Inorganica Chimica Acta 427 (2015) 72–80

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Synthesis, characterization, structures and cytotoxicity of platinum(II) complexes containing dimethylpyrazole based selenium ligands

Suresh M. Chopade, Prasad P. Phadnis, Ananda S. Hodage, Amey Wadawale, Vimal K. Jain *

Chemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085, India

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 27 October 2014
Accepted 16 November 2014
Available online 9 December 2014

Keywords:
Platinum
Selenium
Dimethylpyrazole
X-ray structure
Cytotoxicity

ABSTRACT

A series of water soluble platinum(II) complexes of general formulae $[Pt(en)(L)][NO_3]_2$, $[Pt(NH_3)_2(L)][NO_3]_2$ [en = ethylenediamine; L = $dmpzC_6H_4Se(CH_2)_nCOOH$ or $dmpzCH_2CH_2Se(CH_2)_nCOOH$ (n = 1 and 2)], $[Pt(en)(L)][NO_3][OH]\cdot H_2O$ [L = $dmpzCH_2CH_2Se(CH_2)_nCOOH$ (n = 1 and 2)] and $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2\cdot 2H_2O$ have been synthesized. They were characterized by microanalyses, IR, NMR (^1H, $^{13}C\{^1H\}$, $^{77}Se\{^1H\}$ and $^{195}Pt\{^1H\}$) spectroscopy. Molecular structures of $[Pt(en)(dmpzCH_2CH_2SeCH_2COOH)][NO_3][OH]\cdot H_2O$ and $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2\cdot 2H_2O$ were determined unambiguously by single crystal X-ray diffraction analyses. The cytotoxicity of these complexes has been evaluated against human colon (HT29, Colo205), ovarian (A2780) and bladder (T24) cancer cell lines and compared with the activity of cisplatin and adriamycin.

1. Introduction

Ever since the antitumor activity of cisplatin was reported by Rosenberg et al. [1], a wide variety of platinum complexes, in particular with cis amine ligands, have been synthesized with reference to their use as inorganic antitumor agents [2–4]. Although cisplatin is extensively used as an antitumor drug, its inherent drawbacks, such as limited water solubility, severe toxicity and cellular resistance on prolong use, led to design and development of numerous new platinum complexes particularly with bioactive ligands [4].

The mechanistic and metabolic investigations have revealed that platinum based drugs undergo several non-selective reactions with a variety of biomolecules such as L-methionine before binding to DNA [4]. In fact methionine coordinated platinum complexes have been isolated from urine samples of cisplatin (**I**, Scheme 1) [5] and carboplatin (**II**) [6] treated patients. Platinum thioether complexes are thought to be formed as intermediates prior to DNA platinum binding [7,8]. Accordingly a number of cis-amine platinum complexes containing thio and seleno ligands, have been synthesized (Scheme 1) and evaluated for their cytotoxicity [9–17].

Tumor cells in general have depleted levels of glutathione (GSH) concentration. The GSH is responsible for scavenging reactive oxygen species (ROS) in the cells. Organoselenium compounds are now well known for scavenging ROS and thus help in controlling ROS levels [18]. Keeping this in mind we have recently used organoselenium compounds as ligands for the synthesis of platinum amine complexes to synergize the cytotoxicity of platinum amine fragment and ROS scavenging properties of organoselenium compounds. Accordingly we have reported synthesis of platinum complexes $[Pt(en)(OOC(CH_2)_nSe(CH_2)_nCOOH)][OH]$ and studied their binding with a mononucleotide 5′-guanosine monophosphate (5′-GMP) [19]. Herein we report water soluble platinum complexes with dimethylpyrazole based selenium ligands and their cytotoxic properties.

2. Experimental

2.1. Materials and methods

Elemental selenium (99.99%), sodium borohydride ($NaBH_4$), 2-bromoacetic acid, 3-bromopropionic acid and superhydride ($LiBEt_3H$) were purchased from commercial sources (Aldrich/Fluka). Selenium reagents (Li_2Se and Li_2Se_2) [20], $dmpzC_6H_4Se(CH_2)_nCOOH$ [21] and $dmpzCH_2CH_2Se(CH_2)_nCOOH$ [22] (n = 1, 2) and platinum precursors $[Pt(en)Cl_2]$ [21], $[Pt(NH_3)_2I_2]$ [22],

Abbreviations: en, ethylenediamine; dmpz, 3,5-dimethylpyrazole; ROS, reactive oxygen species; ADR, adriamycin; LC50, concentration of test complex that kills 50% of the cells; TGI, concentration of test complex that produces 100% inhibition of cells; GI50, concentration of test complex that produces 50% inhibition of cells; 5′-GMP, 5′-guanosine monophosphate.

* Corresponding author. Tel.: +91 22 2559 5095; fax: +91 22 2550 5151.
E-mail address: jainvk@barc.gov.in (V.K. Jain).

http://dx.doi.org/10.1016/j.ica.2014.11.017
0020-1693/

$[Pt(en)(NO_3)_2]$ [23,24] and $[Pt(NH_3)_2(NO_3)_2]$ [25] were synthesized by literature methods (supplementary materials). All the reactions were carried out under a nitrogen atmosphere. Solvents were purified and dried by standard procedures and were distilled prior to use. The organoselenium ligands were purified by column chromatography on silica gel 60/120 mesh size. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were carried out on a Thermo-Fischer Flash EA 1112 Series CHNS Analyzer. ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker Avance-II 300 or Bruker Ascend™ 400 MHz spectrometer operating at 300.13 and 400.13 (^1H), 75.47 and 100.61 ($^{13}C\{^1H\}$), while ^{77}Se and ^{195}Pt NMR spectra were recorded on the former operating at 57.25 ($^{77}Se\{^1H\}$) and 64.52 ($^{195}Pt\{^1H\}$) MHz. The ^1H NMR chemical shifts were relative to internal HOD peak at δ 4.79 ppm. $^{13}C\{^1H\}$ and $^{77}Se\{^1H\}$ NMR chemical shifts are relative to external Me_4Si and Me_2Se (secondary reference Ph_2Se_2 in $CDCl_3$ δ 463 ppm), respectively. The $^{195}Pt\{^1H\}$ NMR chemical shifts are relative to external Na_2PtCl_6 in D_2O (δ = 0 ppm).

2.2. Synthesis

2.2.1. *[Pt(en)(dmpzC$_6$H$_4$SeCH$_2$COOH)][NO$_3$]$_2$ (**1**)*

To a stirred aqueous solution (15 ml) of $[Pt(en)(NO_3)_2]$ (100 mg, 0.26 mmol), a methanolic solution (10 ml) of dmpzC$_6$H$_4$SeCH$_2$COOH (82 mg, 0.26 mmol) was added slowly at room temperature. The initial pale yellow solution turns dark yellow after stirring for 2 h. The solution was filtered and the solvent was evaporated *in vacuo* to yield a pale yellow powder (82 mg, 45%), m.p. 125 °C. *Anal.* Calc. for $C_{15}H_{22}N_6O_8Pt_1Se_1$ (FW 688.4): C, 26.17; H, 3.22; N, 12.20. Found: C, 26.21; H, 3.62; N, 12.42%. IR (ν in cm^{-1}): 3065 (OH), 1710 (CO), 1384, 833 (NO_3). ^1H NMR (D_2O) δ: 2.30, 2.45 (each s, 3H, *Me*), 2.67 (s, br, 4H, en N-C*H*$_2$C*H*$_2$), 2.95, 2.96 (each s, 2H, SeC*H*$_2$), 5.77, 5.99 (each br, 2H, en *H*$_2$N-C), 6.50 (s, 1H, *H4*-dmpz), 7.52–7.61 (m, 2H), 7.70–7.76 (m, 1H), 7.81–7.84 (dd, *J* = 7.6, 1.2 Hz, 1H) (C$_6$*H*$_4$); $^{13}C\{^1H\}$ NMR (D_2O) δ: 12.7, 12.9 (dmpz-*Me*), 32.7 (SeCH$_2$, J_{Se-C} = 57 Hz), 47.0, 47.7 (en *C*H$_2$), 111.1 (dmpz-4*C*), 117.1, 127.2, 130.1, 132.5, 132.9, 138.1 (*C*$_6$H$_4$), 146.0, 153.4 (dmpz-3*C* and 5*C*), 169.1 (C=O); $^{77}Se\{^1H\}$ NMR (D_2O) δ: 233 (J_{Pt-Se} = 335 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3238 ppm.

