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

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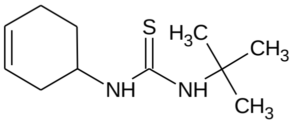
Chemistry

General information

All starting materials, reagents and solvents were purchased as high-grade commercial products and used without further purification. Reactions sensitive to moisture and/or oxygen were carried out under an inert atmosphere of anhydrous argon. Liquid column chromatography was performed using silica gel Acros Organics (40–60 μm). Thin-layer chromatography (TLC) was performed on Silufol-UV254 silica gel sheets and spots were visualized with UV light (λ 254 nm) or stained with iodine vapor or aqueous potassium permanganate solution.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 28°C in $CDCl_3$ at 400 and 100 MHz correspondingly. Chemical shifts (δ) are reported in parts per million (ppm) referenced to residual protonated solvent peak ($CDCl_3$, $\delta = 7.26$ for ^{1}H NMR) or to carbon resonance in the NMR solvent ($CDCl_3$, $\delta = 77.0$ for ^{13}C NMR). Mass spectra were recorded on a Finnigan MAT INCOS 50 spectrometer (electron impact, 70 eV). CHN elemental analysis was performed using a Carlo-Erba ER-20 analyzer. Infrared spectra (IR) were registered on a Thermo Nicolet IR200 apparatus using KBr disks. Melting points were determined using a capillary melting point apparatus and were uncorrected.

1-*tert*-Butyl-3-cyclohex-3-en-1-ylthiourea (4a). Cyclohex-3-en-1-amine trifluoroacetate **3** (407 mg, 1.92 mmol) dissolved in CH_2Cl_2 (15 ml) was treated with $(Pr^i)_2NEt$ (480 μl, 2.76 mmol) and *tert*-butyl isothiocyanate (250 μl, 1.97 mmol). The reaction mass was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 2% methanol in $CHCl_3$) to give **4a** as white solid (387 mg, yield 95%). M.p. 156–158^{0}C.



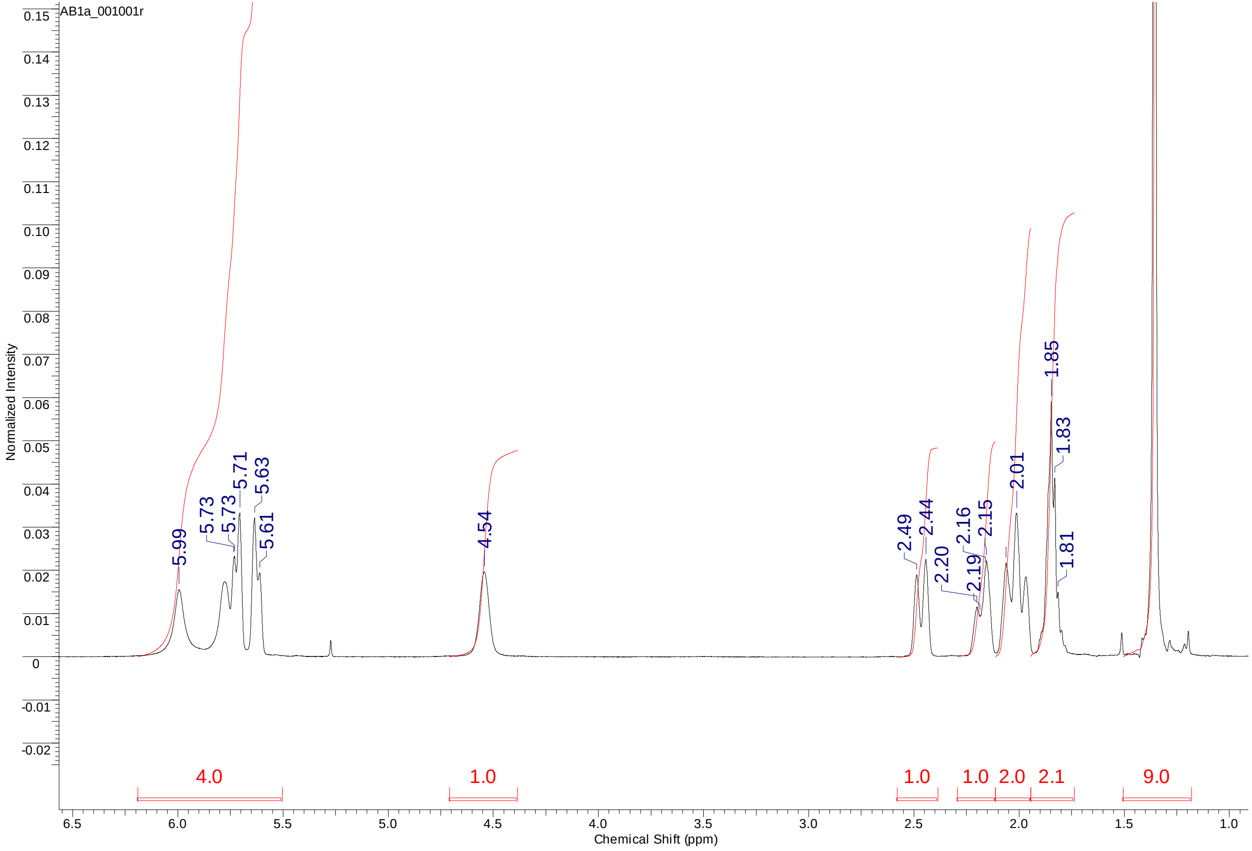
^{1}H NMR (δ, $CDCl_3$): 1.35 (s, 9H, *tert*-Bu), 1.81–1.88 (m, 2H), 1.97–2.06 (m, 2H), 2.14–2.20 (m, 1H), 2.44–2.49 (m, 1H), 4.54 (m, 1H, H^1), 5.61–5.63 (m, J = 10.0 Hz, 1H, H^4), 5.71–5.73 (m, J = 10.0 Hz, 1H, H^3), 5.78 (br s, 1H, NH), 5.99 (br s, 1H, NH).

^{13}C NMR (δ, CDCl$_3$): 22.63 (C^5), 26.71 (C^6), 29.40 (C($\underline{\text{C}}$H$_3$)$_3$), 31.22 (C^2), 49.71 (C^1), 52.41 ($\underline{\text{C}}$(CH$_3$)$_3$), 124.21 (C^3), 127.27 (C^4), 179.78 (C=S).

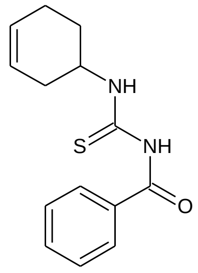
IR (KBr, cm^{-1}): 1135, 1201, 1243, 1319, 1346, 1392, 1540 (C=C), 2836-2979 (C-H), 3029-3116 (=C-H), 3257 br s (N-H).

Anal. Calcd. for $C_{11}H_{20}N_2S$: C, 62.22, H, 9.49, N, 13.19, S, 15.10. Found: C, 62.25, H, 9.52, N, 13.20, S, 15.01.

