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Synthesis and antihypotensive properties of 2-amino-2-thiazoline analogues with enhanced lipophilicity

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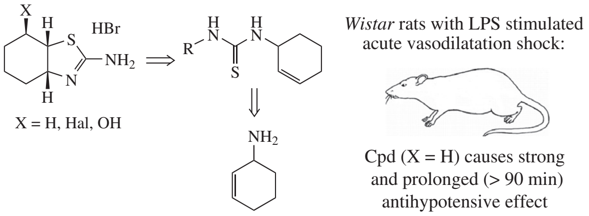
In a search of nitric oxide synthase inhibitors with prolonged vasoconstrictive activity, a series of lipophilic cyclohexa-fused 2-amino-2-thiazolines was obtained *via* cyclization of *tert*-butyl- or benzoyl-substituted *N*-(cyclohex-2-en-1-yl)thioureas. The crystal structure of intermediate *N*-[(3a*RS*,7a*SR*)-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-yl]benzamide hydrobromide was determined by X-ray analysis. One compound was found to cause pronounced and prolonged vasoconstrictive effect after single injection to the Wystar rats with lipopolysaccharide induced acute endotoxic (vasodilatation) shock.

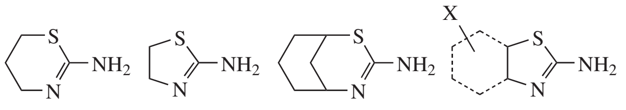


Wistar rats with LPS stimulated acute vasodilatation shock:

Cpd (X = H) causes strong and prolonged (> 90 min) antihypotensive effect

Nitric oxide (NO•) plays a role of signaling molecule in human organism and is involved in many physiological processes, such as vasodilatation, angiogenesis, immune response *etc*.[1] The synthesis of NO• from L-arginine is catalyzed by nitric oxide synthases (NOS), a group of enzymes, which are subdivided into endothelial (*e*NOS), neuronal (*n*NOS) and inducible (*i*NOS) isoforms. Inhibition of *e*NOS and particularly *i*NOS is known to cause antihypotensive effect in the case of septic shock[2] or radioactive irradiation.[3] 2-Amino-5,6-dihydro-4*H*-1,3-thiazine **1** and 2-amino-2-thiazoline **2** (Figure 1) represent the first generation of cyclic isothioureas with high inhibitory activity to NOS[4,5] and pronounced antishock and radioprotective activity (see ref. 6 and citations therein). During last two decades, many analogues of cyclic isothioureas **1** and **2** were obtained as putative NOS inhibitors.[7–15]

The introduction of short alkyl substiutents to the certain positions of thiazine or thiazoline core in compounds **1** and **2** was shown to increase in some cases their inhibitory activity or selectivity to *i*NOS or to improve their pharmacokinetic properties.[4,14] Based on these data, we obtained compound **3**, a bridged analogue of thiazine **1** (see Figure 1), which was much more lipophilic, but maintained high *i*NOS inhibitory activity of initial molecule.[8] This finding stimulated us to apply similar modification to thiazoline **2**. In the present work, we synthesized several cyclohexa-fused 2-amino-2-thiazolines (hexahydro-1,3-benzothiazol-2-amines) of general formula **A** (see Figure 1). As though the increase of lipophilicity often leads to a prolonged action of a compound *in vivo*, we aimed also to test the vasoconstrictive properties of the obtained cyclic isothioureas and duration of their possible hypotensive response.

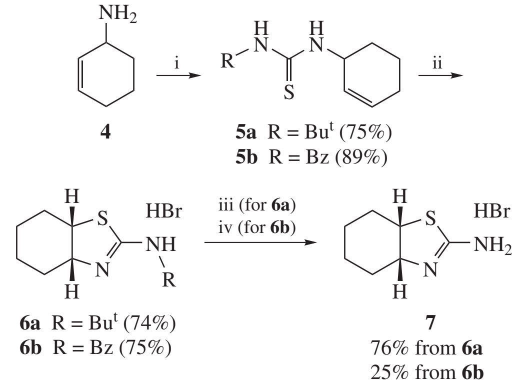


1 **2** **3** (ref. 8) **A** (this work)

Figure 1 *i*NOS inhibitors **1** and **2** and their lipophilic analogues **3** and **A**.

