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Research paper

A comparative study of α-*N*-pyridyl thiosemicarbazones: Spectroscopic properties, solution stability and copper(II) complexation

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ABSTRACT

The effects of methyl substituents at different positions on the 2-formylpyridine thiosemicarbazone (FTSC) core structure on various physico-chemical properties were investigated. Proton dissociation processes, aqueous solution stability, isomer distribution in different solvents, fluorescence properties and lipophilic character of FTSC, pyridine-2-carboxaldehyde N^4,N^4-dimethylthiosemicarbazone (PTSC), 2-acetylpyridine thiosemicarbazone (AcFTSC) and 2-acetylpyridine N^4,N^4-dimethylthiosemicarbazone (AcPTSC) were studied and compared under the same conditions. There are more and more indications that Cu(II) ions play an important role in the biological activity of anticancer thiosemicarbazones. Therefore, the complex formation equilibria of FTSC with Cu(II) ions were studied by pH-potentiometry, UV–visible spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy to determine stoichiometry, stability constants and solution structures of the complexes formed in aqueous solution (with 30% DMSO). Mono-ligand complexes in different protonation states were identified such as $[CuLH]^{2+}$, $[CuL]^{+}$ and [CuL(OH)] with ($N_{pyridyl}$,N,S)(H_2O), ($N_{pyridyl}$,N,S^-)(H_2O) and ($N_{pyridyl}$,N,S^-)(OH) coordination modes, respectively. At ligand excess two kinds of isomers of a bis complex $[CuL_2]$ were detected at pH > 7, in which binding of the ligands via ($N_{pyridyl}$,N,S^-)(N) and ($N_{pyridyl}$,N,S^-)(S^-) donor sets is probable at the equatorial positions. Based on the stability data, $[CuL]^{+}$ complexes of the α-*N*-pyridyl thiosemicarbazones are predominant at pH 7.4 at 1:1 metal-to-ligand ratio possessing such high solution stability that their decomposition is not likely even at biologically relevant micromolar concentrations. In addition, FTSC and all methylated derivatives investigated show similar Cu(II) binding abilities which is in contrast to the respective Fe(II)/(III) complexes where terminal dimethylation distinctly increases the solution stabilities.

1. Introduction

Thiosemicarbazones (TSCs) are versatile compounds regarding their structures, metal binding abilities and pharmacological properties including anticancer activity [1–3]. Among the TSCs the most studied representative is Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP) which has already been evaluated in several clinical phase I and II trials [4–6]. Two novel promising TSCs, namely COTI-2 (an orally available third generation TSC) and di-2-pyridylketone-4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC) have recently entered human clinical studies [7,8]. These compounds (Chart 1) belong to the family of α-*N*-pyridyl TSCs, thus share a common 2-formylpyridine thiosemicarbazone (FTSC, Chart 2) core unit possessing a tridentate ($N_{pyridyl}$,N,S) donor set.

Notably, FTSC was the first discovered representative of this class of compounds with *in vivo* antitumor activity [9]. Ribonucleotide reductase, an iron-containing enzyme catalyzing the rate-determining step in DNA synthesis, is considered as the main target for Triapine and related compounds due to their chelating properties [10–12]. The species being responsible for the inhibition of the enzyme are not the free ligands but their *in vivo* formed redox-active iron complexes [10–12].

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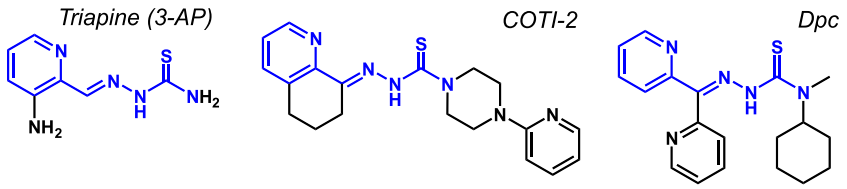
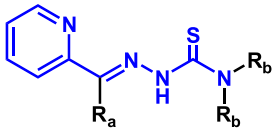


Chart 1. Chemical structures of thiosemicarbazones currently undergoing clinical trials.



