Supplementary Information

Title

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

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Experimental Section	S2-S3
Fig. S1 MALDI-TOF MS and a selected region of ¹ H NMR of 3	S4-S8
Fig. S2 UV-spectra of 3	S9
Fig. S3 Absorbance spectra of 3 in acetonitrile in presence of Fe^{3+}	S10
Fig. S4 Absorbance spectra of $\ 3$ in acetonitrile in presence of Fe ²⁺ .	S11
Fig. S5 Absorbance spectra of $ 3$ in acetonitrile in presence of Ag $$.	S12
Fig. S6 Absorbance spectra of 3 in acetonitrile in presence of Cu2 $^+$.	S13
Fig. S7 Absorbance spectra of 4 in acetonitrile in presence of Zn^{2+} up to 2.0 equivalents.	S14
Fig. S8 Absorbance spectra of 5 in acetonitrile in presence of Zn^{2+} up to 2.0 equivalents.	S15
Fig. S9. Job's plot analysis for the binding Zn^{2+} with 3 .	S16
Fig. S10 MALDI-TOF MS of a mixture of 3 and Zn ²⁺	S16
Fig. S11 Benesi-Hildebrand plot of 1/(A-A ₀) versus 1/[Zn ²⁺]. S17	
Fig. S12 Absorbance of 3 (34.0 μ M) at 327 nm in presence of Zn ²⁺ in acetonitrile.	S18
Fig. S13 UV-Vis spectra of DHICA-ABtz film as prepared and after aerobic oxidation over 16 hours.	S19
Fig. S14 LC-MS profile of methanol fraction DHICA-ABtz polymer film	S19
Fig. S15 Impact of Zn ²⁺ ions on the U-vis profile DHICA-ABtz polymer film	S20
References	S21

Experimental Section

General Information

Pyridine (Py), acetic anhydride (Ac₂O), HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium), HCTU (O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate)), N,N-Diisopropylethylamine (DIPEA) and 2-amino-6-methylbenzothiazole were purchased from Sigma-Aldrich and used without further purification.

DHICA and DAICA were prepared as reported.^{1,2}

Acetonitrile dry was purchased Sigma-Aldrich. All the solvents and reagents used were of analytical grade and spectroscopic grade. The salts used as metal ions source were AgNO₃, CuCl₂, FeCl₃ 6H₂O, FeCl₂ 4H₂O, Zn(CH₃COO)₂ 2H₂O.

¹H, ¹³C, ¹H COSY, ¹H, ¹³C HSQC and ¹H, ¹³C HMBC spectra were recorded with a Bruker DRX-400MHz apparatus (Bruker Corporation, Billerica, MA, USA).

Ultraviolet–visible (UV–Vis) and fluorescence spectra were recorded with JASCO spectrometers (JASCO Inc., Mary's Court, Easton, MD, USA). Attenuated total reflectance (ATR)-FTIR spectra were recorded on a Nicolet 5700 Thermo Fisher Scientific instrument.

Positive Reflectron MALDI spectra were recorded on a AB Sciex TOF/TOF 5800 instrument using 2,5dihydroxybenzoic acid as the matrix.

Melting points were determined in open capillaries with a digital melting point IA9100 series apparatus (Thermo Fischer Scientific).

Optical measurements

Both absorption and emission spectra were collected on the same solutions, in the same conditions, as described in the following. Stock solutions (2.0 mM) of the metal salts were prepared in acetonitrile and a solution of **3** (68.5 μ M) was prepared in the same solvent.

UV-vis and fluorescence titrations of **3** against different concentrations of metal ions were executed by adding 17.1 μ L aliquots of stock salt solution to 2.0 mL of **3** in acetonitrile. After shaking for a few seconds, the spectra were recorded at room temperature. Titration of **3** with metal ions were performed for several times up to 2.0 equivalents of metal ion.

The solutions prepared for UV-vis titrations of DAICA butylamide and ABtz acetamide in acetonitrile were obtained at the same concentrations under the same conditions as those for **3**.

After recorded the spectra of 1.5 mL of sensor (94.5 μ M) in acetonitrile, then 150 μ L of water was added to the cuvette; after mixing these homogeneously, UV-vis were run at room temperature. Addition of water were repeated once with metal ion and up to 1.5 mL for **3**.

Moreover, a series of Britton-Robinson buffer solutions with pH values ranging from 2 to 8 were prepared.³ 0.1 mL of a solution of **3** in methanol were added respectively to 1.4 mL of buffer of each pH. After mixing it homogeneously UV-vis absorption were recorded for all pH ranging from 2 to 8 at room temperature.

The Job's plot measurements were obtained mixing different volumes (from 0.2 to 2.0 mL) of equimolar (68.5 μ M) acetonitrile solutions of **3** and zinc acetate. The molar ratio of metal ion was varied from 0.1 to 0.9, while the total concentration of the adduct and Zn²⁺ remained constant in each solution. After shaking for a few seconds, UV-vis absorption spectra were recorded at room temperature. Similar process was run for blank test with solution of **3** only.

