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Research Article

4-Aminoquinoline-β-Lactam Conjugates: Synthesis, Antimalarial, and Antitubercular Evaluation

Raghu Raj[1], Christophe Biot[2,3], Séverine Carrère-Kremer[4], Laurent Kremer[4,5], Yann Guérardel[2,3], Jiri Gut[6], Philip J. Rosenthal[6] and Vipan Kumar[1,*]

[1]*Department of Chemistry, Guru Nanak Dev University, Amritsar, 143005, India*
[2]*Unité de Glycobiologie Structurale et Fonctionnelle, Université Lille 1, F-59650, Villeneuve d'Ascq, France*
[3]*CNRS, UMR 8576, F-59650, Villeneuve d'Ascq, France*
[4]*Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, UMR 5235 CNRS, Université Montpellier 2, Place Eugène Bataillon, 34095, Montpellier Cedex 05, France*
[5]*INSERM, DIMNP, Place Eugène Bataillon, 34095, Montpellier Cedex 05, France*
[6]*Department of Medicine, University of California, San Francisco, CA, 94143, USA*
**Corresponding author: Vipan Kumar, vipan_org@yahoo.com*

A library of quinoline-β-lactam-based hybrids was synthesized and tested for their antimalarial and antitubercular activities. The present antimalarial data showed the dependence of activity on the nature of linker, *N*-1 substituent of the β-lactam ring as well as the length of alkyl chain. Most of the compounds are not as efficient as chloroquine in inhibiting the culture growth of *Plasmodium falciparum* W2 strain. Nevertheless, the synthesized hybrids showed better antitubercular activities (up to five times) compared with cephalexin (up to three times) and ethionamide.

Key words: 4-aminoquinoline-β-lactam conjugates, antimalarial evaluation, antitubercular evaluation, molecular hybridization, structure–activity relationship

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Malaria is a devastating infectious, parasitic disease estimated to have affected more than 216 million people and roughly 655 000 deaths worldwide in 2010 and continues to be a causative factor to the high mortality rate in tropical and subtropical countries.a The disease remains a major issue in health control, mostly among children and pregnant women with the majority of the cases, and approximately 90% of the malaria deaths occurring in sub-Saharan Africa. A major problem associated with this disease is the development of resistance to the potent and inexpensive chloroquine (CQ), which has a long history in the treatment of malaria (1,2). A significant number of antimalarial drugs such as the antifolate combination of pyrimethamine and sulfadoxine, as well as artemisinin combination therapies, have been considered as the most important drugs for the treatment of malaria. However, these drug regimens are steadily losing their efficacy by the emergence of parasites with reduced vulnerability to these drug combinations (3). The development of drug resistance for the common antimalarials has encouraged considerable research efforts in the development of new drugs using different approaches (4,5), of which the molecular hybridization approach (6,7) is quite an appealing strategy. The advantage of using molecular hybridization is to activate different targets by a single molecule, thereby increasing therapeutic efficacy as well as bioavailability (8). The strategy is particularly interesting in the case of bioactive compounds with high toxicity or pharmacokinetic and pharmacodynamic restrictions. Although there are no examples of antimalarial dual drugs in clinical use, a number of active molecules have been identified using this approach, *viz.* highly active antimalarial trioxaquines developed by covalently linking a 1,2,4-trioxane motif to a 4-aminoquinoline moiety *via* an appropriate spacer (9).

Apart from its well-coveted role as an antimalarial agent, quinoline nucleus has received recent attention because of its antitubercular potential, which can be deduced from the diarylquinoline TMC 207 (ex R207910). The compound has shown bactericidal effect against dormant tubercle bacilli with activity against drug-sensitive and drug-resistant *Mycobacterium tuberculosis* (10,11). The drug is specifically approved for the treatment of multidrug-resistant tuberculosis with minimal inhibitory concentration (MIC) of 0.06 μg/mL and functions by inhibiting ATP synthase subunit C, an energy source for the bacterium (12). In a recent report, Zuazo and coworkers have disclosed the antitubercular potential of a series of tripartite hybrids based on pharmacophores 7-chloroquinoline (CQ), ethylenediamine as a spacer, and phenylurea as a thiourea bioisostere (13). From the observed structure–activity relationship (SAR), it was concluded that the presence of a hetero-aryl group at the 4th position of the quinoline core showed optimal activity against *M. tuberculosis* (14).

