รายงานวิจัยฉบับสมบูรณ์

โครงการ
การค้นคว้าและพัฒนาสาร propargyl glycosides
ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว

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

รุ่งนภา แซ่เอ็งและคณะ

โครงการวิจัยประเภทงบประมาณเงินรายได้
จากเงินอุดหนุนรัฐบาล (งบประมาณแผ่นดิน)
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มหาวิทยาลัยบูรพา

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มหาวิทยาลัยบูรพา

10 กันยายน 2559

กิตติกรรมประกาศ

งานวิจัยนี้ได้รับทุนสนับสนุนการวิจัยจากงบประมาณเงินรายได้จากเงินอุดหนุนรัฐบาล
(งบประมาณแผ่นดิน) ประจำปีงบประมาณ พ.ศ. 2558 มหาวิทยาลัยบูรพา
ผ่านสำนักงานคณะกรรมการการวิจัยแห่งชาติ เลขที่สัญญา 61/2558

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คำนำ

โครงการวิจัย “การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว” ได้รับการสนับสนุนทุนการวิจัยงบประมาณแผ่นดินประจำปีงบประมาณ 2558 มหาวิทยาลัยบูรพา รายงานการวิจัยฉบับนี้เสนอรายละเอียดของการวิจัยซึ่งประกอบด้วยบทนำที่เสนอผลงานวิจัยที่เกี่ยวข้อง ผลการทดลองวิจัย การอภิปรายสรุปผล และการตรวจสอบโครงสร้างของสาร

การวิจัย “การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว” สำเร็จลุล่วงไปด้วยดี โดยผู้วิจัยต้องขอขอบคุณทีมวิจัยซึ่งประกอบด้วยที่ปรึกษาโครงการ ศ.ดร. อภิชาต สุขสำราญ คณะวิทยาศาสตร์ มหาวิทยาลัยรามคำแหง ผู้ร่วมโครงการ ดร. อนันต์ อธิพรชัย ดร. อุทัยวรรณ ศิริอ่อน รวมทั้งนิสิตปริญญาโทและเอกภาควิชาเคมี นางสาวณัฐิยา แซ่หลิม และนางสาวอรอนงค์ ศิริปรุ ขอขอบคุณ คุณสุทธิพร พิกุลทอง มหาวิทยาลัยมหิดล ที่ทำการตรวจสอบ High Resolution Mass ของสารสังเคราะห์ที่ได้ งานวิจัยนี้ได้รับการสนับสนุนจากภาควิชาเคมี คณะวิทยาศาสตร์ และศูนย์นวัตกรรมความเป็นเลิศทางเคมี PERCH-CIC

ผศ. ดร. รุ่งนภา แซ่เอ็ง
อาจารย์ประจำภาควิชาเคมี คณะวิทยาศาสตร์
หัวหน้าโครงการวิจัย

บทคัดย่อ

งานวิจัยนี้ได้ทำการวางแผนและสังเคราะห์ชุดของสาร alkynyl และ propargyl-D-glycosides ชนิดใหม่ และตรวจสอบฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase โดยมีวัตถุประสงค์เพื่อพัฒนาสาร alkynyl และ propargyl-D-glycosides ให้เป็นสารไวท์เทนนิ่งชนิดใหม่เพื่อให้ผิวขาว สารสังเคราะห์อนุพันธ์ alkynyl และ propargyl glycoside จำนวน 20 ชนิดได้ถูกเตรียมขึ้นและศึกษาฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase เพื่อความเข้าใจถึงความสัมพันธ์ของโครงสร้างที่มีผลต่อฤทธิ์ จากผลการศึกษาเบื้องต้นของสารสังเคราะห์ต่อฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase โดยใช้สาร L-tyrosine เป็นซับสเตรทเปรียบเทียบกับ alpha-Arbutin และ Kojic acid พบว่า หมู่แทนที่อัลไคน์บน C-1 และ หมู่แทนที่บน C-6 มีผลต่อฤทธิ์ จากสารสังเคราะห์อนุพันธ์ alkynyl glycoside ทั้งหมดพบว่าสาร **6b** (%tyrosinase inhibition = 97.054) แสดงฤทธิ์ดีกว่าสารอื่นและดีใกล้เคียงกับ kojic acid (%tyrosinase inhibition = 98.239) การค้นพบนี้อาจนำไปสู่สารสารไวท์เทนนิ่งเพื่อผิวขาวชนิดใหม่

Abstract

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. 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. Twenty 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 **6b** (%tyrosinase inhibition = 97.054) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (%tyrosinase inhibition = 98.239). These findings may lead to the discovery of new agent for skin-whitening.

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).

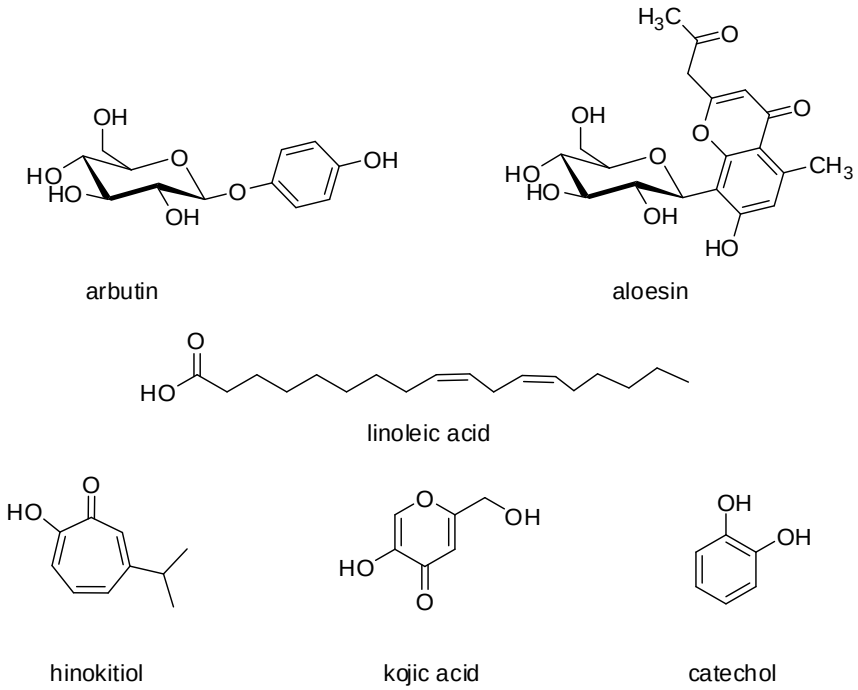


Figure 1.1 Structure of tyrosinase inhibitors

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-octyland 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 structuresand glycoconjugates and development oftyrosinase 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.

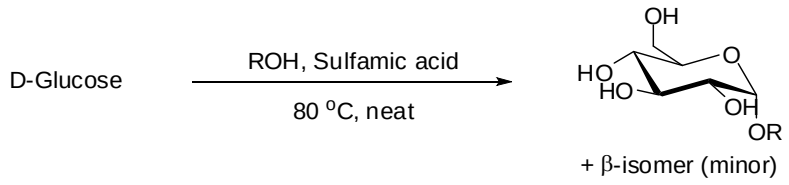


Figure 2-1 Fischer glycosylation of unprotected reducing sugars for the preparation of alkyl glycosides

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.

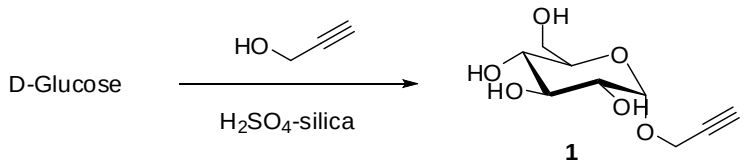


Figure 2-2 Synthesis of propargylglucoside**1**

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 transglycosylation (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%).

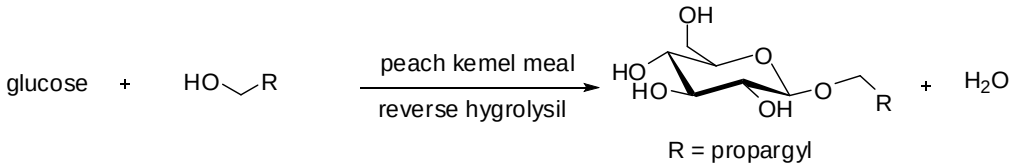


Figure 2-3 Synthesis of *β*-glucosides using fruit kernel meal

Tankam et al. (2007) prepared 4,6-*O*-benzylidene-2,3-di-*O*-propargyl-α-D-gluco-pyranoside (**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**)

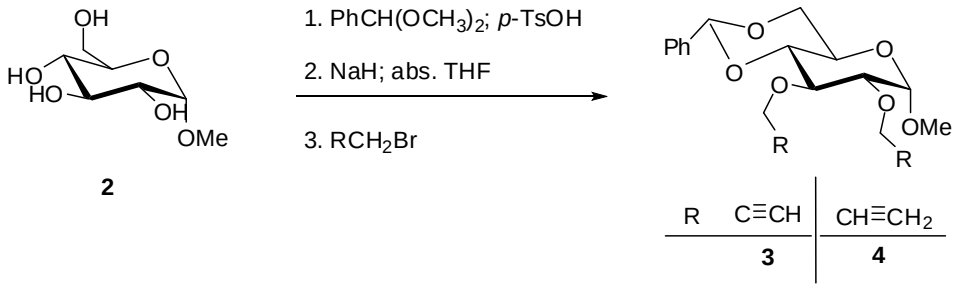


Figure 2-4 Preparation of methyl-4,6-*O*-benzylidene-2,3-di-*O*-propargyl-α-D-gluco-pyranoside(**3**) and methyl-4,6-*O*-benzylidene-2,3-di-*O*-allyl-α-D-glucopyranoside**(4**)

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/toluenein 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.

