EXPERT OPINION

Inhibitors of melanogenesis: a patent review (2009 – 2014)

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Introduction: Melanogenesis is the process of producing the melanin pigment, in which a series of chemical and enzymatic pathways are involved. Modulation at any level of this process would become an important approach in the treatment of hyper- or hypopigmentation-related diseases. Since hyperpigmentation covers important issue in cosmetics, there is a need of such review to understand and update this field to the public domain.

Areas covered: In this review, authors discuss most recent melanogenesis inhibitors published in the patents since 2009. The up-to-date overview of classical catechol-based tyrosinase inhibitors to non-classical melanogenesis inhibitors with different mechanism of action is discussed. Inhibitors including small-interfering RNA and peptides from ~ 30 patents and their associated literature are also discussed.

Expert opinion: Although a huge number of melanogenesis inhibitors have been reported, the future studies should be focused towards the identification of new inhibitors with a clear mechanism. The next breakthrough in the field therefore, is likely to come from the detailed structure–activity relationship studies of thioureas with improved therapeutic profiles. Targeting other parameters such as number or size of melanosomes, maturation of melanosomes and expression of melanogenic enzymes may give the best results to overcome toxicity and other formulation problems.

Keywords: dermatological disorders, freckles, hyperpigmentation, hypopigmentation, inhibitors, melanin, melanocytes, melanogenesis, Parkinson's disease and tyrosinase

Expert Opin. Ther. Patents [Early Online]

1. Introduction

The pigment melanin responsible for human skin and hair color is originated from epidermis, the outermost layer of the skin, where melanocytes are localized to produce it through a process called melanogenesis [1]. Melanin is important in protecting the skin from harmful effects of sunlight, toxic drugs and chemicals [1-4]. Melanin is a heterogeneous polymer which absorbs harmful ultraviolet (UV) radiation and transforms the energy into heat through an internal conversion process [2-4], which keeps the light-induced generation of free radicals at a minimum level. Melanogenesis is a complex pathway involving series of enzymatic and chemical reactions (**Figure 1**) [5-8]. Among them, tyrosinase (TYR), tyrosine-related protein-1 (TRP-1) and TRP-2 (dopachrome tautomerase [DCT]) are mainly involved in the transformation of tyrosine to melanin pigments [9].

1.1 Pathways of melanogenesis

The initial phase of melanogenesis occurs by the formation of dopaquinone (DQ) from the amino acid, tyrosine in two steps. First, the hydroxylation of L-tyrosine to L-DOPA followed by its oxidation to DQ, which in turn serves as a substrate for the synthesis of pheomelanins or eumelanins [6,10]. This first step is the key step in melanin synthesis, and the remainder of the reaction sequence can proceed spontaneously at physiological pH [10]. After its formation, DQ undergoes the

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Article highlights.

- Inhibitors target tyrosinase catalytic activity or down-regulate tyrosinase protein.
- Oligopeptides inhibit melanogenesis with high potency, short sequence length, low toxicity and enhance cell penetration.

intramolecular addition of the amino group generating leukodopachrome (cyclodopa). The redox exchange between leukodopachrome and DQ then give rise to dopachrome and DOPA. DOPA is also a substrate of TYR and oxidized to DQ again by the enzyme. Dopachrome gradually decomposes to give mostly dihydroxyindole (DHI) and to a lesser extent dihydroxyindole-2-carboxylicacid (DHICA). This later process is catalyzed by TRP-2, now known as Dct. Finally, these dihydroxyindoles (DHI and DHICA) are oxidized to eumelanin. TRP-1 is believed to catalyze the oxidation of DHICA to eumelanin. On the other hand, in the presence of cysteine, DQ is converted to 5-*S*-cysteinyldopa and to a lesser extent 2-*S*-cysteinyldopa. Cysteinyldopas are then oxidized to give benzothizine intermediates and finally produce pheomelanin (**Figure 1**).

1.2 Regulation of melanogenesis

Melanogenesis is regulated through a series of intracellular signaling pathways associated with the enzymes TYR, TRP-1 and TRP-2. **Figure 2** shows the three most commonly known signal pathways involved in the regulation of melanogenesis, which involve microphthalmia-associated transcription factor (MITF) in the regulation of melanin synthesis. MITF is the master regulator of melanogenesis in melanocytes *via* binding to the M box of a promoter region and regulating the gene expression of TYR, TRP-1 and TRP-2 [11-15].

cAMP-dependent signaling pathway: Melanocortin 1 receptor (MC1R), the key controller of melanin production in melanocytes is regulated positively by α-melanocyte-stimulating hormone (α-MSH) and negatively by agouti signal protein (ASP) [16]. Once the α-MSH stimulates the MC1R, it results in the production of cAMP by the activation of adenylyl cyclase (AC). cAMP activates protein kinase A (PKA), which in turn activates the gene expression of MITF *via* phosphorylation of the cAMP response element-binding protein (CREB) [17-20]. Finally, MITF regulates transcription of crucial pigmentary genes coding melanogenesis-related proteins (MRPs) through interactions with M- and E-boxes present in the promoter regions of TYR, TYRP-1 and TYRP-2. In addition to α-MSH, other POMC-derived peptides, such as β-MSH, and adrenocorticotropic hormone, also stimulate melanogenesis *via* the same way.

Wnt or β-catenin pathway: WNTs are cysteine-rich secreted glycoproteins with important functions in the embryonic development, especially in neural crest cells like melanocytes [21,22]. In this pathway, WNT binds to G-protein-coupled receptor (called Frizzled) and leads to inactivation of glycogen synthase kinase-3β, followed by the accumulation of β-catenin and its translocation to the nucleus, where it forms a complex to lymphoid enhancer factor and T-cell factor and increases the expression of MITF gene [23,24]. An increased level of nuclear β-catenin increases the expression of MITF, which stimulates melanogenesis [25].

ERK signaling pathway: Otherwise called MAPK signaling pathway and involves many proteins, including MAPK (mitogen-activated protein kinases). The signal starts when a signaling molecule binds to the receptor on the cell surface where Ras activates B-raf kinase and consequently MAP kinases, ERK1 and ERK2. MAP kinases phosphorylate MITF leading to its ubiquitination and degradation, thus removing a major transcriptional regulator of MRP genes expression [26-28]. Activation of Ras oncogene inhibits melanogenesis in normal and malignant melanocytes [27,29]. In addition, the important role of c-Kit in the ERK pathway has been highlighted in some reports [30,31].

2. Modulators of melanogenesis

Melanin is our skin's very own sunscreen which protects it from harmful UV rays from the sun. However, cumulative exposure to UV light can result in an increased risk of skin cancer and skin damage (e.g., premature aging and wrinkles), albinism and leukoderma. Thus, pharmaceutical agents that induce melanogenesis are of medical interest for protecting skin from photodamage or UV exposure. Since 2009, a number of melanogenesis promoters were reported in the patent literatures. For examples, several pyrazoles [32], indole alkaloids [33], cannabinoid derivatives by activating CB1 receptor [34] and 2-bromopolymitate derivative by inhibiting polmitoylation of TYR [35] were reported to stimulate melanin synthesis (melanogenesis) in the skin and hair.

