Materials Science and Engineering C 44 (2014) 1–8

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

# Ruthenium (II) complexes of thiosemicarbazone: Synthesis, biosensor applications and evaluation as antimicrobial agents

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ARTICLE INFO

*Article history:*
Received 4 March 2014
Received in revised form 3 July 2014
Accepted 1 August 2014
Available online 7 August 2014

*Keywords:*
Thiosemicarbazone
Ruthenium
Biosensor
Enzyme immobilization
Antimicrobial activity

ABSTRACT

A conformationally rigid half-sandwich organoruthenium (II) complex [($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl, (**1**) and carbonyl complex [Ru(CO)Cl($PPh_3$)$_2$TSC$^{N-S}$] (**2**) have been synthesized from the reaction of [{($\eta^6$-*p*-cymene)RuCl}$_2$($\mu$-Cl)$_2$] and [Ru(H)(Cl)(CO)($PPh_3$)$_3$] with thiophene-2-carboxaldehyde thiosemicarbazon (TSC) respectively and both novel ruthenium (II) complexes have been characterized by elemental analysis, FT-IR and NMR spectroscopy. The peripheral TSC in the complexes acts as an electrochemical coupling unit providing the ability to carry out electrochemical deposition (ED) and to form an electro-deposited film on a graphite electrode surface. The biosensing applicability of complexes **1** and **2** was investigated by using glucose oxidase (GOx) as a model enzyme. Electrochemical measurements at −0.9 V *versus* Ag/AgCl electrode by following the ED Ru(II) reduction/oxidation due to from the enzyme activity, in the presence of glucose substrate. The designed biosensor showed a very good linearity for 0.01–0.5 mM glucose. The *in vitro* antimicrobial activities of complexes **1** and **2** were also investigated against nine bacterial strains and one fungus by the disc diffusion test method. No activity was observed against the Gram-negative strains and fungus, whereas complex **1** showed moderate antibacterial activities against Gram-positive bacterial strains.



## 1. Introduction

Medicinal inorganic chemistry is defined as a discipline of increasing significance and affluent diversity of coordination chemistry that provides opportunities for the design of new pharmaceuticals [1]. Thiosemicarbazones (TSCs) and their metal complexes are an interesting class of compounds with beneficial biological activity. They have been extensively studied in recent years [2,3] because there are emerging moiety with a wide spectrum of biological activity and having sound scope in research and developing process in the medical and pharmaceutical fields [4–6].

In the past decades, medicinal chemistry has focused on TSCs due to their prominent biological activities demonstrated by various derivatives incorporating the heterocyclic moiety [7–9]. Owning to their chemistry, versatile activity and prospective use as drugs they have an ample interest [10,11]. Many examples of pharmacological applications with thiosemicarbazones and their metal complexes have been evaluated for their antibacterial [12,13], antifungal [14,15], antiviral [16], antiamoebic [9], antimalarial [17] and antitumor [18,19] activities. It is considered to be a reason for biological activity that the TSC molecules to chelates with trace metals in biological system. Versatile biological activities of metal complexes are not similar to ligands and the metal ions [11]. In addition, considering several transition metal complexes, decreased or increased biological activities is reported [20–22]. Moreover, TSCs have also applications in analytical chemistry. Some of the thiosemicarbazones produce highly colored complexes with metal ions. These complexes have been frequently employed for the selective and sensitive quantitative determinations of metal ions [23,24].

Organometallic ruthenium compounds, which are particularly the half of sandwich complexes, are emerging as a very promising class of anti-tumor agents. The geometry of these complexes provides a good scaffold for generating new molecules by changing the coordinated arene, the chelated ligand and the chloride group [1,25]. The chemistry of ruthenium complexes with their thiosemicarbazones that can coordinate with the metal either in the neutral thione form or in the anionic thiolate form, has received attention in recent years primarily because of their diverse coordination mode [26,27]. In particular the use of ruthenium complexes as chemotherapeutic agents for the treatment

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http://dx.doi.org/10.1016/j.msec.2014.08.007
0928-4931/

of cancer is also well established [28]. Principally, there emerge no reports of ruthenium complexes of TSC containing over five-membered heterocyclic ring substituents including thiophene [29]. Recently, oligomer of thiophene-2-carbaldehyde thiosemicarbazone was electrochemically deposited on the graphite surfaces as the matrix for the enzyme immobilization [30]. Electrogenerated film-matrices as biomolecule immobilization platforms provide improvements in biosensor design [31]. The immobilization of enzymes on the modified electrode surface is one of the crucial factors in the biosensor design [32–34]. Enzymes require direct or indirect immobilization on the transducers [35]. A number of researchers have been considerably interested in enzyme-based glucose biosensors because of their practical advantages, such as operational stability, higher selectivity, operational simplicity and suitability for real time detection [36–39].

As an extension of our previous studies on the design and study of useful enzyme-based biosensor applications, herein we describe the synthesis and electrodeposition of the thiophene-2-carbaldehyde thiosemicarbazone with selected transition metal (Ru (II)). The syntheses and full characterization of two TSCs complexes – [($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl (**1**), and [Ru(CO)Cl($PPh_3$)$_2$TSC$^{N-S}$] (**2**) – were elucidated by elemental analysis, a combination of multinuclear NMR spectroscopy and FTIR spectroscopy. We also report the electrochemical dimers of complexes **1** and **2** on the graphite surfaces by using glucose oxidase (GOx) biosensors application as electrogenerated film matrices. Antimicrobial effects of these complexes are also investigated.

## 2. Experimental

### 2.1. Materials and methods

Reactions were carried out under an oxygen-free argon atmosphere using Schlenk techniques. All glassware was oven-dried at 120 °C. All solvents were dried and degassed using standard techniques and stored under nitrogen until used [40]. Acetonitrile (ACN), benzene, petroleum ether, dichloromethane and silica gel were purchased from Merck and $RuCl_3 \cdot 3H_2O$, Glucose oxidase (GOx, from *Aspergillus niger*, EC 1.1.3.4, 50 Units/mg), glutaraldehyde solution (25%, v/v), tetrabutylammonium hexafluorophosphate 98% ($TBAPF_6$) and D (+)-glucose were obtained from Sigma-Aldrich. All chemicals needed for the syntheses of monomer (thiophene-2-carboxaldehyde) were purchased from Sigma-Aldrich.

