

Supplemental data

Manuscript title: 3D-QSAR studies on UGT1A9-mediated 3-O-glucuronidation of natural flavonols using a Pharmacophore based CoMFA model

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Journal name: Journal of Pharmacology and Experimental Therapeutics

Table S1. The conversion factors (K) are showed at detecting wavelengths for flavonol 3-O-glucuronides.

Compd	Name	K	λ , nm
1	3,2'-dihydroxyflavone	1.27 ± 0.08	310
2	3,3',4'-trihydroxyflavone	0.91 ± 0.05	345
3	3,4'-dihydroxyflavone	1.02 ± 0.03	254
4	3,6,4'-trihydroxyflavone	1.15 ± 0.10	254
5	3,6-dihydroxyflavone	1.22 ± 0.18	315
6	3,7-dihydroxyflavone	1.29 ± 0.03	254
7	3-hydroxy-2'-methoxyflavone	1.25 ± 0.11	320
8	3-hydroxy-3'-methoxyflavone	1.08 ± 0.09	320
9	3-hydroxy-4'-methoxyflavone	0.87 ± 0.10	254
10	3-hydroxy-5,7-dimethoxyflavone	1.05 ± 0.13	254
11	3-hydroxy-5-methoxyflavone	0.92 ± 0.23	254
12	3-hydroxy-6,4'-dimethoxyflavone	1.20 ± 0.44	254
13	3-hydroxy-6-methoxyflavone	1.05 ± 0.06	254
14	3-hydroxy-7-methoxyflavone	0.82 ± 0.19	254
15	3-hydroxyflavone	0.96 ± 0.06	254
16	3-hydroxy-6-methyl-3',4'-methylenedioxyflavone	1.06 ± 0.14	360
17	Isorhamnetin	1.28 ± 0.24	354
18	Kaempferol	1.00 ± 0.06	354
19	Morin	0.91 ± 0.08	360
20	Myricetin	1.16 ± 0.04	370
21	Quercetin	1.13 ± 0.16	360
22	Resokaempferol	1.10 ± 0.24	354
23	Rhamnetin	1.08 ± 0.16	370
24	3,3'-dihydroxyflavone	0.90 ± 0.05	320
25	3,5-dihydroxyflavone	1.38 ± 0.03	263
26	3-hydroxy-2',3'-dimethoxyflavone	1.18 ± 0.15	320
27	3-hydroxy-6-methylflavone	0.93 ± 0.03	310
28	3-hydroxy-7,4'-dimethoxyflavone	0.91 ± 0.13	254
29	Fisetin	1.14 ± 0.07	360
30	Galangin	0.71 ± 0.15	354

Figure S1. Characterization on the recombinant UGT1A9 using the probe substrate “propofol”. Panel A: kinetics profile of UGT1A9-mediated glucuronidation with propofol. The kinetics curve was well described by Michaelis-Menten equation with $K_m = 25.8 \pm 4.47 \mu\text{M}$ and $V_{\text{max}} = 148 \pm 7.03 \text{ pmol/mg/min}$. Panel B: the UPLC chromatogram at 273 nm (left) and UV spectra (right) of propofol and its glucuronide. The UPLC sample was generated by incubating propofol at $100 \mu\text{M}$ with UGT1A9 Supersomes ($26.5 \mu\text{g/ml}$) for 60 mins.

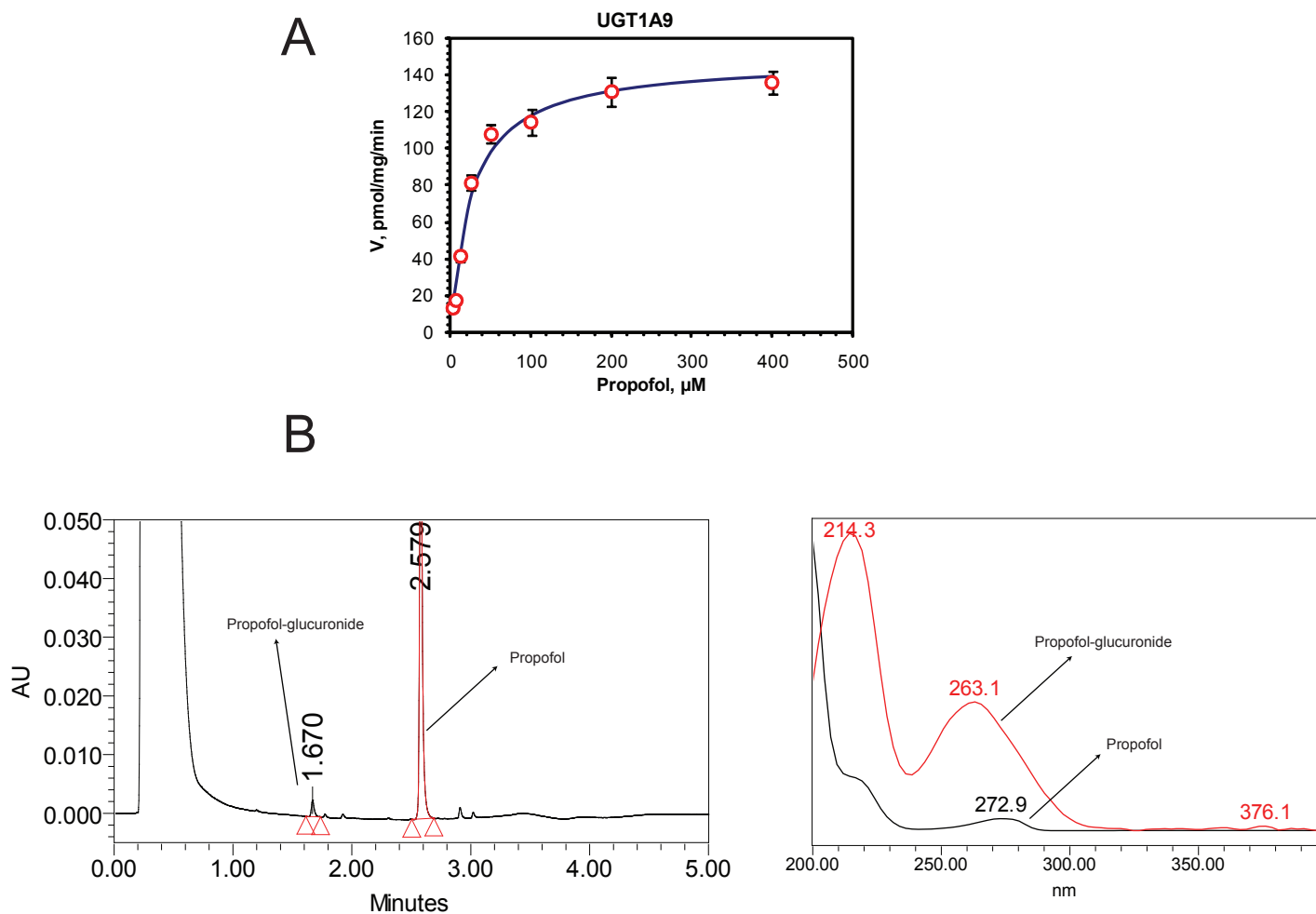


Figure S2. The UPLC chromatograms (left panel) and UV spectra (right panel) of multi-hydroxyl flavonols and their 3-O-glucuronides. The samples were generated by incubating the flavonols at 10 μ M with UGT1A9 (26.5 μ g/ml) for 60 mins. Comparing to the parent compounds, band I of 3-O-glucuronides either disappeared or had large hypsochromic shifts (to shorter wavelengths) (i.e., 14.9~26.7 nm).

