Supplementary Materials

Synthesis and anticancer cytotoxicity of azaaurones overcoming multidrug resistance

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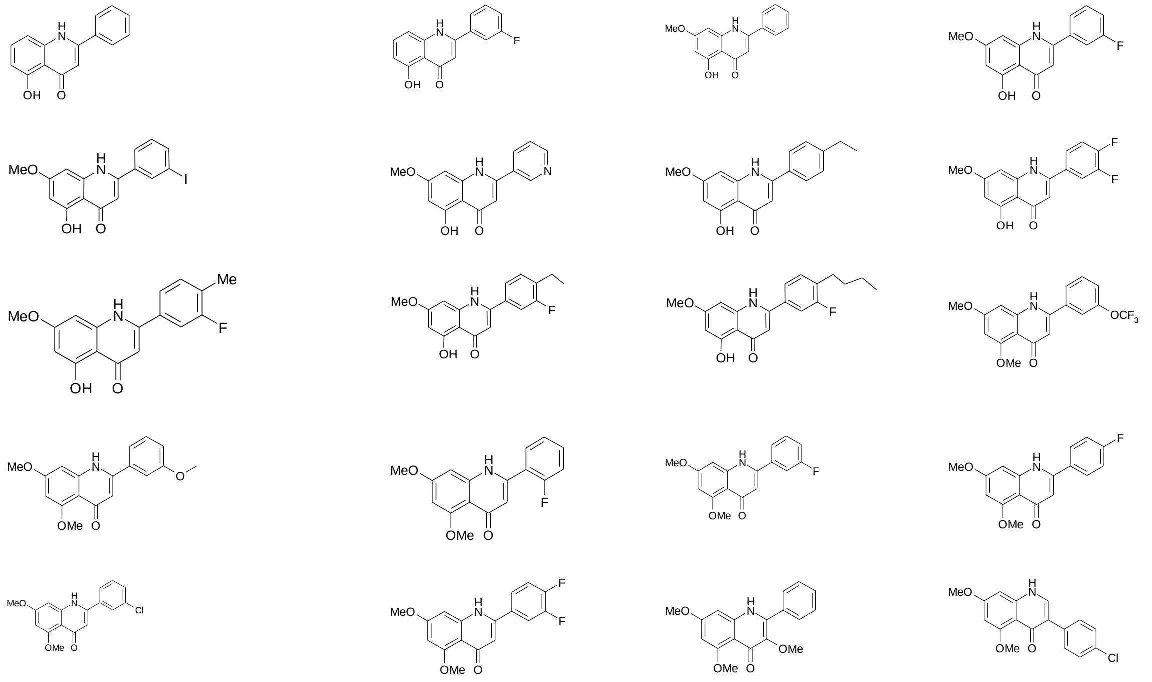
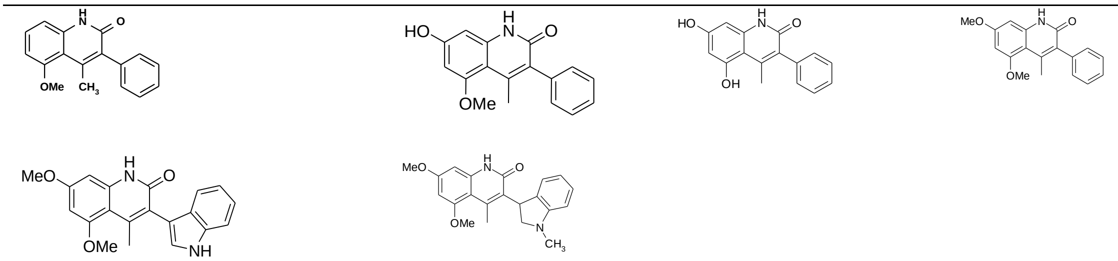
Content

Table S1. Structures of the screened compounds. The screened compounds are from our internal chemical libraries. The syntheses of these compounds were reported in several studies (Hadjeri, M., *J. Med. Chem.* 2**003**; Hadjeri, M., *J. Med. Chem.* **2004**; Doléans-Jordheim, A., *ChemMedChem.* **2013**; Valdameri, G., *J. Med. Chem.* **2012**; Boumendjel, A., *J. Med. Chem.* **2008**; Genoux-Bastide, *ChemMedChem.* **2012**).

