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Supplementary Information

# Promiscuous Halogenase for Derivatization of Flavonoids

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**Content**



## Supplementary Tables

**Table S1.** Bacterial strains, fosmids, plasmids and primers used in this work.

| Bacterial strain | Features | Reference/Source |
|---|---|---|
| *Streptomyces albus* Del14 | Wild-type strain | [1] |
| *Streptomyces albus* 9B-A9 | *S. albus* with BAC 9B-A9 insertion | This work |
| *Streptomyces albus* pSETprom-Hal | *S. albus* with pSETprom-Hal insertion | This work |
| *Escherichia coli* ET12567 pUB307 | Donor strain for intergeneric conjugation | [2] |
| *Escherichia coli* GB2005 | General cloning strain | - |
| **fosmid/plasmid** | **Features** | **Reference/Source** |
| 9B-A9 | BAC containing DklH gene | [1] |
| pSET152 | AmR, cloning vector | - |
| **Primers** | **Sequence (5′-3′)** | **Source** |
| pSETprom-Hal_F | AAAATCTAGACAGGTCGGCTGGTT-GGCTGACAAAGTGCGTAGGATGACTGTCCTCTCG-GACAGGCTGTCGA | Eurofines Scientific |
| pSETprom-Hal_R | AAAAGATATCCTACTGGAGGTTGGCGAGCC | Eurofines Scientific |
| halseqF1 | CTGGAAAAATGCGACGCCG | Eurofines Scientific |
| halseqF2 | CAGCAGAACCAGGGGAAGG | Eurofines Scientific |
| halseqR1 | GACAACAGCGGGTCCAGAA | Eurofines Scientific |
| halseqR2 | TCTCGGCGTTGGTGATTCC | Eurofines Scientific |

## Supplementary Figures

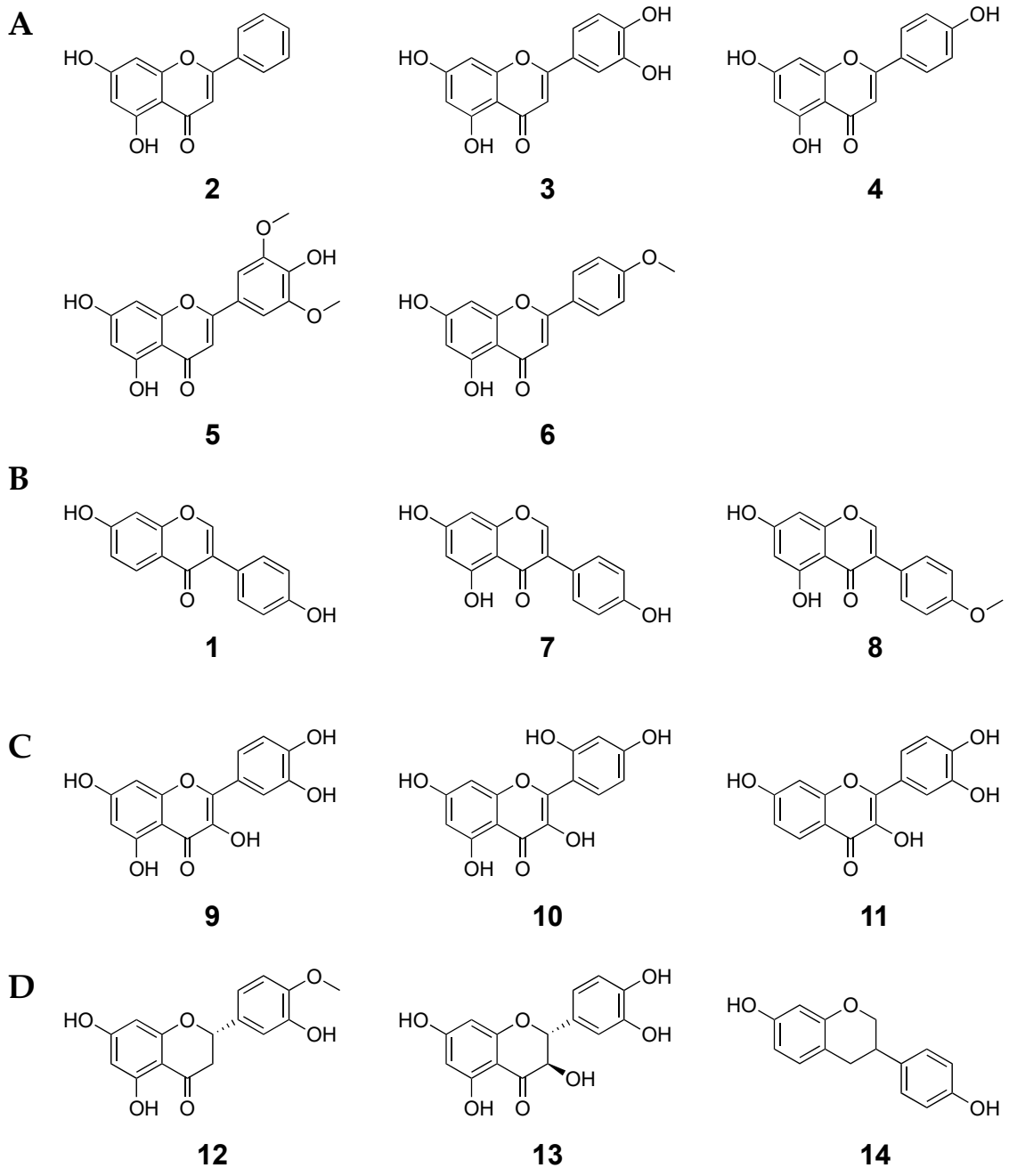


**Figure S1.** Outline of DklH substrates classified by flavonoid substructure. (**A**) Flavone: chrysin[d] **2**, luteolin[b] **3**, apigenin[b] **4**, tricin[c] **5**, acacetin[c] **6**. (**B**) Isoflavone: daidzein[b] **1**, genistein[b] **7**, biochanin A[a] **8**. (**C**) Flavanonole: quercetin[b] **9**, morin[b] **10**, fisetin[a] **11**. (**D**) Flavanone: hesperetin[b] **12**, flavanonole: taxifolin[b] **13** and isoflavandiol: R,S-equol[b] **14**. Suppliers: (a) TCI Germany GmbH, Eschborn, Germany; (b) Santa Cruz Biotechnology, Inc., Dallas, TX, USA; (c) SynInnova Laboratories Inc., Edmonton, Canada; (d) Alfa Aesar, Thermo Fisher Scientific, Kandel, Germany.

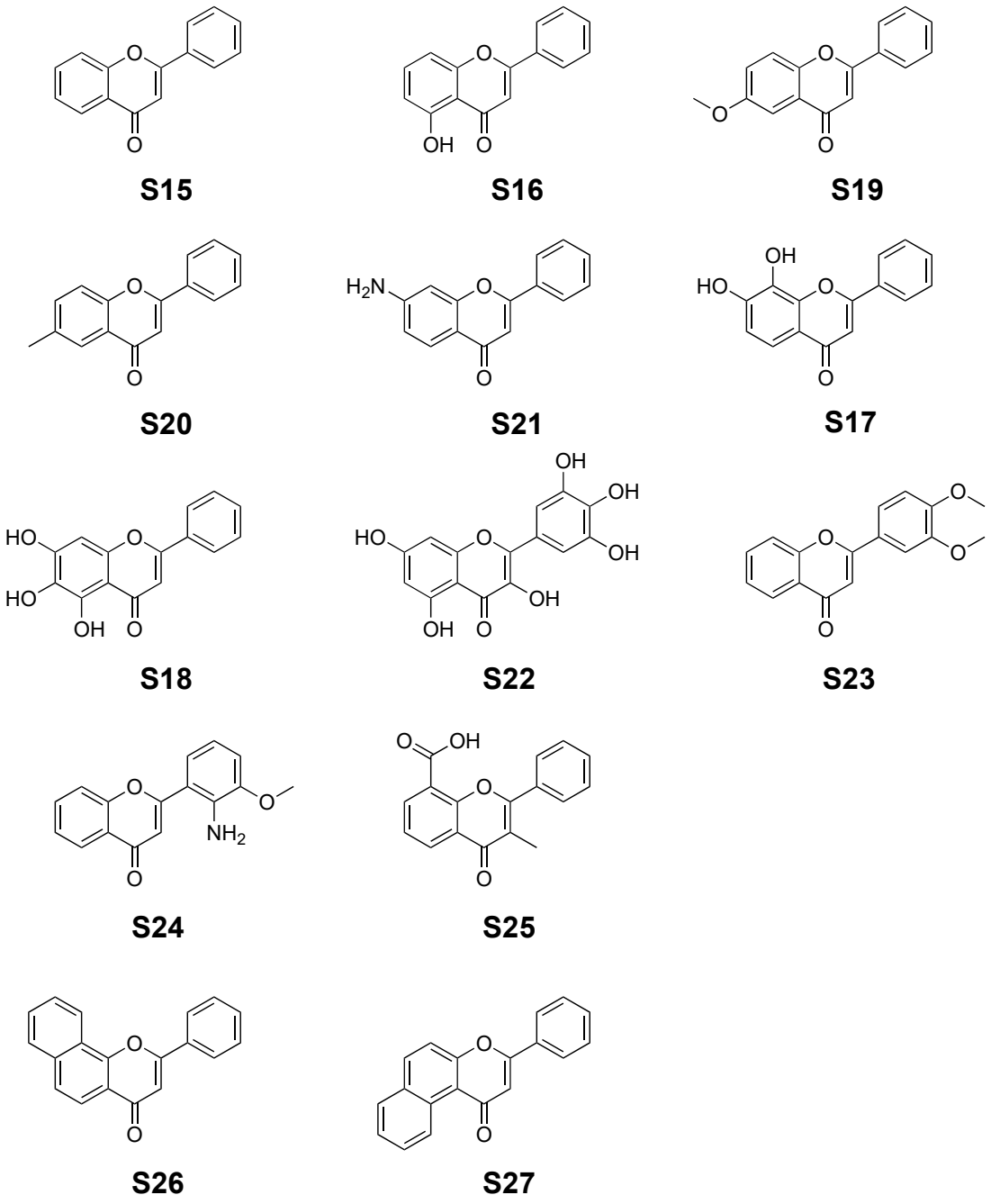


**Figure S2.** Display of flavones and related derivatives not accepted by DklH. Flavone[a] **S15**, 6-hydroxyflavone[a] **S16**, 6-methoxyflavone[a] (**S19**), 6-methylflavone[a] **S20**, 7-aminoflavone[b] **S21**, 7,8-dihydroxyflavone[b] **S17**, baicalein[b] **S18**, myricetin[a] **S22**, 3′,4′-dimethoxyflavone[a] **S23**, 2-(2-amino-3-methoxyphenyl)chromone[a] **S24**, 3-methylflavone-8-carboxylic acid[a] **S25**, α-naphthoflavone[a] **S26**, β-naphthoflavone[a] **S27**. Suppliers: (a) TCI Germany GmbH, Eschborn, Germany; (b) Santa Chruz Biotechnology, Inc., Dallas, TX, USA.

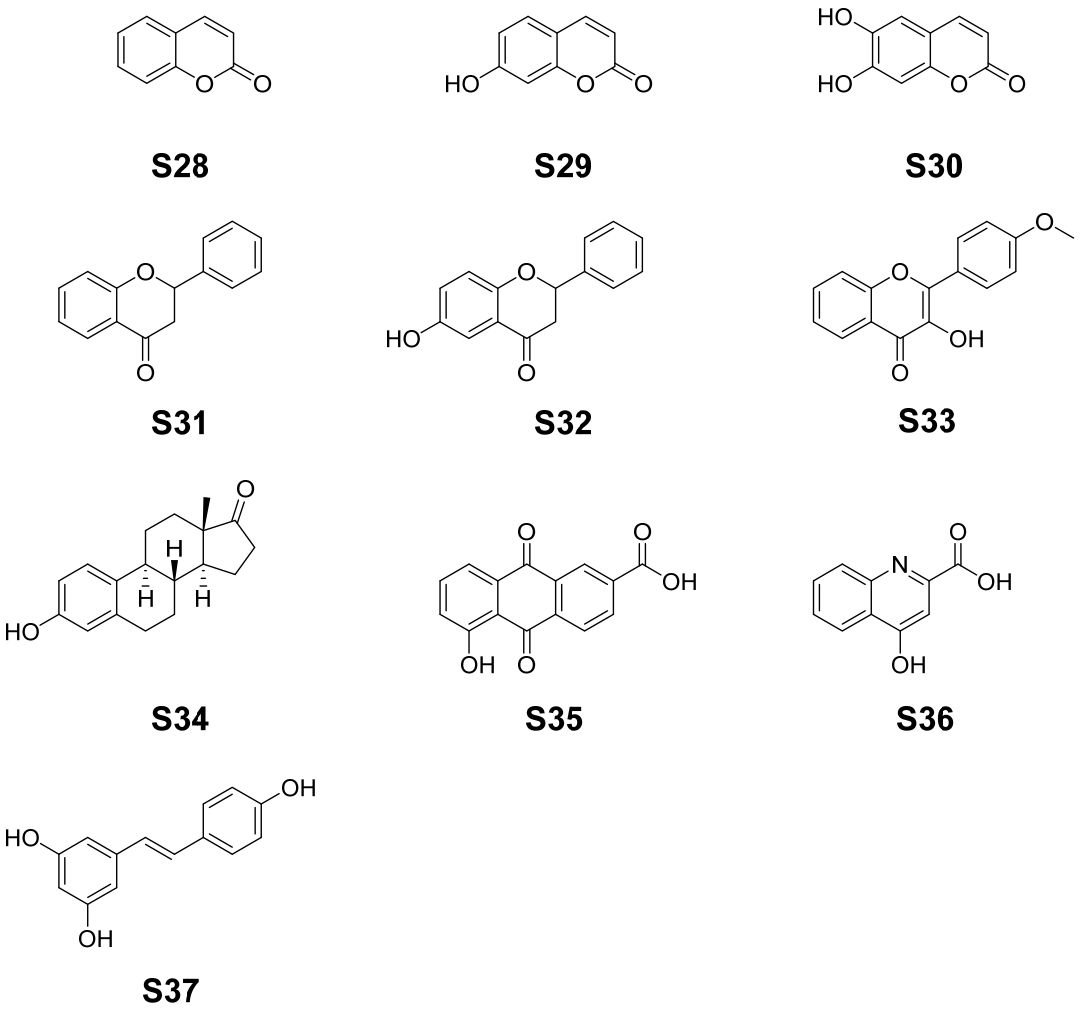


**Figure S3.** Display of compounds not converted by DklH. Coumarin[a] **S28**, umbelliferone[a] **S29**, esculetin[a] **S30**, flavanone[a] **S31**, 6-hydroxyflavanone[a] **S32**, 3-hydroxy-4'-methoxyflavone[a] **S33**, estron[a] **S34**, 4,5-dihydroxyanthraquinone-2-carboxylic acid[a] **S35**, kynurenic acid[a] **S36**, resveratrol[a] **S37**. Suppliers: (a) TCI Germany GmbH, Eschborn, Germnay; (b) Santa Chruz Biotechnology, Inc., Dallas, TX, USA.

