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Coumarin–trioxane hybrids: Synthesis and evaluation as a new class of antimalarial scaffolds ☆

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ABSTRACT

First synthesis of novel coumarin–trioxane hybrids is reported. The synthesis was achieved via condensation of β-hydroxyhydroperoxides with coumarinic-aldehydes in presence of *p*-toluenesulfonic acid in good yields and the novel hybrids were evaluated for their antimalarial activity both in vitro and in vivo.

Malaria, a major parasitic disease affecting around 200–500 million people and causes over 800,000 deaths annually.[1] The main reason for the recent dramatic increase in deaths from malaria is attributed to the spread of *Plasmodium falciparum* strains resistant to the mainstay antimalarial chloroquine.[2] To overcome the challenges of multi-drug resistance in *P. falciparum*, many approaches currently being adopted contain optimization of treatment with available drugs including combination therapy, developing analogs of the existing drugs and evaluation of drug resistance reversers (chemo sensitizers) as well as exploring new chemotherapeutic targets.[3] Recently the concept of hybrid antimalarials[4] has attracted much attention for tackling the alarming problem of drug resistance, as these molecules often act on multiple therapeutic targets because of the presence of two different, covalently fused pharmacophores.[5] Some hybrid molecules like trioxaquine[6a] and ferroquine[6b] comprising 1,2,4-trioxane-(4-aminoquinoline) and ferrocene–chloroquine moieties, respectively, are under clinical trials as hybrid antimalarial agents. Chemical structures of some of the recent hybrid antimalarial agents are shown in Figure 1.

Artemisinin (qinghaosu) is an unusual 1,2,4-trioxane, which has been used clinically in China for the treatment of multidrug-resistant *Plasmodium falciparum* malaria[7] and its semisynthetic derivatives (Fig. 2) are effective against both the chloroquine-sensitive and chloroquine-resistant malaria.[8] The peroxide group, present in the form of 1,2,4-trioxane, is essential for the antimalarial activity of these compounds.[9]

Similarly, Coumarins form an important class of compounds, which occupy a special role in nature. Pharmacologically, they belong to flavonoid class of compounds, which have been found to exhibit a variety of biological activities, usually associated with low toxicity and have raised considerable interest because of their potential beneficial effects on human health.[10,11] They have attracted intense interest in recent years because of their diverse pharmacological properties like anti-HIV,[12] anticoagulant,[13] antibacterial,[14] antioxidant,[15] dyslipidemic,[16] and antimalarial.[17] There are many naturally occurring coumarins which show antimalarial activity like Daphnetin, 5,7-methoxy-8-(3-methyl-1-buten-3-ol)-coumarin, pachyrrhizine (Fig. 2).

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological

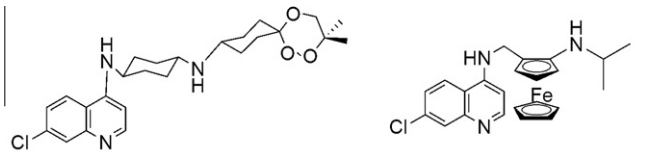

Figure 1. Some representative hybrid antimalarial drugs.

