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Preliminary communication

Synthesis and oxidant properties of novel (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketonethiosemicarbazone

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Abstract

The reaction of 5-bromosalicylaldehyde with 1-mesityl-1-methyl-3-(2-chloro-1-oxoethyl)cyclobutane **(1)** and potassium carbonate was used to prepare (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)methanone **(2)** for the starting reagent purposes. (5-Bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketonethiosemicarbazone **(3)** was synthesized from the reaction of the compound **(2)** with thiosemicarbazide. In the present study, it was aimed to examine the influence of synthetic (5-bromobenzofuran-2-yl)(3-methyl-3-mesityl cyclobutyl)ketonethiosemicarbazone on levels of vitamins (A, E, C), selenium and malondialdehyde in rats. A total of 42 rats were used and the animals were divided into two groups in the study. Only a subcutaneous injection of 250 μl of 75% ethanol was given to the control group every other day. A subcutaneous injection of this compound (25 mg kg^{-1}, dissolved in 250 μl of 75% ethanol) was administered to the other group of rats. After the application of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketonethiosemicarbazone for 20 days, the serum vitamins (A, E, C) and malondialdehyde levels were determined with high performance liquid chromatography, the serum selenium level was determined by using fluorescence spectrophotometer. The serum vitamin A, E, C and selenium levels were significantly decreased compared to control group ($P < 0.005$), whereas serum malondialdehyde levels were higher than control group levels ($P < 0.005$). As a result, it could be suggested that this compound induced a severe stress, and also increased the amount of free radicals depending on the stress.

Keywords: Thiosemicarbazone-substituted benzofuran; Antioxidants; MDA

1. Introduction

Many thiosemicarbazones are already used in medical practice. Thiosemicarbazones are a class of compounds very promising in the treatment of many diseases, cancer in particular, and its development is still in progress [1–3]. The most recognized thiosemicarbazones are the *marboran* and *triapine* compounds **(**Scheme 1A**)**. Thiosemicarbazones are a class of small molecules that have been evaluated over the last 50 years as antivirals and as anticancer therapeutics, as well as for their parasiticidal action against *Plasmodium falciparum* and *Trypanosoma cruzi* which are the causative agents of malaria and Chagas's disease, respectively. Currently, a thiosemicarbazone, *triapine*, is being evaluated in human phase II trials as an antineoplastic therapeutic [4]. Thiosemicarbazones appear to be a structural class with anti-pox virus activity. Isatin derivatives such as *methisazone* (*marboran*), the thiosemicarbazone of *N*-methylisatin, have been described as smallpox chemoprophylactic agents. *Methisazone* decreases morbidity and mortality when given to susceptible contacts, but has no direct therapeutic efficacy vs. variola and is no longer manufactured as a drug substance [5].

Benzofuran derivatives are nowadays an important class of organic compounds that occur in a great number of natural products [6] and synthetic pharmaceuticals [7]. The very well known benzofurans are *ailanthoidol*, *amiodarone* and *bufuralol* compounds **(**Scheme 1B**)**. *Ailanthoidol* has been reported that neolignans and lignans possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, and antifungal and antifeedant activities [8]. *Amiodarone* is a highly effective antiarrhythmic agent with class III activity according to the classification of Vaughan-Williams.

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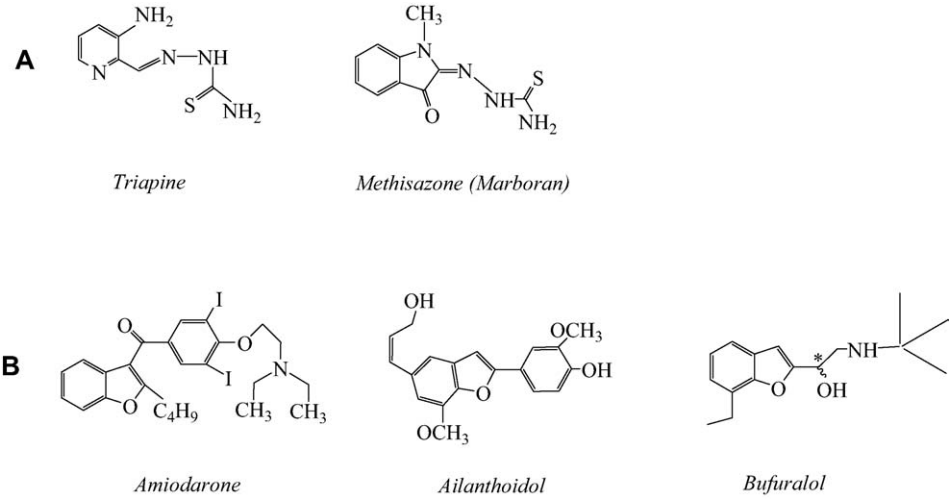


Scheme 1. Thiosemicarbazones and benzofuran containing some biological molecules.