*^{1}H NMR spectrum of compound **4a***



***N*-(Cyclohex-3-en-1-ylcarbamothioyl)benzamide (4b)**. Cyclohex-3-en-1-amine trifluoroacetate **3** (414 mg, 1.96 mmol) dissolved in CH$_2$Cl$_2$ (20 ml) was treated with (Pr^i)$_2$NEt (580 µl, 3.34 mmol) and benzoyl isothiocyanate (270 µl, 2.01 mmol). The reaction mass was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 2% methanol in CHCl$_3$) to give **4b** as white solid (403 mg, yield 79%). M.p. 78–79 ^{0}C.



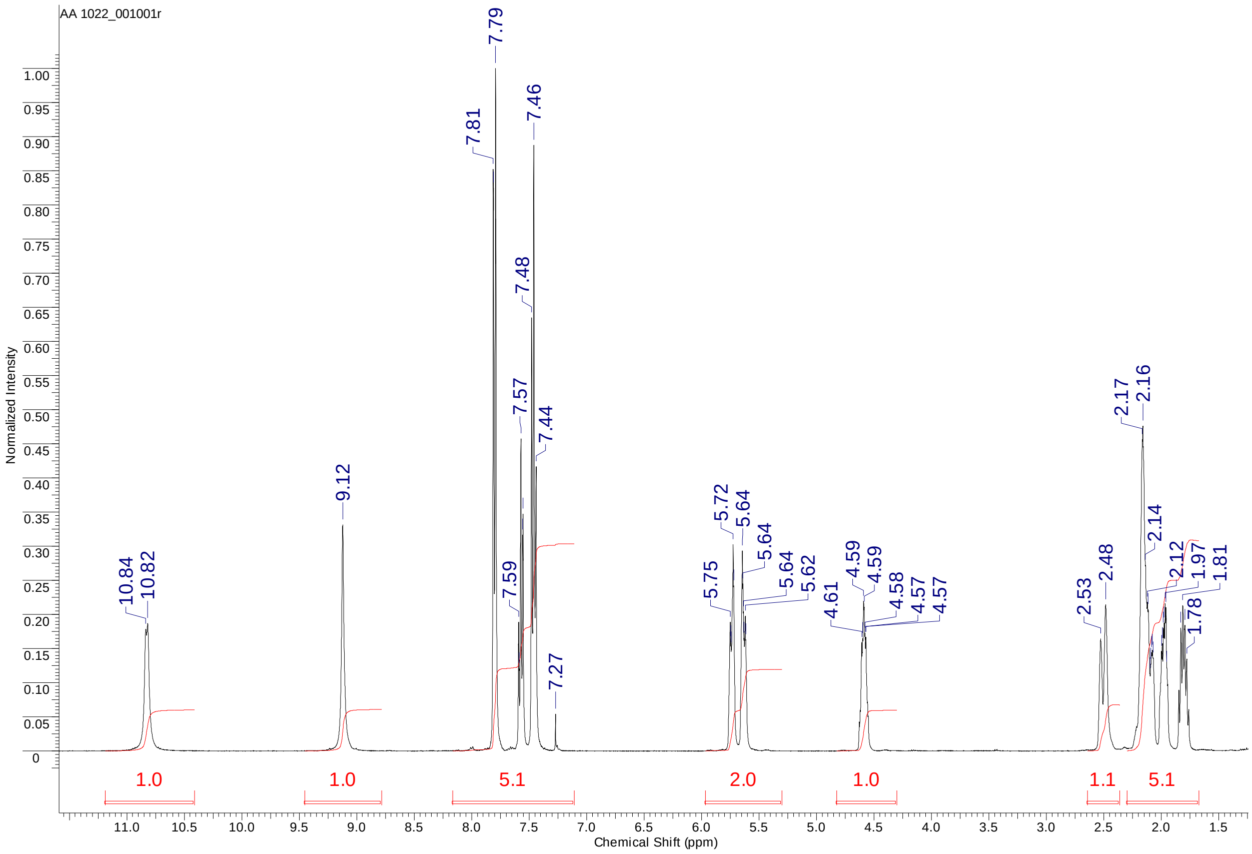
^{1}H NMR (δ, CDCl$_3$): 1.76–1.85 (m, 1H), 1.94 – 2.00 (m, 1H), 2.07–2.21 (m, 3H), 2.48–2.53 (m, 1H), 4.59 (m, 1H, H^1), 5.62–5.64 (m, J = 10.0 Hz, 1H, H^4), 5.72–5.75 (m, J = 10.0 Hz, 1H, H^3), 7.46 (m, 2H, J = 8.2, 7.5 Hz, 3,5-Ph), 7.57 (m, 1H, J = 7.5 Hz, 4-Ph), 7.79 (m, 2H, J = 8.2, 1.1 Hz, 2,6-Ph), 9.12 (s, 1H, NH), 10.83 (d, 1H, J = 6.6 Hz, NH).

^{13}C NMR (δ, CDCl$_3$): 22.77, 26.24, 30.20, 50.39 (C^1), 123,78 (C^3), 126.96 (C^4), 127.30 (2,6-Ph), 128.83 (3,5-Ph), 131.59 (1-Ph), 133.25 (4-Ph), 166.77 (C=O), 178.48 (C=S).

IR (KBr, cm^{-1}): 1159, 1241, 1267, 1523 (C=C), 1540 (C=C), 1673 (C=O), 2844–2962 (C-H), 3033–3099 (=C-H), 3166–3239 (N-H).

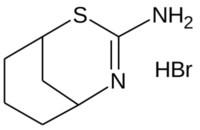
Anal. Calcd. for $C_{14}H_{16}N_2OS$: C, 64.58, H, 6.19, N, 10.76, S, 12.32. Found: C, 64.61, H, 6.16, N, 10.59, S, 12.27.

*^{1}H NMR spectrum of compound **4b***



(1*RS*,5*SR*)-2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine (2) hydrobromide. *Method A.*

A solution of **4a** (50 mg, 0.24 mmol) or **4b** (50 mg, 0.19 mmol) in aqueous HBr (5 ml) was refluxed for 5 h and concentrated under reduced pressure. The residue was purified by column chromatography (eluent: gradient 1–5% methanol in $CHCl_3$) to give **2** (·HBr) as white solid (from **4a**: 6 mg, 12%; from **4b**: 5 mg of impure product after the second column chromatography, crude yield 8%). White crystals, M.p. 220–222 ^{0}C.

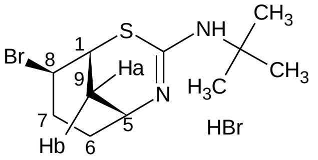


Method B. A solution of **6** (100 mg, 0.341 mmol) in aqueous 10% HBr (5 ml) was refluxed for 3 h. The reaction mass was concentrated under reduced pressure and purified by column chromatography (eluent: CH_2Cl_2, then gradient 1–5% methanol in $CHCl_3$) to give **2** (·HBr) as white solid (69 mg, 85%).

