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4-Thiophenoxy-2-trichloromethyquinazolines display in vitro selective antiplasmodial activity against the human malaria parasite *Plasmodium falciparum*

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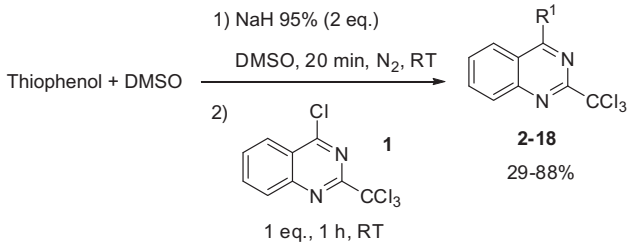
ABSTRACT

A series of original quinazolines bearing a 4-thiophenoxy and a 2-trichloromethyl group was synthesized in a convenient and efficient way and was evaluated toward its in vitro antiplasmodial potential. The series revealed global good activity against the K1-multi-resistant *Plasmodium falciparum* strain, especially with hit compound **5** (IC_{50} = 0.9 μM), in comparison with chloroquine and doxycycline chosen as reference-drugs. Both the in vitro cytotoxicity study which was conducted on the human HepG2 cell line and the in vitro antitoxoplasmic screening against *Toxoplasma gondii* indicate that this series presents an interesting selective antiplasmodial profile. Structure–activity- and toxicity relationships highlight that the trichloromethyl group plays a key role in the antiplasmodial activity and also show that the modulation of the thiophenol moiety influences the toxicity/activity ratio.

Malaria is an infectious erythrocytopathy caused by protozoan parasites belonging to the *Plasmodium* genus. In 2009, according to the WHO, malaria affected about 250 million people worldwide and was responsible for the death of almost 900.000 people, mainly African children aged less than 5 years.[1] *Plasmodium falciparum* is the causative agent of the most severe type of malaria: cerebral malaria. Moreover, many *P. falciparum* strains have developed increasing resistance to most of marketed antimalarial drugs leading to a major public health issue. Thus, the Roll Back Malaria consortium calls for the development of a robust pipeline of new antiplasmodial molecules displaying original mechanisms of action.[2]

In continuation of our research program centred on the design and synthesis of original molecules with anti-infectious properties,[3–5] we focused on the preparation of new antiplasmodial compounds in quinazoline series. Effectively, various original 4-substituted-quinazoline derivatives have recently demonstrated interesting potential as antiplasmodial agents.[6–11] Thus, after identifying that some 4-aryl-,[6] 4-anilino-,[7] and 4-phenoxy-2-trichloromethylquinazolines[8] display good in vitro antiplasmodial activity, we decided to develop new close structural analogues bearing a thiophenoxy moiety at position 4, for the sake of improving the antiplasmodial potency and decreasing the toxicity of the 2-trichloromethylquinazoline scaffold.

The 4-thiophenoxy-2-trichloromethylquinazoline series was obtained from 4-chloro-2-trichloromethyl-quinazoline **1** which we already prepared previously by using an efficient microwave assisted method.[12] Thiophenolates, obtained by reaction of thiophenols with sodium hydride in DMSO, were reacted with



Scheme 1. Simple preparation of compounds **2–18** by a S_NAr reaction.

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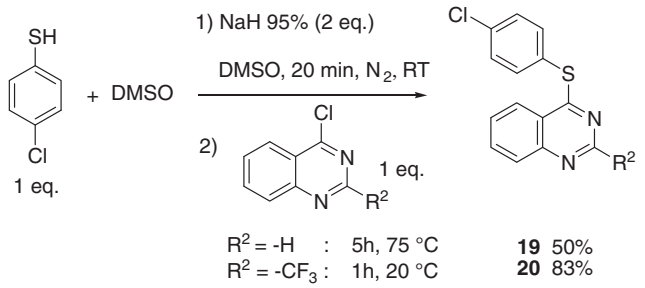


Scheme 2. Preparation of compounds **19** and **20**.

substrate **1** in DMSO, under nitrogen atmosphere at RT, to afford the corresponding target compounds **2–18** (Scheme 1).[13]

Nontrichloromethylated compounds **19**[14] and **20**, which were synthesized for structure–activity relationships study purposes, were prepared by a similar S_NAr reaction approach, with slight modifications, from commercial 4-chloro- or 4-chloro-2-trifluoromethyl-quinazoline, as described in Scheme 2.