It is used in the treatment and prophylaxis of both ventricular and supraventricular arrhythmias, in particular in patients with arrythmia, because it has no significant negative inotropic effect [9]. *Bufuralol* is a nonselective β-adrenoceptor antagonist developed by Hoffman-La Roche. *Bufuralol* is a chiral molecule having an asymmetric carbon in its ethanolamine side chain, yielding the enantiomer 1′*R-bufuralol* and 1′*S-bufuralol*, and the β-adrenoceptor blocking activity resides mainly in 1′*S-bufuralol*. This compound is a good substrate of cytochrome P450 (CYP) and undergoes enantioselective and regioselective oxidations in liver [10].

Thiosemicarbazone derivatives exhibit diverse biological activities possibly due to the presence of –NH–NH–CS–NH_2 moiety. Prompted by this observation and in continuation of our work on the synthesis of substituted benzofurans, we thought it worthwhile to synthesize new compounds of benzofuran having thiosemicarbazone moiety, with the objective of obtaining new biologically active compounds.

In the present study, we synthesized two new benzofuran-substituted compounds and investigated the effect of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketone thiosemicarbazone(thiosemicarbazone-substituted benzofuran: TSBF) administration on the levels of antioxidant vitamins (A, E, C), selenium (Se) and malondialdehyde (MDA), as indicators of lipid peroxidation in rats.

2. Results and discussion

We have synthesized novel compounds of cyclobutane-substituted benzofuran class in this work. 1-Mesityl-1-methyl-3-(2-chloro-1-oxoethyl) cyclobutane **(1)** was prepared following the procedure given in the cited reference [11]. (5-Bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)methanone **(2)** was synthesized from the reaction of 1-mesityl-1-methyl-3-(2-chloro-1-oxoethyl)cyclobutane (**1**) and 3-bromosalicylaldehyde with K_2CO_3 in an acetone/acetonitrile solvent mixture.

It was expected that the synthesized compounds should have two different isomers in the cis and trans configurations, due to the methyl and mesityl groups on the cyclobutane ring. In fact, the starting material, 1-mesityl-1-methyl-3-(2-chloro-1-oxoethyl)cyclobutane **(1)** itself is an 85:15 mixture of cis and trans isomeric structures. Unfortunately, only cis isomer was isolated in our work. It might be as a result of the crystallization technique. CH proton belonging to cyclobutane ring was observed at δ 3.5 ppm integrating for one proton as pentet. This signal was attributed to cis isomer for all the synthesized compounds. In addition to that, CH proton belonging to trans isomer was not observed in ^{1}H- and ^{13}C-NMR spectra. This observation agrees with those available in the literature [11–13]. In the IR spectra of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)methanone **(2)** showed C=O absorption (stretching), which is adjacent to benzofuran ring, at 1675 cm^{-1} and the signals belonging to furan ring C–O–C absorption was observed at 1257 cm^{-1}. In ^{13}C-NMR spectra of this compound the signal belonging to C=O appeared at δ 193.7 ppm.

In the IR spectra of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketonethiosemicarbazone **(3)** displayed broad NH absorption peak between 3472–3205 cm^{-1} and C=N (stretching) absorption peak at 1460 cm^{-1}. NH peak of compound **3** appeared at δ 9.7 ppm (N–H controlled by changing with D_2O). In ^{13}C-NMR spectra this compound C=N signal appeared at δ 139.10 ppm, while the signal belonging to C=O was appeared at δ 158.80 ppm.

Free radicals and other reactive oxygen species (ROS) are generated by all aerobic cells and are known to participate in a wide variety of deleterious reactions [14]. In normal conditions aerobic organisms are protected against oxidative damage by a variety of antioxidant systems. The antioxidant system is divided into two groups as enzymatic and non-enzymatic. Non-enzymatic antioxidant system, which consists of vitamin A, E, C and Se, has been shown to react with organic free radicals and protect biomembranes [15]. In Table 1, it was observed

Table 1
Levels of antioxidant vitamins (A, E and C), selenium and MDA in serum in TSBF and control groups in rats. Results given are mean ± S.D

