











## Cyclic Polyketides with $\alpha$ -Glucosidase Inhibitory Activity from *Endiandra kingiana* Gamble and Molecular Docking Study

Mohamad Nurul Azmi <sup>1\*</sup>, Nur Amirah Saad <sup>1</sup>, Mohamad Hafizi Abu Bakar <sup>2</sup>,  
Mohammad Tasyriq Che Omar <sup>3</sup>, Ahmad Nazif Aziz <sup>4</sup>, Habibah A. Wahab <sup>5</sup>,  
Sadiah Siddiq <sup>6</sup>, M. Iqbal Choudhary <sup>6</sup>, Marc Litaudon <sup>7</sup> and  
Khalijah Awang <sup>8</sup>

<sup>1</sup>School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia

<sup>2</sup>Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia

<sup>3</sup>School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia

<sup>4</sup>Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030, Kuala Nerus  
Terengganu, Malaysia

<sup>5</sup>School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang,  
Malaysia

<sup>6</sup>H.E.J Research Institute of Chemistry, International Centre for Chemical and Biological Sciences,  
University of Karachi, Karachi, Pakistan

<sup>7</sup>Institut de Chimie des Substances Naturelles, CNRS-ICSN UPR2301, Univ. Paris-Sud 11, av. de la  
Terrasse, 91198 Gif-sur-Yvette, France

<sup>8</sup>Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

(Received November 26, 2021; Revised February 07, 2021; Accepted February 21, 2021)

**Abstract:** A phytochemical investigation of the methanolic extract of *Endiandra kingiana* (bark) led to the isolation of four major compounds which are kingianic acid A (**1**), tsangibeilin B (**2**), kingianin A (**3**) and kingianin F (**4**). The structures were determined by 1D- and 2D-NMR analysis in combination with HRMS experiments. The compounds were screened for their *in vitro*  $\alpha$ -glucosidase inhibition activity. Among them, compounds **3-4** showed potent  $\alpha$ -glucosidase inhibition activity with IC<sub>50</sub> value at  $11.9 \pm 2.0 \mu\text{M}$  and  $19.7 \pm 1.5 \mu\text{M}$ , respectively. The molecular docking study found that both compounds were bound into the active site of the N-terminal of MGAM, and thus agreed with the *in vitro*  $\alpha$ -glucosidase enzyme inhibition activity results.

**Keywords:** *Endiandra kingiana*; cyclic polyketides; endiandric acids; kingianins;  $\alpha$ -glucosidase inhibition; Molecular docking. © 2021 ACG Publications. All rights reserved.

\* Corresponding author: E-Mail: [mnazmi@usm.my](mailto:mnazmi@usm.my); Phone: +6046533562; Fax: +6046574854

## 1. Plant Source

The bark of *Endiandra kingiana* (*E. kingiana*) Gamble was collected from the Reserved Forest of Sg. Temau, Pahang, Malaysia. This plant was identified by Teo L. E., the botanist from the University of Malaya. A voucher specimen (KLU 5243) was deposited at the Herbarium, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

## 2. Previous Studies

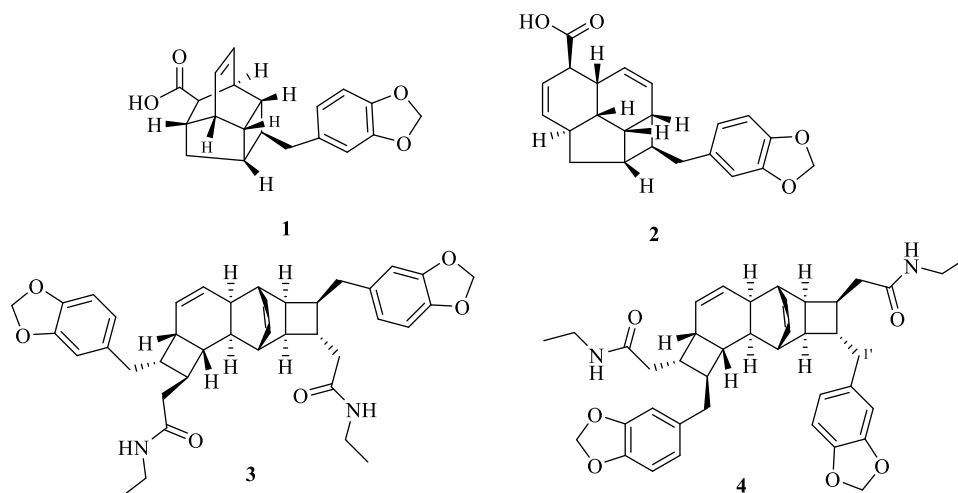
*E. kingiana* Gamble is one of the species in the Lauraceae family which is also known as Medang or Tejur. Lauraceae contains about 68 genera and 2980 species worldwide, mostly in the tropical regions, especially Southeast Asia and tropical America [1]. This family was known to be a source of various types of secondary metabolites, especially cyclic polyketides in the form of endiandric acids, and had been widely used in traditional medicine [2]. Previous phytochemical investigations of this species led to the isolation of kingianins A–Q, kingianic acids A–G, endiandric acid M and tsangibeilin B [3-6]. The Bcl-xL and Mcl-1 binding affinities had been evaluated, and kingianin G showed good results [3-6].