Compounds **1–20** were then assessed for their in vitro antiplasmodial activity against the K1-multi-resistant *P. falciparum* strain by using the SYBR Green I fluorescence-based method.[15] Their inhibitory concentrations 50% (IC_{50}) were then determined and compared with those of chloroquine and doxycycline, chosen as reference-drugs (Table 1). In parallel, their in vitro cytotoxicity (CC_{50}) toward the human HepG2 cell line was evaluated,[16] in order to calculate, for each molecule, the corresponding selectivity index (SI).

As already noted on the *W2 P. falciparum* strain,[6] despite the presence of a trichloromethyl group at position 2, compound **1** did not present any antiplasmodial activity (IC_{50} = 55 μM) while 4-thiophenoxy-2-trichloromethylquinazoline **2** exerts a good activity (IC_{50} = 1.9 μM). The antiplasmodial activity was significantly improved by introducing an electro-attracting group such as a fluorine, chlorine or trifluoromethyl group on the thiophenoxy moiety. More precisely, best activities are achieved with molecules bearing electron-withdrawing groups at the *para* position of the thiophenoxy moiety, which clearly appears by comparing *para*-chloro-substituted hit compound **5** (IC_{50} = 0.9 μM) with less active *ortho*-methyl-substituted compound **12** (IC_{50} = 7 μM). Contrary to the results observed previously in 4-anilino-2-trichloromethylquinazoline series,[7] there was no improvement of activity with compound **18**, bearing two chlorine substituents at positions *ortho* and *para*, indicating that the 4-thiophenoxyquinazoline chemical scaffold may differ from the 4-anilino one as regards of biological profile.

Concerning cytotoxicity, the whole series was rather nontoxic ($12 < CC_{50} < 52$ μM) on the metabolizing HepG2 cell line, in comparison with both doxorubicin toxicity positive control and commercial drug-compounds chloroquine and doxycycline (Table 1). Thus, selectivity indexes of the series range from 7.3 to >28, in between the ones of commercial doxycycline and chloroquine and are close to those obtained in the previously tested 2-trichloromethylquinazoline series. Because of the presence of a trichloromethyl group in the structure of hit compound **5**, its toxicological study was completed by performing Ames test.[17] As presented in Table 2, molecule **5** did not show any mutagenic activity at the tested concentrations.

In order to demonstrate the key-role of the trichloromethyl group in the activity of hit compound **5**, this group was substituted by a hydrogen atom (molecules **19**) or a trifluoromethyl group (molecule **20**). As shown in Table 1, the absence of the trichloromethyl group leads to a 5- to >17-fold reduction of antiplasmodial activity. Such an observation, added to the lack of activity of trichloromethylated compound **1**, indicates that the presence of a CCl_3 group at position 2 of the quinazoline ring is necessary for providing antiplasmodial activity but that this group does not confer activity alone to the quinazoline scaffold when this last does not bear an appropriate substituent at position 4, such as a thiophenoxy group.

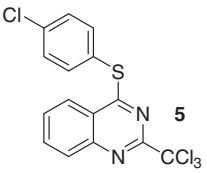
Finally, hit molecule **5** was tested on an other apicomplexan parasite, *Toxoplasma gondii*, in order to assess whether or not such molecule displays a selective action toward the *Plasmodium* genus.[18] As shown in Table 2, in comparison with pyrimethamine used as an antitoxoplasmic drug of reference, compound **5** does not affect the in vitro development of the *Toxoplasma* protozoa (IC_{50} > 31 μM). Then, from this preliminary study, in vitro hit

Table 1
Antiplasmodial activity and human cell toxicity in 4-thiophenoxyquinazoline series

