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Phenyl ethanone and sterols from *Acanthaster planci* as potential PPAR-ligand

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ABSTRACT

*A new phenyl ethanone (1-[4-(1,4-diamino-heptyl)-phenyl]-ethanone (**1**))and two known sterols (stigmasterol (**2**) and β–sitosterol (**3**))were succesfully isolated from methanolic extract of Acanthaster planci collected from the East Coast of Peninsular Malaysia. The chemical structures of compounds **1-3** were deduced on the basis of extensive spectral data (1D and 2D NMR), MS and IR spectroscopy techniques; as well as in comparison the data with those reported in the literature.Compounds **1-3**were not toxic towards HepG2 cells and displayed as a potential peroxissome proliferator activated receptor (PPAR) ligand.*

Keywords: *Acanthaster planci*, PPAR, sterols, phenyl ethanone, luciferase.

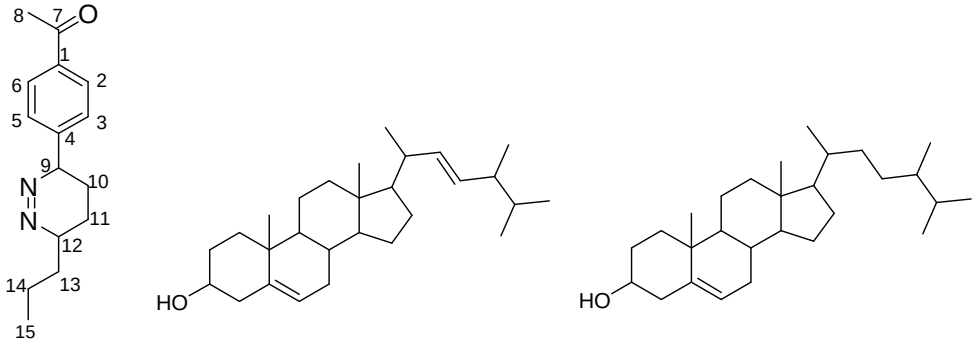
INTRODUCTION

PPARs (peroxisome proliferator activated receptor) are member of steroid hormone family that exists as heterodimers with retinoid acid receptor (RXR) when activated. Each PPAR is activated by natural ligand (such as fatty acids), and synthetic ligand including statins, interleukin and thiazolidinedione (TZD). PGL-3 PPRE is the response element for (PPAR) which exists in three isoforms i.e. PPARγ, PPARδ and PPARα. These PPARs are expressed in different part of body and related to diabetes, obesity and cardiovascular diseases [1,2,3,4]. PPARγ is a transcription factor required to activate many adipose-specific genes and related to lipid metabolism in adipose tissue [5]. TZD is PPARγ agonist and used to treat type-II diabetes. The ability of the ligand to raise HDL level in blood plasma [6] have supported the theory that PPARγ prevent the formation of atherosclerosis. PPARγ have been proved to induce the expression of genes in lipid metabolism and adipogenesis [7].

Acanthaster planci is an echinoderms classified under family Asteroidea [8]. It has been studied in early of 1970's because of the population outbreak and the potential compounds held in their venomous spines. Previous chemical investigation on *Acanthaster planci*have reported the isolation of several compounds including thornasterol [9], steroidal glycosides [10] and carotenoids [11].

Our recent interest on bioactive compounds from marine organism have resulted in the isolation of the new compound **1** along with two known sterols (**2-3**) from the outer layer of *Acanthaster planci*(Figure 1). This paper described the isolation and structure elucidation of compounds **1-3**, and their capability to bind to PPAR*ɣ* compare with the expressions of TZD.

Figure 1: Chemical structures of compounds 1-3



(1) **(2)** **(3)**

EXPERIMENTAL SECTION

General Experimental Procedures

Infrared spectra were recorded on spectrometer Model Perkin Elmer Spectrum 100 Fourier Transform Infra-red (FT-IR) Spectrometer using KBr disc. Mass spectra were obtained on the GCMS (Shimadzu/QP5050A). NMR spectra were acquired on the Bruker Spectrospin-400 (400 MHz) and DMX 600 NMR spectrometers. Melting point was determined using instrument Model Perkin Elmer Pyris 6 Disc (Disc Scanning Calorimeter). Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). The Chromatotron was carried out by preparing the plate with Silica Gel TLC –Gypsum binder.

Sample Preparation

The *A. planci* was collected from the islands of Terengganu in the East Coast of Peninsular Malaysia via scuba diving at the depth of 5 to 10 metres. The samples were kept in ice during transportation to the laboratory and furthered with samples processing. The *A. planci* was separated into two parts; the outer layer (skin and spines) and visceral organs. Subsequently, the samples were freeze-dried and ground to powder formed. The samples were stored at below -80 ^{o}C in labelled and tagged storage bottle.

