

## **Arene ruthenium (II) complexes with 2-acetamidothiazole derived ligands: Synthesis, structural studies, Antifouling and Antibacterial properties**

Keisham S. Singh,<sup>a,\*</sup> Prabha Devi,<sup>a</sup> Sneha G. Sawant,<sup>a</sup> Werner Kaminsky<sup>b</sup>

<sup>a</sup>Bioorganic Chemistry Laboratory, CSIR-National Institute of Oceanography, Goa-403004, India

<sup>b</sup>Department of Chemistry, University of Washington, Seattle, Washington-98195, USA

\* Corresponding author; Tel: +91 0832 2450392; Fax No.: +91 0832 2450607

E-mail: [keisham@nio.org](mailto:keisham@nio.org) or [sarjitkeisham@yahoo.co.in](mailto:sarjitkeisham@yahoo.co.in)

### **Abstract:**

Four related arene ruthenium(II) complexes with antifouling properties of general formula  $[(\eta^6\text{-arene})\text{Ru}(\text{L})\text{Cl}_2]$  have been prepared by the reaction of  $[(\eta^6\text{-arene})\text{RuCl}_2]_2$  with 2-acetamidothiazole derived ligands at room temperature ( $\eta^6\text{-arene} = p\text{-cymene}$ ,  $\text{C}_6\text{Me}_6$ ; and  $\text{L} = 2\text{-acetamidothiazole}$  or  $5\text{-chloro-2-acetamidothiazole}$ ) and were characterized by FTIR,  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and mass spectral analysis. Antifouling properties of these ruthenium(II) complexes have been investigated against marine fouling bacteria and antibacterial properties against clinical pathogens. The complexes showed moderate to good antifouling as well as antibacterial activities. The molecular structure of a representative compound has been established by single crystal X-ray diffraction.

**Keywords:** Marine fouling, Ruthenium(II) complexes, Antifoulants, acetamidothiazole, spectroscopic data, Crystal structure.

### **1. Introduction**

Marine fouling is a natural phenomenon occurring continuously in the marine environment affecting submerged surfaces such as ship hull and sensors. This undesirable build-up of micro- and macroorganisms onto the water structures increases in surface roughness, friction drag and subsequently elevated fuel consumption and reduced maneuverability which ultimately causes loss. Counter measures against this undesired fouling phenomenon included organometallic paint systems comprising of lead, arsenic, and mercury [1-4]. In the early 1960s, tributyltin (TBT) antifoulant a self-polishing coating, based on acrylic and methacrylic was developed which had a broad spectrum of toxicity against most fouling biota and proved to be very effective [5,6]. Subsequently, TBT became a primary antifouling agent used worldwide, however, with the discovery of toxic effects of organotin compounds in marine life in the 1990s, organotin containing

coatings became questionable. Specifically, organotin compounds were found to be responsible for severe shellfish deformity and the bioaccumulation of tin in some ducks, seals and fish [7,8]. This negative impact of TBT on non-targeted organisms prompted the International Marine Organization (IMO) to call for a ban on the application of TBT based antifouling paints in 2003 and later mandatory ban its presence in such paints on the surface of ships from 2008 [9,10].

After TBT was banned, the development of an alternative antifouling paint has been a subject of considerable interest over the past years [11-15]. Several compounds for biocide free antifouling paints [16], including NPA's (Natural product Antifouling) [17-21] and other metal based complexes [22, 23] have been explored as alternatives. Although NPA were claimed to be eco-friendly [17, 24-29], there are several disadvantages, *viz.* supply issue, challenges in purification and characterization of those products responsible for antifouling activity. Until a convenient synthesis protocol is developed to address its supply and ready access, NPA has limited scope for development on vast scale use as antifoulants in near future. In contrast, a relatively simple and straight forward synthetic methods available for organometallic complexes attracted a lot of interest for development as antifoulants. Among the several metal compounds, copper containing antifouling agents have been considered as an alternative to tin based antifouling paint. However, a drawback of copper compounds is that copper is not completely efficient as a biocide on its own, since some of the common marine algae are tolerant to copper. Currently, two metal based antifoulants *viz.*, zinc and copper pyrithione are considered as the best alternative to TBT but little data are available so far about their environmental impact [30]. Thus, there is still scope for the development of other eco-friendly metal based antifouling agents. Noteworthy, some organometallic, ( $\eta^6$ -arene) ruthenium(II) complexes possess antiviral, antimicrobial, anticancer and cytotoxicity properties [31-38]. Well-known biological significant ( $\eta^6$ -arene) ruthenium(II) complexes reported are  $[(\eta^6\text{-arene})\text{Ru}(\text{PTA})\text{Cl}_2]$  by Dyson *et al.* [39] and  $[(\eta^6\text{-arene})\text{Ru}(\text{en})\text{Cl}]^+$  by Sadler *et al.* [34] which showed remarkable anticancer activities. Although ( $\eta^6$ -arene) ruthenium(II) complexes have been studied for various biological properties, to the best of our knowledge, the antifouling potential of organoruthenium(II) complexes have not been explored. As a part of our continuous effort on the study of ( $\eta^6$ -arene) ruthenium(II) complexes [40-42], we wish herein, to report the synthesis of ( $\eta^6$ -arene) ruthenium(II) complexes containing 2-acetamidothiazole derived ligands and for the first time a study of their antifouling properties. In addition, spectroscopic characterization of complexes (**1-4**) and the single crystal structure of a representative complex **2** are also described.

