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GSK-3β inhibition with antiplasmodial activities from the extracts of actinomycetes isolated from Malaysian forest soil

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ABSTRACT

Aims: Glycogen synthase kinase-3 (GSK-3 (EC:2.7.11.1)) is one of the main therapeutic targets for treating cancer, diabetes, neurological illness and parasitic infection. Due to their distinctive structural characteristics and wide-ranging biological actions, small compounds from soil bacteria have been the most sought-after source for GSK-3 inhibitors. This study assessed the activities of soil actinomycetes isolated from Sabah, Malaysia, against human GSK-3β.
Methodology and results: A total of 514 actinomycetes strains were isolated from 144 soil samples. The activities of the crude extracts were evaluated against GSK-3β and its upstream regulators (MKK1 and PP1/GLC7) using yeast-based assays. Eight actinomycetes extracts showed selective human GSK-3β inhibition without affecting MKK1 and PP1/GLC7. The extract from one of these eight isolates, FA013, also showed potent and selective anti-plasmodial activities against *Plasmodium falciparum* 3D7 strain (IC_{50} = 0.18 µg/mL, SI = 13,850) with a non-toxic effect against Chang liver cells.
Conclusion, significance and impact of study: This study identified FA013 as a potential isolate from Malaysian rainforest soil with inhibitory activities against GSK-3β and malaria parasites for future drug development.

Keywords: Anti-plasmodial activities, GSK-3β inhibitors, soil actinomycetes

INTRODUCTION

Glycogen synthase kinase-3 (GSK-3 (EC:2.7.11.1)) is a conserved kinase protein in various organisms. In humans, it is ubiquitously expressed as GSK-3α (51 kDa) and GSK-3β (47 kDa), which are 97% similar in their catalytic domains (Mancinelli *et al*., 2017). GSK-3 is involved in numerous cellular processes, such as glucose metabolism and the Akt (protein kinase B) pathway (Embi *et al*., 1980; Hermida *et al.*, 2017). Dysregulation of GSK3 is regarded as the starting point of diseases such as cancer and Alzheimer's disease, often from the overexpression of GSK-3β. This fatal effect can be reversed by normalising the GSK-3β activity level using inhibitors such as lithium and tideglusib (Alonso and Martinez, 2005; Tolosa *et al.*, 2014). In recent years, GSK-3 has also been indicated as a potential target for parasitic-inflicted diseases, including malaria (Mahmud *et al.*, 2020). *Plasmodium falciparum* GSK-3 (*Pf* GSK-3), the homolog of human GSK-3β (*Hs* GSK-3β), is amongst the most studied *Plasmodium* kinase. *Pf* GSK-3 exists in all parasite life stages and is essential for parasitic growth during the erythrocytes' life cycle. *Pf* GSK-3 and *Hs* GSK-3β showed some degree of differences in their catalytic domains (Masch *et al.*, 2019). Hence, the identification of compounds with a higher selectivity towards *Pf* GSK-3 over *Hs* GSK-3 using known *Hs* GSK-3 inhibitors is possible, or at least, these human kinase inhibitors may be used as valuable starting scaffolds for the rational design of novel anti-parasitic drugs (Osolodkin *et al.*, 2012).

Despite an increasing interest in applying human kinase inhibitors to combat parasite infection, it is challenging to identify a compound with different selectivity between human and plasmodial kinases. Although drug repurposing and the evaluation of bumped-

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kinase inhibitors have previously been suggested as effective strategies for identifying new drug leads against protein kinases (Osolodkin *et al.*, 2012; Cabrera *et al.*, 2018; Knapp, 2018), different scaffolds with unique structural features should be continually explored. Our laboratory has previously shown that *Hs* GSK-3β inhibitors can also be used to treat malarial infections. Lithium chloride suppressed parasitemia development and improved the survivability of the infected animals (Zakaria *et al.*, 2010). We also demonstrated that active substances, including andrographolide, curcumin and methyl-hydroxycinnamate, inhibited GSK-3, suppressed parasitemia infection, increased animal survival and reduced inflammation in infected animals by modulating systemic pro-and anti-inflammatory cytokines levels (Ali *et al.*, 2017; Sudi *et al.*, 2018; Hassan *et al.*, 2019; Ali *et al.*, 2021).

