Electronic Supplementary Information

Ep7GT, a glycosyltransferase with sugar donor flexibility from *Epimedium pseudowushanense*, catalyzes the 7-*O*-glycosylation of baohuoside

Keping Feng,[a, b, c] Ridao Chen,[b , c, d] Kebo Xie, [b, c, d] Dawei Chen, [b, c, d] Jimei Liu, [b, c, d] Wenyu Du, [b, c, d] Lin Yang [a, *] and Jungui Dai [b, c, d, *]

[a] *College of Life and Environmental Sciences, Minzu University of China, 27 Zhong Guan Cun Southern Street, Beijing 100081, China*

[b] *State Key Laboratory of Bioactive Substance and Function of Natural Medicines;* [c] *CAMS Key Laboratory of Enzyme and Biocatalysis of Natural Drugs;* [d] *NHC Key Laboratory of Biosynthesis of Natural Products, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 1 Xian Nong Tan Street, Beijing, 100050, China*

* Corresponding authors.

Tel: +86 10 68936927; fax: +86 10 68936927. E-mail: 15116995486@163.com (L. Y.).

Tel: +86 10 63165195; fax: +86 10 63017757. E-mail: jgdai@imm.ac.cn (J. D.).

Table of Contents

Table S1. The HPLC method used in this study

Time (min)	Solvent A (%) 0.1% formic acid	Solvent B (%) CH_3OH	flow rate (mL min^{-1})
0.00	90.0	10.0	1.0
10.00	50.0	50.0	1.0
30.00	0.0	100.0	1.0
45.00	0.0	100.0	1.0

Table S2. Details of the plasmids and strains used in this study

plasmids or *E. coli* strain	relevant properties
	Plasmids
pET-28a	pBR322 ori, Kan^r
	Primers
pE-7GT forward	CAAATGGGTCGCGGATCCATGGGTTCAGAAACTCAT
pE-7GT reverse	GTGGTGGTGGTGCTCGAGTCAGTGTCCAATAGTTGCAGC
	Strains
S0	Transetta (DE3) harboring empty pET-28a
S1	Transetta (DE3) harboring pE-Ep7GT

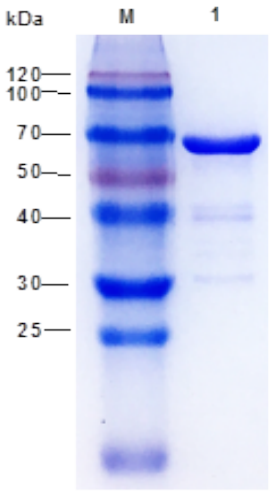

Figure S1. SDS-PAGE of recombinant His_6-Ep7GT purified by affinity chromatography. Lane M: Protein Marker; Lane 1: His-tagged Ep7GT (predicted M.W., 54.1 kDa) purified on Ni Sepharose column chromatography.

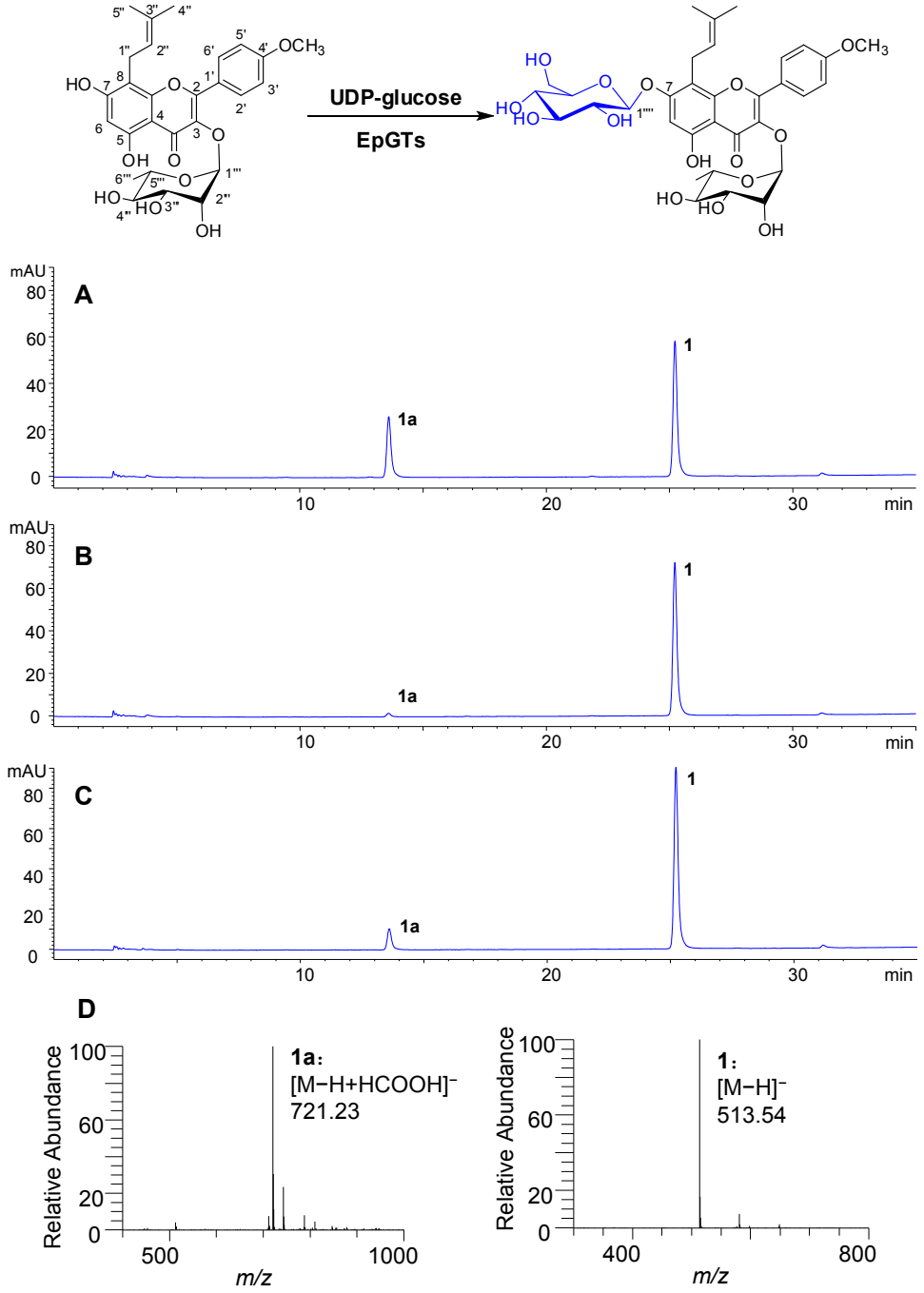

Figure S2. Enzymatic reactions catalyzed by crude extracts of recombinant EpGTs with baohuoside (**1**) as sugar accepter and UDP-glucose as sugar donor. A) EpGT8 (Ep7GT); B) EpGT2; C) EpGT4; D) MS spectra of **1a** and **1** at negative mode.

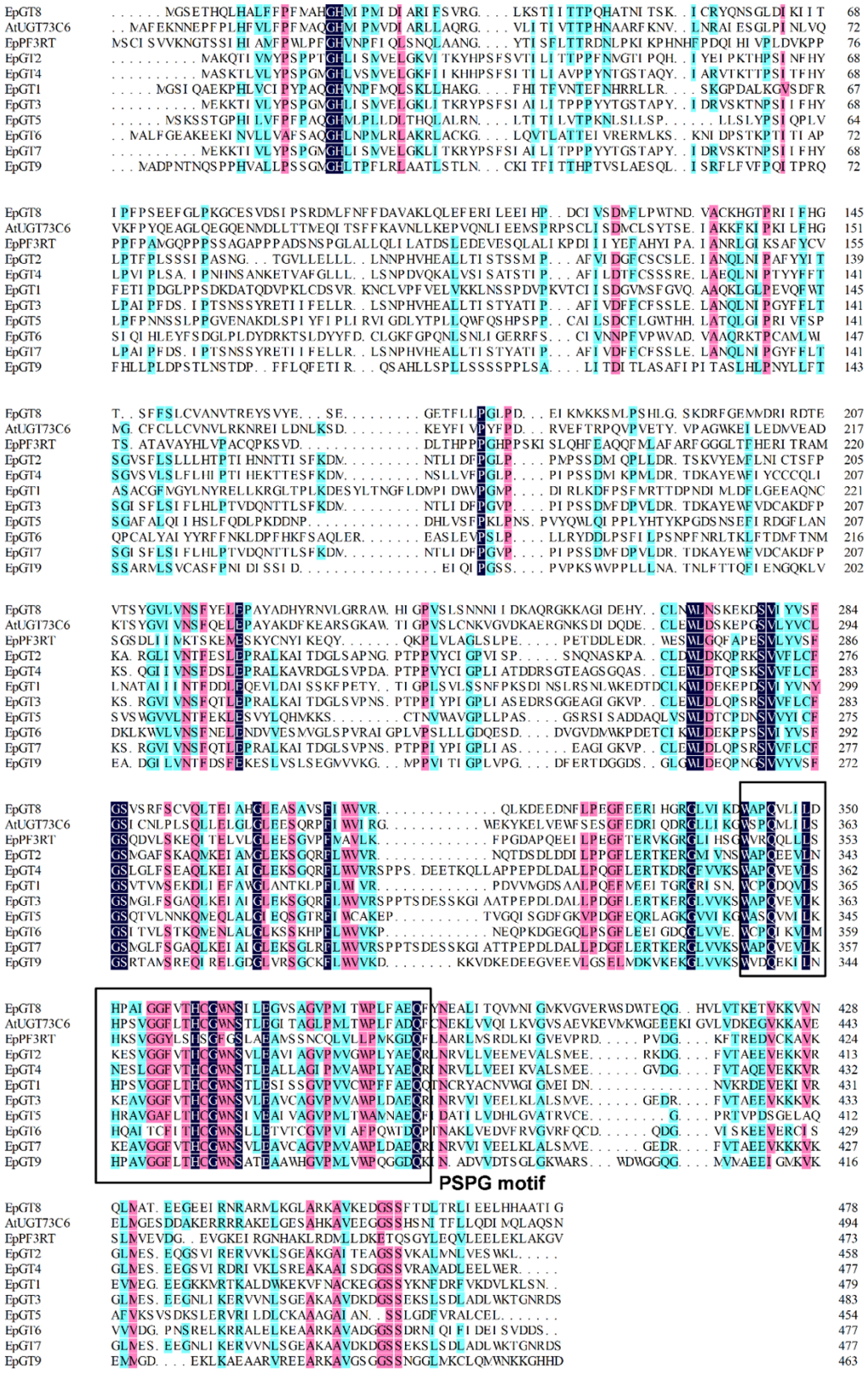

Figure S3. Multiple alignment of the amino acid sequences of EpGT8 (renamed Ep7GT), AtUGT78D1 (GenBank accession number NM_102790, from *Arabidopsis thaliana*) and EpPF3RT (GenBank accession number MG264429, from *E. pseudowushanense*) and other EpGTs.