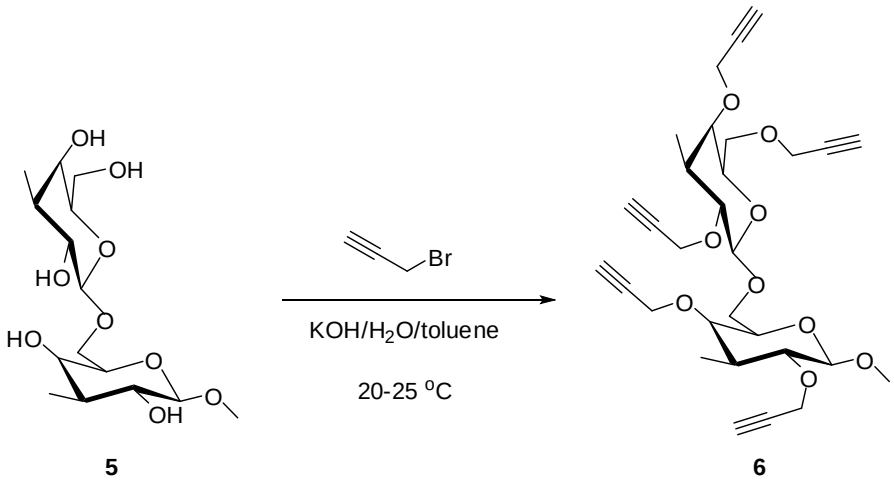


Figure 2-5 Arabinogalactan propargylation with propargyl bromide in the system 30–60% aqueous solution of KOH/toluene

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%).

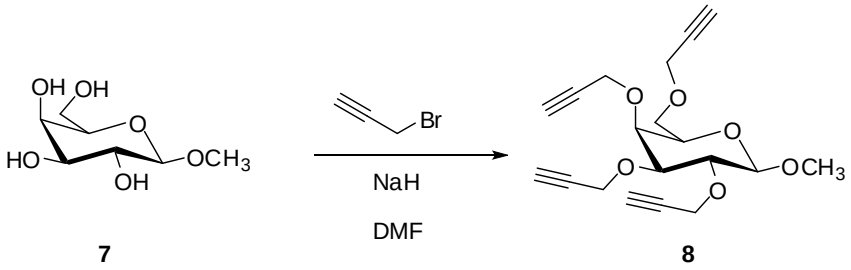


Figure 2-6 Propargylation of methyl-β-D-galactopyranoside **7**

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-dioxoleniumion and also behaves as a Lewis acid to facilitate the attack of the glycosyl acceptor.

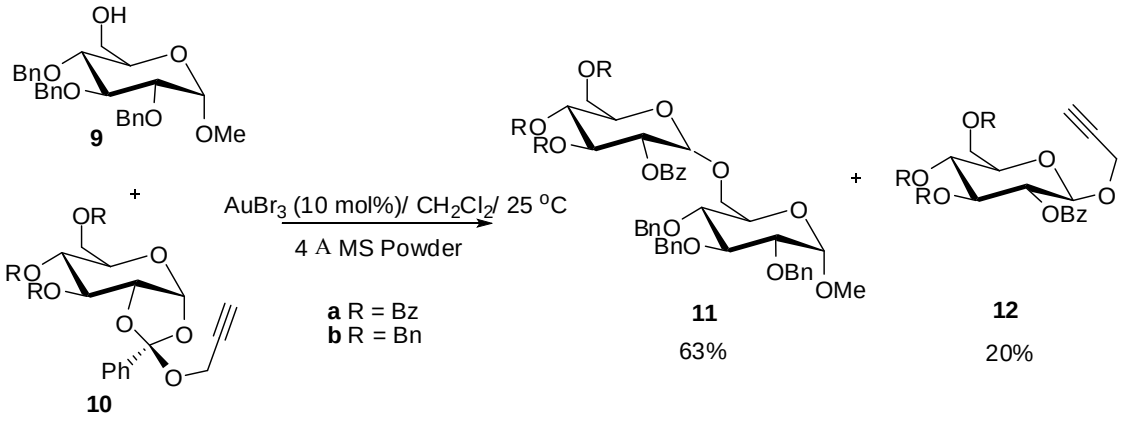


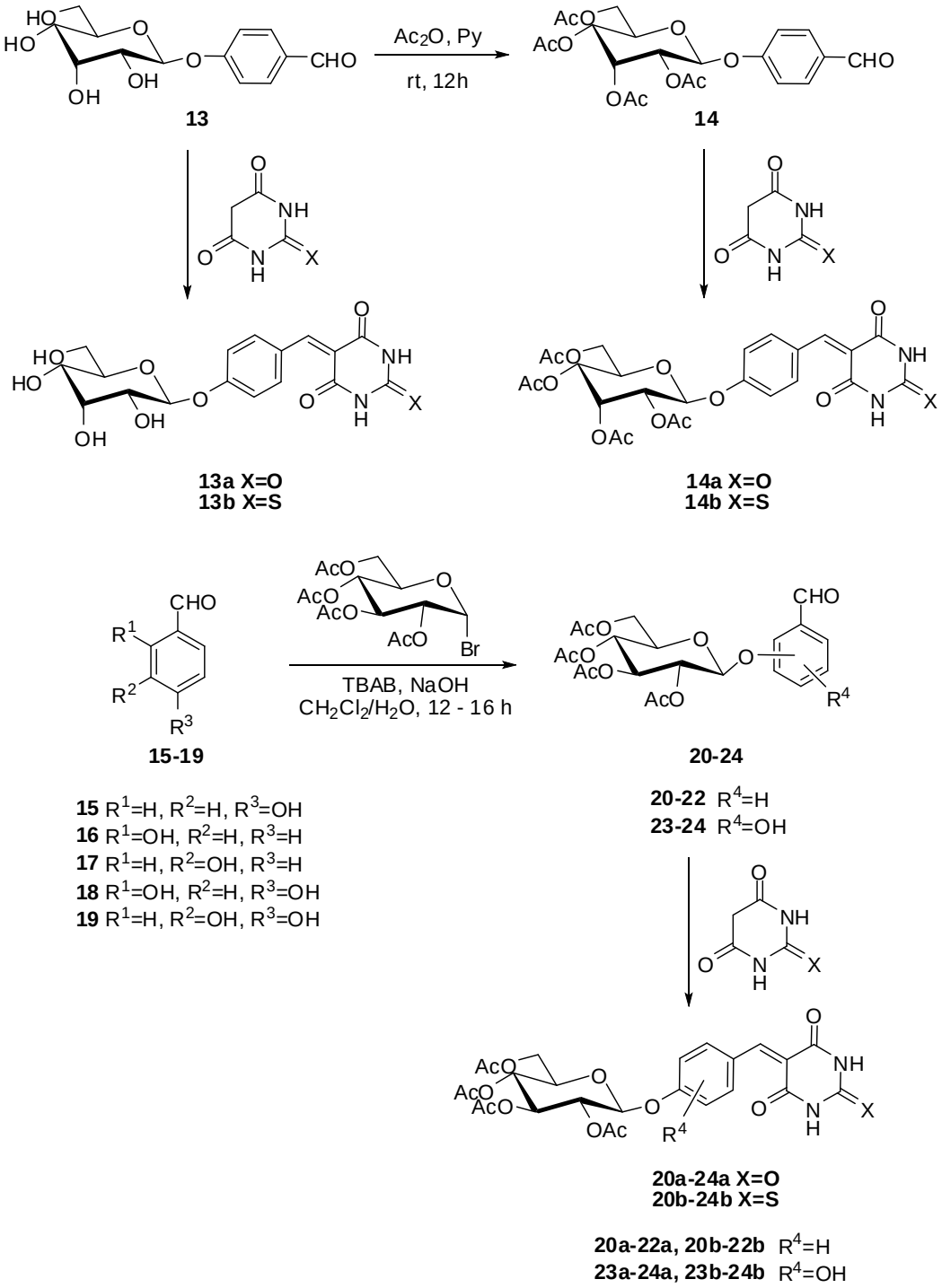
Figure 2-7 Propargyl-1,2-orthoesters as glycosyl donors

Tyrosinase is a copper-containing oxidoreductase, functioning as an important oxidizing agent. This enzyme is responsible for production of melanin, plays an important rolein 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 enzyme catalyzes the oxidation of phenolic compounds found in fruits and vegetable. It pays to the neurodegeneration associatedwith Parkinson's

disease.Thus, the development of noveltyrosinase inhibitors is of interest to agricultural, cosmetic, andmedical 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**.

