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Synthesis of novel 1,2,3-triazole based artemisinin derivatives and their antiproliferative activity

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Abstract

Two series of novel 1,2,3-triazole based artemisinin derivatives were designed, synthesized via copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction and investigated for their antiproliferative activity by MTT assay against various human cancer cell lines. In series 1, compounds **9**, **17**, **18**, and **22** were showed better antiproliferative activity against all the tested cell lines as compared to dihydroartemisinin (DHA, **5**). Compound **9** and compound **17** were the most active, with IC_{50} range from 4.06 to 36.65 μM and 7.16 to 28.21 μM respectively. Compound **9** showed potent antiproliferative activity against the A431 cell lines with IC_{50} 4.06 μM and compound **17** displayed potent activities against A549 cell line with IC_{50} 7.16 μM. In series 2, compounds **24**, **27** and **28** showed better activity than other derivatives. Active compounds **9** and **17** showed significant ($p<0.05$) cell cycle arrest at G2/M phase and apoptosis in skin and lung cancer cells. These molecules significantly ($p<0.05$) induce the ROS formation in tested cell lines. Furthermore the toxicity study on human erythrocyte revealed that these molecules are non-toxic even at the higher tested concentration (100 μg/ml). Some 3α-hydroxydeoxydihydroartemisinin-triazole derivatives (**11a**, **14a**, **15a**, **17a-22a**) were also synthesized along with their peroxy counterparts. The results antiproliferative activity revealed that except compound **11a**, all the compounds with peroxy functionality are more active than their 3α - hydroxydeoxy counterparts.

Keywords: Artemisinin derivatives; 1,2,3-Triazole; Synthesis; Antiproliferative activity; Cell Cycle analysis; ROS; Osmotic fragility

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1. Introduction

Artemisinin (**1**) (qinghaosu), a well-known antimalarial compound was isolated from the plant *Artemisia annua L.* Asteraceae. It is a sesqueterpene lactone endoperoxide having 1,2,4 trioxane system which is essential component for its antimalarial activity.[1,2] Currently, artemisinin and its semisynthetic derivatives such as artemether (**2**), arteether (**3**), and artesunate (**4**) (Figure 1) have been effectively used for malaria treatment. These derivatives are active against both uncomplicated and severe forms of malaria and finally converted to dihydrortemisinin (**5**), the active metabolite of artemsinin inside the body.[3,4]

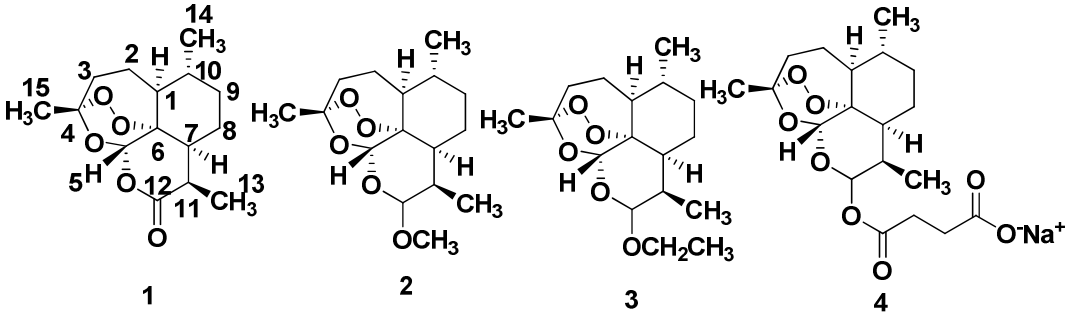

Figure 1. Artemisinin and their clinically used derivatives for malaria treatment.

The anticancer property of artemisinin (**1**) was firstly documented by Woerdenbag et al. in 1993.[5] Interestingly, it was found that the potent anticancer activity of artemisinin (**1**) was also associated with the presence of intact endoperoxide bond. The absence of endoperoxide moiety does not completely abolish the antitumor activity but significantly reduces it as compared to those compounds with the intact endoperoxide bond.[6-9] These residual anticancer activity suggesting that artemisinin compounds may also follow peroxide-independent mechanism.[7] In a general concurrence, free iron or heme/heme-bound proteins have been responsible for the bioreductive activation of artemisinin.[10-12] It has been supposed that iron‐activated artemisinin damage the cancer cells by the formation of highly alkylating carbon‐centered radicals and reactive oxygen species (ROS),[9,13] which is responsible for selective action of artemisinin (**1**) on cancer cells by induced apoptosis, growth arrest, decrease cell proliferation, DNA damage and reducing angiogenesis.[14] It was found that artemisinin (**1**) and its derivatives were active against both the drug‐sensitive and resistant cancer cell lines.[15-17] These compounds are active against highly metastatic and aggressive cancers.[14,18] Furthermore, artemisinin derivatives also show synergistic interaction with other anticancer drugs with minimum toxicity towards normal cells.[19-21] Antitumor activity of

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several artemisinin derivatives including both the monomers and dimers has been document *in vitro* and animal model.[22-24]

1,2,3-triazoles are the nitrogen heterocycles posseses diverse range of pharmacological activities including antiproliferative, antimicrobial, anti-inflammatory, antiretroviral, anticonvulsant and transforming growth factor β1 type 1 receptor inhibition.[25,26] These compounds are generally stable to hydrolysis in acidic and basic condition as well as to oxido-reductive environment which make them stable to metabolic degradation and indicates their high aromatic stabilization. 1,2,3-Triazole moieties have a very high dipole moment (about 5 Debye) and are capable of hydrogen bonding which is favourable condition for binding with the biomolecular targets and also improves their solubility.[27,28] There are number of drugs in market having 1,2,3-triazole moiety such as cefatrizine (antibiotic),[29] tazobactam (antibiotic),[29] rufinamide (anticonvulsant)[30] and many of them are in different phase of clinical trials and may become drugs in future such as carboxyamidotriazole or CAI (anticancer),[31] tert-butyldimethylsilylspiroaminooxathioledioxide or TSAO (HIV reverse transcriptase inhibitor)[32] (Figure 2).

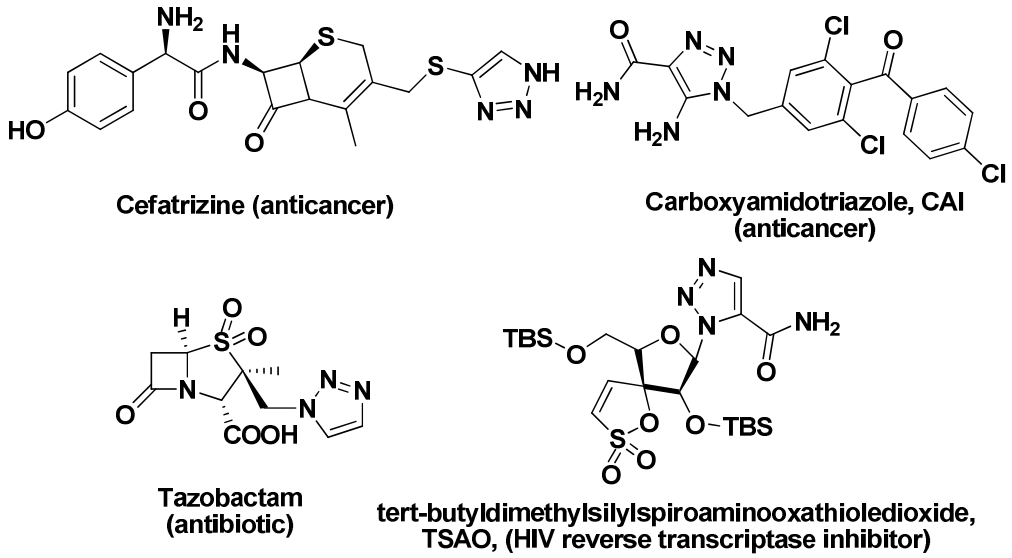

Figure 2. Potential drugs based on 1,2,3-triazole system.

In the present study, we have designed and synthesized two series of novel artemisinin triazole based derivatives and evaluated for their antiproliferative activity against the various organ specific cell lines. Further the mechanism of potential leads was evaluated via cell cycle analysis and ROS production. Furthermore, the toxicity of the potential leads was also estimated by Osmotic fragility assay.

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2. Result and discussion

2.1. Chemistry

We have targeted to synthesized two series of novel artemisinin-azole derivatives. In the first series, synthesized derivatives have artemisinin part attached to the 4 position of 1,2,3-triazole system and in second series, artemisinin part was attached to 1 position of 1,2,3-triazole system (Figure 3).

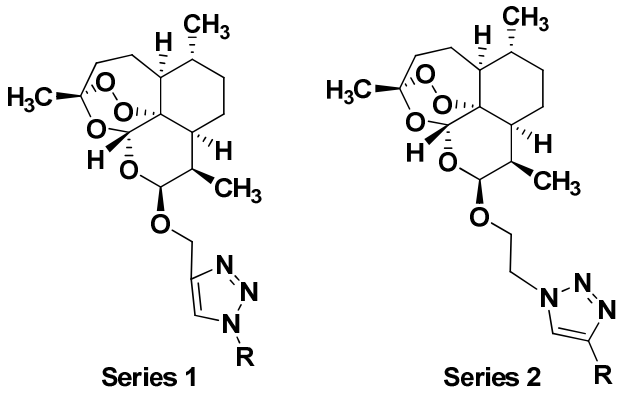

Figure 3. Structures of target molecules.

The synthesis of the series 1 derivatives of artemisinin is depicted in Scheme 1. Artemisinin (**1**) was firstly reduced into DHA (**5**) by using $NaBH_4$ as a reducing agent in methanol at 0 to -5 ºC temperature. Then the propargyl derivative of artemisinin (**6**) was synthesized by the reaction of DHA (**5**) with propargyl alcohol in the presence of $BF_3.OEt_2$ as a catalyst in dry ether at 0-5 ºC temperature. 1,2,3-triazole based artemsinin derivatives were synthesized by Copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) reaction which is the most used click reaction. Targeted compounds (**9-22**) were synthesized by the CuAAC reaction between the compound (**6**) and appropriate aromatic azides (**8a-n**) were carried out in the presence of $CuSO_4.5H_2O$ and sodium ascorbate in dichloromethane and water (5:3). The aromatic azides (**8a-h**) were synthesized by the diazotization of corresponding aromatic amines (**7a-h**) with sodium nitrite followed by the reaction with sodium azide as outline in Scheme 2.

Further the ortho and para substituted azidoacetophenones (**8e-g**) obtained by the above reaction were treated with 2- and 4-substituted trifluoromethyl benzaldehyde to form 2-and 4-substituted azido chalcones (**8i-n**) via the base catalysed Claisen Schmidt condensation reaction (Scheme 2). Generally it was found that 2-trifluoromethyl substituted azido chalcones were formed easily in good yield but the synthesis of 4-trifluoromethyl substituted azido chalcones led to the different side products along with the desired chalcone derivatives. So in that case amino substituted chalcone derivatives were firstly synthesized by Claisen Schmidt reaction of different amino acetophenone with 4-(trifluoromethyl)benzaldehyde,

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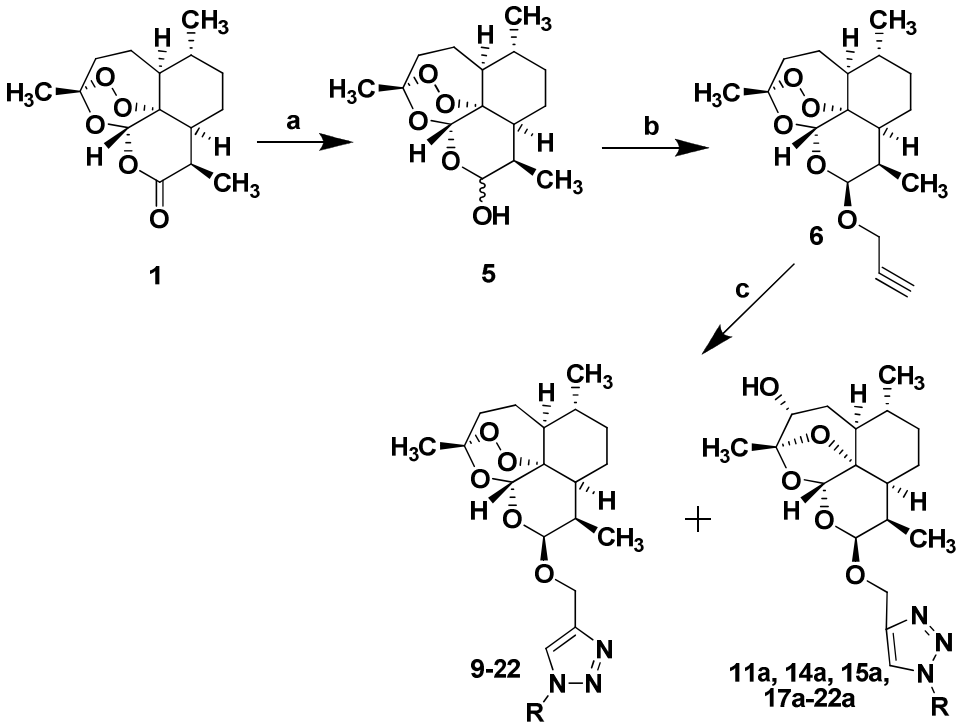

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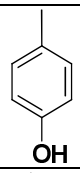
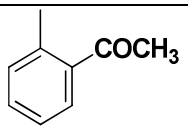
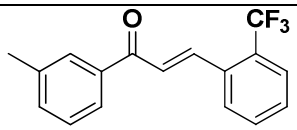
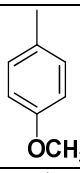
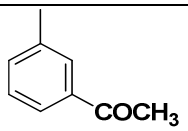
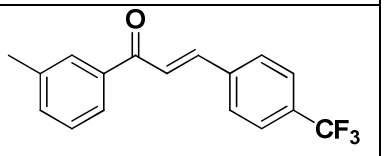
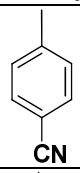
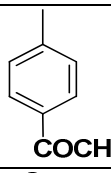
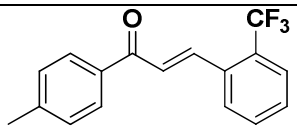
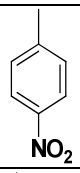
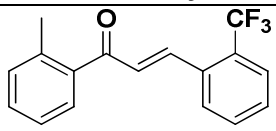
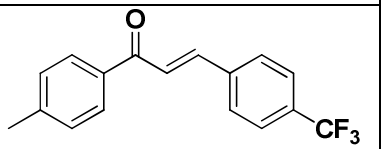
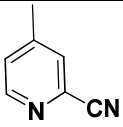
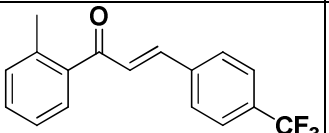
9		14, 14a		19, 19a	
10		15, 15a		20, 20a	
11, 11a		16		21, 21a	
12		17, 17a		22, 22a	
13		18, 18a			

Scheme 1. Reagents and conditions: a) $NaBH_4$, MeOH, 0-5 °C, b) Propargyl alcohol, BF_3Et_2O; c) RN_3, $CuSO_4.5H_2O$, Sodium ascorbate in DCM:H_2O (5:3).

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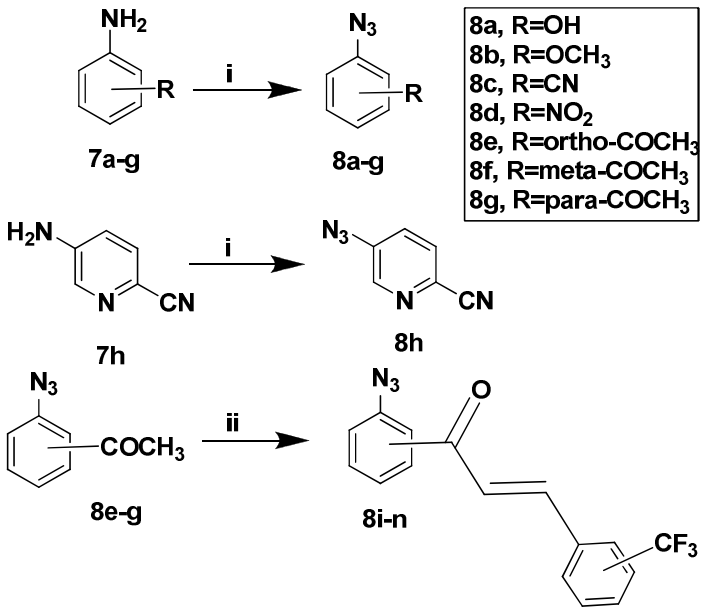

Scheme 2. Reagents and conditions: a) $NaNO_2$, HCl, NaN_3, H_2O:CH_3COOH, b) RCHO, KOH, MeOH

then these 4-trifluoromethyl substituted amino chalcones were converted into the corresponding azides by the diazotization with sodium nitrite followed by the reaction with sodium azide. Synthesis of amino chalcones derivatives from amino acetophenones sometimes required the high temperature (refluxing condition) to complete the reaction.

