Chinese Chemical Letters 32 (2021) 1660–1664

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

Repurposing of antitumor drug candidate Quisinostat lead to novel spirocyclic antimalarial agents

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ARTICLE INFO

Article history:
Received 1 November 2020
Received in revised form 26 November 2020
Accepted 13 December 2020
Available online 20 December 2020

Keywords:
Malaria
*Pf*HDAC inhibitor
Drug repurposing
Epigenetic modulator
Erythrocytic therapy

ABSTRACT

Antimalarial chemotherapies endowed with effectiveness against drug-resistant parasites and good safety are urgently required in clinical. Our previous research revealed that clinical phase II antitumor drug Quisinostat was a promising antimalarial prototype by inhibiting the activity of *Plasmodium falciparum* (*P. falciparum*) histone deacetylase (*Pf*HDAC). Herein, 30 novel spirocyclic linker derivatives were designed and synthesized based on Quisinostat as lead compound, and then their antimalarial activities and cytotoxicity were systematically evaluated. Among them, compounds **8** and **27** could effectively eliminate wild-type and multi-drug resistant *P. falciparum* parasites, and display weakened cytotoxicity and good metabolic stability. Western blot assay demonstrated that they could inhibit *Pf*HDAC activity like Quisinostat. In addition, both **8** and **27** showed certain antimalarial efficacy in rodent malaria model, and the animal toxicity of **8** was significantly improved compared with Quisinostat. Overall, **8** and **27** were structurally novel *Pf*HDAC inhibitors and provided prospective prototype for further antimalarial drug research.

Despite decades of effort to control the disease, malaria is still one of the most threatening problems in global public health governance. According to the statistical data of World Health Organization (WHO) in 2018, malaria has caused 228 million clinical episodes and 405,000 deaths, among them, *Plasmodium falciparum* (*P. falciparum*) induced the highest mortality [1]. Since no malarial vaccines have been approved clinically, chemotherapies are still the mainstays of treating and preventing malaria [2,3]. Unfortunately, the emergence and widespread of multi-drug resistant malaria has severely emasculated the therapeutic efficacy of several early-approved antimalarial agents [4–6]. Especially the prevalence of resistance against artemisinin-based combination therapies (ACTs) has significantly raised concerns about the prospect of malaria control [7–11]. Therefore, discovery of next-generation antimalarial drugs with novel therapeutic targets to overcome the obstacles of drug resistance is an important and urgent task for malaria elimination.

Histone deacetylase (HDAC), selectively hydrolyzing acetyl groups in the amino acid residues of histone, is a critical post-translational modulator. Currently, five *P. falciparum* HDACs (*Pf*HDACs) have been identified which are divided into Zn^{2+}-containing class I (*Pf*HDAC1) and class II (*Pf*HDAC2/3) HDACs, and NAD^{+}-containing class III (*Pf*Sir2A/2B) HDACs [12]. Since *Pf*HDACs play important roles in regulating the acetylation levels of both histone and non-histone malarial proteins, which affect the survival and reproduction of parasite, these enzymes are

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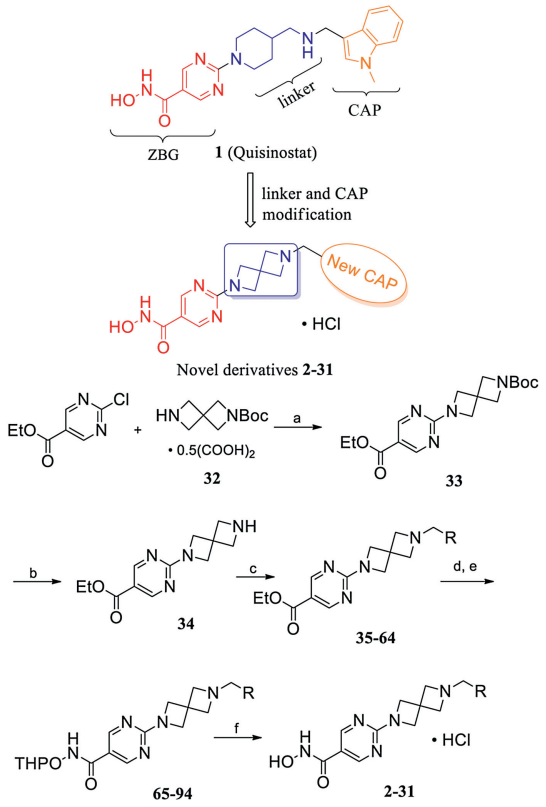
https://doi.org/10.1016/j.cclet.2020.12.023

considered as promising antimalarial drug targets [12–15]. Merck Research Laboratory reported the pioneering *Pf*HDAC inhibitor apicidin in 1996 [16], and several groups subsequently published their researches of *Pf*HDAC inhibitor as potential antimalarial agents [12–15]. However, in spite of more than two decades of research, *Pf*HDAC inhibitors never successfully entered clinical treatment or trail. Although several HDAC inhibitors displayed antimalarial potency, their toxicity has become the major fault of further development [12]. Hence developments of antimalarial HDAC inhibitor in the future should focus on enhancing the therapeutic safety of novel compounds.

