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Design, synthesis and biological evaluation of novel bicyclic β-lactams as potential antimalarials

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Abstract—A series of bicyclic N-substituted and unsubstituted β-lactams were synthesized and evaluated as targeted potential antimalarials. The compounds MNR4 and MNR5 were found to have highest potency against *Plasmodium falciparum* in vitro.

1. Introduction

Malaria has been a serious disease throughout recorded history and it continues to be one of the most widely spread health hazards.[1] It is the major parasitic infection in many tropical and subtropical regions leading to more than two million deaths out of 400 million cases reported each year.[2] *Plasmodium falciparum*, which causes the cerebral form of malaria, is the major contributor towards the mortality. More than half of the world's population lives in areas where they remain at risk of malarial infection. Moreover, during recent years the situation has worsened in many ways mainly due to malarial parasites becoming increasingly resistant to several antimalarial drugs.[3] The emergence of *P. falciparum* strains resistant to chloroquine, the cheapest and most widely used drug to treat malaria has caused global concern as the alternatives available are expensive and mostly beyond the reach of the affected population. Furthermore the control of malaria is becoming more complicated by the parallel spread of resistance in the mosquito vector to the currently available insecticides. Urgent efforts are therefore necessary to identify new classes of antimalarials[4] and develop them as drugs with varied model of action to overcome the problem of resistance.[5] We have initiated the development of a novel class of antimalarials derived from β-lactam.[6] As such the use of β-lactam as chiral building blocks in organic synthesis is well established, after the discovery of penicillin and cephalosporins there has been a remarkable growth in the field of β-lactam chemistry.[7] The need for potent and effective β-lactam antibiotics as well as more effective β-lactamase inhibitors has motivated synthetic organic and medicinal chemist to design new functionalized 2-azetidinones, apart from their clinical use as antibacterial agents. These compounds have also been used as synthons in the preparation of various heterocyclic compounds of biological significance.[8a–d] Because of this general trend of β-lactam use, the search for clinically useful β-lactams that are antibiotics or have other medically importance properties will continue.[9a,b] In the present study we have synthesized and biologically evaluated a series of N-substituted and unsubstituted bicyclic β-lactams against *P. falciparum* in vitro.

2. Chemistry

The synthetic procedure for the β-lactams (S. no 1–10) has been extensively documented in the literature[10] and can be applied to a large range of olefinic or diene containing framework and was therefore ideal for our purposes. The precursors for the synthesis were cyclic alkenes and chlorosulfonyl isocyanate. β-Lactam was prepared by the hydrolysis of an intermediate *N*-chlorosulfonyl β-lactam in 70% yield. *N*-Chlorosulfonyl β-lactam was synthesized by the careful addition of chlorosulfonyl isocyanate to cyclic alkene in dry toluene at 0 °C. In IR, NH stretching vibration was observed at 3214 cm^{-1} along with a sharp peak of carbonyl group at 1730 cm^{-1}. ^1H NMR also showed a broad peak at δ 6.2 ppm due to NH group. The secondary amino moiety in β-lactam was further protected by benzyl group under

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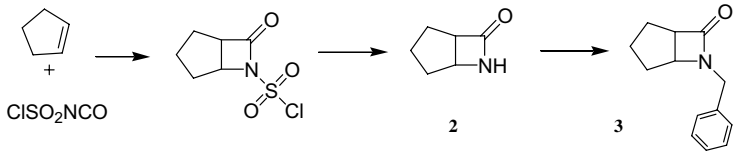


Scheme 1. (a) Cyclopentene and chlorosulfonyl isocyanate (1:1.1), dry toluene, 0 °C for 1 h and then at room temp for 12 h; (b) hydrolysis by aqueous Na_2SO_3; (c) 1:1 mixture of a 50% solution of NaOH, CH_2Cl_2, Bu_4NHSO_4 (0.1 equiv), benzyl bromide (1.2 equiv).

phase transfer conditions, which was proved to be the most efficient method (80% yield). Disappearance of peak in IR at 3214 cm^{-1} and the appearance of peak at 3017, 1495, 1440 cm^{-1} indicated the presence of benzyl groups. In ^1H NMR benzyl protons appeared at δ 7.2 ppm as multiplets also confirms the formation of N-protected lactam and a base peak of tropylium cation at *m/z* 91 in all the compounds were also observed.