[($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl and [Ru(CO)Cl($PPh_3$)$_2$TSC$^{N-S}$] characterized by elemental analyses were performed on a Leco 932 instrument at Technical and Scientific Research Council of Turkey, FT-IR (FT-IR spectra were recorded (KBr pellets) on a Varian 1000 FT spectrophotometer), $^1$H NMR ($^1$H NMR spectra were recorded in DMSO-$d_6$ and $CDCl_3$ on 500 MHz High Performance Digital FT-NMR and chemical shifts were referenced to tetramethylsilane (TMS)).

For the chronoamperometric measurements and cyclic voltammograms (CV), Palm Instrument (PalmSens, Houten, The Netherlands, www.palmsens.com) with three electrode configuration was used. Experiments were carried out in a cell at ambient conditions using a three-electrode configuration consisting of graphite electrode (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) as the working electrode, a platinum electrode (Metrohm, Switzerland, www.metrohm.com) the counter electrode, Ag/AgCl (3.0 M KCl saturated with AgCl as an internal solution) as the reference.

### 2.2. Syntheses

#### 2.2.1. Thiosemicarbazone ligand (TSC$^{N-S}$)

Thiophene-2-carbaldehyde thiosemicarbazone was synthesized by mixing an aqueous solution of thiocarbonylhydrazines (420 mg, 3.0 mmol in 10 mL) and ethanolic solution of thiophene-2-carboxaldehyde (273 mg, 3.0 mmol in 10 mL) at 25 °C for 3 h with continuous stirring. After cooling, the precipitated compound was filtered and recrystallized from an appropriate solvent [41].

#### 2.2.2. [($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl, (1)

The starting metal compound, [{($\eta^6$-*p*-cymene)RuCl}$_2$($\mu$-Cl)$_2$] was synthesized as described in the literature [42]. The metal complex was synthesized using a common procedure: A solution of appropriate TSC$^{N-S}$ (37 mg, 0.2 mmol) in dry methanol (10 mL) was added to a stirred suspension of [{($\eta^6$-*p*-cymene)RuCl}$_2$($\mu$-Cl)$_2$] (34 mg, 0.1 mmol) in hot methanol (10 mL). The obtained mixture was heated and refluxed under argon atmosphere for 24 h during which period starting material dissolved and complex started to separate. After keeping the reaction flask at room temperature for 2 h, the orange-brown solid was filtered, washed with methanol and dried. Yield of (**1**): 57 mg, 63%. Analytical data for **1**: Anal. Calcd for $C_{16}H_{21}Cl_2N_3RuS_2$: C, 39.10; H, 4.31; N, 8.55. Found: C, 40.02; H, 4.07; N, 8.63. FT-IR (KBr, $cm^{-1}$): υ ($NH_2$) 3340 (m); υ (NH) 3149 (m); υ (N–N) 1066 (m); υ (C═N)1584 (s); υ (C═S) 783 (w); υ (C–S–C)$_{thiophen\ ring}$ 843 (m); υ (C–S)$_{sym\ thiophen\ ring}$ 770 (m); υ (C–S)$_{asym\ thiophen\ ring}$ 664 (m); υ (Ru–N) 541 (m). $^1$H NMR (500 MHz, DMSO-$d_6$) δ (ppm) = 11.38 (1H, s, N–H); 8.54 (2H, br, $NH_2$); 8.38 (1H, s, –CH═N); 5.80–5.75 (4H, m, Ar–H); 2.83 (1H, m, C**H**($CH_3$)$_2$); 2.08 (3H, s, $CH_3$); 1.18 (6H, d, $^3J_{HH}$ = 6.81 Hz, CH(C**H$_3$**)$_2$); 7.08–7.59 (3H, m, ($C_4H_3S$)$_{thiophen\ ring}$).

#### 2.2.3. [Ru(CO)Cl($PPh_3$)$_2$TSC$^{N-S}$] (2)

A solution of appropriate TSC$^{N-S}$ (18.5 mg, 0.1 mmol) in benzene (10 mL) was added drop wise into a stirred suspension of [RuH(CO)Cl($PPh_3$)$_3$] (95 mg, 0.1 mmol) in benzene (20 mL). The contents were refluxed under argon atmosphere for 5 h. Then, left to cool to room temperature and the mixture was evaporated in a vacuum. The yellow precipitate washed with dichloromethane/petroleum ether (1:3) and product dried. Yield of (**2**): 58 mg, 75%. Analytical data for **2**: Anal. Calcd for $C_{43}H_{36}ClN_3OP_2RuS_2$: C, 59.14; H, 4.15; N, 4.81. Found: C, 59.43; H, 4.01; N, 4.93. FT-IR (KBr, $cm^{-1}$): υ ($NH_2$) 3343 (m); υ (N–N) 1078 (m); υ (CO) 1938 (s); υ (C═N) 1582 (s), 1542 (s); υ ($PPh_3$) 1417–1456 (m), 1091–1093 (m), 736–796 (m), 516–518 (m); υ (C–S) 747 (m); υ (C–S–C)$_{thiophen\ ring}$ 844 (m); υ (C–S)$_{sym\ thiophen\ ring}$ 765 (m); υ (C–S)$_{asym\ thiophen\ ring}$ 676 (m); υ (Ru–N) 522 (m). $^1$H NMR (500 MHz, $CDCl_3$) δ (ppm) = 8.37 (1H, s, –CH═N); 8.56 (2H, br, $NH_2$); 6.60–7.93 (m, $PPh_3$ ring protons); 7.06–7.59 (3H, m, ($C_4H_3S$)$_{thiophen\ ring}$). $^{31}$P NMR (202 MHz, $CDCl_3$) δ = 36.94.

