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Antimalarial activity of 1-aryl-3,3-dialkyltriazenes

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Abstract—The antimalarial activity of 1-aryl-3,3-dialkyltriazenes to *Plasmodium berghei* NK-65 in infected mice was evaluated at an intraperitoneal dose of 100 mg/kgbw. Some of these compounds were found to possess potent antimalarial activity.

1. Introduction

Malaria, one of the largest tropical diseases, is a protozoal infection that spreads widely in the tropical zone. It generates in the temperate zone particularly and malaria imposes a large burden on the endemic area due to the death of children. According to the World Health Organization (WHO), between 300 and 660 million clinical cases of malaria occur every year.[1] More than 1 million persons are killed by malaria annually. This particularly affects children under the age of six living in sub-Saharan Africa. With the spread in the global scale of insecticide resistant mosquitoes and drug tolerant *Plasmodium*, the treatment and suppression of malaria has become difficult. Chloroquine (CQ) has been used as an antimalarial drug due to its availability, effectiveness, and low toxicity. But CQ is no longer effective in most of the world because of increasing resistance.[2] Thus, it is important to develop a new antimalarial with a new chemical skeleton.[3] We focused on 1-aryl-3,3-dialkyltriazenes as a new candidate antimalarial drug. 1-Aryl-3,3-dialkyltriazenes have been used in many ways in organic syntheses[4] and are known as anticancer drugs represented by dacarbazine (**1**).[5] We explored the additional bioactivities of the triazenes. Until now, 1, 3-diaryltriazenes, Isometamidium (**2**)[6] and Berenil (**3**),[7] are known as chemical compounds with antimalarial activity with the triazene structure. Isometamidium (**2**) is known to inhibit histamine *N*-methyltransferase and diamine oxidase both in vitro and in vivo, which is important for antimalarial activity. And it is also known that Berenil (**3**) is 125 times more active than CQ in antimalarial activity in vivo. But, no report related to antimalarial activities of 1-aryl-3,3-dialkyltriazenes has appeared yet.

Now we report the synthesis of 1-aryl-3,3-dialkyltriazenes and their antimalarial activities against chloroquine-sensitive *Plasmodium berghei* (*P. berghei*) NK-65 strain in vivo.

2. Results and discussion

2.1. Chemistry

The alkyltriazenes were prepared in good yields according to standard conditions.[8] The arylamines were treated with $NaNO_2$ in HCl solution to generate diazonium ion, then the respective secondary amines and potassium carbonate were added to the solution continuously. Resulting mixtures were purified with flash column chromatography to obtain corresponding triazenes (Eq. 1). The results are listed in Table 1. Except for **6**, all compounds were obtained in good to moderate yields.

Keywords: Antimalarial activity; 1-Aryl-3,3-dialkyltriazenes; *Plasmodium berghei* NK-65.
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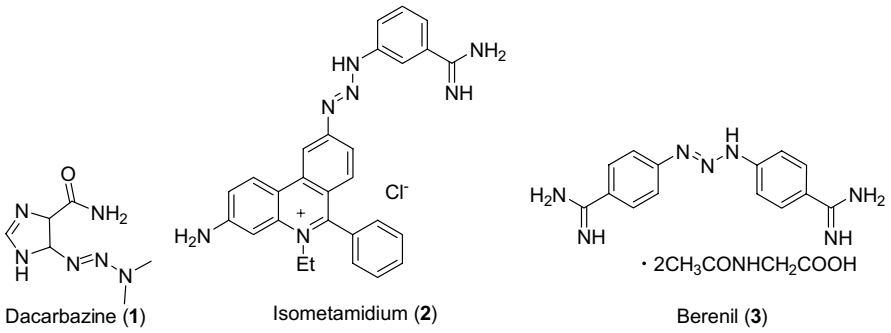
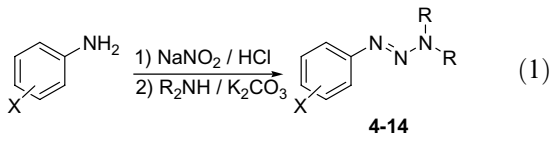




(1)

