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# Accepted Manuscript

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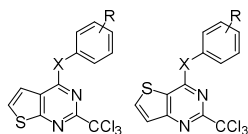
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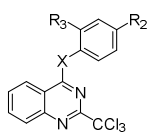
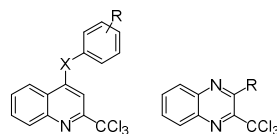
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**Graphical Abstract:****Thienopyrimidine Analogues**SAR  
7 molecules**Initial Antiplasmodial  
Quinazolines**

X = NH, O, S

SAR study  
11 moleculesSAR  
8 molecules**Quinoline and Quinoxaline Analogues**

ACCEPTED MANUSCRIPT

## Discovery of new hit-molecules targeting *Plasmodium falciparum* through a global SAR study of the 4-substituted-2-trichloromethylquinazoline antiplasmodial scaffold

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### Abstract:

From 4 antiplasmodial hit-molecules identified in 2-trichloromethylquinazoline series, we conducted a global Structure-Activity relationship (SAR) study involving 26 compounds and covering 5 molecular regions (I – V), aiming at defining the corresponding pharmacophore and identifying new bioactive derivatives. Thus, after studying the aniline moiety in detail, thienopyrimidine, quinoline and quinoxaline bio-isosters were synthesized and tested on the K1 multi-resistant *P. falciparum* strain, along with a cytotoxicity evaluation on the human HepG2 cell line, to define selectivity indices. SARs first showed that thienopyrimidines and quinolines were globally more cytotoxic, while quinoxaline analogs appeared as active and less cytotoxic than their quinazoline counterparts. Such pharmacomodulation in quinoxaline series not only provided a new antiplasmodial reference hit-molecule ( $IC_{50} = 0.4 \mu M$ , selectivity index = 100), but also highlighted an active ( $IC_{50} = 0.4 \mu M$ ) and quite selective (SI = 265) synthesis intermediate.

### Highlights:

► Antiplasmodial pharmacomodulation of  $CCl_3$ -substituted-nitrogen containing heterocycles was made. ► Thienopyrimidine derivatives appeared more cytotoxic. ► Original 3-substituted-2-trichloromethylquinoxaline analogs were prepared. ► Two quinoxaline derivatives displayed *in vitro*  $IC_{50}$  values of 0.4 and 0.5  $\mu M$  on the K1 multi-resistant *P. falciparum* strain. ► Cytotoxicity was assessed on the human HepG2 cell line showing low cytotoxicity ( $CC_{50} \sim 40 \mu M$ ) and improved selectivity indices (77-100).

**Keywords:** Quinazoline; Quinoline; Quinoxaline; Thienopyrimidine; Trichloromethyl group; *Plasmodium falciparum*; *In vitro* antiplasmodial activity; *In vitro* HepG2 cytotoxicity, Structure-Activity Relationships.

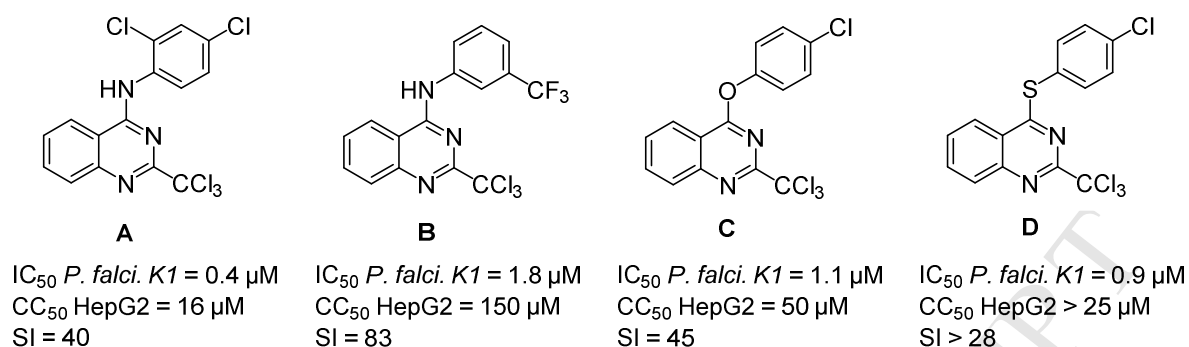
## 1. Introduction

Cerebral malaria, caused by *Plasmodium falciparum*, is the leading cause of death among parasitic infections worldwide. According to the 2015 World Malaria Report [1], 214 million people were infected by *Plasmodium* in 2014, leading to 438.000 deaths out of which about 88% occurred in Africa, mainly in children under five. It is to note that a significant improvement of the situation has been noted since the beginning of the 21st century, thanks to the action of the WHO and the financial involvement of several non-governmental organizations.

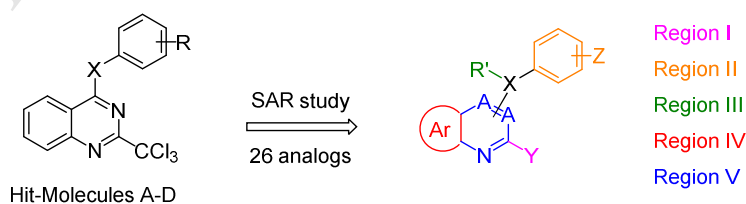
However, the control of the infection is facing worldwide the emergence of drug-resistant strains of the parasite which turns into a major concern for the medical and scientific community. Indeed, the WHO recommended the treatment of *P. falciparum* malaria is based on an artemisinin-combination therapy (ACT), but resistances to artemisinin derivatives are emerging in Asia [2], and it was demonstrated that they were responsible for therapeutic failures in several infected patients [3,4]. Moreover, it was also highlighted that the African *Anopheles gambiae* mosquito could transmit such Asian resistant parasites [5], indicating a major worldwide spreading risk. Thus, research efforts have to be developed, in order to discover new chemical entities presenting novel mechanisms of action, to complete and guaranty the durable efficiency of ACTs.

Several research teams working in the field of antimalarial agents previously reported novel quinazoline derivatives displaying significant antiplasmodial activities, in particular when bearing an amino- [6], alkylamino- [7,8] or an aniline- substituent [9] at position 4 of the quinazoline ring.

The research activity of our group is focused on the synthesis and anti-infective evaluation of new nitrogen-containing heterocycles [10]. We have intensively studied a large series of antiplasmodial derivatives based on the original 2-trichloromethylquinazoline scaffold. Thus, the synthesis and *in vitro* biological evaluation of a large quinazoline chemical library bearing an arylamino- [11,12], aryl- [13], phenoxy- [14], phenylthio- [15], sulfonamido- [16], alkynyl- [17], heteroarylamino- [18], benzyloxy- or alkoxy- [19] substituent at position 4 of the quinazoline ring, revealed several hit-molecules (A-D) which are presented in **Figure 1**.

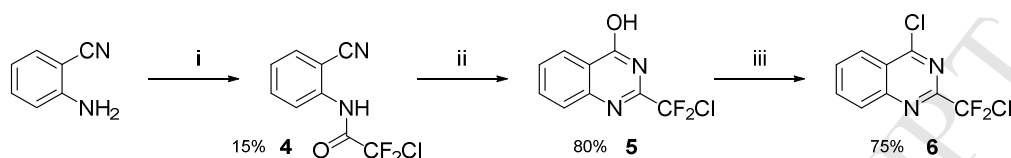
**Figure 1.** Antiplasmodial hit-molecules identified in 2-trichloromethylquinazoline series.

In the light of these hit-molecules, within the aim of defining the antiplasmodial Structure Activity Relationships (SARs) of the corresponding series, 5 key-regions (I-V) were chosen and modulated, as summarized in **Figure 2**. Thus, for region I, we first investigated the role of the  $-CCl_3$  group by replacing it by various substituents including closely related halogenated groups. For region II, we studied the influence of the combination of the halogen atoms on the aniline moiety of hit **A**. The effect of the methylation of the secondary amine nitrogen of hit **A** was then investigated in region III. Further modulations in region IV were made by replacing the quinazoline ring of hits **A-D** by a bio-isosteric thienopyrimidine. Indeed, starting from the natural quinazoline-based antimalarial molecule Febrifugine, such ring replacement was successfully carried out [20]. Moreover, some recent thienopyrimidine derivatives demonstrated promising antiplasmodial activities [21,22]. Finally, in region V, we looked for novel analogs, centered either on the quinoline nucleus, as this heterocycle is encountered in numerous antimalarial drugs such as quinine, chloroquine, mefloquine, amodiaquine, piperazine and primaquine, or on the quinoxaline nucleus, taking advantage of previously reported results obtained in our team in the pyrrolo[1,2-*a*]quinoxaline series [23]. The synthesis of all new derivatives, their *in vitro* biological evaluation and the resulting SARs is presented and discussed herein.

**Figure 2.** Antiplasmodial SAR study involving 5 key-regions (I-V) from the quinazoline hits



(DMAP), affording **4** in low yield (15%). Then, a smooth oxidative hydration of nitrile **4** was operated with H<sub>2</sub>O<sub>2</sub>, followed by a cyclization in alkaline medium [17,28], leading to quinazolinone **5**. This last was further chlorodehydroxylated using POCl<sub>3</sub>, affording the expected intermediate **6** (Scheme 2).

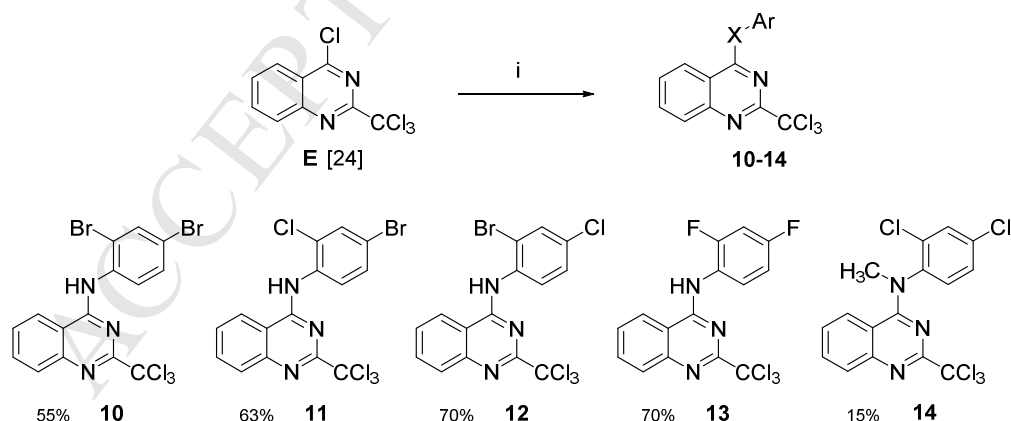


**Scheme 2.** Variation of the region I: Synthesis of intermediate **6**.

*Reagents and conditions* : (i) 2-chloro-2-difluoroacetic acid 1 equiv, EDCI.HCl 1 equiv, DMAP 0.8 equiv, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C then rt, 24 h; (ii) H<sub>2</sub>O<sub>2</sub> 30%, NaOH, EtOH/H<sub>2</sub>O, 0 °C then rt, 90 min; (iii) POCl<sub>3</sub>, 140 °C, MW, 10 min, 800 W.

**2.1.2. Region II: Variation of the halogen atoms at position 2' and 4' of the 4-anilino-substituent**

The synthesis of the anilino derivatives **10-13** was easily performed under classical S<sub>N</sub>Ar reaction conditions by reacting the appropriate 2,4-dihaloaniline with 4-chloro-2-trichloromethylquinazoline **E** in refluxing isopropanol, in 55-70 % range yield (Scheme 3). The results of their *in vitro* biological evaluation are presented in Table 1.



**Scheme 3.** Chemical modulations at regions II and III of the pharmacophore

*Reagents and conditions*: (i) 2,4-dihaloaniline (2 equiv) or 2,4-dichloro-*N*-methylaniline (1.1 equiv), *i*PrOH, reflux, 2 h.



### 2.1.3. Region III: *N*-methylation of the 4-(2',4'-dichlorophenyl)amine substituent

The influence of the hydrogen atom on the secondary amine, in region III, was also studied. For this purpose, a  $S_NAr$  reaction was performed between **E** and *N*-methyl-2,4-dichloroaniline, affording the expected methylated compound **14** in low yield (**Scheme 3**). The biological evaluation of compound **14** is summarized in **Table 1**.

## 2.2. Biological evaluation of the series belonging to regions I, II and III

These new derivatives were evaluated *in vitro* toward both their antiplasmodial activity against the K1 multi-resistant *P. falciparum* strain (determination of the  $IC_{50}$  = inhibitory concentration 50%) and their cytotoxicity (determination of the  $CC_{50}$  = cytotoxic concentration 50%) on the HepG2 human cell line. The results were compared with three commercial antimalarial reference-drugs (atovaquone, chloroquine and doxycycline) and a cytotoxic reference drug (doxorubicine). For all tested compounds, the corresponding selectivity indices (SI) were calculated ( $SI = CC_{50}/IC_{50}$ ). The results are presented in **Table 1**.

The aim of the modulation of region I was to confirm that the trichloromethyl group, at position 2 of the quinazoline ring, was the only substituent providing the antiplasmodial activity. Indeed, the antiplasmodial activity was totally lost when replacing it with other groups, including closely related ones such as  $CF_3$  or  $CHCl_2$ . Looking at the combination of the halogen substituents on the aniline ring at position 4 (region II), the antiplasmodial activity was maintained for brominated compounds **10-12**, in comparison with hit **A** (respectively 0.3, 0.5 and 0.6 vs 0.4  $\mu M$ ), but the cytotoxicity was significantly increased, leading to less selective molecules. Contrary to the results obtained with brominated analogues, fluorinated analogue **13** presented the same cytotoxicity profile as hit **A** but was less active ( $IC_{50} = 3.1 \mu M$ ). Finally in region III, the methylation of the aniline moiety (compound **14**) significantly impaired both antiplasmodial activity and cytotoxicity.