A heterocyclic system gifted with marked biological activities is the β-lactam nucleus. Interest in this class has led to

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the development of classical (e.g. penicillins and cephalosporins) and non-classical (e.g. carbapenams and monobactams) substrates (15–17). Current interest in this family is focused on the synthesis and modification of the β-lactam ring to obtain compounds with diverse pharmacological potential (18). β-lactams target bacterial transpeptidases, termed 'penicillin binding proteins (PBPs)', which are responsible for the introduction of interpeptide cross-links into bacterial peptidoglycan, thereby controlling rigidity of the bacterial cell wall (19). β-lactams have not been widely used for the treatment of *M. tuberculosis* or any other mycobacterial infection. A major obstacle is the presence in mycobacteria of a highly active β-lactamase (20,21). A report on the successful use of the β-lactam meropenem with the β-lactamase inhibitor clavulanic acid against both replicating and non-replicating XDR-TB strains has generated hope of repurposing this class of agents for the treatment of XDR-TB patients (22,23).

In continuation with our endeavor for the synthesis of novel molecular frameworks with biological potential (24–28), we report herein a facile and convenient synthesis of 4-aminoquinoline-β-lactam conjugates tethered *via* alkyl chain linkers as well as through amide functionality as depicted in Figure 1 along with their antimalarial and antitubercular evaluation.

Experimental Section

Materials and methods

Melting points were determined by open capillary using a Veego Precision Digital Melting Point apparatus (MP-D) and are uncorrected. IR spectra were recorded on a Shimadzu D-8001 spectrophotometer. ^{1}H NMR spectra were recorded in deuterochloroform and dimethylsulfoxide-d_6 with a Jeol 300 (300 MHz) spectrometer using TMS as an internal standard. Chemical shift values are expressed as parts per million downfield from TMS, and J values are in hertz. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet; ddd, doublet of a doublet of a doublet; and br, broad peak. ^{13}C NMR spectra were recorded on Jeol 300 (75 MHz) and BRUKER AVANCE II (400 MHz) spectrometers in deuterochloroform and dimethylsulfoxide using TMS as internal standard. High-resolution mass spectra were recorded on Bruker-micrOTOF-Q II spectrometer.

General procedure for the synthesis of 4-aminoquinoline-β-lactam conjugates (5a–5n)

To the stirred solution of **4** (1 mmol) in 10 mL dry DMF, K_2CO_3 (3 mmol) was added at room temperature. The reaction mixture was stirred for 15 min followed by the addition of mesylated product **3**. The reaction mixture was then stirred at 85 °C for 12 h, and progress of the reaction was monitored by TLC. On completion, water (15 mL) was added to the reaction mixture, and extraction was performed using ethyl acetate (2 × 50 mL). Combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure to result in a crude product, which was purified through column chromatography using chloroform/methanol (95:5) mixture.

General procedure for the synthesis of amide-tethered β-lactam-4-aminoquinoline hybrids (7a–7t)

To a solution of 3-amino-azetidin-2-one, **4** (1 mmol) in dry DMF, 4-aminoquinoline-based acids, **6** (1 mmol), Et_3N

