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ScienceDirect
Journal of Inorganic Biochemistry 100 (2006) 1861–1870
Journal of Inorganic Biochemistry
www.elsevier.com/locate/jinorgbio

Synthesis, structural characterization and influence on the phagocytic activity of human neutrophils of thiazoline and thiazine derivative ligands and their zinc(II) complexes

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Received 22 March 2006; received in revised form 14 July 2006; accepted 14 July 2006
Available online 26 July 2006

Abstract

The zinc(II) complexes dichloro[2-(3,4-dichlorophenyl)imino-κ*N*-(2-thiazolin-κ*N*-2-yl)thiazolidine]zinc(II) (**1**) and dichloro[2-(3,4-dichlorophenyl)imino-κ*N*-(4*H*-5,6-dihydro-1,3-thiazin-κ*N*-2-yl)tetrahydrothiazine]zinc(II) (**2**) have been isolated and characterized in the solid state by X-ray diffraction, elemental analysis and IR spectra. In both complexes, the environment around the zinc(II) ion may be described as a distorted tetrahedral geometry, with the metallic atom coordinated to two chlorine atoms [Zn–Cl(1) = 2.218(1) Å; Zn–Cl(2) = 2.221(1) Å], one imino nitrogen [Zn–N(3) = 2.042(2) Å] and one thiazoline nitrogen [Zn–N(1) = 2.022(2) Å] in complex **1** and to two chlorine atoms [Zn–Cl(1) = 2.216(1) Å; Zn–Cl(2) = 2.192(1) Å], one imino nitrogen [Zn–N(3) = 2.045(2) Å] and one thiazine nitrogen [Zn–N(1) = 2.039(2) Å] in complex **2**. In addition, we also report in this study the crystal structure of the 2-(3,4-dichlorophenyl)imino-*N*-(2-thiazolin-2-yl)thiazolidine (TdTn) ligand as well as the synthesis and characterization by X-ray diffraction, ^{1}H and ^{13}C NMR spectra, elemental analysis, IR and electronic spectra of the 2-(3,4-dichlorophenyl)imino-*N*-(4*H*-5,6-dihydro-1,3-thiazin-2-yl)tetrahydrothiazine (TzTz) ligand. Besides, we study the phagocytic function in humans neutrophils treated with each complex and ligand aforementioned.

Keywords: Thiazine; Thiazoline; Thiazolidine; Zinc(II) complex; Phagocytosis

1. Introduction

Neutrophil polymorphonuclear leukocytes are responsible for the non-specific immune response (innate immune response) or the first line of defence of the host to inflammatory and infectious processes [1–3]. They play a determinant role during the initial stages of the immune response through the production of proteases and reactive oxygen intermediates [4]. These cells are capable of adhering to blood vessel walls, emigrating and afterwards reaching the damaged tissues by means of diapedesis (outward passage of blood cells through intact vessel walls) and chemotaxis (directional migration of cells along a chemical gradient). Once in the site of infection, these cells adhere to the germs, phagocytizing and destroying them internally, helped by the enzymes contained in their lysosomes [5].

It is known that antibiotics may also exhibit immunological effects, either inhibiting or stimulating immune response mechanisms [6–10]. This is the case of cefmetazole, cefoxitin and imipenem, β-lactam antibiotics with an unusually broad antibacterial spectrum, that stimulate neutrophils phagocytic capacity [11–14].

In addition, there are coordination compounds that interfere with in vitro phagocytosis, some of them inhibiting

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doi:10.1016/j.jinorgbio.2006.07.007

it, as it is the case of certain diclofenac transition metal complexes [15], and others improving it, like oxoplatinum and cycloplatam [16].

On the other hand, thiazolines, thiazolidines and thiazines are heterocycles present in a great number of substances with biological properties, many of which are antibiotics. Thus, thiazolines are important building blocks in pharmaceutical agents and biologically active natural products with antibiotic properties, like micacocidin [17,18]. Besides, the other five-membered heterocycle present in this work, thiazolidine ring, is a constituent part of penicillins framework [19]. In the same way, 1,3-thiazine ring systems are contained in cephalosporins frame [20], a β-lactam antibiotic family that bases its action mechanism on the inhibition of the bacterial cell wall synthesis [21], in the same way as penicillins, belonging also to this antibiotic family.

We focus on zinc(II) complexes because this metal is one of the most important trace elements and plays a versatile role in biological systems, performing both catalytic and structural functions in many enzymes [22,23]. Besides, it is known that zinc plays an essential role in immune system, exerting a direct stimulatory effect on immune cells upon DNA metabolism because DNA and RNA polymerases are strictly zinc-dependent enzymes [24]. Moreover, it has been demonstrated that zinc deficiency impairs immune response, increasing the risk of infections [25]. Additionally, zinc supplementation together with some antibiotics that present stimulatory effects on polymorphonuclear leukocyte functions has been shown to improve these effects in some cases [26].

