chemistry proceedings

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Proceeding Paper

Structural Analogues of Thyronamines. Some Aspects of the Structure and Bioactivity of the 4-[4-(2-Aminoetoxy)benzyl]aniline †

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† Presented at the 28th International Electronic Conference on Synthetic Organic Chemistry (ECSOC 2024), 15–30 November 2024; Available online: https://sciforum.net/event/ecsoc-28.

Abstract: Molecular modeling of the structure and evaluation of chemical shifts of ^{1}H and ^{13}C nuclei were performed for the 4-[4-(2-aminoethoxy)benzyl]aniline, that is a structural analogue of thyronamines. The intramolecular dynamics of the key structural fragments of 4-[4-(2-aminoethoxy)benzyl]aniline was studied by the PM6-DH2 method as well as at B3LYP/6-31G(d,p) level of theory. Nonspecific solvation with dimethyl sulfoxide was taken into account within the polarized continuum model. Specific solvation by one and two DMSO molecules was considered within supermolecule approximation. Chemical shifts of the ^{1}H and ^{13}C nuclei were estimated for the most stable conformer of 4-[4-(2-aminoethoxy)benzyl]aniline as well as for its solvates with DMSO. Calculated chemical shifts of the ^{1}H and ^{13}C nuclei are in good agreement with the experimental ones obtained for 4-[4-(2-aminoethoxy)benzyl]aniline in DMSO-d_6 solution. Some aspects of bioactivity of the 4-[4-(2-aminoethoxy)benzyl]aniline are discussed.

Keywords: thyronamines; intramolecular dynamics; conformers; DFT calculations; NMR spectroscopy; chemical shift; antioxidant effects

Citation: Eresko, A.B.; Raksha, E.V.; Filimonov, D.A.; Trubnikova, N.N.; Kisilenko, I.A.; Chudoba, D.M. Structural Analogues of Thyronamines. Some Aspects of the Structure and Bioactivity of the 4-[4-(2-Aminoetoxy)benzyl]aniline. *Chem. Proc.* **2024**, *6*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

Tyronamines are endogenous compounds generated from L-thyroxine or its intermediate metabolites by deiodination and decarboxylation. The ability of endogenous thyronamines T0AM and T1AM to induce hypothermia without causing compensatory reactions in the form of chills and piloerection was revealed experimentally. Thyronamines are regulators of thermogenesis and are promising for use as pharmacological inducers of hypothermia [1–3]. As a result of studies aimed at searching for synthetically available structural analogs of thyronamines, 4-[4-(2-aminoethoxy)benzyl]aniline (**1**) and 4-[4-(2-aminoethoxy)benzyl]phenol (**2**) have been proposed (Figure 1). Biological activity of new structural analogues of thyronamines is comparable, or even exceeds the activity of endogenous ones [4,5]. Compounds (**1**) and (**2**) have pleiotropic neuroprotective properties and are being actively studied [1,6].

Experimental studies of the structural features of endogenous thyronamines and their precursors (thyroid hormones) in condensed state were carried out using X-ray diffraction analysis [7–10] and NMR spectroscopy [11,12]. Single crystal X-ray studies of endogenous thyroid hormones and related thyronamines were performed by Mondal S. and Mugesh G. [11] and the conformational changes that take place in their structures upon

deiodination were investigated. Internal molecular dynamic investigations of thyroxine, 3,5,3′-triiodothyronine, and 3,5-diiodothyronin by NMR-spectroscopy revealed that thyroxine and the other thyroid hormones are able to freely move over a moderately large region of conformational space. Moreover, the interaction of endogenous T1AM and its synthetic analogues with the transthyretin was recently studied using NMR spectroscopy (2D ^{1}H-^{15}N TROSY-HSQC and 3D TROSY-HNCA) [13]. It has been shown that thyronamines form a stable complex with transthyretin and thus suppress its acid-induced aggregation. Thus, to correctly plan further detail NMR studies of thyronamines, reliable information on their magnetic properties is necessary, and the ability to predict the magnetic properties of new representatives of this compounds is also important.

