Supplementary Material (ESI) for Dalton Transactions

Supporting information

General Procedures

All experimental manipulations were carried out under a dry nitrogen or argon atmosphere, using standard Schlenk techniques unless otherwise stated. Solvents were dried and distilled prior to use by conventional methods. The precursors *cis*-[(^{t}BuNH)$_2$(μ-N^{t}BuP)$_2$],[1] *cis*-[(MeNC$_4$H$_8$N)$_2$(μ-N^{t}BuP)$_2$],[2] *cis*-[(μ-N^{t}BuP)$_2$(OCH$_2$CH$_2$NMe$_2$)$_2$],[3] *trans*-[(μ-N^{t}BuPO)$_2$(SeCH$_2$CH$_2$NMe$_2$)$_2$][4] and [(Me$_2$S)AuCl][5] were prepared according to the literature procedures. The ^{1}H and ^{31}P{^{1}H} NMR (δ in ppm) spectra were obtained on a Varian VXR 400 spectrometer operating at frequencies of 400 and 162 MHz, respectively. The tetramethylsilane and 85% H_3PO_4 were used as internal and external standards for ^{1}H and ^{31}P{^{1}H} NMR respectively. Positive shifts lie downfield of the standard in all the cases. Microanalyses were carried out on a Carlo Erba Model 1106 elemental analyzer. Melting points of all compounds were determined on Veego melting point apparatus and are uncorrected.

Synthesis of *cis*-[{^{t}BuN(H)P(μ-N^{t}Bu)$_2$P(CH$_3$)N(H)^{t}Bu}(I)] (2).

To a solution of *cis*-[(^{t}BuNH)$_2$(μ-N^{t}BuP)$_2$] (0.50 g, 1.43 mmol) in petroleum ether (10 mL) added 1 mL of MeI also in petroleum ether (15 mL) dropwise over a period of 10 minutes at room temperature during which time the solution turns turbid. The reaction mixture was heated under reflux condition for 2 h. The solution was brought to room temperature and the product was separated by filtration. The product was washed several times with cold petroleum ether and dissolved in dichloromethane, layered with 2 mL of petroleum ether, and placed at -25 °C for 18 h to afford **1** as white crystalline compound. Yield: 90% (0.633 g, 1.29 mmol). Mp: 198-200 °C. ^{1}H NMR (400 MHz, $CDCl_3$, δ):

7.05 (d, $^2J_{PH}$ = 14.4 Hz, *NH*, 1H), 2.30 (d, $^2J_{PH}$ = 13.2 Hz, *NH*, 1H), 1.65 (s, *CH*$_3$, 3H), 1.60 (s, ^{t}*Bu*, 9H), 1.34 (s, ^{t}*Bu*, 18H), 1.32 (s, ^{t}*Bu*, 9H). $^{31}P\{^1H\}$ NMR (161.8 MHz, $CDCl_3$, δ): 80.4 (s), 19.6 (s). Anal. Calcd. for $C_{17}H_{41}N_4P_2I$: C, 41.63; H, 8.42; N, 11.42%. Found: C. 41.47; H, 8.58; N, 11.72%.

Synthesis of *cis*-[{(Me$_2$NC$_4$H$_8$N)P(μ-N^{t}Bu)$_2$P(CH$_3$)(NC$_4$H$_8$NMe$_2$)}(I)$_3$] (4).

Added 2 mL of MeI in 15 mL of dichloromethane to a stirred solution of *cis*-[(μ-N^{t}BuP)$_2$(NC$_4$H$_8$NMe)$_2$] (0.90 g, 2.23 mmol) in 10 mL of dichloromethane over a period of 10 min. The clear solution turns turbid immediately after the addition of methyl iodide. The reaction mixture was allowed to stir at room temperature for further 4 h during which time the compounds get precipitated. The residue was separated by filtration, was washed several times with dichloromethane and dried under vacuum to afford **2** as white crystalline solid. Yield: 85% (1.57 g, 1.90 mmol). Mp: 212-214 °C. ^1H NMR (400 MHz, D_2O, δ): 3.80-3.29 (m, *C*$_4$*H*$_8$, 16H), 3.27 (s, *NMe*$_2$, 6H), 3.19 (s, *NMe*$_2$, 6H), 1.38 (s, ^{t}*Bu,* 18H), 1.37 (s, *CH*$_3$, 3H). $^{31}P\{^1H\}$ NMR (161.8 MHz, D_2O, δ): 87.1 (s), 35.1 (s). Anal. Calcd. for $C_{21}H_{49}P_2N_6I_3$: C, 30.45; H, 5.96; N, 10.15%. Found: C. 30.86; H, 5.68; N, 10.72%.

Synthesis of *cis*-[{(μ-N^{t}BuP)$_2$(OCH$_2$CH$_2$NMe$_3$)$_2$}(I)$_2$] (6).

