PHARMACIA

Pharmacia 66(3): 99–106
DOI 10.3897/pharmacia.66.e37263

Research Article

Synthesis and preliminary hepatotoxicity evaluation of new caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives

Javor Mitkov[1], Magdalena Kondeva-Burdina[2], Alexander Zlatkov[1]

1 *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, 2 Dunav Street, 1000 Sofia, Bulgaria*
2 *Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, 2 Dunav Street, 1000 Sofia, Bulgaria*

Corresponding author: *Javor Mitkov* (javor.mitkov@abv.bg)

Received 13 June 2019 ♦ **Accepted** 29 August 2019 ♦ **Published** 10 October 2019

Citation: Mitkov J, Kondeva-Burdina M, Zlatkov A (2019) Synthesis and preliminary hepatotoxicity evaluation of new caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives. Pharmacia 66(3): 99–106. https://doi.org/10.3897/pharmacia.66.e37263

Abstract

New series of caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives were designed and synthesized. The targed compounds were obtained in yields of 51 to 96% and their structures were elucidated by FTIR, ^{1}H NMR, ^{13}C NMR, MS and microanalyses. All of the compounds were found to be "drug-like" as they fulfill the criteria of drug-likeness, which includes the MDDR-like rule. The tested compounds were subjected to *in silico* prediction of substrate/metabolite specificity and Drug Induced Liver Injury (DILI). The prediction for indicated that the evaluated compounds would most probably act as CYP1A2 substrates. The performed *in vitro* studies didn't reveal statistically significant hepatotoxicity of the tested compounds, probably due to the pro-oxidant effects expressed on sub-cellular (isolated rat liver microsomes) level. The obtained experimental results confirmed the predicted low hepatotoxicity for the tested structures. Based on these results the compounds may be considered as promising structures for design of future molecules with low hepatotoxicity.

Keywords

caffeine, isolated rat liver microsomes, pro-oxidant effects

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring compound and one of the methylxanthines classified as a member of the alkaloid class of natural compounds. It is commonly distributed in plants throughout the world. Caffeine is also a key component of many beverages and food (coffee, tea, cocoa) and is widely consumed for its stimulating effect on the central nervous system. Nowadays, Caffeine is medically considered as the most popular, unregulated, and legal psychoactive drug in approximately all regions in the world (Alsabri et al. 2018). Caffeine and other methylxanthines are used in clinical medicine as diuretics, analgesics, and muscle relaxants, and they can aid in the treatment of brain disorders such as headaches and Parkinson's disease (Al Deeb et al. 2002; Kolayli et al. 2004). Some of the effects of caffeine are due to caffeine-derived metabolites that have their own biological activities (Franco et al. 2013; Hetzler et al. 1990; Welsh et al. 2012). Caffeine and its derivatives in human liver are metabolized by the cytochrome P450 oxidase enzyme system (in particular by the CYP1A2 isoenzyme (Bloomer et al. 1995)). This metabolism

results in the formation of three major dimethylxanthine metabolites (paraxanthine, theobromine and theophylline) and one hydroxylated metabolite (1, 3, 7-trimethyluric acid) (Kot and Daniel 2008a), where paraxanthine is the predominant caffeine-derived metabolite in humans and 1, 3, 7-trimethyluric acid and theophylline are determined as the major metabolites in rodents and monkeys, respectively (Berthou et al. 1992; Kot and Daniel 2008b).

Oxidative processes are very important for living organisms. Under pathological or oxidative stress conditions reactive oxygen species (ROS) are overproduced which results in peroxidation of membrane lipids, leading to accumulation of lipid peroxides. On the other hand, oxidative stress is known to be involved in ageing and in various diseases, including diabetes mellitus, atherosclerosis, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease and cancer (Metro et al. 2017). Some contradictory evidences are available for the influence of caffeine on oxidative stress processes (Acheson et al. 2004). It is known that some of the effects of caffeine lead to protection against cellular damage by producing beneficial antioxidant effects (Pasaoglu et al. 2011), since caffeine has been recognized as an efficient biological antioxidant (Shi et al. 1991). However, some data suggest that this property is observed only when caffeine acts as an effective inhibitor of lipid peroxidation, at millimolar concentrations, in rat liver microsomes (Devasagayam et al. 1996). On the other hand, caffeine shows *in vitro* prooxidant effect (Gulcin 2008) and is capable of inducing oxidative damage by fostering lipid peroxidation in rat liver (Dianzani et al. 1991). The role of caffeine in lipid peroxidation is varied and possibly depends on the dose of caffeine ingested.

Acylhydrazone derivatives are another molecular scaffold, on the basis of which new biologically active compounds can be generated. They are compounds formed from the condensation of an acylhydrazine and a carbonyl reagent. Azomethine group (-NH-N=CH-) contained in their structure is connected with carbonyl group and is responsible for the different pharmacological applications of cited compounds. Hydrazid-hydrazones have, therefore, attracted considerable attention to their wide range of biological activities (Sarıgöl et al. 2015), including antioxidant, anticancer and cytoprotective potency (Hristova-Avakumova et al. 2017; Nikolaevskii et al. 2012).

Microsomes are widely used test systems for investigation of the metabolic stability and metabolic profile of a large number of molecules during the drug discovery and development phases (Barcelos et al. 2014). Microsomal liver fractions combined with the possibility of automation of incubating process are elevated to high-tech applications. High storage stability of microsomes provides an opportunity to create a human bank for hepatic fractions and to study differences in enzyme activities in the population. Correlation analysis is applied to study metabolic pathways in the context of enzymatic topology (Barcelos et al. 2014), where malondialdehyde (MDA) is known to be the most frequently used biomarker of lipid peroxidation and oxidative stress evaluation.

In this study, based on the biological activity profiles of xanthines and acyl hydrazones, we hybridized these two ring systems into one unit thus reporting the synthesis of series novel caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives. Based on some literary data, in addition, we set as purpose to evaluate the effects of the newly synthesized compounds on isolated rat liver microsomes as a model of hepatotoxicity on subcellular level, applying the level of generated malondialdehyde (MDA) in the rat liver microsomes as a measure of the hepatotoxicity.

