



รายงานวิจัยฉบับสมบูรณ์

โครงการ

การค้นคว้าและพัฒนาสาร propargyl glycosides
ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว

Discovery and develop propargyl glycoside
as a new class of skin-whitening agents

รุ่งนภา แซ่เอ็งและคณะ

โครงการวิจัยประเภทงบประมาณเงินรายได้
จากเงินอุดหนุนรัฐบาล (งบประมาณแผ่นดิน)
ประจำปีงบประมาณ 2559
มหาวิทยาลัยบูรพา

รายงานวิจัยฉบับสมบูรณ์

โครงการ

การค้นคว้าและพัฒนาสาร propargyl glycosides
ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว

Discovery and develop propargyl glycoside
as a new class of skin-whitening agents

รุ่งนภา แซ่เอ็งและคณะ
ภาควิชาเคมี คณะวิทยาศาสตร์
มหาวิทยาลัยบูรพา

31 สิงหาคม 2560

คำนำ

โครงการวิจัย “การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว” ได้รับการสนับสนุนทุนการวิจัยงบประมาณแผ่นดินประจำปีงบประมาณ 2559 มหาวิทยาลัยบูรพา รายงานการวิจัยฉบับนี้เสนอรายละเอียดของการวิจัยซึ่งประกอบด้วยบทนำที่เสนอผลงานวิจัยที่เกี่ยวข้อง ผลการทดลองวิจัย การอภิปรายสรุปผล และการตรวจสอบโครงสร้างของสาร

การวิจัย “การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว” สำเร็จลุล่วงไปด้วยดี โดยผู้วิจัยต้องขอขอบคุณทีมวิจัยซึ่งประกอบด้วยที่ปรึกษาโครงการ ศ.ดร. อภิชาติ สุขสำราญ คณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง ผู้ร่วมโครงการ ดร. อนันต์ อธิพรชัย ดร. อุทัยวรรณ ศิริอ่อน รวมทั้งนิสิตปริญญาโทและเอกภาควิชาเคมี นางสาวณัฐิยา แซ่หลิม และนางสาวอรอนงค์ ศิริปัฐ งานวิจัยนี้ได้รับการสนับสนุนจากภาควิชาเคมี คณะวิทยาศาสตร์ และศูนย์นวัตกรรมความเป็นเลิศทางเคมี PERCH-CIC

ผศ. ดร. รุ่งนภา แซ่เอ็ง
อาจารย์ประจำภาควิชาเคมี คณะวิทยาศาสตร์
หัวหน้าโครงการวิจัย

กิตติกรรมประกาศ

งานวิจัยนี้ได้รับทุนสนับสนุนการวิจัยจากงบประมาณเงินรายได้จากเงินอุดหนุนรัฐบาล
(งบประมาณแผ่นดิน) ประจำปีงบประมาณ พ.ศ. 2559 มหาวิทยาลัยบูรพา
ผ่านสำนักงานคณะกรรมการการวิจัยแห่งชาติ เลขที่สัญญา 63/2559

Acknowledgment

This work was financially supported by the Research Grant of Burapha University
through National Research Council of Thailand (Grant no. 63/2559).

บทคัดย่อ

งานวิจัยนี้ได้ทำการวางแผนและสังเคราะห์ชุดของสาร alkynyl และ propargyl-D-glycosides ชนิดใหม่ จำนวนเก้ากลุ่ม และตรวจสอบฤทธิ์การยับยั้งเอนไซม์ tyrosinase โดยมีวัตถุประสงค์เพื่อพัฒนาสาร alkynyl และ propargyl-D-glycosides ให้เป็นสารไวท์เทนนิ่งชนิดใหม่เพื่อให้ผิวขาว สารสังเคราะห์อนุพันธ์ alkynyl และ propargyl glycoside จำนวน 42 ชนิดได้ถูกเตรียมขึ้นและศึกษาฤทธิ์การยับยั้งเอนไซม์ tyrosinase เพื่อความเข้าใจถึงความสัมพันธ์ของโครงสร้างที่มีผลต่อฤทธิ์ จากผลการศึกษาเบื้องต้นของสารสังเคราะห์ต่อฤทธิ์การยับยั้งเอนไซม์ tyrosinase โดยใช้สาร L-tyrosine เป็นซับสเตรทเปรียบเทียบกับ alpha-Arbutin และ Kojic acid พบว่า หมู่แทนที่อัลไคน์บน C-1 และ หมู่แทนที่บน C-6 มีผลต่อฤทธิ์ จากสารสังเคราะห์อนุพันธ์ alkynyl glycoside ทั้งหมดพบว่าสาร **6d** (%tyrosinase inhibition = 99.46) แสดงฤทธิ์ดีกว่าสารอื่นและดีใกล้เคียงกับ kojic acid (%tyrosinase inhibition = 98.24) การค้นพบนี้อาจนำไปสู่สารสารไวท์เทนนิ่งเพื่อผิวขาวชนิดใหม่

Abstract

A series of novel alkynyl and propargyl-D-glycosides bearing benzyl and acetyl groups were designed, synthesized in nine groups and evaluated as a new class of mushroom tyrosinase inhibitors. The purpose of this investigation was to investigate the inhibitory effects on mushroom tyrosinase of synthetic alkynyl and propargyl-D-glycosides, with the aim of developing novel skin whitening agents. 42 synthetic alkynyl and propargyl glycoside analogues were prepared and study for the tyrosinase inhibitory activity to understand the structure activity relationship. The preliminary screening results of our synthetic compounds on mushroom tyrosinase activity with L-tyrosine as a substrate compared with alpha-Arbutin and Kojic acid were studied. The results indicated that C-1 substituted alkyne and C-6 substituted moiety contributed to the inhibitory effects. Of all synthetic alkynyl glycoside analogs, compounds **6d** (%tyrosinase inhibition = 99.46) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (%tyrosinase inhibition = 98.24). These findings may lead to the discovery of new agent for skin-whitening.

สารบัญ

	หน้า
คำนำ	1
Abstract	2
Chapter 1 Introduction and Literature reviews	3
Chapter 2 Results and Discussions	19
Chapter 3 Conclusion	30
References	31
Output/Outcome	33

Chapter 1 Introduction and Literature reviews

Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional coppercontaining enzyme widely distributed in plants and animals. This enzyme catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They also find uses in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms (Shiino et al, 2001). An effective tyrosinase inhibitor should optimally be both safe and potent. For maximal safety, a tyrosinase inhibitor should contain a glycoside moiety. For example, the cosmetics industry, which uses tyrosinase inhibitors as whitening agents, has adopted the use of the two glycosides: arbutin, a hydroquinone glycoside, and aloesin, a C-glycosylated chromone, primarily because other tyrosinase inhibitors such as linoleic acid, hinokitiol, kojic acid, naturally occurring hydroquinones and catechols are known to cause side effects (Seo et al, 2003).

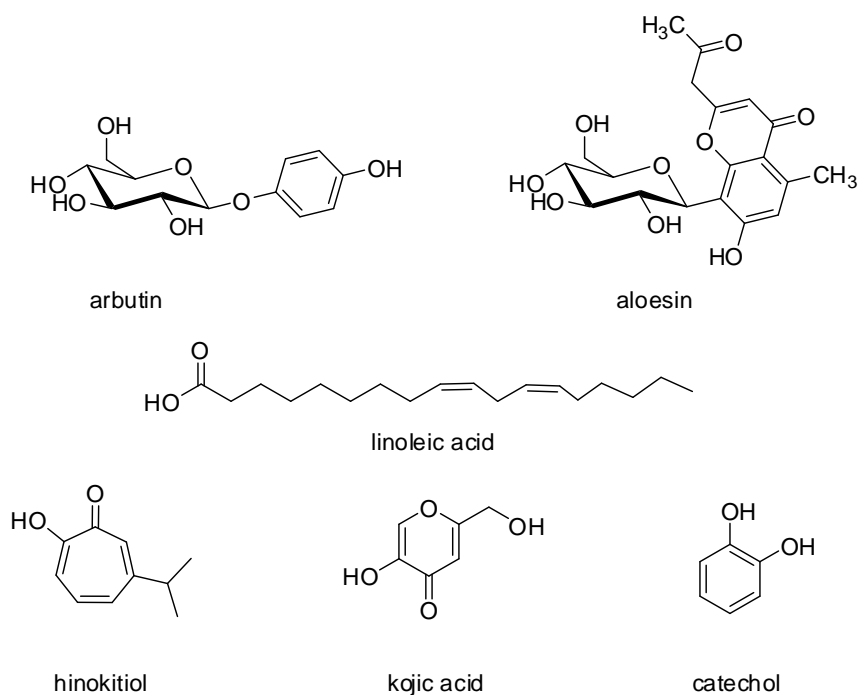


Figure 1.1 Structure of tyrosinase inhibitors

Acetylenic metabolites display important biological activities, namely antitumor, antibacterial, antimicrobial, antifungal, phototoxic, and other chemical and medicinal properties (Dembitsky, 2006, Dembitsky and Levitsky, 2006, Dembitsky et al., 2006, Carballeira, 2008, Minto and Blacklock, 2008, Siddiq and Dembitsky, 2008 and Bador and Paris, 1990).

Acetylene glycoside is a useful precursor for preparation of bioactive polyether natural products. This work was aimed to synthesis of propargyl glycoside with acetylene unit and protection of hydroxyl groups at other position of sugar with benzyl groups for study their biological activity as a tyrosinase inhibitor.

Literature reviews

Carbohydrates in the form of glycosides and glycoconjugates play important roles in many biological processes. As a consequence, the chemistry of glycosides and glycoconjugates has gained much attention for many years. Alkyl glycosides are useful intermediates in the synthesis of complex oligosaccharides and natural products (Guchhait&Misra, 2011).

Simple alkyl and aryl glycosides of free sugars are extremely useful for both synthetic and biological studies. For example, benzyl, allyl or p-methoxybenzylglycosides are used for temporary anomeric protection during oligosaccharide synthesis as they can be removed when needed to make glycoconjugates, whereas long chain alkyl glycosides, for example, n-octyl and n-dodecyl are often used as substrates for enzymatic transformations and other biological studies. Furthermore, acetylene and propargyl glycosides are of great interest for 'Click Chemistry' approaches to mimic various biodynamic carbohydrate structures and glycoconjugates and development of tyrosinase inhibitory activity.

Fischer glycosylation is one of the best choices for preparing these simple alkyl or aryl glycosides from free sugars. Several glycosylation techniques have appeared in the literature as follows.

Selected examples of the synthesis of acetylene and propargyl glycoside analogues.

Guchhait and Misra, (2011) reported a sulfamic acid, mild and environmentally benign catalyst which has been successfully used in the Fischer glycosylation of unprotected sugars for the preparation alkyl glycosides. A diverse range of aliphatic alcohols has been used to prepare a series of alkyl glycosides. The reaction condition is reasonably mild and high yielding.

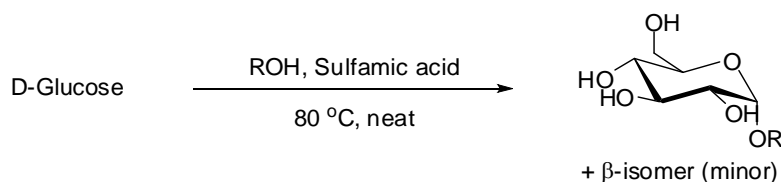


Figure 2-1 Fischer glycosylation of unprotected reducing sugars for the preparation of alkyl glycosides

Roy and Mukhopadhyay (2007) reported the synthesis propargyl glucoside using sulfuric acid immobilized on silica as catalyst for the preparation of various alkyl and aryl

glycosides from free sugars through Fischer type glycosylation using less equivalents of alcohol and shorter reaction times.

