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A structural study of *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine by density functional theory calculations

Kirill V. Timoshchuk[1], Ivan S. Chernov[2], Olga S. Shemchuk[3], Aleksandra V. Protas[4], Olga V. Mikolaichuk[5]✉, Elena A. Popova[6], Oleg E. Molchanov[7], Dmitrii N. Maistrenko[8]

[1] St. Petersburg State Institute of Technology, Saint Petersburg 190013, Russian Federation
[1,2,3,4,6] Pavlov First Saint Petersburg State Medical University, Saint Petersburg, 197022, Russian Federation
[3,5,7,8] Granov Russian Research Center for Radiology and Surgical Technologies, Saint Petersburg, 197758, Russian Federation

[1] kirill.timoshhuk99@mail.ru, https://orcid.org/0009-0003-8862-719X
[2] i.chernov@green-van.ru, https://orcid.org/0009-0009-8950-884X
[3] Olja.shemchuk17@gmail.com, https://orcid.org/0000-0003-3060-2232
[4] aleksandra.protas@mail.ru, https://orcid.org/0000-0002-1920-9360
[5] olgamedchem@gmail.com✉, https://orcid.org/0000-0002-0463-7725
[6] popova_e_a@bk.ru, https://orcid.org/0000-0002-6513-6983
[7] molchanovo@mail.ru, https://orcid.org/0000-0003-3882-1720
[8] st801293@spbu.ru, https://orcid.org/0000-0001-8174-7461

Abstract

Currently, antitumor drug therapy is represented by three directions: chemotherapy, targeted therapy and immunotherapy. Chemotherapy is a non-specific treatment that uses chemicals that inhibit cell proliferation, affect cellular DNA or RNA and cellular metabolism which contributes to the destruction of all dividing cells. Six-membered heterocyclic ring — 1,3,5-triazine and its derivatives are increasingly found in the literature as DNA alkylating agents. One of such triazine derivatives, N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine, previously obtained in our research group, was characterized, and its structure was optimized using the Density Functional Theory (DFT) method, B3LYP functional and 6-31G basis set. The theoretically obtained spectral characteristics were confirmed by practical results with a high degree of convergence. In this work, quantum chemical calculations were performed at different DFT levels using the ORCA software package. The structure of *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine was optimized using the B3LYP functional with the 6-31G basis set. ^{1}H and ^{13}C (DMSO-d_6) Nuclear Magnetic Resonance spectra were recorded on a Bruker 300 Avance instrument at frequencies of 400.0 and 100.0 MHz, respectively. At the first stage of computer modeling, the electronic structure of the molecule was calculated using the DFT method and the geometry was optimized. The calculation was performed in the 6-31G basis set with the B3LYP functional and taking into account the polarization of the solvent (water) with a relative permittivity of 78.54. The charges on the atoms were estimated using the Mulliken scheme. The energy values (eV) for the molecule are: HOMO: –6.279, LUMO: –1.147. The optimized structure was stable, and the charge distribution on the atoms allows us to conclude that there are three possible conformations of *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine. In the next step, for calculations with periodic boundary conditions, 20 studied molecules and approximately $1.3 \cdot 10^5$ water molecules were placed in a cubic box with sides of 16 nm; the distance between *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine molecules was at least 3 nm, and the distance from the molecule to the wall was at least 1.5 nm. The force field for the OPLS-AA/M system was used; the simulation time was 200 ns with a step of 1 fs. Then, in the GROMACS 2023 package in the NVT ensemble with a Berendsen thermostat and a barostat for 400 ps with a time step of 0.1 fs under the condition at temperature T = 298.15 K and pressure P = 100 kPa, solvation of the system, energy minimization and equilibration were carried out. It is shown that when performing the dynamics of association, these molecules do not form aggregates in an aqueous solution. In this work, the synthesis and characterization of *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine by spectroscopic methods are described. The results of the molecular docking studies are consistent with the *in vitro* antitumor activity which showed that the compound exhibits maximum efficiency and show

approximate binding energies in the range from –1.034 to –4.578 kcal·mol^{-1} *N*-(2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine has been demonstrated to have a high affinity for serum albumin, indicating its potential for serum distribution.

