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# Orally active amino functionalized antimalarial 1,2,4-trioxanes☆

Chandan Singh,[a,*] Heetika Malik[a] and Sunil K. Puri[b]

[a]*Division of Medicinal Chemistry, Central Drug Research Institute, Lucknow-226001, India*
[b]*Division of Parasitology, Central Drug Research Institute, Lucknow-226001, India*

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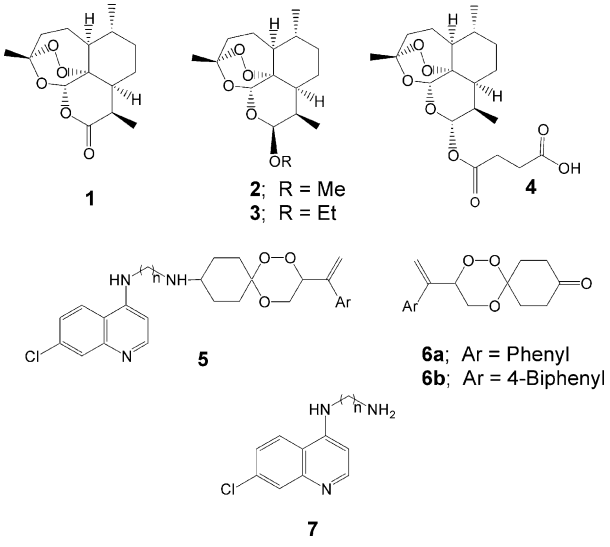
**Abstract**—Using readily available trioxanes **6a–b**, a new series of amino functionalized 1,2,4-trioxanes **8a–e** and **9a–e** have been prepared and evaluated for antimalarial activity against multi-drug resistant *Plasmodium yoelii* in Swiss mice model. Several of these novel trioxanes are orally more active than the parent trioxanes **6a–b**. Antimalarial activity of amino functionalized trioxane **9a**, the most potent compound in the series, is very close to that of β-arteether.


## 1. Introduction

Discovery of Artemisinin **1**, as the active principle of the Chinese traditional drug *Artemisia annua*, has opened new possibilities in malaria chemotherapy.[1] Artemisinin and its more potent derivatives for example, artemether **2**, arteether **3** and artesunic acid **4**, are highly active against both chloroquine-sensitive and resistant malaria. These drugs are fast acting and are well suited for the treatment of cerebral malaria caused by multi-drug resistant *Plasmodium falciparum*.[2] Peroxide group present in the form of 1,2,4-trioxane, is essential for the antimalarial activity of these drugs. Synthesis of a large number of structurally simple trioxanes have been reported, several of which have shown promising in vivo antimalarial activity.[3,4] Though chloroquine is known to antagonise the antimalarial effects of artemisinin derivatives,[5] recently we and others have attempted to improve the antimalarial activity of easily accessible synthetic trioxanes by incorporating 4-aminoquinoline moiety.[6] These hybrid molecules 'trioxaquines' (prototype **5**) prepared by reductive amination of trioxanes **6a–b** with 4-aminoquinolines **7**, show only marginal improvement in antimalarial activity over the parent trioxanes. They also suffer from serious limitations such as poor solubility both in water and oil.[6] Herein we report the preparation and antimalarial activity of **8a–e** and **9a–e**, a new series of amino functionalized trioxanes which are orally more active than the parent trioxanes **6a–b**. While amino functionalized derivatives of artemisinin are known,[7] to the best of our knowledge, this is the first report on amino functionalized synthetic 1,2,4-trioxanes.




## 2. Chemistry

β-Hydroxyhydroperoxides **11a–b** prepared by photooxygenation of allylic alcohols **10a–b** were condensed with 1,4-cyclohexanedione to furnish keto-trioxanes **6a–b** in 42–51% yield.[6] Reductive amination of **6a** with various amines in presence of $NaBH(OAc)_3$ furnished amino functionalized trioxanes **8a–e** as inseparable

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* Corresponding author. Tel.: +91-0522-2224273; fax: +91-0522-2223405; e-mail: chandancdri@yahoo.com

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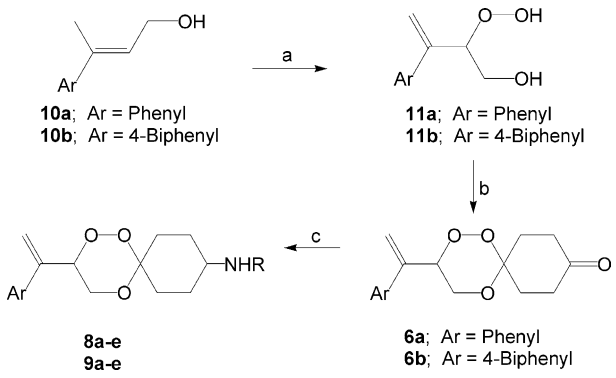
mixture of diastereomers.[8] A similar reductive amination of **6b** furnished amino functionalized trioxanes **9a–e** again as inseparable mixture of diastereomers (Scheme 1, Table 1).[9] Unlike trioxaquines **5**, these amino functionalized trioxanes are stable as free bases and are soluble in groundnut oil, the most commonly used vehicle for antimalarial assessment of artemisinin analogues.

