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Thioamides as radical scavenging compounds: Methods for screening antioxidant activity and detection

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ABSTRACT

Heteroaromatic thiols and thiones attracted the attention of chemists, pharmacologists and biochemists because of participation in the interception of free radicals. For the first time offered independent and reliable methods for evaluating of the antioxidant activity of thioamides-derivatives of pyridine, quinoline, imidazole, triazole, tetrazole, pyrimidine, pyrrolidine and 7-mercapto-4-methylcoumarin -based on kinetic parameters of the thioamide reaction with chromogenic radical (rate constant, M^{-1} min^{-1} and time to decrease concentration of test free radical by 50%, T_{EC50}, min) or thermodynamics of the thioamides reaction with molecular iodine (extent of thioamide conversion, %).

To compare the antioxidant activity of thioamides and widely used standard-antioxidant Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) we have proposed to use a value of relative antioxidant activity constant.

As it was established, the kinetics of interaction between the chromogenic radical and thioamides in the presence of an excess of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) is described by the kinetics of the pseudo first order with respect to the reacting components. A kinetic-spectrophotometric method for the quantification of heteroaromatic thioamides is elaborated and was tested in the analysis of urine. Thioamides were detected at concentrations of 1.53 μg ml^{-1}, RSD=4.6% (2-mercaptoimidazole, V), 2.08 μg ml^{-1}, RSD=1.8% (1-methylimidazoline-2-thione, VI), 1.45 μg ml^{-1}, RSD=4.3% (2-mercaptopyridine, IX).The proposed procedures show good precision and accuracy of the results obtained.

1. Introduction

Antioxidants are substances that can interrupt branched chain of oxidation. In comprehensive scientific literature antioxidants cover a wide range of synthetic and natural compounds, which contains a labile hydrogen atom and can react with active free radical R:

$R^{\bullet} + Het{-}SH \rightarrow RH + Het{-}S^{\bullet}$ $\quad$ $2Het{-}S^{\bullet} \rightarrow Het{-}S{-}S{-}Het$

An important distinction can be made between short and long term antioxidant protection. This is related to the reaction kinetics and the rates at which an antioxidant reacts with a specific radical versus the thermodynamics of the reaction and how completely the antioxidant reacts [1–3].

The analytical strategy for assessing antioxidant activity include: measurement at a fixed time point, measurement of reaction rate, lag phase measurement and integrated rate measurement (which is used where the reaction kinetics are not of a simple order).

In a number of scientific publications [4,5], the role of heteroaromatic thioamides as thyrostatic agents was pointed out; they interact with active species of iodine and play the role of antioxidants. This prophylactic antioxidant protection is attained with the system of the hormonal regulation of the synthesis of thyroid hormones, which excessive concentrations lead to oxidative stress.

It was shown [6,7] that the mechanism of thioamides reaction with molecular iodine (the formation of disulfide, product of desulfurization or iodonium salt) depends on the electron-releasing properties of the thione molecule determined by the nature of heteroaromatic moiety and the solvent used. For example, the molecular structure of oxidation product of pyrrolidine-2-thione by molecular iodine is formed by 5-(2-thioxopyrrolidine-1-yl)-3,4-dihydro-2H-pyrrolium ($C_8H_{13}N_2S^+$) cations and pentaiodide anions I_5^- (Scheme 1) [8].

As used in medical practice, 1-methyl-imidazolin-2-thione exhibiting antithyroidal activity against hyperthyroidism (Graves' disease) is oxidized by the TPO-I system to form disulfide [5]. 5-Trifluoromethyl-pyridine-2-thione forms the iodonium salt through the formation of the three-center 10-electron molecular orbital by the linear $S{-}I^+{-}S$ linker [9]. The redox process involves

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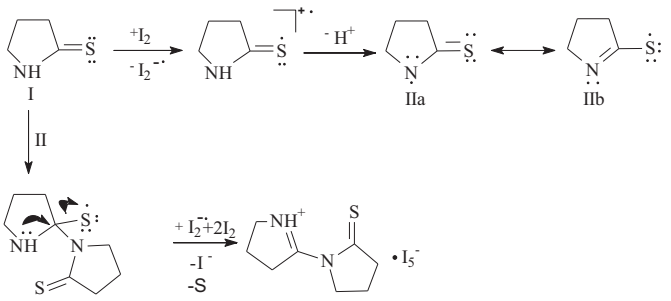

Scheme 1

the formation of a di- and a monocationic disulfide species of methimazol prior to the full transformation to disulfide [10,11]. Hence, knowledge of the oxidation mechanisms of thioamides is important for understanding of their antioxidant activity at the molecular level.

The inhibition activity of the thioamide ligands (derivatives of benzothiazole, thiazolidine, pyridine, pyrimidine, thiouracil, imidazole and benzimidazole) against the catalytic oxidation of iodides by H_2O_2 in the presence of ferric tetra-phenyl-porphyrin chloride was measured as a result of the yield of I_3^- resulting from the oxidation of I^- and was also evaluated theoretically using electronic structure calculation methods (DFT). The kinetic study of inhibition of lactoperoxidase (a model of Thyroid Peroxidase) is based on the assessment of LPO activity in the presence of its inhibitors – antioxidants of the thioamide series. The thioamides lose labile hydrogen atom when interacting with molecular iodine, converting to free radical species that dimerize [12].