## 2. Experimental

2.1. *General Remarks:* All solvent used were of analytical grade and distilled prior to use.  $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$  (Aurora Matthey Limited), 2-acetamidothiazole and 5-chloro-2-acetamidothiazole (Sigma Aldrich) were used as received. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer at 300.13 ( $^1\text{H}$ ), 75.47 MHz ( $^{13}\text{C}$ ) with  $\text{SiMe}_4$  as internal reference and coupling constants are given in Hertz. Infrared spectra were recorded in a diffused reflection spectroscopy (DRS) assembly on a Shimadzu-8201PC spectrometer with sample prepared in KBr. The precursor complexes  $[\{\eta^6\text{-}p\text{-cymene}\}\text{RuCl}_2\}_2]$  and  $[\{\eta^6\text{-C}_6\text{Me}_6\}\text{RuCl}_2\}_2]$  were prepared according to published procedures [43-45].

### 2.2. Synthesis of Ruthenium(II) acetamidothiazole complexes

#### 2.2.1. Preparation of $[\text{RuCl}_2(\text{L})(p\text{-cymene})]$ $\{L = L_1 = 2\text{-acetamidothiazole (1)}; L = L_2 = 5\text{-chloro-2-acetamidothiazole(2)}\}$

A round bottom flask was charged with  $[\text{RuCl}_2(p\text{-cymene})]_2$  (0.05 g, 0.815 mM), thiazole ligand (0.171 mM), and methanol (20 mL). The reaction mixture was stirred at room temperature for 5 h. During this time the red solution turned into an orange cloudy suspension and orange solid appeared. The solid was collected and washed with diethyl ether (2x10 mL).

Yield and spectroscopic data are as follows:

Complex **1**. Yield: 0.055g (75 %); FTIR (KBr,  $\text{cm}^{-1}$ ): 3134, 1703, 1536, 1504;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 12.22 (s, 1H), 7.76 (d, 1H,  $J = 3.9$ ), 6.99 (d, 1H,  $J = 3.9$ ), 5.52 (d, 2H,  $J = 6$ ), 5.30 (d, 2H,  $J = 5.7$ ), 3.03 (sept, 1H), 2.32 (s, 3H), 2.10 (s, 3H), 1.33 (d, 6H,  $J = 7.2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 168.35, 161.75, 141.43, 112.37, 103.96, 97.37, 82.44, 81.86, 30.68, 23.25, 22.20, 18.37; ESI-MS (MeOH):  $m/z$  413  $[\mathbf{1}\text{-Cl}]^+$ ; 377  $[\mathbf{1}\text{-2Cl}]^+$ .

Complex **2**. Yield: 0.053g (67%); FTIR (KBr,  $\text{cm}^{-1}$ ): 3138, 1697, 1543, 1521;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ,  $\delta$ ): 12.28 (s, 1H), 7.63 (s, 1H), 5.54 (d, 2H,  $J = 6$ ), 5.32 (d, 2H,  $J = 6$ ), 3.00 (m, 1H), 2.30 (s, 3H), 2.13 (s, 3H), 1.34 (d, 6H,  $J = 6.9$ );  $^{13}\text{C}$  ( $\text{CDCl}_3$ ,  $\delta$ ): 168.54, 160.14, 138.27, 119.68, 104.04, 97.50, 82.41, 81.79, 30.73, 22.99, 22.19, 18.45; ESI-MS (MeOH):  $m/z$  448  $[\mathbf{2}\text{-Cl}]^+$ ; 413  $[\mathbf{2}\text{-2Cl}]^+$ .

2.2.2. Preparation of  $[RuCl_2(L)(\eta^6-C_6Me_6)]$   $\{L = L_1 = 2\text{-acetamidothiazole (3)}; L = L_2 = 5\text{-chloro-}2\text{-acetamidothiazole (4)}\}$

These complexes were prepared following a similar procedure described for complexes **1** using  $[RuCl_2(\eta^6-C_6Me_6)]_2$  instead of  $[RuCl_2(\eta^6-p\text{-cymene})]_2$  and stirring for 5h.

Complex **3**. Yield: 0.060 g (84%); FTIR (KBr,  $cm^{-1}$ ): 3008, 1697, 1550;  $^1H$ NMR ( $CDCl_3$ ,  $\delta$ ): 12.23 (s, 1H), 7.59 (d, 1H,  $J = 3.6$ ), 6.90 (d, 1H,  $J = 3.6$ ), 2.32 (s, 3H), 2.01 (s, 18H);  $^{13}C$  NMR ( $CDCl_3$ ,  $\delta$ ): 168.23, 160.62, 140.88, 112.46, 91.30, 23.18, 15.68; ESI-MS:  $m/z$  441  $[3-Cl]^+$ , 406  $[3-2Cl]^+$ .