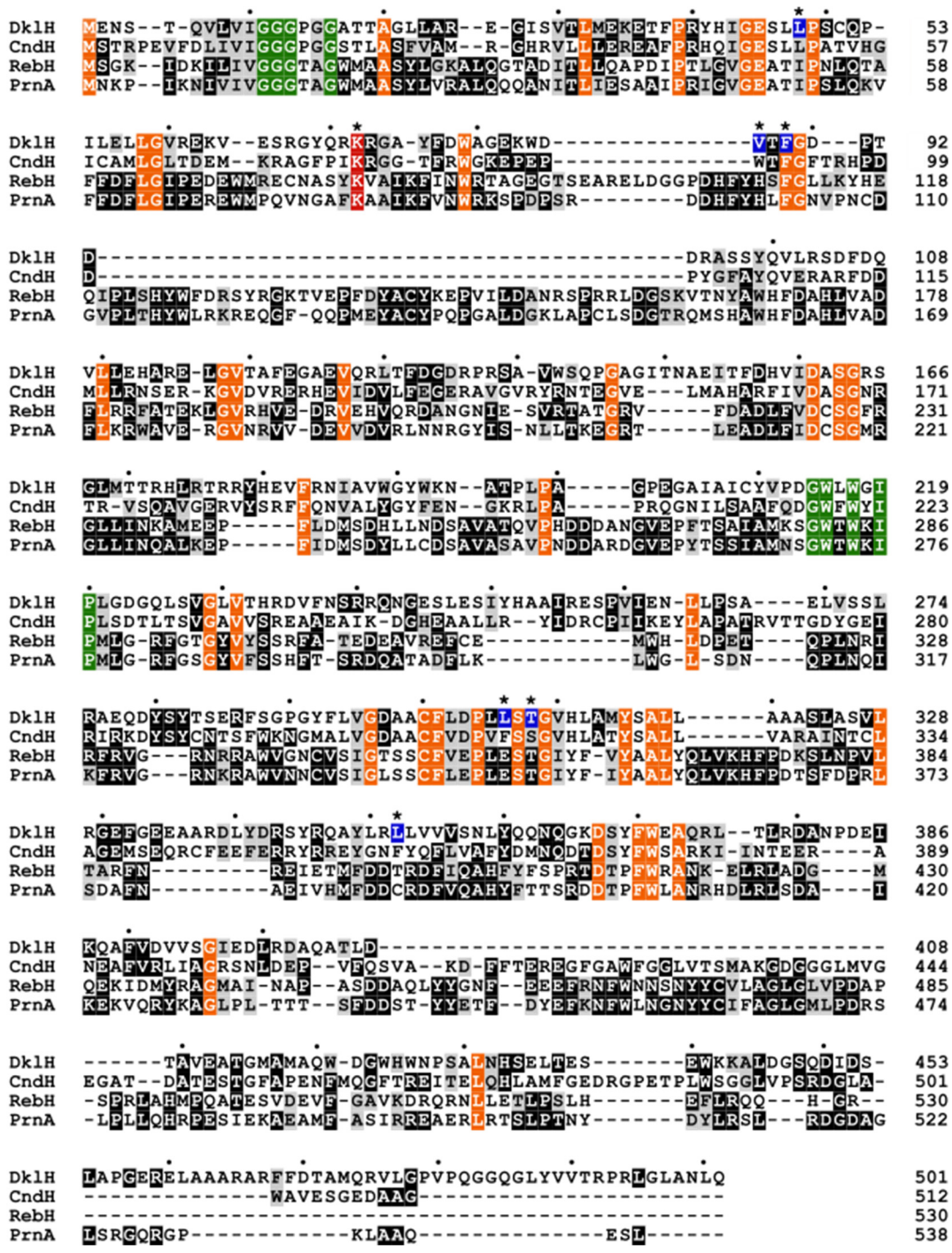


**Figure S4.** Multiple sequence alignment between DklH from *Frankia alni* ACN14a, chondrochloren halogenase. (CndH) from *Chondromyces crocatus* Cm c5, flavin-dependent tryptophan halogenase (RebH) from *Lechevalieria aerocolonigenes* and flavin-dependent tryptophan halogenase (PrnA) from *Pseudomonas fluorescens*. The sequence alignment was performed using the T-Coffee server [3]. The results were visualized by using Boxshade version 3.21. Every tenth residue of DklH is indicated by a dot above. Conserved regions are highlighted by different colors. Overall conserved residues are represented in orange. The FAD binding site is shown in green, while the motif GGGXXG indicates diphosphate binding residues. The FAD isoalloxazine-ring is coordinated by GWXWXIP substructure [4]. Residues that are involved in substrate binding are shown in blue and are additionally labelled with a star above. The lysin-72, which transfers activated chlorine species to substrate, is colored in red.

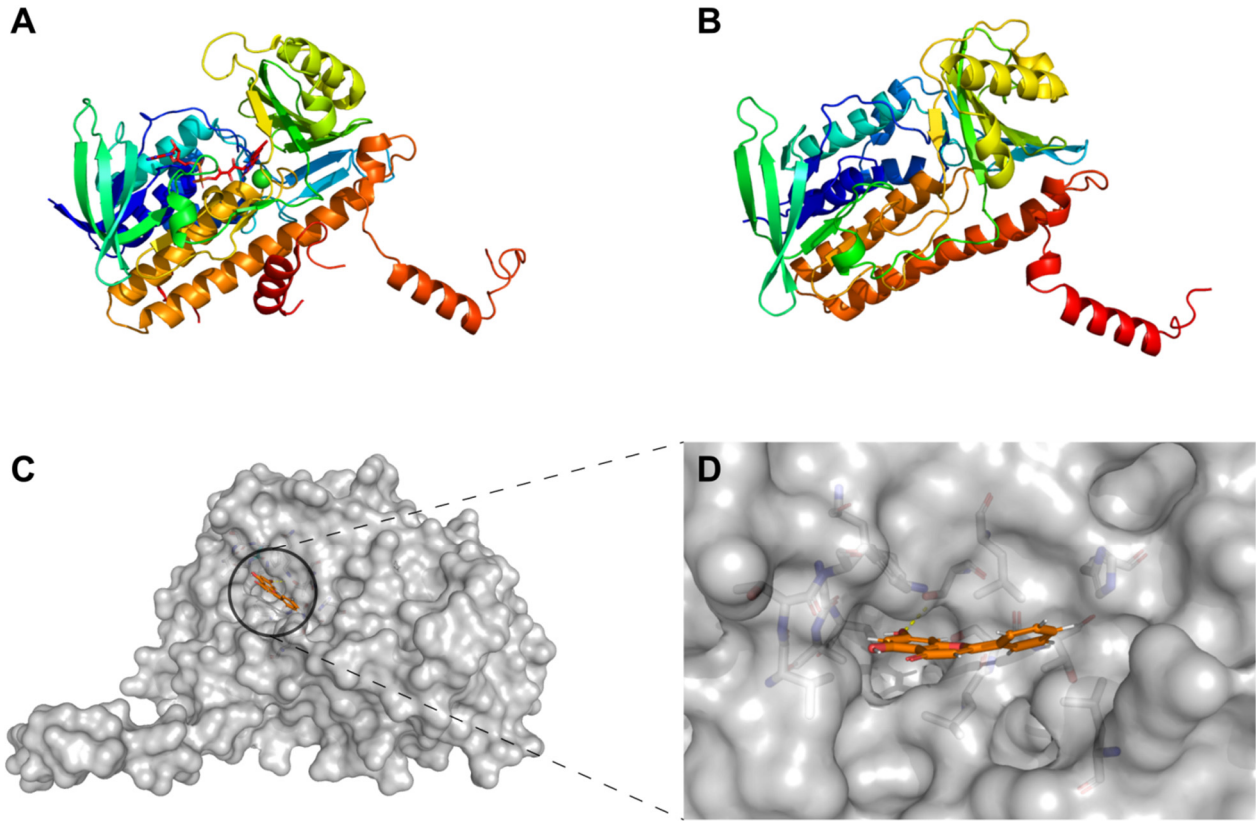


**Figure S5.** (**A**) Crystal structure of chondrochloren halogenase (CndH) from *Chondromyces crocatus* Cm c5 (PDB code 3E1T) in complex with the encased FAD (red) represented as sticks. Chloride (green) is shown as dot near to FAD binding site and active lysin-72 residue. (**B**) Derived homology model of DklH based on the crystal structure of CndH. (**C**) Surface representation of DklH homology model with the bound substrate chrysin. (**D**) Detailed view on substrate binding site of DklH. The ligand **2** (orange) is situated in a non-polar cavity of the protein. The A ring of flavonoid has deeply access to the narrow region near to active lysin-72, which transfers the chlorine to substrate. Therefor the putative binding mode correlates with observed halogenation pattern.

## HPLC-MS data

The LC-MS chromatograms of crude extracts are displayed for the feeding experiments of *S. albus* Del14 9B-A9 cultures supplemented with flavonoid in concentration of 0.02 mg/L in DM media. In the parallel experiment the feeding was also carried out with the controle strains *S. albus* Del14 and *S. albus* Del14 pSET-DklH under the same conditions (data not shown). The chromatograms shown below are represented as extractet ion chromatograms only highlighting the peaks with the corresponding mass of interest. The chlorinated deriviatives were identified by exact mass and isotope peaks. The exact position of halogen was nearly estimated by determination of the relative retention time ($\Delta t_R$) compared to unmodified flavonoid. Therefore the NMR data and chromatograms for chrysin **2** feeding revealed that a relative retention time of $\Delta t_R$ = 0.7 min correlates with halogenation in position 6 (**2a**). Otherwise the relative retention time of $\Delta t_R$ = 1.2 min indicates chlorination in position 8 (**2b**).

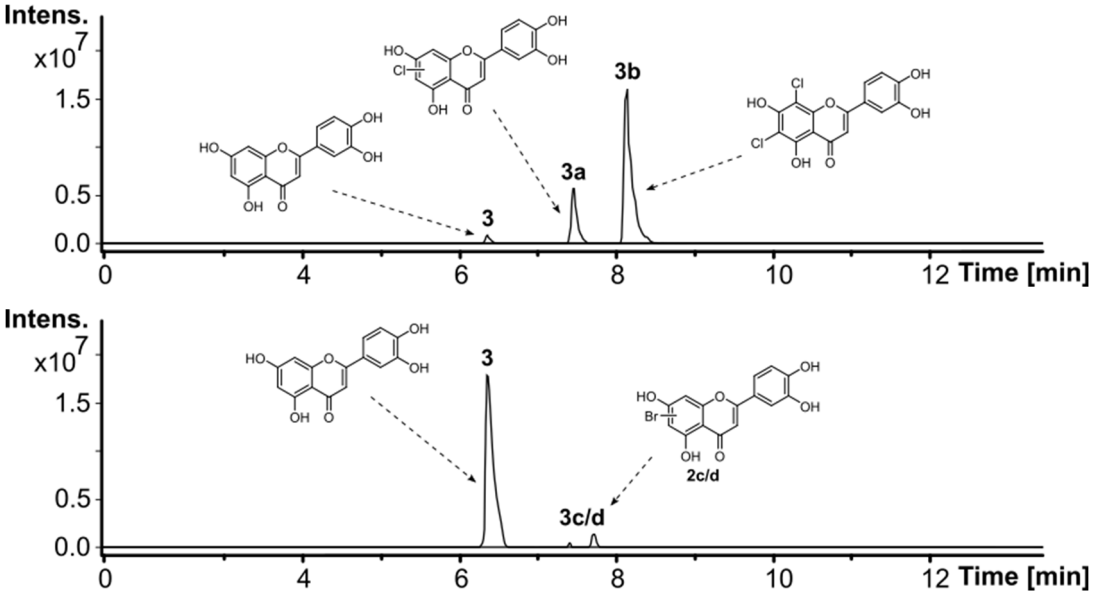


**Figure S6.** Extracted ion chromatogram of luteolin feeding experiment. The unmodified luteolin **3** ($t_R$ = 6.4 min, $[M+H]^+_{cal.}$ = 287.0550 m/z, $[M+H]^+_{obs.}$ = 287.0551 m/z) occurs only in small amounts. Due to relative retention time $\Delta t_R$ = 1.1 min the single chlorinated derivative **3a** ($t_R$ = 7.5 min, $[M+H]^+_{cal.}$ = 321.0160 m/z, $[M+H]^+_{obs.}$ = 321.0160 m/z) may correspond with 8-Cl-luteolin. The doubly chlorinated compound **3b** ($t_R$ = 8.2 min, $[M+H]^+_{cal.}$ = 354.9771 m/z, $[M+H]^+_{obs.}$ = 354.9722 m/z) strongly indicates halogenation in positions 6 and 8. The brominated compounds **3c** ($t_R$ = 7.2 min, $[M+H]^+_{cal.}$ = 364.9655 m/z, $[M+H]^+_{obs.}$ = 364.9661 m/z) and **3d** ($t_R$ = 7.7 min, $[M+H]^+_{cal.}$ = 364.9655 m/z, $[M+H]^+_{obs.}$ = 364.9661 m/z) are shown below.