## 2. Present Study

The present study was designed to evaluate the  $\alpha$ -glucosidase inhibitory activity and its molecular docking study. This is the first report on the biological activity of this plant genus on the *in vitro* inhibition of the carbohydrate hydrolyzing enzyme.

The air-dried bark of *E. kingiana* (1.5 kg) were sliced, ground and extracted with EtOAc (3×1.5 L) followed by MeOH (3×1.5 L) at 40 °C and 100 bar using a static high-pressure Zippertex extractor, developed at the ICSN pilot unit. The methanol extract (40.5 g) was partitioned with EtOAc/H<sub>2</sub>O (1:1, v/v) to afford an EtOAc-soluble fraction (10.5 g) and H<sub>2</sub>O-soluble fraction (28.7 g). The EtOAc-soluble fraction (10.0 g) was subjected to flash column chromatography (Companion<sup>®</sup>, SiO<sub>2</sub>, hexane/dichloromethane/methanol step gradient) to give nine fractions: AM1.Fr.1 – AM1.Fr.9.

Fraction AM1.Fr.6 (0.57 g) was subjected to CC (SiO<sub>2</sub>, 230 – 400 mesh; hexane/AcOEt step gradient) to obtain 8 subfractions based on TLC profile: AM1.Fr.6.1–AM1.Fr.6.8. Fraction AM1.Fr.6.3 (47.9 mg) was separated using Preparative C<sub>18</sub> HPLC to afford kingianic acid A (**1**) (7.3 mg) and tsangibeilin B (**2**) (6.3 mg). Fractions AM1.Fr.6.6 (63.4 mg) and AM1.Fr.6.7 (49.2 mg) were subjected to a semi-preparative C<sub>18</sub> HPLC to give kingianin A (**3**) (4.9 mg) and kingianin F (**4**) (4.1 mg), respectively (see supporting information for details). Four major compounds (Figure 1) were known compounds and have been elucidated in previous studies (see supporting information) [3-6].



**Figure 1.** The structures of four major compounds **1-4**, previously elucidated

Cyclic polyketides from *Endiandra kingiana* Gamble

*α*-glucosidase enzyme activity: Compounds **2-4** were submitted for *α*-glucosidase enzyme inhibition activity (see supporting information for the procedure), and the results are summarized in Table 1. The data indicated that all the tested compounds showed potent inhibition on *α*-glucosidase compared to the positive control, acarbose with  $IC_{50} = 840.0 \pm 1.7 \mu\text{M}$ . Compounds **3** and **4** showed the potent *α*-glucosidase enzyme inhibitory activity with the value of  $IC_{50} 11.9 \pm 2.0 \mu\text{M}$  and  $19.7 \pm 1.5 \mu\text{M}$  respectively, compared to the positive control. Meanwhile, compound **2** exhibited moderate activity with an  $IC_{50}$  value of  $97.4 \pm 0.6 \mu\text{M}$ .

**Table 1.** The  $IC_{50}$  values of isolated compounds against *α*-glucosidase.

Compounds	$IC_{50}$ ( $\mu\text{M}$ )
1	NA
2	$97.4 \pm 0.6 \mu\text{M}$
3	$11.9 \pm 2.0 \mu\text{M}$
4	$19.7 \pm 1.5 \mu\text{M}$
Acarbose (control)	$840.0 \pm 1.7 \mu\text{M}$

NA – the samples not available

*Molecular Docking:* The free binding energy and interaction modes between the residues in the active site of human maltase glucoamylase, MGAM (*α*-glucosidase) and compounds **2-4** were identified by molecular docking studies. AutoDockTools 1.5.6 was used on the crystal structures of the N-terminal (PDB ID: 2QMJ) and C-terminal (PDB ID: 3TOP) subunit of the human MGAM [7-8]. The docking results indicated the binding energy displayed by compounds **2-4** range from  $-5.39$  to  $-7.50$  and  $-7.11$  to  $-10.87$  kcal/mol for the N-terminal and C-terminal subunit of human MGAM, respectively. Compound **3** demonstrated the strongest binding energy among the tested compounds in both active sites. All compounds were expected to bind into the active site of the N-terminal of MGAM than the C-terminal of MGAM since the binding energy pattern (Table 2) was similar to the *in vitro* results. The minimum binding energies obtained from docking of the current potent compounds with *α*-glucosidase enzyme using Autodock4 software were comparable with the free binding energy of reported potent *α*-glucosidase inhibitors that had values from  $-7$  to  $-11$  kcal/mol [9-10].

