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Design of Potential NO-Synthase Inhibitors on the Basis of 2-Amino-5,6-dihydro-4*H*-1,3-thiazine Derivatives

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Abstract—Two spiro analogues of 2-amino-5,6-dihydro-4H-1,3-thiazine, a known NO-synthase inhibitor, were synthesized by means of cyclization of N-2-(1-cyclohexenyl)ethylthioureas.

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Nitrogen oxide NO is an essential neurotransmitter in humans and animals [1]. Its production in cells is regulated by the enzyme NO-synthase, which exists in humans in three isoforms. Two isoforms are constitutive, and the third is induced by some pathologies (e.g., in septic shock). We are now working on design of NO-synthase inhibitors targeted to the induced isoform of this enzyme (iNOS).

2-Amino-5,6-dihydro-4*H*-1,3-thiazine (**1**) is a non-selective NO-synthase inhibitor [2]; a methyl substituent in the position C^6 noticeably increases both the activity and the iNOS selectivity of the inhibitor [1].

This work concerns the synthesis of 2-amino-1-thia-3-azaspiro[5.5]dodec-2-ene (**2**), a spiro analogue of structure **1** in which a cyclohexane moiety is added in the position at C^6 (Scheme 1).

We stress that it is difficult to predict the efficiency of binding of structure **2** with the reactive site of the enzyme. On the one hand, the known 6,6-dimethyl derivative of thiazine **1** has a lower activity than the corresponding monosubstituted analogue; therefore, the activity of compound **2** can be not high. On the other hand, our computer modeling showed that spirothiazine **2** in the catalytic site of the enzyme can have an orientation such that their binding is efficient due to the generation of two hydrogen bonds through the amino hydrogen atoms and due to the interaction between the lone pair of the sulfur atom and the iron atom in the catalytic site of the heme (figure).

Whether this orientation of compound **2** will be implemented is questionable. According to the literature on 2-ethylisothiourea docking to the binding area of the iNOS endogenic substrate (L-arginine), the major ligand-orienting factor in the area in question is its interaction with the Glu377 acid group of iNOS [3]. However, as a result of the possibility of protonation of both the exocyclic nitrogen atom and the nitrogen atom of the thiazine ring in structure **2**, as well as in the base compound **1**, the ligand orientation in the reactive site differs considerably depending on which atom is bound to Glu377. Therefore, to gain more data on the possible position of compound **2** in the catalytic site, we decided to synthesize its N-methyl derivative **3**.[1]

We prepared novel compounds **2** and **3** from N-2-(1-cyclohexenyl)ethylthioureas **4** and **5**, whose cyclization upon boiling with hydrochloric acid generates spiro compounds (Scheme 2).

This method is convenient for preparing spirothiazines **2** and **3** in the form of hydrochlorides with overall yields (from 2-(1-cyclohexenyl)ethylamine) equal to 79 and 68%, respectively. The structure of compounds **2**–**5** was verified by elemental analysis and NMR spectroscopy (see Experimental). Spirothiazines **2** and **3** were sent for in vivo and in vitro tests of their NO-inhibitory activity.

EXPERIMENTAL

For our molecular modeling, we used the model of the L-arginine-binding cavity of the oxidase domain of iNOS taken from the crystal structure database (PDB) and the SYBYL7.3 program package (Tripos) on SGI OCTANE workstations.

^{1}H NMR spectra were recorded on a Bruker DPX-200 spectrometer operating at 200 MHz relative to internal trimethylsilane. The course of the reaction and the purity of the reagents and products were monitored by thin-layer chromatography on Silufol UV-254

