

CATECHOLASE ACTIVITY AND SUBSTITUENT EFFECT OF NEW HOMOLEPTIC COPPER(II) CHALCONE COMPLEXES

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ABSTRACT

Three new neutral complexes of copper(II) containing chalcone ligands derived from 2'-hydroxyacetophenone and 4-substituted benzaldehydes were synthesized. Complexes were prepared by solution synthesis and characterized by spectroscopy. The catalytic activity of complexes was examined in the reaction of 3,5-di-*tert*-butyl catechol (DTBC) oxidation. The kinetics of DTBC catalytic oxidation by copper(II) complexes (1–3) was investigated spectrophotometrically under pseudo-first-order conditions. Catalytic parameters, the maximum reaction rate (v_{max}), Michaelis-Menten constant (K_M), catalytic efficiency, catalytic reaction rate constant (k_{cat}), turnover number (TON), and turnover frequencies (TOF) for complexes 1–3 in DTBC oxidation were collected. The studied complexes 1 and 2 were found to have moderate catalytic activity, while complex 3 does not show catalytic properties.

1. INTRODUCTION

The ability of copper proteins to process dioxygen at ambient conditions has inspired numerous research groups to study their structural, spectroscopic, and catalytic properties. Catechol oxidase (CO), also known as *o*-diphenol oxidase, is a member of the type-3 copper proteins [1]. COs are found in plant tissues and some insects and crustaceans. CO catalyzes exclusively the oxidation of catechols (i.e., *o*-diphenols) to the corresponding *o*-quinones which can rapidly polymerize to form melanin, a dark pigment thought to protect a damaged tissue from pathogens and grants damaged fruits their dark brown coloration [2].

A great number of mononuclear and dinuclear copper(II) complexes have been investigated as biomimetic catalysts for catechol oxidation, regarding the binding of catechol substrate in the first step of the catalytic cycle [3-7]. While no clear relation between the catalytic activity and the redox potential of the copper species has emerged, dinuclear copper complexes are generally found to be more reactive than mononuclear compounds, and a steric match between the dicopper site and the substrate is assumed to be advantageous [8]. The presence of carbonyl oxygen, phenolic oxygen, and/or heteroatom(s) in a heterocyclic ring system makes chalcones excellent chelating ligands for metal coordination. The metal complexes of bidentate chalcone have shown great potential in antiviral, antimalarial, antimicrobial, antioxidant, therapeutic, and catalytic applications [9-13]. Recent studies present that copper(II) chalcone complexes show evidence of catalysis in the oxidation reaction of catechol to *o*-quinone under atmospheric dioxygen [2,14,15]. The catalyzing potential of two novel copper complexes of chalcone derivatives in the oxidation reaction of catechol to *o*-quinone was investigated by Kahrović et al [16].

We report the synthesis of three novel neutral copper(II) complexes (1–3) containing chalcone ligands including the study of their catalytic activity towards the 3,5-di-*tert*-butylcatechol oxidation.

2. EXPERIMENTAL SECTION

2.1 Materials

All used chemicals were commercially available and used as received. Copper(II) acetate hydrate, 2'-hydroxyacetophenone, 4-chlorobenzaldehyde, 4-methylbenzaldehyde, and 4-methoxybenzaldehyde were obtained as reagent-grade chemicals from Sigma. The 3,5-di-*tert*-butyl catechol (DTBC) (98 % *w/w*) was obtained from Sigma. Anhydrous DMSO (water content < 0.005%) and anhydrous methanol (water content < 0.002%) were supplied commercially.

2.2 Physical Measurements

Infrared spectra were recorded as KBr pallets in the 4000 – 400 cm^{-1} region on Perkin Elmer BX FTIR. Electronic spectra were collected in DMSO (5×10^{-5} M) in the 260 – 900 nm range on a Perkin Elmer Lambda 35.

2.3 Ligands Syntheses

All ligands were synthesized by following the standard method of preparation. Chalcones (HL^1 , HL^2 , HL^3) were obtained by the aldol condensation reaction between 2'-hydroxyacetophenone and 4-chlorobenzaldehyde or 4-methylbenzaldehyde or 4-methoxybenzaldehyde with a base catalyst [17].

Synthesis of Chalcone Ligands HL^1 , HL^2 , and HL^3 . In ethanol solution of 4-chlorobenzaldehyde or 4-methylbenzaldehyde or 4-methoxybenzaldehyde (20 mmol) and 2'-hydroxyacetophenone (20 mmol, 2.41 mL) sodium hydroxide (17.2 mL, 5 mol dm^{-3}) was added portion-wise. The reaction mixture was stirred over 24 hours at room temperature. A thick mixture was obtained and acidified by the addition of acetic acid (30% *w/w*) until pH = 6 was reached. The resulting chalcone was isolated by vacuum filtration, washed with water, purified by recrystallization from ethanol solvent, and vacuum dried over silica. Yield: 4.087 g (79%) for HL^1 ; 2.596 g (54%) for HL^2 ; 3.675 g (72%) for HL^3 .

2.4 Syntheses of Copper(II) Complexes, 1–3

Appropriate chalcone ligand (1.0 mmol, 258 mg HL^1 ; 238 mg HL^2 ; 254 mg HL^3) dissolved in methanol (20 mL) was added in a methanolic solution (25 mL) of the dinuclearcopper(II) acetate dihydrate (0.25 mmol, 100 mg). The reaction mixture was refluxed for one hour. After cooling, red substances precipitated. Products were filtered out, washed with ice-cold methanol, and dried under a vacuum.

Complex 1. 176 mg (59%); UV-Vis(DMSO) λ_{max} / nm(log ϵ): 314 (4.26) and 443 (3.53); IR (KBr), ν_{max} / cm^{-1} : 1631 (C=O), 1609 (C=C), 1360 (C–O), 574 (Cu–O).

Complex 2. 163 mg (57%); UV-Vis(DMSO) λ_{max} /nm (log ϵ): 338 (4.55) and 436 (0.55); IR (KBr), ν_{max} / cm^{-1} : 1633 (C=O), 1607 (C=C), 1354 (C–O), 577 (Cu–O).

Complex 3. 102 mg (34%); UV-Vis(DMSO) λ_{max} / nm (log ϵ): 361 (4.68) and 434 (4.36); IR (KBr), ν_{max} / cm^{-1} : 1628 (C=O), 1606 (C=C), 1354 (C–O), 577(Cu–O).

2.5 Catalytic Activity

The catecholase activity of complexes 1–3 was examined by taking DTBC as a model substrate. Catalysis of the DTBC oxidation reaction by copper(II) complexes was estimated spectrophotometrically, in DMSO solution at room temperature under aerobic conditions. Six experiments were performed for each of the complexes, during which the concentration

of the observed complexes ($1.25 \times 10^{-5} \text{ mol dm}^{-3}$, 1000 μL) and the hydrogen peroxide oxidant (30% w/w, 25 μL) was kept constant, while the concentration of the DTBC substrate was varied ($1 \times 10^{-2} \text{ mol dm}^{-3}$, 500 – 25 μL , and DMSO was added up to a volume of 2000 μL). Over 10 minutes, every 30 seconds, an increase in absorption intensity at 400 nm was monitored, originating as a consequence of the formation of 3,5-di-*tert*-butylquinone (DTBQ). Michaelis–Menten method of enzymatic kinetics was applied to obtain Lineweaver–Burk plot and values of K_M , v_{max} , and k_{cat} . The conversion of the reaction rate units from A/s to M/s was done using $\epsilon = 2818 \text{ M}^{-1} \text{ cm}^{-1}$ for 3,5-DTBQ in DMSO with an absorption maximum at 400 nm [18].

3. RESULTS AND DISCUSSION

3.1 Syntheses

Complexes **1–3** contain copper(II) metal centers to which various chalcones (HL^1 , HL^2 or HL^3) are bound as ligands. The synthesis were carried out under reflux in a methanolic solutions, in which both ligand and starting compound, binuclear copper(II) acetate dihydrate, were soluble, while obtained products had poor solubility and therefore can be isolated by filtration. The molar ratio of binuclear copper(II) acetate and appropriate chalcone was 1:4. This stoichiometry was aimed to ensure the coordination of two bidentate anionic chalcone ligands through the carbonyl oxygen atom and the oxygen atom of the deprotonated hydroxyl group (O, O donor atoms) to the copper metal center.

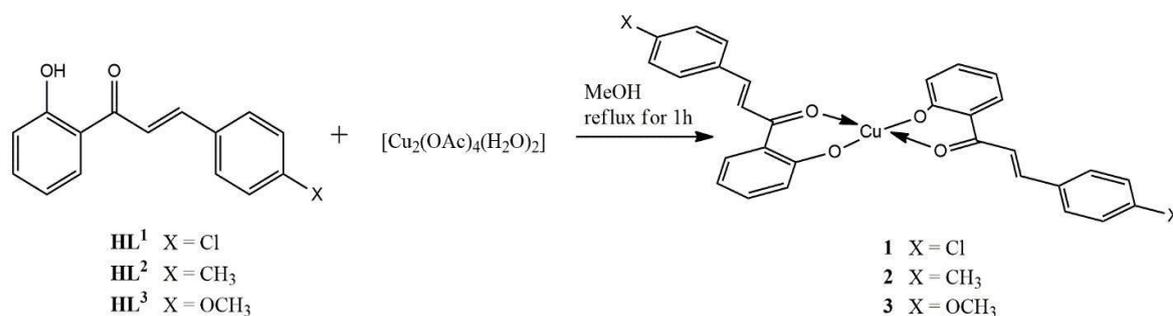


Figure 1. The synthetic route for complexes **1–3**

Complexes of copper(II) with chalcone ligands were characterized based on infrared and electronic spectroscopy.

3.2 Spectroscopic Characterization

Infrared spectra

The infrared spectra of complexes (**1 – 3**) have bands at similar positions, which indicates their structural similarities.

Chalcones are bounded to the copper(II) metal center as bidentate anionic ligands *via* the deprotonated hydroxyl oxygen atom and the carbonyl oxygen atom. After ligand coordination, strong intensity bands assigned to the stretching vibrations of the carbonyl groups (C=O) and C=C stretching vibrations, shifted to lower wave numbers: 1631 and 1609 cm^{-1} for **1**; 1633 and 1607 cm^{-1} for **2**; 1628 and 1606 cm^{-1} for **3**, compared to the free ligands (Figure 2). The coordination of chalcone ligands is further indicated by the shift of the C–O stretching vibrations to higher wave numbers in the spectra of complexes (1360 cm^{-1} for **1** and 1354 cm^{-1} for **2** and **3**) compared to the spectra of free ligands (1339 cm^{-1} for HL^1 , 1341 cm^{-1} for HL^2 and 1344 cm^{-1} for HL^3) [19].

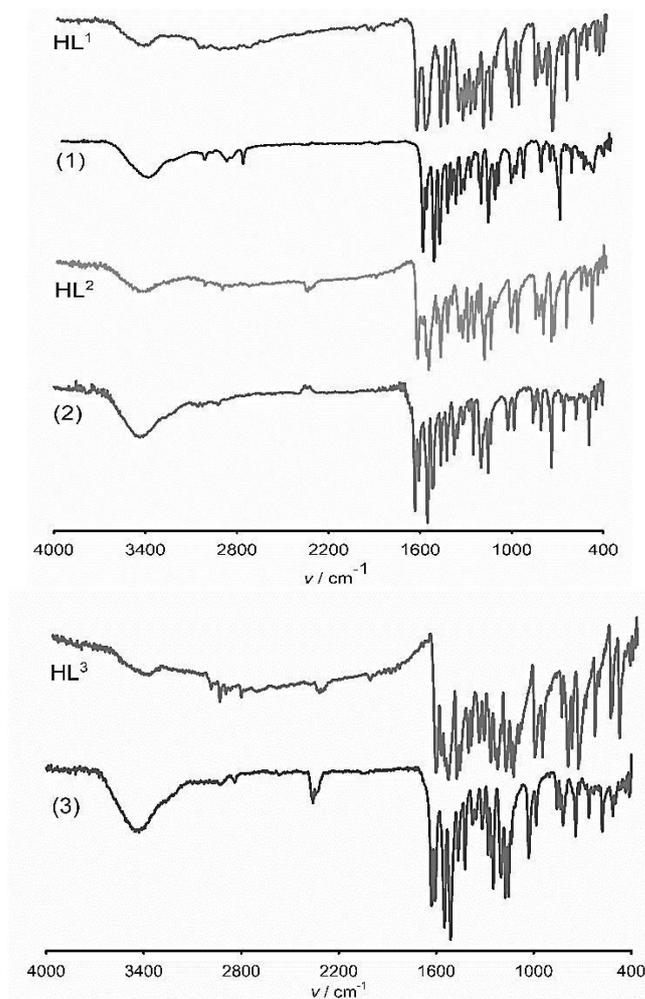


Figure 2. Infrared spectra of copper(II) complexes (1–3) and free chalcone ligands (HL¹ – HL³)

In the low-frequency region in spectra of all complexes, a new band, attributed to stretching vibrations of the newly formed Cu–O bond, appeared. Cu–O stretching vibration appeared at 574 cm⁻¹ for **1**; 577 cm⁻¹ for **2**; 577 cm⁻¹ for **3**.

Table 1. The most important vibrational bands in FTIR spectra of chalcone ligands and complexes of copper(II) 1–3

Compound	$\nu(\text{O-H})$	$\nu(\text{C-H})_{\text{aromatic}}$	$\nu(\text{C-H})_{\text{aliphatic}}$	$\nu(\text{C=O})$	$\nu(\text{C=C})$	$\nu(\text{C-O}_{\text{enol}})$	$\nu(\text{Cu-O})$
cm ⁻¹							
HL ¹	3547	3066, 3021		1641	n.o.	1339	
(1)		3061, 3010	2916, 2884	1631	1609	1360	574
HL ²	3521	3061, 3025		1639	1615	1341	
(2)		3057, 3022	2921, 2869	1633	1607	1354	577
HL ³	3527	3074, 3023		1642	1610	1342	
(3)		3066, 3029	2929, 2842	1628	1606	1354	577

Electronic spectra

Electronic spectra of complexes and corresponding ligands were recorded in DMSO solutions. After coordination of the chalcone ligands HL¹ and HL², the bands ascribed to the carbonyl group (314 nm for HL¹ and 338 nm for HL², Figure 3(a), appeared on similar positions in the spectra of corresponding complexes **1** and **2**, which indicated coordination *via* a carbonyl oxygen atom. The band in the HL³ spectrum at 370 nm, after coordination on the copper(II), shifted to a lower wavelength, 361 nm (complex **3**), also indicating the participation of the carbonyl group in coordination with the metal center. In the spectra of all three complexes, after coordination of the chalcone ligands, new bands appeared with maxima in the 430–450 nm region, Figure 3(b). Based on their positions and extinction coefficients they could be identified as charge transfer bands.

Table 2. Data on electronic spectra of chalcone ligands and corresponding copper(II) complexes **1–3**

Compound	n→π*	CT
	nm (ε)	
HL ¹	314 (4.26)	
(1)	314 (4.17)	443 (3.53)
HL ²	338 (4.35)	
(2)	338 (4.55)	436 (4.04)
HL ³	370 (1.23)	
(3)	361 (4.68)	434 (4.36)

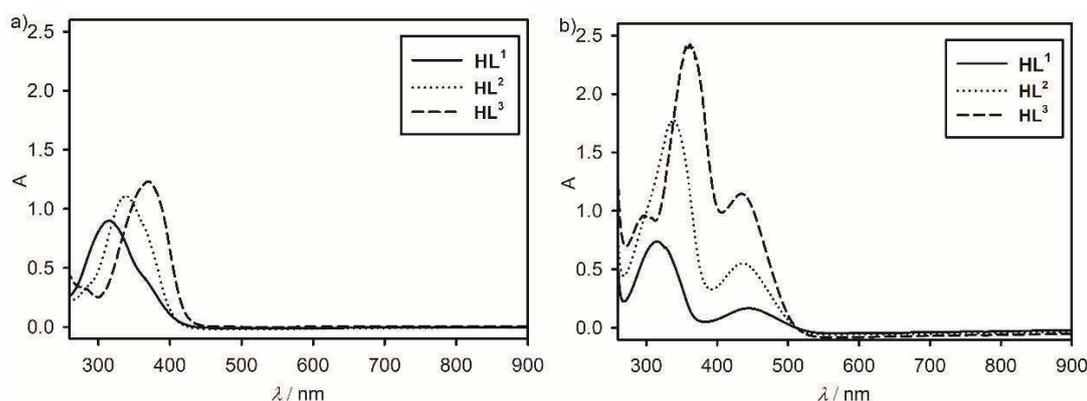


Figure 3. Electronic spectra of free ligands HL¹–HL³(a) and complexes **1–3**(b)

3.3. Spectroscopic Evidence of the Catalysis

Catalytic activities of complexes **1–3** were investigated by spectroscopic measurements. DTBC was used as a substrate in all experiments due to its low oxidation potential and absence of any concomitant or successive reaction upon its oxidation to DTBQ.

In all three cases, the reaction mixture containing DTBC, hydrogen peroxide, and the observed complex showed an absorbance increase of the quinone band near 400 nm ($\lambda_{\text{max}} = 400 \text{ nm}$; $\epsilon = 2818 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$). In the absence of copper(II) complexes, no significant change in the spectrum of the DTBC was observed, which confirms the catalytic nature of this oxidation process. Complexes **1–3** are stable in DMSO solutions and their activity can be ascribed to the originally formulated complex species.

3.4 Kinetic Measurements

Kinetic measurements of the catalytic activity of complexes **1–3** were carried out in a DMSO solution using DTBC as a substrate. The chemical reaction kinetics of the oxidation of DTBC to DTBQ is pseudo-first-order and was investigated using the method of initial rates (Figure 4). The concentrations of the complexes and co-oxidant were kept constant, while the concentration of the substrate was varied.

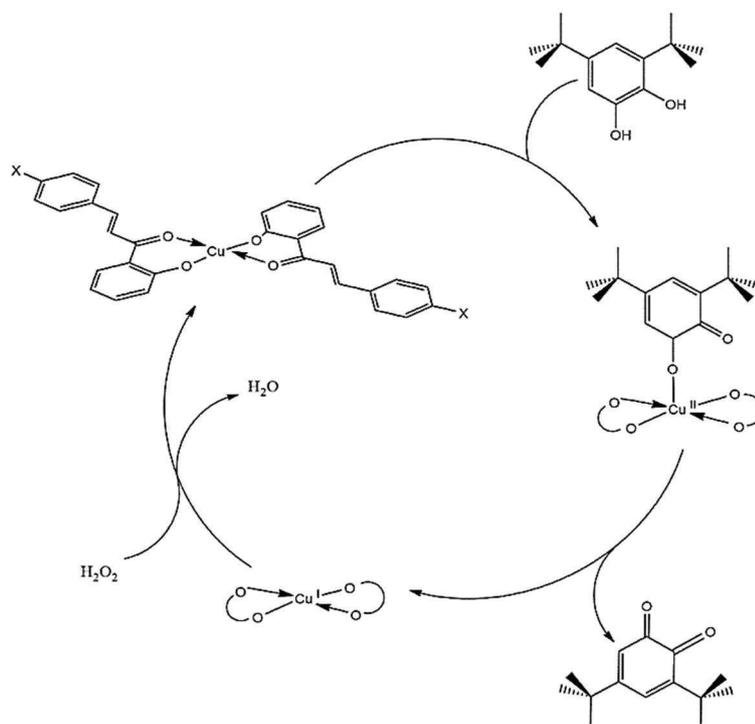


Figure 4. Possible reaction pathway for DTBC oxidation in the presence of complexes **1–3**

Michaelis–Menten method of enzymatic kinetics was applied to obtain Lineweaver–Burk plot and values of catalytic parameters, the maximum reaction rate (v_{\max}), Michaelis–Menten constant (K_M), catalytic efficiency, catalytic reaction rate constant (k_{cat}), turn over number (TON), and turn over frequencies (TOF) for synthesized complexes.

Table 3. Catalytic parameters of complexes **1** and **2** for DTBC oxidation

Complex	$v_{\max} / 10^{-7} \text{ mol dm}^{-3} \text{ s}^{-1}$	$K_M / 10^{-4} \text{ mol dm}^{-3}$	Efficiency / $\text{mol}^{-1} \text{ dm}^{-3} \text{ s}^{-1}$	$k_{\text{cat}} / \text{h}^{-1}$	TON	TOF/ 10^{-2} s^{-1}
1	5.06	4.23	191	291.72	54.3	9.04
2	4.20	2.08	324	241.89	42.1	7.02

Based on the conducted research, it was concluded that complexes **1** and **2** catalyze the oxidation reaction of DTBC to DTBQ and obey Michaelis–Menten kinetics in the range of observed concentrations, while complex **3** did not act as a catalyst for the mentioned reaction. This can be explained as a consequence of the significant substituent effect. Complex **3** containing chalcone ligand with electron-donating methoxy substituent in *para* position HL³ did not show catalytic properties. Complex **2**, including a methyl group as a substituent on the chalcone ligand, showed higher catalytic efficiency and greater affinity for binding to the DTBC substrate compared to complex **1** containing chloro substituent. Reaction rate constants k_{cat} are most often used to compare the catalytic properties of observed catalysts. Complexes **1** and **2** could be compared with other examples of copper complexes possessing catalytic properties (Table 4).

Table 4. Comparison of complexes **1** and **2** with some reported catecholase-like biomimetic complexes

Complex	Solvent	$k_{\text{cat}} / \text{h}^{-1}$	Ref.
$[\text{Cu}_2(\text{L})(\mu\text{-OH})(\text{H}_2\text{O})(\text{ClO}_4)_2]$	DMSO	76	[20]
$[\text{Cu}_2(\text{L}_2)(\mu\text{OAc})](\text{ClO}_4) \times \text{H}_2\text{O} \times (\text{CH}_3)_2\text{CHOH}$	MeOH	183	[15]
$[\text{Cu}_2(\text{HL}_1)_2(\mu\text{HL})_2]$	MeOH	752	[16]
$[\text{Cu}_2(\text{H}_2\text{L}-(\text{CH}_3)_{11})(\text{OH})(\text{H}_2\text{O})(\text{NO}_3)]^{3+}$	MeOH	3.24×10^4	[21]
$[\text{Cu}(\text{phen})(\text{OH}_2)_2(\text{NO}_3)](\text{NO}_3)$	MeOH	3.91×10^3	[22]
$[\text{CuL}(\text{NCO})]$	MeOH	23.5	[23]
1	DMSO	291	This work
2	DMSO	241	This work

The TON value represents the number of moles of substrate that a mole of catalyst can convert before it becomes inactivated and it is given as the ratio of the DTBQ and complex concentration at the defined time (10 min). Although the k_{cat} value for **2** is higher than for **1**, a higher TON value suggests that **1** is the more promising catalyst due to the higher conversion rate of DTBC to DTBQ for 3% as compared to complex **2**.