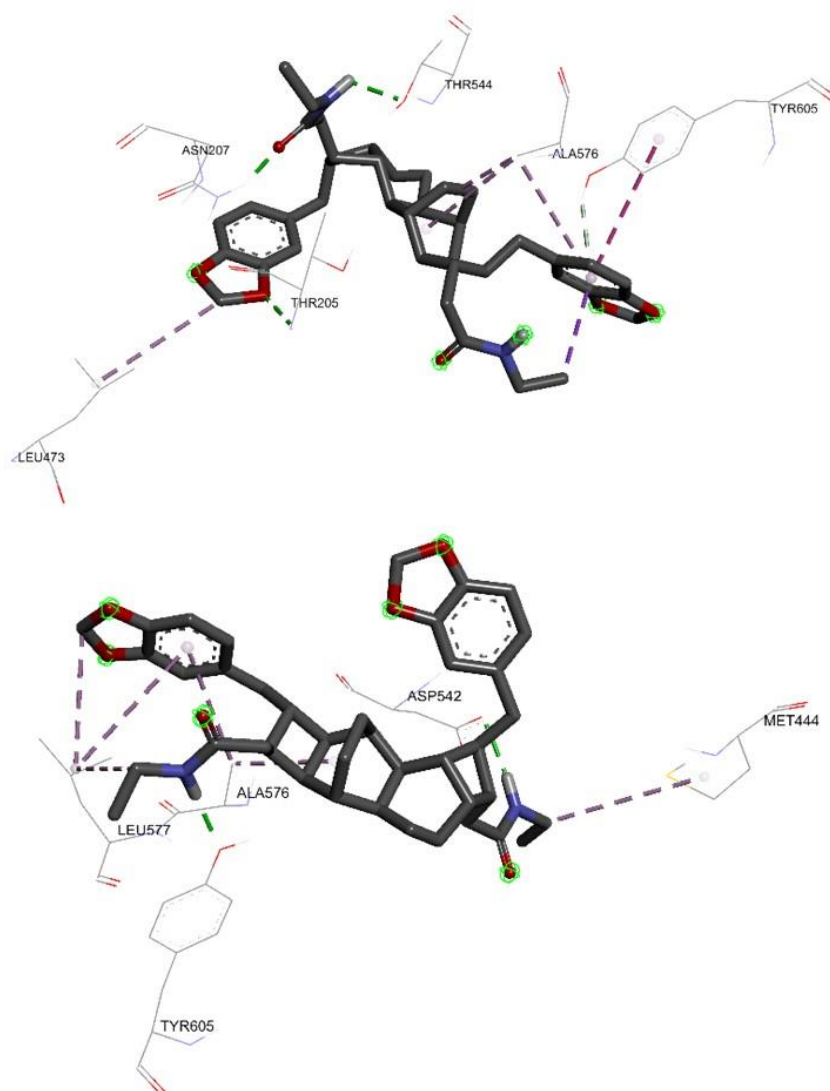
The interactive modes of compounds **3-4** are shown in Figures 2. The details of their interaction at the active site are listed in Table 3. Figure 2 shows that hydrogen atoms of the NH amide group of **3** interacted with THR544 via hydrogen bonding. The oxygen atom of methylenedioxy moiety and C=O amide group also formed a conventional hydrogen bond with THR205 and ASN207, respectively. Non-conventional hydrogen bond occurred between carbon atom of the phenyl ring of **3** with TYR605. Three hydrophobic interactions were formed between atoms methylenedioxy moiety, cyclohexyl ring and N-ethyl amide substitution of **3** with LEU473, ALA576 and TYR605.

