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Anthraquinones from *Morinda longissima* and their insulin mimetic activities *via* AMP-activated protein kinase (AMPK) activation

Phi-Hung Nguyen [a,1], Hong Seok Choi [a], Thi Kim Quy Ha [b], Ji Yeon Seo [b], Jun-Li Yang [b], Da-Woon Jung [c], Darren R. Williams [c], Won-Keun Oh [b,*]

[a] College of Pharmacy, Chosun University, Gwangju 501-759, Republic of Korea
[b] Korea Bioactive Natural Material Bank, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 08826, Republic of Korea
[c] New Drug Targets Laboratory, School of Life Sciences, Gwangju Institute of Science and Technology, 1 Oryong-Dong, Buk-Gu, Gwangju 500-712, Republic of Korea

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ABSTRACT

AMP-activated protein kinase (AMPK) activators are known to increase energy metabolism and to reduce body weight, as well as to improve glucose uptake. During for searching AMPK activators, a new anthraquinone, modasima A (**10**), along with eighteen known analogues (**1**–**9** and **11**–**19**) were isolated from an ethanol extract of the roots of *Morinda longissima* Y. Z. Ruan (Rubiaceae). Using the fluorescent tagged glucose analogues, 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxy-D-glucose (2-NBDG), insulin mimetics were screened with compounds **1**–**19** in 3T3-L1 adipocytes. Among them, compounds **2**, **8** and **10** enhanced significantly glucose uptake into adipocytes and up-regulated the phosphorylated AMPK (Thr^{172}) whereas the glucose uptake enhancing activities of compounds **2**, **8** and **10** were abrogated by treatment of compound C, an AMPK inhibitor. Taken together, these anthraquinones showed the potential action as insulin mimetic to improve glucose uptake via activation of AMPK.

The number of patients with type 2 diabetes (T2D) has been increasing worldwide due to the upsurge in obesity that was caused by sedentary lifestyles and high calorie foods.[1] The AMP-activated protein kinase (AMPK), a heterotrimeric functional enzyme complex, plays a key role in metabolic control and regulating cellular energy homeostasis.[2] The AMPK activation on the phosphorylation of Thr^{172} at the catalytic α subunit[3] can be regulated by calmodulin-dependent protein kinase kinases (CaMKKs) and serine/threonine kinase (LKB1), which are two upstream kinases, and also can be positively influenced by the intracellular AMP/ATP ratio.[4] The activated AMPK is able to regulate lipid homeostasis, and this function has been regarded as a promising strategy for the treatment of type 2 diabetes due to the strong association between the deregulation of the fatty acid metabolism and the development of insulin resistance.[5] In addition, it has been reported the potential of some downstream targets of AMPK in glucose uptake regulation, such as reducing ectopic lipid accumulation for improving insulin sensitivity, suppressing hepatic glucose production in the liver, and stimulating glucose uptake in the muscle.[6] Insulin is the only available agent for both type 1 and type 2 diabetes treatment so far.[7] Some synthetic small molecules and natural products have been reported to mimic the action of insulin and promote glucose uptake in cell culture and animal models of diabetes.[8] Therefore, it is a need to search for new anti-diabetic agents that can mimic insulin.

The genus *Morinda* (Rubiaceae) comprising about 80 species was found in tropical regions worldwide, including vines, shrubs, or trees with aggregated fruits being fleshy or dry. *M. citrifolia* (Noni) is regarded as an important herb plant in Hawaiian, and has been utilized throughout the Pacific Polynesia for a long time.[9] *M. longissima* among these species is closely related to *M. citrifolia* in phytotaxonomy,[10] but there are not many reports on phytochemical and biological investigations of this plant. 2-NBDG assay suggested that an ethanol extract of the roots of *M. longissima* could improve glucose uptake in 3T3-L1 adipocytes. This observation and a recent discovery of four potential insulin mimetics from *M. citrifolia* promoted us to search new insulin mimetics from *M. longissima*.[11] Herein we will describe the isolation and structure elucidation of these constituents and their potential on glucose uptake and their biological effects on AMPK activation.

