PHARMACIA

Pharmacia 69(3): 719–731
DOI 10.3897/pharmacia.69.e86036

Research Article

Synthesis and antioxidant activity of 3-(2-R-ylidenehydrazinyl)-6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones

Yevhenii Novodvorskyi[1], Dmitry Lega[2], Igor Komarov[1], Iryna Zhuravel[2], Oleg Moskalenko[3], Anatolii Demchenko[3]

1 *Taras Shevchenko National University of Kyiv, Kiyv, Ukraine*
2 *National University of Pharmacy of the Ministry of Health of Ukraine, Kharkiv, Ukraine*
3 *Nizhyn Mykola Gogol State University, Nizhyn, Ukraine*

Corresponding author: Yevhenii Novodvorskyi (novodvorskiyps@gmail.com)

Received 1 May 2022 ♦ **Accepted** 19 June 2022 ♦ **Published** 5 August 2022

Citation: Novodvorskyi Y, Lega D, Komarov I, Zhuravel I, Moskalenko O, Demchenko A (2022) Synthesis and antioxidant activity of 3-(2-R-ylidenehydrazinyl)-6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones. Pharmacia 69(3): 719–731. https://doi.org/10.3897/pharmacia.69.e86036

Abstract

Synthesis and structure elucidation of several series of new hydrazones containing 1,2,4-triazine-5-one core and their antioxidant activity are presented. The target compounds have been synthesized *via* interaction of either 4-amino-6-(*tert*-butyl)-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one or 6-(*tert*-butyl)-3-hydrazinyl-1,2,4-triazin-5(2*H*)-one with a wide range of compounds with a carbonyl group in moderate to high yields. Molecular structures of the synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and elemental analyses. The antioxidant activity of these compounds against ascorbic acid was screened to determine their potential as promising oxidative stress suppressors. Our data showed that hydrazones derived from 4-amino-6-(*tert*-butyl)-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one are the most active antioxidants among all tested compounds. Furthermore, 3 compounds of this series have been proved to be twice as active as ascorbic acid does. The conclusions are substantiated for in-depth investigations of these derivatives as promising agents for the treatment of disorders accompanied by oxidative stress.

Keywords

1,2,4-triazine, synthesis, Schiff base, hydrazone, oxidative stress, antioxidant activity

Introduction

Antioxidants (both natural and synthetic) and antioxidant activity have recently become extremely popular in the scientific community and come under the spotlight of many researches, reports, and reviews (Bjelakovic et al. 2007, 2012; Stanner et al. 2007; Jiang et al. 2010). This is a very fast-growing section of chemistry, biochemistry and bioanalysis in particular. According to Scopus, the number of papers containing the word "antioxidants" in journals published during 2000–2018 years is 301,795 (Brainina et al. 2019). Such a state of things is dictated by some reasons. One of them is that antioxidants are widely used in the food industry to protect products from the harmful effects of oxidation (Shahidi 2015). Apart from this is their application in medicine. It is known that some endo- and exogenous factors are exhausting the antioxidant system of a human organism. For instance, the major air pollutants causing public health concerns, such as ozone, nitrogen oxides and particulates are potent oxidants or able

to generate reactive oxygen species (ROS) (Kelly 2003). Moreover, as the body ages, antioxidant levels decline, resulting in a disruption in the balance between antioxidants and prooxidant molecules (Mulgund et al. 2015). All these give rise to oxidative stress and in turn, override the scavenging capacity by antioxidants either due to the diminished availability of antioxidants or excessive generation of ROS and finally trigger redox-sensitive pathways that result in different biological processes such as inflammation and cell death (Lodovici and Bigagli 2011). Moreover, oxidative stress is thought to be associated with various human disorders, such as atherosclerosis (Kaneto et al. 2010; Uno and Nicholls 2010), attention deficit hyperactivity disorder (Joseph et al. 2013), cancer (Halliwell 2006), Parkinson's disease (Hwang 2013), Alzheimer's disease (Valko et al. 2007), etc. Therefore, supplementation with oral oxidants may help to alleviate oxidative stress and its contribution to the pathogenesis of numerous diseases.

Summarizing the above, a search for new molecules possessing antioxidant activity is one of the promising, topical and urgent tasks of synthetic chemistry. Nowadays, heterocyclic chemistry is a deep well of ideas providing solutions to limitless problems accompanying our life (Lipkus et al. 2008; Kolodyazhna et al. 2021). Each of the heterocyclic backbones as well as their countless combinations have been found to be responsible for valuable biological properties allowing their derivatives to be used for the treatment of specific disorders (Garcia-Castro et al. 2016; Grygoriv et al. 2018).

One of these heterocyclic bricks is 1,2,4-triazine moiety which has been known since the middle of the 20th century (Paudler and Barton 1966). During the last half-century 1,2,4-triazine chemistry received considerable attention in the literature. This is reflected in a number of articles and reviews published in the specialized scientific journals, as well as monographs and patents dealing with various aspects of the functionalized 1,2,4-triazine system (Ivanov 2022). It is worth noting that 1,2,4-triazines and their heteroannelated analogues occupy a key position in modern medicinal chemistry because of their high potential for pharmacological activities (Kumar et al. 2014). They possess a wide array of biological actions, including antiviral, antihypertensive, cardioprotective, antimicrobial, antifungal, anxiolytic, anti-HIV, selective carboxylesterase inhibition, analgesic and anti-inflammatory activities (El-Barbary et al. 2005; Khoshneviszadeh et al. 2013; Shchegol'kov et al. 2017). Some derivatives have been proved to be acetylcholinesterase inhibitors (Liu et al. 2014). Many investigations revealed the anticancer potential of triazine derivatives (El Massry et al. 2012; El-Wakil et al. 2019). Moreover, derivatives of the 1,2,4-triazine ring have attracted extensive attention from numerous research groups because of their antioxidant activity (AOA) which can be a supportive mechanism in displaying other bioactivities mentioned above. To the best of our knowledge, most investigations of AOA have employed heteroannelated 1,2,4-triazines (Iwashita et al. 2003; Hamama et al. 2017; Ulomskiy et al. 2020). Our research group has also contributed to this field as we have recently reported results concerning AOA of derivatives of 8*H*-[1,2,4]triazolo[4,3-*b*][1,2,4]triazin-7-ones (Novodvorskyi et al. 2019) where several potent derivatives have been found. However, it should be noted, that by now AOA properties of non-condensed 1,2,4-triazine derivatives have not been studied well and, hence are required in-depth investigations (Yazdani et al. 2019).

Thus, because of these remarks and to fill some gaps in the chemistry and bioactivity of 1,2,4-triazines, in this work we are prompted to synthesize a series of new low-molecular derivatives of 6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones holding different types of functional groups and substituents in order to study their antioxidant properties and in a hope to find molecules with a high level of AOA.

Materials and methods

Chemistry part

The synthetic experiments were accomplished at Nizhyn Mykola Gogol State University. All solvents used in the experiment were purified according to the standard procedures. Carbonyl compounds **5** used in the synthesis of the target ylidenes were obtained from commercial sources and used without further purification. 4-Amino-6-*tert*-butyl-3-methylthio-4*H*-[1,2,4]triazin-5-one (1) was obtained by the method previously described (Schmidt et al. 1982). Hydrazinolysis of 4-amino-6-*tert*-butyl-3-methylthio-4*H*-[1,2,4]triazin-5-one (1) giving compound 2 was carried out by refluxing 3-methylthio derivative 1 with an excess of hydrazine hydrate in propanol-2 (El Massry et al. 2012).

NMR spectra were recorded on a Varian Unity Plus 400 spectrometer (400.4 MHz for ^{1}H and 100.7 MHz for ^{13}C) and Bruker DRX500 (500.13 MHz) in DMSO-d_6 using TMS as an internal standard. Chemical shifts are reported in ppm units with the use of the δ scale. The melting points were measured on a small-sized heating table with the observation device Electrothermal IA 9200 (Electrothermal Engineering Ltd, Great Britain). Elemental analysis was performed on a EuroEA 3000 elemental analyzer.

Synthesis of 6-tert-butyl-3-methylthio-4H-[1,2,4]triazin-5-one (3)

To a solution of 4-amino-6-*tert*-butyl-3-methylthio-4*H*-[1,2,4]triazin-5-one (1) (19.7 g, 0.09 mol) and concentrated HCl (45 mL) in anhydrous EtOH (200 mL) a solution of sodium nitrite (12.7 g, 0.184 mol) in H_2O (50 mL) was dropwise added at 0° C. The resulting mixture was next stirred for 30 min and warmed to the room temperature for an additional 1.5 h. The reaction mixture was concentrated in vacuo and the crude material was crystallized from ethanol to obtain the title compound as an off-white solid. Yield 16.0 g (89%), mp 239–240 °C (EtOH). Anal. Calcd for $C_8H_{13}N_3OS$: N 21.09. Found: N 21.31. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H, C(CH$_3$)$_3$), 2.49 (s, 3H,

SCH_3), 13.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 13.4, 26.8($C(CH_3)_3$), 36.3($C(CH_3)_3$), 151.7 (6-C), 159.1(3-C), 162.3(C = O).

Synthesis of 6-tert-butyl-3-hydrazinyl-2H-[1,2,4]triazin-5-one (4)

To a solution of 6-*tert*-butyl-3-methylthio-4*H*-[1,2,4] triazin-5-one (**3**) (16.0 g, 0.08 mol) in isopropyl alcohol (250 mL) hydrazine hydrate (20 g, 0.5 mol) was added. The reaction mixture was refluxed for 2 h. After cooling, the crystalline solid formed was filtered, washed with isopropyl alcohol, and dried in vacuo to afford the desired product.

Yield 11.6 g (79%), mp 261–262 °C (EtOH). Anal. Calcd for $C_7H_{13}N_5O$: N 38.23. Found: N 38.52. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 4.3 (br. s, 2H, NH_2), 8.3 (s, 1H, NH), 12.0 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.6($C(CH_3)_3$), 36.5($C(CH_3)_3$), 150.8(3-C), 153.7(6-C), 162.0(C = O).

The general procedure for the synthesis of 4-amino-3-(R-ylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-ones 6–8

To a solution of 4-amino-6-*tert*-butyl-3-hydrazinyl-*4H*-[1,2,4]triazin-5-one (2) (1.98 g, 0.01 mol) in ethanol (50 ml) an aldehyde 5 (0.01 mol) was added. The reaction mixture was refluxed for 3 hours and evaporated to the volume of 15 ml. After cooling the residue to the room temperature precipitate of ylidenes 6–8 was formed. The solid was filtered off, washed with ethanol, dried on air, and recrystallized from ethanol or propanol-2.

