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Supporting Information

Unnatural Polyketide Analogues Selectively Target the HER Signaling Pathway in Human Breast Cancer Cells

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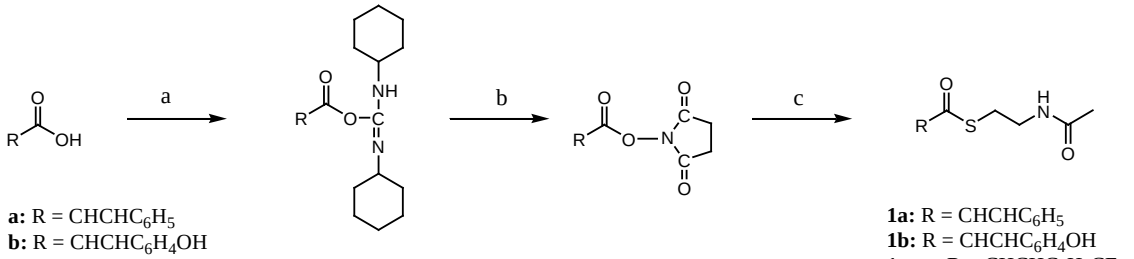
Supporting Information

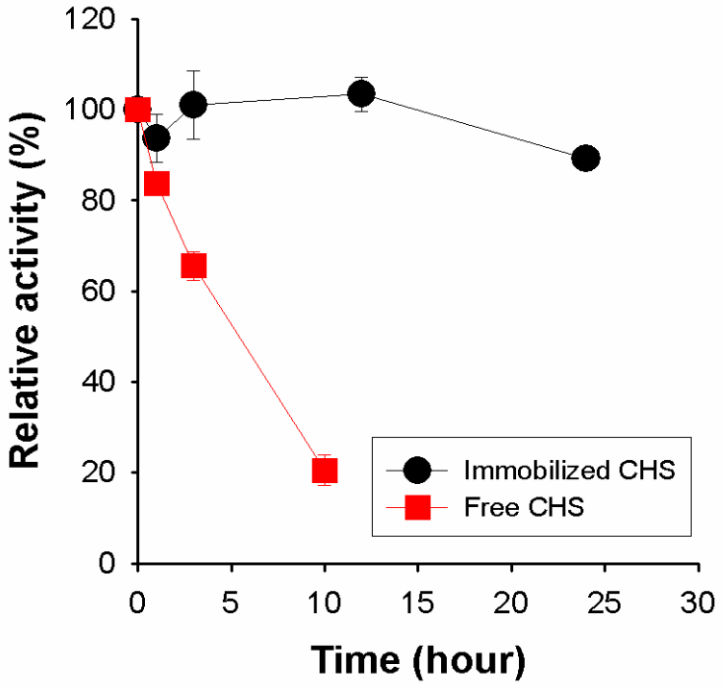


a: R = $CHCHC_6H_5$
b: R = $CHCHC_6H_4OH$
c - e: R = $CHCHC_6H_4CF_3$
f: R = $CHCHC_6H_3CF_3CF_3$
g: R = $CHCHC_6H_3CH_3$
h: R = C_6H_5
i: R = $C_6H_4CH_2OH$
j: R = C_6H_4OH
k: R = $CH_2C_6H_4OH$
l: R = $CH_2CH_2CH_2C_6H_5$

1a: R = $CHCHC_6H_5$
1b: R = $CHCHC_6H_4OH$
1c - e: R = $CHCHC_6H_4CF_3$
1f: R = $CHCHC_6H_3CF_3CF_3$
1g: R = $CHCHC_6H_3CH_3$
1h: R = C_6H_5
1i: R = $C_6H_4CH_2OH$
1j: R = C_6H_4OH
1k: R = $CH_2C_6H_4OH$
1l: R = $CH_2CH_2CH_2C_6H_5$

Figure 1S. Synthesis of N-acetylcysteamine thioester (SNAC) analogues (**1a – l**). Reagents: (a) DCC (dicyclohexyl carboimide), Ethyl acetate; (b) NHS (N-hydroxysuccinimide), Ethyl acetate; (c) NAC (N-acetylcysteamine), Ethyl acetate.

(a)



(b)

