Journal of Molecular Structure 1099 (2015) 215–225

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: http://www.elsevier.com/locate/molstruc

DNA-binding studies and biological activities of new nitrosubstituted acyl thioureas

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ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form
3 June 2015
Accepted 5 June 2015
Available online 17 June 2015

Keywords:
Thiourea
DNA binding studies
Cyclic voltammetry
UV–vis spectroscopy
Antimicrobial activities
Cytotoxicity

ABSTRACT

Four new nitrosubstituted acylthioureas i.e. 1-acetyl-3-(4-nitrophenyl)thiourea (TU1), 1-acetyl-3-(2-methyl-4-nitrophenyl)thiourea (TU2), 1-acetyl-3-(2-methoxy-4-nitrophenyl)thiourea (TU3) and 1-acetyl-3-(4-chloro-3-nitrophenyl)thiourea (TU4) have been synthesized and characterized (by C^{13} and H^1 nuclear magnetic resonance, Fourier transform infrared spectroscopy and single crystal X-ray diffraction). As a preliminary investigation of the anti-cancer potencies of the said compounds, DNA interaction studies have been carried out using cyclic voltammetry and UV–vis spectroscopy along with verification from computational studies. The drug-DNA binding constants are found to be in the order, K_{TU3} 9.04×10^6 M^{-1} > K_{TU4} 8.57×10^6 M^{-1} > K_{TU2} 6.05×10^6 M^{-1} > K_{TU1} 1.16×10^6 M^{-1}. Furthermore, the antioxidant, cytotoxic, antibacterial and antifungal activities have been carried out against DPPH (1,1-diphenyl-2-dipicrylhydrazyl), Brine shrimp eggs, gram positive (*Micrococcus luteus*, *Staphylococcus aureus*) and gram negative (*Bordetella bronchiseptica*, *Salmonella typhimurium*, *Enterobacter aerogens*) and fungal cultures (*Aspergillus fumigatus*, *Mucor* species, *Aspergillus niger*, *Aspergillus flavus*) respectively.

1. Introduction

Thioureas, having a considerably wide range of applications, are the cousin compounds of ureas in which oxygen has been replaced with sulfur. The properties of urea and thiourea differ significantly because of the difference in electronegativity between sulfur and oxygen [1]. Thioureas possess broad spectrum of biological activities including antiviral [2], antibacterial [3], antifungal [4], antitubercular [5], herbicidal [6], insecticidal [7], and pharmacological properties [8], act as corrosion inhibitors, antioxidants and are polymer components [9].

Thiourea derivatives can be easily synthesized in good yield [10], and substituted thioureas have recently gained much interest in the preparation of wide variety of biologically active compounds [11]. Acyl thiourea derivatives are well known for their biological activities like bactericidal, fungicidal, herbicidal, insecticidal and regulation of plant growth [12]. Herein, we have reported, the synthesis of four new nitrosubstituted acylthioureas with complete characterization (FTIR, multinuclear NMR, single crystal XRD) and biological activities (DNA-binding, antioxidant, cytotoxic and antimicrobial activities).

As we previously reported in our review article on the biological applications of selenoureas and their homologs [8], thioureas are an important class of organic compounds with effective anti-cancer applications. However, their UV–vis spectra and cyclic voltammograms are not very helpful in the investigation of their ability to bind with DNA. So, we have synthesized nitrosubstituted thioureas with an aim to quantify the DNA interaction of the synthesized compounds from the results of their UV–vis spectroscopic and cyclic voltammetric analyses.

2. Materials and methods

2.1. General

Melting points were determined in a capillary tube using electrothermal melting point apparatus model MP-D *Mitamura Riken Kogyo (Tokyo, Japan)*. Infrared spectra were taken on a Thermo

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http://dx.doi.org/10.1016/j.molstruc.2015.06.024
0022-2860/

Scientific NICOLET 6700 *Fourier* transform infrared spectroscope (FT-IR). H^1 and C^{13} nuclear magnetic resonance (NMR) were recorded on a Jeol JNM-LA 500 FT-NMR. $Si(CH_3)_4$ was used as internal reference. Suitable single crystal of each nitrosubstituted acyl thiourea was mounted on a glass fiber and the intensity data were collected on a *Brucker* kappa APEXII CCD diffractometer using graphite monochromator having M_o Kα radiation ($\lambda = 0.71073$ Å) at 296 K. The structures were solved by direct methods and refined by full-matrix least squares against F^2 of data using SHELXL97 (Sheldrick, 1997) software [13]. Basic crystal data and description of diffraction experiment are given in Table 1.

The computational study (DFT) was carried out using Gauss-View 5.0 software.

2.2. Synthesis

All chemicals, organic solvents and reagents were purchased from Sigma–Aldrich, Fluka and E. Merck. Organic solvents acetone (99.9% pure), and *n*-hexane (95% pure) were distilled, purified & dried according to reported methods. Potassium thiocyanate (98% pure), acetyl chloride (99% pure), 4-nitroaniline (99% pure), 4-chloro-3-nitroaniline (99% pure), 2-methyl-4-nitroaniline (99% pure) and 2-methoxy-4-nitroaniline (99% pure) were used without further purification.