Complex **4**. Yield: 0.048g (63%); FTIR (KBr,  $cm^{-1}$ ): 3001, 1697, 1543, 1519;  $^1H$ NMR ( $CDCl_3$ ,  $\delta$ ): 12.30 (s, 1H), 7.45 (s, 1H), 2.31 (s, 3H), 2.03 (s, 18H,  $C_6Me_6$ );  $^{13}C$  NMR ( $CDCl_3$ ,  $\delta$ ): 168.43, 159.02, 137.70, 119.55, 91.36, 22.93, 15.73; ESI-MS:  $m/z$  475  $[4Cl]^+$ , 440  $[4-2Cl]^+$ .

### 2.3. Biological assay for determining antifouling and antibacterial activity

Ruthenium(II) complexes were subjected to biological screening to understand their antifouling properties (using fouling bacteria) and antibacterial activity (using clinical bacterial pathogens). The fouling bacteria used for screening were isolated from various substrates collected from the coastal waters of Goa, while the clinical pathogens were generously provided by Goa Medical College. Antibacterial activities against the tested fouling bacteria and clinical isolates for the compounds **1-4** are shown in **Table 1** and **Table 2**. The strains were isolated and identified using standard methods [46-48]. The assay was carried out by the standard disc diffusion method as described earlier [49, 50]. In a typical experiment, ruthenium compounds (**1-4**) to be tested were dissolved in known quantity of methanol and loaded onto sterile paper discs (Whatmann No. 1 GF/C, diameter = 6 mm) at concentrations of 25  $\mu$ g and 100  $\mu$ g per disc for fouling and clinical bacteria, respectively. Selected bacterial culture broth of known concentration ( $\sim 1.2 \times 10^8$  CFU/ml) was spread to form a lawn of individual bacterium on Muller-Hinton agar plates for clinical bacteria and nutrient agar plates for fouling bacteria. Ruthenium(II) complexes (**1-4**) on respective discs were placed aseptically on agar plates and all plates were later incubated at 37°C for 24 hrs. Discs loaded with standard (streptomycin for clinical pathogens and penicillin for fouling bacteria) and methanol were used as positive and negative controls respectively. The zone of growth inhibition formed around each disc was measured in millimeter using a vernier calliper and all the experiments were carried out in triplicate.

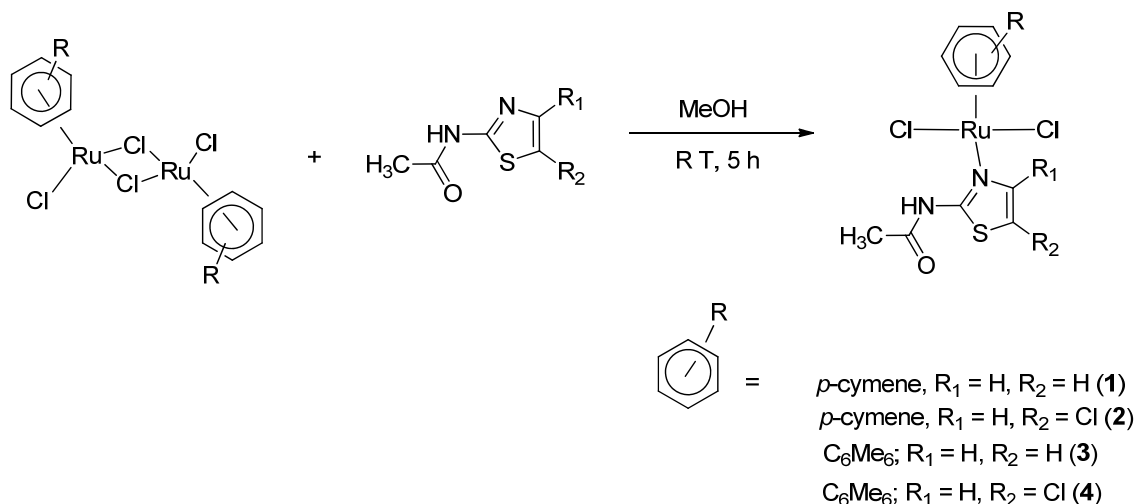
## 2.4. Structure analysis and refinement

