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Short communication

Antitumour activity of novel 1,10-phenanthroline and 5-amino-1,10-phenanthroline derivatives

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ABSTRACT

Synthesis and impact on the tumour growth of palladium(II) complex of 5-amino-1,10-phenanthroline $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ and the protonated dimer $(phen)_2(H^+)(BF_4^-)$ have been described. In the reported experiments a cancerous (100% lethality) myeloid subcutaneous tumour (with Graffi-tumour origin) in hamsters was used. The animals were injected *i.p.* with different doses of the substances. The longest mean survival time (1.65 times longer than the controls) was achieved when the substance $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ was injected into the animals. One of the animals even survived until the 71st day, which is 2.2 times longer than the controls. The compound $(phen)_2(H^+)(BF_4^-)$ prolonged the mean life-time of the animals 1.4 times in comparison to the controls. On the other hand, the Pd(II) complex of 1,10-phenanthroline, $Pd(phen)_2(H_2O)(NO_3)_2$, did not reveal any antitumour activity. Our experience concerning the effect of other drugs on this tumour has shown a survival time no longer than 4–5 d after the death of the controls.

1. Introduction

There is a scarcity of data concerning the anticancer activity of 1,10-phenanthroline (phen) and its derivatives. The synthesis of mixed-ligand complexes of palladium(II) with 1,10-phenanthroline and 5-nitro-1,10-phenanthroline, which possess antitumour activity, has been described [1,2]. There is also a report on anticancer agents containing vanadium and 4,7-dimethyl-1,10-phenanthroline(Me_2-phen) with a composition $VO(Me_2\text{-phen})_2$ [3]. The cation Pd(II) is a structural analogue of the cation Pt(II) (d^8-electron configuration). The platinum drugs represent a unique and important class of antitumour agents. The compound cisplatin and its derivatives have been widely recognized as potent anticancer drugs, especially effective against testicular, ovarian and head tumours [4] due to their interactions with nucleic acid [5–7]. The analogy of Pd(II) with Pt(II) prompted us to synthesize the palladium(II) complexes of 1,10-phenanthroline and 5-amino-1,10-phenanthroline with the composition $Pd(phen)_2(H_2O)(NO_3)_2$ (**1**) and $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ (**2**). The compound $(phen)_2(H^+)(BF_4^-)$ (**3**) was obtained by a process of self-assembly [8] and was not related to any known class of antitumour agents.

We will emphasize on compounds **2** and **3**, which revealed a strong action against a cancerous (100% lethality) myeloid *s.c.* tumour in hamsters, developed by Yakimov et al. [9]. This tumour (*Graffi-tumour-virus*, bought from the "Biological Bank" – Sofia, Bulgaria), was transferred for the first time at the Institute of Experimental Pathology and Parasitology (Sofia) from mice to newborn hamsters after *s.c.* transplantation of tumour cells. Further transplantations led to development of a very fast growing tumour with high lethality [9]. We chose it, because in case we succeeded to prolong the life of the experimental animals, we could be sure that the tested substances were strongly effective.

2. Chemistry

Palladium(II) complexes with 1,10-phenanthroline (phen) and its derivative 5-amino-1,10-phenanthroline ($5\text{-}NH_2$-phen) with a composition of $Pd(phen)_2(H_2O)(NO_3)_2$ and $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ were synthesized. It is known that the palladium(II) cation (d^8-electron configuration) forms very strong covalent bonds with organic bidentate ligands, the complexes having square-planar geometry.

On interaction of 1,10-phenanthroline with $NaBF_4$ in an aqueous solution under specific conditions the protonated dimer $(phen)_2(H^+)(BF_4^-)$ was also obtained. The synthesized compounds were characterized by elemental analysis, FTIR-spectroscopy and FAB-mass spectrometry.

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Table 1
Mean survival time of GTBH (in d) after treatment with different compounds.

Compound/dose	Days
Controls	32
2/0.5 ml	53
2/1.0 ml	33.75
3/0.25 ml	45.3
3/0.5 ml	45.5
3/1.0 ml	41.5

3. Experimental tumour model

The experimental myeloid subcutaneous tumour in hamsters was induced by the *Graffi-virus* in mice, and tumour cells from the developed tumours were transferred to new-born hamsters [9]. The *in vivo* transplantations have to be performed once monthly for keeping the tumour's survival. The tumour is 100% cancerous, and the animals die usually up to the 30th day after transplantation. The sufficient dose is 5×10^4 cells. Our experience has shown that none of the animals, transplanted with this tumour (**G**raffi **t**umour **b**earing **h**amsters, GTBH), could survive too long – they die usually between 20 and 30 d after transplantation.