More than 60% of known drugs from bacteria originated from actinomycetes, mainly from the genus *Streptomyces*. With almost 400 genera known to date, actinomycetes are an inexhaustible supply of active compounds (Barka *et al.*, 2016). Natural products from actinomycetes have been shown to exert GSK-3β inhibitory activity (Omura *et al.*, 1977; Cheenpracha *et al.*, 2009; Pagmadulam *et al.*, 2020). The high biodiversity of the dipterocarp forests with endemic species of flora and fauna in Sabah may contribute to the distribution of novel actinomycetes due to plant-actinomycetes interaction (Ang *et al.*, 2016; Harir *et al*., 2018). Hence, this study aimed to screen for *Hs* GSK-3β inhibitor from soil actinomycetes isolated from Malaysian forests using a yeast-based assay expressing *Hs* GSK-3β and also their anti-plasmodial activity against *P. falciparum* 3D7 (*Pf* 3D7) strain.

MATERIALS AND METHODS

Collection of soil samples, isolation and cultivation of actinomycetes and extraction of metabolites

Soil samples were collected from 2008-2010 from forests in Sabah, Malaysia, at 10-15 cm depth, mostly under leaf litter. As the soil microbial community adapts to specific micro-and macro-environments, different forest types were selected for soil sampling, as described previously (Mahmud *et al.*, 2022a). Actinomycetes were isolated using the method previously described (Ho *et al.*, 2009). Briefly, isolation was performed using modified humic acid agar (HVA, with added vitamin B), actinomycetes isolation agar (AIA) and International Streptomyces Project medium No. 4 (ISP4). The plates were incubated at 28 °C for 1-4 weeks until the sporulation of most colonies was observed.

For extract preparation, 5-7 days old pure actinomycetes cultures were cultivated in 10 mL mannitol-peptone broth at 28 °C and shaken at 210 rpm for three days as the seed culture. It was then inoculated into 30 mL of cultivation media (1% v/v) and incubated for five days. Metabolites were extracted by adding an equal volume of acetone to the culture (1:1 ratio) to break the mycelia. The cell debris was removed by filtration (Whatman No.1) and centrifugation at 12,000× *g* for 5 min. The extracts were freeze-dried and re-dissolved in distilled water or methanol, depending on the extract solubility (for yeast-based assay) and DMSO (for cytotoxic and anti-plasmodial tests) to 100 mg/mL concentration. For the yeast-based assays (disc-diffusion method), the extracts were tested at 2 mg/disc.

Inhibitory activity of the crude extract against human GSK-3β and its upstream regulators

The *Hs* GSK-3β assay was performed using mutant yeast where the GSK-3 homolog genes: *MCK1*, *MDS1 (RIM11)*, *MRK1* and *YOL128c*, were removed to develop a heat-sensitive strain. Subsequently, the mammalian *GSK-3β* gene was inserted and the expression of this gene restored the yeast phenotype at 37 °C (Andoh *et al.*, 2000). To perform the assay, yeast was grown at allowing and defecting temperatures of 28 °C and 37 °C, respectively, as previously described (Chong *et al.*, 2012). Positive *Hs* GSK-3β inhibitory was indicated by a zone of inhibition observed only or more significantly at 37 °C than at 28 °C.

Extracts that showed positive inhibition against GSK-3β were further tested against yeast strains expressing Map Kinase Kinase 1 (MKK1) and Protein Phosphatase 1 (PP1; a homolog to GLC7 in yeast), the upstream regulators of GSK-3β (Goold and Gordon-Weeks 2005; Hernández *et al.*, 2010), for preliminary specificity evaluation. These assays were performed as previously mentioned (Ho *et al.*, 2009; Matawali *et al.*, 2016). In brief, the MKK1 inhibitor was screened using a mutant strain expressing the MKK1P386 gene that is controlled by a strong GAL1 promoter (Andrews and Stark, 2000). Hyperactivation of this promoter in the presence of galactose results in MKK1 overexpression, which is detrimental to yeast cells. Hence, MKK1 inhibition is indicated by yeast growth in the presence of galactose. A mutant yeast strain containing MKK1P386-MSG5 was used to validate MKK1 inhibition. This strain overexpressed tyrosine phosphatase MSG5 (MSG5), suppressing the fatal effect of GAL1 promoter overexpression. The presence of the MKK1 inhibitor was confirmed when there was no growth observed on galactose-supplemented media.