2. Materials and methods

2.1. Materials

All chemicals and solvents were purchased from Merck AG (Merck, Darmstadt, Germany). The chromatographic system for TLC control and purity elucidation is based on an alimunium sheets Silica gel F254 (Merck, Darmstadt, Germany), using the following mobile phases: *Phase 1:* 25% NH_4OH/Acetone/$CHCl_3$/CH_3CH_2OH (1/3/3/4) and *Phase 2:* H_2O/n-butanol/CH_3COOH (5/4/1), with detection at UV 254 nm. Yields were calculated for purified products. A Buchi 535 capillary apparatus (Switzerland) was used to determine the melting points. The IR spectra 400 – 4000cm^{-1} were recorded on a Nicolet iS10 FT-IR Spectrometer using ATR technique with Smart iTR adapter. UV-spectra were recorded on a Jenway 6715 UV/VIS Spectrophotometer, Japan. A Bruker Spectrospin WM250 spectrometer (Faenlanden, Switzerland) was used to acquire ^{1}H-NMR and ^{13}C-NMR spectra at 250 and 75 MHz, respectively. TMS as internal standard and DMSO-d_6 were used as internal standard and solvent, respectively, all OH and NH protons were D_2O exchangeable. The coupling constants (*J*) are expressed in Hertz (Hz). The mass spectra were recorded on a Dionex Ultimate 3000 RSLC Ultrasonic Liquid Chromatography System (Thermo Scientific) equipped of a six-channel SRD-3600 Degaser, a HPG-3400RS High Pressure Binary Gradient Pump, an Automatic Sample Injector WPS-3000TRS and TCC-3000RS column thermostat. The chromatographic system is connected to a Q-Exact Plus mass spectrometer (Thermo Scientific, Bremen, Germany) with Enhanced Resolution Mode enabled (up to 280,000 FWHM at m/z 200). Microanalyses, determined for C, N and H, were within ±0.4% of theoretical values and were performed on Euro EA 3000-Single, EUROVECTOR SpA analyser. All names were generated by using structure–to–name algorithm of ChemBioDraw Ultra software, Version 11.0, CambridgeSoft.

2.2. Chemistry

2.2.1. *Synthesis of initial intermediates 8-bromocaffeine (**1**), caffeine-8-(2-thio)-propanioc acid (**3**), methyl ester of caffeine-8-(2-thio)-propanioc acid (**4**) and caffeine-8-(2-thio)-propanehydrazide (**5**)*

The starting 8-bromocaffeine (**1**) was obtained using oxidative bromination of caffeine according to protocol, described by Mitkov et al (Mitkov et al. 2012). Intermediates **3**, **4** and **5** are obtained in excellent yields and purity,

following the synthetic procedure published in our earlier paper (Mitkov et al. 2019).

2.2.2. *General procedure for syntheses of substituted N'-substituted 2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazides (**6a-i**)*

In a round-bottomed flask equipped with a reflux condenser and an electromagnetic stirrer, the hydrazide **5** (0.0032 mol) and the corresponding ketone (0.004 mol) were mixed in 50 ml of ethanol. The reaction mixture is stirred under reflux. During the reaction, the precipitation of the product begins. Reaction time is determined by exhaustion of the starting reagents (TLC monitoring in phase 2) and varies from 40 minutes to 4 hours. The reaction mixture was cooled and filtered. If necessary, the filtrate is concentrated in vacuum to 10 ml and after several hours additional amount of product is separated. The product is recrystallized from ethanol.

N'-(1-phenylethylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)-propanehydrazide (**6a**)
FTIR (ATR), cm^{-1}: 3176, 1701, 1655, 1601, 1538, 1445, 1159; λ_{max}, nm: 217, 298; ^{1}H-NMR (DMSO-d_6), δ: 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.16 (3H, s, =C-$\underline{CH_3}$), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.32 (1H, t, *J* = 7.4, Ar-H), 7.43 (2H, m, Ar-H), 8.09 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 13.1 (CH-$\underline{C}H_3$), 16.1 (=C-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}H$), 138.3($\underline{C}_{Ar}$-1), 126.1 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 128.7 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 128.9 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 145.4 (N=$\underline{C}$)147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{19}H_{22}N_6O_2S$ (414.48); % calculated: C 55.06, H 5.35, N 20.28, S 7.73; and % found: C 54.98, H 5.25, N 20.47, S 7.70. LC-MS (70 eV) *m/z* (%): 415 (M+1), 416 (M+2).

N'-(1-p-tolylethylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)-propanehydrazide (**6b**)
FTIR (ATR), cm^{-1}: 3178, 1701, 1655, 1601, 1538, 1446, 1159; λ_{max}, nm: 216, 288; ^{1}H-NMR (DMSO-d_6), δ: 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.12 (3H, s, =C-$\underline{CH_3}$), 2.21 (3H, s, Ar-$\underline{CH_3}$), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.13 (2H, m, Ar-H), 7.43 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 13.1 (=C-$\underline{C}H_3$), 16.1 (CH-$\underline{C}H_3$), 21.3 (Ar-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}H$), 138.5 ($\underline{C}_{Ar}$-1), 125.2 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 129.3 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 139.8 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 145.4 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{20}H_{24}N_6O_3S$ (428.51); % calculated: C 56.06, H 5.65, N 19.61, S 7.48; and % found: C 56.03, H 5.45, N 19.47, S 7.20. LC-MS (70 eV) *m/z* (%): 429 (M+1), 430 (M+2).

