Promising Anticancer Activity of [Bis(1,8-quinolato)palladium (II)] Alone and in Combination

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Abstract

Due to similar coordination chemistry of palladium and platinum, a large number of palladium compounds too have been investigated for their anticancer activity. In the present study we describe synthesis, characterization and anticancer activity of palladium complex [Bis(1,8-quinolato)palladium (II)], coded as NH3 against seven different cancer cell lines. NH3 is found to have higher antitumour activity than cisplatin against both parent ovarian A2780 cell line and cisplatin-resistant cell lines. Also, NH3 has the lowest IC50 value against HT-29 colorectal cancer cell line. The higher antitumour activity of NH3 is due to the presence of bulky 8-hydroxy-quinoline ligand thus reducing its reactivity. Proteomic study has identified significantly expressed proteins which have been validated through bioinformatics. NH3 has been found to be less toxic than cisplatin at 2.5 mg/kg and 5 mg/kg dosages on mice models. Binary combinations of NH3 with curcumin and epigallocatechin gallate (EGCG) have demonstrated dose and sequence dependent synergism in ovarian and colorectal cancer models. All of the preclinical studies indicate promising therapeutic potentiality of NH3 [Bis(1,8-quinolato)palladium (II)] as an anticancer drug.

Introduction

Serendipitous discovery of cisplatin by Rosenberg in 1970s opened a new horizon in cancer therapy. Since then, several other metal analogues (e.g. complexes of palladium, ruthenium, gold and copper) along with platinum complexes have been investigated in search for better anticancer drugs. Due to similar coordination chemistry of platinum and palladium, newer palladium complexes have gained much interest. Although initial investigations displayed discouraging results with higher toxicity and lower anticancer activity of palladium complexes than cisplatin, recently higher antitumour activity in palladium complexes with low toxicity has been achieved by introduction of bulky ligands^{1,2}.

8-hydroxyquinoline is a subclass of quinolone family, which has demonstrated a wide variety of biological activities from the distant past. 8-hydroxyquinoline can act as a bidentate chelate that binds with metal ions through the oxygen and nitrogen centres. Many derivatives of 8-hydroxyquinoline have shown neuroprotection, anticancer, anti-HIV and antifungal actions³. In the last few years, a number of research articles from different groups have been published which demonstrate good potential for transition metal complexes containing 8-hydroxy-quinoline derivatives as anticancer agents. Examples include quilamines iron chelator, glycosylated copper(II) ionophores, osmium(VI) nitride complexes, clioquinol copper(II) and zinc(II) complexes, dihalo-8-hydroxyquinoline-metal complexes, hydroxyquinoline derived vanadium (IV and V), copper(II) and iron(II) complexes, platinum and ruthenium(II) complexes^{4,5}. Promising anticancer activity of a platinum complex containing 8-hydroxy-quinoline ligand against ovarian cancer models has also been reported from our laboratory⁶. In the present study we have described synthesis and characterization of a palladium complex [Bis(1,8 quinolato)palladium (coded as NH3)]. Anticancer activity of the designed complex against ovarian, colorectal, breast and cervical cancer models has been studied. Proteomic study has been performed to identify the proteins responsible for the anticancer action of the designed complex. Bioinformatics was also implemented to conduct protein-protein interaction, functional enrichment analysis and survival prediction in ovarian cancer using the proteins identified from proteomics. In addition, preliminary toxicity profile of NH3 has been compared with cisplatin in mice model.

Recently combination of chemotherapeutic drugs has demonstrated better outcome in combating cancer and more specifically in overcoming drug resistance. Since several phytochemicals have shown good antitumour activity and many of them have already entered into clinic, combination of phytochemicals with other chemotherapeutic drugs might explore new horizon in cancer therapy. Earlier, we have shown synergistic activity from combination of platinum and palladium drugs (cisplatin, oxaliplatin, designed compounds) with phytochemicals against different cancer models^{7,8}. In the present study, newly designed complex NH3 has been applied in combination with two naturally derived phytochemicals curcumin and epigallocatechin gallate (EGCG) against ovarian and colorectal cancer models as a function of concentration and sequence of administration.

Results And Discussion

Synthesis: The complex coded as NH3 was synthesized by reacting potassium tetrachloropalladate with 8-hydroxy quinoline ligand. The synthesis scheme of the complex is shown in Figure 2. Synthesis of the same complex was described earlier in 1960s but we have synthesized NH3 using a different method.

