and chloroquine were used as reference antiplasmodial drug compounds.
[e] Compound could not be tested at higher concentrations because of lack of solubility in the culture medium.
[f] No antiplasmodial activity observed at the highest-concentration tested product.
[g] No selectivity index could be determined.

4.2. General procedure and analytical data for compounds **2** and **3**

To a mixture of 4-chloromethyl-2-methylthiazole (500 mg, 3.4 mmol) in water (20 mL) was added the desired sulfinic acid sodium salt (3 equiv). The reaction mixture was irradiated in a microwave oven (ETHOS Synth Lab station) and reaction was carried out under microwave irradiation at 150 °C at 300 W for 0.17 h. After being cooled down, the mixture was poured into water and then extracted with EtOAc (3 × 15 mL). The organic layers were dried over anhydrous sodium sulfate and removed under vacuum. The residue was purified by chromatographic column, eluting with chloroform/petroleum ether/diethyl ether (7/2/1). Recrystallization from *i*-PrOH gave the corresponding required product.

4.2.1. 2-Methyl-4-(phenylsulfonylmethyl)thiazole (**2**)

Brown solid; mp 129 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.59 (s, 3 H), 4.52 (s, 2 H), 7.16 (s, 1 H), 7.44–7.76 (m, 5 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.9 (CH_3), 58.1 (CH_2), 119.8 (CH), 128.5 (2 CH), 128.9 (2 CH), 133.7 (CH), 138.4 (C), 142.6 (C), 166.1 (C). Anal. Calcd for $C_{11}H_{11}NO_2S_2$: C, 52.15; H, 4.38; N, 5.53. Found: C, 52.23; H, 4.48; N, 5.46.

4.2.2. 4-[(4-Chlorophenylsulfonyl)methyl]-2-methylthiazole (**3**)

White solid; mp 111 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.61 (s, 3 H), 4.53 (s, 2 H), 7.21 (s, 1 H), 7.46 (d, $J = 8.6$ Hz, 2 H), 7.66 (d, $J = 8.6$ Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 18.9 (CH_3), 58.1 (CH_2), 120.1 (CH), 129.3 (2 CH), 130.0 (2 CH), 136.8 (C), 140.6 (C), 142.5 (C), 166.3 (C). Anal. Calcd for $C_{11}H_{10}ClNO_2S_2$: C, 45.91; H, 3.50; N, 4.87. Found: C, 46.06; H, 3.51; N, 4.65.

4.3. General procedure and analytical data for compounds **4**–**20**

In a 100 mL round-bottom flask were placed 2-methyl-4-(phenylsulfonylmethyl)thiazole or 2-methyl-4-(tosylmethyl)thiazole or 4-[(4-chlorophenylsulfonyl)methyl]-2-methylthiazole (1.5 equiv), 2-bromobenzaldehyde or 2-bromobenzonitrile (1 equiv), tetrakis(triphenylphosphine) palladium (0.05 equiv), potassium acetate (3 equiv) and dimethylacetamide (5 mL). The vessel was then placed in the synthesis multimode microwave oven cavity (ETHOS Synth Lab station) and reaction was carried out under microwave irradiation at 150 °C at 300 W for an appropriate time. The disappearance of starting materials was monitored by TLC. After being cooled down, the mixture was poured into water and then extracted with EtOAc (5 × 15 mL). The organic layers were dried over anhydrous sodium sulfate and removed under vacuum. The residue was purified by chromatographic column, eluting with EtOAc/cyclohexane (5/5) for all compounds except **7** (chloroform/petroleum ether/ethyl ether (6/2/2)) and **8** (chloroform/EtOAc (9/1)). The residue was then recrystallized from *i*-PrOH to give the corresponding required product.