Entry	R^1	R^2	Activity against K1 *Plasmodium falciparum* IC_{50} (μM)[a]	Cytotoxicity HepG2 CC_{50} (μM)[a]	Selectivity index[b] (SI)
1	Cl–	$-CCl_3$	55.0	>125 [e]	>2.3
2	Ph-S–	$-CCl_3$	1.9	35	18.4
3	2-Cl-Ph-S–	$-CCl_3$	2.8	26	9.3
4	3-Cl-Ph-S–	$-CCl_3$	2.2	16	7.3
5	4-Cl-Ph-S–	**$-CCl_3$**	**0.9**	**>25**[e]	**>28**
6	2-F-Ph-S–	$-CCl_3$	2.3	28	12.2
7	3-F-Ph-S–	$-CCl_3$	1.9	15.5	8.2
8	4-F-Ph-S–	$-CCl_3$	1.2	>30[e]	>25
9	2-CF_3-Ph-S–	$-CCl_3$	2.5	28	11.2
10	3-CF_3-Ph-S–	$-CCl_3$	2.2	17.5	8
11	4-CF_3-Ph-S–	$-CCl_3$	1.0	13	13
12	2-CH_3-Ph-S–	$-CCl_3$	7.0	52	7.4
13	3-CH_3-Ph-S–	$-CCl_3$	1.9	35	18.4
14	4-CH_3-Ph-S–	$-CCl_3$	1.6	>30[e]	>19
15	2-OCH_3-Ph-S–	$-CCl_3$	3.9	32	8.2
16	3-OCH_3-Ph-S–	$-CCl_3$	2.0	12	6
17	4-OCH_3-Ph-S–	$-CCl_3$	2.5	>8[e]	>3.2
18	2,4-di-Cl-Ph-S–	$-CCl_3$	1.5	18.2	12.1
19	4-Cl-Ph-S–	–H	4.4	16.7	3.8
20	4-Cl-Ph-S–	$-CF_3$	>15[e]	>15[e]	ND
Ref.	Chloroquine[c]		0.5	30	60
Ref.	Doxycycline[c]		5.0	20	4
Ref.	Doxorubicin[d]		–	0.2	–

[a] Mean of three independent experiments.
[b] Selectivity index was calculated according to the following formula: $SI_{W2Plasmodium}$ = HepG2 Human cell lineCC_{50}/K1 *Plasmodium falciparum* IC_{50}.
[c] Chloroquine and doxycycline were used as antiplasmodial drug-compounds of reference.
[d] Doxorubicin was used as a drug-compound of reference for human cell toxicity.
[e] Molecule could not be tested at higher concentration because of lack of solubility in the culture medium.

Table 2
Complementary investigations about hit compound **5**

Entry	Activity against *Toxoplasma gondii* IC_{50} (μM)[a]	Ames test[b]
 **5**	>31[d]	Negative (±S9mix)
Pyrimethamine[c]	0.8	–

[a] Mean of three independent experiments.
[b] A product is considered mutagenic when it induces, at least, a twofold increase of the number of revertants in comparison with negative control.
[c] Pyrimethamine was used as an antitoxoplasmic reference-drug.
[d] Molecule **5** could not be tested at higher concentrations because of insufficient solubility.

quinazoline **5** appears to be a new promising candidate for contributing to the identification and development of original antimalarial pharmacophores.

