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3-Hydroxyisoflavanones from the stem bark of *Dalbergia melanoxylon*: Isolation, antimycobacterial evaluation and molecular docking studies

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ABSTRACT

Two new 3-hydroxyisoflavanones, (*S*)-3,4′,5-trihydroxy-2′,7-dimethoxy-3′-prenylisoflavanone (trivial name kenusanone F 7-methyl ether) and (*S*)-3,5-dihydroxy-2′,7-dimethoxy-2″,2″-dimethylpyrano[5″,6″:3′,4′]isoflavanone (trivial name sophoronol-7-methyl ether) along with two known compounds (dalbergin and formononetin) were isolated from the stem bark of *Dalbergia melanoxylon*. The structures were elucidated using spectroscopic techniques. Kenusanone F 7-methyl ether showed activity against *Mycobacterium tuberculosis*, whereas both of the new compounds were inactive against the malaria parasite *Plasmodium falciparum* at 10 μg/ml. Docking studies showed that the new compounds kenusanone F 7-methyl ether and sophoronol-7-methyl ether have high affinity for the *M. tuberculosis* drug target INHA.

1. Introduction

Dalbergia (family Fabaceae) is a large genus of small to medium size trees, shrubs and lianas, with a wide distribution in Central and South America, Africa, Madagascar and Southern Asia (Zheng et al., 2012). This genus has been shown to possess various pharmacological activities including analgesic, antipyretic, anti-inflammatory (Kale et al., 2007; Misar et al., 2005), antimicrobial (Gundidza and Gaza, 1993), antidiarrhoeal (Majumdar et al., 2005), antiulcerogenic (Brito et al., 1997), larvicidal and mosquito repellent (Ansari et al., 2000), antigiardial (Khan et al., 2000), antiplasmodial (Beldjoudi et al., 2003), anti-fertility (Uchendu et al., 2000), antioxidant (Wang et al., 2000) and cancer chemopreventive activities (Ito et al., 2003). The occurrence of isoflavones, isoflavanones, neoflavones, sterols, anthraquinones, cinnamyl esters and triterpenes in this genus has been reported (Vasudeva et al., 2009). 3-Hydroxisoflavanones, which are among the rare flavonoids, have also been described from this genus (Chan et al., 1998); such compounds also occur in a few other related genera such as *Sophora* (Zhang et al., 2009).

Dalbergia melanoxylon is a shrub or small tree mainly found in deciduous woodlands or bushlands, wooded grasslands, often in rocky sites or on black cotton soil (Beentje, 1994). The bark of *D. melanoxylon* has been used traditionally for cleaning wounds while the roots have been used to alleviate abdominal pains, as an anthelmintic and as a part of preparation for the treatment of gonorrhoea (Kokwaro, 1976). The leaves are boiled in soup and drunk to relieve pain in joints (Kareru et al., 2008). The citric acid extract of the bark of *D. melanoxylon* has been reported to have antimicrobial activity (Gundidza and Gaza, 1993). A dihydrofuran (melanoxin), a neoflavone (mellanein) and a quinone (4-hydroxy-4-methoxydalbergione) have previously been reported from this plant (Donnelly et al., 1969). In this paper the isolation and characterization of two new 3-hydroxyisoflavanones (**1** and **2**; Fig. 1) along with two known compounds from the stem bark of *D. melanoxylon* are reported. The antimicrobacterial activity of one of the new compounds (**1**) against *Mycobacterium tuberculosis* is also reported.

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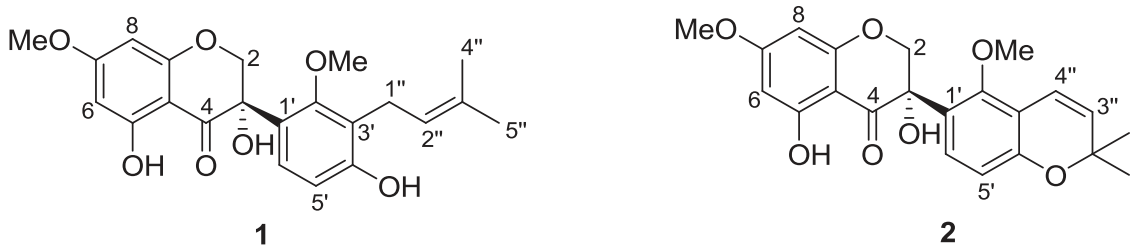

Fig. 1. 3-Hydroxyisoflavanones from *Dalbergia melanoxylon*.

