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Eumelanin pigment precursor 2-carboxy-5,6-dihydroxyindole and 2-amino-6-methylbenzothiazole chromophore integration towards melanin inspired chemoresponsive materials: the case of the Zn²⁺ ion†

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The 2-amino-6-methylbenzothiazole chromophore is introduced at the carboxyl group of the melanin precursor 2-carboxy-5,6-dihydroxyindole achieving a novel dihydroxyindole derivative with metal chelation properties not depending on the catechol moiety. In view of potential exploitation in charge storage systems, systematic investigation of the interaction of the new amide derivative with metal ions is carried out, in comparison with that of the parent 2-carboxy-5,6-dihydroxyindole, and the stoichiometry of the zinc-amide complex is determined.

Introduction

The change in the optical properties of a material following the action of an electric field, as well as chemical interactions, is gaining more and more interest in view of the design and fabrication of active organic layers capable of integrating their actual¹ function into devices, with the monitoring of the operational conditions.^{2–4}

The investigation of chemical physical properties of functional materials when acting into working device, as well as the measurement of their redox state or even the modulation the material conductivity, may take advantage on the selective introduction of chromophores and/or fluorophores into the active material.⁵ More generally, the developing of multicomponent materials to integrate different and independent properties into the hybrid system, as well as to obtain additional properties emerging from the interaction of the components, is a chief goal of the research in organic electronics.^{5,6}

Eumelanins, the black insoluble pigments of human skin and eyes, are emerging as a valuable source for the design and fabrication of active layers for application in organic electronics.^{7,8} Their natural origin and a peculiar set of physico-chemical properties, *i.e.* broadband absorption in the UV-visible

range, intrinsic free radical character, water-dependent hybrid ionic–electronic conductor behavior, are at the root of this interest.⁹

From chemical point of view, eumelanins are the product of oxidative polymerization of 5,6-dihydroxyindole (DHI) and its 2-carboxy derivative (DHICA, **1**).¹⁰ Recent studies disclosed noteworthy potentials of eumelanin integration into organic electronic devices¹¹ and in particular the ability of DHICA derived melanin to act as positive dopant towards PEDOT:PSS conducting layers.¹² Possible exploitation of eumelanin and derivatives as electrodes material within batteries is recently gaining interests in view of possible biocompatible applications.¹³ In this context in particular, because of the difficulty to design an organic polymeric electrode that can act as a Zn²⁺ acceptor,¹⁴ eumelanin derived coatings are under investigation as a cathode material for zinc ion-based batteries.¹⁵

Drawing inspiration from these ideas, herein, we report the synthesis and characterization of a DHICA acetyl derivative (DAICA, **2**) featuring amide linkage between the indole and the widely investigated syntone 2-amino-6-methylbenzothiazole (ABtz).¹⁶ The reported capability of 2-amino-6-methylbenzothiazole to act as metal (*i.e.* zinc)¹⁷ chelating moiety¹⁶ and the deep knowledge of its optical properties make it a choice candidate to investigate as a potential decorating syntone in active eumelanin layers, capable to confer additional properties to the material (*i.e.* metal chelation, fluorescence) and thus to modulate the polymer properties.

Results and discussion

In order to achieve the amide formation (Fig. 1(A)) two different coupling agents were exploited, HATU and HCTU (respectively I

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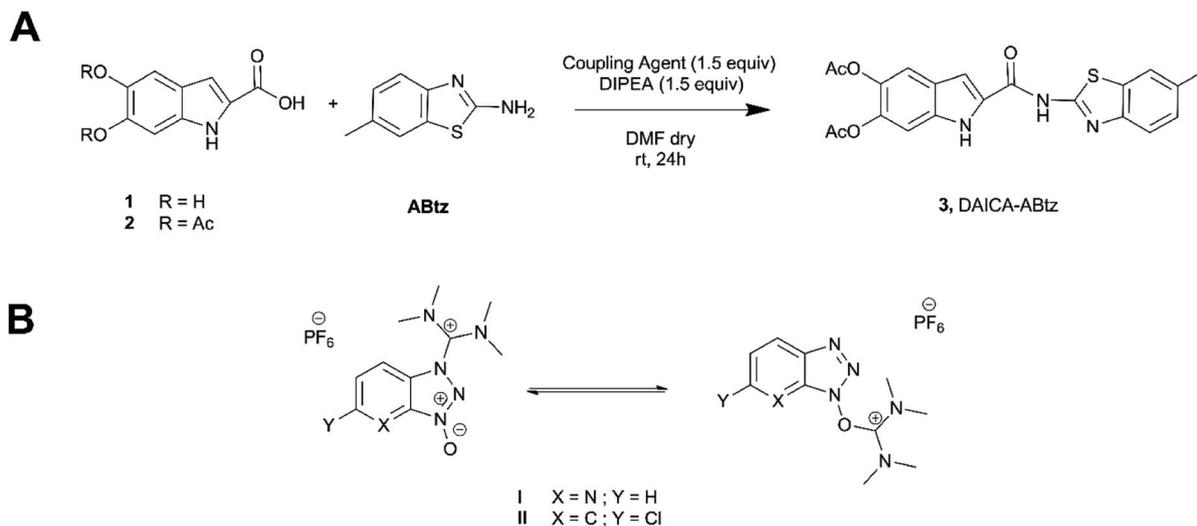


