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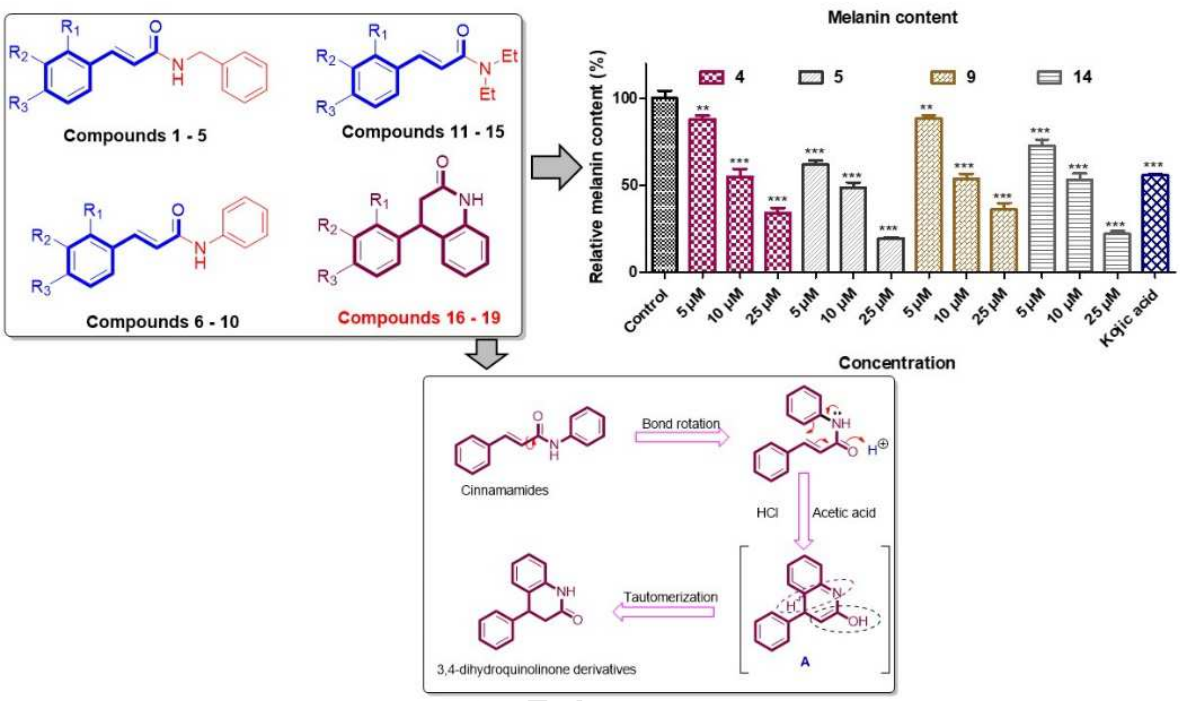
Graphical abstract



Synthesis of cinnamic amide derivatives and their anti-melanogenic effect in α-MSH-stimulated B16F10 melanoma cells

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Abstract

Of the three enzymes that regulate the biosynthesis of melanin, tyrosinase and its related proteins TYRP-1 and TYRP-2, tyrosinase is the most important because of its ability to limit the rate of melanin production in melanocytes. For treating skin pigmentation disorders caused by an excess of melanin, the inhibition of tyrosinase enzyme is by far the most established strategy. Cinnamic acid is a safe natural product with an (*E*)-β-phenyl-α,β-unsaturated carbonyl motif that we have previously shown to play an important role in high tyrosinase inhibition. Since cinnamic acid is relatively hydrophilic, which hinders its absorption on the skin, fifteen less hydrophilic cinnamic amide derivatives (**1** – **15**) were designed as safe and more potent tyrosinase inhibitors and were synthesised through a Horner-Wadsworth-Emmons reaction. The use of *conc*-HCl and acetic acid for debenzylation of the *O*-benzyl-protected cinnamic amides **40** – **54** produced the following three results. 1) Cinnamic amides **43**, **48**, and **53** with a 2,4-dibenzyloxyphenyl group, irrespective of the amine type of the amides, produced complex compounds with high polarity. 2) Cinnamic amides **40** – **42**, **44**, **50** – **52**, and **54** with a benzylamino, or diethylamino group produced the desired debenzylated cinnamic amides **1** – **3**, **5**, **10** – **13**, and **15**. 3) Cinnamic amides **45** – **47**, and **49** with an anilino moiety provided 3,4-dihydroquinolinones **16** – **19** through intramolecular Michael addition of the anilide group. Notably, the use of BBr_3 as an alternative debenzylating agent for debenzylation of cinnamic amides **45** – **49** with the anilino moiety provided our desired cinnamic amides **6** – **10** without inducing the intramolecular Michael addition. Debenzylation of cinnamic amides **43**, **48**, and **53** with a 2,4-dibenzyloxyphenyl group was also successfully accomplished using BBr_3 to give **4**, **9**,

and **14**. Among the nine compounds that inhibited mushroom tyrosinase more potently at 25 μM than kojic acid, four cinnamic amides **4**, **5**, **9**, and **14** showed 3-fold greater tyrosinase inhibitory activity than kojic acid. The docking simulation using tyrosinase indicated that these four cinnamic amides (-6.2 – -7.9 kcal/mol) bind to the active site of tyrosinase with stronger binding affinity than kojic acid (-5.7 kcal/mol). All four cinnamic amides inhibited melanogenesis and tyrosinase activity more potently than kojic acid in α-MSH-stimulated B16F10 melanoma cells in a dose-dependent manner without cytotoxicity. The strong correlation between tyrosinase activity and melanin content suggests that the anti-melanogenic effect of cinnamic amides is due to tyrosinase inhibitory activity. Considering that the cinnamic amides **4**, **9**, and **14**, which exhibited strong inhibition on mushroom tyrosinase and potent anti-melanogenic effect in B16F10 cells, commonly have a 2,4-dihydroxyphenyl substituent, the 2,4-dihydroxyphenyl substituent appears to be essential for high anti-melanogenesis. These results support the potential of these four cinnamic amides as novel and potent tyrosinase inhibitors for use as therapeutic agents with safe skin-lightening efficiency.

Key words: cinnamic amide, anti-melanogenic effect**,** tyrosinase inhibitor, melanin content, docking, B10F16 melanoma cells.

1. Introduction

Melanin is the primary cellular component responsible for eye, hair, and skin colour in humans [1,2] and is biosynthesized in the melanosomes of melanocytes starting from L-tyrosine through complicated chemico-enzymatic processes [3,4]. Mainly three enzymes are involved in the regulation of melanogenesis: tyrosinase and two tyrosinase-related proteins, TYRP1 and TYRP2 [5-8]. Among these three enzymes, tyrosinase (EC 1.14.18.1) is the pivotal enzyme that controls the rate of melanin production, and as the rate-limiting enzyme for melanin biosynthesis is responsible for the conversion of L-tyrosine to dopaquinone via L-dopa using monophenolase and diphenolase activities [1,9,10]. Depending on the presence of thiol materials such as glutathione and L-cysteine, the reactive dopaquinone undergoes two pathways [5,9]. In the presence of thiol materials, the dopaquinone reacts with thiols via Michael addition and is finally converted to eumelanin (pigments responsible for a yellow-red colour) after a series of multi-reactions [8]. On the other hand, in the absence of thiol materials, the dopaquinone is finally transformed to eumelanin (pigments responsible for a brown-black colour) by several reactions [11-15]. The proportion and amount of pheomelanin and eumelanin mainly determine the phenotype of human skin colour [16-19]. Melanin is widely distributed in organisms ranging from bacteria, plants, animals, and humans[19]. Melanin not only plays a positive role in protecting skin cells from UV radiation but also has negative functions involved in hyperpigmentation diseases and browning of fruits and vegetables.

In addition to melanin biosynthesis, tyrosinase is also involved in the defensive and developmental functions of the pests [20,21]. Excessive dopaquinone produced by the

catalysis of tyrosinase has been reported to cause the neurodegeneration associated with Parkinson's disease in the brain [22-26]. Therefore, suitable new tyrosinase inhibitors might be applied to pesticides with a novel mode of action and medications associated with neuronal damages. The abnormal excessive melanin accumulation in a specific region may cause melasma, freckles, age spots and melanoma, a malignant tumour of melanocytes and aesthetic problems [27-34].

Despite the few clinically used whitening agents, a crucial need remains for novel agents to combat some unmet needs, such as side effects of potential carcinogenicity, and low clinical efficacy demonstrated by currently available tyrosinase inhibitors [32,35-38]. Cinnamic acid is a natural, safe substance extracted from balsams or cinnamon oil and is mainly used as a source for fragrances and pharmaceuticals [39]. As part of our ongoing efforts to discover safer and more potent tyrosinase inhibitors, cinnamic amide derivatives that can be prepared by coupling of cinnamic acid with arylamine, arylalkylamine or noncyclic secondary amine, were designed and synthesized. These cinnamic amide derivatives were expected to show strong tyrosinase inhibitory activity due to the presence of the (*E*)-β-phenyl-α,β-unsaturated carbonyl motif. Several of our previous studies have demonstrated that the motif plays an essential role in tyrosinase inhibitory activity [40-49]. The synthesized cinnamic acid analogues were evaluated for their inhibitory activity against mushroom tyrosinase and the cinnamic amides with good mushroom tyrosinase activity were further studied in cell-based experiments and docking simulation.

2. Results and Discussion

2.1. Chemistry

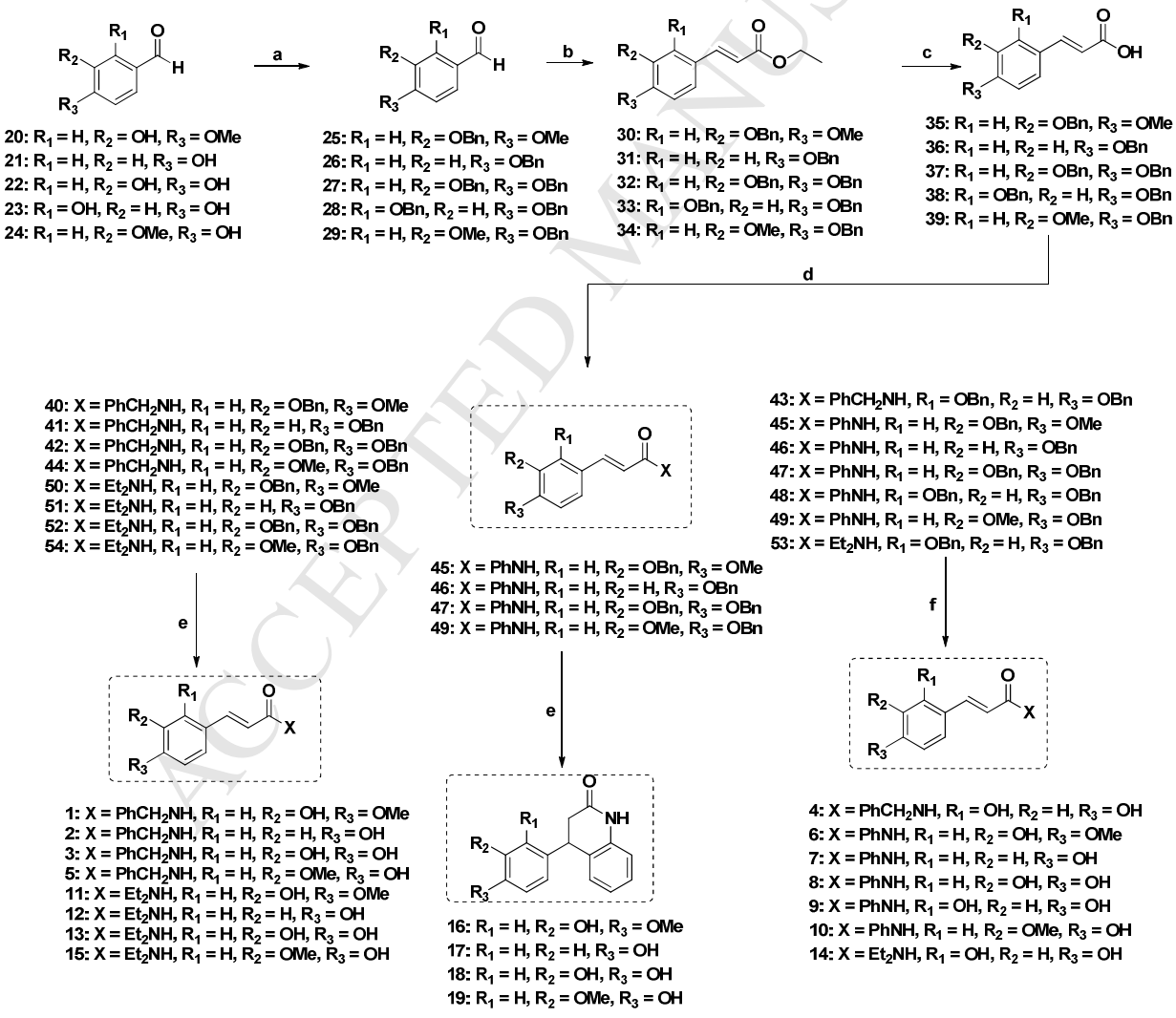
To examine the effect of the amine moiety of cinnamic amide derivatives on tyrosinase inhibition, cinnamic amides having a benzylamino (as a representative of an arylalkylamino

group), anilino (as a representative of an arylamino group), or diethylamino (as a representative of noncyclic secondary amino group) group were designed as our desired compounds. Based on our finding that at least one hydroxyl group on the β–phenyl ring of the (*E*)-β-phenyl-α,β-unsaturated carbonyl motif is required to exhibit high tyrosinase inhibitory activity, cinnamic amide analogues with a substituent, such as 3-hydroxy-4-methoxy, 4-hydroxy, 3,4-dihydroxy, 2,4-dihydroxy, and 4-hydroxy-3-methoxy on the phenyl ring were designed. Five commercially available benzaldehydes were used as starting materials (Scheme 1): isovanillin (**20**), 4-hydroxybenzaldehyde (**21**), 3,4-dihydroxybenzaldehyde (**22**), 2,4-dihydroxybenzaldehyde (**23**) and vanillin (**24**). Reaction of these five benzaldehydes with benzyl bromide in the presence of K_2CO_3 gave the corresponding benzyl ether products **25** – **29**, which, in turn, were subjected to a Horner-Wadsworth-Emmons reaction using triethyl phosphonoacetate, K_2CO_3 (2.0 equiv.) and DBU (1,8-diazabicyclo[5.4.0]undec-7-ene, a catalytic amount) to afford the corresponding ethyl cinnamates **30** – **34**. After saponification under basic conditions using 1.0M-NaOH aqueous solution, in order to activate the carboxylic functionality the resultant carboxylic acids **35** – **39** were converted to the corresponding mixed anhydrides by treatment with isobutyl chloroformate in the presence of morpholine as a base. Reaction of the mixed anhydrides with benzylamine, aniline and diethylamine produced compounds **40** – **54**, and the benzyl ethers of **40** – **54** were removed under acidic conditions using BBr_3 at -40 ^oC, or *conc*-HCl, and acetic acid under reflux.

