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Design, synthesis and biotest of a bicyclo[3.3.1]nonane analogue of 2-amino-5,6-dihydro-4*H*-1,3-thiazine

Olga N. Zefirova,*[a,b] Evgeniya V. Nurieva,[a] Vladimir I. Chupakhin,[b]
Irina S. Semenova,[a] Danil I. Peregud,[c] Mikhail V. Onufriev[d] and Natalia V. Gulyaeva[d]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 0290; olgaz@org.chem.msu.ru*
[b] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. E-mail: chupvl@gmail.com*
[c] *National Research Center on Addictions, 119002 Moscow, Russian Federation. E-mail: peregud_d@yahoo.com*
[d] *Institute for Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, 117485 Moscow, Russian Federation. E-mail: nata_gul@pisem.net*

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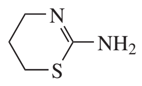
To enhance the lipophilicity of the NO-synthase inhibitor 2-amino-5,6-dihydro-4*H*-1,3-thiazine, its bridgehead analogue was suggested on the basis of molecular modeling; the synthetic routes to new 3-thia-1-azabicyclo[3.3.1]nonane derivatives and the results of their biological testing are presented.

Nitric oxide synthase (NOS) inhibitors,[1] *e.g.* 2-amino-5,6-dihydro-4*H*-1,3-thiazine **1** and its derivatives, possess radioprotective ability.[2] To increase the bioavailability of compound **1**, we suggested to synthesize its more lipophilic analogue by 'insertion' of the parent structure into a bicyclo[3.3.1]nonane core (compound **2**).



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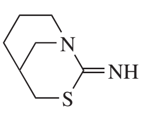

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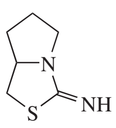

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The molecular docking of compound **2** into the active site of inducible NOS isoform[†] revealed that the bicycle is located in the binding site without steric hindrances. The exocyclic group, being protonated at pH 7.4, forms two important hydrogen bonds with the carboxylic group of Glu377 and the carbonyl group of the main chain of Trp372. Moreover, an additional lipophilic ring of the bicycle is properly positioned in the lipophilic pocket formed by Phe309 and Val352 [Figure 1(*a*)]. The docking of the analogues of compound **2** with bigger cycles shows that their location in the binding site is less beneficial energetically due to the steric hindrances.

Comparison of the positions of compound **2** and its earlier synthesized and almost inactive fused structural analogue **3**[6] in the *i*NOS active site indicates that the lipophilic part of flattened bicycle **3** cannot interact with Phe309 and Val352 if hydrogen bonds to Glu377 and Trp372 are formed [Figure 1(*b*)]. Thus, we expected that beneficial lipophilic interactions of structure **2** would impart its affinity to the enzyme active site and tolerate the loss of one hydrogen bond compared to thiazine **1**.