2. Results and discussion

Chromatographic separation of the dichloromethane/methanol (1:1) extract of the stem bark of *D. melanoxylon* led to the isolation of two new 3-hydroxyisoflavanones (**1** and **2**) along with two known compounds. The known compounds were identified as dalbergin (Chan et al., 1998) and formononetin (Chang et al., 1994) by comparison of their spectroscopic data with those reported in literature.

One of the new compounds (**1**) was obtained as a white amorphous powder. Its molecular formula was established as $C_{22}H_{24}O_7$ from HRESIMS which showed an $[M+H]^+$ peak at *m/z* 401.1589. The UV (λ_{max} 221 and 282 nm) and ^{1}H-NMR data (Table 1) suggested a 3-hydroxyisoflavanone skeleton with two mutually coupled doublets at δ 4.24 and 4.87 (J = 11.6 Hz) being for the oxymethylene protons at C-2, and showing HSQC correlation with the signal at δ_C 74.5 (C-2). The ^{13}C-NMR spectrum further showed an oxygenated quaternary carbon at δ 74.0 (C-3) and a carbonyl at δ_C 196.6 (C-4) which were consistent with a 3-hydroxyisoflavanone skeleton (Zhang et al., 2009). The ^{1}H-NMR and ^{13}C-NMR spectral data also showed the presence of two hydroxyl (one of which being chelated, δ_H 11.70 for OH-5; the presence of the second hydroxyl group was inferred from the presence of five oxygentated sp^2 carbon atoms in the ^{13}C NMR spectrum), two methoxyl (δ_H 3.81 and 3.67; δ_C 56.0 and 62.7) and prenyl (Table 1) substituents on the 3-hydroxyisoflavanone skeleton. The presence of a deshielded singlet at δ_H 11.70 (OH-5) and two *meta*-coupled doublets at δ 6.10 and δ 5.99 (J = 2.0 Hz), for H-6 and H-8, indicated that ring A is 5,7-dioxygenated (Enami et al., 2007).

Two *ortho*-coupled doublets at δ_H 6.61 and 7.22 (J = 8.6 Hz) were assigned to H-5′ and H-6′ of a trisubstituted ring B. In addition to the biogenetically expected oxygenation at C-4′ (δ_C 157.2), further oxygenation at C-2′ (δ_C 157.2) was evident from the ^{13}C-NMR spectrum (Table 1), with the prenyl group placed at C-3′ (She et al., 2009). The two methoxyl groups (δ_H 3.81; δ_C 56.0 and δ_H 3.67; δ_C 62.7) could be placed at C-7, -2′ and/or -4′. The ^{13}C-NMR chemical shift value of one of the methoxyl groups was downfield-shifted (δ_C 62.7) indicating that it is *diortho*-substituted (Xue-Dong et al., 2003), which is consistent with its placement at C-2′. In an NOEDIFF experiment, irradiation of the methoxyl signal at δ_H 3.67 showed enhancement of CH_2-2; whereas irradiation of the second methoxyl group at δ_H 3.81 showed enhancement of H-6 and H-8 (but not H-5′) supporting the placement of the methoxyl groups at C-7 and C-2′. This was confirmed from the HMBC spectrum which showed correlation of the methoxyl protons at δ 3.81 with C-7, while the methoxyl protons at δ 3.67 correlated with C-2′. This new compound was therefore characterized as 3,4′,5-trihydroxy-2′,7-dimethoxy-3′-prenylisoflavanone, for which the trivial name kenusanone F 7-methyl ether is given by relating it to kenusanone F (Iinuma et al., 1993).

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectral data of compounds **1** and **2** (in $CDCl_3$).