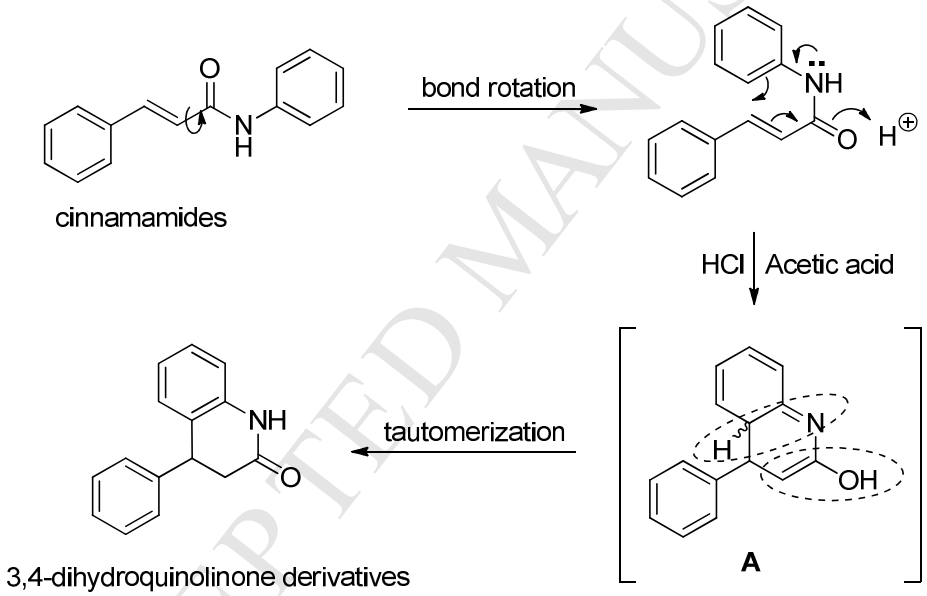
In case of compounds **40** – **44** with a benzylamino group, and **50** – **54** with a diethylamino group, the corresponding debenzylated cinnamic amides **1** – **3**, **5**, **11** – **13**, and **15** were produced during the debenzylation reaction using *conc*-HCl, and acetic acid, except for cinnamic amides **4**, and **14** with a 2,4-dihydroxy substituent. Notably, conversion to acidic

conditions using BBr_3 successfully induced the formation of cinnamic amides **4**, and **14** with a 2,4-dihydroxy substituent. Compounds **45** – **49** having an anilino group also were treated with *conc*-HCl and acetic acid to obtain the desired cinnamic amides **6** – **10**. However, we could not obtain our desired cinnamic amide derivatives **6** – **10**. Instead, 3,4-dihydrocarbostyril compounds, i.e., 3,4-dihydroquinolinones **16** – **19** were obtained from compounds **45** – **47** and **49**, respectively, via a cyclization reaction along with debenzylation. On the other hand, reaction of compound **48** under the same acidic conditions afforded only complex compounds with high polarity. A plausible mechanism for the formation of 3,4-dihydroquinolinone derivatives is depicted in Scheme 2. Protonation of the carbonyl group by HCl/acetic acid may activate the Michael acceptor, an α,β-unsaturated amide and induce a Michael addition of the anilino group to give compound A, which may be spontaneously tautomerised into more stable 3,4-dihydroquinolinone derivatives. Among the four obtained 3,4-dihydroquinolinone derivatives **16** – **19**, two (**17** and **18**) were unknown compounds and although the remaining two (**16** and **19**) are known compounds, these compounds were prepared via a different synthetic method: a reaction of monoanilides of malonic acid with benzaldehydes in trifluoroacetic acid under reflux. Many studies [50-53] have revealed intramolecular cyclization of the anilides of cinnamic acid in the presence of a variety of Bronsted-Lowry acids or Lewis acids: polyphosphoric, hydrobromic, hydroiodic, sulfuric, trifluoromethanesulfonic, and trifluoroacetic acids, and $AlCl_3$. To the best of our knowledge, this study is the first to use HCl/acetic acid for converting cinnamic acid anilides into 3,4-dihydroquinolinone derivatives. A milder acidic reagent and conditions to avoid intramolecular cyclization were required for the desired cinnamic amides **6** – **10**. For this purpose, BBr_3 was chosen because the Lewis acid is a common reagent for debenzylation of phenolic benzyl ether and because a debenzylation reaction can be generally conducted at

low temperatures (-78 ^{o}C ~ 0 ^{o}C). Surprisingly, treatment of **45** – **49** with BBr_3 at -40 ^{0}C provided our desired cinnamic amide analogues **6** – **10** in moderate yields. These results indicate that the intramolecular cyclization of the cinnamic acid anilides requires more vigorous acidic conditions than those for debenzylation. The structures of the fifteen cinnamic amides **1 – 15** and four dihydroquinolinones **16** – **19** were identified by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and mass spectroscopy. The double bond geometry of cinnamic amides **1** – **15** was assigned as an (*E*)-configuration on the basis of *J* values (> 15.0 Hz) of the vinylic protons in the ^{1}H NMR spectra.



Scheme 1. Synthetic scheme of cinnamic amide derivatives 1 – 19. Reagents and conditions: a) BnBr, K_2CO_3, CH_3CN, reflux, 24 h; b) $(EtO)_2P(=O)CH_2CO_2Et$, K_2CO_3, DBU (cat. amount), DCM, DMF, rt for **20**, **22**, and **24**, or 70 ^oC for **21**, and **23**, 24 – 36 h; c) 1N-NaOH aqueous solution, 1,4-dioxane, rt, 48 h; d) *i*-BuO_2CCl, *N*-methylmorpholine, THF, rt, 30 min, and then, benzylamine or aniline and diethylamine, rt, 24 h; and e) *c*-HCl, AcOH, reflux, 40 min; f) 4 – 8 equiv. BBr_3, DCM, -40 ^0C, 30 min.



Scheme 2. A plausible mechanism for the formation of 3,4-dihydroquinolinone derivatives.

2.2.Mushroom tyrosinase activity

The inhibitory activity of cinnamic amide derivatives **1** – **19** against mushroom tyrosinase was investigated at 25 μM. Kojic acid was used as a positive control, as typically done in evaluation of tyrosinase inhibitors. As depicted in Table 1, among the 15 cinnamic amides, cinnamic amides **2**, **7**, and **12** with the 4-hydroxyl group on the β-phenyl ring of the (*E*)-β-phenyl-α,β-unsaturated carbonyl motif did not exhibit tyrosinase inhibitory activity and

cinnamic amides **3**, **8**, and **13** with a catechol group showed no inhibition or weak inhibition against tyrosinase activity. Cinnamic amide derivatives **1** (31.52±2.85% inhibition), **6** (29.68±12.09% inhibition), and **11** (20.66±4.79% inhibition) with a 3-hydroxy-4-methoxy group on the β-phenyl ring exerted similar or slightly stronger tyrosinase inhibition than kojic acid (21.39±5.37% inhibition). On the other hand, cinnamic amides **5**, **10**, and **15**, which have hydroxyl and methoxyl substituents at opposite positions to cinnamic amides **1**, **6**, and **11**, showed 2- to 3-fold greater tyrosinase inhibitory effects (59.05±13.94% to 82.88±3.01% inhibitions) than cinnamic amides **1**, **6**, and **11** with a 3-hydroxy-4-methoxy group. The highest inhibitory activities were observed from cinnamic amides **4**, **9**, and **14** with a resorcinol moiety (85.37±0.54% to 96.20±1.44% inhibition). These results suggest that the degree of tyrosinase inhibition is closely dependent on the chemical structure of the β-phenyl ring of cinnamic amides. Regardless of the amide species, the tyrosinase inhibition of the cinnamic amides decreased in the order of 2,4-dihydroxy < 4-hydroxy-3-methoxy < 3-hydroxy-4-methoxy < 4-hydroxy, 2,3-dihydroxy.

One methylene reduction in the amine residue of cinnamic amides **1** – **5** generally resulted in a reduction in the inhibitory activity as seen in the tyrosinase inhibition of cinnamic amides **6** – **10**. In particular, the phenomenon was clearly found in the cinnamic amide (**5** *vs*. **10**) with a 4-hydroxy-3-methoxyphenyl ring. One exception was found in the cinnamic amide (**4** *vs*. **9**) with a 2,4-dihydroxyphenyl group, and the latter increased tyrosinase inhibition from 82.88% to 93.70%.

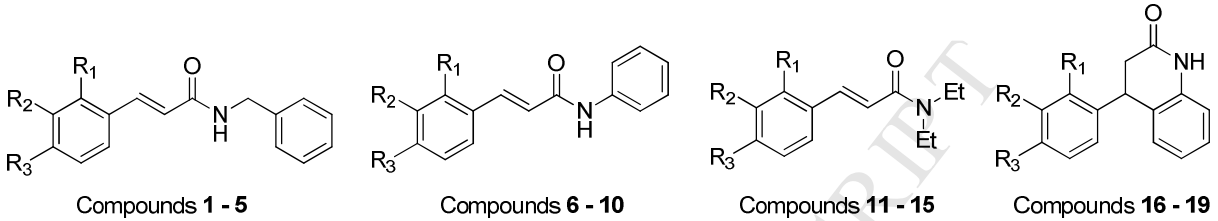
While the cinnamic amides **3**, **8**, and **13** with a 3,4-dihydroxyphenyl substituent showed very low or no tyrosinase inhibition, the cinnamic amides **4**, **9**, and **14** with a 2,4-dihydroxyphenyl substituent showed high inhibition. Presumably, this result means that the former structurally

similar to L-dopa can be rapidly consumed as a substrate for tyrosinase, whereas the latter structurally less similar to L-dopa can act as an inhibitor of tyrosinase rather than a substrate for tyrosinase.

Dihydroquinolinones **16** – **19** obtained through intramolecular cyclization were also evaluated for their tyrosinase inhibitory effects. Only dihydroquinolinone **16** with a 3-hydroxy-4-methoxyphenyl substituent showed similar inhibitory activity to kojic acid, whereas the remaining three dihydroquinolinones **17** – **19** did not show tyrosinase inhibition. Taken together, cinnamic amides having a structural characteristic of the (*E*)-β-phenyl-α,β-unsaturated carbonyl motif exhibited very potent tyrosinase inhibition, whereas dihydroquinolinones showed no or only moderate tyrosinase inhibition, indicating that the motif plays an essential role in tyrosinase inhibition.

Finally, four cinnamic amides **4**, **9**, and **14** with a 2,4-dihydroxyphenyl group and **5** with a 4-hydroxy-3-methoxyphenyl group, were selected for further investigation because they exhibited the most potent tyrosinase inhibitory activities.

Table 1. Substitution patterns, and tyrosinase-inhibitory activities of the synthesized cinnamic amide derivatives **1** – **19**, and kojic acid



Compounds **1 - 5** Compounds **6 - 10** Compounds **11 - 15** Compounds **16 - 19**

Compound	R^1	R^2	R^3	Tyrosinase inhibition (%)[a]	Compound	R^1	R^2	R^3	Tyrosinase inhibition (%)[a]
1	H	OH	OMe	31.52±2.85	**11**	H	OH	OMe	20.66±4.79
2	H	H	OH	NI	**12**	H	H	OH	NI
3	H	OH	OH	10.02±3.55	**13**	H	OH	OH	NI
4	OH	H	OH	85.37±0.54	**14**	OH	H	OH	96.20±1.44
5	H	OMe	OH	82.88±3.01	**15**	H	OMe	OH	61.93±12.34
6	H	OH	OMe	29.68±12.09	**16**	H	OH	OMe	25.94±2.87
7	H	H	OH	NI	**17**	H	H	OH	NI
8	H	OH	OH	NI	**18**	H	OH	OH	NI
9	OH	H	OH	93.70±1.48	**19**	H	OMe	OH	NI
10	H	OMe	OH	59.05±13.94	Kojic acid				21.39±5.37

[a]Tyrosinase inhibition was assayed at 25 μM using L-tyrosine as substrate. The results are presented as mean ± SEMs.

*2.3. Types of inhibition of cinnamic amide derivatives **9** and **14** on mushroom tyrosinase*

Since cinnamic amide derivatives **9** and **14** exhibited the most potent tyrosinase inhibition with the IC_{50} values of 25.6 ± 2.0 nM and 11.2 ± 3.0 nM, respectively, we have determined their inhibitory mode of action by using Lineweaver-Burk double reciprocal plots. The kinetics of the enzyme were shown in Figure 1. The results showed that the plots of 1/V versus 1/[S] gave straight lines with different slopes depending on concentrations of the inhibitor, and the lines intersected on the vertical axis. The Lineweaver-Burk plot showed that V_{max} is the same regardless of concentration of the inhibitor, and K_M increases with increasing concentration of the inhibitor. This behavior indicates that cinnamic amide derivatives **9** and **14** inhibit the enzyme tyrosinase in a competitive manner.