Keywords
1,3,5-triazine, aziridine, NMR, DFT, HSA binding, B3LYP

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УДК 544.18; 544.183; 539.194

Исследование спектральных характеристик *N*-(2-(2-(2-азидоэтокси)этокси) этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амина с помощью методов теории функционала плотности

Кирилл Владимирович Тимощук[1], Иван Сергеевич Чернов[2], Ольга Сергеевна Шемчук[3], Александра Владимировна Протас[4], Ольга Владиславовна Миколайчук[5]✉, Елена Александровна Попова[6], Олег Евгеньевич Молчанов[7], Дмитрий Николаевич Майстренко[8]

[1] Санкт-Петербургский государственный технологический институт, Санкт-Петербург, 190013, Российская Федерация
[1,2,3,4,6] Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация
[3,5,7,8] Российский научный центр радиологии и хирургических технологий им. Гранова, Санкт-Петербург, 197758, Российская Федерация

[1] kirill.timoshhuk99@mail.ru, https://orcid.org/0009-0003-8862-719X
[2] i.chernov@green-van.ru, https://orcid.org/0009-0009-8950-884X
[3] Olja.shemchuk17@gmail.com, https://orcid.org/0000-0003-3060-2232
[4] aleksandra.protas@mail.ru, https://orcid.org/0000-0002-1920-9360
[5] olgamedchem@gmail.com✉, https://orcid.org/0000-0002-0463-7725
[6] popova_e_a@bk.ru, https://orcid.org/0000-0002-6513-6983
[7] molchanovo@mail.ru, https://orcid.org/0000-0003-3882-1720
[8] st801293@spbu.ru, https://orcid.org/0000-0001-8174-7461

Аннотация

Введение. На сегодняшний день лекарственная противоопухолевая терапия представлена тремя направлениями: химиотерапия, таргетная терапия и иммуннотерапия. Химиотерапия является неспецифическим лечением, в котором используются химические вещества, препятствующие пролиферации клеток, воздействующие на клеточную дезоксирибонуклеиновую кислоту (ДНК) или рибонуклеиновую кислоту и клеточный метаболизм, что способствует уничтожению всех делящихся клеток. Шестичленное гетероциклическое кольцо — некоторые производные 1,3,5-триазина встречаются в научных публикациях в качестве алкилирующих ДНК-агентов все чаще. Одно из таких производных триазина, *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амин, полученное на кафедре общей и биоорганической химии ПСПбГМУ им. акад. И.П. Павлова, было синтезировано, а его структура оптимизирована с использованием метода теории функционала плотности Density Functional Theory (DFT), функционала B3LYP и базисного набора 6-31G. Теоретически полученные спектральные характеристики были подтверждены практическими результатами с высокой степенью сходимости. **Метод.** В настоящей работе квантово-химические расчеты проводились на разных уровнях DFT с использованием программного пакета ORCA. Структура *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амина была оптимизирована с применением функционала B3LYP с использованием базисного набора 6-31G. Спектры ядерного магнитного резонанса ^{1}H и ^{13}C (DMCO-d_6) зарегистрированы на приборе Bruker 300 Avance на частотах 400 и 100 МГц соответственно. **Основные результаты.** На первом этапе компьютерного моделирования рассчитана электронная структура молекулы с помощью метода DFT и произведена оптимизация геометрии. Расчет производился в наборе 6-31G с функционалом B3LYP и учетом поляризации растворителя (воды) с относительной диэлектрической постоянной 78,54. Заряды на атомах оценивались по схеме Малликена. Значения энергий для молекулы составило для HOMO: –6,279 эВ, LUMO: –1,147 эВ. Оптимизированная структура была стабильна, а распределение зарядов на атомах позволяет сделать вывод о наличии трех возможных конформациях *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амина. На следующем этапе для расчетов с периодическими граничными условиями 20 исследуемых молекул и примерно 1,3·10^{5} молекул воды были помещены в кубическую коробку со сторонами

16 нм; расстояние между молекулами *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амина составляло не менее 3 нм, расстояние от молекулы до стенки — не менее 1,5 нм. Использовалось силовое поле для системы OPLS-AA/M; время моделирования составляло 200 нс с шагом 1 фс. Далее в пакете GROMACS 2023 в ансамбле NVT с термостатом Берендсена и баростатом в течение 400 пс с шагом по времени 0,1 фс при температуре T = 298,15 К и давлении P = 100 кПа проводили сольватацию системы, минимизацию энергии и уравновешивание. Показано, что при проведении динамики на ассоциацию данные молекулы не образуют агрегатов в водном растворе. **Обсуждение.** В работе описан синтез и характеристика *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амина спектроскопическими методами. Результаты проведенных исследований молекулярного докинга, согласуются с противоопухолевой активностью *in vitro*, которая показала, что соединение проявляет максимальную эффективность и показывают приблизительные значения энергии связывания в диапазоне от –1,034 до –4,578 ккал/моль. Показано, что *N*-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амин обладает высоким сродством к сывороточному альбумину, что указывает на его потенциал для распределения в сыворотке.

