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Antioxidant properties of thio-caffeine derivatives: Identification of the newly synthesized 8-[(pyrrolidin-1-ylcarbonothioyl)sulfanyl] caffeine as antioxidant and highly potent cytoprotective agent

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ABSTRACT

A series of nine thio-caffeine analogues were synthesized and characterised by NMR, FT-IR and MS spectroscopic methods. Molecular structures of four of them were determined using single crystal X-ray diffraction methods. The antioxidant properties of all compounds, at concentration ranges from 0.025 to 0.1 mg/mL, were evaluated by various chemical- and cell-based antioxidant assays. Human erythrocytes were used to examine in vitro haemolytic activity of all compounds and their protective effect against oxidative haemolysis induced by AAPH, one of the commonly used free radical generator. All compounds studied showed no effect on the human erythrocytes membrane structure and permeability with the exception of 8-(phenylsulfanyl)caffeine. Among the nine caffeine thio-analogues tested, the newly synthesized 8-[(pyrrolidin-1-ylcarbonothioyl)sulfanyl]caffeine possessed exceptionally high antioxidant properties. Moreover, it protects human erythrocytes against AAPH-induced oxidative damage as efficiently as the standard antioxidant Trolox. Therefore, 8-[(pyrrolidin-1-ylcarbonothioyl)sulfanyl]caffeine may have a significant cytoprotective potential caused by its antioxidant activity.

Caffeine (**1**), the naturally occurring methylxanthine, is of an unquestionable interest as a leading compound for the development of new derivatives with enhanced activities and/or lower toxicities. It modulates drugs used for curing lung, liver, uterine cervix and breast cancer and enhances gastric secretion and urine production, reduces the risk of developing gallstone disease and also reduces asthma. The anticarcinogenic effect of caffeine has been related to its effect on cell cycle and proliferation.[1] Caffeine easy penetrates through biological membrane[2] and activates the erythrocyte glutathione-*S*-transferase (GST) which is involved in erythrocytes protection.[3] It has been shown that caffeine inhibits eryptosis (suicidal death) of human erythrocytes within the range of its plasma concentration (100 μM)[4] and prevents the accelerated clearance of erythrocytes from circulation and development of anemia. On the other hand, the antiapoptotic effect of caffeine on nucleated cells could be explained by inhibition of the phosphodiesterase and c-AMP production,[5] its inhibitory action on ATM kinase and suppression of p53 phosforylation[6] or suppression of amyloid β-induced caspase-3 activity in neurons.[7] According to Kesavan and co-workers[8–11] caffeine has abilities to scavenge highly reactive free radicals and excited states of oxygen. Antioxidant ability of caffeine is similar to that of the established biological antioxidant glutathione and significantly much higher than that of ascorbic acid. However, other studies demonstrate an absence of antioxidant properties of caffeine using DPPH assay[12–14] or even its pro-oxidant effects.[15,16] Studies have shown that C8-substituted caffeine derivatives are adenosine receptor antagonists,[17–19] acetylcholinesterase inhibitors[20] and monoamine oxidase inhibitors, and that within this group the 8-thiocaffeine analogues belong to a pharmacologically important subclass.[21–24] In general, nearly all organosulfur compounds are considered as antioxidants. Compounds such as allicin, methionine and methylcysteine protect against metal-mediated oxidative DNA damage. Moreover, the results of epidemiological, clinical, in vivo, and in vitro studies have undoubtedly shown the protective effects of sulfur compounds against cellular damage and disease.[25] It therefore came as a surprise to us that although the antioxidant activity of spent coffee extracts rich in caffeine[26] and caffeine alone[27,28] has been extensively examined, similar studies devoted to the thio-caffeine derivatives have not been so farreported. Notably, the

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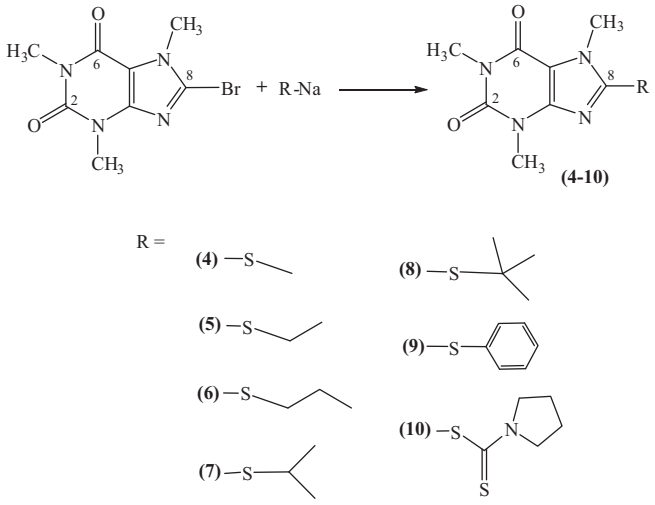
biochemistry of dithiocarbamates is of interest because of their clinical use.[29] Their biological properties include ability to influence oxidative stress, apoptosis and enzyme inhibition.[30–33] Moreover, pyrrolidinedithiocarbamate is widely used as an inhibitor of nuclear factor kappa B(NFkB) and this, or related compounds may have therapeutic potential in inhibiting artheriosclerosis.[34] Relying on the above reports, we expected that there will be some advantage, in terms of biological activity, resulting from a synergism between the biological actions of the caffeine and its sulfur derivatives. We have therefore synthesized and characterised a series of nine structurally diverse thio-caffeine analogues, that included, 6-thiocaffeine, 2,6-dithiocaffeine, 8-thioalkyl derivatives, 8-(phenylsulfanyl)-caffeine and the newly synthesized 8-[(pyrrolidin-1-ylcarbonothioyl)sulfanyl]caffeine, and explored their antioxidant activity as well as their effects on human erythrocytes in vitro. Human red blood cells (RBCs) are widely used in the investigation of antioxidant activity of natural and newly obtained compounds because there are the main targets for free radicals in the circulatory system.[35–38] Although RBCs contain enzymes that are involved in defence against free radicals, namely catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GRd) and glutathione peroxidase (GPx)[39] they are not able to effectively eliminate reactive oxygen species and as a result oxidative haemolysis occurs. The water-soluble free radical generator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) is commonly used for inducing RBCs membrane injury and oxidative haemolysis in vitro. Thus, in the present study, AAPH was employed to examine the oxidative haemolysis in the presence or absence of the thiocaffeine derivatives as well as the standard antioxidants, namely Trolox and butylated hydroxytoluene (BHT).

