Inhibitor protein kinase dari spons Indonesia *Axynissa* sp.

Protein kinase inhibitors from Indonesian Sponge *Axynissa* sp.

Triana Hertiani [1, 2 *)], Ru Angelie Edrada-Ebel [3)], Michael Kubbutat [4)], Rob W.M. van Soest [5)] and Peter Proksch [1,6)]

[1)] Institut für Pharmazeutische Biologie and Biotechnologie, Henrich-Heine Universität Düsseldorf, Universitätsstrasse 1 Geb. 26.23, 40225 Düsseldorf, Germany;
[2)] Pharmaceutical Biology Dept., Faculty of Pharmacy, Gadjah Mada University, Sekip Utara, 55281 Jogjakarta, Indonesia;
[3)] Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, The John Arbuthnott Building, 27 Taylor Street, Glasgow G4 0NR, United Kingdom;
[4)] ProQinase GMBH, Breisacher Str. 117, 79106 Freiburg im Breisgau, Germany; [5]Institute for Systematics and Ecology, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands;
[6)] Corresponding author, Tel. 0049/211-8114163. E-mail: proksch@uni-duesseldorf.de

Abstrak

Telah dilakukan penelitian tentang aktivitas inhibisi beberapa spons laut yang dikoleksi di Indonesia terhadap berbagai protein kinase yang terkait dengan kanker. Penelitian ini bertujuan untuk mengisolasi dan mengidentifikasi struktur dari metabolit spons yang aktif secara biologis.

Pendekatan menggunakan kombinasi antara panduan kimia dan biologi untuk penemuan obat diterapkan dalam penelitian ini. Uji penapisan aktivitas penghambatan protein kinase secara *in vitro* terhadap ekstrak-ekstrak spons dilakukan paralel dengan penggunaan KLT, dan KCKT yang dikopel spektrofotometer UV dan spektrometer massa untuk mengidentifikasi senyawa aktif yang menarik secara kimiawi. Identifikasi struktur dari senyawa aktif dilakukan dengan menggunakan spektroskopi resonansi magnetik inti dan spektrometer massa.

Hasil uji skrining aktivitas inhibisi terhadap berbagai protein kinase yang terkait kanker secara *in vitro* menunjukkan bahwa hanya fraksi *Axynissa* sp. yang menunjukkan aktivitas penghambatan protein kinase. Dua senyawa seskuiterpenoid tipe bisabolen, (+)-curcuphenol (**1**) dan (+)-curcudiol (**2**) diidentifikasi sebagai senyawa aktif dari spons tersebut. Senyawa **1** menunjukkan penghambatan protein kinase SRC dengan nilai IC_{50} 7.8 µg/mL sementara **2** menujukkan penghambatan FAK dengan nilai IC_{50} 9.2 µg/mL.

Kata kunci: Inhibitor protein kinase, *Axynissa* sp.

Abstract

A research about inhibitory activity on various cancer related protein kinases of several marine sponges collected in Indonesia was performed. This study aims were to isolate and to identify structures of biologically active sponge metabolites.

A combination of a chemically-and biologically driven approach for drug discovery was employed. Sponge extracts were tested by protein kinase inhibitory assay by in vitro method in parallel to the usage of TLC, and HPLC coupled to UV spectrophotometry and mass spectrometry to isolate the chemically most interesting substances. Structure identification of active compounds was performed by using NMR spectroscopy and mass spectrometry methods.

This study showed that only *Axynissa* sp. fractions were found active in protein kinase inhibitory assay. Two bisabolene type sesquiterpenoids, (+)-curcuphenol (**1**) and (+)-curcudiol (**2**) were identified as active compounds from the sponge. Compound **1** showed SRC protein kinase inhibition with an IC_{50}value of 7.8 µg/mL while **2** inhibited FAK with an IC_{50} value of 9.2 µg/mL.
Key words: protein kinase inhibitor, *Axynissa* sp.

Introduction

Protein kinases have become a major area for therapeutic intervention due to their fundamental role in signal transduction pathways and their aberrant activity in many diseases, (Scapin, 2002). This includes treatment of a number of diseases including cancer, diabetes, and inflammation (Noble *et al*., 2004). Recently several protein kinase inhibitors have been introduced into cancer chemotherapy (Scapin, 2002).

