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Design and synthesis of small molecular dual inhibitor of falcipain-2 and dihydrofolate reductase as antimalarial agent

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ABSTRACT

Resistance of malaria parasites has quickly developed to almost all used antimalarial drugs. Accordingly, the discovery of new effective drugs to counter the spread of malaria parasites that are resistant to existing agents, especially acting on multi-targets, is an urgent need. The cysteine protease falcipain-2 (FP-2) and dihydrofolate reductase (DHFR) play crucial roles in the *Plasmodium* life cycle. In this study, a series of first-gereration small molecular dual inhibitor of FP-2 and DHFR have been designed and synthesized based on the lead compound **1**, which was randomly identified by screening FP-2 inhibitors in our laboratory. Six compounds (**2f–g**, **2j**, and **2m–o**) showed improved dual inhibitory activities against FP-2 (IC_{50} = 2.7–13.2 μM) and DHFR (IC_{50} = 1.8–19.8 μM), and the inhibitory capability of compound **2o** against FP-2 and DHFR were increased ~8 and ~6 times than that of compound **1**, respectively. Moreover, compound **2o** exhibited moderate in vivo antimalarial activity in a dose dependent fashion, its safety and survival rate were slightly better than that of positive drug. The preliminary SAR was obtained, meanwhile, molecular modeling result provided the key structural information to maintain the dual inhibitory activity, and was helpful for future dual inhibitors design.

Malaria remains one of the most important infectious disease problems in the world, accounting for 225 million clinical cases and up to 0.78 million deaths in 2009.[1] *Plasmodium falciparum* is the most lethal protozoan parasite of the genus, which is responsible for malaria complications such as cerebral malaria or severe anemia.[2] At present, no effective vaccines are available due to the high mutability of the genome of *P. falciparum*,[3] meanwhile, resistance of malaria parasites has also quickly developed to a variety of quinoline analogs (e.g., chloroquine),[4] antifolates (e.g., sulfadoxine-pyrimethamine)[5] and inhibitors of electron transport (e.g., atovaquone).[6] What's worse, resistance to artemisinin has now emerged.[7,8] Accordingly, the discovery of new effective drugs to counter the spread of malaria parasites that are resistant to existing agents, especially acting on multi-targets, is an urgent need.

The cysteine protease falcipain-2 (FP-2) of *P. falciparum* is an essential hemoglobinase of erythrocytic *P. falciparum* trophozoites,[9] and provides the amino acids for the growth and proliferation of *Plasmodium*. Moreover, the degradation of the erythrocyte proteins can create enough space in the internal host for the growth of *Plasmodium*. Many in vitro and in vivo studies have confirmed that inhibitors of FP-2 could block parasite hemoglobin hydrolysis, halt the development of culture parasites, and were effective against murine malaria.[10–16] DHFR has received considerable attention for the prophylaxis and treatment of *P. falciparum* infection.[17,18] Dihydrofolate reductase (DHFR) is one of the key enzymes in the process of DNA replication, and catalyze 7,8-dihydrofolate (7,8-DHF) to transform into tetrahydrofolate (THF).[19] The latter serves as a necessary cofactor in important one-carbon transfer reactions in the pyrimidine, purine, and amino acid biosynthetic pathways.[20] The lower levels of THF result in decreased conversion of glycine to serine, reduced methionine synthesis, and lower thymidylate levels, with a subsequent arrest of DNA replication. Being different from Mammalian, *Plasmodium* cannot absorb the folate from foods, instead, it has to synthesize the folate by its own enzyme systems. Since single-target drug is not effectively used owing to their rapid emergence of resistant *Plasmodium* during treatment, one of the goals of our efforts has been to design dual FP-2 and DHFR inhibitory activity in a single agent. To our knowledge, these dual inhibitors of FP-2 and DHFR have not been reported in previous literatures. Such dual inhibitors could act at two different sites (FP-2 and DHFR) and might be capable of providing 'combination therapy' in a single agent without the pharmacokinetic disadvantages of two separate agents.

