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Synthesis of 3-azabicyclo[3.2.2]nonanes and their antiprotozoal activities

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ABSTRACT

Several bicyclic compounds, 3-azabicyclo[3.2.2]nonanes, have been prepared. The new compounds were tested for their activities against one strain of the causative organism of Malaria tropica, *Plasmodium falciparum* K_1, which is resistant against chloroquine and pyrimethamine. In addition, their cytotoxicity and their activity against the pathogen of the East African form of sleeping sickness, *Trypanosoma brucei rhodesiense*, were investigated. Structure–activity relationships are discussed considering data of readily prepared compounds. For the first time, a distinct in vivo activity was observed against *Plasmodium berghei* in a mouse model. The active compound was further investigated.

The past decade has seen an unprecedented surge in political commitment and international funding for malaria control. Coverage with existing control methods (i.e., vector control and artemisinin-based combination therapy) is increasing, and, in some Asian and African countries, childhood morbidity and mortality from Malaria tropica caused by *Plasmodium falciparum* are starting to decline. But the ability of the parasite to develop resistance to antimalarial drugs and increasing insecticide resistance of the vector threaten to reduce and even reverse current gains. New drugs and insecticides are needed urgently.[1]

Human African trypanosomiasis (sleeping sickness) occurs in sub-Saharan Africa. It is caused by the protozoan parasite *Trypanosoma brucei*, transmitted by tsetse flies. *T. b. rhodesiense* is the causative organism of the East African form of the disease. Its late stage can only be treated with melarsoprol which causes a deadly encephalopathy in more than 5% of the patients.[2] With fewer than 12,000 cases of this disabling and fatal disease reported per year, trypanosomiasis belongs to the most neglected tropical diseases. The clinical presentation is complex, and diagnosis and treatment are difficult. The available drugs are old, complicated to administer, and can cause severe adverse reactions. New diagnostic methods and safe and effective drugs are urgently needed.[3]

We reported the formation of 4-dialkylamino-6,7-diphenylbicyclo[2.2.2]octan-2-ones **1** by a one-pot reaction of cheap acyclic starting material.[4] The ketones **1** and their corresponding alcohols were screened against some causative organisms of tropical diseases like *Leishmania donovani*, *Trypanosoma cruzi*, *T. b. rhodesiense* and *P. falciparum*. Some of them showed moderate activity against the multiresistant K_1-strain of *P. falciparum* and *T. b. rhodesiense*.[5] In the meantime, we prepared a series of derivatives of compounds **1** and screened them for their antitrypanosomal and antiplasmodial activities.[6] An important transformation step was the ring enlargement of ketones **1** to lactames **3** via a Beckman procedure. The reduction products **5** of the latter compounds showed the most promising antiplasmodial activity. For example, 5-dimethylamino-7,8-diphenyl-2-azabicyclo[3.2.2]nonane **5a** exhibits 35% inhibitory activity in an in vivo assay against *Plasmodium berghei* in mice, but no prolongation of the mean survival time compared with uninfected mice was observed.[7] Their bischloro substituted analogues

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6 showed in vitro the highest antitrypanosomal potency (IC_{50} = 0.061–0.066 μM) of all so far prepared compounds in this series however, they had no in vivo efficacy.[8]

The driving force of the present study was the question if the distance of the two nitrogen in the ring skeleton influences the biological activities or not. Compounds with skeleton **A** like the 2-azabicyclo[3.2.2]nonanes have 4 bonds between the nitrogen whereas compounds with skeleton **B** like 3-azabicyclo [3.2.2]nonanes possess only 3 bonds inbetween (Fig. 1).

The present Letter describes therefore the synthesis of several new 1-dialkylamino-6,9-diphenyl-3-azabicyclo[3.2.2]nonanes and their investigation regarding their antiplasmodial and antitrypanosomal activity as well as their cytotoxicity.

The 3-azabicyclo[3.2.2]nonane skeleton is well reported[9,10] and the 3-azabicyclo[3.2.2]non-3-yl residue is the amino component of many compounds with pharmaceutical relevance like antitussives,[11] antiarrhythmics,[12] antimalarials,[13] antivirales,[14] and antibacterials.[15] Furthermore, 3-azabicyclo[3.2.2]nonane derivatives are used as herbicides[16] and as a versatile template for the synthesis of porous silicates.[17] However, most of the compounds contain an unsubstituted 3-azabicyclo[3.2.2]nonane skeleton or the skeleton is part of a condensed ring system. Diaryl- and 1-amino derivatives of 3-azabicyclo[3.2.2]nonanes have not yet been described.

Starting materials for both, 2-azabicyclo[3.2.2]nonanes and 3-azabicyclo[3.2.2]nonanes were 4-dialkylamino-6,7-diarylbi-cyclo[2.2.2]octane-2-ones **1a–d** which were formed from benzylidene acetone and hydrothiocyanates of secondary amines in refluxing toluene.[4] Their reaction with hydroxylamine-*O*-sulphonic acid yielded selectively 5-dialkylamino-7,8-diaryl-2-azabicyclo[3.2.2]nonan-3-ones **3a–d**.[7] When the hydrothiocyanates of ketones **1a–d** reacted with NaN_3 in concentrated sulphuric acid, 5-dialkylamino-7,8-diaryl-3-azabicyclo[3.2.2]nonan-2-ones **7a–e** were obtained with high selectivity (Scheme 1). Only traces of compounds **3** were detected in the reaction mixture. The structure of compounds **7a–e** was proofed by several NMR measurements. The newly formed lactame group was detected at 176 ppm in the carbon spectra of compounds **7**. Moreover, a 2.8 Hz coupling between N–H and H-4 in the ^{1}H NMR spectrum of compounds **7** revealed ring position 3 for the nitrogen atom. In their H,H-correlated spectroscopy (COSY) spectra a small ^4J coupling of 2 Hz from N–H to H-1 was detected.