Marine sponges are known to be rich of novel bioactive natural products, including some potential protein kinase inhibitors (Haefner, 2003; Tasdemir *et al*., 2002; Breton and Chabot-Fletcher, 1997; Roshak *et al*., 1997; Tamaoki *et al*., 1986). As a consequence, sponges may be a potential source in search of lead compounds for novel protein kinase inhibitors from nature. Considering that Indonesia is an archipelago country situated in the tropics, high biodiversity of its marine life including sponges can be already expected. On the other hand, only few reports on bioactive marine metabolites from this region have so far been published (Blunt *et al*., 2006). Therefore, research on bioactive compounds of sponges collected in Indonesia is highly required. In this study several crude extracts and fractions of marine sponges collected from 4 different sites in Indonesia were screened for their bioactive constituents using a biochemical protein kinase assay.

Methodology

Sponge materials

Four sponge specimens (Table I) were collected from 3 different sites in Indonesia i.e., Maluku in 1997, Menjangan Island, Bali in 2003, and Watudodol, Banyuwangi in 2003 at a depth of 10 – 30 m by means of SCUBA. All sponge specimens were directly preserved in ethanol after harvesting. Voucher specimens were deposited in the Zoological Museum in Amsterdam (Table I).

Extraction and preliminary chemical investigation

Dried sponge tissue was ground and extracted exhaustively with methanol. After removing the solvent under reduced pressure, the methanol extract was combined with the ethanol extract to yield crude extracts. Those crude extracts were subjected to a biochemical protein kinase assay.

Parallel to the bioactivity assay, chemical investigation was done by using TLC, DAD HPLC and LC/MS analyses in order to predict chemical constituents of the extracts and to guide the isolation procedure. Chemicals used in the detection and isolation methods were anisaldehyde (4-methoxybenzaldehyde), and concentrated sulphuric acid (all provided by Merck, Darmstadt, Germany). Solvents used for separation techniques were dichloromethane; ethyl acetate; *n*-hexane; methanol. These solvents were purchased from the Institute of Chemistry HHU Düsseldorf. They were distilled before using and special grade were used for spectroscopic measurements. Others solvent used were *n*-butanol and acetonitrile (Fluka, Seelze, Germany), and ethanol (Merck, Darmstadt, Germany).

TLC analysis was carried out on aluminium sheet precoated silica gel 60 F254 or on glass precoated RP-18 F254 plates (Merck, Darmstadt, Germany). DAD HPLC used in this study was as follows. Program used: Chromeleon version 6.3; pump: Dionex P580A LPG; detector: Dionex, Photodiode Arrray Detector UVD 340S; auto-sampler: ASI-100T; column Thermostat: STH 585; column: Knauer, 5.0 mmID; packing material 5 µm;

Table I. List of sponge specimens investigated

No.	Sponge Species	Location Site	Reg. Number
1.	*Agelas nakamurai*	Menjangan Island, Bali	ZMAPOR18297
2.	*Pseudoceratina purpurea*	Watudodol, Banyuwangi	ZMAPOR17800
3.	*Axynissa* sp.	Maluku	ZMAPOR19077
4.	*Mycale phyllophila*	Menjangan Island, Bali	ZMAPOR18344

Table II. Protein kinases, enzymes and substrate used per well for determination inhibitory profiles

