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# Acid-catalyzed synthesis of 10-substituted triazolyl artemisinins and their growth inhibitory activity against various cancer cells

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ABSTRACT

A diastereomeric and regioisomeric library of 10-substituted triazolyl artemisinin compounds (**6a–6h**, **7a–7h**, and **8a–8h**) with a potent growth inhibitory activities against various cancer cell lines was established. These compounds were synthesized by a reaction with dihydroartemisinin (**2**) and various substituted triazoles (**5a–5h**) in methylene chloride using a $BF_3Et_2O$ catalyst. Most of the compounds exhibited a strong potency in the submicromolar range, and, in particular, **6f**, **7f**, and **8f**, which have a pentylphenyltriazole moiety, proved to be promising candidates for preclinical trials.



Since 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 artemether, arteether, artesuic acid, and artelinic acid synthesized from dihydroartemisinin (**2**) are now being used in clinical treatments due to their therapeutic efficacy and non-toxicity.[2–5] Although **2** has been proven to exhibit neurotoxicity in an animal model,[6] clinical trials have been carried out to unearth the anticancer, antiviral, immunosuppressive, and antifungal properties of various artemisinin analogs[7].

We have recently reported that 10-substituted triazolyl artemisinins (**4**) synthesized via Cu(I)-catalyzed Huisgen 1,3-cycloaddition of diastereomeric 10-azidoartemisinins (**3**) with various alkynes exhibit strong growth inhibitory activity against cancer cell lines (Fig. 1).[8] Although we have found some promising anticancer drug candidates by Huisgen 1,3-cycloaddition, we could not synthesize all possible diastereomers, only those with a phenyltriazolyl substituent. The three diastereomers obtained with a phenyltriazolyl group showed strong growth inhibitory effects against cancer cell lines. Therefore, development of a novel synthetic method for establishing a possible diastereomeric 10-substituted triazolyl artemisinin library is essential. As mentioned in our recent report,[8] changes in reaction conditions, such as the copper catalyst and solvent system, are not possible; therefore, we altered the synthesis strategy. Instead of Huisgen 1,3-cycloaddition of **3** with alkynes to yield a series of structure **4**, substituted 1,2,3-triazoles are introduced in the C-10 anomeric position of **2** under acidic conditions (Fig. 2). Rapid equilibration of the oxonium intermediate and 1,2,3-triazoles under acidic conditions result in epimerization at the C-9 and C-10 positions of **2** such that it can form possible two diastereomers (**6** and **8**) and one regioisomer (**7**).[9,10] Thus, diastereomeric and regiomeric derivatives of **2** will have the substituted triazol moieties at the C-10 position.

In order to prepare a complete library, 4-substituted-1-*H*-triazoles (**5a–5h**) were synthesized via Huisgen 1,3-cycloaddition of various alkynes and azidotrimethylsilane ($TMSN_3$) (1.5 equiv) with 5 mol% of CuI in a mixture of DMF and methanol (5:1) shown in Scheme 1.[11,12] Acid ($BF_3Et_2O$, 0.8 equiv)-catalyzed reactions with **2** and 4-substituted-1-*H*-triazoles (**5a–5h**) in methylene chloride resulted in a complex mixture of diastereomers and regioisomers, which included 9-*epi*-10β-(4-substituted triazolyl) artemisinin (**6a–6h**), 10α-(5-substituted triazolyl) artemisinin (**7a–7h**), and 10α-(4-substituted triazolyl) artemisinin (**8a–8h**) (Scheme 1). Contrary to our expectation that epimerization under acidic condition would result in the formation of the diastereomer 4-substituted-10β-pyrazolylartemisinin (**9**) along with the diastereomers **6**, **7**, and **8**, diastereomer **9** was not found in the mixture of products. In order to synthesize **9**, we replaced the Lewis acid $BF_3Et_2O$ with other acids, such as $SnCl_4$, *p*-toluenesulfonic acid, $TiCl_4$, and $AlCl_3$,

