

## **Bioactivities and mode of actions of dibutyl phthalates and nocardamine from *Streptomyces* sp. H11809**

### **ABSTRACT**

Dibutyl phthalate (DBP) produced by *Streptomyces* sp. H11809 exerted inhibitory activity against human GSK-3 $\beta$  (Hs GSK-3 $\beta$ ) and *Plasmodium falciparum* 3D7 (Pf 3D7) malaria parasites. The current study aimed to determine DBP's plausible mode of action against Hs GSK-3 $\beta$  and Pf 3D7. Molecular docking analysis indicated that DBP has a higher binding affinity to the substrate-binding site (pocket 2;  $-6.9$  kcal/mol) than the ATP-binding site (pocket 1;  $-6.1$  kcal/mol) of Hs GSK-3 $\beta$ . It was suggested that the esters of DBP play a pivotal role in the inhibition of Hs GSK-3 $\beta$  through the formation of hydrogen bonds with Arg96/Glu97 amino acid residues in pocket 2. Subsequently, an in vitro Hs GSK-3 $\beta$  enzymatic assay revealed that DBP inhibits the activity of Hs GSK-3 $\beta$  via mixed inhibition inhibitory mechanisms, with a moderate IC<sub>50</sub> of 2.0  $\mu$ M. Furthermore, the decrease in  $K_m$  value with an increasing DBP concentration suggested that DBP favors binding on free Hs GSK-3 $\beta$  over its substrate-bound state. However, the antimalarial mode of action of DBP remains unknown since the generation of a Pf 3D7 DBP-resistant clone was not successful. Thus, the molecular target of DBP might be indispensable for Pf survival. We also identified nocardamine as another active compound from *Streptomyces* sp. H11809 chloroform extract. It showed potent antimalarial activity with an IC<sub>50</sub> of 1.5  $\mu$ M, which is  $\sim 10$ -fold more potent than DBP, but with no effect on Hs GSK-3 $\beta$ . The addition of  $\geq 12.5$   $\mu$ M ferric ions into the Pf culture reduced nocardamine antimalarial activity by 90% under in vitro settings. Hence, the iron-chelating ability of nocardamine was shown to starve the parasites from their iron source, eventually inhibiting their growth.