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Synthesis and antimicrobial activity of tetradentate ligands bearing hydrazone and/or thiosemicarbazone motifs and their diorganotin(IV) complexes

Cristina González-García [a,1], Alejandro Mata [a], Franca Zani [b,*], M. Antonia Mendiola [a], Elena López-Torres [a,*]

[a] Departamento de Química Inorgánica, Universidad Autónoma de Madrid, C/ Francisco Tomás y Valiente 7, 28049 Madrid, Spain
[b] Dipartimento di Farmacia, Università degli Studi di Parma, Parco Area delle Scienze 27/A, 43124 Parma, Italy

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ABSTRACT

Four novel ligands derived from 2,3-butanedione have been synthesized, two dissymmetric thiosemicarbazone/3-hydroxy-2-naphthohydrazone ligands, $\mathbf{H_2L^1}$ (bearing 4-isopropyl-3-thiosemicarbazone) and $\mathbf{H_2L^2}$ (containing 4-cyclohexyl-3-thiosemicarbazone) and the symmetric $\mathbf{H_2L^3}$, diacetyl bis(3-hydroxy-2-naphthohydrazone), and $\mathbf{H_2L^4}$, diacetyl bis(4-cyclohexyl-3-thiosemicarbazone). Their reactivity with SnR_2Cl_2 (R = methyl, *n*-butyl and phenyl) was explored and the resulting complexes were characterized by elemental analysis, molar conductivity, mass spectrometry, IR, ^{1}H, ^{13}C and ^{119}Sn NMR and seven of them also by single crystal X-ray diffraction. The results showed that the reactivity of the dissymmetric ligands is strongly different and while the cyclohexyl derivative is very stable, with isopropyl easily undergoes a symmetrization reaction to yield the corresponding symmetric ligands. The antimicrobial activity of the ligands and the corresponding diorganotin(IV) complexes was investigated *in vitro* against seven species of microorganisms and minimum inhibitory concentrations (MICs) were determined. The results showed that the ligand $\mathbf{H_2L^2}$ and several of its derivatives, together with methyl and phenyl complexes of $\mathbf{H_2L^1}$, have the ability of inhibiting the growth of tested bacteria and fungi to different extents. *Bacillus subtilis* and *Staphylococcus aureus* Gram positive strains were the most sensitive microorganisms.

1. Introduction

The synthesis of hydrazone and thiosemicarbazone complexes have received much attention due to their fascinating structural versatility [1–6] combined with their relevant potential therapeutic activity, such as antitumor, antiviral, antiprotozoal, antibacterial or antifungal agents [7–12]. Therefore, the synthesis of mixed ligands containing both thiosemicarbazone and hydrazone functions is an interesting topic to pursue, although their synthesis is usually hampered by several synthetic problems and an exhaustive control of the reactions conditions must be carried out [13,14]. Concerning to the bioactivity, in many cases the complexes display higher activity than the parent ligands, suggesting that complexation can be an interesting strategy of dose reduction and can also circumvent some side effects [15,16]. This activity is believed to be related to their ability to form stable complexes with a wide range of metal ions which is increased in the case of ligands bearing two of these thiosemicarbazone/hydrazone functions.

On the other hand, organotin(IV) complexes have been widely investigated because of their structural diversity and biological activity, for example as bactericides, fungicides, acaricides, antifouling and anti-tumor agents [17–27]. According to the literature, the biological properties of organotin(IV) complexes depend on the coordination number of the tin atom, the number and nature of the organic groups as well as on the donor system provided by the ligand. Therefore, any modification made in any of these factors could modulate the complex activity.

The discovery of new antimicrobial substances is of considerable biological importance, owing to the dramatic increase in bacterial and fungal resistance observed in the last decades [28–30]. Hence and as continuation of our studies about organotin(IV) complexes with hydrazone ligands endowed with antimicrobial activity [31,32], we have designed two new tetradentate ligands containing two thiosemicarbazone or hydrazone moieties and two dissymmetric bis(thiosemicarbazone/hydrazone) donors, as well as their complexes with SnR_2Cl_2 (R = Me, *n*-Bu, Ph) and SnI_4. The *in vitro* antimicrobial potency of the ligands and their corresponding diorganotin(IV) complexes was evaluated against various strains of bacteria and fungi. For

* Corresponding authors.
E-mail addresses: franca.zani@unipr.it (F. Zani), antonia.mendiola@uam.es (M.A. Mendiola), elena.lopez@uam.es (E. López-Torres).
[1] Present address: Laboratorio de Tecnología de Polímeros, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Madrid, Spain.

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comparison purpose, a similar investigation was performed on tin parent compounds $SnMe_2Cl_2$, $SnBu_2Cl_2$, $SnPh_2Cl_2$ and SnI_4.

