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Full Paper

Design, Synthesis, and Biological Evaluation of Novel 1,2,4-Trioxanes as Potential Antimalarial Agents

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A series of substituted 1,2,4-trioxanes were synthesized and evaluated for their antimalarial potential, *in silico* ADME properties and cytotoxicity on neuronal cell lines. Among the 15 synthesized substituted 1,2,4-trioxanes, two compounds (compound **15**, $IC_{50} = 25.71$ nM; compound **21**, $IC_{50} = 19.6$ nM) exhibited promising *in vitro* antimalarial potential comparable to those of the existing drugs chloroquine and artemisinin. Both of these compounds were found to be nontoxic up to 20 μM concentration in neuronal PC-12 cells. Compound **21** may serve as an optimized lead compound because of its less *in vitro* toxicity and lower probability to cross the blood brain barrier.

Keywords: 1,2,4-Trioxanes / Antimalarial / Drug design / Synthesis

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Introduction

Malaria, one of the most devastating infectious diseases, affects about half of the global population [1]. About 212 million of new cases and 429000 attributed deaths were covered in year 2015 by this disease worldwide [2]. The scenario is aggravated by the persistent tendency of the most common malaria parasite *Plasmodium falciparum*, to rapidly develop resistance against any newly introduced drug [3, 4]. Artemisinin and its analogs are found suitable for the treatment of cerebral malaria caused by multi-drug-resistant *P. falciparum* [5, 6]. The major drawback of all semi-synthetic trioxanes is that they require artemisinin as starting material for their synthesis, but the current production of artemisinin from natural sources is relatively low. In view of it and in search of small molecules incorporating the pharmacophore from this class of compounds, a number of groups have attempted to produce totally synthetic endo-peroxide analogs, some of which have demonstrated remarkable antimalarial activity [7–11].

We have earlier reported a validated quantitative pharmacophore model utilizing this class of molecules [12]. Based on it, a focused virtual library was designed and screened. This led to the identification of simple substituted 1,2,4-trioxane (compound **5a**) having antimalarial activity up to 35 nM (Fig. 1B) with drug likeness property. Encouraged by the results of our previous work, and in order to further improve the antimalarial potential of our above identified hit we herein report the novel substituted 1,2,4-trioxanes as potential antimalarial agent.

Results and discussion

Lead optimization

The judicious addition/replacement in our earlier identified lead trioxane molecule **(5a)** was performed on the basis of different hydrophobic aliphatic and aromatic substituents requirements at site-1 and site-3 (Fig. 1A and B). The *n*-alkyl substituents at site-2 were also evaluated for their potential in

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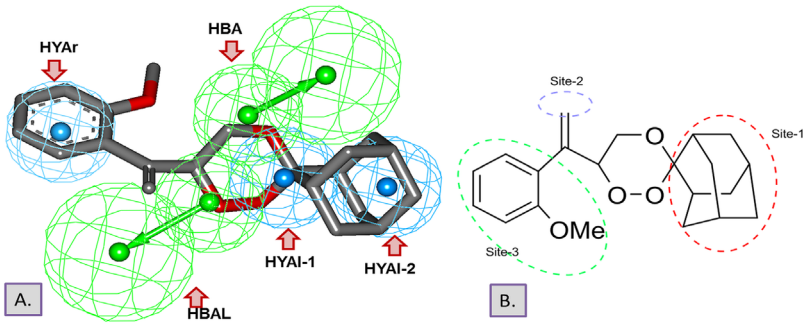


Figure 1. (A) Pharmacophore mapping of the lead compound **5a** previously reported by our research group; H-bond acceptor (HBA) and H-bond acceptor lipid (HBAL) as green, aliphatic hydrophobic (HYAl-1, HYAl-2) as dark blue and aromatic hydrophobic (HYAr) as light blue. (B) Site-1, site-2, and site-3 are the three sites of the lead compound **5a** targeted for the optimization studies presented here.

modulating the antimalarial activity. Table 1 outlines the site-specific modifications carried out at the two sites of the lead compound **5a** as envisaged.

In silico ADME calculation

Various *in silico* ADME properties of these compounds were calculated in the ADME module of acccelrys DS 2.0 software packages [13] and used to predict the pharmacokinetic behavior. Descriptor functions viz. Fast Polar Surface Area (FPSA) and AlogP of the ellipse model were used to predict the blood-brain penetration and intestinal absorption at 95% and 99% confidence. The molecule lying under the region common to both the confidence ellipses for a given property is considered robust with 99.9% confidence.

Chemistry

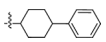
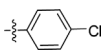
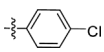
Allylic alcohols **(3a–d)** were prepared from the corresponding ketones (**1a–d**) according to the reported procedure [14] and were photo-oxygenated to give β-hydroxy hydroperoxides (**4a–d**). The photo-oxygenated products of allylic alcohols (β-hydroxy hydroperoxides) were not isolated and were condensed *in situ* where **4a–d** on condensation with different substituted ketones gave trioxanes **5–16** (Scheme 1) in the presence of catalytic amount of HCl to furnish these trioxanes with 49–58% overall yields (Table 1). However, the β-hydroxy hydroperoxides (**4a–c**) on *in situ* condensation with 1,4-cyclohexadiones gave compounds **17–19** which in 48–68% yields. Reductive amination of **17–19** with substituted anilines using $NaBH(OAc)_3$ furnished the amino functionalized trioxanes **20**, **21**, and **22** as mixture of diastereomers [15] in 46–87% yields (Scheme 2).