Molecular docking of the simplest structural templates **A** (X = H) into the three-dimensional structure of *i*NOS active site (PDB ID: 2NSI) revealed that positions of *cis*- and *trans*-isomers in the protein are very close to each other and to the position of co-crystallized *i*NOS inhibitor ethylisothiourea [Figure 2(*a*)]. Interestingly, this result is in accordance with the data[14] for structurally close 4,5-dialkyl-2-aminothiazolidines, whose *i*NOS inhibitory activity was close for *trans*- and *cis*-isomers.

In this work we concentrated on the synthesis of *cis*-ligands and first obtained unsubstituted target compound (Scheme 1). Cyclohex-2-en-1-amine **4** was converted to the corresponding *tert*-butyl- or benzoyl-substituted thioureas **5a**,**b**, which were subjected to cyclization to give hydrobromides of substituted hexahydro-1,3-benzothiazol-2-amines **6a**,**b** (for the synthetic details and characteristics of all novel compounds, see Online Supplementary Materials). The cyclization passed smoothly and



Scheme 1 *Reagents and conditions*: i, RN=C=S (R = Bu^t or Bz), DIPEA, CH_2Cl_2, ~20 °C, 12 h; ii, AcBr + MeOH, CH_2Cl_2, ~20 °C, 12 h; iii, HBr_{aq}, reflux, 3 h; iv, DBU, MeOH, ~20 °C, 12 h, then HBr_{aq}. The marked configuration of the compounds is relative, because they represent racemic mixtures.

stereoselectively: *cis*-configuration of the products was determined by ^{1}H NMR spectra and was unambiguously proved by X-ray analysis [Figure 2(*b*)].[†]

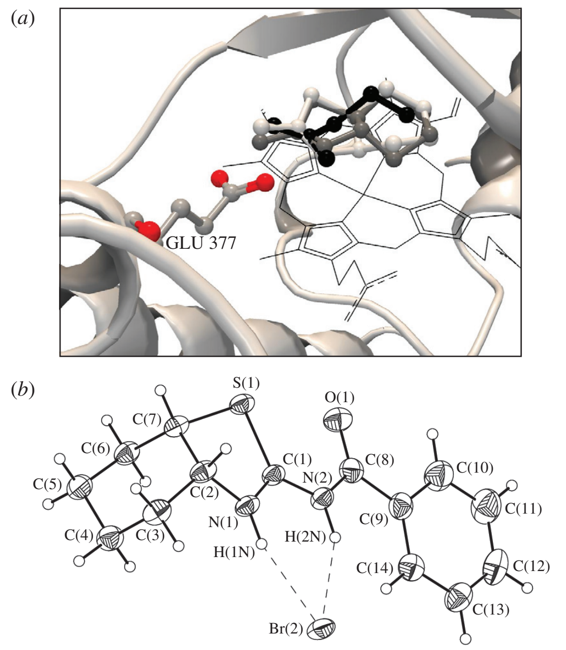
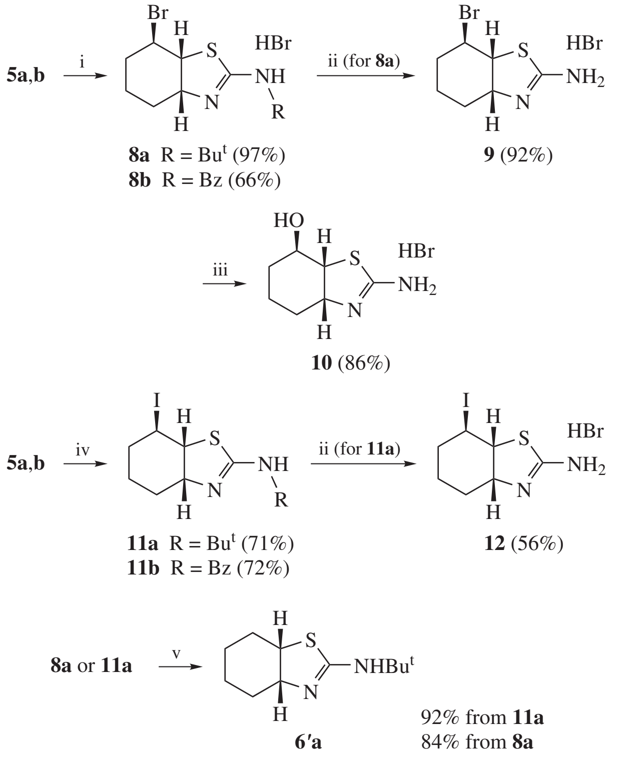