Crystal Structure of NH3

Crystallographic details and selected bond lengths and angles for NH3 are provided in Tables S1 to S3 of the Supplemental Information (SI). An OLEX2 depiction with the numbering scheme and 75% displacement ellipsoids is provided in Figure S1 of the SI. The square planar coordination geometry of the complex molecule is unremarkable. The CCDC deposition numbers for NH3 is 2033432.

Presumably reflecting differences in crystal growth procedures, the crystal structure packing and hence unit cell of NH3 differs from that of the structure reported by Prout and Wheeler in 1966⁹ (CCDC code: HQUIPD) and by Low, Xu, Xiang and Chu in 2011¹⁰ (CCDC code: HQUIPD01). The 1966 and 2011 structures are respectively reported in the non-standard $P2_1/b$ and $P2_1/n$ monoclinic settings, with the latter being an appropriate choice for the 2011 structure in having a beta angle closer to 90 degrees than its $P2_1/c$ standard setting counterpart. The $P2_1/n$ setting would likewise be beneficial for the 1966 structure. In contrast, the NH3 structure reported here is best represented in $P2_1/c$. Differences in the unit cells of the 1966, 2011 and present structure are evident in Table 1, which lists the unit cell constants of all three in the standard $P2_1/c$ setting. The relationship of the $P2_1/c$ unit cell of the NH3 structure reported here and that of the 2011 HQUIPD01 structure is depicted in SI Figure S2. Figures S3 to S6 depict the nature of the packing in these two structures. The differences in the cell constants for the isomorphous 1966 and 2011 structures will at least in part reflect different data collection temperatures.

A key difference is that in the isomorphous HQUIPD and HQUIPD01 structures the complex molecule is located on an inversion centre, whereas in the NH3 polymorph reported here the molecule is located on a general position. The metal to metal distance in the HQUIPD01 structure is 4.7187(3) Å, in contrast to 3.7751(5) Å in the NH3 structure presented here. The inter-planar separation between offset-stacked

molecules is approximately 3.29 Å in the HQUIPD01 structure and 3.42 Å in the NH3 structure. The stacking lateral offset is then approximately 3.38 Å in the HQUIPD01 structure and 1.60 Å in the NH3 structure.

Table 1: Comparison of crystallographic unit cell constants in P2₁/c

Structure	<i>a</i>	<i>b</i>	<i>c</i>	<i>b</i>	Volume	Z	Temp (Kelvin)
HQUIPD	11.49	4.77	15.31	121.9	712.4	2	293
HQUIPD01	11.216	4.719	14.993	120.22	685.7	2	100
NH3	9.340	10.110	14.844	100.77	1385.6	4	150

Antitumour activity of the complex:

MTT reduction assay was used to determine the cytotoxicity of the designed complex NH3 and standard anticancer drug cisplatin against seven (three ovarian i.e. A2780, A2780^{CisR}, A2780^{ZD0473R}; two colorectal HT-29, Caco-2; one cervical Hela and one breast MCF-7) different cancer cell lines. The results of the comparative antitumour activity between NH3 and cisplatin has been displayed in Figure 3. It could be observed that the newly designed complex NH3 demonstrated greater anticancer activity than cisplatin against all tested cell lines.

Among the tested cancer cell lines, NH3 showed 10 times greater activity than cisplatin against parent ovarian A2780 cell line. Anticancer activity of NH3 was even greater in cisplatin resistant cell lines (96 times more in A2780^{CisR} cell line and 82 times more in A2780^{ZD0473R}) compared to cisplatin. The greatest anticancer activity of NH3 in terms of lowest IC₅₀ value was observed in HT-29 colorectal cancer cell line. In other cancer models NH3 exhibited 28 times greater cytotoxicity in Caco-2 cell line; 65 times greater in MCF-7 cell line and 10 times greater in Hela cell line compared to cisplatin. A number of studies have been conducted earlier towards evaluation of anticancer activity of the ligand 8-hydroxyquinoline itself and its derivatives. 8-hydroxyquinoline exhibited significant antitumour activity against Raji (lymphoma), Hela (cervical) and PC-3 (prostate) cancer cell line^{11,12}. Few derivatives of 8-hydroxyquinoline also displayed very high anticancer activity during *in vivo* and *in vitro* model study i.e. clioquinol¹³, nitroxoline¹² and mannich bases of 8-hydroxyquinoline¹⁴. Interestingly, chelation of metals with 8-hydroxyquinoline or its derivatives further increased antitumour activity. Six copper chelated compounds have been reported to show lower IC₅₀ values (1.3 -16 µM) against PC-3 and Hela cell lines¹¹. Promising antitumour activity of platinum chelated compounds also have been described earlier^{4,6}. Palladium compounds containing clioquinol were also reported to possess significant anticancer activity against ovarian cancer model¹⁵. But NH3 is found to show superiority (in terms of IC₅₀ values) over the all previously reported activity of 8-hydroxyquinoline, derivatives of 8-hydroxyquinoline, metal chelated 8-hydroxyquinoline or derivatives against various tumour models.

