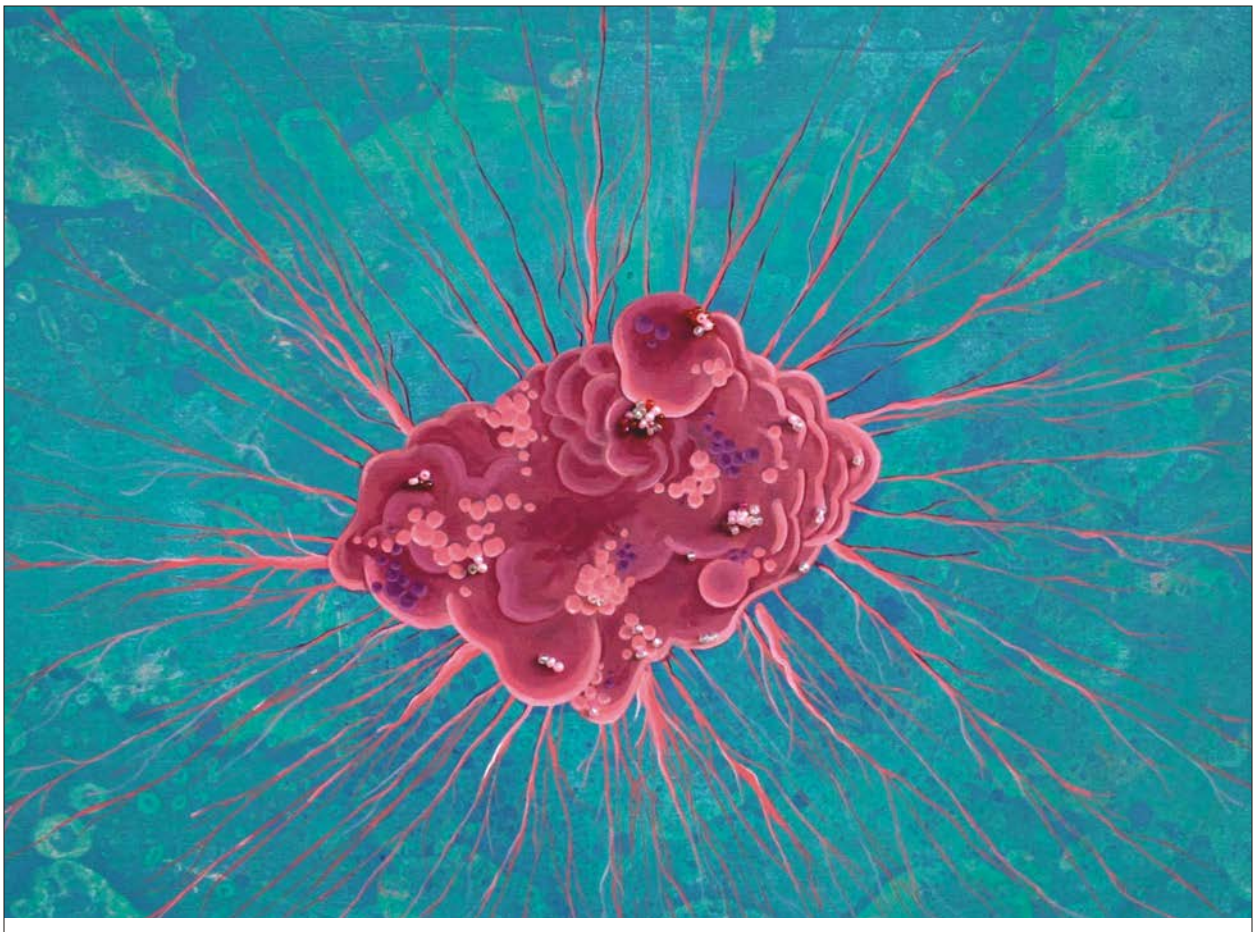

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Antitumour Activity of *cis*-bis{imidazo(1,2-α)-pyridineplatinum (II)

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Abstract: The antitumour activity of *cis*-bis{imidazo(1, 2-α) pyridine} dichloroplatinum(II) (code named AH6) against ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R} has been determined using MTT reduction assay. The activity of AH6 is compared to that of cisplatin and previously reported cisplanaramineplatinum (II) complexes code named AH3, AH4, AH5, AH7 and AH8. The compound is found to be less active than cisplatin against all the three cell lines. However, the decrease in activity of the compound in going from the parent cell line A2780 to the resistance cell line A2780^{cisR} is found to be less marked as compared to that for cisplatin, indicating that at the level of its activity AH6 has been better able to overcome resistance. The variations in activity of AH6 with those of AH3, AH4, AH5, AH7 and AH8 illustrate structure-activity relationships.

