SYNTHESIS AND STRUCTURAL EXPLANATION OF MIXED LIGAND COMPLEXES OF SELENIUM(IV) WITH CAFFEINE AND SOME NITROGEN-BASED LIGANDS

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Abstract

Mixed-ligand complexes of selenium(IV) ion chelating with caffeine (caf) molecule as a primary ligand and N,O donors (L-valine (L-val); L-proline (L-pro); 2,4-diaminophenol (dap)), or nitrogen–base donors (2,2-bipyridine (bpy) and bathophenanthroline (bphen)) as secondary ligand were prepared. These complexes were assigned based on different techniques like molar conductance, UV-Visible (UV-Vis), infrared (FT-IR), ¹H-NMR spectroscopy, thermal analysis (TGA-DTG), X-ray diffraction (XRD), scanning (SEM) and transmission electron microscopes (TEM). The FT-IR and ¹H-NMR spectra deduced that the caf chelate is acts as a monodentate with Se(IV) through the nitrogen atom N9, while secondary ligands (L-val, L-pro, dap, bpy and bphen) coordinated as bi-dentate ligand towards Se(IV) via NO or NN donating atoms. Dependent on the spectroscopic results, it can be confirmed that Se(IV) complexes have an octahedral geometry with general formulas [Se(caf)(L)(Cl)] (where L = L-val, L-pro and dap) I–III and [Se(caf)(L')(Cl)]Cl (where L' = bpy and bphen) IV and V. The conductance data of the synthesized complexes revealed that the [Se(caf)(L')(Cl)]Cl complexes have a non-electrolyte properties, while [Se(caf)(L')(Cl)]Cl complex has a 1:1 electrolyte nature. TGA-DTG analysis shows that the degradation of complexes occurs between 30–800°C. SEM, TEM, and XRD analyses revealed the particle size of the synthesized complexes. All the five complexes in this study were evaluated for their anticancer effect towards two colorectal adenocarcinoma (Caco-2) and breast cancer (Mcf-7) cell lines.

Rezumat

În acest studiu este descris modul de preaparare al complecșilor de chelare ale ionului seleniu(IV) cu o molculă de cafeină (caf) ca ligand primar și donori de N, O (L-valină (L-val); L-prolină (L-pro); 2,4-diaminofenol (dap)), sau donori bazici de azot (2,2-bipiridină (bpy) și batofenantrolină (bphen)) ca ligand secundar. Acești compleși au fost caracterizați prin mai multe metode: conductanța molară, spectroscopie UV-Visibil (UV-Vis), infraroșu (FT-IR), ¹H-RMN, analiză termică (TGA-DTG), difracție de raze X (XRD), SEM și TEM. Spectrele FT-IR și ¹H-RMN au evidențiat cafeină ca ligand monodentat pentru Se(IV) prin atomul de azot N9, în timp ce liganții secundari (L-val, L-pro, dap, bpy și bphen) au acționat ca bidentați pentru Se(IV) prin atomi de donori de NO sau NN. Conform datelor spectroscoptive, se poate confirma că acești compleși au o geometrie octaedrică cu formule generale [Se(caf)(L')(Cl)]Cl (unde L' = bpy și bphen) IV și V. Datele de conductanță ale complecșilor sintetizați au arătat că [Se(caf)(L')(Cl)]Cl prezintă proprietăți non-electrolitice, în timp ce complexes [Se(caf)(L')(Cl)]Cl sunt o natură electrolitică 1:1. Analiza TGA-DTG arată că degradarea complecșilor are loc între 30–800°C. Analizele SEM, TEM și XRD au evidențiat mărimea particulelor complecșilor sintetizați. Toți cei cinci compleși din acest studiu au fost evaluați pentru efectul potențial anticanceros asupra a două linii celulare de adenocarcinom colorectal (Caco-2) și cancer de sân (Mcf-7).

Keywords: caffeine, secondary ligand, mixed-ligand, spectroscopy, anticancer agent