	R_a	R_b
FTSC (FpT)	H	H
PTSC (Fp44mT)	H	CH_3
AcFTSC (HApT)	CH_3	H
AcPTSC (Ap44mT)	CH_3	CH_3

Chart 2. Chemical structures of studied thiosemicarbazones *(in brackets the abbreviations used by D.R. Richardson and coworkers are shown).*

In general, the coordination of α-*N*-pyridyl TSCs to certain metal ions [*e.g.* Cu(II), Pt(II), Pd(II), Ni(II)] can result in complexes with increased anticancer activity and different mechanisms of action [13]. Especially Cu(II) complexes of TSCs often show enhanced antitumor effect where it is generally assumed that their efficacy is frequently based on intracellular reductant-induced reactive oxygen species (ROS) formation [14–16]. Some compounds have been also reported to efficiently inhibit topoisomerase-II α [17,18]. Notably, TSCs which possess a *N*-terminal dimethylation often reveal a more than 100-fold enhanced anticancer activity *in vitro* [19,20]. In our recent work such highly cytotoxic derivatives also showed a very strong synergistic activity with Cu(II) accompanied by induction of ROS production and massive necrotic cell death [19]. This is also true for Richardson type complexes like di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT) or DpC where coordination to cellular Cu(II) seems to play an important role in the mode of action [21,22]. The latter examples reflect that the substituents on the TSC backbone can alter the physico-chemical properties and metal binding abilities which in turn has a strong impact on the bioactivity of the compounds. However, in order to investigate such structure-activity relationships the knowledge of the solution speciation and the most plausible chemical forms of TSCs and their metal complexes under physiological conditions is of primary importance. Characterizations of these compounds are often only performed in solid phase or in the organic solvents, but insufficient information is available in aqueous solution.

Herein we report on a comparative study of various derivatives of the simplest α-*N*-pyridyl TSC (FTSC) to gain insight into the effects of methyl substituents at different positions on various physico-chemical properties: proton dissociation processes, aqueous solution stability, isomer distribution in different solvents, fluorescence properties, lipophilicity and Cu(II) binding ability. As derivatives of FTSC the stepwise methylated pyridine-2-carboxaldehyde N^4,N^4-dimethylthiosemicarbazone (PTSC), 2-acetylpyridine thiosemicarbazone (AcFTSC or AcTSC) and 2-acetylpyridine N^4,N^4-dimethylthiosemicarbazone (AcPTSC) (Chart 2) were investigated and compared under the same conditions. Although several of these data have been already reported in literature, the comparability in most cases is insufficient due to the application of different settings and solvents. Solution chemistry of Cu(II) complexes formed with some α-*N*-pyridyl TSCs has been characterized in some of our former publications [23–25] and also by others research groups [26–30], however surprisingly no comparable stability data are available for the standard thiosemicarbazone compound FTSC. Therefore, stoichiometry, stability and structure of Cu(II) complexes of FTSC were determined in aqueous solution in this study *via* a combined approach using pH-potentiometry, UV–visible (UV–vis) spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy. This enabled now a well-founded comparison of the properties and Cu(II) binding affinities of different biologically active α-*N*-pyridyl thiosemicarbazones.

2. Experimental section

2.1. Chemicals

FTSC, PTSC, AcFTSC and AcPTSC were prepared as described previously [31,32]. 2-Acetylpyridine, 4,4-dimethyl-3-thiosemicarbazide (DMTS), KCl, NaH_2PO_4, Na_2HPO_4 HCl, KOH, 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), dimethyl sulfoxide (DMSO) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma-Aldrich in *puriss* quality. $CuCl_2$ stock solution was made by the dissolution of anhydrous $CuCl_2$ in water and its exact concentration was determined by complexometry through the EDTA complex. All solvents were of analytical grade and used without further purification. Doubly distilled Milli-Q water was used for sample preparation.

2.2. Potentiometric measurements and calculations

The pH-potentiometric measurements for the determination of the proton dissociation constants of FTSC and the overall stability constants of the Cu(II) complexes were carried out at 25.0 ± 0.1 °C in DMSO:water 30:70 (w/w) as solvent and at an ionic strength of 0.10 M (KCl) used in order to keep the activity coefficient constant. The titrations were performed with carbonate-free KOH solution of known concentration (0.10 M). The concentrations of the base and the HCl were determined by pH-potentiometric titrations. An Orion 710A pH-meter equipped with a Metrohm combined electrode (type 6.0234.100) and a Metrohm 665 Dosimat burette were used for the titrations. The electrode system was calibrated to the pH = $-\log[H^+]$ scale in the DMSO/water solvent mixture by means of blank titrations (strong acid *vs.* strong base: HCl *vs.* KOH), similarly to the method suggested by Irving *et al.* [33] in pure aqueous solutions. The average water ionization constant pK_w was 14.52 ± 0.05, which corresponds well to the literature data [23–25,34]. The reproducibility of the titration points included in the calculations was within 0.005 pH unit. The pH-metric titrations were performed in the pH range 2.0–12.5. The initial volume of the samples was 10.0 mL. The ligand concentration was 1 mM and metal ion-to-ligand ratios of 1:1–1:3 were used. The accepted fitting of the titration curves was always less than 0.01 mL. Samples were deoxygenated by bubbling purified argon through them for approximately 10 min prior to the measurements. Argon was also passed over the solutions during the titrations. The exact concentration of the ligand stock solutions together with the proton dissociation constants were determined by pH-potentiometric titrations with the use of the computer