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## **Kinase inhibitory, haemolytic and cytotoxic activity of three deep-water sponges from North Western Australia and their fatty acid composition**

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## Kinase inhibitory, haemolytic and cytotoxic activity of three deep-water sponges from North Western Australia and their fatty acid composition

### Abstract

The c-AMP dependent protein kinase (PKA) inhibition, haemolytic activity, and cytotoxicity of 21 extracts obtained from North Western Australian sponges collected from depths of 84-135 m were investigated. Hexane extracts from *Ircinia/Sarcotragus* sp. and *Geodia* sp. displayed PKA inhibitory activities of 100 and 97% respectively (at 100 µg/mL), while aq. methanol extracts from *Haliclona* sp. exhibited potent haemolytic activity (75%) and hexane extracts from *Geodia* sp. were highly toxic (88%) to the brine shrimp *Artemia franciscana*. As the non-polar extracts gave the greatest PKA inhibition, these were further analysed by GC-MS and 29 fatty acids were identified in the highest proportions in *Ircinia/Sarcotragus* sp. > *Haliclona* sp. > *Geodia* sp. In contrast to shallow-water sponges that are dominated by polyunsaturated fatty acids with a high percentage of long chain fatty acids, LCFAs (C24-C30), the deep-sea sponges investigated herein were all found to be rich in saturated fatty acids, in particular C14-C20 fatty acids, including odd and branched chain fatty acids, with only low levels (0-10%) of LCFAs. Screening of the PKA inhibitory activity of a series of commercially available fatty acids identified C14-C18 fatty acids as possessing significant PKA inhibitory activity that may contribute to the activity observed in the sponges studied.

### Keywords

CMMB

### Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

### Publication Details

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**c-AMP Dependent protein kinase inhibitory and haemolytic activities of  
extracts from five Australian deep-sea sponges from the Orders  
Haplosclerida, Astrophorida and Dictyoceratida**

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Abbreviations: ATP, adenosine triphosphate; BSLA, brine shrimp lethality assay; CDK, cyclin-dependent kinase; DCM, dichloromethane; DMSO, dimethyl sulfoxide; EGFR, epidermal growth factor receptor; GSK, glycogen synthase kinase; MAPKAPK, mitogen activated protein kinase activated protein kinase; MSK; mitogen and stress activated kinase; PBS, phosphate buffered saline; PKA, protein kinase A; PKC, protein kinase C; ROV, remotely operated vehicle.

## Abstract

The c-AMP dependent protein kinase A (PKA) inhibition, haemolytic activity, and brine shrimp toxicity of 28 partitioned extracts obtained from five deep-water sponges were investigated. The sponges, which were collected from the North West Shelf of Australia, were assigned to the genera *Haliclona*, *Sarcotragus*, *Ircinia*, *Cacospongia* and *Geodia*. Hexane extracts from the sponges *Ircinia* sp./*Sarcotragus* sp., *Geodia* sp., and *Cacospongia* sp. were found to display potent PKA inhibitory activity of 100, 97 and 88% respectively (at 100 µg/mL), while dichloromethane and aqueous methanol extracts from *Cacospongia* sp. and aqueous methanol extract from the sponge *Haliclona* sp. exhibited potent haemolytic activities of 100, 90 and 75% respectively. The hexane extract from the sponge *Geodia* sp. also exhibited high toxicity (88%) towards the brine shrimp *Artemia franciscana*. These results indicate that deep-sea sponges from the NW Shelf in Australia are a rich source of PKA inhibitory and haemolytically active substances that warrant further investigation.