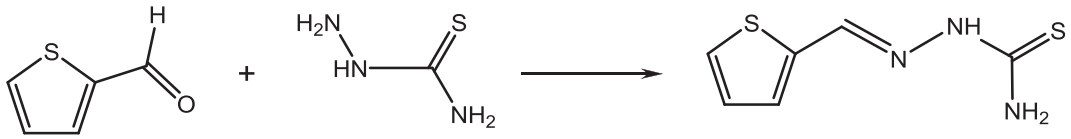
### 2.3. Biosensor application of complexes **1** and **2**

#### 2.3.1. Electrochemical deposition of complexes **1** and **2**

Prior to the matrixes for biomolecule attachment spectroscopic grade graphite rods were polished on emery paper and washed thoroughly with distilled water. Electrochemical depositions (EDs) of complexes **1** and **2** were potentiodynamically carried out between −1.5 and −0.5 V in the presence of 0.005 g complex in ACN medium containing 0.1 M $TBAPF_6$ as the supporting electrolyte on the graphite electrode. A three-electrode cell assembly was used where the working electrode was a spectrographic graphite rod, the counter electrode was a Pt flake and a Ag/AgCl was used as the reference electrode. Electrodeposited Ru(II) complexes were washed with ACN in order to remove excess supporting electrolyte ($TBAPF_6$) and unreacted monomer after the potentiodynamic electrochemical deposition. The EDs of complexes were carried out for fifty cycles at room temperature.

#### 2.3.2. Biomolecule immobilization

For immobilization of enzyme a proper amount of GOx solutions (5.0 mg in 5.0 μL, $50 \times 10^{-3}$ M sodium phosphate buffer, pH 7.0) was spread over the surface ED complex **1** (**ED-1**) and ED complex **2** (**ED-2**) coated graphite electrodes and glutaraldehyde (5.0 μL, 0.1%, in $50 \times 10^{-3}$ M sodium phosphate buffer, pH 7.0) was then added to each other and the electrodes were allowed to stand at ambient



**Scheme 1.** Formation of thiophene-2-carbaldehyde thiosemicarbazone (TSC).

conditions to dry for 1 h prior to use. After immobilization, non-bounded enzyme molecules were removed by rinsing the graphite electrode surface with distilled water and the working buffer solution, respectively.

*2.3.3. Measurements*

All electrochemical experiments were carried out at ambient conditions in an electrochemical cell containing 10 mL of the working buffer ($50 \times 10^{-3}$ M, sodium acetate buffer, pH 4.0). The electrodes were washed with distilled water and kept in working buffer for 5 min after each measurement. The enzyme electrodes were initially equilibrated in buffer and the substrate was added to the reaction cell. The biosensor responses were registered as the current signal (μA) at −0.9 V *vs* Ag/AgCl and Pt as the reference and counter electrodes. The buffer was refreshed after each measurement. The batch biosensor response was registered current signal (μA). The electrochemical behavior of **ED-1**/GOx and **ED-2**/GOx was examined by cyclic voltammetry (CV). In the sample application part, a commercial enzyme assay kit based on spectrophotometric Trinder reaction (Cromatest, Glucose MR, Cat. No. 1129010) was used as a reference method for independent glucose analysis. The measurements were carried out at 25 °C under $N_2$ atmosphere.

*2.4. Biological activities*

*2.4.1. Microorganisms*

The *in vitro* antimicrobial activities of organoruthenium (II) complex **1** and carbonyl complex **2** were tested against laboratory control strains belonging to the American Type Culture Collection (LGC Standards GmbH, Wesel, Germany): *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 23355, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 11774, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615 and one fungus *Candida albicans* ATCC 10231. Clinical isolate *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus agalactiae* were kindly supplied from the Microbiology Department, Faculty of Medicine at the Dicle University (Diyarbakir, Turkey).

*2.4.2. Determination of antimicrobial activity*

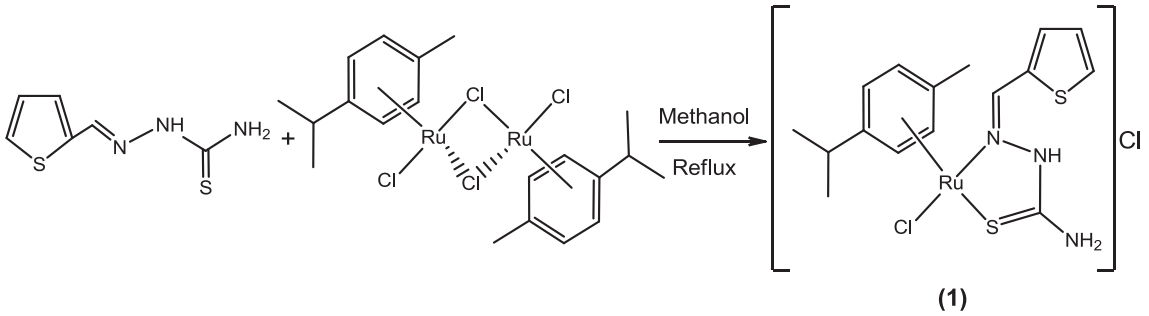
Antimicrobial activities of ruthenium (II) complexes were investigated by the disc diffusion susceptibility test according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [43]. It was performed on Nutrient Agar (NA, Oxoid) plates. Plates were dried at approximately 36 °C for about 30 min in an incubator before inoculation. The bacterial strains were inoculated into 25 mL of Nutrient Broth (NB, Oxoid) medium in a rotary shaker at 180 rpm for 4 to 6 h until a turbidity of 0.5 McFarland ($1 \times 10^8$ CFU/mL) was reached. Final inocula were adjusted to $5 \times 10^5$ CFU/mL. *C. albicans* ATCC 10231 was also inoculated into 25 mL of Sabouraud Dextrose Broth (SDB, Oxoid) in a rotary shaker at 180 rpm for 8 to 10 h until a turbidity of 0.5 McFarland was reached. The final inocula were adjusted to $5 \times 10^5$ CFU/mL using a spectrophotometer [44]. 50 μL of inoculum from the final inoculate was applied to each of agar plates and was uniformly spread with a sterile cotton swab over the surface. Absorption of excess moisture was allowed to occur for 30 min before application of sterile paper discs (6 mm in diameter, Oxoid). The discs were impregnated with 10, 15 and 20 μL of the sample solutions in dichloromethane/methanol (DCM/MeOH, 8:2), 5.0 mg per 1.0 mL of DCM/MeOH and placed on inoculated plates. These plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi. Standard antibiotic discs (all from Oxoid accept for nystatin) of ofloxacin (OFX, 5.0 μg/disc), amoxycillin/clavulanic acid (2:1) (AMC, 30 μg/disc), imipenem (IMP, 10 μg/disc), erythromycin (E, 15 μg/disc) and nystatin (Sigma) (N, 60 μg/disc) were individually used as positive controls, while the discs imbued with 20 μL of DCM/MeOH (8:2) solvent system were accepted as negative control. The diameters of the inhibition zones were measured in millimeters including the diameter of the disc. Studies were performed in triplicate, and the developing inhibition zones were compared with reference antibiotic discs.

