Full Paper

Anti-Tumor Activity of New Artemisinin–Chalcone Hybrids

Lijun Xie, Xin Zhai, Chun Liu, Peng Li, Yangxiong Li, Guoxian Guo, and Ping Gong

Key Laboratory of Original New Drugs Design and Discovery of Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, China

In an attempt to develop potent and selective anti-tumor agents, three new series of artemisinin–chalcone hybrids **10a–10g**, **11a–11g** and **12a–12h** were designed, synthesized and screened for their anti-tumor activity against five cell lines (HT-29, A549, MDA-MB-231, HeLa and H460) *in vitro*. Among compounds **10a–g** and **11a–11g**, most of them displayed enhanced activity and good selectivity toward HT-29 and HeLa cell lines with IC_{50} values ranging from 0.12 to 0.85 μM as compared with DHA (dihydroartemisinin). Compounds **10a** and **11a** are most active toward HeLa cells with IC_{50} values of 0.12 and 0.19 μM. The results revealed that the presence of chalcone moiety is beneficial to their activity and selectivity. In addition, compounds **12a-12h** containing a 'reversed chalcone' moiety showed only slight improvement in activity than those of DHA.

Keywords: Anti-tumor activity / Artemisinin / Chalcone / Hybrid

Received: December 17, 2010; Revised: February 19, 2011; Accepted: February 25, 2011

DOI 10.1002/ardp.201000391

Introduction

The adverse effects of traditional chemotherapy and the emergency of resistance with the newly molecularly targeted anti-tumor drugs have shown the urgent need for development of more effective anti-tumor agents [1]. Thus, the search for novel anti-tumor agents that circumvent these limitations has turned to natural products because of their ability to interact with more to one target [2].

Artemisinin, a sesquiterpene endoperoxide isolated from *Artemisia annua* L., is being widely used as an antimalaria drug [3]. In recent years, the modification of artemisinin has been of increasing interest since some derivatives showed good activity against various cancer cell lines [4–10], even many drug- and radiation-resistant cancer cell lines [11–12]. The mechanism of artemisin analogues seem to be multimodal but mainly rely on formation of free radical inducing apoptosis and antiangiogenic effects [13–17]. Chalcones were also extensively studied for their anti-tumor activity [18–22], which mainly results from the inhibition of tubulin polymerization different from those of artemisinin analogues [23–25]. The observations that both the artemisinin and chalcone groups can act as anti-tumor agents via different molecular mechanisms encourage us to design hybrids containing these two moieties within their structures. They may have improved anti-tumor activity and be less susceptible to the development of drug resistance. Interestingly, a similar strategy had already been reported that the anti-tumor potency of the C-10 acetal artemisinin derivatives could be improved significantly [10]. The potential drawback in these derivatives is the presence of the metabolically susceptible C-10 acetal linkage. Intrigued by these findings, to search for more active artemisinin–chalcone hybrids with increased stability, we thought that the linkage type between artemisinin nucleus and chalcone moieties was an interesting point for further modification. In order to explore the influence of chalcone moiety on bioactivity, a 'reversed' chalcone moiety that the carbonyl and ethylene groups present in chalcone moiety are interchanged was also introduced. With these in mind, we have designed and synthesized three series of new artemisinin derivatives (Fig. 1), in which the chalcone and 'reversed chalcone' groups were combined into the artemisinin nucleus through an amide linkage, respectively.

Chemistry

The synthetic routes of compounds are outlined in Scheme 1 and Scheme 2. Generally, a pair of isomers 10β-azidodihy-

Correspondence: Ping Gong, 103 Wenhua Road, Shenhe District, 110016 Shenyang, Liaoning, P. R. China
E-mail: gongpinggp@126.com
Fax: +86 24 2398-6429

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

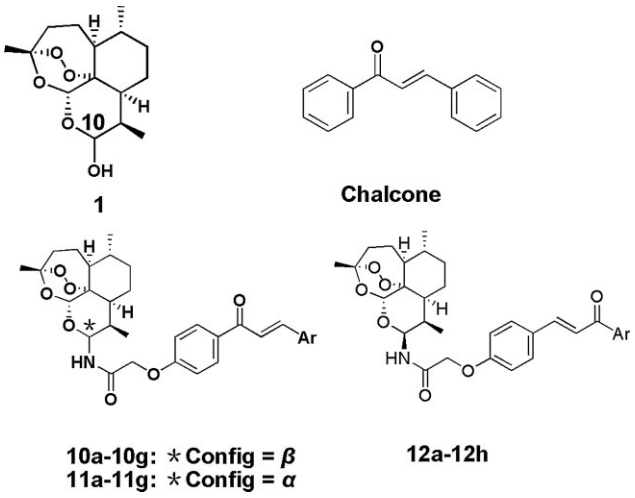
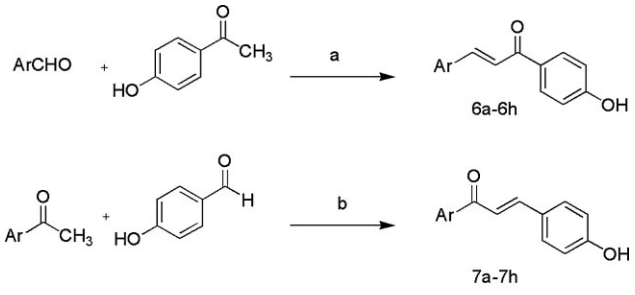


Figure 1. The structures of DHA, chalcone and the target compounds.

droartemisinin (**2**) and 10α-dihydroazidoartemisinin (**3**) was synthesized by treating **1** with trimethylsilyl chloride, sodium azide and sodium iodide at room temperature for 28 h, and individual pure isomer could be separated by column chromatography. The azido compounds (**2**, **3**) were transformed to 10β-aminoartemisinin (**4**) and 10α-aminoartemisinin (**5**) via Staudinger reduction [26], respectively, which subsequently reacted with chloroacetyl chloride to afford two key intermediates (**8**, **9**) as white solid.

On the side chain, the preparation of the chalcone analogues (**6a–6h**, **7a–7h**) was carried out via Claisen-Schmidt condensation [27]. This method for the preparation of chalcones is attractive since it predominantly generates the (*E*)-isomer from simple building blocks, coupling constants (J_{trans} = 15–16 Hz) from the proton nuclear magnetic resonance (^{1}H NMR) spectra of the title compounds indicated clearly that derivatives **6a–6h** and **7a–7h** were both geometrically pure and trans (*E*)-isomers. Thus, appropriate substituted aryl aldehydes were reacted with corresponding inexpensive *p*-hydroxyacetophenone in ethanol/KOH at 0–5°C. Upon completion, the cooled reaction mixture was poured into ice water and treated with HCl (10%) yielded the desired chalcone analogues **6a–6h**. The foregoing method for the preparation of the **6a-6h** was applied to prepare compounds **7a–7h** with substituted acetophenone and *p*-hydroxybenzaldehyde.



Reagents and conditions: (a, b) KOH, ethanol, r.t., 24 h.

Scheme 1. Synthesis of **6a–6h** and **7a–7h**.

Finally, the target compounds **10a–10g**, **11a–11g** and **12a–12h** were successfully prepared via reaction of intermediates **8** and **9** with **6a–6h** and **8** with**7a–7h** in the presence of K_2CO_3 and sodium iodide in DMF at 60°C, respectively. The products were purified by column chromatography on silica gel.

