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# Design, synthesis, and characterization of novel aminoalcohol quinolines with strong *in vitro* antimalarial activity

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## Abstract

Malaria is the fifth most lethal parasitic infections in the world. Herein, five new series of aminoalcohol quinolines including fifty-two compounds were designed, synthesized and evaluated *in vitro* against *Pf3D7* and *PfW2* strains. Among them, fourteen displayed IC<sub>50</sub> values below or near of 50.0 nM whatever the strain with selectivity index often superior to 100. **17b** was found as a promising antimalarial candidate with IC<sub>50</sub> values of 14.9 nM and 11.0 nM against respectively *Pf3D7* and *PfW2* and a selectivity index higher than 770 whatever the cell line is. Further experiments were achieved to confirm the safety and to establish the preliminary ADMET profile of compound **17b** before the *in vivo* study performed on a mouse model of *P. berghei* ANKA infection. The overall data of this study allowed to establish new structure-activity relationships and the development of novel agents with improved pharmacokinetic properties

**Keywords:** Malaria, *P. falciparum*, antimalarial drug resistance, mefloquine analogs, aminoalcohol quinolines.

## 1. INTRODUCTION

Malaria is and remains one of the most important diseases in the world. In 2019, more than 229 million cases and almost 409,000 deaths with 67% of children under 5 years reported, even if associated mortality has decreased by 60% since 2000 [1]. *Plasmodium falciparum* is responsible of the majority of reported malaria cases and is the deadliest species among the different *Plasmodium* parasites able to infect human. *P. vivax* is the second one with nearly 2.85 billion people at risk of infection in the world and contributes to half of all malaria cases outside Africa. An important number of antimalarial drugs is available but *P. falciparum* and *P. vivax* developed (multi-drug) resistance to most of them and spread and fatality rates increase. Today, most antimalarial treatments are composed of two or three drugs in combination to limit the emergence of resistant strains. Artemisinin-based combination therapy (ACT) has been the first officially recommended by the World Health Organization (WHO) since the early 2000's to treat uncomplicated malaria. [2]. The rapid parasitocidal action of artemisinin derivatives, which have short elimination half-life, is completed with the slower action of the partner drug. This one

should have long elimination half-life to clear the remaining parasites and to prevent high recrudescence rates. Unfortunately, the efficiency of these therapies failed due to the emergence of resistant parasites to both the artemisinin derivatives and the partner drugs [3].

Currently, even if the antimalarial pipeline is promising, there is no alternative to ACTs [4,5]. It is therefore important to identify and to develop new drugs to combat resistant parasites and limit the emergence of new resistant strains. Ideally, these novel antimalarial drugs should have a good pharmacokinetic profile and a long duration of action to allow their use in combination with another antimalarial drug and particularly with an artemisinin derivative.

Two protein transporters, the chloroquine resistance transporter (*PfCRT*) and the *P. falciparum* multidrug resistant protein 1 (*PfMDR1*), play a crucial role by conferring resistance to almost all the current antimalarial drugs, including quinolines such as **chloroquine (CQ)**, Figure 1) and **mefloquine (MQ)**, Figure 1). *PfCRT* and *PfMDR1* are both located at the parasite's digestive vacuole membrane. *PfCRT* induces the transport of solutes down its electrochemical gradient while *PfMDR1*, which belonging to the ABC transporter family, transports a large range of substrates notably drugs from the cytosol to the digestive vacuole [6]. Mutations in *PfCRT* (K76T) are known to be particularly involved in CQ resistance [7]. While, the main mechanism of MQ resistance is due to the amplification of the wild-type *pfmdr1* gene encoding for *PfMDR1* [8,9].

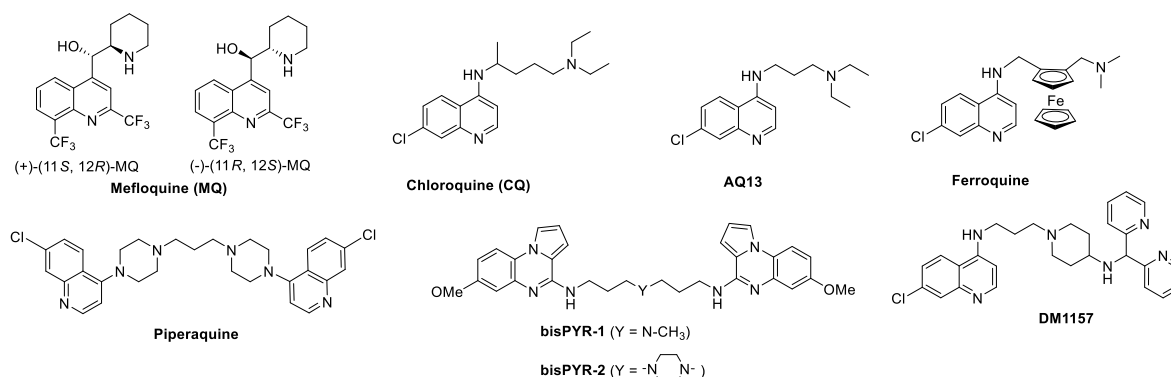
Different strategies can be employed in order to overcome quinoline-resistance mechanism: (i) saturate the transporters *PfCRT* or *PfMDR1* by increasing dosage of antimalarial drug [10], (ii) induce small changes in the antimalarial structure to restore activity against resistant parasites [11–15], (iii) conjugate quinoline either with another quinoline or bioisostere to form bulky bisheterocycle [16–20], (iv) combined covalently quinoline with a reversal agent or an efflux pump inhibitor able to inhibit *PfCRT* or *PfMDR1* [21–23].

Two structurally simple CQ analogues, **AQ-13** [13] (Figure 1) and **ferroquine** [14,15] (Figure 1) as much active against CQ-resistant as CQ-susceptible strains are both in phase 2 clinical trials (NCT01614964, NCT02497612). AQ-13 possesses a shorter diaminoalkane side chain than CQ and ferroquine is a 4-aminoquinoline with a ferrocene moiety in the lateral chain.

The formation of bisquinoline is also a valuable strategy for fighting the resistant parasites [16–20]. Related to their bulky structure, bisheterocycles seem to be less recognized by efflux proteins than heterocycle alone. The most famous bisquinoline is the **piperazine** (Figure 1) developed in the 1960s [24]. Since 1995, this bisquinoline is associated with dihydroartemisinin for uncomplicated *P. falciparum* malaria because of efficacy, bioavailability, tolerability and good pharmacokinetics properties. At least three new piperazine associations are currently in clinical trials (NCT02083380, NCT02198807, NCT02543086). Pyrrolo[1,2-*a*]quinoxalines being considered as quinoline bioisosteres, bispyrrolo[1,2-*a*]quinoxalines were also reported as promising antimalarial candidates to struggle CQ-resistant strains. Previously, we reported both pyrrolo[1,2-*a*]quinoxalines [25–28] and bispyrrolo[1,2-*a*]quinoxalines [29,30] with potent antimalarial activity. Among the best compounds, the bispyrrolo[1,2-*a*]quinoxalines **bisPYR-1** and **bisPYR-2** (Figure 1) were more active than both the corresponding monopyrrolo[1,2-*a*]quinoxalines and CQ against the CQ-resistant strain K1.

Covalent conjugation of quinoline-based antiplasmodial drugs with efflux pump inhibitors or reversal agents can also allow to struggle resistant parasites [21–23]. The first success of this

concept was described in 2006 [21]. Burgess *et al.* combined the 7-chloro-4-aminoquinoline nucleus of CQ with the known *Pf*CRT reversal agent imipramine [21]. Further structural modifications led to **DM1157** (Figure 1), entered into phase I clinical development in July 2018 (NCT03490162) [22].

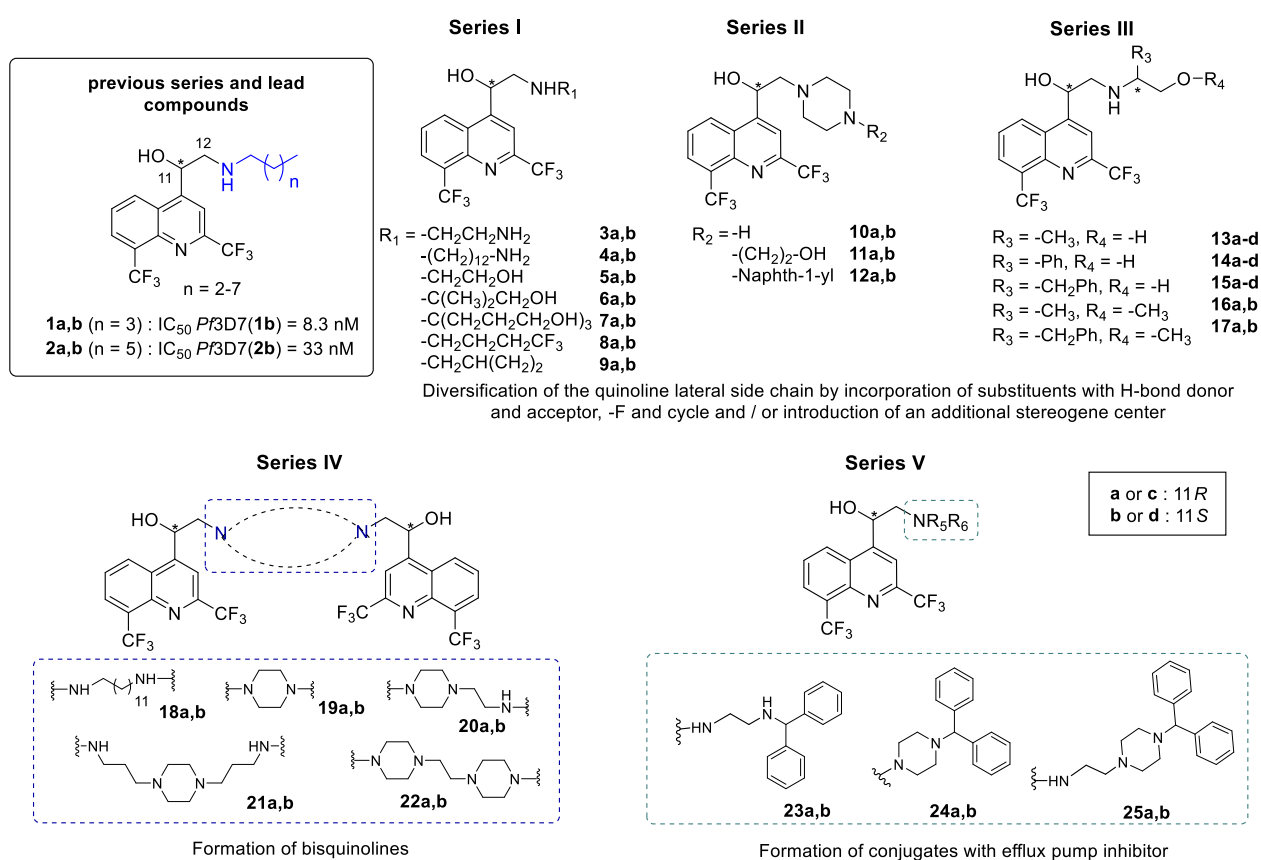


**Figure 1.** Chemical structures of mefloquine (MQ), chloroquine (CQ), AQ-13, ferroquine, piperazine, bispyrrolo[1,2-*a*]quinoxalines bisPYR-1 and bisPYR-2, DM1157.

During our research about novel compounds for antimalarial chemotherapy, we were interested to the MQ and its derivatives [25,31–36]. MQ is a 4-aminoalcohol quinoline which contains two stereocenters and has been commercially available since the 1970s as a racemic mixture ((+)-(11*S*,12*R*)-MQ and (-)-(11*R*,12*S*)-MQ) (Figure 1). Previous studies reported by Karle *et al.* in 1993 [37] and reaffirmed by our team in 2013 [32] have shown the best antimalarial potential of the (+)-(11*S*, 12*R*)-MQ compared to the (-)-(11*R*,12*S*)-MQ. MQ is one of the WHO's model list of essential medicines and is mainly used in combination with artesunate. Although its mechanism of action is not fully understood, several targets were identified which makes the study of its pharmacophore very attractive to fight resistant strains [6,38–43]. However, MQ's neurotoxicity was also reported and unfortunately has limited its use particularly in prophylaxis. Some evidence demonstrated that the least active MQ's enantiomer ((-)-(11*R*, 12*S*)-MQ) accumulates more in the nervous central system [44,45]. (-)-(11*R*, 12*S*)-MQ has also a high affinity for adenosine receptors in the brain suggesting its stronger implication in neurological events associated with MQ usage [46]. Not surprisingly, fairly different pharmacokinetic properties were highlighted for these enantiomers [47]. Thus, several MQ analogues have been already prepared to obtain more potent drugs against *P. falciparum* and non-toxic to human cells [48–50].

In this aim, we designed and prepared novel enantiopure MQ analogs in a five steps synthesis with good yields and excellent enantiomeric excesses (Figure 2) [31,35]. Their antimalarial activities were often superior to MQ against the MQ-reduced susceptibility strain 3D7 and the CQ-resistant strain W2 [33]. As expected, in this series, (*S*)-enantiomers were always more active than their (*R*) counterpart by a factor between 2 and 15, depending on the side chain length (Figure 2) [33]. Further studies were carried out for the lead compounds **1b** and **2b** with the (*S*)-configuration (Figure 2) and confirmed the interest of these kind of compound in antimalarial chemotherapy. They displayed steady *in vitro* activity against several others MQ-resistant strains (HB3, BRE1 and Dd2) and *in vivo* survival rates as good as MQ in a mouse model of *P. berghei* ANKA (*PbA*) infection without hemolytic toxicity. Moreover, their combination with dihydroartemisinin (DHA) has had additive or synergic effect [51].

Motivated by these initial results and based on the above information, we describe herein the synthesis and the study of five new series of enantiopure aminoalcohol quinoline derivatives (Figure 2). The three first series **I-III** aimed to diversify the nature of the lateral side chain of lead compounds **1** and **2** by incorporating substituents: i) with additional H-bond donor and acceptor (**series I-III**), ii) with fluorine group as H isostere (**8**, **series I**), iii) with cyclic group such as methylcyclopropane (**9**, **series I**) or piperazine (**10-12**, **series II**) and / or v) with an another stereogene center (**series III**). The **series IV** was synthesized with the aim of study the influence of a second quinoline moiety on the antimalarial activity. Two 2,8-bis(trifluoromethyl)quinoline cores were connected by various aliphatic polyamine to form symmetric or asymmetric bulky bisquinolines. Finally, six compounds of the **series V** were prepared joining the 2,8-bis(trifluoromethyl)quinoline moiety with reversal agent pharmacophore [52,53].



**Figure 2.** Structures of the previous lead compounds **1** and **2** and **series I-V** compounds.

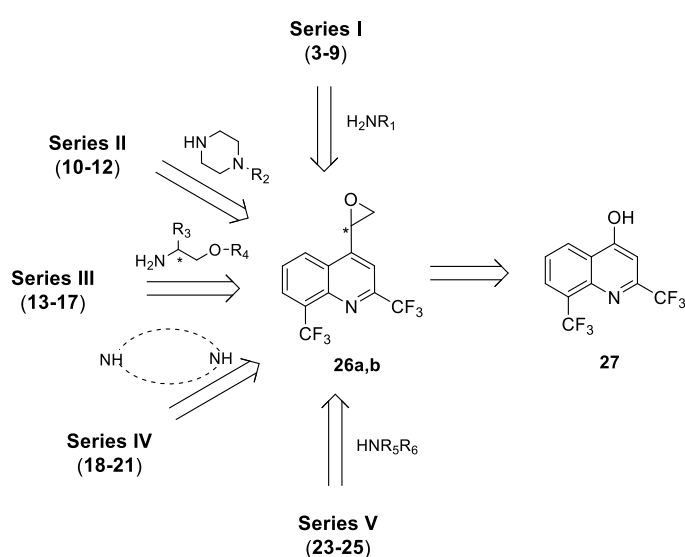
Herein, we report the asymmetric synthesis, the *in vitro* antimalarial activity against two *P. falciparum* strains 3D7 and W2 of the five **series I-V** described above (Figure 2). Some of them were selected for a cytotoxicity study carried out on four different cell lines HepG2, THP-1, CHO and HFF. Finally, further studies were performed with the lead compound (*S,S*)-**17b**. *In vivo* antimalarial activity on *PbA* infected mouse, genotoxicity and pharmacokinetics properties will be described.

## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

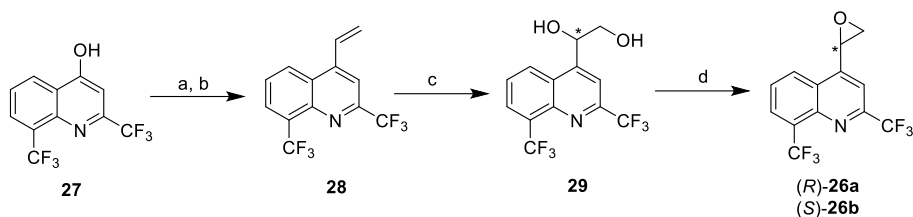
All the compounds were synthesized by a regioselective ring opening of the enantiopure epoxides (*R*)-**26a** or (*S*)-**26b** by commercially available or prepared amines using microwave irradiations (Scheme 1). The syntheses of the two epoxides and the commercially unavailable amines will be described before those of each series of final compounds.

**Scheme 1. Retrosynthesis of compounds of series I-V.**



The key epoxides **26a,b** were prepared in four steps according to the previous published synthesis (Scheme 2) [31]. The 4-hydroxyquinoline **27** was reacted with phosphorus oxybromide to lead to the corresponding 4-bromoquinoline with quantitative yield. A Suzuki-Miyaura reaction in the presence of potassium vinyltrifluoroborate, [1,1'-bis(diphenylphosphino)ferrocene] dichloropalladium(II) as a catalyst and cesium carbonate as base afforded the 4-vinylquinoline **28** in 80% yield. A Sharpless asymmetric dihydroxylation with the commercially available mixture AD-mix  $\beta$  gave the (*R*)-diol **29** with 97% enantiomeric purity while the use of the corresponding mixture AD-mix  $\alpha$  led to the (*S*)-diol **29** with 90% yield and 96% enantiomeric excess [31]. Finally, the epoxides (*R*)-**26a** and (*S*)-**26b** were afforded in a one-pot reaction involving three steps with respectively 80 and 87% yield and enantiomeric excesses superior to 91%.

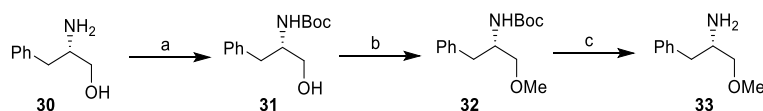
**Scheme 2. Preparation of the key epoxides (*R*)-**26a** and (*S*)-**26b****



**Reagents and conditions:** (a) POBr<sub>3</sub>, 150°C, 100%; (b) CH<sub>2</sub>=CHBF<sub>3</sub>K (1.2 eq), PdCl<sub>2</sub>(dppf).CH<sub>2</sub>Cl<sub>2</sub> (10 mol %), Cs<sub>2</sub>CO<sub>3</sub>, THF/H<sub>2</sub>O, 80%; (c) AD-mix α, K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub> (1 mol %), *t*-BuOH, H<sub>2</sub>O, 20°C, 90% (96% ee, *S*) or AD-mix β, K<sub>2</sub>OsO<sub>2</sub>(OH)<sub>4</sub> (1 mol %), *t*-BuOH, H<sub>2</sub>O, 20°C, 94% (97% ee, *R*); (d) i. trimethyl orthoacetate, APTS (cat.), CH<sub>2</sub>Cl<sub>2</sub>; ii. Me<sub>3</sub>SiCl, CH<sub>2</sub>Cl<sub>2</sub>; iii. K<sub>2</sub>CO<sub>3</sub>, MeOH, 80% (96% ee, *S*) et 87% (92% ee, *R*)

In parallel, different amines non-commercially available were synthesized. The (*S*)-1-methoxy-3-phenylpropan-2-amine **33** was afforded in three steps from the (*S*)-2-amino-3-phenylpropan-1-ol **30** (Scheme 3). This one was protected by a *tert*-butyloxycarbonyl group followed by the methylation of the alcohol function in the presence of silver oxide and methyl iodide. A deprotection with a dichloromethane solution of trifluoroacetic acid led to the desired amine **33** in a 76% global yield.

### Scheme 3. Preparation of the (*S*)-1-methoxy-3-phenylpropan-2-amine **33** for synthesis of series III.

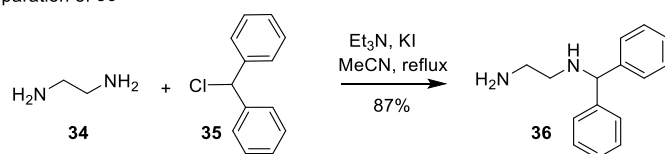


**Reagents and conditions:** (a) Boc<sub>2</sub>O, MeOH, 25°C, 12h, 95%; (b) i. Ag<sub>2</sub>O, DCM, ii. MeI, 25°C, 6 days in the dark, 88%; (c) i. TFA, DCM, 25°C, 12h, ii. NaOHaq 1M, 91%.

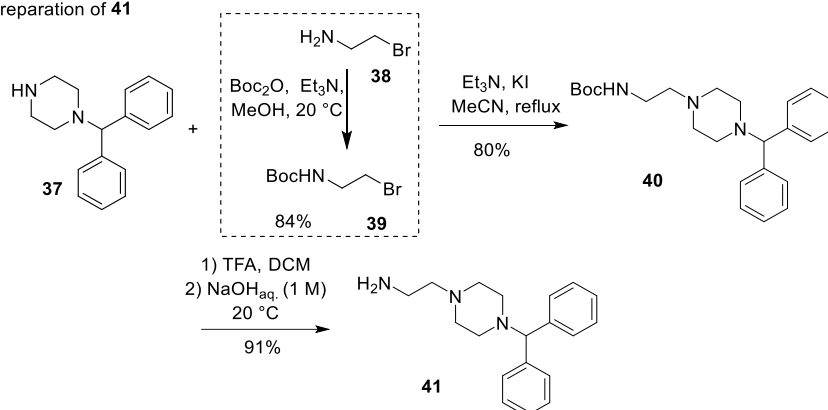
The synthesis of series **V** required the prior preparation of amines **36** and **41** (Scheme 4). To access to **36**, ethylenediamine **34** was reacted with **35** in the presence of potassium iodide as catalyst and triethylamine. A three-step synthesis was used to lead to **36**. After the protection of the 2-bromoethylamine **38**, the resulting compound was reacted with the 1-benzhydrylpiperazine **37** in the same conditions described above. **40** was then deprotected with trifluoroacetic acid to afford **41** with 91% yield.

### Scheme 4. Preparation of the amines **36** and **41** for synthesis of series **V**.

a. preparation of **36**



b. preparation of **41**

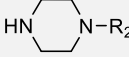

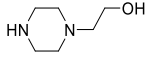

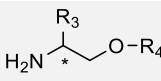


Epoxides **26a** and **26b** were opened, using a regioselective  $S_N2$  ring opening mechanism, with diverse amines commercially available or those previously described in Schemes 3 and 4. To synthesize the lead compounds **1** [31], this reaction had been carried out in classical heating at  $90^\circ\text{C}$  during 24 hours. In this work, this key step was optimized using microwave irradiations and the thirty-four aminoalcohols **1-17** were afforded in 30 minutes (table 1). Ten pairs of enantiomers were synthesized in the **series I** and **II** with yields higher than 70%. The optical rotation was measured for each compound (entries 1-20, table 1) and the enantiomeric excess was determined by chiral high-performance liquid chromatography (HPLC). Enantiomeric purities varied from 86 to 99% depending on the amine. In the **series III**, five families of diastereoisomers were obtained in good yield (entries 21-36, table 1). Compounds **17a** and **17b** were converted to their corresponding chloride salts in quantitative yield by treatment with an anhydrous solution of hydrochloric acid in dioxane.

