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Research Article

The synthesis and the antioxidant activity of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives

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Abstract

New 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives have been designed, synthesized and evaluated for their *in vitro* antioxidant activity under conditions of the artificial oxidative stress using ionol, ascorbic acid and α-tocopherol as the reference drugs. It has been found that 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carbothionic acid derivatives **9b, 9c, 9d, 9e, 9f, 9i** and 1-phenoxymethyl-4-(4[1]-chlorophenyl)-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene-3-carboxylic acid phenylamide **10** reveal a high antioxidant activity and a good *in silico* pharmacokinetic profile. The data obtained allowed us to select the most promising objects from the substances synthesized for further pharmacological screening for the presence of the antioxidant activity *in vivo*.

Keywords

synthesis; derivatives of 5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[cd]azulenes; antioxidant activity; in silico; ADMET properties

Introduction

Damage to cells by free radicals with the subsequent development of the oxidative stress plays a central role in the aging process and the progression of many diseases, for example, oral (Kesarwala et al. 2015) and inflammatory bowel diseases (Piechota-Polanczyk 2014; Dudzińska et al. 2018), atherosclerosis (Kattoor et al.), Alzheimer's disease (Pohanka 2018).

However, a number of studies indicate, with some conformity, that the oxidative stress along with the chronic inflammatory condition pave the way for the development of metabolic and cardiovascular diseases (Holvoet 2008; Matsuda and Shimomura 2014; Rani et al. 2016). Recent studies have shown that the oxidative stress is central in the etiology of male infertility; however, the exact effects of the oxidative stress on the DNA of spermatozoa have not yet been thoroughly studied (Bisht et al. 2017).

The negative impact of the chronic oxidative stress on the body's immune function is also well known (Dhabhar et al. 2012; Glovatchcka et al. 2012).

Antioxidants are our first line of defense against free radical damage, and they are critical to maintaining optimal health and well-being. In the process of the protective action, antioxidants are gradually removed from the body; and therefore, it is necessary to constantly restore their amount with food or pharmaceuticals.

We have already found promising objects for pharmacological screening for the presence of the antihypertensive activity among a number of 1,2,4-triazole derivatives containing piperidine or morpholine fragments in their structure (Perekhoda et al. 2020); it encourages us to continue searching for new biologically active substances among 1,2,4-triazole derivatives. In our opinion, the combination of the 1,2,4-triazole cycle in one molecule with such a well-known pharmacophore as azulene is one of the promising directions for the rational design of new biologically active compounds with the antioxidant action. Functionalization of the compounds obtained makes it possible to achieve the expected pharmacological effect of the "hybrid" molecules synthesized already at the stage of cyclization by radicals of various electronic nature. Each of the cycles of the resulting molecules can exhibit one or another pharmacological activity and is characterized by affinity to various biotargets; it can be considered as an advantage in implementing the concept of multi-target drugs. The use of the combination of *in vitro* and *in silico* studies of the pharmacokinetic profile of the structures obtained is a promising approach for optimizing the targeted search for new antioxidants.

Predicting the pharmacokinetic profile of candidate compounds (ADME/T profiling) can improve the effectiveness of early stages in developing new drugs (Benet et al. 2016; Hardjono et al. 2017; Pires et al. 2018; Han et al. 2019). Substances with undesirable physical and chemical properties are excluded at the early stages of screening, and it significantly reduces the amount of financial investment, labor costs, minimizes time-consuming experiments on animals, contributes to the humanization of the development process as a whole, and most importantly, it significantly increases its efficiency. Therefore, another task of our study was to determine *in silico* what the pharmacokinetic and toxicological (ADMET) properties of the compounds synthesized are.

The possibility of using this approach does not remove the relevance of *in vivo* research; moreover, it has such obvious advantages as cost-effectiveness, a high level of reproducibility, as well as there is no need in the chemical synthesis of a huge number of compounds.

The aim of our work was to synthesize 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives, predict their pharmacokinetic profile *in silico* and search *in vitro* promising antioxidants among them.

Materials and methods

Experimental part

Chemistry

All solvents were purified before use. Reactions were monitored by thin-layer chromatography (TLC) using Fluka silica gel (60 F 254) plates (0.25 mm). Visualization was made with UV light. Melting points of the compounds synthesized were determined by the Kofler method. Elemental analysis was performed on a EuroEA 3000 elemental analyzer. ^{1}H NMR spectra were recorded on a Varian Gemini 400 MHz device in DMSO-d6 using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded on a Varian MR-400 device in DMSO-d_6 using TMS as an internal standard. Chemical shifts were reported in ppm units using the δ scale.

3-Phenoxymethyl-6,7,8,9-tetrahydro-*5H*-[1,2,4]triazolo[4,3-a]azepine **3** was synthesized by the method (Siryi et al. 2009).