4.3.1. 2-Methyl-4-tosylnaphtho[2,1-d]thiazole (**4**)

Yellow crystals; mp 213 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.37 (s, 3 H), 2.91 (s, 3 H), 7.27 (d, $J = 8.2$ Hz, 2 H), 7.56–7.72 (m, 2 H), 7.89–7.93 (m, 1 H), 8.08–8.12 (m, 1 H), 8.18 (d, $J = 8.2$ Hz, 2 H), 8.74 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 21.6 (CH_3), 125.1

(CH), 126.9 (CH), 129.0 (CH), 129.1 (C), 129.2 (2 CH), 129.3 (2 CH), 129.7 (CH), 130.1 (C), 130.6 (CH), 132.7 (C), 135.4 (C), 138.1 (C), 144.1 (C), 145.4 (C), 167.1 (C). Anal. Calcd for $C_{19}H_{15}NO_2S_2$: C, 64.56; H, 4.28; N, 3.96. Found: C, 64.81; H, 4.49; N, 4.11. *m/z* (EI) 354.0617 [M + H^+]. $C_{19}H_{15}NO_2S_2$ required 354.0617.

*4.3.2. 2,8-Dimethyl-4-tosylnaphtho[2,1-d]thiazole (**5**)*

Yellow solid; mp 211 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.38 (s, 3 H), 2.58 (s, 3 H), 2.91 (s, 3 H), 7.27 (d, J = 8.2 Hz, 2 H), 7.42–7.47 (m, 1 H), 7.69 (s, 1 H), 7.99 (d, J = 8.4 Hz, 1 H), 8.17 (d, J = 8.2 Hz, 2 H), 8.69 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 21.6 (CH_3), 22.0 (CH_3), 124.4 (CH), 127.2 (C), 128.9 (CH), 129.1 (3 CH), 129.2 (2 CH), 130.4 (CH), 131.8 (C), 134.7 (C), 137.0 (C), 138.3 (C), 140.4 (C), 144.0 (C), 145.6 (C), 166.9 (C). Anal. Calcd for $C_{20}H_{17}NO_2S_2$: C, 65.37; H, 4.66; N, 3.81. Found: C, 65.36; H, 4.74; N, 3.71.

*4.3.3. 7-Fluoro-2-methyl-4-tosylnaphtho[2,1-d]thiazole (**6**)*

Brown solid; mp 247 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.39 (s, 3 H), 2.91 (s, 3 H), 7.26–7.30 (m, 2 H), 7.42–7.52 (m, 1 H), 7.74 (dd, J = 9.1, 2.5 Hz, 1 H), 7.90–7.97 (m, 1 H), 8.18 (d, J = 8.4 Hz, 2 H), 8.67 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 21.6 (CH_3), 29.7 (C), 114.2 (d, J = 21.2 Hz, CH), 119.7 (d, J = 25.2 Hz, CH), 127.0 (d, J = 1.2 Hz, C), 127.4 (d, J = 8.9 Hz, CH), 128.1 (d, J = 4.6 Hz, CH), 129.2 (2 CH), 129.4 (2 CH), 130.2 (d, J = 9.2 Hz, C), 133.9 (C), 135.7 (C), 137.8 (C), 144.3 (C), 160.9 (d, J = 247.3 Hz, C–F), 166.9 (C). Anal. Calcd for $C_{19}H_{14}FNO_2S_2$: C, 61.44; H, 3.80; N, 3.77. Found: C, 61.32; H, 3.91; N, 3.65.

*4.3.4. 7,8-Dimethoxy-2-methyl-4-tosylnaphtho[2,1-d]thiazole (**7**)*

Yellow solid; mp 282 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.38 (s, 3 H), 2.90 (s, 3 H), 4.04 (s, 3 H), 4.05 (s, 3 H), 7.10 (s, 1 H), 7.27 (d, J = 8.2 Hz, 2 H), 7.37 (s, 1 H), 8.17 (d, J = 8.2 Hz, 2 H), 8.58 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.6 (CH_3), 21.6 (CH_3), 56.1 (CH_3), 56.3 (CH_3), 104.2 (CH), 108.9 (CH), 124.5 (C), 126.1 (C), 127.5 (CH), 129.1 (4 CH), 130.5 (C), 134.1 (C), 138.5 (C), 143.9 (C), 144.7 (C), 149.9 (C), 152.2 (C), 165.9 (C). Anal. Calcd for $C_{21}H_{19}NO_4S_2$: C, 61.00; H, 4.63; N, 3.39. Found: C, 60.88; H, 4.70; N, 3.27.

