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Synthesis and in vitro antiplasmodial evaluation of 4-anilino-2-trichloromethylquinazolines

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ABSTRACT

To identify a new safe antiplasmodial molecular scaffold, an original series of 2-trichloromethylquinazolines, functionalized in position 4 by an alkyl- or arylamino substituent, was synthesized from 4-chloro-2-trichloromethylquinazoline **1**, via a cheap, fast and efficient solvent-free operating procedure. Among the 40 molecules prepared, several exhibit a good profile with both a significant antiplasmodial activity on the W2 *Plasmodium falciparum* strain (IC_{50} values: 0.4–2.2 μM) and a promising toxicological behavior regarding human cells (HepG2/W2 selectivity indexes: 40–83), compared to the antimalarial drug compounds chloroquine and doxycycline. The in vitro antitoxoplasmic and antileishmanial evaluations were conducted in parallel on the most active molecules, showing that these ones specifically display antiplasmodial properties.

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1. Introduction

In the World Malaria Report 2008,[1] the W.H.O. estimated that 247 million people suffered from this parasitic infectious disease world-wide in 2006, with about a million deaths mainly among African children under 5 years of age. This led to the drafting of a series of international recommendations presented in the W.H.O. Global Malaria Action Plan,[2] where in 'opportunities to improve treatments', the W.H.O. requests 'active ingredients with lower cost of goods for treatment of *Plasmodium falciparum* malaria'.

Moreover, anticipating the future emergence of parasites resistant to the current lead treatment for *P. falciparum* malaria (artemisinin-based combination therapies), the W.H.O. calls for 'a robust pipeline of new medicines..., including non-artemisinin-based combinations with novel mechanisms of action'. In such a context it appears that university academic research teams have a major role to play by offering new original therapeutic candidates meeting all these requirements, in order to effectively assist patients in developing countries suffering from malaria.

The trichloromethyl group is already known to confer antiplasmodial activity to various molecular series, such as benzene, triazine or oxadiazole.[3] Recently, some 4-anilino-substituted quinazolines have also shown a quite interesting antiplasmodial activity against the 3D7 chloroquine-sensitive *P. falciparum* strain.[4] Our team, which is particularly involved in the research of new potential anti-infectious agents,[5] showed in 2008 that the 4-aryl-2-trichloromethylquinazoline series (Fig. 1) displays interesting in vitro antiplasmodial properties on the W2 chloroquine-resistant *P. falciparum* strain, combined with a safe toxicological profile on two human cell lines: THP1 (monocyte) and HepG2 (hepatocyte).[6]

Starting from these preliminary encouraging results, we decided to explore the antiplasmodial potential (W2 strain) of the 4-aminosubstituted-2-trichloromethylquinazoline series. In parallel, in order to determine the selectivity indexes of our molecules, we studied their in vitro toxicity toward two human cell

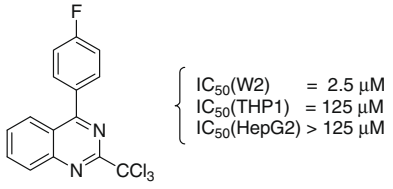

Figure 1. Hit compound in the 4-aryl-2-trichloromethyl-quinazoline series.

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lines: K526 and HepG2. This latter cell line allows a thorough evaluation of the cytotoxic behavior of the tested molecules, following their metabolic activation.[7]

2. Results and discussion

2.1. Chemistry

In order to prepare these molecules, the most adapted chemical starting material appeared to be the 4-chloro-2-trichloromethylquinazoline **1** which was synthesized via a microwave-assisted tetrachlorination protocol that we previously reported.[8]

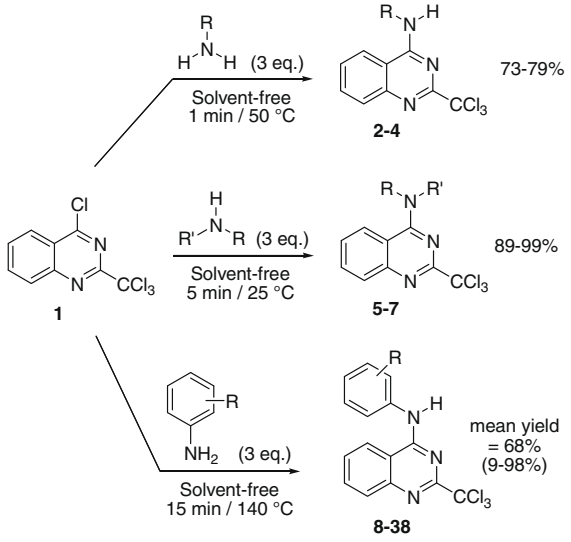
We decided to react **1** successively with 3 primary amines, 3 secondary amines and 3 aromatic amines, so as to compare their antiplasmodial potential and select the most suitable amine category for preparing a complete series of promising derivatives. Focusing on setting up a simple, cheap, fast and efficient synthesis access, we chose to work on the definition of a general operating procedure for each amine category.

Thus, the reaction of **1** with primary amines, consisting in a fusion reaction with an excess of amine, was conducted according to the reaction presented in Scheme 1, reducing as much as possible both reaction time and temperature, so as to preserve the CCl_3 group from reacting. This rapid reaction protocol resulted in the formation of secondary aminoquinazolines **2–4** in good yields.

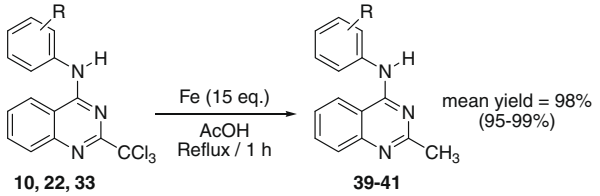
Substrate **1** was reacted very easily with secondary amines, following the procedure as above type of and conducted to the preparation of tertiary aminoquinazolines **5–7** in very good yields (Scheme 1).

Logically, the reaction of **1** with anilines required stronger operating conditions, elevated temperature and longer reaction time to give the expected products in good yields, except for compounds **31** and **36** for which reaction yields were, respectively 25% and 35%, and particularly compound **32**, with a reaction yield of only 9%, probably due to the steric hindrance affecting the amino group on the 2,6-dichloro-bisubstituted-aniline. This reaction was initially carried out for the preparation of compounds **8**–**10** and then applied to prepare a series of 31 molecules (**8**–**38**), as presented in Scheme 1.

Three 2-dehalogenomethylated-quinazolines (**39–41**) were also prepared from the corresponding 2-trichloromethylated-quinazolines (**10, 22, 33**), through a reduction reaction (Scheme 2), in order to compare the biological results and to demonstrate the key role played by the CCl_3 group toward antiplasmodial activity.



Scheme 1. Operating procedures for the reaction of **1** with each category of amines.



Scheme 2. Preparation of compounds **39**–**41** by reductive dehalogenation of the corresponding CCl_3 substrates.

2.2. In vitro antiplasmodial activity

As explained above, the initial step of our study consisted in choosing, through the biological results obtained with the first 9 preliminary synthesized compounds (**2–10**), the most relevant category of amine reagent (I, II, or anilines), so as to prepare an homogenous complete series, with optimal potential.

The antiplasmodial inhibitory concentrations 50% (IC_{50}) of these molecules, toward the W2 *P. falciparum* strain ranged from 5.1 to 14.3, 5.6 to 41.5 and 1.7 to 11 μM, respectively for the compounds derived from primary amines, secondary amines and anilines, pointing out a relative lack of efficiency for molecules **5–7**, obtained via the reaction of **1** with secondary amines (Table 1).

Cytotoxicity experiments conducted on the K562 cell line were not discriminating, with no significant antiproliferative effect measured for any of the tested molecules at the tested concentrations, whereas tests on the HepG2 cell line showed that molecules (**2–4**) derived from primary amines, display some toxicity (Table 1). Thus, compounds **8–10** being overall both the most active and less toxic molecules, we decided to focus our work on the study of the 4-anilino-2-trichloromethylquinazoline series and prepared 31 additional molecules (**11–41**) for structure–activity relationship purposes.

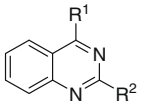
If we consider the W2 antiplasmodial inhibitory concentrations determined in 4-anilino-2-trichloromethylquinazoline series (Table 1), we first note all IC_{50} were located in a narrow range of values, from 0.4 μM (molecule **33**) to 22 μM (molecule **32**). Most of the antiplasmodial IC_{50} values of these compounds are comparable with the ones measured for chloroquine (0.7 μM) or doxycycline (6.5 μM), two antiplasmodial drug compound references presenting separate mechanisms of action.

In comparison with compound **8** which presents an unsubstituted aniline moiety (IC_{50} = 11 μM), it appears that the substitution of the aniline moiety increases effectivity, whatever the substituent or the substitution position, apart for compounds **31** and **32**.

