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Design, synthesis and biological evaluation of artemisinin derivatives containing fluorine atoms as anticancer agents

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ABSTRACT

Ten novel artemisinin derivatives containing fluorine atoms were synthesized and their structures were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS technologies in this study. The *in vitro* cytotoxicity against U87MG, SH-SY5Y, MCF-7, MDA-MB-231, A549 and A375 cancer cell lines was evaluated by MTT assay. Compound **9j** was the most potent anti-proliferative agent against the human breast cancer MCF-7 cells (IC_{50} = 2.1 μM). The mechanism of action of compound **9j** was further investigated by analysis of cell apoptosis and cell cycle. Compound **9j** induced cell apoptosis and arrested cell cycle at G1 phase in MCF-7 cells. Our promising findings indicated that the compound **9j** could stand as potential lead compound for further investigation.

Artemisinin (ART) is the first natural endoperoxide containing sesquiterpene lactone isolated from Chinese herb *Artemisia annua*. Artemisinin and its derivatives (Fig. 1) have been widely used in the treatment of malaria due to their high anti-malarial activity and low toxicity.[1] With the established safety record in millions of malarial patients,[2,3] other activities in addition to antimalaria of artemisinins were also being investigated, such as anti-viral,[4] anti-parasitic,[5] anti-fungal,[6] anti-inflammatory[7] and anti-cancer.[8] In 1993, antitumor activity of artemisinins was firstly reported.[9] Subsequently, many artemisinin derivatives, such as ester,[10] ether,[11] dimer, trimer and tetramer,[12] were synthesized as antitumor drug candidates. Despite the fact that hemiacetal structure provides a chance for the preparation of artemisinin derivatives, current synthetic strategies have only focused on the C-9 and C-10 atoms mainly due to the difficulty to introduce functionalities on the ring systems by conventional chemical methods. Soomro[13] found that C-10 derivatives were more cytotoxic towards cancer cells than C-9 derivatives. Introduction of additional heteroatoms to the skeleton of artemisinin may afford derivatives with novel biological properties. In our previous work, we found artemisinin derivatives with piperazine group at C-10 site displayed potent antitumor activity.[14]

Fluorine has been exploited extensively in drug design and development.[15] An increasing number of fluorinated antimitotic/antitumor agents have now becoming available for cancer treatment.[16] In the design of analogues of bioactivity compounds, replacement of a C—H bond or C—O bond with fluorine has special advantages.[17] Grellepois[18] introduced a trifluoromethyl substituent at C-10 of artemisinin to synthesis artemisinin-like compounds which present excellent anti-malarial activity in oral treatment of infected mice. In our previous research, we found that introduction of fluorine atoms to thiazole exhibited enhanced antitumor activities.[19]

With this background, we attempted to design and synthesize a series of artemisinin derivatives containing fluorine atoms. The *in vitro* cytotoxicity against cancer cell lines (U87MG, SH-SY5Y, MCF-7, MDA-MB-231, A549 and A375) was evaluated. Furthermore, the mechanism of action of compound **9j** against breast cancer cell (MCF-7) was investigated.

Our investigation commenced with the synthesis of key intermediate **4** (Scheme 1). Commercially available aromatic amines were treated with triphosgene (BTC) and trimethylamine to give the aryl isocyanates **2**,[20] which was treated with Boc-protected piperazine to obtain intermediate **3**. Then following deprotection of Boc group of **3** with trifluoroacetic acid (TFA) to provide desired intermediate **4**,[21] which was used to synthesize the target compounds **9h**, **9i** and **9j**.

The target compounds **9a–j** were synthesized starting from artemisinin according to the literature procedure (Scheme 2). Firstly, artemisinin was subjected to $NaBH_4$-mediated reduction to afford dihydroartemisinin (DHA).[22] Then, DHA was treated with trimethylchlorosilane and triethylamine in DCM at 0–5 °C to give DHA *α*-trimethylsilyl ether **7**.[23] The target compounds **9a–j** were obtain from DHA *α*-trimethylsilyl ether **7** with trimethylsilyl bromide (TMSBr), followed by treatment of the intermediate bromide **8** formed *in suit* with the amine nucleophile.[24] The structures of all compounds were confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (Supporting Information).