Figure S2.1. compound 1 (3,2'-dihydroxyflavone)

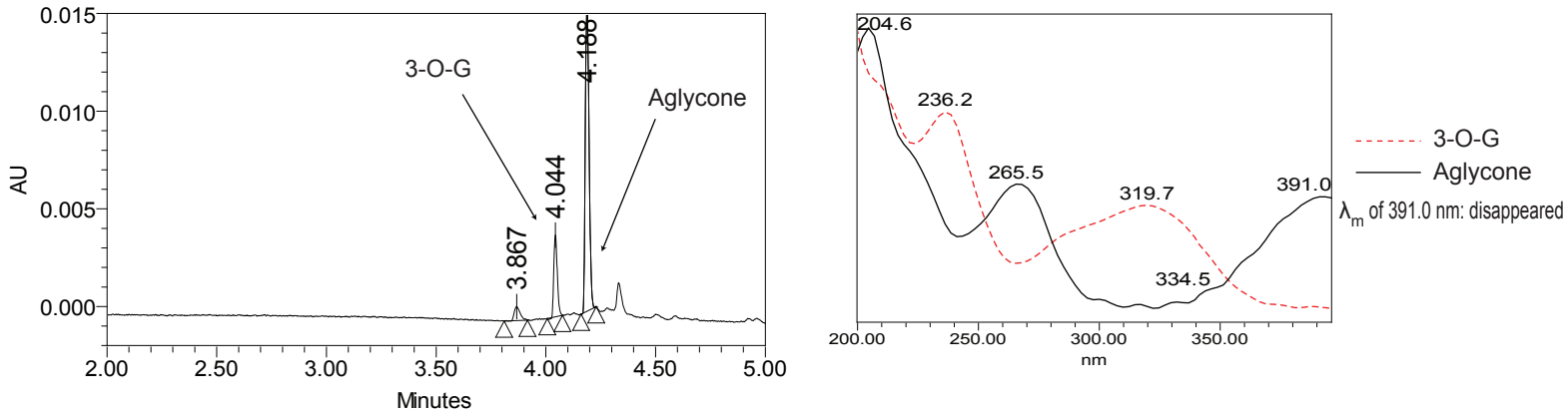


Figure S2.2. compound 2 (3,3',4'-trihydroxyflavone)

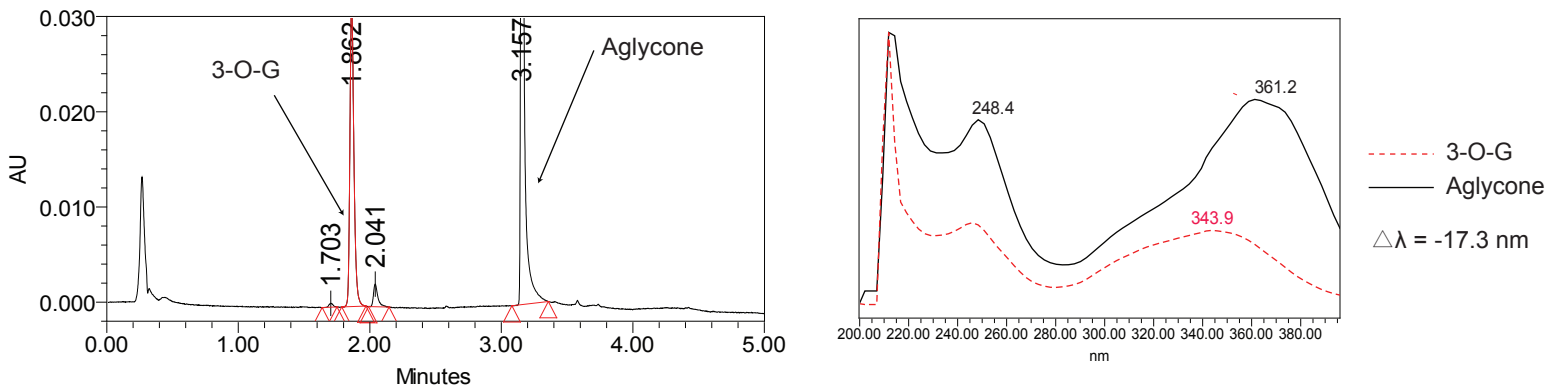


Figure S2.3. compound 3 (3,4'-dihydroxyflavone)

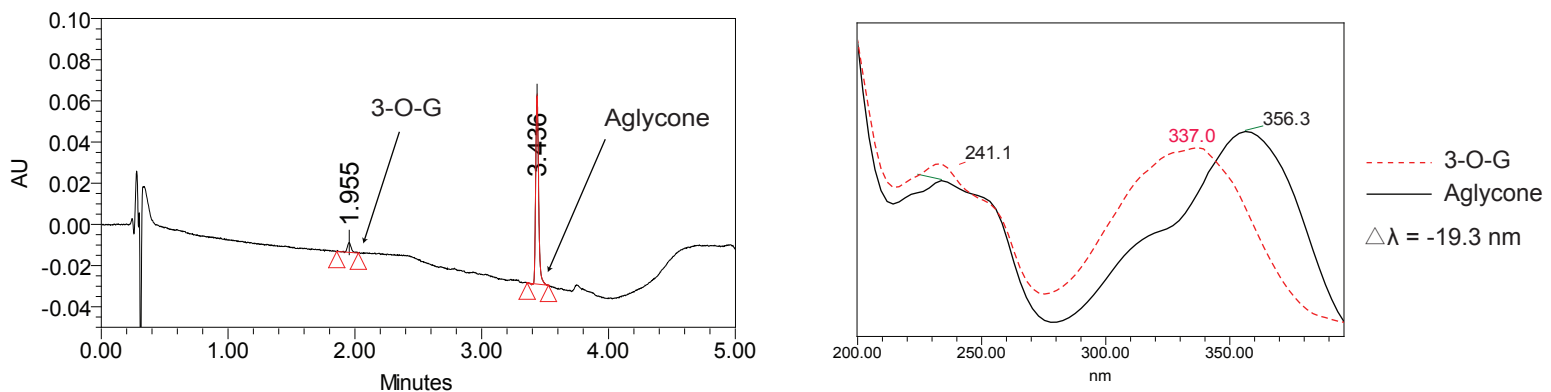


Figure S2.4. compound 4 (3,6,4'-trihydroxyflavone)

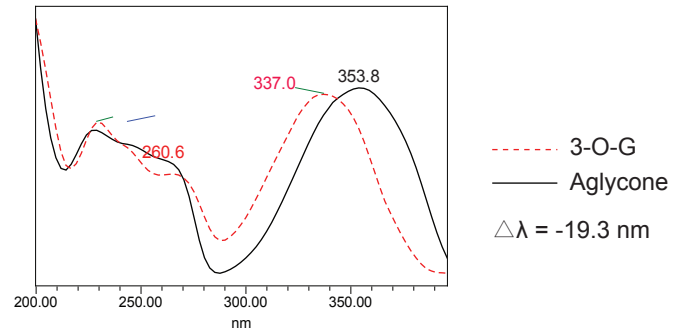
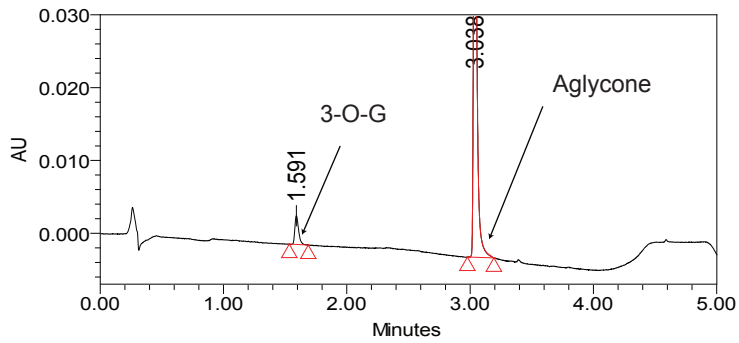