2.2. Antimalarial activity in mouse malaria model

All compounds (**4**–**14**) were evaluated for antimalarial activity in vivo against mouse malaria *P. berghei* NK-65, a chloroquine-susceptible strain of murine malaria parasites. JCL-ICR mice (4–5 weeks, female) were used as the trial animal. Each group contained 5 mice. They were infected intraperitoneally with 3.3×10^6/0.2 ml/mouse parasitized erythrocytes in 1% saline on day 0. The infective dose was calculated by infection number of red cells and the infection rate of protozoa. The 100 mg/kgbw of triazenes was dissolved in arabic gum or corn oil in order to become 1 mL/10 gbw for a dose and administered by subcutaneous injection two times (2 and 48 h after infection). The infected control group was administered 5% arabic gum. CQ was used as the reference antimalarial drug. And the dose of CQ was 50 mg/kgbw. We observed them for 14 days. Survival rate after 14 days from infection, 50% survival day, and parasitemia rate of erythrocytes were calculated and are summarized in Table 2.

After 14 days from infection, the survival rate of the groups treated with compounds **4**, **8**, **10**, **12**, and **13** was over 80%, 60%, 40%, 40%, and 60%, respectively, while it was 0% in the control group. At the 50% survival day, many compounds were active compared to the infected control. Particularly, activities of compounds **4**, **8**, and **12** were high. The activities lasted at least 12 days. And some treatment groups indicated lower percentage than the infected control group in the rate of infected erythrocytes. The parasitemia of the groups treated with compounds **4**, **7**, and **8** was 67.9%, 60.4%, and 62.5%, respectively, after 14 days. In other words, these compounds suppressed proliferation of protozoa. Some acute toxicities of these compounds were observed. The number of white blood cell of the group treated by compounds **4** and **8** increased relatively. And the body weights of all mice decreased. As the above results indicate, the substitution of 3,3-dimethyl and 3,3-tetramethylene groups on the 3-nitrogen of the triazenyl group showed good effects, and *p*-trifluoromethylphenyl, *p*-chlorophenyl and mesityl groups on the 1-nitrogen of the triazenyl group did as well.

3. Conclusions

We have synthesized and revealed antimalarial activity of 1-aryl-3,3-dialkyltriazenes. In particular, 3,3-dimethyl-1-(4-(trifluoromethyl)phenyl)triaz-1-ene (**4**) produced good results in the survival rate, 50% survival day, and % parasitemia. These results indicated that the triazenes are new candidates for antimalarial drugs.

4. Experimental

4.1. General methods

Nuclear magnetic resonance spectra were recorded on JEOL GSX-270 (^{1}H 270 MHz, ^{13}C 67.5 MHz) spectrometer in $CDCl_3$ or C_6D_6 with TMS as an internal standard. Mass spectra (EI) were recorded on a JMS-HX100 spectrometer. Infrared spectra were recorded on a Shimadzu IR-435 spectrophotometer. Unless otherwise noted, all chemicals were obtained from commercial suppliers and used without further purification.

4.2. Preparation of triazenes

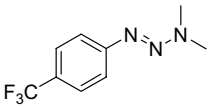
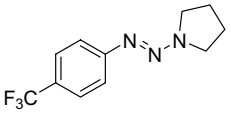
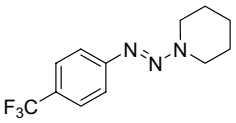
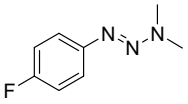
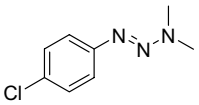
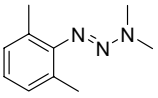
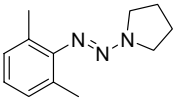
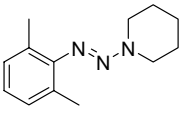
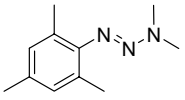
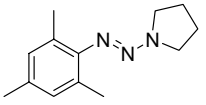
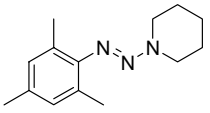
Most 1-aryl-3,3-dialkyltriazenes were synthesized as per the method of literature.[8]

4.3. General procedure for synthesis of triazenes

To a solution of aniline derivative (16.0 mmol) and concd HCl (5.3 mL, 64.5 mmol) in H_2O (30 mL) was added $NaNO_2$ (1.20 g, 17.4 mmol) in portions at 0 °C. After stirring for 0.5 h, the reaction mixture was added to the solution of K_2CO_3 (8.4 g, 60.8 mmol) and the secondary amine (57.7 mmol) in H_2O (53 mL) at 0 °C. Extractive work-up and the subsequent purification with flush column chromatography afforded triazenes.