Series 2 contains mostly dimers (**29-33**) and some monomers (**27, 28**) derivatives of artemisinin were synthesized according to the Scheme 3. Both the monomers and dimers derivatives of artemisinin were prepared by the CuAAc reaction of mono and dipropargyl ether derivatives of different benzene diols (**26a-f**) with 12-β-azidoethoxydihydroartemisinin (**24**). The etherification of benzene diols were accomplished by its reaction with propargyl bromide in the presence of K_2CO_3 as base in acetone under refluxing condition (Scheme 4). For the synthesis of 12-β-azidodihydroartemisinin (**24**), 12-β-(2-bromoethoxy)dihydroartemisinin (**23a** or 12-β-(2-iodoethoxy)dihydroartemisinin, **23b**) was firstly prepared by the etherification of DHA with 2-bromoethanol (or 2-iodoethanol, in case of compound **23b**) in the presence of $BF_3.OEt_2$ as catalyst in dry ether then 12-β(2-bromoethoxy)dihydroartemisinin (**23a**) was reacted with sodium azide in DMSO to afford the 12-β-azidodihydroartemisinin. The CuAAC reaction between the 12-β-azidodihydroartemisinin and different mono and dipropagyl ether derivatives of benzene diols give monomers and dimers derivatives of artemisinin respectively.

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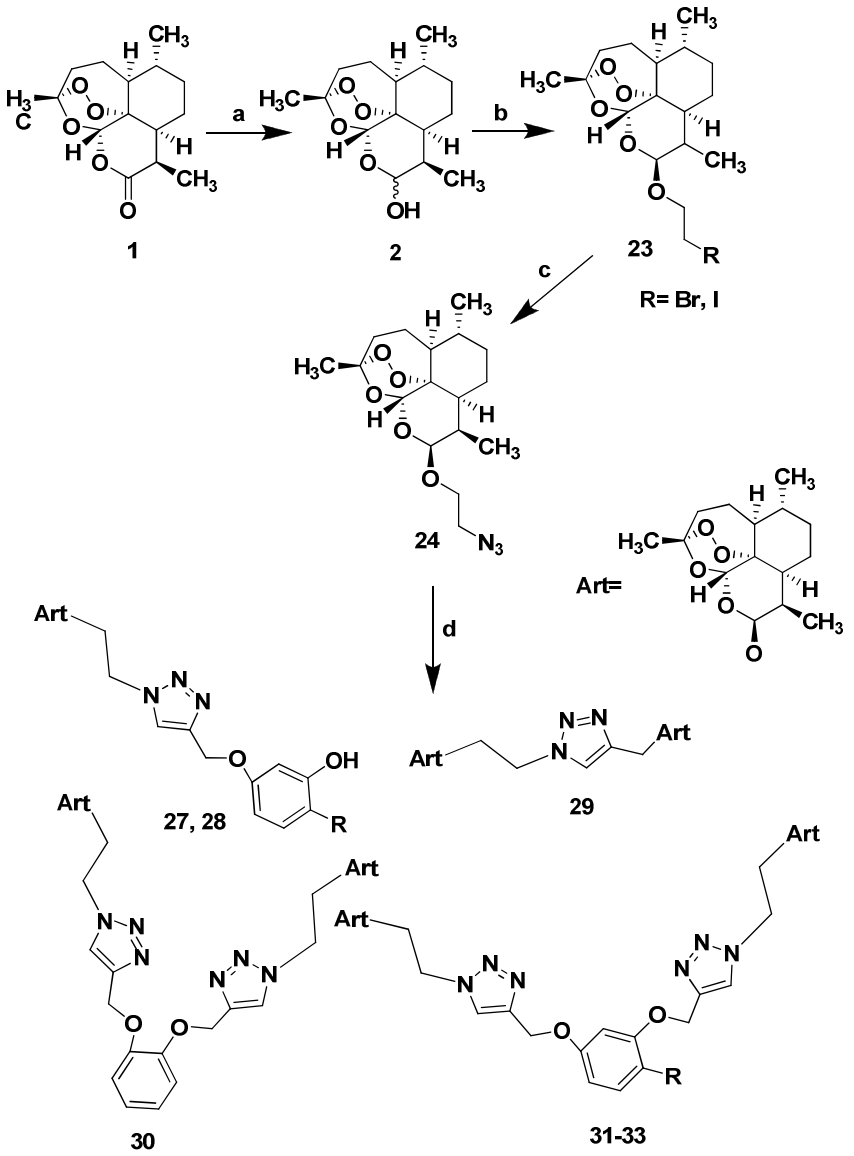

Compounds	R	Compounds	R
27	-H	32	-CHO
28	-CHO	33	-COOH
31	-H		

Scheme 3. Reagents and conditions: a) $NaBH_4$, MeOH, 0 to -5 $^{\circ}C$; b) Bromoethanol (or Iodoethanol), BF_3Et_2O; c) NaN_3, DMSO; d) mono and di propargyl ether derivatives of benzene diol, $CuSO_4.5H_2O$, Sodium ascorbate .

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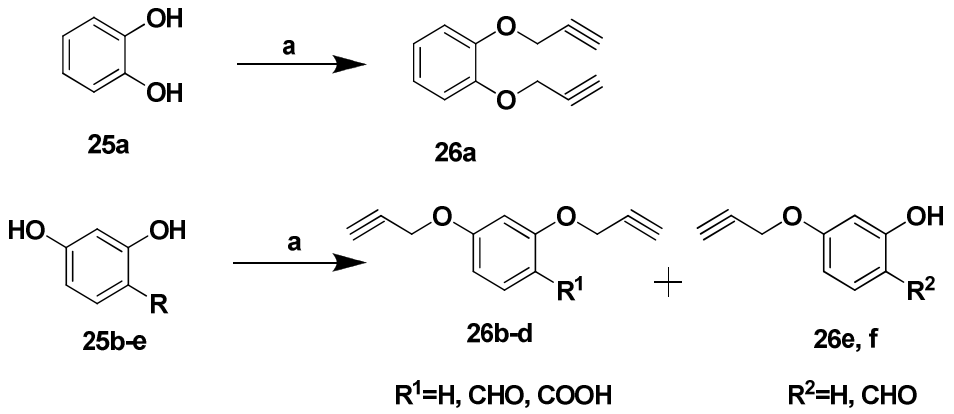

Scheme 4. Reagents and conditions: a) Propargyl bromide, K_2CO_3, acetone, reflux.

The stereochemistry (α or β orientation) of C-12 position of all the synthesized derivatives of artemisinin were determined with the help of ^{1}H NMR spectroscopy. The H-12 proton/s gave a doublet at ~4.90 ppm with the small coupling constant (J~3.5 Hz) which indicated the α orientation of H-12 proton[33-35] so all the synthesized derivatives are 12-β isomers. The high coupling constant values (= ~15.5 Hz) of α-β unsaturated double bond of the compounds having chalcone moiety (**17-22**) indicated the E (trans) isomerism for olefinic double bond.

During the synthesis of artemisinin-triazole derivatives, small amount of 3α-hydroxydeoxydihydroartemisinin ether azole derivatives were also formed whose concentration were increased by increasing the mole equivalent concentration of catalyst ($CuSO_4.5H_2O$ and sodium ascorbate). At 0.5 mole equivalent $CuSO_4.5H_2O$ and 1.5 mole equivalent of sodium ascorbate, peroxy derivatives were completely converted into 3-hydroxydeoxydihydroartemisinin derivatives. They were generally more polar than their peroxy counterpart in TLC analysis. HRMS data indicated that the 3-hydroxydeoxyartemisinin derivatives have the same mass as peroxy derivatives, suggested that they have the same molecular formula. ^{1}H NMR spectra showed a new broad singlet or sometimes doublet peak with small coupling constant (~1.5-2.5 Hz) at around 3.56 ppm suggested that the presence of hydroxyl group. The small coupling constant values of C-3 proton/s indicated its equatorial (β) orientation thus the hydroxyl group has α (axial) orientation.[36-39] ^{13}C and DEPT-135 spectra showed new peak at ~69.50 ppm in place of CH_2 of C-3 carbon peak at ~36.20 ppm indicated that the CH_2 group of C-3 has been hydroxylated into CHOH group at C-3 position. The NMR data of 3α-hydroxydeoxydihydroartemisinin-triazoles derivatives showed quite similarity with those reported for 3α-hydroxydeoxyartemisinin.[36] A plausible mechanism of seteroselective alpha hydroxylation of

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C-3 position of artemisinin derivatives has given in figure 4. Some of the 3-hydroxydeoxyartemisinin triazole derivatives (**11a, 14a, 15a, 17a-22a**) have been synthesized along with their peroxy counterparts to know the effect of endoperoxide bond on antiproliferative activity.

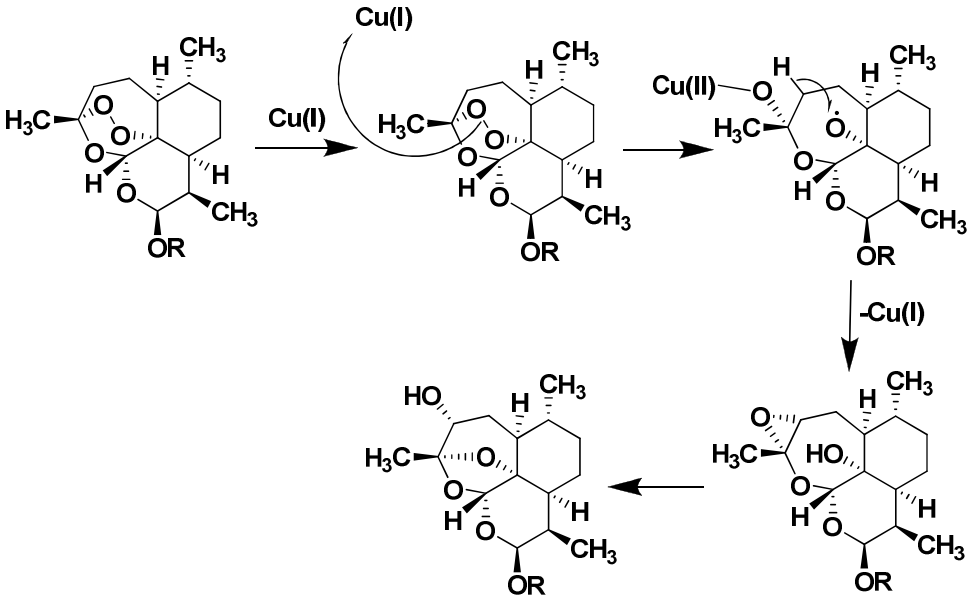

Figure 4. Plausible mechanism of alpha hydroxylation at C-3 position.

2.2. Artemisinin derivatives inhibited the cell proliferation in various cancer cell lines

Antiproliferative effect of artemisinin derivatives series 1 and 2 were analysed using MTT assay in various organ specific cancer cell lines namely K562 (human erythromyeloblastoid leukemia cell line), PC-3 (human prostate carcinoma), A431 (human squamous carcinoma), MDAMB-231 (human breast carcinoma), COLO-205 (human colon carcinoma), A549 (human lung carcinoma) and one normal cell line, HEK-293 (human kidney cells). In series 1, Compound **9**, **15**, **17** and **22** were able to inhibit the proliferation of colon, skin, leukaemia, breast, lung and prostate cancer cells with the IC_{50} ranged from 4.06-89.85 μM (Table 1). Compound **10**, **21** and **22a** were reducing the proliferation of skin and breast cells with the IC_{50} ranged from 17.97-52.86 μM whereas compound **21a** inhibits the proliferation of A431 and PC-3 cell line with the IC_{50} 57.18 and 25.31 μM respectively. Compound **11a** and **13** were selectively inhibiting the skin carcinoma with the IC_{50} values 68.27 μM and 43.42 μM respectively. Compound **17a** showed selectivity for breast cancer cells with IC_{50} of 43.19 μM while rest of the molecules are also inhibit the proliferation of cells with percent inhibition ranged from 3.30–49.60% (Figure 5). In series 2, among all the synthesized compounds only compound **27** was displayed broad spectrum efficiency by inhibiting the growth of lung,

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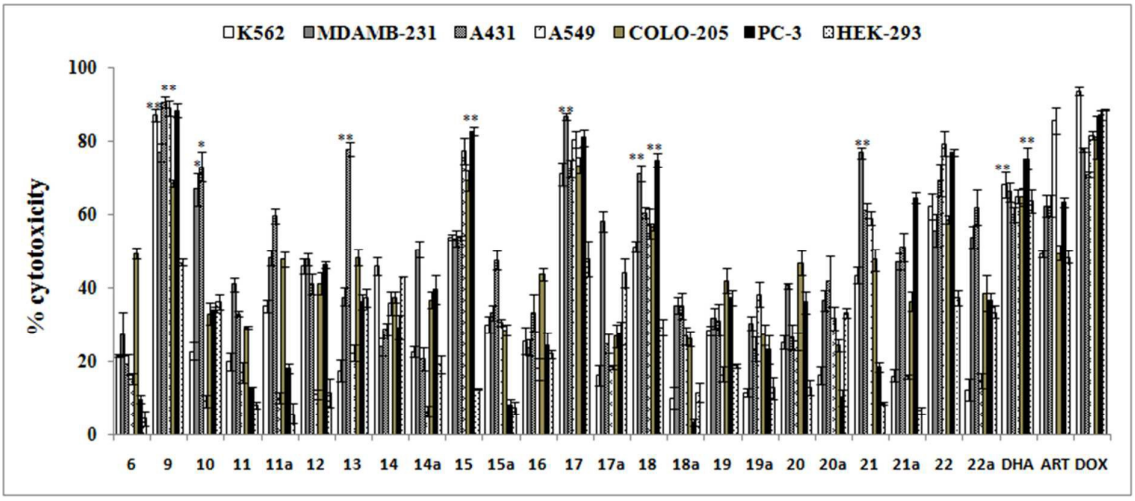

Figure 5. Antiproliferative effect of artemisnin-triazole derivatives (Series 1) on various organ specific cell line at higher tested concentration of 50 µg/ml. ART: Artemisnin, DHA: dihyroartemisnin, DOX: Doxorubicin used as positive control. ART. All values are mean ± SD (n=3). *Comparison of test with artemisnin (Parent). **p<0.05

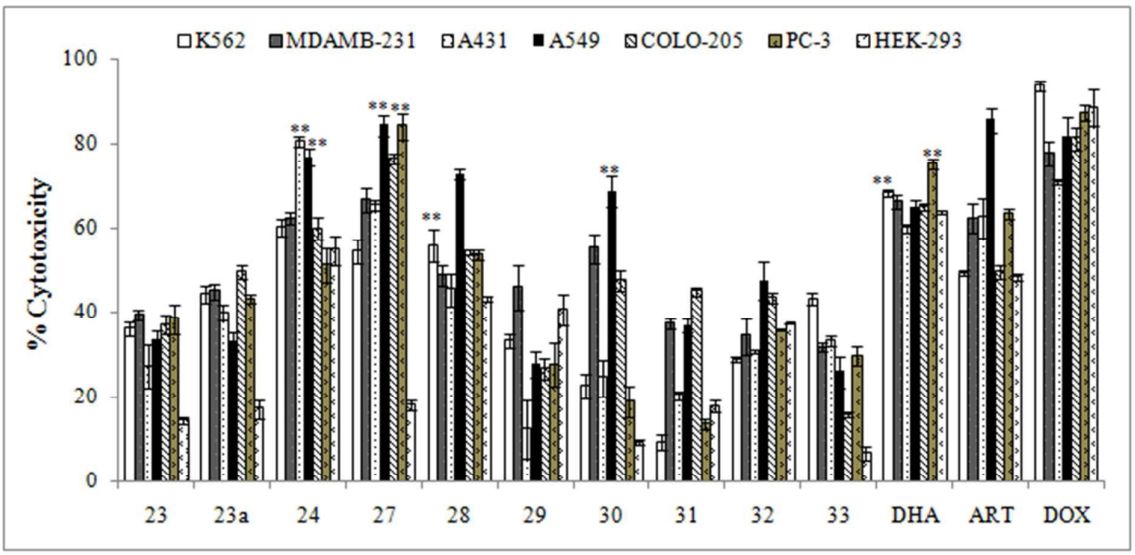

Figure 6. Antiproliferative effect of artemisnin-triazole derivatives (series-2) on various organ specific cell lines at higher tested concentration of 50µg/ml. ART: Artemisnin, DHA: dihyroartemisnin, DOX: Doxorubicin used as positive control. All values are mean ± SD (n=3). *Comparison of test with artemisnin (Parent). **p<0.05

colon, breast, leukaemia, skin and prostate cancer cells with IC_{50} ranged from 16.20-59.93 µM while compound **28** hamper the proliferation of prostate, leukaemia, lung and colon cancer cells (21.45-77.30 µM). Compound **29** was found to be selective for breast (49.98 µM) and lung (20.95 µM) cancer cells whereas rest was inhibited the proliferation up to 47.85 % (Figure 6). Interestingly, all the synthesized derivatives of series 1 and series 2 were

