



A Cipadesin Limonoid and a Tirucallane Triterpene from the Fruit of *Sandoricum koetjape* and their Inhibitory Properties against Receptor Tyrosine Kinases

Dyah Ayu Rachmadhaningtyas¹, Leny Heliawati², Elvira Hermawati¹, and Yana Maolana Syah^{1,*}

¹Organic Chemistry Division, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia

²Department of Chemistry, Pakuan University, Jalan Pakuan PO Box 452, Bogor 16143, Indonesia

Abstract – A new cipadesin limonoid, i.e. 3-epi-cipadonoid C (**1**), and a new tirucallane triterpene, i.e. hispidol B 3-palmitate (**3**), have been isolated from the seeds and fruit peels extract of *Sandoricum koetjape*, respectively. Along with these compounds the known limonoid, cipaferen G (**2**), and two pentacyclic triterpenes, bryonolic (**4**) and bryononic (**5**) acids, were also isolated. The structures of the new compounds were elucidated by the analysis of NMR and mass spectral data. Compounds **1** - **5** were evaluated as the inhibitor of receptor tyrosine kinases (EGFR, Epidermal Growth Factor Receptor; HER2, HER4, Human Epidermal growth factor Receptor 2, -4; IGFR, Insulin-like Growth Factor Receptor; InsR, Insulin Receptor; KDR, Kinase insert Domain Receptor; PDGFR α , and PDGFR β , Platelet-Derived Growth Factor Receptor- α and - β). The results showed only **1** and **3** that have weak activity against InsR.

Keywords – *Sandoricum koetjape*, 3-epi-Cipadonoid C, Hispidol B 3-palmitate, Limonoid, Tirucallane, Receptor tyrosine kinase

Introduction

Cancer is still a difficult disease to treat. One of the traditional approaches to finding anticancer agents is through cytotoxicity evaluation of the candidate compounds against a number of cancer cells.¹ However, in recent years the tyrosine kinases (TKs) become the target in searching for new anticancer agents.^{2,3} In human these enzymes involved in a number of cellular signaling pathways by catalyzing a phosphate transfer from ATP to the tyrosine residue of the other proteins. Lack of TK's function can lead to the formation of generative diseases, while their excessive expression can result in the generation of tumors and/or cancers. TK's are divided into two families, receptor (RTKs) and -non-receptor tyrosine kinases (nRTKs).⁴ Examples of RTKs are epidermal growth factor (EGFR; HER2, HER4, *Human Epidermal growth factor Receptor 2, -4*), insulin (IGFR, *Insulin-like Growth Factor Receptor*; InsR, *Insulin Receptor*), kinase insert domain (KDR) and platelet-derived growth factor (PDGFR α and - β) receptors. These RTKs have been shown to have a relationship to the

formation of various tumors/cancers. Most of the TK's inhibitors are synthetic chemicals, while the inhibitors originated from natural sources, both terrestrial and marine origins, are still very limited.^{5,6}

Sandoricum koetjape Merr. (Meliaceae) is an endemic plant of South East Asia regions and produces edible fruits that have a unique taste.⁷ The only reports on its traditional uses are from the bark part of the plant. The Malaysian people use the aqueous decoction of the bark as a tonic for the women after childbirth, while in Indonesia this preparation is used for the treatment of colic and leukorrhea.⁷ Phytochemical investigation of the plant has isolated a number of terpenoid derivatives, including sesquiterpenes, triterpenes, and limonoids.^{8,9} In searching of RTK's inhibitors from natural sources,^{9,10} we had examined further the seeds extract of the plant which led to the isolation of other limonoid components, namely a cipadesin derivative, 3-epi-cipadonoid C (**1**) (Fig. 1), and a known andirubin, cipaferen G (**2**). In addition, a new tirucallane derivative, hispidol B 3-palmitate (**3**), and two known pentacyclic triterpenes, bryonolic acid (**4**) and bryononic acid (**5**), were also isolated from the fruit pericarps extract of the plant. This paper reports the structure elucidation of the new compounds and inhibitory properties of **1** - **5** against eight RTKs (EGFR, HER2, HER4, IGFR, InsR, KDR, PDGFR α , and PDGFR β).

*Author for correspondence

Yana Maolana Syah, Organic Chemistry Division, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia.
Tel: +62-22-2502103; E-mail: yana@chem.itb.ac.id

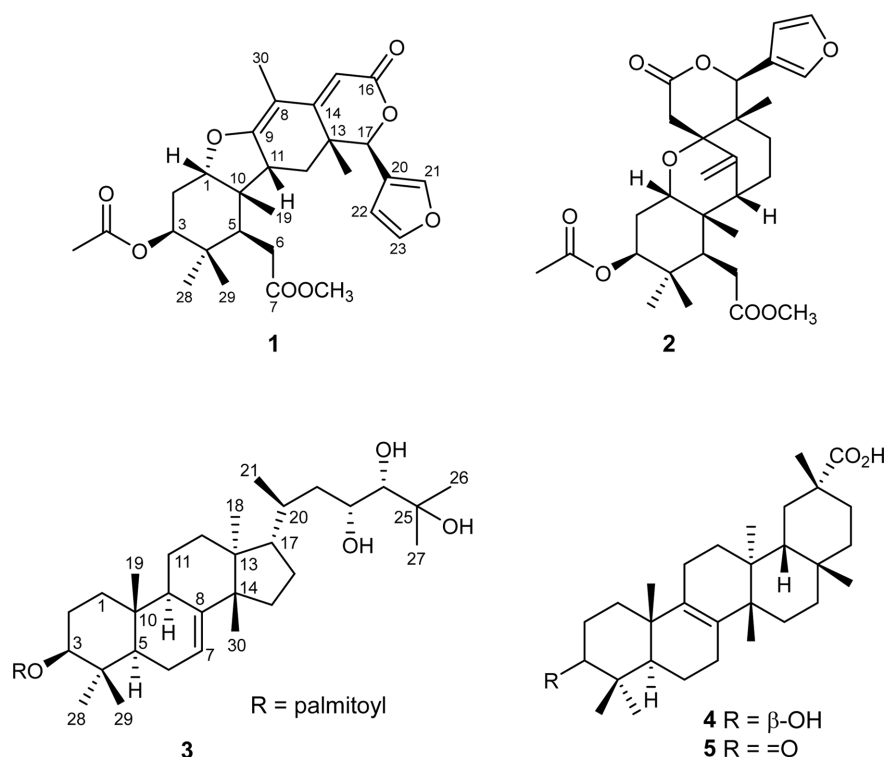


Fig. 1. Structures of the compounds isolated from the seeds and fruit peels of *S. koetjape*.

Experimental

General experimental procedures – UV and IR spectra were measured with a Varian Cary 100 Conc instrument and a Perkin Elmer FTIR Spectrum One spectrometer, respectively. Optical rotation were determined with an Autopol IV Rudolph Research Analytical. High-resolution mass spectra were obtained with an ESI-TOF Waters LCT Premier XE mass spectrometer. ^1H and ^{13}C NMR spectra were measured in CDCl_3 with a spectrometer of Agilent DD2 system operating at 500 and 125 MHz, respectively. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were conducted on Merck silica gel 60 GF254 art. 7731 and 7749, respectively. Thin layer chromatography (TLC) analysis was done using precoated silica gel plates (Merck Kielselgel 60 GF254, 0.25 mm thickness). Spot on TLC were detected by UV irradiation, and sprayed by 10% vanillin in H_2SO_4 , which was followed by heating. Solvents (MeOH, acetone, EtOAc and *n*-hexane) for extraction, fractionation and purification were of technical grades, which were distilled before used. CHCl_3 used in the purification was a pro analysis grade. Kinase Selectivity Profiling System (KSPS) Receptor Tyrosine Kinases TK1 (EGFR, HER2, HER4, IGF1R, InsR, KDR, PDGFR α and PDGFR β) was purchased from Promega and stored at -80°C as one-time

use aliquots. Kinase enzyme assay was done using a pipetmax automatic liquid handler (Gilson), while luminescent measurements were carried out with a GloMax Explorer.

Plant materials – The fruit samples of *S. koetjape* were collected in August 2013 from Pandeglang, Banten Province, Indonesia. The identity of the plant was determined by the staff of Herbarium Bandungense, Institut Teknologi Bandung, and the voucher specimen was deposited at the Herbarium (voucher number 11254).