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General procedure for the preparation of compounds **2–18**: 1 equiv of the appropriate thiophenol was dissolved in DMSO, then added onto 2 equiv of NaH 95%, and stirred under N_2 for 20 min. 1 equiv of 4-chloro-2-trichloromethylquinazoline was dissolved in DMSO, then added to the mixture, and stirred at room temperature for 1 h. The reaction medium was then extracted three times by dichloromethane. The organic layer was washed with water five times, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography on silica gel with appropriate solvent to afford the corresponding nucleophilic aromatic substitution product. Compound **2** was obtained, after purification by column chromatography (eluent: cyclohexane-ethyl acetate 8:2) as a white solid in 65% yield; mp 123 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.44–7.50 (m, 3H), 7.64–7.77 (m, 3H), 7.91–7.99 (m, 1H), 8.09–8.13 (m, 1H), 8.23–8.27 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 97.0 (C), 121.9 (C), 123.7 (CH), 126.4 (C), 129.0 (CH), 129.1 (CH × 2), 129.7 (CH), 129.8 (C), 134.6 (CH), 135.7 (CH × 2), 147.9 (C), 159.7 (C), 173.2 (C). Anal. Calcd for $C_{15}H_9Cl_3N_2S$: C, 50.65; H, 2.55; N, 7.88. Found: C, 50.59; H, 2.49; N, 7.69. Compound **3** was obtained, after purification by column chromatography (eluent: dichloromethane-petroleum ether 1:1) as a white solid in 75% yield; mp 135 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.31–7.49 (m, 2H), 7.57–7.61 (m, 1H), 7.69–7.77 (m, 2H), 7.91–7.99 (m, 1H), 8.12 (dd, J = 0.5 and 8.5 Hz, 1H), 8.25 (dd, J = 0.5 and 8.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.8 (C), 121.9 (C), 123.7 (CH), 126.0 (C), 127.3 (CH), 129.0 (CH), 129.7 (CH), 130.2 (CH), 131.5 (CH), 134.7 (CH), 138.0 (CH), 140.1 (C), 147.9 (C), 159.5 (C), 171.8 (C). Anal. Calcd for $C_{15}H_8Cl_4N_2S$: C, 46.18; H, 2.07; N, 7.18. Found: C, 46.46; H, 2.05; N, 7.10. Compound **4** was obtained, after purification by column chromatography (eluent: dichloromethane-petroleum ether 1:1) as a white solid in 70% yield; mp 147 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.36–7.48 (m, 2H), 7.56 (dd, J = 1.4 and 7.0 Hz, 1H), 7.71–7.78 (m, 2H), 7.93–8.01 (m, 1H), 8.11–8.24 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.9 (C), 121.9 (C), 123.6 (CH), 128.3 (C), 129.2 (CH), 129.8 (CH), 129.9 (CH), 130.0 (CH), 133.5 (CH), 134.6 (C), 134.9 (CH), 135.6 (CH), 148.0 (C), 159.7 (C), 172.3 (C). Anal. Calcd for $C_{15}H_8Cl_4N_2S$: C, 46.18; H, 2.07; N, 7.18. Found: C, 46.52; H, 2.05; N, 7.24. Compound **5** was obtained, after purification by column chromatography (eluent: dichloromethane-petroleum ether 1:1) as a white solid in 68% yield; mp 187 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.42–7.46 (m, 2H), 7.58–7.62 (m, 2H), 7.70–7.78 (m, 1H), 7.92–8.01 (m, 1H), 8.12 (d, J = 8.5 Hz, 1H), 8.22 (d, J = 7.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.9 (C), 121.9 (C), 123.6 (CH), 124.9 (C), 129.2 (CH), 129.3 (CH × 2), 129.8 (CH), 134.8 (CH), 136.2 (C), 136.9 (CH × 2), 147.9 (C), 159.6 (C), 172.6 (C). Anal. Calcd for $C_{15}H_8Cl_4N_2S$: C, 46.18; H, 2.07; N, 7.18. Found: C, 46.58; H, 2.13; N, 7.15. Compound **6** was obtained, after purification by column chromatography (eluent: petroleum ether-ethyl acetate 9:1) as a white solid in 48% yield; mp 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.20–7.29 (m, 2H), 7.48–7.68 (m, 2H), 7.71–7.79 (m, 1H), 7.93–8.01 (m, 1H), 8.11–8.15 (m, 1H), 8.24–8.28 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.8 (C), 113.9 (C, d, J = 18.3 Hz), 116.2 (CH, d, J = 22.7 Hz), 122.0 (C), 123.8 (CH), 124.7 (CH, d, J = 3.6 Hz), 129.1 (CH), 129.8 (CH), 132.6 (CH, d, J = 8.4 Hz), 134.7 (CH), 137.4 (CH), 148.0 (C), 159.6 (C), 163.3 (C, d, J = 250.7 Hz), 171.6 (C). Anal. Calcd for $C_{15}H_8Cl_3FN_2S$: C, 48.22; H, 2.16; N, 7.50. Found: C, 48.35; H, 2.12; N, 7.48. Compound **7** was obtained, after purification by column chromatography (eluent: petroleum ether-ethyl acetate 9:1) as a white solid in 64% yield; mp 130 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.16–7.25 (m, 1H), 7.42–7.47 (m, 3H), 7.71–7.79 (m, 1H), 7.93–8.02 (m, 1H), 8.11–8.15 (m, 1H), 8.20–8.25 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.8 (C), 116.9 (CH, d, J = 20.8 Hz), 121.9 (C), 122.7 (CH, d, J = 23.1 Hz), 123.6 (CH), 128.3 (C, d, J = 8.4 Hz), 129.2 (CH), 129.8 (CH), 130.2 (CH, d, J = 8.1 Hz), 131.2 (CH, d, J = 3.3 Hz), 134.8 (CH), 148.0 (C), 159.7 (C), 162.6 (C, d, J = 248.8 Hz), 172.4 (C). Anal. Calcd for $C_{15}H_8Cl_3FN_2S$: C, 48.22; H, 2.16; N, 7.50. Found: C, 48.60; H, 2.22; N, 7.37. Compound **8** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a pale yellow solid in 88% yield; mp 155 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.12–7.24 (m, 2H), 7.59–7.78 (m, 3H), 7.93–8.01 (m, 1H), 8.10–8.15 (m, 1H), 8.21–8.26 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.9 (C), 116.4 (CH × 2, d, J = 22.0 Hz), 121.6 (C, d, J = 3.7 Hz), 121.9 (C), 123.6 (CH), 129.1 (CH), 129.8 (CH), 134.7 (CH), 138.0 (CH × 2, d, J = 8.8 Hz), 147.9 (C), 159.7 (C), 163.8 (C, d, J = 250.0 Hz), 173.1 (C). Anal. Calcd for $C_{15}H_8Cl_3FN_2S$: C, 48.22; H, 2.16; N, 7.50. Found: C, 48.34; H, 2.25; N, 7.23. Compound **9** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 31% yield; mp 152 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.62–7.67 (m, 2H), 7.73–7.90 (m, 3H), 7.93–8.02 (m, 1H), 8.11–8.15 (m, 1H), 8.25–8.29 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.7 (C), 121.8 (C), 123.8 (CH), 125.3 (C), 127.3 (CH, q, J = 5.0 Hz), 129.2 (CH), 129.7 (C, q, J = 273.0 Hz), 129.8 (CH), 130.3 (CH), 132.2 (CH), 134.4 (C, q, J = 30.0 Hz), 134.8 (CH), 140.4 (CH), 147.9 (C), 159.5 (C), 172.8 (C). Anal. Calcd for $C_{16}H_8Cl_3F_3N_2S$: C, 45.36; H, 1.90; N, 6.61. Found: C, 45.31; H, 2.12; N, 6.65. Compound **10** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 40% yield; mp 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.57–7.65 (m, 1H), 7.73–7.88 (m, 3H), 7.95–8.03 (m, 2H), 8.12–8.17 (m, 1H), 8.22–8.26 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.7 (C), 121.9 (C), 123.5 (CH), 126.5 (CH, q, J = 4.0 Hz), 127.9 (C), 129.1 (C, q, J = 272.0 Hz), 129.3 (CH), 129.5 (CH), 129.9 (CH), 131.6 (C, q, J = 33.0 Hz), 132.8 (CH, q, J = 4.0 Hz), 134.9 (CH), 138.7 (CH), 148.0 (C), 159.6 (C), 172.0 (C). Anal. Calcd for $C_{16}H_8Cl_3F_3N_2S$: C, 45.36; H, 1.90; N, 6.61. Found: C, 45.52; H, 1.89; N, 6.66. Compound **11** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 79% yield; mp 131 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.71–7.86 (m, 5H), 7.95–8.03 (m, 1H), 8.13–8.17 (m, 1H), 8.22–8.27 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.8 (C), 121.9 (C), 123.6 (CH), 123.9 (C, q, J = 272.0 Hz), 125.8 (CH × 2, q, J = 3.6 Hz), 129.3 (CH), 129.9 (CH), 131.4 (C), 131.7 (C, q, J = 33.0 Hz), 134.9 (CH), 135.8 (CH × 2), 148.0 (C), 159.6 (C), 171.9 (C). Anal. Calcd for $C_{16}H_8Cl_3F_3N_2S$: C, 45.36; H, 1.90; N, 6.61. Found: C, 45.48; H, 1.96; N, 6.75. Compound **12** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 69% yield; mp 140 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ:2.42 (s, 3H), 7.22–7.30 (m, 1H), 7.39–7.43 (m, 2H), 7.56–7.61 (m, 1H), 7.70–7.78 (m, 1H), 7.91–8.00 (m, 1H), 8.09–8.14 (m, 1H), 8.27–8.31 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 20.7 (CH_3), 96.9 (C), 122.1 (C), 123.9 (CH), 126.0 (C), 126.6 (CH), 128.9 (CH), 129.7 (CH), 130.5 (CH), 130.7 (CH), 134.5 (CH), 136.6 (CH), 144.0 (C), 147.9 (C), 159.7 (C), 173.1 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2S$: C, 51.98; H, 3.00; N, 7.58. Found: C, 51.54; H, 2.97; N, 7.45. Compound **13** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a pale yellow solid in 31% yield; mp 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.40 (s, 3H), 7.30–7.53 (m, 4H), 7.70–7.77 (m, 1H), 7.92–8.00 (m, 1H), 8.10–8.14 (m, 1H), 8.24–8.28 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.3 (CH_3), 97.0 (C), 122.0 (C), 123.7 (CH), 126.1 (C), 128.9 (CH), 129.0 (CH), 129.7 (CH), 130.5 (CH), 132.3 (CH), 134.6 (CH), 136.3 (CH), 138.9 (C), 147.9 (C), 159.7 (C), 173.3 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2S$: C, 51.98; H, 3.00; N, 7.58. Found: C, 51.46; H, 2.96; N, 7.43. Compound **14** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 30% yield; mp 146 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.43 (s, 3H), 7.16–7.36 (m, 2H), 7.52–7.57 (m, 2H), 7.73–7.76 (m, 1H), 7.91–7.99 (m, 1H), 8.09–8.14 (m, 1H), 8.23–8.28 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 21.4 (CH_3), 97.0 (C), 122.0 (C), 122.9 (C), 123.7 (CH), 128.9 (CH), 129.7 (CH), 129.9 (CH × 2), 134.6 (CH), 135.5 (CH × 2), 139.9 (C), 147.9 (C), 159.7 (C), 173.5 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2S$: C, 51.98; H, 3.00; N, 7.58. Found: C, 51.78; H, 2.98; N, 7.52. Compound **15** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 53% yield; mp 133 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.78 (s, 3H), 7.00–7.08 (m, 2H), 7.46–7.62 (m, 2H), 7.68–7.76 (m, 1H), 7.90–7.98 (m, 1H), 8.08–8.12 (m, 1H), 8.28–8.32 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.8 (OCH_3), 97.1 (C), 111.3 (CH), 114.4 (C), 121.0 (CH), 122.2 (C), 124.1 (CH), 128.8 (CH), 129.6 (CH), 132.2 (CH), 134.4