Table 1. Antiproliferative potential of Arteimisinin and their derivatives

Compound	IC_{50} (μM)						
Series 1	**K562**	**PC-3**	**A431**	**MDAMB-231**	**COLO-205**	**A549**	**HEK-293**
Compound 9	11.34$^{***/\#\#\#}$	10.36$^{\#\#\#}$	4.06$^{***/\#\#\#}$	15.43$^{\#\#\#}$	36.65$^{\#\#\#}$	12.43$^{***/\#\#\#}$	-
Compound 10	-	-	52.81	44.04$^{\#\#\#}$	-	-	-
Compound 11a	-	-	68.27	-	-	-	-
Compound 13	-	-	43.42	-	-	-	-
Compound 15	78.37	21.67$^{\#\#\#}$	73.87	89.85	45.57$^{\#\#\#}$	44.11	-
Compound 17	28.21$^{\#\#\#}$	11.55$^{\#\#\#}$	20.93	13.35$^{\#\#\#}$	13.28$^{\#\#\#}$	7.16$^{***/\#\#\#}$	-
Compound 17a	-	-	-	43.19$^{\#\#\#}$	-	-	-
Compound 18	69.67	21.12$^{\#\#\#}$	37.30	27.32$^{\#\#\#}$	50.58$^{\#\#\#}$	49.49	-
Compound 21	-	-	47.25	17.97$^{\#\#\#}$	-	57.03	-
Compound 21a	-	25.31$^{\#\#\#}$	57.18	-	-	-	-
Compound 22	44.17	17.14$^{\#\#\#}$	31.97	56.59	32.43$^{\#\#\#}$	25.93$^{\#\#\#}$	-
Compound 22a	-	-	37.37	39.20$^{\#\#\#}$	-	-	-
Compound 24	52.65	100.22	42.27	37.80$^{\#\#\#}$	68.81	44.42	106.75
Series 2							
Compound 27	59.93	17.98$^{\#\#\#}$	25.71	38.23$^{\#\#\#}$	28.72$^{\#\#\#}$	16.20$^{\#\#\#}$	-
Compound 28	53.38	77.30	-	-	60.46	21.45$^{\#\#\#}$	-
Compound 29	-	-	-	49.48$^{\#\#\#}$	-	20.95$^{\#\#\#}$	-
DHA	42.44	52.22	15.74	50.99	56.23	43.43	77.19
ART	-	47.67	68.82	39.03	-	-	-
DOX	11.99	1.43	7.81	5.13	5.39	6.36	7.17

DHA- dihyroarteimisnin, ART- Arteimisinin, DOX-doxorubicin. Data represents mean value of two independent evaluations in triplicates. ***Comparison between DOX and compounds. $^{\#\#\#}$ Comparison between DHA and synthesized derivatives

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nontoxic against the normal cell line kidney cells except compound **24** and DHA with IC_{50} values 106.75 and 77.19 μM respectively. Compound **9** (IC_{50} range from 4.06 to 36.65 μM) and **17** (IC_{50} range from 7.16 to 28.21 μM) were the most active, almost 1.5 to 28 times active than parent compound DHA (IC_{50} range from 42.44 to 115.74 μM). Compound **9** has the highest activity against skin carcinoma cell line with IC_{50} 4.06 μM and **17** has the highest activity against lung carcinoma with IC_{50} 7.16 μM therefore these compounds were selected for further mechanistic evaluation.

Regarding the SAR of synthesized derivatives, in Series 1, compound **9** having phenolic OH group at para position of benzene ring, exhibited the highest antiproliferative activity as compared to the other derivatives. Introduction of OCH_3, CN, NO_2 group at para position and $COCH_3$ groups at three different positions (ortho, meta and para) led the reduction in antiproliferative activity relative to that of compound **9**, expect compound **15** having $COCH_3$ group at meta position showed comparable activity against the PC-3 and COLO-205 cell lines. Comparing ortho, meta and para isomers, **14**, **15**, and **16** respectively, compound **14** was found to be the most active among them. Chalcones (**17-22**) with ortho and para substitution on A ring are generally more active than with meta substituted compounds. Compound **17** with ortho substitution at both the ring (A & B ring) is the most active. A comparison of antiproliferative activity of 3-hydroxydeoxydihydroartemisinin ether azole derivatives (**11a, 14a, 15a, 17a-22a**) with derivatives with peroxy functionality suggested that compounds with intact endoperoxide bonds are more active than their deoxy counterparts expect the compound **11a** showed better activity than compound **11**. Our finding showed the strong agreement with the previous reports, where the anticancer activity of artemisinin derivatives were attributed to the presence of endoperoxide linkage and the absence of endoperoxide bond does not completely abolish the anticancer activity but significantly reduce it.[6-9] Compound **11a** might be follow peroxide independent mechanism. In Series 2, monomers were found to be more potent than their dimers. Compounds **27**, **28** and intermediate **24** were more active than other derivatives.

2.3. Compound 9 and 17 arrests G2/M phase and induce apoptosis in A431 and A549 cell line

The effect of most active compound **9** on A431 and **17** on A549 have been studied by cell cycle analysis using PI labelling which is used for pseudohypodiploid cells quantification. Treatment of compound **9** with IC_{50} and double of IC_{50} for 24 h significantly altered the

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phases of cell cycle (Figure 7A). The percentage of sub-diploid cells was increased after the treatment of compound **9** at both the tested concentration with the 6.1 fold and 5.6 fold respectively in A431 cells. Simultaneous reduction of cells at G0/G1 phase was also observed with compound **9** (1.8 to 2.0 fold) at both concentrations with respect to control but there is no significant alteration observed in S phase. Compound **9** significantly increases the number of cells at G2/M phase to 2.5 fold at 4.06 μM and 2.9 fold at 8.12 μM concentration compared to control (Figure 7B). Likewise compound **17** in A549 cells at both the concentration IC_{50} (7.16 μM) and double of IC_{50} (14.32 μM) increase sub-diploid cells 3.4 fold and 6.8 fold respectively. Compound **17** decrease the cells 2.23-2.27 fold in G0/G1phase at both the concentration with compare to control whereas no significant alteration was found in S-phase (Figure 8A). Compound **17** significantly increase to 1.6 fold with 7.16 μM and 1.2 fold with 14.32 μM compared to control at G2/M phase (Figure 8B). Interestingly, at IC_{50} (7.16 μM) concentration, compound **17** significantly arrest the G2/M phase with increase in apoptosis whereas at double of IC_{50} (14.32 μM), cells are arrested in G2/M phase but decrease in the apoptotic cells. From the results, we can conclude that the compounds **9** and **17** inhibited the cell proliferation via the increase in apoptosis and cell cycle arrest at G2/M phase. Artemisinin compounds have been reported to exert both the cytostatic and cytotoxic effects on cancer cells and induced growth arrest in cell cycle phases (A1, A2).[19,40] Our findings are similar with the Yao et al., 2008 and Jiao et al., 2007, who have reported that DHA arrest the cell cycle at G2/M phase against the leukemia (K562 cells) and ovarian cancer cells.[41,42] In another report, 200 μM DHA induced apoptosis in leukemia cells.[21]

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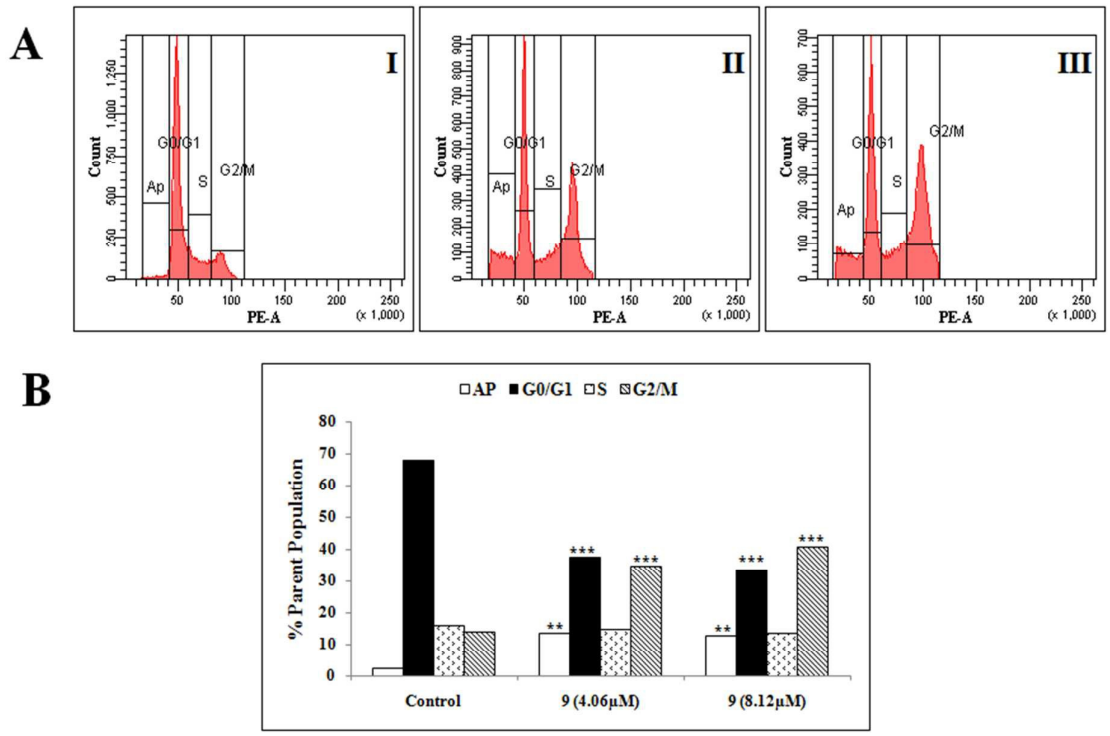

Figure 7. Effect of compound **9** on cell cycle in A431 cell line. Cells were treated with compound **9** at 4.06 µM (IC_{50}) and 8.16 µM (double of IC_{50}) concentrations for 24 h. Cells were collected and stained with Propidium iodide (PI) for flow cytometric analysis. **(A)** Represent the pictorial graphs obtained from cell cycle analysis using FACS Diva software **(B)** Represents percent population in different phase of cell cycle after the treatment with compound **9**. The result is one representative example of two separate experiments. *Comparison of test with control. **p<0.05

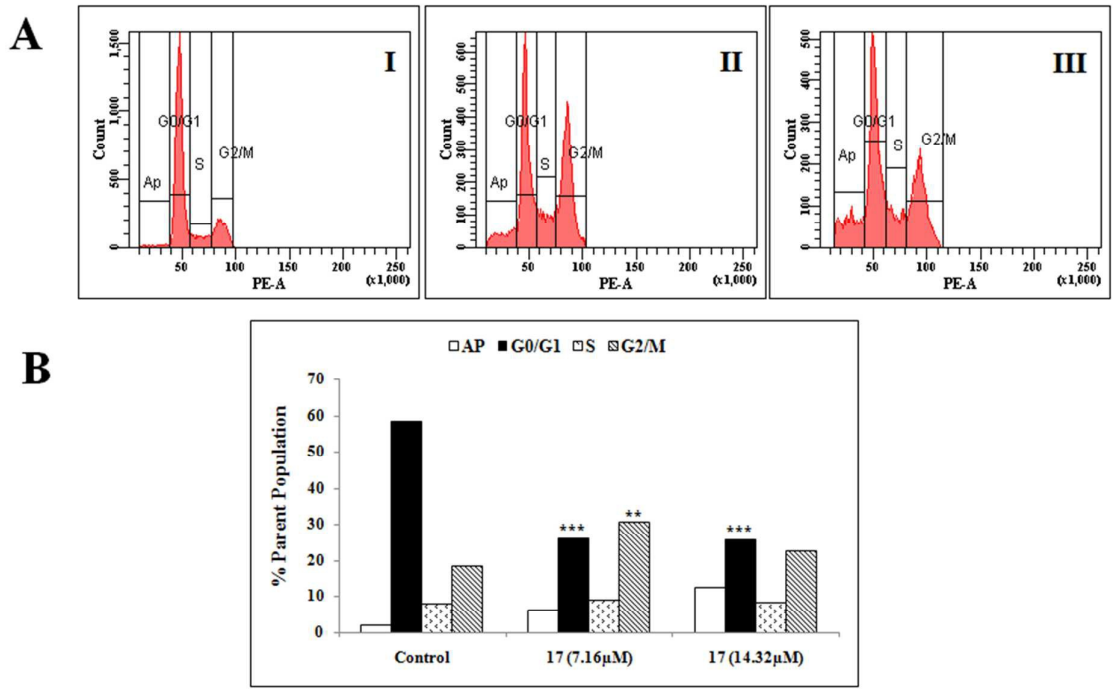

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Figure 8. Cell cycle analysis of compound **17** in A549 cell line. Cells were treated with compound 17 at 7.16 µM (IC_{50}) and 14.32 µM (double of IC_{50}) concentrations for 24 h. Cells were collected and stained with Propidium iodide (PI) for flow cytometric analysis. **(A)** Represent the pictorial graphs obtained from cell cycle analysis using FACS Diva software **(B)** Represents percent population in different phase of cell cycle after the treatment with compound **17**. The result is one representative example of two separate experiments. *Comparison of test with control.

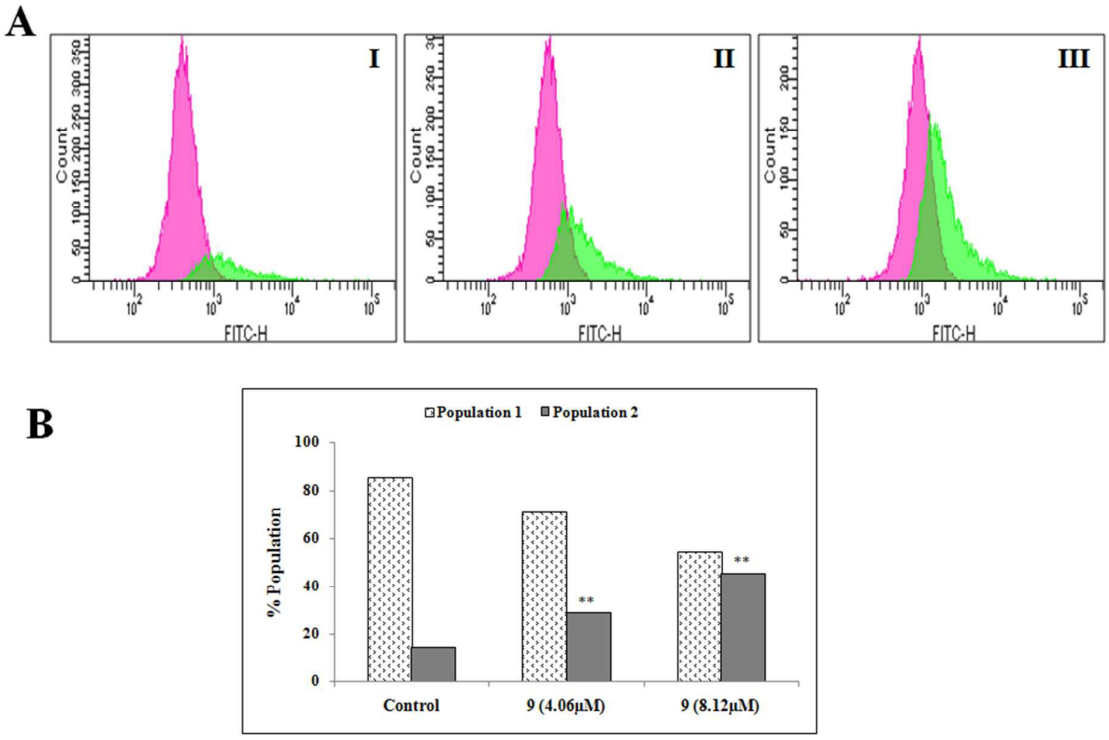

Figure 9. Compound **9** induced ROS generation in skin carcinoma (A431). **(A)** Represents the images obtained during recordings of DCF fluorescence intensity reflecting the ROS level. **(B)** Represents percent population of DCF positive cells shifted towards green channel and non-DCF positive cells. The result is one representative example of two separate experiments. *Comparison of test with control.