2. Experimental

2.1. Measurements

Microanalyses were carried out using a LECO CHNS-932 Elemental Analyzer. IR spectra in the 4000–400 cm^{-1} range were recorded as KBr pellets on a Jasco FT/IR-410 spectrophotometer. The ESI mass spectra in positive mode were recorded on a Q-STAR PULSAR I instrument using a hybrid analyzer QTOF (Quadrupole time-of-flight). Molar conductivity was measured using a freshly prepared DMF or acetone solution (*ca.* 10^{-3} M) at 25 °C with a Crison EC-Meter BASIC 30+ instrument. ^{1}H, ^{13}C and ^{119}Sn NMR spectra were recorded on a spectrometer Bruker AVIII HD-300 MHz using DMSO-d_6 or $CDCl_3$ as solvent and TMS (^{1}H and ^{13}C) or $SnMe_4$ (^{119}Sn) as internal reference. ^{119}Sn CP/MAS NMR spectra were recorded at 298 K in a Bruker AV400WB spectrometer equipped with a 4 mm MAS (magic-angle spinning) NMR probe and obtained using a cross-polarization pulse sequence using spinning rates of 10–14 KHz, pulse delays of 30 s, contact times of 8 ms and two-pulse phase-modulated high power proton decoupling. Chemical shifts are reported relative to $SnMe_4$, using tin(IV) oxide as a secondary reference.

2.2. Synthesis of the compounds

All the chemicals were purchased from standard commercial sources and used as received.

2.2.1. 4-Isopropyl-3-thiosemicarbazide, ^{i}PrTSC

Over a solution of 10.0 mL (93.7 mmol) of isopropylisothiocyanate in 50 mL of diethyl ether cooled in an ice bath, 4.5 mL (93.70 mmol) of monohydrated hydrazine was added dropwise and the mixture was stirred for an hour. The white precipitated formed was filtered off, washed with diethyl ether and vacuum dried (11.78 g, 94%). ^{1}H NMR (DMSO-d_6) δ 8.48 (1H, s, NH), 7.45 (1H, d, $^{3}J = 8.6$ Hz, NH-^{i}Pr), 4.40 (2H, s, NH_2), 4.35 (1H, m, CH), 1.11 (6H, d, $^{3}J = 6.6$ Hz, CH_3).

2.2.2. 4-Cyclohexyl-3-thiosemicarbazide, ChTSC

0.71 mL (14.80 mmol) of monohydrated hydrazine was added dropwise over a solution of 2.00 g (14.70 mmol) of cyclohexylisothiocyanate dissolved in 50 mL of diethyl ether. The mixture was stirred for an hour and the white solid formed was filtered off, washed with diethyl ether and vacuum dried (2.31 g, 93%). ^{1}H NMR (DMSO-d_6) δ 7.35 (1H, d, $^{3}J = 8.2$ Hz, NH), 4.15 (1H, m, CH), 3.86 (2H, br. s, NH_2), 2.08–1.27 (10H, m, CH_2).

2.2.3. Diacetyl-2-(4-isopropyl-3-thiosemicarbazone), HA^{i}PrTSC

To a suspension of 1.00 g (7.52 mmol) of iPrTSC in 20 mL of water with 10 drops conc. Hydrochloric acid was added 1.5 mL (17.6 mmol) of 2,3-butanedione and the mixture was stirred for 1 h. The white solid obtained was filtered off, washed with cold water and methanol and vacuum dried (1.39 g, 92%). ^{1}H NMR (DMSO-d_6) δ 10.56 (1H, s, NH), 8.11 (1H, d, $^{3}J = 8.4$ Hz, NH-^{i}Pr), 4.48 (1H, m, CH), 2.40 (3H, s, CH_3-CO), 1.95 (3H, s, CH_3-CN), 1.23 (6H, d, $J = 6.6$ Hz CH_3-^{i}Pr).

2.2.4. Diacetyl-2-(4-cyclohexyl-3-thiosemicarbazone), HAChTSC

The compound was obtained following the procedure described above but using 1.00 g (6.16 mmol) of ChTSC (1.31 g, 94%). ^{1}H NMR (DMSO-d_6) δ 10.59 (1H, s, NH), 8.1 (1H, d, $^{3}J = 8.3$ Hz, H-Ch), 4.15 (1H, m, CH), 2.38 (3H, s, CH_3-CO), 1.95 (3H, s, CH_3-CN) 1.89–1.28 (10H, m, CH_2).

2.2.5. Diacetyl-2-(4-isopropyl-3-thiosemicarbazone)-3-(3-hydroxy-2-naphthohydrazone), $\mathbf{H_2L^1}$

A suspension of 2.17 g (10.7 mmol) of 3-hydroxy-2-naphthohydrazide in 10.00 mL of ethanol was added to a suspension of 2.00 g (9.94 mmol) of HA^{i}PrTSC in 40 mL of the same solvent with eight drops of conc. hydrochloric acid. The mixture was stirred for 4 h and the cream precipitate was filtered off, washed with ethanol and vacuum dried (3.61 g, 95%). Found C: 59.02, H: 5.94, N: 18.03, S: 8.21. $C_{19}H_{23}N_5O_2S$ requires C: 59.20, H: 6.01, N: 18.17, S: 8.32. ν_{max}(KBr)/cm^{-1} 3522 w (OH), 3359 m, 3342 m, 3178 m (NH), 1645 s (CO), 1628 m, 1523 s, 1496 s (thioamide II + amide II + CN), 813 w (CS). ESI^+ *m/z* 386.10 ($[M]^+$).