(CH), 137.3 (CH), 148.0 (C), 159.7 (C), 160.4 (C), 172.8 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2OS$: C, 49.82; H, 2.87; N, 7.26. Found: C, 49.90; H, 2.95; N, 7.14. Compound **16** was obtained, after purification by column chromatography (eluent: petroleum ether-dichloromethane 1:1) as a white solid in 29% yield; mp 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.84 (s, 3H), 7.01–7.06 (m, 1H), 7.24–7.27 (m, 2H), 7.34–7.42 (m, 1H), 7.69–7.77 (m, 1H), 7.92–8.00 (m, 1H), 8.10–8.14 (m, 1H), 8.23–8.27 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.5 (OCH_3), 97.0 (C), 116.4 (CH), 120.5 (CH), 122.0 (C), 123.7 (CH), 127.4 (C), 127.6 (CH), 129.0 (CH), 129.7 (CH), 129.8 (CH), 134.7 (CH), 147.9 (C), 159.7 (C), 159.9 (C), 173.1 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2OS$: C, 49.82; H, 2.87; N, 7.26. Found: C, 50.09; H, 2.99; N, 7.06. Compound **17** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a white solid in 69% yield; mp 182 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.87 (s, 3H), 6.97–7.02 (m, 2H), 7.54–7.58 (m, 2H), 7.68–7.75 (m, 1H), 7.90–7.98 (m, 1H), 8.08–8.13 (m, 1H), 8.23–8.27 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 55.4 (OCH_3), 97.0 (C), 114.8 (CH × 2), 116.8 (C), 121.9 (C), 123.7 (CH), 128.9 (CH), 129.7 (CH), 134.6 (CH), 137.3 (CH × 2), 147.8 (C), 159.7 (C), 161.0 (C), 173.9 (C). Anal. Calcd for $C_{16}H_{11}Cl_3N_2OS$: C, 49.82; H, 2.87; N, 7.26. Found: C, 49.84; H, 2.82; N, 6.91. Compound **18** was obtained, after purification by column chromatography (eluent: petroleum ether–ethyl acetate 9:1) as a beige solid in 35% yield; mp 117 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.33–7.39 (m, 1H), 7.61–7.69 (m, 2H), 7.72–7.80 (m, 1H), 7.94–8.02 (m, 1H), 8.12–8.16 (m, 1H), 8.22–8.26 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 96.7 (C), 121.9 (C), 123.6 (CH), 124.7 (C), 127.7 (CH), 129.2 (CH), 129.8 (CH), 130.2 (CH), 134.8 (CH), 137.1 (C), 138.6 (CH), 141.0 (C), 148.0 (C), 159.5 (C), 171.3 (C). Anal. Calcd for: $C_{15}H_7Cl_5N_2S$: C, 42.43; H, 1.66; N, 6.60. Found: C, 42.70; H, 1.75; N, 6.37. Compound **19** was described previously:[13] 1 equiv of 4-chlorothiophenol was dissolved in dimethylsulfoxyde (DMSO), then added onto 2 equiv of NaH 95%, and stirred under N_2 for 20 minutes 1 equiv of 4-chloroquinazoline was dissolved in DMSO, then added to the mixture, and stirred at 75 °C for 5 h. The mixture was poured into an ice-water solution and the white solid which precipitated was collected by filtration and then purified by column chromatography (silica gel, eluent: petroleum ether–ethyl acetate 8:2) in 50% yield; mp 139 °C, Lit:[13] 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.45–7.59 (m, 4H), 7.63–7.72 (m, 1H), 7.88–7.96 (m, 1H), 8.03–8.07 (m, 1H), 8.19–8.23 (m, 1H), 8.87 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 123.1 (C), 123.7 (CH), 125.3 (C), 127.9 (CH), 128.4 (CH), 129.8 (CH × 2), 134.4 (CH), 136.4 (C), 137.0 (CH × 2), 147.6 (C), 153.3 (CH), 171.7 (C). Anal. Calcd for: $C_{14}H_9ClN_2S$: C, 61.65; H, 3.33; N, 10.27. Found: C, 61.68; H, 3.31; N, 10.14. Preparation of the compound **20**: 1 equiv of 4-chlorothiophenol was dissolved in DMSO, then added onto 2 equiv of NaH 95%, and stirred under N_2 for 20 min. 1 equiv of 4-chloro-2-trifluoromethylquinazoline was dissolved in DMSO, then added to the mixture, and stirred at 20 °C for 1 h. The mixture was poured into an ice-water solution and the beige solid which precipitated was collected by filtration and then purified by column chromatography (silica gel, eluent: petroleum ether-dichloromethane 1:1) in 83% yield; mp 196 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.43–7.89 (m, 4H), 7.71–7.79 (m, 1H), 7.93–8.01 (m, 1H), 8.09–8.13 (m, 1H), 8.21–8.25 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 119.5 (C, q, J = 276.0 Hz), 123.1 (C), 123.7 (CH), 124.5 (C), 129.5 (CH × 3), 129.6 (CH), 135.0 (CH), 136.4 (C), 136.7 (CH × 2), 147.9 (C), 151.3 (C, q, J = 37.0 Hz), 173.0 (C). Anal. Calcd for $C_{15}H_8ClF_3N_2S$: C, 52.87; H, 2.37; N, 8.22. Found: C, 53.13; H, 2.45; N, 8.17.