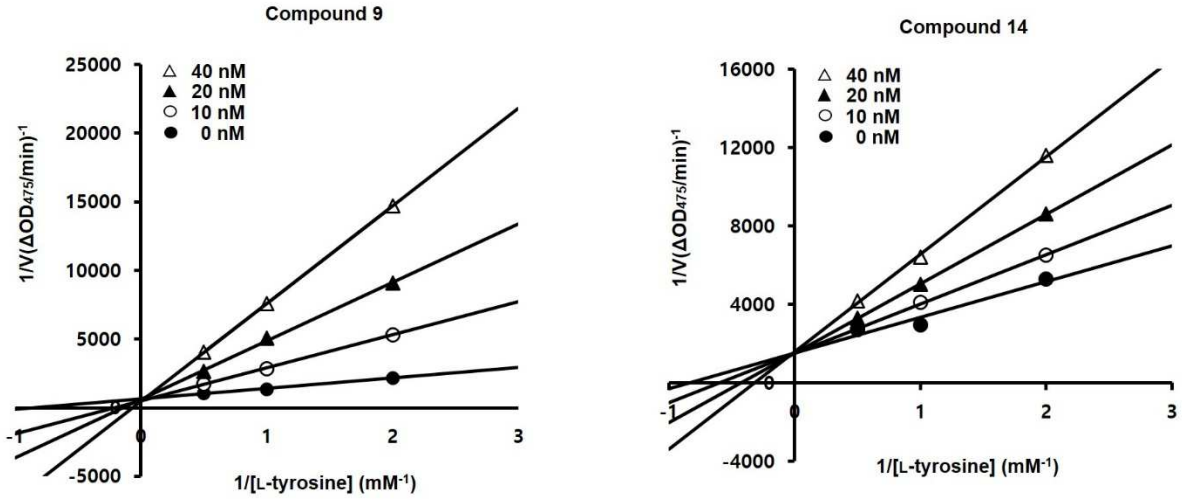
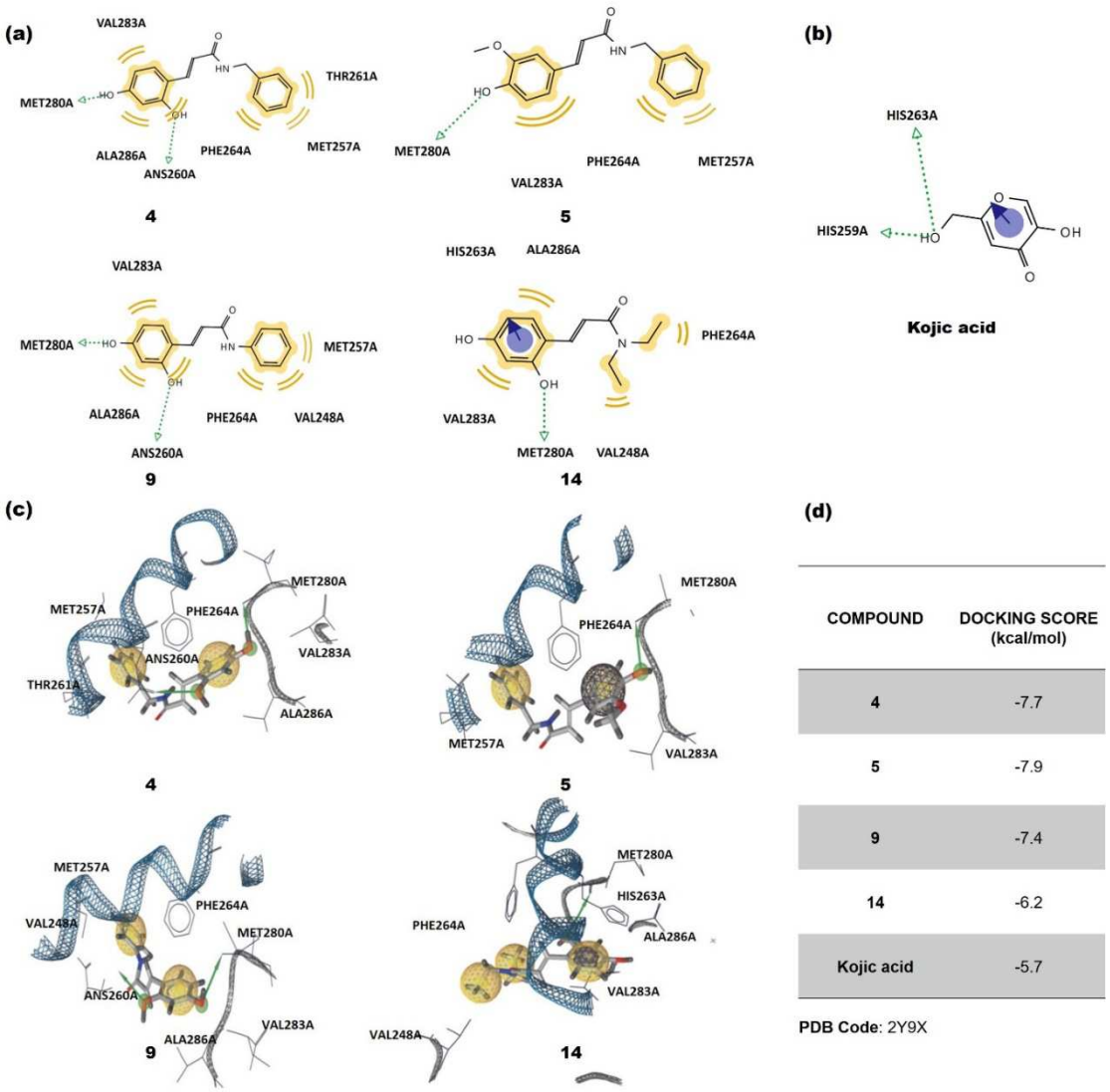


Figure 1. Types of inhibition of cinnamic amide derivatives **9** and **14** against mushroom tyrosinase. Inhibition types were determined using Lineweaver–Burk plots. Results are mean 1/V values, where V is the increase in the absorbance per minute at different L-tyrosine concentrations. The modified Michaelis–Menten equation was utilized: $1/V_{max} = 1/K_M(1 + [S]/K_i)$, where V: reaction velocity, S: L-tyrosine concentration, K_M: Michaelis-Menten constant, K_i: inhibition constant. All experiments were performed in triplicate.

2.4. Docking studies

To determine whether the four selected cinnamic amides are able to bind directly to the active site of tyrosinase, AutoDock Vina 1.1.2 software developed by The Scripps Research Institute was utilised for docking simulation. 3D-structures of the four tyrosinase ligands **4**, **5**, **9**, and **14** were prepared through the energy minimization of the 2D-structures using Chem3D Pro 12.0 software (CambridgeSoft Corporation). The 3D structure of tyrosinase for docking simulation was obtained from *Agaricus bisporus* tyrosinase (Protein Data Bank ID: 2Y9X). Although the correlation between the degree of inhibition of mushroom tyrosinase and the binding affinities of the four ligands was not perfect, all four cinnamic amides showed higher binding affinities (-6.2 ~ -7.9 kcal/mol) than kojic acid (-5.7 kcal/mol), which was used as a reference control (Figure 2d). LigandScout 3.1.2 software showed interactions between the amino acid residues of tyrosinase and ligands. Kojic acid interacts with two amino acid residues (His259 and His263) of tyrosinase as shown in Figure 2b. The branched hydroxyl group of kojic acid creates two hydrogen bonds with both amino acids and the ring of kojic acid interacts with His263 through π-π stacking. The four tested ligands also generate various interactions such as hydrophobic interactions, hydrogen bonding, and π-π stacking, as shown in Figure 2a and 1c. Cinnamic amides **4** and **9** create two hydrogen bonds while cinnamic amides **5** and **14** generate only one hydrogen bond. Cinnamic amide **14** also forms π-π stacking between the β-phenyl ring and His263, which is the same amino acid as the π-π stacking interaction of kojic acid. Interestingly, Met280 creates hydrogen bonds with the hydroxyl group of ligands at different positions, depending on the simulation ligands. Met280 hydrogen bonds with the 4-hydroxyl group on the β-phenyl ring in cinnamic amides **4**, **5**, and **9**, whereas it hydrogen bonds with the 2-hydroxyl group on the β-phenyl ring in cinnamic

amide **14**. Notably, although most hydroxyl substituents form hydrogen bonds, the 4-hydroxyl group in cinnamic amide **14** does not form a hydrogen bond with the amino acids of tyrosinase. Val283 forms hydrophobic interactions with the β-phenyl ring of all four ligands, and Phe264 interacts with both phenyl rings of **4**, **5**, and **9** via hydrophobic interactions. Although the phenyl ring of **14** does not interact with Phe264, one *N*-ethyl group interacts with Phe264 via hydrophobic interaction. In addition to Phe264, the phenyl ring of the amino moiety of the amides **4**, **5**, and **9** forms additional hydrophobic interactions with two amino acids Thr261 and Met257 (compound **4**), one amino acid Met257 (compound **5**), and two amino acids Val248 and Met257 (compound **9**). It is speculated that various hydrophobic interactions and hydrogen bonds between tyrosinase and the ligands may result in stronger binding affinities than kojic acid. Summarising these results, these cinnamic amide ligands have the potential to bind to the active site of tyrosinase and inhibit tyrosinase.



COMPOUND	DOCKING SCORE (kcal/mol)
4	-7.7
5	-7.9
9	-7.4
14	-6.2
Kojic acid	-5.7

PDB Code: 2Y9X

Figure 2. Docking simulation of the cinnamic amide derivatives **4**, **5**, **9**, and **14** and of kojic acid with tyrosinase and pharmacophore analysis. (a, and b) Pharmacophore results for **4**, **5**, **9**, **14** and kojic acid obtained from LigandScout 4.1.0 indicate possible hydrogen-bonding (green arrow), π-π stacking (violet arrow) and hydrophobic (yellow) interactions between the ligands tested and the amino acid residues of tyrosinase. (c) Docking simulation result between cinnamic amides **4**, **5**, **9**, and **14** and mushroom tyrosinase. (d) Docking scores between tyrosinase and **4**, **5**, **9**, **14** or kojic acid.

*2.5. Cell viability of cinnamic amide derivatives **4**, **5**, **9** and **14** in B16F10 melanoma cells*

Cell viabilities were measured by performing WST-8 assay. Treatment of the four cinnamic amide derivatives at four concentrations (0, 5, 10, and 25 µM) in B16F10 melanoma cells, gave no significant cytotoxicity after an incubation of 24 h.

According to Figure 3, negligible cytotoxicity was noted for cinnamic amides **4**, **5**, **9** and **14** at concentrations below 25 µM as compared to the control. On the basis of these findings, further evaluation of the four cinnamic amide derivatives in α-MSH-stimulated B16F10 melanoma cells was carried out at concentrations up to 25 µM.

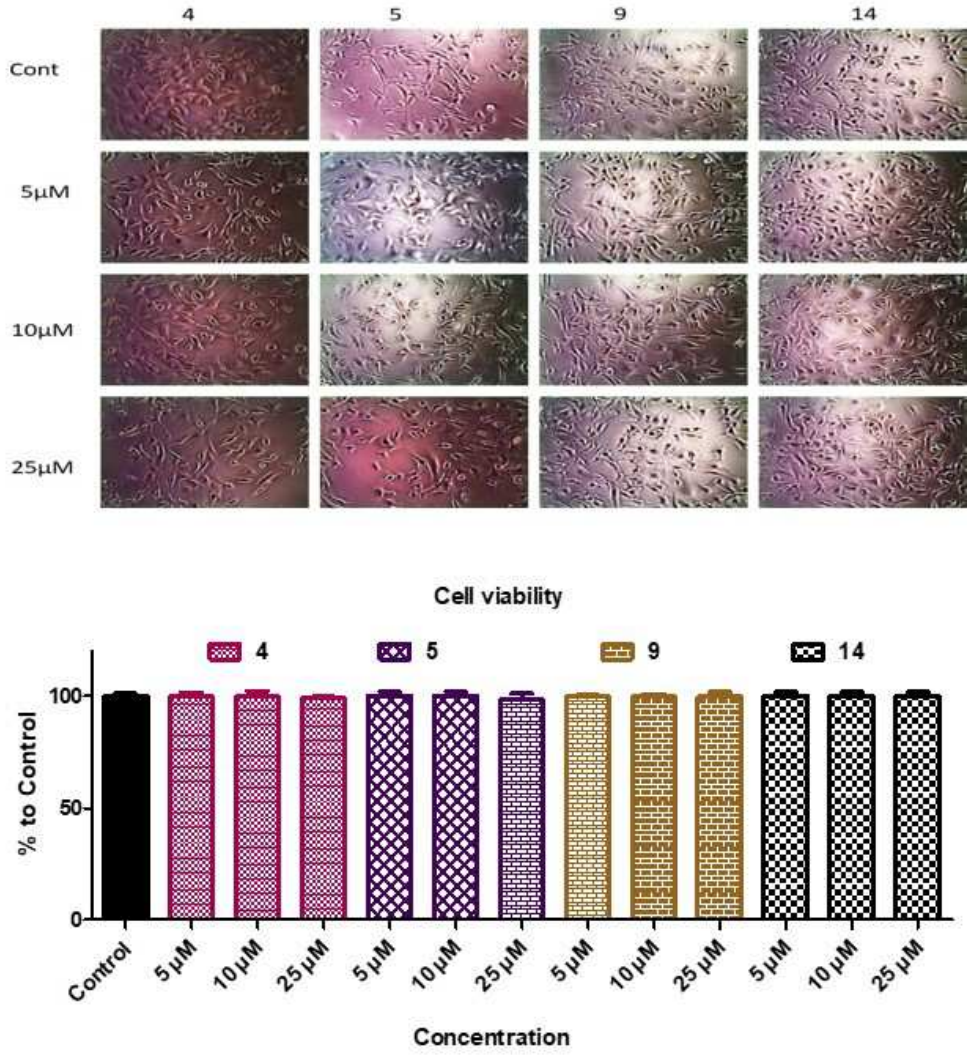


Figure 3. Cell viability assay of cinnamic amide derivatives **4**, **5**, **9** and **14** in B16F10 melanoma cells. Test cinnamic amides were treated at the concentrations of 5, 10, and 25 μM. Viabilities are expressed in % to control, and the bars represent standard errors.

*2.6. Tyrosinase inhibitory activity of cinnamic amide derivatives **4**, **5**, **9** and **14** in α-MSH-stimulated B16F10 melanoma cells*

The tyrosinase inhibition effect of the four cinnamic amides was assessed by using B16F10 melanoma cells stimulated by α-MSH (melanin-stimulating hormone). Once the tyrosinase activity level had been increased by the α-MSH treatment, the cells were treated with four concentrations (0, 5, 10, and 25 μM) of the four cinnamic amides or with two natural tyrosinase inhibitors used as positive controls: kojic acid (25 μM) or arbutin (400 μM). After an incubation of 24 h, the tyrosinase inhibition effect of the cinnamic amides was observed in B16F10 cells by measuring the optical densities spectrophotometrically.