^{1}H NMR (δ, D_2O): 1.56–1.69 (m, 3H), 1.78–1.96 (m, 3H), 2.06 (m, 1H, J = 14.2, 5.4, 2.2 Hz, H^9), 2.23 (ddd, 1H, J = 14.2, 3.5, 3.0 Hz, H^9), 3.74 (m, 1H, J = 3.0 Hz, H^1), 3.91 (m, 1H, H^5). ^{13}C NMR (δ, $CDCl_3$): 15.67, 28.95, 31.54, 32.52, 38.05 (C^1), 45.73 (C^5), 168.89 (C^3). ^{13}C NMR (δ, D_2O): 15.14, 27.94, 30.53, 31.79, 37.77 (C^1), 45.96 (C^5), 168.56 (C^3). The spectral data are in accordance with the data described in [O. N. Zefirova et al. *Mendeleev Commun.*, 2013, **23**, 76]. MS (ESI): 157 $[M+H]^+$.

Anal. Calcd. for $C_7H_{13}BrN_2S$: C, 35.45, H, 5.53, N, 11.81, S, 13.52. Found: C, 35.48, H, 5.43, N, 11,66; S, 13.29.

(1*RS*,5*RS*,8*RS*)-8-bromo-*N*-*tert*-butyl-2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine hydrobromide (5). A solution of **4a** (270 mg, 1.273 mmol) in CH_2Cl_2 (10 ml) was treated with Br_2 (140 μl, 2.712 mmol) and stirred in darkness at room temperature for 24 h. The reaction mass was concentrated and the residue was purified by column chromatography (eluent: CH_2Cl_2, then gradient 1-2% methanol in CH_2Cl_2) to yield **5** as white crystals (360 mg, 76%). M.p. 210–212 ^{0}C. One of the first fractions in CH_2Cl_2 (22 mg, 5%) was subjected to additional column chromatography (eluent: ethyl acetate : petroleum ether (40–70 ^{0}C) 1:7) to yield **1-*tert*-butyl-3-(3,4-dibromocyclohexyl)urea (5')** (12 mg, 3%).

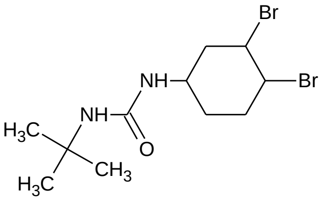


<u>For compound **5**</u>: ^{1}H NMR (δ, $CDCl_3$): 1.41 (s, 9H, *tert*-Bu), 1.82–1.85 (m, 1H, H^6), 1.93–2.03 (m, 3H, $H^{6+7exo+9b}$), 2.09–2.18 (m, 1H, H^{7endo}), 2.78 (m, J = 14.1, 3.1, 2.9 Hz, 1H, H^{9a}), 3.76 (m, 1H, H^1), 3.92 (m, 1H, H^5), 4.58 (m, 1H, H^8), 9.44 (br s, 1H, NH), 10.43 (br s, 1H, NH).

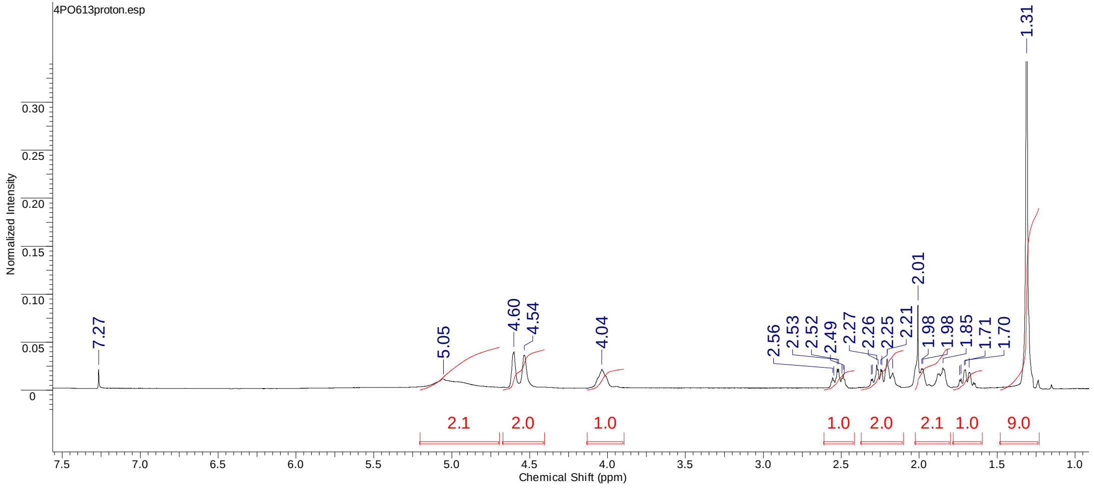
^{13}C NMR (δ, $CDCl_3$): 22.98 (C^9), 24.18 (C^7), 26.22 (C^6), 29.12 ($C(\underline{C}H_3)_3$), 41.41 (C^1), 44.71 (C^5), 50.68 (C^8), 55.37 ($\underline{C}(CH_3)_3$), 164.94 (C^3).

Anal. Calcd. for $C_{11}H_{20}Br_2N_2S$: C, 35.50, H, 5.42, N, 7.53, S, 8.62. Found: C, 35.48, H, 5.60, N, 7.61; S, 8.70.

<u>For compound **5'**</u>: ^{1}H NMR (δ, $CDCl_3$): 1.31 (s, 9H, *t*-Bu), 1.64–1.74 (m, 1H), 1.85–1.88 (m, 1H), 1.98–2.01 (m, 1H), 2.17–2.31 (m, 2H), 2.48–2.65 (m, 1H), 4.04 (m, 1H, H^1), 4.54 (m, 1H, CHBr), 4.60 (m, 1H, CHBr), 4.70–5.10 (br s, 2H, NH).