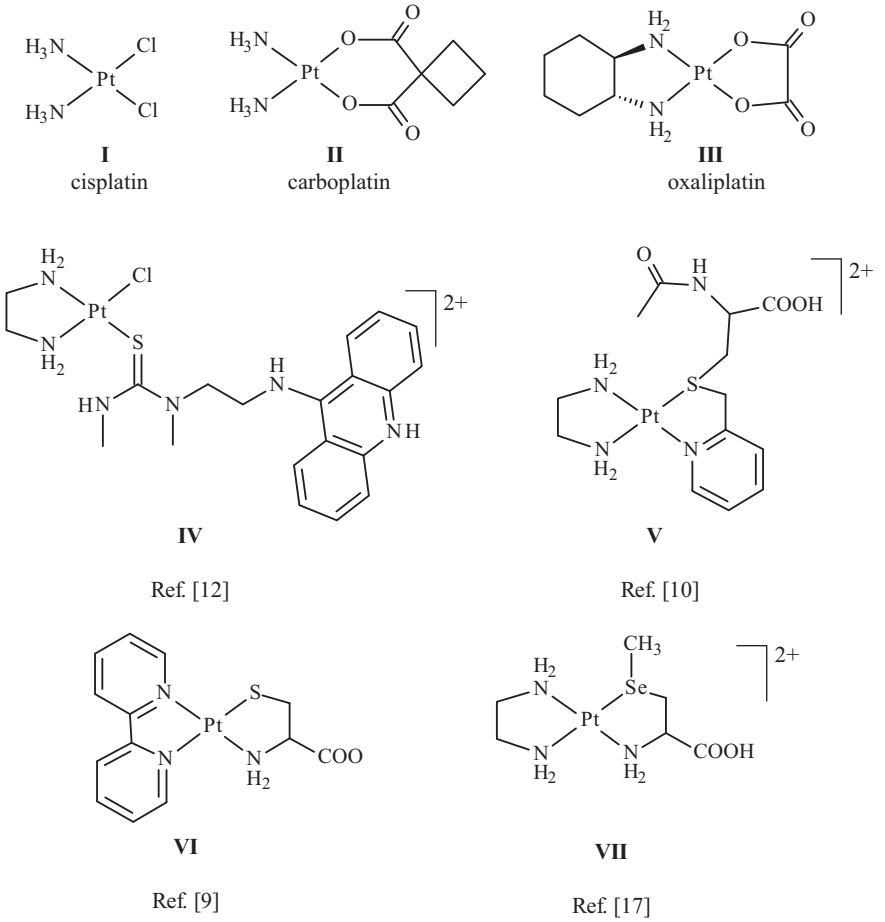
The complexes **2**–**6** were synthesized by following the similar procedure (Scheme 2).

2.2.2. *[Pt(NH$_3$)$_2$(dmpzC$_6$H$_4$SeCH$_2$COOH)][NO$_3$]$_2$ (**2**)*

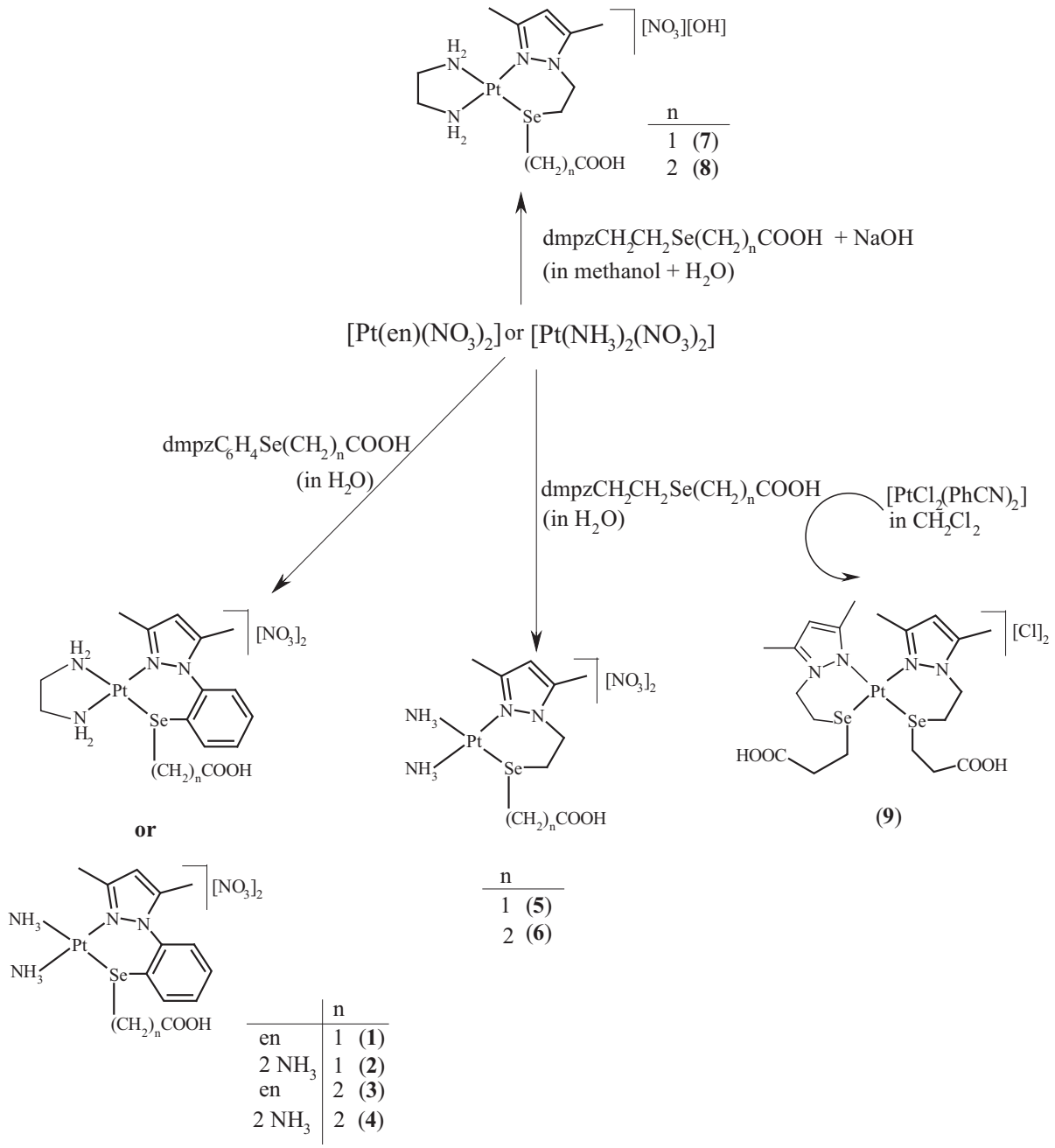
Yellow powder (43%), m.p. 130 °C. *Anal.* Calc. for $C_{13}H_{20}N_6O_8Pt_1Se_1$ (FW 662.4): C, 23.57; H, 3.04; N, 12.68. Found: C, 23.30; H, 3.41; N, 13.13%. IR (ν in cm^{-1}): 3105 (OH), 1715 (CO), 1384, 824 (NO_3). ^1H NMR (D_2O) δ: 2.39, 2.57 (each s, 3H, *Me*), 2.97 (s, 2H, SeC*H*$_2$), 6.59 (s, 1H, *H4*-dmpz), 7.61–7.71 (m, 2H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 7.2 Hz, 1H) (C$_6$*H*$_4$); $^{13}C\{^1H\}$ NMR (D_2O) δ: 13.1, 13.2 (dmpz-*Me*), 34.7 (SeCH$_2$), 111.6 (dmpz-4*C*), 118.1, 127.5, 130.5, 132.7, 133.2, 138.6 (*C*$_6$H$_4$), 146.3, 153.5 (dmpz-3*C* and 5*C*), 170.2 (C=O); $^{77}Se\{^1H\}$ NMR (D_2O) δ: 241 (J_{Pt-Se} = 455 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3038 ppm.