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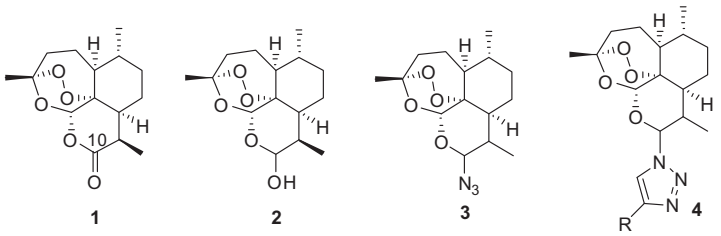


**Figure 1.** Artemisinin and its derivatives.

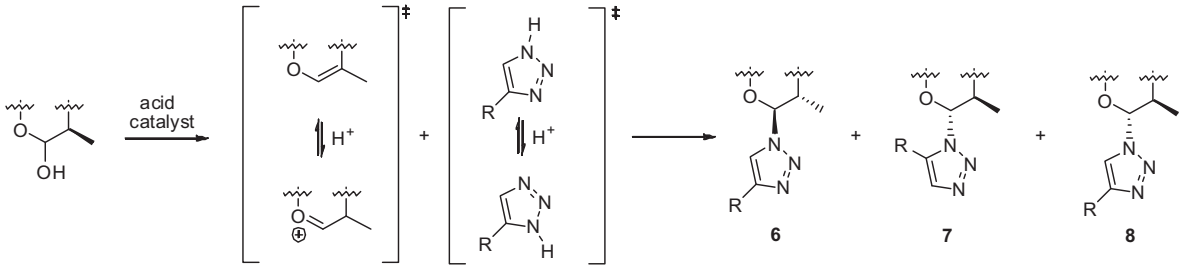


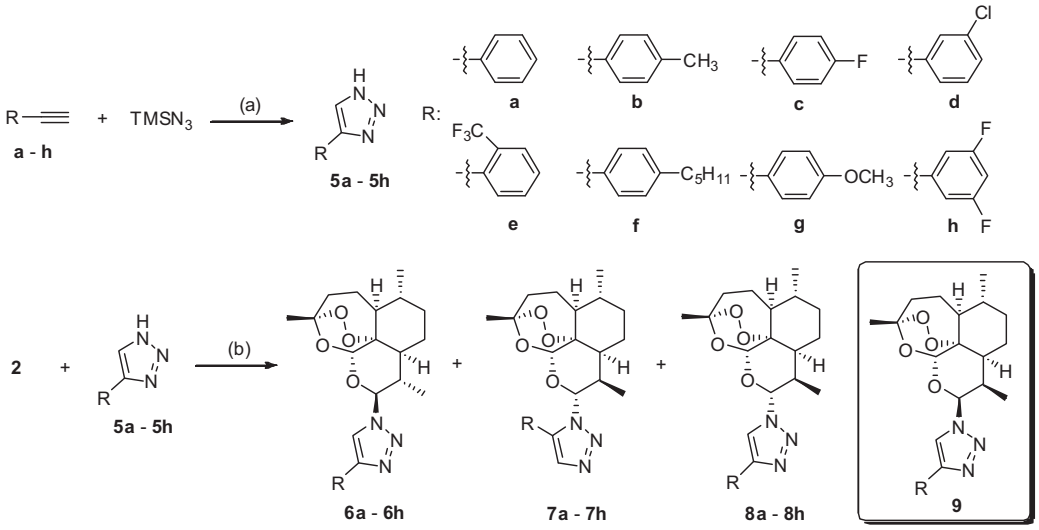
**Figure 2.** Addition of a 1,2,3-triazolyl substituent to the anomeric position of dihydroartemisinin (**2**).

but these attempts were unsuccessful. We confirmed the stereochemistry according to each diastereomeric type on the basis of the H-9/H-10 coupling constants obtained by $^1$H nuclear magnetic resonance (NMR) and the chemical shifts of the 9-methyl group in the $^{13}$C NMR spectra of **6a**, **7a**, and **8a**.[13] **6a** and **8a** were determined to be the same compounds which was synthesized by direct Cu(I)-catalyzed Huisgen 1,3-cycloaddition of 10-azidoartemisinin (**3**) and phenylacetylene (**a**) in our previous report.[8] When the $^1$H NMR and $^{13}$C NMR spectra of **7a** was compared with those of **6a** and **8a**, we found that compound **7a** was the only unknown product synthesized via the new synthetic method.[14] The H-9/H-10 coupling constant ($J$ = 10.7 Hz) and chemical shift (12.2 ppm in $^{13}$C NMR) of the 9-methyl group of **7a** showed that **7a**, like **8a**, possesses 9β-methyl and 10α-triazolyl groups. However we finally differentiated these two compounds by comparing the nuclear Overhauser effect (NOE) between H-9 and the triazole methine proton. In the 1D-NOE experiment, **7a** did not exhibit an NOE while **8a** did; thus, we concluded that **7a** is a 1,5-disubstituted triazole and **8a** is a 1,4-disubstituted triazole. These two compounds (**7a** and **8a**) share a regioisomeric relationship as shown in Scheme 1. On the basis of the stereo- and regio-chemistry of the three products, we summarized the yields and ratios for each product in Table 1.