As shown in Figure 4, all four cinnamic amide derivatives revealed an impressive inhibition of tyrosinase activity, superior to that of kojic acid and arbutin in α-MSH-stimulated B16F10 melanoma cells at 25 μM concentration. Among the four cinnamic amide, **5** (79.17% inhibition) with a 4-hydroxy-3-methoxyphenyl group and **14** (73.69% inhibition) with a 2,4-dihydroxyphenyl group showed the highest tyrosinase inhibition at 25 μM as compared to kojic acid (39.97% inhibition) and arbutin (53.17% inhibition). The other two cinnamic amides **4** (63.11% inhibition) and **9** (60.33% inhibition) also outperformed tyrosinase inhibition at 25 μM, which is superior to kojic acid and arbutin. All cinnamic amides dose-dependently inhibited the activity of tyrosinase. As in our previous report that the 2,4-dihydroxyphenyl group plays an important role in the inhibition of tyrosinase,[42,43,54-56] all three cinnamic amides **4**, **9**, and **14** with a 2,4-dihydroxyphenyl group showed potent inhibition of tyrosinase activity in α-MSH-stimulated B16F10 melanoma cells as well as the

inhibition of mushroom tyrosinase shown in Table 1. Interestingly, cinnamic amides **4** and **5** at a concentration of 10 μM inhibited the activity of tyrosinase more potently than kojic acid at 25 μM.

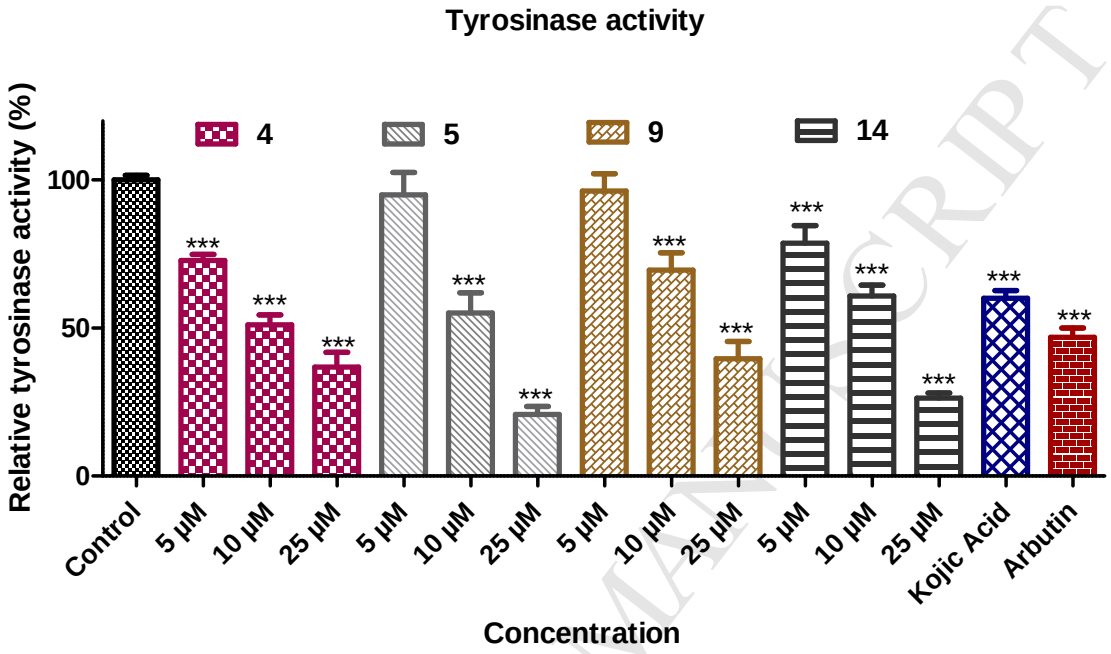


Figure 4. Tyrosinase inhibition activity of cinnamic amide derivatives **4**, **5**, **9** and **14** in α-MSH-stimulated B16F10 melanoma cells that were co-treated with cinnamic amides **4**, **5**, **9**, and **14**, kojic acid (25 μM), or arbutin (400 μM). The asterisks represent the significance difference between the columns: ***, $p<0.001$. The bars represent standard errors.

*2.7. Melanin content inhibition effect of cinnamic amide derivatives **4**, **5**, **9** and **14** in α-MSH-stimulated B16F10 melanoma cells*

To evaluate the melanin content in B16F10 melanoma cells, first, the cells were stimulated by α-MSH and then co-treated with the four cinnamic amides in four concentrations (0, 5, 10, and 25 μM), or kojic acid (25 μM). After an incubation of 24 h, optical densities were measured to evaluate the melanin production inhibitory effect of the cinnamic amides.

The results indicated in Figure 5 revealed that all four cinnamic amide derivatives vigorously and potently decreased the melanin content in α-MSH-stimulated B16F10 melanoma cells as compared to kojic acid at 25 μM concentration. The greatest decreases in melanin content were observed for cinnamic amides **5** (80.43% inhibition) and **14** (78.07% inhibition), followed by **4** (65.84% inhibition) and **9** (63.97% inhibition), as compared to kojic acid (44.26% inhibition). Interestingly, all four cinnamic amides at a concentration of 10 μM reduced the melanin content in α-MSH-stimulated B16F10 melanoma cells to an extent similar to or greater than kojic acid. Of the four cinnamic amides, **5** with a 4-hydroxy-3-methoxyphenyl group exhibited the greatest reduction of melanin content at all tested concentrations. All four cinnamic amides inhibited melanin production in B16F10 melanoma cells in a concentration-dependent manner. The pattern of decrease in melanin content was similar to the inhibition pattern of tyrosinase as shown in Figure 4, suggesting a strong correlation between inhibition of tyrosinase and decreased melanin content.

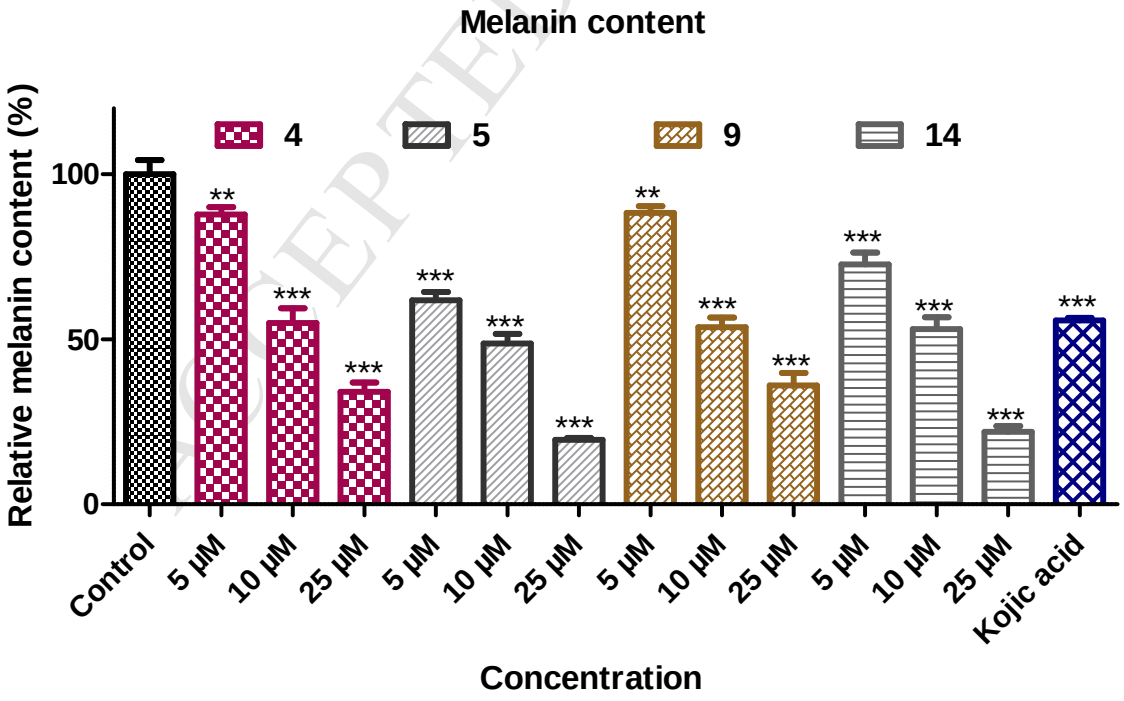


Figure 5. Melanin production inhibitory effect of cinnamic amide derivatives **4**, **5**, **9** and **14**

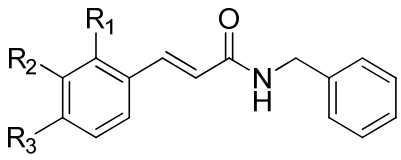
in α-MSH-stimulated B16F10 melanoma cells that were co-treated with cinnamic amides **4**, **5**, **9**, and **14**, or kojic acid (25 μM). The asterisks represent the significant difference between the columns: **, $p<0.01$; and ***, $p<0.001$. The bars represent standard errors.

*2.8. Antioxidant activities of cinnamic amide derivatives **1** – **19** in DPPH radical*

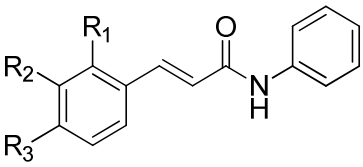
The antioxidant activity of cinnamic amide derivatives **1** – **19** was evaluated by adding 1.0 mM of cinnamic amides, or L-ascorbic acid to a DPPH methanol solution. The decrease in absorbance was measured with a spectrophotometer at 517 nm, indicating the scavenging capacity of the DPPH radical by cinnamic amides. The results are presented in Table 2.

Overall, cinnamic amides **8** (81.88% inhibition), and **13** (84.55% inhibition) with a 3,4-dihydroxyphenyl group, cinnamic amides **10** (82.58% inhibition), and **15** (78.94% inhibition) with a 4-hydroxy-3-methoxyphenyl group, and dihydroquinolinone **19** (83.05% inhibition) with a 4-hydroxy-3-methoxyphenyl group showed high DPPH scavenging activities comparable to L-ascorbic acid (84.64% inhibition) used as a positive control. The DPPH radical scavenging activity of cinnamic amides having a 4-hydroxyphenyl group was very susceptible to their amino moiety and showed very weak to strong DPPH radical scavenging activity (**7** and **12** *vs.* **2**) depending on their structure. The four cinnamic amides **4**, **5**, **9**, and **14** that exhibited strong inhibition of tyrosinase activity and suppression of melanin production in cellular experiments exerted moderate to strong DPPH radical scavenging activity (46.04 ~ 80.60% inhibition). Since ROS and RNS can induce melanogenesis, it is speculated that the DPPH radical scavenging effect of cinnamic amides contributes in part to the anti-melanogenesis effect.

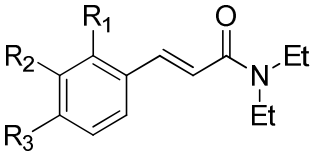
Table 2. DPPH radical scavenging activities of the synthesized cinnamic amide derivatives **1** – **19**, and kojic acid



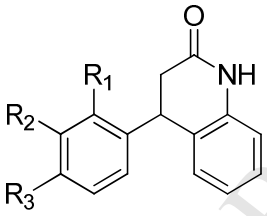
Compounds **1 - 5**



Compounds **6 - 10**



Compounds **11 - 15**



Compounds **16 - 19**

Compound	DPPH radical scavenging activity (%)	Compound	DPPH radical scavenging activity (%)
1	NA	**11**	24.33±0.32
2	81.57±0.48	**12**	3.87±0.47
3	46.67±0.99	**13**	84.55±0.97
4	80.60±0.22	**14**	55.31±0.62
5	49.19±1.21	**15**	78.94±0.51
6	53.27±1.52	**16**	NA
7	16.55±1.06	**17**	81.80±1.19
8	81.88±0.07	**18**	69.21±0.22
9	46.07±0.99	**19**	83.05±0.63
10	82.58±0.04	L-ascorbic acid	84.64±0.32

Radical scavenging activities were determined 30 min after the addition of cinnamic a mides to DPPH in methanol to a final concentration of 1.0 mM. Three independent e xperiments were performed. NA means not active. The results are presented as the m eans±SDs of three experiments.

Conclusion

In summary, fifteen cinnamic amide derivatives **1** – **15** having an (*E*)-β-phenyl-α,β-unsaturated carbonyl motif were synthesized as potential tyrosinase inhibitors, via debenzylation of the *O*-benzyl-protected cinnamic amides **40** – **54** using *conc*-HCl/acetic acid, or BBr_3. Four 3,4-dihydroquinolinones **16** – **19** were also synthesized from cinnamic amides **45** – **47**, and **49** with an anilino moiety during debenzylation using *conc*-HCl and acetic acid, via an intramolecular Michael addition of the anilide group. These nineteen compounds **1** –

19 were evaluated for mushroom tyrosinase inhibition activity. Nine compounds exhibited greater inhibition at 25 μM than kojic acid, indicating that the motif closely affects tyrosinase inhibition. Especially, four of the cinnamic amides (**4**, **5**, **9**, and **14**) showed tyrosinase inhibitory activity three times greater than that of kojic acid. The docking simulation using AutoDock Vina exhibited that these four cinnamic amides (-6.2 – -7.9 kcal/mol) have greater binding affinities to the active site of tyrosinase than kojic acid (-5.7 kcal/mol). Cell-based experiments on B16F10 melanoma cells demonstrated that these four cinnamic amides are not cytotoxic and exhibit more potent anti-melanogenic effect than kojic acid through the inhibition of cellular tyrosinase activity. Notably, a 2,4-dihydroxyphenyl substituent appears to play a major role in the great inhibition of tyrosinase, taking into account that the four cinnamic amides commonly have the substituent. These results imply that the (*E*)-β-phenyl-α,β-unsaturated carbonyl motif plays a key role in tyrosinase inhibition and that cinnamic amide derivatives having the motif are inherently promising candidates for safe and potent tyrosinase inhibitors.

