



SYNTHESIS AND ANTI-TYROSINASE ACTIVITY EVALUATION OF FLUORINATED CHALCONE AND PHENYL BENZYL ETHER DERIVATIVES

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ABSTRACT

Pigmentation is a common skin disorder caused by several factors including UV exposure, skin aging, hormonal imbalance and skin irritation. Ascorbic acid, alpha-arbutin, tranexamic acid, kojic acid, and niacinamide have all been used in skin lightening treatments. This study aimed to develop a new active compound for skin lightening. Ten chalcone and nine phenyl benzyl ether analogues of bibenzyl compounds and bifluranol (9) were synthesized using aldol condensation reaction and Williamson ether synthesis, respectively. Their activity in vitro against tyrosinase, the rate-determining enzyme in melanogenesis, was evaluated at 500 μ M. The results revealed that the chalcone analogues were more active than the phenyl benzyl ethers. The *p*-fluorinated analogues for both chalcone 12d (*p*-F) and ether 15d (*p*-F) inhibited tyrosinase in vitro to the highest extent for each series with inhibitory percentages of 38 and 32 at 500 μ M respectively. These findings might provide useful guidance for further structural development of the more potent tyrosinase inhibitors used in whitening agents.

Keywords: anti-tyrosinase activity, bibenzyl compounds, chalcone, phenyl benzyl ether, whitening agent

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Introduction

Melanogenesis is a complex biological pathway for production of natural polymeric pigments, melanin (eumelanin and pheomelanin), occurring in melanocytes under the animal skin. Melanogenesis plays an important role in pigmentation of skin, hair and eyes. It is stimulated by ultraviolet radiation in order to protect cell and tissue damages from the sun and photocarcinogenesis.¹ Abnormal regulation of melanin production can cause many kinds of skin disorders, such as melasma, esthetics, freckles, senile lentigines, actinic damages, including acanthosis nigricans, poikiloderma, neurodegeneration and skin cancer risk.^{2,3}

The melanin synthesis is catalyzed by a dinuclear copper-containing metalloenzyme called tyrosinase, which is a rate-limiting enzyme.⁴ Furthermore, tyrosinase is responsible for enzymatic browning in vegetables, fruits and fungi.⁵ The process includes oxidation of L-tyrosine to L-Dopa and o-dopaquinone, followed by polymerization to deliver melanin.⁶ Suppression of melanogenesis by tyrosinase inhibition is a key approach to reduce or stop melanin production and browning process resulting in therapy of the hyperpigmentation disorders and prevention of browning in plants.⁷ Thus, effective tyrosinase inhibitors can be developed for whitening agents, medicines and anti-browning agents necessary in cosmetic⁸, pharmaceutical and food industries.⁹

Hence, many organic and medicinal chemists are seeking for potent anti-tyrosinase agents from natural sources, semi-synthetic and synthetic compounds and analogues.^{7,10} Hydroquinone (**1**) was a standard agent for treatment of hyperpigmentation disorders, however, it damages melanosomes and melanocytes permanently. Hydroquinone (**1**) is, therefore, prohibited in cosmetics in many countries.¹¹ Alternatively, kojic acid (**2**) and arbutin (**3**) are used as whitening agents in cosmetics. They are derived from fungal

metabolite and plants, and less toxic than hydroquinone. Unfortunately, their usages are limited due to adverse effects, instability under light and O₂ atmosphere and low skin penetration.¹² Anti-tyrosinase activity of natural and synthetic bibenzyl compounds (**4**) were found to be an effective structure with the potency up to almost 20-fold superior to that of kojic acid.¹³ Various chalcones including chalcone **5** were also identified as tyrosinase inhibitors with IC₅₀ up to 0.17 µM.¹⁰ The results made chalcone structures attractive for further development. 3,5-Dihydroxyphenyl benzyl ether (**6**) showed inhibitory activity towards tyrosinase better than arbutin.¹⁴ Interestingly, thioether **7** exhibited 80-fold higher anti-tyrosinase activity than kojic acid.¹⁰ Moreover, low toxicity and potent anti-tyrosinase activity of bis(4-hydroxybenzyl)sulfide (**7**) were proved in zebrafish model.¹⁵ Recently, it was demonstrated that simple *p*-chlorophenyl benzyl ether (**8**) possessed ca. 2-fold higher activity against mushroom tyrosinase, compared to kojic acid.¹⁴ Furthermore, it was reported that ether **8** showed similar inhibitory activity in cell-based assay, but less toxic than kojic acid.¹⁶ The structures of above mentioned tyrosinase inhibitors are depicted in **Figure 1**.