**Table 1**
Yields and ratios of 10-substituted triazolyl artemisinins synthesized via an acid-catalyzed reaction

| | Yield[a] (%) | Ratio[b] | | | | Yield[a] (%) | Ratio[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **6** | **7** | **8** | | | **6** | **7** | **8** |
| **a** | 79 | 4.3 | 7.1 | 1 | **e** | 80 | 1 | 5.6 | 3.2 |
| **b** | 65 | 2.1 | 5.2 | 1 | **f** | 64 | 3.6 | 4.3 | 1 |
| **c** | 78 | 1 | 4.3 | 2.4 | **g** | 67 | 2.1 | 5.6 | 1 |
| **d** | 87 | 1 | 6.7 | 2.4 | **h** | 75 | 2.4 | 7.8 | 1 |

[a] Isolated yield.
[b] Ratios of each product were calculated by integrating the H-9 peak in the $^1$H NMR of non-purified product mixtures.



**Scheme 1.** Reagents and conditions: (a) azidotrimethylsilane (1.5 equiv), CuI (5 mol%), DMF/MeOH = 5:1, reflux, 24 h. Isolated yields: **5a**, 85%; **5b**, 66%; **5c**, 89%; **5d**, 75%; **5e**, 61%; **5f**, 91%; **5g**, 70%; **5h**, 79%; (b) triazole (**5a**–**5h**, 1 equiv), $BF_3Et_2O$ (0.8 equiv) methylene chloride, rt, 24 h.

**Table 2**
Growth inhibition assay with various cancer cell lines

| | | Growth inhibitory concentration of the derivatives against cancer cells ($GI_{50}$[a], μM) | | | | | |
|---|---|---|---|---|---|---|---|
| | | DLD-1 | U-87 | HeLa | SiHa | A172 | B16 |
| **a** | **6** | 0.28 | 0.15 | 0.03 | 0.14 | 0.11 | 0.20 |
| | **7** | 0.60 | 0.20 | 0.04 | 0.14 | 0.15 | 0.22 |
| | **8** | 0.93 | 0.50 | 0.58 | 0.73 | 0.57 | 0.78 |
| **b** | **6** | 0.16 | 1.34 | 0.73 | 1.58 | 1.74 | 0.70 |
| | **7** | 0.29 | 0.11 | 0.04 | 0.19 | 0.10 | 0.11 |
| | **8** | 0.40 | 0.28 | 0.11 | 0.12 | 0.09 | 0.12 |
| **c** | **6** | 2.15 | 0.63 | 0.41 | 0.96 | 0.56 | 0.84 |
| | **7** | 0.31 | 0.09 | 0.06 | 0.08 | 0.08 | 0.77 |
| | **8** | 0.48 | 0.16 | 0.05 | 0.14 | 0.13 | 0.11 |
| **d** | **6** | 1.17 | 0.35 | 0.21 | 0.41 | 0.33 | 0.28 |
| | **7** | 0.04 | 0.10 | 0.06 | 0.16 | 0.13 | 0.10 |
| | **8** | 0.29 | 0.25 | 0.09 | 0.24 | 0.31 | 0.21 |
| **e** | **6** | 1.07 | 0.53 | 0.35 | 0.62 | 0.40 | 0.48 |
| | **7** | 0.17 | 0.08 | 0.07 | 0.08 | 0.08 | 0.08 |
| | **8** | 2.38 | 0.29 | 0.19 | 0.32 | 0.29 | 0.34 |
| **f** | **6** | 0.84 | 0.27 | 0.24 | 0.44 | 0.26 | 0.34 |
| | **7** | 0.22 | 0.09 | 0.04 | 0.10 | 010 | 0.08 |
| | **8** | 0.14 | 0.07 | 0.09 | 0.08 | 0.07 | 0.07 |
| **g** | **6** | 0.22 | 0.07 | 0.10 | 0.07 | 0.07 | 0.10 |
| | **7** | 0.88 | 0.23 | 0.09 | 0.24 | 0.21 | 0.29 |
| | **8** | 0.49 | 0.39 | 0.13 | 0.36 | 0.30 | 0.13 |
| **h** | **6** | 1.28 | 0.45 | 0.24 | 0.46 | 0.54 | 0.70 |
| | **7** | 0.17 | 0.17 | 0.08 | 0.07 | 0.10 | 0.10 |
| | **8** | 0.30 | 0.13 | 0.05 | 0.12 | 0.14 | 0.13 |
| Taxol | | 0.01 | 0.02 | 0.02 | 0.03 | 0.01 | 0.01 |