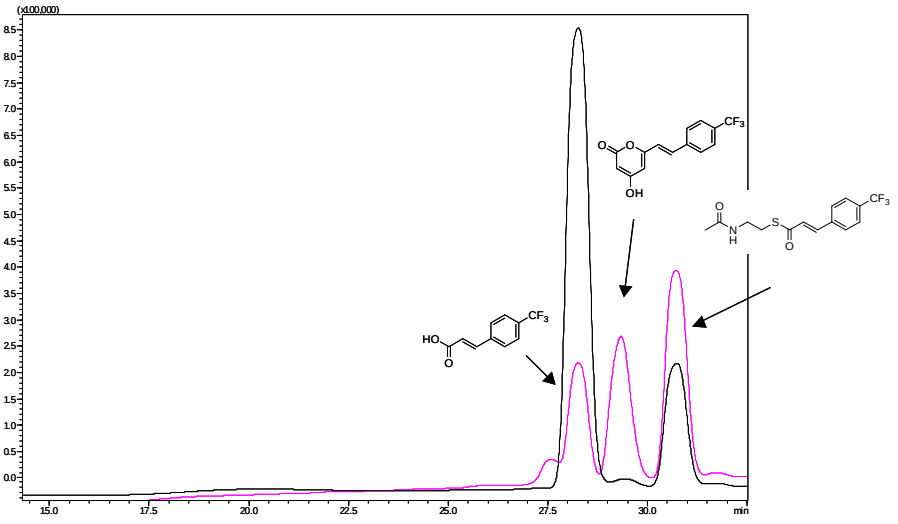


Figure 2S. (a) Stabilization of CHS via immobilization onto Ni-NTA agarose beads at room temperature under shaking at 150 rpm. **(b)** HPLC/MS analysis of 4-trifluoromethylcinnamoyl pyrone (2e) synthesized with free CHS (black line) and immobilized enzyme (pink line) Each peak was identified via LC/MS and NMR.

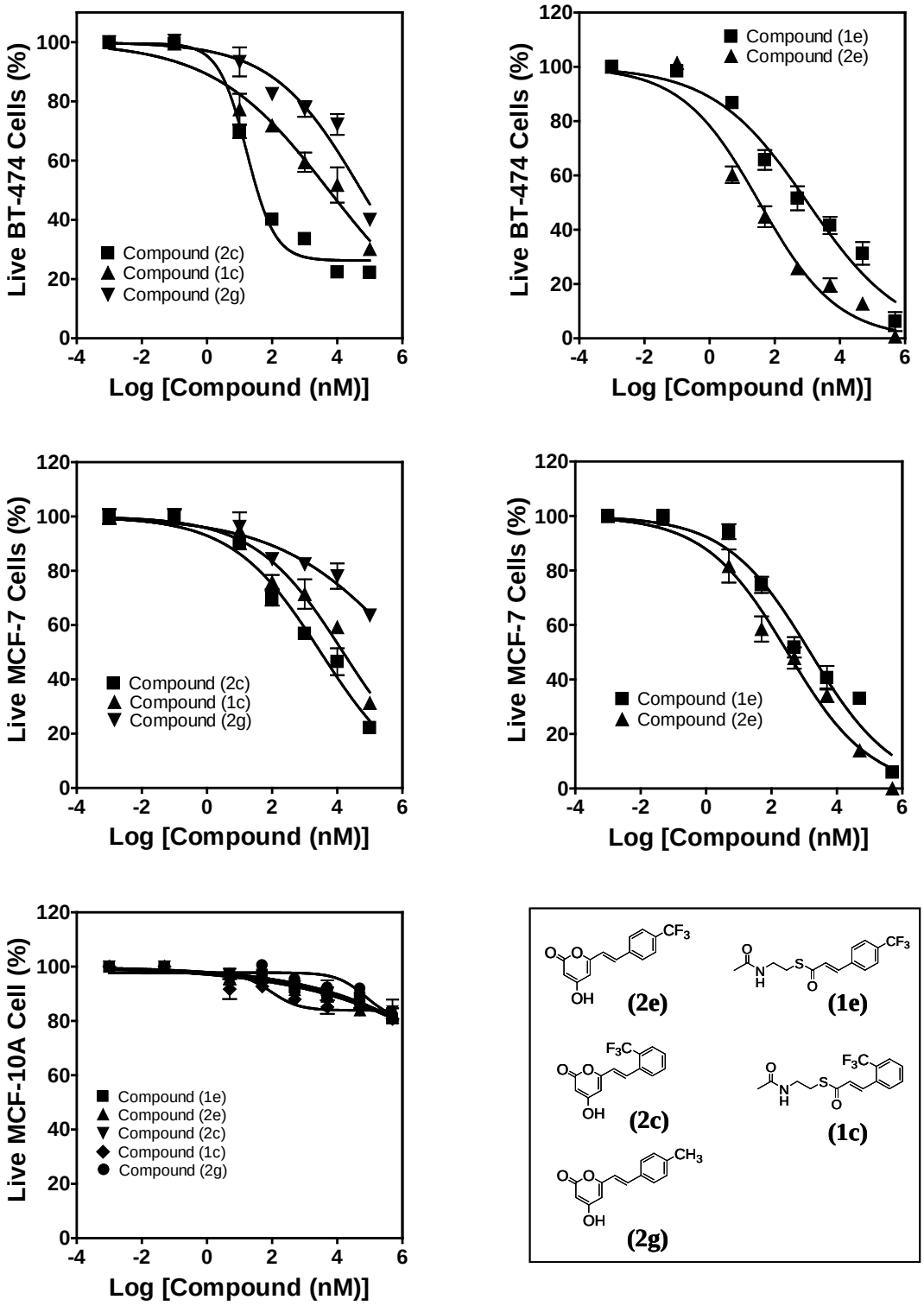


Figure 3S. Dose-response curves for cell growth inhibition on human non-transformed and tumor-derived cell lines by unnatural polyketides and their SNAc substrates.