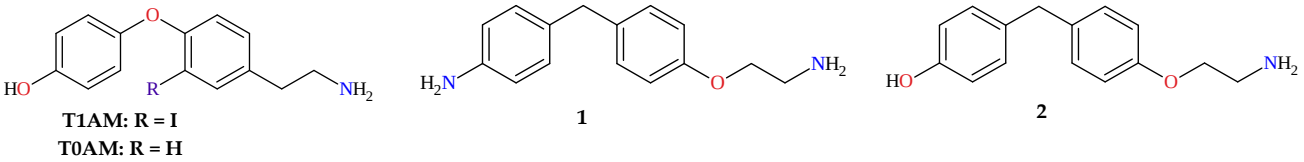

Figure 1. Chemical structures of endogenous thyronamines T0AM and T1AM and their structural analogs: 4-[4-(2-aminoethoxy)benzyl]aniline (**1**) and 4-[4-(2-aminoethoxy)benzyl]phenol (**2**).

DFT methods are actively used to investigate structure and physico-chemical properties of [11,14,15] thyroid hormons as well as their derivatives. To the best of our knowledge new synthetic thyronamines have not been studied and characterized by quantum chemical methods. An integrated approach to the study of the structural features of biologically active compounds using the possibilities of experimental methods of NMR spectroscopy and in silico evaluation of the parameters of NMR spectra provides more reliable results [16]. In this paper we report original research focused on the features of NMR spectra of the new synthetic thyronamine—4-[4-(2-aminoethoxy)benzyl]aniline (**1**) in DMSO-d_6 solution. Antioxidant effects of the 4-[4-(2-aminoethoxy)benzyl]aniline in experimental cerebral ischemia were estimated and discussed.

2. Experimental and Computational Details

2.1. Synthesis of 4-[4-(2-Aminoethoxy)benzyl]aniline

Synthesis of 4-[4-(2-aminoethoxy)benzyl]aniline was carried out in accordance with the procedure reported by Chiellini G. and coworkers [5]. 4-[4-(2-Aminoethoxy)benzyl]aniline hydrochloride was converted to the base by making its aqueous solution alkaline with a saturated aqueous sodium carbonate solution, followed by extraction with methylene chloride, drying of the extract, and evaporation under vacuum.

Yield 62% in the form of dihydrochloride, m.p. 180 °C (with decomposition). NMR ^{1}H spectrum of the base (400 MHz, DMSO-d_6), δ, ppm: 3.03 t (2H, CH_2, J 4.0 Hz), 3.69 s (2H, CH_2), 3.99 t (2H, CH_2, J 4.0 Hz), 5.14 br. s (NH_2 in exchange with water), 6.47 d (2H, H 3′,5′, J 8.0 Hz), 6.80 d (4H, H 2, 6, 2′,6′, J 8.0 Hz), 7.04 d (2H, H 3, 5, J 8.0 Hz). NMR ^{13}C spectrum of the base (100 MHz, DMSO-d_6), δ, ppm: 38.43, 39.71, 64.15, 114.05 (2C), 114.24 (2C), 128.56, 128.74 (2C), 129.20 (2C), 134.86, 146.00, 155.78. Found, %: C 57.19; H 6.37; N 8.98. $C_{15}H_{20}Cl_2N_2O$. Calculated, %: C 57.15; H 6.40; N 8.99. M 315,238.

2.2. NMR ^{1}H and ^{13}C Spectroscopy

NMR ^{1}H and ^{13}C spectra of the 4-[4-(2-aminoethoxy)benzyl]aniline were recorded on a Bruker Avance instrument (400 MHz) in DMSO-d_6.

2.3. Bioactivity of 4-[4-(2-Aminoethoxy)benzyl]aniline

Detailed information on methods used for 4-[4-(2-aminoethoxy)benzyl]aniline antioxidant effects in experimental cerebral ischemia is listed in [17].