*4.3.5. 2-Methyl-4-tosylnaphtho[2,1-d]thiazol-5-amine (**8**)*

Brown needles; mp 206 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.36 (s, 3 H), 2.78 (s, 3 H), 5.57 (br s, 2 H, NH_2), 7.23 (d, J = 8.4 Hz, 2 H), 7.46–7.75 (m, 3 H), 7.93–7.97 (m, 1 H), 8.10 (d, J = 8.4 Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 21.5 (CH_3), 108.6 (C), 122.5 (C), 122.9 (CH), 123.0 (C), 125.8 (CH), 125.9 (CH), 128.1 (2 CH), 128.8 (2 CH), 129.7 (CH), 130.1 (C), 140.5 (C), 143.5 (C), 145.2 (C), 146.7 (C), 165.6 (C). Anal. Calcd for $C_{19}H_{16}N_2O_2S_2$: C, 61.93; H, 4.38; N, 7.60. Found: C, 61.81; H, 4.41; N, 7.49.

*4.3.6. 7-Fluoro-2-methyl-4-tosylnaphtho[2,1-d]thiazol-5-amine (**9**)*

Brown solid; mp 260 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.37 (s, 3 H), 2.79 (s, 3 H), 4.70 (br s, 2 H, NH_2), 7.22–7.26 (m, 2 H), 7.34–7.43 (m, 1 H), 7.61 (dd, J = 10.7, 2.1 Hz, 1 H), 7.72–7.79 (m, 1 H), 8.10 (d, J = 8.2 Hz, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 21.6 (CH_3), 108.1 (d, J = 22.7 Hz, CH), 109.8 (C), 119.2 (d, J = 23.9 Hz, CH), 123.0 (C), 123; 7 (d, J = 7.5 Hz, C), 126.9 (d, J = 1.1 Hz, C), 128.1 (d, J = 8.4 Hz, CH), 128.3 (2 CH), 128.8 (2 CH), 140.3 (C), 143.7 (C), 144.3 (d, J = 4.1 Hz, C), 146.3 (C), 160.7 (d, J = 247.6 Hz, C–F), 165.4 (C). Anal. Calcd for $C_{19}H_{15}FN_2O_2S_2$: C, 59.05; H, 3.91; N, 7.25. Found: C, 59.33; H, 4.10; N, 7.14.

*4.3.7. 2-Methyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazole (**10**)*

Yellow solid; mp 284 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.91 (s, 3 H), 7.43–7.75 (m, 5 H), 7.92–7.96 (m, 1 H), 8.11–8.15 (m, 1 H), 8.30 (dd, J = 8.2, 1.8 Hz, 2 H), 8.77 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 125.1 (CH), 127.0 (CH), 128.5 (2 CH), 129.1 (C), 129.2 (2 CH), 129.3 (CH), 129.8 (CH), 130.2 (C), 130.7 (CH), 132.4 (C), 133.2 (CH), 135.5 (C), 141.1 (C), 145.4 (C), 167.2 (C). *m/z* (EI) 340.0461 [M + H^+]. $C_{18}H_{13}NO_2S_2$ required 340.0460.

