Ru(III) Complexes and Their Ligands Derived from Salicylaldehyde and Halogenated Anilines: Synthesis, Characterisation, and Antioxidant Activity

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Abstract
This work aimed to describe the synthesis and characterisation of two anionic Ru(III) complexes of the general formula Na[RuCl₂(N-4-Cl-Ph-salim)] and Na[RuCl₃(N-3-Br-Ph-salim)], their associated ligands, and determine their antioxidant activity. The ligands N-4-Cl-phenylsalicylideneimine (N-4-Cl-Ph-salimH, HL₁) and N-3-Br-phenylsalicylideneimine (N-3-Br-Ph-salimH, HL₂), Schiff bases, were synthesised from salicylaldehyde and chloroaniline or bromoaniline. The compounds were characterised using IR spectroscopy and ESI ToF mass spectrometry. The following was confirmed: coordination of ligands on the Ru(III) centre, the molecular formulas, and the corresponding M⁻ ions: [C₆H₅N₃O₂Cl₄Ru]⁻ ion, (m/z: 631.9173) and [C₆H₇N₃O₂Cl₄BrRu]⁻ ion, (m/z: 719.8283). The antioxidant activity was determined by the ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. In contrast to the ligands, both complexes proved to be strong scavengers of the ABTS and DPPH radicals with IC₅₀ (half maximal inhibitory concentration) values comparable to those of Trolox. As such, they present valuable candidates for further research related to their biological properties.

Keywords
Ru(III) complexes, Schiff bases, IR spectroscopy, ESI ToF mass spectrometry, antioxidant activity

1 Introduction
Over the last decades, transition metal complexes have gained much attention as attractive candidates in drug development. Among the transition-metal complexes, ruthenium represents a superior choice in biological applications considering its specific biological properties and advantages. The safety profile of ruthenium complexes has been extensively researched leading to the conclusion that these agents are generally characterised by low in vivo toxicity, which is a precondition for medical use. Ruthenium binds easily to biomolecules as it mimics iron in its mode of action, the metal ion is generally hexacoordinated, and its different oxidative states facilitate redox reactions and DNA binding. In addition, the reactivity of Ru complexes can be fine-tuned by using labile or kinetically inert ligands.

Until today, a wide range of different ligands has been chelated to ruthenium, such as Schiff bases, hydrazones, thiosemicarbazones, succinimidato, and phthalimidato ligands, and even flavonoids, as the pharmacological properties of metal complexes depend on both the metal ion and the ligand. Schiff bases are widely used due to their thermal and chemical stability, and their simple synthesis. Alone, or bound to metal ions, Schiff bases are used in many branches of chemistry, including inorganic, organic, polymer chemistry, industrial chemistry, but also in material science and pharmacological studies. The quest for novel pharmacological agents with advanced properties is a never-ending process, where Schiff bases and their metal complexes could play a significant role as they have been reported to exhibit important biological activities, such as antiproliferative, antimicrobial, anti-inflammatory, antioxidant, and cytotoxic activities. Oxidative stress, caused by excessive free radicals and the redox imbalance, is implicated in the majority of diseases. New antioxidant compounds capable of protecting cells and tissues from oxidative stress-induced damage are receiving much attention since they are often multi-modal, exhibiting anticancer, anti-inflammatory, and other biological activities. Recently, several in vitro studies have investigated ruthenium complexes containing diverse ligands for their radical scavenging activity, and compared to a range of standard antioxidant substances, such as ascorbic acid, alpha-tocopherol or butylated hydroxytoluene. A few in vivo studies have also been performed, reporting excellent antioxidant properties of ruthenium complexes that reduced intracellular levels of reactive oxygen species. Mechanistic studies have shown that Ru(III) complexes are more inert in physiological conditions but are, nevertheless, readily reduced to Ru(II) in the acidic, glutathione-rich environment of cancerous cells, thus making water-soluble Ru(III) complexes the preferred targets for the synthesis of bioactive metallo-drugs. Ionic, water-soluble Ru(III) complexes with strategically placed chloride ligands as labile coordination sites are of particular interest for anticancer
research.\textsuperscript{15} These complex species are exploiting the well-known fact that the hydrolysis of Ru-Cl bonds, usually after Ru(III) reduction in the cellular medium, is a major activation pathway for their subsequent cytotoxicity.\textsuperscript{16}