Introduction

Caffeine is one of the nitrogen-base ligand, also, it is one of the main alkanes that form in different types of foods and drinks that we consume in daily life [1]. Caffeine has ability to dissolve many of pharmaceutical drugs, because of it is included two aromatic rings which help in the dissolution of anti-malarial agent [2, 3]. Surely, the caffeine molecule attached to the receptors on the heart muscle cell surface, that increasing the level of cyclic adenosine monophosphate (cAMP) by blocking the enzyme which response to the decomposition of cAMP [4]. The caffeine compound plays an important role in the enhancement of the cisplatin anti-tumour activity [5]. Also from this reason platinum complexes were made from caffeine and studied its cytotoxic activity [6-12]. Caffeine commonly bonded through its nitrogen donor atom or via carbonyl oxygen atom that is a rarity occurs. The metal complexes are monomeric, dimeric, several correlations are stabilized and discussed for coordination
of caffeine and derivatives [13], The Re(l) [14], Cu(II) [15], Zn(II) [16], Au(III) [17], Cu(II) and Zn(II) complexes of caffeine and xanthine compounds [18, 19] have been synthesized and the structures as well as biological efficiency were assigned [20-22]. The caffeine, xanthines and theophylline organic ligands in usual neutral case are coordinated to different metal ions through N(9) donating atom [10-22]. Herein, the scope of this study is aimed to synthesis, biological assessment and spectroscopic characterization of some mixed ligand complexes of caffeine and nitrogen base secondary ligands e.g. L-valine (L-val), L-proline (L-pro), 2,4-diaminophenol (dap), 2,2'-bipyridine (bpy) and bathophenanthroline (bphen) towards selenium(IV) ions.

Materials and Methods

Chemical reagents and analysis
Caffeine, L-valine, L-proline, 2,4-diaminophenol dihydrochloride, 2,2'-bpyridine, selenium tetrachloride and bathophenanthroline disulfonic acid disodium salt hydrate in pure grade form were brought from Sigma-Aldrich Chemical Corporation (St. Louis-Mo-USA). The apparatus and their models used to investigate the synthesized selenium(IV) complexes can be summarized in Table I.

Table I

| Apparatus used for the investigation of the synthesized complexes |
|---------------------------------------------------------------|
| Analyses | Model of apparatus |
|------------------------|-------------------|
| Elemental analysis | Perkin Elmer “CHN 2400” |
| Conductance | Conductivity meter “Jenway 4010” |
| FT-IR spectra | FTIR Spectrophotometer “Bruker” |
| Electronic spectra | UV/Vis Spectrophotometer “UV2 Unicam” |
| Magnetic moment | Balance of Magnetic Susceptibility |
| 1H-NMR | NMR spectrometer “Varian Mercury VX-300 MHz” |
| Thermogravimetric | Shimadzu analyser “TG/DTG-50H” |
| SEM | Quanta equipment “FEG 250” |
| XRD | X ‘Pert PRO PANanalytical |
| TEM | “JEOL 100s microscopy” |

The elemental analyses of the synthesized Se(IV) complexes are presented in Table II.

Table II

| Complex | Colour | Element | Calc. | Found | Calc./Found |
|---------|--------|---------|-------|-------|-------------|
| I | White | %C | 31.50 | 31.23 |
| | | %H | 4.07 | 3.98 |
| | | %N | 14.13 | 14.04 |
| | | %Se | 15.93 | 15.90 |
| II | White | %C | 31.63 | 31.44 |
| | | %H | 3.68 | 3.65 |
| | | %N | 14.19 | 14.04 |
| | | %Se | 16.00 | 15.55 |
| III | Dark Red | %C | 33.45 | 33.32 |
| | | %H | 3.41 | 3.29 |
| | | %N | 16.72 | 16.59 |
| | | %Se | 15.71 | 15.65 |
| IV | Pink | %C | 37.85 | 37.54 |
| | | %H | 3.18 | 3.12 |
| | | %N | 14.71 | 14.43 |
| | | %Se | 13.82 | 13.76 |
| V | White | %C | 51.43 | 51.40 |
| | | %H | 3.51 | 3.49 |
| | | %N | 11.24 | 11.20 |
| | | %Se | 10.57 | 10.54 |

Synthesis of selenium(IV) mixed ligand complexes

0.195 g (1 mmol) of caffeine primary ligand was mixed to SeCl4 (1 mmol) in methanolic solvent (50 mL) with continuously magnetic stirrer. This stage was followed by the addition of 1 mmol of L-valine, L-proline, 2,4-diaminophenol dihydrochloride, 2,2'-bpyridine or bathophenanthroline disulfonic acid disodium salt hydrate secondary ligands with the molar ratio of 1:1:1 (Se⁴⁺:L¹:L²). These mixtures were refluxed for about 3 hrs at 70 - 80°C. The solid precipitates were filtrated, washed by few drops of methanol solvent, and dried over under vacuum. Yields of solid selenium(IV) complexes are within 73 - 75% range with higher melting point above 250°C. The elemental analysis (Calc./Found) data of the synthesized Se(IV) complexes are presented in Table II.