The higher antitumour activity of NH3 can be partially attributed to the presence of the ligand 8-hydroxyquinoline itself. Coordination of the ligand with palladium metal might have resulted in further increase in activity which has been observed in earlier studies with copper and platinum^{4,11}. Four-

coordinated square planar geometry of NH3 where two 8-hydroxyquinoline ligands arrange in the *trans* position with palladium might be in the unique position to bind and DNA and display maximum cell killing in cancer cells. The suggested bio-activation pathway of NH3 for its antitumour activity is given in Figure 4.

Proteomics:

The study has provided information on underlying mechanism on antitumour activity of NH3 alone in different cell lines. Total of 19 (seven from A2780 cell line, nine from A2780^{cisR} cell line and three from HT-29 cell line) proteins have been identified from the present study which underwent significant changes in expression following treatment of NH3 alone. Change in folds of the respective proteins after treatment of NH3 alone is shown in Table 2 (A2780 cell line), Table 3 (A2780^{cisR} cell line) and Table 4 (HT-29 cell line).

Fourteen proteins including: actin cytoplasmic 1 (ACTB), vimentin (VIME), endoplasmin (ENPL), 60 kDa heat shock protein (CH60), 78 kDa glucose-regulated protein (GRP78), polyubiquitin-B (UBB), histone H3.3(H3.3), cofilin-1 (COF1), heat shock cognate 71 kDa protein (HSP7C), 40S ribosomal protein SA (RSSA), keratin type II cytoskeletal 1 (K2C1), citrate synthase (CISY) elongation factor Tu (EFTU) and transitional endoplasmic reticulum ATPase (TERA) have been downregulated and considered to be associated with the anticancer action of NH3 in ovarian cancer. Downregulation of three proteins namely: nucleoside diphosphate kinase B (NDKB), Annexin A1 (ANXA1) and histone H4 (H4) have been esteemed to be related with the anticancer action of NH3 in colorectal cancer. Among the above identified proteins, seven of them was considered to be highly significant (more than 3 folds downregulation) in relation to the antitumour action of NH3 (Figure 5).

Table 2: Protein spots which underwent significant changes in expression after treatment with NH3 alone in A2780 cell line and their identification (name, mass, Da/pI, mascot score, matched peptides, percent of sequence coverage).

Spot No	Change in Expression	Fold change	Protein name	Mass (Da)/pI	Mascot score		No of matched peptides	Sequence coverage (%)
6	Downregulated	4.8	Actin, cytoplasmic 1	41710/5.29	520	16		30
12	Downregulated	1.63	Vimentin	53619/5.06	739	6		45
13	Downregulated	3.23	60 kDa heat shock protein, mitochondrial	61016/5.70	518	19		24
18	Downregulated	4.56	Endoplasmin	92411/4.76	647	36		21
45	Downregulated	2.00	78 kDa glucose-regulated protein	72288/5.07	820	28		27
118	Downregulated	11.39	Polyubiquitin-B	25746	40			
155	Downregulated	6.63	Histone H3.3	15319/11.27	120	11		27

□ Mascot score is insignificant, information not provided.

Table 3: Protein spots which underwent significant changes in expression after treatment with NH₃ alone in A2780^{cisR} cell line and their identification (name, mass, Da/pI, mascot score, matched peptides, percent of sequence coverage).