2.4. Semiempirical and DFT Calculations

At the first stage, the parameters of molecular geometry, electronic structure, and thermodynamic characteristics of thyronamine **1** were calculated by the dispersion corrected semi-empirical method PM6-DH2 [18–20] method using the MOPAC2016 software package [21]. Then the intramolecular dynamics of thyronamine **1** was studied by PM6-DH2 method. Dihedral angles C(8)-C(1)-C(2)-C(3) (α), C(5)-C(7)-O(15)-C(16) (β), O(15)-C(16)-C(17)-N(18) (γ) were used as coordinates of intramolecular rotation (Figure 2). Their mutual arrangement determines the spatial configuration of the molecule. The values of α, β, and γ were varied within 0°–360° with a step of 15°.

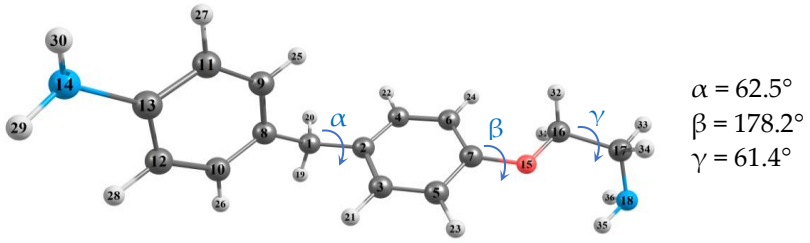

Figure 2. 3D model of the most stable conformer of 4-[4-(2-aminoethoxy)benzyl]aniline (**1**) with atom numbering used for the results discussions (C—dark grey, H—grey, O—red, N—blue).

The molecular geometry and the electronic structure parameters, as well as thermodynamic characteristics of the most stable thyronamine **1** conformer were also calculated using the Orca 5.0.2 software package [22]. The calculations were performed at the B3LYP [23–25] level of the theory with the 6-31G(d,p) basis set. Nonspecific solvation by dimethyl sulfoxide (DMSO) was accounted within the framework of the polarizable continuum model. Specific solvation of thyronamine **1** by one or two molecules of DMSO was also considered within supermolecule approximation. Based on the magnetic shielding constants (χ, ppm) calculated by the gauge-independent atomic orbital (GIAO) method [26] method, the chemical shifts (δ, ppm) of the ^{1}H and ^{13}C nuclei in the studied objects were estimated. Tetramethylsilane was used as a standard, for which full optimization of molecular geometry and calculation of χ were performed using the same level of theory and basis set.

3. Results and Discussions

Investigation of the intramolecular dynamics of key fragments of the 4-[4-(2-aminoethoxy)benzyl]aniline molecule in the PM6-DH2 approximation has resulted in the most stable conformer of this compound. Subsequent optimization of the molecular geometry at the B3LYP/6-31G(d,p)/PCM level with taking into account the nonspecific solvation with DMSO has revealed the synclinal configuration of the aniline and ethylamine fragments in studied thyronamine **1** (Figure 2) with α = and β =. The calculated values of the rotation barriers of thyronamine **1** are within 1.34–10.07 kJ/mol, which makes it possible to characterize the structure of this compound as labile. The largest barriers correspond to the intramolecular dynamics of the oxybenzoic fragment, and the smallest, to the aniline one.

For the most stable conformer of the thyronamine **1**, the magnetic shielding constants of the ^{1}H and ^{13}C nuclei were calculated and the chemical shifts of these nuclei were estimated based on them. Linear correlations were observed between the experimental and calculated values of chemical shifts. Experimental chemical shifts of H(29), H(30) and H(35), H(36) protons (NH_2 groups) are sensitive to the solvent and take part in exchange processes. Therefore, they were not considered on the first step of analysis. Parameters of the others ^{1}H and ^{13}C nuclei are quite correctly reproduced at used level of theory. The value of the mean absolute error (MAE) is 0.16 ppm for ^{1}H nuclei and 4.22 ppm for ^{13}C nuclei, respectively.

In experimental NMR ^{1}H spectrum of thyronamine **1** only one broad singlet at 5.14 ppm was observed for two NH_2 groups protons. Accounting of nonspecific solvation in calculation was not effective for chemical shifts of this groups (values of 3.06 and 0.67 ppm were obtained). Thus we considered a specific solvation of NH_2 groups with one and two molecules of DMSO within structural models presented in Figure 3. Obtained after optimization (B3LYP/6-31G(d,p)/PCM) complexes **1–3** were stabilized by intermolecular hydrogen bonds O···H-N as well as additionally by nonvalent C-H···C interactions.