Structure–activity relationship interpretation

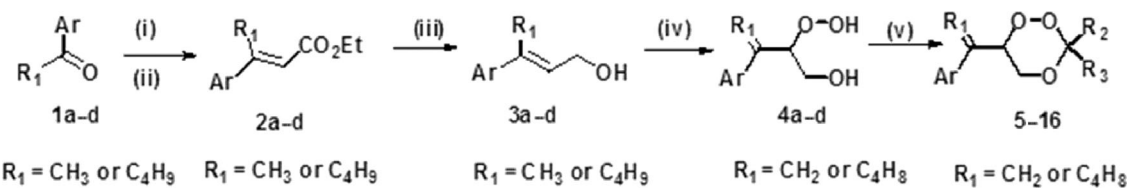
The test results of all the 15 synthesized molecules are summarized in Table 2 where four molecules (**7**, **11**, **15**, and **21**) have shown antimalarial activity less than 40 nM, while two molecules (**15** and **21**) have shown antimalarial activity comparable to the existing drug chloroquine and artemisinin, however, these two molecules were less active than the drug artemether and arteether (Table 2). The earlier developed pharmacophore model was explored to understand the structure–activity relationships (SAR) among these synthesized compounds. Interestingly, the pharmacophore well discriminates the molecules with high and low activity. The error factor between estimated and experimental activity of all the molecules was within ~3 (the uncertainty ratio in the Discovery Studio software) except compound **15** which carries the error factor of ~4.5 (Table 2). It was observed from the mapping of the synthesized molecules to the pharmacophore, that among all these molecules, the 1,2,4-trioxane ring itself provided the HBA and HBAL functionalities to the molecules. Four of the highly active molecules (**7**, **11**, **15**, and **21**) among the 15 synthesized compounds were simple trioxanes where the required pharmacophoric features were better placed than others. The pharmacophore mapping of the two highly active compounds (**15** and **21**) among the 15 synthesized compounds revealed that the di-substituted phenyl ring of the compounds **15** and **21** occupied the HYAr feature of the pharmacophore (Fig. 2A and B). Whereas the admantyl ring attached at position 3′ of the trioxane ring in compound **15** provided the bulky aliphatic hydrophobic functionality and occupied the HYAl-1 and HYAl-2 feature. Addition of amino phenyl ring to the cyclohexyl group of compound **21** does not appear to provide any required pharmacophoric features. However its cyclohexyl group provided the HYAl-1 functionality to the molecule. The high antimalarial activity of this compound is because of total high fit value for the HBA, HBAl, and HYAr feature (Fig. 2A). Similar to compounds **15** and **21** admantyl and cylcohexyl group of compounds **7** and **11** occupied the aliphatic hydrophobic feature (HYAl-1 and 2) while their aromatic rings provided the HYAr feature to the molecule resulting their high activity. The similar study with the two less active compounds (compounds **10** and **13**) revealed that their poor activity may be because of the poor accessibility of the structural features to map the HYAr in case of compound **13** and HYA1-1 and 2 feature in case of compound **10** (Fig. 2C and D).

The inherent problems associated with the most of the artemisinin derivatives as well as current synthetic trioxanes are their poor intestinal absorption and neurotoxicity [16]. Therefore, only those substituted trioxanes with good absorption rate and least neurotoxicity should be forwarded to drug development. The graphical plot analysis revealed that all the clinically used drugs (artemisinin, artemether, arteether) fell under the common region of 95% and 99%

Table 1. **Site-specific modifications of the synthesized compounds (5–22) based on the lead molecule 5a.**

S. N.	Compd. no.	Site-3	Site-2	Site-1	
		Ar	R1	R2/R3	R4
1	**5**	2,4-dimethylphenyl	CH_3		–
2	**6**	2,4-dimethylphenyl	CH_3		–
3	**7**	2,4-dimethylphenyl	CH_3		–
4	**8**	2,4-dichlorophenyl	CH_3		–
5	**9**	2,4-dichlorophenyl	CH_3		–
6	**10**	phenyl	propyl		–
7	**11**	phenyl	propyl		–
8	**12**	phenyl	propyl		–
9	**13**	2,4-difluorophenyl	CH_3		–
10	**14**	2,4-difluorophenyl	CH_3		–
11	**15**	2,4-difluorophenyl	CH_3		–
12	**16**	phenyl	propyl		
13	**20**	phenyl	propyl	–	
14	**21**	2,4-dimethylphenyl	CH_3	–	
15	**22**	2,4-dichlorophenyl	CH_3	–	

confidence ellipses for probability of intestinal absorption and blood-brain penetration, thus showing that these drugs may have a higher risk of neurotoxicity (Fig. 3). Although the value of BBB penetration of two highly active synthetic trioxanes (compounds **15** and **21**) was in close proximity (Table 2) yet they fall under different confidence ellipses. As the compound **15** fell under the common region of 95% and 99% confidence ellipses for both blood-brain penetration and



Scheme 1. Synthesis of the trioxanes **5–16**. Reagents and conditions: (i) Zn, $BrCH_2COOC_2H_5$, C_6H_6 ($CaCl_2$-dried), I_2 (catalyst), reflux (Refortmasky reaction); (ii) pTSA, C_6H_6, reflux, 10% HCl; (iii) LAH, dry ether, N_2 atmosphere, $<0°C$; (iv) O_2, hv, methylene blue, MeCN-CH_2Cl_2, rt; (v) ketones/aldehydes, HCl, rt.