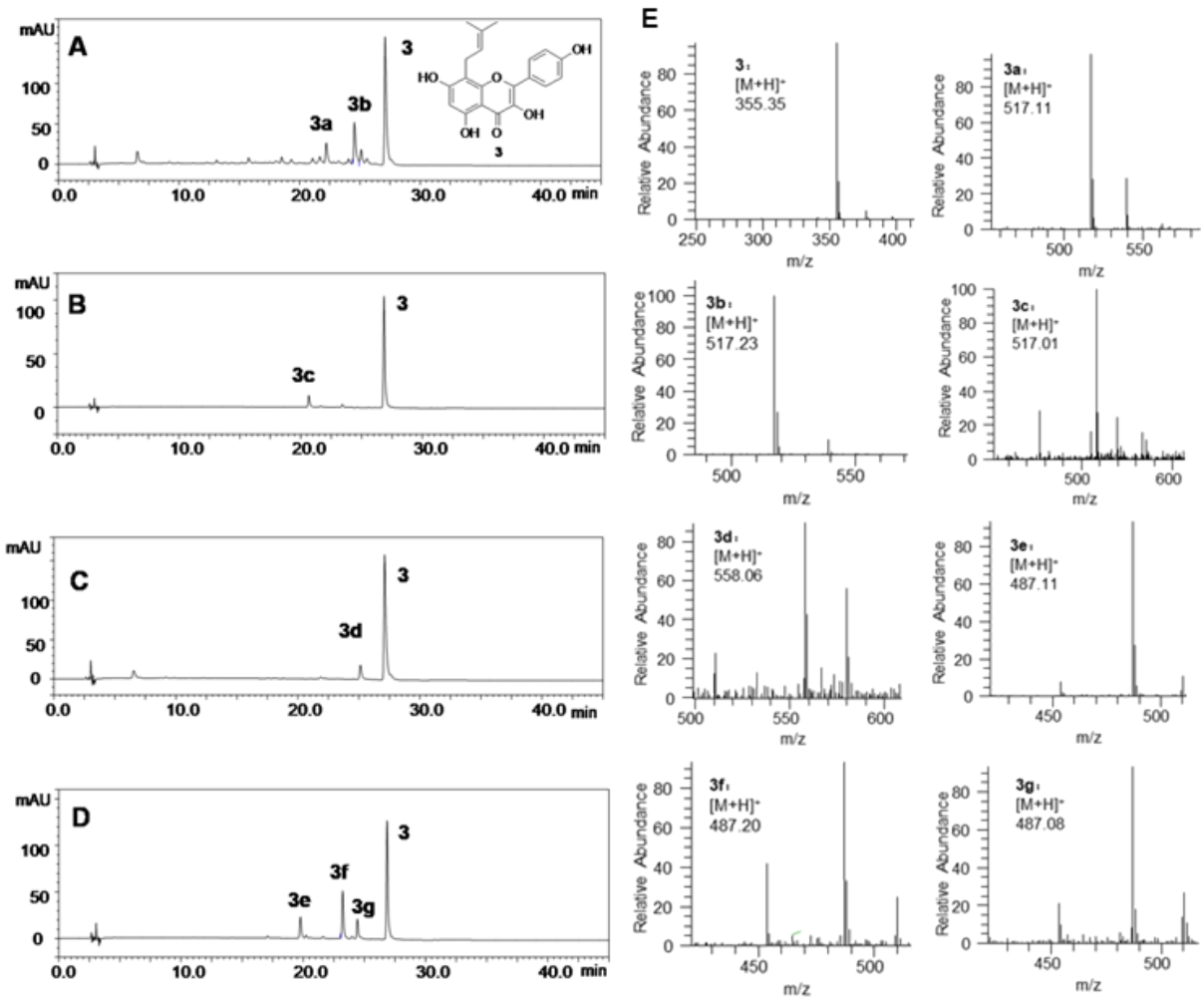

Figure S4. Ep7GT-catalyzed glycosylation of 8-prenylkaempferol (**3**) with different sugar donors. A) UDP-glucose; B) UDP-galactose; C) UDP-*N*-acetylglucosamine; D) UDP-xylose; E) MS spectra of **3** and the main products at positive mode.

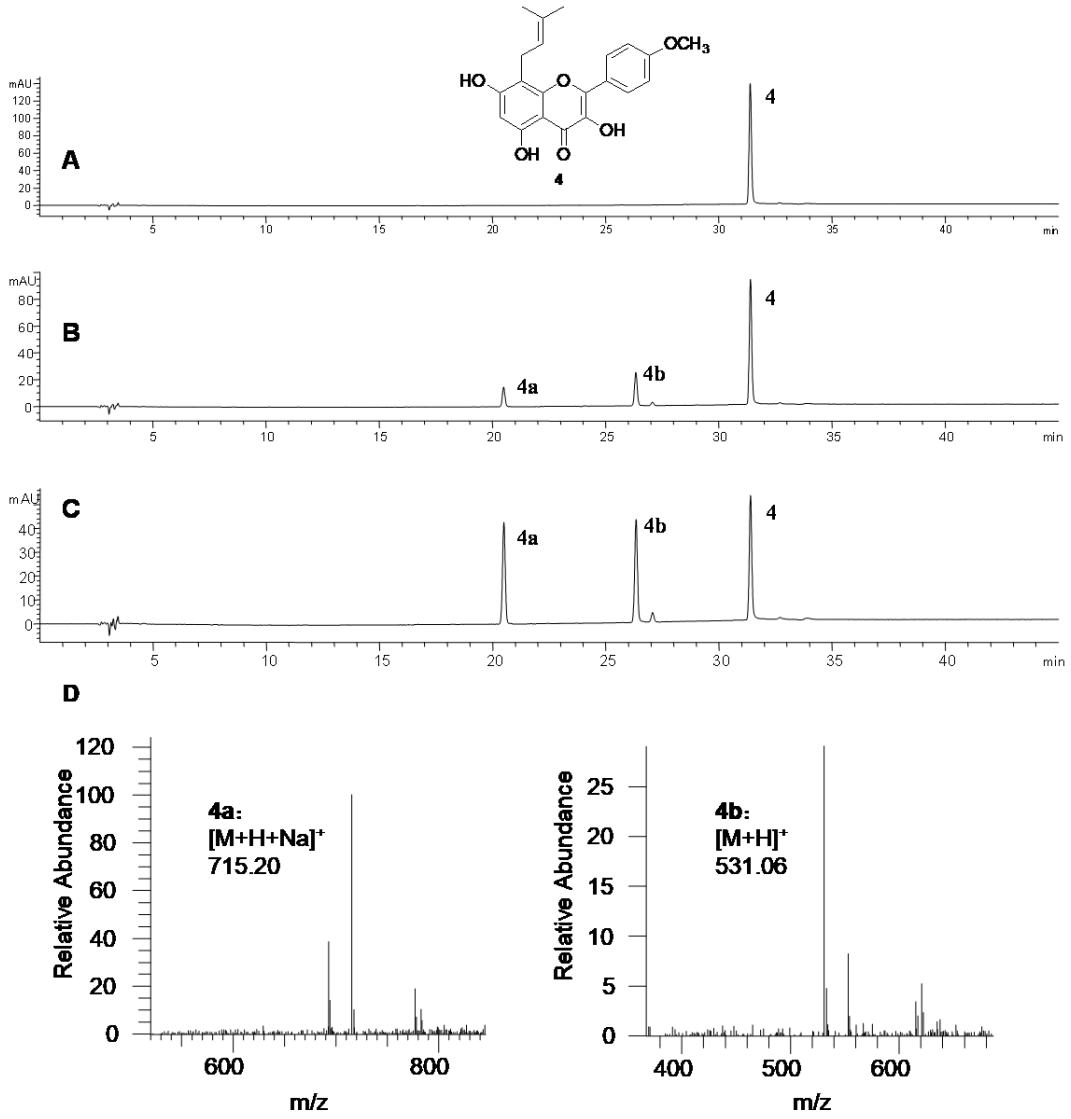

Figure S5. Ep7GT-catalyzed glycosylation of anhydroicaritin (**4**) with different sugar donors. A) Control group; B) UDP-glucose, C) TDP-glucose; D) MS spectra of the main products at positive mode.

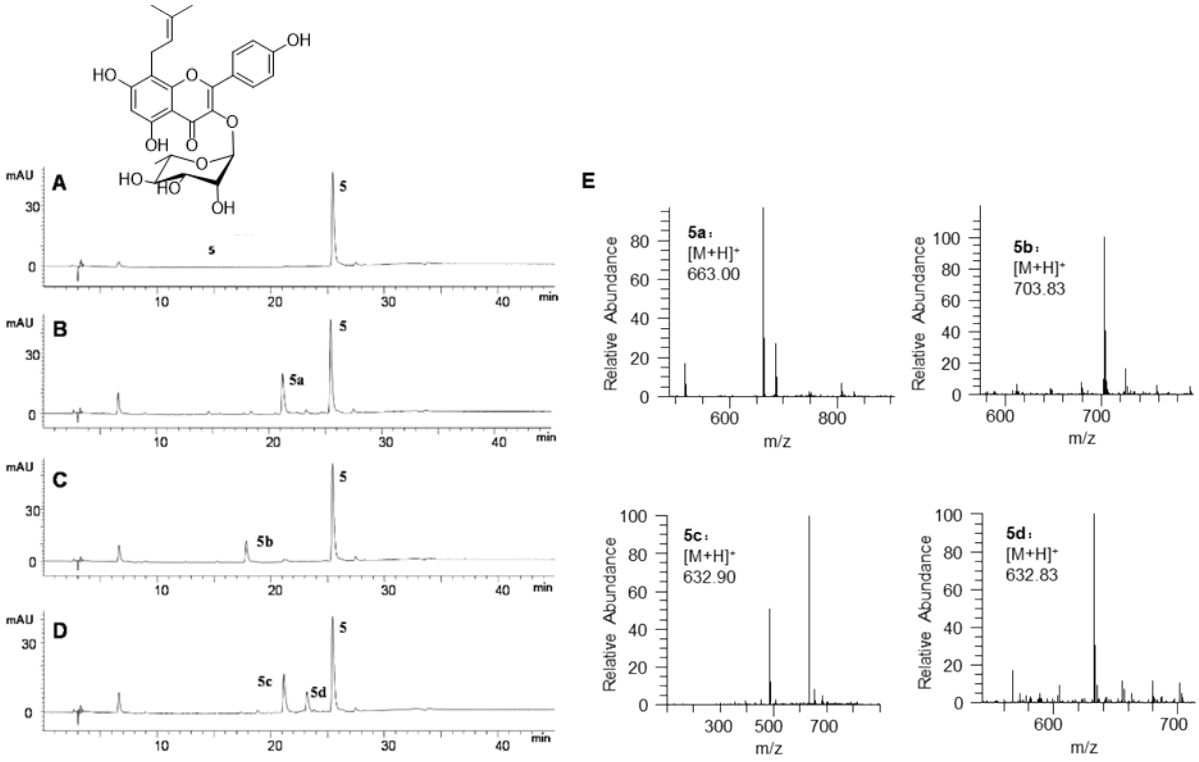

Figure S6. Ep7GT-catalyzed glycosylation of baohuoside II (**5**) with different sugar donors. A) Control group; B) UDP-glucose, C) UDP-*N*-acetylglucosamine; D) UDP-xylose; E) MS spectra of the main products at positive mode.