Extraction and isolation – The dried and powdered seeds of *S. koetjape* (1 kg) were extracted with acetone (3×10 L, overnight) at room temperature to give a dried extract (80 g) after solvent evaporation. A portion of the extract (20 g) was subjected to a VLC (170 g silica gel, gradient elution: *n*-hexane-EtOAc = 10:0 to 1:1, 10% stepwise EtOAc addition, followed by EtOAc and MeOH, each 2×200 mL) to give 18 fractions F1-F18. F9-F16 were combined (3.3 g) and were refractionated using the same method (80 g silica gel, gradient elution: *n*-hexane-EtOAc = 9:1, 4:1, 3:1, 7:3, 1:1, followed by EtOAc, each 2×75 mL) yielding 14 subfractions F916-1 to F916-14. Purification of the F916-9 (200 mg) by CPC (eluent: *n*-hexane-EtOAc = 4:1) afforded **2** (70 mg), while from F916-12 (200 mg) (eluent: *n*-hexane- CHCl_3 = 3:7) gave **1** (14 mg). The same procedures were done to the fruit peels

extract of the plant (20 g) to obtain 18 fractions G1-G18 after VLC fractionation. G8-G9 were left overnight and gave **4** (1.2 g) as white crystals, while G11-G12 (2.6 g) were refractionated (VLC, silica gel 80 g, gradient elution: *n*-hexane-EtOAc = 9:1, 4:1, 3:1, 7:3, 1:1, followed by EtOAc, each 2 × 75 mL) to give 12 subsfraction G1112-1 to G1112-12. Purification of G1112-7 to G1112-10 by CPC gave **3** (50 mg) and **5** (15 mg).

3-*epi*-Cipadonosoid C (1) – White solid. $[\alpha]_D^{20} = +44.0^\circ$ (c 0.1, CHCl₃); UV (CDCl₃): 236 (4.01), 309 (3.62) nm; IR (KBr): 2945, 1734, 1643 cm⁻¹; ¹H NMR (CDCl₃) see Table 1; ¹³C NMR (CDCl₃) see Table 1; HR-ESI-TOF-MS: [M+Na]⁺ ion *m/z* 535.2297 (calcd. [M+Na]⁺ ion for C₂₉H₃₆O₈: *m/z* 535.2302).

Hispidol B 3-palmitate (3) – White solid. $[\alpha]_D^{20} = -27^\circ$ (c 0.27, CHCl₃); IR (KBr): 3342, 1744 cm⁻¹; ¹H NMR (CDCl₃) see Table 2; ¹³C NMR (CDCl₃) see Table 2; HRESITOF-MS: [M+Na]⁺ ion *m/z* 737.6069 (calcd. [M+Na]⁺ ion for C₄₆H₈₂O₅: *m/z* 737.6055).

Kinase enzyme assay – The assay was done according to the previously described.^{8,9} Briefly, the tested compound was made 5% in DMSO and was diluted to a final concentration of 10 μM by 4X kinase buffer and nuclease-free water. Each kinase stock was also diluted with 2.5X reaction buffer (95 μL), while the substrate/cofactor stock was diluted with 80 μM ATP solution (20 μL). The assay was done as follows: the kinase (2 μL), the ATP/substrate

(1 μL), and tested compound (1 μL) were dispensed to each well of the 384-well plate, and then was allowed to react for 1 hour (22 - 25 °C). After this, the ADP-Glo reagent was added (5 μL), and the was incubated for 40 minutes (22 - 25 °C), which was followed by the addition of kinase detection reagent (10 μL) to be incubated again for 30 minutes. After the reaction was completed, the luminescent was measured, which corresponds to the kinase activity. The negative control (100% activity) was the well without the tested compound solution, while the background luminescent (0% activity) was obtained from the well without enzyme solution. The % kinase activity was calculated by subtracting the background luminescent from all kinase reactions (Table 3). This assay used erlotinib as the control positive (1 μM), while the purity of **1 - 5** (>95%) was determined by NMR.

Result and Discussion

Compound **1** was isolated from the seed extract of *S. koetjape* as a white powder, $[\alpha]_D^{20} = -44^\circ$ (CHCl₃). Its molecular formula was determined to be C₂₉H₃₆O₈ based on the HR-ESI-TOF-MS spectrum (positive mode, found *m/z* 535.2297 [M+Na]⁺, calcd. 535.2302). Its UV spectrum exhibited absorptions (λ_{max} 236, 309 nm) assignable to a 3,4,5,5-tetrasubstituted diene ester chromophore,¹¹ while the IR absorptions indicated the presence of a simple ester

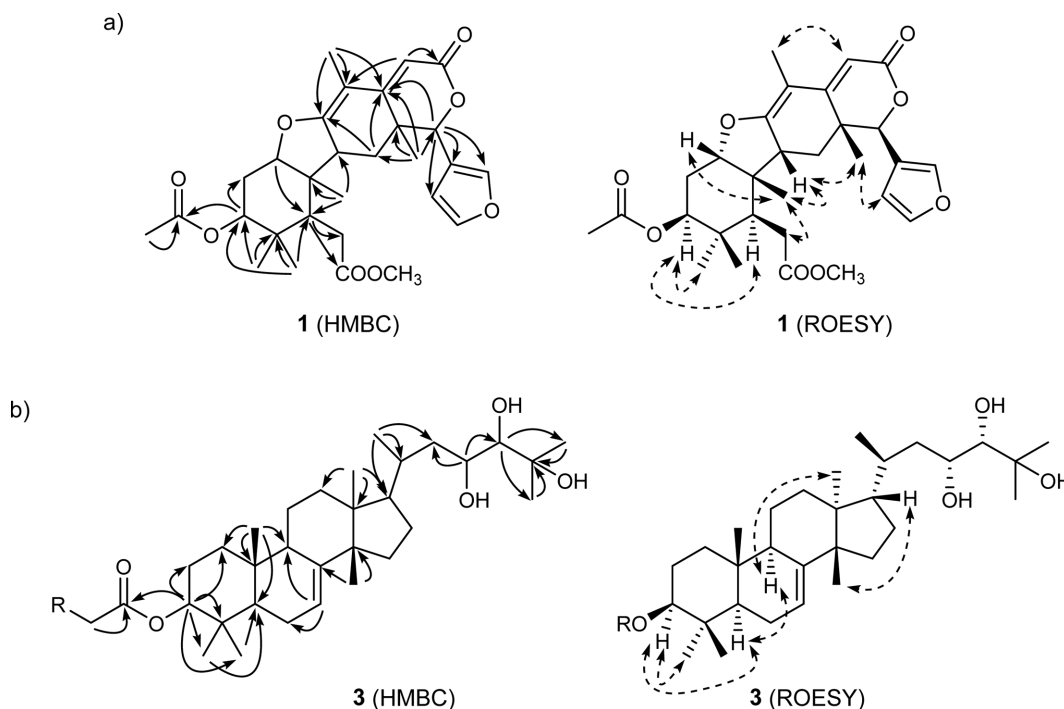


Fig. 2. Selected HMBC and ROESY correlations in a) **1** and b) **3**.

Table 1. NMR data of compound **1** in CDCl₃

C No.	1		6 ¹⁰	
	δ_{H} (<i>mult.</i> , <i>J</i> in Hz)	δ_{C}	δ_{H} (<i>mult.</i> , <i>J</i> in Hz)	δ_{C}
1	4.23 (<i>br t</i> , 3.5)	87.7	4.22 (<i>s</i>)	86.5
2	2.33 (<i>ddd</i> , 14.5, 5.8, 4.2) 1.90 (<i>ddd</i> , 14.5, 12.1, 3.5)	27.0	2.52 (<i>m</i>) 2.10 (<i>s</i>)	25.0
3	4.88 (<i>dd</i> , 12.1, 4.2)	73.5	4.61 (<i>s</i>)	76.0
4	-	38.7	-	37.8
5	1.98 (<i>d</i> , 8.8)	40.0	2.29 (<i>d</i> , 9.0)	35.1
6	2.46 (<i>dd</i> , 17.4, 8.8) 2.08 (<i>d</i> , 17.4)	32.3	2.42 (<i>m</i>) 2.14 (<i>s</i>)	32.2
7	-	173.8	-	174.1
8	-	102.2	-	101.0
9	-	163.3	-	163.2
10	-	44.8	-	44.5
11	2.51 (<i>dd</i> , 12.0, 4.7)	50.4	2.57 (<i>d</i> , 13.0)	50.4
12	1.66 (<i>t</i> , 12.0) 1.36 (<i>dd</i> , 12.0, 4.7)	31.3	1.81 (<i>s</i>) 1.41 (<i>dd</i> , 12.0, 4.0)	31.3
13	-	37.8	-	37.3
14	-	162.6	-	163.9
15	5.63 (<i>s</i>)	104.5	5.59 (<i>s</i>)	103.5
16	-	166.4	-	166.7
17	5.03 (<i>s</i>)	80.1	5.10 (<i>s</i>)	80.1
18	1.05 (<i>s</i>)	16.5	1.06 (<i>s</i>)	16.6
19	1.10 (<i>s</i>)	19.1	1.12 (<i>s</i>)	20.1
20	-	120.5	-	120.4
21	7.34 (<i>br s</i>)	141.0	7.36 (<i>br s</i>)	141.0
22	6.33 (<i>br s</i>)	110.0	6.35 (<i>br s</i>)	109.9
23	7.42 (<i>br s</i>)	143.0	7.44 (<i>br s</i>)	143.0
28	0.98 (<i>s</i>)	15.7	1.01 (<i>s</i>)	21.4
29	0.75 (<i>s</i>)	25.5	0.80 (<i>s</i>)	26.1
30	1.83 (<i>s</i>)	9.6	1.78 (<i>s</i>)	9.5
3-C(=O)CH ₃	-	170.2	-	171.0
3-C(=O)CH ₃	2.07 (<i>s</i>)	21.1	2.06 (<i>s</i>)	21.2
7-OCH ₃	3.73 (<i>s</i>)	52.2	3.79 (<i>s</i>)	52.1