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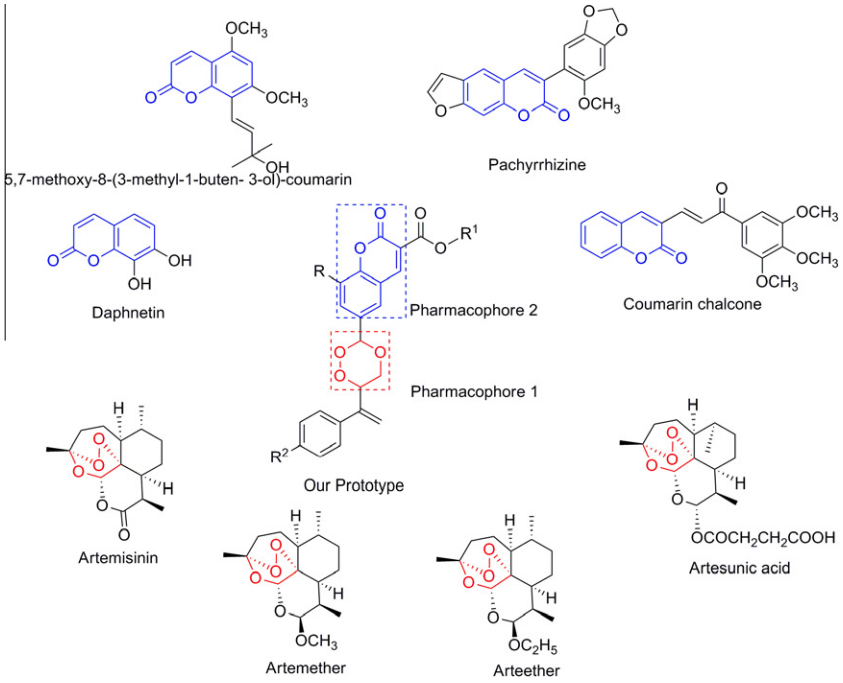
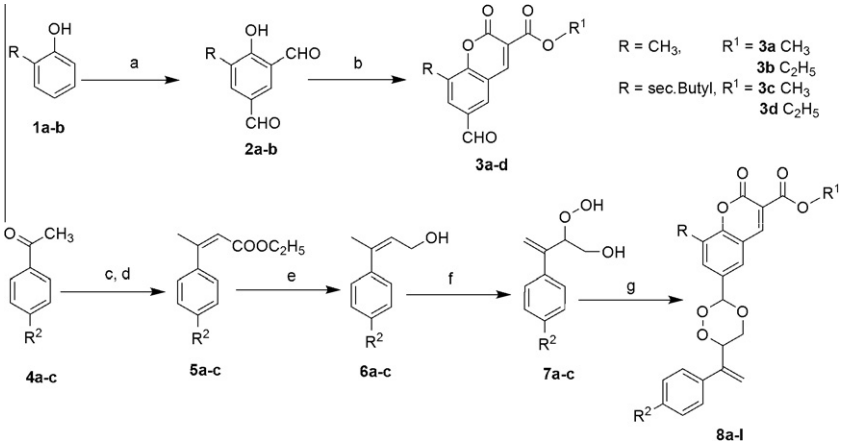


Figure 2. Examples of some potent coumarin and trioxane containing antimalarial compounds.

profiles. Therefore, a single molecule containing more than one pharmacophore, each with different mode of action could be beneficial for the treatment of malaria. Adopting this approach and from our ongoing efforts on finding novel hybrid antimalarials[18] and on oxygenated heterocycles[19] we, therefore, designed, synthesized and evaluated novel hybrid molecules linking a trioxane moiety to coumarin backbone. To the best of our knowledge, the hybridization of these two pharmacophores into novel scaffolds and evaluation of their biological activities have not yet been reported. This report constitutes the first example of a covalently linked trioxane–coumarin hybrid. The route followed for the preparation of coumarin derivatives and coumarin–trioxane hybrids are illustrated in Scheme 1.

The synthetic strategy followed for synthesis of coumarin–trioxane hybrids (**8a**–**l**) is depicted in Scheme 1. They were prepared by reaction between the β-hydroxyhydroperoxides and the appropriate coumarins containing free aldehyde following the previously reported method.[20,21] However, this protocol required the prior synthesis of intermediates (**3a**–**d**) and (**7a**–**c**) (Scheme 1). Coumarinic compounds (**3a**–**d**) were obtained via Knoevenagel-type reaction, by condensation of substituted salicylaldehydes (**2a**–**b**) with different active methylene compounds under basic conditions. These substituted salicylaldehydes (**2a–b**) were obtained by the Duff reaction on substituted phenols (**1a**–**b**). The intermediates (**7a**–**c**) were prepared in three steps (a) the Reformatsky reaction of the substituted acetophenones (**4a**–**c**), with ethyl bromoacetate gave the β-hydroxyester which on dehydration to furnished the α,β-unsaturated ester (**5a**–**c**) in good yield. (b) These α,β-unsaturated esters (**5a**–**c**) were subjected to reduction with $LiAlH_4$ to furnish allylic alcohols (**6a**–**c**). (c) While, the dye sensitized photooxygenation of allylic alcohols (**6a**–**c**) provided β-hydroxyhydroperoxides (**7a**–**c**). Finally, β-hydroxyhydroperoxides (**7a**–**c**) were condensed in situ with coumarinic compounds (**3a**–**d**) to afford the targeted coumarin–trioxane hybrids (**8a**–**l**) in



Scheme 1. Synthesis of coumarin–trioxane hybrids (**8a**–**l**). Reagents and conditions: (a) (i) hexamethylenetetramine/trifluoroacetic acid, 120 °C, 3 h; (ii) 10% H_2SO_4, 90–100 °C, 2 h; (b) piperidine, $CH_2(COOR)_2$, ROH, reflux, 30 min; (c) $BrCH_2COOC_2H_5$, Zn, I_2 (cat), C_6H_6, reflux, 5 h; (d) *p*-toluenesulfonic acid (cat), C_6H_6, reflux, 2 h; (e) $LiAlH_4$, diethyl ether, inert atm, 0 °C, 2 h; (f) O_2, methylene blue, hν, CH_3CN, −10 to 0 °C, 6 h; (g) **3a**–**d**, CH_3CN, concd HCl (cat), rt, 1 h.