**Keywords:** deep-water sponges, Porifera, protein kinase A, kinase inhibitors, haemolysis, brine shrimp toxicity, cytotoxicity

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## 1. Introduction

c-AMP Dependent protein kinase A (PKA) is an important enzyme involved in the regulation of an increasing number of physiological processes including immune, cardiovascular and reproductive functions; steroid biosynthesis; adipocyte metabolism; and a range of exocytotic processes (Tasken et al. 2004). As such, the inhibition of PKA has become an attractive drug target in a number of areas, in particular in immune function (Torgersen et al. 2002) and for memory disorders such as Alzheimer's disease, Parkinson's disease and schizophrenia (Arnsten et al. 2005). The most well known PKA inhibitors are the synthetic isoquinolinesulfonamides such as H-9 and H-89, staurosporine from *Streptomyces* sp., balanol from the fungus *Verticillium balanoides* and plant-derived polyphenols such as ellagic acid and piceatannol. Interestingly, despite PKA being one of the most well characterized of all protein kinases (Taylor et al. 2004), there as yet no PKA inhibitors reported from marine sources.

The deep sea is one of the most biodiverse and species-rich habitats on the planet, and deep-sea organisms are emerging as an important new source of unexplored chemical, genetic and biological diversity (Gage 1996). In particular, deep-sea sponges have provided over 60% of the novel deep-sea natural products reported, with over half of these exhibiting significant toxicity towards a range of human cancer cell lines (Skropeta 2008). Herein, we describe the biological screening of 28 partitioned extracts obtained from five NW Australian deep-sea sponges belonging to the bioactive-rich Orders Haplosclerida, Astrophorida and Dictyoceratida. These extracts were tested for their ability to inhibit purified PKA; to lyse equine erythrocytes; and for their toxicity towards the brine shrimp *Artemia franciscana*.

Four deep-sea sponge samples were collected in the North West Shelf of Australia from depths of 82-135 m using a remotely operated vehicle (ROV) and identified as *Haliclona* sp., *Geodia* sp., *Cacospongia* sp., and a mixed sample of *Sarcotragus* sp. and *Ircinia* sp. Crude dichloromethane and methanol extracts were prepared from each sponge sample and further partitioned using a modified Kupchan method to give seven partially purified extracts in water, butanol, 50% aq. methanol, 10% aq. methanol, dichloromethane and two hexane extracts, one from the original crude methanol extract (referred to as hexane 1) and the other from the original dichloromethane extract (referred to as hexane 2). This gave a total of 28 partitioned extracts, which were tested at a concentration of 100 µg/ml (see supplementary material for experimental methods).

Potent PKA inhibitory activity of 88-100% was observed for the hexane 1 extracts of all the sponges investigated, except that of the *Haliclona* sp. Other non-polar extracts, including the hexane 2 extract from the *Geodia* sp.; the dichloromethane extract from the *Ircinia/Sarcotragus* sp.; and both the hexane 2 and dichloromethane extracts from the *Cacospongia* sp. also exhibited strong PKA inhibitory activities (41-84%), with the remaining extracts exhibiting low to modest activity (Fig. 1, panels A-D). These results are consistent with the large number of non-polar kinase inhibitors described from marine sponges, which include sesterterpenoid mitogen activated protein kinase MSK1 and MAPKAPK-2 inhibitors from *Ircinia* sp. (Buchanan et al. 2001) and a series of prenylhydroquinones isolated from *Sarcotragus muscarum* and *Ircinia fasciculata*, which inhibited multiple protein kinases (Watjen et al. 2009).

Haemolytic activity was found in over half of the extracts, with the greatest activity (>90%) observed for the dichloromethane and 50% methanol extracts of the

sponge *Cacospongia* sp (Fig. 1, panels E-H). Interestingly, all seven extracts from the *Haliclona* sp. showed significant haemolytic activity (Fig. 1, panel E) consistent with reports of *Haliclona* sp. containing a range of haemolytically active substances (Mebs et al. 1985). Of the sponges under investigation, haemolytic activity has only previously been identified from the sponges *Haliclona* sp. and *Geodia corticostylifera* (Rangel et al. 2005).