Bioassay-guided fractionation of the active ethanol extract of the roots of *M. longissima* by column chromatography (silica gel, RP-C_{18}, and prep-HPLC) resulted in the isolation of 19 anthraquinone derivatives (**1**–**19**), including a new that were named modasima A (**10**) (Fig. 1).[12]

* Corresponding author.
E-mail address: wkoh1@snu.ac.kr (W.-K. Oh).
[1] Present address: Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18-Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam.

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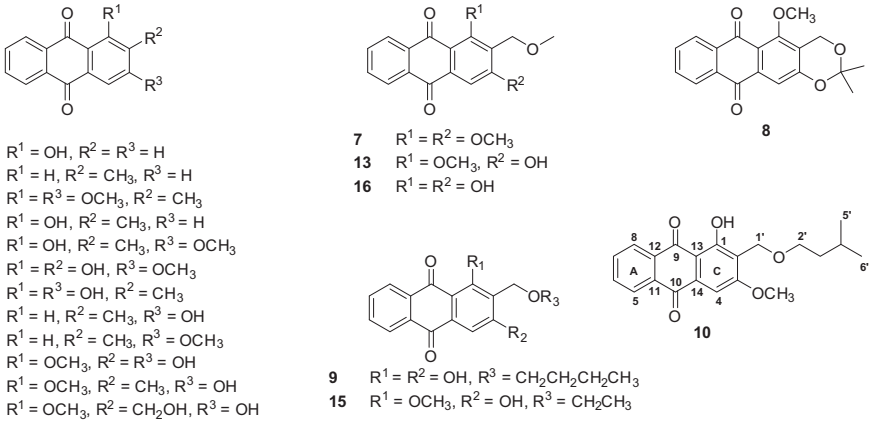

Fig. 1. Chemical structures of compounds **1**–**19** from the roots of *Morinda longissima*.

Compound **10** was isolated as a yellowish amorphous powder. Its molecular formula of $C_{21}H_{22}O_5$ was determined from a HREIMS peak at *m*/*z* 354.1465 $[M]^+$ (calcd for $C_{21}H_{22}O_5$, 354.1467). The IR spectrum suggested the presence of hydroxy (3331 cm^{-1}), unsaturated carbonyl (1670 cm^{-1}), and olefinic (1565 cm^{-1}) functionalities. The UV absorptions at λ_{max} 242, 281, 336, and 352 nm and the ^{13}C NMR signals at δ_C 181.4 and 183.9 implied a 9,10-anthraquinone structure.[13] The ^1H NMR spectrum showed two aromatic broad doublets at δ_H 8.17 (1H, br d, J = 7.6 Hz, H-5) and 8.23 (1H, br d, J = 7.2 Hz, H-8), and two broad triplets at δ_H 7.86 (1H, br t, J = 7.6 Hz, H-6) and 7.89 (1H, br t, J = 7.2 Hz, H-7), which indicated a typical A_2B_2 aromatic system. The above observations together with an aromatic singlet proton at δ_H 7.59 (1H, s, H-4) indicated that **10** was an anthraquinone with a tri-substituted C-ring. A phenolic hydroxyl group demonstrated a proton signal at δ_H 13.2 in the ^1H NMR spectrum measured in $CDCl_3$, which was due to the formation of a hydrogen bond between this hydroxyl group and C(9)═O group. This analysis indicated that a hydroxyl group was located at the *peri* position to the C-9 carbonyl group.[14] An oxygenated methylene (δ_H 4.76, 2H, s, H_2-1′) was bond to C-2 based on HMBC correlations from H-1′ (δ_H 4.76) to C-1 (δ_C 163.9), C-2 (δ_C 126.8), and C-3 (δ_C 163.5). In addition, a ^1H-^1H COSY experiment showed an isopentyloxy group [δ_H 3.63 (2H, t, J = 6.8 Hz), 1.51 (1H, q, J = 6.4 Hz), 1.72 (1H, m), and 0.90 (6H, d, J = 6.4 Hz)], and it was connected to C-1′ from HMBC correlation between H-1′ (δ_H 4.76) and C-2′ (δ_C 70.4). One methoxy group at δ_H 3.94 (3H, s) was located at C-3 due to a cross peak between this methoxy (δ_H 3.94) and C-3 (δ_C 163.5) in HMBC experiment (Fig. 2).

