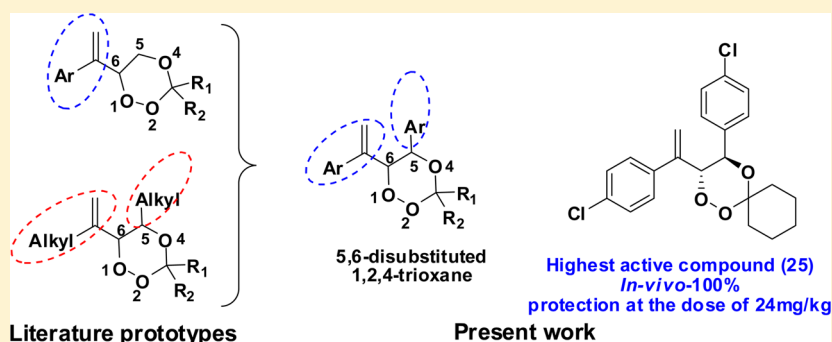


Synthesis and Antimalarial Activity of 3,3-Spiroanellated 5,6-Disubstituted 1,2,4-Trioxanes

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S Supporting Information



ABSTRACT: Novel 3,3-spiroanellated 5-aryl, 6-arylvinyl-substituted 1,2,4-trioxanes 19–34 have been synthesized and appraised for their antimalarial activity against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice by oral route at doses ranging from 96 mg/kg \times 4 days to 24 mg/kg \times 4 days. The most active compound of the series (compound 25) provided 100% protection at 24 mg/kg \times 4 days, and other 1,2,4-trioxanes 22, 26, 27, and 30 also showed promising activity. In this model, β -arteether provided 100 and 20% protection at 48 mg/kg \times 4 days and 24 mg/kg \times 4 days, respectively, by oral route. Compound 25 displayed a similar in vitro pharmacokinetic profile to that of reference drug β -arteether. The activity results demonstrated the importance of an aryl moiety at the C-5 position on the 1,2,4-trioxane pharmacophore.

KEYWORDS: 1,2,4-trioxane, antimalarial, in vivo, orally active

Synthetic trioxanes containing a 1,2,4-trioxane pharmacophore of artemisinin have been a key area of research since the discovery of the antimalarial lead molecule artemisinin from *Artemisia annua*.^{1–5} Malaria is of serious concern in many tropical areas of the world, and the situation has worsened due to drug resistance to common chemotherapeutic agents.⁶ Artemisinin and its derivatives have been thoroughly investigated for their efficacy against malaria parasite and also their peroxide specific mode of action.^{7–17} Since identification of the peroxide group-specific antimalarial activity of artemisinin, many synthetic peroxides have been synthesized and tested for their efficacy and were found to show promising antimalarial activity as compared to artemisinin.^{18–24} For the synthesis and assessment of the antimalarial activity of the 1,2,4-trioxane skeleton, two major prototypes are reported in the literature (Figure 1): (I) aryl vinyl substitution at C-6 position^{25–30} and (II) alkyl vinyl substitution at C-6 and alkyl/cycloalkyl substitution at C-5 position.^{31–33} So far, synthesized trioxanes have a substitution at C-6 because of the ease of synthesis via ene reaction of allylic alcohols with singlet oxygen. A substitution corresponding to the phenyl vinyl part of the molecule has been the subject of an extensive study by Singh et al.,^{25–30} where they have synthesized a number of compounds

based on allylic alcohols derived from Wittig/reformatsky products of various phenyl methyl ketones. They have experimental results to support the importance of an aryl vinyl substitution at C-6 of the 1,2,4-trioxane moiety, along with effects of different substituents in the aromatic ring. The results from Singh et al. motivated us to keep the C-6 phenyl vinyl part as such in our 1,2,4-trioxane (prototype III, Figure 1). For prototype II, Griesbeck et al. utilized diastereoselective photooxygenation to synthesize racemic *threo*- β -hydroperoxy alcohols that were in turn used to synthesize diastereomerically pure aliphatic C-5,6-substituted 1,2,4-trioxane and reported in vitro antimalarial activities against the K1 strain of *P. falciparum*.^{31–33} On the basis of the in vitro activity profile of some of these Griesbeck's 1,2,4-trioxanes, O'Neill et al. reported the synthesis of C-5,6-alkylsubstituted 1,2,4-trioxanes by stereoselective photooxygenation of chiral allylic alcohols.³⁴ From Figure 1, it is clear that approach I is attractive because of promising in vivo activity results, while approach II is attractive for in vitro activity results of C-5,6-dialkyl-substituted 1,2,4-