Herein, we aim to improve the inhibitory potency towards tyrosinase of chalcone and phenyl benzyl ether by introduction of fluorine(s) to the structures, analogous to the structure of bioactive bifluranol (**9**) and bibenzyl compounds (**4**) found in *Dendrobium* orchids.^{17,18} Due to the specific physicochemical properties of fluorine, especially its strong electronegativity and compact size, the presence of fluorine atom(s) may have a great influence on the molecular recognition and affinity for the active site of the enzyme.¹⁹ Moreover, there were several studies that reported superior inhibitory activity towards tyrosinase of fluorinated compounds, such as fluorinated piperidine derivatives (IC₅₀ up to 5.11 µM),¹⁰ fluorinated thiourea derivatives (IC₅₀ up to 6.13 µM),¹⁰

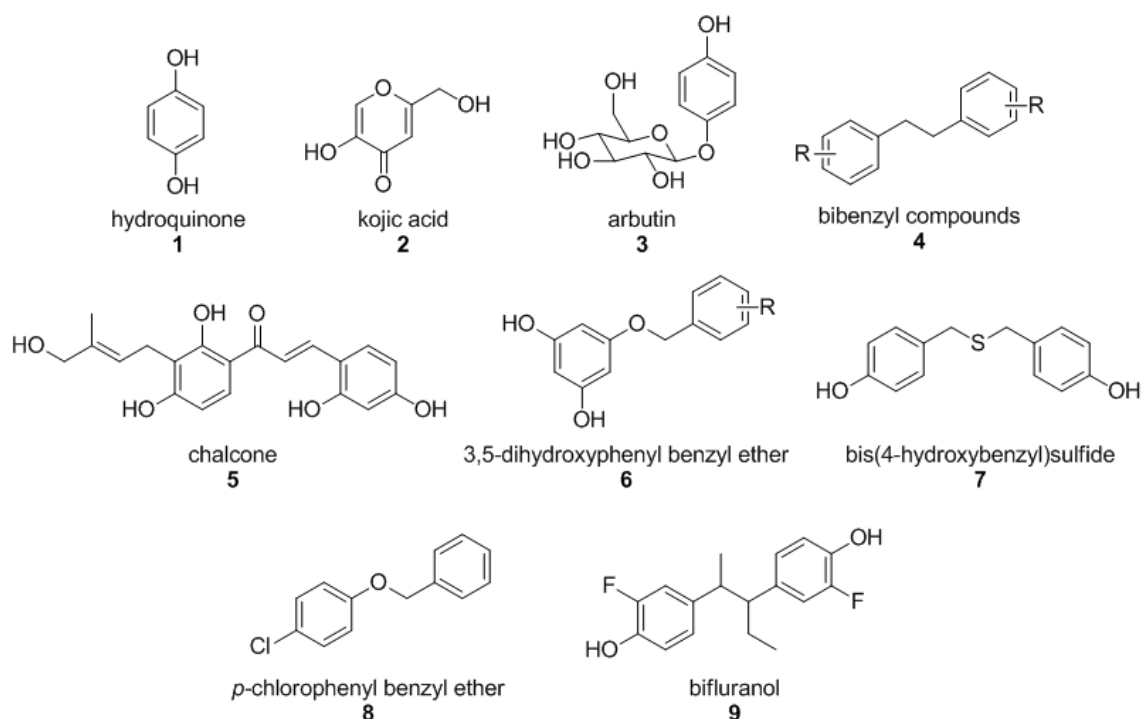


Figure 1 The chemical structures of some potent tyrosinase inhibitors; hydroquinone (1), kojic acid (2), arbutin (3), bibenzyl compounds (4), chalcone (5), 3,5-dihydroxyphenyl benzyl ether (6), bis(4-hydroxybenzyl)sulfide (7), *p*-chlorophenyl benzyl ether (8) and bifluranol (9).

fluorinated thiadiazole (IC_{50} up to 0.77 μM)¹⁰ and fluorinated chalcone derivatives (IC_{50} up to 0.13 μM).²⁰ Hence, various fluorinated chalcones and phenyl benzyl ethers were synthesized, and their anti-tyrosinase activity was investigated *in vitro* in this research. Moreover, the structural feature necessary for the enzyme inhibition was reported here.

