Mini-review on Inhibitors of Human Tyrosinase

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Abstract. Melanin is a major pigment of human skin that protects the skin from harmful ultraviolet radiation, DNA damage and oxidative stress. However, the excess accumulation of melanin may lead to various hyperpigmentation-related diseases. Tyrosinase is a copper containing enzyme that regulates the rate-limiting step of melanin synthesis. So, inhibiting tyrosinase is the crucial target for researchers for the treatment of hyperpigmentation. Unfortunately, almost all the literature is based on mushroom tyrosinase (mTYR) for their application on humans as pure human tyrosinase (hTYR) is difficult to isolate. Since presently used tyrosinase inhibitors are developed using mushroom tyrosinase, they are insufficient to match the affinity, selectivity and efficacy required to target the human tyrosinase. Therefore, there is an urgent need for identifying a selective tyrosinase inhibitor that matches the selectivity and safety standards of human tyrosinase. This mini-review is focused on the tyrosinase inhibitors developed and evaluated using human tyrosinase.

Keywords: Human tyrosinase · Mushroom tyrosinase · Tyrosinase inhibitor · Hyperpigmentation · Melanin

1 Introduction

Melanin is a major pigment that determines the darkening of skin, eyes and hair in humans [1]. It protects the skin from harmful ultraviolet radiations, DNA damage, skin cancer and oxidative stress [2]. Melanin is a mixture of biopolymer pigments that determines the phenotypic shade and appearance of an individual. But the abnormal accumulation of excess melanin may lead to various hyperpigmentation-related diseases like lentigines, melasma, freckles, periorbital hyperpigmentation, senile, ephelides, cervical poikiloderma, etc. [3, 4]. Therefore, melanin reduction is the significant target in developing cosmetic and medicinal products that developed for hyperpigmentation-related diseases [5]. Now-a-days skin whitening agents are gaining more popularity and based on the latest report; by the end of 2022 the global market of skin whitening products is estimated to be over 23,000 million dollars (www.factmr.com).

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Melanogenesis is regulated by several enzymes and among them, inhibition of tyrosinase is a prominent target as it catalyzes the rate-limiting step of melanin biosynthesis [6]. Tyrosinase (EC 1.14.18.1), an enzyme that containsa pair of copper atom at the active site, regulates the initial step of melanogenesis. So, in the past decade, tyrosinase inhibitors are attracting the attention of researchers for the development of skin whitening products [7]. Since human tyrosinase is a membrane-bound enzyme, it is difficult to isolate and its crystal structure is not yet determined. Therefore almost all the reported tyrosinase inhibitors are developed and evaluated using mushroom tyrosinase. However, it has been reported that there is a significant difference in the selectivity and efficacy of mushroom tyrosinase and human tyrosinase. Hence development and evaluation of a novel tyrosinase inhibitor using human tyrosinase are required. In this mini-review, we will be focusing on all the inhibitors which are developed using human tyrosinase [8, 9].

2 Melanin Biosynthesis

Melanin is synthesized in melanocytes by melanosomes. Melanosomes are membrane-bound cell organelle, which is responsible for the synthesising, storing and transportingthe melanin [10]. In humans the functional unit for variation in skin shades is melanin. The skin color is regulated by the number, distribution and size of melanosomes in the cells. In mammalian cells, two chemically-different melanins are synthesized that are eumelanin and pheomelanin. Eumelanin is a pigment that is accountable for black-brown color pigmentation and pheomelanin is accountable for yellow to reddish-browncolor pigmentation; these are together termed as mixed melanin [11]. Melanogenesis is a complicated process that includes a cascade of enzymatic reactions. Among which tyrosinase, tyrosinase-related protein 1 (TRP1), tyrosinase-related protein 2 (TRP2) play an important role in the conversion of tyrosine to melanin [12].

Melanogenesis starts with the conversion of L-tyrosine to dopaquinone (DQ) by enzymatic oxidation which is catalyzed by a copper containing enzyme tyrosinase. Further, DQ forms leukodopachrome by undergoing intramolecular crystallization. The redox reaction that takes place between leukodopachrome and DQ leads to the formation of dopachrome and DOPA, which is converted to DQ by tyrosinase. Gradually dopachrome decomposes by TRP2 and gives rise to dihydroxyindole (DHI) and dihydroxyindole-2-carboxylic acid (DHICA). Finally, eumelanin is formed by the oxidation of DHI and DHICA. TRP1 catalyzes the conversion of DHICA to eumelanin. In parallel, in the presence of cysteine or glutathione DQ is converted to glutothionyldopa or S-cysteinyldopa, which undergoes subsequent oxidation and produces pheomelanin. Though three enzymes are required for the biosynthesis of melanin, tyrosinase is the crucial enzyme as it is involved in rate-limiting step for melanin biosynthesis [6, 13] (Fig. 1).