Although melanin plays an important role in protecting the skin from UV radiation and other environment stimulants, the increased production and accumulation of melanin in different specific parts of the human skin can lead to a large number of dermatological disorders, which include acquired hyperpigmentation such as melasma, freckles, postinflammatory melanoderma, solar lentigo and age spots. Therefore, in recent years, there is a huge demand for melanogenesis inhibitors which provide the impetus for researchers to explore it. Several manuscripts discuss the inhibitors, some well-known and used in the commercial whitening agent, such as hydroquinone (HQ), kojic acid and their derivatives, hydroquinone-*O*-β-glucopyranoside (arbutin), magnesium ascorbyl phosphate, licorice extract, aloesin, azelaic acid, soybean extract and niacinamide [36-42].

The present review surveys melanogenesis inhibitors that have been recently discovered from 2009 to 2014. The inhibitors from both sources natural and chemically synthesized

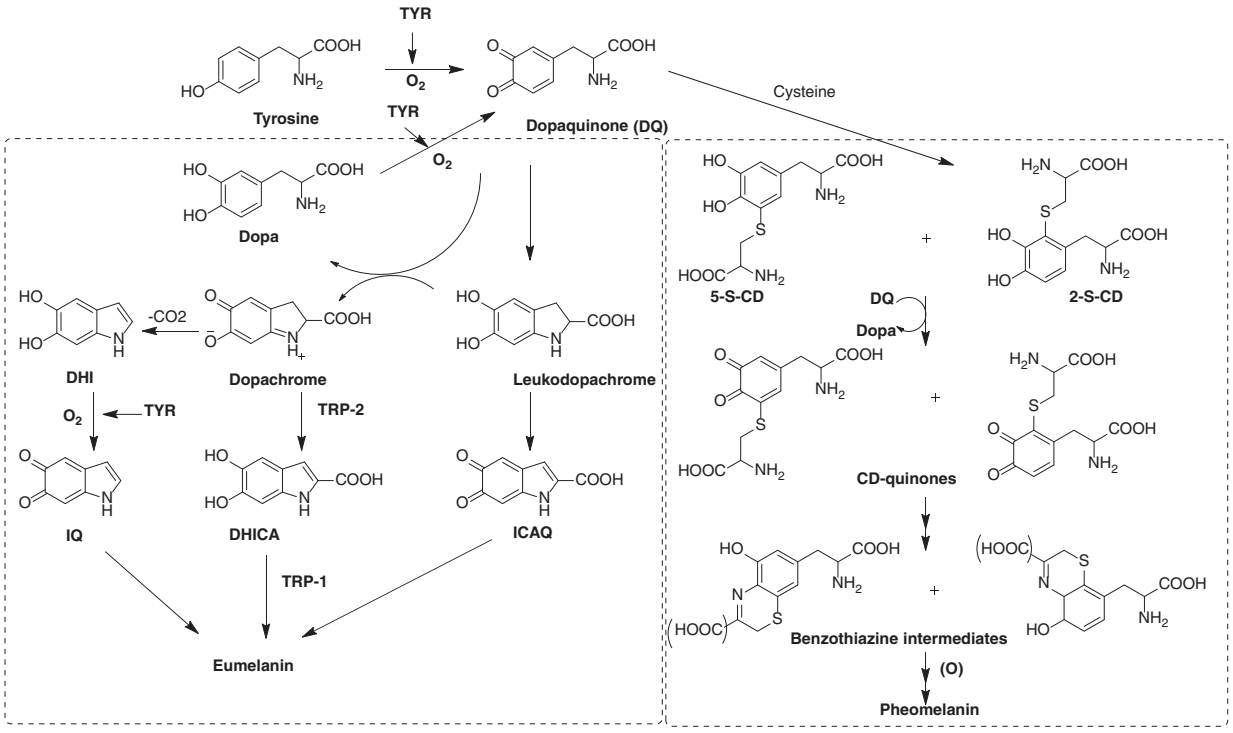


Figure 1. Pathway of melanogenesis (production of eumelanin and pheomelanin).
Adapted from [11].

and particularly whose reaction mechanisms are known to downregulate the TYR, are discussed in detail.

2.1 Melanogenesis inhibitors acting through the downregulation of tyrosinase activity

Although three enzymes (TYR and TRP-1/2) are involved in the melanogenesis signaling pathway, TYR is most critical and rate-limiting enzyme due to its key role in the process. The enzyme is a copper-containing membrane-bound glycoprotein located in the membrane of the melanosome with an inner melanosomal domain that contains the catalytic region, followed by a short transmembrane domain and a cytoplasmic domain [43-45]. Therefore, the inhibition of TYR is expected to provide effective therapeutic means for the treatment of dermatological disorders. Majority of currently known hypopigmenting agents act directly or indirectly on this enzyme *via* several mechanisms.

2.1.1 Benzimidazole-2-thiols, phenylthiols and phenylthioureas as inhibitors

A series of benzimidazole-2-thiol, phenylthiol and phenylthiourea (PTU) compounds are reported to reduce or inhibit the production of melanin by mammalian melanocytes, and these are preferably inhibiting the melanocyte TYR activity [46]. Some selected inhibitors (**1-10**) from each derivative are indicated in (**Table 1**) [46]. These compounds exhibit anti-melanogenesis activities that are equivalent or superior to the known standard skin-bleaching agent, HQ [47] or other known standard TYR inhibitor, methyl gentisate [47,48]. Especially, compound 6-methoxy benzimidazole-2-thiol (**2**) showed excellent activity with an IC_{50} = 0.07 μM and almost no toxicity. The compounds reported herein inhibit the mammalian TYR, yet with minimal cytotoxicity. These compounds are almost selective against melanocyte TYR and thus can be administered systemically by methods including oral, intradermal, transdermal, intravenous and parenteral admirations.

