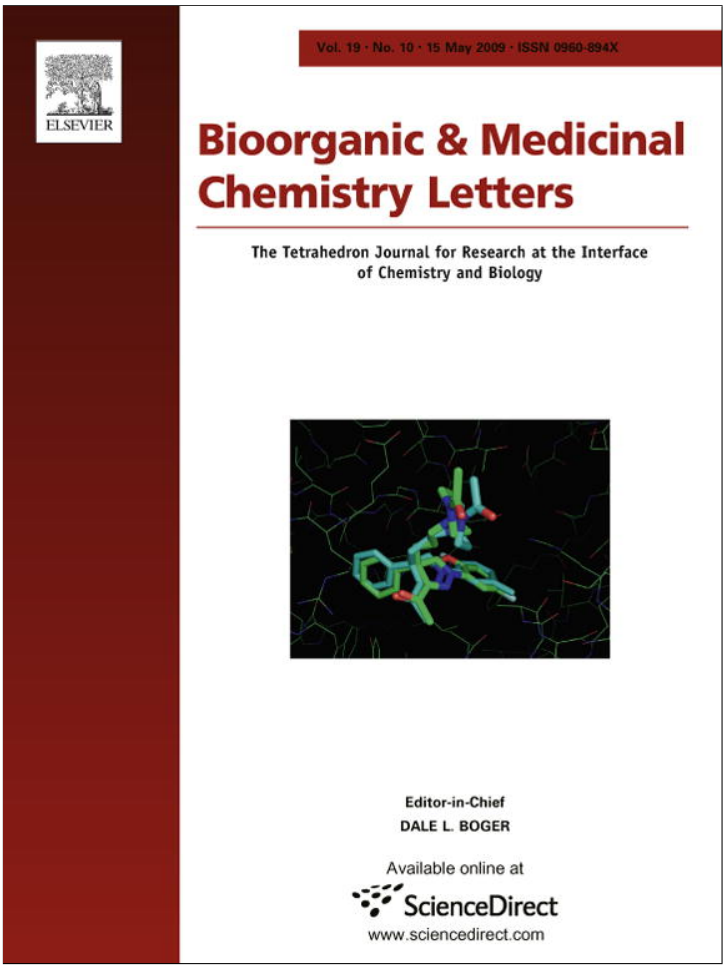

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Cytotoxicity and structure–activity relationships of four α-N-heterocyclic thiosemicarbazone derivatives crystal structure of 2-acetylpyrazine thiosemicarbazone

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ABSTRACT

A series of thiosemicarbazone ligands, HL^1 (2-acetylpyrazine thiosemicarbazone), HL^2 (2-acetylpyrazine *N*(4)-methylthiosemicarbazone), HL^3 (2-benzoylpyridine thiosemicarbazone) and HL^4 (2-benzoylpyridine *N*(4)-methylthiosemicarbazone), have been synthesized. The crystal structure of HL^1 has been determined by single-crystal X-ray diffraction. Hydrogen bonds link the different components to stabilize the crystal structure. The antitumor activity of the four ligands were tested against K562 leucocythemia and BEL7402 liver cancer cell lines. All the thiosemicarbazones showed significant antitumor activity. Different substituents on the ligands show different levels of antitumor activity. By comparison with the other thiosemicarbazone species studied, HL^4 with substitution at N(4) position in thiosemicarbazone along with 2-benzoylpyridine is the most active thiosemicarbazone ligand with IC_{50} = 0.002 μm in the K562 leucocythemia cell line and 0.138 μm in the BEL7402 liver cancer cell line, respectively.

Thiosemicarbazones have been extensively studied because they have a wide range of actual or potential medical applications[1] which include notably antiparasital,[2] antibacterial[3] and antitumor activities.[4,5] Many thiosemicarbazones, such as marboran or triapine, are already used in medical practice. Their mechanism of action is still controversial in many respects, but it is known that heterocyclic thiosemicarbazones act by inhibiting ribonucleotide reductase, a key enzyme in the biosynthesis of DNA precursors.[6–9]

