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Synthesis and antimalarial activity of a new series of trioxaquines☆

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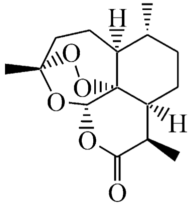
Abstract—Trioxanes **8a–b,** easily accessible in two steps from allylic alcohol **6a–b**, on reductive amination with 4-aminoquinolines **4a–c** furnish a new series of trioxaquines **9a–b**, **10a–b**, **11a–b** in 32–77% yields. Dicitrate salts of these trioxaquines have been evaluated for antimalarial activity against multidrug resistant *Plasmodium yoelii* in mice model.

Following the discovery of antimalarial drug artemisinin **1** from *Artemisia annua*, there has been an intense interest in the synthesis and bioevaluation of its analogues.[1] For the last several years, we have been working in this area with the objective to develop structurally simple synthetic trioxanes as substitutes for artemisinin derivatives. Towards this end we have developed a novel photooxygenation route for the preparation of 1,2,4-trioxanes.[2] Several trioxanes prepared by this route have shown promising antimalarial activity.[3,4] Herein, we report the extension of this methodology for the preparation of a new series of trioxaquines **9a–b**, **10a–b**, and **11a–b**. Trioxaquines[5] are a comparatively new class of antimalarials, which combine the essential structural features of artemisinin and chloroquine **2**.



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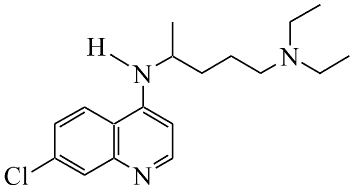

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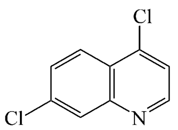
1. Chemistry

4-Aminoquinolines **4a–c** were prepared by condensation of 4,7-dichloroquinoline **3** with appropriate diaminoalkanes[6] and were reacted with citric acid in acetone to furnish dicitrate salts **5a–c**. β-Hydroxyhydroperoxides **7a–b**, prepared by photooxygenation of allylic alcohols **6a–b**, were reacted in situ with 1,4-cyclohexanedione to furnish trioxanes **8a–b** in 42–51% yield (based on allylic alcohols). Reductive amination of **8a** with **4a**, **4b**, and **4c** furnished trioxaquines **9a**, **10a**, and **11a** in more than 70% yield as inseparable mixture of diastereomers. Similar reaction of trioxane **8b** with **4a**, **4b**, and **4c** furnished trioxaquines **9b**, **10b**, and **11b** in 32–44% yield also as inseparable mixture of diastereomers. Since trioxaquines **9a–b**, **10a–b**, and **11a–b** were unstable and were insoluble in oil and water, they were converted to their dicitrate salts **12a–b**, **13a–b**, and **14a–b** which were evaluated for their biological activity (Scheme 1).



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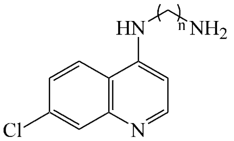
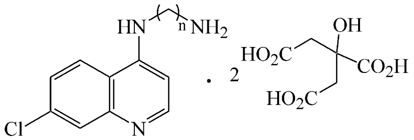