Spot No	Change in Expression	Fold change	Protein name	Mass (Da)/pI	Mascot score		No of matched peptides	Sequence coverage (%)
1	Downregulated	1.51	Actin, cytoplasmic 1	41710 / 5.29	520	16		30
Cn9	Downregulated	2.98	40S ribosomal protein SA	32833 / 4.79	130	10		17
Cn16	Downregulated	1.69	Heat shock cognate 71 kDa protein	70854 / 5.37	686	27		21
Cn23	Upregulated	2.47	Keratin, type II cytoskeletal 1	65999	31	□		□
Cn34	Downregulated	7.86	Elongation factor Tu, mitochondrial	49510 / 7.26	88	13		16
Cn41	Downregulated	2.93	Citrate synthase, mitochondrial	51680 / 8.45	97	14		16
Cn56	Downregulated	3.87	Transitional endoplasmic reticulum ATPase	89266 / 5.14	208	14		10
Cn69	Downregulated	2.27	Cofilin-1	18491 / 8.22	144	10		22
Cn79	Downregulated	2.79	Actin, cytoplasmic 1	41710 / 5.29	231	19		48

□ Mascot score is insignificant, information not provided.

Table 4: Protein spots which underwent significant changes in expression after treatment with NH₃ alone in HT-29 cell line and their identification (name, mass, Da/pI, mascot score, matched peptides, percent of sequence coverage).

Spot No	Change in Expression	Fold change	Protein name	Mass (Da)/pI	Mascot score		No of matched peptides	Sequence coverage (%)
Hn70	Downregulated	2.14	Nucleoside diphosphate kinase B	17287/8.52	308	5(4)		71
Hn89	Downregulated	1.85	Annexin A1	38690/6.57	166	25		54
Hn119	Downregulated	1.91	Histone H4	11360/11.36	58	6		29

Protein-Protein Interaction and Functional Enrichment:

We have carried out protein-protein interaction analysis using the proteins identified in ovarian cancer. In the study we have used protein-protein interaction database “STRING”¹⁶ and NetWorkAnlyst¹⁷ software tools. We found that among the 14 proteins, 11 of them namely: ACTB, HSPD1 (corresponding to CH60), CS (corresponding to CISO), TUFM (corresponding to EFTU), HSP90B1 (corresponding to ENPL), HSPA5 (corresponding to GRP78), HSPA8 (corresponding to HSP7C), RPSA (corresponding to RSSA), VCP (corresponding to TERA), UBB and VIM (corresponding to VIME) are strongly connected together as shown in Figure 6. Hence it appears that these altered proteins bind together with NH3 thus accounting for its antitumour activity.

We have performed signalling pathways enrichment analysis using EnrichR software tools¹⁸ that incorporated several pathways databases including KEEG, WikiPathways, REACTOME, BioCarta, BioPlanet and Panther. Here we have considered the genes corresponding to the altered 14 proteins in the ovarian cancer. Top 50 significant signalling pathways and their -10 logarithmic p-values are shown in Figure 7 and a list of details on pathways is shown in S4 of SI. We have found that there are several significant signalling pathways related to the cancer that included the genes corresponding to the altered 14 proteins in the ovarian cancer. We have also performed Gene Ontology pathways enrichment analysis using the Gene Ontology biological process database¹⁹. Here we have considered the genes corresponding to the altered 14 proteins in the ovarian cancer. Top 50 significant Gene Ontology pathways and their -10 logarithmic p-values are shown in Figure 8 and detailed pathways is shown in S5 of SI. We have found that there are several significant Gene Ontology pathways related to the cancer included the genes corresponding to the altered 14 proteins in the ovarian cancer.

For the colorectal cancer, we have considered the genes corresponding to 3 altered proteins in the colorectal cancer. Top 50 significant signalling pathways and their -10 logarithmic p-values are shown in Figure 10 and

detailed pathways is shown in S6 of SI. We found that there are several significant signalling pathways related to the cancer included the genes corresponding to the altered 3 proteins in the colorectal cancer. We have also performed the Gene Ontology pathways enrichment analysis using the Gene Ontology biological process database. Here we have considered the genes corresponding to the altered 3 proteins in the ovarian cancer. Top 50 significant Gene Ontology pathways and their -10 logarithmic p-values are shown in Figure 10 and detailed pathways is shown in S7 of SI. We found that there are several significant Gene Ontology pathways related to the cancer included the genes.