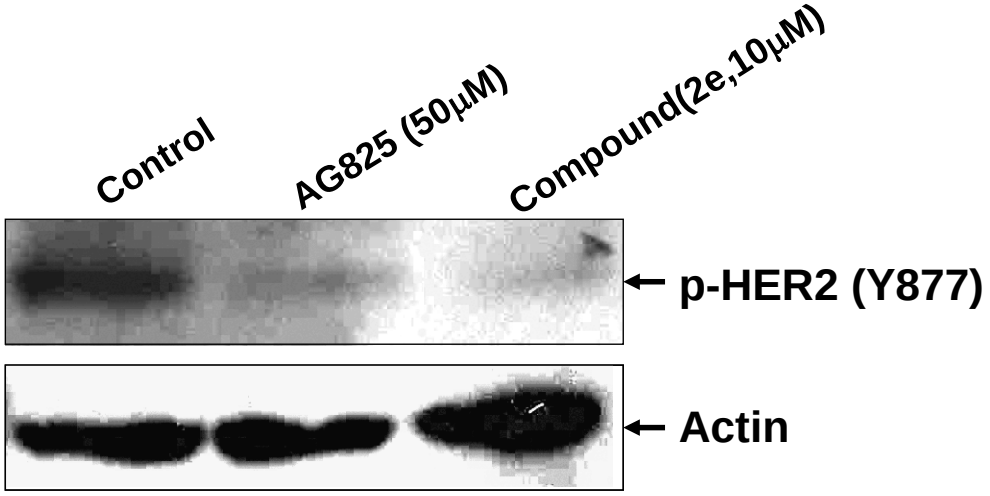


Figure 4S. Effect of 4-trifluoromethyl-cinnamoyl pyrone (**2e**) and AG825 on the phosphorylation of the kinase domain residue Tyr877 of HER2. Whole-cell lysates were subjected to SDS-PAGE analysis and probed with anti-phospho-HER2 (Tyr877) and anti-actin, respectively

Compound characterization

(E)-S-2-acetamidoethyl 3-phenylprop-2-enethioate (cinnamoyl-SNAC) (1a): t_{Ret} = 34.6 min, LC/MS : ESI(+) *m/z* 250$[M+H]^+$, 272$[M+Na]^+$, 499$[2M+H]^+$, 521$[2M+Na]^+$; ESI(+) MS/MS *m/z* 131$[M+H\text{-}CH_3CONHC_2H_4SH]^+$. ^{1}H NMR (500 MHz, CD_3OD): δ= 1.9 (s, 3H), 3.1 (t, 2H, J=7 Hz), 3.3 (t, 2H, J=6.5 Hz), 6.8 (d, 1H, J=16 Hz), 7.3 (m, 3H), 7.6 (m, 3H).

(E)-S-2-acetamidoethyl 3-(4-hydroxyphenyl)prop-2-enethioate (coumaroyl-SNAC) (1b): t_{Ret} = 26 min, LC/MS : ESI(+) *m/z* 266$[M+H]^+$, 288$[M+Na]^+$, 553$[2M+Na]^+$; ESI(-) *m/z* 264$[M-H]^-$, 551$[2M-2H+Na]^-$. 1H NMR (500 MHz, [CD3]DO): δ= 1.9 (s, 3H), 3.0 (t, 2H, J=6.5 Hz), 3.3 (t, 2H, J=7 Hz), 6.6 (d, 1H, J=16 Hz), 6.7 (dd, 2H, J=6.5, 2 Hz), 7.4 (dd, 2H, J=7, 2 Hz), 7.5 (d, 1H, J=15.5).

(E)-S-2-acetamidoethyl 3-(2-(trifluoromethyl)phenyl)prop-2-enethioate (1c): t_{Ret} = 37.2 min, LC/MS : ESI(+) *m/z* 318$[M+H]^+$, 340$[M+Na]^+$; ESI(-) *m/z* 316$[M-H]^-$, 352$[M+Cl-H]^-$, 362$[M+OOCH-H]^-$; ESI(+) MS/MS *m/z* 199$[M+H\text{-}CH_3CONHC_2H_4SH]^+$. ^{1}H NMR (500 MHz, CD_3OD) δ [ppm]: 1.9 (3H, s), 3.1 (2H, t, *J* = 7 Hz), 3.3 (2H, t, *J* = 7 Hz), 6.7 (1H, d, *J* = 15.5), 7.47 (1H, m), 7.56 (1H, m), 7.65 (1H, d, *J* = 7.5 Hz), 7.8 (1H, d, , *J* = 7.5 Hz), 7.84 (1H, m), 7.87 (1H, m). ^{13}C NMR (500 MHz) δ [ppm]: 23.4, 30.2, 40.9, 125.2, 127.3, 128.0, 130.0, 130.7, 132.2, 134.5, 134.6, 137.4, 174.2, 191.2.

