Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Design, modification of phyllanthone derivatives as anti-diabetic and cytotoxic agents

Ngoc-Hong Nguyen, Van-Giau Vo, Hoang-Vinh-Truong Phan, Thanh-The Ngo, Jirapast Sichaem, Thi-Phuong Nguyen, Huu-Hung Nguyen, Duc-Dung Pham, Tien-Cong Nguyen, Van-Kieu Nguyen & Thuc-Huy Duong

To cite this article: Ngoc-Hong Nguyen, Van-Giau Vo, Hoang-Vinh-Truong Phan, Thanh-The Ngo, Jirapast Sichaem, Thi-Phuong Nguyen, Huu-Hung Nguyen, Duc-Dung Pham, Tien-Cong Nguyen, Van-Kieu Nguyen & Thuc-Huy Duong (2020): Design, modification of phyllanthone derivatives as anti-diabetic and cytotoxic agents, Natural Product Research, DOI: 10.1080/14786419.2020.1788023

To link to this article: https://doi.org/10.1080/14786419.2020.1788023

View supplementary material

Published online: 01 Jul 2020.

Submit your article to this journal

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=gnpl20

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1788023

Taylor & Francis
Taylor & Francis Group

Check for updates

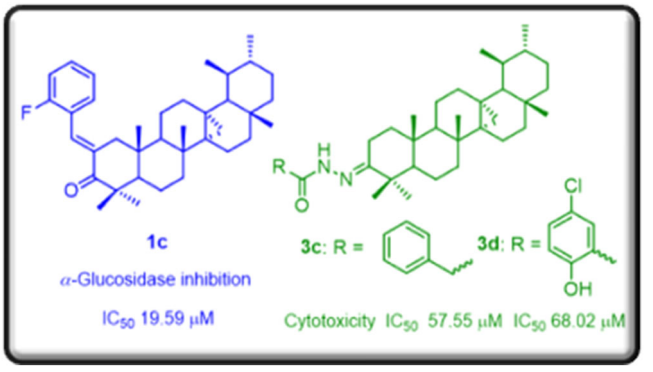
Design, modification of phyllanthone derivatives as anti-diabetic and cytotoxic agents

Ngoc-Hong Nguyen[a], Van-Giau Vo[b], Hoang-Vinh-Truong Phan[c], Thanh-The Ngo[c], Jirapast Sichaem[d], Thi-Phuong Nguyen[e], Huu-Hung Nguyen[f], Duc-Dung Pham[c], Tien-Cong Nguyen[c], Van-Kieu Nguyen[g,h] and Thuc-Huy Duong[c]

[a]CirTech Institute, Ho Chi Minh City University of Technology (HUTECH), Ho ChiMinh City, Vietnam; [b]Department of BionanoTechnology, Gachon Medical Research Institute, Gachon University, Seongnam, Korea; [c]Department of Chemistry, University of Education, Ho Chi Minh City, Vietnam; [d]Research Unit in Natural Products Chemistry and Bioactivities, Faculty of Science and Technology, Thammasat University Lampang Campus, Lampang, Thailand; [e]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam; [f]Faculty of Technology, Van Lang University, Ho Chi Minh City, Vietnam; [g]Institute of Fundamental and Applied Sciences, Duy Tan University, Ho Chi Minh City, Vietnam; [h]Faculty of Natural Sciences, Duy Tan University, Da Nang, Vietnam

ABSTRACT

Twelve benzylidene derivatives, one Baeyer-Villiger oxidative, six imine derivatives were successfully designed and synthesised from phyllanthone. In the search for potential new anti-diabetic agents, phyllanthone along with its benzylidene and oxidation analogues were evaluated for enzyme inhibition against α-glucosidase. In the benzylidene series, most analogues displayed stronger activity than the mother compound. Compound **1c** revealed the strongest activity, outperforming the acarbose positive control with an IC_{50} value of 19.59 μM. Phyllanthone and its derivatives were then tested for cytotoxic activity against the K562 cell line. The imine analogues displayed the most powerful cytotoxic activity with **3c**and **3d** having IC_{50} values of 57.55 and 68.02 μM, respectively.