(ν_{max} 1734 cm⁻¹) and a conjugated ester or lactone (ν_{max} 1643 cm⁻¹) groups. In the limonoid group, these spectral characteristics were reminiscent of the cipadesin class.¹² This was corroborated by the presence of carbon signals at δ_{C} 166.4, 163.3, 162.6, 104.5, 102.2 (Table 1) typical for the signals of C-16, C-9, C-14, C-15, and C-8, respectively, of the cipadesin compounds with a dienelactone group. In addition, a shielded methyl carbon signal (δ_{C} 9.6) was also observed, which is a characteristic for the methyl group attached at C-8.¹³ In fact, the ¹H and ¹³C NMR data of **1** were very close to those cipadonoid C (**6**),¹³ except that proton signal H-3 appeared as a double of doublet ($J = 12.1$ and 4.2 Hz), while this proton signal in **6** is a broad singlet. Thus, the H-3 in **1** should be in the

axial orientation, suggesting that **1** is a 3-epimer of **6**. The HMBC correlations gave more support for the basic structure of **1**, while the NOE correlations from ROESY spectrum established its stereochemistry (Fig. 2). As expected, the NMR parameters of **1** and **6** are different at C-1-C-6, while the rest part of the structure is essentially the same (see Table 1). Therefore, **1** was determined as 3-*epi*-cipadonoid C.

Compound **2** was identified as cipaferen G based on a comparison of its NMR data with those reported in the literature.¹⁴

Compound **3** was isolated from the fruit peels extract of *S. koetjape* also as a white powder, $[\alpha]_{\text{D}}^{20} = -27^{\circ}$ (CHCl₃). The IR spectrum exhibited the absorptions of ester

Table 2. NMR data of compound **3** in CDCl₃

C No.	3		7 ¹³
	δ_{H} (<i>mult.</i> , <i>J</i> in Hz)	δ_{C}	δ_{C}
1	1.67 (<i>m</i>), 1.22 (<i>m</i>)	36.8	38.5
2	1.65 (<i>m</i>), 1.60 (<i>m</i>)	24.2	34.9
3	4.51 (<i>dd</i> , 11.2, 3.9)	80.8	217.0
4	-	37.7	47.8
5	1.41 (<i>m</i>)	50.7	52.3
6	2.12 (<i>m</i>), 1.66 (<i>m</i>)	23.7	24.3
7	5.25 (<i>br t</i> , 4.2)	117.8	117.9
8	-	145.7	145.7
9	2.21 (<i>m</i>)	48.8	48.4
10	-	34.8	35.0
11	1.51 (2H, <i>m</i>)	18.1	18.3
12	1.80 (<i>m</i>), 1.63 (<i>m</i>)	33.8	33.8
13	-	43.6	43.5
14	-	51.2	51.2
15	1.49 (<i>m</i>), 1.44 (<i>m</i>)	34.0	34.0
16	1.99 (<i>m</i>), 1.29 (<i>m</i>)	28.5	28.4
17	1.48 (<i>m</i>)	53.8	53.8
18	0.98 (<i>s</i>)	22.0	22.0
19	0.77 (<i>s</i>)	13.2	12.8
20	1.39 (<i>m</i>)	33.7	33.7
21	0.93 (<i>d</i> , 6.8)	18.9	18.9
22	1.87 (<i>m</i>), 1.18 (<i>m</i>)	40.5	40.4
23	4.12 (<i>br s</i>)	69.8	69.7
24	3.16 (<i>br d</i> , 5.9)	75.0	75.0
25	-	74.4	74.3
26	1.31 (<i>s</i>)	26.3	26.2
27	1.32 (<i>s</i>)	27.2	27.4
28	0.85 (<i>s</i>)	27.6	24.5
29	0.94 (<i>s</i>)	15.9	21.6
30	0.82 (<i>s</i>)	27.4	27.4
1'	-	173.8	
2'	2.30 (<i>t</i> , 7.5)	34.9	
3'	1.61 (<i>m</i>)	25.2	
4'-15'	1.36-1.23 (<i>m</i>)	31.9, 29.7-29.2	
16'	0.88 (<i>t</i> , 7.1)	14.3	

carbonyl (ν_{max} 1744 cm⁻¹) and hydroxy (ν_{max} 3342 cm⁻¹) groups. The NMR spectra of **3** (Table 2) clearly showed proton signals for eight methyl groups (δ_{H} 1.32, 1.31, 0.98, 0.94, 0.93, 0.85, 0.82, 0.77) and four signals for non-oxygenated-*sp*³ carbons (δ_{C} 51.2, 43.6, 37.7, 34.8), indicating that **3** is a tetracyclic triterpene. One of the methyl proton signals was found as a doublet (δ_{H} 0.93, *J*=6.8 Hz), suggesting that **3** has the basic structure of the tirucallane triterpene, as is usually found in the Meliaceae plants.¹² The functional groups found in **3**

include a trisubstituted alkene (δ_{H} 5.25; δ_{C} 145.7, 117.8), one secondary ester (δ_{H} 4.51; δ_{C} 173.8, 80.8), two secondary alcohol (δ_{H} 4.12, 3.16; δ_{C} 75.0, 69.8), and one tertiary alcohol (δ_{C} 74.4) groups.

In the HMBC spectrum (Fig. 2), the olefinic proton signal (δ_{H} 5.25) was correlated with C-9 methine carbon (δ_{C} 48.8), showing the presence of 7,8-double bond in **3**. The HMBC correlations also allowed to place the two secondary alcohols at C-23 and C-24, the tertiary alcohol at C-25, and the ester group at C-3. From these spectroscopic data, **3** is deduced to be the ester derivative of the tirucallane triterpene, hispidol B,¹⁵ based on the presence of large coupling constant (11.2 Hz) at δ_{H} 4.51. The ester was determined to be a saturated fatty acid derivative, as shown by the observation of many methylene carbon signals in the range of δ_{C} 34.9-29.2, as well as a methyl signal at δ_{H} 0.88 (*t*, 7.1 Hz) and δ_{C} 14.3. The HR-ESI-TOF-MS spectrum (positive mode) established the molecular formula C₄₆H₈₂O₅ for this compound (found *m/z* 737.6069 [M+Na]⁺, calcd. 737.6055), and thus identifying the ester part as the palmitoyl group. In addition, support for the tirucallane structure was obtained from the ROESY correlations, as shown in Fig. 2.

The stereochemistry at C-20, C-23, and C-24 was determined by comparing the ¹³C NMR data with those reported for piscidinol A (**7**) (3-oxo derivative of **3**), in which its absolute configurations was determined by x-ray crystallography analysis.¹⁶ The carbon chemical shifts at C-20, C-23, C-24, and C-25 in **3** showed a close agreement with those reported for **7** (Table 2). Compound **3**, therefore, is assigned as hispidol B 3-palmitate.

In addition to **3**, two known pentacyclic triterpenes **4** and **5** were also isolated from the fruit peels extract of *S. koetjape*. Based on their NMR data both compounds were determined as bryonolic and bryononic acids,¹⁷ respectively.

