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Synthesis, crystal structures and biological activities of 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone and its manganese(II) and nickel(II) complexes

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ABSTRACT

2-Acetylpyridine N(4)-cyclohexylthiosemicarbazone (HL) and its manganese(II) and nickel(II) complexes formulated as $[Mn(L)_2]$ (**1**) and $[Ni(L)_2]$ (**2**) have been synthesized and characterized by elemental analysis, infrared spectra, mass spectra, and single-crystal X-ray diffraction studies. In the two complexes, the coordination polyhedron approaches an octahedron, where the two ligands coordinate to the metal via the pyridine nitrogen atom and the nitrogen and sulfur donors of the thiosemicarbazide moiety. Biological studies, carried out *in vitro* against selected bacteria and K562 leukaemia cell line, respectively, have shown that the free ligand and its complexes exhibited distinct differences in the biological activities.

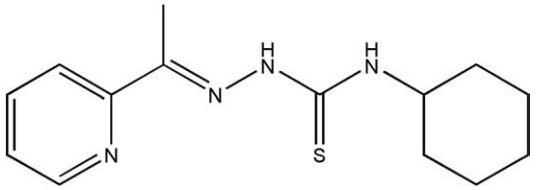
Heterocyclic thiosemicarbazones and their transition metal complexes have received considerable attention due to their coordination chemistry and broad range of pharmacological properties, notable for antiparasital, antibacterial and antitumor activities [1–6]. The best known representative of this class of compounds is 3-aminopyridinecarbaldehyde thiosemicarbazone (Triapine) which is currently undergoing clinical trials [1]. Their mechanism of action is still controversial in many respects and has been identified including ribonucleotide reductase inhibition, metal dependent radical damage, DNA binding, and inhibition of protein synthesis [4]. In general, thiosemicarbazones are obtained by condensation of the corresponding thiosemicarbazide with aldehydes or ketones. The biological activities of thiosemicarbazones often show a high dependence on their substituent. Minor modifications in thiosemicarbazones can lead to significant change in biological activity. Earlier reports on N(4)-substituted thiosemicarbazones have concluded that the presence of a bulky group at the terminal nitrogen considerably increases biological activity [7–9]. Moreover, the biological properties of thiosemicarbazones are often related to metal ion coordination in different ways since some of them increase the biological activity by forming chelates with specific metal ions. Lipophilicity, which controls the rate of entry of molecules into the cell, is modified by coordination, so the metal complex can become more active than the free ligand [10–13].

In recent years we have been working on the structural and biological properties of heterocyclic thiosemicarbazones and their metal complexes [14]. The results have revealed that 2-acetylpyridine N(4)-methylthiosemicarbazone and its Mn(II) and Ni(II) complexes showed significant biological activity *in vitro* against K562 leukaemia cell line [14fg]. The present work is an extension of previously studied 2-acetylpyridine N(4)-substituted thiosemicarbazones with potentially interesting biological activities.

In the present paper, using the screening method, we have tested the biological activities of 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone (Scheme 1) and its Mn(II) and Ni(II) complexes against selected bacteria and K562 leukaemia cell line, respectively. In addition, we also describe synthesis, infrared spectra and single-crystal X-ray crystal structures of the free ligand and its complexes.

The ligand HL was prepared according to the method described [15,16], whereas complexes were synthesized by reacting 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone and $Mn(ClO_4)_2 \cdot 6H_2O$ and $Ni(ClO_4)_2 \cdot 6H_2O$ (2:1 ligand–metal molar ratio) in ethanol [17].

Single-crystal X-ray analysis [18] reveals that the free ligand HL crystallizes in triclinic system, with space group P_{-1}. As shown in Fig. 1, the thione sulfur atom S(1) is trans to the azomethine nitrogen atom N(3) and the pyridine nitrogen atom N(4) is also trans to the



Scheme 1. 2-Acetylpyridine N(4)-cyclohexylthiosemicarbazone, HL.