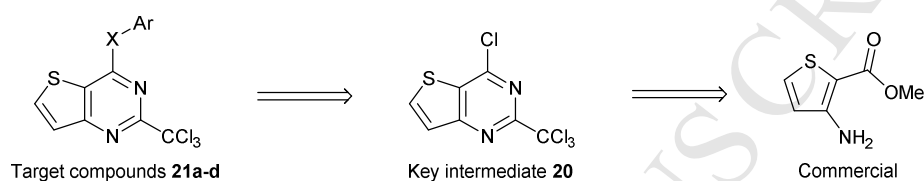
**Table 1.** *In vitro* antiplasmodial and cytotoxicity evaluation of compounds **1-3** and **7-14**: Modulations at regions I, II and III.

Molecule	-R <sub>2</sub>	-R <sub>4</sub>	HepG2 CC <sub>50</sub> ( $\mu$ M)	K1 <i>P. falciparum</i> IC <sub>50</sub> ( $\mu$ M)	Selectivity Index <sup>c</sup>
<b>1</b>	-CH <sub>3</sub>		15	85	0.18
<b>2</b>	-CH <sub>2</sub> Cl		0.5	>12.5 <sup>d</sup>	< 0.04
<b>3</b>	-CHCl <sub>2</sub>		4.7	>50	< 0.09
<b>7</b>	-CF <sub>2</sub> Cl		7	48.4	0.14
<b>8</b>	-CF <sub>3</sub>		>50	>50	-
<b>9</b>	-H		35	>50	<0.7
<b>10</b>	-CCl <sub>3</sub>		2	0.3	6.7
<b>11</b>	-CCl <sub>3</sub>		5	0.5	10
<b>12</b>	-CCl <sub>3</sub>		2.9	0.6	4.8
<b>13</b>	-CCl <sub>3</sub>		19.6	3.1	6.3
<b>14</b>	-CCl <sub>3</sub>		10	3.4	2.9
<b>Hit A</b>	-CCl <sub>3</sub>		16	0.4	40
<b>Doxorubicine<sup>a</sup></b>			0.2	-	-
<b>Atovaquone<sup>b</sup></b>			> 15.6 <sup>d</sup>	0.001	15600
<b>Chloroquine<sup>b</sup></b>			30	0.6	50
<b>Doxycycline<sup>b</sup></b>			20	6.0	3.3

<sup>a</sup>Doxorubicine was used as a cytotoxic reference-drug; <sup>b</sup>Atovaquone, Chloroquinine and doxycycline were used as antimalarial reference-drugs, <sup>c</sup>Selectivity indices were calculated according to the formula : SI = HepG2 CC<sub>50</sub> / K1 IC<sub>50</sub>. <sup>d</sup>Highest concentration tested due to a lack of solubility.

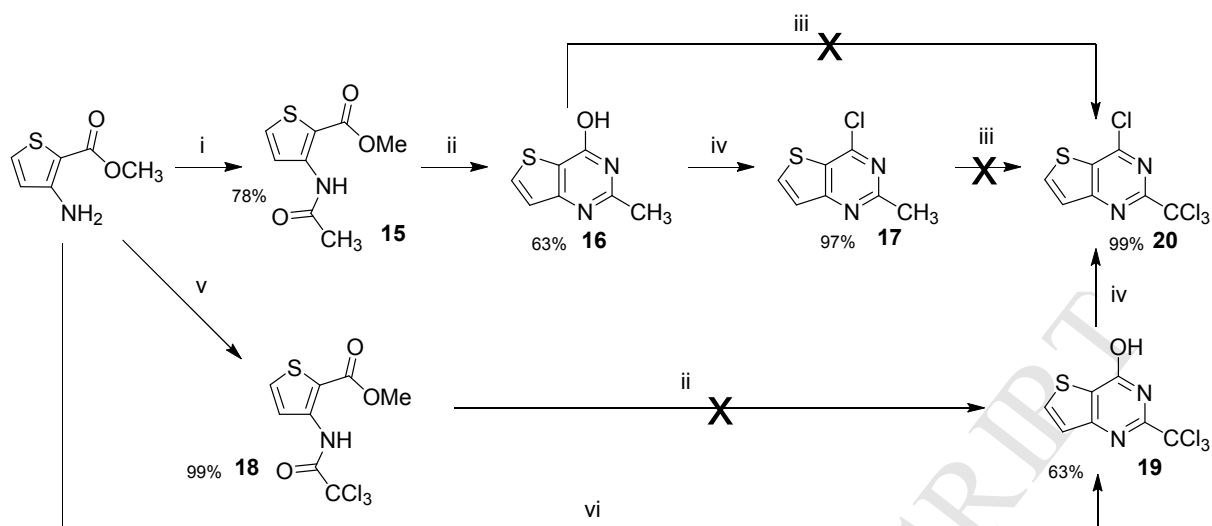
### 2.3. Region IV: Synthesis of thieno[3,2-*d*]pyrimidine series.

The replacement of the phenyl ring of the quinazoline nucleus, by a thiophene nucleus as a bio-isostere, was next studied. We first tried to synthesize 4-substituted-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine derivatives by a  $S_NAr$  reaction between the key chlorinated intermediate **20** and various nucleophilic species, corresponding to the substituents borne by hits **A-D**, respectively 2,4-dichloroaniline **a**, 3-trifluoromethylaniline **b**, 4-chlorophenol **c** and 4-chlorothiophenol **d** (**Figure 3**).



**Figure 3.** Thieno[3,2-*d*]pyrimidines initial retrosynthesis pathway.

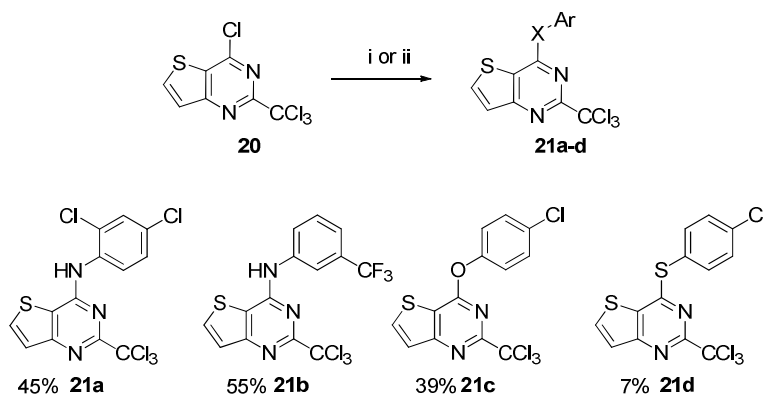
Starting from the commercially available 3-aminothiophene-2-carboxylate, we first acetylated the amino group in presence of  $Ac_2O$ , leading to **15** in 78% yield [29]. Then, the cyclization was made by heating **15** in presence of 25% ammonia in a sealed vial, affording thienopyrimidinone **16** [30] in 63% yield. Unfortunately, in our hands, the chlorination reaction of **16**, to access **20**, did not succeed. Thus, we decided to realize the chlorination reaction in 2 steps. We first synthesized 4-chloro-2-methylthieno[3,2-*d*]pyrimidine **17** [30]. Then, **17** was engaged in a second chlorination reaction with  $PCl_5/POCl_3$ , under classical heating or microwave activation to convert the 2-methyl group into a 2-trichloromethyl substituent. Unfortunately, here again, no trace of the key intermediate **20** was formed, and only inseparable mixtures of polychlorinated compounds were observed by LC-MS analysis (**Scheme 4**).



**Scheme 4.** Synthesis of key intermediate **20**.

*Reagents and conditions:* (i)  $\text{Ac}_2\text{O}$ ,  $\text{AcOH}$  cat.,  $120\text{ }^\circ\text{C}$ , MW, 10 min; (ii)  $\text{NH}_4\text{OH}$  25%,  $105\text{ }^\circ\text{C}$ , 3 h, sealed vial; (iii)  $\text{PCl}_5$  6 equiv,  $\text{POCl}_3$  as solvent,  $100\text{ }^\circ\text{C}$ , MW, 20 min, 800 W; (iv)  $\text{POCl}_3$ , pyridine cat., reflux 24 h (molecule **17**) or 1 h (molecule **20**); (v)  $\text{Et}_3\text{N}$ , trichloroacetyl chloride,  $5\text{ }^\circ\text{C}$ , 30 min; (vi)  $\text{AcOH}$  saturated with gaseous  $\text{HCl}$ , trichloroacetonitrile,  $100\text{ }^\circ\text{C}$ , 18 h, evaporated then refluxed in *i*PrOH, 5 min.

To introduce the  $-\text{CCl}_3$  group, we chose to start again from 3-aminothiophene-2-carboxylate and carried out the acylation reaction, using trichloroacetyl chloride in presence of  $\text{Et}_3\text{N}$ . The corresponding product **18** was obtained in quantitative yield. Unfortunately, the following cyclization step to lactam **19** was unsuccessful under the reaction conditions used (either  $\text{NH}_4\text{OH}$  at  $105\text{ }^\circ\text{C}$  in sealed vial or  $\text{NH}_3$  in  $\text{MeOH}$  at  $120\text{ }^\circ\text{C}$  under MW). Finally, we succeeded in preparing lactam **19** by reacting 3-aminothiophene-2-carboxylate with trichloroacetonitrile in  $\text{AcOH}$ , saturated with gaseous  $\text{HCl}$  [31]. This was a similar approach to the one of Ried [32] who used a more expensive reagent: methyl 2,2,2-trichloroacetimidate. Then, the chlorodehydroxylation of lactam **19** in refluxing  $\text{POCl}_3$  in the presence of a catalytic amount of pyridine, furnished the key intermediate **20** in almost quantitative yield.  $\text{S}_\text{N}\text{Ar}$  reactions between **20** and some N, O or S centered nucleophiles were then performed in a similar way to those operated in quinazoline series. The target 4-substituted-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine derivatives **21a-d** were obtained, however, in low to moderate yields (7-55%) (Scheme 5). The results of the biological evaluation of this series are presented in Table 2.

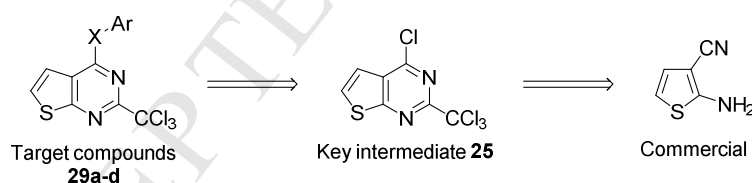


**Scheme 5.** Synthesis of 4-substituted-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine.

*Reagents and conditions:* (i) aniline derivative **a** or **b** 1 equiv, conc. HCl cat., EtOH, 100 °C, 18 h, sealed vial; (ii) NaH 2 equiv, 4-chlorophenol **c** or 4-chlorothiophenol **d** 1 equiv, DMSO, rt or 50 °C, 24 h.

#### 2.4. Region IV: Synthesis of the thieno[2,3-*d*]pyrimidine series.

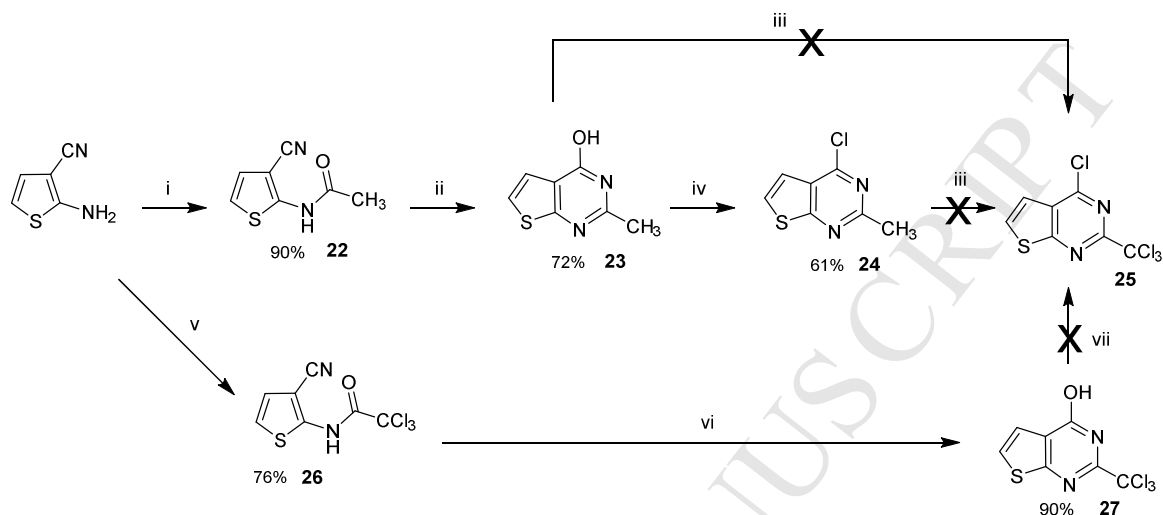
To complete this SAR study with more thiophene-containing bio-isosteres, we decided to synthesize the position isomer analogues in thieno[2,3-*d*]pyrimidine series. This could be performed by a  $S_NAr$  reaction between the desired nucleophiles **a-d** and a key chlorinated intermediate **25**, obtained from 2-aminothiophene-3-carbonitrile (**Figure 4**).



