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Red Hair Benzothiazines and Benzothiazoles: Mutation-Inspired Chemistry in the Quest for Functionality

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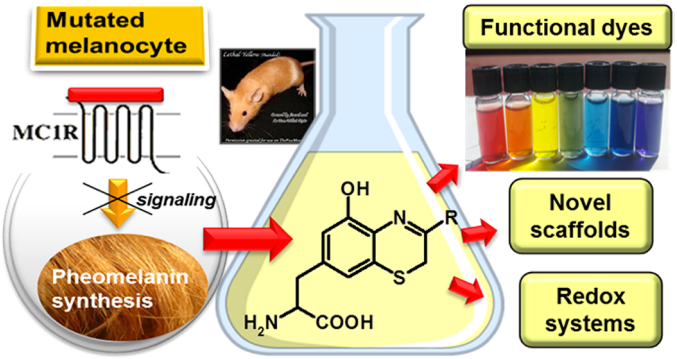

Nature provides a primary source of leads for the design of π-conjugated organic chromophores and other functional molecular systems useful for molecular recognition, light harvesting, photoconversion, and other technological applications. In this Account, we draw attention to a unique group of naturally occurring heterocyclic compounds, the 2*H*-1,4-benzothiazines and related benzothiazole derivatives. Derived from tyrosine and cysteine, these molecules arise from a mutation-induced deviation of the melanin pathway to provide the core structure of the red human hair pigments pheomelanins. Since the elucidation of the biosynthetic pathway of pheomelanins in the 1960s, researchers have focused on 1,4-benzothiazines and red hair pigments. Not only do these molecules have interesting photochemical and molecular recognition properties, they also have compelling biomedical significance. Numerous studies have linked higher levels of pheomelanins and mutations in the pathways that produce these pigments in individuals with red hair and fair skin with an increased sensitivity to UV light and a higher susceptibility to melanoma and other skin cancers.

Prompted by new data about the structure and photochemistry of the bibenzothiazine system, this Account highlights the chemistry of benzothiazines in red-haired individuals as a novel source of inspiration in the quest for innovative scaffolds and biomimetic functional systems. Model studies have gradually shed light on a number of remarkable physical and chemical properties of benzothiazine-based systems. Bibenzothiazine is a robust visible chromophore that combines photochromism and acidichromism. Benzothiazine-based polymers (synthetic pheomelanins) show remarkable photochemical, paramagnetic, and redox cycling properties. Biomimetic or synthetic manipulations of the benzothiazine systems, through decarboxylation pathways controlled by metal ions or unusually facile ring-contraction processes, can produce a diverse set of molecular scaffolds.

Introduction

A most promising, yet so far little explored, concept in Nature-inspired chemical research concerns the development of new molecules and principles from insights into the pathways within which mutated genes operate. The expected outcome is a broad degree of structural diversification within the limits set by the common biogenetic origin and precursor(s).

One of the most familiar examples of chemical diversity induced by altered gene functionality is provided by melanin pigmentation, which accounts for the broad palette of colors and hues of human hair and eyes, mammalian coats and furs, and avian feathers.[1,2]

The two fundamental varieties of melanin pigments, the black-to-brown eumelanins and the reddish pheomelanins,

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both originate from the tyrosinase-catalyzed oxidation of tyrosine; however, the factors controlling prevalence of either pathway have remained unclear for a long time. A fundamental breakthrough came in 1995 with the discovery that people with red hair display mutations in the human melanocortin 1 receptor (MC1R), similarly to what was seen in the case of mouse coat models.[3]

Although extensive epidemiological and biological studies over more than half a century have shown that mutation at several pigmentation genes is involved in predisposition to skin cancer and melanoma,[4] red hair and a fair complexion associated with high levels of pheomelanins have traditionally been regarded as a most important risk factor.[5] This familiar observation has prompted intense studies aimed to understand the (photo)chemical behavior of pheomelanins and to assess the possible causal role of these pigments in UV susceptibility and skin cancer development.

The red–black dualism underpinning human pigmentation has been illustrated in a recent Account by Simon and Peles.[6] These authors provided an overview of the biochemical pathways underlying the formation of eumelanins and pheomelanins and the important implications for skin photoprotection, UV susceptibility, sunburn, and skin cancer risk.

Taking the previous paper as a basis, the present Account has been conceived with a view to stimulating interest into red hair pigments and their benzothiazine-derived systems from the privileged and inspiring perspective of mutation-driven chemistry. Giving only brief mention to the early seminal studies at Naples in the 1960s,[1,7,8] the narrative will develop to show how several naturally derived or bioinspired benzothiazine systems possess most of the crucial properties commonly sought in the current quest for functional materials, including, for example, redox behavior, photochromism, solvatochromism, and paramagnetism. The latter is an important feature of pheomelanins, which has been used for nondestructive pigment characterization by electron paramagnetic resonance (EPR) spectroscopy.[6,9] Revisiting biologically generated structures in relation to specific gene mutations is proposed as a novel paradigm for studies aimed at structure and function discovery in biotechnology-based or diversity-oriented research sectors. Emerging clues picked up from the latest literature in related fields will finally be presented to support the proposed concepts and principles.