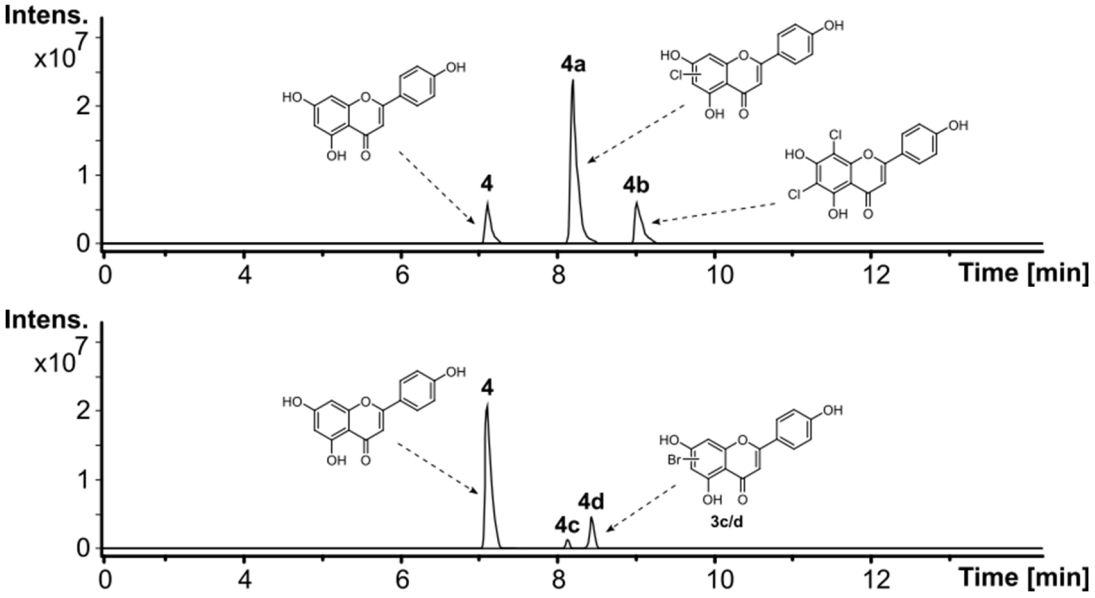


**Figure S7.** Extracted ion chromatogram of apigenin feeding experiment. Compound **4** ($t_R$ = 7.1 min, $[M+H]^+_{cal.}$ = 271.0601 m/z, $[M+H]^+_{obs.}$ = 271.0599 m/z) is equivalent to apigenin. The relative retention time $\Delta t_R$ = 1.1 min of compound **4a** ($t_R$ = 8.2 min, $[M+H]^+_{cal.}$ = 305.0211 m/z, $[M+H]^+_{obs.}$ = 305.0213 m/z) indicates chlorination in position 8. The doubly chlorinated compound **4b** ($t_R$ = 9.0 min, $[M+H]^+_{cal.}$ = 338.9822 m/z, $[M+H]^+_{obs.}$ = 338.9823 m/z) was proposed as 6,8-di-Cl-apigenin. The brominated derivatives of apigenin **4c** ($t_R$ = 8.2 min, $[M+H]^+_{cal.}$ = 348.9706 m/z, $[M+H]^+_{obs.}$ = 348.9712 m/z) and **4d** ($t_R$ = 8.5 min, $[M+H]^+_{cal.}$ = 348.9706 m/z, $[M+H]^+_{obs.}$ = 348.9711 m/z) are shown below.

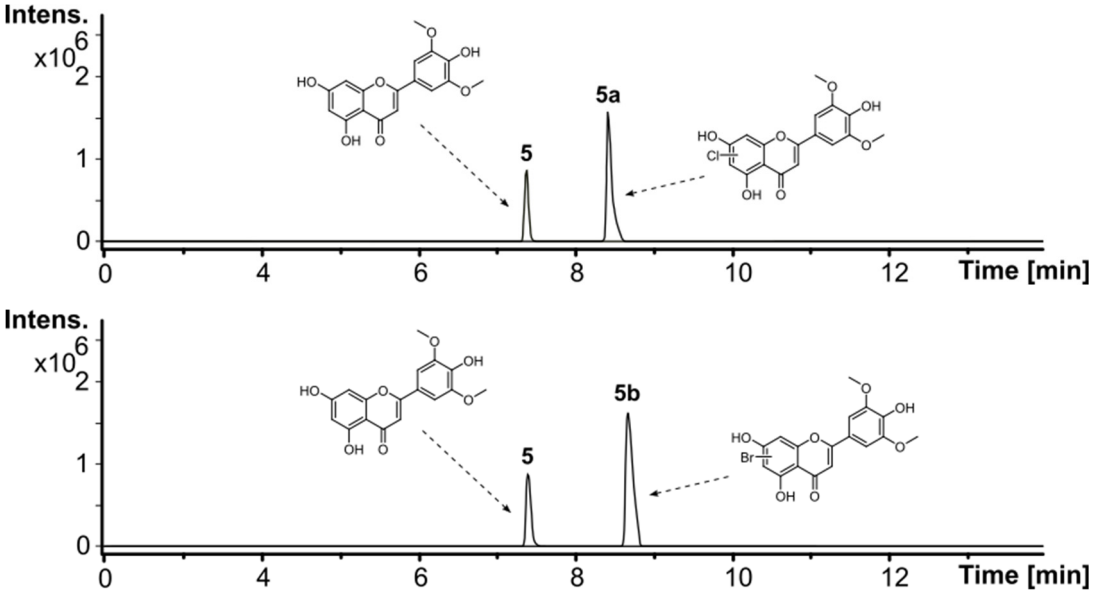


**Figure S8.** Extracted ion chromatogram of tricin feeding experiment. The O-methylated compound **5** ($t_R$ = 7.4 min, $[M+H]^+_{cal.}$ = 331.0812 m/z, $[M+H]^+_{obs.}$ = 331.0818 m/z) was identified as tricin. As chlorination product, only compound **5a** ($t_R$ = 8.4 min, $[M+H]^+_{cal.}$ = 365.0423 m/z, $[M+H]^+_{obs.}$ = 365.0419 m/z) with one chlorine at suggested position 8 ($\Delta t_R$ = 1.0 min) was found. The double chlorinated derivative of tricin is missing. The brominated analog **5b** ($t_R$ = 8.7 min, $[M+H]^+_{cal.}$ = 408.9917 m/z, $[M+H]^+_{obs.}$ = 408.9919 m/z) was obtained in reasonable yield.

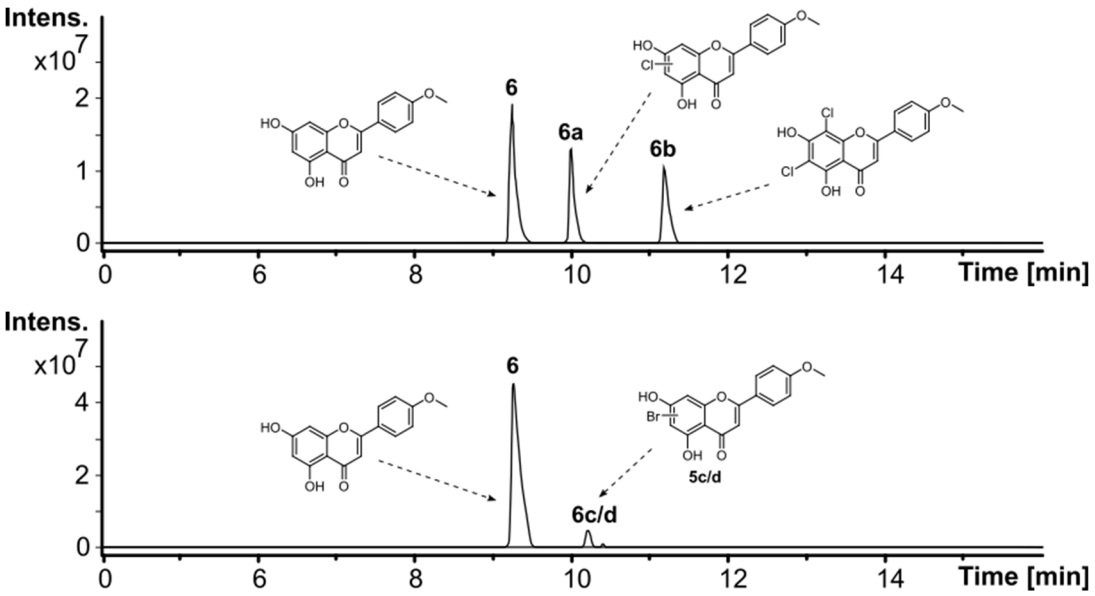


**Figure S9.** Extracted ion chromatogram of acacetin feeding experiment. The peak with strongest intensity corresponds with acacetin **6** ($t_R$ = 9.3 min, $[M+H]^+_{cal.}$ = 285.0757 m/z, $[M+H]^+_{obs.}$ = 285.0763 m/z). Due to calculation of relative retention time $\Delta t_R$ = 0.7 min, the compound **6a** ($t_R$ = 10.0 min, $[M+H]^+_{cal.}$ = 319.0368 m/z, $[M+H]^+_{obs.}$ = 319.0369 m/z) was proposed as 6-Cl-acacetin. The derivative **6b** ($t_R$ = 11.2 min, $[M+H]^+_{cal.}$ = 352.9978 m/z, $[M+H]^+_{obs.}$ = 352.9982 m/z) was therefore identified as doubly chlorinated reaction product 6,8-di-Cl-acacetin. The brominated acacetin analogs **6c** ($t_R$ = 10.2 min, $[M+H]^+_{cal.}$ = 362.9863 m/z, $[M+H]^+_{obs.}$ = 362.9863 m/z) and **6d** ($t_R$ = 10.6 min, $[M+H]^+_{cal.}$ = 362.9863 m/z, $[M+H]^+_{obs.}$ = 362.9860 m/z) are present in very small quantities.

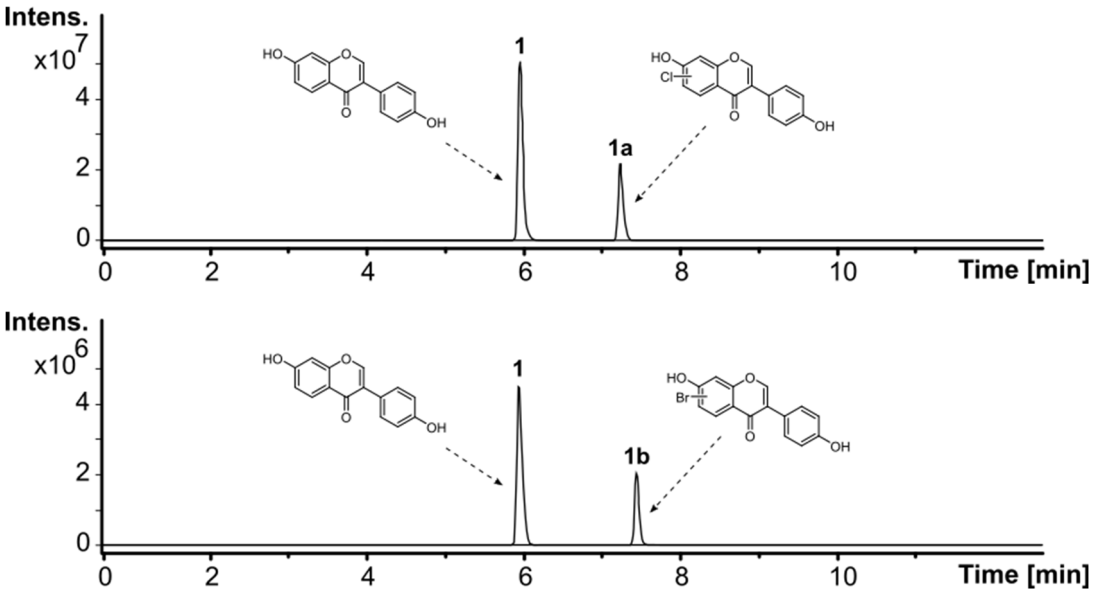


**Figure S10.** Extracted ion chromatogram of daidzein feeding experiment. Compound **1** ($t_R$ = 6.0 min, $[M+H]^+_{cal.}$ = 255.0652 m/z, $[M+H]^+_{obs.}$ = 255.0655 m/z) was identified as daidzein. The structure of chlorine containing derivative **1a** ($t_R$ = 7.2 min, $[M+H]^+_{cal.}$ = 289.0262 m/z, $[M+H]^+_{obs.}$ = 289.0266 m/z) was suggested as 8-Cl-daidzein ($\Delta t_R$ = 1.2 min) and confirmed by NMR analysis. The brominated analog **1b** ($t_R$ = 7.5 min, $[M+H]^+_{cal.}$ = 332.9757 m/z, $[M+H]^+_{obs.}$ = 332.9760 m/z) is represented below.

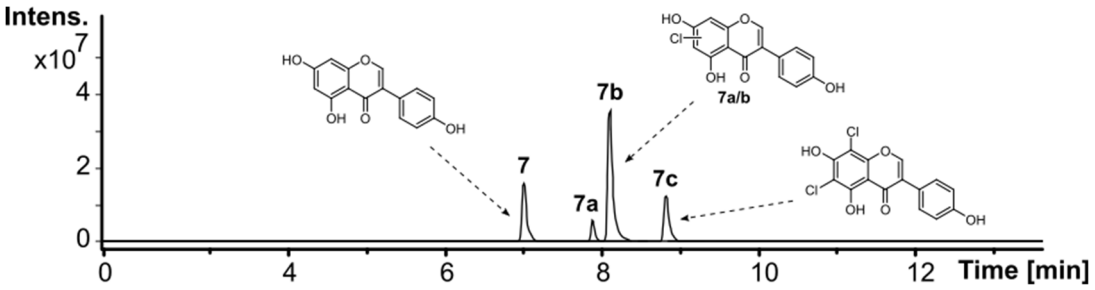


**Figure S11.** Extracted ion chromatogram of genistein feeding experiment. The unmodified compound **7** ($t_R$ = 7.0 min, $[M+H]^+_{cal.}$ = 271.0601 m/z, $[M+H]^+_{obs.}$ = 271.0602 m/z) correlates with genistein. For halogenation two derivatives containing one chlorine were obtained. The calculation of relative retention time $\Delta t_R$ = 0.9 min led to proposal that compound **7a** ($t_R$ = 7.9 min, $[M+H]^+_{cal.}$ = 305.0211 m/z, $[M+H]^+_{obs.}$ = 305.0212 m/z) shows chlorination in position 6. In contrast to that the relative retention time for **7b** ($t_R$ = 8.1 min, $[M+H]^+_{cal.}$ = 305.0211 m/z, $[M+H]^+_{obs.}$ = 305.0213 m/z) revealed 8-Cl-genistein as suggested product. The isotope peaks for derivative **7c** ($t_R$ = 8.8 min, $[M+H]^+_{cal.}$ = 338.9822 m/z, $[M+H]^+_{obs.}$ = 338.9824 m/z) indicates double chlorination.