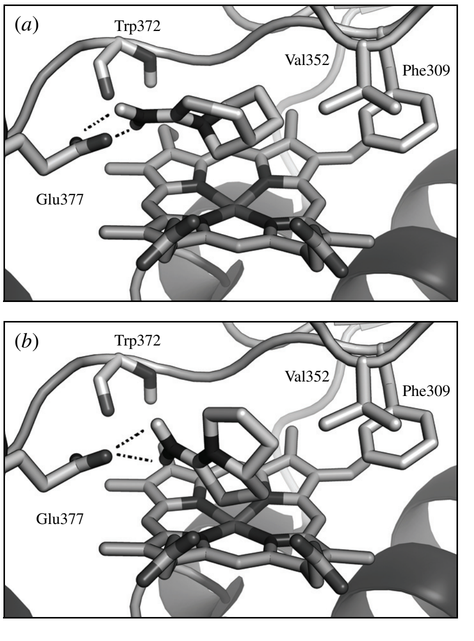


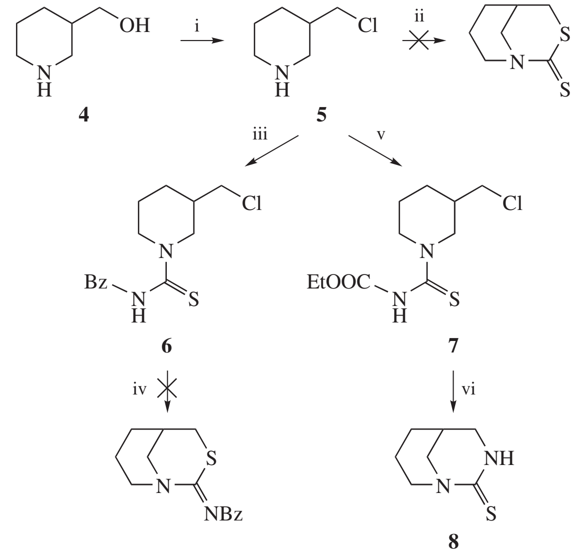
Figure 1 Docking of structures (*a*) **2** and (*b*) **3** into the *i*NOS active site (hydrogen bonds are indicated by dashed lines; some hydrogen atoms are not indicated for clarity).

Bridged dihydrothiazine bicycles have not been reported yet, and the synthesis of compound **2** met some difficulties. Thus, an attempt to access it by an approach used for compound **3**, *i.e.*, by the reaction of 3-(chloromethyl)piperidine hydrochloride **5** with carbon disulfide[6] failed under various reaction conditions and did not afford the corresponding bicyclic thione (Scheme 1).[‡]

The interaction of compound **5** with benzoyl isothiocyanate[8,9] gave benzamide derivative **6**, but the heating of the latter under basic conditions did not lead to a bicyclic product. An analogous reaction with ethoxycarbonyl-substituted thiourea **7** (obtained by heating of **5** with ethoxycarbonyl isothiocyanate)

[†] There are three NOS isoforms – two constitutive versions (*n*NOS and *e*NOS) and an inducible one, which is synthesized as a response to irradiation. Docking in the three-dimensional structure of *i*NOS active site (PDB ID: 2NSI) was carried out with the help of Dock v. 6.3 program[3] with regard to the ligands flexibility (3D coordinates were calculated with Molconverter Marvin 5.3.0[4]). Protonated states of the ligands at pH 7.4 were used (charge scheme MMFF94). Ligands–enzyme complexes with the best values of the Grid score scoring function calculated by Dock v. 6.3 were chosen and visualized using PyMOL v. 0.99.[5]

[‡] For the synthetic details and characteristics of all the compounds, see Online Supplementary Materials.

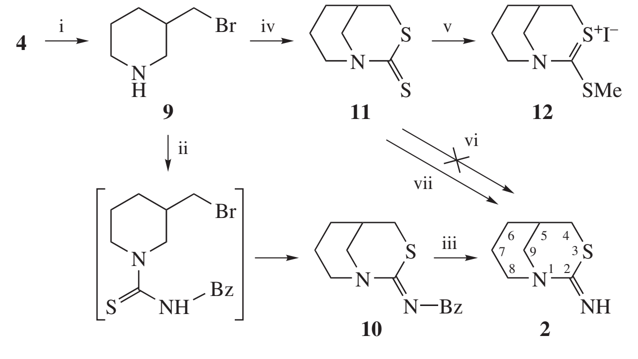


Scheme 1 *Reagents and conditions*: i, $SOCl_2$, HCl_{gas}, $CHCl_3$, 82%; ii, (1) CS_2, Et_3N, room temperature, 24 h,[6] (2) CS_2, Et_3N, 105 °C, sealed ampule, 24 h, (3) CS_2, DMF, K_2CO_3, room temperature;[7] iii, Bz–NCS, Et_3N, acetone, reflux, 6 h, 52%; iv, $NaHCO_3$, EtOH, H_2O, reflux, 12 h; v, EtO_2C–NCS, Et_3N, EtOAc, reflux, 2 h, 25%; vi, $NaHCO_3$, EtOH, H_2O, reflux, 12h, 23%.

afforded a product with a molecular mass of 156, corresponding to the structure **2**. However, ^{13}C NMR spectral data of the synthesized compound revealed low-field chemical shifts of C^2 (195 ppm) and C^4 (49 ppm) carbon atoms that proved the formation of 1,3-diazabicyclo[3.3.1]nonane-2-thione **8**, a product of N- rather than S-cyclization.