N'-(1-(4-methoxyphenyl)ethylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6c**)
FTIR (ATR), cm^{-1}: 3171, 1701, 1652, 1603, 1538, 1446, 1159; λ_{max}, nm: 216,294; ^{1}H-NMR (DMSO-d_6), δ: 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.12 (3H, s, =C-$\underline{CH_3}$), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.79 (3H, s, O$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.40 (2H, m, Ar-H), 7.28 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 13.1 (=C-$\underline{C}H_3$), 16.1 (CH-$\underline{C}H_3$), 55.5 (O$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}H$), 138.5 ($\underline{C}_{Ar}$-1), 128.7 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 114.1 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 160.4 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 145.4 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{20}H_{24}N_6O_4S$ (444.51); % calculated: C 54.04, H 5.44, N 18.91, S 7.21; and % found: C 54.03, H 5.42, N 18.87, S 7.18. LC-MS (70 eV) *m/z* (%): 445 (M+1), 446 (M+2), 447 (M+3).

N'-(1-(2-hydroxyphenyl)ethylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6d**)
FTIR (ATR), cm^{-1}: 3176, 1699, 1654, 1612, 1538, 1489, 1447, 1157; λ_{max}, nm: 216, 288; ^{1}H-NMR (DMSO-d_6), δ: 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.19 (3H, s, =C-$\underline{CH_3}$), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.53 (1H, d, *J* = 7.3 Hz, Ar-H), 7.23 (1H, d, *J* = 8.8 Hz, Ar-H), 7.16 (1H, s, Ar-H), 6.95 (2H, d, *J* = 8.8 Hz, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 13.7 (=C-$\underline{C}H_3$), 16.1 (CH-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}H$), 120.5 ($\underline{C}_{Ar}$-1), 156.7 ($\underline{C}_{Ar}$-2), 117.2 ($\underline{C}_{Ar}$-3), 131.9 ($\underline{C}_{Ar}$-4), 118.7 ($\underline{C}_{Ar}$-5) 128.1 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 148.6 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{19}H_{22}N_6O_4S$ (430.48); % calculated: C 53.01, H 5.15, N 19.52, S 7.45; and % found: C 52.95, H 5.05, N 19.49, S 7.38. LC-MS (70 eV) *m/z* (%): 431 (M+1), 432 (M+2).

N'-(1-(4-hydroxyphenyl)propylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6e**)
FTIR (ATR), cm^{-1}: 3274, 3173, 1694, 1652, 1607, 1538, 1514, 1447, 1157; λ_{max}, nm: 216, 308; ^{1}H-NMR (DMSO-d_6), δ: 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.16 (3H, s, =C-$\underline{CH_3}$), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.47 (2H, d, *J* = 7.3 Hz, Ar-H), 7.18 (2H, d, *J* = 8.8 Hz, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 13.1 (=C-$\underline{C}H_3$), 16.3 (CH-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}H$), 138.5 ($\underline{C}_{Ar}$-1), 128.7 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 116.9 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 157.8 ($\underline{C}_{Ar}$-4), 145.4 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{20}H_{24}N_6O_4S$ (444.51); % calculated: C 54.04, H 5.44, N 18.91, S 7.21; and % found: C 53.98, H 5.37, N 18.48, S 7.22. LC-MS (70 eV) *m/z* (%): 445 (M+1), 446 (M+2).

N'-(1-phenylpropylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6f**)
FTIR (ATR), cm^{-1}: 3175, 1700, 1654, 1603, 1539, 1446, 1159; λ_{max}, nm: 216, 288; ^{1}H-NMR (DMSO-d_6), δ: 1.05 (3H, t, *J* = 7.3 Hz, -CH_2-$\underline{CH_3}$), 1.30 (3H, d, *J* = 6.7 Hz, -CH-$\underline{CH_3}$), 2.44 (2H, q, *J* = 7.3 Hz, =C-$\underline{CH_2}$-), 3.30 (s; 3H; N1-$\underline{CH_3}$), 3.39 (s; 3H; N3-$\underline{CH_3}$), 3.69 (s; 3H; N7-$\underline{CH_3}$), 3.82 (1H, q, *J* = 6.7 Hz, S-$\underline{CH}$-), 7.27 (2H, m, Ar-H), 7.43 (2H, m, Ar-H), 8.09 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 25.1 (=C-$\underline{C}H_2$), 10.2 (CH_2-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$),

29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}$H), 137.1 ($\underline{C}_{Ar}$-1), 126.9 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 128.7 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 129.8 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 157.4 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{20}H_{24}N_6O_3S$ (428.51); % calculated: C 56.06, H 5.65, N 19.61, S 7.48; and % found: C 56.01, H 5.55, N 19.57, S 7.35. LC-MS (70 eV) *m/z* (%): 429 (M+1), 430 (M+2).

N'-(1-phenylbutylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6g**)
FTIR (ATR), cm^{-1}: 3179, 1699, 1654, 1603, 1538, 1447, 1159; λ_{max}, nm: 216, 306; ^{1}H-NMR (DMSO-d_6), δ: 1.00 (3H, t, *J* = 6.7 Hz, -CH_2-$\underline{C}H_3$), 1.33 (3H, d, *J* = 6.7 Hz, -CH-$\underline{C}H_3$), 1.67 (2H, m, -$\underline{C}H_2$-), 2.43 (2H, q, *J* = 7.3 Hz, -$\underline{C}H_2$-CH_3), 3.30 (s; 3H; N1-$\underline{C}H_3$), 3.39 (s; 3H; N3-$\underline{C}H_3$), 3.69 (s; 3H; N7-$\underline{C}H_3$), 3.88 (1H, q, *J* = 6.7 Hz, S-$\underline{C}$H-), 7.27 (1H, m, Ar-H), 7.43 (2H, m, Ar-H), 8.22 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 28.4 (=C-$\underline{C}H_2$), 13.8 (CH_2-$\underline{C}H_3$), 21.1 (-$\underline{C}H_2$-), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}$H), 137.1 ($\underline{C}_{Ar}$-1), 126.9 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 128.7 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 128.9 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 157.4 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{21}H_{26}N_6O_3S$ (442.54); % calculated: C 57.00, H 5.92, N 18.99, S 7.24; and % found: C 56.95, H 5.55, N 18.77, S 7.30. LC-MS (70 eV) *m/z* (%): 443 (M+1), 444 (M+2).