Brine shrimp toxicity has been reported for a family of cerebroside from *Haliclona* sp. (Mansoor et al. 2007); three sulphated polyprenylhydroquinones from *Ircinia spinosula* (DeRosa et al. 1995); and a novel tricyclic sesterterpene, lintenone, from *Cacospongia cf. linteiformis* (Fattorusso et al. 1992). Herein, we found the only extract to exhibit high toxicity (88% lethality) towards the brine shrimp *A. franciscana*, was the hexane 1 extract from the *Geodia* sp. (see Table 1); the first report of such activity for this genus.

A diverse range of bioactive metabolites have been reported from shallow-water species from the five sponge genera under investigation, a small fraction of which include sesterterpenes, pyrrolo- and furanoterpenoids from *Sarcotragus* sp. (Liu et al. 2002; Wang et al. 2008); macrolides from *Ircinia* sp. (Chevallier et al. 2006) and *C. mycofijiensis*; and cytotoxic alkaloids and peptides from both *Haliclona* sp. and *Geodia* sp. (Fusetani et al. 1989; Hedner et al. 2008). However, only a small handful of metabolites have been reported from related deep-water species including triterpenoids (Ponomarenko et al. 1998) and sesterterpene sulfates from the deep-water sponges *Sarcotragus spinulosus* and *Ircinia* sp. (Wright et al. 1989), and the nematocidal polyketide geodin A (Capon et al. 1999) and antibacterial alkaloid barettin (Lidgren et al.

1986) from two deep-water *Geodia* species. Thus, the results described herein are the first report on the biological activity of deep-water sponges belonging to the genera *Haliclona* and *Cacospongia*.

In summary, potent PKA inhibitory and haemolytic activities have been found in extracts obtained from a series of deep-sea sponges belonging to the genera *Haliclona*, *Sarcotragus*, *Ircinia*, *Cacospongia* and *Geodia*, collected by ROV from the NW Shelf of Australia. Further studies are currently underway to characterize the bioactive constituents responsible for the observed PKA inhibitory and haemolytic activities.

### **Acknowledgments**

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### **Conflict of interest**

The authors declare that there are no conflicts of interest.

**Supporting Information Available:** Supplementary data associated with this article including experimental details, maps, and specimen photographs can be found at doi:....