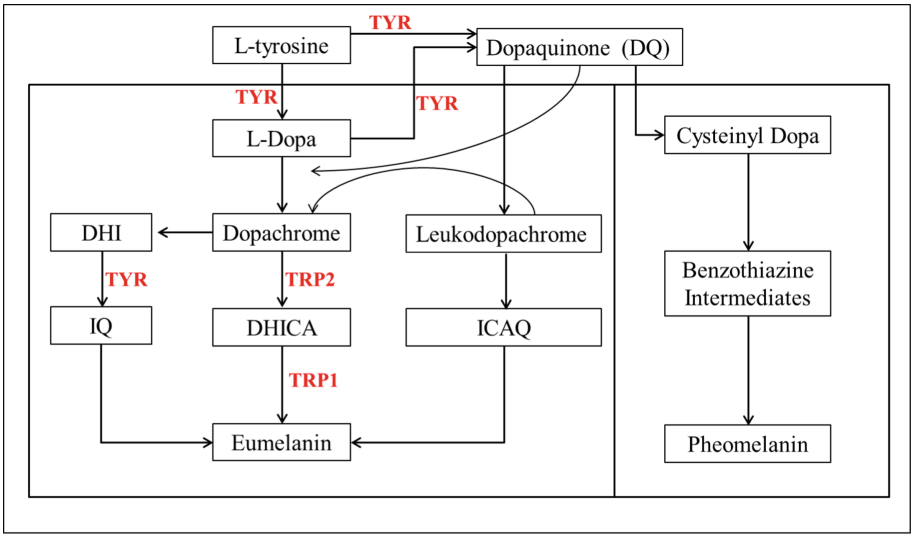


Fig. 1. Biosynthesis of melanin [14].

3 Tyrosinase

Tyrosinase is a multifunctional enzyme that plays a key role in melanin biosynthesis. Based on the structural perspective, it is included in the family of type-3 copper protein because it contains a coupled copper center at the active site [15]. The copper ions of tyrosinase are enclosed by six histidine residues and are directly accountable for its catalytic activities. The substrates of tyrosinase are phenols and catechols which undergo monooxygenation and oxidation respectively to form *ortho*-quinones. During the activity of tyrosinase oxidation state of both copper, ions change that gives different forms to the enzyme. The active site of tyrosinase is found in three states that are, *oxy, met* and *deoxy*forms [16].

Although tyrosinase from many of the sources has been sequenced, only aa small number of them have been isolated and characterized, like human. Mushroom (*Agaricusbisporus*) tyrosinase is one of the well-studied and characterized tyrosinase which is easily available in the market [17]. The availability of mushroom tyrosinase plays a decisive role in the identification of tyrosinase inhibitors and most of the studies have been performed using it. But it can be problematic since human tyrosinase differs from mushroom tyrosinase in various aspects. Mushroom tyrosinase is present in tetramer form, whereas human tyrosinase is highly glycosylated and has amonomer shape. In addition, human tyrosinase is bound to the cell membrane; however mushroom tyrosinase is a cytosolic enzyme. Apart from it, mushroom and human tyrosinase vary in substrate specificity, efficacy and safety standards [18]. Human tyrosinase and mushroom tyrosinase only have 23% identity in its protein sequence [19].

Since mushroom tyrosinase is present in cytosol, it is easy to isolate however human tyrosinase is bound to the membrane and is highly hydrophobic which makes it difficult

to isolate and characterize [20]. The most prominent target for inhibition of melanin production is tyrosinase and it is most popular because it is only produced by melanocytes. Tyrosinase inhibitors are highly specific and do not have other side effects [21].

