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Supporting information for article:

The relationship between Hirshfeld potential and cytotoxic activity: a study along a series of flavonoid and chromanone derivatives

Magdalena Małecka, Joachim Kusz, Lars Eriksson, Angelika Adamus-Grabicka and Elżbieta Budzisz

S 2.1. Analytical data for compounds **I-VI**

Analytical data for compound **I**: yield: 85% m.p: 103–105 °C. IR (KBr) ν(cm^{-1}): 3068, 2932, 1668, 1604, 1577, 1506, 1492, 1472, 1461, 1142. ^{1}H-NMR (600 MHz, DMSO-d_6) δ (ppm):7.81 - 6.99 (m, 14H, Ar-H); 7.69 (s, 1H, =CH); 6.30 (s, 1H, C2-H). ^{13}C-NMR (600 MHz, $CDCl_3$) δ (ppm): 182.7; 158.9; 139.5; 138.1; 136.1; 134.1; 132.5; 129.9; 128.9; 128.7; 127.6; 122.2; 121.8; 118.7; 77.3. Analysis calculated for $C_{22}H_{16}O_2$: C, 84.59; H, 5.16; O, 10.25. Found: C, 84.55; H, 5.18; O, 10.27.

Analytical data for compound **II**: yield: 61% m.p: 153.0–155.0 °C. IR (KBr) ν(cm^{-1}): 3061, 3025, 2907, 1662, 1606, 1590, 1460, 1182, 1141. ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.01 (s, 1H, =CH); 7.82 - 7.05 (m, 13H, Ar-H); 6.75 (s, 1H, C2-H); 3.19 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ (ppm): 181.3; 158.3; 138.9; 137.7; 136.7; 134.6; 131.8; 130.1; 129.0; 128.8; 127.4; 126.9; 122.2; 121.7; 118.8; 116.1; 115.1; 77.0; 21.0. Analysis calculated for $C_{23}H_{18}O_2$: C, 84.64; H, 5.56; O, 9.8. Found: C, 84.64; H, 5.58; O, 9.78.

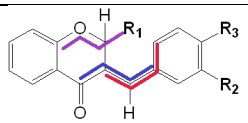
Analytical data for compound **III**: yield: 61% m.p: 108.8–109.3 °C. IR (KBr) ν(cm^{-1}): 3031, 2945, 1668, 1614, 1601, 1580, 1462, 1176, 1140. ^{1}H-NMR (DMSO-d_6) δ (ppm): 8.11 (s, 1H, =CH), 7.81 - 6.99 (m, 13H, Ar-H), 6.74 (s, 1H, C2-H), 2.36 (s, 3H, CH_3). ^{13}C-NMR ($CDCl_3$) δ (ppm): 181.3, 158.3, 138.9, 137.7, 136.7, 134.6, 131.8, 130.1, 129.0, 128.8, 127.4, 126.9, 122.2, 121.5, 118.8, 116.1, 115.1, 77.2, 21.3. Analysis calculated for $C_{23}H_{18}O_2$: C, 84.64; H; 5.56; O, 9.8. Found: C, 84.79; H, 5.45; O, 9.76.

Analytical data for compound **IV**: yield: 41% m.p: 139.8–141.3 °C. IR (KBr) ν(cm^{-1}): 3030, 2945, 2910, 1675, 1602, 1578, 1506, 1304, 1172, 1148. ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.99 (s, 1H, =CH), 7.81 – 7.04 (m, 13H, Ar-H), 6.74 (s, 1H, C2-H), 2.28 (s, 3H, OCH_3). ^{13}C-NMR ($CDCl_3$) δ (ppm): 183.0, 158.9, 158.6, 140.0, 138.1, 136.0, 132.5, 128.6, 127.7, 126.0, 122.3, 121.7, 118.6, 116.2, 77.9, 29.7. Analysis calculated for $C_{23}H_{18}O_3$: C, 80.68; H, 5.26; O, 14.06. Found: C, 80.60; H, 5.25; O, 14.15.

Analytical data for compound **V**: yield: 85% m.p: 111–112 °C. IR (KBr) ν(cm^{-1}): 3056, 3028, 2904, 2854, 1666, 1600, 1572, 1461, 1454, 1306, 1144. ^{1}H-NMR (DMSO-d_6) δ (ppm): 7.99 – 7.05 (m, 9H, Ar-H), 7.59 (d, 2H, J_{AB} = 14.22 Hz, C2-H), 5.43 (s, 1H, =CH). ^{13}C- NMR ($CDCl_3$ δ (ppm): 182.3, 155.9, 154.5, 137.5, 134.5, 131.1, 129.9, 129.5, 128.6, 125.2, 121.9, 119.3, 108.2, 67.6. Analysis calculated for $C_{16}H_{12}O_2$: C, 81.33; H, 5.08; O, 13.59. Found: C, 81.03; H, 5.10; O, 13.87.