By following the above proposed scheme a series of bicyclic β-lactams have been synthesized[11] and biologically evaluated (compounds **1**–**10**). Several of the new compounds have achieved the IC_{50} in the range of 1.6–13.5 μM and provide lead for the development of novel class of ant parasitic drugs with improved biological and pharmacological properties (Scheme 1).

3. In vitro evaluation of antimalarial activity

Two strains of chloroquine sensitive strain and one strain of *P. falciparum* isolated from patients from Jagadalpur region of the country was adapted to and maintained in vitro. The cultures were maintained as per the standard culture procedures described earlier.[12] The parasites were grown in O +ve human RBCs with the addition of RPMI 1640 culture media with 10% human serum as supplement. The cells were incubated at 37 °C at 5% CO_2 atmosphere and the parasitaemia was checked after 24 h and media changed. When parasitaemia exceeded 10% parasitized cells the culture was subcultured with the addition of fresh RBC. The parasite growth was synchronized by the sorbitol lysis method and synchronized ring stage parasites were used for testing. The in vitro testing was done in 100 μL complete media per well with the addition of 10 μL of erythrocytes with 2% of ring stages of parasites. All the tests were run in duplicates with in 96 well flat bottomed tissue culture plate and double dilutions were made for each of the test compound with individual control wells only with the RPMI 1640 and human serum supplement. The growth of the parasites in the presence of each of the test compound, chloroquine and control wells were monitored by the examination of the giemsa stained blood smears made after 24 h of incubation. The counting was done for the presence of mature schizonts among 200 asexual parasites and the average schizont maturation inhibition was calculated by the formula $(1 - N_t/N_c) \times 100$ where in N_t and N_c represent the number of schizont present in the test and control respectively. The IC_{50}, IC_{90} and IC_{99} values were calculated by using the commercial statistical package Sigmastat.

Compounds MNR4 and MNR5 showed the highest potency among the compounds screened as both had IC_{50} values below 2 μM and IC_{99} values of 6.1 and 3.2 μM respectively against chloroquine susceptible strain of *P. falciparum*. The values remained almost similar with the chloroquine resistant strain of *P. falciparum*. Since

Table 1. Structures and in vitro antimalarial activities of compounds 1–10 against *Plasmodium falciparum*

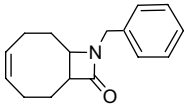
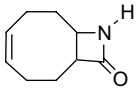
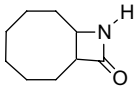
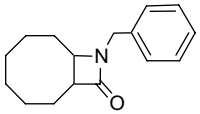
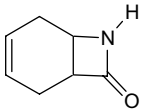
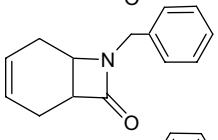
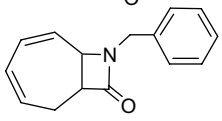
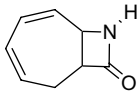
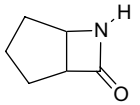
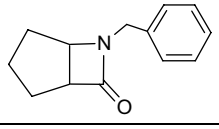
S. no	Compound code	Structure	IC_{50} (μM) (chloroquine sensitive strain)
1	MNR00		5.4
2	MNR0		6.8
3	MNR1		8.1
4	MNR2		8.1
5	MNR3		9.1
6	MNR4		1.5
7	MNR5		1.8
8	MNR6		5.9
9	MNR7		13.5
10	MNR11		6.5

Table 2. Antimalarial activity of compounds by schizont maturation assay against chloroquine sensitive (CS) and chloroquine resistant (CR) strains of *P. falciparum* (μM)