No.	Kinase	Kinase ng/50µL	Substrate	Substrate ng/50µL
1.	AKT1	100	GSK3(14-27)	1000
2.	ARK5	100	Autophosphorylation	-
3.	Aurora-A	50	tetra(LRRWSLG)	500
4.	Aurora-B	200	Tetra(LRRWSLG)	250
5.	B-RAF-VE	20	MEK1-KM (Lot 011)	250
6.	CDK2/CycA	100	Histone H1	125
7.	CDK4/CycD1	50	Rb-CTF (SP009)	500
8.	CK2-alpha1	200	p53-CTM 11.6.99	250
9.	EGF-R	25	$Poly(Glu, Tyr)_{4:1}$	125
10.	EPHB4	10	$Poly(Glu, Tyr)_{4:1}$	125
11.	ERBB2	200	$Poly(Glu, Tyr)_{4:1}$	125
12.	FAK	200	$Poly(Glu, Tyr)_{4:1}$	125
13.	IGF1-R	25	$Poly(Glu, Tyr)_{4:1}$	125
14.	SRC	10	$Poly(Glu, Tyr)_{4:1}$	125
15.	VEGF-R2	50	$Poly(Glu, Tyr)_{4:1}$	125
16.	VEGF-R3	100	$Poly(Glu, Tyr)_{4:1}$	125
17.	COT	400	Autophosphorylation	-
18.	PLK1	50	Casein	250
19.	SAK	200	Autophosphorylation	-
20.	TIE2	200	$Poly(Ala,Glu,Lys,Tyr)_{6:2:5.1}$	250
21.	FLT3	100	$Poly(Ala,Glu,Lys,Tyr)_{6:2:5.1}$	125
22.	INS-R	25	$Poly(Ala,Glu,Lys,Tyr)_{6:2:5.1}$	125
23.	MET	10	$Poly(Ala,Glu,Lys,Tyr)_{6:2:5.1}$	125
24.	PDGFR-beta	50	$Poly(Ala,Glu,Lys,Tyr)_{6:2:5.1}$	125

Eurospher-100 C-18. Column was initially equilibrated isocratically with 10:9 [methanol: acidic-nanopure water (adjusted to pH 2 with phosphoric acid)] in 5 minutes then the solvent was gradually changed to 100% methanol in 30 minutes which was continued to 40 minutes with 100% methanol. Injection volume was 20 µL. Compounds having chromophores were detected by UV-Vis diode array detector at 240 nm, 254 nm, 280 nm and 365 nm.

LC/MS equipment used in this study was the Finnigan LCQ-DECA (mass spectrometer); Agilent 1100 series for HPLC system (pump, detector and autosampler): column Knauer, 125 mm L, 2 mm ID, prepacked with Eurospher-100 C18 (5 µm) and with integrated pre-column. As standard analytical program, column was initially equilibrated isocratically with 10:9 [acetonitrile: acidic-nanopure water (containing1% formic acid)] in 5 minutes then the solvent was gradually changed to acetonitrile 100% in 30 minutes which was continued to 10 minutes with 100% acetonitrile. HPLC was run on a Eurospher C-18 reversed phase column.

Purification

Axynissa sp. extract was fractionated by vacuum liquid chromatography (VLC) on silica gel (0.040-0.063 mm; Merck, Darmstadt, Germany). The crude extract was subjected to silica gel G60 vacuum liquid chromatography (VLC) by using mobile phase with increasing polarity from 100% *n*-hexane to 100 % ethyl acetate followed by 100% dichloromethane to 100% MeOH, resulting to 10 fractions. Fraction 2 (hexane:ethyl-acetate, 3:1) was later identified as **(+)-curcuphenol** (**1**, 19 mg) and fraction 3 (hexane:ethyl-acetate, 1:1) as **(+)-curcudiol** (**2**, 24 mg).

Structure elucidation

Structure elucidation was performed using mass spectrometry data as well as 1D and 2D NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were

recorded at 300°K on Bruker DPX 300, ARX 400, 500 or AVANCE DMX 600 NMR spectrometers using deuterated methanol or DMSO-d_6 (Eurisotop, France) as solvents. All 1D and 2D spectra were obtained using the standard Bruker software. ESI mass spectra were obtained from the LC/MS equipment. EIMS was measured on Mass spectrometer type Finnegan MAT 8200.

Optical rotation was determined on a Perkin-Elmer 241 MC polarimeter by measuring the angle of rotation at a wavelength of 589 nm of a Na/Hg vapour lamp. Samples were measured in a 0.5 mL cuvette with 0.1 dm length and unless stated different was run at room temperature (25° C). Solvent used in this method were spectroscopy grade of chloroform (Sigma-Aldrich, Steinheim, Germany).

