

Article

Synthesis and Antimalarial Activity of Novel Dihydro-Artemisinin Derivatives

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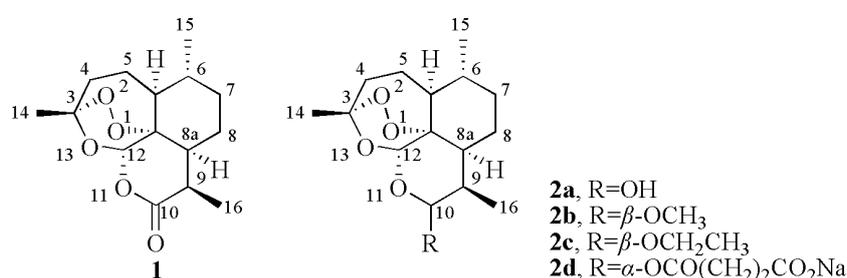
Abstract: The *Plasmodium falciparum* cysteine protease falcipain-2, one of the most promising targets for antimalarial drug design, plays a key role in parasite survival as a major peptide hydrolase within the hemoglobin degradation pathway. In this work, a series of novel dihydroartemisinin derivatives based on (thio)semicarbazone scaffold were designed and synthesized as potential falcipain-2 inhibitors. The *in vitro* biological assay indicated that most of the target compounds showed excellent inhibition activity against *P. falciparum* falcipain-2, with IC₅₀ values in the 0.29–10.63 μM range. Molecular docking studies were performed to investigate the binding affinities and interaction modes for the inhibitors. The preliminary SARs were summarized and could serve as a foundation for further investigation in the development of antimalarial drugs.

Keywords: falcipain-2 inhibitors; dihydroartemisinin derivatives; inhibition activity; molecular docking studies; SARs

1. Introduction

Malaria is the most common parasitic disease in the World. *Plasmodium falciparum* is the major cause of severe malaria and death and has developed resistance to most available antimalarial drugs [1]. Artemisinin (**1**), isolated from the *Artemisia annua* L., and its derivatives **2a-d** (Figure 1) are effective antimalarial drugs against multidrug-resistant *P. falciparum* [2]. The WHO has recommended artemisinin-based combination therapies (ACTs) as first-line treatment for uncomplicated *P. falciparum* malaria since 2001 [3]. However, as *P. falciparum* resistance to artemisinin has emerged [4], development of antimalarial drugs against new targets is an urgent priority.

Figure 1. Structures of artemisinin and its derivatives.



Among the promising new targets, *P. falciparum* cysteine protease falcipain-2, which plays a key role for the parasite survival as a major peptide hydrolase within the hemoglobin degradation pathway, is one of the most attractive targets for antimalarial drug design [5]. Falcipain-2 inhibitors can block parasite protein biosynthesis by preventing host hemoglobin hydrolysis [6]. Therefore, discovery of new falcipain-2 inhibitors with acceptable properties is a meaningful work.

Iron is essential for the biological activities of some plasmodial proteins and due to the metal chelating properties, the (thio)semicarbazone moiety has shown activity as a potential falcipain-2 inhibitor aimed at preventing the growth of malarial parasites [7-9].

In this work, a series of novel dihydroartemisinin derivatives **10a-l** and **12a-f** were designed and synthesized by incorporating the above two biologically active scaffolds, dihydroartemisinin and (thio)semicarbazone. The *in vitro* biological assay was carried out against *P. falciparum* falcipain-2. The preliminary SARs were summarized and could benefit to the subsequent drug design process.

