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Diisooctyl Phthalate Isolated from *Rourea mimosoides:* A Partial PPARγ Agonist Potently Blocks Adipocyte Differentiation

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ABSTRACT

Type II diabetes mellitus (T2DM) is a metabolic disorder due to the development of insulin resistant caused by the modification of insulin signalling, resulting in a reduction in glucose uptake in myocytes, hepatocytes, adipocytes, and elevation of blood glucose levels. Thiazolidinediones (TZDs) are medications used in the treatment of T2DM. The primary target of TZDs is the peroxisome proliferator-activated receptor-gamma (PPAR-γ), a key regulator of adipogenesis and glucose homeostasis. In this study, extracts from Malaysian plants were tested in a screening platform which includes tests for adipocyte differentiation, insulin-stimulated glucose uptake and PPAR-γ transactivation. A total of 122 plant extracts collected from Royal Belum Rainforest, Malaysia were tested on adipocyte differentiation, glucose uptake and PPAR-γ transcription assays. *Rourea mimosoides* was found to inhibit the adipocyte differentiation and showed the ability to enhance the glucose uptake in C2C12 myoblast cells as well as active to promote the transcription of PPAR-γ. A bioactive compound designated as compound **1** was isolated from this plant and was identified as Diisooctyl phthalate. The biological activities (adipocyte differentiation and PPAR-γ transactivation) of this compound were demonstrated and compound **1** was found to possess the ability to trigger expression of PPAR-γ in HepG2 cells.

Keywords: Type II Diabetes mellitus, PPAR-γ, Adipocyte Differentiation, *Rourea mimosoides*, Diisooctyl phthalate.

Introduction

The Peroxisome Proliferator Activator Receptors (PPARs) belong to a family of ligand activated nuclear transcription factors which mainly function to control the expression of gene networks involved in adipogenesis, lipid metabolism, and inflammation as well as to maintain the metabolic homeostasis.[1-3] There are three main subtypes of PPARs which have been identified; α, β, and γ.[4] These subtypes are found in different tissues where they carry out specific functions.[5] In the human body, PPAR-γ plays an important role in lipid metabolism and glucose homeostasis, modulates metabolism and inflammation in immune cells, as well as controls cell proliferation.[6] Based on previous report, PPAR-γ is also the molecule target for TZD which is a known type 2 anti-diabetic drug. Thiazolidinediones regulates insulin sensitivity, by activating PPAR-γ which in turn increases production of proteins involved in glucose uptake, and hence resulting in a decrease of insulin resistance in muscle and adipose tissue. It also plays a significant role in reducing hepatic glucose production by improving insulin sensitivity.[7] By utilizing the huge natural resources provided by Malaysian forest biodiversity, an established research platform which include adipocyte differentiation, glucose uptake and PPAR-γ transcription assays were used to discover and to evaluate the effect of these natural products against Type 2 diabetes mellitus.[8] This research could provide new candidate(s) that can give the least effect on the adipocyte differentiation but effectively enhance glucose uptake and transactivate PPAR-γ ligand thereby increasing insulin sensitivity. A forest plant identified as *Rourea mimosoides (R. mimosoides)* collected from Royal Belum Rainforest was perfectly found to possess the desirable binding activities as PPAR-γ ligand. *R. mimosoides* is a very vigorous climbing shrub producing shoots that can be 50 metres long and 10 cm in diameter. This species is usually found in lowland and hill forest at elevation up to 750 metres. Its habitats cover from beach forest, bamboo forest and shrubberies, to secondary and primary forest. *R. mimosoides* has a morphology similar to curry leaf; leaflets are often many and paired. In addition, the leaves of this plant are alternately arranged and there is petiolate in between the leaf and stem. This unique species is mostly found in Indonesia, India, and Malaysia.

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Materials and Methods

Preparation of crude extracts

A total of 122 plant samples were collected from Royal Belum Forest Reserve in Perak, in June 2010. The plant samples were recorded as ID Code: IP350 – IP472 and deposited at the Malaysian Institute of Pharmaceuticals and Nutraceuticals, Malaysia. The dried leaves powder (100 g) of each plant were soaked separately in 500 mL of methanol and shaken in water bath at 50ºC for 1 hour. The extracts were filtered through a filter paper (Whatman No. 1). The extracts were concentrated by using rotary vacuum evaporator (Brand: Eyela Digital Water Bath, SB-1000) at 40ºC under reduced pressure. The concentrated plant extracts were placed in the oven at 40ºC for 48

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hours to dry. The obtained plant extracts were kept at 4ºC before used for screening purposes.