Figure S2.5. compound 5 (3,6-dihydroxyflavone)

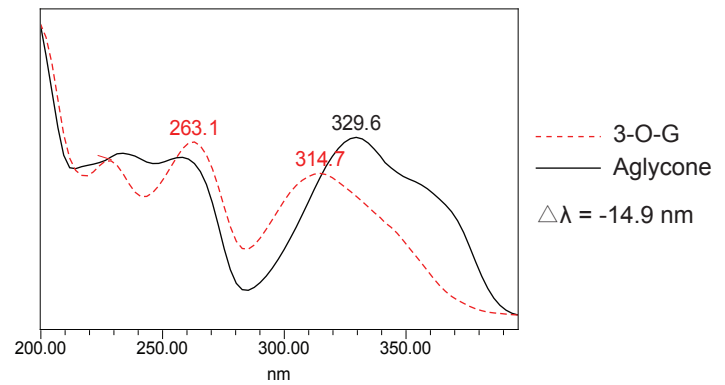
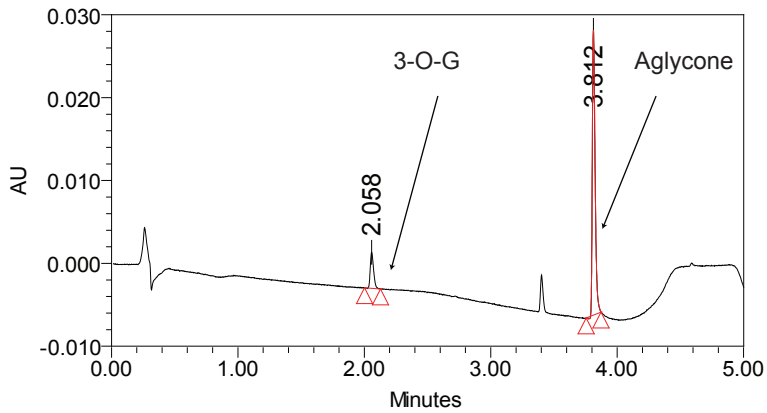


Figure S2.6. compound 6 (3,7-dihydroxyflavone)

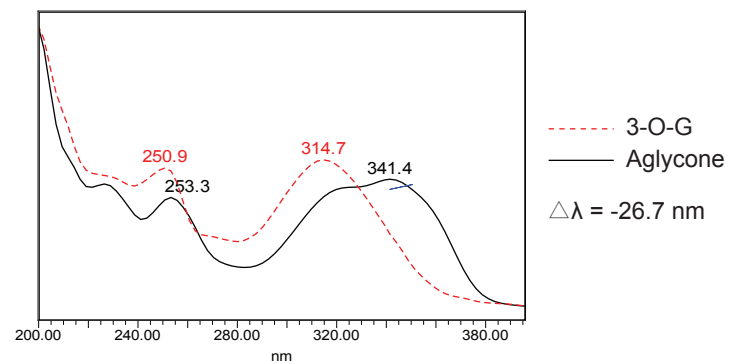
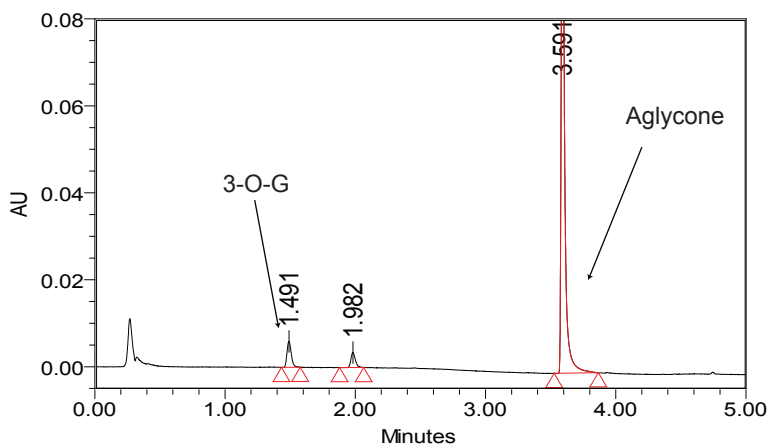


Figure S2.7. compound 17 (isorhamnetin)

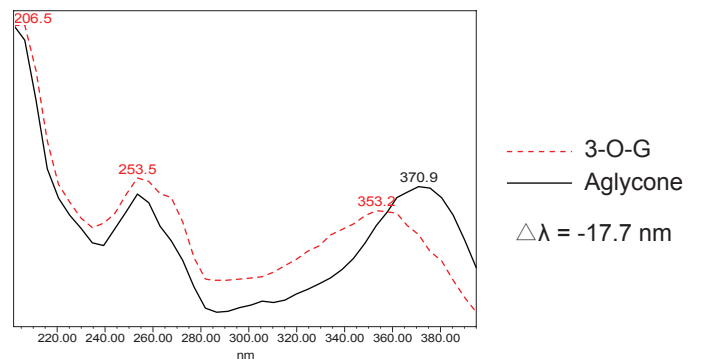
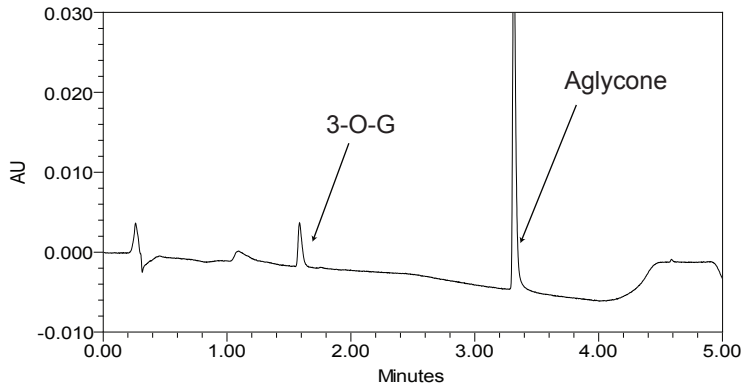


Figure S2.8. compound 18 (kaempferol)

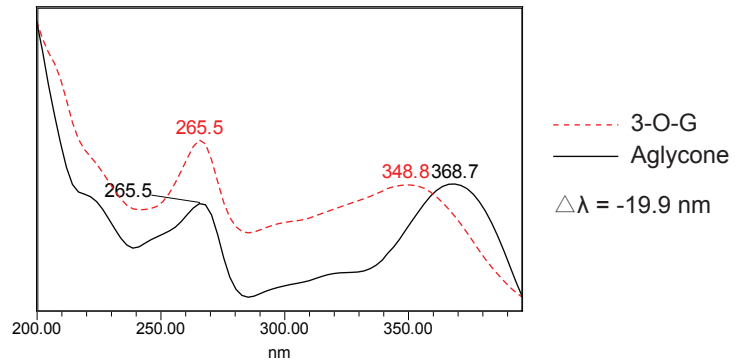
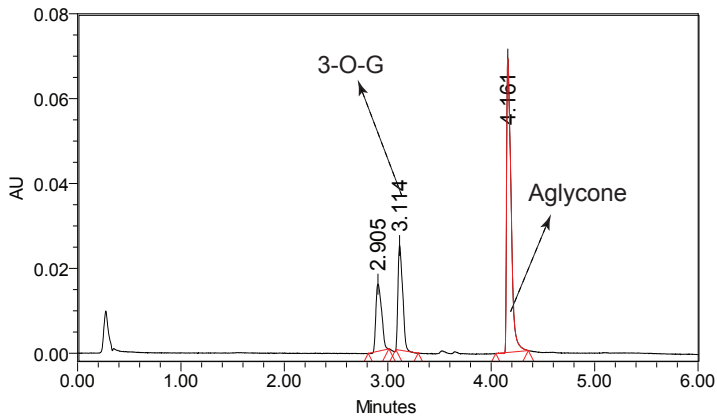


