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SYNTHESIS, SPECTRAL CHARACTERISTICS AND MOLECULAR DOCKING STUDIES OF *N*-(2,2,2-TRICHLORO-1-(2-(PHENYLCARBAMOTHIOYL)HYDRAZINE-1-CARBOTHIOAMIDO)ETHYL)CINNAMAMIDE AS A POTENTIAL ANALOG OF SALUBRINAL

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In this work, we synthesized N-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide, which is a new potential inhibitor of the GADD34:PP1 holoenzyme. This compound was obtained by reacting equimolar amounts of N-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide with *N*-phenylhydrazinecarbothioamide in acetonitrile. The reaction was carried out at reflux for two minutes. The yield of the product after recrystallization from acetonitrile was 79%, and ^{1}H NMR, ^{13}C NMR, and IR spectroscopy reliably proved its structure. The ability of the obtained compound to inhibit the activity of the GADD34:PP1 holoenzyme was assessed using molecular docking. We selected a known GADD34:PP1 inhibitor Salubrinal as a standard. According to the results obtained, the synthesized compound can effectively interact with the active site of the target. It was shown that its molecule, like the Salubrinal molecule, formed an intermolecular hydrogen bond with the Tyr 272 amino acid. The compound we obtained was almost as good as the standard in terms of the energy value of the complex with GADD34:PP1.

Keywords: Salubrinal, GADD34:PP1, cinnamamide, inhibitor, molecular docking, endoplasmic reticulum stress, active site.

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Introduction

Protein biosynthesis is a key process for the vital activity of an organism, which includes the stage of synthesis of the polypeptide chain and the stage of maturation of protein molecules [1]. The first stage occurs on ribosomes with the participation of mRNA and tRNA molecules, and the second one occurs in the internal space of the granular endoplasmic reticulum (ER). In the granular ER, «immature» protein molecules are subject to folding, i.e. they take the correct spatial conformation. All incorrectly folded proteins are destroyed since their excessive accumulation can lead to a functional overload of the ER. This phenomenon is called ER stress. It can lead to a disruption of normal cell functioning, or even to its death through the apoptosis system, and it also causes many diseases [2].

To date, it is known about the role of ER stress in the development of oncological, endocrine, cardiovascular, and nervous system diseases. In addition, this phenomenon underlies some diseases of the kidneys, lungs, and bones [3]. The occurrence of ER stress has an important role in the pathogenesis of infectious diseases, including those caused by Mycobacterium tuberculosis, hepatitis B virus, herpes simplex virus, HIV-1, and others [4].

In this regard, the molecular mechanisms of ER stress are intensively investigated nowadays. Currently, three main ER stress signal-sensor systems are known, namely PERK, ATF6, and IRE1. They inhibit translation processes by phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), which is a key participant in protein translation. It is eIF2α that is responsible for binding the ribosomal

40S subunit to $tRNA_{imet}$, which recognizes the start codon of mRNA and initiates the synthesis of the peptide chain. When ER stress occurs, the activity of eIF2α is inhibited by its phosphorylation of PERK. In this case, eIF2α passes into the inactive form eIF2αP. However, the holoenzyme complex GADD34:PP1 dephosphorylates eIF2αP converting it back into the active form eIF2α, thereby triggering protein synthesis processes and increasing ER stress [2].

Recently, several selective inhibitors of GADD34:PP1 have been developed that weaken protein synthesis, help maintain homeostasis in the ER and thereby save cells from apoptosis. Such drugs include Salubrinal [5], Sal003 [6], and some analogs [7,8] (Fig.1). These substances are widely used in experimental medicine, as well as biochemical and cytological studies to investigate the molecular mechanisms of ER stress and the degree of its pathophysiological impact on various organs and systems. Now we can confidently talk about the prospects of drugs based on them for treating heart, kidney, and bone diseases as well as fighting malignant tumors and diabetes mellitus [9].

In this paper, we report the synthesis of a new structural analog of Salubrinal in which a phenylthiourea fragment replaces the quinoline cycle. In addition we report *in silico* evaluation of the obtained *N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide as a potential GADD34:PP1inhibitor.