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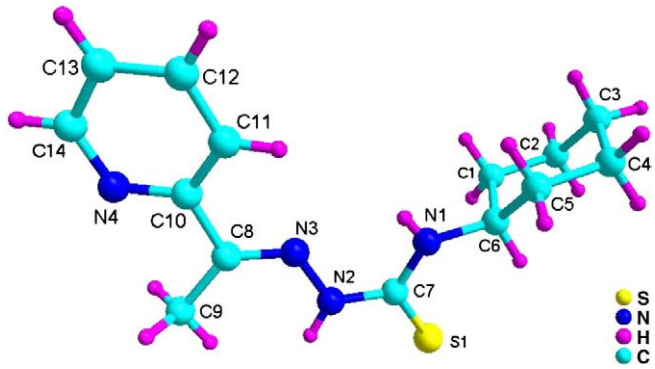

Fig. 1. The molecular structure of HL ligand with atomic numbering scheme. Selected bond lengths (Å) and angles (°): N(1)–C(6) 1.458(3), N(1)–C(7) 1.332(3), S(1)–C(7) 1.679(2), N(2)–C(7) 1.323(3), N(3)–N(2) 1.395(3), and N(3)–C(8) 1.280(3); and C(7)–N(1)–C(6) 125.5(2), C(7)–N(2)–N(3) 119.3(2), and C(8)–N(3)–N(2) 120.2(2).

azomethine nitrogen atom N(3). Thus, the configuration of the ligand as observed in the solid state is not suitable for its coordination with a metal ion as a N_2S tridentate chelating agent. However, rotation of the N(1)–C(7)–S(1) fragment by 180° about the C(7)–N(2) bond and a simultaneous rotation of the pyridine ring by 180° about the C(8)–C(10) bond orients the donor atoms in the correct position for tridentate coordination as found for other tridentate thiosemicarbazones [21]. From the imine and the thioamide bond distances, along with the rest of the structural parameters of the free ligand, we may conclude that the ligand exists in thione form in its solid state as observed in other free unsubstituted thiosemicarbazides [22,23]. On the other hand, the molecules of the free ligand are held together in the crystal packing through intermolecular hydrogen bonds involving the hydrazine nitrogen atom N(2) and the sulfur atom S(1) with N(2) ⋯ S(1) 3.624(2) Å (symmetry code: −x, −y+2, −z) (Fig. 2).

In view of the structural similarity of $[Mn(L)_2]$ (**1**) (Fig. 3) and $[Ni(L)_2]$ (**2**) (Fig. 4) [24,25], only complex **1** was described in some detail. As shown in Fig. 3, the manganese(II) ion is in a slightly distorted octahedral environment, where two 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone units deprotonated act as N_2S tridentate ligands coordinated to the central manganese atom via the pyridine nitrogen, azomethine nitrogen and sulfur atoms. One sulfur atom, one imine nitrogen atom and one pyridine nitrogen atom from one ligand and one imine nitrogen atom from another ligand occupy the equatorial positions, the two remaining positions in the octahedral geometry are the axial ones which are occupied by one sulfur atom and one pyridine nitrogen atom from different ligands. The pseudo-macrocyclic coordination mode of each ligand affords two five-membered chelate rings, the dihedral angles between the chelate rings in the two ligands are 2.7° and 13.9°, respectively.

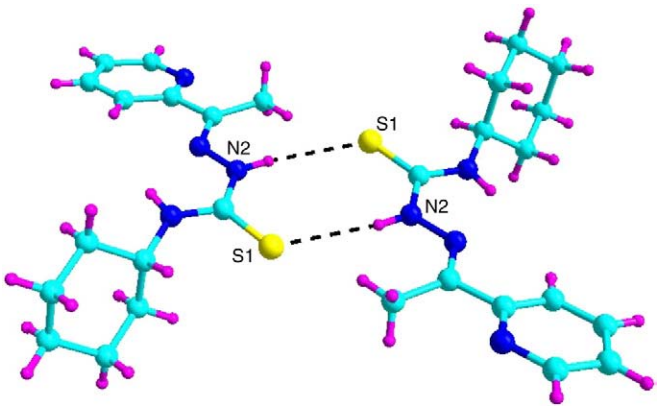

Fig. 2. Hydrogen bond in dashed lines in HL.