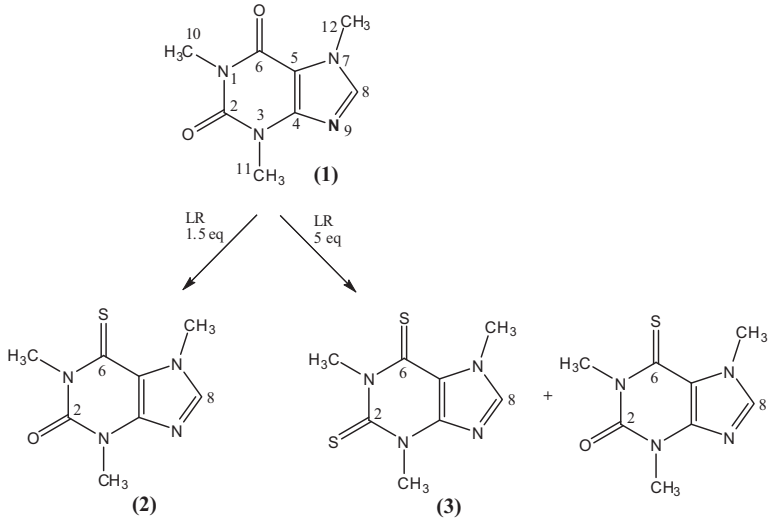
The 6-thiocaffeine (**2**) and 2,6-dithiocaffeine (**3**) were synthesized by reaction of caffeine with Lawesson's reagent in toluene (Scheme 1). Reaction of 8-bromocaffeine with an appropriate sodium thiolate reagent in ethanol solution gives the C8 thiocaffeine analogues (**4**–**10**).[40] The synthetic routes of these target compounds are outlined in Scheme 2. All obtained compounds were structurally (^{1}H NMR, ^{13}C NMR, FTIR, ESI-MS) characterised (see Supplementary materials). For four of them the crystal structures were determined.

The most noticeable differences in the NMR spectra were the downfield shifts of signals corresponding to positions C5, C6 in 6-thio- or C2, C5 and C6 in 2,6-dithiocaffeine, as compared with caffeine. The IR spectrum of **2** showed an absorption band near



Scheme 2. Reaction and conditions: 1 equiv 8-bromocaffeine, 4 equiv sodium thiolate, ethanol, reflux, 2–48 h. Time for completion of the reaction at reflux as indicated by TLC.

1675 cm^{-1} associated to the carbonyl group and absorption band near 1110 cm^{-1} associated to the thiocarbonyl group, whereas in the spectrum of **3** we can observed absorption bands of two thiocarbonyl groups at 1110 and 1070 cm^{-1}. The IR spectrum of compound **5** as representative of the series (**4**–**8**), showed an absorption band at 1319 and 1037 cm^{-1} associated to the thioalkyl group and absorption bands at 1702 and 1656 cm^{-1} associated to the carbonyl groups of the caffeine fragment. The IR spectrum of compound **9** showed an absorption band at 3060 and 1440–1578 cm^{-1} associated to the aromatic ring, whereas in the IR spectrum of **10** the bands in 1400–1100 cm^{-1} region are associated with N—C═S—S stretching vibrations. In the ^{1}H NMR spectra of compounds **4**–**10**, three singlets in the range of 3.37–3.97 ppm indicated the presence of the three methyl groups from caffeine unit. Aromatic protons of compound **9** are present in the range of 7.60–7.30 ppm, while signals at 3.61 and 1.90 ppm present in the spectra of **10** are connected with presence of pyrrolidine ring. The ^{13}C NMR spectrum of compound **9** showed signals at 132–127 ppm corresponding to the phenyl ring. Signal at about



Scheme 1. Reaction and conditions: Caffeine (1), 1.5 or 5 equiv LW (Lawesson's reagent), toluene, reflux at 25 h. Time for completion of the reaction at reflux as indicated by TLC.