A distinct advantage of the screening systems is the focus on mammalian TYR, as opposed to mushroom TYR often reported. Three bioassays characterize the compounds with regard to mammalian TYR enzyme inhibition (cell free), pigmentation in melanocyte cultured cells and cytotoxicity of mammalian-cultured cells. Both cell-based pigmentation and cell-free enzymatic-based assays have been developed using the mammalian melanocyte cell line Mel-Ab, a C57BL/6 mouse-derived cell lines those produces high levels of melanin. Also, Mel-Ab melanocyte can serve as adequate surrogate for human melanocytes (HEMns) for many pharmacologic purposes. For the enzymatic assay, multi-well plate assays have been demonstrated and for pigmentation assays on cultured Me-Ab cells. This assay can detect in apparent loss in pigmentation resulting from their either inhibition of *de novo* synthesis (e.g., *via* inhibition of TYR, or the adenylate cyclase pathway, or another pathway) or a

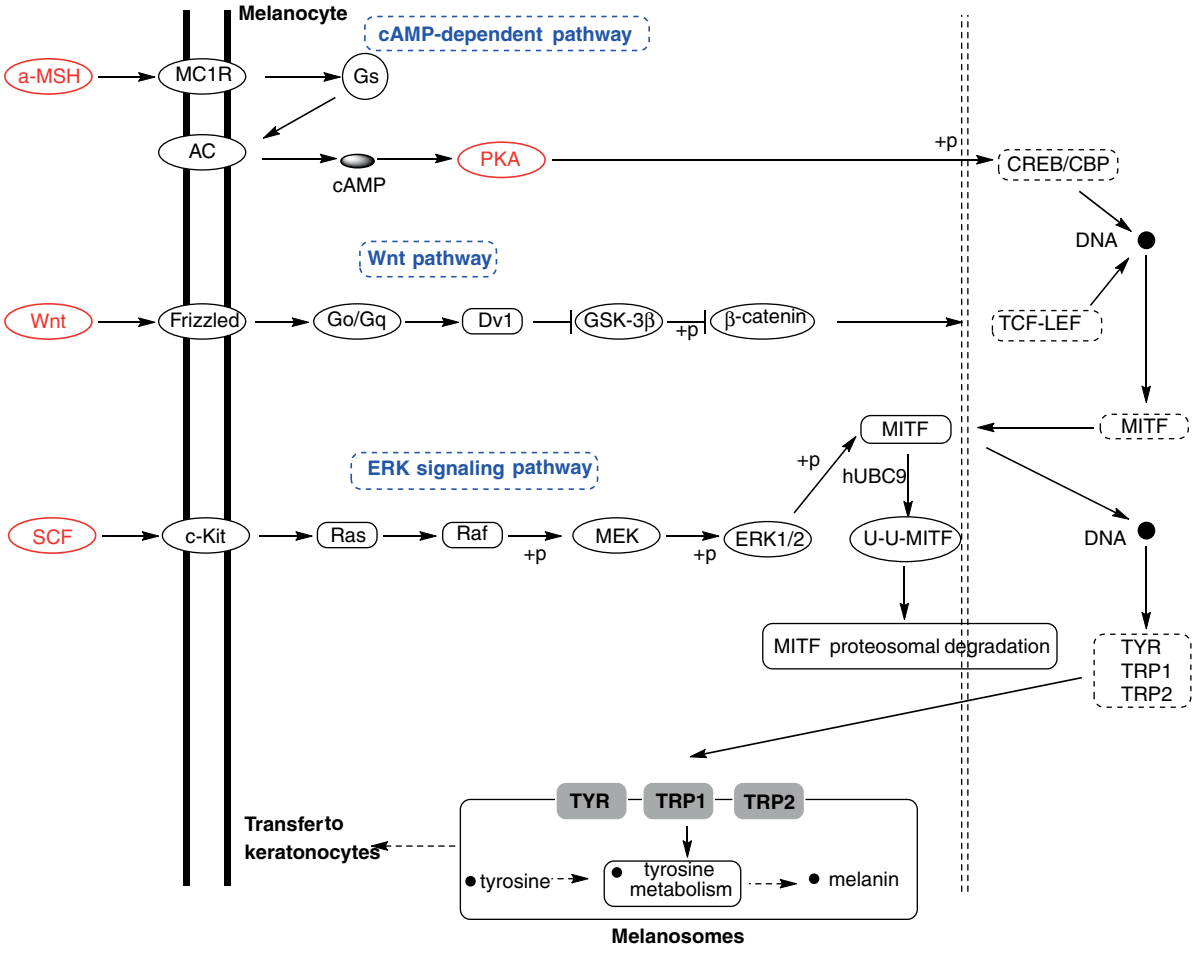


Figure 2. Regulation of melanogenesis through three main signaling pathways.
Reproduced from [11].

cytostatic/cytotoxicity mechanism. It is used in parallel with the TYR enzymatic assay to determine whether an inhibitor of pigmentation at the cellular is acting primarily at the enzyme level. To determine cytotoxicity, crystal violet or other staining methods may be used to quantify adherent cell numbers following period of treatment by an agent HQ used as a positive control in the assays. These compounds can be formulated along with other active and functional ingredients such as organic or inorganic sunscreens, antioxidants, anti-inflammatory or antibiotics.

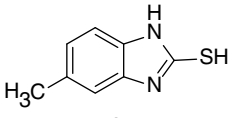
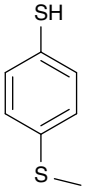
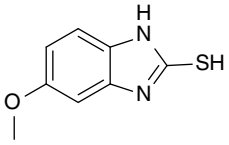
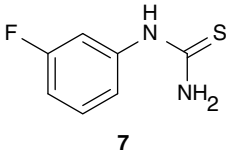
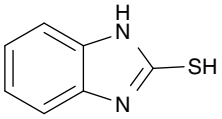
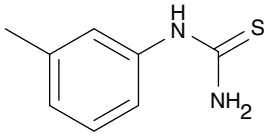
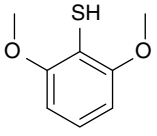
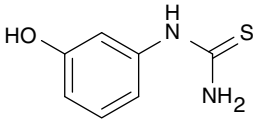
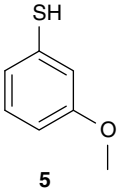
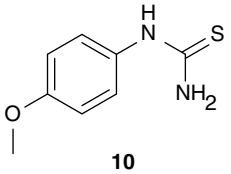
2.1.2 4-Phenylimidazole-2-thiones as inhibitors

From the screening of compounds on B16F1 (murine melanoma cell line), it has recently been discovered that certain 4-phenylimidazole-2-thiones (**Figure 3A**) exhibit a very good TYR inhibitory activity in a dose-dependent manner with a very low cytotoxicity [50-52]. Therefore, these compounds could find applications in human medicine, particularly in dermatology and cosmetics. The general structure of the inhibitor and selected inhibitors from this with an activity profile are illustrated in **Figure 3** (**11-21**). Structure–activity relationship (SAR) studies prove the imidazole-2-thione unit is a pharmacophore and aryl or heteroaryl substitution on the 5-membered unit led to enhance the TYR inhibitory potency. However, replacement of aminohydrogen with substituent decreases potency (compare **16** and **21** in **Figure 3**) [51,52]. The compositions for topical application have a concentration of a compound generally ranging from 0.001% to 10% by weight, preferably from 0.01% to 5% by weight, with respect to total weight of the composition.