Analytical data for compound **VI**: yield: 55% m. p: 133.8–134.8 °C. IR (KBr) ν(cm^{-1}): 3038, 3000, 2958, 2866, 1665, 1603, 1568, 1510, 1477,1463, 1146, 1112. ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.03 – 6.96 (m, 8H, Ar-H), 5.38 (d, 2H, J_{AB} =16.60 Hz, C2-H), 3.87 (s, 1H, =CH), 1.58 (s, 3H, OCH_3). ^{13}C-NMR ($CDCl_3$) δ (ppm): 182.3, 160.1, 137.4, 135.7, 132.1, 128.9, 127.9, 127.0, 121.9, 117.9, 114.3, 67.8, 55.5. Analysis calculated for $C_{17}H_{14}O_3$: C, 76.67; H, 5.25; O, 18.08. Found: C: 76.68; H, 5.20; O, 18.12.

Table S1. Specified torsion angles showing whether the substituent is perpendicular or parallel (violet shows torsion angle for substituent at C2, and blue and red substituent at C3.



Torsion angle [°]			
Compound I	84.6(2)	-179.9(2)	-26.0(3)
Compound II	78.28(12)	177.54(12)	-21.3(2)
Compound III	73.50(12)	-179.62(11)	-39.5(2)
Compound IV_a	85.06(12)	-179.20(12)	-18.2(2)
IV_b	85.35(11)	177.83(12)	-20.9(2)
IV_c	79.67(12)	-177.97(12)	-21.0(2)
Compound V	-	177.04(13)	-29.7(2)
Compound VI_a	-	-177.8(2)	33.6(4)
VI_b	-	179.1(2)	-35.7(4)

Table S2. Ring puckering parameters with asymmetry parameters. Q, θ, Φ puckering parameters calculated according to Cremer & Pople, ΔCs, ΔC2 – asymmetry parameters calculated due to Duax & Norton

compound	Q (Å)	θ (°)	Φ (°)	asymmetry parameter (°)	conformation
I	0.366(2)	115.6(3)	233.7(4)	ΔCs(C2)=5.5(2)	E
II	0.394(2)	61.3(2)	43.6(2)	ΔC2(O1-C2)=9.7(2)	S/B
III	0.446(2)	61.0(2)	57.9(2)	ΔCs(C2)=3.2(2)	E
IV_a	0.339(2)	120.9(2)	220.2(2)	ΔCs(C2)=7.7(2)	E
IV_b	0.338(2)	119.5(2)	226.0(2)	ΔC2(O31-C32)=10.2(2)	S/B
IV_c	0.374(2)	61.4(2)	41.9(2)	ΔC2(O61-C62)=7.9(2)	S/B
V	0.423(2)	57.4(2)	60.1(2)	ΔCs(C2)=1.6(2)	E

VI_a	0.425(2)	116.3(3)	225.3(4)	ΔC2(O1-C2)=13.0(2)	S/B
VI_b	0.428(2)	62.6(4)	49.5(4)	ΔCs(C22)=10.4(2)	E

S 3.3. Determination of Lipophilicity

The RP-TLC experiments were performed on TLC plates (5 × 10 cm) RP-18 F254S (Merck, Darmstadt, Germany). The synthesized compounds were dissolved in *N*,*N*-dimethylformamide DMF (2 mg/mL). DMF grade was purchased from Chempur (Piekary Slaskie, Poland). The solutions of each compound in DMF were spotted on the plates, and observed under UV light at λ = 254 nm. A DMF-water solvent system was used as mobile phase. The composition of the solvent system was changed from 50%:50% to 95%:5%. All experiments were performed at room temperature. The log*P* parameter was calculated using the equation from the calibration curve. Theoretical values of lipophilicity were calculated using Molinspiration Cheminformatics (miLog*P*). The research also compares the experimental and theoretical values of Log*P* obtained from RP-TLC method and Molinspiration Cheminformatics programme (miLog*P*) (Table 3).