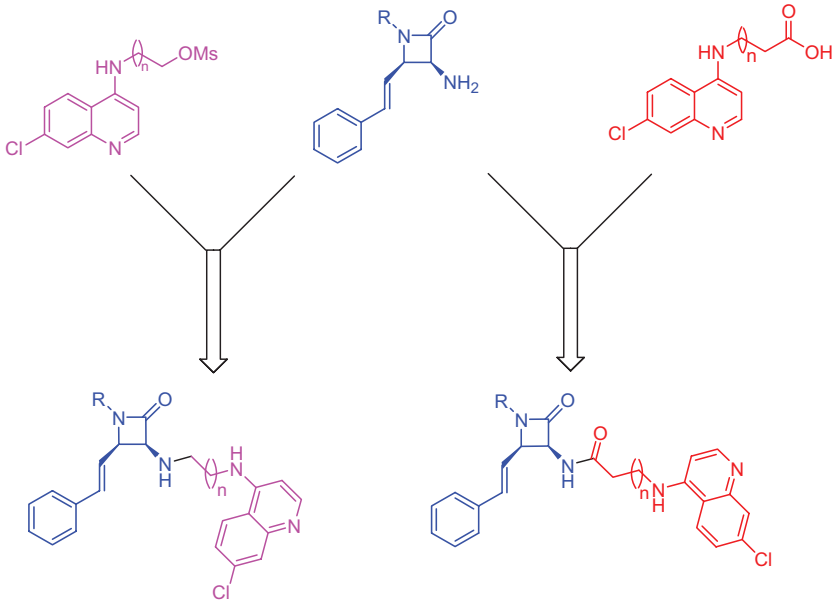


Figure 1: General structure of target 4-aminoquinoline-β-lactam hybrids.

(1.5 mmol), HOBt (1.2 mmol), and DCC (1.1 mmol) were added, and the reaction mixture was stirred overnight at room temperature. Aq. Na_2CO_3 was added into the reaction mixture, and pH of the aqueous layer was adjusted to 6.5–9.0 and extracted with ethyl acetate (EtOAC). The organic layer was separated and dried over Na_2SO_4. The solvent was evaporated affording crude product, which was purified by column chromatography on silica gel with eluent $CHCl_3$/MeOH (95:5) resulting in the desired product **7**.

Biological evaluations

Methods for the assessment of antimalarial activity of test compounds

The W2 strain of *P. falciparum* was cultured in RPMI-1640 medium with 10% human serum, following standard methods, and parasites were synchronized with 5% D-sorbitol (29). Beginning at the ring stage, microwell cultures were incubated with different concentrations of compounds for 48 h. The compounds were added from DMSO stocks; the maximum concentration of DMSO used was 0.1%. Controls without inhibitors included 0.1% DMSO. After 48 h, when control cultures had progressed to new rings, the culture medium was removed, and cultures were incubated for 48 h with 1% formaldehyde in PBS, pH 7.4, at room temperature. Fixed parasites were then transferred to 0.1% Triton X-100 in PBS containing 1 nM YOYO-1 dye (molecular probes). Parasitemia was determined from dot plots (forward scatter versus fluorescence) acquired on a FACSort flow cytometer using CellQuest software (Beckton Dickinson, San Jose, CA, USA). IC_{50} values for growth inhibition were determined from plots of percent control parasitemia over inhibitor concentration using the Prism 3.0 program (GraphPad Software, San Diego, CA, USA), with data from duplicate experiments fitted by nonlinear regression (30).

In vitro *antitubercular activity*

Bacterial strains and growth conditions

Mycobacterium tuberculosis $mc^2$7000, an unmarked version (31) of $mc^2$6030, was grown at 37°C in Sauton's medium supplemented with 20 μg/mL of pantothenic acid.

Drug susceptibility testing

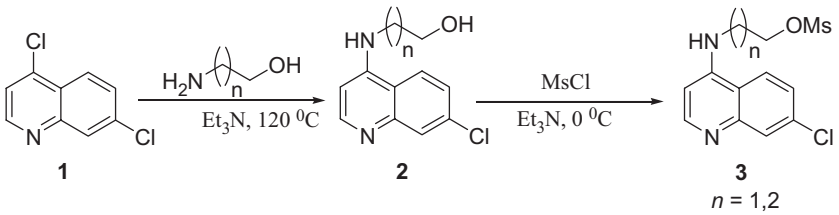
The susceptibility of *M. tuberculosis* to the various compounds was determined as reported previously (32). In brief, Middlebrook 7H10 solid medium containing oleic-albumin-dextrose-catalase enrichment (OADC) and 20 μg/mL of pantothenic acid was supplemented with increasing concentrations of the chemical analogues. Serial 10-fold dilutions of each actively growing culture were plated and incubated at 37 °C for 2–3 weeks. The MIC was defined as the minimum concentration required to inhibit 99% of the growth.