Ключевые слова

1,3,5-триазин, азиридин, ЯМР, молекулярная динамика, альбумин, B3LYP

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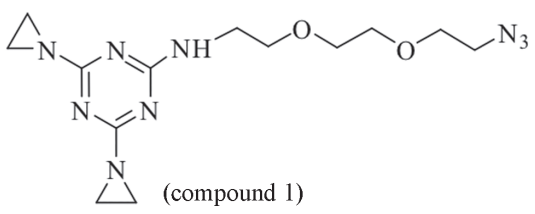
Introduction

Heterocycles that contain nitrogen are essential structural elements of many contemporary medications that have long-term therapeutic effects [1]. The synthesis of extremely effective cytostatics is one of the heterocycles promising uses in medicine, including 1,3,5-triazines [2]. For the theoretical study of different physical and thermochemical parameters of molecules, including nitrogen-containing heterocycles, Density Functional Theory (DFT) is a computational quantum mechanical modeling technique that has grown to be a potent tool. A synthesis and DFT analysis of certain pyrazole derivatives, specifically thiophenes, 1,3-thiazoles, and 1,3,4-thiadiazoles, were reported by Rodrigo et al. [3] and Asmaa et al. [4]. Zhou et al. [5] reported on the connection between structure and biological activity of pyrazole carboxamide derivatives in the HOMO-LUMO study. Dhonnar and associates [6] used the 6-311++G(d,p) template to study the synthesis and theoretical analysis of 2-(aryl)-5-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,3,4-Oxadiazoles using the DFT and MD calculations methods. Therefore, DFT is regarded as the most significant and fruitful approach in the field of pharmaceuticals and pharmaceutical chemistry. Initially, researchers must identify the crucial rarity property in the DFT. All the molecules other characteristics, including its ground energy state, can be readily ascertained using the electron density principle. In a previous study we reported synthetic methods of *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazine-2-amine (Compound 1) (Fig. 1) [7].



(compound 1)

Fig. 1. Structure of *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazine-2-amine

In the current study, Compound 1 is synthesized and characterized by ^{1}H Nuclear Magnetic Resonance (NMR), ^{13}C NMR. The geometric optimization is done by using the B3LYP functional with 6-31G basis set. The computer simulation of the process of Compound 1 association in aqueous solution was carried out using GROMACS 2023. Molecular docking simulations were applied to comprehend the binding of Compound 1 to the molecular target of Human Serum Albumin (HSA). Obtained results are in good correlations with experimental data regarding binding affinity by HSA.

Materials and Methods

Experimental NMR measurements

^{1}H and ^{13}C{1H} NMR spectra were obtained on a Bruker Avance III 400 spectrometer (Germany) (400.13 MHz for ^{1}H and 100.61 MHz for ^{13}C) in DMSO-d_6 at 298.15 K.

DFT and MD calculations

In the first stage of computational modeling, the optimized geometry and electronic structure of the molecule was calculated using DFT. The calculations were performed using the 6-31G basis set with the B3LYP functional, considering the polarization of the solvent (water) with a relative permittivity of 78.54. Computer modeling NMR was performed using basis and functional 6-31G B3LYP and DMSO-d_6 polarization was taken into account. For the calculations with periodic boundary

conditions, 20 studied molecules and approximately $1.3 \cdot 10^5$ water molecules were placed in a cubic box with sides of 16 nm; the distance between *N*-(2-(2-(2-azidoethoxy) ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine molecules was at least 3 nm, and the distance from the molecule to the wall was at least 1.5 nm. The Optimized Potentials for Liquid Simulations (OPLS) force field for the OPLS-AA/M system was used; the simulation time was 200 ns with a step of 1 fs. Next, in the GROMACS 2023 package in the NVT ensemble with a Berendsen thermostat and a barostat for 400 ps with a time step of 0.1 fs under the condition at temperature $T = 298.15$ K and pressure $P = 100$ kPa, solvation of the system, energy minimization and equilibration were carried out.