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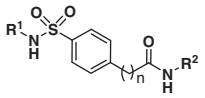
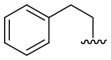
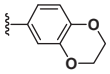
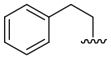
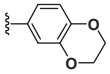
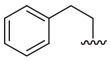
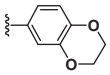
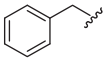
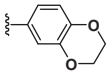
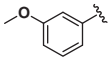
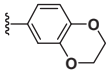
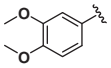
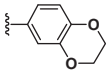
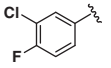
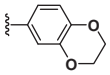
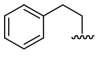
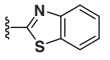
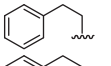
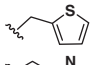
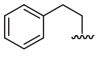
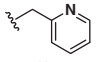
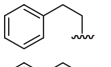
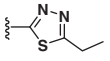
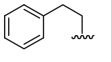
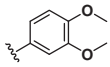
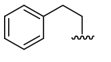
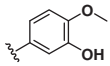
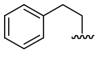
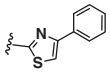
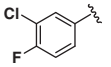
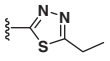
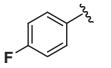
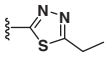
Table 1
Chemical structures of compounds **1** and **2a–o** and their activities against FP-2 and DHFR



Compds	R^1	R^2	*n*	Inhibition ratio (%) at 10 μM		IC_{50}[a] (μM)	
				FP-2	DHFR	FP-2	DHFR
1			2	23.0	26.5	54.2	35.5
2a			1	<5	68.0		5.0
2b			0	<5	11.8		
2c			2	<5	15.6		
2d			2	17.9	9.3		
2e			2	6.3	23.2		
2f			2	32.7	46.5	13.2	16.8
2g			2	41.5	37.9	8.9	15.1
2h			2	15.2	10.8		
2i			2	10.7	17.9		
2j			2	46.5	31.6	8.1	19.8
2k			2	15.0	51.2		9.4
2l			2	13.6	46.1		12.6
2m			2	94.5	34.8	2.7	14.6
2n			2	45.1	70.6	9.8	1.8
2o			2	72.9	67.5	7.0	6.3
E64				97.4		0.017	
Pyrimethamine					98.9		0.020

[a] Data are means of three independent experiments.

To identify quickly novel dual inhibitors of FP-2 and DHFR, we firstly test the DHFR inhibitory activity of all FP-2 inhibitors in our laboratory. Fortunately, one compound (**1**, Table 1) exhibited inhibitory activity against both FP-2 and DHFR, with IC_{50} values of 54.2 and 35.5 μM, respectively. Considering the biological potency and synthetic accessibility of compound **1**, it may serve as a reasonable lead compound for further developments. In this study, we have synthesized a total of 15 derivatives (**2a–o**) of compound **1**.

Firstly, we varied the chain length of phenyl alkanamide moiety and obtained analogs **2a–b** (Table 1). Secondly, changing the substituents on sulfamide nitrogen, we designed compounds **2c–f** (Table 1). Thirdly, compounds **2g–m** (Table 1) were achieved by replacing the substituents on amide nitrogen with other electronic and hydrophobic substituted aryl or aralkyl groups. Finally, we designed two compounds **2n–o** (Table 1) by modifying simultaneously the substituents on amide nitrogen and sulfamide nitrogen. Scheme 1 depicts the synthetic route for the preparation of compounds **1**, **2a** and **2c–o**. Esterification of phenyl alkanoic acid **3** with methanol under reflux quickly afforded phenyl alkanoate **4**, followed by the chlorosulfonation with $ClSO_3H$ to get methyl 4-(chlorosulfonyl)-phenyl alkanoate **5**. Compound **6** was prepared by coupling **5** with substituted primary amine in pyridine. Hydrolysis of **6** using LiOH at room temperature gave carboxylic acid