2. Results and Discussion

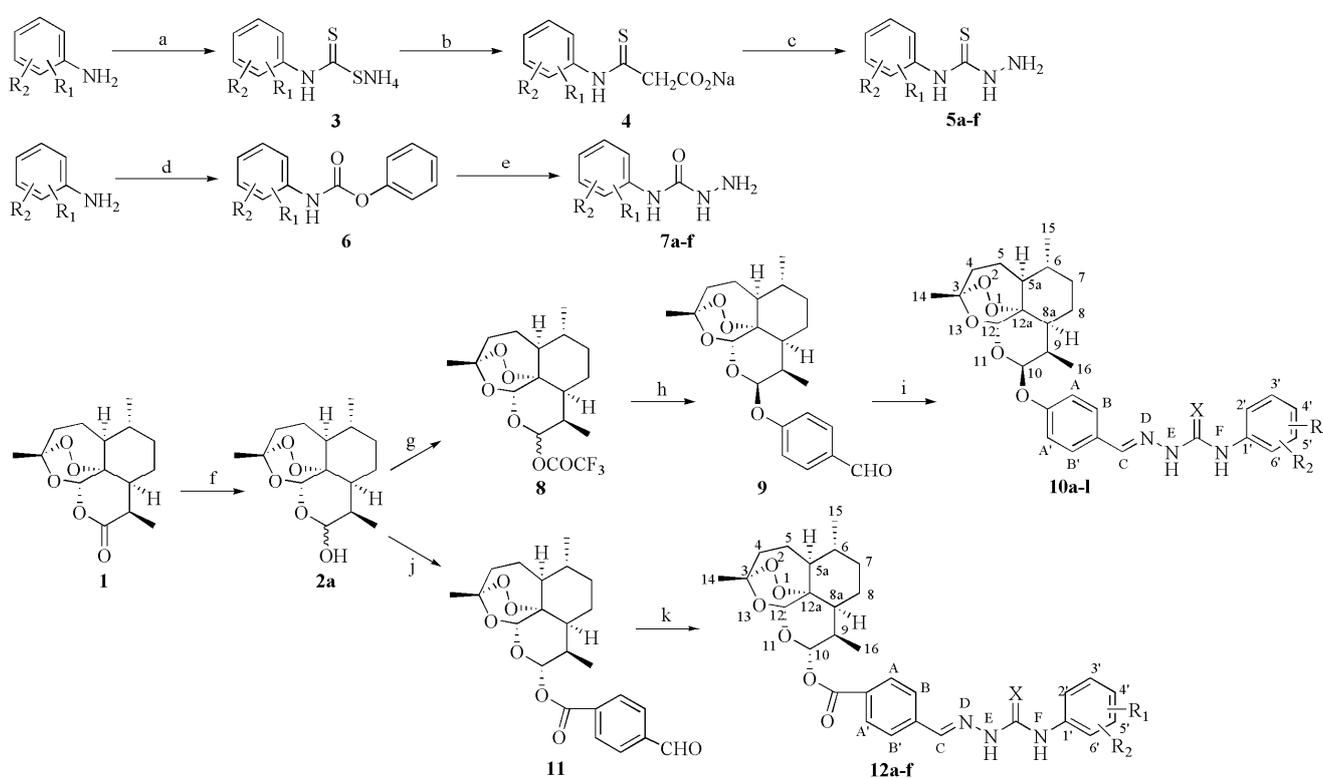
2.1. Chemistry

The target compounds **10a-l** and **12a-f** were synthesized through a general condensation procedure of 4-substituted benzaldehydes **9** and **11** with thiosemicarbazides **5a-f** or semicarbazides **7a-f** respectively (Scheme 1).

Compounds **9** and **11** were the key intermediates of the synthetic process. Compound **9** was synthesized by an indirect method rather than the generally direct etherification catalyzed by BF₃·Et₂O [10]. Artemisinin (**1**) was reduced with NaBH₄ based on an effective route to dihydroartemisinin (**2a**) [11] which was activated by trifluoroacetic anhydride (TFAA) in the presence of Et₃N to form trifluoroacetate **8**. Without separation, compound **8** reacted with 4-hydroxybenzaldehyde to

give compound **9** which was purified on a silica gel column. Compound **11** was synthesized directly through the esterification of dihydroartemisinin **2a** and 4-formylbenzoic acid catalyzed by dicyclohexylcarbodiimide (DCC) and 4-*N,N*-dimethylaminopyridine (DMAP) and was also purified on a silica gel column [12]. Thiosemicarbazides **5a-f** were obtained from different anilines by a literature method [13]. The anilines reacted with carbon disulphide in the presence of concentrated ammonia to yield dithiocarbamates **3**. These compounds on treatment with sodium monochloroacetate followed by condensation with hydrazine hydrate gave **5a-f**. Semicarbazides **7a-f** were also obtained from anilines through acylation with phenyl chloroformate to give carbamates **6**, which were condensed with hydrazine hydrate to yield **7a-f** [14].

Scheme 1. The synthetic routes for compounds **10a-l** and **12a-f**.



Reagents and conditions: (a) CS₂, NH₃·H₂O, EtOH, rt, 1 h; (b) ClCH₂COONa, rt, 30 min; (c) NH₂NH₂·H₂O, rt, 1 h; (d) phenyl chloroformate, pyridine, CH₂Cl₂, ice-water bath, rt, 4 h; (e) NH₂NH₂·H₂O, reflux, 12 h; (f) NaBH₄, CH₃OH, 0–5 °C, 3 h; (g) TFAA, Et₃N, CH₂Cl₂, –5–0 °C, 1 d; (h) 4-hydroxybenzaldehyde, –5–0 °C, 12 h; (i) **5a-f** or **7a-f**, CH₃COOH, EtOH, rt, 2 h; (j) 4-formylbenzoic acid, DCC, DMAP, ice-water bath, rt, 1 d; (k) **7a-f**, CH₃COOH, EtOH, rt, 2 h.

All the target compounds were identified as single isomers due to the configuration of compounds **9** and **11** confirmed by ¹H-NMR. The configuration at C-10 was assigned based on the coupling constant between H-9 and H-10. A small coupling constant ($J = 3.6$ Hz) was observed in β -arteether, while a large coupling constant ($J = 9.2$ Hz) was observed in α -arteether [11] and a similar rule was found in dihydroartemisinin aromatic ethers in other reports [15,16]. In compounds **9** and **11**, these coupling constants were 3.3 Hz and 9.6 Hz, respectively, which indicated the former was a β -isomer and the latter was an α -isomer.

2.2. Biological Assay in Vitro

The *in vitro* *P. falciparum* falcipain-2 inhibition assay against was carried out according to a previously reported method by taking *N*-(*L*-3-*trans*-carboxyoxiran-2-carbonyl)-*L*-leucyl)-amido(4-guanido)butane (**E-64**) as a positive control and DMSO as a negative control [17]. Falcipain-2 could hydrolyse the fluorogenic substrate benzyloxycarbonyl-Leu-Arg-7-amino-4-methylcoumarin (*Z*-Leu-Arg-AMC) to produce coumarin and its activity could be evaluated by monitoring the fluorescence of the coumarin. Activity of all the tested compounds is shown in Table 1.