Cipadesin class is among the minor limonoid found in Meliaceae and the genus *Cipadessa* is the only source of this compound class.¹² The presence of **1** in *S. sandoricum* is the only example of cipadesin limonoid that occurs outside this genus and supports further the close relationship chemotaxonomically between the two genera *Cipadessa* and *Sandoricum*.⁸ Fang et al. has proposed a biogenetic relationship between andirobin and trijugin in one hand, and with cipadesin on the other through the rearrangement of the andirobin's skeleton from C-8 to C-11 and C-10 to C-11, respectively.¹³ The key compound is 10-hydroxy derivative of andirobin, which is found in both genera. However, the terpenoid resource in the *S. koetjape* is not allocated to the limonoid metabolites, but also to the tetracyclic and pentacyclic triterpenes, such as **3** - **5**.

Table 3. Tyrosine kinase activity of compounds **1 - 5** at concentration 10 μ M

Compounds	% Activity ^b							
	EGFR	HER2	HER4	IGF1R	InsR	KDR	PDGFR α	PDGFR β
1	103 \pm 1.3	135 \pm 0.8	98 \pm 1.7	92 \pm 3.3	67 \pm 4.0	101 \pm 0.3	104 \pm 3.2	84 \pm 3.1
2	118 \pm 1.3	130 \pm 1.8	106 \pm 3.2	87 \pm 3.7	120 \pm 3.8	110 \pm 3.9	105 \pm 2.3	83 \pm 2.7
3	80 \pm 2.6	120 \pm 1.8	102 \pm 2.1	95 \pm 3.7	65 \pm 2.3	109 \pm 2.0	108 \pm 1.5	85 \pm 1.8
4	116 \pm 2.8	114 \pm 3.4	101 \pm 3.9	98 \pm 2.9	118 \pm 0.4	126 \pm 3.6	104 \pm 2.7	123 \pm 1.1
5	122 \pm 0.9	109 \pm 4.1	96 \pm 5.4	94 \pm 3.6	115 \pm 1.9	113 \pm 4.8	101 \pm 5.7	110 \pm 5.8
Erlotinib ^a	0 \pm 0.9	41 \pm 2.5	44 \pm 1.4	97 \pm 0.4	42 \pm 0.6	6 \pm 1.0	26 \pm 3.0	23 \pm 0.6

^a positive control at concentration 1 μ M.

^b strong: <20%, moderate: 20 - 60%, weak or not active: >60%.

Compounds **1 - 5** were screened as the inhibitor for the phosphoryl transfer of eight receptor tyrosine kinases (RTKs), including EGFR, HER2, HER4, IGF1R, InsR, KDR, and PDGFR α . The results are shown in Table 3, with erlotinib was used as the positive control. Compounds **1** (3-*epi*-cipadesin C) and **3** (hispidol B 3-palmitate) were the only compounds that showed weak inhibitory properties (33-35%) against InsR. Other compounds (**2**, **4 - 5**) were practically not active against to all tested TKRs. InsR is the insulin receptor that involves in the homeostasis of glucose in the cells.¹⁸ The binding of insulin to InsR activates its tyrosine kinase activity leading to recruitment of downstream effectors which end up in the transport of glucose into the cell. Compounds that can act as the InsR agonist are useful for antidiabetics, while the InsR antagonists are potential candidates for anticancer agents.

In conclusion, 2-*epi*-cipadonoid C (**1**) (cipadesin limonoid) and hispidol B 3-palmitate (**3**) (tirucallane derivative) have been isolated from the seeds and fruit peels extract of *S. koetjape*, respectively, along with the known limonoid, cipaferen G (**2**), and pentacyclic triterpenes, bryonolic (**4**) and bryononic (**5**) acids. Chemotaxonomically the secondary metabolites of *S. koetjape* bear a close relationship with those contained in the species of *Cipadessa*. Testing these compounds against eight RTKs only resulted in weak activities of **1** and **3** against InsR. This paper is the first report for the limonoid and triterpene derivatives to be tested against TKRs.

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References

- (1) Meegan, M. J.; O'Boyle, N. M. *Pharmaceuticals* **2019**, *12*, 134.
- (2) Pottier, C.; Fresnais, M.; Gilon, M.; Jérusalem, G.; Longuespée, R.; Sounni, N. E. *Cancers* **2020**, *12*, 731.
- (3) Ferguson, F.; Gray, N. S. *Nat. Rev. Drug Discov.* **2018**, *17*, 353-377.
- (4) Jiao, Q.; Bi, L.; Ren, Y.; Song, S.; Wang, Q.; Wang, Y. S. *Mol. Cancer* **2018**, *17*, 36.
- (5) Yin, B.; Fang, D. M.; Zhou, X. L.; Gao, F. *Eur. J. Med. Chem.* **2019**, *182*, 111664.
- (6) Pejini, B.; Jovanović, K. K.; Mojović, M.; Savić, A. G. *Curr. Top. Med. Chem.* **2013**, *13*, 2745-2766.
- (7) Efdi, M.; Ninomiya, M.; Suryani, E.; Tanaka, K.; Ibrahim, S.; Watanabe, K.; Koketsu, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4242-4245.
- (8) Bumi, M. B.; Heliawaty, L.; Hermawati, E.; Syah, Y. M. *J. Nat. Med.* **2019**, *73*, 641-647.
- (9) Heliawati, L.; Khatimah, H.; Hermawati, E.; Syah, Y. M. *Nat. Prod. Sci.* **2020**, *26*, 345-350.
- (10) Hermawati, E.; Ellita, S. D.; Juliawaty, L. D.; Hakim, E. H.; Syah, Y. M.; Ishikawa, H. *J. Nat. Med.* **2021**, *75*, 217-222.
- (11) Mistry, B. D. *Ultraviolet spectroscopy in A handbook of spectroscopic data chemistry*; Oxford Book Company: India, **2009**, pp 3-5.
- (12) Tan, Q. G.; Luo, X. D. *Chem. Rev.* **2011**, *111*, 7437-7522.
- (13) Fang, X.; Zhang, Q.; Tan, C. J.; Mu, S. Z.; Lu, Y.; Lu, Y. B.; Zheng, Q. T.; Di, Y. T.; Hao, X. J. *Tetrahedron* **2009**, *65*, 7408-7414.
- (14) Siva, B.; Poornima, B.; Venkanna, A.; Prasad, K. R.; Sridhar, B.; Nayak, V. L.; Ramakrishna, S.; Babu, K. S. *Phytochemistry* **2014**, *98*, 174-182.
- (15) Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Bates, R. B. *J. Org. Chem.* **1981**, *46*, 4085-4088.
- (16) MacChesney, J. D.; Dou, J.; Sindelar, R. D.; Goins, D. K.; Walker, L. A.; Rogers, R. D. *J. Chem. Cryst.* **1997**, *27*, 283-290.
- (17) Sim, K. Y.; Lee, H. T. *Phytochemistry* **1972**, *11*, 3341-3343.
- (18) De Meyts, P.; Whittaker, J. *Nat. Rev. Drug Discov.* **2002**, *1*, 769-783.

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by Dyah Ayu Rachmadhaningtiyas, Leny Heliawati, Elvira Hermawati And Yana Maolana Syah

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A Cipadesin Limonoid and a Tirucallane Triterpene from the Fruit of *Sandoricum koetjape* and their Inhibitory Properties against Receptor Tyrosine Kinases

Dyah Ayu Rachmadhaningtyas¹, Leny Heliawati², Elvira Hermawati¹, and Yana Maolana Syah^{1,*}

¹Organic Chemistry Division, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia

²Department of Chemistry, Pakuan University, Jalan Pakuan PO Box 452, Bogor 16143, Indonesia

Abstract – A new cipadesin limonoid, i.e. 3-epi-cipadonoid C (**1**), and a new tirucallane triterpene, i.e. hispidol B 3-palmitate (**3**), have been isolated from the seeds and fruit peels extract of *Sandoricum koetjape*, respectively. Along with these compounds the known limonoid, cipaferen G (**2**), and two pentacyclic triterpenes, bryonolic (**4**) and bryononic (**5**) acids, were also isolated. The structures of the new compounds were elucidated by the analysis of NMR and mass spectral data. Compounds **1** - **5** were evaluated as the inhibitor of receptor tyrosine kinases (EGFR, Epidermal Growth Factor Receptor; HER2, HER4, Human Epidermal growth factor Receptor 2, -4; IGFR, Insulin-like Growth Factor Receptor; InsR, Insulin Receptor; KDR, Kinase insert Domain Receptor; PDGFR α , and PDGFR β , Platelet-Derived Growth Factor Receptor- α and - β). The results showed only **1** and **3** that have weak activity against InsR.

