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Synthesis of a Series of *cis*-Diamminedichloro-platinum (II) Complexes Linked to Uracil and Uridine as Candidate Antitumor Agents.

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The search for platinum (II)-based compounds with improved therapeutic properties was prompted to design and synthesize a new family of water-soluble, third generation cis-diamminedichloroplatinum (II) complexes linked to uracil and uridine. Six heretofore undescribed uracil and uridine-platinum (II) complexes are; [N-(2-aminoethyl)uracil-5-carboxamide]dichloroplatinum (II) (**3a**), [N-(2-aminoethyl)uracil-6-carboxamide]dichloroplatinum (II) (**3b**), [5-(2-aminoethyl)carbamoyl-2',3',5',-tri-O-acetyluridine] dichloroplatinum (II) (**6a**), [6-(2-aminoethyl) carbamoyl-2',3',5',-tri-O-acetyluridine] dichloroplatinum (II) (**6b**), [5-(2-aminoethyl)carbamoyluridine]dichloroplatinum (II) (**7a**), [6-(2-aminoethyl)carbamoyluridine]dichloroplatinum (II) (**7b**).

These analogues were prepared from the key starting materials, 5-carboxyuracil (**1a**) and 6-carboxyuracil (**1b**) which were reacted with ethylenediamine to afford the respective N-(2-aminoethyl)uracil-5-carboxamide (**2a**) and N-(2-aminoethyl)uracil-6-carboxamide (**2b**). The cisplatin complexes **3a** and **3b** were obtained through the reaction of the respective **2a** and **2b** with potassium tetrachloroplatinate (II). The heterocyclic nucleic acid bases **1a** and **1b** were efficiently introduced on the β-D-ribose ring via a Vorbruggen-type nucleoside coupling procedure with hexamethyldisilazane, trimethylchlorosilane and stannicchloride under anhydrous acetonitrile to yield the stereospecific β-anomeric 5-carboxy-2',3',5'-tri-0-acetyluridine (**4a**) and 6-carboxy-2',3',5'-tri-0-acetyluridine (**4b**), respectively. The nucleosides **4a** and **4b** were coupled with ethylenediamine to provide the respective 5-(2-aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine (**5a**) and 6-(2-aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine (**5b**). The diamino-uridines **5a** and **5b** were reacted with potassium tetrachloroplatinate (II) to give the novel nucleoside complexes, **6a** and **6b**, respectively which were deacetylated into the free nucleosides, **7a** and **7b** by the treatment with CH_3ONa. The antitumor activities were evaluated against three cell lines (K-562, FM-3A and P-388).

Key words : cis-Diamminedichlorplatinum (II), [N-(2-Aminoethyl)uracil-5-carboxamide]dichloroplatinum (II), [N-(2-Aminoethyl)uracil-6-carboxamide]dichloroplatinum (II), [5-(2-Aminoethyl) carbamoyl-2',3',5'-tri-0-acetyluridine] dichloroplatinum (II), [6-(2-Aminoethyl)carbamoyl-2',3', 5'-tri-0-acetyluridine] dichloroplatinum (II), [5-(2-Aminoethyl)carbamoyl uridine]dichloroplatinum (II), [6-(2-Aminoethyl)carbamoyl uridine]dichloroplatinum (II) β-anomeric 5-carboxy-2',3', 5'-tri-0-acetyluridine, 6-carboxy-2',3',5'-tri-0-acetyluridine, 5-(2-Aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine, 6-(2-Aminoethyl)carbamoyl-2',3',5'-tri-0-acetyl)uridine, antitumor activities, Human chronic myelogenous leukemia cell (K562), Mouse lymphoid neoplasma cell (P-388), Mouse mammary carcinoma cell (FM-3A)