3. Materials and Methods

4.1. General methods

All the chemicals and reagents were obtained commercially and used without further purification. Thin layer chromatography (TLC) and column chromatography were conducted on Merck precoated $60F_{245}$ plates and MP Silica 40-63, 60 Å, respectively. All anhydrous solvents were distilled over CaH and Na/benzophenone. High resolution mass spectroscopy data were obtained on an Agilent accurate Mass quadruple time of flight (Q-TOF) liquid

chromatography (LC) mass spectrometer (Agilent, Santa Clara, CA, USA) in electrospray ionization (ESI) negative mode while low-resolution mass data were obtained in ESI positive mode on an Expression CMS spectrometer (Advion Ithaca, NY, USA). NMR spectra were recorded on a Varian Unity INOVA 400 spectrometer, or a Varian Unity AS500 spectrometer (Agilent technologies, Santa Clara, CA, USA) for 400 MHz or 500 MHz ^{1}H NMR and 100 MHz ^{13}C NMR. $CDCl_3$, DMSO-d_6, and $CDCl_3$+CD_3OD were used as an NMR solvent for NMR samples. All chemical shifts were measured in parts per million (ppm) versus residual solvent or deuterated peaks (δ_H 7.24 and δ_C 77.0 for $CDCl_3$, δ_H 2.50 and δ_C 39.7 for DMSO-d_6). Coupling constant (*J*) values were measured in hertz (Hz). The following abbreviations are used for ^{1}H NMR: s (singlet), brs (broad singlet), d (doublet), brd (broad doublet), dd (doublet of doublets), t (triplet), brt (broad triplet), td (triplet of doublets), q (quartet), brq (broad quartet), and m (multiplet).

*4.1.1. General procedure for the synthesis of compounds **25** – **29** [57].*

Benzaldehydes **20 – 24** (5.00 g), benzyl bromide (1.0 equiv.), potassium carbonate (1.0 equiv.) and acetonitrile (50 mL) were added to a 250 mL round-bottom flask and the reaction mixture was refluxed for 24 h. Acetonitrile was evaporated on completion of the reaction and the residues were partitioned between dichloromethane and water. The dichloromethane layer was dried with anhydrous $MgSO_4$ and evaporated *in vacuo* to give benzyl-protected benzaldehydes **25 - 29** as a white or grey solid in yields of 95 – 97%.

*4.1.1.1 3-(Benzyloxy)-4-methoxybenzaldehyde (**25**).* White solid, 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.82 (s, 1 H, CHO), 7.47 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6-H), 7.47 – 7.44 (m, 3 H, 2-H, 2′-H, 6′-H), 7.38 (t, 2 H, *J* = 7.5 Hz, 3′-H, 5′-H), 7.32 (t, 1 H, *J* = 7.5 Hz, 4′-H), 7.00 (d, 1 H, *J* = 8.0 Hz, 5-H), 5.19 (s, 2 H, benzylic H), 3.96 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz,

$CDCl_3$) δ 191.0, 155.2, 148.9, 136.5, 130.2, 128.8, 128.3, 127.7, 127.1, 111.6, 111.0, 71.1, 56.4.

*4.1.1.2. 4-(Benzyloxy)benzaldehyde (**26**).* White solid, 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 (s, 1 H, CHO), 7.84 (d, 2 H, *J* = 8.5 Hz, 2-H, 6-H), 7.44 (d, 2 H, *J* = 8.0 Hz, 2′-H, 6′-H), 7.41 (t, 2 H, *J* = 8.0 Hz, 3′-H, 5′-H), 7.35 (t, 1 H, *J* = 7.5 Hz, 4′-H), 7.08 (d, 2 H, *J* = 8.5 Hz, 3-H, 5-H), 5.15 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 163.9, 136.1, 132.2, 130.3, 128.9, 128.5, 127.7, 115.3, 70.5.

*4.1.1.3. 3,4-Bis(benzyloxy)benzaldehyde (**27**).* White solid, 96% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.81 (s, 1 H, CHO), 7.49 (d, 1 H, *J* = 2.0 Hz, 2-H), 7.48 – 7.36 (m, 9 H, 6-H, 2′-H, 3′-H, 5′-H, 6′-H, 2″-H, 3″-H, 5″-H, 6″-H), 7.34 – 7.30 (m, 2 H, 4′-H, 4″-H), 7.02 (d, 1 H, *J* = 8.0 Hz, 5-H), 5.26 (s, 2 H, benzylic H), 5.22 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.0, 154.4, 149.4, 136.7, 136.4, 130.5, 128.9, 128.8, 128.3, 128.2, 127.5, 127.2, 126.9, 113.3, 112.5, 71.2, 71.0.

*4.1.1.4. 2,4-Bis(benzyloxy)benzaldehyde (**28**).* Grey solid, 95 % yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.39 (s, 1 H, CHO), 7.84 (d, 1 H, *J* = 9.0 Hz, 6-H), 7.45 – 7.38 (m, 8 H, 2′-H, 3′-H, 5′-H, 6′-H, 2″-H, 3″-H, 5″-H, 6″-H), 7.37 – 7.34 (m, 2 H, 4′-H, 4″-H), 6.64 (dd, 1 H, *J* = 9.0, 2.0 Hz, 5-H), 6.60 (d, 1 H, *J* = 2.0 Hz, 3-H), 5.14 (s, 2 H, benzylic H), 5.11 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.5, 165.4, 162.9, 136.1, 136.1, 130.7, 129.0, 128.9, 128.6, 128.5, 127.8, 127.5, 119.7, 107.2, 100.3, 70.6, 70.4.

*4.1.1.5. 4-(Benzyloxy)-3-methoxybenzaldehyde (**29**).* White solid, 97% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (s, 1 H, CHO), 7.45 – 7.42 (m, 3 H, 2-H, 2′-H, 6′-H), 7.40 – 7.37 (m, 3 H, 6-H, 3′-H, 5′-H), 7.33 (t, 1 H, *J* = 7.5 Hz, 4′-H), 6.99 (d, 1 H, *J* = 8.0 Hz, 5-H), 5.25 (s, 2 H, benzylic H), 3.95 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.1, 153.8, 150.3, 136.2,

130.5, 128.9, 128.4, 127.4, 126.8, 112.6, 109.5, 71.1, 56.3.

*4.1.2. General procedure for the synthesis of cinnamic acid derivatives **35** – **39** via ethyl cinnamates **30** – **34**.*

To a stirred solution of compounds **25** – **29** (2.00 g), triethyl phosphonoacetate (1.1 equiv.), and potassium carbonate (2.0 equiv.) in dichloromethane/*N*,*N*-dimethylformamide (2:1, 15 mL) was added a catalytic amount of DBU (0.03 equiv.). The reaction mixture was stirred at room temperature (compounds **25**, **27**, and **29**) or 70 ^{0}C (**26**, and **28**) for 24 – 36 h. After removal of dichloromethane by evaporation, ice water was added to the reaction mixture which was then stirred for 30 min to give precipitates. After filtration, the filter cake was washed with plenty of water, and dried to produce ethyl cinnamates **30** – **34** as a white or grey solid in yields of 95 – 98%. The ethyl cinnamates were used directly in the next reaction without characterization.

Ethyl cinnamates **30** – **34** (2.57 to 4.11 g) were added to a 100 mL round-bottom flask and 1,4-dioxane (20 mL) and 1N-NaOH aqueous solution (8.0 – 16.0 equiv.) were added subsequently. The reaction mixture was stirred at room temperature for 48 h. After completion of the reaction, the reaction mixture was acidified until pH 2 using 2N-HCl aqueous solution. The reaction mixture was stirred at room temperature for 30 min and then the resulting solid was filtered. The filter cake was washed with a plenty of water, and dried to give the cinnamic acid derivatives **35** – **39** as a white or grey solid in yields of 100%.

*4.1.2.1. (E)-3-(3-(Benzyloxy)-4-methoxyphenyl)acrylic acid (**35**).* White solid, 100% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.19 (brs, 1 H, COOH), 7.49 (d, 1 H, J = 16.0 Hz, 3-vinylic H), 7.45 (d, 2 H, J = 7.5 Hz, 2′-H, 6′-H), 7.41 (d, 1 H, J = 2.0 Hz, 2′-H), 7.38 (t, 2 H, J = 8.0

Hz, 3′-H, 5′-H), 7.32 (t, 1 H, *J* = 7.5 Hz, 4′-H), 7.20 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6´-H), 6.98 (d, 1 H, *J* = 8.0 Hz, 5´-H), 6.40 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.12 (s, 2 H, benzylic H), 3.78 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 151.7, 148.6, 144.7, 137.6, 129.1, 128.6, 128.5, 127.6, 123.7, 117.4, 112.5, 112.4, 70.5, 56.3.

*4.1.2.2. (E)-3-(4-(Benzyloxy)phenyl)acrylic acid (**36**).* White solid, 100% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.25 (brs, 1 H, COOH), 7.61 (d, 2 H, *J* = 9.0 Hz, 2-H, 6-H), 7.52 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.43 (d, 2 H, *J* = 7.5 Hz, 2′-H, 6′-H), 7.38 (t, 2 H, *J* = 7.5 Hz, 3′-H, 5′-H), 7.31 (t, 1 H, *J* = 7.5 Hz, 4′-H), 7.02 (d, 2 H, *J* = 8.0 Hz, 3-H, 5-H), 6.36 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.14 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 160.6, 144.3, 137.4, 130.6, 129.1, 128.6, 128.4, 127.7, 117.4, 115.8, 70.0.

*4.1.2.3. (E)-3-(3,4-Bis(benzyloxy)phenyl)acrylic acid (**37**).* White solid, 100% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.22 (brs, 1 H, COOH), 7.47 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.46 -7.41 (m, 5 H, 2-H, 2′-H, 6′-H, 2″-H, 6″-H), 7.37 (t, 2 H, *J* = 7.5 Hz, 3′-H, 5′-H), 7.36 (t, 2 H, *J* = 7.5 Hz, 3″-H, 5″-H), 7.30 (t, 2 H, *J* = 7.5 Hz, 4′-H, 4″-H), 7.17 (dd, 1 H, *J* = 8.5, 2.0 Hz, 6-H), 7.05 (d, 1 H, *J* = 8.5 Hz, 5-H), 6.40 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.18 (s, 2 H, benzylic H), 5.16 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 150.7, 148.9, 144.6, 137.8, 137.6, 129.1, 129.1, 128.5, 128.5, 128.3, 128.1, 123.5, 117.7, 117.5, 114.5, 113.5, 70.6, 70.5.

*4.1.2.4. (E)-3-(2,4-Bis(benzyloxy)phenyl)acrylic acid (**38**).* Grey solid, 100% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.10 (s, 1 H, COOH), 7.77 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.62 (d, 1 H, *J* = 9.0 Hz, 6-H), 7.45 – 7.31 (m, 10 H, 2 ×Ph), 6.81 (d, 1 H, *J* = 2.0 Hz, 3-H), 6.65 (dd, 1 H, *J* = 9.0, 2.0 Hz, 5-H), 7.38 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.19 (s, 2 H, benzylic H), 5.14 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.8, 162.1, 158.8, 139.3,

137.3, 137.3, 130.6, 129.2, 129.1, 128.7, 128.6, 128.5, 128.4, 117.3, 116.6, 107.8, 101.2, 70.5, 70.2.

*4.1.2.5. (E)-3-(4-(Benzyloxy)-3-methoxyphenyl)acrylic acid (**39**).* White solid, 100% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.17 (brs, 1 H, COOH), 7.50 (d, 1 H, J = 16.0 Hz, 3-vinylic H), 7.42 (d, 2 H, J = 7.5 Hz, 2′-H, 6′-H), 7.38 (t, 2 H, J = 7.5 Hz, 3′-H, 5′-H), 7.32 (t, 1 H, J = 7.5 Hz, 4′-H), 7.32 (d, 1 H, J = 2.0 Hz, 2-H), 7.16 (dd, 1 H, J = 8.0, 2.0 Hz, 6-H), 7.04 (d, 1 H, J = 8.0 Hz, 5-H), 6.43 (d, 1 H, J = 16.0 Hz, 2-vinylic H), 5.11 (s, 2 H, benzylic H), 3.80 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.5, 150.3, 149.9, 144.7, 137.4, 129.1, 128.6, 128.5, 128.0, 123.1, 117.5, 113.7, 111.2, 70.4, 56.3.

*4.1.4. General procedure for the preparation of compounds **40** – **54*** [58].

Cinnamic acid derivatives **35 – 39** (100 mg), isobutyl chloroformate (2.00 equiv.), *N*-methyl morpholine (2.5 equiv.), and anhydrous THF (5 mL) were added to a 25 mL round-bottom flask and stirred at 25 ^{0}C for 30 min. Then benzylamine, aniline or diethylamine (2.00 equiv.) was added to the reaction flask which was then stirred at room temperature for 24 h. After completion of the reaction, the reaction mixture was partitioned between ethyl acetate and H_2O. The ethyl acetate layer was washed with brine, dried with $MgSO_4$ and evaporated under reduced pressure. The resultant residue was purified by column chromatography using methylene chloride and methanol (30 – 70:1) as the eluent. The products **40** – **54** were obtained as a white or yellow solid in yields of 75 – 96%.

*4.1.3.1. (E)-N-Benzyl-3-(3-(benzyloxy)-4-methoxyphenyl)acrylamide (**40**).* Yellowish white solid, 91% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, 1 H, J = 15.5 Hz, 3-vinylic H), 7.42 (d, 2 H, J = 7.5 Hz, 2″-H, 6″-H), 7.36 (t, 2 H, J = 7.5 Hz, 3′-H, 5′-H), 7.34 – 7.28 (m, 6 H, 4′-H, Ph), 7.08 (dd, 1 H, J = 8.5, 1.5 Hz, 6-H) 7.03 (d, 1 H, J = 1.5 Hz, 2-H), 6.85 (d, 1 H, J =

8.5 Hz, 5-H), 6.21 (d, 1 H, *J* = 15.5 Hz, 2-vinylic H), 5.93 (brt, 1 H, *J* = 5.0 Hz, NH), 5.13 (s, 2 H, benzylic H), 4.55 (d, 2 H, *J* = 5.0 Hz, *N*-benzylic H), 3.89 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 151.5, 148.4, 141.6, 138.4, 136.9, 128.9, 128.8, 128.2, 128.1, 127.8, 127.7, 127.5, 122.5, 118.2, 112.8, 111.7, 71.2, 56.2, 44.1.

*4.1.3.2. (E)-N-benzyl-3-(4-(benzyloxy)phenyl)acrylamide (**41**).* Yellowish solid, 96% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1 H, *J* = 15.5 Hz, 3-vinylic H), 7.44 – 7.27 (m, 12 H, 2-H, 6-H, 2 ×Ph), 6.94 (d, 2 H, *J* = 8.5 Hz, 3-H, 5-H), 6.30 (d, 1 H, *J* = 15.5 Hz, 2-vinylic H), 6.00 (brt, 1 H, *J* = 5.0 Hz, NH), 5.08 (s, 2 H, benzylic H), 4.56 (d, 2 H, *J* = 5.0 Hz, *N*-benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 160.3, 141.5, 138.4, 136.7, 129.7, 128.9, 128.9, 128.3, 128.1, 127.8, 127.7, 118.0, 115.3, 70.2, 44.1.