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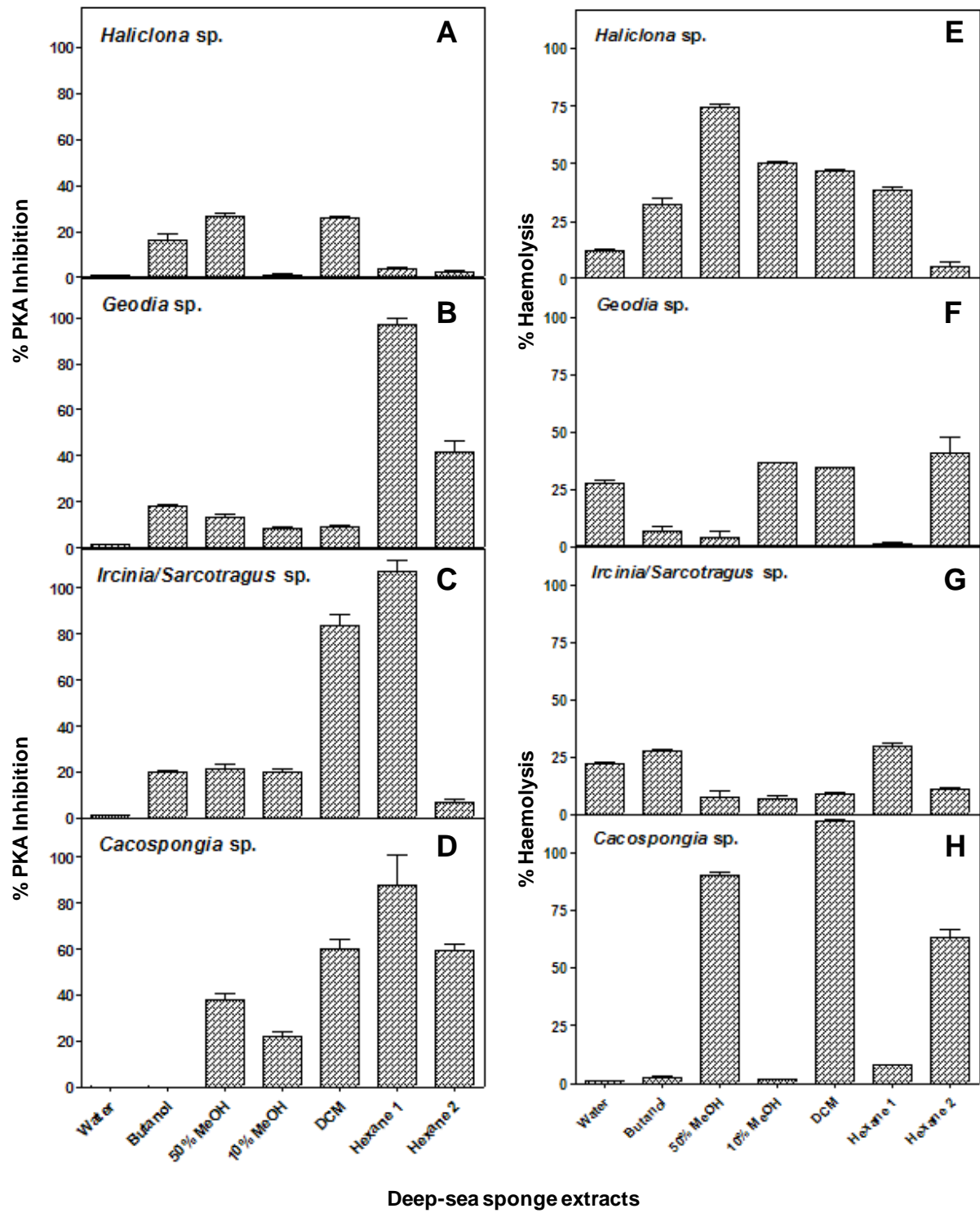
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## Figure Captions

Fig. 1 % PKA inhibition and haemolysis activity of seven partitioned extracts obtained from four NW Australian deep-sea sponge samples. All measurements were performed in triplicate at a final concentration of 100  $\mu\text{g/mL}$  and data are presented as the mean  $\pm$  S.E.M. MeOH = methanol, DCM = dichloromethane. See the supplementary material for further details.

Figure 1



**Table 1**

% Brine shrimp lethality of extracts from four NW Australian deep-sea sponge samples.

<i>Sponge species</i>	Extraction solvent						
	Water	BuOH	50%	10%	DCM	Hexane 1 <sup>†</sup>	Hexane 2 <sup>‡</sup>
			MeOH	MeOH			
<i>Haliclona</i> sp.	+	-	+	-	-	++	+
<i>Geodia</i> sp.	+	+	-	-	+	++++	-
<i>Sarcotragus/Ircinia</i> sp.	+	-	+	+	-	+	+
<i>Cacospongia</i> sp	+	-	-	-	-	-	-

(-) No activity, (+) 1-25% activity, (++) 26-50% activity, (+++) 51-75% activity, (++++) 76-100% activity.

\* Tested in triplicate at a concentration of 100 µg/mL.

<sup>†</sup> Obtained from crude methanol extract. <sup>‡</sup> Obtained from crude dichloromethane extract.

BuOH, butanol; MeOH, methanol; DCM, dichloromethane. See supplementary material for further details.