Survival Prediction of the Ovarian Cancer Proteins:

We have considered mRNA and clinical data from the TCGA database to predict the survival of the genes corresponding to the altered 14 proteins identified in ovarian cancer²⁰. The Kaplan-merrier plots for the 16 genes corresponding to the 14 proteins of the ovarian cancer are shown in Figure 11. We observed that 12 genes (ACTB, HSPD1, HSP90B1, GRP78, HSPA5, H3F3A, H3F3B, HSPA8, TERA, KET1, VCP, UBB) among the 16 genes showed significant effect on the ovarian cancer survival.

Altered proteins associated with the Cancer:

We have analysed gene-disease association studies using the DisGeNET database that contains validated gene markers for each disease²¹. Carcinoma and neoplasm diseases associated with the genes corresponding to the altered 14 proteins in the ovarian cancer and their significance levels are shown in Figure 12. Similarly, carcinoma and neoplasm diseases associated with the genes corresponding to the altered 3 proteins in the colorectal cancer and their significance level are shown in Figure 13. We found that there are many cancer or associated diseases are already found for our altered proteins.

In vivo toxicological study. Twenty days toxicity study on swiss albino mice have shown that NH3 is less toxic compared to clinically used drug cisplatin in both lower (2.5 mg/kg) and higher doses (5 mg/kg). All mice were alive in control group and NH3 treated group at the lower dose, but only one mouse was alive in cisplatin treated group till the end of the experiment at the same dose. The rest of the mice of cisplatin treated group died within 15 days of experiment at the lower dose. Similarly, at the higher dose of cisplatin none of the mice was alive till the end of the experiment and most of them died within ten days of the experiment. However, all mice were alive in control group and only one mouse died following administration of NH3 at the higher dose. The change in mean body weight of mice in different treatment groups is depicted in Figure 14, showing that body weight has been decreased drastically in cisplatin treated group at both lower and higher doses.

Combination study:

Binary sequenced combinations of NH3 with curcumin and EGCG have been investigated in both ovarian and colorectal cancer models using four different cell lines. In terms of synergistic outcome, combination of NH3 with curcumin proved to be more beneficial than that with EGCG. In ovarian cancer models, the degree of synergism observed from combination of NH3 with curcumin is greater in parent A2780 cell line than in resistant A2780^{cisR} cell line. Degree of synergism is found to increase with the increase in added

concentrations for 0/4 and 4/0 sequences of administration of NH3 with curcumin against A2780^{cisR} cell line. But with bolus additions the converse result is observed (Table 5). Taking together, combination of NH3 with curcumin at lower concentration is best for 0/4 sequence of administration followed by 4/0 sequence in regards to synergistic outcome against ovarian cancer model.

While NH3 in combination with curcumin against HT-29 cell line has displayed synergism at all added concentrations for the 4/0 and bolus administrations. But antagonism is observed at all concentrations for the 0/4 sequence of administration. Greater synergism is shown at lower concentrations than at higher added concentrations for the 4/0 sequence but the converse is true for the bolus administration in HT-29 cell line. Against Caco-2 cell line, synergistic effect is produced at ED₅₀ level irrespective of the sequence of administration. But at ED₇₅ level the synergism is found to decrease for all sequences of administration and it is actually additive for 0/4 combination of NH3 with Cur in Caco-2 cell line (Table 6). With increase in concentration to ED₉₀, all sequences of administration are found to display antagonism.

Table 5: CI values (at ED₅₀, ED₇₅, ED₉₀) and dose-effect parameters (median effect dose D_m, the exponent defining the shape of the dose effect curve m, correlation coefficient r) applying to combinations of NH3 with curcumin and EGCG in the A2780 and A2780^{cisR} cell line