**Table 1. Preparation of compounds of series I, II and III**

Entry	Amine	Compd	Config. <sup>a</sup>	Yield (%)	<i>Ee</i> or <i>Ed</i> (%) <sup>b</sup>	$\alpha_D^{T_c}$
<b>Series I</b>						
	$\text{H}_2\text{NR}_1$					
1	$\text{H}_2\text{N}-(\text{CH}_2)_2\text{NH}_2$	<b>3a</b>	<i>R</i>	82	ND <sup>c</sup>	-10
2	$\text{H}_2\text{N}-(\text{CH}_2)_2\text{NH}_2$	<b>3b</b>	<i>S</i>	91	ND	+12
3	$\text{H}_2\text{N}-(\text{CH}_2)_{12}\text{NH}_2$	<b>4a</b>	<i>R</i>	70	ND	-27
4	$\text{H}_2\text{N}-(\text{CH}_2)_{12}\text{NH}_2$	<b>4b</b>	<i>S</i>	87	ND	+30
5	$\text{H}_2\text{N}-(\text{CH}_2)_2\text{OH}$	<b>5a</b>	<i>R</i>	73	ND	-54
6	$\text{H}_2\text{N}-(\text{CH}_2)_2\text{OH}$	<b>5b</b>	<i>S</i>	71	ND	+60
7	$\text{H}_2\text{N}-\text{C}(\text{CH}_3)_2\text{CH}_2\text{OH}$	<b>6a</b>	<i>R</i>	71	80	-33
8	$\text{H}_2\text{N}-\text{C}(\text{CH}_3)_2\text{CH}_2\text{OH}$	<b>6b</b>	<i>S</i>	87	89	+43

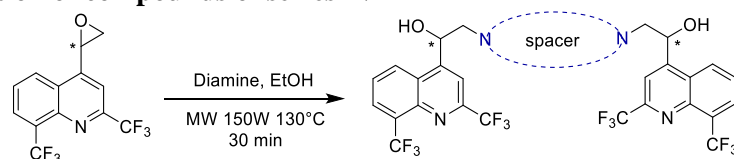


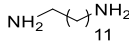
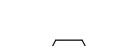


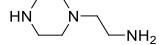

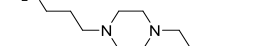
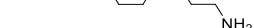
9		<b>7a</b>	<i>R</i>	70	96	-55
10	H <sub>2</sub> N-C((CH <sub>2</sub> ) <sub>3</sub> OH) <sub>3</sub>	<b>7b</b>	<i>S</i>	97	90	+47
11		<b>8a</b>	<i>R</i>	83	92	-27
12	H <sub>2</sub> N-(CH <sub>2</sub> ) <sub>3</sub> -CF <sub>3</sub>	<b>8b</b>	<i>S</i>	90	89	+34
13		<b>9a</b>	<i>R</i>	79	94	-71
14	H <sub>2</sub> N-CH <sub>2</sub> -CH(CH <sub>2</sub> ) <sub>2</sub>	<b>9b</b>	<i>S</i>	82	91	+58
<b>Series II</b>						
						
15		<b>10a</b>	<i>R</i>	88	ND	-57
16		<b>10b</b>	<i>S</i>	Qt <sup>d</sup>	ND	+80
17		<b>11a</b>	<i>R</i>	92	97	-66
18		<b>11b</b>	<i>S</i>	94	95	+59
19		<b>12a</b>	<i>R</i>	57	89	-56
20		<b>12b</b>	<i>S</i>	90	85.5	+44
<b>Series III</b>						
						
21		<b>13a</b>	<i>R,R</i>	92	91 <sup>f</sup>	-63
22		<b>13b</b>	<i>S,S</i>	73	81 <sup>f</sup>	+68
23	H <sub>2</sub> N-C*H(CH <sub>3</sub> )CH <sub>2</sub> OH	<b>13c</b>	<i>R,S</i>	Qt	96 <sup>f</sup>	-37
24		<b>13d</b>	<i>S,R</i>	93	95 <sup>f</sup>	+42
25		<b>14a</b>	<i>R,R</i>	98	100 <sup>f</sup>	-70
26		<b>14b</b>	<i>S,S</i>	87	100 <sup>f</sup>	+68
27	H <sub>2</sub> N-C*H(Ph)CH <sub>2</sub> OH	<b>14c</b>	<i>R,S</i>	Qt	100 <sup>f</sup>	-15
28		<b>14d</b>	<i>S,R</i>	Qt	100 <sup>f</sup>	+13
29		<b>15a</b>	<i>R,R</i>	90	95 <sup>f</sup>	-39
30		<b>15b</b>	<i>S,S</i>	Qt	91 <sup>f</sup>	+39
31	H <sub>2</sub> N-C*H(Bn)CH <sub>2</sub> OH	<b>15c</b>	<i>R,S</i>	Qt	89 <sup>f</sup>	-53
32		<b>15d</b>	<i>S,R</i>	Qt	86 <sup>f</sup>	+28
33		<b>16a</b>	<i>R,S</i>	80	91 <sup>f</sup>	-43
34	H <sub>2</sub> N-C*H(CH <sub>3</sub> )CH <sub>2</sub> OMe	<b>16b</b>	<i>S,S</i>	88	91 <sup>f</sup>	+70
35		<b>17a</b>	<i>R,S</i>	93	97 <sup>f</sup>	-35
36	H <sub>2</sub> N-C*H(Bn)CH <sub>2</sub> OMe	<b>17b</b>	<i>S,S</i>	83	91 <sup>f</sup>	+35

<sup>a</sup>Absolute configuration. <sup>b</sup>Enantiomeric or diastereoisomeric excess determined by HPLC. Conditions in experimental part. <sup>c</sup>Optical rotation. Concentration, solvent and T in experimental part. <sup>d</sup>Quantitative yield. <sup>e</sup>Not determined. <sup>f</sup>In this case, diastereoisomeric excess was done.

The conjugates **18-21** (**series IV**) were synthesized in one step by reacting piperazine, 3,3'-(piperazine-1,4-diyl)*bis*(propan-1-amine), 2-(piperazin-1-yl)ethan-1-amine or dodecane-1,12-diamine with two equivalents of epoxides **26a** or **26b** in the same conditions described above (table 2). Thus, four pairs of enantiomers with the same 2,8-bis(trifluoromethyl)quinoline core but different amine spacer were obtained in a yield ranging from 25 to 99% depending on both the diamine and the configuration of the epoxide used. The measured enantiomeric excesses were superior or equal to 79 % (entries 3-6).

**Table 2. Preparation of compounds of series IV**

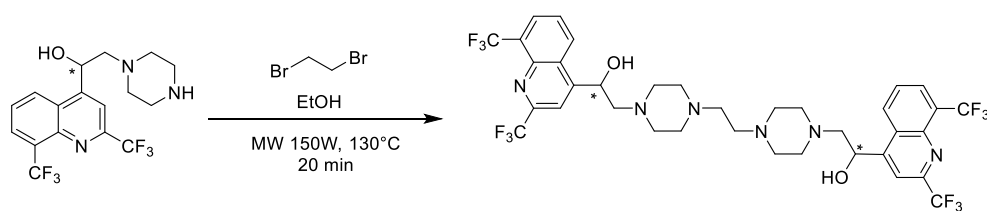


Entry	Diamine	Compd	Config. <sup>a</sup>	Yield (%)	Ee (%) <sup>b</sup>	$\alpha_D^{T_c}$
1		<b>18a</b>	<i>R,R</i>	25	ND <sup>d</sup>	-43
2		<b>18b</b>	<i>S,S</i>	30	ND	+39
3		<b>19a</b>	<i>R,R</i>	73	99.5	-63
4		<b>19b</b>	<i>S,S</i>	57	100	+63
5		<b>20a</b>	<i>R,R</i>	32	89	-39
6		<b>20b</b>	<i>S,S</i>	65	79	+41
7		<b>21a</b>	<i>R,R</i>	53	ND	-53
8		<b>21b</b>	<i>S,S</i>	65	ND	+49

<sup>a</sup>Absolute configuration. <sup>b</sup>Enantiomeric excess determined by HPLC, conditions in experimental part. <sup>c</sup>Optical rotation. Concentration, solvent and T in experimental part. <sup>d</sup>Not determined.

For bisquinolines **22a** and **22b**, the synthesis was achieved by reacting **10a** and **10b** (entries 15 and 16, table 1) with 1,2-dibromoethane in ethanol using microwave irradiations (Scheme 5).

#### Scheme 5. Synthesis of the conjugates **22a,b** (series IV)



**10a** (*R,R*)

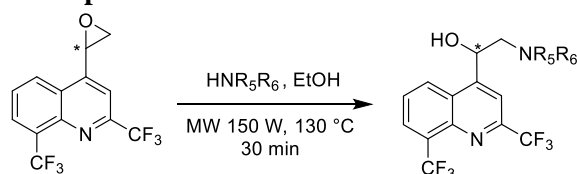
**10b** (*S,S*)

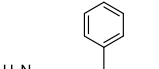
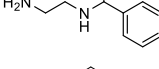
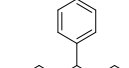
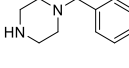
**22a** (*R,R*): 56%,  $[\alpha]_D^{19}$  - 40 (c 0.25, MeOH)

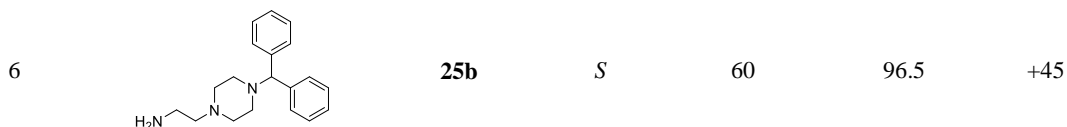
**22b** (*S,S*): 56%,  $[\alpha]_D^{19}$  + 45 (c 0.25, MeOH)

Finally, three enantiomers pairs **23a,b**, **24a,b** and **25a,b** were obtained in series V in moderate yield (Table 3).

**Table 3. Preparation of compounds of series V**

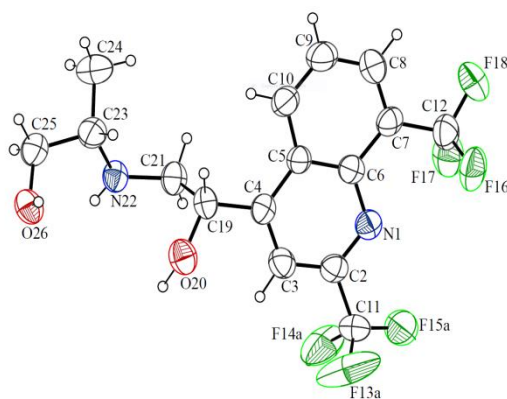


Entry	HNR <sub>5</sub> R <sub>6</sub>	Compd	Config. <sup>a</sup>	Yield (%)	Ee (%) <sup>b</sup>	$\alpha_D^{T_c}$
1		<b>23a</b>	<i>R</i>	80	94	-55
2		<b>23b</b>	<i>S</i>	79	88	+49
3		<b>24a</b>	<i>R</i>	65	93	-59
4		<b>24b</b>	<i>S</i>	56	96	+52
5		<b>25a</b>	<i>R</i>	75	91	-48



<sup>a</sup>Absolute configuration. <sup>b</sup>Enantiomeric excess determined by HPLC, conditions in experimental part. <sup>c</sup>Optical rotation. Concentration, solvent and T in experimental part.

All final compounds were fully characterized using infrared (IR) spectroscopy, melting points, nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS). Their optical rotation was measured and when possible, their enantiomeric purity was determined by chiral HPLC. Some final compounds were crystallized and analyzed by X-Ray diffraction as previously for intermediate **26a** or **28** [31,54]. Thus, the structure of **13b** was confirmed by collecting the crystallographic data on a Bruker Kappa CCD diffractometer using monochromatic Mo- $K\alpha$  radiation (Figure 3,[55]).



**Figure 3.** Crystal structure of **13b**.

## 2.2. Biological evaluation

Two different *in vitro* methods were used to assess the *P. falciparum* parasite growth: the SYBR Green I fluorescence-based method and the HRP-2-based method. The first one measures the incorporation of the fluorescent SYBR Green I dye into the parasite's DNA and was first used to screen the drug candidates **3-25**. Indeed, this test allows on a single step of DNA staining and has thus the advantage of being less intensive labor compared to HRP-2-based method which uses colorimetric ELISA to measure *P. falciparum* HRP-2 protein. However, in some case, non-specific fluorescence background attributed to SYBR Green I binding to human DNA of white blood cells may reduce sensitivity of parasite detection [56]. Thus, thirteen lead compounds identified with the SYBR Green I method were also assessed using the HRP-2-based method. *In vitro* cytotoxicity study was then carried out on four cell lines (HepG2, CHO, THP-1, HFF) for these compounds and **17b** displaying the best selective index (SI) was selected for genotoxic assay. Its *in vitro* profile was completed measuring its both trans-epithelial intestinal permeability (Papp) and human Ether-a-go-go-Related Gene (*hERG*) activity. Finally, **17b** was assessed *in vivo* on mouse model of *PbA* infection and metabolic stability study was performed.

### 2.2.1. *In Vitro* Antiplasmodial Activity

For the two methods quoted above, the reference strains used were culture-adapted *P. falciparum* 3D7 (*Pf3D7*) and W2 (*PfW2*). The first strain is susceptible to CQ but displays a decreased susceptibility to MQ, while the latter is considered as resistant to CQ and susceptible to MQ.

In the first assay using the SYBR Green I method, CQ and MQ were used as controls: CQ showed IC<sub>50</sub> values of 75.9 nM and 198.8 nM respectively against *Pf3D7* and *PfW2* while MQ involved IC<sub>50</sub> values of 79.7 nM (*Pf3D7*) and 31.8 nM (*PfW2*).

In the **series I**, compounds **3**, **5** and **7** with the lower clogP (1.15, 1.47 and 2.27) were those with IC<sub>50</sub> values higher than 1,000 nM (Table 4). Increasing the hydrophobic nature of compounds by rising the length of the aliphatic chain (entries 3-4 vs 1-2) or by introducing methyl groups (entries 7-8 vs 5-6) had a positive impact on antimalarial activity. For example, **6a** (clogP = 2.36, entry 7) with two methyl groups was at least 10-fold more active (IC<sub>50</sub> *Pf3D7* = 99.3 nM) than the corresponding non-methylated **5a** (IC<sub>50</sub> *Pf3D7* > 1,000 nM, clogP = 1.47, entry 5). The best compounds of this **series I** were the (*S*)-enantiomers of **8** (**8b**) carrying a 1,1,1-trifluorobutan-4-yl group (IC<sub>50</sub> *Pf3D7* = 11.4 nM, entry 12) and **9** (**9b**) with a cyclopropanemethyl substituent (IC<sub>50</sub> *Pf3D7* = 4.1 nM, entry 14). As observed for the previous series (Figure 2),[33] enantiomers's activities were different with eudysmic ratios between 0.11 to 0.39 often in favor of the (*S*)-enantiomer. Incorporation of piperazine group (entries 15-20, **series II**) had deleterious effect on antimalarial activity since the IC<sub>50</sub> values were always superior to one micromolar. However, the introduction of both second stereogenic center and a hydrophobic group (methyl, phenyl and benzyl) significantly increased the antimalarial activity (entries 21-36 vs 5-6). Indeed, **series III** compounds were often more active than the reference on the two strains with IC<sub>50</sub> varying between 11.7 to 166.4 nM on *Pf3D7* and 3.3 to 54.3 against *PfW2*. They were more efficient against the CQ-resistant strain *PfW2* than against *Pf3D7* by a factor between 1.1 (entry 36) and 10.3 (entry 28).

Only one hydrophobic substituent in a defined spatial configuration is required: **13a** and **13c** (entries 21 and 23) were 3-fold more active than **6a** (**series I**, entry 7) on *PfW2* with IC<sub>50</sub> values respectively of 10.1 and 10.0 nM. Compared with the non-substituted **5a** (entry 5), the factor is higher than 20 in favor of **13a** and **13b**. Replacement of methyl group with phenyl (**14a-d**, entries 25-28) or benzyl (**15a-d**, entries 29-31) afforded to compounds often more active against *Pf3D7* and as active on *PfW2*. Benzyl substitution led to the best compounds against both *Pf3D7* (**15b**, IC<sub>50</sub> = 5.6 nM), and *PfW2* (**15d**, IC<sub>50</sub> = 3.3 nM).

Finally, increasing the hydrophobicity of **13** and **15** by the methylation of their primary alcohol to afford **16** and **17** had a positive impact on antimalarial activity, more particularly on the *Pf3D7* activity. In this third series, the eudysmic ratios were less important against *Pf3D7* and were ranged from 0.10 to 0.63 on *PfW2*. Once again, (*S*) enantiomers were often more active than their (*R*) counterpart.

**Table 4. Antiplasmodial activity of compounds of series I, II and III against 3D7 and W2 *Plasmodium falciparum* strains using SYBR Green I fluorescence-based method**

Entry	Compd	Config. <sup>a</sup>	IC <sub>50</sub> (nM) <sup>b</sup>		IC <sub>50</sub> <i>Pf3D7</i> /IC <sub>50</sub> <i>PfW2</i>	Eudysmic ratio <sup>e</sup>		cLogP <sup>f</sup>
			<i>Pf3D7</i> <sup>c</sup>	<i>PfW2</i> <sup>d</sup>		<i>Pf3D7</i> <sup>c</sup>	<i>PfW2</i> <sup>d</sup>	
<b>References</b>								
	chloroquine		75.9 ± 3.0	198.8 ± 27.0	0.4	ND <sup>g</sup>	ND	4.33
	mefloquine		79.7 ± 8.5	31.8 ± 1.0	2.5	ND	ND	3.47
<b>Series I</b>								
1	<b>3a</b>	<i>R</i>	> 1,000	> 1,000	ND	ND	ND	1.15

2	<b>3b</b>	<i>S</i>	> 1,000	> 1,000	ND			
3	<b>4a</b>	<i>R</i>	176.3 ± 9.5	379.2 ± 43.2	0.5			
4	<b>4b</b>	<i>S</i>	23.9 ± 1.8	43.8 ± 3.1	0.55	0.13 ( <i>S</i> )	0.11 ( <i>S</i> )	4.64
5	<b>5a</b>	<i>R</i>	> 1,000	ND	ND			
6	<b>5b</b>	<i>S</i>	> 1,000	ND	ND	ND	ND	1.47
7	<b>6a</b>	<i>R</i>	99.3 ± 16.1	31.5 ± 4.0	3.15			
8	<b>6b</b>	<i>S</i>	254.1 ± 27.9	136.9 ± 16.2	1.9	0.39 ( <i>R</i> )	0.23 ( <i>R</i> )	2.36
9	<b>7a</b>	<i>R</i>	> 1,000	ND	ND			
10	<b>7b</b>	<i>S</i>	> 1,000	ND	ND	ND	ND	2.27
11	<b>8a</b>	<i>R</i>	57.6 ± 3.7	61.0 ± 5.2	0.9			
12	<b>8b</b>	<i>S</i>	11.4 ± 1.0	8.55 ± 2.0	1.3	0.20 ( <i>S</i> )	0.14 ( <i>S</i> )	2.91
13	<b>9a</b>	<i>R</i>	23.0 ± 2.2	16.8 ± 2.1	1.4			
14	<b>9b</b>	<i>S</i>	4.1 ± 0.3	4.1 ± 0.2	1.0	0.18 ( <i>S</i> )	0.24 ( <i>S</i> )	3.32
<b>Series II</b>								
15	<b>10a</b>	<i>R</i>	> 1,000	ND	ND			
16	<b>10b</b>	<i>S</i>	> 1,000	ND	ND	ND	ND	2.34
17	<b>11a</b>	<i>R</i>	> 1,000	ND	ND			
18	<b>11b</b>	<i>S</i>	> 1,000	ND	ND	ND	ND	2.62
19	<b>12a</b>	<i>R</i>	> 1,000	ND	ND			
20	<b>12b</b>	<i>S</i>	> 1,000	ND	ND	ND	ND	4.70
<b>Series III</b>								
21	<b>13a</b>	<i>R,R</i>	48.2 ± 8.5	10.1 ± 0.8	4.8			
22	<b>13b</b>	<i>S,S</i>	166.4 ± 24.3	ND	ND	0.29 ( <i>R</i> )	ND	
23	<b>13c</b>	<i>R,S</i>	68.5 ± 8.6	10.0 ± 1.6	6.85			2.20
24	<b>13d</b>	<i>S,R</i>	138.4 ± 13.9	51.7 ± 6.7	2.7	0.49 ( <i>R</i> )	0.19 ( <i>R</i> )	
25	<b>14a</b>	<i>R,R</i>	136.2 ± 18.1	29.9 ± 3.75	4.55			
26	<b>14b</b>	<i>S,S</i>	94.4 ± 7.5	9.8 ± 2.5	9.6	0.69 ( <i>S</i> )	0.32 ( <i>S</i> )	
27	<b>14c</b>	<i>R,S</i>	103.9 ± 5.15	15.9 ± 2.7	6.5			2.82
28	<b>14d</b>	<i>S,R</i>	103.0 ± 6.9	10.1 ± 2.0	10.3	1.0 ( <i>S</i> )	0.63 ( <i>S</i> )	
29	<b>15a</b>	<i>R,R</i>	36.2 ± 6.3	54.3 ± 17.1	0.7			
30	<b>15b</b>	<i>S,S</i>	30.2 ± 3.8	5.6 ± 0.8	5.4	0.83 ( <i>S</i> )	0.10 ( <i>S</i> )	
31	<b>15c</b>	<i>R,S</i>	35.6 ± 2.5	10.3 ± 0.9	3.5			2.97
32	<b>15d</b>	<i>S,R</i>	30.9 ± 2.9	3.3 ± 0.5	9.4	0.87 ( <i>S</i> )	0.32 ( <i>S</i> )	
33	<b>16a</b>	<i>R,S</i>	14.4 ± 1.4	5.1 ± 0.7	2.8			
34	<b>16b</b>	<i>S,S</i>	11.7 ± 1.7	57.2 ± 3.7	0.2	ND	ND	2.69

35	<b>17a</b>	<i>R,S</i>	17.4 ± 2.4	11.4 ± 2.8	1.5		ND	ND	3.47
36	<b>17b</b>	<i>S,S</i>	15.1 ± 1.7	13.2 ± 0.4	1.1				

<sup>a</sup>Absolute configuration. <sup>b</sup>Mean of 2 independent experiments. <sup>c</sup>Susceptible to CQ but displays a decreased susceptibility to MQ. <sup>d</sup>Resistant to CQ and sensitive to MQ. <sup>e</sup>The eudysmic ratio was calculated using the following formula: IC<sub>50</sub> eutomer /IC<sub>50</sub> distomer. The eutomer configuration is between bracket. <sup>f</sup>Calculated with Pallas 3.5. <sup>g</sup>Not determined

Unfortunately, bisquinolines **18-22** (**series IV**, entries 1-10, Table 5) were always less active than the reference CQ and MQ whatever the amino spacer except **20b** which was more active than CQ on *PfW2* (IC<sub>50</sub> = 61.8 nM, entry 6, Table 5). **18b** was 10 to 19-fold less active than the corresponding monoquinoline **4b** depending on the strains. Thus, the strategy consisting to duplicate the heterocycle for fighting resistant strains is inefficient with the 2,8-bis(trifluoromethyl)quinoline core.