Adipocyte cell differentiation assay

The adipocyte cell differentiation assay was performed following the standard protocol with slight modifications.[9] Briefly, 3T3-L1 cells were harvested with 2 mL of Trypsin-express when it reached 80% of confluency. Cells were then centrifuged at 2000 rpm and the pellet were resuspended with DMEM supplemented L-Glutamine, 10% (v/v) of FBS, 1% (v/v) of penicillin-streptomycin, 1% (v/v) of sodium pyruvate and 2% (v/v) of sodium bicarbonate. The cells were plated into 96 well plates at cell density of approximately 40,000 cells/well. The cells were then incubated at 37°C humidified incubator supplemented with 5% (v/v) of carbon dioxide (CO_2) for 72 hours prior to differentiation. After 72 hours of incubation, the medium in each well was discarded and the cells were washed twice with 100 µL of PBS. 3T3-L1 was then differentiated with differentiation medium which consists of DMEM supplemented L-Glutamine, 10% (v/v) of FBS, 1% (v/v) of penicillin-streptomycin, 1% (v/v) of sodium pyruvate, 2% (v/v) of sodium bicarbonate, 1 µM of Dexamethasone, 0.5 mM of IBMX and 5 µg/mL of insulin. The cells were incubated in an incubator supplemented in 5% (v/v) of CO_2 at 37°C for another 2 days. The cells were allowed to differentiate in treatment medium which consisted of DMEM supplemented L-Glutamine, 10% (v/v) of FBS, 1% (v/v) of penicillin-streptomycin, 1% (v/v) of sodium pyruvate, 2% (v/v) of sodium bicarbonate, 5 µg/mL of insulin, extracts and compound which dissolved in 0.5% (v/v) of DMSO in a final concentration range from 0.078 µg/mL to 20 µg/mL. The cells were then incubated at 37°C humidified incubator supplemented with 5% (v/v) CO_2 for another 4 days. After 4 days of treatment, the medium was discarded and the cells were cultured in 3T3-L1 cell post treatment medium which consisted of DMEM supplemented L-Glutamine, 10% (v/v) of FBS, 1% (v/v) of penicillin-streptomycin, 1% (v/v) of sodium pyruvate, 2% (v/v) of sodium bicarbonate and extract. The cells were incubated at 37°C, in the 5% (v/v) CO_2 incubator for another 4 days. After 10 days of differentiation and treatment, the medium in each well were discarded and the cells were washed twice with 100 µL of PBS prior to staining the lipid droplet. Adipose cells were fixed with 100 µL of 4% (v/v) of PFA, followed by 30 minutes of incubation at 37°C, 5% (v/v) CO_2 incubator. After 30 minutes of incubation, PFA solution was discarded, and the cells were washed twice with 100 µL of ice-cold PBS. 50 µL of Oil Red O stain was added into each well to stain the lipid droplet and the cells were incubated at room temperature for another 1 hour. Next, the Oil Red O stain was discarded, and the cells were washed twice with 100 µL of ice-cold PBS followed by 60% (v/v) of room temperature isopropanol. The stained lipid droplets were then observed under inverted phase microscope at lens power of X40.

Insulin-stimulated glucose uptake assay

Glucose uptake studies were categorized into two parts, the first part was the qualitative analysis, and the second part was the semi-quantitative analysis. For both analyses, C2C12 myotubes cells were selected as the model system to study the glucose uptake by the cells treated with the test compounds.[10] Differentiated C2C12 myotubes cells cultured in 96-well plates were serum starved overnight and treated with the compounds in four double dilutions, with starting concentration 6.25 µg/mL (i.e 3.13, 1.56 and 0.78 µg/mL) for 24 hours. For controls, C2C12 myotubes cells were treated with 0.5 % (v/v) DMSO for 24 hours and insulin for 30 minutes. After the treatment, the C2C12 myotubes cells were washed using KRPH buffer supplemented with 0.1 % (w/v) BSA and incubated with 100µM 2-NBDG for 15 minutes at 37ºC and 5 % (v/v) CO_2. Subsequently, the cells were washed 3 times for 5-10 minutes each. After the final wash, cells were lysed using 60 µL passive lysis buffer (PLB) for 5-10 minutes. Then, the cell lysates were transferred into a 96-well black plate using a multichannel pipettor. Finally, the relative fluorescence units (RFU) were read at 465/540 nm using a microplate reader.