*4.3.8. 2,8-Dimethyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazole (**11**)*

Yellow needles; mp 245 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.58 (s, 3 H), 2.90 (s, 3 H), 7.42–7.58 (m, 4 H), 7.69 (s, 1 H), 8.00 (d, J 8.2 Hz, 1 H), 8.29 (dd, J = 8.2, 1.7 Hz, 2 H), 8.70 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 22.0 (CH_3), 124.4 (CH), 127.1 (C), 128.4 (2 CH), 129.0 (CH), 129.1 (3 CH), 130.3 (CH), 130.4 (C), 131.3 (C), 133.1 (CH), 134.7 (C), 140.5 (C), 141.2 (C), 145.5 (C), 167.0 (C). Anal. Calcd for $C_{19}H_{15}NO_2S_2$: C, 64.95; H, 4.44; N, 3.96. Found: C, 64.95; H, 4.44; N, 3.85.

*4.3.9. 7-Fluoro-2-methyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazole (**12**)*

Yellow solid; mp 312 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.91 (s, 3 H), 7.43–7.60 (m, 4 H), 7.76 (dd, J = 9.1, 2.4 Hz, 1 H), 7.91–7.98 (m, 1 H), 8.27–8.31 (m, 2 H), 8.69 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 114.2 (d, J = 22.0 Hz, CH), 119.8 (d, J = 25.1 Hz, CH), 127.1 (d, J = 1.5 Hz, C), 127.5 (d, J = 8.9 Hz, CH), 128.2 (d, J = 4.4 Hz, CH), 128.6 (2 CH), 129.3 (2 CH), 130.3 (d, J = 9.2 Hz, C), 133.4 (CH), 133.5 (C), 135.7 (d, J = 1.1 Hz, C), 140.8 (C), 145.0 (d, J = 1.8 Hz, C), 160.9 (d, J = 247.8 Hz, C–F), 167.0 (C). *m/z* (EI) 358.0364 [M + H^+]. $C_{18}H_{12}FNO_2S_2$ required 358.0366.

*4.3.10. 7,8-Dimethoxy-2-methyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazole (**13**)*

Yellow solid; mp 310 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.89 (s, 3 H), 4.05 (s, 3 H), 4.06 (s, 3 H), 7.10 (s, 1 H), 7.38 (s, 1 H), 7.42–7.58 (m, 3 H), 8.29 (dd, J = 8.3, 1.9 Hz, 2 H), 8.60 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.6 (CH_3), 56.1 (CH_3), 56.3 (CH_3), 104.2 (CH), 108.9 (CH), 124.5 (C), 126.2 (C), 127.7 (CH), 128.5 (2 CH), 129.1 (2 CH), 130.1 (C), 133.0 (CH), 134.1 (C), 141.4 (C), 144.7 (C), 150.0 (C), 152.3 (C), 166.0 (C). Anal. Calcd for $C_{20}H_{17}NO_4S_2$: C, 60.13; H, 4.29; N, 3.51. Found: C, 60.34; H, 4.38; N, 3.42.

*4.3.11. 2-Methyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazol-5-amine (**14**)*

Yellow needles; mp 278 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.78 (s, 3 H), 4.06 (br s, 2 H, NH_2), 7.39–7.67 (m, 5 H), 7.75–7.79 (m, 1 H), 7.96–8.00 (m, 1 H), 8.19–8.23 (m, 2 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 108.3 (C), 122.5 (C), 122.9 (CH), 123.0 (C), 125.9 (CH), 126.1 (CH), 128.1 (4 CH), 129.9 (CH), 130.3 (C), 132.7 (CH), 143.4 (C), 145.4 (C), 146.8 (C), 165.6 (C). Anal. Calcd for $C_{18}H_{14}N_2O_2S_2$: C, 60.99; H, 3.98; N, 7.90. Found: C, 60.85; H, 4.00; N, 7.80.

