SYNTHESIS OF SOME MIXED LIGAND COMPEXES OF CADMIUM AND THEIR BIOMEDICINAL STUDIES

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Abstract

The present manuscript deals with the synthesis of some novel mixed ligand complexes of Cadmium and their characterization on the basis of melting point, elemental and spectral analysis. The newly synthesized complexes were also tested for their antibacterial, antifungal and antitumor activity in-vitro and their results of screening clearly indicating them as potent biomedicinal agents.

Introduction

There is an enormous potential for the application of metals in medicines and the selection of metal ions offer the possibility for the discovery of metallodrugs with novel mechanism of action[1]. The importance of metal based drugs lies in the fact that they are essential components for various physico-chemical processes occurring in living system[2-6]. The spectrum of the chemotherapeutic values of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds exhibiting antimicrobial activity against different kinds of microbial strains along with anti-inflammatory, cardiovascular, trypanosomal, anti-herpes and anti-tubercular activity[7-11].

Coordination chemistry forms the backbone for the major part of the current researches in chemistry[12-15]. This can be seen from large number of books, review articles and international conferences which are held every year. The importance of coordination compounds and their role in numerous systems of chemical and biological significance can easily be recognized when one realizes that chlorophyll, which is vital to photosynthesis in plants, is- a complex of magnesium and that hemoglobin, which carries oxygen to animal cells is an iron complex[16-19]. Coordinated metal ions incorporated in the structure of enzymes are responsible for their action. The transition metal ions, Zn, Co, Pe, etc. are known to persist in biological systems by coordination with numerous enzymes containing the heme, and related structures such as catalases, peroxidases and cytochromes. The iron containing proteins, ferritin, transferrin and hemosiderin are known to predominate in biological systems[20-22]. Zinc complex as sine insulin and Beryllium salt or complex, as lymphocyte activator are- known for their- importance.

The present manuscript contains the details of the synthesis of some mixed ligand complexes of Cd and their biomedicinal studies.

Experimental

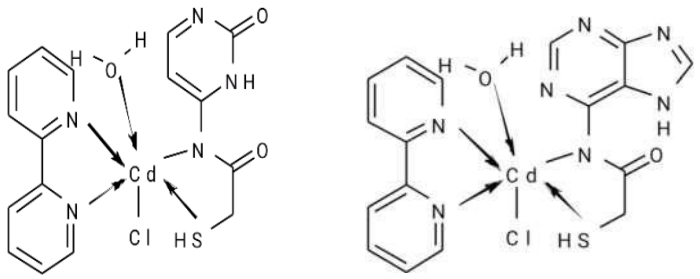
The primary ligands i.e. 2, 2'-bipyridyl and 1,10- phenanthroline will be taken as such (AR grade) and will be used while preparing the mixed ligand complexes Cd(II) salts while secondary Ligands (Amides) will be synthesized by taking 1:1 molar ratio of thio-glycolic acid and 2-aminopyridine , 2- amino- adinine, 2-amino – guanine and 2- amino cytosine by refluxing the components for appropriate periods and the resultant product will be crystallized.

Binary complexes of Cd(II) with corresponding thio-amides of 2-aminopyridine, 2-aminoadinine, 2-aminoguanine, and 2- amino-cytosine will be synthesized by taking 1:2 molar ratio of metal ion and the amide and refluxing the components for suitable period and crystallized.

The procedural details have been described for one system, while for others only modifications used in the procedure are given:

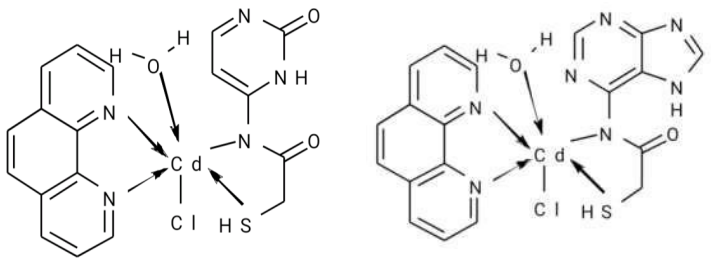
Cd (II)-Bipy-thioamide:

Calculated amount of $CdCl_2$ (0.005 mole) and Bipridyl (0.005 mole) were dissolved separately in 25 ml double distilled water and in 25 ml ethanol (95%) respectively. The solutions were warmed and mixed, in a 250 ml round bottomed flask. 30 ml of ethanolic solution of thioamide (0.005 mole) was added to the contents in round bottomed flask and a requisite amount of 10/6 sodium acetate solution was added so as to adjust pH of the contents between 4.0 and. 5.0. The contents in round bottomed flask were refluxed for 1-2 hours. This resulted in separation of granular white precipitate. The contents ware cooled to room temperature and filtered under vacuum suction. The residue was washed with water and then 3-4 times with ethanol to remove the unreacted metal ion and ligands if any, and finally it was washed with ether and dried in an oven.



Cd(II)-Phenanthroline-thioamide:

Calculated amount of $CdCl_2$ (0.005 mole) and phenanthroline (0.005 mole) were dissolved separately in 25 ml double distilled water and in 25 ml ethanol (95%) respectively. The solutions were warmed and mixed, in a 250 ml round bottomed flask. 30 ml of ethanolic solution of thioamide (0.005 mole) was added to the contents in round bottomed flask and a requisite amount of 10/6 sodium acetate solution was added so as to adjust pH of the contents between 4.0 and. 5.0. The contents in round bottomed flask were refluxed for 1-2 hours. This resulted in separation of granular white precipitate. The contents ware cooled to room temperature and filtered under vacuum suction. The residue was washed with water and then 3-4 times with ethanol to remove the unreacted metal ion and ligands if any, and finally it was washed with ether and dried in an oven.



Determination of molecular weight:

Cryoscopy method was used in determining molecular weights of all mixed-ligand complexes. Purified glacial acetic acid was used as solvent. For this purpose it was allowed to freeze in an ice bath.

ANTI-BACTERIAL ACTIVITY

Antibacterial activity of these compounds was determined by disc-diffusion method (22). In this technique, the filter paper (Whatmann No. 1) sterile discs of 5 mm diameter, impregnated with the test compounds (10µg/ml of ethanol) were placed on the nutrient agar plate at 37°C for 24 hours. The inhibition zones around the dried impregnated discs were measured after 24 hrs. The activity was classified as "highly active" (diameter= >14mm); "moderately active" (diameter = 10-14 mm) and "slightly active" (diameter 6-10 mm). The diameter less than 6 mm was regarded as inactive.

ANTI-FUNGAL ACTIVITY

The antifungal activity of these compounds was determined by agar plate method; (23) using four concentrations *viz*; 10, 20, 50 and 100 µg/ml of test compounds against human pathogenic fungal strains, *Aspergillusflavus* and *Aspergillusnigar*. One 1 ml of each compound was poured into a petri-dish having about 20-25 ml of molten potato-dextose agar medium. As the medium gets solidify, petridishes were inoculated separately with the fungal isolates and kept at 27° for 96 hrs. All the values (% inhibition) were recorded. The percentage inhibition of various organoantimony compounds were calculated by using following mathematical equations (24).

$$\text{Percentage(\%) Inhibition} = \frac{C - T}{C} \times 100$$

Here, C = Diameter of fungus in control ; T = Diameter of fungus in test compounds

ANTITUMOR ACTIVITY:

The human Breast Cancer cell line (MCF-7) was co-incubated with the test compounds at 1 µg/ml doses for 96 hrs and the cell growth count was measured by MTT assay as described below (25). Here 17β estradiol as positive control and culture medium as negative control was used.

Results & Discussion

The mixed-ligand complexes of Zn(II), Cd(II), Cr(II) and Ni(II) ions are appreciably soluble in ethanol and slightly soluble in nitrobenzene, acetone etc. These complexes dissolve readily in acetic acid. All these complexes do not melt sharply but decomposes above 150°C. Their conductance measurements in glacial acetic acid indicate that these mixed ligand complexes are non-electrolyte. Molecular weight determinations of these complexes correspond to their monomeric nature in solution. The mixed-ligand complexes of Co(II) ion are insoluble in common organic solvents and do not, melt up to 250°C. This may be indicative of their

polymeric nature. The complexes show proper ratios of elements as in their data of elemental analysis.