4-Amino-6-tert-butyl-3-(furan-2-ylmethylenehydrazono)-3,4-dihydro-2H-[1,2,4]triazin-5-one (6a). Yield 1.52 g (55%), mp 196–197 °C (*i*PrOH). Anal. Calcd for $C_{12}H_{16}N_6O_2$: N 30.42. Found: N 30.63. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 5.72 (s, 2H, NH_2), 6.58–7.72 (m, 3H, furyl), 8.13 (s, 1H, = CH), 12.1 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.4($C(CH_3)_3$), 37.8($C(CH_3)_3$), 112.4, 118.6, 134.4, 144.1(3-C), 148.8, 150.7, 153.1(3-C), 159.6(C = O).

4-Amino-6-tert-butyl-3-(thiophen-2-ylmethylenehydrazono)-3,4-dihydro-2H-[1,2,4]triazin-5-one (*6b*). Yield 1.66 g (57%), mp 177–178 °C (propanole-2). Anal. Calcd for $C_{12}H_{16}N_6OS$: N 28.75. Found: N 28.68. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 5.97 (s, 2H, NH_2), 7.16 (t, *J* 4.4 Hz, 1H, H-4 thienyl), 7.56 (d, *J* 3.9 Hz, 1H, thienyl), 7.77 (d, *J* 5.1 Hz, 1H, thienyl), 8.06 (s, 1H, = CH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.7($C(CH_3)_3$), 36.7($C(CH_3)_3$), 124.0, 126.7, 128.3, 130.2 (C = N), 144.6, 151.2(6-C), 153.0(3-C), 159.4(C = O).

4-Amino-3-(2-benzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7a). Yield 1.86 g (65%), mp 136–137 °C (*i*PrOH). Anal. Calcd for $C_{14}H_{18}N_6O$: N 29.35. Found: N 29.11. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.36 (s, 9H, $C(CH_3)_3$), 5.77 (s, 2H, NH_2), 7.34–7.93 (m, 5H, ArH), 8.27 (s, 1H, = CH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.6($C(CH_3)_3$), 37.1($C(CH_3)_3$), 127.3, 128.6, 131.7, 133.1, 151.9(6-C), 158.0(C = N), 160.1(3-C), 162.3(C = O).

4-Amino-3-(4-bromobenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7b). Yield 2.59 g (71%), mp 203–204 °C (EtOH). Anal. Calcd for $C_{14}H_{17}BrN_6O$: N 23.01. Found: N 23.30. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (s, 9H, $C(CH_3)_3$), 6.14 (s, 2H, NH_2), 7.01 (d, *J* 8.3 Hz, 2H, ArH), 7.43 (d, *J* 8.3 Hz, 2H, ArH), 8.95 (s, 1H, = CH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.5($C(CH_3)_3$), 36.7($C(CH_3)_3$), 123.8, 128.1, 130.6, 131.9, 150.7(6-C), 154,8(3-C), 157.7(C = N), 163.4(C = O).

4-Amino-3-(4-hydroxybenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7c). Yield 2.08 g (69%), mp 203–204 °C (EtOH). Anal. Calcd for $C_{14}H_{18}N_6O_2$: N 27.80. Found, %: N 27.58. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (s, 9H, $C(CH_3)_3$), 5.60 (s, 2H, NH_2), 6.75 (d, *J* 8.8 Hz, 4H, ArH), 7.65 (d, *J* 8.8 Hz, 4H, ArH), 8.15 (s, 1H, = CH), 9.28 (s, 1H, OH), 11.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.8($C(CH_3)_3$), 37.6($C(CH_3)_3$), 116.1, 125.7, 130.9, 149.8(6-C), 153.7(3-C), 159.1(C = N), 160.3(C = O), 164.5(C-OH).

4-Amino-3-(2-hydroxybenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7d). Yield 1.81 g (60%), mp 163–164 °C (EtOH). Anal. Calcd for $C_{14}H_{18}N_6O_2$: N 27.80. Found: N 27.93. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (s, 9H, $C(CH_3)_3$), 5.79 (s, 2H, NH_2), 6.83–7.57 (m, 4H, ArH), 8.49 (s, 1H, = CH), 9.91 (s, 1H, OH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.6($C(CH_3)_3$), 36.8($C(CH_3)_3$), 116.9, 117.8, 121.9, 125.7, 132.8, 145.7(C-OH), 150.7(6-C), 153.8(3-C), 156.2(C = N), 159.7(C = O).

4-Amino-3-(4-hydroxy-3-methoxybenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7e). Yield 2.16 g (65%), mp 215–216 °C (EtOH). Anal. Calcd for $C_{15}H_{20}N_6O_3$: N 25.29. Found, %: N 25.61. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 3.86 (s, 3H, OCH_3), 5.77 (s, 2H, NH_2), 6.77 (d, *J* 7.9 Hz, 1H, ArH), 7.10 (d, *J* 7.9 Hz, 1H, ArH), 7.76 (s, 1H, H-2 benzylidene), 8.15 (s, 1H, = CH), 8.86 (s, 1H, OH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.6($C(CH_3)_3$), 38.4($C(CH_3)_3$), 58.1(OCH_3), 111.4, 116.5, 121.7, 129.4, 143.5(C-OCH_3), 147.8(C-OH), 149.4(C = N), 150.7(6-C), 153.1(3-C), 158.7(C = O).

4-Amino-3-(3-hydroxy-4-methoxybenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7f). Yield 2.09 g (63%), mp 166–167 °C (EtOH). Anal. Calcd for $C_{15}H_{20}N_6O_3$: N 25.29. Found: N 25.45. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 3.82 (s, 3H, OCH_3), 5.76 (s, 2H, NH_2), 6.94 (d, *J* 8.2 Hz, 1H, ArH), 7.23 (d, *J* 8.2 Hz, 1H, ArH), 7.47 (s, 1H, H-2 benzylidene), 8.17 (s, 1H, = CH), 9.36 (s, 1H, OH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 28.4($C(CH_3)_3$), 37.7($C(CH_3)_3$), 56.0(OCH_3), 111.7, 115.7, 122.1, 131.6, 146.1(C-OCH_3), 147.1(C-OH), 150.6(C = N), 151.7(6-C), 153.1 (3-C), 158.6(C = O).

Methyl 4-[(4-amino-6-(tert-butyl)-5-oxo-4,5-dihydro-1,2,4-triazin-3(2H)-ylidene)hydrazono)methyl]benzoate (7g). Yield 2.09 g (68%), mp 204–205 °C (MeOH). Anal. Calcd for $C_{16}H_{20}N_6O_3$: N 24.40. Found: N 24.63. ^{1}H NMR

(400 MHz, DMSO-d_6) δ 1.33 (s, 9H, $C(CH_3)_3$), 3.87 (s, 3H, CO_2CH_3), 5.84 (s, 2H, NH_2), 7.97 (d, *J* 8.4 Hz, 2H, ArH), 8.13 (d, *J* 8.4 Hz, 2H, ArH), 8.37 (s, 1H, = CH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.5($C(CH_3)_3$), 37.4($C(CH_3)_3$), 51.3($COOCH_3$), 128.3, 130.7, 132.4, 138.5, 151.8(6-C), 153.7(3-C), 159.4(5-C = O), 162.4(C = N), 165.8($COOCH_3$).

4-Amino-3-(2-nitrobenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7h). Yield 2.32 g (70%), mp 227–228 °C (EtOH). Anal. Calcd for $C_{14}H_{17}N_7O_3$: N 29.59. Found: N 29.86. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 5.85 (s, 2H, NH_2), 7.61–8.74 (m, 4H, ArH), 8.59 (s, 1H, = CH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.3($C(CH_3)_3$), 35.7($C(CH_3)_3$), 122.8, 128.8, 130.1, 131.7, 134.4, 143.1(C = N), 147.5(C-NO_2), 150.7(6-C), 152.5(3-C), 158.5(C = O).

4-Amino-3-(3-nitrobenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7i). Yield 2.42 g (73%), mp 146–147 °C (EtOH). Anal. Calcd for $C_{14}H_{17}N_7O_3$: N 29.59. Found: N 29.52. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 5.83 (s, 2H, NH_2), 7.67–8.92 (m, 4H, ArH), 8.44 (s, 1H, = CH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 36.8($C(CH_3)_3$), 120.6, 126.4, 128.5, 132.8, 134.6, 146.2(C-NO_2), 148.4(C = N), 151.7(6-C), 153.5(3-C), 157.5(C = O).

4-Amino-3-(4-nitrobenzylidenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (7j). Yield 2.55 g (77%), mp 194–195 °C (EtOH). Anal. Calcd for $C_{14}H_{17}N_7O_3$: N 29.59. Found: N 29.91. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 (s, 9H, $C(CH_3)_3$), 5.85 (s, 2H, NH_2), 7.83 (d, *J* 8.8 Hz, 2H, ArH), 8.31 (d, *J* 8.8 Hz, 2H, ArH), 8.46 (s, 1H, = CH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 38.1($C(CH_3)_3$), 123.4, 123.5, 125.4, 125.5, 138.2, 150.1(C-NO_2), 150.6(6-C), 153.5(3-C), 157.5(C = N), 162.5(C = O).

4-Amino-3-(2-hydroxynaphthalene-1-ylmethylenehydrazono)-6-tert-butyl-3,4-dihydro-2H-[1,2,4]triazin-5-one (8). Yield 2.26 g (64%), mp 234–235 °C (*i*PrOH). Anal. Calcd for $C_{18}H_{20}N_6O_2$: N 23.85. Found, %: N 23.58. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 (s, 9H, $C(CH_3)_3$), 5.91 (s, 2H, NH_2), 7.21–8.39 (m, 6H, naphthyl), 9.34 (s, 1H, = CH), 11.5 (s, 1H, OH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.6($C(CH_3)_3$), 36.6($C(CH_3)_3$), 107.4, 117.5, 120.6, 122.7, 126.0, 127.5, 128.6, 132.7, 133.4, 142.7(C = N), 150.2(6-C), 152.4(3-C), 158.4(C = O), 171.4(C-OH).

Synthesis of 6-tert-butyl-4-((2-methoxybenzylidene) amino)-3-(2-methoxybenzylidenehydrazono)-3,4-dihydro-2H-[1,2,4]triazin-5-one (9). To a solution of 4-amino-6-*tert*-butyl-3-hydrazinyl-*4H*-[1,2,4]triazin-5-one (3) (1.98 g, 0.01 mol) in ethanol (50 ml) 2-methoxybenzaldehyde (2.72 g, 0.02 mol) was added**.** The reaction mixture was refluxed for 5 hours and evaporated to the volume of 15 ml. After cooling the residual solution to the room temperature, the precipitate of 9 was formed. The solid was filtered off, washed with ethanol, dried on air and recrystallized from ethanol.