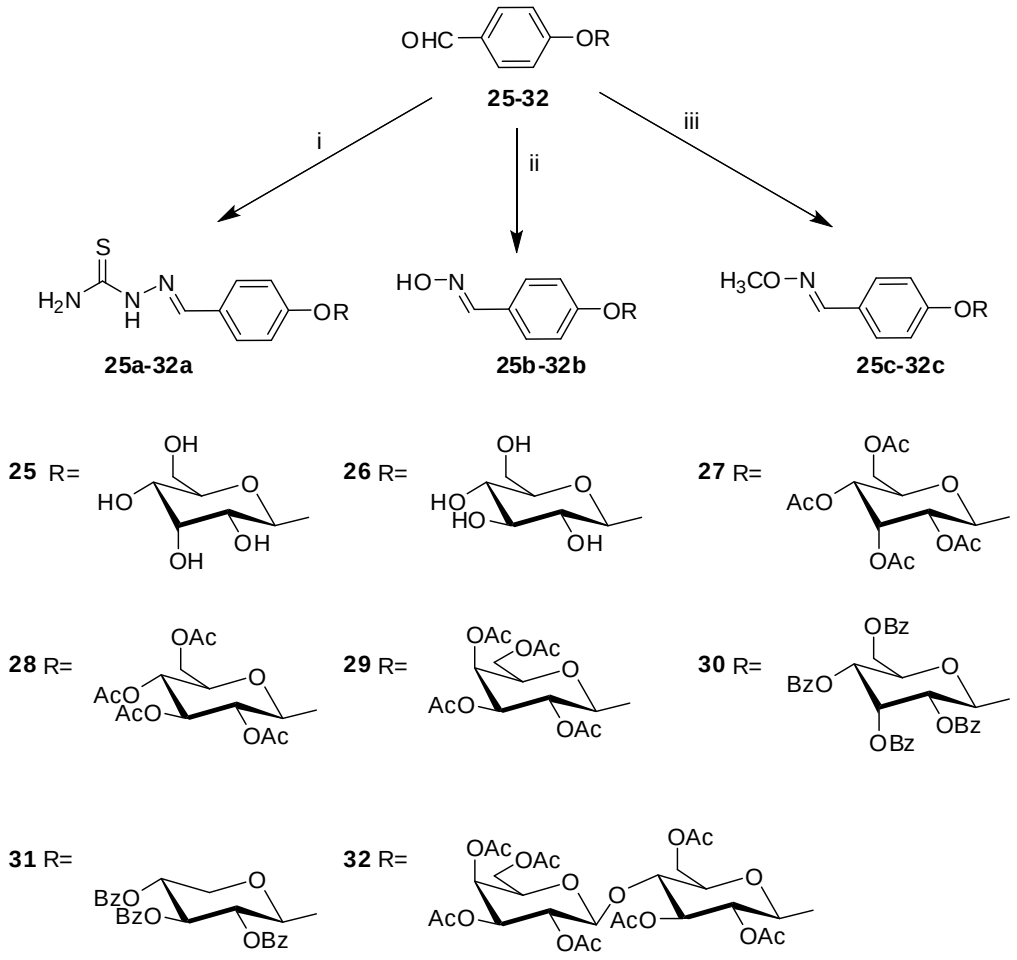
They were found that compound **24b** has the most potent tyrosinase inhibitor with IC_{50} value of 0.05 mmol/L.SARs analysis. The results indicated that 5-benzylidene thiobarbiturate substructures were efficacious for the inhibitory activity and the lipophilic property of acetylated sugar moiety facilitated inhibitory potency. In addition, the hydroxyl

group of 3′-configuration contributed to the increase ofinhibitory 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-glycosidesas 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 methyloximemoiety. 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 study the inhibitory effect and mechanismon mushroom tyrosinase.



Scheme 2.2 Synthesis of 4-functionalized phenyl-*O*-β-D-glycosides **25a–32a, 25b–32b** and **25c–32c**. Reagents and conditions: (i) $H_2NHCSNH_2$/EtOH/reflux, 5–12 h; (ii)NH_2OH:HCl/EtOH, pH 6–7, 45 ^oC, 2–6 h; (iii) NH_2OCH_3:HCl/EtOH, pH 6–7, 45 ^oC, 2–6 h.

The inhibition of 4-functionalized phenyl-*O*-β-D-glycosideson the diphenolase activity of mushroom tyrosinase was investigated by usual procedure and compared with Arbutin. The IC_{50} values of all obtained compounds were summarized in **Table 2.2**. They were found that compounds **25a–32a** bearing a thiosemicarbazide moiety exhibited potent activities with IC_{50}values range from 0.31 to 52.8 μM. Particularly,compound **28a** containing acetylated glucose moiety was found to be the most active molecule with an IC_{50} value of 0.31 μM. SARs analysis suggested that the thiosemicarbazide moiety remarkably contributed to the increase of inhibitory effects on tyrosinase. In addition, the configuration and bond type of sugar moiety also played a very important role in determining their inhibitory activities. The inhibition kinetic sand inhibition mechanism study revealed that compound

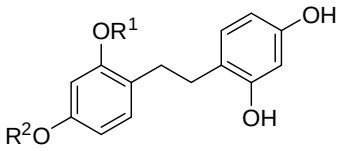
28a was reversible and competitive type inhibitor, whereas compound **32a** was reversible and competitive–uncompetitive mixed-II type inhibitor.

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

Compounds	IC_{50}[a] (μM)	Compounds	IC_{50} (μM)
25a	3.61±0.55	**29b**	>200
26a	2.96±0.62	**30b**	>200
27a	3.41±0.28	**31b**	>200
28a	0.31±0.12	**32b**	>200
29a	0.41±0.09	**25c**	>200
30a	52.8±2.2	**26c**	>200
31a	36.5±3.8	**27c**	>200
32a	0.65±0.21	**28c**	>200
25b	>200	**29c**	>200
26b	>200	**30c**	>200
27b	>200	**31c**	>200
28b	>200	**32c**	>200
Arbutin	7300±600		

[a] IC_{50} = mean ± SEM. SEM: standard error of mean.

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



33: R^1 = Xyl, R^2 = H
34: R^1 = H, R^2 = Xyl
35: R^1 = R^2 = Xyl
36: R^1 = H, R^2 = Glc
37: R^1 = H, R^2 = Cel
38: R^1 = H, R^2 = Mal
39: R^1 = R^2 = H

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

All ofthe 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.

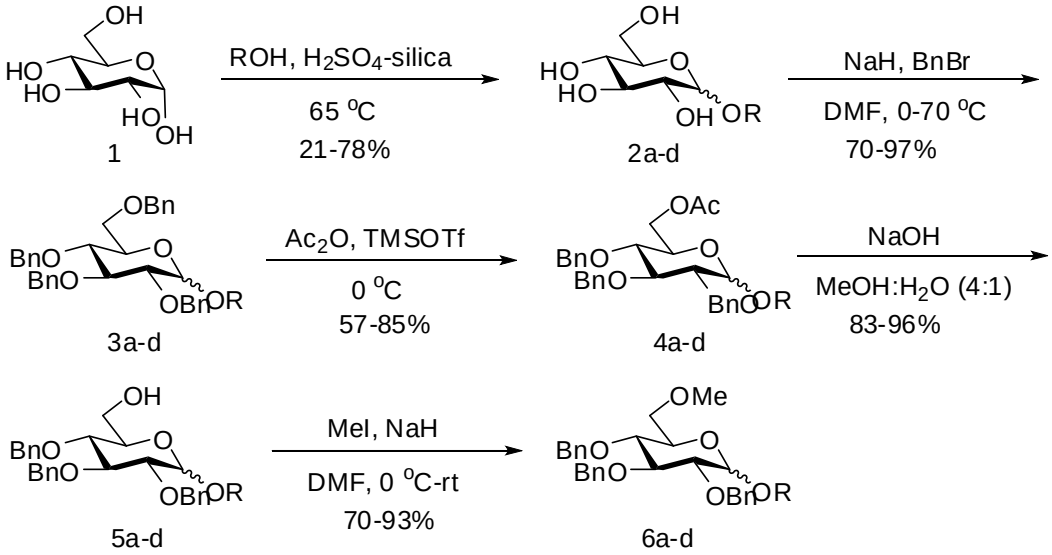
Compounds tested	IC_{50} (μM)
33	1.6±0.43[a]
34	0.43±0.18
35	0.73±0.11
36	0.77±0.04
37	0.68±0.05
38	0.83±0.06
39	0.37±0.06
Kojic acid	7.4±1.4

[a] The IC_{50} values represent means ± SE of three different experiments.

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 propargyl glycoside analogues and study for the tyrosinase inhibitory activity (Scheme 1).

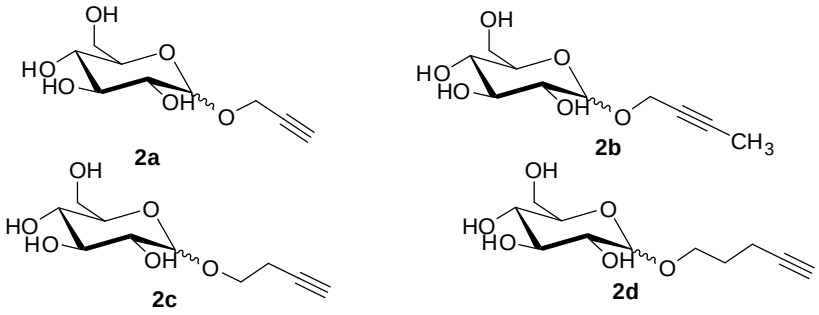
The synthesis of propargyl glycoside analogues started with glycosylation at C-1 position of glucose with propargyl, bytynyl and pentynyl alcohols using sulfuric acid on silica support. The reaction was performed at 65^{o}C to generate acetylene glycosides **2a-2d** in 21-78 %yields. Benzylation of hydroxyl 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 ^{o}C for 2 h to give alkynyl benzyl glycoside analogues **3a-3d** in high yields (70-97%). Regioselective debenzylation and acetylation at C-6 of analogues **3a-3d** by TMSOTf (0.4 equiv.) and acetic anhydride gave C-6 acetyl benzyl glycosides **4a-4d** in high yields (70-97%). Deacetylation at C-6 of glycosides **4a-4d** gave glycosides **5a-5d** in high yields using sodium hydroxide in methanol/water. In the final step, methylation of hydroxyl at C-6 of glycosides using NaH (1.2 equiv.) and iodomethane (2.0 equiv.) gave **6a-6d**.



The synthetic propargyl glycoside analogues can be devided in five groups to study for the tyrosinase inhibitory activity and to understand the structure activity relationship.

