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Note

Thiopyridyl triazine derivatives and their platinum complexes: a new class of potential antitumor agents

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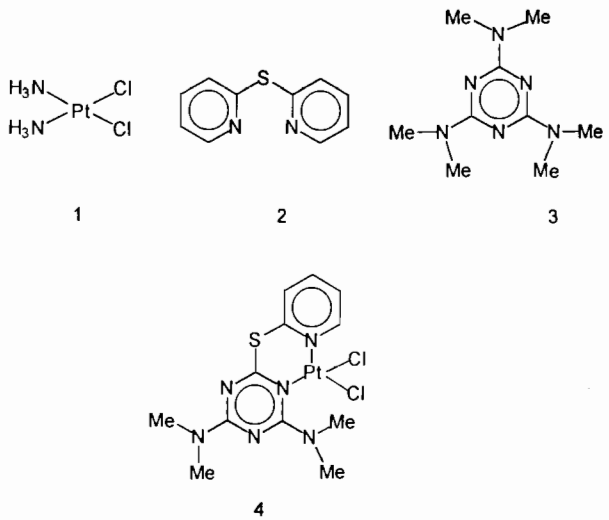
Abstract

A novel series of thiopyridyl triazine ligands and their metal(II) complexes have been synthesized. The X-ray structure of the mononuclear copper(II) complex of the ligand 6-thiopyridyl-2,4-bis(dimethylamino)-1,3,5-triazine (L5) has also been determined. The in vitro cytotoxic potency against the human colon cancer cell line (HT-29) has been determined for the ligands and their platinum complexes. All compounds including their ligands have been found to be highly potent against the HT-29 cell line. The ligand 2,4,6-trithiopyridyl-1,3,5-triazine (L2) and its platinum complex were found to be 10–30 times more potent than cisplatin.

Keywords: Antitumor agents; Platinum complexes; Thiopyridyl triazine complexes

1. Introduction

The use of chemotherapy to treat cancer has become increasingly important in recent years, because cancer is one of the most common diseases in humans, being the primary cause of death in children and the second most common cause of death in adults. Successful treatment of cancer with chemotherapy requires the preparation and characterization of new drugs with novel actions. Ideal drugs would be water soluble, more effective and less toxic. Though remarkable progress has been made in the treatment of certain malignancies with chemotherapy, there are still forms of cancer for which chemotherapy is relatively ineffective; colon cancer [1] is one of them. The chemotherapy for colon cancer is limited to 5-fluorouracil (5-FU). Its use is restricted owing to its toxicity and efficacy and the survival rate (20%) [2,3]. However, combinations of 5-FU with levamisole or leucovorin are more effective than 5-FU alone [4]. In addition to 5-FU, the only other active drugs are fluorodeoxyuridine (FUDR), nitrosourea, lomustine and mitomycin [1]. These drugs have response rates of the same order as 5-FU but have greater toxicity. Another common reason for the failure of chemotherapy is the development of multidrug resistance (MDR) during treatment [5,6]. Therefore,



1 2 3

4

Scheme 1.

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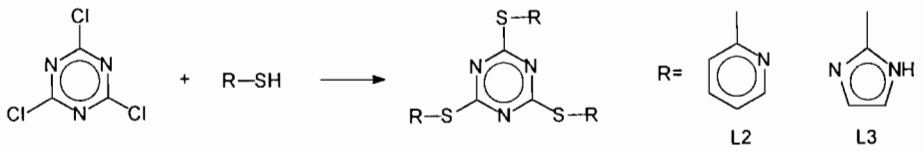
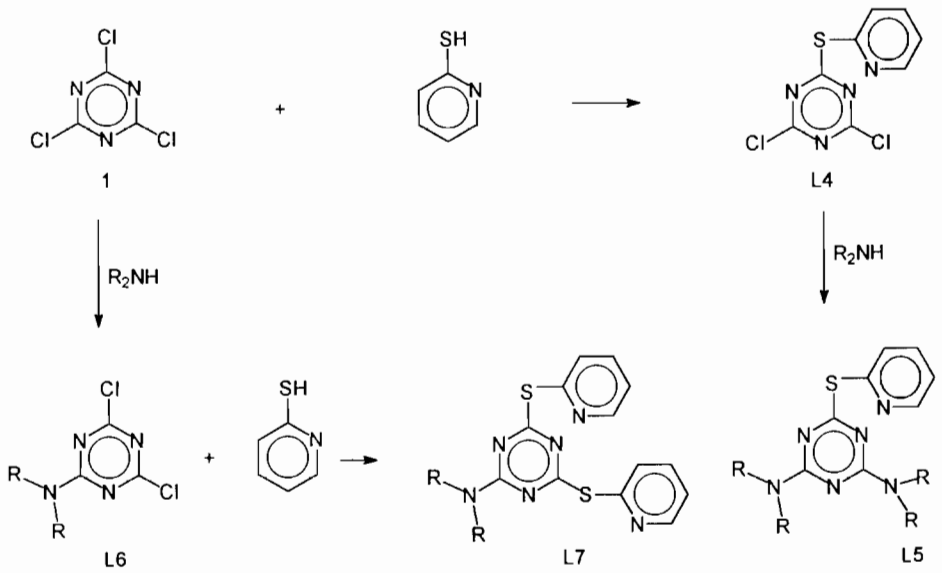