X-ray quality crystals of the complex **2** were grown by slow diffusion of hexane into a dichloromethane solution of **2**. The X-ray diffraction data was collected at 100K on a Bruker APEX II single crystal X-ray diffractometer, using MoK $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). Crystal-to-detector distance was 40 mm and exposure time was 10 seconds per frame. Data collection was 100% complete to  $25^\circ$  in  $\theta$ . observed reflections = 59975, 4455 were unique [ $I > 2\sigma(I)$ ] and were used in the final refinement. SHELXS, SIR97 [51] and (SHELXL 97) [52] were used for structure solution and full matrix least squares refinement on  $F^2$ . The weighting scheme used was  $W = 1/[\sigma^2(F^2_o) + 0.0490P^2 + 0.0000P]$  where  $P = (F^2_o + 2F^2_c)/3$ . All non-hydrogen atoms were refined anisotropically while hydrogen atoms were placed in geometrically idealized positions and constrained to ride on their parent atoms with C--H distances in the range 0.95-1.0  $\text{\AA}$ . Refinement converged at a final  $R_1 = 0.0202$  (for observed data  $F$ ), and  $wR_2 = 0.0415$  (for unique data  $F^2$ ). Further details of the data collection and refinement of complex **2** are summarized in **Table 3**.

## 3. Results and discussion

### 3.1. Synthesis and characterization of ( $\eta^6$ -arene) ruthenium(II) complexes

Arene ruthenium(II) complexes containing 2-acetamidothiazole ligands (L) have been prepared by the reaction of precursor complex  $[\text{RuCl}_2(\eta^6\text{-arene})]_2$  with 2 fold excess of ligand in methanol at room temperature (Scheme 1). All these new complexes **1-4** are neutral and adopted piano stool structure. The complexes have been characterized by FTIR, NMR and mass spectral data. However, our attempt to synthesis a chelated and cationic complex of the type  $[\text{RuCl}(\text{L})(\eta^6\text{-arene})]^+$  by reaction the of  $[\text{RuCl}_2(\eta^6\text{-arene})]_2$  with the ligands (L) in the presence of  $\text{NaBF}_4$  or  $\text{NH}_4\text{PF}_6$  also gave the neutral complexes **1-4** was unsuccessful even by increasing reaction time and in refluxing condition instead the reaction also gave the identical neutral complexes **1-4**. The above results indicated that the reaction of  $[\text{RuCl}_2(\eta^6\text{-arene})]_2$  with acetamidothiazole ligands lead to the the formation of neutral complexes, where the ligand coordinated to the ruthenium centre in a mono-dentate fashion but did not give a cationic complex of the type  $[\text{RuCl}(\text{L})(\eta^6\text{-arene})]^+$  in which the ligand coordinated in a bi-dentate fashion. The Spectroscopic data of complexes **1-4** are well matched with the formulation of these complexes. The molecular structure of a representative complex **2** has been established by single crystal X-ray analysis.

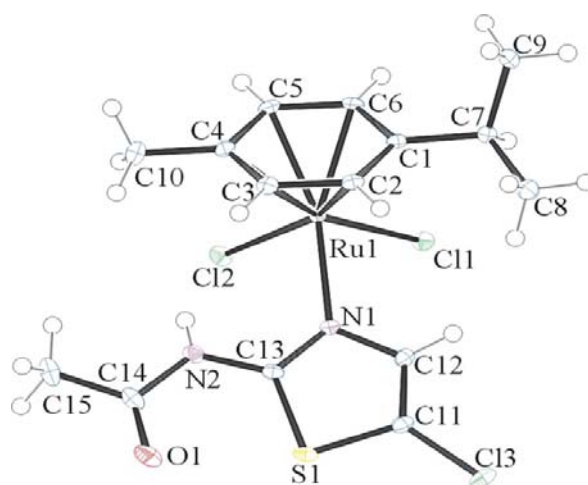


Scheme 1. Reaction pathways for the preparation of Ruthenium(II) Acetamidothiazole complexes

The FTIR spectra of complexes **1-4** showed absorption peaks in the range of 1697-1703  $\text{cm}^{-1}$  corresponding to  $\nu(\text{C}=\text{O})$  of the coordinating 2-acetamidothiazole ligands.  $^1\text{H}$ NMR spectrum of **1** showed a singlet at  $\delta$  12.23 assignable to -NH proton of coordinating ligand whereas the protons of thiazole ring ligand were displayed as doublets at around  $\delta$  7.76 and 6.99, respectively. In addition, the spectrum also showed the characteristic signals for *p*-cymene ring protons (see experimental section). The  $^{13}\text{C}$  NMR spectrum of **1** displayed 12 distinct signals of which the signal for C=O appeared at  $\delta$  168.35 while quaternary carbon of thiazole ring appeared at  $\delta$  161.75. The proton NMR spectral pattern of complex **2** is similar to that of complex **1** and the spectral data accorded the composition of the complex. The  $^1\text{H}$  NMR spectrum of  $\eta^6$ -hexamethylbenzene complexes **3** and **4** displayed signals at  $\delta$  2.01-2.23 region due to proton of  $\eta^6$ -hexamethylbenzene ligand in addition to the signals for the coordinated ligands. Mass spectral analysis of the complexes **1-4** further support the formation of these complexes. For instance, mass spectrum of **1** displayed peak at  $m/z$  449, 413 and 377 corresponding to  $[\text{M}+1]^+$ ,  $[\text{M}-\text{Cl}]^+$  and  $[\text{M}-2\text{Cl}]^+$ , respectively. Mass spectra of  $\eta^6$ -hexamethylbenzene complexes have also shown a similar pattern. Complex **3** displayed peak at  $m/z$  477  $[\text{M}+1]^+$  and 439  $[\text{M}-\text{Cl}]^+$  (see experimental section). Spectroscopic data of these complexes are matched well with the proposed structures.