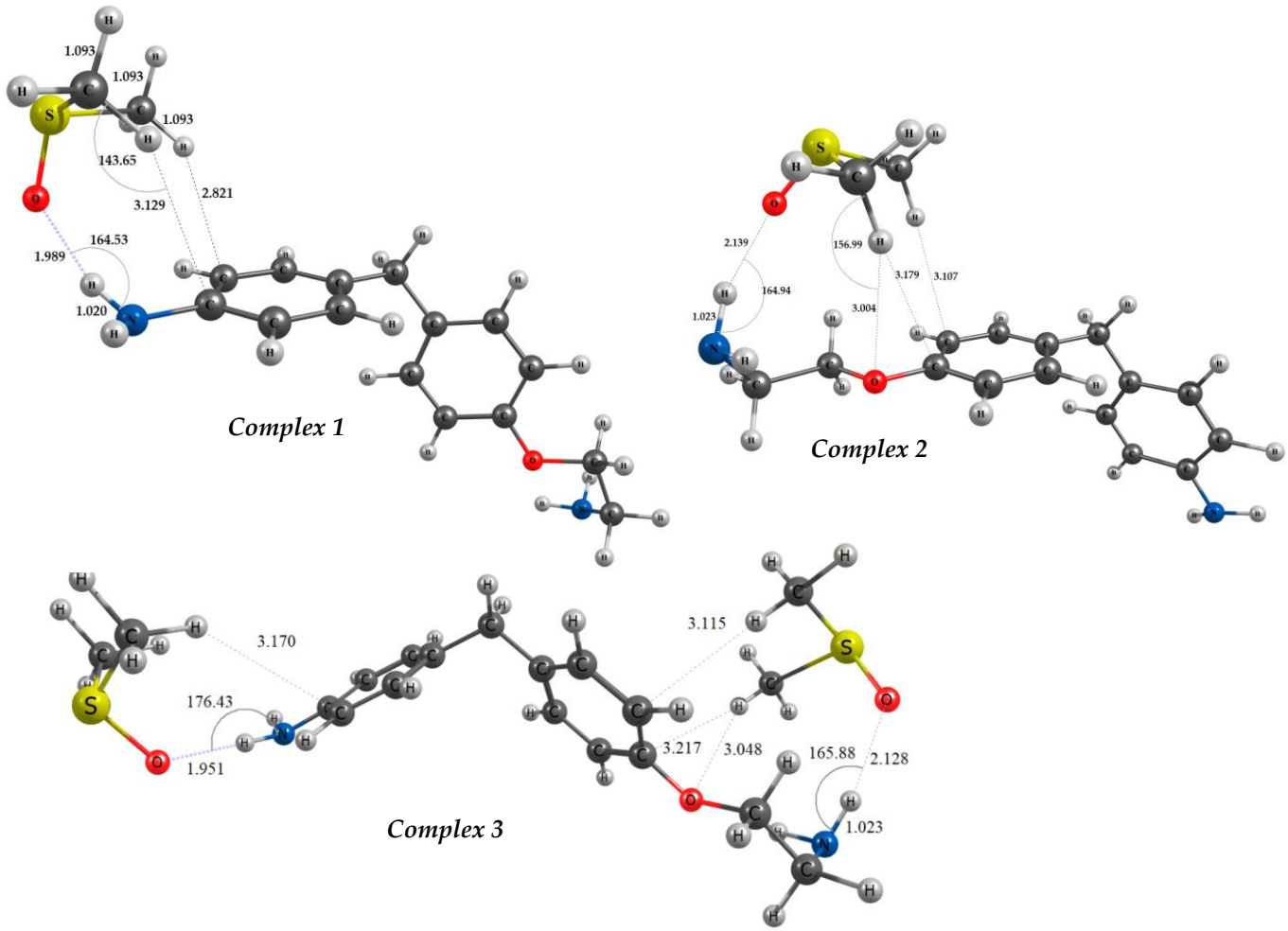

Figure 3. Structure of solvate complexes of 4-[4-(2-aminoethoxy)benzyl]aniline with DMSO molecules (B3LYP/6-31G(d,p)/PCM).