The formation of 10β-azidoartemisinin (**2**) and 10α-azidoartemisinin (**3**) were confirmed by ^{1}H-NMR and LC-MS data. In our previous report, the stereochemistry of **2** had been determined by single crystal X-ray diffraction studies [28]. The structure of **3** was elucidated by ^{1}H-NMR and LC-MS analysis. Specifically, the configuration of **3** at the C-10 position was assigned as 10α based on the H-9/H-10 coupling constant (J = 10.1 Hz) and chemical shift H-10 (4.86 ppm).

The formation of two important intermediates (**8**, **9**) was confirmed by ^{1}H-NMR and LC-MS data, The formation of the title compounds **10a–10g**, **11a–11g** and **12a–12h** were evidenced by ^{1}H-NMR spectral, IR, LC-MS and element analysis data, which were explained in experimental part.

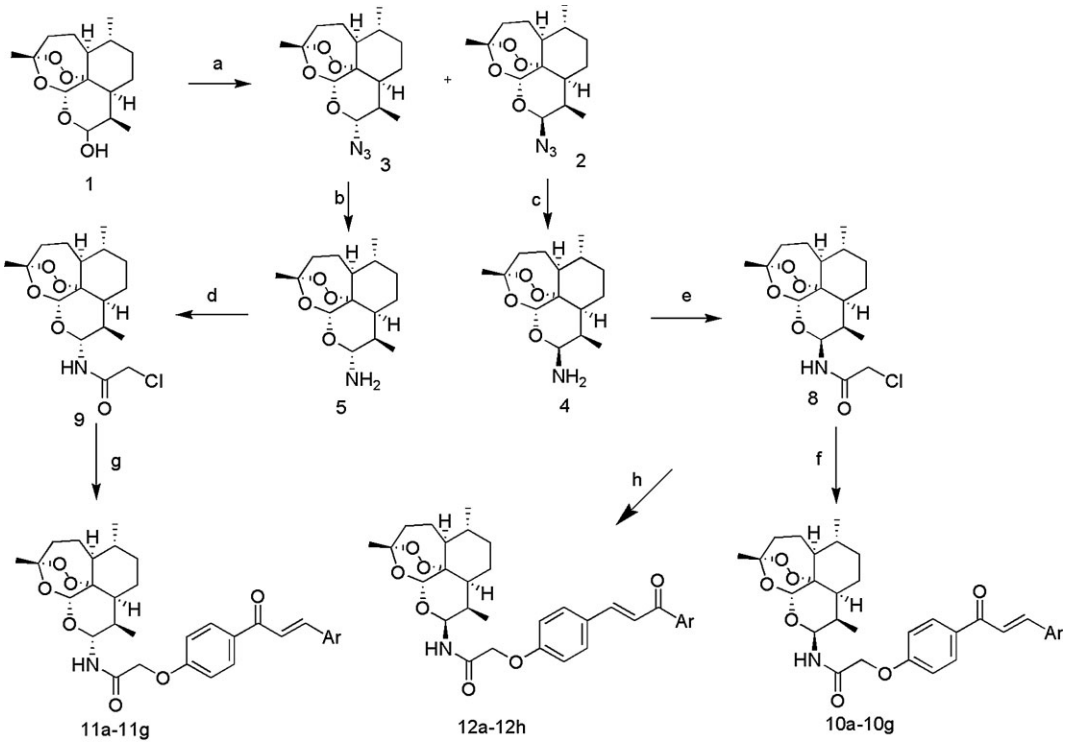
Results and Discussion

Biological evaluation

All compounds **10a–10g**, **11a–11g** and **12a–12h** were evaluated for their anti-tumor activity on human colorectal cancer cell line (HT-29), human non-small-cell lung cancer cell line (A549), human breast cancer cell line (MDA-MB-231), human cervical carcinoma cell line (HeLa) and human lung cancer cell line (H460) by the MTT assay [29], using DHA as a positive control. The results expressed as IC_{50} were summarized in Table 1, and the IC_{50} values were the average of at least two independent experiments.

As shown in Table 1, the artemisinin-chalcone hybrids **10a–10g** and **11a–11g** were more active than the corresponding DHA in most cases, suggesting that the presence of chalcone moiety enhanced their anti-tumor activity significantly. In addition, it was worth to mention that most of the tested compounds (**10a–10g**, **11a–11g**) against HT-29 and HeLa cell lines were several- to ten-fold more potent than against MDA-MB-231, A549 and H460 cell lines, respectively. The results demonstrated that the introduction of chalcone moiety could enhance their selectivity for HT-29 and HeLa cell lines significantly at the same time. Unfortunately, compounds **12a–12h** showed only moderate activity with IC_{50} values

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim



Reagents and conditions: (a) Me_3SiCl, NaN_3, NaI, dichloromethane, r.t., 28 h; (b, c) PPh_3, H_2O, THF, 60°C, 6 h; (d, e) 2-chloroacetyl chloride, Et_3N, 0-10°C, 3 h; (f, g) compound **6**, K_2CO_3, DMF, 60°C, 5 h; (h) compound **7**, K_2CO_3, DMF, 60°C, 5 h.

Scheme 2. Synthesis of **10a–10g**, **11a–11g** and **12a–12h**.

ranging from 1.5 to 27 μM, which were just slight improvement compared with those of DHA. The results revealed that the introduction of a 'reversed' chalcone moiety was probably unsuitable in enhancing their activity and selectivity.

The data in Table 1 revealed that substitution pattern on the phenyl rings linked to the *β*-position of the enone system affected their potency significantly. For compounds **10a–10g**, specifically with the halogen substituted derivatives (**10b**, **c**, **f**, **g**), the dihalogen-substituted derivatives (**10c**, **f**, **g**) possessed almost the same activity were favored over mono-substituted derivative (**10b**). It was also observed that the presence of electron-donating methoxyl group (**10a**) brought about enhanced activity and selectivity against HeLa cells with IC_{50} value of 0.12 μM. Turning to the effects of **10d** containing heteroaromatic chalcone moiety, interestingly, the bioisosteric replacement of substituted phenyl with pyridine ring (**9d**) resulted in a decrease of the anti-tumor activity on HT-29 cells.

Regarding the anti-tumor activity of **11a–11h**, bearing a same molecular structure but only different C-10 stereochemistry, showed few differences in inhibitory effect as compared with **10a–10h**. The results revealed that the configuration of C-10 was not essential for their activity. Compound **11a**, the most active compound of this series towards HeLa, possess 2,5-dimethoxyl group on the phenyl ring, which might be played a very vital role in determine the activity and selectivity.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

Table 1. Anti-tumor activity of the compounds against HT-29, A549, MDA-MB-231, HeLa and H460 cell lines *in vitro*

Compd. no	IC_{50} (μM)[a]				
	HT-29 [b]	A549 [b]	MDA-MB-231 [b]	HeLa [b]	H460 [b]
10a	0.45	19	2.3	0.12	2.4
10b	0.55	2.3	1.4	0.59	1.7
10c	0.55	3.2	2.1	0.33	3.6
10d	0.97	1.6	2.4	0.74	1.2
10e	0.53	3.6	1.1	1.9	0.8
10f	0.68	5.3	6.9	0.48	n.d [c]
10g	0.42	2.3	3.9	0.38	4.0
11a	0.55	12.0	1.7	0.19	4.1
11b	0.59	3.7	1.4	0.95	2.3
11c	0.69	1.7	1.0	0.83	5.8
11d	0.84	2	1.0	1.3	1.7
11e	1.2	1.7	0.68	0.77	0.84
11f	0.86	2.0	1.3	2.1	2.7
11g	1.3	n.d [c]	5.8	0.21	4.0
12a	3.8	5.0	1.7	8.5	6.3
12b	1.6	11.0	4.8	1.5	n.d [c]
12c	1.8	4.4	3.3	n.d [c]	6.7
12d	4.2	n.d [c]	6.5	2.6	4.1
12e	8.4	7.8	5.0	2.9	27.0
12f	5.1	4.6	13	n.d [c]	3.9
12g	3.2	3.8	9.3	6.2	6.8
12h	1.7	3.0	6.9	6.3	7.1
DHA [d]	5.6	15.6	9.8	6.3	13.0

[a] IC_{50}: Concentration of the compound (μM) producing 50% cell growth inhibition after 72 h of drug exposure, as determined by the MTT assay. Each experiment was run at least two times, and the results are presented as average values.[b] HT-29, human colon cancer cell line; A549, non-small-cell lung adenocarcinoma cell line; MDA-MB-231, human breast cancer cell line; HeLa, human cervical carcinoma cell line; H460, lung cancer cell line.[c] n.d.: not determined.[d] Used as a positive control.