Position	**1**		HMBC correlation	**2**		HMBC correlation
	δ_C	δ_H (J in Hz)	H→C	δ_C	δ_H (J in Hz)	H→C
1						
2	74.5	4.87 *d* (11.6) 4.24 *d* (11.6)	C4, C5′ C4, C9, C1′	74.6	4.81 *d* (11.8) 4.25 *d*(11.8)	C4, C5′ C3, C4, C9, C1′
3	74.0			73.8		
4	196.6			196.5		
5	164.7			164.8		
6	95.7	6.10 *d* (2.0)	C4a, C8	94.6	6.00 *d* (2.3)	C4a, C8
7	168.4			168.4		
8	94.6	5.99 *d* (2.0)	C4a, C6	95.7	6.10 *d*(2.3)	C4a, C6
9	162.9			162.9		
10	101.9			101.9		
1′	123.7			123.2		
2′	157.2			155.2		
3′	121.0			115.1		
4′	157.2			154.7		
5′	112.1	6.61 *d* (8.6)	C3	112.5	6.57 *dd* (0.4, 8.6)	C1′
6′	126.4	7.22 *d* (8.6)	C2′, C4′,	127.7	7.27 *d* (8.6)	C2′, C4′
1″	24.1	3.37 *d* (6.4)	C2′,C4′,C3″			
2″	121.4	5.22 *t* (6.4)	3″	76.0		
3″	136.3			130.5	5.65 *d* (10)	C2″, C3′
4″	18.2	1.80 s		117.7	6.48 *dd* (0.4, 10)	C3′
5″	26.0	1.76 s		28.1	1.43 s	
6″				27.7	1.41 s	
5-OH		11.70 s			11.73 s	
7-OCH_3	56.0	3.81 s	C7	56.0	3.81 s	C7
2′-OCH_3	62.7	3.67 s	C2′	63.0	3.70 s	C2′

Table 2
Configuration of 3-hydroxyisoflavanones in literature and proposed changes.

3-Hydroxyisoflavanone	Cotton effect for $n\rightarrow\pi^*$ transition	Assigned designation	Configuration in chemical structure drawn	Proposed configuration
2′,4′-Dimethoxy-3,7-dihydroxyisoflavanone (Lee et al., 2013)	Negative at 341 nm	*3S*	*3R*	*3R*
2′,4′,5′-Trimethoxy-7-hydroxyisoflavanone (Lee et al., 2013)	Negative at 324 nm	*3S*	*3R*	*3R*
Sophoranone (Atta-ur-Rahman Haroone et al., 2012)	Positive at 297 nm	*3R*	*3S*	*3S*
Sophoronols A-F (Zhang et al., 2009)	Negative at 317–330 nm	*3S*	*3S*	*3R*
3,7,2′,3′-Tetrahydroxy-4′-methoxy-5′-prenyl-isoflavanone (Vila et al., 1998)	Negative at 331 nm	*3S*	*3S*	*3R*
3,7,2′-Trihydroxy-4′-methoxy-5′-prenyl-isoflavanone (Nguyen et al., 2011)	Negative at 331 nm	*3S*	*Not shown*	*3R*

The octant rule modified for the cyclic arylketone predicts a positive Cotton effect in the range between *ca.* 320 and 350 nm for the $n\rightarrow\pi^*$ carbonyl transition for (3*R*)-isoflavanones with the B ring in the favoured equatorial position (Slade et al., 2005; Won et al., 2008). Compound **1** showed a positive Cotton effect at 348 nm and hence should have the same configuration as (3*R*)-isoflavanones, but the designation at C-3 will be *S* for formal reasons (according to the Cahn–Ingold–Prelog rules, the priority order changes when hydrogen at C-3 in isoflavanones is replaced with hydroxyl group in 3-hydroxyisoflavanones). A negative Cotton effect for the $n\rightarrow\pi^*$ transition (which corresponds to (*S*)-isoflavanones) will then correspond to (3*R*)-3-hydroxyisoflavanone. In this regard there appear to be misinterpretation (of assignment of configuration and/or designation) in literature on the transfer of the octant rule, as applied to isoflavanones, to 3-hydroxyanthraquinones. Some examples and proposed corrections are shown in Table 2.