Figure S2.9. compound 19 (morin)

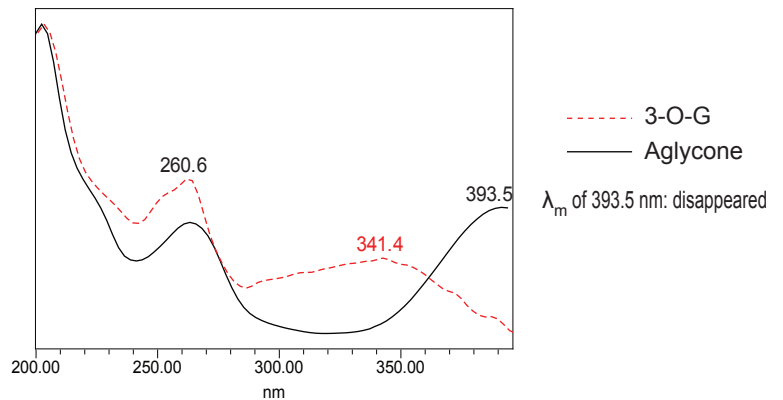
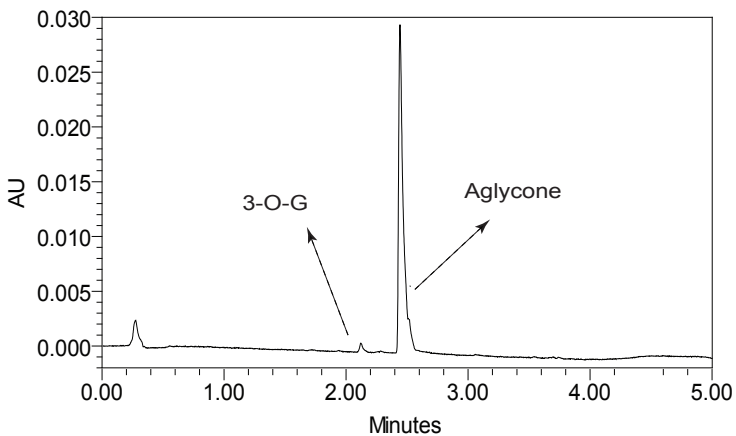


Figure S2.10. compound 20 (Myricetin)

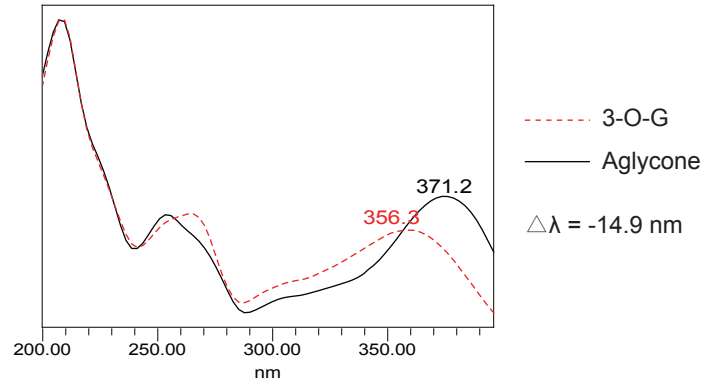
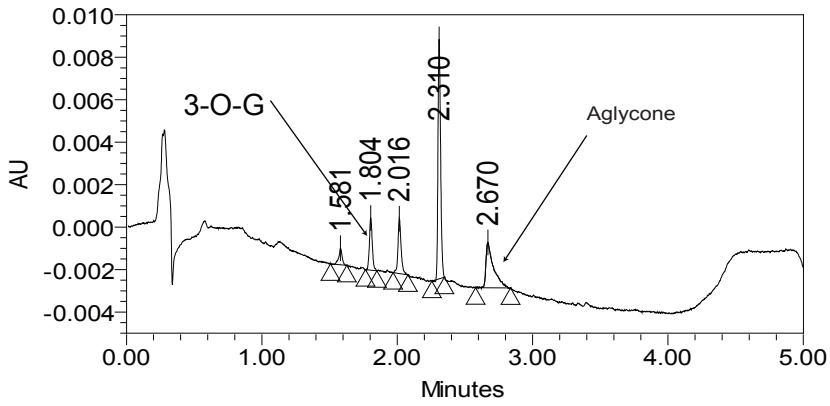


Figure S2.11. compound 21 (Quercetin)

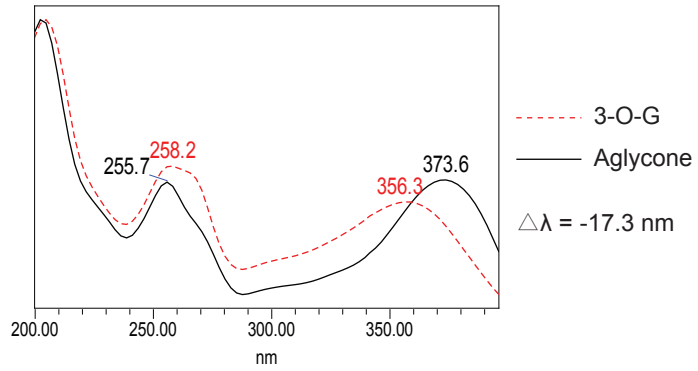
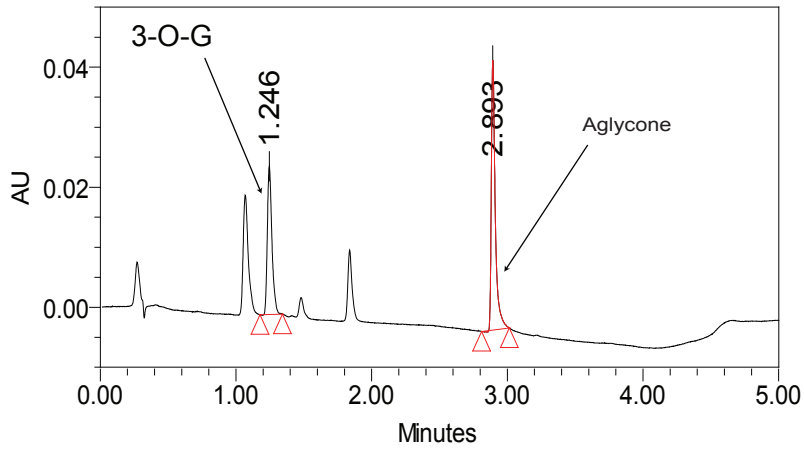


Figure S2.12. compound 22 (resokaempferol)

