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ELECTRONIC SUPPLEMENTARY INFORMATION

In vivo studies of a platinum(II) metallointercalator

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Experimental

Instrumentation

Polarimetric studies were conducted using an Optical Activity POLAAR 2001 automatic polarimeter. Solutions were prepared in distilled water or hydrochloric acid (Ajax, 32 %), as specified. Rotation measurements were recorded at the sodium-D line (589 nm) at ambient temperature using a 1 dm cell. The specific optical rotation was calculated using Equation S1:

$$[\alpha]_D = \frac{100 \times \alpha}{l \times c} \qquad \textbf{Equation S1}$$

where α is the observed optical rotation (°), l is the length of tube (dm), and c is the concentration (g/100 cm^3).

^1H NMR and $^{13}C\{^1H\}$ NMR analyses were carried out on a Bruker AC200 spectrometer (200 MHz). NMR grade deuterated solvents were obtained commercially: $CDCl_3$ (Merck, 99.8 %), DMSO-d_6 (Aldrich, 99.5+ %) and D_2O (Cambridge Isotope Laboratories, 99.9 %). Tetramethylsilane (TMS) (Aldrich, >99.9 %) was added as an internal standard

for samples prepared in $CDCl_3$ and DMSO-d_6. For samples prepared in D_2O, DMSO-d_6 was added as the internal calibrant.[1]

Diffuse Reflectance Infrared Fourier Transform Spectra (FT-IR) of the platinum(II) complexes were collected on a BIO-RAD FTS-40 spectrophotometer using either caesium iodide (Aldrich, 99.9 %) or potassium bromide (Riedel-de Haen, 99.5 %) as background matrices[2].

Mass spectrometric analyses of the platinum(II) complexes were carried out on a Finnigan LCQ mass spectrometer by Dr Keith Fisher (USyd). Spectra were collected in the range m/z = 100 and 1000 using electrospray ionisation (ESI) at a voltage of 4 kV. The loop injection was carried out in a 1:1 mixture of H_2O/MeOH with a flow rate of 0.20 $mL{\cdot}min^{-1}$. The nitrogen sheath gas pressure was 60 psi and the capillary temperature was 200 °C.

UV-Visible spectra of the water-soluble platinum(II) complex were recorded on either a CARY 5E UV-Vis-NIR spectrophotometer. Molar extinction coefficients (ε, $M^{-1}{\cdot}cm^{-1}$) were determined from the absorption maxima and shoulders obtained between 200-400 nm. Stock solutions were prepared in water and further diluted to a minimum of ten different concentrations. Samples were analysed in a 1 cm quartz cuvette at room temperature. The absorbance of each solution was recorded and a line-of-best fit was used to determine ε values for each complex at the wavelengths specified (experiments were conducted in duplicate with independent weighing).

Circular dichroism measurements of the platinum(II) complexes were conducted using a JASCO J-710 spectropolarimeter equipped with J-700 analysis software for Windows to calculate the respective $\Delta\varepsilon$ ($mol^{-1}{\cdot}dm^3{\cdot}cm^{-1}$) values (Equation S2). The instrument was calibrated prior to use with (+)-10-camphorsulfonic acid (1 $mg{\cdot}mL^{-1}$; λ_{max} = 290 nm,

[1] *On addition of 3-(trimethylsilyl)-1-propane sulfonic acid (TPS), a 'typical' internal standard for D_2O solutions, the platinum(II) chloride complexes precipitated out of solution. Hence, TPS could not be used as a reference.*

[2] *CsI and KBr are IR transparent within the range 4000-250 cm^{-1} and 4000-400 cm^{-1}, respectively.*

θ = 335 mdeg). Samples were prepared in either DMSO or water (as specified) and measurements were performed using a cylindrical 1 cm quartz cell. Spectra were collected between 200-400 nm (five accumulations per sample) at a speed of 50 nm·min^{-1}, using a sensitivity setting of 50 mdeg, a spectral band width of 1 nm and a N_2 flow-rate of 4 L·min^{-1}.

$$\Delta\varepsilon = \frac{\theta}{32980 \times c \times l} \qquad \textbf{Equation S2}$$

where θ is the angle of rotation (mdeg), c is the concentration (mol/L)[†], and l is the path length (cm).

