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Biologically inspired one-pot access routes to 4-hydroxybenzothiazole amino acids, red hair-specific markers of UV susceptibility and skin cancer risk

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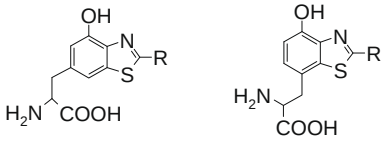
ABSTRACT

The first practical access to 4-hydroxy-6-(2-amino-2-carboxyethyl)benzothiazole and 4-hydroxy-7-(2-amino-2-carboxyethyl)benzothiazole (**1b** and **2b**) and the corresponding 2-carboxy-derivatives **1a** and **2a** is reported, involving one-pot sequential Zn^{2+}-assisted biomimetic oxidation of L-dopa and L-cysteine, 5-*S*-cysteinyldopa or 2-*S*-cysteinyldopa.

4-Hydroxybenzothiazole amino acids **1a** and **2a** are specific structural markers of pheomelanins, the characteristic sulfur-containing pigments of red human hair.[1–3] They can be obtained in small amounts by direct oxidative degradation of red hair[4,5] by a process that is at the basis of innovative microanalytical methodologies for pheomelanin determination in biological tissues.[6,7] The generation of 4-hydroxybenzothiazole compounds from pheomelanin degradation reflects the biogenetic origin of the pigments from tyrosine (or dopa) and cysteine via tyrosinase-catalyzed conversion to 5-*S*- and 2-*S*-cysteinyldopa conjugates (Scheme 1).[8,9] Oxidative cyclization of the latter gives 4-hydroxybenzothiazine intermediates,[10,11] from which 4-hydroxybenzothiazole-containing units would be generated via a ring-contraction process.[4,12]



1a, R = COOH
1b, R= H

2a, R = COOH
2b, R= H

The biomedical interest of 4-hydroxybenzothiazole amino acids is related to the familiar association between the red-haired pheomelanic phenotype and elevated susceptibility to actinic damage, premature aging and skin cancers, including melanoma.[13,14] The possibility that pheomelanins may act as potent UV photosensitizers, generating on degradation toxic radical species,[15] has stimulated intense investigations of the photophysical and photochemical behaviour of the 4-hydroxybenzothiazole system as a model for pheomelanin photodegradation.[16,17] Studies of metal-binding properties of 4-hydroxybenzothiazoles have also been reported.[18–20] Despite their pivotal importance for studies of pheomelanin phototoxicity and for prediction of UV susceptibility, 4-hydroxybenzothiazole amino acids have never been investigated because of the lack of practical, operationally simple protocols for their preparation in sufficient amounts for analytical and photochemical studies. Available syntheses of the 4-hydroxybenzothiazole system are lengthy and require several steps (from six to eight to obtain **1a** or **2a**).[20–22] This is a most critical gap since compounds **1a** and **2a** appear to be selective markers for red-haired individuals[5,6] at higher risk for skin cancer, and the current lack of straightforward synthetic routes has impeded their use for routine population screenings, and for genetic and epidemiologic correlation studies. For biomedical research purposes, synthetic procedures for analytical marker preparation should in fact be facile, cheap and easily executable even by non-chemists.

Prompted by the urgent and increased demand for these markers, and capitalizing on recent advances in the mechanisms of pheomelanin synthesis,[3] we have explored the feasibility of the biosynthetic pathway leading to 4-hydroxybenzothiazole amino acids as a practical access route to **1a** and **2a** as well as to their decarboxylated derivatives **1b** and **2b**. Herein, we report the successful achievement of this goal.

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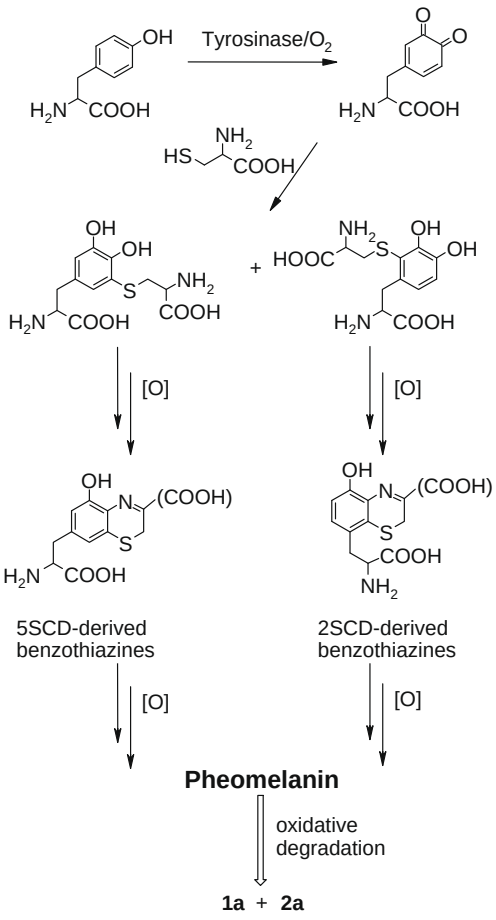
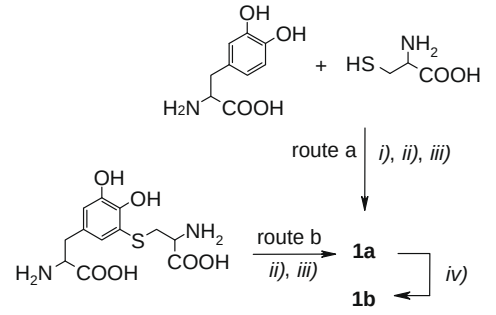