## SUPPLEMENTARY MATERIAL

# Kinase Inhibitory, Haemolytic and Cytotoxic Activity of Three Deep-water Sponges from North Western Australia and their Fatty Acid Composition

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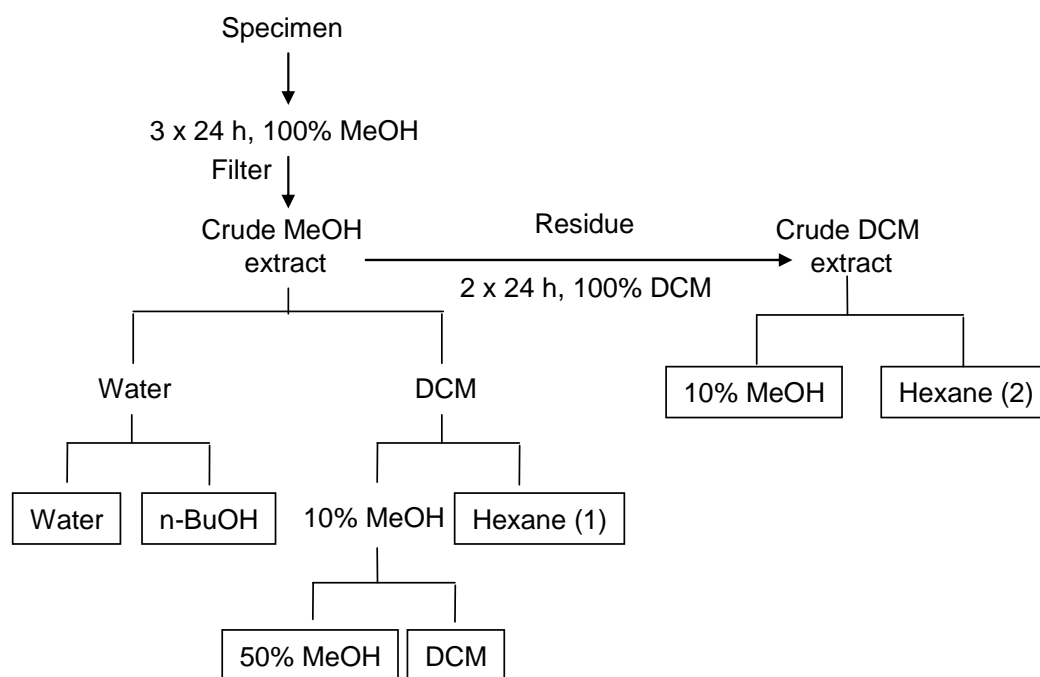
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## Experimental