In previous work, we discovered that the clinical antitumor HDAC inhibitor Quisinostat (**1**) was a promising antimalarial agent with potent *Pf*HDAC1 inhibition, but the severe toxic effects limited its further application [17,18]. Previous work also explored the structure–activity relationship (SAR) of Quisinostat, and revealed that replacing the 4-aminomethyl piperidine linker with other diamine linkers could significantly regulate the bioactive properties [18]. In order to reduce the toxicity and discover new chemical prototype of antimalarial agent, we envisaged a novel series of *Pf*HDAC inhibitors based on displacing the original diamine linker by a spirocyclic 2,6-diazaspiro[3.3]heptane linker (Scheme 1). Modification of the CAP fragment was then carried out to jointly improve the safety. Therefore, 30 novel spirocyclic linker derivatives with various CAP groups were designed and synthesized, and their antimalarial activities and cytotoxicity were systematically evaluated in this work.



Scheme 1. Chemical modification strategy of novel *Pf*HDAC inhibitors and their synthetic routes. Reagents and conditions: (a) DIPEA, DCM, 25 °C; (b) HCl/1,4-dioxane (4 mol/L), DCM, 25 °C; (c) R-CHO, HOAc, $NaBH(OAc)_3$, DCE, 25 °C; (d) K_2CO_3, MeOH, H_2O, 65–70 °C; (e) $THPONH_2$, EDCI, HOBt, TEA, DMF, 25 °C; (f) HCl/1,4-dioxane (4 mol/L), DCM, 25 °C.

The general synthetic routes of compounds **2**–**31** were depicted in Scheme 1 [19]. Briefly, 2,6-diazaspiro[3.3]heptane-2-carboxylic acid *tert*-butyl ester half oxalate (**32**) underwent nucleophilic substitution reaction with ethyl 2-chloropyrimidine-5-carboxylate under basic condition to produce intermediate **33**. The *tert*-butoxycarbonyl (Boc) group in **33** was removed under acidic condition and the resultant free amine **34** underwent reductive amination to produce **35**–**64**. After basic hydrolysis of ethyl ester in the **35**–**64**, the corresponding acid underwent condensation with *O*-(tetrahydro-*2H*-pyran-2-yl)hydroxylamine to produce **65**–**94**. Finally compounds **2**–**31** were prepared by removing the 2-tetrahydropyranyl (THP) group in **65**–**94** under treatment with organic hydrogen chloride solution.

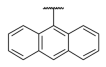
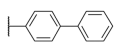
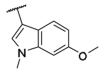
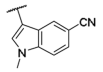
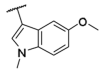
In vitro plasmodium-killing assay of compounds **2**–**31** against drug-sensitive (3D7) and chloroquine-resistant (Dd2) *P. falciparum* strains, and their cytotoxicity against two human cell lines (HepG2 and 293T) were systematically evaluated with Quisinostat and dihydroartemisinin (DHA) as positive controls. Analysis of the data summarized in Table 1 revealed some noteworthy observations from the SAR study of compounds **1**–**31**: (1) diamine group displacement could slightly attenuate antimalarial potency and significantly improve the selectivity (**1** *vs.* **2**); (2) small monocyclic and large aromatic CAP groups could retain or attenuate potency, but the selectivity was not improved or even greatly reduced (**3**–**6** and **25**–**26** *vs.* **2**); (3) bicyclic CAP groups, such as naphthalyl, quinolyl, isoquinolyl, piperonyl, dihydrobenzofuryl and other azaindolyl groups were disadvantageous to potency, while benzofuryl, benzothiophenyl and indazolyl groups slightly enhanced potency although the selectivity was compromised (**10**–**24** *vs.* **2**); (4) the *N*-methyl group in the indolyl CAP groups was critical to potency and 2-indolyl group was superior in improving both potency and selectivity (**7**–**9** *vs.* **2**); (5) introducing methoxyl group to 3-indolyl group undermined the potency, while introducing cyano and halogen groups could retain or enhance potency (**27**–**31** *vs.* **2**). Encouragingly, the two most potent compounds **8** and **27** also significantly inhibited three multi-drug resistant *P. falciparum* strains (see Table S2 in Supporting information) [20]. Further investigations revealed that the metabolic stability of **8** and **27** was enhanced since their half-life ($T_{1/2}$) toward mouse liver microsomes *in vitro* was longer than that of **1** (Table 2). Western blot assay also displayed that **8** or **27** could also induce hyperacetylation of parasite histone H3 compared with the blank group, which indicated that **8** and **27** were *Pf*HDAC inhibitors the same as compound **1** (Fig. 1).