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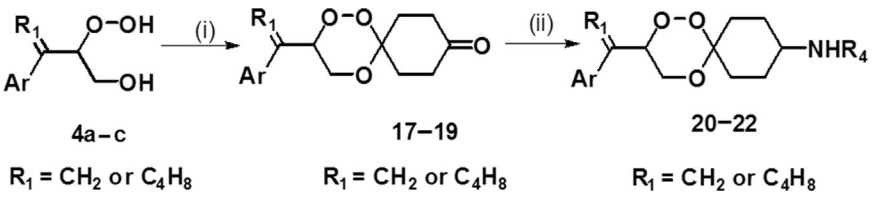

Scheme 2. Synthesis of the fuctionalized trioxanes **20–22**. Reagents and conditions: (i) 1,4-Cyclohexadione, HCl, rt; (ii) R_4NH_2, $NaBH(OAc)_3$, C_6H_6, 0°C, 1 h.

intestinal absorption properties, it was expected it may have good intestinal absorption but may cause neuronal effect because of its permeability to BB barrier.

On the other hand, though the compound **21** did not fall under common region of 95% and 99% confidence ellipses for blood-brain penetration and intestinal absorption properties, thus the compound **21** may have some poorer intestinal absorption than compound **15** yet it may not cross the BB barrier to cause neuronal effects.

In vitro cytotoxic evaluation on neuronal cell lines

The trioxane molecules are known to carry inherent neurotoxic properties causing severe energy depletion in brainstem neurons, and paradoxial increase in the reactive oxygen species at therapeutic concentrations [17, 18]. *In vitro* study on neuronal cells using arteether, artemether, and dihydroartemisinin (DHA) at 300 nM drug concentrations was found to significantly inhibit neurite outgrowth and MTT metabolism [19]. Another *in vitro* study where neurotoxicity was monitored by uptake of radiolabeled leucine into mouse neuroblastoma–rat glioma hybrid NG108-15 cells growing in cultures suggested artemisinin as least neurotoxic with an IC_{50} value of 100 μM whereas DHA and artesunic acid with IC_{50} value of 0.46 μM as most neurotoxic compound among clinically active trioxanes [20]. *In vivo* neurohistopathological studies on clinically active trioxanes including arteether, artemether, DHA, artelinic acid, artesunate indicated to produce manifest neurotoxicity [21].

Table 2. Experimental and predictive *in vitro* antimalarial activity of compounds against 3D7 strain of *P. falciparum* and their predictive ADME properties.

Comp. no./name	Antimalarial activity			ADMET properties			
	Experimental IC_{50} (nM)	Predicted IC_{50} (nM)	Error[a)]	BBB[b)]	Hepatotoxicity probability	A logP	PSA[c)]
5	110	61.61	−1.79	0.92	0.50	4.86	26.79
6	201	168.50	−1.19	1.06	0.44	5.31	26.79
7	36	41.70	1.16	1.27	0.74	5.97	26.79
8	68.1	44.28	−1.54	1.17	0.35	5.67	26.79
9	229	243.24	1.06	1.38	0.69	6.33	26.79
10	667	577.01	−1.16	1.03	0.37	5.20	26.79
11	39.4	29.80	−1.32	1.17	0.36	5.65	26.79
12	142	218.04	1.54	1.37	0.70	6.31	26.79
13	3271.4	1137.58	−2.88	0.46	0.34	3.36	26.79
14	286.85	214.07	−1.34	0.89	0.37	4.75	26.79
15	25.71	118.24	4.60	1.09	0.71	5.41	26.79
16	168.6	209.12	1.24	1.57	0.37	6.94	26.79
20	364	362.98	−1.00	–	0.40	7.08	39.6
21	19.6	40.00	2.04	1.10	0.20	6.07	39.6
22	311.9	239.51	−1.30	–	0.48	7.09	39.6
Artemether	2.86	8.2	2.86	0.12	0.158	3.17	44.65
Arteether	3.27	7.4	2.26	0.23	0.132	3.52	44.65
Artemisinin	11.2	6.8	−1.63	−0.023	0.90	3.14	53.02
Chloroquine	16.3	–	–	0.76	0.25	4.35	27.42

[a)] Values in the error column represent the ratio of estimated activity to experimental activity, or its negative inverse if the ratio is less than 1; [b)]BBB, blood brain barrier; [c)]PSA, polar surface area.