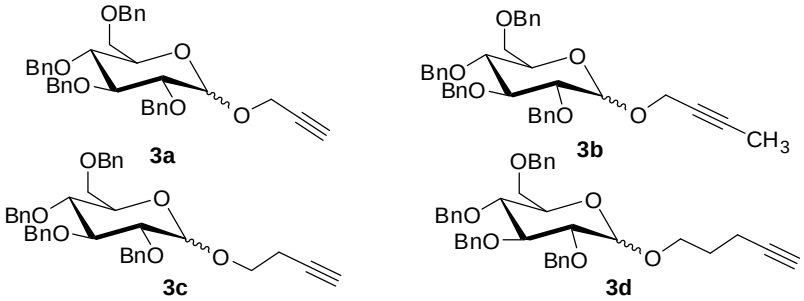
Group 1. *O*-alkynyl glycoside

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



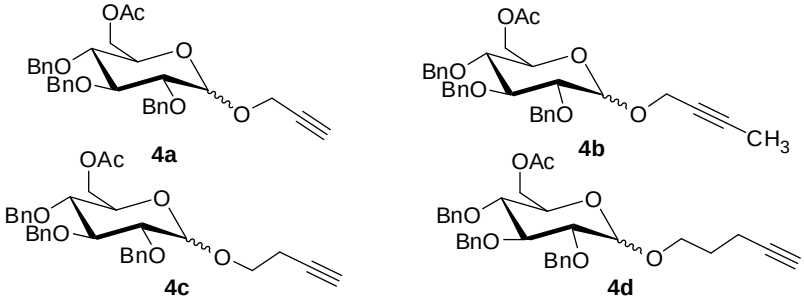
Group 2. *O*-alkynyl benzylglycoside

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



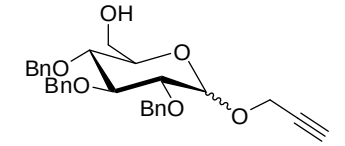
Group 3. *O*-alkynyl-6-acetyl-benzylglycoside

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

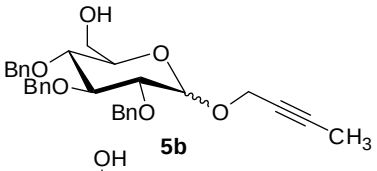


Group 4. *O*-alkynyl-6-hydroxyl-benzylglycoside

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



5a



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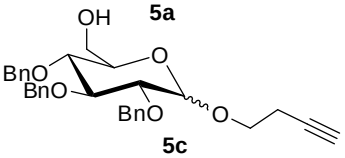

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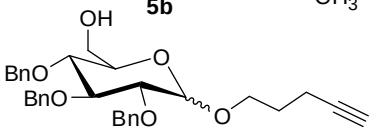
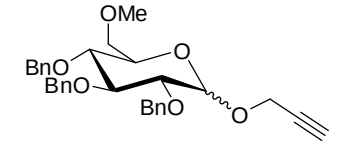


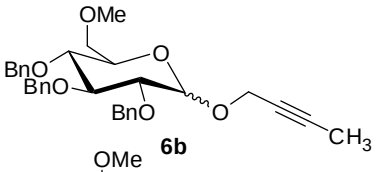
5d

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

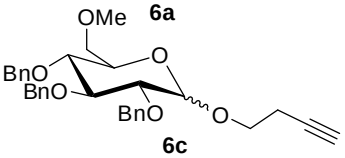
O-propargyl-6-methoxy-benzylglycoside **6a**, *O*-butynyl-6-methoxy-benzylglycoside **6b** (internal alkyne), *O*-butynyl-6-methoxy-benzylglycoside **6c** (terminal alkyne), *O*-pentynyl-6-methoxy-glycoside **6d**



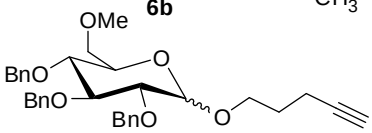
6a



6b

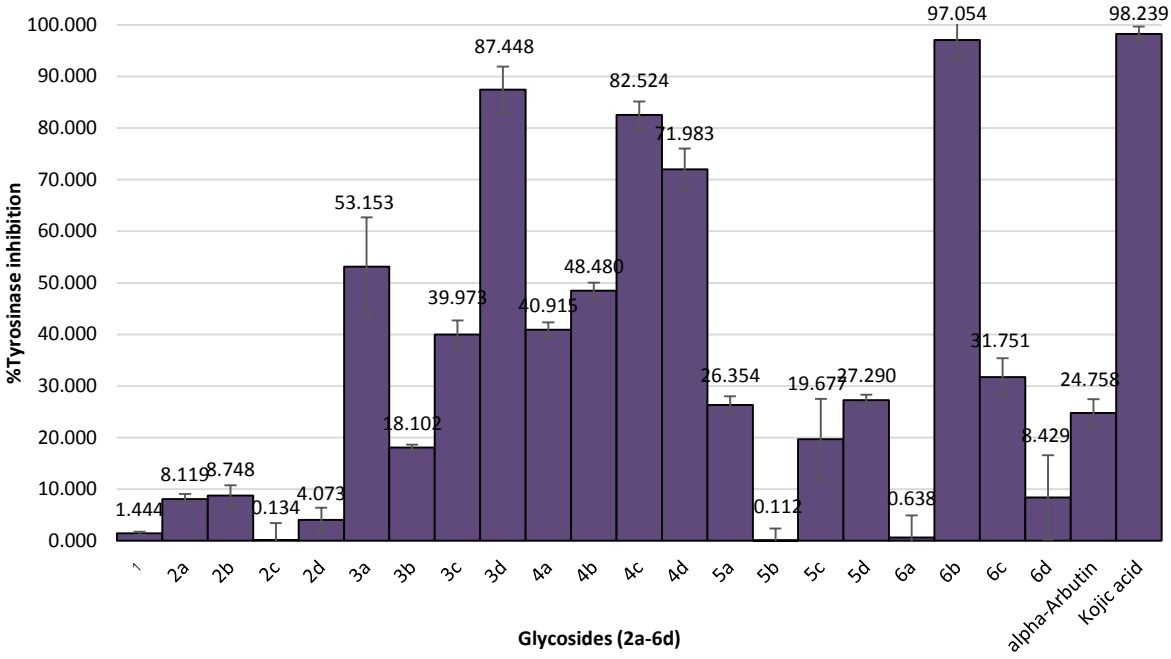


6c



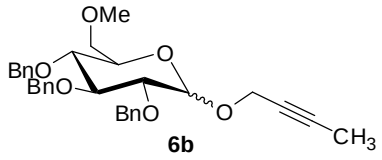
6d

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



The preliminary screening results of the effect of our twenty synthetic alkynyl and propargyl-D-glycosides on mushroom tyrosinase activity with L-tyrosine as a substrate are summarized in Chart 1. 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 no effects on the activity in this group. *O*-benzylglycosides **2a-2d** in group 2 showed moderate to good inhibitory activity against tyrosinase (%tyrosinase inhibition = 39.973-87.448) and showed better activity than alpha-Arbutin, skin whitening agent (%tyrosinase inhibition = 24.758) except compound **3b** bearing butynyl group (internal alkyne) showed low activity. *O*-alkynyl-6-acetyl-benzylglycoside **4a-4b** demonstrated significant tyrosinase inhibitory effects better than alpha-arbutin with %tyrosinase inhibition = 40.915-82.524. These results suggested that the acetyl moiety at C-6 position of sugar ring contributed to the increase of inhibitory effects. *O*-alkynyl-6-hydroxyl-benzylglycoside **5a-5b** exhibited sharply decreased inhibitory effects comparing with *O*-alkynyl-6-acetyl-benzylglycoside **4a-4b**. These results indicated that the C-6 substituted moiety contributed to the inhibitory effects. Of all synthetic alkynyl

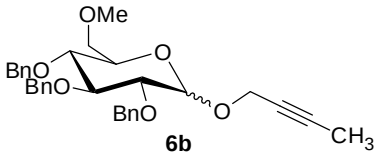
glycoside analogs, compounds 6b (%tyrosinase inhibition = 97.054) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (%tyrosinase inhibition = 98.239) whereas other 6-methoxy-benzylglycoside in the same group showed low activity.



6b

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. Twenty synthetic alkynyl and propargyl glycoside analogues were prepared and devided in five 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 **6b** (%tyrosinase inhibition = 97.054) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (%tyrosinase inhibition = 98.239). These findings may lead to the discovery of therapeutically potent agents against clinically dermatological disorders including hyperpigmentation as well as skin melanoma.

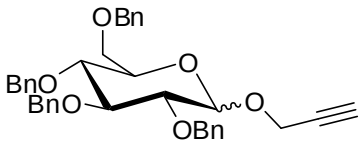


6b

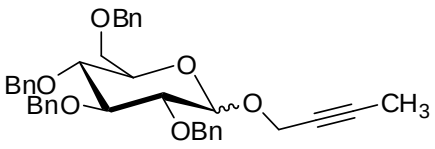
Chapter 4 Compound characterizations

General

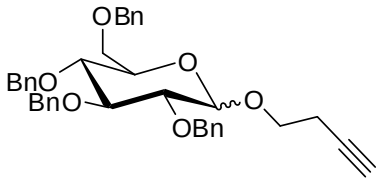
Proton NMR spectra were recorded on a BRUKER AVANCE (400 MHz). All spectra were measured in $CDCl_3$ solvent and chemical shifts are reported as δ values in parts per million (ppm) relative to tetramethylsilane (δ 0.00) or $CDCl_3$ (δ 7.26) as internal standard. Data are reported as follows; chemical shift (multiplicity, integrate intensity or assignment, coupling constants in Hz, assignment).