The observation of compounds for which the aniline ring is monosubstituted indicates a global better activity for *para* or *meta*-aniline-substituted compounds, except those with nitro or methoxy groups for which no effect of the substitution position was noted on the antiplasmodial activity (compounds **11**, **16**, **17**, **24–26**). Among these *para* or *meta*-aniline-substituted molecules, it also appears that bromine, chlorine and trifluoromethyl groups are the substituents which most contribute to increasing the antiplasmodial activity (1.5–3 μM).

Then, if we have a look at the molecules in which the aniline moiety is bisubstituted (molecules **30–36**), we surprisingly note that this subgroup is very heterogeneous, including both the best and the worst activity values (respectively compounds **33** and **32**) and, except for compound **33** (the most potent in the series), the double substitution of the aniline ring does not improve antiplasmodial activity, in comparison with monosubstituted derivatives, even when the most promising groups (Cl or CF_3) are combined on the two *meta*-aniline positions (molecules **34** and **36**).

Table 1
Antiplasmodial activity and human cell toxicity of the studied molecules



Molecule	R_1-	R_2-	W2 Antiplasmodial IC_{50} activity (μM)	Human cell toxicity[a] (μM)		W2 strain selectivity index toward K562[b]	W2 strain selectivity index toward HepG2[b]
				K562 IC_{50}	HepG2 IC_{50}		
2	*n*-Butyl-NH–	$-CCl_3$	14.3	>50[f]	16	>3.5	1.1
3	Cycloheptyl-NH–	$-CCl_3$	5.1	>50[f]	19	>9.8	3.7
4	Benzyl-NH–	$-CCl_3$	6.9	>50[f]	13	>7.2	1.9
5	*N*-Morpholinyl–	$-CCl_3$	14.9	>125[f]	63	>8.4	>2
6	*N*-Pyrrolidinyl–	$-CCl_3$	41.5	>125[f]	>250[f]	>3	>6
7	4-CH_3-piperazin-1-yl–	$-CCl_3$	5.6	65	75	11.6	13.4
8	Ph-NH–	$-CCl_3$	11	>50[f]	>50[f]	>4.5	>4.5
9	4-F-Ph-NH–	$-CCl_3$	5.4	>125[f]	>50[f]	>23.1	>9.3
10	**3-Cl-Ph-NH–**	**$-CCl_3$**	**1.7**	**>50**[f]	**>100**[f]	**>29.4**	**>58.8**
11	2-NO_2-Ph-NH–	$-CCl_3$	3	>60[f]	>100[f]	>20	>33.3
12	2-Cl-Ph-NH–	$-CCl_3$	4.6	>50[f]	25	>10.9	5.5
13	4-Cl-Ph-NH–	$-CCl_3$	3	14	9	4.7	3
14	2-F-Ph-NH–	$-CCl_3$	9	30	10	3.3	1.1
15	3-F-Ph-NH–	$-CCl_3$	4	15	16	3.8	4
16	3-NO_2-Ph-NH–	$-CCl_3$	3	5	10	1.7	3.3
17	4-NO_2-Ph-NH–	$-CCl_3$	2.8	5	6	1.8	2.1
18	2-Br-Ph-NH–	$-CCl_3$	3	>30[f]	>50[f]	>10	>16.7
19	**3-Br-Ph-NH–**	**$-CCl_3$**	**2.2**	**>50**[f]	**>100**[f]	**>22.7**	**>45.5**
20	4-Br-Ph-NH–	$-CCl_3$	1.5	15	10	10	6.7
21	2-CF_3-Ph-NH–	$-CCl_3$	7.4	30	10	4.1	1.4
22	**3-CF_3-Ph-NH–**	**$-CCl_3$**	**1.8**	**>125**[f]	**150**[f]	**>69.4**	**83.3**
23	4-CF_3-Ph-NH–	$-CCl_3$	1.6	4	6	2.5	3.8
24	2-OCH_3-Ph-NH–	$-CCl_3$	6	60	60	10	10
25	3-OCH_3-Ph-NH–	$-CCl_3$	4	9	0.5	2.3	0.1
26	4-OCH_3-Ph-NH–	$-CCl_3$	6	8	7	1.3	1.2
27	2-CH_3-Ph-NH–	$-CCl_3$	7	11	24	1.6	3.4
28	3-CH_3-Ph-NH–	$-CCl_3$	2.3	9	12	3.9	5.2
29	4-CH_3-Ph-NH–	$-CCl_3$	2.4	6	8	2.5	3.3
30	3,4-Di-Cl-Ph-NH–	$-CCl_3$	5	9	8	1.8	1.6
31	2,3-Di-Cl-Ph-NH–	$-CCl_3$	14	>60[f]	>100[f]	>4.3	7.1
32	2,6-Di-Cl-Ph-NH–	$-CCl_3$	21.9	10	23	0.5	1.1
33	**2,4-Di-Cl-Ph-NH–**	**$-CCl_3$**	**0.4**	**10**	**16**	**25**	**40**
34	3,5-Di-Cl-Ph-NH–	$-CCl_3$	4	9	10	2.2	2.5
35	2,5-Di-Cl-Ph-NH–	$-CCl_3$	1.7	>8[f]	30	>4.7	17.6
36	3,5-Di-CF_3-Ph-NH–	$-CCl_3$	3	10	3	3.3	1
37	4-OCF_3-Ph-NH–	$-CCl_3$	2.9	6	6	2.1	2.1
38	1-Naphthyl-NH–	$-CCl_3$	6	5	15	0.8	2.5
39	3-Cl-Ph-NH–	$-CH_3$	55	27	40	0.5	0.7
40	3-CF_3-Ph-NH–	$-CH_3$	42.5	10	20	0.2	0.5
41	2,4-Di-Cl-Ph-NH–	$-CH_3$	85	15	15	0.2	0.2
Ref.	Chloroquine[c]		0.7	32	30	45.7	42.9
Ref.	Doxycycline[d]		6.5	15	20	2.3	3.1
Ref.	Doxorubicin[e]		—	0.06	0.2	—	—

In bold: hit compounds in the series.
[a] Mean of three independent experiments.
[b] Selectivity index was calculated according to the following formula: $SI_{W2 Plasmodium}$ = Human cell IC_{50}/W2 *Plasmodium* IC_{50}.
[c,d] Chloroquine and doxycycline were used as antiplasmodial drug compounds of reference.
[e] Doxorubicin was used as a drug compound of reference for human cell toxicity.
[f] No toxicity noted at the highest concentration tested.

In the 4-anilino-2-trichloromethylquinazoline series, the most important structural element for conferring activity remains the essential influence of the trichloromethyl group, as demonstrated with dehalogenated molecules **39–41** which have completely lost antiplasmodial activity (IC_{50} = 42.5–85 μM).

2.3. In vitro human cell toxicity assessment

Contrary to the results obtained for the compounds derived from primary amines (**2–4**), for which no toxicity was noted on the K562 cell line while a light toxicity was detected on the HepG2 cell line, the results reported on these two distinct cell lines in 4-anilino-2-trichloromethylquinazoline series were quite similar. This indicates that the metabolism of these derivatives by HepG2 cells does not modify their toxicological behavior.

Thus, considering together HepG2 and K562 cell lines, the spectrum of IC_{50} values appears rather broad, in the 0.5–150 μM range, traducing a large diversity in the toxicological profile inside the 4-anilino-2-trichloromethylquinazoline series. In the same series, 9 molecules presented IC_{50} values superior to 50 μM on both human cell lines in comparison with the two antiplasmodial reference drugs tested for which toxicity values are comprised in between 15 and 32 μM on the two same human cell lines.

Among molecules for which the aniline ring is monosubstituted, toxicity seems to be essentially influenced by the substitution position on the aniline ring and little by the nature of substituents, even if the methyl group seems to promote cytotoxicity. In this way, apart for compound **9**, the *para* position is involved in elevated toxicity whereas the *ortho* position appears as related with the less toxic molecules, apart for compounds **14** and **21**.

In the bisubstituted aniline moiety subgroup, only one molecule (**31**) does not display any toxicity toward these two human cell lines.

2.4. Hit molecules in the series

Taking into account the antiplasmodial activity and the toxicity of the synthesized molecules, their selectivity indexes (ratio IC_{50} human cell line/IC_{50}W2 *P. falciparum*.) were calculated to identify compounds with therapeutic potential. In the target 4-anilino-2-trichloromethylquinazoline series, 5 molecules reveal selectivity indexes included in the >23–83 range, considering the two human cell lines tested, in comparison with chloroquine and doxycycline which, respectively display selectivity indexes of 43–46 and 2.3–3.1.

As a logical consequence in matching both activity and toxicity structural criterias, the molecules substituted by a bromine, chlorine or CF_3 group on the *meta* position of the aniline moiety are the most promising (molecules **10**, **19** and **22**).

Despite a non negligible toxicity, compound **33**, maintains good selectivity indexes (25 and 40) because of its high antiplasmodial activity (IC_{50}W2 = 0.4 μM).

The conversion from trichloromethyl to methyl group, operated with molecules **39–41** for comparison purposes, leads to the complete inversion of the selectivity indexes (0.2–0.7), which clearly vouches for its crucial role in the antiplasmodial scaffold.