## 3. Results and discussion

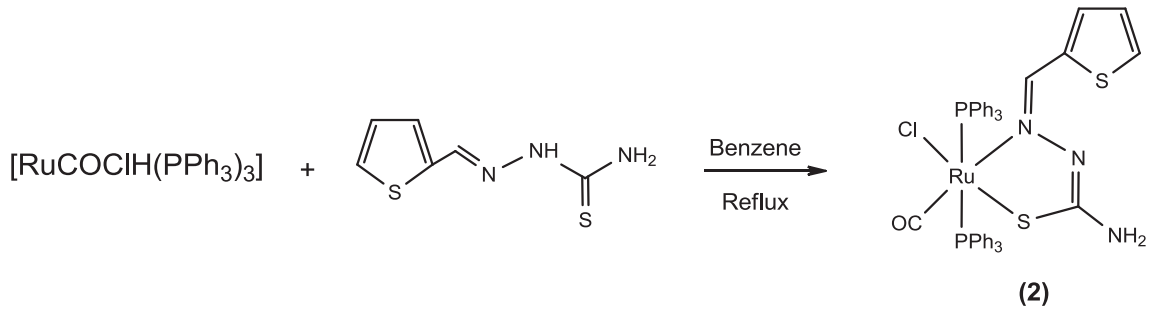
*3.1. Synthesis and characterization*

A conformationally rigid half-sandwich organoruthenium (II) complex [($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl, (**1**) and carbonyl complex [Ru(CO)Cl($PPh_3$)$_2$TSC$^{N-S}$] (**2**) have been synthesized from the reaction of thiophene-2-carboxaldehydethiosemicarbazon (TSC) with [{($\eta^6$-*p*-cymene)RuCl}$_2$(μ-Cl)$_2$] and [Ru(H)(Cl)(CO)($PPh_3$)$_3$] respectively and both ruthenium (II) complexes have been characterized by elemental analysis, FT-IR, NMR spectroscopic methods.

Thiophene-2-carboxaldehydethiosemicarbazon (TSC) is shown in Scheme 1. It was synthesized by aqueous solution of thiocarbonylhydrazines and ethanolic solution of thiophene-2-carboxaldehyde. The complex **1** was synthesized from [{($\eta^6$-*p*-cymene)RuCl}$_2$(μ-Cl)$_2$] by reaction with two equivalents of the thiosemicarbazone in methanol at room temperature under argon. The orange-brown solid that was obtained was very soluble in DMSO but less soluble in alcohols. The complex **2** was synthesized from [RuH(CO)Cl($PPh_3$)$_3$] by reaction




**Scheme 2.** [($\eta^6$-*p*-cymene)RuClTSC$^{N-S}$]Cl, (**1**).




**Scheme 3.** $[Ru(CO)Cl(PPh_3)_2TSC^{N-S}]$ (**2**).

with the thiosemicarbazone in benzene at room temperature under argon. The complexes were obtained in powder form. Various attempts have been made to obtain the single crystals of the complexes but it has been unsuccessful. So for the ligand and the complexes are stable at room temperature, non-hygroscopic and insoluble in water and soluble in $CH_2Cl_2$, $CHCl_3$, DMF, DMSO and $CH_3CN$.

Based on elemental analysis and spectroscopic data we propose that the complexes are best formulated as $[(\eta^6\text{-}p\text{-cymene})RuClTSC^{N-S}]Cl$, (**1**) (Scheme 2) and $[Ru(CO)Cl(PPh_3)_2TSC^{N-S}]$ (**2**) (Scheme 3). The spectroscopic data reasonably support the formula of the compound.

The IR spectra of the free ligands were compared with those of the metal complexes in order to study the binding modes of the thiosemicarbazone ligand to metal. The highest frequency bands present around 3340 $cm^{-1}$ in the spectrum of TSC ligand are assigned to $\nu_{asym}$ and $\nu_{sym}$ vibration of the terminal $NH_2$ group. This band is also present in the spectra of the complexes **1** and **2** indicating the non-involvement of this group in coordination. The $\nu$(N–N) band of the ligand are present around 1058 $cm^{-1}$. The increase in frequency of this band 1066 and 1078 $cm^{-1}$ in the spectra of the complexes (**1**) and (**2**), respectively provides evidence for the coordination *via* the azomethine nitrogen [45,46].

Thiosemicarbazone ligands can coordinate in a number of different manners. In general, they can bind two tautomeric forms; a neutral thione form and the anionic thiolate form [1,27]. Infrared spectrophotometry was used to confirm coordination as the thione form for the complex (**1**) and the thiol form for the complex (**2**). The TSC ligand and complex (**1**) showed intense, strong bands at 820 and 783 $cm^{-1}$ respectively, due to $\nu$(C═S) stretch. No band near 2570 $cm^{-1}$ due to $\nu$(C–SH) suggesting that these ligands remain in the thione form in the solid-state in complex (**1**). The downward (C═S) band shift (37 $cm^{-1}$) in the complex (**1**) suggested the coordination of thiocarbonyl sulfur. A band appeared at 820 $cm^{-1}$ in free TSC ligand due to vibration of the C═S double bond which disappeared in the spectra of the complex (**2**) and a new band C–S appeared at 747 $cm^{-1}$ indicating that the other coordination is through thiolate sulfur after enolization followed by deprotonation on sulfur [47]. The band observed at 3149 $cm^{-1}$, due to the NH group stretching vibration in free TSC ligand also remains unaffected in the complex (**1**). This band is not observed in the spectrum of the complex (**2**). The spectra of all the thiosemicarbazones exhibit a strong band at 1582–1584 $cm^{-1}$ region due to the $\nu$(C═N) stretch of the azomethine linkage. In the complexes this band shifted (2–4 $cm^{-1}$) to lower frequency. This lowering of the $\nu$(C═N) stretch on complexation may be attributed to lowering of the C═N bond order as a result of the M–N band formation [41]. A medium sharp band at 1586 $cm^{-1}$ due to the azomethine C═N stretching frequency of the free ligand was shifted to lower frequency in the spectra of the complexes at 1584 and 1582 $cm^{-1}$ indicating that the coordination through N atom [48]. Bands are assigned to the $\nu$(M–N) band which further supports the coordination of the azomethine nitrogen. In the complexes (**1**) and (**2**) the medium intensity band in the region 541 and 522 $cm^{-1}$ respectively, is attributed the Ru–N [49]. The bands observed at 843, 770, 664 for complex (**1**) and 844, 765, 676 $cm^{-1}$ for complex (**2**) are attributed to the thiophene ring deformation modes in the ligands. These thiophen ring deformation vibrations are not affected in the complexes [41].