PPAR- γ transactivation assay

Near confluent, cultures of HepG2 cells were harvested with 2 mL of Trypsin-express. Cells were then centrifuged at 2000 rpm and the pellet were resuspended with MEM supplemented with % (v/v) of FBS, 1% (v/v) of penicillin-streptomycin, 1% (v/v) of sodium pyruvate and 1% (v/v) of non-essential amino acids. The cells were then plated into 96 well plates at cell density of approximately 6000 cells/well. The cells were then incubated at 37°C humidified incubator supplemented with 5% (v/v) of CO_2 for 24 hours. After 24 hours of incubation, 11 µg of plasmid DNA (PPAR-γ, PPRE, RXR-α) and 0.22 µg of pRL-CMV were diluted in 150 µL of Opti-MEM solution. Then 25 µL of Fugene reagent was added into the mixture and incubated at room temperature for 10-15 minutes according to the manufacturer's protocols. Subsequently 25 µL of the genes solution was added into each well and incubated at 37°C incubator supplemented with 5% of CO_2 for another 24 hours prior to plant extracts/compounds treatment. After 24 hours of transfection, cells were treated with plant extracts and compounds dissolved in 0.5% (v/v) of DMSO in a final concentration ranging from 0.078 µg/mL to 20 µg/mL. The cells were then incubated at 37°C incubator supplemented with 5% of CO_2 for another 24 hours. After 24 hours luminescent intensity produced through gene expression was measured by using luminometer.

Flash Column Chromatography of Ethyl Acetate extract of R. mimosoides

Methanol extract of *R. mimosoides* was partitioned between n-hexane, ethyl acetate and butanol. All organic layers obtained were concentrated using rotary vacuum evaporator (Brand: Eyela Digital Water Bath, SB-1000) at 40ºC under reduced pressure. All organic extracts obtained were then subjected to PPAR-γ transactivation assay. The result obtained from PPAR-γ transactivation assay showed that ethyl acetate extract possessed the best activity. Therefore, the ethyl acetate extract was proceeded for compound isolation using Flash Column Chromatography. Seven hundred milligram of ethyl acetate extract of *R. mimosoides* were dissolved with 80% (v/v) of methanol and loaded into the column. The extract was then allowed for diffusion step into the stationary phase and form a layer of sample zone with the highest edge of stationary phase. The mobile phase was flushed to the top of column by the aid of the pressure in flash column chromatography. The extract was separated into few fractions by using gradient elution with increasing polarity of hexane, ethyl acetate, chloroform and methanol solvent system. A total of 104 fractions were collected and were combined based on their thin layer chromatography (TLC) profile to obtain 14 fractions. Based on the TLC profile of the 14 fractions, a single compound from fraction 2 was obtained when developed with chloroform and ethyl acetate as mobile phase in the ratio of 2:8. The single compound designated as Compound **1** was subjected for NMR, LCMS and IR analysis to elucidate the chemical structure.

Statistical analysis

The data were presented as the mean of three replicates ± standard deviation. All experiments were repeated in triplicate.

Results and Discussion

One hundred and twenty-two (122) plant species were collected and were screened for anti-diabetic properties. A screening platform that allows the identification of PPAR-γ agonists with little or no adipogenic potential was used in this study. In the first screening step, only 17 out of 122 plant extracts showed less or no adipogenic potential in the adipocyte differentiation assay (Table 1). The 17 extracts with less adipogenic potential were then screened for their glucose uptake activity. From this bioassay system, two plant extracts (IPharm423 and IPharm426) were found to increase the rosiglitazone-stimulated glucose uptake in C2C12 myotubes cell by 7.8-fold at concentration of 1.25 µg/mL and 4.3-fold at concentration of 5 µg/mL, respectively. Subsequently, when the 2 extracts were subjected to the PPAR- γ transactivation assay, only the methanolic extract of IPharm423 was found to activate PPAR-γ by 6.84-fold at concentration of 0.625 µg/mL.

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Table 1: The plant extracts tested for potential antidiabetic properties in the screening platform

Location	Plant Code*	Adipocyte Differentiation	Glucose Uptake	PPARγ Transactivation	Plant Identification
Hutan Belum, Perak	IPharm 395	Negative	Negative	ND	ND
	IPharm 396	Negative	Negative	ND	ND
	IPharm 397	Negative	Negative	ND	ND
	IPharm 398	Negative	Negative	ND	ND
	IPharm 399	Negative	Negative	ND	ND
	IPharm 400	Negative	Negative	ND	ND
	IPharm 401	Negative	Negative	ND	ND
	IPharm 402	Negative	Negative	ND	ND
	IPharm 403	Negative	Negative	ND	ND
	IPharm 405	Negative	Negative	ND	ND
	IPharm 406	Negative	Negative	ND	ND
	IPharm 412	Negative	Negative	ND	ND
	IPharm 416	Negative	Negative	ND	ND
	IPharm 419	Negative	Negative	ND	ND
	IPharm 422	Negative	Negative	ND	ND
	IPharm 423	**Negative**	**Positive**	**Positive**	***Rourea mimosoides***
	IPharm 426	Negative	Positive	Negative	ND

ND: Not Determined *Plant codes for the remaining 107 are not shown as they showed adipogenic effect.