Extraction and Isolation

The outer layer of *A. planci* (128.80g) were macerated with methanol at room temperature for several times. The solvent was evaporated under reduced pressure to give 7.56 g of methanolic crude extract. About 5.40 g of the methanolic crude extract was subjected to Vacuum Liquid Chromatography to yield 10 fractions (A1 to A10). Fraction A3 (810 mg) was subjected to chromatotron, eluted successively with gradient hexane-methanol mixtures of increasing polarity and separated into 10 fractions (B1 to B10). Fraction B1 (60 mg) was further subjected to chromatotron, eluting with $CHCl_3$-MeOH-hexane (9.0:5.0:0.5) to yield compound **1** (**9.1** mg) and compound **2** (**7.5** mg).

Another 2.16 g of the crude extract was suspended in H_2O and then successively partitioned with diethyl ether and n-BuOH. The n-BuOH extract was evaporated in vacuo, and the crude extract (981 mg) was subjected to a chromatotron, eluting with $CHCl_3$-MeOH (9.5:0.5) to yield 6 fractions (C1-C6). Fraction C1 (15 mg) was recrystallized with methanol to afford compound **3** (**5.5** mg).

1-[4-(1,4-diamino-heptyl)-phenyl]-ethanone (1) : Yellowish oil, positive with Dragendorff reaction. IR (KBr) ν_{max}: 3044, 2916, 2847, 1672, 1606, 1446, 1416 and 1270 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): 7.85 (2*H*,*d*, *J*=7.8 Hz), 7.25 (2*H*, d, *J*=7.8 Hz), 2.58 (3*H*, *s*), 2.53-2.55 (1*H*, *m*), 1.05-1.09 (2H, *m*), 1.44-1.50 (2*H*, *m*), 1.29-1.40 (1H, *m*),

1.21-1.26 (2*H*, *m*), 1.32-1.37 (2*H*, *m*), 0.91 (3*H*, *t*, *J*=7.2 Hz). ^{13}C NMR (125 MHz, CD_3OD): 14.4, 20.0, 26.6, 39.7, 36.9, 33.4, 34.0, 44.8, 127.1, 128.5, 135.1 and 153.7 ppm. EI-MS *m/z*: 245.7 $[M + H]^+$ (calculated for $C_{15}H_{20}N_2O$).

Stigmasterol (2): White powder; mp: 169-171°C. IR (KBr) v_{max}: 3423, 2932, 2853, 1600, 1384, 1351 and 1041 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 0.56 (3H, s), 0.80 (3H, d, *J*=2.4 Hz), 0.82 (3H, d, *J*=4.0 Hz), 0.85 (3H, s), 0.96 (3H, d, J=5.6Hz), 1.20-2.25 (m), 3.58-3.61 (1H, m), 5.18-5.19 (1H, m), 5.36-5.37 (1H, m), 5.37 (1H, br s). ^{13}C NMR (125 MHz, CD_3OD): 11.8, 13.1, 18.9, 18.9, 19.0, 19.0, 20.5, 23.9, 23.9, 27.9, 29.7, 30.7, 31.5, 31.5, 36.2, 36.6, 37.2, 39.6, 40.3, 43.4, 43.4, 49.5, 55.1, 55.1, 71.1, 117.4, 129.4, 130.3, 139.6. LCMSQ-TOF *m/z*: 415.2131 $[M^+]$ (calculated for $C_{29}H_{48}O$).

ß-Sitosterol (3): White powder; mp: 139-141°C. IR (KBr) v_{max}: 3392, 2955, 2872, 1446, 1382 and 1040 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 0.56 (3H, s), 0.80 (3H, d, *J*=2.4 Hz), 0.82 (6H, d, *J*=4.0 Hz), 0.85 (3H, s), 0.96 (3H, d, *J*=5.6 Hz), 0.90-1.90 (m), 3.65 (1H, m), 5.21 (1H, br s). ^{13}C NMR (125 MHz, CD_3OD): 11.9, 12.1, 18.3, 18.9, 19.0, 19.6, 20.5, 23.0, 23.9, 27.1, 27.9, 29.7, 30.4, 30.7, 31.5, 33.7, 36.2, 36.7, 37.2, 39.6, 40.3, 43.3, 43.4, 49.5, 55.1, 55.2, 71.1, 117.4 and 139.6 ppm. LCMSQ-TOF *m/z*: 415.2136 $[M-2H]^+$ (calculated for $C_{29}H_{50}O$).

Biological Evaluation

Cytotoxic Screening Assay.