### *Extract preparation*

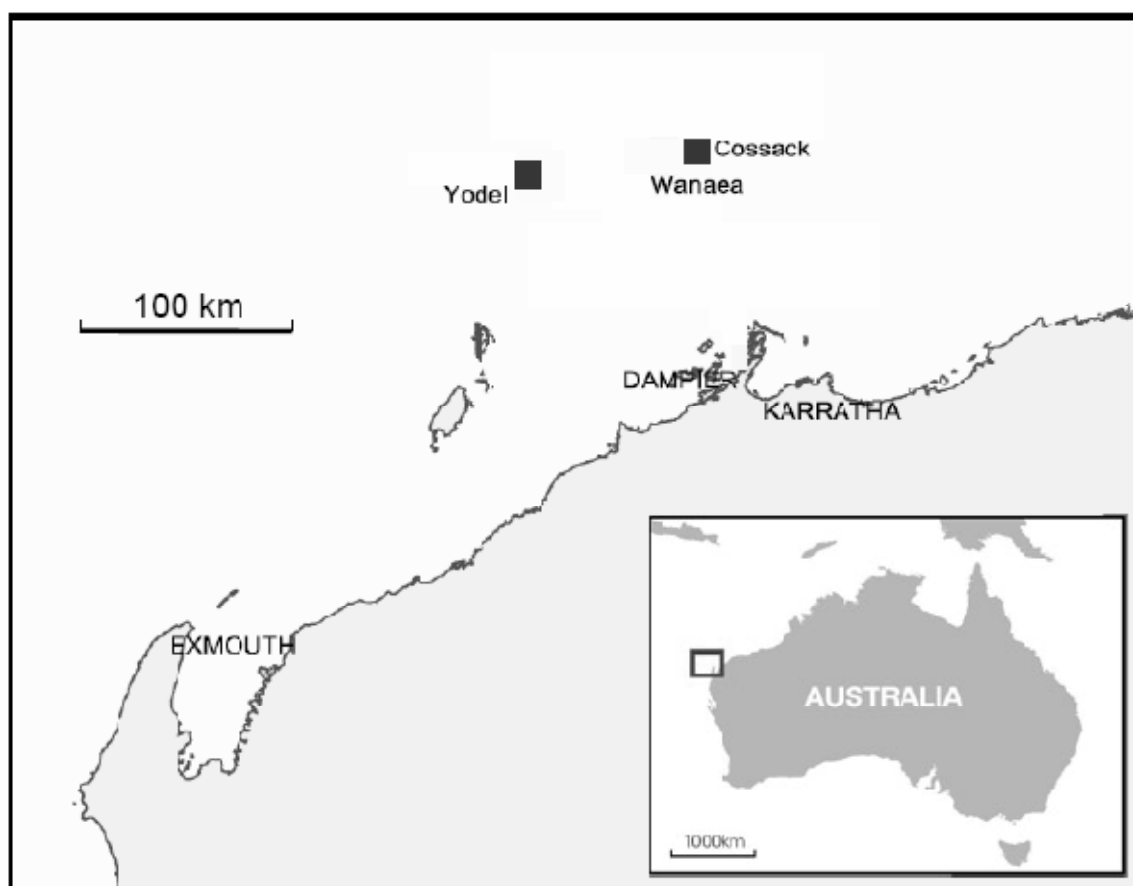
Crude extracts of the sponges were prepared from methanol and dichloromethane respectively, followed by fractionation using a modified Kupchan partitioning method. The frozen sponge samples (500 g) were cut into small pieces and suspended in 100% MeOH (300 ml) for 24 hours at 4°C. The suspension was filtered, the residue returned to the flask and the process repeated twice, and the three methanol filtrates then combined and the solvent removed. This crude methanol extract was then partitioned between water and dichloromethane, and the latter concentrated and partitioned between 10% methanol and hexane. The 10% methanol fraction was adjusted to 50% methanol and partitioned with dichloromethane. The original water fraction was partitioned with n-butanol. The solid residue from the original methanol filtration was suspended in 100% dichloromethane for 24 hours at 4°C and filtered, the residue returned to the flask and the process repeated. The two dichloromethane filtrates were combined and concentrated to give a crude dichloromethane extract, which was partitioned between 10% methanol and hexane. This gave a total of seven extracts for each sponge: water, butanol, 50% aq. methanol, 10% aq. methanol, dichloromethane, hexane (1) and hexane (2), where hexane (1) was derived from the crude methanol extract and hexane (2) from the crude dichloromethane extract (see **Fig. S1**).



**Figure S1.** Modified Kupchan partitioning method used in the preparation of the sponge extracts.

Crude methanol and dichloromethane extracts were prepared for each sponge sample. A modified Kupchan partitioning method was then used to generate seven different polarity extracts (shown in boxes) for each sponge as described in the experimental section (**Fig. S1**). MeOH = methanol, BuOH = butanol, DCM = dichloromethane.





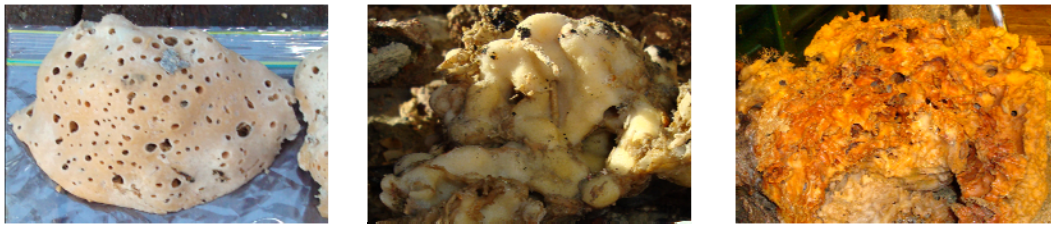
**Figure S2.** Map of sponge collection sites (North West Shelf, Australia).