*The general procedure of the synthesis of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd] azulene **5a, 5b***. To the solution of 2.43 g (0.01 mol) of 3-phenoxymethyl-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-*a*]azepine **3** in 50 ml of ethyl acetate add the solution of 0.01 mol of the corresponding α-bromoacetophenone in 25 ml of ethyl acetate while stirring. Reflux the reaction mixture for 2 hours. After cooling, decant the solvent from oil-like salts **4a,b** used later without isolation and identification. Add 15 ml of 10% NaOH aqueous solution to the residue and reflux for 1 hour. After cooling filter the crystalline compounds formed, wash with water, and dry. Purify from benzene by crystallization.

*1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene **5a.*** Yield 49%, mp 176–178 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.97 (m, 2H, 6-CH_2), 2.09 (m, 2H, 7-CH_2), 2.79 (m, 2H, 5-CH_2), 4.06 (m, 2H, 8-CH_2), 5.24 (s, 2H, CH_2O), 7.16 (s, 1H, 3-H), 6.96–7.45 (m, 10H, 2Ph). Anal. calcd for $C_{22}H_{21}N_3O$, % N 12.2. Found, % N 12.4. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.76, 25.79, 26.78, 46.26, 70.17, 96.18, 113.3, 121.3, 121.8, 124.2, 124.3, 126.4, 128.9, 131.5, 136.5, 144.8, 148.0, 156.7.

*1-Phenoxymethyl-4-(4^{1}-chlorophenylphenyl)-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene **5b.*** Yield 52%, mp 198-199 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.98 (m, 2H, 6-CH_2), 2.08 (m, 2H, 7-CH_2), 2.79 (m, 2H, 5-CH_2), 4.07 (m, 2H, 8-CH_2), 5.23 (s, 2H, CH_2O), 7.19 (s, 1H, 3-H), 6.90–7.46 (m, 9H, C_6H_4+Ph). Anal. calcd for $C_{22}H_{20}ClN_3O$, % Cl 9.39, N 11.1. Found, % Cl 9.47, N 11.3. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.83, 25.71, 26.84, 46.31, 70.25, 98.24, 113.2, 119.9, 121.7, 124.2, 128.8, 129.9, 130.1, 130.2, 135.9, 144.7, 147.9, 156.6.

*General procedure of the synthesis of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[cd] azulene-3-carbothionic acid amides **9a-9k.*** The mixture 0.005 mole of 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5 a** and 0.005