3.1. Experimental procedure

Golden Syrian hamsters (150–200 g) were randomly assigned to groups of 6 animals.

The impact of the substances **1**–**3** on the tumour growth was evaluated *in vivo* after *s.c.* injections of 5×10^4 cells and simultaneously *i.p.* with 1.0, 0.5 or 0.25 ml of each substance (5 mg/ml suspension). Substance **1** revealed no activity in the preliminary experiments, and was excluded from further studies.

The animals were divided into the following groups:

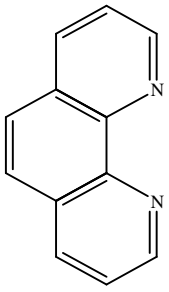
a) Injected with the substance **2** – the first group received 0.5 ml of the suspension and the second group 1 ml of it.
b) Injected with the substance **3** – the first group received 0.25 ml of the suspension, the second group 0.5 ml, and the third group 1 ml of it.

The control group GTBH received *s.c.* only tumour cells. The following biometrical parameters were examined:

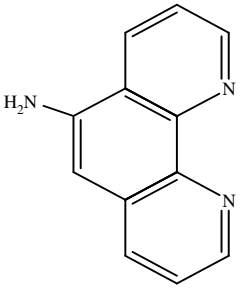
a) Transplantation ability – measured as number of animals which developed tumours from the whole number of transplanted animals.
b) Mean survival time – the mean time in d (arithmetic average), in which the transplanted animals developed the tumour after the d 0 (day of the transplantation) and died compared to the controls.
c) Inhibition of the tumour growth – following transplantation of the tumour cells and treatment by each substance after 5, 10, 15 d, etc., the size of the developed tumours was measured and compared to that of the control animals. The delay of the tumour growth under the influence of a given substance demonstrated that it has a suppressive effect on the cancer development.

4. Results

The newly synthesized compounds **1** and **2** are coordination complexes of Pd(II) with 1,10-phenanthroline and 5-amino-1,10-phenanthroline.



1,10-phenanthroline (phen)



5-amino-1,10-phenanthroline (5-NH_2-phen)

The longest mean survival time of the experimental animals was achieved after injecting substance **2** (53 d), but one of the animals survived until the 71st day, which is 2.2 times longer than the controls. It should be emphasized that these animals received a lower dose of 2.5 mg (Tables 1 and 2). Substance **3** also prolonged the live of the experimental animals up to 46 d (Table 1). Substance **1** did not demonstrate any antitumour activity.

Mortality experiments (expressed in %) are demonstrated in Table 2. Again, most of the animals, treated with **2** (2.5 mg), survived longer than the controls (75% of them survived up to the 65th day). The higher doses of this compound had an insignificant effect compared to the controls. As far as compound **3** is concerned, the effect of the given doses decreases in the following order: 2.5 mg > 1.25 mg > 5 mg.

Transplantability of the tumours, treated with experimental substances **2** and **3** is evident from Table 3. It is obvious that until the 15th day the animals so treated did not develop any tumour, while in the control animals tumours appeared quite visibly. Immediately after this period, tumours were observed in all animals. Differences were seen concerning the highest dose (1.0 ml), which did not limit further growth of the tumours, and they almost reached the size of the controls. On the contrary, lower doses of **3** (0.25 ml and 0.5 ml) prevented fast tumour development.

The tumour size (statistical data by Student's *t*-test showed $p < 0.05$) in the different experimental treatings is demonstrated in Table 4. Substance **3** strongly delayed the tumour growth when lower doses were injected (0.5 and 0.25 ml per animal), and the tumours were as large as 2/3 of the tumours in the GTBH.

Table 2
Mortality of GTBH (in %) after treatment with different compounds.

Compound/dose	Days								
	30	35	40	45	50	55	60	65	70
Controls	50	100							
2/0.5 ml	0	25	25	75	75	75	75	75	100
2/1.0 ml	25	75	100						
3/0.25 ml	33	33	33	66	66	66	100		
3/0.5 ml	0	25	25	75	75	75	75	100	
3/1.0 ml	50	49.8	49.8	49.8	83	100			

Table 3
Transplantability of GTBH (in %) after treatment with different compounds.

Compound/dose	Days				
	10	15	20	25	30
Controls	50	100			
2/0.5 ml	0	50	100		
2/1.0 ml	0	50	100		
3/0.25 ml	0	50	50	100	
3/0.5 ml	0	50	50	50	100
3/1.0 ml	0	50	100		

Table 4
Tumour size (in mm) in GTBH after treatment with different compounds.