Table 1
Antimalarial activity (IC_{50}, ng/mL) of novel coumarin–trioxane hybrids

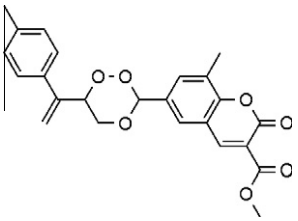
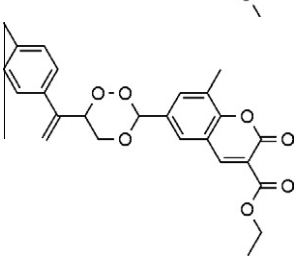
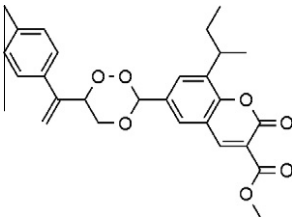
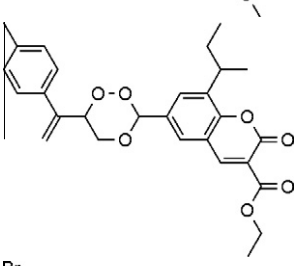
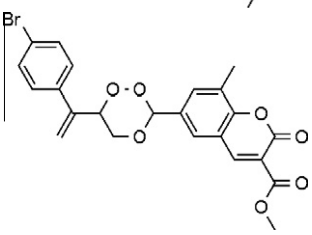
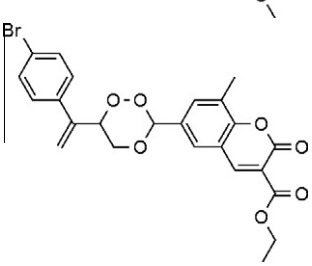
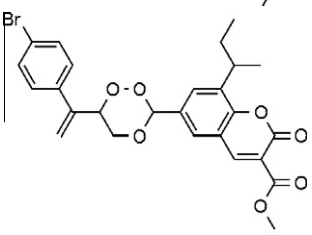
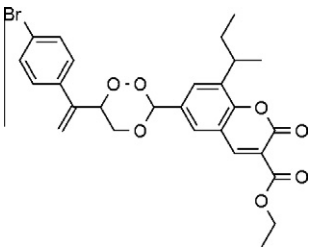
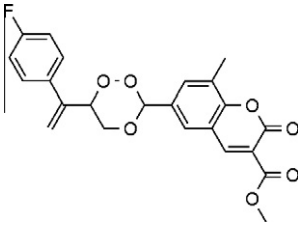
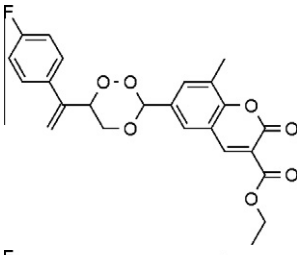
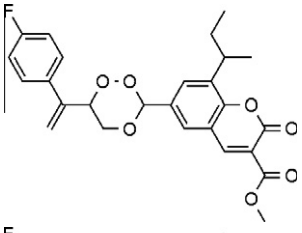
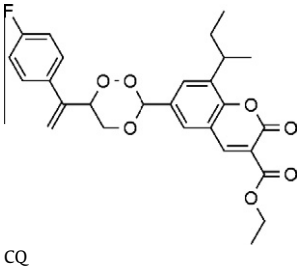
Compounds	Structure	In vitro antimalarial activity IC_{50}[a] (ng/mL)	*C*log *P*[b]
8a		360.53	4.00
8b		262.17	4.53
8c		131.99	5.46
8d		39.28	5.99
8e		305.92	4.36
8f		245.52	4.89
8g		76.01	5.82

Table 1 (*continued*)

Compounds	Structure	In vitro antimalarial activity IC_{50}[a] (ng/mL)	Clog*P*[b]
8h		110.12	6.35
8i		>500	3.64
8j		>500	4.17
8k		203.00	5.10
8l		116.97	5.63
	CQ	5.20	
	Artemether	0.40	

[a] IC_{50}: concentration corresponding to 50% growth inhibition of chloroquine-sensitive strain 3D7 of *P. falciparum*.
[b] *C*log*P* value were calculated using the software chemdraw.

good yield (Scheme 1).[22] All compounds were characterized using ^{1}H NMR, ^{13}C NMR, 2D NMR, mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis.