Conclusion

In this study, three series of artemisinin–chalcone hybrids were synthesized and evaluated for their anti-tumor activity. The results demonstrated that most of compounds exhibited potent activity. Especially, compound **10a**, **11a** bearing 2,5-dimethoxyl group on the phenyl ring were found to be the most potent anti-tumor agents with the IC_{50} value of 0.12 and 0.19 μM to HeLa-29 cells.

From the above results, some interesting structure activity relationships can be disclosed that: (1) The configuration of the C-10 position has no evident effect on the anti-tumor activity. (2) The introduction of chalcone moiety was crucial for improving their activity and selectivity, while a 'reversed' chalcone moiety had little impact on the activity. (3) The substitution pattern had substantial impact on their potency. Generally, the presence of a number of methoxy groups seems to be a fundamental requirement to obtain potent and selective anti-tumor agents. (4) The replacement of substituted phenyl ring with pyridine ring might be detrimental to the activity. All these data suggested that these hybrids might be utilized for the development of new anti-tumor candidates. Especially, the most potent and selective hybrids **10a** and **11a** deserve further evaluation to study their metabolic stability and anti-tumor activity *in vivo*, and the research results will be reported in due course.

Experimental

Chemistry

All melting points were obtained on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland) and were uncorrected. Mass spectra (MS) were taken in ESI mode on Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Proton (1H) nuclear magnetic resonance spectroscopy was performed using Bruker ARX-300, 300MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker IFS 55 instrument (Bruker). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Unless otherwise noted, all the materials were obtained from commercially available sources and were used without further purification.

*Preparation of 10β-azidodihydroartemisinin **2** and 10α-azidodihydroartemisinin **3***

Trimethylchlorosilane (300 mmol, 38.1 mL) was added gradually to the mixture of dihydroartemisinin (200 mmol, 56.8 g) and sodium azide (300 mmol, 19.5 g) in dry dichloromethane (300 mL), then sodium iodide (5 mmol, 3.0 g) was added to the reaction mixture at 0–5°C. The reaction mixture was stirred at room temperature for 28 h, and then the mixture was quenched with a saturated $NaHCO_3$ solution (100 mL) and diluted with dichloromethane. The organic phase was washed with brine, then collected and dried overnight over anhydrous Na_2SO_4. After filtering, the solvent was evaporated under reduced pressure. The crude mixture was purified by column chromatography (silica, 1%–5% ethyl acetate/hexanes) to furnish the compound **2** (29.0 g, 47%) and compound **3** as white solid (3.0 g, 5%).

Compound **2**: M.p.: 41–43°C; MS (ESI) *m/z*: 332.2 $(M + Na)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.76 (s, 1H), 5.54 (d, J = 3.6 Hz, 1H), 2.46 (m, 1H), 2.26–2.12 (m, 1H), 2.07–1.96 (m, 1H), 1.87–1.78 (m, 1H), 1.77–1.65 (m, 1H), 1.58 (m, 2H), 1.48–1.33 (m, 3H), 1.31 (s, 3H), 1.16 (m, 1H), 0.89 (d, J = 6.3 Hz, 3H), 0.84 (d, J = 7.3 Hz, 3H).

Compound **3**: M.p.: 101–103°C; MS (ESI) *m/z*: 332.2 $(M + Na)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.53 (s, 1H), 4.86 (d, J = 10.1 Hz, 1H), 2.29–2.06 (m, 2H), 2.05–1.94 (m, 1H), 1.87–1.76 (m, 1H), 1.65–1.33 (m, 5H), 1.30 (s, 3H), 1.24–1.10 (m, 1H), 0.89 (d, J = 6.2 Hz, 3H), 0.81 (d, J = 7.1 Hz, 3H).

*Preparation of 10β-aminodihydroartemisinin **4***

A 100-mL round-bottomed flask was charged with compound **2** (16.0 g, 52 mmol) and THF (130 mL). To this solution, triphenyl phosphine (20.4 g, 78 mmol) was added slowly and the reaction mixture was stirred for 2 h at 60°C. Distilled water (160 mmol, 3.0 mL) was added and the resulting suspension was stirred for 6 h. The mixture was concentrated under reduced pressure. The

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

crude mixture was purified by flash column chromatography (dichloromethane/methanol, 200:1) to afford the desired compound **4** (9.1 g, 62%) as yellow oil. MS (ESI) *m/z*: 284.1 $(M + H)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.34 (s, 1H), 4.21 (d, $J = 9.7$ Hz, 1H), 2.44–2.31 (m, 1H), 2.30–2.17 (m, 1H), 2.08–1.94 (m, 4H), 1.94–1.83 (m, 2H), 1.81–1.61 (m, 3H), 1.60–1.46 (m, 2H), 1.44 (s, 3H), 0.97 (d, $J = 6.2$ Hz, 3H), 0.94 (d, $J = 7.2$ Hz, 3H).

*Preparation of 10α-aminodihydroartemisinin **5***

This compound was prepared according to compound **4** from compound **3** (5.0 g, 16.2 mmol), triphenyl phosphine (6.4 g, 24.5 mmol) and water (52.0 mmol, 1 mL). Purification of the resulting oil afforded **5** as colorless crystal (3.2 g, 70%); mp: 46°C; MS (ESI) *m/z*: 284.1 $(M + H)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.31 (s, 1H), 4.08 (d, $J = 9.6$ Hz, 1H), 2.23 (s, 2H, NH_2, D_2O-exchangable), 2.21–2.10 (m, 1H), 2.08–1.89 (m, 2H), 1.86–1.72 (m, 1H), 1.65–1.52 (m, 2H), 1.45–1.29 (m, 4H), 1.26 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.79 (d, $J = 7.2$ Hz, 3H).

*Preparation of (10R)-N-chloroacetyl-aminodihydroartemisinin **8***

Triethylamine (3.2 g, 31.8 mmol) was added to a solution of compound **4** (6.0 g, 21.2 mmol) in dichloromethane (80 mL) and then 2-chloroacetyl chloride (3.1 g, 27.6 mmol) was added drop-wise to the reaction mixture at 0–10°C. The reaction mixture was stirred at room temperature for 1–3 h. After completion of reaction as indicated by TLC, the mixture was quenched with H_2O (5 mL) and diluted with dichloromethane (50 mL). The organic layer was washed with brine (200 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resultant solid products were collected by filtration and recrystallized from methanol to give compound **8** (5.9 g, 78%). M.p.: 171–173°C; MS (ESI) *m/z*: 382.1 $(M + Na)^+$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.96 (d, $J = 9.1$ Hz, 1H), 5.47 (s, 1H), 5.08 (t, $J = 9.8$ Hz, 1H), 4.09 (s, 2H), 2.40–2.25 (m, 1H), 2.18 (td, $J = 14.0$, 3.7 Hz, 1H), 2.04–1.93 (m, 1H), 1.87–1.75 (m, 1H), 1.66–1.30 (m, 7H), 1.27 (s, 3H), 1.17 (m, 1H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.72 (d, $J = 7.1$ Hz, 3H).