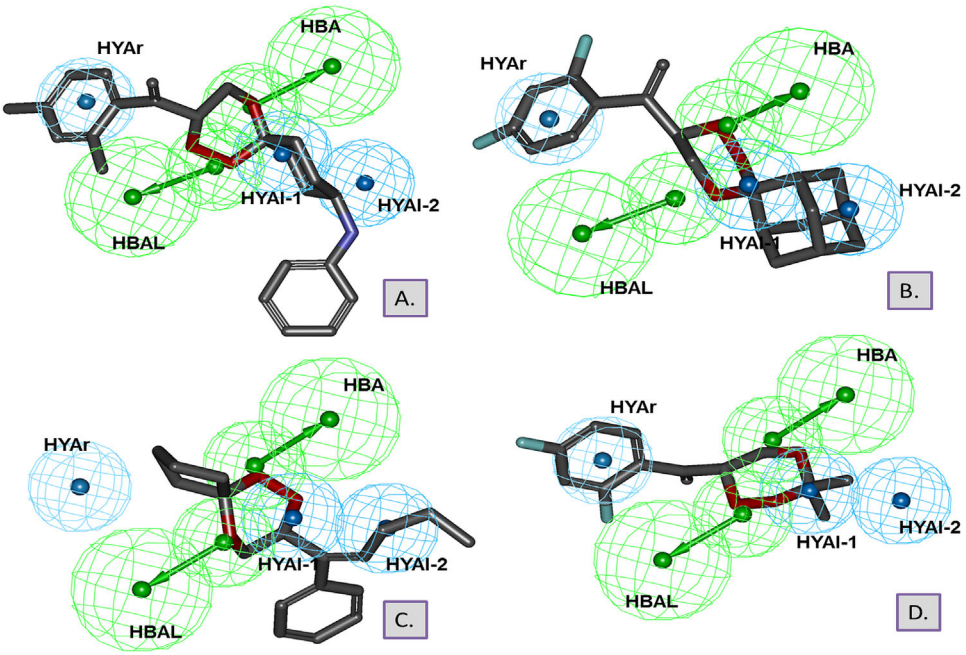

Figure 2. Pharmacophore mapping of the two highly active compounds (A) **21** and (B) **15** and of the two less active compounds (C) **13** and (D) **10**.

Therefore, the four highly active molecules (**7**, **11**, **15**, and **21**) among the 15 synthesized trioxanes were evaluated for their neurotoxicity on neuronal PC-12 cell lines. Results of percent cell viability of neuronal PC-12 cells exposed to various concentrations (1–80 μM) of compounds (**7**, **11**, **15**, and **21**) for 24 h are summarized in Fig. 4 where compounds **15** and **21** showed better results in terms of cell viability at higher dose than other molecules. At 40 μM concentration compounds **15** and **21** were found to be cytotostatic with **21** having less cytotostatic effect, as this concentration seems to be comparatively less lethal and causes a physiological stress to the cells. A total of 80 μM concentration was found to be cytotoxic for both compounds **15** and **21**; however, compound **21** was less toxic than **15** at this concentration. For compounds **7**, **11**, and **15** the concentration of 40 μM was found to be cytotoxic while 20 μM concentration was found cytostatic, thus confirming the *in silico* predictions for the compounds **15** and **21**. The rest of the lower doses (1 and 10 μM) were found non-cytotoxic for all the molecules used in this study.

Conclusion

In conclusion, in this study, we embarked on the design, synthesis and biological evaluation of novel 1,2,4-trioxanes as potential antimalarial agents where the optimization of our earlier discovered lead compound was performed. For this, we have synthesized and evaluated a series of novel trioxanes which led to the identification of the two promising novel antimalarial compounds **15** ($IC_{50} = 25.71$ nM) and **21** ($IC_{50} = 19.6$ nM) having *in vitro* antimalarial potential comparable to the existing drugs chloroquine and artemisinin. Compound **21** has been identified as optimized lead for further studies due to its less neurotoxicity and less probability to cross the blood brain barrier among the compounds tested.

Experimental

Chemistry

General

Melting points were determined in open capillaries on a COMPLAB melting point apparatus and are uncorrected. IR spectra were recorded on a PerkinElmer RXI FT-IR spectrophotometer. ^{1}H NMR and 75 MHz for ^{13}C NMR was recorded on a Bruker Supercon Magnet DPX-200 (operating at 200 MHz for ^{1}H) spectrometer using deuterated as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR. Multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). Reactions were monitored on silica gel TLC plates. All the compounds tested were 95% pure. Column chromatography was performed over silica gel (60–120 mesh) procured from Qualigens (India) using freshly distilled solvents. All the chemicals and reagents were obtained from Aldrich (USA), Lancaster