INTRODUCTION

The clinical utility of the presently widely used antitumor agent cisplatin (cis-diamminedichloplatinum (II)) is well established (Korolkovas,1988; Nicolini, 1988), but their widespread clinical use is limited by inherent resistance (limited activity against many common human cancers), by intrinsic or acquired drug resistance (reduc-ed efficacy upon repeated treatment) and by their relative toxic side effects (Andrews, 1992). The carboplatin (cis-diammine-(1,1-cyclobutane dicarboxylato)platinum (II) is the only clinically succesful second generation platinum (II) complexes (Harland, *et al.*, 1984). It does not exhibit significant nephrotoxicity and emesis, and its relatively

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lower toxicities as compared to those of cisplatin have been related to the greater pharmacokinetic stability of its 1,1-cyclobutane-carboxylate ligand in solution (Bitha, *et al.*, 1989). Nevertheless, it still has two other draw- backs. Just like cisplatin,it only effects a narrow range of tumors and causes the development of resistance in the tumor cell. In considering a third generation platinum complexes for clinical development, a key focus of drug design will be the ability of an agent to improve a broader spectrum of activity, increased clinical efficcacy less severe side-effects, lack of cross-resistance to cisplatin and enhanced water-solubility for systematic use (Burchenal, *et, al.*, 1979).

The need for platinum (II) complexes with improved characteristics stimulate our drug design of several biologically important nucleic acid base (uracil and uridine)-based ligands that may have greater water-solubility and less general systematic toxicity than cisplatin. We have synthesized six heretofore undescribed platinum (II) complexes of the amino analogues of uracil and uridine ; uracil analogues, [N-(2-Amonoethyl)uracil-5-carboxamide]dichloroplatinum (II) (**3a**) and [N-(2-Aminoethyl)uracil-6-carboxamide]dichloroplatinum (II) (**3b**), and uridine nucleoside analogues, [5-(2-Aminoethyl) carbamoyl-2',3',5'-tri-0-acetyluridine]dichloroplatinum (II) (**6a**),[6-(2-Aminoethyl)carbomoyl-2',3',5'-tri-0-acetyluridine]dichloroplatinum (II) (**6b**), [5-(2-Aminoethyl)carbamoyl-uridine]dichloroplatinum (II) (**7a**), and [6-(2-Aminoethyl)carboomoyluridine]dichloroplatinum (II) (**7b**). Antitumor activities of those synthesized compounds were evaluated against the following three cell lines;

(a) human chronic myelogenous leukemia cell(K-562);

(b) mouse lymphoid neoplasma cell(P-388);

(c) mouse mammary carcinoma call(FM-3A).

MATERIALS AND METHODS

Melting points were determined on electrothermal capillary melting point apparatus and are uncorrected. TLC was performed on glass plates coated with silica gel (silica gel 60 F_{254}) and compounds were visualized using an UV lamp. Proton magnetic resonance spectra were obtained with Varian EM-360A spectrophotometer and Varian Gemini 200 MHz (solution in dimethylsulfoxide-d_6 with tetramethyl-silane as internal standard). Ultraviolet spectral data were measured with Hitachi 124 spectrometer. The organic solvents and chemicals were obtained from the commercial and purified by the appropriate methods before use.

N-(2-Aminoethyl)uracil-5-carboxamide (2a).

5-carboxyluracil (0.3 g,1.72 mmol) was added under N_2 to a stirred solution of dry ethylenediamine (20 ml) and heated at 120°C for 15 hours. The reaction mixture was evaporated in vaccuo to give light brown solids which were recrystallized from CH_3OH, brown solid (82%): m.p >300°C; Mass m/z 199 (M^+); IR (KBr) 3159 cm^{-1} (N-H), 3029 cm^{-1} (C-H), 1677 cm^{-1} (C=O) ; ^{1}H-NMR (D_2O) δ 3.37 (t, 2H, $NHCH_2$), δ 3.15 (t, 2H, CH_2NH_2), 7,61 (s, 1H, H_6).