Kinase inhibitory assay

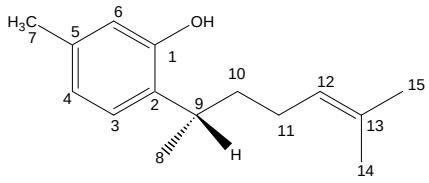
The inhibitory profile of the samples tested was determined using 24 protein kinase assay (^{33}PanQinase® Activity Assay). The protein kinases used for determination of inhibitory profiles as well as the amounts of enzyme and substrate used per well as described in Table II. Stock solutions were dissolved in DMSO (1 x 10^{-03} g/mL) in column 3 – 12 of one 96-well micronic box. All compounds were tested at a final assay concentration of 1 x 10^{-06} g/mL in 1% DMSO in singlicate. All kinase assays were performed in 96-well FlashPlates TM from Perkin Elmer/NEN (Boston, MA, USA) in a 50 µL reaction volume. All assays were performed with a BeckmanCoulter/Sagian robotic system. The residual activity (in %) for each well of a particular plate was calculated by using the following formula:

Res. Activity (%) = 100 X [(cpm of compound-low control) / (high control-low control]

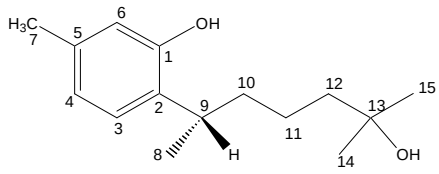
Results And Discussion

Result of the protein kinase inhibitory assay on sponge extracts showed no inhibitions detected. Therefore further isolation procedures to those extracts were guided by chemical profile data to obtain pure compounds which will be then later tested to biological assays. Since only compounds from the *Axynissa* sp. sponge were active on the protein kinase inhibitory assay, only those compounds will be discussed further in this report.

DAD-HPLC results of fractions 2 and 3 obtained from VLC technique of the *Axynissa* sp. crude extract showed that each fraction contains a single major compound, **1** and **2**, respectively. Both compounds showed a similar UV absorption pattern, i.e., λ_{max} at 209.5 and 277.0 nm (**1**); λ_{max} at 207.6 and 276.5 nm (**2**) but differed in retention times. Peak of **1** appeared at 32.20 min while **2** appeared at 28.21 min in the HPLC chromatogram. Further investigation on the chemical structures of **1** and **2** revealed that both compounds were bisabolene type sesquiterpenoids.



1



2

Compound **1** was isolated as a yellow brownish oily substance with a yield of 19 mg (0.95% of the sponge crude extract). EIMS revealed its molecular weight of 218 g/mol. ^{1}H-NMR spectrum of **1** in $CDCl_3$ indicated a phenolic derivative of α-curcumene as described by McEnroe and Fenical (1978). It revealed the presence of a terminal isopropylidene group as indicated by a vinyl proton signal at δ_H 5.13 (1H, t, J = 7.1 Hz, H-12) and two vinyl methyl singlets at δ_H 1.68 (3H, s, H_3-14) and δ_H 1.54 (3H, s, H_3-15). The presence of an aromatic methyl group was showed at δ_H 2.28 (3H, s, H_3-7) as well as the presence a benzyl substituted secondary methyl group shown by a signal at δ_H 1.22 (3H, d, J = 6.9 Hz, H_3-8) which coupled to a methine multiplet at δ_H 2.96 (1H, m, H-9). Signals arising from a 1,2,4-trisubstituted aromatic ring were observed at δ_H 7.03 (1H, d, J = 7.9 Hz, H-3), 6.73 (1H, d, J = 7.6 Hz, H-4) and 6.58 (1H, s, H-6).

^{13}C-NMR and DEPT experiments supported the proposed substructure by showing the carbon resonances of the 1,2,4 trisubstituted aromatic ring and the dimethyl-

Table III. NMR data of compounds **1** and **2**

No	Compound 1 [a]			Compound 2 [b]		
	^{1}H-NMR δ	H, multiplicity, *J* in Hz	^{13}C-NMR δ, DEPT	^{1}H-NMR δ	H, multiplicity, *J* in Hz	^{13}C-NMR δ, DEPT
1	-	-	152.8, C	-	-	152.8, C
2	-	-	129.9, C	-	-	128.6, C
3	7.03	1H, d, 7.9	126.8, CH	7.03	1H,d, 7.6	126.8, CH
4	6.73	1H, d, 7.6	121.7, CH	6.73	1H, d, 7.6	121.7, CH
5	-	-	136.5, C	-	-	136.4, C
6	6.58	1H, s	116.1, CH	6.58	1H, s	116.3, CH
7	2.28	3H, s	21.1, CH_3	2.28	3H, s	21.3, CH_3
OH	5.38	2H, m	-	5.35	1H, s	-
OH	-	-	-	3.68	1H, s	-
8	1.22	3H, d, 6.9	20.9, CH_3	1.22	3H, d, 6.9	21.5, CH_3
9	2.96	1H, sextet	31.3, CH	3.06	1H, q, 7.1	31.1, CH
10a	1.62	2H, m	37.2, CH_3	1.64	1H, m	38.1, CH_2
10b				1.52	1H, m	
11	1.93	3H, d, 6.9	26.0, CH_2	1.32	2H, m	22.5, CH_2
12a	5.13	1H, sextet	124.6, CH	1.54	1H, m	43.7, CH_2
12b		2H, m		1.49	1H, m	
13	-	-	132.0, C	-	-	71.4, C
14	1.68	3H, s	25.7, CH_3	1.19	3H, s	29.4, CH_3
15	1.54	3H, s	17.7, CH_3	1.16	3H, s	28.6, CH_3