Compound 3a:A pale yellow oil (4.8777 g, 70 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ: 7.40-7.10(40H, m, Ph, isomer A, isomer B), 5.08(1H, d,*J* = 3.6 Hz, H-1, isomer A), 4.98(1H,d,*J* =10.8 Hz, CH-Ph), 4.97(1H,d, *J* =10.8 Hz, CH-Ph),4.93(1H,d, *J* =10.8 Hz, CH-Ph),4.84(1H,d, *J* =10.8 Hz, CH-Ph),4.83(1H,d,*J* =10.8 Hz, CH-Ph),4.81(1H,d, *J* =10.8 Hz, CH-Ph),4.77(1H,d, *J* =10.8 Hz, CH-Ph),4.76(1H,d, *J* =12.0 Hz, CH-Ph),4.71(1H,d, *J* =12.0 Hz, CH-Ph),4.70(1H,d, *J* =12.0 Hz, CH-Ph),4.68(1H,d, *J* =10.8 Hz, CH-Ph), 4.63 (1H,d, *J* =10.8 Hz, CH-Ph),4.62 (1H,d, *J* =10.8 Hz, CH-Ph),4.61 (1H,d, *J* =10.8 Hz, CH-Ph),4.55 (1H, d, *J* = 8.0 Hz, H-1, isomer B), 4.52 (1H, d, *J* = 10.8 Hz, CH-Ph), 4.49 (1H, dd, *J* = 10.5, 2.5 Hz, H-6b, isomer B), 4.48 (1H, d, *J* = 10.8 Hz, CH-Ph), 4.39-4.33 (1H, m, H-5, isomer B), 4.27 (4H, d, *J* = 2.4 Hz, CH_2-≡, isomer A, isomer B), 3.98 (1H, t, *J* = 9.2 Hz, H-3, isomer A), 3.79 (1H, ddd, *J* = 10.4, 3.6,2.0 Hz, H-5, isomer A), 3.76 (1H, dd, *J* = 10.5, 2.0 Hz, H-6a, isomer B), 3.72 (1H, dd, *J* = 10.4, 3.6 Hz, H-6b, isomer A), 3.70 (1H, t, *J* = 8.0 Hz, H-3, isomer B), 3.68 (1H, t, *J* = 8.0 Hz, H-4, isomer B), 3.67 (1H, t, *J* = 9.2 Hz, H-4, isomer A), 3.65 (1H, dd, *J* = 10.4, 2.0 Hz, H-6a, isomer A), 3.60 (1H, dd, *J* = 10.4, 3.6 Hz, H-2, isomer A), 3.49 (1H, t, *J* = 8.0 Hz, H-2, isomer B), 2.43(2H, t, *J* = 2.4 Hz, ≡-H, isomer A, isomer B)

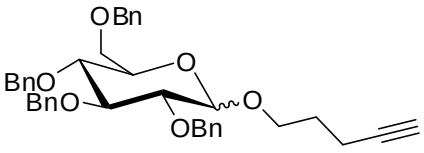


Compound 3b:A colorless oil (2.4861 g, 97 %yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35-7.04 (40H, m, Ph, isomer A, isomer B), 5.00 (1H, d,*J* = 3.5 Hz, H-1,isomer A), 4.92 (1H,d,*J* =10.5 Hz, CH-Ph), 4.91 (1H,d, *J* =10.5 Hz, CH-Ph),4.86 (1H,d, *J* =10.5 Hz, CH-Ph),4.76 (1H,d, *J* =10.5 Hz, CH-Ph),4.74 (1H,d,*J* =10.5 Hz, CH-Ph),4.73 (1H,d, *J* =10.5 Hz, CH-Ph),4.71 (1H,d, *J* =10.5 Hz, CH-Ph),4.69 (1H,d, *J* =12.0 Hz, CH-Ph),4.66 (1H,d, *J* =12.0 Hz, CH-Ph),4.63 (1H,d, *J* =12.0 Hz, CH-Ph),4.61 (1H,d, *J* =10.5 Hz, CH-Ph),4.55 (1H, d, *J* =8.0 Hz, H-1, isomer B),4.54 (1H,d, *J* =12.0 Hz, CH-Ph),4.53 (1H,d, *J* =12.0 Hz, CH-Ph),4.49 (1H, d, *J* = 10.5 Hz, CH-Ph), 4.46 (1H, dd, *J* = 11.5, 3.5 Hz, H-6b, isomer B), 4.39 (1H, d, *J* = 10.5 Hz, CH-Ph), 4.38 (1H, d, *J* = 12.0 Hz, CH-Ph), 4.33 (1H, ddd, *J* = 11.5, 3.5, 2.0 Hz, H-5, isomer B), 4.15 (4H, dd, *J* = 3.5, 1.5 Hz, CH_2-≡, isomer A, isomer B), 3.92 (1H, t, *J* = 9.0 Hz, H-3, isomer A), 3.71(1H, ddd, *J* = 10.0, 3.5, 1.5 Hz, H-5, isomer A), 3.67 (1H, dd, *J* = 11.5, 2.0 Hz, H-6a, isomer B), 3.65 (1H, dd, *J* = 10.5, 3.5 Hz, H-6b, isomer A), 3.63 (1H, t, *J* = 8.0 Hz, H-3, isomer B), 3.61 (1H, t, *J* = 8.0 Hz, H-4, isomer B), 3.57 (1H, t, *J* = 9.0 Hz, H-4, isomer A), 3.55 (1H, dd, *J* = 10.5, 1.5 Hz, H-6a, isomer A), 3.52 (1H, dd, *J* = 9.0, 3.5 Hz, H-2, isomer A), 3.41 (1H, t, *J* = 8.0 Hz, H-2, isomer B), 1.78 (6H, s, ≡-CH_3, isomer A, isomer B)

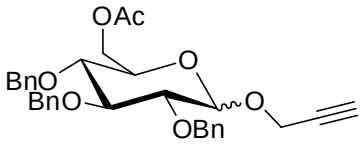


Compound 3c: A colorless oil (1.3449 g, 88 %yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.40-7.10 (40H, m, Ph, isomer A, isomer B),5.00 (1H,d,*J* =5.5 Hz, CH-Ph),4.98 (1H,d,*J* =5.5 Hz, CH-Ph),4.92 (1H,d,*J* =5.5 Hz, CH-Ph),4.83 (1H,d,*J* =5.5 Hz, CH-Ph),4.82 (1H,d,*J* =7.5 Hz, CH-Ph),4.81 (1H,d,*J* =5.5 Hz, CH-Ph),4.79 (1H,d,*J* =5.5 Hz, CH-Ph),4.78 (1H, d,*J* = 3.5 Hz, H-1, isomer A),4.71 (1H,d,*J* =5.5 Hz, CH-Ph),4.68 (1H,d,*J* =5.5 Hz, CH-Ph),4.65 (1H,d,*J* =5.5 Hz, CH-Ph),4.60 (1H,d,*J* =5.5 Hz, CH-Ph),4.57 (1H,d,*J* =8.0 Hz, H-1, isomer B),4.54 (1H,d,*J* =5.5 Hz, CH-Ph),4.52 (1H,d,*J* =5.5 Hz, CH-Ph),4.47 (1H,d,*J* =5.5 Hz, CH-Ph),4.46 (1H,d,*J* =7.5 Hz, CH-Ph),4.45 (1H,d,*J* =5.5 Hz, CH-Ph),4.08-4.00 (1H, m, H-5, isomer B), 3.98 (1H, t, *J* = 4.5 Hz, H-3, isomer A), 3.85-3.80 (1H, m, H-5, isomer A), 3.77-3.70 (4H, m, O-CH_2, isomer A, isomer B),3.67 (1H, t, *J* = 5.0 Hz, H-4, isomer A), 3.66 (2H, dd, *J* = 5.0, 2.5 Hz, H-6, isomer B), 3.64 (1H, t, *J* = 4.0 Hz, H-3, isomer B), 3.62 (2H, dd, *J* = 4.0, 1.5 Hz, H-6, isomer A), 3.57 (1H, t, *J* = 4.0 Hz, H-4, isomer B), 3.56 (2H, dd, *J* = 4.5, 1.5 Hz, H-2,

isomer A), 3.46 (1H, t, *J* = 4.0 Hz, H-2, isomer B), 2.60-2.49 (4H, m, CH_2-≡, isomer A, isomer B),1.96 (2H, brs, ≡-H, isomer A, isomer B)

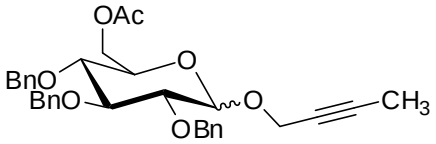


Compound 3d: A pale yellow oil (1.8660 g, 81 %yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ:7.40-7.12 (40H, m, Ph, isomer A, isomer B),4.98 (1H,d,*J* =11.0 Hz, CH-Ph),4.98 (1H,d,*J* =11.0 Hz, CH-Ph),4.92 (1H,d,*J* =11.0 Hz, CH-Ph),4.91 (1H,d,*J* =11.0 Hz, CH-Ph),4.83 (1H,d,*J* =11.0 Hz, CH-Ph),4.81 (1H,d,*J* =11.0 Hz, CH-Ph),4.78 (1H,d,*J* =11.0 Hz, CH-Ph),4.77 (1H, d,*J* = 3.0 Hz, H-1, isomer A),4.76 (1H,d,*J* =11.0 Hz, CH-Ph),4.71 (1H,d,*J* =11.0 Hz, CH-Ph),4.64 (1H,d,*J* =12.0 Hz, CH-Ph),4.62 (1H,d,*J* =12.0 Hz, CH-Ph),4.59 (1H,d,*J* =11.0 Hz, CH-Ph),4.57 (1H, d,*J* =8.5 Hz, H-1, isomer B),4.55 (1H,d,*J* =12.0 Hz, CH-Ph),4.53 (1H,d,*J* =11.0 Hz, CH-Ph),4.48 (1H,d,*J* =11.0 Hz, CH-Ph),4.46 (1H,d,*J* =11.0 Hz, CH-Ph),4.40 (1H,d,*J* =8.0 Hz, CH-Ph), 4.09-4.00 (1H, m, H-5, isomer B), 3.97 (1H, t, *J* = 9.0 Hz, H-3, isomer A), 3.81-3.75 (1H, m, H-5, isomer A), 3.75-3.70 (4H, m, O-CH_2, isomer A, isomer B), 3.69 (1H, t, *J* = 9.0 Hz, H-4, isomer A), 3.66 (1H, t, *J* = 8.0 Hz, H-3, isomer B), 3.63 (2H, dd, *J* = 9.0, 3.0 Hz, H-6, isomer A), 3.57 (2H, dd, *J* = 9.0, 3.0 Hz, H-6, isomer B),3.55 (2H, dd, *J* = 9.0, 3.0 Hz, H-2, isomer A), 3.50 (1H, t, *J* = 8.0 Hz, H-4, isomer B), 3.45 (1H, t, *J* = 8.0 Hz, H-2, isomer B), 2.38-2.23 (4H, m, CH_2-≡, isomer A, isomer B), 1.94(1H, t,*J* = 2.5 Hz, ≡-H, isomer A), 1.93 (1H, t,*J* = 2.4 Hz, ≡-H, isomer B), 1.90-1.75 (4H, m, -CH_2-, isomer A, isomer B)