Table 1. Inhibition activity of compounds **10a-l** and **12a-f** against *P. falciparum* falcipain-2 *in vitro*.

Compd.	X	R ₁	R ₂	Inhibition rate ^a (%)	IC ₅₀ ^b (μM)
10a	S	H	2-F	72.48	2.51
10b	S	H	4-F	59.76	2.17
10c	S	H	4-OCH ₂ CH ₃	87.42	0.52
10d	S	2-CH ₃	5-CH ₃	75.28	0.55
10e	S	3-CH ₃	5-CH ₃	74.06	1.35
10f	S	3-Cl	2-CH ₃	75.62	0.984
10g	O	H	2-F	76.74	2.25
10h	O	H	4-F	84.60	1.02
10i	O	H	4-OCH ₂ CH ₃	72.70	0.7
10j	O	2-CH ₃	5-CH ₃	59.01	2.28
10k	O	3-CH ₃	5-CH ₃	87.88	1.03
10l	O	3-Cl	2-CH ₃	81.82	2.54
12a	O	H	2-F	79.32	0.47
12b	O	H	4-F	69.25	0.65
12c	O	H	4-OCH ₂ CH ₃	75.89	0.29
12d	O	2-CH ₃	5-CH ₃	76.61	2.96
12e	O	3-CH ₃	5-CH ₃	71.62	0.57
12f	O	3-Cl	2-CH ₃	74.67	10.63
E-64				100.0	0.0197
DMSO				0	

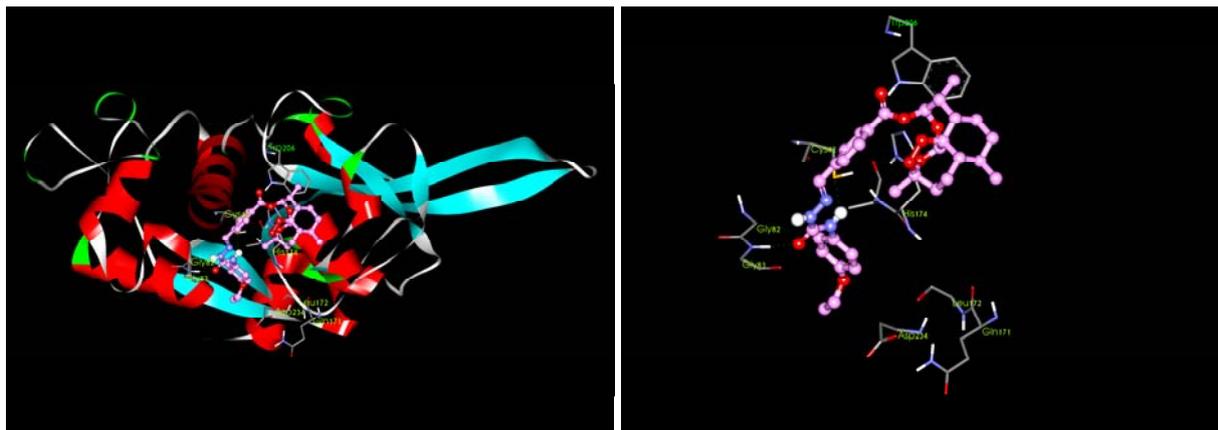
^a Inhibition rates were evaluated at 10 μM concentration level; ^b IC₅₀ values were determined if above inhibition rates were larger than 20%.

All the tested compounds were found to be inhibitors of falcipain-2 with inhibition rates greater than 20% at 10 μM concentration level and IC₅₀ values ranging from 0.29 to 10.63 μM. The preliminary SARs showed that the ester linker in compounds **12a-f** was more effective than the ether linkers in compounds **10a-l** with the same substitution on the aromatic ring (e.g., **12a** and **10a**) and the latter displayed equivalent efficacy between semicarbazones and thiosemicarbazones (e.g., **10e** and **10k**). Furthermore, in the same kind of scaffolds, the introduction of fluoro or ethoxyl groups at the C-4 position as monosubstituents on the aromatic ring could improve the activity (e.g., **12b** and **10c**) and among disubstituted compounds, the ones with methyl group disubstitution at the C-3 and C-5 positions on the aromatic ring presented better activities (e.g., **10k** and **12e**). These implications would benefit a drug design in future research.

2.3. Molecular Docking Studies

Molecular docking studies were performed to investigate the binding affinities and interaction modes for the inhibitors using AutoDock 3.05 [18]. As illustrated in Figure 2, residues Trp206, Cys42, His174, Gly82, Gly83, Leu172, Asp234 and Gln171 form a pocket for ligand binding, and the most potent compound **12c** forms hydrogen bonds with Cys42 and Gly83.

Figure 2. Proposed binding mode of compound **12c** in the active site of falcipain-2.



3. Experimental

3.1. Instruments and Reagents

Melting points were taken on glass slides with X-4 digital display microscopic melting point apparatus and were presented uncorrected. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded using a Bruker ARX-300 spectrometer with CDCl_3 as solvent and TMS as internal standard. Electrospray ionization mass spectra (ESIMS) were recorded on a Waters Quattro micro API. All reagents were commercially available and were used with purification as needed. Chromatography was carried on silica gel (200–300 mesh). All reactions were monitored by TLC (silica gel plates with fluorescence F_{254} were used).

