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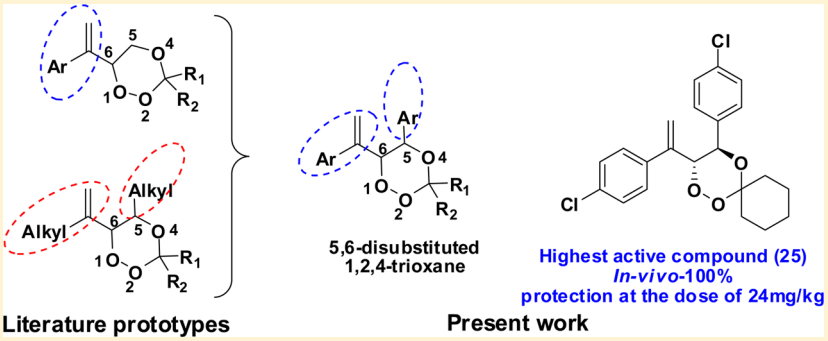
Synthesis and Antimalarial Activity of 3,3-Spiroanellated 5,6-Disubstituted 1,2,4-Trioxanes

Ranjani Maurya,[†] Awakash Soni,[‡] Devireddy Anand,[†] Makthala Ravi,[†] Kanumuri S. R. Raju,[§] Isha Taneja,[§] Niraj K. Naikade,[†] S. K. Puri,[‡] Wahajuddin,[§] Sanjeev Kanojiya,[∥] and Prem P. Yadav*[,†]

[†]Division of Medicinal & Process Chemistry, [‡]Division of Parasitology, [§]Division of Pharmacokinetics and Metabolism, and [∥]Sophisticated Analytical Instrument Facility, Central Drug Research Institute, Lucknow-226001, India

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 × 4 days to 24 mg/kg × 4 days. The most active compound of the series (compound **25**) provided 100% protection at 24 mg/kg × 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 × 4 days and 24 mg/kg × 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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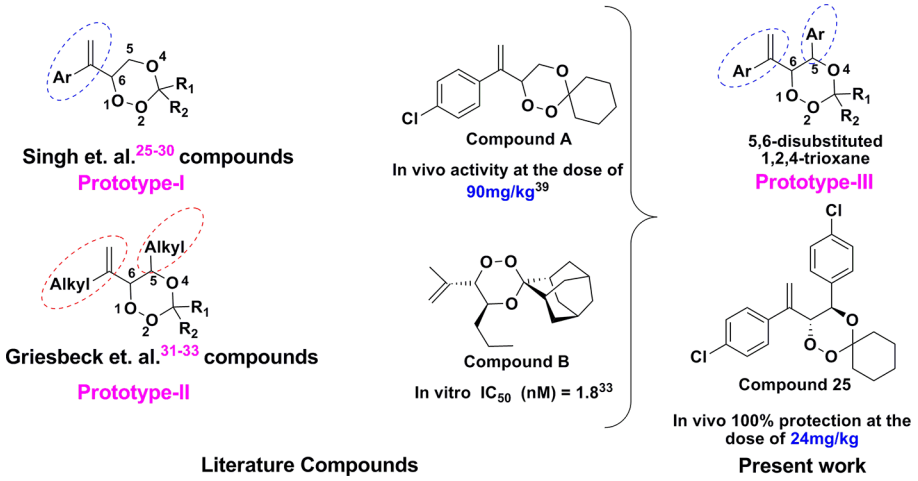
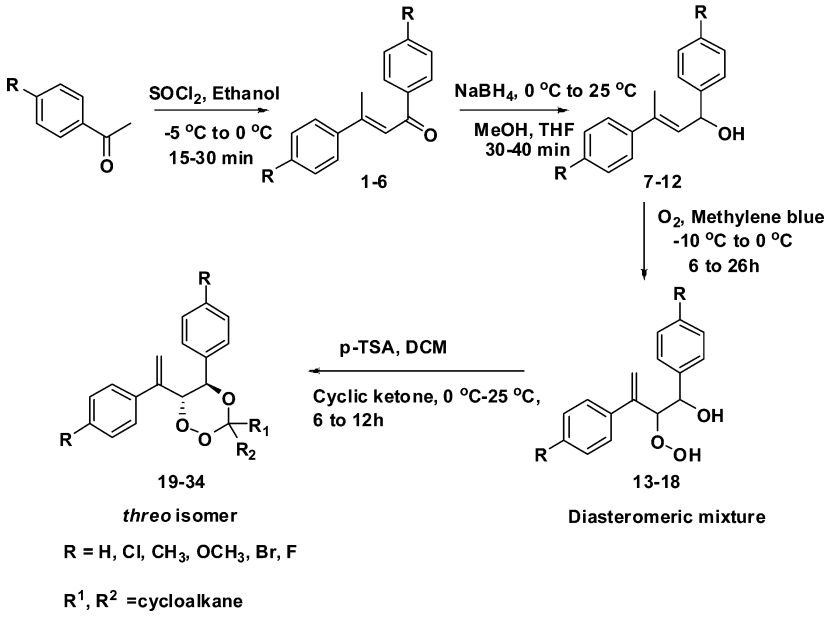