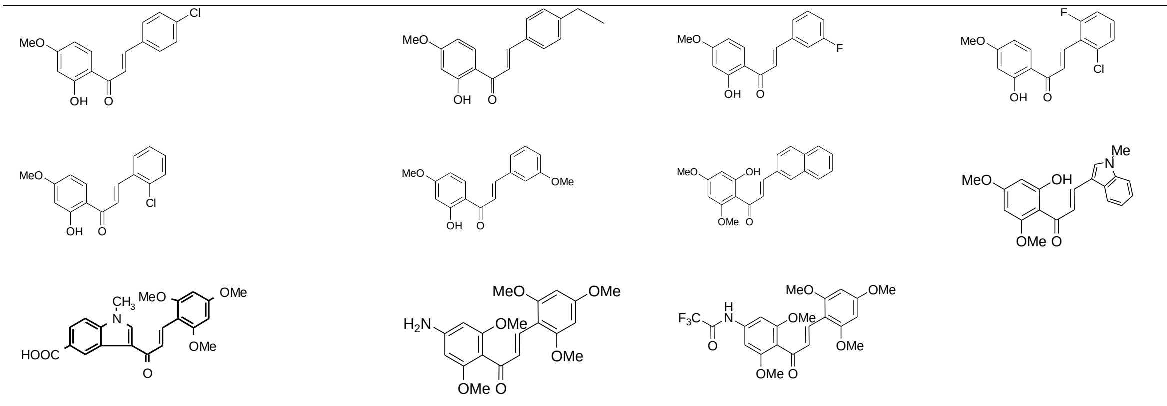
4-Quinolones (20 compounds)



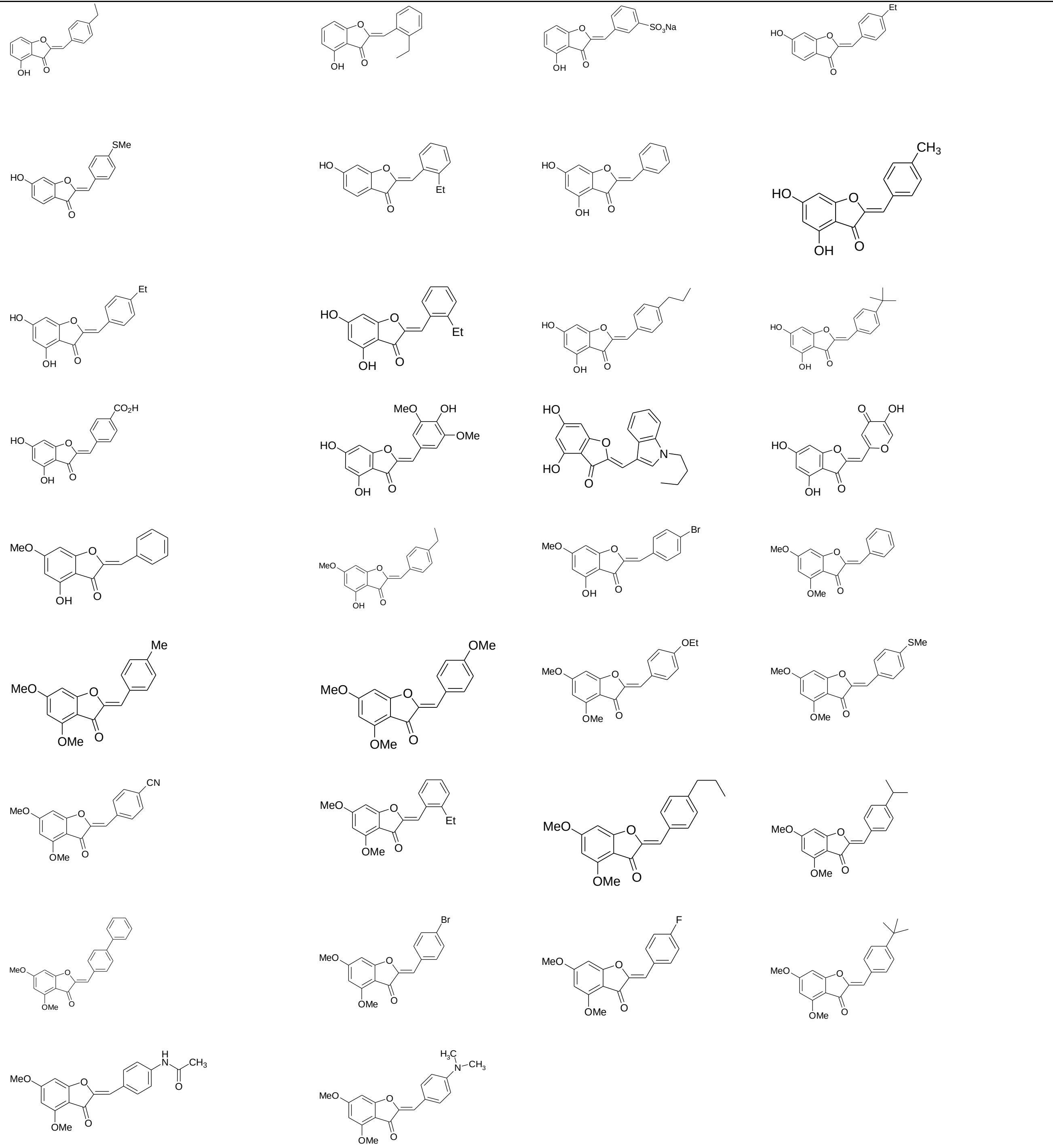
2-Quinolones (6 compounds)