[a] $GI_{50}$ values were calculated by non-linear regression analysis using the GraphPad Prism software ($R^2 > 0.95$).

ble 1. In general, the yield of 1,5-disubstituted triazole type artemisinins (**7**) is higher than that of the others (**6** and **8**).[15]

In order to evaluate the growth inhibitory effect of the members of the synthetic 10-substituted triazolyl artemisinin library, we examined the effect of these derivatives against cancer cell lines such as DLD-1, U-87, HeLa, SiHa, A172, and B16[16] using the MTT colorimetric method.[17] These results are summarized in Table 2.

The growth inhibitory activity of the compounds (**6a**, **8a–8d**, and **8f–8h**) reported in our previous manuscript were re-evaluated in the same batch system used for obtaining the novel synthetic artemisinin library. Although there were some unintentional deviations in the DLD-1 cell lines, most of the antiproliferation activities of the previous compounds observed in this test were in accordance with those observed in previous screening test results.[8] The two newly obtained artemisinin group, namely, 9-*epi*-10β-(4-substituted triazolyl) artemisinins (**6a–6h**) and 10α-(5-substituted triazolyl) artemisinins (**7a–7h**), showed a similar potency as 10α-(4-substituted triazolyl) artemisinin (**8a–8h**) at submicromolar concentrations. Based on this preliminary structure–activity relationship, there were a little differences of growth inhibitory effect according to the stereo- and regio-chemistry. As far as we are concerned, the 10α-(5-substituted triazolyl) artemisinin group (**7a–7h**) is more or less as potent as the other groups (**6** and **8**). Although at this stage we cannot comment on the activity difference with changes in functional group, three compounds, namely, **6f**, **7f**, and **8f**, with a pentylbenzene group were found to be the most active anticancer molecules.

In conclusion, we have established a 10-substituted triazolyl artemisinins library (**6a–6h**, **7a–7h**, and **8a–8h**) comprising compounds that strongly inhibit the growth of cancer cell lines, such as DLD-1, U-87, HeLa, SiHa, A172, and B16. These compounds could be synthesized by an acid-catalyzed reaction with **2** and various substituted triazoles (**5a–5h**) in methylene chloride at room temperature. Compounds **6f**, **7f**, and **8f**, which have a pentylphenyltriazole moiety, exhibit potent activity; therefore, these compounds will be analyzed in preclinical trials to evaluate their in vivo anticancer activity and as well as toxicity.