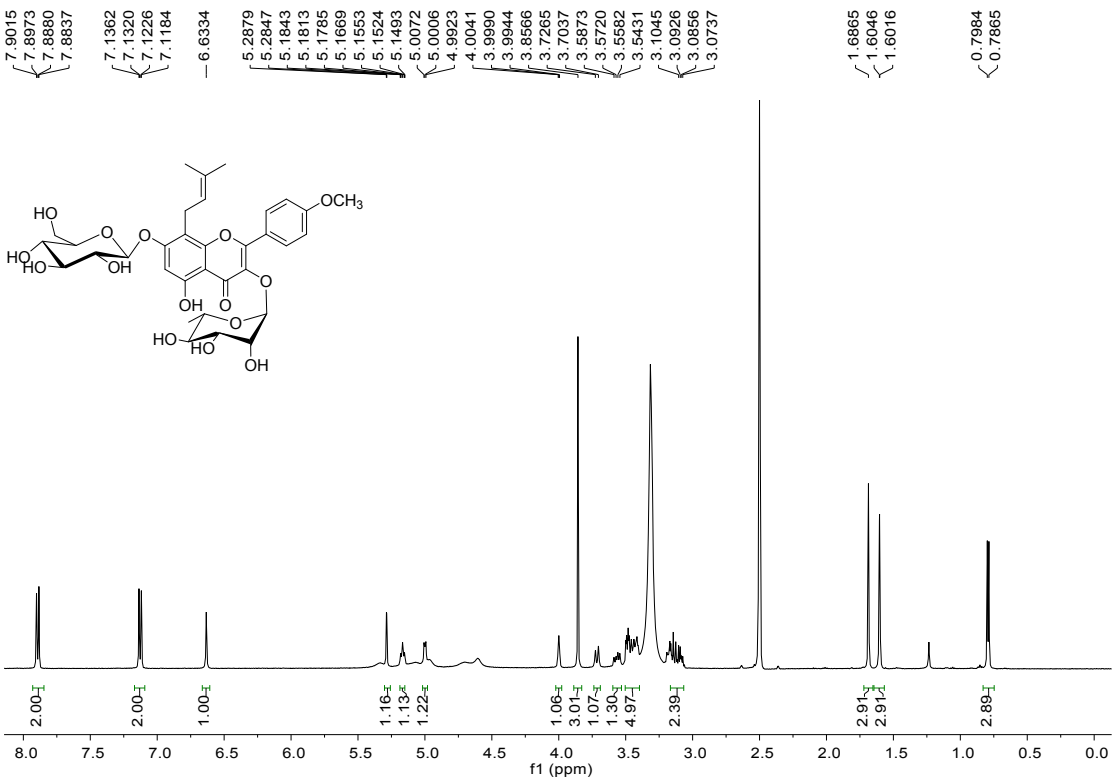

Figure S7. ^{1}H NMR spectrum of icariin (**1a**) in DMSO-d_6

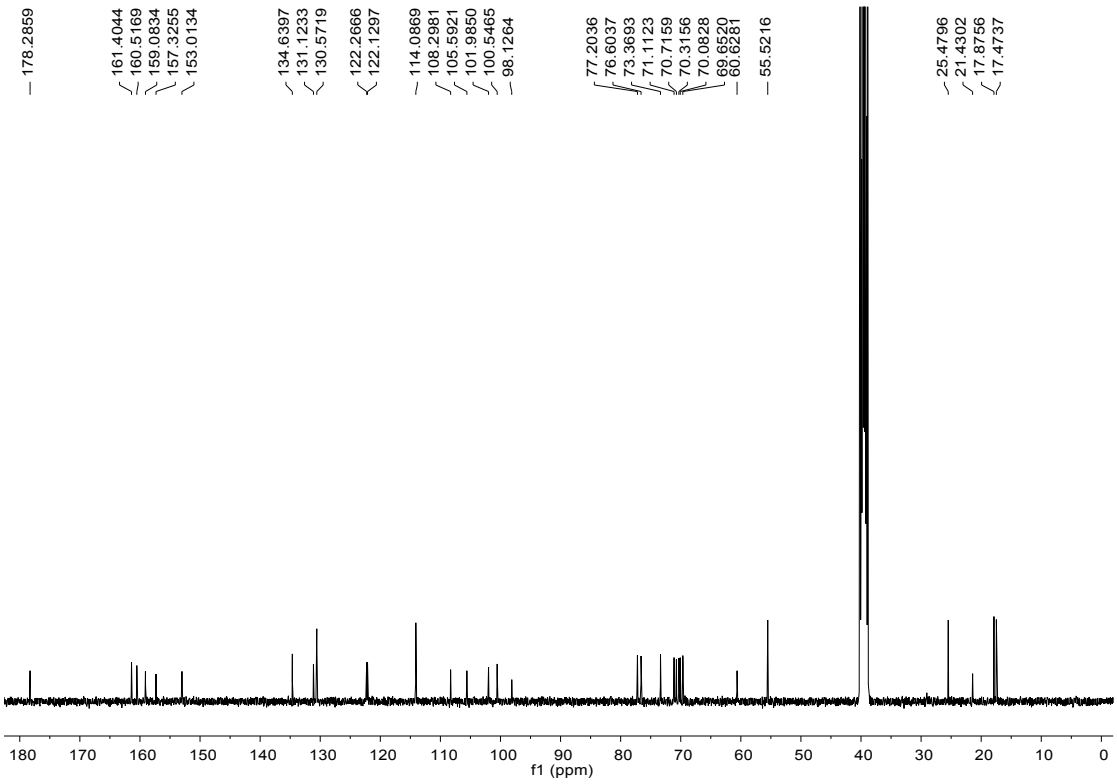

Figure S8. ^{13}C NMR spectrum of icariin (**1a**) in DMSO-d_6

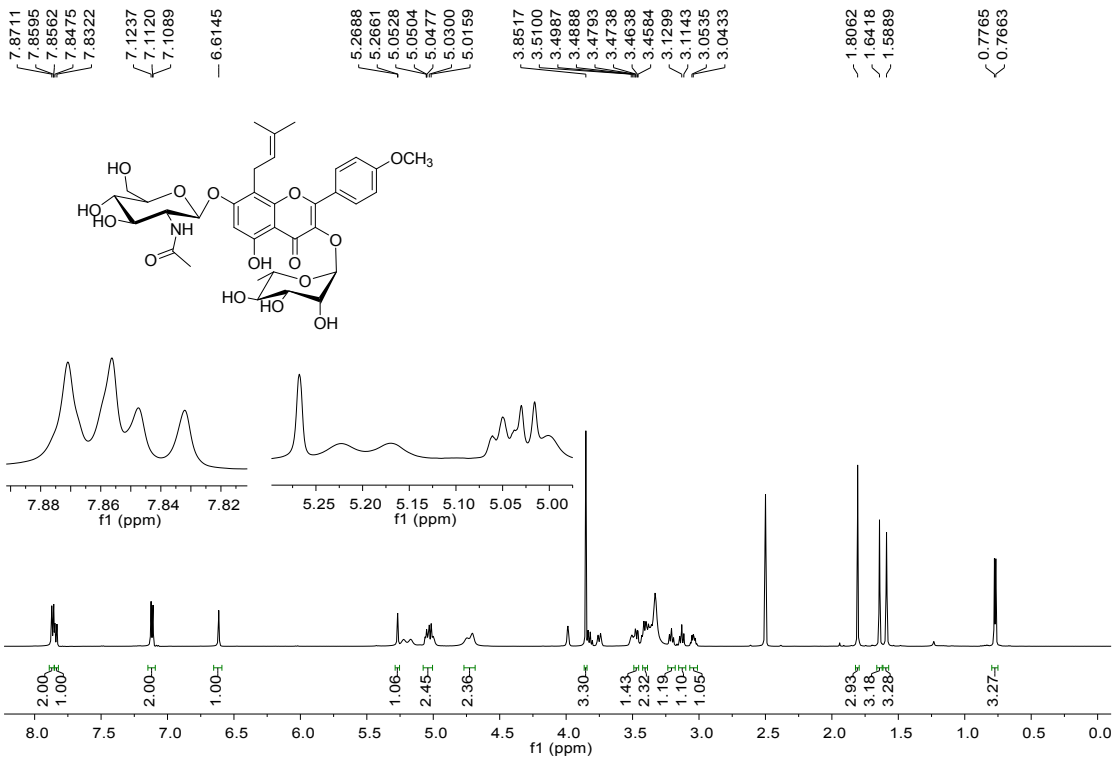

Figure S9. ^{1}H NMR spectrum of **1b** in DMSO-d_6

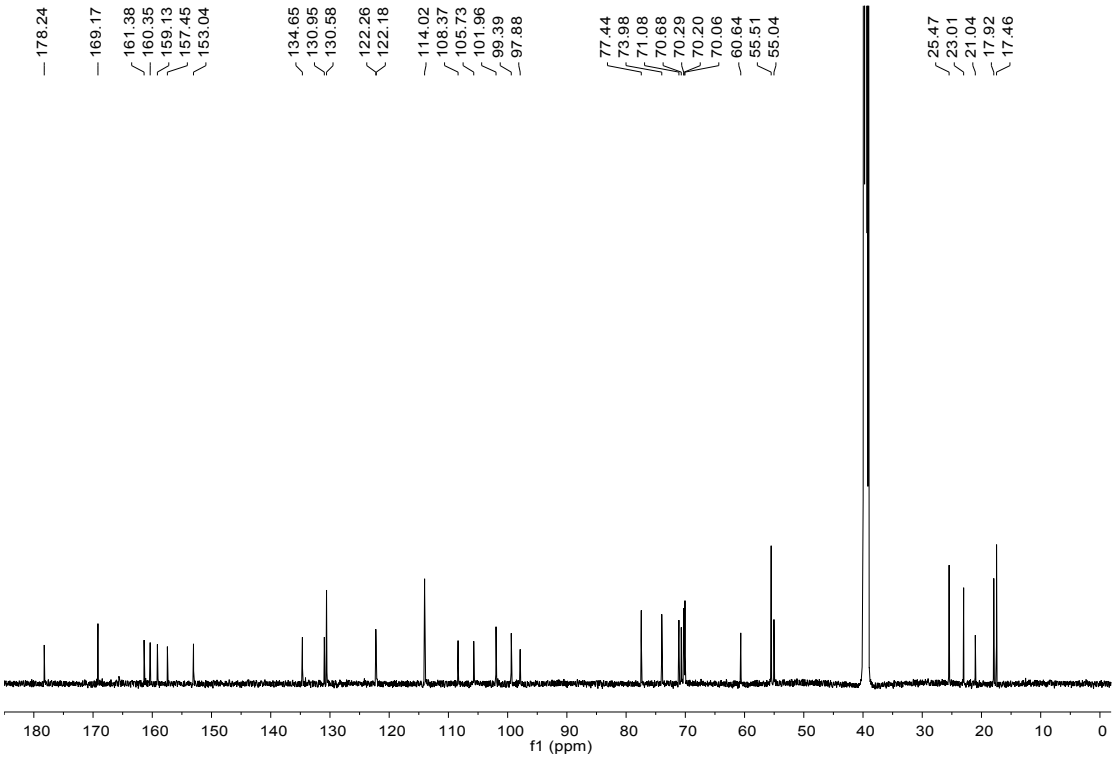

Figure S10. ^{13}C NMR spectrum of **1b** in DMSO-d_6

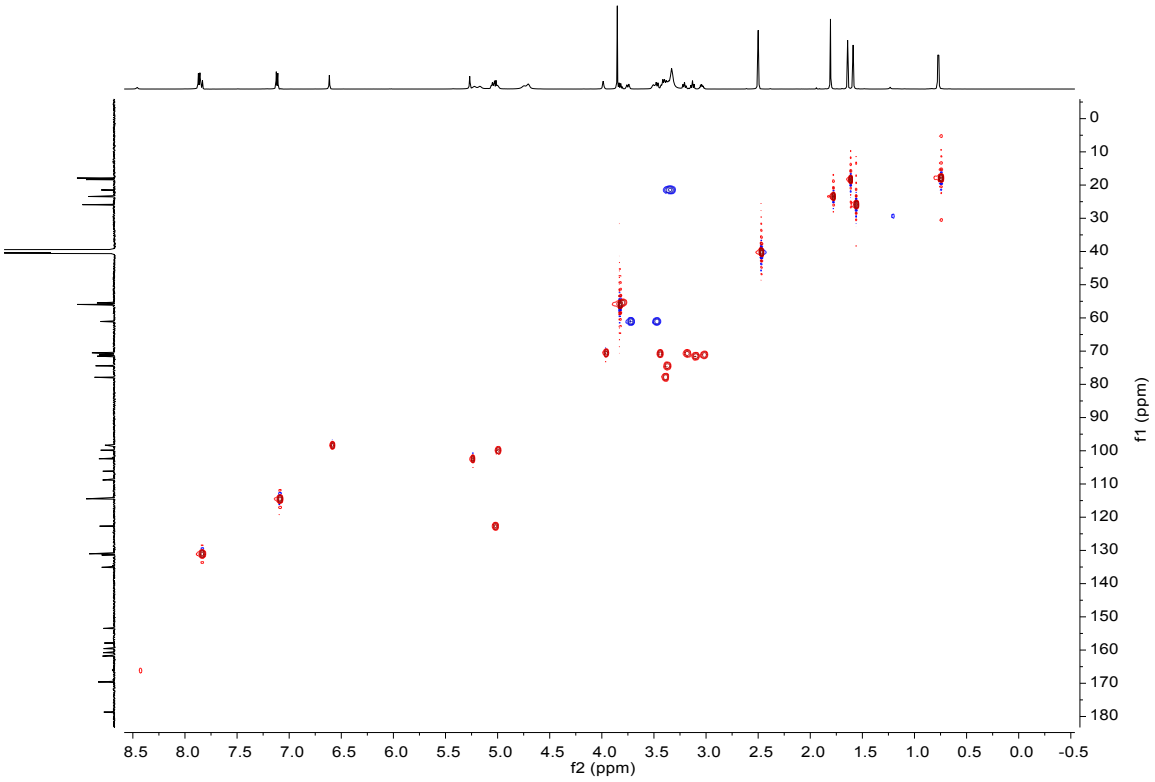

Figure S11. HSQC spectrum of **1b** in DMSO-d_6

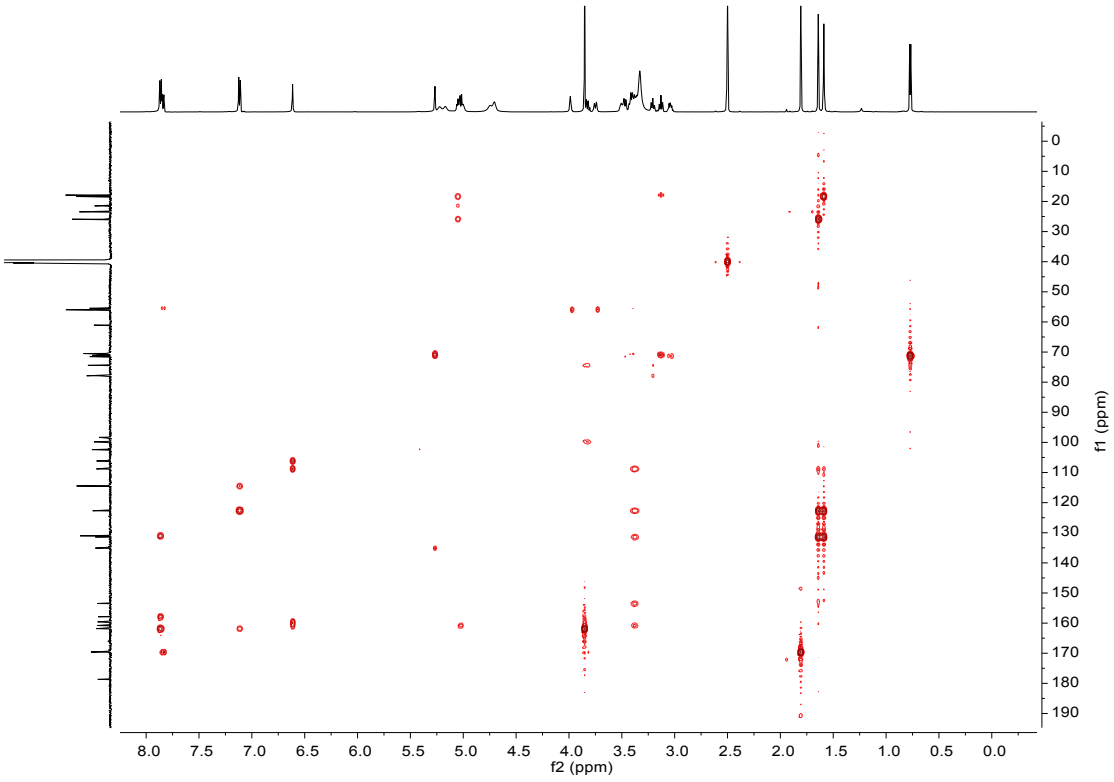