N'-((4-methoxyphenyl)(phenyl)methylene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio) propanehydrazide (**6h**)
FTIR (ATR), cm^{-1}: 3272, 3175, 1699, 1652, 1603, 1538, 1445, 1157; λ_{max}, nm: 216, 288; ^{1}H-NMR (DMSO-d_6), δ: 3.82 (3H, s, -O-$\underline{C}H_3$), 1.33 (3H, d, *J* = 6.7 Hz, -CH-$\underline{C}H_3$), 3.30 (s; 3H; N1-$\underline{C}H_3$), 3.39 (s; 3H; N3-$\underline{C}H_3$), 3.69 (s; 3H; N7-$\underline{C}H_3$), 3.88 (1H, q, *J* = 6.7 Hz, S-$\underline{C}$H-), 7.32 (1H, m, Ar-H), 7.32 (2H, m, Ar-H), 7.43 (2H, m, Ar-H), 8.20 (2H, m, Ar-H), 8.22 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 55.5 (O-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}$H), 133.4 ($\underline{C}_{Ar}$-1), 126.4 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 128.7 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 128.9 ($\underline{C}_{Ar}$-6), 133.4 ($\underline{C}_{Ar}$-1'), 131.2 ($\underline{C}_{Ar}$-2' and $\underline{C}_{Ar}$-6'), 114.1 ($\underline{C}_{Ar}$-3' and $\underline{C}_{Ar}$-5'), 160.4 ($\underline{C}_{Ar}$-6'), 107.1 (C-5), 153.8 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{25}H_{26}N_6O_4S$ (506.58); % calculated: C 59.28, H 5.17, N 16.59, S 6.33; and % found: C 59.18, H 5.14, N 16.87, S 6.25. LC-MS (70 eV) *m/z* (%): 507 (M+1).

N'-(2-methyl-1-phenylpropylidene)-2-(1,3,7-trimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purin-8-ylthio)propanehydrazide (**6i**)
FTIR (ATR), cm^{-1}: 3218, 1694, 1655, 1607, 1522, 1465, 1158; λ_{max}, nm: 218, 294; ^{1}H-NMR (DMSO-d_6), δ: 1.29 (6H, d, *J* = 6.7 Hz, 2 x-CH-$\underline{C}H_3$), 1.33 (3H, d, *J* = 6.7 Hz, -CH-$\underline{C}H_3$), 2.45 (1H, m, =C-$\underline{C}$H-), 3.30 (s; 3H; N1-$\underline{C}H_3$), 3.39 (s; 3H; N3-$\underline{C}H_3$), 3.69 (s; 3H; N7-$\underline{C}H_3$), 3.88 (1H, q, *J* = 6.7 Hz, S-$\underline{C}$H-), 7.37 (1H, m, Ar-H), 7.43 (2H, m, Ar-H), 8.35 (2H, m, Ar-H); ^{13}C NMR (DMSO-d_6), δ: 30.3 (=C-$\underline{C}$H), 21.0 (2 x CH-$\underline{C}H_3$), 27.9 (N^1-$\underline{C}H_3$), 29.8 (N^3-$\underline{C}H_3$), 32.5(N^7-$\underline{C}H_3$), 33.7 (S-$\underline{C}$H), 132.6 ($\underline{C}_{Ar}$-1), 127.2 ($\underline{C}_{Ar}$-2 and $\underline{C}_{Ar}$-6), 128.7 ($\underline{C}_{Ar}$-3 and $\underline{C}_{Ar}$-5), 128.9 ($\underline{C}_{Ar}$-6), 107.1 (C-5), 153.8 (N=$\underline{C}$), 147.0 ($\underline{C}$-8), 148.8 ($\underline{C}$-4), 151.4 ($\underline{C}$-2), 154.7 ($\underline{C}$-6), 167.3 ($\underline{C}$O-NH); $C_{21}H_{26}N_6O_3S$ (442.54); % calculated: C 57.00, H 5.92, N 18.99, S 7.24; and % found: C 56.88, H 5.85, N 18.87, S 7.18. LC-MS (70 eV) *m/z* (%): 443 (M+1), 444 (M+2).

2.3. Biological evaluation

Animals

Male Wistar rats (body weight 200–250 g) were used. The rats were housed in plexiglass cages (3 per cage) in a 12/12 light/dark cycle, under standard laboratory conditions (ambient temperature 20 °C ± 2 °C and humidity 72 % ± 4 %) with free access to water and standard pelleted rat food 53-3, produced according to ISO 9001:2008.

Animals were purchased from the National Breeding Center, Sofia, Bulgaria. At least 7 days of acclimatization was allowed before the commencement of the study. The health was monitored regularly by a veterinary physician. The vivarium (certificate of registration of farm № 0072/01.08.2007) was inspected by the Bulgarian Drug Agency in order to check the husbandry conditions (№ A-11-1081/03.11.2011). All performed procedures were approved by the Institutional Animal Care Committee and made according Ordinance № 15/2006 for humaneness behavior to experimental animals.

Isolation and incubation of microsomes

The liver microsomes were isolated by ultracentrifugation, using Beckman L8-M centrifuge with a 70Ti rotor, for 1 hour, following Guengerich's methodology (Guengerich 1989). The protein measurement was carried out using method of Lowry et al (Lowry et al. 1951).

Measurement of malondialdehyde (MDA) in isolated rat microsomes

The concentration of MDA was determined spectrophotometrically at 535 nm by the Debby& Gutier method (Deby and Goutier 1990).