**3.2. Crystal structure:** In order to confirm the solid state structure of the complexes, crystal structure analysis of a representative complex **2** has been carried out by X-ray diffraction (**Fig. 1**). Details of crystallography data collection are given in **Table 3** and selected bond lengths and angles are presented in **Table 4**. The complex **2** crystallizes in monoclinic space group  $\text{P}2_1/\text{c}$  with an average ruthenium to six membered ring bond distance of 2.1874(16) Å. The complex adopts the familiar

“piano stool” structure as evident by the nearly 90° bond angles for N(1)-Ru(1)-Cl(1) (85.01 (4)°) and N(1)-Ru(1)-Cl(2) (86.85(4)°) [42]. The geometry around the ruthenium atom can be regarded as pseudooctahedral with the *p*-cymene ligands occupying three coordination sites while the remaining coordination sites are occupied by the N-atom of thiazole ligand and by the two chlorine atoms. There is no significant difference in the Ru-Carbon bond distance of six membered *p*-cymene ring carbon indicating planarity of the six member ring. As far as ruthenium and nitrogen bond length is concerned, the Ru-N bond length (2.1506 Å) is close to the reported values of Ru-N bond length [38,40-42]. Further, the pi-bond in the *p*-cymene ring carbon showed considerable delocalisation as evident from the insignificant different of C-C bond distance of *p*-cymene ring.



**Figure 1.** ORTEP diagram of complex **2** showing thermal ellipsoids at the 50% probability level.

**3.3. Biological assay:** Antibacterial properties of ruthenium(II) complexes **1-4** against 10 marine fouling bacteria have been investigated for the first time employing disc diffusion method using *penicilium* as standard and 9 clinical bacterial pathogens using *streptomycin* as standard (**Table 1** & **Table 2**) [47,48]. As evident from **Table 1**, all the ruthenium(II) complexes **1-4** showed antifouling activity against one or more fouling bacteria except for fouling bacteria *Aeromonas hydrophila* ATCC7966 which was resistant to all the ruthenium(II) complexes while *Pseudoalteromonas* sp. was only mildly sensitive to complex **2**. In order to ascertain the role of free ligands, antifouling activities of uncoordinated acetamidothiazole ligands viz. 2-acetamidothiazole (**L**<sub>1</sub>) and 5-chloro-2-acetamidothiazole (**L**<sub>2</sub>) have been evaluated against all the 10 fouling bacteria (**Table 1**). The results showed that both the free ligands **L**<sub>1</sub> and **L**<sub>2</sub> did not show any activities against all the tested fouling bacteria indicating ruthenium(II) complexes **1-4** are the responsible for their antifouling activities. Fouling bacteria *Aeromonas hydrophila* sp. subsp. *Hydrophila* was most sensitive to all these ruthenium(II) complexes showing inhibition zone

between 11-20mm around experimental discs. This was closely followed by *Alcanivorax sp.* which was more active to the ruthenium(II) complexes **1-4**(11-20mm inhibition zone) as compared to the standard penicillin (1-10mm inhibition zone). Screening of these complexes **1-4** against *Erythrobacteria litoralis* showed a weak activity but fairly good activity was observed against *Alivibrio salmonicida* with complexes **2** and **4**. In most of the cases activities of ruthenium(II) complexes bearing *p*-cymene ligand **1** and **2** were comparable to that of ruthenium(II) complexes **3** and **4** bearing hexamethylbenzene ligand. However, difference in the activities was observed in the assay against the marine fouling bacteria *Aeromonas salmonicida* where the complexes **3** and **4** showed moderate activity but no activity was observed with complexes **1** and **2**.

As far as antibacterial activity against the clinical pathogens are concerned, *Staphylococcus aureus* was sensitive to **2**, **3** and **4** while *Shigella flexineri* was sensitive to **3** and **4**, showing growth inhibition zone between 11-20mm (**Table 2**). As observed in antifouling assay, the free ligands 2-acetamidothiazole(L<sub>1</sub>) and 5-chloro-2-acetamidothiazole (L<sub>2</sub>) showed inactive against all the pathogens except a very weak activity observed against *Escheichia Coli*, *staphylococcus aureus* and *Shigella flexineri* (**Table 2**). Compound **1** showed moderate activity (12 mm) against *Klebsiella pneumonia* whereas mild activity was observed for complex **3** and **4**(1-10mm). Similar mild activity was shown by **2** and **4**(1-10mm) against *Escherichia coli* while *Acinetobacter sp.* was mildly sensitive to complex **3**. Some of the clinical pathogenic bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholera* and Methicillin Resistant *Staphylococcus aureus* were totally resistant to any of these four ruthenium (II) complexes.