The yeast strains PAY704-1 (expressing wild-type GLC7) and PAY700-4 (expressing GLC7-10, a heat-sensitive GLC7 mutant) were used to screen for PP1/GLC7 inhibitors. The PP1/GLC7 of PAY704-1 is not affected by high temperature (37 °C). Meanwhile, the PAY700-4 strain underwent lysis at 37 °C due to a compromised cell wall, as GLC7-10 has lost its function. However, the diminished PP1/GLC7 role in PAY700-4 at 37 °C can be compensated in YPD media supplemented with 1 M sorbitol, which increases media osmolarity and prevents cell lysis. Hence, the presence of a PP1/GLC7 inhibitor is indicated by the growth defects observed only on the PAY704-1 strain grown in YPD media at 37 °C (Andrews and Stark, 2000).

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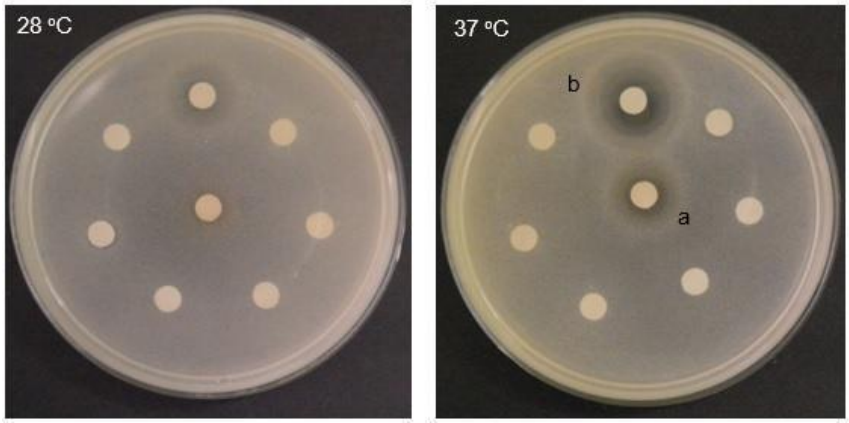

Figure 1: The inhibitory activity of actinomycetes crude extracts against *Hs* GSK-3 was evaluated using a yeast-based assay. Samples exerted zone of inhibition only (a: positive control) or more significant (b: crude extracts of FA013, the most potent crude extract in this study) at 37 °C, indicating the presence of GSK-3 inhibitors.

Cytotoxicity test of selected extracts against Chang liver cells

Chang liver cell line was obtained from the American Type Culture Collection (ATCC). The cytotoxicity of the extracts was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphyenyltetrazolium bromide (MTT) assay as previously mentioned (Dahari *et al.*, 2016). Briefly, 2×10^4 cells/mL of Chang liver cells (cultured in RPMI with 10% FBS) were exposed to diluted extracts (10 to 0.01 µg/mL) for 48 h. MTT-PBS (5 mg/mL) solution in culture medium was added to each well, followed by 3 h of incubation at 37 °C. The medium was removed and replaced with 100 µL of DMSO to dissolve the MTT formazan product. The solution was measured at 540 nm. The 50% inhibition concentration (IC_{50}) was estimated from a non-linear regression (GraphPad Prism). IC_{50} of >100 µg/mL is considered as non-toxic.

Anti-plasmodial assessment of selected extracts against *P. falciparum* 3D7

Extracts that inhibited *Hs* GSK-3β with no cytotoxic effect were further tested against *P. falciparum* 3D7 (*Pf* 3D7, chloroquine-sensitive) for antiplasmodial evaluation as previously described (Vejanan *et al.*, 2012; Wong *et al.*, 2015). One hundred microliters per well of asynchronised *Pf* cultures (grown in 2% hematocrit and 2% parasitemia) were exposed to 1000 to 0.0001 µg/mL of extracts and incubated at 37 °C for 48 h. After incubation, plates were frozen at -80 °C, followed by three 30 min of freeze/thaw cycles to lyse the erythrocytes. A plasmodium lactate dehydrogenase (pLDH) assay was measured at 650 nm. The IC_{50} values were determined by non-linear regression (GraphPad Prism). The extract selectivity indexes (SI) were determined by a ratio of values from IC_{50} of cytotoxicity over anti-plasmodial tests. In general, extracts with SI values greater than 10 indicate selective antiplasmodial agents (Memvanga *et al.*, 2015).