Materials and methods

Materials

All chemicals and reagents used in this work were purchased from Tokyo Chemical Industry (TCI), Sigma-Aldrich and Arcos Organics. They were analytical grade and used as received without purification. 3,4-Dihydroxyphenylalanine (L-dopa), kojic acid and mushroom tyrosinase were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water used

in the experiments was deionized. The chromatographic separations were performed on silica gel 63-200 μm purchased from Merck. All reactions and column chromatographic separations were monitored by TLC analysis using precoated silica gel 60 TLC sheets with fluorescent indicator (254 nm) from Merck and visualized with a UV lamp (254 and 365 nm).

Instrumentation

Melting points were measured on a Büchi Melting Point B-545 apparatus. All products were characterized by nuclear magnetic resonance spectroscopy (NMR). 1H NMR (300 MHz), ^{13}C NMR (75 MHz) and ^{19}F NMR (282 MHz) spectra were recorded on a Bruker AVANCE 300 using $CDCl_3$ on 5 mm diameter tubes. The chemical shifts in ppm were quoted relative to either internal standard TMS (δ_H

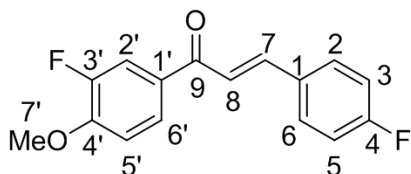
0.00 ppm) or CF_3COOH (δ_{F} -76.55 ppm) or residual signals of CDCl_3 (δ_{H} 7.26 ppm, or δ_{C} 77.22 ppm). The signals were reported as follows: chemical shifts, multiplicity, coupling constant. The multiplicities were given as s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet, br = broad. The coupling constants (J) were recorded in Hertz (Hz).

Synthesis of fluorinated chalcones 12a-12j

General procedure A for the syntheses of fluorinated chalcone derivatives 12a-12j

A stirred mixture of 3-fluoro-4-methoxyacetophenone (**10**) (1.00 eq.) and various substituted aldehyde **11a-11j** (1.00 eq.) in methanol (ca. 0.3 M) was treated with 3 M aq. NaOH (1.10 eq.) at room temperature. After stirring at room temperature overnight, the mixture was filtered and the obtained precipitate was washed excessively with cold methanol to deliver the target chalcone derivatives 12a-12j as a solid.

Synthesis of 3-fluoro-4-methoxy-4-fluorochalcone (12d)



The synthesis of 3-fluoro-4-methoxy-4-fluorochalcone (**12d**) was performed successfully using the *general procedure A*. 3-Fluoro-4-methoxyacetophenone (**10**) (103.5 mg, 0.615 mmol, 1.00 eq), 4-fluorobenzaldehyde (**11d**) (76.4 mg, 0.616 mmol, 1.00 eq), MeOH (2.00 mL) and 3M NaOH (3 mL, 0.70 mmol, 1.10 eq.) were used. The product was obtained as a white solid (145.3 mg, 0.530 mmol, 86% yield). Mp. = 136-137 °C; ^1H NMR (300 MHz, CDCl_3) δ (ppm): 7.84 (ddd, J = 8.6, 2.1, 1.1 Hz, 1H, H-6'), 7.79 (dd, J = 11.7, 2.1 Hz, 1H, H-2'), 7.78 (d, J = 15.8 Hz, 1H, H-8), 7.64 (dd, J = 14.0, 8.6 Hz, 2H, H-3 and H-5), 7.42 (d, J = 15.6 Hz, 1H, H-7), 7.12 (t, J = 8.6 Hz, 2H, H-2 and H-6),