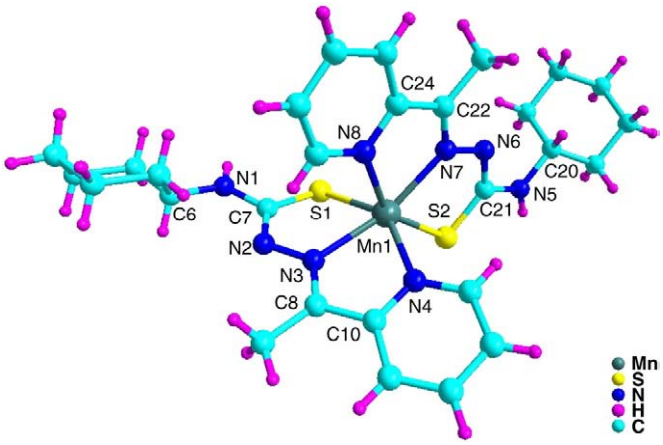

Fig. 3. Structure of complex **1** with atomic numbering scheme. Selected bond lengths (Å) and angles (°): Mn(1)–N(3) 2.257(2), Mn(1)–N(4) 2.280(2), Mn(1)–N(7) 2.232(2), Mn(1)–N(8) 2.336(1), Mn(1)–S(1) 2.519(1), Mn(1)–S(2) 2.549(1), S(1)–C(7) 1.747(2), S(2)–C(21) 1.733(2), N(3)–C(8) 1.301(2), and N(7)–C(22) 1.298(2); and N(3)–Mn(1)–N(4) 71.03(5), N(3)–Mn(1)–S(1) 74.61(4), N(3)–Mn(1)–N(7) 156.0(1), N(4)–Mn(1)–N(7) 106.7(1), and N(8)–Mn(1)–S(2) 147.7(1).

The C(7)–S(1) and C(21)–S(2) bond lengths of 1.747(2) and 1.733(2) Å, respectively, are within the normal range of C–S single bonds, indicating that the thiosemicarbazone moieties adopt the thiol tautomeric form [26]. The C–N and N–N bond lengths in L^- are intermediate between formal single and double bonds, pointing to an extensive electron delocalization over the entire molecular skeleton. The two thiosemicarbazone ligands have slightly different Mn–N (pyridine) bond distances and they are longer than the Mn–N(imine) distances, this may be attributed to the fact that the imine nitrogen is a stronger base compared with the pyridine nitrogen [22].

Complex **1** is stabilized by intermolecular hydrogen bonds (Fig. 5). The hydrogen bond involves the uncoordinated nitrogen atom N(1) and sulfur atom S(2). The uncoordinated nitrogen atom N(1) acts as a hydrogen bond donor while sulfur atom S(2) acts as an acceptor with N(1) ⋯ S(2) 3.431(2) Å and the angle N(1)–H(1A) ⋯ S(2) being 153.8° (symmetry code: −x+1, −y+2, −z+1).

The infrared spectral bands most useful for determining the mode of coordination of the ligand are the ν(C N), ν(N−N) and ν(C S) vibrations. The ν(C N) band of the free ligand at 1581 cm^{-1} shifts to

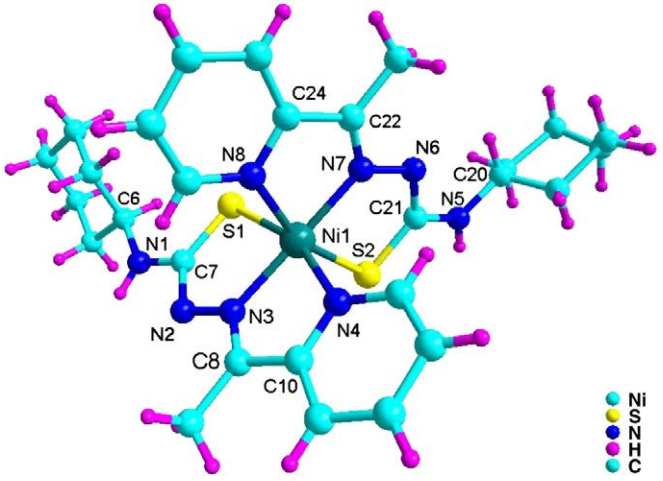

Fig. 4. Structure of complex **2** with atomic numbering scheme. Selected bond lengths (Å) and angles (°): Ni(1)–N(3) 2.038(4), Ni(1)–N(4) 2.105(4), Ni(1)–N(7) 2.029(4), Ni(1)–N(8) 2.108(5), Ni(1)–S(1) 2.397(2), Ni(1)–S(2) 2.403(2), S(1)–C(7) 1.702(5), S(2)–C(21) 1.729(5), N(3)–C(8) 1.301(7), N(7)–C(22) 1.289(7), and N(3)–Ni(1)–N(4) 77.84(2); and N(3)–Ni(1)–S(1) 81.53(13), N(3)–Ni(1)–N(7) 172.3(2), N(4)–Ni(1)–N(7) 96.88(2), and N(8)–Ni(1)–S(2) 159.0(1).