Fig. 1 (A) Formation of DHICA-ABtz amide adduct. (B) Coupling agents investigated.

and **II** in Fig. 1(B))^{18–20} to activate the carboxyl group of DAICA (2). After optimization of reaction condition, HATU catalyzed DAICA amidation was chosen allowing to obtain the pure product with 69% isolation yield by simple water induced precipitation of the adduct from the reaction medium and subsequent acidic removal of residual **ABtz**, as detailed in experimental procedure (ESI[†]).

The structure of **3** was confirmed by 1D and 2D NMR and mass analysis (Fig. S1a and b, ESI[†]) and the disappearance of the NH₂ signal observed in the 1H spectrum of **ABtz** paired by the appearance of the amide signal (11.3 ppm) in the spectrum of **3**. In the ¹H NMR spectrum, worthy of note is also the observed resonance shift for H-3 on indole moiety from 7.24 in **2** to 7.78 ppm in final product **3** following a deshielding contribution of the formed amide. The MALDI-MS spectrum revealed the presence of signals at *m/z* 446 and 462, ascribable respectively to the ions [M + Na]⁺, [M + K]⁺ of **3**.

The UV-vis spectra of **3** were collected in different solvents (Fig. S2, ESI[†]).

In acetonitrile, a solvent selected in view of further development of the study in the field of energy storage,²¹ the absorption profile showed two main bands at λ 218 and 327 nm with a small shoulder in the region around at 250 nm. The optical behavior of **3** toward several metal ions, such as Zn²⁺, Fe³⁺, Fe²⁺, Ag⁺, Cu²⁺, was evaluated, also in acetonitrile, at different concentrations of the cations up to 2 equiv. (Fig. S3–S6, ESI[†]).

Exposition to Zn²⁺ ions produced marked changes of UV-vis spectrum profile of **3** (Fig. 2) and the phenomenon was investigated at different Zn²⁺/3 ratios. Indeed after increasing the Zn²⁺ concentration, the 218 nm band remained unaffected while the peak at 327 nm decreased in intensity and, at same time, two new bands at 344 and 362 nm were observed suggesting the attitude of **3** to chelate Zn²⁺ ions and the formation of a complex.

For comparison, the impact Zn²⁺ on the absorption profiles of two related amides containing the precursors of **3** (acetamide of **ABtz**, **4**, and butylamide of DAICA, **5**, represented in Fig. 3) was investigated.

The lack of changes in the spectral features of both **4** and **5** after exposition to Zn²⁺ ions (Fig. S7 and S8, ESI[†]) concurs to sustain the central role of the peculiar amide-mediated linking of the two heterocyclic systems in determining the affinity of **3**