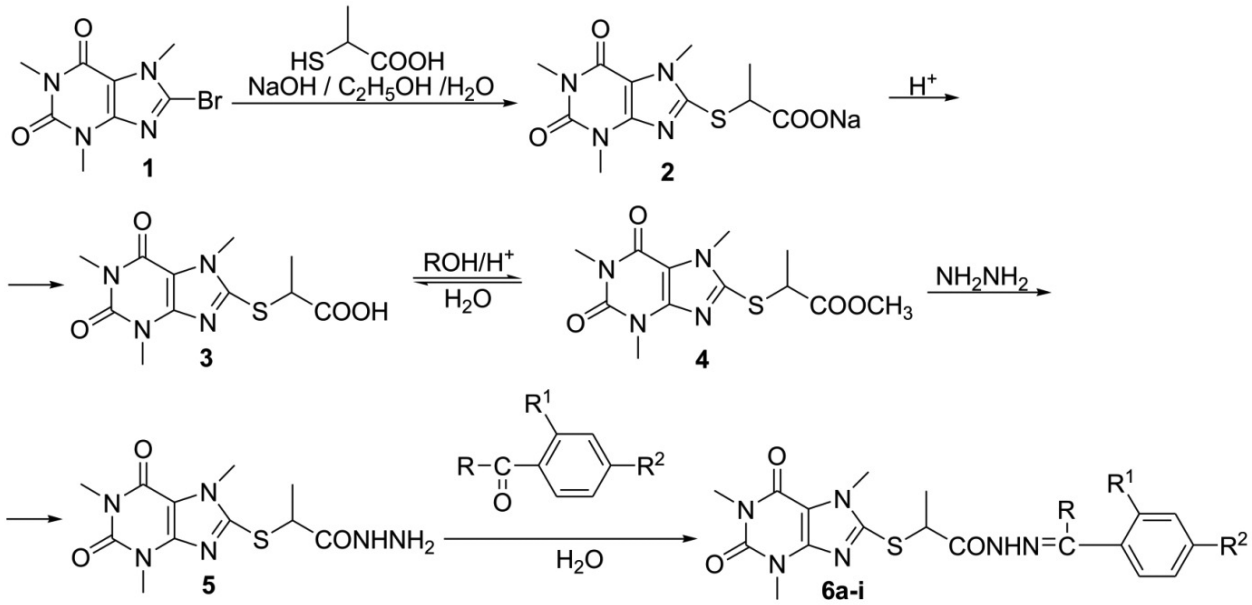
Statistical analysis

Statistical analysis was performed using statistical program "MEDCALC". Results are expressed as mean ± SEM for 7 experiments. The significance of the data was assessed using the nonparametric Mann–Whitney test. Values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ were considered statistically significant. Three parallel samples were used.

3. Results and discussion

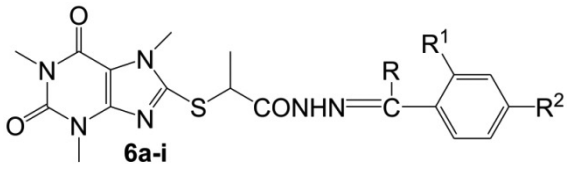
3.1. Chemistry

The synthesis of described hydrazid-hydrazones was carried out following a procedure outlined on Scheme 1. The starting 8-bromocaffeine (**1**) was obtained using oxidative bromination of caffeine according to protocol, described



Scheme 1. General pathway for synthesis of a new caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives **6a–i**.

Table 1. ID, structures, m.p. and yields of newly synthesized hydrazones.



Compound	R	R^1	R^2	M.p. (°C)	Yield (%)
6a	CH_3	H	H	242–243	51
6b	CH_3	H	CH_3	248–250	69
6c	CH_3	H	OCH_3	24–249	73
6d	CH_3	OH	H	239–243	68
6e	C_2H_5	H	OH	228–230	80
6f	C_2H_5	H	H	231–233	95
6g	C_3H_7	H	H	242–246	96
6h	C_6H_5	H	OCH_3	219–221	62
6i	$CH(CH_3)_2$	H	H	243–245	82

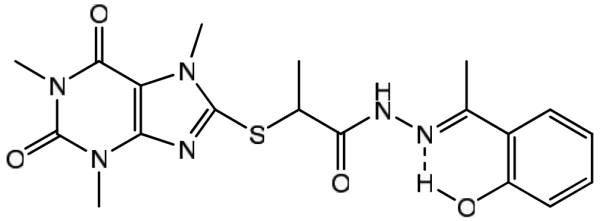


Figure 1. Formation of a 6-membered ring in **6d** due to an intramolecular hydrogen bond.

by Mitkov et al (Mitkov et al. 2012). Intermediates **3**, **4** and **5** are obtained in excellent yields and purity, following the synthetic procedure published in our earlier paper (Mitkov et al. 2019). The final products **6a-i** were prepared via condensation of the hydrazide **5** with the corresponding ketone in a molar ratio of 1: 1.25 in an ethanol medium. The compounds **6a-i** were obtained with purity (within ± 0.4% of the calculated value), evaluated by microanalysis. The structures of the target compounds were confirmed on the basis of their FT-IR, ^{1}H-, ^{13}C NMR and mass spectral data. The physical data of all synthesized compounds **6a-i** are given in Table 1.

In the performed spectral analysis a slight discrepancy in the expected and the measured IR spectra for compound **6d** was observed. This may be due to the intramolecular hydrogen bond which may be found in **6d** resulting in formation of a stable 6-membered ring as shown in structure below (Figure 1).

Thus no absorption bands due to the free O-H stretching vibration were found in the infrared spectra of any of the salicylidene anilines. Instead, a broad, weak band having some fine structure is found in the region from 2700 to 3100 cm^{-1} as is the case with **6d**.

Also, a slight absorption band at 1157–1159 cm^{-1}, which is not observed in the spectra of starting xanthines **1** and **3**, is clearly visible in the spectra of studied compounds **6a-i**. We consider this band to be due to N-N stretching vibration from the hydrazide residue. The identifications of this stretching vibration is a difficult task, but there is literary evidence to support our assumption (Bharanidharan et al. 2017; Gulaczyk et al. 2003).

Prediction of toxicity and drug-likeness

The toxicity and drug-likeness of compounds were predict by using PROTOX II ("ProTox-II") web server. The ProTox-II web server provides several advantages over existing computational models. It includes both chemical and molecular target knowledge. The prediction scheme is classified into different levels of toxicity such as oral toxicity as well as organ toxicity (hepatotoxicity). ProTox-II incorporates molecular similarity and pharmacophore based fragment propensities (Banerjee et al. 2018b).

