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Bicyclic bridged isothioureas: synthesis and evaluation of activity in a model of lipopolysaccharide-induced septic shock

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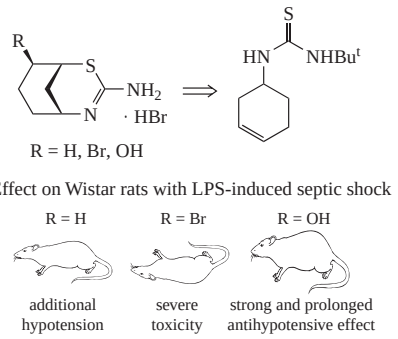
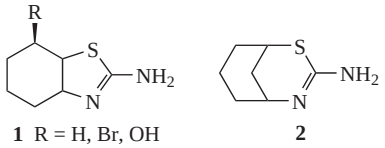
A convenient synthetic pathway to bridged isothioureas *via* cyclization of *tert*-butyl-substituted cyclohex-3-en-1-ylthiourea by the action of bromine has been developed. The effective use of *tert*-butyl protective group was demonstrated, and the crystal structure of synthesized (3a*RS*,7*SR*,7a*RS*)-7-bromo-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine hydrobromide was determined by X-ray analysis. (1*RS*,5*SR*)-2-Thia-4-azabicyclo[3.3.1]non-3-en-3-amine as a strong inducible nitric oxide synthase inhibitor *in vitro* surprisingly caused additional hypotension in LPS-induced acute endotoxic shock model while its C[8]-hydroxy-derivative displayed prominent and durable vasoconstrictive effect.



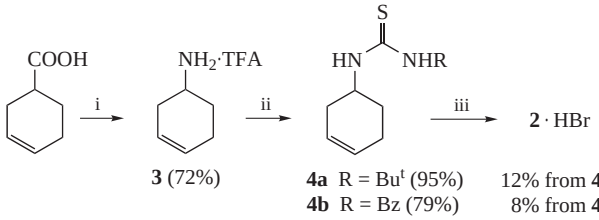
Septic shock or ionizing irradiation-induced damage are accompanied by severe symptoms including in particular the blood pressure reduction.[1,2] Antihypotensive effect for the treatment of these conditions can be achieved by inhibition of the nitric oxide synthase (NOS) enzyme especially in its inducible isoform (*i*NOS) and in part in its endothelial isoform (*e*NOS).[2,3] Monocyclic thioureas such as 2-amino-5,6-dihydro-4*H*-1,3-thiazine or 2-amino-2-thiazoline are known to display pronounced but short-term antishock and radioprotective effect *in vivo*.[4] An attempt to enhance the duration of this effect led us earlier to the synthesis of more lipophilic bicyclic isothioureas, *e.g.* **1** and **2**.[5,6]



Compound **1** (R = H) exhibited prolonged vasoconstrictive effect *in vivo*[5] while bridged thiazine **2** had strong *i*NOS inhibitor properties *in vitro*.[6] However, the multistep synthesis of compound **2** (*via* cyclization of *trans*-3-bromocyclohexylamine with carbon disulfide followed by thiocarbonyl modification) in total yield less than 2%[6] did not allow scaling up. This led to a search of convenient procedure for the synthesis of thiazine **2** and its derivatives in amounts sufficient for their biotesting *in vivo*.

In this work we firstly investigated the synthetic pathway of the Scheme 1 using the described procedures.[5]

Trifluoroacetate of cyclohex-3-en-1-amine **3** (obtained from cyclohex-3-ene-1-carboxylic acid *via* the Curtius rearrangement[7]) was converted to *tert*-butyl- or benzoyl-substituted thioureas **4a**,**b** by reaction with the corresponding isothiocyanates.[†] Attempts to carry out the cyclization of compounds **4a**,**b** under mild conditions by treatment with AcBr/MeOH[5] failed, therefore more rigorous conditions were used. The reflux of **4a** or **4b** with aqueous HBr resulted in cyclization with simultaneous hydrolysis of *tert*-butyl or benzoyl groups and gave the target bridged isothiourea **2** as hydrobromide (Scheme 1). In the case of the *tert*-butyl-substituted derivative **4a** the pure final product **2**·HBr was isolated in 8% total yield from cyclohex-3-ene-1-carboxylic acid. Though better than described above,[6] this synthetic scheme

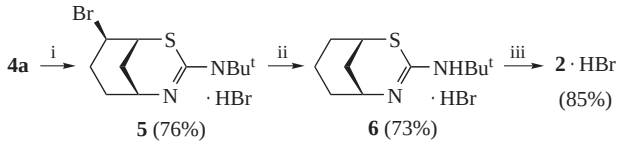


Scheme 1 *Reagents and conditions:* i, NaN_3, Bu_4NBr, $Zn(OTf)_2$, THF, Boc_2O, then CF_3COOH, room temperature, 12 h; ii, RNCS (R = Bu^t or Bz), Pr^i_2NEt, CH_2Cl_2, room temperature, 12 h; iii, HBr aq., reflux, 5 h.