3.2. General Procedure for the Synthesis of N^4 -(Substitued Phenyl)thiosemicarbazides **5a-f**

To an EtOH solution (50 mL) of aniline (0.1 mol), concentrated ammonia (20 mL) was slowly added. The mixture was cooled below $20\text{ }^\circ\text{C}$ and CS_2 (8 mL) was added dropwise during a period of 15 min. After 1 h, sodium monochloroacetate (14 g, 0.12 mol) was added and vigorously stirred for 30 min followed by adding hydrazine hydrate (80%, 12.5 mL). After 1 h, the mixture was cooled overnight and the crude reaction mixture was filtered and recrystallized from EtOH.

3.3. General Procedure for the Synthesis of N^4 -(Substitued Phenyl)semicarbazides **7a-f**

To a mixture of aniline (0.05 mol), pyridine (5 mL) and CH_2Cl_2 (30 mL), phenyl chloroformate (6.3 mL, 0.05 mol) was added dropwise under ice-water bath and reacted at room temperature for 4 h. The mixture was then evaporated under reduced pressure and the residue was poured into saturated

NaCl solution for salting out. The precipitate was filtered and dried followed by refluxing in hydrazine hydrate (80%, 10 mL) for 12 h. After cooling, the crude reaction mixture was filtered and recrystallized from EtOH.

3.4. General Procedure for the Synthesis of Dihydroartemisinin (2a)

To a stirred solution of artemisinin (**1**, 2 g, 7.08 mmol) in CH₃OH, NaBH₄ (0.40 g, 10.62 mmol) was added at 0–5 °C over a period of 20 min. After being stirred for an additional 3 h under the same condition, the mixture was neutralized with glacial acetic acid while the temperature was maintained at 0–5 °C, concentrated by evaporating most CH₃OH, diluted with cold water (100 mL) and stirred for 15 min at room temperature. The precipitate was collected, washed with water (100 mL × 3) and dried. Yield 97.15%, m.p. 143–145 °C.

3.5. General Procedure for the Synthesis of 4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde N⁴-(substitued phenyl)(thio)semicarbazones 10a-l