Overall, the complexes contain NS coordinated thiosemicarbazones. In addition, the characteristic absorption bands due to triphenylphosphine were also observed for the complex (**2**) in their expected regions. The characteristic bands due to $PPh_3$ is also present at around 1417–1456 $cm^{-1}$, 1091–1093 $cm^{-1}$, 736–796 $cm^{-1}$ and 516–518 $cm^{-1}$ in complex (**2**). A strong band in the 3159 $cm^{-1}$ region attributed to $\nu$(N–H) group of –NH–N═C– found in the spectra of free ligand is not present in the spectra of the complex (**2**). However, new bands are present at 1542 and 747 $cm^{-1}$ which are assigned to –C═N

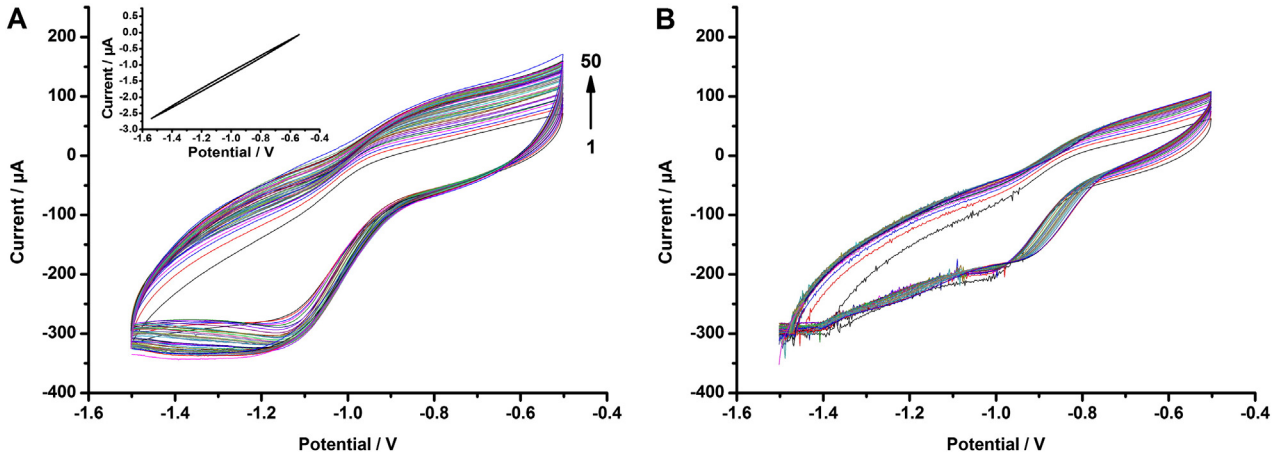



**Fig. 1.** Repeated potential-scan electro depositions of complex **1** (A) and complex **2** (B) in 0.1 M ACN/$TBAPF_6$ solvent–electrolyte system at a scan rate of 0.1 V·$s^{-1}$ on graphite (up to fifty cycles) and cyclic voltammogram of bare graphite electrode (inset A).

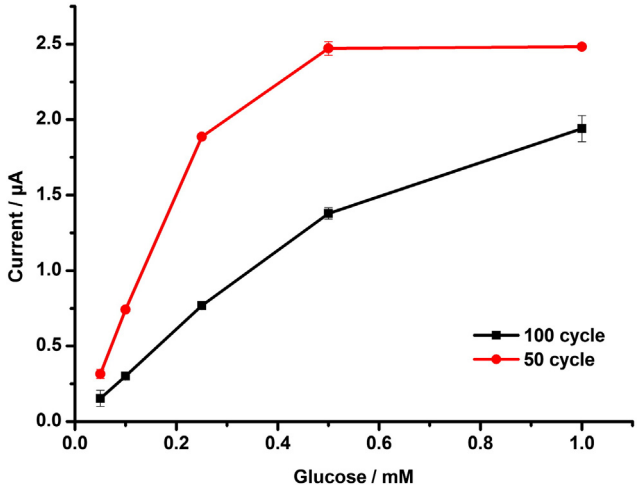



**Fig. 2.** Effect of the scan number on the biosensor response of the **ED-1**/GOx biosensor (in $50 \times 10^{-3}$ M, sodium acetate buffer, pH 4.0, 25 °C, −0.9 V). Error bars show the SD of three measurements.

and −C−S, respectively. These observations indicate the enolization of the −NH−C═S group and subsequent deprotonation before coordination to the metal. In the complex (**2**) the band due to free ν(C═O) group is present at 1938 $cm^{-1}$. The replacement of the hydride ion in the starting complexes by the TSC ligand has been confirmed by the absence of a band around 2020 $cm^{-1}$ in the complex (**2**) [50]. The ligand to metal bonding is further supported by $^1$H NMR spectra. The TSC ligand and the complexes are very soluble in DMSO and $CHCl_3$ and so their NMR spectra were obtained in DMSO-$d_6$ and $CDCl_3$. The NMR spectrum of the thiosemicarbazone ligand and ruthenium (II) compounds confirm the complex formation. In the spectra of the complexes (**1**) (Fig. S1) and (**2**) (Fig. S2) a sharp singlet appeared at 8.38 and 8.37 ppm, respectively, has been assigned to azomethine proton (−HC═N). The positions of azomethine signal in the complexes are down field compared to that of the free ligand observed at 8.04 ppm, indicating coordination through the azomethine nitrogen atom. In the case of complex (**1**) for the ligand the absence of the signal at 4.00 ppm that can be ascribed to −SH [51] is consistent with the idea that in solution, as in the solid state, the ligand exists as the thione tautomer. The *p*-cymene protons resonate at frequencies typically seen for this group [1,52]. A multiplet is seen near 5.80–5.75 ppm attributed to the ring protons; the isopropyl methine is found at 2.83 ppm with the methyls occurring as a doublet at 1.18 ppm. The