*Preparation of (10S)-N-chloroacetyl-aminodihydroartemisinin **9***

The product was prepared from **5** (3.0 g, 10.6 mmol) in a similar manner as described for the preparation of compound **8**. The resultant solid products were collected by filtration and recrystallized from methanol to give compound **9** (3.1 g, 81%). M.p.: 175–177°C; MS (ESI) *m/z*: 382.2 $(M + Na)^+$; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 5.45 (s, 1H), 5.36 (t, $J = 10.1$ Hz, 1H), 4.09 (s, 2H), 2.55–2.41 (m, 1H), 2.41–2.32 (m, 1H), 2.11–2.00 (m, 1H), 1.98–1.86 (m, 1H), 1.85–1.70 (m, 2H), 1.71–1.46 (m, 4H), 1.45 (s, 3H), 1.39–1.24 (m, 2H), 0.99 (d, $J = 6.0$ Hz, 3H), 0.88 (d, $J = 7.2$ Hz, 3H).

*General procedure for preparation of compounds **6a–6h***

A stirred solution of *p*-hydroxyacetophenone (8.1 g, 0.06 mol) in ethanol (200 mL) was added to 60% KOH (6.7 g, 0.12 mol) solution at 0°C, the mixture was stirred at 0–5°C for 1 h. The substituted aryl aldehyde (0.06 mol) was added to the mixture and the resulting mixture was stirred at room temperature for 24 h. The aqueous mixture was neutralized by the addition of aqueous 10% HCl solution. The light yellow solid thus obtained was filtered, washed with water and dried. The residue was recrystallized from ethanol to afford pure compounds **6a–6h**.

*General procedure for preparation of compounds **7a–7h***

The foregoing method for the preparation of the **6a–6h** was applied to prepare the **7a–7h** except that the starting materials were substituted acetophenone and *p*-hydroxybenzaldehyde instead of substituted aryl aldehydes and *p*-hydroxyacetophenone.

*General procedure for preparation of compounds **10a–10g**, compounds **11a–11g** and compounds **12a–12h***

K_2CO_3 (0.12 g, 0.84 mmol) was added to a stirred solution of compound **7** or compound **8** (0.2 g, 0.57 mmol) and substituted chalcone (0.57 mmol) in DMF (6 mL). The reaction mixture was heated to 60°C for 3–5 h. The mixture was poured into ice water, stirred for 1 h and separated by filtration to give crude compounds, which purified by column chromatography on silica gel using (1%–5% petroleum ether/ethyl acetate) to obtain **10a–10g**, **11a–11g** and **12a–12h**.

*(10R)-N-{4-[3-(2,5-Dimethoxyphenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin **10a***

This compound was obtained as yellow solid in 82% yield. M.p.: 96–98°C; MS (ESI) *m/z*: 645.7 $(M + K)^+$; IR (KBr) cm^{-1}: 3240.7, 2925.9, 2872.9, 1692.9, 1661.2, 1605.6, 1254.2, 1218.8, 1172.0, 1089.1, 1038.3, 818.0, 751.8, 509.9. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.93 (d, $J = 9.0$ Hz, 1H), 8.16 (2d, $J = 8.8$ Hz, 2H), 8.01 (d, $J = 15.7$ Hz, 1H), 7.90 (d, $J = 15.8$ Hz, 1H), 7.55 (s, 1H), 7.10 (d, $J = 8.8$ Hz, 2H), 7.07–7.00 (m, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.7$ Hz, 1H), 4.71 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H), 2.45–2.37 (m, 1H), 2.25–2.12 (m, 1H), 2.04–1.94 (m, 1H), 1.86–1.75 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 6.2$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{34}H_{41}NO_9$ (in %): C, 67.20; H, 6.80; N, 2.30; Found: C, 67.17; H, 6.91 and N, 2.23.

*(10R)-N-{4-[3-(2-Fluorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin **10b***

This compound was obtained as pale yellow solid in 86% yield. M.p.: 106–109°C; MS (ESI) *m/z*: 588.3 $(M + Na)^+$; IR (KBr) cm^{-1}: 3422.1, 2926.1, 2873.1, 1684.4, 1664.0, 1605.3, 1538.0, 1508.1, 1257.9, 1217.6, 1172.5, 1038.5, 838.5, 761.9, 584.4; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.94 (d, $J = 9.4$ Hz, 1H), 8.17 (d, $J = 8.6$ Hz, 2H), 8.14–8.09 (m, 1H), 8.00 (d, $J = 16.0$ Hz, 1H), 7.81 (d, $J = 15.9$ Hz, 1H), 7.58–7.47 (m, 1H), 7.39–7.27 (m, 2H), 7.12 (d, $J = 8.7$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 10.0$ Hz, 1H), 4.72 (s, 2H), 2.45–2.34 (m, 1H), 2.25–2.11 (m, 1H), 2.05–1.93 (m, 1H), 1.87–1.73 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 6.1$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{32}H_{36}FNO_7$ (in %): C, 67.95; H, 6.42; N, 2.48; Found: C, 67.92; H, 6.56 and N, 2.42.

*(10R)-N-{4-[3-(3,4-Difluorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin **10c***

This compound was obtained as pale yellow solid in 79% yield. M.p.: 112–115°C; MS (ESI) *m/z*: 606.5 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.4, 2927.0, 2874.0, 1691.8, 1661.4, 1602.2, 1513.0, 1276.1, 1173.3, 1038.5, 878.2, 823.8, 596.9, 558.9; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.93 (d, $J = 8.8$ Hz, 1H), 8.19 (d, $J = 8.7$ Hz, 2H), 8.15–8.08 (m, 1H), 7.99 (d, $J = 15.7$ Hz, 1H), 7.79–7.72 (m, 1H),

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

7.68 (d, J = 15.9 Hz, 1H), 7.59–7.46 (m, 1H), 7.11 (d, J = 8.9 Hz, 2H), 5.47 (s, 1H), 5.17 (t, J = 9.7 Hz, 1H), 4.72 (s, 2H), 2.45–2.34 (m, 1H), 2.26–2.11 (m, 1H), 2.05–1.93 (m, 1H), 1.87–1.74 (m, 1H), 1.28 (s, 3H), 0.89 (d, J = 6.0 Hz, 3H), 0.71 (d, J = 7.0 Hz, 3H); Anal. calcd. for $C_{32}H_{35}F_2NO_7$ (in %): C, 65.85; H, 6.04; N, 2.40; Found: C, 65.79; H, 6.15; N, 2.40.