**Figure 4.** Thieno[2,3-*d*]pyrimidines initial retrosynthesis pathway.

Thus, 2-aminothiophene-3-carbonitrile was acetylated with  $Ac_2O$  to give **22** [33]. Then, the cyclization was made in the presence of  $H_2O_2$  30% in alkaline medium (NaOH) [17,28] leading to thienopyrimidinone **23** [34] which was then chlorodehydroxylated into **24**. Unfortunately, as previously observed in the thieno[3,2-*d*]pyrimidine series, we did not succeed in synthesizing **25**, neither by the *gem*-trichloromethylation of **24** [35], nor by the tetrachlorination of **23** with in a  $PCl_5/POCl_3$  mixture. (**Scheme 6**). Acylation of 2-aminothiophene-3-carbonitrile was then realized with trichloroacetyl chloride, to give **26**. Cyclization into lactam **27** was next conducted with a mixture of polyphosphoric acid (PPA)

and phosphoric acid. Nevertheless, once again, it remained impossible to isolate key intermediate **25** via a chlorodehydroxylation reaction. It seemed that **25** was too unstable to be isolated, indeed some traces were detected by LC-MS, but direct engagement of crude **25** in  $S_NAr$  reaction did not afford target compounds.

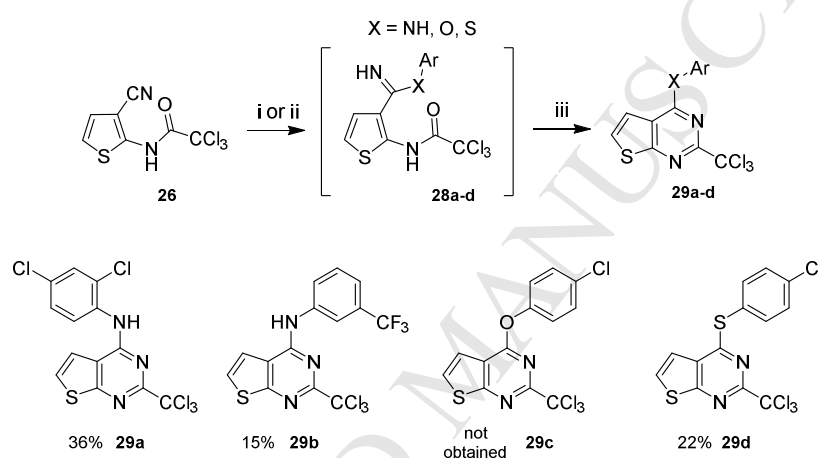


**Scheme 6.** Various synthesis routes investigated for the synthesis of key intermediate **25**.

*Reagents and conditions:* (i)  $Ac_2O$ ,  $AcOH$  cat.,  $120\text{ }^\circ C$ , MW, 10 min; (ii)  $H_2O_2$  35%,  $NaOH$ ,  $EtOH/H_2O$ , 0 to  $55\text{ }^\circ C$ , 15 min; (iii)  $PCl_5$  6 equiv,  $POCl_3$  as solvent,  $100\text{ }^\circ C$ , MW, 20 min, 800 W; (iv)  $POCl_3$ , pyridine cat., reflux 24 h; (v)  $Et_3N$ , trichloroacetyl chloride, rt, 36 h; (vi)  $H_3PO_4$ , polyphosphoric acid,  $70\text{ }^\circ C$ , 3 h; (vii)  $POCl_3$  or  $PCl_5$  or oxalyl chloride or  $P_2O_5$ /tetrabutylammonium chloride (TBACl) or  $SOCl_2$ .

Facing the difficulty to prepare the intermediate **25** and in order to access to the target thieno[2,3-*d*]pyrimidine derivatives, we tried another synthesis pathway based on the cyclization of thiophene derivatives which could be cyclized into thieno[2,3-*d*]pyrimidine rings bearing already both the  $CCl_3$  group and an aniline (or phenol or thiophenol) moiety (**Scheme 7**). Thus the synthesis of amidines **28a** and **28b** was conducted by reacting nitrile **26** with appropriate anilines in presence of  $AlCl_3$  at room temperature in  $CH_2Cl_2$  [36]. The crudes residues obtained were a mixture of unreacted starting material **26** (even after 72 h at rt), the expected amidines **28a-b** and cyclized target derivatives **29a** and **29b**. When the temperature was increased to improve the conversion, undesirable side products appeared. Refluxing the obtained amidines **28a-b** in ethanol led to the target compounds **29a** and **29b**, respectively in 36 and 15% yields [37]. For phenol **c** and thiophenol **d**, the same conditions using  $AlCl_3$  did not afford the expected derivatives. We then considered the synthesis work of Baati and al.,

describing the addition of thiols onto nitrile groups in presence of gaseous HBr in ether at 0 °C [38]. Indeed, to our delight, by using these reaction conditions, thioimide **28d** was produced, and further cyclization in refluxing ethanol afforded compound **29d** in low yield (**Scheme 7**). Unfortunately, the same reaction conditions were inefficient to obtain compounds **28c** and **29c**. Other conditions were attempted in order to produce the imide **28c** *via* the addition of 4-chlorophenol into the nitrile group, in the presence of NaH [39], Na [40], K<sub>2</sub>CO<sub>3</sub> [41] or gaseous HCl [42], but all of them conducted to the same disappointing result. The results of the biological evaluation are presented in **Table 2**.



**Scheme 7.** Synthesis of 4-substituted-2-(trichloromethyl)thieno[2,3-*d*]pyrimidine **29a-d**.

*Reagents and conditions:* (i) anilines **a** or **b**, AlCl<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 72 h; (ii) 4-chlorothiophenol, gaseous HBr, Et<sub>2</sub>O, - 20 °C, 30 min then 18 h at rt; (iii) *i*PrOH, reflux, 5 min.

### 2.5. Modulation of region IV: Biological evaluation

In region IV, the replacement of the quinazoline ring by thienopyrimidine scaffolds led to molecules displaying similar antiplasmodial activity as hit-molecules **A-D**, with IC<sub>50</sub> values in the submicromolar range (0.4 to 0.9 μM) (**Table 2**). However, all the thienopyrimidine derivatives, belonging either to the [3,2-*d*] or [2,3-*d*] series, appeared more cytotoxic (CC<sub>50</sub> = 0.7 to 13.3 μM) than the corresponding hit-molecules, leading to lower selectivity indices. Globally, SARs indicate that there is no significant difference between the two thienopyrimidine series, regarding both activity and cytotoxicity.

**Table 2.** *In vitro* antiplasmodial and cytotoxicity evaluation of compounds **21a-d** and **29a-d**: modulations at region IV

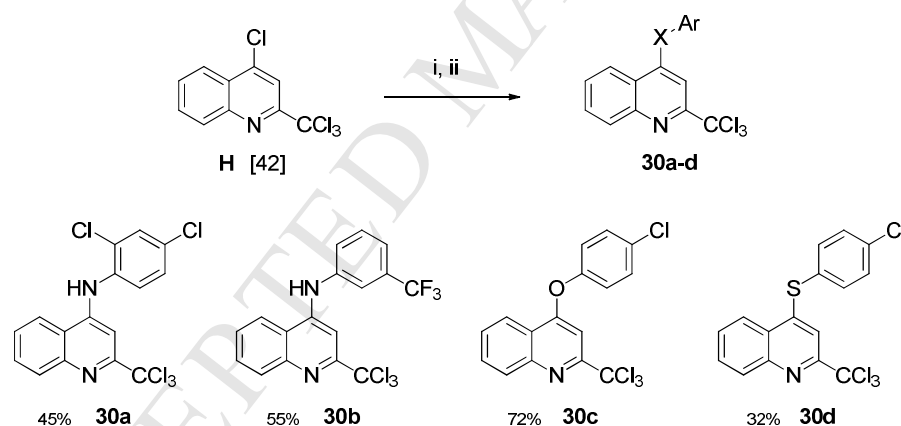
Molecule	Structure	HepG2 CC <sub>50</sub> ( $\mu$ M)	K1 <i>P.falciparum</i> IC <sub>50</sub> ( $\mu$ M)	Selectivity Index <sup>c</sup>
<b>21a</b>		3.2	0.6	5.3
<b>21b</b>		0.7	0.9	0.8
<b>21c</b>		4.3	0.6	7.2
<b>21d</b>		6.9	0.4	17.2
<b>29a</b>		6.2	0.5	12.4
<b>29b</b>		4.0	0.6	6.7
<b>29d</b>		13.3	0.8	16.6
<b>Hit A</b>		16	0.4	40
<b>Hit B</b>		150	1.8	83
<b>Hit C</b>		50	1.1	45
<b>Hit D</b>		>25 <sup>d</sup>	0.9	>28
<b>Doxorubicine<sup>a</sup></b>		0.2	-	-
<b>Atovaquone<sup>b</sup></b>		> 15.6 <sup>d</sup>	0.001	15600
<b>Chloroquine<sup>b</sup></b>		30	0.6	50
<b>Doxycycline<sup>b</sup></b>		20	6.0	3.3

<sup>a</sup>Doxorubicine was used as a cytotoxic reference-drug; <sup>b</sup>Atovaquone, Chloroquinine and doxycycline were used as antimalarial reference-drugs, <sup>c</sup>Selectivity indices were calculated according to the formula : SI = HepG2 CC<sub>50</sub> / K1 IC<sub>50</sub>. <sup>d</sup>Highest concentration tested due to a lack of solubility.



## 2.6. Region V: Synthesis of quinoline series.

In order to evaluate the influence of the pyrimidine moiety of the quinazoline ring onto the biological profile, we chose to synthesize some analogs in 4-substituted-2-trichloromethylquinoline series bearing, at position 4, the same substituents **a-d** as the hit-molecules. Thus, starting from 4-chloro-2-trichloromethylquinoline **H** obtained by chlorination of 2-methylquinolin-4(1*H*)-one with  $\text{PCl}_5$  and  $\text{POCl}_3$  [42], we first tried solvent-free  $\text{S}_{\text{N}}\text{Ar}$  reaction conditions [11] with anilines but without any conversion. Compounds **30a** and **30b** were finally obtained by using a catalytic amount of conc. HCl in refluxing EtOH to obtain the desired compounds in 45 and 55% yields respectively. For the phenol **c** and thiophenol **d**, we generated the corresponding anions using NaH before reacting **H**. Thus, phenol derivative **30c** and thiophenol derivative **30d** were obtained, respectively, in 72 and 32% yields (**Scheme 8**). The biological evaluation of these molecules is summarized in **Table 3**.



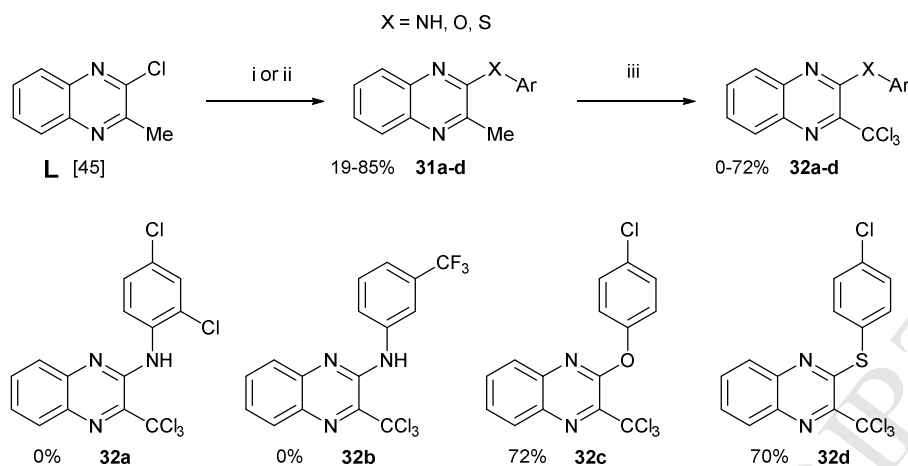
**Scheme 8.** Synthesis of 4-substituted-2-trichloromethylquinoline series.

*Reagents and conditions* : (i) aniline derivative 1 equiv, conc. HCl cat., EtOH, 100 °C, 18 h, sealed vial; (ii) NaH 2 equiv, 4-chlorophenol or 4-chlorothiophenol reagent 1 equiv, DMSO, rt or 50 °C, 24 h.

## 2.7. Region V: Synthesis of quinoxaline derivatives.

Previously, we reported that 2-trichloromethylquinoxaline **I** displayed an *in vitro* antiplasmodial activity ( $\text{IC}_{50} = 1.5 \mu\text{M}$ ), hindered by a significant *in vitro* cytotoxicity ( $\text{CC}_{50} = 3.1 \mu\text{M}$ ) [23]. Moreover, the introduction of a phenyl substituent at position 3 (compound **J**) gave a more selective derivative **J** ( $\text{SI} = 17.5$  versus 2.1, respectively), thanks to a better





### Scheme 9. Synthesis of 2-substituted-3-trichloromethylquinoxaline series.

*Reagents and conditions:* (i) 2,4-dichloroaniline, 4-chlorophenol or 4-chlorothiophenol 1 equiv,  $\text{Cs}_2\text{CO}_3$  1 equiv, anh. DMF, 70 °C, 12 h, sealed vial,  $\text{N}_2$ ; (ii) 3-trifluoromethylaniline 7 equiv, 140 °C, MW, 45 min, sealed vial; (iii)  $\text{PCl}_5$  6 equiv,  $\text{POCl}_3$  as solvent, 100 °C, MW, 20 min, 800 W.

### 2.8. Modulation of region V: biological evaluation.

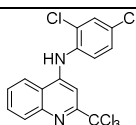
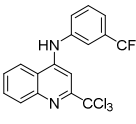
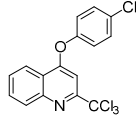
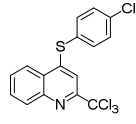
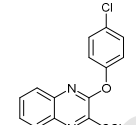
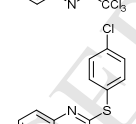
Thus, the ring variation of region V afforded two series of compounds: a quinoline and a quinoxaline series.

Among the four tested compounds in quinoline series, it appeared that all exerted an antiplasmodial activity globally similar to the ones of **hits A-D**. However, the cytotoxicity toward the HepG2 human cell line was increased for all of the quinoline series, in comparison to the Hits **A-D** and particularly for aniline **b** (6.5  $\mu\text{M}$  vs 150  $\mu\text{M}$  for **hit B**). It must be pointed out that for quinoline **3d**, a lack of solubility in the biological media was observed, as previously reported for **hit D**. Thus, the substitution of the quinazoline ring by a quinoline ring was globally detrimental.