Finally, all compounds of **series V** (entries 11-16, Table 5) displayed IC<sub>50</sub> values always below than those of the reference CQ and MQ. The two enantiomers of **23** were the most active of this series whatever the strain with IC<sub>50</sub> values of 35.2 and 35.8 nM on *Pf3D7* and 29.6 and 34 nM against *PfW2*. Thus, efflux pump inhibitor scaffold such as diphenylmethyl moiety seems to be an effective pharmacophore for antimalarial drug.

**Table 5. Antiplasmodial activity of compounds of series IV and V against 3D7 and W2 *Plasmodium falciparum* strains using SYBR Green I fluorescence-based method**

Entry	Compd	Config. <sup>a</sup>	IC <sub>50</sub> (nM) <sup>b</sup>		IC <sub>50</sub> Pf3D7 / IC <sub>50</sub> PfW2	Eudysmic ratio <sup>e</sup>		cLogP <sup>f</sup>
			Pf3D7 <sup>c</sup>	PfW2 <sup>d</sup>		Pf3D7 <sup>c</sup>	PfW2 <sup>d</sup>	
<b>References</b>								
	chloroquine		75.9 ± 3.0	198.8 ± 27.0	0.4	ND <sup>g</sup>	ND	4.33
	mefloquine		79.7 ± 8.5	31.8 ± 1.0	2.5	ND	ND	3.47
<b>Series IV</b>								
1	<b>18a</b>	<i>R,R</i>	> 1,000	> 1,000	ND	< 0.25 ( <i>S,S</i> )	< 0.84 ( <i>S,S</i> )	5.87
2	<b>18b</b>	<i>S,S</i>	248.7 ± 24.43	837.1 ± 238.2	0.3			
3	<b>19a</b>	<i>R,R</i>	> 1,000	> 1,000	ND	< 0.95 ( <i>S,S</i> )	< 0.71 ( <i>S,S</i> )	4.35
4	<b>19b</b>	<i>S,S</i>	953.1 ± 51.1	707.1 ± 129.2	1.35			
5	<b>20a</b>	<i>R,R</i>	453.0 ± 151.9	182.0 ± 47.6	2.5	0.80 ( <i>S,S</i> )	0.34 ( <i>S,S</i> )	4.25
6	<b>20b</b>	<i>S,S</i>	361.1 ± 98.1	61.8 ± 9.0	5.8			
7	<b>21a</b>	<i>R,R</i>	> 1,000	321.0 ± 48.7	> 3.1	ND	0.60 ( <i>R,R</i> )	1.75
8	<b>21b</b>	<i>S,S</i>	> 1,000	534.0 ± 80.2	> 1.9			
9	<b>22a</b>	<i>R,R</i>	146.9 ± 16.6	> 1,000	> 0.15	0.20 ( <i>R,R</i> )	ND	4.59
10	<b>22b</b>	<i>S,S</i>	726.0 ± 13.22	ND	ND			
<b>Series V</b>								
11	<b>23a</b>	<i>R</i>	35.8 ± 11.1	34.0 ± 11.2	1.05	1.0 ( <i>S</i> )	0.87 ( <i>S</i> )	4.60
12	<b>23b</b>	<i>S</i>	35.2 ± 7.5	29.6 ± 18.5	1.2			
13	<b>24a</b>	<i>R</i>	53.6 ± 5.1	29.3 ± 4.9	1.8	0.73 ( <i>S</i> )	0.80 ( <i>R</i> )	5.44
14	<b>24b</b>	<i>S</i>	39.0 ± 2.0	36.7 ± 5.3	1.1			
15	<b>25a</b>	<i>R</i>	45.1 ± 5.4	76.5 ± 5.1	0.6	0.59 ( <i>R</i> )	0.82 ( <i>S</i> )	4.90
16	<b>25b</b>	<i>S</i>	75.7 ± 9.6	63.0 ± 3.7	1.2			

<sup>a</sup>Absolute configuration. <sup>b</sup>Mean of 2 independent experiments. <sup>c</sup>Susceptible to CQ but displays a decreased susceptibility to MQ. <sup>d</sup>Resistant to CQ and sensitive to MQ. <sup>e</sup>The eudysmic ratio was calculated using the following formula: IC<sub>50</sub> eutomer / IC<sub>50</sub> distomer. The eutomer configuration is between bracket. <sup>f</sup>Calculated with Pallas 3.5. <sup>g</sup>Not determined.

For the second screening using the HRP-2-based method (Table 6), ten compounds in series **I-III** with IC<sub>50</sub> values below or near of 50.0 nM (**8a-b**, **9a-b**, **15a-d**, **17a-b**) and three compounds of series **V** (**23a**, **23b** and **25b**) were selected.

With respect to the first screening, four other controls such as monodesethylamodiaquine, lufefantrine, dihydroartemisinin and quinine were added. On *Pf3D7*, the compounds displayed IC<sub>50</sub> values generally slightly above those obtained with the SYBR Green I fluorescence-based method. However, the best compounds **8b**, **9b** and **17b** kept the same with IC<sub>50</sub> values of 15.4, 12.9 and 14.9 nM respectively (entries 2, 4 and 10, Table 6).

**Table 6. Antiplasmodial activity of the lead compounds against 3D7 and W2 *Plasmodium falciparum* strains using the HRP-2-based ELISA method**

Entry	Compd	Config. <sup>a</sup>	IC <sub>50</sub> (nM) <sup>b</sup>		IC <sub>50</sub> Pf3D7 / IC <sub>50</sub> PfW2	Eudysmic ratio <sup>e</sup>	
			Pf3D7 <sup>c</sup>	PfW2 <sup>d</sup>		Pf3D7 <sup>c</sup>	PfW2 <sup>d</sup>
<b>References</b>							
	chloroquine		23.9 ± 4.0	469.2 ± 49.9	0.05	ND <sup>f</sup>	ND
	mefloquine		54.1 ± 10.3	28.3 ± 3.9	1.9	ND	ND
	monodesethylamodiaquine		24.7 ± 3.2	100.0 ± 29.0	0.25	ND	ND
	lumefantrine		28.7 ± 3.5	39.9 ± 2.8	0.7	ND	ND
	dihydroartemisinin		2.8 ± 0.7	3.0 ± 0.9	0.9	ND	ND
	quinine		74.2 ± 25.6	473.9 ± 48.4	0.2	ND	ND
<b>Lead compounds</b>							
1	<b>8a</b>	R	227.4 ± 72.4	229.0 ± 63.3	1.0	0.07 (S)	0.08 (S)
2	<b>8b</b>	S	15.4 ± 3.3	18.2 ± 4.8	0.85		
3	<b>9a</b>	R	17.9 ± 3.6	15.3 ± 2.2	1.2	0.72 (S)	0.97 (S)
4	<b>9b</b>	S	12.9 ± 2.5	15.8 ± 2.9	0.8		
5	<b>15a</b>	R,R	106.5 ± 29.5	63.8 ± 2.4	1.7	0.54 (S)	0.62 (S)
6	<b>15b</b>	S,S	58.1 ± 4.8	39.4 ± 4.0	1.5		
7	<b>15c</b>	R,S	102.2 ± 3.0	82.7 ± 8.4	1.2	0.73 (S)	0.75 (S)
8	<b>15d</b>	S,R	74.8 ± 8.9	61.9 ± 10.1	1.2		
9	<b>17a</b>	R,S	23.2 ± 3.9	15.4 ± 3.2	1.5	ND	ND
10	<b>17b</b>	S,S	14.9 ± 5.0	11.0 ± 1.1	1.35		
11	<b>23a</b>	R	49.8 ± 4.8	20.7 ± 8.5	2.4	0.48 (S)	0.68 (S)
12	<b>23b</b>	S	23.9 ± 8.2	14.1 ± 2.7	1.7		
13	<b>25b</b>	S	116.2 ± 12.5	89.3 ± 22.8	1.3	ND	ND

<sup>a</sup>Absolute configuration. <sup>b</sup>Mean of 5 independent experiments <sup>c</sup>Susceptible to CQ but displays a decreased susceptibility to MQ.

<sup>d</sup>Resistant to CQ and sensitive to MQ. <sup>e</sup>The eudysmic ratio was calculated using the following formula: IC<sub>50</sub> eutomer / IC<sub>50</sub> distomer. The eutomer configuration is between bracket. <sup>f</sup>Not determined

### 2.2.2. *In Vitro* Toxicity and First Pharmacokinetics Parameters

The cytotoxicity of fourteen selected compounds **8a-b**, **9a-b**, **15a-d**, **17a-b**, **23a-b** and **25a-b** was evaluated *in vitro* on four cell lines (Table 7). Three complementary cancer adherent cell lines HepG2, CHO and THP-1 were used. The human-derived hepatocarcinoma cell line HepG2, which is particularly discriminating since it expresses many of the hepatocyte-specific metabolic enzymes, allows to measure the impact of metabolic activation of the tested drug on cell viability [57]. The human monocytic cell line THP-1, derived from an acute monocytic leukemia patient, is commonly used to investigate immune-modulating effects of tested drug in



both activating and resting conditions of cells [58]. Finally, the epithelial cell line CHO, derived from the ovary of the Chinese hamster, does not show any metabolic activation or immune modulation. Moreover, a non-cancer adherent cell line such as human fibroblast cell line HFF derived from normal foreskin was used [59]. The cellular viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) spectrophotometric test regardless of the adherent cell line used. SI were calculated and defined as the ratio between IC<sub>50</sub> values on the different tested cell lines (HepG2, CHO, THP-1 or HFF) and those obtained against *Pf3D7* (Table 6).

All the tested compounds were always less cytotoxic than the reference doxorubicin whatever the cell lines was (Table 7). Generally, all compounds displayed IC<sub>50</sub> values superior to 25 μM on CHO cell line except for compounds of **series V** (**23a,b** and **25a**) which, with IC<sub>50</sub> values close to 10 μM, nevertheless appear three times less cytotoxic than doxorubicin used as reference. Besides, all compounds displayed IC<sub>50</sub> values higher than 10 μM against non-cancer HFF cells. SI values were often superior to 100 except on HFF for **8a** (entry 1), on THP-1 for **15a,c** (entries 5 and 7), on HepG2 for **25a** (entry 13) and on both HepG2 and THP-1 for **25b** (entry 14). Compound **17b** had the best SI ranged from 778 to 3,174 depending on the cell lines (entry 10).

**Table 7.** *In vitro* cytotoxicity results for the lead compounds against HepG2, CHO, THP-1 and HFF cell lines.

Entry	Compd	Con-fig. <sup>a</sup>	IC <sub>50</sub> (μM) <sup>b</sup>				IC <sub>50</sub> <i>Pf3D7</i> (nM) <sup>g</sup>	Selectivity Index (SI) <sup>h</sup>			
			HepG2 <sup>c</sup>	CHO <sup>d</sup>	THP-1 <sup>e</sup>	HFF <sup>f</sup>		HepG2 2 /3D7	CHO /3D7	THP1 /3D7	HFF /3D7
<b>Reference</b>											
	<b>doxorubicin</b>		0.06 ± 0.01	3.6 ± 0.5	0.020 ± 0.001	ND <sup>i</sup>	ND	ND	ND	ND	ND
<b>Lead compounds</b>											
<b>1</b>	<b>8a</b>	<i>R</i>	29.7 ± 1.6	45.4 ± 4.2	32.75 ± 3.0	14.6	227.4 ± 72.4	130	199	144	64
<b>2</b>	<b>8b</b>	<i>S</i>	7.6 ± 0.9	39.5 ± 6.2	> 125 <sup>j</sup>	35.6	15.4 ± 3.3	493	2,564	> 8,116	2,311
<b>3</b>	<b>9a</b>	<i>R</i>	6.6 ± 1.9	25.4 ± 2.6	12.4 ± 3.5	48.4	17.9 ± 3.6	369	1,418	693	2,704
<b>4</b>	<b>9b</b>	<i>S</i>	4.3 ± 0.9	31.0 ± 2.9	6.9 ± 1.3	18.5	12.9 ± 2.5	333	2,403	534	1,434
<b>5</b>	<b>15a</b>	<i>R,R</i>	16.8 ± 2.3	26.6 ± 1.6	7.8 ± 0.4	37.35	106.5 ± 29.5	158	250	73	351
<b>6</b>	<b>15b</b>	<i>S,S</i>	10.9 ± 1.9	29.7 ± 3.3	7.2 ± 1.25	25.4	58.1 ± 4.8	188	511	124	437
<b>7</b>	<b>15c</b>	<i>S,R</i>	15.4 ± 2.6	35.8 ± 4.55	7.5 ± 1.4	45.4	102.2 ± 3.0	151	345	73	444
<b>8</b>	<b>15d</b>	<i>R,S</i>	22.7 ± 4.1	35.2 ± 3.1	9.2 ± 0.9	53.7	74.8 ± 8.9	303	470	123	718
<b>9</b>	<b>17a</b>	<i>R,S</i>	9.6 ± 1.4	37.6 ± 2.8	16.9 ± 1.1	36.7	23.2 ± 3.9	414	1,621	728	1,582
<b>10</b>	<b>17b</b>	<i>S,S</i>	11.6 ± 1.2	47.3 ± 4.3	16.2 ± 0.4	44.2	14.9 ± 5.0	778	3,174	1,087	2,966

11	23a	R	8.8 ± 1.4	11.6 ± 0.9	5.0 ± 0.35	43.9	49.8 ± 4.8	177	233	100	881
12	23b	S	6.4 ± 2.0	9.0 ± 2.6	8.9 ± 0.8	16.6	23.9 ± 8.2	268	377	372	694
13	25a	R	3.3 ± 0.9	10.2 ± 2.9	7.6 ± 1.9	13.9	45.1 ± 5.4 <sup>k</sup>	73	226	168	308
14	25b	S	7.0 ± 1.0	24.8 ± 5.4	10.75 ± 2.4	13.3	116.2 ± 12.5	60	213	93	114

<sup>a</sup>Absolute configuration. <sup>b</sup>IC<sub>50</sub> values were measured on the HepG2, CHO, THP-1 and HFF cell lines. The IC<sub>50</sub> (μM) values for HepG2, CHO and THP-1 correspond to the mean ± standard deviation from three independent experiments while the IC<sub>50</sub> (μM) values for HFF correspond to one experiment. <sup>c</sup>HepG2 is a hepatocarcinoma cell line purchased from ATCC, ref HB-8065. <sup>d</sup>CHO is a Chinese Hamster Ovarian cell line purchased from ATCC, ref CCL-61. <sup>e</sup>THP-1 is an acute monocytic leukemia cell line purchased from ATCC, ref TIB-202. <sup>f</sup>HFF is a Human Foreskin Fibroblast cell line purchased from ATCC, ref SCRC-1041.1. <sup>g</sup>Values obtained by the HRP-2 method, Table 6. <sup>h</sup>Selectivity Index (SI) was defined as the ratio between the IC<sub>50</sub> value on the HepG2, CHO, THP-1 or HFF cell line and the IC<sub>50</sub> value against the *Pf3D7* strain. <sup>i</sup>Not determined. <sup>j</sup>Highest concentration tested due to the compound's limit of solubility in the cell culture medium. <sup>k</sup>Value obtained using the SYBR Green I fluorescence-based method.

The safety profile of **17b** was then completed carrying out a genotoxic study. Its mutagenic potential (without metabolic activation) and that of its associated metabolites (with metabolic activation) were evaluated through two different tests. The first one detects the mutagenic activity by the bacterial reverse mutation assay (Ames Salmonella/microsome mutagenicity assay, OECD guideline N° 471) while the second one measures the cytogenetic effect by the *in vitro* micronucleus assay (MNvit, OECD guideline N° 487). In these experimental conditions, no significant mutagenic, clastogenic and/or aneugenic activities were highlighted for **17b** and its metabolites.

Prior the beginning of the *in vivo* study, Papp as well as *h*ERG activity were measured for **17b**, the previous *in vivo* leads **1a-b**, **2a-b** [51] and the two enantiopure *erythro*-MQ enantiomers (Table 8, [32]).

Papp was determined using the Caco-2/TC7 cell line (Table 8). Except **2a**, all compounds exhibited Papp values above the threshold value of 20 nm/s. The (*S*)-enantiomers **1b** and **2b** possessed a better intestinal permeability compared to their (*R*)-antipode whatever the chain length (entries 2,4 vs 1,3) while the two MQ enantiomers had the same Papp values (Papp = 45 nm/s, entries 6,7). **17b** exhibited favorable characteristics for good intestinal absorption in humans (Papp = 61 nm/s, entry 5) encouraging a future *in vivo* study. Moreover, it inhibited the *h*ERG current with IC<sub>50</sub> value generally superior to those of the previous leads **1** and **2** (except for **2a**, entry 3) and close to the two MQ-enantiomers (IC<sub>50</sub> = 17.3 μM vs 13.6-15 μM, entry 5 vs entries 6,7).

**Table 8. ClogP, Caco-2/TC7 permeability and *h*ERG activity for 1a-b, 2a-b, 17b and *erythro*-MQ enantiomers.**

Entry	Compound	MW (g/mol)	clogP <sup>a</sup>	Caco-2/TC7 Permeability		<i>h</i> ERG
				Papp (nm/s) <sup>b</sup>	(% recovery)	IC <sub>50</sub> (μM)
1	<b>1a</b>			37	86	5.6
2	<b>1b</b>	394	3.84	114	99	8.8
3	<b>2a</b>			5	90	>30
4	<b>2b</b>	424	4.70	53	65	7
5	<b>17b</b>	470	3.47	61	81	17.3
6	<i>R,S</i> -MQ			45	85	13.6
7	<i>S,R</i> -MQ	378	3.47	45	91	15.0

<sup>a</sup>Calculated with Pallas 3.5. <sup>b</sup>Apparent permeability rate coefficient.

### 2.2.3. *In Vivo* Antimalarial Activity and *in Vitro* Metabolic Stability Profile of **17b**

Based on its overall *in vitro* profile, the *in vivo* efficacy of **17b** was evaluated in a mouse model infected by *PbA*. All experiments were carried out according to the French guidelines for animal research and were approved by the Ethics Committee for Animal Experimentation n°5. As for prior work,[51] the BALB/c female mice were infected with *PbA* parasites by intraperitoneal (*ip*) inoculation of donor mouse blood. The treatment was started when the parasitaemia of receiver mice was higher than 1% (Day 0). Three groups of ten mice were randomly distributed and received a daily *ip* dose for five days with MQ.HCl at 9 mg/kg, with **17b.HCl** salt at 10 mg/kg equivalent MQ or with the vehicle for the control group. Mice were monitored for their survival during thirty days and survival curves were used to study the likelihood of death in treated and control mice [60]. Unfortunately, the clearance of blood parasites was observed only in the infected mice treated with MQ within less than hundred hours. For **17b**, the parasitemia increased regularly, in the same manner that in the control group, until it reaches a maximum the day 15. After this day, the survival rate drastically decreased in both the control and the **17b** arms while it stayed constant in the MQ arm (data not shown).

So, to better understand the difference between the strong *in vitro* activity of **17b** and its *in vivo* inactivity, we have searched to characterize its *in vivo* behavior through metabolic stability study (Table 9). **17b** and MQ enantiomers were incubated on three species of liver microsomes (mouse, rat and human). MQ enantiomers were found stable in liver microsomes whatever the species with intrinsic clearance below to 20  $\mu\text{L}/\text{min}/\text{mg}$  (entries 1,2, Table 9). This weak clearance in human liver microsomes was confirmed in human hepatocytes for both MQ enantiomers ( $\text{Cl}_{\text{int}} = 0.002\text{-}0.004 \text{ mL}/\text{h}/10^6 \text{ cells}$ ). As assumed, **17b** exhibited a high turnover in all microsomes ( $\text{Cl}_{\text{int}} > 1,000 \text{ }\mu\text{L}/\text{min}/\text{mg}$ ) which can explain, partly, its *in vivo* inactivity. Moreover, **17b** were identified as potent CYP3A4 inhibitor with  $\text{IC}_{50}$  values of 2.8  $\mu\text{M}$  and 2.9  $\mu\text{M}$ , using midazolam and testosterone, as probe substrates, respectively. A mechanism-based inhibition was also highlighted by  $\text{IC}_{50}$  values shift observed after pre-incubation with ( $\text{IC}_{50} = 5.9 \text{ }\mu\text{M}$ ) and without ( $\text{IC}_{50} = 2.8 \text{ }\mu\text{M}$ ) NADPH cofactor using midazolam as substrate.

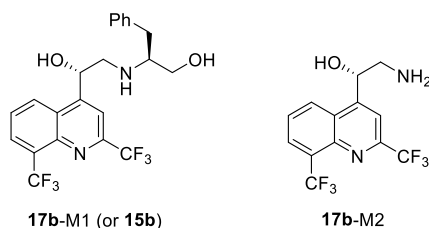
**Table 9. Metabolic stability on both microsomes and hepatocytes and CYP3A4 activity for **17b** and erythro-MQ enantiomers**

Entry	compound	Metabolic stability in liver microsomes			Metabolization in human hepatocytes	CYP3A4 activity	
		$\text{Cl}_{\text{int}} (\mu\text{L}/\text{min}/\text{mg})$				$\text{Cl}_{\text{int}} (\text{mL}/\text{h}/10^6 \text{ cells})$	$\text{IC}_{50} (\mu\text{M})$
		Mouse	Rat	Human			Midazolam
1	<b>R,S-MQ</b>	< 20	< 20	< 20	0.004	45 (43) <sup>a</sup>	19 (12) <sup>a</sup>
2	<b>S,R-MQ</b>	< 20	< 20	< 20	0.002	33 (30)	19 (18)
3	<b>17b</b>	> 1,000	> 1,000	> 1,000	ND <sup>b</sup>	2.8 (5.9)	2.9 (2.3)

<sup>a</sup> $\text{IC}_{50}$  value after pre-incubation without and with (in brackets) NADPH cofactor. <sup>b</sup>Not determined

In order to identify associated metabolites and metabolic hot spot of **17b**, the microsomal stability was monitored by LC-MS (see supporting information) in three microsomal models (mouse, rat and human). After 5 minutes in the presence of mouse microsomes, **17b** was mostly converted in two metabolites (Figure 4): i) **17b-M1**, a *O*-demethylated product and ii) **17b-M2**, a *N*-dealkylated compound. An oxidative product of **17b** was also identified when rat or human microsomes were used. The antimalarial activity of **17b-M1** and **17b-M2** was assessed in this study (**15b**, entry 30, Table 4) and by Milner *et al.*[61]. While **17b-M1** (or **15b**) possessed a good antiplasmodial activity on the two *Pf* strains ( $\text{IC}_{50} \text{ Pf3D7} = 30.2 \text{ nM}$ ,  $\text{IC}_{50} \text{ PfW2} = 5.6 \text{ nM}$ ), the racemic **17b-M2** was described as an inactive compound ( $\text{IC}_{90} \text{ PfW2} = 1,459 \text{ nM}$ ). Thus, the *in vitro* antimalarial activity of **17b** associated metabolites is not the only criterion to explain

the *in vivo* inactivity of **17b** in the mouse model. Also, further studies are currently under progress to better understand the *in vivo* behavior of **17b** and others analogs are designed with highlighted metabolic hot spots in mind.