mole of appropriate arylisothiocyanate **6 a,b** or **7 c-k** was refluxed in 50 ml of dry benzene during 2 hours. After cooling the solid **9 a-k** was filtered, washed with benzene, then dry on air and recrystallize from benzene or propanol-2.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid allyl amide ***9a.*** Yield 68%, mp 154-155 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.96 (m, 2H, 6-CH_2), 2.08 (m, 2H, 7-CH_2), 2.45 (m, 2H, 5-CH_2), 4.19 (m, 2H, 8-CH_2), 4.33 (m, 2H, CH_2-CH=CH_2), 5.19 (m, 2H, CH_2-CH=CH_2), 5.39 (s, 2H, CH_2O), 5.96 (m, 1H, CH_2-CH=CH_2), 7.00–7.35 (m, 10H, 2Ph), 9.09 (t, 1H, NH). Anal. calcd for $C_{26}H_{26}N_4OS$, % N 12.7; S 7.23. Found, % N 12.8; S 7.34. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.77, 25.82, 26.80, 42.25, 48.36, 70.15, 90.31, 113.3, 115.5, 121.8, 123.0, 125.6, 128.9, 130.5, 131.3, 134.5, 138.6, 139.5, 146.5, 152.4, 156.7, 191.3.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid benzylamide ***9b.*** Yield 71%, mp 146-147 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.92 (m, 2H, 6-CH_2), 2.09 (m, 2H, 7-CH_2), 2.41 (m, 2H, 5-CH_2), 4.19 (m, 2H, 8-CH_2), 4.94 (d, 2H, $NHCH_2$), 5.37 (s, 2H, CH_2O), 6.98–7.33 (m, 15H, 3Ph), 9.30 (t, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4OS$, % N 11.4; S 6.50. Found, % N 11.5; S 6.67. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.74, 25.78, 26.77, 46.23, 54.39, 70.16, 94.89, 113.2, 212.7, 122.5, 125.5, 127.6, 128.8, 130.0, 130.4, 131.2, 138.8, 139.1, 140.2, 146.5, 152.4, 156.6, 195.0.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid phenylamide ***9c.*** Yield 81%, mp 188-189 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.94 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.48 (m, 2H, 5-CH_2), 4.20 (m, 2H, 8-CH_2), 5.40 (s, 2H, CH_2O), 7.00–7.59 (m, 15H, 3Ph), 10.5 (s, 1H, NH). Anal. calcd for $C_{29}H_{26}N_4OS$, % N 11.7; S 6.69. Found, % N 11.9; S 6.84. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.70, 25.73, 26.81, 46.25, 70.17, 95.73, 113.4, 114.6, 121.8, 124.9, 126.6, 128.4, 128.9, 131.1, 131.6, 136.1, 139.6, 146.6, 149.7,156.7, 193.1.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid o-tolylamide ***9d.*** Yield 79%, mp 192-193 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.96 (m, 2H, 6-CH_2), 2.10 (m, 2H, 7-CH_2), 2.21 (s, 3H, CH_3), 2.47 (m, 2H, 5-CH_2), 4.22 (m, 2H, 8-CH_2), 5.41 (s, 2H, CH_2O), 6.99–7.49 (m, 14H, 2Ph+C_6H_4), 10.3 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4OS$, % N 11.4; S 6.50. Found, % N 11.1; S 6.63. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 17.90, 21.73, 25.81, 26.79, 46.26, 70.16, 95.69, 113.3, 114.5, 121.7, 123.5, 123.8, 125.7, 128.9, 131.0, 131.5, 131.8, 136.5, 139.6, 144.9, 146.5, 149.6, 156.6, 192.8.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid m-tolylamide ***9e.*** Yield 67%, mp 173-174 °C, ^{1}H NMR (500 MHz, DMSO-d6), δ: 1.94 (m, 2H, 6-CH_2), 2.10 (m, 2H, 7-CH_2), 2.30 (s, 3H, CH_3), 2.48 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.41 (s, 2H, CH_2O), 6.92–7.41 (m, 14H, 2Ph+C_6H_4), 10.4 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4OS$, % N 11.4; S 6.50. Found, % N 11.5; S 6.68.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid p-tolylamide ***9f.*** Yield 75%, mp 185-186 °C, ^{1}H NMR (500 MHz, DMSO-d6), δ: 1.94 (m, 2H, 6-CH_2), 2.10 (m, 2H, 7-CH_2), 2.29 (s, 3H, CH_3), 2.49 (m, 2H, 5-CH_2), 4.20 (m, 2H, 8-CH_2), 5.41 (s, 2H, CH_2O), 7.00–7.47 (m, 14H, 2Ph+C_6H_4), 10.4 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4OS$, % N 11.4; S 6.50. Found, % N 11.6; S 6.39.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid (2,4-dimethylphenyl)amide ***9g.*** Yield 63%, mp 188-189 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.92 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.15 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.43 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.40 (s, 2H, CH_2O), 6.95–7.39 (m, 13H, 2Ph+C_6H_3), 10.4 (s, 1H, NH).Anal. calcd for $C_{31}H_{30}N_4OS$, % N 11.1; S 6.32. Found, % N 11.3; S 6.44. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 17.49, 20.65, 21.74, 25.79, 26.80, 46.21, 70.21, 95.77, 113.2, 114.6, 121.8, 122.0, 123.6, 125.6, 128.8, 131.1, 131.6, 131.7, 138.5, 139.5, 145.6, 146.6, 149.7, 156.7, 192.7.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid (2,5-dimethylphenyl)amide ***9h.*** Yield 65%, mp 175-176 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.93 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.15 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.43 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.41 (s, 2H, CH_2O), 6.96–7.36 (m, 13H, 2Ph+C_6H_3), 10.2 (s, 1H, NH). Anal. calcd for $C_{31}H_{30}N_4OS$, % N 11.1; S 6.32. Found, % N 10.9; S 6.19.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid (3,4-dimethylphenyl)amide ***9i.*** Yield 71%, mp 197-198 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.93 (m, 2H, 6-CH_2), 2.13 (m, 2H, 7-CH_2), 2.14 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 2.43 (m, 2H, 5-CH_2), 4.22 (m, 2H, 8-CH_2), 5.43 (s, 2H, CH_2O), 6.98–7.41 (m, 13H, 2Ph+C_6H_3), 10.3 (s, 1H, NH). Anal. calcd for $C_{31}H_{30}N_4OS$, % N 11.1; S 6.32. Found, % N 11.2; S 6.39.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid (2-methoxyphenyl)amide ***9j.*** Yield 60%, mp 164-165 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.93 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.41 (m, 2H, 5-CH_2), 3.80 (s, 3H, OCH_3), 4.21 (m, 2H, 8-CH_2), 5.41 (s, 2H, CH_2O), 6.89–8.60 (m, 14H, 2Ph+C_6H_4), 10.5 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4O_2S$, % N 11.0; S 6.29. Found, % N 11.3; S 6.43.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carbothioic acid (4-methoxyphenyl)amide ***9k.*** Yield 60%, mp 205-205 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.94 (m, 2H, 6-CH_2), 2.10 (m, 2H, 7-CH_2), 2.47 (m, 2H, 5-CH_2), 3.74 (s, 3H, OCH_3), 4.20 (m, 2H, 8-CH_2), 5.40 (s, 2H, CH_2O), 6.92–7.45 (m, 14H, 2Ph+C_6H_4), 10.4 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4O_2S$, % N 11.0; S 6.29. Found, % N 11.1; S 6.37. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.71, 25.77,

26.81, 46.23, 55.64, 70.18, 95.74, 113.3, 114.5, 121.7, 125.5, 128.9, 131.1, 131.5, 134.7, 136.1, 139.6, 146.5, 149.6, 155.6, 193.1.