N-(2-Aminoethyl)uracil-6-carboxamide (2b)

The same procedure described above in compound **2a** was employed for the preparation of **2b** to give a yellow solid (79%): m.p >300°C; Mass m/z 199(M^+); IR (KBr) 3135 cm^{-1} (N-H), 2939 cm^{-1} (C-H), 1651 cm^{-1} (C=O); ^{1}H-NMR(D_2O) δ 3.41 (t, 2H, $NHCH_2$), δ 3.23 (t, 2H, CH_2NH_2), δ 5.72 (s, 1H, H_5).

[N-(2-Aminoethyl)uracil-5-carboxamide]dichloroplatinum (II) (3a)

To a stirred solution of K_2PtCl_4 (0.21 g,0.5 mmol) in distilled H_2O (2 ml) was added under N_2 **2a** (0.1 g,0.5 mmol) in deionized H_2O (2 ml). The basic, homogenous reaction mixture (pH=8) was continously stirred at 70°C for 48 hours (pH=4 was achieved), then 5% aqueous KCl (10 ml) was added and the mixture was stirred for an additional one hour. The precipitate was collected, washed several times with deioniged water, and dried to give grey solid (35%) An analytical sample was obtained by chromatograaphy on silica gel and elution with CH_2Cl_2-CH_3OH (10 : 1): m.p>300°C; Mass m/z 472(M^+); IR (KBr) 3160 cm^{-1} (N-H), 3050 cm^{-1} (C-H), 1685 cm^{-1} (C=0); ^{1}H-NMR (DMSO-d_6 δ 3.46(t,2H, $NHCH_2$) δ 3.28 (t, 2H, CH_2NH_2), δ 7.80 (s, 1H, H_6).

[N-(2-Aminoethyl)uracil-6-carboxamide]dichlotoplatinum (II) (3b)

The same procedure described above in compound **3a** was employed for the preparation of **3b** to give grey solid (32%): m.p>300°C Mass m/z 472(m^+); IR (KBr) 3134 cm^{-1} (N-H), 2939 cm^{-1} (C-H), 1660 cm^{-1} (C =O); ^{1}H-NMR (DMSO-d6) δ 3.54 (t, 2H, $NHCH_2$), δ 3. 37 (t, 2H, CH_2NH_2), 5.60 (s, 1H, H_5).

5-Carboxy-2',3',5'-tri-O-acetyluridine (4a)

To a stirred mixed solution of 5-carboxyuracil (0.3 g, 1.72 mmol) and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (0.55g,1.72 mmol) in anhydrous CH_3CN (30 ml) under N_2 was added hexamethyldisilazane and trimethylsilyl chloride, followed by $SnCl_4$. The reaction mixture was stirred at room temperature for 24 hours, and evaporated in vaccuo to give yellow sirupy residues. The residues were dissolved in CH_2Cl_2 (30 ml) and washed with saturated $NaHCO_3$ (2×20

ml) and H_2O (2×20 ml), and the organic CH_2Cl_2 solution was dried over anhydrous $MgSO_4$. Filtration, evaporation and chromatography on silica gel (CH_2Cl_2-CH_3OH (20 : 1)) gave yellow sirupy residue (42%): IR (KBr) 3177 cm^{-1} (br,O-H), 1700 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ 2.11 (s, 9H, CH_3CO_2-), δ 4.05-4.71 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$), δ 5.31 (m, 1H, $H_{4'}$), δ 5.89 (d, 1H, H_6), δ7.28 (s, 1H, H_6).

6-Carboxy-2',3',5'-tri-O-acetyluridine (4b)

The same procedure described above in compound 4a was employed for the preparation of 4a to give yellow sirupy residue (39%): IR (KBr) 3177 cm^{-1} (br, O-H), 1700 cm^{-1} (C=O) δ 2.13 (s, 9H, CH_3CO_2-), δ 4. 05-4.48 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$), δ 5.31-5.97 (m, 3H, $H_{1'}$, $H_{4'}$, H_5).