Chemistry from a Mutation

Melanin pigmentation is believed to be one of the main determinants of sensitivity to UV radiation and sun damage. Remarkably, only the melanocortin 1 receptor gene (*mc1r*) is known to account for variation in skin and hair pigmentation and in skin cancer incidence. The *mc1r* gene encodes a 317-amino acid G-coupled receptor, MC1R. Human *mc1r* sequence variants are associated with red hair and fair skin in the Caucasian population.[10–12] These variant alleles are extremely common, and in northern European populations <50% of the *mc1r* genes encode the "wild-type" or consensus protein. Three alleles in particular, Arg151Cys, Arg160Trp, and Asp294His together make up 22% of the *mc1r* genes and account for 60% of all cases of red hair.[12] Thus, a single locus can contribute significantly to human pigmentary variation.

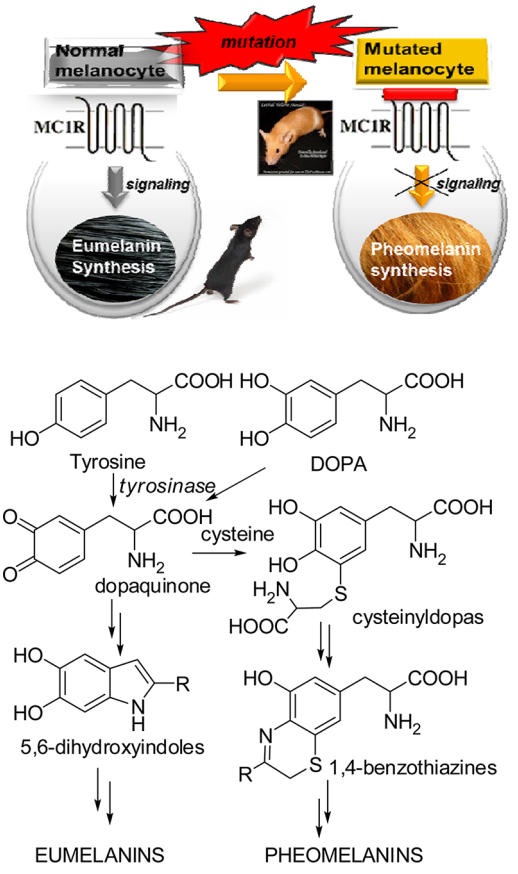


FIGURE 1. The melanocortin 1 receptor (MC1R), the tyrosine/tyrosinase pathway, and their role in human and mammalian pigmentation.

In wild-type eumelanic subjects, MC1R activation induces eumelanin synthesis via tyrosinase activation (Figure 1). Among red haired individuals, homozygous for alleles of the *mc1r* gene can be found that show varying degrees of diminished function. The main consequence is a decrease in the amount of eumelanin pigments with prevalence of the pheomelanin variant. At the biochemical level, this change is the result of the drop in tyrosinase activity favoring the concomitant intervention of cysteine in the pathway.[6] Nonenzymatic addition of the SH group to the oxidation

product of tyrosine, dopaquinone, leads to the formation of isomeric cysteinyldopas.[13−15] As a result, the intramolecular cyclization pathway of 5,6-dihydroxyindole formation leading to eumelanin polymers is inhibited, and an alternate 1,4-benzothiazine route to pheomelanins and trichochromes becomes dominant.

Chemical analysis of hair melanins showed that the switching mechanism does not operate on an all-or-nothing basis but leads rather to the generation of mixed melanins responsible for phenotypes with variable shades of color.[13] Thus, a characteristic of the MC1R controlled pathway is that it can lead to a broad spectrum of products that differ for nearly gradual variations in the eumelanin-to-pheomelanin ratios.

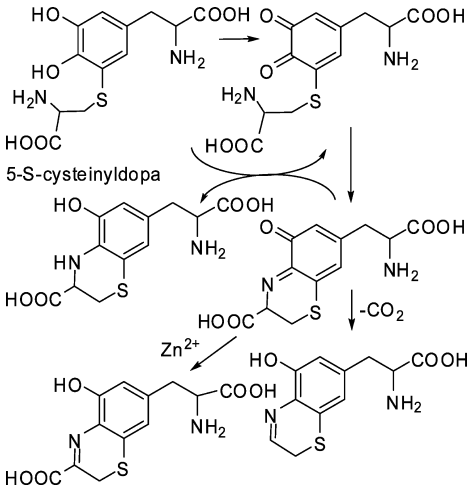
Highlight 1*: Genetic control over the melanin pathway does not result in all-or-nothing switch between eumelanin and pheomelanin synthesis but may lead to mixed pigments with variable compositions. Bioproduct properties may thus be finely tuned by specific gene mutations coupled with a sufficient biochemical supply of cysteine.*