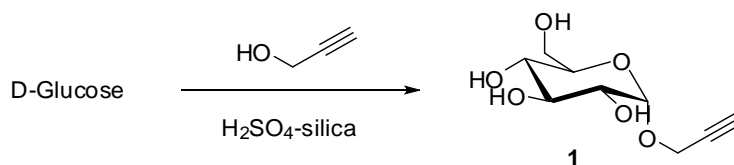


Figure 2-2 Synthesis of propargyl glucoside **1**

The enzymatic approach, by virtue of its mildness, high selectivity, and acceptance of unprotected sugars as substrates, is increasing importance in the synthesis of glycosides. Lu et al. (2010) studied the glycosidase, mediating the cleavage glycosidic bonds *in vivo* which can be used for glycoside synthesis via reverse hydrolysis (thermodynamic control) or trans glycosylation (kinetic control). The latter process is attractive due to ability of synthesis of glycosides from unprotected and unactivated sugars in one step. For introduction of propargyl, a highly reactive functional group which is sensitive to heat and light. The content of propargyl alcohol should not be higher than 50% (v/v) in performing the reaction, to maintain the activity of the enzyme. When 50% (v/v) of solvent was added, acetonitrile can achieve the best result (yield, 35%).

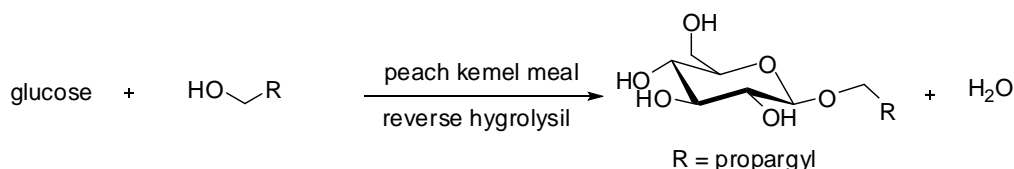


Figure 2-3 Synthesis of β -glucosides using fruit kernel meal

Tankam et al. (2007) prepared 4,6-*O*-benzylidene-2,3-di-*O*-propargyl- α -D-glucopyranoside (**2**) by protection of the hydroxyl of sugar in a first step to exclude interference with protic groups and to simplify isolation and purification of the products. Two neighboring reactive groups should give a first impression on the efficiency of multiple reactions, on decoupling of the relative reactivities from the sugar core, and possible interactions between the two acetylenic groups in close proximity. Therefore, the 2,3-di-*O*-propargyl-glucoside was chosen as the simplest appropriate, but adequately complex model compound. Since partly derivatized polysaccharides inevitably are complex mixtures, their characterization is difficult and limited. To prepare acetylene derivative **2**, methyl- α -D-glucoside (**1**) was reacted with benzaldehydedimethylacetal, and the resulting intermediate subsequently

etherified in THF by treatment with NaH followed by reaction with propargyl bromide give to (2)

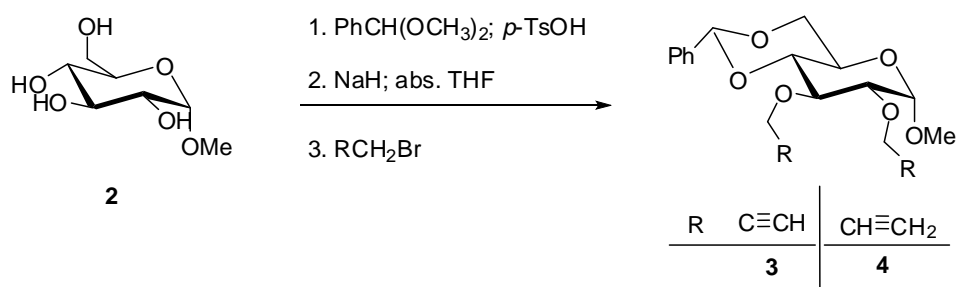


Figure 2-4 Preparation of methyl-4,6-*O*-benzylidene-2,3-di-*O*-propargyl- α -D-glucopyranoside(3) and methyl-4,6-*O*-benzylidene-2,3-di-*O*-allyl- α -D-glucopyranoside(4)

Lyudmila et al. (2013) studied the propargylation of arabino-3,6-galactan (AG) with propargyl bromide (PB) in the two-phase system 30–60% KOH aqueous solution/toluene in the presence of triethylbenzylammonium chloride (TEBAC) or without catalyst (ambient temperature, 1–24 h) to obtain the degree of substitution (DS) of arabinogalactan propargyl ethers in 20–87% yields. The highest yields have been reached using TEBAC as phase-transfer catalyst, though the non-catalytic version proves to be also efficient (DS 2.8, 70% yield). The propargylation of AG is less effective in the systems MOH (M = Na, K)/DMSO. DS of propargyl AG reaches 1.8 (70% yield) when propargyl chloride is used as propargylating agent.

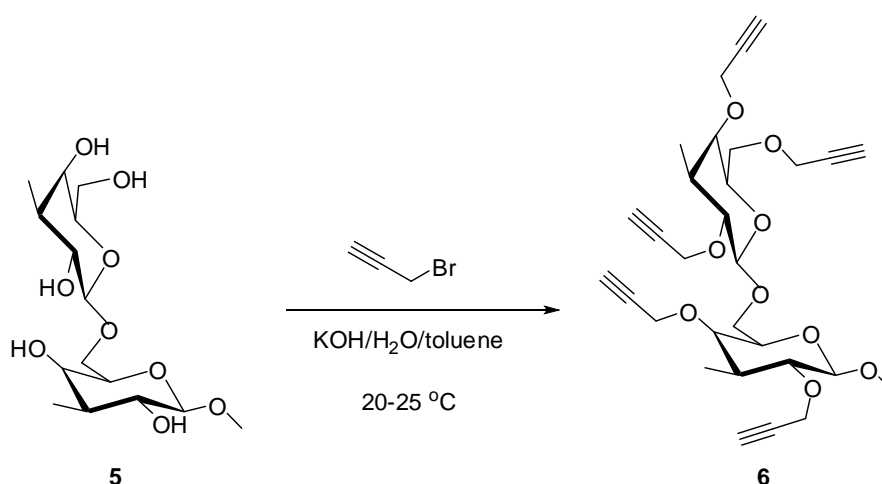


Figure 2-5 Arabinogalactanpropargylation with propargyl bromide in the system 30–60% aqueous solution of KOH/toluene

Yongjun et al. (2005) showed the transformation of Unprotected methyl- β -D-galactopyranoside to methyl-2,3,4,6-tetra-*O*-propargyl- β -D-galactopyranoside using propargyl bromide and NaH in DMF to give product **8** in high yield (80%).

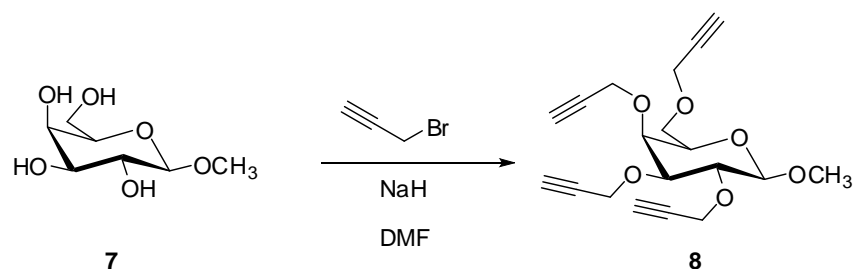


Figure 2-6 Propargylation of methyl- β -D-galactopyranoside **7**

Sureshkumar G., Hotha S. (2007) have developed a new stereoselectively *O*-glycosylation method that enables the synthesis of 1,2-*trans* glycosides from propargyl-1,2-orthoesters. They have demonstrated the scope and utility of propargyl-1,2-orthoesters (**10**) as glycosyl donors in the syntheses of glycosides and disaccharides by using AuBr_3 as the promoter. AuBr_3 may activate the alkyne resulting in the formation of a 1,2-dioxolenium ion and also behaves as a Lewis acid to facilitate the attack of the glycosyl acceptor.

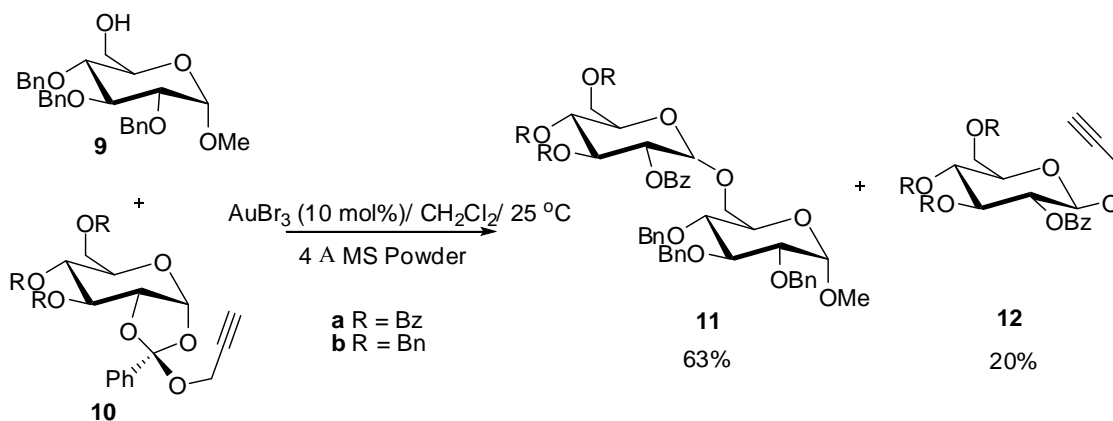


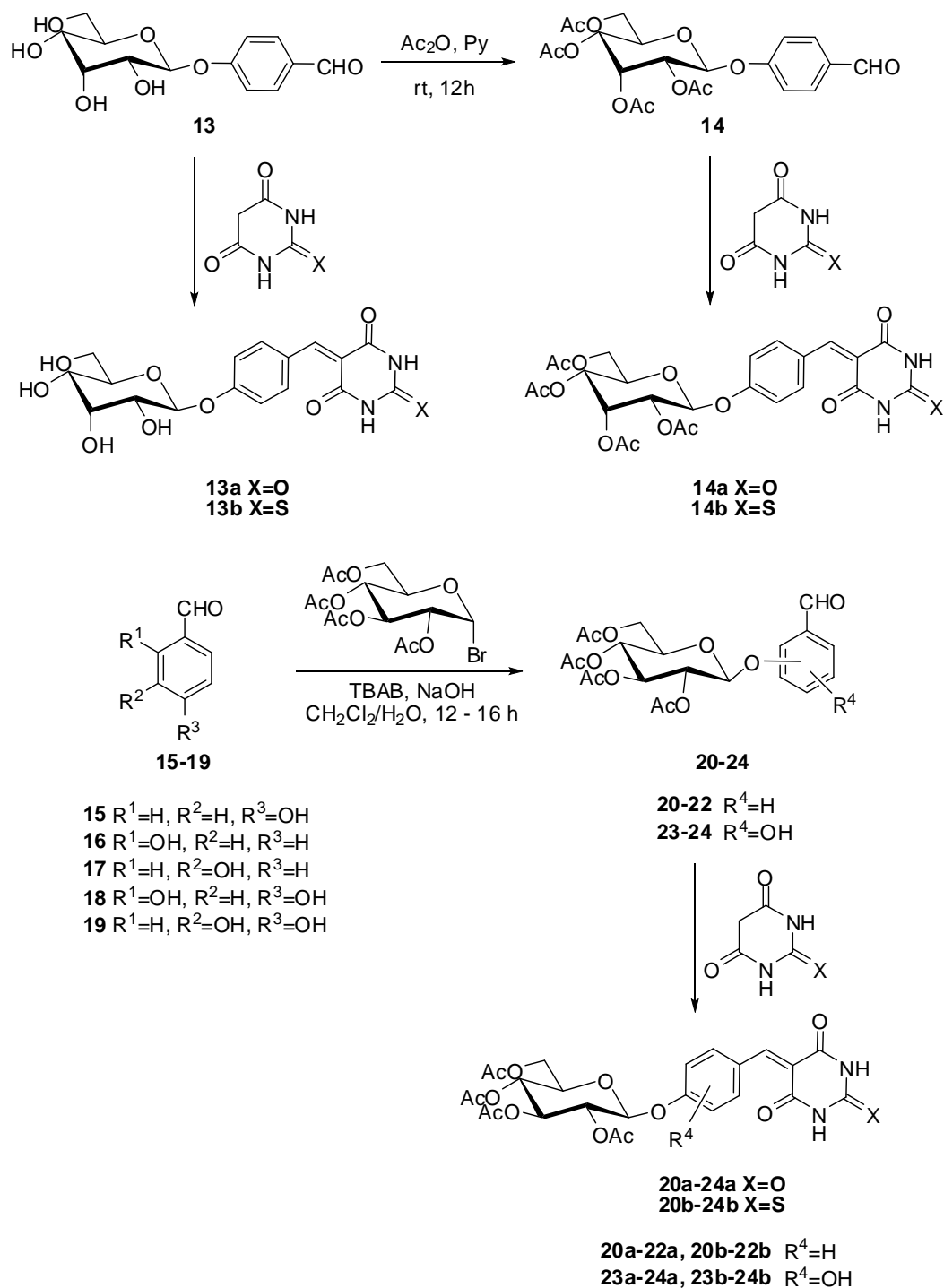
Figure 2-7 Propargyl-1,2-orthoesters as glycosyl donors