The quantum yield of **3** was measured in the absence and in the presence of zinc ions in using quantum yield 0.546, for quinine sulfate dihydrate as standard.^{4,5}

Synthesis of DAICA butylamide and ABtz acetamide

ABtz (500 mg) was acetylated with acetic anhydride (5 mL) and pyridine (250 µL) for 24 h. After dried a powder light in color was obtained (yield 98%).

DAICA (100 mg, 0.3607 mmol) was dissolved in 1.3 mL DMF dry, then HATU (206 mg, 0.5411 mmol) were added simultaneously with 94 μ L of DIPEA. The reaction is carried out in Ar atmosphere. After about 30 minutes, a DMF dry solution of butylamine 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 amide. The product was isolated by filtration as a light brown solid (108 mg, 90%).

Fabrication of DHICA and 3 polymer thin films

The films were prepared by spreading a fresh prepared methanol solution of DHICA or DHICA-ABtz (obtained by 3 after potassium tert-butoxide treatment in methanol). Blank films were also prepared, in acetonitrile in presence of the same volume of a solution of zinc acetate 3 mM in the same solvent. The deposited volume for spreading on 2 x 2 cm quartz substrates was 150 μ L.

After the sample has been spread, films were annealed at 50°C for 30 min on a hot plate in the inert atmosphere.

Films were exposed to air-equilibrated gaseous ammonia, from an ammonia solution (28% in water) inside a sealed chamber at 1 atm pressure and at a controlled temperature (c.a. 25 °C). ⁸

To check the impact of Zn²⁺ ions the films were immersed in an aqueous solution of EDTA 3 mM for 30 min or in a solution of zinc acetate 3 mM in acetonitrile for the same time and UV-vis spectra registered.



Fig. S1_a MALDI-TOF MS (up) and a selected region of ¹H NMR of 3 (down).



DAICA





B)



C)

S6











E)

Fig. S1_b Mono- (¹H and ¹³C - A)-B) and bi-dimensional (HH-COSY - C), HSQC - D), HMQC - E)) NMR spectra of 3. For comparison also 1H spectra are reported for DHICA and ABTZ A.1)



Fig. S2 Uv-spectra of 3 recorded in methanol (green), ethanol (blue), acetonitrile (black) and diethyl ether (red).

 Fe^{3+} and Fe^{2+} had little effect when added to the amide, producing no variation in the original absorption spectrum, but a slightly red-shifted for both the ions (Fig. S3 and S4). After the addition of Cu^{2+} and Ag^+ no changes in the absorption profiles were observed (Fig. S5 and S6).



Fig. S3 Absorbance spectra of **3** in acetonitrile (grey) with different concentrations of Fe³⁺ up to 2.0 equivalents (pink).



Fig. S4 Absorbance spectra of **3** in acetonitrile with different concentrations of Fe²⁺ up to 2.0 equivalents.



Fig. S5 Absorbance spectra of **3** in acetonitrile with different concentrations of Ag⁺ up to 2.0 equivalents.



Fig. S6 Absorbance spectra of **3** in acetonitrile with different concentrations of Cu²⁺ up to 2.0 equivalents.



Fig. S7 Absorbance spectra of **4** in acetonitrile with different concentrations of Zn²⁺ up to 2.0 equivalents.



Fig. S8 Absorbance spectra of **5** in acetonitrile with different concentrations of Zn²⁺ up to 2.0 equivalents.



Fig. S9. Job's plot analysis for the binding Zn^{2+} with 3.



Fig. S10 The MALDI-TOF MS of a mixture of 3 and Zn²⁺

The strength of **3**-Zn²⁺ complex was investigated by determining the association constant and the detection and quantification limits for Zn²⁺, respectively LoD and LoQ. Basing on Benesi-Hildebrand equation⁶ the constant was calculated as log Ka = 4.88, (or a double-reciprocal plot) below (Fig. S11), while LoD and LoQ, were determined in acetonitrile by the calibration curve method, resulted 0.7 μ M and 2.2 μ M (Fig. S12).



Fig. S11 Benesi-Hildebrand plot of 1/(A-A₀) versus 1/[Zn²⁺].



Fig. S12 Absorbance of 3 (34.0 μ M) at 327 nm with different concentration of Zn²⁺ in acetonitrile.



Fig. S13 UV-Vis spectra of DHICA-ABtz film as prepared and after aerobic oxidation over 16 hours.



Fig. S14 LC-MS/MS chromatogram revealing oligomer species in the methanol soluble fraction of DHICA-ABtz polymer film. ions-promoted autoxidation. The mixture also contains unreacted of DHICA-ABtz (423 + H+) and a dimer 844 + H+)



Fig. S15 Absorption intensity changes of DHICA-ABtz polymer film after alternate exposition to zinc ions and EDTA

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