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# Synthesis and antitumor activity of aza-chalcones and their Pt(IV) complexes

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## ABSTRACT

Eight aza-chalcones $L_{1-8}$ and eight new platinum(IV) complexes $C_{1-8}$ with aza-chalcones were synthesized. The complexes have been characterized by elemental anaylsis, IR spectra, electronic spectrum and $^1$H NMR. The cytotoxicity was tested by MTT assays and compared with the cytotoxicity of cisplatinum. The results indicate that the complexes $C_{1-3}$ and $C_{5-8}$ have cytotoxicity against tested A-549 cell line; moreover, the cytotoxicity of complexes $C_6$ approached cisplatinum. The complexes $C_3$, $C_4$ and $C_6$ have cytotoxicity against tested Hela cell line, but the cytotoxicity of these complexes is lower than cisplatinum. Most of the coorespounding ligands have no cytotoxicity against tested two cell lines, and the cytotoxicity of all ligands is lower than complexes.The results suggest that the cytotoxicity of all complexes against A-549 cell line is better than against Hela cell line.



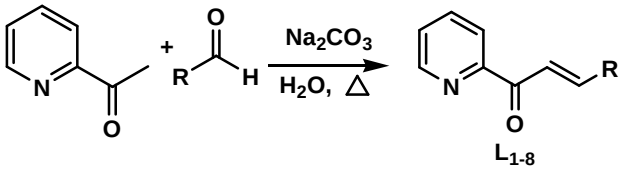
## KEYWORDS

Platinum (IV) complexes;
Aza-chalcones;
Synthesis;
Cytotoxicity.

## INTRODUCTION

Cisplatin is widely applied in the treatment of a various types of cancer such as germ cell tumors, ovarian, lung, hed and neck, bladder carcinomas, etc[1,2]. However, it has severe side effects such as nephrotoxicity, nausea, ototoxicity, neurotoxicity and myelotoxicity in low dose. In addition to these, the therapeutic efficacy is also limited by resistant tumor cell sub-populations. To overcome these disadvantages, people have explored to prepare many new platinum complexes and studied their anti-tumor activity in recent decades. Several Pt(IV) complexes have been synthesized recently and shown high activtity *in vitro* and in clinical testing[3]. Pt(IV) complexes are a new class of platinum complexes that exhibits less toxicity than cisplatin. This kind of complex has some advantages such as allowing oral administration, reduced toxicity, decrease in the amount of the complex that is lost or deactivated on the path to the target cell and a low cross-resistance to cisplatin[4,5].

Chalcone is a kind of natural organic compound which is widely in plants such as liquorice, safflower and so on. The molecule of chalcones is flexible; it can




**Scheme 1 : The synthesis of aza-chalcones**

be combined with many biological receiver. So that, this kind of compounds have many biological activities, such as, antitumor activity, anti-HIV activity, anti anti-bacterial activity, antioxidant activity, etc[6-15].

It contains pyridinyl in the structure of aza-chalcone, the N atom of pyridinyl has strong coordination ability with various metal ions. Medvecky et al.[16] reported that aza-chalcone has higher cytotoxic. Based on the above reasons, eight platinum(IV) complexes were synthesized, anticancer activity *in vitro* were also studied in the present work.

## RESULTS AND DISSCUSION

### Synthesis and spectroscopic data of aza-chalcones

All reagents and solvents were of analytical grade.

2-Acetyl pyridine (2.10 mmol) and aromatic aldehyde(2.00 mmol) was added to water with stirring, then the sodium carbonate solution was added to the mixture. The reactions were followed by thin layer chromatography (TLC). After completion of the reaction, the products were purified by column chromatography on silica gel (200-300 mesh, eluted with a mixture of petroleum ether and ethyl acetate) (Scheme 1).

The yields of aza-chalcones $L_{1-8}$ were shown in TABLE 1.