To a CH₂Cl₂ solution (50 mL) of dihydroartemisinin (**2a**, 11.36 g, 40 mmol) and triethylamine (11.08 mL, 80 mmol), TFAA (11.12 mL, 80 mmol) in CH₂Cl₂ (30 mL) was added dropwise at –5–0 °C. After 1 d, 4-hydroxybenzaldehyde (9.76 g, 80 mmol) was added and stirred for 12 h at the same temperature. The reaction solution was quenched with saturated NaHCO₃ solution and washed by saturated NaHCO₃ solution (50 mL × 5) and water (50 mL × 5), respectively. The organic layer was dried (Na₂SO₄) and concentrated in vacuo. The residue was purified on silica gel column with petroleum ether/ethyl acetate (8:1) to afford **9**. Yield 40.29%, m.p. 88–90 °C. MS (ESI) *m/z*: 411 (M+Na); ¹H-NMR (300 MHz, CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.34–2.44 (1H, m, 4-H), 2.83–2.88 (1H, m, 9-H), 5.44 (1H, s, 12-H), 5.62 (1H, d, *J* = 3.3 Hz, 10-H), 7.23 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.84 (2H, d, *J* = 8.7 Hz, BB'-2H), 9.90 (1H, s, C-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 191.17, 162.65, 132.20, 131.03, 116.95, 104.63, 100.40, 88.65, 81.11, 52.74, 44.52, 37.69, 36.57, 34.85, 31.10, 26.29, 24.88, 24.67, 20.56, 13.13. Equimolar quantities of **9** (2 mmol) and **5a-f** (2 mmol) or **7a-f** (2 mmol) was dissolved in EtOH (20 mL) catalyzed by glacial acetic acid (3 drops) and stirred for 2 h at room temperature. The precipitate was filtered and purified on silica gel column with petroleum ether/ethyl acetate (3:1) to afford **10a-f** or with CH₂Cl₂/CH₃OH (80:1) to afford **10g-l**.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde N⁴-(2-fluorophenyl)thiosemicarbazone (**10a**). Yield 19.90%, m.p. 108–109 °C. MS (ESI) *m/z*: 556 (M+H); ¹H-NMR (300 MHz, CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.34–2.44 (1H, m, 4-H), 2.82–2.87 (1H, m, 9-H), 5.47 (1H, s, 12-H), 5.57 (1H, d, *J* = 3.0 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.63 (2H, d, *J* = 9.0 Hz, BB'-2H), 7.84 (1H, s, C-H), 9.37 (1H, s, E-H), 9.53 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 176.32, 159.78, 143.30, 136.74, 134.31, 130.96, 129.30, 127.53, 127.22, 126.77, 117.25, 104.63, 100.58, 88.63, 81.25, 52.79, 44.61, 37.72, 36.63, 34.90, 31.21, 26.36, 24.92, 24.73, 20.62, 13.22.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde *N*⁴-(4-fluorophenyl)thiosemicarbazone (**10b**). Yield 42.29%, m.p. 117–118 °C. MS (ESI) *m/z*: 556 (M+H); ¹H-NMR (300 MHz, CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.44 (3H, s, 14-CH₃), 2.34–2.44 (1H, m, 4-H), 2.82–2.87 (1H, m, 9-H), 5.46 (1H, s, 12-H), 5.57 (1H, d, *J* = 3.0 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.62 (2H, d, *J* = 9.0 Hz, BB'-2H), 7.85 (1H, s, C-H), 9.07 (1H, s, E-H), 9.68 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 175.82, 159.98, 143.54, 136.74, 131.76, 129.45, 129.17, 126.81, 126.11, 117.28, 104.64, 100.57, 88.64, 81.23, 52.78, 44.60, 37.73, 36.62, 34.90, 31.20, 26.35, 24.92, 24.74, 20.60, 13.20.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde *N*⁴-(4-ethoxyphenyl)thiosemicarbazone (**10c**). Yield 31.80%, m.p. 114–116 °C. MS (ESI) *m/z*: 580 (M-H); ¹H-NMR (300 MHz, CDCl₃) δ: 0.96 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.02 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.44 (3H, s, 14-CH₃), 2.33–2.44 (1H, m, 4-H), 2.81–2.86 (1H, m, 9-H), 4.05 (2H, q, *J* = 6.9 Hz, 4'-OCH₂CH₃), 5.46 (1H, s, 12-H), 5.55 (1H, d, *J* = 3.3 Hz, 10-H), 7.14 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.60 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.91 (1H, s, C-H), 9.02 (1H, s, E-H), 10.25 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 176.53, 159.79, 158.31, 143.24, 131.07, 129.34, 127.22, 117.22, 114.34, 104.62, 100.57, 88.62, 81.25, 55.77, 52.79, 44.62, 37.72, 36.63, 34.90, 31.21, 26.35, 24.92, 24.73, 20.60, 19.33, 13.20.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde *N*⁴-(2,5-dimethylphenyl)thiosemicarbazone (**10d**). Yield 38.00%, m.p. 120–122 °C. MS (ESI) *m/z*: 566 (M+H); ¹H-NMR (300 MHz, CDCl₃) δ: 0.96 (3H, d, *J* = 5.7 Hz, 16-CH₃), 1.02 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.44 (3H, s, 14-CH₃), 2.32 (3H, s, 2'-CH₃), 2.37 (3H, s, 5'-CH₃), 2.81–2.86 (1H, m, 9-H), 5.46 (1H, s, 12-H), 5.55 (1H, d, *J* = 3.3 Hz, 10-H), 7.14 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.60 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.90 (1H, s, C-H), 8.93 (1H, s, E-H), 10.13 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 176.91, 159.74, 143.29, 137.45, 134.48, 134.14, 131.69, 129.28, 127.64, 127.47, 127.29, 117.23, 104.62, 100.59, 88.62, 81.24, 52.79, 44.63, 37.72, 36.64, 34.92, 31.21, 26.36, 24.92, 24.74, 21.42, 20.61, 18.28, 13.21.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde *N*⁴-(3,5-dimethylphenyl)thiosemicarbazone (**10e**). Yield 16.19%, m.p. 125–127 °C. MS (ESI) *m/z*: 566 (M+H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 5.7 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.44 (3H, s, 14-CH₃), 2.35 (6H, s, 3'-CH₃, 5'-CH₃), 2.81–2.86 (1H, m, 9-H), 5.46 (1H, s, 12-H), 5.56 (1H, d, *J* = 3.3 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.61 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.80 (1H, s, C-H), 9.08 (1H, s, E-H), 9.38 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 176.56, 159.77, 143.10, 137.43, 135.80, 135.15, 130.18, 129.32, 127.19, 126.46, 122.87, 117.23, 104.62, 100.58, 88.61, 81.24, 52.80, 44.62, 37.72, 36.64, 34.91, 31.21, 27.21, 26.35, 24.92, 24.73, 20.60, 19.68, 13.20.

4-[(10S)-Dihydroartemisinin-10-oxy]benzaldehyde *N*⁴-(3-chloro-2-methylphenyl)thiosemicarbazone (**10f**). Yield 26.44%, m.p. 150–152 °C. MS (ESI) *m/z*: 584 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.44 (3H, s, 14-CH₃), 2.39 (3H, s, 2'-CH₃), 2.81–2.86 (1H, m, 9-H), 5.46 (1H, s, 12-H), 5.56 (1H, d, *J* = 3.3 Hz, 10-H), 7.15 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.61 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.89 (1H, s, C-H), 8.95 (1H, s, E-H), 10.00 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 177.06, 159.74, 143.29, 138.08, 136.65, 133.88, 129.28, 127.32,

126.04, 117.22, 104.62, 100.58, 88.62, 81.25, 52.79, 44.62, 37.72, 36.64, 34.91, 31.21, 26.36, 24.93, 24.74, 20.84, 20.62, 14.33, 13.22.