Yield 2.43 g (56%), mp 198–199 °C (EtOH). Anal. Calcd for $C_{23}H_{26}N_6O_3$: N 19.34. Found, %: N 19.60. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (s, 9H, $C(CH_3)_3$), 3.79 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.96 (t, *J* 7.5 Hz, 1H, ArH), 7.02 (d, *J* 8.4 Hz, 1H, ArH), 7.13 (t, *J* 7.5 Hz, 1H, ArH), 7.22 (d, *J* 8.4 Hz, 1H, ArH), 7.36 (t, *J* 8.4 Hz, 1H, ArH), 7.64 (t, *J* 7.8 Hz 1H, ArH), 8.07 (d, *J* 7.8 Hz, 1H, ArH), 8.38 (d, *J* 7.8 Hz, 1H, ArH), 8.45 (s, 1H, = CH), 8.84 (s, 1H, = CH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 29.3($C(CH_3)_3$), 36.7($C(CH_3)_3$), 55.4(OCH_3), 110.5, 115.9, 120.6, 131.6, 132.5, 142.7, 145.1, 150.5(6-C), 152.8(3-C), 156.5(C-OCH_3), 164.2(C = O).

The general procedure for the synthesis of 3-(2-(het)arylidenehydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-ones 10–12 and 14

To a solution of 6-*tert*-butyl-3-hydrazinyl-*4H*-[1,2,4]triazin-5-one (4) (1.83 g, 0.01 mol) in ethanol (50 ml) an aldehyde 5 or a methyl ketone 13 (0.01 mol) was added**.** The reaction mixture was refluxed for 3 hour and evaporated to the volume of 15 ml. After cooling of the residual solution to the room temperature the precipitate of a corresponding ylidene 10–12, 14 was formed. The solid was filtered off, washed with ethanol, dried on air and recrystallized from methanol, DMF or acetic acid.

6-tert-Butyl-3-(2-(furan-2-ylmethylene)hydrazinyl)-2H-[1,2,4]triazin-5-one (10a). Yield 1.59 g (61%), mp 253–254 °C (MeOH). Anal. Calcd for $C_{12}H_{15}N_5O_2$: N 26.80. Found, %: N 26.64. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 6.59–7.72 (m, 3H, furyl), 7.97 (s, 1H, = CH), 11.4 (s, 1H, NH), 12.4 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 37.5($C(CH_3)_3$), 114.4, 117.7, 135.3(C = N), 142.5(5-furan), 149.5(2-furan), 152.4(6-C), 153.4(3-C), 164.4(C = O).

6-tert-Butyl-3-(2-(thiophen-2-ylmethylene)hydrazinyl)-2H-[1,2,4]triazin-5-one (10b). Yield 1.80 g (65%), mp > 260 °C (MeOH). Anal. Calcd for $C_{12}H_{15}N_5OS$: N 25.25. Found: N 25.54. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H, $C(CH_3)_3$), 7.11 (t, *J* 3.9 Hz, 1H, H-5 thienyl), 7.49 (d, *J* 3.9 Hz, 1H, thienyl), 7.66 (d, *J* 5.0 Hz, 1H, thienyl), 8.23 (s, 1H, = CH), 11.6 (s, 1H, NH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.6($C(CH_3)_3$), 39.5($C(CH_3)_3$), 124.7, 126.5, 128.5, 132.6, 143.7(2-thiophene), 150.6(6-C), 152.8(3-C), 164.4(C = O).

3-(2-Benzylidenehydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11a). Yield 1.87 g (69%), mp > 260 °C (MeOH). Anal. Calcd for $C_{14}H_{17}N_5O$: N 25.81. Found: N 25.56. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (s, 9H, $C(CH_3)_3$), 7.39–7.96 (m, 5H, ArH), 8.08 (s, 1H, = CH), 11.6 (s, 1H, NH), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.8($C(CH_3)_3$), 36.5($C(CH_3)_3$), 124.4, 124.5, 127.5, 127.6, 130.7, 132.8, 150.8(3-C), 153.7(6-C), 161.1(C = N), 161.8(C = O).

3-(2-(4-Bromobenzylidene)hydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11b). Yield 2.73 g (78%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{16}BrN_5O$: N 20.00. Found: N 20.22. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 7.62 (d, *J* 8.5 Hz, 2H, ArH), 7.89 (d, *J* 8.5 Hz, 2H, ArH), 8.01 (s, 1H, = CH), 11.7 (s, 1H, NH), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ

25.6($C(CH_3)_3$), 35.2($C(CH_3)_3$), 123.9, 126.7, 129.5, 130.5, 150.9(3-C), 153.6(6-C), 160.5(C = N), 163.5(C = O).

3-(2-(4-Hydroxybenzylidene)hydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11c). Yield 2.18 g (76%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{17}N_5O_2$: N 24.38. Found: N 24.70. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 6.77 (d, *J* 8.8 Hz, 2H, ArH), 7.69 (d, *J* 8.8 Hz, 2H, ArH), 7.94 (s, 1H, = CH), 9.64 (s, 1H, OH), 11.3 (s, 1H, NH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.6($C(CH_3)_3$), 37.0($C(CH_3)_3$), 114.6, 126.7, 132.5, 150.3(3-C), 152.9(6-C), 160.8(C = N), 162.3(C = O), 164.1(C-O).

3-(2-(2-Hydroxybenzylidene)hydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11d). Yield 1.52 g (53%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{17}N_5O_2$: N 24.38. Found: N 24.63. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 6.82–8.12 (m, 4H, ArH), 8.37 (s, 1H, = CH), 9.89 (s, 1H, OH), 11.5 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 35.6($C(CH_3)_3$), 117.5, 118.6, 121.0, 127.7, 132.9, 145.7, 150.8(3-C), 154.8(6-C), 158.2(C-O), 164.5(C = O).

3-(2-(4-Hydroxy-3-methoxybenzylidene)hydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11e). Yield 1.78 g (56%), mp > 260 °C (DMF). Anal. Calcd for $C_{15}H_{19}N_5O_3$: N 22.07. Found: N 22.00. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 3.83 (s, 3H, OCH_3), 6.85–8.09 (m, 3H, ArH), 8.41 (s, 1H, = CH), 9.83 (s, 1H, OH), 11.3 (s, 1H, NH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 35.8($C(CH_3)_3$), 53.6(OCH_3), 112.8, 116.2, 122.0, 130.7, 145.9, 148.6(C-OCH_3), 151.0(C-OH), 151.1(3-C), 152.6(6-C), 165.4(C = O).

3-(2-(3-Hydroxy-4-methoxybenzylidene)hydrazinyl)-6-tert-butyl-2H-[1,2,4]triazin-5-one (11f). Yield 1.87 g (59%), mp > 260 °C (DMF). Anal. Calcd for $C_{15}H_{19}N_5O_3$: N 22.07. Found: N 22.41. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H, $C(CH_3)_3$), 3.82 (s, 3H, OCH_3), 6.95 (d, *J* 8.5 Hz, 1H, ArH), 7.22 (d, *J* 8.5 Hz, 1H, ArH), 7.42 (s, 1H, H-2 ArH), 7.90 (s, 1H, = CH), 8.94 (s, 1H, OH), 11.4 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.6($C(CH_3)_3$), 34.9($C(CH_3)_3$), 58.7(OCH_3), 112.9, 114.9, 121.9, 130.7, 146.8(C = N), 148.6(C-OH), 150.6(C-OCH_3),, 152.0(3-C), 153.8(6-C), 163.7(C = O).

Methyl 4-((2-(6-(tert-butyl)-5-oxo-2H-1,2,4-triazin-3-yl)hydrazono)methyl)benzoate (11g). Yield 2.17 g (66%), mp > 260 °C (DMF). Anal. Calcd for $C_{16}H_{19}N_5O_3$: N 21.26. Found: N 21.6. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 3.88 (s, 3H, CO_2CH_3), 7.97 (d, *J* 8.4 Hz, 2H, ArH), 8.03 (d, *J* 8.4 Hz, 2H, ArH), 8.10 (s, 1H, = CH), 11.7 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.0($C(CH_3)_3$), 38.6($C(CH_3)_3$), 52.7($COOCH_3$), 127.6, 129.6, 131.8, 138.5, 149.6(3-C), 150.6(6-C), 160.7(C = N), 162.8(5-C = O), 168.5($COOCH_3$).

6-tert-Butyl-3-(2-(2-nitrobenzylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (11h). Yield 2.28 g (72%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{16}N_6O_3$: N 26.57. Found: N 26.48. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 7.61 (t, *J* 8.7 Hz, 1H, ArH), 7.73 (t, *J* 8.7 Hz, 1H, ArH), 8.01 (d, *J* 8.7 Hz, 1H, ArH), 8.50 (s, 1H, = CH), 8.68 (d, *J* 8.7 Hz, 1H, ArH), 11.8 (s, 1H, NH), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.5($C(CH_3)_3$), 37.2($C(CH_3)_3$), 123.0, 126.9, 128.9, 130.9, 132.6, 142.9, 147.7(C-NO_2), 151.9(3-C), 152.7(6-C), 163.6(C = O).

6-tert-Butyl-3-(2-(3-nitrobenzylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (11i). Yield 2.40 g (76%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{16}N_6O_3$: N 26.57. Found: N 26.81. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 7.69 (t, *J* 8.4 Hz, 1H, H-5 ArH), 8.17 (s, 1H, = CH), 8.19 (d, *J* 8.4 Hz, 1H, ArH), 8.24 (d, *J* 8.4 Hz, 1H, ArH), 8.86 (s, 1H, H-2 ArH), 11.7 (s, 1H, NH), 12.9 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.5($C(CH_3)_3$), 35.6($C(CH_3)_3$), 120.6, 124.9, 127.8, 132.6, 133.4, 144.9(C = N), 147.5(C-NO_2), 151.1(3-C), 152.8(6-C), 164.2(C = O).

6-tert-Butyl-3-(2-(4-nitrobenzylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (11j). Yield 2.56 g (81%), mp > 260 °C (DMF). Anal. Calcd for $C_{14}H_{16}N_6O_3$: N 26.57. Found: N 26.67. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, $C(CH_3)_3$), 8.14 (s, 1H, = CH), 8.18 (d, *J* 8.7 Hz, 2H, ArH), 8.23 (d, *J* 8.7 Hz, 2H, ArH), 11.8 (s, 1H, NH), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.7($C(CH_3)_3$), 39.4($C(CH_3)_3$), 124.7, 124.9, 137.5, 149.7, 151.9(3-C), 154.9(6-C), 160.8(C = N), 163.6(C = O).

6-tert-Butyl-3-(2-(2-methoxybenzylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (11k). Yield 1.87 g (62%), mp > 260 °C (DMF). Anal. Calcd for $C_{15}H_{19}N_5O_2$: N 23.24. Found: N 23.53. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H, $C(CH_3)_3$), 3.83 (s, 3H, OCH_3), 6.98 (t, *J* 7.7 Hz, 1H, ArH), 7.06 (d, *J* 8.2 Hz, 1H, ArH), 7.38 (t, *J* 8.2 Hz, 1H, ArH), 8.31 (d, *J* 7.7 Hz, 1H, ArH), 8.40 (s, 1H, = CH), 11.5 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 24.1($C(CH_3)_3$), 35.7($C(CH_3)_3$), 56.0(OCH_3), 109.7, 116.2, 120.6, 131.7, 132.0, 145.1(C = N), 150.2(3-C), 152.7(6-C), 156.9(C-OCH_3), 160.5(C = O).