2.2.3. *[Pt(en)(dmpzC$_6$H$_4$SeCH$_2$CH$_2$COOH)][NO$_3$]$_2$ (**3**)*

Pale yellow powder (52%), m.p. 75 °C. *Anal.* Calc. for $C_{16}H_{24}N_6O_8Pt_1Se_1$ (FW 702.4): C, 27.36; H, 3.44; N, 11.96. Found: C, 26.89; H, 3.71; N, 11.55%. IR (ν in cm^{-1}): 3065 (OH), 1725 (CO), 1384,



Scheme 1. Platinum(II) complexes exhibiting antitumor activity.



Scheme 2. Synthesis of platinum complexes with dimethylpyrazole based selenium ligands.

833 (NO_3). ^{1}H NMR (D_2O) δ: 2.37, 2.55 (each s, 3H, *Me*), 2.67–2.71 (br, 6H, en N-CH_2CH_2 + $SeCH_2$), 5.75–5.99 (br, 4H, en H_2N-C), 6.54 (s, 1H, *H4*-dmpz), 7.55–7.65 (m, 2H), 7.72 (t, J = 7.8 Hz, 1H), 7.82 (d, J = 7.2 Hz, 1H) (C_6H_4); ^{13}C{^{1}H} NMR (D_2O) δ: 12.6, 12.9 (dmpz-*Me*), 27.1 (J_{Se-C} = 52 Hz, $SeCH_2$), 31.5 ($SeCH_2CH_2$), 46.9, 47.7 (en CH_2), 110.9 (dmpz-4*C*), 117.5, 127.2, 129.9, 132.2, 133.0, 138.2 (C_6H_4), 145.6, 153.2 (dmpz-3*C* and 5*C*), 174.3 (C=O); ^{77}Se{^{1}H} NMR (D_2O) δ: 238 (J_{Pt-Se} = 366 Hz); ^{195}Pt{^{1}H} NMR (D_2O) δ: −3252 ppm.

*2.2.4. [Pt(NH$_3$)$_2$(dmpzC$_6$H$_4$SeCH$_2$CH$_2$COOH)][NO$_3$]$_2$ (**4**)*

Yellow powder (44%), m.p. 110 °C. *Anal.* Calc. for $C_{14}H_{22}N_6O_8Pt_1Se_1$ (FW 676.4): C, 24.86; H, 3.28; N, 12.42. Found: C, 24.81; H, 3.59; N, 12.06%. IR (ν in cm^{-1}): 3090 (OH), 1715 (CO), 1385, 825 (NO_3). ^{1}H NMR (D_2O) δ: 2.39, 2.59 (each s, 3H, *Me*), 2.66–2.70 (br, 2H, $SeCH_2$), 6.57 (s, 1H, *H4*-dmpz), 7.58–7.69 (m, 2H), 7.80 (t, J = 7.8 Hz, 1H), 7.88 (d, J = 7.2 Hz, 1H) (C_6H_4); ^{13}C{^{1}H} NMR (D_2O) δ: 12.6, 12.8 (dmpz-*Me*), 27.5 ($SeCH_2$), 31.7 ($SeCH_2C$), 111.1 (dmpz-4*C*), 117.8, 127.1, 129.9, 132.2, 132.9, 138.2 (C_6H_4), 145.5, 153.0 (dmpz-3*C* and 5*C*), 174.7 (C=O); ^{77}Se{^{1}H} NMR (D_2O) δ: 254 (J_{Pt-Se} = 481 Hz); ^{195}Pt{^{1}H} NMR (D_2O) δ: −3031 ppm.

*2.2.5. [Pt(NH$_3$)$_2$(dmpzCH$_2$CH$_2$SeCH$_2$COOH)][NO$_3$]$_2$ (**5**)*

White powder (40%), m.p. 55 °C. *Anal.* Calc. for $C_9H_{20}N_6O_8Pt_1Se_1$ (FW 614.1): C, 17.59; H, 3.28; N, 13.68. Found: C, 17.06; H, 3.48; N, 13.96%. IR (ν in cm^{-1}): 3175 (OH), 1760 (CO), 1389, 825 (NO_3). ^{13}C{^{1}H} NMR (D_2O) δ: 10.6, 12.4 (each s, dmpz-*Me*), 29.0 ($SeCH_2$ CH_2N), 33.0, (J_{Se-C} = 53 Hz, $SeCH_2CO$), 48.6 (J_{Se-C} = 78 Hz, $SeCH_2CH_2$ N), 108.0 (dmpz-4*C*), 144.5, 150.5 (dmpz-3*C* and 5*C*), 170.4 (C=O);

$^{77}Se\{^1H\}$ NMR (D_2O) δ: 218 (J_{Pt-Se} = 502 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3145 ppm.

2.2.6. [Pt(NH$_3$)$_2$(dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH)][NO$_3$]$_2$ (**6**)

Sticky white solid (45%). *Anal.* Calc. for $C_{10}H_{22}N_6O_8Pt_1Se_1$ (FW 628.4): C, 19.11; H, 3.52; N, 13.37. Found: C, 18.97; H, 3.05; N, 13.11%. IR (ν in cm^{-1}): 3125 (OH), 1720 (CO), 1385, 825 (NO_3). ^1H NMR (D_2O) δ: 2.39, 2.41 (each s, 6H, *Me*), 2.74–2.79 (br, m, 4H), 3.34–3.43 (br, m, 1H) (SeC*H*$_2$C*H*$_2$CO and SeC*H*$_2$C*H*$_2$N), 6.19 (s, 1H, *H4*-dmpz); $^{13}C\{^1H\}$ NMR (D_2O) δ: 10.6, 12.4 (each s, dmpz-*Me*), 27.9 (SeCH$_2$CH$_2$CO), 29.3 (SeCH$_2$C*H*$_2$N), 32.6 (SeCH$_2$CH$_2$CO), 48.8 (J_{Se-C} = 74 Hz, SeC*H*$_2$C*H*$_2$N), 107.9 (dmpz-4*C*), 144.4, 150.2 (dmpz-3*C* and 5*C*), 174.9 (C=O); $^{77}Se\{^1H\}$ NMR (D_2O) δ: 221 (J_{Pt-Se} = 501 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3162 ppm.

