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Synthesis of 10-substituted triazolyl artemisinins possessing anticancer activity via Huisgen 1,3-dipolar cylcoaddition

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ABSTRACT

Most of the 10-substituted triazolylartemisinin synthesized via the Huisgen 1,3-dipolar cylcoaddition of diastereomeric 10-azidoartemisinin (**5**, **6**, and **7**) with various alkynes (**a**–**h**) exhibit strong growth inhibition activity, even at sub-micromolar concentrations, against various cancer cell lines such as DLD-1, U-87, Hela, SiHa, A172, and B16. In particular, **10b** and **10f** showed a highly strong cytotoxicity.

Naturally occurring endoperoxide sesquiterpene lactone artemisinin (**1**) isolated from *Artemisia annua L.* has been used as an important lead compound for antimalarial drug development.[1] Semisynthetic antimalarial agents, including artmether, arteether, artesuic acid, and artelinic acid, which are synthesized from dihydroartemisinin (**2**), are now being used in clinical treatments because of their therapeutic efficacy and nontoxicity,[2–5] although **2** has been proven to exhibit neurotoxicity in an animal model.[6] Recently, clinical trials have been carried out to unearth the anticancer, antiviral, immunosuppressive, and antifungal properties of various artemisinin derivatives and other antimalarial drugs.[7]

We have previously reported that sulfide and sulfonyl derivatives of artemisinins (**3**) inhibit the proliferation of human umbilical vein endothelial cells (HUVEC) in response to various growth factors and selectively control tumor-related angiogenesis.[8,9] Introduction of a sulfur atom to an artemisinin moiety afforded a new derivative with unique biological properties that had not been observed previously. Ziffer synthesized 11-azaartemisinin and its alkyl derivatives with enhanced antimalarial activity,[10,11] while Haynes recently synthesized artemisone (10-alkylaminoartemisinin), *N*-sulfonyl-11-azaartemisinin, and *N*-carbonyl-11-azaartemisinin.[12,13] Information on the abovementioned molecules and their synthesis methods is of immense value to medicinal chemists and helps them obtain a novel chemical library from natural lead compounds (Fig. 1).

With this background, we attempted to introduce nitrogen atoms with functional groups to a specific position in **1** via the Cu(I)-catalyzed Huisgen 1,3-cylcoaddition between azides and alkynes.[14] This reaction is a simple and efficient method for synthesizing various derivatives for biomedical applications.[15,16] Since the C-10 position of **2** has a cyclic hemiacetal functionality, it can be regarded as an sugar-anomeric center that can form a carbon–nitrogen glycoside bond. Hence, we attempted to use the reported click reaction between a 1-azido-sugar and an alkyne[17,18] for synthesizing novel artemisinin derivatives.

On the basis of the assumption that the introduction of additional heteroatoms to the C-10 position of **1** will afford derivatives with novel biological properties, we designed and synthesized various 10-substituted triazolylartemisinins and tested their proliferation inhibition effect on various cancer cell lines such as DLD-1, U-87, Hela, SiHa, A172, and B16.

As shown in Scheme 1, a separable diastereomeric mixture of 9α,10β-azidoartemisinin (**5**), 10β-azidoartemisinin (**6**), and 10α-azidoartemisinin (**7**) was obtained by reacting **2** with trimethylsilyl bromide (2.2 equiv) and sodium azide (3 equiv) at room temperature for 12 h according to a modified Haynes' method.[13] Rapid equilibrium of the oxonium ions under acidic conditions led to epimerization at the C-9 position of **2** to afford the three diastereomers (**5**, **6**, and **7**).[19,20] The stereochemistry of **5**, **6**, and **7** was