The data of NMR, IR and MS spectra for the new compounds are provided as follows:

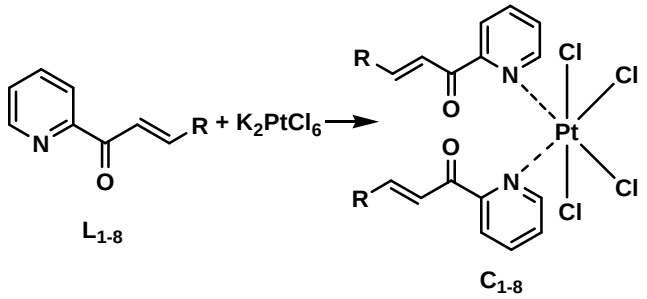
**$L_1$**: yellow solid; $^1$H NMR (600 MHz, $CDCl_3$), δ: 6.97-7.07 (m, 2H, CH-CH), 7.27-7.30 (m, 2H, Ph-H), 7.34 (d, 1H, J=13 Hz, CH=CH), 7.40-7.46 (m, 3H, Ph-H), 7.68-7.74 (m, 1H, Py-H), 7.78-7.82 (m, 2H, Py-H), 8.13 (d, 1H, J=13 Hz, CH=CH), 8.69 (d, 1H, Py-H); $^{13}$C NMR, δ: 122.81, 124.60, 126.80, 127.35, 127.47, 128.85, 129.22, 136.18, 137.05, 142.10, 144.78, 148.75, 154.16, 189.30; IR (KBr), $\nu_{max}$: 1660, 1577, 989 $cm^{-1}$; m/z (ESI) calcd. for $C_{16}H_{13}NO$ $[M+Na]^+$ 258.1.

**$L_3$**: yellow solid; $^1$H NMR (600 MHz, $CDCl_3$), δ: 3.79 (s, 3H, $OCH_3$), 6.88 (d, 2H, Ph-H), 7.42-7.44 (m, 1H, Py-H), 7.64 (d, 2H, Ph-H), 7.80-7.83 (m, 1H, Py-H), 7.87 (d, J=16 Hz, 1H, CH=CH), 8.14-8.17 (m, 2H, 1CH=CH and 1Py-H), 8.69 (d, 1H, Py-H); $^{13}$C NMR, δ: 55.37, 114.33, 118.49, 121.03, 122.84, 126.75, 127.38, 127.92, 130.66, 137.01, 144.67, 148.77, 154.41, 161.74, 189.31; IR (KBr), $\nu_{max}$: 1663, 1598, 1565, 1257, 1029, 986 $cm^{-1}$; m/z (ESI) calcd. for $C_{15}H_{13}NO_2$ $[M+Na]^+$ 262.1.

**$L_7$**: slight yellow solid; $^1$H NMR (600 MHz, $CDCl_3$), δ: 7.28-7.51 (m, 4H, Ph-H), 7.85 (d, J=13 Hz, 1H, CH=CH), 7.89-7.91 (m, 1H, Py-H), 8.19 (d, J=13 Hz, 1H, CH=CH), 8.28-8.37 (m, 2H, Py-H), 8.73-8.74 (m, 1H, Py-H); $^{13}$C NMR, δ: 123.0, 123.3, 126.8, 127.0, 128.1, 130.2, 131.2, 133.4, 135.7, 137.3, 140.2, 148.6, 153.8, 188.8; IR (KBr), $\nu_{max}$: 1672, 1602, 1578, 1027, 996 $cm^{-1}$; m/z (ESI) calcd. for $C_{14}H_{10}ClNO$ $[M+Na]^+$ 266.1.

### Synthesis spectroscopic data of aza-chalcone platinum(IV) complexes

### Synthesis of aza-chalcone platinum(IV) complexes




**Scheme 2 : The synthesis of aza-chalcone platinum(IV) complexes**

All reagents and solvents were of analytical grade.

The aza-chalcone platinum(IV) complexes have been prepared by the reaction of $K_2PtCl_6$ with aza-chalcones: adding aza-chalcones and $K_2PtCl_6$ to a solution of water and ethanol, then the mixture was stirred at 40°C. The reactions were followed by thin layer chromatography (TLC). After completion of the reaction, the products were separated by centrifugal effect, then washed with the mixture of water-ethanol, and dried under vacuum condition (Scheme 2).