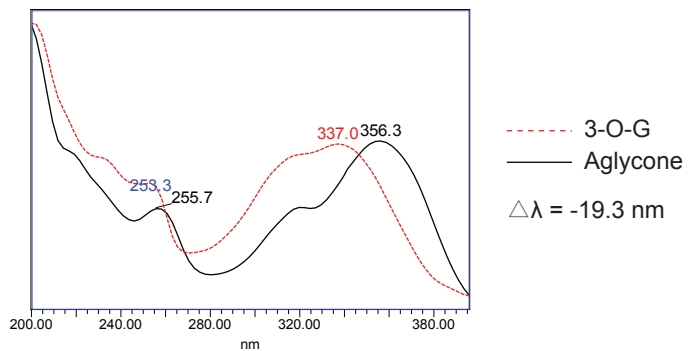
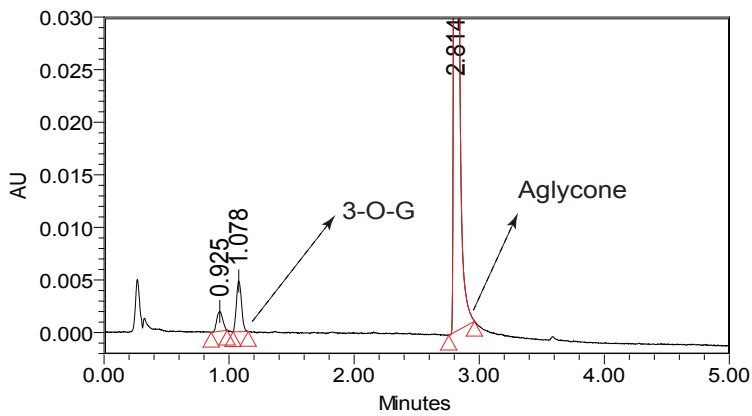


Figure S2.13. compound 23 (rhamnetin)

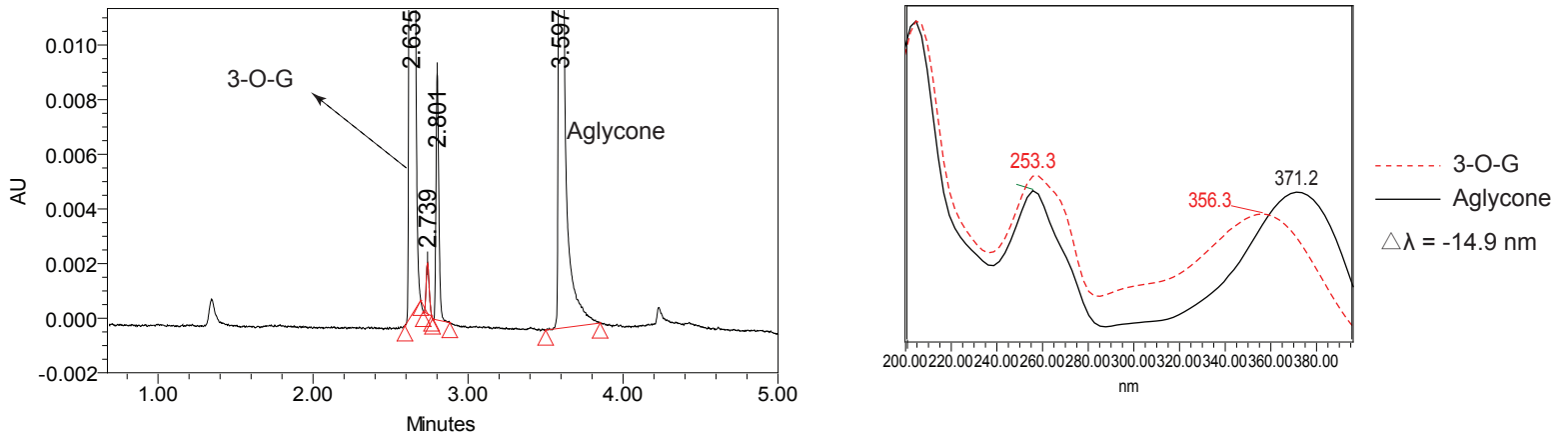


Figure S2.14. compound 24 (3,3'-dihydroxyflavone)

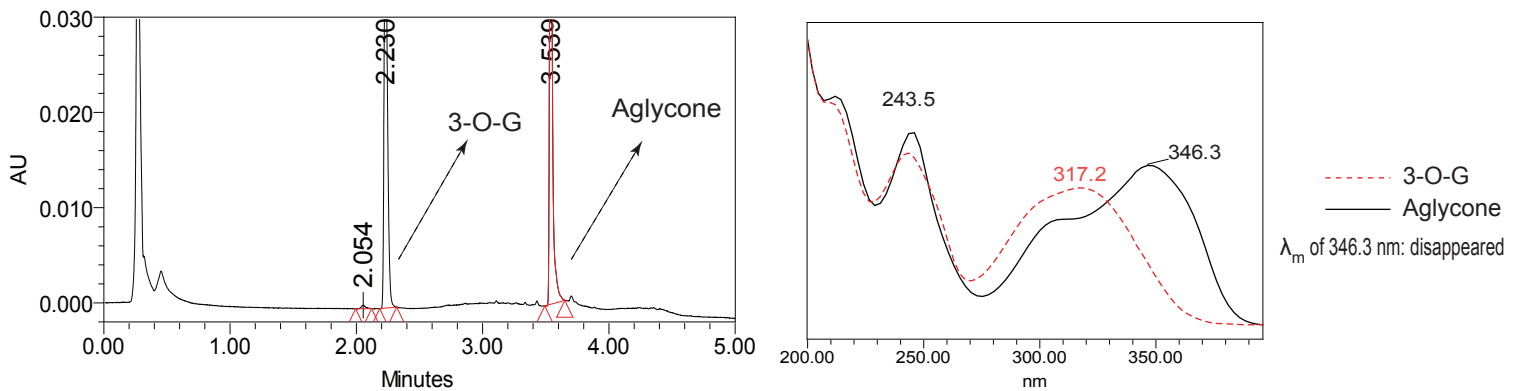


Figure S2.15. compound 25 (3,5-dihydroxyflavone)

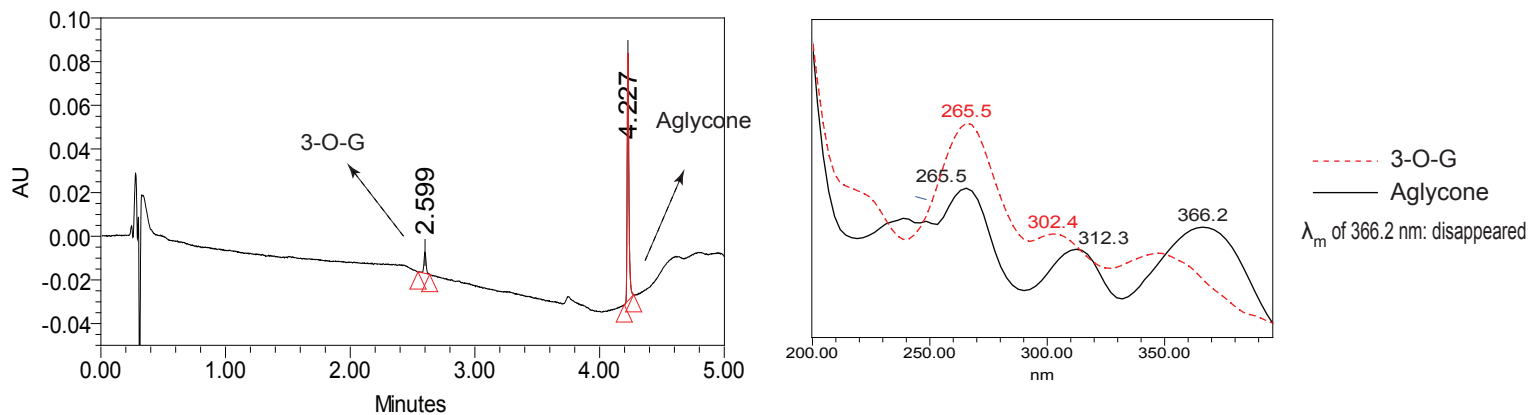


Figure S2.16. compound 29 (Fisetin)