Microanalysis for C, H and N of the platinum(II) complexes were conducted by the Microanalytical Unit of the Australian National University, Canberra (ANU).

Reagents

All commercially available chemicals utilised in the synthesis were used without further purification unless specified. Hydrochloric acid (32 %), lithium perchlorate (99 %) and sodium chloride (99.9 %) were obtained from Ajax. *Trans*-1,2-diaminocyclohexane (99 %), 1,10-phenanthroline monohydrate (99 %), and D-(−)-tartaric acid (99 %) were obtained from Aldrich. Sodium sulfate (anhydrous, 99 %) and sulfuric acid (98 %) were purchased from APS Finechem. Benzene (99.5 %) was obtained from BDH. Activated charcoal (GR), dimethyl sulfoxide (98 %) and lithium chloride (99 %) were purchased from Merck. Potassium tetrachloroplatinate(II) (99 %) was purchased from Precious Metals Online. Sodium hydroxide pellets (98.5 %) were obtained from Riedel-de Haen. The IRA-400(Cl) Amberlite ion exchange resin (AR) used to convert perchlorate salt complexes to chloride salts was obtained from BDH. Before use, the resin was thoroughly washed with water, 1 M HCl, and finally water to remove any residual impurities. Solvents such as acetone, dichloromethane, diethyl ether and ethanol were of general laboratory grade and were used without further purification.

[†] *1 L = 1000 cm^3 = 1 dm^3*

Syntheses

Preparation of *trans*-(1*S*,2*S*)-1,2-diaminocyclohexane (*S,S*-dach):

A mixture containing D-(−)-tartaric acid (37.4 g, 0.25 mol) and *trans*-1,2-diaminocyclohexane (28.5 g, 0.25 mol) in water (300 mL) was heated to boiling. The solution was cooled to room temperature and then put on ice where a white crystalline solid formed. The precipitate was filtered, washed with a minimal amount of ice-cold water, followed by ethanol and diethyl ether, and then air dried. The filtrate was treated with activated charcoal (1 g/100 mL) to remove dark-coloured impurities before being reduced in volume by heating to obtain a second crop. The crystalline product was collected and treated as described above. The combined crops of the diamine-tartrate salt were recrystallised from boiling water (10 mL/1 g) until constant specific rotation was achieved. Yield: 24.0 g, 36 %. $[\alpha]_D = -11.1°$ (c = 1, H_2O) {lit. −12.5° (c = 4, H_2O)}; ^1H NMR (200 MHz) D_2O, ppm: 1.40 (*br m*, 4H), 1.75 (*m*, 2H), 2.10 (*m*, 2H), 3.32 (*m*, 2H), 4.28 (*s*, 2H).

The obtained *S,S*-dachH_2(−)-tartrate (10.0 g, 0.04 mol) was then dissolved in a minimal amount of NaOH solution (2 M) with stirring. The solution was cooled on ice and made basic (pH = 14) with NaOH solution (10 M). The isolated diamine was extracted with dichloromethane (DCM) (7 × 50 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation to obtain a dark yellow oil. Residual water was removed from the oil azeotropically with benzene (5 mL). The oil was stored overnight at 4 °C under nitrogen, where a cream coloured crystalline solid formed. Yield: 3.28 g, 76 %. $[\alpha]_D = +22.5°$ (c = 1.1, 1 M HCl) {lit. +25.0° (c = 5, 1 N HCl)}; ^1H NMR (200 MHz) $CDCl_3$, ppm: 1.20 (*br m*, 4H), 1.67 (*s*, 6H), 1.87 (*d*, 2H), 2.25 (*m*, 2H); $^{13}C\{^1H\}$ NMR (200 MHz) $CDCl_3$, ppm: 25.5, 35.7, 57.5.