	Controls (*N*: 21)	TSBF (*N*: 21)	*P* value
Vitamin A (μg ml^{-1})	0.71 ± 0.13	0.48 ± 0.10	< 0.005
Vitamin E (μg ml^{-1})	8.01 ± 1.40	6.0 ± 1.13	< 0.005
Vitamin C (μg ml^{-1})	9.28 ± 1.70	6.18 ± 1.05	< 0.005
Selenium (ng ml^{-1})	82.45 ± 8.57	61.15 ± 7.06	< 0.005
MDA (nmol ml^{-1})	2.05 ± 0.42	4.46 ± 0.82	< 0.005

that the rats subcutaneously injected TSBF (25 mg kg^{-1}, dissolved 250 μl in ethanol 75%) had the low levels of antioxidant vitamins A, E, C and Se in blood serum when compared with the control group ($P < 0.005$). On the other hand, MDA levels clearly increased according to the control group ($P < 0.005$).

Lipid peroxidation has a free radical chain reaction which causes degeneration of cell membranes. Free radical species affect all the important components of cells such as lipids, proteins, carbohydrates and nucleic acids. Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation [16,17]. (–)1-(benzofuran-2-yl)-2-propylaminopentane effects as a hydroxyl radical scavenger [18] and 2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-*tert*-butylbenzofuran has been acted as a novel radical-scavenging antioxidant against lipid peroxidation [19]. On the other hand, antioxidant action of 2,2,4,6-tetra-substituted 2,3-dihydro-5-hydroxybenzofuran against lipid peroxidation effects of substituents and side chain [20]. Nevertheless, substituted thiosemicarbazones have shown to be able to produce free radicals in biological medium [21]. In our study, it was observed that the injection of TSBF to the rats significantly increased serum MDA levels according to control group ($P < 0.005$).

Selenium (Se) has effect on preventing decomposition, absorption and biological activity of α-tocopherol [22]. At the same time, Se protects the cell by inhibiting free oxygen radical production [23]. α-Tocopherol stops lipid peroxidation by trapping the free radicals. In this process α-tocopherol is converted to α-tocopheroxyl radical. Vitamin C regenerates α-tocopherol from α-tocopheroxyl radical [24,25]. Moreover, an important antioxidant—vitamin E—is transported by selenoproteins as a free radical scavenger; ascorbate works lipid rich areas of the cell, interacting with vitamin E in the later medium. The same property of vitamin C prevents the formation of nitrosamines from nitrites and nitrates [26]. Vitamin C inhibits division and growth of cell through the production of hydrogen peroxide, which damages the cells probably through an unidentified free radical(s) generation/mechanism [27]. In this study, it was found that the levels of antioxidant vitamins A, E, C and Se decreased in TSBF-injected rats in comparison to control group ($P < 0.005$). The environmental chemicals and some drugs can reduce the antioxidant levels of the organism and could thus lead to cancer and various diseases [28]. Therefore, drugs including antioxidant compounds (such as vitamin A, E and C) can be required from external sources.

3. Experimental

3.1. Chemistry

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemical Co. (Darmstadt, Germany). Bidistilled water was used to in the all studies. Melting points (uncorrected) were determined with a Gallenkamp apparatus. The IR spectra were measured with Mattson 1000 FT-IR spectrophotometer (potassium bromide disks). The ^{1}H-NMR spectra were recorded on a Varian-Gemini 200 MHz spectrometer and are reported in ppm (δ) relative to tetramethylsilane (TMS) as the internal standard and ^{13}C-NMR (50.34 MHz) is referenced to deuterochloroform ($CDCl_3$). Elemental analysis was determined on a LECO CHNSO-932 auto elemental analysis apparatus.

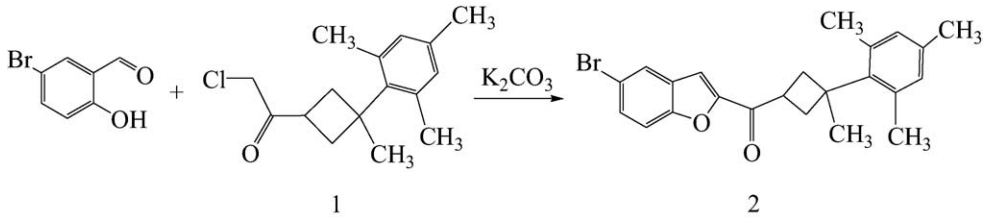
3.1.1. Synthesis of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)methanone (2)

5-Bromosalicylaldehyde (2.01 g, 10 mmol), potassium carbonate (1.38 g, 10 mmol) and dry acetonitrile (75 ml) were placed in a 250 ml two-necked flask fitted with a reflux condenser, and the mixture was stirred for 2 h at room temperature. To this solution, 1-mesityl-1-methyl-3-(2-chloro-1-oxoethyl)cyclobutane **(1)** (2.64 g, 0.101 mol, dissolved in 50 ml acetonitrile) was added and the mixture refluxed for 2 h. The course of the reaction was monitored using IR spectroscopy. The mixture was allowed to cool to room temperature and the solvent was evaporated by low pressure. The solid was extracted by diethyl ether and recrystallized from acetonitrile. Yield 3.66 g, 89%; m.p. 208–209 °C **(**Scheme 2**)**.