Synthesis and Reactivity of Hydroxy-2*H*-1,4-benzothiazines

Formation of cysteinyldopas by spontaneous nucleophilic trapping of enzymatically generated dopaquinone by cysteine SH group is generally recognized as the primary mechanism by which eumelanin synthesis is regulated. This is demonstrated by the presence of detectable levels of cysteinyldopas in the blood and urine of healthy dark-skinned individuals lacking significant pheomelanin synthesis.[16] However, under conditions of defective MC1R signaling secondary to *mc1r* mutation, pheomelanin synthesis may be triggered following the onset of an oxidative stress condition. Oxidation of cysteinyldopas followed by intramolecular cyclization would then lead to the generation of a transient *o*-quinoneimine, which can either undergo redox exchange with the parent cysteinyldopa to give a dihydrobenzothiazine or isomerize with or without concomitant decarboxylation to give 2*H*-1,4-benzothiazine derivatives (Scheme 1).[17,18] Notably, when the reaction is carried out in the presence of zinc ions, decarboxylation of the quinoneimine is substantially inhibited, and the 3-carboxyl derivative is the main product, which persists in the reaction medium for relatively long periods of time due to the stabilizing effect of the metal.[19−21]

Highlight 2*: Zinc ions are potent regulators of the pheomelanin pathway in vitro through stabilization of the labile 3-carboxybenzothiazine. This effect can be attributed to the formation of a stable chelate, which shifts bathochromically the benzothiazine chromophore and affects reaction pathways, chemical composition, and physicochemical properties of the resulting products.*

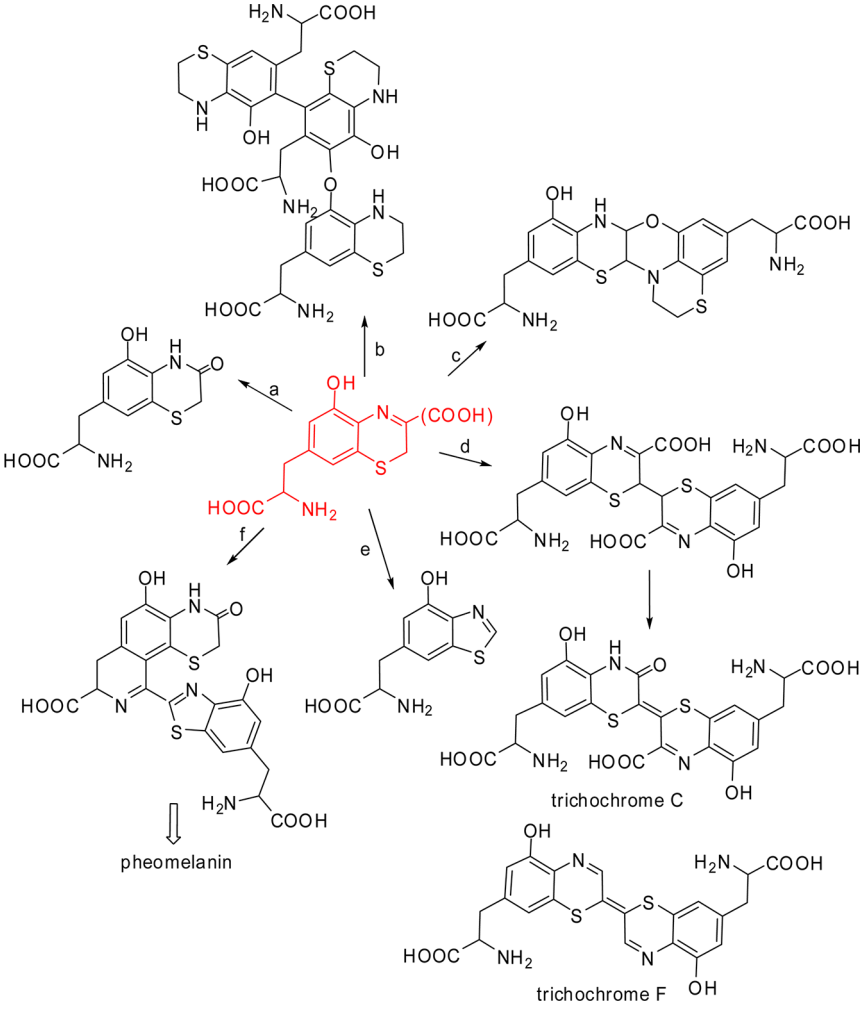
SCHEME 1. Origin of the Benzothiazine System via Intramolecular Cyclization of Cysteinyldopa



Just formed, the 2*H*-1,4-benzothiazine derivatives may follow diverse routes reflecting the presence or the absence of the carboxyl group on the 3-position, and the specific reaction conditions. Scheme 2 illustrates the variety of structural scaffolds that have been identified by oxidative conversion of cysteinyldopa or of related 1,4-benzothiazines under different biomimetic conditions. Alkaline or hydrogen peroxide treatment of 1,4-benzothiazines leads to a stable 3-oxo derivative as in path a.[20,22,23] Complex oligomers like those indicated in path b are produced by peroxidase/H_2O_2 oxidation of cysteinyldopa after reductive treatment, suggesting formation of benzothiazine intermediates that would then couple through C−C and C−O bonds at the benzene moieties.[24]