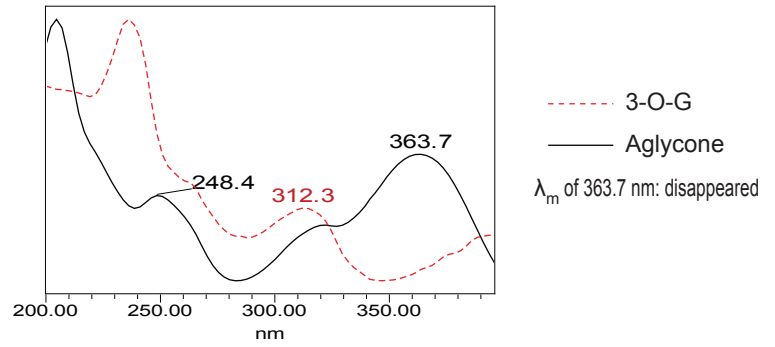
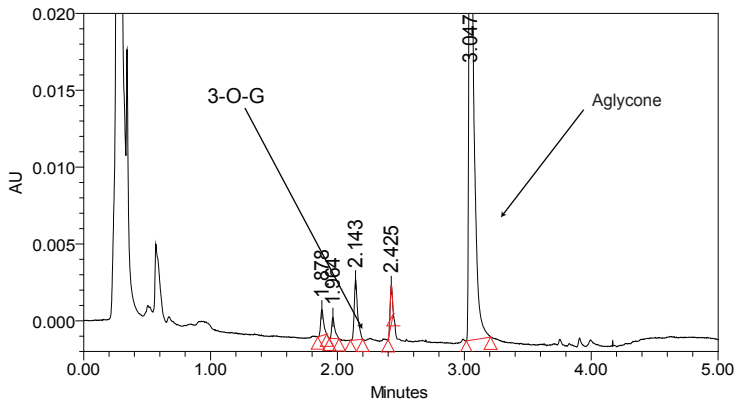


Figure S2.17. compound 30 (galangin)

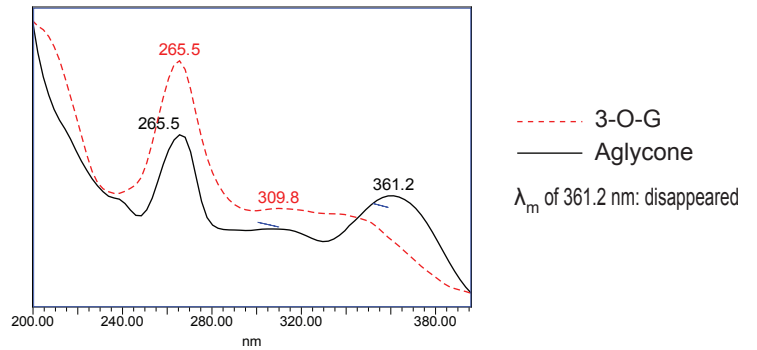
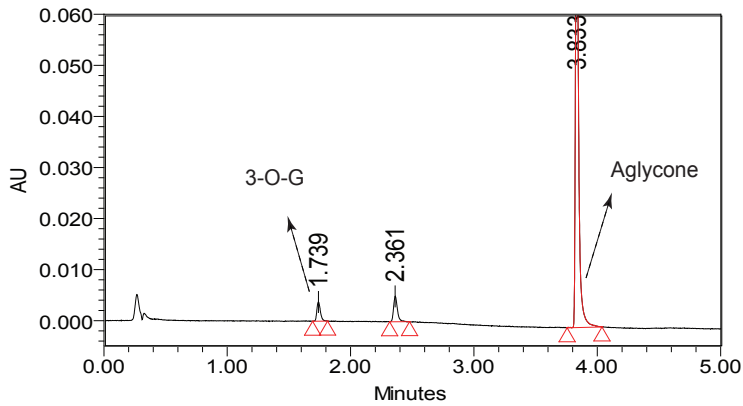
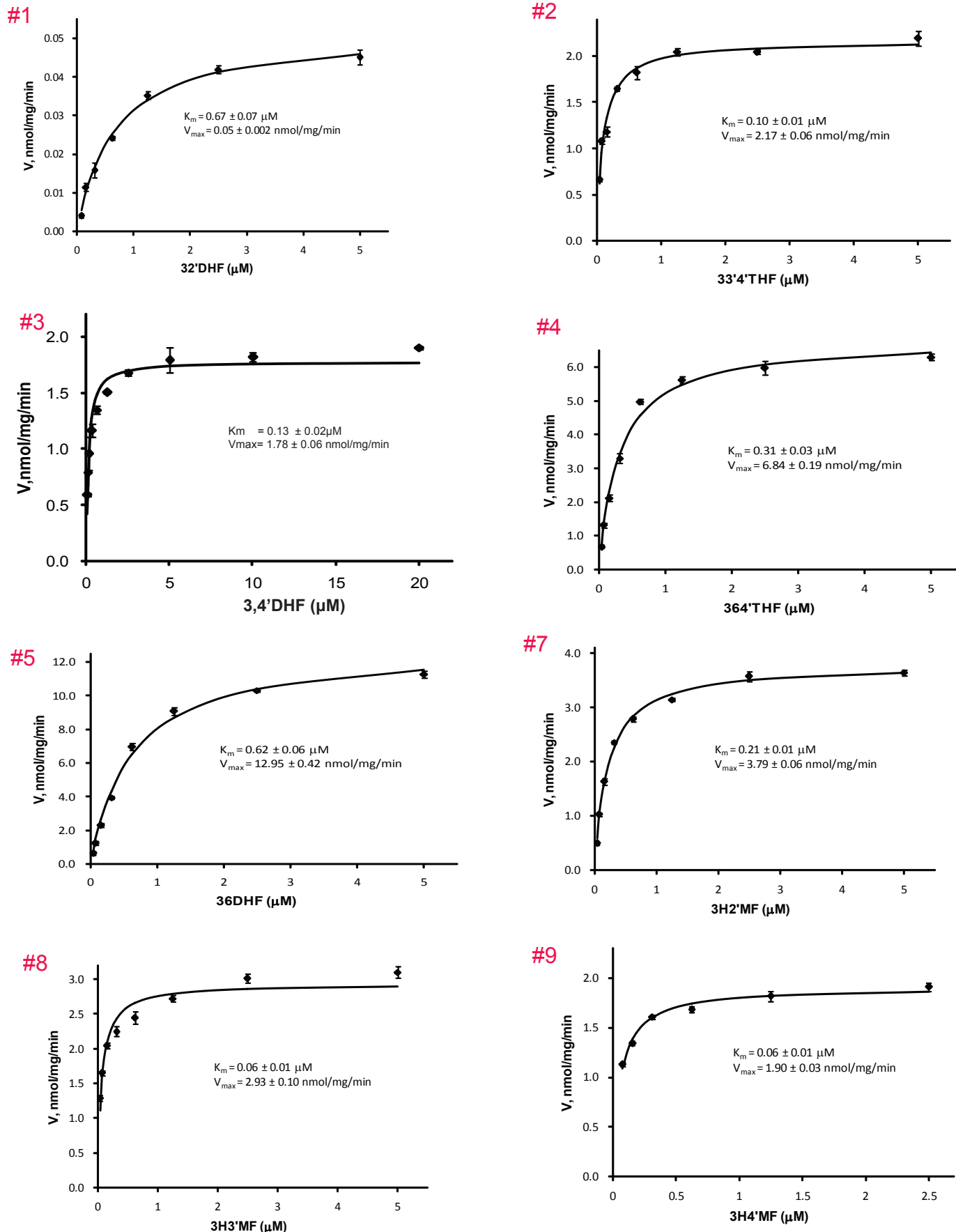
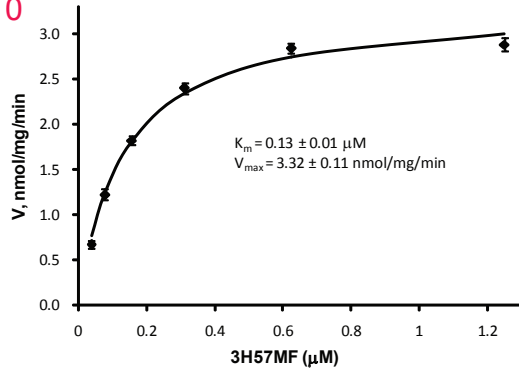