The results obtained indicated that IPharm423 can act as PPAR-γ natural ligand with less adipogenic potential but stimulated glucose uptake. Recollection of active plant (IPharm 423) was carried out from the same collection site of Royal Belum Rainforest. Herbarium specimen was prepared and identified by botanist, Encik Kamarudin at Forest Research Institute Malaysia (FRIM). The voucher specimen IPharm/Herb423 was deposited at Division of Bio-screening, IPharm and identified as *Rourea mimosoides*, a plant species under the family of Connaraceae. In the past, *R. mimosoides* was reported as a traditional remedy to treat cold in children, bloody diarrhoea as well as used as diuretic agent.[11] In addition, *R. mimosoides* was reported to be used for the treatment of tuberculosis by the Jakun community in Kampung Peta, Johor, south of Peninsular Malaysia.[12]

The extracts obtained were subjected to PPAR-γ transcriptional activity assay. It was found that ethyl acetate extract increased the PPAR-γ transcription by 46.97 ± 11.00-fold at the concentration of 0.625 µg/mL which was higher than the hexane extract (25.33± 2.73-fold at concentration of 10 µg/mL) (Figure 1). Butanol extract did not increase the transcription of PPAR-γ activity (< 5-fold within concentration ranged from 20 µg/mL to 0.313 µg/mL). The hexane extract and ethyl acetate extract of *R. mimosoides* were further tested for rosiglitazone-stimulated glucose uptake. Hexane extract was found to increase the rosiglitazone-stimulated glucose uptake by 2.65-fold at concentration of 10 µg/mL while the ethyl acetate extract was found to increase the glucose uptake by 3.05-fold at concentration of 0.625 µg/mL (Figure 2). The ethyl acetate extract of *R. mimosoides* was subjected to flash column chromatography due to its positive activities on adipocyte differentiation, insulin-stimulated glucose uptake and PPAR-γ transactivation at extract level. The extract was separated into few fractions by using gradient elution with increasing polarity of hexane, ethyl acetate, chloroform and methanol solvent system. A total of 104 fractions were collected and were combined based on their thin layer chromatography (TLC) profile to obtain 14 fractions. Based on the TLC profile of the 14 fractions, one single compound from fraction 2 was obtained when developed with chloroform and ethyl acetate as mobile phase in the ratio of 2:8.

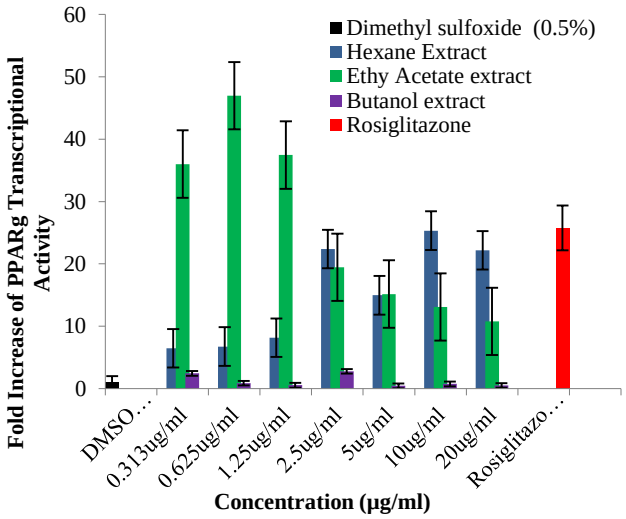

Figure 1: PPAR-γ transcriptional activity of n-hexane, ethyl acetate and butanol extract of *R. mimosoides*. Extract concentration ranged from 20 µg/mL to 0.313 µg/mL.

The single compound was isolated in 1.41% yield (46.32 mg) as brown-yellowish gummy form from ethyl acetate fraction and was designated as Compound **1**. Compound **1** has the melting and boiling points of 45°C and 365°C, respectively. In the mass spectrum of compound **1**, a signal was observed at [M+H] at m/z (relative intensity) 390 which corresponds to a molecular formula of $C_{24}H_{38}O_4$. This compound was subjected to spectroscopic analysis using IR, ^{1}H-NMR^{13}C-NMR, DEPT as well as 2D NMR spectral, ^{1}H-^{1}H COSY, NOESY, HMBC and HMQC. The IR band showed an aliphatic aldehyde peak at 1724 cm^{-1}; aliphatic hydrocarbon at 2858 cm^{-1}, 2926 cm^{-1} and 2957 cm^{-1}; ortho substituted aromatic hydrocarbon at 741 cm^{-1}.