RESULTS

Preliminary screening of actinomycetes extracts against human GSK-3β activity and its upstream regulator, MKK1 and PP1/GLC7

Yeast strains expressing mammalian kinase and phosphatase, including *Hs* GSK-3β, are routinely used for screening in our laboratory. In this study, 514 actinomycetes strains were isolated from 144 soil samples. Subsequently, we identified nine actinomycetes extracts (MI4701, FA013, FH038, FH039(b), H17(1)-2, H17(2)-1, SA3H-1, SA8A-2 and SM0307) that showed differences in growth inhibition on yeasts at 37 °C compared to 28 °C, indicating selective *Hs* GSK-3β inhibition activity (Mahmud *et al.*, 2022a) (Figure 1). In the presence of selective *Hs* GSK-3β inhibitor, Rog1 remains in phosphorylated form, and its accumulation causes yeast growth defects at 37 °C Since the activity of *Hs* GSK-3β at 28 °C is not vital for yeast survival, inhibition of *Hs* GSK-3β at this temperature exerted no or minimal yeast growth inhibition (Andoh *et al.*, 2000).

To further evaluate the selectivity of the extracts, the active extracts were tested against kinase (MKK1) and phosphatase (MSG5 and GCL7) proteins using yeast-based assays. Eight extracts (MI4701, FA013, FH038, FH039(b), H17(1)-2, H17(2)-1, SA3H-1 and SA8A-2) exerted no activity on MKK1 and PP1/GLC7. Hence, *Hs* GSK-3β inhibition exerted by these extracts is selective towards *Hs* GSK-3β without affecting the upstream regulatory proteins. Meanwhile, SM0307 inhibited *Hs* GSK-3β activity but exerted a toxic effect, indicating poor selectivity (Table 1).

Cytotoxic and anti-plasmodial effects of selected actinomycetes extracts

Using Chang liver cells, extracts that demonstrated selective and consistent *Hs* GSK-3β (inhibitory activity;

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Table 1: Activities of actinomycetes crude acetone extract against kinase and phosphatase proteins.

Strain	GSK-3β		MKK1		MSG5 (MKK1 Confirmation assay)		PP1/GLC7								Strain
							PAY704-1 (wild-type, GLC7)				PAY700-4 (mutant, GLC7-10)				
							Inhibition zone (mm)								
	Inhibition zone (mm)		Inhibition zone (mm)	Yeast growth (mm)	Inhibition zone (mm)		28 °C		37 °C		28 °C		37 °C		
	28 °C	37 °C	Glu	Gal	Glu	Gal	YPD	YPD + 1 M sorbitol	YPD	YPD + 1 M sorbitol	YPD	YPD + 1 M sorbitol	YPD	YPD + 1 M sorbitol	
MI4701	10.33 ± 0.58	15.33 ± 0.58	0	0	0	0	0	0	0	0	0	0	-	0	Selective GSK-3β inhibitor, no effect on upstream GSK-3β regulators
FA013	10.67 ± 0.58	16.67 ± 0.58	0	0	0	0	0	0	0	0	0	0	-	0	
FH038	0.00 ± 0.00	8.33 ± 0.58	0	0	0	0	0	0	0	0	0	0	-	0	
FH039b	0.00 ± 0.00	8.00 ± 0.00	0	0	0	0	0	0	0	0	0	0	-	0	
H17(1)-2	7.67 ± 0.58	11.00 ± 0.58	0	0	0	0	0	0	0	0	0	0	-	0	
H17(2)-1	8.00 ± 0.00	12.00 ± 0.00	0	0	0	0	0	0	0	0	0	0	-	0	
SA3H-1	10.67 ± 0.58	13.00 ± 0.00	0	0	0	0	0	0	0	0	0	0	-	0	
SA8A-2	0.00 ± 0.00	7.67 ± 0.58	0	0	0	0	0	0	0	0	0	0	-	0	
SM0307	10.00 ± 1.73	14.67 ± 0.58	19.83 ± 0.29	0	20.00 ± 0.00	20.33 ± 0.58	12.00 ± 0.00	14.83 ± 0.29	9.67 ± 0.58	10.33 ± 0.58	15.50 ± 0.50	14.83 ± 0.76	-	13.33 ± 0.58	Non-selective GSK-3β inhibitor, toxic effects observed

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Table 2: Cytotoxic and anti-plasmodial activities of selected actinomycetes isolates.