Key words: Cisplatin, transplatin, imidazo(1,2-α)pyridine, anticancer activity, MTT, resistance

Introduction

Although transplatin is inactive but toxic, mainly because of its high reactivity, *trans*-platinum complexes with bulky planar ligands are found to be active in both murine and human cisplatin-resistant tumour cell lines (Farrell *et al*., 1992). In our laboratory also a number of *trans*-planaramineplatinum (II) complexes of the forms: *trans*-$PtCl_2NH_3L$ and *trans*-$PtCl_2L_2$, where L stands for a planaramine ligand, have been prepared which have shown significant anticancer activity (Huq *et al*., 2004a, b; Chowdhury *et al*., 2005). It has also been found that *cis*-planaramineplatinum(II) complexes such as ZD0D473 show significant antitumour activity against a number of cancer cell lines (Holford *et al*., 1998). Recently we reported on the synthesis, binding with DNA and activity of the following *cis*-planaramineplatinum (II) complexes: *cis*-(3-hydroxypyridine) (ammine) dichloroplatinum (II) (code named AH3, *cis*-bis(3-hydroxypyridine) dichloroplatinum (II) (code named AH4), *cis*-{imidazo(1,2-α)pyridine}(ammine)dichloroplatinum (II) (code named AH5), *cis*-(2,3-diaminopyridine)(ammine)dichloroplatinum (II) (code named AH7) and *cis*-bis(2,3-diaminopyridine)diiodoplatinum (II) (code named AH8) (Huq *et al*., 2006a; Abdullah *et al*., 2006a-c). In the present study, we report on the antitumour activity of *cis*-bis{imidazo(1,2-α)pyridineplatinum(II) (Fig. 1) (code named AH6) and compare it with those of cisplatin and AH3, AH4, AH5, AH7 and AH8 (Huq *et al*., 2006b, c; Abdullah *et al*., 2006b, c).

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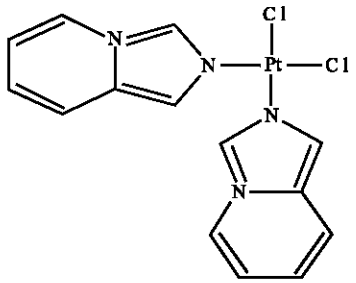

Fig. 1: Structure of AH6

Materials and Methods

Materials

Potassium tetrachloroplatinate ($K_2[PtCl_4]$), cisplatin, transplatin, N,N-dimethylformamide [DMF] [C_3H_7NO] and imidazo(1,2-α)pyridine were obtained from Sigma Aldrich Chemical Company Milwaukee USA; acetone [$(CH_3)_2CO$] and silver nitrate [$AgNO_3$] were obtained from Ajax Chemicals Auburn NSW Australia; methanol [CH_3OH] and ethanol [C_2H_5OH] were obtained from Merck Ptv. Limited Kilsyth VIC Australia.

Synthesis

AH6 has been synthesized and characterized as described previously according to modified Dhara (1970) method. Briefly 415 mg (1 mmol) of $K_2[PtCl_4]$ was dissolved in 10 mL of mQ water to which was added 2 g (about 12 mmol) of KI. This was followed by the addition with stirring of 200 μL (2 mmol) of imidazo(1,2-α)pyridine to the mixture kept in ice. The mixture, kept in ice, was stirred for 5 h following which it was left standing at room temperature for 12 h. The yellow precipitate of *cis*-Pt{imidazo(1,2-α)pyridine}$_2I_2$ was collected by filtration, washed with ice-cold water and ethanol and air-dried. The mass of the precipitate was 613 mg (0.9 mmol). The precipitate was suspended in 5 mL of mQ water to which was added 301 mg (1.77 mmol) of $AgNO_3$ with stirring in the dark. The mixture was kept in ice and stirred for 24 h following which it was centrifuged to collect the yellow supernatant to which was added 140 mg (1.88 mmol) of KCl with stirring at room temperature. The yellow precipitate of *cis*-{imidazo(1,2-α)pyridine}$_2$dichloroplatinum(II) formed immediately. The mixture was kept in ice and stirred for 24 h. The precipitate was collected by filtration, washed with ice-cold water and ethanol and air-dried. The weight of the final product was 250 mg (0.48 mmol) corresponding to 48% yield.