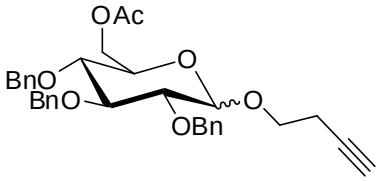


Compound 4a: A pale yellow oil (2.0299 g, 57 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ: 7.40-7.23 (30H, m, Ph, isomer A, isomer B), 5.05 (1H, d,*J* = 3.5 Hz, H-1, isomer A),5.01 (1H,d,*J* =11.0 Hz, CH-Ph),4.89 (1H,d,*J* =12.0 Hz, CH-Ph),4.88 (1H,d,*J* =11.0 Hz, CH-Ph),4.83 (1H,d,*J* =11.0 Hz, CH-Ph),4.82 (1H,d,*J* =11.0 Hz, CH-Ph),4.76 (1H,d,*J* =12.0 Hz, CH-Ph),4.74 (1H,d,*J* =11.0 Hz, CH-Ph),4.71 (1H,d,*J* =12.0 Hz, CH-Ph),4.69 (1H,d,*J* =11.0 Hz, CH-Ph), 4.65 (1H, d,*J* =8.0 Hz, H-1, isomer B), 4.64 (1H,d,*J* =11.0 Hz, CH-Ph),4.57 (1H,d,*J*

=11.0 Hz, CH-Ph),4.56 (1H,d,*J* =11.0 Hz, CH-Ph),4.48-4.32 (1H, m, H-5, isomer B), 4.30-4.20 (12H, m, H-6, CH_2-≡, isomer A, isomer B), 4.02 (1H, t, *J* = 9.5 Hz, H-3, isomer A), 3.90-3.82 (2H, m,H-5, isomer A, H-3, isomer B),3.72-3.64 (2H, m, H-4, isomer B), 3.58 (1H, dd, *J* =9.5, 3.5 Hz, H-2, isomer A),3.57 (1H, t, *J* = 9.5 Hz, H-4, isomer A), 3.50 (1H, t, *J* = 9.5 Hz, H-2, isomer B),2.45 (2H, brs,≡-H, isomer A,isomer B), 2.02 (6H, brs, OAc, isomer A, isomer B)

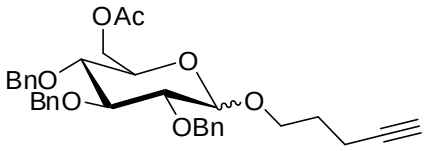


Compound 4b: A pale yellow oil (0.1066 g, 85 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ: 7.40-7.24 (40H, m, Ph, isomer A, isomer B), 5.06 (1H, d,*J* = 3.5 Hz, H-1, isomer A), 5.02 (1H,d, *J* =10.5 Hz, CH-Ph),4.99 (1H,d, *J* =10.5 Hz, CH-Ph),4.89 (1H,d, *J* =10.5 Hz, CH-Ph),4.87 (1H,d, *J* =10.5 Hz, CH-Ph),4.83 (1H,d, *J* =10.5 Hz, CH-Ph),4.82 (1H,d, *J* =10.5 Hz, CH-Ph),4.79 (1H, d, *J* =8.5 Hz, H-1, isomer B),4.77 (1H,d, *J* =12.0 Hz, CH-Ph),4.74 (1H,d, *J* =10.5 Hz, CH-Ph),4.71 (1H,d, *J* =12.0 Hz, CH-Ph),4.64 (1H,d, *J* =12.0 Hz, CH-Ph),4.57 (1H,d, *J* =10.5 Hz, CH-Ph),4.55 (1H,d, *J* =10.5 Hz, CH-Ph),4.30-4.16 (7H, m, H-6, CH_2-≡, isomer A, isomer B), 4.02 (1H, t, *J* = 9.5 Hz, H-3, isomer A), 3.97 (1H, t, *J* = 9.5 Hz, H-3, isomer B),3.95-3.90 (1H, m, H-5, isomer B),3.89-3.82 (1H, m, H-5, isomer A),3.67 (1H, dd, *J* = 9.5, 4.0 Hz, H-6a, isomer A),3.57 (1H, dd, *J* = 9.5, 3.5 Hz, H-2, isomer A),3.54 (1H, t, *J* = 9.5 Hz, H-4, isomer B),3.49 (1H, t, *J* = 9.5 Hz, H-2, isomer B),2.02 (6H, brs, OAc, isomer A, isomer B),1.86(6H, s, ≡-CH_3, isomer A, isomer B)

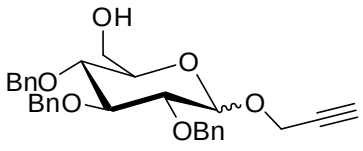


Compound 4c: A pale yellow oil (0.3710 g, 83 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.23 (30H, m, Ph, isomer A, isomer B),5.01 (1H,d,*J* =10.5 Hz, CH-Ph),5.00 (1H,d,*J* =10.5 Hz, CH-Ph),4.96 (1H,d,*J* =10.5 Hz, CH-Ph),4.88 (1H,d,*J* =10.5 Hz, CH-Ph),4.83 (1H,d,*J* =12.0 Hz, CH-Ph),4.79 (1H,d,*J* =12.0 Hz, CH-Ph),4.76 (1H, d,*J* = 3.5 Hz, H-1, isomer A),4.75 (1H,d,*J* =12.0 Hz, CH-Ph),4.72 (1H,d,*J* =10.5 Hz, CH-Ph),4.69 (1H,d,*J* =12.0 Hz, CH-Ph),4.66 (1H,d,*J* =12.0 Hz, CH-Ph),4.56 (1H,d,*J* =10.5 Hz, CH-Ph),4.55 (1H,d,*J* =10.5

Hz, CH-Ph),4.44 (1H, d, *J* =8.0 Hz, H-1, isomer B),4.35-4.28 (1H, m, H-5, isomer B),4.27-4.20 (3H, m, H-6,isomer A, isomer B),4.01 (1H, t, *J* = 9.0 Hz, H-3, isomer A),3.95-3.90 (1H, m, H-5, isomer A), 3.75-3.68 (4H, m, O-CH_2, isomer A, isomer B), 3.65 (1H, t, *J* = 9.0 Hz, H-4, isomer A),3.62 (1H, t, *J* = 9.5 Hz, H-3, isomer B),3.54 (2H, dd, *J* = 9.0, 3.5 Hz, H-2, isomer A),3.52 (1H, dd, *J* = 8.5, 3.0 Hz, H-6a, isomer A),3.47 (1H, t, *J* = 9.5 Hz, H-4, isomer B),3.45 (1H, t, *J* = 9.5 Hz, H-2, isomer B),2.56-2.50 (4H, m, CH_2-≡, isomer A, isomer B), 2.04 (2H, brs, ≡-H, isomer A, isomer B),2.02 (6H, brs, OAc, isomer A, isomer B)

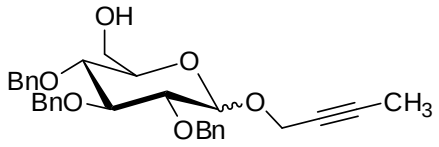


Compound 4d: A pale yellow oil (0.3763g, 38 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.25 (30H, m, Ph, isomer A, isomer B),5.01 (1H,d,*J* =10.5 Hz, CH-Ph),4.95 (1H,d,*J* =10.5 Hz, CH-Ph),4.92 (1H,d,*J* =10.5 Hz, CH-Ph),4.88 (1H,d,*J* =10.5 Hz, CH-Ph),4.86 (1H,d,*J* =10.5 Hz, CH-Ph),4.85 (1H,d,*J* =10.5 Hz, CH-Ph),4.82 (1H,d,*J* =11.0 Hz, CH-Ph),4.80 (1H,d,*J* =11.0 Hz, CH-Ph),4.78 (1H, d,*J* = 3.5 Hz, H-1, isomer A),4.74 (1H,d,*J* =11.0 Hz, CH-Ph),4.71 (1H,d,*J* =10.5 Hz, CH-Ph),4.65 (1H,d,*J* =11.0 Hz, CH-Ph),4.56 (1H,d,*J* =10.5 Hz, CH-Ph),4.41 (1H, d,*J* =8.0 Hz, H-1, isomer B),4.35-4.29 (1H, m, H-5, isomer B),4.29-4.20 (4H, m, H-6,isomer A, isomer B),4.00 (1H, t, *J* = 9.0 Hz, H-3, isomer A),3.89-3.82 (1H, m, H-5, isomer A), 3.80-3.63 (4H, m, O-CH_2, isomer A, isomer B),3.54 (2H, dd, *J* = 9.0, 3.5 Hz, H-2, isomer A),3.51 (1H, t, *J* = 9.0 Hz, H-4, isomer A),349 (1H, t, *J* = 9.0 Hz, H-3, isomer B),3.46 (1H, t, *J* = 9.0 Hz, H-4, isomer B),3.44 (1H, t, *J* = 9.0 Hz, H-2, isomer B),2.37-2.30 (4H, m, CH_2-≡, isomer A, isomer B),2.05 (3H, s, OAc, isomer A),2.02 (3H, s, OAc, isomer B)**,** 1.96(2H, t,*J* = 2.5 Hz, ≡-H, isomer A, isomer B),1.90-1.80 (4H, m, -CH_2-, isomer A, isomer B)