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15. In this study, a K1 culture-adapted *P. falciparum* strain (clone of *W2*), resistant to chloroquine, pyrimethamine, and proguanil was used in an in vitro culture. Cultures were maintained in fresh A+ human erythrocytes at 2.5% haematocrit 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 5% O_2, 5% CO_2, and 90% N_2). Parasitaemia 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 synchronised 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 (5% O_2 and 5% CO_2) at 37 °C, in 96-well flat bottom plates. Triplicate assays were performed for each sample. After incubation, 170 μL supernatant was discarded and cells were washed with 150 μL 1× PBS. 15 μL re-suspended cells were transferred to 96-well flat bottom nonsterile black plates (Greiner Bio-one) already containing 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). Negative control, treated by solvents (DMSO or H_2O) and positive controls (chloroquine, doxycycline) were added to each set of experiments. Plates were incubated for 15 min 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 of parasite growth (IC_{50} K1) were calculated from three independent experiments.

16. The evaluation of the tested molecules cytotoxicity was conducted on the HepG2 (purchased from ATCC, ref HB-8065) and HFF cell lines. Briefly, cells in 100 μL of complete medium, [RPMI supplemented with 10% foetal 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 with 95% air atmosphere. After a 24 h incubation, 100 μL of medium with various product concentrations was added and the plates were incubated for 72 h. At the end of the treatment and incubation, each plate-well was microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 10 μL of MTT solution (5 mg MTT/mL in PBS) were then added to each well with 100 μL of medium without foetal calf 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 removed and DMSO (100 μL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength with a microplate spectrophotometer. DMSO was used as blank and doxorubicine as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration was determined from the dose–response curve.