6-tert-Butyl-3-(2-(4-methoxybenzylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (11l). Yield 1.87 g (62%), mp > 260 °C (MeOH). Anal. Calcd for $C_{15}H_{19}N_5O_2$: N 23.24. Found: N 23.03. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 3.80 (s, 3H, OCH_3), 7.93 (d, *J* 8.8 Hz, 2H, ArH), 8.71 (d, *J* 8.8 Hz, 2H, ArH), 8.00 (s, 1H, = CH), 11.3 (s, 1H, NH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.4($C(CH_3)_3$), 37.7($C(CH_3)_3$), 58.2(OCH_3), 113.6, 125.9, 130.2, 148.5(3-C), 153.8(6-C), 160.7(C = N), 162.7(C = O), 165.5(C-OCH_3),

6-tert-Butyl-3-(2-(3-ethoxy-4-hydroxybenzylidene) hydrazinyl)-2H-[1,2,4]triazin-5-one (11m). Yield 1.99 g (60%), mp 258–259 °C (MeOH). Anal. Calcd for $C_{16}H_{21}N_5O_3$: N 21.13. Found: N 21.34. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, $C(CH_3)_3$), 1.37 (t, *J* 6.8 Hz, 3H, CH_2CH_3), 4.11 (s, *J* 6.8 Hz, 2H, CH_2CH_3), 6.78 (d, *J* 8.0 Hz, 1H, ArH), 7.03 (d, *J* 8.0 Hz, 1H, ArH), 7.67 (s, 1H, H-2 ArH), 7.90 (s, 1H, = CH), 9.37 (s, 1H, OH), 11.4 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.6(OCH_2CH_3),, 26.3($C(CH_3)_3$), 36.9($C(CH_3)_3$), 64.3(OCH_2CH_3), 111.4, 117.5, 121.5, 130.2, 143.5(C = N), 147.5(C-OCH_2CH_3), 150.2(3-C), 152.8(6-C), 155.2(C-OH), 163.5(C = O).

6-tert-Butyl-3-(2-((2-hydroxynaphthalen-1-yl)methylene)hydrazinyl)-2H-[1,2,4]triazin-5-one (12). Yield 2.33 g (69%), mp > 260 °C (DMF). Anal. Calcd for $C_{18}H_{19}N_5O_2$: N 20.7. Found: N 20.4. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, C(CH$_3$)$_3$), 7.19 (d, *J* 8.9 Hz, 1H, naphthyl), 7.38 (t, *J* 8.9 Hz, 1H, naphthyl), 7.55 (t, *J* 8.9 Hz, 1H, naphthyl), 7.81 (d, *J* 8.0 Hz, 1H, naphthyl), 7.83 (d, *J* 8.9 Hz, 1H, naphthyl), 8.41 (d, *J* 8.0 Hz, 1H, naphthyl), 9.09 (s, 1H, = CH), 10.5 (s, 1H, OH), 11.4 (s, 1H, NH), 12.8 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.8(C(CH$_3$)$_3$), 38.1(C(CH$_3$)$_3$), 107.9, 117.2, 120.1, 123.9, 125.0, 127.5, 127.9, 131.1, 132.6, 141.1(C = N), 151.8(3-C), 151.9(6-C), 161.7(C = O), 174.5(C-OH).

6-tert-Butyl-3-(2-isopropylidenehydrazinyl)-2H-[1,2,4]triazin-5-one (14a). Yield 1.09 g (49%), mp > 260 °C (MeOH). Anal. Calcd for $C_{10}H_{17}N_5O$: N 31.37. Found: N 31.70. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, C(CH$_3$)$_3$), 1.92 (s, 3H, CH$_3$), 2.00 (s, 3H, CH$_3$), 10.3 (s, 1H, NH), 12.1 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 18.6, 24.5, 27.9(C(CH$_3$)$_3$), 36.2(C(CH$_3$)$_3$), 151.4(3-C), 153.0(C = N), 152.7(6-C), 163.2(C = O).

6-tert-Butyl-3-(2-(1-(1-hydroxynaphthalene-2-yl)ethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14b). Yield 2.00 g (57%), mp > 260 °C (MeOH). Anal. Calcd for $C_{19}H_{21}N_5O_2$: N 19.93. Found: N 19.98. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9H, C(CH$_3$)$_3$), 2.49 (s, 3H, CH$_3$), 7.38–8.30 (m, 6H, naphthyl), 10.4 (s, 1H, OH), 11.2 (s, 1H, NH), 12.3 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.6, 26.4(C(CH$_3$)$_3$), 35.2(C(CH$_3$)$_3$), 112.5, 120.7, 124.3, 124.6, 127.4, 127.5, 127.7, 128.6, 136.4, 150.8(3-C), 152.7(6-C), 161.1(C = N), 163.2(C = O), 167.5(C-OH).

6-tert-Butyl-3-(2-(1-phenylethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14c). Yield 1.57 g (55%), mp 251–252 °C (AcOH). Anal. Calcd for $C_{15}H_{19}N_5O$: N 24.54. Found: N 24.72. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.32 (s, 9H, C(CH$_3$)$_3$), 2.30 (s, 3H, CH$_3$), 7.78–8.07 (m, 5H, ArH), 11.4 (s, 1H, NH), 12.6 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 16.2, 27.5(C(CH$_3$)$_3$), 36.5(C(CH$_3$)$_3$), 125.7, 127.6, 131.9, 137.8, 147.5(C = N), 151.6(3-C), 153.2(6-C), 163.9(C = O).

6-tert-Butyl-3-(2-(1-(p-tolyl)ethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14d). Yield 1.70 g (57%), mp 227–228 °C (MeOH). Anal. Calcd for $C_{16}H_{21}N_5O$: N 23.39. Found: N 23.11. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (s, 9H, C(CH$_3$)$_3$), 2.29 (s, 3H, CH$_3$), 2.37 (s, 3H, CH$_3$), 7.34 (d, *J* 8.3 Hz, 2H, ArH), 7.91 (d, *J* 8.3 Hz, 2H, ArH), 10.7 (s, 1H, NH), 12.4 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.4, 21.0, 27.2(C(CH$_3$)$_3$), 37.7(C(CH$_3$)$_3$), 126.0, 128.2, 133.3, 135.1, 148.8(C = N), 150.7(3-C), 152.7(6-C), 163.6(C = O).

6-tert-Butyl-3-(2-(1-(4-tert-butylphenyl)ethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14e). Yield 1.74 g (51%), mp > 260 °C (MeOH). Anal. Calcd for $C_{19}H_{27}N_5O$: N 20.51. Found: N 20.29. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.33 (s, 9H, C(CH$_3$)$_3$), 1.34 (s, 9H, C(CH$_3$)$_3$), 2.30 (s, 3H, CH$_3$), 7.14 (d, *J* 8.4 Hz, 2H, ArH), 7.90 (d, *J* 8.4 Hz, 2H, ArH), 10.7 (s, 1H, NH), 12.4 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.4, 26.7(C(CH$_3$)$_3$), 31.1, 34.5, 36.6(C(CH$_3$)$_3$), 125.6, 128.2, 133.1, 145.5(C = N), 149.1(C-*t*Bu), 152.2(3-C), 152.3(6-C), 163.3(C = O).

6-tert-Butyl-3-(2-(1-(4-chlorophenyl)ethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14f). Yield 1.95 g (61%), mp 262–263 °C (MeOH). Anal. Calcd for $C_{15}H_{18}ClN_5O$: N 21.90. Found: N 22.21. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (s, 9H, C(CH$_3$)$_3$), 2.29 (s, 3H, CH$_3$), 7.43 (d, *J* 8.7 Hz, 2H, ArH), 8.10 (d, *J* 8.7 Hz, 2H, ArH), 10.8 (s, 1H, NH), 12.7 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.1, 28.1(C(CH$_3$)$_3$), 36.3(C(CH$_3$)$_3$), 125.5, 127.8, 135.7, 136.1, 148.5(C = N), 150.6(3-C), 154.1(6-C), 161.5(C = O).

6-tert-Butyl-3-(2-(1-(4-bromophenyl)ethylidene)hydrazinyl)-2H-[1,2,4]triazin-5-one (14g). Yield 2.58 g (71%), mp > 260 °C (MeOH). Anal. Calcd for $C_{15}H_{18}BrN_5O$: N 19.23. Found: N 19.50. ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (s, 9H, C(CH$_3$)$_3$), 2.30 (s, 3H, CH$_3$), 7.48 (d, *J* 8.8 Hz, 2H, ArH), 7.99 (d, *J* 8.8 Hz, 2H, ArH), 10.8 (s, 1H, NH), 12.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.8, 28.4(C(CH$_3$)$_3$), 36.3(C(CH$_3$)$_3$), 124.9, 127.1, 134.1, 134.6, 149.2(C = N), 150.6(3-C), 153.1(6-C), 162.9(C = O).

Screening of antioxidant activity

Antioxidant activity (AOA) of the synthesized compounds was studied at Nizhyn Mykola Gogol State University.

The primary assessment of AOA is usually carried out using *in vitro* experiments as this way is quick, cheap, and does not require the use of animals (Albert 1971; Hubskyi et al. 2001).

To study the structure-activity relationship in this work all of the types of synthesized compounds – 4-amino- 6–8 and 4-desamino-derivatives 10–12, 14 as well as bis-Schiff base **9** – were employed to screen for the presence of AOA. Structure variations of the tested representatives make it possible to trace regularities of how various substituents affect the level of AOA and thus to plan further research in this field.

The antioxidant activity of the substances obtained in the work was assessed in the experiments *in vitro* on the model of the artificial oxidative stress using an emulsion of yolk lipoproteins as a substrate sensitive to oxidation (Perekhoda et al. 2017). During the assay pH value of the culture medium was maintained at the level of 7.5 which is an optimal one for most living organisms. The model system chosen has a number of advantages, namely, it is affordable and cheap, the separation of lipoproteins is quite easy, the model is reproducible and stable during storage, and, simultaneously, characterized by high oxidizability as the yolk contains two types of lipid-protein complexes corresponding to lipoproteins of very low and low density of the blood plasma by their lipid and protein composition.

To prepare the model system, the yolk was separated from a chicken egg, then it was mixed with an equal volume of the phosphate buffer solution (40 mM KH_2PO_4 and 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 drug (ascorbic acid) were prepared in the form of DMSO solutions with an initial concentration of 3 mg/ml.