4.3.1. 3,3-Dimethyl-1-(4-(trifluoromethyl)phenyl)triaz-1-ene (4). Oil, 92% ^{1}H NMR ($CDCl_3$) δ: 7.53 (m, 4H), 3.50 (br s, 3H), 3.28 (br s, 3H); ^{13}C NMR ($CDCl_3$) δ: 153.54, 126.83 (q, J = 32.3 Hz), 125.97 (q, J = 3.9 Hz), 124.52 (q, J = 271.9 Hz), 120.53, 43.24, 35.78; IR (neat) cm^{-1}: 2900 (w), 1650 (m), 1480 (m), 1380 (m), 1320 (s), 1160 (s), 1090 (s), 1060 (s), 840 (m); MS (EI) (*m*/*z*, %): 217 (M^+, 14), 133 (32), 105 (100), 91 (36), 79 (38); HRMS (*m*/*z*): 217.0837 (Calcd for $C_9H_{10}F_3N_3$, 217.0827).

Table 1. Yields of 1-aryl-3,3-dialkyltriazenes

Entry	Structure	Compound	Yield (%)
1		**4**	92
2		**5**	79
3		**6**	47
4		**7**	83
5		**8**	Quant.
6		**9**	85
7		**10**	83
8		**11**	82
9		**12**	91
10		**13**	77
11		**14**	64

4.3.2. 1-((4-(Trifluoromethyl)phenyl)diazenyl)pyrrolidine (5). Yellow crystal, 79%; mp 104–107 °C (recryst from hexane); ^{1}H NMR ($CDCl_3$) δ: 7.56 (d, J = 9.0 Hz, 2H), 7.48 (d, J = 9.0 Hz, 2H), 3.93 (br s, 2H), 3.70 (br s, 2H), 2.05 (s, 4H); ^{13}C NMR ($CDCl_3$ at 60 °C) δ: 154.26, 126.75 (q, J = 32.4 Hz), 125.90 (q, J = 3.7 Hz), 124.63 (q, J = 264.3 Hz), 120.49, 48.92, 23.67; IR (Nujol) cm^{-1}: 2850 (s), 1600 (m), 1470 (w), 1300 (m), 1090 (w), 1060 (w), 830 (m); MS (EI) (*m*/*z*, %): 243 (M^+, 12), 173 (16), 145 (100), 125 (4.5), 95 (5.8); HRMS (*m*/*z*): 243.0972 (Calcd for $C_{11}H_{12}F_3N_3$, 243.0983).

4.3.3. 1-((4-(Trifluoromethyl)phenyl)diazenyl)piperidine (6). Brown oil, 47%; ^{1}H NMR ($CDCl_3$) δ: 7.58 (d, J = 9.0 Hz, 2H), 7.49 (d, J = 9.0 Hz, 2H), 3.83 (s, 4H), 1.72 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 153.76, 127.25 (q, J = 32.3 Hz), 125.97 (q, J = 3.7 Hz), 125.28 (q, J = 269.8 Hz), 120.65, 48.28, 25.39, 24.34; IR (neat) cm^{-1}: 2900 (S), 2850 (S), 1650 (s), 1400 (w), 1260 (w), 1220 (w), 1060 (w), 950 (m), 920 (w); MS (EI) (*m*/*z*, %): 257 (M^+, 22), 238 (5.2), 173 (33), 145 (100), 125 (6.7), 84 (10); HRMS (*m*/*z*): 257.1141 (Calcd for $C_{12}H_{14}F_3N_3$, 257.1140).

4.3.4. 1-(4-Fluorophenyl)-3,3-dimethyltriaz-1-ene (7). Yellow oil, 83%; ^{1}H NMR ($CDCl_3$) δ: 7.38 (m, 2H), 7.05 (m, 2H), 3.32 (br s, 6H); ^{13}C NMR ($CDCl_3$) δ: 160.92 (d, J = 243.5 Hz), 147.43, 121.75 (d, J = 8.5 Hz), 115.28 (d, J = 22.6 Hz), 38.96; IR (neat) cm^{-1}: 2850 (m), 1645 (m), 1425 (s), 1300 (s), 1190 (m), 1050 (br m), 810 (m); MS (EI) (*m*/*z*, %): 167 (M^+, 18), 123 (27), 95 (75), 75 (13), 58 (33), 43 (100); HRMS (*m*/*z*): 167.0849 (Calcd for $C_8H_{10}FN_3$, 167.0859).