Scheme 2.



Scheme 3.

the search for new anti-colon-cancer drugs to overcome such problems is particularly important.

The focus of the present work is on developing simple, potent organic molecules and their platinum complexes to improve the activity against cell lines which are resistant to a number of chemotherapeutic agents.

The simple coordination compound cisplatin **1** (Scheme 1) is a synthetic antitumor agent with significant activity against several human tumors [7–12]. Several thiobispyridine derivatives have been found to possess a wide range of pharmacological activities. For example, 2,2′-thiobispyridine **2** (Scheme 1) shows pronounced antitumor activity in addition to other biological activities [13,14]. In general, sulfur-containing compounds are known to antagonize the toxic effects of the drugs [15]. Also, triazine derivatives have been shown to overcome MDR and possess antitumor activity [16–19]. For instance, hexamethylmelamine **3** (Scheme 1) is a potent agent against breast and ovarian cancer. Thus, we endeavored to combine the three structures **1–3** (Scheme 1) into a single unit similar to compound **4** (Scheme 1). We expected that such a compound might be more potent and perhaps have fewer toxic side effects and at the same time be able to overcome MDR. To the best of our knowledge, no information is available concerning the possible anticancer activity of such thiopyridyl triazine derivatives.

We describe here the synthesis of four new ligands and their metal complexes, and their in vitro biological activity against the HT-29 cell line.

2. Experimental

The ligands were synthesized by a straightforward one- or two-step procedure, as shown in Schemes 2 and 3. Ligand L2 (2,4,6-trithiopyridyl-1,3,5-triazine) was prepared by reacting a threefold excess of 2-mercaptopyridine and cyanuric chloride in acetone for 2 h at room temperature. The solid product that formed almost immediately was separated, washed with acetone, dissolved in water, neutralized with aqueous ammonia and extracted with chloroform. The chloroform extract was dried over anhydrous Na_2SO_4, chromatographed over alumina and eluted with $CHCl_3$. Ligand L2 was obtained in 65% yield as a white crystalline solid. Ligand L3 (Scheme 2) was obtained in 50% yield by the procedure described above. Ligand L3 was recrystallized from methanol.

The synthetic route leading to 2,4-dithiopyridyl-6-dimethylamino-1,3,5-triazine (ligand L7) is outlined in Scheme 3. 2,4-Dichloro-6-dimethylamino-1,3,5-triazine (L6) [20] was converted to 2,4-dithiopyridyl-6-dimethylamino-1,3,5-triazine (L7) by refluxing a mixture of L6 and a twofold excess of 2-mercaptopyridine in acetone for 2 h. The solid product was separated, washed with

acetone and isolated following the procedure described for L2; ligand L7 was obtained in 58% yield as a white crystalline solid. Synthesis of ligand L5, 6-thiopyridyl-2,4-bis(dimethylamino)-1,3,5-triazine, was carried out as shown in Scheme 3. The reaction of 2-mercaptopyridine with cyanuric chloride in acetone at −30 °C afforded the intermediate ligand L4 (Scheme 3) as the solid hydrochloride salt, which was shown to be hygroscopic. Ligand L4 was separated, washed with acetone and quickly transferred, covered with acetone, treated with an excess of dimethylamine solution and stirred at room temperature for 4 h. Removal of the solvent under reduced pressure gave the crude product. Ligand L5 was isolated following the same general procedure as described above. Ligand L5 was obtained in 60% yield (based on cyanuric chloride) as a white crystalline solid.