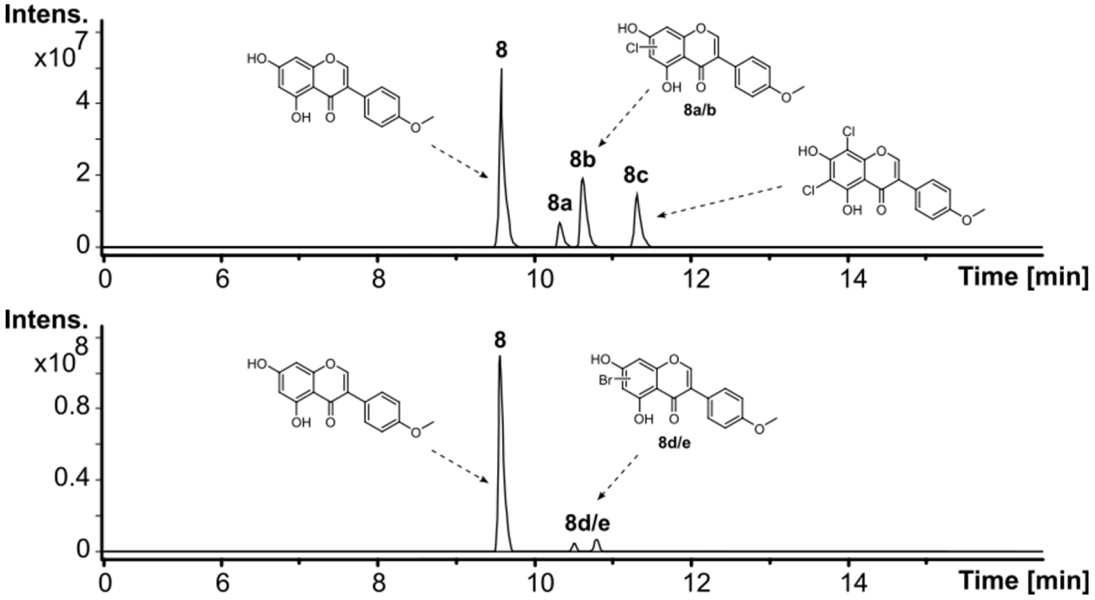


**Figure S12.** Extracted ion chromatogram of biochanin A feeding experiment. The peak with strongest intensity was identified as biochanin A **8** ($t_R$ = 9.6 min, $[M+H]^+_{cal.}$ = 285.0757 m/z, $[M+H]^+_{obs.}$ = 285.0770 m/z). Due to relative retention time of $\Delta t_R$ = 0.7 min the compound **8a** ($t_R$ = 10.3 min, $[M+H]^+_{cal.}$ = 319.0368 m/z, $[M+H]^+_{obs.}$ = 319.0372 m/z) strongly corresponds with one chlorine containing derivative and halogenation in position 6. Compared to that compound **8b** ($t_R$ = 10.6 min, $[M+H]^+_{cal.}$ = 319.0368 m/z, $[M+H]^+_{obs.}$ = 319.0376 m/z) was found in larger amount. The relative retention time of $\Delta t_R$ = 1.0 min for this peak correlates with proposed 8-Cl-biochanin A. The derivative **8c** ($t_R$ = 11.3 min, $[M+H]^+_{cal.}$ = 352.9978 m/z, $[M+H]^+_{obs.}$ = 352.9979 m/z) showed double incorporation of chlorine in the suggested position 6 and 8. The compounds **8d** ($t_R$ = 10.5 min, $[M+H]^+_{cal.}$ = 362.9863 m/z, $[M+H]^+_{obs.}$ = 362.9867 m/z) and **8e** ($t_R$ = 10.8 min, $[M+H]^+_{cal.}$ = 362.9863 m/z, $[M+H]^+_{obs.}$ = 362.9865 m/z) were identified as brominated analogs according to isotope peaks.

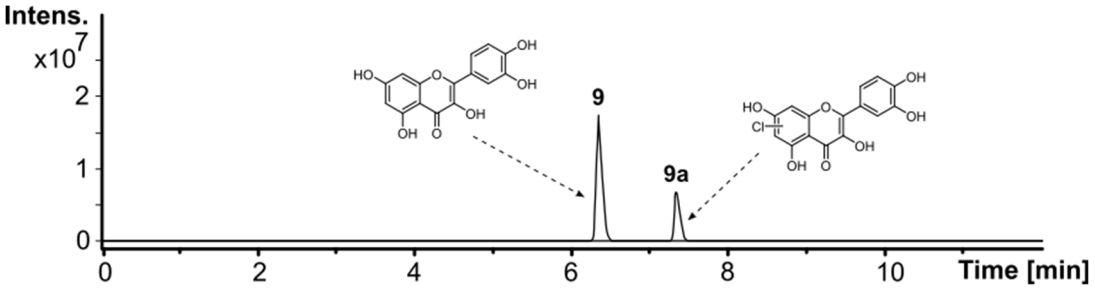


**Figure S13.** Extracted ion chromatogram of quercetin feeding experiment. The unmodified compound **9** ($t_R$ = 6.3 min, $[M+H]^+_{cal.}$ = 303.0499 m/z, $[M+H]^+_{obs.}$ = 303.0500 m/z) was identified as quercetin. Chlorine containing derivative **9a** ($t_R$ = 7.3 min, $[M+H]^+_{cal.}$ = 337.0110 m/z, $[M+H]^+_{obs.}$ = 337.0107 m/z) presumably corresponds with 8-Cl-quercetin ($\Delta t_R$ = 1.0 min).

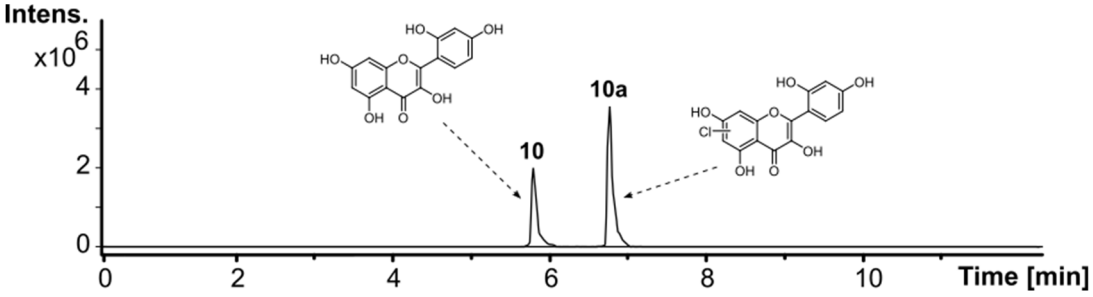


**Figure S14.** Extracted ion chromatogram of morin feeding experiment. Compound **10** ($t_R$ = 5.8 min, $[M+H]^+_{cal.}$ = 303.0499 m/z, $[M+H]^+_{obs.}$ = 303.0501 m/z) was identified as morin. The related derivative **10a** ($t_R$ = 6.8 min, $[M+H]^+_{cal.}$ = 337.0110 m/z, $[M+H]^+_{obs.}$ = 337.0108 m/z) with relative retention time of $\Delta t_R$ = 1.0 min correlates with 8-Cl-morin.

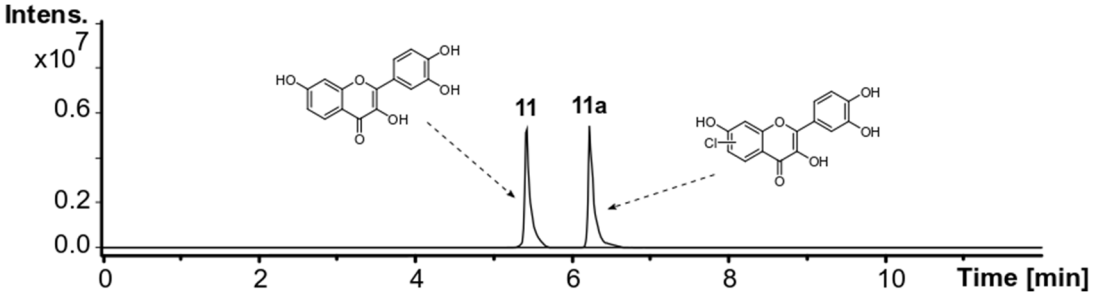


**Figure S15.** Extracted ion chromatogram of fisetin feeding experiment. For fisetin **11** ($t_R$ = 5.4 min, $[M+H]^+_{cal.}$ = 287.0550 m/z, $[M+H]^+_{obs.}$ = 287.0550 m/z) the chlorine containing derivative **11a** ($t_R$ = 6.2 min, $[M+H]^+_{cal.}$ = 321.0160 m/z, $[M+H]^+_{obs.}$ = 321.0162 m/z) was obtained in equal amounts. The proposed halogenation position 8 was identified due to relative retention time of $\Delta t_R$ = 1.2 min.

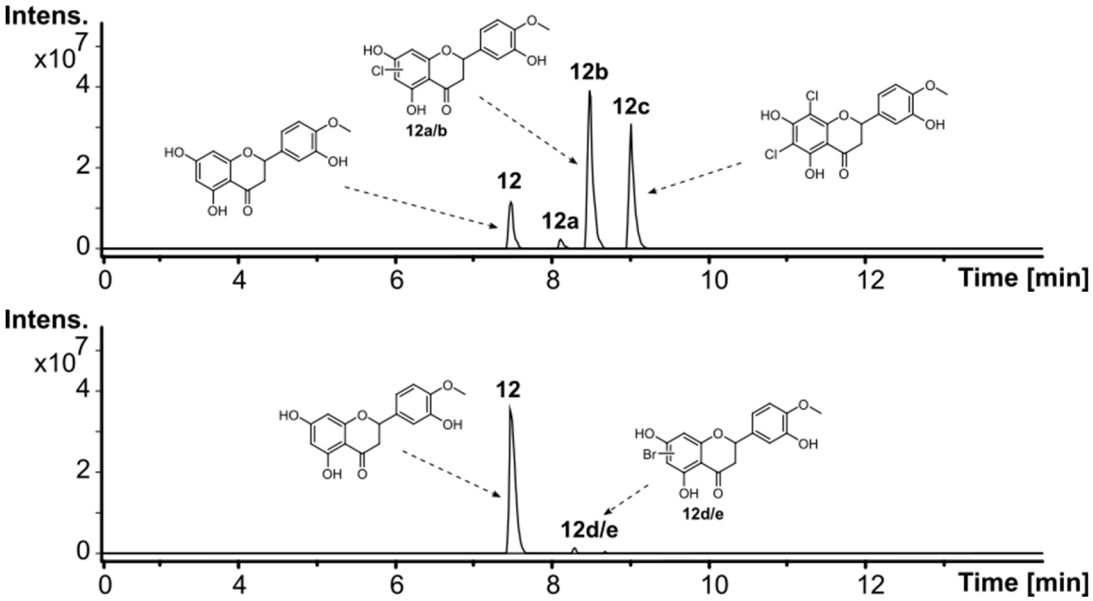


**Figure S16.** Extracted ion chromatogram of hesperetin feeding experiment. The first peak with small intensity **12** ($t_R$ = 7.5 min, $[M+H]^+_{cal.}$ = 303.0863 m/z, $[M+H]^+_{obs.}$ = 303.0866 m/z) was identified as hesperetin. For halogenation two derivatives containing one chlorine in different positions were obtained. With aide of relative retention time of $\Delta t_R$ = 0.6 min compound **12a** ($t_R$ = 8.1 min, $[M+H]^+_{cal.}$ = 337.0473 m/z, $[M+H]^+_{obs.}$ = 337.0475 m/z) corresponds with proposed 6-Cl-hesperetin. Moreover derivative **12b** ($t_R$ = 8.5 min, $[M+H]^+_{cal.}$ = 337.0473 m/z, $[M+H]^+_{obs.}$ = 337.0479 m/z) shows a relative retention time of $\Delta t_R$ = 1.0 min which indicates chlorination in position 8. For double incorporation of chlorine compound **12c** ($t_R$ = 9.0 min, $[M+H]^+_{cal.}$ = 371.0084 m/z, $[M+H]^+_{obs.}$ = 371.0088 m/z) was identified. The bromine containing derivatives **12d** ($t_R$ = 8.3 min, $[M+H]^+_{cal.}$ = 380.9968 m/z, $[M+H]^+_{obs.}$ = 380.9966 m/z) and **12e** ($t_R$ = 8.7 min, $[M+H]^+_{cal.}$ = 380.9968 m/z, $[M+H]^+_{obs.}$ = 380.9969 m/z) were detected in very small amounts.

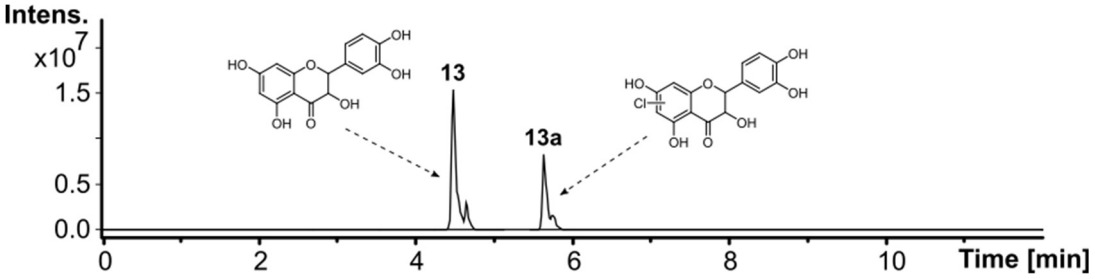


**Figure S17.** Extracted ion chromatogram of taxifolin feeding experiment. The first peak indicates presence of unmodified taxifolin **13** ($t_R$ = 4.5 min, $[M+H]^+_{cal.}$ = 305.0656 m/z, $[M+H]^+_{obs.}$ = 305.0655 m/z). Compound **13a** ($t_R$ = 5.7 min, $[M+H]^+_{cal.}$ = 339.0266 m/z, $[M+H]^+_{obs.}$ = 339.0267 m/z) was identified as 8-Cl-taxifolin according to relative retention time of $\Delta t_R$ = 1.2 min.

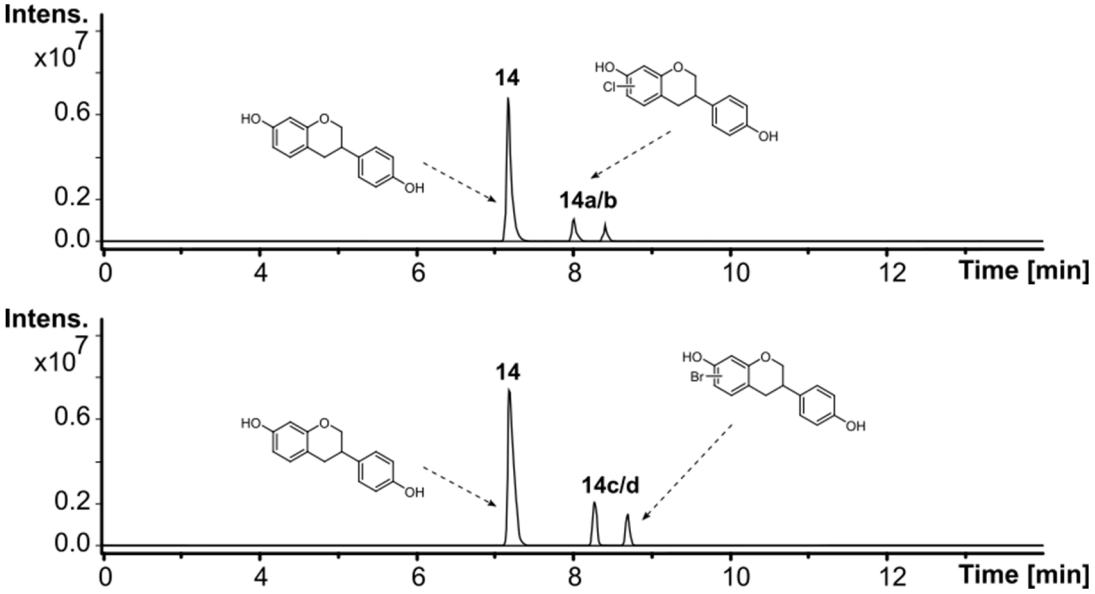


**Figure S18.** Extracted ion chromatogram of *R,S*-equol feeding experiment. The peak with strongest intensity **14** ($t_R$ = 7.2 min, $[M+H]^+_{cal.}$ = 243.1016 m/z, $[M+H]^+_{obs.}$ = 243.1016 m/z) was identified as *R,S*-equol. Two reactions products were obtained for chlorination experiment as well as for bromination. Due to relative retention time of $\Delta t_R$ = 0.8 min the compound **14a** ($t_R$ = 8.0 min, $[M+H]^+_{cal.}$ = 277.0626 m/z, $[M+H]^+_{obs.}$ = 277.0624 m/z) correlates with proposed R,S-6-Cl-equol. The derivative **14b** ($t_R$ = 8.4 min, $[M+H]^+_{cal.}$ = 277.0624 m/z, $[M+H]^+_{obs.}$ = 277.0624 m/z) with relative retention time of $\Delta t_R$ = 1.2 min shows proposed chlorination in position 8. The compounds **14c** ($t_R$ = 8.3 min, $[M+H]^+_{cal.}$ = 321.0121 m/z, $[M+H]^+_{obs.}$ = 321.0115 m/z) and **14d** ($t_R$ = 8.7 min, $[M+H]^+_{cal.}$ = 321.0121 m/z, $[M+H]^+_{obs.}$ = 321.0120 m/z) represents the bromine containing flavonoids.