2.5. Complementary investigations

After establishing the precise structure of our in vitro antiplasmodial pharmacophore, designed as 2-trichloromethyl-*N*-(3-substituted-phenyl)quinazolin-4-amine with Br, Cl or CF_3 as substituents, we wanted to further investigate its toxicological profile by testing its mutagenic potential. Compounds **10**, **19**, **22** and **33** were evaluated for their mutagenicity via the Ames test, at a concentration of 5 mM, on 4 distinct *Salmonella typhimurium* strains, in two different conditions including metabolic activation (S9 mix). None of these molecules presented mutagenic activity (Table 2).

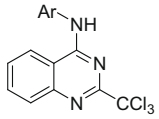
We then wanted to explore the antiparasitic spectrum of this molecular scaffold toward other protozoa. Thus, molecules **10**, **19**, **22** and **33** were evaluated in vitro against the *Leishmania donovani* promastigotes. None of them displayed any antileishmanial activity, their IC_{50} values being superior to 50 μM (Table 2).

Then, the same molecules were tested on *Toxoplasma gondii*. Once again, none of these 4 molecules exerted any activity against this apicomplexan related to *Plasmodium*, (IC_{50} >50 μM, Table 2). This last information is more surprising than the lack of efficacy toward *Leishmania*, as several antimalarial drug compounds such as atovaquone[9,10] or doxycycline[11] are well-known for being used in the treatment of toxoplasmosis, particularly in immunosuppressed patients. Therefore, this new antiprotozoal molecular scaffold shows its selective antiplasmodial activity.

3. Conclusion

We identified a new cheap antiplasmodial molecular scaffold, quickly and easily prepared in two steps from commercially available reagents, in the 4-anilino-2-trichloromethylquinazoline series. Depending on the aniline moiety substitution, the structure–activity relationships reveal that 4 molecules (**10**, **19**, **22** and **33**) in the series present both a potent antiplasmodial activity on the W2 chloroquine-resistant *P. falciparum* strain (IC_{50} = 0.4–2.2 μM) in comparison with chloroquine (IC_{50} = 0.7 μM) and doxycycline (IC_{50} = 6.5 μM) and a safe toxicological profile toward two human cell lines, affording high selectivity indexes (>23–83). In this scaffold, the trichloromethyl group appears to be key to both potent activity and low toxicity. Moreover, none of these molecules presents any mutagenic activity via the Ames test, at a concentration of 5 mM. Furthermore, these molecules present a specific action on *P. falciparum*. Considering the partial ATP-competitive structure of our scaffold,[12] *Plasmodium* kinases appear interesting targets to investigate so as to determine the antiplasmodial mechanism of action of our molecules. Such hypothesis is currently under active investigation.

Table 2
Survey of the antiparasitic profile displayed by the most promising compounds



Molecule	Ar-	W2 Antiplasmodial activity IC_{50} (μM)	Antitoxoplasmic activity IC_{50} (μM)	Antileishmanial activity Promastigotes IC_{50} (μM)	Human cell toxicity[a] (μM)		Mutagenicity (AmesTest)
					K562 IC_{50}	HepG2 IC_{50}	
10	3-Cl-Ph–	1.7	>50[g]	>50[g]	>50[h]	>100[h]	Negative
19	3-Br-Ph–	2.2	>50[g]	>50[g]	>50[h]	>100[h]	Negative
22	3-CF_3-Ph–	1.8	>50[g]	>50[g]	>125[h]	150	Negative
33	2,4-Di-Cl-Ph–	0.4	>50[g]	>50[g]	10	16	Negative
Ref.	Chloroquine[b]	0.7	—	—	32	30	—
Ref.	Doxycycline[c]	6.5	—	—	15	20	—
Ref.	AmphotericinB[d]	—	—	0.08	17	10	—
Ref.	Pyrimethamine[e]	—	2	—	5	30	—
Ref.	Doxorubicin[f]	—	—	—	0.06	0.2	—

[a] Mean of three independent experiments.
[b,c] Chloroquine and doxycycline were used as antiplasmodial drug compounds of reference.
[d] Amphotericin B was used as the antileishmanial drug compound of reference.
[e] Pyrimethamine was used as the antitoxoplasmic drug compound of reference.
[f] Doxorubicin was used as the drug compound of reference for human cell toxicity.
[g] No activity noted at the highest concentration tested.
[h] No toxicity noted at the highest concentration tested.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points were determined in open capillary tubes with a Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined on a Bruker Avance 200 MHz instrument, at the Faculté de Pharmacie de Marseille. Chemical shifts are given in δ values referenced to the solvent. High resolution mass spectra were recorded on a QStar Elite or a JEOL JMS GCMate spectrometer at the Spectropôle department of the Faculté des Sciences et Techniques de St Jérôme or at the Centre d'Etudes et de Recherche sur le Médicament de Normandie. Elemental analyses were carried out with a Thermo Finnigan EA 1112 apparatus at the Spectropôle department of the Faculté des Sciences et Techniques de St Jérôme. Silica Gel 60 (Merck 70–230) was used for column chromatography. The progress of the reactions was monitored by thin layer chromatography on using Kieselgel 60 F254 (Merck) plates.

4.1.2. 4-Chloro-2-trichloromethylquinazoline (1)

Compound **1** was synthesized according to a microwave-assisted procedure that we previously reported,[8] and obtained as a white solid in 75% yield; mp 127 °C (Lit.[13] mp 125 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.82–7.90 (m, 1H), 8.03–8.12 (m, 1H), 8.22 (dd, J = 8.5 and 0.5 Hz, 1H), 8.36 (dd, J = 8.5 and 0.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 164.0 (C), 159.9 (C), 150.2 (C), 135.9 (CH), 130.6 (CH), 129.7 (CH), 126.0 (CH), 122.9 (C), 95.9 (C). Anal. Calcd for $C_9H_4Cl_4N_2$: C, 38.34; H, 1.43; N, 9.94. Found: C, 38.25, H, 1.41; N, 9.60.

4.1.3. General procedure for the preparation of compounds (2) and (3)

Three equivalents of the appropriate primary amine reagent were added dropwise onto 1 equiv of 4-chloro-2-trichloromethylquinazoline **1**. The solvent-free reaction mixture was then stirred at 50 °C for 1 min. The crude residue was then extracted with dichloromethane. The organic layer was washed with water three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the corresponding nucleophilic aromatic substitution product.

4.1.4. *N*-Butyl-2-(trichloromethyl)quinazolin-4-amine (2)

Compound **2** was obtained as a white solid in 73% yield; mp 113 °C (Lit[14] mp = 113–114 °C). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 0.91 (t, J = 7.2 Hz, 3H), 1.30–1.48 (m, 2H), 1.60–1.75 (m, 2H), 3.70 (q, J = 6.7 Hz, 2H), 6.57 (br s, 1H), 7.39–7.47 (m, 1H), 7.63–7.71 (m, 1H), 7.85–7.89 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.3 (C), 160.4 (C), 148.8 (C), 133.0 (CH), 128.7 (CH), 127.1 (CH), 121.0 (CH), 113.5 (C), 98.2 (C), 41.1 (CH_2), 31.0 (CH_2), 19.9 (CH_2), 13.7 (CH_3). Anal. Calcd for $C_{13}H_{14}Cl_3N_3$: C, 49.00; H, 4.43; N, 13.19. Found: C, 49.50, H, 4.62; N, 13.32.

4.1.5. *N*-Cycloheptyl-2-trichloromethylquinazolin-4-amine (3)

Compound **3** was obtained as a white solid in 78% yield; mp 151 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.60–1.65 (m, 8H), 2.15–2.19 (m, 4H), 4.43–4.44 (m, 1H), 5.82–5.85 (m, 1H), 7.48–7.56 (m, 1H), 7.70–7.80 (m, 2H), 7.94–7.98 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.4 (C), 159.2 (C), 149.1 (C), 133.0 (CH), 129.5 (CH), 127.2 (CH), 120.4 (CH), 113.6 (C), 98.2 (C), 52.6 (CH), 34.4 (CH_2^*2), 28.1 (CH_2^*2), 24.4 (CH_2^*2). Anal. Calcd for $C_{16}H_{18}Cl_3N_3$: C, 53.58; H, 5.06; N, 11.71. Found: C, 53.35, H, 5.13; N, 12.00.