General procedure of the synthesis of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[cd] azulene-3-carboxylic acid amides ***9l-9o, 10.*** The mixture 0.005 mole of 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a** or **5b** and 0.005 mole of appropriate arylisocyanate **8l-o** was reflaxed in 50 ml of dry benzene during 2 hours. After cooling the solid **9a-k** was filtered, washed with benzene, then dry on air and recrystallize from benzene or ethanol.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carboxylic acid phenylamide ***9l.*** Yield 85%, mp 215-216 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.96 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.53 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.46 (s, 2H, CH_2O), 6.98–7.52 (m, 15H, 3Ph), 9.40 (s, 1H, NH). Anal. calcd for $C_{29}H_{26}N_4O$, % N 12.1. Found, % N 12.4. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.41, 25.80, 26.78, 46.23, 70.15, 95.52, 113.3, 117.3, 120.4, 121.8, 123.2, 124.2, 128.5, 128.8, 129.8, 131.5, 138.7, 142.8, 149.0, 152.4, 156.6, 162.2.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carboxylic acid (3-chlorophenyl)amide ***9m.*** Yield 88%, mp 190-191 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.96 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.53 (m, 2H, 5-CH_2), 4.20 (m, 2H, 8-CH_2), 5.48 (s, 2H, CH_2O), 7.00–7.80 (m, 14H, 2Ph+C_6H_4), 9.51 (s, 1H, NH). Anal. calcd for $C_{29}H_{25}ClN_4O_2$, % N 11.3. Found, % N 11.5. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.39, 25.74, 26.77, 46.25, 70.21, 95.57, 113.4, 117.4, 118.4, 119.6, 121.7, 123.6, 124.1, 128.9, 129.1, 129.7, 129.9, 131.4, 132.7, 138.6, 140.0, 149.1, 152.3, 156.7, 162.4.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carboxylic acid (2-methoxyphenyl)amide ***9n.*** Yield 72%, mp 180-181 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.94 (m, 2H, 6-CH_2), 2.12 (m, 2H, 7-CH_2), 2.46 (m, 2H, 5-CH_2), 3.79 (s, 3H, OCH_3), 4.22 (m, 2H, 8-CH_2), 5.42 (s, 2H, CH_2O), 6.79–8.55 (m, 14H, 2Ph+C_6H_4), 10.5 (s, 1H, NH). Anal. calcd for $C_{30}H_{28}N_4O_3$, % N 11.4. Found, % N 11.2. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.42, 25.79, 26.79, 46.26, 56.41, 70.17, 95.53, 114.4, 113.3, 117.3, 118.7, 119.9, 121.6, 124.2, 125.0, 128.4, 128.9, 129.8, 131.5, 138.6, 145.9, 149.1, 152.4, 156.7, 161.2.

1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carboxylic acid (3,4-dichlorophenyl)amide ***9o.*** Yield 91%, mp 195-196 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.96 (m, 2H, 6-CH_2), 2.11 (m, 2H, 7-CH_2), 2.54 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.48 (s, 2H, CH_2O), 7.01–7.99 (m, 13H, 2Ph+C_6H_3), 9.57 (s, 1H, NH). Anal. calcd for $C_{29}H_{24}Cl_2N_4O_2$, % N 10.5. Found, % N 10.8.

1-Phenoxymethyl-4-(4^1-chlorophenyl)-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[cd]azulene-3-carboxylic acid phenylamide ***10.*** Yield 80%, mp 228-229 °C, ^{1}H-NMR (500 MHz, DMSO-d6), δ: 1.97 (m, 2H, 6-CH_2), 2.12 (m, 2H, 7-CH_2), 2.57 (m, 2H, 5-CH_2), 4.21 (m, 2H, 8-CH_2), 5.47 (s, 2H, CH_2O), 6.98–7.54 (m, 14H, 2Ph+C_6H_4), 9.48 (s, 1H, NH). Anal. calcd for $C_{29}H_{25}ClN_4O_2$, % N 11.3. Found, % N 11.6. ^{13}C-NMR (100 MHz, DMSO-d6) δ: 21.39, 25.75, 26.83, 46.27, 70.19, 95.45, 113.2, 117.3, 120.5, 121.7, 123.3, 127.6, 128.5, 128.9, 130.6, 132.5, 138.1, 142.7, 149.1, 152.4, 156.7, 162.1.

Antioxidant and antiradical activity

To study the antioxidant activity (AOA) of potential drugs, especially at the initial stages of their biological screening, it is feasible to use methods of the primary assessment of the antioxidant and antiradical activity of compounds in the experiments *in vitro* (Albert 1971; Hubskyi et al. 2001).

At the same time, it is advisable to conduct pharmacological studies of AOA of new substances on several models of initiation of free radical reactions in the experiments *in vitro* showing different stages of the complex chain process of activation of free radical oxidation.

To study the structure-activity relationship, the initial substance – 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a** was the object of screening for the presence of the antiradical and antioxidant activity *in vitro*, in addition to the target carbothioic and carboxylic acids derivatives containing 5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene moiety **9a-9o, 10**.

The 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives **9a-9o, 10** under research differ in the presence and nature of substituents in a thioamide (compounds **9a-9k**) or amide (compounds **9l-9o, 10**) fragment of a molecule.