Therefore, this study aimed to describe the synthesis and characterisation of two Ru(III) complexes, containing the anionic [RuCl\(_6\)(k\(^2\)-N,0-L\(^{18}\))]\(^+\) species, with salicylaldehyde-derived Schiff base ligands, and determine their radical scavenging ability against the DPPH and ABTS free radicals.

2 Experimental

2.1 Chemicals

All reagents used in this study were of analytical grade, obtained from commercial sources, and used as received without further purification. All solvents were distilled prior to use.

2.2 Methods

Infrared spectra were recorded on a Perkin Elmer spectrum BX FTIR System in the range of 4000–400 cm\(^{-1}\) as KBr pellets. Mass spectra of the synthesised complexes were recorded on a 6210 Time-of-Flight LC/MS system (Agilent Technologies, Santa Clara, California, USA) equipped with an ESI interface (ESI–ToF–MS). Mass spectra were recorded in the range 100–2000 m/z in negative ESI ionisation mode (capillary voltage 4000 V, fragmentor voltage 140 V, skimmer voltage 60 V, OCT RF voltage 250 V); atomising gas (nitrogen): pressure 45 min, flows 15 µl, injection volume of the samples dissolved in acetonitrile (719.8156). UV/Vis (DCM) \([\nu(C–Cl)]\), UV/Vis (DCM) \(\lambda_{\text{max}}/\text{nm} (\log e)\): 272 (4.15), 320 (4.12), 343 (4.15).

N-3-Br-phenylsalicylidenimine(HL\(^b\)): orange crystalline solid; yield (87%). M. p. 106 °C. FT-IR (KBr, cm\(^{-1}\)): 3010 w [\(\nu(O–H)\)], 1616 s [\(\nu(C=N)\)], 1589 m [\(\nu(C–C)_{\text{arom.}}\)], 1532 s [\(\delta(C–O–H) + (C=N) + (H–C=N)\)], 1457 s [\(\nu(C=N)\)] 1279 m [\(\nu(C=O)\)], 753 s [\(\delta(C–H)_{\text{arom.}}\)], 650 m [\(\nu(C–Br)\)]. UV/Vis (DCM) \(\lambda_{\text{max}}/\text{nm} (\log e)\): 272 (4.22), 319 (4.09), 342 (4.13).

2.2.2 Synthesis of complexes

A warm ethanolic solution (40 ml) of the appropriate Schiff base ligand HL\(^a\) or HL\(^b\) (1 mmol; 232 mg or 276 mg, respectively) was added to a freshly prepared solution of RuCl\(_2\)·3 H\(_2\)O (0.5 mmol, 131 mg) in 10 ml of absolute ethanol. The resulting dark green solution was stirred magnetically and heated in a water bath maintained at 70 °C for 3 h. The reaction mixture was allowed to cool to room temperature, and then treated with an equimolar amount of a saturated, aqueous NaCl solution (0.5 mmol, 58 mg in 170 µl deionised water). After 24 h at room temperature, the mixture was cooled in an ice bath for 3 h. The fine precipitated solids were vacuum filtered, washed with deionised water, ethanol, and finally three 10 ml portions of ether. The crude products were recrystallised from dichloromethane/ethanol DCM/EtOH (1:1), filtered, and dried in a vacuum desiccator overnight.

