Synthesis and Antitumour Properties of Complexes with Heavy Transition Metals and Thioproline

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Metal coordination complexes, particularly those of platinum, have now been proved to be active anticancer agents in animals and man [1], but other heavy metals, as Pd(II) [2, 3], Rh(III) [2, 3] and Au(III) [4–6], have also shown promising activities in this field.

These findings prompted us to synthesize and to test as antitumour agents some complexes with heavy transition metals employing as ligand the thiazolidine-4-carboxylic acid or thioproline. This ligand was chosen because of good activity found in complexes between Pt(II), Pd(II), Rh(I) and Rh(III) and some thiazole derivatives [7–9]. Moreover the thioproline in particular aroused considerable interest as a compound capable of inducing 'reverse transformation' in tumour cells, since it caused tissue cells in culture to lose the characteristics of tumoral state [10, 11]. Clinical trials in advanced human cancer showed therapeutic activity [12], even if these results recently have been not confirmed [13].

Experimental

Preparation of Neutral Complexes

cis-*$M(L)_2X_2$ [M = Pt(II) or Pd(II), L = thioproline, X = Cl, Br, I)]*

cis-$Pt(L)_2Cl_2$ was obtained by mixing a solution of 1 g $K_2[PtCl_4]$ in 50 ml water with thioproline (molar ratio 1·2), dissolved in 25 ml of a water–ethanol mixture at 60 °C. In order to obtain the iodo-derivative, 3 g of KI in 25 ml water were added to the $K_2[PtCl_4]$ solution before ligand addition. It is possible to obtain the bromo-complex by the same procedure working with KBr instead of KI or by direct reaction of $K_2[PtBr_4]$ with the ligand. An alternative synthesis, which yields a complex of high purity, involves the reaction of KBr with the aquo ions obtained by suspending *cis*-$Pt(L)_2Cl_2$ in water in the presence of $AgNO_3$. *cis*-$Pd(L)_2X_2$ complexes were obtained by the analogous procedure using $K_2[PdX_4]$ instead of $K_2[PtX_4]$. Concentration of the reaction mixture under vigorous stirring led to precipitation of *cis*-$M(L)_2X_2$ complexes, which were filtered and washed subsequently with water, ethanol and few drops of ether. These complexes are easily soluble in DMSO or DMF, but are poorly soluble in water.

Preparation of Pt(IV), Rh(III) and Au(III) Complex Salts

$[PtX_6](LH)_2$ complex salts (X = Cl, Br) were obtained by reaction of thioproline, dissolved in 25 ml of a hot 1:1 mixture of ethanol and 6 *N* HCl, and the corresponding amount (Pt:L = 1:2) of $H_2[PtCl_6]\cdot 6H_2O$ or $K_2[PtBr_6]$ respectively dissolved in a hot 6*N* HX solution (X = Cl, Br). $[AuCl_4](LH)$ was obtained by the same procedure using $H[AuCl_4]$ in Au:L = 1:1 stoichiometry. The Rh(III) complex salts were obtained by the reaction of 1 g $Na_3[RhX_6]$, dissolved in hot 6*N* HX (respectively X = Cl, Br), with 4 equivalents of thioproline, dissolved in 50 ml ethanol set to pH 5 by adding 1*N* HCl. Concentration of the reaction mixture (under vigorous stirring) to 1/3–1/4 of its initial volume and subsequent cooling to room temperature led to the precipitation of complex salts, which were filtered and washed with ethanol and ether. All the complex salts are almost insoluble in water but quite soluble in DMSO or DMF, except for $[AuCl_4](LH)$, poorly soluble in DMSO or DMF. Yields for complexes and complex salts were in the range 70–80%; only Rh(III) derivatives were synthesized in 40–50% yields.

All these new compounds are stable in air and light exposure. Microanalyses were carried out by Dr. J. Shohet at the "ABIC" Ltd. Pharmaceutical and Chemical Laboratories (Tel-Aviv University, Israel) and are reported in Table I. The IR spectra were recorded on a Perkin Elmer 457 IR grating spectrophotometer (4000–250 cm^{-1}) in KBr, KCl or KI pellets. Nujol mulls were also employed.

Procedure for in vitro *and* in vivo *Studies of Growth Inhibition*

The cytostatic activity was evaluated on KB cells according to Geran *et al.* [14]. Minimal Eagle's Medium (MEM) [15], supplemented with 10% non

Abbreviations: BES, (N,N-bis[2-Hydroxyethyl]-2-aminoethane sulfonic acid); HEPES, (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid); TES, (N-tris[Hydroxymethyl]-methyl-2-aminoethanesulfonic acid); DMSO = dimethylsulfoxide; DMF = N,N-dimethylformamide.

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TABLE I. Analytical Data[a] and IR Spectra of the Complexes (cm^{-1}).

