SHORT REPORT

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Cyclic Polyketides with α-Glucosidase Inhibitory Activity from *Endiandra kingiana* Gamble and Molecular Docking Study

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

[1]*School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*

[2]*Bioprocess Technology Division, School of Industrial Technology, Universiti Sains Malaysia*

[3]*School of Distance Education, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*

[4]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030, Kuala Nerus Terengganu, Malaysia*

[5]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia*

[6]*H.E.J Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan*

[7]*Institut de Chimie des Substances Naturelles, CNRS-ICSN UPR2301, Univ. Paris-Sud 11, av. de la Terrasse, 91198 Gif-sur-Yvette, France*

[8]*Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*

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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* α-glucosidase inhibition activity. Among them, compounds **3**-**4** showed potent α-glucosidase inhibition activity with IC_{50} value at 11.9 ± 2.0 µM and 19.7 ± 1.5 µM, 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* α-glucosidase enzyme inhibition activity results.

Keywords: *Endiandra kingiana*; cyclic polyketides; endiandric acids; kingianins; α-glucosidase inhibition; Molecular docking.

* Corresponding author: E-Mail: mnazmi@usm.my; Phone: +6046533562; Fax: +6046574854

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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 α-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_2O (1:1, v/v) to afford an EtOAc-soluble fraction (10.5 g) and H_2O-soluble fraction (28.7 g). The EtOAc-soluble fraction (10.0 g) was subjected to flash column chromatography (Companion®, SiO_2, 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_2, 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_{18} 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_{18} 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].

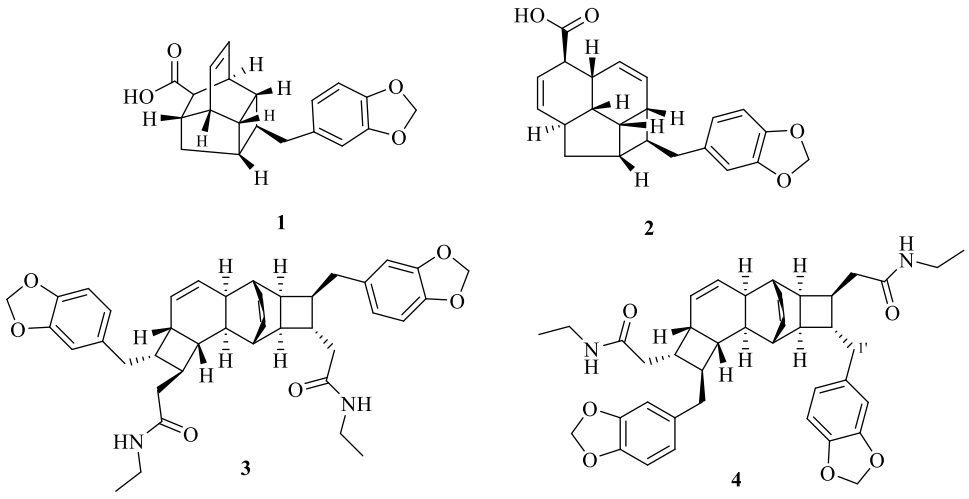

Figure 1. The structures of four major compounds **1**-**4**, previously elucidated

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 ± 1.7 µM. Compounds **3** and **4** showed the potent α-glucosidase enzyme inhibitory activity with the value of IC_{50} 11.9 ± 2.0 µM and 19.7 ± 1.5 µM respectively, compared to the positive control. Meanwhile, compound **2** exhibited moderate activity with an IC_{50} value of 97.4 ± 0.6 µM.

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

Compounds	**IC_{50} (µM)**
1	NA
2	97.4 ± 0.6 µM
3	11.9 ± 2.0 µM
4	19.7 ± 1.5 µM
Acarbose (control)	840.0 ± 1.7 µM

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 alpha-glucosidase enzyme using Autodock4 software were comparable with the free binding energy of reported potent alpha-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 methylenedioxyl 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 methylenedioxyl 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 methylenedioxyl 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