S 3.4. Biologial Assay. Cells Cultures and Cytotoxicity Assay by MTT

The compounds were tested for biological activity on four cell lines: HL-60 (human leukemia cell line), NALM-6 (human peripheral blood leukemia cell line), WM-115 (melanoma cell line, ECACC, Salisbury, UK) and COLO-205 (human colon adenocarcinoma cells). The cell lines COLO-205, WM-115 and HL-60 used in this work came from the ATCC American Type Culture Collection (Manassas, VA, USA), whereas the NALM-6 cell line was purchased from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). The leukaemia cells and colon adenocarcinoma were cultured in RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and gentamicin (25 µg/mL; KRKA, Novo Mesto, Slovenia). For melanoma WM-115 cells, Dulbecco`s minimal essential medium (DMEM; Invitrogen) was used. All cell lines were cultured at 37 ^{0}C in a humidified atmosphere of 5% CO_2 in air. The compounds were dissolved in DMSO (Sigma-Aldrich) and were further diluted in culture medium to obtain <0.1% DMSO concentration. In each experiment, controls with and without 0.1% DMSO were performed. The cytotoxicity of all compounds and of the reference compounds 4-chromanone and 3-benzylideneflavanone was determined by the MTT assay, i.e., 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO, USA), which measures cellular dehydrogenase activity (Hansen *et al.*, 1989). Exponentially growing cells were seeded a day before the experiment onto a 96-well microplates (Nunc, Roskilde, Denmark) at a density up to 6–8 × 10^3 cells/well (depending on the cell line). Subsequently, various concentrations of the

studied compounds freshly prepared in DMSO and diluted with complete culture medium were added. All compounds were tested for their cytotoxicity at a final concentration of 10^{-7}–10^{-3} M. After 46 h of incubation with the studied compounds, the cells were treated with the MTT reagent and incubation was continued for another two hours. MTT – formazan crystals were dissolved in 20% sodium dodecyl sulphate (SDS, Sigma-Aldrich) and 50% DMF (Sigma-Aldrich) at pH 4.7; following this, absorbance was read at 570 nm on a multifunctional Victor ELISA-plate reader (Perkin Elmer, Turku, Finland). The IC_{50} values, i.e., the concentration of the test compound required to reduce the cell survival fraction to 50% of controls, were calculated from concentration-response curves and used as a measure of the sensitivity of the cells to a given treatment. As a control, cultured cells were grown in the absence of drugs. The data points represent the means of at least five to ten repeats ± standard deviation (S.D.).

Table S3. The cytotoxic activity of tested compounds against cancer cell lines. The results are presented as IC_{50} values in µM range.

Compound	IC50 (µM)			
	HL-60	NALM-6	WM-115	COLO-205
I	33.74±3.87	27.02±3.10	47.41±4.62	77.1 ± 1.9
II	40.0±6.1	51.4±3.8	60.1±2.4	>250
III	48.82±4.79	48.45±5.46	44.81±4.50	32.64±6.61
IV	53.38±2.49	49.81± 2.35	58.13±4.93	51± 2.3
V	5.4±0.6	6.2±0.2	53.7±4.3	31± 1.4
VI	45.36±4.77	30.07±3.15	23.53±1.71	193.1 ± 2.9
4-chromanone	676.7 ± 32.6	673.7 ± 22.5	>1000	721.5 ± 6.4
Flavanone (Kupcewicz et al., 2013)	51.1 ± 1.7	57.6 ± 8.6	71.2 ± 3.2	-

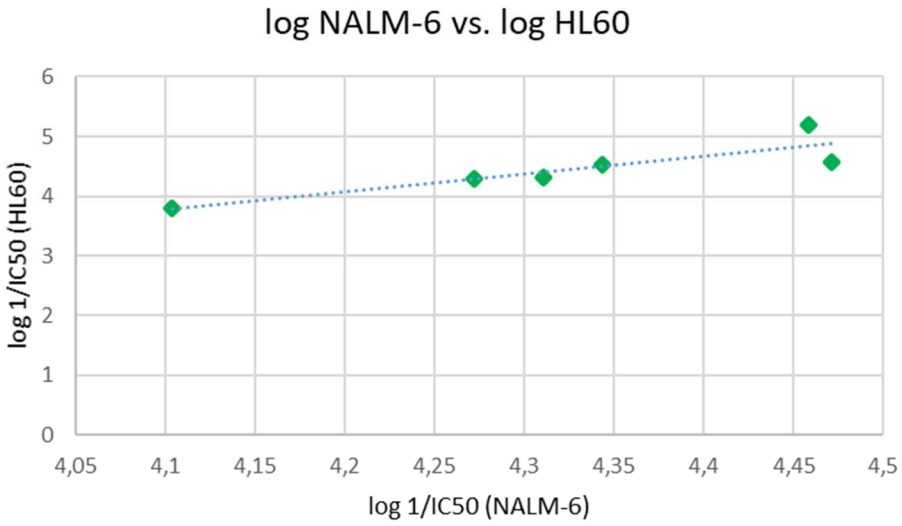


Figure S1. The relationship between cytotoxic activity (log $1/IC_{50}$) towards HL-60 and NALM-6 cell lines.