ARTICLE HISTORY
Received 28 March 2020
Accepted 11 June 2020

KEYWORDS
Phyllanthus (Phyllanthaceae); phyllanthone derivatives; α-glucosidase inhibition; cytotoxic activity

CONTACT Van-Kieu Nguyen nguyenvankieu2@duytan.edu.vn; Thuc-Huy Duong huydt@hcmue.edu.vn
Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1788023
© 2020 Informa UK Limited, trading as Taylor & Francis Group

1. Introduction

Diabetes mellitus (DM), characterised by the high blood sugar level is associated with long-term damage, dysfunction, and failure of various organs, i.e. the eyes, kidneys, nerves, heart and blood vessels if left untreated (Tahlan and Verma 2017). There are three main types of DM: type I, type II and gestational diabetes. α-Glucosidase inhibitors demonstrated large clinical importance in the treatment of type II diabetes, and this therapeutic class is mainly sustained by acarbose, miglitol and voglibose, the three of them being derived from natural products. This enzyme converted disaccharide into glucose to catalyse the breakage of the α-1,4-glycosidic bonds (Van Beers et al. 1995; Sun et al. 2015). Thus, the inhibition of this enzyme led to a decrease in the rate of glucose absorption for controlling the level of glucose in type II diabetes (Sun et al. 2015; Yousefi et al. 2015).

Triterpenoids are a large class of natural isoprenoids, which exhibit a wide range of biological activities including anti-inflammatory, anticancer, antiviral, antibacterial, antimycotic, antiulcerotic, virostatic, cytotoxic properties (Dzubak et al. 2006), and enzyme inhibitory properties (Ohigashi et al. 1986; Tokuda et al. 1986; Lee et al. 1994; Sohn et al. 1995; Hsu et al. 1997; Cárdenas et al. 2004; Harmand et al. 2005; Dalla Vechia et al. 2009). Ursane-type triterpenoids and their analogues are highly bioactive, acting as a cytotoxin (Yonemoto et al. 2014) and antimicrobials (Wang et al. 1993; Woldemichael et al. 2003). They act as anti-HIV agents (Kashiwada et al. 2000), inhibitors of tumorigenesis (Huang et al. 1994) and enzymes including PTP1B (Na et al. 2010), 5-lipoxygenase (Schweizer et al. 2000), DNA polymerase β lyase (Chaturvedula et al. 2004), cyclooxygenase-2 (Ringbom et al. 1998), elastase (Sun et al. 2006). However, anti-diabetic activity against α-glucosidase has not yet been reported.

The genus *Phyllanthus* (Phyllanthaceae), comprising approximately 900 species (Unander et al. 1995; Calixto et al. 1998) widely used in folk medicine to treat kidney problems, urinary bladder disturbances, intestinal infections, diabetes and the hepatitis B virus (Calixto et al. 1998) is a rich source of triterpenoids (Calixto et al. 1998). Phyllanthone and phyllanthol, ursane-type triterpenoids having a 3-membered ring at C-13/C-14 (Chiang et al. 2001; Ndlebe et al. 2008), are common in two members of the genus *Phyllanthus*: *Phyllanthus polyanthus* (Ndlebe et al. 2008) and *Phyllanthus acidus* (Duong et al. 2019; Sengupta and Mukhopadhyay 1966). To the best of our knowledge, very few compounds containing a cyclopropyl component at C-13/C-14 have been found in nature (Kojima et al. 1988; Chiang et al. 2001; Ndlebe et al. 2008; Ali et al. 2015). To date, only 3-acetylphyllanthol, a derivative of phyllanthol, has been synthesised (Ndlebe et al. 2008) and no phyllanthone derivatives have been reported. In this study, we prepared phyllanthone analogues *via* aldolization, Baeyer-Villiger oxidation, and imine formation. Furthermore, the products were evaluated the biological activities as α–glucosidase inhibition using Baker's yeast method of Nguyen et al. (Nguyen et al. 2011) and cytotoxic activity towards the cancer cell line K562 using MTT method (Nguyen et al. 2019) to investigate the new anti-diabetic and cytotoxic agents.