Figure S12. HMBC spectrum of **1b** in DMSO-d_6

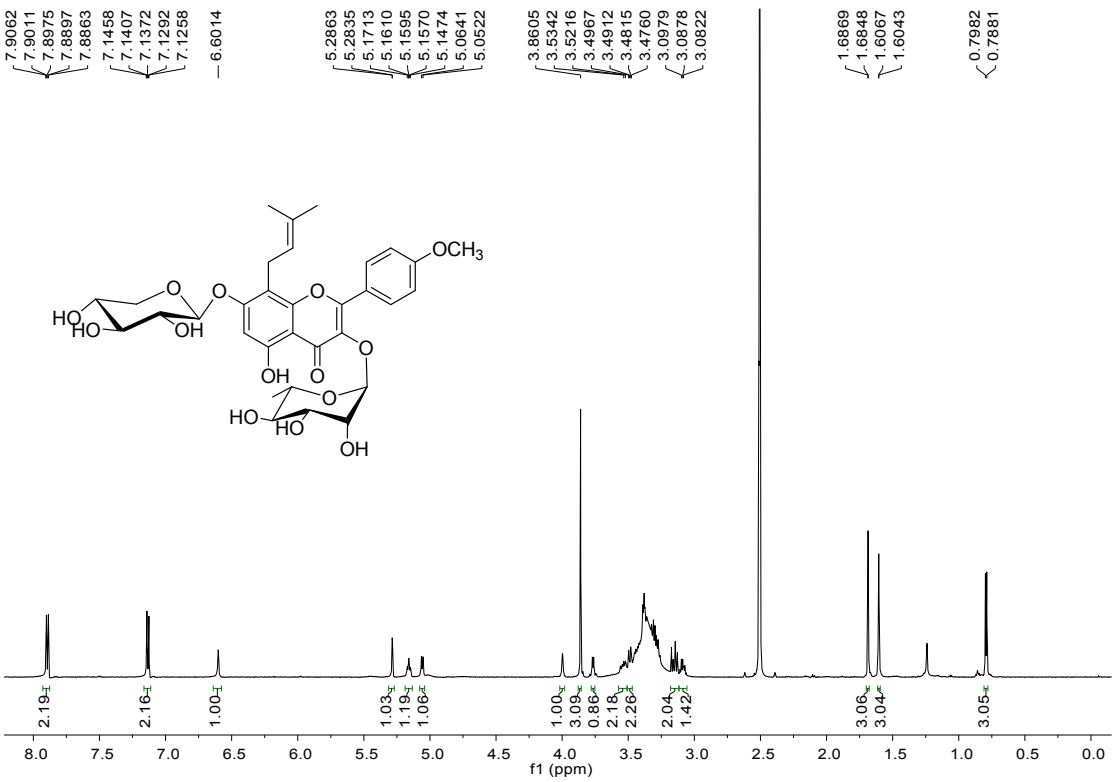

Figure S13. ^{1}H NMR spectrum of **1c** in DMSO-d_6

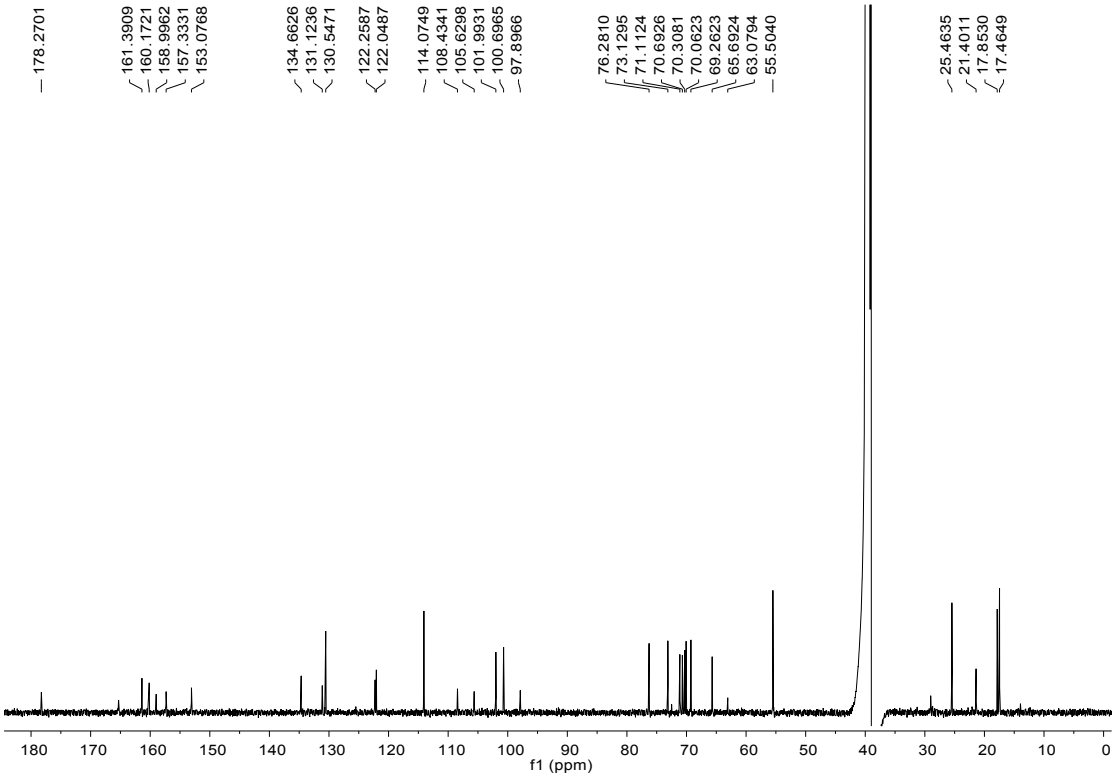

Figure S14. ^{13}C NMR spectrum of **1c** in DMSO-d_6

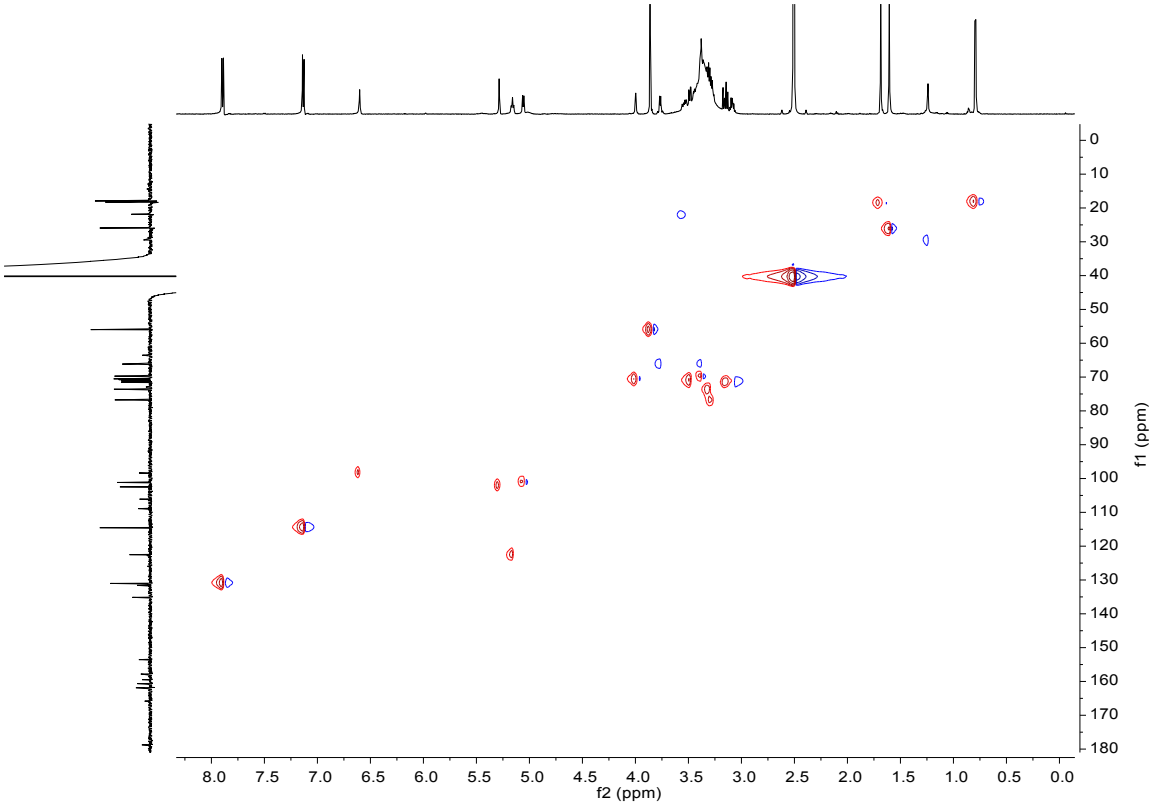

Figure S15. HSQC spectrum of **1c** in DMSO-d_6

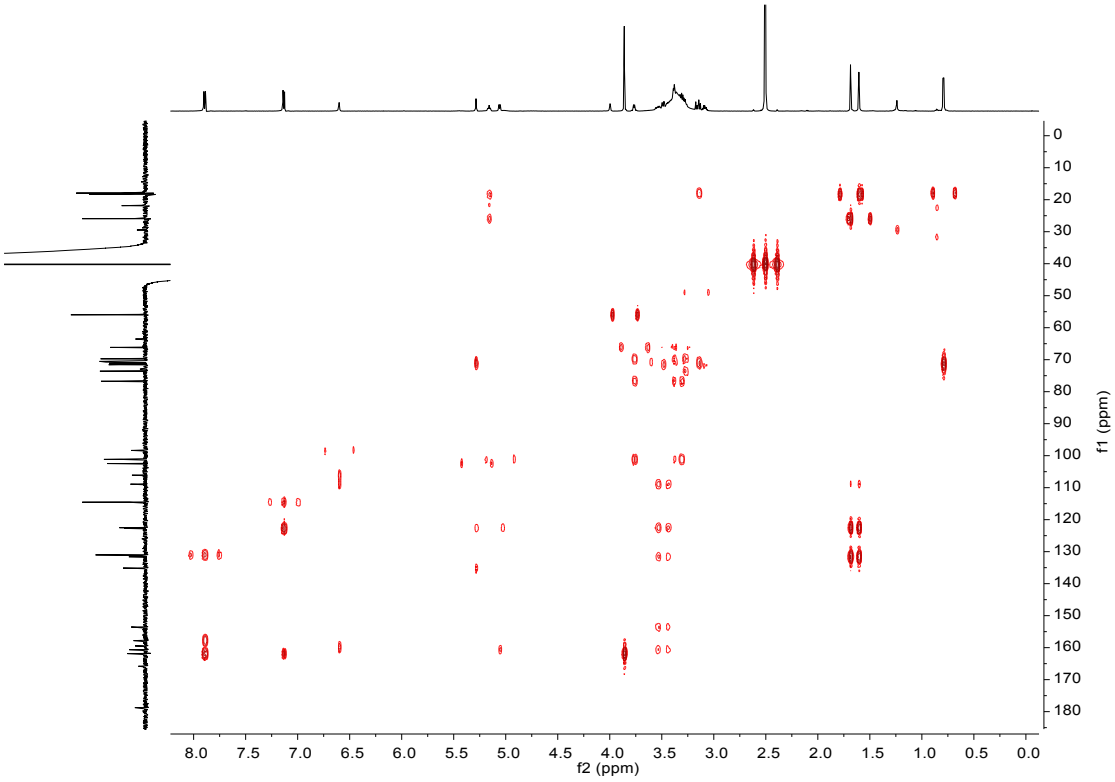

Figure S16. HMBC spectrum of **1c** in DMSO-d_6

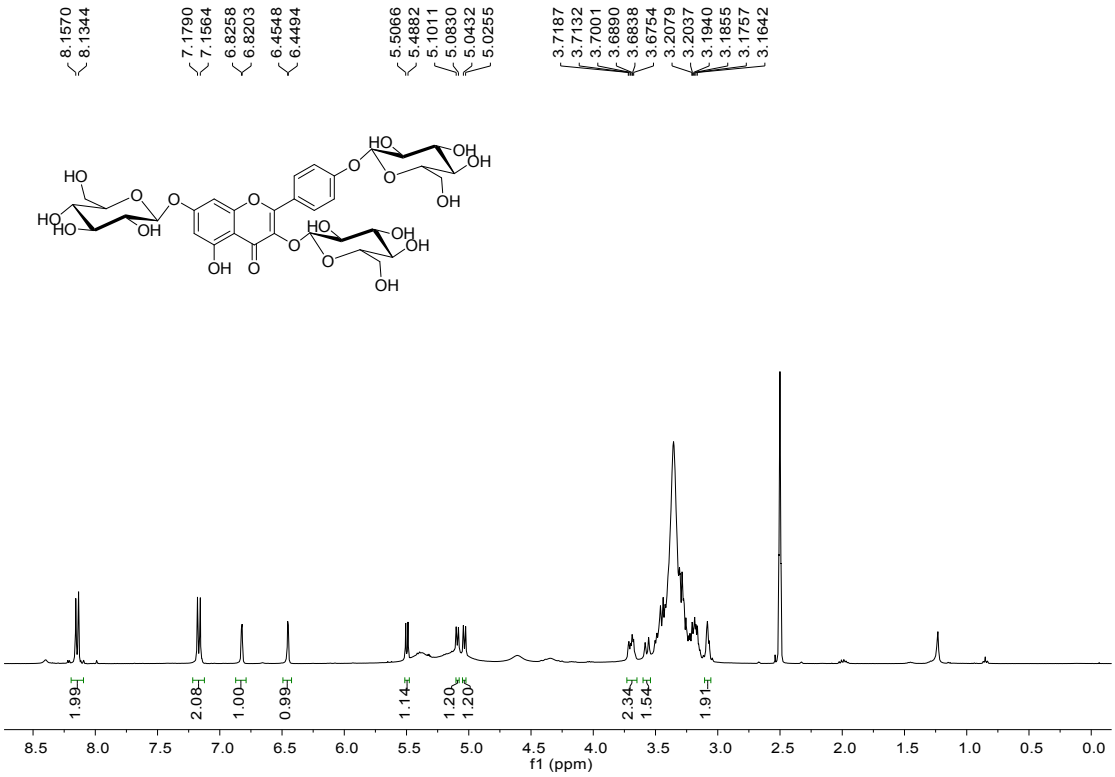

Figure S17. ^{1}H NMR spectrum of **2a** in DMSO-d_6