2.4. Compound 9 and 17 induced ROS formation in A431 and A549 cell line

ROS involved in various stages in the apoptotic pathway such as caspase activation, release of mitochondrial apoptogenic factors, DNA damage etc.[43,44] Therefore we further investigate whether compound **9** in A31 and Compound **17** in A549 exert its apoptotic effect by inducing the ROS, the ROS levels was measured using DCFDA dye. In flow cytometric analysis, the ROS level at IC_{50} (4.06 µM) and double of IC_{50} (8.12 µM) of compound **9** was significantly increased which indicated by number of DCF positive cells (Figure 9A & B). The DCF positive cells were 28.7% and 45.2% at 4.06 µM and 8.12 µM respectively with respect to

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control (14.3%). Similarly Compound **17** in A549 cells at both the concentration IC_{50} (7.16µM) and double of IC_{50} (14.32 µM) increased ROS level 23.2% and 38.8% respectively in compare to control (15.4%, Figure 10A & B). Mercer et al., 2007 and 2011 reported that reactive oxygen species and free radicals (especially highly alkylated carbon centered free radical) released from iron activated artemisinin which leads to cell alterations.[9,13] Results showed that both the compounds **9** and **17** are able to induce the ROS production therefore might be responsible for apoptosis. Previous reports also in accordance to our findings, Xu et al., 2011 & Bostwick et al., 2000 reported that increased oxidative stress due to ROS production associated with the artemisinin cytotoxicity and might be responsible for the selective action of artemisinin on cancer cells.[14,45]

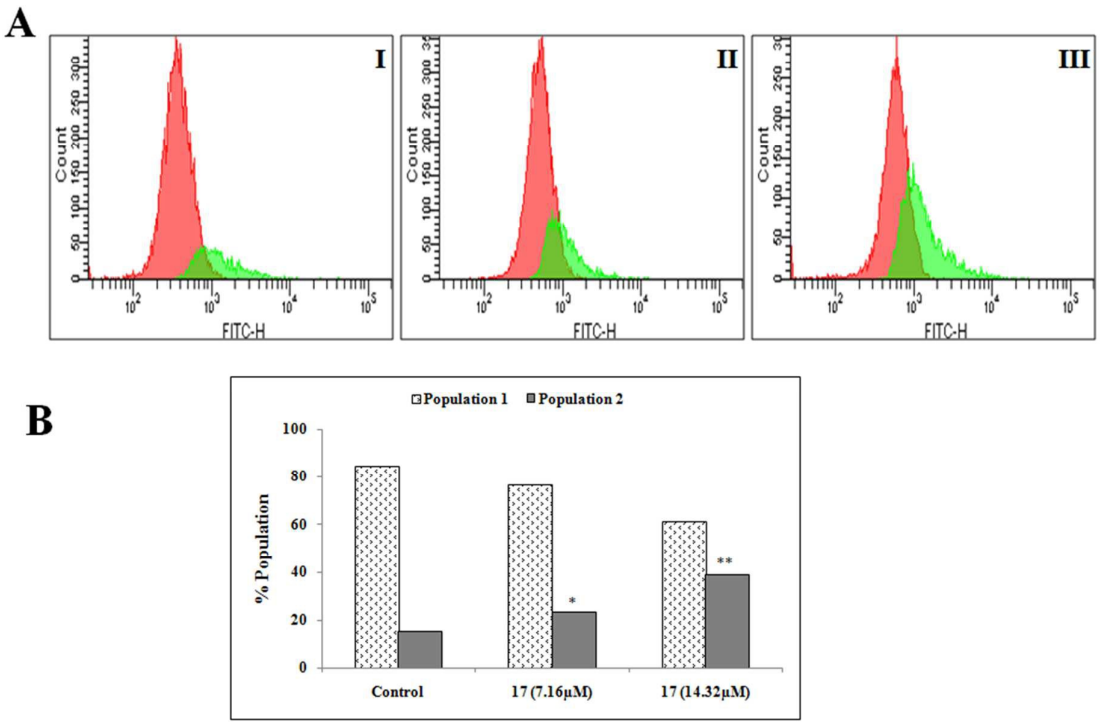

Figure 10. Compound **17** induced ROS generation in lung cancer cells (A549). (A) Represents the images obtained during recordings of DCF fluorescence intensity reflecting the ROS level. (B) Represents percent population of DCF positive cells shifted towards green channel and non-DCF positive cells. The result is one representative example of two separate experiments. *Comparison of test with control

2.5. Compound 9 and 17 were non-toxic to human erythrocytes

The toxicity of the both the potent compounds **9** and **17** were also determined with erythrocyte osmotic fragility evaluation (Figure 11). Both the compounds **9** and **17** were found to be non-toxic to human erythrocytes even at higher concentrations (100 µg/ml) with lower MEF_{50} 0.57 and 0.55 respectively with respect to PDT (MEF_{50} 0.68; Table 2).

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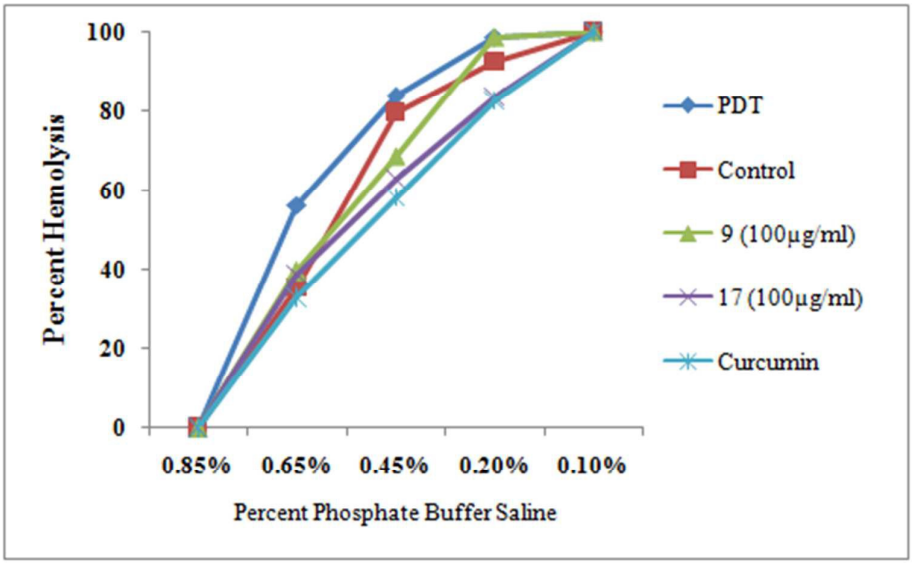

Figure 11. Erythrocyte osmotic fragility curve of Artemisnin derivatives compound **9** and **17**, Curcumin and Podophyllotoxin (PDT).

Table 2. Mean Erythrocyte Fragility (MEF) of active derivatives of Arteimisinin (Compound 9 & 17)

Samples	Concentration	MEF_{50}
Compound 9	100µg/ml	0.57
Compound 17	100µg/ml	0.55
Control	-	0.58
Podophyllotoxin	100µg/ml	0.67
Curcumin	100µg/ml	0.51

3. Conclusion

In conclusion, two novel series of artemisinin-1,2,3-triazole based derivatives have been synthesized and explored as potent antiproliferative agents. The compound **9** and **17** exhibited potent antiproliferative activity. Both the compounds induced apoptosis along with the cell cycle arrest. The active compounds **9** and **17** are also able to induce ROS in tested cell lines which suggested that the inhibition cellular proliferation by compound **9** and **17** possibly linked with their ROS generation. Interestingly, both the compounds were non-toxic for normal kidney cells (HEK-293). Additionally, Erythrocyte osmotic fragility assay confirms the non-toxic nature of both the compounds **9** and **17** at higher concentration of 100 µg/ml. This study may contribute to further design and development of potential artemisinin-triazole based derivatives in future with higher therapeutic index. Since toxicity is the major concern for existing anticancer drugs and our results suggested that compounds **9** and **17** are

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non toxic at higher concentration so these compounds could be taken up as a lead for further evaluation and validation against skin and lung cancer.

4. Experimental:

4.1. General materials & methods

Melting points were recorded on a Toshniwal melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance, 500 MHz instrument using $CDCl_3$, Acetone-d_6 as a solvent and TMS as an internal standard. The chemical shift values (δ) are determined in ppm and coupling constants values are reported in Hz. HRMS spectra were recorded on Agilent 6545 Q-TOF LC/MS mass spectrometer. TLC analyses were carried out on Merck aluminum sheet TLC plate*s* using silica gel coated with flourescent indicator F254 plates. The compounds on TLC plates were visualized by vanillin–sulfuric acid spraying reagent. Reaction products were purified by column chromatography using 60-120 mesh size Silica-gel. The structures of synthesized compounds were established by their ^{1}H, ^{13}C, DEPT-135 and COSY NMR spectroscopic analysis. All the chemicals and dried solvents used for the reaction work were purchased from Sigma Aldrich India Ltd and Meck India Ltd and used without further purification. DMEM (Dulbecco's modified essential eagle medium), RPMI-1640() and FBS (fetal bovine serum) were purchased from Gibco, India. MTT dye, trypsin-EDTA, Propidium iodide (PI), 2',7'-dichlorofluorescin diacetate (DCFDA), antibiotic/antimycotic (Ab/Am) Sodium di-hydrogen phosphate (NaH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4) and phosphate buffer saline (PBS) were obtained from Sigma Aldrich, India Ltd. Sodium bicarbonate, Heparin HEPES were acquired from Himedia, India. Solvents such as Dimethyl sulphoxide (DMSO), ethanol, formaldehyde, isopropanol were purchased from Merck, India Ltd.

4.2. Synthesis of 12-β-(prop□2□yn□1□yloxy)-dihydroartemisinin

To a solution of DHA (5 gram, 17.59 mmol) in dry Et_2O at 0 ºC temperature was added 1.97 gram, 35.18 mmol of propargyl alcohol and 8 ml of $BF_3.OEt_2$ dropwise. The reaction mixture was stirred at same temperature for 6 hrs. After completion of reaction, the reaction mixture was washed with 5 % $NaHCO_3$ solution, then water and brine solution, dried over anhydrous Na_2SO_4. The organic layer was concentrated in vaccum. The residue was purified by column chromatography using 60-120 mesh size silica gels as absorbent.

12-β-(prop□2□yn□1□yloxy)-dihydroartemisinin: White crystalline solid; yield 82%; mp 95-97 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.40 (1H, s, H-5), 4.96 (1H, d, J=3.5 Hz, H-12),

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4.30 (1H, d, J=1.5 Hz, H-1b'), 4.29 (1H, d, J=1.5 Hz, H-1a'), 2.65 (1H, m, H-11), 2.38 (1H, s, H-3'), 1.43 (3H, s, H_3-15), 0.93 (3H, d, J=6.5 Hz, H_3-14), 0.92 (3H, d, J=2.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=104.16 (C-4), 100.61 (C-12), 88.07 (C-5), 81.06 (C-6), 79.77 (C-2'), 73.97 (C-3'), 54.96 (C-1'), 52.54 (C-1), 44.34 (C-7), 37.41 (C-10), 36.40 (C-3), 34.61 (C-9), 30.59 (C-11), 26.14 (C-15), 24.68 (C-2), 24.45 (C-8), 20.34 (C-14), 12.81 (C-13).

4.3. General procedure for synthesis of aromatic azides, 8a-h

7.40 mmol of aromatic amines were dissolved in 5 ml of concentrated HCl and 5 ml of CH_3COOH solution and the reaction mixture was kept at 0ºC. Then the solution of 11.10 mmol of sodium nitrite ($NaNO_2$) dissolved in 5 ml water was added dropwise into the reaction mixture. A reddish brown coloured gas was evolved. The reaction mixture was stirred for 30 minutes at 0ºC. Then 14.8 mmol of Sodium azide (NaN_3) dissolved in 5ml of water was added into the reaction mixture dropwise. After that reaction mixture was stirred for 2-4 hrs at room temp. After completion of reaction, the reaction mixture was extracted with dichloromethane and the combined organic layer was washed with water, then brine solution and dried over anhydrous sodium sulphate. The crude product was obtained almost as a single product and used in further step without purification.

4.4. General procedure for the synthesis of different substituted azido chalcones, 8i-8n:

For the synthesis of different substituted azidochalcones, 6.21 mmol of 2, 3 & 4 substituted azido acetophenones were dissolved in 10 ml of methanol at room temperature and 6.83 mmol of 2 or 4-substituted trifluoromethyl benzaldehyde was added into the reaction mixture. Then 62.10 mmol of KOH was added and the reaction mixture was stirred for 2-4 hrs. After completion of reaction, the resulting mixture was poured into ice –cold water and acidified by addition of 1 N HCl. The crude product was extracted with dichloromethane (50 ml x 3) and the organic layer was washed with cold water, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on 60-120 mesh size silica gels to get the desired products for further reaction.

Synthesis of chalcones with 4-trifluoromethyl benzaldehyde gave different side product so in that case chalcones were formed by the Clasien Schmidt reaction of different amino substituted acetophenones with 4-trifluoromethyl benzaldehydes followed by the diazotisation and then azide formation of the product as the procedure described for the synthesis of aromatic azides.

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4.5. General procedure for the synthesis of Series 1 type of artemisinin-azole derivatives, 9-22

A mixture of Compound **6** (0.621 mmol), appropriate aromatic azide (**8a-8i**, 0.931 mmol) and hexadecyltrimethylammonium bromide (0.310 mmol) was taken in dichloromethane (5ml) and stirred for 5 min at room temperature. $CuSO_4.5H_2O$ (0.0621 mmol, 10 mol%) and sodium ascorbate (0.156 mmol, 25 mol%) were dissolved in 3 ml of water and added to the reaction mixture. The resulting solution was stirred for 1-2 hrs at room temperature. After completion of reaction, the reaction mixture was extracted with dichloromethane (25ml x 3 times) and organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified on silica gel (60-120 mesh size) column chromatography using EtOAc/Hexane as eluent to afford the target compounds (comp **9** to **22**). For the synthesis of 3α-hydroxydeoxydihydroartemisinin derivatives (**11a, 14a, 15a, 17a-22a),** the same procedure as described above was followed except 0.310 mmol of $CuSO_4.5H_2O$ and 0.776 mmol of sodium ascorbate were used.

4-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenol (9): White solid; yield 66%; mp 125-128 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.84 (1H, s, H-3'), 7.56 (2H, m, H-5', H-9'), 7.01 (2H, m, H-6', H-8'), 6.72 (1H, brs, OH, D_2O exchangeable), 5.46 (1H, s, H-5), 4.99 (1H, d, J=12.5 Hz, H-1b'), 4.95 (1H, d, J=3.5 Hz, H-12), 4.74 (1H, d, J=12.5 Hz, H-1a'), 2.67 (1H, m, H-11), 1.45 (3H, s, H_3-15), 0.92 (1H, d, J=6.5 Hz, H_3-14), 0.90 (3H, d, J=7.5 Hz, H-13).^{13}C NMR (125 MHz, $CDCl_3$): δ=156.75 (C-7'), 145.34 (C-2'), 130.25 (C-4'), 122.49 (C-5', C-9'), 121.13 (C-3'), 116.42 (C-6', C-8'), 104.25 (C-4), 101.74 (C-12), 88.06 (C-5), 81.17 (C-6), 61.51 (C-1'), 52.54 (C-1), 44.39 (C-7), 37.36 (C-10), 36.43 (C-3), 34.56 (C-9), 30.87 (C-11), 26.14 (C-15), 24.68 (C-2), 24.47 (C-8), 20.32 (C-14), 12.99 (C-13); HRMS (ESI) calcd for $C_{24}H_{31}N_3O_6$ $[M + H]^+$ 458.2291, found 458.2289.