## 3. Antimalarial activity

Trioxanes **6a–b** and amino functionalized trioxanes **8a–e** and **9a–e** were tested against multi-drug resistant *Plasmodium yoelii* in Swiss mice initially at 96 mg/kg both by oral and intramuscular (im) routes.[10] Compound **9a** which showed 100% protection at 96 mg/kg by oral route was also tested at 48 mg/kg and 24 mg/kg. β-Arteether **3**, which provides 100% protection at 48 mg/kg by oral route, served as positive control. The results are summarized in Table 2.

## 4. Results and discussion

We have earlier tried to improve the antimalarial activity of trioxanes **6a–b** by incorporating 4-aminoquinoline moieties **7**. But the resulting hybrid molecules 'trioxaquines' (represented by prototype **5**) showed only marginal improvement in activity. These trioxaquines were also unstable as free bases and had serious solubility problem. Amino functionalized trioxanes **8a–e** and




**Scheme 1.** Reaction conditions: (a) hv, $O_2$, methylene blue, MeCN, −10 to 0 °C, 4 h. (b) 1,4-cyclohexanedione, concd HCl, 5 °C, 18 h. (c) $RNH_2$, $NaBH(OAc)_3$, $CH_2Cl_2$, rt, 4 h.

**Table 1.** Amino functionalized 1,2,4-trioxanes

| Compd | Ar | R | Yield (%) |
|---|---|---|---|
| **8a** | Phenyl | Phenyl | 81 |
| **8b** | Phenyl | 4-Methoxyphenyl | 61 |
| **8c** | Phenyl | 3,5-Dichlorophenyl | 62 |
| **8d** | Phenyl | 4-Acetylaminophenyl | 88 |
| **8e** | Phenyl | 1-Naphthyl | 78 |
| **9a** | 4-Biphenyl | Phenyl | 84 |
| **9b** | 4-Biphenyl | 4-Methoxyphenyl | 91 |
| **9c** | 4-Biphenyl | 3,5-Dichlorophenyl | 44 |
| **9d** | 4-Biphenyl | 4-Acetylaminophenyl | 61 |
| **9e** | 4-Biphenyl | 1-Naphthyl | 41 |

**Table 2.** In vivo antimalarial activity against *P. yoelii* in Swiss mice

| Compd | Dose (mg/kg/day) | Route | % Suppression on day 4[a] | Mice alive on day 28 | Mean survival time[b] (day) ± SE |
|---|---|---|---|---|---|
| **6a** | 96 | oral | 7 | 0/5 | 7.2 ± 0.38 |
| | 96 | im | 99 | 0/5 | 11.6 ± 1.08 |
| **6b** | 96 | oral | 92 | 0/5 | 10.2 ± 0.58 |
| | 96 | im | 100 | 1/5 | 17.7 ± 1.97 |
| **8a** | 96 | oral | 95 | 0/5 | 12.2 ± 0.73 |
| | 96 | im | 60 | 0/5 | 13.4 ± 1.50 |
| **8b** | 96 | oral | 93 | 0/5 | 12.4 ± 1.12 |
| | 96 | im | 63 | 0/5 | 10.8 ± 1.50 |
| **8c** | 96 | oral | 100 | 2/5 | 16.5 ± 2.31 |
| | 96 | im | 62 | 0/5 | 12.4 ± 0.93 |
| **8d** | 96 | oral | 77 | 0/5 | 9.8 ± 0.37 |
| | 96 | im | 98 | 0/5 | 9.0 ± 0.45 |
| **8e** | 96 | oral | 100 | 0/5 | 19.5 ± 1.85 |
| | 96 | im | 44 | 0/5 | 9.2 ± 1.68 |
| **9a** | 96 | oral | 100 | 5/5 | > 28 |
| | 48 | oral | 100 | 3/5 | 14.5 ± 2.5 |
| | 24 | oral | 98 | 0/5 | 13.4 ± 1.14 |
| | 96 | im | 72 | 0/5 | 10.7 ± 1.25 |
| **9b** | 96 | oral | 94 | 0/5 | 11.1 ± 1.06 |
| | 96 | im | 57 | 0/5 | 8.4 ± 2.4 |
| **9c** | 96 | oral | 65 | 0/5 | 10.2 ± 2.03 |
| | 96 | im | 46 | 0/5 | 7.0 ± 0.55 |
| **9d** | 96 | oral | 49 | 0/5 | 8.4 ± 0.75 |
| | 96 | im | 90 | 0/5 | 11.6 ± 1.69 |
| **9e** | 96 | oral | 100 | 0/5 | 14.8 ± 1.06 |
| | 96 | im | nil | 0/5 | 6.6 ± 0.24 |
| β-Arteether | 48 | oral | 100 | 5/5 | > 28 |
| | 24 | oral | 100 | 1/5 | 17.75 ± 2.46 |
| Chloroquine | 48 | oral | 100 | 2/5 | 17.6 ± 1.33 |
| | 24 | oral | 100 | 0/5 | 14.8 ± 2.24 |
| Vehicle control | — | — | — | 0/15 | 7.07 ± 0.10 |

