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Synthesis and *In Silico* Research of Derivatives of 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazole-2-imine

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ABSTRACT

The synthesis of those new derivatives of 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazole-2-imine was conducted which consist substituted aromatic radicals. Structure of the compounds synthesized has proved by means of a comprehensive use of modern physical and chemical methods of analysis. In silico research has been conducted of the potential molecular mechanisms of cardioprotective action of the obtained substances with the method of a soft molecular docking, and recommendations to the rational design of the ligands have been made.

Keywords: Derivatives of 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazole-2-imine, Synthesis, Molecular docking

INTRODUCTION

Those thiazoles which contain main nitrogen atom are of great practical and fundamental interest. Analysis of the scientific literature indicates that numerous of their derivatives can be applied as medicines for the treatment of infectious [1], autoimmune, inflammatory, oncological [2], neurogenetic [3], cardiovascular [4] and other diseases. Therefore, the search for new biologically active substances among biogenic thiazoles, which have aliphatic, aromatic, heterocyclic radicals in their structure, the development of promising techniques for their synthesis, the study of physical and chemical properties, pharmacological activity is an urgent task of modern medical chemistry. Currently, a search for selective inhibitors of biomasks is actively being carried out in order to find new medicines with cardioprotective action, cerebral and angio-protectants. *In silico* methods allow shorten time, expenditures and reduce the number of laboratory animals by performing a procedure similar to high-performance biological screening.

MATHERIALS AND METHODS

The starting, auxiliary substances and solvents used in the work have been obtained and purified by standard procedures. Propene-3-amine, 4-R-acetophenone were purchased from AcrosOrganics and used without further purification. Anisometric thioureas 1a-h was obtained by interaction of R-phenylisothiocyanates and propen-3-amine in a dry dioxan medium [5]. The synthesis of R-phenylisothiocyanates was carried out by treatment of aromatic amines with tetramethylthiuram disulfide followed by destruction of intermediate product $N_{(1)}$-aryl-N,N-dimethylthiourea by concentrated HCl [6]. α-Bromo-4-R-acetophenone 2 was prepared by bromination of the 4-R-acetophenone [7]. The forming of the final products of reaction was monitored by thin layer chromatography through the use of Fluka silica gel (60 F254) plates (0.25 mm). The visualization was carried out by UV-radiation. The melting points of the synthesized compounds were determined by means of the Kofler device. The elemental analysis of the nitrogen content was determined by the method of Dumas. ^{1}H-NMR spectra were recorded on a VarianGemini 400 MHz device in DMSO-d_6, Tetramethylsilane (TMS) was used as an internal standard.

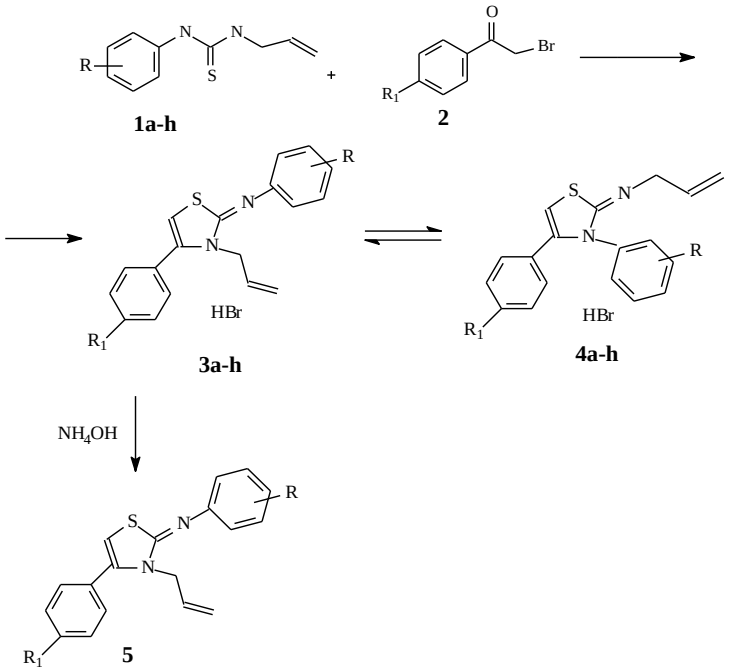
For the receptor-oriented flexible docking, the Autodock 4.2.6 software package was used [8-11]. The preparation of ligands was carried out with the help of programs Vega ZZ (command line) [9] and MGL Tools 1.5.6 [8]. To perform calculations in the Autodock 4.2.6 program, the original formats of the receptor and ligands data were converted into a special format PDBQT. Formation of PDBQT files, calculation of dihedral angles and removal of hydrogen atoms in nonpolar atoms for the investigated ligands was cconducted in the program Vega ZZ (command line). As a biological target for the docking, the active center of macromolecule was used from Protein Data Bank (PDB) of the gamma butirobetaine hydroxylase enzyme (PDB ID: 3O2G) [10]. The choice of biotarget is conditioned by the data in the literature on the mechanism of action of the reference preparations mildronate and levocarnitine. The receptor's cards were prepared in MGL Tools and AutoGrid programs. From the PDB-file ID: 3O2G molecules of water, ions and ligand were removed. The following docking parameters were set up: a step of propulsion was equal to 2 Å, an angle of quaternion was 50°, a dihedral angle was 50°. The torsial degree of freedom and coefficient were 2 and 0.274, respectively. The cluster tolerance was 2 Å. The external energy of the breaker plate was 1000, the maximum initial energy was 0 and the maximum number of efforts was 10,000.