In general, thiosemicarbazones are obtained by condensation of the corresponding thiosemicarbazide with aldehydes or ketones. Thiosemicarbazones possess a wide range of biological activity depending on the parent aldehyde or ketone. Earlier reports on N(4)-substituted thiosemicarbazones have concluded that the presence of bulky groups at the N(4) position of the thiosemicarbazone moiety greatly enhances biological activity.[10–14]

Previous studies from our laboratories have shown that 2-acetylpyrazine thiosemicarbazone, 2-benzoylpyridine thiosemicarbazone and their cobalt, zinc complexes exhibit antitumor activities against A549 lung cancer cell line and different substituents on the ligands show different levels of antitumor activity.[15,16]

In view of these encouraging results we have extended our studies to other derivatives of thiosemicarbazones. In the present work, we study the biological activities of four thiosemicarbazone ligands with different substituents (Scheme 1) against K562 leucocythemia and BEL7402 liver cancer cell lines, respectively. Our objective is to study the variation in antitumor activity by changing parent ketone from 2-acetylpyrazine to 2-benzoylpyridine and the N(4) substituent from NH_2 group to $NHCH_3$ group. Our interest in searching for systems with antitumor activity and also in finding structure-activity relationships, led us to evaluate each of these compounds discussed in this paper and analyze the differences in antitumor activity. In our experiment, we have found that the four ligands with different substituents show significantly different levels of antitumor activity. It is worth noting that HL^4 with substitution at N(4) position in the thiosemicarbazone along with 2-benzoylpyridine is proved to be particularly effective thiosemicarbazone ligand against the two studied cell lines. In addition, to the best of our knowledge, there is no structural information of 2-acetylpyrazine thiosemicarbazone, we also describe synthesis and single crystal X-ray crystal structure of 2-acetylpyrazine thiosemicarbazone here.

The ligand HL^1 has been prepared by refluxing condensation of 2-acetylpyrazine and thiosemicarbazide (1:1 molar ratio) with an acetic catalyst in ethanol.[17]The ligand HL^2, HL^3 and HL^4 were prepared according to the literature method.[18–20] Elem. Anal. Calcd for HL^1 (%): C, 43.06; H, 4.65; N, 35.87. Found: C, 43.02; H, 4.61; N, 35.85.

Single-crystal X-ray diffraction structure analysis[21] shows that 2-acetylpyrazine thiosemicarbazone ligand exists in the thione

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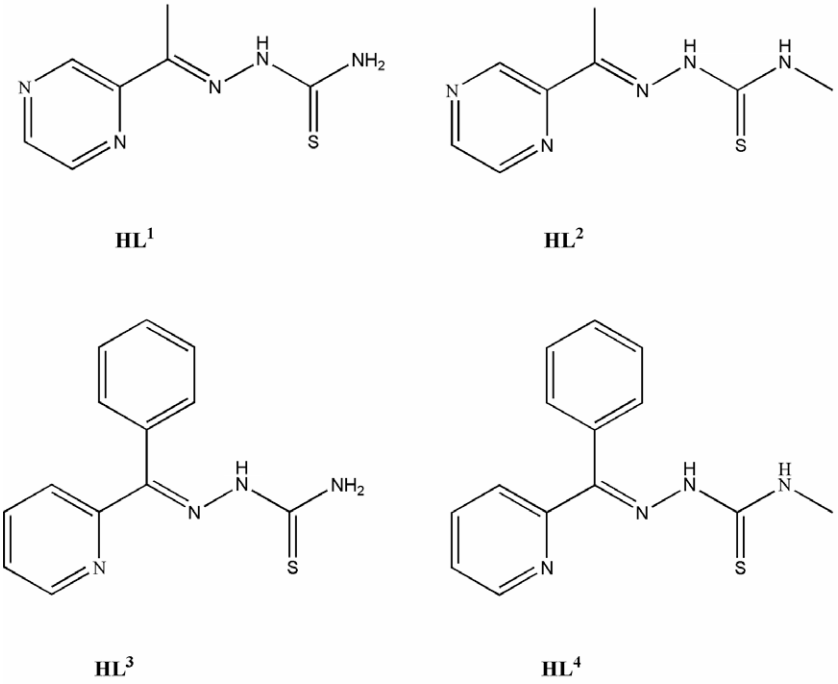

Scheme 1. Thiosemicarbazone ligands.