Preparation of [PtCl_2(phen)]:

Sodium chloride (0.53 g, 9.07 mmol) and potassium tetrachloroplatinate(II) (0.42 g, 1.02 mmol) were dissolved in water (400 mL) in a large evaporating basin. The diimine, 1,10-phenanthroline monohydrate (0.20 g, 1.00 mmol), in a minimal amount of water (~ 2 mL) and hydrochloric acid (10 M, 0.8 mL) was added to the initial solution with stirring. The

reaction mixture was left to slowly evaporate on a steam bath for 4 hr, after which a flocculent yellow product formed. The product was collected *via* suction filtration, washed with ice-cold water (4 × 5 mL), ethanol (5 mL) and diethyl ether (5 mL), and then air dried. Additional crops were obtained by further concentration of the filtrate. Yield: 0.41 g, 90 % (yellow solid). ESI-MS (EtOH/H_2O) *m/z*: +ve, 468.8 ($\{Na^+ + [PtCl_2(phen)]\}^+$); IR (CsI) cm^{-1}: 3078 (m), 1438 (s), 848 (vs), 703 (vs), 352 (s), 258 (vw); ^1H NMR (200 MHz) DMSO-d_6, ppm: 8.19 (*dd*, 2H), 8.29 (*s*, 2H), 9.06 (*d*, 2H), 9.79 (*d*, 2H).

Preparation of [Pt(*S,S*-dach)(phen)](ClO_4)$_2$:

The intermediate [$PtCl_2$(phen)] (0.22 g, 0.49 mmol) was suspended in water (100 mL) and a solution of the diamine, *S,S*-dach (0.13 g, 1.09 mmol), in water (10 mL) was added. The reaction mixture was refluxed for 24 hr, or until the solution had turned clear. The clear yellow solution was then cooled to room temperature and filtered through a Sartorius™ Minisart® filter (0.45 μm). The volume of the solution was reduced to approx. 50 mL under vacuum and the solution was re-filtered through a Sartorius™ Minisart® filter (0.45 μm). The complex was precipitated by the dropwise addition of saturated $LiClO_4$ solution.[3] The product was then collected *via* suction filtration through a small sintered-glass filter. The microcrystalline solid was washed with ice-water (2 × 5 mL), ethanol (5 mL) and diethyl ether (5 mL), and then air dried. The product was further dried overnight *in vacuo* over silica. Yield: 0.30 g, 90 %. Colour: cream (microcrystalline solid). $PtC_{18}H_{22}N_4Cl_2O_8$, % Calc.: C: 31.4, H: 3.22, N: 8.14; % found: C: 31.1, H: 3.20, N: 7.93 (ANU); ESI-MS (MeOH/DMF) *m/z*: +ve, 244.7 ($\{[Pt(S,S\text{-dach})(phen)]^{2+}\}^{2+}$), 488.3 ($\{[Pt(S,S\text{-dach})(phen)]^{2+}-H^+\}^+$), 588.9 ($\{[Pt(S,S\text{-dach})(phen)]^{2+}ClO_4^-\}^+$); IR (CsI) cm^{-1}: 3060 (m, vbr), 2948 (m, br), 1440 (m), 1082 (vs), 866 (m), 716 (m), 634 (s), 258 (w); ^1H NMR (200 MHz) DMSO-d_6, ppm: 1.25 (*m*, 2H), 1.47 (*br m*, 2H), 1.65 (*m*, 2H), 2.11 (*d*, 2H), 2.59 (*m*, 2H), 6.55 (*m*, 2H), 7.15 (*m*, 2H), 8.28 (*dd*, 2H), 8.37 (*s*, 2H), 9.15 (*m*, 2H), 9.20 (*s*, 2H); $^{13}C\{^1H\}$ NMR (200 MHz) DMSO-d_6,

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[3] *CAUTION: Perchlorate salts are potentially explosive and must be handled with great care - only small amounts of compounds should be prepared.*

ppm: 24.2, 32.1, 60.9, 125.3, 126.5, 129.7, 139.9, 145.7, 150.5; CD (DMSO) λ, nm (Δε, $mol^{-1}\cdot dm^3\cdot cm^{-1}$): 322 (–0.48), 343 (−1.39).