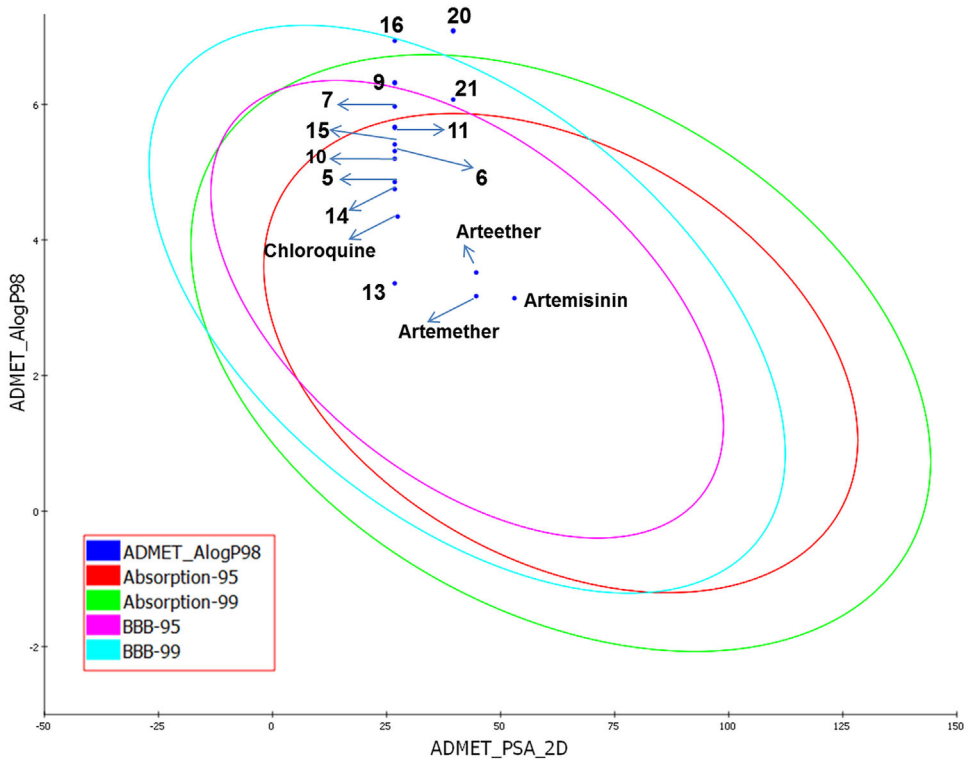

Figure 3. Plot of polar surface area (PSA) versus ALogP for the synthesized compounds along with marketed drugs showing 95% and 99% confidence limit ellipses corresponding to the blood brain barrier and intestinal absorption models.

(England), or Spectrochem (India) and were used without purification.

The InChI codes of the investigated compounds are provided as Supporting Information.

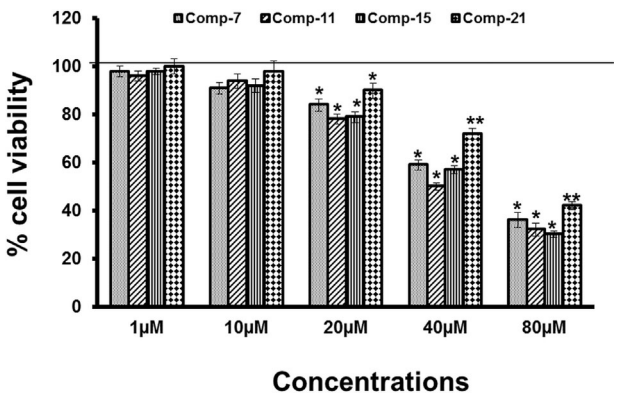

Figure 4. MTT assay of compounds **7**, **11**, **15**, and **21** on the neuronal PC-12 cell line. Error bars in the graph represent the SEM for the values obtained from at least three independent experiments. The values of $^{*}p < 0.05$ were considered as significant and of $^{**}p < 0.01$ as more significant.

Trioxane 5

ESMS (*m/z*): 274 $[M]^+$, IR (KBr, cm^{-1}) 3015.8, 2960.8, 2873.3, 2368.5, 1449.2, 1332.3, 1216.9, 1110, 971.5, 922.6, ^{1}H NMR (300 MHz.$CDCl_3$) 1.60–1.89 (m,8H); 2.28 (s,3H); 2.33 (s,3H); 3.71–3.79 (m,2H); 4.99–5.03 (m,1H); 5.18 (s,1H); 5.47 (s,1H); 6.9–7.35 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.85, 21.05, 23.01, 25.01, 32.7, 37.5, 63.1, 81.2, 102.4, 118.1, 126.31, 128.69, 131.11, 135.26, 136.13, 137.47, 144.01.

Trioxane 6

ESMS (*m/z*): 288 $[M]^+$, IR (KBr, cm^{-1}) 3018, 2963, 2365, 1449.6, 1340.5, 1218.4, 1103.5, 984.3, 925.6, ^{1}H NMR (300 MHz.$CDCl_3$) 1.28–1.60 (m,10H); 2.28 (s,3H); 2.32 (s,3H); 3.66–3.71 (m,1H); 3.87–3.95 (m,1H); 4.76–4.79 (m,1H); 5.17 (s,1H); 5.48 (s,1H); 6.94–7.02 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.90, 21.62, 22.25, 26.4, 29.6, 32.5, 63.3, 81.5, 102.5, 116.9, 126.3, 128.72, 131.16, 135.29, 136.15, 137.50, 144.02.

Trioxane 7

ESMS (*m/z*): 340 $[M]^+$; IR (KBr, cm^{-1}) 3429.2, 3015.7, 2922.8, 2369.1, 1451.9, 1219.3, 1111.2, 924.4; ^{1}H NMR (300 MHz. $CDCl_3$) 1.43–2.06 (m,14H); 2.20 (s,3H); 2.30 (s,3H); 3.63–3.70 (m,1H); 3.81–3.93 (m,1H); 4.90–4.97 (m,1H); 5.15 (s,1H); 5.44 (s,1H); 6.9–7.0 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.87, 21.2, 27.9, 32.9, 36.4, 37.1, 63.4, 81.6, 112.6, 118.1, 126.4, 128.6, 131.2, 135.4, 136.2, 137.6, 144.02.