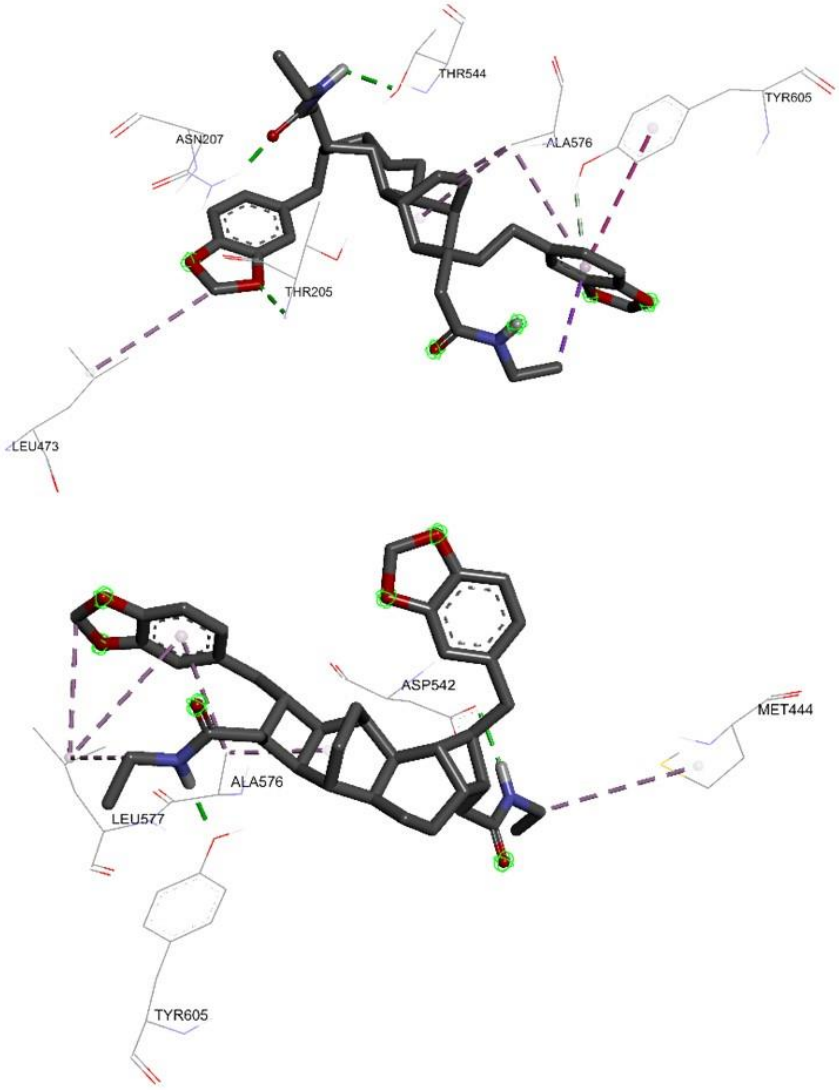

Figure 2. The three-dimensional binding modes of compound **3** (above) and compound **4** (below) at the active site of the N-terminal of human MGAM

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	**3**	−7.50	Methylenedioxyl	THR205	H-bond
			C=O amide	ASN207	H-bond
			Methylenedioxyl	LEU473	alkyl
			NH amide	THR544	H-bond
			Cyclohexyl	ALA576	π-alkyl
			Phenyl	TYR605	H-bond carbon
			-CH_2CH_3 amide		π-π T-shaped
	4	−6.80	-CH_2CH_3 amide	MET444	alkyl
			NH amide	ASP542	H-bond
			Cyclohexyl	ALA576	π-alkyl
			Methylenedioxyphenyl	LEU577	π-alkyl
			-CH_2CH_3 amide		alkyl
			NH amide	TYR605	H-bond
CtMGAM	**3**	−10.87	Cyclohexyl	PRO1159	alkyl
			Methylenedioxyl	LYS1164	H-bond
			Phenyl	ASP1420	π-anion
			C=O amide	LYS1460	H-bond
			Cyclohexyl	PHE1560	π-alkyl
			NH amide	THR1586	H-bond
	4	−10.48	Phenyl	TRP1355	π-π stacked
			Phenyl	TRP1369	π-π T-shaped
			Cyclohexyl		π-alkyl
			Methylenedioxyl	ARG1377	H-bond
			Phenyl	MET1421	π-sulfur
			C=O amide	LYS1460	H-bond
			Phenyl	ASP1526	π-anion
			Cyclohexyl	PHE1560	π-alkyl
			NH amide	GLY1588	Unfavorable donor-donor

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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
Nur Amirah Saad :0000-0002-3105-7419
Mohamad Hafizi Abu Bakar :0000-0001-8064-695X
Mohammad Tasyriq Che Omar :0000-0002-1294-7520
Ahmad Nazif Aziz :0000-0001-8816-2626
Habibah A. Wahab :0000-0002-8353-8679
Sadia Siddiq : 0000-0002-5352-8533
M. Iqbal Choudhary :0000-0001-5356-3585
Marc Litaudon :0000-0002-0877-8234
Khalijah Awang :0000-0001-5971-6570

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