[a] Percent suppression = [(C − T)/C] × 100; where C = parasitaemia in control group, and T = parasitaemia in treated group.
[b] MST calculated for the mice which died during 28-day observation period and the mice which survive beyond 28 days are excluded.

**9a–e**, on the other hand, are stable as free bases and are freely soluble in groundnut oil, the most commonly used vehicle for antimalarial testing of artemisinin derivatives. As can be seen from Table 2, several of these novel compounds are orally more active than the parent trioxanes. Thus, while trioxane **6a** is almost completely inactive at 96 mg/kg by oral route, all the amino functionalized trioxanes derived from it except **8d** show 93–100% clearance of parasitaemia on day 4 by oral route. Amino functionalized trioxane **8c**, which shows 100% inhibition of parasitaemia on day 4, also provides 40% protection in 28-day survival assay. Keto-trioxane **6b** shows 92% inhibition of parasitaemia on day 4 when given orally at 96 mg/kg. Its derivative **9a**, shows 100% clearance of parasitaemia at 96 mg/kg by oral route and all the treated mice survived beyond day 28. Even at 48 mg/kg by oral route **9a** shows 100% clearance of parasitaemia on day 4 and 60% of treated mice survived beyond day 28. Thus activity profile of **9a** by oral route is very close to that of β-arteether. Rests of the compounds derived from **6b** have either comparable activity (**9b** and **9e**) or are less active than **6b**. However none of the amino functionalized trioxanes is more active than the parent trioxanes **6a–b** by im route. Thus introduction of the amino moiety improves absorption by oral route only.

## 5. Conclusion

Using easily available trioxanes **6a–b**, for the first time we have prepared a series of amino functionalized trioxanes **8a–e** and **9a–e** in good yield. Several of these trioxanes show better activity by oral route than the parent trioxanes. The activity profile of **9a**, the most potent compound of the series, is very close to that of β-arteether, one of the clinically useful antimalarial drugs belonging to artemisinin class.