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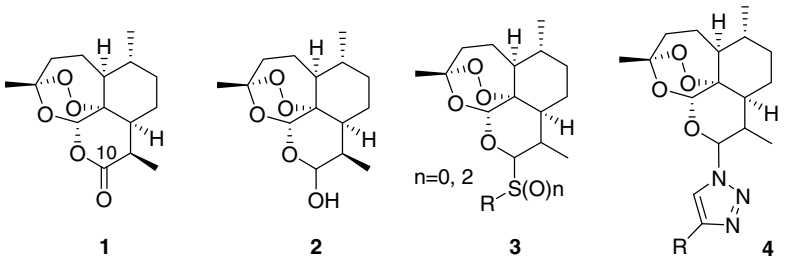
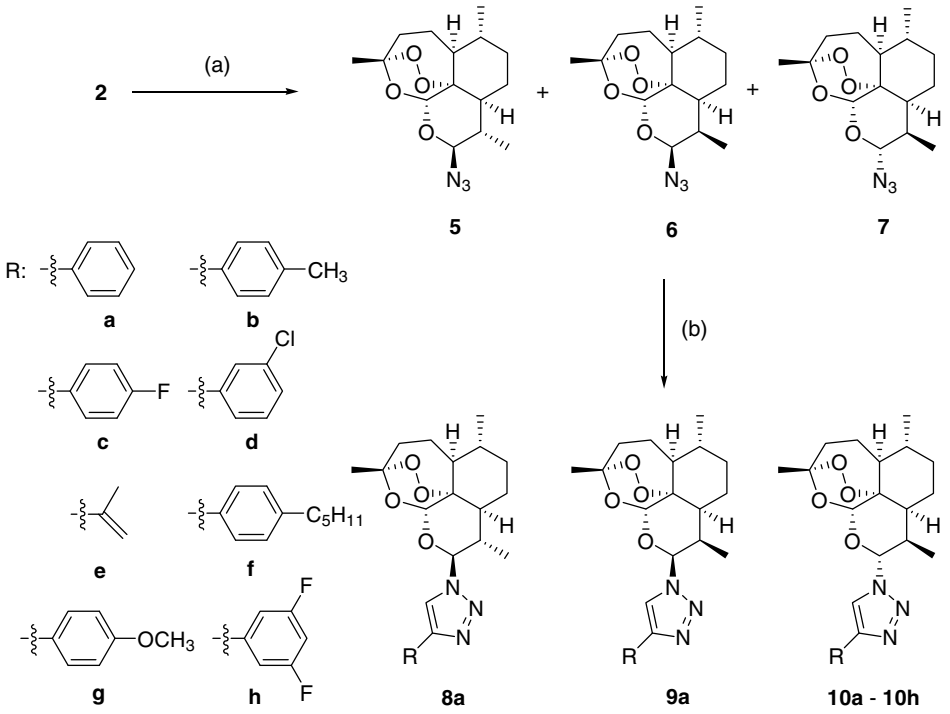


Figure 1.



Scheme 1. Reagents and conditions: (a) trimethylsilyl bromide (2.2 equiv), sodium azide (3 equiv), methylene chloride, rt, 12 h. Isolated yields **5**: 21%, **6**: 17%, **7**: 49%; (b) Copper sulfate (1.1 equiv), sodium ascorbate (2.8 equiv), methylene chloride:water (1:1), rt, 48 h. Isolated yields **8a**: 43%, **9a**: 20%, **10a**: 75%, **10b**: 60%, **10c**: 57%, **10d**: 63%, **10e**: 71%, **10f**: 65%, **10g**: 69%, **10h**: 66%.