Contrary to the quinoline series, the quinoxaline series appeared very promising. Thus, derivative **32c** showed both a better antiplasmodial activity than its analog **hit C** (0.4  $\mu\text{M}$  vs 1.1  $\mu\text{M}$ ) and a great improvement in the cytotoxic profile, its SI reaching 100, compared to 45 for **hit C**. Similarly, the same profile was observed for compound **32d** with a 2-fold activity increase compared to **hit D** (0.45  $\mu\text{M}$  vs 0.9  $\mu\text{M}$ ) and a SI of 77 without any solubility impairment, as observed for **hit D**.

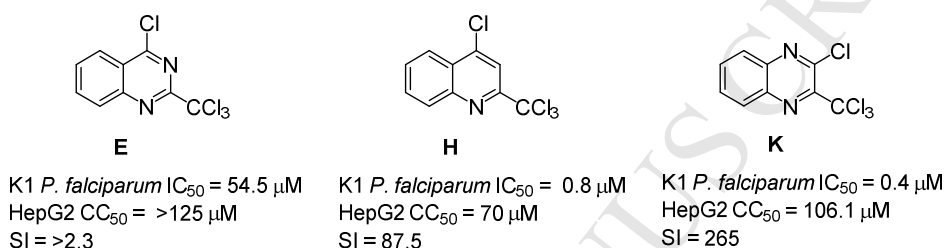
To conclude, we noted that for the region V, the suppression of nitrogen *N*-3 of the quinazoline led to generally more cytotoxic compounds while the replacement of the quinazoline moiety by a quinoxaline one was a significant improvement of the biological profile, compound **32c** reaching a SI of 100.

**Table 3.** *In vitro* antiplasmodial and cytotoxicity evaluation of compounds **30a-d**, and **32c-d**: modulations at region V.

Molecule	Structure	HepG2 CC <sub>50</sub> (μM)	K1 <i>P.falciparum</i> IC <sub>50</sub> (μM)	Selectivity Index <sup>c</sup>
<b>30a</b>		11.7	1.3	9.0
<b>30b</b>		6.5	1.3	5.0
<b>30c</b>		31	1.5	20.7
<b>30d</b>		>15.6 <sup>d</sup>	1.1	>14.2
<b>32c</b>		40.2	0.4	100.5
<b>32d</b>		38.6	0.5	77.2
<b>Hit A</b>		16	0.4	40
<b>Hit B</b>		150	1.8	83
<b>Hit C</b>		50	1.1	45
<b>Hit D</b>		>25 <sup>d</sup>	0.9	>28
<b>Doxorubicine<sup>a</sup></b>		0.2	-	-
<b>Atovaquone<sup>b</sup></b>		> 15.6 <sup>d</sup>	0.001	15600
<b>Chloroquine<sup>b</sup></b>		30	0.6	50
<b>Doxycycline<sup>b</sup></b>		20	6.0	3.3

<sup>a</sup>Doxorubicine was used as a cytotoxic reference-drug; <sup>b</sup>Atovaquone, Chloroquine and doxycycline were used as antimalarial reference-drugs, <sup>c</sup>Selectivity indices were calculated according to the formula : SI = HepG2 CC<sub>50</sub> / K1 IC<sub>50</sub>. <sup>d</sup>Highest concentration tested due to a lack of solubility.

Finally, when comparing the biological activities of the synthetic intermediates prepared for studying region V, it was interesting to note that the intermediate **E** was not active ( $IC_{50} = 54.5 \mu\text{M}$ ) while intermediates **H** and **K** displayed a significant  $IC_{50}$  (0.8 and 0.4  $\mu\text{M}$  respectively). Noticeably, the intermediate **K** possessed a very good SI (265) thanks to a moderate value of its  $CC_{50}$ . Moreover, the presence of a substituent at position 3 appeared to be mandatory for displaying a good antiplasmodial profile by comparison of compound **I** which displayed a SI of only 2 (**Figure 6**). It is to note that both derivatives **32c** ( $IC_{50} = 0.15 \mu\text{g/mL}$ ) and **K** ( $IC_{50} = 0.11 \mu\text{g/mL}$ ) meet the hit to lead *in vitro* TDR criteria [46] for “selectively active antiplasmodial agents”:  $IC_{50} < 0.2 \mu\text{g/mL}$ ,  $SI > 100$ .

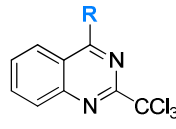
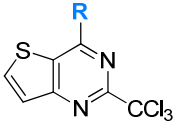
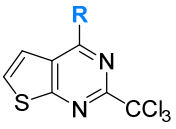
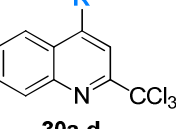
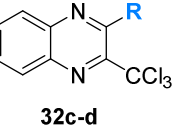
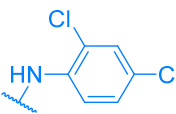
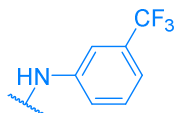
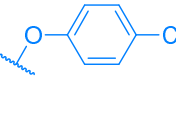
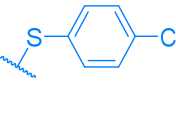


**Figure 6.** Biological assessment of synthetic intermediates.

### 3. Conclusion

From previously identified antiplasmodial hits **A-D**, in 2-trichloromethyl-4-substituted-quinazoline series, new derivatives were synthesized in order to study the SARs in 5 precisely defined regions (I-V). The modulation of region I showed that the  $-CCl_3$  group was mandatory for providing antiplasmodial activity in quinazoline series. In the same series, the study of regions II and III demonstrated that the optimal substitution of the aniline moiety by halogens atoms was 2,4-dichloro and that the *N*-methylation was detrimental toward activity. Then, by modulating regions IV and V, it respectively appeared that the replacement of the quinazoline ring by a quinoline ring led to activity levels similar to the ones of Hits **A-D** but also to increased cytotoxicities values, while the replacement of the quinazoline ring by a quinoxaline ring highlighted derivatives **32c-d**, displaying both a preserved activity and a reduced cytotoxicity, in comparison with hits **A-D**, as summarized in **Table 4**. These molecules now appear as new reference antiplasmodial hits. Finally, the great antiplasmodial potential of intermediate **K** was revealed. Both derivatives **32c** ( $IC_{50} = 0.15 \mu\text{g/mL}$ ) and **K** ( $IC_{50} = 0.11 \mu\text{g/mL}$ ) meet the hit to lead *in vitro* TDR criteria [46] for “selectively active antiplasmodial agents”:  $IC_{50} < 0.2 \mu\text{g/mL}$ ,  $SI > 100$ , opening the way to the synthesis of novel lead-compounds.

**Table 4.** Summary of SAR data at regions IV and V.

R						
		Previous hits A-D	21a-d	29a,b,d	30a-d	32c-d
	IC <sub>50</sub>	<b>0,4</b>	<b>0.6</b>	<b>0.5</b>	<b>1.3</b>	-
	CC <sub>50</sub>	<b>16</b>	<b>3.2</b>	<b>6.2</b>	<b>11.7</b>	-
	SI	40	5.3	12.4	9.0	-
	IC <sub>50</sub>	<b>1,8</b>	<b>0.9</b>	<b>0.6</b>	<b>1.3</b>	-
	CC <sub>50</sub>	<b>150</b>	<b>0.7</b>	<b>4.0</b>	<b>6.5</b>	-
	SI	83	0.8	6.7	5.0	-
	IC <sub>50</sub>	<b>1.1</b>	<b>0.6</b>	-	<b>1.5</b>	<b>0.4</b>
	CC <sub>50</sub>	<b>50</b>	<b>4.3</b>	-	<b>31</b>	<b>40.2</b>
	SI	45	7.2	-	20.7	<b>100.5</b>
	IC <sub>50</sub>	<b>0.9</b>	<b>0.4</b>	<b>0.8</b>	<b>1.1</b>	<b>0.5</b>
	CC <sub>50</sub>	<b>&gt;25</b>	<b>6.9</b>	<b>13.3</b>	<b>&gt;15.6*</b>	<b>38.6</b>
	SI	<b>&gt;28</b>	17.2	16.6	<b>&gt;14.2</b>	<b>77.2</b>

\*Highest concentration tested due to a lack of solubility.

In bold, newly identified hit-molecules

## 4. Experimental

### 4.1. Chemistry

Commercial reagents were used as received without additional purification. Melting points were determined on a Kofler bench and are uncorrected. Elemental analysis and HRMS were carried out at the Spectropole, Faculté des Sciences et Techniques de Saint-Jérôme, Marseille, France. NMR spectra were recorded on a Bruker ARX 200 spectrometer or a Bruker AV 250 spectrometer at the Faculté de Pharmacie de Marseille or a BRUKER Avance III nanobay 400 at the the Spectropole, Faculté des Sciences et Techniques de Saint-Jérôme, Marseille (<sup>1</sup>H-NMR: 200, 250 or 400 MHz, <sup>13</sup>C-NMR: 50, 63 or 100 MHz). NMR references were the following: <sup>1</sup>H: CHCl<sub>3</sub> δ = 7.26, DMSO-*d*<sub>6</sub> δ = 2.50 and <sup>13</sup>C: CHCl<sub>3</sub> δ = 76.9, DMSO-*d*<sub>6</sub> δ = 39.5. Solvents were dried by conventional methods. The following adsorbent was used for column chromatography: silica gel 60 (Merck, particle size 0.063–0.200 mm, 70–230 mesh ASTM). TLC was performed on 5 cm × 10 cm aluminium plates coated with silica gel 60F-

254 (Merck) in an appropriate eluent. Visualization was made with ultraviolet light (234 nm). HRMS spectra were recorded on QStar Elite (Applied Biosystems SCIEX) spectrometer. PEG was the matrix for HRMS. The experimental exact mass was given for the ion which has the maximum isotopic abundance. Purity of synthesized compounds was checked with LC-MS analyses which were realized at the Faculté de Pharmacie de Marseille with a Thermo Scientific Accela High Speed LC System<sup>®</sup> coupled with a single quadrupole mass spectrometer Thermo MSQ Plus<sup>®</sup>. The RP-HPLC column used is a Thermo Hypersil Gold<sup>®</sup> 50 × 2.1 mm (C18 bounded), with particles of 1.9 μm diameter. The volume of sample injected on the column was 1 μL. The chromatographic analysis, total duration of 8 min, is made with the gradient of following solvents: t = 0 min, water/methanol 50/50; 0 < t < 4 min, linear increase in the proportion of water to a ratio water/methanol 95/5; 4 < t < 6 min, water/methanol 95/5; 6 < t < 7 min, linear decrease in the proportion of water to return to a ratio 50/50 water/methanol; 6 < t < 7 min, water/methanol 50/50. The water used was buffered with 5 mM ammonium acetate. The retention times (*t<sub>R</sub>*) of the molecules analyzed are indicated in min. The preparation of 4-chloro-2-trichloromethylquinazoline **E** [24], 2-methyl-N-(2,4-dichlorophenyl)quinazolin-4-amine **1** [10], N-(2,4-dichlorophenyl)quinazolin-4-amine **9** [27], 4-chloro-2-trichloromethylquinoline **H** [43], methyl 3-acetamidothiophene-2-carboxylate **15** [28], 4-chloro-2-chloromethylquinazoline **F** [24], 4-chloro-2-dichloromethylquinazoline **G** [26], 4-chloro-2-trifluoromethylquinazoline [47], 2-chloro-3-trichloromethylquinoxaline **K** [44] and 2-chloro-3-methylquinoxaline **L** [45] was achieved as described in the literature and characterization were consistent as reported in literature.

#### **4.1.1. General procedure for the preparation of N-aryl-2-trichloromethylquinazolin-4-amine (2, 3, 7, 8 and 14)**

To a mixture of appropriate 4-chloroquinazoline (1 equiv) in isopropanol (10 mL) and appropriate aniline (0.8 equiv) were added a few drops of concentrated HCl. The vial was then sealed and heated at 70 °C for 2 h. The mixture was allowed to cool to room temperature and was poured into 20 mL of iced water. The mixture was then extracted twice with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were washed twice with water, then dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 1/1).

##### **4.1.1.1. 2-Chloromethyl-N-(2,4-dichlorophenyl)quinazolin-4-amine (2)**

Starting from 4-chloro-2-chloromethylquinazoline [25] (500 mg, 2.35 mmol) and 2,4-dichloroaniline (304 mg, 1.88 mmol).

Yield 71%. White powder. mp 171 °C.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.97 (d,  $J$  = 8.9 Hz, 1H), 8.21 (bs, 1H), 7.99-7.82 (m, 3H), 7.68-7.60 (m, 1H), 7.47-7.35 (m, 2H), 4.75 (s, 2H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 160.9, 157.1, 149.9, 133.6, 128.9, 128.7, 128.0, 127.6, 127.5, 123.9, 122.9, 120.1, 113.9, 47.6. A quaternary C was not observed under these experimental conditions. Anal. Calcd for  $\text{C}_{15}\text{H}_{10}\text{Cl}_3\text{N}_3$ : C, 53.20; H, 2.98; N, 12.41. Found: C, 53.60; H, 3.12; N, 11.61. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.21 min,  $m/z$  338  $[\text{M}+\text{H}]^+$ .

#### 4.1.1.2. 2-Dichloromethyl-*N*-(2,4-dichlorophenyl)quinazolin-4-amine (3)

Starting from 4-chloro-2-dichloromethylquinazoline **G** [26] (500 mg, 2.02 mmol) and 2,4-dichloroaniline (259 mg, 1.62 mmol).