Strains	Cytotoxicity test, IC_{50} (µg/mL ± SD)	Anti-plasmodial test, IC_{50} (µg/mL ± SD)	Selectivity Index (SI) = (IC_{50} MTT/IC_{50} pLDH)
FA013	2492.97 ± 332.72	0.18 ± 0.03	13850
FH039b	3189.00 ± 1.34	14.30 ± 12.69	223
FH038	414.50 ± 0.43	142.30 ± 0.16	3
MI4701	3189.00 ± 1.34	2.65 ± 0.89	1203
Chloroquine[1]		0.05 ± 0.001	66000

Note: [1]Anti-malarial reference drug (Control); Chang liver cells IC_{50} >100 µg/mL are non-toxic; IC_{50} threshold for anti-plasmodial activity; Potent ≤10 µg/mL, Moderate = 10-50 µg/mL, Weak = 50-100 µg/mL, No activity ≥100 µg/mL.

FA013, MI4701, FH038 and FH039b) were chosen for cytotoxic testing. These extracts exerted no cytotoxic effects (IC_{50} >100 µg/mL), which warrants subsequent anti-plasmodial activity against *Pf* 3D7. It was revealed that FA013 and MI4701 showed potent antiplasmodial activities with IC_{50} values at 0.18 and 2.65 µg/mL, respectively. Meanwhile, FH039b and FH038 showed moderate and no antiplasmodial activity, respectively (Table 2). FA013 was initially screened against *Hs* GSK-3β. SI analysis revealed that the anti-plasmodial activity of FA013 extract was highly selective towards *P. falciparum* (SI = 13,850), indicating that this isolate possessed significant antimalarial activity.

DISCUSSION

Several *Hs* GSK-3β inhibitors, both natural and synthetic, are now in the preclinical and clinical stages of development, primarily for cancer treatment. The mode of action of these inhibitors can be divided into non-ATP-competitive, ATP-competitive and substrate-competitive inhibitors (Cervello *et al.*, 2017). GSK-3 is also one of the most studied *Plasmodium* essential kinases and is indicated as a promising target for antimalarial development. Some studies showed subtle structural differences between human and parasite GSK-3 active sites, including the ATP-binding site, a general feature shared by all kinases across different species, which indicates the development of inhibitors with different selectivity between GSK-3β and *Pf* GSK3 is achievable (Osolodkin *et al.*, 2011; Mahmud *et al.*, 2020).

GSK-3β yeast-based assay as applied in this study was more sensitive toward non-ATP-competitive inhibitors such as TDZD-8, which is known to inhibit *Hs* GSK-3β modulated by Ser9 phosphorylation. However, some ATP-competitive inhibitors, such as staurosporine, can exert observable inhibitory activity but are 10-fold less effective than TDZD-8 (Abdul Aziz *et al.*, 2017). Using this yeast-based assay, we previously reported the identification of several *Hs* GSK-3β inhibitors, including three new amides and several known compounds (Cheenpracha *et al.*, 2009; Dahari *et al.*, 2016). Dibutyl phthalates were identified from *Streptomyces* sp. H11809, isolated from Sabah, Malaysia, inhibited *Hs* GSK-3β in yeast-based assay with potent anti-plasmodial activities *in vitro* and *in vivo*. Dibutyl phthalates was shown to bind on *Hs* GSK-3β substrate-binding site (via mixed inhibition) and to increase GSK-3 Ser9 phosphorylation, hence deactivating the enzyme (Dahari *et al.*, 2016; Mahmud *et al.*, 2022b).