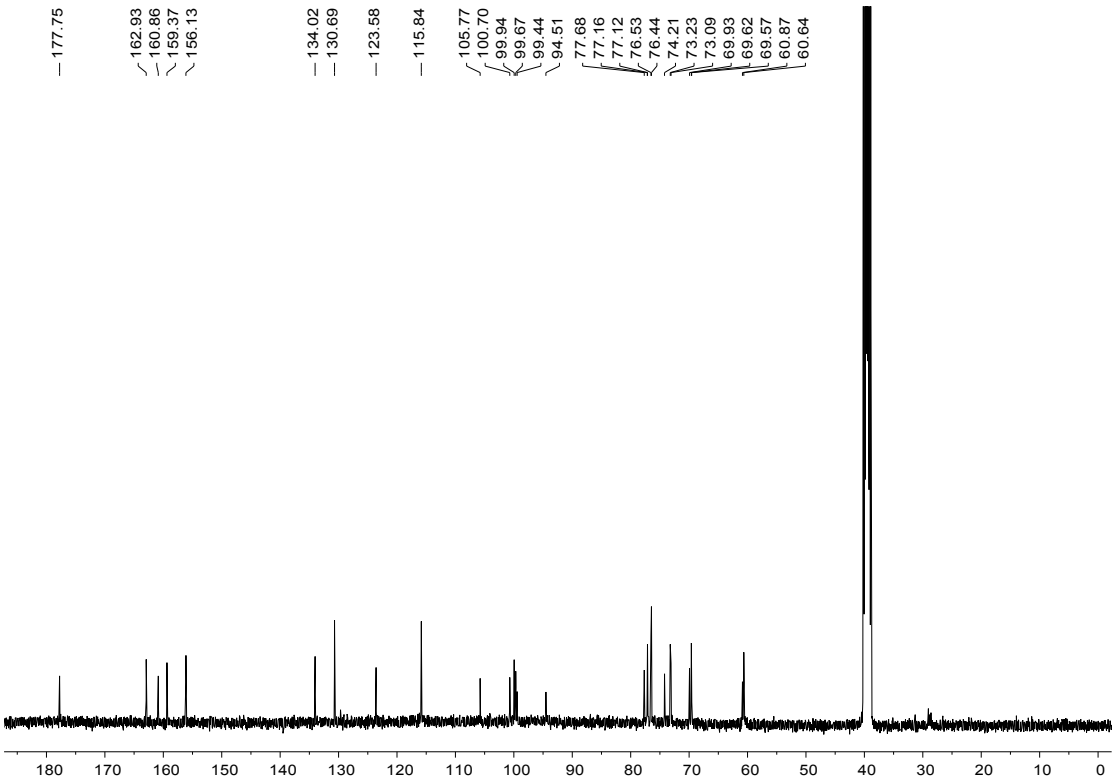

Figure S18. ^{13}C NMR spectrum of **2a** in DMSO-d_6

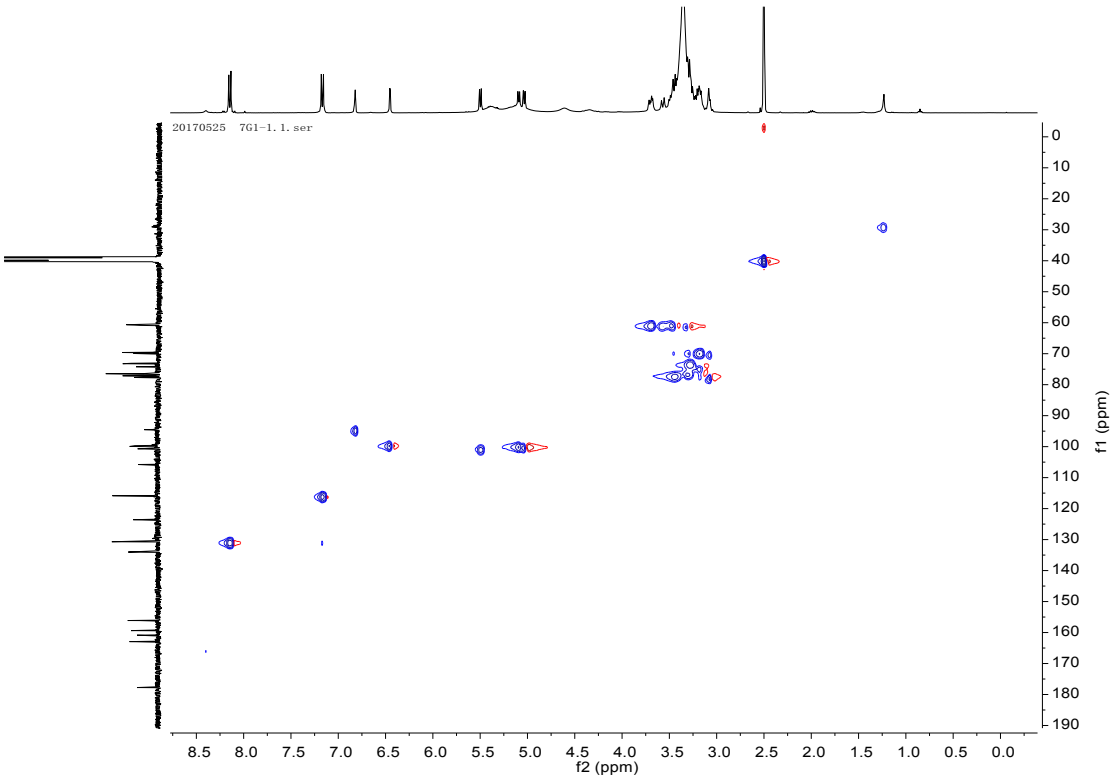

Figure S19. HSQC spectrum of **2a** in DMSO-d_6

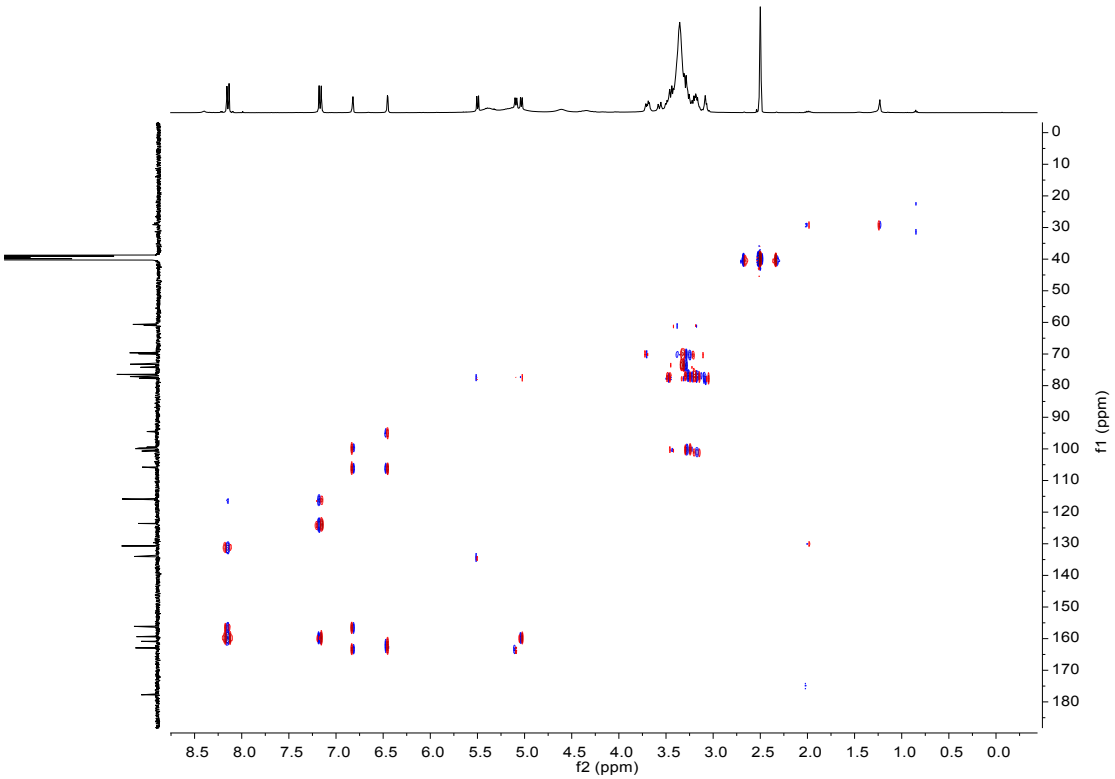

Figure S20. HMBC spectrum of **2a** in DMSO-d_6

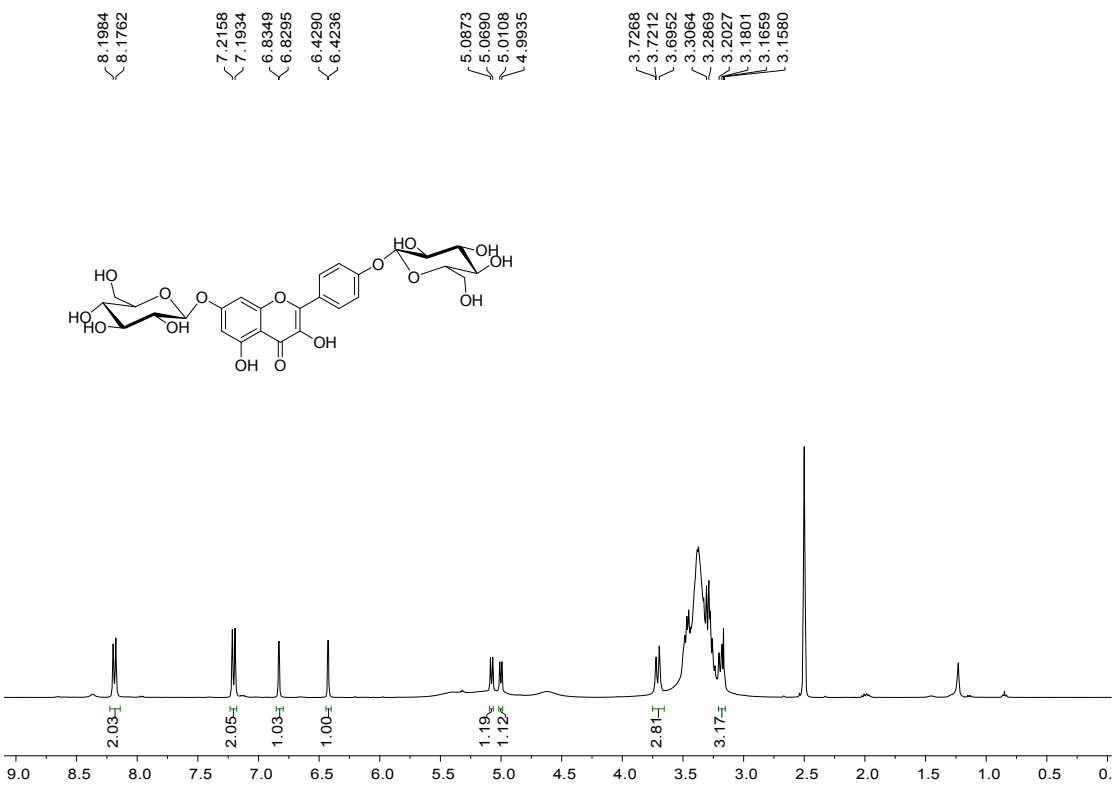

Figure S21. ^{1}H NMR spectrum of **2b** in DMSO-d_6

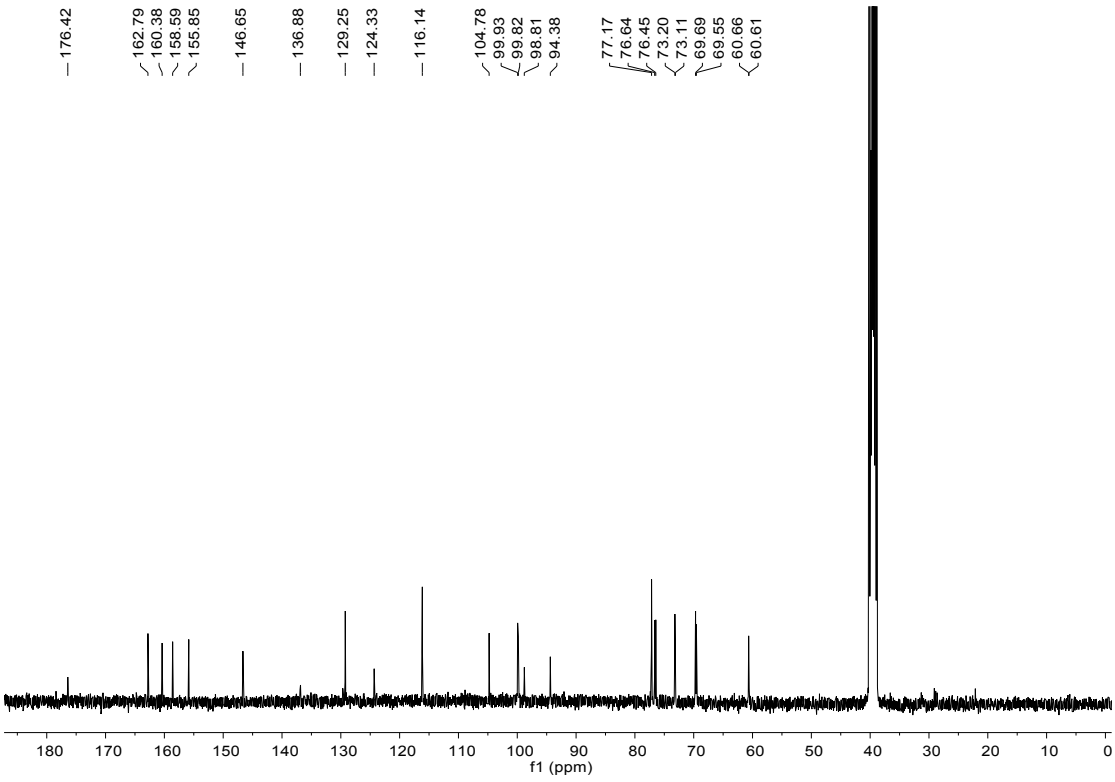

Figure S22. ^{13}C NMR spectrum of **2b** in DMSO-d_6

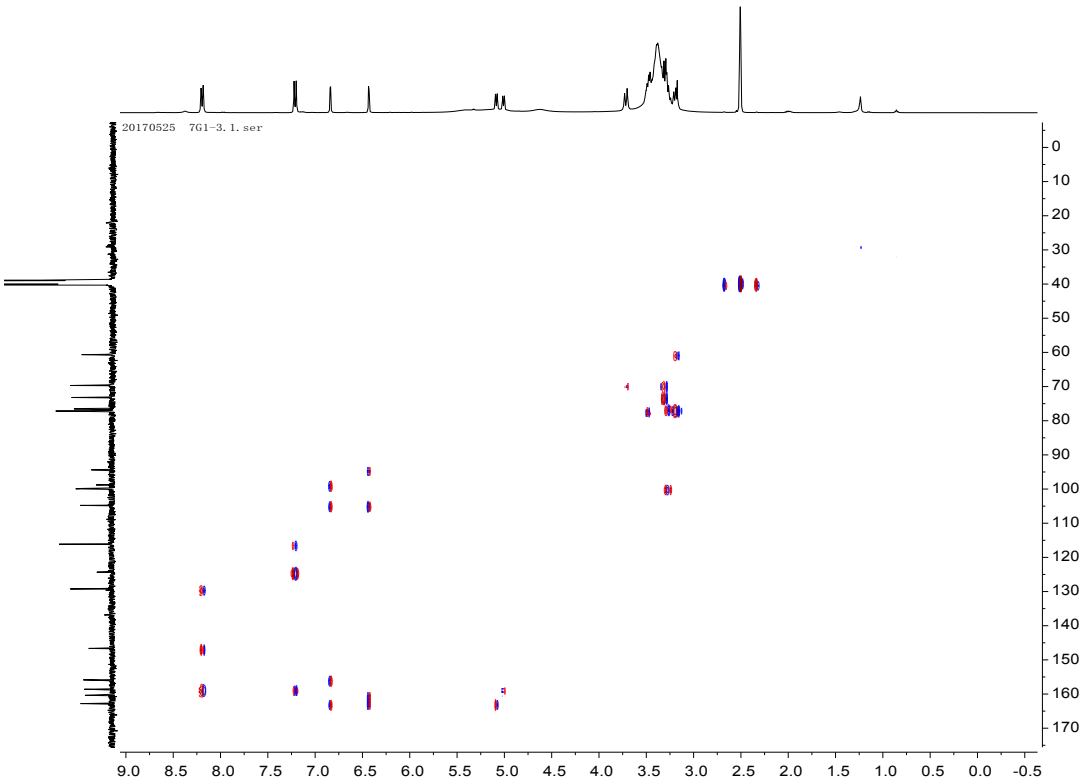