The oxidative stress was modelled as follows. To 1.0 ml of YLP emulsion, 0.5 ml of the solution of a test substance, 0.5 ml of 0.5 mM iron (II) sulphate solution (the reactive oxygen species (ROS) generation system), and 3 ml of the phosphate buffer solution (40 mM KH_2PO_4 + 105 mM KCl, pH 7.5) were sequentially added. The resulting solution was mixed and incubated for 30 minutes at 37 °C in a water thermostat.

After incubation, the solution was cooled and used to determine the amount of the products of lipids oxidation. ROS degrade polyunsaturated lipids, forming several low-molecular-weight end products, one of them is malondialdehyde (MDA) (Pryor and Stanley 1975). MDA is a reactive aldehyde and one of the reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts (Farmer and Davoine 2007). The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in the organism (Moore and Roberts 1998). The thiobarbituric acid (TBA) test is used extensively as a spectrophotometric method for the detection of MDA and other TBA-reactive products as well (Nair et al. 2008). The red pigment (TBA-MDA adduct) formed is a mixture of equilibrating structures with an absorption maximum of 532 nm (Scheme 1).

With the purpose to determine the content of MDA and other TBA-reactive products, 2 ml of cooled 20% trichloroacetic acid (TCA) and 0.05 ml of disodium EDTA (50 mg/l) 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, 2 ml of freshly prepared 1% thiobarbituric acid solution was added to the supernatant. The solution obtained was incubated for 35 minutes at 95 °C. In the acidic medium MDA reacts with 2-thiobarbituric acid to form the coloured derivative (Scheme 1). After cooling, 4 ml of butanol-1 was added, and the optical density of the butanol extract was measured, using an SF-46 spectrophotometer at a wavelength of 532 nm.

The antioxidant properties of the compounds studied were 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 ascorbic acid as the reference drug according to the equation:

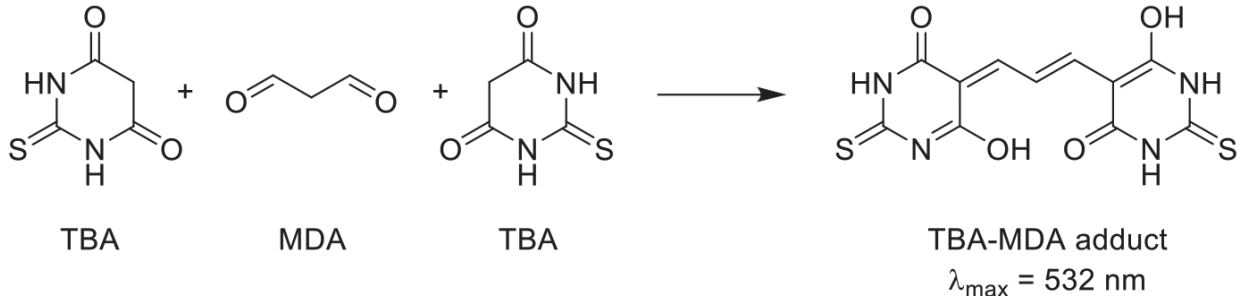
$$AOA,\% = \frac{D_{DMSO} - D_{AC}}{D_{DMSO} - D_{\text{vitac}}} \times 100\%, \quad \text{where}$$

D_{DMSO} – average value of absorption coefficient for sample with DMSO without the compound analysed;

D_{AC} – average value of absorption coefficient for analysing compound;

$D_{vitaminC}$ – average value of absorption coefficient for ascorbic acid.

The content of MDA was calculated by the following equation (Bohacheva et al. 2016):



Scheme 1. Formation of TBA-MDA adduct.

$$C_{mda} = \frac{D_{532} \times 10^6 \times K_p}{1.56 \times 10^5}, \quad \text{where}$$

C_{mda} – concentration of MDA, nmol/ml;

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

10^6 – coefficient of conversion from μmol/l to nmol/ml;

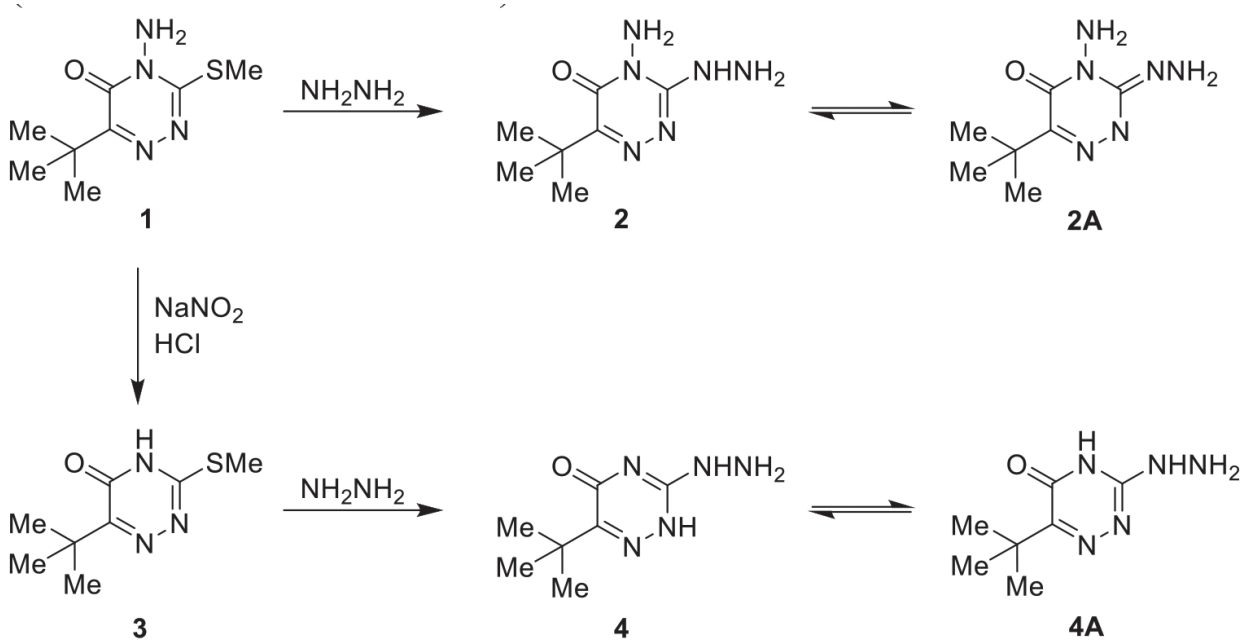
$1{,}56 \times 10^5$ – molar attenuation coefficient of TBA-MDA complex at 532 nm, L mol^{-1} cm^{-1};

K_p – coefficient of the sample dilution.

Results and discussion

Nowadays, hydrazones play a significant role in a medical chemistry for new drug development, and importance of this class is rising day by day (Narang et al. 2012). Many research groups are employed in the synthesis of hydrazones and evaluation of their biological activities (Ajani et al. 2010). Thus, hydrazones have been confirmed to display such activities as antimicrobial (Rollas et al. 2002), anticonvulsant (Dimmock et al. 2000), analgesic (Lima et al. 2000), anti-inflammatory and antiplatelet (Silva et al. 2004), etc. According to the above, it is considered appropriate to develop a series of 1,2,4-triazinohydrazones with the aim of investigating *in vitro* antioxidant properties.

As starting compounds for the current research, we chose 6-*tert*-butyl-3-hydrazinyl-4*H*-[1,2,4]triazin-5-ones 2 and 4. The synthesis of the starting 1,2,4-triazinohydrazine **2** was carried out by the reaction of hydrazinolysis of 6-*tert*-butyl-3-methylthio-4*H*-[1,2,4]triazin-5-one (1) according to a known procedure (El Massry et al. 2012) (Scheme 2). To obtain another initial 1,2,4-triazinohydrazine 4 4-amino group in compound 1 was firstly removed by means of a simple diazotization reaction followed by interaction of the product 3 with hydrazine hydrate. It should be noted, that literature data provide different isomeric forms for compounds similar to 2 and 4. Some



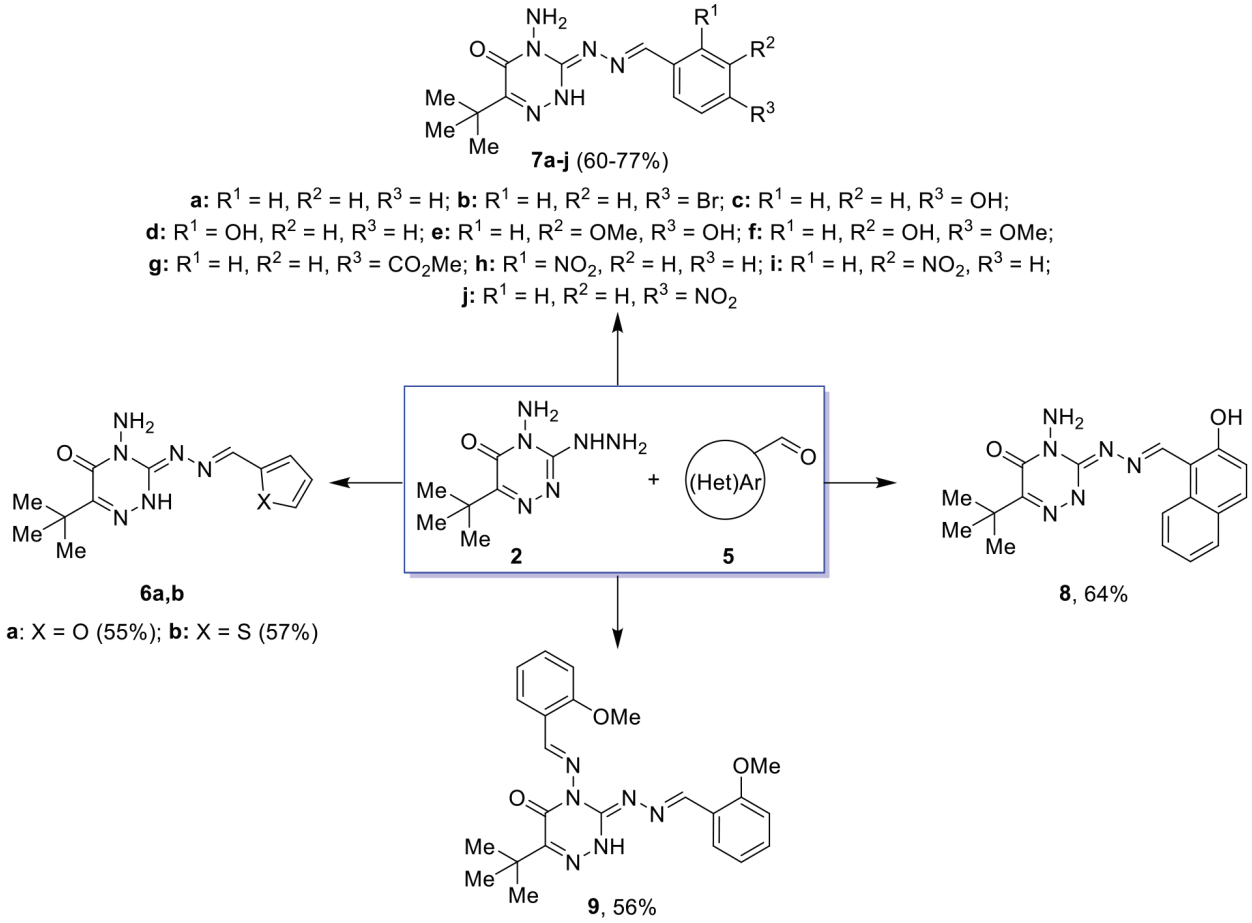
Scheme 2. The synthesis of the starting 4-amino-6-*tert*-butyl-3-hydrazinyl-4*H*-[1,2,4]triazin-5-one (2) and 6-*tert*-butyl-3-hydrazino-2*H*-[1,2,4]triazin-5-one (4).

papers indicate tautomeric transformations for these derivatives, without strong evidence though. Thus, it was claimed that compound 2 could exist in the solution together with hydrazonotriazine 2A (Hamama et al. 2018). The structure of 4 was also reported ambiguously as 2*H*- 4 and 4*H*- 4A isomers in which the distribution of electron density on the ring nitrogen atoms is significantly different. Yet it should be acknowledged that when carrying out the reaction the starting triazine 4 exists principally in the 2*H*-form as the ring closure occurs at the N(2) atom (Mironovich and Ivanov 2003).