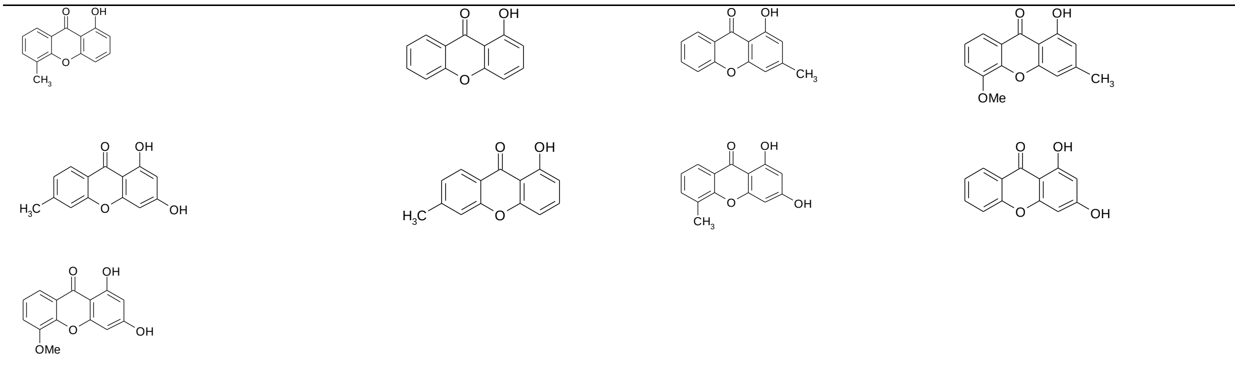
Chalcones (11 compounds)



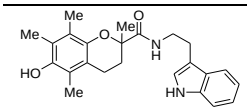
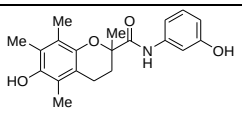
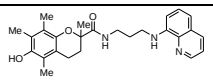
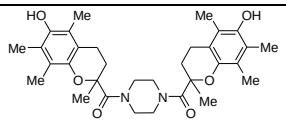
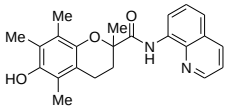
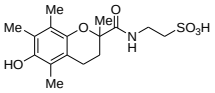
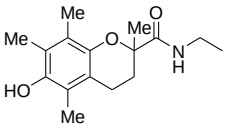
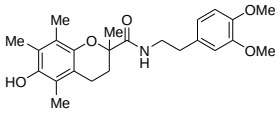
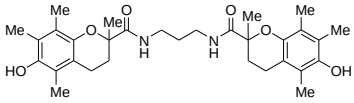
Aurones (34 compounds)