### Elemnetal analysis

The elemental analysis showed that all the complexes were of high purity (TABLE 2). There is good agreement between caculated and found values for the complexes.

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TABLE 1 : The yields of aza-chalcones $L_{1-8}$

| Product | R | Time, h | Yield, % | M.P.,°C | M.P.,°C[Lit] |
|---|---|---|---|---|---|
| $L_1$ | $C_6H_5CH=CH$ | 5 | 83 | 86-88 | - |
| $L_2$ | 4-$CH_3C_6H_4$ | 6 | 79 | 84-86 | 84.8-85.3[17] |
| $L_3$ | 2-$OCH_3C_6H_4$ | 6 | 76 | 180-182 | - |
| $L_4$ | 4-$OCH_3C_6H_4$ | 4.5 | 83 | 84-86 | 84.6-85.2[17] |
| $L_5$ | 3,4-$O_2CH_2C_6H_3$ | 4 | 80 | 150-151 | 150.3-150.8[17] |
| $L_6$ | 4-$NO_2C_6H_4$ | 0.5 | 97 | 156-158 | 158.2-158.5[17] |
| $L_7$ | 2-$ClC_6H_4$ | 5 | 85 | 92-96 | - |
| $L_8$ | 4-$ClC_6H_4$ | 5 | 89 | 100-102 | 102.2-102.5[17] |

TABLE 2 : Elemental analysis values of platinum(IV) complexes

| Complexes | R | Mf. of complexes | Found. (Calcd.)/% | | |
|---|---|---|---|---|---|
| | | | C | H | N |
| $C_1$ | $CH=CHC_6H_5$ | $Pt(L_1)Cl_4$ | 47.53 (47.57) | 3.67 (3.22) | 3.44 (3.47) |
| $C_2$ | 4-$CH_3C_6H_4$ | $Pt(L_2)Cl_4$ | 45.74 (45.97) | 3.76 (3.32) | 3.59 (3.58) |
| $C_3$ | 2-$OCH_3C_6H_4$ | $Pt(L_3)Cl_2{\cdot}2H_2O$ | 42.05 (42.30) | 3.47 (3.52) | 3.37 (3.29) |
| $C_4$ | 4-$OCH_3C_6H_4$ | $Pt(L_4)Cl_4$ | 42.29 (42.30) | 3.41 (3.52) | 3.32 (3.29) |
| $C_5$ | 3,4-$O_2CH_2C_6H_3$ | $Pt(L_5)Cl_4{\cdot}H_2O$ | 41.88 (41.81) | 3.04 (2.55) | 3.29 (3.25) |
| $C_6$ | 4-$NO_2C_6H_4$ | $Pt(L_6)Cl_4{\cdot}C_2H_5OH$ | 40.43 (40.40) | 3.07 (2.92) | 6.57 (6.28) |
| $C_7$ | 2-$ClC_6H_4$ | $Pt(L_7)Cl_4{\cdot}C_2H_5OH$ | 42.08 (42.05) | 3.13 (3.30) | 3.32 (3.16) |
| $C_8$ | 4-$ClC_6H_4$ | $Pt(L_8)Cl_4{\cdot}C_2H_5OH$ | 42.11 (42.05) | 3.09 (3.30) | 3.41 (3.16) |

## IR spectra and electronic spectrum

As listed in TABLE 3, the IR spectra of the complexes are similar; the main bands with tentative assignments are listed in Table. The bands of $\nu_{Py}$, $\nu_{C=O}$ and $\nu_{HC=CH}$ in these complexes shift to lower frequencies than the corresponding aza-chalcones. Thus it indicates that the aza-chalcones are coordinated with platinum.

Pyridinyl in different ligands have strong absorption peaks at 240-260 nm. Aftre coordinated with platinum, the absorption peaks of complexes have appeared at 263-265 nm. That means the absorption peaks red shift by ca. 5-25 nm for the complexes compared with ligands. That means the pyridinyl in ligands is coordinated with platinum.