Tyrosinase is a copper-containing oxidoreductase, functioning as an important oxidizing agent. This enzyme is responsible for production of melanin, plays an important role in the protection of the skin against ultraviolet (UV) rays owing to its ability to absorb and reflect UV energy, and responsible for the color of skin (Kang et al, 2013). In addition, tyrosinase is known to be an enzyme that catalyzes the oxidation of phenolic compounds found in fruits and vegetables. It plays a role in the neurodegeneration associated with Parkinson's

disease. Thus, the development of novel tyrosinase inhibitors is of interest to agricultural, cosmetic, and medical fields (Li et al, 2013). In the literature there are several reports of tyrosinase inhibitors for treatment of skin.

Selected examples of the synthesis of glycoside derivatives and their tyrosinase inhibitory activity.

Qinet et. al. (2009), synthesized a series of 5-benzylidene(thio)barbiturate- β -D-glycosides bearing lipophilic glycoyl group and cyclic urea or thiourea moiety. The procedure for the preparation of products was outlined in **scheme 2.1**. The 5-benzylidene(thio)barbiturate- β -D-glycosides derivatives were screened for the inhibitory effect and mechanism on mushroom tyrosinase.



Scheme 2.1 The synthesis of 5-benzylidene(thio)barbiturate-β-D-glycosides **13-24**, **13a-24a** and **13b-24b**.

They were found that compound **24b** has the most potent tyrosinase inhibitor with IC₅₀ value of 0.05 mmol/L. SARs analysis. The results indicated that 5-benzylidene thiobarbiturate substructures were efficacious for the inhibitory activity and the lipophilic property of acetylated sugar moiety facilitated inhibitory potency. In addition, the hydroxyl group of 3'-configuration contributed to the increase of inhibitory effects. The inhibition

mechanism study revealed that 5-benzylidene thiobarbiturate- β -D-glycosides were irreversible inhibitors.

Table 2.1 Inhibitory effects on mushroom tyrosinase of 5-benzylidene(thio)barbiturate- β -D-glycosides as compared with arbutin

Compounds	IC ₅₀ (mM)
13	2.62±0.055
13a	1.63±0.029
13b	1.16±0.021
14	0.78±0.015
14a	1.50±0.028
14b	0.34±0.007
20	>3.0
20a	1.27±0.039
20b	0.23±0.005
21	>3.0
21a	>3.0
21b	0.43±0.010
22	>3.0
22a	>3.0
22b	0.87±0.019
23	>3.0
23a	1.73±0.034
23b	0.28±0.006
24	0.43±0.011
24a	0.13±0.025
24b	0.05±0.002
Arbutin	8.4

In the same year, Weiet al. designed and synthesized a series of 4-functionalized phenyl-*O*- β -D-glycosides bearing thiosemicarbazide, oxime and methyloxime moiety. The process for the preparation of compounds **25a–32a**, **25b–32b** and **25c–32c** is shown in **scheme 2.2**. The 4-functionalized phenyl-*O*- β -D-glycosides and its analogues were studied for their inhibitory effect and mechanism on mushroom tyrosinase.

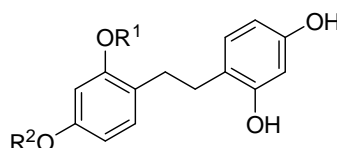
28a was reversible and competitive type inhibitor, whereas compound **32a** was reversible and competitive–uncompetitive mixed-II type inhibitor.

Table 2.2 Inhibitory effects on mushroom tyrosinase of 4-functionalized phenyl-*O*- β -D-glycosides as compared with arbutin.

Compounds	IC ₅₀ ^a (μ M)	Compounds	IC ₅₀ (μ M)
25a	3.61 \pm 0.55	29b	>200
26a	2.96 \pm 0.62	30b	>200
27a	3.41 \pm 0.28	31b	>200
28a	0.31 \pm 0.12	32b	>200
29a	0.41 \pm 0.09	25c	>200
30a	52.8 \pm 2.2	26c	>200
31a	36.5 \pm 3.8	27c	>200
32a	0.65 \pm 0.21	28c	>200
25b	>200	29c	>200
26b	>200	30c	>200
27b	>200	31c	>200
28b	>200	32c	>200
Arbutin	7300 \pm 600		

^a IC₅₀ = mean \pm SEM. SEM: standard error of mean.

Reikoet al. (2011), designed and synthesized bibenzyl glycosides **33-39** from 2,4-dihydroxybenzaldehyde and xylose, glucose, cellobiose or maltose via Wittig reaction and trichloroacetimidate glycosylation. Several bibenzyl glycosides derivatives were study for their tyrosinase inhibitory activity.



- 33:** R¹ = Xyl, R² = H
34: R¹ = H, R² = Xyl
35: R¹ = R² = Xyl
36: R¹ = H, R² = Glc
37: R¹ = H, R² = Cel
38: R¹ = H, R² = Mal
39: R¹ = R² = H

Figure 2-8 Structure of bibenzyl derivatives **33-39**

All of the synthesized bibenzyl glycosides showed greater activity than the common inhibitor kojic acid, a reference standard. Bibenzylxyloside **34** is particularly potent inhibitor

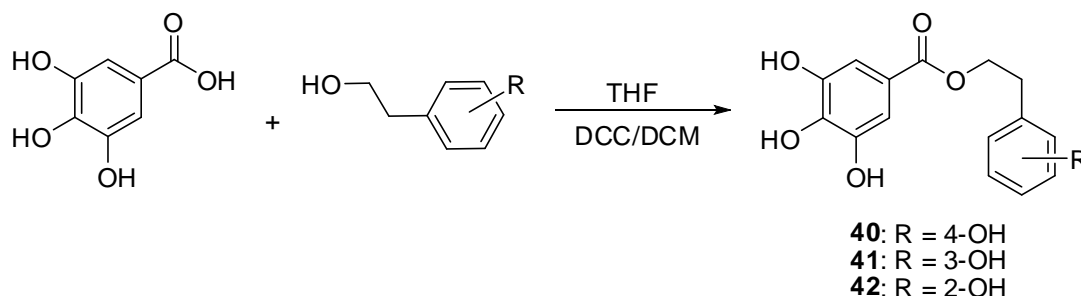
($IC_{50} = 0.43$ mM, 17 times higher than that of kojic acid). Glycosylation at the C-4 position (R^2) with a large sugar moiety appears to influence inhibitory activity and hydrophilic.

Table 2.2 Tyrosinase inhibitory activities of bibenzyl derivatives **33-39** and kojic acid.

Compounds tested	IC_{50} (μ M)
33	1.6 ± 0.43^a
34	0.43 ± 0.18
35	0.73 ± 0.11
36	0.77 ± 0.04
37	0.68 ± 0.05
38	0.83 ± 0.06
39	0.37 ± 0.06
Kojic acid	7.4 ± 1.4

^a The IC_{50} values represent means \pm SE of three different experiments.

Lee et al. (2007), designed and synthesized a three tyrosyl gallate derivatives (**40-42**) by position of hydroxyl substituent at the aromatic ring of tyrosol and evaluated as potent inhibitors on tyrosinase activity and melanin formation in melanin cells.



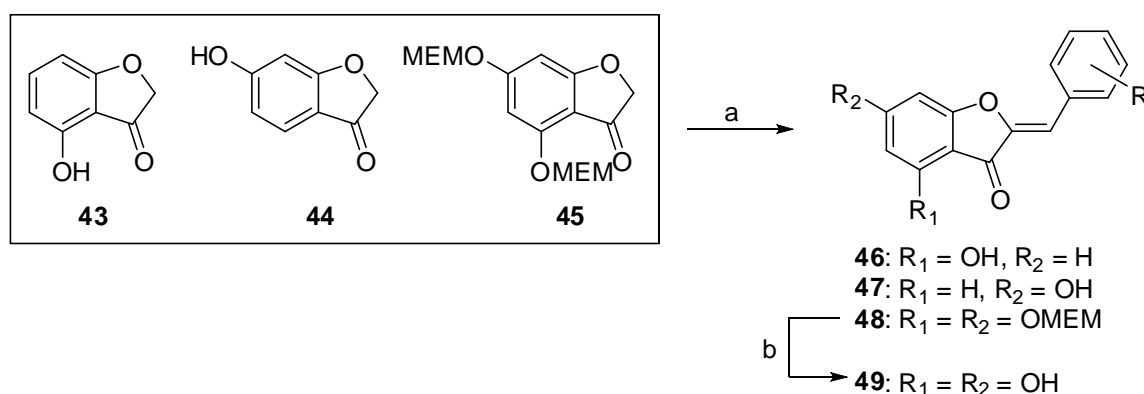
Scheme 2.3 Synthesis of tyrosyl gallate derivatives (**40-42**).

The three compounds (**40-42**) exhibited significant inhibitory effect on tyrosinase activity. As shown in **Table 2.3**, all compound were more potent than arbutin with tyrosinase activity IC_{50} values of 30.26 μ M whereas compounds **40** did not show the inhibitory activity in melanin formation. From the structural-activity point of view, they have found that 4-hydroxyphenethyl 3,4,5-trihydroxybenoate (**40**) with a para-configuration of hydroxyl group may contribute to the inhibitory activity against tyrosinase enzyme.