Conversion of perchlorate salt to [Pt(*S,S*-dach)(phen)]$Cl_2\cdot 1.5H_2O\cdot 0.5HCl$:

The complex, [Pt(*S,S*-dach)(phen)]$(ClO_4)_2$, was suspended in water (50 mL) with IRA-400(Cl) Amberlite ion exchange resin (1 g) added. The mixture was stirred continuously at room temperature until the complex had fully dissolved. The solution was filtered *via* suction filtration through a sintered-glass microfilter, followed by filtration through a Sartorius™ Minisart® filter (0.45 µm). The resultant solution was frozen at liquid N_2 temperature in a 250 mL round-bottom flask and then freeze-dried at approximately −48 °C for 72 hr to produce a flocculent pale yellow powder. Yield: 75 %. $PtC_{18}H_{22}N_4Cl_2\cdot 1.5H_2O\cdot 0.5HCl$, % Calc.: C: 35.7, H: 4.24, N: 9.25; % found: C: 35.5, H: 4.26, N: 9.08 (ANU); ESI-MS ($MeOH/H_2O$) *m/z*: +ve, 244.7 ({[Pt(*S,S*-dach)(phen)]$^{2+}$}$^{2+}$), 488.2 ({[Pt(*S,S*-dach)(phen)]$^{2+}$–H^+}$^+$); −ve, 595.0 ({[Pt(*S,S*-dach)(phen)]Cl_2+Cl^-}$^-$; IR (CsI) cm^{-1}: 3320 (w, br), 3047 (s, br), 2926 (m), 1520 (w), 1438 (m), 1034 (w), 875 (m), 719 (m), 263 (w); ^1H NMR (200 MHz) D_2O, ppm: 1.33 (*m*, 2H), 1.56 (*m*, 2H), 1.76 (*m*, 2H), 2.31 (*d*, 2H), 2.82 (*m*, 2H), 8.08 (*dd*, 2H), 8.19 (*s*, 2H), 8.93 (*s*, 2H), 8.99 (*d*, 2H); $^{13}C\{^1H\}$ NMR (200 MHz) D_2O, ppm: 24.5, 32.8, 62.3, 126.9, 128.5, 131.3, 141.4, 147.7, 151.5; UV-Vis (H_2O) λ, nm (ε, $M^{-1}\cdot cm^{-1}$): 227 (38960), 277 (33907), 326 (2550, sh), 341 (1939), 358 (1598); CD (H_2O) λ, nm (Δε, $mol^{-1}\cdot dm^3\cdot cm^{-1}$): 309 (−1.06), 333 (+0.66).

In vivo Studies

Three different studies were conducted to examine the adverse effects of [Pt(*S,S*-dach)(phen)]$Cl_2\cdot 1.5H_2O\cdot 0.5HCl$ (PHENSS) and cisplatin (CDDP) (positive control) in mice.[4] All *in vivo* experiments were conducted by ICP Firefly Pty Ltd, an Animal Research Establishment accredited with NSW Agriculture (Ref. No. AW96/042). All work undertaken by ICP Firefly Pty Ltd complies with the NSW Government Legislation

[4] *Specific Pathogen Free ARC(Swiss) naive mice and nude mice were selected as the test species as they are widely accepted and recognised by international regulatory authorities as an appropriate experimental model for these types of studies.*

(The Animal Research Act 1985 and Regulation 1995) and was approved by the Animal Ethics Committee (#E0819) constituted by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 1997 (National Health and Medical Research Council). The experimental protocols used for these studies are summarised below. Statistical analyses of all animal testing data were conducted by ICP Firefly Pty Ltd.

Test animals:

Specific Pathogen Free ARC(Swiss) female naive mice and nude mice were purchased from the Animal Resources Centre, Western Australia, and acclimatised to laboratory conditions for at least 5 days before the commencement of each test.

Preparation of stock and test solutions:

A freshly prepared stock solution of PHENSS was submitted for testing. The solution was prepared by dissolving an accurately weighed amount of PHENSS (~ 160 mg) in a sterile saline solution (0.9 % *w/v* NaCl, 25 mL) to give a concentration of approximately 4 mg/mL. The solution was stored at 4 ºC until required.

The stock solution of CDDP was purchased from DBL as a 'cisplatin injection' (specified to contain: 10 mg cisplatin, 90 mg sodium chloride, 10 mg mannitol, water to 10 mL) and was stored at room temperature (as per instructions on the vial) until required.

The stock solutions of PHENSS and CDDP were further diluted to the required test concentrations with sterile saline, which was also used as a test solution (negative control) where specified. Test solutions were heated to 37 ºC before injection.

General testing protocols:

Upon treatment of the test solutions, the mice were observed for specific periods, after which they were sacrificed. During the test period, skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns were monitored. Particular attention was paid to signs of tremor, convulsion, salivation, diarrhoea, lethargy, sleep and coma.