**Table S2.** LC-MS quantification and AUC determination for the chlorinated flavonoids. The data observed from the feeding experiment for the strain *S. albus* Del14 9B-A9 were calculated form UV absorption at 280 nm.

| Flavonoid | 6-$Cl_1$ [AUC] | 8-$Cl_1$ [AUC] | 6,8-$Cl_2$ [AUC] | Unmodified [AUC] |
|---|---|---|---|---|
| Chrysin (**2**) | 40043 | 2669 | 6874 | 16617 |
| Luteolin (**3**) | 201684 | n.d. | 1420409 | 18617 |
| Apigenin (**4**) | 136715 | n.d. | 29021 | 12521 |
| Tricin (**5**) | 12525 | n.d. | n.d. | 7521 |
| Acacetin (**6**) | 27410 | n.d. | 37091 | 26545 |
| Daidzein (**1**) | 93633 | n.d. | n.d. | 284223 |
| Genistein (**7**) | 12100 | 107345 | 58138 | 28119 |
| Biochanin A (**8**) | 16755 | 63109 | 73077 | 129896 |
| Quercetin (**9**) | 19172 | n.d. | n.d. | 35531 |
| Morin (**10**) | 111159 | n.d. | n.d. | 42814 |
| Fisetin (**11**) | 10371 | n.d. | n.d. | 11142 |
| Hesperetin (**12**) | 23933 | 1216164 | 1127255 | 238997 |
| Taxifolin (**13**) | 15699 | n.d. | n.d. | 86157 |
| R,S-Equol (**14**) | 166125 | 56245 | n.d. | 1322615 |

**Table S3.** Yield [%] determination for the chlorinated flavonoids based on the estimated AUC

| Flavonoid | 6-$Cl_1$ [%] | 8-$Cl_1$ [%] | 6,8-$Cl_2$ [%] | Unmodified [%] |
|---|---|---|---|---|
| Chrysin (**2**) | 60.8 | 4.0 | 10.3 | 24.9 |
| Luteolin (**3**) | 12.2 | n.d. | 86.6 | 1.1 |
| Apigenin (**4**) | 76.7 | n.d. | 16.3 | 7.0 |
| Tricin (**5**) | 62.5 | n.d. | n.d. | 37.5 |
| Acacetin (**6**) | 30.1 | n.d. | 40.7 | 29.2 |
| Daidzein (**1**) | 24.8 | n.d. | n.d. | 75.2 |
| Genistein (**7**) | 5.9 | 52.2 | 28.3 | 13.7 |
| Biochanin A (**8**) | 5.9 | 22.3 | 25.8 | 45.9 |
| Quercetin (**9**) | 35.0 | n.d. | n.d. | 65.0 |
| Morin (**10**) | 72.2 | n.d. | n.d. | 27.8 |
| Fisetin (**11**) | 48.2 | n.d. | n.d. | 51.8 |
| Hesperetin (**12**) | 9.2 | 46.7 | 43.3 | 9.2 |
| Taxifolin (**13**) | 15.4 | n.d. | n.d. | 84.6 |
| R,S-Equol (**14**) | 10.7 | 3.6 | n.d. | 85.6 |

**Table S4.** LC-MS quantification and AUC determination for the brominated flavonoids. The data observed from the feeding experiment for the strain *S. albus* Del14 9B-A9 were calculated form UV absorption at 280 nm.

| Flavonoid | 6-Br1 [AUC] | 8-Br1 [AUC] | Unmodified [AUC] |
|---|---|---|---|
| Chrysin (**2**) | 391945 | 53510 | 1000756 |
| Luteolin (**3**) | 894661 | 6332011 | 78843016 |
| Apigenin (**4**) | 24609 | 206991 | 750257 |
| Tricin (**5**) | 28030 | n.d. | 7521 |
| Acacetin (**6**) | 907588 | 0.09 | 8277759 |
| Daidzein (**1**) | 81874 | n.d. | 124570 |
| Genistein (**7**) | Not tested | Not tested | Not tested |
| Biochanin A (**8**) | 10723 | 14782 | 265075 |
| Quercetin (**9**) | Not tested | Not tested | Not tested |
| Morin (**10**) | Not tested | Not tested | Not tested |
| Fisetin (**11**) | Not tested | Not tested | Not tested |
| Hesperetin (**12**) | 26609 | 15665 | 1054121 |
| Taxifolin (**13**) | Not tested | Not tested | Not tested |
| R,S-Equol (**14**) | 138946 | 93490 | 701993 |

**Table S5.** Yield [%] determination for the chlorinated flavonoids based on the estimated AUC

| Flavonoid | 6-Br1 [%] | 8-Br1 [%] | Unmodified [%] |
|---|---|---|---|
| Chrysin (**2**) | 27.1 | 3.7 | 69.2 |
| Luteolin (**3**) | 1.0 | 7.4 | 91.6 |
| Apigenin (**4**) | 2.5 | 21.1 | 76.4 |
| Tricin (**5**) | 78.8 | n.d. | 21.2 |
| Acacetin (**6**) | 9.9 | - | 90.1 |
| Daidzein (**1**) | 39.7 | n.d. | 60.3 |
| Genistein (**7**) | Not tested | Not tested | Not tested |
| Biochanin A (**8**) | 3.7 | 5.1 | 91.2 |
| Quercetin (**9**) | Not tested | Not tested | Not tested |
| Morin (**10**) | Not tested | Not tested | Not tested |
| Fisetin (**11**) | Not tested | Not tested | Not tested |
| Hesperetin (**12**) | 2.4 | 1.4 | 96.1 |
| Taxifolin (**13**) | Not tested | Not tested | Not tested |
| R,S-Equol (**14**) | 14.9 | 10.0 | 75.1 |

## NMR data

**Table S6.** $^{13}$C-NMR (125 MHz) and $^{1}$H-NMR (500 MHz) data of 8-Cl-daidzein and reference date from the literature [5].

| 8-Cl-daidzein (**6a**) | | | Lit. 8-Cl-daidzein | |
|---|---|---|---|---|
| **pos.** | **δC (ppm)** | **δH (ppm, *m*, *J* Hz)** | **δC (ppm)** | **δH (ppm, *m*, *J* Hz)** |
| **CH-2** | 154.7 | 8.26 (1H, s) | 152.9 | 8.25 (1H, s) |
| **C-3** | 126.3 | | 124.9 | |
| **C-4** | 177.9 | | 176.4 | |
| **C-4a** | 109.0 | | 107.5 | |
| **CH-5** | 126.1 | 8.00 (1H, d J = 8.9) | 124.2 | 7.97 (1H, d, J = 9.0) |
| **CH-6** | 116.2 | 7.06 (1H, d J = 8.9) | 115.9 | 7.01 (1H, d, J = 9.0) |
| **C-7** | 160.9 | | 161.9 | |
| **C-8** | 119.1 | | 116.2 | |
| **C-8a** | 155.5 | | 154.2 | |
| **C-1'** | 124.0 | | 122.6 | |
| **CH-2'/6'** | 131.6 | 7.37-7.41 (2H, m) | 130.1 | 7.42-7.40 (2H, m) |
| **CH-3'/5'** | 116.4 | 6.84-6.87 (2H, m) | 114.8 | 6.88-6.86 (2H, m) |
| **C-4'** | 159.1 | | 157.4 | |