Table 2.3 Inhibitory effects of the synthetic tyrosyl gallate derivatives on tyrosinase and melanin formation in melanin cells

Compound	Tyrosinase IC ₅₀ (μM)	Melanin formation IC ₅₀ (μM)	Cytotoxicity IC ₅₀ (μM)
40	4.93	-	>10
41	15.21	8.94	>50
42	14.50	13.67	>50
Arbutin	30.26	118.05	>1500

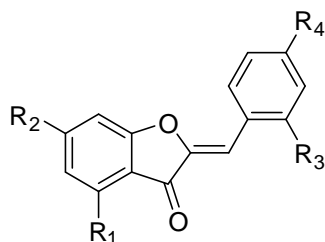
This work was investigated naturally occurring aurones (*Z*-benzylidenebenzofuran-3(2H)-one) and analogues as human tyrosinase inhibitors. Okombi et al. (2006), synthesized aurones (*Z*-benzylidenebenzofuran-3(2H)-one) bearing hydroxyl groups on A-ring and different substituents on B-ring and evaluated as inhibitors of human melanocyte-tyrosinase by an assay which measures tyrosinase-catalyzed L-Dopa oxidation.



Scheme 2.4 Synthesis of aurones (*Z*-benzylidenebenzofuran-3(2H)-one) **46-49**. Reagents and conditions: (a) benzaldehyde derivative, KOH, MeOH/H₂O, 60 °C; (b) HCl (1 M in Et₂O), 60 °C, 2 h.

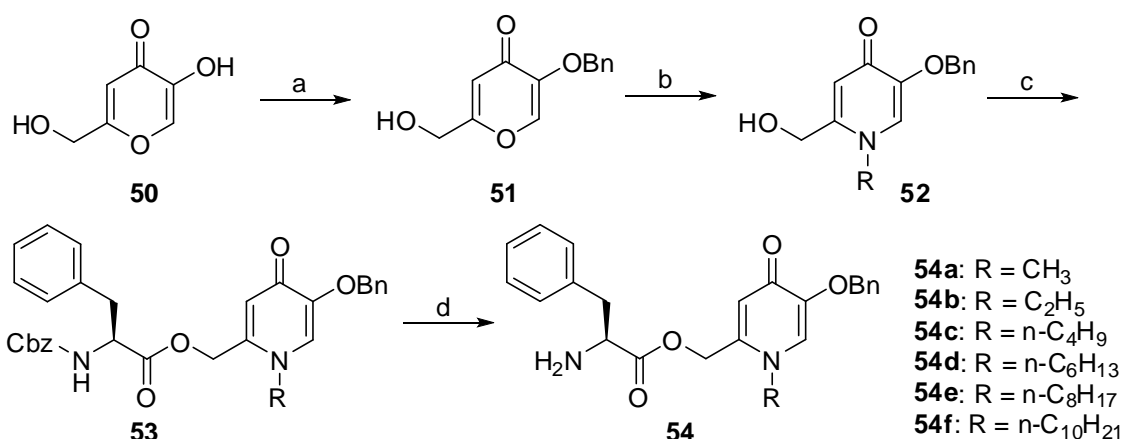
They found that unsubstituted aurones showed weak inhibitors; however, derivatives with two or three hydroxyl groups preferably at 4,6 and 4' positions are able to induce significant tyrosinase inhibition. The most potent aurone was found to be the naturally occurring 4,6,4'-trihydroxyaurone which induces 75% inhibition at 0.1 mM concentration and is highly effective comparing to kojic acid.

Table 2.4 Tyrosinase inhibition results of aurones (*Z*-benzylidenebenzofuran-3(2H)-one) derivatives.



compound	R ₁	R ₂	R ₃	R ₄	inhibition % at 0.1 mM	IC ₅₀ (μ M)
46a	H	H	H	OH	39 \pm 6	
46b	OH	H	H	OH	71 \pm 7	31.7 \pm 2.6
47a	H	OH	H	H	0 \pm 23	
47b	H	OH	H	OH	69 \pm 3	38.4 \pm 2.6
47c	H	OH	H	Et	0 \pm 29	
49a	OH	OH	H	H	3 \pm 3	
49b	OH	OH	H	OH	75 \pm 8	38.0 \pm 2.9
49c	OH	OH	Et	Et	nd	
49d	OH	OH	OH	H	0 \pm 34	
49e	OH	OH	H	H	4 \pm 9	
49f	OH	OH	H	OMe	11 \pm 4	
49g	OH	OH	H	OEt	5 \pm 8	
49h	OH	OH	H	<i>n</i> -Pr	5 \pm 3	
49i	OH	OH	H	<i>t</i> -Bu	0 \pm 14	
49j	OH	OH	H	3'-OMe, 4'-OH	4 \pm 4	
apigenin (5,7,4'-trihydroxyflavone)						0 \pm 2
kojic acid (evaluated at 7 mM)						20 \pm 5

Li et al. (2013), synthesized 3-hydroxypyridin-4-one derivatives (**54**) with a hydrophobic alkyl group at the N-1 position from kojic acid and screened for their tyrosinase inhibitory activities.



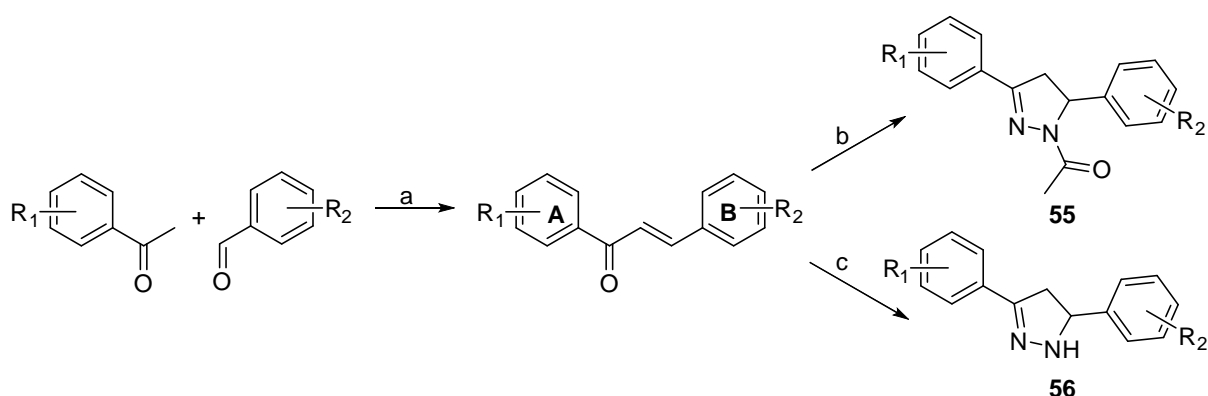
Scheme 2.5 Synthesis of 3-hydroxypyridin-4-one derivatives **54**. Reagents and conditions: (a) BnCl, MeOH/H₂O, 70 °C, 6 h; (b) RNH₂, EtOH/H₂O, NaOH, reflux, 3 h, 72–85% yield; (c) EDC, DMAP; (d) H₂ (30 psi), Pd/C, EtOAc/MeOH (1:1), room temperature, 5 h, 88–93% yield.

Table 2.5 Inhibitory Effect of Hydroxypyridinone-L-phenylalanine Conjugates (**54**) against Monophenolase and Diphenolase Activity of Mushroom Tyrosinase

comp.	C log <i>P</i>	IC ₅₀ (μM) (against monophenolase activity)	IC ₅₀ (μM) (against diphenolase activity)	<i>K</i> _I (μM)	<i>K</i> _{IS} (μM)
54a	0.238	2590 ± 55.8			
54b	0.614	1150 ± 31.5			
54c	1.676	190 ± 6.4			
54d	2.686	19.2 ± 0.8	42.8 ± 2.3	55.5 ± 1.5	193.5 ± 12.5
54e	3.697	12.6 ± 0.3	4.0 ± 0.1	4.0 ± 0.03	8.36 ± 0.53
54f	4.707	1650 ± 41.3			
kojic â	-0.888	26.8 ± 1.2	20	13.0	100.0

It was found that compound **54e** showed the best potent inhibitory effect against mushroom tyrosinase an IC₅₀ value of 12.6 μM, which was better than that of kojic acid (IC₅₀ = 26.8 μM). It was also demonstrated that these conjugates are mixed-type inhibitors, suggesting they could bind to both the free enzyme and the enzyme–substrate complexes. MTT assay indicated that **54e** was nontoxic to three cell lines. This compound may find applications in food preservation and cosmetics.

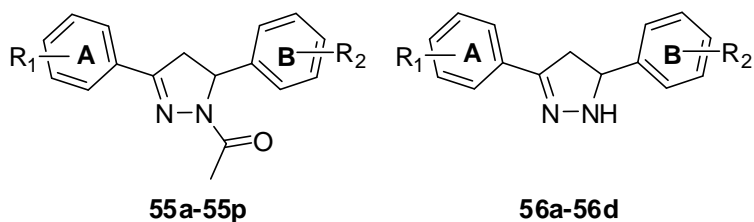
In the same year, Wei et al. designed and synthesized a series of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives (**55a–55p**, **56a–56d**) according to the routes in Scheme 2.6 by vary substituted on ring A and ring B.



Scheme 2.6 Synthesis of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives. Reagents and conditions: Reagents and conditions: (a) KOH, neutral Al₂O₃, microwave irradiation, 30–70 s; (b) acetic acid, hydrazine hydrate, reflux, 50–90 min; (c) ethanol, hydrazine hydrate, reflux, 5 h.

The results showed that among the compounds, 1-(5-(3,4-dihydroxyphenyl)-3-(4-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethanone **55d** was found to be the most potent tyrosinase inhibitor with IC₅₀ value of 0.301 μM. Kinetic study revealed that these compounds were competitive inhibitors of tyrosinase. The structure–activity relationships were studied and investigated that the chemical groups between the two hydroxylated benzene rings of the compounds also influenced the inhibitory activity on tyrosinase, addition of a heterocycle could increase inhibitory activity.

Table 2.6 Inhibition effect of 3,5-diaryl-4,5-dihydro-1*H*-pyrazole derivatives on mushroom tyrosinase activities



comp.	R ₁	R ₂	IC ₅₀ (μM)
55a	2,4-OH	3',4'-OH	2.74 ± 0.21
55b	2,4-OH	4'-OH	1.69 ± 0.03
55c	2,4-OH	3'-OH	0.863 ± 0.102
55d	2,4-OH	3'-OMe, 4'-OH	0.301 ± 0.090
55e	2,4-OH	3'-NO ₂ , 4'-OH	>200
55f	4-OH	3',4'-OH	1.07 ± 0.14
55g	4-OH	4'-OH	3.51 ± 0.04
55h	4-OH	3'-OH	147 ± 5
55i	4-OH	3'-OMe, 4'-OH	>200
55j	2-OH	3',4'-OH	>200
55k	2-OH	4'-OH	13.1 ± 0.8
55l	2-OH	3'-OH	>200
55m	2-OH	3'-OMe, 4'-OH	13.4 ± 0.3
55n	2-OH	2'-OMe, 4'-OH	>200
55o	3,5-OH	4'-OH	161 ± 16
55p	3,5-OH	3'-OH	>200
56a	2,4-OH	4'-OH	0.882 ± 0.057
56b	2,4-OH	3'-OH	0.837 ± 0.148
56c	2,4-OH	3'-OMe, 4'-OH	2.43 ± 0.41
56d	2-OH	4'-OH	11.8 ± 1.5
kojic â			18.3 ± 0.7