Result and Discussions

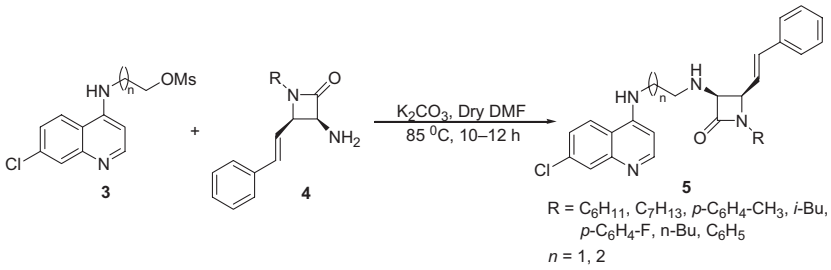
Chemistry

Methanesulfonic acid 2-(7-chloro-quinolin-4-ylamino)-ethyl/propyl esters **3,** required for the synthesis of the desired hybrids **5**, were prepared by initially reacting 4,7-dichloroquinoline **1** with ethanolamine or 1-propanol amine at 120 °C for 12 h, followed by mesylation at 0 °C in dry $CHCl_3$ for 2–3 h (Scheme 1). The target scaffolds **5** were synthesized by initial stirring of 3-amino-2-azetidinones **4**, prepared *via* Staudinger reaction of substituted 1-azadienes with azidoketene generated *in situ* from azidoacetic acid and *p*-toluene sulfonyl chloride in the presence of triethylamine followed by reduction using Zn/NH_4Cl protocol (33), with K_2CO_3 in dry DMF at room temperature for 15 min., followed by the addition of mesylated product **3** (Scheme 2). The reaction mixture was stirred at 85 °C for 12 h, and the progress of reactions was monitored using TLC. On completion, the solvent was evaporated under reduced pressure, and the crude product obtained was purified by column chromatography using a chloroform/methanol (95:5) mixture. The structures of synthesized hybrids were assigned on the basis of spectral data and analytical evidence, with the *cis*-stereochemical relationship between H^3 and H^4 confirmed on the basis of the coupling constant J = 5.4 Hz.

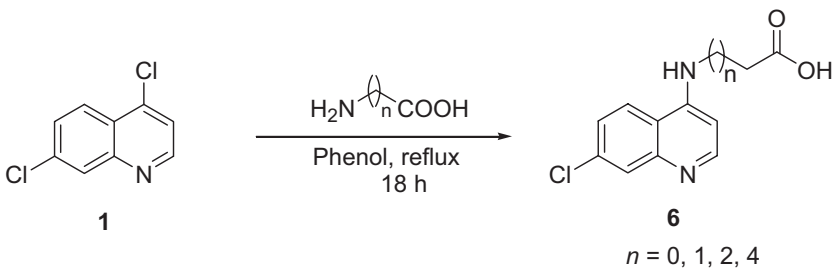
For the synthesis of conjugates **7**, the synthetic approach involved an initial preparation of 4-aminoquinoline amino acids **6** by refluxing of 4,7-dichloroquinoline **1** with corresponding amino acids in phenol for 18 h (Scheme 3), followed by acidic workup and extraction using ethyl acetate (34). Synthesis of desired hybrids **7** was carried out *via*



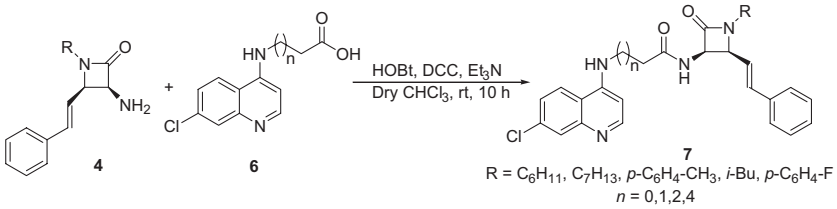
Scheme 1: Synthesis of 4-aminoquinoline-based mesylated products **3a**–**3b**.



Scheme 2: Synthesis of 4-aminoquinoline-β-lactam hybrids linked through non-ionizable covalent linker **5a**–**5n**.



Scheme 3: Synthesis of 4-aminoquinoline amino acids **6a**–**6d**.



Scheme 4: Synthesis of amide-tethered 4-aminoquinoline-β-lactam conjugates **7a**–**7t**.

DCC-mediated coupling of the precursors **4** and **6** in the presence of HOBt in dry DMF as shown in Scheme 4.