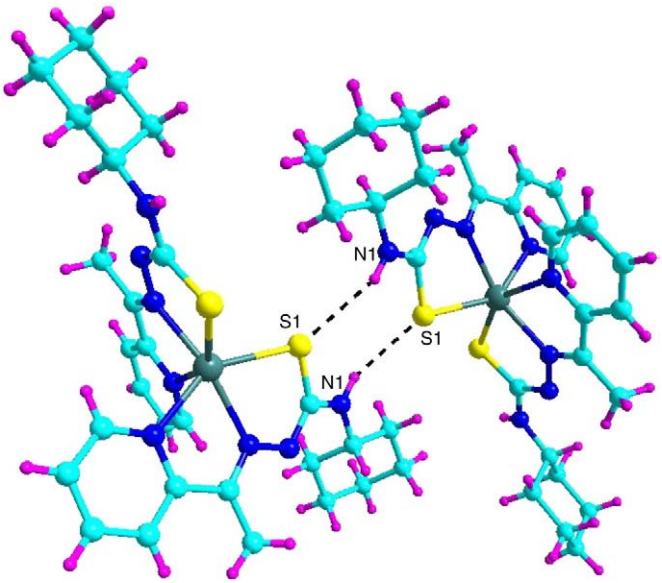

Fig. 5. Hydrogen bond in dashed lines in complex **1**.

1516 and 1507 cm^{-1} in the spectra of complexes **1** and **2**, respectively, a clear sign of coordination via the imine nitrogen atom [14g,27]. In 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone, a band at 866 cm^{-1} is assigned to ν(C S), whereas in its complexes this band is shifted to lower frequency (826 cm^{-1} for **1**, and 832 cm^{-1} for **2**), indicating the coordination of sulfur [28]. The increase in the frequency of ν(N–N) band of the thiosemicarbazone in the spectra of complexes is due to the increase in the bond strength, again confirms the coordination via the imine nitrogen [29]. The breathing motion of the pyridine ring is shifted to a higher frequency upon complexation and is consistent with pyridine ring nitrogen coordination [14g]. These observations have also been confirmed by X-ray single-crystal structure analysis.

In view of the antimicrobial activity of thiosemicarbazone [30,31], we have tested the ability of the free ligand and its complexes against representative Gram positive bacteria *B. subtilis* and Gram negative bacteria *P. aeruginosa* by the disc diffusion method [32]. Based on the minimum inhibitory concentration (Table 1), the remarkable antimicrobial activities are observed for the free ligand and complex **1** against the tested microorganism with an MIC value of 15.6 μg/mL. As can be expected where the more bulky N(4) substituent leads to more activity, 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone shows enhanced antibacterial activity than 2-acetylpyridine N(4)-methylthiosemicarbazone [14f]. In addition, complex **2** display more inhibitory properties against Gram positive bacteria *B. subtilis* than against Gram negative bacteria *P. aeruginosa*. A further evaluation of mechanism will be investigated in the future.

Heterocyclic substituted thiosemicarbazones and their metal complexes show particularly effective antitumor activity, due to the NNS tridentate system [33]. Therefore, we have tested the ability of the compounds to inhibit tumor cell growth against K562 leukaemia cell line [34]. In our experiments, IC_{50} values (compound concentration that produces 50% of cell death) in micro molar units were calculated.

Table 1
In vitro antibacterial activities of the compounds.

Compound	MIC μg/mL	
	B. subtilis	*P. aeruginosa*
HL	15.6	15.6
1	15.6	15.6
2	15.6	62.5

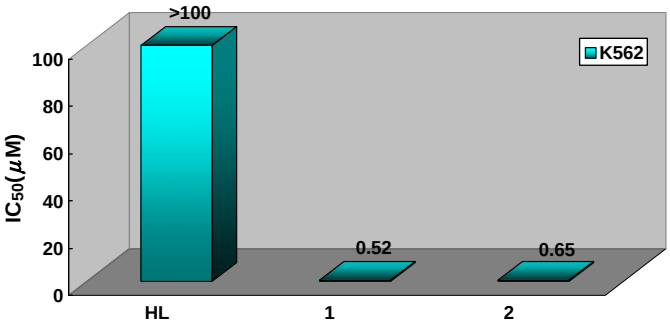

Fig. 6. The antitumor activities of the compounds against K562 leukaemia cell line.