The presence of the antiradical and antioxidant activity of the substances synthesized was studied in the experiments *in vitro* when initiating free radical processes by modeling the artificial oxidative stress using an emulsion of yolk lipoproteins (Perekhoda et al. 2017.) placed in the culture medium with an optimal pH value of 7.5 for biological systems as an oxidation substrate. The model system chosen has a number of the following advantages. It is affordable; the release of lipoproteins is easy; the model is stable during storage, and, at the same time, it has a high oxidizability since the yolk of chicken eggs contains two types of lipid-protein complexes that correspond to lipoproteins of very low and low density of the blood plasma by their lipid and protein composition.

The experiment was performed under simulated conditions; the variants of the experiment included the control (dimethyl sulfoxide (DMSO) as a solvent), solutions of reference drugs (ionol, ascorbic acid and α-tocopherol) and the compounds synthesized with a titer of 0.3 mg/mL in the incubation medium.

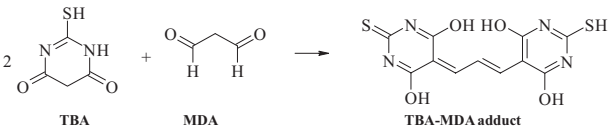
To prepare the model system, the yolk was isolated from a chicken egg, then it was mixed with an equal volume of the potassium phosphate buffer solution (40 mM KH_2PO_4 + 105 mM KCl, pH 7.5). The resulting emulsion of yolk lipoproteins (YLP) was 25 times diluted with the

same buffer solution before use. The test compounds, as well as the reference drugs, were prepared in the form of DMSO solutions with the initial titer of 3 mg/mL.

The oxidative stress was modeled as follows. To 1 ml of YLP emulsion sequentially 0.5 ml of solutions of the test substances, 0.5 ml of 0.5 mM Ferum (II) sulfate solution (the ROS generation system) and 3 mL the potassium phosphate buffer solution (40 mM KH_2PO_4 + 105 mM KCl, pH 7.5) were added. The resulting solution was mixed and incubated for 30 minutes at 37° C in a Water Thermostat ITJ-0-03, and then irradiated with a 40 W fluorescent light source.

After incubation the solution was cooled and used to determine the products of lipid peroxidation, their intensity was estimated by the accumulation of TBA-reactive products, in particular malonyldialdehyde (MDA) by its reaction with thiobarbituric acid (Bohacheva et al. 2016). To do this, 2 ml of cooled 20% trichloroacetic acid (TCA) and 0.1 ml of 0.01 m alcohol ionol solution were added to the solution obtained after incubation. The resulting solution was placed in the refrigerator for 12 hours, after that the samples were centrifuged at 4000 rpm. Then, 1 ml of 0.8% thiobarbituric acid solution freshly prepared in 0.3% sodium dodecyl sulfate solution was added to 2.5 ml of the TCA extract and placed in a boiling water bath for 10 minutes. It is known (Bohacheva et al. 2016) that in the acidic medium MDA reacts with 2-thiobarbituric acid to form a colored azomethine complex with an absorption maximum of 532 nm (Scheme 1). The resulting complex was extracted with alcohols; thus, after cooling, 4 ml of butanol-1 was added to the sample, and the optical density of butanol extracts was measured using a SF-46 spectrophotometer at a wavelength of 532 nm.

The antioxidant properties of the compounds studied was calculated taking into account the formation of TBA-active adducts formed during the interaction of TBA with MDA according to Scheme 1 in control samples containing DMSO, samples of the test compounds and inhibition of the formation of TBA adducts by the reference drugs according to the Formula 1:



Scheme 1. The interaction between malonyldialdehyde and thiobarbituric acid.

$$\%AOA_{\text{substance}} = \frac{A_{DMSO} \bullet A_{\text{substance}}}{A_{DMSO} \bullet A_{reference\_drug}} \bullet 100\%$$

where A_{DMSO} – is the average value of the optical density of solutions containing DMSO as a solvent;

$A_{substance}$ – is the average value of the optical density of solutions containing DMSO solutions of the test compounds;

$A_{reference\_drug}$ – is the average value of the optical density of solutions containing DMSO solutions of the reference drugs.

The content of malonyldialdehyde was calculated by the formula 2 (Bohacheva et al. 2016):

$$[\text{MDA}] = \frac{A_{532} \cdot 10^6 \cdot 5.25}{1.56 \cdot 10^5 \cdot 1\text{ml}};$$

where [MDA] – is the concentration of malonyldialdehyde, nmol/mL;

A_{532} – is the optical density of the solution at 532 nm;

10^6 – is the conversion factor to nmol/ml or μm/L;

$1.56 \cdot 10^5$ – is the molar optical density coefficient of the trimethine complex at 532 nm, $mole^{-1} \cdot cm^{-1}$;

5.25 – is the sample dilution factor.

The mathematical processing of the data obtained was performed by calculating the unpaired t-test (Lakyn 1990). In all cases, the analytical repeatability was 5 (n=5). The probable effect of the compounds studied on the inhibition of the formation of TBA-reactive products was assessed for the significance level $p<0.05$ by comparing the content of MDA in solutions of the test compounds and solutions of ionol, ascorbic acid and α-tocopherol.