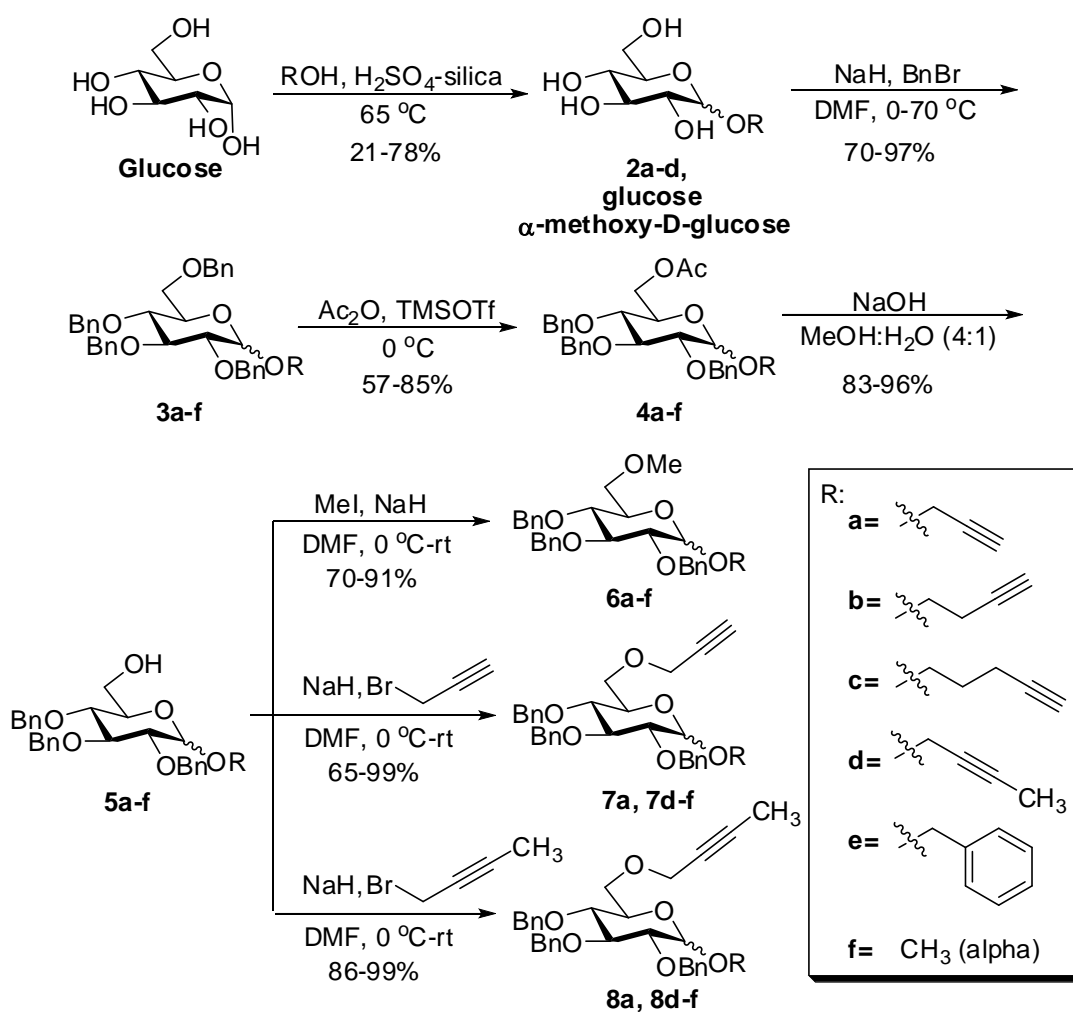
Chapter 2: Results and Discussions

Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They also find uses in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms. In this work, we designed to synthesize acetylene glycoside analogues and study for the tyrosinase inhibitory activity (Scheme 1).

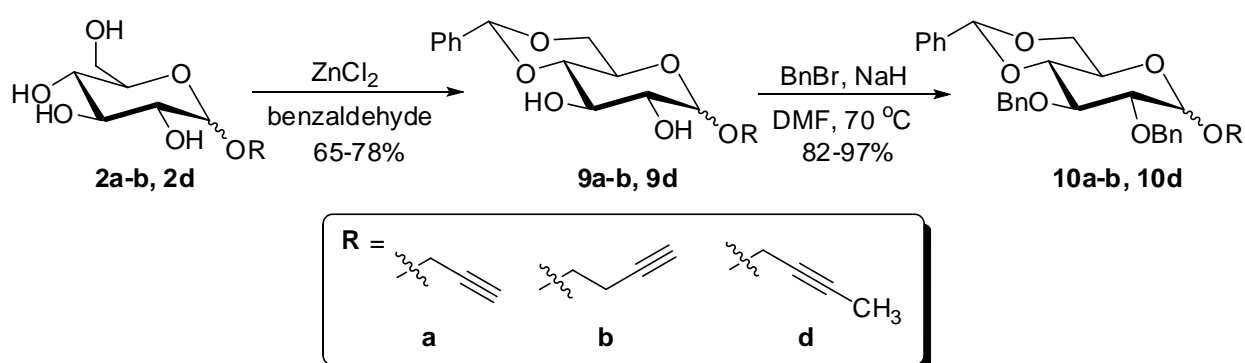
The synthesis of acetylene glycoside analogues started with glycosylation at C-1 position of glucose with propargyl, butynyl and pentynyl alcohols using sulfuric acid on silica support. The reaction was performed at 65°C to generate acetylene glycosides **2a-2d** in 21-78 % yields. Benzoylation of hydroxyl groups at C-2, C-3, C-4 and C-6 by using NaH (5.0 equiv.) and benzyl bromide (5.0 equiv.) and the reaction was heated at 70 °C for 2 h to give alkynyl benzyl glycoside analogues **3a-3f** in high yields (70-97%). Regioselective debenzoylation and acetylation at C-6 of analogues **3a-3f** by TMSOTf (0.4 equiv.) and acetic anhydride gave C-6 acetyl benzyl glycosides **4a-4f** in high yields (70-97%). Deacetylation at C-6 of glycosides **4a-4f** gave glycosides **5a-5f** in high yields using sodium hydroxide in methanol/water. Methylation of hydroxyl at C-6 of glycosides using NaH (1.2 equiv.) and iodomethane (2.0 equiv.) gave **6a-6f** in high yields (83-96%). In the second series, alkylation of hydroxyl at C-6 of glycosides under basic condition by using propargyl bromide (1.5 equiv.) gave **7a, 7d-f** and in the third series, 1-bromo-2-butyne (1.5 equiv.) gave **8a, 8d-f** in high yields.

In addition, protection of dihydroxyl groups of acetylene glycosides with benzylidene group was studied (scheme 2).

The protection of hydroxyl groups at C-6 and C-4 with benzylidene group was performed using benzaldehyde (5.0 equiv.) and ZnCl₂ (1.5 equiv.) gave 4,6-benzylidene-2,3-dihydroxyl acetylene glycosides **9a-b, 9d** in moderate yields. After that, protection of hydroxyl groups at other position of sugar with benzyl groups by benzylation to obtain 4,6-benzylidene-2,3-dibenzyl acetylene glycosides **10a-b, 10d** in high yields.



Scheme 1 Synthesis of acetylene glycoside analogues (**2-8**)

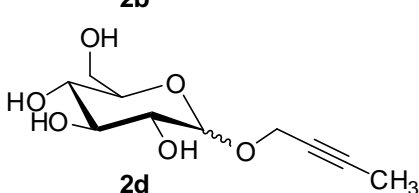
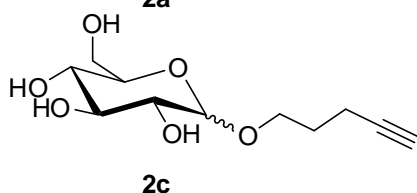
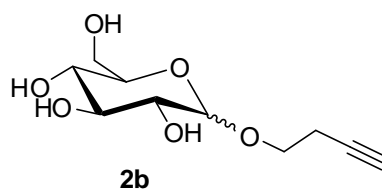
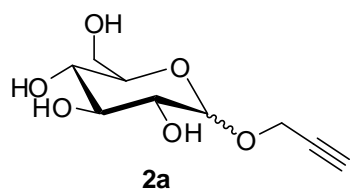


Scheme 2 Synthesis of 4,6-benzylidene acetylene glycoside analogues (**9-10**)

The synthetic acetylene glycoside analogues can be divided in nine groups to study for the tyrosinase inhibitory activity and to understand the structure activity relationship.

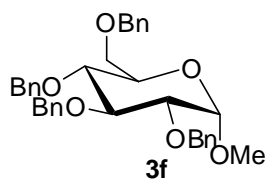
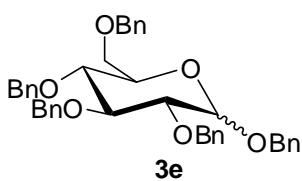
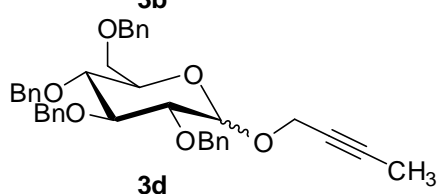
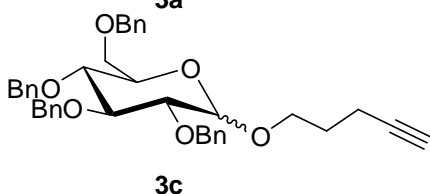
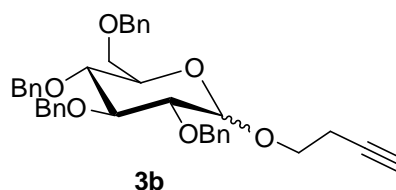
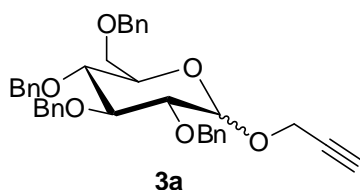
Group 1. *O*-alkynyl glycoside

O-propargyl glycoside **2a**, *O*-butynyl glycoside **2b** (terminal alkyne), *O*-pentynyl glycoside **2c**, *O*-butynyl glycoside, **2d** (internal alkyne)



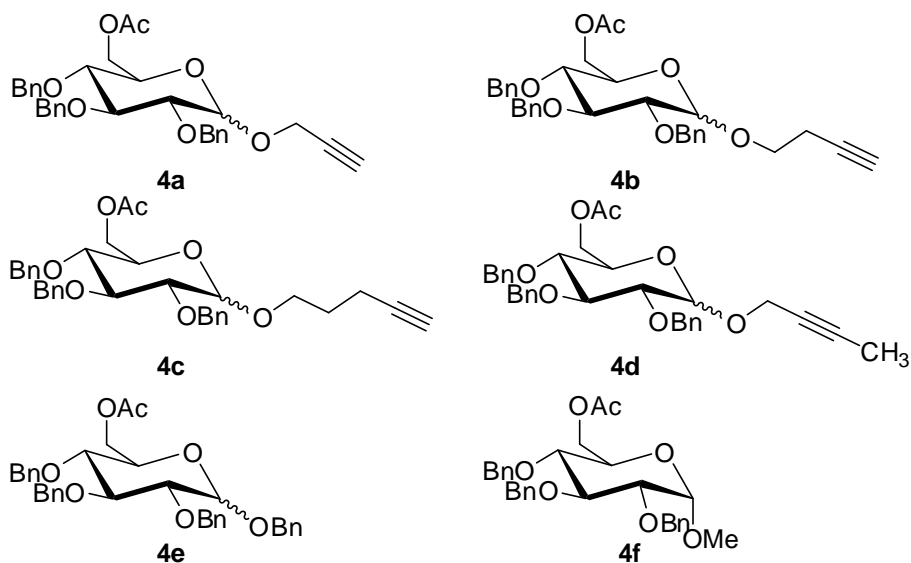
Group 2. *O*-alkynyl benzylglycoside

O-propargyl benzylglycoside **3a**, *O*-butynyl benzylglycoside **3b** (terminal alkyne), *O*-pentynyl glycoside **3c**, *O*-butynyl benzylglycoside **3d** (internal alkyne), benzylglycoside **3e**, *O*-methyl benzylglycoside **3f**