Figure 2 (*a*) Positions of (3a*R*,7a*S*)- (shown in light gray) and (3a*S*,7a*S*)-(shown in gray) isomers of 3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine **6b** in *i*NOS active site as predicted by automated docking (AutoDock 4.2; visualized using CLC Drug Discovery Workbench). Location of ethylisothiourea molecule (co-crystallized with the protein, PDB ID: 2NSI) is shown in black for comparison. All displayed molecules may form important hydrogen bond with Glu377. An interaction of sulfur atoms in all compounds with the iron atom in heme (shown with thin lines) is also possible (hydrogen atoms are omitted for clarity). (*b*) The general view of one of the independent molecules of *N*-[(3a*RS*,7a*SR*)-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-yl]benzamide hydrobromide **6b** in representation of atoms by thermal ellipsoids (p = 50%).

Cleavage of both *tert*-butyl group in compound **6a** and benzoyl group in **6b** led to the target amine (as hydrobromide **7**), but in the case of **6a** the yield was better (see Scheme 1). It should be mentioned that analogous to **7** hydroiodide was obtained earlier by a different method,[16] however no characteristics except low-resolution ^{1}H NMR spectra were presented. In our case spectral data indicate the presence of only one tautomer of compound **7**, but do not allow us to assign it to the specific form.

To synthesize the substituted hexahydro-1,3-benzothiazol-2-amines of general formula **A** (see Figure 1), we carried out the cyclization of thioureas **5a**,**b** in the presence of bromine or iodine (Scheme 2). This reaction led to the individual diastereomers of compounds **8a**,**b** and **11a**,**b** (only one set of signals was observed in their ^{1}H NMR spectra).



Scheme 2 *Reagents and conditions*: i, Br_2, CH_2Cl_2, ~20 °C, 24 h; ii, HBr_{aq}, reflux, 3 h; iii, PbO, H_2O, 50 °C, 10 h; iv, I_2, K_2CO_3, Et_2O, ~20 °C, 24 h; v, Bu_3SnH, AIBN, toluene, 100 °C, 10 h. The marked configuration of the compounds is relative, because they represent racemic mixtures.

Characteristic signal of H^{7a} proton is displayed as doublet of doublets with $J_{7a,7}$ ~ 10 Hz and $J_{7a,3a}$ ~ 5 Hz, confirming *cis*-fusion of rings and *trans*-orientation of the halogen substituent towards thiazolidine cycle. Removal of *tert*-butyl substituent in **8a**,**b** gave bromo-substituted hexahydro-1,3-benzothiazol-2-amine **9** (as hydrobromide), which was then converted to the corresponding alcohol **10** by treating with the suspension of PbO in water[17] (see Scheme 2).[‡] Iodo-substituted hexahydro-1,3-benzothiazol-2-amine **12** (as hydrobromide) was obtained similarly to its bromo analogue **9**, but in a smaller yield. Bromide **8a** and iodide **11a** bearing *N*-*tert*-butyl substituent were reductively dehalogenated by treatment with tri-*n*-butyltin hydride (Bu_3SnH) to afford compound **6′a** which is a free base of salt **6a** (see

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[†] *Crystal data for* **6b**: $C_{14}H_{17}BrN_2OS$, ($0.5CCl_4$, Br), M = 418.17 (from CCl_4–MeOH, 9 : 1), monoclinic, space group $P2_1/n$, at 120(2) K: a = 17.7348(4), b = 8.5127(2) and c = 26.4539(6) Å, β = 106.0947(10)°, V = 3837.24(15) Å^3, Z = 8 (Z' = 2), d_{calc} = 1.448 g cm^{-3}, μ(CuKα) = 65.08 cm^{-1}. Intensities of 41817 reflections were measured with Bruker APEX-II CCD [λ(CuKα) = = 1.54178 Å, 2θ < 144.5°) and 7420 independent reflections (R_{int} = 0.0411) were used in the further refinement. The structure was solved by direct method and refined by the full-matrix least-squares technique against F^2 in the anisotropic–isotropic approximation. The refinement converged to wR_2 = 0.1139 and GOF = 1.057 for all independent reflections [R_1 = = 0.0410 was calculated against F for 6373 observed reflections with $I > 2\sigma(I)$]. All calculations were performed using SHELXTL-2014/6.