Cell line	Drug or drug combination	Sequence (h)	Molar Ratio	CI values at					
				ED ₅₀	ED ₇₅	ED ₉₀	D _m	m	r
A2780	NH3		1:63.55	N/A	N/A	N/A	0.18	1.97	0.94
	Curcumin			N/A	N/A	N/A	8.63	1.25	1.00
	NH3+Curcumin	0/0		0.59	0.92	1.48	0.05	0.93	1.00
	NH3+Curcumin	0/4		0.30	0.71	1.74	0.02	0.69	0.99
	NH3+Curcumin	4/0		0.46	0.80	1.42	0.04	0.86	1.00
	NH3		1:64.2	N/A	N/A	N/A	0.18	1.97	0.94
	EGCG			N/A	N/A	N/A	10.34	0.94	0.95
	NH3+EGCG	0/0		0.44	0.89	1.97	0.04	0.71	0.94
	NH3+EGCG	0/4		1.22	0.93	0.77	0.10	1.95	0.97
	NH3+EGCG	4/0		1.34	1.02	0.84	0.11	1.97	0.99
A2780 ^{CisR}	NH3		1:76.07	N/A	N/A	N/A	0.23	2.05	0.99
	Curcumin			N/A	N/A	N/A	16.74	1.56	1.00
	NH3+Curcumin	0/0		0.83	0.77	0.72	0.09	2.02	0.94
	NH3+Curcumin	0/4		0.55	0.66	0.80	0.06	1.37	0.97
	NH3+Curcumin	4/0		0.86	0.81	0.76	0.10	1.99	0.94
	NH3		1:64.2	N/A	N/A	N/A	0.23	2.05	0.99
	EGCG			N/A	N/A	N/A	8.90	0.93	0.95
	NH3+EGCG	0/0		1.15	0.85	0.69	0.11	1.99	0.97
	NH3+EGCG	0/4		0.91	0.67	0.55	0.09	1.99	0.92
	NH3+EGCG	4/0		0.95	1.01	1.20	0.09	1.19	0.98

Table 6: CI values (at ED₅₀, ED₇₅, ED₉₀) and dose-effect parameters (median effect dose D_m, the exponent defining the shape of the dose effect curve m, correlation coefficient r) applying to combinations of NH3 with curcumin and EGCG in the HT-29 and Caco-2 cell line

Cell line	Drug or drug combination	Sequence (h)	Molar Ratio	CI values at					
				ED ₅₀	ED ₇₅	ED ₉₀	D _m	m	r
HT-29	NH3		1:199.97	N/A	N/A	N/A	0.38	2.06	0.98
	Curcumin			N/A	N/A	N/A	16.63	1.25	0.97
	NH3+Curcumin	0/0		0.98	0.86	0.77	0.07	1.62	1.00
	NH3+Curcumin	0/4		1.21	1.29	1.40	0.08	1.27	0.99
	NH3+Curcumin	4/0		0.50	0.57	0.68	0.03	1.16	1.00
	NH3		1:278.26	N/A	N/A	N/A	0.38	2.06	0.98
	EGCG			N/A	N/A	N/A	3.67	0.39	0.96
	NH3+EGCG	0/0		5.69	1.02	0.46	0.07	1.29	0.96
	NH3+EGCG	0/4		9.00	1.33	0.50	0.11	1.67	1.00
	NH3+EGCG	4/0		7.54	1.24	0.52	0.10	1.43	1.00
Caco-2	NH3		1:61.25	N/A	N/A	N/A	0.56	1.96	0.93
	Curcumin			N/A	N/A	N/A	21.90	1.08	1.00
	NH3+Curcumin	0/0		0.38	0.65	1.19	0.08	0.81	1.00
	NH3+Curcumin	0/4		0.89	1.06	1.33	0.19	1.11	1.00
	NH3+Curcumin	4/0		0.71	0.93	1.28	0.15	1.01	1.00
	NH3		1:177.17	N/A	N/A	N/A	0.56	1.96	0.93
	EGCG			N/A	N/A	N/A	107.58	1.32	0.94
	NH3+EGCG	0/0		0.45	0.69	1.09	0.13	0.98	0.95
	NH3+EGCG	0/4		0.34	0.73	1.58	0.10	0.77	0.99
	NH3+EGCG	4/0		0.90	1.15	1.49	0.26	1.19	0.98

Prospective benefits of synchronized addition of curcumin and cisplatin have been described against various cancers e.g. lymphoma²², lung cancer^{23,24} and bladder cancer in literature²⁵. Curcumin in combination with platinum drugs (cisplatin, oxaliplatin) was reported to show sequence and dose dependent synergism in ovarian cancer models in earlier studies from host laboratory as well²⁶. Ulukaya *et al.* reported synergism obtained from combination of a tumour active palladium compound with curcumin²⁷. The mechanism behind the synergistic outcome from combination of NH3 with curcumin could be associated with the role of the latter as a prooxidant^{25,28,29}. Due to prooxidant activity, oxidative stress would be generated after entry of curcumin into the cells through dysregulation of glutathione and glutathione-S-transferase levels. Oxidative stress would confer the cells ability to produce signals for MEK activation