(E)-S-2-acetamidoethyl 3-(3-(trifluoromethyl)phenyl)prop-2-enethioate (1d): t_{Ret} = 37.7 min, LC/MS : ESI(+) *m/z* 318$[M+H]^+$, 340$[M+Na]^+$; ESI(-) *m/z* 316$[M-H]^-$, 352$[M+Cl-H]^-$, 362$[M+OOCH-H]^-$; ESI(+) MS/MS *m/z* 199$[M+H\text{-}CH_3CONHC_2H_4SH]^+$.

(E)-S-2-acetamidoethyl 3-(4-(trifluoromethyl)phenyl)prop-2-enethioate (1e) : t_{Ret} = 38 min, LC/MS : ESI(+) *m/z* 318$[M+H]^+$, 340$[M+Na]^+$; ESI(-) *m/z* 316$[M-H]^-$,

352[M+Cl-H]$^-$, 362[M+OOCH-H]$^-$; ESI(+) MS/MS *m/z* 199[M+H-$CH_3CONHC_2H_4SH$]$^+$. ^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.9 (3H, s), 3.1 (2H, t, *J* = 6.4 Hz), 3.5 (2H, dd, *J* = 12.5 Hz, *J* = 6.1 Hz), 6.7 (1H, d, *J* = 15.9), 7.63 (1H, d, *J* = 15.9), 7.64-7.68 (4H, m).

(E)-S-2-acetamidoethyl 3-(3,5-bis(trifluoromethyl)phenyl)prop-2-enethioate (**1f**)**:** t_{Ret} = 39 min, LC/MS : ESI(+) *m/z* 386[M+H]$^+$, 408[M+Na]$^+$; ESI(-) *m/z* 384[M-H]$^-$.

(E)-S-2-acetamidoethyl 3-p-tolylprop-2-enethioate (**1g**): t_{Ret} = 36 min, LC/MS : ESI(+) *m/z* 264[M+H]$^+$, 286[M+Na]$^+$; ESI(-) *m/z* 262 [M-H]$^-$.

S-2-acetamidoethyl benzothioate (**1h**)**:** t_{Ret} = 29 min, LC/MS : ESI(+) *m/z* 224[M+H]$^+$, 246[M+Na]$^+$, 469[2M+Na]$^+$. ^{1}H NMR (500 MHz, CD_3OD): δ= 1.93 (s, 3H), 3.1 (t, 2H, J=7 Hz), 3.4 (t, 2H, J=7 Hz), 7.4 (m, 2H), 7.5 (m, 1H), 7.9 (m, 2H).

S-2-acetamidoethyl 4-(hydroxymethyl)benzothioate (**1i**)**:** t_{Ret} = 20 min, LC/MS : ESI(+) *m/z* 254[M+H]$^+$, 276[M+Na]$^+$, 529[2M+Na]$^+$. 1H NMR (500 MHz, [CD3]DO): δ= 2.2 (s, 3H), 2.9 (t, 2H, J=6.5 Hz), 3.1 (t, 2H, J=6.5 Hz). 3.3 (t, 2H, J=6 Hz), 7.4 (m, 2H), 7.9 (m, 2H).

S-2-acetamidoethyl 4-hydroxybenzothioate (**1j**)**:** t_{Ret} = 21 min, LC/MS : ESI(+) *m/z* 239[M+H]$^+$, 262[M+Na]$^+$, 501[2M+Na]$^+$; ESI(-) *m/z* 238[M-H]$^-$. ^{1}H NMR (500 MHz, CD_3OD): δ= 1.9 (s, 3H), 3.1 (t, 2H, J=7 Hz), 3.3 (t, 2H, J=6.5 Hz), 6.8 (d, 2H, J=9.5), 7.8 (d, 2H, J=9).

S-2-acetamidoethyl 2-(4-hydroxyphenyl)ethanethioate (**1k**)**:** t_{Ret} = 22 min, LC/MS : ESI(+) *m/z* 254[M+H]$^+$, 276[M+Na]$^+$, 529[2M+Na]$^+$; ESI(-) *m/z* 252[M-H]$^-$. 1H NMR (500 MHz, CD3DO): δ= 1.9 (s, 3H), 2.9 (t, 2H, J=7 Hz), 3.2 (t, 2H, J=7 Hz), 3.7 (s, 2H), 6.7 (dd, 2H, J=6.5, 2 Hz), 7.0 (dd, 2H, J=6.5, 2 Hz).