In a manner identical to that used for the synthesis of **7**, *cis*-[(μ-N^{t}BuP)$_2$(OCH$_2$CH$_2$NMe$_2$)$_2$] (1.12 g, 2.94 mmol) and MeI of 3 mL were allowed to react. Yield: 95% (1.86 g, 2.79 mmol). Mp: 148-150 °C. ^1H NMR (400 MHz, D_2O, δ): 4.35 (t, J_{HH} = 9.8 Hz, *OCH*$_2$, 4H), δ 3.62 (t, J_{HH} = 8.4 Hz, *NCH*$_2$, 4H), 3.21 (s, *Nme*, 18H), 1.31 (s, ^{t}*Bu*, 18H). $^{31}P\{^1H\}$ NMR (161.8 MHz, D_2O, δ): 132.9 (s). Anal. Calcd. for $C_{18}H_{44}N_4P_2O_2I_2$: C, 32.54; H, 6.67; N, 8.43%. Found: C. 32.86; H, 6.86; N, 8.72%.

Synthesis of *cis*-[{^{t}BuN(H)P(AuI)(μ-N^{t}Bu)$_2$P(CH$_3$)N(H)^{t}Bu}(Cl)] (7).

To a solution of *cis*-[{^{t}BuN(H)P(μ-N^{t}Bu)$_2$P(CH$_3$)N(H)^{t}Bu}(I)] (0.034 g, 0.069 mmol) in 4 mL of dichloromethane added a solution of [(Me$_2$S)AuCl] (0.020 g, 0.069 mmol) also in dichloromethane (8 mL) at room temperature. The reaction mixture was stirred for 4 h. The clear solution obtained was concentrated to a small bulk under reduced pressure and layered with petroleum ether to get **11** as colorless crystalline solid. Yield: 85% (0.043 g, 0.059 mmol). Mp: 190-192 °C (dec). ^{1}H NMR (400 MHz, CDCl$_3$, δ): 8.11 (d, $^2J_{PH}$ = 9.6 Hz, *NH*, 1H), 2.23 (d, $^2J_{PH}$ = 15.2 Hz, *NH*, 1H), 1.69 (s, *CH$_3$*, 3H), 1.63 (s, ^{t}*Bu*, 18H), 1.60 (s, ^{t}*Bu*, 9H), 1.52 (s, ^{t}*Bu*, 9H). ^{31}P{^{1}H} NMR (161.8 MHz, CDCl$_3$, δ): 82.4 (s), 30.0 (d, $^2J_{PP}$ = 5.5 Hz). ES (EI): 687.1 (m/e – Cl). Anal. Calcd. for C$_{17}$H$_{41}$N$_4$P$_2$AuICl : C, 28.24; H, 5.71; N, 7.75%. Found: C. 27.91; H, 5.88; N, 7.43%.

Synthesis of *cis*-[{(Me$_2$NC$_4$H$_8$N)P(AuI)(μ-N^{t}Bu)$_2$P(CH$_3$)(NC$_4$H$_8$NMe$_2$)}(I)$_2$(Cl)] (8).

To a solution of *cis*-[{(Me$_2$NC$_4$H$_8$N)P(μ-N^{t}Bu)$_2$P(CH$_3$)(NC$_4$H$_8$NMe$_2$)}(I$_3$)] (0.034 g, 0.041 mmol) in 6 mL of methanol was added a solution of [(Me$_2$S)AuCl] (0.012 g, 0.041 mmol) in dichloromethane (4 mL). The reaction mixture was stirred at room temperature for 4 h. All the volatiles were removed under vacuum and the residue obtained was extracted with 5 mL dichloromethane and cooled to 0 ^{o}C to afford **6** as analytically pure crystalline solid. Yield: 78% (0.034 g, 0.0319 mmol). Mp: 190-192 °C (dec). ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 3.66-3.44 (m, *C$_4$H$_8$*, 16H), 2.44 (s, *NMe$_2$*, 6H), 2.43 (s, *NMe$_2$*, 6H), 1.52 (s, *CH$_3$*, 3H), 1.36 (s, ^{t}*Bu*, 18H). ^{31}P{^{1}H} NMR (161.8 MHz, DMSO-d$_6$): δ 88.5 (s), 37.7 (s). Anal. Calcd. for C$_{21}$H$_{49}$P$_2$N$_6$AuClI$_3$: C, 23.77; H, 4.65; N, 7.92%. Found: C. 23.58; H, 5.96; N, 8.51%.

Synthesis of *cis*-[{(μ-N^{t}BuP)$_2$(OCH$_2$CH$_2$NMe$_3$)$_2$Au$_2$I$_2$}(Cl)$_2$] (9).

In a manner identical to that used for the synthesis of **12**, *cis*-[{(μ-N^{t}BuP)$_2$(OCH$_2$CH$_2$NMe$_3$)$_2$}(I)$_2$] (0.025 g, 0.038 mmol) and [(Me$_2$S)AuCl] (0.022 g, 0.075 mmol) were allowed to react. Yield: 88% (0.038 g, 0.034 mmol). Mp: 168-170 °C (dec). ^{1}H NMR (400 MHz, D$_2$O, δ): 4.70 (t, J_{HH} = 8.2 Hz, *OCH$_2$*, 4H), 3.82 (t, J_{HH} = 9.6 Hz, *NCH$_2$*, 4H), 3.18 (s, *Nme*, 18H), 1.57 (s, ^{t}*Bu*, 18H). ^{31}P{^{1}H} NMR (161.8 MHz, DMSO-d$_6$, δ): 110.3 (s). Anal. Calcd. for C$_{18}$H$_{44}$N$_4$P$_2$O$_2$Au$_2$Cl$_2$I$_2$: C, 19.14; H, 3.92; N, 4.96%. Found: C. 19.25; H, 3.78; N, 4.72%.