5-(2-Aminoethyl)carbamoyl-2',3',5'-tri-O-acetyluridine (5a)

5-Carboxy-2',3',5'-tri-0-acetyluridine (0.3 g,0.78 mmol) was added under N_2 to a stirred solution of dry ethylenediamine (0.8 ml,7.8 mmol), and refluxed for 24 hours. The reaction mixture was evaporated in vaccuo to afford yellow oily residues which were chromatographed on silica gel and elution with CH_2Cl_2-CH_3OH (20 : 1), light brown solid (73%) : m.p> 300°C; Mass m/z 469 (M^+) : IR (KBr) 3185 cm^{-1} (N-H), 2943 cm^{-1} (C-H), 1659 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ 2.14 (s, 9H, CH_3CO_2-), δ 3.12-3.31 (m, 4H, CH_2CH_2-),; 4.06-4.20 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$), 5.07-5.28 (m, 2H, $H_{1'}$, $H_{4'}$), δ 7.24 (s, 1H, H_6).

6-(2-Aminoethyl)carbamoyl-2',3',5'-tri-0-acetylusidine (5b)

The same procedure described above in compound **5a** was employed for the preparation of **5b** to give yellow solid (81%) : m.p>300°C; Mass m/z 469(M^+); IR (KBr) 3179 cm^{-1} (N-H), 3008 cm^{-1} (C-H), 1648 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ 2.19 (s, 9H, CH_3CO_2-), δ 2.97-3.15 (m, 4H, CH_2CH_2-), δ 3.24-4.18 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$), δ 4.61-5.54 (m, 3H, $H_{1'}$, $H_{4'}$, H_5).

[5-(2-Aminoethyl)carbamoyl-2',3',5'-tri-0-acetylusidine]dichloroplatinum(II) (6a)

To a stirred solution of K_2PtCl_4 (0.27 g,0.68 mmol) in deionized H_2O (3 ml) was slowly added under N_2 **5a** (0.1 g, 0.68 mmol) in deioniged H_2O (2 ml). The basic, homogenous reaction mixture was continously stirred at 70°C for 36 hours until pH of 4 was achieved, then 5% aqueous KCl (20 ml) was added and the mixture was stirred for an additional one hour. The precipitate was collected, washed several times with deioniged H_2O (10 ml), and dried to give brown solid (40%) : m.p >300°C; Mass m/z 724 (M^+); IR(KBr) 3188 cm^{-1} (N-H), δ 2913 cm^{-1} (C-H), 1665 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ 2.10 (s, 9H, CH_3CO_2-), δ 3.25-3.48 (m, 4H, CH_2CH_2-), δ 4.11-4.52 (m, $H_{2'}$, $H_{3'}$, $H_{5'}$), δ 5. 10-5.35 (m, 2H, $H_{1'}$, $H_{4'}$), δ 7.24(s, 1H, H_6).

[6-(2-Aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine]dichloroplatinum (II) (6b)

The same procedure described above in compound **6a** was employed for the preparation of **6b** to give light reddish solid (38%) : m.p>300°C; Mass m/z 724 (M^+); IR (KBr) 3190 cm^{-1} (N-H), 3009 cm^{-1} (C-H), 1654 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ 2.10 (s, 9H, CH_3CO_2-), δ 3.25-3.41 (m, 4H, CH_2CH_2-) δ 3.82-4.41 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$), δ 4.64-5.52 (m, 3H, $H_{1'}$, $H_{4'}$, H_5).

[5-(2-Aminoethyl)carbamoyluridine]dichloroplatinum (II) (7a)

To a stirred solution of 6a (0.1 g,0.25 mmol) in a mixed solvent of DMSO (10 ml) and CH_3OH(10 ml) was added CH_3ONa (0.25 mmol), and the reaction mixture was stirred at room temperature for 15 hours. On the end of the deacetylation, Dowex-50 (H^+) ion exchange resin (5 ml) was added, and the mixture was filtered and the resin was washed with CH_3OH (3×10 ml). The combined filtrate and washings were concentrated in vaccuo to give brown residues which were crystallized from CH_3OH, brown solid (70%) : m. p>300°C; ^{1}H-NMR (DMSO-d_6) δ 3.22-3.86 (m, 6H, -CH_2CH_2-, $H_{2'}$, $H_{3'}$), δ 4.28-5.10 (m, 4H, $H_{1'}$, $H_{4'}$, $H_{5'}$), δ 7.26 (s, ^{1}H, H_6).