Spectral Analysis:

The spectral analysis of complexes covers its IR, and NMR analysis as per standard norms. The Infrared absorption spectra of Bipridyl, Phenanthroline, thioamide and all mixed-ligand complexes under investigation have been obtained in KBr pallet using Perkin-Elmer model-577 IR absorption spectrophotometer.

IR spectra of 2.2'-Bipridyl, 1, 10-Phenanthroline:

These ligands and their metal complexes have been extensively studied through infrared absorption spectroscopy. By looking to the structure one would expect characteristic vibrations due to heterocyclic rings in Bipy, and due to aromatic heterocyclic rings in Phenanthroline. These characteristic vibrations are given in Tables. Heterocyclic aromatic compounds such as pyridine have been reported to show somewhat similar set of bands. -C-H stretching vibrations, in pyridine has been reported to occur at near 3070-3020 cm^{-1}. 2,2'-Bipyridyl shows absorption band near 3060 cm^{-1} due to characteristic CH stretching vibration. Aromatic C=C stretching vibrations have been reported to occur in the region 1650-1450 cm^{-1}, actual position of these band;-, show wide violations. In aromatic compounds band at 1580 cm^{-1} is reported to be very weak and difficult to detect but its intensity is enhanced by external conjugation. Bipy shows a band of medium intensity at 1582 cm^{-1} probably it has derived its intensity from interaction of p-electrons of the nitrogen atom of the ring. In other words 1582 cm^{-1} absorption band carries some contribution due to ring C=N vibrations also. 1,10- Phenanthroline shows absorption band at 1590 cm^{-1}. This may also carry contribution due to ring C=N vibrations. Another characteristic C=C, stretching vibration has been reported to occur near 1450 cm^{-1}. Bipy and Phenanthroline show absorption band at 1455 and 1450 cm^{-1} respectively due to C=C stretching vibrations. An absorption band in the region 1525-1475 cm^{-1} usually close to 1500 cm^{-1} has been reported to be due to C=C stretching vibration. Phenanthroline shows absorption band at 1495 cm^{-1}.

^{1}H and ^{13}C NMR Spectra:

^{1}H NMR spectra of the mixed ligand complexes was recorded in $CDCl_3$ using TMS as an internal reference at 25°C. The details of signals of only one series of mixed ligand complexes have been explained here which clearly indicates the presence of aromatic hydrogens, amide hydrogen as per signals. The details are as under.

Complex-1:^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.59-7.12 (12H, m, 2-pyridine CH) 3.33 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 166.1, 149.8, 144.4 123.6, 135.7,123.6, 117.5, 110.8, 77.8, 59.8, 41.9, 35.0.

Complex-2: $C_{21}H_{29}ClN_7O_2SCd$**^{1}H-NMR** in ppm (400MHz; Solvent: $CDCl_3$): δ 8.55-7.14 (12H, m, 2-pyridine CH), 7.50 (2H, s, CH, aldimine) 3.4 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 166.1, 149.8, 144.4 125.6, 137.7,126.6, 118.5, 79.8, 57.8, 42.9.

Complex-3: $C_{22}H_{35}ClN_7O_2SCd$**^{1}H-NMR** in ppm (400MHz; Solvent: $CDCl_3$): δ 8.59-7.12 (12H, m, 2-pyridine CH), 7.50 (2H, s, CH, aldimine), 3.33 (2H, s, methylene) 1.5 (1H, s, methine)**^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 166.1, 149.8, 144.4 123.6, 135.7,123.6, 117.5, 110.8,71.1, 64.4, 48.2.

Complex-4: ^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.0 (1H, s, NH sec. amide) 7.50 (2H, CH, aldimine) 8.59-7.12 (12H, m, 2-pyridine CH) 3.33 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 166.1,, 165.5163.1, 149.8, 144.4 123.6, 135.7,123.6, 117.5, 110.8, 77.8, 59.8, 41.9, 35.0.