Results and discussion

We used *N*-(2,2,2-trichloro-1-hydroxyethyl)cinnamamide (**3**) as a starting compound, previously obtained by condensation of chloral hydrate (**1**) and cinnamic acid amide **(2)** [10]. Then, we obtained *N*-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide (**5**) through the stage of chlorine derivative formation **4** [11]. Addition of *N*-phenylhydrazinecarbothioamide (**6**) to the latter resulted in the formation of the target *N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide (**7**). This reaction was carried out by refluxing for two minutes in acetonitrile (Scheme 1). The yield of compound **7** was 79%.

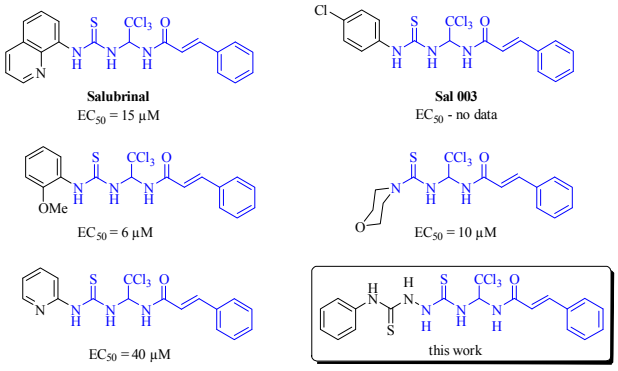
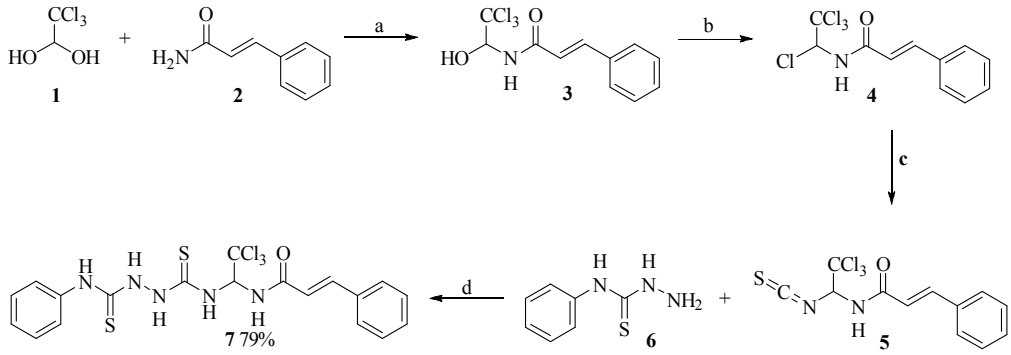


Fig. 1. Structures of some GADD34:PP1 inhibitors and the compound under investigation.

^{1}H, ^{13}C NMR, and IR spectroscopy data reliably proved the structure of compound **7**. In the ^{1}H NMR spectrum, signals of five NH protons were observed, which appeared in the 9.90–8.73 ppm region. In this case, NH protons of the NHCHNH system appeared as two doublets at 9.90 and 8.73 ppm with the same spin-spin interaction constant equal to 7.3 Hz. The signal of the adjacent CH proton appeared at 7.09 ppm as a doublet of doublets with J=7.3 Hz. The remaining three NH protons of the *N*-phenylhydrazinecarbothioamide fragment appeared as two broadened singlets at 9.65 (1NH) and 9.14 (2NH) ppm. The protons of the phenyl and styryl substituents appeared as four multiplet systems in the region from 7.65 to 6.96 ppm. In the ^{13}C NMR spectrum, the most indicative signals were those of two C=S carbons, which appeared at 182.7 and 181.9 ppm. The carbon of the amide group (C=O) appeared at 164.9 ppm, and the signals of the CCl_3 group and CH carbon were at 101.9 and 70.2 ppm, respectively. The signals of two carbon atoms of the ethene fragment appeared at 141.7 and 121.0 ppm, and the signals of the aromatic ring carbons were located between them in the 139.2 and 125.5 ppm regions. The bands of NH group stretching vibrations were located in the IR spectrum at 3280–3200 cm^{-1}. The band corresponding to vibrations of the C=O bond of the amide group appeared at 1670 cm^{-1}.

The potential ability of compound **7** to inhibit the activity of the GADD34:PP1 holoenzyme was assessed using molecular docking implemented in the ArgusLab 4.0.1 software. We selected Salubrinal as a comparison structure, for which we had previously conducted similar investigations [9]. Before docking, the structure of compound **7** was optimized using the semiempirical PM3 method. As in the case of Salubrinal [9], the most energetically favorable conformation for compound **7** was the one stabilized by an intramolecular hydrogen bond formed between the oxygen atom of the amide group and the NH proton of the thiourea fragment. The bond length was 2.6 Å (Fig. 2,a).