(10R)-N-{4-[3-(4-Pyridyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***10d***

This compound was obtained as brown solid in 71% yield. M.p.: 99–101°C; MS (ESI) m/z: 571.3 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.0, 2927.1, 2844.0, 1661.8, 1631.4, 1602.6, 1511.0, 1256.1, 1153.3, 1038.6, 876.2, 821.8, 566.9, 538.9; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.94 (d, J = 8.8 Hz, 1H), 8.67 (d, J = 5.1 Hz, 2H), 8.25–8.11 (m, 3H), 7.84 (d, J = 5.3 Hz, 2H), 7.65 (d, J = 15.9 Hz, 1H), 7.12 (d, J = 8.7 Hz, 2H), 5.47 (s, 1H), 5.17 (t, J = 9.6 Hz, 1H), 4.73 (s, 2H), 2.45–2.34 (m, 1H), 2.26–2.11 (m, 1H), 2.05–1.93 (m, 1H), 1.86–1.75 (m, 1H), 1.28 (s, 3H), 0.89 (d, J = 5.7 Hz, 3H), 0.71 (d, J = 7.2 Hz, 3H); Anal. calcd. for $C_{31}H_{36}N_2O_7$ (in %): C, 67.87; H, 6.61; N, 5.11; Found: C, 67.79; H, 6.73; N, 5.12.

(10R)-N-{4-[3-(4-Methylsulfonylphenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***10e***

This compound was obtained as pale yellow solid in 71% yield. M.p.: 116–118°C; MS (ESI) m/z: 648.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.6, 2918.9, 2862.8, 1665.8, 1609.5, 1552.5, 1508.4, 1428.6, 1266.3, 1230.1, 1161.8, 1028.5, 776.5, 598.3; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.10 (d, J = 8.9 Hz, 2H), 8.05–7.97 (m, 3H), 7.65 (d, J = 15.7 Hz, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.00 (d, J = 8.9 Hz, 2H), 5.52–5.40 (m, 1H), 4.62 (s, 2H), 3.11 (s, 3H), 2.53–2.31 (m, 2H), 2.13–1.99 (m, 1H), 1.98–1.86 (m, 1H), 1.83–1.70 (m, 2H), 1.45 (s, 3H), 0.99 (d, J = 5.8 Hz, 3H), 0.82 (d, J = 7.3 Hz, 3H); Anal. calcd. for $C_{33}H_{39}NO_9S$ (in %): C, 63.34; H, 6.28; N, 2.24; Found: C, 63.32; H, 6.34; N, 2.23.

(10R)-N-{4-[3-(4-Chloro-2-fluorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***10f***

This compound was obtained as pale yellow solid in 71% yield. M.p.: 111–113°C; MS (ESI) m/z: 622.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.7, 2925.2, 2853.0, 1663.9, 1627.4, 1601.9, 1509.0, 1258.4, 1152.9, 1038.4, 856.1, 811.6, 530.1; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.94 (d, J = 9.1 Hz, 1H), 8.32 (dd, J = 8.7, 6.3 Hz, 1H), 8.19 (d, J = 8.7 Hz, 2H), 8.01 (d, J = 15.6 Hz, 1H), 7.94 (d, J = 15.5 Hz, 1H), 7.59 (dd, J = 8.8, 2.4 Hz, 1H), 7.38 (td, J = 8.6, 2.6 Hz, 1H), 7.11 (d, J = 8.7 Hz, 2H), 5.47 (s, 1H), 5.16 (t, J = 9.8 Hz, 1H), 4.72 (s, 2H), 2.46–2.34 (m, 1H), 2.19 (td, J = 14.5, 3.8 Hz, 1H), 2.04–1.93 (m, 1H), 1.88–1.74 (m, 1H), 1.28 (s, 3H), 0.89 (d, J = 6.2 Hz, 3H), 0.71 (d, J = 7.0 Hz, 3H); Anal. calcd. for $C_{32}H_{35}ClFNO_7$ (in %): C, 64.05; H, 5.88; N, 2.33; Found: C, 64.02; H, 5.93; N, 2.35.

(10R)-N-{4-[3-(2,3-Dichlorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***10g***

This compound was obtained as pale yellow solid in 81% yield. M.p.: 111–113°C; MS (ESI) m/z: 638.2 $(M + Na)^+$; IR (KBr) cm^{-1}: 3420.2, 2926.9, 2874.0, 1655.8, 1598.5, 1531.5, 1500.4, 1421.4, 1226.8, 1220.4, 1173.9, 1038.5, 773.4, 596.3; ^{1}H-NMR (300 MHz, CDCl3) δ: 8.18 (d, J = 15.7 Hz, 1H), 8.08 (d, J = 8.9 Hz, 2H), 7.67 (dd, J = 7.9, 1.3 Hz, 1H), 7.53 (dd, J = 8.0, 1.4 Hz, 1H), 7.47 (d, J = 15.7 Hz, 1H), 7.12–7.03 (m, 3H), 5.52–5.40 (m, 2H), 4.62 (s, 2H), 2.52–2.32 (m, 2H), 2.11–2.01 (m, 1H), 1.97–1.86 (m, 1H), 1.83–1.71 (m, 2H), 1.45 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.82 (d, J = 7.2 Hz, 3H); Anal. calcd. for $C_{32}H_{35}Cl_2NO_7$ (in %): C, 62.34; H, 5.72; N, 2.27; Found: C, 62.33; H, 5.78; N, 2.25.

(10S)-N-{4-[3-(2,5-Dimethoxyphenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***11a***

This compound was obtained as yellow solid in 91% yield. M.p.: 106–109°C; MS (ESI) m/z: 630.5 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.0, 2925.9, 1687.6, 1657.7, 1601.9, 1496.1, 1260.1, 1225.1, 1171.1, 1112.8, 1039.5, 802.3, 551.8; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.14–8.03 (m, 3H), 7.60 (d, J = 15.8 Hz, 1H), 7.06 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 3.6 Hz, 1H), 6.89 (d, J = 3.7 Hz, 1H), 6.74 (s, 1H), 5.50–5.42 (m, 2H), 4.62 (s, 2H), 3.90 (s, 3H), 3.73 (s, 3H), 2.53–2.27 (m, 2H), 2.13–1.98 (m, 1H), 1.98–1.85 (m, 1H), 1.84–1.71 (m, 2H), 1.44 (s, 3H), 0.99 (d, J = 5.7 Hz, 3H), 0.81 (d, J = 6.9 Hz, 3H); Anal. calcd. for $C_{34}H_{41}NO_9$ (in %): C, 67.20; H, 6.80; N, 2.30; Found: C, 67.13; H, 6.93; N, 2.26.

(10S)-N-{4-[3-(2-Fluorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***11b***

This compound was obtained as pale yellow solid in 86% yield. M.p.: 96–98°C; MS (ESI) m/z: 588.3 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.9, 2926.1, 2873.5, 1663.6, 1605.1, 1575.8, 1531.4, 1333.5, 1257.6, 1217.6, 1172.4, 1038.5, 761.9, 584.7; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.09 (d, J = 8.9 Hz, 2H), 7.92 (d, J = 15.9 Hz, 1H), 7.72–7.61 (m, 2H), 7.46–7.35 (m, 1H), 7.27–7.12 (m, 3H), 7.07 (d, J = 8.9 Hz, 2H), 5.52–5.41 (m, 2H), 4.62 (s, 2H), 2.53–2.32 (m, 2H), 2.11–1.99 (m, 1H), 1.98–1.86 (m, 1H), 1.84–1.70 (m, 2H), 1.45 (s, 3H), 0.99 (d, J = 5.9 Hz, 3H), 0.82 (d, J = 7.1 Hz, 3H); Anal. calcd. for $C_{32}H_{36}FNO_7$ (in %): C, 67.95; H, 6.42; N, 2.48; Found: C, 67.95; H, 6.53; N, 2.46.