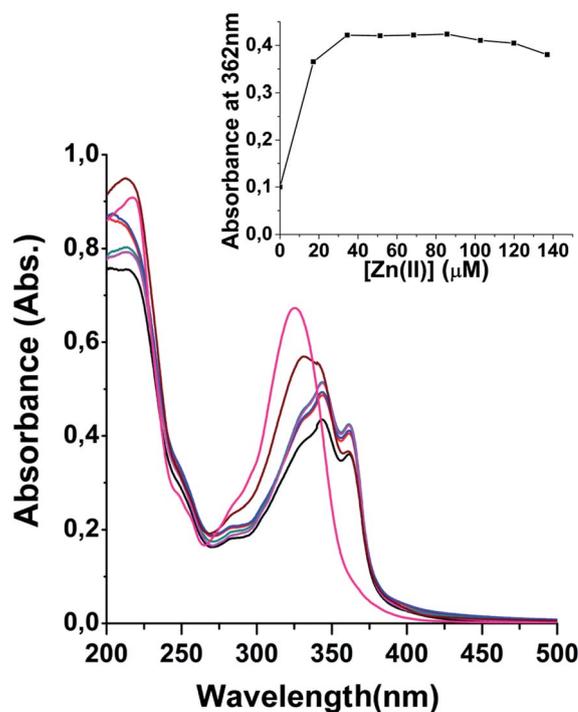


Fig. 2 Absorption intensity changes of **3** (68.5 μM) upon addition of Zn²⁺ (0–2.0 equiv.: 0 (pink) – 0.25 (brown) – 1.0 (turquoise) – 1.25 (purple) – 1.5 (blue) – 1.75 (red) – 2.0 (black)) in 2.0 mL of acetonitrile solution. Inset: variation of absorption at 362 nm.



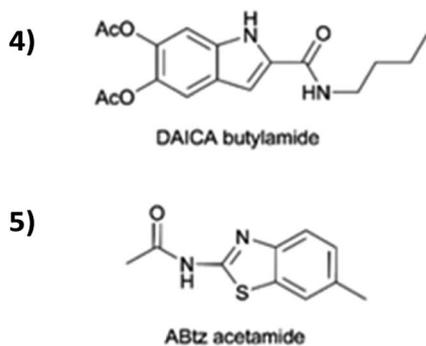


Fig. 3 Structures of two amides containing respectively the acid (4) and amino (5) precursors of 3.

towards Zn^{2+} . This observation pushed the deepening of the study of 3- Zn^{2+} interaction.

After the Job's plot analysis²² (Fig. S9, ESI[†]) 1 : 2 stoichiometry was proposed as binding mode of amide with Zn^{2+} .²³ This stoichiometry was confirmed by MALDI-TOF MS (Fig. S10, ESI[†]) which provided direct evidence for the formation of a coordinate complex containing two molecules of DAICA-ABtz with one Zn^{2+} ion whose strength was determined as reported in ESI (Fig. S11 and S12[†]) obtaining the value of $\log K_a = 4.88$.

Upon excitation at 325 nm, the amide displays an emission at 417 nm in acetonitrile. After the addition of different metal ions, a relevant enhancement in emission intensity, in the case of Zn^{2+} with respect to other cations, was observed as reported in Fig. 4.

Fluorescence measurements were carried out up to 2 equiv. of the Zn^{2+} ion showing a larger than eight-fold increase of the emission compared to the free amide (inset Fig. 5), reaching a maximum at 0.75 equiv. of the cation (Fig. 5).

The quantum yield of 3 was measured in the absence and in the presence of zinc ions in acetonitrile (see ESI[†] for details) obtaining 0.035 and 0.12 as the quantum yields of 3 and 3 + Zn^{2+} (0.75 equiv.), respectively. According to the following formula:

$$\Phi_S = \Phi_{\text{Ref}} \left(\frac{\text{Grad}_S}{\text{Grad}_{\text{Ref}}} \right) \left(\frac{\eta_S^2}{\eta_{\text{Ref}}^2} \right)$$

where the subscripts Ref and S stand for standard and sample, Φ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs. absorbance, and η the refractive index of the solvent.

The rationale of functionalizing a melanin precursor includes future developments addressing the exploitation of polymer forms of the new amide as cation (Zn^{2+}) exchanger for energy storage applications. Such a long-term goal requires the ability of a solid layer of the polymer to reversibly exchange Zn^{2+} when interfacing with appropriate electrolytes. In this perspective preliminary studies were carried out to investigate the ability of 3 and its polymers to complex Zn^{2+} in solid phase.

Thin films of deacetylated form of 3 were allowed to oxidize according to literature protocols^{11,12} and oxidation progress was followed by the evolution of the UV-vis profile which presented typical features of melanin-like polymer formation²⁴ (Fig. S13, ESI[†]). Qualitative inspection of the composition of the oxidized film, was achieved by LC-MS analysis of the methanol soluble fraction, observing signals at the m/z values expected for oligomeric species (Fig. S14, ESI[†]).

Basing on these preliminary results concerning the oxidative polymerization of new amide, thin films of the deacetylated form of 3 were investigated, as prepared as well as after exposition to oxidizing atmosphere, to achieve information about the polymer- Zn^{2+} interaction.