2.2.7. [Pt(en)(dmpzCH$_2$CH$_2$SeCH$_2$COOH)][NO$_3$][OH]·H$_2$O (**7**·H$_2$O)

To a stirred methanolic solution (15 ml) of dmpzCH$_2$CH$_2$SeCH$_2$COOH (71 mg, 0.23 mmol), aqueous NaOH solution (0.13 M, 2 ml, 10 mg, 0.25 mmol) was added and stirred at room temperature for 15 min. To this [Pt(en)(NO_3)$_2$] (103 mg, 0.27 mmol) was added whereupon the reaction mixture turned clear within 10 min. The stirring continued for 2 h. The solvent was evaporated *in vacuo* to afford a white powder (85 mg, 54%) which was re-crystallized from water (5%) acetone-ether mixture to give plate like colorless crystals, m.p. 190 °C. *Anal.* Calc. for $C_{11}H_{23}N_5O_6Pt_1Se_1\cdot H_2O$ (FW 613.4): C, 21.54; H, 4.11; N, 11.41. Found: C, 21.42; H, 3.61; N, 11.30%. IR (ν in cm^{-1}): 3450 (H_2O), 1635 (CO), 1366, 826 (NO_3). ^1H NMR (CD_3OD) (400 MHz) δ: 2.46, 2.48 (each s, 6H, *Me*), 2.78–2.80 (br, 5H, en N-C*H*$_2$C*H*$_2$ + SeC*H*$_2$), 6.27 (s, 1H, *H4*-dmpz); $^{13}C\{^1H\}$ NMR (D_2O) δ: 13.2, 15.2 (each s, dmpz-*Me*), 30.3 (s, SeCH$_2$CO), 39.6 (s, SeCH$_2$CH$_2$N), 49.0, 49.1 (SeCH$_2$C*H*$_2$), 49.2, 50.2, 50.3, 50.4, 51.44 (–CH$_2$C*H*$_2$–), 110.3 (dmpz-4*C*), 147.0, 153.0 (dmpz-3*C* and 5*C*), 175.0 (C=O); $^{77}Se\{^1H\}$ NMR (D_2O) δ: 184 (J_{Pt-Se} = 397 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3358 ppm.

2.2.8. [Pt(en)(dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH)][NO$_3$][OH]·H$_2$O (**8**·H$_2$O)

Prepared in an analogous manner to **7** and isolated as a white powder (43%), m.p. 210 °C (deco.). *Anal.* Calc. for $C_{12}H_{25}N_5O_6Pt_1Se_1\cdot H_2O$ (FW 627.4): C, 22.97; H, 4.34; N, 11.16. Found: C, 22.53; H, 4.02; N, 11.52%. IR (ν in cm^{-1}): 3430 (H_2O), 3060 (OH), 1762 (CO), 1387, 825 (NO_3). ^1H NMR (D_2O) δ: 2.37, 2.38 (each s, 6H, *Me*), 2.61 (br, s, 2H), 2.79 (br, 6H) (SeC*H*$_2$C*H*$_2$CO + SeC*H*$_2$C*H*$_2$N), 3.31 (s, 1H, unidentified), 5.59–5.98 (br, m, 4H, en N-C*H*$_2$C*H*$_2$), 6.17 (s, 1H, *H4*-dmpz); $^{13}C\{^1H\}$ NMR (400 MHz) (D_2O) δ: 10.7, 12.8 (dmpz-*Me*), 28.6 ($^1J_{Se-C}$ = 55 Hz, SeCH$_2$CH$_2$CO), 29.6 (SeCH$_2$CH$_2$N), 34.9 (SeCH$_2$CH$_2$CO), 46.8 and 48.0 (each t, en CH$_2$), 49.1 ($^1J_{Se-C}$ = 77 Hz, SeCH$_2$CH$_2$N), 107.8 (dmpz-4*C*), 144.6, 150.7 (dmpz-3*C* and 5*C*), 177.1 (C=O); $^{77}Se\{^1H\}$ NMR (D_2O) δ: 202 (J_{Pt-Se} = 392 Hz); $^{195}Pt\{^1H\}$ NMR (D_2O) δ: −3363 ppm.

2.2.9. [Pt(dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH)$_2$][Cl]$_2$ (**9**)

To a stirred CH_2Cl_2 solution (20 ml) of [$PtCl_2$(PhCN)$_2$] (128 mg, 0.27 mmol), dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH (76 mg, 0.29 mmol) was added slowly with stirring at room temperature, initial clear solution turned turbid. The reaction was further stirred for 2 h and the precipitate was filtered through a G3 filtering unit and washed with CH_2Cl_2 and the residue was dried *in vacuo* and recrystallized from methanol to give pale yellow crystals of the title complex (158 mg, 72%), m.p. 130 °C. *Anal.* Calc. for $C_{20}H_{32}Cl_2N_4O_4Pt_1Se_2$ (FW 816.5): C, 29.42; H, 3.95; N, 6.86. Found: C, 29.54; H, 3.89; N, 6.48%. IR (ν in cm^{-1}): 3455 (H_2O), 3122 (OH), 1746, 1715 (CO). ^1H NMR (CD_3OD) δ: 1.94, 2.58 (each s, 6H, *Me*), 2.85–2.96 (br, m, 4H), 3.11–3.19 (br, m, 2H), 3.61, 3.65 (each br s, 1H), 4.99–5.54 (br, m, 7H), 6.33 (s, 1H, *H*4-dmpz); $^{13}C\{^1H\}$ NMR (CD_3OD) δ: 12.4, 13.7 (each s, dmpz-*Me*), 32.5 (SeCH$_2$CH$_2$CO), 33.0 (SeCH$_2$CH$_2$CO), 34.2 (NCH$_2$CH$_2$), 51.4 ($^1J_{Se-C}$ = 68 Hz, NCH$_2$CH$_2$), 110.3 (dmpz-4*C*), 147.1, 152.6 (dmpz-3*C* and 5*C*), 175.6 (C=O), $^{77}Se\{^1H\}$ NMR (CD_3OD) δ: 230 ($^1J_{Pt-Se}$ = 564 Hz); $^{195}Pt\{^1H\}$ NMR (CD_3OD) δ: −3759 ($^1J_{Pt-Se}$ = 583 Hz) ppm.

Table 1
Crystallographic and structural refinement data for the compounds [Pt(en)(dmpzCH$_2$CH$_2$SeCH$_2$COOH)][NO_3][OH]·H$_2$O (**7**·H$_2$O) and [Pt(dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH)$_2$][Cl]$_2$·2H$_2$O (**9**·2H$_2$O).