Given the remarkable *in vitro* antimalarial potency of compounds **8** and **27** against multiple *P. falciparum* strains along with alleviative cytotoxicity and stable metabolic properties, we intended to evaluate their *in vivo* blood-stage antimalarial activities. Since *P. falciparum* cannot directly infect rodent animal model [21], we selected a widely-accepted rodent *P. yoelii* malaria infection model with piperaquine phosphate (PPQ) as positive control [20,22]. The animal experiment was approved by Animal Welfare and Committee of Institut Pasteur of Shanghai, Chinese Academy of Sciences (IACUC Issue No. A2018009). Female BALB/c mice were randomly divided into 5 groups and each one was inoculated with 1×10^5 parasites on day 0. Subsequently, tested drug solution or solvent alone (for blank group) were administered intraperitoneally once daily since day 1 to day 5. As shown in Fig. 2, the mice in blank group finally died from the hyperparasitemia, and the mice in compound **1** administration group died quickly from the compound toxicity. Compound **27** displayed strong *in vivo* efficacy in killing malaria parasites, but there was still a problem of toxicity. Although compound **8** was unable to eliminate the parasites thoroughly, it still delayed the progress of disease and exhibited the inhibition rate of 84% on day

Table 1
In vitro antimalarial activity and cytotoxicity of compounds **2**–**31**.[a]

Compound	CAP	Erythrocytic IC_{50} (nmol/L)[a]		Cytotoxicity IC_{50} (nmol/L)[a] and SI[b]			
		3D7	Dd2	HepG2	SI	293T	SI
1	–	5.2 ± 1.6	7.09 ± 0.01	42 ± 10	8	48 ± 1	9
2		24.9 ± 2.9	15.7 ± 2.6	14735 ± 1803	592	22080 ± 2036	886
3		13.7 ± 1.3	21.9 ± 2.3	4464 ± 1027	635	4667 ± 605	340
4		198.6 ± 14.1	242.9 ± 11.0	7015 ± 139	35	8694 ± 878	44
5		24.1 ± 1.8	35.6 ± 2.9	1738 ± 185	72	3977 ± 120	111
6		24.1 ± 0.8	34.8 ± 2.2	1135 ± 163	47	17545 ± 3500	732
7		191.5 ± 18.9	105.2 ± 2.6	18875 ± 1195	99	> 40000	> 209
8		8.6 ± 0.9	22.8 ± 5.2	10153 ± 929	1175	25455 ± 1973	2945
9		56.57 ± 0.09	90.7 ± 1.5	5692 ± 765	101	11000 ± 1414	194
10		24.2 ± 2.1	37.4 ± 0.5	3340 ± 325	138	4223 ± 1276	174
11		15.0 ± 1.0	15.9 ± 1.2	2046 ± 143	137	5417 ± 781	362
12		10.2 ± 1.5	8.4 ± 1.3	890 ± 169	88	1109 ± 130	109
13		17.4 ± 1.6	16.8 ± 1.5	1084 ± 25	62	9745 ± 1083	561
14		11.1 ± 0.8	8.2 ± 1.0	608 ± 35	55	1065 ± 206	96
15		88.3 ± 1.8	33.4 ± 1.8	9473 ± 313	107	22820 ± 148	258
16		76.9 ± 0.1	39.7 ± 3.9	> 40000	> 520	> 40000	> 520
17		42.1 ± 6.1	50.9 ± 5.5	8495 ± 829	202	8743 ± 432	208

Table 1 (*Continued*)

Compound	CAP	Erythrocytic IC_{50} (nmol/L)[a]		Cytotoxicity IC_{50} (nmol/L)[a] and SI[b]			
		3D7	Dd2	HepG2	SI	293T	SI
18		60.7 ± 2.2	28.3 ± 1.5	7714 ± 394	127	11415 ± 1266	188
19		23.8 ± 8.9	51.4 ± 8.6	7818 ± 354	328	13765 ± 573	577
20		10.3 ± 0.6	14.1 ± 0.1	549 ± 49	53	5453 ± 887	528
21		77.3 ± 4.2	131.8 ± 4.0	> 40000	> 518	> 40000	> 518
22		46.3 ± 4.5	102.5 ± 29.7	10725 ± 49	231	21115 ± 3189	456
23		162.5 ± 132.7	27.5 ± 0.4	704 ± 175	4	2810 ± 344	17
24		325.3 ± 70.6	64.8 ± 4.9	18720 ± 28	58	26775 ± 940	82
25		39.3 ± 2.2	72.9 ± 1.0	1044 ± 238	27	2627 ± 272	67
26		131.9 ± 0.8	60.1 ± 3.6	4960 ± 699	38	7279 ± 484	55
27		3.9 ± 0.5	6.8 ± 0.2	598 ± 61	152	1440 ± 61	213
28		24.4 ± 0.1	21.9 ± 5.7	4156 ± 602	170	7123 ± 1136	292
29		53.6 ± 2.1	34.1 ± 0.7	9499 ± 949	177	15430 ± 2814	288
30		28.0 ± 1.6	25.9 ± 1.1	6238 ± 756	223	4702 ± 601	168
31		172.5 ± 22.6	74.3 ± 1.7	10255 ± 290	59	10857 ± 1616	63
DHA	–	2.7 ± 0.2	2.68 ± 0.02	ND			