Na[RuCl\(_6\)(l\(^a\))]: black solid; yield (37%). M. p. > 200 °C. FT-IR (KBr, cm\(^{-1}\)): 1602 s [\(\nu(C=N)\)], 1584 m [\(\nu(C–C)_{\text{arom.}}\)], 1532 s [\(\delta(C–N) + (H–C=N)\)], 1434 m [\(\nu(C–N)\)] 1294 m [\(\delta(C–O)\)], 760 s [\(\delta(C–H)_{\text{arom.}}\)], 700 m [\(\nu(C=Cl)\)], 531 m [\(\nu(C–Ru)\)], 432 m [\(\nu(C–O)\)]; ESI-ToF MS for \([C\(_9\)H\(_6\)N\(_2\)O\(_2\)Cl\(_2\)Ru\]^+\) Found (Calc.): 631.9173 (631.9166). UV/Vis (DCM) \(\lambda_{\text{max}}/\text{nm} (\log e)\): 252 (4.33), 322 (3.98), 622 (3.85).

Na[RuCl\(_6\)(l\(^b\))]: black solid; yield (21%). M. p. > 200 °C. FT-IR (KBr, cm\(^{-1}\)): 1603 s [\(\nu(C=N)\)], 1585 s [\(\nu(C–C)_{\text{arom.}}\)], 1529 s [\(\delta(C–N) + (H–C=N)\)], 1437 m [\(\nu(C–N)\)] 1300 m [\(\delta(C–O)\)], 762 s [\(\delta(C–H)_{\text{arom.}}\)], 646 m [\(\delta(C–Br)\)], 537 m [\(\nu(C–Ru)\)], 422 m [\(\nu(C–O)\)]; ESI-ToF MS for \([C\(_9\)H\(_6\)N\(_2\)O\(_2\)Cl\(_2\)Br\(_2\)Ru\]^+\) Found (Calc.): 719.8283 (719.8156). UV/Vis (DCM) \(\lambda_{\text{max}}/\text{nm} (\log e)\): 252 (4.33), 319 (3.96), 549 (3.76).

2.2.3 ABTS and DPPH assay

For both assays, samples were prepared in various concentrations, dissolved in dichloromethane, and then in methanol. The ABTS assay was carried out using the method by Re et al.\textsuperscript{17} Briefly, ABTS\(^+\) reagent (1200 µl) was mixed with sample or negative control (30 µl) and the absorbance at 734 nm was measured 6 min after the initial mixing, using 95 % ethanol as the blank.

The DPPH assay was performed following the method described by Blois.\textsuperscript{18} 1000 µl of DPPH solution (0.05 mmol L\(^{-1}\) in methanol) were added to 100 µl of sample solution or negative control. The reaction mixture was allowed to stand for 30 min at room temperature in the dark, and
the absorbance was measured at 517 nm, using methanol as the blank.

For both assays, the radical scavenging ability was expressed as IC$_{50}$ (μg ml$^{-1}$ and mg ml$^{-1}$) and calculated using the Eq. (1).

\[
\text{scavenging activity (\%)} = \left[ \frac{(A_0 - A_1)}{A_0} \right] \times 100
\]

where A0 is the absorbance of the control, and A1 is the absorbance of the sample/standard solution. Trolox, with a final concentration range of 10–80 μg ml$^{-1}$, was prepared as a standard.

3 Results and discussion

The ligands HL$^a$ and HL$^b$, imines of halogenated anilines and salicylaldehyde were prepared by the usual procedure for the synthesis of such Schiff bases (Fig. 1) via acid-catalysed condensation of salicylaldehyde with an equimolar amount of the appropriate aniline in ethanol.$^{19,20}$

The complexes, proposed as sodium dichlorido-bis[(N-(3-bromophenyl)-2-oxy-κO-benzilidenimine-κN-(1-)]ruthenate(III) and sodium dichlorido-bis[(N-(4-chlorophenyl)-2-oxy-κO-benziliden-imine-κN-(1-)]ruthenate(III), abbreviated as Na[RuCl$_2$(L$^a$)$_2$] and Na[RuCl$_2$(L$^b$)$_2$], respectively, were synthesised according to literature with slight modifications.$^{21}$ The starting RuCl$_3$·3H$_2$O and the corresponding Schiff base ligand were reacted in a 1 : 2 molar ratio in absolute ethanol, and the resulting anionic complexes precipitated as their sodium salts by the addition of aqueous NaCl solution (Fig. 2).