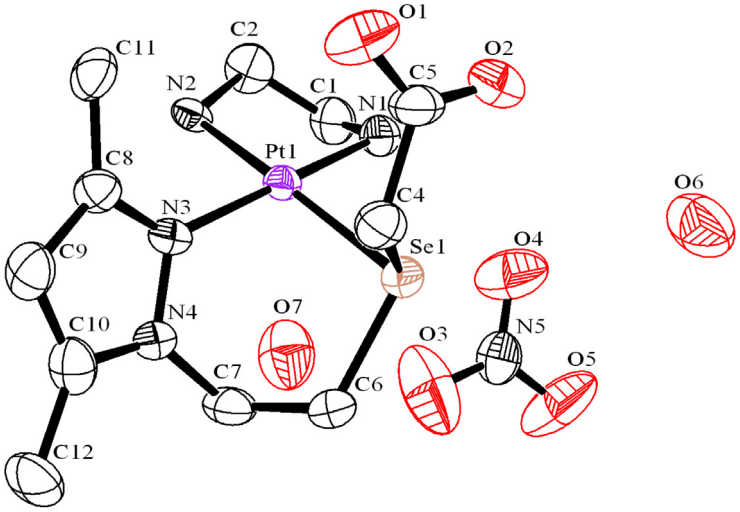
Compound	**7**·H$_2$O	**9**·2H$_2$O
Chemical formula	$C_{11}H_{25}N_5O_7Pt_1Se_1$	$C_{20}H_{36}Cl_2N_4O_6Pt_1Se_2$
Formula weight	613.41	852.44
Color	Colorless	Colorless
Crystal size (mm)	0.25 × 0.15 × 0.05	0.25 × 0.15 × 0.10
Crystal system/space group	triclinic/$P\bar{1}$	monoclinic/$C2/c$
Unit cell dimensions		
a (Å)	7.5633 (17)	17.900(2)
b (Å)	11.000 (2)	9.891(2)
c (Å)	12.141 (5)	32.225(3)
α (°)	72.37 (2)	90.000
β (°)	80.43 (3)	92.940(8)
γ (°)	81.665 (18)	90.000
V (Å^3)	944.4 (5)	5697.5(15)
Z	2	8
D_{calc} (g cm^3)	2.157	1.988
μ(Mo Kα) (mm^{-1})/F(000)	9.398/588	7.709/3296
Limiting indices	$-9 \leqslant h \leqslant 9$	$-12 \leqslant h \leqslant 23$
	$-8 \leqslant k \leqslant 14$	$-12 \leqslant k \leqslant 7$
	$-15 \leqslant k \leqslant 15$	$-41 \leqslant k \leqslant 41$
θ range for data collection	2.74–27.52	2.53–27.51
No. of reflections collected	4336	6543
No. of independent reflections	3013	3357
Data/restraints/parameters	4336/1/235	6543/0/313
R indices [$I > 2\sigma(I)$]	R_1 = 0.0483, wR_2 = 0.1122	R_1 = 0.0810, wR_2 = 0.2008
R indices (all data)	R_1 = 0.0993, wR_2 = 0.1319	R_1 = 0.1776, wR_2 = 0.2402
$(\Delta/\sigma)_{max}$	0.001	0.001
$(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ (Å^{-3})	1.690, −3.069	2.863, −3.163
Goodness-of-fit on F^2	1.020	1.069

2.3. X-ray crystallography

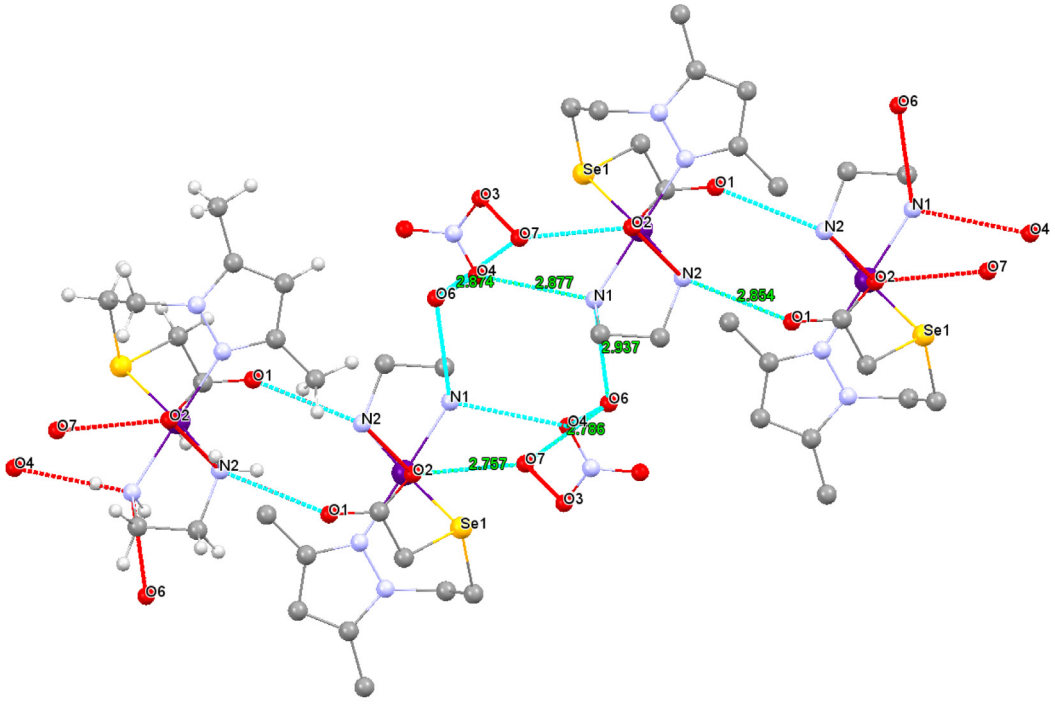
Single crystal X-ray diffraction data for [Pt(en)(dmpz$CH_2CH_2SeCH_2COOH$)][NO_3][OH]·H_2O (**7**·H_2O) and [Pt(dmpz$CH_2CH_2SeCH_2CH_2COOH)_2$][Cl]$_2$·$2H_2O$ (**9**.$2H_2O$) were collected at room tempera ture (298 ± 2 K) on a Rigaku AFC 7S diffractometer using graphite monochromated Mo Kα (λ = 0.71069 Å) radiation so that θ_{max} = 27.52°. The unit cell parameters (Table 1) were determined from 25 reflections measured by a random search routine. The intensity data were corrected for Lorenz, polarization and absorption effects with an empirical procedure [26]. The structures were solved by direct methods [27] and refined by full-matrix least-square methods [28]. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were fixed in their calculated positions. The molecular structures were drawn by ORTEP [29].

2.4. In vitro evaluation of cytotoxicity of platinum complexes against human colon, ovarian and bladder cancer cell lines

The cytotoxicity of platinum(II) complexes was evaluated against human colon, ovarian and bladder cancer cell lines by Sulforhodamine B assay method [30]. Two types of human colon cancer cell lines *viz.*, HT29 and Colo205 as well as ovarian (A2780) and bladder (T24) (5×10^3 cells/well on a plate) cell lines obtained from NCI, USA were treated *in vitro* with the complexes, cisplatin and a positive control drug, adriamycin (ADR) in concentrations



(a)



(b)

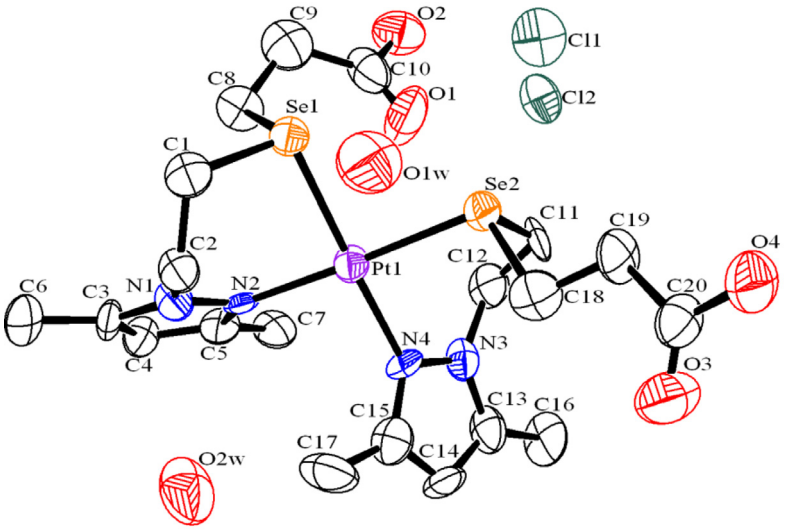
Fig. 1. (a) ORTEP diagram of [Pt(en)(dmpz$CH_2CH_2SeCH_2COOH$)][NO_3][OH]·H_2O (**7**·H_2O). (The ellipsoids were drawn at the 50% probability.) (b) Hydrogen bonding interactions.

