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New luminescent ruthenium probes for detection of diacetyl

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ABSTRACT

We introduce two water-soluble ruthenium complexes, RuPD and RuPP, and show their potential as probes for luminescent recognition of diacetyl. The recognition involves the reaction of diacetyl with a diamine moiety to convert the almost nonluminescent complex into a stronger emitting species with an up to 31-fold increase of luminescence. The incubation time could be reduced by a factor of 2.5 with respect to our former rhodamine B-based probe for diacetyl. Moreover, RuPD is the first probe that permits determination of diacetyl in aqueous buffer at neutral pH and (even more sensitive) at acidic pH in micromolar concentrations. RuPD reacts more selectively with diacetyl than with other carbonyls and shows longwave emission at 625 nm. This provides an assay for diacetyl that is hardly prone to co-excited background luminescence in biological environments as shown by its application in spiked samples of cell nutrition medium.

1. Introduction

Diacetyl (2,3-butanedione) is a constituent of many foods and beverages, frequently produced by bacterial fermentation [1–4]. Long-term inhalation of diacetyl is suspected to be hazardous and toxic responses, such as lung disease, Alzheimer's disease, mutagenesis, and carcinogenesis [5,6] are discussed. The substance is mutagenic in the Ames Test [7], has tumor-promoting activities and may also have tumor-initiating activities in carcinogenesis in the glandular stomach [8]. Moreover, diacetyl was found as a volatile organic compound specifically in the breath of lung cancer patients [9]. Therefore, it is necessary to develop efficient methods for the determination of diacetyl.

Spectrophotometric methods for determination of diacetyl are based on chromogenic reactions. One uses the conversion of diacetyl into dimethylglyoxime, followed by conversion into a colored metal complex or formation of a colored condensation product with urea. The slow formation of dimethylglyoxime under prolonged heating prevents the use *in vivo* [10]. The Westerfeld method [11] is used to measure diacetyl levels in blood. Here, diacetyl and acetoin can form a chromogenic compound with creatine in the presence of α-naphthol. The more rapid reaction of diacetyl than that of acetoin allows the determination of diacetyl, even in presence of acetoin [10,12,13]. Later, the use of chromatography (GC and GC–MS) and solid-phase microextraction became very popular for diacetyl determination [14–16], and HPLC is used [17], as well. Pre-column fluorescent derivatization contributed to further improve the sensitivity of chromatography [18]. Here, compounds such as 2,3-diaminobenzene [19,20] are commonly used. Hydrazide-containing fluorophores are another convenient way of derivatizing ketones and aldehydes fluorescently [21,22].

The drawbacks of existing fluorescent reagents for diacetyl are mostly the very short (UV) excitation wavelengths, such as in the case of 2,3-diaminobenzene, ABD-H or DBD-H [19–21]. This can cause interference by scatter and tissue absorbance because the therapeutic window of tissue (with more reduced absorbance) opens at wavelengths longer than 600 nm. Hence, probes with excitation wavelengths in the visible region and emission in the red would represent a substantial progress because less luminescence would be reabsorbed by the matrix. Among the long-wavelength emitting probes, ruthenium complexes are widely used due to their good photostability, water solubility and emission maxima in the range of 610–650 nm [23,24]. Moreover, these probes show large Stokes' shifts of not less than 150 nm and long luminescence decay times. Recently, we have presented a probe based on rhodamine B hydrazide (RBH) which shows a pink-colored fluorescence at 586 nm in weakly acidic media. This enabled quantitation of diacetyl down to the low μmolar range [25].

In this paper, we show red emitting luminescent ruthenium complexes as improved probes with less luminescent background as in the case of RBH. The preparation of the almost non-luminescent complexes (RuPD and RuPP) is shown and a method to detect diacetyl was developed. Both complexes react with diacetyl at neutral to slightly acidic pH and undergo an increase in luminescence intensity. Conditions, such as pH, buffer and reaction time were optimized to yield a novel luminescent assay for diacetyl determination at neutral pH. The selectivity for diacetyl was studied with respect to an application in physiological fluids. The potential of the new probes

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for determination of diacetyl in cancerous cells is shown by standard addition experiments in a strong matrix (cell nutrition medium).