According to the molecular docking results, compound **7** effectively interacts with the active site of GADD34:PP1. After docking, the Salubrinal and compound **7** molecules are very closely located in the active site of GADD34:PP1 (Figure 2b,c). In addition,



Scheme 1. Synthesis of *N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide (**7**). Reagents and conditions: (a) solvent-free, melt heating, 20 min; (b) $SOCl_2$, CCl_4, reflux 4 h; (c) KSCN, CH_3CN, r.t., 1h; (d) CH_3CN, reflux, 2 min

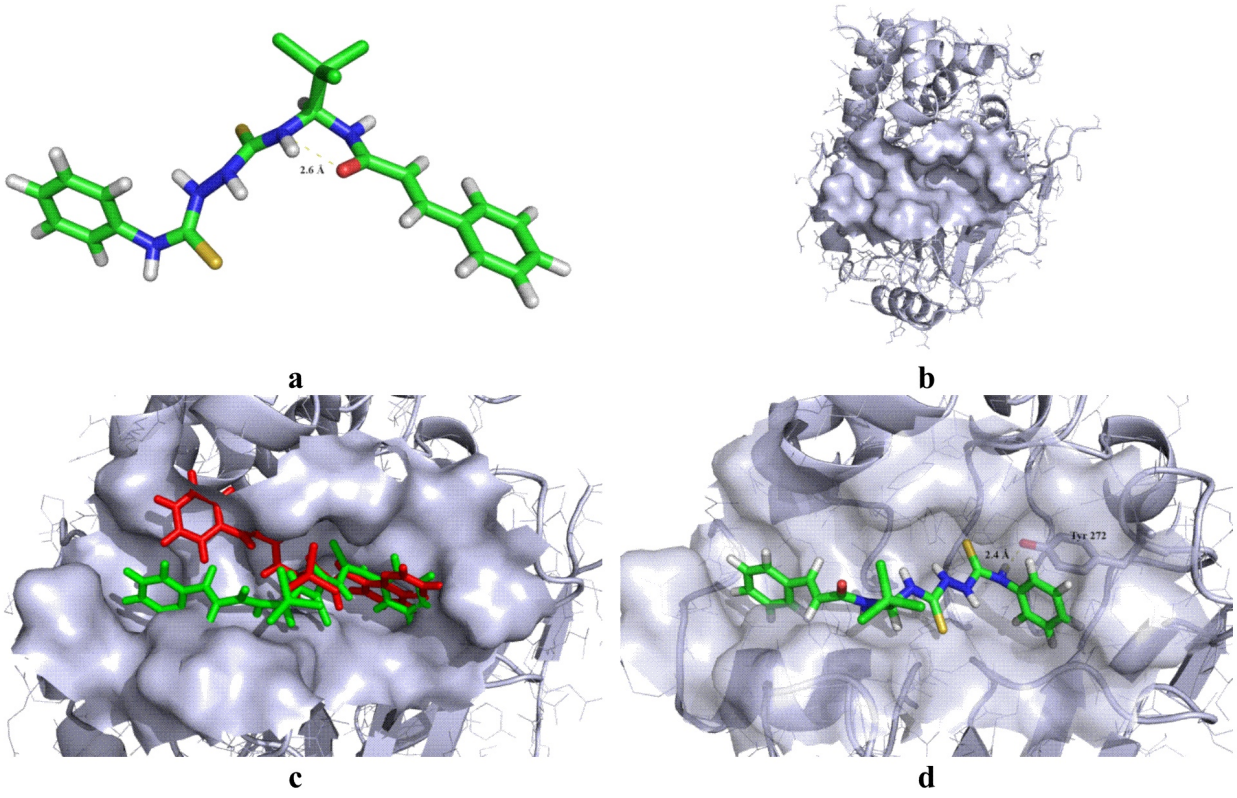


Fig. 2. Results of *in silico* experiment for *N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide (**7**): (a) calculated optimal structure of compound **7** (PM3 method); (b) active site of GADD34:PP1 (shown as a solid surface); (c) superposition of Salubrinal and compound **7** molecules in the active site of GADD34:PP1; (d) position of the compound **7** molecule in the active site of GADD34:PP1. Visualization in PyMOL

Salubrinal and compound **7** are additionally fixed in the active site by forming a hydrogen bond with Tyr 272. Moreover, in the case of compound **7**, the hydrogen bond is shorter than for Salubrinal [9] by about 1 Å and is about 2.4 Å. The energy of the GADD34:PP1, **7** complex, is −12.0497 kcal/mol, which in absolute value is inferior to Salubrinal by less than 0.2 kcal/mol.