2.1.3 Tibolone metabolites as inhibitors

Tibolone (17-hydroxy-7α-methyl-19-norpregn-5(10)-en-20-yn-3-one) is a synthetic steroid that combines estrogenic and progestogenic properties with androgenic property, which mimic the action of a male sex hormone. Several new metabolites (**Table 2**, **22-29**) of tibolone were obtained by incubation of tibolone with various fungi such as *Rhizopus stolonifera*, *Fusarium lini*, *Cunninghamella elegans* and *Gibherella fujikuroi* [53]. The compounds were primarily screened for the diphenolase inhibitory activity of TYR using L-DOPA as substrate. The active compounds from the primary

Table 1. Melanogenesis inhibitory activities of benzimidazole-2-thiols, phenylthiols and phenylthioureas (1-10) [46].

Structure	*IC_{50} (μM)	‡IC_{50} (μM)	§IC_{50} (μM)	Structure	*IC_{50} (μM)	‡IC_{50} (μM)	§ IC_{50} (μM)
 **1**	0.12	2.4	> 1000	 **6**	0.24	115	126
 **2**	0.07	1.6	> 1000	 **7**	1.2	1.78	> 1000
 **3**	8	-	-	 **8**	0.82	2.28	> 1000
 **4**	500	200	200	 **9**	4	8	> 1000
 **5**	53	85	202	 **10**	8	30	60

*Cell-free melanin enzyme assay.
‡Mammalian melanocyte culture.
§Cytotoxicity.

screening were subjected to IC_{50} studies against mushroom TYR. As a result, metabolites, such as 17α-ethynyl steroids, were identified as strong inhibitors of TYR enzyme and thus useful in producing skin lightning and preserving fruits and vegetables. Especially, hydroxyl metabolites (**23**, **24** and **28**) of tibolone exhibited significant inhibitory activity against enzyme TYR (**Table 2**). The composition generally contain about 0.00001% to about 10% of compounds of tibolone metabolites.

2.1.4 Resorcinol derivatives as inhibitors

Among the phenols or HQ derivatives described in literature, resorcinol derivatives have drawn a considerable attraction as depigmenting agents. Unsubstituted 4-alkylresorcinols, which have the ability to reduce skin pigmentation, are described in many publications: WO99/15148 (4-cycloalkylresorcinol), WO2006/097223 (4-cycloalkylmethylresorcinols), FR 2704428 (4-haloresorcinol) and EO2004/052330 (4-(1,3) dithian-2-ylresorcinols). More particularly patent EP 0341 664 discloses the use of *n*-alkyl resorcinols as depigmenting agents, among them 4-*n*-butyl resorcinol (**30**), also known as rucinol, which forms part of the composition of a depigmenting cream sold under the trade name Iklen®. Novel compounds with a 4-(azacycloalkyl)benzene-1,3-diol structure have recently been discovered with a good TYR inhibitory activity and a very low cytotoxicity (**Table 3**) [54]. The general structure A and selected compounds are exemplified in **Figure 4**. These compounds, in particular the compound **34**,

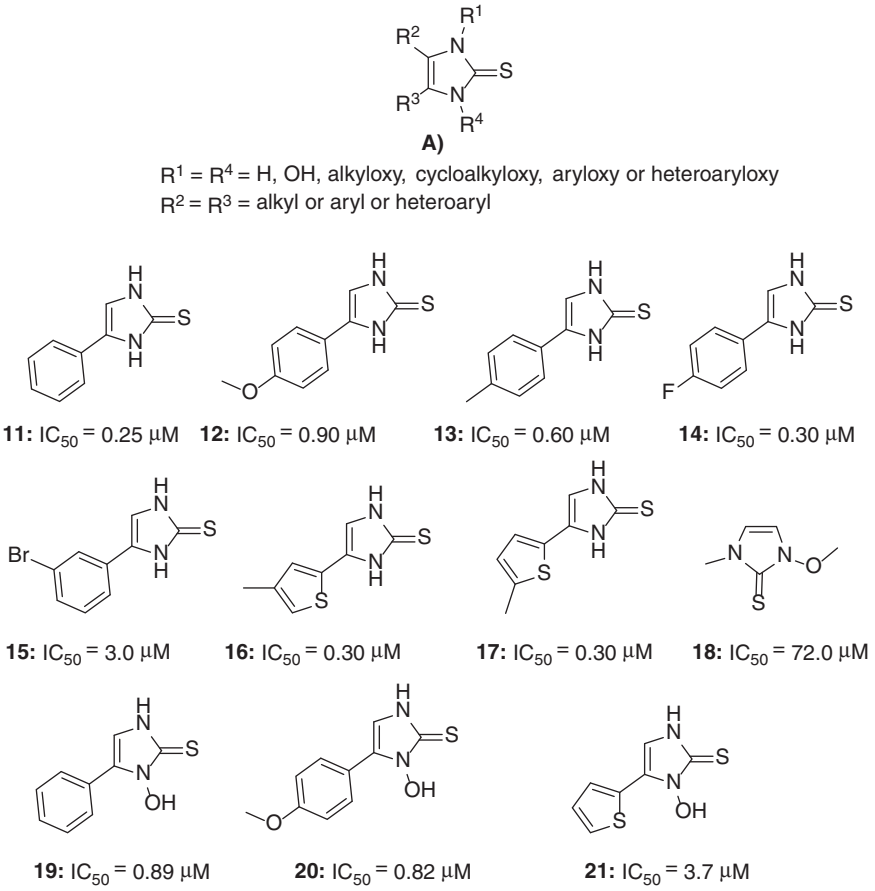


Figure 3. General chemical structure and tyrosinase inhibitory activity of imidazole-2-thiones (19-29) [50-52].

have a greater TYR inhibitory potency (**34**, IC_{50} = 0.5 μM) than that of rucinol (**30**, IC_{50} = 3.0 μM), while being less cytotoxic than rucinol (**Table 3**, **30-34**).

Surprisingly, it has now been found that some substituted 4-alkylresorcinols have excellent skin lightening properties [55], for example, compound **33** in **Figure 4**. The inhibition of compounds against TYR was assessed using TYR from fungi and L-DOPA as substrate and Kojic acid or 4-hexyl resorcinol as a positive control. The skin lightener compounds described in this section (**Table 3**, **30-34**) can be formulated with other ingredients and used as a cream, ointment and lotion.