Trioxane 8
ESMS (*m/z*): 329 $[M]^+$; IR (KBr, cm^{-1}) 3021.1, 2940.3, 2359.0, 1448.3, 1216.7, 1041.7, 927.8; ^1H NMR (300 MHz.$CDCl_3$) 0.94–2.31 (m,10H); 3.79–3.83 (m,1H); 3.92–3.99 (m,1H); 5.03–5.07 (m,1H); 5.34 (s,1H); 5.61 (s,1H); 7.1–7.43 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.23, 26.6, 29.5, 32.5, 63.5, 81.7, 102.7, 116.5, 125.31, 127.9, 129.2, 130.16, 133.29, 133.9, 144.03.

Trioxane 9
ESMS (*m/z*): 380 $[M]^+$; IR (KBr, cm^{-1}) 3452.5, 3020.9, 2359.5, 1449.4, 1216.6, 1042.6, 925.7; ^1H NMR (300 MHz.$CDCl_3$) 1.63–2.44 (m,14H); 3.54–3.59 (m,1H); 3.93–3.96 (m,1H); 5.49–5.53 (m,1H); 5.86–5.91 (m,2H); 7.34–7.39 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.9, 32.7, 36.4, 37.5, 63.4, 81.6, 112.4, 116.1, 125.41, 127.6, 129.6, 130.6, 133.6, 133.6, 144.03.

Trioxane 10
ESMS (*m/z*): 289 $[M+1]^+$, IR (KBr, cm^{-1}) 3019.7, 2961.8, 2872.6, 2361.3, 1448.3, 1331.2, 1216.2, 1111.5, 970.1; ^1H NMR (300 MHz.$CDCl_3$) 1.42 (t,3H, $J = 6$ Hz); 1.39–1.81 (m,8H); 2.35–2.41 (m,2H); 2.98 (t,2H, $J = 9$ Hz); 3.58–3.62 (m,1H); 3.83–3.90 (m,1H); 5.48–5.50 (m,1H); 5.80–5.85 (m,1H); 7.3–7.99 (m,3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.8, 20.5, 23.01, 25.01, 32.7, 37.5, 63.2, 81.2, 102.4, 117.7, 126.9, 128.5, 128.9, 139.4, 139.1, 144.01.

Trioxane 11
ESMS (*m/z*): 303 $[M+1]^+$, IR (KBr, cm^{-1}) 3018.5, 2935.7, 2871.6, 2361.2, 2338.9, 1448.8, 1329.2, 1216.5, 1086.4, 924.2; ^1H NMR (300 MHz.$CDCl_3$) 0.95 (t,3H, $J = 6$ Hz); 0.95–1.60 (m,10H); 2.32–2.36 (m,2H); 2.97 (t,2H, $J = 12$ Hz); 3.50–3.51 (m,1H); 3.85–3.96 (m,1H); 5.39–5.43 (m,1H); 5.80–5.84 (m,1H); 7.26–7.99 (m,5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.8, 20.5, 22.30, 26.1, 30.7, 33.2, 63.2, 81.2, 102.4, 118.1, 126.9, 128.5, 128.9, 135.4, 144.01.

Trioxane 12
ESMS (*m/z*): 354 $[M]^+$; IR (KBr, cm^{-1}) 3020, 2928.6, 2872.6, 2360.4, 1455.9, 1217.4, 1111.5, 1038.4, 920.9; ^1H NMR (300 MHz. $CDCl_3$) 0.99 (t,3H, $J = 6$ Hz); 1.25–1.80 (m,14H); 2.0–2.17 (m,2H); 2.80–2.88 (m,2H); 3.44–3.52 (m,1H); 3.83–3.94 (m,1H); 5.40–5.47 (m,1H); 5.77–5.80 (m,1H); 7.61–7.98 (m,5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.6, 20.4, 27.8, 32.7, 36.7, 37.8, 63.4, 81.4, 112.4, 118.1, 127.01, 128.6, 129.0, 135.4, 144.01.

Trioxane 13
ESMS (*m/z*): 256 $[M]^+$; IR (KBr, cm^{-1}) 3450.5, 3019.5, 2361.5, 1450.4, 1211.6, 1042.6, 923.7; ^1H NMR (300 MHz.$CDCl_3$) 1.60 (s,3H); 1.63 (s,3H); 3.81–3.97 (m,2H); 5.07–5.11 (m,1H); 5.44 (s,1H); 5.55 (s,1H); 6.80–6.90 (m,2H); 7.27–30 (m,1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 25.38, 63.10, 81.23, 102.4, 118.1, 120.1, 120.9, 122.9, 126.8, 144.01, 153.6, 156.5.

Trioxane 14
ESMS (*m/z*): 296 $[M]^+$; IR (KBr, cm^{-1}) 3455.5, 3022.9, 2361, 1451, 1215, 1040.4, 924.5; ^1H NMR (300 MHz.$CDCl_3$) 1.10–2.19 (m,10H); 3.66–3.99 (m,2H); 5.08–5.12 (m,1H); 5.34 (s,1H); 5.44 (s,1H); 6.81–6.90 (m,2H); 7.27–30 (m,1H); ^{13}C NMR ($CDCl_3$, 75 MHz) 22.24, 26.4, 29.8, 32.7, 63.10, 81.23, 102.4, 118.1, 120.3, 121.2, 122.9, 127.2, 144.01, 153.5, 155.9.