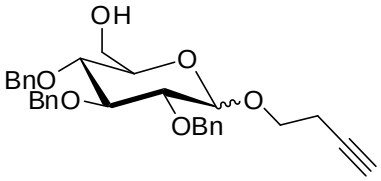


Compound 5a: A pale yellow oil (0.7148 g, 83 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.35-7.22 (30H, m, Ph, isomer A, isomer B),5.01 (1H, d,*J* = 3.6 Hz, H-1, isomer A),4.96 (1H,d,*J* =10.8 Hz, CH-Ph),4.92 (1H,d,*J* =10.8 Hz, CH-Ph),4.90 (1H,d,*J* =10.8 Hz, CH-Ph),4.85

(1H,d,*J* =10.8 Hz, CH-Ph),4.83 (1H,d,*J* =10.8 Hz, CH-Ph),4.79 (1H,d,*J* =10.8 Hz, CH-Ph),4.77 (1H,d,*J* =10.8 Hz, CH-Ph),4.76 (1H,d,*J* =10.8 Hz, CH-Ph),4.70 (1H,d,*J* =10.8 Hz, CH-Ph),4.67 (1H,d,*J* =10.8 Hz, CH-Ph),4.66 (1H, d, *J* = 8.0 Hz, H-1, isomer B),4.65 (1H,d,*J* =10.8 Hz, CH-Ph),4.60 (1H,d,*J* =10.8 Hz, CH-Ph),4.23 (4H, d, *J* = 2.4 Hz, CH_2-≡, isomer A, isomer B),3.98 (1H, t, *J* = 9.6Hz, H-3, isomer A),3.72 (2H, dd, *J* = 12.8, 3.6 Hz, H-2, isomer A),3.70-3.62 (4H, m, H-4, H-5, isomer A, isomer B),3.53-3.48 (5H, m, H-3,isomer B, H-6,isomer A, isomer B),3.42 (1H, t, *J* = 9.0 Hz, H-2, isomer B),2.43 (2H, t, *J* = 2.4 Hz, ≡-H, isomer A, isomer B)

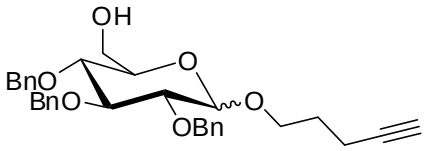


Compound 5b: A pale yellow oil (0.1490 g, 85 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.26 (30H, m, Ph, isomer A, isomer B),5.05 (1H, d,*J* = 3.6 Hz, H-1, isomer A),5.00 (2H,d,*J* =11.2 Hz, CH_2-Ph),5.00 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.89 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.83 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.81 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.78 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.75 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.71 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.64 (2H,d,*J* =11.2 Hz, CH_2-Ph),4.55 (1H, d, *J* =8.0 Hz, H-1, isomer B),4.23 (4H, dd, *J* = 3.5, 1.5 Hz, CH_2-≡, isomer A, isomer B),4.02 (2H, t, *J* = 9.2 Hz, H-3, isomer A, isomer B),3.77-3.67 (6H, m, H-2, H-5, H-6a, isomer A, isomer B),3.56-3.51 (4H, m, H-4, H-6b, isomer A, isomer B),1.86(6H, s, ≡-CH_3, isomer A, isomer B)**.**

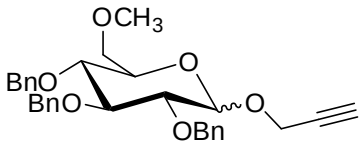


Compound 5c: A pale yellow oil (0.1590 g, 82 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.26 (30H, m, Ph, isomer A, isomer B),5.00(1H,d,*J* =10.5 Hz, CH-Ph),4.98(1H,d,*J* =10.5 Hz, CH-Ph),4.94(1H,d,*J* =10.5 Hz, CH-Ph),4.90(1H,d,*J* =11.0 Hz, CH-Ph),4.87(1H,d,*J* =10.5 Hz, CH-Ph),4.85(1H,d,*J* =10.5 Hz, CH-Ph),4.81(1H,d,*J* =11.0 Hz, CH-Ph),4.80(1H,d,*J* =11.0 Hz, CH-Ph),4.76(1H, d,*J* = 3.5 Hz, H-1, isomer A),4.72(1H,d,*J* =10.5 Hz, CH-Ph),4.67(1H,d,*J* =11.0 Hz, CH-Ph),4.65(1H,d,*J* =10.5 Hz, CH-Ph),4.64(1H,d,*J* =10.5 Hz, CH-Ph),4.49 (1H, d,

J =8.0 Hz, H-1, isomer B),4.05-3.97 (2H, m, H-3, H-5, isomer A),3.87 (1H, dd, *J* = 11.5, 2.5 Hz, H-6b, isomer A),3.80-3.65 (5H, m, O-CH_2, isomer A, isomer B,H-6, isomer B),3.62 (1H, t, *J* = 8.0 Hz, H-4, isomer B),3.60 (1H, dd, *J* = 7.0, 3.0 Hz, H-6a, isomer B),3.59 (1H, t, *J* = 9.5 Hz, H-4, isomer A),3.56 (2H, dd, *J* = 9.5, 3.5 Hz, H-2, isomer A), 3.51 (1H, t, *J* = 8.0 Hz, H-3, isomer B), 3.52 (1H, dd, *J* = 11.5, 3.0 Hz, H-6a, isomer A),3.43 (1H, t, *J* = 4.0 Hz, H-2, isomer B),3.40-3.35 (1H, m, H-5, isomer B),2.58-2.50 (4H, m, CH_2-≡, isomer A, isomer B), 1.98 (2H, dd, ≡-H, *J* = 4.0, 2.0 Hz isomer A, isomer B)

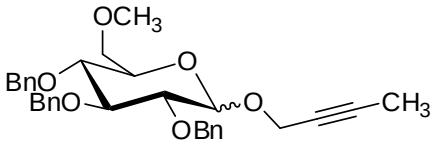


Compound 5d: A pale yellow oil (0.2210 g, 96 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.36-7.22 (30H, m, Ph, isomer A, isomer B),4.97(2H,d,*J* =11.2 Hz, CH_2-Ph),4.86(2H,d,*J* =11.2 Hz, CH_2-Ph),4.81(2H,d,*J* =11.2 Hz, CH_2-Ph),4.78(2H,d,*J* =11.2 Hz, CH_2-Ph),4.75(2H,d,*J* =11.2 Hz, CH_2-Ph),4.68(1H, d,*J* = 3.6 Hz, H-1, isomer A), 4.62(2H,d,*J* =11.2 Hz, CH_2-Ph),4.41 (1H, d,*J* =8.0 Hz, H-1, isomer B),3.97 (2H, t, *J* = 9.2 Hz, H-3, isomer A, isomer B),3.76-3.71(4H, m,H-6b, isomer A, isomer B),3.69-3.65 (4H, m,O-CH_2, isomer A, isomer B),3.52-3.43 (6H, m, H-2, H-4, H-6a, isomer A, isomer B),2.34-2.28 (4H, m, CH_2-≡, isomer A, isomer B), 1.96(2H, t,*J* = 2.5 Hz, ≡-H, isomer A, isomer B),1.90-1.80 (4H, m, -CH_2-, isomer A, isomer B)

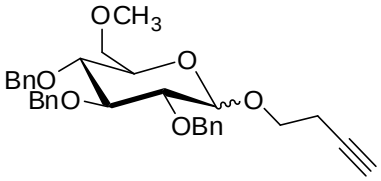


Compound 6a: A pale yellow oil (0.0460 g, 85 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.35-7.22 (30H, m, Ph, isomer A, isomer B),5.02(1H, d,*J* = 3.6 Hz, H-1, isomer A),4.96 (1H,d,*J* =11.2 Hz, CH-Ph),4.94(1H,d,*J* =11.2 Hz, CH-Ph),4.90(1H,d,*J* =11.2 Hz, CH-Ph),4.85 (1H,d,*J* =11.2 Hz, CH-Ph),4.84(1H,d,*J* =11.2 Hz, CH-Ph),4.79(1H,d,*J* =11.2 Hz, CH-Ph),4.74(1H,d,*J* =11.2 Hz, CH-Ph),4.73(1H,d,*J* =11.2 Hz, CH-Ph),4.70(1H,d,*J* =11.2 Hz, CH-Ph),4.66 (1H, d, *J* = 8.0 Hz, H-1, isomer B),4.61(1H,d,*J* =11.2 Hz, CH-Ph),4.57 (1H,d,*J* =11.2 Hz, CH-Ph),4.56(1H,d,*J* =11.2 Hz, CH-Ph),4.23 (4H, d, *J* = 2.0 Hz, CH_2-≡, isomer A, isomer B),3.96 (1H, t, *J* = 9.2Hz, H-3, isomer A),3.72-3.70 (2H, m, H-5, isomer A,isomer

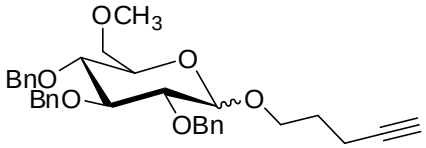
B),3.61-3.54(6H, m, H-2, H-4, H-3, H-4, H-6b,isomer A, isomer B),3.50 (2H, m, H-6a,isomer A, isomer B),3.30(6H, s, OCH_3,isomer A, isomer B), 2.41 (2H, t, *J* = 2.4 Hz, ≡-H, isomer A, isomer B)