form as shown in Figure 1 which was confirmed by the presence of two double bonds in the thiosemicarbazone moiety viz. C(1)–S(1) 1.686(2) Å and C(2)–N(3) 1.289(2) Å which are consistent with the previously reported cases.[22] The N–N distance is 1.375(2) Å, showing the single N–N bond character, and agrees with the 2-acetylpyrazine 4-methyl thiosemicarbazone.[18] The planarity in the thiosemicarbazone is revealed by a smaller dihedral angle of the N(3)–N(2)–C(1)–S(1) (0.034°) skeleton. The smaller dihedral angles between the pyrazine ring and the methyl of the 2-acetylpyrazine moiety and thiosemicarbazone moiety C(3)–C(2)–C(4)–C(7) (0.03°) and N(2)–N(3)–C(2)–C(4) (0.01°), is due to a resonance effect between the π systems.

Since the thiosemicarbazone moieties have both the hydrogen bond donors and the hydrogen bond acceptors, the species provide the possibility of forming hydrogen bonds in the crystal.[23–25] As shown in Figure 2, the molecules are held together in the crystal packing through intermolecular hydrogen bonds involving the amino nitrogen N(1), hydrazine nitrogen N(2) atoms and sulfur

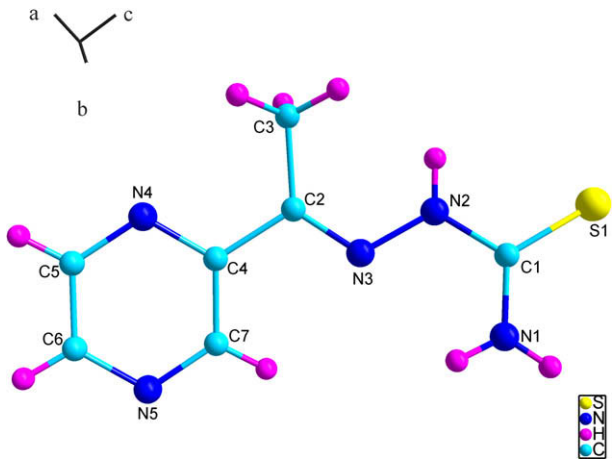

Figure 1. The molecular structure of the HL^1 along with the atom numbering scheme.

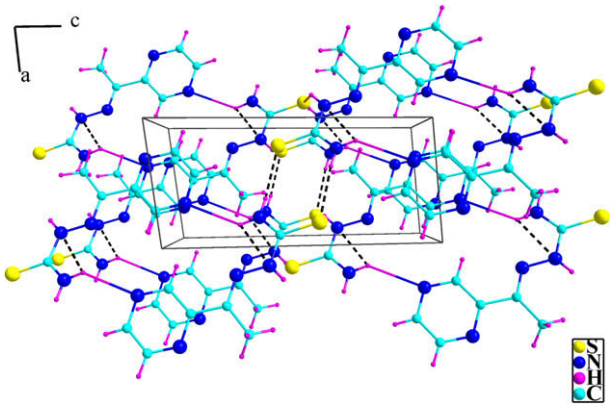

Figure 2. The space-filling packing projected along the *b* axis of the crystal.

atom S(1). The terminal N(1) hydrogen atom on the 2-acetylpyrazine thiosemicarbazone ligand participates in forming two hydrogen bonds. One is with pyrazine nitrogen atom N(5) (symmetry code: $-x, -y+2, -z$) interaction (N(1)···N(5) 3.236(2) Å) and the other with the S(1) (symmetry code: $-x-1, -y+2, -z+1$) interaction (N(1)···S(1) 3.458(2) Å). The hydrazine nitrogen atoms of thiosemicarbazone moieties act as H-bond donors while the sulfur atoms as acceptors with the N(2)···S(1) separations of 3.522(2) Å (symmetry code: $-x, -y+1, -z+1$).