HSA preparation with Maestro 11.5

Crystal structures of the target protein (HSA) were taken from the Protein Data Bank digital library. The structures were selected according to the best binding constants and high resolution of the structural data (4L9K, 5GIX, 2BXD, 3LU6, 2BXF, 1E7A). The resulting structures were prepared using the Maestro 11.5 protein preparation wizard (Maestro, v. 11.5 Schrodinger, LLC, USA). Bond orders in the protein were restored and water molecules were removed. OPLS force field was used to optimize the protein. The ligand structure was preliminarily established by quantum-chemical calculations and then saved in 3D format. The compound was further prepared using LigPrep 3.6, it was used to create the conformational structure of the ligands by removing ions and adding hydrogen molecules and ionization at pH (7.0 ± 2.0). Energy minimization was performed with an OPLS force field using conventional atomic mechanics energy capacity and Root Mean Square Deviation (RMSD) cutoff of 0.001 nm to create an isomer with minimal energy. The modeled 3D structure of the human Prostate Specific Antigen (PCA) was prepared using the Protein Preparation panel on the Schrödinger platform. Protein preparation methods include many steps, such as adding protons, determining bond order, optimizing protonation states and hydrogen bond networks, and minimizing the protein structure. Protons were added to meet structural requirements, and side chains were optimized either near the binding cavity or near the active center or salt bridges. Hydrogen atoms were added to the structure, in the form of hydroxyl and thiol hydrogen atoms, in protonation states and tautomers of histidine residues, and as "flipped" asparagine, glutamic acid, and histidine residues. The optimized structure was minimized using the OPLS-AA force field until the RMSD of the non-hydrogen atoms reached 0.03 nm. A docking study was performed using a grid-based method to analyze the interaction of the selected compound with Carbamylated form of Human Serum Albumin (ChSA). The receptor grid was created to ensure that other amino acids are involved in the interaction of the compound with ChSA. For the receptor, a 3 × 3 × 3 × 3 nm grid was created with a default inner core (1 × 1 × 1 × 1 nm) centered on the corresponding ligand. After grid creation, all prepared conformations of the selected compound were docked to the binding site using GlideXP docking which allows free docking of compounds. The compounds were selected for further evaluation based on various docking parameters such as docking score, binding energy and physical parameters such as hydrogen bond interactions. Maestro 11.5 software was used as a visualization tool for the docked ligands.

Results and discussion

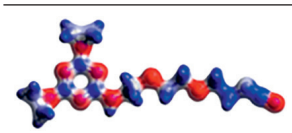
DFT Calculation

DFT calculations using the 6-31G basis set provide a computationally efficient approach for predicting atomic charges, offering a good balance between accuracy and computational cost for routine structural characterization. Atomic charges were estimated using the Malliken scheme. The energy values (in electronvolts) for the molecule are as follows: HOMO: –6.279, LUMO: –1.147. The obtained data are consistent with previously obtained values for triazines [8]. The results of the spatial distribution of the charge density surface are presented in Table 1.

As can be seen from the obtained results, a significant negative charge is concentrated on the nitrogen and oxygen atoms; however, the nitrogen atom designated as N8 exhibits a positive charge, which is consistent with previous data [9]. Furthermore, thermodynamic functions for Compound 1 were obtained (Table 2).

The molecular dynamics method was used to study the connection of molecules of Compound 1 with each other in an aquatic environment. The charges on the atoms, estimated using the Mulliken scheme, are used based on quantum chemical calculations. The OPLS-AA/M force field was used for the calculations; the simulation time was 200 ns with a time step of 1 fs. The 200 ns simulation time represents an optimal compromise between sufficient duration for observing associative processes, correspondence to experimental time scales and comparable to the relaxation time in NMR measurements. Subsequently, in the GROMACS 2023 package, NVT and NPT ensembles with the Berendsen thermostat was used for 400 ps with a time step of 0.1 fs, under conditions of $T = 298.15$ K and $P = 100$ kPa to perform system solvation, energy minimization, and equilibration. Molecular dynamics were used to further investigate possible aggregation pathways of the Compound 1 molecules. During the modeling of molecule association in water, it was found

Table 1. Red color is the region of increased negative charge; blue color is the region of positive charge. Charges on atoms, eV

Compound	N1	N2	N3	N4	N5	N6	N7	N8	N9	O1	O2
	–0.477	–0.432	–0.477	–0.441	–0.448	–0.656	–0.273	0.130	–0.146	–0.570	–0.581

that the approach of these molecules to each other is quite problematic; by 150 ns, clusters do not form in the system. Further, by 200 ns, no associates were formed in water (Fig. 2). At the end of the study, Compound 1 is released as individual molecules in water. The potential for clustering in water is also indicated by the low control panel graph the values of which continue to be maintained throughout the entire dynamic (Fig. 3). At the end of the simulation in water, the 1,3,5-triazine molecules remained as individual molecules. The low clustering potential in water is also evidenced by the surface area reduction plot, whose values remain constant throughout the entire dynamics (Fig. 2 and 3).

NMR Analysis

It should be noticed that the 6-31G basis set is particularly well-suited for organic molecules as it adequately describes the valence electrons of carbon and hydrogen atoms while maintaining reasonable computational demands, making it a good choice for NMR shift predictions in medium-sized organic systems. NMR spectroscopy is still one of the most powerful techniques available for the detection and investigation of the behavior of such dynamic organic compounds for medical applications in several time scales. Using computer modeling nuclear magnetic resonance for Compound 1 was calculated using the DFT method and geometry optimization was performed. Excellent linear regression correlation coefficient ($R^2 > 0.998$) is obtained for the obtained results (Table 2). Neutralizer polarization of solvent (DMSO) was considered.