As shown in Fig. 6, the free ligand exhibited poor antitumor activity while its Mn(II) and Ni(II) complexes exhibited remarkable antitumor activity with $IC_{50} = 0.52$ and 0.65 μm, respectively. But reversed as expected where the more bulky N(4) substituent leads to more activity, 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone shows remarkably lower antitumor activity than both 2-acetylpyridine thiosemicarbazone and 2-acetylpyridine N(4)-methylthiosemicarbazone, in a similar way to that observed with 2-benzoylpyridine N(4)-methylthiosemicarbazone and 2-benzoylpyridine N(4)-phenylthiosemicarbazone[14g]. The biochemical mechanism of the notable exception is not understood and deserves more studies. In addition, it is clearly observed that complexation with metals has a synergetic effect on the antitumor activity of these compounds. The enhancement of antitumor activity of these metal complexes can be related to an increase in the lipophilicity so they can penetrate into the cells more easily [35]. It has also been suggested that metal complexation may be a vehicle for activation of the ligand as the cytotoxic agent [36].

In summary, 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone and its manganese(II) and nickel(II) complexes were synthesized and fully characterized. Biological studies showed that the title three compounds exhibited important and different biological activities. These promising results are encouraging further screening *in vitro* and/or *in vivo*. Our continuing and detailed studies of the toxicity of these compounds, as well as mechanism of action are in process, which will be essential for medical practice.

Acknowledgements

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Appendix A. Supplementary material

CCDC 783954, 783955 and 783956 contain 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.