Experimental

Chemistry

IR spectra were recorded on a Spectrum BX II spectrometer in KBr tablets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured for solutions in DMSO-d_6 on a Varian Agilent VNMRS

400 MHz spectrometer. Elemental analysis was performed on LECO CHNS-900. The progress of the reaction and the purity of the resulting compounds were monitored by TLC on Silufol UV-254 plates, using a chloroform/acetone mixture (3:1) as an eluent. Melting points were determined in open capillaries and were not corrected.

Synthesis of N-(2,2,2-trichloro-1-hydroxyethyl)cinnamamide (3) [7,10]

Compound **3** was obtained by condensing chloral hydrate with cinnamamide in the melt according to the procedure described in ref. [10].

Synthesis of N-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide (5) [7]

Isothiocyanate **5** was obtained according to the procedure described in ref. [11].

Synthesis of N-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide (7)

An equimolar amount (1.67 g) of *N*-phenylhydrazinecarbothioamide (**5**) [12] was added to 10 mmol of *N*-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide (**5**) [7] in 30 mL of acetonitrile. The mixture was brought to boiling, filtered warm through a paper filter, and left at room temperature for 14 hours. The resulting precipitate was filtered, washed with acetonitrile (2×10 mL) and dried. The product was purified by recrystallization from acetonitrile. White solid; yield 79% (3.97 g); mp. 171–173^{0}C (MeCN); R_f=0.47. IR (KBr) ν_{max}: 3278, 3201 (NH), 2926, 2854 (CH), 1670 (C=O), 1628, 1598, 1548, 1497, 1449, 1378, 1342, 1290, 1242, 1194, 1145, 1118, 1072, 1024, 976, 934, 877, 856, 815, 764, 744, 692, 637, 624, 608, 567, 521 cm^{-1}. ^{1}H NMR δ: 9.90 (d, *J*=7.3 Hz, 1H, NH), 9.65 (brs, 1H, NH), 9.14 (brs, 2H, NH), 8.73 (d, *J*=7.3 Hz, 1H, NH), 7.65–7.53 (m, 5H, 4H$_{arom.}$+=CH-*cis*), 7.45–7.40 (m, 3H, 3H$_{arom.}$), 7.31–7.25 (m, 2H, 1H$_{arom.}$+=CH-*trans*), 7.09 (dd, *J*=7.3 Hz, 1H, CH), 7.02–6.96 (m, 1H, H$_{arom.}$). ^{13}C NMR: δ 182.7 (C=S), 181.9 (C=S), 164.9 (C=O), 141.7 (CH=$\underline{C}$HC$_6$H$_5$), 139.2, 134.7, 130.5, 129.5, 128.7, 128.2, 125.9, 125.5 (C$_{arom.}$), 121.0 ($\underline{C}$H=CHC$_6$H$_5$), 101.9 (CCl$_3$), 70.2 (CH). Anal. Calcd (%) for $C_{19}H_{18}Cl_3N_5OS_2$ (502.86): C 45.38; H 3.61; N 13.93; S 12.75. Found: C 45.31; H 3.52; N 14.00; S 12.79.

In silico experiment

The structure of compound **7** was optimized using the semiempirical PM3 method [13] and the ArgusLab 4.0.1 software package[1]. The three-dimensional crystal structure of the GADD34:PP1 holoenzyme (PDB ID: 4XPN) was downloaded in .pdb format from the Protein Data Bank (http://www.rcsb.org). Hydrogen atoms were added throughout the protein structure before molecular docking. Before docking, all non-protein molecules, except for the phosphoric acid residue 403 PO4, were removed from the structure. Based on this phosphoric acid residue, a ligand group called Ligand_X-ray was created. Based on this group, a three-dimensional model of the binding site was created, the dimensions of which were set manually and were 40 Å along the X-axis, 40 Å along the Y-axis, and 40 Å along the Z-axis. The flexible ligand parameter was set during docking. The results were evaluated by the semi-empirical AScore function based on the XScore function [14]. The cell resolution was set at 0.250 Å. Calculation type – Dock; Docking Engin – ArgusLab. The results were visualized using the PyMOL 0.99rc6 program.