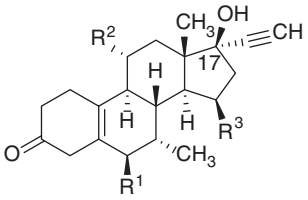
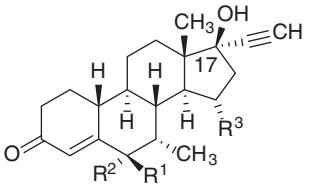
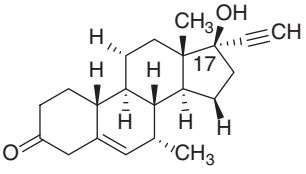
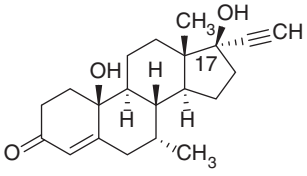
2.1.5 siRNA oligomers as inhibitors

Another powerful tool used to study gene function in mammalian cells is the process of small interfering RNA (siRNA)-mediated gene silencing. The process uses double-stranded RNA that is less than 30 base pairs long and has a sequence complementary to the mRNA targeted. Safe, effective and new compositions containing siRNA oligomers to reduce or inhibit and/or improve hyperpigmentation would be advantageous for the formulation of treatments and products for the skin. Mechanistically, the siRNA inhibit production of TYR protein by binding to a specific complement sequence found in the TYR mRNA and therefore, by inhibiting TYR, melanin and pigmentation decreases. The siRNA oligomers exert their effectiveness by preferably crossing the plasma membrane of skin cells, wherein the siRNA and any sequence exactly like TYR mRNA will be destroyed and degraded at the site of application, for example, skin of the face, neck, arms and layers of the skin where hair follicles/melanocytes interact. The sequences for the siRNA oligomers are a total of 21 base pairs long and are homologous found in both human and mouse forms of TYR [56-58]. These siRNAs can be stabilized by including thymidine or uridine nucleotide 3' overhangs and few examples of siRNA are indicated in **Figure 5** [59]. The siRNA oligomers comprise compositions which include topically applied sunscreens, antioxidants, anti-inflammatories and cosmetics

2.1.6 Oligopeptides as inhibitors

Some new oligopeptides were reported to inhibit TYR with improved properties such as high potency, short sequence length, low toxicity and enhance cell penetration [59]. Short peptides between 6 and 12 amino acids, preferably 8 amino

Table 2. Tyrosinase inhibitory activity of tibolone metabolites (22-29) [53].

Metabolites	*Tyrosinase inhibitory activity IC_{50} (μM)	Metabolites	Tyrosinase inhibitory activity IC_{50} (μM)
			
Delta^4 -Tibolone (**22**); $R^1 = R^2 = R^3 = H$ 6β -Hydroxytibolone (**23**); $R^1 = R^2 = H$, $R^3 = OH$ 11$^{\alpha}$,15β-Dihydroxytibolone (**24**); $R^1 = H$, $R^2 = R^3 = OH$		15β-Hydroxytibolone (**25**) ; $R_1 = R_2 = R_3 = H$ 6β-Hydroxy-delta4-Tibolone (**26**); $R_1 = OH$, $R_2 = R_3 = H$ 6β-Methoxy-delta4-Tibolone (**27**); $R_1 = OH$, $R_2 = R_3 = H$	
			
11$^{\alpha}$,15β-Dihydroxy-delta5-Tibolone (**28**)		10β-Hydroxy-delta 4 -Tibolone (**29**)	
22	8.19	**27**	25.15
23	7.45	**28**	7.10
24	5.12	**29**	119.4
25	50.86	Kojic acid	16.67
26	36.14	L-Mimosine	3.68

*Tyrosinase was assessed using tyrosinase from mushroom [53]. Kojic acid and L-mimosine are standard tyrosinase inhibitors.

acids (peptides **35**-**37**) are also disclosed and shown to have inhibition against TYR (**Table 4**) [59]. These peptides are synthetically designed using naturally occurring amino acids, and therefore biologically safe. Most importantly, these peptides do not cause cancer or are not toxic to skin cells as does HQ, and since they are derived from naturally occurring amino acids, are easily degraded intracellularly upon inactivation of TYR. Based on the requirements in physiochemical properties for the formulation and for overcoming the toxicity, the present peptides may also be conjugated to other small molecule inhibitors such as kojic acid or gentol [60].

The inhibitory activity against mushroom TYR was illustrated in **Table 4**, and these peptides were identified as dose-dependent. The inhibitory activity of **35**-**37** against human TYR was also demonstrated using L-DOPA as the substrate. The octapeptides were significantly more potent inhibitors of human TYR relative to HQ. For example, at 200 mM, human TYR activity was reduced by 30% for **35**, 12% for **36** and 25% for **37** compare to only 5% of HQ. These octapeptides possess adequate cell penetration and appropriately translocate to melanosomes to exert their inhibitory effect on TYR. The MTT cell viability and proliferation assay showed very high toxicity for HQ towards primary HEMns, keratinocytes and fibroblasts, whereas less than 10% toxicity was observed for the octapeptides.

2.2 Inhibitors of adaptor protein (AP)-complexes

It was reported that some specific siRNA of a subunit of adaptor protein-1 (AP-1) adaptor complex have a significant effect on the production of melanin pigment and can thus be effectively used as depigmenting agents. During the melanin biosynthesis, the pigmented mature melanosomes is obtained by the addition of enzymes that are keys to melanin synthesis and of effectors (e.g., kinasins) necessary to its transport towards the periphery. The enzymes involved in melanogenesis are thus synthesized in the Golgi apparatus and then transferred in the pre-melanosomes. This type of transfer requires the participation of AP complexes, which are having the role of recruiting the enzymes in the transport vesicles. There are four of these complexes, named to AP1–4. Raposa *et al.* discovered that synthesis of melanin pigments could be decreased by means of an inhibitor of a kinesin interacting with AP-1 adaptor complex, in particular Kif13A, an inhibitor of a subunit of AP-1 adaptor complex [61]. The inhibitor

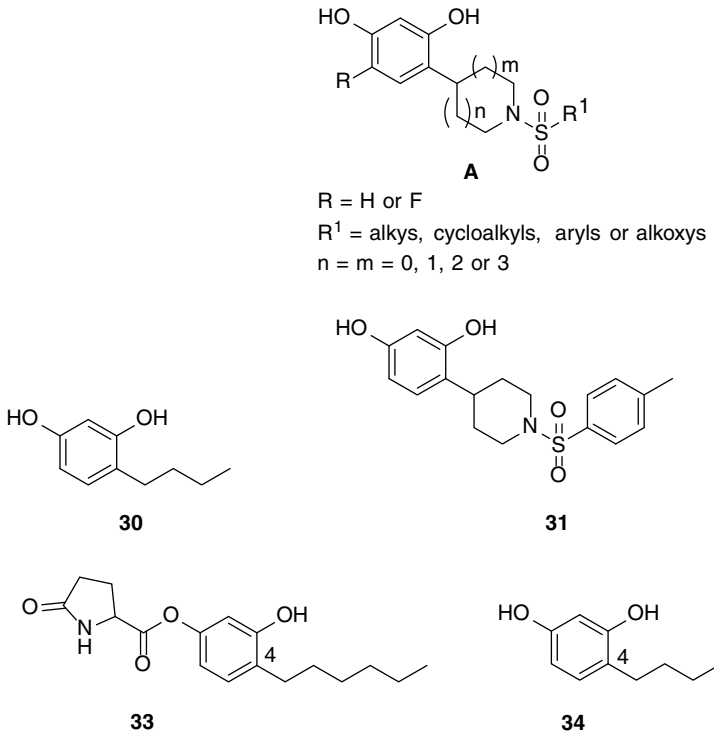


Figure 4. Chemical **structure of resorcinol derivatives [55].**

Table 3. Resorcinol derivatives (30-34) and their melanogeneis inhibitory activity [55].