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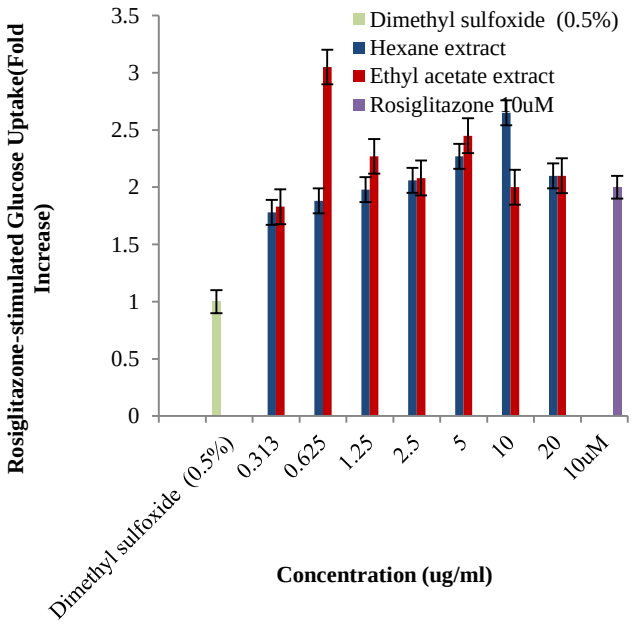

Figure 2: Rosiglitazone-stimulated glucose uptake of hexane and ethyl acetate extract of *R. mimosoides*. Extract concentration ranged from 20 µg/mL to 0.313 µg/mL.

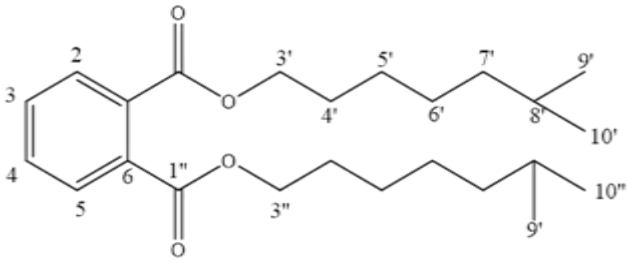

Figure 3: Compound 1: Diisooctyl phthalate

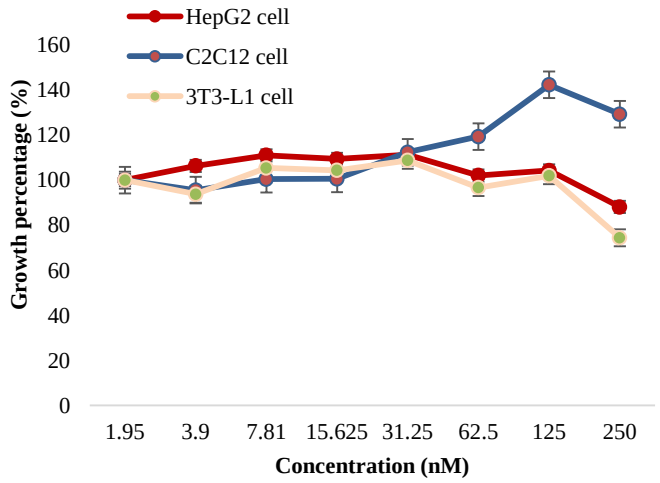

Figure 4: Cytotoxic activity of compound 1 on C2C12, HepG2 and 3T3-L1 cell lines.

