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In Vivo Active Antimalarial Isonitriles†

Chandan Singh,[a,*] Naveen Chandra Srivastav[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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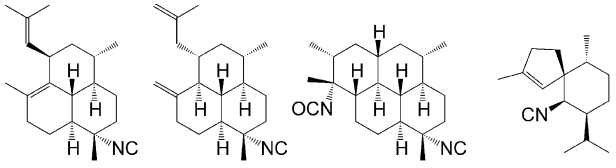
Abstract—Building on the lead from antimalarial isonitriles **1**–**4** of marine origin, several easily accessible synthetic isonitriles were assessed for their antimalarial activity against *Plasmodium falciparum* (in vitro) and multidrug resistant *Plasmodium yoelii* in Swiss mice model (in vivo). Isonitrile **11** has shown promising activity in both these assays. © 2002 Elsevier Science Ltd. All rights reserved.

Malaria is endemic in many parts of world. Around 300–500 million cases of malaria are reported every year, of which more than a million die of severe and complicated malaria.[1] This problem has acquired a new dimension with the rapid emergence of malaria parasite resistant to the contemporary antimalarial drugs. It is against this background that the isolation of sesquiterpene peroxide, artemisinin, as the active principle of the Chinese traditional drug against malaria, *Artemisia annua*, has been a welcome development.[2] Artemisinin and its derivatives are the only antimalarial drugs against which clinically relevant resistance has not been reported. However, a recent report on artemisinin resistant strain of *Plasmodium yoelii* suggests that it is only a matter of time before clinically important resistant to artemisinin and its derivatives is observed.[3] Thus there is an urgent need to develop a second line of antimalarials.

Recently a series of terpene isonitriles, for example, **1**, **2**, **3**, and **4**, isolated from marine sponges, have been reported to show significant antimalarial activity in vitro.[4–6] The antimalarial activity of these isonitriles has been correlated with their ability to inhibit heme polymerization.[7] Recently some synthetic analogues of **1** and **2** have been prepared and shown to exhibit moderate antimalarial activities against *Plasmodium falciparum*, in vitro.[8] We have assessed a series of easily accessible synthetic isonitriles (**5**–**14**), against *P. falciparum* (in vitro) and multidrug resistant *P. yoelii* (in vivo). Isonitrile **11** has shown very promising activity in both these assays. Herein, we report the preliminary results of this study. To the best of our knowledge, this is the first report on in vivo active antimalarial isonitriles.



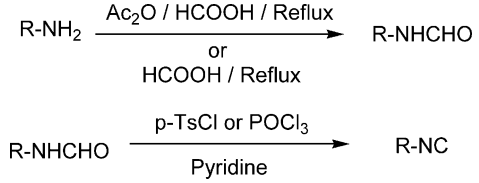
1 **2** **3** **4**

Isonitriles **5**–**14** were prepared from the easily accessible amines using established procedures[9] (Scheme 1) and were characterized by IR, ^{1}H NMR and MS.[10]

Antimalarial Activity

In vitro[15]

Compounds **5**–**14** were evaluated against *P. falciparum* (strain NF-54) using minor modification to technique of



Scheme 1. Synthetic procedure for isonitriles.

*Corresponding author. Tel.: +91-522-212414x4385; fax: +91-522-223405; e-mail: chandancdri@yahoo.com
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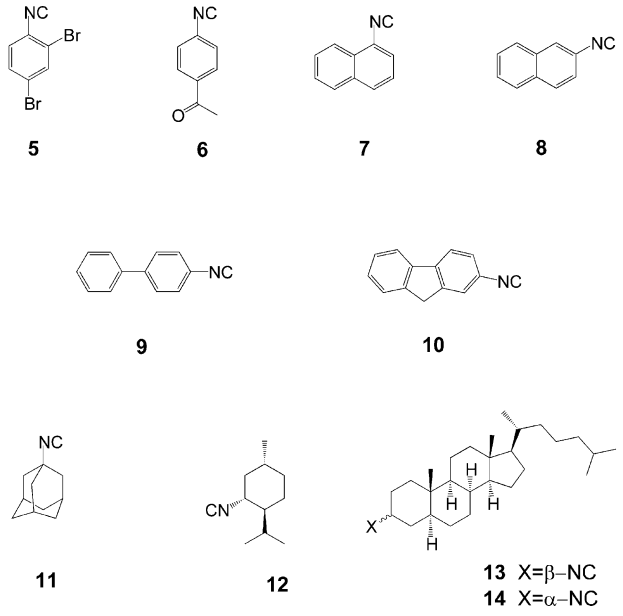
Rieckmann and co-workers.[16] The results are summarized in Table 1.