Specific solvation of aniline fragment (complex **1** and **3**) resulted in shift of δ(NH_2) up to 4.48 and 4.49 ppm, whereas solvation of aminoethoxy fragment (complex **2** and **3**) yields δ(NH_2) values 1.22 and 1.26 ppm. But in experimental NMR ^{1}H spectrum of thyronamine **1** in DMSO-d_6 there were no signals in the region about 0.1–2.4 ppm. All chemical shifts obtained for thyronamine **1** and considered complexes **1–3** are listed in Supplementary (Tables S1 and S2). The best agreement between the calculated and experimental values of the ^{1}H nuclei in DMSO was obtained for the complex **1** with specific solvation of aniline fragment.

Antioxidant effects of the thyronamine **1** in experimental cerebral ischemia were estimated. Permanent ligation of the right common carotid artery was performed to simulate acute cerebral ischemia in white rats. The animals were divided into two groups: the control group receiving vehicle (0.5 mL DMSO solution + 0.5 mL NaCl 0.9% solution) and the experimental group, to which the thyronamine **1** was intraperitoneally administrated (75 mg/kg of the rat's body weight, dissolved in vehicle). After 24 h the rat was

decapitated, and the cerebral cortex tissue was extracted for biochemical analysis. The following LP indicators were determined by spectrophotometry: malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx). When administering the thyronamine **1**, a significant (2-fold) decrease in MDA levels was observed in the ischemic hemisphere (p = 0.022), along with the 2.49-fold increase in the GPx activity in the brain tissue (p = 0.004) of the intact hemisphere and the 2.65-fold increase in its activity (p = 0.021) in the ischemic hemisphere, as well as the 1.23-fold increase in SOD activity in the ischemic hemisphere (p = 0.042) [17].

4. Conclusions

According to our results one can conclude that the experimentally observed chemical shift of the NH_2 group of the aniline fragment (5.14 ppm) in DMSO-d_6 is due to its specific solvation. And for the amino group of aminoethoxy fragment, it is apparently necessary to take into account possible protonation in the presence of trace amounts of water. This will be the subject of our further studies. The 4-[4-(2-aminoethoxy)benzyl]aniline has a great potential in terms of activation of the antioxidant protection mechanisms in the cerebral cortex of white laboratory rats under conditions of acute hemispheric ischemia.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Experimental (δ_{exp}, ppm) and calculated (δ_{calc}, ppm) chemical shifts of the ^{13}C nuclei of 4-[4-(2-aminoethoxy)benzyl]aniline and its complexes with DMSO (B3LYP/6-31G(d,p)/PCM); Table S2: Experimental (δ_{exp}, ppm) and calculated (δ_{calc}, ppm) chemical shifts of the ^{1}H nuclei of 4-[4-(2-aminoethoxy)benzyl]aniline and its complexes with DMSO (B3LYP/6-31G(d,p)/PCM).

Author Contributions: Conceptualization, A.B.E., E.V.R. and D.A.F.; methodology, A.B.E., E.V.R. and D.A.F.; software, E.V.R. and D.M.C.; validation, E.V.R., N.N.T. and I.A.K.; formal analysis, E.V.R., N.N.T. and I.A.K.; investigation, A.B.E., E.V.R. and D.A.F.; resources, A.B.E., D.M.C. and D.A.F.; data curation, E.V.R., N.N.T. and I.A.K.; writing—original draft preparation, A.B.E., E.V.R., D.A.F. and N.N.T.; writing—review and editing, A.B.E., E.V.R., D.M.C. and D.A.F.; visualization, E.V.R.; supervision, A.B.E., D.M.C. and D.A.F.; project administration, A.B.E., D.M.C. and D.A.F.; funding acquisition, A.B.E., D.M.C. and D.A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the FEDERAL STATE BUDGETARY INSTITUTION "V.K. GUSAK INSTITUTE OF EMERGENCY AND RECONSTRUCTIVE SURGERY" OF THE MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION (protocol No. 3; 23 November 2023).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available within the paper and from the corresponding author upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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