4-(12-β-dihydroartemisinoxymethyl)-1-(4-methoxyphenyl)-1H-1,2,3-triazole (10): Light brown solid; yield 67%; mp 84-86 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.85 (1H, s, H-3'), 7.60 (2H, d, J=8.5 Hz, H-5', H-9'), 7.00 (2H, d, J=8.5 Hz, H-6', H-8'), 5.43 (1H, s, H-5), 4.98 (1H, d, J=12.5 Hz, H-1b'), 4.95 (1H, d, J=3.5 Hz, H-12), 4.73 (1H, d, J=12.5 Hz, H-1a'), 3.85 (3H, s, OCH_3), 2.67 (1H, m, H-11), 1.44 (3H, s, H_3-15), 0.91 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.82 (C-7'), 145.54 (C-2'), 130.57 (C-4'), 122.23 (C-5', C-9'), 120.96 (C-3'), 114.77 (C-6', C-8'), 104.15 (C-4), 101.81 (C-12), 88.01 (C-5), 81.13 (C-6), 61.71 (C-1'), 55.64 (OCH_3), 52.55 (C-1), 44.41 (C-7), 37.37 (C-10), 36.43 (C-3), 34.58

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(C-9), 30.87 (C-11), 26.17 (C-15), 24.68 (C-2), 24.47 (C-8), 20.32 (C-14), 13.01 (C-13); HRMS (ESI) calcd for $C_{25}H_{33}N_3O_6$ $[M+H]^+$ 472.2448, found 472.2439.

4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)benzonitrile (11): White solid; yield 68%; mp 159-161 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.03 (1H, s, H-3'), 7.92 (2H, d, J=8.5 Hz, H-6', H-8'), 7.84 (2H, d, J=8.5 Hz, H-5', H-9'), 5.42 (1H, s, H-5), 5.00 (1H, d, J=12.5 Hz, H-1b'), 4.96 (1H, d, J=3.5 Hz, H-12), 4.77 (1H, d, J=12.5 Hz, H-1a'), 2.68 (1H, m, H-11), 1.47 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MH_Z, $CDCl_3$): δ=146.60 (C-2'), 139.86 (C-4'), 133.94 (C-6', C-8'), 120.60 (C-3'), 120.50 (C-5', C-9'), 117.70 (CN), 112.42 (C-7'), 104.24 (C-4), 102.04 (C-12), 88.03 (C-5), 81.09 (C-6), 61.57 (C-1'), 52.49 (C-1), 44.33 (C-7), 37.40 (C-10), 36.39 (C-3), 34.54 (C-9), 30.85 (C-11), 26.17 (C-15), 24.68 (C-2), 24.48 (C-8), 20.32 (C-14), 13.01 (C-13); HRMS (ESI) calcd for $C_{25}H_{30}N_4O_5$ $[M+H]^+$ 467.2294, found 467.2275.

4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)benzonitrile (11a): White solid; yield 64%; mp 178-180 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.99 (1H, s, H-3'), 7.91 (2H, dd, J=8.5, 2.0 Hz, H-6', H-8'), 7.83 (2H, dd, J=8.5, 2.0 Hz, H-5', H-9'), 5.31 (1H, s, H-5), 5.00 (1H, d, J=12.5 Hz, H-1b'), 4.93 (1H, d, J=4.5 Hz, H-12), 4.72 (1H, d, J=12.5 Hz, H-1a'), 3.57 (1H, m, H-3), 2.49 (1H, m, H-11), 1.34 (3H, s, H_3-15), 0.94 (3H, d, J=6.5 Hz, H_3-14), 0.87 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=146.70 (C-2'), 139.83 (C-4'), 133.95 (C-6', C-8'), 120.56 (C-5', C-9'), 120.23 (C-3'), 117.69 (CN), 112.43 (C-7'), 108.07 (C-4), 99.55 (C-12), 93.90 (C-5), 84.12 (C-6), 69.58 (C-3), 61.49 (C-1'), 42.43 (C-1), 40.63 (C-7), 34.88 (C-10), 34.69 (C-9), 30.34 (C-2, C-11), 25.06 (C-8), 20.98 (C-14), 18.81 (C-15), 12.29 (C-13); HRMS (ESI) calcd for $C_{25}H_{30}N_4O_5$ $[M+H]^+$ 467.2294, found 467.2293.

4-(12-β-dihydroartemisinoxymethyl)-1-(4-nitrophenyl)-1H-1,2,3-triazole (12): Yellow solid; yield 67%; mp 128-130 ºC; ^{1}H NMR (500 MHz, Acetone d_6): δ=8.76 (1H, s, H-3'), 8.47 (2H, dd, J=7.0, 1.2 Hz, H-5', H-9'), 8.24 (2H, dd, J=7.0, 1.2 Hz, H-6', H-8'), 5.47 (1H, s, H-5), 4.97 (1H, d, J=12.5 Hz, H-1b'), 4.92 (1H, d, J=3.5 Hz, H-12), 4.73 (1H, d, J=12.5 Hz, H-1a'), 2.65 (1H, m, H-11), 1.33 (3H, s, H_3-15), 0.92 (3H, d, J=6.5 Hz, H_3-14), 0.89 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, Acetone d_6): δ=148.23 (C-7'), 147.36 (C-2'), 142.51 (C-2'), 126.39 (C-6', C-8'), 122.84 (C-3'), 121.64 (C-5', C-9'), 104.57 (C-4), 102.06 (C-12), 88.64 (C-5), 81.62 (C-6), 61.54 (C-1'), 53.65 (C-1), 45.50 (C-7), 38.15 (C-10), 37.25

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(C-3), 35.53 (C-9), 31.83 (C-11), 26.30 (C-15), 25.63 (C-2), 25.31 (C-8), 20.69 (C-14), 13.32 (C-13); HRMS (ESI) calcd for $C_{24}H_{30}N_4O_7$ $[M+H]^+$ 487.2193, found 487.2196.

4-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)picolinonitrile (13): Brown solid; yield 69%; mp 140-142 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=9.14 (1H, d, J=3.5 Hz, H-5'), 8.34 (1H, dd, J=8.5, 2.5 Hz, H-8'), 8.12 (1H, s, H-3'), 7.90 (1H, d, J=8.5 Hz, H-9'), 5.40 (1H, s, H-5), 5.00 (1H, d, J=12.5 Hz, H-1b'), 4.92 (1H, d, J=3.5 Hz, H-12), 4.81 (1H, d, J=12.5 Hz, H-1a'), 2.68 (1H, m, H-11), 1.46 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=147.21 (C-2'), 142.25 (C-7'), 135.33 (C-4'), 133.28 (C-6'), 129.32 (C-8'), 128.08 (C-5'), 120.56 (C-3'), 116.33 (CN), 104.27 (C-4), 102.31 (C-12), 88.05 (C-5), 81.05 (C-6), 61.65 (C-1'), 52.48 (C-1), 44.30 (C-7), 37.42 (C-10), 36.37 (C-3), 34.53 (C-9), 30.87 (C-11), 26.14 (C-15), 24.67 (C-2), 24.48 (C-8), 20.29 (C-14), 12.98 (C-13); HRMS (ESI) calcd for $C_{24}H_{29}N_5O_5$ $[M + H]^+$ 468.2247, found 468.2242.

1-(2-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone (14): Yellow solid; yield 65%; mp 40-42 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 7.82 (1H, s, H-3'), 7.70 (1H, dd, J=7.5, 1.5 Hz, H-6'), 7.64 (1H, dd, J=7.5, 1.5 Hz, H-8'), 7.58 (1H, dd, J=7.5, 1.5 Hz, H-7'), 7.48 (1H, dd, J=7.5, 1.5 Hz, H-9'), 5.43 (1H, s, H-5), 5.01 (1H, d, J=12.5 Hz, H-1b'), 4.97 (1H, d, J=3.5 Hz, H-12), 4.78 (1H, d, J=12.5 Hz, H-1a'), 2.66 (1H, m, H-11), 2.17 (3H, s, $COCH_3$), 1.44 (3H, s, H_3-15), 0.92 (3H, d, J=6.5 Hz, H_3-14), 0.90 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=199.52 (C-10), 145.90 (C-2'), 136.44 (C-4'), 134.49 (C-5'), 131.87 (C-8'), 129.88 (C-7'), 128.97 (C-6'), 125.68 (C-9'), 124.05 (C-3'), 104.17 (C-4), 101.85 (C-12), 88.02 (C-5), 81.12 (C-6), 61.58 (C-1'), 52.56 (C-1), 44.39 (C-7), 37.37 (C-10), 36.43 (C-3), 34.59 (C-9), 30.85 (C-11), 29.18 ($COCH_3$), 26.16 (C-15), 24.67 (C-2), 24.47 (C-8), 20.32 (C-14), 13.00 (C-13). HRMS (ESI) calcd for $C_{26}H_{33}N_3O_6$ $[M+H]^+$ 484.2448, found 484.2450.

1-(2-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone (14a): Brown viscous liquid; yield 62%; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.80 (1H, s, H-3'), 7.69 (1H, dd, J=7.5, 1.5 Hz, H-6'), 7.61 (1H, dd, J=7.5, 1.5 Hz, H-8'), 7.58 (1H, dd, J=7.5, 1.5 Hz, H-7'), 7.46 (1H, dd, J=7.5, 1.5 Hz, H-9'), 5.29 (1H, s, H-5), 4.97 (1H, d, J=12.5 Hz, H-1b'), 4.92 (1H, d, J=4.5 Hz, H-12), 4.73 (1H, d, J=12.5 Hz, H-1a'), 3.56 (1H, d, J=2.5 Hz, H-3), 2.52 (1H, m, H-11), 2.16 (3H, s, $COCH_3$), 1.55 (3H, s, H_3-15), 0.92 (3H, d, J=7.5 Hz, H_3-14), 0.85 (3H, d, J=6.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=199.52 (C=O), 145.99 (C-2'), 136.42 (C-4'), 134.46 (C-5'), 131.89 (C-8'), 129.89 (C-7'),

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128.94 (C-6'), 125.72 (C-9'), 123.99 (C-3'), 108.04 (C-4), 99.83 (C-12), 93.73 (C-5), 84.20 (C-6), 69.61 (C-3), 61.62 (C-1'), 42.54 (C-1), 40.68 (C-7), 34.77 (C-10), 34.74 (C-9), 30.35 (C-2), 30.32 (C-11), 29.18 ($COCH_3$), 25.02 (C-8), 21.02 (C-14), 18.81 (C-15), 12.38 (C-13); HRMS (ESI) calcd for $C_{26}H_{33}N_3O_6$ $[M+H]^+$ 484.2448, found 484.2441.

1-(3-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone (15): Light brown solid; yield 66%; mp 115-118 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 8.29 (1H, dd, J=2.4, 1.2 Hz, H-5'), 8.04 (1H, s, H-3'), 8.02 (2H, m, H-7', H-9'), 7.65 (1H, t, J=8.0 Hz, H-8'), 5.44 (1H, s, H-5), 5.01 (1H, d, J=13.0 Hz, H-1b'), 4.98 (1H, d, J=3.5 Hz, H-12), 4.77 (1H, d, J=13.0 Hz, H-1a'), 2.69 (1H, m, H-11), 2.68 (3H, s, $COCH_3$), 1.46 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=196.66 (C-10), 146.18 (C-2'), 138.55 (C-6'), 137.53 (C-4'), 130.23 (C-8'), 128.43 (C-7'), 124.85 (C-9'), 120.83 (C-3'), 119.75 (C-5'), 104.23 (C-4), 102.05 (C-12), 88.03 (C-5), 81.12 (C-6), 61.74 (C-1'), 52.54 (C-1), 44.39 (C-7), 37.39 (C-10), 36.42 (C-3), 34.56 (C-9), 30.88 (C-11), 26.76 (C-15, $COCH_3$), 24.69 (C-2), 24.49 (C-8), 20.32 (C-14), 13.02 (C-13); HRMS (ESI) calcd for $C_{26}H_{33}N_3O_6$ $[M+H]^+$ 484.2448, found 484.2446.

1-(3-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone (15a): Brown solid; yield 63%; mp 90-92 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.26 (1H, s, H-3'), 8.01 (1H, s, H-5'), 7.99 (2H, dd, J=8.0, 1.5 Hz, H-7', H-9'), 7.63 (1H, dd, J=6.5, 6.5 Hz, H-8'), 5.31 (1H, s, H-5), 4.98 (1H, d, J=12.5 Hz, H-1b'), 4.92 (1H, d, J=4.0 Hz, H-12), 4.70 (1H, d, J=12.5 Hz, H-1a'), 3.56 (1H, d, J=1.5 Hz, H-3), 2.52 (1H, m, H-11), 1.59 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=196.74 (C=O), 146.20 (C-2'), 138.51 (C-6'), 137.46 (C-4'), 130.25 (C-8'), 128.43 (C-7'), 124.79 (C-9'), 120.65 (C-3'), 119.70 (C-5'), 108.05 (C-4), 99.54 (C-12), 93.85 (C-5), 84.14 (C-6), 69.57 (C-3), 61.55 (C-1'), 42.44 (C-1), 40.65 (C-7), 34.84 (C-10), 34.69 (C-9), 30.35 (C-2, C-11), 26.75 ($COCH_3$), 25.05 (C-8), 20.98 (C-14), 18.80 (C-15), 12.32 (C-13); HRMS (ESI) calcd for $C_{26}H_{33}N_3O_6$ $[M+H]^+$ 484.2448, found 484.2451.

1-(4-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)ethanone (16): Light brown solid; yield 71%; mp 135-137 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.10 (2H, d, J=8.5 Hz, H-6', H-8'), 8.01 (1H, s, H-3'), 7.85 (2H, d, J=8.5 Hz, H-5', H-9'), 5.41 (1H, s, H-5), 4.99 (1H, d, J=12.5 Hz, H-1b'), 4.95 (1H, d, J=3.5 Hz, H-12), 4.75 (1H, d, J=12.5 Hz, H-1a'), 2.65 (1H, m, H-11), 2.63 (3H, s, $COCH_3$), 1.41 (3H, s, H_3-15), 0.90 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=196.55 (C=O), 146.26 (C-2'), 140.12 (C-7'), 136.85

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(C-4'), 130.10 (C-6', C-8'), 120.63 (C-3'), 120.08 (C-5', C-9'), 104.21 (C-4), 102.00 (C-12), 88.03 (C-5), 81.11 (C-6), 61.64 (C-1'), 52.52 (C-1), 44.37 (C-7), 37.39 (C-10), 36.41 (C-3), 34.56 (C-9), 30.87 (C-11), 26.68 ($COCH_3$), 26.17 (C-15), 24.68 (C-2), 24.48 (C-8), 20.32 (C-14), 13.01 (C-13); HRMS (ESI) calcd for $C_{26}H_{33}N_3O_6$ $[M+H]^+$ 484.2448, found 484.2441.

(E)-1-(2-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (17): Yellow solid; yield 64%; mp 95-97 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 7.77 (1H, s, H-3'), 7.72 (1H, m, H-6'), 7.68 (1H, dd, J=7.5, 1.5 Hz, H-15', H-18'), 7.63 (1H, d, J=16.0 Hz, H-12'), 7.59 (1H, m, H-17'), 7.58 (1H, dd, J=8.0, 1.5 Hz, H-8'), 7.54 (1H, m, H-16'), 7.45 (2H, m, H-7', H-9'), 6.53 (1H, d, J=16.0 Hz, H-11'), 5.37 (1H, s, H-5), 4.93 (1H, d, J=12.5 Hz, H-1b'), 4.85 (1H, d, J=3.5 Hz, H-12), 4.64 (1H, d, J=12.5 Hz, H-1a'), 2.58 (1H, m, H-11), 1.43 (3H, s, H_3-15), 0.90 (3H, d, J=6.5 Hz, H_3-14), 0.80 (3H, d, J=7.0 Hz, H_3-13); ^{13}C NMR (125 MHZ, $CDCl_3$): δ=192.34 (C=O), 146.08 (C-2'), 140.56 (C-12'), 134.92, 134.80 (C-4'/C-13'), 132.92 (C-5'), 132.25, 132.00 (C-8'/C-17'), 130.04 (C-6'), 129.80, 129.78, 129.09 (C-9'/C-16'/C-18'), 127.91 (C-15'), 126.20, 126.15 (C-7'/C-11'), 125.15 (CF_3), 124.15 (C-3'), 104.14 (C-4), 101.82 (C-12), 87.95 (C-5), 81.04 (C-6), 61.66 (C-1'), 52.52 (C-1), 44.32 (C-7), 37.32 (C-10), 36.41 (C-3), 34.51 (C-9), 30.73 (C-11), 26.14 (C-15), 24.65 (C-2), 24.38 (C-8), 20.30 (C-14), 12.86 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2646.