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[15] M. Joseph, V. Suni, C.R. Nayar, M.R.P. Kurup, H.-K. Fun, J. Mol. Struct. 705 (2004) 63.
[16] Synthesis of HL: Cyclohexyl isothiocyanate (1.41 g, 10 mmol) and hydrazine hydrate (0.50 g, 10 mmol), each dissolved in 20 ml methanol were mixed with constant stirring. The stirring was continued for 1 h and the white product, N(4)-cyclohexyl thiosemicarbazide formed was filtered, washed, dried and recrystallized from methanol. A methanolic solution of 2-acetylpyridine (0.36 g, 3 mmol) was added dropwise to a methanolic solution (30 mL) of N(4)-cyclohexyl thiosemicarbazide (0.52 g, 3 mmol) with five drops of acetic acid as catalyst. After refluxed for 4 h with stirring, the resultant solution was filtered. Colorless crystals suitable for X-ray studies were obtained by slow evaporation of its methanol solution. Anal. Calcd. for C14H20N4S: C 60.78, H 7.24, N 20.26; found C 60.53, H 7.06, N 20.74; ESI-MS (m/z): 277.2 [HL + H^+].
[17] Synthesis of complex **1**: An ethanol solution containing $Mn(ClO_4)_2{\cdot}6H_2O$ (0.18 g, 0.5 mmol) was added dropwise to an ethanol solution (30 mL) of 2-acetylpyridine N(4)-cyclohexylthiosemicarbazone (0.28 g, 1.0 mmol) and NaOAc (0.08 g, 1.0 mmol). After refluxed for 2 h with stirring, the resultant solution was filtered. Dark-red Crystals suitable for X-ray studies were obtained by slow evaporation of its ethanol solution. Anal. Calcd. for $C_{28}H_{38}MnN_8S_2$: C 55.47, H 6.27, N 18.49; found C 54.98, H 6.04, N 18.11; ESI-MS (*m/z*): 606.6 [$Mn(L)_2 + H^+$]. Complex **2** was prepared by a similar procedure to that of complex **1** using $Ni(ClO_4)_2{\cdot}6H_2O$ in place of $Mn(ClO_4)_2{\cdot}6H_2O$. By evaporation of the solvent, violet–red crystals suitable for X-ray work are separated. Anal. Calcd. for $C_{28}H_{38}NiN_8S_2$: C 55.13, H 6.23, N 18.37; found C 55.92, H 6.34, N 18.03; ESI-MS (*m/z*): 610.3 [$Ni(L)_2 + H^+$].
[18] Crystal data for HL: C14H20N4S (276.40), Crystal dimensions $0.37\times0.19\times0.17$ mm^3, triclinic, space group P−1, a = 5.889(1) Å, b = 10.236 (1) Å, c = 12.383(1) Å, α = 94.628(2)° β = 90.468(2)° γ = 90.987(2)° V = 743.86(13) Å 3, Z = 2, ρcalcd = 1.234 Mg·m−3, 2595 reflections collected, 2099 unique (Rint = 0.0454), R1 = 0.0608 [I>2σ (I)], and wR2 = 0.1850 (all data). The data were collected on a Bruker APEX-II CCD diffractometer (MoKa, λ = 0.71073 Å) at 296(2) K. All structures were solved by the direct method and refined by the full-matrix least-squares on F2 using the SHELXL-97 [19,20]. All of the non-hydrogen atoms were refined anisotropically.
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[24] Crystal data for complex 1: C28H38MnN8S2 (605.72), Crystal dimensions $0.22\times0.19\times0.18$ mm3, triclinic, space group P−1, a = 10.515(4) Å, b = 12.533 (2) Å, c = 13.177(2) Å, α = 115.923(2)° β = 98.045(3)° γ = 99.434(3)° V = 1496.2(6) Å 3, Z = 2, ρcalcd = 1.344 Mg·m−3, 5238 reflections collected, 4283 unique (Rint = 0.0177), R1 = 0.0375 [I>2σ (I)], and wR2 = 0.1084 (all data). The data were collected on a Bruker APEX-II CCD diffractometer (MoKa, λ = 0.71073 Å) at 296(2) K. All structures were solved by the direct method and refined by the full-matrix least-squares on F2 using the SHELXL-97. All of the non-hydrogen atoms were refined anisotropically.
[25] Crystal data for complex 2: C28H38NiN8S2 (609.49), Crystal dimensions $0.30\times0.27\times0.22$ mm3, monoclinic, space group P21/c, a = 11.697(1) Å, b = 20.844(1) Å, c = 12.129(1) Å, β = 92.946(1)° V = 2953.3(3) Å 3, Z = 4, ρcalcd = 1.371 Mg·m−3, 5140 reflections collected, 4179 unique (Rint = 0.0417), R1 = 0.0737 [I>2σ (I)], and wR2 = 0.2411 (all data). The data were collected on a Bruker APEX-II CCD diffractometer (MoKa, λ = 0.71073 Å) at 296(2) K. All structures were solved by the direct method and refined by the full-matrix least-squares on F2 using the SHELXL-97. All of the non-hydrogen atoms were refined anisotropically.
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[32] The minimal inhibitory concentrations (MIC, μg/mL) were estimated by the disk diffusion method. The final concentration of all cultures in Mueller-Hinon agar (MHA) for bacteria was adjusted to 106 CFU/mL and used for inoculation in the MIC test. Serial dilutions of the test compounds (dissolved in DMSO) were prepared at concentrations of 0–2000 μg/mL. Each plate was inoculated with 0.1 mL of the prepared bacterial cultures. Similarly, each plate carried a blank disc, with solvent DMSO only in the center to serve as negative control. The inoculated plates were then incubated at 37 °C for 18–20 h. The minimal inhibitory concentration (MIC) was detected as the lowest concentration of drug in plate for which no visible growth took place by macroscopic evaluation. All determinations were performed in triplicate and confirmed by three separate experiments.
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[34] K562 leukaemia cell line, (purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS) was cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U mL^{-1} of penicillin, 100 μg (200 μL per well) of streptomycin at 37 °C in humid air atmosphere of 5% CO_2. Cell cytotoxicity was assessed by the MTT assay. Briefly, cells were placed into a 96-well-plate (5×103 cells per well). The next day the compound diluted in culture medium at various concentrations was added (200 μL per well) to the wells. 48 h later 20 μL of MTT (0.5 mg mL^{-1} MTT in PBS) was added and cells were incubated for a further 4 h. 200 μL of DMSO were added to each culture to dissolve the MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a micro plate reader. Then the inhibitory percentage of each compound at various concentrations was calculated, and the IC50 value was determined.
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