Table 2. The values of the calculated molecular descriptors of tested compounds **6a–i**.

Comp.	Molweight	Num. of hydrogen bond acceptors	Num. of hydrogen bond donors	Num. of atoms	Num. of bonds	Num. of rings	Num. of rotable bonds	Total charge	Molecular Polar Surface Area
Cff	194.19	3	0	14	15	2	0	0	61.82
3	294.37	3	0	20	21	2	3	0	90.28
4	312.34	5	0	21	22	2	4	0	113.42
5	312.35	6	0	21	22	2	4	0	142.24
6a	414.48	6	0	29	31	3	6	0	128.58
6b	428.51	6	0	30	32	3	6	0	128.58
6c	444.51	7	0	31	33	3	7	0	137.81
6d	430.48	7	0	30	32	3	6	0	148.81
6e	428.51	6	0	30	32	3	7	0	128.58
6f	444.51	7	0	31	33	3	7	0	148.81
6g	442.53	6	0	31	33	3	8	0	128.58
6h	506.58	7	0	36	39	4	8	0	137.81
6i	442.53	6	0	31	33	3	7	0	128.58

Table 3. Results of toxicity prediction with ProTox-II webserver.

Comp.	Hepatotoxicity prediction	Probability	Predicted LD_{50} [mg/kg b.w.]	Predicted toxicity class
Cff	Inactive	0.97	127	3
3	Active	0.54	910	4
4	Inactive	0.65	196	3
5	Active	0.50	550	3
6a	Inactive	0.56	550	4
6b	Inactive	0.56	550	4
6c	Inactive	0.53	150	3
6d	Inactive	0.52	150	3
6e	Inactive	0.55	660	4
6f	Inactive	0.51	150	3
6g	Inactive	0.54	790	4
6h	Inactive	0.53	3000	5
6i	Inactive	0.56	660	4

An MDDRlike rule published by Oprea et al. (Oprea 2000; Oprea et al. 2000) was applied for drug-likeness evaluation of the newly synthesized compounds. The values of the calculated descriptors (Table 2) indicate that all compounds can be defined as "drug-like" as they fulfill the criteria of drug-likeness which includes the MDRP filter. MDDR-like rule (Oprea 2000; Oprea et al. 2000) is using as molecular descriptors in the means of number of rings, number of rigid bonds and number of rotatable bonds, whit the probability of finding a 'druglike' compound being higher in the ranges of No. Rings ≥ 3, No. Rigid bonds ≥ 18, No. Rotatable bonds ≥ 6, while the probability of finding a 'nondrug-like' compound being higher in the ranges of No.Rings ≤ 2, No. Rigid bonds ≤ 17, No. Rotatable bonds ≤ 5.

Drug-induced hepatotoxicity is a significant cause of acute liver failure and one of the major reasons for the withdrawal of drugs from the market. Drug-induced liver injury (DILI) is either a chronic process or a rare event. However, prediction of DILI is important and one of the safety concerns for the drug developers, regulators and clinicians (Liu et al. 2015). The data for the prediction of DILI are taken from DILIrank (Chen et al. 2016) and the NIH LiverTox database (Thakkar et al. 2018). The ProTox-II hepatotoxicity prediction model has a balanced accuracy of 82.00% on cross validation and 86.00% on external validation. The AUC– ROC scores of cross-validation and external validation are 0.86 and 0.91 respectively. The kappa value is 0.69 for the model (Banerjee et al. 2018b). The model applied to predict hepatotoxicity is described in detail in the literature (Banerjee et al. 2018a).

The results of prediction are shown in Table 3. All the compounds appeared to be low toxic belonging to the category III and IV of GHS (Globally Harmonized System of Classification and Labeling of Chemicals) categories ("GHS-unece").

Prediction of substrate/metabolite specificity

The evaluated set of compounds was subjected to a prediction of substrate/metabolite specificity, using an SMP web-service (Bezhentsev et al. 2016) for *in silico* prediction of the substrate/metabolite specificity. This service provides the possible interaction of the tested group of compounds with 18 cytochrome P450 and UGT isoforms: CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, UGT1A10, UGT1A1, UGT2B7, UGT1A7, UGT2B15, UGT1A8, UGT1A4, UGT2B17, UGT2B10, UGT1A3, UGT1A9, UGT1A6, UGT2B4; defined as the most probable targets. The performed prediction is based on the PASS algorithm and is defined in the software Multilevel Neighborhoods of Atoms (MNA) descriptors. The obtained data

Table 4. "Probability to be Active" (Pa) values for the substrate based prediction result of **6a–i**, **3, 4, 5** and **Caffeine**.

Cmpnd	1A2	2A6	2E1	2C8
Cff	0.912	0.942	0.921	0.770
3	0.631	0.654	0.693	0.772
4	0.650	0.714	0.701	0.781
5				0.526
6a	0.672	0.697	0.687	0.594
6b	0.635	0.732	0.729	0.606
6c	0.679	0.705	0.695	0.629
6d	0.667		0.521	0.539
6e	0.754	0.794	0.788	0.673
6f	0.798	0.621	0.688	0.546
6g	0.822	0.858	0.839	0.623
6h	0.636	0.657	0.659	0.704
6i	0.721	0.764	0.765	0.712

Table 5. "Probability to be Active" (Pa) values for the metabolite based prediction result of **6a–i**, **3, 4, 5** and **Caffeine**.