S-2-acetamidoethyl 4-phenylbutanethioate (**1l**)**:** t_{Ret} = 35 min, LC/MS : ESI(+) *m/z* 266[M+H]$^+$, 288[M+Na]$^+$, 553[2M+Na]$^+$. ^{1}H NMR (500 MHz, [CD_3]DO): δ= 1.8 (s,

3H), 1.86-1.92 (m, 2H), 2.51 (t, 2H, J=8 Hz). 2.57 (t, 2H, J=8 Hz), 2.9 (t, 2H, J=7 Hz), 3.2 (t, 2H, J=7 Hz), 7.1 (m, 3H), 7.2 (m, 2H)

4-Hydroxy-6-styryl-pyran-2-one (**2a**)**:** t_{Ret} = 36 min, Yield = 12%; LC/MS : ESI(+) *m/z* 215$[M+H]^+$; ESI(-) *m/z* 213$[M-H]^-$; ESI(-) MS/MS *m/z* 169$[M-H-CO_2]^-$. ^{1}H NMR (500 MHz, $[CD_3]DO$): δ= 5.4 (s, 1H), 6.0 (s, 1H), 6.7 (d, 1H, J=16 Hz), 7.2 (m, 2H), 7.3 (t, 2H, J=7.5 Hz), 7.5 (d, 2H, J=7.5 Hz).

4-Hydroxy-6-[2-(2-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**2c**)**:** t_{Ret} = 37.3 min, LC/MS : ESI(+) *m/z* 283$[M+H]^+$; ESI(-) *m/z* 281$[M-H]^-$; ESI(-) MS/MS *m/z* 237$[M-H-CO_2]^-$. ^{1}H NMR (500 MHz, CD_3OD) δ [ppm]: 5.5 (1H, s), 6.2 (1H, s), 6.9 (1H, d, *J* = 16 Hz), 7.5 (1H, t, *J* = 8), 7.6 (1H, t, *J* = 7.5), 7.7 (2H, m), 7.9 (1H, d, *J* = 8 Hz). ^{13}C NMR (500 MHz, CD_3OD) δ [ppm]: 105.9, 125.4, 127.9, 129.5, 130.9, 131.9, 134.4, 136.3, 160.9, 167.9, 174.1.

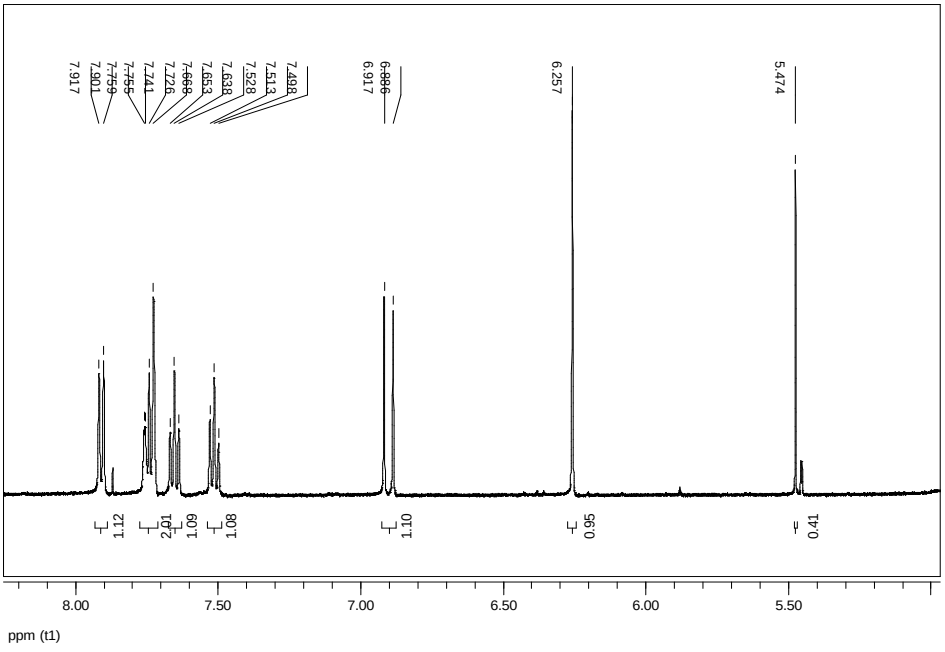


Figure 5S. ^{1}H NMR of 4-Hydroxy-6-[2-(2-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**2c)**

4-Hydroxy-6-[2-(3-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**2d**) **:** t_{Ret} = 37.3 min, LC/MS : ESI(+) *m/z* 283$[M+H]^+$; ESI(-) *m/z* 281$[M-H]^-$; ESI(-) MS/MS *m/z* 237$[M-H-CO_2]^-$.

4-Hydroxy-6-[2-(4-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**2e**)**:** t_{Ret} = 37.3 min, LC/MS : ESI(+) *m/z* 283$[M+H]^+$; ESI(-) *m/z* 281$[M-H]^-$; ESI(-) MS/MS *m/z* 237$[M-H-CO_2]^-$. ^{1}H NMR (500 MHz, CD_3OD) δ [ppm]: 5.4 (1H, s), 6.2 (1H, s), 7.0 (1H, d, *J* = 16.5 Hz), 7.4 (1H, d, *J* = 16 Hz), 7.6 (2H, m), 7.7 (2H, m). ^{13}C NMR (500 MHz, CD_3OD) δ [ppm]: 106.5, 107, 124.1, 127.6, 127.7, 129.8, 135, 141.6, 160.9, 168.6, 175.4.