17. Compounds were assessed for mutagenicity by a modified version of the liquid incubation assay of the classical Ames test at five concentrations (25–125 nM). *Salmonella* tester strains (TA97a, TA98, TA100 and TA102 with and without S9mix) were grown overnight in a Nutrient Broth n°2 (Oxoid, France). After this period, products dissolved in DMSO (Sigma) were added to 0.1 mL of culture and incubated for 1 h at 37 °C with shaking. Each sample was assayed in duplicate. After incubation, 2 mL of molten top agar were mixed gently with the pre-incubated solution and poured onto Vogel-Bonner minimal agar plates. After 48 h at 37 °C, the number of spontaneous- and drug-induced revertants per plate was determined for each dose with a bacterial colony counter. A product was considered mutagenic when it induced a two-fold increase of the number of revertants, compared with the spontaneous frequency (negative control). For each *Salmonella* strain, a specific positive- and solvent-control were performed with and without S9mix.

18. The effects of the tested compounds on the growth of *Toxoplasma gondii* tachyzoites (PRU-β-Gal strain) were assessed by a colorimetric microtiter assay. Briefly, tachyzoites were maintained by serial passage in confluent monolayer of human foreskin fibroblasts HFF (ATCC, Manassas, USA). For assay, 96-well microtiter plates were seeded with 3.10^4 HFF cells per well and allowed to grow to confluence in RPMI 1640 (without phenol red) supplemented with 10% FCS and 1% L-glutamine/penicillin-streptomycin mix at 37 °C with 6% CO_2. Cell monolayers were infected with 1.10^4 parasites per well and incubated for 3 h at 37 °C with 6% CO_2. Then, various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v) were incorporated in triplicate. Appropriate controls treated by DMSO or the reference drug pyrimethamine were added to each set of experiments. Negative control consisted in cell monolayers incubated without parasite and drug. After a 72 h incubation period at 37 °C with 6% CO_2, cell medium was removed and 100 μL of a 1 mM chlorophenol red-β-D-galactopyranoside (CPRG) solution were added to each well. The plates were incubated at 37 °C with 6% CO_2 for 6 h, at which time β-galactosidase activity was measured by reading plates at 570 and 630 nm on a Biotek microtiter plate reader. Blanking was made on the negative-control wells. The concentration of compounds required to induce a 50% decrease of parasite growth (IC_{50}) was calculated by nonlinear regression analysis processed on dose-response curves, using the Table Curve software 2D v.5.0. IC_{50} values represent the mean value calculated from three independent experiments.