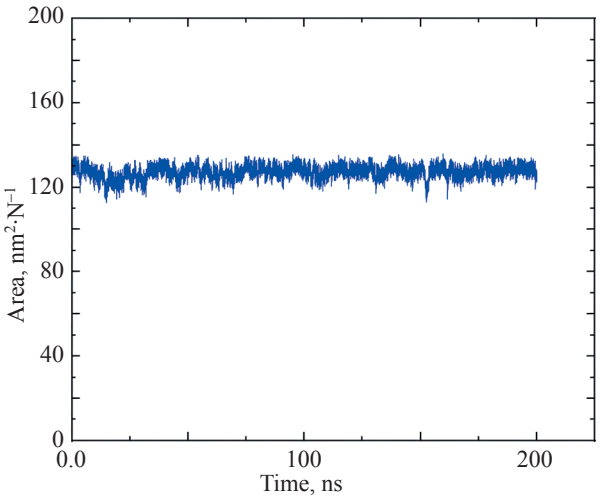


Fig. 2. Graph of surface area, interaction with solvent (water), where N represents number of molecules of 1,3,5-triazine

The data given in Table 3 shows that the Signed Deviations (SD) are typically positive, indicating that the computed ^{13}C NMR chemical shifts are exclusively higher than those of the experimental ones.

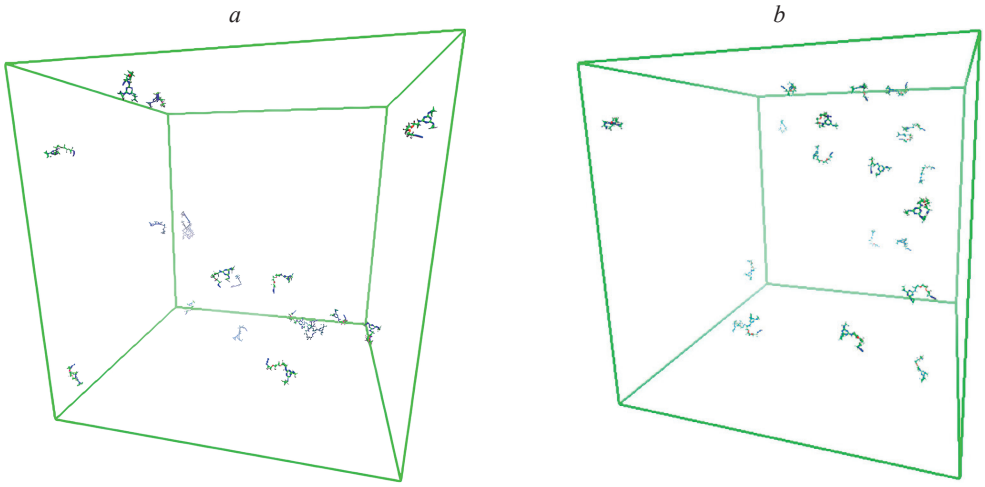


Fig. 3. Molecular dynamics visualization: association of 1,3,5-triazine derivative molecules in water after 150 ns (*a*) and 200 ns (*b*)

Table 2. NMR shifts

NMR	Group	DMSO-d_6, ppm	
		DFT method	Practice
^{1}H	$CH_{2\ peg}$	3.88, 3.67, 3.45, 3.38	3.66, 3.62, 3.43–3.37
^{13}C	$C_{triazine}$	170.98, 170.85, 169.26	174.05, 169.62, 166.43
	$CH_{2\ aziridine}$	33.54, 32.96, 32.84, 32.70	28.88, 27.43, 27.21, 26.21
	$CH_{2\ peg}$	63.38, 59.95	62.18, 59.98
	CH_2–O–	71.92, 71.92	70.12, 70.44
	CH_2–N_3	72.68	70.60
	CH_2–NH–	40.71	40.62

Table 3. The SD values for 13C NMR shifts

Group	SD, ppm
$C_{triazine}$	+0.096
$CH_{2\ aziridine}$	+4.320
$CH_{2\ peg}$	+0.585
CH_2–O–	+1.640
CH_2–N_3	+2.080
CH_2–NH–	+0.090

Table 4. Values of the ultimate binding energy in the docking process

Glide docking score, kcal/mol	IA	IIA	IIIA	IB	IIB	IIIB
XP GScore	–2.477	–1.034	–4.127	–3.808	–4.578	–2.128

Based on the data provided in Table 3, the lowest convergence is found in the signed deviations of aziridine ring from the NMR shift. The SD for the ^{1}H NMR chemical shifts weren't calculated due to appearance of many signals as multiplets (doublets, triplets, etc.), making it difficult to select a single representative value for each proton environment for statistical analysis. This approach ensures consistency in our comparison between experimental and DFT-calculated chemical shifts by avoiding arbitrary selection of specific peaks within multiplet patterns.