All of the ligands form mononuclear and multinuclear metal complexes, depending on the reaction conditions. Multinuclear platinum(II) complexes were prepared by mixing stoichiometric amounts of the appropriate ligand in methanol and K_2PtCl_4 in 0.5 M aqueous NaCl (30:70). Upon standing, the platinum complexes crystallized. Mononuclear metal complexes were prepared by mixing stoichiometric amounts of the appropriate ligand in methanol and the appropriate metal salt in water. One such compound was characterized by X-ray structure analysis. All compounds were confirmed by elemental analysis, IR, NMR and MS spectral data.

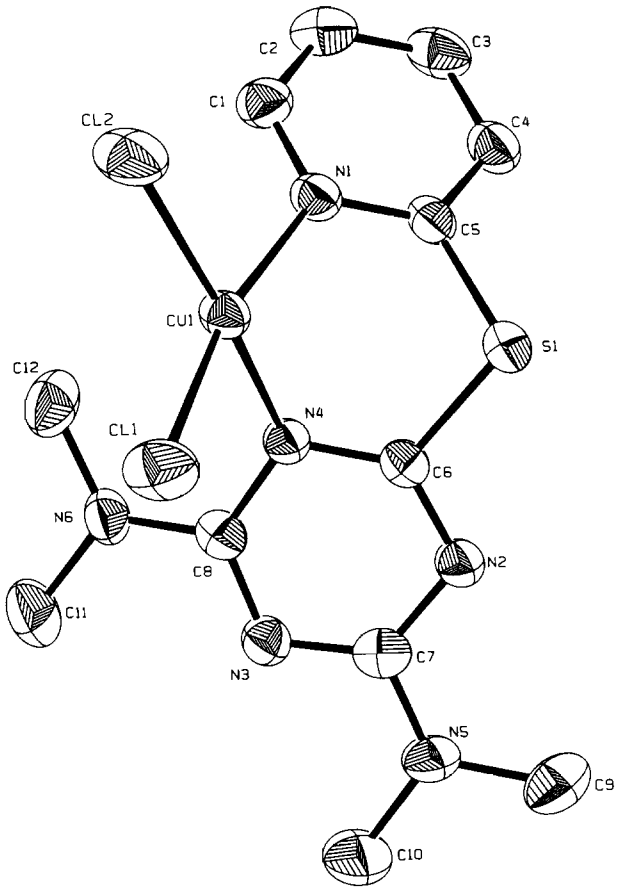


Fig. 1. The structure of [L5CuCl$_2$] with the atomic numbering system.

3. Results and discussion

The molecular structure[1] of L5CuCl$_2$ is illustrated in Fig. 1; Fig. 2 shows its packing diagram. The metal is coordinated by one triazine nitrogen atom, one pyridine nitrogen atom and two chlorine atoms, completing a CuN_2Cl_2 chromophore. The two Cu–N bond lengths are approximately equal (~2.052 Å) and are comparable to the ones found in other Cu–N containing complexes. Similarly, the two Cu–Cl bond lengths are almost equal (~2.228 Å).

The new compounds have been synthesized and screened for cytotoxicity against the human colon cancer cell line HT-29. The biological results are shown in Table 1. Preliminary screening tests showed that the metal complexes listed in Table 1 have cytotoxic activity, and all of the metal-free ligands have cytotoxic activity