2. Results and discussion

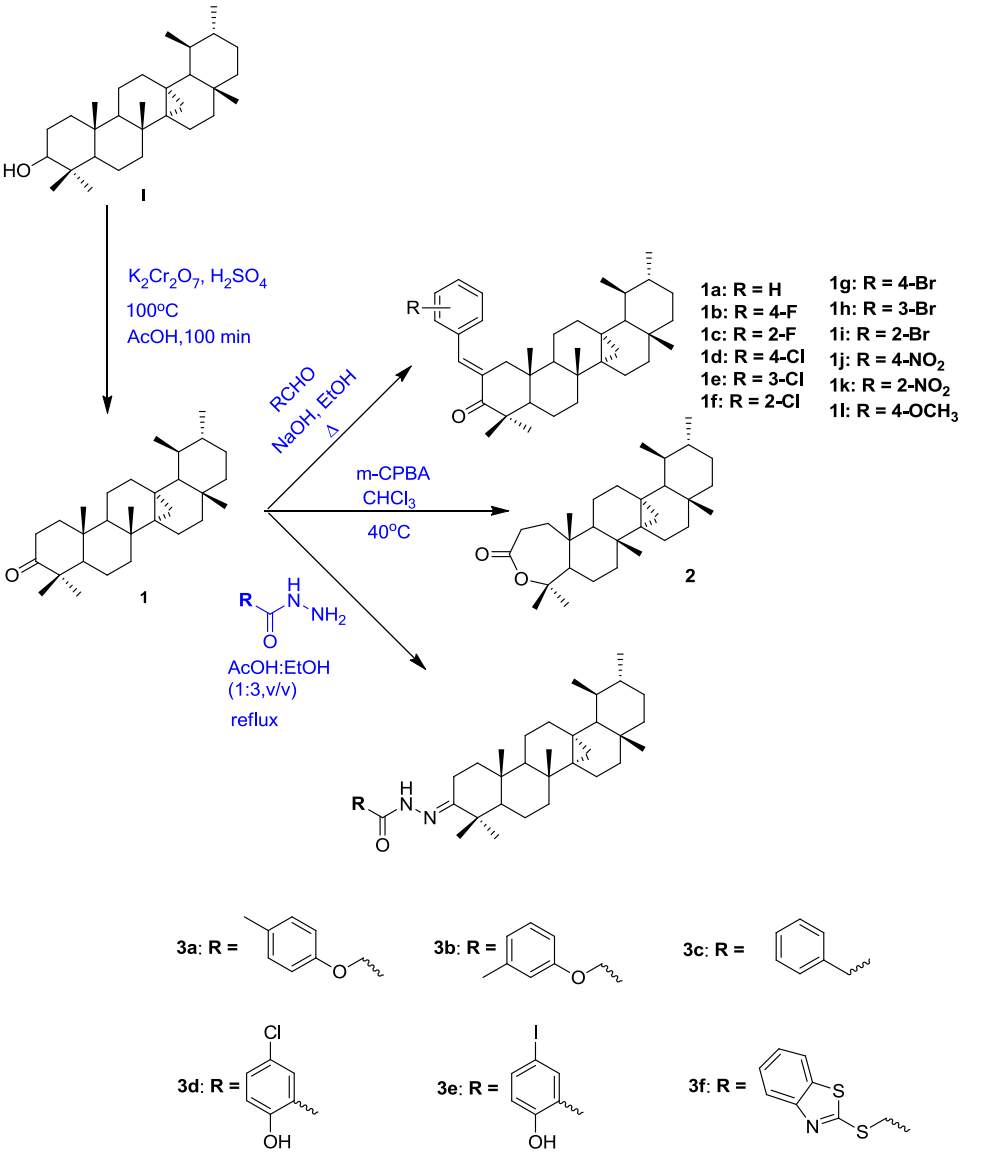
In this study, 19 new derivatives (**1a–1l**, **2**, and **3a–3f**) from phyllanthone (**1**) were successfully synthesised *via* three different routes (Scheme 1). The first route yielded benzylidene derivatives of phyllanthone, **1a–1l**. Phyllanthone (**1**) was subjected to aldol condensation with aromatic aldehydes, including benzaldehyde, 4-fluorobenzaldehyde, 2-fluorobenzaldehyde, 4-chlorobenzaldehyde, 3-chlorobenzaldehyde, 2-chlorobenzaldehyde, 4-bromobenzaldehyde, 3-bromobenzaldehyde, 2-bromobenzaldehyde, 4-nitrobenzaldehyde, 2-nitrobenzaldehyde, and 4-methoxybenzaldehyde. This produced **1a**–**1l** with isolated yields of 36–75.2%. Compound **1a** was readily identified from its 1D-NMR spectra. Comparison of the ^{1}H-NMR of **1a** and **1** showed that the diastereotopic methylene at δ_H 2.94 (*d*, 1H, $J = 16.5$ Hz, H-1a) and 2.18 (*d*, 1H, $J = 16.0$ Hz, H-1b), assignable to 1-CH_2 in **1a**, were replaced by the methylenes, 1-CH_2 and 2-CH_2 in **1**. The NMR data of synthetic compounds **1b**–**1l** were consistent with those of **1** and were in full accordance with their illustrated structures. The synthesis routes are shown as Scheme 1.

