

**Identification of *Aspergillus fumigatus* UDP-
galactopyranose mutase inhibitors
(Supporting Data)**

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Table S1. Summary and classification of hits purchased from independent companies.

Anisotropy readings from screening were normalized to negative control (protein and 30 nM ADP-TAMRA). Inhibition against UGM was assayed at 20 and 200 μ M with 20 μ M UDP-Galf and 10 mM sodium dithionite using the UPLC method.

Family	Name	Normalized anisotropy (%)	Inhibition ^a > 50 %	Concentration effect ^b
Flavonoids	Hesperetin	17	+	+
	Naringenin	23	+	+
	Chrysin	48	+	+
	Kaempferol ^c	38	+	+
Non-Flavonoids	Nicorandil	27	-	-
	Diclazuril	37	+	+
	Diffraitaic acid	37	-	-
	Febuxostat	39	+	+
	Olsalazine sodium	48	-	-
	Ellagic acid ^d	50	-	-
	Aurin tricarboxylic acid	14	-	-
	Nimesulide	28	-	-
	Suramin hexasodium	41	-	-

^a Inhibition of product formation was determined by UPLC with 200 μ M of compound.

^b Inhibition of product formation at 200 μ M compound concentration > inhibition at 20 μ M compound concentration.

^c The K_D curve of kaempferol (30 nM ADP-TAMRA) plateaued at around 50 % anisotropy. Similar behavior was observed on the IC_{50} curve, that plateaued at around 70 % enzyme activity.

^d Due to low solubility, inhibition assays were performed with 6 and 12 μ M ellagic acid with 2% DMSO.

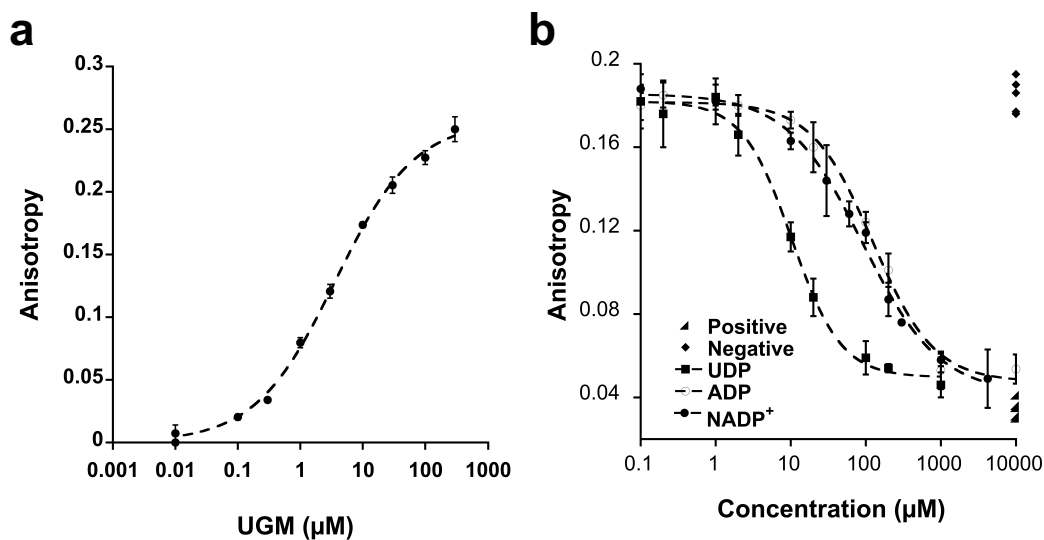


Fig. S1. (a) Binding isotherm of ADP-TAMRA (30 nM) to UGM. (b) Competitive binding of UDP, ADP, and NADP^+ . The K_D values are $10 \pm 1 \mu\text{M}$, $124 \pm 16 \mu\text{M}$, and $92 \pm 13 \mu\text{M}$, respectively. Negative control samples consisted of UGM and ADP-TAMRA (30 nM) with no other ligand (complex with high anisotropy value). The positive control contained only ADP-TAMRA (free chromophore with low anisotropy value).

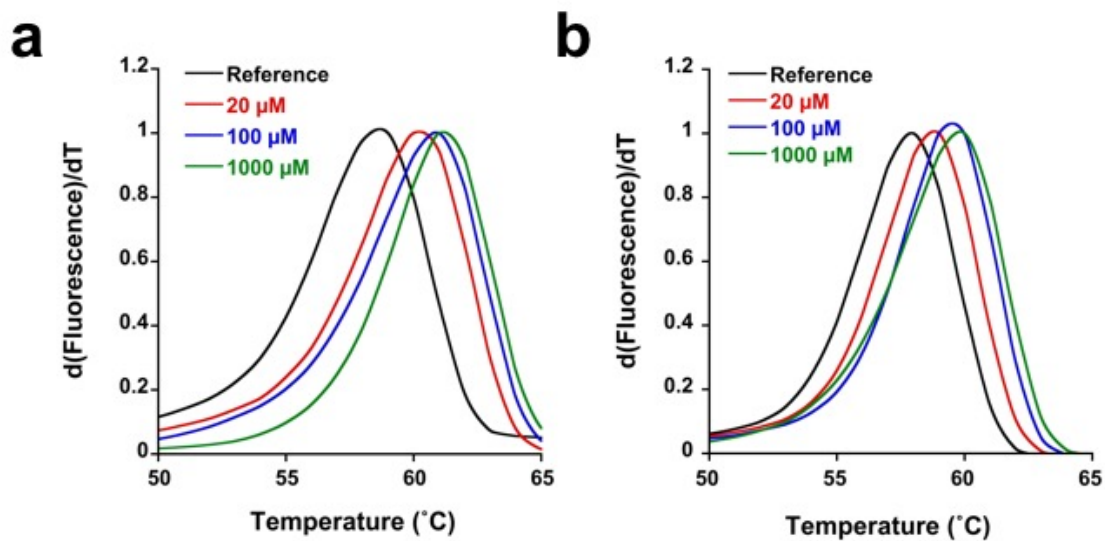


Fig. S2. Melting curves obtained with the ThermoFAD method for UGM at different concentrations of **(a)** (2*S*)-hesperetin and **(b)** (2*S*)-naringenin. As a reference, UGM with 2% DMSO was used.