4 Studies on Human Tyrosinase Inhibitors

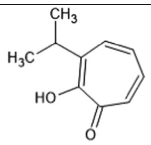
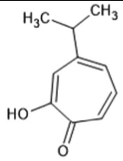
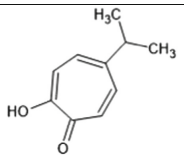
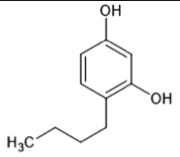
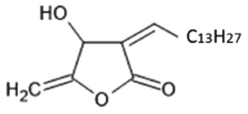
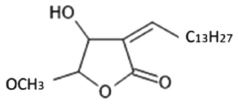
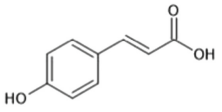
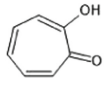
Recently, researchers are developing tyrosinase inhibitors that target hTYR. Yoshimori et al. (2014) assessed the effect of α, β, and γthujaplicin on hTYR using kojic acid as control as shown in Table 1. The IC_{50} value for thujaplicins was evaluated on a malignant melanoma cell line of a human (G-361). α, β, γthujaplicin, and kojic acid showed the IC_{50} value $>$ 1000 μM, 8.98 μM, 1.15 μM, and 571.17 μM respectively on hTYR which was far more than the IC_{50} value on mTYR. Further for the interaction studies, they predicted the structure of hTYR using the crystal structure of *Streptomyces castaneoglobisporus*tyrosinase with PDB code 1WX2. The structure was analyzed by RAMPAGE software which showed that 94.8% residues were present in the favored region and the allowed region of Ramachandran plot. The result of the docking study showed that His367, Ile368, and Val377 are the hot spot residues. It was also revealed that some of the active site residues of mTYRdiffer from that of hTYR. Overall the result showed that γ-thujaplicin was the most effective inhibitor among the thujaplicins [22, 23].

Okubo et al. (1995) studied the effect of and 4-n-butylresorcinol on B16 melanoma cells. It was seen that 42% of melanin decreased per cell by the action of 10 μM of 4-n-butylresorcinol [24]. Kolbe and coworkers evaluated the inhibitory effect of kojic acid, hydroquinone, arbutin, and 4-n-butylresorcinol (Table 1) on hTYR and MelanoDerm skin culture. The result showed that arbutin and hydroquinone inadequately inhibit hTYR with IC_{50} value in the range of millimolars. However, kojic acid and 4-n-butylresorcinol gave an IC_{50} value of 500 μmol/L and 21 μmol/L respectively against hTYR. Invivo testing of 4-n-butylresorcinol was performed and the subject with age spots was treated twice with 4-n-butylresorcinol and within 2 months, the appearance of age spots was found to be reduced while control showed no difference. The study showed that 4-n-butylresorcinol is morepotent inhibitor of hTYR than the standard inhibitors [25].

In another study, Wang et al. demonstrated the influence of the inhibitorssubamolide A and linderanolide B (Table 1) on the zebrafish and human melanocyte, keratinocyte, epidermaland dermal fibroblast cell cultures. The outcome showed that subamolide A andlinderanolide Bshowed 50% and 40% reduction in HEMn-MP cells respectively in 48 h without any toxicity. Linderanolide B and subamolide A also showed remarkable inhibition of the pigmentation of zebrafish. The structure of hTYR was predicted using the crystal structure of *Octopus dofleini*hemocyanin (PDB ID 1JS8) and binding modes for both the inhibitors was proposed [3]. In the year 2010 An et al. compared the inhibitory effect of p-coumaric on mushroom, murine and hTYRusing arbutinand kojic acid as the positive control. It was seen the inhibitory effect of p-coumaricwas weak on mTYR as compared to its effect on murine and hTYR. Furthermore, its inhibitory effect was found to be much more than that of kojic acid and arbutin [26].

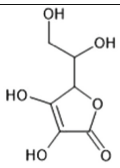
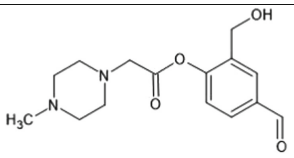
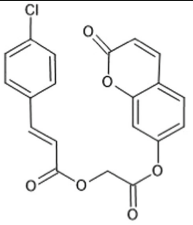
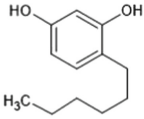
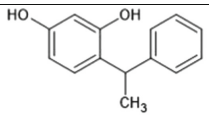
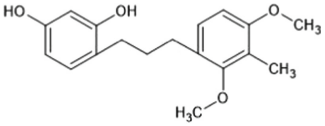
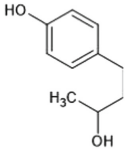
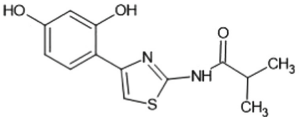
Nokinsee et al. presented the difference between the active site and interactions of mushroom, bacterial and hTYR by molecular docking and molecular dynamics (MD)

Table 1. Compounds evaluated and developed using human tyrosinase.