The kinetic and thermodynamic parameters of the hydrogen atom transfer from a donor to an acceptor (radical R)˙ are vastly presented in the scientific literature [13,14]. The values of free energies and constants of hydrogen transfer and their relation to the protolytic constants in the system of heteroaromatic thione-solvent were considered in [15].

The measurement of antioxidant activity of certain compounds *in vitro* requires the definition of free radical which is formed. Expression of antioxidant activity appears to be dependant on the methods of measurement. These, in turn, include direct or indirect measurements of the rate or extent of substrate decay; determination of the volume of absorbed oxygen; concentration of formed or decayed probe free radicals; study of oxidation products formed.

The 2,2′-diphenyl-1-picrylhydrazyl radical is often used in the evaluation of the general radical scavenging abilities of antioxidant. The stable free radical absorbs at 517 nm in a substrate-free system and antioxidant activity can be readily determined by monitoring the decrease in this absorbance [16].

The works [17,18] report for the first time the total antioxidant capacity (TAC) assay of various biothiols (such as glutathione, cysteine and homocysteine) using CUPric Reducing Antioxidant Capacity (CUPRAC) and other spectrophotometric methods. Trolox was used as a control standard.

Thiols are essential in various biological processes, especially for maintenance of reductive environments through scavenging intracellular reactive oxygen species during oxidative stress, since unbalanced environments may cause damage to cells and induce undesired cell death [19].

The aim of this work was to suggest the ways to evaluate the antioxidant activity of thioamides based on reliable and precise kinetic parameters of the thioamide reaction with chromogenic radical (rate constant and time to decrease concentration of test free radical by 50%, T_{EC50}) and the thermodynamics of the reaction with molecular iodine (extent of reaction conversion, ω, %). The aim also is to elaborate a kinetic-spectrophotometric method for quantification of the heteroaromatic thioamides.

2. Experimental

2.1. Chemicals and instruments

The following chemical substances of analytical reagent grade were supplied from the corresponding sources:7-mercapto-4-methylcoumarine (≥ 97%), 1-methyltetrazoline-5-thione (98%), 5-(trifluoromethyl)-pyridine-2-thione (97%), 3,4,5,6-terahydro-2-pyrimidinethiol (≥ 99%), 2,2′-diphenyl-1-picrilhydrazil, 2-thiouracile (99%), quinoline-2-thione (97%) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (97%): Sigma-Aldrich; 2-mercaptoimidazole (> 98%), 1-methyl-imidazoline-2-thione (98%), 4-methyl-1,2,4-triazoline-3-thione (97%), 2-mercaptopyridine (99%), 2-thiobarbituric acid (99%) and pyrrolidine-2-thione (97%): Alfa Aesar; dithiouracil (95%)-Acros Organics; 8-mercaptoquinolinate Na (II) (pure for analysis) was purchased from Chemical Line.

The absorption spectra were recorded in quartz cuvettes with a length of 1.0 cm using a Varian Cary 50 spectrophotometer.

An electrode system consisting of a glass electrode and a reference electrode with liquid junction, connected to a digital pH-meter with multi-point calibration, was used for determination of pK_a thioamides.

For evaluation the extent of thioamides reaction conversion (ω) was used ionomer in voltmeter mode with platinum indicator electrode and a reference electrode with liquid junction.

2.2. Sample preparation

For the DPPH test of thioamides the stock solutions of substances (I–XIV) with the concentration 4.0×10^{-4} M and of 2,2′-diphenyl-1-picrylhydrazyl with the concentration 4.0×10^{-4} M were prepared by dissolving accurately weights in ethanol (with an additive of acetic acid (0.04 vol %)), acetonitrile and carbon tetrachloride respectively. In order to estimate second order rate constants, solutions with isomolar concentrations of the reaction components with 8.0×10^{-5} M were used.

For determination of the pK_a value a stock potassium hydroxide solution (about 0.1 mol l^{-1}) was prepared from potassium hydroxide standard titre, and was standardized by titration with H_2SO_4 solution.

For evaluation the extent of thioamides reaction conversion a stock thiosulfate solution (~0.1 mol l^{-1}) was prepared from sodium thiosulfate standard titre, and was standardized by iodometric titration. The iodine stock solutions (about 0.025 mol l^{-1}) were standardized by titration with sodium thiosulfate.

All standard solutions were prepared using deionized water.

2.3. Determination of pK_a thioamides

pK_a values were obtained from the data of pH titration of a 25.0 mL $8.00 \cdot 10^{-3}$ M of thioamide in water: ethanol (1:1) solution with standardized aqueous KOH. Mainstream equipment was used for pK_a measurement – a pH-meter with 0.04 pH unit resolution. A burette with 5 mL capacity was used for titration. Titration was carried out in a cell thermostated to 23 °C and using a magnetic stirrer. Ionic strength of solutions (μ) was 0.1 M (KCl). Titration was repeated triply.

2.4. Procedure for evaluation extent of thioamide reaction conversion

The extent of thioamides reaction conversion (ω) was determined by potentiometric titration of excess of iodine (0.75 mmol)