Tyrosinase-catalyzed oxidation of cysteinyldopa follows an alternate pathway, as in route c, in which an unusual cycloaddition process apparently takes place involving both benzothiazine and quinoneimine intermediates.[25] It should be noticed that routes b and c have so far been observed only in biomimetic chemical studies, so their interest would be mainly in relation to the reaction behavior of cysteinyldopa-derived benzothiazine systems rather than to a possible relevance to natural pheomelanin buildup. An intriguing oxidative dimerization process (path d) can be observed by spontaneous oxidation under mild conditions.[19] Further oxidative steps lead to unusual bibenzothiazine derivatives commonly referred to as the trichochromes. These latter

SCHEME 2. Overall View of the Molecular Scaffolds That May Originate from Biomimetic Chemistry of 1,4-Benzothiazine Compounds



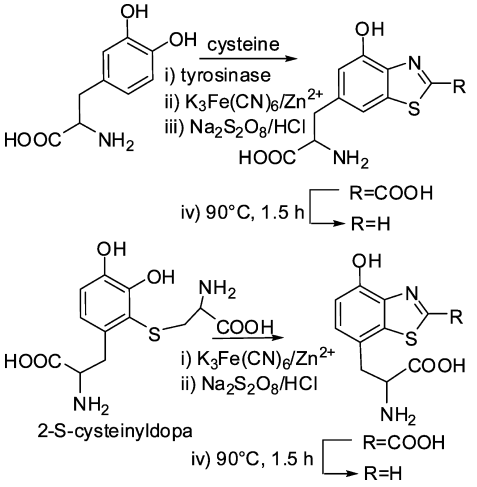
comprise four main variants, two of which consist of a symmetric bibenzothiazine derivative (trichochromes E and F) while the other two are mixed systems (trichochromes B and C).[8] The trichochromes were originally discovered in red human hair and are known to arise from cysteinyldopas, mainly the 5-*S*-isomer, but their mode of formation, the direct precursors, and actual biological relevance remain little defined. Trichochrome formation by oxidation of 5-*S*-cysteinyldopa has been reported to be a minor process that is dramatically enhanced under strongly acidic, non-natural conditions, suggesting a possible artifactual generation under the harsh acidic conditions used for pheomelanin extraction from red hair. Model studies[19] showed that trichochrome generation from 5-*S*-cysteinyldopa is favored in the presence of zinc ions, which are typically found in skin and hair, suggesting a possible role of this metal *in vivo*.

By far, one of the most typical, chemically and biologically relevant characteristics of the 2*H*-1,4-benzothiazines is their tendency to undergo ring contraction either spontaneously or following UV irradiation to give benzothiazole products as in path e.[26] The biosynthetic and biological relevance of the ring contraction process has been recently addressed in a number of papers[27−30] and will not be discussed further here.

Facile conversion to benzothiazoles is an important feature of benzothiazine chemistry. Pending mechanistic issues should incite investigation of this transformation, which is triggered or mediated by a variety of factors including UV light, oxidizing agents, and metal cations. The latter, for example, zinc ions, provide the key ingredient of a biomimetic one-pot approach to benzothiazoles, illustrating the potential of biomimetic chemistry for small scale synthesis of natural heterocycles with biosynthesis-dictated substitution patterns (Scheme 3).[31]

The chemistry of cysteinyldopa-related benzothiazoles has remained so far largely unexplored. It is worth mentioning here the importance of the benzothiazole-carboxylic acids as

SCHEME 3. Biomimetic Synthesis of Benzothiazoles[31]



markers of high levels of hair pheomelanin and increased risk of skin cancer and melanoma.[29,32,33]

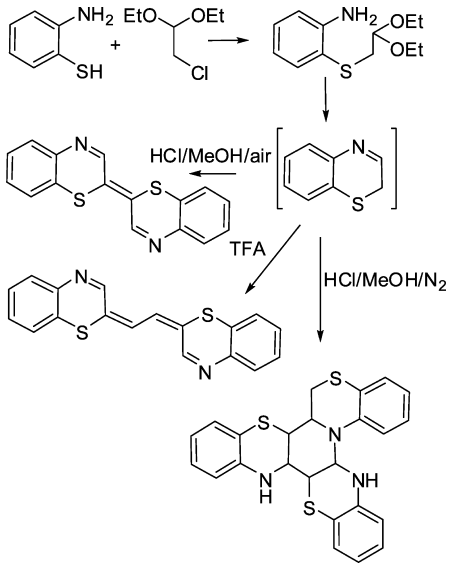
Pathway f in Scheme 2 entails an outstanding sequence of spontaneous chemical processes, which is probably triggered by the coupling of two oxidized benzothiazine species.[23] The main dimeric products, featuring isoquinoline and benzothiazole moieties, have been shown to provide the most important building blocks of natural pigments from red hair and chicken feathers.[34] Current evidence indicates that most red hair pheomelanin properties, including UV–visible chromophore and chemical degradation marker profile, are matched fairly well by the unusual dimer generated as in path f. Moreover, a recent investigation of red hair melanosomes by 2D solid state NMR spectroscopy provided strong evidence in support of the isoquinoline-containing dimer as a key structural component.[35] Finally, 2*H*-1,4-benzothiazine intermediates derived from 5-*S*-cysteinyldopa may give rise to semiquinone-like one-electron oxidation products detectable by EPR spectroscopy and putatively responsible for the free radical properties of natural pheomelanins and their synthetic mimics.[9] It is an important research goal to assess whether UV or oxidatively induced benzothiazine-derived free radical species are involved in the known association between red hair and melanoma.