CCDC 1821378 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

[‡] An attempt to synthesize **9** from unprotected *N*-(cyclohex-2-en-1-yl)-thiourea[18] led to a complex mixture of products, in which the target compound **9** was detected by spectroscopy in trace amounts, but could not be isolated.

Scheme 1). These transformations may serve as additional approval of the structures obtained.

It is noteworthy, that alcohol **10** (see Scheme 2) was obtained as individual diastereomer with the same *trans*-orientation of hydroxyl towards fused thiazolidine core as in the parent bromo derivatives **8a** and **9**. According to ^{1}H NMR spectroscopy, the values of vicinal spin–spin coupling constants ($J_{7a,7}$ 9.6 Hz and $J_{7a,3a}$ ~ 5.3 Hz) of the doublet of doublets corresponding to H^{7a} proton of **10** (at δ 3.42 ppm) remained almost the same as in compounds **8a** and **9**. Configuration of hydroxyl in alcohol **10** was also confirmed based on the Overhauser effect: irradiation of the H^{3a} proton resonance at 4.27 ppm did not lead to the signal response of H^7 proton, but caused an increase in the intensity of the peak of H^{7a}. Interestingly, though nucleophilic reactions with retention of configuration are well known (see, for example, the hydrolysis of α-chloro carboxylic acids with water/Ag_2O[19]), literature search did not reveal examples of similar reactions for isothiourea derivatives.

Elaborated synthetic scheme to target cyclic thioureas was effective and operationally convenient and allowed us to scale-up compounds **7**, **9** and **10** for biotesting *in vivo*. Antihypotensive properties of hexahydro-1,3-benzothiazol-2-amines **7**, **9** and **10** (as racemic mixtures) were tested on Wistar rats with lipopolysaccharide stimulated acute endotoxic (vasodilatation) shock (see Online Supplementary Materials).[§] Injection of compound **7** caused prolonged and positive action: systolic blood pressure (SBP) since the second minute till the end of experiment (90 min) increased to 120–130% from the initial shock blood pressure (BP) level (or 82% from the initial background). Diastolic blood pressure (DBP) in the first five minutes increased to 140% and was then fluctuated within 105–130% from the initial shock BP level. Heart rate was raised to the initial values of healthy animals in 5–10 minutes, and then (since 20th minute till the end of experiment) was at the level of 95% from physiological standards. Injection of compound **9** led to short-term SBP increase (from the second to 20th minute), and then continuous downward trend was registered. Heart rate dropped to 90% since the fifth minute and DBP dropped to 90% from initial values in 20 min and to 70% in 30 min. Respiratory rate was stable. Compound **10** caused additional hypotension at 10th minute after injection. In 30 min the values of the SBP and DBP were 75% and 65% from the initial ones, respectively. Heart rate first moderately increased (105–107%), but after 40 min lowered to 95%. Respiratory rate increased within 40–50 min, then the breathing became abrupt and dyspnea developed.

These data indicate that the type of substituent at C^7 of the synthesized hexahydro-1,3-benzothiazol-2-amines plays a crucial role in their vasoconstrictive properties. Antihypotensive effect of C^7-bromo substituted compound **9** is short-termed and weak, while its hydroxy analogue **10** causes additional hypotension. The best result is observed for unsubstituted bicyclic isothiourea **7**, which causes strong vasoconstriction (equal to that of compounds **1** and **2**) which lasts for at least 90 min and is prolonged in comparison with the lead molecules.[20]

In conclusion, a series of lipophilic analogues of NOS inhibitor 2-amino-2-thiazoline **2** was synthesized using new and effective procedure, and (3a*RS*,7a*SR*)-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine hydrobromide **7** demonstrated pronounced and prolonged antihypotensive activity in the experiments *in vivo*.

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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.2018.07.016.

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[§] *In vivo* trials were conducted according to current legislation in Russian Federation concerning humanitarian handling of laboratory animals, namely GOST P53434-2009, 'Principles of Laboratory Practices' and Russian Ministry of Health and Social Development Order of Aug. 23, 2010, No. 708n 'On Approval of Laboratory Practice Rules'.