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## References and notes

1. Klayman, D. L. *Science* **1985**, *228*, 1049.
2. Brewer, T. G.; Peggins, J. O.; Grate, S. J.; Petras, J. M.; Levine, B. S.; Weina, P. J.; Swearengen, J.; Heiffer, M. H. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, 33.
3. Lin, A. J.; Lee, M.; Klayman, D. L. *J. Med. Chem.* **1989**, *32*, 1249.
4. Lin, A. J.; Klayman, D. L.; Milhous, W. K. *J. Med. Chem.* **1987**, *30*, 2147.
5. Lin, A. J.; Miller, R. E. *J. Med. Chem.* **1995**, *38*, 764.
6. Genovese, R. F.; Newman, D. B.; Brewer, T. G. *Pharmacol. Biochem. Behav.* **2000**, *67*, 37.
7. Lee, S. *Mini-Rev. Med. Chem.* **2007**, *7*, 411.
8. Cho, S.; Oh, S.; Um, Y.; Jung, J.-H.; Ham, J.; Shin, W. S.; Lee, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 382.
9. Chorki, F.; Crousse, B.; Bonnet-Deipon, D.; Bégué, J. P.; Brigaud, T.; Portella, C. *Tetrahedron Lett.* **2001**, *42*, 1487.
10. Chorki, F.; Grellepois, F.; Crousse, B.; Ourévitch, M.; Bonnet-Deipon, D.; Bégué, J. P. *J. Org. Chem.* **2001**, *66*, 7858.
11. Jin, T.; Kamijo, S.; Yamamoto, Y. *Eur. J. Org. Chem.* **2004**, 3789.
12. The procedure for the synthesis of 4-phenyl-1-*H*-1,2,3-triazole (**5a**): Ethynylbenzene (500 mg, 4.9 mmol) and azidotrimethylsilane (847 mg, 7.4 mmol) were added to a solution of DMF and MeOH (6 mL, 5:1) under $N_2$ atmosphere. After adding the CuI (46.7 mg, 0.25 mmol), the reaction mixture was refluxed for 12 h, which was monitored by TLC, and then quenched with water (10 mL). The mixture was extracted with dichloromethane (50 mL, two times), dried with $MgSO_4$, and then evaporated under reduced pressure. The residue was purified on silica-gel chromatography with ethyl acetate/hexane (5:2) to afford **5a**.
13. Lee, S.; Oh, S. *Tetrahedron Lett.* **2002**, *43*, 2891.
14. The procedure for the synthesis of 10-(phenyltriazolyl) artemisinin (**6a**, **7a**, and **8a**): To a solution of dihydroartemisinin (**2**, 1.0 g, 3.5 mmol) and 4-phenyl-1*H*-1,2,3-triazole (**a**, 0.51 g, 3.5 mmol) in dichloromethane (85 mL) was added $BF_3Et_2O$ (0.40 g, 2.8 mmol) at room temperature, and then stirred for 12 h. If starting materials were disappeared on TLC, the reaction mixture was diluted with water (100 mL), and extracted with dichloromethane (50 mL, three times) dried with $MgSO_4$, and then evaporated under reduced pressure. The residue

was purified on silica-gel chromatography with ethyl acetate/hexane (5:2) to afford **6a**, **7a**, and **8a**. The spectral data of **6a** and **8a** were previously reported in Ref. 8. **7a**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.95 (1H, s, triazole), 7.82 (2H, d, $J$ = 6.9 Hz, phenyl), 7.42 (2H, d, $J$ = 7.3 Hz, phenyl), 7.37 (1H, t, $J$ = 7.3 Hz, phenyl), 5.80 (1H, d, $J$ = 10.7 Hz, H-10), 5.57 (1H, s, H-12), 3.42 (1H, m), 2.42 (1H, td, $J$ = 14.6, 4.1 Hz), 2.06 (1H, m), 1.42 (3H, s, 3-$CH_3$), 1.00 (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, $CDCl_3$) $\delta$ 148.0, 132.2, 128.8, 128.5, 126.1, 104.6, 92.2, 88.6, 79.8, 51.6, 45.4, 37.3, 36.2, 34.0, 31.6, 25.8, 21.7, 20.2, 12.2 ppm.