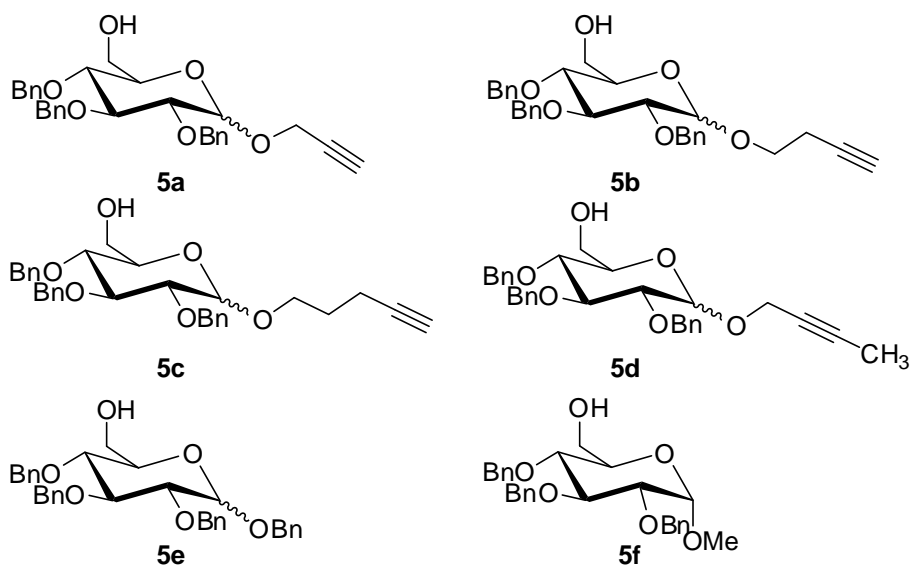
Group 3. *O*-alkynyl-6-acetyl-benzylglycoside

O-propargyl-6-acetyl-benzylglycoside **4a**, *O*-butynyl-6-acetyl-benzylglycoside **4b** (terminal alkyne), *O*-pentynyl-6-acetyl-glycoside **4c**, *O*-butynyl-6-acetyl-benzylglycoside **4d** (internal alkyne), 6-acetyl-benzylglycoside **4e**, *O*-methyl-6-acetyl-benzylglycoside **4f**



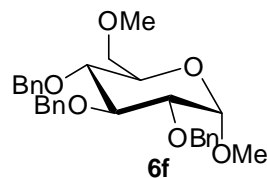
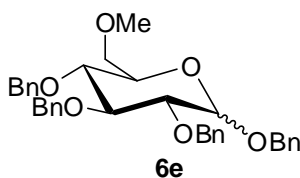
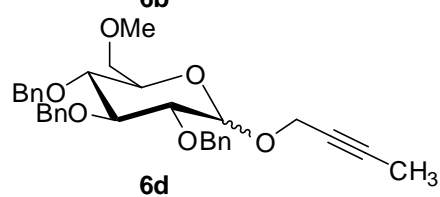
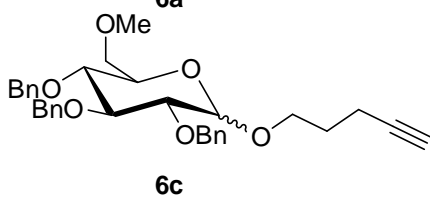
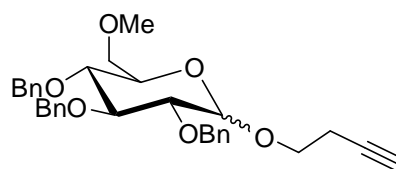
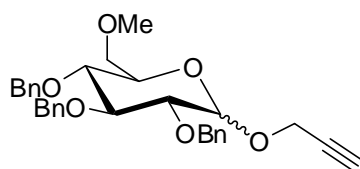
Group 4. *O*-alkynyl-6-hydroxyl-benzylglycoside

O-propargyl-6-hydroxyl-benzylglycoside **5a**, *O*-butynyl-6-hydroxyl-benzylglycoside **5b** (terminal alkyne), *O*-pentynyl-6-hydroxyl-glycoside **5c**, *O*-butynyl-6-hydroxyl-benzylglycoside **5d** (internal alkyne), 6-hydroxyl-benzylglycoside **5e**, *O*-methyl-6-hydroxyl-benzylglycoside **5f**



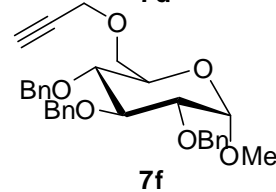
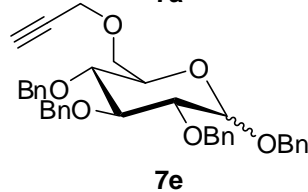
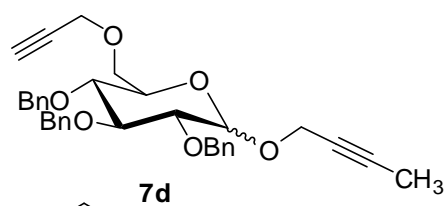
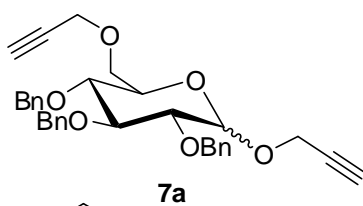
Group 5. *O*-alkynyl-6-methoxy-benzylglycoside

O-propargyl-6-methoxy-benzylglycoside **6a**, *O*-butynyl-6-methoxy-benzylglycoside **6b** (terminal alkyne), *O*-pentynyl-6-methoxy-glycoside **6c**, *O*-butynyl-6-methoxy-benzylglycoside **6d** (internal alkyne), 6-methoxy-benzylglycoside **6e**, *O*-methyl-6-methoxy-benzylglycoside **6f**



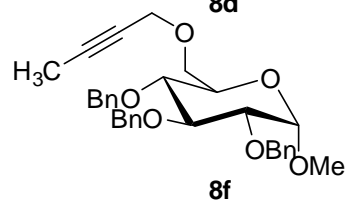
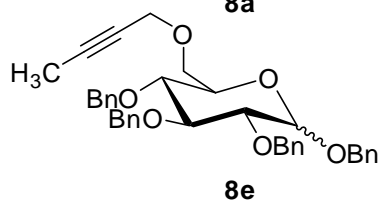
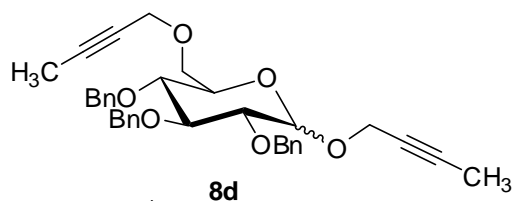
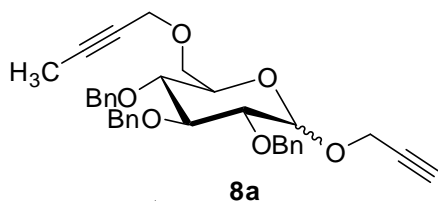
Group 6. *O*-alkynyl-6-propargyl-benzylglycoside

O-propargyl-6-propargyl-benzylglycoside **7a**, *O*-butynyl-6-propargyl-benzylglycoside **7d** (internal alkyne), 6-propargyl-benzylglycoside **7e**, *O*-methyl-6-propargyl-benzylglycoside **7f**



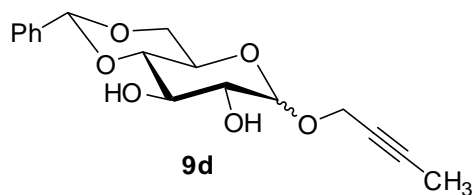
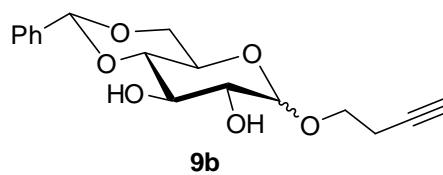
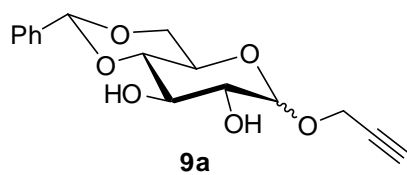
Group 7. *O*-alkynyl-6-butynyl-benzylglycoside

O-propargyl-6-butynyl-benzylglycoside **8a**, *O*-butynyl-6-butynyl-benzylglycoside **8d** (internal alkyne), 6-butynyl-benzylglycoside **8e**, *O*-methyl-6-butynyl-benzylglycoside **8f**



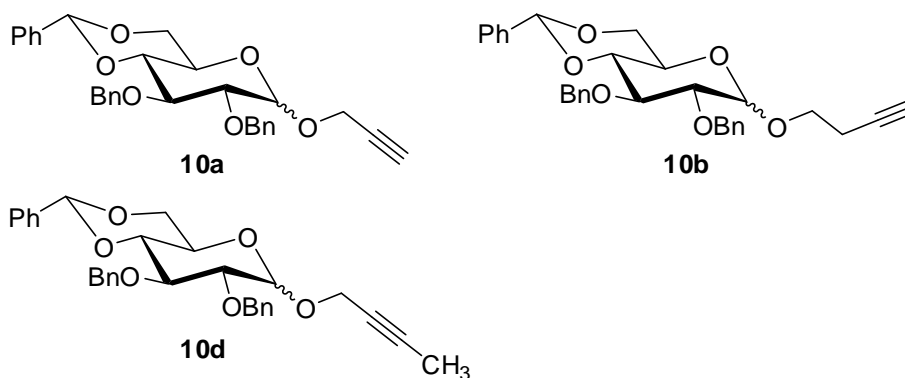
Group 8. *O*-alkynyl-4,6-benzylidene glycoside

O-propargyl-4,6-benzylidene-glycoside **9a**, *O*-butynyl-4,6-benzylidene-glycoside **9b** (terminal alkyne), *O*-butynyl-4,6-benzylidene-glycoside **3d** (internal alkyne)



Group 9. *O*-alkynyl-4,6-benzylidene-benzylglycoside

O-propargyl-4,6-benzylidene-benzylglycoside **10a**, *O*-butynyl-4,6-benzylidene-benzylglycoside **10b** (terminal alkyne), *O*-butynyl-4,6-benzylidene-benzylglycoside **10d** (internal alkyne)



The preliminary screening results of the effect of our forty-two synthetic alkynyl and propargyl-D-glycosides on mushroom tyrosinase activity with L-tyrosine as a substrate are summarized in Chart 1.