The number of structures in the population was 300, the maximum number of stages of the energy assessment was 850,000, the maximum number of generations was 27,000, the number of structures which were going over the next generation-1, the level of genome mutation was 0.02, the crossover's level was 0.8, the crossover's method was arithmetic. α-parameter of the Gaus distribution was equal to 0, β-parameter of the Gaus distribution-1. The number of interactions of the genetic algorithm- Lamarck search was 50 for each ligand. A visual analysis of the complexes of substances in the active center of the enzyme of gamma butirobetaine hydroxylase was carried out using the program Discovery Studio Visualizer 4.0 [12].

RESULTS AND DISCUSSION

A new series of hydrobromide derivatives of 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazole-2-imine (3a-h) were obtained by interaction derivatives of asymmetric thioureas (1a-h) with α-brom-4-R-acetophenones 2 in equimolar amounts by boiling in ethanol for 3 h. 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazol-2-imine 5 was obtained by neutralizing the corresponding hydrobromide with a 10% solution of NH_4OH. As a result of the reaction, two isomeric structures 3a-h or 4a-h are possible to be formed (Scheme 1).

The obtained hydro bromides of 3-allyl-4-(R-phenyl)-N-(R_1-phenyl)-thiazol-2-imine (3a-h) are crystalline solvents of white and light yellow colour, soluble in water, methanol, ethanol, propanol-2, slightly soluble in heptane, and insoluble in chloroform. The synthesized compound 5 is a white crystalline substance which is soluble in methanol, ethanol, propanol-2, slightly soluble in heptane, insoluble in chloroform, water.



3aR=CH_3, R_1=OCH_3; 3bR=CH_3, R_1=Cl; 3cR=CH_3, R_1=2',4'-$(Cl)_2$; 3dR=Br, R_1=Cl; 3eR=H, R_1=Cl; 3fR=Br, R_1=NO_2; 3gR=CH_3, R_1=CH_3; 3hR=CF_3, R_1=OCH_3; 5R=CH_3, R_1=OCH_3

Scheme 1: Isomeric structures

The structure of compounds which have been obtained is confirmed by the comprehensive use of modern physicochemical methods of analysis: ^{1}H-NMR spectroscopy, 2DNMR spectroscopy (NOEZY, ROEZY), elemental analysis, thin layer chromatography.