(10S)-N-{4-[3-(3,4-Difluorophenyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***11c***

This compound was obtained as pale yellow solid in 82% yield. M.p.: 108–111°C; MS (ESI) m/z: 606.2 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.3, 2926.9, 2874.0, 1662.9, 1602.3, 1514.8, 1276.3, 1222.4, 1173.4, 1115.3, 1038.4, 823.6, 519.1; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.08 (d, J = 8.9 Hz, 2H), 7.73 (d, J = 15.6 Hz, 1H), 7.46 (d, J = 15.6 Hz, 1H), 7.42–7.31 (m, 1H), 7.25–7.13 (m, 2H), 7.07 (d, J = 8.9 Hz, 2H), 5.47 (t, J = 10.2 Hz, 1H), 4.62 (s, 2H), 2.53–2.27 (m, 2H), 2.13–1.98 (m, 1H), 1.99–1.84 (m, 1H), 1.84–1.70 (m, 2H), 1.45 (s, 3H), 1.00 (d, J = 5.9 Hz, 3H), 0.82 (d, J = 7.2 Hz, 3H); Anal. calcd. for $C_{32}H_{35}F_2NO_7$ (in %): C, 65.85; H, 6.04; N, 2.40; Found: C, 65.77; H, 6.09; N, 2.36.

(10S)-N-{4-[3-(4-Pyridyl)-2-(E)-propenoyl]phenyloxyacetyl}aminodihydroartemisinin ***11d***

This compound was obtained as pale yellow solid in 69% yield. M.p.: 119–121°C; MS (ESI) m/z: 571.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3422.5, 2923.7, 2864.3, 1662.7, 1612.8, 1524.8, 1276.3, 1235.4, 1161.6, 1125.3, 1038.4, 828.6, 519.3; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.71 (d, J = 5.1 Hz, 2H), 8.11–8.05 (m, 3H), 7.67 (d, J = 16.8 Hz, 1H), 7.49 (d, J = 5.0 Hz, 2H), 7.07 (d, J = 8.9 Hz, 2H), 5.52–5.41 (m, 2H), 4.62 (s, 2H), 2.52–2.31 (m, 2H), 2.11–2.00 (m, 1H), 1.97–1.86 (m, 1H), 1.83–1.70 (m, 2H), 1.44 (s, 3H), 0.98 (d, J = 5.9 Hz, 3H), 0.81 (d, J = 7.2 Hz, 3H); Anal. calcd. for $C_{31}H_{36}N_2O_7$ (in %): C, 67.87; H, 6.61; N, 5.11; Found: C, 67.82; H, 6.73; N, 5.08

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

(10S)-N-{4-[3-(4-Methylsulfonylphenyl)-2-(E)-propenoyl]-phenyloxyacetyl}aminodihydroartemisinin ***11e***

This compound was obtained as pale yellow solid in 81% yield. M.p.: 111–113°C; MS (ESI) *m/z*: 648.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.9, 2925.9, 2872.9, 1662.1, 1598.7, 1533.7, 1380.5, 1307.4, 1222.6, 1171.5, 1148.2, 1038.2, 825.9, 548.6; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.06–7.96 (m, 3H), 7.84 (d, $J = 8.3$ Hz, 2H), 7.65 (d, $J = 15.9$ Hz, 1H), 7.58 (d, $J = 8.4$ Hz, 2H), 7.00 (d, $J = 8.8$ Hz, 2H), 5.46 (t, $J = 3.5$ Hz, 1H), 4.59 (s, 2H), 3.03 (s, 3H), 2.52–2.31 (m, 2H), 2.11–2.00 (m, 1H), 1.98–1.87 (m, 1H), 1.85–1.71 (m, 2H), 1.44 (s, 3H), 0.99 (d, $J = 5.9$ Hz, 3H), 0.80 (d, $J = 7.3$ Hz, 3H); Anal. calcd. for $C_{33}H_{39}NO_9S$ (in %): C, 63.34; H, 6.28; N, 2.24; Found: C, 63.27; H, 2.37; N, 2.16.

(10S)-N-{4-[3-(4-Chloro-2-fluorophenyl)-2-(E)-propenoyl]-phenyloxyacetyl}aminodihydroartemisinin ***11f***

This compound was obtained as pale yellow solid in 89% yield. M.p.: 98–101°C; MS (ESI) *m/z*: 622.2 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.6, 2927.0, 2873.8, 1663.0, 1599.6, 1532.7, 1508.3, 1487.3, 1235.8, 1172.9, 1115.2, 1039.9, 878.6, 542.3; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.13 (d, $J = 15.8$ Hz, 1H), 8.07 (d, $J = 8.8$ Hz, 2H), 7.92 (d, $J = 8.9$ Hz, 1H), 7.83–7.73 (m, 1H), 7.45 (d, $J = 15.7$ Hz, 1H), 7.07 (d, $J = 8.8$ Hz, 2H), 6.94 (d, $J = 8.9$ Hz, 1H), 5.51–5.39 (m, 2H), 4.62 (s, 2H), 2.52–2.32 (m, 2H), 2.11–2.00 (m, 1H), 1.98–1.86 (m, 1H), 1.83–1.71 (m, 2H), 1.45 (s, 3H), 0.99 (d, $J = 5.9$ Hz, 4H), 0.81 (d, $J = 7.3$ Hz, 3H); Anal. calcd. for $C_{32}H_{35}ClFNO_7$ (in %): C, 64.05; H, 5.88; N, 2.33; Found: C, 64.03; H, 5.98; N, 2.26.

(10S)-N-{4-[3-(2,3,4-Trimethoxyphenyl)-2-(E)-propenoyl]-phenyloxyacetyl}aminodihydroartemisinin ***11g***

This compound was obtained as yellow solid in 87% yield. M.p.: 103–105°C; MS (ESI) *m/z*: 660.2 $(M + Na)^+$; IR (KBr) cm^{-1}: 3419.9, 2926.4, 2874.7, 1682.6, 1661.5, 1608.3, 1502.0, 1317.6, 1216.1, 1168.2, 1117.5, 1038.58, 767.7, 560.1; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.07 (d, $J = 8.8$ Hz, 2H), 8.01 (d, $J = 15.8$ Hz, 1H), 7.57 (d, $J = 15.8$ Hz, 1H), 7.41 (d, $J = 8.8$ Hz, 1H), 7.05 (d, $J = 8.9$ Hz, 2H), 6.74 (d, $J = 8.8$ Hz,1H), 5.52–5.41 (m, 2H), 4.61 (s, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.91 (s, 3H), 2.54–2.32 (m, 2H), 2.11–2.00 (m, 1H), 1.98–1.85 (m, 1H), 1.83–1.71 (m, 2H), 1.45 (s, 3H), 0.99 (d, $J = 5.9$ Hz, 3H), 0.81 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{35}H_{43}NO_{10}$ (in %): C, 65.92; H, 6.80; N, 2.20; Found: C, 65.87; H, 6.82; N, 2.18.

(10R)-N-{4-[(1E)-3-Oxo-3-(4-chlorophenyl)prop-1-en-1-yl]-phenyloxyacetyl}aminodihydroartemisinin ***12a***

This compound was obtained as pale yellow solid in 75% yield. M.p.: 106–109°C; MS (ESI) *m/z*: 604.4 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.1, 2924.4, 1688.5, 1662.9, 1593.6, 1508.7, 1463.0, 1256.3, 1201.9, 1038.8, 828.4, 705.9, 548.7; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.89 (d, $J = 9.3$ Hz, 1H), 8.17 (d, $J = 8.6$ Hz, 2H), 7.88 (d, $J = 8.8$ Hz, 2H), 7.83 (d, $J = 15.6$ Hz, 1H), 7.73 (d, $J = 15.5$ Hz, 1H), 7.64 (d, $J = 8.6$ Hz, 2H), 7.04 (d, $J = 8.8$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.47–2.37 (m, 1H), 2.19 (td, $J = 14.3$, 3.8 Hz, 1H), 2.04–1.94 (m, 1H), 1.86–1.75 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{32}H_{36}ClNO_7$ (in %): C, 66.03; H, 6.23; N, 2.41; Found: C, 66.04; H, 6.31; N, 2.32.