(E)-1-(2-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (17a): Yellow solid; yield 58%; mp 53-55 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.75 (1H, s, H-3'), 7.72 (1H, dd, J=8.0, 1.5 Hz, H-5'), 7.68 (2H, dd, J=8.0, 1.5 Hz, H-15', H-18'), 7.64 (1H, d, J=16.0 Hz, H-12'), 7.63 (1H, dd, J=7.5, 1.0 Hz, H-17'), 7.59 (1H, dd, J=8.0, 1.0 Hz, H-8'), 7.52 (1H, m, H-16'), 7.45 (2H, m, H-7', H-9'), 6.53 (1H, d, J=16.0 Hz, H-11'), 5.24 (1H, s, H-5), 4.90 (1H, d, J=12.5 Hz, H-1b'), 4.82 (1H, d, J=4.0 Hz, H-12), 4.60 (d, J=12.5 Hz, H-1a'), 3.55 (1H, br s, H-3), 2.47 (1H, m, H-11), 1.57 (3H, s, H_3-15), 0.83 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=192.29 (C=O), 146.15 (C-2'), 140.63 (C-12'), 134.93, 134.85 (C-4'/C-13'), 132.96 (C-5'), 132.21, 132.00 (C-8'/C-17'), 130.02 (C-6'), 129.77 (C-16'), 129.07 (C-9', C-18'), 127.93 (C-15'), 126.18, 126.13 (C-7'/C-11'), 125.20 (CF_3), 124.16 (C-3'), 108.03 (C-4), 99.83 (C-12), 93.65 (C-5), 84.16 (C-6), 69.60 (C-3), 61.66 (C-1'), 42.53 (C-1), 40.63 (C-7), 34.77 (C-10), 34.68 (C-9), 30.31 (C-2), 30.23 (C-11), 24.96 (C-8), 20.99 (C-14), 18.79 (C-15), 12.25 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2635.

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(E)-1-(2-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (18): Yellow solid; yield 61%; mp 124-126 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.76 (1H, s, H-3'), 7.71 (2H, t, J=8.0 Hz, H-15', H-16'), 7.63 (2H, t, J=8.0 Hz, H-7', H-9'), 7.59 (2H, t, J=8.0 Hz, H-14', H-18'), 7.45 (2H, d, J=7.0 Hz, H-15', H-17'), 7.35 (1H, d, J=16.0 Hz, H-12'), 6.60 (1H, d, J=16.0 Hz, H-11'), 5.37 (1H, s, H-5), 4.92 (1H, d, J=12.5 Hz, H-1b'), 4.84 (1H, d, J=3.0 Hz, H-12), 4.64 (1H, d, J=12.5 Hz, H-1a'), 2.58 (1H, m, H-11), 1.43 (3H, s, H_3-15), 0.92 (3H, d, J=6.5 Hz, H_3-14), 0.75 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=192.30 (C-10'), 146.00 (C-2'), 143.76 (C-12'), 137.40 (C-13'), 135.15 (C-4'), 134.90 (C-5', C-16'), 132.05 (C-8'), 129.96, 129.73, 129.61 (C-6'/C-14'/C-18'), 128.52 (C-15', C-17'), 126.97 (C-7'), 125.94, 125.91, 125.68 (C-9'/C-11'/CF_3), 124.40 (C-3'), 104.17 (C-4), 101.63 (C-12), 87.95 (C-5), 81.03 (C-6), 61.42 (C-1'), 52.49 (C-1), 44.35 (C-7), 37.36 (C-10), 36.39 (C-3), 34.53 (C-9), 30.70 (C-11), 26.13 (C-15), 24.65 (C-2), 24.40 (C-8), 20.32 (C-14), 12.87 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2632.

(E)-1-(2-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (18a): Yellow solid; yield 59%; mp 68-70 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.75 (1H, s, H-3'), 7.70 (2H, m, H-6', H-8'), 7.63 (2H, m, H-7', H-9'), 7.58 (2H, d, J=8.0 Hz, H-14', H-18'), 7.45 (2H, d, J=8.0 Hz, H-15', H-17'), 7.34 (1H, d, J=16.0 Hz, H-12'), 6.60 (1H, d, J=16.0 Hz, H-11'), 5.24 (1H, s, H-5), 4.88 (1H, d, J=13.0 Hz, H-1b'), 4.82 (1H, d, J=4.0 Hz, H-12), 4.60 (1H, d, J=13.0 Hz, H-1a'), 3.55 (1H, brs, H-3), 2.39 (1H, m, H-11), 1.55 (3H, s, H_3-15), 0.84 (3H, d, J=6.5 Hz, H_3-14), 0.80 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=192.24 (C=O), 146.12 (C-2'), 143.69 (C-12'), 137.44 (C-13'), 135.18 (C-4'), 134.91 (C-5', C-16'), 132.03 (C-8'), 129.94 (C-6'), 129.60 (C-14', C-18'), 128.50 (C-15', C-17'), 127.01 (C-7'), 125.90 (C-11'), 125.87 (CF_3), 125.64 (C-9'), 124.33 (C-3'), 108.02 (C-4), 99.58 (C-12), 93.75 (C-5), 84.10 (C-6), 69.59 (C-3), 61.51 (C-1'), 42.46 (C-1), 40.61 (C-7), 34.80 (C-10), 34.68 (C-9), 30.32 (C-2), 30.24 (C-11), 24.96 (C-8), 20.96 (C-14), 18.79 (C-15), 12.19 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2635.

(E)-1-(3-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (19): Yellow solid; yield 62%; mp 90-92 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.34 (2H, dd, J=2.0, 1.5 Hz, H-5'), 8.19 (1H, dd, J=15.5, 2.0 Hz, H-12'), 8.07 (1H, brs, H-3'), 8.05 (2H, dd, J=7.5, 1.5 Hz, H-15', H-18'), 7.86 (1H, d, J=7.5 Hz, H-8'), 7.74 (2H, d, J=7.5 Hz, H-7'), 7.69 (1H, m, H-17'), 7.63 (1H, m, H-9'), 7.52 (1H,

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m, H-16'), 7.45 (1H, d, J=15.5 Hz, H-11'), 5.45 (1H, s, H-5), 5.03 (1H, d, J=13.0 Hz, H-1b'), 4.98 (1H, d, J=3.5 Hz, H-12), 4.77 (1H, d, J=13.0 Hz, H-1a'), 2.68 (1H, m, H-11), 1.47 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.89 (C=O), 146.20 (C-2'), 141.41 (C-12'), 139.19 (C-6'), 137.58 (C-4', C-13'), 133.56 (C-14'), 132.24 (C-17'), 130.30 (C-8'), 128.64, 128.06 (C-16'/C-18'), 126.41, 126.36 (C-9'/C-15'), 124.74 (C-7', CF_3), 120.84, 120.22 (C-3'/C-5'/C-11'), 104.23 (C-4), 102.02 (C-12), 88.03 (C-5), 81.13 (C-6), 61.73 (C-1'), 52.53 (C-1), 44.37 (C-7), 37.38 (C-10), 36.40 (C-3), 34.55 (C-9), 30.88 (C-11), 26.16 (C-15), 24.68 (C-2), 24.49 (C-8), 20.33 (C-14), 13.04 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2630.

(E)-1-(3-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (19a): Yellow solid; yield 58%; mp 48-50 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 8.31 (1H, dd, J=2.0, 1.5 Hz, H-5'), 8.17 (1H, d, J=15.5 Hz, H-12'), 8.05 (1H, dd, 8.0, 2.5 Hz, H-15'), 8.04 (1H, s, H-3'), 8.02 (1H, dd, J=8.0, 2.5 Hz, H-18'), 7.84 (1H, d, J=7.5 Hz, H-8'), 7.72 (1H, d, J=8.0 Hz, H-7'), 7.67 (1H, m, H-17'), 7.62 (1H, dd, J=7.5, 2.1 Hz, H-9'), 7.52 (1H, m, H-16'), 7.44 (1H, d, J=15.5 Hz, H-11'), 5.31(1H, s, H-5), 4.99 (1H, d, J=12.5 Hz, H-1b'), 4.92 (1H, d, J=4.0 Hz, H-12), 4.70 (1H, d, J=12.5 Hz, H-1a'), 3.19 (1H, brs, H-3), 2.53 (1H, m, H-11), 1.52 (3H, s, H_3-15), 0.91 (3H, d, J=7.5 Hz, H_3-14), 0.83 (3H, d, J=6.0 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.84 (C=O), 146.23 (C-2'), 141.36 (C-12'), 139.15 (C-6'), 137.52 (C-4', C-13'), 133.57 (C-14'), 132.55 (C-17'), 130.95 (C-8'), 128.61, 128.05 (C-16'/C-18'), 126.36, 126.33 (C-9'/C-15'), 125.68 (C-7'), 124.68 (CF_3), 122.83 (C-5'), 120.65 (C-11'), 120.15 (C-3'), 108.05 (C-4), 99.53 (C-12), 93.84 (C-5), 84.13 (C-6), 69.54 (C-3), 61.07 (C-1'), 40.65 (C-7), 42.43 (C-1), 34.84 (C-10), 34.69 (C-9), 30.34 (C-11), 30.37 (C-2), 24.83 (C-8), 20.47 (C-14), 18.60 (C-15), 12.32 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2629.

(E)-1-(3-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (20): Yellow solid; yield 58%; mp 69-71 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.38 (1H, t, J=1.7 Hz, H-3'), 8.09 (2H, m, H-7', H-9'), 8.03 (2H, dd, J=2.5 Hz, 2.0, H-5'), 7.77 (2H, d, J=8.0 Hz, H-15', H-17'), 7.70 (2H, d, J=8.0 Hz, H-14', H-18'), 7.69 (1H, d, J=7.0 Hz, H-8'), 7.67 (1H, d, J=15.5 Hz, H-12'), 7.61 (1H, d, J=15.5 Hz, H-11'), 5.45 (1H, s, H-5), 5.01 (1H, d, J=13.0 Hz, H-1b'), 4.97 (1H, d, J=3.5 Hz, H-12), 4.78 (1H, d, J=13.0 Hz, H-1a'), 2.65 (1H, m, H-11), 1.47 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.59 (C=O), 146.20 (C-2'), 143.99 (C-

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12'), 139.34 (C-6'), 137.87, 137.63 (C-4'/C-13'), 132.44 (C-16'), 130.33 (C-8'), 128.73 (C-14', C-18'), 128.48 (C-7'), 126.04, 126.01 (C-17'/C-15'), 125.98, 124.74 (C-9'/CF_3), 123.39 (C-3'), 120.88, 120.18 (C-5'/C-11'), 104.23 (C-4), 101.99 (C-12), 88.03 (C-5), 81.12 (C-6), 61.66 (C-1'), 52.54 (C-1), 44.38 (C-7), 37.39 (C-10), 36.41 (C-3), 34.56 (C-9), 30.88 (C-11), 26.16 (C-15), 24.69 (C-2), 24.50 (C-8), 20.32 (C-14), 13.03 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2635.

(E)-1-(3-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (20a): Yellow solid; yield 55%; mp 52-54 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 8.37 (1H, dd, J=2.0, 1.5 Hz, H-5'), 8.04 (1H, brs, H-3'), 8.02 (2H, dd, J=8.0, 1.2 Hz, H-7', H-8'), 7.89 (1H, d, J=15.5 Hz, H-12'), 7.77 (2H, d, J=8.0 Hz, H-14', H-18'), 7.70 (2H, d, J=8.0 Hz, H-15', H-17'), 7.62 (1H, d, J=15.5 Hz, H-11'), 5.33 (1H, s, H-5), 5.02 (1H, d, J=12.5 Hz, H-1b'), 4.95 (1H, d, J=4.5 Hz, H-12), 4.74 (1H, d, J=12.5 Hz, H-1a'), 3.55 (1H, m, H-3), 2.52 (1H, m, H-11), 1.55 (3H, s, H_3-15), 0.96 (3H, d, J=7.5 Hz, H_3-14), 0.87 (3H, d, J=6.5 Hz, H_3-13). ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.58 (C=O), 146.26 (C-2'), 144.01 (C-12'), 139.32 (C-6'), 137.87, 137.59 (C-4'/C-13'), 132.44 (C-16'), 130.34 (C-8'), 128.73, 128.48 (C-14'/C-18'), 126.03, 126.00 (C-7'/C-15'/C-17'), 124.85, 124.69 (C-9'/CF_3), 123.37 (C-3'), 120.66, 120.13 (C-5'/C-11'), 108.06 (C-4), 99.52 (C-12), 93.88 (C-5), 84.16 (C-6), 69.60 (C-3), 61.55 (C-1'), 42.46 (C-1), 40.67 (C-7), 34.86 (C-10), 34.71 (C-9), 30.37 (C-2), 30.35 (C-11), 25.07 (C-8), 20.98 (C-14), 18.80 (C-15), 12.32 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2632.

(E)-1-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (21): Yellow solid; yield 65%; mp 93-95 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.16 (3H, m, H-6', H-8', H-12'), 8.07 (1H, s, H-3'), 7.91 (2H, dd, J=7.5, 1.2 Hz, H-5', H-9'), 7.84 (1H, m, H-15'), 7.73 (1H, dd, J=7.5, 1.5 Hz, H-18'), 7.62 (1H, m, H-17'), 7.52 (1H, m, H-16'), 7.44 (1H, d, J=15.5 Hz, H-11'), 5.44 (1H, s, H-5), 5.01 (1H, d, J=12.5 Hz, H-1b'), 4.97 (1H, d, J=3.5 Hz, H-12), 4.78 (1H, d, J=12.5 Hz, H-1a'), 2.66 (1H, m, H-11), 1.45 (3H, s, H_3-15), 0.91 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHZ, $CDCl_3$): δ=188.83 (C-10), 146.27 (C-2'), 141.05 (C-12'), 140.06 (C-7'), 137.41 (C-4'), 133.69 (C-13'), 132.20 (C-17'), 130.62 (C-16'), 130.47 (C-6', C-8'), 130.01 (C-18'), 128.53 (C-14'), 128.00 (C-15'), 126.35 (CF_3), 120.71, 120.17 (C-3'/C-11'), 120.66 (C-5', C-9'), 104.21 (C-4), 102.02 (C-12), 88.03 (C-5), 81.12 (C-6), 61.65 (C-1'), 52.52 (C-1), 44.37 (C-7), 37.39 (C-10), 36.41 (C-3), 34.56 (C-9), 30.88 (C-11), 26.17 (C-15), 24.67 (C-2), 24.49

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(C-8), 20.31 (C-14), 13.01 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2629.

(E)-1-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(2-(trifluoromethyl)phenyl)prop-2-en-1-one (21a): Orange solid; yield 62%; mp 48-50 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.15 (3H, m, H-6', H-8', H-12'), 8.04 (1H, s, H-3'), 7.90 (2H, dd, J=7.5, 1.2 Hz, H-5', H-9'), 7.83 (2H, d, J=7.5 Hz, H-15', H-18'), 7.73 (1H, m, H-17'), 7.52 (1H, m, H-16'), 7.41 (1H, d, J=15.5 Hz, H-11'), 5.31 (1H, s, H-5), 4.94 (1H, m, H-1b'), 4.93 (1H, d, J=3.0 Hz, H-12), 4.73 (1H, brs, H-1a'), 3.57 (1H, br s, H-3), 2.49 (1H, m, H-11), 1.52 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.81 (C=O), 146.33 (C-2'), 141.05 (C-12'), 140.01 (C-7'), 137.42 (C-4'), 133.69 (C-13', C-14'), 132.20 (C-17'), 130.62 (C-16'), 130.47 (C-6', C-8'), 130.01 (C-18'), 127.99 (C-15'), 125.92 (CF_3), 120.13 (C-5', C-9'), 120.49, 119.86 (C-3'/C-11'), 108.06 (C-4), 99.50 (C-12), 93.88 (C-5), 84.13 (C-6), 69.55 (C-3), 61.47 (C-1'), 42.41 (C-1), 40.64 (C-7), 34.86 (C-10), 34.69 (C-9), 30.35 (C-2), 30.34 (C-11), 25.05 (C-8), 20.98 (C-14), 18.80 (C-15), 12.31 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2635.