#### 4. Conclusion

This paper described preparation and characterization of four related arene ruthenium(II) complexes **1-4** of general formula  $[(\eta^6\text{-arene})\text{Ru}(\text{L})\text{Cl}_2]$ , ( $\eta^6\text{-arene}$  = *p*-cymene, C<sub>6</sub>Me<sub>6</sub> and L = 2-acetamidothiazole derive ligands). The spectroscopic characterization by FT-IR, NMR, mass spectral data and crystal structure determination of one representative complex have been presented, confirming the proposed structures. Antifouling and antibacterial properties of these ruthenium(II) complexes were evaluated using ten different marine fouling bacteria and 9 clinical bacterial pathogens. Ruthenium(II) complexes showed antifouling activity against one or more fouling bacteria except for the fouling bacteria *Aeromonas hydrophila* ATCC7966 which was resistant to all the ruthenium(II) complexes. Clinical pathogenic bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae* and Methicillin Resistant *Staphylococcus aureus* were totally resistant to any of the four ruthenium(II) complexes. Screening of activities for free ligand and



ruthenium(II) complexes revealed that ruthenium complexes are responsible for the activities. This result shows that ruthenium(II) complexes have potential for antifouling/antibacterial properties and may serve as an alternative to toxic tributyl tin oxide (TBTO), for exploration as antifouling agents.

### **Supplementary Materials**

Crystallographic data for this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1048636. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

### **Acknowledgements**

This work was supported by the Department of Science and Technology (grant no. SR/FT/CS-001/2010) and the Council of Scientific and Industrial Research. We thank the Director, National Institute of Oceanography for providing necessary facilities.

### **References**

- [1] M. Callow, Chem. Ind. 5 (1990) 123.
- [2] Woods Hole Oceanographic Institution (WHOI), US Naval Institute, Annapolis, Islin, COD (1952).
- [3] I. Lunn, Antifouling: A Brief Introduction to the Origines and Developments of the Marine Antifouling Industry, BCA Publications, Thame, UK (1974) 68.
- [4] M. Callow, Chem. Ind. 5 (1990) 123.
- [5] A. Milne, G. Hails, G. B. Patent, International Paint 457 (1976) 590.
- [6] C. J. Evans, S. Karpel, Journal of Organometallic Chemistry, Vol. 16, Elsevier: Amsterdam, (1985) 135.
- [7] J. Strand, J.A. Jacobsen, Sci. Total Environ. 350 (2005) 72.
- [8] S.M. Evans, T. Leksono, P.D. McKinnell, Mar. Pollut. Bull. 30 (1995) 14.
- [9] M. A. Champ, Mar. Pollut. Bull. 46 (2003) 935.
- [10] S. M. Evans, Biofouling 14 (1999) 117.
- [11] I. Omae, Chem. Rev. 103 (2003) 3431.

- [12] A. I. Railkin, *Marine Biofouling Colonization Processes and Defenses*, CRC Press LLC (2004).
- [13] D. M. Yebra, S. Kiil, K. Dam-Johansen, *Prog. Org. Coat.* 50 (2004) 75.
- [14] STMGT Sea Technology Group, *Warpaint*, Issue 20 (2005).
- [15] B. Watermann, *Alternative Antifouling Techniques: Present and Future*, Report, Limno Marine, Hamburg, Germany (1999).
- [16] B. Watermann, B. Daehne, S. Sievers, R. Dannenberg, J. C. Overbeke, J.W. Klijnstra, O. Heemken, *Chemosphere* 60 (2005) 1530.
- [17] S. Abarzua, S. Jakubowski, *Mar. Ecol. Prog. Ser.* 123 (1995) 301.
- [18] S. Abarzua, S. Jakubowski, S. Eckert, P. Fuchs, *Bot. Mar.* 42 (1999) 459.
- [19] A. S. Clare, *Biofouling* 9 (1996) 211.
- [20] N. Fusetani, *Nat. Prod. Rep.* 21 (2004) 94.
- [21] P.Y. Qian, Y. Xu, N. Fusetani, *Biofouling* 26 (2010) 223.
- [22] P. A. Turley, R. J. Fenn, J. C. Ritter, *Biofouling* 15 (2000) 175.
- [23] I. Omae, *Appl. Organomet. Chem.* 17 (2003) 81.
- [24] M. Wahl, *Mar. Ecol. Prog. Ser.* 58 (1989) 175.
- [25] P. Y. Qian, Y. Xu, N. Fusetani, *Biofouling* 26 (2010) 223-234.
- [26] N. Fusetani, *Nat Prod. Rep.* 21 (2004) 94.
- [27] S. Abarzua, S. Jakubowski, *Mar. Ecol. Prog. Ser.* 123 (1995) 301.
- [28] A. Terlizzi, S. Fraschetti, P. Gianguzza, M. Faimali, F. Boero, *Aquat. Conserv. Mar. Freshw. Ecosyst.* 11 (2001) 311.
- [29] M.A. Champ, *Sci. Total Environ.* 258 (2000) 21.
- [30] S. M. Evans, A.C. Birhenough, M.S. Brancato, *Mar. Pollut. Bull.* 40 (2000) 204.
- [31] C. S. Allardyce, P. J. Dyson, D. J. Ellis, P. A. Salter, R. Scopelliti, *J. Organomet. Chem.* 668 (2003) 35.
- [32] C. Scolaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurency, T. J. Geldbach, Gianni Sava, P. J. Dyson, *J. Med. Chem.* 48(2005)4161.
- [33] W. H. Ang, E. Daldini, C. Scolaro, R. Scopelliti, L. Juillerat-Jeannerat, P. J. Dyson, *Inorg. Chem.* 45 (2006) 9006.
- [34] Y. K. Yan, M. Melchart, A. Habtemariam, P. J. Sadler, *Chem. Commun.* (2005) 4764.