[6-(2-Aminoethyl)carbamoyluridine]dichloroplatinum (II) (7b).

The same procedure described above in compound **7a** was employed for the preparation of **7b** to give grey solid (82%) : m.p>300°C; ^{1}H-NMR (DMSO-d_6) δ 3.15-3.81 (m, 6H, -CH_2CH_2-, $H_{2'}$, $H_{3'}$), δ 4.49-5.61 (m, 5H, $H_{1'}$, $H_{4'}$, $H_{5'}$, H_5).

Evaluation of Antitumor Activity

The antitumor effect of the synthesized compounds was determined by the modified method (Mosmann, *et al.*, 1983; Carmichael *et al.*, 1987; Kim, *et al.*, 1994 a-c); MTT-Microculture Tetrazolium Assay. The assay is dependent on the cellular reduction of water-soluble MTT (Sigma Chemical Co., St. Louis, M.O) by the mitochondrial dehydrogenase of vial cells to a blue water-nonsoluble formazan crystal product which can be measured spectrophotometrically.

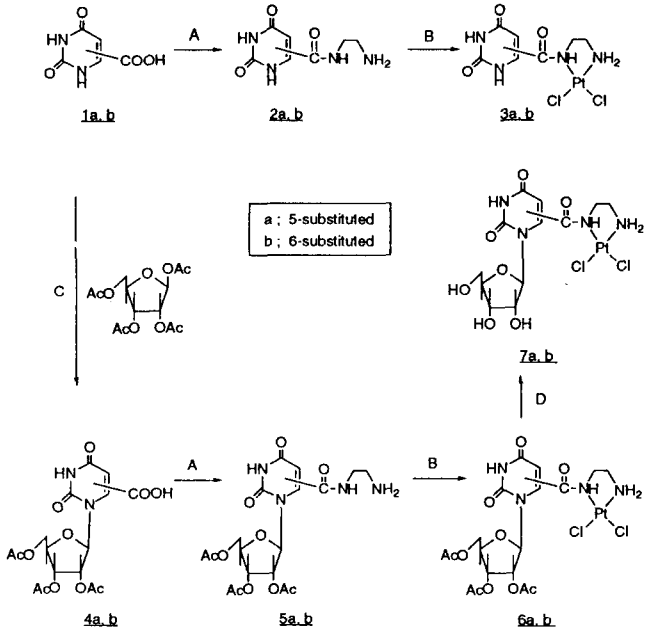
Following approproate incubation of cells (P-388, FM-3A and K-562 cells) in the presence or absence of synthesized-compounds, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added to each well and incubated at 37°C for further

4 h before processing as described below. For cell growth, serially increasing cell numbers were plated in different columns across 96-well microtiter plates. Well growing cells were harvested,counted and inoculated at the concentration of 2×10^4 cells/ml into 96-well microtiter plates. After 24h,synthesized compounds were applied to triplicate culture wells and culture were incubated an 37°C for 3 days. Following this incubation, 20 μl of MTT solution (5 mg/ml in phosphate buffer solution; KCl 0.2 g, KH_2PO_4 0.2 g, NaCl 8.0 g, Na_2HPO_4 1.15 g, $MgCl_2$ 0.101 g/l, pH-7.4) was added to microculture wells. After 4 h incubation at 37°C, the supernatant was removed from each well and 100 μl of 100% DMSO was added to solubilize the formazan crystals which were formed by the cellular reduction of MTT. After thorough mixing with a mechanical plate mixer, absorbance spectra was read on ELISA Processor Microplate Reader (Behring Co.) at a wavelength of 570 nm and a reference wavelength of 650 nm (absorbance peak for DMSO). All measurements were carried out in triplicates.There was good reproducibility between replicate wells with standard errors +10% (Table I).