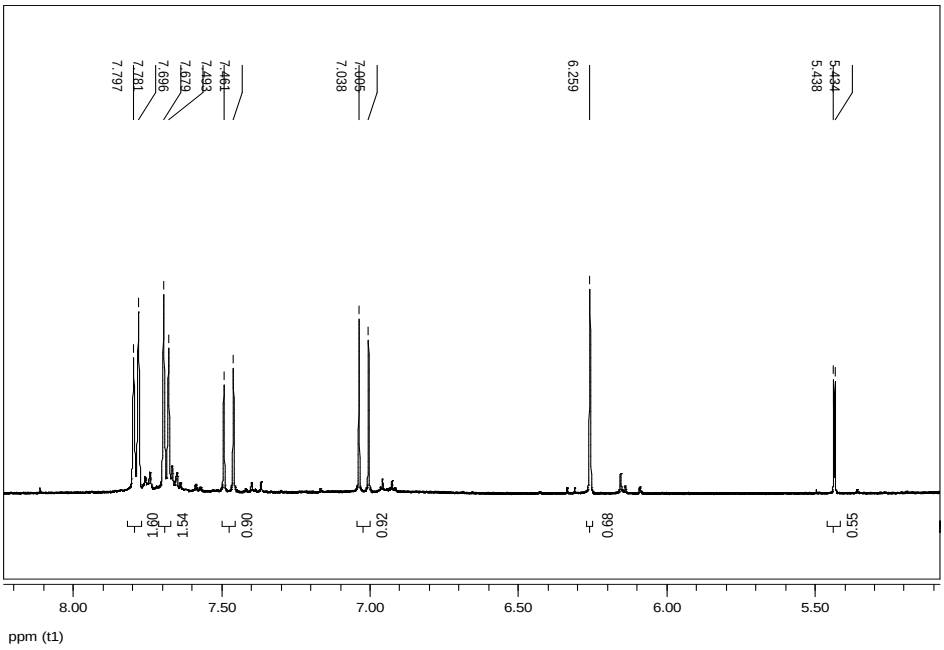


Figure 6S. ^{1}H NMR of 4-Hydroxy-6-[2-(4-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**2e**)

4-Hydroxy-6-[2-(4-methyl-phenyl)-vinyl]-pyran-2-one (**2g**)**:** Yield = 17%; ESI(+) *m/z* 229$[M+H]^+$; ESI(-) *m/z* 227$[M-H]^-$. ^{1}H NMR (500 MHz, CD_3OD) δ [ppm]: 2.3 (3H, s), 5.4 (1H, d, *J* = 2 Hz), 6.1 (1H, s), 6.7 (1H, d, *J* = 16 Hz), 7.2 (2H, d, *J* = 8 Hz), 7.41

(1H, d, *J* = 16 Hz), 7.47 (2H, d, *J* = 8 Hz) (Figure 5S). ^{13}C NMR (500 MHz, CD_3OD) δ [ppm]: 22, 91, 103, 119, 129.3, 131.3, 134.6, 137.5, 141.7, 162, 168, 173.

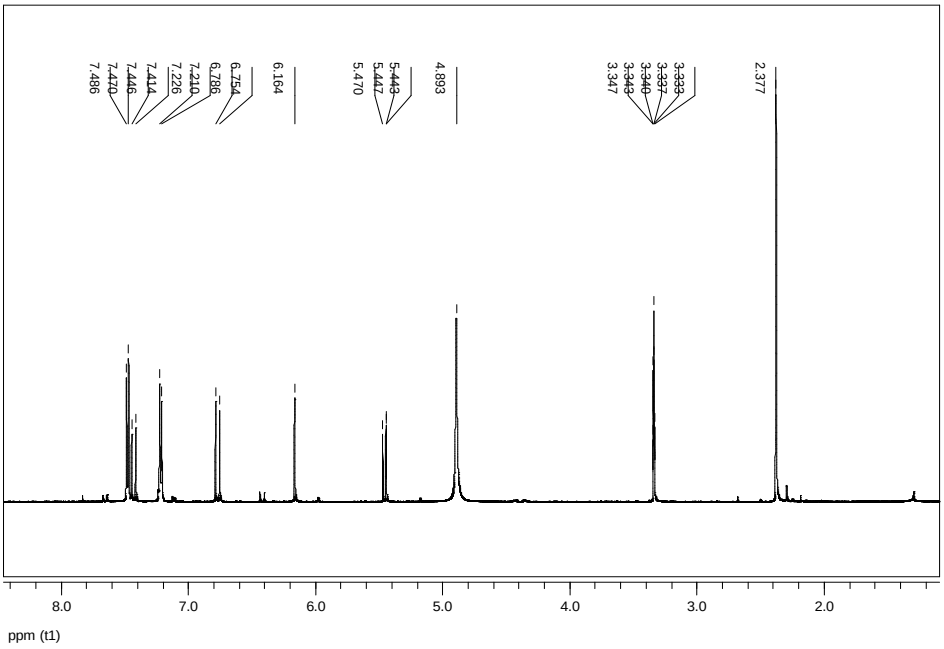


Figure 7S. ^{1}H NMR of 4-Hydroxy-6-[2-(4-methyl-phenyl)-vinyl]-pyran-2-one (**2g**)