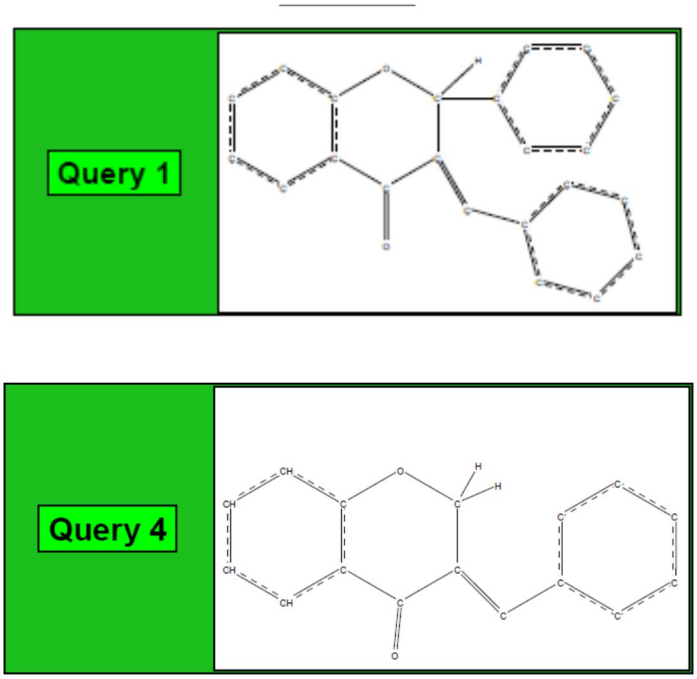
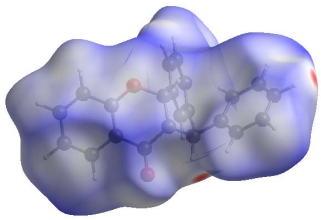
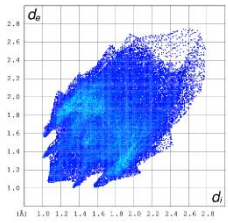
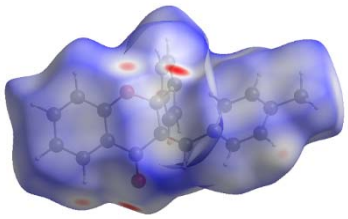
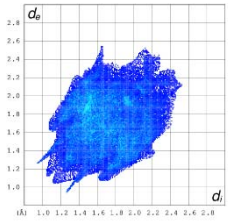
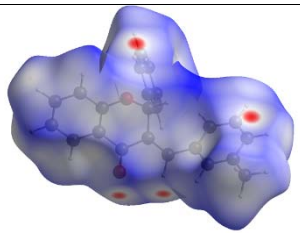
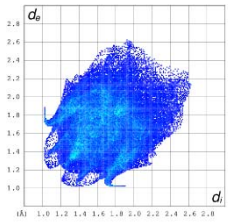
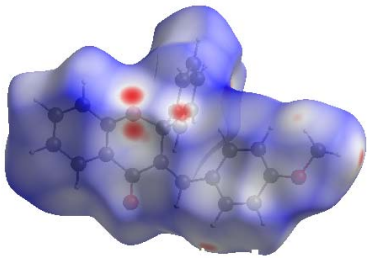
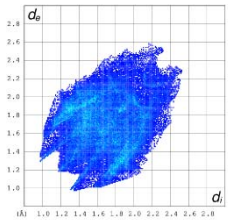
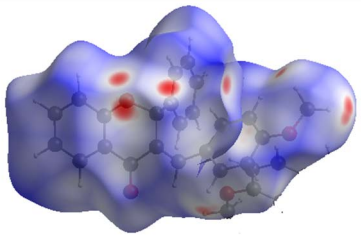
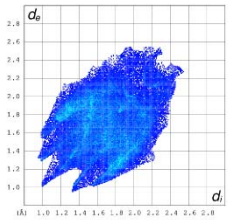
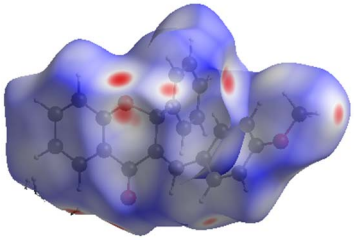
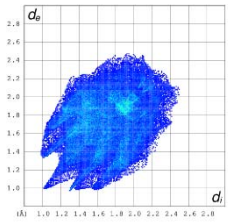
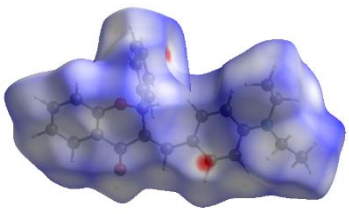
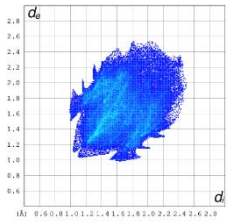
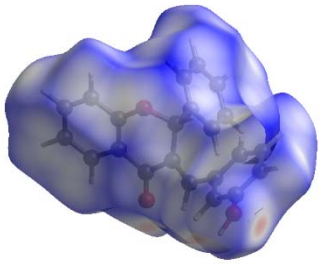
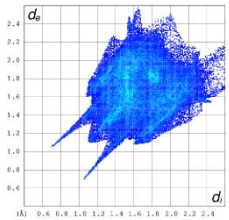
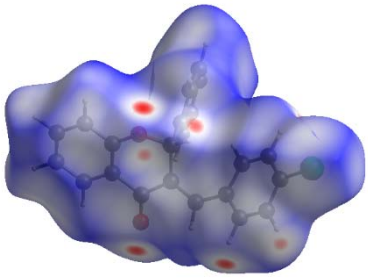
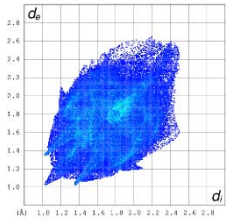