Figure 1. Basis for the synthesis of 3,3-spiroanellated 5,6-disubstituted 1,2,4-trioxane.

Scheme 1. Synthesis of 3,3-Spiroanellated 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 ($NaBH_4$) in a mixture of methanol and tetrahydrofuran (THF) to give the corresponding allylic alcohol **7**[36]**−12** (55−66%) yields. Allylic alcohols are a very useful intermediates for the synthesis of 1,2,4-trioxanes via peroxyketalization 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 (CH_3CN) and chloroform ($CHCl_3$) with methylene blue as a sensitizer furnished a diasteromeric 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. Peroxyketalization 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 Peroxyketalization of hydroperoxyalcohol **13−18** with admantanone were carried out in presence of *p*-toulene 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 peroxyketalization product from erythro (minor) *β*-hydroperoxyalcohol could not be isolated in all cases. This is the first

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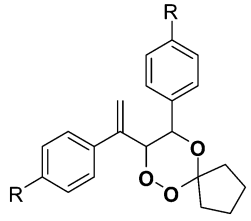
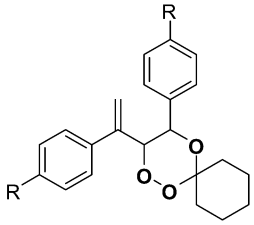
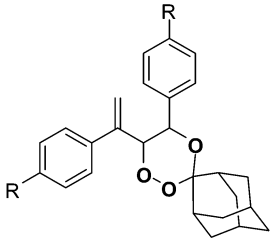
Table 1. In Vivo Antimalarial Activity of Compounds 19−34 against Multidrug-Resistant *P. yoelii nigeriensis* in Swiss Mice by oral and im Routes

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
				24	98.58	0/5
	23	Fluoro	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
	26	Methyl		96	100	4/5
			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

[a]Percent suppression = $[(c - t)/c] \times 100$, where c = parasitaemia in control group, and t = parasitaemia in treated group. [b]100% 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 parasitemia[37] at 48 mg/kg × 4 days, and all of the treated mice survived beyond day 28. At 24 mg/kg × 4 days, *β*-arteether provides only 20% protection to the treated mice. Therefore, all of the trioxanes were initially tested at 96 mg/kg × 4 days orally, double the effective dose of *β*-arteether. Compounds **22**, **25**, and **26** provided 100% protection[38] at 96 mg/kg × 4 days dose and hence were further screened at lower doses of 48, 24, and 12 mg/kg × 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 × 4 days, while at 12 mg/kg × 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 × 4 days, while at 24 mg/kg × 4 days,

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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 × 4 days dose, whereas only 89.43% suppression of parasitaemia on day 4 was observed at 48 mg/kg × 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 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 (IC_{50} > 100 μM) (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]

Table 2. Biopharmaceutical Properties of Compound 25

drug-likeness property	compound **25**	β-arteether[43]
solubility, distilled water (S)	13 μM	practically insoluble
distribution coefficient (Log D), pH 7.4	4.23	3.6
in situ permeability (P_{eff}, cm/s)	11.28 ± 1.68 × 10^{-5}	NA
plasma protein binding (% free)	0.8	1−2
metabolic stability,		
half-life ($T_{1/2}$, min)	23.00	47.36
clearance (CLint, μL/min/mg)	60.26	36.58

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, ^{1}H and ^{13}C 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.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +91-522-2612411-4496. Fax: +91-522-2623405, 2623938. E-mail: pp_yadav@cdri.res.in.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

$NaBH_4$, sodium borohydride; THF, tetrahydrofuran; CH_3CN, acetonitrile; $CHCl_3$, chloroform; PTSA, *p*-toulene sulfonic acid

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