*4.1.3.3. (E)-N-Benzyl-3-(3,4-bis(benzyloxy)phenyl)acrylamide (**42**).* White solid, 90% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.44 – 7.28 (m, 15 H, 2 ×Ph), 7.08 (d, 1 H, J = 1.5 Hz, 2-H), 7.03 (dd, 1 H, *J* = 8.5, 1.5 Hz, 6-H), 6.89 (d, 1 H, *J* = 8.5 Hz, 5-H), 6.22 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.91 (brt, 1 H, J = 5.0 Hz, NH), 5.17 (s, 2 H, benzylic H), 5.15 (s, 2 H, benzylic H), 4.55 (d, 2 H, *J* = 5.0 Hz, *N*-benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 150.8, 149.0, 141.6, 138.3, 137.1, 137.0, 129.0, 128.8, 128.8, 128.3, 128.1, 128.1, 127.8, 127.5, 127.4, 122.6, 118.4, 114.5, 114.0, 71.5, 71.1, 44.1.

*4.1.3.4. (E)-N-Benzyl-3-(2,4-bis(benzyloxy)phenyl)acrylamide (**43**).* White solid, 88% yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.39 – 7.24 (m, 16 H, 3 ×Ph, 6-H), 6.54 – 6.51 (m, 2 H, 3-H, 5-H), 6.46 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.87 (brt, 1 H, J = 5.2 Hz, NH), 5.06 (s, 2 H, benzylic H), 5.00 (s, 2 H, benzylic H), 4.51 (d, 2 H, *J* = 5.2 Hz, *N*-benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 161.3, 158.9, 138.6, 137.1, 136.6, 130.9, 128.9, 128.9, 128.9, 128.4, 128.3, 128.1, 127.8, 127.7, 127.4, 119.2, 117.6,

106.6, 100.9, 70.6, 70.4, 44.0.

*4.1.3.5. (E)-N-Benzyl-3-(4-(benzyloxy)-3-methoxyphenyl)acrylamide (**44**).* Yellowish white solid, 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, 1 H, *J* = 15.6 Hz, 3-vinylic H), 7.39 (d, 2 H, J = 7.2 Hz, 2′-H, 6′-H), 7.34 (t, 2 H, J = 7.2 Hz, 3′-H, 5′-H), 7.29 – 7.25 (m, 6 H, 4′-H, Ph), 6.99 (s, 1 H, 2-H), 6.96 (d, 1 H, *J* = 8.0 Hz, 6-H), 6.80 (d, 1 H, *J* = 8.0 Hz, 5-H), 6.29 (d, 1 H, *J* = 15.6 Hz, 2-vinylic H), 6.19 (brd, 1 H, J = 5.2 Hz, NH), 5.14 (s, 2 H, benzylic H), 4.51 (d, 2 H, *J* = 5.2 Hz, *N*-benzylic H), 3.84 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 150.0, 149.8, 141.6, 138.3, 136.8, 128.9, 128.8, 128.2, 128.1, 127.8, 127.4, 122.0, 118.4, 113.6, 110.4, 71.0, 56.2, 44.1. `

*4.1.3.6. (E)-3-(3-(Benzyloxy)-4-methoxyphenyl)-N-phenylacrylamide (**45**).* Yellow solid, 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1 H, NH), 7.65 (d, 2 H, *J* = 8.0 Hz, 2″-H, 6″-H), 7.49 – 7.26 (m, 9 H, 2-H, 3-vinylic H, Ph, 3″-H, 5″-H), 7.17 (d, 1 H, *J* = 8.4 Hz, 6-H), 7.00 (m, 2 H, 5-H, 4″-H), 6.66 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.10 (s, 2 H, benzylic H), 3.77 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 151.3, 148.5, 140.9, 140.1, 137.5, 129.4, 129.1, 128.6, 128.6, 128.0, 123.8, 122.6, 120.5, 119.8, 112.7, 112.6, 70.5, 56.3.

*4.1.3.7. (E)-3-(4-(Benzyloxy)phenyl)-N-phenylacrylamide (**46**).* Yellow solid, 85% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.08 (s, 1 H, NH), 7.67 (d, 2 H, *J* = 8.5 Hz, 2-H, 6-H), 7.56 (d, 2 H, *J* = 8.5 Hz, 2″-H, 6″-H), 7.52 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.44 (d, 2 H, *J* = 7.5 Hz, 2′-H, 6′-H), 7.39 (t, 2 H, *J* = 7.5 Hz, 3′-H, 5′-H), 7.32 (t, 1 H, 4′-H), 7.31 (t, 2 H, J = 8.0 Hz, 3″-H, 5″-H), 7.07 (d, 2 H, *J* = 8.5 Hz, 3-H, 5-H), 7.04 (t, 1 H, *J* = 8.0 Hz, 4″-H), 6.67 (s, 1 H, *J* = 16.0 Hz, 2-vinylic H), 5.14 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 160.3, 140.5, 140.0, 137.4, 130.0, 129.4, 129.1, 128.6, 128.4, 128.1, 123.8, 120.4, 119.8, 115.9, 70.0.

*4.1.3.8. (E)-3-(3,4-Bis(benzyloxy)phenyl)-N-phenylacrylamide (**47**).* Yellowish white solid, 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 – 7.58 (m, 3 H, 2-vinylic H, 2″-H, 6″-H), 7.47 – 7.29 (m, 13 H, NH, 2 ×Ph, 3″-H, 5″-H), 7.13 – 7.10 (m, 2 H, 2-H, 4″-H), 7.06 (d, 1 H, *J* = 8.5 Hz, 6-H), 6.90 (d, 1 H, *J* = 8.5 Hz, 5-H), 6.36 (d, 1 H, *J* = 15.5 Hz, 2-vinylic H), 5.18 (s, 2 H, benzylic H), 5.16 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.7, 151.0, 149.1, 142.4, 138.3, 137.0, 136.9, 129.3, 128.8, 128.3, 128.2, 127.5, 127.4, 124.5, 122,8, 120.1, 119.0, 114.4, 114.1, 71.5, 71.1.

*4.1.3.9. (E)-3-(2,4-Bis(benzyloxy)phenyl)-N-phenylacrylamide (**48**).* Yellowish white solid, 75% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.04 (s, 1 H, NH), 7.81 (d, 1 H, *J* = 16.0 Hz, 3-vinylic H), 7.66 (d, 2 H, *J* = 7.5 Hz, 2″-H, 6″-H), 7.53 (d, 1 H, *J* = 9.0 Hz, 6-H), 7.46 - 7.28 (m, 12 H, 2 ×Ph, 3″-H, 5″-H), 7.02 (t, 1 H, *J* = 7.5 Hz, 4″-H), 6.18 (d, 1 H, *J* = 2.0 Hz, 3-H), 6.71 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 6.70 (dd, 1 H, *J* = 9.0, 2.0 Hz, 5-H), 5.22 (s, 2 H, benzylic H), 5.13 (s, 2 H, benzylic H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8, 161.7, 158.5, 140.0, 137.4, 137.3, 135.4, 129.4, 129.2, 129.1, 128.6, 128.6, 128.5, 128.2, 123.8, 120.4, 119.7, 117.2, 107.8, 101.3, 70.3, 70.2.

*4.1.3.10. (E)-3-(4-(Benzyloxy)-3-methoxyphenyl)-N-phenylacrylamide (**49**).* Yellow solid, 81% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 – 7.52 (m, 3 H, 3-vinylic H, 2″-H, 6″-H), 7.35 (d, 2 H, *J* = 7.5 Hz, 2′-H, 6′-H), 7.29 (t, 2 H, J = 7.5 Hz, 3′-H, 5′-H), 7.26 – 7.21 (m, 4 H, NH, 4′-H, 3″-H, 5″-H), 7.15 – 7.05 (m, 2 H, 6-H, 4″-H), 6.99 (d, 1 H, J = 8.5 Hz, 6-H), 6.80 (d, 1 H, *J* = 8.5 Hz, 5-H), 6.47 (d, 1 H, *J* = 15.5 Hz, 2-vinylic H), 5.09 (s, 2 H, benzylic H), 3.83 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz $CDCl_3$) δ 164.5, 149.8, 149.7, 141.8, 138.6, 136.7, 129.0, 128.7, 128.4, 128.1, 127.4, 124.2, 122.0, 120.1, 119.2, 113.6, 110.7, 71.0, 56.0.

*4.1.3.11. (E)-3-(3-(Benzyloxy)-4-methoxyphenyl)-N,N-diethylacrylamide (**50**).* Yellowish

white solid, 91% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, 1 H, J = 15.5 Hz, 3-vinylic H), 7.44 (d, 2 H, J = 7.5 Hz, 2′-H, 6′-H), 7.36 (t, 2 H, J = 7.5 Hz, 3′-H, 5′-H), 7.29 (t, 1 H, J = 7.5 Hz, 4′-H), 7.11 (dd, 1 H, J = 8.5, 2.0 Hz, 6-H), 7.03 (d, 1 H, J = 2.0 Hz, 2-H), 6.87 (d, 1 H, J = 8.5 Hz, 5-H), 6.55 (d, 1 H, J = 15.5 Hz, 2-vinylic H), 5.18 (s, 2 H, benzylic H), 3.91 (s, 3 H, OCH_3), 3.46 (brq, 4 H, J = 7.0 Hz, 2 ×NCH_2), 1.20 (t, 6 H, J = 7.0 Hz, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 151.5, 148.3, 143.4, 137.1, 128.8, 128.2, 127.5, 122.6, 117.5, 114.8, 113.3, 111.7, 71.5, 56.2, 42.2, 14.7.

*4.1.3.12. (E)-3-(4-(Benzyloxy)phenyl)-N,N-diethylacrylamide (**51**).* Yellowish white solid, 90% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, 1 H, J = 15.0 Hz, 3-vinylic H), 7.47 (d, 2 H, J = 8.5 Hz, 2-H, 6-H), 7.42 (d, 2 H, J = 7.5 Hz, 2′-H, 6′-H), 7.39 (t, 2 H, J = 7.5 Hz, 3′-H, 5′-H), 7.33 (t, 1 H, J = 7.5 Hz, 4′-H), 6.96 (d, 2 H, J = 8.5 Hz, 3-H, 5-H), 6.69 (d, 1 H, J = 15.0 Hz, 2-vinylic H), 5.08 (s, 2 H, benzylic H), 3.48 (q, 4 H, J = 7.0 Hz, 2 ×NCH_2), 1.22 (t, 6 H, J = 7.0 Hz, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 160.3, 143.3, 136.7, 129.7, 128.9, 128.4, 128.3, 127.7, 115.3, 114.7, 70.3, 42.2, 14.0.

*4.1.3.13. (E)-3-(3,4-Bis(benzyloxy)phenyl)-N,N-diethylacrylamide (**52**).* White solid, 87% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (d, 1 H, J = 15.0 Hz, 3-vinylic H), 7.46 – 7.29 (m, 10 H, 2 ×Ph), 7.08 (d, 1 H, J = 2.0 Hz, 2-H), 7.07 (dd, 1 H, J = 8.0, 2.0 Hz, 6-H), 6.90 (d, 1 H, J = 8.0 Hz, 5-H), 6.57 (d, 1 H, J = 15.0 Hz, 2-vinylic H), 5.19 (s, 4 H, 2 ×benzylic H), 3.46 (q, 4 H, J = 7.0 Hz, 2 ×NCH_2), 1.20 (t, 6 H, J = 7.0 Hz, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 150.8, 149.0, 143.7, 137.3, 137.0, 128.8, 128.8, 128.1, 128.1, 127.5, 127.5, 122.7, 115.3, 114.7, 114.6, 71.8, 71.2, 42.2, 13.9.

*4.1.3.14. (E)-3-(2,4-Bis(benzyloxy)phenyl)-N,N-diethylacrylamide (**53**).* White solid, 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, 1 H, J = 16.0 Hz, 3-vinylic H), 7.48 – 7.33 (m,

11 H, 6-H, 2 ×Ph), 6.95 (d, 1 H, *J* = 16.0 Hz, 2-vinylic H), 6.64 (d, 1 H, J = 1.5 Hz, 3-H), 6.59 (dd, 1 H, *J* = 8.5, 1.5 Hz, 5-H), 5.07 (s, 2 H, benzylic H), 5.05 (s, 2 H, benzylic H), 3.48 (q, 4 H, J = 7.0 Hz, 2 ×NCH_2), 1.25 (t, 6 H, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.2, 161.1, 159.3, 139.6, 136.7, 136.4, 133.2, 128.9, 128.9, 128.5, 128.4, 128.4, 127.8, 118.0, 116.6, 106.3, 100.7, *70.8*, *70.4*, *41.7*, *14.2*.

*4.1.3.15. (E)-3-(4-(Benzyloxy)-3-methoxyphenyl)-N,N-diethylacrylamide (**54**).* Yellowish solid, 89% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, 1 H, *J* = 15.0 Hz, 3-vinylic H), 7.42 (d, 2 H, *J* = 7.5 Hz, 2′-H, 6′-H), 7.36 (t, 2 H, *J* = 7.5 Hz, 3′-H, 5′-H), 7.30 (t, 1 H, *J* = 7.5 Hz, 4′-H), 7.06 (dd, 1 H, *J* = 8.0, 1.5 Hz, 6-H), 7.05 (d, 1 H, *J* = 1.5 Hz, 2-H), 6.86 (d, 1 H, *J* = 8.5 Hz, 5-H), 6.65 (d, 1 H, *J* = 15.0 Hz, 2-vinylic H), 5.18 (s, 2 H, benzylic H), 3.92 (s, 3 H, OCH_3), 3.48 (q, 4 H, *J* = 7.0 Hz, 2 ×NCH_2), 1.22 (t, 6 H, *J* = 7.0 Hz, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 149.9, 149.8, 143.9, 136.9, 128.8, 128.8, 128.2, 127.4, 121.8, 114.7, 113.7, 111.3, 71.1, 56.3, 42.2, 14.0.