In the current study, the extract of FA013 isolate was identified to exert the most potent inhibitory activity against *Hs* GSK-3β. Interestingly, this extract showed no effect on MKK1, which shares a high sequence identity with human MEK, a kinase involved in the MAPK pathway that activates *Hs* GSK-3β (Goold and Gordon-Weeks 2005; Hernández *et al.*, 2010). This result suggests a selective inhibitory activity against *Hs* GSK-3β, and it may not affect the ATP-binding sites of both kinases. Selectivity is a prime feature for clinically applicable kinase inhibitors as it minimises off-target possibility in cells for safer use (Bello and Gujral, 2018).

FA013 extract was further indicated as non-cytotoxic against Chang liver cells, suggesting its possible application for *Hs* GSK-3β-related diseases. In addition, subsequent evaluation of FA013 extracts against *P. falciparum* culture activity indicates significant antimalarial activity with high SI, which suggests the extract directly inhibits the parasite. Although the relation between *Pf* GSK-3 inhibition and antimalarial activity was not established, selective *Hs* GSK-3β and *P. falciparum* inhibition exerted by FA013 extract provide enough evidence for this isolate to be investigated further for the isolation and identification of active compound(s) that may provide future drug candidates for GSK-3-related disease treatment.

CONCLUSION

Due to their pivotal roles in cellular processes in humans and microorganisms, GSK-3 is an exciting target for drug development. This study revealed a promising potential of actinomycetes from the rainforest of Sabah to discover GSK-3β inhibitors and antiplasmodial agents. The yeast-based assay expressing *Hs* GSK-3β applied in this study offers an easy method to identify crude extracts exerting *Hs* GSK-3β inhibitors without affecting its upstream regulator. Although the connection between antimalarial activity and *Pf* GSK-3 inhibition is yet to be established, the yeast-based assay could narrow down sample selection for potent antimalarial activity. FA013 was suggested as the most potent strain in this study, which exerted selective *Hs* GSK-3β, no cytotoxic effect, and potent antimalarial activity with a high SI index. The ongoing research focuses on further purifying the active compound(s) from FA013 to understand the exact

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inhibitory mechanism against both human and plasmodial GSK-3.

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CONFLICTS OF INTEREST

All the authors declare that they have no conflict of interest.

REFERENCES

Abdul Aziz, D. N. A. F., Mahmud, F., Sidek, H. M., Embi, N., Andoh, T. T. and Lee, P. C. (2017). Assessment of the inhibitory mechanism of action for a yeast cell-based screening system targeting glycogen synthase kinase-3α (GSK-3α). *Journal of Biological Sciences* **17(1), 47-51.**

Ali, A. H., Sudi, S., Basir, R., Embi, N. and Sidek, H. M. (2017). The antimalarial effect of curcumin is mediated by the inhibition of glycogen synthase kinase-3β. *Journal of Medicinal Food* **20(2), 152-161.**

Ali, A. H., Sudi, S., Shi-Jing, N., Hassan, W. R. M., Basir, R., Agustar, H. K. *et al.* (2021). Dual anti-malarial and GSK3β-mediated cytokine-modulating activities of quercetin are requisite of its potential as a plant-derived therapeutic in malaria. *Pharmaceuticals* **14(3), 248.**

Alonso, M. and Martinez, A. (2005). GSK-3 inhibitors: Discoveries and developments. *Current Medicinal Chemistry* **11(6), 755-763.**

Andoh, T., Hirata, Y. and Kikuchi, A. (2000). Yeast glycogen synthase kinase 3 is involved in protein degradation in cooperation with Bul1, Bul2, and Rsp5. *Molecular and Cellular Biology* **20(18), 6712-6720.**

Andrews, P. D. and Stark, M. J. R. (2000). Type 1 protein phosphatase is required for maintenance of cell wall integrity, morphogenesis and cell cycle progression in *Saccharomyces cerevisiae*. *Journal of Cell Science* **113, 507-520.**

Ang, C. C., O'Brien, M. J., Ng, K. K. S., Lee, P. C., Hector, A., Schmid, B. *et al.* (2016). Genetic diversity of two tropical tree species of the Dipterocarpaceae following logging and restoration in Borneo: High genetic diversity in plots with high species diversity. *Plant Ecology and Diversity* **9(5-6), 459-469.**

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Meier-Kolthoff, J. P. *et al.* (2016). Taxonomy, physiology, and natural products of actinobacteria. *Microbiology and Molecular Biology Reviews* **80(1), 1-43.**