Cytotoxicity

Cytotoxicity of AH6 against human ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R} along with that for cisplatin was determined using MTT growth inhibition assay (Freshney, 1994; Mosmann, 1983). Briefly, between 5000 to 9000 cells seeded into the wells of the flat-bottomed 96-well culture plate in 10% FCS/RPMI 1640 culture medium, were incubated for 24 h at 37°C in a humidified atmosphere. Platinum complexes were first dissolved in a minimum volume of DMF, then diluted to the required concentrations by adding mQ water and finally filtered to sterilize. A serial fivefold dilutions of the drugs ranging from 0.02 to 62.5 μM in 10% FCS/RPMI 1640 medium were prepared and added to equal volumes of cell culture in quadruplicate wells, then left to incubate under normal growth conditions for 72 h. The inhibition of the cell growth was determined using MTT assay (Mosmann, 1983). Four hour after the addition of MTT (50 μL per well of 1 mg mL^{-1} MTT solution), the cells were dissolved in 150 mL of DMSO and read using Bio-Rad Model 3550 Microplate Reader. The IC_{50} values were obtained from results of quadruplicate determinations of at least three independent experiments.

Results and Discussion

Figure 2 gives the cell survival curves for AH6 along with those for cisplatin as applied to human ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD047R}. Table 1 gives the IC_{50} values for AH6 along with those for AH3, AH4, AH5, AH7 and cisplatin against the cell lines: A2780, A2780^{cisR} and A2780^{ZD047R}. It was reported that the IC_{50} values of AH8 could not be determined as it showed very little activity. It can be seen that although AH6 is less active than cisplatin, it is more active than two *cis*-planaramineplatinum(II) complexes AH7 and AH8 against all the three cell lines. The variations in activity of AH3, AH4, AH5, AH6, AH7, AH8 and cisplatin against the three cell lines A2780, A2780^{cisR} and A2780^{ZD047R} are described more fully as follows.

As applied to A2780 cell line, the order of the IC_{50} values from highest to lowest was AH7 > AH4 > AH6 > AH3 > AH5 > cisplatin, indicating that among the *cis*-planaramineplatinum(II) complexes AH5 and AH3 are most active whilst AH7 is least active. Although all of the *cis*-planaramineplatinum (II) complexes were found to be less active than cisplatin, the activity of AH3 and AH5 was significant (about a half of that of cisplatin). As applied to A2780^{cisR} cell line, the order of the IC_{50} values from highest to lowest was AH4 > AH7 > AH6 > AH3 > AH5 > cisplatin, indicating that among the *cis*-planaramineplatinum(II) complexes AH5 and AH3 were most active whilst AH4 was least active. As applied to A2780^{ZD0473R} cell line, the order of the IC_{50} values from highest to lowest was AH4 > AH7 > AH6 > AH5 > AH3 > cisplatin, indicating that among the *cis*-planaramineplatinum (II) complexes once again AH5 and AH3 are most active whilst AH4 is least active. It can be seen that order of activity of the *cis*-planaramineplatinum (II) complexes is slightly different for different cell lines, indicating that the cell lines must differ to some extent in their mechanisms of resistance and that the compounds differ in their ability to overcome resistance. A number of mechanisms of resistance (such as reduced cell uptake, increased deactivation by cellular

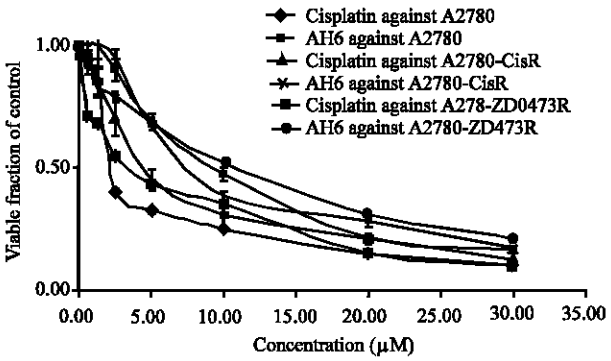

Fig. 2: Cell survival curves for AH6 and cisplatin as applied to the cell lines A2780, A2780^{cisR} and A2780^{ZD0473R}