4a; n=2
4b; n=3
4c; n=4



5a; n=2
5b; n=3
5c; n=4

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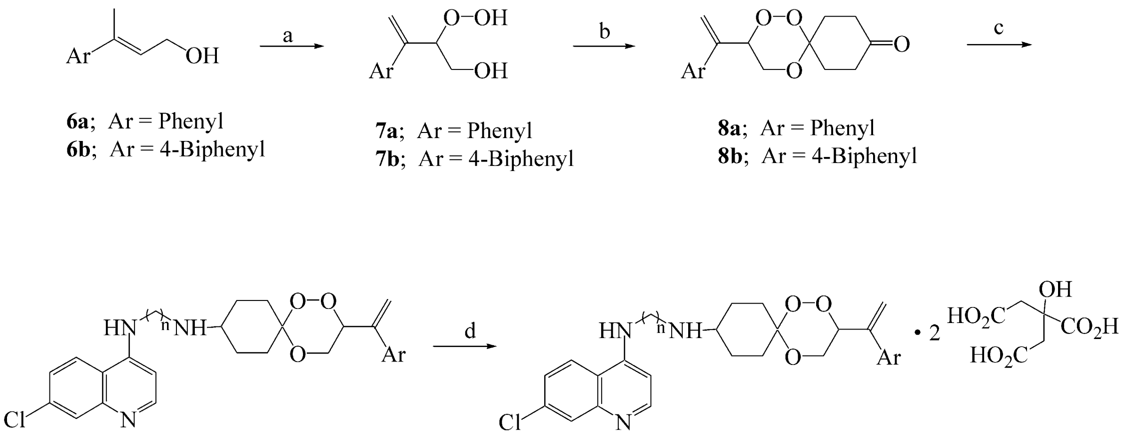

6a; Ar = Phenyl
6b; Ar = 4-Biphenyl

7a; Ar = Phenyl
7b; Ar = 4-Biphenyl

8a; Ar = Phenyl
8b; Ar = 4-Biphenyl

9a; n = 2, Ar = Phenyl
9b; n = 2, Ar = 4-Biphenyl
10a; n = 3, Ar = Phenyl
10b; n = 3, Ar = 4-Biphenyl
11a; n = 4, Ar = Phenyl
11b; n = 4, Ar = 4-Biphenyl

12a; n = 2, Ar = Phenyl
12b; n = 2, Ar = 4-Biphenyl
13a; n = 3, Ar = Phenyl
13b; n = 3, Ar = 4-Biphenyl
14a; n = 4, Ar = Phenyl
14b; n = 4, Ar = 4-Biphenyl

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

2. Antimalarial activity

4-Aminoquinoline dicitrates **5a–c**, trioxanes **8a–b** and trioxaquine dicitrates **12a–b**, **13a–b**, and **14a–b** were tested against multidrug resistant *Plasmodium yoelii* in Swiss mice at 96 mg/kg by both oral and intramuscular (im) routes. Artemisinin, which provides 100% protection at 48 mg/kg served as positive control. The results are summarized in Table 1.

As can be seen from Table 1, trioxaquine dicitrates **12a**, **13a**, and **14a**, all derived from trioxane **8a**, do not show any improvement in activity over the parent compound (100% suppression of parasitaemia on day 4 by **12a**, **13a**, and **14a** as compared to 99% inhibition by **8a**) when given by the intramuscular route. All these compounds, however, show significant improvement in activity over the parent trioxane when given by oral route (89–94% suppression of parasitaemia by **12a–14a** as compared with only 7% suppression of parasitaemia by **8a**). On the other hand, trioxaquine dicitrates **12b**, **13b**, and **14b** are comparable in activity to the parent trioxane **8b** when administered either by oral or im route. Biphenyl substituted trioxane **8b** is orally more active than the phenyl substituted trioxane **8a**. This is because of the higher lipophilicity of biphenyl group as compared with phenyl group. The same trend is maintained in the trioxaquine series, biphenyl substituted trioxaquines **12b**, **13b**, and **14b** are orally more active than **12a**, **13a**, and **14a**. None of the trioxaquine dicitrates, however, provides significant protection to the treated mice in 28-day survival assay.

3. Conclusion

Using easily accessible trioxanes **8a** and **8b** we have prepared a new series of antimalarial trioxaquines, some of which are orally more active than the parent trioxanes and 4-aminoquinoline moieties. These hybrid molecules, however, suffer from serious limitations such as poor stability and poor solubility both in oil and water. Their formation as inseparable mixture of diastereomers is another serious limitation. Our current efforts in this area are directed to address these problems.