4.1.6. *N*-Benzyl-2-trichloromethylquinazolin-4-amine (4)

Compound **4** was prepared by reacting 570 mg (5.34 mmol, 3 equiv) of benzylamine with 500 mg (1.78 mmol, 1 eq.) of 4-chloro-2-trichloromethylquinazoline **1**, without any solvent, at 50 °C for 1 min. The reaction mixture was then extracted with dichloromethane. The organic layer was washed with water three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford a crude residue with was purified by chromatography on a silica gel, eluting with dichloromethane. **4** was obtained as a white solid in 79% yield; mp 102 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.94–4.96 (m, 2H), 6.59 (br s, 1H), 7.30–7.41 (m, 3H), 7.47–7.52 (m, 3H), 7.71–7.81 (m, 2H), 8.00–8.04 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.9 (C), 160.1 (C), 148.5 (C), 137.8 (C), 133.4 (CH), 129.1 (CH), 128.8 (CH^*2), 128.6 (CH^*2), 127.9 (CH), 127.6 (CH), 120.8 (CH), 113.4 (C), 97.6 (C), 45.7 (CH_2). Anal. Calcd for $C_{16}H_{12}Cl_3N_3$: C, 54.49; H, 3.43; N, 11.92. Found: C, 54.31, H, 3.53; N, 11.72.

4.1.7. General procedure for the preparation of compounds (5), (6) and (7)

Three equivalents of the appropriate secondary amine reagent were added dropwise onto 1 equiv of 4-chloro-2-trichloromethylquinazoline **1**. The solvent-free reaction mixture was then stirred at room temperature for 5 min. The crude residue was then extracted with dichloromethane. The organic layer was washed with water three times, dried over anhydrous Na_2SO_4 and concentrated in vacuo to afford the corresponding nucleophilic aromatic substitution product.

4.1.8. 4-(2-Trichloromethylquinazolin-4-yl)morpholine (5)

Compound **5** was obtained as a yellow solid in 99% yield; mp 101 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.86–3.98 (m, 8H), 7.50–7.58 (m, 1H), 7.76–7.85 (m, 1H), 7.89–7.93 (m, 1H), 8.02–8.07 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 164.6 (C), 160.0 (C), 151.6 (C), 133.1 (CH), 129.6 (CH), 126.7 (CH), 124.5 (CH), 114.8 (C), 97.6 (C), 66.5 (CH_2^*2), 50.0 (CH_2^*2). Anal. Calcd for $C_{13}H_{12}Cl_3N_3O$: C, 46.94; H, 3.64; N, 12.63. Found: C, 46.62, H, 3.67; N, 12.71.

4.1.9. 4-(Pyrrolidin-1-yl)-2-trichloromethylquinazoline (6)

Compound **6** was obtained as a white solid in 89% yield; mp 130 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.05–2.11 (m, 4H), 3.98–4.05 (m, 4H), 7.41–7.50 (m, 1H), 7.70–7.77 (m, 1H), 7.94–7.98 (m, 1H), 8.18–8.22 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 160.1 (C), 151.5 (C), 132.4 (CH), 129.1 (CH), 125.8 (CH), 125.1 (CH), 115.2 (C), 98.2 (C), 51.0 (CH_2^*2), 25.6 (CH_2^*2). Anal. Calcd for $C_{13}H_{12}Cl_3N_3$: C, 49.32; H, 3.82; N, 13.27. Found: C, 49.58, H, 3.92; N, 13.27.

4.1.10. 4-(4-Methylpiperazin-1-yl)-2-trichloromethylquinazoline (7)

Compound **7** was obtained as a brown solid in 99% yield; dec. 90 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.32 (s, 3H), 2.55–2.60 (m, 4H), 3.90–3.95 (m, 4H), 7.41–7.50 (m, 1H), 7.68–7.75 (m, 1H), 7.83–7.87 (m, 1H), 7.92–7.97 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 164.3 (C), 159.9 (C), 151.6 (C), 132.9 (CH), 129.4 (CH), 126.5 (CH), 124.7 (CH), 114.8 (C), 97.7 (C), 54.6 (CH_2^*2), 49.2 (CH_2^*2), 45.9 (CH_3). HR MS: m/z 344.0364. Calcd for $C_{14}H_{15}Cl_3N_4$: 344.0362.

4.1.11. General procedure for the preparation of compounds (8)–(38)

Three equivalents of the appropriate aromatic amine reagent were added dropwise onto 1 equiv of 4-chloro-2-trichloromethylquinazoline 1. The solvent-free reaction mixture was then stirred at 140 °C for 15 min, cooled down and then extracted with dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was purified by chromatography on a silica gel, eluting with dichloromethane to afford the corresponding nucleophilic aromatic substitution product.

4.1.12. *N*-Phenyl-2-trichloromethylquinazolin-4-amine (8)

Compound **8** was obtained as a beige solid in 62% yield; mp 150 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.15–7.23 (m, 1H), 7.40–7.48 (m, 2H), 7.62–7.71 (m, 2H), 7.84–7.88 (m, 1H), 7.93–7.99 (m, 3H), 8.07–8.11 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.0 (C), 157.7 (C), 149.6 (C), 138.0 (C), 133.6 (CH), 130.1 (CH), 129.1 (CH*2), 128.2 (CH), 124.6 (CH), 120.9 (CH*2), 120.2 (CH), 113.8 (C), 97.7 (C). Anal. Calcd for $C_{15}H_{10}Cl_3N_3$: C, 53.20; H, 2.98; N, 12.41. Found: C, 52.94, H, 2.97; N, 12.01.

4.1.13. *N*-(4-Fluorophenyl)-2-trichloromethyl-quinazolin-4-amine (9)

Compound **9** was obtained as a beige solid in 88% yield; mp 165 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.05–7.14 (m, 2H), 7.58–7.67 (m, 2H), 7.80–7.92 (m, 4H), 8.02–8.06 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.9 (C), 159.4 (d, J = 244.5 Hz, C), 157.7 (C), 149.5 (C), 133.9 (d, J = 2.9 Hz, C), 133.7 (CH), 129.9 (CH), 128.2 (CH), 122.7 (d, J = 7.6 Hz, CH*2), 120.2 (CH), 115.7 (d, J = 22.3 Hz, CH*2), 113.6 (C), 97.8 (C). Anal. Calcd for $C_{15}H_9Cl_3FN_3$: C, 50.52; H, 2.54; N, 11.78. Found: C, 50.46, H, 2.56; N, 11.64.

4.1.14. *N*-(3-Chlorophenyl)-2-trichloromethyl-quinazolin-4-amine (10)

Compound **10** was obtained as a beige solid in 90% yield; mp 174 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.11–7.17 (m, 1H), 7.25–7.37 (m, 1H), 7.63–7.75 (m, 3H), 7.85–7.95 (m, 2H), 8.06–8.11 (m, 1H), 8.21–8.23 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.5 (C), 149.6 (C), 139.1 (C), 134.8 (C), 133.8 (CH), 130.0 (CH), 129.9 (CH), 128.4 (CH), 124.4 (CH), 121.0 (CH), 120.1 (CH), 118.6 (CH), 113.6 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9Cl_4N_3$: C, 48.29; H, 2.43; N, 11.26. Found: C, 48.26, H, 2.46; N, 11.42.

4.1.15. *N*-(2-Nitrophenyl)-2-trichloromethylquinazolin-4-amine (11)

Compound **11** was obtained as a yellow solid in 94% yield; mp 181 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.20–7.29 (m, 1H), 7.76–7.83 (m, 2H), 7.93–8.00 (m, 1H), 8.16 (dd, J = 2.4 and 8.2 Hz, 2H), 8.35 (dd, J = 1.5 and 8.5 Hz, 1H), 9.58 (d, J = 8.7 Hz, 1H), 11.77 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.4 (C), 157.7 (C), 149.9 (C), 136.5 (C), 136.4 (CH), 135.9 (C), 134.2 (CH), 130.2 (CH), 129.3 (CH), 126.2 (CH), 122.9 (CH), 122.7 (CH), 120.9 (CH), 114.8 (C), 97.5 (C). Anal. Calcd for $C_{15}H_9Cl_3N_4O_2$: C, 46.96; H, 2.36; N, 14.60. Found: C, 47.14, H, 2.36; N, 14.52.

4.1.16. *N*-(2-Chlorophenyl)-2-trichloromethyl-quinazolin-4-amine (12)

Compound **12** was obtained as a white solid in 63% yield; mp 164 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.06–7.15 (m, 1H), 7.37–7.49 (m, 2H), 7.68–7.76 (m, 1H), 7.87–7.99 (m, 2H), 8.11 (d, J = 7.7 Hz, 1H), 8.45 (br s, 1H), 9.12 (dd, J = 1.5 and 8.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.3 (C), 149.6 (C), 134.7 (C), 133.9 (CH), 130.2 (CH), 129.0 (CH), 128.6 (CH), 128.1 (CH), 124.4 (CH), 123.2 (C), 122.0 (CH), 120.2 (CH), 114.2 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9Cl_4N_3$: C, 48.29; H, 2.43; N, 11.26. Found: C, 48.28, H, 2.48; N, 11.09.

4.1.17. *N*-(4-Chlorophenyl)-2-trichloromethyl-quinazolin-4-amine (13)

Compound **13** was obtained as a yellow solid in 66% yield; mp 208 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.36–7.43 (m, 2H), 7.64–7.71 (m, 2H), 7.85–7.95 (m, 4H), 8.07–8.11 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.5 (C), 149.5 (C), 136.5 (C), 133.8 (CH), 130.0 (CH), 129.5 (C), 129.1 (CH*2), 128.3 (CH), 122.1 (CH*2), 120.1 (CH), 113.7 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9Cl_4N_3$: C, 48.29; H, 2.43; N, 11.26. Found: C, 48.10, H, 2.41; N, 11.06.