Complex-5:^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.57-7.22 (12H, m, 2-pyridine CH) 3.35 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 168.1, 148.8, 143.4 125.6, 133.7, 3520.

Complex-6: ^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.51-7.18 (9H, m, aromatic CH), 7.52(2H, s, CH, aldimine) 3.4 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 168.1, 149.8, 144.4 125.6, 137.7,126.6, 118.5, 72.8, 57.8, 44.9.

Complex-7: ^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.59-7.12 (9H, m, phenanthroline, CH), 7.49(2H, s, CH, aldimine), 3.35 (2H, s, methylene) 1.7 (1H, s, methine)**^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 168.1, 149.8, 144.4 123.6, 135.7,123.6, 117.5, 110.8,71.1, 64.4, 48.2.

Complex-8: ^{1}H-NMR in ppm (400MHz; Solvent: $CDCl_3$): δ 8.2 (1H, s, NH sec. amide)8.59-7.12 (9H, m, aromatic CH), 7.50 (2H, CH, aldimine) 3.33 (2H, s, methylene) **^{13}C-NMR** in ppm (100 MHz; Solvent: $CDCl_3$): δ 168.1,, 165.5163.1, 149.8, 144.4 123.6, 135.7,123.6, 117.5, 110.8, 77.8, 59.8, 41.9, 35.0.

Anti-bacterial activity of Mixed Ligand Complexes of Cd:

The antibacterial activity of this series of mixed ligand complexes was tested against three bacterial strains, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiela pneumonia*: (*Human pathogens*) using 10 μg/ml concentration of the compounds.

Compounds **3** show highest antibacterial activity against *Staphyloccusaureus,* complex**1,3,5,7,** and **8**in case of*Klebsiela Pneumonia* and complex **2, 5** and **6** in case of *pseudomonas aeruginosa* respectively. The rest of the compounds show moderate antibacterial activity. The compounds having higher sulfur content exhibit higher activity. Results of the activity are given in Table-2.

E) Anti-fungal Activity of Mixed Ligand Complexes of Cd:

The fungicidalactivity of the mixed ligand complexes were tested against the fungal strains, *Aspergillusflavus* and *Aspergillusnigar* and the percentage inhibition of the compounds calculated. The fungicidal activity was carried out using four different concentrations *viz*. 10, 20, 50, 100 μg/ml of test compounds. At 10 μg/ml concentration of the compounds they shows moderate to low fungicidal activity but compound **1, 4, 6** and **8** shows higher/moderate activity against *Aspergillusflavus* and complex **1, 3** and **8** shows higher activity against *Aspergillusnigar* strains. At 20 μg/ml of concentration, the compounds **5** and **6** shows higher activity against *Aspergillusflavus* and rest of the compounds shows moderate to low activity against. Against *Aspergillusnigar* compound **3** shows higher activity while the rest of the compounds show moderate activity. At 50 μg/ml of concentration of test compound the compounds **1, 5** and **6** shows higher percentage inhibition than the rest of the compounds in case of *Aspergillusflavus* while the compounds **3, 6** and **7** are more potentially active against the *Aspergillusnigar*. At 100 μg/ml concentration generally all the compounds shows approximately above 90% of inhibition against both the fungal strains. A variation in activity appears due to different ligands in the complexes. The results are given in table-3 (A-D).

E) *In-vitro* antitumor activity of mixed ligand Complexes of Cd:

The antitumor activity of mixed ligand complexes of Cd were tested against human breast adenocarcinoma cell line. The cell count of the compound was measured. It was found that the compound 1, **2, 3, 6** and **8** shows moderate antitumor activity against MCF-7 tumor cell line. These compounds inhibit the growth of tumor cells about 25-30%. The rest of compounds were inactive against tumor cell line. The variation in activity is due to

presence of different ligand in the molecules as well as presence of nitrogen and sulfur content.