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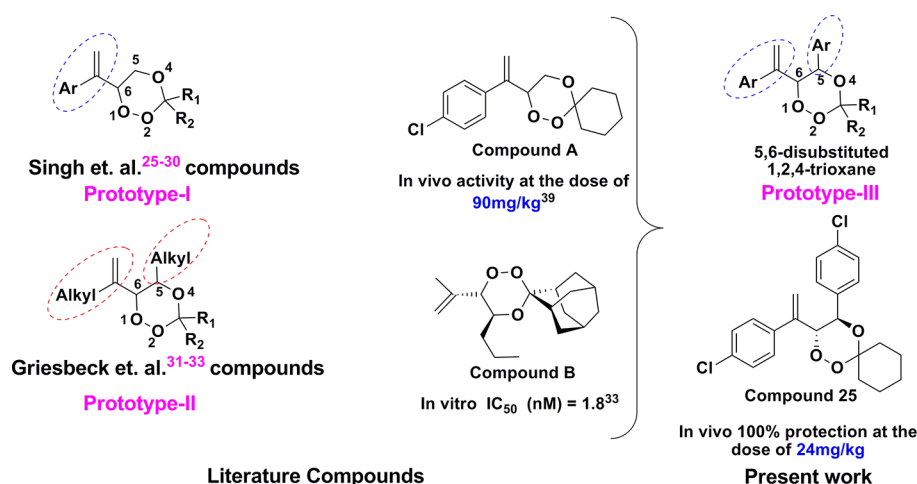
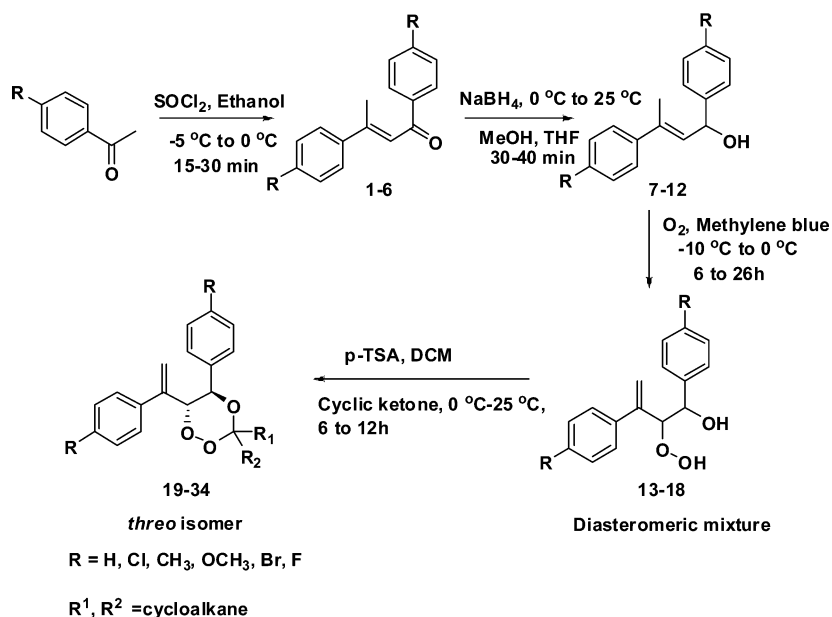


Figure 1. Basis for the synthesis of 3,3-spiroannellated 5,6-disubstituted 1,2,4-trioxane.

Scheme 1. Synthesis of 3,3-Spiroannellated 5,6-Disubstituted 1,2,4-Trioxanes (19–34)



trioxanes. Taking in to consideration the importance of a phenyl substituent on the synthetic 1,2,4-trioxanes evident from work by Singh et al. and in vitro activity results of C-5,6-disubstituted 1,2,4-trioxanes, we envisage a new route for aryl substitution at C-5 and aryl vinyl substitution at C-6 positions of the 1,2,4-trioxane moiety to assess their in vivo antimalarial potential. (Figure 1, prototype III).