4.3.5. 1-(4-Chlorophenyl)-3,3-dimethyltriaz-1-ene (8). Yellow crystal, Quant.; mp 56 °C (recryst from hexane); ^{1}H NMR ($CDCl_3$) δ: 7.35 (m, 2H), 7.27 (m, 2H), 3.33 (br s, 6H); ^{13}C NMR ($CDCl_3$) δ: 149.48, 130.42, 128.81, 121.65, 42.14; IR (KBr) cm^{-1}: 2850 (m), 1645 (m), 1425 (s), 1300 (s), 1190 (m), 1050 (br m), 810 (m); MS (EI) (*m*/*z*, %): 183 (M^+, 25), 139 (43), 111 (100), 75 (23); HRMS (*m*/*z*): 183.0552 (Calcd for $C_8H_{10}ClN_3$, 183.0563).

4.3.6. 1-(2,6-Dimethylphenyl)-3,3-dimethyltriaz-1-ene (9). Brown oil, 85%; ^{1}H NMR ($CDCl_3$) δ: 6.98 (m, 3H), 3.30 (s, 6H), 2.17 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 148.91, 130.07, 128.39, 124.62, 38.87, 18.37; IR (neat) cm^{-1}: 2900 (m), 1460 (s), 1400 (s), 1300 (s), 1190 (w), 1070 (s), 760 (m); MS (EI) (*m*/*z*, %): 177 (M^+, 25), 139 (43), 111 (100), 75 (23); HRMS (*m*/*z*): 177.1261 (Calcd for $C_{10}H_{15}N_3$, 177.1266).

4.3.7. 1-((2,6-Dimethylphenyl)diazenyl)pyrrolidine (10). Brown oil, 83%; ^{1}H NMR ($CDCl_3$) δ: 6.96 (m, 3H), 3.75 (t, J = 6.5 Hz, 4H), 2.19 (s, 6H), 2.01 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 149.14, 129.99, 128.28, 124.42, 48.29, 23.81, 18.44; IR (neat) cm^{-1}: 2950 (m), 1580 (w), 1420 (s), 1320 (s), 1200 (m), 1150 (m), 1090 (m), 1020 (w), 760 (m) ; MS (EI) (*m*/*z*, %): 203 (M^+, 14), 174 (8.5), 133 (24), 105 (100), 91 (22), 79 (28); HRMS (*m*/*z*): 203.1418 (Calcd for $C_{12}H_{17}N_3$, 203.1422).

4.3.8. 1-((2,6-Dimethylphenyl)diazenyl)piperidine (11). Brown oil, 82%; ^{1}H NMR ($CDCl_3$) δ: 6.98 (m, 3H), 3.72 (t, J = 5.5 Hz, 4H), 2.18 (s, 6H), 1.68 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 148.79, 129.87, 128.32, 124.70, 47.90, 25.05, 24.46, 18.40; IR (neat) cm^{-1}: 2900 (s), 1740 (m), 1590 (w), 1440 (s), 1360 (s), 1290 (s), 1260 (m), 1160 (s), 1090 (s), 980 (m), 850 (m), 760 (m); MS (EI) (*m*/*z*, %): 217 (M^+, 8), 133 (24), 105 (100), 91 (52), 77 (19); HRMS (*m*/*z*): 217.1584 (Calcd for $C_{13}H_{19}N_3$, 217.1579).

4.3.9. 1-Mesityl-3,3-dimethyltriaz-1-ene (12). Brown oil, 91%; ^{1}H NMR ($CDCl_3$) δ: 6.83 (s, 2H), 3.29 (s, 6H), 2.25 (s, 3H), 2.14 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 146.39,

Table 2. Antimalarial activity of triazenes against *P. berghei* NK-65 in mice infected with blood parasites, treated by subcutaneous route