Biological tests

The biological assessments of synthesized selenium(IV) complexes were scanned dependent on the disc diffusion method [23]. The bacterial strains used in this study are Klebsiella, Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis. The cytotoxic test which applied on the five Se(IV) complexes were performed against colorectal adenocarcinoma Caco-2 and breast cancer MCF-7 cancer cell lines using neutral red uptake assay [24].

Results and Discussion

Micro analytical and conductance measurements

The synthesized selenium(IV) mixed ligand complexes were isolated in solid state forms, soluble in selected organic solvents (dimethylformamide (DMF) and dimethylsulfoxide (DMSO)) with gently warming. The lower data (2 - 12 cm²/ohm x mol) of observed molar conductance in DMSO regarding [Se(caf)(L)(Cl)]₃ complexes I-III (where L = L-val, L-pro and dap) indicates the non-electrolyte nature [25], while the molar conductance values of [Se(caf)(L')(Cl)]₃Cl
Infrared spectral studies

The FTIR spectra of the free primary (caf) and secondary ligands (L-val, L-pro, dap, bpy and bphen) as well as their selenium(IV) complexes were scanned as shown in Figure 2, also the assignments of distinguish infrared frequencies are summarized in Table III. The vibration spectra of caffeine carbonyl group in case of Se(IV) complexes I–V exhibit an absorption band with strong intensity at 1699 - 1712 cm\(^{-1}\) range due to \(\nu(\text{CO})\) asymmetric, and the another strong band at the 1661 cm\(^{-1}\) attributed to \(\nu(\text{CO})\) stretching symmetry. These vibration bands are observed in the same wavenumbers compared with the free caffeine ligand, this assigned to that oxygen of carbonyl group don’t sharing in the selenium(IV) complexation. The stretching \(\nu(\text{C}≡\text{N})\) band is shifted to lower wavenumbers at 1548 - 1566 cm\(^{-1}\) in comparable with the free caf ligand (1658 cm\(^{-1}\)). This confirm the chelation of the caffeine via the nitrogen atom in position N(9) [26]. An essential infrared spectral band at \(\sim > 3400 \text{ cm}^{-1}\) for the [Se(caf)(L)(Cl)]\(_3\) complexes I–III due to the \(\nu(\text{OH})\) stretching vibration of the –COOH and –OH groups of L-val, L-pro and dap secondary ligands are disappear. This result revealed that the complex formation occurs through the deprotonation of the hydroxyl group of L-val, L-pro and dap moieties. In the infrared spectra of the free L-val, L-pro and dap ligands, the \(\nu(\text{CO})\) frequencies are observed in the 1171 - 1198 cm\(^{-1}\).
In case of selenium(IV) complexes I-III, the stretching vibration ν(NH) of –NH₂ group is observed in the 3118 - 2950 cm⁻¹ range in comparison with the L-val, L-pro and dap ligands (3036 - 3149 cm⁻¹). This lower shift suggests the coordination of nitrogen atom of amino group towards selenium metal ion. The existing absorption bands at 1480 - 1496 cm⁻¹ and 1279 - 1293 cm⁻¹ in case of complexes I and II are assigned to νₐ(COO) and νₐ(COO) respectively, in present study the difference frequencies Δν are much greater than the ionic complexes (200 cm⁻¹) (Δν = νₐ(COO) - νₐ(COO)) suggesting the uni-dentate binding of carboxylato group [26].

Table III
Distinguish infrared spectral bands of free ligands and complexes I-V

| Assignments          | caf | L-val | L-pro | dap | bpy | bphen | I   | II  | III | IV  | V   |
|----------------------|-----|-------|-------|-----|-----|-------|-----|-----|-----|-----|-----|
| ν(O–H)               | -   | -     | -     | 3435| -   | -     | -   | -   | -   | -   | -   |
| ν(N–H)               | -   | 3149  | 3058  | 3036| -   | -     | -   | 3118| 2951| 2950| -   |
| ν(C=O)               | 1700| 1700  | 1625  | -   | -   | -     | 1703| 1712| 1699| 1708| 1708|
|                   | 1659|       |       |     |     |       | 1661| 1661| 1661| 1661| 1661|
| δ(NH₂)               | -   | 1613  | 1618  | 1641| -   | -     | -   | -   | -   | -   | -   |
| ν(C=N)               | 1584| 1584  | 1614  | 1566| 1548| 1555  | 1557| 1557| -   | -   | -   |
| νₐ(COO)              | -   | -     | -     | -   | -   | -     | 1496| 1480| -   | -   | -   |
| νₐ(COO)              | -   | -     | -     | -   | -   | -     | 1293| 1279| -   | -   | -   |
| ν(C=O)               | 1198| 1171  | 1171  | 1198| -   | -     | 1034| 1030| 1026| -   | -   |
| Δν                   | -   | -     | -     | -   | -   | -     | 203 | 201 | -   | -   | -   |
| ν(M-O)+ν(M-N)        | -   | -     | -     | -   | -   | -     | 618 | 609 | 618 | 609 | 551 |
|                      |     |       |       |     |     |       | 495 | 419 | 429 | 428 | 419 |