4-hydroxy-6-phenyl-2H-pyran-2-one (**2h**): Yield = 7.7%; LC/MS : ESI(+) *m/z* 189$[M+H]^+$; ESI(-) *m/z* 187$[M-H]^-$.^{1}H NMR (500 MHz, $[CD_3]DO$): δ= 1.8 (s, 3H), 1.86-1.92 (m, 2H), 2.51 (t, 2H, J=8 Hz). 2.57 (t, 2H, J=8 Hz), 2.9 (t, 2H, J=7 Hz), 3.2 (t, 2H, J=7 Hz), 7.1 (m, 3H), 7.2 (m, 2H).

4-Hydroxy-6-(3-phenyl-propyl)-pyran-2-one (**2l**) **:** t_{Ret} = 35.3 min, Yield = 6%; LC/MS : ESI(+) *m/z* 231$[M+H]^+$; ESI(-) *m/z* 229$[M-H]^-$. 4-Hydroxy-6-(2-oxo-4-phenyl-but-3-enyl)-pyran-2-one (**3a**) **:** t_{Ret} = 35 min, LC/MS : ESI(+) *m/z* 257$[M+H]^+$; ESI(-) *m/z* 255$[M-H]^-$. ^{1}H NMR (500 MHz, $[CD_3]DO$): δ= 3.3 (m, 2H), 5.6 (s, 1H), 6.0 (s, 1H), 6.7 (d, 1H, J=16 Hz), 7.2 (m, 2H), 7.3 (t, 2H, J=8 Hz), 7.5 (d, 2H, J=7.5 Hz).

4-Hydroxy-6-[4-(4-hydroxy-phenyl)-2-oxo-but-3-enyl]-pyran-2-one (**3b**)**:** t_{Ret} = 16 min, LC/MS : ESI(+) *m/z* 273$[M+H]^+$; ESI(-) *m/z* 271$[M-H]^-$; ESI(-) MS/MS *m/z* 227 $[M-H-CO_2]^-$.

4-Hydroxy-6-[2-oxo-4-(2-trifluoromethyl-phenyl)-but-3-enyl]-pyran-2-one (**3c**)**:** t_{Ret} = 36.1 min, LC/MS : ESI(+) *m/z* 325$[M+H]^+$, 347$[M+Na]^+$; ESI(-) *m/z* 323$[M-H]^-$, 346$[M+Na]^-$; ESI(-) MS/MS *m/z* 279 $[M-H-CO_2]^-$.

4-Hydroxy-6-[2-oxo-4-(3-trifluoromethyl-phenyl)-but-3-enyl]-pyran-2-one (**3d**)**:** t_{Ret} = 36.1 min, LC/MS : ESI(+) *m/z* 325$[M+H]^+$, 347$[M+Na]^+$; ESI(-) *m/z* 323$[M-H]^-$, 346$[M+Na]^-$; ESI(-) MS/MS *m/z* 279 $[M-H-CO_2]^-$.

4-Hydroxy-6-[2-oxo-4-(4-trifluoromethyl-phenyl)-but-3-enyl]-pyran-2-one (**3e**)**:** t_{Ret} = 36.1 min, LC/MS : ESI(+) *m/z* 325$[M+H]^+$, 347$[M+Na]^+$; ESI(-) *m/z* 323$[M-H]^-$, 346$[M+Na]^-$; ESI(-) MS/MS *m/z* 279 $[M-H-CO_2]^-$.

4-Hydroxy-6-(2-oxo-2-phenyl-ethyl)-pyran-2-one (**3h**)**:** t_{Ret} = 35 min, Yield = 21%; LC/MS : ESI(+) *m/z* 231$[M+H]^+$, 253$[M+Na]^{+,}$ 483$[2M+Na]^+$; ESI(-) *m/z* 229$[M-H]^-$.]$^-$. ^{1}H NMR (500 MHz, CD_3OD) δ [ppm]: 3.4 (2H, t, *J* = 7 Hz), 5.4 (1H, s), 6.0 (1H, s), 7.4 (3H, m), 7.9 (2H, m).

4-Hydroxy-6-[2-(4-hydroxymethyl-phenyl)-2-oxo-ethyl]-pyran-2-one (**3i**)**:** t_{Ret} = 32 min, LC/MS : ESI(+) *m/z* 261$[M+H]^+$; ESI(-) *m/z* 259$[M-H]^-$.

4-Hydroxy-6-[2-(4-hydroxy-phenyl)-2-oxo-ethyl]-pyran-2-one (**3j**)**:** t_{Ret} = 36 min, Yield = 15.7%; LC/MS : ESI(+) *m/z* 247$[M+H]^+$; ESI(-) *m/z* 245$[M-H]^-$. ^{1}H NMR (500 MHz, $[CD_3]DO$): δ= 3.7 (m, 2H), 5.5 (m, 2H), 6.8 (m, 2H), 7.8 (m, 2H).