Table 1: IC_{50} values and resistance factors for AH6 along with those for AH3, AH4, AH5, AH7, AH8 and cisplatin as applied to cell lines: A2780, A2780^{cisR}, A2780^{ZD0473R}

	A2780 IC_{50} (μM)	A2780^{cisR} IC_{50} (μM)	IC_{50}A2780^{cisR} / IC_{50}A2780 RF	A2780^{ZD0473R} IC_{50} (μM)	IC_{50}A2780^{ZD0473R} / IC_{50}A2780 RF
Cisplatin	2.2±0.5	4.5±0.4	2.05	3.5±0.6	1.59
AH3	4.1±1.0	7.1±1.0	1.73	8.0±1.0	1.95
AH4	10.8±0.6	20.6±0.5	1.91	18.4±0.6	1.7
AH5	4.0±0.5	6.5±0.7	1.63	9.6±0.6	2.4
AH6	8.6±0.6	8.0±0.5	0.93	10.6±0.6	1.23
AH7	13.7±1.4	9.9±1.3	0.72	13.2±1.0	0.96
AH8	N/A	N/A	N/A	N/A	N/A

platinophiles and increased DNA repair) (with one playing a greater than another) may be operating simultaneously in a given cell line and that a given compound is better able to overcome one mechanism than another. It should be noted that AH3 and AH5 are consistently found to be more active than AH4 and AH7. The planaramine ligand present in AH7 (as noted earlier) is 2,3-diaminopyridine, that in AH5 it is imidazo(1,2-α)pyridine and in AH3 and AH4 it is 3-hydroxypyridine. Whereas AH3 has one 3-hydroxypyridine ligand, AH4 has two 3-hydroxypyridine ligands. Much lower activity of AH4 in all the three cell lines is believed to be due to a greater stearic crowding (and hence a greater shielding of platinum) provided by the two planaramine ligands so that the formation of intra strand Pt(1,2-GG) adduct is hindered. Much lower activity of AH7 and much higher activity of AH5 in all the three cell lines suggests that whereas 2,3-diaminopyridine is a deactivating ligand, imidazo(1,2-α)pyridine is an activating one. It was reported that among four *trans*-planaramineplatnum(II) complexes of the form: *trans*-PtL(NH_3)Cl_2 where L stands for a planaramine ligand, the *trans*-analogue of AH5 code named YH12 also was most active against the cell lines: A2780 and A2780^{cisR} (Huq *et al*., 2004b). It thus appears that the presence of imidazo(1,2-α)pyridine rather than that of 3-hydroxypyridine and 2,3-diaminopyridine confers a greater antitumour activity in both *cis*- and *trans*-complexes of the type: PtL(NH_3)$Cl_2$where L is a planaramine ligand. The greater activity conferred by imidazo(1,2-α)pyridine ligand, is believed to be associated at least in part with non-covalent interactions such as stacking interaction between the ligand and nucleobases. It is generally accepted that the activity of platinum-based anticancer drugs is associated with their binding with DNA although in a recent review Bose suggests that multiple mechanisms including drug-DNA binding may determine the activity of platinum-based anticancer drugs (Bose, 2002). Although among AH3, AH4, AH5, AH6 and AH7, AH7 (which has 2,3-diaminopyridine ligand) was found to be least active against the cell line A2780, it was found to be slightly more active (much more active than AH4) against the cell line A2780^{cisR} indicating that at the level of its activity the compound was better able to overcome mechanisms of resistance (better than AH4) operating in A2780^{cisR} cell line.

Figures 3-6 give the levels of platinum accumulation (nanomoles Pt per 3×10^6 cells) as applied to the compounds AH3, AH4, AH5, AH6, AH7, AH8 and cisplatin in A2780, A2780^{cisR}, A2780^{ZD0473R} cells, respectively.