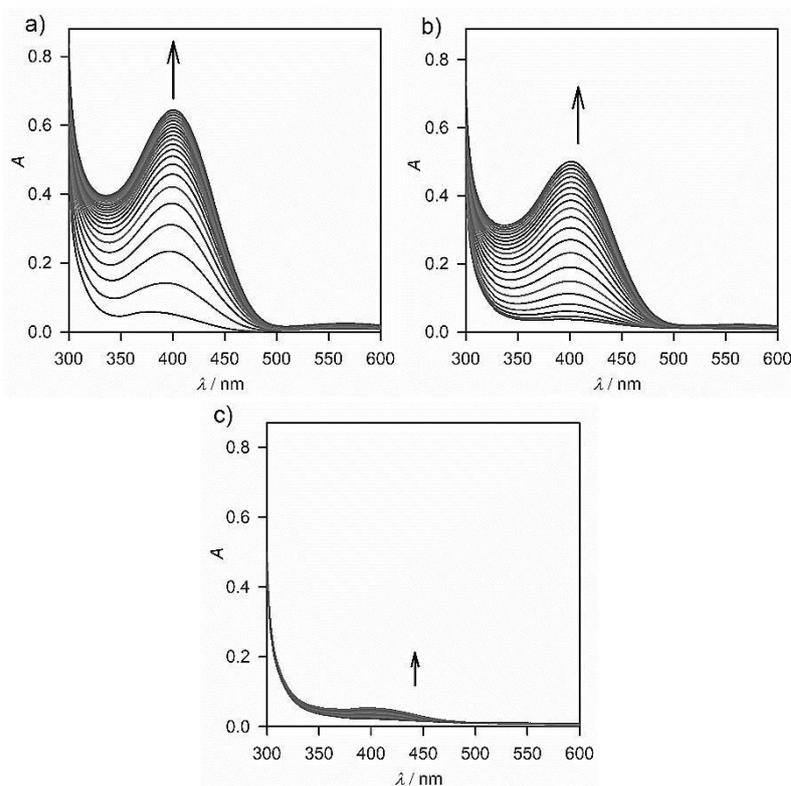


Figure 5. Changes in the absorption spectra in the course of catalytic oxidation of DTBC ($2.5 \times 10^{-3} \text{ mol dm}^{-3}$) by complexes **1** (a), **2** (b) and **3** (c) ($6.28 \times 10^{-6} \text{ mol dm}^{-3}$) in the presence of hydrogen peroxide (0.12 mol dm^{-3}) in DMSO at room temperature during 10 minutes

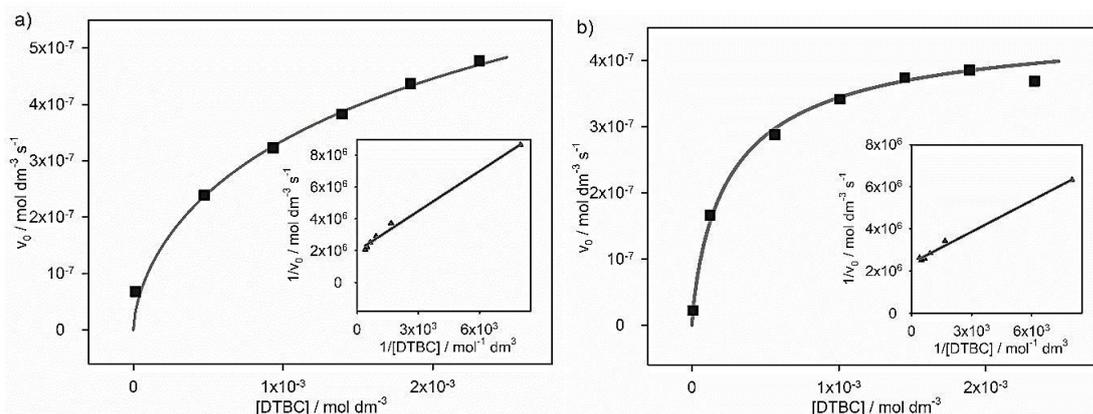


Figure 6. Michaelis-Menten saturation curve: Dependence of the initial rate of oxidation vs DTBC concentration in the presence of the complexes **1** (a) and **2** (b). Inset: Lineweaver-Burk plot

4. CONCLUSION

Three new copper(II) complexes were prepared by solution synthesis from copper(II) acetate and appropriate chalcone ligands (HL¹, HL² and HL³). Infrared spectroscopy suggested coordination of the chalcones as bidentate anionic ligands to the metal center *via* the deprotonated hydroxyl oxygen atom and the carbonyl oxygen atom. Electron spectroscopy showed the formation of new bands in the spectra of products that were assigned to charge transfer bands of the new copper(II) chalcone complex species. Catalytic measurements revealed moderate catalytic properties of complexes **1** and **2**, while complex **3** did not catalyze the DTBC oxidation reaction. TON and TOF values suggested that **1** is the more promising catalyst due to the higher conversion rate of DTBC to DTBQ as compared to complex **2**.

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