Compounds	Melanogenesis inhibitory activity IC_{50} (μM)	*Tyrosinase inhibitory activity IC_{50} (μM)	Cytotoxicity IC_{50} (μM)
30	15	3.0	55
31	-	1.5	-
32	0.2	-	> 999
33	-	19	-
34	-	0.5	-
Kojic acid	-	12	-

*Tyrosinase was assessed using tyrosinase from fungi.
-: Not determined.

Table 4. Oligopeptides (35-37) and their tyrosinase inhibitory activities [59].

C.No	Peptides	Mushroom tyrosinase inhibitory activity IC_{50} (μM)	Human tyrosinase inhibitory activity (%)
35	Arg-Arg-Trp-Arg-Arg-Tyr-Tyr	238	30
36	Arg-Arg-Arg-Tyr-Trp-Tyr-Tyr-Arg	398	12
37	Arg-Arg-Tyr-Trp-Tyr-Trp-Arg-Arg	282	26
	HQ	560	5

C.No: Compound number; HQ: Hydroquinone.

is a nucleic acid comprising or consisting of hybridizing specifically with a gene or mRNA coding for a subunit of AP-1 adaptor complex. They indeed observed that such inhibitor was not only able to disrupt the transport of melanogenesis enzyme towards the melanosomes, thus blocking the maturation of these organelles, but also able to decrease the expression of melanogenic enzymes.

2.3 Miscellaneous inhibitors of melanin formation

Nontoxic natural products useful in the formulation of cosmetics and pharmaceuticals are considered of significant interest. WO 0135971 discloses the water/methanol extract of a plant belonging *to Rosaceae pyracantha* effectively inhibits TYR enzyme. According to the recent invention, two new compounds are isolated and purified from the same plant [62]. These two compound are 3,4-dihydroxy-5-methoxybiphenyl-2'-*O*-β-D-glucopyranoside (**38** or MHL-1) and 3,6-dihydroxy-2,4-dimethoxy-dibenzofuran (**39** or MHL-2, **Figure 6**). The ability to inhibit TYR activity of these compounds was measured on HEMn cells using arbutin as a positive control. The result shows that MHL-2 has TYR inhibitory activity similar to that of arbutin, whereas MHL-1 has superior

5'-UAGGACCUGCCAGUGCUCUtt-3'

3'-tt AUCCUGGACGGUCACGAGA-5'

5'-UAGGACCUGCCAGUGCUCUtt-3'

3'-ttAUCCUGGACGGUCACGAGA-5'

5'-UCCUGGAAACCAUGACAAAtt-3'

3'-ttAGGACCUUUGGUACUGUUU-5'

5'-CACACCUGUCUUUGUCUUAtt-3'

3'-ttGUGUGGACAGAAACAGAAC-5'

Figure 5. Structure of tyrosinase inhibitors siRNA oligomers [59].

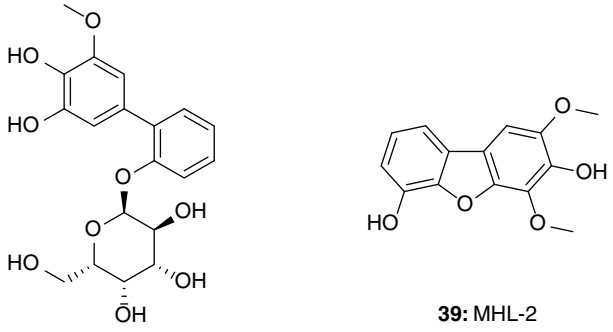


Figure 6. Chemical structure of MHL-1 and MHL-2 [62].

activity compared to arbutin. MHL-1 and MHL-2 inhibit around 40 and 20% TYR activity, respectively.

It has been long time known that *p*-aminobenzoic acid (PABA) is one of the additives in sunscreens for its capacity to absorb UV radiation. Now, it has been discovered that PABA acts as a potent inhibitor of melanogenesis and the mechanism of action study indicated that PABA was a potent inhibitor of TYR [63].

A novel series of quinoline [64], benzopyrone [65] and coumarin [66] were identified to have melanogenesis inhibitory activity in the cell-based assays. Especially in the condition that are casually related to aberrant melanogenesis activity such as pigmentation abnormalities and hyperpigmentation. The IC_{50} values of quinolines (**Figure 7**, **40-45**) [64] and percentage of control melanin remaining after treatment of inhibitors of benzopyrone (**46-50**) [65] and coumarin (**51-61**) [66] derivatives are indicated in **Figure 8** and **Table 5**. Among the quinolines (**40-45**), a compound with phosphate salt (**41**) exhibited an excellent melanogenesis inhibitory activity. The ability of such compounds to decrease melanin synthesis in mammalian cells may be used as advantage to decrease the melanin contents of melanocytes, which in turn, results in decrease pigmentation or lightening skin and hair.

New alkaloids pipperlongumine and piperlonguminine from the roots of *Piper longum L.* were reported for melanogenesis inhibition [67]. The coffee oil (from Coffea Robusta) extracted by super critical carbondioxide was found to inhibit melanogenesis in melanoma B16F1 cells [68]. An effective dosage of coffee oil wherein said dosage of 10 mg/l of treating oil, the total melanin content was decreased over 25%. The coffee oil inhibits the early period of melanogenesis which was proved by the