[a] Data were recorded in $CDCl_3$, at 500 MHz, multiplicities and coupling constant are given in Hz

isopropylidene terminal side chain. Four carbon *sp*^{2} signals derived from the aromatic carbons at δ_C 126.8 (C-3), 121.7 (C-4), and 116.1 (C-6), as well as from the vinylic carbon at δ_C 124.6 (C-12) were observed. Four carbon *sp* signals appeared at δ_C 152.8 (C-1), 129.9 (C-2), 136.5 (C-5), and 132.0 (C-13). The presence of four methyl units was confirmed by signals at δ_C 25.7 (C-14), 17.7 (C-15), 21.1 (C-7) and at 20.9 (C-8). Two methylene signals of the isoprenoid side chain were assigned to δ_C 37.2 (C-10) and 26.0 (C-11), as well as a methine carbon at δ_C 31.3 (C-9).

^{1}H–^{1}H COSY spectrum showed two spin systems. One spin system indicated the aromatic protons as well as the aromatic methyl group, while the second spin system was derived from the isoprenoid C-8 side chain. ^{1}H–^{13}C HMBC experiment revealed cross peaks correlated the aromatic methyl protons to C-4, C-5 and C-6 implying its substitution at C-5. Cross peaks of H-9 to C-3 as well as H-8 to C-2 further confirmed the connection of the isoprenoid side chain to the aromatic ring. These findings established compound **1** as curcuphenol.

Considering that the chiral center at C-9 of curcuphenol can give rise to two possible stereoisomers which both occur in nature (Cichewicz *et al*., 2005), an optical rotation measurement of **1** was performed. The result showed a $[\alpha]_D^{25}$ value of +8.9° ± 0.5° (*c* 0.52, $CHCl_3$), indicating that **1** is a (+)-curcuphenol. According to Harrison and Crews (1997), all known sponge-derived bisabolene derivatives possess 9*S* configuration while other marine (e.g. gorgonian coral) and terrestrial metabolites exhibit 9*R* configuration. (+)-Curcuphenol was previously isolated from marine sponges of the genus *Didiscus* (Wright *et al*., 1987; Tasdemir *et al*., 2003) and from *Axynissa aculeata* (Murti, 2006), while its antipode, (-)-curcuphenol was

reported from the gorgonian coral *Pseudopterogorgia rigida* by McEnroe and Fenical (1978).

Compound **2** was isolated as a yellow oily substance at a yield of 24 mg (1.2% of sponge crude extract). ESIMS revealed a molecular weight of 236 g/mol. As it showed similar UV absorption pattern to **1** and a molecular weight difference of 18 mU, **2** was suggested to be a hydroxyl derivate of **1**. This suggestion was supported by ^{1}H and ^{13}C-NMR spectra of both compounds which showed almost identical spectra in the aromatic region (Table III). The primary difference found was with regard to the resonances observed for the C-12—C-13 olefin of **1** which were replaced by the resonances of a quaternary carbon bearing oxygen at δ_C 71.4 (C-13) and of a methylene group at δ_C 43.7 (C-12) in **2**. As a result, two geminal methyl functions are shifted upfield in comparison to those of **1**. Therefore **2** was identified as the known compound curcudiol, a hydrogenation product of curcuphenol. Like already found for **1**, compound **2** exhibited 9*S* configuration with an optical rotation of $[\alpha]_D^{25} = +3.4^o \pm 0.5^o$ (*c* 0.41, $CHCl_3$).