4. Experimental

Melting points were taken in open capillaries on Complab melting point apparatus and are uncorrected. IR spectra (cm^{-1}) were recorded on Perkin–Elmer RX-1 in KBr disc. ^1H NMR and ^{13}C NMR were recorded on Bruker Supercon Magnet DPX-200/DRX-300 MHz using $CDCl_3$/DMSO-d_6 as solvent. Tetramethylsilane (δ 0.0 ppm) was used as an internal standard in ^1H NMR and $CDCl_3$ (δ 77.0 ppm) was used in ^{13}C NMR. Position of NH proton in ^1H NMR was determined by D_2O exchange. Fast Atom Bombardment Mass Spectra (FABMS) were obtained on Jeol (Japan)/SX-102 spectrometer using glycerol or *m*-nitrobenzyl alcohol as matrix. The Electro Spray Mass Spectra (ESMS) were recorded on a Micromass Quattro II triple quadruple mass spectrometer. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer and values were within ±0.5% of the calculated values. Some dicitrate salts are hygroscopic so during calculation of elemental analysis one water molecule is added in their molecular formula. The progress of the reaction was monitored by silica gel thin layer chromatography with detecting agents: iodine vapours, spraying with an aq solution of vanillin in 10% sulphuric acid followed by heating at 150 °C, or by spraying with Dragendorff reagent. Chromatographic purification was performed over silica gel (60–120 mesh). All chemicals and reagents were obtained from Aldrich (USA), Lancaster (UK) or

Table 1. In vivo antimalarial activity against multidrug resistant *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] (MST in days)±SE
5a	96	Oral	74	0/5	11.0±1.34
	96	im	87	0/5	10.0±0.58
5b	96	Oral	87	0/5	12.8±1.59
	96	im	97	0/5	16.4±2.16
5c	96	Oral	65	0/5	9.6±0.81
	96	im	92	0/5	11.2±1.07
8a	96	Oral	7	0/5	7.2±0.38
	96	im	99	0/5	11.6±1.08
8b	96	Oral	92	0/5	10.2±0.58
	96	im	100	1/5	17.7±1.97
12a	96	Oral	89	0/5	8.6±0.24
	96	im	100	0/5	14.8±0.73
12b	96	Oral	94	0/5	11.2±0.20
	96	im	97	0/5	10.7±0.25
13a	96	Oral	96	0/5	10.6±1.21
	96	im	100	0/5	14.6±0.75
13b	96	Oral	99	0/5	11.2±1.07
	96	im	100	0/5	15.2±1.24
14a	96	Oral	94	0/5	9.2±0.96
	96	im	100	0/5	13.2±0.85
14b	96	Oral	94	0/5	11.8±1.62
	96	im	93	0/5	14.4±0.93
Artemisinin	48	im	100	5/5	>28
	24	im	100	3/5	16.0±0.00
Chloroquine	96	Oral	100	4/5	20.0±0.00
	48	Oral	100	2/5	17.6±1.33
Vehicle control	—	—	—	0/15	7.07±0.10

im, intramuscular.

[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 survived beyond 28-day are excluded.

Spectrochem Pvt. Ltd. (India) and were used without further purification. Allylic alcohols **6a** and **6b** were prepared by known procedures.[2,7]

4.1. N^1-(7-Chloroquinolin-4-yl)ethane-1,2-diamine dicitrate (5a)

A mixture of 4,7-dichloroquinoline **3** (5 g, 25 mmol) and 1,2 diaminoethane (6.10 g, 100 mmol) was heated slowly from room temperature to 80 °C over 1 h with stirring and subsequently at 130–140 °C for 6 h with continued stirring. The reaction mixture was cooled to room temperature; 1 N NaOH (30 mL) was added, warmed to 40 °C and extracted with EtOAc (300 mL). EtOAc extract was washed with water, dried over anhyd. Na_2SO_4 and concentrated. Crude product was purified by column chromatography over silica gel using chloroform–methanol–water (65:35:30, lower layer) to furnish **4a** (4.86 g, 86.17% yield). To a solution of N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine **4a** (0.30 g, 1.35 mmol) in acetone (5 mL) was added a solution of citric acid (0.52 g, 2.70 mmol, 2 equiv) in acetone (5 mL) at room temperature. The trioxaquine dicitrate precipitated spontaneously, which after centrifugation was washed first with acetone, then with diethyl ether and dried under vacuum to furnish **5a** (0.49 g, 72% yield); mp 132–135 °C; FT-IR (KBr, cm^{-1}) 3412.6; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.52–2.63 (m, 8H), 2.70 (s, 1H, NH), 3.15 (t, 2H, J = 5.60 Hz), 3.60 (t, 2H, J = 5.60 Hz), 6.66 (d, 1H, J = 5.69 Hz), 7.59 (d, 1H, J = 8.93 Hz), 7.87 (s, 1H), 8.29 (d, 1H, J = 8.93 Hz), 8.50 (d, 1H, J = 5.69 Hz); ESMS (positive mode) 222 and 224 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{23}H_{28}N_3O_{14}Cl$: C 45.58%, H 4.65%, N 6.93%; Found C 45.37%, H 4.79%, N 7.38%.