followed by ERK1/2 activation which would lead towards apoptotic and necrotic cell death²⁵. Additionally, depletion of glutathione and GSTs would cause the reactive species (Figure 15) to interact with DNA in an efficient manner for maximum cell killing. The idea is supported from palladium-DNA binding study as well in HT-29 cell line where 3 folds increase in palladium-DNA binding level from combined treatment of NH₃ with curcumin is observed than single treatment of NH₃ (data not shown). Literature suggests that prooxidant activity of curcumin is predominant mainly at a lower concentration³⁰ which is also corroborated with the results from the present study where it has been observed that CI values are more synergistic at ED₅₀ level than ED₇₅ and ED₉₀ levels. Moreover, generation of reactive species from metabolism of NH₃ would cause oxidative stress from other side and would lead towards cell death using multiple signalling pathways.

Current literature shows that EGCG inhibits cancer cells growth *in vitro* and *in vivo* synergistically in combination with ascorbic acid, curcumin, 6-gingerol, quercetin, sulforaphane, raphasatin, proanthocyanidins and other natural small molecules³¹. Synergism from combination with chemotherapeutics is also evident through the ability of EGCG to sensitise cancer cells towards chemotherapeutic drugs such as: cisplatin³², bleomycin³³, docetaxel, capecitabine, paclitaxel³⁴ and doxorubicin. Sequence and dose dependent synergism was also reported from the host laboratory from combination of EGCG with cisplatin and designed palladiums³⁵. A number of molecular pathways have been linked with the antitumour activity of EGCG, through which it display synergism in combination with other phytochemicals and chemotherapeutics^{36,37}.

Experimental

Chemistry:

Reagents and chemicals: Potassium tetrachloropalladate (K₂[PdCl₄]); 8-hydroxyquinoline (Sigma Chemical Company, St. Louis, MO, USA); HCl (Ajax chemicals, Auburn, NSW, Australia); ethanol (Merck Pty. Ltd., Kilsyth, Australia).

Synthesis of [Bis(1,8-quinolato)palladium (II)] coded as NH3:

0.5 millimole of potassium tetrachloropalladate (0.163 g) was dissolved in 7.5 mL of mQ water to which 0.25 mL of concentrated HCl was added. Five millimoles of 8-hydroxyquinoline (0.726 g) dissolved in 7.5 mL of ethanol, was added drop wise over 1 h to the solution of potassium tetrachloropalladate at 40°C. The reaction mixture was stirred at room temperature for 2 weeks. 4 mL of 0.25 M hydrochloric acid was added to the mixture and stirring was continued for 1 week at room temperature. The mixture was centrifuged at 5500 rpm for 10 min to collect precipitate of NH₃. The crude product was purified by dissolving in 0.05 M HCl, followed by filtration and collected after washing successively with ice-cold water and ethanol. The purified product was air dried and weighed. Vapour diffusion technique was used during production of suitable crystals of NH₃ for crystallography using methanol and diethyl ether as solvents.

Elemental and Spectral Characterization:

Elemental microanalysis of the designed compound for C, H, and N was determined using microanalysis facility available at the Macquarie University. Model PE2400 CHNS/O (PerkinElmer, Shelton, CT, USA) analyser was used for the determination of C, H, and N. Palladium content was determined by graphite furnace atomic absorption spectroscopy (AAS) available at University of Sydney. IR spectrum of NH3 was recorded using a PerkinElmer FT-IR spectrometer. To obtain mass spectra, solution of NH3 was made in 10% DMF and 90% methanol and then 2 μ L was transferred into a drawn glass capillary pre sputter coated with silver and inserted into a nanospray holder attached to a Thermo Orbitrap mass spectrometer available at the Bioanalytical mass spectrometry facility at the University of New South Wales. The ^1H NMR spectrum of NH3 (dissolved in deuterated DMSO) were recorded on a Bruker DPX400 spectrometer using a 5 mm high-precision Wilmad NMR tube at 300 K (± 1 K).