Based on the comparison of its spectral data with the reported data of Fadipe *et al.*,[13] compound **1** was identified as Diisooctyl phthalate or 1,2-Benzenedicarboxylic acid, diisooctyl ester (Figure 3). The spectroscopic data of compound **1** is presented as follows:
Diisooctyl phthalate: brown-yellowish emulsion; m.p. -45°C; b.p. 360-370°C; IR(ATR) υmax cm^{-1}: 741, 1724, 2858, 2969 and 2957. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.84 (3H, t, H-9'/9", H-10'/10"), δ1.18 – 1.28 (m, overlapping region of CH_2, H-4'/4", H-5'/5", H-6'/6", H-7'/7"), δ1.62 (2H, pentet, H-8'/8"), δ4.15 (4H, septet, H- 3'/3"), δ7.42 (2H, d, *J* = 9.0 Hz, H- 3, H-4), δ7.64 (2H, d, *J* = 9.0 Hz, H-2; H-5); ^{13}C-NMR ($CDCl_3$, 100MHz); δ10.98 (C-10'/10") , δ14.08 (C-9'/9"), δ38.72 (C-8'/8"), δ23.00 (C-7'/7"), δ23.74 (C-6'/6"), δ28.93(C-5'/5"), δ30.36(C- 4'/4"), δ68.16(C- 3'/3"), δ128.81(C-3;C-4), δ130.89 (C-2, C-5), δ132.46(C-1, C-6), δ167.77(C-1'/1").
Compound **1** was further analyzed for its cytotoxicity, adipocyte cell differentiation and insulin-stimulated glucose uptake activities. Cytotoxicity of Compound **1** was evaluated on 3T3-L1 preadipocyte fibroblast, HepG2 and C2C12 myoblast cells. Each cell-line was treated with Compound **1** at concentration ranged from 1.95 nM at 2-fold increment to the highest concentration of 250nM. A growth inhibition curve was plotted for each treated cell-line as presented in Figure 5. Based on the growth curve, Compound **1** showed IC_{50} value above 250nM (IC_{50} > 250 nm) and therefore was not toxic against 3T3-L1 preadipocyte fibroblast, HepG2 and C2C12 myoblast cells (Figure 4).
In the adipocyte cell differentiation assay, pre-adipocyte cells (3T3-L1) were treated with Compound **1** at different molarity which ranged from 0.39nM to the highest molarity of 25nM. Data for this assay was collected qualitatively. The lipid droplets appeared as red-ring form after stained with Oil Red O solution as shown in Figure 5.
It was observed that, Compound **1** showed the ability to induce PPARγ gene expression in adipocyte cells in dose dependent manner as indicated by the formation of lipid droplets in mature adipose cell at molarity of 0.39 nM to 12.5nM. However, adipocyte differentiation activity of Compound **1** decreased at the concentration of 12.5nM onward. The inhibition of the adipocyte differentiation activity was due to the presence of PPARγ in mature adipocyte cell, which played a central role in fats and carbohydrate metabolism controlled by marker protein, adiponectin.[14,15]
As presented in Figure 6, compound **1** showed PPAR-γ transactivation activity in dose-dependent manners and exhibited highest PPAR-γ ligand binding activity by 17.91-fold at molarity of 1.56 nM, more potent than standard drug, Rosiglitazone (16.72-fold at molarity of 10 µM). Thus, compound **1** possesses the potential to be developed as anti T2DM due to its ability to enhance the transcription of PPAR-γ gene. Phthalate esters have long been discovered as PPARs transcription factor.[16] For example, studies showed Monobenzyl phthalate (MBzP), Mono-*sec*-butyl phthalate (MBuP**)** and Mono-(2-ethylhexyl)-phthalate (MEHP), belongs to phthalate groups, were reported to stimulate the activation of endogenous PPAR-α target genes.[17] To the best of our knowledge, there is no report on the activity of PPAR-γ binding of Diisooctyl phthalate. Apart from that, Diisooctyl phthalate has been reported to possess activities on antibacterial, antimelanogenetic, antialga and cytotoxicity properties.[18] In a report by Human Health Hazard Assessment on Diisooctyl phthalate, the bulk of labelled phthalates ingested by humans were eliminated in urine within the first 24 hours and there was no significant tissue accumulation of this compound, therefore, Diisooctyl phthalate is not injurious to health.[19]

Conclusion

A total of 122 plant species collected from Royal Belum Rainforest, Malaysia were tested on adipocyte differentiation, glucose uptake and PPAR-γ transcription assays. *R. mimosoides* was found to inhibit the adipocyte differentiation, and showed the ability to enhance the glucose uptake in C2C12 cells as well as active to promote the transcription of PPAR-γ. A bioactive compound designated as compound **1** was isolated from this plant and was identified as Diisooctyl phthalate. The biological activities of this compound were demonstrated and have the potential to bind to PPAR-γ and may have the ability to trigger the down-stream regulation of glucose homeostasis and up-stream regulation of adipogenesis in human. This partial agonist of PPAR-γ is a promising therapeutic candidate for the management of T2D with minimum side effect including significant weight gain lost and adverse drug reaction.