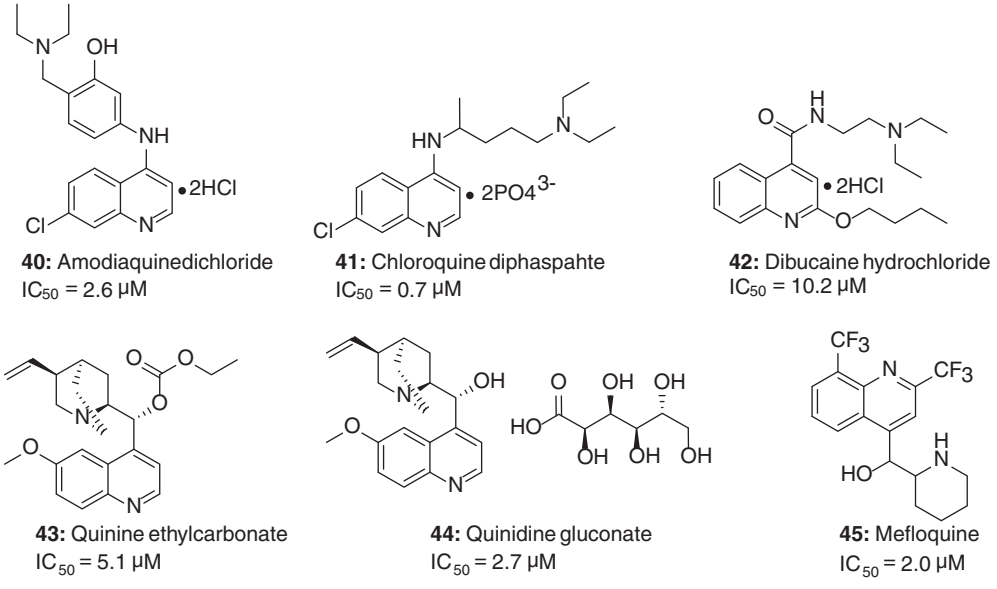


Figure 7. Quinolines and their inhibitory activity against melanogenesis [64].

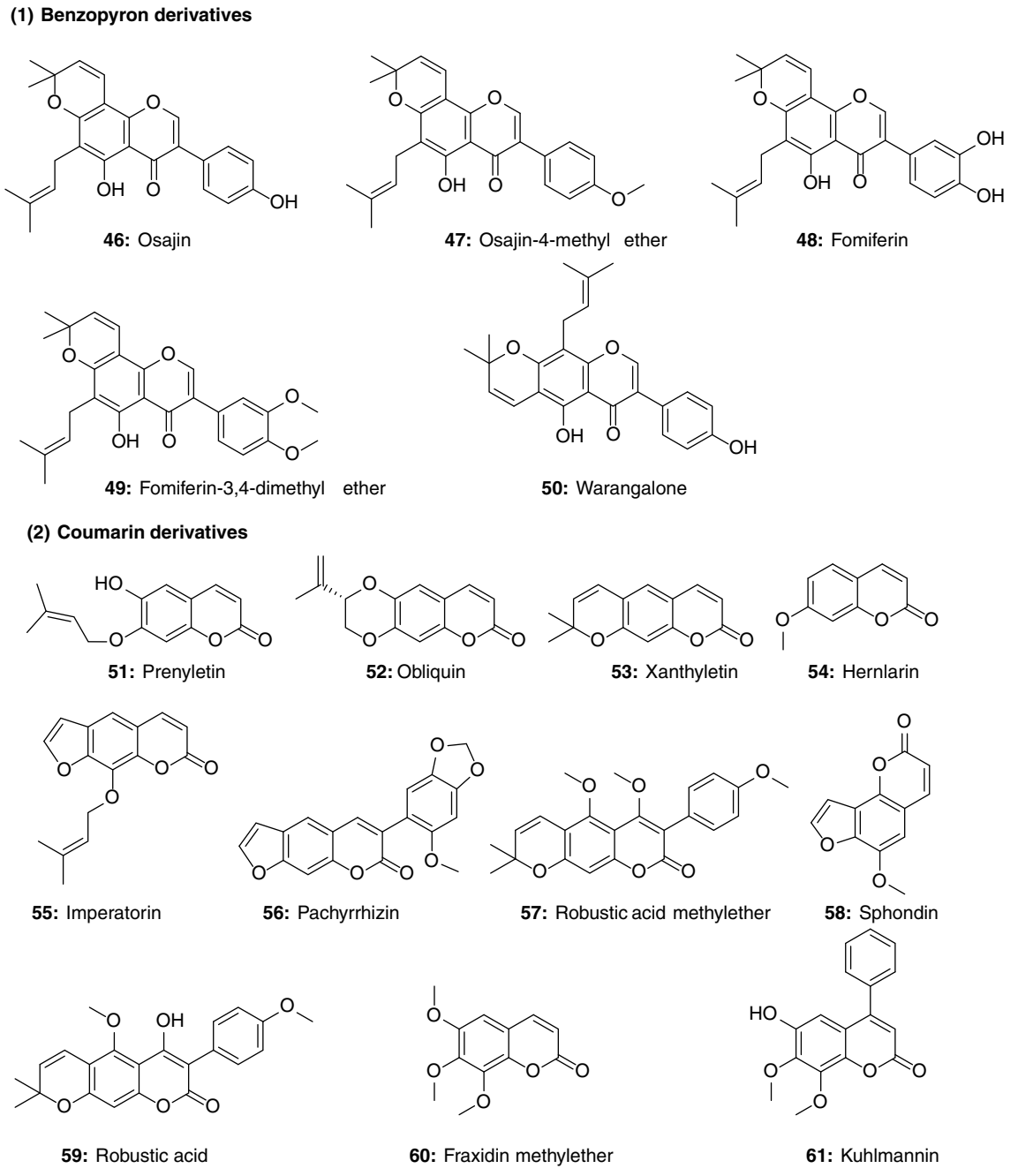


Figure 8. Structure of selected benzofurans and coumarins on melanogenesis [65,66].

downregulation of eight identified proteins including α-MSH, MC1R, ACTHR, MITF, PKC, SCF, TYR and TRP-2 [68].

The extracts of *Laminaria saccharina* are described to be used in cosmetic compositions as an osmoprotector, free-radical scavenger, or against the effects of skin aging. A cosmetic composition sold under the brand name SK-II Facial clear solution (Procter & Gamble, Cincinnati, Ohio) has a concentration of Phlorogine about 1.25%. It was able to identify the mechanism of action in whitening effect due to inhibition of TYR [69].

3. Conclusion

In the last few years, knowledge of melanocyte biology and the processes underlying melanin synthesis have made remarkable progress, opening new paths in the identification of melanogenesis inhibitors and novel approaches to treat diseases caused by melanocyte dysfunction. Since 2009, the most patent applications were claiming that melanogenesis inhibitors are targeting TYR, either by directly inhibiting the

Table 5. Inhibitory activity data of benzofurans (46-50) [65] and coumarins (51-61) [66] on melanogenesis.

Compound no.	Compounds	% of control melanin*	Compound no.	Compounds	% of control melanin*
46	Osajin	99	**54**	Hemlarin	84
47	Osajin-4-methyl ether	99	**55**	Imperatorin	91
48	Fomiferin	53	**56**	Pachyrrihzin	75
49	Fomiferin-3,4-dimethylether	80	**57**	Robustic acid methyl ether	69
50	Warangalone	100	**58**	Sphondin	81
51	Prenyletin	79	**59**	Robustic acid	69
52	Obliquin	84	**60**	Fraxidin methylether	87
53	Xanthyletin	94	**61**	Kuhlmannin	81

*Screening of compounds in cultured murine melanocytes and the percentage of control melanin reaming after treatment at 1 μM.