(10R)-N-{4-[(1E)-3-Oxo-3-(4-fluorophenyl)prop-1-en-1-yl]-phenyloxyacetyl}aminodihydroartemisinin ***12b***

This compound was obtained as pale yellow solid in 70% yield. M.p.: 111–113°C; MS (ESI) *m/z*: 588.4 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.2, 2925.8, 1693.6, 1663.7, 1591.1, 1508.3, 1447.2, 1253.6, 1189.5, 1038.4, 819.3, 707.8, 547.3; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.89 (d, $J = 9.2$ Hz, 1H), 8.29–8.21 (m, 2H), 7.87 (d, $J = 8.8$ Hz, 2H), 7.84 (d, $J = 15.4$ Hz, 1H), 7.72 (d, $J = 15.5$ Hz, 1H), 7.39 (t, $J = 8.9$ Hz, 2H), 7.04 (d, $J = 8.8$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.48–2.37 (m, 1H), 2.19 (td, $J = 14.1$, 3.5 Hz, 1H), 2.04–1.95 (m, 1H), 1.86–1.75 (m, 1H), 1.29 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{32}H_{36}FNO_7$ (in %): C, 67.95; H, 6.42; N, 2.48; Found: C, 67.89; H, 6.53; N, 2.42.

(10R)-N-{4-[(1E)-3-Oxo-3-(3-chlorophenyl)prop-1-en-1-yl]-phenyloxyacetyl}aminodihydroartemisinin ***12c***

This compound was obtained as pale yellow solid in 78% yield. M.p.: 114–116°C; MS (ESI) *m/z*: 604.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3420.1, 2925.3, 1692.4, 1663.2, 1596.7, 1508.1, 1443.6, 1253.4, 1208.9, 1175.9, 1039.0, 827.9, 703.3, 547.8; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.90 (d, $J = 9.1$ Hz, 1H), 8.19 (s, 1H), 8.10 (d, $J = 7.8$ Hz, 1H), 7.90 (d, $J = 8.8$ Hz, 2H), 7.85 (d, $J = 15.5$ Hz, 1H), 7.80–7.71 (m, 2H), 7.60 (t, $J = 7.9$ Hz, 1H), 7.04 (d, $J = 8.8$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.47–2.36 (m, 1H), 2.19 (td, $J = 13.8$, 3.8 Hz, 1H), 2.04–1.94 (m, 1H), 1.87–1.75 (m, 1H), 1.29 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{32}H_{36}ClNO_7$ (in %): C, 66.03; H, 6.23; Cl, 6.09; N, 2.41; Found: C, 66.00; H, 6.34; N, 2.32.

(10R)-N-{4-[(19E)-3-Oxo-3-phenylprop-1-en-1-yl]-phenyloxyacetyl}aminodihydroartemisinin ***12d***

This compound was obtained as pale yellow solid in 73% yield. M.p.: 103–105°C; MS (ESI) *m/z*: 570.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.5, 2923.8, 1684.5, 1658.3, 1596.5, 1508.4, 1448.9, 1252.2, 1216.6, 1174.4, 1038.1, 828.3, 696.7, 546.4; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.90 (d, $J = 9.1$ Hz, 1H), 8.14 (d, $J = 7.4$ Hz, 2H), 7.87 (d, $J = 8.1$ Hz, 2H), 7.83 (d, $J = 15.6$ Hz, 1H), 7.72 (d, $J = 15.7$ Hz, 1H), 7.72–7.62 (m, 1H), 7.62–7.52 (m, 2H), 7.04 (d, $J = 8.5$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.44–2.36 (m, 1H), 2.19 (td, $J = 13.6$, 2.5 Hz, 1H), 2.04–1.94 (m, 1H), 1.87–1.74 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 5.6$ Hz, 3H), 0.71 (d, $J = 6.9$ Hz, 3H); Anal. calcd. for $C_{32}H_{37}NO_7$ (in %): C, 70.18; H, 6.81; N, 2.56; Found: C, 70.16; H, 6.85; N, 2.52.

(10R)-N-{4-[(1E)-3-Oxo-3-(4-trifluoromethylphenyl)prop-1-en-1-yl]phenyloxyacetyl}aminodihydroartemisinin ***12e***

This compound was obtained as pale yellow solid in 81% yield. M.p.: 118–121°C; MS (ESI) *m/z*: 638.4 $(M + Na)^+$; IR (KBr) cm^{-1}: 3422.1, 2925.6, 1690.3, 1666.1, 1597.4, 1593.8, 1508.2, 1451.5, 1251.6, 1202.3, 1170.4, 1037.8, 829.0, 542.9; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.90 (d, $J = 9.0$ Hz, 1H), 8.32 (d, $J = 8.1$ Hz, 2H), 7.94 (d, $J = 8.4$ Hz, 2H), 7.89 (d, $J = 8.8$ Hz, 2H), 7.84 (d, $J = 15.7$ Hz, 1H), 7.76 (d, $J = 15.6$ Hz, 1H), 7.05 (d, $J = 8.7$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.7$ Hz, 1H), 4.66 (s, 2H), 3.27–3.27 (m, 1H), 2.47–2.36 (m, 1H), 2.19 (td, $J = 14.2$, 3.7 Hz, 1H), 2.05–1.94 (m, 1H), 1.85–1.75 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{33}H_{36}F_3NO_7$ (in %): C, 64.38; H, 5.89; N, 2.28; O, 18.19 Found: C, 64.32; H, 5.93; N, 2.26.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

(10R)-N-{4-[(1E)-3-Oxo-3-(3-methoxyphenyl)prop-1-en-1-yl]phenyloxyacetyl}aminodihydroartemisinin ***12f***

This compound was obtained as yellow solid in 75% yield. M.p.: 117–119°C; MS (ESI) *m/z*: 600.1 $(M + Na)^+$; IR (KBr) cm^{-1}: 3420.6, 2926.4, 1691.5, 1661.6, 1592.5, 1508.4, 1450.2, 1252.5, 1174.4, 1037.3, 827.8, 740.2, 549.9; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.89 (d, $J = 9.1$ Hz, 1H), 7.88 (d, $J = 8.7$ Hz, 2H), 7.81 (d, $J = 15.6$ Hz, 1H), 7.78–7.67 (m, 2H), 7.60 (s, 1H), 7.49 (t, $J = 8.0$ Hz, 1H), 7.23 (dd, $J = 8.2$, 2.0 Hz, 1H), 7.04 (d, $J = 8.7$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 3.86 (s, 3H), 2.47–2.38 (m, 1H), 2.26–2.12 (m, 1H), 2.05–1.92 (m, 1H), 1.86–1.75 (m, 1H), 1.29 (s, 3H), 0.89 (d, $J = 6.2$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{33}H_{39}NO_8$ (in %): C, 68.61; H, 6.80; N, 2.42; Found: C, 68.60; H, 6.83; N, 2.42.

