Synthesis of 2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes via LiAlH4-induced reductive cyclization of 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines and evaluation of their antimalarial activity

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ABSTRACT

2-(4-Chloro-2-cyano-2-phenylbutyl)aziridines were employed for the one-step stereoselective construction of both *endo*- and *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes as new azaheterobicyclic scaffolds *via* a double LiAlH4-induced reductive cyclization protocol. Antiplasmodial assessment of these 1-azabicyclo[2.2.1]heptanes revealed moderate to good activities in the micromolar range, with the *exo*-isomers being the most promising structures. Furthermore, the proposed mode of action was supported by ligand docking studies, pointing to a strong binding interaction with the enzyme plasmepsin II.

***Keywords***: aziridines, 1-azabicyclo[2.2.1]heptanes, ring transformation, antimalarial agents, plasmepsin II, ligand docking

An important challenge in modern health care relates to the control of malaria, as this disease poses a major threat to African children and pregnant women with 216 million clinical cases and roughly 655.000 deaths worldwide in 2010.[[2]](#endnote-1) Encouraged by the increasing resistance of malaria parasites against current drugs such as chloroquine, antimalarial research is more and more focused on new drugable parasite targets.[[3]](#endnote-2) In that respect, a family of aspartic proteinases (plasmepsins) has been identified in the digesting vacuole of *Plasmodium falciparum* several years ago,[[4]](#endnote-3) and further studies in that direction revealed the occurrence of three aspartic proteinases (PMI, PMII, and PMIV) and the related histo-aspartic proteinase HAP (PMIII).[[5]](#endnote-4)

Although antimalarial drug design is still dominated by the search for new quinoline derivatives,[[6]](#endnote-5) functionalized piperidines have emerged as interesting alternatives due to their potentially different mode of actions as compared to quinoline systems. Whereas quinolines are known to interact with the toxic heme, thus preventing its crystallization into hemozoin, a number of (bicyclic) piperidines has been shown to inhibit the above-mentioned plasmepsin enzymes,[[7]](#endnote-6) which are involved in the early steps of hemoglobin degradation. The piperidine ring indeed comprises a key structural motif in a broad variety of natural products and biologically active agents and, as a consequence, many efforts are devoted to the synthesis of new piperidine derivatives as potential lead compounds in drug discovery programs.[[8]](#endnote-7)

In the present study, a new synthetic approach for the construction of the 1-azabicyclo[2.2.1]heptane scaffold was developed based on a one-step double LiAlH4-induced reductive cyclization protocol of 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines. Both *endo*- and *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes were thus prepared and evaluated in terms of their antiplasmodial activity. Furthermore, ligand docking was performed to support the proposed interaction of the test compounds with the enzyme plasmepsin II.

Aziridines are generally acknowledged as versatile building blocks in organic synthesis.[[9]](#endnote-8) Although 2-(2-cyanoethyl)aziridines have recently been used by us as synthons for the development of straightforward strategies toward a variety of piperidines[[10]](#endnote-9) and cyclopropanes,[[11]](#endnote-10) their chemistry still remains a scarcely investigated field of research in the literature.[[12]](#endnote-11) A challenge to be addressed concerns the deployment of the cyano group in 2-(2-cyanoethyl)aziridines as a nucleophile upon double hydride reduction which, through consecutive selective attack at either the aziridine moiety or at an additional electrophilic carbon atom in the side chain, might provide a convenient access to a new and biologically relevant azaheterobicyclic skeleton. To that end, the required 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines **1** andtheir diastereomeric counterparts **4** were prepared by treatment of the corresponding 2-(bromomethyl)aziridines[[13]](#endnote-12) with α-lithiated phenylacetonitrile in THF, followed by a lithium diisopropylamide-mediated coupling with 1-bromo-2-chloroethane.10a It should be noted that the correct relative stereochemistry of racemic aziridines **1** and **4** had previously been assigned via initial transformation of these aziridines into the corresponding 2-(chloromethyl)piperidines, followed by X-ray diffraction analysis of the latter six-membered azaheterocycles.10a

In continuation of our interest in the construction of novel azaheterocyclic systems, 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines(2*S*\*,2’*S*\*)-**1** were thus treated with two molar equivalents of LiAlH4 in THF for two hours under reflux, affording *endo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** in excellent yields (90-95%) as single reaction products (Scheme 1). Furthermore, aziridines (2*S*\*,2’*R*\*)-**4**, the diastereomeric counterparts of aziridines **1**, were subjected to the same reaction conditions (LiAlH4, THF, reflux, 2h),furnishing *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **18** in 80-83% yield (Scheme 1). These observations point to the conclusion that this LiAlH4-mediated rearrangement of 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines **1** and **4** allows the straightforward and elegant construction of stereodefined 1-azabicyclo[2.2.1]heptanes as valuable scaffolds in organic and medicinal chemistry.

**Scheme 1. LiAlH4-mediated transformation of aziridines 1 and 4 into 1-azabicyclo[2.2.1]heptanes 2 and 5.**



The reaction mechanism of this new approach to the 1-azabicyclo[2.2.1]heptane skeleton involves an initial addition of hydride across the cyanide moiety, followed by an iminyl anion-induced 5-*exo-tet* ring closure towards 1-pyrrolines **7** (Scheme 2).[[14]](#endnote-13) Finally, the pyrrolidine nitrogen regiospecifically opens the aziridine ring in a SN2 fashion at the more substituted carbon atom in a second cyclization reaction (5-*exo-tet*) after initial reduction of the cyclic imine **7** toward pyrrolidine **8**, resulting in the envisaged 2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** in excellent yields as single reaction products (Scheme 2). During the second reductive cyclization step, LiAlH4 acts as a Lewis acid to activate the aziridine ring which then becomes susceptible to ring-opening reactions.[[15]](#endnote-14) According to Baldwin’s rules, the 5-*exo-tet* ring closure is favored and the 6-*endo*-*tet* is disfavored, which can explain the regiospecificity of the final aziridine ring opening.

**Scheme 2. Proposed reaction mechanism for the formation of 1-azabicyclo[2.2.1]heptanes 2.**



The assignment of the correct structure of 2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** and **5** was supported by detailed NMR analysis. For example, the appearance of a coupling in the HMBC spectrum between the benzylic hydrogen atoms and the exocyclic NHCH2 carbon atom, and between the hydrogen atoms of the exocyclic NHCH2 and the NCH carbon is in line with the proposed structure. Furthermore, bicyclic piperidine **2b** was dissolved in dry ether, and gaseous HCl was bubbled through the solution affording a white crystalline bicyclic piperidinehydrochloride salt **3** after recrystallisation from ethanol (Scheme 1). The structure of 2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** was then unambiguously assigned through X-ray diffraction analysis of hydrochloride **3** (see Supporting Information; CCDC deposition number 849691).

To summarize, the net conversion of this methodology concerns a novel synthetic transformation of 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines **1** and **4** into stereodefined *endo*- and *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** and **5** *via* a double reductive cyclization protocol. 1,2-Diamines such as bicyclic piperidines **2** and **5** can be seen as valuable substrates for the synthesis of new cisplatin [*cis*-diaminodichloroplatinum(II)] derivatives known for their anticancer activities.[[16]](#endnote-15) Moreover, very little information regarding these conformationally constrained piperidine systems bearing an aromatic ring at the bridgehead position is available in the literature, pointing to the novelty of this approach. In addition to a few examples of 4-heteroaryl-1-azabicyclo[2.2.1]heptanes,[[17]](#endnote-16),[[18]](#endnote-17) only a few other examples have been reported concerning 1-azabicyclo[2.2.1]heptanes substituted with a phenyl group at the bridgehead position, obtained in low to moderate yields and as isomeric mixtures through either intramolecular addition of *N*,*N*-disubstituted hydroxylamines to unactivated olefins or *via* gold-catalyzed tandem cyclization reactions of 1,6-diynes.[[19]](#endnote-18)