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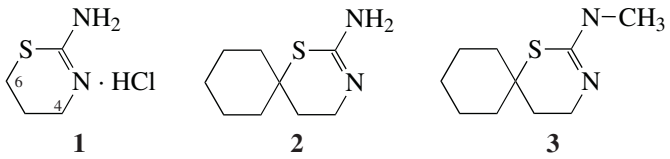
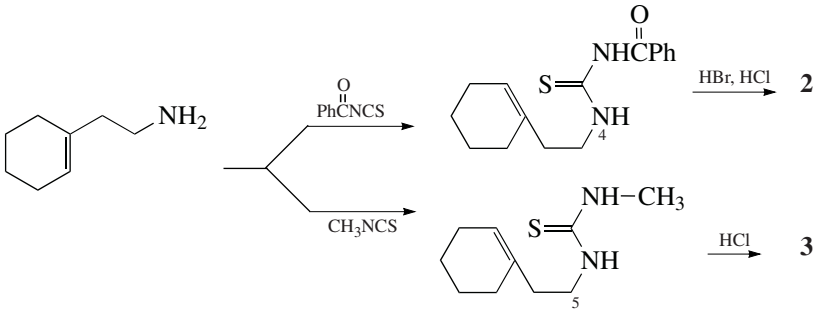
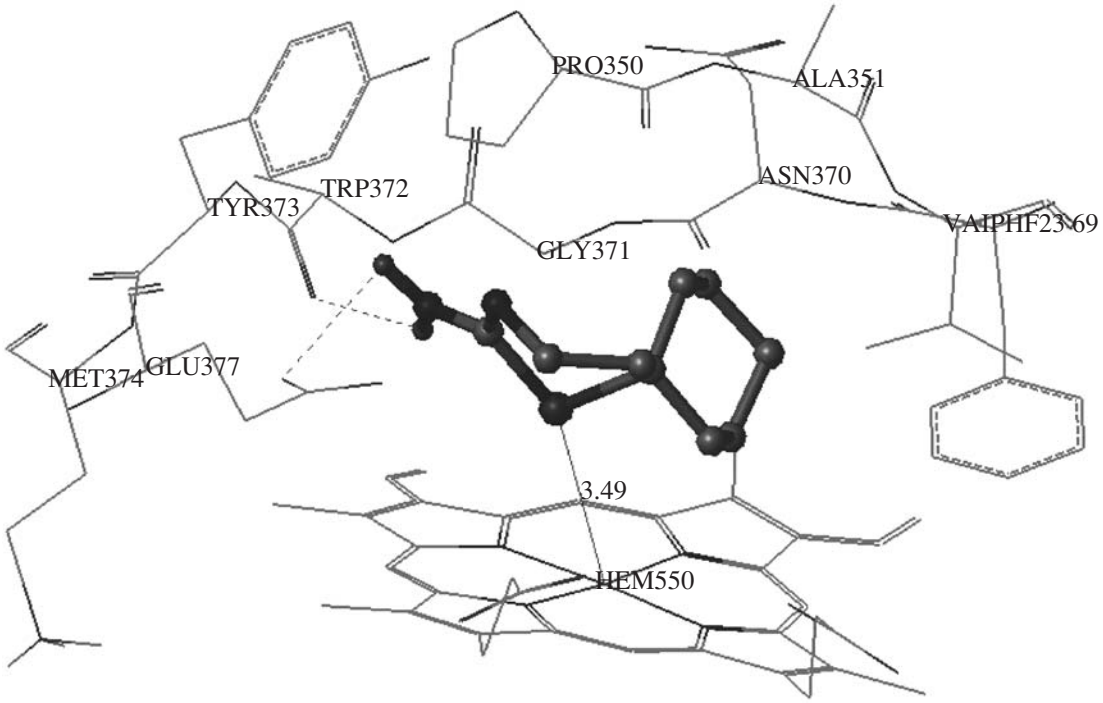
[1] Patent [2] claims a spiro analogue of compound **1** in position 4 as an NO-synthase inhibitor, although neither the synthesis method nor the activity of this compound is specified. It is, however, evident that, from the chemical standpoint, this compound strongly differs from structure **2** we consider in this work.



Scheme 1.



Scheme 2.



Suggested version of the interaction of compound *2* with the reactive site of iNOS.

plates. Melting temperatures were determined on a Boethius heating unit.