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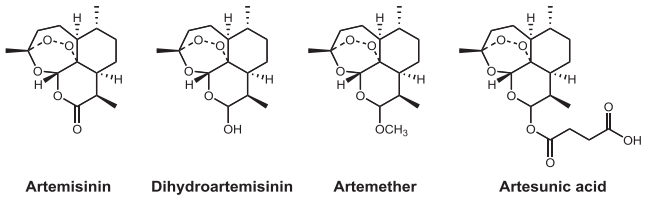
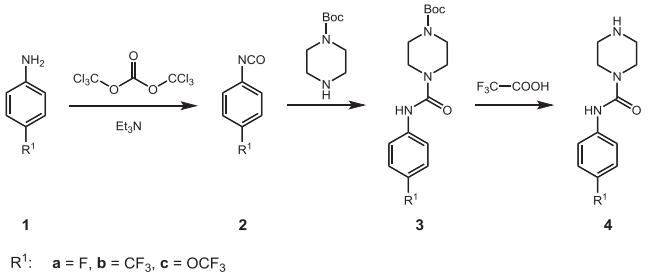
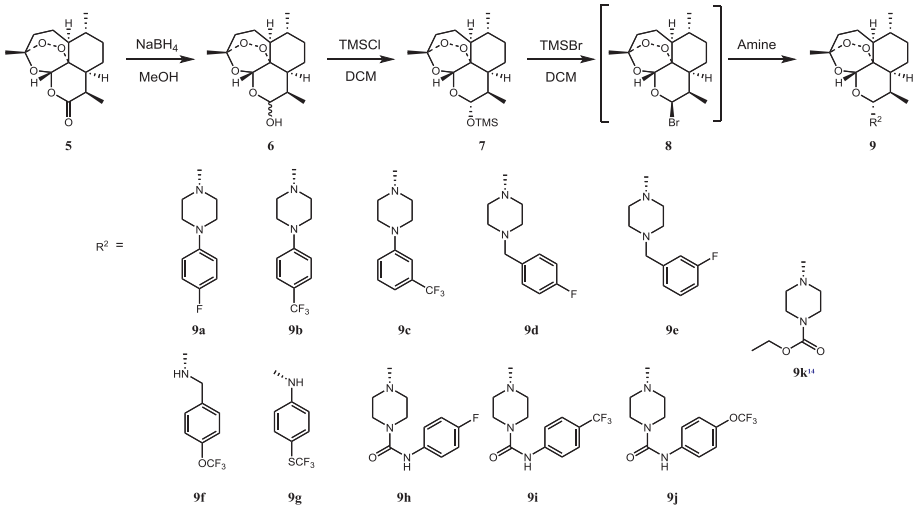


Fig. 1. The structures of artemisinin and its analogues.



Scheme 1. The synthetic routes of compound **4a–c**.

The *in vitro* cytotoxicity of artemisinin derivatives were determined against U87MG, SH-SY5Y, MCF-7, MDA-MB-231, A549, A375 by using MTT assay. Artemisinin, dihydroartemisinin, doxorubicin (DOX) and temozolomide (TMZ) were used as positive controls. The IC_{50} for each compound with respect to these cell lines were calculated and the results were summarized in Table 1. Artemisinin derivatives **9a–j** were generally more effective inhibiting cancer cells growth than the parent compound artemisinin, but presented lower cytotoxicity than doxorubicin against human normal liver cell line L02. While regarding to human neuroblastoma cell lines U87, compounds **9a**, **9f**, **9g**, **9h**, **9i** and **9j** have moderate antiproliferation activity (IC_{50} values from 15.2 μM to 28.7 μM), while **9b, 9c**, **9d** and **9e** have no pronounced inhibition activity (IC_{50} > 50 μM). For SH-SY5Y cells, compounds **9f, 9h**, **9i** and **9j** showed potent cytotoxic activities with IC_{50} values of 5.0, 5.0, 3.2 and 3.6 μM, respectively. Among human breast cancer cell MCF-7, compounds **9a–j** exhibited significantly higher cytotoxicity compared with artemisinin (IC_{50} values from 2.1 μM to 28.8 μM). It is remarkable that compound **9j** presented the strongest cytotoxic activities (IC_{50} = 2.1 μM) against MCF-7 cells. For MDA-MB-231cells, compounds **9i** and **9j** showed significantly higher cytotoxicity compared with artemisinin (the IC_{50} values were 5.8 μM and 8.4 μM, respectively).