(10R)-N-{4-[(1E)-3-Oxo-3-(3.4-dichlorophenyl)prop-1-en-1-yl]phenyloxyacetyl}aminodihydroartemisinin ***12g***

This compound was obtained as yellow solid in 69% yield. M.p.: 113–115°C; MS (ESI) *m/z*: 639.9 $(M + Na)^+$; IR (KBr) cm^{-1}: 3423.4, 2923.1, 1691.5, 1665.8, 1595.3, 1588.7, 1508.4, 1255.6, 1193.7, 1038.3, 828.6, 736.1, 538.0; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.90 (d, $J = 9.2$ Hz,1H), 8.40 (d, $J = 2.0$ Hz, 1H), 8.11 (d, $J = 2.0$ Hz, 1H), 7.94–7.82 (m, 4H), 7.76 (d, $J = 15.5$ Hz, 1H), 7.05 (d, $J = 8.8$ Hz, 1H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.47–2.35 (m, 1H), 2.19 (td, $J = 14.0$, 3.8 Hz, 1H), 2.04–1.94 (m, 1H), 1.86–1.75 (m, 1H), 1.28 (s, 3H), 0.89 (d, $J = 6.3$ Hz, 3H), 0.71 (d, $J = 7.1$ Hz, 3H); Anal. calcd. for $C_{32}H_{35}Cl_2NO_7$ (in %): C, 62.34; H, 5.72; N, 2.27; Found: C, 62.30; H, 5.78; N, 2.25.

(10R)-N-{4-[(1E)-3-Oxo-3-(3-pyridinyl)prop-1-en-1-yl]-phenyloxyacetyl}aminodihydroartemisinin ***12h***

This compound was obtained as pale yellow solid in 61% yield. M.p.: 105–117°C; MS (ESI) *m/z*: 571.2 $(M + Na)^+$; IR (KBr) cm^{-1}: 3421.8, 2924.7, 1686.4, 1662.8, 1593.2, 1593.5, 1507.5, 1252.4, 1198.3, 1038.1, 839.5, 719.0, 516.0; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.32 (s, 1H), 8.90 (d, $J = 9.2$ Hz, 1H), 8.82 (d, $J = 3.4$ Hz, 1H), 8.46 (d, $J = 8.1$ Hz, 1H), 7.98–7.82 (m, 3H), 7.76 (d, $J = 15.7$ Hz, 1H), 7.68–7.53 (m, 1H), 7.05 (d, $J = 8.5$ Hz, 2H), 5.47 (s, 1H), 5.17 (t, $J = 9.8$ Hz, 1H), 4.66 (s, 2H), 2.44–2.34 (m, 1H), 2.26–2.12 (m, 1H), 2.08–1.93 (m, 1H), 1.88–1.74 (m, 1H), 1.29 (s, 3H), 0.89 (d, $J = 6.1$ Hz, 3H), 0.71 (d, $J = 7.0$ Hz, 3H); Anal. calcd. for $C_{31}H_{36}N_2O_7$ (in %): C, 67.87; H, 6.61; N, 5.11; Found: C, 67.79; H, 6.73; N, 5.11.

Anti-tumor activity assay *in vitro*

The anti-tumor activity of compounds **10a–10g**, **11a–11g** and **12a–12h** were evaluated with HT-29, A549, MDA-MB-231, HeLa, and H460 cell lines by the standard MTT assay *in vitro*, with DHA as the positive control. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS).

Approximately 4×10^3 cells, suspended in MEM, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The test compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL and incubated with cells at 37°C for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested twice in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of two determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

This work was supported by the National S&T Major Project of China (No. 2009ZX09301-012)

The authors have declared no conflict of interest.

References

[1] I. K. Mellinghoff, C. L. Sawyers, *Pharmacogenomics* **2002**, *3*, 603–623.
[2] M. E. Wall, M. C. Wani, *Cancer. Res.* **1995**, *55*, 753–760.
[3] V. Dhingra, R. K. Vishweshwar, N. M. Lakshmi, *Life Sci.* **2000**, *66*, 279–300.
[4] T. Efferth, A. Benakis, M. R. Romero, *et al*., *Free. Radic. Biol. Med.* **2004**, *37*, 998–1009.
[5] G. H. Posner, P. Ploypradith, M. H. Parker, *et al*., *J. Med. Chem.* **1999**, *42*, 4275–4280.
[6] S. Cho, Y. Um, J. H. Jung, *et al*., *Bioorg. Med. Chem. Lett.* **2009**, *19*, 382–385.
[7] A. S. Rosenthal, X. C. Chen, J. O. Liu, *et al*., *J. Med. Chem.* **2009**, *52*, 1198–1203.
[8] A. M. Galal, W. Gul, D. Slade, *et al., Bioorg. Med. Chem.* **2009**, *17*, 741–751.
[9] N. P. Singh, H. Lai, *Life. Sci.* **2001**, *70*, 49–56.
[10] X. L. Yang, W. Wang, J. Tan, *et al., Bioorg. Med. Chem. Lett.* **2009**, *19*, 4385–4388.
[11] T. Efferth, *Drug. Resist. Update* **2005**, *8*, 85–97.
[12] D. Sadava, T. Phillips, S. E. Kane, *Cancer. Lett.* **2002**, *179*, 151–156.
[13] A. E. Mercer, J. L. Maggs, X. M. Sun, *et al., J. Biol. Chem.* **2007**, *282*, 9372–9382.
[14] W. Nam, J. Tak, J. K. Ryu, *et al., Head Neck* **2006**, *29*, 335–340.
[15] H. H. Chen, H. J. Zhou, G. D. Wu, *et al., Pharmacology* **2004**, *71*, 1–9.
[16] S. Oh, I. H. Jeong, C. M. Ahn, *et al., Bioorg. Med. Chem.* **2004**, *12*, 3783–3790.
[17] M. Jung, J. Tak, W. Chung, *et al., Bioorg. Med. Chem. Lett.* **2006**, *16*, 1227–1230.
[18] F. Herencia, M. L. Ferrandiz, A. Ubeda, *et al., Bioorg. Med. Chem. Lett.* **1998**, *8*, 1169–1174.
[19] S. Ducki, R. Forrest, J. A. Hadfield, *et al., Bioorg. Med. Chem. Lett.* **1998**, *8*, 1051–1056.
[20] H. Tsuchiya, M. Sato, M. Akagiri, *et al., Pharmazie* **1994**, *49*, 756–758.
[21] M. L. Go, X. Wu, X. L. Liu, *et al., Curr. Med. Chem.* **2005**, *12*, 483–499.
[22] M. L. Edwards, D. M. Stemerick, P. S. Sunkara, *J. Med. Chem.* **1990**, *33*, 1948–1954.
[23] Y. L. Hsu, P. L. Kuo, W. S. Tzeng, *et al., Food Chem. Toxicol.* **2006**, *44*, 704–713.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

[24] O. Nerya, R. Musa, S. Khatib, *et al., Phytochemistry* **2004**, *65*, 1389–1395.

[25] O. Sabzevari, G. Galati, M. Y. Moridani, *et al., Chem. Biol. Interact.* **2004**, *14*, 857–867.

[26] K. J. Wallace, R. Hanes, E. Anslyn, *et al*., *Synthesis* **2005**, *12*, 2080–2083.

[27] Y. K. Rao, S. H. Fang, Y. M. Tzeng, *Bioorg. Med. Chem.* **2004**, *12*, 2679–2686.

[28] L. J. Xie, X. Zhai, J. Zuo, *et al., Acta Cryst., E* **2010**, *66*, o1839–o1839.

[29] S. Y. Xu, R. L. Bian, X. Chen, *Method of Pharmacology*, Public Health Publishing House, Beijing **2002**, pp. 1784–1786.

© 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim