ORIGINAL ARTICLE



Synthesis and Characterization of Two Novel Oxazol-5-ones Derivatives and Their Multifunctional Properties; pH Sensitivity, Electropolymerizability and Antiproliferative Activity

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Abstract

The aim of this study is to synthesize oxazol-5-one derivatives, which have multi-functional properties. Nomenclatures of newly synthesized molecules are 4-(4-N,N-diethylaminophenylmethylene)-2-(3-thienyl)oxazol-5-one (4a) and 4-(4-(1,4,7,10-tetraoxa-13-azacyclopentadecyl)phenylmethylene)-2-(3-thienyl)oxazol-5-one (4b). These two novel derivatives contain pH sensitive and polymerizable groups. 3-Thienyl group was attached to position-2 of the oxazol-5-one ring to provide electrochemical polymerization capability. pH sensing properties were provided by attaching p-N,N-diethylaminophenylmethylene and p-aza-15-crown-5-phenylmethylene groups to the arylmethylene moiety at position-4 of the ring. Target molecules were synthesized by classical process known as Erlenmeyer-Plöchl Azlactone Synthesis Erlenmeyer (Justus Liebigs Ann Chem 275:1-12, 1893), Rodrigues et al. (J Chem Educ 92:1543-1546, 2015). After structural characterization of 4a and 4b, absorption and emission characteristics were determined in solvents that have different polarities. Difference in maximum absorption and emission wavelengths of the molecules related to solvent polarities were observed at around 6–7 nm and 35–36 nm respectively. In pH studies of the target derivatives in PVC polymer matrix, ratiometric changes were observed at isosbestic point around 398 nm. Polymeric depositions of the molecules (4a, 4b) were proved by using cyclic voltammetry, electrochemical impedance spectrometry studies and scanning electron microscope images. MTT assay studies showed significant results like, 4b derivative's strong cytotoxic activity on PC-3 (cancerous cell line) with IC₅₀ value of 12.57 \pm 0.41 μ g/ml without exhibiting any cytotoxic effect on HEK293 (healthy cell line).

Keywords Oxazol-5-one · Thiophene · Electropolymerization · Cytotoxicity

Introduction

In this study, two novel oxazol-5-one derivatives, containing ionophor group and polymerizable group were synthesized according to Erlenmeyer Plöchl method [1–5]. These compounds,

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biosensors [6, 7], fluorophores [8], electro photographic photo-receptors and in non-linear optical materials [8–10].
As is known in the literature, aza-crown ethers are susceptible to specific cations [11–15]. For example, the monoazacrown structures bound to fluorophores as an iono-

phore group were synthesized and their ability to complex with various cations were investigated for many years [16–22].

known as unsaturated oxazol-5-one class, are considered as cyclic derivatives of the amino acids. Due to their favourable

optical properties, they have been used in many studies on

In the articles published, studies on development of pH, carbon dioxide and cation-sensitive optical sensors were performed using different oxazol-5-one derivatives. For example, transparent films of the sol-gel matrix / polyvinyl chloride (PVC) matrix of 4-(p-N,N-dimethylaminophenylmethylene)-2-phenyl-5-oxazolone were prepared and optical pH / carbon dioxide sensors were developed [8–10, 23]. In addition, carbon dioxide sensor was developed in ethyl cellulose matrix by using carbazole side group containing oxazole-5-one derivative [24]. Furthermore, the photo physical and photochemical properties of the 4-(2-furylmethylene)-2-phenyl-5-oxazolone derivative in different solvents and in solid matrix of polyvinyl chloride (PVC) have been investigated. Determination of Fe³⁺ ion has been carried out by using this derivative [25]. In addition, biosensor applications with oxazole-5-one derivatives bearing monoaza-15-crown-5 group were studied. Determination of acetylcholine (ACh), glucose and Donepezil concentrations were performed on PVC and solgel matrices [6, 8].

Sensor applications generally based on process of doping the molecules into a polymeric matrix contained plasticizer [26-32]. The biggest challenge in sensor studies using fluorophore organic dyes is the reduction in molecular response capacity due to sensor preparation. The main causes of matrix incompatibility are aggregation, damping by energy-electron transfer to the matrix components and reduction of sensitivity of the formed matrix films.

Besides oxazol-5-ones' sensor application in polymer matrices, the properties of being super capacitor have also become the focus of recent studies. Electropolymerization studies of polythiophene-based derivatives are dramatically increasing due to their potential to be used as electrode material for super capacitors. Polythiophenes, which are electrically conductive polymers, have been modified with organic molecules to improve electrochemical energy storage properties and to increase specific capacitances that are crucial to alternative energy storage systems. Organic molecular polymerization studies are also being carried out by using thiopheneoxazol-5-one-thiophene conjugating systems for capacitance enhancement studies [33–35].

Based on the studies mentioned above, synthesis of new, polymerizable group containing oxazole-5-one derivatives (4a, 4b) that can be used as pH probes has been decided in this project. In this respect, disadvantages of polymer matrix can be eliminated by direct polymerization of the molecules without the need for any immobilization process in polymer matrices was thought.

Besides to widely studied sensor and spectroscopic applications, oxazole-5-one derivatives show important biological activities such as antifungal, antimicrobial, anticancer, neuroleptic [36–40] as they are ring derivatives of Nacylaminoacids. Oxazol-5-one derivatives with antitumor properties pose special interest and have been studied in recent years [37]. Main importance of oxazol-5-one derivatives cytotoxic activity, although their lesser potency, is that unlike most of commercial chemotherapy agents, oxazol-5-one derivatives can have specific inhibition to only one kind of cancerous cell line and have very small or none cytotoxicity towards healthy human cell line.

As a result, sensor probe properties against pH, responses to electropolymerization and cytotoxic activities of 4a and 4b had been examined. These multifunctional novel derivatives have been shown in Fig. 1.

Experimental

Material and Instrumentation

All chemicals and PVC membrane components were purchased from Carlo Erba, Merck, Fluka and Sigma Aldrich. Polyester support (Mylar type) provided by Du Pont. PVC matrices were prepared according to the previous literature [9]. All solvents used were at spectroscopic grade. Melting points were determined by Barnstead Electrothermal 9,100 instrument. Mass spectra were carried out with TripleTOF®4600 system. FT-IR spectra were recorded by Perkin-Elmer Spectrum BX FTIR spectrometer using KBr pellets. NMR data were collected by Varian 3.2 400 MHz spectrometer in CDCl₃ solutions and chemical shifts expressed in ppm downfield from tetramethylsilane standard. Absorption spectra and emission-excitation spectra were recorded by Shimadzu UV-1601 spectrophotometer and Varian-Cary Eclipse spectrofluorometer, respectively. SEM Image analyses were done by Carl Zeiss 300VP SEM instrumentation. Voltammetric analyses were done by Autolab PGSTAT 204. Electrochemical impedance spectrometric (EIS) measurements were carried out with the AUTOLAB PGSTAT-30 electrochemical analysis system (Eco Chemieans The Netherlands). The triple electrode system











was completed with the pencil graphite electrode, the Ag / AgCl reference electrode and the Pt auxiliary electrode.

Synthesis

3-thienylcarbonylchloride (1) and N-(3-thienylcarbonyl) glycine (2)

N-(3-thienylcarbonyl)glycine, which is a derivative of hippuric acid, was synthesized according to literature [41, 42]. (1): 3.0 g (23 mmol) 3-thiophenecarboxylic acid, 23 mL toluene 1.73 mL (30 mmol) thionylchloride. Synthesized crude product of 1 was directly used without purification to synthesize 2 (Fig. 2). (2): 3-thienylcarbonylchloride (crude product),23.0 mL 10.0% NaOH, 1.73 g (23 mmol) glycine, 2N HCl (for neutralisation). Product was collected as white opaque crystals. Yield 54%, m.p.: 181-183 °C, FT-IR (KBr, v (cm^{-1}) : 3400 – 2400 broad (-O-H_{stretch}), 3107 (= C-H_{stretch}), 1681 (C = O_{stretch}), 1277 (O-C = O_{stretch}), ¹H NMR (DMSOd₆, CDCl₃, 400 MHz, δ (ppm)): 12.25 (1H, br s, -COOH), 8.04 (1H, s, -NH), 7.97 (1H, dd, thiophenyl), 7.43 (1H, dd, thiophevl), 7.24 (1H, dd, thiophenvl), 3.97 (2H, d, J = 5.8 Hz, -CH₂), ¹³C NMR (DMSO-*d*₆, CDCl₃, 400 MHz, δ (ppm)): 176.49 (-COOH), 167.81 (-CONH-), 142.01 (thiophenyl-C₃), 133.55 (thiophenyl-C₂), 131.69 (thiophenyl-C₅), 130.72 (thiophenyl-C₄), 46.01 (-CH₂-).

4-formylbenzo-aza-15-crown-5 (3)

The commercially available N-phenyl-aza-15-crown-5 with phosphoroxychloride in dimethylformamide (DMF) was used to attach the aldehyde group to the para position of the benzene ring. This reaction was performed according to the article that studied by Mateeva N. et al. [43]. Structural

Fig. 3 Synthetic scheme of 3 iii): POCl3/DMF "Vilsmeier-Haake Reaction"

characterization of the compound 3 was carried out only by ¹H NMR technique which is enough for identification of the structure. 0.31 mL (3.4 mmol) POCl₃ was added dropwise to precooled (-10.0 °C), 1.0 g (3.4 mmol) of N-phenyl-(aza-15-crown-5) dissolved in 3.0 mL (40 mmol) DMF. The mixture was first stirred at room temperature, and then it was heated to 100.0 °C for four hours until the reaction completed (Fig. 3). After work up process, the product was directly used in next step. Yield 90.5%, m.p.: 85-87°C, ¹H NMR (CDCl₃, 400 MHz, δ , (ppm)): 9.65 (1H, s, aldehyde), 7.62–7.68 (2H,d, J = 8.8 Hz, phenyl), 6.62–6.68, (2H, d, J = 9.2 Hz, phenyl), 3.6–3.65, (4H, m, crown ether), 3.71–3.76, (4H, m, crown ether), 3.6–3.59, (4H, s, crown ether).

Synthesis of 4 Derivatives (4a and 4b)

Aldehyde (1 eq.), glycine derivative (1 eq.), sodium acetate (1 eq.) and acetic anhydride (3 eq.) mixture was stirred at room temperature for 10 min then at 85–90 °C for 4–6 h. After this step, mixture cooled down to room temperature and products were precipitated by using cold ethanol. Obtained precipitates were filtered and washed with cold ethanol then with hot water.

4-(4-N,N-diethylaminophenylmethylene)-2-(3-thienyl) oxazol-5-one (4a)

0,72 g, (4.1 mmol) 4-N,N-dimethylaminobenzaldehyde, 0.75 g (4.1 mmol) 3-thienylcarbonyl glycine, 0.34 g (4.1 mmol) sodium acetate and 6 mL acetic anhydride (Fig. 4). Product was collected as orange crystal. Yield 56%, m.p: 197–200 °C, ¹H NMR (DMSO- d_{6} -CDCl₃, 400 MHz, δ , (ppm)): 8.09 (1H, dd, J=3.2 Hz, 1.2 Hz, thiophenyl), 8.07



Fig. 4 Synthetic scheme of 4a vi): (CH3CO)2O/CH3COONa



(2H, d, J = 9.2 Hz, phenyl), 7.72 (1H, dd, J = 4.8 Hz, 1.2 Hz, thiophenyl), 7.41 (1H, dd, J = 4.8 Hz, 1.2 Hz, thiophenyl), 7.17 (1H, s, =C<u>H</u>-, methine), 6.71 (2H, d, J = 9.2 Hz, phenyl), 3.45 (4H, q, J = 7.2 Hz, -C<u>H</u>₂-CH₃), 1.23 (6H, t, J = 7.2 Hz, -CH₂-C<u>H₃), ¹³C NMR (DMSO-*d*₆,-CDCl₃, 400 MHz, δ , (ppm): 168.44 (-<u>C</u>₅ oxazolone), 156.78 (-<u>C</u>₂ oxazolone), 150.03 (-<u>C</u>₄ oxazolone), 135.01 (phenyl), 133.10 (thiophenyl), 129.48 (thiophenyl), 128.95 (phenyl), 127.49 (phenyl), 129.54 (=<u>C</u>H-) 44.60 (-<u>C</u>H₂-CH₃), 12.56 (-CH₂-<u>C</u>H₃), 1C-ESI-QTOF MS: m/z Found: [M + H]⁺ 327.1253; 'molecular formula C₁₈H₁₈N₂O₂S' requires [M + H]⁺ 327.1167.</u>

4-(4-(1,4,7,10-tetraoxa-13-azacyclopentadecyl) phenylmethylene)-2-(3-thienyl)oxazol-5-one (4b)

1.0 g, (3.1 mmol), 4-(1,4,7,10-tetraoxa-13azacyclopentadecyl) benzaldehyde, 0.57 g (3.1 mmol) 3thienyl carbonyl glycine, 0.25 g (3.1 mmol) sodium acetate and 5 mL acetic anhydride (Fig. 5). Product has orange-red crystal appearance. Yield 48%, m.p: 247-249 °C, FT-IR (KBr, v,(cm⁻¹)): 1771 (<u>C = O_{stretch}</u>), 1602 (<u>C = N_{stretch}</u>), 1124 (-<u>C-O_{stretch}</u>), ¹H NMR (DMSO-*d₆*,-CDCl3, 400 MHz, δ, (ppm)): 8.10 (1H, dd, thiophenyl), 8.07 (2H, d, phenyl), 7.72 (1H, dd, thiophenyl), 7.42 (1H, dd, thiophenyl), 7.17 (1H, d, =CH-, methine), 6.72 (2H, d, phenyl), 3.80 (4H, t, crown ether), 3.69 (4H, t, crown ether), 3.67 (8H, s, crown ether), 3.63 (4H, s, crown ether), 13 C NMR (DMSO- d_6 ,-CDCl₃,400 MHz, δ, (ppm)): 168.35 (-C₅ oxazolone), 157.14 (-C2 oxazolone), 150.13 (-C4 oxazolone), 134.82 (phenyl), 132.82 (thiophenyl), 128.89 (thiophenyl), 128.02 (phenyl-<u>C1</u>), 121.74 (phenyl-<u>C4</u>), 126.92 (thiophenyl), 126.35 (thiophenyl), 111.72 (phenyl), 129.70 (= CH-), 71.31 (2C,

Fig. 5 Synthetic scheme of 4b vi): (CH3CO)2O/CH3COONa

crown ether), 70.29 (2C, crown ether), 69.99 (2C, crown ether), 68.25 (2C, crown ether), 52.83 (2C, crown ether), LC-ESI-QTOF MS: m/z Found: $[M + H]^+$ 473.1760; 'molecular formula $C_{24}H_{28}N_2O_6S$ ' requires $[M + H]^+$ 473.1746.

Spectrophotometric Studies

Absorption, emission and excitation spectra of synthesized derivatives 4 in six different solvents were recorded and maximum wavelength values were determined. The combined absorption spectra of the derivative 4a in six different solvents are given in Fig. 6. Stokes' shift values, molar absorption coefficients, fluorescence quantum yields, radiative lifetimes, fluorescence lifetimes, radiative and non-radiative rate constants were calculated (Table 1). Fluorescence quantum yields (Φ_F) were determined by the comparative method [44, 45]:

$$\Phi F = \Phi std \times (F \operatorname{Astd} \eta^2) / (F \operatorname{std} A \eta \operatorname{std}^2), \tag{1}$$

where F and F_{std} are the areas under the fluorescence emission curves of the samples and the standard, respectively. A and A_{std} are the absorbances of the samples and standard at the excitation wavelength and η and η_{std} are the refractive indexes of solvents used for the samples and standard, respectively. Rhodamine 101 (in ethanol) ($\Phi = 1$) [46, 47] was employed as the standard.

The radiative lifetime, τ_0 , was estimated by the formula [48, 49] assuming the lowest singlet state is the only fluorescent state:

$$\tau_0 = 3.5 \times 10^8 / \nu max^2 \varepsilon max \Delta \nu_{1/2} \tag{2}$$





Fig. 6 UV-Vis absorption spectra of 4a and 4b in six different solvents

where ν_{max} is the wave number in cm⁻¹, ε_{max} is the molar extinction coefficient at the selected absorption wavelength, and $\Delta \nu_{1/2}$ is the half width of the selected absorption in wavenumber units of cm⁻¹. The fluorescence lifetime (τ_f) is calculated by the formula given below:

$$\Phi F = \tau f / \tau_0, \tag{3}$$

The radiative (k_r) and non-radiative (k_{nr}) rate constants were calculated from the fluorescence quantum yields and radiative lifetimes by using:

$$\mathbf{kr} = \Phi \mathbf{F} / \tau \mathbf{f} = 1 / \tau_0 \text{ and } \mathbf{knr} = (1 - \Phi \mathbf{F}) / \tau \mathbf{f}, \tag{4}$$

In this part of the work, polyvinylchloride (PVC) films were prepared to determine the response of derivatives 4 to hydrogen ion, according to previous conventional studies [9] and the performance of the responses against pH was demonstrated. The absorption studies of derivatives 4 prepared in PVC matrix in $H_2PO_4^-$ / HPO_4^{2-} buffer at pH 1.0–7.0 are given in Fig. 7.

Voltammetric Studies

Firstly, 0.14 g sodium perchlorate monohydrate was dissolved in 10 mL acetonitrile. Thereafter 3.6 mg 4a was added to mixture and dissolved. 2.5 mL of this solution was transferred into the voltammetric cell where a pencil graphite electrode (Faber castel, 2B, 0.7 mm) was the working electrode. The three-electrode system was completed with Ag/AgCl as the reference electrode platinum wire as the counter electrode. Electropolymerization process was carried out by cycling the potential in range of -0.5–1.5 V at a scan rate of 50 mV/s and polymeric film depositions were inspected by electrochemical impedance spectroscopic (EIS) measurements. SEM studies were also carried out for further surface inspection of polymeric film deposition.

Cytotoxicity Studies

PC-3 (human prostate adenocarcinoma), A549 (human alveolar adenocarcinoma), SHSY-5Y (human neuroblastoma) and as a normal cell line HEK293 (human embryonic kidney cells) were used for testing cytotoxicity of 4a and 4b derivatives. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium F12 (DMEM/F12), supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/mL of penicillin and 100 μ g/mL of streptomycin (Lonza, Visp, Switzerland). The cells were incubated at 37 °C in a humidified atmosphere of 5% CO₂. The cells were sub-cultured twice a week and cells in the exponential growth phase were used in the experiments.

Cytotoxicity of samples were determined using a modified MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide)] assay, which detects the activity of mitochondrial reductase of viable cells [50]. In order to perform the cytotoxicity assay, all cell lines were cultivated for 24 h in 96-well microplates with an initial concentration of 1×10^5 cells/mL in a humidified atmosphere with 5% CO₂ at 37 °C. Then, the cultured cells were treated with different dilutions of samples (4a and 4b derivatives) (0.5, 5, 50 µg/ml) followed by incubation for 48 h at 37 °C. Doxorubicin was used as positive control. The optical density of the dissolved material was measured at 570 nm spectrophotometer (Thermo Multiskan Spectrum). The viability (%) was determined by the following formula:

% Viable cells= [(absorbance of treated cells) (absorbance of blank)] / [(absorbance of control) – (absorbance of blank)] \times 100.

Determination of half-minimal inhibitory concentration (IC₅₀), cytotoxicity was expressed as mean percentage increase relative to the unexposed control \pm SD. Control values was set to 0% cytotoxicity. The IC₅₀ values were reported at \pm 95% confidence intervals (\pm 95% CI). The analyses were performed by using Graph Pad Prism 5 software (San Diego, ca., USA).

Table 1 Maximum wavelengths (λ : nm,), Stokes' shift values ($\Delta\lambda$: nm), molar extinction coefficients (ε : mol⁻¹dm³cm⁻¹), fluorescence quantum yields (ϕ_F), radiative lifetimes (τ_0 : ns), fluorescence lifetimes

 $(\tau_f; p_s)$, radiative (k_r) and nonradiative (k_{nr}) rate constants obtained from absorption, emission and excitation spectra of 4a and 4b in six different solvents

	Solvent	λ_{\max}^{abs} . (nm)	λ_{\max}^{f} (nm)	λ_{\max}^{ex} (nm)	Δλ (nm)	$\epsilon mol^{-1}dm^{3} cm^{-1}x(10^{4})$	$\varphi_{\rm F}$	$\tau_0(ns)$	$\tau_f(ps)$	k _r	k _{nr}
4a	THF	460	511	478	51	7.29	0.0065	0.067	0.434	14.92	2287.2
	DCM	467	517	481	50	6.31	0.0046	0.077	0.353	12.92	2820.8
	ACN	460	527	479	67	8.92	0.0041	0.056	0.228	17.97	4375.6
	DMF	467	530	481	63	6.75	0.0074	0.077	0.570	13.00	1742.0
	Ethanol	461	517	481	56	5.09	0.0044	0.101	0.446	9.92	2230.4
	Toluene	461	494	472	33	7.38	0.0040	0.061	0.243	16.52	4094.2
4b	THF	456	509	472	53	8.69	0.0052	0.050	0.261	19.90	3815.1
	DCM	460	514	479	54	9.57	0.0037	0.051	0.190	19.57	5256.6
	ACN	453	521	459	68	9.14	0.0040	0.049	0.194	20.52	5134.2
	DMF	462	527	480	65	8.68	0.0063	0.055	0.346	18.15	2872.4
	Ethanol	455	514	476	59	8.68	0.0032	0.053	0.172	18.72	5811.6
	Toluene	457	492	568	35	9.36	0.0040	0.040	0.158	25.10	6313.9



Fig. 7 Absorption spectra of 4a and 4b at various pH values

Results and Discussions

Within the scope of this study, at first pH sensitivity and probe characteristics of the newly synthesized derivatives 4 have been investigated by UV-Vis absorption and emission spectroscopies, secondly electropolymerization and copolymerization studies have been carried out by cyclic voltammetry. Moreover, biological activities have been determined by cytotoxicity tests. Since derivatives 4 contain groups with similar electronic effects, no significant changes dependent to solvent polarities were observed in extracted data. Differences in maximum absorption and emission wavelengths of the molecules related to solvent polarities were observed at around 6–7 nm and 35–36 nm respectively. The longest absorption and emission wavelengths were detected in DMF and the highest Stokes' shifts were determined in ACN (Table 1).

In pH studies of the target derivatives in PVC polymer matrix, ratiometric changes were observed at isosbestic point around 398 nm. While the absorption intensity at 450–480 nm

was decreasing, a new absorption band occurred at 330–390 nm depending on the pH value. With the results obtained from pH studies it has been proven that these molecules can be used in preparation of pH-sensor probe.

Voltammetric Behaviour of 4a Compound

Figure 8 shows voltammogram obtained by 7 consecutive cycles. In these voltammograms, two oxidation peaks were observed at 0.8 and 1.3 V in anodic scan. Small reduction peaks were observed at 1.2 V, 0.9 and 0.4 V in cathodic scan indicating a quasi-reversible redox mechanism is involved. In addition, a polymeric deposition of the species can be established from the successive increase in the peak currents.

Another proof of the polymeric film deposition can be obtained by electrochemical impedance spectroscopic (EIS) measurements. Deposition of a vast range of coatings including polymeric structures on metallic or carbon based surfaces can be tested via EIS measurements. The free ions in the deposition solution penetrate into the polymeric structure and alter the insulating structure of the polymer, which modifies the impedance characteristics of the overall conductive surface/polymer element. In the present study, the EIS measurements have utilized to monitor the charge transfer resistance (R_{ct}) of bare electrode before and after the electropolymerization. The Rct value of bare electrode about 1299 ohm decreased to 106 ohm in the presence of 4a. This can be interpreted as the conductive polymer deposited on the surface significantly reduces the surface resistance. Similar results have been obtained in the presence of thiophene (178 ohm) and EDOT (14 ohm) (Table 2).

Close inspection of the surface has revealed the porous and clustered polymeric structure of the deposit (Fig. 9a). In addition to the homopolymer of 4a, its copolymers with thiophene and EDOT were synthesized electrochemically on a pencil graphite electrode by applying the same procedure. SEM images of these co-polymers can be seen in Fig. 9b and c, respectively.

For the former species, the 4a polymeric clusters can be detected on the cauliflower-like porous structure of polythiophene and for the latter, it was more apparent on the finely distributed deposits of PEDOT co-polymers. Similar studies were repeated for molecule 4b and negligible differences has been observed compared to the results of 4a.