2.2.6. Diacetyl-2-(4-cyclohxyl-3-thiosemicarbazone)-3-(3-hydroxy-2-naphthohydrazone), $\mathbf{H_2L^2}$

To a suspension of 3.21 g (13.6 mmol) of HAChTSC in 20 mL of ethanol with six drops of conc. hydrochloric acid was added a suspension of 2.76 g (13.70 mmol) of 3-hydroxy-2-naphthohydrazide dissolved in 10 mL of ethanol. The mixture was stirred for 1 h and the cream solid was filtered off, washed with ethanol and vacuum dried (5.61 g, 94%). Found C: 62.18, H 6.37, N: 16.75, S: 7.42. $C_{22}H_{27}N_5O_2S$ requires C: 62.09, H: 6.40, N: 16.46, S: 7.53. ν_{max}(KBr)/cm^{-1} 3444 sh (OH), 3352 m, 3255 m (NH), 1672s (CO), 1627 m, 1528 s (thioamide II + amide II + CN), 832 m (CS). ESI^+ *m/z* 425.19 ($[M]^+$).

2.2.7. Diacetyl bis(3-hydroxy-2-naphthohydrazone), $\mathbf{H_2L^3}$

To a suspension of 2.00 g (9.89 mmol) of 3-hydroxy-2-naphthohydrazide in 30 mL of ethanol was added 0.40 mL (4.95 mmol) of 2,3-butanedione and eight drops of conc. hydrochloric acid. The mixture was stirred during 24 h and the cream solid was filtered off, washed with ethanol and vacuum dried (2.06 g, 92%). Found C: 68.42, H 5.03, N: 11.98. $C_{26}H_{22}N_4O_4$ requires C: 68.71, H: 4.88, N: 11.96. ν_{max}(KBr)/cm^{-1} 3411 w (OH), 3220 m (NH), 1649 s (CO), 1631 m (amide II + CN). ESI^+ *m/z* 454.16 ($[M]^+$).

2.2.8. Diacetyl bis(4-cyclohexyl-3-thiosemicarbazone), $\mathbf{H_2L^4}$

0.25 mL (3.10 mmol) of 2,3-butanedione was added to a suspension of 1.00 g (5.95 mmol) of ChTSC in 20 mL of ethanol with 10 drops of conc. hydrochloric acid and the mixture was refluxed for 3 h. The white precipitate was filtered off, washed with ethanol and vacuum dried (1.00 g, 85%). Found C: 54.38, H 8.33, N: 21.09, S: 15.99. $C_{18}H_{32}N_6S_2$ requires C: 54.51, H: 8.13, N: 21.19, S: 16.17. ν_{max}(KBr)/cm^{-1} 3336 m, 3185 m (NH), 1525 s, 1490 s (thioamide II + CN), 867 m (CS). ESI^+ *m/z* 396.21 ($[M]^+$).

2.2.9. [$SnMe_2L^1$] **1**

To a boiling suspension of 100 mg (0.26 mmol) of $\mathbf{H_2L^1}$ and 22 mg (0.52 mmol) of $LiOH\cdot H_2O$ in 8 mL of ethanol was added a solution of 57 mg (0.26 mmol) of $SnMe_2Cl_2$ in 5 mL of the same solvent. The mixture was stirred for 5 min and the resulting orange solution was immediately cooled in the fridge. After 3 h the orange precipitate formed was filtered off, washed with cold ethanol and vacuum dried (110 mg, 80%). Found C: 47.22, H: 4.93, N: 13.06, S: 5.90. $SnC_{21}H_{27}N_5O_2S$ requires C: 47.39, H: 5.11, N: 13.16, S: 6.02. ν_{max}(KBr)/cm^{-1} 3431 s (OH), 3266 m (NH), 1642 m (CO), 1530 m (thioamide II + amide II + CN), 821 w (CS). ESI^+ *m/z* 534.09 ($[M + H]^+$).

2.2.10. [$SnBu_2L^1$] **2**

This complex was obtained following the same procedure described for the synthesis of **1** but adding 79 mg (0.26 mmol) of $SnBu_2Cl_2$ (red, 141 mg, 88%). Slow evaporation of a solution in DMF yielded crystals suitable for single crystal X-ray diffraction. Found C: 52.36, H: 6.28, N: 11.19, S: 5.12. $SnC_{27}H_{39}N_5O_2S$ requires C: 52.58, H: 6.38, N: 11.36, S: 5.20. ν_{max}(KBr)/cm^{-1} 3437 m (OH), 3266 m (NH), 1641 m (CO), 1536 s (thioamide II + amide II + CN), 808 w (CS). ESI^+ *m/z* 618.19 ($[M + H]^+$).

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