The structures of synthesized hybrids were assigned on the basis of spectral data and analytical evidence, the details of which have been provided in the Appendix S1, while the salient features are discussed here. The compound, for example, **7a** showed a molecular ion peak $[M + H]^+$ at 497.1660, along with the characteristic peaks in ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum exhibited the presence of a singlet at δ 2.21 corresponding to methyl protons along with a triplet at δ 3.96 corresponding to methylene protons and the characteristic quinoline ring protons. The presence of the required number of carbons in the ^{13}C NMR spectrum along with the two characteristic peaks at δ 160.0 and 169.6 corresponding to β-lactam ring carbonyl and amide carbonyl, respectively, further corroborated the assigned structure.

In vitro antimalarial activity and antitubercular evaluation

The synthesized hybrids were evaluated for their antimalarial profiles against the CQ-resistant W2 strain of *P. falciparum* (Table 1). Analysis of activity data in case of alkyl chain-tethered conjugates **5a**–**5n** revealed an interesting structure–activity relationship (SAR), with association between activity and the length of the alkyl chain as well as the substituent at *N*-1 of the β-lactam ring. Compounds having an ethyl linker as spacer showed better activity profiles than those with a propyl linker irrespective of the substituent on the *N*-1 of β-lactam ring, except in the case of **5k** and **5m**. In the case of conjugates with ethyl spacers (**5a**, **5c**, **5e**, **5g**, **5i**, **5k**, **5 m**), the test compounds with *N*-aryl substituents on the β-lactam ring (5**a**, **5c**, **5e)** showed better activities compared with counterparts with *N*-alkyl substituents. In the case of conjugates tethered *via* a propyl linker (**5b**, **5d**, **5f**, **5h**, **5j**, **5l**, **5n**), the scaffolds **5h** and **5j**, with *N*-cycloalkyl substituents (cyclohexyl and cycloheptyl), exhibited better activity compared with *N*-aryl substituted compounds. The compound **5c,** with an optimum combination of aryl (*p*-fluorophenyl) substituent at *N*-1 of the β-lactam ring and an ethyl linker, exhibited the best activity of the test compounds, with an IC_{50} of 59.6 nM.

Analyzing the antimalarial activity among conjugates **7a**–**7t** showed an overall loss of antimalarial profile with the

Table 1: Antimalarial and antitubercular activity results of test compounds

Code	*R*	*n*	*Plasmodium falciparum* W2 (CQ-R) strain IC_{50} (nM)	*Mycobacterium tuberculosis* $mc^2$7000 strain MIC (μg/mL)
5a	*p*-C6H4-CH3	1	94.3	6–7
5b	*p*-C6H4-CH3	2	164.1	5–6
5c	*p*-C6H4-F	1	59.6	8–10
5d	*p*-C6H4-F	2	204.2	10
5e	C6H5	1	135.3	7–8
5f	C6H5	2	188.1	ND
5g	C6H11	1	138.9	10
5h	C6H11	2	143.1	6–7
5i	C7H13	1	122.3	ND
5j	C7H13	2	176.0	5
5k	*n*-Bu	1	611.8	10–15
5l	*n*-Bu	2	397.7	ND
5m	*i*-Bu	1	424.6	ND
5n	*i*-Bu	2	410.3	ND
7a	*p*-C6H4-CH3	0	482.7	>100
7b	*p*-C6H4-CH3	1	112.5	5
7c	*p*-C6H4-CH3	2	79.5	>100
7d	*p*-C6H4-CH3	4	89.8	>100
7e	*p*-C6H4-F	0	400.9	80
7f	*p*-C6H4-F	1	148.2	6–7
7g	*p*-C6H4-F	2	142.1	8–10
7h	p-C6H4-F	4	187.4	10–15
7i	C6H11	0	570.3	10–20
7j	C6H11	1	432.6	7.5
7k	C6H11	2	556.7	10
7l	C6H11	4	186.7	7.5–10
7m	C7H13	0	1145	ND
7n	C7H13	1	378.1	5–7.5
7o	C7H13	2	275.8	5
7p	C7H13	4	152.0	7.5–10
7q	*i*-Bu	0	1027.5	20
7r	*i*-Bu	1	565.3	15–20
7s	*i*-Bu	2	675.6	10–15
7t	*i*-Bu	4	355.1	15
Chloroquine			99.0	
Cephalexin				25
Isoniazid (INH)				0.4
Rifampicin (RIF)				0.1
Ethionamide (ETH)				15