To explore for further derivatives, the carbonyl carbon at C-3 was modified through oxidation using *m*-CPBA of **1** *via* Baeyer-Villiger oxidation, followed by nucleophilic addition with different hydrazides: 2-(*p*-tolyloxy)acetohydrazide, 2-(*m*-tolyloxy)acetohydrazide, 2-phenylacetohydrazide, 5-chloro-2-hydroxybenzohydrazide, 2-hydroxy-5-iodobenzohydrazide, and 2-(benzo[d]thiazol-2-ylthio)acetohydrazide. Seven products, **2** and **3a**–**3f**, were obtained. NMR data showed **2** to be similar to **1** with certain important differences. These include the appearances of carbon signals at δ_C 174.3 and 85.7, assignable to C-3 and C-4, respectively, in place of the δ_C 217.9, 47.6 in **1**, indicating 7-member lactonization. This chemical feature was further reflected in the molecular formula deduced from the HRESIMS: $C_{30}H_{48}O_2$.

The ^{1}H-NMR spectra of the series **3a-3f** were expected to be complex due to *cis*-to-*trans* thermal relaxation of hydrazide derivatives (Kitaev et al. 1970; Todeschini et al. 1998; Jakusová et al. 2014; Esguerra and Lumb 2017). However, the 1D-NMR data for **3a** were very similar to those of **1**. The observation of a carbon signal at δ_C 167.3, rather than that of the ketone carbon at δ_C 217.9, indicating C = N imine formation between the NH_2 group of the hydrazide reagent and the C = O group of **1**. This was further supported by the HMBC correlation of CH_3-23 (δ_H 1.16) and CH_3-24 (δ_H 1.12) to C-3 (δ_C 167.3). The NMR data for synthetic compounds **3b-3f** were consistent with those for **3a**, and were in full accordance with their described structures.