Compounds **5b** and **5c** were also prepared by the above procedure.

4.2. N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine dicitrate (5b)

Yield 72%; mp 95–98 °C; FT-IR (KBr, cm^{-1}) 3250.0; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.94 (t, 2H, J = 6.81 Hz), 2.49–2.59 (m, 8H), 2.67 (s, 1H, NH), 2.94 (t, 2H, J = 7.24 Hz), 3.37–3.45 (m, 2H), 6.60 (d, 1H, J = 5.58 Hz), 7.53 (d, 1H, J = 8.96 Hz), 7.83 (s, 1H), 8.31 (d, 1H, J = 8.96 Hz), 8.46 (d, 1H, J = 5.58 Hz); ESMS (positive mode) 236 and 238 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{24}H_{30}N_3O_{14}Cl.H_2O$: C 45.18%, H 5.05%, N 6.58%; Found C 45.37%, H 5.45%, N 6.72%.

4.3. N^1-(7-Chloroquinolin-4-yl)butane-1,4-diamine dicitrate (5c)

Yield 74%; mp 125–127 °C; FT-IR (KBr, cm^{-1}) 3420.1; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.61–1.73 (m, 4H), 2.50–2.58 (m, 8H), 2.65 (s, 1H, NH), 2.84–2.87 (m, 2H), 3.33-3.36 (m, 2H), 6.58 (d, 1H, J = 5.59 Hz), 7.52 (d, 1H, J = 8.67 Hz), 7.82 (s, 1H), 8.31 (d, 1H, J = 8.67 Hz), 8.43 (d, 1H, J = 5.59 Hz); ESMS (positive mode) 250 and 252

(M^+), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{25}H_{32}N_3O_{14}Cl$: C 47.35%, H 5.08%, N 6.62%; Found C 47.58%, H 5.48%, N 6.29%.

4.4. 3-(1-Phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-one (8a)

A solution of allylic alcohol **6a** (1 g, 6.75 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. 1,4-Cyclohexanedione (1.15 g, 10.13 mmol) and concd HCl (five drops) 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** (0.94 g, 51% yield, based on allylic alcohol **6a**); mp 70–71 °C; 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, 2H), 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. calcd for $C_{16}H_{18}O_4$: C 70.05%, H 6.61%; found C 69.77%, H 6.86%.

4.5. 3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-one (8b)

Compound **8b** was prepared by the above procedure. Yield 42%; mp 104–105 °C; FT-IR (KBr, cm^{-1}) 1711.5; ^1H NMR (200 MHz, $CDCl_3$): δ 2.07 (t, 2H, $J=7.06$ Hz), 2.33–2.69 (m, 6H), 3.91 (dd, 1H, $J=12.02$, 3.52 Hz), 4.02 (dd, 1H, $J=12.02$, 9.79 Hz), 5.38 (dd, 1H, $J=9.79$, 3.52 Hz), 5.41 and 5.59 (2×s, 2H), 7.31–7.62 (m, 9H); FABMS (*m*/*z*) 351 ($M^+ + 1$). Anal. calcd for $C_{22}H_{22}O_4$: C 75.40%, H 6.32%; Found C 75.41%, H 6.68%.