Keywords – *Sandoricum koetjape*, 3-epi-Cipadonoid C, Hispidol B 3-palmitate, Limonoid, Tirucallane, Receptor tyrosine kinase

Introduction

Cancer is still a difficult disease to treat. One of the traditional approaches to finding anticancer agents is through cytotoxicity evaluation of the candidate compounds against a number of cancer cells.¹ However, in recent years the tyrosine kinases (TKs) become the target in searching for new anticancer agents.^{2,3} In human these enzymes involved in a number of cellular signaling pathways by catalyzing a phosphate transfer from ATP to the tyrosine residue of the other proteins. Lack of TK's function can lead to the formation of generative diseases, while their excessive expression can result in the generation of tumors and/or cancers. TK's are divided into two families, receptor (RTKs) and -non-receptor tyrosine kinases (nRTKs).⁴ Examples of RTKs are epidermal growth factor (EGFR; HER2, HER4, Human Epidermal growth factor Receptor 2, -4), insulin (IGFR, Insulin-like Growth Factor Receptor; InsR, Insulin Receptor), kinase insert domain (KDR) and platelet-derived growth factor (PDGFR α and - β) receptors. These RTKs have been shown to have a relationship to the

formation of various tumors/cancers. Most of the TK's inhibitors are synthetic chemicals, while the inhibitors originated from natural sources, both terrestrial and marine origins, are still very limited.^{5,6}

Sandoricum koetjape Merr. (Meliaceae) is an endemic plant of South East Asia regions and produces edible fruits that have a unique taste.⁷ The only reports on its traditional uses are from the bark part of the plant. The Malaysian people use the aqueous decoction of the bark as a tonic for the women after childbirth, while in Indonesia this preparation is used for the treatment of colic and leukorrhea.⁷ Phytochemical investigation of the plant has isolated a number of terpenoid derivatives, including sesquiterpenes, triterpenes, and limonoids.^{8,9} In searching of RTK's inhibitors from natural sources,^{9,10} we had examined further the seeds extract of the plant which led to the isolation of other limonoid components, namely a cipadesin derivative, 3-epi-cipadonoid C (**1**) (Fig. 1), and a known andirubin, cipaferen G (**2**). In addition, a new tirucallane derivative, hispidol B 3-palmitate (**3**), and two known pentacyclic triterpenes, bryonolic acid (**4**) and bryononic acid (**5**) were also isolated from the fruit pericarp extract of the plant. This paper reports the structure elucidation of the new compound and inhibitory properties of **1** - **5** against eight RTKs (EGFR, HER2, HER4, IGFR, InsR, KDR, PDGFR α , and PDGFR β).

* Author for correspondence

Yana Maolana Syah, Organic Chemistry Division, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia.
Tel: +62-22-2502103; E-mail: yana@chem.itb.ac.id

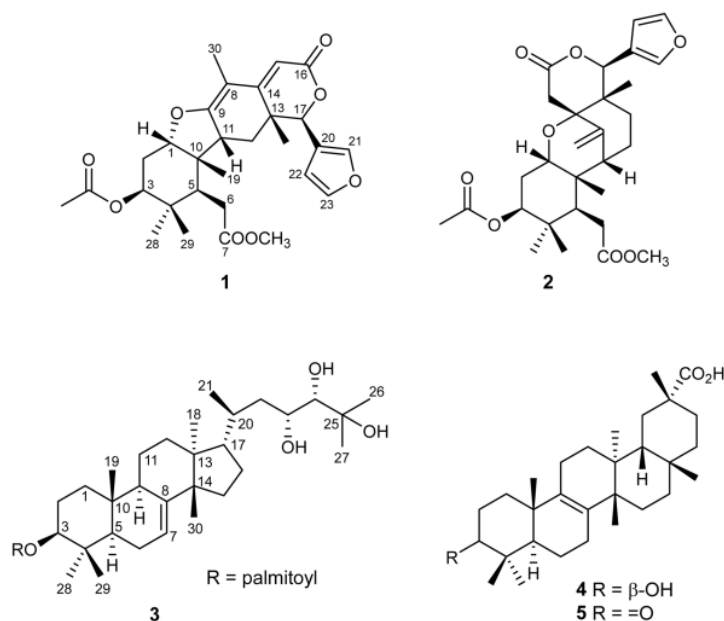


Fig. 1. Structures of the compounds isolated from the seeds and fruit peels of *S. koetjape*.

Experimental

General experimental procedures – UV and IR spectra were measured with a Varian Cary 100 Conc instrument and a Perkin Elmer FTIR Spectrum One spectrometer, respectively. Optical rotation were determined with an Autopol IV Rudolph Research Analytical. High-resolution mass spectra were obtained with an ESI-TOF Waters LCT Premier XE mass spectrometer. ^1H and ^{13}C NMR spectra were measured in CDCl_3 with a spectrometer of Agilent DD2 system operating at 500 and 125 MHz, respectively. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were conducted on Merck silica gel 60 GF254 art. 7731 and 7749, respectively. Thin layer chromatography (TLC) analysis was done using precoated silica gel plates (Merck Kielselgel 60 GF254, 0.25 mm thickness). Spot on TLC were detected by UV irradiation, and sprayed by 10% vanillin in H_2SO_4 , which was followed by heating. Solvents (MeOH, acetone, EtOAc and *n*-hexane) for extraction, fractionation and purification were of technical grades, which were distilled before used. CHCl_3 used in the purification was a pro analysis grade. Kinase Selectivity Profiling System (KSPS) Receptor Tyrosine Kinases TK1 (EGFR, HER2, HER4, InsR, KDR, PDGFR α and PDGFR β) was purchased from Promega and stored at -80°C as one-time

use aliquots. Kinase enzyme assay was done using a pipetmax automatic liquid handler (Gilson), while luminescent measurements were carried out with a GloMax Explorer.

Plant materials – The fruit samples of *S. koetjape* were collected in August 2013 from Pandeglang, Banten Province, Indonesia. The identity of the plant was determined by the staff of Herbarium Bandungense, Institut Teknologi Bandung, and the voucher specimen was deposited at the Herbarium (voucher number 11254).

Extraction and isolation – The dried and powdered seeds of *S. koetjape* (1 kg) were extracted with acetone ($3 \times 10\text{ L}$, overnight) at room temperature to give a dried extract (80 g) after solvent evaporation. A portion of the extract (20 g) was subjected to a VLC (170 g silica gel, gradient elution: *n*-hexane-EtOAc = 10:0 to 1:1, 10% stepwise EtOAc addition, followed by EtOAc and MeOH, each $2 \times 200\text{ mL}$) to give 18 fractions F1-F18. F9-F16 were combined (3.3 g) and were refractionated using the same method (80 g silica gel, gradient elution: *n*-hexane-EtOAc = 9:1, 4:1, 3:1, 7:3, 1:1, followed by EtOAc, each $2 \times 75\text{ mL}$) yielding 14 subfractions F916-1 to F916-14. Purification of the F916-9 (200 mg) by CPC (eluent: *n*-hexane-EtOAc = 4:1) afforded **2** (70 mg), while from F916-12 (200 mg) (eluent: *n*-hexane- CHCl_3 = 3:7) gave **1** (14 mg). The same procedures were done to the fruit peels

extract of the plant (20 g) to obtain 18 fractions G1-G18 after VLC fractionation. G8-G9 were left overnight and gave **4** (1.2 g) as white crystals, while G11-G12 (2.6 g) were refractionated (VLC, silica gel 80 g, gradient elution: *n*-hexane-EtOAc = 9:1, 4:1, 3:1, 7:3, 1:1, followed by EtOAc, each 2 × 75 mL) to give 12 subfraction G1112-1 to G1112-12. Purification of G1112-7 to G1112-10 by CPC gave **3** (50 mg) and **5** (15 mg).

3-*epi*-Cipadonosoid C (1) – White solid. $[\alpha]_D^{20} = +44.0^\circ$ (c 0.1, CHCl₃); UV (CDCl₃) λ_{max} 236 (4.01), 309 (3.62) nm; IR (KBr): 2945, 1734, 1643 cm⁻¹; ¹H NMR (CDCl₃) see Table 1; ¹³C NMR (CDCl₃) see Table 1; HR-ESI-TOF-MS: [M+Na]⁺ ion *m/z* 535.2297 (calcd. [M+Na]⁺ ion for C₂₉H₃₆O₈: *m/z* 535.2302).