The second new compound (**2**) was obtained as an amorphous powder. Its molecular formula was established as $C_{22}H_{22}O_7$ by the HRESIMS ion at *m*/*z* 399.1447 for $[M+H]^+$. The UV (λ_{max} 227and 283 nm), ^{1}H- and ^{13}C-NMR (Table 1) spectra of **2** suggested a 3-hydroxyisoflavanone skeleton with a similar substitution pattern to **1**. In fact the only difference between compounds **1** and **2** is that the signals for the prenyl group in **1** are replaced by those of a 2,2-dimethylpyran ring in **2** (Zhang et al., 2009). The appearance of a double doublet signal for H-4″ in the 2,2-dimethylpyran ring (Table 1) is indicative of long range coupling (^5J) with H-5′ along with vicinal coupling with H-3″. The HMBC spectrum showed correlation between H-3″ and C-2″, H-3″ and C-3′, and H-4″ and C-3′, thus confirming the attachment of the dimethylpyran ring between C-3′ and C-4′ of ring B. As in compound **1**, the ^{13}C-NMR downfield chemical shift position of one of the methoxyl signals (δ_C 63.0) is in agreement with its placement at C-2′; the second methoxyl group (δ_H 3.81, δ_C 56.0) was placed at C-7 from NOE (methoxy protons with H-6 and H-8) and HMBC (methoxy protons with C-7) experiments. In the CD spectrum of compound **2**, a positive Cotton effect at 350 nm is consistent with the *S* configuration at C-3 as in **1**. This new compound was therefore characterized as (3*S*)-3,5-dihydroxy-2′,7-dimethoxy-2″,2″-dimethylpyrano[5″,6″:3′,4′]isoflavanone, for which the trivial name sophoronol-7-methyl ether is given.

When tested for antimycobacterial activity against replicating cultures of the *M. tuberculosis* H_{37}Rv strain, compound **1** exhibited 96% inhibition at 128 μM with an MIC of 80.3 μM (compound **2** was not tested due to paucity of the sample). The new compounds were also tested for antiplasmodial activity against the D10 strain of the human malaria parasite *Plasmodium falciparum*, based on the fact that related compounds possess antiplasmodial activity (Zhang et al., 2009). However the compounds did not show significant activity up to 10 μg/mL.

3. Molecular docking of 3-hydroxyisoflavanones

Recently we reported a computational (*in silico*) study in which the chemical space, based on physicochemical properties and structural features, of a diverse group of natural products with reported *in vitro* activity against different *M. tuberculosis* strains were investigated. This was compared to the chemical space occupied by drugs currently recommended for the treatment of various forms of tuberculosis (TB) as well as compounds in preclinical and clinical development (Espinoza-Moraga et al., 2013). As part of this work we also carried out molecular docking studies exploring possible binding affinities and modes of the natural products on two different *Mycobacterial* targets, which included the oxidoreductase of *Mtb* INHA, a validated target of the drugs ethionamide and isoniazide, which inhibit the biosynthesis of mycolic acid and have been used for many years for the treatment of TB. This work was undertaken with a view to providing a rational basis for selecting natural products predicted to bind and/or interact with INHA for *in vitro* testing against this target (Espinoza-Moraga et al., 2013). Encouraged by this work, we extended the molecular docking studies to 3-hydroxyisoflavones **1** and **2**.

Docking calculations using GLIDE software were carried out on 2-ethyl-isonicotinic-acyl-nicotinamide-adenine-dinucleotide (ETH-NAD) adduct enzyme INHA (PDB ID 2H91), *T. aquaticus* RNA polymerase–rifampicin complex (PDB ID 1YNN) and the *C*-terminal domain of the arabinosyltransferase *M. tuberculosis* EmbC (PDB ID 3PTY). Compounds **1** and **2** showed favourable binding to all three enzymes (Fig. 2). However, both compounds had a higher affinity for INHA than for 3PTY and 1YNN (Table 3). The docked conformations were stabilized by a dense network of hydrogen bonding. Compound **1** formed H-bonds with Gly192, Thr196 and Ser94 whilst compound **2** bonded to Ser20, Ser94 and Ile21. Compound **2** shares some interaction sites (Ser20 and Ile21) with ETH-NAD though the binding affinities differ by a factor of 2 (−14.212 and −6.637). Since the important residues for the activation of the enzymes are known, these results could provide a starting point for structure based optimization of these new compounds based on their interactions with the enzymes and their binding modes.