## Acknowledgements

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8. In all cases the ratio of the two isomers as measured by HPLC (Shimadzu-CLC Reversed phase $C_{18}$ column; MeOH:$H_2O$ 80:20, Flow rate 0.8 mL/min) is around 60:40.
9. Selected spectral data: Compound **6a**: FT-IR (KBr, $cm^{-1}$) 1717.4; $^1H$ NMR (200 MHz, $CDCl_3$) δ 2.05 (t, 2H, $J=7.06$ Hz), 2.32–2.67 (m, 6H), 3.85 (dd, 1H, $J=11.87$, 2.91 Hz), 3.96 (dd, 1H, $J=11.87$, 10.16 Hz), 5.31 (dd, 1H, $J=10.16$, 2.91 Hz), 5.36 and 5.53 (2×s, 2×1H), 7.32–7.40 (m, 5H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 27.79 (t), 33.57 (t), 36.79 (t), 36.95 (t), 63.72 (t), 80.83 (d), 101.50 (s), 117.10 (t), 126.81 (2×d), 128.71 (d), 129.04 (2×d), 138.84 (s), 143.59 (s), 210.00 (s); FABMS (*m*/*z*) 275 ($M^+ + 1$). Anal. calculated: C 70.05%, H 6.61%; found C 69.77%, H 6.86%. Compound **8c**: FT-IR (KBr, $cm^{-1}$) 1590.7, 3418.0; $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.47–2.01 (m, 7H), 2.58–2.86 (bm, 1H), 3.34 (bs, 1H), 3.70 (bm, 1H, NH), 3.78 (dd, 1H, $J=11.88$, 3.78 Hz), 3.85–4.04 (m, 1H), 5.26 (dd, 1H, $J=10.70$, 3.78 Hz), 5.32 and 5.51 (2×s, 2×1H), 6.42 and 6.43 (2×s, 1H), 6.63 (s, 1H), 7.25–7.39 (m, 6H); FABMS (*m*/*z*) 420, 422 and 424 ($M^+ + 1$). Anal. calculated: C 62.86%, H 5.51%, N 3.33%; found C 62.59%, H 5.46%, N 2.89%. Compound **8e**: FT-IR (Neat, $cm^{-1}$) 1581.8, 3423.9; $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.43–2.17 (m, 7H), 2.60–2.90 (bm, 1H), 3.60–3.65 (bm, 1H), 3.80 (dd, 1H, $J=11.90$, 2.77 Hz), 3.89–4.06 (m, 1H), 4.24 (bs, 1H, NH), 5.28 (dd, 1H, $J=9.82$, 2.77 Hz), 5.33 and 5.51 (2×s, 2×1H), 6.63 (d, 1H, $J=7.28$ Hz), 7.19–7.47 (m, 9H), 7.75–7.79 (m, 2H); FABMS (*m*/*z*) 402 ($M^+ + 1$). Anal. calculated: C 77.77%, H 6.77%, N 3.48%; found C 77.83%, H 6.46%, N 3.25%. Compound **9a**: FT-IR (KBr, $cm^{-1}$) 1602.0, 3408.4; $^1H$ NMR (200 MHz, $CDCl_3$) δ 1.49–2.04 (m, 7H), 2.61–2.85 (bm, 1H), 3.40–3.64 (bm, 2H), 3.83 (dd, 1H, $J=11.96$, 2.97 Hz), 3.90–4.09 (m, 1H), 5.31 (dd, 1H, $J=9.98$, 2.97 Hz), 5.35 and 5.57 (2×s, 2×1H), 6.57–6.71 (m, 2H), 7.12–7.20 (m, 2H), 7.25–7.60 (m, 10H); $^{13}C$ NMR (50 MHz, $CDCl_3$) δ 27.03 and 27.60 (t), 28.48 and 28.74 (t), 28.98 and 29.09 (t), 32.62 and 33.52 (t), 50.81 and 51.14 (d), 63.23 and 63.52 (t), 80.69 (d), 102.35 and 102.43 (s), 113.69 (2×d), 116.76 and 116.84 (t), 117.69 (d), 127.20 (2×d), 127.43 (2×d), 127.70 (2×d), 127.93 (d), 129.25 (2×d), 129.75 (2×d), 137.81 and 137.85 (s), 140.84 (s), 141.51 (s), 143.33 and 143.37 (s), 147.60 (s); FABMS (*m*/*z*) 428 ($M^+ + 1$). Anal. calculated: C 78.65%, H 6.93%, N 3.27%; found C 78.25%, H 7.26%, N 3.61%. Compound **9d**: FT-IR (KBr, $cm^{-1}$)

1612.0, 3305.5; $^{1}$H NMR (200 MHz, $CDCl_3$) δ 1.33–2.07 (m, 7H), 2.12 (s, 3H), 2.59–2.85 (bm, 1H), 3.31–3.41 (bm, 2H), 3.83 (dd, 1H, $J = 11.89$, 3.06 Hz), 3.90–4.08 (m, 1H), 5.30 (dd, 1H, $J = 10.01$, 3.06 Hz), 5.34 and 5.57 (2×s, 2×1H), 6.55 (d, 2H, $J = 8.62$ Hz), 7.02 (s, 1H, NH), 7.24 (d, 2H, $J = 8.62$ Hz) 7.31–7.60 (m, 9H); $^{13}$C NMR (50 MHz, $CDCl_3$) δ 24.52 (q), 27.02 and 27.56 (t), 28.42 and 28.66 (t), 29.00 (t), 32.59 and 33.48 (t), 51.18 and 51.49 (d), 63.20 and 63.49 (t), 80.68 (d), 102.36 and 102.44 (s), 113.99 (2×d), 116.86 (t), 122.98 (d), 127.19 (2×d), 127.41 (2×d), 127.69 (2×d), 127.95 (2×d), 129.26 (2×d), 137.82 (s), 140.79 (2×s), 141.47 (s), 143.27 (s), 144.74 (s), 168.90 (s); FABMS ($m/z$) 485 ($M^+ + 1$). Anal. calculated: C 74.44%, H 6.66%, N 5.78%; found C 74.57%, H 6.67%, N 5.38%.

10. The in vivo efficacy of compounds was evaluated against *Plasmodium yoelii* (MDR) in Swiss mice model. The mice were inoculated with $1 \times 10^6$ parasitised RBC on day zero and treatment was administered to a group of five mice at each dose, from day 0 to 3, in two divided doses daily. The required drug dilutions were prepared in groundnut oil and 0.1 mL volume was administered intramuscularly and orally for each dose. Parasitaemia level were recorded from thin blood smears between day 4–28.[11] Mice treated with arteether and chloroquine served as positive controls.
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