- [35] A. Habtemariam, M. Melchart, R. Fernández, S. Parsons, I. D. H. Oswald, A. Parkin, F. P. A. Fabbiani, J. E. Davidson, A. Dawson, R. E. Aird, D. I. Jodrell, P. J. Sadler, *J. Med. Chem.* 49(2006)6858.
- [36] T. Bugarcic, A. Habtemariam, J. Stepankova, P. Heringova, J. Kasparikova, R. J. Deeth, R. D. L. Johnstone, A. Prescimone, A. Parkin, S. Parsons, V. Brabec, P. J. Sadler, *Inorg. Chem.* 47 (2008) 11470.
- [37] C. A. Vock, A. K. Renfrew, R. Scopelliti, L. Juillerat-Jeanneret, P. J. Dyson, *Eur. J. Inorg. Chem.*(2008) 1661.
- [38] T. Stringer, B. Therrien, D. T. Hendricks, H. Guzgay, G. S. Smith, *Inorg. Chem. Commun.* 14 (2011) 956.
- [39] G. Süss-Fink, *Dalton Trans.* 30 (2010) 1673.
- [40] K. S. Singh, W. Kaminsky, *Polyhedron* 68 (2014) 279.
- [41] K. S. Singh, V. Svitlyk, Y. Mozharivskyj, *Dalton Trans.* 40 (2011) 1020.
- [42] K. S. Singh, W. Kaminsky, *Inorg. Chim. Acta*, 365 (2011) 487.
- [43] M. A. Bennett, T. N. Huang, T. W. Matheson, A. K. Smith, *Inorg. Synth.* 21 (1982) 74.
- [44] M. A. Bennett, A. K. Smith, *J. Chem. Soc. Dalton Trans.* (1974) 233.
- [45] M. A. Bennett, T. N. Huang, T. W. Matheson, A. K. Smith, P. A. Tucker, *Inorg. Chem.* 19 (1980) 1014.
- [46] M. Allegrucci, K. Sauer, *J. Bacteriol.* 189 (2007) 2030.
- [47] H. M. Dalton, L. K. Poulsen, P. Halasz, M. L. Angles, A. E. Goodman and K. C. Marshall, *J. Bacteriol.* 176 (1994) 6900.
- [48] C. Bollet, M. J. Gevaudan, X. Lamballerie, C. Zandotti, P. Micco, *Nucleic Acids Res.* 19 (1955) 4101.
- [49] W. M. M. Kirby, G. M. Yoshihara, K. S. Sundsted, J. H. Warren, *Antibiot. Annu.* (1957) 892.
- [50] Prabha Devi, S. Wahidullah, T. Kamat, L. D. Souza, I., *J. Geo.Mar.Sci.* 40 (2011) 338.
- [51] SIR97: A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Cryst.* 32 (1999) 115.
- [52] G.M. Sheldrick, *SHELXL97*, Program for the Refinement of Crystal Structures, University of Gottingen, Germany (1997).

**Table 1.** Results of antifouling screening showing zones of growth-inhibition values for compounds **1-4** against fouling bacteria

Fouling Bacteria	Inhibition zone diameter in mm for Ruthenium(II) complexes and std.						
	L <sub>1</sub>	L <sub>2</sub>	1	2	3	4	Std.
<i>Alcanivorax sp.</i>	-	-	12	11	11	12	8
<i>Planococcus donghaensis</i>	-	-	12	8	8	12	22
<i>Aeromonas hydrophila</i> ATCC7966	-	-	-	-	-	-	26
<i>Aeromonas hydrophila</i> subsp. <i>Hydrophila</i>	-	-	11	12	14	12	22
<i>Erythrobacter litoralis</i>	-	-	8	6	8	8	12
<i>Pseudomonas mendocina</i>	-	-	6	-	12	6	22
<i>Alcanivorax borkumensis</i>	-	-	4	-	12	-	26
<i>Alivibrio salmonicida</i>	-	-	8	14	8	14	22
<i>Pseudoalteromonas sp.</i>	-	-	-	4	-	-	26
<i>Vibrio furnisii</i>	-	-	8	8	8	10	30

The data are expressed as a measure of the inhibition zones (mm) at concentration 25  $\mu\text{g}/\text{disc}$  for all the compounds and standard (penicillin). Data given are the mean of three replicates. (-) Inactive, 1-10 mm, mild activity; 11-20 mm, moderate activity; 21-30 mm, strong activity.