Conclusions

We have synthesized *N*-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide, which is a new potential inhibitor of the GADD34:PP1 holoenzyme. The synthesis of the target compound has been carried out by the addition reaction of *N*-phenylhydrazinecarbothioamide to *N*-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide. Complex spectral investigations have proven the structure of the obtained compound.

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СИНТЕЗ, СПЕКТРАЛЬНІ ХАРАКТЕРИСТИКИ І МОЛЕКУЛЯРНИЙ ДОКІНГ N-(2,2,2-ТРИХЛОР-1-(2-(ФЕНИЛКАРБАМОТІОЇЛ)ГІДРАЗИНО-1-КАРБОТІОАМІДО)ЕТИЛ)АМІДУ КОРІЧНОЇ КИСЛОТИ ЯК ПОТЕНЦІЙНОГО АНАЛОГУ САЛУБРІНАЛУ

В.В. Павлова, П.В.Задорожній, В.В. Кисельов, О.В. Охтіна, О.В. Харченко

У даній роботі нами було здійснено синтез N-(2,2,2-трихлор-1-(2-(фенілкарбамотіоіл)гідразин-1-карботіоамідо)етил)циннаміду, який є новим потенційним інгібітором холоферменту GADD34:PP1. Цю сполуку було одержано взаємодією еквімолярних кількостей N-(2,2,2-трихлор-1-ізотіоціанатоетил)циннаміду з N-фенілгідразинкарботіоамідом в ацетонітрилі. Реакцію проводили при кип'ятінні зі зворотним холодильником протягом двох хвилин. Вихід продукту після перекристалізації з ацетонітрилу становив 79%. Будова цільової сполуки була надійно доведена даними ЯМР ^{1}H, ЯМР ^{13}C та ІЧ спектроскопії. Оцінювання здатності одержаної сполуки інгібувати активність холоферменту GADD34:PP1 була здійснена з використанням молекулярного докінгу. Як еталон нами було обрано відомий інгібітор GADD34:PP1 – Salubrinal. Згідно з отриманими результатами синтезована сполука може ефективно взаємодіяти з активним сайтом мішені. Показано, що її молекула, як і молекула Salubrinal, утворює з амінокислотою Tyr 272 міжмолекулярний водневий зв'язок. За значенням енергії комплексу з GADD34:PP1 запропонована нами сполука не значно поступається еталону.

Ключові слова: салубрінал, GADD34:PP1, амід коричної кислоти, інгібітор, молекулярний докінг, стрес ендоплазматичного ретикулума, активний сайт.

SYNTHESIS, SPECTRAL CHARACTERISTICS AND MOLECULAR DOCKING STUDIES OF N-(2,2,2-TRICHLORO-1-(2-(PHENYLCARBAMOTHIOYL)HYDRAZINE-1-CARBOTHIOAMIDO)ETHYL)CINNAMAMIDE AS A POTENTIAL ANALOG OF SALUBRINAL

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In this work, we synthesized N-(2,2,2-trichloro-1-(2-(phenylcarbamothioyl)hydrazine-1-carbothioamido)ethyl)cinnamamide, which is a new potential inhibitor of the GADD34:PP1 holoenzyme. This compound was obtained by reacting equimolar amounts of N-(2,2,2-trichloro-1-isothiocyanatoethyl)cinnamamide with *N*-phenylhydrazinecarbothioamide in acetonitrile. The reaction was carried out at reflux for two minutes. The yield of the product after recrystallization from acetonitrile was 79%, and ^{1}H NMR, ^{13}C NMR, and IR spectroscopy reliably proved its structure. The ability of the obtained compound to inhibit the activity of the GADD34:PP1 holoenzyme was assessed using molecular docking. We selected a known GADD34:PP1 inhibitor Salubrinal as a standard. According to the results obtained, the synthesized compound can effectively interact with the active site of the target. It was shown that its molecule, like the Salubrinal molecule, formed an intermolecular hydrogen bond with the Tyr 272 amino acid. The compound we obtained was almost as good as the standard in terms of the energy value of the complex with GADD34:PP1.

Keywords: Salubrinal; GADD34:PP1; cinnamamide; inhibitor; molecular docking; endoplasmic reticulum stress; active site.

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