Acetyl chloride was added to the solution containing potassium thiocyanate in dry acetone and stirred for about 3 h then respective nitroaniline was added into the reaction mixture and further refluxed for about 5 h to obtain the desired thioureas [14].

2.3. DNA-binding study by cyclic voltammetry

Commercial *Salmon* DNA was solubilized in doubly distilled water to prepare a stock solution of 6×10^{-4} M from which working concentrations of DNA were prepared. Concentration of the stock solution was measured by UV absorbance (with *Shimadzu* 1800 spectrphotometer) at 260 nm using an epsilon value of 6600 M^{-1} cm^{-1}. This DNA was protein free because A260/A280 > *1.8*. Solution preparation for DNA binding studies was carried out according to our previously reported method [15,16]. Cyclic voltammetry was performed on Biologic SP-300 cyclic voltammeter running with EC-Lab Express V 5.40 software (made in *France*). Before every reading, the working electrode was polished with alumina powder and rinsed with distilled water. Analytical grade KCl was used as supporting electrolyte and nitrogen gas (99.9%) was purged through the mixture to avoid interference from oxygen. A setup having three electrodes system, i.e. working (platinum disc electrode with a geometric area of 0.071 cm^2 s^{-1}), reference Ag/AgCl and auxiliary electrode (platinum electrode with geometric area much greater than working electrode) was used for cyclic voltammetric studies. The decrease in the peak current provided information about DNA binding constant whereas the shifts in the peak potentials were useful for the determination of the mode of interaction of the drug with DNA. Drug-DNA binding constant was determined with the help of following equation:

$$\log\ (1/[\mathrm{DNA}]) = \log \mathrm{K} + \log\ (\mathrm{I}/\mathrm{I_o} - \mathrm{I}) \quad (1)$$

where K is the binding constant and I_o and I are the peak currents of free drug and DNA-bound drug respectively [14].

2.4. DNA-binding study by UV–vis spectroscopy

For the DNA binding constants with the help of UV–vis spectroscopy at first the spectra of the analytes before the addition of DNA were recorded by taking solvent in the reference cell and solution of the analyte in the sample cell. Then the spectra were recorded by adding different concentration of DNA, in solutions having constant concentration of the compound. The whole experiment was carried out by keeping the volume and concentration of the compound constant while varying the amount of DNA [16]. The equilibrium constants (binding constant) is calculated by fitting data in the *Benesi-Hildebrand* equation (2).

$$\mathrm{A_o}/\mathrm{A} - \mathrm{A_o} = (\varepsilon_G/\varepsilon_{H-G} - \varepsilon_G) + (\varepsilon_G/\varepsilon_{H-G} - \varepsilon_G)\ (1/\mathrm{K}[\mathrm{DNA}]) \quad (2)$$

where A_o and A are the absorbance of the free compound and of the compound–DNA complex, εG and εH-G are the molar extinction coefficients of free compound and of the compound–DNA complex respectively.

2.5. Antioxidant assay

Oxidant reducing abilities of all the four nitrosubstituted thioureas were determined with the help of 1,1-diphenyl-2-picrylhydrazyl radical in DMSO to produce 1,1-diphenyl-2-picrylhydrazine. The decrease in the absorption of 1,1-diphenyl-2-

Table 1
Crystal diffraction data of TU1, TU2, TU3 and TU4.

	TU1	TU2	TU3	TU4
Empirical formula	$C_9H_9N_3O_3S$	$C_{10}H_{11}N_3O_3S$	$C_{20}H_{22}N_6O_8S_2$	$C_9H_8ClN_3O_3S$
Formula weight	239.25	253.28	538.55	273.69
Temperature (K)	296(2)	296(2)	296(2)	296(2)
Wavelength (Å)	0.71073	0.710173	0.710173	0.710173
a [Å]	5.1519(4)	8.7665(11)	3.9876(12)	4.484(2)
b [Å]	9.0702(7)	7.7560(7)	8.973(3)	23.617(11)
c [Å]	11.9097(9)	17.384(2)	16.899(6)	10.749(5)
α [degree]	71.175(4)	90	81.083(12)	90
β [degree]	82.499(4)	100.680(3)	88.322(13)	97.151(14)
γ [degree]	86.865(5)	90	79.505(13)	90
Volume (°A3)	522.20(7)	1161.5(2)	587.3(3)	1129.5(9)
Crystal system	triclinic	monoclinic	triclinic	monoclinic
Space group	P-1	P 21/c	P-1	P 21/c
Index ranges	$-6 \le h \le 6$, $-11 \le k \le 11$, $-15 \le l \le 15$	$-11 \le h \le 11$, $-9 \le k \le 10$, $-22 \le l \le 21$	$-5 \le h \le 4$, $-11 \le k \le 11$, $-22 \le l \le 21$	$-5 \le h \le 5$, $-29 \le k \le 21$, $-13 \le l \le 13$
Absorption coefficient (μ)	0.305	0.279	0.287	0.522
F(000)	248	528	280	560
Goodness-of-fit on F2 (S)	1.044	1.035	0.922	0.968
R factor (%); R_1, R_2.	0.0652, 0.0425	0.0602, 0.0422	0.2252, 0.0650	0.1452, 0.0556