Hispidol B 3-palmitate (3) – White solid. $[\alpha]_D^{20} = -27^\circ$ (c 0.27, CHCl₃); IR (KBr): 3342, 1744 cm⁻¹; ¹H NMR (CDCl₃) see Table 2; ¹³C NMR (CDCl₃) see Table 2; HRESITOF-MS: [M+Na]⁺ ion *m/z* 737.6069 (calcd. [M+Na]⁺ ion for C₄₆H₈₂O₅: *m/z* 737.6055).

Kinase enzyme assay – The assay was done according to the previously described.^{8,9} Briefly, the tested compound was made 5% in DMSO and was diluted to a final concentration of 10 μM by 4X kinase buffer and nuclease-free water. Each kinase stock was also diluted with 2.5X reaction buffer (95 μL), while the substrate/cofactor stock was diluted with 80 μM ATP solution (20 μL). The assay was done as follows: the kinase (2 μL), the ATP/substrate

(1 μL), and tested compound (1 μL) were dispensed to each well of the 384-well plate, and then was allowed to react for 1 hour (22 - 25 °C). After this, the ADP-Glo reagent was added (5 μL), and the was incubated for 40 minutes (22 - 25 °C), which was followed by the addition of kinase detection reagent (10 μL) to be incubated again for 30 minutes. After the reaction was completed, the luminescent was measured, which corresponds to the kinase activity. The negative control (100% activity) was the well without the tested compound solution, while the background luminescent (0% activity) was obtained from the well without enzyme solution. The % kinase activity was calculated by subtracting the background luminescent from all kinase reactions (Table 3). This assay used erlotinib as the control positive (1 μM), while the purity of **1** - **5** (>95%) was determined by NMR.

Result and Discussion

Compound **1** was isolated from the seed extract of *S. koetjape* as a white powder. $[\alpha]_D^{20} = -44^\circ$ (CHCl₃). Its molecular formula was determined to be C₂₉H₃₆O₈ based on the HR-ESI-TOF-MS spectrum (positive mode, found *m/z* 535.2297 [M+Na]⁺, calcd. 535.2302). Its UV spectrum exhibited absorptions (λ_{max} 236, 309 nm) assignable to a 3,4,5,5-tetrasubstituted diene ester chromophore,¹¹ while the IR absorptions indicated the presence of a simple ester

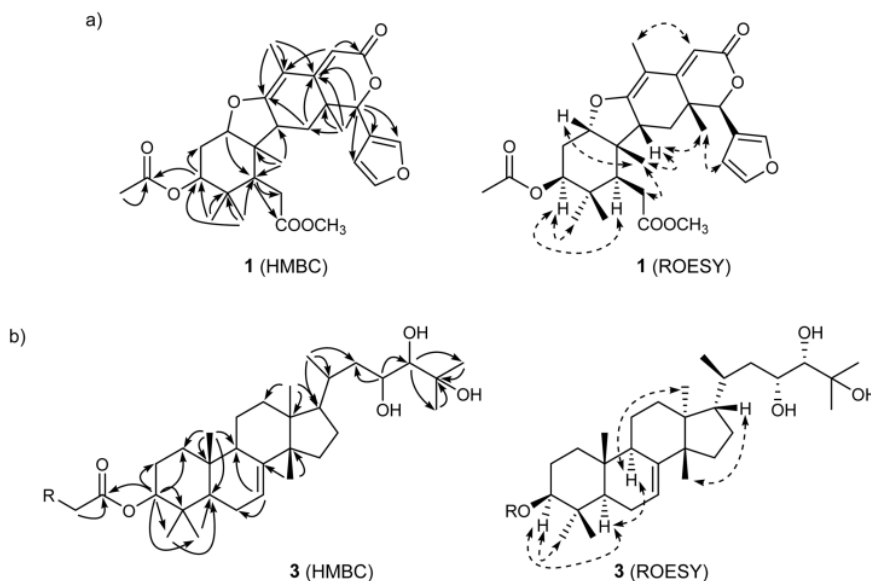


Fig. 2. Selected HMBC and ROESY correlations in a) **1** and b) **3**.

Table 1. NMR data of compound **1** in CDCl₃

C No.	1		6 ¹⁰	
	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)	δ_{C}
1	4.23 (<i>br t</i> , 3.5)	87.7	4.22 (<i>s</i>)	86.5
2	2.33 (<i>ddd</i> , 14.5, 5.8, 4.2) 1.90 (<i>ddd</i> , 14.5, 12.1, 3.5)	27.0	2.52 (<i>m</i>) 2.10 (<i>s</i>)	25.0
3	4.88 (<i>dd</i> , 12.1, 4.2)	73.5	4.61 (<i>s</i>)	76.0
4	-	38.7	-	37.8
5	1.98 (<i>d</i> , 8.8)	40.0	2.29 (<i>d</i> , 9.0)	35.1
6	2.46 (<i>dd</i> , 17.4, 8.8) 2.08 (<i>d</i> , 17.4)	32.3	2.42 (<i>m</i>) 2.14 (<i>s</i>)	32.2
7	-	173.8	-	174.1
8	-	102.2	-	101.0
9	-	163.3	-	163.2
10	-	44.8	-	44.5
11	2.51 (<i>dd</i> , 12.0, 4.7) 1.66 (<i>t</i> , 12.0)	50.4	2.57 (<i>d</i> , 13.0) 1.81 (<i>s</i>)	50.4
12	1.36 (<i>dd</i> , 12.0, 4.7)	31.3	1.41 (<i>dd</i> , 12.0, 4.0)	31.3
13	-	37.8	-	37.3
14	-	162.6	-	163.9
15	5.63 (<i>s</i>)	104.5	5.59 (<i>s</i>)	103.5
16	-	166.4	-	166.7
17	5.03 (<i>s</i>)	80.1	5.10 (<i>s</i>)	80.1
18	1.05 (<i>s</i>)	16.5	1.06 (<i>s</i>)	16.6
19	1.10 (<i>s</i>)	19.1	1.12 (<i>s</i>)	20.1
20	-	120.5	-	120.4
21	7.34 (<i>br s</i>)	141.0	7.36 (<i>br s</i>)	141.0
22	6.33 (<i>br s</i>)	110.0	6.35 (<i>br s</i>)	109.9
23	7.42 (<i>br s</i>)	143.0	7.44 (<i>br s</i>)	143.0
28	0.98 (<i>s</i>)	15.7	1.01 (<i>s</i>)	21.4
29	0.75 (<i>s</i>)	25.5	0.80 (<i>s</i>)	26.1
30	1.83 (<i>s</i>)	9.6	1.78 (<i>s</i>)	9.5
3-C(=O)CH ₃	-	170.2	-	171.0
3-C(=O)CH ₂	2.07 (<i>s</i>)	21.1	2.06 (<i>s</i>)	21.2
7-OCH ₃	3.73 (<i>s</i>)	52.2	3.79 (<i>s</i>)	52.1

(ν_{max} 1734 cm^{-1}) and a conjugated ester or lactone (ν_{max} 1643 cm^{-1}) groups. In the limonoid group, these spectral characteristics were reminiscent of the cipadesin class.¹² This was corroborated by the presence of carbon signals at δ_{C} 166.4, 163.3, 162.6, 104.5, 102.2 (Table 1) typical for the signals of C-16, C-9, C-14, C-15, and C-8, respectively, of the cipadesin compounds with a dienelactone group. In addition, a shielded methyl carbon signal (δ_{C} 9.6) was also observed, which is a characteristic for the methyl group attached at C-8.¹³ In fact, the ¹H and ¹³C NMR data of **1** were very close to those cipadonoid C (**6**),¹³ except that proton signal H-3 appeared as a doublet ($J = 12.1$ and 4.2 Hz), while this proton signal in **6** is a broad singlet. Thus, the H-3 in **1** should be in the

axial orientation, suggesting that **1** is a 3-epimer of **6**. The HMBC correlations gave more support for the basic structure of **1**, while the NOE correlations from ROESY spectrum established its stereochemistry (Fig. 2). As expected, the NMR parameters of **1** and **6** are different at C-1-C-6, while the rest part of the structure is essentially the same (see Table 1). Therefore, **1** was determined as 3-*epi*-cipadonoid C.