S.no.	Compound	Structure	Inhibitor Target	Inhibition Mechanism	Reference
1.	α-Thujaplicin		Human malignant melanoma cell line (G-361)	Competative Inhibition	[22, 23]
2.	β-Thujaplicin		Human malignant melanoma cell line (G-361)	Competative Inhibition	[22, 23]
3.	γ-Thujaplicin		Human malignant melanoma cell line (G-361)	Competative Inhibition	[22, 23]
4.	4-Butyl Resorcinol		B16 melanoma cells and MelanoDerm	Competative Inhibition	[24, 25]
5.	Linderanolide B		Human epidermal melanocytes cells (HEMn-MP)	Competative Inhibition	[3]
6.	Subamolide A		Human epidermal melanocytes cells (HEMn-MP)	Competative Inhibition	[3]
7.	p-Coumaric Acid		Human Epidermal Melanocytes	Competative Inhibition	[25]
8.	Tropolone		Predicted structure of hTYR	-	[27]

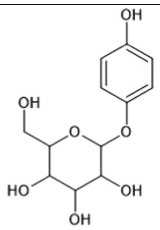
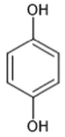
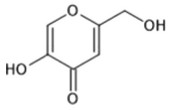
(*continued*)

Table 1. (*continued*)

9.	Ascorbic Acid		Predicted structure of hTYR	-	[27]
10.	Vanillin Derivative (V7)		Predicted structure of hTYR	-	[28]
11.	Coumarin Derivative (C9)		Predicted structure of hTYR	-	[28]
12.	4-Hexyl Resorcinol		Recombinant hTYR expressed in HEK293 cells	Competative Inhibition	[9]
13.	4-Phenylethyl Resorcinol		Recombinant hTYR expressed in HEK293 cells	Competative Inhibition	[9]
14.	Dimethoxypropyl Resorcinol		Recombinant hTYR expressed in HEK293 cells	Competative Inhibition	[9]
15.	Rhododendrol		Recombinant hTYRexpressed in HEK293 cells	Competative Inhibition	[9]
16.	Thiamidol		Recombinant hTYR expressed in HEK293 cells	Competative Inhibition	[9]

(*continued*)

Table 1. (*continued*)

17.	Arbutin (Control)		MelanoDerm; Human Epidermal Melanocytes; Recombinant hTYRexpressed in HEK293 cells	Competative Inhibition	[9, 25, 28]
18.	Hydroquinone (Control)		MelanoDerm; Recombinant hTYRexpressed in HEK293 cells	Competative Inhibition	[9, 25]
19.	Kojic Acid (Control)		Human malignant melanoma cell line (G-361);MelanoDerm;HEMn-MP cells;Human Epidermal Melanocytes: Recombinant hTYRexpressed in HEK293 cells	Competative Inhibition	[3, 22, 23, 25, 28]

simulation using standard tyrosinase inhibitors (kojic acid, tropolone, ascorbic acid and arbutin) shown in Table 1. The crystal structure of hTYR was made using a protein template of *B. megaterium*tyrosinase(PDB code 3NQ1). On the evaluation of the predicted structure, 81.6% and 12.6% residues were found in the favored region and additionally allowed region of Ramachandran plot respectively. On performing docking and MD simulation, it was found that amino acid residue M280, N81, H263, and N260 are responsible for the binding of inhibitors to mTYR. Whereas, S265, E230, H252, S245, V262, and N249amino acid residue of hTYR are important for the inhibitor binding [27].

Recently, Hassan et al. (2018) performed an in-silico study for the effect of derivatives of coumarin (C1–C9), vanillin (V1–V8) and thymol (T1–T8) on hTYR. The 3-D structure of hTYRwas predicted using the crystal structure of *Bacillus megaterium*tyrosinase as a template sequence. The validation of the predicted structure indicated that 95.0% residues were present in the favored region of the Ramachandran plot. The docking studies revealed that V7 and C9 derivatives (Table 1) showed reliable binding energy that are −7.79 kcal/moland −7.40 kcal/mol respectively which was much more than kojic acid and arbutin. Simulation outcomes validated that the V7 compound could be used as