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Table-1: Physico-chemical analysis of Mixed ligand Complexes of Cd:

S.N.	Complexes formula	Molecular Weight	Exact Weight	Melting/ Decomposition Point(ºC)	Elemental Analysis			
					% C	% H	% N	% S
1	$C_{17}H_{17}ClN_4O_2SCd$	489.27	489.97	164	41.73	3.50	11.45	6.55
2	$C_{17}H_{16}ClN_7O_2SCd$	530.28	530.98	149	38.50	3.04	18.49	6.05
3	$C_{17}H_{18}ClN_7O_2SCd$	532.29	532.99	148	38.36	3.41	18.42	6.02
4	$C_{16}H_{16}ClN_5O_3SCd$	506.25	506.96	154	37.96	3.19	13.83	6.33
5	$C_{19}H_{17}ClN_4O_2SCd$	513.29	513.97	152	44.46	3.34	10.92	6.25
6	$C_{19}H_{16}ClN_7O_2SCd$	554.30	554.98	146	41.17	2.91	17.69	5.78
7	$C_{19}H_{18}ClN_7O_2SCd$	556.32	556.99	145	41.02	3.26	17.62	5.76
8	$C_{18}H_{16}ClN_5O_3SCd$	530.28	530.99	148	40.77	3.04	13.21	6.05

Table-2: Antibacterial Activity of Mixed Ligand complexes of Cd

S. N.	Compounds	Control	*Pseudomonas aeruginosa*	*Staphylococcus aurerus*	*Klebsiela pneumonia*
1.	$C_{17}H_{17}ClN_4O_2SCd$	–	++	++	+++
2.	$C_{17}H_{16}ClN_7O_2SCd$	–	+++	+	++
3.	$C_{17}H_{18}ClN_7O_2SCd$	–	++	++	+++

4.	$C_{16}H_{16}ClN_5O_3SCd$	–	++	+++	++
5.	$C_{19}H_{17}ClN_4O_2SCd$	–	+++	++	+++
6.	$C_{19}H_{16}ClN_7O_2SCd$	–	+++	++	++
7.	$C_{19}H_{18}ClN_7O_2SCd$	–	++	++	+++
8.	$C_{18}H_{16}ClN_5O_3SCd$	–	++	++	+++

+ = 6-10 mm; +++ = > 14 mm; ++ = 10-14 mm; – = Inactive (Control)

Table-3: Anti-Fungal Activity of Mixed Ligand complexes of Cd

(A)	**Activity at 10 μg/ml of conc. of test compounds**				
S. N.	**Compounds**	***Aspergillusflavus***		***Aspergillusnigar***	
		Colony dia (mm)	**% Inhibition A. nigar**	**Colony dia (mm)**	**% Inhibition A. flavus**
1.	$C_{17}H_{17}ClN_4O_2SCd$	1.2	60.0	1.0	50.0
2.	$C_{17}H_{16}ClN_7O_2SCd$	1.4	53.3	1.5	25.0
3.	$C_{17}H_{18}ClN_7O_2SCd$	1.4	53.3	1.0	50.0
4.	$C_{16}H_{16}ClN_5O_3SCd$	1.2	60.0	1.4	30.0
5.	$C_{19}H_{17}ClN_4O_2SCd$	1.2	60.0	1.5	25.0
6.	$C_{19}H_{16}ClN_7O_2SCd$	0.8	73.3	1.4	30.0
7.	$C_{19}H_{18}ClN_7O_2SCd$	1.4	53.3	1.5	25.0
8.	$C_{18}H_{16}ClN_5O_3SCd$	1.2	60.0	1.0	50.0
9.	Control	3.0	–	2.0	–
(B)	**Activity at 20 μg/ml of conc. of test compounds**				
S. N.	**Compounds**	***Aspergillusflavus***		***Aspergillusnigar***	
		Colony dia (mm)	**% Inhibition A. nigar**	**Colony dia (mm)**	**% Inhibition A. flavus**
1.	$C_{17}H_{17}ClN_4O_2SCd$	1.0	66.6	1.0	50.0