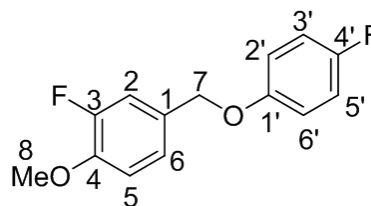
7.04 (t, J = 8.3 Hz, 1H, H-5'), 3.98 (s, 3H, H-7'); ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 187.8 (d, J = 1.50 Hz, C-9), 164.1 (d, J = 249.8 Hz, C-4), 152.1 (d, J = 246.0 Hz, C-3'), 151.9 (d, J = 11.3 Hz, C-4'), 143.6 (C-8), 131.1 (d, J = 10.3 Hz, C-1'), 131.1 (d, J = 1.8 Hz, C-1), 130.4 (d, J = 8.6 Hz, 2C, C-3 and C-5), 125.8 (d, J = 3.3 Hz, C-6'), 120.8 (d, J = 2.2 Hz, C-7), 116.2 (d, J = 18.8 Hz, C-2'), 116.2 (d, J = 21.8 Hz, 2C, C-2 and C-6), 112.4 (d, J = 1.50 Hz, C-5'), 56.3 (C-7'); ^{19}F NMR (282 MHz, CDCl_3) δ (ppm): -109.8 (s, 1F, F-4), -134.8 (s, 1F, F-3').

Synthesis of Phenyl Benzyl Ether 15a-15i

General Procedure B for syntheses of fluorinated phenyl benzyl ether derivatives 15a-15i

A mixture of 3-fluoro-4-methoxybenzyl chloride (**13**) (1.00 eq.), various substituted phenol **14a-14i** (1.00 eq.) and Cs_2CO_3 (0.70 eq.) in acetonitrile (MeCN) (3.0 mL) was stirred at 80 °C for 3 h. After this time, the reaction mixture was diluted with H_2O (5 mL) and extracted with EtOAc (3 x 30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 3% EtOAc in hexane) to obtain the target phenyl benzyl ether derivatives 15a-15i.

Synthesis of 2-fluoro-4-(4-fluorophenoxy)methyl-1-methoxybenzene (15d)



The synthesis of 2-fluoro-4-(4-fluorophenoxy)methyl-1-methoxybenzene (**15d**) was performed successfully using the *general procedure B*. 3-Fluoro-4-methoxybenzyl chloride (**13**) (101.1 mg, 0.579 mmol, 1.00 eq), 4-fluorophenol (**14d**) (64.9 mg, 0.579 mmol, 1.00 eq) and Cs_2CO_3 (132.1 mg, 0.405 mmol, 0.70 eq) were used. The desired product was obtained as a

white solid (101.0 mg, 0.404 mmol, 70%). Mp. = 63-64 °C; ^1H NMR (300 MHz, CDCl_3) δ (ppm): 7.15 (dd, J = 11.9, 2.0 Hz, 1H, H-2), 7.13-7.08 (m, 1H, H-6), 6.96 (dd, J = 9.3, 8.1 Hz, 2H, H-2' and H-6'), 6.97-6.91 (m, 1H, H-5), 6.87 (dd, J = 13.8, 9.3 Hz, 2H, H-3' and H-5'), 4.92 (s, 2H, H-7), 3.88 (s, 3H, H-8); ^{13}C NMR (75 MHz, CDCl_3) δ (ppm): 157.4 (d, J = 237.1 Hz, C-4'), 154.7 (d, J = 2.0 Hz, C-1'), 152.4 (d, J = 244.7 Hz, C-3), 147.5 (d, J = 10.6 Hz, C-4), 129.8 (d, J = 6.1 Hz, C-1), 123.5 (d, J = 3.5 Hz, C-6), 116.0 (d, J = 3.9 Hz, 2C, C-2' and C-6'), 115.8 (d, J = 11.0 Hz, 2C, C-3' and C-5'), 115.6 (d, J = 18.7 Hz, C-2), 113.3 (d, J = 2.1 Hz, C-5), 69.8 (d, J = 1.2 Hz, C-7), 56.3 (C-8); ^{19}F NMR (282 MHz, CDCl_3) δ (ppm): -124.2 (s, 1F, F-4'), -135.5 (s, 1F, F-3).