Yield 42%. White powder. mp 203 °C.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  = 9.11 (d,  $J$  = 8.9 Hz, 1H), 8.30 (bs, 1H), 8.02-7.85 (m, 3H), 7.73-7.66 (m, 1H), 7.49-7.39 (m, 2H), 6.78 (s, 1H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 160.6, 157.6, 149.6, 133.9, 133.6, 129.5, 128.7, 128.3, 128.2, 123.7, 122.9, 120.1, 114.5, 71.8. A quaternary C was not observed under these experimental conditions. Anal. Calcd for  $\text{C}_{15}\text{H}_9\text{Cl}_4\text{N}_3$ : C, 48.29; H, 2.43; N, 11.26. Found: C, 48.41; H, 2.29; N, 11.03. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.71 min,  $m/z$  372  $[\text{M}+\text{H}]^+$ .

#### 4.1.1.3. 2-(chlorodifluoromethyl)-*N*-(2,4-dichlorophenyl)quinazolin-4-amine (7)

Starting from 4-chloro-2-chlorodifluoromethylquinazoline **6** (500 mg, 2.01 mmol) and 2,4-dichloroaniline (293 mg, 1.8 mmol).

Yield 30%. White powder. mp 195 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.93 (d,  $J$  = 9.0 Hz, 1H), 8.38 (bs, 1H), 8.11 (d,  $J$  = 8.3 Hz, 1H), 8.00-7.92 (m, 2H), 7.79-7.74 (m, 1H), 7.48 (d,  $J$  = 2.5 Hz, 1H), 7.42-7.35 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  = 157.3, 155.6 (t,  $J$  = 29 Hz), 149.4, 134.2, 133.2, 130.0, 129.2, 129.0, 128.8, 128.2, 123.9, 122.9 (t,  $J$  = 292 Hz), 122.8, 120.1, 114.9. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{15}\text{H}_8\text{Cl}_3\text{F}_2\text{N}_3$   $[\text{M}+\text{H}]^+$ : 374.9908. Found: 374.9909. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.58 min,  $m/z$  376  $[\text{M}+\text{H}]^+$ .

#### 4.1.1.4. *N*-(2,4-dichlorophenyl)-2-(trifluoromethyl)quinazolin-4-amine (8)

Starting from 4-chloro-2-trifluoromethylquinazoline [45] (500 mg, 2.15 mmol) and 2,4-dichloroaniline (279 mg, 1.72 mmol).

Yield 37%. White powder. mp 198 °C.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.88 (d,  $J$  = 9.0 Hz, 1H), 8.29 (bs, 1H), 8.11-8.07 (m, 1H), 7.97-7.89 (m, 2H), 7.78-7.70 (m, 1H), 7.47-7.35 (m, 2H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 157.3, 152.1 (q,  $J$  = 36 Hz), 149.5, 134.2, 133.2, 130.1, 129.2, 129.1, 128.8, 128.2, 123.9, 122.8, 120.1, 119.8 (q,  $J$  = 275 Hz), 115.2. Anal. Calcd for  $\text{C}_{15}\text{H}_8\text{Cl}_2\text{F}_3\text{N}_3$ : C, 50.30; H, 2.25; N, 11.73. Found: C, 50.39; H, 2.21; N, 11.80.



#### 4.1.1.5. *N*-(2,4-dichlorophenyl)-*N*-methyl-2-trichloromethylquinazolin-4-amine (14)

Starting from 4-chloro-2-(trichloromethyl)quinazoline **E** [24] (500 mg, 1.77 mmol) and *N*-methyl-2,4-dichloroaniline (188  $\mu$ L, 1.95 mmol).

Yield 15%. Colorless oil.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.12-8.08 (m, 1H), 7.61-7.60 (m, 2H), 7.35-7.17 (m, 3H), 6.93-6.89 (m, 1H), 3.63 (s, 3H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 161.8, 160.1, 150.9, 143.1, 134.3, 133.1, 133.0, 131.3, 129.9, 129.5, 129.1, 127.1, 124.4, 114.9, 97.4, 41.3. HRMS (ESI):  $m/z$  calcd. for  $\text{C}_{16}\text{H}_{10}\text{Cl}_5\text{N}_3$   $[\text{M}+\text{H}]^+$ : 418.9317. Found: 418.9319. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 5.18 min,  $m/z$  419.7  $[\text{M}+\text{H}]^+$ .

#### 4.1.2. Synthesis of 4-chloro-2-chlorodifluoromethylquinazoline (6)

##### 4.1.2.1. 2-Chloro-*N*-(2-cyanophenyl)-2,2-difluoroacetamide (4)

In a 250 mL round-bottom flask was successively introduced 2-aminobenzonitrile (2.0 g, 16.9 mmol), 4-(*N,N*-dimethylamino)pyridine (DMAP) (1.7 g, 13.3 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI.HCl) (3.4 g, 17.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL). Then, 2-chloro-2,2-difluoroacetic acid (1.4 mL, 16.9 mmol) was added into the mixture and it was stirred for 24 h at rt. The solvent was evaporated under reduced pressure and water (200 mL) was added to the residue. The mixture was gently heated at 40  $^\circ\text{C}$  until a homogeneous solution was obtained. After cooling down to rt, conc. HCl was added to adjust the pH = 1. The resulting precipitate was filtered, thoroughly washed with water and dried in an oven leading 2-chloro-*N*-(2-cyanophenyl)-2,2-difluoroacetamide.

Yield 15%. White powder. mp 84  $^\circ\text{C}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.37 (d,  $J$  = 8.5 Hz, 2H), 7.71 (t,  $J$  = 7.6 Hz, 2H), 7.36 (t,  $J$  = 7.6 Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  = 157.1 (t,  $J$  = 30 Hz), 137.6, 134.5, 132.7, 126.3, 121.6, 121.5, (t,  $J$  = 300 Hz), 115.3, 103.7. Anal. Calcd for  $\text{C}_9\text{H}_5\text{ClF}_2\text{N}_2\text{O}$ : C, 46.88; H, 2.19; N, 12.15. Found: C, 46.98; H, 2.13; N, 12.35. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 0.85 min,  $m/z$  229  $[\text{M}-\text{H}]^-$ .

##### 4.1.2.2. 2-Chlorodifluoromethylquinazolin-4(3*H*)-one (5)

In a 100 mL round-bottom flask was successively introduced 2-chloro-*N*-(2-cyanophenyl)-2,2-difluoroacetamide **4** (500 mg, 2.17 mmol), 30%  $\text{H}_2\text{O}_2$  (2 mL) and a mixture of EtOH (6 mL) and water (8 mL). Then, NaOH (140 mg, 3.47 mmol) was added at 0  $^\circ\text{C}$ . The mixture was stirred at rt for 90 min and the volatiles were removed under vacuum. Water (100 mL) was added to the residue and the resulting mixture was acidified to pH = 4 with conc. HCl.

The precipitate obtained was filtered, thoroughly washed with water and dried in an oven gave the 2-chlorodifluoromethylquinazolin-4(3*H*)-one (**5**).

Yield 80%. Beige powder. mp 246 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 10.44 (bs, 1H), 8.37 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 3.7 Hz, 2H), 7.67-7.61 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 135.6, 129.2, 128.7, 126.9. Five quaternary C were not observed under these experimental conditions. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.66 min, *m/z* 229 [M-H]<sup>-</sup>.

#### 4.1.2.3. 4-Chloro-2-chlorodifluoromethylquinazoline (**6**)

In a 20 mL vial was successively introduced 2-chlorodifluoromethylquinazolin-4(3*H*)-one **5** (500 mg, 2.17 mmol) and POCl<sub>3</sub> (1 mL, 10.8 mmol). The mixture was stirred at 140 °C for 10 min under microwave irradiation (800 W). After cooling at rt, the crude was purified by silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>) to afford 4-chloro-2-chlorodifluoromethylquinazoline.

Yield 75%. Beige powder. mp 90 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ = 8.38 (d, *J* = 8.3 Hz, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 8.12-8.08 (dt, *J* = 8.2, 1.2 Hz, 1H), 7.89 (dt, *J* = 8.2, 1.2 Hz, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ = 164.2, 155.0 (t, *J* = 30 Hz), 150.4, 136.2, 130.9, 129.6, 126.1, 123.8, 122.0 (t, *J* = 292 Hz). Anal. Calcd for C<sub>9</sub>H<sub>4</sub>Cl<sub>2</sub>F<sub>2</sub>N<sub>2</sub>: C, 43.40; H, 1.62; N, 11.25. Found: C, 43.29; H, 1.56; N, 11.42. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 3.42 min, *m/z* 249 [M+H]<sup>+</sup>.

#### 4.1.3. Representative procedure for the preparation of *N*-(2,4-dihalogenophenyl)-2-trichloromethylquinazolin-4-amine (**10-13**)

A 100 mL round-bottomed single-neck flask equipped with a condenser and a magnetic stir bar was charged with 4-chloro-2-trichloromethylquinazoline **E** [24] (500 mg, 1.77 mmol), 2 equiv. of the appropriated dihalogeno-aniline (3.54 mmol) and 30 mL of isopropanol. The mixture was refluxed for 48 h. After cooling, water was added (50 mL), and the resulting solution was extracted three times with CH<sub>2</sub>Cl<sub>2</sub>. Then, the combined organic layers were washed with water and dried (Na<sub>2</sub>SO<sub>4</sub>). Finally, the solvent was evaporated under reduced pressure. The residue was purified by trituration with CH<sub>2</sub>Cl<sub>2</sub> and filtration yielding the *N*-(2,4-dihalogenophenyl)-2-trichloromethylquinazolin-4-amines (**10-13**).

##### 4.1.3.1. *N*-(2,4-dibromophenyl)-2-trichloromethylquinazolin-4-amine (**10**)

Yield 55%. White powder. mp 243 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 10.32 (bs, 1H), 8.55 (d, *J* = 8.2 Hz, 1H), 8.02- 7.91 (m, 3H), 7.82-7.59 (m, 3H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ = 160.0, 159.5, 148.9, 136.3, 134.7, 134.4, 131.1, 130.8, 128.5, 128.3, 123.1, 122.5, 119.5, 113.5, 97.7. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>Br<sub>2</sub>Cl<sub>3</sub>N<sub>3</sub>: C, 36.59; H, 1.62; N, 8.46. Found: C, 36.16; H, 1.53; N, 8.50. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.41 min, *m/z* 494 [M+H]<sup>+</sup>.

**4.1.3.2. *N*-(4-bromo-2-chlorophenyl)-2-trichloromethylquinazolin-4-amine (11)**

Yield 63%. White powder. mp 222 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 10.38 (bs, 1H), 8.62 (d, *J* = 8.3 Hz, 1H), 8.07-7.97 (m, 2H), 7.93 (s, 1H), 7.87-7.80 (m, 1H), 7.75 - 7.65 (m, 2H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ = 160.2, 159.7, 149.1, 135.0, 134.6, 132.1, 131.8, 130.8, 130.6, 128.7, 128.5, 123.4, 119.3, 113.7, 97.9. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>BrCl<sub>4</sub>N<sub>3</sub>: C, 39.86; H, 1.78; N, 9.30. Found: C, 39.83; H, 1.70; N, 9.45. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.41 min, *m/z* 450 [M+H]<sup>+</sup>.

**4.1.3.3. *N*-(2-bromo-4-chlorophenyl)-2-trichloromethylquinazolin-4-amine (12)**

Yield 70%. White powder. mp 223 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 9.09 (d, *J* = 9.0 Hz, 1H), 8.38 (s, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 8.00-7.89 (m, 2H), 7.77-7.70 (m, 1H), 7.63 (d, *J* = 2.3 Hz, 1H), 7.44 (dd, *J* = 9.0, 2.3 Hz, 1H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ = 160.6, 157.2, 149.6, 134.5, 134.0, 131.7, 130.3, 129.2, 128.8, 128.7, 122.8, 120.1, 120.0, 114.1, 97.5. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>BrCl<sub>4</sub>N<sub>3</sub>: C, 39.86; H, 1.78; N, 9.30. Found: C, 40.01; H, 1.69; N, 9.26. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.82 min, *m/z* 450 [M+H]<sup>+</sup>.

**4.1.3.4. *N*-(2,4-difluorophenyl)-2-trichloromethylquinazolin-4-amine (13)**

Yield 70%. White powder. mp 165 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 9.00-8.87 (m, 1H), 8.12-8.08 (m, 1H), 7.95-7.66 (m, 4H), 7.07-6.92 (m, 2H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ = 160.7, 157.4, 153.2 (d, *J* = 260 Hz), 152.9 (d, *J* = 260 Hz), 149.6, 133.9, 130.2, 128.5, 123.5 (d, *J* = 8.8 Hz), 123.1 (dd, *J* = 9.5, 4.1 Hz), 120.1, 113.8, 111.4 (dd, *J* = 21.6, 3.6 Hz), 104.3-103 (m, 1CH), 97.6. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>Cl<sub>3</sub>F<sub>2</sub>N<sub>3</sub>: C, 48.09; H, 2.15; N, 11.22. Found: C, 48.45; H, 1.99; N, 11.25. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.58 min, *m/z* 374 [M+H]<sup>+</sup>.

**4.1.4. 2-Methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one (16)**

A solution of methyl 3-acetamidothiophene-2-carboxylate **15** [29] (760 mg, 3.81 mmol) in NH<sub>4</sub>OH 25% (9.5 mL) was heated, in a sealed vial, at 105 °C for 3 h. After cooling to room temperature, concentrated HCl was added until pH 8. The precipitate thus formed was filtered, washed with water and dried in a vacuum oven at 40 °C to afford 2-methylthieno[3,2-*d*]pyrimidin-4(3*H*)-one. No further purification necessary.

Yield 63%. White solid. mp 91 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 12.31 (bs, 1 H, NH), 8.12 (d, *J* = 5.2 Hz, 1 H), 7.29 (d, *J* = 5.2 Hz, 1 H), 2.36 (s, 3 H). <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ = 181.0, 158.8, 158.0, 156.3, 133.9, 124.5, 21.1. LC-MS (ESI, 35eV): *t*<sub>R</sub> = 0.64 min, *m/z* 167 [M+H]<sup>+</sup>.