4.6. *N*-(7-Chloroquinolin-4-yl)-*N*¹-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-ethane-1,2-diamine (9a)

To a mixture of trioxane **8a** (0.50 g, 1.22 mmol) and 4-aminoquinoline **4a** (0.60 g, 2.73 mmol) in dichloromethane (20 mL) was added sodium triacetoxyborohydride (0.58 g, 2.73 mmol) and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water and extracted with CH_2Cl_2 (2×15 mL). Combined organic layer was dried over anhyd Na_2SO_4, concentrated under vacuum and the crude product was chromatographed over silica gel using chloroform–methanol–water (65:35:40, lower layer) as eluant to furnish pure trioxaquine **9a** (0.69 g, 77% yield); mp 68–70 °C; FT-IR (KBr, cm^{-1}) 1584.0, 3407.2; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28–2.05 (m, 8H), 2.60–2.89 (m, 1H, cyclohexyl and 1H, NH), 3.11 (t, 2H, $J=5.36$ Hz), 3.44 (t, 2H, $J=5.36$ Hz), 3.72–4.01 (m, 2H), 5.23 (dd, 1H, $J=9.67$, 3.23 Hz), 5.30 and 5.49 (2×s, 2H), 6.23 (d, 1H, $J=5.60$ Hz), 6.39 (s, 1H, NH), 7.28–7.37 (m, 6H), 7.79–7.84 (m, 2H), 8.34 (d, 1H, $J=5.60$ Hz); FABMS (*m*/*z*) 480 and 482 ($M^+ + 1$).

Trioxaquines **9b**, **10a**, **10b**, **11a**, **11b** were also prepared by the procedure described for **9a**.

4.7. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*¹-(7-chloroquinolin-4-yl)-ethane-1,2-diamine (9b)

Yield 34%; mp 72–74 °C; FT-IR (KBr, cm^{-1}) 1589.5, 3395.4; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28–2.03 (m, 8H), 2.63–2.90 (m, 1H, cyclohexyl, and 1H, NH), 3.05 (t, 2H, $J=5.24$ Hz), 3.31 (t, 2H, $J=5.24$ Hz), 3.78–4.08 (m, 2H), 5.29 (dd, 1H, $J=10.05$, 3.98 Hz), 5.34 and 5.57 (2×s, 2H), 6.00 (s, 1H, NH), 6.36 (d, 1H, $J=5.37$ Hz), 7.25–7.72 (m, 11H), 7.93 and 7.94 (2×s, 1H), 8.49 (d, 1H, $J=5.37$ Hz); FABMS (*m*/*z*) 556 and 558 ($M^+ + 1$).

4.8. *N*-(7-chloroquinolin-4-yl)-*N*¹-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-propane-1,3-diamine (10a)

Yield 76%; mp 75–78 °C; FT-IR (KBr, cm^{-1}) 1580.0, 3292.0; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28–2.05 (m, 10H), 2.69–2.90 (m, 1H, cyclohexyl, and 1H, NH), 2.94–2.96 (m, 2H, becomes triplet with $J=5.31$ Hz on D_2O exchange), 3.39–3.42 (m, 2H, becomes triplet with $J=5.31$ Hz on D_2O exchange), 3.73–4.13 (m, 2H), 5.21–5.28 (m, 1H), 5.29 and 5.31 (2×s, 1H), 5.47 and 5.50 (2×s, 1H), 6.28 (d, 1H, $J=5.62$ Hz), 6.83 (bs, 1H, NH), 7.26–7.38 (m, 6H), 7.82–7.91 (m, 2H), 8.44 (d, 1H, $J=5.62$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 26.18 (t), 26.69 and 26.91 (t), 27.39 (t), 32.37 (t), 33.23 (t), 42.05 (t), 44.20 and 44.44 (t), 56.27 (d), 63.09 and 63.44 (t), 80.69 (d), 101.66 and 101.79 (s), 116.91 and 117.20 (t), 123.82 (d), 125.30 (d), 125.98 (d), 126.70 (2×d), 128.60 (d), 128.96 (3×d), 136.53 (s), 138.84 (s), 143.61 (s), 145.66 (s), 148.67 and 148.83 (d), 152.48 (2×s); FABMS (*m*/*z*) 494 and 496 ($M^+ + 1$).