Highlight 3*: Water-soluble benzothiazine and benzothiazole derivatives are easily accessible from cysteinyldopas through biomimetic routes. They provide useful building blocks for constructing a diversity of structural scaffolds for several possible applications.*

The Trichochromes: New Clues for Functional Chromophore Design

The discovery of trichochromes marked one of the most important landmarks in melanin research, because studies

SCHEME 4. Synthesis of the Unstable 2*H*-1,4-Benzothiazine and Its Conversion to Bibenzothiazine Scaffolds or to Cyclic Trimers



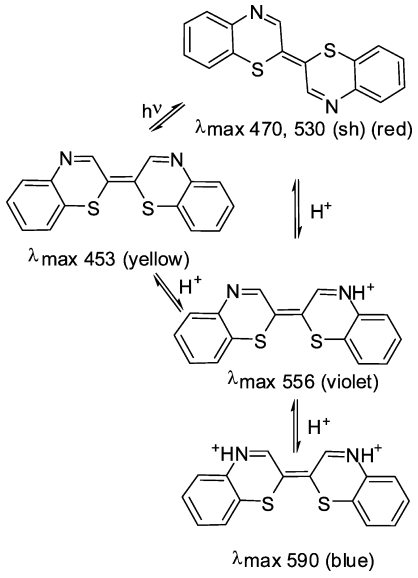
of these pigments provided valuable breakthroughs in the origin and properties of pheomelanins. Originally regarded as the sole isolable components from natural pheomelanin preparations of well-defined chemical structure, the trichochromes are characterized by a distinct visible absorption band and a marked insolubility that has hindered in-depth insights. Both the origin of trichochromes as independent entities from the pheomelanin pathway and their chemical and biological properties have been addressed in previous reports, and the interested reader is referred to the original papers and reviewing articles for further information.[8,16,36,37]

Time-resolved spectroscopic techniques have been used to quantify the energetics and dynamics of the primary photoprocesses of trichochromes following excitation into the lowest excited singlet state. Trichochromes are efficient quenchers of singlet oxygen, exhibiting a bimolecular rate constant comparable with vitamin C and suggesting that they could serve a protective role in pheomelanin pigments.[38]

The indigo-like visible chromophore of trichochromes prompted Prota and co-workers in the 1970s to embark on a series of studies directed to characterize the parent 2*H*-1,4-benzothiazine and its reaction behavior (Scheme 4).[39–42]

The synthesis of the parent benzothiazine was based on the reaction of *o*-aminobenzenethiol with chloroacetaldehyde in the acetal-protected form. Treatment of the resulting aminoacetal with acids leads to the rapid formation of the parent heterocycle, which as formed dimerizes under acid conditions to form the isomeric bibenzothiazines in good yield.[41] Notably, when the same aminoacetal is reacted in

SCHEME 5. Photochromism and Acidichromism in $\Delta^{2,2'}$-Bibenzothiazine, a Unique Bioinspired Four-State System



trifluoroacetic acid, an intriguing reaction occurs leading to a highly colored homologue with a cyanine dye structure.[40] Depletion of oxygen from the medium favors an alternate aldol-type reaction leading to an unusual cyclic trimer derivative.[39] Related synthetic studies expanded the scope of benzothiazine synthesis to include the stable 3-phenyl derivative, which dimerizes only under forcing oxidative conditions.[42]

Not Only Red

Early studies of trichochromes and related derivatives[41−43] disclosed photochromism (shift from yellow to red color upon exposure to sunlight) and acidichromism (marked bathochromic shift at low pH) as most peculiar characteristics of the bibenzothiazine chromophore. Unambiguous structural characterization of the stable yellow species of unsubstituted $\Delta^{2,2'}$-bibenzothiazine by X-ray analysis was precluded by the failure to grow suitable crystals. However, although the X-ray diffraction spectrum of the 3-phenyl derivative[42] indicated a *cis* configuration about the central double bond, it was assumed that the most stable form of bibenzothiazine was the *trans* isomer based on classic chemical arguments. Recently, a detailed structural reexamination of the stable yellow isomer of $\Delta^{2,2'}$-bibenzothiazine by an integrated 2D NMR and theoretical approach revealed that the stable yellow species is in fact the *cis* isomer.[44] In the same study, it was also found that under strongly acidic conditions the initially formed violet species (λ_{max} 556 nm), corresponding to the protonated derivative,