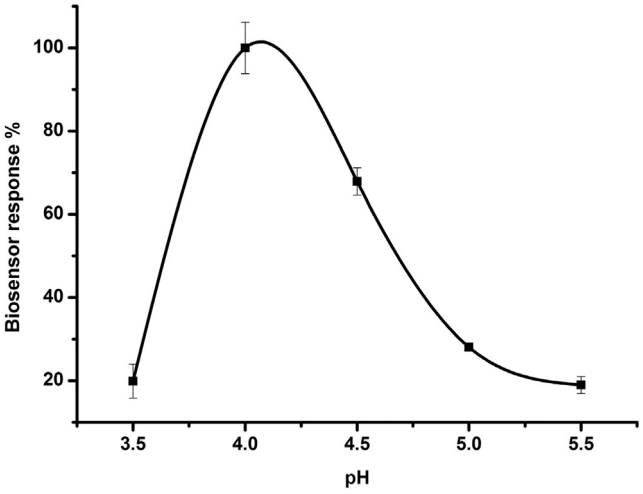



**Fig. 3.** The effect of the pH on the biosensor response of the **ED-1**/GOx biosensor (in $50 \times 10^{-3}$ M, sodium acetate buffer, 25 °C, −0.9 V). Error bars show the SD of three measurements.

singlet signal due to the methyl on the ring is observed at 2.08 ppm. The $NH_2$ and NH signals in the complexes attributed to the ligand are practically unchanged from the free ligands. The $NH_2$ group generates broad signal at 8.54 ppm in the complex (**1**) and 8.56 ppm in the complex (**2**). The N−H resonates as a sharp singlet at 11.38 ppm in complex (**1**). All the other protons resonate in regions commonly expected. The $^1$H NMR spectra of the complex (**1**) is essentially a direct combination of the signals from the ligands plus those from the *p*-cymene moiety. In the $^1$H NMR spectra of complex (**1**) all indications are that the ligands remain neutral form (as evidenced by the presence of the NH protons). Enolization of thiocarbonyl group is indicated by the singlet present at 10.4 ppm in the spectra of the ligand, which are attributed to −C−SH protons of thioamide group. The absence of thionyl group in the complex (**2**) indicates deprotonation of this group of the thiosemicarbazone ligands on complexation and coordination to ruthenium through thionyl sulfur.

The terminal $-NH_2$ and −NH protons in the complexes are seen in the positions with slight deviation as in the ligands spectrum confirming the non-involvement of this group in coordination with the metal [46]. Multiplets are observed at around 6.60–7.93 ppm in all the complexes and have been assigned to the aromatic protons of triphenylphosphine and thiophen of the thiosemicarbazone ligands. Furthermore, in all the complexes largest Δδ are observed for the protons that are located close to the coordinating atoms. So, the deshielding effect of the metal is apparent to such protons [53]. The $^{31}$P NMR spectra were recorded for the complex (**2**) to confirm the presence of triphenylphosphine group in TSC ligand (Fig. S3). The signal appeared at 36.94 ppm attributed to the two phosphine ligands are trans to each other in this complex. Initially, we synthesized a conformationally rigid half-sandwich organoruthenium (II) complex [($\eta^6$-*p*-cymene)$RuClTSC^{N-S}$]Cl, (**1**) and carbonyl complex $[Ru(CO)Cl(PPh_3)_2TSC^{N-S}]$ (**2**) from the reaction of $[\{(\eta^6\text{-}p\text{-cymene})RuCl\}_2(\mu\text{-}Cl)_2]$ and $[Ru(H)(Cl)(CO)(PPh_3)_3]$ with thiophene-2-carboxaldehyde thiosemicarbazon (TSC) respectively. Both ruthenium (II) complexes have been characterized by elemental analysis, FT-IR and NMR spectroscopy. According to the spectral data, it can be said that the TSC ligand behaves as bidentate thione (**1**) and thiol (**2**) form coordinate to the metal center *via* its sulfur and imine nitrogen atom. The TSC ligand must act as a 4-electron donor in order to satisfy the 18-electron rule.

### 3.2. Biosensing applications

Before electrodeposition, spectroscopic grade graphite rods were polished on emery paper and washed thoroughly with distilled water. ED-1 and ED-2 were deposited potentiodynamically on graphite electrodes in 0.1 M $TBAPF_6$ (in ACN solvent) electrolyte couple with repeated scan interval between −1.5 and −0.5 V (*vs* Ag/AgCl reference electrode) with a scan rate of 0.1 V $s^{-1}$. After the polymerization, the surface of the electrode was rinsed with distilled water to remove impurities.

Because of the electroactive thiophene ring on the TSC group, both complexes **1** and **2** can be oxidatively electrodimerized. The electrodeposited film matrices were carried out in ACN solution. We controlled the highest potentials in the ED process before the oxidation potential of the metal center to protect it, and display the ED curves of complex **1** and complex **2** in Fig. 1. While repetitive CV scans between choosing potentials continue, both signals of oxidation and reduction of dimeric TSCs appear at −0.87 and −1.14 V, respectively. Meanwhile, the reversible couples gradually decrease in potential but become more prominent with each scan. ED curves of complex **1** are displayed in Fig. 1A shows electrochemistry property similar to complex **2** (Fig. 1B). The inset (Fig. 1A) also shows cyclic voltammogram of bare graphite electrode.