ESI-ToF mass spectrometry confirmed the formulated complexes by detection of the corresponding [C$_{26}$H$_{18}$N$_2$O$_2$Cl$_2$Ru]$^-$ and [C$_{26}$H$_{18}$N$_2$O$_2$Cl$_2$Br$_2$Ru]$^-$ ions with the observed peaks at 631.9173 m/z and 719.8283 m/z, respectively (Fig. 3, Table 1). Both spectra showed the authentic isotopic pattern characteristic of stable ruthenium isotopes.$^{22}$

The infrared spectra of the synthesised complexes showed a high degree of similarity, suggesting an identical structure. Both Schiff base ligands functioned as O- and N-donating phenolates, indicated by the band shifts in the phenolic and azomethine groups of the complexes as opposed to the free ligands (Figs. 4 and 5).

Coordination via the azomethine nitrogen was confirmed by a shift of the C=N stretching vibration to lower wavenumbers for 8–13 cm$^{-1}$ in the spectra of the prepared complexes (1602–1603 cm$^{-1}$) compared to the spectra of the free ligands (1610–1613 cm$^{-1}$). Both free ligands showed a prominent band at around 1568 cm$^{-1}$, which
Fig. 3 – Mass spectrum of Na[RuCl₂(Lₐ)₂] (top), and Na[RuCl₂(Lₐ)₂] (bottom)
Slika 3 – Maseni spektri Na[RuCl₂(Lₐ)₂] (gore) i Na[RuCl₂(Lₐ)₂] (dolje)

Table 1 – ESI ToF MS data for molecular ions of the complex compounds
Tablica 1 – ESI ToF MS podatci za molekularne ione kompleksnih spojeva

<table>
<thead>
<tr>
<th>Complex compounds</th>
<th>Molecular ion</th>
<th>Ion mass / m/z</th>
<th>Measured mass / m/z</th>
<th>Error / ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na[RuCl₂(Lₐ)₂]</td>
<td>[C₂₆H₁₈N₂O₂Cl₂Ru]⁻</td>
<td>631.9166</td>
<td>631.9173</td>
<td>1.11</td>
</tr>
<tr>
<td>Na[RuCl₂(Lₐ)₂]</td>
<td>[C₂₆H₁₈N₂O₂Cl₂Br₂Ru]⁻</td>
<td>719.8156</td>
<td>719.8283</td>
<td>17.63</td>
</tr>
</tbody>
</table>
Turbeville and Dutta (1990) assigned to a mode centred on the conjugated HOCCCN region due to its high sensitivity to isotopic effect (both deuteration and $^{15}$N substitution). This band disappeared from the spectra of the ruthenium complexes and was replaced by a less intense band at around 1530 cm$^{-1}$ owing to the coordination of the azomethine nitrogen, as well as the deprotonation and subsequent coordination of the phenolic oxygen.

This conclusion is furthermore indicated based on the shift of the phenolic $\nu$(C−O) vibration to higher wavenumbers for ≈ 21 cm$^{-1}$ in the spectra of the complexes (1272–1279 cm$^{-1}$) versus the spectra of free the ligands (1294–1300 cm$^{-1}$), and a regular decrease in band intensities following this increase in band wavenumbers. Additionally, the aniline $\nu$(C−N) vibration of the free ligands found at around 1457 cm$^{-1}$ decreased in intensity and shifted to lower wavenumbers for 20–24 cm$^{-1}$ in the spectra of the complexes (1434–1437 cm$^{-1}$). The mentioned observations are consistent with substituted salicylidene anilines figuring as phenolate ligands.