Entry	Compound	Survival rate[a] (%)	50% Survival day[b]	% Parasitemia (% infected erythrocytes)		
				Day 3	Day 7	Day 14
1	**4**	80	>12.6	4.8	17.6	67.9
2	**5**	0	7.5	2.9	66.3	—
3	**6**	0	8.0	2.3	44.6	—
4	**7**	20	9.4	1.7	53.5	60.4
5	**8**	60	>14.0	4.8	27.0	62.5
6	**9**	0	10.3	1.7	53.9	—
7	**10**	40	10.4	5.6	54.6	81.7
8	**11**	0	7.0	4.2	76.4	—
9	**12**	40	>12.4	4.4	12.4	73.3
10	**13**	60	11.4	4.1	38.5	74.6
11	**14**	0	7.0	4.2	76.4	—
12	CQ	100	>14.0	ND	(<1.0)	(8.5)
Infected control	5% Arabic gum	0	7.0	4.8	76.5	—
Normal control		100	>14.0	NT	NT	NT

[a] Survival rate (%) = (the number of survival mice/5) × 100.
[b] 50% Survival day = the total survival day of five mice/5.

133.90, 129.84, 129.06, 38.89, 20.79, 18.40; IR (neat) cm^{-1}: 2800 (s), 1590 (w), 1480 (s), 1410 (s), 1310 (s), 1200 (m), 1080 (s), 850 (m); MS (EI) (*m*/*z*, %): 191 (M^+, 20), 147 (27), 119 (100), 105 (64), 91 (44), 77 (21); HRMS (*m*/*z*): 191.1434 (Calcd for $C_{11}H_{17}N_3$, 191.1422).

4.3.10. 1-(Mesityldiazenyl)pyrrolidine (13). Brown oil, 77%; ^1H NMR ($CDCl_3$) δ: 6.81 (s, 2H), 3.72 (t, $J = 6.5$ Hz, 4H), 2.23 (s, 3H), 2.16 (s, 6H), 1.97 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 146.62, 133.54, 129.67, 128.89, 48.20, 23.71, 20.66, 18.37; IR (neat) cm^{-1}: 2900 (s), 1600 (w), 1430 (s), 1300 (s), 1200 (s), 1140 (m), 1020 (w), 840 (m); MS (EI) (*m*/*z*, %): 217 (M^+, 2), 147 (15), 119 (100), 105 (60), 91 (42), 77 (25); HRMS (*m*/*z*): 217.1575 (Calcd for $C_{13}H_{19}N_3$, 217.1579).

4.3.11. 1-(Mesityldiazenyl)piperidine (14). Brown oil, 64%; ^1H NMR ($CDCl_3$) δ: 6.82 (m, 2H), 3.70 (m, 4H), 2.24 (s, 3H), 2.15 (s, 6H), 1.68 (m, 6H); ^{13}C NMR ($CDCl_3$) δ: 146.35, 134.02, 129.74, 129.05, 47.94, 25.06, 24.52, 20.75, 18.40; IR (neat) cm^{-1}: 2900 (s), 1610 (w), 1460 (s), 1360 (s), 1290 (m), 1170 (m), 1090 (m); MS (EI) (*m*/*z*, %): 231 (M^+, 3), 147 (22), 119 (100), 105 (36), 91 (25), 84 (12); HRMS (*m*/*z*): 231.1737 (Calcd for $C_{14}H_{21}N_3$, 231.1735).

4.3.12. Antimalarial activity in vivo. In vivo assays of the antimalarial activity were performed in mice (4–5 weeks, female; JCL-ICR mouse CLEA Japan, Inc., Japan) infected with *P. berghei* NK-65, a chloroquine-susceptible pathogen. Each group contained 5 mice. At day 0, they were infected intraperitoneally with an erythrocytic form of *P. berghei* obtained from a heavily infected donor mouse. A 1:100 dilution of the blood in sterilized saline was inoculated at 0.2 mL/mouse by intraperitoneal route. The untreated group received 5% arabic gum solution. The infection dose was shown by the number of protozoa-infected red blood cells, and the blood smears (rate of infection) and number of red blood cells were counted. IP inoculation was administered twice (after 2 and 48 h). The clinical condition (piloerection, anemia, fatality, etc.) was monitored daily after infection. Survival rate (%) and 50% survival day were obtained during the test period. At days 3, 7, and 14, thin smears of tail blood were made, fixed with methanol, and stained with Giemsa (2%, for 2 h). The level of parasitemia (percentage of infected erythrocytes) was determined microscopically by examination of cells. The samples were prepared at 0.1 mL/10 gbw of given dose, dissolved in sterilized saline of 5% gum arabic emulsion to the solid compounds, and in cone oil to oily compound similarly. The doses of samples were 100 mg/kgbw; only the dose of chloroquine was 50 mg/kgbw.

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