In vitro anti-tyrosinase activity assay

Tyrosinase activity inhibition was determined by the method as described previously by measuring the DOPACHrome formed due to the action of tyrosinase enzyme on DOPA substrates.²¹ In brief, the 96-well plate was set up in the following order; 120 μL of 50 mM phosphate buffer (pH 6.8), 20 μL of sample and 20 μL of 400 units/mL mushroom tyrosinase in 50 mM phosphate buffer (pH 6.8). After incubation at 25 °C for 10 min, reaction was initiated by adding 40 μL of 3.0 mM

DOPA substrate solutions to each well and incubated further at 25 °C for 10 min. The enzyme activity was determined by measuring the absorbance at 492 nm using microplate reader spectrophotometer. Kojic acid was used as a positive control. Negative control without extracts was set up in parallel. The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = 1 - \frac{(C - D)}{(A - B)} \times 100$$

Where A is the absorbance of the control with the enzyme, B is the absorbance of the control without the enzyme, C is the absorbance of the test sample with the enzyme and D is the absorbance of the test sample without the enzyme.

Results and Discussion

Chemistry

Ten chalcone analogues were synthesized in one step via aldol condensation between 3-fluoro-4-methoxyacetophenone (**10**) and various substituted aldehydes **11a-11j** under basic conditions to deliver the target chalcones **12a-12j** with yields of 69-94% as illustrated in Figure 2.

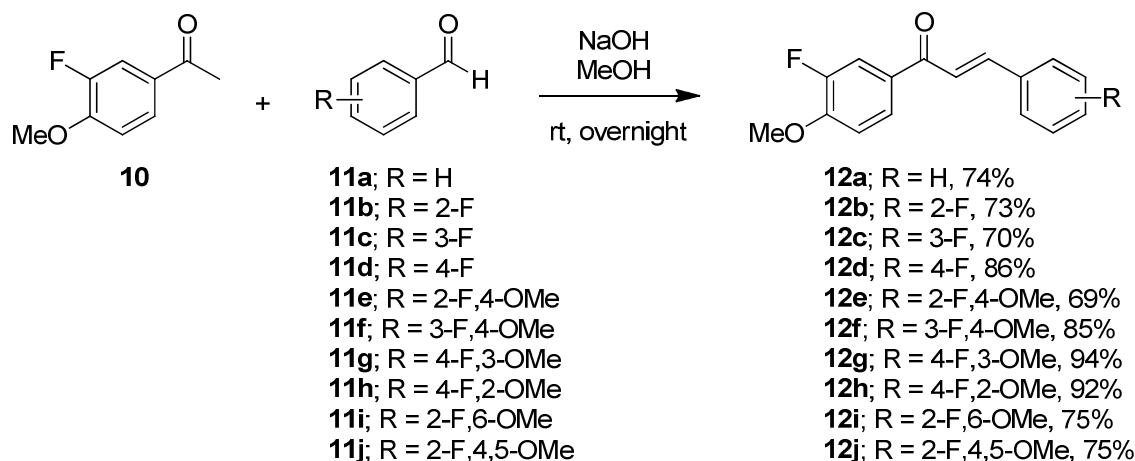


Figure 2 Syntheses of various fluorinated chalcones **12a-12j** via aldol condensation using 3-fluoro-4-methoxyacetophenone (**10**) and various substituted aldehydes **11a-11j** with yields.

For phenyl benzyl ethers **15a-15i**, 3-fluoro-4-methoxybenzyl chloride (**13**) reacted with various substituted phenols **14a-14i** in the presence of Cs_2CO_3 to generate the desired phenyl benzyl ethers **15a-15i** with yields of 63 to quantitative yield as depicted in **Figure 3**. The structures of all target synthetic compounds were confirmed by nuclear magnetic resonance (NMR).

Structure and *in vitro* anti-tyrosinase activity relationship

The structures of synthetic chalcone and phenyl benzyl ether analogues were derived from bioactive bibenzyl compounds (**4**) and bifluranol (**9**). They contained a 3-fluoro-4-methoxyphenyl and 3-fluoro-4-methoxybenzyl fragments for chalcones and ethers, respectively, whereas another phenyl or phenoxy rings were substituted with a fluorine atom and/or methoxy group(s) in order to study the effects of the substituent(s) on their anti-tyrosinase activity. All 19 synthesized inhibitors were investigated on their *in vitro* anti-tyrosinase activity. The results are

expressed as inhibition percentage at 500 μM as shown in **Table 1**.