10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M. The treatments were performed thrice and the percentage growth of the target cell lines was calculated from average of three experiments and plotted against concentration of tested complexes. The concentration of complex which produces 50% inhibition (GI50), total inhibition (TGI) and a concentration which kills 50% (LC50) of cells was calculated from the plot (Supplementary materials).

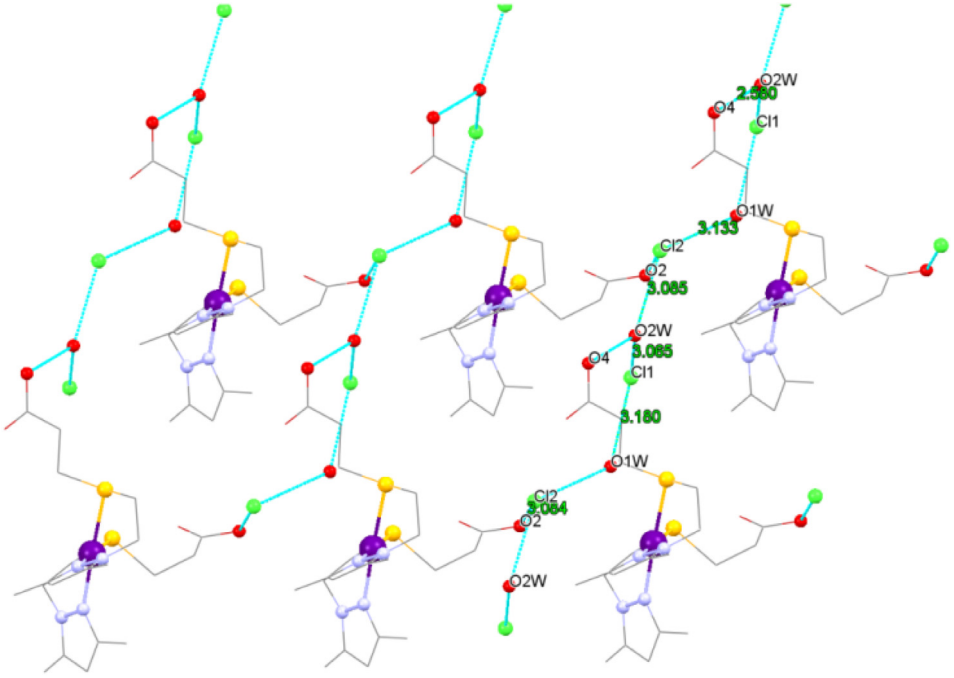
3. Results and discussion

3.1. Synthesis

Treatment of $[Pt(en)(NO_3)_2]$ or $[Pt(NH_3)_2(NO_3)_2]$ with $dmpzC_6H_4Se(CH_2)_nCOOH$ or $dmpzCH_2CH_2Se(CH_2)_nCOOH$ (Se∩N) in 1:1 stoichiometry afforded cream to colorless water soluble complexes of composition $[Pt(en)(Se\cap N)][NO_3]_2$ or $[Pt(NH_3)_2(Se\cap N)][NO_3]_2$ (**1**–**6**) in 45–55% yield (Scheme 2). These complexes are stable in aqueous solution for several days as the ^{195}Pt NMR spectrum of $[Pt(en)(dmpzCH_2CH_2SeCH_2COOH)][NO_3]_2$ remained unchanged for about two weeks. A similar reaction between $[Pt(en)(NO_3)_2]$ and $dmpzCH_2CH_2Se(CH_2)_nCOOH$ in the presence of one equivalent of aqueous NaOH solution at room temperature gave $[Pt(en)(dmpzCH_2CH_2Se(CH_2)_nCOOH)][NO_3][OH]$ (n = 1 (**7**) or 2 (**8**)) in moderate yield and re-crystallized with a molecule of water. When $[PtCl_2(PhCN)_2]$ was treated with $dmpzCH_2CH_2SeCH_2CH_2COOH$ in 1:1 or 1:2 stoichiometry, $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2$ was formed invariably which recrystallized with two water molecules. All the complexes were characterized by microanalyses, IR and NMR spectroscopy (Section 3.2) while molecular structures



(a)



(b)

Fig. 2. (a) ORTEP diagram of $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2{\cdot}2H_2O$ (**9**·$2H_2O$). (The ellipsoids were drawn at the 50% probability.) (b) Hydrogen bonding interactions.

of the complexes **7**·H_2O and **9**.$2H_2O$ were established by single crystal X-ray diffraction analyses (Section 3.3).

3.2. Spectroscopy

The IR spectra of **1**–**6** displayed a broad band in the region 3065–3175 cm^{-1} due to ν OH absorptions while complexes **7**–**9** showed a very broad band at ~3440 cm^{-1} attributable to coordinated water molecule. The carbonyl stretching for all the complexes appeared in the region 1710–1760 cm^{-1} indicative of non-coordinated carboxylic acid group. The absorptions at ~1385 and 830 cm^{-1} have been assigned to ionic nitrate group [31].

The ^1H and $^{13}C\{^1H\}$ NMR spectra showed expected resonances and peak multiplicities. The dmpz methyl and CH-4 proton resonances are deshielded in the complexes with respect to the corresponding signals for the free ligands. Similarly CH-4 carbon resonances for the dmpz group are considerably deshielded with respect to the resonances for the free ligand indicating coordination of the pyrazolyl nitrogen to platinum. The carbonyl carbon resonances appeared in the region 169.1–177.1 ppm. The $^{13}C\{^1H\}$ NMR carbon resonances for $SeCH_2$ carbon of dmpz$SeCH_2CH_2Se$ fragment is deshielded considerably (7–10 ppm) as compared to free ligand.

The $^{77}Se\{^1H\}$ NMR spectra of these complexes displayed a single resonances in the region 184–254 ppm which were flanked by platinum satellites with ^1J(Pt–Se) varying between 335 and 564 Hz. The resonances for complexes (**1**–**4**) derived from aromatic ligand were shielded with respect to signals observed for the corresponding free ligands. Whereas resonances for complexes (**5**–**9**) containing aliphatic ligands were deshielded, except for **7**. Among **1**–**4** the shielding in amine complexes are less than the corresponding en derivatives while the ^1J(Pt–Se) is larger in the former than the latter. Smaller magnitude of ^1J(Pt–Se) in the latter may be due to steric crowding of chelated en ligand as compared to the monodentate NH_3 group in **1** and **3**. The magnitude of ^1J(Pt–Se) for these complexes is in accord with the values reported in the literature [32,33].

The $^{195}Pt\{^1H\}$ NMR spectra of complexes (**1**–**8**) displayed a single resonance in the region δ −3031 to −3363 ppm, while for **9** it is further shielded (δ −3759 ppm). The observed resonances for amine complexes are highly shielded with respect to the resonances for the precursors ($[Pt(NH_3)_2(H_2O)_2][NO_3]_2$ ^{195}Pt δ −1585 ppm); $[Pt(en)(NO_3)_2]$ ^{195}Pt δ −1922 ppm; lit [34,35] δ −1915 ppm). The observed shielding may be attributable to the coordination of selenoether ligands to platinum. Such shielding of $^{195}Pt\{^1H\}$ NMR spectra are reported for chalcogenoether complexes such as $[Pt(en)\{OOC(CH_2)_nSe(CH_2)_nCOOH\}][OH]$ or $[Pt(en)\{OOC(CH_2)_nSe(CH_2)_nCOOH\}][OH]$ (n = 1 or 2) (δ −2616 to −2904 ppm) [19] and $[Pt(NH_3)_2(dmso)Cl]^+$ (δ −3147 ppm) [36].