4-Hydroxy-6-(2-oxo-5-phenyl-pentyl)-pyran-2-one (**3l**)**:** t_{Ret} = 34.5 min, LC/MS : ESI(+) *m/z* 273$[M+H]^+$; ESI(-) *m/z* 271$[M-H]^-$.

5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one (4b): t_{Ret} = 31 min, Yield = 4.5%; LC/MS : ESI(+) *m/z* 273$[M+H]^+$; ESI(-) *m/z* 271$[M-H]^-$. ^{1}H NMR (500 MHz, $[CD_3]DO$): δ= 2.7 (dd, 1H, J=3, 17 Hz), 3.1 (dd, 1H, J=13, 17.5 Hz), 5.3 (dd, 1H, J=3, 13 Hz), 5.8 (m, 2H), 6.8 (d, 2H, J=8.5 Hz), 7.3 (d, 2H, J=9 Hz)

4-Hydroxy-3,5-dimethyl-6-styryl-pyran-2-one (**5a**)**:** t_{Ret} = 37 min, LC/MS : ESI(+) *m/z* 243$[M+H]^+$; ESI(-) *m/z* 241$[M-H]^-$; ESI(-) MS/MS *m/z* 182$[M-H-CO_2-CH_3]^-$, 197$[M-H-CO_2]^-$.

4-Hydroxy-3,5-dimethyl-6-[2-(2-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**5c**)**:** t_{Ret} = 38.9 min, LC/MS : ESI(+) *m/z* 311$[M+H]^+$; ESI(-) *m/z* 309$[M-H]^-$; ESI(-) MS/MS *m/z* 250$[M-H-CO_2-CH_3]^-$, 265$[M-H-CO_2]^-$.

4-Hydroxy-3,5-dimethyl-6-[2-(3-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**5d**)**:** t_{Ret} = 38.9 min, LC/MS : ESI(+) *m/z* 311$[M+H]^+$; ESI(-) *m/z* 309$[M-H]^-$; ESI(-) MS/MS *m/z* 250$[M-H-CO_2-CH_3]^-$, 265$[M-H-CO_2]^-$.

4-Hydroxy-3,5-dimethyl-6-[2-(4-trifluoromethyl-phenyl)-vinyl]-pyran-2-one (**5e**)**:** t_{Ret} = 38.9 min, LC/MS : ESI(+) *m/z* 311$[M+H]^+$; ESI(-) *m/z* 309$[M-H]^-$; ESI(-) MS/MS *m/z* 250$[M-H-CO_2-CH_3]^-$, 265$[M-H-CO_2]^-$.

4-Hydroxy-3,5-dimethyl-6-phenyl-pyran-2-one (**5h**)**:** t_{Ret} = 40 min, LC/MS : ESI(+) *m/z* 217$[M+H]^+$, 239$[M+Na]^+$; ESI(-) *m/z* 215$[M-H]^-$.

4-Hydroxy-3,5-dimethyl-6-(3-phenyl-propyl)-pyran-2-one (**5l**)**:** t_{Ret} = 37 min, LC/MS : ESI(+) *m/z* 259$[M+H]^+$; ESI(-) *m/z* 257$[M-H]^-$.

4-Hydroxy-3,5-dimethyl-6-(1-methyl-2-oxo-4-phenyl-but-3-enyl)-pyran-2-one (**6a**)**:** t_{Ret} = 31 min, LC/MS : ESI(+) *m/z* 299$[M+H]^+$; ESI(-) *m/z* 297$[M-H]^-$; ESI(-) MS/MS *m/z* 119$[M-H-CO_2-HOC_6H_4CHCO]^-$, 176$[M-H-HOC_6H_4CO]^-$, 253$[M-H-CO_2]^-$.

4-Hydroxy-6-[4-(4hydroxy-phenyl)-1-methyl-2-oxo-but-3-enyl]-3,5-dimethyl-pyran-2-one (**6b**)**:** t_{Ret} = 28 min, LC/MS : ESI(+) *m/z* 315$[M+H]^+$; ESI(-) *m/z* 313$[M-H]^-$.

6-[4-(3,5-Bis-trifluoromethyl-phenyl)-1-methyl-2-oxo-but-3-enyl]-4-hydroxy-3,5-dimethyl-pyran-2-one (**6f**)**:** t_{Ret} = 35 min, LC/MS : ESI(+) *m/z* 435$[M+H]^+$; ESI(-) *m/z* 433$[M-H]^-$.

4-Hydroxy-6-[3-(4-hydroxy-phenyl)-1-methyl-2-oxo-propyl]-3,5-dimethyl-pyran-2-one (**6k**)**:** t_{Ret} = 24 min, LC/MS : ESI(+) *m/z* 325$[M+Na]^+$; ESI(-) *m/z* 301$[M-H]^-$; ESI(-) MS/MS *m/z* 123$[M-H-CO_2-HOC_6H_4CHCO]^-$, 167$[M-H-HOC_6H_4CO]^-$, 257$[M-H-CO_2]^-$.