a potential inhibitor of hTYR [28]. Mann et al. used recombinant hTYR and screened a library with 5000 compounds for their function as the skin whitening agent. Using molecular docking and simulation; thiamidol, hydroquinone, 4-butylresorcinol, rhododendrol, 4-hexylresorcinol,dimethoxytolylpropyl resorcinol, kojic acid, 4-phenylethylresorcinol, and arbutin (Table 1) was evaluated. Among which thiamidol was identified as the most potential inhibitor of hTYR. The IC_{50} value ofthiamidol was found to be 1.1 μmol/L and 108 μmol/L for hTYR and mTYR respectively. Clinical studies showed that thiamidol was able to reduce the age spots within a month and after 3 months some spots were indistinguishable from the skin color which showed thiamidol can be a potential inhibitor of hTYR [9]. Further Mann et al. studied the inhibition mechanism of thiazolylresorcinols with human tyrosinase and their structural interaction using invivo approach. From the result of docking and simulation it was concluded that both the rings of thiazolyl resorcinol are important for efficient inhibition of human tyrosinase. It was proposed that the interaction between the sulphur atom f the inhibitor thiazolyl resorcinol and the conserved residue of human tyrosinase N364 plays an important role in the inhibition of human tyrosinase and melanin biosynthesis [29].

5 Conclusion

Hyperpigmentation is an important problem that needs to be addressed and the most potential target for inhibition of hyperpigmentation-related disease is tyrosinase as it catalyzes the rate-limiting step. Nowadays, used skin whitening cosmetics contain harmful bleaching agents and toxins like mercury which can have an adverse effect on the skin. These harmful bleaching agents can be easily replaced by tyrosinase inhibitors, therefore, tyrosinase inhibitors, are gaining popularity in the cosmetics and pharmaceutical industries. Currently, organic cosmetics and creams are attracting consumers and in this regard also tyrosinase inhibitors have an advantage as most of the identified tyrosinase inhibitors were extracted from natural sources. A large number of tyrosinase inhibitors are already identified but most of them were evaluated using mushroom tyrosinase. Even the computational studies for the tyrosinase inhibitors were carried out using mushroom tyrosinase because the crystal structure of human tyrosinase is not yet available. Therefore, th ecurrently used skin whitening agents do not match the safety standards like effectiveness, solubility, absorption, etc.

In this mini-review, we have discussed the tyrosinase inhibitors which are developed using human tyrosinase. Since human tyrosinase is difficult to isolate, some researchers have studied the effect of tyrosinase inhibitors using human cell lines as an alternative. The outcome obtained by using different human cell lines is more reliable as compared to the result we get using mushroom tyrosinase. For, computational studies, researchers can use the predicted structure of human tyrosinase rather than the crystal structure of mushroom tyrosinase for studying the effect of tyrosinase inhibitors. Scientists can evaluate already identified mushroom tyrosinase inhibitors using human tyrosinase for assessing the safety standards of inhibitors for human use. Despite a lot of research, there is a long way to go for identifying an effective human tyrosinase inhibitor.