*4.3.12. 7-Fluoro-2-methyl-4-(phenylsulfonyl)naphtho[2,1-d]thiazol-5-amine (**15**)*

Brown solid; mp 295 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.77 (s, 3 H), 7.37–7.53 (m, 4 H), 7.62 (dd, J = 10.5, 2.5 Hz, 1 H), 7.75–7.82 (m, 1 H), 8.20–8.23 (m, 2 H). NH_2 not visible under these conditions. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 108.1 (d, J = 22.7 Hz, CH), 109.4 (C), 115.5 (C), 119.4 (d, J = 24.5 Hz, CH), 123.0 (C), 127.0 (d, J = 1.5 Hz, C), 128.1 (2 CH), 128.2 (2 CH), 128.4 (CH), 132.9 (CH), 143.2 (C), 144.5 (d, J = 4.0 Hz, C), 146.3 (d, J = 1.4 Hz, C), 160.7 (d, J = 247.4 Hz, C–F), 165.5 (C). Anal. Calcd for $C_{18}H_{13}FN_2O_2S_2$: C, 58.05; H, 3.52; N, 7.52. Found: C, 58.46; H, 3.74; N, 7.33.

*4.3.13. 4-(4-Chlorophenylsulfonyl)-2-methylnaphtho[2,1-d]thiazole (**16**)*

Brown solid; mp 244 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.92 (s, 3 H), 7.41–7.47 (m, 2 H), 7.60–7.76 (m, 2 H), 7.92–7.96 (m, 1 H), 8.10–8.15 (m, 1 H), 8.21–8.26 (m, 2 H), 8.75 (s, 1 H). ^{13}C NMR

(50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 125.2 (CH), 127.1 (CH), 128.8 (2 CH), 129.1 (C), 129.3 (CH), 130.0 (CH), 130.3 (C), 130.7 (CH), 130.8 (2 CH), 131.9 (C), 135.5 (C), 139.5 (C), 139.9 (C), 145.2 (C), 167.4 (C). *m/z* (EI) 374.0074 [M + H^+]. $C_{18}H_{12}ClNO_2S_2$ required 374.0071.

4.3.14. 4-(4-Chlorophenylsulfonyl)-7-fluoro-2-methylnaphtho[2,1-d]thiazole (**17**)

Yellow solid; mp 307 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.91 (s, 3 H), 7.44–7.54 (m, 3 H), 7.76 (dd, *J* = 9.1, 2.3 Hz, 1 H), 7.91–7.99 (m, 1 H), 8.24 (d, *J* = 8.6 Hz, 2 H), 8.68 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.5 (CH_3), 114.3 (d, *J* = 21.3 Hz, CH), 120.0 (d, *J* = 25.3 Hz, CH), 127.1 (d, *J* = 1.1 Hz, CH), 127.5 (d, *J* = 8.8 Hz, CH), 128.3 (d, *J* = 4.7 Hz, C), 128.9 (2 CH), 130.2 (d, *J* = 9.6 Hz, C), 130.9 (2 CH), 133.1 (C), 135.8 (C), 139.7 (d, *J* = 45.7 Hz, C), 144.8 (d, *J* = 1.8 Hz, C), 161.0 (d, *J* = 249.6 Hz, C–F), 167.3 (C), 171.3 (C). Anal. Calcd for $C_{18}H_{11}ClFNO_2S_2$: C, 55.17; H, 2.83; N, 3.57. Found: C, 55.42; H, 3.10; N, 3.39.

4.3.15. 4-(4-Chlorophenylsulfonyl)-7,8-dimethoxy-2-methylnaphtho[2,1-d]thiazole (**18**)

Yellow solid; mp 281 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.90 (s, 3 H), 4.05 (s, 3 H), 4.06 (s, 3 H), 7.11 (s, 1 H), 7.38 (s, 1H), 7.44 (d, *J* = 8.6 Hz, 2 H), 8.22 (d, *J* = 8.6 Hz, 2 H), 8.59 (s, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.6 (CH_3), 56.2 (CH_3), 56.3 (CH_3), 104.2 (CH), 108.9 (CH), 124.4 (C), 126.4 (C), 127.7 (CH), 128.8 (2 CH), 129.7 (C), 130.7 (2 CH), 134.2 (C), 139.7 (C), 139.9 (C), 144.5 (C), 150.1 (C), 152.5 (C), 166.2 (C). *m/z* (EI) 434.0281 [M + H^+]. $C_{20}H_{16}ClNO_4S_2$ required 434.0282.