## $^1$H NMR

The chemical shift of the complexes was listed as follows:

**$C_1$**: red solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 6.97-7.07 (m, 4H, CH-CH), 7.27-7.30 (m, 4H, Ph-H), 7.34 (d, 2H, J=16 Hz, CH=CH), 7.40-7.46 (m, 6H, Ph-H), 7.68-7.74 (m, 2H, Py-H), 7.78-7.82 (m, 4H, Py-H), 8.13 (d, 2H, J=16 Hz, CH=CH), 8.69 (d, 2H, Py-H). **$C_2$**: brown solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 3.23 (s, 6H, $2CH_3$), 6.93 (d, 4H, 4Ph-H), 7.62 (d, J=16 Hz, 2H, 2CH=CH), 7.68-7.71 (m, 2H, 2Py-H), 7.81 (d, 4H, 4Ph-H), 8.04-8.07 (m, 2H, 2Py-H), 8.16 (d, J=16 Hz, 2H, 2CH=CH), 8.23 (d, 2H, 2Py-H), 8.81 (d, 2H, 2Py-H).

**$C_3$**: red solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 3.91 (s, 6H, $2CH_3$), 7.46-7.49 m, 2H, 2Ph-H), 7.50-7.53 (m, 2H, 2Ph-H), 7.75-7.77 (m, 2H, 2Py-H), 7.78 (d, J=16 Hz, 2H, 2CH=CH), 7.84 (d, 2H, 2Ph-H), 8.02 (d, 2H, 2Ph-H), 813-8.15 (m, 2H, 2Py-H), 8.24-8.25 (m, 2H, 2Py-H), 8.30 (d, J=16 Hz, 2H, 2CH=CH), 8.81 (d, 2H, 2Py-H).

**$C_4$**: yellow solid; 3.83 (s, 6H, $2CH_3$), 6.93 (d, 4H, 4Ph-H), 7.62 (d, J=16 Hz, 2H, 2CH=CH), 7.68-7.71 (m, 2H, 2Py-H), 7.81 (d, 4H, 4Ph-H), 8.04-8.07 (m, 2H, 2Py-H), 8.16 (d, J=16 Hz, 2H, 2CH=CH), 8.23 (d, 2H, 2Py-H), 8.81 (d, 2H, 2Py-H).

**$C_5$**:dark red solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 6.12 (s, 4H, $2CH_2$), 7.64 (d, J=16 Hz, 2H, 2CH=CH), 7.70 (s, 2H, 2Ph-H), 7.73-7.75 (m, 2H, 2Py-H), 7.81 (d, J=16 Hz, 2H, 2CH=CH), 8.01 (d, 2H, 2Ph-H), 8.07 (d, 2H, 2Ph-H), 8.12 (d, 2H, 2Py-H), 8.23-8.25 (m, 2H, 2Py-H), 8.80 (m, 2H, 2Py-H).

**$C_6$**:dark yellow solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 7.73-7.75 (m, 2H, 2Py-H), 7.96 (d, J=16 Hz, 2H, 2CH=CH), 8.07-8.10 (m, 2H, 2Py-H), 8.13 (d, 4H, 4Ph-H), 8.15 (d, 2H, 2Py-H), 8.31 (d, 4H, 4Ph-H), 8.44 (d, 2H, J=16 Hz, 2CH=CH).

**$C_7$**: brown solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 7.28-7.51 (m, 8H, Ph-H), 7.85 (d, J=16 Hz, 2H, CH=CH), 7.89-7.91 (m, 2H, Py-H), 8.19 (d, J=16 Hz, 2H, CH=CH), 8.28-8.37 (m, 4H, Py-H), 8.73-8.74 (m, 2H, Py-H).

**$C_8$**:dark yellow solid; $^1$H NMR (600 MHz, $d_6$-DMSO), δ: 7.50-7.52 (m, 2H, 2Py-H), 7.55 (d, 4H, 4Ph-H), 7.70-7.72 (m, 2H, 2Py-H), 7.87 (d, J=16 Hz, 2H, 2CH=CH), 7.88 (d, 4H, 4Ph-H), 7.95 (d, 2H, 2Py-H), 8.13 (d, 2H, 2Py-H), 8.29 (d, J=16 Hz, 2H, 2CH=CH).