The HepG2 cell was treated with a serial dilution of compounds, from the highest concentration of 100µg/ml to the lowest concentration of 0.39µg/ml. The treatments were carried out in eight replicates to ensure the accuracy of the results. The negative control consist of 20% DMSO and 80% of Modified Eagle Media (MEM). The positive control was made using vincristine sulphate, the standard drug in treatment of liver cancer [13]. The cells were incubated for 72 hours in 5% carbon dioxide incubator at 37°C. The cytotoxicity of the compounds were determined using CellTiter 96®AQ_{ueous} One Solution Cell Proliferation Assay (MTS) where only 20µL of the solution were transferred in each well and left incubated for 1.5 hour in 37°C. The cell viability was measured using Glomax Multi-detection (Promega) at absorbance 490nm. The value of IC_{50}, the effective concentration of drug that is required for 50% inhibition of the cell was determined with non-linear regression.

PPAR ligand potential screening assay

Plasmid (pGL-3 PPRE) extraction was conducted using Qiagen Midi Kit (Qiagen, Germany) and the transfection took place within HepG2 cells. All compounds were prepared as in the toxicity assay, but the highest concentration of sample in this assay was 25µg/ml. The thiazolidinediones (TZD), a standard drug to observe PPARγ activity was used as positive control with optimized concentration of 0.08µg/ml. The working concentration of negative control consist of 20% DMSO and 80% of Modified Eagle Media (MEM). Then, the cells were incubated for 24 hours in 5% carbon dioxide incubator at 37°C. The plate was removed from the incubator after 24 hours of treatment. 50µl of the media in each well were pipette out from the plate and measured for the firefly luciferase activity and the *Renilla* luciferase activity according to protocols provided by manufacture (Promega, Germany).

RESULTS AND DISCUSSION

Chemistry

Compound **1** was obtained in yellowish oil, showing a positive orange in colour with Dragendorff reagent. The EI-MS spectrum of this compound displayed molecular ion at m/z 244.2 (calculated for $[M]^+$), corresponding to a molecular formula of $C_{15}H_{20}N_2O$.

The IR spectrum showed the absorption bands of carbonyl group (1672cm^{-1}), and aromatic ring (1606cm^{-1} and 1446cm^{-1}) functionalities.

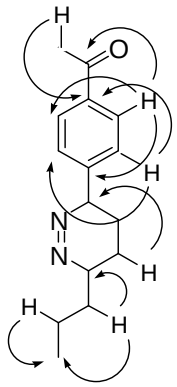
The ^{1}H NMR spectrum revealed signals of para substituted benzene atδ_H[7.29 (2H, d, *J*=7.8 *Hz*) and 7.90 (2H, d, *J*=7.8 *Hz*)]. In addition, two methyl groups at δ_H[2.58 (3H, s) and 0.91(3H, t, *J*= 7.2 *Hz*)], four methylene proton [CH_2]at δ_H[1.05-1.09 (2H, m); 1.44-1.50 (2H, m); 1.21-1.26 (2H, m) and 1.32-1.37 (2H, m)] and two methine(CH) proton at δ_H[2.53-2.55 (1H, m) and 1.29-1.40 (1H, m)] were observed.

The ^{13}C and the HSQC spectra showed 15 carbon resonance ascribed to a three quaternary, two methyl, four methylene (CH_2) and six methine (CH) C atoms.

The spectroscopic data indicated that compound **1**exhibited para substituted benzyl with cyclic diamine skeleton. The COSY spectrum reveals correlation of H-15/H-13/H-14 signified the position of protons were neighbouring to each other.

The HMBC correlation of H-2 with C-4 (δ_C153.7) and C-7 (δ_C 197.9)confirmed the position of C=O group at C-7 and suggested that the moiety is para substituted benzene (Figure 2, Table 1).In addition, correlation observed between H-10with C-9 (δ_C 44.8), C-11 (δ_C 33.4), C-12 (δ_C 36.9) and C-13 (δ_C39.7) ppm, confirmed the presence of diamine cyclic skeleton. On the basis of these data, the suggested structure for this compound is1-[4-(1,4-diamino-heptyl)-phenyl]-ethanone as shown in Figure 2.

Figure 2: HMBC correlation of compound 1



Compound **2** and **3** were deduced as sterol skeletal structure; the stigmasterol and ß-sitosterol, respectively by comparison of their spectral data with those reported in the literature [12].

In addition, LCMS-Tof spectroscopy of **2** and **3** showed the molecular ion at m/z 415.2131 [M^+] (calculated for $C_{29}H_{48}O$) and m/z 415.2136 $[M\text{-}2H]^+$ (calculated for $C_{29}H_{50}O$), respectively.