[†] For synthetic details and characteristics of new compounds as well as additional characteristics of earlier described compounds, see Online Supplementary Materials.

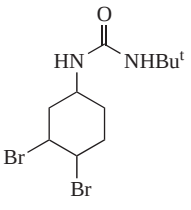
was hardly applicable for scaling up due to the very low yield of the target compound **2** at the last step.

Another synthetic pathway developed towards bridged isothiourea **2** was close to the pathway studied for annelated thioureas **1**[5] and initially included reaction of *tert*-butylcyclohexenylthiourea **4a** with bromine (Scheme 2).‡ Simultaneous bromine addition and cyclization resulted in the individual diastereomer of the major product **5** as was determined by NMR spectroscopy. The data on the Overhauser effect for compound **5** showed that irradiation of the characteristic resonance of proton HC^8Br at 4.58 ppm did not lead to the signal response for C^9H^{9b} proton but caused an increase in the intensity of the of C^6H^{endo} peak (see Online Supplementary Materials). Reductive debromination of bromine derivative **5** in the presence of tri-*n*-butyltin afforded compound **6** and the following reflux of the latter in aqueous HBr gave hydrobromide of the target bridged isothiourea **2** (see Scheme 2). Total yield of **2**·HBr in this five-step synthetic pathway (from cyclohex-3-ene-1-carboxylic acid) was 32%, which was much better than those for both methods mentioned above, namely, studied in ref. 6 (2%) and depicted in Scheme 1 (8%).



Scheme 2 *Reagents and conditions:* i, Br_2, CH_2Cl_2, room temperature, 24 h; ii, Bu_3SnH, AIBN, toluene, 100 °C, 6 h; iii, HBr aq., reflux, 3 h. The depicted configuration of the compounds is relative (they are racemic mixtures).

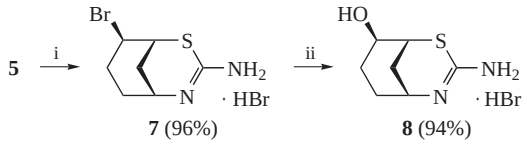
It is noteworthy that a mixture of by-products with identical molecular masses was formed in 5% overall yield along with the main compound **5** in the reaction of **4a** with bromine. Using column chromatography we isolated one of these by-products. Its ^1H and ^{13}C NMR spectra combined with mass spectrometry data (*i.e.*, the isotopic pattern of dibrominated molecule, see Online Supplementary Materials) corresponded completely to the structure **5′**, which might form as a result of bromine addition to the double bond of **4a** and simultaneous conversion of thiourea moiety to urea under atmospheric moisture conditions.[8,9]



5′

The derivatives of bridged isothiourea **2** were obtained by reflux of compound **5** with diluted aqueous HBr, which led to hydrobromide of (1*RS*,5*RS*,8*RS*)-8-bromo-2-thia-4-azabicyclo-[3.3.1]non-3-en-3-amine **7**, and the following replacement of bromine atom in compound **7** with hydroxyl group by treatment with PbO in water[5] (Scheme 3). The hydrobromide of alcohol **8** was isolated as individual diastereomer with the same splitting pattern and width (~8–9 Hz) of resonance peaks in the ^1H NMR weak field range (3–5 ppm) as for the initial bromo derivative **7**. So, analogously to the corresponding annelated bicyclic thiourea

‡ Each bridged bicyclic compound mentioned in the present study was obtained as individual tautomer, but the spectral data did not allow us to assign them to a specific form. In Figure 1 and Schemes 2 and 3 the most probable bioactive tautomers are shown.

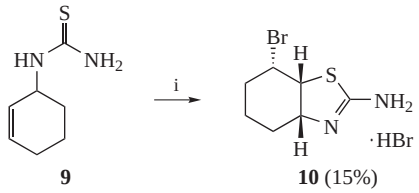


Scheme 3 *Reagents and conditions:* i, HBr aq., reflux, 3 h; ii, PbO, H_2O, 50 °C, 10 h. The depicted configuration of the compounds is relative (they are racemic mixtures).

1 (R = Br),[5] the nucleophilic substitution in **7** under the action of PbO in water proceeds with retention of configuration.

It should be emphasized that the use of protective groups (*e.g.*, Bu^t or Bz) in the synthesis of bicyclic isothioureas of the types **1** or **2** is rather important: the attempted reaction of unsubstituted cyclohex-2-enylthiourea **9** with equivalent amount of bromine at 0 °C (Scheme 4) led to a complex mixture of products which we failed to separate by recrystallization from ethanol as described.[10] Fractional precipitation allowed us to isolate only one compound, namely (3a*RS*,7*SR*,7a*RS*)-7-bromo-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine hydrobromide **10**, its structure has been proved by X-ray analysis (Figure 1).§

The developed synthetic pathways to bridged thioureas (see Schemes 2 and 3) were effective and convenient as well as allowed



Scheme 4 *Reagents and conditions:* i, Br_2, $CHCl_3$, 0 °C, 0.5 h. The depicted configuration of compound **10** is relative (it is racemic mixture).