confirmed from the H-9/H-10 coupling constants in their ^{1}H NMR spectra and the chemical shifts of the 9-methyl group in their ^{13}C NMR spectra. The H-9/H-10 coupling constant (J = 7.6 Hz) and chemical shift (19.3 ppm) of the 9-methyl group of **5** showed that it possesses 9α-methyl and 10β-azido groups. Similarly, **6** was confirmed to possess 9β-methyl (13.2 ppm) and 10β-azido (J = 4.0 Hz) groups, while **7** was found to contain 9β-methyl (12.9 ppm) and 10α-azido (J = 10.2 Hz) groups. [21,22] The ratio of the diastereomers was confirmed by comparing the H-12 chemical shifts in the ^{1}H NMR spectrum of the crude product (**5**:**6**:**7** = 1:1:3). Then, we attempted to synthesize novel 10-substituted triazolyl artemisinin analogs via the Huisgen cylcoaddition reaction between each of the synthesized 10-azidoartemisinins (**5**, **6**, and **7**) and various terminal alkynes (**a**–**h**) in the presence of copper sulfate (1.1 equiv) and sodium ascorbate (2.8 equiv) at room temperature. First, we attempted to carry out the 1,3-dipolar cycloaddition of the major precursor **7** with various alkynes in a co-solvent system of ethanol and water (1:1);[17,23] however, we could not obtain the desired products, probably because of the low solubility of two starting materials in the co-solvent system and the steric hindrance at the C-10 position of **1**. Hence, in order to identify and set the optimum reaction conditions for obtaining the designed triazolylartemisinin library, we carried out the cycloaddition reaction in several reported co-solvent systems.[24] Among the tested solvent systems, methylene chloride/water system was found to be the most effective, which was used in our subsequent synthesis. Huisgen 1,3-dipolar cylcoaddition carried out in this system afforded 10α-substituted triazolylartemisinins (**10a–h**), albeit in moderate yields. [25] Although this co-solvent system could be successfully used for the 1,3-dipolar cycloaddition reaction of **7**, it was not as effective as expected in the case of **5** and **6**. The 1,3-dipolar cycloaddition reaction of **5** and **6** in methylene chloride/water proceeded only with phenylacetylene (**a**) to afford 9α,10β-phenyl-triazolylartemisinin (**8a**) and 10β-phenyl-triazolylartemisinin (**9a**) in a low yield, respectively, as shown in Scheme 1.[26] Trial reactions of **5**, and **6** in several other co-solvent systems were unsuccessful.[24] The three-dimensional structures models of **5**, **6**, and **7** showed that the azido groups in **5** and **6** are in the axial position in the slightly distorted cyclohexane ring of artemisinin, whereas those in **7** are in the equatorial position. Hence, we concluded that **7** readily underwent cycloaddition because its equatorial azido groups were sterically less hindered than those in **5** and **6**, and thus, they could be easily attacked the acetylene compound and Cu(I) catalyst.

Table 1
Proliferation inhibition assay against various cancer cell lines

	Growth inhibition concentration against cancer cells (GI50[a], μM)					
	DLD-1	U87	Hela	SiHa	A172	B16
5	>50	11.23	>50	9.07	16.81	>50
6	9.44	1.97	0.49	1.13	2.31	>50
7	>50	1.06	1.93	1.47	0.89	1.56
8a	29.67	2.86	0.72	3.22	2.50	8.78
9a	0.52	0.23	0.13	0.26	0.22	0.30
10a	12.10	0.43	0.21	0.54	0.45	1.25
10b	0.08	0.11	0.05	0.15	0.33	0.24
10c	0.14	0.34	0.60	0.35	0.42	0.43
10d	14.22	0.16	0.08	0.15	0.37	0.06
10e	23.01	0.83	0.31	1.13	1.31	1.32
10f	0.03	0.08	0.03	0.09	0.10	0.08
10g	6.68	1.22	0.56	0.35	1.21	0.90
10h	22.92	0.14	0.14	0.20	0.29	0.16
Taxol	0.01	0.02	0.02	0.03	0.01	0.01

[a] GI_{50} values were calculated from nonlinear regression using GraphPad Prism software. ($R^2 > 0.95$).