**Figure 4.** Structures of **17b-M1** and **17b-M2**, the potential metabolites of **17b** in mouse microsomes.

### 3. CONCLUSION

Based on our previous study which highlighted two lead compounds **1b** and **2b** as enantiopure MQ analogs, five new **series I-V** were explored. Fifty-two compounds were synthesized in five to eight steps with good yields and excellent enantiomeric excesses. Their *in vitro* antimalarial activity was evaluated against *Pf3D7* and *PfW2* using two different methods (SYBR Green and HRP-2). The best compounds of **series I** were the (*S*)-enantiomers **8b** and **9b** carrying respectively a 1,1,1-trifluorobutan-4-yl group or a cyclopropanemethyl substituent. They displayed  $IC_{50}$  values of 11.4 nM (**8b**) and 4.1 nM (**9b**) against *Pf3D7*. The introduction of both a second stereogenic center and hydrophobic groups (**series III**) significantly increased the antimalarial activity. Indeed, **series III** compounds displayed  $IC_{50}$  values between 11.7 to 166.4 nM on *Pf3D7* and 3.3 to 57.2 nM against *PfW2*. Compounds of **series V** with a diphenylmethyl moiety as potential efflux pump inhibitor were also highly active with  $IC_{50}$  values lower than both CQ and MQ. A cytotoxicity study carried out on fourteen compounds displaying  $IC_{50}$  values below or close to 50 nM on both strains established that most compounds possessed SI superior to 100. Finally, compound **17b** possessing SI between 778 to 3,174 depending on the cell line was selected for further studies. It demonstrated none mutagenic, clastogenic and/or aneugenic activities. Unfortunately, **17b** was inactive *in vivo* in the mouse model infected by *PbA* which could be explained partly by a weak microsomal stability. SAR studies are currently carried out in order to: (i) remove metabolically hot spots on **17b**, (ii) exploit other potential efflux pump inhibitors in **series V**.

## 4. MATERIALS AND METHODS

### 4.1. Chemistry

Chemicals and solvents were purchased from the Aldrich Chemical Co., VWR, Acros, Fluo-rochem, and Alfa Aesar. They were used as received unless additional purification. The enantiopure epoxides **26a,b** were prepared according to the previous method described by Jonet *et al* [31]. Anhydrous tetrahydrofuran, methanol, acetonitrile, *N,N*-dimethylformamide and dichloromethane were dried using a solvent dryer Pure Solv-Innovative Technology PS-MD-5 purchased from Serlabo Technologies. Air- and moisture-sensitive reactions were carried out under an inert atmosphere of argon in oven-dried glassware. Microwave reactor is a microwave Discover SP reactor purchased from CEM. All the reactions were monitored by thin layer chromatography (TLC) on silica gel on aluminium plates 60F<sub>254</sub> purchased from Merck. The TLC plates were observed by ultra-violet (UV) with a wavelength of 254 nm, before to be revealed chemically by phosphomolybdic acid in ethanol followed by a heating to obtain a maximal

coloration. Metallo-catalysed reactions were monitored using Gas Chromatography (GC) coupled to Mass Spectrometry (MS) Shimadzu CGSM-QP2010S equipped with SLB-5 ms column. LC-MS analyses were performed on an ACQUITY UPLC H-Class system (Waters-Micromass, Manchester, UK) coupled with a SYNAPT G2-Si Q-TOF hybrid quadrupole time-of-flight instrument (Waters-Micromass, Manchester, UK), equipped with an electrospray (ESI) ionization source (Z-spray) and an additional sprayer for the reference compound (Lock Spray, Torrance, CA, USA) heated at 50 °C. Compounds were purified using flash chromatography on silica gel Kieselgel 60 (40-63 μm) purchased from Macherey-Nagel. Melting points were determined using Stuart SMP3 device. Enantiomeric excesses were determined by High Pressure Liquid Chromatography (HPLC) Shimadzu LC-20AD equipped with both an UV detector and two injection pumps SPD-10AS. Depending of the compound structure, different chiral columns were used: Chiralpak IA, IB, ID or IG. Optical rotations ( $[\alpha]_D^T$ ) were measured using a Jasco P-1010 polarimeter or a Jasco P-2000 polarimeter with Peltier thermostated cell hold PTC-262. Specific rotations are reported in 10<sup>-1</sup> deg.cm<sup>2</sup>.g<sup>-1</sup> and concentrations in g per 100 mL. <sup>1</sup>H NMR spectra (300, 400 or 600 MHz) and <sup>13</sup>C NMR spectra (75, 100 or 150 MHz) were recorded on a Bruker 300, 400 or 600 Ultrashield <sup>13</sup>C/<sup>1</sup>H (300, 400 or 600 MHz) spectrometer using deuterated chloroform (CDCl<sub>3</sub>) or deuterated methanol (CD<sub>3</sub>OD). Chemical shifts (δ) are expressed in ppm using the residual solvent as the internal reference. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), or a combination thereof. Coupling constants (*J*) are quoted to the nearest 0.1 Hz. Infrared spectrometry (IR) analyses were performed using FT/IR-4200 Jasco spectrometer equipped with ATR-Golden gate system permitting us to analyze solid and liquid compounds. High Resolution Mass Spectrometry (HRMS) have been carried out using Micromass Q-TOF Ultima with positive electrospray ionization mode. Mass Spectrometry (MS) analyses were carried out using a Shimadzu LCMS-2020 spectrometer.

#### 4.1.1. Preparation of amines **31-33**, **36**, **39-41**.

4.1.1.1. (*S*)-*Tert*-butyl-(1-hydroxy-3-phenylpropan-2-yl)carbamate (**31**). To a solution, under argon and at 0°C, of (*S*)-2-amino-3-phenylpropan-1-ol (2.00 g, 13.2 mmol, 1 eq.) in 40 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub> was added di-*tert*-butyl dicarbonate (3.48 g, 15.8 mmol, 1.2 eq.). The solution was stirred at 25°C for 12 h, washed with 50 mL of a 10% aqueous acetic acid solution and with 40 mL of brine. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in *vacuo*. The residue was purified by flash chromatography (Cyclohexane/AcOEt 2:1) to afford **31** as a white solid. Yield: 95%. R<sub>f</sub> 0.45 (Cyclohexane/AcOEt 2:1).  $[\alpha]_D^{20}$  - 16 (*c* 0.1, AcOEt). Mp 90.4-92.0 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.29-7.18 (m, 5H), 3.80-3.75 (m, 1H), 3.50 (d, *J* = 5.2 Hz, 2H), 2.89 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.68 (dd, *J* = 13.2, 8.0 Hz, 1H), 1.39 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 156.9, 138.8, 129.0, 127.9, 125.8, 80.1, 64.7, 55.5, 38.6, 28.7. IR  $\nu_{\text{max}}$ : 3346, 2934, 1681, 1447, 1314, 1259 cm<sup>-1</sup>. MS (ESI+) *m/z*: 252 (M+H)<sup>+</sup>. HRMS calcd for C<sub>14</sub>H<sub>21</sub>NO<sub>3</sub>Na (M+Na)<sup>+</sup>, 274.1419 found 274.1420.

4.1.1.2. (*S*)-*Tert*-butyl (1-methoxy-3-phenylpropan-2-yl)carbamate (**32**). To a solution, under argon and in the dark, of **31** (1.67 g, 6.61 mmol, 1 eq.) in 32 mL of anhydrous CH<sub>2</sub>Cl<sub>2</sub> were added silver oxide (7.30 g, 33.0 mmol, 5 eq.) and iodomethane (3.90 mL, 63.0 mmol, 10 eq.). The suspension was stirred at 25°C and in the dark for 6 days. The mixture was filtered through a celite pad. The filtrate was concentrated in *vacuo* before to purify the residue by flash chromatography (Cyclohexane/AcOEt 5:1) to afford 465 mg of **32** as a yellow oil. Yield: 88%. R<sub>f</sub> 0.70 (Cyclohexane/AcOEt 5:1).  $[\alpha]_D^{20}$  - 20 (*c* 0.1, AcOEt). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.28-7.16 (m, 5H), 3.28 (s, 3H), 3.27-3.25 (m, 4H), 2.84 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.68 (dd, *J* =

13.2, 8.0 Hz, 1H), 1.37 (s, 9H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  158.1, 140.1, 139.6, 129.4, 127.4, 80.1, 75.0, 59.3, 53.8, 38.9, 28.9. IR  $\nu_{\text{max}}$ : 3350, 2928, 1682, 1451, 1324, 1252  $\text{cm}^{-1}$ . MS (ESI $^+$ )  $m/z$ : 266 (M+H) $^+$ . HRMS calcd for  $\text{C}_{15}\text{H}_{23}\text{BrNO}_3\text{Na}$  (M+Na) $^+$ , 288.1576 found 288.1576.

4.1.1.3. (*S*)-1-Methoxy-3-phenylpropan-2-amine (**33**). To a solution, under argon, of **32** (378 mg, 1.42 mmol, 1 eq.) in 6 mL of anhydrous  $\text{CH}_2\text{Cl}_2$  were added 4 mL of 40% TFA solution in  $\text{CH}_2\text{Cl}_2$ . The solution was stirred at 25°C for 12 h. An aqueous solution of NaOH (1M) was added to pH 14. The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **33** as a yellow oil. Yield: 91%.  $R_f$  0.60 (AcOEt/MeOH/aqueous ammonia 8:1:1).  $[\alpha]_{\text{D}}^{20} = -6$  ( $c$  0.1; AcOEt).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.32-7.19 (m, 5H), 3.34 (s, 3H), 3.28-3.26 (m, 2H), 3.19-3.08 (m, 1H), 2.75 (dd,  $J = 12.8, 4.4$  Hz, 1H), 2.61 (dd,  $J = 13.2, 7.2$  Hz, 1H), 2.15 (sl, 2H,  $\text{NH}_2$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  140.2, 130.7, 129.9, 127.8, 77.6, 59.5, 53.7, 41.3. IR  $\nu_{\text{max}}$ : 3016, 1591, 1453, 1111, 915, 828, 744, 699  $\text{cm}^{-1}$ . MS (ESI $^+$ )  $m/z$ : 166 (M+H) $^+$ . HRMS calcd for  $\text{C}_{10}\text{H}_{16}\text{NO}$  (M+H) $^+$ , 166.1232 found 166.1235.

4.1.1.4. *N*-benzhydrylethane-1,2-diamine (**36**). To a solution of chloro(phenyl)methylbenzene (877  $\mu\text{L}$ , 4.93 mmol, 1 eq.) in 20 mL of anhydrous acetonitrile, under argon, were added successively ethylenediamine (2.13 mL, 39.44 mmol, 8 eq.),  $\text{Et}_3\text{N}$  (825  $\mu\text{L}$ , 5.92 mmol, 1.2 eq.) and KI (42 mg, 0.25 mmol, 0.05 eq.). The resulted mixture was stirred for 16 h at reflux. After an addition of water (20 mL) at room temperature, the product was extracted with AcOEt (3 x 30 mL). The organic phases were brought together, washed with brine (20 mL), dried with  $\text{Na}_2\text{SO}_4$  and concentrated. The obtained residue was then purified by a flash chromatography (DCM/MeOH/aqueous ammonia 95/5/0.5) to afford **36** as a yellow oil. Yield: 87%.  $R_f$  0.18 (DCM/MeOH/aqueous ammonia 95/5/0.5).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42-7.38 (m, 4H), 7.31-7.27 (m, 4H), 7.23-7.17 (m, 2H), 4.82 (s, 1H), 2.81 (t,  $J = 5.8$  Hz, 2H), 2.65 (t,  $J = 5.8$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  144.0, 128.6, 127.4, 127.2, 67.4, 49.2, 41.4 ppm. IR  $\nu_{\text{max}}$ : 3084, 2932, 2832, 1597, 1491, 1451, 1265, 734, 696  $\text{cm}^{-1}$ . MS (ESI $^+$ )  $m/z$ : 227 (M+H) $^+$ .

4.1.1.5. *Tert*-butyl (2-bromoethyl) carbamate (**39**). A solution of 3-bromoethan-1-amine **38** (6.0 g, 29.3 mmol, 1 eq.) in anhydrous methanol (125 mL) was cooled at 0 °C under argon atmosphere.  $\text{Boc}_2\text{O}$  (9.59 g, 43.9 mmol, 1.5 eq.) then triethylamine (6.06 mL, 43.9 mmol, 1.5 eq.) drop to drop were added. The mixture was stirred at 20°C during 12 h. The methanol was evaporated *in vacuo* and water (60 mL) was added to the resulting residue. Extraction was realized with ethyl acetate (3x50 mL). The organic layers were gathered, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated after filtration. The residue was purified by flash chromatography (Cyclohexane/AcOEt 80/20) to afford **39** as a colourless oil. Yield: 84%.  $R_f$  0.60 (Cyclohexane/ AcOEt 8:2).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  4.94 (s, NH), 3.58-3.49 (m, 2H), 3.49-3.38 (m, 2H), 1.45 (s, 9H)  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  155.7, 80.0, 42.5, 33.0, 28.5. IR  $\nu_{\text{max}}$ : 3343, 2977, 2933, 1688, 1508, 1366, 1248, 1161, 1116, 1071, 661.  $\text{cm}^{-1}$ . MS (ESI $^+$ )  $m/z$ : 125.9 (M-Boc) $^+$ .

4.1.1.6. *Tert*-butyl (2-(4-benzhydrylpiperazin-1-yl)ethyl)carbamate (**40**). To a solution, under argon, of 1-benzhydrylpiperazine **37** (1.07 g, 4.24 mmol, 1 eq.) in 20 mL of anhydrous acetonitrile were added *tert*-butyl (2-bromoethyl) carbamate **39** (1.90 g, 8.48 mmol, 2 eq.), KI (70 mg, 0.42 mmol, 0.1 eq.) and triethylamine (886  $\mu\text{L}$ , 6.36 mmol, 1.5 eq.). The mixture was heated at reflux during 8 h. At 25 °C, water was added, the aqueous layer was extracted with ethyl acetate. The organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The residue was purified

by flash chromatography (DCM/MeOH 95:5) to afford **40** as a yellow oil. Yield: 80%.  $R_f$  0.71 (DCM/MeOH 95:5).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.42-7.37 (m, 4H), 7.30-7.22 (m, 4H), 7.22-7.14 (m, 2H), 5.08 (s, 1H), 4.23 (s, 1H), 3.31-3.12 (m, 2H), 2.62-2.30 (m, 10H), 1.43 (s, 9H).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  156.9, 142.7, 128.6, 128.0, 127.1, 79.3, 76.3, 57.3, 53.3, 51.6, 37.1, 28.6. IR  $\nu_{\text{max}}$ : 3083, 2973, 2881, 2810, 1597, 1491, 1451, 1364, 1248, 1152, 1007  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{24}\text{H}_{34}\text{N}_3\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 396.2651 found 396.2640.

4.1.1.7. 2-(4-Benzhydrylpiperazin-1-yl)ethan-1-amine (**41**). To a suspension of **40** (1.395 g, 3.53 mmol) in DCM (20 mL) was added trifluoroacetic acid (5 mL). The mixture was stirred at 20 °C for 2h30. Then an aqueous solution of NaOH (1 M) was added dropwise until pH 12. The aqueous layer was extracted with DCM. The organic layers were dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. **41** was obtained as a yellow oil. Yield: 91%.  $R_f$  0.18 (DCM/MeOH 95:5).  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.46-7.34 (m, 4H), 7.30-7.21 (m, 4H), 7.21-7.11 (m, 2H), 4.21 (s, 1H), 2.79 (t,  $J = 6.2$  Hz), 2.61-2.19 (m, 12H).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ )  $\delta$  142.7, 128.5, 127.7, 127.6, 127.3, 76.1, 53.9, 51.9, 51.5, 35.8. IR  $\nu_{\text{max}}$ : 3307, 2807, 1655, 1448, 1301, 1280, 1134, 1002, 852, 751, 700  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{19}\text{H}_{26}\text{N}_3$  ( $\text{M}+\text{H}$ ) $^+$ , 296.2127 found 296.2124.

#### 4.1.2. General procedure for synthesis of compounds 3-25

To a solution of epoxide **26a** or **26b** (1 eq.) in ethanol (1 mL) was added the appropriate amine. The reaction mixture was heated to 130°C using microwave irradiation ( $P = 150$  W) for 30 min. The obtained solution was concentrated *in vacuo*. The residue was purified by flash chromatography.

4.1.2.1. (R)-2-((2-Aminoethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol (**3a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with ethylenediamine (0.44 mL, 6.5 mmol, 10 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **3a** as a solid gum. Yield: 82 %.  $R_f$  0.20 (AcOEt/MeOH/aqueous ammonia 8:1:1).  $[\alpha]_{\text{D}}^{20} -10$  ( $c$  0.09, MeOH).  $^1\text{H NMR}$  (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.52 (d,  $J = 8.2$ , 1H), 8.22 (d,  $J = 7.1$  Hz, 1H), 8.16 (s, 1H), 7.88-7.80 (m, 1H), 5.69 (dd,  $J = 8.5$ , 2.9 Hz, 1H), 3.02 (dd,  $J = 12.6$ , 3.1 Hz, 1H), 2.90-2.75 (m, 5H);  $^{13}\text{C NMR}$  (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  154.8, 149.3 (q,  $J = 34.9$  Hz), 144.8, 130.2 (q,  $J = 5.5$  Hz), 129.1, 128.6, 128.0, 125.1 (q,  $J = 272.9$  Hz), 122.9 (q,  $J = 274.6$  Hz), 115.7 (q,  $J = 1.8$  Hz), 69.6, 57.2, 51.1, 41.4. IR  $\nu_{\text{max}}$ : 3326, 1640, 1312, 1027, 607  $\text{cm}^{-1}$ . MS (ESI $^+$ )  $m/z$ : 368 ( $\text{M}+\text{H}$ ) $^+$ .

4.1.2.2. (S)-2-((2-Aminoethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol (**3b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with ethylenediamine (0.44 mL, 6.5 mmol, 10 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **3b** as a solid gum. Yield: 91 %.  $[\alpha]_{\text{D}}^{20} +12$  ( $c$  0.10, MeOH).  $R_f$  NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **3a**. HRMS calcd for  $\text{C}_{15}\text{H}_{15}\text{F}_6\text{N}_3\text{ONa}$  ( $\text{M}+\text{Na}$ ) $^+$ , 390.1017 found 390.1000.

4.1.2.3. (R)-2-((12-Aminododecyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**4a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with decane-1,10-diamine (391 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **4a** as a yellow oil. Yield: 70 %.  $R_f$  0.50 (AcOEt/MeOH/aqueous ammonia 8:1:1).  $[\alpha]_{\text{D}}^{23} -27$  ( $c$  0.10, MeOH).  $^1\text{H NMR}$  (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  8.52 (d,  $J = 8.7$ , 1H), 8.23 (d,  $J = 6.9$  Hz, 1H), 8.15 (s, 1H), 7.88-7.82 (m, 1H), 5.67 (dd,  $J = 9.1$ , 2.4 Hz, 1H), 2.96 (dd,  $J = 12.5$ , 3.0 Hz, 1H), 2.78 (dd,  $J = 12.5$ , 8.9 Hz,

1H), 2.82-2.56 (m, 4H), 1.55-1.44 (m, 4H), 1.29-1.27 (m, 16H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 154.9, 149.3 (q, *J* = 37.5 Hz), 144.8, 130.2 (q, *J* = 7.5 Hz), 129.0, 128.6, 127.9, 125.1 (q, *J* = 277.5 Hz), 122.8 (q, *J* = 270.0 Hz), 115.6 (q, *J* = 2.2 Hz), 69.4, 57.5, 50.5, 42.4, 33.4, 30.7, 30.6, 30.55, 28.3, 28.0. IR  $\nu_{\text{max}}$ : 3338, 2921, 2850, 1587, 1305, 1158, 1124, 891, 835, 771 cm<sup>-1</sup>. HRMS calcd for C<sub>25</sub>H<sub>36</sub>F<sub>6</sub>N<sub>3</sub>O (M+H)<sup>+</sup>, 508.2763 found 508.2756.

4.1.2.4. (*S*)-2-((12-Aminododecyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**4b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with decane-1,10-diamine (391 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **4b** as a yellow oil. Yield: 87 %.  $[\alpha]_{\text{D}}^{23}$  +30 (*c* 0.10, MeOH). *R<sub>f</sub>*, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **4a**. HRMS calcd for C<sub>25</sub>H<sub>36</sub>F<sub>6</sub>N<sub>3</sub>O (M+H)<sup>+</sup>, 508.2763 found 508.2762.

4.1.2.5. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((2-hydroxyethyl)amino)ethanol (**5a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with 2-aminoethan-1-ol (391 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **5a** as a yellow solid. Yield: 73 %. *R<sub>f</sub>* 0.37 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 128.9-130.0 °C.  $[\alpha]_{\text{D}}^{20}$  -54 (*c* 0.25, MeOH). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 8.51 (d, *J* = 8.2, 1H), 8.19 (d, *J* = 7.2 Hz, 1H), 8.16 (s, 1H), 7.84-7.77 (m, 1H), 5.69 (dd, *J* = 9.0, 2.5 Hz, 1H), 3.70 (t, *J* = 5.7 Hz, 2H), 3.01 (dd, *J* = 12.5, 3.0 Hz, 1H), 2.89-2.71 (m, 3H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 154.7, 149.3 (q, *J* = 34.0 Hz), 144.8, 130.2 (q, *J* = 3.75 Hz), 130.1 (q, *J* = 29.8 Hz), 129.0, 128.6, 127.9, 125.1 (q, *J* = 272.9 Hz), 122.8 (q, *J* = 274.7 Hz), 115.6 (q, *J* = 1.9 Hz), 69.5, 61.7, 57.2, 52.1. IR  $\nu_{\text{max}}$ : 3295, 2841, 2379, 1306, 1103, 891, 835, 768 cm<sup>-1</sup>. HRMS calcd for C<sub>15</sub>H<sub>15</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 369.1038 found 369.1049.

4.1.2.6. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((2-hydroxyethyl)amino)ethanol (**5b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with 2-aminoethan-1-ol (391 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **5b** as a yellow oil. Yield: 71 %.  $[\alpha]_{\text{D}}^{20}$  +60 (*c* 0.25, MeOH). *R<sub>f</sub>*, Mp, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **5a**. HRMS calcd for C<sub>15</sub>H<sub>15</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 369.1038 found 369.1022.

4.1.2.7. (*R*)-2-((2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-2-methylpropan-1-ol (**6a**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with 2-amino-2-methylpropan-1-ol (0.93 mL, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **6a** as a yellow solid. Yield: 71 %. *R<sub>f</sub>* 0.54 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 168-169 °C. *E<sub>e</sub>*: 80 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA (96:4:0.1 v/v/v); flow 0.7 mL/min, *t<sub>r</sub>*(**6a**) = 50.8 min, *t<sub>r</sub>*(**6b**) = 62.4 min.  $[\alpha]_{\text{D}}^{24}$  -33 (*c* 0.10, MeOH). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.53 (d, *J* = 9.0 Hz, 1H), 8.24 (d, *J* = 7.3 Hz, 1H), 8.14 (s, 1H), 7.89-7.83 (m, 1H), 5.59 (dd, *J* = 9.0, 2.9 Hz, 1H), 3.41-3.28 (m), 2.91 (dd, *J* = 11.8, 3.0 Hz, 1H), 2.74 (dd, *J* = 11.9, 9.0 Hz, 1H), 1.04 (d, *J* = 4.3 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 154.9, 149.3 (q, *J* = 35.0 Hz), 144.8, 130.2 (q, *J* = 31.0 Hz), 130.2 (q, *J* = 5.0 Hz), 129.1, 128.7, 128.0, 125.1 (q, *J* = 271.0 Hz), 122.8 (q, *J* = 273.0 Hz), 115.5 (q, *J* = 2.0 Hz), 70.2, 69.2, 54.9, 50.1, 24.1, 23.0. IR  $\nu_{\text{max}}$ : 3395, 2376, 1603, 1310, 1190, 1142, 1103, 1042, 889, 767, 661 cm<sup>-1</sup>. HRMS calcd for C<sub>17</sub>H<sub>19</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 397.1351 found 397.1348.