15. Spectral data for **6b**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.91 (1H, s, triazole), 7.72 (2H, d, $J$ = 8.07 Hz, phenyl), 7.23 (2H, d, $J$ = 8.61 Hz, phenyl), 6.49 (1H, d, $J$ = 10.1 Hz, H-10), 5.65 (1H, s, H-12), 3.40 (1H, m), 2.58 (1H, td, $J$ = 10.1, 2.0 Hz), 2.38 (3H, s, tosyl), 1.49 (3H, s, 3-$CH_3$), 0.99 (3H, d, $J$ = 7.0 Hz, 9-$CH_3$), 0.73 (3H, d, $J$ = 7.0 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 148.5, 131.8, 129.5, 127.9, 104.6, 91.1, 82.4, 51.2, 47.1, 45.4, 39.9, 37.3, 36.3, 36.2, 25.9, 25.8, 24.7, 24.6, 21.7, 12.4 ppm; **7b**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (1H, s, triazole), 7.70 (2H, d, $J$ = 8.0 Hz, phenyl), 7.23 (2H, d, $J$ = 7.87 Hz, phenyl), 5.78 (1H, d, $J$ = 10.6 Hz, H-10), 5.57 (1H, s, H-12), 3.41 (1H, m), 2.38 (3H, s, tosyl), 1.41 (3H, s, 3-$CH_3$), 1.00 (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, $CDCl_3$) $\delta$ 148.1, 138.5, 132.2, 129.5, 126.1, 104.6, 92.9, 91.0, 88.5, 79.8, 51.7, 45.4, 37.4, 36.2, 34.1, 31.8, 25.9, 21.3, 20.2, 18.4, 12.3 ppm; **6c**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.89 (1H, s, triazole), 7.81 (2H, m, phenyl), 7.12 (2H, t, $J$ = 7.87 Hz, phenyl), 6.49 (1H, d, $J$ = 10.26 Hz, H-10), 5.64 (1H, s, H-12), 2.57 (1H, m), 2.37 (1H, m), 1.51 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.9 Hz, 9-$CH_3$), 0.94 (3H, d, $J$ = 7.1 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 171.2, 131.7, 128.1, 128.0, 115.9, 115.7, 91.1, 89.6, 82.4, 60.4, 51.2, 47.1, 39.9, 37.3, 36.3, 34.0, 31.7, 25.8, 24.7, 21.1, 19.9, 18.2, 16.7, 15.3, 14.2 ppm; **7c**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.92 (1H, s, triazole), 7.74 (2H, m, phenyl), 7.12 (2H, t, $J$ = 8.97 Hz, phenyl), 6.46 (1H, d, $J$ = 9.9 Hz, H-10), 5.76 (1H, s, H-12), 3.41 (1H, m), 1.41 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.85 Hz, 9-$CH_3$), 0.93 (3H, d, $J$ = 5.67 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 172.8, 132.0, 127.9, 127.6, 115.9, 115.6, 109.5, 104.6, 102.9, 99.9, 91.1, 86.6, 82.2, 79.8, 44.0, 42.2, 39.6, 37.3, 35.5, 34.1, 33.7, 31.2, 24.2, 22.1, 20.2, 18.7, 12.3 ppm; **6d**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (1H, s, triazole), 7.70 (1H, d, $J$ = 6.75 Hz, phenyl), 7.36 (1H, t, $J$ = 8.04 Hz, phenyl), 7.33 (1H, d, $J$ = 3.3 Hz, phenyl), 6.50 (1H, d, $J$ = 10.23 Hz, H-10), 5.65 (1H, s, H-12), 2.57 (1H, m), 2.38 (1H, td, $J$ = 12.99, 4.02 Hz), 1.49 (3H, s, 3-$CH_3$), 0.99 (3H, d, $J$ = 5.67 Hz, 9-$CH_3$), 0.98 (3H, d, $J$ = 6.93 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 147.1, 134.8, 132.0, 130.1, 128.6, 126.3, 124.3, 102.9, 91.9, 89.7, 82.4, 60.4, 51.2, 47.1, 39.9, 37.3, 36.3, 34.0, 31.7, 25.8, 24.7, 21.1, 19.8, 18.2, 14.2 ppm; **7d**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.83 (1H, s, triazole), 7.68 (1H, d, $J$ = 6.96 Hz, phenyl), 7.36 (1H, t, $J$ = 8.07 Hz, phenyl), 7.33 (1H, d, $J$ = 3.3 Hz, phenyl), 5.79 (1H, d, $J$ = 10.62 Hz, H-10), 5.58 (1H, s, H-12), 