To observe the biosensor responses, electrodes were prepared using two different scan numbers (50 and 100) during the electrochemical deposition process, and the corresponding biosensor responses were

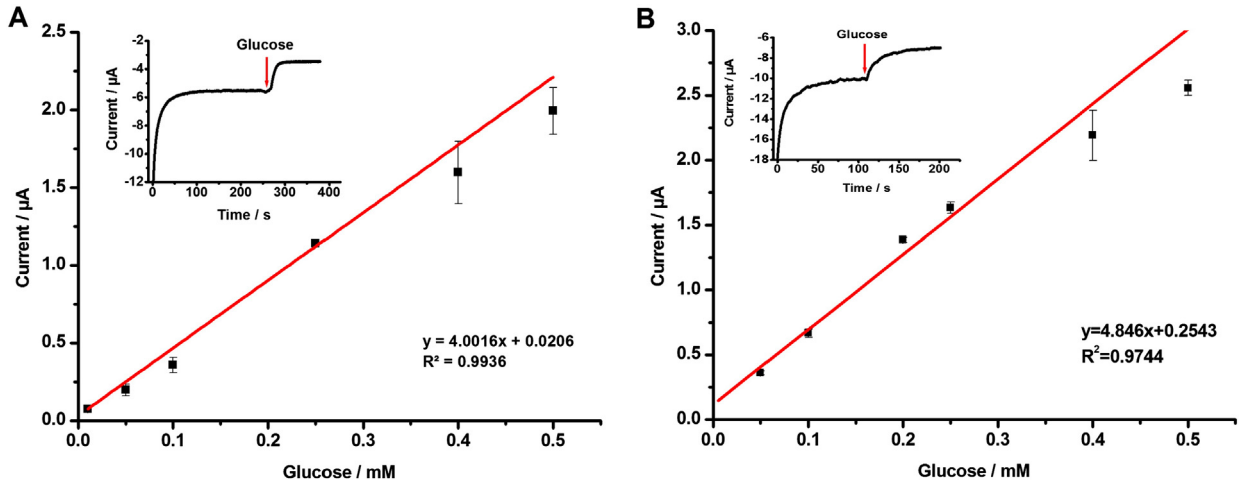



**Fig. 4.** Calibration curves for the detection of glucose of the **ED-1**/GOx biosensor (A) and the **ED-2**/GOx biosensor (B) (in $50 \times 10^{-3}$ M, sodium acetate buffer, 25 °C, −0.9 V). Error bars show the SD of three measurements.

recorded (Fig. 2). 25 scan numbers were also applied during the electrochemical deposition process, but a suitable and significant biosensor response was not observed. The lower current responses were observed with 100 cycles. The decrease in responses could be related to the conducting film layer thickness after 100 scans of ED. When the thickness of the conducting film layer is increased, the diffusion distance becomes longer causing lower charge transfer rates. 50 cycles of ED may provide an appropriate interface for biomolecule immobilization and stabilization, as reported in previous works [54–56].

Dimeric mediators have been successfully used to wire enzymes and applied for construction of biosensor. The influence of pH on the response signals of **ED-1**/GOx biosensor was investigated over a pH range of 3.5–5.5 with sodium acetate buffers (50 mM) in the presence of 0.5 mM glucose as the substrate. The biosensor revealed the best result at pH 4.0 as given in Fig. 3. It was also reported that the free GOx is acidic (with pI; 4.2) and shows activity over a wide range of pH values (pH 3.0–8.0) [31,57,58]. Further experiments were conducted with the pH 4.0 using 50 mM acetate buffer.

As to the analytical characterizations, the dependence of current signals on the glucose concentration and linear ranges were shown in Fig. 4. A perfect linearity for **ED-1**/GOx biosensor was obtained for $0.01–0.5 \times 10^{-3}$ M glucose in 50 mM sodium acetate buffer (pH 4.0) and defined by the equation of $y = 4.002x + 0.021$ and $R^2 = 0.994$ (response time given as inset in Fig. 4A). Also, a good linearity for **ED-2**/GOx biosensor was obtained for $0.05–0.5 \times 10^{-3}$ M glucose in same reaction conditions and defined by the equation of $y = 4.846x + 0.254$ and $R^2 = 0.974$ (response time given as inset in Fig. 4B).

Dimeric mediated **ED-1**/GOx biosensors were also tested on real samples. Initially, the samples were degassed, diluted with working buffer and then, injected into the carrier buffer instead of the substrate. The calibration curves for glucose were used to determine the glucose contents in the samples. The **ED-1**/GOx biosensor was applied for the glucose analysis in three different commercial beverages. Afterwards, the obtained results were compared by a commercial enzyme assay kit based on the spectrophotometric method as a reference method. Table 1 shows the results of the recoveries of the two different methods. As a result of the good correlation between them, it can be claimed that the **ED-1**/GOx biosensor provides a similar result of the reference method without affecting the sample matrices.

**Table 1**
Results of the total glucose using the **ED-1**/GOx biosensor and spectrophotometric method in commercial beverages.

| Sample | Glucose[a] [M] | | |
|---|---|---|---|
| | **ED-1**/GOx biosensor | Spectrophotometric method | Recovery (%) |
| Fruit juice | 0.101 ± 0.0088 | 0.100 ± 0.0026 | 101.0 |
| Orange fizzy drink | 0.036 ± 0.0081 | 0.032 ± 0.0016 | 110.4 |
| Fizzy drink | 0.061 ± 0.0027 | 0.063 ± 0.0110 | 96.80 |

[a] Values are the mean of three measurements ± SD.

### 3.3. Antimicrobial activity of ruthenium (II) complexes

The antimicrobial activities of ruthenium (II) complexes were studied using a disc diffusion assay recommended by NCCLS. In this study, one fungus and nine different bacterial strains were used to screen for the antimicrobial activity. The disc diffusion susceptibility method is simple, practical and extensively used to investigate the antimicrobial activity testing, and has been well-standardized in routine clinical microbiology laboratories [59–61]. Table 2 shows the results of the antibacterial activities of the standard antibiotics and complexes **1** and **2** against tested microorganisms. The DCM/MeOH (8:2) (20 μL) negative control showed no inhibiting effect. All microorganisms were evaluated as susceptible for IPM and OFX.

None of the ruthenium complexes showed any activity against Gram-negative bacteria and fungus at the concentration evaluated in this work. Although the antimicrobial activity of carbonyl complex **2** did not show antimicrobial activities against Gram-positive and Gram-negative bacteria, organoruthenium (II) complex **1** with contain of *p*-cymene, surprisingly exhibited moderate antimicrobial activities against Gram-positive *B. subtilis* ATCC 11774, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 and clinical isolates *S. aureus* and *S. agalactiae*. Our data revealed that standard ATCC strains of Gram-positive bacteria were more sensitive than Gram-negative ones towards antimicrobial activities studied. This is likely because Gram-positive bacteria lack the outer membrane of Gram-negative ones, which acts as a barrier for penetration of numerous molecules [62]. The variation of susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the ruthenium complexes.