HSA-Binding Assay

To design better therapeutics, it is important to understand how a molecule interacts with protein. Thus, great interest is paid to the understanding of the binding mechanism of molecules with carrier proteins like HSA. It has been extensively utilized as a model protein for decoding protein-ligand interactions. Computational methods have evolved as an effective tool for predicting the properties of newly synthesized organic compounds and the nature of interactions in a protein-ligand complex. After successful exploration of probable mode of fluorescence quenching [10], an attempt has been made to explore the presence the various non-covalent forces such as van der Waals forces, hydrophobic interactions, hydrogen bonding and electrostatic interactions that are involved in the binding of the proteins with ligands. The value of Gibb's free energy change was calculated to analyze the nature of binding interactions between Compound 1 and HSA. The spontaneous nature of the interaction was confirmed by the negative value of Gibb's free energy (XP GScore, Table 4). It was shown that when binding to HSA, the 1,3,5-triazine derivative has a higher affinity for subdomain IIB. Furthermore, data obtained is consistent with the experimental data published before [11].

Conclusion

Various characterizations calculated by Density Functional Theory (DFT) method were considered to predict the ^{1}H and ^{13}C-NMR chemical shifts in *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine (Compound 1). The computational results were predicted using the experimental shifts as a reference using several statistical descriptors. From the calculated data, excellent linear correlations were obtained between the DFT calculated and experimental ^{1}H Nuclear Magnetic Resonance (NMR) chemical shifts for all the chemical characterizations tested. Based on the NMR shift, we also discovered that the B3LYP functional with 6-31G basis had the lowest convergence for the small aziridine ring. What makes the B3LYP functional with 6-31G basis set method inapplicable for calculating NMR chemical shifts of methylene groups of small rings. Molecular docking simulations were applied to comprehend the binding of Compound 1 to the molecular target of Human Serum Albumin. Compound 1 has been demonstrated to have a high affinity for serum albumin's subdomain IIB XP GScore –4.578 kcal/mol, indicating its potential for serum distribution.

References

1. Kumar A., Singh A.K., Singh H., Vijayan V., Kumar D., Naik J., Thareja S., Yadav J.P., Pathak P., Grishina M., et al. Nitrogen containing heterocycles as anticancer agents: a medicinal chemistry perspective. *Pharmaceuticals*, 2023, vol. 16, no. 2, pp. 299. https://doi.org/10.3390/ph16020299
2. Lim H.Y., Dolzhenko A.V. 1,3,5-Triazine as a promising scaffold in the development of therapeutic agents against breast cancer. *European Journal of Medicinal Chemistry*, 2024, vol. 276, pp. 116680. https://doi.org/10.1016/j.ejmech.2024.116680
3. Faundez-Gutierrez R., Macleod-Carey D., Zarate X., Bustos C., Molins E., Schott E. Synthesis, characterization and DFT study of a new family of pyrazole derivatives. *Polyhedron*, 2014, vol. 81, pp. 414–420. https://doi.org/10.1016/j.poly.2014.06.003

Литература

1. Kumar A., Singh A.K., Singh H., Vijayan V., Kumar D., Naik J., Thareja S., Yadav J.P., Pathak P., Grishina M., et al. Nitrogen containing heterocycles as anticancer agents: a medicinal chemistry perspective // Pharmaceuticals. 2023. V. 16. N 2. P. 299. https://doi.org/10.3390/ph16020299
2. Lim H.Y., Dolzhenko A.V. 1,3,5-Triazine as a promising scaffold in the development of therapeutic agents against breast cancer // European Journal of Medicinal Chemistry. 2024. V. 276. P. 116680. https://doi.org/10.1016/j.ejmech.2024.116680
3. Faundez-Gutierrez R., Macleod-Carey D., Zarate X., Bustos C., Molins E., Schott E. Synthesis, characterization and DFT study of a new family of pyrazole derivatives // Polyhedron. 2014. V. 81. P. 414–420. https://doi.org/10.1016/j.poly.2014.06.003