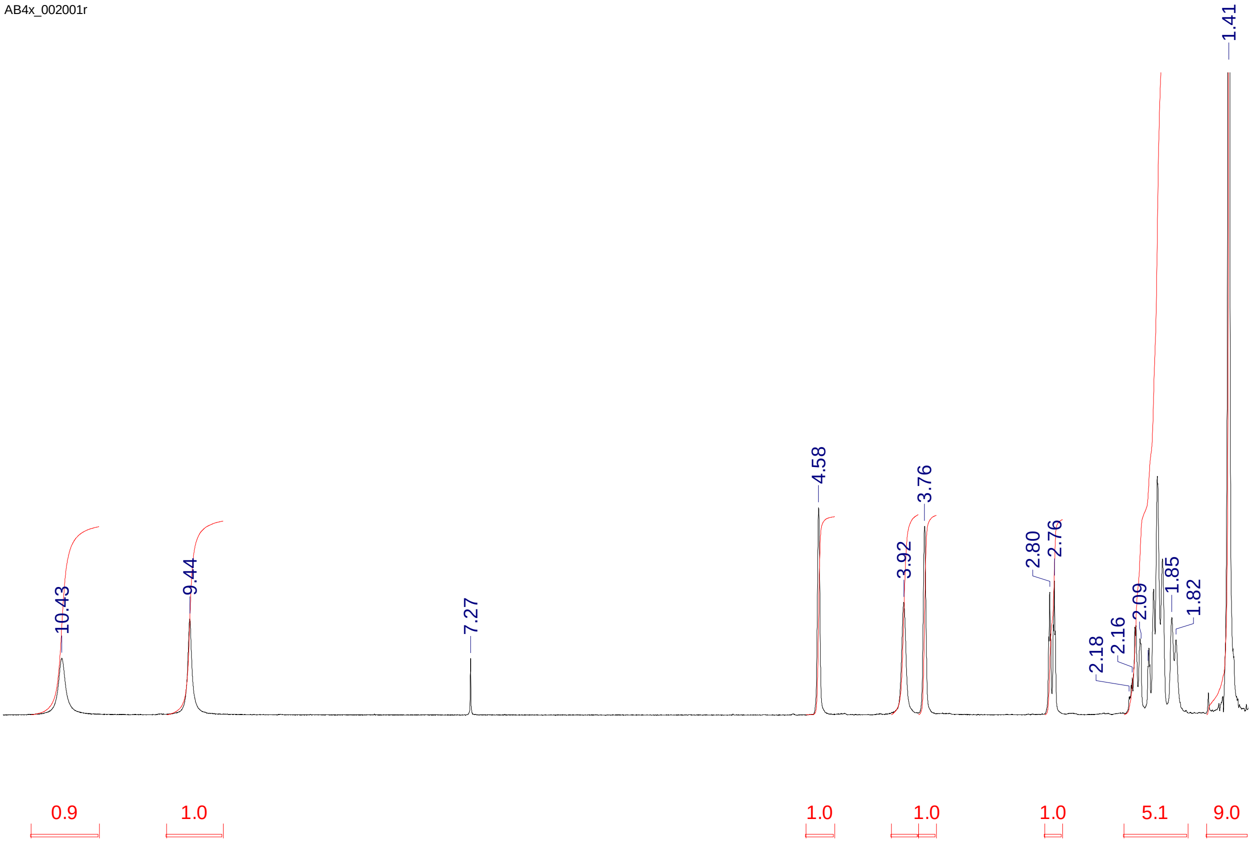


*^{1}H NMR spectrum of **5'** in CDCl$_3$:*



^{13}C NMR (δ, CDCl$_3$): 28.16, 28.73, 29.55 (C($\underline{\text{C}}$H$_3$)$_3$), 36.03 (C^2), 44.15 (CHBr), 50.16 (C^1), 52.19 (CHBr), 52.78 ($\underline{\text{C}}$(CH$_3$)$_3$), 157.11 (C=O). MS (ESI, m/z, I%): 359 ([M+4+H]$^+$, 54), 357 ([M+2+H]$^+$, 100), 355 ([M+H]$^+$, 58) [three molecular ion peaks with relative intensity ~ 1:2:1 each separated by two mass units correspond to the isotopic pattern of dibrominated molecule], 303 ([M-C$_4$H$_8$+4+H]$^+$, 37), 301 ([M-C$_4$H$_8$+2+H]$^+$, 78), 299 ([M-C$_4$H$_8$+H]$^+$, 40).

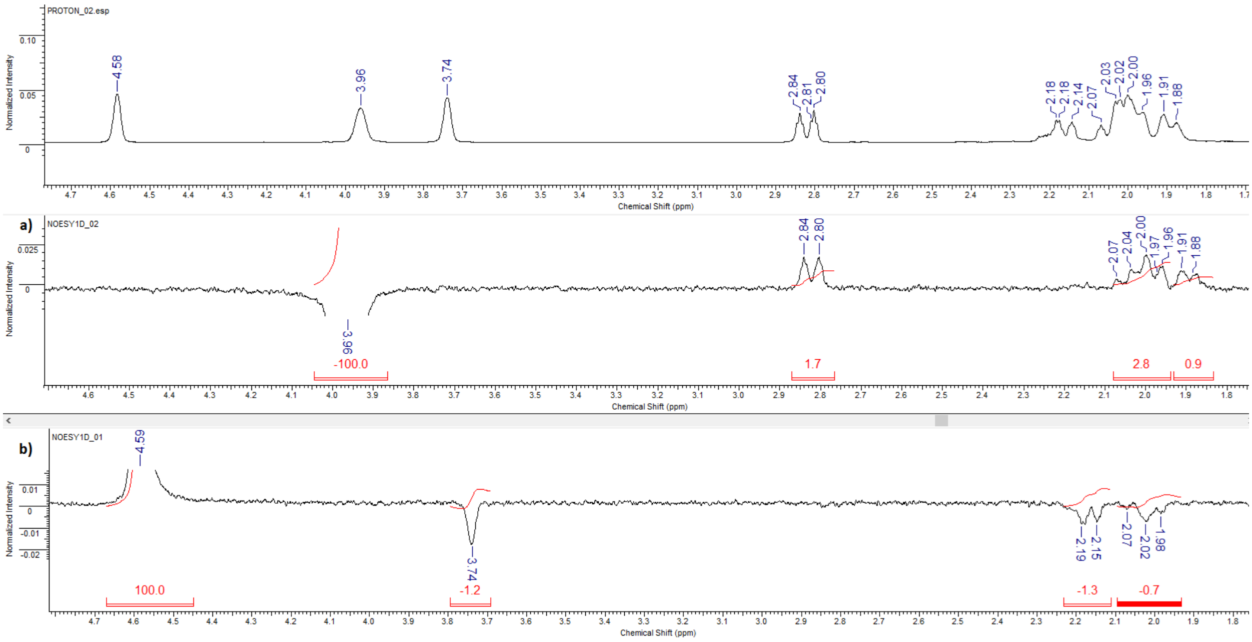
*^{1}H NMR spectrum of compound **5***



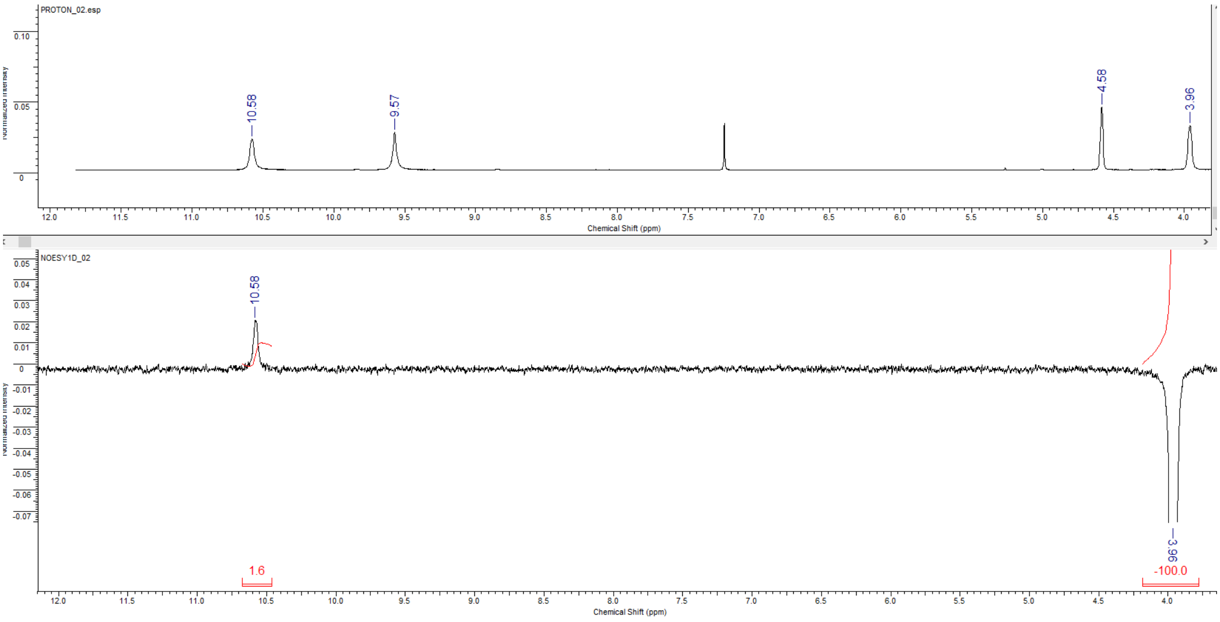
For compound 5:

NOESY 1D: a) H^{5}–H^{9a} (1.4%), H^{5}–$H^{6+7exo+9b}$ (2.8%), H^{5}–H^{6} (0.9%), interaction with H^{7endo} at 2.17 ppm is not observed.

b) H^{8}–H^{1} (1.2%), H^{8}–H^{7endo} (1.3%), H^{8}–$H^{6endo+7exo}$ (0.7%), interaction with H^{9b} (peak resonances at 2.00-1.96 ppm) is not observed

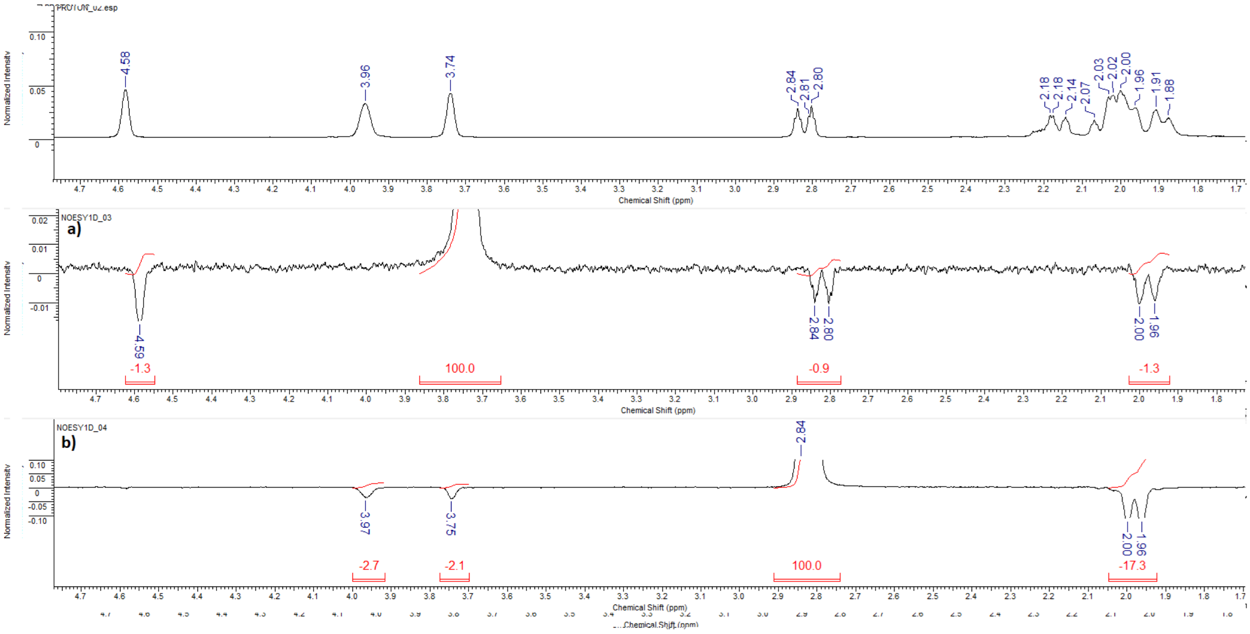


NOESY 1D: H^{5}–H^{NH} (1.6%)

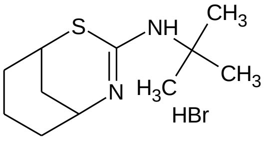


NOESY 1D: a) H^{1}–H^{8} (1.3%), H^{1}–H^{9a} (0.9%), H^{1}–H^{9b} (1.3%).

b) H^{9a}–H^{5} (2.7%), H^{9a}–H^{1} (2.4%), H^{9a}–H^{9b}(17.3%).



***N*-*tert*-butyl-2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine hydrobromide (6)** A solution of **5** (130 mg, 0.349 mmol) in toluene (20 ml) was treated with tri-*n*-butyltin hydride (190 µl, 0.706 mmol) and azobisisobutyronitrile (AIBN) (2 mg, 0.12 mmol). The mixture was heated at 100^{0}C in a sealed pressure valve under argon atmosphere during 6 h, then concentrated under reduced pressure and purified by column chromatography (eluent: CH_2Cl_2, then 2% methanol in $CHCl_3$) to yield **6** as white solid (75 mg, 73%). M.p. 84–86 ^{0}C.