(E)-1-(4-(4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (22): Yellow solid; yield 67%; mp 139-141 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.20 (2H, dd, J=7.0, 2.0 Hz, H-6', H-8'), 8.07 (1H, s, H-3'), 7.94 (2H, dd, J=7.0, 2.0 Hz, H-5', H-9'), 7.86 (1H, d, J=15.5 Hz, H-12'), 7.77 (2H, d, J=8.5 Hz, H-15', H-17'), 7.69 (2H, d, J=8.5 Hz, H-14', H-18'), 7.61 (1H, d, J=15.5, H-11'), 5.45 (1H, s, H-5), 5.02 (1H, d, J=13.0 Hz, H-1b'), 4.98 (1H, d, J=3.5 Hz, H-12), 4.78 (1H, d, J=13.0 Hz, H-1a'), 2.66 (1H, m, H-11), 1.46 (3H, s, H_3-15), 0.92 (6H, m, H_3-13, H_3-14); ^{13}C NMR (125 MHZ, $CDCl_3$): δ=188.43 (C-10'), 146.29 (C-2'), 143.63 (C-12'), 140.12 (C-7'), 137.99, 137.54 (C-4'/C-13'), 132.35 (C-16'), 130.36 (C-6', C-8'), 128.66 (C-14', C-18'), 126.03, 126.00 (C-15'/C-17'), 123.52 (CF_3), 120.67, 120.08 (C-3'/C-11'), 120.23 (C-5', C-9'), 104.23 (C-4), 101.98 (C-12), 88.04 (C-5), 81.12 (C-6), 61.61 (C-1'), 52.51 (C-1), 44.36 (C-7), 37.40 (C-10), 36.41 (C-3), 34.56 (C-9), 30.87 (C-11), 26.19 (C-15), 24.68 (C-2), 24.50 (C-8), 20.33 (C-14), 13.03 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2631.

(E)-1-(4-(4-(12-β-(3α-hydroxy-dihydroartemisinoxy)methyl)-1H-1,2,3-triazol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (22a): Yellow solid; yield 65%; mp 110-112 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.20 (2H, dd, J=7.0, 2.0 Hz, H-6', H-8'),

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8.03 (1H, s, H-3'), 7.93 (2H, dd, J=7.0, 2.0 Hz, H-5', H-9'), 7.86 (1H, d, J=15.5 Hz, H-12'), 7.77 (2H, d, J=8.5 Hz, H-14', H-18'), 7.69 (2H, d, J=8.5 Hz, H-15', H-17'), 7.60 (1H, d, J=15.5 Hz, H-11'), 5.32 (1H, s, H-5), 5.00 (1H, d, J=12.5 Hz, H-1b'), 4.94 (1H, d, J=4.0 Hz, H-12), 4.74 (1H, d, J=12.5 Hz, H-1a'), 3.58 (1H, d, J=2.5 Hz, H-3), 2.52 (1H, m, H-11), 1.55 (3H, s, H_3-15), 0.95 (3H, d, J=7.5 Hz, H_3-14), 0.86 (3H, d, J=6.0 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=188.45 (C=O), 146.38 (C-2'), 143.65 (C-12'), 140.08 (C-7'), 137.98, 137.57 (C-4'/C-13'), 131.89 (C-16'), 130.36 (C-6', C-8'), 128.65 (C-14', C-18'), 126.03, 126.00 (C-15'/C-17'), 123.55 (CF_3), 120.44, 120.08 (C-3'/C-11'), 120.20 (C-5', C-9'), 108.07 (C-4), 99.52 (C-12), 93.90 (C-5), 84.26 (C-6), 69.61 (C-3), 61.50 (C-1'), 42.45 (C-1), 40.66 (C-7), 34.88 (C-10), 34.71 (C-9), 30.37 (C-2), 30.35 (C-11), 25.07 (C-8), 20.98 (C-14), 18.81 (C-15), 12.31 (C-13); HRMS (ESI) calcd for $C_{34}H_{36}F_3N_3O_6$ $[M+H]^+$ 640.2634, found 640.2635.

4.6. Synthesis of 12-β-(2□bromoethoxy)-dihydroartemisinin and 12-β-(2□iodoethoxy)-dihydroartemisinin

For the synthesis of 12-β-(2□bromoethoxy)-dihydroartemisinin, 8 ml of BF_3OEt_2 was added dropwise to the mixture of DHA (5 gram, 17.59 mmol) and 2-bromomethanol (4.39 gram, 35.18 mmol) in dry Et_2O at 0-5 ºC temperature. The reaction mixture was stirred at the same temperature for 4-5 hrs. After completion of reaction, reaction mixture was washed with 5 % $NaHCO_3$, water and dried over anhydrous Na_2SO_4. The crude thus obtained was purified by column chromatography. In case of synthesis of 12-β-(2□iodoethoxy)-dihydroartemisinin, 2-iodoethanol (6.04 gram, 35.18 mmol) had been taken in place of 2-bromoethanol.

12-β-(2□bromoethoxy)-dihydroartemisinin (23a): White crystalline solid; yield 89%; mp 129-131ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.48 (1H, s, H-5), 4.83 (1H, d, J=3.0 Hz, H-12), 4.11 (1H, m, H-1b'), 3.78 (1H, m, H-1a'), 3.50 (2H, m, H-2a', H-2b'), 2.64 (1H, m, H-11), 1.42 (3H, s, H_3-15), 0.94 (3H, d, J=6.0 Hz, H_3-14), 0.93 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=104.12 (C-4), 102.07 (C-12), 88.16 (C-5), 81.10 (C-6), 68.19 (C-1'), 52.60 (C-1), 44.38 (C-7), 37.40 (C-10), 36.42 (C-3), 34.68 (C-9), 31.41 (C-2'), 30.90 (C-11), 26.15 (C-15), 24.66 (C-2), 24.37 (C-8), 20.37 (C-14), 12.98 (C-13); HRMS (ESI) calcd for $C_{17}H_{27}BrO_5$ $[M+H]^+$ 391.1120, found 391.2823.

12-β-(2□iodoethoxy)-dihydroartemisinin (23b): White crystalline solid; yield 88%; mp 125-127ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.49 (1H, s, H-5), 4.83 (1H, d, J=3.0 Hz, H-12), 4.05 (1H, m, H-1b'), 3.73 (1H, dd, J=11.0, 6.5 Hz, H-1a'), 3.29 (2H, m, H-2a', H-2b'), 2.64

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(1H, m, H-11), 1.44 (3H, s, H_3-15), 0.94 (3H, d, J=7.0 Hz, H_3-14), 0.91 (3H, d, J=7.0 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=104.12 (C-4), 101.95 (C-12), 88.32 (C-5), 81.10 (C-6), 68.94 (C-1'), 52.62 (C-1), 44.33 (C-7), 37.38 (C-10), 36.43 (C-3), 34.69 (C-9), 30.93 (C-11), 26.13 (C-15), 24.67 (C-2), 24.46 (C-8), 20.36 (C-14), 13.04 (C-13), 3.85 (C-2').

4.7. Synthesis of 12-β-(2□azidoethoxy)-dihydroartemisinin

To a solution of 12-β-(2□bromoethoxy)-dihydroartemisinin (**23a**, 4 gram, 10.22 mmol) in DMSO (25 ml), NaN_3 (1.66 gram, 25.55 mmol) and NaI (756.93 mg, 5.11 mmol) were added and the reaction mixture was stirred at 50 ºC for 3-4 hrs. After completion of reaction, 150 ml of water was added to the reaction mixture and the resulting mixture was extracted with DCM (150 ml x 3). The organic layer was washed with water, brine and dried over Na_2SO_4. The crude product was obtained in almost pure state which was further purified by column chromatography. Compound **24** can also be synthesized by 12-β-(2□iodoethoxy)-dihydroartemisinin (**23b**). In this case, there is no need to add NaI.

(12-β-(2□azidoethoxy)-dihydroartemisinin (24): White solid; yield 90%; mp 92-94 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=5.44 (1H, s, H-5), 4.83 (1H, d, J=3.0 Hz, H-12), 4.03 (1H, m, H-1b'), 3.57 (1H, m, H-1a'), 3.38 (2H, dd, J=11.0, 6.5 Hz, H-2'), 2.63 (1H, m, H-11), 1.46 (3H, s, H_3-15), 0.95 (3H, d, J=6.5 Hz, H_3-14), 0.93 (3H, d, J=8.0 Hz, H_3-13). ^{13}C NMR (125 MHz, $CDCl_3$): δ=104.18 (C-4), 102.45 (C-12), 87.99 (C-5), 81.06 (C-6), 67.29 (C-1'), 52.57 (C-1), 51.18 (C-2'), 44.40 (C-7), 37.43 (C-10), 36.42 (C-3), 34.60 (C-9), 30.78 (C-11), 26.16 (C-15), 24.68 (C-2), 24.41 (C-8), 20.35 (C-14), 12.92 (C-13).

General procedure for synthesis of mono and dipropargyl ether derivatives of benzene diols (26a-f)

For the synthesis of monopropargyl ether derivatives of benzene diol, a solution of appropriate benzene diols (9.09 mmol) in dry acetone was taken in a round botton flask and 13.6 mmol of K_2CO_3 and 9.27 mmol of propargyl bromide were added. For dipropargyl ether derivatives of benzene diol, a mixture of 9.09 mmol of benzene diols, 27.27 mmol of K_2CO_3 and 22.72 mmol of propargyl bromide was taken in a round bottom flask. The reaction mixture was refluxed for 4-6 hrs. After completion of reaction, acetone was removed under reduced pressure and reaction mixture was extracted with DCM, washed with water and brine then dried over Na_2SO_4.

4.9. Synthesis of Compound 29

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A mixture of compound **6** (200 mg, 0.621 mmol), compound **24** (219 mg, 0.621 mmol) and hexadecyltrimethylammonium bromide (113 mg, 0.310 mmol) was dissolved in 5 ml of DCM and stirred at room temperature for 5 min. $CuSO_4.5H_2O$ (15.5 mg, 0.0621 mmol, 10 mol%) and sodium ascorbate (28 mg, 0.141 mmol, 25 mol%) were dissolved in 3 ml of water and added to the reaction mixture. The resulting solution was stirred for 1-2 hrs at room temperature. After completion of reaction, the reaction mixture was extracted with dichloromethane (25ml x 3 times) and organic layer was washed with water, and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified on silica gel (60-120 mesh size) column chromatography using EtOAc/Hexane as eluent to afford the compound **29**.

1-(2-(12-β-dihydroartemisinoxy)ethyl)-4-(12-β-dihydroartemisinoxymethyl)-1H-1,2,3-triazole (29): Light brown solid; yield 57%; mp 124-126 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 7.52 (1H, s, H-3'), 5.42, 5.19 (2H, s, H-5a, H-5b), 4.96, 4.61 (2H,d, J=12.5 Hz, H-1b'', H-1a''), 4.90, 4.75 (2H, d, J=3.5 Hz, H-12a, H-12b), 4.59 (1H, m, H-1b'), 4.51 (1H, m, H-1a'), 4.27 (1H, m, H-2b'), 3.80 (1H, m, H-2a'), 2.36, 2.33 (2H, m, H-11a, H-11b), 1.43, 1.41 (6H, s, H_3-15a, H_3-15b), 0.93, 0.92 (6H, d, J=6.0, H_3-14a, H_3-14b), 0.88, 0.79 (6H, d, J=7.5, H_3-13a, H_3-13b); ^{13}C NMR (125 MHz, $CDCl_3$): 145.05 (C-4'), 122.91 (C-3'), 104.21, 104.16 (C-4a, C-4b), 102.13, 101.73 (C-12a, C-12b), 87.96, 87.87 (C-5a, C-5b), 81.08, 80.84 (C-6a, C-6b), 66.45 (C-5'), 61.82 (C-1', C-1''), 52.54, 52.42 (C-1a, C-1b), 50.31 (C-2'), 44.37, 44.10 (C-7a, C-7b), 37.38, 37.34 (C-10a, C-10b), 36.41, 36.38 (C-3a, C-3b), 34.57, 34.49 (C-9a, C-9b), 30.78, 30.61 (C-11a, C-11b), 26.17, 26.09 (C-15a, C-15b), 24.68, 24.63 (C-2a, C-2b), 24.47, 24.33 (C-8a, C-8b), 20.35 (C-14a, C-14b), 12.99, 12.87 (C-13a, C-13b).

4.10. General procedure for the synthesis of Series 2 type of artemisinin-azole derivatives

For the synthesis of monomers (**27, 28**), a mixture of Compound **24** (0.565 mmol), appropriate mono propargyl ether derivatives of benezene diols (**26e & 26f**, 0.621 mmol) and hexadecyltrimethylammonium bromide (0.282 mmol) was taken in dichloromethane (5ml) and stirred for 5 min at room temperature. $CuSO_4.5H_2O$ (0.0565 mmol, 10 mol%) and sodium ascorbate (0.156 mmol, 25 mol%) were dissolved in 3 ml of water and added to the reaction mixture. The resulting solution was stirred for 1-2 hrs at room temperature. After completion of reaction, the reaction mixture was extracted with dichloromethane (25ml x 3 times) and organic layer was washed with water, and dried over anhydrous Na_2SO_4 and

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concentrated under reduced pressure. The crude product was purified on silica gel (60-120 mesh size) column chromatography using EtOAc/Hexane as eluent. For the synthesis of dimers (**30-33**), 0.310 mmol of dipropargyl derivatives of benzene diol (**26a-d**) were taken in place of mono propargyl ethers derivatives of benzene diols.

3-((1-(2-(12-β-dihydroartemisinoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenol (27): Brown viscous liquid; yield 62%; ^{1}H NMR (500 MHz, CDCl3): δ= 7.66 (1H, s, H-3'), 7.10 (1H, t, J=8.0 Hz, H-10'), 6.54 (1H, dd, J=8.0, 2.0 Hz, H-11'), 6.50 (1H, m, H-9'), 6.49 (1H, dd, J=1.5, 1.5 Hz, H-7'), 5.17 (2H, s, H-5'), 5.13 (1H, s, H-5), 4.73 (1H, d, J=3.5 Hz, H-12), 4.61 (1H, m, H-1b'), 4.50 (1H, m, H-1a'), 4.24, 3.80 (2H, m, H-2a', H-2b'), 2.58 (1H, m, H-11), 1.40 (3H, s, H_3-15), 0.90 (3H, d, J=6.0 Hz, H_3-14), 0.77 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.38 (C-6'), 157.40 (C-8'), 144.31 (C-4'), 130.19 (C-10'), 123.34 (C-3'), 108.62 (C-11'), 106.87 (C-9'), 104.29 (C-4), 102.40, 102.21 (C-12/C-7'), 87.93 (C-5), 80.88 (C-6), 66.48 (C-5'), 61.86 (C-1'), 52.41 (C-1), 50.52 (C-2'), 44.07 (C-7), 37.33 (C-10), 36.32 (C-3), 34.44 (C-9), 30.63 (C-11), 26.04 (C-15), 24.59 (C-2), 24.32 (C-8), 20.31 (C-14), 12.80 (C-13); HRMS (ESI) calcd for $C_{26}H_{35}N_3O_7$ $[M+H]^+$ 502.2553, found 502.2553.

4-((1-(2-(12-β-dihydroartemisinoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-2-hydroxybenzaldehyde (28): Light brown viscous liquid; yield 57%; mp 84-86 ºC; ^{1}H NMR (500 MHz, $CDCl_3$): δ=11.42 (1H, s, OH), 9.72 (1H, s, CHO), 7.81 (1H, d, J=9.0 Hz, H-10'), 7.69 (1H, s, H-3'), 7.43 (1H, d, J=9.0 Hz, H-11'), 6.52 (1H, d, J=2.0 Hz, H-7'), 5.30 (1H, s, H-5'), 5.27 (1H, s, H-5), 4.78 (1H, d, J=2.5 Hz, H-12), 4.75 (1H, m, H-1b'), 4.74 (1H, m, H-1a'), 4.27 (1H, m, H-2b'), 3.82 (1H, m, H-2a'), 2.57 (1H, m, H-11), 1.38 (3H, s, H_3-15), 0.91 (3H, d, J=6.0 Hz, H_3-14), 0.74 (3H, d, J=7.5 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 187.92 (CHO), 164.39 (C-6'), 162.07 (C-8'), 143.01 (C-4'), 135.45 (C-10'), 123.45 (C-3'), 119.88 (C-9'), 108.42 (C-11'), 104.22 (C-4), 102.20, 102.16 (C-12/C-7'), 87.88 (C-5), 80.79 (C-6), 66.40 (C-5'), 62.28 (C-1'), 50.51 (C-2'), 52.39 (C-1), 44.06 (C-7), 37.35 (C-10), 36.32 (C-3), 34.46 (C-9), 30.59 (C-11), 26.07 (C-15), 24.61 (C-2), 24.33 C-8), 20.33 (C-14), 12.80 (C-13); HRMS (ESI) calcd for $C_{27}H_{36}N_3O_8$ $[M+H]^+$ 530.2502, found 530.2507.