4. Fahim A.M., Farag A.M., Shabaan M.R., Ragab E.A. Synthesis and DFT study of novel pyrazole,thiophene, 1,3-thiazole and 1,3,4-thiadiazole derivatives. *European Journal of Chemistry*, 2018, vol. 9, no. 1, pp. 30–38. https://doi.org/10.5155/eurjchem.9.1.30-38.1675
5. Mu J.X., Shi J.X., Yang M.Y., Sun Z.H., Liu X.H., Li B.J., Sun N.B. Design, synthesis, DFT study and antifungal activity of pyrazolecarboxamide derivatives. *Molecules*, 2016, vol. 21, no. 1, pp. 68. https://doi.org/10.3390/molecules21010068
6. Dhonnar S.L., Buchude S.S., Sargade V.N., Adole V.A., Sadgir N.V. Synthesis, DFT, in silico ADME, and antimicrobial study of new 2-(aryl)-5-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,3,4-oxadiazoles. *ChemistrySelect*, 2024, vol. 9, no. 46, pp. e202402534. https://doi.org/10.1002/slct.202402534
7. Molchanov O.E., Popova E.A., Maistrenko D.N., Semenov K.N, Protas A.V., Mikolaichuk O.V., Popova A.A, Sharoiko V.V, Shemchuk O.S, Golub V.M. N-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-4,6-di(aziridin-1-yl)-1,3,5-triazin-2-amine, having cytotoxic. *Patent RU* № 2825828C1. 2024. (in Russian)
8. Al-Rasheed H.H., AL-khamis S.A., Barakat A., El-Faham A., Haukka M., Soliman S.M. Synthesis and characterizations of novel isatin-s-triazine hydrazone derivatives; X-ray structure, hirshfeld analysis and DFT calculations. *Crystals*, 2023, vol. 13, no. 2, pp. 305. https://doi.org/10.3390/cryst13020305
9. Yoshikawa N., Yamazaki S., Nishiyama A., Yamashita Y., Kanehisa N., Tohnai N., Nakata E., Takashima H. Structures, atomic charges, emission properties and DFT studies of biquinoline derivatives induced by protonation of a nitrogen atom. *Journal of Molecular Structure*, 2022, vol. 1251, pp. 131990. https://doi.org/10.1016/j.molstruc.2021.131990
10. Hiremath K.B., Shivashankar M., Chandrasekaran N. Multispectroscopic studies on HSA interaction, DFT calculations, molecular docking, and antimicrobial activities of imine-functionalized tris(hydroxymethyl)aminomethane derivatives. *ChemistrySelect*, 2023, vol. 8, no. 25, pp. e202301772. https://doi.org/10.1002/slct.202301772
11. Alanazi R.L.B., Zaki M., Bawazir W.A. Synthesis and characterization of new metal complexes containing Triazino[5,6–b]indole moiety: In vitro DNA and HSA binding studies. *Journal of Molecular Structure*, 2021, vol. 1246, pp. 131203. https://doi.org/10.1016/j.molstruc.2021.131203

4. Fahim A.M., Farag A.M., Shabaan M.R., Ragab E.A. Synthesis and DFT study of novel pyrazole,thiophene, 1,3-thiazole and 1,3,4-thiadiazole derivatives // European Journal of Chemistry. 2018. V. 9. N 1. P. 30–38. https://doi.org/10.5155/eurjchem.9.1.30-38.1675
5. Mu J.X., Shi J.X., Yang M.Y., Sun Z.H., Liu X.H., Li B.J., Sun N.B. Design, synthesis, DFT study and antifungal activity of pyrazolecarboxamide derivatives // Molecules. 2016. V. 21. N 1. P. 68. https://doi.org/10.3390/molecules21010068
6. Dhonnar S.L., Buchude S.S., Sargade V.N., Adole V.A., Sadgir N.V. Synthesis, DFT, in silico ADME, and antimicrobial study of new 2-(aryl)-5-(3-(difluoromethyl)-1-methyl-1H-pyrazol-4-yl)-1,3,4-oxadiazoles // ChemistrySelect. 2024. V. 9. N 46. P. e202402534. https://doi.org/10.1002/slct.202402534
7. Молчанов О.Е., Попова Е.А., Майстренко Д.Н., Семенов К.Н, Протас А.В., Миколайчук О.В., Попова А.А, Шаройко В.В, Шемчук О.С, Голуб В.М. N-(2-(2-(2-азидоэтокси)этокси)этил)-4,6-ди(азиридин-1-ил)-1,3,5-триазин-2-амин, обладающий цитотоксическим действием. Патент РФ № 2825828C1. Бюл. 2024. № 25.
8. Al-Rasheed H.H., AL-khamis S.A., Barakat A., El-Faham A., Haukka M., Soliman S.M. Synthesis and characterizations of novel isatin-s-triazine hydrazone derivatives; X-ray structure, hirshfeld analysis and DFT calculations // Crystals. 2023. V. 13. N 2. P. 305. https://doi.org/10.3390/cryst13020305
9. Yoshikawa N., Yamazaki S., Nishiyama A., Yamashita Y., Kanehisa N., Tohnai N., Nakata E., Takashima H. Structures, atomic charges, emission properties and DFT studies of biquinoline derivatives induced by protonation of a nitrogen atom // Journal of Molecular Structure. 2022. V. 1251. P. 131990. https://doi.org/10.1016/j.molstruc.2021.131990
10. Hiremath K.B., Shivashankar M., Chandrasekaran N. Multispectroscopic studies on HSA interaction, DFT calculations, molecular docking, and antimicrobial activities of imine-functionalized tris(hydroxymethyl)aminomethane derivatives // ChemistrySelect. 2023. V. 8. N 25. P. e202301772. https://doi.org/10.1002/slct.202301772
11. Alanazi R.L.B., Zaki M., Bawazir W.A. Synthesis and characterization of new metal complexes containing Triazino[5,6–b]indole moiety: In vitro DNA and HSA binding studies // Journal of Molecular Structure. 2021. V. 1246. P. 131203. https://doi.org/10.1016/j.molstruc.2021.131203