Figure 2.
Infrared spectra of Se(IV) complexes I, II, III, IV, and V
Electronic spectra studies

The electronic absorption spectra of the free ligands caf (275, 316 & 365 nm), L-val (205 and 325 nm), L-pro (280 and 332 nm), dap (275 and 475 nm), bpy (235 and 280 nm) and bphen (280 and 320 nm) may be assigned to $\pi \rightarrow \pi^*$ and n→$\pi^*$ electronic transitions (Table IV) of the C≡N and C=O groups. The absorption bands at 511 and 354 nm in case of complexes III and V are attributed to the metal-to-ligand or ligand-to-metal electron transfer M-L$_{CT}$ transitions [27, 28].

| Compounds | Colour | n-$\pi^*$ | $\pi$-$\pi^*$ | M-L$_{CT}$ |
|-----------|--------|-----------|---------------|------------|
| I         | White  | 274       | 287           | -          |
| II        | White  | 275       | 288           | -          |
| III       | Red    | 286       | 387           | 511        |
| IV        | White  | 274       | 286           | -          |
| V         | Pink   | 276       | 311           | 354        |

$^1$H-NMR spectral studies

$^1$H-NMR chemical shift data of the free primary and can be listed as follows: $^1$H-NMR of caf: $\delta$: 3.39 (3H, N3-CH$_3$), 3.57 (3H, N1-CH$_3$), 4.00 (3H, N7-CH$_3$) and 7.53 ppm (1H, C8-H); L-val: $\delta$: 3.61 (1H, CH-NH$_2$), 2.26 (1H, CH$_2$(CH$_3$)$_2$), 1.05 (3H, CH$_3$) and 1.00 ppm (3H, CH$_3$); L-pro: $\delta$: 12.80 (1H, COOH), 7.93 (1H, NH) and 4.07 - 1.97 ppm (7H, pyrrolidine); dap: $\delta$: 6.41 - 6.70 (3H, Ar); bpy: $\delta$: 7.12-8.59 (8H, 2Ar); bphen: $\delta$: 7.10-8.40 (16H, 5Ar).

$^1$H-NMR spectrum of the caf ligand in DMSO-d$_6$ has protons at 3.39, 3.57, 400 and 7.53 ppm due to N3-CH$_3$, N1-CH$_3$, N7-CH$_3$(methyl) groups and C8-H respectively. Regarding of the selenium(IV) complexes I-V, the protons of N3-CH$_3$, N1-CH$_3$ and N7-CH$_3$ are practically shifted. The proton of C8-H was shifted to $\delta$ 7.98 - 8.01 ppm in case of the Se(IV) complexes. This downfield shift was assigned to the involvement of N9 in complexity. The $^1$H-NMR spectrum of the [Se(caf)(L-val)(Cl)$_2$] complex (I) has displayed 0.90 (3H, CH$_3$) and 8.40 (16H, 5Ar). The prominent feature of the $^1$H-NMR spectra show downfield shifts of the C8-H (caf) and protons of aromatic rings (dap, bpy and bphen) signals for the synthesized complexes. This indicates coordination through the N9, while secondary ligands (L-val, L-pro, dap, bpy and bphen) act as a bidentate ligand, coordinated to Se$^{4+}$ ion through the NO or NN atoms. The $^1$H-NMR data confirm that the important chemical shifts for primary and secondary ligands have changed by coordination.

Thermogravimetric measurements

The profiles of the TGA curves (Figure 3) of the five selenium(IV) complexes I-V showed the same thermal behavior and that the mass loss of the complexes occurs in one sharp decomposition stage, this stage is in the range of 209 - 317°C at maximum differential thermogravimetric peaks DTG$_{max}$ = 283, 267, 254, 275 and 240°C with mass loss 100, 100, 100, 100 and 92%, respectively attributed to sublimation and vaporization of the complexes [29, 30]. As for complex V, it was found that the mass loss percentage was 92%, and it can be explained that the 8% ratio is assigned to few carbon atoms as a residue after thermal cracking due to the large molecular weight and the presence of 32 carbon atoms.