Figure S23. HMBC spectrum of **2b** in DMSO-d_6

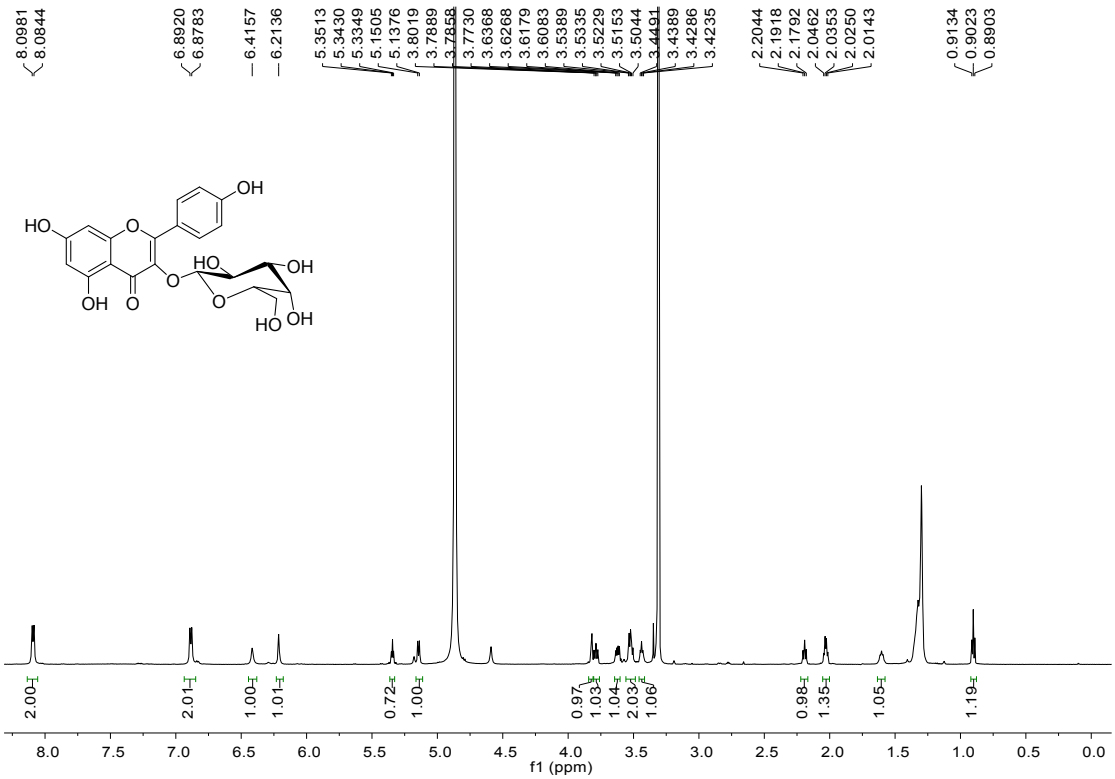

Figure S24. ^{1}H NMR spectrum of **2c** in Methanol-d_4

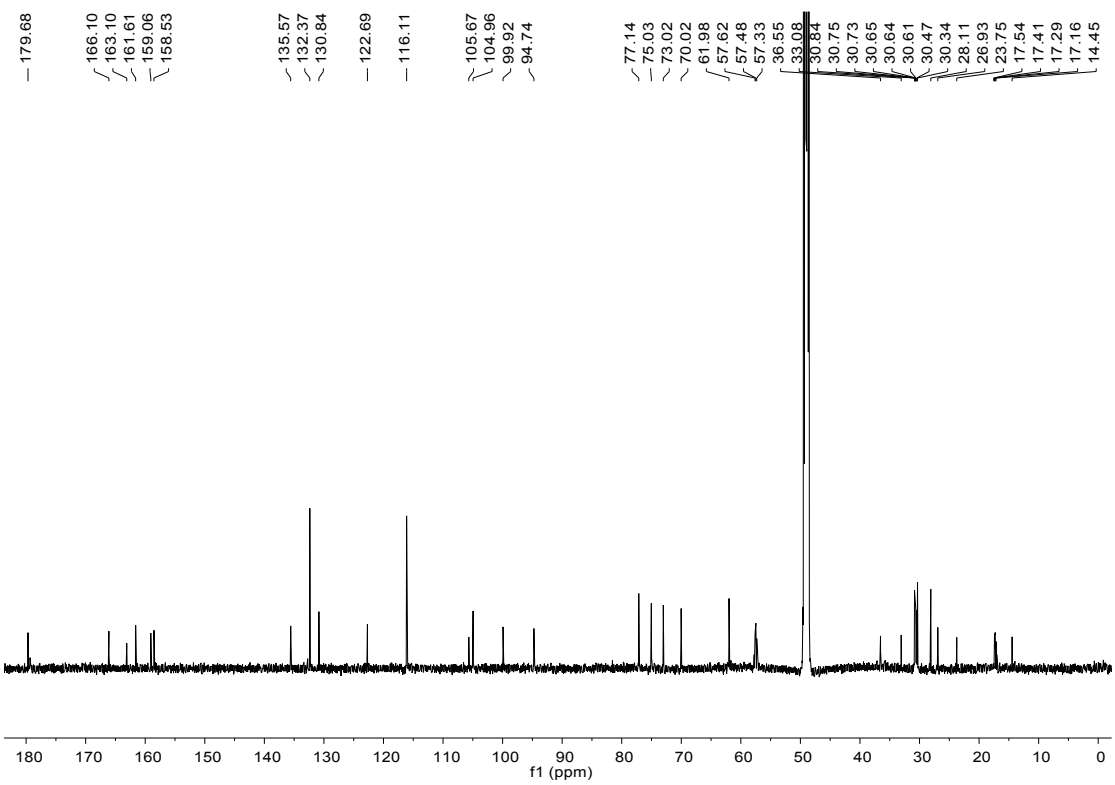

Figure S25. ^{13}C NMR spectrum of **2c** in Methanol-d_4

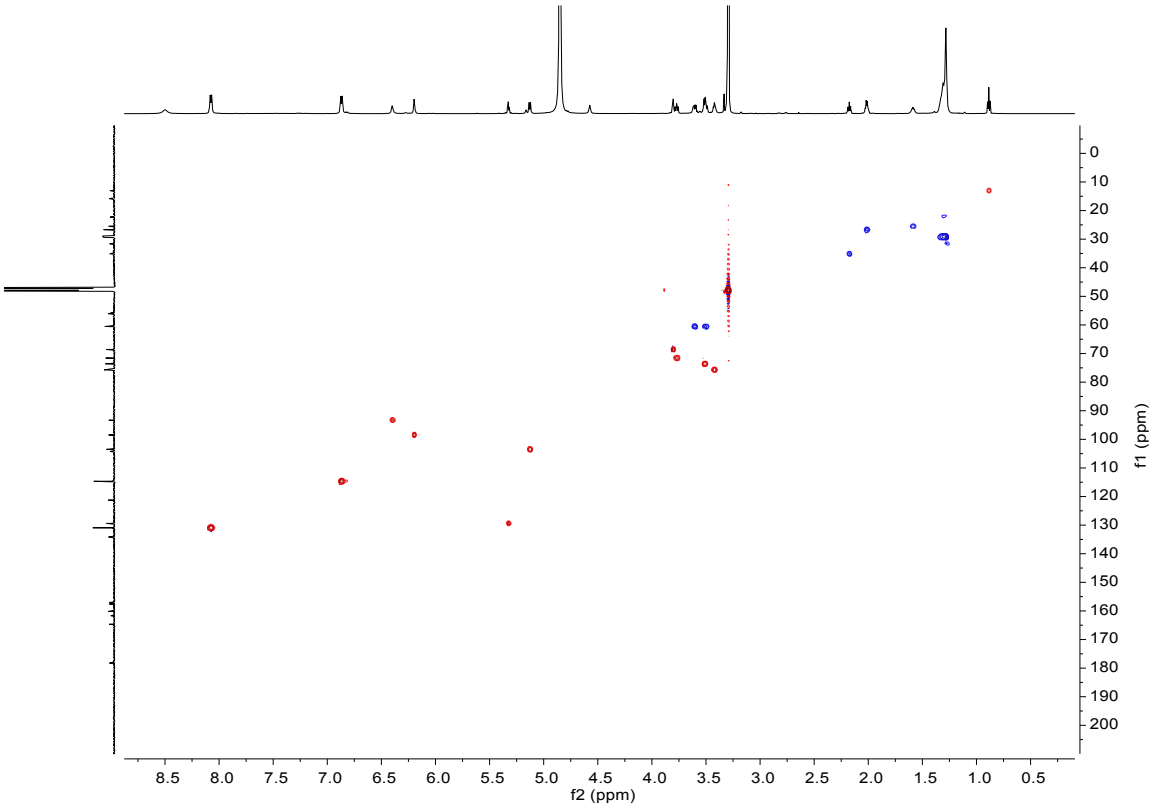

Figure S26. HSQC spectrum of **2c** in Methanol-d_4

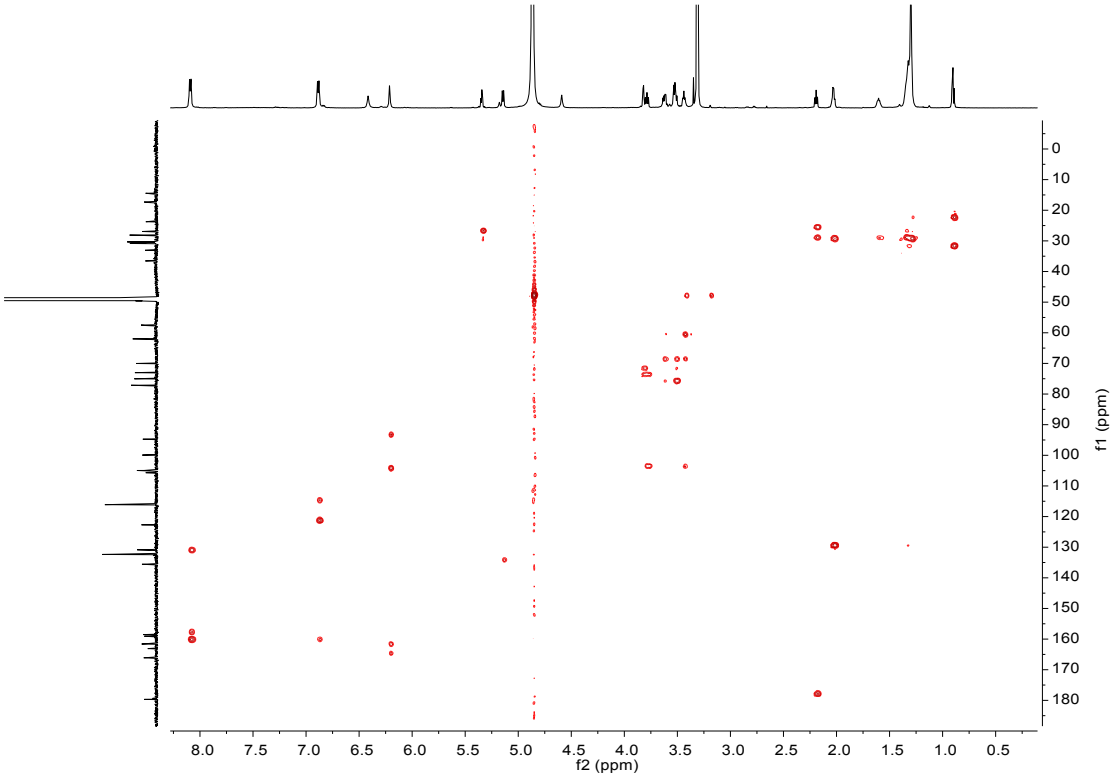

Figure S27. HMBC spectrum of **2c** in Methanol-d_4

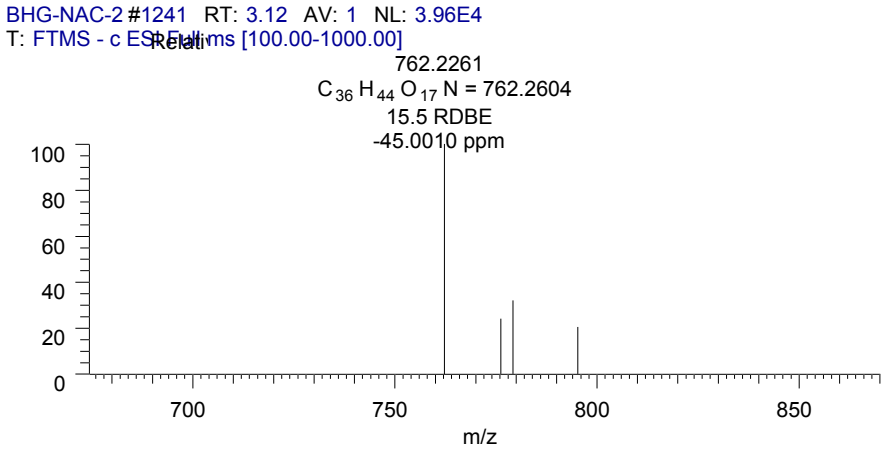

Figure S28. The HRESIMS spectrum of **1b**

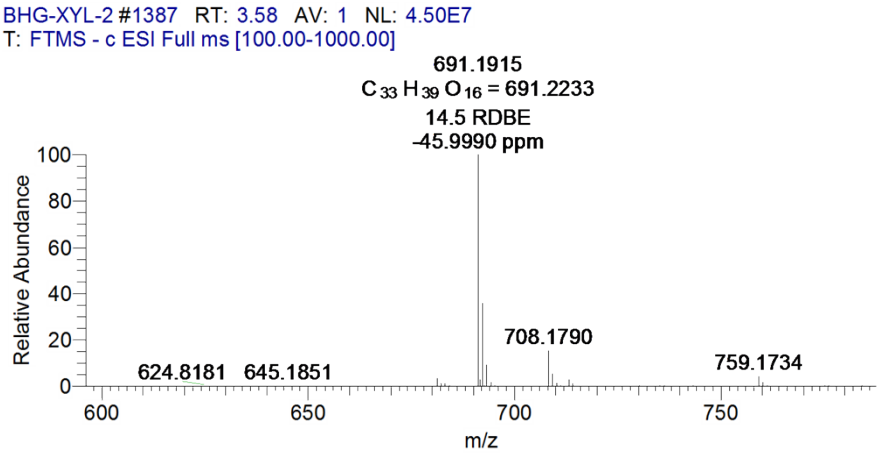