**Table 2.** Results of antibacterial screening of ruthenium (II) complexes **1-4** against clinical pathogens.

<i>Clinical pathogens</i>	Inhibition diameter in mm for Ruthenium(II) complexes and std.						
	<b>L<sub>1</sub></b>	<b>L<sub>2</sub></b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>Std.</b>
<i>Escherichia coli</i>	2	3	-	8	8	8	18
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	22
<i>Staphylococcus aureus</i>	1	1	-	14	8	14	18
<i>Shigella flexineri</i>	1	1	-	-	12	12	14
<i>Salmonella typhi</i>	-	-	-	-	-	-	26
<i>Vibrio cholerae</i>	-	-	-	-	-	-	26
<i>Klebsiella pneumonia</i>	-	-	12	-	8	8	14
<i>Acinetobacter sp.</i>	-	-	-	-	-	-	14
<i>Methicillin Resistant Staphylococcus aureus</i>	-	-	-	-	-	-	26

The data are expressed as a measure of the inhibition zones (mm) at concentration 100  $\mu\text{g}/\text{disc}$  for **1-4** and 25  $\mu\text{g}$  for standard (streptomycin); Inactive (-); mild activity (1-10); moderate activity (11-20); 21-30 (strong activity).

**Table 3.** Summary of crystal structure determination and refinement for **2**

Empirical formula	C <sub>15</sub> H <sub>19</sub> Cl <sub>3</sub> N <sub>2</sub> ORuS
CCDC	1048636
Formula weight	482.80
Temperature	100(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2 <sub>1</sub> /c
Unit cell dimensions	a = 9.6159(4) Å    α = 90° b = 27.0693(11) Å    β = 104.460(2)° c = 6.9752(3) Å    γ = 90°
Volume	1758.10(13) Å <sup>3</sup>
Z	4
Density (calculated)	1.824 Mg/m <sup>3</sup>
Absorption coefficient	1.470 mm <sup>-1</sup>
F(000)	968
Crystal size	0.10 x 0.08 x 0.02 mm <sup>3</sup>
Theta range for data collection	2.19 to 28.51°
Index ranges	-12 ≤ h ≤ 12, -36 ≤ k ≤ 36, -9 ≤ l ≤ 9
Reflections collected	59975
Independent reflections	4455 [R <sub>(int)</sub> = 0.0456]
Completeness to theta = 25.00°	100.0 %
Max. and min. transmission	0.9712 and 0.8669
Refinement method	Full-matrix least-squares on F <sup>2</sup>
Data / restraints / parameters	4455 / 0 / 215
Goodness-of-fit on F <sup>2</sup>	1.038
Final R indices [I > 2σ(I)]	R <sub>1</sub> = 0.0202, wR <sub>2</sub> = 0.0415
R indices (all data)	R <sub>1</sub> = 0.0261, wR <sub>2</sub> = 0.0436
Largest diff. peak and hole	0.431 and -0.388 e.Å <sup>-3</sup>

**Table 4.** Selected bond lengths (Å) and bond angles (°) for **2**

---

<i>Bond lengths</i>			
Ru(1)-C(1)	2.2028(16)	Ru(1)-C(2)	2.1762(16)
Ru(1)-C(3)	2.1858(16)	Ru(1)-C(4)	2.2110(16)
Ru(1)-C(5)	2.1787(16)	Ru(1)-C(6)	2.1700(16)
C(1)-C(2)	1.409(2)	C(2)-C(3)	1.424(2)
C(3)-C(4)	1.402(2)	C(4)-C(5)	1.433(2)
C(5)-C(6)	1.397(2)	C(6)-C(1)	1.436(2)
Ru(1)-N(1)	2.1506(14)	C(11)-Cl(13)	1.7126(17)
Ru(1)-Cl(1)	2.4073(4)	Ru(1)-Cl(2)	2.4355(4)
C(13)-N(1)	1.314(2)	C(12)-N(1)	1.382(2)
C(13)-S(1)	1.7304(17)	C(11)-S(1)	1.7319(18)
<i>Bond angles</i>			
N(1)-Ru(1)-Cl(1)	85.01(4)	N(1)-Ru(1)-Cl(2)	86.85(4)
C(12)-N(1)-C(13)	110.95(14)	C(11)-S(1)-C(13)	87.69(8)
N(1)-C(13)-S(1)	115.19(13)	C(11)-C(12)-N(1)	114.01(15)
Cl(3)-C(11)-S(1)	120.98(10)	C(12)-C(11)-Cl(3)	126.87(14)

---