4. Experimental

4.1. General

Melting points were determined using a Reichert-Jung Thermovar hot stage microscope apparatus. Analytical TLC was carried out using Merck pre-coated silica gel 60 F_{254} plates, while column chromatography was done on silica gel impregnated with oxalic acid. Gel filtration was carried out on Sephadex LH-20. Optical rotations were determined using a Perkin-Elmer 343 polarimeter at 20 °C and the concentration *c* is expressed in g/100 mL. CD data were obtained using a JASCO Corp. J-715 CD spectrometer. UV was on a Varian Cary 100 conc. spectrometer. ^{1}H-NMR (600 MHz or 200 MHz) and ^{13}C-NMR (150 MHz or 50 MHz) were carried out on Bruker or Varian-Mercury spectrometers. HSQC and HMBC spectra were acquired using the

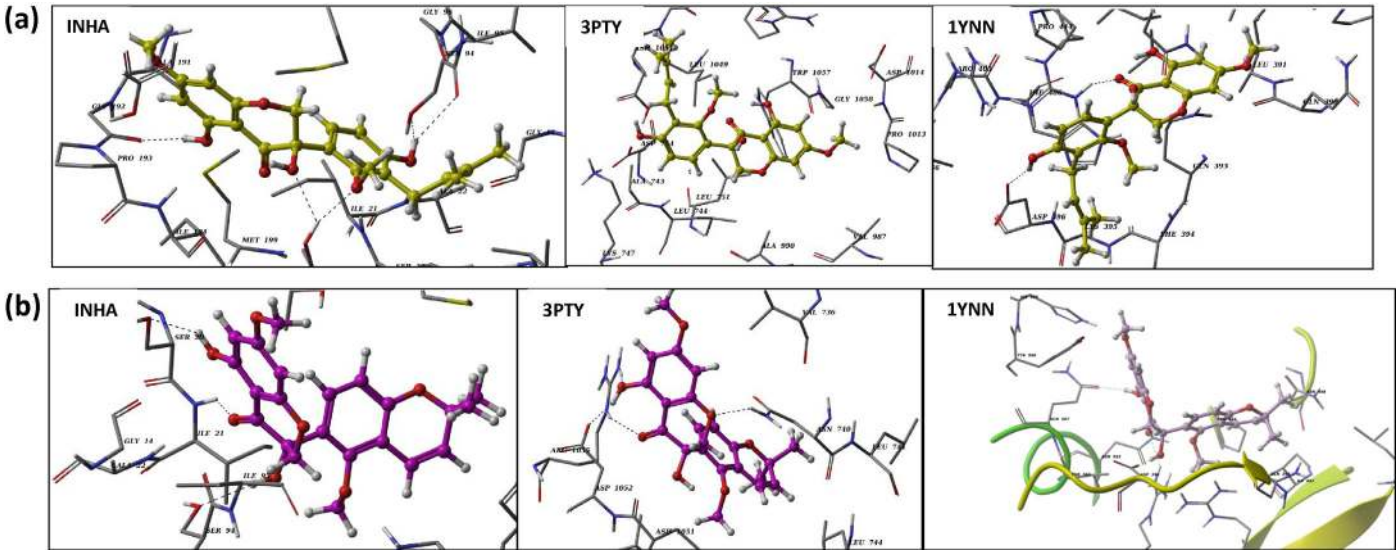

Fig. 2. Molecular docking of compounds **1** (2a) and **2** (2b).

standard Bruker software. HRESIMS were obtained using a Waters API Q-TOF Ultima mass spectrometer.

4.2. Plant material

The stem bark of *D. melanoxylon* was collected from Kaya Muhaka forest, Kwale district, the Coastal region of Kenya in July 2005 and a voucher specimen (voucher number AYT-024-2005) has been deposited at the Herbarium, School of Biological Sciences, University of Nairobi.

Table 3
Docking studies of compounds **1** and **2** in comparison with the co-crystallized ligands.

	Target site	Docking score	Interactions
	INHA		
Ligand-ETH-NAD		−14.212	Tyr158A Phe149A Gly14A Ile95A Ile21A Lys165A Ser20A
1		−7.937	Gly 192 Thr 196 Ser 94
2		−6.637	Ser20 Ser94 Ile21
	1YNN		
Ligand-rifampicin		−6.936	None
1		−4.846	None
2		−4.427	Glu567 Asp396 Arg405
	3PTY		
Ligand-AFO		−2.086	Val736A Asn740A
1		−5.270	None
2		−5.453	Asn740 Arg1055 Asp1052

AFO, arabinosyltransferaseM.tbEmbC; ETH-NAD, 2-ethyl-isonicotinic-acyl-nicotinamide-adenine-dinucleotide.