4.9. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*¹-(7-chloroquinolin-4-yl)-propane-1,3-diamine (10b)

Yield 32%; mp 78–80 °C; FT-IR (KBr, cm^{-1}) 1589.9, 3401.4; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28–2.09 (m, 10H), 2.62–2.78 (m, 1H, cyclohexyl, and 1H, NH), 2.97 (t, 2H, $J=5.41$ Hz), 3.17 (bs, 1H, NH), 3.40 (t, 2H, $J=5.48$ Hz), 3.78–4.08 (m, 2H), 5.26–5.34 (m, 2H), 5.55 and 5.57 (2×s, 1H), 6.27 (d, 1H, $J=5.64$ Hz), 7.28–7.60 (m, 10H), 7.83 (2×d, 1H, $J=8.80$ Hz each), 7.94 (m, 1H), 8.44 (d, 1H, $J=5.64$ Hz); FABMS (*m*/*z*) 570 and 572 ($M^+ + 1$).

4.10. *N*-(7-chloroquinolin-4-yl)-*N*¹-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-butane-1,4-diamine (11a)

Yield 78%; mp 72–74 °C; FT-IR (KBr, cm^{-1}) 1576.7, 3276.2; ^1H NMR (200 MHz, $CDCl_3$): δ 1.31–2.03 (m, 12H), 2.50 (s, 1H, NH), 2.53–2.60 (m, 1H), 2.69 (t, 2H, $J=6.42$ Hz), 3.28 (t, 2H, $J=5.85$ Hz), 3.75–4.03 (m, 2H), 5.25 (dd, 1H, $J=10.03$, 3.18 Hz), 5.31 and 5.32 (2×s, 1H), 5.49 and 5.51 (2×s, 1H), 5.86 (bs, 1H, NH), 6.34 (d, 1H, $J=5.55$ Hz), 7.26–7.36 (m, 6H), 7.92–7.94 (m, 2H), 8.47 (d, 1H, $J=5.55$ Hz); FABMS (*m*/*z*) 508 and 510 ($M^+ + 1$).

4.11. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*^{1}-(7-chloroquinolin-4-yl)-butane-1,4-diamine (11b)

Yield 45%; mp 65–70 °C; FT-IR (KBr, cm^{-1}) 1584.6, 3397.0; ^{1}H NMR (200 MHz, CDCl$_3$): δ 1.28–2.06 (m, 12H), 2.58–2.74 (m, 1H, cyclohexyl, and 1H, NH), 2.79–2.82 (m, 2H, becomes triplet with $J=5.24$ Hz on D$_2$O exchange), 3.27–3.29 (m, 2H, becomes triplet with $J=5.24$ Hz on D$_2$O exchange), 3.48 (bs, 1H, NH), 3.77–4.06 (m, 2H), 5.26 (dd, 1H, $J=9.43$, 3.25 Hz), 5.32 and 5.55 (2×s, 2H), 6.30 (2×d, 1H, $J=5.57$ each), 7.29–7.63 (m, 10H), 7.77–7.98 (m, 2H), 8.41 (2×d, 1H, $J=5.57$ each); ^{13}C NMR (75 MHz, CDCl$_3$) δ 23.2 and 23.8 (t), 25.2 and 25.8 (t), 26.5 and 26.7 (t), 27.2 and 27.7 (t), 29.6 (t), 38.4 and 39.0 (t), 42.7 and 43.1 (t), 45.5 and 45.8 (t), 56.8 (d), 63.0 (t), 80.1 (d), 101.6 (s), 116.4 and 117.0 (t), 121.8 (d), 122.3 (d), 125.4 (d), 126.7 (d), 126.9 (3×d), 127.2 (2×d), 127.5 (d), 128.8 (3×d), 135.4 (s), 137.2 (s), 141.0 (s), 142.7 (s), 147.3 (d), 150.2 (s), 150.5 (s), 150.8 (2×s); FABMS (*m*/*z*) 584 and 586 (M$^+$+1).