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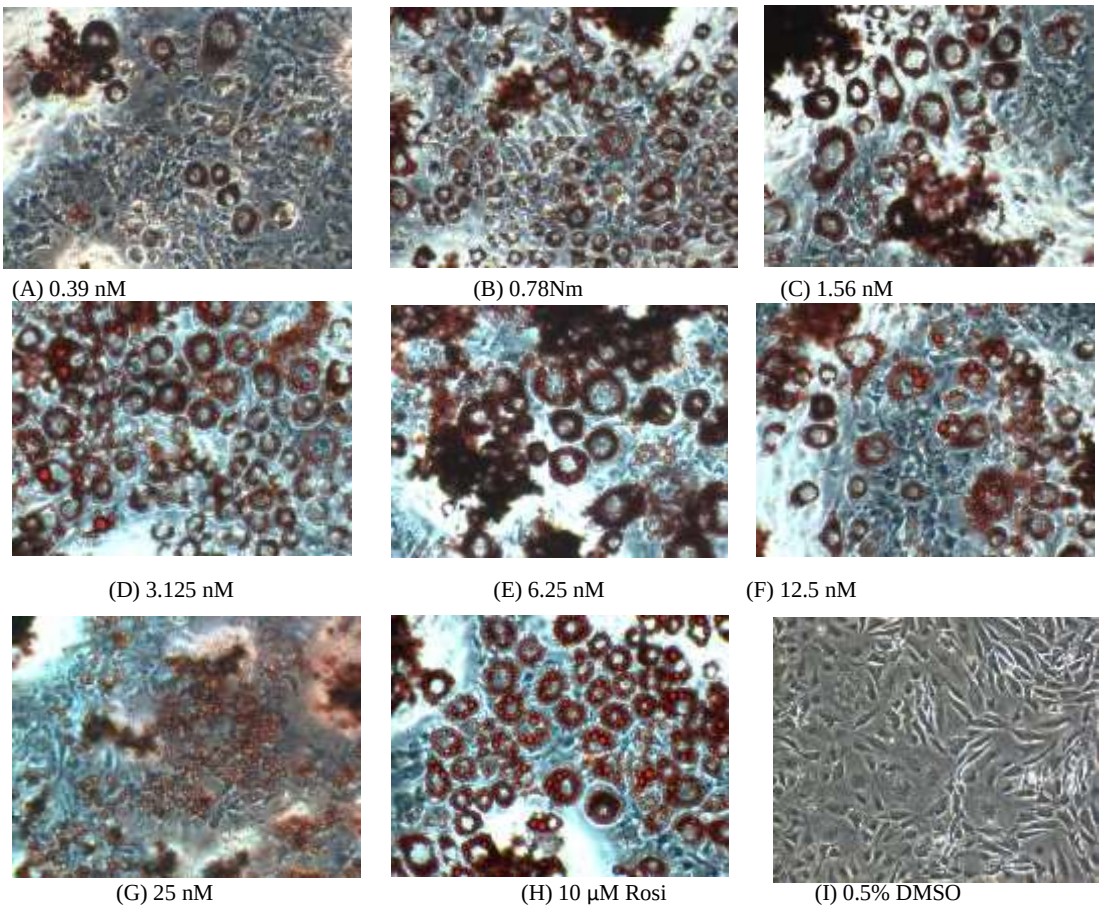

Figure 5: Lipid droplet staining in Compound 1 treated 3T3-L1 cell line. The red-ring like droplets are lipid droplets which were stained with Oil Red O solution. (A) – (G) 3T3-L1 cell line treated with Compound 1; (H) 3T3-L1 cell line treated with 10µM of Rosiglitazone (Synthetic PPARγ ligand); (I) 3T3-L1 cell line treated with 0.5% DMSO.