Indeed, after Zn^{2+} ions exposition the films of the amide polymer did exhibit a marked modification of the UV-vis profile akin the one observed in solution for the monomer (Fig. S15, ESI[†]). Moreover, a reversible exchange of Zn^{2+} ions was demonstrated by alternating treatment of a thin film with ethylenediaminetetraacetic acid (EDTA) in water and Zn^{2+} in acetonitrile. For comparison the polymer- Zn^{2+} interaction was

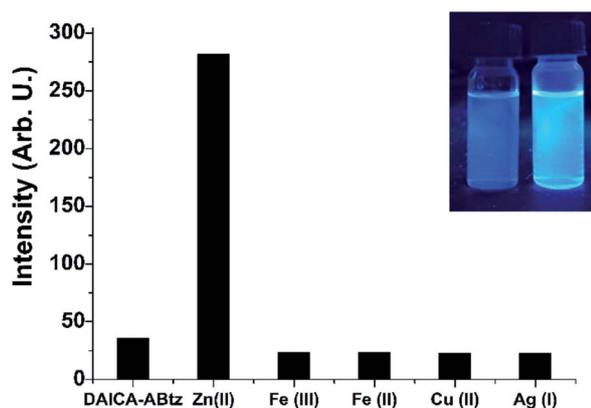


Fig. 4 Fluorescence intensity of 3 68.5 μM in acetonitrile ($\lambda_{\text{ex}} = 325$ nm) after the addition of different metal ions (n equiv. = 0.75). Inset: fluorescent changes of 3 (left) upon the addition of Zn^{2+} ions (equiv. = 0.75) (right).

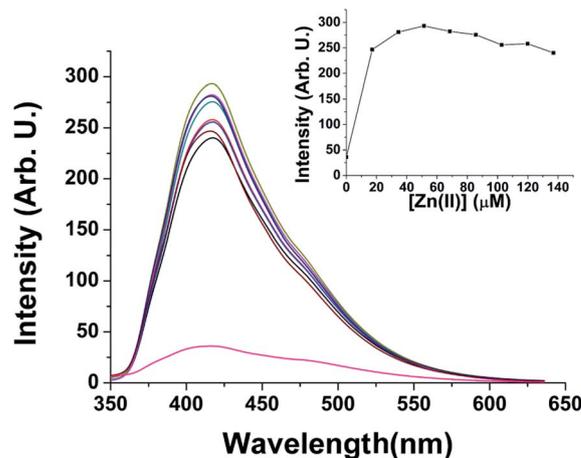


Fig. 5 Fluorescence intensity changes of 3 (68.5 μM) upon addition of Zn^{2+} (0–2.0 equiv.: 0 (pink) – 0.25 (turquoise) – 1.0 (purple) – 1.25 (red) – 1.5 (teal) – 1.75 (brown) – 2.0 (black)) in 2 mL of acetonitrile solution. Inset: changes of emission intensity at 417 nm. $\lambda_{\text{ex}} = 325$ nm.



also investigated for thin films of the sole DHICA polymer which did not show any appreciable modification after exposition to Zn^{2+} ions.

In Fig. 6 the observed reversibility is highlighted reporting the absorbance of the film at the maxima of the amide (359 nm) and of the complex (387) at each treatment cycle (Zn^{2+} /EDTA). After an initial larger increase, the exposition of the film to the Zn^{2+} solution results in a constant increase of the absorbance at 387 nm followed, after EDTA treatment, by a parallel decrease featuring complete reversibility up to ten cycles. In this picture the absorbance difference observed in presence of Zn^{2+} from the 0 cycle to the next ones has to be attributed to some absorbed EDTA in the film and thus competing with the polymer for zinc chelation.

Experimental

All commercially available reagents were used as received and all the solvents were of analytical grade. 2-Carboxy-5,6-dihydroxyindole (DHICA) were prepared according to a reported procedure⁷

Details about the analytical techniques used to characterize the materials and about the tests are reported in the ESI section.†

Synthesis of 1*H*-indole-2-carboxamide-5,6-bis(acetyloxy)-*N*-(6-methyl-2-benzothiazolyl) (3)

DAICA (50 mg, 0.1804 mmol) was dissolved in 650 μ L DMF dry, then coupling agent (0.2708 mmol) were added simultaneously with 47 μ L of DIPEA. The reaction is carried out in inert atmosphere. After about 30 minutes, a DMF dry solution of **ABtz** was added.

The progress of the reaction was monitored by TLC. After completion of the reaction, water was added to the solution to promote the precipitation of the adduct; the precipitate is then taken up with ethyl acetate and it was treated with acetic acid. Without the amine, the product precipitates from the organic phase as a white solid and isolated by filtration (76.3 mg, 69%).