CQR, chloroquine-resistant; ND, not determined.

introduction of amide functionality except **7c** and **7d**, which have shown comparable activity with CQ. An interesting SAR was revealed that among conjugates **7a**–**7t** with the compounds (**7a**, **7e**, **7i**, **7m**, **7q**), having a methyl spacer showed poor activity irrespective of the substituent on the *N*-1 atom of the 2-azetidinone ring, while the replacement of the methyl linker with an ethyl linker improved the activity (**7b**, **7f**, **7j**, **7n**, **7r)**. The conjugates **7b** and **7f** with *N*-aryl substituted 2-azetidinones showed better activity than *N*-alkyl analogues, *viz*. **7j**, **7n**, and **7r**. Further, an increase in chain length *via* introduction of a propyl linker improved antiplasmodial activity, with effects more pronounced in the case of **7c** and **7g**, with an IC_{50} values of 79.5 and 142.1 nM, respectively. The introduction of an *n*-pentyl chain as a spacer substantially improved the antimalarial activity of all the conjugates, *viz*. **7d**, **7h**, **7l**, **7p**, and **7t** irrespective of the substituent at *N*-1 of the 2-azetidinone ring. The hybrid **7d** with a *p*-tolyl substituent at *N*-1 and an *n*-pentyl linker exhibited the best activity among the test compounds, with an IC_{50} of 89.8 nM. The antiplasmodial data of the precursor, *viz.* 3-amino-β-lactam, were recently published by our group, and the observed improvement in antimalarial efficacy of the synthesized conjugates ascertained the significance of hybridization strategy in the present context (35).

The synthesized scaffolds were also evaluated for their antitubercular activity (Table 1). The MIC values were calculated and compared with those for the standard drugs

isoniazid, rifampicin, ethionamide, and cephalexin. When analyzing the antitubercular profile among **5a**–**5n**, whereas the present compounds are not as active as isoniazid or rifampicin, most of the compounds exhibited better antitubercular activity than ethionamide (up to 3 times) and cephalexin (up to five times) (36). A closer inspection of the series revealed that antitubercular profiles were independent of the nature of substituents at the *N*-1 atom of the 2-azetidinone ring as well as the length of the alkyl chain. Similar comparison between amide-tethered conjugates **7a**–**7t** revealed the dependence of activity on the substituent at *N*-1 position with a preference for alkyl substituent as evidenced by compounds **7i**–**7t**. The *N*-aryl substituted conjugates proved inactive in inhibiting the growth of *M. tuberculosis* even at the highest tested concentration (**7a**, **7c**, and **7d**). Furthermore, the observed antitubercular efficacy in the amide series seems to be independent of the alkyl chain length as was observed in the conjugates **5a**–**5n**. The effects of cephalexin and the test compounds against *M. tuberculosis* $mc^2$7000 are illustrated in electronic supplementary information.

Conclusion

In conclusion, a series of 4-aminoquinoline-β-lactam conjugates were synthesized and evaluated for their antimalarial and antitubercular profiles. On comparing the activities among alkyl chain and amide-tethered series, the alkyl chain-linked conjugates showed better efficacy in inhibiting the growth of *P. falciparum* and *M. tuberculosis,* while the introduction of an amide functionality adversely affected the activity profiles. Most of the synthesized conjugates showed poor antiplasmodial profile except **5a**, **7c,** and **7d**, while their anti-TB efficacy was better than ethionamide and cephalexin. Further work on the extension of above protocol *via* introduction of urea, thiourea, and oxalamide functionalities is currently underway in the laboratory and will soon be communicated.

Acknowledgment

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Conflict of Interest

The authors declare no competing financial interest.

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Note

[a]World Health Organization, World Malaria Report (2011) http://www.who.int/malaria/world_malaria_report_2011/en/.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The characterization of compounds **5a**–**5n** and **7a**–**7t**, scanned ^{1}H and ^{13}C spectra of **5a**, **5b**, **5e**, **7a**, **7i**, **7p** along with the figures of Petri dishes depicting the inhibition of mc^{2}7000 strain of *M. tuberculosis* by cephalexin and test compounds.