The second most potent compound (**4**) bound to the N-terminal of human MGAM showed that the hydrogen atoms of both NH amide groups of **4** interacted with ASP542 and TYR605, respectively via hydrogen bonds. Three hydrophobic interactions were formed between atoms of N-ethyl amide substitution, cyclohexyl ring and methylenedioxy moiety of **4** with MET44, ALA576 and LEU577, respectively (Figure 2). Recently, Alqahtani *et al.* showed that 3-oxolupenal and katononic acid formed a complex with *α*-glucosidase [11]. This was confirmed *via* static quenching mechanism through stabilization by a network of two to three hydrogen bonds and five to ten hydrophobic interactions. The results of molecular docking of compounds **3** and **4** at the C-terminal of human MGAM range between the number of networks of hydrogen bonds and hydrophobic interactions as previously reported [7-8,11]. Moreover, these findings agreed with the *in vitro* *α*-glucosidase enzyme inhibition activity of compounds **3** and **4**.

**Table 2.** The binding energy and relative cluster rank of compounds 2-4

PDB ID	Protein	Compounds	BE (kcal/mol)	DFER	Best model (cluster rank)	Number of conformations in cluster rank
2QMJ	NtMGAM	2	-5.39	-5.39 to 4.91	56 (1)	2
		3	-7.50	-7.50 to -6.13	94 (1)	4
		4	-6.80	-6.80 to -5.54	92 (1)	2
		Acarbose	-3.23	-3.23	49 (1)	1
3TOP	CtMAGM	2	-7.11	-7.11 to -6.14	53 (1)	81
		3	-10.87	-10.87 to -9.93	76 (1)	3
		4	-10.48	-10.48 to -8.44	47 (1)	3
		Acarbose	-10.17	-10.17	92 (1)	1

BE: Binding Energy; DFER: Docked free energy range



**Figure 2.** The three-dimensional binding modes of compound 3 (above) and compound 4 (below) at the active site of the N-terminal of human MGAM

Cyclic polyketides from *Endiandra kingiana* Gamble**Table 3.** The detail of the binding interactions details of compounds **3** and **4** docked into N-terminal and C-terminal of human MGAM

Protein	Compound	Free energy of binding	Interacting unit of compounds	Protein Residue	Type of interaction
NtMGAM	<b>3</b>	-7.50	Methylenedioxy	THR205	H-bond
			C=O amide	ASN207	H-bond
			Methylenedioxy	LEU473	alkyl
			NH amide	THR544	H-bond
			Cyclohexyl	ALA576	$\pi$ -alkyl
	<b>4</b>	-6.80	Phenyl	TYR605	H-bond carbon
			-CH <sub>2</sub> CH <sub>3</sub> amide		$\pi$ - $\pi$ T-shaped
			-CH <sub>2</sub> CH <sub>3</sub> amide	MET444	alkyl
			NH amide	ASP542	H-bond
			Cyclohexyl	ALA576	$\pi$ -alkyl
CtMGAM	<b>3</b>	-10.87	Methylenedioxyphenyl	LEU577	$\pi$ -alkyl
			-CH <sub>2</sub> CH <sub>3</sub> amide		alkyl
			NH amide	TYR605	H-bond
			Cyclohexyl	PRO1159	alkyl
			Methylenedioxy	LYS1164	H-bond
	<b>4</b>	-10.48	Phenyl	ASP1420	$\pi$ -anion
			C=O amide	LYS1460	H-bond
			Cyclohexyl	PHE1560	$\pi$ -alkyl
			NH amide	THR1586	H-bond
			Phenyl	TRP1355	$\pi$ - $\pi$ stacked
<b>4</b>	-10.48	Phenyl	TRP1369	$\pi$ - $\pi$ T-shaped	
		Cyclohexyl		$\pi$ -alkyl	
		Methylenedioxy	ARG1377	H-bond	
		Phenyl	MET1421	$\pi$ -sulfur	
		C=O amide	LYS1460	H-bond	
<b>4</b>	-10.48	Phenyl	ASP1526	$\pi$ -anion	
		Cyclohexyl	PHE1560	$\pi$ -alkyl	
		NH amide	GLY1588	Unfavorable donor-donor	

**Acknowledgments**

This research was financially funded through Universiti Sains Malaysia Research Grant RUI (1001.PKIMIA.8012310) and Ministry of Higher Education FRGS (FRGS/1/2019/STG01/UMT/03/2). This research was conducted within the frame of the collaboration between USM-CNRS (LoI) and auspices of CNRS-UM; Associated International Laboratory (LIA) under FM-NatProLab.