4.1.18. *N*-(2-Fluorophenyl)-2-trichloromethyl-quinazolin-4-amine (14)

Compound **14** was obtained as a pink solid in 45% yield; mp 155 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.04–7.30 (m, 3H), 7.65–7.73 (m, 1H), 7.85–7.96 (m, 3H), 8.09 (d, J = 8.1 Hz, 1H), 8.96–9.05 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.3 (C), 152.9 (d, J = 243.0 Hz, C), 149.5 (C), 133.8 (CH), 130.1 (CH), 128.5 (CH), 126.7 (d, J = 8.7 Hz, C), 124.8 (d, J = 4.0 Hz, CH), 124.1 (d, J = 7.7 Hz, CH), 122.3 (CH), 120.1 (CH), 114.6 (d, J = 19.0 Hz, CH), 113.9 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9Cl_3FN_3$: C, 50.52; H, 2.54; N, 11.78. Found: C, 50.68, H, 2.79; N, 11.57.

4.1.19. *N*-(3-Fluorophenyl)-2-trichloromethyl-quinazolin-4-amine (15)

Compound **15** was obtained as a white solid in 82% yield; mp 182 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 6.82–6.92 (m, 1H), 7.29–7.41 (m, 1H), 7.46–7.51 (m, 1H), 7.64–7.71 (m, 2H), 7.85–7.95 (m, 2H), 8.06–8.14 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 165.5 (C), 160.7 (d, J = 2.3 Hz, C), 157.5 (C), 149.5 (C), 139.5 (d, J = 11.3 Hz, C), 133.8 (CH), 130.1 (d, J = 9.6 Hz, CH), 130.0 (CH), 128.4 (CH), 120.1 (CH), 115.8 (d, J = 3.0 Hz, CH), 113.7 (C), 111.1 (d, J = 21.6 Hz, CH), 108.5 (d, J = 28.4 Hz, CH), 97.6 (C). Anal. Calcd for $C_{15}H_9Cl_3FN_3$: C, 50.52; H, 2.54; N, 11.78. Found: C, 50.64, H, 2.65; N, 11.67.

4.1.20. *N*-(3-Nitrophenyl)-2-trichloromethylquinazolin-4-amine (16)

Compound **16** was obtained as a yellow solid in 63% yield; mp 227 °C. ^{1}H NMR (200 MHz, DMSO-d6) δ: 7.66–7.74 (m, 1H), 7.78–7.86 (m, 1H), 7.99–8.01 (m, 3H), 8.41 (dd, J = 1.1 and 8.3 Hz, 1H), 8.70 (d, J = 8.3 Hz, 1H), 9.21 (s, 1H), 10.55 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 159.9 (C), 158.5 (C), 149.1 (C), 148.1 (C), 140.2 (C), 134.7 (CH), 130.0 (CH), 128.9 (CH), 128.7 (CH), 127.6 (CH), 123.5 (CH), 118.4 (CH), 116.1 (CH), 114.1 (C), 97.8 (C). Anal. Calcd for $C_{15}H_9Cl_3N_4O_2$: C, 46.96; H, 2.36; N, 14.60. Found: C, 46.55, H, 2.32; N, 14.32.

4.1.21. *N*-(4-Nitrophenyl)-2-trichloromethylquinazolin-4-amine (17)

Compound **17** was obtained as a yellow solid in 77% yield; mp 294 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 7.81–7.89 (m, 1H), 8.00–8.08 (m, 2H), 8.27–8.39 (m, 4H), 8.74 (d, J = 8.3 Hz, 1H), 10.65 (s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 159.8 (C), 158.4 (C), 149.3 (C), 145.4 (C), 142.6 (C), 134.9 (CH), 129.0 (CH), 128.9 (CH), 124.8 (CH*2), 123.7 (CH), 121.4 (CH*2), 114.4 (C), 97.7 (C). Anal. Calcd for $C_{15}H_9Cl_3N_4O_2$: C, 46.96; H, 2.36; N, 14.60. Found: C, 46.56, H, 2.34; N, 14.38.

4.1.22. *N*-(2-Bromophenyl)-2-trichloromethyl-quinazolin-4-amine (18)

Compound **18** was obtained as a white solid in 60% yield; mp 191 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.00–7.09 (m, 1H), 7.42–7.50 (m, 1H), 7.62–7.76 (m, 2H), 7.89–7.96 (m, 1H), 8.01 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.47 (br s, 1H), 9.12 (d, J = 8.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.4 (C), 149.6 (C), 135.7 (C), 133.8 (CH), 132.3 (CH), 130.2 (CH), 128.7 (CH), 128.6 (CH), 124.9 (CH), 122.2 (CH), 120.3 (CH), 114.3 (C), 114.1 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9BrCl_3N_4$: C, 43.15; H, 2.17; N, 10.06. Found: C, 43.33, H, 2.20; N, 9.96.

4.1.23. *N*-(3-Bromophenyl)-2-trichloromethyl-quinazolin-4-amine (19)

Compound **19** was obtained as a beige solid in 93% yield; mp 181 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.25–7.29 (m, 2H), 7.63–7.80 (m, 3H), 7.84–7.95 (m, 2H), 8.06–8.10 (m, 1H), 8.38–8.39 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.5 (C), 149.6

(C), 139.2 (C), 133.9 (CH), 130.2 (CH), 130.1 (CH), 128.4 (CH), 127.3 (CH), 123.9 (CH), 122.8 (C), 120.1 (CH), 119.1 (CH), 113.7 (C), 97.6 (C). Anal. Calcd for $C_{15}H_9BrCl_3N_4$: C, 43.15; H, 2.17; N, 10.06. Found: C, 43.53, H, 2.26; N, 10.36.

4.1.24. *N*-(4-Bromophenyl)-2-trichloromethyl-quinazolin-4-amine (20)

Compound **20** was obtained as green crystals in 59% yield; mp 228 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.59 (d, J = 8.9 Hz, 2H), 7.74–7.81 (m, 1H), 7.91–8.02 (m, 4H), 8.66 (d, J = 8.2 Hz, 1H), 10.28 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.1 (C), 158.4 (C), 149.1 (C), 138.3 (C), 134.4 (CH), 131.5 (CH*2), 128.8 (CH), 128.4 (CH), 123.9 (CH*2), 123.4 (CH), 116.0 (C), 114.1 (C), 98.0 (C). HR MS (+ESI): m/z 415.9117 ($M+H^+$). Calcd for $C_{15}H_9BrCl_3N_3$: 414.9045.

4.1.25. 2-Trichloromethyl-*N*-(2-trifluoromethylphenyl)-quinazolin-4-amine (21)

Compound **21** was obtained as a white solid in 40% yield; mp 128 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.24–7.32 (m, 1H), 7.63–7.76 (m, 3H), 7.85 (d, J = 8.4 Hz, 1H), 7.91–7.96 (m, 1H), 8.08–8.12 (m, 2H), 8.90 (d, J = 8.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.7 (C), 149.7 (C), 135.8 (C), 134.0 (CH), 133.0 (CH), 130.2 (CH), 128.8 (CH), 126.2 (q, J = 5.5 Hz, CH), 124.5 (q, J = 273.0 Hz, C4), 124.4 (CH), 124.2 (CH), 119.9 (CH), 119.8 (q, J = 29.0 Hz, C4), 114.0 (C), 97.5 (C). Anal. Calcd for $C_{16}H_9Cl_3F_3N_3$: C, 47.26; H, 2.23; N, 10.33. Found: C, 47.24, H, 2.18; N, 10.18.

4.1.26. 2-Trichloromethyl-*N*-(3-trifluoromethylphenyl)-quinazolin-4-amine (22)

Compound **22** was obtained as a beige solid in 86% yield; mp 139 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.41–7.58 (m, 2H), 7.66–7.74 (m, 1H), 7.82–7.96 (m, 1H), 8.00–8.13 (m, 4H), 8.53 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.6 (C), 149.3 (C), 138.5 (C), 134.0 (CH), 131.6 (q, J = 32.6 Hz, C4), 129.9 (CH), 129.5 (CH), 128.5 (CH), 123.9 (q, J = 272.0 Hz, C4), 123.7 (CH), 121.0 (q, J = 3.7 Hz, CH), 120.3 (CH), 118.0 (q, J = 4.0 Hz, CH), 113.6 (C), 97.3 (C). Anal. Calcd for $C_{16}H_9Cl_3F_3N_3$: C, 47.26; H, 2.23; N, 10.33. Found: C, 47.08, H, 2.21; N, 10.36.