The research was carried out through the example of hydrobromide of 3-allyl-4-(4'-methoxyphenyl)-N-(4-methylphenyl)-thiazole-2-imine 3a. Herewith, it was discovered that the ^{1}H-NMR spectrum of the examined substance contain one singlet of an allyl fragment at 4.66 ppm, and 2 multiplets at 4.9-5.3 and 5.83-5.95 ppm, a singlet of the thiazole proton at 7.0 ppm, two multiplets for two AA'XX'-systems of two para-substituted aromatic nuclei. Fixation of the signals of the one aromatic nuclei proton at 7.05-7.16 ppm and the other one at 7.35-7.38 ppm was done on the basis of the data of NOESY experiment. In addition, the ROESY experiment has shown the presence of cross peaks between the signal of 4.66 ppm of allyl protons and ortho-protons of both aromatic nuclei (signals at 7.05 and 7.35 ppm), that indicates the location of allyl fragment between these two nuclei and, therefore, allows declare that the examined structure is 3-allyl-4-(4'-methoxyphenyl)-N-(4-methylphenyl)thiazol-2-imine 3a. To search for new biologically active substances as potential cardioprotective agents of metabolic action, a gamma butirobetaine hydroxylase enzyme was chosen as a biotarget for docking studies (PDBID: 3O2G) [10].

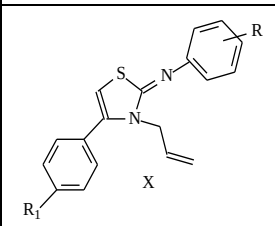
In order to determine the main interactions, a docking was carried out in the active center of gamma butyrobetaine hydroxylase of the reference preparations mildronate and L-carnitine. Under the same conditions, docking of 9 substances of the study sample was performed. All results were ranked by means of the scoring function of the Autodock 4.2.6 program and visually assessed by the presence of key interactions with the active site of the gamma butirobetaine hydroxylase enzyme.

The results of the study were characterized by a high level of affinity of substances to the active site of the enzyme, which is commensurate with classical inhibitors. The values of scoring functions of molecular docking for the studied compounds are presented in the Table 1. The results obtained were compared with the results of the control experiment, namely, with the scoring functions of the reference preparations of mildronate and L-carnitine: EDoc -5.68 kcal/mol and EDoc-5.41 kcal/mol, respectively.

Scoring functions for all examined substances have negative values and are comparable or exceed scoring functions for reference preparations in absolute values, that indicates a high thermodynamic probability of inhibitory activity towards this enzyme.

An analysis of the geometric arrangement of molecules of the substances synthesized in the active center of gamma butirobetaine hydroxylase enzyme indicates the formation of stable complexes through hydrogen bridges between ligands and aminoacid residues of tyrosine Tyr205, asparagine Asn191 and asparagine Asn292.

Table 1: Results of soft molecular docking of synthesized compounds with the gamma butirobetaine hydroxylase enzyme (PDBID: 3O2G)

Code of compound	Structure	R	R_1	X	EDoc kcal/mol
3a		CH_3	OCH_3	HBr	-9.74
3b		CH_3	Cl	HBr	-10.44
3c		CH_3	2',4'-$(Cl)_2$	HBr	-9.63
3d		Br	Cl	HBr	-8.99
3e		H	Cl	HBr	-9.77
3f		Br	NO_2	HBr	-9.35
3g		CH_3	CH_3	HBr	-6.95
3h		CF_3	OCH_3	HBr	-10.80
5		CH_3	OCH_3	-	-8.26

The hydrophobic contacts with tyrosine Tyr177 and tryptophan Trp181 contributed to stabilization of the complexes formed. Also, possibly, the complexes might be further stabilized by the π-H, π-cation or π-π interaction of phenyl rings of the examined compounds with Tyr177 and Tyr205. The control trials with mildronate and L-carnitine have confirmed significance of these interactions. The type of binding of compound 3 h to the acceptor site of gamma butirobetaine hydroxylase (PDBID: 3O2G) is represented in the Figure 1.

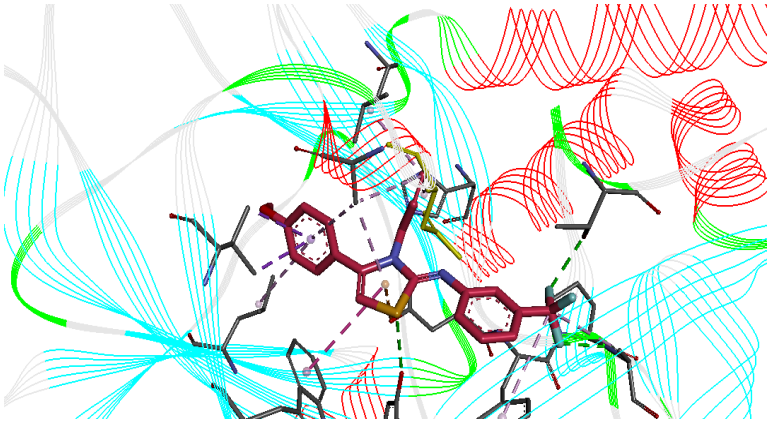


Figure 1: Super position of binding of compound 3h to the acceptor site of gamma butirobetaine hydroxylase