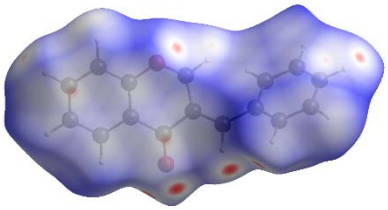
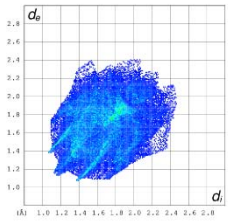
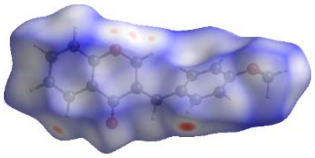
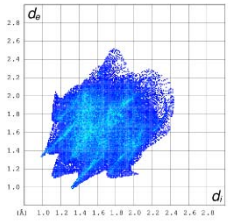
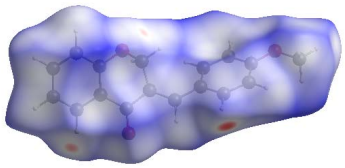
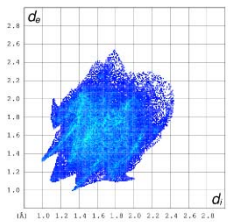
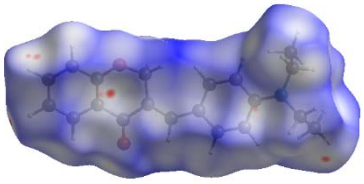
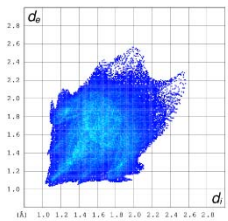
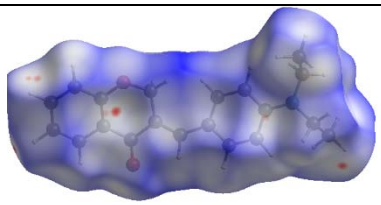
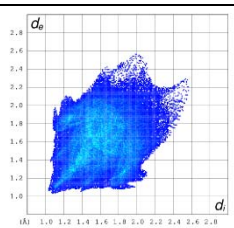
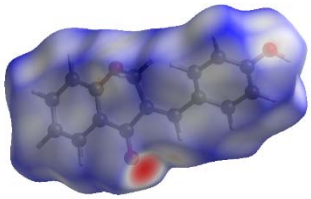
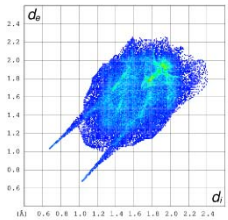
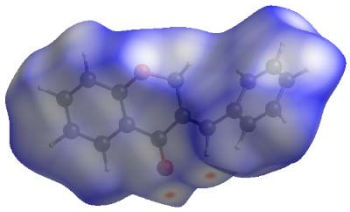
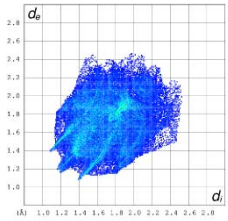
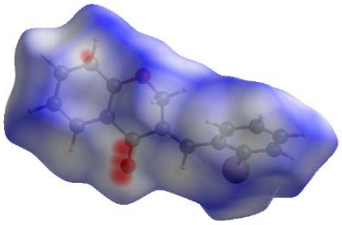
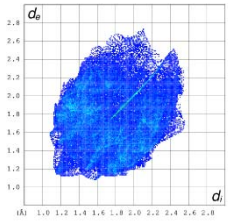
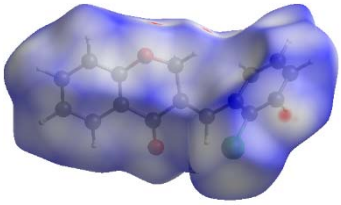
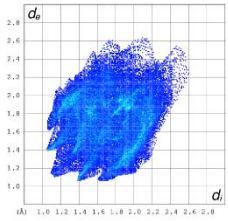
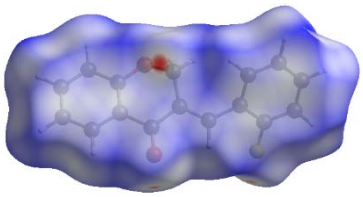