4.1.27. 2-Trichloromethyl-*N*-(4-trifluoromethylphenyl)-quinazolin-4-amine (23)

Compound **23** was obtained as a white solid in 61% yield; mp 157 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 7.76–7.81 (m, 3H), 7.98–7.99 (m, 2H), 8.30 (d, J = 8.4 Hz, 2H), 8.72 (d, J = 7.9 Hz, 1H), 10.46 (br s, 1H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 160.3 (C), 158.9 (C), 149.5 (C), 142.9 (C), 134.9 (CH), 129.2 (CH), 128.9 (CH), 126.3 (q, J = 3.7 Hz, CH*2), 124.9 (q, J = 272.0 Hz, C4), 124.2 (q, J = 32.0 Hz, C4), 123.9 (CH), 122.0 (CH*2), 114.5 (C), 98.2 (C). HR MS (+ESI): m/z 405.9881 ($M+H^+$). Calcd for $C_{16}H_9F_3N_3Cl_3$: 404.9814.

4.1.28. *N*-(2-Methoxyphenyl)-2-trichloromethyl-quinazolin-4-amine (24)

Compound **24** was obtained as a yellow solid in 86% yield; mp 199 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 4.01 (s, 3H), 6.98–7.12 (m, 3H), 7.61–8.06 (m, 4H), 8.62 (br s, 1H), 9.03–9.09 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.4 (C), 157.2 (C), 149.5 (C), 148.3 (C), 133.4 (CH), 129.9 (CH), 128.1 (CH), 127.8 (C), 123.6 (CH), 121.4 (CH), 120.5 (CH), 120.3 (CH), 114.4 (C), 109.9 (CH), 97.9 (C), 56.1 (OCH_3). Anal. Calcd for $C_{16}H_{12}Cl_3N_3O$: C, 52.13; H, 3.28; N, 11.40. Found: C, 52.23, H, 3.31; N, 11.33.

4.1.29. *N*-(3-Methoxyphenyl)-2-trichloromethyl-quinazolin-4-amine (25)

Compound **25** was obtained as a green solid in 90% yield; mp 142 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.89 (s, 3H), 6.71–6.76 (m, 1H), 7.14–7.33 (m, 2H), 7.66–7.70 (m, 2H), 7.84–7.93 (m, 2H), 8.05–8.12 (m, 2H). ^{13}C NMR (50 MHz, CDCl3) δ: 160.8 (C), 160.3 (C), 157.6 (C), 149.4 (C), 139.3 (C), 133.6 (CH), 130.1 (CH), 129.6 (CH), 128.2 (CH), 120.1 (CH), 113.8 (C), 112.3 (CH), 111.0 (CH), 106.0 (CH), 97.7 (C), 55.6 (OCH_3). Anal. Calcd for $C_{16}H_{12}Cl_3N_3O$: C, 52.13; H, 3.28; N, 11.40. Found: C, 52.35, H, 3.36; N, 11.07.

4.1.30. *N*-(4-Methoxyphenyl)-2-trichloromethyl-quinazolin-4-amine (26)

Compound **26** was obtained as a beige solid in 98% yield; mp 149 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 3.81 (s, 3H), 6.90–6.94 (m, 2H), 7.59–7.63 (m, 2H), 7.79–7.88 (m, 4H), 8.00–8.04 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.9 (C), 157.6 (C), 156.5 (C), 149.1 (C), 133.5 (CH), 131.0 (C), 129.5 (CH), 128.0 (CH), 122.6 (CH*2), 120.5 (CH), 114.1 (CH*2), 113.7 (C), 97.7 (C), 55.5 (OCH_3). HR MS (+ESI): m/z 368.0112 ($M+H^+$). Calcd for $C_{16}H_{12}N_3OCl_3$: 367.0046.

4.1.31. *N*-*o*-Tolyl-2-trichloromethylquinazolin-4-amine (27)

Compound **27** was obtained as a white solid in 80% yield; mp 149 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.42 (s, 3H), 7.10–7.18 (m, 1H), 7.27–7.35 (m, 2H), 7.54 (br s, 1H), 7.59–7.68 (m, 1H), 7.82–7.90 (m, 2H), 8.03–8.08 (m, 1H), 8.30–8.34 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.1 (C), 158.1 (C), 149.6 (C), 136.0 (C), 133.5 (CH), 130.6 (CH), 130.0 (CH), 129.3 (C), 128.1 (CH), 126.9 (CH), 125.2 (CH), 123.1 (CH), 120.1 (CH), 113.9 (C), 97.7 (C), 18.0 (CH_3). Anal. Calcd for $C_{16}H_{12}Cl_3N_3$: C, 54.49; H, 3.43; N, 11.92. Found: C, 54.05, H, 3.55; N, 11.77.

4.1.32. *N*-*m*-Tolyl-2-trichloromethylquinazolin-4-amine (28)

Compound **28** was obtained as a pale beige solid in 87% yield; mp 150 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.40 (s, 3H), 6.96–7.00 (m, 1H), 7.30–7.33 (m, 1H), 7.60–7.74 (m, 3H), 7.85–7.91 (m, 3H), 8.03–8.07 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.1 (C), 157.7 (C), 149.6 (C), 139.0 (C), 137.9 (C), 133.6 (CH), 130.0 (CH), 128.9 (CH), 128.1 (CH), 125.3 (CH), 121.5 (CH), 120.2 (CH), 117.8 (CH), 113.8 (C), 97.9 (C), 21.6 (CH_3). Anal. Calcd for $C_{16}H_{12}Cl_3N_3$: C, 54.49; H, 3.43; N, 11.92. Found: C, 54.27, H, 3.46; N, 11.73.

4.1.33. *N*-*p*-Tolyl-2-trichloromethylquinazolin-4-amine (29)

Compound **29** was obtained as a yellow solid in 71% yield; mp 146 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.23 (s, 3H), 7.03–7.07 (m, 2H), 7.43–7.52 (m, 1H), 7.69–7.83 (m, 5H), 7.91–7.95 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.0 (C), 157.6 (C), 149.5 (C), 135.4 (C), 134.1 (C), 133.5 (CH), 129.8 (CH), 129.5 (CH*2), 128.0 (CH), 120.8 (CH*2), 120.2 (CH), 113.7 (C), 97.8 (C), 20.9 (CH_3). Anal. Calcd for $C_{16}H_{12}Cl_3N_3$: C, 54.49; H, 3.43; N, 11.92. Found: C, 54.11, H, 3.45; N, 11.74.

4.1.34. *N*-(3,4-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (30)

Compound **30** was obtained as a white solid in 80% yield; mp 223 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.46 (d, J = 8.8 Hz, 1H), 7.62–7.74 (m, 3H), 7.87–7.95 (m, 2H), 8.07–8.13 (m, 1H), 8.35 (d, J = 2.5 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.4 (C), 149.8 (C), 137.4 (C), 134.0 (CH), 133.0 (C), 130.5 (CH), 130.3 (CH), 128.5 (CH), 127.6 (C), 122.7 (CH), 119.9 (CH), 119.8 (CH), 113.6 (C), 97.5 (C). Anal. Calcd for $C_{15}H_8Cl_5N_3$: C, 44.21; H, 1.98; N, 10.31. Found: C, 44.35; H, 2.00; N, 10.19.

4.1.35. *N*-(2,3-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (31)

Compound **31** was obtained as a white solid in 25% yield; mp 231 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.25–7.41 (m, 2H), 7.71–7.79 (m, 1H), 7.90–8.01 (m, 2H), 8.13 (d, J = 7.8 Hz, 1H), 8.50 (br s, 1H), 9.08 (dd, J = 1.7 and 8.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.3 (C), 149.7 (C), 136.3 (C), 134.0 (CH), 132.8

(C), 130.3 (CH), 128.9 (CH), 128.1 (CH), 125.0 (CH), 121.8 (C), 120.1 (CH), 119.9 (CH), 114.2 (C), 97.5 (C). Anal. Calcd for $C_{15}H_8Cl_5N_3$: C, 44.21; H, 1.98; N, 10.31. Found: C, 44.31, H, 1.96; N, 10.26.

4.1.36. *N*-(2,6-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (32)

Compound **32** was obtained as a white solid in 9% yield; mp 164 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.22–7.33 (m, 2H), 7.44–7.48 (m, 2H), 7.66–7.74 (m, 1H), 7.87–7.96 (m, 1H), 8.02 (d, J = 8.2 Hz, 1H), 8.11 (d, J = 8.2 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.9 (C), 159.0 (C), 150.0 (C), 133.9 (CH), 133.8 (C4*2), 132.7 (C), 130.0 (CH), 128.6 (CH), 128.5 (CH*2), 128.2 (CH), 120.9 (CH), 113.7 (C), 97.3 (C). HR MS (+ESI): m/z 405.9237 ($M+H^+$). Calcd for $C_{15}H_8Cl_5N_3$: 404.9161.