4.3.16. 4-(4-Chlorophenylsulfonyl)-2-methylnaphtho[2,1-d]thiazol-5-amine (**19**)

Brown solid; mp 194 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.78 (s, 3 H), 7.41 (d, *J* = 8.5 Hz, 2 H), 7.47–7.68 (m, 2 H), 7.76–7.80 (m, 1 H), 7.95–7.99 (m, 1 H), 8.16 (d, *J* = 8.5 Hz, 2 H). NH_2 not visible under these conditions. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 107.5 (C), 122.4 (C), 123.0 (CH), 126.0 (CH), 126.2 (CH), 128.4 (2 CH), 128.9 (C), 129.7 (2 CH), 130.1 (CH), 133.0 (C), 139.3 (C), 141.8 (C), 145.6 (C), 146.0 (C), 166.2 (C). Anal. Calcd for $C_{18}H_{13}ClN_2O_2S_2$: C, 55.59; H, 3.37; N, 7.20. Found: C, 55.80; H, 3.49; N, 7.02.

4.3.17. 4-(4-Chlorophenylsulfonyl)-7-fluoro-2-methylnaphtho[2,1-d]thiazol-5-amine (**20**)

Brown solid; mp 253 °C (*i*-PrOH) ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.77 (s, 3 H), 7.35–7.46 (m, 3 H), 7.61(dd, *J* = 10.6, 2.2 Hz, 1 H), 7.73–7.80 (m, 1 H), 8.15 (d, *J* = 8.7 Hz, 2 H). NH_2 not visible under these conditions. ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.2 (CH_3), 108.2 (d, *J* = 23.0 Hz, CH), 108.8 (C), 119.5 (d, *J* = 24.2 Hz, CH), 123.0 (d, *J* = 1.1 Hz, C), 123.6 (d, *J* = 7.7 Hz, C), 127.0 (d, *J* = 1.9 Hz, C), 128.2 (d, *J* = 8.4 Hz, CH), 128.4 (2 CH), 129.8 (2 CH), 139.4 (C), 141.6 (C), 144.6 (d, *J* = 4.4 Hz, C), 146.0 (d, *J* = 1.9 Hz, C), 160.7 (d, *J* = 247.4 Hz, C), 165.7 (C). *m/z* (EI) 407.0088 [M + H^+]. $C_{18}H_{12}ClFN_2O_2S_2$ required 407.0086.

4.4. X-ray structure determination of compounds **4** and **8**

4.4.1. Crystal data for compound **4**

$C_{19}H_{15}NO_2S_2$, yellow prism (0.2 × 0.08 × 0.06 mm^3), MW = 353.44, monoclinic, space group *P*21/n (*T* = 293 K), *a* = 7.3669 (2) Å, *b* = 14.4698 (4) Å, *c* = 15.5892 (6) Å, α = 90°, β = 91.674 (1)°, γ = 90°; *V* = 1661.06 (9) $Å^3$, *Z* = 4, μ = 0.331 mm^{-1}, *F*(000) = 736, index ranges $0 \le h \le 9$, $0 \le k \le 19$, $-20 \le l \le 20$; θ range = 3.1–28.18°, 219 variables and 0 restraints, were defined for 2315 independent reflections with $I \ge 2\sigma(I)$ to *R1* = 0.0698, *wR2* = 0.1238, *GooF* = 1.117.

4.4.2. Crystal data for compound **8**

$C_{19}H_{16}N_2O_2S_2$, yellow prism (0.25 × 0.25 × 0.08), MW = 368.46, monoclinic, space group *P*21/c (*T* = 293 K), *a* = 11.4979 (2) Å, *b* = 7.7289 (1) Å, *c* = 19.6273 (6) Å, α = 90°, β = 91.674 (1)°, γ = 90°; *V* = 1661.06 (4) $Å^3$, *Z* = 4, μ = 0.327, *F*(000) = 768, index ranges $0 \le h \le 15$, $0 \le k \le 9$, $-26 \le l \le 25$; θ range = 1.81–28.63°, 228 variables and 0 restraints, were defined for 3287 independent reflections with $I \ge 2\sigma(I)$ to *R1* = 0.0607, *wR2* = 0.1578, *GooF* = 1.237.