The new compounds were evaluated for their in vitro antimalarial activities against CQ sensitive 3D7 strain of *P. falciparum*.[23] The in vitro antimalarial activity, and *C*log*P* values of above synthesized compounds are shown in Table 1. Among all the synthesised compounds, some of which showed moderate antimalarial activity with IC_{50} in the range of 39.28–360.53 ng/mL. It is interesting to note that the presence of bulky aliphatic group (*sec*-butyl group) on C-8 position as in the compounds **8c**, **8d**, **8g**, **8h**, **8k** and **8l** tend to increase the antimalarial activity. Surprisingly, the substitution at phenyl ring with either electron donating group (**8a**–**d**) or by the electron withdrawing as in the case of compounds (**8e**–**l**) seem to have no effect, as the activity was conserved in both the cases. These compounds were further evaluated for their in vivo potency against multi-drug-resistant *P. yoelii nigeriensis* in mice at 96 mg/kg/day for 4 days by oral route using Peters's procedure.[24] Among all the synthesised compound **8b** showed an in vivo suppression of 41.14% on day 4 against multidrug-resistant *Plasmodium yoelii* in Swiss mice by oral route.

In conclusion, we have prepared a new series of coumarin–trioxane hybrids (**8a**–**l**) by using new methodology. The synthesized hybrids have been evaluated for their antimalarial activity against CQ sensitive 3D7 strain of *P. falciparum* and some of which exhibited the moderate activity. These hybrid molecules, however, suffer from serious limitations such as poor solubility both in oil and water. Our current efforts in this area are directed to address these problems.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.100.

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22. *Representative procedure for the synthesis of compound* ***8a*** *(methyl 8-methyl-2-oxo-6-(6-(1-p-tolylvinyl)-1,2,4-trioxan-3-yl)-2H-chromene-3-carboxylate)*: A solution of allylic alcohol **7a** (1 g, 6.18 mmol) and methylene blue (30 mg) in acetonitrile (100 mL) was irradiated with a 500 W tungsten–halogen lamp at −10 to 0 °C while oxygen was bubbled slowly into the reaction mixture for 4 h. Methyl 6-formyl-8-methyl-2-oxo-2*H*-chromene-3-carboxylate **3a** (2.28 g, 9.26 mmol) and concd HCl (0.2 mL) were added, and the reaction mixture was left at 5 °C for 18 h. Usual workup followed by chromatography over silica gel furnished trioxane **8a**.
White solid, yield: 65%; mp 136–138 °C; IR (KBr): 3023, 1760, 1619, 1520, 1021 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.50 (s, 1H), 7.63 (s, 1H), 7.59 (s, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 7.8 Hz, 2H), 6.20 (s, 1H), 5.52 (s, 1H), 5.44 (d, J = 10.1 Hz, 1H), 5.31 (s, 1H), 4.23 (dd, J = 11.68 Hz and 2.4 Hz, 1H), 3.94 (t, J = 10.8 Hz, 1H), 3.94 (s, 3H), 2.45 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 163.7, 156.5, 154.4, 149.2, 142.5, 138.4, 135.5, 134.1, 130.6, 129.4, 126.9, 126.4, 126.1, 118.2, 117.5, 116.6, 102.8, 80.8, 69.9, 53.0, 21.2, 15.5; ESI-MS (m/z): 423 $(M+H)^+$; Anal. Calcd for $C_{24}H_{22}O_7$: C, 68.24; H, 5.25, found: C, 68.31; H, 5.30.
23. *Evaluation of antimalarial activity*: Chloroquine sensitive 3D7 strain of *Plasmodium falciparum* was used to evaluate in vitro antimalarial activity of compounds. The assay used was Malaria SYBR Green-I nucleic acid staining dye based fluorescence (MSF) assay (Smilkstein et al., 2004). The compounds were tested in 96 well plates in duplicate wells. The compounds were incubated for 72 h with 1% parasitized cell suspension containing 0.8–1% initial parasitaemia at 37 °C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture. Finally SYBR Green-I was added to each well and incubated for one hour at 37 °C. The plates were examined at 485 ± 20 nm of excitation and 530 ± 20 nm of emission (FLX800, BIO-TEK). Chloroquine was used as standard antimalarial. To obtain 50% inhibitory concentration (IC_{50}) of compounds Data were first transferred into a graphic programme (e.g., EXCEL) and expressed as percentage of the untreated controls and then evaluated by Logit regression analysis using pre-programmed Excel spreadsheet (Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803.
24. 100% suppression of parasitemia means, number of parasites if at all present, are below the detection limit. The parasites present below the detection limit can multiply and eventually can be detected. In such cases though the drug is providing near 100% suppression of the parasitemia but will not provide full protection to the treated mice. Multi-drug-resistant *Plasmodium yoelii nigeriensis* used in this study is resistant to chloroquine, mefloquine and halofantrine.