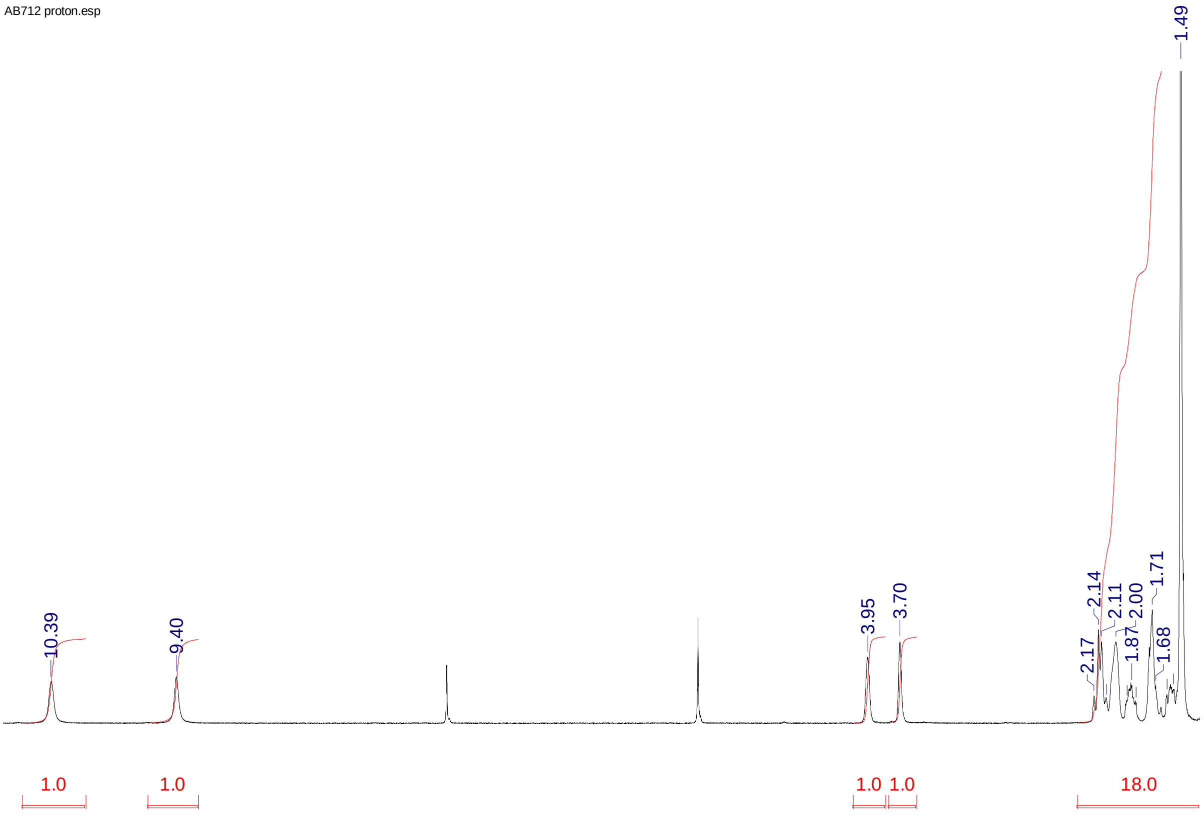


^{1}H NMR (δ, $CDCl_3$): 1.49 (s, 9H, *tert*-Bu), 1.52–1.60 (m, 1H), 1.68–1.75 (m, 2H), 1.84–1.92 (m, 1H), 1.95–2.03 (m, 2H), 2.07–2.17 (m, 2H), 3.70 (m, 1H, H^1), 3.95 (m, 1H, H^5), 9.40 (br s, 1H, NH), 10.39 (br s, 1H, NH).

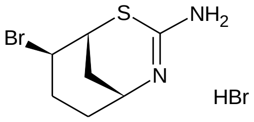
^{13}C NMR (δ, $CDCl_3$): 15.81, 28.56, 29.26 ($C(\underline{C}H_3)_3$), 31.38, 32.3, 38.30 (C^{1}), 45.33 (C^5), 55.14 ($\underline{C}(CH_3)_3$), 166.66 (C^3).

Anal. Calcd. for $C_{11}H_{21}BrN_2S$: C, 45.05, H, 7.22, N, 9.55, S, 10.93. Found: C, 45.18, H, 7.32, N, 9,66; S, 10.29.

^{1}H NMR spectrum of compound ***6***



(1*RS*,5*RS*,8*RS*)-8-bromo-2-thia-4-azabicyclo[3.3.1]non-3-en-3-amine hydrobromide (7). A solution of **5** (80 mg, 0.215 mmol) was refluxed in aqueous 10% HBr (5 ml) for 3 h. The reaction mass was concentrated under reduced pressure and purified by column chromatography on silica gel (eluent: 5% methanol in $CHCl_3$) to give **7** as white solid (65 mg, 96%). M.p. 190–193^{0}C.



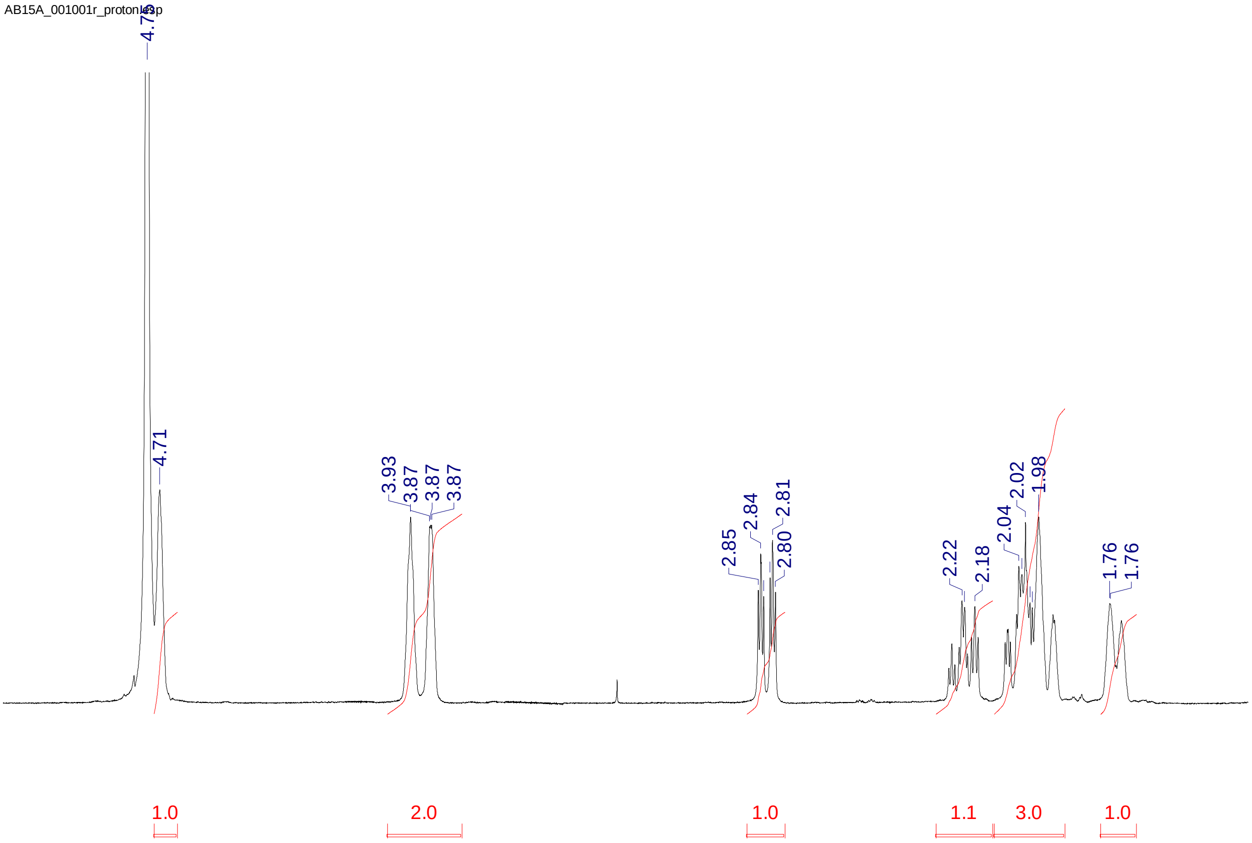
^{1}H NMR (δ, D_2O): 1.72–1.76 (m, J = 14.2 Hz, 1H), 1.93–2.08 (m, 3H), 2.17–2.26 (m, J = 14.7, 4.1, 3.7 Hz, 1H), 2.80–2.85 (m, J = 14.7, 3.8, 2.9 Hz, 1H,), 3.87 (m, 1H, H^1), 3.93 (m, 1H, H^5), 4.71 (m, 1H, H^8).

^{13}C NMR (δ, D_2O): 22.85, 23.73, 25.71, 40.90 (C^1), 45.38 (C^5), 51.95 (C^8), 167.19 (C^3).