**4.1.5. 4-Chloro-2-methylthieno[3,2-*d*]pyrimidine (17) [30]**

To a solution of compound **16** (200 mg, 1.20 mmol) in POCl<sub>3</sub> (5.0 mL) were added a few drops of pyridine. The mixture was then refluxed for 24 h. After cooling down to room temperature, the solution was poured on ice and a saturated Na<sub>2</sub>CO<sub>3</sub> solution was added until the excess of POCl<sub>3</sub> was neutralized. The aqueous phase was then extracted with EtOAc, the combined organic phases were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 8/2) to afford 4-chloro-2-methylthieno[3,2-*d*]pyrimidine.

Yield 97%. White solid. mp 84 °C. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ = 7.96 (d, *J* = 5.5 Hz, 1H), 7.45 (d, *J* = 5.5 Hz, 1H), 2.78 (s, 3H). <sup>13</sup>C NMR (63 MHz, CDCl<sub>3</sub>) δ = 164.7, 162.6, 154.7, 136.7, 127.9, 124.8, 25.5. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 1.74 min, *m/z* 185 [M+H]<sup>+</sup>.

**4.1.6. Methyl 3-(2,2,2-trichloroacetamido)thiophene-2-carboxylate (18)**

To a solution of methyl 3-aminothiophene-2-carboxylate (500 mg, 3.18 mmol) and triethylamine (440 μL, 3.18 mmol) in tetrahydrofuran (4.0 mL) at 5 °C was added trichloroacetyl chloride (350 μL, 3.18 mmol). The reaction was stirred at 5 °C for 30 min. Water (5.0 mL) was then added and the aqueous phase was extracted with EtOAc. The organic phase was then washed, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated to afford methyl 3-(2,2,2-trichloroacetamido)thiophene-2-carboxylate. No further purification necessary.

Yield 99%. White solid. mp 244 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 11.42 (bs, 1 H), 8.06 (d, *J* = 5.4 Hz, 1 H), 7.89 (d, *J* = 5.4 Hz, 1 H), 3.88 (s, 3 H). <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ = 163.4, 158.1, 140.9, 133.5, 121.0, 113.6, 91.8, 52.2. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.11 min, *m/z* 300 [M+H]<sup>+</sup>.

**4.1.7. 2-(Trichloromethyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one (19)**

HCl<sub>(g)</sub> was bubbled into acetic acid (1.23 mL) for 30 minutes. Then, nitrogen was swept across the solution. This solution was added to a mixture of methyl 3-aminothiophene-2-carboxylate (3.0 g, 19.08 mmol) and trichloroacetonitrile (0.85 mL, 8.48 mmol) in acetic acid (1.86 mL). The reaction was then heated at 100 °C for 18 h. After cooling down to rt, the acetic acid was evaporated under reduced pressure and isopropanol (4.5 mL) was added. This solution was refluxed for 5 min, then refrigerated. The precipitate thus formed was filtered, and then purified by silica gel column chromatography (eluent: Petroleum Ether 100% + 10

mL of EtOAc every 100 mL of eluent) to afford 2-(trichloromethyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one.

Yield 63%. White solid. mp 237 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 10.50 (bs, 1 H), 7.93 (d, *J* = 5.3 Hz, 1 H), 7.51 (d, *J* = 5.3 Hz, 1 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 157.7, 155.7, 151.8, 135.7, 126.1, 123.7, 92.0. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.78 min, *m/z* 267 [M-H]<sup>-</sup>.

#### 4.1.8. 4-Chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine (20)

To a solution of 2-(trichloromethyl)thieno[3,2-*d*]pyrimidin-4(3*H*)-one **19** (200 mg, 0.74 mmol) in POCl<sub>3</sub> (3.0 mL) were added a few drops of pyridine. The reaction was refluxed for 1 h. After cooling to room temperature, the solution was poured on ice and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 8/2) to yield 4-chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine.

Yield 99%. White solid. mp 99 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.21 (d, *J* = 5.5 Hz, 1 H), 7.74 (d, *J* = 5.5 Hz, 1 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 161.9, 161.7, 155.6, 139.0, 130.7, 125.6, 96.0. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.05 min (no ionization).

#### 4.1.9. *N*-(2,4-Dichlorophenyl)-2-(trichloromethyl)thieno[3,2-*d*]pyrimidin-4-amine (21a)

To a solution of 4-chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine **20** (190 mg, 0.66 mmol) and 2,4-dichloroaniline (106 mg, 0.66 mmol) in ethanol (3.0 mL) were added a few drops of concentrated HCl. The vial was sealed and the reaction was heated at 100 °C for 24 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2 + 10 mL of CH<sub>2</sub>Cl<sub>2</sub> every 100 mL of eluent) to afford *N*-(2,4-dichlorophenyl)-2-(trichloromethyl)thieno[3,2-*d*]pyrimidin-4-amine.

Yield 45%. White solid. mp 197 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.72 (d, *J* = 8.9 Hz, 1 H), 7.96 (d, *J* = 5.4 Hz, 1 H), 7.66 (d, *J* = 5.4 Hz, 1 H), 7.61 (s, 1 H), 7.48 (d, *J* = 2.3 Hz, 1 H), 7.37 (dd, *J* = 8.9, 2.3 Hz, 1 H). <sup>13</sup>C NMR (200 MHz, CDCl<sub>3</sub>) δ = 161.6, 161.0, 154.6, 134.1, 133.2, 130.0, 129.1, 128.3, 126.2, 125.1, 124.0, 116.2, 97.1. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.90 min, *m/z* 410 [M-H]<sup>-</sup>. Anal. Calcd for C<sub>13</sub>H<sub>6</sub>Cl<sub>5</sub>N<sub>3</sub>S: C, 37.76; H, 1.46; N, 10.16. Found: C, 38.03; H, 1.26; N, 10.30.

#### 4.1.10. 2-(Trichloromethyl)-*N*-[3-(trifluoromethyl)phenyl]thieno[3,2-*d*]pyrimidin-4-amine (21b)

To a solution of 4-chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine **20** (150 mg, 0.52 mmol) and 3-(trifluoromethyl)aniline (87  $\mu$ L, 0.52 mmol) in ethanol (2.5 mL) were added a few drops of concentrated HCl. The vial was sealed and the reaction was heated at 100°C for 24 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 8/2) to yield 2-(trichloromethyl)-*N*-[3-(trifluoromethyl)phenyl]thieno[3,2-*d*]pyrimidin-4-amine.

Yield 55%. White solid. mp 141 °C.  $^1\text{H}$  NMR (200MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.42 (s, 1 H), 7.88-7.83 (m, 2 H), 7.58-7.39 (m, 4 H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 161.9, 161.0, 155.0, 138.3, 133.9, 131.9, 131.3, 129.7, 125.7, 124.3, 121.3, 118.6, 115.7, 97.4. Anal. Calcd for  $\text{C}_{14}\text{H}_7\text{Cl}_3\text{F}_3\text{N}_3\text{S}$ : C, 40.75; H, 1.71; N, 10.18. Found: C, 40.99; H, 1.80; N, 10.39. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.87 min,  $m/z$  412  $[\text{M}+\text{H}]^+$

#### 4.1.11. 4-(4-Chlorophenoxy)-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine (21c)

To a solution of 4-chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine **20** (210 mg, 0.73 mmol) and 4-chlorophenol (281 mg, 2.19 mmol) in acetonitrile (5.0 mL) was added  $\text{K}_2\text{CO}_3$  (302 mg, 2.19 mmol). The reaction was heated at 160 °C for 1h10 under microwave irradiation. The mixture was poured onto a NaOH 10% solution. The aqueous phase was extracted with EtOAc. The organic phases were combined, washed with brine, dried ( $\text{Na}_2\text{SO}_4$ ) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (eluent: Petroleum Ether/ $\text{CH}_2\text{Cl}_2$  8/2 + 10 mL of  $\text{CH}_2\text{Cl}_2$  every 100 mL of eluent) to yield 4-(4-chlorophenoxy)-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine.

Yield 39%. White solid. mp 161 °C.  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  = 8.09 (d,  $J$  = 5.4 Hz, 1 H), 7.70 (d,  $J$  = 5.4 Hz, 1 H), 7.45-7.31 (m, 4 H).  $^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  = 163.5, 163.1, 161.5, 150.3, 136.9, 131.4, 129.9, 129.5, 125.2, 122.9, 117.7, 116.4, 96.4. Anal. Calcd for  $\text{C}_{13}\text{H}_6\text{Cl}_4\text{N}_2\text{OS}$ : C, 41.08; H, 1.59; N, 7.37. Found: C, 41.28; H, 1.72; N, 7.45. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 5.08 min,  $m/z$  379  $[\text{M}+\text{H}]^+$ .

#### 4.1.12. 4-[(4-Chlorophenyl)thio]-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine (21d)

To a solution of 4-chlorothiophenol (100 mg, 0.69 mmol) in DMSO (0.5mL), NaH (33 mg, 1.39 mmol) was added portionwise under inert atmosphere. The mixture was stirred at room temperature for 20 mi. 4-Chloro-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine **20** (200 mg, 0.69 mmol) was then added portionwise and the reaction was stirred for 24 h at rt. Water (1.0 mL) was added and the aqueous phase was then extracted with  $\text{CH}_2\text{Cl}_2$ . The organic phase was washed with water, brine and then dried ( $\text{Na}_2\text{SO}_4$ ). The solvent was evaporated and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether 100% +

10 mL of CH<sub>2</sub>Cl<sub>2</sub> every 100 mL of eluent) to afford 4-[(4-chlorophenyl)thio]-2-(trichloromethyl)thieno[3,2-*d*]pyrimidine.

Yield 7%. White solid. mp not determined. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.02 (d, *J* = 5.5 Hz, 1 H), 7.67-6.64 (m, 3 H), 7.45 (d, *J* = 8.8 Hz, 2 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$  = 164.5, 161.1, 159.9, 137.0, 136.6, 136.4, 129.5, 127.5, 125.0, 124.7, 96.8. Anal. Calcd for C<sub>13</sub>H<sub>6</sub>Cl<sub>4</sub>N<sub>2</sub>S<sub>2</sub>: C, 39.41; H, 1.53; N, 7.07. Found: C, 39.75; H, 1.72; N, 7.20. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.36 min, *m/z* 395 [M+H]<sup>+</sup>.

#### 4.1.13. *N*-(3-Cyanothiophen-2-yl)acetamide (22)

To a solution of 2-aminothiophene-3-carbonitrile (1.020 g, 8.21 mmol) in acetic anhydride (3.0 mL) were added a few drops of acetic acid. The reaction was heated at 120 °C for 10 min under microwave irradiation. The brown solid thus obtained was filtered, washed with water and dried in a vacuum oven at 40 °C to afford *N*-(3-cyanothiophen-2-yl)acetamide. No further purification necessary.

Yield 90%. Brown solid. mp 210 °C (litt. 210-211 °C). <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 11.70 (bs, 1 H), 7.13-7.12 (m, 2 H), 2.20 (s, 3 H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 168.4, 149.4, 124.7, 118.7, 114.9, 91.8, 22.5. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.81 min, *m/z* 165 [M-H]<sup>-</sup>.

#### 4.1.14. 2-Methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one (23)

To a solution of *N*-(3-cyanothiophen-2-yl)acetamide **22** (1.232 g, 7.41 mmol) and H<sub>2</sub>O<sub>2</sub> 30% (6.5 mL) in ethanol (22 mL) and water (4 mL), at 0 °C, was added NaOH in pellets (0.495 g, 12.4 mmol). The reaction was stirred at room temperature for 15 min and then heated at 55 °C until a homogeneous solution was obtained. After cooling to rt, the solvent was evaporated under reduced pressure. Water (10 mL) was added and the mixture was heated to 50 °C. After cooling to rt, a HCl 1N solution was added until pH 4. The precipitate was filtered, washed with water and dried in a vacuum oven at 40 °C to afford 2-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one. No further purification necessary.

Yield 72%. White solid. mp 210 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 7.90 (s, 1 H), 7.39 (d, *J* = 5.8 Hz, 1 H), 6.93 (d, *J* = 5.8 Hz, 1 H), 2.19 (s, 3 H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  = 166.9, 166.8, 145.8, 123.0, 115.8, 114.8, 23.3. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.65 min, *m/z* 167 [M+H]<sup>+</sup>.

#### 4.1.15. 4-Chloro-2-methylthieno[2,3-*d*]pyrimidine (24)

To a solution of 2-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-one **23** (300 mg, 1.80 mmol) in POCl<sub>3</sub> (5.0 mL) were added a few drops of pyridine. The mixture was refluxed for 24 h and, after

cooling to rt, was poured onto ice. A saturated Na<sub>2</sub>CO<sub>3</sub> solution was added until complete neutralization of the excess of POCl<sub>3</sub>. The aqueous phase was extracted with EtOAc. The combined organic phases were washed with brine, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 8/2) to yield 4-chloro-2-methylthieno[2,3-*d*]pyrimidine.

Yield 61%. Yellow solid mp 93 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 7.48 (d, *J* = 6.0 Hz, 1 H), 7.35 (d, *J* = 6.0 Hz, 1 H), 2.79 (s, 3 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 169.2, 163.0, 154.4, 126.8, 126.6, 119.5, 25.5. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 1.84 min, *m/z* 185 [M+H]<sup>+</sup>.