The above conclusion was also supported by the HMBC correlations from the aromatic singlet H-4 (δ_H 7.59, 1H) to C-2 (δ_C 126.8), C-3 (δ_C 163.5), C-10 (δ_C 183.9), C-13 (δ_C 119.8), and C-14 (δ_C 137.7) (Fig. 2). Thus, compound **10** was elucidated as 1-hydroxy-2-((isopentyloxy)methyl)-3-methoxy-9,10-anthraquinone, and named modasima A.[15]

By detailed analysis and comparison of the spectroscopic and optical rotation values with literature data, the known compounds were characterized to be 1-hydroxy-anthraquinone (**1**),[16] tectoquinone (**2**),[17] rubiadin-dimethyl ether (**3**),[17] 1-hydroxy-2-methyl-9,10-anthraquinone (**4**),[17] rubiadin-3-methyl ether (**5**),[17] 1,2-dihydroxy-3-methoxy-anthraquinone (**6**),[17] 1,3-dimethoxy-2-methoxymethylanthraquinone (**7**),[17] 1-methoxy-2′,2′-dimethyl-dioxine-(5′,6′:2,3)-anthraquinone (**8**),[18] lucidin-ω-butyl ether (**9**),[18] rubiadin (**11**),[13] 3-hydroxy-2-methylanthraquinone (**12**),[13] 1-methoxy-3-hydroxy-2-methoxymethylanthraquinone (**13**),[13] 2-methoxy-3-methyl-anthraquinone (**14**),[19] damnacanthol-ω-ethyl ether (**15**),[20] lucidin-ω-methyl ether (**16**),[20] 1-methoxy-2,3-dihydroxy-anthraquinone (**17**),[20] rubiadin-1-methyl ether (**18**),[20] and damnacanthol (**19**).[20]

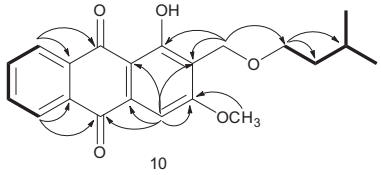

Fig. 2. Key HMBC (⟶) and ^1H–^1H COSY (▬) correlations for modasima A (**10**).

All of the isolates (**1**–**19**) were evaluated for their potential on glucose metabolism and insulin mimetic action. 2-NBDG has been used as a fluorescent glucose analog widely used for monitoring glucose uptake in cells, and it was established to be a useful reagent for discovering insulin mimetic compounds.[21] Herein we examined the stimulatory effects of compounds **1**–**19** on 2-NBDG uptake in 3T3-L1 adipocyte cells by using an *in vitro* assay (2-NBDG assay).[22] The 3T3-L1 adipocytes were chosen for this assay as fat represents one of the major body tissues that is sensitive to insulin. 3T3-L1 fibroblasts were induced to differentiate into adipocytes,[23] and then isolated compounds were treated to the differentiated 3T3-L1 adipocytes with 2-NBDG.[11,24] DMSO and insulin (01. μM) were used as negative and positive controls in this assay, respectively.

As shown in Fig. 3A, anthraquinones **2**, **7**–**10**, **12**–**14** and **17**–**18** significantly enhanced 2-NBDG uptake at a concentration of 10 μM. Compared with insulin, compounds **2** and **8** showed the most potent stimulatory activities. Detailed analysis of structure–activity relationships (SARs) of anthraquinones **1**–**19** indicated the key role of the substitution at C-1′ in their stimulatory effects on glucose uptake. Attachment of ether bond at C-1′ gave the most important positive influence on the 2-NBDG uptake, such as compounds **8**–**10**. There are no functional groups attached to C-1′ in compounds **2**–**5**, **11**, **12**, **14** and **18**. Among them, compound **2** showed the strongest stimulatory effect on 2-NBDG uptake. This observation indicated that more electronegative functionalities (OH/OMe) in 9,10-anthraquinones may be responsible for decreasing the stimulation on glucose uptake.

To confirm the transportation efficacy of 2-NBDG into the cells by compounds **2**, **8** and **10**, we further measured fluorescent signals in adipocytes after compound treatment using fluorescence microscopy. Compared to control group (treated with DMSO), compounds **2**, **8** and **10** showed higher intensity of fluorescent signals from the adipocytes at a concentration of 10 μM (Fig. 3B), and these fluorescence intensities were as much as insulin (0.1 μM). The results indicated the potential of compounds **2**, **8** and **10** to improve glucose uptake.