4.12. *N*-(7-chloroquinolin-4-yl)-*N*^{1}-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-ethane-1,2-diamine dicitrate (12a)

To a solution of trioxaquine **9a** (0.35 g, 0.70 mmol) in acetone (5 mL) was added a solution of citric acid (0.28 g, 1.45 mmol, 2 equiv) in acetone (5 mL) at room temperature. The trioxaquine dicitrate precipitated spontaneously which after centrifugation was washed first with acetone, then with diethyl ether and dried under vacuum to furnish trioxaquine dicitrate **12a** (0.60 g, 97% yield); mp 165–170 °C; FT-IR (KBr, cm^{-1}) 1584.6, 3397.0; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.48–1.97 (m, 8H), 2.27–2.66 (m, 8H), 2.78–2.82 (m, 1H), 3.07–3.13 (m, 2H), 3.39–3.45 (m, 2H), 3.83–4.06 (m, 2H), 5.20–5.23 (bm, 1H), 5.40 and 5.62 (2×s, 2H), 6.62 (2×d, 1H, $J=5.42$ Hz each), 7.27–7.46 (m, 5H), 7.55 (d, 1H, $J=8.70$ Hz), 7.84 (s, 1H), 8.31 (d, 1H, $J=8.70$ Hz), 8.47 (d, 1H, $J=5.42$ Hz); ESMS (positive mode) 480 and 482 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{39}H_{46}N_3O_{17}Cl$: C 54.19%, H 5.36%, N 4.86%; found C 54.44%, H 5.78%, N 4.72%. Trioxaquine dicitrates **12b**, **13a**, **13b**, **14a**, **14b** were also prepared by the above method.

4.13. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*^{1}-(7-chloroquinolin-4-yl)-ethane-1,2-diamine dicitrate (12b)

Yield 51%; mp 108–110 °C; FT-IR (KBr, cm^{-1}) 1596.8, 3414.8; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.52–2.00 (m, 8H), 2.23–2.67 (m, 8H), 2.77–2.90 (m, 1H), 3.23 (bs, 2H), 3.50–3.54 (m, 2H), 3.79–3.91 (m, 1H), 3.99–4.11 (m, 1H), 5.25–5.28 (m, 1H), 5.43 and 5.70 (2×s, 2H), 6.65 (2×d, 1H, $J=5.58$ Hz each), 7.35–7.70 (m, 10H), 7.85 and 7.86 (2×s, 1H), 8.26 (2×d, 1H, $J=8.40$ Hz each), 8.49 (d, 1H, $J=5.58$ Hz); ESMS (positive mode) 556 and 558 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{45}H_{50}N_3O_{17}Cl.H_2O$: C 56.33%, H 5.46%, N 4.38%; Found C 56.58%, H 5.38%, N 4.42%.

4.14. *N*-(7-chloroquinolin-4-yl)-*N*^{1}-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-propane-1,3-diamine dicitrate (13a)

Yield 88%; mp 160–165 °C; FT-IR (KBr, cm^{-1}) 1609.8, 3427.6; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.50–2.32 (m, 10H), 2.39–2.67 (m, 8H), 2.79–2.84 (m, 1H), 3.23–3.25 (m, 2H), 3.63–3.65 (m, 2H), 3.83–4.07 (m, 2H), 5.20–5.23 (m, 1H), 5.40 and 5.62 (2×s, 2H), 6.65 (2×d, 1H, $J=4.81$ Hz each), 7.28–7.46 (m, 5H), 7.57 (d, 1H, $J=8.70$ Hz), 7.85 (s, 1H), 8.25 (2×d, 1H, $J=8.70$ Hz each), 8.49 (d, 1H, $J=4.81$ Hz); ESMS (positive mode) 494 and 496 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{40}H_{48}N_3O_{17}Cl$: C 54.69%, H 5.50%, N 4.78%; found C 54.74%, H 5.58%, N 5.17%.