In order to explore the potential of these novel azabicyclic systems as antimalarial agents, compounds **2** and **5** were screened for their *in vitro* antiplasmodial activity. All samples were tested against a chloroquine-sensitive strain of *P. falciparum* (D10). Subsequently, those samples showing promising antiplasmodial activity were tested against a chloroquine-resistant strain of *P. falciparum* (Dd2) and screened for *in vitro* cytotoxicity against a mammalian cell-line, Chinese Hamster Ovarian (CHO) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay. Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen,[[20]](#endnote-19) and quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.[[21]](#endnote-20) The samples were tested in triplicate on one occasion.[[22]](#endnote-21) The MTT-assay was used as a colorimetric assay for cellular growth and survival, and compares well with other available assays.[[23]](#endnote-22) The tetrazolium salt MTT was used to measure all growth and chemosensitivity. The samples were tested in triplicate on one occasion.[[24]](#endnote-23)

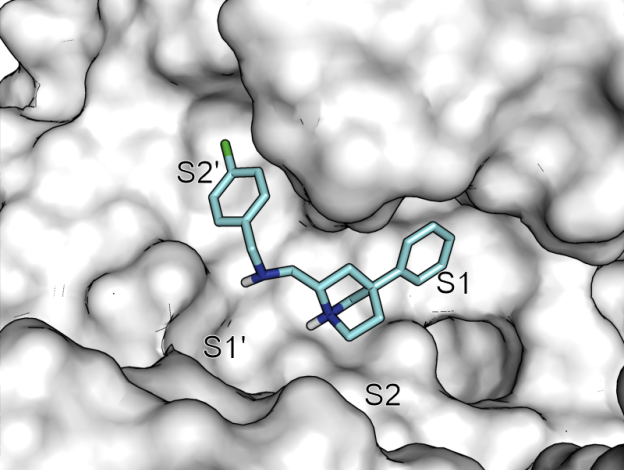
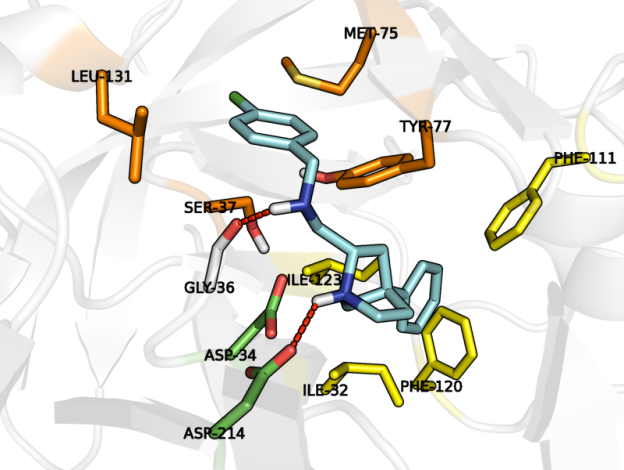
**Table 1. IC50-values of piperidines 2 and 5 tested for *in vitro* antimalarial activity and cytotoxicity**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **compound** | **R** | **D10 IC50 (µM)** | **Dd2 IC50 (µM)** | **CHO IC50 (µM)** | **RIa** | **SIb** |
| **2a** | H | 18.45 | 78.49 | >300 | 4.4 | ND |
| **2b** | 4-Me | 10.34 | 15.88 | 162.83 | 1.5 | 15.7 |
| **2c** | 4-Cl | 6.01 | 11.75 | 90.38 | 2.0 | 15.1 |
| **5a** | H | 2.63 | 24.21 | 230.79 | 9.6 | 91.2 |
| **5b** | 4-Me | 0.81 | 10.00 | 160.44 | 12.5 | 200.0 |
| **5c** | 4-Cl | 1.39 | 2.40 | 71.21 | 1.7 | 51.1 |
| **Chloroquine** |  | 0.03 | 0.36 |  | 7 |  |
| **Emetine** |  |  |  | 0.33 |  |  |

ND = not determined; a RI (Resistance Index) = IC50 (Dd2)/IC50 (D10); b SI (Selectivity Index) = IC50 (CHO)/IC50 (D10)

The results of the biological evaluation, expressed as D10 IC50, Dd2 IC50, CHO IC50, RI and SI, are summarized in Table 1. From these data, it is clear that *endo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **2** display less pronounced bioactivities as compared to their *exo*-counterparts **5**. e*xo*-2-Aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptane **5b** appeared to be the most active compound against the chloroquine-sensitive strain of *P. falciparum* D10 with an IC50-value of 810 nM, and showed no cytoxicity (SI = 200). However, this compound was found to be considerably less active against the chloroquine-resistant strain of *P. falciparum* Dd2, with an IC50-value of 10 µM. Also compound **5a** showed to be several times less active against strain Dd2 as compared to strain D10. On the other hand, the chloro-substituted e*xo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptane **5c** was considered to be a promising compound, as it exhibited a good activity against both the chloroquine-sensitive and the chloroquine-resistant strain with IC50-values of 1.39 and 2.40 µM, respectively, and a good selectivity index of 51.1.