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References

1. Adams, D.R., et al., *One-year pilot study on the effects of nitisinone on melanin in patients with OCA-1B.* JCI insight, 2019. **4**(2).
2. Abbas, K., M.I. Qadir, and S. Anwar, *The role of melanin in skin cancer.* Critical Reviews™ in Eukaryotic Gene Expression, 2019. **29**(1).
3. Wang, H.M., C.Y. Chen, and Z.H. Wen, *Identifying melanogenesis inhibitors from Cinnamomum subavenium with in vitro and in vivo screening systems by targeting the human tyrosinase.* Experimental dermatology, 2011. **20**(3): p. 242–248.
4. Kim, Y.-J. and H. Uyama, *Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future.* Cellular and molecular life sciences CMLS, 2005. **62**(15): p. 1707–1723.
5. Zolghadri, S., et al., *A comprehensive review on tyrosinase inhibitors.* Journal of enzyme inhibition and medicinal chemistry, 2019. **34**(1): p. 279–309.
6. Masum, M.N., K. Yamauchi, and T. Mitsunaga, *Tyrosinase inhibitors from natural and synthetic sources as skin-lightening agents.* Reviews in Agricultural Science, 2019. **7**: p. 41–58.
7. Zaidi, K.U., et al., *Natural tyrosinase inhibitors: Role of herbals in the treatment of hyperpigmentary disorders.* Mini Reviews in Medicinal Chemistry, 2019. **19**(10): p. 796–808.
8. Roulier, B., B. Pérès, and R. Haudecoeur, *Advances in the design of genuine human tyrosinase inhibitors for targeting melanogenesis and related pigmentations.* Journal of Medicinal Chemistry, 2020. **63**(22): p. 13428–13443.
9. Mann, T., et al., *Inhibition of human tyrosinase requires molecular motifs distinctively different from mushroom tyrosinase.* Journal of Investigative Dermatology, 2018. **138**(7): p. 1601–1608.
10. Qian, W., et al., *Natural skin-whitening compounds for the treatment of melanogenesis.* Experimental and therapeutic medicine, 2020. **20**(1): p. 173–185.
11. Schallreuter, K.U., et al., *Regulation of melanogenesis–controversies and new concepts.* Experimental dermatology, 2008. **17**(5): p. 395–404.
12. Chen, Y.-M., et al., *Anti-melanogenesis of novel kojic acid derivatives in B16F10 cells and zebrafish.* International journal of biological macromolecules, 2019. **123**: p. 723–731.
13. El-Nashar, H.A., et al., *Insights on the Inhibitory Power of Flavonoids on Tyrosinase Activity: A Survey from 2016 to 2021.* Molecules, 2021. **26**(24): p. 7546.
14. Yamauchi, K. and T. Mitsunaga, *Melanogenesis and melanosome transportation modulators from medicinal plants.* Letters in Drug Design & Discovery, 2016. **13**(8): p. 742–751.
15. Mukherjee, P.K., et al., *Validation of medicinal herbs for anti-tyrosinase potential.* Journal of Herbal Medicine, 2018. **14**: p. 1–16.
16. Panzella, L. and A. Napolitano, *Natural and bioinspired phenolic compounds as tyrosinase inhibitors for the treatment of skin hyperpigmentation: Recent advances.* Cosmetics, 2019. **6**(4): p. 57.
17. Obaid, R.J., et al., *Natural and synthetic flavonoid derivatives as new potential tyrosinase inhibitors: A systematic review.* RSC Advances, 2021. **11**(36): p. 22159–22198.
18. Chang, T.-S., *Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity.* Materials, 2012. **5**(9): p. 1661–1685.
19. Li, J., et al., *Recent advances in the design and discovery of synthetic tyrosinase inhibitors.* European Journal of Medicinal Chemistry, 2021. **224**: p. 113744.
20. Lai, X., et al., *Structure and function of human tyrosinase and tyrosinase-related proteins.* Chemistry–A European Journal, 2018. **24**(1): p. 47–55.

21. Chang, T.-S., *An updated review of tyrosinase inhibitors.* International journal of molecular sciences, 2009. **10**(6): p. 2440–2475.
22. Yoshimori, A., et al., *Structure–activity relationships of the thujaplicins for inhibition of human tyrosinase.* Bioorganic & medicinal chemistry, 2014. **22**(21): p. 6193–6200.
23. Pillaiyar, T., M. Manickam, and S.-H. Jung, *Downregulation of melanogenesis: drug discovery and therapeutic options.* Drug Discovery Today, 2017. **22**(2): p. 282–298.
24. Okubo, T., et al., *The inhibitory effects of 4-N-butyl-resorcinol on melanogenesis.* Journal of Dermatological Science, 1995. **1**(10): p. 88.
25. Kolbe, L., et al., *4-n-butylresorcinol, a highly effective tyrosinase inhibitor for the topical treatment of hyperpigmentation.* Journal of the European Academy of Dermatology and Venereology, 2013. **27**: p. 19–23.
26. An, S.M., J.S. Koh, and Y.C. Boo, *p-coumaric acid not only inhibits human tyrosinase activity in vitro but also melanogenesis in cells exposed to UVB.* Phytotherapy Research, 2010. **24**(8): p. 1175–1180.
27. Nokinsee, D., et al., *Estimation of inhibitory effect against tyrosinase activity through homology modeling and molecular docking.* Enzyme research, 2015. **2015**.
28. Hassan, M., et al., *Exploration of novel human tyrosinase inhibitors by molecular modeling, docking and simulation studies.* Interdisciplinary sciences: computational life sciences, 2018. **10**(1): p. 68–80.
29. Mann, T., et al., *Structure-activity relationships of thiazolyl resorcinols, potent and selective inhibitors of human tyrosinase.* International journal of molecular sciences, 2018. **19**(3): p. 690.