The two identic chains were discriminated as follows: the ortho protons of the phenyl ring in position 7 showed through-space couplings (3%) to H-7 and H-8. In heteronuclear multiple bond correlation (HMBC) spectra crosspeaks were observed from these ortho protons to the corresponding ipso carbon as well as to C-7 (Fig. 2).

Finally the structure of compounds **7** was shown by a single crystal structure analysis of compound **7b**.[18]

The 2-azanonanes **5a–d** have been obtained from **3a–d** using lithium aluminium hydride ($LiAlH_4$) as reductive agent in boiling diethylether.[7] In a similar way 3-azanonanes **9a–e** were prepared

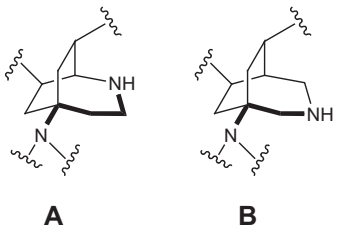
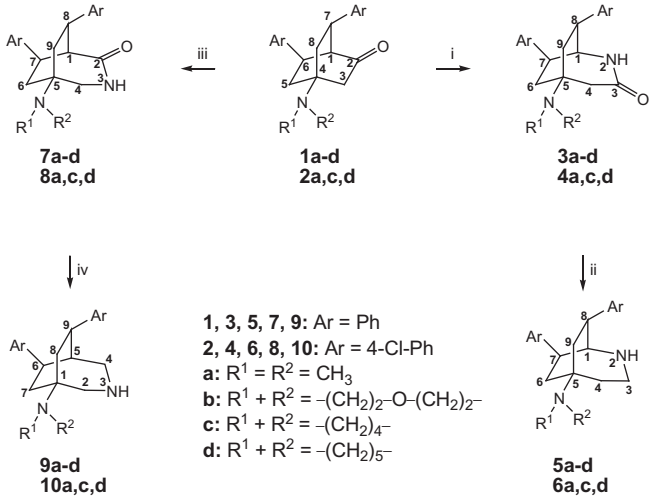


Figure 1. Bicyclic skeletons **A** and **B** with differing distance between the two nitrogens.



Scheme 1. Synthesis of compounds **1**–**10**. Reagents and conditions: (i) hydroxylamine-*O*-sulphonic acid, glacial acetic acid, reflux, 145 °C, 18 h; (ii) lithium aluminium hydride, ether, reflux, 55 °C, 48 h; (iii) NaN_3, H_2SO_4, 10 min; (iv) lithium aluminium hydride, ether, reflux, 55 °C, 18 h.

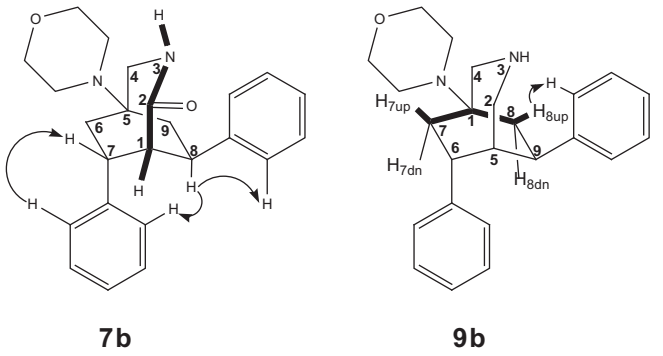


Figure 2. Compounds **7b** and **9b**: through-space couplings (nuclear overhauser effects (NOE's), arrows) and w-couplings (bold).

from **7a–e**. Again, the two identic chains were determined using NMR spectroscopy: The resonance of H-8_{up} of **9b** was detected by a through-space coupling (6%) to an aromatic ortho proton and a w-coupling of 2.2 Hz to H-7_{up} (Fig. 2). The aryl substituted analogues **10** were prepared via 3-azabicyclo-nonan-2-ones **8** as described above. Compounds **6** were prepared in a similar way from 2-azabicyclo-nonan-3-ones **4**.

The new bicyclic diamines were tested in vitro against bloodstream form trypomastigotes of *T. b. rhodesiense* (STIB900) and *P. falciparum* K_1, which is resistant to chloroquine and pyrimethamine, using [3*H*]-hypoxanthine incorporation assay[19] and alamar blue-based assay,[20] respectively. The cytotoxicity was assessed against rat myoblast cells (L-6). All results are presented in Table 1. Since the lactames **3a–d** and **7b** showed in general low antiprotozoal activities compared to those of bicyclic diamines, we did not test further lactames. All compounds were tested as hydrochlorides.

The in vitro antitrypanosomal activities of 2-azanonanes **5a–d** (median inhibitory concentration, IC_{50} = 0.60–9.44 μM)[7] and of 3-azanonanes **9a–d** (IC_{50} = 1.00–3.84 μM) against *T. b. rhodesiense* are only moderate. The activity is influenced by aromatic substitution. The *p*-chloro analogues **6** and **10** show at least fourfold and in case of **6d** 100-fold antitrypanosomal activity than their unsubstituted parent compounds. The most potent *p*-chloro substituted compounds **6a,c,d** ($IC_{50} \approx 0.06$ μM) were more toxic than **5a,c,d**