#### 4.1.16. 2,2,2-Trichloro-*N*-(3-cyanothiophen-2-yl)acetamide (26)

To a solution of 2-aminothiophene-3-carbonitrile (1.08 g, 8.0 mmol) and trichloroacetyl chloride (0.89 mL, 8.0 mmol) in dioxane (15 mL), triethylamine (1.67 mL, 12.0 mmol) was added dropwise. The reaction was stirred at room temperature for 36 h. The aqueous phase was extracted with diethyl ether and the combined organic phases were washed with brine and dried (Na<sub>2</sub>SO<sub>4</sub>). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 9/1) to afford 2,2,2-trichloro-*N*-(3-cyanothiophen-2-yl)acetamide.

Yield 76%. White solid. mp 127 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 9.48 (s, 1 H), 7.08 (s, 2 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 158.7, 147.0, 124.8, 120.5, 113.4, 96.8, 90.8. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.80 min, *m/z* 267 (M). HRMS (ESI): *m/z* calcd. for C<sub>7</sub>H<sub>3</sub>Cl<sub>3</sub>N<sub>2</sub>OS [M+H]<sup>+</sup>: 268.9104. Found: 268.9110.

#### 4.1.17. 2-(Trichloromethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (27)

Compound 2,2,2-trichloro-*N*-(3-cyanothiophen-2-yl)acetamide **26** (809 mg, 3.0 mmol) was added to a mixture of phosphoric acid and polyphosphoric acid (60 mL, 1:1). The solution was stirred at rt for 1 h and then heated at 70 °C for 3 h. After cooling to room temperature, the mixture was poured onto ice. The precipitate was filtered, washed with ice-cold water and dried in a vacuum oven at 40 °C to yield 2-(trichloromethyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one. No further purification necessary.

Yield 90%. White solid. mp 195 °C. <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>) δ = 8.23 (s, 1 H), 7.56 (d, *J* = 5.8 Hz, 1 H), 7.25 (d, *J* = 5.8 Hz, 1 H). <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>) δ = 167.4, 158.8, 144.6, 124.1, 119.2, 118.5, 91.6. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 0.69 min, *m/z* 267 [M-H]<sup>-</sup>.

#### 4.1.18. *N*-(2,4-Dichlorophenyl)-2-(trichloromethyl)thieno[2,3-*d*]pyrimidin-4-amine (29a)



To a solution of 2,2,2-trichloro-*N*-(3-cyanothiophen-2-yl)acetamide **27** (200 mg, 0.74 mmol) and 2,4-dichloroaniline (118 mg, 0.74 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.3 mL), AlCl<sub>3</sub> (100 mg, 0.74 mmol) was added portionwise. The mixture was stirred at rt for 72 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/EtOAc 7/3) to afford a white solid **28a** (LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.00 min, *m/z* 430 (M+H)<sup>+</sup>) which was dissolved in ethanol (2 mL) and the resulting solution was refluxed for 4 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2 to 5/5) to afford *N*-(2,4-dichlorophenyl)-2-(trichloromethyl)thieno[2,3-*d*]pyrimidin-4-amine.

Yield 36%. White solid. mp 181 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.94 (d, *J* = 9.0 Hz, 1 H), 7.77 (bs, 1 H), 7.64 (d, *J* = 6.0 Hz, 1 H), 7.47 (d, *J* = 2.3 Hz, 1 H), 7.37 (dd, *J* = 5.4, 2.3 Hz, 2 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 167.3, 160.2, 153.8, 133.5, 128.9, 128.8, 128.2, 127.9, 123.4, 122.6, 116.8, 116.2, 96.3. Anal. Calcd for C<sub>13</sub>H<sub>6</sub>Cl<sub>5</sub>N<sub>3</sub>S: C, 37.76; H, 1.46; N, 10.16. Found: C, 37.95; H, 1.52; N, 10.39. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.26 min, *m/z* 412 [M+H]<sup>+</sup>.

#### 4.1.19. 2-(Trichloromethyl)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidin-4-amine (29b)

To a solution of 2,2,2-trichloro-*N*-(3-cyanothiophen-2-yl)acetamide **26** (320 mg, 1.18 mmol) and 3-(trifluoromethyl)aniline (200 μL, 1.18 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.3 mL), AlCl<sub>3</sub> (160 mg, 1.18 mmol) was added portionwise. The mixture was stirred at rt for 72 h. The solvent was evaporated under reduced pressure and the residue **28b** (LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.99 min, *m/z* 430 [M+H]<sup>+</sup>) was dissolved in ethanol (2.0 mL) and the solution was refluxed for 18 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2 to 5/5) to afford 2-(Trichloromethyl)-*N*-(3-(trifluoromethyl)phenyl)thieno[2,3-*d*]pyrimidin-4-amine.

Yield 15%. Pale blue solid. mp 124 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.42 (s, 1 H), 7.92 (d, *J* = 8.1 Hz, 1 H), 7.60 (d, *J* = 5.9 Hz, 1 H), 7.52 (t, *J* = 7.9 Hz, 1 H), 7.48-7.40 (m, 2 H), 7.35 (d, *J* = 5.9 Hz, 1 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 167.4, 160.4, 154.3, 138.7, 132.0, 131.3, 129.7, 127.6, 123.4, 120.9, 117.7, 116.6, 116.4, 97.2. Anal. Calcd for C<sub>14</sub>H<sub>7</sub>Cl<sub>3</sub>F<sub>3</sub>N<sub>3</sub>S: C, 40.75; H, 1.71; N, 10.18. Found: C, 41.03; H, 1.86; N, 10.24. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.22 min, *m/z* 412 [M+H]<sup>+</sup>.

#### 4.1.20. 4-((4-Chlorophenyl)thio)-2-(trichloromethyl)thieno[2,3-*d*]pyrimidine (29d)

HBr<sub>(g)</sub> was bubbled into a solution of 2,2,2-trichloro-*N*-(3-cyanothiophen-2-yl)acetamide **26** (270 mg, 1.0 mmol) and 4-chlorothiophenol (145 mg, 1.0 mmol) in diethyl ether (2.5 mL), at -20 °C, for 30 min. The bubbling was stopped when the mixture became homogeneous (bright yellow solution). The reaction was then stirred at rt for 18 h. Water (2.0 mL) was then added and the aqueous phase was extracted with EtOAc. The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2 + 5 mL of CH<sub>2</sub>Cl<sub>2</sub> every 100 mL of eluent) to afford a white solid **28d** (86 mg) (LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 4.46 min, *m/z* 413 [M+H]<sup>+</sup>) which was dissolved in ethanol (1.0 mL) and the resulting solution was refluxed for 5 minutes. The solvent was evaporated under reduced pressure to yield 4-((4-chlorophenyl)thio)-2-(trichloromethyl)thieno[2,3-*d*]pyrimidine. No further purification necessary.

Yield 22%. White solid. mp 150 °C (dec.). <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 7.70 (d, *J* = 6.0 Hz, 1 H), 7.61 (d, *J* = 8.6 Hz, 2 H), 7.46-7.42 (m, 3 H). <sup>13</sup>C NMR (50, MHz, CDCl<sub>3</sub>) δ = 166.5, 164.6, 159.5, 136.8, 136.3, 129.8, 129.5, 126.7, 125.1, 118.8, 96.7. Anal. Calcd for C<sub>13</sub>H<sub>6</sub>Cl<sub>4</sub>N<sub>2</sub>S<sub>2</sub>: C, 39.41; H, 1.53; N, 7.07. Found: C, 39.61; H, 1.61; N, 7.16. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.45 min, *m/z* 395 [M+H]<sup>+</sup>.

#### 4.1.21. *N*-(2,4-Dichlorophenyl)-2-trichloromethylquinoline-4-amine (30a)

To a solution of 4-chloro-2-(trichloromethyl)quinoline **H** [43] (281 mg, 1.0 mmol) and 2,4-dichloroaniline (160 mg, 1.0 mmol) in ethanol (5.0 mL) were added a few drops of concentrated HCl. The vial was then sealed and heated at 100 °C overnight. The mixture was allowed to cool to rt and the organic phase was then washed with a solution of NaOH 2N. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (gradient: Petroleum Ether 100% + 10 mL of EtOAc every 100 mL of eluent) to afford 158 mg of the expected *N*-(2,4-dichlorophenyl)-2-trichloromethylquinoline-4-amine.

Yield 39%. Orange powder. mp 121 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.22 (d, *J* = 8.3 Hz, 1 H), 8.02 (d, *J* = 8.3 Hz, 1 H), 7.84-7.76 (m, 1 H), 7.68-7.64 (m, 2 H), 7.54-7.51 (m, 1 H), 7.47 (s, 1 H), 7.35-7.29 (m, 1 H), 7.11 (s, 1 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 158.6, 147.5, 147.0, 135.4, 131.0, 130.3, 129.7, 128.3, 127.8, 126.9, 122.5, 119.8, 99.7, 98.1. Anal. Calcd for C<sub>16</sub>H<sub>9</sub>Cl<sub>5</sub>N<sub>2</sub>: C, 47.27; H, 2.23; N, 6.89. Found: C, 47.01; H, 2.28; N, 6.99. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.10 min, *m/z* 403 [M-H]<sup>-</sup>.

**4.1.22. 2-(Trichloromethyl)-N-[3-(trifluoromethyl)phenyl]quinoline-4-amine (30b)**

To a solution of 4-chloro-2-(trichloromethyl)quinoline **H** [43] (281 mg, 1.0 mmol) and 3-(trifluoromethyl)aniline (170  $\mu$ L, 1.0 mmol) in ethanol (5.0 mL) were added a few drops of concentrated HCl. The vial was then sealed and heated at 100 °C overnight. The mixture was allowed to cool to rt and the organic phase was then washed with a solution of NaOH 2N. The aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phases were dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated under reduced pressure. The residue was then purified by silica gel column chromatography (gradient: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2 + 5 mL of CH<sub>2</sub>Cl<sub>2</sub> every 100 mL of eluent) to afford 2-(trichloromethyl)-N-[3-(trifluoromethyl)phenyl]quinoline-4-amine.

Yield 73%. Yellow powder. mp 159 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.22 (d, *J* = 8.3 Hz, 1 H), 8.12 (d, *J* = 8.3 Hz, 1 H), 7.75 (t, *J* = 7.7 Hz, 1 H), 7.69 (s, 1 H), 7.57-7.42 (m, 6 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$  = 157.8, 149.0, 146.4, 140.0, 132.8, 131.3, 130.6, 130.1, 127.6, 125.3, 121.7, 120.3, 119.3, 119.1, 119.0, 98.8, 97.5. Anal. Calcd for C<sub>17</sub>H<sub>10</sub>Cl<sub>3</sub>F<sub>3</sub>N<sub>2</sub>: C, 50.34; H, 2.48; N, 6.91. Found: C, 50.73; H, 2.69; N, 6.66. LC-MS (ESI, 35 eV) *t*<sub>R</sub> = 5.09 min, *m/z* 403 [M-H]<sup>-</sup>.

**4.1.23. 4-(4-Chlorophenoxy)-2-(trichloromethyl)quinoline (30c)**

To a solution of 4-chlorophenol (141 mg, 1.1 mmol) in DMSO (1.0 mL), NaH (48 mg, 2.0 mmol) was added portionwise under inert atmosphere. The mixture was stirred at rt for 20 min. 4-chloro-2-(trichloromethyl)quinoline **H** [43] (281 mg, 1.0 mmol) was then added portionwise and the reaction was stirred for 24 h at 50 °C. The aqueous phase was then extracted with CH<sub>2</sub>Cl<sub>2</sub> and the organic phase was washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated. The residue was then purified by silica gel column chromatography (eluent Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 6/4) to afford 4-(4-chlorophenoxy)-2-(trichloromethyl)quinolone.

Yield 72%. White solid. mp 166 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  = 8.34 (d, *J* = 8.3 Hz, 1 H), 8.20 (d, *J* = 8.6 Hz, 1 H), 7.89-7.81 (m, 1 H), 7.71-7.64 (m, 1 H), 7.48 (d, *J* = 8.8 Hz, 2 H), 7.22 (s, 1 H), 7.18 (d, *J* = 8.8 Hz, 2 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$  = 162.6, 158.4, 152.6, 147.4, 131.3, 130.6, 130.0, 128.0, 122.2, 121.6, 121.0, 100.6, 97.7. Anal. Calcd for C<sub>16</sub>H<sub>9</sub>Cl<sub>4</sub>NO: C, 51.51; H, 2.43; N, 3.75. Found: C, 51.69; H, 2.53; N, 3.81. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.52 min, *m/z* 372 [M+H]<sup>+</sup>.

**4.1.24. 4-((4-Chlorophenyl)thio)-2-(trichloromethyl)quinoline (30d)**

To a solution of 4-chlorothiophenol (159 mg, 1.1 mmol) in DMSO (1.0 mL), NaH (48 mg, 2.0 mmol) was added portionwise under inert atmosphere. The mixture was stirred at rt for 20

min. 4-Chloro-2-(trichloromethyl)quinoline **H** [43] (281 mg, 1.0 mmol) was then added portionwise and the reaction was stirred for 24 h at rt. The aqueous phase was then extracted with CH<sub>2</sub>Cl<sub>2</sub> and the organic phase was washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated. The residue was then purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 8/2) to afford 4-((4-chlorophenyl)thio)-2-(trichloromethyl)quinolone.

Yield 32%. White powder. mp 151 °C. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) δ = 8.24-8.18 (m, 2 H), 7.87-7.78 (m, 1 H), 7.72-7.64 (m, 1 H), 7.57-7.46 (m, 5 H). <sup>13</sup>C NMR (50 MHz, CDCl<sub>3</sub>) δ = 156.8, 150.2, 145.4, 136.4, 136.1, 131.0, 130.8, 130.5, 129.3, 128.4, 127.7, 125.6, 123.2, 114.1, 97.7. Anal. Calcd for C<sub>16</sub>H<sub>9</sub>Cl<sub>4</sub>NS: C, 49.39; H, 2.33; N, 3.60. Found: C, 49.49; H, 2.23; N, 3.53. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.76 min, *m/z* 388 [M+H]<sup>+</sup>.