Phyllanthone (**1**) and its derivatives **1a–1l** and **2** were tested for inhibition of α-glucosidase (Table S1) as well as cytotoxic activity against the K562 cell line (Table S2). In the α-glucosidase inhibition assay, most products exhibited higher IC_{50} values than the positive control (IC_{50} 162.54 ± 0.19 μM). The benzylidene derivatives bearing *ortho*-halogenated groups (**1c**, **1f** and **1i**) or none substituent (**1a**) (Table S1) exhibited more powerful α-glucosidase inhibition than both the original (**1**, IC_{50} >200 μM) and the positive control. Among three derivatives **1c**, **1f**, and **1i**, the rank-order of potency reflected the electronic effect of the R-substituent: fluorinated > chlorinated > brominated with the IC_{50} values of **1c** (19.59 μM), **1f** (129.74 μM), and **1i** (154.81 μM), respectively.

In the cytotoxic activity assay, most derivatives displayed weaker activity (IC_{50} >100 μM) than the original. Compounds **1j**, **1 l**, **3c**, and **3d** had lower IC_{50} values



Scheme 1. General synthesis route towards analogues **1a**–**1l**, **2**, and **3a**–**3f**.

against the K562 cell line than the starting material (**1**, IC_{50} 98.82 ± 5.19 μM). This suggested that activity reflected the role played by the specific substituents that replaced the ketone group.

3. Experimental section

3.1. General

Solvents were distilled before use. All reagents were purchased from Sigma-Aldrich. All reactions were monitored by silica gel 60 F254 TLC plates (Merck). Column

chromatography was performed on silica gel 60–120 mesh. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker 500 and 400 MHz spectrometers using TMS as the internal standard. Mass spectra were obtained on an Agilent ESI-QTOF instrument.

3.2. Isolation of phyllanthol

Air-dried *P. acidus* twigs and leaves (5.5 kg) were ground into a powder and exhaustively extracted at room temperature with methanol (3 × 10 L). The filtered solution was evaporated under reduced pressure to afford a residue (225 g). This crude extract was subsequently reextracted using solvents of *n*-hexane, *n*-hexane–ethyl acetate (1:1), and ethyl acetate to yield *n*-hexane (54.5 g), *n*-hexane–ethyl acetate (16.3 g) and ethyl acetate (25.8 g) extracts. The *n*-hexane extract was subjected to silica gel column chromatography (CC) using a solvent system of *n*-hexane–ethyl acetate (8:2 to 0:1) to obtain six fractions (H1-H6). Fraction H3 was resuspended in methanol to afford phyllanthol (8.1 g) as a white amorphous powder.

3.3. Preparation of phyllanthone from phyllanthol

Phyllanthol (**I**, 200 mg, 0.485 mmol) dissolved in acetic acid (37.4 mL) was treated with 50 mL of Jones reagent (Shittu et al. 2016) prepared by adding $K_2Cr_2O_7$ (0.45 g) and 16.8 mL H_2SO_4 3 M to 100 mL water. The mixture was heated at 100 °C for 3 hours under stirring. The organic layer gained after extracting of the mixture with ethyl acetate–water (1:1) was washed with brine and dried over anhydrous Na_2SO_4. The residue was subjected to silica gel column chromatography (CC) eluted with *n*-hexane–ethyl acetate–acetone–acetic acid (8:0.2:0.1:0.01) to yield phyllanthone (**1**).

3.4. General procedure for the synthesis of benzaldehyde derivatives of phyllanthone

A solution of phyllanthone (**1**, 70 mg, 0.165 mmol), NaOH (35 mg, 0.875 mmol) in ethanol (7 mL) was stirred at 55 °C for 15 min. Appropriate aromatic aldehydes (0.33 mmol) and ethanol (7 mL) were added. The mixture was stirred for 2 h at 55 °C. The reaction mixture was partitioned between ethyl acetate–water (1:1) to yield an organic layer. This was pooled, washed with brine and dried over anhydrous Na_2SO_4. The residue was subjected to silica gel column chromatography eluted with *n*-hexane–ethyl acetate (100:1) to afford the products.