2. Experimental

2.1. Apparatus

Absorption spectra were recorded on a Cary 50 Bio UV–vis spectrophotometer (Varian, Australia, www.varian.com). Luminescence spectra were recorded on an Aminco-Bowman AB 2 luminescence spectrometer (www.thermo.com) equipped with a 150-W continuous wave xenon lamp as excitation light source. All spectra are uncorrected. Luminescence measurements for the calibration plot were performed on a Tecan GENios Plus microplate reader (www.tecan.com) at $\lambda_{exc} = (430 \pm 17.5)$ nm and $\lambda_{em} = (595 \pm 17.5)$ nm in transparent flat bottom 96-well plates from Greiner (www.gbo.com). pH was measured with a pH meter CG 842 from Schott (www.schott.com) at room temperature. The ESI mass spectra were taken on a ThermoQuest TSQ 7000 (www.thermo.com) mass spectrometer. The ^{1}H NMR spectra were acquired on an Avance 300 MHz NMR Spectrometer (Bruker-BioSpin GmbH, www.bruker-biospin.com).

2.2. Reagents

Chemicals were purchased from commercial sources (Sigma-Aldrich, Fluka). Diacetyl was distilled before use. Stock solutions of RuPD and RuPP (100 mmol/L) were prepared by dissolving an appropriate quantity of the complexes in ethanol. Diacetyl stock solution was prepared by dissolving an appropriate quantity in ethanol. MEM medium was from Sigma (M2279).

2.3. Synthesis of RuPD and RuPP

RuPD and RuPP were synthesized according to a method reported in literature [26]. More specifically, the bis-bipyridyl or bis-phenanthrolyl-ruthenium dichlorides, respectively, were reacted with 5-amino-6-nitro-1,10-phenanthroline [27]. The resulting complexes were then subjected to subsequent reduction using hydrazine in the presence of palladium on charcoal (Scheme 1) to convert the nitro groups into amino groups. The work-up of the products yielded the complexes as their hexafluorophosphate hydrates with a diamine moiety as recognition unit for diacetyl.

2.3.1. Synthesis of **3**

$Ru(bpy)_2Cl_2$ (0.271 g, 0.560 mmol) and 5-amino-6-nitro-1,10-phenantroline (0.150 g, 0.624 mmol) in deoxygenated ethanol (40 mL) were stirred under reflux for 4 h. After cooling, the reaction mixture was filtered and concentrated. Purification on silica using MeCN–$MeCN/NH_4PF_6$ as eluent followed by subsequent re-dissolving in DCM and concentration afforded the product as an orange solid (0.200 g, 38%). ^{1}H NMR (CD_3CN) δ = 7.21–7.31 (2H, m); 7.40–7.48 (2H, m); 7.55–7.61 (3H, m); 7.75–7.80 (4H, m); 7.79–8.15 (6H, m); 8.17 (1H, dd, J = 1.1 Hz, J = 5.2 Hz); 8.45–8.54 (4H, dt, J = 1.1 Hz, J = 8.2 Hz); 8.81 (1H, dd, J = 1.1 Hz, J = 8.5 Hz); 8.97 (1H, dd, J = 1.1 Hz, J = 8.8 Hz).