4-[(10*S*)-Dihydroartemisinin-10-*oxy*]benzaldehyde *N*⁴-(2-fluorophenyl)semicarbazone (**10g**). Yield 72.28%, m.p. 175–177 °C. MS (ESI) *m/z*: 538 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.04 (3H, d, *J* = 7.5 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.34–2.45 (1H, m, 4-H), 2.81–2.86 (1H, m, 9-H), 5.48 (1H, s, 12-H), 5.56 (1H, d, *J* = 3.3 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.61 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.77 (1H, s, C-H), 8.49 (1H, s, E-H), 8.98 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 159.21, 154.37, 141.72, 136.54, 130.66, 128.57, 128.05, 128.00, 127.17, 124.02, 121.44, 117.25, 104.60, 100.69, 88.63, 81.26, 52.82, 44.66, 37.73, 36.65, 34.93, 31.25, 26.39, 24.94, 24.75, 20.63, 13.25.

4-[(10*S*)-Dihydroartemisinin-10-*oxy*]benzaldehyde *N*⁴-(4-fluorophenyl)semicarbazone (**10h**). Yield 44.63%, m.p. 124–126 °C. MS (ESI) *m/z*: 538 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.04 (3H, d, *J* = 7.5 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.34–2.45 (1H, m, 4-H), 2.82–2.87 (1H, m, 9-H), 5.48 (1H, s, 12-H), 5.56 (1H, d, *J* = 3.0 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.59 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.74 (1H, s, C-H), 8.08 (1H, s, E-H), 8.76 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 159.27, 154.12, 142.40, 136.95, 129.28, 128.78, 127.72, 121.17, 117.20, 104.61, 100.63, 88.62, 81.26, 52.81, 44.64, 37.73, 36.64, 34.92, 31.24, 26.37, 24.94, 24.74, 20.61, 13.23.

4-[(10*S*)-Dihydroartemisinin-10-*oxy*]benzaldehyde *N*⁴-(4-ethoxyphenyl)semicarbazone (**10i**). Yield 52.15%, m.p. 158–159 °C. MS (ESI) *m/z*: 564 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 5.7 Hz, 16-CH₃), 1.04 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.42 (3H, t, *J* = 7.2 Hz, 4'-OCH₂CH₃), 1.45 (3H, s, 14-CH₃), 2.34–2.44 (1H, m, 4-H), 2.81–2.86 (1H, m, 9-H), 4.03 (2H, q, *J* = 6.9 Hz, 4'-OCH₂CH₃), 5.48 (1H, s, 12-H), 5.55 (1H, d, *J* = 3.3 Hz, 10-H), 7.15 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.58 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.73 (1H, s, C-H), 7.97 (1H, s, E-H), 8.81 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 159.18, 156.34, 154.57, 141.83, 131.32, 128.66, 128.03, 122.24, 117.15, 114.53, 104.58, 100.64, 88.60, 81.27, 55.82, 52.81, 44.66, 37.72, 36.65, 34.93, 31.25, 26.37, 24.93, 24.74, 20.61, 19.50, 13.23.

4-[(10*S*)-Dihydroartemisinin-10-*oxy*]benzaldehyde *N*⁴-(2,5-dimethylphenyl)semicarbazone (**10j**). Yield 80.05%, m.p. 172–173 °C. MS (ESI) *m/z*: 548 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 5.7 Hz, 16-CH₃), 1.03 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.33 (3H, s, 2'-CH₃), 2.36 (3H, s, 5'-CH₃), 2.81–2.86 (1H, m, 9-H), 5.48 (1H, s, 12-H), 5.55 (1H, d, *J* = 3.3 Hz, 10-H), 7.16 (2H, d, *J* = 8.7 Hz, AA'-2H), 7.57 (2H, d, *J* = 8.7 Hz, BB'-2H), 7.76 (1H, s, C-H), 8.12 (1H, s, E-H), 8.95 (1H, s, F-H); ¹³C-NMR (75 MHz, CDCl₃) δ: 159.15, 154.62, 141.61, 133.83, 131.36, 128.57, 127.63, 122.14, 117.22, 104.59, 100.70, 88.62, 81.26, 52.83, 44.67, 37.72, 36.65, 34.95, 31.25, 26.38, 24.74, 21.12, 20.62, 18.34, 13.24.

4-[(10*S*)-Dihydroartemisinin-10-*oxy*]benzaldehyde *N*⁴-(3,5-dimethylphenyl)semicarbazone (**10k**). Yield 84.60%, m.p. 169–171 °C. MS (ESI) *m/z*: 548 (M-H); ¹H-NMR (CDCl₃) δ: 0.97 (3H, d, *J* = 6.0 Hz, 16-CH₃), 1.04 (3H, d, *J* = 7.2 Hz, 15-CH₃), 1.45 (3H, s, 14-CH₃), 2.33 (6H, s, 3'-CH₃, 5'-CH₃), 2.81–2.86 (1H, m, 9-H), 5.48 (1H, s, 12-H), 5.56 (1H, d, *J* = 3.3 Hz, 10-H), 7.16 (2H, d,

$J = 8.7$ Hz, AA'-2H), 7.59 (2H, d, $J = 8.7$ Hz, BB'-2H), 7.76 (1H, s, C-H), 8.05 (1H, s, E-H), 9.01 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 159.19, 154.33, 141.79, 137.44, 135.94, 132.01, 130.30, 128.67, 128.04, 121.58, 117.71, 117.17, 104.59, 100.67, 88.60, 81.27, 52.83, 44.67, 37.73, 36.66, 34.94, 31.26, 26.37, 24.94, 24.75, 20.61, 20.24, 19.03, 13.23.