#### 4.1.25. *N*-(2,4-Dichlorophenyl)-3-methylquinoxalin-2-amine (31a)

To a solution of 2-chloro-3-methylquinoxaline **L** [45] (1.0 g, 5.6 mmol) and 2,4-dichloroaniline (0.91 g, 5.6 mmol) in anhydrous DMF (10 mL), Cs<sub>2</sub>CO<sub>3</sub> (1.82 g, 5.6 mmol) was added under inert atmosphere. The mixture was stirred at 70 °C for 24 h. After cooling, water then CH<sub>2</sub>Cl<sub>2</sub> were added. The organic layer was then washed five times with water and dried with Na<sub>2</sub>SO<sub>4</sub>. After filtration and evaporation, the resulting solid was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 7/3 then 1/1) to afford *N*-(2,4-dichlorophenyl)-3-methylquinoxalin-2-amine.

Yield 19%. White powder. mp 152 °C. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ = 9.06 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.67- 7.57 (m, 1H), 7.57- 7.30 (m, 4H), 2.78 (s, 3H). <sup>13</sup>C NMR (63 MHz, CDCl<sub>3</sub>) δ = 147.5, 145.2, 140.4, 138.0, 135.3, 129.6, 128.8, 128.4, 128.0, 127.6, 127.0, 126.4, 123.2, 121.4, 21.1. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.49 min, *m/z* 304 [M+H]<sup>+</sup>.

#### 4.1.26. 3-Methyl-*N*-(3-(trifluoromethyl)phenyl)quinoxalin-2-amine (31b)

A solution of 2-chloro-3-methylquinoxaline **L** [45] (500 mg, 2.8 mmol) and 3-trifluoromethylaniline (2.5 mL, 20 mmol) in anhydrous DMF (10 mL) was heated at 140 °C in a sealed vial under microwave irradiation. After completion of the reaction (45 min), CH<sub>2</sub>Cl<sub>2</sub> was added, and the organic phase was washed successively with 1M HCl and brine. The organic layer was then dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The resulting solid was purified by silica gel column chromatography (eluent: CH<sub>2</sub>Cl<sub>2</sub>) to afford 3-Methyl-*N*-(3-(trifluoromethyl)phenyl)quinoxalin-2-amine.

Yield 69%. Amber powder. mp 98 °C. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ = 8.24 (s, 1H), 7.93 (d, *J* = 8.1 Hz, 1H), 7.87 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.78 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.64 –

7.53 (m, 1H), 7.52-7.39 (m, 2H), 7.37- 7.27 (m, 1H), 6.77 (bs, 1H, NH), 2.65 (s, 3H).  $^{13}\text{C}$  NMR (63 MHz,  $\text{CDCl}_3$ )  $\delta$  = 147.7, 144.6, 140.3 (d,  $J$  = 3.2 Hz), 137.9, 131.5 (q,  $J$  = 33.1 Hz), 129.4 (d,  $J$  = 6.0 Hz), 128.2, 126.9, 126.1, 124.3 (q,  $J$  = 272.9 Hz), 122.8, 119.6 (d,  $J$  = 3.8 Hz), 116.6 (d,  $J$  = 3.4 Hz), 21.03. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.75 min,  $m/z$  304  $[\text{M}+\text{H}]^+$ .

#### 4.1.27. 2-(4-Chlorophenoxy)-3-methylquinoxaline (31c)

To a solution of 2-chloro-3-methylquinoxaline **L** [45] (650 mg, 3.6 mmol) and 4-chlorophenol (0.37 mL, 3.6 mmol) in anhydrous DMF (15 mL),  $\text{Cs}_2\text{CO}_3$  (1.19 g, 3.6 mmol) was added under inert atmosphere. The mixture was stirred at 70 °C overnight. After completion of the reaction, water was added, leading to a precipitate which was separated by filtration. The resulting precipitate was then thoroughly washed with water. The precipitate was dissolved in  $\text{CH}_2\text{Cl}_2$  and dried with  $\text{Na}_2\text{SO}_4$ . After filtration and evaporation, the resulting solid was purified by silica gel column chromatography (eluent: Petroleum Ether/ $\text{CH}_2\text{Cl}_2$  1/1) to afford 2-(4-chlorophenoxy)-3-methylquinoxaline.

Yield 85%. Off-white powder. mp 108 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.69 (dd,  $J$  = 6.1, 3.7 Hz, 1H), 7.57 (dd,  $J$  = 6.3, 3.5 Hz, 2H), 7.46-7.38 (m, 2H), 7.28 – 7.19 (m, 2H), 2.81 (s, 3H).  $^{13}\text{C}$  NMR (63 MHz,  $\text{CDCl}_3$ )  $\delta$  = 155.8, 151.5, 147.9, 139.6, 139.4, 130.6, 129.7, 129.4, 128.1, 127.6, 127.4, 123.3, 20.7. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 4.35 min,  $m/z$  271  $[\text{M}+\text{H}]^+$ .

#### 4.1.28. 2-((4-Chlorophenyl)thio)-3-methylquinoxaline (31d)

To a solution of 2-chloro-3-methylquinoxaline **L** [45] (500 mg, 2.8 mmol) and 4-chlorothiophenol (405 mg, 2.8 mmol) in anhydrous DMF (10 mL),  $\text{Cs}_2\text{CO}_3$  (912 mg, 2.8 mmol) was added under inert atmosphere. The mixture was stirred at 70 °C overnight. After completion of the reaction, water was added, leading to a precipitate which was separated by filtration. The resulting precipitate was then thoroughly washed with water. The precipitate was dissolved in  $\text{CH}_2\text{Cl}_2$  and dried with  $\text{Na}_2\text{SO}_4$ . After filtration and evaporation, the resulting solid was purified by silica gel column chromatography (eluent: Petroleum Ether/ $\text{CH}_2\text{Cl}_2$  1/1) to afford 2-((4-chlorophenyl)thio)-3-methylquinoxaline.

Yield 83%. Beige powder. mp 118 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  = 7.96-7.92 (m, 1H), 7.72-7.68 (m, 1H), 7.60-7.54 (m, 4H), 7.44 (d,  $J$  = 6.6 Hz, 2H), 2.77 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  = 155.3, 151.4, 141.4, 140.0, 136.7, 135.6, 129.5, 129.2, 128.6, 128.3, 128.1, 127.2, 22.4. LC-MS (ESI, 35 eV):  $t_{\text{R}}$  = 5.51 min,  $m/z$  287  $[\text{M}+\text{H}]^+$ .

#### 4.1.29. 2-(4-Chlorophenoxy)-3-(trichloromethyl)quinoxaline (32c)

To a solution of 2-(4-chlorophenoxy)-3-methylquinoxaline **31c** (624 mg, 2.8 mmol) and  $\text{PCl}_5$  (2.88 g, 16.8 mmol),  $\text{POCl}_3$  was added to make a slurry (ca 5 mL). The mixture was then

heated in a multimode microwave oven at 100 °C, 800 W for 20 min. After completion of the reaction, the mixture was poured into ice and was then neutralized with Na<sub>2</sub>CO<sub>3</sub>. The resulting solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> and dried with Na<sub>2</sub>SO<sub>4</sub>. After filtration and evaporation, the resulting solid was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 7/3) to afford 2-(4-chlorophenoxy)-3-(trichloromethyl)quinoxaline.

Yield 72%. White powder. mp 108 °C. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ = 8.11 (d, *J* = 8.0 Hz, 1H), 7.72-7.63 (m, 3H), 7.38 (dd, *J* = 6.6, 3.5 Hz, 2 H), 7.22 (dd, *J* = 6.6, 3.5 Hz, 2 H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ = 152.5, 151.2, 142.4, 141.2, 137.0, 132.3, 131.1, 129.9, 129.8, 128.8, 127.1, 123.2, 94.9. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 5.63 min, (no ionization). HRMS (ESI): *m/z* calcd. for C<sub>15</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>2</sub>O [M+H]<sup>+</sup>: 372.9464. Found: 372.9461. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>2</sub>O: C, 48.16; H, 2.16; N, 7.49. Found: C, 48.37; H, 2.32; N, 7.42.

#### 4.1.30. 2-((4-Chlorophenyl)thio)-3-(trichloromethyl)quinoxaline (32d)

To a solution of 2-((4-chlorophenyl)thio)-3-methylquinoxaline **31d** (623 mg, 2.2 mmol) and PCl<sub>5</sub> (2.7 g, 13.0 mmol), POCl<sub>3</sub> was added to make a slurry (ca 6 mL). The mixture was then heated in a multimode microwave oven at 100 °C, 800 W for 20 min. After completion of the reaction, the mixture was poured into ice and was then neutralized with Na<sub>2</sub>CO<sub>3</sub>. The resulting solution was extracted with CH<sub>2</sub>Cl<sub>2</sub> and dried with Na<sub>2</sub>SO<sub>4</sub>. After filtration and evaporation, the resulting solid was purified by silica gel column chromatography (eluent: Petroleum Ether/CH<sub>2</sub>Cl<sub>2</sub> 9/1) to afford 2-((4-chlorophenyl)thio)-3-(trichloromethyl)quinoxaline.

Yield 72%. Yellow powder. mp 92 °C. <sup>1</sup>H NMR (250 MHz, CDCl<sub>3</sub>) δ = 8.15- 8.04 (m, 1H), 7.76-7.64 (m, 3H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.43 (d, *J* = 8.6 Hz, 2H). <sup>13</sup>C NMR (63 MHz, CDCl<sub>3</sub>) δ = 152.9, 147.7, 142.0, 137.2, 137.0, 136.0, 132.1, 129.9, 129.8, 129.5, 128.7, 127.8, 96.7. LC-MS (ESI, 35 eV): *t*<sub>R</sub> = 6.59 min, *m/z* 389 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>15</sub>H<sub>8</sub>Cl<sub>4</sub>N<sub>2</sub>S: C, 46.18; H, 2.07; N, 7.18. Found: C, 46.33; H, 2.26; N, 6.97.

## 4.2. Biology

### 4.2.1. *In vitro* Antiplasmodial evaluation

In this study, a K1 culture-adapted *P. falciparum* strain resistant to chloroquine, pyrimethamine and proguanil was used in an *in vitro* culture. Maintenance in continuous culture was done as described previously by Trager and Jensen [48]. Cultures were maintained in fresh A+ human erythrocytes at 2.5% hematocrit in complete medium (RPMI 1640 with 25 mM HEPES, 25 mM NaHCO<sub>3</sub>, 10% of A+ human serum) at 37 °C under reduced O<sub>2</sub> atmosphere (gas mixture 10% O<sub>2</sub>, 6% CO<sub>2</sub>, and 84% N<sub>2</sub>). 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 [49] using a 96-well fluorescence plate reader. Compounds, previously dissolved in DMSO (final concentration less than 0.5% v/v) were incubated in a total assay volume of 200  $\mu$ L of synchronized culture suspension (2% hematocrit and 0.4% parasitaemia) for 72 h in a humidified atmosphere (84% N<sub>2</sub>, 10% O<sub>2</sub> and 6% CO<sub>2</sub>) at 37 °C, in 96-well flat bottom plates. Duplicate assays were performed for each sample. After incubation, 125  $\mu$ L supernatant was discarded and cells were washed twice with 125  $\mu$ L 1X PBS. 15  $\mu$ L re-suspended cells were transferred to 96-well flat bottom nonsterile black plates (Greiner Bio-one) already containing 15  $\mu$ L of the SYBR Green I lysis buffer (2X SYBR Green I, 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<sub>2</sub>O) and positive controls (atovaquone, chloroquine and 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 485 and 535 nm, respectively. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC<sub>50</sub> K1) were calculated from three independent experiments.

#### **4.2.2. *In vitro* Cytotoxicity evaluation**

HepG2 cell line was maintained at 37 °C, 6% CO<sub>2</sub> with 90% humidity in RPMI supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL) / streptomycin (100  $\mu$ g/mL) (complete RPMI medium). The evaluation of the tested molecules cytotoxicity on the HepG2 (hepatocarcinoma cell line purchased from ATCC, ref HB-8065) cell line was performed according to the method of Mosmann [50] with slight modifications. Briefly, 5.10<sup>3</sup> cells in 100  $\mu$ L of complete medium were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO<sub>2</sub>. After 24 h incubation, 100  $\mu$ L of medium with various product concentrations dissolved in DMSO (final concentration less than 0.5% v/v) were added and the plates were incubated for 72 h at 37 °C. Triplicate assays were performed for each sample. Each plate-well was then microscope-examined for detecting possible precipitate formation before the medium was aspirated from the wells. 100  $\mu$ L of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (0.5 mg/mL in medium without FCS) were then added to each well. Cells were incubated for 2 h at 37 °C. After this time, the MTT solution was removed and DMSO (100  $\mu$ L) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (700 rpm) for 10 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength using a

BIO-TEK ELx808 Absorbance Microplate Reader. DMSO was used as blank and doxorubicin (purchased from Sigma Aldrich) as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration (CC<sub>50</sub>) was determined from the dose–response curve by using the TableCurve software 2D v.5.0. CC<sub>50</sub> values represent the mean value calculated from three independent experiments.

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**Highlights:**

► Antiplasmodial pharmacomodulation of CCl<sub>3</sub>-substituted-nitrogen containing heterocycles was made. ► Thienopyrimidine derivatives appeared more cytotoxic. ► Original 3-substituted-2-trichloromethylquinoxaline analogs were prepared. ► Two quinoxaline derivatives displayed *in vitro* IC<sub>50</sub> values of 0.4 and 0.5 μM on the K1 multi-resistant *P. falciparum* strain. ► Cytotoxicity was assessed on the human HepG2 cell line showing low cytotoxicity (CC<sub>50</sub> ~ 40 μM) and improved selectivity indices (77-100).