The obtained results indicate the possibility of stable complexes formation of substituted thiazole-2-imines with a biological target, in which the arrangement of ligands in the active center of receptor and amino acid residues of the side chains participating in the noncovalent bonds formation are similar to the geometry and types of binding of L-carnitine and mildronate, established on the basis of crystallographic studies.

The derivatives of thiazole-2-imine can be considered as a promising scafold for formation of the combinatorial libraries of potential biological active substances with cardiotropic action, namely by introducing new pharmacophore centers in the 3 position of the thiazole cycle, as evidenced by the results of virtual screening procedures.

General procedure for the synthesis

Hydrobromides of 3-allyl-4-(R-phenyl)-N-(R'-phenyl)-thiazole-2-imine (3a-h) were obtained by boiling equimolar amounts of unsymmetrical substitutes of thioureas in ethanol with α-brom-4-R-acetophenones for 3 h. 3-allyl-4-(4'-methoxyphenyl)-N-(4-methylphenyl)thiazol-2-imine 5 was obtained through neutralization of the corresponding hydrobromide by NH_4OH 10% solution. The obtained compounds were filtered, washed with water and crystallized from ethanol.

Hydrobromide of 3-allyl-4-(4'-methoxyphenyl)-N-(4-methylphenyl)-thiazole-2-imine (3a)

Yield 85%, MP=212-214°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.36 (s, 3H, OCH_3), 3.84 (s, 3H, CH_3), 4.66 (s, 2H, CH_2–CH=CH_2) 4.9-5.3 (m, 1H, CH_2–CH=CH_2), 5.83-5.95 (m, 2H, CH_2–CH=CH_2), 7.0 (s, 1H, thiazole), 7.05-7.5 (m, 8H, Ar-H), 10.80 (s, 1H, NH^+). Calculated for $C_{20}H_{20}N_2OS$ HBr N-6.71%. Measured, %: N-6.96.

Hydrobromide of 3-allyl-4-(4'-chlorophenyl)-N-(4-methylphenyl)-thiazole-2-imine (3b)

Yield 81%, MP=190-192°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.65 (s, 3H, CH_3), 4.5 (s, 2H, CH_2–CH=CH_2) 4.8-5.2 (m, 1H, CH_2–CH=CH_2), 5.9-6.0 (m, 2H, CH_2–CH=CH_2), 7.0 (s, 1H, thiazole), 7.1-7.6 (m, 8H, Ar-H), 10.80 (s, 1H, NH^+). Calculated for $C_{19}H_{17}N_2SCl$ HBr N-6.64%. Measured, %: N 7.14.

Hydrobromide of 3-allyl-4-(2',4'-dichlorophenyl)-N-(4-methylphenyl)-thiazole-2-imine (3c)

Yield 86%, MP=197-199°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.63 (s, 3H, CH_3), 4.3 (s, 2H, CH_2–CH=CH_2) 4.58-4.95 (m, 1H, CH_2–CH=CH_2), 5.51-6.05 (m, 2H, CH_2–CH=CH_2), 6.98 (s, 1H, thiazole), 7.12-7.39 (m, 7H, Ar-H), 10.70 (s, 1H, NH^+). Calculated for $C_{19}H_{16}N_2SCl_2$ HBr N 6.14%. Measured, %: N 6.44.

Hydrobromide of 3-allyl-4-(4'-chlorophenyl)-N-(4-bromophenyl)-thiazole-2-imine (3d)

Yield 82%, MP=210-212°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=4.43 (s, 2H, CH_2–CH=CH_2) 4.52-4.78 (m, 1H, CH_2–CH=CH_2), 5.38-5.96 (m, 2H, CH_2–CH=CH_2), 7.15 (s, 1H, thiazole), 7.19-7.48 (m, 8H, Ar-H), 10.80 (s, 1H, NH^+). Calculated for $C_{18}H_{14}N_2SClBr$ HBr N 5.76%. Measured, %: N 6.01.