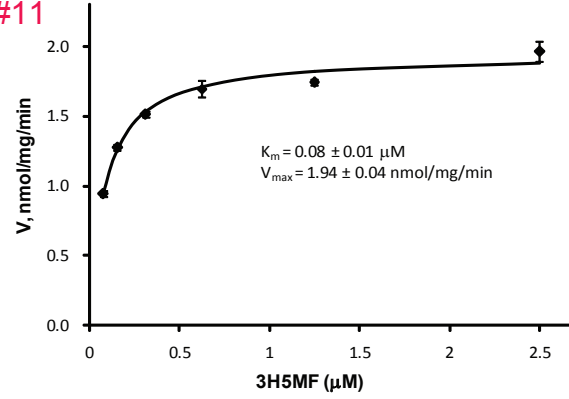
Figure.S3 Kinetics profiles of UGT1A9 with 26 flavonols (3-O-glucuronidation alone). K_m and V_{max} or K_s values were derived by fitting the data to Michaelis-Menten or substrate inhibition equations (Luukkanen et al., 2005). The kinetics profiles of additional 4 flavonols (#6, 37DHF; #18, 3574'QHF; #22, 374'THF; #30, 357THF) were summarized somewhere else (Wu and Hu, in preparation). The graphs are ordered by compound number (#) that corresponds the flavonols numbering in Table 1 or Table 2.