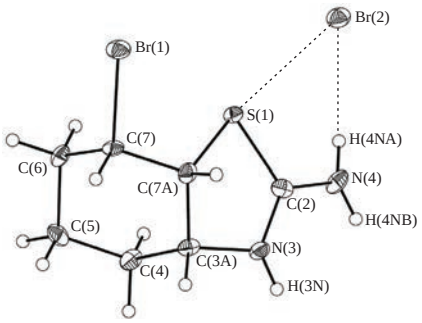


Figure 1 The general view of (3a*RS*,7*SR*,7a*RS*)-7-bromo-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine hydrobromide **10** with representation of atoms by thermal ellipsoids (p = 50%). Bromine anion participates not only in N–H···Br interactions [N···Br 3.255(6)–3.554(6) Å], but also in the formation of Br(2)···S(1)–C(7A) halogen bond [S(1)···Br(2) 3.672(2) Å, C(7a)S(1)Br(2) 173.0°].

§ *Crystal data for* **10**: $C_7H_{12}Br_2N_2S$, M = 316.05 (from CCl_4–MeOH, 9:1), monoclinic, space group $P2_1/c$, T = 120(2) K, a = 6.5020(5), b = = 9.0751(7) and c = 17.4075(14) Å, β = 94.0156(19)°, V = 1024.63(14) Å^3, Z = 4, d_{calc} = 2.049 g cm^{-3}, μ(MoKα) = 80.65 cm^{-1}. Intensities of 9776 reflections were measured with a Bruker APEX-II CCD diffractometer [λ(MoKα) = 0.71073 Å, 2θ < 58°] and 2776 independent reflections (R_{int} = 0.0329) 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 = 0.1386 and GOF = 1.022 for all independent reflections [R_1 = 0.0455 was calculated against F for 2398 observed reflections with $I > 2\sigma(I)$]. All calculations were performed using SHELXTL-2014/6.

CCDC 1857783 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.

us to scale-up compounds **2**·HBr, **7** and **8** for biotesting *in vivo*. Antihypotensive effect of these compounds (as racemic mixtures) was investigated on Wistar rats with lipopolysaccharide (LPS)-stimulated acute endotoxic (vasodilatation) shock (for procedure, see Online Supplementary Materials).[¶] Results of this study were intriguing and unexpected.

Injection of compound **2**·HBr initially had no effect, but in 15–20 min an additional hypotension was observed: systolic blood pressure (SBP) lowered to 85% and diastolic blood pressure (DBP) to 70% of the initial shock blood pressure level. Negative dynamics was accompanied by a significant decrease in heart and respiratory rates, which lasted till the end of monitoring. Compound **7** caused even more severe effect: from the 5th minute after its injection an additional drop in SBP, DBP and heart rate was detected. Between 15th and 20th minutes the convulsions and severe muscular spasms were registered. Accompanied by hard and abrupt breath, the blood pressure reduced to 40–50% of the initial shock level. This led to a death of one animal from the studied group. Contrary to that, the injection of compound **8** caused prolonged positive effect: from the 2nd minute an increase of blood pressure was observed, which reached 120% of the initial shock background level by the 5th minute. Till the end of the experiment (90 min after injection) SBP remained at the level of 120%, while DBP and heart rate were about 105%.

Therefore, the most prominent result of strong and prolonged vasoconstriction with no toxic side effects was observed for the hydroxy-substituted bridged isothiourea **8**. Surprisingly, compound **2**·HBr, though being an effective *i*NOS inhibitor *in vitro*, caused additional hypotension in the model of acute endotoxic shock. An additional test for this compound on the different model of prolonged endotoxemia (refractory vasoplegia) demonstrated the absence of vasoconstrictive effect as well: the SBP and DBP values for the animals that received isothiourea **2**·HBr as single intraperitoneal injection of 20 mg kg^{-1} were below the initial shock level by 10–15% during 60 min of monitoring. In addition, these animals showed symptoms of heart failure and their breathing was more jerky and uneven compared to the control group. The unexpected behavior *in vivo* for the active *in vitro* *i*NOS inhibitor may be related to the difficulty for isothiourea **2**·HBr in cell penetration as well as its possible interaction with different molecular target.

Interestingly, the structure–activity relationship for compounds of annelated[5] and bridged thiourea series do not match each other, though for both series the substituent in the cycloaliphatic part of bicyclic system plays crucial role in the ability of the compounds to prevent Wistar rats from septic shock.

In conclusion, an effective procedure for the synthesis of bridged isothioureas was developed and prolonged antihypotensive effect was revealed for (1*RS*,5*RS*,8*RS*)-3-amino-2-thia-4-azabicyclo[3.3.1]non-3-en-8-ol hydrobromide **8** in the model of LPS-induced acute endotoxic shock.

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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.2019.01.003.

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[¶] *In vivo* trials were conducted in accordance with the current legislation of Russian Federation on humane treatment and management of laboratory animals: GOST P 53434-2009, 'Principles of Good Laboratory Practice'; Russian Ministry of Health and Social Development, order no. 708n 'On Approval of Laboratory Practice Rules,' August 23, 2010.