3.5. Synthesis of phyllanlactone

Phyllanthone (**1**, 35.5 mg, 0.084 mmol) was added to a solution of *m*-CPBA (16 mg, 0.093 mmol) in chloroform (3.6 mL). The mixture was stirred at 40 °C for 3.5 h. After extraction with ethyl acetate–water (1:1 v/v), an organic layer was obtained. This was washed with brine and dried over anhydrous Na_2SO_4. The residue was purified by normal phase silica gel column chromatography with *n*-hexane–ethyl acetate–acetone–acetic acid (10:0.4:0.1:0.01) as an eluent to yield phyllanlactone (**2**).

3.6. General procedure for the synthesis of derivatives 3a–3f

Hydrazides were prepared using the methods described in Supplementary material. To a solution of phyllanthone (**1**, 30 mg, 0.071 mmol) in ethanol–acetic acid (5.4 mL, 3:1, v/v (was added appropriate hydrazides (0.212 mmol). The mixture was refluxed at 60 °C for 2 h 30 min under stirring. The mixture partitioned between chloroform-ethanol-water (1:1:0.5 v/v/v). Organic layers were pooled, washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed and the organic layers were dissolved in methanol. The precipitate was finally washed with ethyl acetate–methanol (1:1) to afford a pure product.

4. Conclusions

Phyllanthone analogues were prepared *via* aldolization, Baeyer-Villiger oxidation and imine formation. We tested the products for α–glucosidase inhibition as well as cytotoxic activity towards the cancer cell line K562. The modification of phyllanthone to benzylidene enhanced activity against α-glucosidase. The benzylidene analogues of phyllanthone displayed stronger α-glucosidase inhibition. The 2-fluorobenzylidene analogue (**1c**) was demonstrated to be a promising α-glucosidase inhibitor, having an IC_{50} value of 19.59 μM. The imine derivatives were more cytotoxic to the K562 cell line than the benzylidene analogues. Among the products, compound **3c** exhibited the strongest activity (IC_{50} 57.55 μM). Further investigation into α-glucosidase inhibition of the derivatives (**3a**–**3f**) as well as cytotoxicity of the potential products are necessary for completing biological data.

Supplementary material

Supplementary material relating to this article is available online, alongside Tables S1–S2 and Figures S1–S71.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Ali L, Ahmad R, Ur Rehman N, Latif Khan A, Hassan Z, Shamim Rizvi T, Al-Harrasi A, Khan Shinwari Z, Hussain J. 2015. A new cyclopropyl-triterpenoid from *Ochradenus arabicus*. HCA. 98(9):1240–1244.

Calixto JB, Santos ARS, Filho VC, Yunes RA. 1998. A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 18(4): 225–258.

Cárdenas C, Quesada AR, Medina MA. 2004. Effects of ursolic acid on different steps of the angiogenic process. Biochem Biophys Res Commun. 320(2):402–408.

Chaturvedula VP, Gao Z, Jones SH, Feng X, Hecht SM, Kingston DG. 2004. A new ursane triterpene from *Monochaetum vulcanicum* that inhibits DNA polymerase beta lyase. J Nat Prod. 67(5):899–901.

Chiang YM, Su JK, Liu YH, Kuo YH. 2001. New cyclopropyl-triterpenoids from the aerial roots of *Ficus microcarpa*. Chem Pharm Bull. 49(5):581–583.

Dalla Vechia L, Gnoatto SC, Gosmann G. 2009. Derivados oleananos e ursanos e sua importância na descoberta de novos fármacos com atividade antitumoral, anti-inflamatória e antioxidante. Quím Nova. 32(5):1245–1252.

Duong T-H, Beniddir MA, Genta-Jouve G, Nguyen H-H, Nguyen D-P, Nguyen T-A-T, Mac D-H, Boustie J, Nguyen K-P-P, Chavasiri W, et al. 2019. Further terpenoids from *Euphorbia tirucalli*. Fitoterapia. 135:44–51.