Scheme 1. Biogenetic origin of pheomelanins from L-tyrosine and L-cysteine via tyrosinase-catalyzed conversion to 5-*S*- and 2-*S*-cysteinyldopa conjugates and generation of **1a** and **2a** from pigment degradation.

The protocol to **1a** involves a rationally devised sequence of enzymatic and chemical steps that have been elaborated into a remarkable one-pot procedure. The reported methodology involves (i) oxidation of L-dopa and L-cysteine in 0.05 M phosphate buffer (pH 7.4) with mushroom tyrosinase; (ii) treatment of the mixture with potassium ferricyanide/zinc sulfate and then (iii) acidification and oxidation with sodium persulfate. HPLC purification eventually gives the desired compound in pure form (57% yield) (Scheme 2, route a).[23] By a simple additional operation, that is (iv) heating of the final mixture at 90 °C for 1.5 h, the decarboxylated derivative **1b** can be obtained instead in similar (55%) yield, after HPLC purification.[24]



Scheme 2. Reagents and conditions: (i) tyrosinase, O_2; (ii) $K_3Fe(CN)_6/ZnSO_4$; (iii) $Na_2S_2O_8/HCl$; (iv) 90 °C,1.5 h.

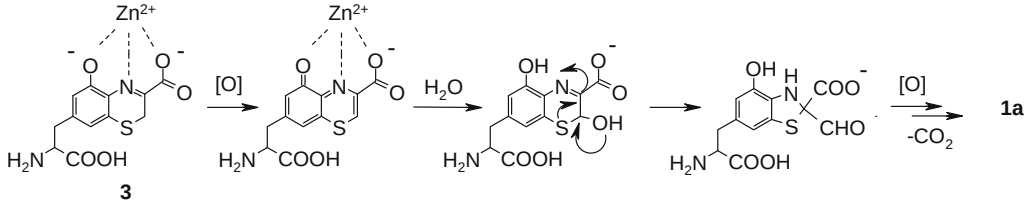
Three critical steps underpin the synthetic methodology: (a) the enzymatic conversion of L-dopa to dopaquinone followed by the addition of L-cysteine to give 5-*S*-cysteinyldopa (the prevalent isomer); (b) the ferricyanide-promoted oxidative cyclization of 5-*S*-cysteinyldopa to a benzothiazine intermediate stabilized by Zn^{2+} ions and (c) the persulfate-induced oxidative ring contraction of the benzothiazine-Zn^{2+} complex **3** to give **1a**. (Scheme 3).[12]

Consistent with the proposed mechanism is an alternate procedure to **1a/b** which has been developed in the course of this study based on the oxidation of 5-*S*-cysteinyldopa, prepared following a reported procedure,[25] according to the above steps (ii) and (iii)[26] (and iv,[27] if appropriate) to give the products in quite similar yields (Scheme 2, route b).

The successful conversion of L-dopa and L-cysteine to **1a/b** reflects a careful choice of oxidation methods and sequential operation times that have been optimized within the one-pot protocol after a thorough and careful screening of oxidizing systems and experimental conditions. Thus, for example, commercially available mushroom tyrosinase proved to be the most efficient oxidizing system for in situ conjugation of L-cysteine to L-dopa,[28] but was ineffective in promoting subsequent oxidation of cysteinyldopa. On the other hand, ferricyanide was effective on the latter step but not in the initial conjugate formation.