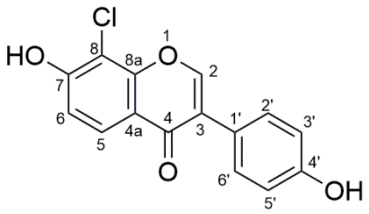


**Figure S19**. Structure of 8-Cl-daidzein.

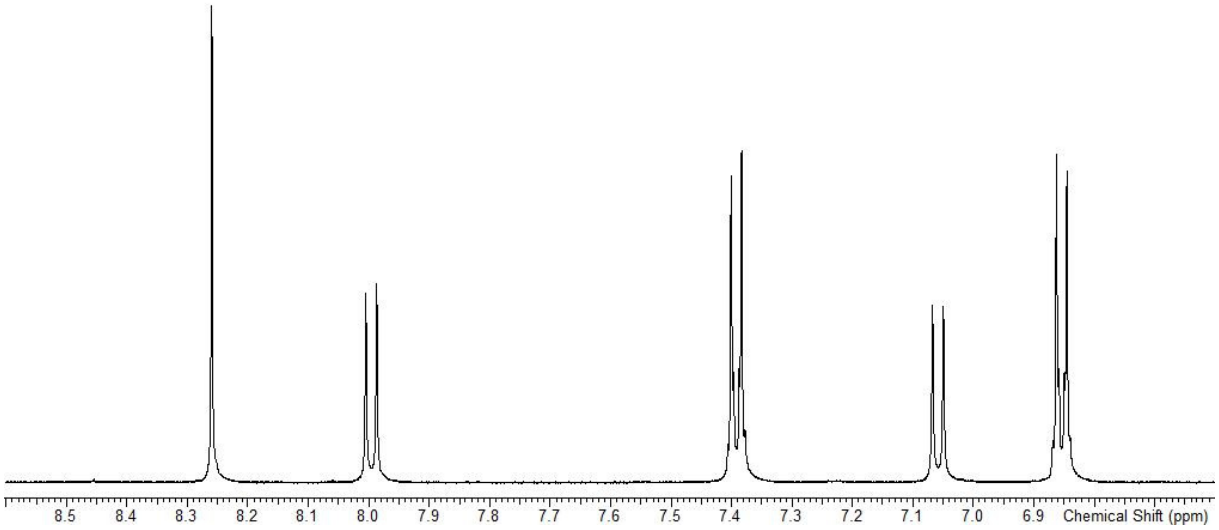


**Figure S20.** $^{1}$H-NMR spectrum of 8-Cl-daidzein (DMSO-$d_6$, 500 MHz).

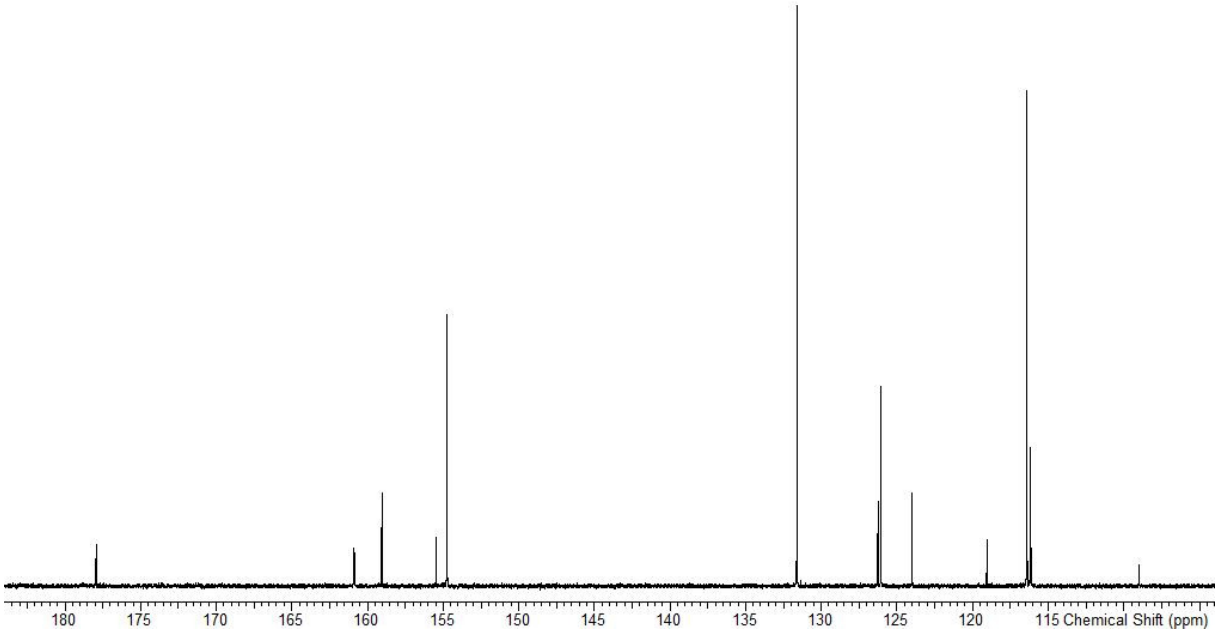


**Figure S21.** $^{13}$C-NMR spectrum of 8-Cl-daidzein (DMSO-$d_6$, 125 MHz).

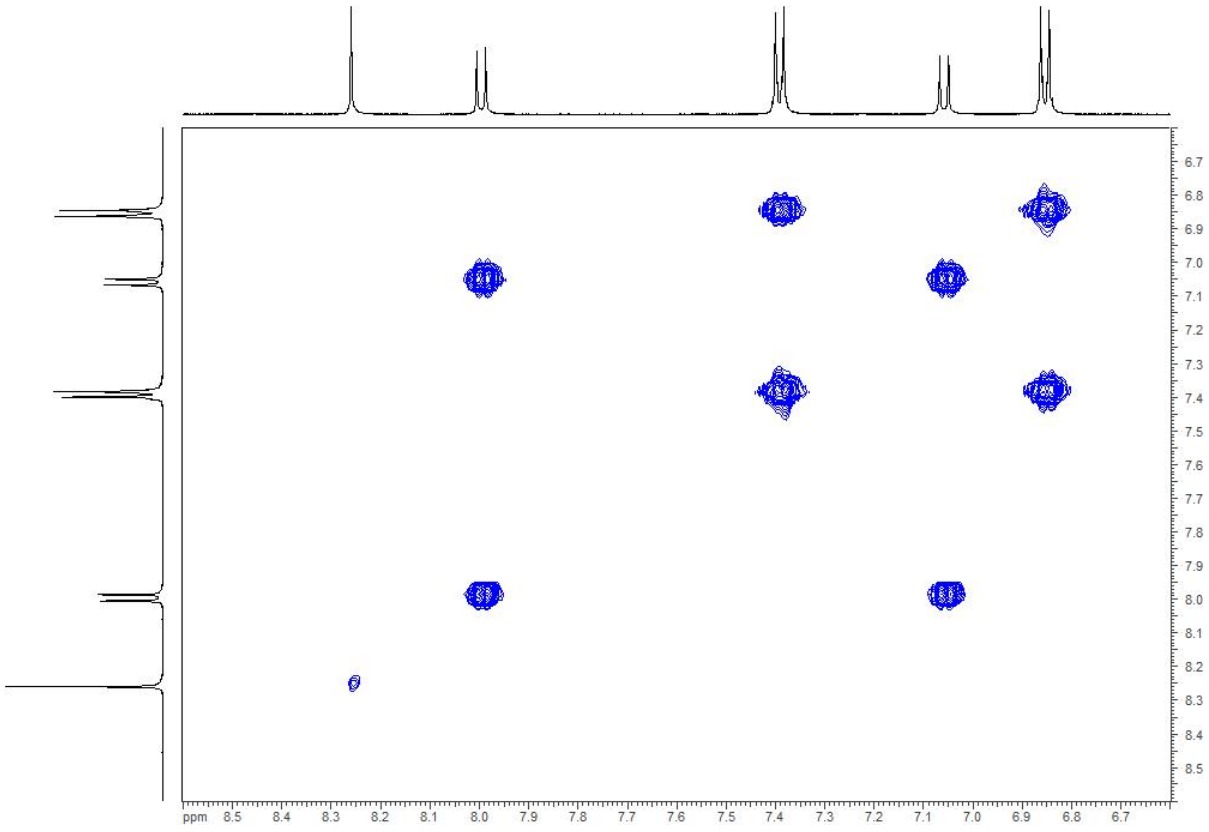


**Figure S22.** COSY spectrum of 8-Cl-daizein (DMSO-$d_6$, 500 MHz).

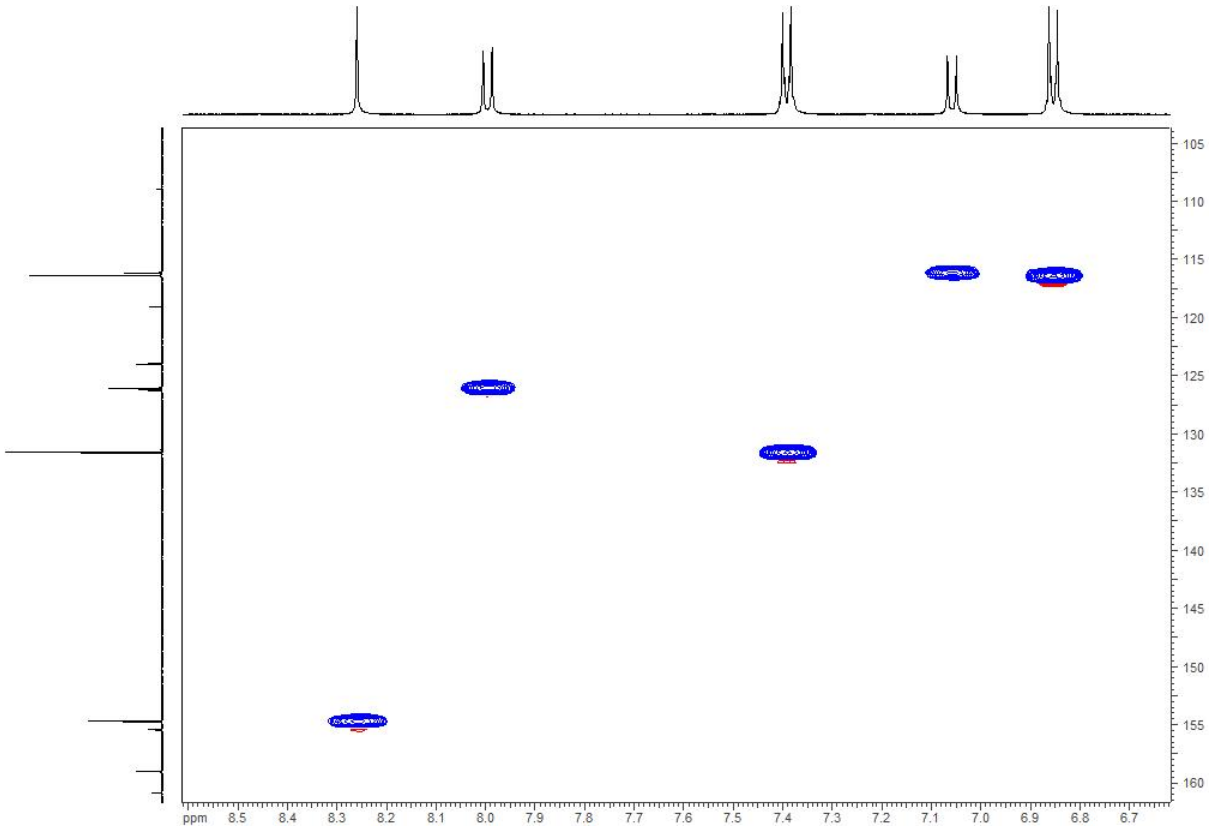


**Figure S23.** HSQC spectrum of 8-Cl-daidzein (DMSO-$d_6$, 500 MHz).

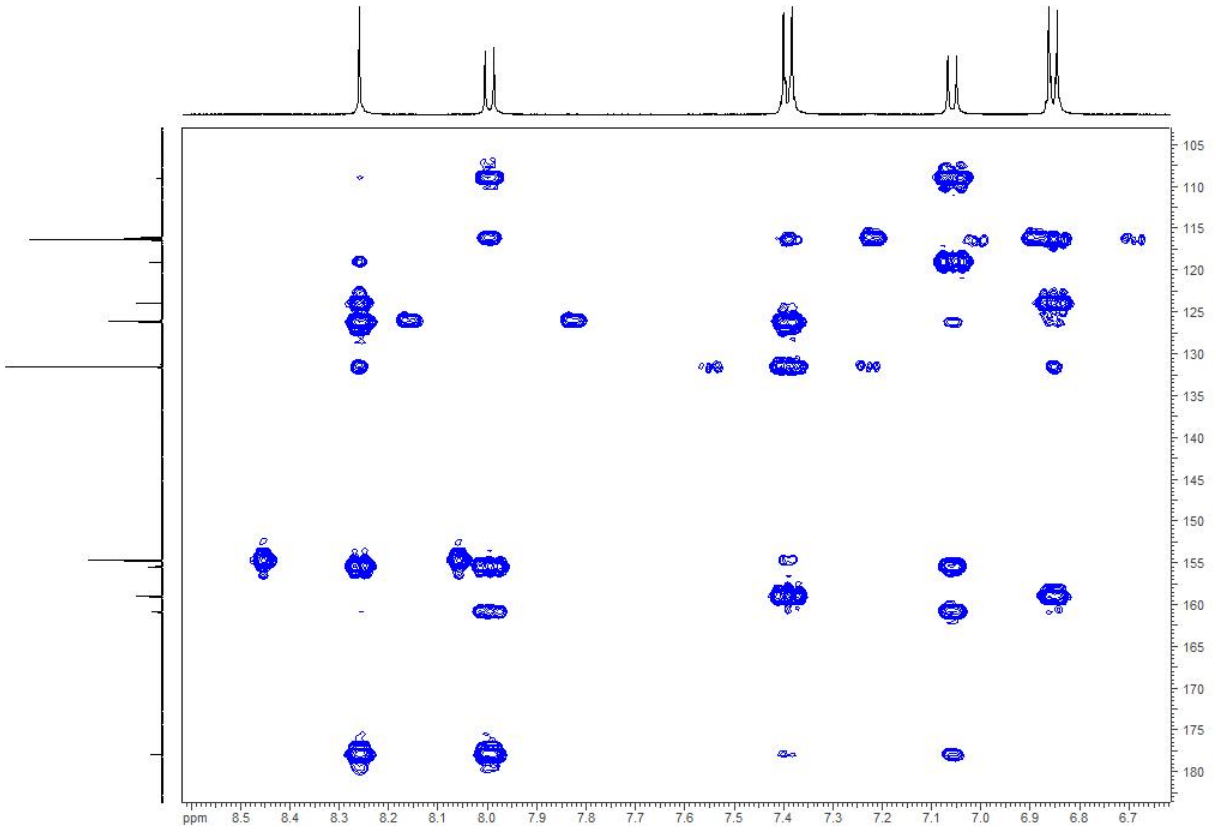


**Figure S24.** HMBC spectrum of 8-Cl-daidzein (DMSO-$d_6$, 500 MHz).

**Table S7**. $^{13}$C-NMR (125 MHz) and $^{1}$H-NMR (500 MHz) data of 6-Cl-chrysin **2a** and the minor side product 8-Cl-chrysin **2b** including their associated HOSE (Hierarchical Organisation of Spherical Environments) code predictions from ACD.

| | 6-Cl-chrysin (**2a**) | | HOSE 6-Cl-chrysin | | 8-Cl-chrysin (**2b**) | | HOSE 8-Cl-chrysin | |
|---|---|---|---|---|---|---|---|---|
| **pos.** | **δc (ppm)** | **δH (ppm, *m*, *J* Hz)** | **δc (ppm)** | **δH (ppm, *m*, *J* Hz)** | **δc (ppm)** | **δH (ppm, *m*, *J* Hz)** | **δc (ppm)** | **δH (ppm, *m*, *J* Hz)** |
| **C-2** | 163.8 | | 164.4 | | 163.1 | | 163.3 | |
| **CH-3** | 105.3 | 7.04 (1H, *s*) | 105.5 | 6.51(1H, *s*) | n.a** | 7.09 (1H, ovl.) | 104.9 | 6.80 (1H, *s*) |
| **C-4** | 181.9 | | 180.9 | | 182.0 | | 180.3 | |
| **C-5** | 157.2 | | 155.8 | | 160.8 | | 161.4 | |
| **C/CH-6** | 103.4 | | 103.6 | | n.a** | 6.41 (1H, ovl.) | 100.0 | 6.61 (1H, *s*) |
| **C-7** | 160.9 | | 158.1 | | 161.2 | | 160.1 | |
| **C/CH-8** | 94.8 | 6.72 (1H, *s*) | 95.6 | 6.70 (1H, *s*) | 98.3 | | 100.5 | |
| **C-9** | 155.5 | | 157.1 | | n.a** | | 153.5 | |
| **C-10** | 104.1 | | 102.1 | | 104.7 | | 105.0 | |
| **C-1'** | 130.9 | | 131.1 | | n.a** | | 131.1 | |
| **CH-2'/6'** | 126.9 | 8.08 (2H, *d*, *J* = 7.3) | 126.5 | 7.94 (2H, *d*, *J* = 7.2) | n.a** | ovl.* | 126.0 | 7.91 (2H, *d*, *J* = 7.2) |
| **CH-3'/5'** | 129.6 | 7.58 (2H, *t*, *J* = 7.3) | 129.2 | 7.53, (2H, *t*, *J* = 7.3) | n.a** | ovl.* | 129.1 | 7.49 (2H, *t*, *J* = 7.3) |
| **CH-4'** | 132.6 | 7.62 (1H, *t*, *J* = 7.3) | 132.0 | 7.55 (1H, *t*, *J* = 7.3) | 130.9 | ovl.* | 131.7 | 7.51 (1H, *t*, *J* = 7.3) |

* overlapping signals ** not available

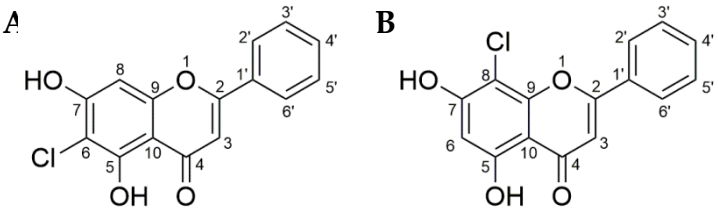


**Figure S25.** Structure of 6-Cl-chrysin (**A**) and 8-Cl-chrysin (**B**).

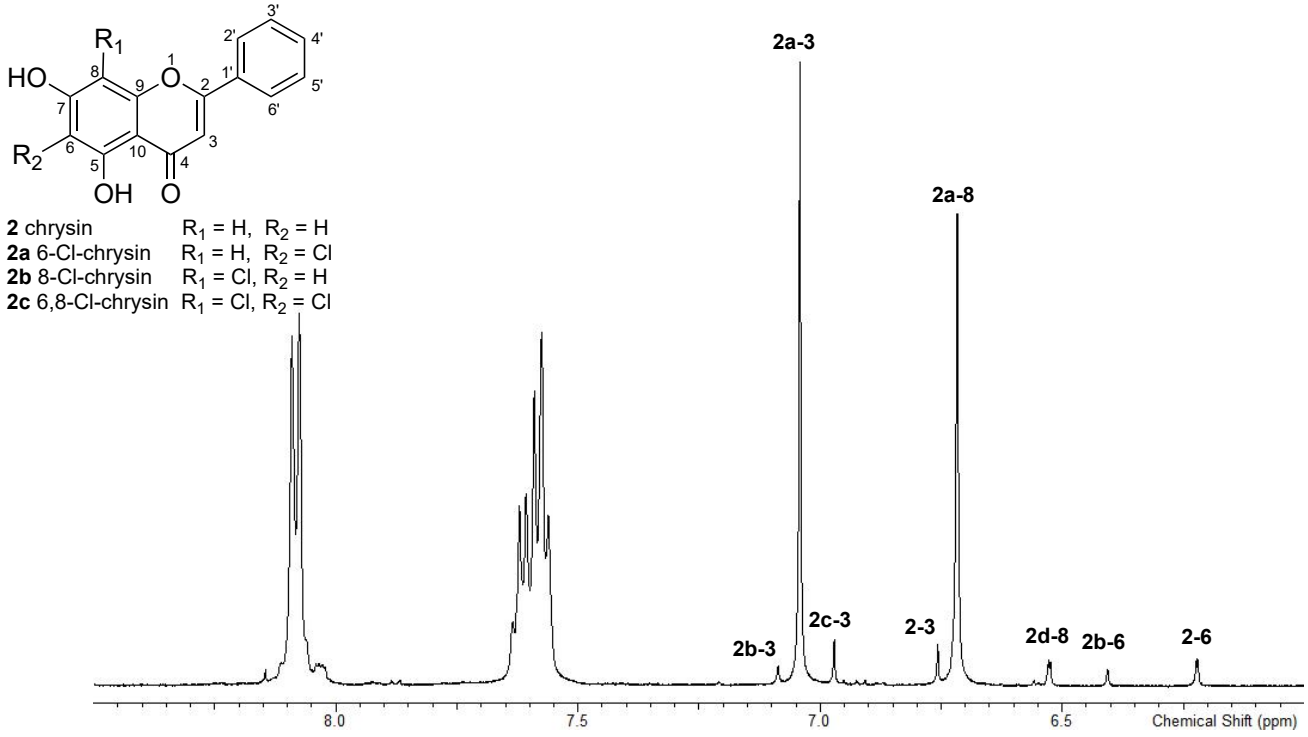


**Figure S26**. [1]H-NMR spectrum showing the compounds A-D and the signals of protons 3, 6 and 8 of **2a**, **2b**, **2c** and the precursor **2** (DMSO-d6, 500 MHz).