In silico studies of ADMET properties

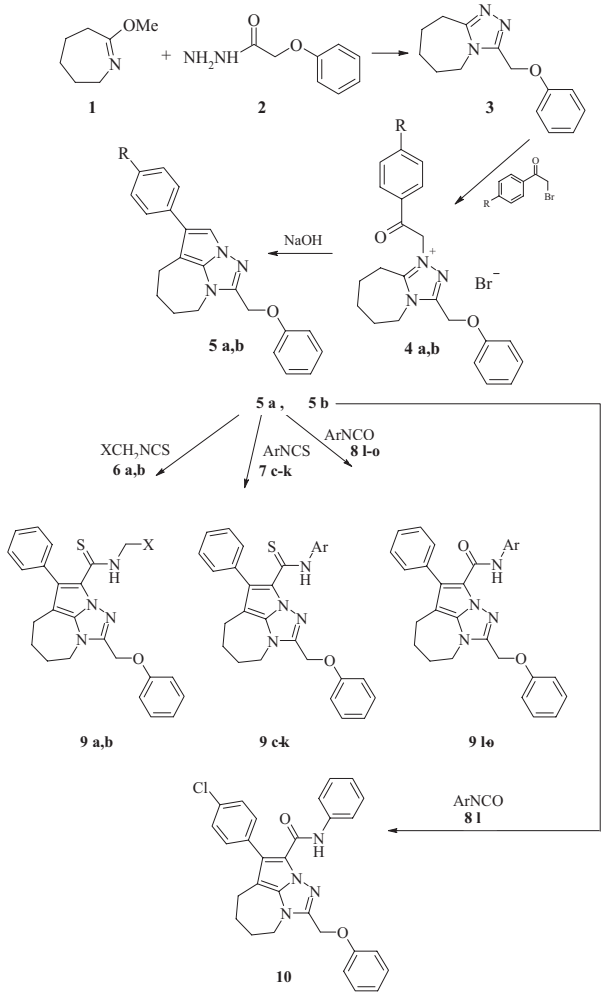
Prediction of the pharmacokinetic properties (ADME: absorption, distribution, metabolism, and excretion) and toxicity of the carbothioic and carboxylic acids derivatives containing 5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*] azulene moiety **9a-9o, 10** and 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a** was performed using the pkCSM online tool, i.e., firstly, the test compounds were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then stored as * .sdf file. Secondly, all of the compounds tested were translated into the SMILES format using SMILES Translator Online Help. In the SMILES format, the compounds were processed using the pkCSM online tool (pkCSM) to predict the ADME and toxicity of the compounds. The properties involved in distribution, metabolism, excretion and toxicity, such as volume of distribution (VDss) and central nervous system (CNS) permeability; CYP450 inhibitors; total clearance; and hepatotoxicity, respectively, were analyzed through this server.

Results and discussion

3-(Phenoxymethyl)-6,7,8,9-tetrahydro-5*H*-[1,2,4]triazolo[4,3-a]azepine **3** was obtained by the cyclization of 2-methoxy-3,4,5,6-tetrahydro-7*H*-azepine **1** with 2-phenoxyacetohydrazide **2** and further alkylation of compound **3** by the corresponding α-halogen ketones in the ethyl acetate medium. 1-Phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a,b** were isolated as bases under the action the solution of sodium hydroxide since the corresponding hydrobromides

were oily precipitates. The synthesis of the target compounds – new 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives **9a-9o, 10** was carried out *via* formation of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a, b** via several stages according to Scheme 2:

It should be noted that the reaction of the formation of target compounds occurred more easily by the interaction between the 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a** and the corresponding arylisocyanates. It was confirmed by higher yields (72–91%) of carboxylic acid derivatives **9l-9o, 10**, while the yield of most carbothionic acid derivatives (**9a,e,g,k**) was relatively low (60–68%).



Scheme 2. The synthesis of the 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[cd]azulene-3-carboxylic (or carbothionic) acid derivatives **9a-9o, 10.** Where **6-9 a)** X= -CH=CH_2, **b)** X= Ph, **c), l** Ar=Ph, **d)** 2MeC_6H_4, **e)** 3MeC_6H_4, **f)** 4MeC_6H_4, **g)** 2,4$Me_2C_6H_3$, **h)** 2,5$Me_2C_6H_3$, **i)** 3,4$Me_2C_6H_3$, **j)** 2$MeOC_6H_4$, **k)** 4$MeOC_6H_4$, **m)** 3ClC_6H_4, **n)** 2$MeOC_6H_4$, **o)** 3,4$Cl_2C_6H_3$.

The values of C, H and N calculated in the compounds newly synthesized were in excellent agreement with the experimental values found from elemental analysis results. The structure of these compounds proposed was further confirmed by ^{1}H NMR- and ^{13}C NMR spectral data.

Antioxidant Activity *in vitro*. To assess the ability of the test compounds to exhibit antioxidant effects and the level of their activity against ionol, ascorbic acid, and α-tocopherol, the percentage of inhibition of the formation of TBA-reactive products, namely the content of MDA, was calculated (Tab. 1).