Figure S29. The HRESIMS spectrum of **1c**

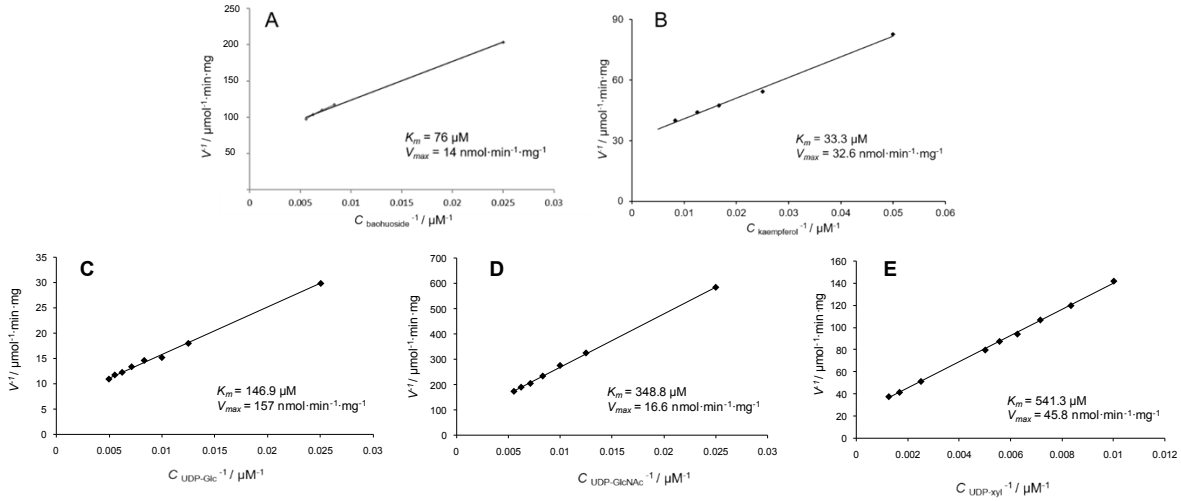

Figure S30. The apparent K_m values of recombinant Ep7GT for baohuoside (A), kaempferol (B), UDP-glucose (C), UDP-*N*-acetylglucosamine (D) and UDP-xylose (E).

Table S3. The apparent K_m, K_{cat} and K_{cat}/K_m values of recombinant Ep7GT for different sugar donors with **1** as the accepter.

Sugar donors	K_m (μM)	K_{cat} (S^{-1})	K_{cat}/K_m (S^{-1} mM^{-1})
UDP-glucose	146.9	0.13	0.88
UDP-*N*-acetylglucosamine	348.0	0.014	0.04