Compound **2** was identified as cipaferen G based on a comparison of its NMR data with those reported in the literature.¹⁴

Compound **3** was isolated from the fruit peels extract of *S. koetjape* also as a white powder, $[\alpha]_{\text{D}}^{20} = -27^{\circ}$ (CHCl₃). The IR spectrum exhibited the absorptions of ester

Table 2. NMR data of compound **3** in CDCl₃

C No.	3		7 ¹³
	δ_{H} (mult., <i>J</i> in Hz)	δ_{C}	δ_{C}
1	1.67 (<i>m</i>), 1.22 (<i>m</i>)	36.8	38.5
2	1.65 (<i>m</i>), 1.60 (<i>m</i>)	24.2	34.9
3	4.51 (<i>dd</i> , 11.2, 3.9)	80.8	217.0
4	-	37.7	47.8
5	1.41 (<i>m</i>)	50.7	52.3
6	2.12 (<i>m</i>), 1.66 (<i>m</i>)	23.7	24.3
7	5.25 (<i>br t</i> , 4.2)	117.8	117.9
8	-	145.7	145.7
9	2.21 (<i>m</i>)	48.8	48.4
10	-	34.8	35.0
11	1.51 (2H, <i>m</i>)	18.1	18.3
12	1.80 (<i>m</i>), 1.63 (<i>m</i>)	33.8	33.8
13	-	43.6	43.5
14	-	51.2	51.2
15	1.49 (<i>m</i>), 1.44 (<i>m</i>)	34.0	34.0
16	1.99 (<i>m</i>), 1.29 (<i>m</i>)	28.5	28.4
17	1.48 (<i>m</i>)	53.8	53.8
18	0.98 (<i>s</i>)	22.0	22.0
19	0.77 (<i>s</i>)	13.2	12.8
20	1.39 (<i>m</i>)	33.7	33.7
21	0.93 (<i>d</i> , 6.8)	18.9	18.9
22	1.87 (<i>m</i>), 1.18 (<i>m</i>)	40.5	40.4
23	4.12 (<i>br s</i>)	69.8	69.7
24	3.16 (<i>br d</i> , 5.9)	75.0	75.0
25	-	74.4	74.3
26	1.31 (<i>s</i>)	26.3	26.2
27	1.32 (<i>s</i>)	27.2	27.4
28	0.85 (<i>s</i>)	27.6	24.5
29	0.94 (<i>s</i>)	15.9	21.6
30	0.82 (<i>s</i>)	27.4	27.4
1'	-	173.8	
2'	2.30 (<i>t</i> , 7.5)	34.9	
3'	1.61 (<i>m</i>)	25.2	
4'-15'	1.36-1.23 (<i>m</i>)	31.9, 29.7-29.2	
16'	0.88 (<i>t</i> , 7.1)	14.3	

carbonyl (ν_{max} 1744 cm⁻¹) and hydroxy (ν_{max} 3342 cm⁻¹) groups. The NMR spectra of **3** (Table 2) clearly showed proton signals for eight methyl groups (δ_{H} 1.32, 1.31, 0.98, 0.94, 0.93, 0.85, 0.82, 0.77) and four signals for non-oxygenated-*sp*³ carbons (δ_{C} 51.2, 43.6, 37.7, 34.8), indicating that **3** is a tetracyclic triterpene. One of the methyl proton signals was found as a doublet (δ_{H} 0.93, *J* = 6.8 Hz), suggesting that **3** has the basic structure of the tirucallane triterpene, as is usually found in the Meliaceae plants.¹² The functional groups found in **3**

include a trisubstituted alkene (δ_{H} 5.25; δ_{C} 145.7, 117.8), one secondary ester (δ_{H} 4.51; δ_{C} 173.8, 80.8), two secondary alcohol (δ_{H} 4.12, 3.16; δ_{C} 75.0, 69.8), and one tertiary alcohol (δ_{C} 74.4) groups.

In the HMBC spectrum (Fig. 2), the olefinic proton signal (δ_{H} 5.25) was correlated with C-9 methine carbon (δ_{C} 48.8), showing the presence of 7,8-double bond in **3**. The HMBC correlations also allowed to place the two secondary alcohols at C-23 and C-24, the tertiary alcohol at C-25, and the ester group at C-3. From these spectroscopic data, **3** is deduced to be the ester derivative of the tirucallane triterpene, hispidol B,¹⁵ based on the presence of large coupling constant (11.2 Hz) at δ_{H} 4.51. The ester was determined to be a saturated fatty acid derivative, as shown by the observation of many methylene carbon signals in the range of δ_{C} 34.9-29.2, as well as a methyl signal at δ_{H} 0.88 (*t*, 7.1 Hz) and δ_{C} 14.3. The HR-ESI-TOF-MS spectrum (positive mode) established the molecular formula C₄₆H₈₂O₅ for this compound (found *m/z* 737.6069 [M+Na]⁺, calcd. 737.6055), and thus identifying the ester part as the palmitoyl group. In addition, support for the tirucallane structure was obtained from the ROESY correlations, as shown in Fig. 2.

The stereochemistry at C-20, C-23, and C-24 was determined by comparing the ¹³C NMR data with those reported for piscidinol A (**7**) (3-oxo derivative of **3**), in which its absolute configurations was determined by x-ray crystallography analysis.¹⁶ The carbon chemical shifts at C-20, C-23, C-24, and C-25 in **3** showed a close agreement with those reported for **7** (Table 2). Compound **3**, therefore, is assigned as hispidol B 3-palmitate.

In addition to **3**, two known pentacyclic triterpenes **4** and **5** were also isolated from the fruit peels extract of *S. koetjape*. Based on their NMR data both compounds were determined as bryonolic and bryononic acids,¹⁷ respectively.

Cipadesin class is among the minor limonoid found in Meliaceae and the genus *Cipadessa* is the only source of this compound class.¹² The presence of **1** in *S. sandoricum* is the only example of cipadesin limonoid that occurs outside this genus and supports further the close relationship chemotaxonomically between the two genera *Cipadessa* and *Sandoricum*.⁸ Fang et al. has proposed a biogenetic relationship between andirobin and trijugin in one hand, and with cipadesin on the other through the rearrangement of the andirobin's skeleton from C-8 to C-11 and C-10 to C-11, respectively.¹³ The key compound is 10-hydroxy derivative of andirobin, which is found in both genera. However, the terpenoid resource in the *S. koetjape* is not allocated to the limonoid metabolites, but also to the tetracyclic and pentacyclic triterpenes, such as **3** - **5**.

Table 3. Tyrosine kinase activity of compounds **1** - **5** at concentration 10 μ M

Compounds	% Activity ^b							
	EGFR	HER2	HER4	IGF1R	InsR	KDR	PDGFR α	PDGFR β
1	103 \pm 1.3	135 \pm 0.8	98 \pm 1.7	92 \pm 3.3	67 \pm 4.0	101 \pm 0.3	104 \pm 3.2	84 \pm 3.1
2	118 \pm 1.3	130 \pm 1.8	106 \pm 3.2	87 \pm 3.7	120 \pm 3.8	110 \pm 3.9	105 \pm 2.3	83 \pm 2.7
3	80 \pm 2.6	120 \pm 1.8	102 \pm 2.1	95 \pm 3.7	65 \pm 2.3	109 \pm 2.0	108 \pm 1.5	85 \pm 1.8
4	116 \pm 2.8	114 \pm 3.4	101 \pm 3.9	98 \pm 2.9	118 \pm 0.4	126 \pm 3.6	104 \pm 2.7	123 \pm 1.1
5	122 \pm 0.9	109 \pm 4.1	96 \pm 5.4	94 \pm 3.6	115 \pm 1.9	113 \pm 4.8	101 \pm 5.7	110 \pm 5.8
Erlotinib ^a	0 \pm 0.9	41 \pm 2.5	44 \pm 1.4	97 \pm 0.4	42 \pm 0.6	6+1.0	26 \pm 3.0	23+0.6

^a positive control at concentration 1 μ M.

^b strong: <20%, moderate: 20 - 60%, weak or not active: >60%.

Compounds **1** - **5** were screened as the inhibitor for the phosphoryl transfer of eight receptor tyrosine kinases (RTKs), including EGFR, HER2, HER4, IGF1R, InsR, KDR, and PDGFR α . The results are shown in Table 3, with erlotinib was used as the positive control. Compounds **1** (3-*epi*-cipadesin C) and **3** (hispidol B 3-palmitate) were the only compounds that showed weak inhibitory properties (33-35%) against InsR. Other compounds (**2**, **4** - **5**) were practically not active against to all tested TKRs. InsR is the insulin receptor that involves in the homeostasis of glucose in the cells.¹⁸ The binding of insulin to InsR activates its tyrosine kinase activity leading to recruitment of downstream effectors which end up in the transport of glucose into the cell. Compounds that can act as the InsR agonist are useful for antidiabetics, while the InsR antagonists are potential candidates for anticancer agents.