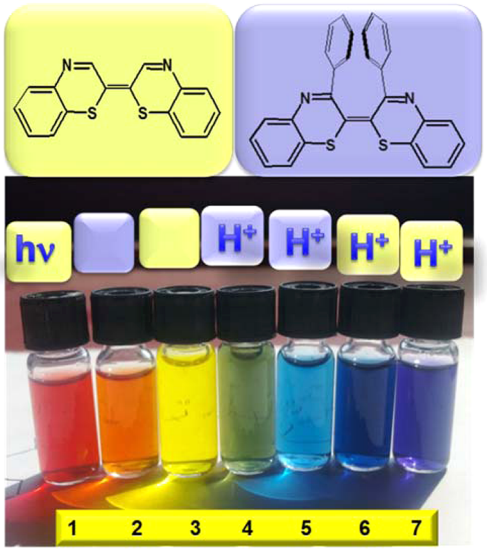


FIGURE 2. The bibenzothiazine rainbow. Yellow labels, $\Delta^{2,2'}$-bi(2*H*-1,4-benzothiazine) (vials 1,3,6,7); pale blue labels, $\Delta^{2,2'}$-bi(3-phenyl-2*H*-1,4-benzothiazine) (vials 2,4,5). Symbols in labels refer to treatments: vial 1, photoexcited; vials 4 and 5, mono- and diprotonated; vials 6 and 7, di- and monoprotonated; no symbol, neutral compound in methanol.

undergoes further protonation to give a blue species (λ_{max} 590 nm), identified as the dication (Scheme 5).

Similar work on the 3-phenyl and cyanine derivatives demonstrated the unique potential of bibenzothiazine as a light- and acid-sensitive system with a broad palette of visible chromophores (Figure 2).

***Highlight 4**: $\Delta^{2,2'}$-Bibenzothiazines are easily accessible π-electron platforms with light - and acid-tunable visible chromophores. Rational structure manipulation coupled with proper selection of inputs may provide a versatile means of producing the entire palette of color outputs for diverse applications.*

From UV Susceptibility to Novel Technologies: Pheomelanin at Work

Being widely implicated in the abnormal susceptibility of red haired individuals to sunburn, skin cancer, and melanoma, natural pheomelanin pigments and their synthetic variants have been extensively investigated for their photosensitizing and prooxidant properties by a range of approaches including flash photolysis and pulse radiolysis.[45−49] A detailed coverage of the topic from the biological and biophysical perspective is out of the scope of this Account. Here, mention will be given to the classic paper by Chedekel and co-workers who first reported photodestruction of human red hair pheomelanin following UV irradiation.[45] In the same study, it was shown that photoexcited pheomelanin generates superoxide, a finding that supported a photosensitization mechanism

as the key to UV-induced cell damage in red-haired individuals. Subsequent studies showed that UVA excitation of pheomelanin results in the activation of molecular oxygen through the production of the superoxide anion, $O_2^{-\bullet}$, and this outcome is associated with the scavenging reaction between O_2 and solvated electrons produced by one-photon ionization of the pigment.[21,46] Free-electron laser photoelectron emission microscopy, femtosecond absorption spectroscopy carried out using different excitation wavelengths, and electron paramagnetic resonance (EPR) measurements of oxygen photoconsumption confirmed that the photoionization threshold of pheomelanin is <326 nm, consistent with the activation of oxygen observed for UVA excitation of pheomelanin.[47]

Work on the photophysical and photochemical properties of several model compounds, including the parent benzothiazole, contributed to elucidation of the abnormal photoreactivity of pheomelanin polymers and related metabolites compared with their eumelanin counterparts.[50] A role of iron, zinc, and other metal cations in pheomelanin photosensitizing properties was suggested.[21,51] Studies of model pigment chromophores along with chemical degradation experiments[20,27,34,52] supported the view that pheomelanins originally consist for the most part of benzothiazine moieties that gradually turn into benzothiazoles with UV irradiation or simply on aging. The dual origin of red hair pigment from 5-*S*-cysteinyldopa- and 2-*S*-cysteinyldopa-derived building blocks was recently highlighted[28] along with the higher photostability of the latter minor components.