It can be seen that for all the compounds: cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8, the level of platinum accumulation in all the three cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R} increases significantly with the increase in duration of incubation from 0 to 24 h. The increase is more pronounced for cisplatin, AH7 and AH8 but unlike cisplatin (which is highly active against the cell line), AH4, AH6, AH7 and AH8 display much lower activity or no activity at all against A2780 cell

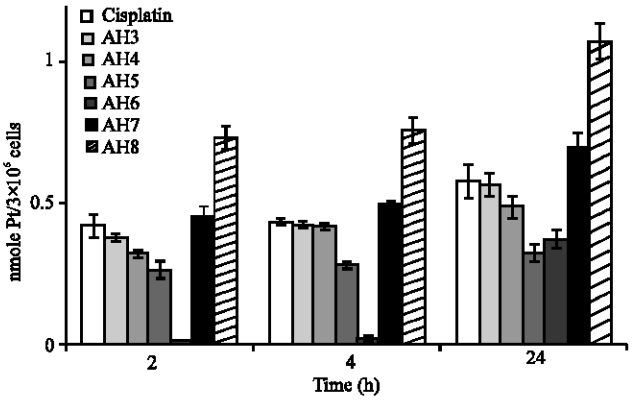

Fig. 3: Pt accumulation in A2780 cell line as applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

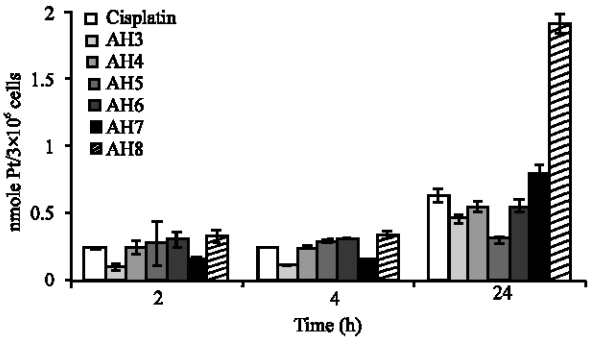

Fig. 4: Pt accumulation in A2780^{cisR} cell line as applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

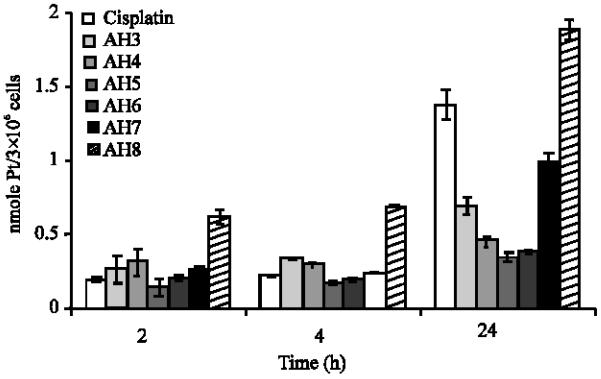

Fig. 5: Pt accumulation in A2780^{ZD0473} cell line as applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

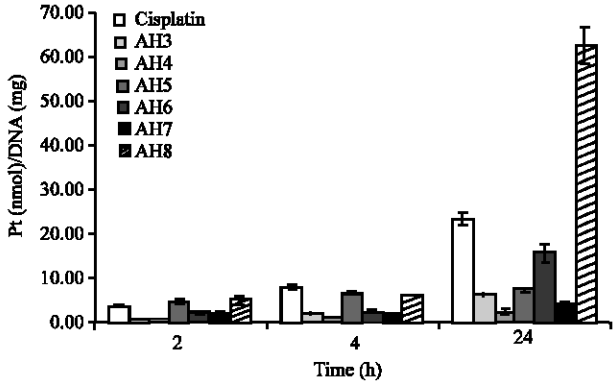

Fig. 6: Level of Pt-DNA binding in A2780 as applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

line. However, as noted earlier, AH7 is found to be more active against A2780^{cisR} cell than the parent cell line A2780. The results show that the level of platinum accumulation per se may not provide a measure of activity of platinum-based anticancer drugs. Even the level of platinum-DNA binding may not provide an accurate picture of the activity of the compounds or lack of it. For example, AH8