Interestingly, **1** and **2** were discovered to inhibit different protein kinases, as **1** inhibited SRC protein kinase with an IC_{50} value of 7.8 µg/mL while **2** inibited FAK (focal adhesion kinase) with an IC_{50} value of 9.2 µg/mL. Both SRC and FAK are belonging to the tyrosine kinase family. FAK is an important component of the focal adhesion signalling complex (Zachary and Rozengurt, 1992; Hanks and Polte, 1997) which is implicated in several fundamental cellular biological functions including migration, adhesion, survival, embryonic development and cell-cycle control (Hanks and Polte, 1997; Illic *et al.*, 1995; Richardson and Parson, 1996; Gilmore and Romer, 1996; Frisch *et al.*, 1996; Hungerford *et al.*, 1996; Zhao *et al.*, 1998; Abu Ghazaleh *et al.*, 2001). Activation of the tyrosine kinases Src and FAK is known to be one of the earliest steps in transducing extracellular cues through integrins to the cytoskeleton (Schwartz *et al.*, 1995; Giancotti and Tarone, 2003; Brown *et al.*, 2005).

Conclusion

This research revealed **1** and **2** as undescribed inhibitors of protein kinases but their activities are not sufficient to warrant further studies. Nevertheless marine sources are interesting for drug discovery also of new kinase inhibitors as shown in this initial study. Further research to search for more potent protein kinase inhibitors from marine environment will be conducted in the future in order to find more promising substances.

Acknowledgements

This report is part of PhD. thesis of T. Hertiani at the University of Düsseldorf, Germany, 2007 (Supervisor: Prof. Dr. P. Proksch). A DAAD scholarship granted to T.H. is gratefully acknowledged. This study was supported by a grant of BMBF (Federal Ministry of Education and Research) Germany.

References

Abu Ghazaleh, R., Kabir, J., Jia, H., Lobo, M., and Zachary, I., 2001, Src mediates stimulation by vascular endothelial growth factor of the phosphorylation of focal adhesion kinase at tyrosine 861, and migration and anti-apoptosis in endothelial cells, *Biochem. J.*, **360**, 255-64

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T., and Prinsep, M.R., 2006, Marine natural products, *Nat. Prod. Rep.*, **23**, 26 -78

Breton, J., and Chabot-Fletcher, M.C, 1997, The natural product hymenialdisine inhibits interleukin-8 production in U937 cells by inhibition of nuclear factor-KB, *J. Pharmacol. Exp. Ther.*, **282**, 459-466

Brown, M.C., Cary, L.A., Jamieson, J.S., Cooper, J.A., and Turner, C.E., 2005, Src and FAK kinases cooperate to phosphorylate paxillin kinase linker, stimulate its Focal Adhesion Localization, and regulate cell spreading and protrusiveness, *Molecular Biology of the Cell*, **16**, 4316-438

Cichewicz, R.H., Clifford, L.J., Lassen, P.R., Cao, X., Freedman, T.B., Nafie, L.A., Deschamps, J.D., Kenyon, V.A., Flanary, J.R., Holman, T.R., Crews, P., 2005, Stereochemical determination and bioactivity assessment of (*S*)-(+)-curcuphenol dimers isolated from the marine sponge *Didiscus aceratus* and synthesized through laccase biocatalysis, *Bioorganic, and Medicinal Chemistry*, **13**(19), 5600-5612

Frisch, S.M., Vuori, K., Ruoslahti, E., and Chan-Hui, P.Y., 1996, Control of adhesion dependent cell survival by focal adhesion kinase, *J. Cell Biol.*, **134**(3), 739-799

Giancotti, F.G., and Tarone, G., 2003, Positional control of cell fate through joint integrin/receptor protein kinase signalling, *Annu. Rev. Cell. Dev. Biol.*, **19**, 173-206

Gilmore, A.P., and Romer, L.H., 1996, Inhibition of focal adhesion kinase (FAK) signalling in focal adhesions decreases cell motility and proliferation, *Mol. Biol. Cell.*, **7**, 1209-1224

Haefner, B., 2003, Drugs from the deep: marine natural products as drug candidates, *DDT*, **8**(12), 536-544

Hanks, S.K., and Polte, T.R., 1997, Signalling through focal adhesion kinase, *Bioassays*, **19**, 137-145