Highlight 5*: Natural or synthetic pheomelanins are potent photosensitizers that can release electrons to oxygen upon photoexcitation, inducing formation of superoxide and related reactive oxygen species. Rational design of pheomelanin-based materials may inspire novel efficient systems for photoconversion, light harvesting, and optoelectronic applications.*

From the preceding discussion, it appears that studies of pheomelanin properties have traditionally been focused on the light-induced reactivity of these pigments in relation to UV susceptibility and skin photocarcinogenesis in red haired individuals. However, a remarkable UV-unrelated property of model synthetic pheomelanins of both biological and technological relevance has been disclosed recently.[53] The key observation was that in the presence of a suspension of 5-*S*-cysteinyldopa pheomelanin in phosphate buffer, pH 7.4, DOPA as well as dopamine were converted to black eumelanin polymers at a much faster rate than in the absence of pheomelanin (Figure 3, lower right corner image). Kinetic, chemical and scanning electron microscopy (SEM) evidence

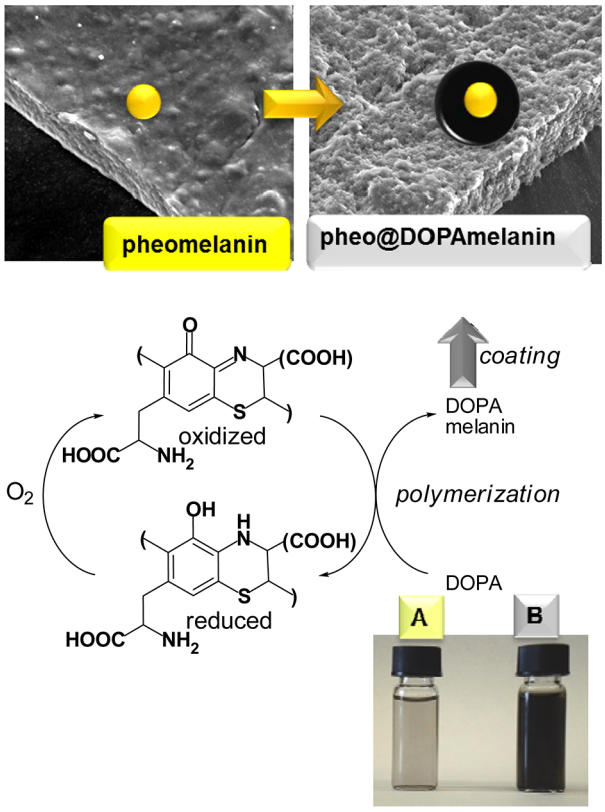


FIGURE 3. Scheme showing eumelanin deposition by aerial oxidation of DOPA promoted by synthetic pheomelanin. SEM images refer to synthetic pheomelanin before (left) and after (right) polymerization of DOPA and deposition of eumelanin-type polymer. Lower right corner, vial A, autoxidative conversion of DOPA to black eumelanin-type pigment as determined after 24 h; vial B, same as vial A but in the presence of 5-*S*-cysteinyldopa pheomelanin.

indicated the rapid conversion of DOPA into a black insoluble polymer encapsulating the redox-active pheomelanin core and gradually inactivating the redox-active moieties (Figure 3).

DOPA or dopamine oxidation occurs on the surface of finely suspended CD-melanin particles and involves apparently redox exchange processes leading to polycatecholamine coatings. This interaction results in a remarkable core–shell material and would mimic the natural casing process of melanosome assembly.[54–56]

Highlight 6*: Pheomelanin-type polymers behave as redox cycling systems promoting oxidative polymerization of catecholamine substrates. Structural units (benzothiazines) apparently can shuttle between oxidized and reduced forms accepting electrons from the catecholamines and transferring them to molecular oxygen sustaining the redox cycle. This peculiar property can be utilized to achieve controlled surface coating with polydopamine and other catecholic polymers and to engineer novel materials with redox exchange behavior.*

Inspiration and Opportunities beyond Melanin Pigmentation

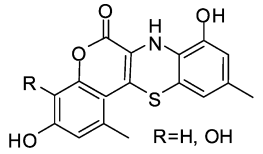
Bioengineering benzothiazine-based metabolites is proposed herein as a valuable strategy for the discovery of bioactivity, functionality, and molecular diversity. Support for this view is provided by the recent report of cytotoxic metabolites, pheofungins (Chart 1), with a benzothiazine chromophore strikingly similar to pheomelanins, which have been engineered via deletion of a gene that is required for global protein N-acetylation in *Aspergillus nidulans*.[57] Notably, the pheofungin chromophore is similar to pheomelanins in that it embodies a benzothiazine ring system. The production of unprecedented structures from a metabolic switch induced in an engineered mutant may open new avenues in the quest for innovative systems and functionality may lead to the discovery of natural products with potential therapeutic applications. A full assessment of the actual potential of gene mutation driven product discovery awaits that the powerful tools of genetics and molecular biology on one side and the methods of modern organic chemistry and biophysics on the other side are combined synergically to generate and tailor structural and functional diversity in a rationally predictable fashion.

As an aside to the above examples of mutated gene products, attention is called here to the recent identification of heronamycin A,[58] a novel component of benzothiazine ansamycins, from a *Streptomyces* sp. (CMB-M0392).