Bello, T. and Gujral, T. S. (2018). KInhibition: A kinase inhibitor selection portal. *iScience* **8, 49-53.**

Cabrera, D. G., Horatscheck, A., Wilson, C. R., Basarab, G., Eyermann, C. J. and Chibale, K. (2018). Plasmodial kinase inhibitors: License to cure? *Journal of Medicinal Chemistry* **61(18), 8061-8077.**

Cervello, M., Augello, G., Cusimano, A., Emma, M. R., Balasus, D., Azzolina, A. *et al.* (2017). Pivotal roles of glycogen synthase-3 in hepatocellular carcinoma. *Advances in Biological Regulation* **65, 59-76.**

Cheenpracha, S., Zhang, H., Mar, A. M. N., Foss, A. P., Sek, H. F., Lai, N. S. *et al.* (2009). Yeast glycogen synthase kinase-3β pathway inhibitors from an organic extract of *Streptomyces* sp. *Journal of Natural Products* **72(8), 1520-1523.**

Chong, C. J., Wen, L. H., Sani, H. A., Latip, J., Gansau, J. A., Chin, L. P. *et al.* (2012). Hypoglycemic effects of *Gynura procumbens* fractions on streptozotocin-induced diabetic rats involved phosphorylation of GSK3β (Ser-9) in liver. *Sains Malaysiana* **41, 969-975.**

Dahari, D. E., Salleh, R. M., Mahmud, F., Chin, L. P., Embi, N. and Mohd Sidek, H. (2016). Antimalarial activities of two soil actinomycete isolates from Sabah via inhibition of glycogen synthase kinase 3β. *Tropical Life Sciences Research* **27(2), 53-71.**

Embi, N., Rylatt, D. B. and Cohen, P. (1980). Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *European Journal of Biochemistry* **107(2), 519-527.**

Goold, R. G. and Gordon-Weeks, P. R. (2005). The MAP kinase pathway is upstream of the activation of GSK3β that enables it to phosphorylate MAP1B and contributes to the stimulation of axon growth. *Molecular and Cellular Neuroscience* **28(3), 524-534.**

Harir, M., Bendif, H., Bellahcene, M., Fortas, Z. and Pogni, R. (2018). *Streptomyces* secondary metabolites. *In*: Basic Biology and Applications of Actinobacteria. Enany, S. (ed.). IntechOpen Limited, London. **pp. 99-116.**

Hassan, W. R. M., Basir, R., Ali, A. H., Embi, N. and Sidek, H. M. (2019). Antimalarial and cytokine-modulating effects of andrographolide in a murine model of malarial infection. *Tropical Biomedicine* **36(3), 776-791.**

Hermida, M. A., Kumar, J. D. and Leslie, N. R. (2017). GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. *Advances in Biological Regulation* **65, 5-15.**

Hernández, F., Langa, E., Cuadros, R., Avila, J. and Villanueva, N. (2010). Regulation of GSK3 isoforms by phosphatases PP1 and PP2A. *Molecular and Cellular Biochemistry* **344(1-2), 211-215.**

Ho, W. L., Chan, K. W., Bernadr, T. Z. V., Lim, S. H., Ngao, W. C., Tong, M. L. *et al.* (2009). Screening for potential microbial inhibitors against prokaryotic and eukaryotic signal transduction and isocitrate lyase in mycobacterium from Danum Valley, Sabah. *Sabah Society Journal* **26, 1-91.**

Knapp, S. (2018). New opportunities for kinase drug repurposing and target discovery. *British Journal of Cancer* **118(7), 936-937.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Mahmud, F., Maili, S., Abdul Aziz, D. N. A. F., Marajin, L., Jembun, M., Eng How, S. *et al.* (2022a). The isolation rate of culturable actinomycetes from Malaysian Borneo forests and their activity against mammalian GSK-3β. *Current Applied Science and Technology* **22(4), 10.55003/cast.2022.04.22.004.**

Mahmud, F., Lai, N. S., How, S. E., Gansau, J. A., Mustaffa, K. M. F., Leow, C. H. *et al.* (2022b). Bioactivities and mode of actions of dibutyl phthalates and nocardamine from *Streptomyces* sp. H11809. *Molecules* **27(7), 2292.**