Trioxane 15
ESMS (*m/z*): 348 $[M]^+$; IR (KBr, cm^{-1}) 3453, 3021, 2360, 1451, 1217, 1043, 920.7; ^1H NMR (300 MHz.$CDCl_3$) 1.60–2.0 (m,14H); 3.78–3.97 (m,2H); 5.09–5.13 (m,1H); 5.44 (s,1H); 5.55 (s,1H); 6.79–6.89 (m,2H); 7.21–29 (m,1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 27.8, 32.7, 36.8, 37.9, 63.2, 81.3, 112.4, 118.3, 120.4, 121.3, 123.4, 125.6, 144.01, 153.6, 156.5.

Trioxane 16
ESMS (*m/z*): 378 $[M]^+$; IR (KBr, cm^{-1}) 3448.5, 3019, 2360.5, 1444, 1215.6, 1040.6, 920.7; ^1H NMR (300 MHz.$CDCl_3$) 0.98 (m,3H), 1.45–2.13 (m,10H); 2.95 (m,1H); 3.54–3.65 (m,2H); 5.54–5.59 (m,1H); 6.14 (m,1H); 6.50–7.56 (m,10H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.3, 20.5, 63.5, 81.32, 111.4, 115.1, 127.2, 127.3, 127.6, 127.6, 128.3, 128.9, 137.7, 139.4, 144.01.

Trioxane 20
ESMS (*m/z*): 428 $[M+1]^+$; IR (KBr, cm^{-1}) 3019, 2960, 2930, 2401, 1497.8, 1216, 1089, 929.9; ^1H NMR (300 MHz.$CDCl_3$) 0.99 (t,3H, $J = 6$ Hz); 1.3–1.99 (m,10H); 2.31–3.37 (m,2H); 3.51–3.54 (m,1H); 3.90–3.97 (m, 1H); 4.22–4.25 (m,1H); 5.42–5.46 (m,1H); 5.80–5.86 (m,1H); 6.49–6.53 (m,2H); 7.1–7.12 (m,2H); 7.5–7.99 (m,5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.7, 20.5, 22.3, 26.3, 26.6, 62.01, 63.5, 81.32, 102.4, 111.6, 115.1, 126.2, 126.5, 128.2, 128.8, 129.9, 140.1, 144.01, 145.9.

Trioxane 21
ESMS (*m/z*): 367 $[M]^+$; IR (KBr, cm^{-1}) 3020.6, 2923.1, 2358.9, 1430.8, 1216.5, 1105.2, 1042.9, 928.9; ^1H NMR (300 MHz. $CDCl_3$) 1.50–1.80 (m,8H); 2.27 (s,3H); 2.33 (s,3H); 2.78 (m, 1H); 3.60–3.70 (m,1H); 3.72–3.96 (m,1H); 4.94–4.97 (m,1H); 5.19 (s,1H); 5.47 (s,1H); 6.7–7.30 (m,8H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.8, 20.09, 22.4, 27.6, 61.8, 63.9, 81.23, 102.27, 111.2, 118.07, 120.6, 126.31, 128.4, 129.6, 131.1, 135.26, 136.13, 137.4, 144.01, 146.3.

Trioxane 22
ESMS (*m/z*): 453 $[M]^+$; IR (KBr, cm^{-1}) 3423, 2941, 2363.0, 1500.4, 1217.0, 1043.7, 931.0; ^1H NMR (300 MHz.$CDCl_3$) 0.88–1.66 (m,8H); 2.69–2.73 (m,1H): 3.33–3.38 (m,1H); 3.85–3.90 (m,2H); 5.08–5.10 (m,1H); 5.32 (s,1H); 5.36 (s,1H); 5.61 (s,1H); 6.55–6.60 (m,2H); 7.12–7.17 (m,4H); 7.43 (s,1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 22.9, 27.5, 62.01, 63.10, 81.23, 102.4, 118.07, 120.6, 126.31, 126.9, 129.6, 131.2, 132.3, 135.26, 136.13, 144.01, 145.4.

Pharmacology

Antimalarial screening
All the 15 synthesized compounds along with the standard drugs artemether, arteether, and chloroquine were evaluated for their *in vitro* antimalarial activity against chloroquine (CQ) sensitive 3D7 strain of *P. falciparum* using a

standardized inexpensive assay based on SYBER Green I (Table 2) [22]. The IC_{50} values were calculated from experiments carried out in triplicate. The compounds were dissolved in DMSO at 5 ng/mL. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 μL of highest starting concentration (500 ng/mL) was dispensed in duplicate wells in row B of 96-well tissue culture plates. The highest concentration of chloroquine was 25 ng/mL. Subsequently, twofold serial dilutions were prepared up to row H (seven concentrations). Finally, 50 μL of 2.5% parasitized cell suspension containing 0.5% parasitemia was added to each well except four wells in row "A" which received a non infected cell suspension. These wells containing non-infected erythrocytes in the absence of drugs served as negative controls, while parasitized erythrocytes in the presence of CQ served as positive control. After 72 h of incubation, 100 μL of lysis buffer (20 mM Tris [pH 7.5], 5 mM EDTA, 0.008% [wt/vol] saponin, and 0.08% [vol/vol] Triton X-100) containing 1× concentration of SYBER Green I (Invitrogen) was added to each cell. The plates were re-incubated for 1 h at room temperature and examined for the relative fluorescence units (RFUs) per well using the FLUO star, BMG lab technologies. The 50% inhibitory concentration (IC_{50}) has been determined using nonlinear regression analysis dose response curves from experiments carried out in triplicate.