In order to evaluate the cytotoxicity of the synthetic 10-substituted triazolylartemisinin library, we tested the cell proliferation inhibitory activity of these derivatives against cancer cell lines such as DLD-1, U-87, Hela, SiHa, A172, and B16[27] using the MTT colorimetric method.[28] These results are summarized in Table 1. To begin with, we found that the inhibitory effects of **5**, **6**, and **7** (at micromolar concentrations) differed with the type of cancer cell line, but their antiproliferation effects against cancer cells increased far when considering that the anticancer activity of artemisinin and dihydroartemisinin is quite weak.[29,30] Although the mechanism underlying the antiproliferative effect was not clear, we could improve their biological properties by introducing a nitrogen atom at the C-10 position. As expected, via the Huisgen 1,3-dipolar cylcoaddition reaction, we could successfully synthesize 10-substituted triazolylartemisinins with an additional binding group at C-10 and remarkably improved cytotoxicity. The majority of the 10α-substituted triazolylartemisinins (**10a–h**) synthesized in this study exhibited a strong growth inhibition effect on cancer cell growth even at submicromolar concentrations. In particular, the cytotoxicity of **10f**, which has a pentylbenzene group, was found to be comparable to that of taxol, positive control drug. We could not confirm the structure–activity relationship from the results of this study because we were unable establish a complete library containing every possible diastereomer set of the derivatives obtained from **5**, **6**, and **7**; nevertheless, we can state that triazolyl artemisinins will be promising candidates for anticancer agents.

In conclusion, in vitro screening of 10-substituted triazolylartemisinins (synthesized by the Huisgen 1,3-dipolar cylcoaddition of diastereomeric 10-azidoartemisinin with various acetylenes) for their proliferation inhibitory effect revealed the strong anticancer activity of some of these derivatives. Further research is currently underway for establishing a complete library that includes all possible diastereomers synthesized from **5** and **6**.