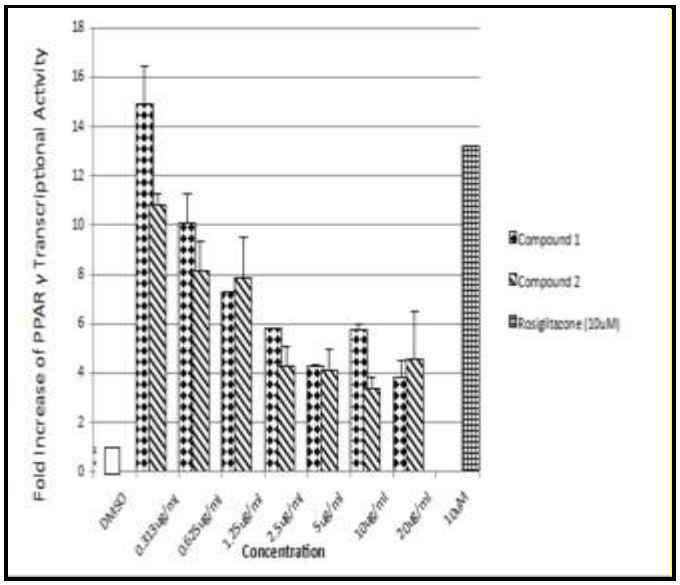

Figure 6: PPAR-γ transcriptional activity of Compound 1

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Wang L, Waltenberger B, Pferschy-Wenzig E-M, Blunder M, Liu X, Malainer C, Blazevic T, Schwaiger S, Rollinger JM, Heiss EH, Schuster D, Kopp B, Bauer R, Stuppner H, Dirsch VM, Atanasov AG. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review. Biochem Pharmacol. 2014; 92(1):73-89.
2. Van Marrewijk LM, . Polyak SW, Hijnen M, Kuruvilla D, Chang MR, Shin Y, Kamenecka TM, Griffin PR, Bruning JB. SR2067 Reveals a Unique Kinetic and Structural Signature for PPARγ Partial Agonism. ACS Chem. Biol. 2016; 11:273-283.
3. Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, Evans RM. PPARγ signaling and metabolism:

the good, the bad and the future. Nature Med. 2013; 19:557-566.
4. Hyo JG, Yong-S C, Hua L, Yoon-J K, Jae-H R, Jeon R. Identification of a Novel PPAR-γ Agonist through a Scaffold Tuning Approach. Int J Mol Sci. 2018; 19: 3032.
5. Grygiel-Górniak B. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications - a review. Nutr J. 2014; 13:17.
6. Soonkyu C, Young JK, Soo JY, Yunkyoung L, Myoungsook L. Nutrigenomic Functions of PPARs in Obesogenic Environments. PPAR Res. 2016; 2016: Article ID 4794576:17 pages.
7. Kroker A J and Bruning JB. Review of the Structural and Dynamic Mechanisms of PPARγ Partial Agonism. PPAR Res. 2015; 2015: Article ID 816856:15 pages.
8. Christensen LP and El-Houri RB. Development of an In Vitro Screening Platform for the Identification of Partial PPARγ Agonists as a Source for Antidiabetic Lead Compounds. Mol. 2018; 23:2431.
9. Natural Compound 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al from Momordica charantia Acts as PPARγ Ligand. Ahmad Noruddin N A, Hamzah M F, Rosman Z, Salin N H, Shu-Chien A C and Tengku Muhammad T S. Mol. 2021; 26:2682.
10. Wong CY, Al-Salamia H, Crispin R. Dassa. C2C12 cell model: its role in understanding of insulin resistance at the molecular level and pharmaceutical development at the preclinical stage. J Pharm Pharmacol. 2020; 72(12):1667.
11. Osman CP, Zahari Z, Adenan MI, Mohd Zohdi R. A review on traditional uses, phytochemistry, and pharmacology of the genus *Rourea*. J Appl Pharm Sci. 2019; 9(09):125.
12. Sabran SF, Mohamed M, Abu Bakar MF. Ethnobotany: A Living Science for Alleviating Human Suffering. Evid-Based Compl Altern Med. 2016; 2016, Article ID 2850845:12 pages.
13. Fadipe LA, Haruna K, Mohammed I. Antibacterial activity of 1, 2- benzenedicarboxylic acid, dioctyl ester isolated from the ethyl acetate soluble sub-portion of the unripe fruits of Nauclea latifolia. Int J Appl Biosci. 2014; 2(1):223.
14. Aranaz P, Navarro-Herrera D, Zabala M, Miguéliz I, Romo-Hualde A, López-Yoldi MJ. Martínez A, Vizmanos JL, Milagro FI, González-Navarro CJ. Phenolic Compounds Inhibit 3T3-L1 Adipogenesis Depending on the Stage of Differentiation and Their Binding Affinity to PPARγ. Mol. 2019; 24(6):1045.
15. Fan H, Pan S, Yuan G, Pengfei X, Yonggong Z. PPARs as Nuclear Receptors for Nutrient and Energy Metabolism. Mol. 2019; 24(14), 2545.
16. Desvergne B, Feige JN, Casals-Casas C. PPAR-mediated activity of phthalates: A link to the obesity epidemic? Mol Cellul Endocrinol. 2009; 304:43-48.
17. Hurst CH and Waxman DJ. Activation of PPARα and PPARγ by Environmental Phthalate Monoesters. Toxicol Sci. 2003; 74(2):297-308.
18. Roy RN. Bioactive natural derivatives of phthalate ester. Crit Rev Biotechnol. 2020; 40(7):913-929.
19. Carlson KR and Patton LE. Report Commission pursuant to contract CPSC-D-06-0006. [Online]. 2010 [cited 2021 Nov 2]. Available from: https://www.researchgate.net/publication/281275585_Toxicity_review_of_ditridecyl_phthalate_DTDP.