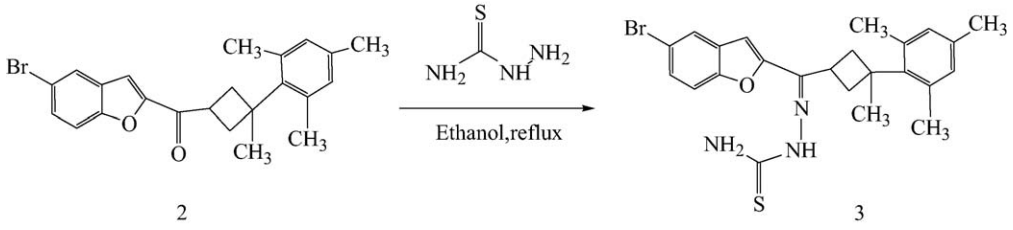
(5-Bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl) methanone (**2**). IR (KBr) (υ, cm^{-1}), 3100–2983 (aromatic C–H stretching), 2983–2858 (aliphatic C–H stretching), 1675 (C=O stretching), 1640 (C=C stretching), 1257 (C–O–C benzofuran stretching), 977–725 (benzofuran plane-out stretching), 770–700 (monosubstituted benzene plane-out stretching), ^{1}H-NMR (DMSO-d_6, 200 MHz) (ppm) δ; 1.7 (s, 3H, CH_3), 2.2 (s, 9H, mesitylene CH_3), 2.5–2.9 (m, 4H, cyclobutane CH_2), 4.1 (p, 1H, cyclobutane CH), 6.7 (s, 2H, aromatic mesitylene protons), 7.42–7.80 (m, 4H, aromatic benzofuran protons); ^{13}C-NMR (DMSO-d_6) δ : 36.90, 41.90 (2C), 42.80 (2C), 113.40, 115.90, 118.90, 127.60, 131.10, 132.30 (2C), 132.40, 136.80, 136.90 (2C), 145.20, 155.90, 156.30, 193.7; Anal. Calcd. for $C_{23}H_{23}BrO_2$: C, 67.16; H, 5.64; Found: C, 67.10; H,5.80.

3.1.2. Synthesis of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)ketonethiosemicarbazone (3)

(5-Bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl) methanone **(2)** (4.11 g, 10 mmol), thiosemicarbazide (0.911 g, 10 mmol), *p*-toluenesulfonic acid (0.01 mg) and dry ethanol (80 ml) were refluxed for 8 h. The course of the reaction was monitored with IR spectroscopy. The reaction mixture was poured into 500 ml water and reprecipitated twice from water.



Scheme 2. Synthesis of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl)methanone (**2**).



Scheme 3. Synthesis of (5-bromobenzofuran-2-yl)(3-methyl-3-mesitylcylobutyl)ketonethiosemicarbazone.

The solid was filtered and recrystallized from ethanol. Yield 4.11 g, 85%; m.p. 267–268 °C (Scheme 3).

(5-Bromobenzofuran-2-yl)(3-methyl-3-mesitylcyclobutyl) ketonethiosemicarbazone(3): IR(KBr) (υ, cm^{-1}), 3472–3205 (N–H stretching), 3141–3025 (aromatic C–H stretching), 2951–2858 (aliphatic C-H stretching), 1689 (C=O stretching), 1610–1460 (C=C and C=N stretchings), 1256 (C–O–C stretching); ^{1}H-NMR (DMSO-d_6, 200 MHz) (ppm) δ; 1.7 (s, 3H, CH_3), 2.2 (s, 9H, mesitylene CH_3), 2.5–2.7 (m, 4H, cyclobutane CH_2), 3.5 (p, 1H cyclobutane CH), 6.7–7.8 (m, 8H, aromatic benzofuran protons and NH_2 protons), 9.71 (s, 1H, NH proton); ^{13}C-NMR (DMSO-d_6) δ : 22.30, 23.10, 26.90, 35.60, 42.60, 42.90, 43.90 (2C), 110.30, 114.90, 115.20, 119.50, 126.10, 130.60, 131.00, 132.00, 136.00, 136.80, 137.00, 139.10, 145.70, 152.16, 155.90, 158.80, Anal. Calcd. for $C_{24}H_{26}BrN_3O_2$: C, 61.54; H,5.60; N, 8.97. Found: C, 61.23; H, 5.58; N, 9.02.