Cmpnd	1A2	2E1	3A4
Cff	0.944	0.858	
3	0.677	0.677	
4	0.833		0.504
5	0.735	0.520	
6a	0.808	0.568	0.554
6b	0.818	0.557	0.544
6c	0.855		0.539
6d	0.667	0.583	
6e	0.859	0.686	
6f	0.781	0.742	
6g	0.903	0.762	0.515
6h	0.833		
6i	0.841	0.637	

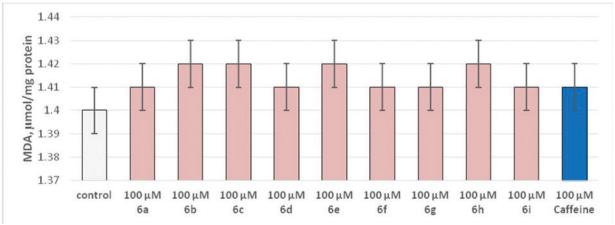


Figure 2. Effect of the hydrazid-hydrazones **6a–i**, administered alone at concentration 100 µM, on isolated rat liver microsomes.

for the substrate based predictions are presented in Table 4 for compounds **6a–i**, **3, 4, 5** and **Caffeine**. The results for the corresponding metabolite based predictions for the same set of structures are presented in Table 5. Based on the obtained data we consider the tested compounds to perform most probably CYP4501A2 substrate activity.

In vitro evaluation of effect on isolated rat liver microsomes

It is a well known fact that Caffeine is metabolized by CYP1A2. It is of interest to establish whether during their metabolism the the newly synthesized caffeine derivatives **6a-i** would be biotransformed into reactive metabolites and by this to reveal possible pro-oxidative properties.

Thus we decided to evaluate their effect on isolated rat liver microsomes, as an *in vitro* system, which contains most of the isoforms of the cytochrome P450 system.

Administered alone, all of the tested compounds didn't reveal statistically significant pro-oxidant effects on isolated rat liver microsomes (Figure 2). Caffeine (as positive control) also didn't induce lipid peroxidation in statistically significant manner on isolated microsomes.

4. Conclusion

The present study describes the synthesis of series new caffeine-8-(2-thio)-propanoic hydrazid-hydrazone derivatives which are subjected to *in silico* prediction of substrate/metabolite specificity. Based on the obtained data we consider the tested compounds to perform most probably as CYP1A2 substrates. The newly synthesized hydrazid-hydrazones were also subjected to *in silico* prediction of the Drug Induced Liver Injury (DILI). The prediction for all compounds indicates that they would not show hepatotoxicity. The carried out *in vitro* studies confirmed that the tested compounds didn't reveal statistically significant hepatotoxicity, due to pro-oxidant effects, on sub-cellular (isolated rat liver microsomes) level. These results underlined the tested molecules as promising structures for design of future compounds with low hepatotoxicity.

References

Acheson KJ, Gremaud G, Meirim I, Montigon F, Krebs Y, Fay LB, Gay L-J, Schneiter P, Schindler C, Tappy L (2004) Metabolic effects of caffeine in humans: lipid oxidation or futile cycling? American Journal of Clinical Nutrition 79. https://doi.org/10.1093/ajcn/79.1.40

Al Deeb SA, AlMoutaery KA, Arshaduddin M, Biary N, Tariq M (2002) Effect of acute caffeine on severity of harmaline induced tremor in rats. Neuroscience Letter 325: 219–218. https://doi.org/10.1016/S0304-3940(02)00042-3

Alsabri S, Mari W, Younes S, Alsadawi M, Oroszi T (2018) Kinetic and dynamic description of caffeine. Journal of Caffeine and Adenosine Research 8: 3–9. https://doi.org/10.1089/caff.2017.0011

Banerjee B, Dehnbostel FO, Preisner R (2018a) Prediction is a balancing act: importance of sampling methods to balance sensitivity and specificity of predictive models based on imbalanced chemical data sets. Frontier in Chemistry 6: 1–11. https://doi.org/10.3389/fchem.2018.00362

Banerjee P, Eckert AO, Schrey AK, Preissner R (2018b) ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Acids Research 46: W257–W263. https://doi.org/10.1093/nar/gky318

Barcelos R, Souza M, Amaral G, Stefanello S, Bresciani G, Fighera M, Soares F, Barbosa N (2014) Caffeine supplementation modulates oxidative stress markers in the liver of trained rats. Life Sciences 96: 40–45. https://doi.org/10.1016/j.lfs.2013.12.002

Berthou F, Guillois B, Riche C, Dreano Y, Jacqz-Aigrain E, Beaune PH (1992) variations in caffeine metabolism related to cytochrome P4501A enzymes. Xenobiotica 22: 671–680. https://doi.org/10.3109/00498259209053129

Bezhentsev VM, Tarasova OA, Dmitriev AV, Rudik AV, Lagunin AA, Filimonov DA, Poroikov VV (2016) Computer-aided prediction of xenobiotics metabolism in the human organism. Russ Chem Rev 85: 854–879. https://doi.org/10.1070/RCR4614

Bharanidharan S, Saleem H, Subashchandrabose S, Suresh M, Ramesh Babu N (2017) FT-IR, FT-Raman and UV-Visible Spectral Analysis on (E)-N'-(thiophen-2-ylmethylene) Nicotinohydrazide. Archives in Chemical Research 1: 1–14. https://doi.org/10.21767/2572-4657.100007

Bloomer JC, Clarke SE, Chenery RJ (1995) Determination of P4501A2 activity in human liver microsomes using [3-'4C-methyl]caffeine. Xenobiotica 25: 917–927. https://doi.org/10.3109/00498259509046663

Chen M, Suzuki A, Thakkar S, Yu K, Hu C, Tong W (2016) DILIrank: The largest reference drug list ranked by the risk for developing drug-induced liver injury in humans. Drug Discovery Today 21: 648–653. https://doi.org/10.1016/j.drudis.2016.02.015

Deby C, Goutier R (1990) New perspectives on the biochemistry of superoxide anion and the efficiency of SOD. Biochem Pharmacol 39: 399–405. https://doi.org/10.1016/0006-2952(90)90043-K

Devasagayam TP, Kamat JP, Mohan H, Kesavan PC (1996) Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. Biochimica et biophysica acta 1282: 63–70. https://doi.org/10.1016/0005-2736(96)00040-5

Dianzani MU, Muzio G, Biocca ME, Canuto RA (1991) Lipid peroxidation in fatty liver induced by caffeine in rats. International Journal of Tissue reactions 13: 79–85.