Figure 3.

TGA curves of Se(IV) complexes I-V

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To obtain further acquaintance about the particle size of the metal complexes XRD spectra was performed as given in Figure 4. The XRD patterns indicate a crystalline behaviour for the I-IV complexes, while the XRD pattern of [(caf)(bphen)(Cl)]3.Cl complex (V) is very less crystalline phase. The estimation of d-spacing values of reflections were resulted using the Bragg’s equation (nλ = 2d sinθ). The average size of the particles was calculated to be 15 - 60 nm from the half-width at half-maximums of diffraction peaks by using the Debye Scherrer equation (D = λ/(βCosθ)) [31], which is in good agreement with the result of the TEM image.

The morphology of the selenium metal complexes I-V have been referred by the scanning electron micrography. Figure 5 describes the SEM photographs of the synthesized Se(IV) mixed ligand complexes. It is to note that there is a homogeneous phase and a uniform matrix of the synthesized complexes in the photographs. A parallel plate package, package of parallel wide plates, group of snowballs, slices and snow block like shapes are observed in case of the Se(IV) complexes I-V respectively. The well-defined crystalline homogeneous nature of the metal complexes is observed from the XRD and SEM analysis. The TEM image of Se(IV) complexes I-V have been illustrated in Figure 6. TEM images show the complexes II and V have black spherical shape with average size of 15 - 60 nm.

Figure 4.
XRD pattern of selenium(IV) complexes I-V

Figure 5.
SEM images of selenium(IV) complexes I-V
Biological results
In this study, a screening test for the antibacterial property for five selenium(IV) complexes \([\text{Se}(\text{caf})(L)(\text{Cl})_3]\) (where \(L = L\)-val, \(L\)-pro and dap \(\text{III}\)) and \([\text{Se}(\text{caf})(L')(\text{Cl})_3]\)Cl (where \(L' = \text{bpy IV}\) and \(\text{bphen V}\)) was done using disc diffusion method on four selected bacteria species. The results showed four complexes out of five have slightly lower antibacterial activity only on Klebsiella bacteria. The inhibition zones were 0.2, 0.1, 0.1 and 0.1 mm for \(\text{I, II, III and V}\) complexes respectively, as compared with the inhibition zone of gentamicin which was 0.3 mm. Complex \([\text{Se}(\text{caf})(\text{bpy})(\text{Cl})_3]\)Cl showed no antibacterial activity against all tested bacteria. All the five complexes in this study were evaluated for their cytotoxic effect against colorectal adenocarcinoma cell line (Caco-2) and breast cancer cell line (Mcf-7). The anticancer potency was determined by IC\(_{50}\) values using the neutral red assay. The negative control was represented by untreated cells with media only and the positive standard control was doxorubicin HCl (8 \(\mu\)g/mL). As shown in Table V, complexes \(\text{I, II and IV}\) showed moderate cytotoxic activity against Caco-2 cell line only with IC\(_{50}\) values of 79.6, 65 and 79 \(\mu\)g/mL respectively. However, No cytotoxicity was seen in all tested complexes against breast cancer cell line (Mcf-7). The viability of the cells indicated by IC\(_{50}\) values were more the 100 \(\mu\)g/mL.

![Figure 6. TEM images of selenium(IV) complexes A: III and B: V](image)

Table V

| Complexes | IC\(_{50}\) (\(\mu\)g/mL) |
|-----------|-------------------------|
|           | (CaCo-2) | (MCF-7) |
| I         | 79.6 | > 100 |
| II        | 65  | > 100 |
| III       | -   | -     |
| IV        | 79  | > 100 |
| V         | -   | > 100 |

IC\(_{50}\) values are the average of 4 replicates

Conclusions
This paper includes synthesis and characterization of mixed ligand complexes derived from caf as a primary ligand and (\(L\)-val, \(L\)-pro, dap, bpy and \(bphen\)) as a secondary ligands using selenium(IV) metal ions. The synthesized complexes have been characterized with the help of molar conductance, magnetic susceptibility measurements, thermogravimetric analysis and spectral techniques such as IR, \(^1\)H-NMR and electronic spectra. The synthesized complexes are also screened for their antibacterial and cytotoxic activities against colorectal adenocarcinoma and breast cell lines activities. The studies made on these complexes proposed a six-coordinated octahedral geometry for all these complexes.

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Conflict of interest
The authors declare no conflict of interest.

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