Different substituent position on the phenyl ring on chalcones and phenoxy ring of phenyl benzyl ethers resulted significant difference in anti-tyrosinase activity. For chalcone derivatives, *p*-fluorophenyl analogue **12d** showed 38.3% inhibition, which was greater than having no substituent (**12a**) or *o*-fluorophenyl (**12b**) and *m*-fluorophenyl ones (**12c**). Adding a fluorine atom at the *para*-position on the phenyl ring of chalcone derivatives could improve anti-tyrosinase activity ca. 4-fold compared to **12a**, whereas the addition at *ortho*- and *meta*-position delivered lower activity at the same concentration. For phenyl benzyl ether derivatives, *m*- **15c** and *p*-fluorophenyl benzyl ether **15d** exhibited 18.5% and 32.1% inhibition respectively, which were greater than the unsubstituted compound **15a**. The results revealed that fluorination at *para*-position was crucial to enhance the anti-tyrosinase activity for both chalcone and phenyl benzyl ether derivatives.

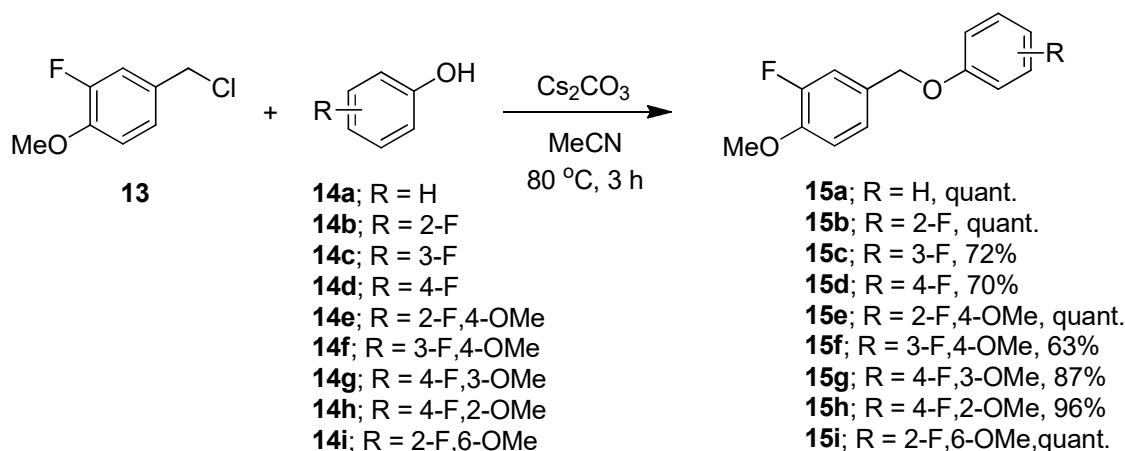
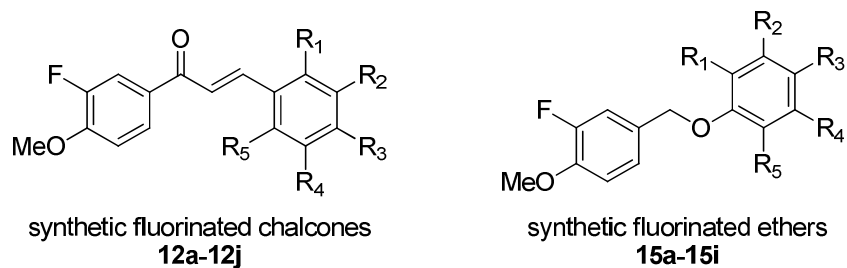


Figure 3 Syntheses of various fluorinated phenyl benzyl ethers **15a-15i** via Williamson ether synthesis starting from 3-fluoro-4-methoxybenzyl chloride (**13**) and various substituted phenols **14a-14i** with yields.

Table 1 The chemical structures of the synthetic fluorinated chalcones **12a-12j** and phenyl benzyl ethers **15a-15i** and their inhibition percentage against mushroom tyrosinase at the concentration of 500 μ M.