Compound	Colour	Metal %	N %	Hal.%	νC=N	νC–S	νC=O	$\nu N^{+}-N$	νM–X
Thioproline	white	–	–	–	1648	670	1720	–	–
cis-$Pt(L)_2Cl_2$	brownish-yellow	36.8 (36.9)	5.2 (5.3)	13.1 (13.4)	1625	670	1725	–	320, 325
cis-$Pt(L)_2Br_2$	brownish–yellow	36.6 (35.5)	4.9 (5.1)	29.0 (29.3)	1630	670	1720	–	280, 270
cis-$Pt(L)_2I_2$	brownish–yellow	27.0 (27.5)	3.9 (3.9)	35.0 (35.7)	1628	670	1720	–	250, 255
cis-$Pd(L)_2Cl_2$	brownish–yellow	44.1 (44.5)	6.1 (6.4)	16.1 (16.2)	1620	675	1720	–	310, 315
cis-$Pd(L)_2Br_2$	brownish–yellow	20.6 (20.1)	5.1 (5.3)	30.1 (30.3)	1615	675	1720	–	250, 255
$[PtCl_6](LH)_2$	yellow–orange	29.0 (29.0)	4.1 (4.2)	31.0 (31.7)	–	675	1720	2700	330
$[PtBr_6](LH)_2$	yellow–orange	20.8 (20.8)	2.0 (2.9)	52.3 (51.1)	–	670	1720	2740	280
$[AuCl_4](LH)$	brown–yellow	41.2 (41.8)	2.7 (3.0)	30.1 (30.3)	–	670	1720	2680	480
$[Rh(L)_4Cl_2]^{+}Cl^{-}$	brown–yellow	13.8 (13.9)	7.5 (7.7)	14.2 (14.5)	1630	670	1720	–	340
$[Rh(L)_4Br_2]^{+}Cl^{-}$	brown–yellow	11.7 (11.8)	6.2 (6.5)	27.3 (27.7)	1630	670	1720	–	320

[a]The calculated values are in parentheses.

TABLE II. Antitumour Activity against the L1210 and P388 Systems and Cytotoxicity against KB Cells.

Compounds	Vehicle	Dose (mg/Kg)	T/C (%)		KB ID_{50}[a] (μg/ml)
			L1210	P388	
Thioproline	–	–	–	–	$>10^2$
cis-$Pt(L)_2Cl_2$	saline	400	115	119	1.87
	slurry	200	100	115	
cis-$Pt(L)_2Br_2$	DMSO	400	109	102	5.09
cis-$Pt(L)_2I_2$	DMSO	400	100	100	36.86
cis-$Pd(L)_2Cl_2$	DMSO	400	105	101	55.77
cis-$Pd(L)_2Br_2$	DMSO	400	102	100	59.90
$[PtCl_6](LH)_2$	DMSO	400	120	122	10.67
		200	105	115	
$[PtBr_6](LH)_2$	DMSO	400	107	105	20.40
		200	104	100	
$[AuCl_4](LH)$	DMSO	400	100	100	$>10^2$
	suspension	200	100	100	
$[Rh(L)_4Cl_2]^{+}Cl^{-}$	DMSO	400	118	105	$>10^2$
		200	106	98	
$[Rh(L)_4Br_2]^{+}Br^{-}$	DMSO	400	103	102	$>10^2$
	slurry	200	102	100	

[a]All the compounds were dissolved in sterile DMSO immediately before use, except the Au(III) complex salt whixh was tested as fine DMSO suspension. Final DMSO concentration in MEM (0.5%) was tested for non-cytotoxicity. For ID_{50} determination five dose levels were used.

essential amino acids and 10% calf serum was used. The medium was buffered with TES (3 m*M*), HEPES (3 m*M*), BES (3 m*M*) and Tricine (3 m*M*) [16]. As positive control, 6-mercaptopurine was always included ($ID_{50} \cong 0.1$ μg/ml). The significance of the results was established by use of Student's *t* test ($p < 0.01$).

Antitumour activity of the new compounds was estimated against mice bearing the established L 1210 and P 388 leukaemias. The screening was carried out by the National Cancer Institute (Bethesda) according to protocols 1100 and 1200 [14]. The mean survival time of treated (T) and control (C) groups was calculated and the antitumour activity was expressed as T/C %. Values of T/C exceeding 120 were taken as indicating effectiveness.

Results and Discussion

The IR spectra of the free ligand and its complexes are listed in Table I. The presence of two intensive bands in the range 325–250 (cm^{-1}) can be an indication of *cis*-configuration for neutral complexes [17]. These bands are of course absent in the IR spectra of the pure ligand. IR spectra of the complex salts present a medium intensive band, which confirms the metal–halogen bond. The frequent shifts observed in IR spectra of neutral complexes as well as in Rh(III) complex salts for the C–N bond stretching vibration account for the coordination through tertiary N atom of ligand, as observed by Dehand for other thiazole derivatives [7]. The presence of a broad band in IR spectra of Au(III) and Pt(IV) complex salts (in the region near 2700 cm^{-1}) confirms the protonation of the N tertiary atom and the establishment of Intramolecular Hydrogen Bonding (IHB).

In Table II are reported the results obtained by screening the new compounds for antitumour activity. Results of *in vitro* assay are expressed as concentration of the compound required to inhibit growth by 50% (ID_{50}). In general the complexes with thioproline showed moderate *in vitro* cytotoxic effects; only the Pt(II) neutral complexes and Pt(IV) complex salts developed significant cytostatic activity. It is possible to note a progressive increase in ID_{50} values employing as leaving group Cl^-, Br^- or I^- respectively according to previous observations [2]. None of the compounds presented significant antitumour activity *in vivo*; only the $[PtCl_6](LH)_2$ complex salt displayed marginal antitumour effects in both test systems.

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