5,6-Disubstituted 1,2,4-trioxanes 19–34 were prepared by the procedure given in Scheme 1 and starts with the formation of homochalcones. The desired homochalcones 1–6 were synthesized by the thionyl chloride-catalyzed self-condensation of substituted acetophenones in ethanol in the temperature range from –5 to 0 °C (21–42% yields), following the reported procedure.³⁵ Homochalcones 1–6 were reduced by sodium borohydride (NaBH₄) in a mixture of methanol and tetrahydrofuran (THF) to give the corresponding allylic alcohol 7³⁶–12 (55–66%) yields. Allylic alcohols are a very useful intermediates for the synthesis of 1,2,4-trioxanes via peroxycetalization of hydroperoxyalcohol. The synthesis of allylic alcohol-derived hydroperoxides has been the key step toward

development of many synthetic 1,2,4-trioxanes. Photooxygenation of allylic alcohols 7–12 in acetonitrile (CH₃CN) and chloroform (CHCl₃) with methylene blue as a sensitizer furnished a diastereomeric mixture of *threo* (major) and *erythro* (minor) β -hydroperoxyalcohol 13–18 (44–66%). The *threo*-selectivity is explained on the basis of a hydroxyl-directing effect of allylic alcohol, which is in turn strongly affected by competing hydrogen bond acceptors. Peroxycetalization of hydroperoxyalcohol 13–15, 17, and 18 with cyclopentanone and cyclohexanone was carried out in the presence of catalytic acid to furnish exclusively the *threo*-1,2,4-trioxanes 19–23 (17–26%) and 24–28 (15–28%), respectively. Similarly Peroxycetalization of hydroperoxyalcohol 13–18 with adamantane were carried out in presence of *p*-toluene sulfonic acid (PTSA) to furnish the *threo*-1,2,4-trioxanes 29–34 (14–28%). The relative configuration of the product was determined to be *threo* based on ³J_{H5–H6} observed in the proton spectrum of product (³J_{H5–H6} = 9.6 Hz for compound 25).^{31,32} The corresponding peroxycetalization product from *erythro* (minor) β -hydroperoxyalcohol could not be isolated in all cases. This is the first

Table 1. In Vivo Antimalarial Activity of Compounds 19–34 against Multidrug-Resistant *P. yoelii nigeriensis* in Swiss Mice by oral and im Routes

General structure	Compound	R	Dose	%Suppression of parasitaemia on day 4 ^{a,b}	Mice alive on day28	
	19	H	Oral 96	23.78	0/5	
			i.m. 96	46.91	0/5	
	20	Chloro	Oral 96	63.56	0/5	
	21	Methyl	Oral 96	100	0/5	
	22	Bromo	96	100	5/5	
			Oral 48	100	1/5	
23	Fluoro	24	98.58	0/5		
		Oral 96	22.47	0/5		
	24	H	Oral 96	34.52	0/5	
			i.m. 96	22.31	0/5	
	25	Chloro	96	100	5/5	
			48	100	5/5	
			Oral 24	100	5/5	
			12	100	0/5	
			i.m. 96	3.45	0/5	
			96	100	4/5	
	26	Methyl	Oral 48	89.43	0/5	
			24	49.59	0/5	
			i.m. 96	16.56	0/5	
	27	Bromo	Oral 96	100	2/5	
28	Fluoro	Oral 96	42.47	0/5		
	29	H	Oral 96	32.41	0/5	
			i.m. 96	44.79	0/5	
	30	Chloro	Oral 96	77.22	1/5	
	31	Methyl	Oral 96	36.7	0/5	
			i.m. 96	5.76	0/5	
	32	Methoxy	Oral 96	24.56	0/5	
	33	Bromo	Oral 96	18.08	0/5	
	34	Fluoro	Oral 96	16.71	0/5	
	β -arteether	-	-	Oral 48	100	5/5
				Oral 24	100	1/5

^aPercent suppression = $[(c - t)/c] \times 100$, where c = parasitaemia in control group, and t = parasitaemia in treated group. ^b100% suppression of parasitaemia means that the number of parasites, if present, is below the detection limit.

report for the synthesis of 5-aryl, 6-aryl vinyl-substituted 1,2,4-trioxanes and their antimalarial activities.