Among the reference drugs, ionol (0.79 nmol MDA/ml of the YLP emulsion) showed the highest degree of inhibition of the formation of TBA-reactive products, while ascorbic acid (2.38 nmol MDA/ml of the YLP emulsion) demonstrated the lowest degree of inhibition in relation to the control containing DMSO. α-Tocopherol had an intermediate value of AOA (1.49 nmol MDA/ml of the YLP emulsion.

Table 1. The antioxidant activity of derivatives containing the 5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene moiety (in % of inhibition of the formation of TBA-reactive products).

The variant of the experiment	Substituent, or Ar	The MDA content (nmol/ml of the yolk lipoprotein emulsion)	AOA (% in relation to ionol)	AOA (% in relation to ascorbate)	AOA (% in relation to α-tocopherol)
DMSO		3.857 ± 0,04	–	–	–
Ionol		0.787 ± 0,01	–	–	–
Ascorbate		2.38 ± 0,03	–	–	–
α–Tocopherol		1.49 ± 0,02	–	–	–
5 a	–	0.82 ± 0,019	98.70	205.41	128.27
9 a	$CH_2CH=CH_2$	2.79 ± 0,014	34.74	72.30	45.15
9 b	CH_2Ph	1.6 5 ± 0,017	71.75	149.32	93.25
9 c	Ph	1.14 ± 0,031	88.60	183.78	114.77
9 d	2MeC_6H_4	1.25 ± 0,014	85.02	176.35	110.13
9 e	3MeC_6H_4	1.36 ± 0,015	81.43	168.92	105.49
9 f	4MeC_6H_4	1.45 ± 0,032	78.50	162.83	101.69
9 h	2,5$Me_2C_6H_3$	1.85 ± 0,014	65.47	135.81	84.81
9 g	2,4$Me_2C_6H_3$	1.69 ± 0,004	70.68	146.62	91.56
9 i	3,4$Me_2C_6H_3$	1.36 ± 0,008	81.43	168.92	105.49
9 j	2$MeOC_6H_4$	1.86 ± 0,037	65.15	135.14	84.39
9 k	4$MeOC_6H_4$	1.02 ± 0,007	92.50	191.89	119.83
9 l	Ph	2.87 ± 0,008	32.24	66.89	41.77
9 m	3ClC_6H_4	1.88 ± 0,003	64.50	133.78	83.54
9 n	2$MeOC_6H_4$	1.15 ± 0,050	88.27	183.11	114.35
9 o	3,4$Cl_2C_6H_3$	2.55 ± 0,010	42.67	88.51	55.27
10	4ClC_6H_4	1.88 ± 0,003	90.55	187.84	117.30

According to the results of the primary pharmacological screening *in vitro*, all substances tested are able to exhibit the antioxidant activity in one way or another. The carbothionic acid derivative with an allyl substitute in the phenyl fragment of the molecule (compound **9a**), the carboxylic acid derivative with non-substituted phenyl and dichloro-substituted phenyl substituents (compounds **9l** and **9o,** respectively) demonstrated the lowest percentage of inhibition of the formation of TBA-reactive products, and therefore, the lowest activity at the level of 32.24–42.67%, 66.89–88.51% and 41.77–55.27% in relation to ionol, ascorbic acid and α-tocopherol, respectively. It is interesting to note that during the experiment all compounds with chloro-, benzylidene and methyl substituents in the phenyl fragment of the molecule (compounds **9m, 9b, 9d-9k**, respectively) showed a moderate antioxidant and antiradical activity in the range of 64.50–85.02%,

133.78–176.35% and 83.54–110.13%, in relation to each of the reference drugs.

The initial compound **5a** had the highest ability to inhibit the formation of TBA-reactive products among all substances; it showed the antioxidant and antiradical activity approaching the level of the ionol effect (98.70%), while its activity exceeded the activity of ascorbic acid by 105% (205.41%), and the activity of α-tocopherole by 28% (128.27%).

The interaction between pharmacokinetics, toxicity, and potency is crucial for effective drugs. PkCSM can predict how molecules are distributed within the body based on their structure. The volume of distribution (VD) is the calculated volume that the whole quantity of a drug will be circulated at an equal level of blood plasma. The higher the VD is, the larger the amount of the drug is distributed to the tissue rather than plasma. This model is set from the estimation of the steady-state volume of distribution (VDss), which is then revealed as log L/kg. According to Pires et al. (Pires et al. 2015), VDss higher than 2.81 L/kg (log VDss > 0.45) is categorized as high, whereas VDss lower than 0.71 L/kg (log VDss < -0.15) is categorized as low. It can be seen that the VDss values of the test compounds range from 0.0038 to 0.688; therefore, it can be predicted that all these compounds can be distributed evenly providing an equal level of the blood plasma. It should be noted that compounds **9a** (log VDss = 0.688), **5a** (log VDss = 0.596), **9m** (log VDss = 0.523), **9b** (log VDss = 0.507), **9c** (log VDss = 0.446) had the highest value of VDss. According to the data obtained all test compounds can be well distributed in the body.