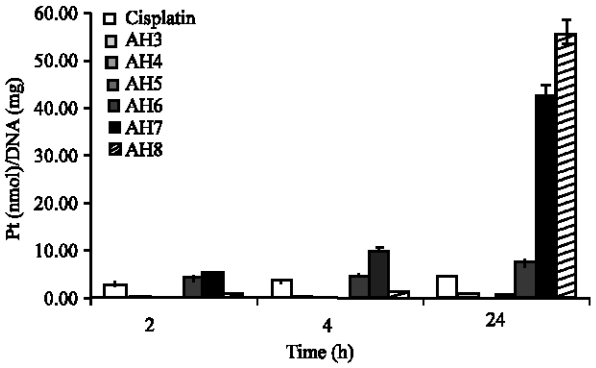

Fig. 7: Level of Pt-DNA binding in A2780^{cisR} applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

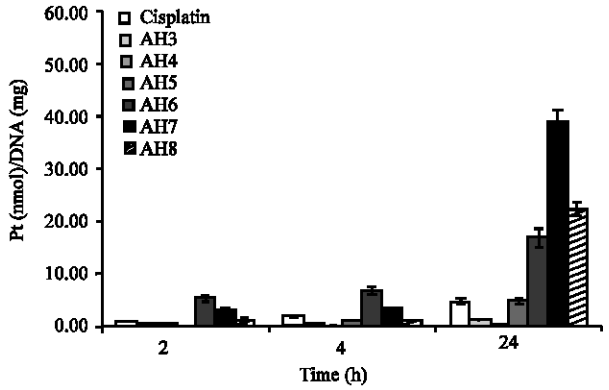

Fig. 8: Level of Pt-DNA binding in A2780^{ZD0473R} as applied to cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in 2, 4 and 24 h, respectively

(which was found to be totally inactive) had a higher cell uptake and a higher platinum-DNA binding than cisplatin as applied to all the three cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R}. Figure 6-8 give the levels of platinum-DNA binding as applied to the compounds: cisplatin, AH3, AH4, AH5, AH6, AH7 and AH8 in A2780, A2780^{cisR}, A2780^{ZD0473R} cells, respectively

As applied to the cell line A2780, the order of Pt-DNA binding in 24 h from highest to lowest was: AH8 > cisplatin > AH6 > AH5 > AH3 > AH7 > AH4. The low level of Pt-DNA binding observed for AH7 and AH4 are in line with their low activity against the cell line. However, moderately high level of Pt-DNA binding observed for AH6 and exceptionally high level Pt-DNA binding observed for AH8 do not correlate with their low activity. It was suggested that the high cell uptake of AH8 was associated with the nature of the compound. AH8 is less dissociated than the other compounds (AH3, AH4, AH5, AH6 and AH7) because of a greater covalent character of the Pt-I bond than Pt-Cl bond and also because of a greater stearic crowding provided by two planaramine ligands (Huq *et al*., 2006a-c). It is suggested that the greater level of Pt-DNA binding of AH8 does not equate to the formation of a greater level of critical intra strand Pt(1,2-GG) adduct that is responsible for the bending of a DNA strand. It is likely that in the case of AH8, most of the Pt-DNA lesions will be Pt(G) and Pt(A) monofunctional adducts. Because of the bulky nature of the planaramine ligands, AH3, AH4, AH5, AH6 and AH7 may not form intra strand bifunctional Pt(1,2-GG) adducts as well

as cisplatin so that a significant proportion of Pt-DNA adducts in all the *cis*-planaramineplatinum (II) complexes of the present study may be either monofunctional adducts and/or DNA-Pt-protein cross links, as it was found in other such compounds (Gonzalez *et al*., 2001). As applied to the cell line $A2780^{cisR}$, the order of Pt-DNA binding in 24 h from highest to lowest was: AH8 > AH7 > AH6 > Cisplatin > AH3 > AH5 > AH4. The lowest level of Pt-DNA binding observed for AH4 is in line with its low activity and as noted before highest level of Pt-DNA binding observed for AH8 does not correlate with its lowest activity. As applied to the cell line $A2780^{ZD0473R}$, the order of Pt-DNA binding in 24 h from highest to lowest was: AH7 > AH8 > AH6 > AH5> Cisplatin > AH3 > AH4. Once again, the lowest level of Pt-DNA binding observed for AH4 is in line with its low activity but the high level of Pt-DNA binding observed for AH8 does not correlate with its lowest activity.