At the 100 μg concentrations, complex **1** was found to be highly active against *B. subtilis* ATCC 11774, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 and clinical isolates *S. aureus* and *S. agalactiae* with a zone diameter of 17, 10, 12, 13 and 12 mm respectively (Fig. 5).

## 4. Conclusion

In conclusion, new ruthenium (II) thiosemicarbazone complexes derived from thiophene-2-carboxaldehyde represent an interesting

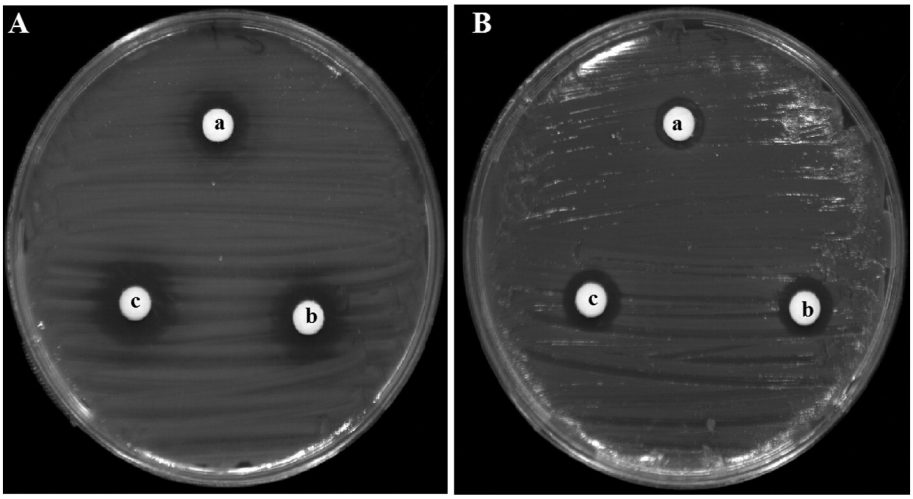



**Fig. 5.** Antimicrobial activity of organoruthenium (II) complex (**1**) against the used indicator microorganisms *B. subtilis* ATCC 11774 (A) and *S. aureus* (clinical isolate) (B), a: 50 μg/disc, b: 75 μg/disc and c: 100 μg/disc.

new family of compounds to control growth of *B. subtilis* ATCC 11774, *S. aureus* ATCC 25923, *S. pyogenes* ATCC 19615 and clinical isolates *S. aureus* and *S. agalactiae*. The biological behavior revealed that the complex **1** shows significant activity against the selected Gram-positive bacterial test organisms. Complex **2** did not show any antimicrobial activities against to test organisms. These results can be also utilized in the design of a new type of metal-based drug with selective antimicrobial activities. The biosensing applicabilities of dimeric ruthenium complexes were also investigated by using glucose oxidase (GOx) as a model enzyme to detect glucose. The biosensors were successfully applied for the glucose analysis without requiring any pre-treatment.

**Table 2**
Antimicrobial activities of complexes **1**, **2** and standard antibiotics.

| Test microorganisms | μg/6 mm paper disc | Zones of inhibition (mm)[c] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | complexes | | standard antibiotics | | | | |
| | | **1** | **2** | OFX (5)[a] | AMC (30)[a] | IPM (10)[a] | E (15)[a] | N (60)[a] |
| *Escherichia coli* | 50 | – | – | 26 | 16 | 20 | 10 | NT |
| ATCC 25922 | 75 | – | – | | | | | |
| | 100 | – | – | | | | | |
| *Enterobacter cloacae* | 50 | – | – | 28 | 18 | 22 | – | NT |
| ATCC 23355 | 75 | – | – | | | | | |
| | 100 | – | – | | | | | |
| *Pseudomonas aeruginosa* | 50 | – | – | 14 | R | 22 | – | NT |
| ATCC 27853 | 75 | – | – | | | | | |
| | 100 | – | – | | | | | |
| *Pseudomonas aeruginosa*[b] | 50 | – | – | 22 | R | 24 | – | NT |
| | 75 | – | – | | | | | |
| | 100 | – | – | | | | | |
| *Bacillus subtilis* | 50 | 13 | – | 26 | 28 | >30 | >30 | NT |
| ATCC 11774 | 75 | 15 | – | | | | | |
| | 100 | 17 | – | | | | | |
| *Staphylococcus aureus* | 50 | 8 | – | 22 | 30 | >30 | 20 | NT |
| ATCC 25923 | 75 | 9 | – | | | | | |
| | 100 | 10 | – | | | | | |
| *Staphylococcus aureus*[b] | 50 | 10 | – | 22 | 16 | >30 | 20 | NT |
| | 75 | 12 | – | | | | | |
| | 100 | 13 | – | | | | | |
| *Streptococcus pyogenes* | 50 | 8 | – | 16 | 24 | 28 | 24 | NT |
| ATCC 19615 | 75 | 10 | – | | | | | |
| | 100 | 12 | – | | | | | |
| *Streptococcus agalactiae*[b] | 50 | 8 | – | 22 | >30 | >30 | 28 | NT |
| | 75 | 10 | – | | | | | |
| | 100 | 12 | – | | | | | |
| *Candida albicans* | 50 | – | – | NT | NT | NT | NT | 22 |
| ATCC 10231 | 75 | – | – | | | | | |
| | 100 | – | – | | | | | |

OFX: ofloxacin; AMC: amoxycillin/clavulanic acid (2:1); IPM: imipenem; E: erythromycin; N: nystatin; NT: not tested; and (–) not active.
[a] μg/6 mm paper disc.
[b] Clinical isolates.
[c] Results are expressed as means of three independent experiments, with each experiment run in triplicate.

## Acknowledgments

This study was supported by project number 2011.KB.FEN.047 and 2012.KB.FEN.047 from Dokuz Eylul University Rectorship, Scientific Research Project Coordination Center. We also thank the Graduate School of Natural and Applied Sciences, Dokuz Eylul University, EBILTEM, Ege University for NMR analysis and TUBITAK, for the elemental analysis.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.msec.2014.08.007.

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