[a] Results are presented as mean ± SD collected from two independent biological repeats. DHA, dihydroartemisinin; ND, not determined.
[b] Selectivity index is calculated as IC_{50} (human cell)/IC_{50} (3D7).

Table 2
In vitro metabolic stability of compounds **1**, **8** and **27**.

Compound	$T_{1/2}$ (min)	CL (mL min^{-1} kg^{-1})
1	13.31	410.10
8	30.12	181.19
27	59.76	91.33

$T_{1/2}$, half-life; CL, clearance rate.

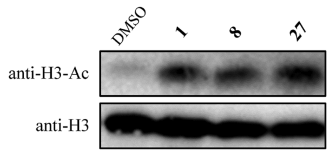


Fig. 1. Hyperacetylation analysis of *P. falciparum* histone H3 by compounds **1**, **8** and **27**.

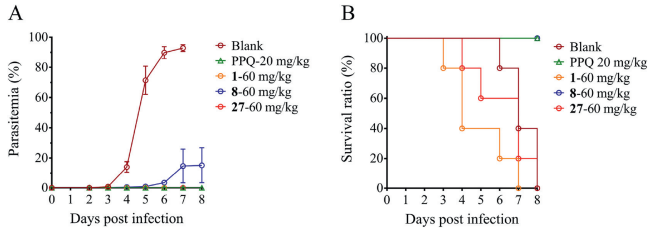


Fig. 2. *In vivo* erythrocytic antimalarial activity of compounds **1**, **8** and **27**. Percentage of parasitemia (A) and survival ratio (B) of compounds **1**, **8** and **27** at doses of 60 mg/kg. Blank is the solvent-treated group. Each group had five mice. Piperaquine phosphate (PPQ) was the positive control. Tail vein blood smears were prepared on the indicated days, and the percentage of parasitemia was determined *via* microscopic examination of 5000 red bold cells and presented as mean ± SD.

8 while resulting in no mouse death. Therefore, both compounds **8** and **27** inhibited the *in vivo* parasite growth and compound **8** further displayed significantly reduced toxicity compared to compound **1**.

In summary, based on the structure of the lead compound **1**, we designed and synthesized 30 novel spirocyclic linker derivatives (**2**–**31**), and their antimalarial activity and cytotoxicity were systematically evaluated. An explicit SAR was acquired and revealed that introduction of the 2,6-diazaspiro[3.3]heptane linker could remarkably reduce cytotoxicity, and modifications of CAP fragment could further attenuate cytotoxicity and regulate antimalarial activity. Among all the derivatives, compounds **8** and **27** displayed potent antimalarial activity with half maximal inhibitory concentration (IC_{50}) values below 10 nmol/L. These compounds also exhibited strong killing efficacy against several multi-drug resistant clinical *P. falciparum* isolates, and showed enhanced metabolic stability against mouse liver microsomes *in vitro* compared to **1**. Western blot analysis demonstrated that compounds **8** and **27** are *Pf*HDAC inhibitors the same as lead compound **1**. Moreover, both compounds **8** and **27** showed killing efficacy against parasites in animal experiment, and the toxicity of compound **8** in animals was significantly improved compared with **1**. These results implied that the risks of potential toxic effects could be reduced by reasonable structural modification. Overall, **8** and **27** had the potential to be new lead compounds for further development.

Declaration of competing interest

The authors report no declarations of interest.

Acknowledgments

This work was supported by the National Key R&D Program of China (Nos. 2017YFB0202600, 2018YFA0507300), the National Mega-project for Innovative Drugs of China (No. 2019ZX09721001-004-003), the National Natural Science Foundation of China (Nos. 81872747, 81903457, 31972169), the National Science and Technology Major Project (No. 2018ZX10101004003001), the Innovative Research Team of High-level Local Universities in Shanghai, the National Special Fund for State Key Laboratory of Bioreactor Engineering (No. 2060204), the Shanghai Sailing Program (No. 19YF1412600) and the Shanghai Morning Light Program (No. 18CG33).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.12.023.

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