## Cytotoxic studies

As listed in TABLE 4, the complexes $C_{1-3}$ and $C_{5-8}$

**TABLE 3 : The IR spectrum data of Pt(IV) complexes**

| Entry | R | Compounds | $\nu_{max}$ | | |
|---|---|---|---|---|---|
| | | | Py | C=O | HC=CH |
| 1 | $CH=CHC_6H_5$ | $C_1$ | 1625 | 1706 | 1066 |
| | | $L_1$ | 1577 | 1660 | 993 |
| 2 | $4\text{-}CH_3C_6H_4$ | $C_2$ | 1631 | 1709 | 1044 |
| | | $L_2$ | 1595 | 1662 | 995 |
| 3 | $2\text{-}OCH_3C_6H_4$ | $C_3$ | 1626 | 1702 | 1044 |
| | | $L_3$ | 1598 | 1663 | 986 |
| 4 | $4\text{-}OCH_3C_6H_4$ | $C_4$ | 1604 | 1706 | 1031 |
| | | $L_4$ | 1598 | 1663 | 986 |
| 5 | $3,4\text{-}O_2CH_2C_6H_3$ | $C_5$ | 1613 | 1693 | 1035 |
| | | $L_5$ | 1595 | 1661 | 990 |
| 6 | $4\text{-}NO_2C_6H_4$ | $C_6$ | 1634 | 1713 | 1048 |
| | | $L_6$ | 1605 | 1668 | 991 |
| 7 | $2\text{-}ClC_6H_4$ | $C_7$ | 1641 | 1705 | 1040 |
| | | $L_7$ | 1602 | 1672 | 993 |
| 8 | $4\text{-}ClC_6H_4$ | $C_8$ | 1614 | 1702 | 1052 |
| | | $L_8$ | 1587 | 1667 | 990 |

**TABLE 4 : The cytotoxicity of Pt(IV) complexes or coorespo-nding ligands to A-549 and Hela cells**

| Entry | Compounds | $IC_{50}$(μM) ($\bar{x} \pm s$) | |
|---|---|---|---|
| | | A-549 | Hela |
| 1 | CDDP | 19.41±5.67 | 20.01±1.36 |
| 2 | $C_1$ | 39.48±8.01 | 87.38±3.11 |
| | $L_1$ | 68.23±6.23 | 308.37±9.38 |
| 3 | $C_2$ | 43.67±3.03 | 129.59±4.06 |
| | $L_2$ | 249.46±25.56 | 203.43±0.67 |
| 4 | $C_3$ | 39.46±2.93 | 43.38±2.29 |
| | $L_3$ | 190.66±17.34 | 237.42±20.66 |
| 5 | $C_4$ | 79.35±3.38 | 53.28±2.39 |
| | $L_4$ | 262.95±19.35 | 175.89±8.82 |
| 6 | $C_5$ | 42.46±6.67 | 48.92±1.86 |
| | $L_5$ | 81.38±9.15 | 259.06±4.66 |
| 7 | $C_6$ | 21.70±1.87 | 39.18±0.08 |
| | $L_6$ | 45.19±2.99 | 200.60±8.00 |
| 8 | $C_7$ | 45.16±3.17 | 110.88±4.57 |
| | $L_7$ | 149.58±30.39 | 586.84±19.40 |
| 9 | $C_8$ | 41.82±6.68 | 90.48±1.87 |
| | $L_8$ | 255.95±19.53 | 530.21±10.57 |