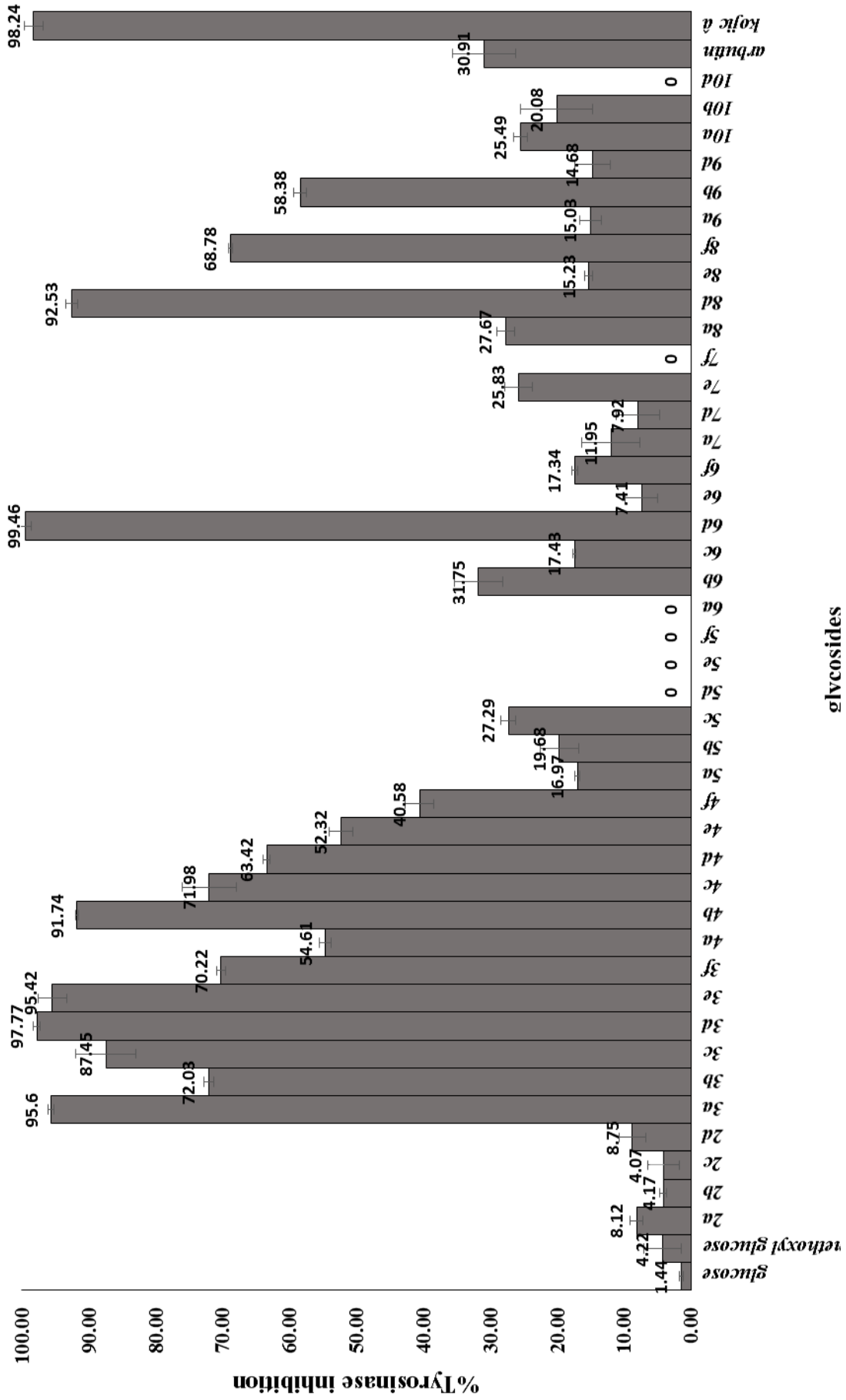
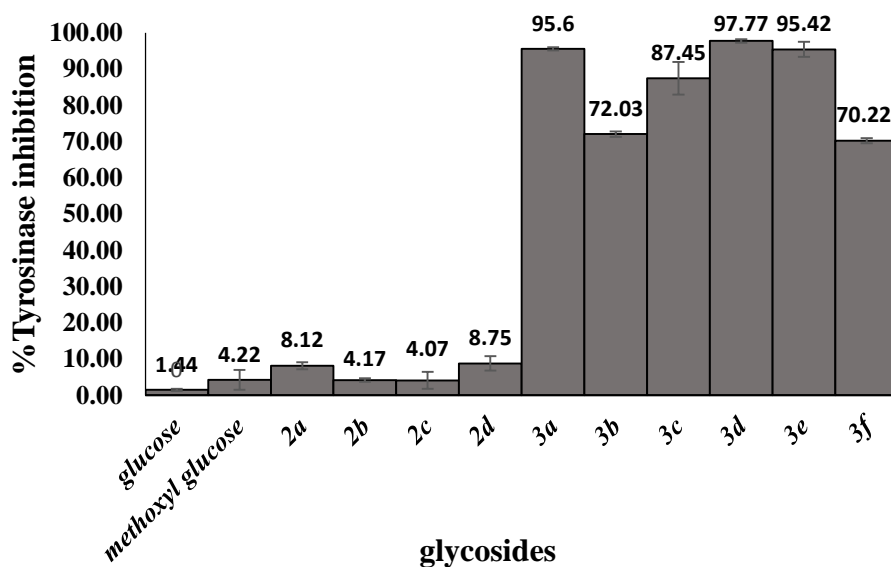
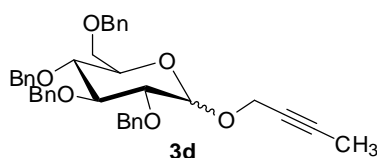


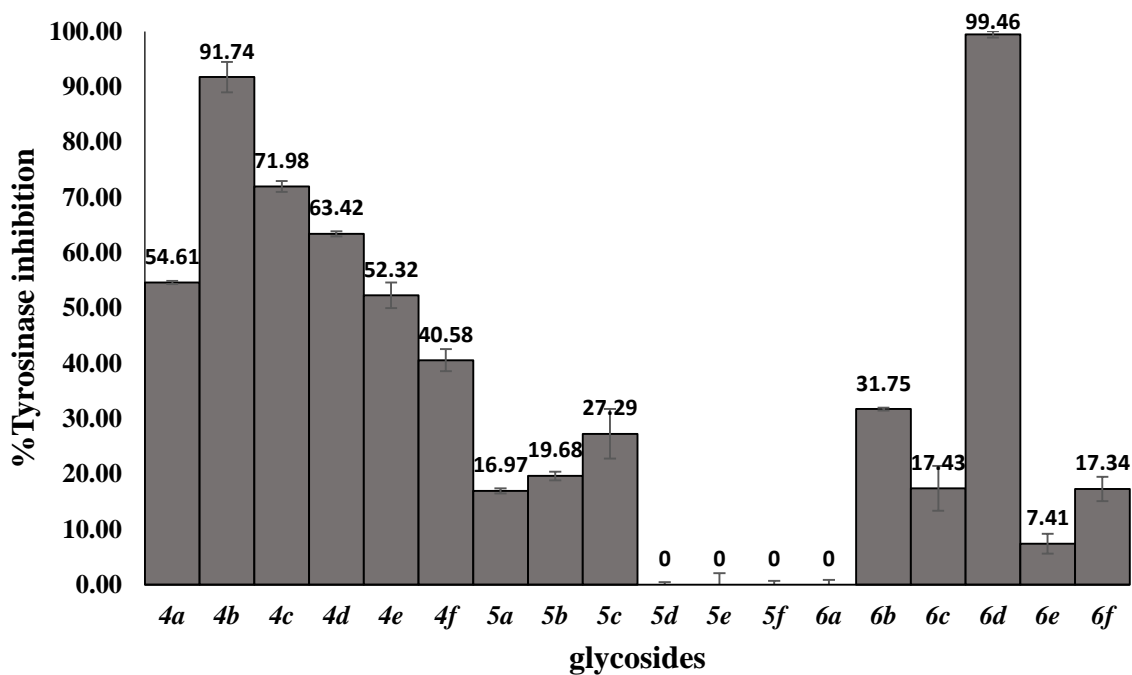
Chart 1: The effect of various glycosides on mushroom tyrosinase activity with L-tyrosine as a substrate.

The inhibitory activity against mushroom tyrosinase was investigated by usual procedure and compared with alpha-Arbutin and Kojic acid (Kojic acid is an excellent skin whitening agent but has been accused of serious side effects, such as cytotoxicity, skin cancer, dermatitis, and has been banned in cosmetics in many countries).

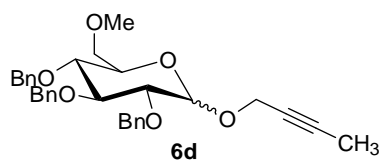
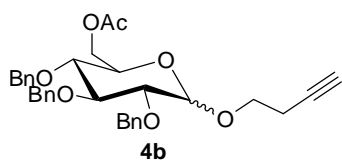


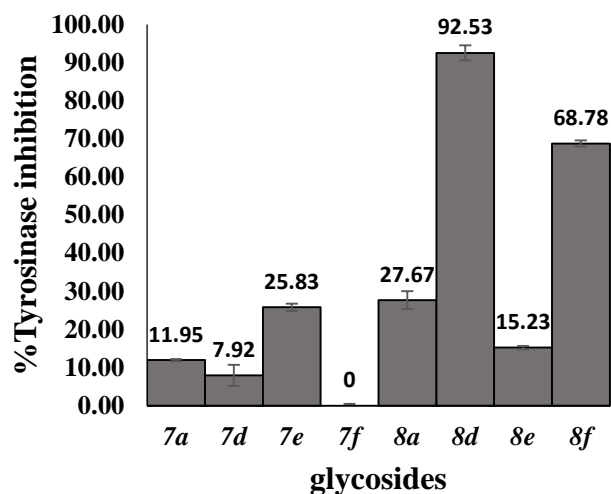
Compounds **2a-2d** in group 1 showed low inhibitory activity against tyrosinase. The results indicated that C-1 substituted alkyne of hydroxyl sugar showed little effects on the activity in this group. *O*-benzylglycosides **3a-3f** in group 2 showed moderate to good inhibitory activity against tyrosinase (% Tyrosinase inhibition = 70.22-97.77) and showed better activity than alpha-Arbutin, skin whitening agent (% Tyrosinase inhibition = 30.91). Particularly, compound **3d** bearing butynyl group (internal alkyne) showed high activity which indicated that benzyl group are very important for activity.



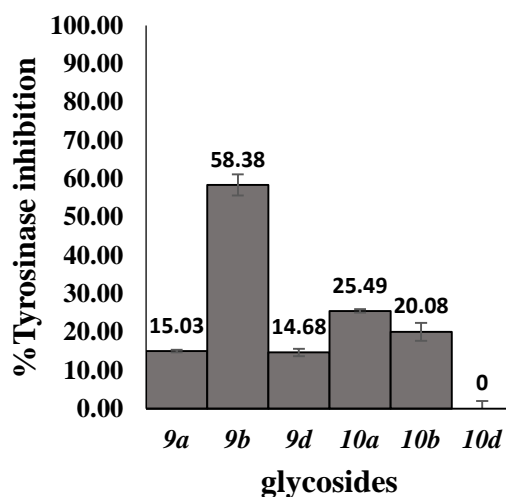
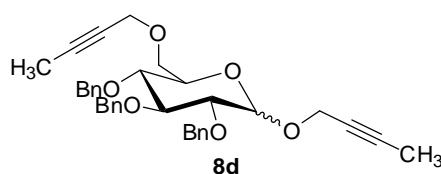


O-alkynyl-6-acetyl-benzylglycoside **4a-4f** demonstrated significant tyrosinase inhibitory effects better than alpha-arbutin with % Tyrosinase inhibition = 40.58-91.74. *O*-alkynyl-6-hydroxyl-benzylglycoside **5a-5f** and *O*-alkynyl-6-methoxy-benzylglycoside **6a-6f** exhibited sharply decreased inhibitory effects except compound **4b** (% Tyrosinase inhibition = 91.74) bearing butynyl group (terminal alkyne) and compounds **6d** (% Tyrosinase inhibition = 99.46) bearing butynyl group (internal alkyne) showed better activity than *O*-benzylglycosides. These results indicated that the C-6 substituted moiety contributed to the inhibitory effects.





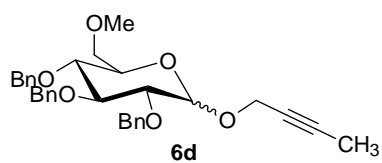
6-Propargyl compounds **7a**, **7d-f** in group 6 and 6-butynyl compounds **8a**, **8d-f** in group 7 showed low inhibitory activity except compound **8d** (% Tyrosinase inhibition = 92.53) bearing dibutynyl group (internal alkyne) but lower than 6-benzyl-benzylglycoside.