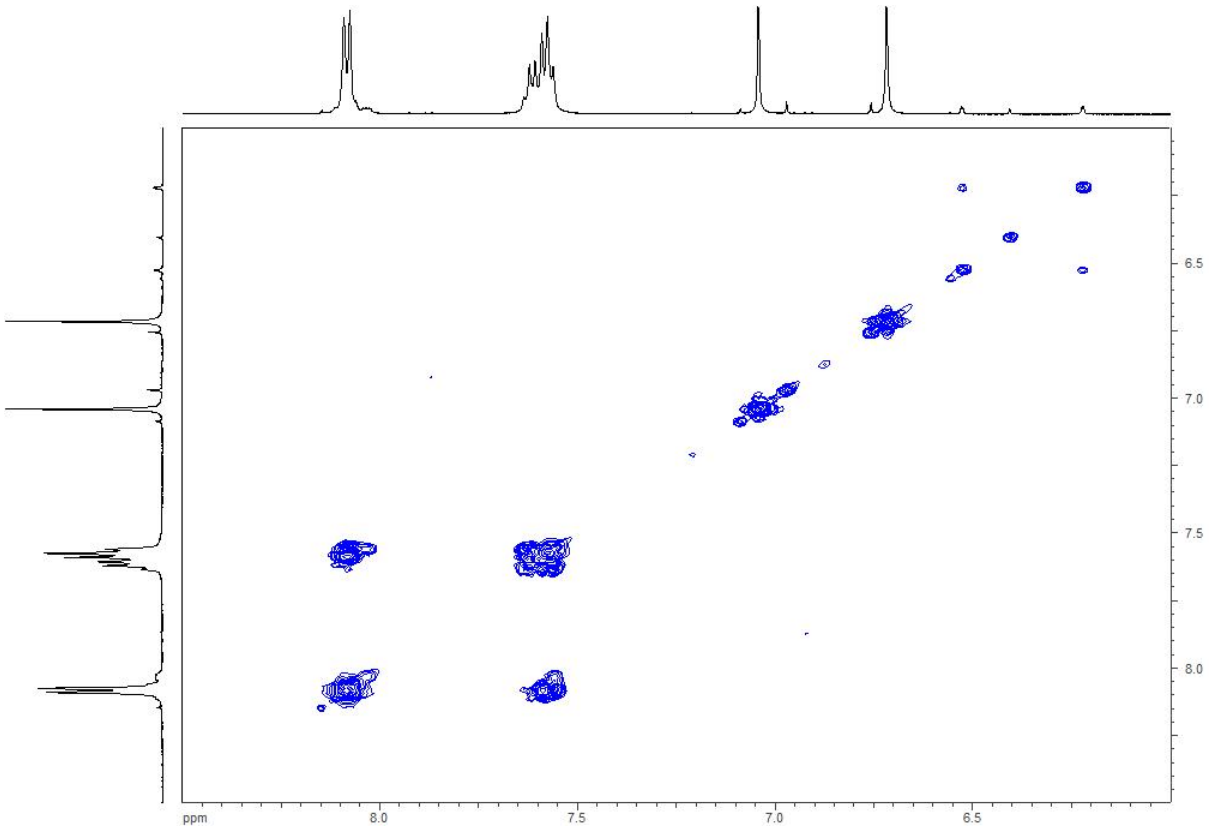


**Figure S27.** COSY spectrum of 6-Cl-chrysin including side products (DMSO-$d_6$, 500 MHz).

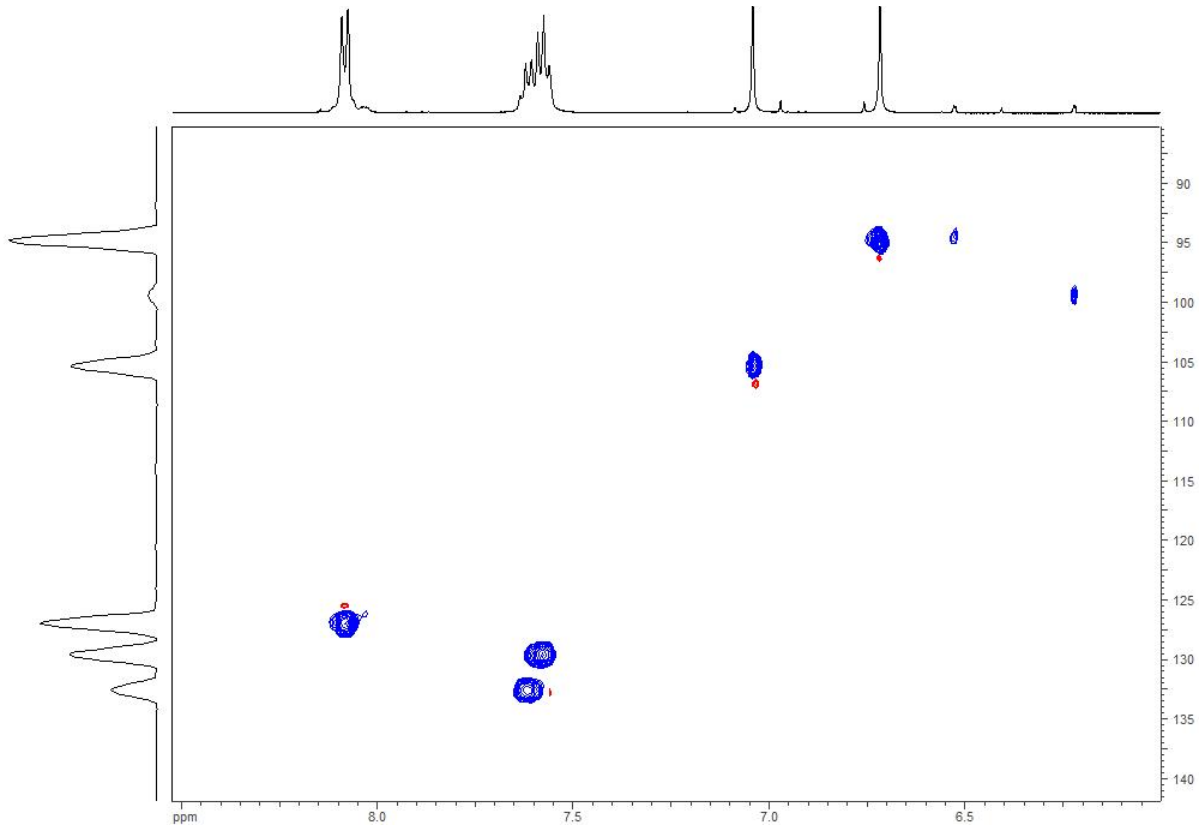


**Figure S28.** Edited-HSQC spectrum of 6-Cl-chrysin including side products (DMSO-$d_6$, 500 MHz).

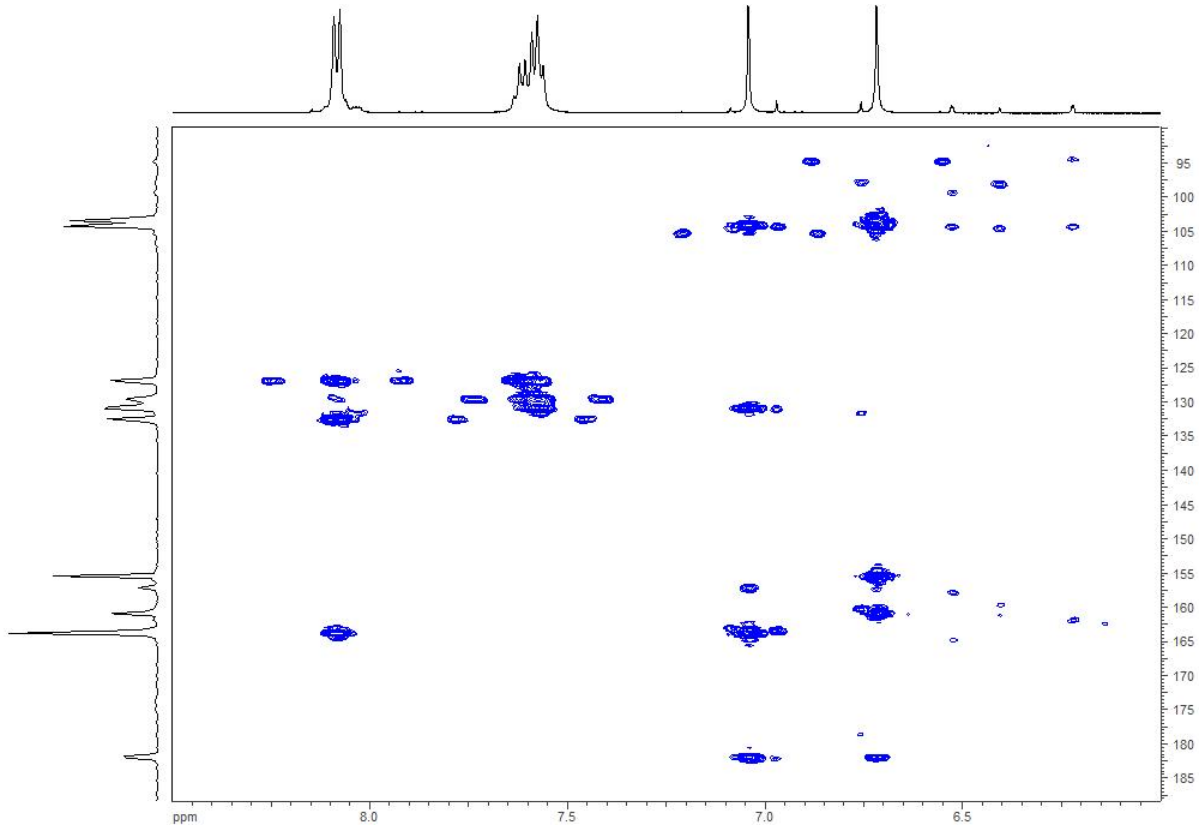


**Figure S29.** HMBC spectrum of 6-Cl-chrysin including side products (DMSO-$d_6$, 500 MHz).

**Table S8.** $^{13}$C-NMR (125 MHz) and $^{1}$H-NMR (500 MHz) data of 6,8-Cl-chrysin **2c** including the associated HOSE (Hierarchical Organisation of Spherical Environments) code predictions from ACD.

| | 6,8-Cl-chrysin (**2c**) | | HOSE 6,8-Cl-chrysin | |
|---|---|---|---|---|
| **pos.** | **δC (ppm)** | **δH (ppm, *m*, *J* Hz)** | **δC (ppm)** | **δH (ppm, *m*, *J* Hz)** |
| **C-2** | 161.626 | | 163.81 | |
| **CH-3** | 104.655 | 6.965(1H, ovl.*) | 106.41 | 6.83 (1H, *s*) |
| **C-4** | 180.003 | | 179.59 | |
| **C-5** | 155.015 | | 155.92 | |
| **C-6** | 105.397 | | 105.84 | |
| **C-7** | 155.018 | | 155.45 | |
| **C-8** | 99.594 | | 100.13 | |
| **C-9** | 151.113 | | 152.42 | |
| **C-10** | 100.814 | | 101.61 | |
| **C-1'** | 130.816 | | 131.07 | |
| **CH-2'/6'** | 126.119 | 8.068 (2H, ovl.*) | 126.00 | 7.91 (2H, *d*, *J* = 7.2) |
| **CH-3'/5'** | 129.246 | 7.575 (2H, ovl.*) | 129.12 | 7.49 (2H, *d*, *J* = 7.3) |
| **CH-4'** | 131.928 | 7.598 (1H, ovl.*) | 131.71 | 7.51 (1H, *d*, *J* = 7.3) |

* overlapping signals

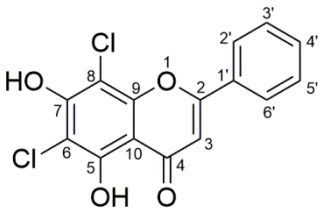


**Figure S30.** Structure of 6,8-Cl-chrysin.

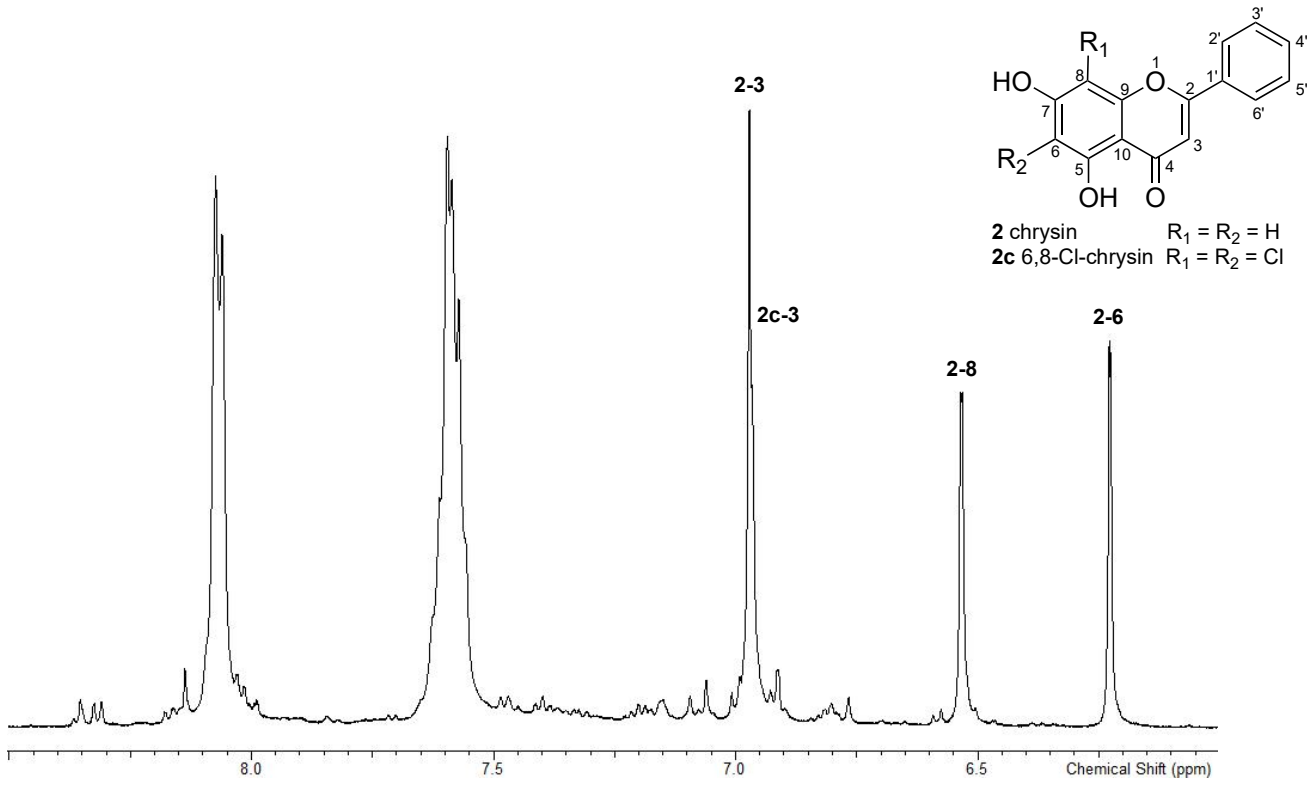


**Figure S31.** [1]H-NMR spectrum showing the compounds 6,8-Cl-chrysin (**2c**) and the precursor chrysin (**2**) including the signals of their protons 3, 6 and 8(DMSO-d6, 500 MHz).