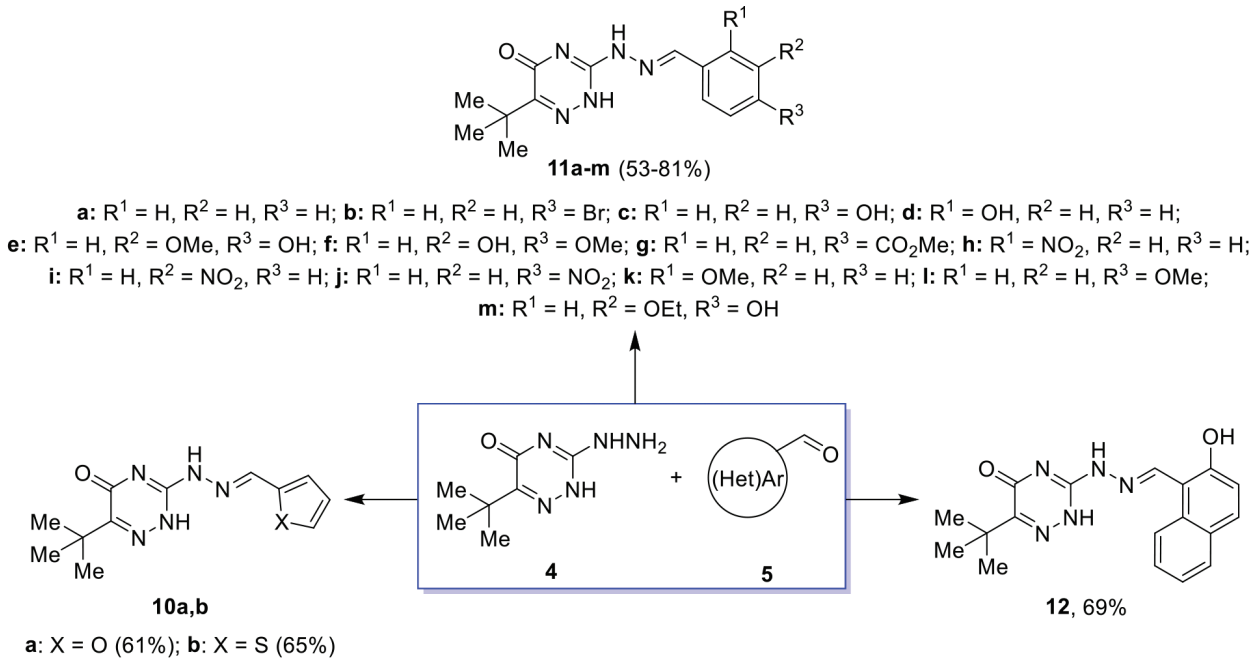
The next step of the research was to synthesize hydrazones of compounds 2 and 4 *via* classical reaction of Schiff bases formation and to prove the structure of the compounds isolated. For this purpose, various aromatic and heteroaromatic aldehydes, as well as different ketones, were applied to the reaction. Evidently, the "carbonyl" part of the Schiff bases constitutes an additional pharmacophore in the products. Therefore, different nature and the substitution patterns of the starting carbonyl compounds would allow us to trace some regularities of "structure-bioactivity" relationships.

Interaction of 4-aminotriazinone 2 with a series of (het) arenecarbaldehydes **5** (ratio 1:1) in refluxing ethanol for 3 hours led to the corresponding hydrazones 6–8 with yields 55–77% (Scheme 3). As we expected more reactive NH_2-group of the exocyclic hydrazine moiety was involved in the reaction. Additionally, we showed that another NH_2-fragment can also be employed in the formation of the Schiff base when 2 equiv of an aldehyde is used. With this, utilization of 2 equiv of 2-methoxybenzaldehyde resulted in the isolation of bis-Schiff base **9**. The structure of compounds 6–9 was confirmed by elemental analysis, ^{1}H and ^{13}C NMR spectroscopy methods. ^{1}H NMR spectra of 6–8 are characterized by singlets arisen from *t*-butyl (1.31–1.44), NH_2 (5.60–6.14), ylidene = CH (8.06-), and NH (11.6–12.7) protons. ^{1}H NMR spectrum of bis-derivative 9 features the absence of NH_2 singlet and the presence of two ylidene = CH signals at 8.45 and 8.84 ppm as well as NH singlet at 12.3 ppm. A quite interesting fact was stated in the work of El-Barbary et al. (2010). On the basis of ^{1}H NMR spectra evidence the authors reported a ring-chain tautomerism for arylidenehydrazones of 4-amino-3-hydrazinyl-6-(4-methoxystyryl)-1,2,4-triazin-5(4*H*)-one between acyclic hydrazone and bicyclic 2,8-dihydro-[1,2,4]triazolo[4,3-*b*][1,2,4]triazin-7(3*H*)-one forms coexisting in a dynamic equilibrium. However, our results obtained in this work do not support this statement as we did not observe additional signals in ^{1}H NMR relating to the cyclic form. One should also note that the products are apparently formed in an isomeric = N-N = form with a displacement of the endocyclic double bond outside the ring as it was reported before on the basis of X-ray diffraction analysis of similar structures (Ghassemzadeh et al. 2012).

Condensation of equimolar quantities of 6-*tert*-butyl-3-hydrazinyl-1,2,4-triazin-5(2*H*)-one (4) with (het)arenecarbaldehydes 5 afforded the target hydrazones 10–12 in 53–81% yields (Scheme 4). The reaction was carried out under the same conditions as for the 4-amino derivative in refluxing ethanol for 3 hours. However, attention should be paid to the conditions used as catalytic amounts of a base can push the reaction further promoting cyclization of hydrazonotriazines to [1,2,4]triazolo[4,3-*b*][1,2,4]triazin-7(1*H*)-one heterocyclic system (El-Shehry et al. 2020). The structure of compounds 10–12 was confirmed by elemental analysis and spectral evidence. The ^{1}H NMR spectra showed the structure of 10–12 fitted with the recorded data. Thus, all of the ^{1}H NMR spectra of these derivatives showed characteristic singlets within the following ranges: 1.30–1.33 corresponding to *t*-Bu protons, 7.90–9.09 corresponding to the ylidene = CH proton, 11.3–11.8 and 12.4–12.9 for exo- and endocyclic NH groups, respectively.

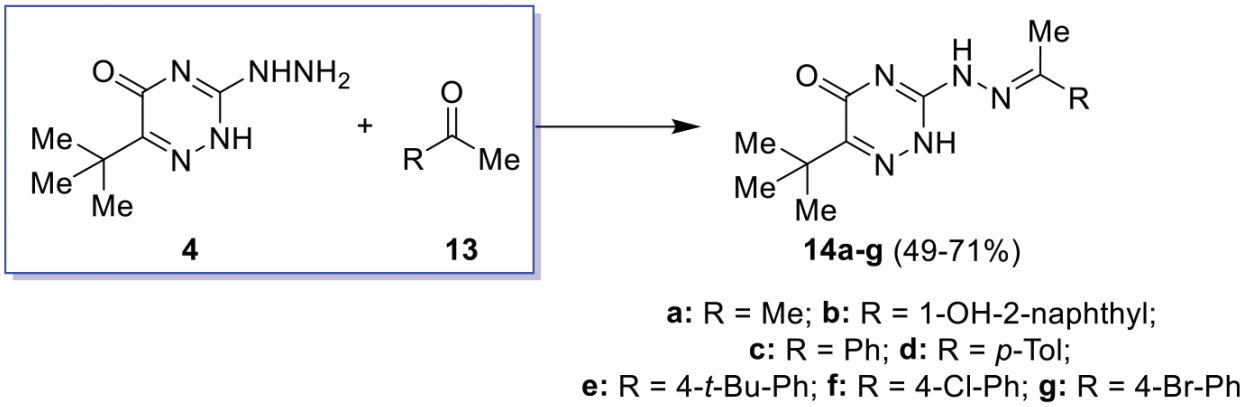


Scheme 3. The synthesis of 4-amino-3-(2-(het)arylidenehydrazinyl)-6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones 6–9 (conditions: EtOH, reflux, 3 hours).



Scheme 4. Application of aldehydes in the synthesis of the derivatives of 3-(2-R-ylidenehydrazinyl)-6-*tert*-butyl-*2H*-[1,2,4]triazin-5-ones 10–12 (conditions: EtOH, reflux, 3 hours).

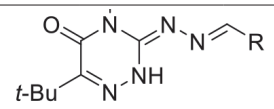
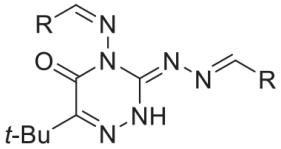
Several works published earlier include results on the application of ketones in reaction with 6-R-3-hydrazinyl-2*H*-[1,2,4]triazin-5-ones. Pyruvic acid, isatins, ethyl acetoacetate and acetone were successfully used for the synthesis of the corresponding hydrazones (Mironovich and Ivanov 2003; Ashour et al. 2013; El-Wakil et al.



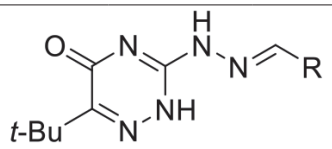
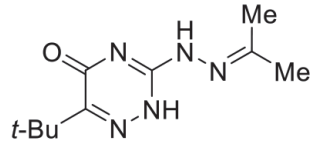
Scheme 5. Application of ketones in the synthesis of the derivatives of 3-(2-R-ylidenehydrazinyl)-6-*tert*-butyl-2*H*-[1,2,4]triazin-5-ones 14 (conditions: EtOH, reflux, 3 hours).