have cytotoxicity against A-549 with lower $IC_{50}$ value (<50 μM), however, complex $C_4$ have no cytotoxicity against A-549 with a higher $IC_{50}$ value (79.35±3.38 μM). Complexes $C_3$, $C_5$ and $C_6$ have cytotoxicity against Hela cell line with lower $IC_{50}$ value (43.38±2.29 μM, 48.92±1.86 μM and 39.18±0.08 μM), but the other five complexes ($C_1$, $C_2$, $C_4$, $C_7$ and $C_8$) have no cytotoxicity against Hela with a higher $IC_{50}$ value (>50 μM). Complex $C_6$ has the best cytotoxicity among the eight complexes against tested carcinoma cell lines with $IC_{50}$ value of 21.70±1.87 μM and 39.18±0.08 μM, moreover, the cytotoxicity of $C_6$ against A-549 approached cisplatinum ($IC_{50}$ = 19.41 ± 5.67 μM). The corresponding ligand $L_6$ has cytotoxicity against A-549 cell line with $IC_{50}$ value of 45.19±2.99 μM, but has no cytotoxicity against Hela cell line with a higher $IC_{50}$ (>50 μM). The other corresponding ligands have no cytotoxicity against both A-549 and Hela cell lines ($IC_{50}$>50 μM). Compared with corresponding ligands $L_{1\text{-}8}$, the complexes $C_{1\text{-}8}$ have better cytotoxicity against both A-549 and Hela cell lines.

## CONCLUSION

In our present work, several Pt(IV) complexes have been synthesized from aza-chalcones and $K_2PtCl_6$, and characterized the structure by elemental analysis, $^1H$ NMR, IR spectra and electronic spectra. The results showed that all the complexes were of high purity. The cytotoxicity of the complexes was tested by MTT assays. The results showed that complexes have activity against A-549 cell line with lower $IC_{50}$ (<50 μM) except complex $C_4$ with $IC_{50}$ value of 79.35±3.38 μM. The complex $C_3$, $C_5$ and $C_6$ have activity against Hela cell line with $IC_{50}$ values of 43.38±2.29 μM, 48.92±1.86 μM and 39.18±0.08 μM.

## EXPERIMENTAL PROTOCOLS

### Instrumentation and measurement

Elemental analyses were determined on an EA-1110 elemental analyzer. Molar conductances at room temperature were measured in $10^{-3}$ mol/L DMF solutions using a DFS-1 type conductivity meter. The IR spectra were recorded using KBr pellets and a PerkineElmer Model-683 spectrophotometer. The electronic spectra in DMSO were measured on an UV-3400 Toshniwal

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spectrophotometer. The $^1$H NMR spectra were measured on a Bruker AV-400 NMR spectrometer in dimethyl sulfoxide-d6 with solvent peaks as references, and in chloroform with TMS as internal standard. ESIMS data were measured with Thermo Finnigan-LCQ. The thermal analysis was conducted using RI-GAKU 8150 meter (Ar, 10°C min$^{-1}$, $Al_2O_3$). The optical density (OD) at 570 nm was measured on a microplate spectrophotometer (Bio-Rad Model 680, USA).

### Cell culture

Two different human carcinoma cell lines were used for cytotoxicity determination: A-549 (human lung adenocarcinoma cell line) and Hela (human cervical cancer cell line). They were obtained from the American Type Culture Collection (ATCC) and were cultured in RPMI-1640mediumsupplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 mg/ml of streptomycin. Cells were maintained at 37C in a humidified atmosphere of 5% $CO_2$ in air.

### Cytotoxicity analysis

The cells harvested from exponential phase were seeded equivalently into a 96-well plate, complexes were then added to the wells to achieve final concentrations. Control wells were prepared by addition of culture medium. Wells containing culture medium without cells were used as blanks. The plates were incubated at 37°C in a 5% $CO_2$ incubator for 48 h. The MTT assay was performed as described by Mosmann[18]. Upon completion of the incubation, stock MTT dye solution (20 ml, 5 mg/ml) was added to each well. After 4 h incubation, 2-propanol (100 ml) was added to solubilize the MTT formazan. The OD of each well was then measured on a microplate spectrophotometer at a wavelength of 570 nm. The SRB assay was performed as previously described[19]. Upon completion of the incubation, the cells were fixed in 10% trichloroacetic acid (100 ml) for 30 min at 4°C, washed five times in tap water and stained with 0.1% SRB in 1% acetic acid (100 ml) for 15 min. The cells were washed four times in 1% acetic acid and air-dried. The stain was solubilized in 10 mM unbuffered Tris base (100 ml) and OD was measured at 540 nm as above. The $IC_{50}$ value was determined from plot of % viability against dose of compounds added.

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