*4.1.4. General procedure for the preparation of cinnamic amide derivatives **1** – **3**, **5**, **11** – **13**, **15**, **16** – **19**.*

Compounds **40** – **43**, **45** – **49**, and **51** – **54** (80 mg) were added in a 25 mL round-bottom flask along with concentrated HCl and acetic acid (1:1, 2 mL) and the reaction mixture was refluxed for 40 min. After completion of the reaction, the mixture was neutralised by 2 N NaOH aqueous solution and partitioned between methylene chloride and water. The organic layer was dried with anhydrous $MgSO_4$, and filtered and the filtrate was evaporated in reduced pressure. The resulting residue was purified by column chromatography using methylene chloride and methanol (10 – 30:1) as the eluent. The cinnamic amides **1** – **3**, **5**, **11** – **13**, and **15** – **19** were produced as a yellow or white solid in yields of 65 – 91%.

*4.1.4.1. (E)-N-Benzyl-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**1**).* Yellowish white solid, 85% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.51 (d, 1 H, J = 16.0 Hz, 3-H), 7.31 – 7.21 (m, 5 H, Ph) 7.06 (d, 1 H, J = 2.0 Hz, 2′-H), 6.93 (dd, 1 H, J = 8.5, 2.0 Hz, 6′-H), 6.77 (d, 1 H, J = 8.5 Hz, 5′-H), 6.28 (d, 1 H, J = 16.0 Hz, 2-H), 4.49 (s, 2 H, benzylic CH_2), 3.85 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 167.3, 149.2, 146.4, 142.1, 138.4, 129.1, 128.3, 128.0, 127.9, 122.2, 118.1, 113.2, 111.2, 56.3, 44.2; LRMAS (ESI-) *m/z* 282 $(M-H)^-$.

*4.1.4.2. (E)-N-Benzyl-3-(4-hydroxyphenyl)acrylamide (**2**).* Yellowish white solid, 88% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.52 (d, 1 H, J = 15.5 Hz, 3-H), 7.30 – 7.21 (m, 7 H, Ph, 2′-H, 6′-H), 6.75 (d, 2 H, J = 9.0 Hz, 3′-H, 5′-H), 6.26 (d, 1 H, J = 15.5 Hz, 2-H), 4.48 (s, 2 H, benzylic CH_2); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 167.5, 159.1, 142.2, 138.0, 129.9, 128.9, 128.0, 127.7, 126.4, 116.5, 116.0, 44.0; LRMAS (ESI-) *m/z* 252 $(M-H)^-$.

*4.1.4.3. (E)-N-Benzyl-3-(3,4-dihydroxyphenyl)acrylamide (**3**).* White solid, 78% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.37 (d, 1 H, J = 16.0 Hz, 3-H), 7.24 – 7.14 (m, 5 H, Ph), 6.93 (d, 1 H, J = 2.0 Hz, 2′-H) 6.80 (dd, 1 H, J = 8.0, 2.0 Hz, 6′-H), 6.69 (d, 1 H, J = 8.0 Hz, 5′-H), 6.22 (d, 1 H, J = 16.0 Hz, 2-H), 4.40 (s, 2 H, benzylic CH_2); ^{13}C NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 167.8, 147.2, 144.9, 142.1, 138.1, 128.7, 127.8, 127.5, 127.1, 121.7, 116.8, 115.4, 113.9, 43.8; LRMAS (ESI-) *m/z* 268 $(M-H)^-$.

*4.1.4.4. (E)-N-Benzyl-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**5**).* Yellowish white solid, 87% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, 1 H, J = 15.5 Hz, 3-H), 7.35 – 7.26 (m, 5 H, Ph), 7.04 (dd, 1 H, J = 8.0, 2.0 Hz, 6′-H), 6.97 (d, 1 H, J = 2.0, 2′-H) 6.89 (d, 1 H, J = 8.0 Hz, 5′-H), 6.29 (d, 1 H, J = 15.5 Hz, 2-H), 6.03 (brt, 1 H, J = 4.5 Hz, NH), 4.56 (d, 2 H, J =

4.5 Hz, benzylic CH_2), 3.88 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 147.4, 146.9, 142.0, 138.3, 128.9, 128.1, 127.8, 127.4, 122.4, 117.8, 114.9, 109.8, 56.1, 44.1; LRMAS (ESI-) *m/z* 282 $(M-H)^-$; HRMS (ESI+) *m/z* $C_{17}H_{18}NO_3$ $(M+H)^+$ calcd 284.1281, obsd 284.1288.

*4.1.4.5. (E)-N,N-Diethyl-3-(3-hydroxy-4-methoxyphenyl)acrylamide (**11**).* Yellow solid, 91% yield. ^1H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, 1 H, *J* = 15.5 Hz, 3-H), 7.18 (d, 1 H, *J* = 2.0 Hz, 2′-H), 7.00 (dd, 1 H, *J* = 8.5, 2.0 Hz, 6′-H), 6.81 (d, 1 H, *J* = 8.5 Hz, 5′-H), 6.67 (d, 1 H, *J* = 15.5 Hz, 2-H), 3.89 (s, 3 H, OCH_3), 3.47 (q, 4 H, *J* = 7.0 Hz, 2 ×NCH_2), 1.21 (t, 6 H, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 148.4, 146.1, 143.0, 129.0, 121.9, 115.4, 112.9, 110.8, 56.2, 42.0, 14.2; LRMAS (ESI-) *m/z* 248 $(M-H)^-$.

*4.1.4.6. (E)-N,N-Diethyl-3-(4-hydroxyphenyl)acrylamide (**12**).* Yellow solid, 90% yield. ^1H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.49 (d, 1 H, *J* = 15.5 Hz, 3-H), 7.30 (d, 2 H, *J* = 8.5 Hz, 2′-H, 6′-H), 6.73 (d, 2 H, *J* = 8.5 Hz, 3′-H, 5′-H), 6.55 (d, 1 H, *J* = 15.5 Hz, 2-H), 3.38 (q, 4 H, *J* = 7.0 Hz, 2 ×NCH_2), 1.17 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 1.08 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 167.2, 159.0, 143.4, 129.7, 126.7, 115.9, 113.6, 42.7, 41.5, 14.8, 13.1; LRMAS (ESI-) *m/z* 218 $(M-H)^-$.

*4.1.4.7. (E)-3-(3,4-Dihydroxyphenyl)-N,N-diethylacrylamide (**13**).* Yellow solid, 88% yield. ^1H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.41 (d, 1 H, *J* = 16.0 Hz, 3-H), 6.95 (d, 1 H, *J* = 2.0 Hz, 2′-H), 6.84 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6′-H), 6.71 (d, 1 H, *J* = 8.0 Hz, 5′-H), 6.53 (d, 1 H, *J* = 16.0 Hz, 2-H), 3.37 (q, 4 H, *J* = 7.0 Hz, 2 ×NCH_2), 1.16 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3), 1.08 (t, 3 H, *J* = 7.0 Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 167.3, 147.1, 145.0, 143.8, 127.4, 121.2, 115.4, 114.4, 113.7, 42.8, 41.6, 14.8, 13.1; LRMAS (ESI-) *m/z* 234 $(M-H)^-$.

*4.1.4.8. (E)-N,N-Diethyl-3-(4-hydroxy-3-methoxyphenyl)acrylamide (**15**).* Yellow solid, 91% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, 1 H, J = 15.5 Hz, 3-H), 7.10 (dd, 1 H, J = 8.0, 2.0 Hz, 6′-H), 6.97 (d, 1 H, J = 2.0 Hz, 2′-H), 6.91 (d, 1 H, J = 8.0 Hz, 5′-H), 6.67 (d, 1 H, J = 15.5 Hz, 2-H), 3.92 (s, 3 H, OCH_3), 3.49 (q, 4 H, J = 7.0 Hz, 2 ×NCH_2), 1.22 (brt, 6 H, J = 7.0 Hz, 2 ×CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 147.6, 146.9, 143.4, 128.0, 121.8, 115.0, 114.8, 110.5, 56.2, 42.5, 41.5, 14.7, 13.8; LRMAS (ESI-) *m/z* 248 $(M-H)^-$.

*4.1.4.9. 4-(4-Hydroxy-3-methoxyphenyl)-3,4-dihydroquinolin-2(1H)-one (**16**).* White solid, 70% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.14 (s, 1 H), 8.87 (brs, 1 H), 7.14 (t, 1 H, J = 8.0 Hz), 6.93 (d, 1 H, J = 7.0 Hz), 6.89 (d, 1 H, J = 7.5 Hz), 6.88 (t, 1 H, J = 7.5 Hz), 6.82 (d, 1 H, J = 8.0 Hz, 5′-H), 6.56 (d, 1 H, J = 2.0 Hz, 2′-H), 6.53 (dd, 1 H, J = 8.0, 2.0 Hz, 6″-H), 4.13 (t, 1 H, J = 6.5 Hz, 4-H), 3.70 (s, 3 H, OCH_3), 2.75 (dd, 1 H, J = 16.5, 6.5 Hz, 3-Ha), 2.63 (dd, 1 H, J = 16.5, 6.5 Hz, 3-Hb); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 147.1, 147.1, 138.6, 135.7, 128.8, 128.1, 127.4, 122.8, 118.6, 116.0, 115.3, 113.0, 56.3, 40.9, 38.7; LRMAS (ESI-) *m/z* 268 $(M-H)^-$.

*4.1.4.10. 4-(4-Hydroxyphenyl)-3,4-dihydroquinolin-2(1H)-one (**17**).* White solid, 68% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.00 (t, 1 H, J = 8.0 Hz), 6.81 (d, 2 H, J = 8.5 Hz, 2′-H, 6′-H), 6.78 (t, 1 H, J = 7.5 Hz, 6-H), 6.74 (d, 1 H, J = 7.5 Hz), 6.71 (d, 1 H, J = 8.0 Hz), 6.59 (d, 2 H, J = 8.5 Hz, 3′-H, 5′-H), 4.02 (dd, 1 H, J = 8.5, 6.5Hz, 4-H), 2.68 (dd, 1 H, J = 16.0, 6.5 Hz, 3-Ha), 2.62 (dd, 1 H, J = 16.0, 8.5 Hz, 3-Hb); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 171.7, 155.9, 136.9, 132.3, 128.7, 128.2, 127.8, 127.3, 123.4, 115.7, 115.6, 41.0, 38.5; LRMAS (ESI-) *m/z* 238 $(M-H)^-$.

*4.1.4.11. 4-(3,4-Dihydroxyphenyl)-3,4-dihydroquinolin-2(1H)-one (**18**).* White solid, 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.11 (td, 1 H, J = 7.0, 2.5 Hz),

6.92 – 6.88 (m, 2 H), 6.77 (d, 1 H, *J* = 8.0 Hz), 6.69 (d, 1 H, *J* = 8.0 Hz, 5′-H), 6.56 (d, 1 H, *J* = 2.0 Hz, 2′-H), 6.45 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6′-H), 4.07 (dd, 1 H, *J* = 8.0, 6.0 Hz, 4-H), 2.80 (dd, 1 H, *J* = 16.0, 6.0 Hz, 3-Ha), 2.73 (dd, 1 H, *J* = 16.0, 8.0 Hz, 3-Hb); ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.9, 145.8, 144.7, 138.6, 134.0, 128.8, 128.0, 127.6, 122.7, 118.7, 116.2, 115.9, 115.4, 40.8, 38.3; LRMAS (ESI-) *m/z* 254 $(M-H)^-$.

*4.1.4.12. 4-(4-Hydroxy-3-methoxyphenyl)-3,4-dihydroquinolin-2(1H)-one (**19**).* White solid, 72% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.03 (t, 1 H, *J* = 7.5 Hz), 6.80 (t, 1 H, *J* = 7.5 Hz), 6.77 (d, 1 H, *J* = 7.5 Hz), 6.74 (d, 1 H, *J* = 7.5 Hz), 6.64 (d, 1 H, *J* = 8.0 Hz, 5′-H), 6.53 (s, 1 H, 2′-H), 6.46 (d, 1 H, *J* = 8.0 Hz, 6′-H), 4.04 (t, 1 H, *J* = 7.5 Hz, 4-H), 3.65 (s, 3 H, OCH_3), 2.69 (m, 2 H, 3-Ha, 3-Hb); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 171.7, 147.6, 145.1, 136.9, 133.0, 128.3, 127.9, 127.2, 123.5, 120.4, 115.7, 115.2, 110.8, 55.7, 41.5, 38.5; LRMAS (ESI-) *m/z* 268 $(M-H)^-$.

*4.1.5. General procedure for the preparation of cinnamic amide derivatives **4**, **6** – **10** and **14**.*

To a 25 mL round-bottom flask, a solution of compounds **43**, **45** – **49** and **53** (80 mg) in anhydrous methylene chloride (3 mL) was added under a nitrogen atmosphere. BBr_3 (4.0 equiv.) was added to the reaction mixture at -40 ^{0}C which was then stirred for 30 min. After completion of the reaction, the reaction mixture was neutralised by the addition of an equal amount of pyridine to the used BBr_3, and then an equal amount of methanol was added to the reaction mixture. The reaction mixture was evaporated under reduced pressure. The resultant residue was purified by column chromatography using dichloromethane and methanol (10 – 30:1) as the eluent. Cinnamic amides **4**, **6** – **10** and **14** were obtained as a white solid in yields of 63-85 %.

*4.1.5.1. (E)-N-Benzyl-3-(2,4-dihydroxyphenyl)acrylamide (**4**).* White solid, 75% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.87 (s, 1 H, OH), 9.64 (s, 1 H, OH), 8.39 (t, 1 H, J = 6.0 Hz, NH), 7.56 (d, 1 H, J = 15.5 Hz, 3-H), 7.32 – 7.20 (m, 6 H, Ph, 6′-H), 6.50 (d, 1 H, J = 15.5 Hz, 2-H), 6.32 (d, 1 H, J = 2.0 Hz, 3′-H), 6.24 (dd, 1 H, J = 8.5, 2.0 Hz, 5′-H), 4.35 (d, 2 H, J = 6.0 Hz, benzylic CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.8, 160.4, 158.4, 140.5, 135.8, 130.1, 128.9, 127.9, 127.4, 118.3, 114.0, 108.1, 103.2, 42.8; LRMAS (ESI-) *m/z* 268 $(M-H)^-$; HRMS (ESI-) *m/z* $C_{16}H_{14}NO_3$ $(M-H)^-$ calcd 268.0979, obsd 268.0991.