Dzubak P, Hajduch M, Vydra D, Hustova A, Kvasnica M, Biedermann D, Markova L, Urban M, Sarek J. 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. Nat Prod Res. 23(3):394–411.

Esguerra KVN, Lumb JP. 2017. Synthesis of ortho-azophenols by formal dehydrogenative coupling of phenols and hydrazines or hydrazides. Chemistry. 23(36):8596–8600.

Harmand PO, Duval R, Delage C, Simon A. 2005. Ursolic acid induces apoptosis through mitochondrial intrinsic pathway and caspase-3 activation in M4Beu melanoma cells. Int J Cancer. 114(1):1–11.

Hsu H-Y, Yang J-J, Lin C-C. 1997. Effects of oleanolic acid and ursolic acid on inhibiting tumor growth and enhancing the recovery of hematopoietic system postirradiation in mice. Cancer Lett. 111(1–2):7–13.

Huang MT, Ho CT, Wang ZY, Ferraro T, Lou YR, Stauber K, Ma W, Georgiadis C, Laskin JD, Conney AH. 1994. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. Cancer Res. 54(3):701–708.

Jakusová K, Cigáň M, Donovalová J, Gáplovský M, Sokolík R, Gáplovský A. 2014. Light initiated *E*–*Z* and *Z*–*E* isomerization of isatinphenylsemicarbazones: Tautomeric equilibrium effect. J Photochem Photobiol A Chem. 288:60–69.

Kashiwada Y, Nagao T, Hashimoto A, Ikeshiro Y, Okabe H, Cosentino LM, Lee KH. 2000. Anti-AIDS agents 38. Anti-HIV activity of 3-*O*-acyl ursolic acid derivatives. J Nat Prod. 63(12): 1619–1622.

Kitaev YP, Buzykin BI, Troepol'skaya TV. 1970. The structure of hydrazine. Russ Chem Rev. 39(6): 441–456.

Kojima H, Tominaga H, Sato S, Takayanagi H, Ogura H. 1988. Two novel hexacyclic triterpenoids from *Prunella vulgaris*. Phytochemistry. 27(9):2921–2925.

Lee HY, Chung HY, Kim KH, Lee JJ, Kim KW. 1994. Induction of differentiation in the cultured F9 teratocarcinoma stem cells by triterpene acids. J Cancer Res Clin Oncol. 120(9):513–518.

Na M, Thuong PT, Hwang IH, Bae K, Kim BY, Osada H, Ahn JS. 2010. Protein tyrosine phosphatase 1B inhibitory activity of 24-norursane triterpenes isolated from *Weigela subsessilis*. Phytother Res. 24(11):1716–1719.

Ndlebe VJ, Crouch NR, Mulholland DA. 2008. Triterpenoids from the African tree *Phyllanthus polyanthus*. Phytochemistry Lett. 1(1):11–17.

Nguyen TH, Um BH, Kim SM. 2011. Two unsaturated fatty acids with potent α-glucosidase inhibitory activity purified from the body wall of sea cucumber (*Stichopus japonicus*). J Food Sci. 76(9):H208–H214.

Nguyen VK, Sichaem J, Nguyen HH, Nguyen XH, Huynh TTL, Nguyen TP, Niamnont N, Mac DH, Pham DD, Chavasiri W, et al. 2019. Synthesis and cytotoxic evaluation of usnic acid benzylidene derivatives as potential anticancer agents. Nat Prod Res. [accessed 2020 May 31]:[10 p.]. https://www.tandfonline.com/doi/full/10.1080/14786419.2019.1639176.

Ohigashi H, Takamura H, Koshimizu K, Tokuda H, Ito Y. 1986. Search for possible antitumor promoters by inhibition of 12-*O*-tetradecanoylphorbol-13-acetate-induced Epstein-Barr virus activation; ursolic acid and oleanolic acid from an anti-inflammatory Chinese medicinal plant, *Glechoma hederaceae* L. Cancer Lett. 30(2):143–151.