2.	$C_{17}H_{16}ClN_7O_2SCd$	1.0	66.6	1.0	50.0
3.	$C_{17}H_{18}ClN_7O_2SCd$	1.2	60.6	0.8	60.0
4.	$C_{16}H_{16}ClN_5O_3SCd$	1.0	66.6	1.0	50.0
5.	$C_{19}H_{17}ClN_4O_2SCd$	0.7	76.6	1.2	40.0
6.	$C_{19}H_{16}ClN_7O_2SCd$	0.6	80.0	1.2	40.0
7.	$C_{19}H_{18}ClN_7O_2SCd$	1.2	60.6	1.5	25.0
8.	$C_{18}H_{16}ClN_5O_3SCd$	1.0	66.6	1.4	30.0
9.	Control	3.0	–	2.0	–
(C)	**Activity at 50 μg/ml of conc. of test compounds**				
S. N.	**Compounds**	***Aspergillusflavus***		***Aspergillusnigar***	
		Colony dia (mm)	**% Inhibition A. nigar**	**Colony dia (mm)**	**% Inhibition A. flavus**
1.	$C_{17}H_{17}ClN_4O_2SCd$	0.6	80.0	0.5	75.0
2.	$C_{17}H_{16}ClN_7O_2SCd$	0.7	76.6	0.6	70.0
3.	$C_{17}H_{18}ClN_7O_2SCd$	1.0	66.6	0.5	75.0
4.	$C_{16}H_{16}ClN_5O_3SCd$	0.8	73.3	0.8	60.0
5.	$C_{19}H_{17}ClN_4O_2SCd$	0.5	83.3	0.8	60.0
6.	$C_{19}H_{16}ClN_7O_2SCd$	0.4	86.7	0.5	75.0
7.	$C_{19}H_{18}ClN_7O_2SCd$	1.0	66.6	0.5	75.0
8.	$C_{18}H_{16}ClN_5O_3SCd$	0.7	76.6	0.8	60.0
9.	Control	3.0	–	2.0	–

(D)	**Activity at 100 μg/ml of conc. of test compounds**				
S. N.	**Compounds**	***Aspergillusflavus***		***Aspergillusnigar***	
		Colony dia (mm)	**% Inhibition A. nigar**	**Colony dia (mm)**	**% Inhibition A. flavus**

1.	$C_{17}H_{17}ClN_4O_2SCd$	0.4	86.7	0.2	90.0
2.	$C_{17}H_{16}ClN_7O_2SCd$	0.4	86.7	0.1	95.0
3.	$C_{17}H_{18}ClN_7O_2SCd$	0.8	73.3	0.2	90.0
4.	$C_{16}H_{16}ClN_5O_3SCd$	0.5	83.3	0.4	80.0
5.	$C_{19}H_{17}ClN_4O_2SCd$	0.1	96.7	0.5	75.0
6.	$C_{19}H_{16}ClN_7O_2SCd$	0.2	93.3	0.2	90.0
7.	$C_{19}H_{18}ClN_7O_2SCd$	0.8	73.3	0.5	75.0
8.	$C_{18}H_{16}ClN_5O_3SCd$	1.0	66.6	0.6	70.0
9.	Control	3.0	–	2.0	–

Table-4: *In-vitro* anti-tumor activity of Mixed Ligand complexes of Cd

S. N.	Compounds	Cell No. x 10^4	Activity
1.	$C_{17}H_{17}ClN_4O_2SCd$	9.35±0.61	+
2.	$C_{17}H_{16}ClN_7O_2SCd$	9.17±0.90	+
3.	$C_{17}H_{18}ClN_7O_2SCd$	9.17±0.87	+
4.	$C_{16}H_{16}ClN_5O_3SCd$	12.34±1.05	–
5.	$C_{19}H_{17}ClN_4O_2SCd$	11.89±1.05	–
6.	$C_{19}H_{16}ClN_7O_2SCd$	9.25±0.86	+
7.	$C_{19}H_{18}ClN_7O_2SCd$	11.69 ± 1.04	-
8.	$C_{18}H_{16}ClN_5O_3SCd$	9.17 ± 0.90	+
9.	Negative control	10.21±1.01	–
10.	Positive control	40.26±3.23	–

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