Taking into account that thiosemicarbazone molecules exhibit cytotoxic activity,[26] we have tested the ability of the four ligands to inhibit tumor cell growth. In our experiment, IC_{50} values (compound concentration that produces 50% of cell death) in micro molar units were calculated for ligands HL^1–HL^4 against K562 leucocythemia cell line and BEL7402 liver cancer cell line, respectively.[27] Different IC_{50} values are found for different compounds as shown in Figure 3. We found that the four thiosemicarbazones all show higher antitumor activities against K562 leucocythemia cell line indicating the conclusion that the antitumor activities can be increased when the thiosemicarbazone contain a pyridine ring or

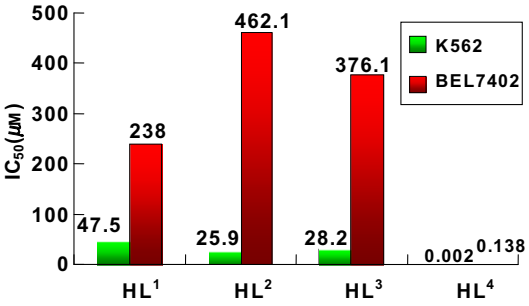

Figure 3. The antitumor activities of the HL^1, HL^2, HL^3, and HL^4 against K562 leucocythemia cell line and BEL7402 liver cancer cell line.

derivatives giving rise to NNS tridentate system.[28] It is worth noting that the comparison of cytotoxic activity of the four compounds against K562 leukemia cell line indicates that the HL^2 show a lower IC_{50} value (25.9 μm) than the HL^1 (47.5 μm), and the HL^4 show a lower IC_{50} value (0.002 μm) than the HL^3 (28.2 μm), which indicate that the presence of bulky groups at position N(4) of the thiosemicarbazone moiety enhanced the antitumor activities.[10–14] In addition, HL^3 show a lower IC_{50} value (28.2 μm) than the HL^1 (47.5 μm) and HL^4 show a lower IC_{50} value (0.002 μm) than the HL^2 (25.9 μm) against K562 leukemia cell line indicating importance of the substituent group of the parent ketone in thiosemicarbazones derivatives on antitumor activities.[29–32] On the other hand, the lowest IC_{50} value was found for HL^4 with substitutions at N(4) position in thiosemicarbazones along with 2-benzoylpyridine in the two cell lines which shows it is the most active thiosemicarbazone among all the ligands. It should be emphasized that HL^4 effectively inhibit K562 leucocythemia cell line at concentrations more than 14,100-fold lower and inhibit BEL7402 liver cancer cell line at concentrations more than 2725-fold lower than HL^3 only by the presence of bulky group CH_3 at position N(4) of the thiosemicarbazone moiety. Therefore, changing the substituent group in the thiosemicarbazones must play a significant role in these compounds growth inhibitory activity.

In summary, compounds discussed in this article, represent a good model for comparison to establish a good correlation of structure and activity. The relationship between structural and biological properties has been explored which could be helpful in designing more potent antiamoebic agents for therapeutic use. Detailed studies of the toxicity of the four ligands, as well as mechanism of action will be investigated in the future.

Acknowledgments

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Supplementary data

CCDC 701838 contains the supplementary data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.135.

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21. *Crystal data for* HL^1: $M = 195.26$, triclinic, space group $P\bar{1}$, $a = 5.410(1)$ Å, $b = 7.451(1)$ Å, $c = 11.779(1)$ Å, $\alpha = 81.87(1)°$, $\beta = 82.64$ (1)°, $\gamma = 77.30(1)°$, $V = 456.23(12)$ Å^3, $Z = 2$, $D_c = 1.421$ Mg m^{-3}, $\mu = 0.314$ mm^{-1}, $F(000) = 204$, $T = 293(2)$ K. A crystal with approximate dimensions of $0.20 \times 0.18 \times 0.10$ mm^3 was mounted on a glass fiber in a random orientation. Crystallographic data were collected with a Siemens Smart-CCD diffractometer with graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å). A total of 4264 reflections was measured by ω scan technique at 293(2) K within $1.76 \leqslant \theta \leqslant 25.00°$, of which 1377 were independent with $R_{int} = 0.0303$, and 1577 were observed with $I \geqslant 2\sigma(I)$. The structure was solved by Direct Methods and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for all non-hydrogen atoms using SHELXTL-97 program package. The hydrogen atoms were added in idealized geometrical positions. Final R indices [$I \geqslant 2\sigma(I)$]: $R_1 = 0.0368$, $wR_2 = 0.1393$.
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