4.1.37. *N*-(2,4-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (33)

Compound **33** was obtained as a white solid in 75% yield; mp 204 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.40 (dd, J = 2.4 and 9.0 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.69–7.77 (m, 1H), 7.89–7.98 (m, 2H), 8.07–8.14 (m, 1H), 8.35 (br s, 1H), 9.10 (d, J = 9.0 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.6 (C), 157.2 (C), 149.6 (C), 134.0 (CH), 133.4 (C), 130.3 (CH), 128.9 (C), 128.8 (CH), 128.7 (CH), 128.2 (CH), 123.6 (C), 122.7 (CH), 120.0 (CH), 114.1 (C), 97.5 (C). Anal. Calcd for $C_{15}H_8Cl_5N_3$: C, 44.21; H, 1.98; N, 10.31. Found: C, 44.15, H, 1.96; N, 10.25.

4.1.38. *N*-(3,5-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (34)

Compound **34** was obtained as a white solid in 76% yield; mp 216 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.14–7.15 (m, 1H), 7.65–7.72 (m, 2H), 7.86–7.95 (m, 2H), 8.00 (d, J = 1,4 Hz, 2H), 8.09 (d, J = 8.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.4 (C), 149.8 (C), 139.7 (C), 135.3 (C4*2), 134.0 (CH), 130.2 (CH), 128.6 (CH), 124.2 (CH), 120.0 (CH), 119.1 (CH*2), 113.6 (C), 97.5 (C). Anal. Calcd for $C_{15}H_8Cl_5N_3$: C, 44.21; H, 1.98; N, 10.31. Found: C, 44.14, H, 2.00; N, 10.19.

4.1.39. *N*-(2,5-Dichlorophenyl)-2-trichloromethyl-quinazolin-4-amine (35)

Compound **35** was obtained as a white solid in 47% yield; mp 176 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.06–7.12 (m, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.71–8.16 (m, 4H), 8.41 (br s, 1H), 9.35–9.36 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.7 (C), 157.1 (C), 149.8 (C), 135.4 (C), 134.1 (CH), 133.9 (C), 130.3 (CH), 129.5 (CH), 128.8 (CH), 124.2 (CH), 122.1 (CH), 121.1 (C), 121.0 (CH), 114.1 (C), 97.5 (C). Anal. Calcd for $C_{15}H_8Cl_5N_3$: C, 44.21; H, 1.98; N, 10.31. Found: C, 44.18, H, 1.92; N, 10.11.

4.1.40. *N*-[3,5-bis(Trifluoromethyl)phenyl]-2-trichloro-methylquinazolin-4-amine (36)

Compound **36** was obtained as a white solid in 35% yield; mp 179 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.67–7.77 (m, 2H), 7.90–7.97 (m, 2H), 8.02 (d, J = 8.2 Hz, 1H), 8.12 (d, J = 8.2 Hz, 1H), 8.56–8.57 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.6 (C), 157.4 (C), 149.8 (C), 139.4 (C), 134.3 (CH), 132.4 (q, J = 33.6 Hz, C4*2), 130.2 (CH), 128.9 (CH), 123.1 (q, J = 273.0 Hz, C4*2), 120.6 (q, J = 3.3 Hz, CH), 120.0 (CH), 117.5 (q, J = 3.7 Hz, CH*2), 113.5 (C), 97.2 (C). Anal. Calcd for $C_{17}H_8Cl_3F_6N_3$: C, 43.02; H, 1.70; N, 8.85. Found: C, 43.36, H, 1.84; N, 8.93.

4.1.41. 2-Trichloromethyl-*N*-(4-trifluoromethoxy-phenyl)quinazolin-4-amine (37)

Compound **37** was obtained as a white solid in 70% yield; mp 153 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.25–7.29 (m, 2H), 7.62–7.69 (m, 2H), 7.83–8.08 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 160.8 (C), 157.5 (C), 149.5 (C), 145.3 (C), 136.6 (C), 133.8 (CH), 130.1 (CH), 128.4 (CH), 121.8 (CH*2), 121.7 (CH*2), 120.5 (q, J = 257.0 Hz, C4), 120.1 (CH), 113.6 (C), 97.6 (C). Anal. Calcd for $C_{16}H_9Cl_3N_3O$: C, 45.47; H, 2.15; N, 9.94. Found: C, 45.51; H, 2.37; N, 9.86.

4.1.42. *N*-(Naphtalen-1-yl)-2-trichloromethylquinazolin-4-amine (38)

Compound **38** was obtained as a pale pink solid in 33% yield; mp 179 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.54–7.80 (m, 5H), 7.86–8.13 (m, 6H), 8.41 (d, J = 7.1 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 161.1 (C), 158.9 (C), 149.8 (C), 134.2 (C), 133.7 (CH), 132.6 (C), 130.1 (CH), 129.0 (CH), 128.2 (CH), 127.4 (C), 126.5 (CH), 126.1 (CH), 126.0 (CH), 125.8 (CH), 121.1 (CH), 120.5 (CH), 120.4 (CH), 114.0 (C), 97.6 (C). Anal. Calcd for $C_{19}H_{12}Cl_3N_3$: C, 58.71; H, 3.11; N, 10.81. Found: C, 58.96; H, 3.15; N, 10.64.

4.1.43. General procedure for the preparation of compounds (39)–(41)

To a refluxing mixture of acetic acid and iron (15 equiv), 1 equiv of the corresponding trichloromethylated precursor reactant (respectively compounds **10**, 22 and **33**) was added. The reaction mixture was then stirred for 1 h and filtered over Celite. Acetic acid was evaporated under reduced pressure, giving an orange residue which was dissolved in dichloromethane. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was purified by chromatography on a silica gel, eluting with ethyl acetate to afford the corresponding reduction product.

4.1.44. *N*-(3-Chlorophenyl)-2-methylquinazolin-4-amine (39)

Compound **39** was obtained as a beige solid in 99% yield; mp 171 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H), 7.11 (dd, J = 0.9 and 8.0 Hz, 1H), 7.32 (dd, J = 8.0 and 8.2 Hz, 1H), 7.50 (dd, J = 0.9 and 8.0 Hz, 1H), 7.64–7.88 (m, 5H), 8.01 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.9 (C), 156.9 (C), 150.4 (C), 139.8 (C), 134.6 (C), 133.1 (CH), 129.9 (CH), 128.2 (CH), 126.0 (CH), 123.9 (CH), 121.0 (CH), 120.0 (CH), 118.9 (CH), 113.0 (C), 26.4 (CH_3). HR MS (+ESI): m/z 270.0793 ($M+H^+$). Calcd for $C_{15}H_{12}ClN_3$: 269.0720.

4.1.45. 2-Methyl-*N*-(3-trifluoromethylphenyl)-quinazolin-4-amine (40)

Compound **40** was obtained as a beige solid in 99% yield; mp 178 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.72 (s, 3H), 7.40 (d, J = 7.7 Hz, 1H), 7.52 (dd, J = 7.3 and 7.7 Hz, 2H), 7.74–8.06 (m, 5H), 8.23 (br s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.7 (C), 157.1 (C), 149.9 (C), 139.1 (C), 133.3 (C), 131.4 (q, J = 32.6 Hz, C4), 129.5 (CH), 127.8 (CH), 126.2 (CH), 124.1 (CH), 123.9 (q, J = 272.2 Hz, C4), 120.6 (q, J = 3.6 Hz, CH), 120.3 (CH), 118.0 (q, J = 4.1 Hz, CH), 112.9 (C), 26.2 (CH_3). HR MS (+ESI): m/z 304.1058 ($M+H^+$). Calcd for $C_{16}H_{12}F_3N_3$: 303.0983.

4.1.46. *N*-(2,4-Dichlorophenyl)-2-methylquinazolin-4-amine (41)

Compound **41** was obtained as a beige solid in 95% yield; mp 154 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.75 (s, 3H), 7.32–7.38 (m, 1H), 7.45–7.46 (m, 1H), 7.52–7.60 (m, 1H), 7.77–7.85 (m, 1H), 7.90–7.94 (m, 2H), 8.19 (br s, 1H), 8.88 (d, J = 8.3 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ: 163.6 (C), 156.6 (C), 149.9 (C), 134.0 (C), 133.3 (CH), 128.8 (CH), 128.4 (C), 128.0 (CH), 127.8 (CH), 126.4 (CH), 124.0 (C), 122.9 (CH), 120.1 (CH), 113.3 (C), 26.3 (CH_3). HR MS (+ESI): m/z 304.0398 ($M+H^+$). Calcd for $C_{15}H_{11}Cl_2N_3$: 303.0330.

4.2. Biology/material and methods

4.2.1. General

Cell culture medium (RPMI 1640), foetal calf serum (FCS), L-glutamine, non essential amino acids and other medium additives were from Eurobio (Paris, France). All other chemicals were of highest chemical purity and were purchased from Sigma except contrary mention. Stock solutions of quinazoline derivatives were prepared in DMSO. Stock solutions of reference drugs (doxorubicin, pyrimethamine, amphotericin B, chloroquine and doxycycline) were prepared in ultrapure H_2O or DMSO. Flow cytometry was performed at the Faculté de Pharmacie de Marseille, using a FACSort flow cytometer apparatus (Beckton Dickinson, Paris, France), equipped with an argon laser (power of 15 mW, and wavelength of 488 nm).