3.2. Animals

A total of 42 male Wistar rats (14–16-week old, 200–220 g) were used in this study. Upon arrival, the animals were allowed to acclimatize for two weeks. The rats were housed in a temperature controlled room (22–25 °C) with a 12:12 light-dark cycle; water and food were given ad libitum. Animals were divided into two groups; one control group ($N = 21$) and an experimental group ($N = 21$). The experimental group was administered the TSBF. While only an injection of 250 μl of 75% ethanol was given to the control group, TSBF (25 mg kg^{-1}, dissolved in 250 μl of 75% ethanol) was injected to the other group of rats, the every other day. Injections continued for 20 days. All animals were on a normal diet throughout the experimental period. Blood samples were harvested and kept at –20 °C until analyzed.

3.3. Determination of vitamin A and E levels in serum

Blood samples were centrifuged at 4000 rpm for 3 min at 4 °C and the serum was separated. In this serum, MDA and antioxidant vitamins (A, E and C) levels were determined by using HPLC. Determination of the amounts of vitamin A and E was performed according to cited reference [29]. Separations were carried out at room temperature with the Cecil liquid chromatography system (Series 1100) consisting a sample injection valve (Cotati 7125) with a 20 μl sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174), 326 and 296 nm for vitamin A and E, respectively. Integrator (HP 3395) and a Techsphere ODS-2 packed (5 μm particle and 80°Å pore size) column (25 0 × 4.6 ID) with a methanol/acetonitrile/chloroform (47:42:11, v/v) as a mobile phase at 1.0 ml min^{-1} flow rate.

3.4. Determination of vitamin C and MDA levels in serum

Vitamin C and free MDA were extracted [30], the supernatant was filtered and then the vitamin C level was determined according to cited reference [31]. The MDA level was determined by using the method Karatas et al. [32]. The Supelcosil LC-18-DB HPLC reversed-phase column (3 μm particle size and 25 0 × 3.9 ID) was utilized for the detection of vitamin C and MDA levels. While mobile phase (3.7 mM phosphate buffer, pH 4.0) used 1.0 ml min^{-1} flow rate to vitamin C level, the free MDA level was determined with mobile phase 30 mM KH_2PO_4 buffer, pH 4 with H_3PO_4 and methanol (65–35% v/v) at 1.5 ml min^{-1} flow rate.

3.5. Determination of selenium levels in serum

The serum samples for Se determination were treated as follows: to 1.0 ml serum samples 1.5 ml nitric acid/perchloric

acid (1:5, v/v) was added and the mixture was held in an Teflon bomb at 100 °C for 12 h for breaking the organic material and then cooled down to room temperature. Mixture was transferred into tubes and a 4.0 N HCl concentration was achieved by adding concentrated HCl (about 2.0 ml). The mixture was held to reduce Se (VI) to Se (IV) at 90 °C for 15 min. To this mixture 2.0 ml 2.5 M formic acid, 5.0 ml 0.1 M EDTA and 1.5 ml freshly prepared 3,3-diaminobenzidine (DAB) solution (1.0 mg ml^{-1}) was added for and the pH of mixture was adjusted to 1.7 with 4 N NH_3 and let stand in dark for 1.0 h for the formation of a metal-ligand complex. The mixture volume was adjusted to 50 ml by adding H_2O and 5 ml toluene, and was mixed for 2.0 min. The mixture was transferred into a volumetric separation funnel and let stand at room temperature for 2 min for phase separation. Selenium was separated in toluene phase and its level was determined fluorometrically by a Perkin Elmer 100 fluorescence spectrophotometer at 570 nm using standard addition method [33].

3.6. Statistical analysis

The SPSS software (SPSS, Chicago, IL, USA) was used for statistical analyses. Results for the groups are expressed as mean ± S.D. Differences between the groups were analyzed for significance using the student's *t*-test. Statistical significance was defined as $P < 0.05$

4. Conclusion

It can be suggested that TSBF produces more free radicals and in turn this decreases the level of antioxidant vitamins in the serum of rats. From these results, we could suggest that, antioxidant vitamins such as vitamin A, E, and C should be taken along with the medicine containing TSBF groups to compensate the potential vitamin deficiency caused by these drugs.

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