Compound 6b: A pale yellow oil (0.0287 g, 93 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.22 (30H, m, Ph, isomer A, isomer B),5.03 (1H, d,*J* = 3.6 Hz, H-1, isomer A),4.98(2H,d,*J* =11.2 Hz, CH_2-Ph),4.86(2H,d,*J* =11.2 Hz, CH_2-Ph),4.81(2H,d,*J* =11.2 Hz, CH_2-Ph),4.76(2H,d,*J* =12.0 Hz, CH_2-Ph),4.69(2H,d,*J* =12.0 Hz, CH_2-Ph),4.57(2H,d,*J* =12.0 Hz, CH_2-Ph),4.55 (1H, d, *J* =8.0 Hz, H-1, isomer B),4.21 (4H, dd, *J* = 4.4, 2.0 Hz, CH_2-≡, isomer A, isomer B),3.98 (2H, t, *J* = 9.6 Hz, H-3, isomer A, isomer B),3.75-3.72 (2H, m, H-5, isomer A,isomer B),3.62-3.54(6H, m, H-2, H-4, H-6a, isomer A, isomer B),3.52-3.49(2H, m, H-6a, isomer A, isomer B),3.32(6H, s, OCH_3,isomer A, isomer B), 1.84(6H, t,*J* =2.0 Hz,≡-CH_3, isomer A, isomer B)



Compound 6c: A pale yellow oil (0.0434 g, 70 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.40-7.26 (30H, m, Ph, isomer A, isomer B),5.01(1H,d,*J* =10.8 Hz, CH-Ph),4.99(1H,d,*J* =10.8 Hz, CH-Ph),4.94(1H,d,*J* =10.8 Hz, CH-Ph),4.93(1H,d,*J* =10.8 Hz, CH-Ph),4.88 (1H,d,*J* =10.8 Hz, CH-Ph),4.84(1H,d,*J* =10.8 Hz, CH-Ph),4.83(1H,d,*J* =10.8 Hz, CH-Ph),4.81(1H,d,*J* =10.8 Hz, CH-Ph),4.79(1H,d,*J* =10.8 Hz, CH-Ph),4.77(1H, d,*J* = 3.5 Hz, H-1, isomer A),4.72(1H,d,*J* =10.8 Hz, CH-Ph),4.68(1H, d,*J* = 3.5 Hz, H-1, isomer A),4.66 (1H, d,*J* = 3.5 Hz, H-1, isomer A),4.60(1H, d,*J* = 3.5 Hz, H-1, isomer A),4.45 (1H, d, *J* =8.0 Hz, H-1, isomer B),4.01-3.96 (2H, m, H-3, H-5, isomer A),3.82-3.71 (4H, m,H-6b, O-CH_2, isomer A, isomer B),3.65-3.52 (7H, m,H-2, isomer A, H-3, H-5, isomer B, H-4, H-6a, isomer A, isomer B),3.44 (1H, t, *J* = 4.0 Hz, H-2, isomer B),3.34 (6H, s, OCH_3, isomer A, isomer B),2.55-2.52 (4H, m, CH_2-≡, isomer A, isomer B), 1.97 (2H, t, ≡-H, *J* = 2.4 Hz isomer A, isomer B)



Compound 6d: A pale yellow oil (0.0290 g, 88 %yield), ^{1}H NMR (400 MHz, $CDCl_3$)δ:7.30-7.20 (30H, m, Ph, isomer A, isomer B),4.91(1H,d,*J* =11.2 Hz, CH-Ph),4.85(1H,d,*J* =11.2 Hz, CH-Ph),4.80(1H,d,*J* =11.2 Hz, CH-Ph),4.78(1H,d,*J* =11.2 Hz, CH-Ph),4.75(1H,d,*J* =11.2 Hz, CH-Ph),4.73(1H,d,*J* =11.2 Hz, CH-Ph),4.70(1H,d,*J* =11.2 Hz, CH-Ph),4.67(1H, d,*J* = 3.6 Hz, H-1, isomer A),4.66(1H,d,*J* =11.2 Hz, CH-Ph),4.65(1H,d,*J* =11.2 Hz, CH-Ph),4.63(1H,d,*J* =11.2 Hz, CH-Ph),4.56(1H,d,*J* =11.2 Hz, CH-Ph),4.53(1H,d,*J* =11.2 Hz, CH-Ph),4.32(1H, d,*J* =8.0 Hz, H-1, isomer B),3.91-3.87 (2H, m, H-3, H-5, isomer A),3.71-3.65 (4H, m, H-6b, O-CH_2, isomer A, isomer B),3.57-3.32 (8H, m, H-3, H-5, isomer B,H-2, H-4, H-6a, isomer A, isomer B), 3.26 (6H, s, OCH_3, isomer A, isomer B),2.27-2.23 (4H, m, CH_2-≡, isomer A, isomer B), 1.88(2H, t,*J* = 2.5 Hz, ≡-H, isomer A, isomer B),1.81-1.74(4H, m, -CH_2-, isomer A, isomer B)

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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.

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

การผลิตบัณฑิต

นิสิตปริญญาโท ภาคเคมี คณะวิทยาศาสตร์ ม.บูรพา จำนวน 1 คน

นางสาวอรอนงค์ ศิริปรุ

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

นางสาวณัฐิยา แซ่หลิม

รายงานสรุปการเงิน

เลขที่โครงการระบบบริหารงานวิจัย 177520 สัญญาเลขที่ 61/2558

โครงการวิจัยประเภทงบประมาณเงินรายได้จากเงินอุดหนุนรัฐบาล (งบประมาณแผ่นดิน)

ประจำปีงบประมาณ พ.ศ. 2558

มหาวิทยาลัยบูรพา

ชื่อโครงการ: การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว

ชื่อหัวหน้าโครงการวิจัยผู้รับทุน ผศ.ดร. รุ่งนภา แซ่เอ็ง

รายงานในช่วงตั้งแต่วันที่ 1 มกราคม 2557 ถึง วันที่ 1 กันยายน 2559

ระยะเวลาดำเนินการ 1 ปี ตั้งแต่วันที่ 1 ตุลาคม 2557 ถึง วันที่ 30 กันยายน 2558

จำนวนเงินที่ได้รับ

งวดที่ 1 (50% มหาวิทยาลัยหัก 10% ออกแล้ว) 387,000 บาท เมื่อ 13/01/58

งวดที่ 2 (50% มหาวิทยาลัยหัก 10% ออกแล้ว) 309,600 บาท เมื่อ 11/08/58

งวดที่ 3 -

รวม 696,600 บาท

<u>รายจ่าย</u>

หมวด	งบประมาณที่ตั้งไว้	งบประมาณที่ใช้จริง	จำนวนเงินคงเหลือ/เกิน
1. ค่าตอบแทน	84,000	84,000	-
2. ค่าจ้าง	187,200	187,200	-
3. ค่าวัสดุ	357,800	357,800	-
4. ค่าใช้สอย	145,000	145,000	-
5. ค่าครุภัณฑ์	-	-	-
6. ค่าใช้จ่ายอื่นๆ -ค่าธรรมเนียม 10%	86,000	86,000	-
รวม	860,000	860,000	-

(ผศ.ดร. รุ่งนภา แซ่เอ็ง)

หัวหน้าโครงการวิจัยผู้รับทุน

บทสรุปสำหรับผู้บริหาร
(Executive Summary)

ข้าพเจ้า ผศ. ดร. รุ่งนภา แซ่เอ็ง ได้รับทุนสนับสนุนโครงการวิจัย จากมหาวิทยาลัยบูรพา ประเภทงบประมาณเงินรายได้ จากเงินอุดหนุนรัฐบาล (งบประมาณแผ่นดิน) มหาวิทยาลัยบูรพา โครงการวิจัยเรื่อง การค้นคว้าและพัฒนาสาร propargyl glycosides ให้เป็นสารกลุ่มใหม่สำหรับเครื่องสำอางค์ผิวขาว
Discovery and develop propargyl glycoside as a new class of skin-whitening agents
รหัสโครงการ 177520 สัญญาเลขที่ 61/2558 ได้รับงบประมาณรวมทั้งสิ้น 860,000 บาท (แปดแสนหกหมื่นบาทถ้วน)

ระยะเวลาดำเนินการ 1 ปี ตั้งแต่วันที่ 1 ตุลาคม 2557 ถึง วันที่ 30 กันยายน 2558

บทคัดย่อ

งานวิจัยนี้ได้ทำการวางแผนและสังเคราะห์ชุดของสาร alkynyl และ propargyl-D-glycosides ชนิดใหม่ และตรวจสอบฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase โดยมีวัตถุประสงค์เพื่อพัฒนาสาร alkynyl และ propargyl-D-glycosides ให้เป็นสารไวท์เทนนิ่งชนิดใหม่เพื่อให้ผิวขาว สารสังเคราะห์อนุพันธ์ alkynyl และ propargyl glycoside จำนวน 20 ชนิดได้ถูกเตรียมขึ้นและศึกษาฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase เพื่อความเข้าใจถึงความสัมพันธ์ของโครงสร้างที่มีผลต่อฤทธิ์ จากผลการศึกษาเบื้องต้นของสารสังเคราะห์ต่อฤทธิ์การยับยั้งเอ็นไซม์ tyrosinase โดยใช้สาร L-tyrosine เป็นซับสเตรทเปรียบเทียบกับ alpha-Arbutin และ Kojic acid พบว่า หมู่แทนที่อัลไคน์บน C-1 และ หมู่แทนที่บน C-6 มีผลต่อฤทธิ์ จากสารสังเคราะห์อนุพันธ์ alkynyl glycoside ทั้งหมดพบว่าสาร **6b** (%tyrosinase inhibition = 97.054) แสดงฤทธิ์ดีกว่าสารอื่นและดีใกล้เคียงกับ kojic acid (%tyrosinase inhibition = 98.239) การค้นพบนี้อาจนำไปสู่สารสารไวท์เทนนิ่งเพื่อผิวขาวชนิดใหม่

Abstract

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. 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. Twenty 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 **6b** (%tyrosinase inhibition = 97.054) exhibited the best activity over other compounds and demonstrated inhibitory potential on mushroom tyrosinase comparable to kojic acid (%tyrosinase inhibition = 98.239). These findings may lead to the discovery of new agent for skin-whitening.

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.

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

การผลิตบัณฑิต

นิสิตปริญญาโท ภาคเคมี คณะวิทยาศาสตร์ ม.บูรพา จำนวน 1 คน

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

นางสาวณัฐิยา แซ่หลิม