*4.1.5.2. (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-phenylacrylamide (**6**).* Yellowish white solid, 78% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.07 (s, 1 H), 9.22 (s, 1 H), 7.67 (d, 2 H, J = 7.5 Hz, 2″-H, 6″-H), 7.42 (d, 1 H, J = 16.0 Hz, 3-H), 7.31 (t, 2 H, J = 8.0 Hz, 3″-H, 5″-H), 7.04 – 7.01 (m, 3 H, 2′-H, 6′-H, 4″-H), 6.95 (d, 1 H, J = 7.5, Hz, 5′-H), 6.59 (d, 1 H, J = 16.0 Hz, 2-H), 3.79 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.5, 150.1, 147.4, 141.0, 140.1, 129.4, 128.2, 123.8, 121.3, 120.1, 119.7., 114.0, 112.7, 56.2; LRMAS (ESI-) *m/z* 268 $(M-H)^-$.

*4.1.5.3. (E)-3-(4-Hydroxyphenyl)-N-phenylacrylamide (**7**).* Yellowish white solid, 75% yield. ^{1}H NMR (500 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 7.48 (d, 2 H, J = 7.5 Hz, 2″-H, 6″-H), 7.46 (d, 1 H, J = 15.5 Hz, 3-H), 7.26 (d, 2 H, J = 8.0 Hz, 2′-H, 6′-H), 7.15 (t, 2 H, J = 7.5 Hz, 3″-H, 5″-H), 6.93 (t, 1 H, J = 7.5 Hz, 4″-H), 6.67 (d, 2 H, J = 8.0 Hz, 3′-H, 5′-H), 6.39 (d, 1 H, J = 15.5, 2-H); ^{13}C NMR (100 MHz, $CDCl_3$ + a few drops of CD_3OD) δ 165.9, 158.9, 141.9, 138.6, 129.7, 128.9, 126.5, 124.1, 120.1, 117.7, 115.8; LRMAS (ESI-) *m/z* 238 $(M-H)^-$.

*4.1.5.4. (E)-3-(3,4-Dihydroxyphenyl)-N-phenylacrylamide (**8**).* White solid, 70% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1 H, NH) 9.42 (brs, 1 H, OH), 9.17 (brs, 1 H, OH), 7.66 (d, 2 H, J = 7.5 Hz, 2″-H, 6″-H), 7.39 (d, 1 H, J = 15.5 Hz, 3-H), 7.29 (t, 2 H, J = 7.5 Hz,

3″-H, 5″-H), 7.02 (t, 1 H, *J* = 7.5 Hz, 4″-H), 6.99 (d, 1 H, *J* = 2.0 Hz, 2′-H), 6.89 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6′-H), 6.76 (d, 1 H, *J* = 8.0 Hz, 5′-H), 6.53 (d, 1 H, *J* = 15.5 Hz, 2-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.7, 148.4, 146.2, 141.4, 140.1, 129.4, 126.8, 123.7, 121.5, 119.7, 119.0, 116.4, 114.6; LRMAS (ESI-) *m/z* 254 $(M-H)^-$.

*4.1.5.5. (E)-3-(2,4-Dihydroxyphenyl)-N-phenylacrylamide (**9**).* White solid, 71% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.00 (s, 1 H), 9.96 (s, 1 H), 9.72 (s, 1 H), 7.67 (d, 2 H, *J* = 7.5 Hz, 2″-H, 6″-H) 7.65 (d, 1 H, *J* = 15.5 Hz, 3-H), 7.29 – 7.26 (m, 3 H, 6′-H, 3″-H, 5″-H) 7.00 (t, 1 H, *J* = 7.5 Hz, 4″-H), 6.67 (d, 1 H, *J* = 15.5 Hz, 2-H), 6.36 (d, 1 H, *J* = 2.0 Hz, 3′-H), 6.27 (dd, 1 H, *J* = 8.5, 2.0 Hz, 5′-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.5, 160.8, 158.8, 140.4, 137.3, 130.8, 129.3, 123.5, 119.7, 118.5, 113.9, 108.2, 103.3; LRMAS (ESI-) *m/z* 254 $(M-H)^-$; HRMS (ESI+) *m/z* $C_{15}H_{13}NO_3$ $(M+H)^+$ calcd 256.0968, obsd 256.0973.

*4.1.5.6. (E)-3-(4-Hydroxy-3-methoxyphenyl)-N-phenylacrylamide (**10**).* Yellowish white solid, 79% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1 H), 9.50 (s, 1 H), 7.67 (d, 2 H, *J* = 7.5 Hz, 2″-H, 6″-H), 7.47 (d, 1 H, *J* = 15.5 Hz, 3-H), 7.30 (t, 2 H, *J* = 7.5 Hz, 3″-H, 5″-H), 7.17 (d, 1 H, *J* = 2.0 Hz, 2′-H), 7.04 (dd, 1 H, *J* = 8.0, 2.0 Hz, 6″-H), 7.03 (t, 1 H, *J* = 7.5 Hz, 4″-H), 6.80 (d, 1 H, *J* = 8.0 Hz, 5″-H), 6.63 (d, 1 H, *J* = 15.5 Hz, 2-H), 3.81 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.6, 149.3, 148.5, 141.3, 140.2, 129.4, 126.8, 123.7, 122.6, 119.7, 119.5, 116.3, 111.4, 56.1; LRMAS (ESI-) *m/z* 268 $(M-H)^-$.

*4.1.5.7. (E)-3-(2,4-Dihydroxyphenyl)-N,N-diethylacrylamide (**14**).* Yellow solid, 85% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (brs, 1 H, OH), 9.65 (brs, 1 H, OH), 7.64 (d, 1 H, *J* = 15.5 Hz, 3-H), 7.40 (d, 1 H, *J* = 8.0 Hz, 6′-H), 6.83 (d, 1 H, *J* = 15.5 Hz, 2-H), 6.32 (d, 1 H, *J* = 2.5 Hz, 3′-H), 6.23 (dd, 1 H, *J* = 8.0, 2.5 Hz, 5′-H), 3.42 (brq, 2 H, *J* = 7.0 Hz, NCH_2), 3.32 (brq, 2 H, *J* = 7.0 Hz, NCH_2), 1.12 (brt, 3 H, *J* = 7.0 Hz, CH_2CH_3), 1.03 (brt, 3 H, *J* = 7.0 Hz,

CH_2CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.1, 160.5, 158.4, 137.7, 130.3, 114.4, 114.4, 108.0, 103.1, 42.1, 40.8, 15.8, 14.0; LRMAS (ESI-) *m/z* 234 $(M-H)^-$; HRMS (ESI-) *m/z* $C_{13}H_{16}NO_3$ $(M-H)^-$ calcd 234.1136, obsd 234.1139.

4.2. Biological studies

4.2.1. Mushroom tyrosinase assay

The mushroom tyrosinase inhibitory assay for the synthesized cinnamic amide derivatives **1** – **19** was carried out according to a previously reported method with slight modifications [59]. In brief, a 200 µL mixture containing 20 µL of tyrosinase solution, 10 µL of the test sample (final concentration: 25 µM), and 170 µL of substrate solution, comprising 14.7 mM potassium phosphate buffer and 293 µM L-tyrosine solution (1:1, v/v) was added to a 96-well microplate and incubated for 30 min at 37 ^{0}C. The absorbance of dopachrome produced during incubation was measured at 450 nm using a microplate reader (VersaMax^{TM}, Molecular Devices, Sunnyvale, CA, USA). Kojic acid (25 µM) and arbutin (400 µM) were used as positive controls. All experiments were triplicated. The following formula was used to calculate the tyrosinase inhibition:

%Inhibition = [1-(A/B) X 100)]

where A represents the absorbance of the test samples B the absorbance of the non-treated control.

4.2.2. Kinetic analysis of tyrosinase inhibition

An aqueous mushroom tyrosinase solution (200 units, 20 µl) and a test sample [10, 20, and 40 nM cinnamic amide, each 10 µl] were added to a 96-well plate containing an assay

mixture (170 µl) consisting of an aqueous solution of various concentration of L-tyrosine (0.03125 to 16 mM) substrate, 50 mM potassium phosphate buffer (pH 6.5) and a distilled water in a ratio of 10:10:9. The initial rate of DOPA chrome formation in the reaction mixture was examined by the increase in absorbance at 475 nm per min (ΔOD_{475}/min) read by a microplate reader. The Michaelis-Menten constant (K_M) and maximal velocity (V_{max}) of tyrosinase activity were determined by using Lineweaver-Burk plots with various concentrations of L-tyrosine. The plots show the inverse of reaction velocity (1/V) versus the inverse of substrate concentration (1/[S]). On the basis of the point of convergence of lines on the plot, an inhibitory mechanism could be determined.

*4.2.3. In silico docking simulation of cinnamic amide derivatives **4**, **5**, **9**, and **14** and tyrosinase*

The docking studies were carried out between tyrosinase and the four cinnamic amides or kojic acid according to the previously used method with slight modification [60,61]. In brief, the 3D structures of the four cinnamic amides or kojic acid were created on Chem3D Pro 12.0 software. AutoDock Vina 1.1.2, and Chimera softwares were used for the docking score calculation between cinnamic amides and tyrosinase enzyme. The 3D structure of tyrosinase (*Agaricus bisporus*) was obtained from Protein Data Bank (ID: 2Y9X). Pharmacophore models were created on a LigandScout 4.1.0, indicating possible interactions between ligands and the amino acid residues of tyrosinase.

4.2.4. Cell culture

B16F10 murine melanoma cells purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) were used for cell viability assays of the four cinnamic amides. Cells were cultured in Dulbecco's modified Eagle's medium containing 10%

fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL streptomycin obtained from Gibco/Thermo Fisher Scientific (Carlsbad, CA, USA) at 37 ºC and 5% CO_2. Further assay experiments for cell viability, melanin content and tyrosinase activity were done on these cells cultured in 96-well plates or 24-well dishes according to an experimental guideline. All experiments were performed in triplicate.

*4.2.5. Cell viability assay of cinnamic amide derivatives **4**, **5**, **9** and, **14** in B16F10 melanoma cells*

The WST-8 assay was used to determine the cell viability in a 96-well plate following the standard method [62]. Briefly, test cinnamic amides at four concentrations of 0, 5, 10, and 25 μM were added to the pre-cultured cells and further incubated for 24 h. After 24 h, WST-8 reagents (EZ-3000, Daeil Lab Service, Seoul, Korea) were added to the treated and control cells and incubated for a further 30 min to 2 hrs. The cell viability was assessed by measuring absorbances at 450 nm. All experiments were performed in triplicate.

*4.2.6. Tyrosinase inhibitory activity of cinnamic amide derivatives **4**, **5**, **9** and **14** in B16F10 melanoma cells*

The tyrosinase inhibitory activity of the four cinnamic amides in B16F10 melanoma cells was assayed using the standard protocols with slight modification [40]. B16F10 cells were treated with 1 μM α-MSH to activate the melanin production in the cells. Either the cinnamic amides at four concentrations of 0, 5, 10, and 25 μM or 25 μM kojic acid was added to the cells. After 24 h of incubation, the cells were washed with PBS 2-3 times, and then broken with 100 μL of lysis buffer, consisting of 50 mM PBS (90 μL, pH 6.8), 0.1 mM PMSF (5 μL), and 1% Triton X-100 (5 μL). The obtained lysis cells were frozen at -80 °C for 30 min, followed by centrifugation at 12,000 rpm for 30 min at 4 °C. Then 80 μL of the lysate supernatant was mixed with 20 μL of 10 mM L-dopa and incubated at 37 °C for 30 min in 96-

well plates. A microplate reader (Tecan, Männedorf, Switzerland) was used to measure the optical densities at 500 nm. Experiments were performed in triplicate.

*4.2.7. The effect of cinnamic amide derivatives **4**, **5**, **9** and **14** on melanin production in B16F10 melanoma cells*

Melanin content assay was performed to evaluate the inhibitory effect of the four test cinnamic amides on melanogenesis using the standard method with slight modification [63]. Briefly, cells were cultured in 24-well plates for 24 h and activated by 1 µM α-MSH to increase melanin production. Next, the cells were treated either with four concentrations of the test cinnamic amides (0, 5, 10, and 25 µM), or kojic acid (25 µM) and incubated for 24 h under the standard humidified conditions. The following day, the cells were washed with PBS buffer 2-3 times to remove the media content and incubated with 200 µL of 1N NaOH solution for dissolving the melanin content. The absorbance of the dissolved melanin was obtained using a microplate reader at 405 nm. All experiments were performed in triplicate.

*4.2.8. DPPH radical scavenging activity assay of cinnamic amide derivatives **1** – **19**.*

The DPPH radical scavenging activities of the test cinnamic amides **1** – **15** and the test dihydroquinolinones **16** – **19** were determined using a previously reported method with slight modification [64]. Briefly, in 96-well plates, 180 µL of a DPPH methanol solution (0.2 mM) and 20 µL of test cinnamic amides **1** – **15** or test dihydroquinolinones **16** – **19** (10 mM in DMSO) were mixed. L-Ascorbic acid was used as standard reference material. The 96-well plates were then incubated for 30 min in dark conditions. The absorbances were measured using a VersaMax^{TM} microplate reader at 517 nm. All experiments were performed in triplicate.

The DPPH radical scavenging activities of **1** – **19** were calculated using the following formula:

$$\text{Scavenging activity (\%)} = \left[\frac{(\text{Ac} - \text{As})}{\text{Ac}}\right] \times 100$$

where Ac is the absorbance of the non-treated control and As is the test sample absorbance.

4.2.9. Statistical analysis

The statistical analyses were performed using Graph Pad Prism 5 software (La Jolla, CA, USA). Calculated results are presented as means ± SEMs. Intergroup significance differences were determined by using one-way ANOVA and Tukey's test. Statistical significance was considered at < 0.05 of two-sided *P*-values.

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Highlights

- Fifteen **cinnamic amide** derivatives were designed and synthesized via Horner-Wadsworth-Emmons reaction.
- Their anti-melanogenic effect were evaluated in mushroom tyrosinase and α-MSH-stimulated B16F10 melanoma cells.
- Compound **4**, **5**, **9** and **14** revealed excellent anti-melanogenic effect as compared to kojic acid, used as positive control.
- The docking simulation study expressed that cinnamic amides, **4**, **5**, **9** and **14** bind to the active site of tyrosinase with stronger binding affinity than kojic acid.