Bands ascribed to stretching vibrations of the aromatic skeleton at around 1585 cm$^{-1}$, and the out-of-plane C−H bending vibration of ortho-substituted benzene rings at around 755 cm$^{-1}$, as well as the carbon halogen stretching vibrations (650 and 698 cm$^{-1}$ for C−Br and C−Cl, respectively), remained almost identical between the spectra of the ligands and complexes. The infrared spectra of the complexes displayed increased complexity in the region below 600 cm$^{-1}$, most likely caused by new ruthenium-heteroatom bonds. The most interesting, new bands in both the complexes appeared at 531–537 cm$^{-1}$ and 422–432 cm$^{-1}$, which may have arisen due to Ru−N and Ru−O stretching vibrations, respectively.

$^{25}$
The electronic absorption spectra of the ligands and complexes were taken in DCM in the spectral range of 240–800 nm. Ligand-centred transitions ranged from 250 to 400 nm, and are attributable to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the aromatic ring and the double bond of the azomethine group, the latter being affected by coordination to ruthenium. Broad bands of low-intensity peaking at 600 nm arose from spin-allowed d–d transitions of the t$_{2g}^5$ Ru(III) centre and can be denoted as ($^3t_{2g} \rightarrow ^3A_{2g}$). The obtained IC$_{50}$ values revealed a high radical scavenging ability of both complexes, which closely resembled the antioxidant activity of Trolox. This is particularly well illustrated by the IC$_{50}$ value of Na[RuCl$_2$(L$_2$)$_2$] (131.00 $\mu$g ml$^{-1}$) in comparison to the standard antioxidant (124.35 $\mu$g ml$^{-1}$) in the ABTS assay. The ligands were found to be less effective antioxidants with IC$_{50}$ values in the mg ml$^{-1}$ range. According to the ABTS assay, the IC$_{50}$ values ranged between 5.67 mg ml$^{-1}$ (HL$^a$) and 7.24 mg ml$^{-1}$ (HL$^b$). The IC$_{50}$ values in the DPPH assay were experimentally found to be above 8 mg ml$^{-1}$.

The results presented in Fig. 6 clearly suggest a strong antioxidant activity of the complexes and a low antioxidant activity of the ligands. Similar results were described by Soh et al. who found the arene Ru(II) complexes to be more effective in the neutralisation of free radicals than their free ligands or even positive controls as in the case of azo-containing Schiff base Ru(II) complexes. The better scavenging activity of the complexes may be due to chelation of the metal ions with the ligands; however, this cannot be considered a general rule. The biological activity of metal complexes depends on a number of factors, including the nature of the metal ion and its oxidation state, the type and number of chelated ligands and isomers present.

In conclusion, the finding of this study is in line with several other studies of ruthenium complexes with different Schiff bases suggesting their strong antioxidant activities. The synthesised complexes could be valuable candidates in future in vitro and in vivo research that would extend to their antimicrobial, antiproliferative, and other biological activities.

4 Conclusion

In the present study, we describe the synthesis of two Ru(III) complexes with Schiff base ligands derived from substituted anilines and salicylaldehyde. The synthesised complexes were characterised by FT-IR spectroscopy, UV/Vis spectrometry, and ESI ToF mass spectrometry, based on which we propose their structures to be sodiated ruthenate(III) complexes. The antioxidant activity was determined using the ABTS and DPPH assays and expressed as IC$_{50}$ values in the mg ml$^{-1}$.

In the present study, we describe the synthesis of two Ru(III) complexes and Their Ligands Derived from Salicylaldehyde, their antimicrobial, antiproliferative, and other biological activities. The synthesised complexes were characterised by FT-IR spectroscopy, UV/Vis spectrometry, and ESI ToF mass spectrometry, based on which we propose their structures to be sodiated ruthenate(III) complexes. The antioxidant activity was determined using the ABTS and DPPH assays and expressed as IC$_{50}$ values in the mg ml$^{-1}$.