Another point to note is that although AH4 and AH5 are less active than cisplatin, the decrease in activity of the compounds in going from cisplatin-responsive cell line: A2780 to the resistant cell lines: $A2780^{cisR}$ and $A2780^{ZD0473R}$ is less marked than that for cisplatin. The results suggest that at the level of their activity, the compounds have been able to overcome mechanisms of resistance operating in $A2780^{cisR}$ and $A2780^{ZD0473R}$ cell lines. AH4 is found to be slightly more active in $A2780^{cisR}$ cell line than in A2780 cell line. As stated earlier, like cisplatin, AH4 and AH5 are expected to form monofunctional and intra strand bifunctional adducts with DNA. It is possible that due to the presence of one or two planaramine ligands, the reactivity of AH4 and AH5 is reduced as compared to cisplatin. Also, AH4 and AH5 may undergo intercalation with DNA.

When we compare the IC_{50} values of AH4 and AH5 in A2780 cell line, it is found that the value for AH5 (which has one planaramine ligand) is significantly lower than that for AH4 which has two planaramine ligands per molecule. However, for the resistant cell lines, the IC_{50} values for the compounds are comparable. Because the ligands: 3-hydroxypyridine and imidazo(1,2-α)pyridine are different in size (the former one has one ring whilst the latter has two fused rings) and presence of functional groups (e.g., whilst the former has a hydroxyl group the latter does not), the differences in activity of the compounds cannot be explained based on stearic factors alone. It would be interesting to find out whether *cis*-(ammine)(3-hydroxypyridine)dichloroplatinum(II) (that would contain one 3-hydroxypyridine ligand) is more active or not than AH4 that contains two 3-hydroxypyridine ligands.

Further Comment on the Difference in Activity of cis- and trans-isomers

As stated earlier, one of the two *cis*-planaramineplatinum (II) complexes in the present study (AH5) is found to be less active than its *trans*-counterpart *trans*-{imidazo(1,2-a)pyridine} (ammine)dichloroplatinum (II) (Huq *et al*., 2004a, b), the *trans*-isomer being about 2.2 times as active as the *cis*-isomer against $A2780^{cisR}$ ovarian cell line. AH4 is found to be less active than its *trans*-counterpart (Chowdhury *et al*., 2005) (the *trans*-isomer being 1.59 times more active) against A2780 cell line but marginally more active than its *trans*-counterpart (about 1.3 times more) against the cell line $A2780^{cisR}$. The results suggest that both AH4 and AH5 are better able to overcome resistance in $A2780^{cisR}$ cell line than their *trans*-counterparts.

Since the isomers differ in the nature of bifunctional adducts that can be formed (intra strand in the case of *cis*-isomer and interstrand in the case of *trans*-isomer), the results can be seen to provide support to the idea that a possible mechanism of cisplatin resistance in $A2780^{cisR}$ cell line is associated with repair of platinum-DNA lesions.

Conclusions

The *cis*-planaramineplatinum(II) complex: [*cis*-bis{imidazo(1,2-α)pyridine}dichloroplatinum (II)] (code named AH6) is found to be less active than cisplatin against the three ovarian cell lines: A2780, $A2780^{cisR}$ and $A2780^{ZD0473R}$ but more active than some other *cis*-planaramineplatinum(II) complexes. The variations in activity of AH6 with those of AH3, AH4, AH5, AH7 and AH8 shown structure-activity relationships.

Abbreviations

AH3: *cis*-(3-hydroxypyridine)(ammine)dichloroplatinum(II)
AH4: *cis*-bis(3-hydroxypyridine)dichloroplatinum(II)
AH5: *cis*-{imidazo(1,2-α)pyridine}(ammino)dichloroplatinum(II)
AH6: *cis*-bis{imidazo(1,2-α)pyridineplatinum(II)
AH7: *cis*-(2,3-diaminopyridine)(ammine)dichloroplatinum(II)
AH8: *cis*-bis(2,3-diaminopyridine)diiodoplatinum(II)
Cisplatin: *cis*-dichlorodiamminplatinum(II)
DMSO: Dimethyl sulfoxide
AAS: Atomic Absorption Spectrophotometry
Adenine: A
Guanine: G
YH9: *trans*-(2-hydroxypyridine)(ammine)dichloroplatinum(II)
YH10: *trans*-(imidazole)(ammine)dichloroplatinum(II)
YH11: *trans*-(3-hydroxypyridine)(ammine)dichloroplatinum(II)
YH12: *trans*-(imidazo(1,2-a-pyridine))(ammine)dichloroplatinum(II)
HMG: High-mobility Group.

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