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22. *Spectral data for* ***5***: ^{1}H NMR(300 MHz, $CDCl_3$) δ 5.51(1H, s, H-12), 5.30(1H, d, J = 7.6 Hz, H-10), 2.31(1H, m), 2.03(1H, td, J = 14.6, 4.0 Hz), 2.05(1H, m), 1.44(3H, s, 3-CH_3), 0.96(3H, d, J = 6.2 Hz, 9-CH_3), 0.90(3H, d, J = 6.4 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 103.0, 90.3, 89.9, 81.8, 51.5, 46.6, 40.6, 37.3, 36.4, 34.1, 31.5, 25.8, 24.7, 19.9, 19.3 ppm; for **6**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 5.54(1H, s, H-12), 5.37(1H, d, J = 4.0 Hz, H-10), 2.72(1H, m), 2.37(1H, td, J = 14.6, 4.1 Hz), 1.92(1H, m), 1.43(3H, s, 3-CH_3), 1.16(3H, d, J = 7.1 Hz, 9-CH_3), 0.96(3H, d, J = 5.8 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 104.5, 91.8, 88.6, 80.7, 52.5, 44.1, 37.3, 36.3, 34.5, 30.2, 26.0, 24.6, 23.5, 20.3, 13.2 ppm; for **7**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 5.39(1H, s, H-12), 4.61(1H, d, J = 10.2 Hz, H-10), 2.42(1H, m), 2.37(1H, m), 2.04(1H, m), 1.45(3H, s, 3-CH_3), 0.97(3H, d, J = 5.9 Hz, 9-CH_3), 0.92(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 104.5, 91.7, 87.7, 80.0, 51.6, 45.2, 37.3, 36.1, 34.0, 32.5, 25.9, 24.7, 21.7, 20.2, 12.9 ppm.
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25. Spectral data for **10a**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 8.10(1H, s, triazol), 7.88(2H, d, J = 7.0 Hz, phenyl), 7.44(2H, t, J = 7.1 Hz, phenyl), 7.34(H, t, J = 7.3 Hz, phenyl), 5.95(1H, d, J = 10.7 Hz, H-10), 5.58(1H, s, H-12), 2.87(1H, m), 2.44(1H, td, J = 14.5, 3.8 Hz), 2.08(1H, m), 1.66(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.70(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 148.4, 130.5, 128.8, 128.2, 125.8, 117.1, 104.8, 92.1, 85.9, 78.0, 51.5, 45.5, 37.3, 36.1, 33.9, 33.8, 25.8, 24.6, 21.5, 20.1, 12.3 ppm; **10b**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 8.05(1H, s, triazol), 7.77(2H, d, J = 8.1 Hz, phenyl), 7.24(2H, d, J = 7.9 Hz, phenyl), 5.93(1H, d, J = 10.7 Hz, H-10), 5.58(1H, s, H-12), 2.87(1H, m), 2.44(1H, td, J = 14.4, 3.8 Hz), 2.38(3H, s, toluyl), 2.10(1H, m), 1.60(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.71(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 138.0, 129.5, 127.8, 125.7, 116.8, 104.8, 92.1, 85.9, 78.0, 51.5, 45.5, 37.4, 36.1, 34.0, 33.9, 25.8, 24.6, 21.6, 21.3, 20.1, 12.3 ppm; **10c**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 8.06(1H, s, triazol), 7.85(2H, m, phenyl), 7.13(2H, t, J = 8.8 Hz, phenyl), 5.94(1H, d, J = 10.7 Hz, H-10), 5.58(1H, s, H-12), 2.86(1H, m), 2.44(1H, td, J = 14.5, 3.8 Hz), 2.08(1H, m), 1.45(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9Hz, 9-CH_3), 0.71(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 161.1, 147.5, 127.6, 127.5, 116.9, 115.9, 115.6, 104.8, 92.1, 85.9, 79.9, 51.5, 45.4, 37.3, 36.1, 33.9, 29.7, 25.7, 24.6, 21.5, 20.1, 12.3 ppm; **10d**: ^{1}H NMR(300MHz, $CDCl_3$) δ 8.11(1H, s, triazol), 7.89(1H, t, J = 1.7 Hz, phenyl), 7.77(1H, dt, J = 7.4, 1.5Hz, phenyl), 7.37(1H, t, J = 7.9 Hz, phenyl), 7.31(1H, t, J = 1.5 Hz, phenyl), 5.95(1H, d, J = 10.8 Hz, H-10), 5.58(1H, s, H-12), 2.86(1H, m), 2.44(1H, td, J = 14.5, 3.8 Hz), 2.08(1H, m), 1.45(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.70(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 147.1, 134.8, 132.3, 130.1, 128.2, 125.9, 123.9, 117.6, 104.9, 92.2, 86.0, 79.9, 51.5, 45.6, 37.3, 36.1, 34.0, 33.9, 25.8, 24.6, 21.5, 20.2, 12.3 ppm; **10e**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 7.80(1H, s, triazol), 5.90(1H, d, J = 10.7 Hz, H-10), 5.77(1H, broad s, vinyl), 5.56(1H, s, H-12), 5.12(1H, broad s, vinyl), 2.82(1H, m), 2.43(1H, td, J = 14.6, 3.8 Hz), 2.16(3H, s, triazol-CH_3), 2.04(1H, m), 1.44(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.67(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 133.5, 112.8, 104.8, 92.1, 85.8, 80.0, 51.5, 45.5, 37.4, 36.1, 34.0, 33.8, 29.7, 25.8, 24.6, 21.6, 20.7, 20.2, 12.3 ppm; **10f**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 8.05(1H, s, triazol), 7.78(2H, d, J = 8.3 Hz, phenyl), 7.26(2H, d, J = 8.4 Hz, phenyl), 5.93(1H, d, J = 10.6 Hz, H-10), 5.58(1H, s, H-12), 2.86(1H, m), 2.63(2H, t, J = 7.5 Hz, benzyl), 2.44(1H, td, J = 13.2, 3.7 Hz), 2.10(1H, m), 1.48(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.69(3H, d, J = 7.1 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 148.5, 143.1, 128.9, 127.9, 125.7, 116.7, 104.8, 100.5, 92.1, 85.6, 79.9, 51.5, 45.5, 37.3, 36.1, 35.7, 33.9,