4.15. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*^{1}-(7-chloroquinolin-4-yl)-propane-1,3-diamine dicitrate (13b)

Yield 55%; mp 138–142 °C; FT-IR (KBr, cm^{-1}) 1597.9 and 3421.8; ^{1}H NMR (200 MHz, DMSO-*d*$_6$): δ 1.52–2.32 (m, 10H), 2.50–2.58 (m, 4H), 2.61–2.68 (m, 4H), 2.78–2.88 (m, 1H), 2.97–3.20 (m, 2H), 3.33–3.46 (m, 2H), 3.70–4.06 (m, 2H), 5.25–5.29 (m, 1H), 5.43 and 5.70 (2×s, 2H), 6.64 (d, 1H, $J=5.47$ Hz), 7.34–7.71 (m, 10H), 7.85 (s, 1H), 8.35 (d, 1H, $J=8.83$ Hz), 8.47 (d, 1H, $J=5.47$ Hz); ESMS (Positive mode) 570 and 572 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191(citrate). Anal. calcd for $C_{46}H_{52}N_3O_{17}Cl$: C 57.88%, H 5.49%, N 4.40%; found C 58.01%, H 5.82%, N 4.41%.

4.16. *N*-(7-chloroquinolin-4-yl)-*N*^{1}-[3-(1-phenyl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-butane-1,4-diamine dicitrate (14a)

Yield 88%; mp 160–163 °C; FT-IR (KBr, cm^{-1}) 1576.7 and 3276.2; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.28–2.18 (m, 12H), 2.44–2.72 (m, 8H), 2.84–2.88 (m, 1H), 3.05 (bm, 2H), 3.50 (bs, 2H), 3.84–4.07 (m, 2H), 5.21–5.23 (m, 1H), 5.40 and 5.62 (2×s, 2H), 6.77 (d, 1H, $J=4.82$ Hz), 7.25–7.55 (m, 6H), 7.64 (d, 1H, $J=8.10$ Hz), 7.93 (s, 1H), 8.48 (bs, 1H), 8.79 (bs, 1H, NH); ESMS (positive mode) 508 and 510 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191 (citrate). Anal. calcd for $C_{41}H_{50}N_3O_{17}Cl$: C 55.18%, H 5.64%, N 4.70%; found C 55.56%, H 5.42%, N 4.81%.

4.17. *N*-[3-(1-Biphenyl-4yl-vinyl)-1,2,5-trioxaspiro[5.5]undec-9-yl]-*N*^{1}-(7-chloroquinolin-4-yl)-butane-1,4-diamine dicitrate (14b)

Yield 50%; mp 106–108 °C; FT-IR (KBr, cm^{-1}) 1584.6, 3397.0; ^{1}H NMR (300 MHz, DMSO-*d*$_6$): δ 1.49–2.40 (m, 12H), 2.49–2.65 (m, 8H), 2.78–2.86 (m, 1H), 2.90–3.04 (m, 2H), 3.32–3.53 (m, 2H), 3.86–4.08 (m, 2H), 5.23–5.26 (m, 1H), 5.41 and 5.68 (2×s, 2H), 6.63 (d, 1H, $J=5.70$ Hz) 7.33–7.68 (m, 10H), 7.85 (s, 1H), 8.00 (bs, 1H, NH), 8.38 (d, 1H, $J=8.98$ Hz), 8.45 (d, 1H, $J=5.70$ Hz); ESMS (positive mode) 584 and 586 (M$^+$), M corresponding to the protonated base compound; ESMS (negative mode) 191(citrate). Anal. calcd for $C_{47}H_{54}N_3O_{17}Cl$: C 58.29%, H 5.62%, N 4.33%; found C 57.89%, H 5.88%, N 4.22%.

5. In vivo antimalarial efficacy test

The in vivo efficacy of test compounds was evaluated against *P. yoelii* (MDR) in Swiss mice model at 96 mg/kg/day. The mice (25 ± 2 g) were inoculated with 1×10^6 parasitised RBC on day 0 and treatment was administered to a group of five mice from day 0 to 3, in two divided doses daily. The required dilutions of the compounds **5a–c** were prepared in water; of the two trioxanes **8a–b** in groundnut oil and aqueous suspensions of **12a–b**, **13a–b**, and **14a–b** were prepared with a few drops of Tween 80. The required drug dosage was administered in 0.1 mL volume via oral and intramuscular route. Parasitaemia level were recorded from thin blood smears between days 4 and 28.[8]

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