Compounds 19–34 were screened for antimalarial activity against multidrug-resistant *Plasmodium yoelii nigeriensis* in Swiss mice via oral route using Peter's procedure.³⁷ In this model, β -arteether (standard drug) showed 100% suppression of parasitemia³⁷ at 48 mg/kg \times 4 days, and all of the treated mice survived beyond day 28. At 24 mg/kg \times 4 days, β -arteether provides only 20% protection to the treated mice. Therefore, all of the trioxanes were initially tested at 96 mg/kg \times 4 days orally, double the effective dose of β -arteether. Compounds 22, 25, and 26 provided 100% protection³⁸ at 96 mg/kg \times 4 days dose and hence were further screened at lower

doses of 48, 24, and 12 mg/kg \times 4 days. The results are summarized in Table 1.

As evident from Table 1, some of the tested compounds displayed activity comparable with or better than that of β -arteether by oral route. Compound 25, the most active compound of the series, showed 100% parasite suppression on day 4 and 100% survival on day 28 of treatment at a dose of 24 mg/kg \times 4 days, while at 12 mg/kg \times 4 days, 100% suppression of parasitaemia on day 4 was shown but provided poor protection in terms of survival of the treated mice. Compound 25 is twice as active as β -arteether by oral route.

Compound 22, the next best compound, showed 20% protection at 48 mg/kg \times 4 days, while at 24 mg/kg \times 4 days,

98.58% suppression of parasitaemia on day 4 was shown and provided poor protection in terms of survival of the treated mice. Compound **26** showed 80% protection at 96 mg/kg \times 4 days dose, whereas only 89.43% suppression of parasitaemia on day 4 was observed at 48 mg/kg \times 4 days dose. Compound **27** showed 100% suppression of parasitaemia on day 4 at 96 mg/kg and provided 40% protection in terms of survival of the treated mice. The compounds **22**, **26**, **27**, and **30** were inactive at further lower doses. Activity results given in Table 1 and a comparative study in Figure 1^{33,39} clearly indicated that aryl substitution at C-5 resulted in highly active 1,2,4-trioxanes where the best active compound, that is, compound **25**, was twice as active as the reference drug β -arteether and 3.75 times more active as compared to the compound **A** (Figure 1) without C-5 aryl substitution. Biologically the most promising molecule of this series, compound **25** was assessed for its biopharmaceutical properties (Table 2). It could be categorized

Table 2. Biopharmaceutical Properties of Compound 25

drug-likeness property	compound 25	β -arteether ⁴³
solubility, distilled water (S)	13 μ M	practically insoluble
distribution coefficient (Log D), pH 7.4	4.23	3.6
in situ permeability (P_{eff} , cm/s)	$11.28 \pm 1.68 \times 10^{-5}$	NA
plasma protein binding (% free)	0.8	1–2
metabolic stability,		
half-life ($T_{1/2}$, min)	23.00	47.36
clearance (CL _{int} , μ L/min/mg)	60.26	36.58

as a class II drug as per the Biopharmaceutical Classification System based on its low solubility and high permeability.⁴⁰ It was found to be highly protein bound, 50% metabolically stable after 30 min on incubation with rat liver microsomes, and was not an inhibitor of rat CYPs 3A, 2D4, 1A2, or 2C11 (IC₅₀ > 100 μ M) (Supporting Information, Experimental Section). When compared to the reference drug, β -arteether, compound **25** showed a similar in vitro pharmacokinetic profile; however, it was found to be less metabolically stable. The biopharmaceutical properties obtained meet most of the criteria set by MMV Compound Progression Criteria 2008; thus, it may be a potential candidate for lead selection and optimization.^{41,42}

In conclusion, the C-5 aryl and C-6 arylvinyl-substituted 1,2,4-trioxanes were synthesized using a simple methodology starting from self-condensation products of acetophenones, that is, homochalcones, in the least possible number of steps. Compound **25** was identified as the most active compound of the series, which is twice as active as β -arteether. The activity results demonstrated the importance of an aryl moiety at the C-5 position on the 1,2,4-trioxane pharmacophore. Further work on C-5-substituted 1,2,4-trioxanes with better aqueous solubility, oral bioavailability, and efficacy is under progress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed spectroscopic data, ¹H and ¹³C NMR and mass spectra of synthesized compounds, and experimental procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NaBH₄, sodium borohydride; THF, tetrahydrofuran; CH₃CN, acetonitrile; CHCl₃, chloroform; PTSA, *p*-toluene sulfonic acid

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