Authors

Kirill V. Timoshchuk — PhD Student, Assistant, St. Petersburg State Institute of Technology, Saint Petersburg 190013, Russian Federation; Specialist, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, 197022, Russian Federation, https://orcid.org/0009-0003-8862-719X, kirill.timoshhuk99@mail.ru

Ivan S. Chernov — PhD Student, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, 197022, Russian Federation, https://orcid.org/0009-0009-8950-884X, i.chernov@green-van.ru

Olga S. Shemchuk — Scientific Researcher, Granov Russian Research Center for Radiology and Surgical Technologies, Saint Petersburg, 197758, Russian Federation; Specialist, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, 197022, Russian Federation, SC 57203429632, https://orcid.org/0000-0003-3060-2232, Olja.shemchuk17@gmail.com

Aleksandra V. Protas — PhD (Chemistry), Associate Professor, Pavlov First Saint Petersburg State Medical University, Saint Petersburg, 197022, Russian Federation, https://orcid.org/0000-0002-1920-9360, aleksandra.protas@mail.ru

Olga V. Mikolaichuk — PhD (Chemistry), Senior Researcher, Granov Russian Research Center for Radiology and Surgical Technologies, Saint Petersburg, 197758, Russian Federation, SC 57200216315, https://orcid.org/0000-0002-0463-7725, olgamedchem@gmail.com

Elena A. Popova — D.Sc. (Chemistry), Associate Professor, Professor, Pavlov First Saint Petersburg State Medical University, Saint Petersburg,

Авторы

Тимощук Кирилл Владимирович — аспирант, ассистент, Санкт-Петербургский государственный технологический институт, Санкт-Петербург, 190013, Российская Федерация; специалист, Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация, https://orcid.org/0009-0003-8862-719X, kirill.timoshhuk99@mail.ru

Чернов Иван Сергеевич — аспирант, Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация, https://orcid.org/0009-0009-8950-884X, i.chernov@green-van.ru

Шемчук Ольга Сергеевна — научный сотрудник, Российский научный центр радиологии и хирургических технологий им. Гранова, Санкт-Петербург, 197758, Российская Федерация; специалист, Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация, SC 57203429632, https://orcid.org/0000-0003-3060-2232, Olja.shemchuk17@gmail.com

Протас Александра Владимировна — кандидат химических наук, доцент, Первый Санкт-Петербургский государственный медицинский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация, https://orcid.org/0000-0002-1920-9360, aleksandra.protas@mail.ru

Миколайчук Ольга Владиславовна — кандидат химических наук, старший научный сотрудник, Российский научный центр радиологии и хирургических технологий им. Гранова, Санкт-Петербург, 197758, Российская Федерация, SC 57200216315, https://orcid.org/0000-0002-0463-7725, olgamedchem@gmail.com

Попова Елена Александровна — доктор химических наук, доцент, профессор, Первый Санкт-Петербургский государственный медицин-

197022, Russian Federation, https://orcid.org/0000-0002-6513-6983, popova_e_a@bk.ru

Oleg E. Molchanov — D.Sc., (Medicine), Head of Department, Granov Russian Research Center for Radiology and Surgical Technologies, Saint Petersburg, 197758, Russian Federation, https://orcid.org/0000-0003-3882-1720, molchanovo@mail.ru
Dmitrii N. Maistrenko — D.Sc., (Medicine), Director, Granov Russian Research Center for Radiology and Surgical Technologies, Saint Petersburg, 197758, Russian Federation, https://orcid.org/0000-0001-8174-7461, st801293@spbu.ru

ский университет имени академика И.П. Павлова, Санкт-Петербург, 197022, Российская Федерация, https://orcid.org/0000-0002-6513-6983, popova_e_a@bk.ru
Молчанов Олег Евгеньевич — доктор медицинских наук, руководитель отдела, Российский научный центр радиологии и хирургических технологий им. Гранова, Санкт-Петербург, 197758, Российская Федерация, https://orcid.org/0000-0003-3882-1720, molchanovo@mail.ru
Майстренко Дмитрий Николаевич — доктор медицинских наук, директор, Российский научный центр радиологии и хирургических технологий им. Гранова, Санкт-Петербург, 197758, Российская Федерация, https://orcid.org/0000-0001-8174-7461, st801293@spbu.ru

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