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In conclusion, the finding of this study is in line with several other studies of ruthenium complexes with different Schiff bases suggesting their strong antioxidant activities. The synthesised complexes could be valuable candidates in future in vitro and in vivo research that would extend to their antimicrobial, antiproliferative, and other biological activities.
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Popis kratica
List of abbreviations

ABTS – 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
2,2’-azino-bis(3-ethylbenzothiazoline)-5-sulfonic acid
kiselnina
DPPH – 1,1-diphenyl-2-picrylhydrazyl
1,1-diphenyl-2-piridilhidrazil
HL\textsuperscript{a} – N-4-Cl-phenylsalicylidenedimine
N-4-Cl-phenylsalicylidenedimimine
HL\textsuperscript{b} – N-3-Br-phenylsalicylidenedimine
N-3-Br-phenilsalicylidenedimimine
Na[RuCl\textsubscript{3}](L\textsubscript{2})\textsuperscript{2+} – sodium bis[N-4-Cl-phenylsalicylideneiminato-N,O]dichlororuthenate(II)
natrij bis[N-4-Cl-phenylsalicylidenedimimato-N,O]diklororutenat(II)
Na[RuCl\textsubscript{3}](L\textsubscript{3})\textsuperscript{2+} – sodium bis[N-3-Br-phenylsalicylideneiminato-N,O]dichlororuthenate(II)
natrij bis[N-3-Br-phenilsalicylidenedimimato-N,O]diklororutenat(II)
DCM – dichloromethane
dikloromethan
EtOH – ethanol
diklorometan
e thanol
IR – infrared
infra crveno
ESI ToF – electrospray ionisation time-of-flight
elektrosprej ionizacija vrijeme leta
MS – mass spectrometry
spektrometrija masa
FT-IR – Fourier-transform infrared spectroscopy
infra crvena spektroskopija s Fourierovom transformacijom
IC\textsubscript{50} – half maximal inhibitory concentration
koncentracija koja postiže 50 % inhibicije

References
Literatura

SAŽETAK

Ru(III) kompleksi i njihovi ligandi izvedeni iz salicilaldehida i halogeniranih anilina: sinteza, karakterizacija i antioksidativno djelovanje

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Ovaj rad je imao cilj opisati sintezu i karakterizaciju dvaju anionskih Ru(III) kompleksa općenito opšte formule Na[RuCl4(N-(4-Cl-PH-salim))2] i Na[RuCl4(N-(3-Br-PH-salim))2], njihove pridružene ligande i određene njihovu antioksidacijsku aktivnost. Ligandi N-4-Cl-fenilsalicilididen (N-4-Cl-PH-salimH2), N-3-Br-fenilsalicilididen (N-3-Br-PH-salimH2), Schiffove baze, sintetizirani su iz salicilaldehida i kloranilina ili bromanilina. Spojevi su karakterizirani primjenom IR spektroskopije i ESI ToF spektrometrije masa. Potvrđena je koordinacija liganada na Ru(III) centru, molekulске formule i odgovarajućih M⁺ ionima: [C6H4ClNO2Ru]⁺ ion (m/z: 631.9173) i [C6H4BrNO2ClBrRu]⁺ ion (m/z: 719.8283). Antioksidacijska aktivnost određena je metodama ABTS (2,2’-azino-bis(3-ethylbenzotiazolin-6-sulfonska kiselina) i DPPH (1,1-difenil-2-pikrilhidrazil). Za razliku od liganda, oba kompleksa pokazala su se jakim hvatačima ABTS i DPPH radikala s IC50 vrijednostima (koncentracija koja postiže 50% inhibicije) usporedbom s onima od Troloxa. Kao takvi, vrijedni su kandidati za daljnja istraživanja vezana uz njihova biološka svojstva.

Ključne riječi

Ru(III) kompleksi, Schiffove baze, IR spektroskopija, ESI ToF spektrometrija masa, antioksidativno djelovanje

Izvorni znanstveni rad