Ringbom T, Segura L, Noreen Y, Perera P, Bohlin L. 1998. Ursolic acid from Plantago major, a selective inhibitor of cyclooxygenase-2 catalyzed prostaglandin biosynthesis. J Nat Prod. 61(10):1212–1215.

Schweizer S, von Brocke AF, Boden SE, Bayer E, Ammon HP, Safayhi H. 2000. Workup-dependent formation of 5-lipoxygenase inhibitory boswellic acid analogues. J Nat Prod. 63(8):1058–1061.
Sengupta P, Mukhopadhyay J. 1966. Terpenoids and related compounds—VII: triterpenoids of *Phyllanthus acidus* Skeels. Phytochemistry. 5(3):531–534.
Shittu AA, Ayodeji OA, Datsugwai M. 2016. Bioethanol production from rice winery cake using lactic acid bacteria and yeasts by the process of simultaneous saccharification and fermentation. Int J Microbiol Biotechnol. 1(1):33–39.
Sohn KH, Lee HY, Chung HY, Young HS, Yi SY, Kim KW. 1995. Anti-angiogenic activity of triterpene acids. Cancer Lett. 94(2):213–218.
Sun H, Fang WS, Wang WZ, Hu C. 2006. Structure-activity relationships of oleanane- and ursane-type triterpenoids. Bot Stud. 47(4):339–368.
Sun H, Li Y, Zhang X, Lei Y, Ding W, Zhao X, Wang H, Song X, Yao Q, Zhang Y, et al. 2015. Synthesis, α-glucosidase inhibitory and molecular docking studies of prenylated and geranylated flavones, isoflavones and chalcones. Bioorg Med Chem Lett. 25(20):4567–4571.
Tahlan S, Verma PK. 2017. Biological potential of thiazolidinedione derivatives of synthetic origin. Chem Cent J. 11(1):130–159.
Todeschini AR, de Miranda ALP, da Silva KCM, Parrini SC, Barreiro EJ. 1998. Synthesis and evaluation of analgesic, anti-inflammatory and antiplatelet properties of new 2-pyridylarylhydrazone derivatives. Eur J Med Chem. 33(3):189–199.
Tokuda H, Ohigashi H, Koshimizu K, Ito Y. 1986. Inhibitory effects of ursolic and oleanolic acid on skin tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Lett. 33(3): 279–285.
Unander DW, Webster GL, Blumberg BS. 1995. Usage and bioassays in *Phyllanthus* (Euphorbiaceae). IV. Clustering of antiviral uses and other effects. J Ethnopharmacol. 45(1): 1–8.
Van Beers EH, Büller HA, Grand RJ, Einerhand AW, Dekker J. 1995. Intestinal brush border glycohydrolases: structure, function, and development. Crit Rev Biochem Mol Biol. 30(3):197–262.
Wang S, Lai J, Huang K. 1993. Studies on the constituents of the leaves of *Alyxia insularis* kanehira & sasaki. China Pharm J. 45(4):329–336.
Woldemichael GM, Singh MP, Maiese WM, Timmermann BN. 2003. Constituents of antibacterial extract of *Caesalpinia paraguariensis* Burk. Z Naturforsch C J Biosci. 58(1–2):70–75.
Yonemoto R, Shimada M, Gunawan-Puteri MD, Kato E, Kawabata J. 2014. α-Amylase inhibitory triterpene from *Abrus precatorius* leaves. J Agric Food Chem. 62(33):8411–8414.
Yousefi A, Yousefi R, Panahi F, Sarikhani S, Zolghadr AR, Bahaoddini A, Khalafi-Nezhad A. 2015. Novel curcumin-based pyrano[2,3-d]pyrimidine anti-oxidant inhibitors for α-amylase and α-glucosidase: implications for their pleiotropic effects against diabetes complications. Int J Biol Macromol. 78:46–55.