Hydrobromide of 3-allyl-4-(4'-chlorophenyl)-N-phenyl-thiazole-2-imines (3e)

Yield 84%, MP=195-197°C ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=4.38 (s, 2H, CH_2–CH=CH_2) 4.62-4.93 (m, 1H, CH_2–CH=CH_2), 5.63-5.95 (m, 2H, CH_2–CH=CH_2), 7.23 (s, 1H, thiazole), 7.25-7.87 (m, 9H, Ar-H), 10.80 (s, 1H, NH^+). Calculated for $C_{18}H_{15}N_2SClHBr$ N 6.87%. Measured, %: N 7.11.

Hydrobromide of 3-allyl-4-(4'-nitrophenyl)-N-(4-bromophenyl)-thiazole-2-imine (3f)

Yield 83%, MP=202-204°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=4.4 (s, 2H, CH_2–CH=CH_2) 4.5-4.81 (m, 1H, CH_2–CH=CH_2), 5.43-5.95 (m, 2H, CH_2–CH=CH_2), 7.02 (s, 1H, thiazole), 7.05-7.41 (mm, 8H, Ar-H), 10.70 (s, 1H, NH^+). Calculated for $C_{18}H_{14}N_3SO_2Br$ HBr N 8.45%. Measured, %: N 8.75.

Hydrobromide of 3-allyl-4-(4'-methylphenyl)-N-(4-methylphenyl)-thiazole-2-imine (3g)

Yield 87%, MP=196-198°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.47 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 4.45 (s, 2H, CH_2–CH=CH_2) 4.51-4.86 (m, 1H, CH_2–CH=CH_2), 5.29-5.87 (m, 2H, CH_2–CH=CH_2), 6,97 (s, 1H, thiazole), 7.01-7.69 (m, 8H, Ar-H), 10,70 (cs, 1H, NH^+). Calculated for $C_{20}H_{20}N_2S$ HBr N 6.98%. Measured, %: N 7.38.

Hydrobromide of 3-allyl-4-(4'-methoxy)-N-(3-trifluoromethylphenyl)-thiazole-2-imine (3h)

Yield 82%, MP=220-222°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.32 (s, 3H, OCH_3), 4.61 (s, 2H, CH_2–CH=CH_2), 4.82-5,17 (m, 1H, CH_2–CH=CH_2), 5.29-5.73 (m, 2H, CH_2–CH=CH_2), 6.95 (s, 1H, thiazole), 6.98-7.55 (m, 8H, Ar-H), 10.70 (s, 1H, NH^+). Calculated for $C_{20}H_{17}N_2SOF_3$ HBr N 5.94%. Measured, %: N 6.34.

3-allyl-4-(4'-methoxyphenyl)-N-(4-methylphenyl)-thiazole-2-imine (5)

Yield 82%, MP=100-102°C (ethanol). ^{1}H-NMR (400 MHz, DMSO-d_6) δ=2.34 (s, 3H, OCH_3), 3.82 (s, 3H, CH_3), 4.68 (s, 2H, CH_2–CH=CH_2) 4.87-5.26 (m, 1H, CH_2–CH=CH_2), 5.8-5.97 (m, 2H, CH_2–CH=CH_2), 7.02 (s, 1H, thiazole), 7.05-7.53 (m, 8H, Ar-H). Calculated for $C_{19}H_{18}N_2OS$ N 8.69%. Measured, %: N 8.94.

CONCLUSION

The synthesis of derivatives of 3-allyl-4-(R-phenyl)-N-(R'-phenyl)-thiazole-2-imine has conducted in the conditions of Hantzsch reaction. The structure of compounds synthesized has been confirmed by the comprehensive use of modern physical and chemical methods of analysis. The results of docking experiments allow to declare that new derivatives of 3-allyl-4-(R-phenyl)-N-(R'-phenyl)-thiazole-2-imine are prospective cardioprotective agents with metabolic action. The results obtained indicate the possibility of stable complexes formation of substituted thiazole-2-imines with biological target, in which the ligands arrangement in the active center of receptor and the amino acid residues of the side chains participating in the non-covalent bonds formation are similar to the geometry and types of L-carnitine and Mildronate, established on the basis of crystallographic studies.

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