4.3. Extraction and isolation

The ground stem bark of *D. melanoxylon* (1.0 kg) was macerated in a 1:1 mixture of dichoromethane and methanol to yield a gummy extract (45 g). A 40 g portion of the extract was subjected to column chromatography on oxalic acid impregnated silica gel (600 g) eluting with *n*-hexane containing increasing amounts of ethyl acetate. The fraction eluted with 2% ethyl acetate in *n*-hexane was subjected to column chromatography on Sephadex LH-20 (eluent CH_2Cl_2:CH_3OH; 1:1) and further purification on preparative TLC (*n*-hexane:ethyl acetate; 3:2) yielded compound **1** (14 mg). The fraction eluted using 3% ethyl acetate in *n*-hexane was further purified by column chromatography (oxalic acid impregnated silica gel, dichloromethane/acetone) to yield compound **2** (8 mg) and dalbergin (41 mg). Formononetin (23 mg) was obtained from the 20% ethyl acetate in n-hexane fraction which was purified by column chromatography on silica gel (eluted with 3% methanol in dichloromethane).

4.4. Kenusanone F 7-methyl ether (*1*)

White amorphous powder; m.p. 155–158 °C, UV λ_{max} (MeOH)nm: 221, 282; $[\alpha]_D$ +68.57° (*c* 0.7, $CHCl_3$); ^{1}H- and ^{13}C-NMR (Table 1); EI-MS *m/z* (rel. int.): 400 (8, $[M]^+$) 219 (12), 217 (37), 168 (9), 167 (100); HRMS *m/z* 401.1589 $[M+H]^+$ for $C_{22}H_{25}O_7$) (calculated for 401.1600).

4.5. Sophoronol-7-methy ether (*2*)

White amorphous powder; mp 162–165 °C, UV λ_{max} (MeOH)nm: 227, 283; $[\alpha]_D$ +52.5° (*c* 0.4, $CHCl_3$); ^{1}H- and ^{13}C-NMR (Table 1); EI-MS *m/z* (rel. int.): 398 (16, $[M]^+$), 365 (25), 232 (15), 217 (100), 215 (27), 187 (12), 167(37); HRMS *m/z* 399.1447 $[M+H]^+$ for $C_{22}H_{23}O_7$ (calculated for 399.1444).

4.6. Antimycobacterial activity

The compounds were tested for antimycobacterial activity against *M. tuberculosis* H37Rv (ATCC 27294) using the Microtiter-Alamar Blue assay (MABA) as previously described (Collins and Franzblau, 1997) but using 7H12 media instead of 7H9, glycerol, casitane and OADC. Cultures were incubated for 7 days at 37 °C. Alamar blue and Tween 80 were added and incubation continued

for 24 h at 37 °C. Fluorescence was determined at excitation/emission wavelengths of 530/590 nm, respectively. The MIC was defined as the lowest concentration effecting a reduction in fluorescence (or luminescence) of 90% relative to controls. Rifampicin exhibited 97% inhibition with an MIC of 0.06 μM and isoniazid exhibited 93% inhibition with an MIC of 0.21 μM.

4.7. Antiplasmodial activity

The samples were tested in triplicate on two separate occasions against a chloroquine-sensitive (CQS) strain of *P. falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained as described by Trager and Jensen (1976). Quantitative assessment of *in vitro* antiplasmodial activity was determined using the parasite lactate dehydrogenase assay (Makler et al., 1993). Chloroquine diphosphate was used as the reference drug in all antiplasmodial experiments. Samples were tested according to a well-established method (Pillay et al., 2007).

4.8. Docking experiments

Docking calculations were performed using GLIDE 5.7 (Glide, 2011), a well known and widely used tool for docking small molecules. The three dimension crystal structures of *M. tuberculosis* target proteins were obtained from the PDB database. Before docking, the protein was prepared using the protein preparation wizard on Maestro (Maestro, 2011) by removal of water and addition of hydrogen. The tool used for minimization was Impact version 57111. Energy minimization steps were increased to 5000 and the default GLIDE parameters were used. Ligprep was used to generate molecules of different ionization state at pH 7.0 ± 2.0. ConfGen version 2.3 on Maestro generated different conformations of the compound and Standard Precision calculations were carried out. To validate the docking studies, the co-crystallized inhibitors were redocked to their respective targets. After validation compounds **1** and **2** were docked into the target sites and Maetsro was used to view and analyze the best docked conformations.