Cell culture and cell proliferation assay.

HeLa cells were grown in minimal essential medium (Hi Media, Mumbai) supplemented with 10 % (v/v) fetal bovine serum and sodium bicarbonate (2.0 mg/mL) in the presence of antibiotics.[6] HCT-116 (Human colon carcinoma cell line containing wild type p53) cells were grown in DMEM containing 10% (v/v) fetal bovine serum and sodium bicarbonate (2.0 mg/mL) in the presence of antibiotics. The effects of different compounds on HeLa cell proliferation were determined in 96-well tissue culture plates by SRB assay. DMSO (0.1 %) alone was used as a vehicle control.

Immunofluorescence microscopy.

HCT-116 cells (6 X 10^4 cells/mL) were seeded on glass coverslips in 24- well tissue culture plates and treated with different drugs dissolved in 100 % DMSO. The final DMSO concentration in all the experiments was 0.1 %. After 24 h of the drug treatment, cells were fixed in 3.7 % formaldehyde for 30 minutes at 25 °C and processed for immunofluorescence microscopy to visualize p53.[7] The processed coverslips were observed by using a 40 X objective in a Nikon Eclipse TE 2000U microscope

(Kanagawa, Japan). The images were analyzed by using Image Pro-Plus software (Media Cybernetics, Silver Spring, MD).

Annexin V/Propidium iodide staining.

HeLa cells (6 X 10^4 cells/mL) were seeded on glass coverslips in 24- well tissue culture plates. Twenty four hours after seeding the old media was replaced with fresh media containing no compound or 20 μM of compound 411 and 320 and incubated for an additional 24 h. The cells were then centrifuged in a Labofuge 400R cytospin (Heraeus, Hanau/Germany) for 10 min to attach the floating cells on the coverslips. The cells were stained using the annexin V apoptosis kit (Santa Cruz Biotechnology, CA, USA) according to the instructions provided with the kit and observed immediately using the Nikon Eclipse TE-2000 U microscope.[8]

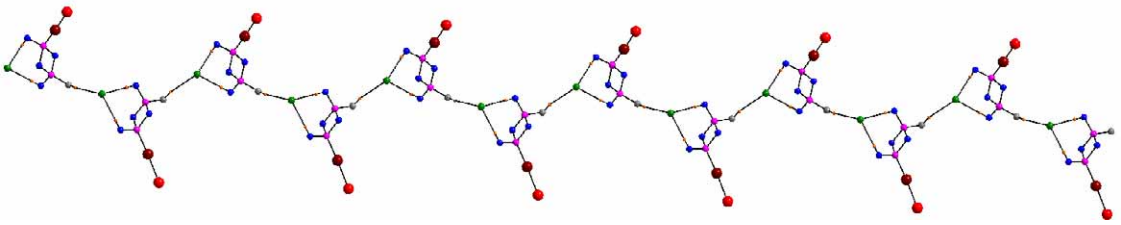

Fig 1. Ball and stick representation of one-dimensional polymeric sheet like structure of **7** due to inter- and intramolecular hydrogen bonding.

Table 1 Crystallographic Data for **2** and **7**

	2	**7**
formula	$C_{17}H_{41}N_4P_2$, I	$C_{17}H_{41}AuIN_4P_2$, Cl
fw	490.38	722.80
crystal system	Orthorhombic	Monoclinic
space group	Pbca (No. 61)	$P2_1/a$ (No. 14)
a, Å	14.090(3)	15.5625(7)
b, Å	25.9449(18)	11.9084(13)
c, Å	27.452(3)	15.895(2)
α, deg	90	90
β, deg	90	118.376(3)
γ, deg	90	90
V, Å^3	10036(3)	2591.9(5)
Z	16	4
ρ_{calc}, g cm^{-3}	1.298	1.852
μ (MoKa), mm^{-1}	1.411	7.104
F (000)	4064	1400
crystal size mm^3	0.09 × 0.16 × 0.33	0.21 × 0.26 × 0.33
T, K	150	293
2θ range, deg	3.0, 25.0	3.1, 25.0
Total no. reflns	61951	14699
No. of indep. reflns	8818, [$R_{(int)}$ = 0.060]	4359, [$R_{(int)}$ =0.085]
R1[a]	0.0296	0.0432
wR_2[b]	0.0606	0.1229
GOF (F^2)	0.831	1.071

[a] $R = \Sigma||F_o|-|Fc||/\Sigma|F_o|$.

[b] $wR_2 = \{[\Sigma w(F_o^2-F_c^2)/\Sigma w(F_o^2)^2]\}^{1/2}$; $w = 1/[\sigma^2(F_o^2)+(xP)^2]$ where $P = (F_o^2+2F_c^2)/3$

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