Structure	Compound	Substituent(s)					Inhibition percentage at 500 μ M
		R ₁	R ₂	R ₃	R ₄	R ₅	
Chalcone derivatives	12a	H	H	H	H	H	9.63 $\pm$ 0.38
	12b	F	H	H	H	H	3.16 $\pm$ 0.62
	12c	H	F	H	H	H	0.98 $\pm$ 0.38
	12d	H	H	F	H	H	38.3 $\pm$ 0.10
	12e	F	H	OCH ₃	H	H	8.25 $\pm$ 0.27
	12f	H	F	OCH ₃	H	H	26.0 $\pm$ 0.56
	12g	H	OCH ₃	F	H	H	5.68 $\pm$ 0.37
	12h	OCH ₃	H	F	H	H	22.8 $\pm$ 0.71
	12i	F	H	H	H	OCH ₃	21.1 $\pm$ 0.56
	12j	F	H	OCH ₃	OCH ₃	H	20.3 $\pm$ 0.54
Phenyl benzyl ether derivatives	15a	H	H	H	H	H	1.01 $\pm$ 0.82
	15b	F	H	H	H	H	0.69 $\pm$ 0.29
	15c	H	F	H	H	H	18.5 $\pm$ 0.11
	15d	H	H	F	H	H	32.1 $\pm$ 0.29
	15e	F	H	OCH ₃	H	H	5.51 $\pm$ 0.33
	15f	H	F	OCH ₃	H	H	15.5 $\pm$ 0.77
	15g	H	OCH ₃	F	H	H	10.5 $\pm$ 0.29
	15h	OCH ₃	H	F	H	H	1.27 $\pm$ 0.77
	15i	F	H	H	H	OCH ₃	1.58 $\pm$ 0.55
Kojic acid ⁽¹⁴⁾	2						78.0 $\pm$ 0.25

The effect of multi-substitution on the phenyl ring was also investigated. Attachment of methoxy group(s) on *o*-fluorophenyl (**12e**, **12i**, **12j**) and *m*-fluorophenyl (**12f**) chalcone derivatives showed higher % inhibition compared to those without this

substitution at these positions (**12b**, **12c**). On the other hand, % inhibition was decreased when methoxy group was added on *p*-fluorophenyl ring of chalcone (**12g**, **12h**). For phenyl benzyl derivatives, the addition of methoxy group on *o*-fluorophenoxy

compounds (**15e**, **15i**) increased the potency of anti-tyrosinase activity compared to having no methoxy substitution (**15b**), but the addition on *m*- and *p*-fluorophenoxy derivatives (**15f**, **15g**, **15h**) decreased the activity. Thus, addition of methoxy group could enhance the inhibitory activity in some cases.

Identical modifications on different core structure showed significantly different inhibitory activity. In most cases, chalcone derivatives exhibited higher % inhibition compared to phenyl benzyl ether derivatives, except in the case of *m*-fluorophenyl and 4-fluoro-3-methoxy compounds. For both classes of compounds, addition of one fluorine atom at R₃-position helped to improve the activity significantly, superior to substitution at R₁- and R₂-positions. Multi-substituted systems consisting of fluorine atom and methoxy groups exhibited higher inhibitory percentage compared to unsubstituted one, nevertheless they were still less active than *p*-fluoro version and the positive control, kojic acid. Although we could identify *p*-fluorinated chalcone **12d** and ether **15d** as the potent tyrosinase inhibitors, they inhibited tyrosinase *in vitro* with ca. 2-fold less efficiently than kojic acid. However, our experimental results revealed that the addition of only one fluorine atom at the *para*-position on the phenyl and phenoxy part of both series led to higher efficiency of anti-tyrosinase activity. This could be the result from the strong electronegativity property and appropriate size of one single fluorine atom which provided high affinity for the active site of the enzyme.¹⁹ In addition, this result agreed with our previous studies that the *p*-substituted analogues, especially *p*-chlorophenyl benzyl ether, showed significantly higher inhibitory potency compared to the corresponding analogues substituted at *ortho*- and *meta*-position.¹⁴ However, the interaction of the analogues on the active binding site of tyrosinase remained unknown and would be investigated in the future work. The evidences from this work may be a useful guidance for further development of novel tyrosinase inhibitors. Moreover, 3-fluoro-4-

methoxyphenyl fragment of both classes might not be an appropriate structure for anti-tyrosinase agents. For further structural development, it could be replaced with other substituted aromatic parts for the better activity.

Conclusion

Ten fluorinated chalcones and nine fluorinated phenyl benzyl ethers, which were analogues of bibenzyl compounds found in *Dendrobium* orchids, were prepared successfully with good to excellent yields. The assessment of anti-tyrosinase activity revealed that the synthetic chalcones showed superior inhibitory activity than the synthetic ethers, although the difference of both was only the linker between two identical substituted benzene rings. Among the derivatives, monofluorination at *para*-position on the phenyl ring of chalcone provided the most effective inhibitors. The findings from this study suggested that the type, amount, and position of substituents on different core structure are the important keys for further structural development of the more potent tyrosinase inhibitors for whitening agents.