Table 1.^{1}H, ^{13}C NMR data and HMBC correlations of compound 1

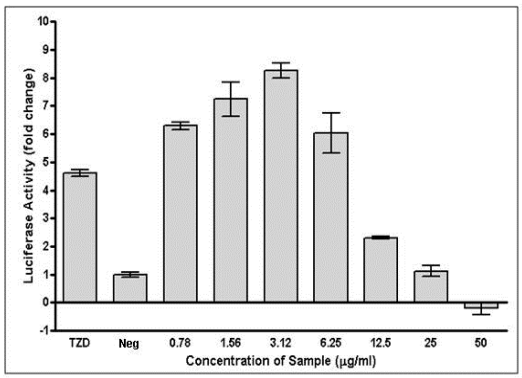
Position	^{1}H NMR (δ in ppm, *J in* Hz) (600MHz in $CDCl_3$)	^{13}C NMR (δ in ppm) (125MHz in $CDCl_3$)	HMBC correlation	
			2*J*	3*J*
1	-	135.1	-	H-3,H-8
2	7.85 (2*H*,*d*, *J*=7.8)	128.5	-	-
3	7.25 (2*H*, d, *J*=7.8)	127.1	-	H-9
4	-	153.7	H-9	H-2
5	7.25 (2*H*, d, *J*=7.8)	127.1	-	H-3,H-9
6	7.85 (2H,d, J=7.8)	128.5	-	H-2
7	-	197.9	H-8	H-2
8	2.58 (3*H*,*s*)	26.6	-	-
9	2.53 – 2.55 (1*H*, *m*)	44.8	H-10	H-3, H-10, H-11
10	1.05 – 1.09 (*m*)	34.0	H-9, H-11	-
11	1.44 - 1.50 (2*H*, *m*)	33.4	H-10	H-13, H-14
12	1.29 – 1.40 (*m*)	36.9	H-10,H-11, H-13,H-14	H-10,H-13, H-14
13	1.21 - 1.26 (2*H*, *m*)	39.7	H-14,H-12	H-15
14	1.32 - 1.37 (2*H*, *m*)	20.0	H-15, H-13	-
15	0.91 (3*H*, *t*, *J*=7.2)	14.4	H-13,H-14	H-13,H-14

Biological Assay

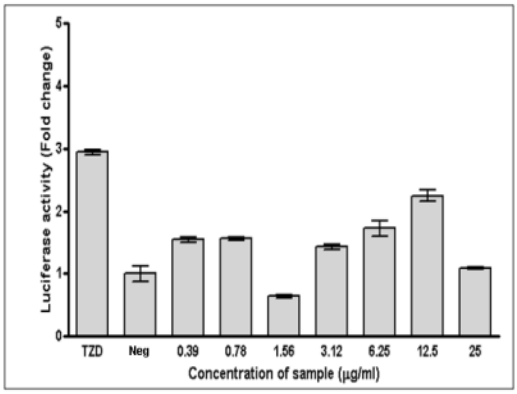
All three isolates were tested for toxicity activity against Hepatocellular carcinoma liver cancer cells (HepG2). Result indicated that these compounds did not possess the anticancer properties against the cells tested (IC_{50} value higher than 30µg/ml).

These compounds were further evaluated for their potential as PPAR ligands. Our investigation showed that compounds **1-3**possessed as a potential PPAR ligands similar to TZD.

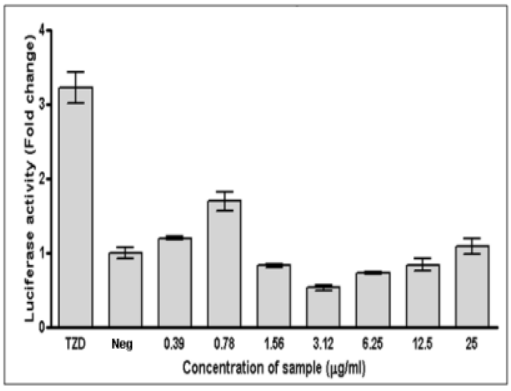
Figure 3: Luciferase activity of transfected HepG2 cells against various concentrations of compounds 1-3



Compound **1**



Compound **2**



Compound **3**

Compound **1** showed the highest level expression of the luciferase gene at optimum concentration of 3.12µg/ml (Figure 3). The concentration was higher than positive control, TZD (0.08µg/ml) indicated that **1** possessed better properties as ligand to PPAR compared to TZD. The decreased expression in concentration higher than 3.12µg/ml might be due to the limited ligand binding site of PPAR as the excess concentration of compounds.

Compounds **2** and **3** showed moderate activity as compared to TZD. Compound **2** showed biphasic pattern. The first peak was at concentrations 0.39 to 0.78µg/ml and drastically dropped at 1.56 µg/ml. The second peak was at concentration 3.12 to 12.5µg/ml. The highest activity of luciferase gene was at concentration of 12.5 µg/ml (Figure 3). Compound **3** exhibited the highest activity at 0.78µg/ml. Luciferase activity was not activated in higher than this

concentrations. This phenomenon might due to the properties possessed by these compounds were different than TZD. Although compounds **2** and **3** showed moderate expression of luciferase enzyme, the ability to express the genes indicating their binding potential to PPAR at certain concentrations.

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