In vitro cytotoxic evaluation on neuronal PC-12 cell lines

Since the trioxane molecules are known to have inherent neurotoxicity therefore, the cytotoxicity of four high active molecules (**7**, **11**, **15,** and **21**) among the 15 synthesized trioxanes were evaluated through MTT assay on neuronal PC-12 cell lines. In brief, cells (1×10^4) were seeded in poly-L-lysine pre-coated 96-well culture plates and allowed to adhere properly for 24 h at 37°C in a CO_2 incubator (5% CO_2/95% atmospheric O_2 under humid conditions). After the respective exposure of compounds, MTT (5 mg/mL of stock in PBS) was added (10 μL/well in 100 μL of cell suspension), and plates were incubated for 4 h. At the end of incubation period, the reaction mixture was carefully taken out, and 200 μL of dimethyl sulfoxide (DMSO) was added to each well by pipetting up and down several times until the contents became solubilized. After 10 min, absorbance was taken at 550 nm, using a multiwell microplate reader (Synergy HT, BioTek). Untreated sets were run simultaneously under identical conditions and served as basal control.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) for the values obtained from three independent experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA) using GraphPad Prism (Version 5.0) software. The values $^{*}p < 0.05$ were considered as significant and $^{**}p < 0.01$ more significant.

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References

[1] W. Fang, J. V. Rodriguez, A. K. Ghosh, M. J. Lorena, A. Kang, R. J. St Leger, *Science* **2011**, *331*, 1074–1077.
[2] World Malaria Report World Health Organization: Geneva **2016**.
[3] F. J. Gamo, L. M. Sanz, J. Vidal, C. de Cozar, E. Alvarez, J. Lavandera, D. E. Vanderwall, D. V. S. Green, V. Kumar, S. Hasan, J. R. Brown, C. E. Peishoff, L. R. Cardon, J. F. Garcia-Bustos, *Nature* **2010**, *465*, 305–310.
[4] J. N. Burrows, K. Chibale, T. N. Wells, *Curr. Top. Med. Chem*. **2011**, *11*, 1226–1254.
[5] D. L. Klayman, *Science* **1985**, *228*, 1049–1055.
[6] N. J. White, *Science* **2008**, *320*, 330–334.
[7] G. H. Posner, W. Chang, L. Hess, L. Woodard, S. Sinishtaj, A. R. Usera, W. Maio, A. S. Rosenthal, A. S. Kalinda, J. G. D'Angelo, K. S. Petersen, J. Stohle, Chollet, J. Santo-Tomas, C. Snyder, M. Rottmann, S. Wittlin, R. Brun, T. A. Shapiro, *J. Med. Chem*. **2008**, *51*, 1035–1042.
[8] C. Singh, V. P. Verma, N. K. Naikade, A. S. Singh, M. Hassam, S. K. Puri, *J. Med. Chem*. **2008**, *51*, 7581–7592.
[9] P. M. O'Neill, A. Mukhtar, S. A. Ward, J. F. Bickley, J. Davies, M. D. Bachi, P. A. Stocks, *Org. Lett*. **2004**, *18*, 3035–3038.
[10] O. D. Cabaret, F. B. Vical, C. Loup, A. Robert, H. Gornitzka, A. Bonhoure, H. Vial, J. F. Magnaval, J. P. Seguela, B. Meunier, *Chem. Eur. J*. **2004**, *10*, 1625–1636.
[11] A. G. Griesbeck, T. T. El-Idreesy, L. O. Höinck, J. Lex, R. Brun, *Bioorg. Med. Chem. Lett*. **2005**, *15*, 595–597.
[12] A. K. Gupta, S. Chakroborty, K. Srivastava, S. K. Puri, A. K. Saxena, *J. Chem. Inf. Model*. **2010**, *50*, 1510–1520.
[13] Discovery Studio, version F 2.0; Accelrys Software Inc.: San Diego, CA, **2001**.
[14] C. Singh, R. Kanchan, D. Srivastava, S. K. Puri, *Bioorg. Med. Chem. Lett*. **2006**, *16*, 584–586.
[15] C. Singh, H. Malik, S. K. Puri, *Bioorg. Med. Chem. Lett*. **2004**, *14*, 459–462.
[16] C. J. Woodrow, R. K. Haynes, S. Krishna, *Postgrad. Med. J*. **2005**, *81*, 71–78.
[17] G. Schmuck, E. Roehrdanz, R. K. Haynes, R. Kahl, *Antimicrob. Agents Chemother*. **2002**, *46*, 821–827.
[18] P. F. Chinnery, C. Elliott, G. R. Green, A. Rees, A. Coulthard, D. M. Turnbull, T. D. Griffiths, *Brain* **2000**, *123*, 82–92.

[19] S. L. Smith, J. L. Maggs, G. Edwards, S. A. Ward, B. K. Park, W. G. McLean, *Neurotoxicology* **1998**, *19*, 557–559.
[20] D. L. Wesche, M. A. DeCoster, F. C. Tortella, T. G. Brewer, *Antimicrob. Agents Chemother*. **1994**, *38*, 1813–1819.
[21] S. Toovey, *Toxicol. Lett*. **2006**, *166*, 95–104.
[22] M. Smilkstein, N. Sriwilaijaroen, J. X. Kelly, P. Wilairat, M. Riscoe, *Antimicrob. Agents Chemother*. **2004**, *48*, 1803–1806.