In vivo[17]

Isonitriles **6**, **9**, **10** and **11** which showed activity in the range of 0.40 to 2.00 μg/mL in vitro, were tested against multi-drug resistant strain of *P. yoelii* in Swiss mice on 50 and 100 mg/kg by im route. The results are summarized in Table 2.

Isonitrile **11**, which has shown 100% inhibition of parasitaemia on day 4 at 50 mg/kg, was evaluated at lower doses to determine its ED_{50} and ED_{90} values. Results are summarized in Table 3.

The ED_{50} and ED_{90} values as determined by log dose probit analysis showed values of 10.7 and 19.6 mg/kg, respectively.

In conclusion, we have prepared a series of structurally simple isonitriles using known methods and evaluated them for their antimalarial activity both in vitro and in vivo. Several of these isonitriles have shown good antimalarial activity in vitro. Isonitrile **11**, the best compound in the series has shown promising activity in vivo also. Although compound **11** cannot be taken for further development, as its therapeutic index is poor, it provides a good lead for further study.

Table 1. In vitro antimalarial activity of isonitriles **5**–**14** against *P. falciparum* (strain **NF-54**)

Compd	MIC[a,b] μg/mL
5	50.00
6	2.00
7	50.00
8	50.00
9	1.00
10	2.00
11	0.40
12	50.00
13	>50.00
14	>50.00
Artemisinin	0.03
Chloroquine	0.04

[a]MIC, minimum concentration inhibiting development of ring stage parasites into the schizonts.
[b]50.00 μg/mL is the highest concentration used in this study.

Table 2. In vivo antimalarial activity against multi-drug resistant strain of *P. yoelii* in Swiss mice

Compd	Dose (mg/kg/day)	Percent suppression on day 4	No. of mice cured
6	100	35.72	0/5
	50	Nil	0/5
9	100	62.79	0/5
	50	32.98	0/5
10	100	21.35	0/5
	50	6.34	0/5
11	100	Toxic	0/5
	50	100	2/5

Table 3. In vivo antimalarial activity of compound **11** against multi-drug resistant strain of *P. yoelii* in Swiss mice

Dose mg/kg/day	Percent suppression on day 4	No. of mice cured	Mean survival time[a] ±S.D.	Mice alive on day 28
50	100	2/5	16.3±1.0	2
25	99.8	0/5	12.8±4.4	1
12.5	73	0/5	11.8±5.9	0
6.25	19	0/5	8.2±1.3	0
Control	—	0/6	7.5±0.8	0

[a]MST calculated for animals which died during 28-day observation period.

Acknowledgements

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10. Isonitriles **5** and **6** are new compounds;[11] synthesis of isonitriles **7**,[9] **8**,[9] **9**,[12] **10**,[9] **11**,[13] **12**,[14] **13**,[9] and **14**[9] has been reported earlier.
11. Isonitrile **5**: FT-IR (KBr): 2121.6 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.30 (d, 1H, $J=7.6$ Hz), 7.49 (d, 1H, $J=7.6$ Hz), 7.84 (s, 1H); EI–MS (m/z): 261 (M^+). Isonitrile **6**: FT-IR (KBr): 2127.6 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 2.62 (s, 3H), 7.48 (d, 2H, $J=6.8$ Hz), 7.99 (d, 2H, $J=6.8$ Hz); EI–MS (m/z): 145 (M^+).
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15. In vitro antimalarial efficacy test. The asynchronous parasites obtained from cultures of *P. falciparum* were synchronized after 5% sorbitol treatment so as to contain only ring stage parasites.[18] Parasite suspension in medium RPMI 1640 at 1–2% parasitaemia and 3% hematocrit was dispensed into wells of sterile 96-well plates. Test compounds were serially diluted in duplicate wells to obtain final test concentration. The culture plates were incubated in a candle jar at 37 °C for 36–40 h. Thin blood smears from each well prepared at the end of incubation period were microscopically examined and the concentration, which inhibited the maturation of rings into schizonts stage, was recorded as MIC.
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