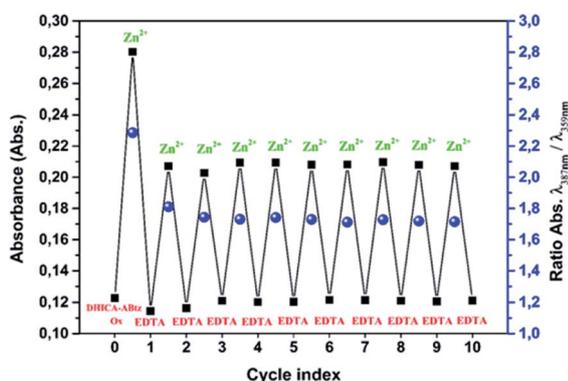


Fig. 6 Absorbance of a thin film of polymerized **3** at 387 nm (left axis, black squares) as prepared (cycle 0) and after alternating expositions to Zn^{2+} and EDTA solutions. Ratios of the absorbance at 387/359 nm (right axis, blue dots).

Mp, 277–279 °C. ¹H NMR (CD_3COCD_3 -d₆, 400 MHz, δ , ppm): 2.31 (s, 3H), 2.32 (s, 3H), 2.47 (s, 3H), 7.29 (dd, $J = 10$ Hz, $J = 1.5$ Hz, 1H), 7.48 (s, 3H), 7.58 (s, 3H), 7.66 (d, $J = 10$ Hz, 1H), 7.77 (t, $J = 1.5$ Hz, 1H), 7.78 (dd, $J = 2.75$, 0.75 Hz, 1H), 11.00 (s, 1H), 11.29 (s, 1H).

¹³C NMR (CD_3COCD_3 -d₆, 100 MHz, δ , ppm), 20.09, 20.15, 20.95, 106.45, 107.01, 116.02, 120.74, 121.46, 125.43, 127.89, 131.25, 132.81, 134.01, 135.48, 138.32, 141.78, 147.26, 157.58, 159.77, 168.58 and 168.85. MS (MALDI) m/z : 446 (75% [$M + Na$]⁺), 462 (25% [$M + K$]⁺). FTIR: $\nu = 1200, 1268, 1380, 1460, 1560, 1715, 1759, 3235, 3383$.

Conclusion

The integration of melanin precursor DHICA and the 2-aminobenzothiazole synthon allowed to achieve a chelation depending fluorophore exhibiting noteworthy selectivity to Zn^{2+} ions capable to form a complex in 2 : 1 molar ratio. The value of K_a (7.6×10^4) indicates the high stability of 3–Zn complex.

Notably, although the amide bond linking the two aromatic π -conjugated systems could have been associated to a cross-conjugation involving the indole and benzothiazole rings,²⁵ the presence of a band at 327 nm in the absorption profile of **3** reflects a unexpected communication between two moieties. Most likely this can be the consequence of a synergistic delocalization through the amide bond, assisted by the potential hydrogen bond with the atom of N and/or S of the benzothiazole ring.²⁶ Moreover, the amide bond linking the two aromatic bicycle systems acts as intramolecular rotation joint capable to provide a dissipation channel for excited state decay. These structural features are responsible for a very high sensitivity in the micromolar range (0.7 μ M) towards Zn^{2+} ions and for the remarkable chelating enhanced fluorescence,²⁷ which allowed to achieve up to eight fold increase of the emission intensity after the exposition of the amide to Zn^{2+} ions.

The presence in the structure of **3** of the dihydroxyindole melanin precursor and the characteristic of the amide to operate zinc chelation, not involving the catechol system, preserves the oxidative polymerization attitude of DHICA moiety within **3**. This feature opens to the fabrication of decorated melanin polymers, for biointerfaces as well as bioelectronics applications, integrating the expected functionalities of the polymer and the sensing ones of the novel amide expanding scope of eumelanin exploitation in bioelectronics.

Moreover, on a wider perspective, the capability of the polymer of **3** to chelate Zn^{2+} was investigated verifying the attitude of thin films of polymer to reversibly chelate Zn^{2+} ions. This finding provides a key element supporting the developments of a melanin derived solid polymer electrolyte²⁸ for design and fabrication of non aqueous Zn batteries²⁹ as well as COFs-based electrochemical energy storage.³⁰

Author contributions

A. P. and U. C. conceptualization, A. P. and F. M. methodology and investigation, A. P., U. C. and F. M. writing.



Conflicts of interest

There are no conflicts of interest to declare.

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