2019). In this research we expanded the range of ketones to aryl methyl ketones, acetone was also examined. It was found out that their application allows the reaction to proceed smoothly in refluxing ethanol with the formation of hydrazones 14 in 49–71% yields (Scheme 5). The identity of hydrazones 14 was proved by elemental analysis as well as NMR data. ^{1}H NMR of products 14 revealed the presence of *t*-butyl group (1.30–1.34), exo- (10.3–11.4) and endocyclic (12.1–12.7) NH, the methyl group of the starting ketone (1.92–2.49). ^{13}C NMR spectra are also fully consistent with the structures of hydrazones 14.

Table 1. Results of the antioxidant activity investigation of the synthesized compounds.

Cmp	R	Absorbance (mean of 3 measurements)	MDA content (nmol/ml of the yolk lipoprotein emulsion)	AOA, % (with reference to vitamin C)
DMSO	–	0,14	0,92	–
Ascorbic acid	–	0,09	0,56	–
				
6a	2-furyl	0.35	2.24	-363.5%
7c	Ph	0.03	0.20	199.4%
7d	4-Br-Ph	0.12	0.74	51.2%
7e	4-HO-Ph	0.09	0.56	98.8%
7i	4-MeO_2C-Ph	0.11	0.69	63.5%
7j	2-NO_2-Ph	0.04	0.27	180.0%
7k	3-NO_2-Ph	0.03	0.16	210.0%
7l	4-NO_2-Ph	0.08	0.48	121.8%
8	2-HO-1-naphthyl	0.03	0.19	201.2%
				

On the next stage antioxidant potency of the synthesized hydrazones 6–12, 14 were evaluated in *in vitro* tests. As we mentioned above similar 6-substituted 4*H*-[1,2,4] triazin-5-ones were already obtained, but their antioxidant properties still remain overlooked. Therefore, the antioxidant activity of the new compounds obtained in this work was evaluated in in vitro experiments on a model of artificial oxidative stress using an emulsion of yolk lipoproteins as a substrate sensitive to oxidation. The level of their activity against ascorbic acid and the percentage of inhibition of the formation of TBA-reactive products, namely the content of MDA, were calculated (Table 1).

Cmp	R	Absorbance (mean of 3 measurements)	MDA content (nmol/ml of the yolk lipoprotein emulsion)	AOA, % (with reference to vitamin C)
9	2-MeO-Ph	0.07	0.42	138.8%
				
10a	2-furyl	0.38	2.44	-417.1%
11c	Ph	0.14	0.90	5.3%
11d	4-Br-Ph	0.24	1.54	-169.4%
11e	4-HO-Ph	0.04	0.28	178.2%
11i	4-MeO_2C-Ph	0.13	0.83	25.9%
11j	2-NO_2-Ph	0.14	0.87	14.1%
11k	3-NO_2-Ph	0.13	0.86	16.5%
11l	4-NO_2-Ph	0.15	0.95	-7.7%
11n	4-MeO-Ph	0.16	1.01	-23.5%
12	2-HO-1-naphthyl	0.06	0.36	155.3%
				
14	–	0.19	1.24	-87.1%

According to the results of the primary pharmacological screening *in vitro*, there is a distinctive difference in AOA between hydrazones of 6-*tert*-butyl-4-amino-4*H*-[1,2,4]triazin-5-ones 6–9 and 6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones 10–12. Thus, 4-amino derivatives, in general, turned out to be more active as compared to the corresponding 4-desamino ones, and almost all displayed positive AOA. Obviously, this conclusion is due to the reducing and radical scavenging properties of primary amines (Suzuki et al. 2013). In addition, it was found before that introducing $-NH_2$ group into the compound can improve its antioxidant activity owing to the outstanding activities of amino-substituents (Wang et al. 2019). The common feature of both classes was the significant prooxidative properties of furyl-containing derivatives 6a and 10a. 6-*Tert*-butyl-2*H*-[1,2,4]triazin-5-ones 11d (R = 4-Br-Ph), 11l (R = 4-NO_2-Ph), 11n (R = 4-MeO-Ph), 14 (R = isopropyliden) were found to be inactive in the experimental conditions. In contrast to them, more than half of 6-*tert*-butyl-4-amino-4*H*-[1,2,4]triazin-5-ones **7**c (R = Ph), 7j (R = 2-NO_2-Ph), 7k (R = 3-NO_2-Ph), 7l (R = 4-NO_2-Ph), 8 (R = 2-HO-1-naphthyl), 6-*tert*-butyl-4*H*-[1,2,4]triazin-5-ones 11e (R = 4-HO-Ph), 12 (R = 2-HO-1-naphthyl) as well as bis-Schiff derivative 9 showed AOA exceeding AOA of the reference drug ascorbic acid. What is more, 7c, 7k and 8 showed significant AOA, which was twice as much as the AOA of ascorbic acid and, hence, can be regarded as promising substances for further pharmacological investigations.

Conclusion

In conclusion, 34 new hydrazone derivatives of 4-amino-6-(*tert*-butyl)-3-hydrazinyl-1,2,4-triazin-5(4*H*)-one and 6-(*tert*-butyl)-3-hydrazinyl-1,2,4-triazin-5(2*H*)-one have been synthesized with moderate to high yields. The structure and purity of the compounds obtained have been confirmed by elemental analysis, ^{1}H and ^{13}C NMR spectroscopy. Among synthesized ones, 21 compounds were tested for the antioxidant activity under conditions of the artificial oxidative stress *in vitro*. Pharmacological experiments have revealed 8 compounds displaying a higher level of AOA than ascorbic acid does; among them, 3 compounds have been proved to be twice as active as ascorbic acid. The fact that most of the active compounds belong to 6-*tert*-butyl-4-amino-4*H*-[1,2,4]triazin-5-one derivatives makes them promising objects for in-depth investigations of antioxidant properties and disorders accompanied by oxidative stress.

Funding

The authors have no funding to report.

Conflict of interests

The authors have no conflict of interests to declare.

References

Ajani OO, Obafemi CA, Nwinyi OC, Akinpelu DA (2010) Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives. Bioorganic & Medicinal Chemistry 18: 214–221. https://doi.org/10.1016/j.bmc.2009.10.064

Albert E (1971) Yzbyratelnaia Toksychnost. Myr, Moskva, 431 pp.

Ashour HM, El-Wakil MH, Khalil MA, Ismail KA, Labouta IM (2013) Synthesis of some (E)-6-[2-(furan-2-yl)ethenyl]-1,2,4-triazin-5-ones and their biological evaluation as antitumor agents. Medicinal Chemistry Research 22: 1909–1924. https://doi.org/10.1007/s00044-012-0192-x

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis. JAMA 297: 842–857. https://doi.org/10.1001/jama.297.8.842

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2012) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database of Systematic Reviews. https://doi.org/10.1002/14651858.CD007176.pub2

Brainina K, Stozhko N, Vidrevich M (2019) Antioxidants: Terminology, Methods, and Future Considerations. Antioxidants 8: 297. https://doi.org/10.3390/antiox8080297

Dimmock JR, Vashishtha SC, Stables JP (2000) Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds. European Journal of Medicinal Chemistry 35: 241–248. https://doi.org/10.1016/S0223-5234(00)00123-9

El Massry AM, Asal AM, Khattab SN, Haiba NS, Awney HA, Helmy M, Langer V, Amer A (2012) Synthesis and structure elucidation of novel fused 1,2,4-triazine derivatives as potent inhibitors targeting CYP1A1 activity. Bioorganic & Medicinal Chemistry 20: 2624–2637. https://doi.org/10.1016/j.bmc.2012.02.041

El-Barbary AA, Sakran MA, El-Madani AM, Nielsen C (2005) Synthesis, characterization and biological activity of some 1,2,4-triazine derivatives. Journal of Heterocyclic Chemistry 42: 935–941. https://doi.org/10.1002/jhet.5570420528

El-Barbary AA, Hafiz YA, Abdel-Wahed MS (2010) Glycosylation, Sugar Hydrazones, and Antimicrobial Evaluation of Some 6-Substituted-1,2,4-Triazines. Nucleosides, Nucleotides & Nucleic Acids 29: 55–71. https://doi.org/10.1080/15257770903454482

El-Shehry MF, El-Hag FAA, Ewies EF (2020) Synthesis and Antimicrobial Study of New Fused Thiazolo[3,2-b]triazine, Triazolo[4,3-b]triazine, and 1,2,4-Triazinone Derivatives. Russian Journal of Organic Chemistry 56: 129–136. https://doi.org/10.1134/S1070428020010200

El-Wakil MH, El-Yazbi AF, Ashour HMA, Khalil MA, Ismail KA, Labouta IM (2019) Discovery of a novel DNA binding agent via design and synthesis of new thiazole hybrids and fused 1,2,4-triazines as potential antitumor agents: Computational, spectrometric and in silico

studies. Bioorganic Chemistry 90: e103089. https://doi.org/10.1016/j.bioorg.2019.103089

Farmer EE, Davoine C (2007) Reactive electrophile species. Current Opinion in Plant Biology 10: 380–386. https://doi.org/10.1016/j.pbi.2007.04.019

Garcia-Castro M, Zimmermann S, Sankar MG, Kumar K (2016) Scaffold Diversity Synthesis and Its Application in Probe and Drug Discovery. Angewandte Chemie International Edition 55: 7586–7605. https://doi.org/10.1002/anie.201508818

Ghassemzadeh M, Rezaeirad B, Bahemmat S, Neumüller B (2012) Syntheses, characterization and crystal structures of new mono- and bis-Schiff base compounds derived from 1,2,4-triazine and the silver(I) complexes containing mono-Schiff base ligands. Journal of the Iranian Chemical Society 9: 285–296. https://doi.org/10.1007/s13738-011-0023-x

Grygoriv GV, Lega DA, Chernykh VP, Zaprutko L, Gzella AK, Pawełczyk A, Shemchuk LA (2018) 1,2-Benzoxathiin-4(3H)-one 2,2-dioxide – new enol nucleophile in three-component interaction with benzaldehydes and active methylene nitriles. RSC Advances 8: 37295–37302. https://doi.org/10.1039/c8ra06801a

Halliwell B (2006) Oxidative stress and cancer: have we moved forward? Biochemical Journal 401: 1–11. https://doi.org/10.1042/bj20061131

Hamama W, El-Bana G, Shaaban S, Habib OMO, Zoorob H (2017) An Easy Access to Construct Some New Fused 1,2,4-Triazines with Ring Junction Nitrogen Systems and Their Biological Evaluation. Journal of Heterocyclic Chemistry 54: 1384–1390. https://doi.org/10.1002/jhet.2718

Hamama WS, El-Bana GG, Shaaban S, Zoorob HH (2018) Synthetic Approach to Some New Annulated 1,2,4-Triazine Skeletons with Antimicrobial and Cytotoxic Activities. Journal of Heterocyclic Chemistry 55: 971–982. https://doi.org/10.1002/jhet.3127

Hubskyi YuI, Levytskyi YeL, Horiushko HH, Marchenko OM, Prymak RH, Danylenko VP, Ovrutskyi VM (2001) Biohimichni mekhanizmi genomozahisnoï diï novih pohidnih piridinkarbonovih kislot za urazhennya zaurazhennya klitin tetrahlormetanom. Ukrainskyi biokhimichnyi zhurnal 73(5): 100–107.