Similar procedures were carried out for 3-(bromomethyl)-piperidine **9**, synthesized as hydrobromide from alcohol **4** and phosphorus tribromide.[7] In the reaction of compound **9** with benzoylisothiocyanate, the intermediate thiourea underwent cyclization to give desired bicyclic product **10** in a low yield (Scheme 2). The composition of **10** ($C_{14}H_{16}N_2SO$) was confirmed by elemental analysis. Its spectral characteristics proved the formation of a bicyclic structure being different from that of noncyclic product **6**, particularly in specific chemical shifts of signals of carbonyl and imino carbons at 175 and 176 ppm in ^{13}C NMR spectrum (chemical shifts of the corresponding atoms in compound **6** are 163 and 179 ppm) and the absence of an NH proton signal from the ^1H NMR spectrum (8.6 ppm for compound **6**).

The treatment of compound **10** by concentrated hydrochloric acid followed by neutralization led to crude product **2** with a touch of by-products (hard to separate by column chromatography).



Scheme 2 *Reagents and conditions*: i, HBr, C_6H_6, then PBr_3, 100 °C, 10 h, 65%; ii, Bz–NCS, Et_3N, acetone, 20% for **9**; iii, conc. HCl, heating, then $NaHCO_3$, H_2O, 23%; iv, CS_2, DMF, K_2CO_3, 36%; v, MeI, CH_2Cl_2, then NH_3, MeOH, 90%; vi, NH_3, THF, $HgCl_2$, 50 °C;[10] vii, $SOCl_2$, CH_2Cl_2, then NH_3, MeOH, 38%.

Parallel cyclization of compound **9** with carbon disulfide proceeded well and led to thione **11** (Scheme 2). The low field shifts of signals of carbon atoms adjacent to nitrogen (56 and 58 ppm) in the ^{13}C NMR spectrum of **11**, as compared to the signals of corresponding atoms in the spectrum of starting bromide **9** (43 and 47 ppm), gave evidence for bicycle structure formation. The signal at 208 ppm in ^{13}C NMR spectrum of compound **11** corresponds to alicyclic thiocarbonyl carbon.

The following modification of the thiocarbonyl group in **11** to the imino group was not completed neither by reaction with gaseous ammonia in the presence of mercury dichloride,[10] nor by the consequent treatment with methyl iodide and ammonia methanol solution.[6] In the latter case, the formed intermediate was resistant to the action of ammonia. The only isolated product according to mass-spectrometric data was an iodine salt, its structure being determined as **12** on the basis of the ^1H NMR spectrum (singlet of methyl protons at 2.36 ppm and two broad signals at 4.49 and 5.31 assigned to methylene H^4 protons).

Product **2** was obtained by the reaction of thione **11** with thionyl chloride and the following treatment with an ammonia solution in methanol.[11] The molecular formula of the target compound is $C_7H_{12}N_2S$, as determined by elemental analysis and mass spectrometry data. A proton signal at 4.7 ppm (NH) in ^1H NMR and carbon atom signal at 158 ppm (C=NH) in ^{13}C NMR spectra confirmed the structure of compound **2**. The synthesis of bicyclic thiazine **2** *via* thione **11** (in 14% yield from **9**) is more convenient operationally than the above way *via* benzoyl derivative **10**, and it allowed us to isolate the final product in an individual state.

The experiments on the evaluation of the inhibiting activity to two nitric oxide synthase isoforms (inducible *i*NOS and neuronal *n*NOS) were carried out for the bicyclic thiazine **2** and thione **11** (both as racemic mixtures). NOS activity was determined *in vitro* by a radiometric method[12,13] from the rate of [^{3}H]-L-citrulline accumulation in the NOS-catalyzed oxidation of NOS substrate [^{3}H]-L-arginine.[§] The binding results revealed that both of the tested compounds were inactive as the inhibitors of NOS isoforms (Figure 2).