UDP-xylose	541.3	0.038	0.07

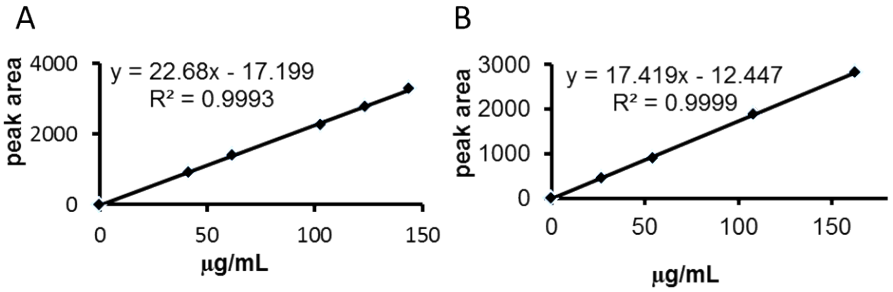

Figure S31. The linear regression models and the regression equations of the external standard method established for the quantitative analysis of baohuoside (A) and icariin (B).

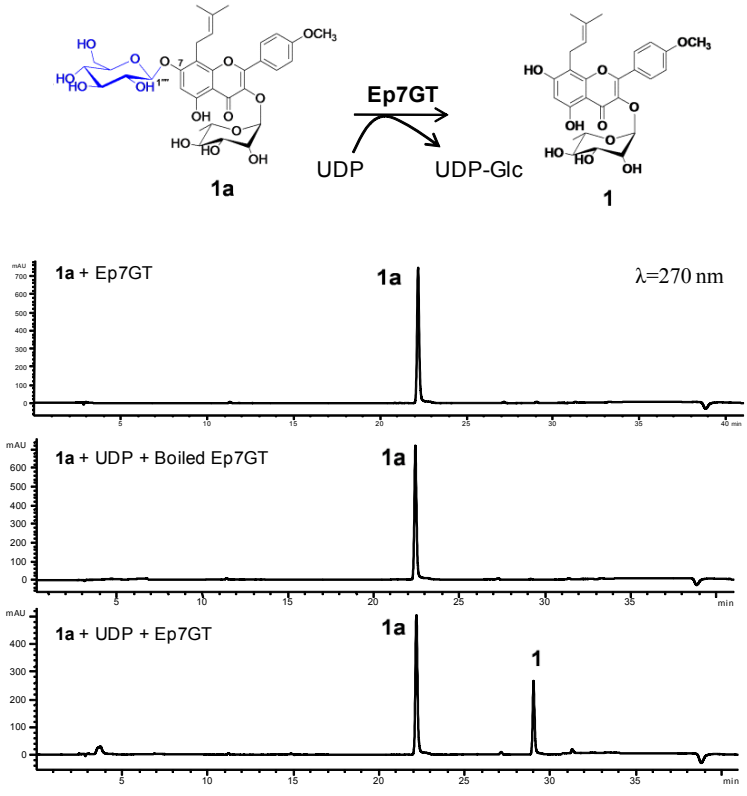

Figure S32. Exploring the catalytic reversibility of Ep7GT when **1a** and UDP were used as substrate.

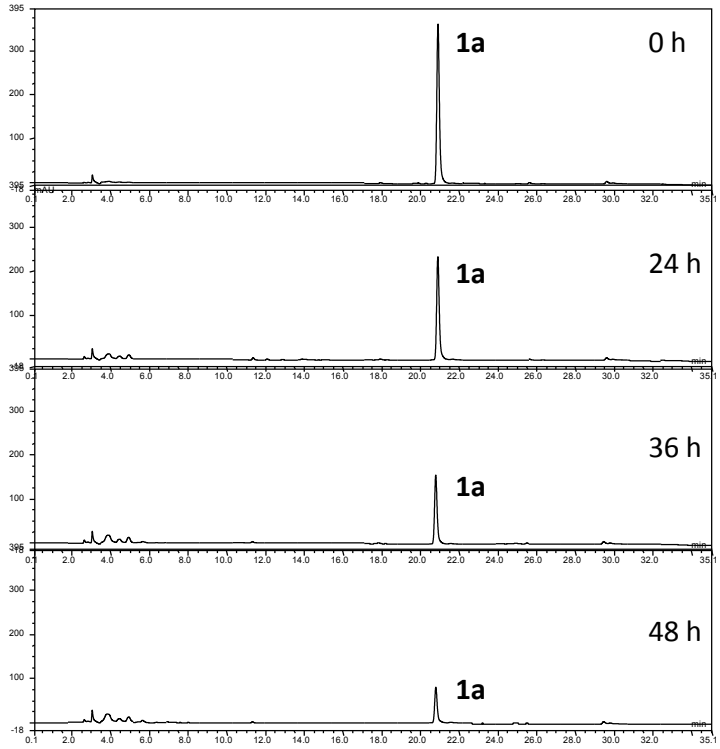

Figure S33. Time course assay of **1a** with whole cell catalyst of engineered *E. coli*.