1-(2-(12-β-dihydroartemisinoxy)ethyl)-5-((2-((1-(2-(12-β-dihydroartemisinoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenoxy)methyl)-1H-1,2,3-triazole (30): Brown viscous liquid; yield 55%; ^{1}H NMR (500 MHz, CDCl3): δ= 7.73 (2H, s, H-3a', H-3b'), 7.03 (2H, m, H-9', H-10'), 6.91 (2H, m, H-8', H-11'), 5.26, 5.24 (4H, s, H-5', H-5''), 5.14 (2H, s, H-5a,

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H-5b), 4.74 (2H, d, J=2.5, H-12a, H-12b), 4.58 (2H, m, H-1b', H-1b''), 4.51 (2H, m, H-1a', H-1a''), 4.26 (2H, m, H-2b', H-2b''), 3.79 (2H, m, H-2a', H-2a''), 2.52 (2H, m, H-11a, H-11b), 1.24, 1.39 (6H, s, H_3-15a, H_3-15b), 0.89 (6H, d, J=6.0, H_3-14a, H_3-14b), 0.73 (6H, d, J=7.0, H_3-13a, H_3-13b); ^{13}C NMR (125 MHZ, $CDCl_3$): δ=148.43 (C-6', C-7'), 144.31 (C-4a', C-4b'), 123.51 (C-3a', C-3b'), 122.14 (C-9', C-10'), 115.28 (C-8', C-11'), 104.18 (C-4a, C-4ab), 102.13 (C-12a, C-12b), 87.87 (C-5a, C-5b), 80.86 (C-6a, C-6b), 66.38 (C-5a', C-5b'), 63.41 (C-1', C-1''), 53.79, 52.41 (C-1a, C-1b), 50.38 (C-2', C-2''), 44.08 (C-7a, C-7b), 37.29 (C-10), 36.33 (C-3a, C-3b), 34.46 (C-9a, C-9b), 30.61 (C-11a, C-11b), 26.09 (C-15a, C-15b), 24.60 (C-2a, C-2b), 24.29 (C-8), 20.33 (C-14a, C-14b), 12.81 (C-13a, C-13b); HRMS (ESI) calcd for $C_{46}H_{64}N_6O_{12}$ $[M+H]^+$ 893.4660, found 893.4650.

1-(2-(12-β-dihydroartemisinoxy)ethyl)-4-((3-((1-(2-(12-β-dihydroartemisinoxy) ethyl)-4,5-dihydro-1H-1,2,3-triazol-4-yl)methoxy)phenoxy)methyl)-1H-1,2,3-triazole (31): Brown viscous liquid; yield 58%; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 7.67 (2H, s, H-3', H-3''), 7.17(1H, m, H-10'), 6.58 (3H, m, H-7', H-9', H-11'), 5.17, 5.14 (2H, s, H-5a, H-5b), 5.16 (4H, s, H-5', H-5''), 4.74 (2H, d, J=3.5 Hz, H-12a, H-12b), 4.61 (2H, m, H-1b', H-1b''), 4.51 (2H, m, H-1a', H-1a''), 4.27 (2H, m, H-2b', H-2b''), 3.78 (2H, m, H-2a', H-2a''), 2.58 (2H, m, H-11a, H-11b), 1.39 (6H, s, H_3-15a, H_3-15b), 0.89 (6H, d, J=6.0 Hz, H_3-14a, H_3-14b), 0.78 (6H, d, J=7.0 Hz, H_3-13a, H_3-13b); ^{13}C NMR (125 MHz, $CDCl_3$): δ=159.50 (C-6', C-8'), 144.12 (C-4', C-4''), 130.11 (C-10'), 123.98, 123.28 (C-3', C-3''), 107.46 (C-9', C-11'), 104.19 (C-4a, C-4b), 102.16 (C-7'), 102.08 (C-12a, C-12b), 87.87 (C-5a, C-5b), 80.84 (C-6a, C-6b), 66.41 (C-5', C-5''), 62.08 (C-1', C-1''), 52.65, 52.41 (C-1a, C-1b), 50.43 (C-2', C-2''), 44.07 (C-7a, C-7b), 37.31 (C-10a, C-10b), 36.32 (C-3a, C-3b), 34.45 (C-9a, C-9b), 30.94, 30.62 (C-11a, C-11b), 26.08 (C-15a, C-15b), 24.60 (C-2a, C-2b), 24.33 (C-8a, C-8b), 20.34 (C-14a, C-14b), 12.83 (C-13a, C-13b); HRMS (ESI) calcd for $C_{46}H_{66}N_6O_{12}$ $[M+H]^+$ 893.4660, found 893.4661.

2,4-bis((1-(2-(12-β-dihydroartemisinoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy) benzaldehyde (32): White solid; yield 54%; mp 62-64 ºC; ^{1}H NMR (500 MHz, CDCl3): δ= 10.26 (1H, s, CHO), 7.78 (1H, d, J=8.0, H-10'), 7.75, 7.73 (2H, s, H-3', H-3''), 6.79 (1H, d, J=8.0, H-7'), 6.67 (1H, d, J=8.0, H-11'), 5.28, 5.26 (4H, s, H-5', H-5''), 5.14, 5.13 (2H, s, H-5a, H-5b), 4.74, 4.73 (2H, d, J=3.5, H-12a, H-12b), 4.63 (2H, m, H-1b', H-1b''), 4.62 (2H, m, H-1a', H-1a''), 4.27 (2H, m, H-2b', H-2b''), 3.81 (2H, m, H-2a', H-2a''), 2.58 (2H, m, H-11a, H-11b), 1.40, 1.39 (6H, s, H_3-15a, H_3-15b), 0.90, 0.89 (6H, d, J=6.0, H_3-14a, H_3-14b), 0.77, 0.74 (6H, d, J=7.5, H_3-13a, H_3-13b); ^{13}C NMR (125 MHz, $CDCl_3$): δ=187.93 (C-12'),

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164.63 (C-6'), 162.21 (C-8'), 143.15, 143.06 (C-4', C-4''), 130.77 (C-10'), 123.64, 123.54 (C-3', C-3''), 119.48 (C-9'), 107.62 (C-11'), 104.22 (C-4a, C-4b), 102.18, 102.14 (C-12a, C-12b), 99.67 (C-7'), 87.87 (C-5a, C-5b), 80.80 (C-6a, C-6b), 66.38, 66.35 (C-5', C-5''), 62.56, 62.18 (C-1', C-1''), 52.37 (C-1a, C-1b), 50.53, 50.50 (C-2', C-2''), 44.03, 44.01 (C-7a, C-7b), 37.34 (C-10a, C-10b), 36.30, 36.27 (C-3a, C-3b), 34.46, 34.43 (C-9a, C-9b), 30.94, 30.59, (C-11a, C-11b), 26.08, 26.05 (C-15a, C-15b), 24.61 (C-2a, C-2b), 24.33, 24.32 (C-8a, C-8b), 20.35, 20.31 (C-14a, C-14b), 12.82, 12.77 (C-13 a, C-13b).

2,4-bis((1-(2-(12-β-dihydroartemisinoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy) benzoic acid (33): Light brown viscous liquid; yield 49%; ^{1}H NMR (500 MHz, $CDCl_3$): δ= 10.85 (1H, s, COOH), 7.73, 7.66 (2H, s, H-3', H-3''), 7.71 (1H, d, J=9.0 Hz, H-10'), 6.49 (1H, d, J=2.5 Hz, H-7'), 6.44 (1H, d, 9.0 Hz, H-11'), 5.17 (2H, s, H-5a, H-5b), 5.44, 5.41 (4H, d, J=12.5 Hz, H-5b', H-5b''), 5.11 (4H, d, J=13 Hz, H-5a', H-5a''), 4.72, 4.71 (2H, d, J=3.5 Hz, H-12a, H-12b), 4.62 (2H, m, H-1b', H-1b''), 4.48 (2H, m, H-1a', H-1a''), 4.27 (2H, m, H-2b', H-2b''), 3.77 (2H, m, H-2a', H-2a''), 2.56 (2H, m, H-11a, H-11b), 1.38, 1.37 (6H, s, H_3-15a, H_3-15b), 0.90, 0.89 (6H, each d, J=6.0 Hz, J=5.5 Hz, H_3-14a, H_3-14b), 0.75, 0.71 (6H, d, J=7.5 Hz, H_3-13a, H-13b); ^{13}C NMR (125 MHZ, $CDCl_3$): δ=169.66 (COOH), 164.35 (C-6'), 163.84 (C-8'), 142.48, 143.23 (C-4'/C-4''), 131.64 (C-10'), 123.40 (C-3', C-3''), 121.84 (C-9'), 107.75 (C-11'), 105.75, 104.18, (C-4a/C-4b), 124.82 (C-7'), 102.14, 101.68 (C-12a/C-12b), 87.85 (C-5a, C-5b), 80.80 (C-6a, C-6b), 66.38 (C-5', C-5''), 62.10, 58.02 (C-1'/ C-1''), 52.37 (C-1a, C-1b), 50.65, 50.47 (C-2'/C-2''), 44.02 (C-7a, C-7b), 37.34, 37.29 (C-10a, C-10b), 36.29 (C-3a, C-3b), 34.42 (C-9a, C-9b), 30.58, 30.56 (C-11a, C-11b), 26.06, 26.04 (C-15a, C-15b), 24.58 (C-2a, C-2b), 24.32, 24.30 (C-8a/C-8b), 20.32, 20.31 (C-14a, C-14b), 12.80, 12.74 (C-13a, C-13b).

4.11. MTT assay

The cell lines used in the present study were procured from National centre for cell science (NCS) pune, India and maintained in a cell incubator with 5% CO_2, 85% relative humidity at 37^0C. MTT assay was performed as per our previously reported work Singh et al., 2017.[46] Cells 0.5–2×10^4 cells/ml was prepared in fresh complete growth media. The cells were seeded on the wells; by inoculating the 100 µL cell suspension to each well of the plate at the desired density of cells and incubated for 24 hours with 5% and 85% relative humidity at 37^0C. After that, the treatments were performed with different concentrations (0.4, 2, 10, and 50 µg/ml) and incubated for 24 h. After that, 5 mg/ml MTT dye was added and further

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incubated in dark for 4 h. After incubation, the dye was removed and 100 µl/well DMSO was added to the plates and mixed well with the shaker to solublize the formazon crystals. Doxorubicin was used as positive control. The absorbance was measured at 570 nm using microplate reader (Multiscan^{TM} Go Skanlt Software 4.0 version, Thermo Fisher Scientific Waltham USA) and percentage cytotoxicity was calculated as reported earlier Maurya et al., 2017.[47]

4.12. Cell Cycle analysis

Cell cycle analysis was done by Propidium iodide PI dye according to Krishan, 1975; Crissman & Steinkamp, 1973.[48,49] The cells were seeded on the wells in a final concentration of 5–7×10^5 cells/ml and incubated for 24 hours at 37 °C with 5% CO_2 and 85% relative humidity. After 24 h, different concentrations of tested compounds were added. After that cells were collected with the trypsin-EDTA and prepare single cell suspension using PBS and ice cold absolute ethanol by gently vortexing. After washing, cells were resuspended in PBS and then DNA extraction buffer (0.2M Na_2HPO_4, 0.1M citric acid and 0.1% Triton X, adjust pH 7.8) was added. After 5 min incubation cells were centrifuge at 5000rpm for 5min at 4^0C and pellet was dissolve in 50ug/mL PI staining solution followed by 10mg/ml of RNase-A treatment and further incubate for 30 min at room temperature. Cell cycle analysis was performed in maximum excitation of PI bound to DNA at 536nM and emission is at 617 nM by using flow cytometer (BD biosciences, USA). The data was analyzed by using FACS-Diva software (BD-Biosciences, USA).

4.13. Intracellular ROS estimation

Intracellular ROS level was measured according to Hamidullah et al., 2015 using DCF-DA dye by flow-cytometry.[50] Briefly, cells seeded to a density of 1-2 × 10^6 per well in 6 well plate and incubated with compound in a different concentration for 24 h at 37°C. After incubation, 10 µM DCF-DA dye was added followed by further incubation for 30 min at 37°C and washing (twice) with ice cold PBS. ROS levels were calculated in cells by measuring the formation of DCF using analyzed using LSRII Flow-Cytometer equipped with 488 nm argon lasers as light source at 485nm and 520nm for excitation and emission respectively. The cells were analyzed using LSRII Flow-Cytometer (BD Biosciences). Mean fluorescence intensity was calculated using FACS Diva analysis software (BD Biosciences).

4.14. Osmotic fragility evaluation

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The osmotic fragility assay was performed according to Luqman et al., 2004.[51] The experiments were performed in compliance with the guidelines laid by Ethical Guidelines for Biomedical Research on Human Subjects (2000), Indian Council of Medical Research (ICMR, New Delhi, India 2006), Central Ethics Committee on Human Research (CECHR) Policy Statement of Ethical Considerations involved in Research on Human Subject and Ministry of Health and Family Welfare (MHFW), Government of India (ICMR Ethical Guidelines, 2017). Blood was collected from the median cubical vein of healthy volunteers in 10U/ml heparin by a recognized pharmacist in the presence of Medical Officer in the dispensary of the Institute, subsequent to informed consent. The heparinized blood was incubated with compounds (100 µg/ml and 50 µg/ml) for 60 min at 37 °C. 10% phosphate buffer saline stock was used to prepare working standards of 0.10–0.85%. After incubation, the blood was transferred to tubes containing different concentration of PBS and further incubated for 30 min at 37 °C with mild shaking. The cells were centrifuged at 5000 rpm for 5 min. The supernatant was collected and absorbance was recorded at 540 nm. Osmotic fragility curve was constructed by plotting the percentage lysis against the concentration of saline solutions as reported earlier Ponnam et al., 2014.[52]

4.15. Statistical analysis

One way analysis of variance (ANOVA) was performed using Graphpad prism 4 by employing post hoc Dunnett's test. All the data expressed as mean ± SD (n=3). P value<0.05 was considered statistically significant.

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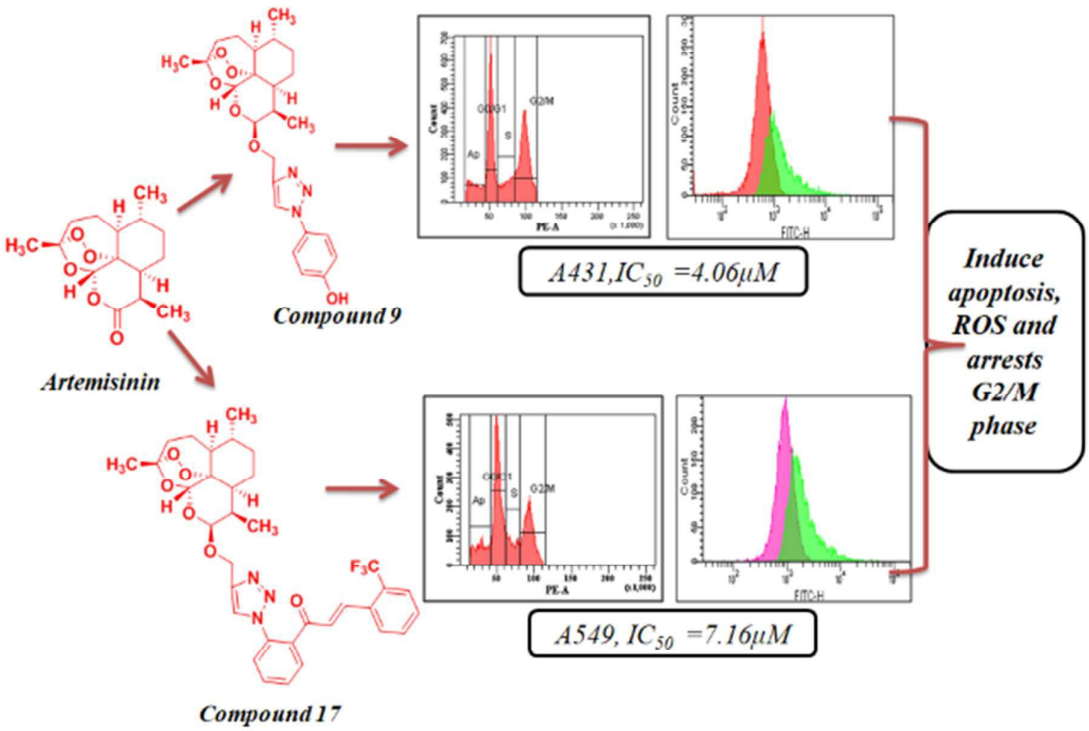

Novel artemisinin-1,2,3-triazole derivatives showed significant antiproliferative activity and induce apoptosis, ROS generation and arrest cell cycle at G2/M phase.