3.3. Molecular structures

ORTEP diagrams of $[Pt(en)(dmpzCH_2CH_2SeCH_2COOH)][NO_3][OH]\cdot H_2O$ (**7**·H_2O) and $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2\cdot 2H_2O$ (**9**·$2H_2O$) (crystals obtained from ethanol–water mixture) of these complexes with atomic numbering scheme are shown in Figs. 1 and 2 and selected inter-atomic parameters are summarized in Tables 2 and 3. Both complexes comprise of distorted square planar platinum center. The Pt–Se and Pt–N distances compare well with $[Pt(en)(OOCCH_2SeCH_2COOH)][OH]$ and are in accord with the literature values [19,24,37,38]. The coordination environment around platinum in **7**·H_2O is defined by three nitrogen atoms and selenium from chelating en and seleno ligands. The Pt–N distance trans to selenium atom is slightly longer (Pt1–N2 = 2.068(8) Å) than the other two Pt–N distances due to strong trans influence of the selenide ligand. The five membered Pt-en ring is puckered.

The packing diagrams show the formation of distinct 2-D planar blocks (Supplementary Fig. S36) in which molecules are held together through a complex network of hydrogen bonding between the oxygen atoms of water molecules (O6) nitrate ions (O3 and O4), hydroxyl ions (O7) and nitrogen atoms of en group (N1 and N2). The O1 and O2 oxygen atoms of the acetate group of a molecule show hydrogen bonding with nitrogen atom N2 of en group of two different neighboring molecules (O2…N2 = 2.880, O1…N2 = 2.854). The contact distances (Å) are given in Table 2.

The square planar platinum in **9**·$2H_2O$ is coordinated with two chelated seleno ligands in which two selenium and two nitrogen atoms are cis disposed. The six membered chelate rings are puckered. The two acetate groups are oriented differently. One of them is directed away from the central platinum whereas the other is directed towards it. The distance between the carbonyl oxygen O1 of the later acetate group and Pt1 is 3.159 Å suggesting short interaction between the two. The molecular packing diagram show chains of alternative chloride ions and water molecules linked through hydrogen bonding (…Cl2…O1W…Cl1…O2w…Cl2…O1w…). The molecules of **9**·$2H_2O$ are held together in the middle of these chains by hydrogen bonds between oxygen atoms O2 and O4 of acetate groups with Cl2 and O2W of the chloride ion-water molecule chains respectively, thus forming 2-D hydrogen bonded network. The molecules in these 2-D networks are

Table 2
Selected bond lengths (Å), angles (°), torsion angles (°) and contact distances (Å) for $[Pt(en)(dmpzCH_2CH_2SeCH_2COOH)][NO_3][OH]\cdot H_2O$ (**7**·H_2O).

Pt1–N1	2.038(9)	Pt1–Se1	2.3955(13)
Pt1–N2	2.068(8)	Se1–C4	1.947(11)
Pt1–N3	2.024(9)	Se1–C6	1.947(11)
N1–Pt1–N2	83.1(4)	Pt1–N1–C1	108.3(7)
N1–Pt1–N3	176.3(3)	Pt1–N2–C2	109.2(7)
N1–Pt1–Se1	94.1 (3)	Pt1–N3–N4	120.0(7)
N2–Pt1–N3	94.3(3)	Pt1–N3–C8	132.5(7)
N2–Pt1–Se1	175.1(3)	Pt1–Se1–C4	100.8(3)
N3–Pt1–Se1	88.3(2)	Pt1–Se1–C6	100.5(3)
Torsion angles (°)			
C4–Se1–Pt1–N3	56.2(4)	C7–C6–Se1–C4	103.2(9)
C4–Se1–Pt1–N2	178(3)	Pt1–N1–C1–C2	46.8(12)
C4–Se1–Pt1–N1	126.5(4)	Pt1–N2–C2–C1	30.1(11)
Contacts (Å)			
O7…O3	2.870	O6…N1	2.937
O7…O6	2.786	O4…N1	2.877
O7…O2	2.757	O2…N2	2.880
O4…O6	2.874	O1…N2	2.854

Table 3
Selected bond lengths (Å), angles (°) and contact distances (Å) for $[Pt(dmpzCH_2CH_2SeCH_2CH_2COOH)_2][Cl]_2\cdot 2H_2O$ (**9**·$2H_2O$).

Pt1–Se2	2.382(2)	Se2–C11	1.960(17)
Pt1–Se1	2.3974(19)	Se2–C18	1.95(2)
Pt1–N2	2.050(13)	Se1–C1	1.958(19)
Pt1–N4	2.020(13)	Se1–C8	1.983(18)
Se2–Pt1–Se1	89.52(7)	C11–Se2–Pt1	100.8(5)
N2–Pt1–Se2	177.7(4)	C18–Se2–Pt1	104.8(7)
N4–Pt1–Se2	89.3(4)	C1–Se1–Pt1	100.1(5)
N2–Pt1–Se1	88.8(3)	C8–Se1–Pt1	102.1(6)
N4–Pt1–Se1	177.3(4)	C18–Se2–C11	97.2(9)
N2–Pt1–N4	92.3(5)	C1–Se1–C8	100.0(9)
Contacts (Å)			
O2…Cl2	3.084	Cl1…O2w	3.065
O4…O2w	2.580	O2W…Cl2	3.085
O1W…Cl1	3.180	Cl2…O1W	3.133

Table 4
Evaluation of cytotoxicity of platinum complexes against human colon (HT29 and Colo205), ovarian (A2780) and bladder (T24) cell lines.

Complex No.	Concentration of test complexes (μM)											
	HT29			Colo205			A2780			T24		
	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50	LC50	TGI	GI50
1	>100	82.4	42.6	>100	>100	54.3	#	#	#	#	#	#
2	>100	86.6	45.1	>100	>100	67.7	#	#	#	#	#	#
3	>100	>100	>100	>100	>100	>100	>100	>100	58.5	>100	95.9	25.5
4	>100	68.1	36.1	>100	>100	53.1	#	#	#	#	#	#
5	#	#	#	#	#	#	#	#	#	#	#	#
6	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
7	#	#	#	#	#	#	>100	>100	76.6	>100	>100	>100
8	#	#	#	#	#	#	>100	>100	98.9	>100	>100	77.9
9	#	#	#	#	#	#	>100	76.7	36.8	>100	>100	69.1
Cisplatin	>100	90.5	40.6	>100	>100	43.6	>100	55.9	<0.1	>100	87.5	14.9
Adriamycin	>100	17.6	<0.1	>100	>100	<0.1	58.5	<0.1	<0.1	55.5	<0.1	<0.1

Not evaluated for cytotoxicity.

arranged in such a manner that the contact oxygen atoms of the acetate groups O2 and O4 face toward the contact atoms O2 and O4 of the opposite 2-D network (Supplementary Fig. S37). Thus the mean planes formed by Pt atoms are alternatively parallel spaced with distance 8.482 and 7.609 Å, respectively.