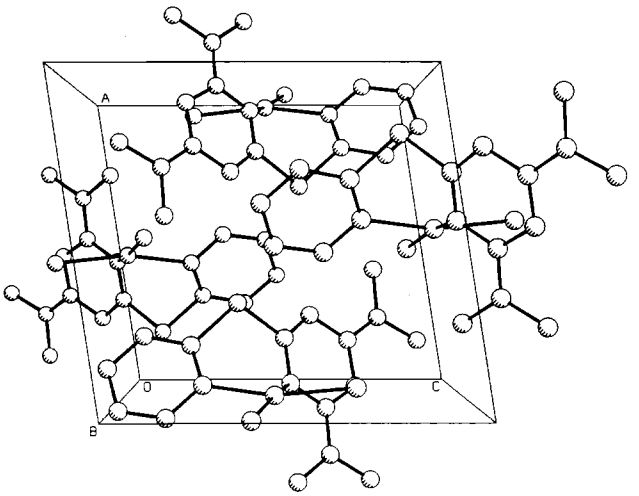


Fig. 2. Packing diagram of [L5CuCl$_2$].

in their own right, but an initial comparison of the activities of the ligands compared with the metal complexes indicates that the complexes exhibit a somewhat greater cytotoxic activity than the metal-free ligands. As shown in Table 1, ligand L2 is 125 times more potent than ligand L7 and 300 times more potent than ligand L5. Apparently, the cytotoxic activity of the

[1] Crystal data for $C_{12}H_{16}N_6SCuCl_2$: green, irregular, monoclinic, space group $P2_1/n$, $a=9.138(4)$, $b=18.819(4)$, $c=9.718(2)$ Å, $\beta=98.50(3)°$, λ (Mo Kα) = 0.71069 Å, $T=298$ K, $V=1652.8(9)$ Å^3, $Z=4$, $D_{calc}=1.651$ g cm^{-3}. 3222 independent reflections were collected in the range $37.46 < 2\theta < 50.0°$ on a Rigaku AFC6S diffractometer with graphite monochromatized Mo Kα radiation, and 2172 unique reflections were used in the analysis. Final residuals of $R=0.034$ and $R_w=0.031$ were obtained for significant reflections.

Table 1
In vitro activity of compounds against the HT-29 cell line

Compound	IC50 (μM)
Cisplatin	9.8
L2	1.0
$(L2)_2Pt_3Cl_6$	0.3
L7	125.1
$(L7)_3Pt_2Cl_4$	1.2
L5	301.1
$(L5)_5Pt_4Cl_8$	4.8
L3	69.1

ligands may be associated in some way with either the thio group or the pyridyl nitrogen group, because ligand L2 contains three thiopyridyl groups and exhibits greater potency than ligand L7, which contains two thiopyridyl groups; ligand L7 is in turn more potent than ligand L5, which contains only one thiopyridyl group. The potency of ligand L3 (which is an analogue of L2), with an IC50 value of 69 μM, established that thioheterocycles are preferred for activity enhancement. Although ligands L5 and L7 are not as potent as ligands L2 or L3, their platinum complexes have a significant potency enhancement.

The IC50 values of ligand L2 and cisplatin were found to be 1.0 and 9.8 μM, respectively. Thus, a comparison of the IC50 values reveals that the cytotoxic effect of ligand L2 by itself is 10 times greater than that of cisplatin, and the cytotoxic effect of the platinum complex of ligand L2 (IC50 = 0.3 μM) is 32 times greater than that of cisplatin. To evaluate the biological activity, the compounds were dissolved in a small volume of DMSO at room temperature prior to in vitro testing and diluted immediately with RPMI medium to a final DMSO concentration of lower than 0.5%. Although the compounds were dissolved in DMSO prior to in vitro testing, the cytotoxic activity of the compounds remained at almost the same order of magnitude even after long exposure to the mixed medium (DMSO and RPMI).

Systematic replacement of the thiopyridyl group by a dimethyl amine group in ligand L2 resulted in a significant reduction of the activity. The reasons for the enhanced activity of ligand L2 and its platinum complex are under investigation.

The results of the present study indicate for the first time that the thiopyridyl triazine derivatives form a new class of compounds with significant cytotoxic activity. At present, thiopyridyl triazine chemistry is largely unexplored for this purpose. We believe our results may lead to the development of a new class of antitumor agents.

4. Supplementary material

The experimental details, including the preparation of the ligands and the metal complexes, melting points, analytical data, NMR and mass spectral data, determination of drug sensitivity in vitro and the X-ray crystal data (25 pages), are available upon request. Ordering information is given on any current masthead page.

Acknowledgements

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