Scheme 2. The synthetic routes of artemisinin derivatives containing fluorine atoms.

Table 1
Cytotoxic activity of **9a–j** and control drugs against cell lines.

Compound	IC_{50} Values(μM)[a]						
	U87	SH-SY5Y	MCF-7	MDA-MB-231	A549	A375	L02
9a	25.5 ± 2.7	> 50	28.8 ± 0.1	> 50	> 50	> 50	11.2 ± 1.6
9b	> 50	> 50	27.0 ± 5.7	> 50	> 50	40.9 ± 3.1	20.5 ± 0.5
9c	> 50	11.2 ± 0.9	7.5 ± 0.5	12.5 ± 0.3	12.9 ± 0.1	20.0 ± 4.6	9.1 ± 1.0
9d	> 50	15.0 ± 2.7	3.5 ± 0.2	12.0 ± 0.7	35.9 ± 0.3	12.8 ± 0.1	17.1 ± 1.6
9e	> 50	> 50	6.8 ± 1.4	32.9 ± 2.9	44.4 ± 4.0	46.5 ± 3.0	23.2 ± 4.0
9f	17.7 ± 0.5	5.0 ± 0.4	10.2 ± 0.8	21.8 ± 3.0	17.9 ± 0.7	10.9 ± 0.1	35.7 ± 2.8
9g	28.0 ± 1.1	20.3 ± 1.9	5.3 ± 03	22.9 ± 3.4	41.8 ± 2.4	26.5 ± 1.5	9.3 ± 2.1
9h	28.7 ± 0.3	5.0 ± 0.2	5.5 ± 0.1	10.9 ± 2.5	17.6 ± 3.2	9.1 ± 0.5	15.6 ± 2.2
9i	15.2 ± 2.4	3.2 ± 1.7	2.7 ± 0.1	5.8 ± 0.7	21.1 ± 3.3	15.0 ± 1.1	4.0 ± 0.2
9j	19.7 ± 2.6	**3.6 ± 2.3**[d]	**2.1 ± 0.2**[d]	**8.4 ± 1.6**[d]	19.9 ± 2.6	**3.4 ± 0.7**[d]	6.7 ± 0.2
9k[b]	> 50	> 50	15.2 ± 1.6	19.2 ± 0.7	19.9 ± 1.3	NT[c]	> 50
artemisinin	> 50	> 50	> 50	> 50	> 50	> 50	> 50
dihydroartemisinin	> 50	> 50	> 50	45.7 ± 2.7	46.8 ± 0.7	38.5 ± 2.6	34.0 ± 1.6
doxorubicin	NT[c]	NT[c]	1.0 ± 0.1	2.8 ± 0.5	2.3 ± 0.2	0.4 ± 0.1	0.9 ± 0.1
temozolomide	> 50	> 50	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]

[a] IC_{50} values are indicated as the mean ± SD (standard error) of at least three independent experiments. The cells were continuously treated with compounds for 72 h.
[b] Reported in Ref. 14.
[c] NT means not tested.
[d] The IC_{50} values of compound **9j** against SH-SY5Y, MCF-7, MDA-MB-231 and A375 cells (IC_{50} < 10 μM).