4.1.2.8. (*S*)-2-((2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-2-methylpropan-1-ol (**6b**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with 2-amino-2-methylpropan-1-ol (0.93 mL, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **6b** as a yellow solid. Yield: 87%. Ee: 89 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 96:4:0.1; flow 0.7 mL/min,  $t_r(\mathbf{6a}) = 50.8$  min,  $t_r(\mathbf{6b}) = 61.3$  min.  $[\alpha]_D^{24} +43$  (c 0.10, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectra were identical to those of **6a**. HRMS calcd for  $\text{C}_{17}\text{H}_{19}\text{F}_6\text{N}_2\text{O}_2$  (M+H) $^+$ , 397.1351 found 397.1338.

4.1.2.9. (*R*)-4-((2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-4-(3-hydroxypropyl)heptane-1,7-diol (**7a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with 4-amino-4-(3-hydroxypropyl)heptane-1,7-diol (401 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **7a** as a white solid. Yield: 70 %.  $R_f$  0.38 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 140-144 °C. Ee: 96 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 1 mL/min,  $t_r(\mathbf{7b}) = 14.3$  min,  $t_r(\mathbf{7a}) = 16.7$  min.  $[\alpha]_D^{20} -55$  (c 0.25, MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.53 (d,  $J = 8.8$  Hz, 1H), 8.23 (d,  $J = 7.2$  Hz, 1H), 8.15 (s, 1H), 7.88-7.82 (m, 1H), 5.61 (dd,  $J = 8.6, 2.8$  Hz, 1H), 3.57-3.45 (m, 6H), 2.90 (dd,  $J = 11.8, 3.1$  Hz, 1H), 2.73 (dd,  $J = 11.8, 8.5$  Hz, 1H), 1.58-1.32 (m, 12H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  154.9, 149.3 (q,  $J = 35.1$  Hz), 144.7, 130.2 (q,  $J = 27.7$  Hz), 130.2 (q,  $J = 5.25$  Hz), 129.1, 128.6, 128.0, 125.1 (q,  $J = 270.7$  Hz), 122.8 (q,  $J = 273.0$  Hz), 115.7 (q,  $J = 1.5$  Hz), 70.4, 63.4, 57.0, 49.3, 33.1, 27.2. IR  $\nu_{\text{max}}$ : 3301, 3119, 2938, 1311, 1132, 1103, 1052, 1005, 772  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{23}\text{H}_{31}\text{F}_6\text{N}_2\text{O}_4$  (M+H) $^+$ , 513.2188 found 513.2192.

4.1.2.10. (*S*)-4-((2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-4-(3-hydroxypropyl)heptane-1,7-diol (**7b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with 4-amino-4-(3-hydroxypropyl)heptane-1,7-diol (401 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **7b** as a white solid. Yield: 97 %. Ee: 90 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 1 mL/min,  $t_r(\mathbf{7b}) = 14.0$  min,  $t_r(\mathbf{7a}) = 16.8$  min.  $[\alpha]_D^{20} +47$  (c 0.25, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **7a**. HRMS calcd for  $\text{C}_{23}\text{H}_{31}\text{F}_6\text{N}_2\text{O}_4$  (M+H) $^+$ , 513.2188 found 513.2186.

4.1.2.11. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4,4,4-trifluorobutyl)amino)ethan-1-ol (**8a**). Compound **26a** (99 mg, 0.32 mmol, 1 eq.) was treated with 4,4,4-trifluorobutan-1-amine (110  $\mu\text{L}$ , 0.97 mmol, 3 eq.) according to general procedure (0.65 M in EtOH). The residue was purified by flash chromatography (DCM/MeOH 95:5) to afford **8a** as a white solid. Yield: 83%.  $R_f$  0.63 (DCM/MeOH 9:1). Mp 96.4-101.4 °C. Ee: 92%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 95:5; flow 1 mL/min,  $t_r(\mathbf{8a}) = 6.8$  min,  $t_r(\mathbf{8b}) = 13.9$  min.  $[\alpha]_D^{25} -27$  (c 0.10, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.20 (d,  $J = 8.4$  Hz, 1H), 8.16 (d,  $J = 7.6$  Hz, 1H), 8.11 (s, 1H), 7.73 (dd,  $J = 8.0, 7.6$  Hz, 1H), 5.49 (dd,  $J = 3.2, 8.8$  Hz, 1H), 3.18 (dd,  $J = 3.6, 12.8$  Hz, 1H), 2.86-2.72 (m, 3H), 2.25-2.13 (m, 2H), 1.83-1.75 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.0, 148.6 (q,  $J = 35.3$  Hz), 143.7, 129.5 (q,  $J = 29.9$  Hz), 128.8 (q,  $J = 5.7$  Hz), 127.2, 126.7 (q,  $J = 275.2$  Hz), 126.6, 124.0 (q,  $J = 273.7$  Hz), 123.6 (q,  $J = 273.6$  Hz), 114.6 (q,  $J = 2.3$  Hz), 67.7, 55.6, 48.2, 31.4 (q,  $J = 29.0$  Hz), 22.8 (q,  $J = 5.0$  Hz). IR  $\nu_{\text{max}}$ : 3131, 2876, 1602, 1445, 1308, 1248, 1133, 1101, 1020, 889, 770  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_2\text{OF}_9$  (M+H) $^+$ , 435.1119 found 435.1117.

4.1.2.12. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4,4,4-trifluorobutyl)amino)ethan-1-ol (**8b**). Compound **26b** (115 mg, 0.37 mmol, 1 eq.) was treated with 4,4,4-trifluorobutan-1-amine (128  $\mu$ L, 1.12 mmol, 3 eq.) according to general procedure (0.65 M in EtOH). The residue was purified by flash chromatography (DCM/MeOH 95:5) to afford **8b** as a white solid. Yield: 90%. Ee: 89%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 95:5; flow 1 mL/min,  $t_r$ (**8a**) = 6.8 min,  $t_r$ (**8b**) = 13.9 min.  $[\alpha]_D^{23} +34$  (*c* 0.06, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra, were identical to those of **8a**. HRMS calcd for  $\text{C}_{17}\text{H}_{16}\text{N}_2\text{OF}_9$  ( $\text{M}+\text{H}$ ) $^+$ , 435.1119 found 435.1117.

4.1.2.13. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((cyclopropylmethyl)amino)ethan-1-ol (**9a**). Compound **26a** (153 mg, 0.50 mmol, 1 eq.) was treated with cyclopropylmethanamine (347 mg, 1.49 mmol, 3 eq.) according to general procedure (0.65 M in EtOH). The residue was purified by flash chromatography (DCM/MeOH 96:4) to afford **9a** as a white solid. Yield: 79%.  $R_f$  0.36 (DCM/MeOH 96:4). Mp 147.1-149.0  $^\circ\text{C}$ . Ee: 94%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 95:5; flow 1 mL/min,  $t_r$ (**9a**) = 6.9 min,  $t_r$ (**9b**) = 18.0 min.  $[\alpha]_D^{22} -71$  (*c* 0.12, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.23 (d,  $J = 8.0$  Hz, 1H), 8.17-8.14 (m, 2H), 7.72 (dd,  $J = 7.9, 7.2$  Hz, 1H), 5.50 (dd,  $J = 9.1, 3.4$  Hz, 1H), 3.21 (dd,  $J = 12.5, 3.4$  Hz, 1H), 2.75 (dd,  $J = 12.5, 9.1$  Hz, 1H), 2.65 (dd,  $J = 12.4, 6.9$  Hz, 1H), 2.52 (dd,  $J = 12.4, 7.0$  Hz, 1H), 1.02-0.92 (m, 1H), 0.55-0.50 (m, 2H), 0.19-0.12 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.5, 148.5 (q,  $J = 35.3$  Hz), 143.7, 128.6 (q,  $J = 5.7$  Hz), 127.0, 126.9, 126.6, 123.7 (q,  $J = 224.6$  Hz), 123.5 (q,  $J = 213.3$  Hz), 114.5 (q,  $J = 2.2$  Hz), 67.5, 55.3, 54.5, 11.3, 3.5, 3.4. IR  $\nu_{\text{max}}$ : 3075, 2691, 1585, 1311, 1101, 927, 769, 664  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{17}\text{H}_{17}\text{N}_2\text{OF}_6$  ( $\text{M}+\text{H}$ ) $^+$ , 379.1245 found 379.1259.

4.1.2.14. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((cyclopropylmethyl)amino)ethan-1-ol (**9b**). Compound **26b** (101 mg, 0.36 mmol, 1 eq.) was treated with cyclopropylmethanamine (347 mg, 1.08 mmol, 3 eq.) according to general procedure (0.65 M in EtOH). The residue was purified by flash chromatography (DCM/MeOH 96:4) to afford **9b** as a white solid. Yield: 82%. Ee: 91%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 95:5; flow 1 mL/min,  $t_r$ (**9a**) = 6.9 min,  $t_r$ (**9b**) = 17.4 min.  $[\alpha]_D^{22} +58$  (*c* 0.12, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **9a**. HRMS calcd for  $\text{C}_{17}\text{H}_{17}\text{N}_2\text{OF}_6$  ( $\text{M}+\text{H}$ ) $^+$ , 379.1245 found 379.1260.

4.1.2.15. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(piperazin-1-yl)ethan-1-ol (**10a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with piperazine (168 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **10a** as a white solid. Yield: 88%.  $R_f$  0.36 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 135-136  $^\circ\text{C}$ .  $[\alpha]_D^{22} -57$  (*c* 0.09, MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.52 (d,  $J = 8.6$  Hz, 1H), 8.23 (d,  $J = 7.2$  Hz, 1H), 8.15 (s, 1H), 7.88-7.82 (m, 1H), 5.74 (dd,  $J = 7.1, 4.7$  Hz, 1H), 2.88-2.83 (m, 4H), 2.71-2.58 (m, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  155.35, 149.5 (q,  $J = 35.25$  Hz), 145.0, 130.4 (q,  $J = 5.25$  Hz), 130.4 (q,  $J = 30.3$  Hz), 129.61, 128.8, 128.3, 125.3 (q,  $J = 271.5$  Hz), 123.05 (q,  $J = 273.8$  Hz), 116.0 (q,  $J = 1.5$  Hz), 68.4, 67.1, 55.4, 46.5. IR  $\nu_{\text{max}}$ : 2805, 1433, 1310, 1191, 1133, 1096, 837, 772  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{17}\text{H}_{18}\text{F}_6\text{N}_3\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 394.1354 found 394.1351.

4.1.2.16. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(piperazin-1-yl)ethan-1-ol (**10b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with piperazine (168 mg, 1.95 mmol,

3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **10b** as a white solid. Yield: Qt.  $[\alpha]_D^{22} +80$  (c 0.09, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **10a**.

4.1.2.17. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanol (**11a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with 2-(piperazin-1-yl)ethan-1-ol (0.240 mL, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **11a** as a yellow solid. Yield: 92 %.  $R_f$  0.46 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 105-110 °C. Ee: 97 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r(\mathbf{11b}) = 28.0$  min,  $t_r(\mathbf{11a}) = 29.9$  min.  $[\alpha]_D^{19} -66$  (c 0.25, MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.48 (d,  $J = 8.6$  Hz, 1H), 8.19 (d,  $J = 7.2$  Hz, 1H), 8.16 (s, 1H), 7.84-7.77 (m, 1H), 5.71 (dd,  $J = 7.2, 4.5$  Hz, 1H), 3.68 (t,  $J = 6.0$  Hz, 2H), 2.75-2.50 (m, 12H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  155.0, 149.7 (q,  $J = 34.5$  Hz), 144.7, 130.2 (q,  $J = 5.25$  Hz), 130.2 (q,  $J = 30.3$  Hz), 129.3, 128.4, 128.0, 125.0 (q,  $J = 271.5$  Hz), 122.7 (q,  $J = 273.0$  Hz), 115.7, 68.2, 66.15, 61.2, 59.7, 54.3. IR  $\nu_{\text{max}}$ : 3170, 3147, 2828, 1588, 1427, 1310, 1190, 1126, 1098, 1032, 890, 766, 665  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{19}\text{H}_{22}\text{F}_6\text{N}_3\text{O}_2$  (M+H) $^+$ , 438.1616 found 438.1610.

4.1.2.18. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanol (**11b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with 2-(piperazin-1-yl)ethan-1-ol (0.240 mL, 1.95 mmol, 3 eq.). The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **11b** as a yellow solid. Yield: 94 %. Ee: 95 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r(\mathbf{11b}) = 26.1$  min,  $t_r(\mathbf{11a}) = 31.0$  min.  $[\alpha]_D^{19} +59$  (c 0.25, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **12a**. HRMS calcd for  $\text{C}_{19}\text{H}_{22}\text{F}_6\text{N}_3\text{O}_2$  (M+H) $^+$ , 438.1616 found 438.1611.

4.1.2.19. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)ethan-1-ol (**12a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with 1-(naphthalen-1-ylmethyl)piperazine (442 mg, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 95:5) to afford **12a** as a white solid. Yield: 57 %.  $R_f$  0.69 (DCM/MeOH 95:5). Mp 81.2-85.4 °C. Ee: 89%. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 1 mL/min,  $t_r(\mathbf{12b}) = 8.1$  min,  $t_r(\mathbf{12a}) = 9.8$  min.  $[\alpha]_D^{22} -56$  (c 0.13, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.48 (dd,  $J = 8.8, 1.2$  Hz, 1H), 8.27 (d,  $J = 8.0$  Hz, 1H), 8.22 (d,  $J = 7.2$  Hz, 1H), 8.13 (s, 1H), 7.87-7.75 (m, 3H), 7.52-7.35 (m, 4H), 5.71 (dd,  $J = 7.2, 4.8$  Hz, 1H), 3.92 (s, 2H), 2.70-2.57 (m, 10H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  151.4, 148.6 (q,  $J = 34.5$  Hz), 133.9, 133.8, 132.57, 129.4 (q,  $J = 30.0$  Hz), 128.8 (q,  $J = 5.25$  Hz), 128.5, 128.1, 127.5, 127.1, 127.0, 126.6, 123.6 (q,  $J = 271.5$  Hz), 121.4 (q,  $J = 273.75$  Hz), 114.5 (q,  $J = 2.25$  Hz), 65.2, 64.6, 61.0, 53.2. IR  $\nu_{\text{max}}$ : 3451, 2939, 2817, 1593, 1307, 1133, 1103, 1004, 904, 768, 727  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{28}\text{H}_{26}\text{F}_6\text{N}_3\text{O}_2$  (M+H) $^+$ , 534.1980 found 534.1989.

4.1.2.20. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)ethan-1-ol (**12b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with 1-(naphthalen-1-ylmethyl)piperazine (442 mg, 1.95 mmol, 3 eq.). The residue was purified by flash chromatography (DCM/MeOH 95:5) to afford **12b** as a white solid. Yield: 90 %. Ee: 85.5%. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 1 mL/min,  $t_r(\mathbf{12b}) = 8.1$  min,  $t_r(\mathbf{12a}) = 9.8$  min.  $[\alpha]_D^{22} +44$  (c 0.11, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra

were identical to those of **12a**. HRMS calcd for C<sub>28</sub>H<sub>26</sub>F<sub>6</sub>N<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 534.1980 found 534.1977.

4.1.2.21. (*R*)-2-(((*R*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)propan-1-ol (**13a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with (*R*)-2-aminopropan-1-ol (0.152 mL, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **13a** as a yellow solid. Yield: 92 %. R<sub>f</sub> 0.47 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 130-133 °C. Ed: 91 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 0.8 mL/min, t<sub>r</sub>(**13a**) = 38.2 min, t<sub>r</sub>(**13d**) = 60.0 min. [α]<sub>D</sub><sup>20</sup> -63 (c 0.25, MeOH). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 8.52 (d, *J* = 8.7 Hz, 1H), 8.22 (d, *J* = 7.2 Hz, 1H), 8.16 (s, 1H), 7.88-7.80 (m, 1H), 5.68 (dd, *J* = 8.4, 2.9 Hz, 1H), 3.53 (dd, *J* = 10.9, 4.7 Hz, 1H), 3.41 (dd, *J* = 10.9, 6.9 Hz, 1H), 3.04 (dd, *J* = 12.6, 3.2 Hz, 1H<sub>b</sub>), 2.93-2.81 (m, 2H), 1.04 (d, *J* = 6.5 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 154.8, 149.3 (q, *J* = 33.75 Hz), 144.8, 130.2 (q, *J* = 5.25 Hz), 130.2 (q, *J* = 30.3 Hz), 129.0, 128.6, 128.0, 125.1 (q, *J* = 270.8 Hz), 122.8 (q, *J* = 273.0 Hz), 115.7 (q, *J* = 1.8 Hz), 69.4, 66.8, 55.3, 54.4, 16.7. IR ν<sub>max</sub>: 3304, 1428, 1309, 1126, 1103, 1045, 925, 764 cm<sup>-1</sup>. HRMS calcd for C<sub>16</sub>H<sub>17</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 383.1194 found 383.1207.

4.1.2.22. (*S*)-2-(((*S*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)propan-1-ol (**13b**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with (*S*)-2-aminopropan-1-ol (0.152 mL, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **13b** as a yellow solid. Yield: 73 %. Ed: 81 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 0.8 mL/min, t<sub>r</sub>(**13c**) = 39.1 min, t<sub>r</sub>(**13b**) = 59.3 min. [α]<sub>D</sub><sup>20</sup> +68 (c 0.25, MeOH). R<sub>f</sub>, Mp, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **13a**. HRMS calcd for C<sub>16</sub>H<sub>17</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 383.1194 found 383.1209.

4.1.2.23. (*S*)-2-(((*R*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)propan-1-ol (**13c**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with (*S*)-2-aminopropan-1-ol (0.152 mL, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **13c** as a yellow solid. Yield: Qt. R<sub>f</sub> 0.40 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 130-133 °C. Ee: 96 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 0.8 mL/min, t<sub>r</sub>(**13c**) = 37.3 min, t<sub>r</sub>(**13b**) = 60.1 min. [α]<sub>D</sub><sup>20</sup> -37 (c 0.25, MeOH). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) δ 8.53 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 7.2 Hz, 1H), 8.16 (s, 1H), 7.88-7.82 (m, 1H), 5.65 (dd, *J* = 9.5, 2.4 Hz, 1H), 3.58 (dd, *J* = 10.9, 4.2 Hz, 1H), 3.38 (dd, *J* = 10.9, 7.8 Hz, 1H), 3.09 (dd, *J* = 12.1, 2.8 Hz, 1H), 2.83-2.74 (m, 1H), 2.68 (dd, *J* = 12.1, 9.5 Hz, 1H), 1.01 (d, *J* = 6.3 Hz, 3H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD) δ 154.9, 149.3 (q, *J* = 35.25 Hz), 144.8, 130.2 (q, *J* = 5.6 Hz), 130.2 (q, *J* = 30.3 Hz), 129.1, 128.7, 127.8, 125.1 (q, *J* = 271.5 Hz), 122.1 (q, *J* = 274.8 Hz), 115.5 (q, *J* = 1.9 Hz), 69.9, 66.9, 56.0, 55.2, 16.6. IR ν<sub>max</sub>: 3304, 1428 1309, 1126, 1103, 1045, 925, 764 cm<sup>-1</sup>. HRMS calcd for C<sub>16</sub>H<sub>17</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 383.1194 found 383.1177.

4.1.2.24. (*R*)-2-(((*S*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)propan-1-ol (**13d**). Compound **26b** (200 mg, 0.65 mmol, 1 eq.) was treated with (*R*)-2-aminopropan-1-ol (0.152 mL, 1.95 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) as eluent to afford **13d** as a yellow solid. Yield: 93 %. Ee: 95 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 0.8 mL/min, t<sub>r</sub>(**13a**) = 39.0 min, t<sub>r</sub>(**13d**) = 57.4 min. [α]<sub>D</sub><sup>20</sup> +42 (c 0.25, MeOH).

R<sub>f</sub>, Mp, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **13c**. HRMS calcd for C<sub>16</sub>H<sub>17</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 383.1194 found 383.1207.

4.1.2.25. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*R*)-2-hydroxy-1-phenylethyl)amino)ethanol (**14a**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with (*R*)-2-amino-2-phenylethan-1-ol (134 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **14a** as a yellow solid. Yield: 98 %. R<sub>f</sub> 0.52 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 123-124 °C. Ed: 100 %. HPLC analysis: Chiralpak IB column, Heptane/*i*-PrOH/EDA 96:4:0.1; flow 0.5 mL/min, t<sub>r</sub>(**14a**) = 75.7 min. [α]<sub>D</sub><sup>24</sup> -70 (c 0.10, MeOH). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.13 (d, *J* = 7.2 Hz, 1H), 8.12 (s, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.60-7.55 (m, 1H), 7.22-7.30 (m, 5H), 5.69 (dd, *J* = 8.2, 2.7 Hz, 1H), 4.03 (dd, *J* = 8.4, 4.4 Hz, 1H), 3.67 (dd, *J* = 10.8, 4.4 Hz, 1H), 3.57 (dd, *J* = 10.8, 8.5 Hz, 1H), 2.97 (dd, *J* = 13.3, 3.0 Hz, 1H), 2.67 (dd, *J* = 13.3, 8.3 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 154.9, 149.2 (q, *J* = 35.0 Hz), 144.6, 141.0, 130.0 (q, *J* = 30.0 Hz), 129.9 (q, *J* = 5.0 Hz), 129.6, 128.9, 128.85, 128.8, 128.2, 127.8, 125.1 (q, *J* = 272.0 Hz), 122.8 (q, *J* = 273.0 Hz), 115.6 (q, *J* = 2.0 Hz), 68.4, 68.0, 64.5, 54.0. IR √<sub>max</sub>: 3300, 2849, 1664, 1309, 1132, 1100, 1032, 808, 682 cm<sup>-1</sup>. HRMS calcd for C<sub>21</sub>H<sub>19</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 445.1351 found 445.1358.

4.1.2.26. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-2-hydroxy-1-phenylethyl)amino)ethanol (**14b**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-2-amino-2-phenylethan-1-ol (134 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **14b** as a yellow solid. Yield: 87 %. Ed: 100 %. HPLC analysis: Chiralpak IB column, Heptane/*i*-PrOH/EDA 96:4:0.1; flow 0.5 mL/min, t<sub>r</sub>(**14b**) = 83.3 min. [α]<sub>D</sub><sup>24</sup> +68 (c 0.10, MeOH). R<sub>f</sub>, Mp, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **14a**. HRMS calcd for C<sub>21</sub>H<sub>19</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 445.1351 found 445.1356.