3.41 (1H, m), 2.43 (1H, td, $J$ = 13.92, 3.84 Hz), 2.05 (1H, m), 1.42 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.85 Hz, 9-$CH_3$), 0.69 (3H, d, $J$ = 9.84 Hz, 6-$CH_3$) ppm; $^{13}$C NMR(75 MHz, $CDCl_3$) $\delta$ 146.8, 134.8, 132.3, 132.0, 130.1, 128.6, 126.2, 124.2, 104.7, 92.3, 88.7, 79.8, 51.6, 45.4, 37.4, 36.2, 34.0, 31.6, 25.9, 24.6, 21.8, 20.2, 12.3 ppm; **6e**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85 (1H, s, triazole), 7.77 (1H, d, $J$ = 7.68 Hz, phenyl), 7.73 (1H, d, $J$ = 7.5 Hz, phenyl), 7.63 (1H, t, $J$ = 7.5 Hz, phenyl), 6.52 (1H, d, $J$ = 10.26 Hz, H-10), 5.66 (1H, s, H-12), 2.54 (1H, m), 2.37 (1H, m), 1.48 (3H, s, 3-$CH_3$), 0.99 (3H, d, $J$ = 4.95 Hz, 9-$CH_3$), 0.97 (3H, d, $J$ = 6.93 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 145.6, 134.7, 132.1, 128.7, 102.9, 91.2, 89.7, 82.4, 51.2, 47.0, 40.3, 37.3, 36.3, 34.0, 31.7, 25.8, 24.7, 19.8, 18.0, 14.2; **7e**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.86 (1H, s, triazole), 7.77 (1H, d, $J$ = 7.68 Hz, phenyl), 7.71 (1H, d, $J$ = 7.68 Hz, phenyl), 7.61 (1H, t, $J$ = 6.78 Hz, phenyl), 7.51 (1H, t, $J$ = 7.35 Hz, phenyl), 5.81 (1H, d, $J$ = 10.62 Hz, H-10), 5.58 (1H, s, H-12), 3.38 (1H, m), 2.25 (1H, td, $J$ = 14.5, 3.84 Hz), 2.07 (1H, m), 1.44 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.88 Hz, 9-$CH_3$), 0.66 (3H, d, $J$ = 7.14 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 145.0, 134.9, 132.1, 128.7, 126.2, 104.7, 92.3, 88.7, 79.8, 51.6, 45.4, 37.4, 36.2, 34.0, 32.0, 25.9, 24.6, 21.7, 20.2, 12.0 ppm; **6f**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.91 (1H, s, triazole), 7.74 (1H, d, $J$ = 8.04 Hz, phenyl), 7.24 (1H, d, $J$ = 8.25 Hz, phenyl), 6.47 (1H, d, $J$ = 10.26 Hz, H-10), 5.65 (1H, s, H-12), 2.63 (1H, t, $J$ = 7.5 Hz, ben-$CH_2$–), 2.57 (1H, m), 2.37 (1H, td, $J$ = 12.99, 3.84 Hz), 1.49 (3H, s, 3-$CH_3$), 0.99 (3H, d, $J$ = 3.84 Hz, 9-$CH_3$), 0.97 (3H, d, $J$ = 6.96 Hz, 6-$CH_3$), 0.89 (3H, t, $J$ = 6.78 Hz, $CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 148.5, 143.5, 131.8, 128.8, 127.6, 126.2, 102.8, 91.1, 89.5, 82.4, 51.2, 47.1, 39.9, 37.3, 36.3, 35.7, 34.0, 31.7, 31.4, 31.1, 25.8, 24.7, 22.5, 19.9, 18.3, 14.0 ppm; **7f**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (1H, s, triazole), 7.72 (1H, d, $J$ = 8.22 Hz, phenyl), 7.24 (1H, d, $J$ = 8.4 Hz, phenyl), 5.78 (1H, d, $J$ = 10.62 Hz, H-10), 5.57 (1H, s, H-12), 3.40 (1H, m), 2.63 (1H, t, $J$ = 7.5 Hz, ben-$CH_2$–), 2.43 (1H, td, $J$ = 12.99, 3.84 Hz), 2.04 (1H, m), 1.42 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.85 Hz, 9-$CH_3$), 0.96 (3H, t, $J$ = 6.96 Hz, $CH_3$), 0.68 (3H, d, $J$ = 6.93 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 148.1, 143.5, 132.2, 128.9, 127.6, 126.1, 104.6, 92.9, 88.5, 79.8, 51.7, 45.4, 37.4, 36.2, 35.7, 34.1, 31.7, 31.5, 31.1, 25.9, 24.7, 22.5, 21.8, 20.2, 14.0, 12.3 ppm; **6g**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.84 (1H, s, triazole), 7.74 (2H, d, $J$ = 8.76 Hz, phenyl), 6.96 (2H, d, $J$ = 8.70 Hz, phenyl), 5.74 (1H, d, $J$ = 11.94 Hz H-10), 5.53 (1H, s, H-12), 3.83 (3H, s, –$OCH_3$), 2.53 (1H, m), 1.49 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.9 Hz, 9-$CH_3$), 0.87 (3H, d, $J$ = 7.1 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 161.2, 148.2, 131.4, 127.0, 114.7, 104.5, 103.6, 101.4, 99.0, 92.1, 81.0, 60.4, 55.3, 52.0, 45.6, 37.1, 37.0, 32.5, 31.1, 26.8, 20.5, 15.7, 14.6 ppm; **7g**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.89 (1H, s, triazole), 7.74 (2H, d, $J$ = 8.79 Hz, phenyl), 6.96 (2H, d, $J$ = 8.76 Hz, phenyl), 5.80 (1H, d, $J$ = 11.91 Hz, H-10), 5.57 (1H, s, H-12), 3.85 (3H, s, –$OCH_3$), 3.40 (1H, m), 1.43 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.88 Hz, 9-$CH_3$), 0.86 (3H, d, $J$ = 7.14 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 160.0, 147.9, 131.8, 127.5, 114.2, 104.6, 103.8, 101.5, 98.7, 90.6, 81.2, 60.4, 55.3, 51.7, 45.4, 37.4, 37.3, 32.9, 31.6, 26.2, 20.3, 14.2, 12.3 ppm; **6h**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93 (2H, s, triazole), 7.37 (1H, d, $J$ = 8.25 Hz, phenyl), 6.80 (1H, s, phenyl), 6.49 (1H, d, $J$ = 10.26 Hz, H-10), 5.65 (1H, s, H-12), 2.55 (1H, m), 2.37 (1H, td, $J$ = 12.99, 4.02 Hz), 1.49 (3H, s, 3-$CH_3$), 1.00 (3H, d, $J$ = 5.67 Hz, 9-$CH_3$), 0.98 (3H, d, $J$ = 6.96 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 171.2, 165.1, 161.6, 132.1, 108.9, 102.9, 91.1, 89.8, 82.4, 60.4, 51.2, 47.1, 39.9, 37.3, 36.3, 34.0, 31.7, 25.8, 19.8, 18.2, 14.2 ppm; **7h**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.94 (1H, s, triazole), 7.35 (2H, d, $J$ = 8.25 Hz, phenyl), 6.80 (1H, s, phenyl), 5.43 (1H, d, $J$ = 11.73 Hz, H-10), 5.58 (1H, s, H-12), 3.4 (1H, m), 2.4 (1H, td, $J$ = 14.46, 3.66 Hz), 1.49 (3H, s, 3-$CH_3$), 1.01 (3H, d, $J$ = 5.88 Hz, 9-$CH_3$), 0.69 (3H, d, $J$ = 7.14 Hz, 6-$CH_3$) ppm; $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 171.2, 165.0, 161.8, 132.4, 109.1, 108.9, 104.7, 99.9, 92.3, 79.8, 60.4, 51.6, 45.3, 42.2, 37.4, 36.2, 34.0, 31.6, 20.2, 18.7, 12.3 ppm.

16. DLD-1: human colorectal adenocarcinoma; U-87 and A172: human glioma; HeLa, and SiHa: human cervical carcinoma; B16: mouse melanoma.

17. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55. Growth inhibitory activity of the artemisinin derivatives was evaluated with the MTT assay. Cancer cells were plated in 96-well culture plates at a density of $5 \times 10^3$ cells/well in a final volume of 100 μL of DMEM 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 and DMSO (100 μL) was added to dissolve the formed formazan crystals. The plate was then read at 570 nm in a microplate spectrophotometer (SpectraMax 250, Molecular Devices, CA, USA). Each assay was performed in triplicate. $GI_{50}$ was calculated by non-linear regression analysis from a sigmoidal dose–response curve using the GraphPad Prism software ver 3.0 (GraphPad Software, CA, USA) when $R^2 > 0$.