The crucial experimental expedient favouring efficient benzothiazole formation is the addition of Zn^{2+} which is known to stabilize 3-carboxy-4-hydroxybenzothiazines by forming a chelate complex, such as **3**, as evidenced by the typical absorption maximum at around 390 nm.[3,11] Thus, in the presence of Zn^{2+}, the key benzothiazine intermediate can be accumulated in suitable amounts for the oxidative ring contraction. The latter step was efficiently achieved by using persulfate combined with acid conditions while other oxidants or reaction media proved to be much less effective. Interestingly, the acid-promoted ring contraction of a remarkably stable 2-alkoxy-7-hydroxy-2*H*-[1,4]benzothiazine intermediate is the key step in a strategically similar synthesis of 6-hydroxybenzothiazole-2-carboxylic acid.[29]

Extension of the L-dopa+L-cysteine coupling route to the synthesis of **2a/b** was precluded by the low formation ratio of 2-*S*-cysteinyldopa/5-*S*-cysteinyldopa (1:5) in the tyrosinase-promoted conjugation.[28] After extensive unfruitful efforts to orient the coupling reaction, we eventually resorted to use 2-*S*-cysteinyldopa as the precursor. This was made possible by the facile synthesis recently described for this melanogen.[30] As expected, oxidation of 2-*S*-cysteinyldopa as in Scheme 2, route b, furnished the desired **2a/b** in similar yields (52–57%).



Scheme 3. Proposed route of formation of **1a** from benzothiazine-Zn^{2+} complex **3**.

Besides providing an expedient access to predictive markers of skin cancer and melanoma, the reported methodologies feature several aspects of chemical interest. The proposed route to **1a/b** is efficient, applicable up to a hundred milligram scale, environmentally sound (use of organic solvents or toxic reagents is avoided) and attractive for routine preparation of small standard samples. By executing sequential oxidation and, where appropriate, decarboxylation steps in a single pot and including only a final purification step, this procedure avoids time-consuming isolation of intermediates, including the labile benzothiazines, with significantly decreased operating costs. Although the protocol depends on an enzymatic step which, however efficient, may limit its scalability, it provides a simple unprecedented means of constructing a naturally occurring benzothiazole skeleton from two commercially available amino acids. Use of biologically relevant Zn^{2+} for the stabilization and further manipulation of in situ generated 3-carboxy-4-hydroxybenzothiazine compounds is also a chemical highlight of this study.

Acknowledgements

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23. A solution of L-dopa (158 mg, 0.80 mmol) in 0.05 M phosphate buffer (pH 7.4) (34 mL) was sequentially treated with L-cysteine (194 mg, 1.60 mmol) and mushroom tyrosinase (77,800 units) and the mixture was kept under vigorous stirring at rt. After 2 h the mixture was treated with a solution of zinc sulfate heptahydrate (275 mg, 0.96 mmol) in water (4 mL) and a solution of potassium ferricyanide (260 mg, 0.80 mmol) in 0.05 M phosphate buffer (pH 7.4) (4 mL); after 20 min, sodium persulfate (570 mg, 2.40 mmol) and 12 M HCl (40 mL) were added to the oxidation mixture and, after additional 20 min, the reaction mixture was treated with sodium disulfite (305 mg, 1.60 mmol). The resulting mixture was fractionated by preparative HPLC (10 μm particle size 250 × 22 mm Econosil C18, 0.2% trifluoroacetic acid/methanol 65:35, 20 mL/min). After evaporation of the solvent the residue was dissolved in 0.1 M HCl and taken to dryness to afford **1a**[4] as hydrochloride salt (145 mg, 57% yield, purity >98% as determined by ^{1}H NMR analysis).
24. The oxidation mixture obtained as above[23] was heated at 90 °C for 1.5 h. The fraction obtained by preparative HPLC (column and solvent rate as above, eluant trifluoroacetic acid/methanol 75:25) was taken to dryness and treated with 0.1 M HCl to afford **1b**[12] as hydrochloride salt (120 mg, 55% yield, purity >98% as determined by ^{1}H NMR analysis).
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26. The reaction of 5-*S*-cysteinyldopa or 2-*S*-cysteinyldopa (250 mg, 0.80 mmol) was carried out in 0.05 M phosphate buffer (pH 7.4) (34 mL) using zinc sulfate, potassium ferricyanide and sodium persulfate at the same reagent/substrate ratios indicated in Ref. 23 followed by preparative HPLC. The residue was dissolved in 0.1 M HCl and taken to dryness to afford **1a** (152 mg, 60% yield, purity >98% as determined by ^{1}H NMR analysis) or **2a**,[5] (145 mg, 57% yield, purity >98% as determined by ^{1}H NMR analysis) respectively, as hydrochloride salt.
27. The mixture obtained by oxidation of 5-*S*-cysteinyldopa or 2-*S*-cysteinyldopa[26] was heated at 90 °C for 1.5 h, and purified by preparative HPLC under the conditions described in Ref. 24. Treatment of the residue with 0.1 M HCl afforded **1b** (125 mg, 57% yield, purity >98% as determined by ^{1}H NMR analysis) or **2b,**[21] (114 mg, 52% yield, purity >98% as determined by ^{1}H NMR analysis) respectively, as hydrochloride salt.
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