4.1.2.27. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-2-hydroxy-1-phenylethyl)amino)ethanol (**14c**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-2-amino-2-phenylethan-1-ol (134 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **14c** as a yellow oil. Yield: Qt. R<sub>f</sub> 0.58 (AcOEt/MeOH/aqueous ammonia 8:1:1). Ed: 100 %. HPLC analysis: Chiralpak IB column, Heptane/*i*-PrOH/EDA 96:4:0.1; flow 0.5 mL/min, t<sub>r</sub>(**14c**) = 114.9 min. [α]<sub>D</sub><sup>20</sup> -15 (c 0.08, MeOH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.16-8.14 (m, 2H), 8.10 (s, 1H), 7.68 (t, *J* = 7.9 Hz, 1H), 7.33-7.19 (m, 5H), 5.54 (dd, *J* = 8.3, 3.5 Hz, 1H), 3.76 (dd, *J* = 8.9, 4.2 Hz, 1H), 3.65 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.59 (dd, *J* = 10.8, 9.0 Hz, 1H), 2.81 (dd, *J* = 12.5, 3.7 Hz, 1H), 2.75 (dd, *J* = 12.5, 8.4 Hz, 1H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 154.8, 149.2 (q, *J* = 35.0 Hz), 144.7, 141.6, 130.0 (q, *J* = 30.0 Hz), 129.9 (q, *J* = 5.0 Hz), 129.6, 129.0, 128.8, 128.75, 127.9, 125.1 (q, *J* = 272.0 Hz), 122.8 (q, *J* = 273.0 Hz), 115.4 (q, *J* = 2.0 Hz), 70.6, 67.7, 66.8, 55.7. IR √<sub>max</sub>: 3300, 2849, 1664, 1309, 1132, 1100, 1032, 808, 682 cm<sup>-1</sup>. HRMS calcd for C<sub>21</sub>H<sub>19</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 445.1351 found 445.1355.

4.1.2.28. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*R*)-2-hydroxy-1-phenylethyl)amino)ethanol (**14d**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with (*R*)-2-amino-2-phenylethan-1-ol (134 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to

afford **14d** as a yellow oil. Yield: Qt. Ed: 100 %. HPLC analysis: Chiralpak IB column, Heptane/*i*-PrOH/EDA 96:4:0.1; flow 0.5 mL/min,  $t_r(\mathbf{14d}) = 51.8$  min.  $[\alpha]_D^{20} +13$  (*c* 0.10, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **14c**. HRMS calcd for  $\text{C}_{21}\text{H}_{19}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 445.1351 found 445.1348.

4.1.2.29. (*R*)-2-(((*R*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-3-phenylpropan-1-ol (**15a**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with (*R*)-2-amino-3-phenylpropan-1-ol (148 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **15a** as a yellow solid. Yield: 90 %.  $R_f$  0.62 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 121-122 °C. Ed: 95 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 0.6 mL/min,  $t_r(\mathbf{15a}) = 25.7$  min,  $t_r(\mathbf{15d}) = 36.3$  min.  $[\alpha]_D^{23} -39$  (*c* 0.10, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.43 (d,  $J = 8.5$  Hz, 1H), 8.22 (d,  $J = 7.2$  Hz, 1H), 8.09 (s, 1H), 7.84-7.79 (m, 1H), 7.22-7.05 (m, 5H), 5.60 (dd,  $J = 8.2, 3.1$  Hz, 1H), 3.55 (dd,  $J = 11.0, 4.5$  Hz, 1H), 3.44 (dd,  $J = 11.0, 6.1$  Hz, 1H), 3.09 (dd,  $J = 12.4, 3.3$  Hz, 1H), 2.93-2.87 (m, 1H), 2.82-2.70 (m, 2H), 2.67 (dd,  $J = 13.6, 7.3$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  154.5, 149.2 (q,  $J = 35.0$  Hz), 144.75, 140.0, 130.1 (q,  $J = 6.0$  Hz), 130.2, 130.1 (q,  $J = 30.0$  Hz), 129.45, 129.1, 128.6, 127.9, 127.3, 125.1 (q,  $J = 271.0$  Hz), 122.8 (q,  $J = 273.0$  Hz), 115.6 (q,  $J = 2.0$  Hz), 69.6, 64.3, 62.0, 55.0, 38.7. IR  $\nu_{\text{max}}$ : 3299, 2838, 1310, 1138, 1106, 1051, 1028, 928, 837, 754, 698  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{22}\text{H}_{21}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 459.1507 found 459.1503.

4.1.2.30. (*S*)-2-(((*S*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-3-phenylpropan-1-ol (**15b**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-2-amino-3-phenylpropan-1-ol (148 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **15b** as a yellow oil. Yield: Qt. Ed: 91 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 0.6 mL/min,  $t_r(\mathbf{15c}) = 27.4$  min,  $t_r(\mathbf{15b}) = 41.5$  min.  $[\alpha]_D^{23} +39$  (*c* 0.10, MeOH).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **15a**. HRMS calcd for  $\text{C}_{22}\text{H}_{21}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 459.1507 found 459.1522.

4.1.2.31. (*S*)-2-(((*R*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-3-phenylpropan-1-ol (**15c**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-2-amino-3-phenylpropan-1-ol (148 mg, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **15c** as a yellow oil. Yield: Qt.  $R_f$  0.62 (AcOEt/MeOH/aqueous ammonia 8:1:1). Ed: 89 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 0.6 mL/min,  $t_r(\mathbf{15c}) = 26.9$  min,  $t_r(\mathbf{15b}) = 41.5$  min.  $[\alpha]_D^{23} -53$  (*c* 0.11, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.46 (d,  $J = 8.5$  Hz, 1H), 8.23 (d,  $J = 7.2$  Hz, 1H), 8.09 (s, 1H), 7.86-7.81 (m, 1H), 7.22-7.08 (m, 5H), 5.59 (dd,  $J = 8.6, 3.2$  Hz, 1H), 3.57 (dd,  $J = 11.1, 4.1$  Hz, 1H), 3.41 (dd,  $J = 11.1, 6.8$  Hz, 1H), 3.03 (dd,  $J = 12.3, 3.4$  Hz, 1H), 2.94-2.87 (m, 1H), 2.80 (dd,  $J = 12.3, 8.7$  Hz, 1H), 2.70-2.67 (m, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  154.7, 149.2 (q,  $J = 35.0$  Hz), 144.75, 140.0, 130.1 (q,  $J = 6.0$  Hz), 130.2, 130.1 (q,  $J = 30.0$  Hz), 129.4, 129.2, 128.6, 128.0, 127.3, 125.1 (q,  $J = 271.0$  Hz), 122.8 (q,  $J = 273.0$  Hz), 115.6 (q,  $J = 2.0$  Hz), 69.8, 64.2, 62.3, 55.3, 38.9. IR  $\nu_{\text{max}}$ : 3299, 2838, 2383, 1310, 1138, 1106, 1051, 1028, 928, 837, 754, 698  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{22}\text{H}_{21}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 459.1507 found 459.1518.

4.1.2.32. (*R*)-2-(((*S*)-2-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)-3-phenylpropan-1-ol (**15d**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with (*R*)-2-amino-3-phenylpropan-1-ol (148 mg, 0.98 mmol, 3 eq.) according to general procedure. The

residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **15c** as a yellow oil. Yield: Qt. Ed: 86 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 90:10:0.1; flow 0.6 mL/min,  $t_r(\mathbf{15a}) = 25.3$  min,  $t_r(\mathbf{15d}) = 34.5$  min.  $[\alpha]_D^{23} +28$  (*c* 0.11, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **15c**. HRMS calcd for  $\text{C}_{22}\text{H}_{21}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 459.1507 found 459.1510.

4.1.2.33. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxypropan-2-yl)amino)ethan-1-ol (**16a**). Compound **26a** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-1-methoxypropan-2-amine (0.103 mL, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 9:1) to afford **16a** as a yellow solid. Yield: 80 %.  $R_f$  0.35 (DCM/MeOH 9:1). Mp 111.4-113.0 °C. Ed: 91 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 0.8 mL/min,  $t_r(\mathbf{16b}) = 16.2$  min,  $t_r(\mathbf{16a}) = 19.8$  min.  $[\alpha]_D^{23} -43$  (*c* 0.13, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.22 (d,  $J = 8.5$  Hz, 1H), 8.14 (d,  $J = 7.6$  Hz, 1H), 8.11 (s, 1H), 7.69 (dd,  $J = 8.0, 7.6$  Hz, 1H), 5.47 (dd,  $J = 8.8, 3.2$  Hz, 1H), 3.39 (dd,  $J = 9.4, 3.7$  Hz, 1H), 3.34 (s, 3H), 3.30-3.23 (m, 2H), 3.00-2.93 (m, 1H), 2.71 (dd,  $J = 12.7, 8.5$  Hz, 1H), 1.10 (d,  $J = 6.5$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.8, 148.6 (q,  $J = 34.0$  Hz), 143.8, 129.4 (q,  $J = 31.0$  Hz), 128.8 (q,  $J = 5.0$  Hz), 127.2, 127.1, 126.7, 123.6 (q,  $J = 272.0$  Hz), 121.4 (q,  $J = 274.0$  Hz), 114.6 (q,  $J = 2.0$  Hz), 77.0, 68.4, 59.1, 53.3, 53.1, 17.8. HRMS calcd for  $\text{C}_{17}\text{H}_{19}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 397.1351 found 397.1353.

4.1.2.34. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxypropan-2-yl)amino)ethan-1-ol (**16b**). Compound **26b** (100 mg, 0.32 mmol, 1 eq.) was treated with (*S*)-1-methoxypropan-2-amine (0.103 mL, 0.98 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 9:1) to afford **16b** as a yellow solid. Yield: 88 %.  $R_f$  0.37 (DCM/MeOH 9:1). Mp 111.6-113.2 °C. Ed: 91 %. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH/EDA (95:5:0.1 v/v/v); flow 0.8 mL/min,  $t_r(\mathbf{16b}) = 15.9$ ,  $t_r(\mathbf{16a}) = 20.0$  min.  $[\alpha]_D^{23} +70$  (*c* 0.13, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.24 (d,  $J = 8.8$  Hz, 1H), 8.13 (m, 1H), 8.14 (s, 1H), 7.71-7.67 (m, 1H), 5.47 (dd,  $J = 9.2, 2.9$  Hz, 1H), 3.42-3.35 (m, 4H), 3.29-3.19 (m, 2H), 3.02-2.93 (m, 1H), 2.68 (dd,  $J = 12.4, 9.5$  Hz, 1H), 1.07 (d,  $J = 6.4$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  151.5, 148.6 (q,  $J = 34.0$  Hz), 143.7, 129.6 (q,  $J = 31.0$  Hz), 128.8 (q,  $J = 5.0$  Hz), 127.2, 126.8, 123.6 (q,  $J = 272.0$  Hz), 121.4 (q,  $J = 274.0$  Hz), 114.6 (q,  $J = 2.0$  Hz), 77.1, 68.0, 59.1, 53.4, 52.7, 17.4; HRMS calcd for  $\text{C}_{17}\text{H}_{19}\text{F}_6\text{N}_2\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 397.1351 found 397.1348.

4.1.2.35. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)ethan-1-ol (**17a**). Compound **26a** (84 mg, 0.27 mmol, 1 eq.) was treated with (*S*)-1-methoxy-3-phenylpropan-2-amine **33** (136 mg, 0.82 mmol, 3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH/aqueous ammonia 8:1:1) to afford **17a** as a yellow oil. Yield: 93 %.  $R_f$  0.58 (DCM/MeOH /aqueous ammonia 8:1:1). Ed: 97 %. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 98.5:1.5; flow 1 mL/min,  $t_r(\mathbf{17b}) = 59.1$  min,  $t_r(\mathbf{17a}) = 91.9$  min.  $[\alpha]_D^{23} -35$  (*c* 0.12, MeOH).  $^1\text{H}$  NMR  $\delta$  (ppm) (400 MHz,  $\text{CD}_3\text{OD}$ ): 8.50 (d,  $J = 8.8$  Hz, 1H), 8.26 (d,  $J = 7.2$  Hz, 1H), 8.11 (s, 1H), 7.87 (dd,  $J = 8.0, 7.6$  Hz, 1H), 7.27-7.21 (m, 2H), 7.16-7.12 (m, 3H), 5.60 (dd,  $J = 8.4, 3.6$  Hz, 1H), 3.40 (dd,  $J = 9.6, 3.6$  Hz, 1H), 3.34 (s, 3H), 3.28 (dd,  $J = 9.6, 6.8$  Hz, 1H), 3.08 (dd,  $J = 12.4, 3.6$  Hz, 1H), 3.06-3.01 (m, 1H), 2.81 (dd,  $J = 12.4, 8.8$  Hz, 1H), 2.72 (dd,  $J = 13.4, 6.7$  Hz, 1H), 2.70 (dd,  $J = 13.4, 6.7$  Hz, 1H).  $^{13}\text{C}$  NMR  $\delta$  (ppm) (100 MHz,  $\text{CD}_3\text{OD}$ ): 153.4, 148.0 (q,  $J = 35.2$  Hz), 143.5, 138.6, 128.9, 128.1, 127.9, 128.4 (q,  $J = 45.3$  Hz), 127.3, 126.6, 126.0, 123.7 (q,  $J = 272.6$  Hz), 121.6 (q,  $J = 274.6$  Hz), 114.2, 73.9, 68.2, 58.5, 57.8, 53.8, 37.6. IR  $\nu_{\text{max}}$ : 3231,

2754, 1586, 1431, 1309, 1131, 1104, 903, 773, 702 cm<sup>-1</sup>. HRMS calcd for C<sub>23</sub>H<sub>23</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 473.1664 found 473.1655.

4.1.2.36. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)ethan-1-ol (**17b**). Compound **26b** (130 mg, 0.42 mmol, 1 eq.) was treated with (*S*)-1-methoxy-3-phenylpropan-2-amine **33** (210 mg, 1.27 mmol, 3 eq.) according to general procedure (0.42 M in EtOH). The residue was purified by flash chromatography (DCM/MeOH/aqueous ammonia 8:1:1) to afford **17b** as a yellow oil. Yield: 83 %. R<sub>f</sub> 0.50 (dichloromethane/methanol/ammonia aqueous 8:1:1 v/v/v). Ed: 91 %. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 98.5:1.5; flow 1 mL/min, t<sub>r</sub>(**17b**) = 58.2 min, t<sub>r</sub>(**17a**) = 90.1 min. [α]<sub>D</sub><sup>25</sup> +35 (c 0.08, MeOH). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ 8.44 (d, *J* = 8.7 Hz, 1H), 8.23 (d, *J* = 7.2 Hz, 1H), 8.09 (s, 1H), 7.83 (dd, *J* = 8.0, 7.6 Hz, 1H), 7.24-7.21 (m, 2H), 7.16-7.13 (m, 3H), 5.58 (dd, *J* = 8.4, 2.8 Hz, 1H), 3.36-3.26 (m, 5H), 3.08 (dd, *J* = 12.4, 3.3 Hz, 1H), 3.02-2.97 (m, 1H), 2.80 (dd, *J* = 12.4, 8.4 Hz, 1H), 2.71 (d, *J* = 6.9 Hz, 2H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ 153.3, 147.7 (q, *J* = 35.2 Hz), 143.4, 138.5, 128.9, 128.8, 128.1, 127.8, 127.2, 126.6, 126.0, 123.5 (q, *J* = 272.6 Hz), 121.5 (q, *J* = 273.6 Hz), 114.3 (q, *J* = 8.0 Hz), 73.9, 68.3, 58.5, 57.8, 53.7, 37.4; HRMS calcd for C<sub>23</sub>H<sub>23</sub>F<sub>6</sub>N<sub>2</sub>O<sub>2</sub> (M+H)<sup>+</sup>, 473.1664 found 473.1646.

4.1.2.37. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)ethan-1-ol hydrochloride (**17a.HCl**). The product **17a** (99 mg, 0.21 mmol, 1 eq.) was solubilized in diethyl ether (5 mL) before addition of hydrochloride solution 4M in dioxane (58 μL, 0.23 mmol, 1.1 eq.). The precipitate was filtrated to afford **17a.HCl** as a yellow solid. Yield: Qt. Mp 183.9-184.6 °C. <sup>1</sup>H NMR δ (ppm) (400 MHz, CD<sub>3</sub>OD): 8.67 (d, *J* = 8.8 Hz, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.28 (s, 1H), 7.98 (dd, *J* = 8.0 Hz, 1H), 7.38-7.24 (m, 5H), 6.04 (dd, *J* = 8.0, *J* = 3.2 Hz, 1H), 3.82-3.76 (m, 1H), 3.63-3.57 (m, 2H), 3.43 (dd, *J* = 10.8, 5.2 Hz, 1H), 3.39-3.35 (m, 4H), 3.25 (dd, *J* = 14.0, 5.2 Hz, 1H), 3.06 (dd, *J* = 13.2, 10.4 Hz, 1H). <sup>13</sup>C NMR δ (ppm) (100 MHz, CD<sub>3</sub>OD): 150.6, 147.9 (q, *J* = 35.2 Hz), 143.5, 135.6, 129.2, 129.1 (q, *J* = 35.4 Hz), 129.1, 128.7, 128.0, 127.4, 127.1, 126.3, 123.6 (q, *J* = 272.6 Hz), 121.4 (q, *J* = 274.6 Hz), 114.3, 68.0, 65.3, 59.5, 58.2, 50.4, 33.6. IR √<sub>max</sub>: 3231, 2754, 1586, 1431, 1309, 1131, 1104, 903, 773, 702 cm<sup>-1</sup>. HRMS, HPLC analysis were identical to those of **17a**.

4.1.2.38. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(((*S*)-1-methoxy-3-phenylpropan-2-yl)amino)ethan-1-ol hydrochloride (**17b.HCl**). The product **17b** (99 mg, 0.21 mmol, 1 eq.) was solubilized in diethyl ether (5 mL) before addition of hydrochloride solution 4M in dioxane (58 μL, 0.23 mmol, 1.1 eq.). The precipitate was filtrated to afford **17b.HCl** as a yellow solid. Yield: Qt. Mp 180.9-183.6 °C. <sup>1</sup>H NMR δ (ppm) (400 MHz, CD<sub>3</sub>OD): 8.56 (d, *J* = 8.8 Hz, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.25 (s, 1H), 7.97 (dd, *J* = 8.0, 7.6 Hz, 1H), 7.40-7.27 (m, 5H), 5.98 (dd, *J* = 10.0, 2.8 Hz 1H), 3.76-3.68 (m, 1H), 3.60-3.50 (m, 2H), 3.45 (dd, *J* = 12.0, *J* = 8.0 Hz, 1H), 3.40 (s, 3H), 3.36-3.30 (m, 1H), 3.25 (dd, *J* = 13.6, 4.8 Hz, 1H), 3.04 (dd, *J* = 13.2, 10.0 Hz, 1H). <sup>13</sup>C NMR δ (ppm) (100 MHz, CD<sub>3</sub>OD): 150.5, 148.3 (q, *J* = 35.2 Hz), 143.5, 135.6, 129.2, 129.1, 129.0 (q, *J* = 36.2 Hz), 128.7, 128.0, 127.4, 127.1, 126.3, 123.5 (q, *J* = 272.6 Hz), 121.0 (q, *J* = 274.6 Hz), 114.8, 68.0, 65.3, 59.5, 58.0, 50.5, 33.6. IR √<sub>max</sub>: 3231, 2754, 1586, 1431, 1309, 1131, 1104, 903, 773, 702 cm<sup>-1</sup>. HRMS, HPLC analysis were identical to those of **17b**.

4.1.2.39. (*1R,1'R*)-2,2'-(Dodecane-1,12-diylbis(azanediyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol) (**18a**). Compound **26a** (195 mg, 0.38 mmol) was treated with (*R*)-2-((12-aminododecyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol **4a** (118 mg, 0.38 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **18a** as a yellow oil. Yield: 25 %. R<sub>f</sub> 0.74



(AcOEt/MeOH/aqueous ammonia 8:1:1).  $[\alpha]_{\text{D}}^{25}$  -43 (*c* 0.12, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.51 (d, *J* = 8.8 Hz, 2H), 8.23 (d, *J* = 7.2 Hz, 2H), 8.145 (s, 2H), 7.85 (dd, *J* = 8.0, 7.3 Hz, 2H), 5.67 (d, *J* = 7.0 Hz, 2H), 2.96 (dd, *J* = 12.4, 2.4 Hz, 2H), 2.77 (dd, *J* = 12.4, 8.8 Hz, 2H), 2.73-2.56 (m, 4H), 1.58-1.46 (m, 4H), 1.38-1.21 (m, 16H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  154.8, 149.3 (q, *J* = 35.0 Hz), 144.8, 130.2 (q, *J* = 5.0 Hz), 130.1 (q, *J* = 30.0 Hz), 129.7, 129.05, 128.7, 128.0, 125.1 (q, *J* = 271 Hz), 122.9 (q, *J* = 273 Hz), 115.6, 69.4, 57.3, 50.5, 30.5, 28.3; IR  $\nu_{\text{max}}$ : 2923, 1306, 1105, 767, 629  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{38}\text{H}_{43}\text{F}_{12}\text{N}_4\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 815.3194 found 815.3155.

4.1.2.40. (*1S,1'S*)-2,2'-(Dodecane-1,12-diylbis(azanediyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol) (**18b**). Compound **26b** (196.8 mg, 0.39 mmol) was treated with (*S*)-2-((12-aminododecyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol **4b** (119 mg, 0.39 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **18b** as a yellow oil. Yield: 30 %.  $[\alpha]_{\text{D}}^{25}$  +39 (*c* 0.12, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **18a**. HRMS calcd for  $\text{C}_{38}\text{H}_{43}\text{F}_{12}\text{N}_4\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 815.3194 found 815.3215.

4.1.2.41. (*1R,1'R*)-2,2'-(Piperazine-1,4-diyl)bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**19a**). Compound **26a** (200 mg, 0.65 mmol, 2 eq.) was treated with piperazine (0.28 mg, 0.33 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 9:1) to afford **19a** as a white solid. Yield: 73 %.  $R_f$  0.76 (DCM/MeOH 9:1). Mp 176 °C. Ee: 99,5 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r$ (**19b**) = 13.5 min,  $t_r$ (**19a**) = 19.1 min.  $[\alpha]_{\text{D}}^{23}$  -63 (*c* 0.20, DMSO).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.50 (d, *J* = 8.6 Hz, 1H), 8.21 (d, *J* = 7.5 Hz, 1H), 8.16 (s, 1H), 7.85-7.79 (m, 1H), 5.72 (dd, *J* = 7.3, 4.6 Hz, 1H), 2.81-2.64 (m, 6H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  155.1, 149.4 (q, *J* = 35.0 Hz), 144.8, 130.2 (q, *J* = 30.3 Hz), 130.2 (q, *J* = 5.5 Hz), 129.4, 128.5, 128.1, 125.1 (q, *J* = 273.4 Hz), 122.9 (q, *J* = 275.0 Hz), 115.8 (q, *J* = 2.25 Hz), 68.3, 66.2, 54.5. IR  $\nu_{\text{max}}$ : 3406, 2932, 2834, 1591, 1428, 1370, 1307, 1129, 1103, 1006, 904, 766, 669  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{30}\text{H}_{25}\text{F}_{12}\text{N}_4\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 701.1786 found 701.1791.

4.1.2.42. (*1S,1'S*)-2,2'-(Piperazine-1,4-diyl)bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**19b**). Compound **26b** (200 mg, 0.65 mmol, 2 eq.) was treated with piperazine (0.28 mg, 0.33 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 9:1) as eluent to afford **19b** as a white solid. Yield: 57 %. Ee: 100 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r$ (**19b**) = 13.1 min.  $[\alpha]_{\text{D}}^{23}$  +63 (*c* 0.20, DMSO).  $R_f$ , Mp, NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **19a**. HRMS calcd for  $\text{C}_{30}\text{H}_{25}\text{F}_{12}\text{N}_4\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 701.1786 found 701.1761.