Hwang O (2013) Role of Oxidative Stress in Parkinson's Disease. Experimental Neurobiology 22: 11–17. https://doi.org/10.5607/en.2013.22.1.11

Ivanov SM (2022) 9.02 - 1,2,4-Triazines and Their Benzo Derivatives. In: Black DS, Cossy J, Stevens CV (Eds) Comprehensive Heterocyclic Chemistry IV. Elsevier, Oxford, 29–180. https://doi.org/10.1016/B978-0-12-818655-8.00062-7

Iwashita A, Maemoto T, Nakada H, Shima I, Matsuoka N, Hisajima H (2003) A Novel Potent Radical Scavenger, 8-(4-Fluorophenyl)-2-((2*E*)-3-phenyl-2-propenoyl)-1,2,3,4-tetrahydropyrazolo[5,1-*c*][1,2,4]triazine (FR210575), Prevents Neuronal Cell Death in Cultured Primary Neurons and Attenuates Brain Injury after Focal Ischemia in Rats. Journal of Pharmacology and Experimental Therapeutics 307: 961–968. https://doi.org/10.1124/jpet.103.056572

Jiang L, Yang K-h, Tian J-h, Guan Q-l, Yao N, Cao N, Mi D-h, Wu J, Ma B, Yang S-h (2010) Efficacy of Antioxidant Vitamins and Selenium Supplement in Prostate Cancer Prevention: A Meta-Analysis of Randomized Controlled Trials. Nutrition and Cancer 62: 719–727. https://doi.org/10.1080/01635581.2010.494335

Joseph N, Zhang-James Y, Perl A, Faraone SV (2013) Oxidative Stress and ADHD: A Meta-Analysis. Journal of Attention Disorders 19: 915–924. https://doi.org/10.1177/1087054713510354

Kaneto H, Katakami N, Matsuhisa M, Matsuoka T-a (2010) Role of Reactive Oxygen Species in the Progression of Type 2 Diabetes and Atherosclerosis. Mediators of Inflammation 2010: e453892. https://doi.org/10.1155/2010/453892

Kelly FJ (2003) Oxidative stress: its role in air pollution and adverse health effects. Occupational and Environmental Medicine 60: 612–616. https://doi.org/10.1136/oem.60.8.612

Khoshneviszadeh M, Ghahremani MH, Foroumadi A, Miri R, Firuzi O, Madadkar-Sobhani A, Edraki N, Parsa M, Shafiee A (2013) Design, synthesis and biological evaluation of novel anti-cytokine 1,2,4-triazine derivatives. Bioorganic & Medicinal Chemistry 21: 6708–6717. https://doi.org/10.1016/j.bmc.2013.08.009

Kolodyazhna TI, Lega DA, Suikov SY, Kyrylchuk AA, Vovk MV, Chernykh VP, Shemchuk LA (2021) Some Aspects of 4H-Pyrans Synthesis Based on 4-Chloro-1-ethyl-1H-benzo[c][1,2]thiazine-3-carbaldehyde 2,2-dioxide: Antimicrobial Activity of the Compounds Synthesized. ChemistrySelect 6: 14005–14012. https://doi.org/10.1002/slct.202104103

Kumar R, Sirohi TS, Singh H, Yadav R, Roy RK, Chaudhary A, Pandeya SN (2014) 1,2,4-Triazine Analogs as Novel Class of Therapeutic Agents. Mini-Reviews in Medicinal Chemistry 14: 168–207. http://dx.doi.org/10.2174/1389557514666140131111837

Lima PC, Lima LM, da Silva KCM, Léda PHO, de Miranda ALP, Fraga CAM, Barreiro EJ (2000) Synthesis and analgesic activity of novel N-acylarylhydrazones and isosters, derived from natural safrole. European Journal of Medicinal Chemistry 35: 187–203. https://doi.org/10.1016/S0223-5234(00)00120-3

Lipkus AH, Yuan Q, Lucas KA, Funk SA, Bartelt WF, Schenck RJ, Trippe AJ (2008) Structural Diversity of Organic Chemistry. A Scaffold Analysis of the CAS Registry. The Journal of Organic Chemistry 73: 4443–4451. https://doi.org/10.1021/jo8001276

Liu S, Shang R, Shi L, Wan DC-C, Lin H (2014) Synthesis and biological evaluation of 7H-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives as dual binding site acetylcholinesterase inhibitors. European Journal of Medicinal Chemistry 81: 237–244. https://doi.org/10.1016/j.ejmech.2014.05.020

Lodovici M, Bigagli E (2011) Oxidative Stress and Air Pollution Exposure. Journal of Toxicology 2011: e487074. https://doi.org/10.1155/2011/487074

Mironovich LM, Ivanov MA (2003) The Reactions of 6-(*tert*-Butyl)-3-hydrazino-1,2,4-triazin-5(2*H*)-one with Carbonyl Compounds. Chemistry of Heterocyclic Compounds 39: 1207–1209. https://doi.org/10.1023/B:COHC.0000008267.69391.d6

Moore K, Roberts LJ (1998) Measurement of Lipid Peroxidation. Free Radical Research 28: 659–671. https://doi.org/10.3109/10715769809065821

Mulgund A, Doshi S, Agarwal A (2015) Chapter 25 - The Role of Oxidative Stress in Endometriosis. In: Watson RR (Ed.) Handbook of Fertility. Academic Press, San Diego, 273–281. https://doi.org/10.1016/B978-0-12-800872-0.00025-1

Nair V, O'Neil CL, Wang PG (2008) Malondialdehyde. Encyclopedia of Reagents for Organic Synthesis. https://doi.org/10.1002/047084289X.rm013.pub2

Narang R, Narasimhan B, Sharma S (2012) A Review on Biological Activities and Chemical Synthesis of Hydrazide Derivatives. Current medicinal chemistry 19: 569–612. http://dx.doi.org/10.2174/092986712798918789

Paudler WW, Barton JM (1966) The Synthesis of 1,2,4-Triazine. The Journal of Organic Chemistry 31: 1720–1722. https://doi.org/10.1021/jo01344a013

Perekhoda L, Yeromina H, Drapak I, Kobzar N, Smolskiy O, Demchenko N (2017) The antioxidant properties of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives under conditions of artificial oxidative stress *in vitro*. Saudi Journal of Medical and Pharmaceutical Sciences 3(1): 55–59.

Pryor WA, Stanley JP (1975) Suggested mechanism for the production of malonaldehyde during the autoxidation of polyunsaturated fatty acids. Nonenzymic production of prostaglandin endoperoxides during autoxidation. The Journal of Organic Chemistry 40: 3615–3617. https://doi.org/10.1021/jo00912a038

Rollas S, Gulerman N, Erdeniz H (2002) Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines. Il Farmaco 57: 171–174. https://doi.org/10.1016/S0014-827X(01)01192-2

Schmidt T, Wittig A, Sehnem H-P, Kratzer H, Singer R-J (1982) Preparation of 4-amino-6-tert-butyl-3-alkylthio-1,2,4-triazin-5(4H)-ones. US4309538A, C07D 253/06 ed. Bayer Aktiengesellschaft, United States, 7 pp.

Shahidi F (2015) 1 - Antioxidants: Principles and applications. In: Shahidi F (Ed.) Handbook of Antioxidants for Food Preservation. Woodhead Publishing, 1–14. https://doi.org/10.1016/B978-1-78242-089-7.00001-4

Shchegol'kov EV, Makhaeva GF, Boltneva NP, Lushchekina SV, Serebryakova OG, Rudakova EV, Kovaleva NV, Burgart YV, Saloutin VI, Chupakhin ON, Bachurin SO, Richardson RJ (2017) Synthesis, molecular docking, and biological activity of polyfluoroalkyl dihydroazolo[5,1-c][1,2,4]triazines as selective carboxylesterase inhibitors. Bioorganic & Medicinal Chemistry 25: 3997–4007. https://doi.org/10.1016/j.bmc.2017.05.045

Silva GA, Costa LMM, Brito FCF, Miranda ALP, Barreiro EJ, Fraga CAM (2004) New class of potent antinociceptive and antiplatelet 10H-phenothiazine-1-acylhydrazone derivatives. Bioorganic & Medicinal Chemistry 12: 3149–3158. https://doi.org/10.1016/j.bmc.2004.04.009

Stanner SA, Hughes J, Kelly CNM, Buttriss J (2007) A review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutrition 7: 407–422. https://doi.org/10.1079/PHN2003543

Suzuki K, Watanabe T, Murahashi S-I (2013) Oxidation of Primary Amines to Oximes with Molecular Oxygen using 1,1-Diphenyl-2-picrylhydrazyl and WO3/Al2O3 as Catalysts. The Journal of Organic Chemistry 78: 2301–2310. https://doi.org/10.1021/jo302262a

Ulomskiy EN, Ivanova AV, Gorbunov EB, Esaulkova IL, Slita AV, Sinegubova EO, Voinkov EK, Drokin RA, Butorin II, Gazizullina ER, Gerasimova EL, Zarubaev VV, Rusinov VL (2020) Synthesis and biological evaluation of 6-nitro-1,2,4-triazoloazines containing polyphenol fragments possessing antioxidant and antiviral activity. Bioorganic & Medicinal Chemistry Letters 30: e127216. https://doi.org/10.1016/j.bmcl.2020.127216

Uno K, Nicholls SJ (2010) Biomarkers of inflammation and oxidative stress in atherosclerosis. Biomarkers in Medicine 4: 361–373. https://doi.org/10.2217/bmm.10.57

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. The International Journal of Biochemistry & Cell Biology 39: 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

Wang L, Yang F, Zhao X, Li Y (2019) Effects of nitro- and amino-group on the antioxidant activity of genistein: A theoretical study. Food Chemistry 275: 339–345. https://doi.org/10.1016/j.foodchem.2018.09.108

Yazdani M, Edraki N, Badri R, Khoshneviszadeh M, Iraji A, Firuzi O (2019) Multi-target inhibitors against Alzheimer disease derived from 3-hydrazinyl 1,2,4-triazine scaffold containing pendant phenoxy methyl-1,2,3-triazole: Design, synthesis and biological evaluation. Bioorganic Chemistry 84: 363–371. https://doi.org/10.1016/j.bioorg.2018.11.038