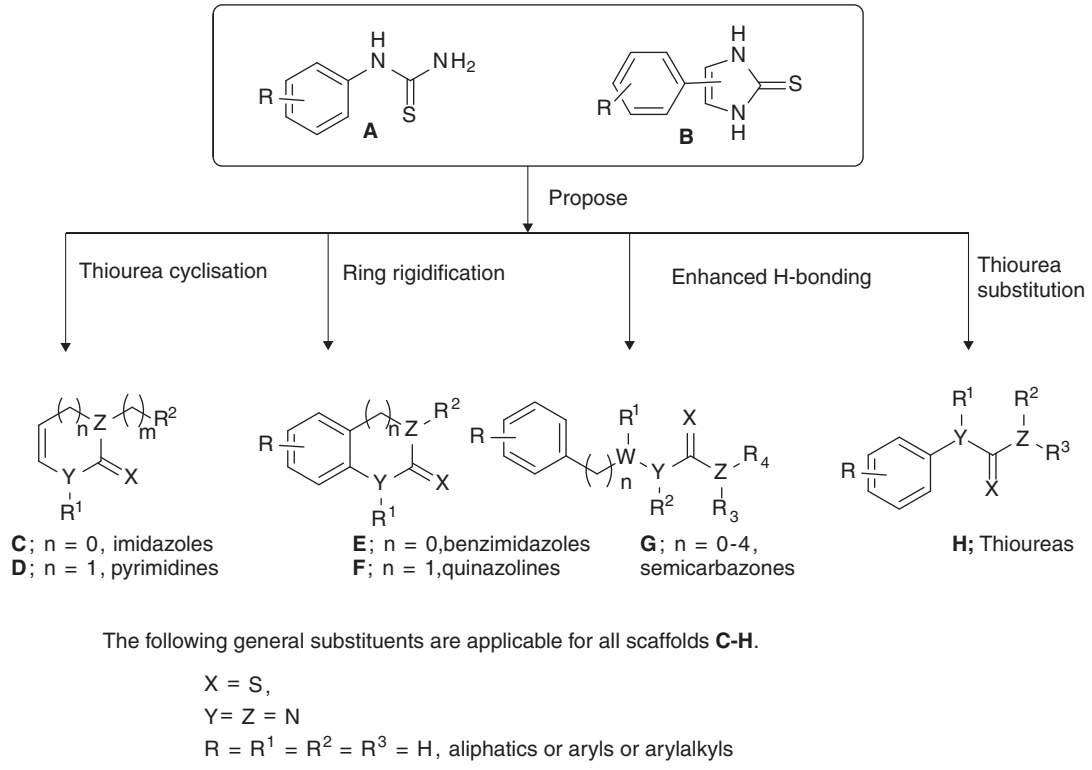


Figure 9. Structure of reported thioureas **A** and **B** and proposed molecules (**C** – **F**) for SARs.

catalytic activity or by down-regulating TYR production. The findings of oligopeptides [59] are ideal therapeutic candidates for cutaneous hyperpigmentation disorders due to their potent inhibitor activity against TYR and relative absence of melanocyte and fibroblast toxicity. In addition to the direct inhibition of TYR catalytic activity, other approach to use siRNA to inhibit the production of TYR protein by binding to a specific complement sequence found in the TYR mRNA are also established and therefore melanin and pigmentation decreases. Safe, effective and new compositions containing siRNA oligomers to inhibit hyperpigmentation would be advantageous for the formulation of treatments and products for the skin. Most inhibitors described in the present review have been incorporated in topically applied cosmetics in the forms of cream, ointment and lotion.

4. Expert opinion

The majority of known hypopigmenting agents are phenols and HQ derivatives. These compounds target TYR, but

most of them are cytotoxic to melanocytes. Compounds that could inhibit melanogenesis while remaining low in cytotoxicity or devoid of toxicity towards melanocytes would be of particular interest.

Although some recent findings described in the present review proved to have potent inhibitory activity with less toxicity against melanogenesis, none of them had become as a commercial whitening product, probably due to a lack of *in vivo* hypopigmentation activity in human clinical trials. To address the above shortcomings, future studies should be focused towards the identification of new inhibitors with the clear mechanism of action.

Most of the inhibitors targeting TYR or melanocyte cells are known to be toxic and therefore targeting other melanogenic parameters such as number or size of melanosomes, maturation of melanosomes and expression of melanogenic enzymes may give best results to overcome the toxicity and other formulation problems. The approach is to inhibit kinesin interacting with AP-1 adaptor complex; particularly, Kif13A would be one of the promising approaches in the future research towards melanogenic disorders, especially for hyperpigmentation disorders.

One of the interesting findings from this topic is *N*-phenylthoiurea and 4-phenylimidazole-2-thione derivatives have accomplished moderate inhibitory activity against mammalian or murine TYR exhibit a low toxicity (see Sections 2.1.1. and 2.1.2.) *N*-PTU has long been known as reference inhibitor against TYR. The crystal structure of PTU-bound catechol oxidase establishes that the sulfur atom of PTU binds to both copper ions in the active site of the enzyme. Although the crystal structure of PTU-bound TYR has not been known, the binding mode of this compound would probably be very much similar. The next breakthrough in field is, therefore, likely to come from the detailed SAR studies of thioureas, which will offer many novel molecules with improved therapeutic profiles. Based on the thioureas (**A** and **B**) reported herein, we have proposed a series of other possible derivatives comprising, imidazole (**C**), pyrimidine (**D**), quinazoline (**E**), benzimidazole (**F**), thiosemicarbazone (**G**) and thioureas (**H**) scaffolds as illustrated in **Figure 9**. The following key points to rationalize the designed scaffolds **C**-**H** can be explained as follows: i) imidazoles (**C**) and pyrimidine (**D**) can be designed by cyclizing the PTU (**A**); ii) quinazoline (**E**) and benzimidazole (**F**) can be designed by rigidifying the phenyl thioimidazole scaffold (**B**); iii) thiosemicarbazone (**G**) can be designed by introducing additional hydrogen bond interaction from phenyl thiourea (**A**); and iv) introducing various substitutes on the PTU (**A**) leads to another proposed structure **H**. Especially, substituents at various positions at phenyl group of PTU may give a valuable SAR to improve the melanogenesis inhibitory activity potency.

The study of melanogenesis has revealed different kinds of interaction not only between melanocytes and other cells like keratinocytes but also with systems like CNS, immune, inflammatory, endocrine and endocannabinoid, and eventually, raises the role of skin as a neuroendocrine organ [70]. Moreover, investigating their mechanisms is important for understanding pigmentation defects and for a competent development of potential therapeutic agents.

This box summarizes key points contained in the article.

Acknowledgment

Special thanks to Prof. Te-sheng Chang for stimulating discussions and help with the preparation of the manuscript.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Te-sheng Chang assisted with the preparation and discussion of this.

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