O-alkynyl-4,6-benzylidene glycoside **9a-b**, **9d** in group 8 showed low to moderate inhibitory activity against tyrosinase (% Tyrosinase inhibition = 14.68-58.38). After protect hydroxyl groups of sugar with benzyl groups as compounds **10a-b**, **10d** in group 9 provide little effects on the activity

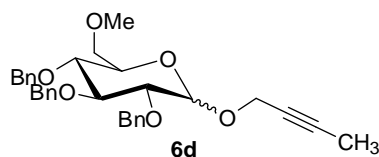
Of all synthetic alkynyl glycoside analogs, compounds **6d** (%tyrosinase inhibition = 99.46) exhibited the best activity over other compounds and demonstrated inhibitory potential

on mushroom tyrosinase comparable to kojic acid (% Tyrosinase inhibition = 98.24) whereas other 6-methoxy-benzylglycoside in the same group showed low activity.



Chapter 3 Conclusion

A series of novel alkynyl and propargyl-D-glycosides bearing benzyl and acetyl groups were designed, synthesized and evaluated as a new class of mushroom tyrosinase inhibitors with the aim of developing novel potent tyrosinase inhibitors. Forty-two synthetic alkynyl and propargyl glycoside analogues were prepared and divided in nine groups to study for the tyrosinase inhibitory activity and to understand the structure activity relationship. The preliminary screening results of the effect of our synthetic alkynyl and propargyl-D-glycosides on mushroom tyrosinase activity with L-tyrosine as a substrate compared with alpha-Arbutin and Kojic acid were studied. The results indicated that C-1 substituted alkyne and C-6 substituted moiety contributed to the inhibitory effects. Of all synthetic alkynyl glycoside analogs, compounds **6d** (% Tyrosinase inhibition = 99.46) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (% Tyrosinase inhibition = 98.24). These findings may lead to the discovery of therapeutically potent agents against clinically dermatological disorders including hyperpigmentation as well as skin melanoma.



REFERENCES

- Bimalendu, R. & Balaram, M. N. (2007), Sulfuric acid immobilized on silica: an excellent catalyst for Fischer type glycosylation, *Tetrahedron Letters*, 48, 3783-3787.
- Gao, Y., Eguchi, A., Kakehi, K., & Lee, Y. C. (2005). Synthesis and molecular recognition of carbohydrate-centered multivalent glycoclusters by a plant lectin RCA120. *Bioorg Med Chem*, 13(22), 6151-6157.
- Gopalsamy, S. & Srinivas, H. (2007), Propargyl 1,2-orthoesters as glycosyl donors: stereoselective synthesis of 1,2-*trans* glycosides and disaccharides, *Tetrahedron Letters*, 48, 6564-6568.
- Goutam, G. & Anup, K. M. (2011), Efficient glycosylation of unprotected sugars using sulfamic acid: A mild eco-friendly catalyst, *Catalysis Communications*, 14, 52-57.
- Grischenko, L. A., Parshina, L. N., Kanitskaya, L. V., Larina, L. I., Novikova, L. N., & Trofimov, B. A. (2013). Propargylation of arabinogalactan with propargyl halides--a facile route to new functionalized biopolymers. *Carbohydr Res*, 376, 7-14.
- Guchhait, G., & Misra, A. K. (2011). Efficient glycosylation of unprotected sugars using sulfamic acid: A mild eco-friendly catalyst. *Catalysis Communications*, 14(1), 52-57.
- Lee, C. W., Son, E. M., Kim, H. S., Xu, P., Batmunkh, T., Lee, B. J., & Koo, K. A. (2007). Synthetic tyrosyl gallate derivatives as potent melanin formation inhibitors. *Bioorg Med Chem Lett*, 17(19), 5462-5464.
- Li, D. F., Hu, P. P., Liu, M. S., Kong, X. L., Zhang, J. C., Hider, R. C., & Zhou, T. (2013). Design and synthesis of hydroxypyridinone-L-phenylalanine conjugates as potential tyrosinase inhibitors. *J Agric Food Chem*, 61(27), 6597-6603.
- Lu, W.-Y., Sun, X.-W., Zhu, C., Xu, J.-H., & Lin, G.-Q. (2010). Expanding the application scope of glycosidases using click chemistry. *Tetrahedron*, 66(3), 750-757.
- Lyudmila, A. G., Lidiya, N. P., Lyudmila, V. K., Lyudmila, I. L., Lyubov, N. N., & Boris, A. T. (2013), Propargylation of arabinogalactan with propargyl halides-a facile route to new functionalized biopolymers, *Carbohydrate Research*, 376, 7-14.
- Pascal, F. T., Petra, M., Henning, H. & Peter, G. J. (2007), Modification of methyl *O*-propargyl-D-glucosides: model studies for the synthesis of alkynyl based functional polysaccharides, *Carbohydrate Research*, 342, 2031-2048.
- Qin, Y., Rihui, C., Wei, Y., Liang, Y., Zhiyong, C., Lin, M. & Huacan, S. (2009), Synthesis and evaluation of 5-benzylidene(thio)barbiturate-b-D-glycosides

- as mushroom tyrosinase inhibitors, *Bioorganic & Medicinal Chemistry Letters*, *19*, 4055-4058.
- Reiko, T., Hiromi, O., Seiichi, M., Saori, T., Yukari, M., Hiroyuki, N., Yoichi, Y., Noriyoshi, M., & Ken-ichi, N. (2011), Synthesis and evaluation of bibenzyl glycosides as potent tyrosinase inhibitors, *European Journal of Medicinal Chemistry*, *46*, 1374-1381.
- Roy, B., & Mukhopadhyay, B. (2007). Sulfuric acid immobilized on silica: an excellent catalyst for Fischer type glycosylation. *Tetrahedron Letters*, *48*(22), 3783-3787.
- Sureshkumar, G., & Hotha, S. (2007). Propargyl 1,2-orthoesters as glycosyl donors: stereoselective synthesis of 1,2-trans glycosides and disaccharides. *Tetrahedron Letters*, *48*(37), 6564-6568.
- Tajima, R., Oozeki, H., Muraoka, S., Tanaka, S., Motegi, Y., Nihei, H., Nihei, K. (2011). Synthesis and evaluation of bibenzyl glycosides as potent tyrosinase inhibitors. *Eur J Med Chem*, *46*(4), 1374-1381.
- Tankam, P. F., Mischnick, P., Hopf, H., & Jones, P. G. (2007). Modification of methyl O-propargyl-D-glucosides: model studies for the synthesis of alkynyl based functional polysaccharides. *Carbohydr Res*, *342*(14), 2031-2048.
- Wen-Ya, L., Xing-Wen, S., Chen, Z., Jian-He, X., & Guo-Qiang, L. (2010), Expanding the application scope of glycosidases using click chemistry, *Tetrahedron*, *66*, 750-757.
- Wei, Y., Rihui, C., Huan, W., Qin, Y., Binhua, Z., Lin, M., & Huacan S. (2009), Discovery of 4-functionalized phenyl-O-β-D-glycosides as a new class of mushroom tyrosinase inhibitors, *Bioorganic & Medicinal Chemistry Letters*, *19*, 6157-6160.
- Yan, Q., Cao, R., Yi, W., Yu, L., Chen, Z., Ma, L., & Song, H. (2009). Synthesis and evaluation of 5-benzylidene(thio)barbiturate-β-D-glycosides as mushroom tyrosinase inhibitors. *Bioorg Med Chem Lett*, *19*(15), 4055-4058.
- Yi, W., Cao, R., Wen, H., Yan, Q., Zhou, B., Ma, L., & Song, H. (2009). Discovery of 4-functionalized phenyl-O-β-D-glycosides as a new class of mushroom tyrosinase inhibitors. *Bioorg Med Chem Lett*, *19*(21), 6157-6160.
- Yongjun, G., Atsuko, E., Kazuaki, K., & Yuan, M. L. (2005), Synthesis and molecular recognition of carbohydrate-centered multivalent glycoclusters by a plant lectin RCA₁₂₀, *Bioorganic & Medicinal Chemistry*, *13*, 6151-6157.
- Zhou, Z., Zhuo, J., Yan, S., & Ma, L. (2013). Design and synthesis of 3,5-diaryl-4,5-dihydro-1H-pyrazoles as new tyrosinase inhibitors. *Bioorg Med Chem*, *21*(7), 2156-2162.

Output / Outcome

International Publications

Rungnapha Saeeng, Onanong Siripru, Uthaiwan Sirion, IBr-Catalyzed *O*-Glycosidation of D-Glucals: Facile Synthesis of 2,3-Unsaturated-*O*-glycosides. *Heterocycles*, **2015**, *91*, 849-861.

ผลงานที่ขอจดสิทธิบัตรการประดิษฐ์ จำนวน 1 เรื่อง

การสังเคราะห์อนุพันธ์ของอะเซทิลีนไกลโคไซด์และการตรวจสอบฤทธิ์ในการยับยั้งการทำงานของเอนไซม์ไทโรซิเนส (Synthesis of acetylene glycoside analogues and evaluation for their tyrosinase inhibitory activity) เลขที่คำขอ 1701000127

การนำเสนอผลงานวิจัยแบบโปสเตอร์

Natthiya Saehlim, Anan Athipornchai, and Rungnapha Saeeng* Synthesis of Acetylene Glycoside Analogues and Evaluation for their Tyrosinase Inhibitory Activity, The 12th International Symposium on Organic Reactions and The 6th German – Japanese Symposium on Electrosynthesis, April 22-24, 2016, Kyoto, Japan.

Natthiya Saehlim, Anan Athipornchai, and Rungnapha Saeeng* Synthesis and biological evaluation of dialkynyl glycosides analogues as inhibitors of mushroom tyrosinase, The international conference on Scientific Frontiers in Natural Product Based Drugs 2017, Medical Drive, Singapore.

การนำเสนอผลงานวิจัยแบบบรรยาย

Natthiya Saehlim, Anan Athipornchai, and Rungnapha Saeeng* Design and Synthesis of Acetylene Glycoside Analogues for Study the Tyrosinase Inhibitory Activity, The 1st International Conference on Natural Medicine: From Local Wisdom to International Research, August 5-6, 2017, Bangkok, Thailand.

การผลิตบัณฑิต

นิสิตปริญญาโท ภาคเคมี คณะวิทยาศาสตร์ ม.บูรพา จำนวน 1 คน

นางสาวอรอนงค์ ศิริปรุ

นิสิตปริญญาเอก ภาคเคมี คณะวิทยาศาสตร์ ม.บูรพา จำนวน 1 คน (กำลังศึกษา)

นางสาวณัฐยา แซ่หลิม

ประวัติหัวหน้าโครงการฯ

ผู้ช่วยศาสตราจารย์ดร. รุ่งนภา แซ่เอ็ง

Assistant Prof. Rungnapha Saeeng

หน่วยงานที่อยู่ที่สามารถติดต่อได้สะดวก

ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยบูรพา

ต. แสนสุข ชลบุรี 20131

โทร 0-3810-3039มือถือ 08-1656-7524 โทรสาร 0-3839-3494

E-mail rungnaph@buu.ac.th

ประวัติการศึกษา

ปริญญาตรี เคมี มหาวิทยาลัยมหิดล พ.ศ. 2532

ปริญญาโท เคมีอินทรีย์ มหาวิทยาลัยมหิดล พ.ศ. 2538

(ทุนมูลนิธิ ศ.ดร. แถบ นีละนิธิ)

ปริญญาเอก เคมีอินทรีย์ Nagoya University พ.ศ. 2542

(ทุนรัฐบาลญี่ปุ่น Monbucho)