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References

Ansari, M.A., Razdan, R.K., Tandon, M., Vasudevan, P., 2000. Larvicidal and repellent actions of *Dalbergia sissoo* Roxb (F-Leguminosae) oil against mosquitoes. Bioresour. Technol. 73, 207–211.

Atta-ur-Rahman Haroone, M.S., Tateen, R.B., Mesaik, M.A., Jan, S., Abbaskhan, A., Asif, M., Gulzar, T., Al-majid, A.M., Yousuf, S., Choudhary, M.I., 2012. Secondary metabolites of *Sophora mollis* subsp. *griffithii* (Stocks) Ali. Phytochemistry. Lett. 5, 613–616.

Beldjoudi, N., Mambu, L., Labaied, M., Grellier, P., Ramanitrahasimbola, D., Rosoanaivo, P., Martin, M.T., Frappier, F., 2003. Flavanoids from *Dalbergia louvelii* and their antiplasmodial activity. J. Nat. Prod. 66, 1447–1450.

Beentje, H.J., 1994. Kenya Trees Shrubs and Lianas. National Museums of Kenya, , pp. 293.

Brito, A.R.M.S., Cota, R.H.S., Nunes, D.S., 1997. Gastric Antiulcerogenic Effects *of Dalbergia monetaria* L in rats. Phytother. Res. 11, 314–316.

Chan, S-C., Chang, Y-S., Wang, J-P., Chen, S-C., Kuo, S-C., 1998. Three new flavonoids and antiallergic, anti-inflammatory constituents from the heartwood of *Dalbergia odorifera*. Planta Med. 64, 153–158.

Chang, Y-C., Nair, M.G., Santell, R.C., Helferich, W.G., 1994. Microwave mediated synthesis of anticarcinogenic isoflavones from soybeans. J. Agric. Food. Chem. 42, 1869–1871.

Collins, L., Franzblau, S.G., 1997. Microplate alamar blue assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob. Agents Chemother. 41, 1004–1009.

Donnelly, B.J., Donnelly, D.M.X., O'Sullivan, A.M., Prendergast, J.P., 1969. Dalbergia species VII: The isolation and structure of melanoxin a new dihydrobenzofuran from *Dalbergia melanoxylon* Guill. and Perr. (Leguminoseae). Tetrahedron 25, 4409–4414.

Enami, S.A., Ramimeh, E.A.A., Ahi, A., Kashy, M.R.B., Schneider, B., Iranshahi, M., 2007. Prenylated flavonoids and flavonostilbenes from *Sophora pachycarpa* roots. Pharm. Biol. 45, 435–457.

Espinoza-Moraga, M., Njuguna, N., Mugumbate, G., Caballero, J., Chibale, K., 2013. In silico comparison of anti mycobacterial natural products with known antituberculosis drugs. J. Chem. Inf. Model. 53, 649–660.

Schrödinger, Inc., New York, NY.

Gundidza, M., Gaza, N., 1993. Antimicrobial activity of *Dalberga melanoxylon* extracts. J. Ethnopharmacol. 40, 127–130.

Iinuma, M., Ohyama, M., Tanaka, T., Mizuno, M., Hong, S-K., 1993. Five flavonoid compounds from *Echinosophora koreensis*. Phytochemistry 33, 1241–1245.

Ito, C., Itoigawa, M., Kanematsu, T., Ruangrungsi, N., Higashihara, H., Takuda, H., Nishino, H., Furakawa, H., 2003. New cinnamylphenols from *Dalbergia* species with cancer chemopreventive activity. J. Nat. Prod. 66, 1574–1577.

Kale, M., Misar, A.V., Dave, V., Joshl, M., Majumdar, A.M., 2007. Anti-inflammatory activity of *Dalbergia lanceolaria* bark ethanolic extract in mice and rats. J. Ethnopharmacol. 112, 300–304.

Kareru, P.G., Gachanja, A.N., Keriko, J.M., Kenji, G.M., 2008. Antimicrobial activity of some medicinal plants used by herbalists in Eastern province, Kenya. Afr. J. Trad. CAM 5, 51–55.