NH3: Yield: 0.163 g (82.32%). Anal. Calcd. for $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2]$ (394.73 g/mol): C=54.77%, H=3.06%, N=7.10%, Pd=26.96%. Found: C=55.15%, H=3.67%, N=6.94%, Pd=26.64%. Selected IR data (KBR, cm^{-1}): ν =3055, 2360, 2341, 1572, 1497, 1461, 1372, 1316, 1283, 1214, 1172, 1114, 824, 738, 657, 529. ^1H NMR (400 MHz, D_2O): δ = 8.58 (d, due to C_2H); 8.47 (d, due to C_2H); 7.67 (q, due to C_6H); 7.46 (t, due to C_3H); 7.11 (d, due to C_4H); 6.93 (d, due to C_7H); 3.69 (s, due to water); 2.49 (s, due to DMSO). MS (ESI) m/z (%): 395.00 (100) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2]$, 789.99 (18) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2]$, 811.97 (33) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{H}_2\text{O} + 4\text{H}]$, 827.71 (7) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + 2\text{H}_2\text{O} + 2\text{H}]$, 416.98 (18) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{H}_2\text{O} + 3\text{H}]$, 445.35 (13) = $[\text{PdC}_{18}\text{H}_{12}\text{N}_2\text{O}_2 + \text{Cl} + \text{H}_2\text{O} - 3\text{H}]$

Biological activity:

Antitumour activity and proteomics: Cytotoxicity of NH3 against seven cancer cell lines along with that for cisplatin (used as reference) was determined using the MTT reduction assay^{38,39}. The method for determining single drug cytotoxicity and activity of drugs in sequenced combination was the same as described in our previous articles⁷. Proteomic study was conducted following same procedure mentioned before in our published research article⁸.

Toxicity study: Swiss-albino-male mice has been used to conduct the preliminary toxicological study. The mice were divided into six groups (each containing six mice) and drugs (DMSO/NH3/cisplatin) were administered subcutaneously. First group and second group considered as control which was administered with the solvent DMSO at a dose 2.5 mg/kg and 5 mg/kg, respectively. Third group was administered with the investigational drug (NH3) at a dose 2.5 mg/kg, fourth group was administered with NH3 at a dose of 5 mg/kg. Fifth and sixth group was administered with standard anticancer drug cisplatin at a dose of 2.5 mg/kg and 5 mg/kg, respectively. During the experiment, NH3/DMSO/cisplatin was administered from 6th to 10th day and 16th to 20th day at corresponding dose to corresponding group. During 1st to 5th and 11th to 15th days, no drug was administered to any mice. Body weight of each mouse was taken every day and other physical changes were observed. At the end of the experiment, all alive mice were sacrificed. The mice model preliminary toxicity study was approved by the Biosafety, Biosecurity and Ethical Committee [Approval Number: BBEC-JU/M2019(12)1] of Jahangirnagar University, Savar, Dhaka, Bangladesh.

Conclusions

Palladium complex [Bis(1,8-quinolato)palladium (II)] coded as NH3 has been synthesized and characterized followed by studies on its activity alone and in combination with ovarian and colorectal cancer cell lines. Proteomic studies were carried out to determine key proteins associated with antitumour activity of NH3. Theoretical studies have also been carried out on protein-protein interaction.

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Figures

Figure 1

Schematic for the structure of the synthesized complex NH3

Figure 2

Flow diagram for the synthesis of the compound NH3

Figure 3

IC50 values (µM) against a variety of human cancer cell lines (After 72 h, MTT assay, 3 repeats)

Figure 4

Proposed bio-transformation pathway of NH3 based on the reported mechanism of oxaliplatin
Proposed bio-transformation pathway of NH3 based on the reported mechanism of oxaliplatin

Figure 5

Figure 6

Protein-Protein Interaction of the proteins Identified in the ovarian cancer cell line

Figure 7

Pathway enrichment of the genes corresponding to the altered 14 proteins in the ovarian cancer.

Figure 8

Gene Ontology enrichment of the genes corresponding to the altered 14 proteins in the Ovarian cancer.

Figure 9

Pathway enrichment of the genes corresponding to the altered 3 proteins in the colorectal cancer.

Figure 10

Gene Ontology enrichment of the genes corresponding to the altered 3 proteins in the colorectal cancer.

Figure 11

Survival prediction of the 16 genes corresponding to the altered 14 proteins in the ovarian cancer.

Figure 12

Diseases those are associate with the genes corresponding to the altered 14 proteins in the ovarian cancer

Figure 13

Diseases associated with the genes corresponding to the altered 3 proteins in the colorectal cancer.

Figure 14

Change in body weight of mice in different treatment groups