Following sacrifice, all mice were subjected to a full gross necropsy. This included the detailed examination of the external surface of the body, the thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, gonads and spleen were weighed wet immediately following dissection. Animals that died during the studies were preserved in formalin (10 %) until the end of each experiment when they were necropsied with the other test animals.

Study 1 - Acute intraperitoneal toxicity sighting study in mice:

A total of 30 female Specific Pathogen Free ARC(S) naive mice weighing between 17 and 23 g and aged 6-8 weeks at the beginning of the experiment (Day 1 of acclimatisation) were used for the test. The test compounds PHENSS and CDDP were administered intraperitoneally to ten groups containing three mice each (designated Groups 1 to 10). Six groups of mice were given PHENSS at dose levels of either 2, 4, 8, 12, 16 or 32 mg/kg. The remaining four groups of mice were treated with CDDP at dose levels of 2, 4, 8 or 12 mg/kg.

The mice were observed for signs of toxicity (including piloerection, i.e. rough coat) and abnormal behaviour at least once daily for 14 days following Day 1 of test compound administration. Body weights of each mouse were determined immediately prior to administration, and again on Days 4, 8 and 15. A gross necropsy, including the recording of wet weights of major organs, was performed on each mouse at sacrifice. No negative control animals were included in this study.

Study 2 - Repeated-dose intraperitoneal toxicity sighting study in nude mice:

Thirty female Specific Pathogen Free ARC(S) nude mice weighing between 17 and 23 g and aged 4-5 weeks at the beginning of the experiment (Day 1 of acclimatisation) were used for the test. The study was conducted over a period of 20 days. The test compounds, PHENSS and CDDP, were administered intraperitoneally to 10 groups containing three mice each (designated Groups 1 to 10). Four groups of mice were given PHENSS at dose levels of 4, 8, 12 or 16 mg/kg which were administered every two days (Groups 1-4) (10 doses in total). An additional four groups of nude mice were treated with PHENSS at

dose levels of either 4, 8, 12 or 16 mg/kg which were administered every four days (Groups 5-8) (5 doses in total). The remaining two groups of mice were treated with CDDP at two dose levels of 4 or 8 mg/kg every 6 days (Groups 9-10) (4 doses in total). The dose levels were selected following the results of the acute sighting study (Study 1).

The body weights of the mice were measured immediately prior to the first administration of the test compound, weekly thereafter, and at sacrifice. All animals were observed for signs of toxicity and abnormal behaviour at least once daily during the 20-day experimental period post-treatment. A gross necropsy, including the recording of wet weights of major organs, was performed on each mouse at sacrifice. No negative control animals were included in this study.

Study 3 - Twenty day repeated-dose intraperitoneal efficacy and toxicity study in nude mice bearing PC3 tumours:

Eighteen female Specific Pathogen Free ARC(S) nude mice were used in the study. Each mouse weighed between 18 and 24 g and aged 6-8 weeks upon receipt. The eighteen mice were divided into three groups of six (designated Groups 1-3).

On Day 1, all mice (Groups 1-3) were injected subcutaneously on the dorsal aspect with PC3 cell suspension[5] (0.1 mL, 2×10^7 cells/mL) in a 50:50 mix of growth medium:BD Matrigel Basement Membrane Matrix.

On Day 3, Groups 1, 2 and 3 were injected intraperitoneally with a volumetric dose equivalent (20 mL/kg) of PHENSS, CDDP (positive control) and sterile saline vehicle (negative control), respectively. Group 1 was given a dose of PHENSS at 16 mg/kg and this was repeated every two days commencing Day 3 (8 doses in total). Group 2 was given a dose of CDDP at 8 mg/kg and this treatment was repeated every six days, starting Day 3 (3 doses in total). Group 3 was treated only with sterile saline, given every two days, beginning Day 1. The doses were given over a twenty day period and dosage levels were based on the results of the repeated-dose sighting study (Study 2).

[5] *PC3 is a hormone-independent cell line derived from human prostate metastasis.*

Tumours, once palpable, were measured daily using a vernier calliper until completion of study and tumour volumes (*TV*, mm^3) were calculated using Equation S3:

$$TV = \frac{(d^2 \times D)}{2}$$ **Equation S3**

where d and D represent the shortest and longest diameter measurements (mm) of the tumour, respectively.