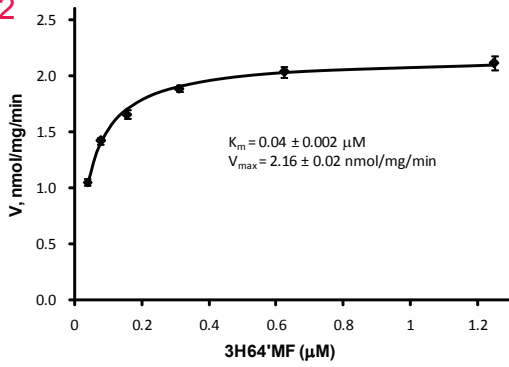
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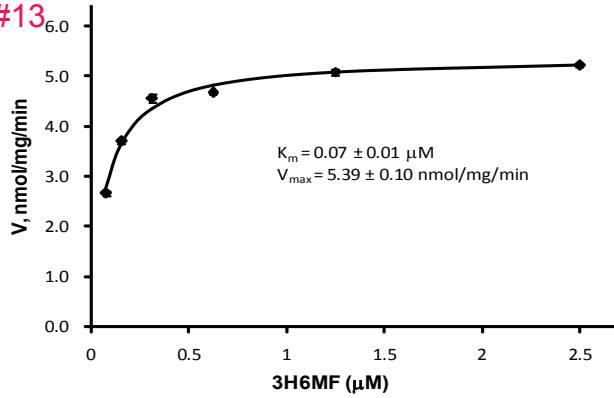
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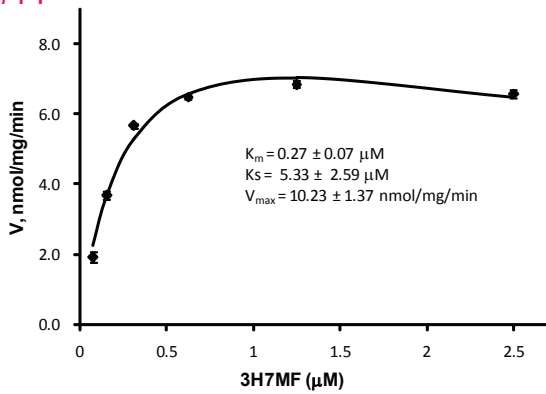
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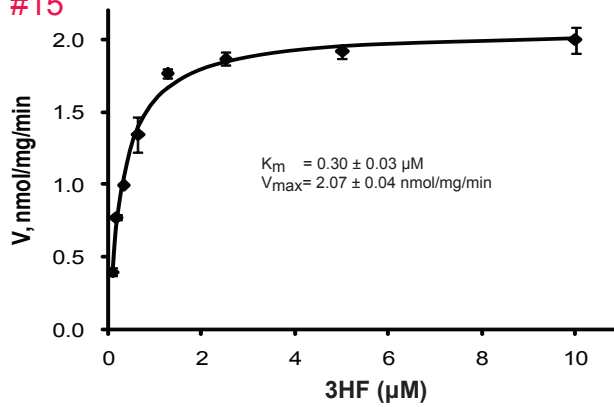
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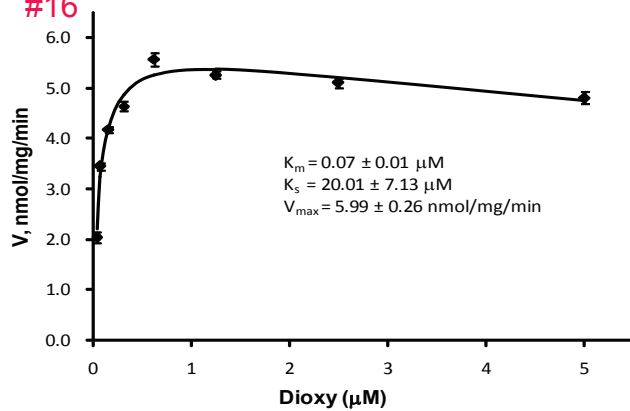
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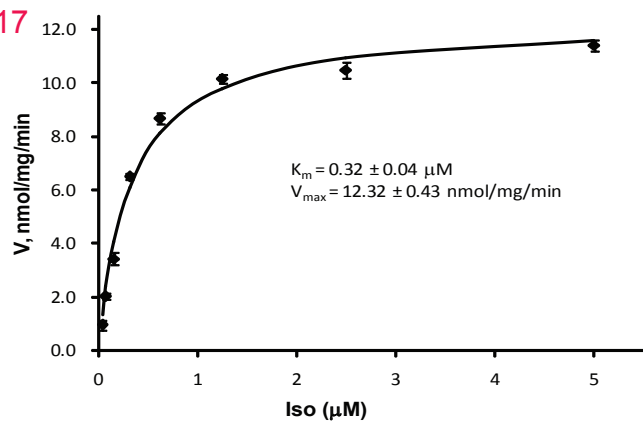
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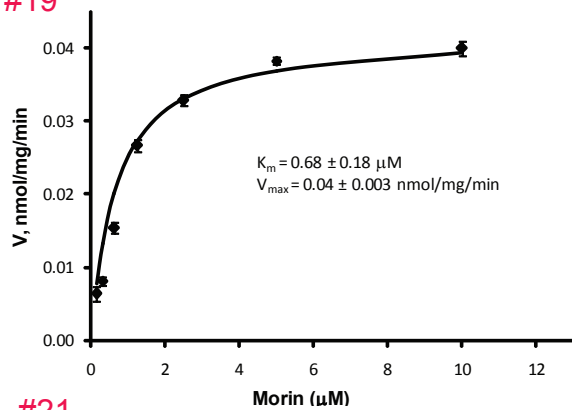
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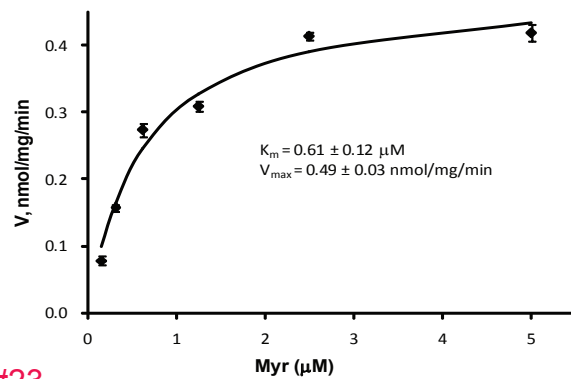
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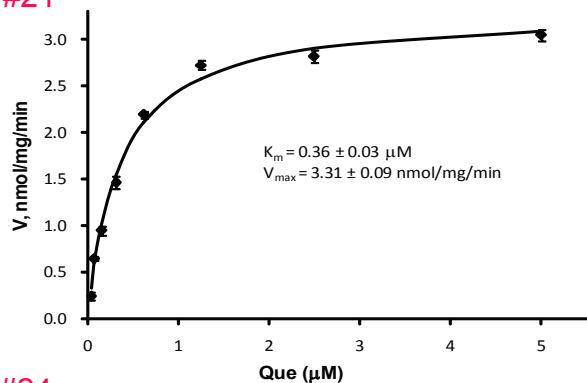
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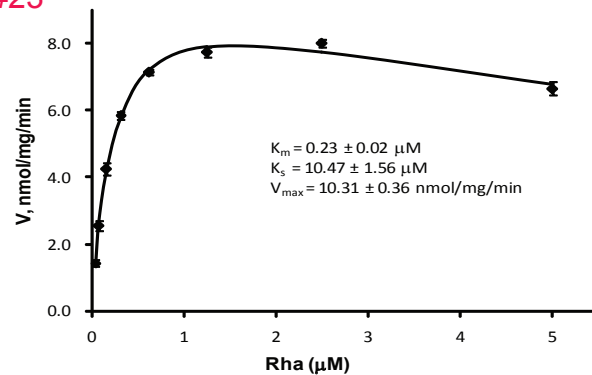
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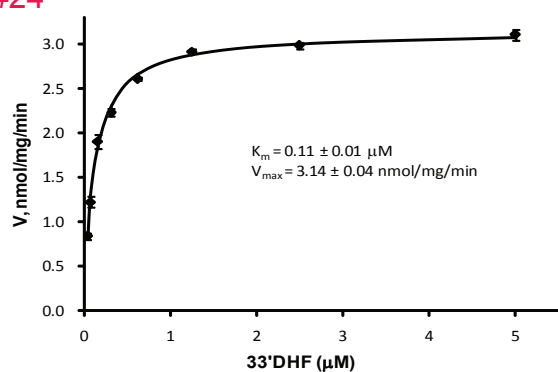
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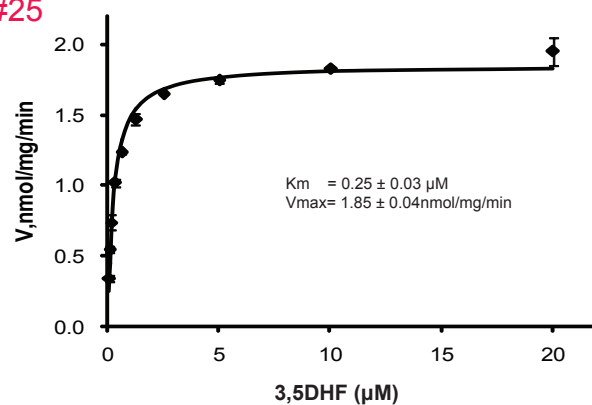
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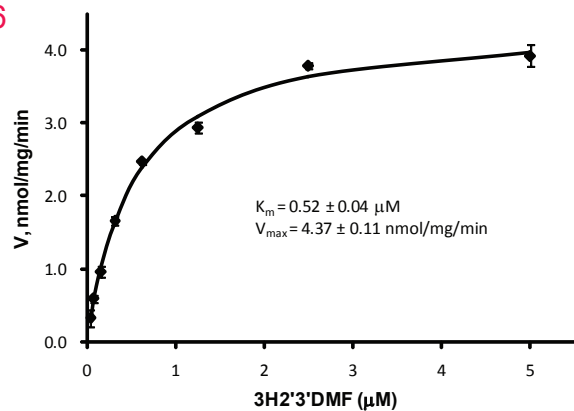
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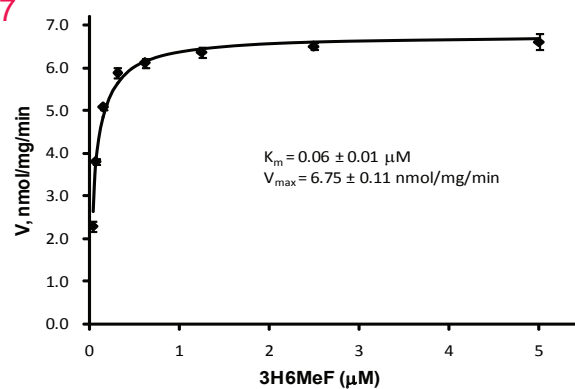
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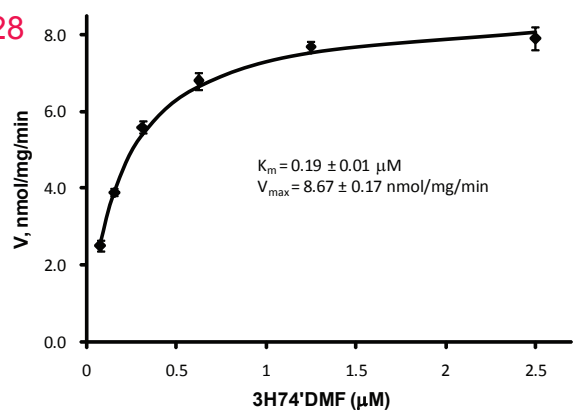
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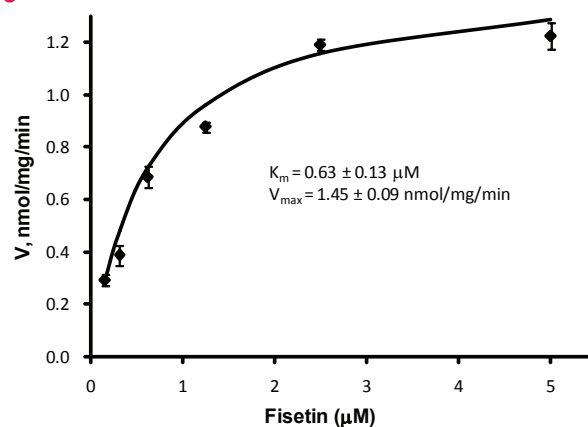
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References:

Luukkanen L, Taskinen J, Kurkela M, Kostainen R, Hirvonen J and Finel M (2005) Kinetic characterization of the 1A subfamily of recombinant human UDP-glucuronosyltransferases. *Drug Metab Dispos* 33(7):1017-1026.