In the final part of this study, a possible interaction of *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes **5** with the enzyme plasmepsin II was investigated by means of ligand docking. The most interesting compound **5c** was selected to perform this analysis. Because of the acidic environment within the food vacuole, resulting in the *in situ* formation of ammonium salts, the *N*-protonated version of molecule **5c** was used to represent the actual situation. This structure showed the most optimal fit in the active site with its phenyl and 4-chlorophenyl groups in subsite S1 and S2’, respectively (Figure 1). In that orientation, good overlap can also be observed with the binding of the known inhibitor rs370, as determined by crystallographic analysis.[[25]](#endnote-24) The piperidine nitrogen atom of compound **5c** is pointing toward the catalytic residues Asp34 and Asp214, enabling strong hydrogen bonds with these residues. In addition, a hydrogen bond is observed between the backbone oxygen atom of Gly36 and the secondary amine nitrogen atom of the ligand. The highly hydrophobic subsite S1 comprises the side chains of Ile32, Tyr77, Phe111, Phe120 and Ile123, thus providing an ideal environment to accommodate the phenyl ring of compound **5c**. In turn, the 4-chlorophenyl group is positioned in subsite S2’, where it interacts with the side chains of Ser37, Met75, Tyr77 and Leu131.



(b)

(a)

**Figure 1. Docking of compound 5c (in blue) in the active site of plasmepsin II: (a) stick representation of the most important residues, and (b) surface representation with designation of subsites.**

The strong binding capacity of e*xo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptane **5c** with respect to plasmepsin II corroborates the hypothesis that this new class of compounds could (at least partially) act as novel plasmepsin II inhibitors, although the observed antimalarial activity might be due to other mode of actions as well. The findings described in this Letter thus provide a platform for more elaborate studies based on rational design and synthesis of more potent representatives of this new class of azaheterobicyclic systems, which, in combination with further optimization of drug-relevant molecular properties, should result in promising new lead structures.

In conclusion, a straightforward and highly efficient synthetic route toward *endo*- and *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes is presented, involving an unprecedented one-step LiAlH4-mediated transformation of 2-(4-chloro-2-cyano-2-phenylbutyl)aziridines. The good *in vitro* activities of *exo*-2-aminomethyl-4-phenyl-1-azabicyclo[2.2.1]heptanes 5 and their *in silico* binding affinity with respect to plasmepsin II provide interesting opportunities for the design of new antimalarial agents in the fight against the emerging resistance.

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22. The test samples were prepared to a 20 mg/mL stock solution in 100% DMSO. Stock solutions were stored at -20 °C. Further dilutions were prepared in complete medium on the day of the experiment. Chloroquine (CQ) was used as the reference. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC50-value). Test samples were tested at a starting concentration of 100 µg/mL, which was then serially diluted 2-fold in complete medium to give 10 concentrations, the lowest concentration being 0.2 µg/mL. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/mL against a CQS strain. The highest concentration of solvent to which the parasites were exposed to, had no measurable effect on the parasite viability. [↑](#endnote-ref-21)
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24. The test samples were prepared to a 2 mg/mL stock solution in 10% DMSO and were tested as a suspension. Test compounds were stored at -20 °C until use. Dilutions were prepared on the day of the experiment. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 µg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/mL. The same dilution technique was applied to all test samples. The highest concentration of solvent to which the cells were exposed to, had no measurable effect on the cell viability. The 50% inhibitory concentration (IC50) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software. [↑](#endnote-ref-23)
25. Asojo, O. A.; Afonina, E.; Gulnik, S. V.; Yu, B.; Erickson, J. W.; Randad, R.; Medjahed, D.; Silva, A. M.; *Acta Crystallogr. D* **2002**, *58*, 2001. [↑](#endnote-ref-24)