4.2.2. Antiplasmodial activity

In this study, the W2 culture-adapted *P. falciparum* reference strain was used. It is resistant to chloroquine, pyrimethamine and proguanil. Maintenance in continuous culture was done as described previously by Trager and Jensen.[15] Parasites were cultivated in 75 cm^2-flasks containing RPMI 1640 (20 mL) supplemented with 25 mM HEPES, 25 mM $NaHCO_3$, 10% of A+ human serum and 1 mL of washed erythrocytes (final haematocrit 2.5%). Parasitaemia was maintained daily between 1% and 6%. Dilutions used non-infected A+ erythrocytes. Cultures were incubated at 37 °C, 10% O_2, 6% CO_2, 84% N_2, with 90% humidity. Cultures were monitored daily by microscopic examination of blood smears fixed with methanol and stained with 10% Giemsa stain. Parasite growth was assessed by flow cytometry according to a methodology previously described using hydroethidine (HE, Interchim, Montluçon, France) that is converted by metabolically active parasites into ethidium.[16] After incubation with hydroethidine, parasitized and uninfected erythrocytes were all identified on the basis of fluorescence intensity and size. Triplicate assays were performed in 96-well plates (Nunc Brand products, Fisher, Paris, France) containing 200 μL of asynchronous parasite cultures at 2% of parasitaemia and 2% haematocrit, and 5 μL of the appropriate extract dissolved in DMSO or ultrapure H_2O. Negative control, treated by solvents (DMSO or H_2O) and positive controls (chloroquine and doxycycline) were added to each set of experiments. After 48 h incubation without medium change, plates were centrifuged and the upper liquids were replaced with 200 μL hydroethidine solution [0.05 mg/ml in phosphate buffered saline (PBS)]. Plates were incubated 20 min in the dark at 37 °C and washed three times with PBS. Finally, cells were suspended in 1 mL of PBS to allow determination of the number of cell events (around 300 per s) and parasitaemia by flow cytometry using a FACSort flow cytometer. Settings were: Forward Scatter (FSC-H), size: Voltage E-1, gain 1, mode Log; Side Scatter (SSC-H), granulosity: Voltage 250, gain 1, mode Log; Fluorescence 2 (FL2), red fluorescence: Voltage 459, gain 1, mode Log. The concentrations of compounds required to induce a 50% decrease of infected erythrocytes (IC_{50}W2) were calculated from three independent experiments.

4.2.3. Antileishmanial activity

The effects of the tested compounds on the growth of *L. donovani* promastigotes (GFP-transfected strain HOM/IN/01/2001, kindly provided by Dr. N. Singh, Lucknow, India) were assessed by flow cytometry as described previously by Singh and Dube.[17] Briefly, promastigotes in log-phase in M199 medium supplemented with 10% FCS and 500 μg/mL of G418 were incubated at an average density of 10^5 parasites/mL in 24-well plates with various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v) incorporated in duplicate. Amphotericin B was used as the reference drug. Appropriate controls, treated by DMSO or amphotericin B, were added to each set of experiments. After a 72-h incubation period at 27 °C, parasite growth was determined using a FACSort flow cytometer, equipped with an argon laser (power of 15 mW, and wavelength of 488 nm). Settings were: Forward Scatter (FSC-H), size: Voltage E-1, gain 1, mode Lin; Side Scatter (SSC-H), granulosity: Voltage 489, gain 1, mode Lin; Fluorescence 1 (FL1), green fluorescence: Voltage 505, gain 1, mode Log. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC_{50}) were calculated from three independent experiments.

4.2.4. Antitoxoplasmic activity

The effects of the tested compounds on the growth of *T. gondii* tachyzoites (PRU-β-Gal strain, kindly provided by Pr. I. Villena, Reims, France) were assessed by a colorimetric microtiter assay according to the method of Mc Fadden et al.[18] Briefly, tachyzoites were maintained by serial passage in confluent human foreskin fibroblast (HFF) monolayer (a gift of Pr. I. Dimier-Poisson, Tours, France). For assay, 96-well microtiter plates were seeded with 10^4 HFF cells and allowed to grow to confluence in RPMI 1640 (without phenol red) supplemented with 10% FCS and 1% L-glutamine/penicillin-streptomycin mix at 37 °C with 6% CO_2. Cell monolayers were infected with 2.10^3 parasites per well and incubated for 3 h at 37 °C with 6% CO_2. Then, various concentrations of compounds dissolved in DMSO (final concentration less than 0.5% v/v) were incorporated in triplicate. Pyrimethamine was used as the reference drug compound. Appropriate controls treated by DMSO or pyrimethamine were added to each set of experiments. Negative control consisted in cell monolayers incubated without parasite and drug. After a 96-h incubation period at 37 °C with 6% CO_2, 20 μL of chlorophenol red-β-D-galactopyranoside (CPRG) were added to each well to give a final concentration of 500 μM. The plates were incubated at 37 °C with 6% CO_2 for an additional 24 h, at which time β-galactosidase activity was measured by reading plates at 570 and 630 nm on a Biotek microtiter plate reader. Blanking was made on the negative-control wells. The concentrations of compounds required to induce a 50% decrease of parasite growth (IC_{50}) were calculated from three independent experiments.

4.2.5. Cytotoxic assays on human myeloid leukemic K562

Cytotoxicity was assessed on chemosensitive subline K562 derived from a chronic myeloid leukemia and purchased from Dr J. Boutonnat (UMR-CNRS 5525, Université Joseph Fourier, La Tronche, France).[19] Late log-phase K562 cells were incubated in RPMI 1640 (without phenol red) supplemented with 10% FSC, 2% L-glutamine and 1% penicillin-streptomycin mix (complete RPMI medium) and a range of compound concentrations incorporated in duplicate (final DMSO concentration less than 0.5%). Appropriate controls treated with or without solvent (DMSO), and various concentrations of doxorubicin (positive control), chloroquine, doxycycline, amphotericin B and pyrimethamine were added to each set of experiments. After 72 h incubation at 37 °C and 6% CO_2, cell growth was measured by flow cytometry after staining monocytes with 5 μL of propidium iodide. Antiproliferative activity was evaluated by counting the number of live cells in a 100 μL sample. Inhibitory concentration 50% (IC_{50} K562) was defined as the concentration of drug required to induce a 50% in the K562 cell proliferation compared to the control. IC_{50} was calculated by non-linear regression analysis processed on dose–response curves, using the Table Curve software 2D v.5.0. IC_{50} values represent the mean value calculated from three independent experiments.

4.2.6. MTT assays

The evaluation of the tested molecules' cytotoxicity on the HepG2 cell line was done according to the method of Mosmann[20] with slight modifications. Briefly, cells in 100 μL of complete med-

ium, [RPMI supplemented with 10% foetal bovine serum, 1% L-glutamine (200 mM) and penicillin (100 U/mL)/streptomycin (100 μg/mL)] were inoculated into each well of 96-well plates and incubated at 37 °C in a humidified 6% CO_2 with 95% air atmosphere. After 24 h incubation, 100 μl of medium with various product concentrations was added and the plates were incubated for 72 h. At the end of the treatment and incubation, the medium was aspirated from the wells and 10 μL MTT solution (5 mg MTT/mL in PBS) was added to each well with 100 μl of medium without foetal calf serum. Cells were incubated for 2 h at 37 °C to allow MTT oxidation by mitochondrial dehydrogenase in the viable cells. After this time, the MTT solution was aspirated and DMSO (100 μL) was added to dissolve the resulting blue formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with 630 nm as reference wavelength with a microplate spectrophotometer. DMSO was used as blank and doxorubicine as positive control. Cell viability was calculated as percentage of control (cells incubated without compound). The 50% cytotoxic concentration was determined from the dose–response curve.

4.2.7. Selectivity index (SI)

The selectivity indexes that are presented correspond to the ratios between, respectively, the toxicity on K562 or HepG2 human cell lines and the W2 antiplasmodial activity. They are calculated as follows: SI W2 = IC_{50}(K562) or (HEPG2)/IC_{50}(W2).

4.2.8. Ames test

All samples were assessed for mutagenicity by a modified version of the liquid incubation assay of the classical Ames test.[21,22] *S. typhimurium* tester strains (TA97a, TA98, TA100 and TA102) were grown overnight in an Oxoid nutrient broth. After this period, 5×10^{-3} M DMSO solutions of the tested drugs were added to 0.1 mL of culture and incubated with and without 4% S9 mix for 1 h at 37 °C with shaking. After this period, 2 mL of molten top agar were added and the mixture was transferred onto Vogel-Bonner agar plates. After 48 h at 37 °C in the dark, the number of spontaneous and drug induced revertants per plate was determined for each dose with a laser bacterial colony counter. A product is considered mutagenic when it induces a twofold increase of the number of revertants compared with the spontaneous frequency.

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