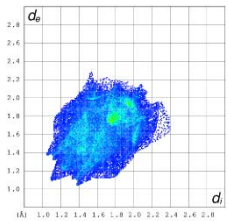
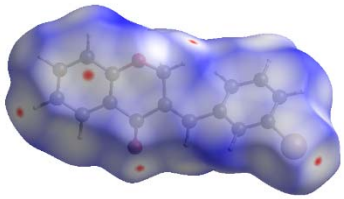
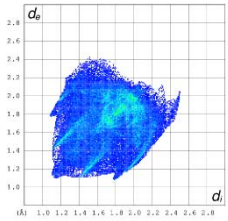
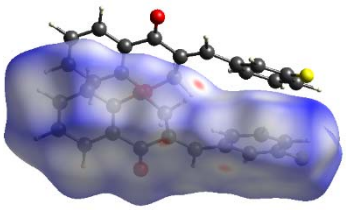
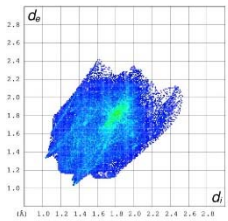
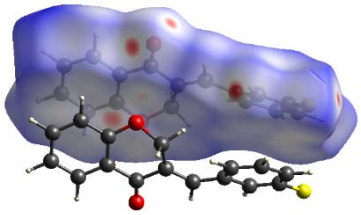
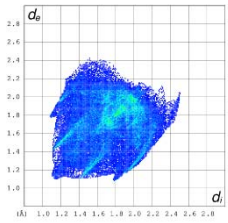
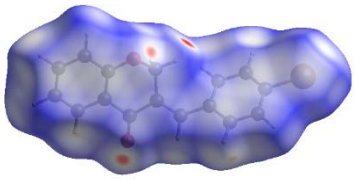
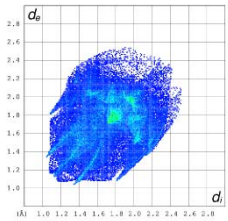
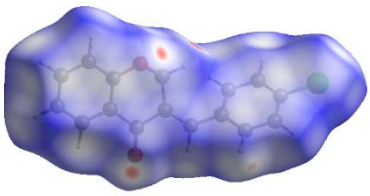
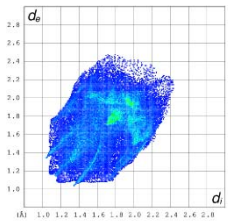
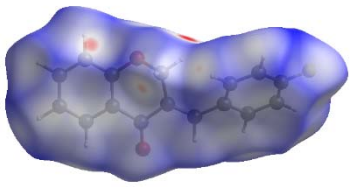
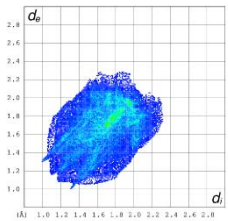
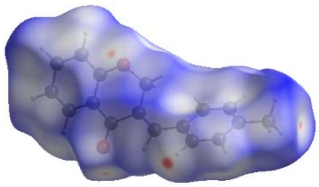
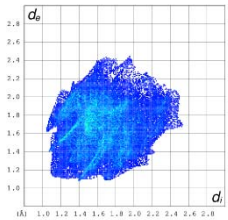
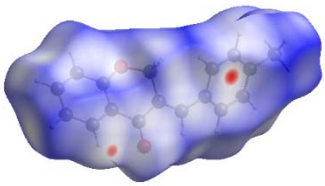
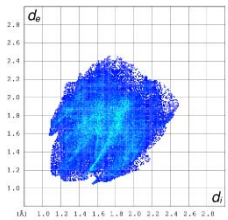
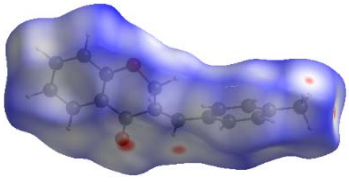
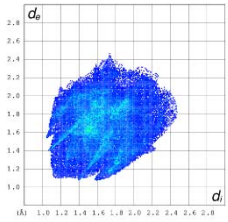