Khan, I.A., Avery, M.A., Burandt, C.L., Goins, D.K., Mikell, J.R., Nash, T.E., Azadegan, A., Walker, L.A., 2000. Antigiardial activity of isoflavones from *Dalbergia frutescens* Bark. J. Nat. Prod. 63, 1414–1416.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa, first ed. East African Literature Bureau, , pp. 135.

Schrödinger, LLC, New York, NY.

Lee, C., Lee, J.W., Jin, Q., Jang, D.J., Lee, S.-J., Lee, D., Hong, J.T., Kim, Y., Lee, M.K., Bang Yeon Hwang, B.Y., 2013. Inhibitory constituents of the hearwood of Dalbergia odorifera on nitric oside production in RAW 264.7 macrophages. Bioorg. Med. Chem. Lett. 23, 4263–4266.

Majumdar, A.M., Misar, A.V., Upadhye, A.S., 2005. Antidiarrhoeal activity of ethanolic extract of the bark of *Dalbergia lanceolaria*. J. Ethnopharmacol. 102, 213–216.

Makler, M.T., Ries, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L., Hinrichs, D.J., 1993. Parasite lactate dehydrogenase as an assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg. 48, 739–741.

Misar, A.V., Kale, M., Joshi, M., Majumdar, A.M., 2005. Analgesic activity of *Dalbergia lanceolaria* bark extract in Swiss albino mice. Pharm. Biol. 45, 723–725.

Nguyen, P.H., Dao, T.T., Kim, J., Phong, D.T., Ndinteh, D.T., Mbafor, J.T., Oh, W.K., 2011. New 5-deoxyflavonoids and their inhibitory effects on protein tyrosine phosphatase 1B (PTP1B) activity. Bioorg. Med. Chem. 19, 3378–3383.

Pillay, P., Vleggaar, R., Maharaj, V.J., Smith, P.J., Lategan, C.A., Chouteau, F., Chibale, K., 2007. Antiplasmodial hirsutinolides from *Vernonia staehelinoides* and their utilization towards a simplified pharmacophore. Phytochemistry 68, 1200–1205.

She, G., Guo, Z., Lv, H., She, D., 2009. New flavonoid glycosides from *Esholtzia rugulosa* Hemsl. Molecules 14, 4190–4196.

Slade, D., Ferreira, D., Marais, J.P.J., 2005. Circular dichroism. A powerful tool for the assignment of absolute configuration of flavonoids. Phytochemistry 66, 2177–2215.

Trager, W., Jensen, J.B., 1976. Human malaria parasites in continuous culture. Science 193, 673–675.

Uchendu, C.N., Kamalu, T.N., Asuzu, I.U., 2000. A preliminary evaluation of antifertility activity of a triterpenoid glycoside (DSS) from *Dalbergia lanceolaria* in female witsar rats. Pharmacolog. Res. 41, 521–525.

Vasudeva, N., Vats, M., Sharma, S.K., Sardana, S., 2009. Chemistry and biological activities of the genus Dalbergia – a review. Phcog. Rev. 3, 307–319.

Vila, J., Balderrama, L., Bravo, J.L., Alma, G., 1998. Prenylisoflavanones from *Geoffroea decorticans*. Phytochemistry 49, 2525–2528.

Wang, W., Weng, X., Cheng, D., 2000. Antioxidant activities of natural phenolic components from *Dalbergia odorifera* T. Chen. Food Chem. 71, 45–49.

Won, D., Shin, B-K., Han, J., 2008. Synthesis and the absolute configurations of isoflavanone enantiomers. J. Appl. Biol. Chem. 51, 17–19.

Xue-Dong, Y., Li-Zhein, X., Shi-Lin, Y., 2003. Two Xanthones from the stems of *Securidaca inappendiculata*. Acta Bot. Sin. 45, 365–368.

Zhang, G., Xiao, Z.Y., Rafique, J., Arfan, M., Smith, P.J., Lategan, C.A., Hu, L.H., 2009. Antiplasmodial isoflavanones from the roots of *Sophora mollis*. J. Nat. Prod. 72, 1265–1268.

Zheng, L., Xing, H., Li, W., Zhengxing, C., 2012. Physicochemical properties, chemical composition and antioxidant activity of *Dalbergia odorifera* T. Chen seed oil. J. Am. Oil Chem Soc. 89, 883–890.