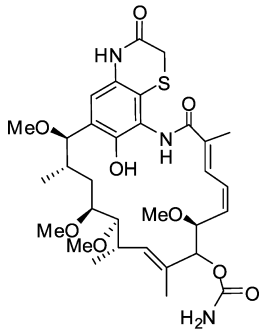
Biosynthetic studies revealed that the benzothiazine nucleus arises from condensation of the corresponding benzoquinone with cysteine to form an imine, followed by intramolecular cyclization and decarboxylation.[59] Awaiting new insights into the biogenetic origin of the benzothiazine derivatives of bacterial origin to pave the way to possible biotechnological approaches to their large scale production and application, those studies would prompt parallel approaches to structural and functional diversity based on cysteine-quinone and benzothiazine chemistry downstream to gene-controlled pathways. Mention should go in this connection to the efficient one-pot synthesis of 1,4-benzothiazines by Baker's yeast-catalyzed condensation of 2-aminobenzenethiols and 1,3-dicarbonyls using ultrasonication to increase reaction rate.[60]

Highlight 7*: Access to benzothiazine-based molecular scaffolds may be offered by new advances in the control of gene mutation products in biotechnological settings, by post-synthetic manipulation of quinone metabolites through inclusion of cysteine or related sulfhydryl compounds in the culture medium, and by conventional synthesis through biocatalytic systems.*

CHART 1. Benzothiazine-Containing Natural Scaffolds from Fungi and Bacterial Sources

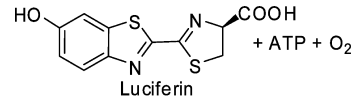


Pheofungins

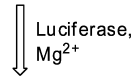


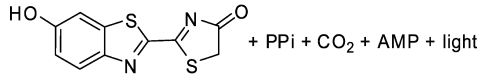
Heronamycin A

SCHEME 6. The Luciferin–Luciferase Reaction



Luciferin





Oxyluciferin

Benzothiazoles: A Tale of Fireflies

Although benzothiazoles figure prominently in pheomelanins and red hair pigments, the most outstanding example of naturally occurring benzothiazole derivatives is provided by the luciferins that fireflies use to generate light (bioluminescence) in a multistep process mediated by luciferases (Scheme 6).[61]

Because the firefly luciferase-catalyzed light emission from D-luciferin is widely used as a reporter of gene expression and enzymatic activity both *in vitro* and *in vivo*, the design and synthesis of enzyme–substrate pairs that combine an intense burst of light with high levels of sustained light output is a desirable goal for application. An interesting approach toward this goal has been based on the creation of mutant luciferases that yield improved sustained light emission with suitable luciferin derivatives, for example, aminoluciferins, in both lysed and live mammalian cells, allowing the use of aminoluciferins for cell-based bioluminescence experiments.[62,63]

Red Hair Time Has Come

Red hair pigments provide a paradigmatic example of how genetic mutations at a critical receptor controlling melanin pigmentation may produce unpredictable structural diversity associated with interesting chemical and physical properties. Research directed to translate nature-derived products and principles into new materials and new chemistry-based technological solutions continues to progress at a rapid pace, and new sources of inspiration are expected from a reappraisal of red hair-derived 2*H*-1,4-benzothiazines and related molecular systems beyond the traditional boundaries of photoprotection and skin cancer risk. Benzothiazine-based bioinspired systems can suit a range of purposes and can be used to design innovative *cis/trans*-isomerizing, pH-responsive, high extinction chromophores, for example, for sensors, for molecular calculators, or for photoconversion, and as efficient redox-cycling systems promoting controlled surface functionalization and coating.

Epilogue

When this Account was almost completed, Professor Ernesto Fattorusso, one of the pioneers of melanin chemistry at Naples, suddenly passed away. Together with his colleagues Rodolfo A. Nicolaus, Giuseppe Prota, Luigi Minale, and many others, he set the scene for an exciting age in the chemistry of natural products. Thanks to their brilliant intuition and brave efforts, the intractable and elusive melanin pigments have gradually been forced to disclose their secrets, and new important advances are expected to spring from the early studies on 1,4-benzothiazine compounds, hopefully contributing to novel discoveries in the ever expanding field of bioinspired chemistry.

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BIOGRAPHICAL INFORMATION

Alessandra Napolitano is Professor of Organic Chemistry at Naples University "Federico II" and a member of the Board of Directors of the European Society for Pigment Cell Research since 2006. Her research interests lie in the field of heterocyclic compounds, with special reference to hydroxyindoles and benzothiazines, chemistry of natural pigments, including pheomelanins, oxidative chemistry of phenolic natural products, food chemistry, and lipid peroxidation.

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Marco d'Ischia is Professor of Organic Chemistry at Naples University "Federico II" since 2001. He is currently member of the Council of the Italian Chemical Society, Division of Organic Chemistry, and scientific coordinator of the EuMelaNet group for melanin research of the European Society for Pigment Cell Research (ESPCR). His main current interests focus on melanin chemistry (for his studies in the field he was awarded the Raper Medal by the ESPCR and the International Federation of Pigment Cell Societies) and the design and synthesis of bioinspired organic systems for biomedical and technological applications.

FOOTNOTES

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The authors declare no competing financial interest.

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