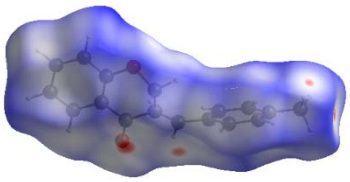
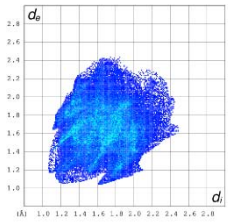
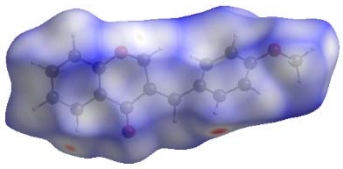
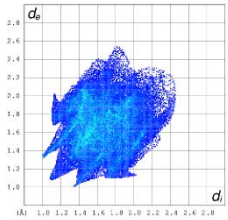
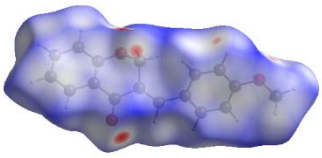
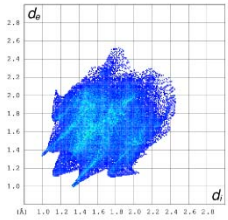
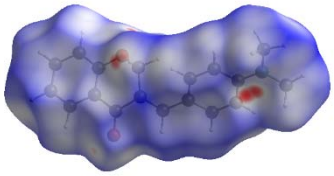
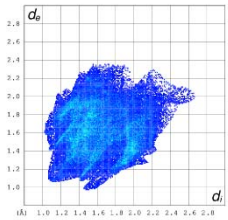
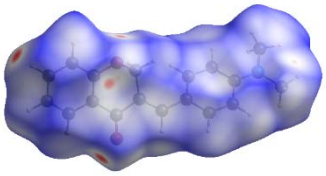
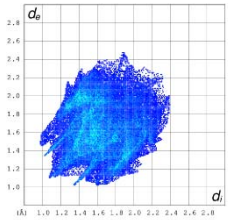
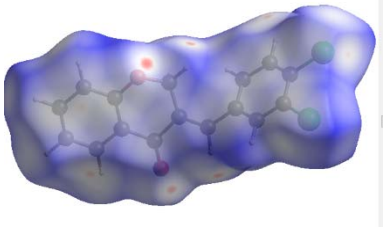
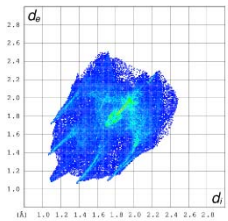
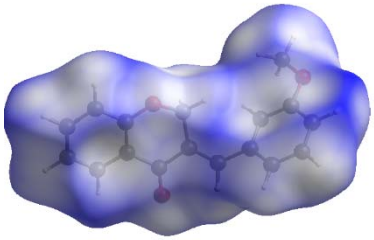
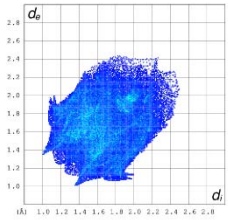
Figure S2. Reference moieties for database survey