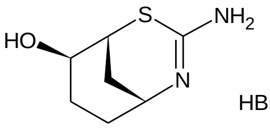
MS (EI): 236 $(M+2)^+$, 234 $(M)^+$, 155 $(M\text{-}Br)^+$, 128 $(C_5H_8N_2S)^+$, 113 $(C_4H_5N_2S)^+$, 79 $(Br)^+$, 80 $(Br)^+$, 43 $(C_3H_7)^+$.

Anal. Calcd. for $C_7H_{12}Br_2N_2S$: C, 26.60, H, 3.83, N, 8.86, S, 10.15. Found: C, 26.32, H, 3.91, N, 8.54; S, 10.10.

*^{1}H NMR spectrum of compound **7***



(1*RS*,5*RS*,8*RS*)-3-amino-2-thia-4-azabicyclo[3.3.1]non-3-en-8-ol hydrobromide (8). A suspension of **7** (164 mg, 0.519 mmol) and PbO (202 mg, 0.906 mmol) in water (10 ml) was stirred at 50 ^{0}C for 10h. The reaction mass was filtered, the filtrate was concentrated under reduced pressure, triturated with Et_2O (10 ml), the solution was decanted and the residue was dried to give pure **8** as almost white solid (124 mg, 94%). M.p. 188–190 ^{0}C.



^{1}H NMR (δ, $DMSOd_6$): 1.48–1.52 (m, 1H), 1.60–1.72 (m, 3H), 1.80–1.88 (m, 1H), 2.41–2.44 (m, *J* = 13.8, 3.4, 3.0 Hz, 1H), 3.43 (m, 1H, H^8), 3.79 (m, 1H, H^1), 3.89 (m, 1H, H^5), 5.38 (m, 1H).

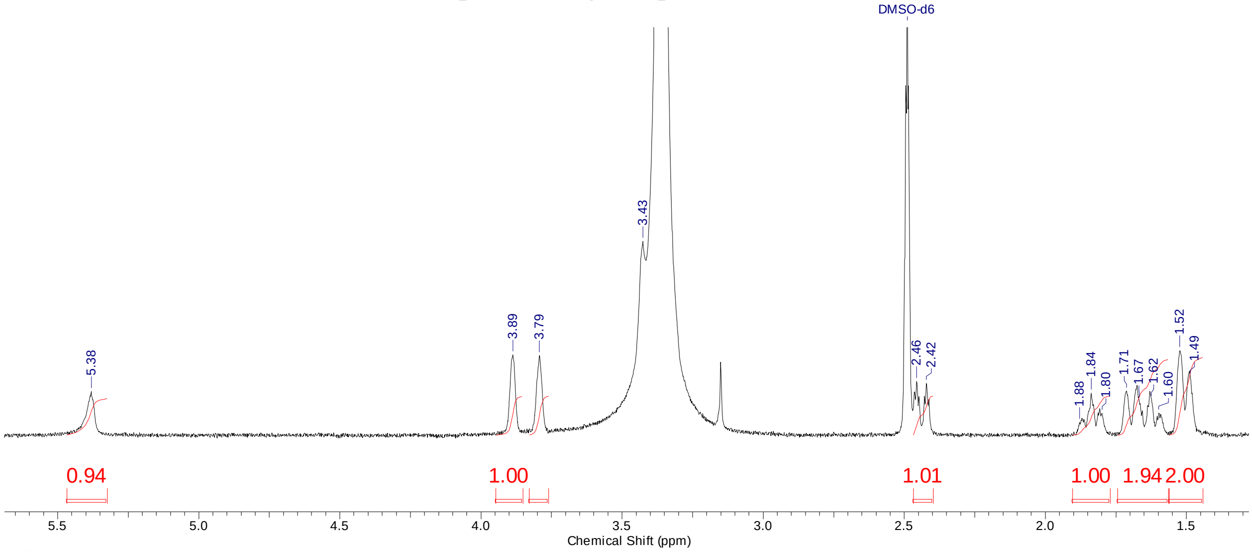
^{1}H NMR (δ, D_2O): 1.62–1.67 (m, 2H), 1.81–1.92 (m, 3H), 2.41–2.46 (m, *J* = 14.4, 3.8, 2.9 Hz, 1H), 3.49 (m, 1H, H^8), 3.89 (m, 1H, H^1), 4.11 (m, 1H, H^5).

^{13}C NMR (δ, D_2O): 21.26, 21.79, 25.20, 39.12 (C^1), 45.52 (C^5), 68.72 (C^8), 168.13 (C^3).

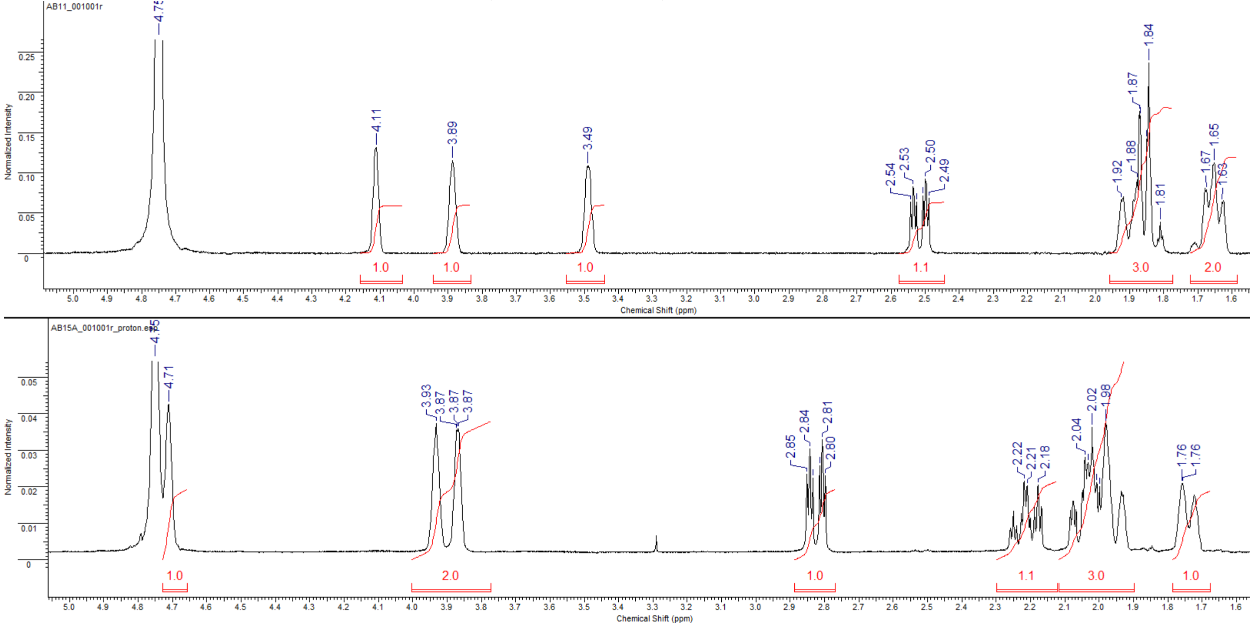
MS (ESI): 173 $(M+H)^+$.

Calcd. for $C_7H_{13}BrN_2OS$: C, 33.21, H, 5.18, N, 11.07, S, 12.67. Found: C, 33.11, H, 5.28, N, 11.08; S, 12.10.