Table I. IC_{50} Values for Uracil-Platinum (II) complexes **3a**, and **3b** and Uridine-Platinum (II) complexes **6a**, **6b**, **7a** and **7b**

Compounds	IC_{50} (μg/ml)[a]		
	K-562[b]	FM-3A[c]	P-388[d]
3a	52	39	41
3b	34	31	32
6a	69	73	71
6b	34	34	34
7a	34	43	34
7b	19	49	30

[a] mean values of triplicate runs. The concentratation of synthesized compounds required to reduce cell number to 50% of controls in a growth inhibition assay.
[b] Human chronic meylogenous leukemia cell.
[c] Mouse mammary carcinoma cell
[d] Mouse lymphoid neoplasma cell



[a] Reagents : A ethylenediamine, reflux, 24hrs ; B K_2PtCl_4, H_2O, 78°C, 48hrs, N_2 C HMDS, TMS-Cl, CH_3CN, $SnCl_4$; D i) CH_3ONa, DMSO-CH_3OH, ii) Dowex-50 (H^+)

Scheme[a]: Synthesis of Cisplatin Complexes-bearing Uracils and Uridines, **3a, b, 6a, b, 7a, b**

RESULTS AND DISCUSSION

A number of uracil platinum (II) complexes, **3a** and **3b**, and uridine nucleoside platinum (II) complexes **6a**, **6b**, **7a** and **7b**, have been synthesized by the treatment of the diamine-uracils, **3a** and **3b**, or diamine-uridine nucleosides, **5a** and **5b** with the appropriate molar ratio of potassium tetrachloroplatinate in deionized water at 78°C for 48 hours. The starting 5-carboxyuracil **1a** and 6-carboxyuracil (orotic acid) **1b** were reacted with ethylenediamine to afford the respective N-(2-aminoethyl)uracil-5-carboxamide **2a** and N-(2-aminoethyl)uracil-6-carboxamide **2b** outlined in Scheme. The reactions of the starting materials **1a** and **1b** with 1,2,3,5-tetra-0-acetyl-β-D-ribofuranose under hexamethyldisilagane, and trimethylsilyl chloride followed by the addition of stannic chloride, afforded 5-carboxy-2',3',5'-tri-0-acetyluridine **4a** and 6-carboxy-2',3',5'-tri-0-acetyluridine **4b**, respectively.

The uridine nucleosides **4a** and **4b** were reacted with ethylenediamine to yield the respective 5-(2-aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine **5a** and 6-(2-aminoethyl)carbamoyl-2',3',5'-tri-0-acetyluridine **6a**. The uridine nucleoside ethylenediamine ligands **5a** and **5b** were reacted with potassium tetrachloroplatinate to yield the nucleoside uridine-platinum (II) complexes **6a** and **6b**, and the complexes were deacetylated with CH_3ONa to afford [5-(2-aminoethyl)carbamoyluridine]dichloroplatinum (II) **7a**, and [6-(2-aminoethyl)carbamoyluridine]dichloroplatinum (II) **7b**, respectively. The synthesized compounds were identified by the FT-IR, ^{1}H-NHR,UV and mass spectra. Six heretofore unreported uracil-platinum (II) complexes **3a** and **3b**, and uridine nucleoside platinum (II) complexes **6a**, **6b**, **7a** and **7b** were evaluated for antitumor efficacy against the following three cell lines ;

(a) human chronic myelogenous cell (K-562);
(b) mouse lymphoid neoplasma cell (P-388);
(c) mouse mammary carcinoma cell (FM-3A),

and none of our synthesized compounds showed any siginificant antitumor activity against above the three cell lines (Table I).

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