3.4. Cytotoxic activity against human colon, ovarian and bladder cancer cell lines

To assess cytotoxicity of these complexes, their activity was tested against human colon (HT29 and Colo205), ovarian (A2780) and bladder (T24) cancer cell lines and their activity was compared with cisplatin and adriamycin under similar conditions (Table 4). The results indicate that the concentration of the tested complexes as well as cisplatin and adriamycin to kill 50% cancer cells (LC50) are >100 μM. Hence the results are analyzed by comparison of the concentration required to inhibit 50% cell growths (GI50). As it is evident from Table 4, cytotoxicity of platinum complexes depends on nature of human cancer cell lines and also on the dose of test complex. The GI50 values for platinum complexes (**1**–**4**) towards HT29, Colo205 and T24 cancer cell lines are similar to cisplatin but poor for A2780 cell lines. Among the tested complexes the highest activity comparable to cisplatin was observed for **3** in case of only T24 cell lines (GI50 25.5 μM) while in other cell lines (HT29, Colo205 and A2780) it was inactive (GI50 >100 μM). The complex **6** is inactive against all the cell lines. The complexes **1**, **2** and **4** exhibited cytotoxicity comparable to cisplatin in case of colon (HT29, Colo205) cell lines. The structurally characterized complexes **7**·H_2O and **9**·$2H_2O$ exhibited poor cytotoxicity against A2780 and T24 cell lines. The following trend of cytotoxicity for these complexes can be noted from Table 4: adriamycin > cispl atin > **3** > **4** > **1** > **2** > **9** > **8** > **7** > **6**.

4. Conclusions

The water soluble platinum(II) complexes with dimethylpyrazole based selenides were synthesized and characterized by microanalyses, IR and NMR spectroscopy. The molecular structures of representative complexes **7**·H_2O and **9**·$2H_2O$ were determined by single crystal X-ray diffraction analyses. Although the *in vitro* cytotoxicity of platinum complexes are poor than adriamycin, values are comparable to cisplatin.

Acknowledgements

One of the authors (ASH) is grateful to Board of Research in Nuclear Sciences (BRNS), Department of Atomic Energy (DAE) for the award of a Senior Research Fellowship (SRF). The cytotoxicity of platinum complexes against cancer cell lines was tested at Anticancer Drug Screening Facility, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, India. The facility provided by ACTREC for tests and help in analysis of results is gratefully acknowledged.

Appendix A. Supplementary material

CCDC 982059 for [Pt(en)(dmpzCH$_2$CH$_2$SeCH$_2$COOH)][NO_3][OH]·H_2O (**7**·H_2O) and 982060 for [Pt(dmpzCH$_2$CH$_2$SeCH$_2$CH$_2$COOH)$_2$][Cl]$_2$·$2H_2O$ (**9**·$2H_2O$) contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2014.11.017.

References

[1] B. Rosenberg, L. van Camp, T. Krigas, Nature 205 (1965) 698.
[2] D. Gibson, Dalton. Trans. (2009) 10681.
[3] N.J. Wheate, S. Walker, G.E. Craig, R. Oun, Dalton. Trans. 39 (2010) 8113.
[4] S. van Zutphen, J. Reedjik, Coord. Chem. Rev. 249 (2005) 2845.
[5] C.M. Riley, L.A. Sternson, A.J. Repta, S.A. Slyter, Anal. Biochem. 130 (1983) 203.
[6] K.J. Barnham, M.I. Djuram, P. del, S. Muldoch, J.D. Ranford, J. Sadler, Inorg. Chem. 35 (1996) 1065.
[7] C. Li, R. Huang, Yi Ding, E. Sletten, F. Arnesano, M. Losacco, G. Natile, Y. Liu, Inorg. Chem. 50 (2011) 8168.
[8] J.-M. Teuben, M.R. Zubiri, J. Reedijk, J. Chem. Soc., Dalton Trans. (2000) 369.
[9] K.A. Mitchell, C.M. Jensen, Inorg. Chem. 34 (1995) 4441.
[10] T. Rau, R. Alsfasser, A. Zahl, R. Van Eldik, Inorg. Chem. 37 (1998) 4223.
[11] U. Bierbach, J.D. Roberts, N. Farrell, Inorg. Chem. 37 (1998) 717.
[12] R. Guddneppanavar, U. Bierbach, Anti-cancer Agents Med. Chem. 7 (2007) 125.
[13] M. Carland, B.E. Abrahams, T. Rede, J. Stephenson, V. Murray, W.A. Denny, W.D. McFadyen, Inorg. Chim. Acta 359 (2006) 3252.
[14] C. Rothenburger, M. Galanski, V.B. Arion, H. Görls, W. Weigand, B.K. Keppler, Eur. J. Inorg. Chem. (2006) 3746.
[15] C. Li, Z. Li, E. Sletten, F. Arnesano, M. Losacco, G. Natile, Y. Liu, Angew. Chem., Int. Ed. 48 (2009) 8497.
[16] K.M. Williams, C. Rowan, J. Mitchell, Inorg. Chem. 43 (2004) 1190.
[17] K.M. Williams, R.P. Dudgeon, S.C. Chmely, S.R. Robey, Inorg. Chim. Acta 368 (2011) 187.
[18] N. Traverso, R. Ricciarelli, M. Nitti, B. Marengo, A.L. Furfaro, M.A. Pronzato, U.M. Marinari, C. Domenicotti, Oxid. Med. Cell. Longev. 2013 (2013) 1; D.M. Townsend, K.D. Tew, H. Tapiero, Biomed. Pharmacother. 57 (2003) 145; M.Y. Abdalla, J. Biol. Sci. 4 (2011) 119.
[19] S.M. Chopade, P.P. Phadnis, A. Wadawale, A.S. Hodage, V.K. Jain, Inorg. Chim. Acta 385 (2012) 185.
[20] D.P. Thompson, P. Boudjouk, J. Org. Chem. 53 (1988) 2109.
[21] A.S. Hodage, P.P. Phadnis, A. Wadawale, K.I. Priyadarsini, V.K. Jain, Phosphorus, Sulfur Silicon Relat. Elem. 187 (2012) 1419.
[22] A.S. Hodage, P.P. Phadnis, A. Wadawale, K.I. Priyadarsini, V.K. Jain, Org. Biomol. Chem. 9 (2011) 2992.
[23] F.D. Rochon, A. Moeneau, Mag. Res. Chem. 29 (1991) 120.
[24] V.M. Djinovic, M. Galanski, V.B. Arion, B.K. Keppler, Dalton Trans. 39 (2010) 3633.
[25] S.C. Dhara, Indian J. Chem. 8 (1970) 193.

[26] T. Higashi, ABSCOR-Empirical Absorption Correction based on Fourier Series Approximation, Rigaku Corporation, 3-9-12 Matsubara, Akishima, Japan, 1995.
[27] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, J. Appl. Crystallogr. 27 (1994) 435.
[28] G.M. Sheldrick, Acta Crystallogr., Sect. A 64 (2008) 112.
[29] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[30] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107.
[31] N.F. Curtis, Y.M. Curtis, Inorg. Chem. 4 (1965) 804.
[32] J.A. Dimmer, M. Hornung, K. Eichele, L. Wesemann, Dalton Trans. 41 (2012) 8989.
[33] S. Narayan, V.K. Jain, B. Varghese, J. Chem. Soc., Dalton Trans. (1998) 2359.
[34] T.V. O'Halloran, S.J. Lippard, T.J. Richmond, A. Krug, J. Mol. Biol. 194 (1987) 705.
[35] I.M. Ismail, P.J. Sadler, Platinum, Gold and Other Metal Chemotherapeutic Agents, American Chemical Society, Washington DC, 1983. pp. 171–190.
[36] P.S. Pregosin, Annu. Rep. NMR Spectrosc. 17 (1986) 285.
[37] L.B. Kumbhare, V.K. Jain, P.P. Phadnis, M. Nethaji, J. Organomet. Chem. 692 (2007) 1546.
[38] M. Risto, S. Kantola, R. Oilenkaiemi, R.S. Laitinen, Acta Crystallogr., Sect. E 62 (2006) M3442.