Compound/dose	Days					
	10	15	20	25	30	35
Controls	0.50 ± 0.1	1.0 ± 0.28	1.84 ± 0.32	2.51 ± 0.66	3.35 ± 0.47	3.66 ± 0.64
2/0.5 ml	0	0.62 ± 0.39	$0.75 \pm 0.28^{***}$	$1.57 \pm 0.26^{**}$	$2.47 \pm 0.66^{*}$	3.28 ± 0.45
2/1.0 ml	0	$0.56 \pm 0.16^{**}$	$1.11 \pm 0.6^{*}$	2.06 ± 0.37	$2.38 \pm 0.35^{**}$	3.50 ± 0.5
3/0.25 ml	0	1.16 ± 0.26	$1.40 \pm 0.33^{*}$	2.13 ± 0.57	$1.75 \pm 0.66^{**}$	$2.50 \pm 0.5^{*}$
3/0.5 ml	0	$0.50 \pm 0.4^{*}$	$1.07 \pm 0.64^{*}$	$1.38 \pm 0.56^{**}$	$2.32 \pm 0.22^{***}$	$2.40 \pm 0.28^{***}$
3/1.0 ml	0	$0.30 \pm 0.3^{**}$	$0.78 \pm 0.3^{***}$	$1.28 \pm 0.22^{***}$	$2.33 \pm 0.44^{**}$	3.41 ± 0.38

Statistical analysis was performed by the *t*-test for the different groups. "*p*" is given with asterisk: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

5. Discussion

It is interesting to note that substance **1**, which is a palladium(II) complex of 1,10-phenanthroline, did not show any antitumour activity. This fact provides evidence that in **2** the presence of amino group, attached to the 1,10-phenanthroline aromatic system, is essential for the activity as the mean survival time of the animals, treated with this substance, was the longest. The amino group is an electron donor substituent, thus increasing the electron density in the aromatic ring. It is known that the palladium cation, Pd(II), which contains 8 d-electrons in the outer shell, forms very stable complexes with bidentate ligands. The structure studies indicate that square-planar geometry is typical of them. We believe that the compounds obtained by us are not an exception.

The structure of **3** is quite different from that of **1** and **2**. It is a protonated dimer of 1,10-phenanthroline compensated by BF_4^- anion. It has been found that BF_4^- can well be encapsulated within the interior of coordination cages containing aromatic systems [10]. By analogy with this finding, we might suggest that in **3** the tetrafluoroborate anion is inserted between the two phenanthroline molecules. The positively charged proton most probably contributes to decreasing the electrostatical repulsion.

Unfortunately, detailed structure studies by X-ray analysis were not possible because of the difficulties in obtaining suitable single crystals.

Our experience with this tumour when studying other antitumour drugs demonstrated survival time no longer than 4–5 d after the controls. We would like to express our strong expectations for discovering new very effective antitumour agents, which might be helpful in treatment of human diseases.

Finally, it is important to note that the three tested compounds lack an acute toxicity, as reported by Dimitrov et al. [11].

6. Conclusions

The complex $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ is a newly synthesized compound which together with the substance $(phen)_2(H^+)(BF_4^-)$ demonstrated a strong effect on a cancerous (100% lethality) myeloid *s.c.* tumour in hamsters. The longest mean survival time was achieved when the complex **2** was injected into the animals (1.65 times longer than the controls). One of the animals even survived until the 71st day (2.2 times longer than the controls). The mean survival time of the protonated dimer **3** was 46 d, which is 1.4 times longer than that of the controls. The palladium(II) complex of the unsubstituted 1,10-phenanthroline, $Pd(phen)_2(H_2O)(NO_3)_2$, did not show any activity, which indicates the dominant effect of the electron donor amino group in the 5th position in **2**.

7. Experimental protocols

7.1. Chemistry

7.1.1. Chemicals and apparatus

1,10-Phenanthroline·H_2O and $NaBF_4$ were obtained from Merck, Darmstadt. 5-Amino-1,10-phenanthroline·H_2O was prepared in our laboratory from 5-nitro-1,10-phenanthroline by reduction with hydrazine hydrate using Raney-nickel as a catalyst. All other chemicals used were of the highest available quality. Elemental analyses were performed with VarioEl (Elemental Analyser Systeme GmbH). FAB-mass spectra were taken on a VG-autospec mass spectrometer. The samples were dissolved in NBA and lactic acid. Infrared spectra were recorded on a Perkin–Elmer 1600 Series FTIR spectrophotometer in the range of 4400–450 cm^{-1}, resolution 4 cm^{-1} in KBr pellets.