4.1.2.43. (*R*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(2-(((*R*)-2-(2,8-bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)ethyl)piperazin-1-yl)ethanol (**20a**). Compound **26a** (200 mg, 0.65 mmol, 1 eq.) was treated with 2-(piperazin-1-yl)ethan-1-amine (0.085 mL, 0.65 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) as eluent to afford **20a** as a solid gum. Yield: 32 %.  $R_f$  0.17 (AcOEt/MeOH/aqueous ammonia 8:1:1). Ee: 89 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r$ (**20a**) = 21.6 min,  $t_r$ (**20b**) = 27.9 min.  $[\alpha]_{\text{D}}^{20}$  -39 (*c* 0.25, MeOH).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.53 (d, *J* = 8.1 Hz, 1H), 8.50 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 7.3 Hz, 2H), 8.19 (s, 2H), 7.87-7.81 (m, 2H), 5.75-5.65 (m, 2H), 3.05 (dd, *J* = 12.6, 3.3 Hz, 1H), 2.87 (dd, *J* = 12.6, 8.2 Hz, 1H), 2.84-2.44 (m, 14H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  155.1, 154.7, 149.3 (q, *J* = 35.6 Hz), 144.8, 130.2 (q, *J* = 5.4 Hz), 130.2

(q,  $J = 30.6$  Hz), 129.4, 129.1, 128.7, 128.5, 128.1, 125.1 (q,  $J = 271.5$  Hz), 122.8 (q,  $J = 273.0$  Hz), 115.8, 69.5, 68.3, 66.2, 58.1, 57.0, 54.4, 54.0, 46.6. IR  $\nu_{\max}$ : 2824, 1666, 1307, 1129, 1101, 770  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{32}\text{H}_{30}\text{F}_{12}\text{N}_5\text{O}_2$  ( $\text{M}+\text{H}$ )<sup>+</sup>, 744.2208 found 744.2218.

4.1.2.44. (*S*)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(4-(2-(((*S*)-2-(2,8-bis(trifluoromethyl)quinolin-4-yl)-2-hydroxyethyl)amino)ethyl)piperazin-1-yl)ethan-1-ol (**20b**). Compound **26b** (100 mg, 0.33 mmol, 2 eq.) was treated with 2-(piperazin-1-yl)ethan-1-amine (0.021 mL, 0.16 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **20b** as a solid gum. Yield: 65 %. Ee: 79 %. HPLC analysis: Chiralpak ID column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r$ (**20a**) = 20.6 min,  $t_r$ (**20b**) = 27.5 min.  $[\alpha]_{\text{D}}^{20} +41$  ( $c$  0.25, MeOH).  $R_f$ , NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **20a**. HRMS calcd for  $\text{C}_{32}\text{H}_{30}\text{F}_{12}\text{N}_5\text{O}_2$  ( $\text{M}+\text{H}$ )<sup>+</sup>, 744.2208 found 744.2226.

4.1.2.45. (1*R*,1'*R*)-2,2'-((Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(azanediyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**21a**). Compound **26a** (200 mg, 0.65 mmol, 2 eq.) was treated with 3,3'-(piperazine-1,4-diyl)bis(propan-1-amine) (0.067 mL, 0.33 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **21a** as a white solid. Yield: 53 %.  $R_f$  0.32 (AcOEt/MeOH/aqueous ammonia 8:1:1). Mp 69.3-70 °C.  $[\alpha]_{\text{D}}^{20} -53$  ( $c$  0.25, MeOH). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  8.52 (d,  $J = 8.5$  Hz, 2H), 8.25 (d,  $J = 7.2$  Hz, 2H), 8.16 (s, 2H), 7.87 (t,  $J = 8.0$  Hz, 2H), 5.69 (dd,  $J = 8.5, 2.7$  Hz, 2H), 3.01 (dd,  $J = 12.5, 3.0$  Hz, 2H), 2.88-2.29 (m, 18H), 1.79-1.64 (m, 4H); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD)  $\delta$  154.7, 149.3 (q,  $J = 34.6$  Hz), 144.8, 130.2 (q,  $J = 30.1$  Hz), 130.2 (q,  $J = 5.4$  Hz), 129.1, 128.7, 128.0, 125.1 (q,  $J = 272.9$  Hz), 122.9 (q,  $J = 275.0$  Hz), 115.7 (q,  $J = 2.25$  Hz), 69.4, 57.8, 57.1, 53.9, 49.1, 26.9. IR  $\nu_{\max}$ : 3214, 2823, 1669, 1586, 1307, 1132, 1103, 835, 770  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{36}\text{H}_{39}\text{F}_{12}\text{N}_6\text{O}_2$  ( $\text{M}+\text{H}$ )<sup>+</sup>, 815.2943 found 815.2958.

4.1.2.46. (1*S*,1'*S*)-2,2'-((Piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(azanediyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**21b**). Compound **26b** (200 mg, 0.65 mmol, 2 eq.) was treated with 3,3'-(piperazine-1,4-diyl)bis(propan-1-amine) (0.28 mg, 0.33 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **21b** as a white solid. Yield: 65 %.  $[\alpha]_{\text{D}}^{20} +49$  ( $c$  0.25, MeOH).  $R_f$ , Mp, NMR (<sup>1</sup>H and <sup>13</sup>C) and IR spectra were identical to those of **21a**. HRMS calcd for  $\text{C}_{36}\text{H}_{39}\text{F}_{12}\text{N}_6\text{O}_2$  ( $\text{M}+\text{H}$ )<sup>+</sup>, 815.2943 found 815.2905.

4.1.2.47. (1*R*,1'*R*)-2,2'-(4,4'-(Ethane-1,2-diyl)bis(piperazine-4,1-diyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**22a**). Compound **10a** (200 mg, 0.51 mmol, 2 eq.) was treated with 1,2-dibromoethane (0.022 mL, 0.25 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography using (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **22a** as a solid gum. Yield: 56 %.  $R_f$  0.37 (AcOEt/MeOH/aqueous ammonia 8:1:1).  $[\alpha]_{\text{D}}^{19} -40$  ( $c$  0.25, MeOH). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  8.52 (d,  $J = 8.6$  Hz, 2H), 8.25 (d,  $J = 7.2$  Hz, 2H), 8.15 (s, 2H), 7.86 (t,  $J = 8.0$  Hz, 2H), 5.74 (dd,  $J = 7.0, 4.6$  Hz, 2H), 2.80-2.50 (m, 24H); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD)  $\delta$  155.1, 149.3 (q,  $J = 35.0$  Hz), 144.8, 130.2 (q,  $J = 6.0$  Hz), 130.1 (q,  $J = 30.0$  Hz), 129.4, 128.5, 128.1, 125.1 (q,  $J = 271$  Hz), 122.9 (q,  $J = 273$  Hz), 115.8 (q,  $J = 2.0$  Hz), 68.4, 66.2, 56.2, 54.4, 54.3; HRMS calcd for  $\text{C}_{36}\text{H}_{37}\text{F}_{12}\text{N}_6\text{O}_2$  ( $\text{M}+\text{H}$ )<sup>+</sup>, 813.2786 found 813.2798.

4.1.2.48. (1*S*,1'*S*)-2,2'-(4,4'-(Ethane-1,2-diyl)bis(piperazine-4,1-diyl))bis(1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethanol) (**22b**). Compound **10b** (100 mg, 0.25 mmol, 2 eq.) was treated with 1,2-dibromoethane (0.011 mL, 0.13 mmol, 1 eq.) according to general procedure. The residue was purified by flash chromatography (AcOEt/MeOH/aqueous ammonia 8:1:1) to afford **22b** as a solid gum. Yield: 56 %.  $[\alpha]_{\text{D}}^{19} +45$  (*c* 0.25, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectra were identical to those of **22a**. HRMS calcd for  $\text{C}_{36}\text{H}_{37}\text{F}_{12}\text{N}_6\text{O}_2$  ( $\text{M}+\text{H}$ ) $^+$ , 813.2786 found 813.2792.

4.1.2.49. (*R*)-2-((2-(Benzhydrylamino)ethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**23a**). Compound **26a** (60 mg, 0.19 mmol, 1 eq.) was treated with *N*<sup>1</sup>-benzhydrylethane-1,2-diamine (129 mg, 0.57 mmol, 3 eq.) according to general procedure. The obtained residue was first purified by flash chromatography (DCM/MeOH 98/2 to 96/4) to afford the **23a** as a yellow oil. Yield: 80%.  $R_f$  0.65 (DCM/MeOH 95:5), Ee: 94 %. HPLC analysis: Chiralpak IF column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r(\mathbf{23a}) = 10.0$  min,  $t_r(\mathbf{23b}) = 19.6$  min.  $[\alpha]_{\text{D}}^{20} -55$  (*c* 0.10, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.50 (d,  $J = 8.5$  Hz, 1H), 8.24 (d,  $J = 7.3$  Hz, 1H), 8.15 (s, 1H), 7.83 (t,  $J = 8.0$  Hz, 1H), 7.37 (dd,  $J = 7.7$ , 1.8 Hz, 4H), 7.27 (t,  $J = 7.6$  Hz, 4H), 7.18 (t,  $J = 7.3$  Hz, 2H), 5.67 (dd,  $J = 8.6$ , 3.4 Hz, 1H), 4.82 (s, 1H), 2.97 (dd,  $J = 12.6$ , 3.3 Hz, 1H), 2.92-2.72 (m, 3H), 2.74-2.66 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  153.3, 147.9 (q,  $J = 35.2$  Hz), 143.6, 143.4, 128.8 (q,  $J = 5.8$  Hz), 128.8 (q,  $J = 30.3$  Hz), 128.1, 127.7, 127.3, 127.0, 126.7, 126.6, 123.7 (q,  $J = 272.7$  Hz), 121.4 (q,  $J = 274.5$  Hz), 114.3 (q,  $^3J = 2.5$  Hz), 68.0, 67.1, 55.5, 48.1, 46.3 ppm IR  $\nu_{\text{max}}$ : 3403, 3201, 1547, 1409, 609  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{28}\text{H}_{26}\text{F}_6\text{N}_3\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 534.1980 found 534.1979.

4.1.2.50. (*S*)-2-((2-(Benzhydrylamino)ethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**23b**). Compound **26a** (60 mg, 0.19 mmol, 1 eq.) was treated with *N*<sup>1</sup>-benzhydrylethane-1,2-diamine (129 mg, 0.57 mmol, 3 eq.) according to general procedure. The obtained residue was first purified by flash chromatography (DCM/MeOH 98/2 to 96/4) followed a second one (DCM/MeOH/aqueous ammonia 99/1/0.5 to 98/2/0.5) to afford the **23b** as a yellow oil. Yield: 79%. Ee: 88 %. HPLC analysis: Chiralpak IF column, Heptane/*i*-PrOH/EDA 95:5:0.1; flow 1 mL/min,  $t_r(\mathbf{23a}) = 10.3$  min,  $t_r(\mathbf{23b}) = 20.1$  min.  $[\alpha]_{\text{D}}^{20} +49$  (*c* 0.10, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **23a**. HRMS calcd for  $\text{C}_{28}\text{H}_{26}\text{F}_6\text{N}_3\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 534.1980 found 534.1979.

4.1.2.51. (*R*)-2-(4-Benzhydrylpiperazin-1-yl)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**24a**). Compound **26a** (150 mg, 0.488 mmol, 1 eq.) was treated with 1-benzhydrylpiperazine (184 mg, 0.73 mmol, 1.5 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 100:0 to 95:5) to afford **24a** as a colorless oil. Yield: 65%.  $R_f$  0.39 (DCM/MeOH 98:2). Ee: 93 %. HPLC analysis: Chiralpak IF column, Heptane/*i*-PrOH 98:2; flow 1 mL/min,  $t_r(\mathbf{24b}) = 23.9$  min,  $t_r(\mathbf{24a}) = 29.4$  min.  $[\alpha]_{\text{D}}^{20} -59$  (*c* 0.10, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.49 (dd,  $J = 8.8$ , 1.2 Hz, 1H), 8.24 (d,  $J = 7.6$  Hz, 1H), 8.13 (s, 1H), 7.83 (dd,  $J = 8.4$ , 7.2 Hz, 1H), 7.43 (dd,  $J = 8.4$ , 1.6 Hz, 4H), 7.26 (ddd,  $J = 7.6$ , 7.2, 1.2 Hz, 4H), 7.16 (m, 2H), 5.72 (dd,  $J = 7.2$ , 4.0 Hz, 1H), 4.24 (s, 1H), 2.73-2.65 (m, 6H), 2.45-2.55 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  155.2, 149.3 (q,  $J = 34.7$  Hz), 144.8, 144.1, 130.2 (q,  $J = 5.7$  Hz), 130.1 (q,  $J = 30.3$  Hz), 129.5, 129.4, 129.0, 128.5, 128.1, 125.1 (q,  $J = 272.7$  Hz), 122.8 (q,  $J = 274.5$  Hz), 115.7 (q,  $J = 2.0$  Hz), 77.7, 68.2, 66.4, 54.9, 53.0. HRMS calcd for  $\text{C}_{30}\text{H}_{28}\text{F}_6\text{N}_3\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 560.2137 found: 560.2150.

4.1.2.52. (*S*)-2-(4-Benzhydrylpiperazin-1-yl)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**24b**). Compound **26b** (150 mg, 0.488 mmol, 1 eq.) was treated with 1-benzhydrylpiperazine (184 mg, 0.73 mmol, 1.5 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 100:0 to 95:5) to afford **24b** as a colorless oil. Yield: 56%. Ee: 96%. HPLC analysis: Chiralpak IA column, Heptane/*i*-PrOH 98:2; flow 1 mL/min,  $t_r(\mathbf{24b}) = 23.4$  min,  $t_r(\mathbf{24a}) = 29.1$  min.  $[\alpha]_D^{20} +52$  (*c* 0.10, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) spectra were identical to those of **24a**. HRMS calcd for  $\text{C}_{30}\text{H}_{28}\text{F}_6\text{N}_3\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 560.2137 found: 560.2151.

4.1.2.53. (*R*)-2-((2-(4-Benzhydrylpiperazin-1-yl)ethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**25a**). Compound **26a** (75 mg, 0.24 mmol, 1 eq.) was treated with 2-(4-benzhydrylpiperazin-1-yl)ethan-1-amine (94 mg, 0.32 mmol, 1.3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 100:0 to 95:5) to afford **25a** as a colorless oil. Yield: 75%.  $R_f$  0.29 (DCM/MeOH 98:2). Ee: 91%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 98:5; flow 1 mL/min,  $t_r(\mathbf{25a}) = 15.6$  min,  $t_r(\mathbf{25b}) = 23.1$  min.  $[\alpha]_D^{20} -48$  (*c* 0.10, MeOH).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.14 (dd,  $J = 8.8, 1.2$  Hz, 1H), 8.07-8.04 (m, 2H), 7.60 (dd,  $J = 8.0, 7.6$  Hz, 1H), 7.35-7.28 (m, 4H), 7.21-7.15 (m, 4H), 7.14-7.07 (m, 2H), 5.45 (dd,  $J = 8.4, 3.2$  Hz, 1H), 4.14 (s, 1H), 3.09 (dd,  $J = 13.2, 3.2$  Hz, 1H), 2.82-2.64 (m, 3H), 2.57-2.28 (m, 10H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  152.0, 148.6 (q,  $J = 35.0$  Hz), 143.7, 142.7, 129.6 (q,  $^3J = 30.1$  Hz), 128.5, 128.0, 127.0, 127.0, 126.7, 124.3 (q,  $J = 275.4$  Hz), 121.5 (q,  $^1J = 273.4$  Hz), 115.0 (q,  $^3J = 1.9$  Hz), 76.3, 68.6, 57.4, 55.4, 53.4, 51.8, 45.3. IR  $\nu_{\text{max}}$ : 3389, 3258, 1606, 1433, 1100, 676  $\text{cm}^{-1}$ . HRMS calcd for  $\text{C}_{32}\text{H}_{33}\text{F}_6\text{N}_4\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 603.2559 found 603.2559.

4.1.2.54. (*S*)-2-((2-(4-Benzhydrylpiperazin-1-yl)ethyl)amino)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)ethan-1-ol (**25b**). Compound **26b** (75 mg, 0.24 mmol, 1 eq.) was treated with 2-(4-benzhydrylpiperazin-1-yl)ethan-1-amine (94 mg, 0.32 mmol, 1.3 eq.) according to general procedure. The residue was purified by flash chromatography (DCM/MeOH 100:0 to 95:5) to afford **25b** as a colorless oil. Yield: 60%. Ee: 96.5%. HPLC analysis: Chiralpak IG column, Heptane/*i*-PrOH 98:5; flow 1 mL/min,  $t_r(\mathbf{25a}) = 16.0$  min,  $t_r(\mathbf{25b}) = 22.1$  min.  $[\alpha]_D^{20} +45$  (*c* 0.10, MeOH).  $R_f$ , NMR ( $^1\text{H}$  and  $^{13}\text{C}$ ) and IR spectra were identical to those of **25a**. HRMS calcd for  $\text{C}_{32}\text{H}_{33}\text{F}_6\text{N}_4\text{O}$  ( $\text{M}+\text{H}$ ) $^+$ , 603.2559 found 603.2559.

## 4.2. Biological evaluation

### 4.2.1. *In vitro* SYBR Green I fluorescence-based method.

Two *Plasmodium falciparum* strains were used : (i) *Pf3D7*, susceptible to chloroquine but displays a decreased susceptibility to mefloquine (cloned from NF54, Netherlands, Europe) and (ii) *PfW2*, susceptible to mefloquine and resistant to chloroquine (cloned from Indo III/CDC, Indochina, Asia). The cultivation of these two strains (obtained from the collection of the National Museum of Natural History, Paris, France) was carried out in a Roswell Park Memorial Institute (RPMI) medium (Sigma–Aldrich, Lyon, France) supplemented with 0.5% Albumax I (Life Technologies corporation, Paisley, United Kingdom), hypoxanthine (Sigma–Aldrich) and gentamicin (Sigma–Aldrich) with human erythrocytes. As previously described [62], the parasites were incubated at 37°C with 5 %  $\text{CO}_2$ .

The *P. falciparum* drug susceptibility was assessed using a 96-well flat bottom sterile plates under a final volume of 250  $\mu\text{L}$ . Drugs were dissolved in DMSO then diluted in sterile water in order to obtain a range of concentration from 39 nM to 40  $\mu\text{M}$  for a first screening. A second

range of concentration from 1 nM to 1  $\mu$ M was carried out when the molecule efficacy was warranted on the wide range. Negative (DMSO and H<sub>2</sub>O) and positive (chloroquine and mefloquine) controls were added to each set of experiments. After 48 h incubation at 37°C, plates containing drugs and cultured parasites were frozen and stored at -20°C until quantities of *P. falciparum* DNA in each well were determined according to the SYBR Green I (Sigma–Aldrich) fluorescence-based method [63–65].

Briefly, after thawing the plates for 2 h at room temperature, 100  $\mu$ L of the homogenized culture were additionning with 100  $\mu$ L of the SYBR Green I lysis buffer (which consisted of 2xSYBR Green, 20 mM Tris base pH 7.5, 5 mM EDTA, 0.008% w/v saponin, 0.08% w/v Triton X-100) in a 96-well flat bottom sterile black plates (Nunc Inc.). These plates were then incubated in the dark for 1 h at room temperature and the fluorescence intensity was measured with a fluorescence plate reader (Tecan, Austria) with excitation and emission wavelengths of 485 and 535 nm, respectively.

The concentrations at which the screening drug or antimalarial is capable of inhibiting 50% of parasitic growth (IC<sub>50</sub>) are calculated from a sigmoid inhibition model Emax with an estimate of IC<sub>50</sub> by non-linear regression (IC Estimator version 1.2) and are reported as means calculated from two independent experiments [64].

#### **4.2.2. *In vitro* HRP2-based ELISA method**

Drugs were dissolved in methanol then diluted in sterile water to obtain a range of concentration from 0.1 nM to 500 nM. Antiplasmodial activity of drugs was compared to reference lumefantrine (LMF, Novartis Pharma, Bâle, Switzerland), chloroquine (CQ, Sigma, Saint-louis, MO, USA), mefloquine (MQ, Roche, Paris, France), monodesethylamodiaquine, the active metabolite of amodiaquine (MDAQ, OMS, Genève, Switzerland) and dihydroartemisinin, the active metabolite of artemisinin (DHA, Sigma). MDAQ, MQ and DHA were dissolved in methanol then diluted in sterile water to obtain a concentration range between 1.9 nM to 1988 nM for MDAQ, 1.5 nM to 392 nM for MQ and 0.1 nM to 107 nM for DHA. LMF was dissolved and diluted in ethanol to obtain a concentration range between 0.6 nM to 310 nM. CQ was dissolved and diluted in water to obtain a concentration range from 6 nM to 3231 nM.

The clones *Pf*3D7 (isolated in West Africa, obtained from MR4, Manassas, VA, USA) and *Pf*W2 (isolated in Indochina, obtained from MR4, Manassas, VA, USA), maintained in continuous cultivation, were synchronized by sorbitol method before use [66]. Clonality of these clones was controlled each 15 days (genotypic polymorphism markers and microsatellites) and annually by an independent laboratory (Worldwide antimalarial resistance network) [67,68]. These two clones were maintained in continuous culture at 1.5% hematocrit in the presence of healthy red blood cells of group A + (Etablissement Français du sang, Marseille, France), in a RPMI 1640 (Invitrogen, Paisley, RU) buffered with HEPES (25 mM) and NaHCO<sub>3</sub> (25 mM) and supplemented with 10% human serum (Abcys, Paris, France) in controlled atmosphere at 10% O<sub>2</sub>, 6% CO<sub>2</sub> and 84% N<sub>2</sub>.

The *P. falciparum* drug susceptibility was assessed using a 96-well flat bottom sterile plates under a final volume of 225  $\mu$ L. For the assay, 200  $\mu$ L of parasitized erythrocyte suspension (0.5% final parasitaemia and 1.5% hematocrit) were distributed in each wells of the plate of 96 wells pre-dosed with the different concentrations of compounds to be evaluated. Plates were incubated 72 h in controlled atmosphere at 10% O<sub>2</sub>, 6% CO<sub>2</sub> and 84% N<sub>2</sub>. After freezing and hemolysis, then dilution of the parasitaemia of the control without drugs at 72 h to the equivalent of 0.02%, the HRP2 concentration (Histidine-rich protein 2) of the supernatants was evaluated by the commercial test HRP2 ELISA (Malaria Ag Celisa, Cellabs PTY LTD, Brookvale,

Australia). The optical density (OD) of each well, correlated with HRP2 concentration and parasitic growth, was measured by spectrophotometry (Saphir 2, Tecan, Lyon, France).

The concentration which the screening drug or antimalarial was capable of inhibiting 50% of parasitic growth (IC<sub>50</sub>) was calculated from a sigmoid inhibition model Emax with an estimate of IC<sub>50</sub> by non-linear regression (ICEstimator version 1.2) (<http://www.antimalarial-icestimator.net>) and was reported as means calculated from five independent experiments.

#### **4.2.3. *In vitro* cytotoxic studies**

The cytotoxicity was assessed according to the slightly modified method previously reported by Mosmann to calculate the IC<sub>50</sub> values [69]. Doxorubicin purchased from Sigma Aldrich was used as cytotoxic reference. These assays were performed toward four adherent cell lines: i) the human HepG2 cells (hepatocarcinoma cell line purchased from ATCC, ref HB-8065), ii) the human monocytic THP-1 cells (acute monocytic leukemia cell line purchased from ATCC, ref TIB-202), iii) the mammal CHO cells (Chinese Hamster Ovarian cell line purchased from ATCC, ref CCL-61), iv) the human fibroblast HFF cells (Human Foreskin Fibroblast cell line purchased from ATCC, ref SCRC-1041.1).