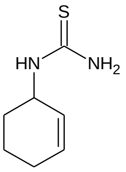
*^{1}H NMR spectrum of compound **8** in DMSOd$_6$*



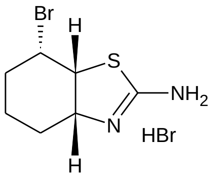
*^{1}H NMR spectrum of compound **8** (at the top) in comparison with the spectrum of compound **7** (at the bottom) in D$_2$O*



1-(Cyclohex-2-en-1-yl)thiourea (9) was prepared according to the procedure described in [D. W. Emerson, J. K. Booth, *JOC*, 1965, **30**, 2480]. Melting point of compound **9** was 135^{0}C and corresponded to the literature data (134–136^{0}C). NMR spectral data have not been published earlier. ^{1}H NMR (DMSO-d$_6$): 1.45–1.99 (m, 3H), 1.77–1.76 (m, 1H), 1.95 (m, 2H), 4.62 (m, 1H, H^{1}), 5.58 (m, *J* = 7.8 Hz, 1H, H^2), 5.80 (m, *J* = 7.8 Hz, 1H, H^3), 6.87 (br s, 2H, NH$_2$), 7.59 (d, *J* = 6.5 Hz, 1H, NH). ^{13}C NMR (DMSO-d$_6$): 19.54, 24.43, 28.83, 48.76 (C^{1}), 128.17 (C^3), 129.76 (C^2), 182.14 (C=S).



(3a*RS*,7*SR*,7a*RS*)-7-Bromo-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-amine hydrobromide (10). A solution of Br$_2$ (34 µl, 0.66 mmol) in CHCl$_3$ (5 ml) was added dropwise to a solution of **9** (100 mg, 0.64 mmol) in $CHCl_3$ (30 ml) with ice cooling. After 30 min the precipitate was filtered, washed with $CHCl_3$ (5 ml) and dried to give yellow solid (65 mg). Fractional precipitation from ethanolic solution of the product with diethyl ether yielded white crystals, that were filtered, washed with diethyl ether and dried (32 mg, 15%). M.p. 198–200 ^{0}C.



For X-ray data see the manuscript.

^{1}H NMR (DMSO-d_6): 1.31 (m, 1H, *Jgem* = 13.2, *J* = 10.6, 9.7, 3.1, H^{ax5}), 1.45 (m, 1H, *Jgem* = 12.8, *J* = 10.6, 4.0, 3.1, H^{ax4}), 1.67 (m, 1H, *Jgem* = 13.2, *J* = 4.0, 3.1 H^{eq5}), 1.87 (m, 1H, *Jgem* = 12.8, *J* = 5.3, 3.1, H^{eq4}), 1.91 (m, 1H, *Jgem* = 12.8, *J* = 12.4, 7.2, 4.4, H^{ax6}), 2.12 (m, 1H, *Jgem* = 12.8, *J* = 9.7, 4.4, 3.1, H^{eq6}), 4.13 (ddd, 1H, *J* = 10.6, 5.7, 5.3, H^7), 4.75 (ddd, 1H, *J* = 12.4, 7.2, 4.4, H^{3a}), 4.98 (dd, 1H, *J* = 7.2, 5.3, H^{7a}), 9.30 (br s), 9.67 (br s), 10.03 (br s).

^{1}H NMR (D_2O): 1.33 (m, 1H, *Jgem* = 13.3, *J* = 9.9, 6.0, 3.2, H^{ax5}), 1.66 (m, 1H, *Jgem* = 13.8, *J* = 11.0, 3.4, H^{ax4}), 1.78 (m, 1H, *Jgem* = 13.3, *J* = 3.9, 3.5 H^{eq5}), 1.95 (m, 1H, *Jgem* = 13.8, *J* = 4.6, H^{eq4}), 2.05 (m, 1H, *Jgem* = 13.0, *J* = 9.7, 9.1, 3.2, H^{ax6}), 2.20 (m, 1H, *Jgem* = 13.0, *J* = 8.7, 4.4, 3.3, H^{eq6}), 4.17 (ddd, 1H, *J* = 11.0, 5.3, 5.1, H^7), 4.58 (ddd, 1H, *J* = 12.1, 7.1, 4.1, H^{3a}), 5.01 (dd, 1H, *J* = 7.1, 5.3, H^{7a}).

^{13}C NMR (DMSO-d_6): 21.62 (C^5), 26.34 (C^4), 31.42 (C^6), 47.04 (C^7), 57.32 (C^{7a}), 58.31 (C^{3a}), 170.26 (C^2). ^{13}C NMR (D_2O): 21.47 (C^5), 25.82 (C^4), 31.08 (C^6), 45.74 (C^7), 57.90 (C^{7a}), 58.72 (C^{3a}), 171.87 (C^2).

MS (EI, m/z, I%): 236 ($[M+2]^+$, 15), 234 ($[M]^+$, 14), 203 ($[M+2-SH]^+$, 2), 201 ($[M-SH]^+$, 1), 155 (100), 126 (7), 128 (7), 113 (26), 81 ($[Br]^+$, 6), 79 ($[Br]^+$, 6).

Biotesting *in vivo*. The vasopressor action of compounds **2** (·HBr), **7** and **8** was studied on Wistar rats (KYO, males, 3–4 months, mass 240–270 g) with induced acute severe septic shock. Compound **2** (·HBr) was also tested on the model of prolonged endotoxemia.

Before the operative intervention each animal was anesthetized with sodium thiopental (60 mg/kg, intraperitoneally). A tracheostome was placed, catheterization was performed with the jugular vein and carotid arteries, invasive sensors of blood pressure and electrodes of ECG registration were connected. Heparin (100 units/ml) was injected intravenously. When the state of the animals stabilized, the following initial parameters were registered using PowerLab 8/30 system (ADInstruments, Australia): the heart rate (beats per minute), respiratory rate (breaths per minute), systolic (SBP) and diastolic (DBP) blood pressure (mm Hg) in the left carotid artery.

The acute endotoxic (vasodilatation) shock was induced by intravenous injection of lipopolysaccharide *E. coli* (0111:B4; Sigma–Aldrich, USA) in a dose 18 mg/kg.

To model the ***prolonged endotoxemia (refractory vasoplegia)*** LPS was administered twice intraperitoneally at a dose of 5 mg/kg in 0.5 ml solution: the first injection was done 18 hours before the start of the study, the second one – after connecting the sensors and recording the baseline values in the anesthetized animal.

After development of a stable hypotension – in 20–30 minutes after the last lipopolysaccharide injection (blood pressure reduction to 60–70% of initial values or the values of intact animals) – the physiologic parameters were repeatedly measured. Tested compounds **2** (·HBr), **7** and **8** were dissolved in a drop (10 μl) of ethyl alcohol and then in isotonic sodium chloride solution and were syringed intraperitoneally in one dose 20 mg/kg (0.1 ml per 100 g of body weight). Further monitoring of the parameters was being continued within the following 90 min. The rats survived in the end of the tests were deprived from the experience by air embolism.