N-Benzoyl-N'-2-(1-cyclohexenyl)ethylthiourea (4). To a solution of 2-(1-cyclohexenyl)ethylamine (2.52 g, 0.02 mol) in diethyl ether (40 mL), a solution of benzoyl isothiacyanate (3.26 g, 0.02 mol) in diethyl ether (20 mL) was dropped. A precipitate was filtered and washed with diethyl ether. Compound **4** was obtained in 96% yield (5.54 g). T_m = 86–88°C.

For compound **4** anal. calcd. (%): C, 66.63; H, 6.99; N, 9.71.

Found (%): C, 66.97; H, 6.87; N, 9.88.

^{1}H NMR spectrum (DMSO-d_6; δ, ppm): 11.20 (1H, s, NH), 11.00 (1H, t, NH), 8.00 (2H, d, Ar–H), 7.50 (3H, m, Ar–H), 5.60 (1H, br s, C=H), 3.70 (2H, q, CH_2N), 2.30 (2H, t, $\underline{CH_2}CH_2N$), 2.00 (4H, m, $(CH_2)_2$), 1.70 (4H, m, $(CH_2)_2$).

N-Methyl-N'-2-(1-cyclohexenyl)ethylthiourea (5) was prepared as described above. Yield: 93%. T_m = 75–77°C.

For $C_{10}H_{18}N_2S$ anal. calcd. (%): C, 60.56; H, 9.15; N, 14.12.

Found (%): C, 60.83; H, 9.38; N, 14.40.

^{1}H NMR spectrum ($CDCl_3$; δ, ppm): 6.70 (1H, br s, C=H), 6.30 (1H, br s, NH), 5.15 (1H, br s, C=H), 3.20 (2H, q, CH_2N), 2.40 (3H, d, CH_3), 1.80 (2H, t, $\underline{CH_2}CH_2N$), 1.70 (4H, m, $(CH_2)_2$), 1.30 (4H, m, $(CH_2)_2$).

2-Amino-1-thia-3-azaspiro[5.5]dodec-2-ene hydrochloride (2). To compound **4** (2.88 g, 0.01 mol), concentrated HBr (10 mL) was added, and the mixture was refluxed for 30 h. The reaction mixture was cooled, a benzoic acid precipitate was filtered and diluted with cold water (40–50 mL), and Na_2CO_3 was added with stirring to bring pH to 8. Extraction was carried out with methylene chloride (2 × 30 mL). The organic layer was dried, concentrated, and dissolved in isopropanol (20 mL). Strong hydrochloric acid (1 mL) was added with stirring. The precipitate was filtered and dried. Compound **2** was obtained in 82% yield (1.81 g). T_m = 70–72°C.

^{1}H NMR spectrum (D_2O; δ, ppm; *J*, Hz): 3.55 (2H, t, *J* = 6.7, CH_2N), 2.05 (2H, t, *J* = 6.7, $\underline{CH_2}CH_2N$), 2.00–1.20 (10H, m, $(CH_2)_5$).

2-Methylamino-1-thia-3-azaspiro[5.5.]dodec-2-ene hydrochloride (3). To compound **5** (1.98 g, 0.01 mol), strong HCl (20 mL) was added, and the mixture was refluxed for 3 h. The reaction mixture was cooled, excess hydrochloric acid was eliminated on a rotary evaporator, the residue was diluted with cold water (40–50 mL), and Na_2CO_3 (2 × 30 mL) was added with stirring to adjust pH to 8. Extraction was performed with methylene chloride (2 × 30 mL). The organic layer was dried, concentrated, and dissolved in isopropanol (20 mL). Strong hydrochloric acid (1 mL) was added with stirring. The precipitate was filtered and dried. Compound **3** was obtained in 73% yield (1.71 g). T_m = 140–142°C.

^{1}H NMR spectrum (DMSO-d_6; δ, ppm; *J*, Hz): 10.55 (H, m, NH), 9.70 (H, m, NH), 3.60 (3H, s, NCH_3), 3.50 (2H, t, *J* = 6.7, CH_2N), 1.70–1.25 (12H, m, $(\underline{CH_2})_5$, $\underline{CH_2}CH_2N$).

ACKNOWLEDGMENTS

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