**Supporting Information**

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

**ORCID** Mohamad Nurul Azmi : [0000-0002-2447-0897](https://orcid.org/0000-0002-2447-0897)Nur Amirah Saad : [0000-0002-3105-7419](https://orcid.org/0000-0002-3105-7419)Mohamad Hafizi Abu Bakar : [0000-0001-8064-695X](https://orcid.org/0000-0001-8064-695X)Mohammad Tasyriq Che Omar : [0000-0002-1294-7520](https://orcid.org/0000-0002-1294-7520)Ahmad Nazif Aziz : [0000-0001-8816-2626](https://orcid.org/0000-0001-8816-2626)Habibah A. Wahab : [0000-0002-8353-8679](https://orcid.org/0000-0002-8353-8679)Sadiah Siddiq : [0000-0002-5352-8533](https://orcid.org/0000-0002-5352-8533)M. Iqbal Choudhary : [0000-0001-5356-3585](https://orcid.org/0000-0001-5356-3585)Marc Litaudon : [0000-0002-0877-8234](https://orcid.org/0000-0002-0877-8234)Khalijah Awang : [0000-0001-5971-6570](https://orcid.org/0000-0001-5971-6570)**References**

- [1] I. H. Burkill (1966). A Dictionary of the Economic Products of the Malay Peninsular (2nd ed.). Government of Malaya and Singapore, Kuala Lumpur, Malaysia
- [2] B. N. Lenta, J. R. Chouna, P. A. Nkeng-Efouet and N. Sewald (2015). Endiandric acid derivatives and other constituents of plants from the genera *Beilschmiedia* and *Endiandra* (Lauraceae), *Biomolecules*, **5**, 910-942.
- [3] A. Leverrier, K. Awang., F. Guéritte and M. Litaudon (2011). Pentacyclic polyketides from *Endiandra kingiana* as inhibitors of the Bcl-xL/Bak interaction, *Phytochemistry* **72**, 1443-1452.
- [4] A. Leverrier, M.E.T.H. Dau, P. Retailleau, K. Awang, F. Guéritte and M. Litaudon (2010). Kingianin A: a new natural pentacyclic compound from *Endiandra kingiana*, *Org. Lett.* **12**, 3638-3641.
- [5] M. N. Azmi, C. Gény, A. Leverrier, M. Litaudon, V. Dumontet, N. Birlirakis, F. Guéritte, K.H. Leong, S.N.A Halim, K. Mohamad and K. Awang (2014). Kingianic acids A–G, endiandric acid analogues from *Endiandra kingiana*, *Molecules* **19**, 1732-1747.
- [6] M. N. Azmi, T. Péresse, C. Remeur, G. Chan, F. Roussi, M. Litaudon and K. Awang (2016). Kingianins O–Q: Pentacyclic polyketides from *Endiandra kingiana* as inhibitor of Mcl-1/Bid interaction, *Fitoterapia* **109**, 190-195.
- [7] L. Sim, R. Quezada-Calvillo, E. E. Sterchi, B. L. Nichols, and D. R. Rose Sim (2008). Human intestinal maltase–glucoamylase: crystal structure of the N-terminal catalytic subunit and basis of inhibition and substrate specificity, *J. Mol Biol.* **375**, 782-792.
- [8] L. Ren, X. Qin, X. Cao, L. Wang, F. Bai, G. Bai and Y. Shen (2011). Structural insight into substrate specificity of human intestinal maltase-glucoamylase, *Protein Cell.* **2**, 827-836.
- [9] A. Bhatia, B. Singh, R. Arora and S. Arora (2019). In vitro evaluation of the  $\alpha$ -glucosidase inhibitory potential of methanolic extracts of traditionally used antidiabetic plants, *BMC Complement. Altern. Med.* **19**, 74.
- [10] P. Khanal and B. M. Patil (2020).  $\alpha$ -Glucosidase inhibitors from *Duranta repens* modulate p53 signaling pathway in diabetes mellitus, *Adv. Tradit. Med.* 1-12.
- [11] A. S. Alqahtani, S. Hidayathulla, M. T. Rehman, A. A. ElGamal, S. Al-Massarani, V. Razmovski-Naumovski, M. S. Alqahtani, R. A. El Dib, and M. F. AlAjmi (2020). Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia*, *Biomolecules* **10**, 61, doi: 10.3390/biom10010061.
- [12] E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng and T.E. Ferrin (2004). UCSF Chimera-a visualization system for exploratory research and analysis, *J. Comput. Chem.* **25**, 1605-1612.
- [13] L. D. Mendelsohn (2004). ChemDraw 8 ultra, windows and macintosh versions, *J. Chem. Inform. Comput. Sci.* **44**, 2225-2226.
- [14] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function, *J. Comput. Chem.* **19**, 1639-1662.
- [15] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.* **30**, 2785-2791

**A C G**  
publications

© 2021 ACG Publications