This result was expected for thione **11** due to the absence of hydrogens able to bind to Glu377 and Trp372 (Figure 3).

The inability of compound **2** to inhibit the enzyme despite the molecular modeling prediction signifies either realization of not the most beneficial conformation of the compound at the *i*NOS active site, or insufficiency of the lipophilic interactions

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[§] The activity of NO synthase was measured for two samples: *i*NOS, isolated from lipopolysaccharide-stimulated mouse macrophages produced by Cayman Chemical Co., USA (22.43 units mg^{-1}, 4.97 mg ml^{-1}, 111.5 mg ml^{-1}, catalogue no. 60862) and a soluble fraction of rat cerebellum homogenate (NO synthase activity detected in this sample is represented mainly by calcium-dependent *n*NOS). NOS activity was assessed as the rate of conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline as described by Bredt and Snyder[12] with minor modifications.[13] Reaction was started by adding supernatant (final enzyme content 2 U ml^{-1} for *i*NOS; final protein concentration 0.15 mg ml^{-1} for cerebellar extract) to the reaction media containing 50 mM HEPES (pH 7.2), 2 μCi ml^{-1} [^{3}H]-L-arginine, 1 μM L-arginine, 1 mM $MgCl_2$ for *i*NOS or 1 mM $CaCl_2$ for *n*NOS, 5 mM FAD, 5 mM FMN, 0.2 mM NADPH, 50 mM BH_4 (final volume 50 μl). After 10 min (for *n*NOS) or 5 min (for *i*NOS) of the sample incubation at 37 °C, the reaction was stopped by the addition of 300 μl ice-cold Dowex 50WX8-400 (Na^+-form) suspension in 20 mM HEPES (pH 7.4), containing 2 mM EDTA, and incubated for 15 min to remove non-reacted arginine. Radioactivity of [^{3}H]-L-citrulline was counted in a 1414 Winspectral liquid scintillation counter (Wallac Oy, Finland). NOS enzymatic activity was expressed as the content of accumulated content of [^{3}H]-L-citrulline in cpm for 10 min for *n*NOS (or 5 min for *i*NOS) between samples without inhibitors and those containing inhibitors. The binding results were compared with the standard, 2-amino-5,6-dihydro-4*H*-1,3-thiazine.

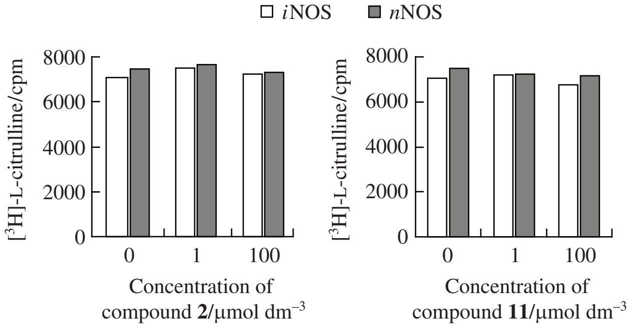

Figure 2 Effects of compounds **2** and **11** on the NOS activity.

with Phe309 and Val352 for the compensation of a hydrogen bond lost in compound **2** in comparison with thiazine **1**. If the latter conclusion is correct, then a bicyclo[3.3.1]nonane analogue of **1** with an additional cycle attached to the positions at C^4 and C^6 should possess inhibitory activity and is worth synthesizing and testing. This work is now in progress and its results will be reported in a due course.

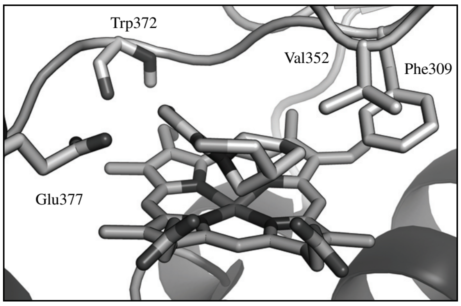

Figure 3 Docking of structure **11** into the *i*NOS active site (no hydrogen bonds are formed).

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2010.11.007.

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