4-[(10S)-Dihydroartemisinin-10-oxyl]benzaldehyde N^t-(3-chloro-2-methylphenyl)semicarbazone (10l). Yield 92.97%, m.p. 177–179 °C. MS (ESI) m/z : 568 (M-H); $^1\text{H-NMR}$ (CDCl_3) δ : 0.97 (3H, d, $J = 5.7$ Hz, 16- CH_3), 1.03 (3H, d, $J = 7.5$ Hz, 15- CH_3), 1.45 (3H, s, 14- CH_3), 2.81–2.86 (1H, m, 9-H), 5.48 (1H, s, 12-H), 5.56 (1H, d, $J = 3.3$ Hz, 10-H), 7.57 (2H, d, $J = 8.7$ Hz, BB'-2H), 7.76 (1H, s, C-H), 8.20 (1H, s, E-H), 8.98 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 159.15, 154.79, 141.71, 137.49, 136.14, 128.56, 128.24, 126.43, 126.24, 120.86, 117.21, 104.59, 100.69, 88.62, 81.26, 52.83, 44.67, 37.73, 36.66, 34.94, 31.25, 26.38, 24.94, 24.74, 21.03, 20.62, 13.73, 13.24.

3.6. General Procedure for the Synthesis of 4-[(10S)dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(substitued phenyl)semicarbazones **12a-f**

To a CH_2Cl_2 solution (100 mL) of dihydroartemisinin (**2a**, 11.36 g, 40 mmol) and 4-formylbenzoic acid (7.21 g, 48 mmol), DCC (9.90 g, 48 mmol) and DMAP (1.47 g, 12 mmol) were added at 0–5 °C for 1 h. After 1 d reaction at room temperature, the mixture was filtered and filtrate was concentrated in vacuo. The residue was purified on silica gel column with petroleum ether/ethyl acetate (6:1) to afford **11**. Yield 40.46 %, m.p. 81–82 °C. MS (ESI) m/z : 439 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.99 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.35–2.45 (1H, m, 4-H), 2.74–2.81 (1H, m, 9-H), 5.54 (1H, s, 12-H), 6.03 (1H, d, $J = 9.6$ Hz, 10-H), 7.97 (2H, d, $J = 8.1$ Hz, AA'-2H), 8.28 (2H, d, $J = 8.1$ Hz, BB'-2H), 10.12 (1H, s, C-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 190.89, 168.31, 162.30, 133.17, 117.52, 104.61, 100.73, 88.56, 81.13, 52.69, 44.62, 37.70, 36.63, 34.89, 31.10, 26.34, 24.72, 23.67, 20.60, 13.11. Equimolar quantities of **11** (2 mmol) and **7a-f** (2 mmol) was dissolved in EtOH (20 mL) catalyzed by glacial acetic acid (3 drops) and stirred for 2 h at room temperature. The precipitate was filtered and purified on silica gel column with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (80:1) to afford **12a-f**.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(2-fluorophenyl)semicarbazone (12a). Yield 17.62%, m.p. 145–147 °C. MS (ESI) m/z : 590 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.99 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.44 (3H, s, 14- CH_3), 2.35–2.46 (1H, m, 4-H), 2.74–2.81 (1H, m, 9-H), 5.55 (1H, s, 12-H), 6.02 (1H, d, $J = 9.9$ Hz, 10-H), 7.72 (2H, d, $J = 8.4$ Hz, AA'-2H), 7.89 (1H, s, C-H), 8.14 (2H, d, $J = 8.4$ Hz, BB'-2H), 8.20 (1H, s, E-H), 9.46 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 166.32, 158.65, 154.63, 142.01, 136.62, 130.66, 128.63, 128.14, 128.03, 127.22, 124.03, 121.35, 117.33, 104.62, 100.70, 88.45, 81.27, 52.80, 44.51, 37.67, 36.73, 34.89, 31.20, 36.37, 24.90, 24.71, 20.59, 13.12.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(4-fluorophenyl)semicarbazone (12b). Yield 81.92%, m.p. 139–141 °C. MS (ESI) m/z : 590 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.93 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.99 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.35–2.45 (1H, m, 4-H), 2.73–2.81 (1H, m, 9-H), 5.55 (1H, s, 12-H), 6.02 (1H, d, $J = 9.9$ Hz, 10-H), 7.71 (2H, d, $J = 8.4$ Hz,