33.9, 31.5, 31.1, 25.8, 24.6, 22.5, 21.5, 20.2, 14.0, 12.3 ppm; **10g**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 8.01(1H, s, triazol), 7.80(2H, d, J = 8.8 Hz, phenyl), 6.97(2H, d, J = 8.8 Hz, phenyl), 5.93(1H, d, J = 10.7 Hz, H-10), 5.58(1H, s, H-12), 3.85(3H, s, $-OCH_3$), 2.87(1H, m), 2.44(1H, td, J = 14.3, 3.7 Hz), 2.10(1H, m), 1.45(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.70(3H, d, J = 7.0 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 159.6, 148.3, 127.1, 123.3, 116.3, 114.2, 104.8, 92.1, 85.9, 80.0, 55.3, 51.5, 45.5, 37.3, 36.1, 33.9, 33.8, 25.8, 24.6, 21.5, 20.2, 12.3 ppm; **10h**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 7.97(1H, s, triazol), 7.38(2H, m, phenyl), 6.82(1H, m, phenyl), 5.86(1H, d, J = 10.6 Hz, H-10), 5.59(1H, s, H-12), 2.86(1H, m), 2.44(1H, td, J = 14.5, 3.4 Hz), 2.10(1H, m), 1.46(3H, s, 3-CH_3), 1.01(3H, d, J = 5.9 Hz, 9-CH_3), 0.71(3H, d, J = 6.8 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 161.8161.7, 146.2, 133.3, 132.4, 118.1, 108.8, 105.0, 103.9, 92.1, 86.1, 79.9, 51.4, 45.4, 37.6, 36.1, 34.0, 33.9, 25.8, 24.6, 21.5, 20.2, 12.3 ppm.

26. Spectral data for **8a**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 7.96(1H, s, triazol), 7.85(2H, d, J = 7.2 Hz, phenyl), 7.44(2H, t, J = 7.0 Hz, phenyl), 7.33(H, t, J = 7.1 Hz, phenyl), 6.47(1H, d, J = 10.0 Hz, H-10), 5.63(1H, s, H-12), 2.37(1H, m), 1.46(3H, s, 3-CH_3), 1.07(3H, d, J = 6.8 Hz, 9-CH_3), 1.01(3H, d, J = 5.4 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 148.4, 130.6, 128.8, 128.1, 125.8, 118.5, 102.9, 91.0, 86.5, 82.3, 51.1, 47.1, 41.8, 37.3, 36.2, 33.9, 31.8, 25.7, 24.7, 19.8, 18.4 ppm; **9a**: ^{1}H NMR(300 MHz, $CDCl_3$) δ 7.84(1H, s, triazol), 7.78(2H, d, J = 7.0 Hz, phenyl), 7.44(2H, t, J = 7.1 Hz, phenyl), 7.36(H, t, J = 7.3 Hz, phenyl), 6.48(1H, s, H-12), 6.29(1H, d, J = 5.7 Hz, H-10), 3.20(1H, m), 2.43(1H, td, J = 14.5, 4.0 Hz), 2.10(1H, m), 1.48(3H, s, 3-CH_3), 0.99(3H, d, J = 6.2 Hz, 9-CH_3), 0.92(3H, d, J = 7.5 Hz, 6-CH_3)ppm; ^{13}C NMR(75 MHz, $CDCl_3$) δ 147.1, 130.3, 128.6, 128.4, 125.9, 104.3, 91.3, 90.5, 80.8, 52.6, 43.7, 37.2, 36.2, 34.4, 30.8, 25.9, 24.6, 22.4, 20.3, 13.2 ppm.
27. DLD-1: human colorectal adenocarcinoma; U87 and A172: human glioma; HeLa, and SiHa: human cervical carcinoma; B16: mouse melanoma.
28. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.; Growth inhibition activity test of artemisinin derivatives was evaluated by MTT assay. Cancer cells were plated in 96-well culture plates at a density of 5×10^3 cells/well in a final volume of 100 μl of DMEM medium containing 10%FBS, preincubated for 4 h, and treated with serial concentrations of artemisinin derivatives for 72 h. After treatment, the cells were incubated for 4 h at 37 °C with a solution of MTT at a concentration of 1 mg/mL. The culture supernatant was aspirated, DMSO (100 μl) was added to dissolve the formed formazan crystals. The plate was read in a microplate spectrophotometer (SpectraMax 250, Molecular Devices, CA, U.S.A.) at 570 nm. Each assay was performed in triplicate. Calculation of GI_{50} was performed by non liner regression analysis from sigmoidal dose–response curve using GraphPad Prism software ver 3.0 (GraphPad Software, CA, U.S.A.) when $R^2 > 0.95$.
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