Cytotoxic Activity Studies

Since it's also among the study targets that synthesized derivatives 4 would have biological activities due to the functional groups they contain (five membered heterocyclic ring, 4-N,Ndialkylaminophenyl and thiophene moieties), cytotoxicity



Fig. 8 Cycling voltammogram of 4a compound

tests were done. Cytotoxic activities of 4a and 4b derivatives were performed on PC-3 (human prostate adenocarcinoma), A-549 (human lung carcinoma), SHSY5Y (human neuroblastoma cell line) and HEK293 (normal cell line) cells (Table 3; Fig. 10) by MTT assay.

Significant results were obtained on SHSY5Y and PC-3 cells. The MTT assay results demonstrated that while derivative 4a did not show any cytotoxicity on A549 and PC-3, it exhibited cytotoxic activity on SHSY5Y and HEK293 with an IC₅₀ values of 23.38 ± 1.99 and $27.89 \pm 6.62 \mu$ g/ml, respectively. Unlike 4a, the derivative 4b only showed strong cytotoxic activity on PC-3 with IC₅₀ value of $12.57 \pm 0.41 \mu$ g/ml without exhibiting any cytotoxic effect on HEK293. It is an important result that the derivative 4b does not show cytotoxicity on the HEK293 at tested maximum concentration (50 µg/ml). As a result, derivative 4b can be used as fluorescent biocompatible material in cell imaging systems and can be considered as a promising anti-cancer agent for human prostate adenocarcinoma to furtherly studied.

Conclusions

Within the scope of this study, multifunctional dye molecules have been synthesized, pH sensitivity of the target 4

 Table 2
 Electrochemical Impedance Spectroscopic (EIS)

 Measurements
 Figure 1

4a	Z (ohm)	4b	Z (ohm)
Bare Electrode	1299	Bare Electrode	1299
Thiophene	454	Thiophene	454
4a	106	4b	388
4a + Thiophene	177,8	4b + Thiophene	222,6
4a + EDOT	13,95	4b + EDOT	305



Fig. 9 SEM images taken at the magnitude of 100x, 500x and 1000x respectively left to right of a) homopolymerization of 4a b) copolymerization of 4a + thiophene c) copolymerization of 4a + EDOT

derivatives in PVC polymer matrix has been revealed and pH dependent ratiometric changes have been observed which are extremely important in sensor applications. To avoid disadvantages of polymer matrices, electropolymerization and copolymerization studies have been carried out on the thiophene group of target molecules, which mentioned as superiority of the synthesized structures and it has been proved that polymeric coating can be performed on the electrode. The



Fig. 10 The effect of 4a and 4b molecules on various cancer cell lines (A549, PC-3, SHSY5Y) and normal cell line (HEK293)

Table 3 Evaluation of the effect of 4a, 4b and Doxorubicin on PR 2		A549	PC3	НЕК293	SHSY-5Y
A-549, SHSY5Y) and normal cell	4a	50 µg/ml<	50 μg/ml<	$23.38\pm1.99~\mu\text{g/ml}$	$27.89 \pm 6.62 \ \mu g/ml$
line (HEK293) by MTT assay	4b	-	$12.57\pm0.41~\mu\text{g/ml}$	-	-
(IC ₅₀ values (μ g/ml))	Doxorubicin	$4.96\pm1.54~\mu\text{g/ml}$	$5.52\pm0.21~\mu\text{g/ml}$	$1.19\pm0.92~\mu g/ml$	$5.77\pm2.82~\mu g/ml$

derivatives 4 can be directly coated on the electrode surface in the form of polymer and copolymer films without the need of a different polymer matrix or immobilization process.

As the electropolymerisability of these dye molecules is proven by voltammetric studies, it can be concluded that pH sensor applications are promising for voltammetric methods as much as spectroscopic methods. The most important result of studied derivatives as cytotoxic agents is that they are promising molecules in anti-cancer application fields.

It is expected that some molecules may show selective cytotoxicity on PC3 cells due to the difference in membrane structure of PC3 cells compared to HEK293. Cytotoxic agents may cause toxicity on cells via different mechanisms such as destruction of cell membranes, prevention of protein synthesis, irreversible binding to receptors, inhibition of polydeoxynucleotide elongation or enzymatic reactions.

As the 4 molecules show selective cytotoxic activity on cancer cells they need further investigation for elucidation of their bioactive cytotoxic mechanisms and this study about newly synthesized oxazole-5-one derivatives may shed light on future studies in this field.

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