AA'-2H), 7.91 (1H, s, C-H), 8.12 (2H, d, $J = 8.1$ Hz, BB'-2H), 8.13 (1H, s, E-H), 9.99 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 166.02, 159.32, 154.34, 142.36, 136.89, 129.33, 128.78, 128.91, 121.20, 117.15, 104.61, 100.60, 88.63, 81.32, 52.77, 44.56, 37.73, 36.55, 34.90, 31.22, 26.41, 24.91, 24.70, 20.62, 13.16.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(4-ethoxyphenyl)semicarbazone (12c). Yield 42.11%, m.p. 152–153 °C. MS (ESI) m/z : 616 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.93 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.98 (3H, d, $J = 5.1$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.35–2.45 (1H, m, 4-H), 2.73–2.80 (1H, m, 9-H), 4.04 (2H, q, $J = 6.9$ Hz, 4'- OCH_2CH_3), 5.54 (1H, s, 12-H), 6.01 (1H, d, $J = 9.6$ Hz, 10-H), 7.70 (2H, d, $J = 8.1$ Hz, AA'-2H), 7.90 (1H, s, C-H), 8.03 (1H, s, E-H), 8.12 (2H, d, $J = 8.1$ Hz, BB'-2H), 10.11 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 166.87, 159.52, 154.62, 142.78, 131.31, 128.70, 128.03, 122.17, 117.22, 114.51, 104.59, 100.60, 88.56, 81.32, 55.83, 52.79, 44.65, 37.71, 36.63, 34.90, 31.21, 26.36, 24.90, 24.72, 20.60, 19.45, 13.21.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(2,5-dimethylphenyl)semicarbazone (12d). Yield 85.69%, m.p. 155–156 °C. MS (ESI) m/z : 600 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.99 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.73–2.80 (1H, m, 9-H), 5.54 (1H, s, 12-H), 6.02 (1H, d, $J = 9.6$ Hz, 10-H), 7.67 (2H, d, $J = 8.4$ Hz, AA'-2H), 7.89 (1H, s, C-H), 8.11 (1H, s, E-H), 8.13 (2H, d, $J = 8.4$ Hz, BB'-2H), 9.68 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 167.14, 159.02, 154.61, 141.70, 134.23, 131.41, 128.48, 127.55, 121.11, 117.20, 104.62, 100.60, 88.56, 81.31, 52.78, 44.72, 37.66, 36.69, 34.90, 31.24, 26.42, 24.67, 21.10, 18.33, 13.21.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(3,5-dimethylphenyl)semicarbazone (12e). Yield 63.18%, m.p. 154–155 °C. MS (ESI) m/z : 600 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.93 (3H, d, $J = 7.2$ Hz, 16- CH_3), 0.98 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.73–2.80 (1H, m, 9-H), 5.54 (1H, s, 12-H), 6.01 (1H, d, $J = 9.9$ Hz, 10-H), 7.68 (2H, d, $J = 8.4$ Hz, AA'-2H), 7.93 (1H, s, C-H), 8.08 (1H, s, E-H), 8.11 (2H, d, $J = 8.4$ Hz, BB'-2H), 10.06 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 167.71, 159.22, 154.31, 141.77, 134.90, 132.01, 130.86, 128.66, 128.02, 121.56, 117.71, 117.20, 104.61, 100.68, 88.61, 81.32, 52.80, 44.73, 37.67, 36.70, 34.86, 31.20, 26.43, 24.91, 24.73, 20.60, 20.16, 19.21, 13.21.

4-[(10S)-Dihydroartemisinin-10-oxycarbonyl]benzaldehyde N^t-(3-chloro-2-methylphenyl)semicarbazone (12f). Yield 63.54%, m.p. 173–174 °C. MS (ESI) m/z : 620 (M+Na); $^1\text{H-NMR}$ (CDCl_3) δ : 0.94 (3H, d, $J = 6.9$ Hz, 16- CH_3), 0.98 (3H, d, $J = 5.7$ Hz, 15- CH_3), 1.43 (3H, s, 14- CH_3), 2.73–2.80 (1H, m, 9-H), 5.54 (1H, s, 12-H), 6.01 (1H, d, $J = 9.9$ Hz, 10-H), 7.67 (2H, d, $J = 8.4$ Hz, AA'-2H), 7.92 (1H, s, C-H), 8.12 (2H, d, $J = 8.1$ Hz, BB'-2H), 8.21 (1H, s, E-H), 10.19 (1H, s, F-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) δ : 166.32, 159.14, 154.81, 141.68, 137.11, 136.10, 128.61, 128.15, 126.42, 126.21, 120.94, 117.20, 104.56, 100.72, 88.61, 81.26, 52.80, 44.66, 37.70, 36.74, 34.92, 31.31, 26.42, 24.89, 24.70, 21.01, 20.64, 13.71, 13.20.

3.7. Inhibition Assays

Recombinant falcipain-2 (30 nM) was incubated for 30 min at room temperature in 100 mM NaOAc, pH 5.5, 10 mM dithiothreitol (DTT), with different concentrations of **10a-l** and **12a-f**. These solutions were prepared from stock in DMSO (maximum concentration of DMSO in the assay was 1%). After incubation, the substrate Z-Leu-Arg-AMC with the same buffer was added to a final concentration of 25 μ M. The increase in fluorescence (excitation at 355 nm and emission at 460 nm) was monitored for 30 min at room temperature with an automated microplate spectrofluorimeter (Biotek, Synergy 2). IC₅₀ values were determined from plots of percent activity over compound concentration using the GraphPad Prism software if inhibition rates were larger than 20% at 10 μ M concentration level.

4. Conclusions

In conclusion, a total of 18 dihydroartemisinin derivatives, **10a-l** and **12a-f**, were synthesized from the natural product artemisinin (**1**) and anilines through simplified procedures. All target compounds were obtained as single isomers as confirmed by ¹H-NMR through the key intermediates **9** and **11**. The biological evaluation and molecular docking studies indicated that this new class of dihydroartemisinin derivatives could be identified as potential falcipain-2 inhibitors and the preliminary SARs could serve as a foundation for further investigation of antimalarial drugs.

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Sample Availability: Samples of the compounds **9**, **11**, **10a-l** and **12a-f** are available from the authors.