Harrison, B., and Crews, P., 1997, The structure and probable biogenesis of helianane, a heterocyclic sesquiterpene, from the Indo-Pacific sponge *Haliclona fascigera, J. Org. Chem.*, **62**(8), 2646-2648

Hungerford, J.E., Compton, M.T., Matter, M.L., Hoffstrom, B.G., and Otey, C.A., 1996, Inhibition of pp125FAK in cultured fibroblasts results in apoptosis, *J. Cell Biol.*, **135**(5), 1383-1390

Illic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., Yamamoto, T., and Aizawa, S., 1995, Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice, *Nature* (London), **377**, 539-544

McEnroe, F.J., and Fenical, W., 1978, Structures and synthesis of some new antibacterial sesquiterpenoids from the gorgonian coral *Pseudopterogorgia rigida, Tetrahedron*, **34** (11), 1661-1664

Murti, Y.B., Isolation and Structure Elucidation of Bioactive Secondary metabolites from sponges collected at Ujung Pandang and in the Bali Sea, Indonesia, 2006, *Dissertation*, Heinrich-Heine Universitaet, Duesseldorf, Germany, 61 - 64

Noble, M.E.M., Endicott, J.A., and Johnson, L.N., 2004, Protein kinase inhibitors: insights into drug design from structure, *Drug Discovery*, **303**, 1800-1805

Richardson, A., and Parsons, J.T., 1996, A mechanism for regulation of the adhesion-associated protein tyrosine kinase pp125^{FAK}, *Nature* (London), **380**, 538-540

Roshak, A., Jackson, J.R., Chabot-Fletcher, M., and Marshall, L.A., 1997, Inhibition of NFκB-mediated interleukin-1β-stimulated prostaglandin E_2 formation by the marine natural product Hymenialdisine, *J. Pharmacol. Exp. Ther.*, **283**, 955-961

Scapin, G., 2002, Structural biology in drug design: selective protein kinase inhibitors, *DDT*, **7**(11), 601-611

Schwartz, M.A., Schaller, M.D., and Ginsberg, M.H., 1995, Integrins: emerging paradigms of signal transduction, *Annu. Rev. Cell. Dev. Biol.*, **11**, 549-599

Tamaoki, T., Nomoto, H., Takahashi, I., Kato, Y., Morimoto, M., and Tomita, F., 1986, Staurosporine, a potent inhibitor of phospholipid/Ca^{++} dependent protein kinase, *Biochem. Biophys. Res. Commun.*, **135**(2), 397-402

Tasdemir, D., Mallon, R., Greenstein, M., Feldberg, L.R., Kim, S.C., Collins, K., Wojciechowicz, D., Mangalindan, G.C., Concepcion, G.P., Harper, M. K., Ireland, C.M. 2002, Aldisine alkaloids from the Philippine sponge *Stylissa massa* are potent inhibitors of mitogen-activated protein kinase kinase-1 (MEK-1), *J. Med. Chem.*, **45**(2), 529-532

Tasdemir, D., Bugni, T.S., Mangalindan, G.C., Concepción, G.P., Harper, M.K., Ireland, C.M., 2003, Bisabolane type sesquiterpenes from a marine Didiscus sponge, *Turk. J. Che*m., **27**, 273-279

Wright, A.E., Pomponi, S.A., McConnell., O.J., Kohmoto, S., and McCarthy, P.J., 1987, (+)-Curcuphenol and (+)-curcudiol, sesquiterpene phenols from shallow and deep water collections of the marine sponge *Didiscus flavus*, *J. Nat. Prod.*, **50**(5), 976-978

Zachary, I., and Rozengurt, E., 1992, Focal adhesion kinase (p125^{FAK}): a point of convergence in the action of neuropeptide, integrins and oncogenes, *Cell,* **71**, 891-894

Zhao, J.-H., Reiske, H., and Guan, J.-L., Regulation of the cell cycle by focal adhesion kinase, *J. Cell Biol.*, 1998, **143**, 1997-2008

* Korespondensi: Dr. Triana Hertiani, S.Si., M.Si., Apt.
Bagian Biologi Farmasi,Fakultas Farmasi UGM
Jl. Sekip Utara Yogyakarta,55281
Email : hadna3ana@yahoo.com