7.1.2. Synthesis of palladium(II) complex of 1,10-phenanthroline $Pd(phen)_2(H_2O)(NO_3)_2$ (**1**)

0.386 g (1.95 mmol) of 1,10-phenanthroline hydrate, 0.444 g (2.5 mmol) of $PdCl_2$, 0.590 g (2.5 mmol) of $Ca(NO_3)_2\cdot 4H_2O$ and 10 ml of 96% ethanol were dissolved in 80 ml of distilled water. The solution was heated up to boiling temperature for 5 min, then hot filtered. The filtrate stayed in the refrigerator at 2–3 °C for 24 h. The yellow solid, which was formed, was filtered off, washed with distilled water, then dried in a desiccator over P_4O_{10}. Yield: 0.040 g; 6.75%.

Anal. calcd. for $C_{24}H_{18}N_6O_7Pd$ (C, H, N).

Analyses, indicated by the symbols of the elements, were within ±0.4% of the theoretical values.

FTIR (cm^{-1}): 3447, 1516, 1433, 1384, 841, 740.

FAB-mass (*m*/*z*): 181 $(phen)H^+$, 287 $[Pd(phen)\text{-}H^+]^+$, 466 $[Pd(phen)_2\text{-}H^+]^+$, 528 $[Pd(phen)_2(NO_3)]^+$.

7.1.3. Synthesis of palladium(II) complex of 5-amino-1,10-phenanthroline $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ (**2**)

0.100 g (0.474 mmol) of 5-amino-1,10-phenanthroline·H_2O, 0.166 g (0.703 mmol) $Ca(NO_3)_2\cdot 4H_2O$, 0.126 g (0.704 mmol) of $PdCl_2$, and 3.0 ml of 96% ethanol were dissolved in 24 ml of distilled water. The solution was heated up to boiling temperature for 5 min and hot filtered. The filtrate stayed in a refrigerator at 2–3 °C for 24 h. Orange-brown crystals of $Pd(5\text{-}NH_2\text{-}phen)_2(NO_3)_2$ were formed. They were filtered off, washed with distilled water then dried in a desiccator over P_4O_{10}. Yield: 0.030 g; 21.4%.

Anal. calcd. for $C_{24}H_{18}N_8O_6$ Pd (C, H, N).

Analyses, indicated by the symbols of the elements, were within ±0.4% of the theoretical values.

FTIR (cm^{-1}): 3342, 3227, 1635, 1598, 1514, 1386, 822, 731.

FAB-mass (*m*/*z*): 196 $[(5\text{-}NH_2\text{-}phen)H]^+$, 301 $[Pd(5\text{-}NH_2\text{-}phen)\text{-}H^+]^+$, 496 $[Pd(5\text{-}NH_2\text{-}phen)_2\text{-}H^+]^+$.

7.1.4. Synthesis of compound $(phen)_2(H^+)(BF_4^-)$ (**3**)

The compound was synthesized as described in [8] introducing minor modification. 0.386 g (1.95 mmol) of 1,10-phenanthroline

hydrate, 0.549 g (5.0 mmol) of $NaBF_4$ and 10 ml ethanol were dissolved in 70 ml of distilled water. Then the solution was brought to ambient temperature and water added to 100 ml. After 20 d of stay in a refrigerator at 2–3 °C, the colourless crystals formed were filtered off, washed with water and ethyl ether, and dried in a desiccator over P_4O_{10}. Yield: 0.214 g; 49.76%.

Anal. calcd. for $C_{24}H_{17}N_4BF_4$ (C, H, N).

Analyses, indicated by the symbols of the elements, were within ±0.4% of the theoretical values.

FTIR (cm^{-1}): 3431, 1618, 1597, 1546, 1054 (BF_4^-), 840, 717.

FAB-mass (*m*/*z*): 181 (phen)H^+, 361 $[(phen)_2H^+]^+$, 449 $[(phen)_2(H^+)_2(BF_4^-)]^+$.

7.2. Experimental animals

The experiments followed a protocol approved by the Committee of Ethics for Animal Experiments at the Institute of Experimental Pathology and Parasitology (Bulgarian Academy of Sciences). All experiments comply with the Bulgarian National Veterinary Service (Reg. No. 11130007/19.01.2007–26.01.2012) according to the requirements of the National Institute of Health (NIH) Guide for the Care and Treatment of Animals (Bethesda, MD, USA).

Golden Syrian hamsters (150–200 g) were provided by the breading house of the Bulgarian Academy of Sciences. Animals were given standard pellet diet and tab water *ad libitum* and kept in rooms with controlled 12/12 h light and dark cycle and temperature (22 ± 2 °C), humidity 60 ± 10%. All experiments were carried out between 09.00 a.m./13.00 p.m. The animals were randomly assigned to groups of 6, and only those with normal motor function were used.

7.3. Statistical analysis

Statistical analysis was performed by the Student's *t*-test for the different groups. Data are expressed as mean ± SD.

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