The culture of each adherent cell line was carried out in a culture medium (RPMI for CHO and THP-1 cells, DMEM for HFF cells and MEM for HepG2 cells) supplemented with 10% FCS, 1% L-glutamine (200 mM) and penicillin (100 U/mL) / streptomycin (100 µg/mL) (complete culture medium).

The *in vitro* cytotoxicity evaluation was performed according to a similar protocol whatever the cell line was. In 96-well plates, 100 µL of a cell culture adjusted to 5.10<sup>4</sup> cells/mL was dispensed into each well and the plates were then incubated at 37 °C in a humidified 5% CO<sub>2</sub> for 24 h to allow the cells to adhere to the bottom of the wells. After microscopic verification of the adherent cells, complete culture medium with various product concentrations, previously dissolved in DMSO (final concentration less than 0.5% v/v), was added at a rate of 100 µL/well. Controls were represented by DMSO as blank, doxorubicin as positive control and a cell viability control (none compound). Duplicate assays were performed for each product and control. The plates were incubated at 37 °C in a humidified 5% CO<sub>2</sub>. After a 24 h (CHO cell line) to 72 h (THP-1, HFF and HepG2 cell lines) incubation, the plates were examined microscopically, well by well, to detect any precipitates. The wells observed with precipitates were identified and not taken into account for the determination of the IC<sub>50</sub> value. Then, the supernatant from each well was removed before the addition of 100 µL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution (0.5 mg/mL in medium without FCS) per well. After a 2 h incubation, the supernatant from each well was removed and 100 µL of DMSO was added to dissolve the resulting blue formazan crystals accumulated at the bottom of the wells. The wells were homogenized by shaking the plates (600 rpm) for 10 min, and the absorbance of each well was measured at 570 nm with 630 nm as reference wavelength spectrophotometer using a Bio-Tek ELx808 Absorbance Microplate Reader. The IC<sub>50</sub> values were determined by non-linear regression analysis processed on dose-response curves, using the Table Curve software 2D v.5.0 and represent the mean value calculated from three independent experiments.

#### **4.2.4. *In vitro* mutagenicity assays**

##### *4.2.4.1. In vitro bacterial reverse mutation assay*

This experiment was carried out according to the OECD guideline N°471. Few crystals of the frozen working strains were taken with a sterile pipette, and seeded in 10 ml of nutrient broth

(NB N°2, Oxoid). Strains were grown overnight at 37 °C with shaking in Nutrient Broth No. 2, with ampicillin (25 µg/mL) for TA1535, TA97a, TA98, and TA 100, or ampicillin (25 µg/mL) plus tetracyclin (2 µg/mL) for TA 102. At the end of the incubation period, 0.1 mL of each overnight culture was mixed to 0.1 mL S9 Mix or 0.1 mL phosphate-buffered saline (PBS, pH 7.4) in 5 mL plastic tubes and various volumes of **17b** were added to the mixtures. Each experiment included triplicate plates of four tested doses. A solvent (DMSO, 10 µL) control was added to determine the spontaneous frequency of revertants (quadruple plates). Five positive controls were also included to ensure the performance of the tester strains and the S9 Mix. After a 60 min pre-incubation period at 37 °C, 2 mL of melted top agar, complemented with 40 µg/mL histidine and 48.8 µg/mL biotin mix, was added to each tube and contents were poured immediately onto Vogel-Bonner minimal agar plates. After hardening, the plates were incubated at 37 °C for 48 h.

At the end of the incubation period, revertants were counted with a laser colony counter equipped with a bacterial enumeration program (Spiral System Instrument Inc., Bethesda, MD, USA). The Dunnett test was performed to determine a significant difference between the mean number of induced revertants and the mean number of spontaneous revertants. The assay was considered positive if:

- A dose-response relationship was obtained between the rate revertants and the doses,
- At least one of these doses induced a statistically significant increase ( $P < 0.05$ ) in the number of revertants as compared to the negative control.

#### 4.2.4.2. *In vitro* micronucleus assay

This experiment was carried out according to the OECD guideline N°487. All the assays using protocol based on a short term exposure were conducted in duplicate. The CHO-K1 cells, suspended in Mac Coys'5A medium, were transferred into Labteck wells at a concentration of 100,000 cells/mL, and incubated for 24 h at 37°C in CO<sub>2</sub> (5%). When the test was performed without metabolic activation, **17b** was added into cell cultures at 1 mM, 0.01 mM and 0.001 mM. A negative control containing culture medium, a solvent control containing 1% DMSO and a positive control containing 0.6 µg/mL of mitomycin C were added. When the assay was performed in the presence of metabolic activation, S9 mix metabolizing mixture was added to cell cultures at a concentration of 10%. Then **17b** was added to the cell cultures at 1 mM, 0.01 mM and 0.001 mM. A negative control containing culture medium, a solvent control containing 1% DMSO and a positive control containing 5 µg/mL of benzo-a-pyrene were added. After 3 h of incubation at 37 °C in CO<sub>2</sub> (5%), the culture medium was removed, the cells were rinsed with phosphate buffered saline (PBS), and then returned to culture in McCoy's 5A medium containing 3 µg/mL of cytochalasin B. After a 21-h incubation period at 37°C, cells were rinsed with phosphate buffered saline (PBS), fixed with methanol and stained with 10% Giemsa for 20 minutes.

Then, the protocol based on a long-term exposure was conducted. All the assays were conducted in duplicate. The CHO-K1 cells, suspended in Mac Coys'5A medium, were transferred into Labteck wells at a concentration of 100,000 cells/mL, and incubated for 24 h at 37°C in CO<sub>2</sub> (5%). The test was performed without metabolic activation: **17b** was added into cell cultures at 1 mM, 0.01 mM and 0.001 mM. A negative control containing culture medium, a solvent control containing 1% DMSO and a positive control containing 0.6 µg/mL of mitomycin C were added. After 24 h of incubation at 37 °C in CO<sub>2</sub> (5%), in culture medium containing 3µg/ml of cytochalasine B. After a 24 h incubation period at 37°C, cells were rinsed with phosphate buffered saline (PBS), fixed with methanol and stained with 10% Giemsa for 20 min.

The analysis of results was performed under a microscope at x1000 magnification. The anti-proliferative activity of test substances was estimated by counting the number of binucleated cells relative to the number of mononucleated cells on a total of 500 cells for each dose (250 cells counted per well). The proliferation index (Cytokinesis Blocked Proliferative Index CBPI) was calculated using the following formula:

$$CBPI = \frac{2 * BI + MONO}{500}$$

*BI* : number of binucleated cells

*MONO* : number of mononucleated cells

The cytostasis index (CI%), i.e. the percentage of cell replication inhibition, was calculated using the following formula:

$$CI\% : 100 - \{ 100 \times (CBPI_{\text{test material}} - 1) / (CBPI_{\text{solvent control}} - 1) \}$$

After this step, only the doses inducing a decrease of less than 55±5% of CI% as compared to the negative control were taken into account for counting micronuclei.

The rates of micronuclei were evaluated for the presence of independent nuclear core entities in 1000 binucleated cells per well, which corresponds to 2000 cells examined by test substance dose. Micronuclei were identified as small nuclei well differentiated from cell nucleus, stained in the same manner and having a diameter less than one third of that of the cell nucleus.

Micronuclei rates obtained for different doses of test substances were compared to the negative control by a  $\chi^2$  test. The assay was considered positive if:

- A dose-response relationship was obtained between the rate of micronuclei and the doses tested,
- At least one of these doses induced a statistically significant increase ( $P < 0.05$ ) in the number of micronucleated cells as compared to the negative control.

#### **4.2.5. *In vivo* antimalarial evaluation**

The BALB/c female mice used in this assay were five weeks old and pathogen-free (Charles River Laboratories, L'arbresle, France). Mice were housed during one week without any manipulation but under daily visual surveillance, after delivery They were housed under standard and controlled constant laboratory conditions (19-22 °C, relative humidity around 60%), with access to food and water ad libitum. Mice were monitored for general health, weight loss and anaemia/parasitemia throughout infections to ensure they did not reach IACUC endpoints. All experiments adhered to the French guidelines for animal research and were approved by the Ethics Committee for Animal Experimentation n°5 (registration number 05104.02). All efforts were made to minimize animal suffering.

The *PbA* line was graciously provided by Dr Salah Méchéri (Institut Pasteur, Paris, France). Four mice (average body weight 18 g) of six weeks old were infected with a *PbA* strain in order to make a first pass allowing a parasitic multiplication. The plasmodial strain, previously cryo-preserved with 30% parasitaemia in glycerolysis, was quickly thawed in a 37°C water bath. 100 µL of the strain was administered by intraperitoneal (*ip*) injection to each of the four mice. Parasitaemia was then monitored regularly with blood smears. After a week, when parasitaemia reached about 10%, the blood was taken retro-orbitally, after a general anesthesia procedure.



30 mice used in the experiment (second pass) were infected intraperitoneally with 200  $\mu\text{L}$  of a 0.9% NaCl solution containing  $1 \times 10^7$  *PbA* parasitized murine erythrocytes. These mice had seven weeks old and their average body weight was between 19 and 20 grams.

The solutions were prepared the day before each week of experience and stored at +4 °C. The protocol was as follows. Exactly weighted masses of compounds in its hydrochloride forms were dissolved in a solution composed finally of 40% ethanol (Sigma-Aldrich), 20% Tween 20 (Sigma-Aldrich), and 40% water for injectable preparation. The prepared solutions were administered intraperitoneally at a volume of 150  $\mu\text{L}$  per 20 grams of body mass.

The antimalarial activity of **17b** was assessed at 10 mg/kg/day equivalent MQ for 5 days. A control group (10 mice) received an *ip* of the vehicle. Afterwards, the parasitic growth was evaluated every two days by blood smears and mice were monitored for their survival up to Day 30.

The parasite multiplication rate (PMR) was used to normalize the activity of the studied compounds on the parasite growth (treated), to the natural parasite growth (control) [60]. Survival curves were used to study the likelihood of death in treated and control mice. They were fitted using Graph- Pad Prism® software version 5.04 (GraphPad Software Inc, 2012, La Jolla, CA). The survival analysis was performed according the method of Kaplan-Meier, which determines the probability of survival when at least one ‘death’ is recorded [70].

### 4.3. *In vitro* ADMET study

#### 4.3.1. Permeability in Caco-2/TC7 cells for **1a,b**, **2a,b**, **17b**, **R,S-MQ** and **S,R-MQ**

The trans-epithelial intestinal permeability ( $P_{\text{app}}$ ) of **1a,b**, **2a,b**, **17b**, **R,S-MQ** and **S,R-MQ** was investigated using the Caco-2/TC7 cell line. Caco-2/TC7 cells were maintained at 37°C, with 10% CO<sub>2</sub> and 95% relative humidity; in DMEM culture medium containing 20% inactivated fetal calf serum, 1% non-essential amino acids, 1% Glutamax and 100 IU/mL Penicillin and 100  $\mu\text{g/mL}$  Streptomycin. At 90% confluence, Caco-2/TC7 cells were washed with Ca<sup>2+</sup> and Mg<sup>2+</sup> free PBS and dissociated with 0.5 g/L Trypsin solution (1:250) containing 0.2 g/L EDTA. For transport experiments, Caco-2/TC7 cells were seeded onto permeable filter inserts in 96-well plates (Corning HTS Transwell-96 Permeable Support with 1.0  $\mu\text{m}$  pore polyester membrane, Costar CLS3380). Apical and basal volumes of culture medium were 50  $\mu\text{L}$  and 350  $\mu\text{L}$ , respectively. All transport experiments were conducted from day 15 to day 30 after cell seeding. The integrity of the cell monolayer was monitored by measuring the passive permeability (paracellular route) of Mannitol (20  $\mu\text{M}$ ) across the epithelium under same experimental conditions as test compounds. For apical to basolateral (A $\rightarrow$ B) permeability, compounds at 20  $\mu\text{M}$  were added to the apical (A) side and amount of permeation was determined on the basolateral (B) side. Caco-2/TC7 cells were incubated for 2 h and the receiver side buffer removed for analysis of compounds by LC-MS/MS after protein precipitation with acetonitrile and their removal by centrifugation. Data were expressed as coefficient of permeability ( $P_{\text{app}}$ ):  $P_{\text{app}} = (dQ/dt)/(C_0.A)$ , where  $dQ/dt$  is the rate of permeation,  $C_0$  is the initial concentration of the compound, and  $A$  is the monolayer area. Compounds exhibiting  $P_{\text{app}}$  values above the threshold value of  $20 \times 10^{-7}$  cm/s were defined as highly transported compounds and predicted to have a good absorption in humans.

#### 4.3.2. *h*ERG inhibition studies for **1a,b**, **2a,b**, **17b**, **R,S-MQ** and **S,R-MQ**

The effect of the test compounds on *h*ERG current was investigated *in vitro* using Patch-Clamp technique in the whole-cell configuration on CHO cells stably transfected with the human gene of ERG.

Test compounds were dissolved at a final concentration of 30 mM in DMSO (dimethylsulfoxide, SDS). Appropriate volumes of the 30 mM solution were sampled to obtain the final concentrations (up to 30  $\mu$ M) to be tested and volumes of DMSO were added to the final solutions in order to adjust them to 0.1 % DMSO (maximal solvent concentration).

The cell line (Chinese Hamster Ovary, CHO) stably expressing the *h*ERG potassium channel (Cytomyx CYL3002) was cultured in a 75-cm<sup>2</sup> flask with the  $\alpha$ -Minimum Essential Medium containing 10 % fetal bovine serum and 1 % Geneticin. Confluent cells were unplated with EDTA medium (Versene) and the resulting cell suspension was centrifuged. Part of cells was seeded in cell culture dishes and incubated at 28°C in a humidified atmosphere containing 5 % CO<sub>2</sub>/95 % air. These cells were then used (from 4 to 72 h) for patch-clamp experiments. Patch-clamp experiments were carried out at 35°C. The chambers containing the cell preparation were placed on the stage of an inverted microscope and were superfused with the extracellular solution PATCH (NaCl, 138 mM; KCl, 4 mM; CaCl<sub>2</sub>, 1.8 mM; MgCl<sub>2</sub>, 1 mM; glucose, 5.6 mM, HEPES, 10 mM). The pH was adjusted to 7.3 with NaOH. The osmolarity was adjusted to 285 mOsm. *h*ERG currents were recorded in a whole-cell configuration on CHO-*h*ERG using suction pipettes containing the internal pipette solution (KCl, 140 mM; MgCl<sub>2</sub>, 2 mM; CaCl<sub>2</sub>, 2 mM; EGTA(K), 11 mM; HEPES, 10 mM). The pH was adjusted to 7.25 with KOH. Glass micropipette electrodes with tip resistances of 3-5 M $\Omega$  were connected to a voltage clamp amplifier. No leak subtraction was applied. Current signals were filtered (1 kHz) then acquired using an analogue-to-digital converter connected to a PC and analysed using Pclamp software. The *h*ERG current was activated in response to voltage steps (2 s) from a holding potential of -80 mV to a test potential of +20 mV followed by a repolarization to -100 mV. Voltage steps were applied at 20 s intervals. *h*ERG current was measured as the tail deactivating current at -100 mV. After a 2-3 min period for baseline measurements, drug effects were measured 4-6 min after the change of solution. At the end of each experiment, the superfusion of a specific *h*ERG-blocker, i.e. Risperidone (which exhibits an IC<sub>50</sub> around 0.3  $\mu$ M), at a 10  $\mu$ M concentration allowed to measure a 100 % inhibition of *h*ERG.

The currents were analysed using Clampfit (Pclamp software, Axon Instruments). The linear rundown of currents was extrapolated from baseline measurement period and the percentage of residual *h*ERG current after drug effect was calculated as followed:

$$\text{Residual } h\text{ERG current (\%)} = (I - I_{\text{blocker}}) / (I_{\text{baseline}} - I_{\text{blocker}}) \times 100$$

where: "I" is the current measured after drug effect ; "I<sub>blocker</sub>" is the current measured after the effect of the specific *h*ERG-blocker ; "I<sub>baseline</sub>" is the current measured before the superfusion of drug. IC<sub>50</sub> (effective concentration to inhibit 50 % of drug-sensitive *h*ERG current), confidence interval and coefficient of variation (CV) were calculated by using the Biostat Speed software (internal software).

#### 4.3.3. Metabolic stability in liver microsomes across species for 17b, *R,S*-MQ and *S,R*-MQ

17b, *R,S*-MQ and *S,R*-MQ (10 mM stock dissolved in DMSO, final concentration of DMSO in the incubation was less than 0.2% [v/v]) were assessed for metabolic stability in liver microsomes from mouse, rat, and human. Briefly, incubations (0.1 mL total volume) containing liver microsomes (0.25 mg protein/mL), NADPH (1 mM), compounds (0.5  $\mu$ M) in potassium phosphate buffer (0.1 M, pH 7.4) were performed at 37°C under shaking. Incubation aliquots were

taken at time 0, 5, 10, 20 and 30 min and added directly to 100  $\mu\text{L}$  of acetonitrile containing the internal standard. Positive substrate control incubations were performed with verapamil. Quenched samples were mixed, followed by centrifugation at 10 000 rpm for 10 min. Supernatants were analyzed by LC-MS/MS. Peak area ratios of tested compound/IS from each time point were natural log transformed as the percent remaining relative to time zero [71]. The slope ( $k$  expressed in  $\text{min}^{-1}$ ) was transformed to in vitro half-life ( $t_{1/2}$ ), where  $t_{1/2} = -0.693/k$ . The calculated  $\text{CL}_{\text{int}}$  (expressed as  $\mu\text{L}/\text{min}/\text{mg}$  protein) was determined from the relationship:

$$\text{CL}_{\text{int}} = k \cdot \text{Volume of incubation in } \mu\text{L}/\text{mg microsome protein.}$$

#### 4.3.4. Metabolic stability in human hepatocytes for *R,S*-MQ and *S,R*-MQ

A metabolic stability study was conducted in fully metabolically characterized cryopreserved human hepatocytes in primary (PHH) culture ( $n = 2$  donors). Cryopreserved PHH were thawed using cryopreserved hepatocyte recovery medium (ThermoFisher, Waltham, MA) and centrifuged 10 min at 100 g. Viability for all used human hepatocyte preparations was greater than 95% as measured by Trypan blue exclusion test. Before seeding, cell concentration was adjusted to obtain a final density of  $160 \times 10^3$  cells/well (Collagen I-coated 48-well plate, Corning, Tewksbury, MA). After seeding and a cell attachment period of around 4 h, the plating medium was removed. Cells were then incubated overnight with culture medium in a  $37^\circ\text{C}$  thermostatically controlled incubator, using 5%  $\text{CO}_2$  and 95% relative. The study was initiated the day after seeding with a final incubation volume of 100  $\mu\text{L}$ /well of 5  $\mu\text{M}$  dosing solution. Positive substrate control incubations were performed with midazolam, dextromethorphan, phenacetin and tolbutamide. At appropriate time points (up to 24 h) the reaction was terminated by addition of quench solution (acetonitrile/water 50/50, [v/v]). Following removal of proteins by centrifugation, supernatants (pooled intra + extra-cellular compartments) were analysed by LC-MS/MS.

The *in vitro* intrinsic clearance ( $\text{CL}_{\text{int}}$  expressed in  $\text{mL}/\text{h}/10^6$  hepatocytes) was calculated as follows:  $\text{CL}_{\text{int, in vitro}} = k_e \cdot V$  with  $V =$  incubation volume containing  $10^6$  cells and  $k_e =$  elimination rate constant [calculated *via* Phoenix, with a compartmental modeling analysis (one compartment, intravenous bolus, no lag time, first order elimination)], determined using the equation:  $C = C_0 \cdot e^{-k_e \cdot t}$ , with  $C_0 =$  initial substrate concentration.

#### 4.3.5. CYP3A4 activity for 17b, *R,S*-MQ and *S,R*-MQ

The aim was to evaluate the inhibitory potency (direct and time-dependency) of the different compounds in human hepatic microsomal fractions on CYP3A4 midazolam 1'-hydroxylase and testosterone 6  $\beta$ -hydroxylase activities by determination of  $\text{IC}_{50}$  after pre-incubation with and without NADPH cofactor [72].

Briefly, human hepatic microsomal fractions (0.1 mg/mL) were incubated at  $37^\circ\text{C}$  for 20 min with increasing concentrations of test compounds (up to 50 or 100  $\mu\text{M}$ ) in  $\text{KH}_2\text{PO}_4$  0.05 mM pH 7.4 buffer in the absence and presence of 1 mM NADPH cofactor. At the end of the pre-incubation period, the reactions are transferred into fresh wells containing both cofactors and CYP3A4/5 specific substrates (ie., midazolam (3  $\mu\text{M}$ ) and testosterone (50  $\mu\text{M}$ )) for a secondary incubation. Incubation duration was 10 min for assessment of 1'-hydroxymidazolam metabolite and 6 $\beta$ -hydroxytestosterone metabolite formation. The reactions were quenched and the resulting CYP activity assessed by analyzing supernatant fluids by LC-MS/MS. An  $\text{IC}_{50}$  curve of each test compound for each condition (+NADPH and -NADPH) was generated from its concentration curve using the Enzyme Kinetics module of SigmaPlot (Systat Software, Inc, San Jose, CA, USA). The  $\text{IC}_{50}$  ratio of the two conditions was then calculated to assess TDI

liability, indicated by an “IC<sub>50</sub> Shift”:  $IC_{50}\text{-shift} = IC_{50}(-NADPH) / IC_{50}(+NADPH)$ . If  $IC_{50}\text{-Shift} \geq 2.0$ , the compound was considered as a potential time-dependent inhibitor.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Abbreviations

ACN	Acetonitrile
ACT	Artemisinin-based combination therapy
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
AD-mix $\alpha$	Asymmetric dihydroxylation mixture $\alpha$
AD-mix $\beta$	Asymmetric dihydroxylation mixture $\beta$
BOC	tert-Butyloxycarbonyl
CHO	Chinese hamster ovarian cell line
CL <sub>int</sub>	Intrinsic Clearance
DCM	Dichloromethane
DHA	Dihydro-artemisinine
DNA	Deoxyribonucleic acid
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
GTPase	Guanosine triphosphate hydrolase
HEPES	4-(2-Hydroxyethyl)-1-Piperazineethanesulfonic Acid
HepG2	Hepatocarcinoma cell line
<i>h</i> ERG	Ether-a-go-go-Related Gene
HFF	Human Foreskin Fibroblast cell line
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HRP-2	Histidine-Rich Protein 2

IC <sub>50</sub>	Half Maximal Inhibitory Concentration
IR	Infrared
LC-MS	Liquid Chromatography-Mass Spectroscopy
LogP	Logarithm of Partition-Coefficient
MDAQ	Amodiaquine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear Magnetic Resonance
Papp	Trans-epithelial intestinal permeability
<i>PbA</i>	<i>P. berghei</i> ANKA
<i>PfACBP</i>	<i>P. falciparum</i> acyl-CoA binding proteins
<i>PfCRT</i>	<i>P. falciparum</i> chloroquine resistance transporter
<i>PfMDR1</i>	<i>P. falciparum</i> multidrug resistant protein 1
PK	Pharmacokinetics
Qt	Quantitative
SAR	Structure-Activity Relationship
SI	Selectivity index
SYBR Green I	<i>N,N</i> -dimethyl- <i>N</i> -[4-[( <i>E</i> )-(3-methyl-1,3-benzothiazol-2-ylidene)methyl]-1-Phenylquinolin-1-ium-2-yl]- <i>N</i> -propylpropane-1,3-diamine
THP-1	Human leukemia monocytic cell line
WHO	World Health Organization

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