The body weights of each mouse were determined on Days 1, 8, 15 and at sacrifice or death where possible. All animals were observed for signs of toxicity and abnormal behaviour at least once daily during the 20 day experimental period. Animals that died during the study were necropsied at the end of the experiment. On Day 20, all remaining animals were sacrificed, subjected to a gross necropsy examination and the liver, kidneys, adrenals, gonads and spleen were weighed wet. The observations and assessments of the treated groups (Groups 1 and 2) were compared with the negative control group (Group 3).

Unpaired two-tail Student's t-tests were used to compare the body and organ weights, and tumour volumes and weights obtained from the treated and untreated mice (where possible). The significance level was preset at $P < 0.05$ (confidence levels of 95 %). Results not deemed statistically significant may still be considered biologically significant if they met the criterion of approximately 40 % difference following treatment.

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Results

Table S1. Mean body weights of SPF Swiss mice (Study 1) treated with various doses of PHENSS or CDDP (no. mice per group = 3)

	Mean Body Weight (g) (± SD) PHENSS						Mean Body Weight (g) (± SD) CDDP			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day	**2 mg/kg**	**4 mg/kg**	**8 mg/kg**	**12 mg/kg**	**16 mg/kg**	**32 mg/kg**	**2 mg/kg**	**4 mg/kg**	**8 mg/kg**	**12 mg/kg**
1	23.2 ± 2.1	23.7 ± 2.1	22.7 ± 1.1	23.5 ± 1.9	22.3 ± 2.5	25.3 ± 2.0	22.2 ± 1.8	23.6 ± 0.4	23.6 ± 0.1	23.5 ± 1.0
4	23.8 ± 2.6	23.4 ± 1.3	22.5 ± 1.6	23.0 ± 1.7	23.0 ± 2.1	25.6 ± 2.4	22.1 ± 1.6	23.1 ± 0.2	23.8 ± 0.2	21.4 ± 0.3
8	23.0 ± 3.0	24.0 ± 1.5	23.3 ± 1.5	23.3 ± 1.6	23.0 ± 2.3	25.6 ± 3.0	22.4 ± 0.9	24.5 ± 0.1	24.2 ± 0.4	21.6 ± 0.3
15	24.7 ± 3.0	25.0 ± 2.4	24.3 ± 2.4	24.9 ± 2.0	24.0 ± 2.2	27.5 ± 3.5	22.6 ± 0.8	24.4 ± 0.6	25.5 ± 0.8	24.2 ± 0.6

Table S2. Mean body weights of SPF Swiss mice (Study 2) treated with various doses of PHENSS or CDDP (no. mice per group = 3)

	Mean Body Weight (g) (± SD) PHENSS (treated every 2 days)				Mean Body Weight (g) (± SD) PHENSS (treated every 4 days)				Mean Body Weight (g) (± SD) CDDP (treated every 6 days)	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Day	**4 mg/kg**	**8 mg/kg**	**12 mg/kg**	**16 mg/kg**	**4 mg/kg**	**8 mg/kg**	**12 mg/kg**	**16 mg/kg**	**4 mg/kg**	**8 mg/kg**
1	21.2 ± 0.6	21.0 ± 1.7	21.5 ± 2.2	21.1 ± 1.3	19.9 ± 2.8	22.0 ± 0.6	22.5 ± 1.8	21.1 ± 0.8	21.5 ± 1.2	21.2 ± 2.4
8	23.0 ± 0.7	23.2 ± 1.6	24.8 ± 2.2	23.4 ± 1.6	22.3 ± 3.0	24.4 ± 0.3	24.6 ± 1.8	22.5 ± 0.4	23.7 ± 1.6	22.1 ± 2.1
15	24.1 ± 0.1	25.1 ± 1.9	26.3 ± 3.4	25.6 ± 1.3	23.8 ± 2.9	25.7 ± 0.9	26.0 ± 1.7	23.8 ± 0.5	25.9 ± 0.6	22.8 ± 1.3
21	24.6 ± 0.7	25.7 ± 2.0	26.7 ± 3.5	26.3 ± 1.3	24.1 ± 2.3	27.1 ± 1.1	27.5 ± 2.0	24.7 ± 0.6	27.0 ± 0.3	21.8 ± 0.3