Acknowledgments

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References

1. Lee CH, Wu SB, Hong CH, Yu HS, Wei YH. Molecular mechanisms of UV-induced apoptosis and its effects on skin residential cells: the implication in UV-based phototherapy. *Int J Mol Sci.* 2013;14:6414-35.
2. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol.* 2008;84:539-49.
3. Dogra S, Sarangal R. Pigmentary disorders: an insight. *Pigment Int.* 2014;1(1):5-7.

4. Park HY, Kosmadaki M, Yaar M, Gilchrist BA. Cellular mechanisms regulating human melanogenesis. *Cell Mol Life Sci.* 2009;66(9):1493-506.
5. Kilimnik A, Dembitsky VM. Anti-melanoma agents derived from fungal species. *Mathews J Pharm Sci.* 2016;1(1):002.
6. Videira IF, Moura DF, Magina S. Mechanisms regulating melanogenesis. *An Bras Dermatol.* 2013;88(1):76-83.
7. Lee SY, Baek N, Nam TG. Natural, semisynthetic and synthetic tyrosinase inhibitors. *J Enzyme Inhib Med Chem.* 2016;31(1):1-13.
8. Subongkot T. Anti-dark circle eye and anti-bag eye active ingredients in cosmetic products. *Thai Bull Pharm Sci.* 2017;12(1):63-76. (in Thai)
9. Halaoui S, Asther M, Kruus K, Guo L, Hamdi M, Sigoillot JC, et al. Characterization of a new tyrosinase from *Pycnoporus* species with high potential for food technological applications. *J Appl Microbiol.* 2005;98:332-43.
10. Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. *J Enzyme Inhib Med Chem.* 2019;34(1):279-309.
11. Wester RC, Melendres J, Hui X, Cox R, Serranzana S, Zhai H, et al. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. *J Toxicol Environ Health A.* 1998;54(4):301-17.
12. Mohammad NM, Yamauchi K, Mitsunaga T. Tyrosinase inhibitors from natural and synthetic sources as skin-lightening agents. *Rev Agric Sci.* 2019;7:41-58.
13. Tajima R, Oozeki H, Muraoka S, Tanaka S, Motegi Y, Nihei H, et al. Synthesis and evaluation of bibenzyl glycosides as potent tyrosinase inhibitors. *Eur J Med Chem.* 2011;46:1374-81.
14. Suttisintong K, Palakhachane S, Athipornchai A, Pimtong W, Limpachayaporn P. Synthesis and evaluation of anti-tyrosinase activity of phenyl benzyl ether derivatives: effects of functional groups and their positions. *SEHS.* 2018;12(2):111-23.
15. Chen WC, Tseng TS, Hsiao NW, Lin YL, Wen ZH, Tsai CC, et al. Discovery of highly potent tyrosinase inhibitor, T1, with significant anti-melanogenesis ability by zebrafish *in vivo* assay and computational molecular modeling. *Sci Rep.* 2015;5:7995.
16. Riam-Amatakun W, Limpachayaporn P, Pizon JRL, Opanasopit P, Nuntharatanapon N. Anti-melanogenic activity of p-chlorophenyl benzyl ether in α -MSH-induced mouse melanoma B16F10 cells. *Key Eng Mater.* 2019;819:118-23.
17. Gong YQ, Fan Y, Wu DZ, Yang H, Hu ZB, Wang ZT. *In vivo* and *in vitro* evaluation of erianin, a novel anti-angiogenic agent. *Eur J Cancer.* 2004;40:1554-65.
18. Zhang X, Xu JK, Wang J, Wang NL, Kurihara H, Kitanaka S, et al. Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*. *J Nat Prod.* 2007;70:24-8.
19. Bégué JP, Bonnet-Delpon D. Bioorganic and medicinal chemistry of fluorine. Hoboken (NJ): John Wiley & Sons; 2008.
20. Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *J Enzyme Inhib Med Chem.* 2017;32(1):403-25.
21. Chompoo J, Upadhyay A, Fukuta M, Tawata S. Effect of *Alpinia zerumbet* components on antioxidant and skin diseases-related enzymes. *BMC Complement Altern Med.* 2012;12:106.