Compound	IC_{50}		IC_{90}		IC_{99}	
	CS	CR	CS	CR	CS	CR
MNR00	5.4	5.8	10.7	11.4	13.3	14.5
MNR0	6.8	7.3	25.4	26.1	43.4	48.7
MNR1	8.1	8.4	33.7	33.9	51.2	54.8
MNR2	8.1	7.9	18.8	19.4	25.2	27.4
MNR3	9.1	8.7	19.3	21.2	23.2	25.3
MNR4	1.5	1.6	5.6	6.1	6.1	6.4
MNR5	1.8	1.8	2.8	2.9	3.2	3.3
MNR6	5.9	4.6	14.5	15.4	20.1	24.8
MNR7	13.5	12.4	31.9	34.5	42.2	48.7
MNR11	6.5	7.4	11.2	12.4	12.3	14.5
Chloroquine	0.02	0.03	0.05	0.5		

both these compounds are active against the chloroquine sensitive as well as the resistant strains of *P. falciparum* this class of compounds has the potential as antimalarial. The normal human RBC's used for the culture when exposed at all the concentrations showed no cell lysis which suggests that the test compounds have no lytic activity on the normal human cells. Compounds MNR4 and MNR5 that showed maximum activity in the schizont maturation assay were also highly active in the total growth inhibition assay indicates that the possible mode of action of these compounds is on the total growth inhibition rather than slowing down the cell division cycle. Its also noteworthy to mention that although these compounds are active against the chloroquine resistant strain *P. falciparum* at much higher concentrations than chloroquine (which is six times more potent than the lactams) or other natural products like artemesinin, the potency of these compounds can be enhanced by further structural modifications. From the results it has been clearly indicated that the introduction of benzyl group on the 2-azetidine ring has some effect towards the antimalarial activity. In conclusion, the result obtained established that N-benzylated lactam (Table 1 S. no 1–10) has in vitro antimalarial activities against both chloroquine resistant and choloroquine sensitive malarial strains (Table 2).

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11. Compounds (**1**–**10**) were fully characterized by IR, MS, ^{1}H NMR and ^{13}C NMR.
For example compound number **3** code MNR1. Mp 105–106 °C (dec) IR 3214 cm^{-1}, 1731 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.99 (td, 1H, 9 Hz, 4 Hz), 1.30 (m, 2H), 1.50 (m, 2H), 3.07 (m, 1H), 6.1 (br, NH, 1H). ^{13}C ($CDCl_3$, 100 MHz) NMR δ 24.9, 26.8, 29.8, 30.01, 31.09, 36.1, 45.5, 48.7, 171.30. ESI-MS 154 (M+H).
Compound number **4** code MNR2. Mp 62–63 °C (dec). IR 1767, 1730, 1656, 3011, 1485, 1440 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (m, 2H), 1.29 (m, 8H), 1.51 (m, 2H), 1.53 (m, 2H), 2.85 (m, 1H), 3.03 (td, 1H, 9 Hz, 4 Hz), 3.98, 4.46 (dd, 2H) AB Coupling J^{AB} 16 Hz, 7.06 (m, 2H), 7.33 (m, 2H), 7.07 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.2, 26.8, 29.6, 30.0, 31.12, 33.9, 43.3, 52.5, 53.3, 128.3, 126.8, 137.2, 176.7. ESI-MS 244 (M+H).
Compound number **5** code MNR3. Mp 98–99 °C (dec). IR 3210, 1729 cm^{-1} ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.18 (m, 2H), 2.20 (m, 2H), 3.10 (m, 1H), 5.59–5.74 (td, 2H, J = 8.0, 2.8 Hz), 6.20 (br, NH, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.3, 35.0, 35.3, 47.7, 131.7, 172.54 ESI-MS 124 (M+H).
Compound number **6** code MNR4.Mp 58–63 °C (dec). IR 1760, 1732, 1650, 3017, 1495, 1440 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.18 (m, 2H), 2.20 (m, 2H), 3.07 (m, 1H), 2.89 (m, 1H), 4.07 (d, J = 5.4 Hz, 1H), 4.55 (d, J = 15.4 Hz, 1H), 5.66–5.72 (m, 2H), 7.06 (m, 3H), 7.14 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 29.6, 33.1, 45.5, 53.4, 54.9, 131.7, 128.1–128.45, 137.2, 174.1 ESI-MS 244 (M+H).
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