CCDC 826725 (for compound **4**) and CCDC 826726 (for compound **8**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.cdcc.cam.ac.uk/data_request/cif of from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: + 44 (1223) 336033; email: deposit@ccdc.cam.ac.uk.

4.5. MTT assays

The tested compounds' cytotoxicity on the HepG2 cell line (purchased from ATCC, ref HB-8065) was evaluated according to the method of Mosmann with slight modifications. Briefly, cells 5×10^3 in 100 μL of complete medium [RPMI supplemented with 10% fœtal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 μg/mL)] were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO_2, 14% O_2, 80% N_2 atmosphere. After 24 h incubation, 100 μL of medium with various product concentrations was added and the plates were incubated for 72 h. Duplicate assays were performed for each sample. At the end of the treatment and incubation, the medium was aspirated from the wells and 10 μL MTT solution (5 mg MTT/mL in PBS) was added to each well with 100 μL of medium without fœtal bovine serum. Cells were incubated for 2 h at 37 °C to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. After this time, the MTT solution was aspirated and DMSO (100 μL) was added to each well to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for a few minutes. The absorbance was measured at 570 nm with 630 nm as reference wavelength, using a microplate spectrophotometer. DMSO was used as blank and doxorubicin as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration (CC_{50}) was determined by non-linear regression analysis processed on dose–reponse curves, using the Table Curve software 2D v.5.0. CC_{50} values represent the mean value calculated from three independent experiments.

4.6. In vitro evaluation of antiplasmodial activity

The evaluation of the less cytotoxic compounds' antiplasmodial activity was conducted on a K1 *in vitro* culture-adapted *P. falciparum* strain (clone of W2), resistant to chloroquine, pyrimethamine and proguanil. Cultures were maintained in fresh A+ human erythrocytes at 2.5% hematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM $NaHCO_3$, 10% of A+ human serum) at 37 °C under reduced O_2 atmosphere (gas mixture 6% CO_2, 14% O_2, 80% N_2). Parasitemia was maintained daily between 1% and 6%. The *P. falciparum* drug susceptibility test was carried out by comparing quantities of DNA in treated and control cultures of parasite in human erythrocytes according to a SYBR Green I fluorescence-based method using a 96-well fluorescence plate reader. Parasite culture was synchronized at ring stage with 5% sorbitol. Compounds were incubated in a total assay volume of 200 μL (RPMI, 2% haematocrit and 1% parasitaemia) for 72 h in a humidified atmosphere (6% CO_2, 14% O_2, 80% N_2) at 37 °C, in 96-well flat-bottom plates. Duplicate assays were performed for each sample.

After incubation, 125 μL supernatant was discarded and cells were washed twice with 150 μL 1X PBS. 15 μL re-suspended cells were transferred to 96-well flat bottom non- sterile black plates (Greiner Bio-one). 15 μL of the SYBR Green lysis buffer (2XSYBR Green, 20 mM Tris base pH 7.5, 20 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100) was added to each well. Negative control (treated by DMSO) and positive controls (doxycycline and chloroquine) were added to each set of experiments. Plates were incubated for 0.25 h at 37 °C and then read on a TECAN Infinite F-200 spectrophotometer with excitation and emission wavelengths at 497 and 520 nm respectively. The concentrations of compounds required to induce a 50% decrease in parasite growth (K1 IC_{50}) were calculated from three independent experiments.

4.7. Selectivity index (SI)

The selectivity indexes presented correspond to the ratios between, respectively, the toxicity on HepG2 human cell line and the K1 antiplasmodial activity. They are calculated as follows: SI K1 = CC_{50} (HepG2)/IC_{50} (K1).

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