For all these reasons, we have studied in the present paper the influence of thiazoline, thiazolidine and thiazine derivatives, and their zinc(II) complexes on the phagocytic activity of human neutrophils. Results are reported on the isolation and characterization of TzTz, as well as a study through elemental analysis, IR and X-ray diffraction of the solid phases obtained by reaction of TdTn [27] and TzTz with zinc(II). In addition, we report the crystal structure of the TdTn ligand, determined by X-ray single-crystal diffractometry, together with the study of the effect that TdTn, TzTz and their complexes with zinc(II) have on neutrophils phagocytic function.

2. Experimental

2.1. Preparation of 2-(3,4-dichlorophenyl)imino-*N*-(2-thiazolin-2-yl)thiazolidine (TdTn)

The preparation of TdTn was performed as described by Outcalt [27] and was recrystallized from ethanol 96% (2 g; 74%).

2.2. Preparation of 2-(3,4-dichlorophenyl)imino-N-(4H-5,6-dihydro-1,3-thiazin-2-yl)tetrahydro-1,3-thiazine (TzTz)

First, 3,4-dichlorophenyl isothiocyanate (3 mL, 21.0 mmol) was treated with 1.5 mL (19.6 mmol) of 3-amine-1-propanol in 15 mL of dichloromethane. The resulting thiourea was heated to reflux in 100 mL of 3 N hydrochloric acid for 3 h, cooled and the solution made basic with a solution of 12.5 g of sodium hydroxide in 50 mL of water. Extraction with dichloromethane, drying over anhydrous magnesium sulfate and concentration gave 5.3 g of a white solid that corresponded to the precursor 2-(3,4-dichlorobenzylamino)-4*H*-5,6-dihydro-1,3-thiazine. After this step, a solution of 2.12 g (8.1 mmol) of this precursor and 1.09 mL (10.2 mmol) of 3-chloropropylisothiocyanate [28] in 30 mL of chloroform were stirred and heated to reflux for 4 h. At this point, a white precipitate had separated and the reaction mixture was allowed to cool to room temperature. A solution of 1.62 g (15.3 mmol) of sodium carbonate in 20 mL of water was added and the mixture was stirred until dissolution of the solid was complete. The phases were separated and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated under reduced pressure to give a solid that was recrystallized from ethanol 96% (1.7 g; 58%).

^{1}H NMR (deuteriochloroform): δ 7.32 (doublet (d), $J = 8.36$ Hz, 1H), 7.04 (d, $J = 2.35$ Hz, 1H), 6.77 (doublet of a doublet (dd), $J = 2.37$, 8.52 Hz, 1H), 3.91 (triplet (t), $J = 6.24$ Hz, 2H), 3.76 (t, $J = 5.63$ Hz, 2H), 2.97 (t, $J = 6.24$ Hz, 2H), 2.94 (t, $J = 6.46$ Hz, 2H), 2.19 (multiplet (m), $J = 6.41$ Hz, 2H), 1.89 (m, $J = 5.98$ Hz, 2H); ^{13}C NMR (deuteriochloroform): δ 154.16, 152.01, 147.83, 132.22, 130.31, 126.55, 123.38, 121.32, 47.01, 46.13, 27.22, (2C), 24.48, 21.07. Found: C, 46.39; H, 4.19; N, 11.70; S, 17.56%. Calc. for $C_{14}H_{15}Cl_2N_3S_2$: C, 46.67; H, 4.19; N, 11.66; S, 17.80%. UV–visible (UV–Vis) (EtOH): λ_{max}, nm; (ε, M^{-1} cm^{-1}) 202 (8227), 239 (3890), 293 (1648).

2.3. Preparation of [$ZnCl_2$(TdTn)]

This complex was isolated by adding an ethanol solution of $ZnCl_2$ (1 mL) (41.0 mg, 0.3 mmol) to another ethanol solution of TdTn (15 mL) (100 mg, 0.3 mmol). The resulting solution was allowed to evaporate slowly at room temperature. After a few days, colourless crystals were isolated from the solution (114 mg, 81%). The crystals were separated by filtration, washed with cold ether and air-dried. Found: C, 30.80; H, 2.32; N, 8.98; S, 13.64%. Calc. for $C_{12}H_{11}Cl_4ZnN_3S_2$: C, 30.76; H, 2.37; N, 8.97; S, 13.69%.

2.4. Preparation of [$ZnCl_2$(TzTz)]

This complex was prepared by reacting an ethanol solution (1 mL) of $ZnCl_2$ (37.8 mg, 0.3 mmol) with an ethanol solution (15 mL) of TzTz (100 mg, 0.3 mmol), being obtained colourless crystals after a slow evaporation of the solution at room temperature (83 mg, 60%). The crystals were filtered and washed with cold ether and air-dried. Found: C, 33.72; H, 3.20; N, 8.37; S, 13.03%. Calc. for $C_{14}H_{15}Cl_4ZnN_3S_2$: C, 33.86; H, 3.04; N, 8.46; S, 12.91%.