**Table S1:** Description of collection sites.<sup>†</sup>

Sponge species	Well Site	Depth (m)*	Latitude	Longitude
<i>Haliclona</i> sp.	Yodel 1	135	19°44'40''S	115°43'12"E
<i>Geodia</i> sp.	Yodel 1	135	19°44'40''S	115°43'12"E
<i>Sarcotragus</i> sp. / <i>Ircinia</i> sp.	Wanaea 5	84	19°35'13"S	116°24'41"E

<sup>†</sup> All locations and depths from Geoscience Australia (<http://dbforms.ga.gov.au/www/npm.well.search>).

\* Below mean sea level.



**Figure S3.** Above water photographs of the deep-sea sponges *Haliclona* sp. (A), *Geodia* sp. (B) and mixed *Ircinia* sp. / *Sarcotragus* sp. (C).

Photographs of the three deep-sea sponge samples comprising four different sponge species collected from the NW Shelf of Australia are shown in **Fig. S3**.

**Table S2:** % Brine shrimp lethality of extracts from three NW Australian deep-sea sponges.

<i>Sponge species</i>	Extraction solvent						
	Water	BuOH	50% MeOH	10% MeOH	DCM	Hexane 1 <sup>†</sup>	Hexane 2 <sup>‡</sup>
<i>Haliclona</i> sp.	+	-	+	-	-	++	+
<i>Geodia</i> sp.	+	+	-	-	+	++++	-
<i>Sarcotragus/Ircinia</i> sp.	+	-	+	+	-	+	+

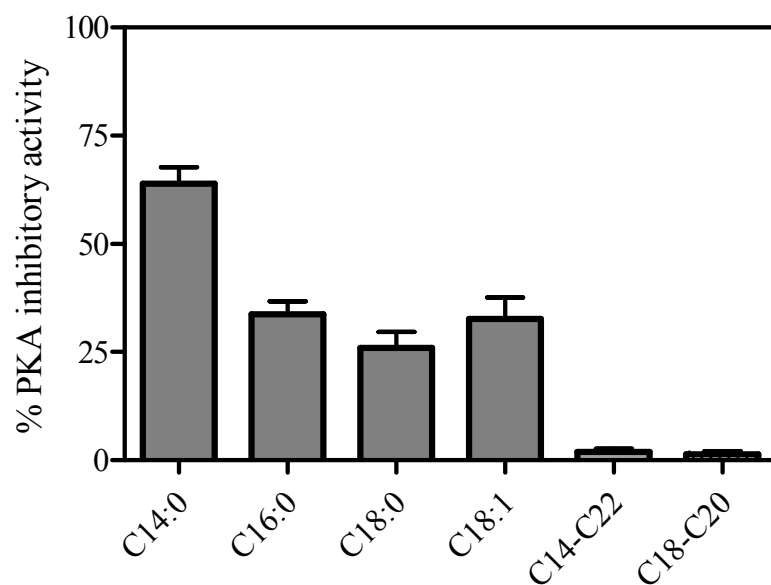
(-) No activity, (+) 1-25% activity, (++) 26-50% activity, (+++) 51-75% activity, (++++) 76-100% activity.

\* Tested in triplicate at a concentration of 100 µg/mL.

<sup>†</sup> Obtained from crude methanol extract.

<sup>‡</sup> Obtained from crude dichloromethane (DCM) extract.

See Figure S1 for further details.



**Figure S4:** PKA inhibitory activity of fatty acids typically found in marine organisms. Measurements were performed in triplicate at 100  $\mu\text{g/mL}$  and data are presented as means  $\pm$  S.E.M. DCM, dichloromethane.