Franco R, Onatibia-Astibia A, Martinez-Pinilla E (2013) Health benefits of methylxanthines in cacao and chocolate. Nutrients 5: 4159–4173. https://doi.org/10.3390/nu5104159

GHS-unece (2019) GHS-unece. http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html [accessed January 29.2019]

Guengerich FP (1989) Analysis and characterizaton of enzymes. In: Hayes AW (Ed.) Principles and methods of toxicology. New York Raven Press, 777–814.

Gulaczyk I, Krezglewski M, Valentin A (2003) The N–N stretching band of hydrazine. Journal of Molecular Spectroscopy 220: 132–136. https://doi.org/10.1016/S0022-2852(03)00106-1

Gulcin I (2008) In vitro prooxidant effect of caffeine. Journal of Enzyme Inhibition and Medicinal Chemistry 23: 149–152. https://doi.org/10.1080/14756360701306404

Hetzler RK, Knowlton RG, Somani SM, Brown DD, Perkins RM (1990) Effect of paraxanthine on FFA mobilization after intravenous caffeine administration in humans. Journal of Applied Physiology 68: 44–47. https://doi.org/10.1152/jappl.1990.68.1.44

Hristova-Avakumova N, Yoncheva K, Nikolova-Mladenova B, Traykov T, Momekov G, Hadjimitova V (2017) 3-Methoxy aroylhydrazones – free radicals scavenging, anticancer and cytoprotective potency. Redox Report 22: 408–417. https://doi.org/10.1080/13510002.2016.1276256

Kolayli S, Ocak M, Kucuk M, Abbasoglu R (2004) Does caff eine bind to metal ions? Food Chemistry 84: 383–388. https://doi.org/10.1016/S0308-8146(03)00244-9

Kot M, Daniel WA (2008a) The relative contribution of human cytochrome P450 isoforms to the four caffeine oxidation pathways: an in vitro comparative study with cDNA-expressed P450s including CYP2C isoforms. Biochemical Pharmacolology 76: 543–551. https://doi.org/10.1016/j.bcp.2008.05.025

Kot M, Daniel WA (2008b) Relative contribution of rat cytochrome P450 isoforms to the metabolism of caffeine: the pathway and concentration dependence. Biochemical Pharmacolology 75: 1538–1549. https://doi.org/10.1016/j.bcp.2007.12.017

Liu J, Mansouri K, Judson RS, Martin MT, Hong H, Chen M, Xu X, Thomas RS, Shah I (2015) Predicting hepatotoxicity using ToxCast in vitro bioactivity and chemical structure. Chemical Research in Toxicology 28: 738–751. https://doi.org/10.1021/tx500501h

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193: 265–275.

Metro D, Cernano V, Santoro D, Papa M, Buemi M, Benvenga S, Manasseri L (2017) Beneficial effects of oral pure caffeine on oxidative stress. Journal of Clinical & Translational Endocrinology 10: 22–27. https://doi.org/10.1016/j.jcte.2017.10.001

Mitkov J, Georgieva M, Zlatkov A (2012) Development of an optimized synthetic approach for synthesis of caffeine-8-thioglycolic acid and its ester derivatives. Pharmacia 59: 17–23.

Mitkov J, Kasabova-Angelova A, Kondeva-Burdina M, Tzankova V, Tzankova D, Georgieva M, Zlatkov A (2019) Design, Synthesis and Evaluation of 8-Thiosubstituted 1,3,7-Trimethylxanthine Hydrazones with In Vitro Neuroprotective and MAO-B Inhibitory Activities. Medicinal Chemistry 15. https://doi.org/10.2174/1573406415666190531121927

Nikolaevskii AN, Kniga OP, Khizhan EI, Tikhonova GA, Vinogradov VV, Khizhan AI (2012) Antioxidant activity of hydrazones with sterically hindered phenol fragments. Russian Journal of Physical Chemistry A 86: 1816–1820. https://doi.org/10.1134/S0036024412120205

Oprea TI (2000) Property distribution of drug-related chemical databases. Journal of Computer-Aided Molecular Design 18: 251–264. https://doi.org/10.1023/A:1008130001697

Oprea TI, Gottfries J, Sherbukhin V, Svensson P, Kiihler TC (2000) Chemical information management in drug discovery: Optimizing the computational and combinatorial chemistry interfaces. Journal of Molecular Graphics and Modelling 18: 512–524. https://doi.org/10.1016/S1093-3263(00)00066-8

Pasaoglu H, Demir FEO, Demirtas CY, Hussein A, Pasaoglu OT (2011) Th e eff ect of caff eine on oxidative stress in liver and heart tissues of rats. Turkish Journal Of Medical Sciences 41: 665–671.

ProTox-II (2019) ProTox-II. http://tox.charite.de/protox_II [accessed January 28.2019]

Sarıgöl D, Yüksel D, Okay G, Uzgören-Baran A (2015) Synthesis and structural studies of acyl hydrazone derivatives having tetrahydrocarbazole moiety. Journal of Molecular Structure 1086: 146–152. https://doi.org/10.1016/j.molstruc.2014.12.092

Shi X, Dalal NS, Jain AC (1991) Antoxidant behaviour of caffeine: efficient scavenging of hydroxyl radicals. Food and Chemical Toxicology 29: 1–6. https://doi.org/10.1016/0278-6915(91)90056-D

Thakkar S, Chen M, Fang H, Liu Z, Roberts R, Tong W (2018) The Liver Toxicity Knowledge Base (LKTB) and drug-induced liver injury (DILI) classification for assessment of human liver injury. Expert Review of Gastroenterology and Hepatology 12: 31–38. https://doi.org/10.1080/17474124.2018.1383154

Welsh E, Bara A, Barley E, Cates CJ (2012) Caffeine for asthma. John Wiley & Sons, Ltd. https://doi.org/10.1002/14651858.CD001112.pub2