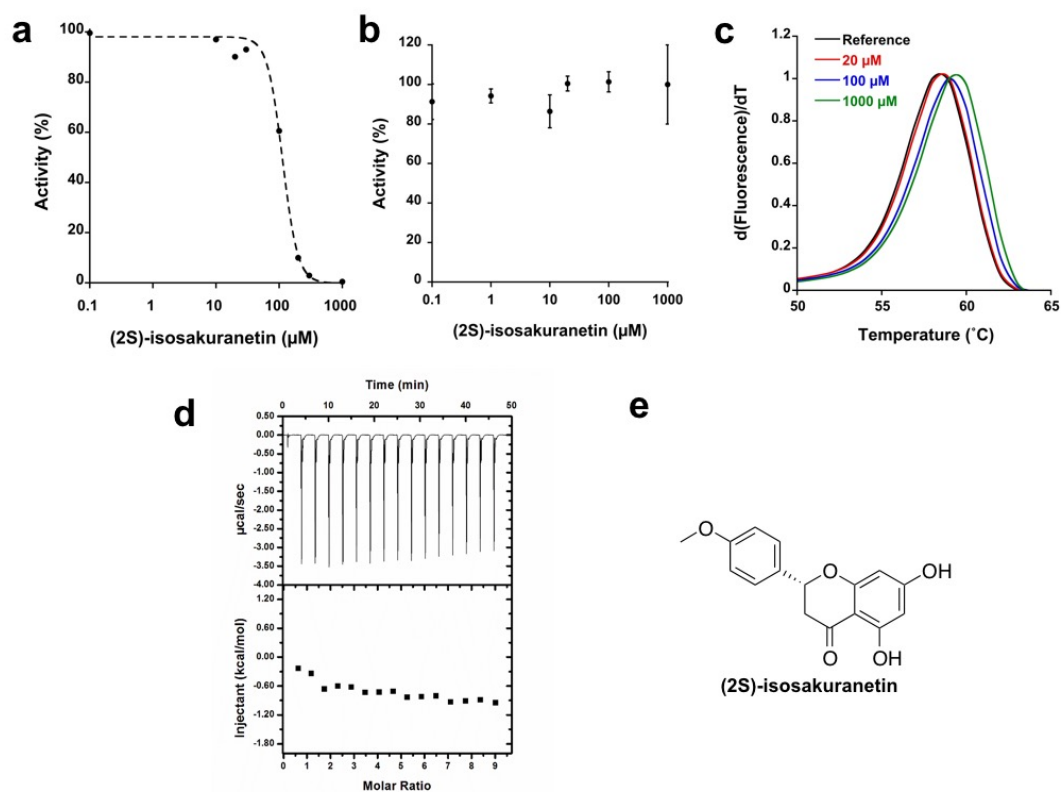


Fig. S3. Characterization of (2*S*)-isosakuranetin. **(a)** IC₅₀ curve obtained using the UPLC activity assay. **(b)** NADPH (250 μM) oxidation by UGM at various concentrations of (2*S*)-isosakuranetin. **(c)** Melting curves of UGM in the presence of 20, 100, and 1000 μM (2*S*)-isosakuranetin and 2 % DMSO. **(d)** ITC results; no binding of (2*S*)-isosakuranetin was observed. **(e)** Structure of (2*S*)-isosakuranetin.

Time	Reference	(2S)-hesperetin		(2S)-naringenin	
		600μM	1000 μM	600μM	1000 μM
24 h					
30 h					
48 h					

Fig. S4. *A. fumigatus* in PDA medium grown at 37 °C in the presence of 600 and 1000 μM (2S)-hesperetin or (2S)-naringenin. These experiments show that under optimal growth conditions these compounds are not toxic.

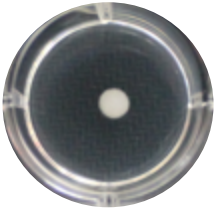
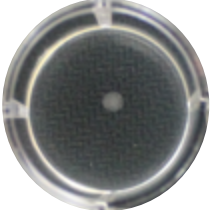
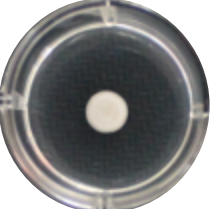
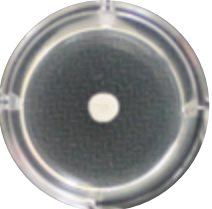
Spores	Reference	(2S)-hesperetin	(2S)-naringenin
1×10^3			
1×10^5			

Fig. S5. Cultures of *A. fumigatus* in PDA medium containing 1000 μ M (2S)-hesperetin or (2S)-naringenin. Agar was spot inoculated with 1×10^3 or 1×10^5 spores and incubated at 50 °C for 48 h. The reference consisted of PDA with DMSO.