Mahmud, F., Lee, P. C., Wahab, H., Mustaffa, K. M. F., Leow, C. H., Rasul, A. *et al.* (2020). ATP gatekeeper of *Plasmodium* protein kinase may provide the opportunity to develop selective antimalarial drugs with multiple targets. *Asian Pacific Journal of Tropical Medicine* **13(8), 350-357.**

Mancinelli, R., Carpino, G., Petrungaro, S., Mammola, C. L., Tomaipitinca, L., Filippini, A. *et al.* (2017). Multifaceted roles of GSK-3 in cancer and autophagy-related diseases. *Oxidative Medicine and Cellular Longevity* **2017, Article ID 4629495.**

Masch, A., Nasereddin, A., Alder, A., Bird, M. J., Schweda, S. I., Preu, L. *et al.* (2019). Structure-activity relationships in a series of antiplasmodial thieno[2,3-b]pyridines. *Malaria Journal* **18, 89.**

Matawali, A., Chin, L. P., Eng, H. S., Boon, L. H. and Gansau, J. A. (2016). *In-vitro* evaluation of anti-kinase, anti-phosphatase and cytotoxic activities of *Mikania micrantha* HBK (asteraceae) from Malaysia. *Journal of Chemical and Pharmaceutical Sciences* **9(2), 696-701.**

Memvanga, P. B., Tona, G. L., Mesia, G. K., Lusakibanza, M. M. and Cimanga, R. K. (2015). Antimalarial activity of medicinal plants from the Democratic Republic of Congo: A review. *Journal of Ethnopharmacology* **169, 76-98.**

Omura, S., Iwai, Y., Hirano, A., Nakagawa, A., Awaya, J., Tsuchiya, H. *et al.* (1977). A new alkaloid AM-2282 of Streptomyces origin. Taxonomy, fermentation, isolation and preliminary characterisation. *The Journal of Antibiotics* **30(4), 275-282.**

Osolodkin, D. I., Palyulin, V. A. and Zefirov, N. S. (2012). Glycogen synthase kinase 3 as an anticancer drug target: Novel experimental findings and trends in the design of inhibitors. *Current Pharmaceutical Design* **19(4), 665-679.**

Osolodkin, D. I., Zakharevich, N. V., Palyulin, V. A., Danilenko, V. N. and Zefirov, N.S. (2011). Bioinformatic analysis of glycogen synthase kinase 3: Human versus parasite kinases. *Parasitology* **138(6), 725-735.**

Pagmadulam, B., Tserendulam, D., Rentsenkhand, T., Igarashi, M., Sawa, R., Nihei, C. *et al.* (2020). Isolation and characterization of antiprotozoal compound-producing *Streptomyces* species from Mongolian soils. *Parasitology International* **74, 101961.**

Sudi, S., Hassan, W. R. M., Ali, A. H., Basir, R., Embi, N. and Sidek, H. M. (2018). A derivative of cinnamic acid, methyl-4-hydroxycinnamate modulates inflammatory cytokine levels in malaria-infected mice through inhibition of GSK3β. *Malaysian Applied Biology* **47(3), 153-157.**

Tolosa, E., Litvan, I., Höglinger, G. U., Burn, D., Lees, A., Andrés, M. V. *et al.* (2014). A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. *Movement Disorders* **29(4), 470-478.**

Vejanan, V., Latip, J., Chin, L. P., Embi, N. and Sidek, H. M. (2012). *In vitro* and *in vivo* anti-plasmodial activities of *Gynura procumbens*. *Sains Malaysiana* **41(12), 1535-1542.**

Wong, S. K., Jann, M. L. S., Sudi, S., Hassan, W. R. B. M., Chin, L. P., Embi, N. *et al.* (2015). Antimalarial and anti-inflammatory effects of *Gynura procumbens* are mediated by kaempferol via inhibition of glycogen synthase kinase-3β (GSK3β). *Sains Malaysiana* **44(10), 1489-1500.**

Zakaria, N. A., Embi, N. and Sidek, H. M. (2010). Suppression of *Plasmodium berghei* parasitemia by LiCl in an animal infection model. *Tropical Biomedicine* **27(3), 624-631.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538