2.3.2. Synthesis of **4**

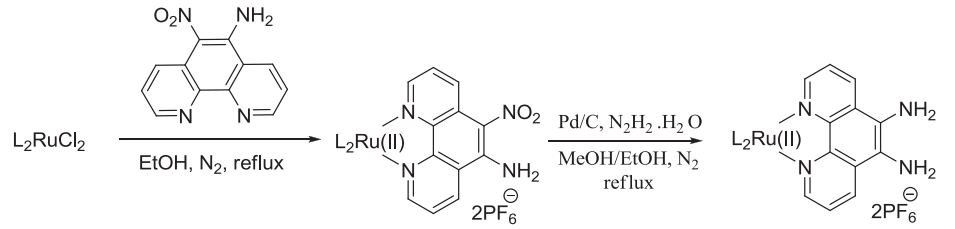
$Ru(phen)_2Cl_2$ (0.055 g, 0.096 mmol) and 5-amino-6-nitro-1,10-phenantroline (0.028 g, 0.116 mmol) in deoxygenated ethanol (10 mL) were stirred under reflux for 4 h. After cooling, the reaction mixture was filtered and concentrated. Purification on silica using MeCN–$MeCN/NH_4PF_6$ as eluent followed by subsequent re-dissolving in DCM and concentration afforded the product as a reddish-orange solid (0.022 g, 23%). ^{1}H NMR (CD_3CN) δ = 7.47 (1H, dd, J = 5.2 Hz, J = 8.8 Hz); 7.54–7.71 (6H, m); 7.92 (2H, dt, J = 1.4 Hz, J = 6.6 Hz); 7.98–8.10 (5H, m); 8.22 (4H, s); 8.55 (2H, dt, J = 1.4 Hz, J = 8.2 Hz); 8.60 (2H, dt, J = 1.1 Hz, J = 8.2 Hz); 8.76 (1H, dd, J = 1.1 Hz, J = 8.5 Hz); 8.92 (1H, dd, J = 1.1 Hz, J = 8.8 Hz).

2.3.3. Synthesis of **5**

To a refluxing solution of compound **3** (0.180 g, 0.190 mmol) in MeOH/EtOH (1:1) (40 mL) and 65 mg Pd/C (10%), hydrazine monohydrate (0.091 mL, 1.87 mmol) in MeOH–EtOH (3 mL) was added rapidly. After 30 min, another portion of hydrazine-hydrate was added and the reaction mixture was refluxed for an additional 30 min. After cooling to room temperature the mixture was filtered through Celite, washed with MeOH and diluted with toluene. The solvent was concentrated in vacuo (temperature of water bath kept below 30 °C) until a reddish-orange precipitate appeared. The precipitate was collected by filtration, washed with toluene and dried in vacuo (0.155 g, 88%). 1H NMR (CD_3CN) δ = 4.76 (4H, s); 7.18–7.24 (2H, m); 7.38–7.45 (2H, m); 7.50 (2H, ddd, J = 0.8 Hz, J = 1.7 Hz, J = 5.7 Hz); 7.55–7.61 (2H, m); 7.78–7.83 (4H, m); 7.97 (2H, dd. J = 1.6 Hz, J = 7.9 Hz); 8.07 (2H, dd, J = 1.6 Hz, J = 8.2 Hz); 8.44–8.53 (6 H, m), m/z (ESI-MS, HRMS) for M^{2+} ($C_{32}H_{26}N_8Ru$), calculated: 312,0662, found: 312,0608.

2.3.4. Synthesis of **6**

To a refluxing solution of compound **4** (0.022 g, 0.021 mmol) in MeOH/EtOH (1:1) (40 mL) and 10 mg Pd/C (10%), hydrazine monohydrate (0.01 mL, 0.218 mmol) in MeOH–EtOH (3 mL) was added rapidly. After 30 min, another portion of hydrazine-hydrate was added and the reaction mixture was refluxed for an additional 30 min. After cooling to room temperature the mixture was filtered through Celite, washed with MeOH and diluted with toluene. Solvent was concentrated in vacuo (temperature of water bath kept below 30 °C) until a reddish-orange precipitate appeared. The precipitate was collected by filtration, washed with toluene and dried in vacuo (0.018 g, 83%. ^{1}H NMR (CD_3CN) δ = 4.76 (4H, bs); 7.48 (2H, dd, J = 5.1 Hz, J = 8.8 Hz); 7.57–7.63 (4H, m); 7.75 (2H, dd, J = 1.1 Hz, J = 5.2 Hz); 7.97–8.01 (4H, m); 8.23 (4H, s); 8.45 (2H, dd, J = 1.1 Hz,



1 (L = Bpy)

2 (L = Phen)

3: 38% (L = Bpy)

4: 23% (L = Phen)

5: 88% (L = Bpy; RuPD)

6: 83% (L = Phen; RuPP)

Scheme 1. Synthesis of RuPD and RuPP with yields, as obtained after purification.