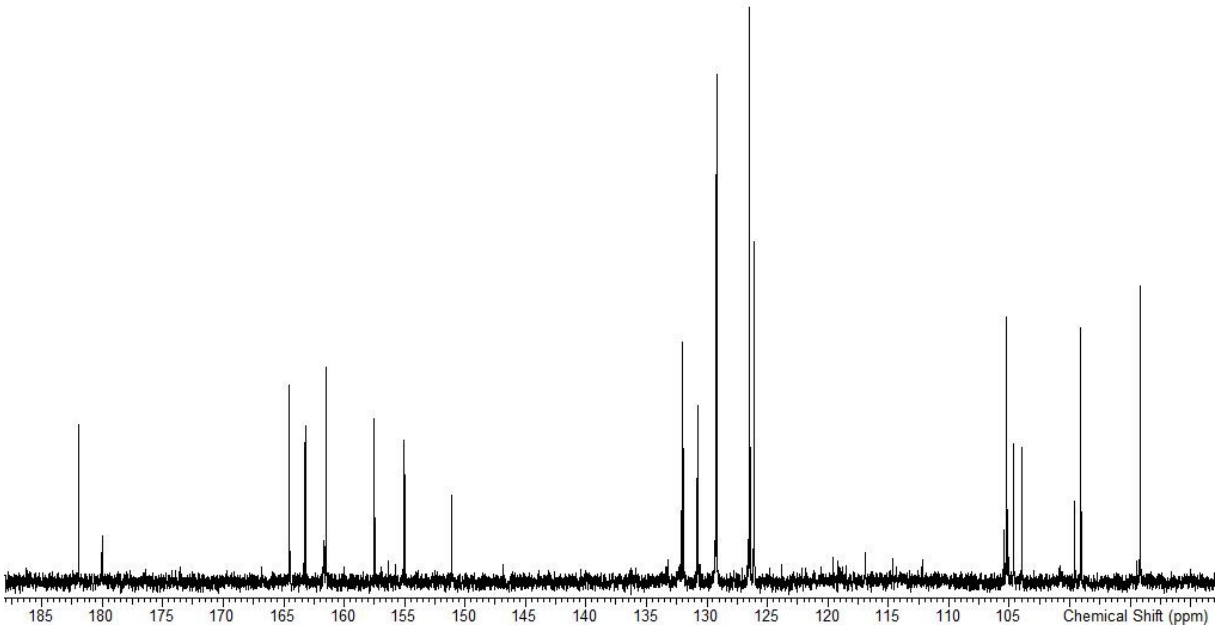


**Figure S32.** [13]C-NMR spectrum of 6,8-Cl-chrysin and the precursor chrysin (DMSO-d6, 125 MHz).

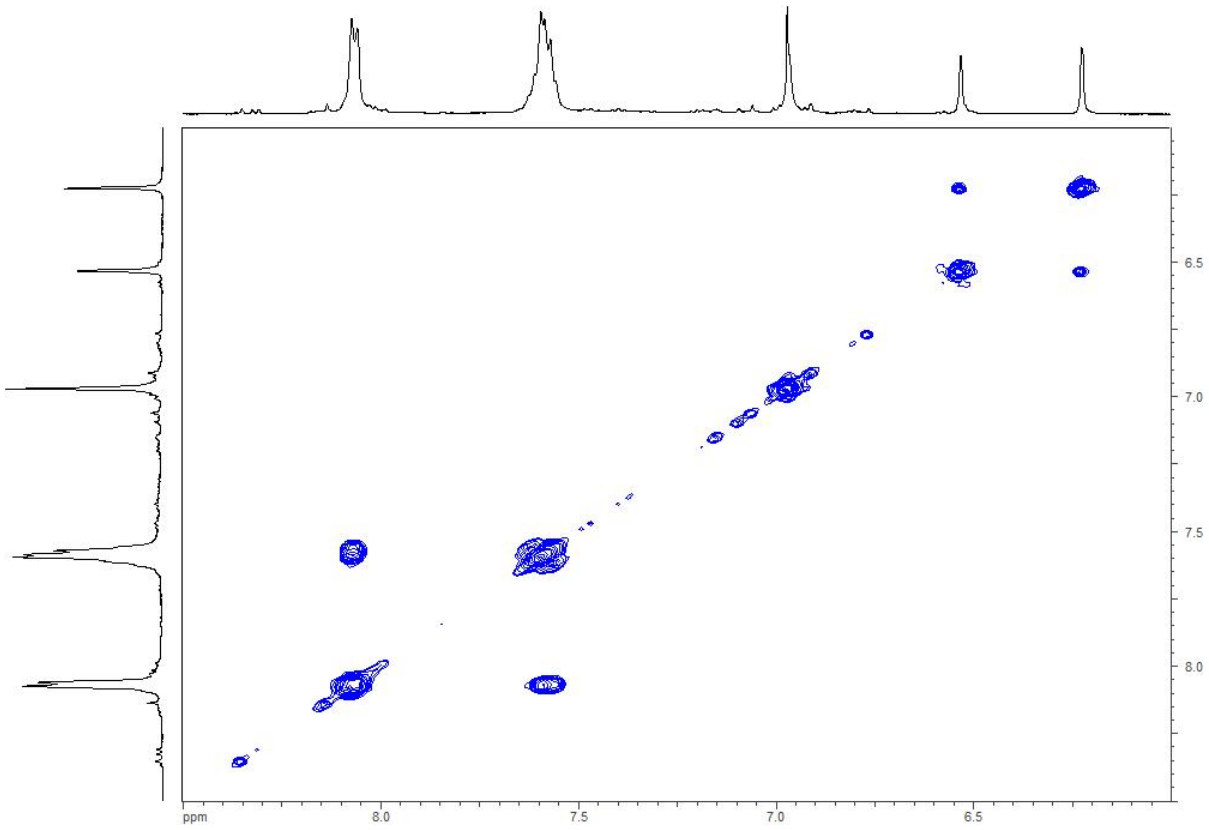


**Figure S33.** COSY spectrum of 6,8-Cl-chrysin and the precursor chrysin (DMSO-d6, 500 MHz).

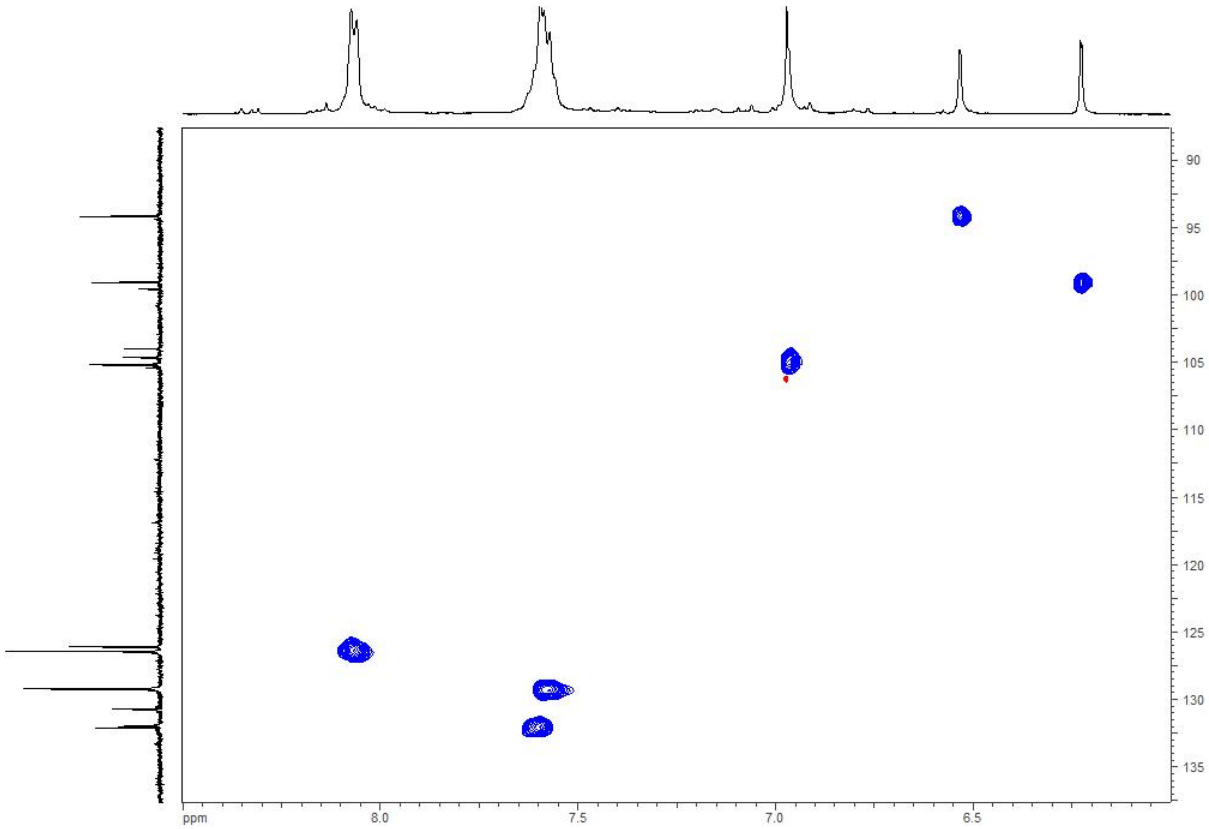


**Figure S34.** Edited HSQC spectrum of 6,8-Cl-chrysin and the precursor chrysin (DMSO-$d_6$, 500 MHz).

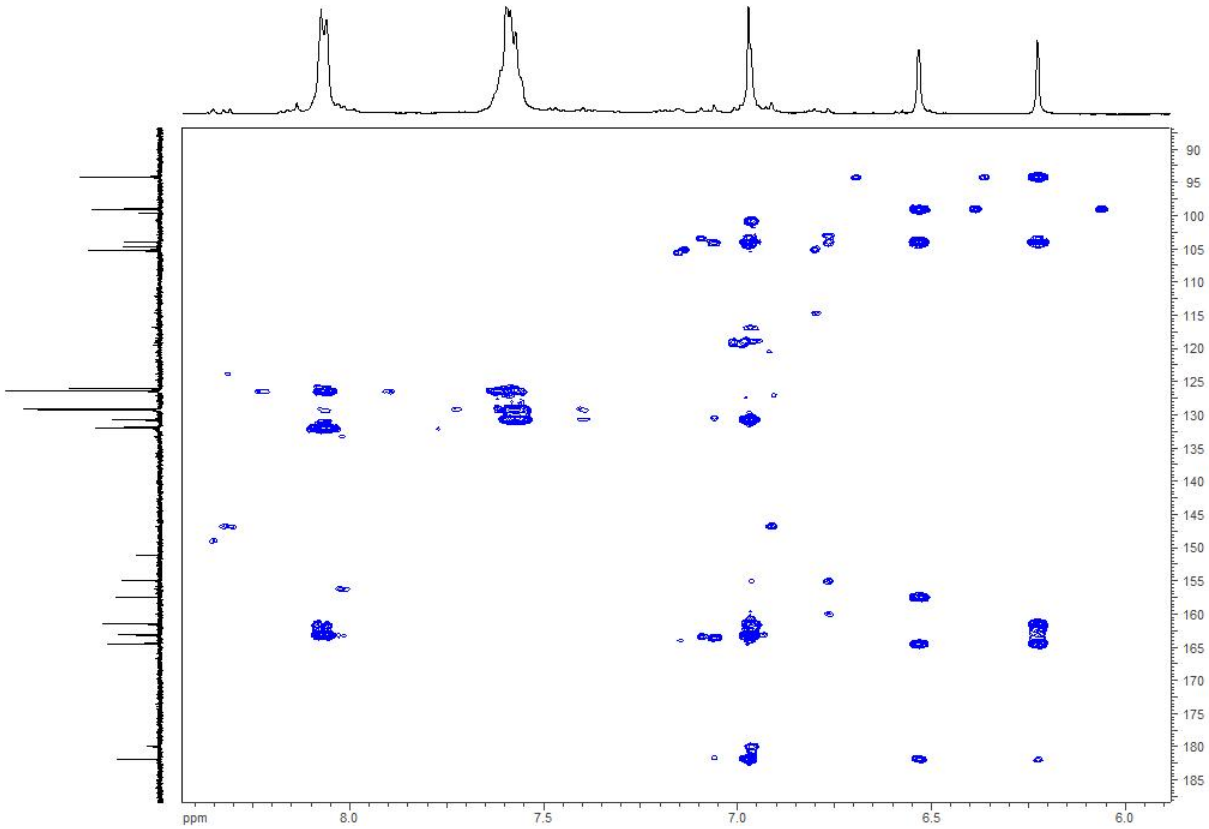


**Figure S35.** HMBC spectrum of 6,8-Cl-chrysin and the precursor chrysin (DMSO-$d_6$, 500 MHz).

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