Compound I		
Compound II		
Compound III		
Compound IV_a		
Compound IV_b		
Compound IV_c		
FLAV_I		

FLAV_II		
FLAV_III		
Compound V		
Compound VI_a		
Compound VI_b		
BCHR_I_a		
BCHR_I_b		

BCHR_II		
FAVVIH		
NAMXIK		
NAMXOQ		
NAMXUW		
NAMYEH		
NAMYOR_A		

NAMYOR_B		
NAMYUX		
NAMZEI		
NAMZOS		
NAMZUY_a		
NAMZUY_b		
NAMZUY_c		

NAMZUY_d		
NANBAH_a		
NANBAH_b		
NENDOB_a		
NENDOB_b		
SECBAF		
YAWLEP		

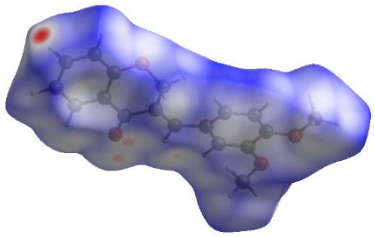
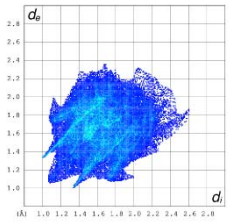
YEJPIO		

Figure S3. Hirshfeld surfaces mapped with d_{norm} (left) and full fingerprint plots for all tested compounds including compounds studied by us and from CSD database: FLAV_I, BCHR_I, (Adamus-Grabicka *et al.,* 2018); FLAV_II, BCHR_II, (Suchojad *et al.,* 2019); FLAV_III, (Kupcewicz, *et al.,* 2013); FAVVIH, (Katrusiak *et al.,* 1987); NAMXIK, NAMXOQ, NAMXUW,NAMYEH, NAMYOR, NAMYUX, NAMZEI, NAMZOS, NAMZUY, NANBAH, (Cheng et al., 2011); NANDOB, (Valkonen et al., 2012); SECBAF, (Gopaul et al., 2012a); YAWLEP, (Gopaul et al., 2012b); YEJPIO, (Gopaul et al., 2012c)

Table S4. Percentage contribution of close contact to the Hirshfeld surface with log*P*theor value for all compounds.

	O…H	O…C	C…H	H…H	C…C	C…C&C…H	logPteor
I	10	1,2	39,6	47,6	0,6	40,2	5,2
II	11,9	0,1	29,9	54	4,1	34	5,65
III	10,1	1,6	32,6	53,1	2,4	35	5,63
IV_a	18,2	0,2	35,3	43,1	3,2	38,5	5,26
IV_b	17,9	0,2	26,8	49	6,2	33	5,26
IV_c	17,3	0,1	32,4	47,1	3,2	35,6	5,26
FLAV_I	11,2	0	32,7	55,5	0,3	33	6,05
FLAV_II	16	0	33,1	45,5	1,7	34,8	4,7
FLAV_III	12,5	0	25,6	39,2	5,2	30,8	5,33
FIHMAM	12,5	0	25,6	39,2	5,2	30,8	5,33
V	17,5	1,3	26,8	46,6	7,7	34,5	3,62
VI_a	22,1	0,9	28,1	43,5	5	33,1	3,68
VI_b	22,7	0,8	28	43,1	5,2	33,2	3,68
BCHR_I_a	15,6	0	26,9	53,1	1,2	28,1	4,47
BCHR_I_b	15,3	0	27,2	53,2	1,2	28,4	4,47
BCHR_II	24,5	0	15,1	45,2	13,1	28,2	3,14
FAVVIH	17,2	1,3	25,7	48,2	7,5	33,2	3,62
NAMXIK	13,2	4,4	33,9	30,3	1,2	35,1	4,05
NAMXOQ	14,5	2,5	30,3	32,2	3,7	34	4,01
NAMXUW	15,4	3,1	14,8	41,6	12,1	26,9	3,56
NAMYEH	14	4,3	14,3	38,4	10,4	24,7	4,4
NAMYOR_A	13,7	3,4	17,8	41,9	11	28,8	3,76
NAMYOR_B	14,9	3,5	17,2	34,7	11	28,2	3,76
NAMYUX	14,2	3,8	15,9	36,7	10,5	26,4	4,43
NAMZEI	14,4	3,6	15,7	37,3	11,1	26,8	4,3
NAMZOS	14,8	3,9	15,5	39,5	11,3	26,8	3,78
NAMZUY_A	13,8	3,4	32,1	48,3	2	34,1	4,07
NAMZUY_B	15,1	2,3	29,9	48,5	3,7	33,6	4,07
NAMZUY_C	14,9	2,1	30,8	48,1	3,5	34,3	4,07
NAMZUY_D	14,4	3	31,4	49	1,7	33,1	4,07
NANBAH_A	22,3	0,9	27,8	43,8	4,9	32,7	3,68
NANBAH_B	22,8	0,8	27,8	43,2	5,1	32,9	3,68
NENDOB_A	12,6	2,4	31,1	51,3	1,3	32,4	3,72
NENDOB_B	15,2	2	31,4	48,7	1,3	32,7	3,72
SECBAF	13,4	4	25,8	26,4	10,3	36,1	4,9
YAWLEP	22,1	1,4	29,6	43,4	3,2	32,8	3,65
YEJPIO	23,2	3,9	22,7	42,7	2,4	25,1	3,27