In conclusion, 2-*epi*-cipadonoid C (**1**) (cipadesin limonoid) and hispidol B 3-palmitate (**3**) (tirucallane derivative) have been isolated from the seeds and fruit peels extract of *S. koetjape*, respectively, along with the known limonoid, cipaferen G (**2**), and pentacyclic triterpenes, bryonolic (**4**) and bryononic (**5**) acids. Chemotaxonomically the secondary metabolites of *S. koetjape* bear a close relationship with those contained in the species of *Cipadessa*. Testing these compounds against eight RTKs only resulted in weak activities of **1** and **3** against InsR. This paper is the first report for the limonoid and triterpene derivatives to be tested against TKRs.

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References

- (1) Meegan, M. J.; O'Boyle, N. M. *Pharmaceuticals* **2019**, *12*, 134.
- (2) Pottier, C.; Fresnais, M.; Gilon, M.; Jérusalem, G.; Longuespée, R.; Soumni, N. E. *Cancers* **2020**, *12*, 731.
- (3) Ferguson, F.; Gray, N. S. *Nat. Rev. Drug Discov.* **2018**, *17*, 353-377.
- (4) Jiao, Q.; Bi, L.; Ren, Y.; Song, S.; Wang, Q.; Wang, Y. S. *Mol. Cancer* **2018**, *17*, 36.
- (5) Yin, B.; Fang, D. M.; Zhou, X. L.; Gao, F. *Eur. J. Med. Chem.* **2019**, *182*, 111664.
- (6) Pejin, B.; Jovanović, K. K.; Mojović, M.; Savić, A. G. *Curr. Top. Med. Chem.* **2013**, *13*, 2745-2766.
- (7) Efdi, M.; Ninomiya, M.; Suryani, E.; Tanaka, K.; Ibrahim, S.; Watanabe, K.; Koketsu, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4242-4245.
- (8) Bumi, M. B.; Heliawaty, L.; Hermawati, E.; Syah, Y. M. *J. Nat. Med.* **2019**, *73*, 641-647.
- (9) Heliawaty, L.; Khatimah, H.; Hermawati, E.; Syah, Y. M. *Nat. Prod. Sci.* **2020**, *26*, 345-350.
- (10) Hermawati, E.; Ellita, S. D.; Juliawaty, L. D.; Hakim, E. H.; Syah, Y. M.; Ishikawa, H. *J. Nat. Med.* **2021**, *75*, 217-222.
- (11) Mistry, B. D. *Ultraviolet spectroscopy in A handbook of spectroscopic data chemistry*; Oxford Book Company: India, **2009**, pp 3-5.
- (12) Tan, Q. G.; Luo, X. D. *Chem. Rev.* **2011**, *111*, 7437-7522.
- (13) Fang, X.; Zhang, Q.; Tan, C. J.; Mu, S. Z.; Lu, Y.; Lu, Y. B.; Zheng, Q. T.; Di, Y. T.; Hao, X. J. *Tetrahedron* **2009**, *65*, 7408-7414.
- (14) Siva, B.; Poornima, B.; Venkanna, A.; Prasad, K. R.; Sridhar, B.; Nayak, V. L.; Ramakrishna, S.; Babu, K. S. *Phytochemistry* **2014**, *98*, 174-182.
- (15) Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Bates, R. B. *J. Org. Chem.* **1981**, *46*, 4085-4088.
- (16) MacChesney, J. D.; Dou, J.; Sindelar, R. D.; Goins, D. K.; Walker, L. A.; Rogers, R. D. *J. Chem. Cryst.* **1997**, *27*, 283-290.
- (17) Sim, K. Y.; Lee, H. T. *Phytochemistry* **1972**, *11*, 3341-3343.
- (18) De Meyts, P.; Whittaker, J. *Nat. Rev. Drug Discov.* **2002**, *1*, 769-783.

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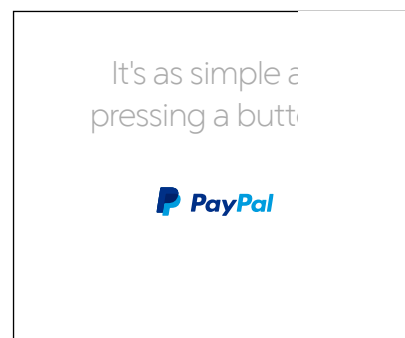
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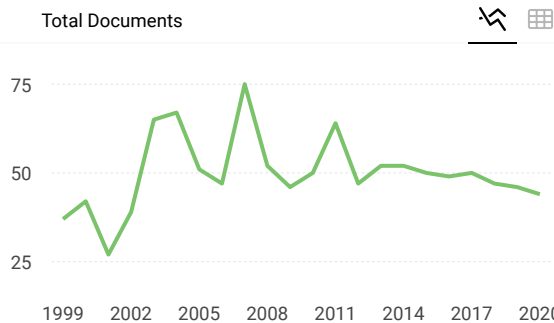
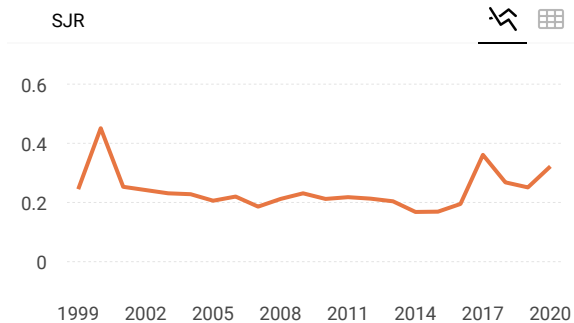
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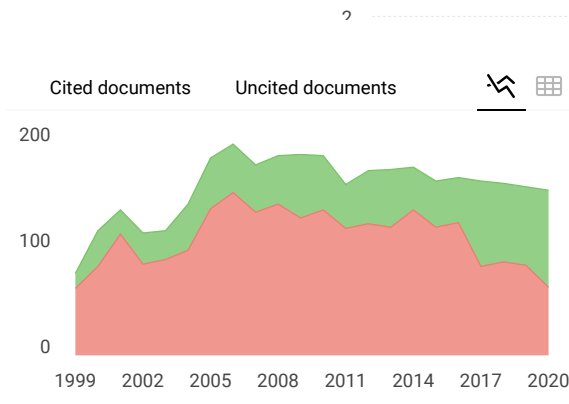
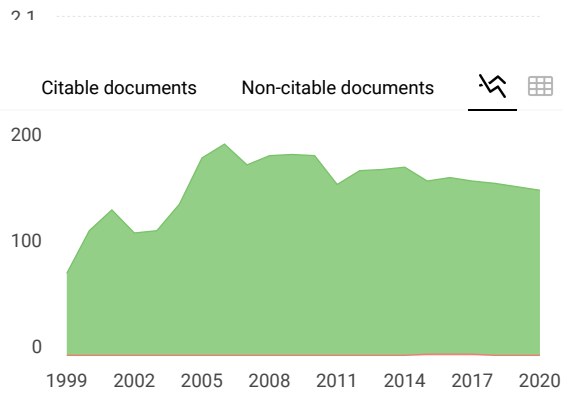
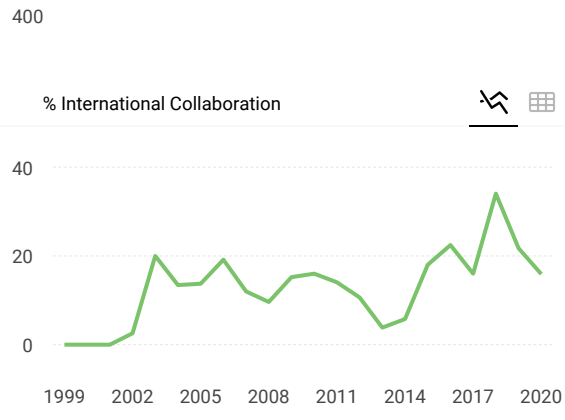
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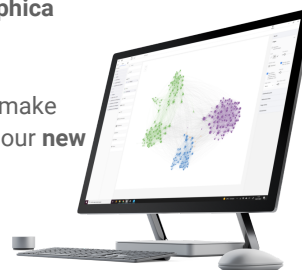
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