Evaluating the ability of potential antioxidants to pass through the blood-brain barrier (BBB) is essential at the early stages of their development. The distribution of potential antioxidants in the structures of the brain, its cellular elements and subcellular fractions is very important since hypoxia is the basis of many neurological diseases (Andreeva 2009).

Based on the pkCSM result it could be assumed as well that all test compounds would be well distributed to the brain (besides the initial compound, 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene **5a** and 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene-3-carbothioic acid (4-methoxyphenyl)amide **9k**).

Especially 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carbothionic acid derivatives **9b, 9c, 9d, 9e, 9g, 9i** have high values of LogBB and LogPS, thus, they are predicted to readily cross the blood-brain barrier and penetrate directly into the central nervous system.

Based on the *in silico* study of the pharmacokinetic profiles of the test compounds using pkCSM online tool it can be concluded that, generally, they can be well absorbed through oral administration (% absorption more than 92%).

The degree of binding to blood proteins for all test compounds is high and amounts to more than 82%.

Cytochrome P450 is an important detoxification enzyme in the body, mainly found in the liver. It oxidizes xenobiotics to facilitate their excretion. Many drugs are deactivated by cytochrome P450, but some can be activated by it. Cytochrome P450 is responsible for the metabolism of many drugs. However, inhibitors of it can dramatically alter the pharmacokinetics of these drugs; therefore, it is important to evaluate whether a given compound is likely to be a cytochrome P450 substrate. Two main isoforms, which are responsible for the drug metabolism, are P2D6 cytochrome (CYP2D6) and P3A4 cytochrome (CYP3A4). All test compounds can be substrates or inhibitors of these isoforms.

The results also showed that all test compounds could not be the substrates of Organic Cation transporter 2 (OCT2), a renal uptake transporter playing an important role in drug elimination through the kidney. From the above result, it can be concluded that all the compounds studied are excreted through the kidneys by a mechanism other than OCT2.

The potential toxicity of prospective compounds should be assessed. The acute toxicity and relative toxicity of all compounds can be determined by the lethal dose value.

The maximum tolerated dose for all test substances, with the exception of compounds **9n, 9g** and **9j,** is in the dose range from 0.413 to 0.755. The maximum tolerated dose of compounds **9n, 9g** and **9j** is less than 0.413, which is an unfavorable parameter.

The value of LD_{50}, the average lethal dose of a substance causing the death of 50% of the experimental animals when administered in one dose, was also predicted. The value of LD_{50} characterizing the acute toxicity in rats in oral administration of the compounds studied was in the range of 2.29–3.4 mol/kg; moreover, it should be noted that this dose was the lowest for the initial compound **5a**, and the highest for 1-phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene-3-carboxylic acid (2-methoxyphenyl)amide **9n**.

The dose of substances causing the chronic toxicity in rats when administered orally for most compounds is 0.16–0.66, while for compounds **5a, 9g, 9i, 9m, 9n** it is 0.706–1.636 (log mg/kg of the body weight per day), which is a safer parameter.

Regarding the toxicological properties of the derivatives containing the 5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene moiety it should be noted that according to the forecast all compounds (with the exception of **9a**) have a 50% probability of cardiotoxicity considering their ability to suppress the hERG II gene, and the inability to suppress the hERG I. 1-Phenoxymethyl-4-phenyl-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene-3-carbothionic acid allyl amide **9a** probably will not have cardiotoxicity. Moreover, these molecules displayed promising results in the synthetic accessibility assessment and *in silico* ADMET evaluations.

Summing up the results obtained, it can be concluded that it is promising to conduct further experimental biological tests for the presence of the antioxidant activity of 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carbothionic acid derivatives **9b, 9c, 9d, 9e, 9f, 9i** and 1-phenoxymethyl-4-(4^1-chlorophenyl)-5,6,7,8-tetrahydro-2,2a,8-triazacyclopenta[*cd*]azulene-3-carboxylic acid phenylamide **10** in the experiments *in vivo*. Despite the high activity of compounds **5a**, **9k**, **9n**

in vitro it is impractical to conduct the *in vivo* study taking into account the probably moderate ability of compounds **5a**, **9k** to be distributed in the brain and the probable too high maximum tolerated dose of compound **9n**.

We expect that the present analysis and the database would allow identification of potential antioxidant molecules, which follow ADMET properties and act as a valuable lead for the drug development.

Conclusion

1. Fourteen new 1-phenoxymethyl-4-aryl-5,6,7,8-tetrahydro-2a,4a,8a-triazacyclopenta[*cd*]azulene-3-carboxylic (or carbothionic) acid derivatives have been synthesized, their structure and purity have been confirmed by ^{1}H NMR- and ^{13}C NMR-spectroscopy.
2. All compounds synthesized were tested for the antioxidant activity under conditions of the artificial oxidative stress *in vitro*.
3. When individually applying the parameters in each of ADMET group compounds **9b, 9c, 9d, 9e, 9f, 9i** and **10** that are absorbable, distributable, metabolized, excreted and nontoxic have been identified.
4. The molecules showing the promising *in vitro* and *in silico* results have been selected for further research *in vivo*.

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