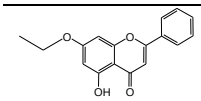
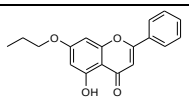
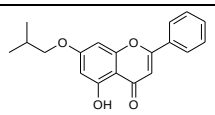
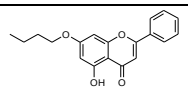
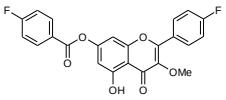
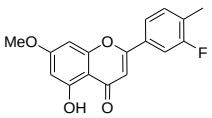
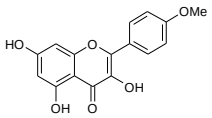
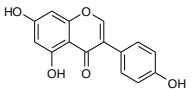
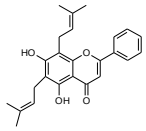
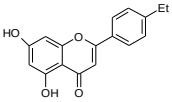
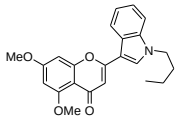
Xanthones (9 compounds)



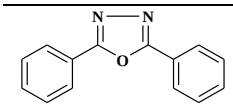
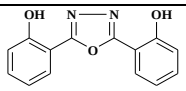
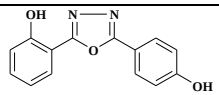
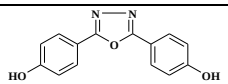
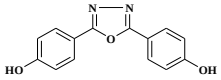
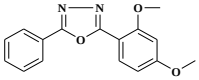
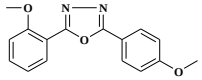
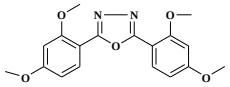
Dihydrobenzopyranes (9 compounds)

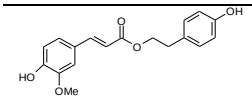
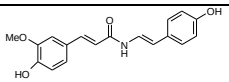
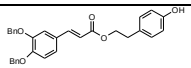
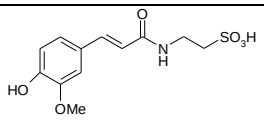
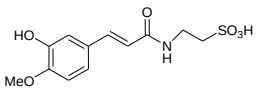
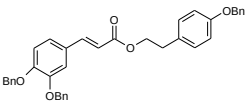
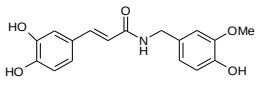
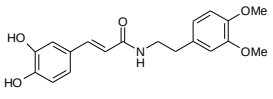
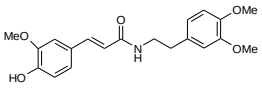
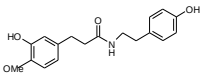
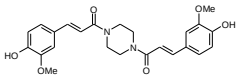
Flavones (11 compounds)

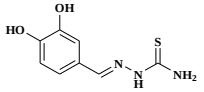
Oxadiazoles (8 compounds)

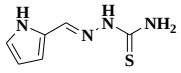
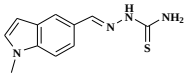
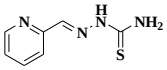
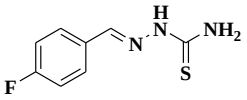
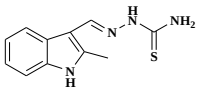
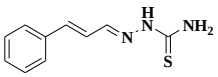
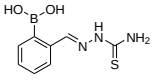
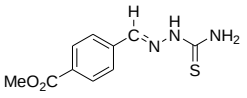
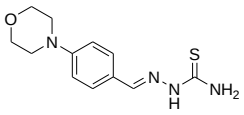
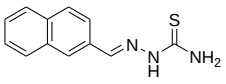
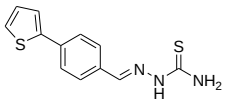









Ferulic acid derivatives (11 compounds)

Thiosemicarbazides (15 compounds)



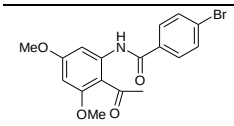
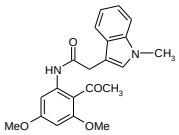
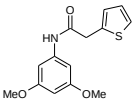
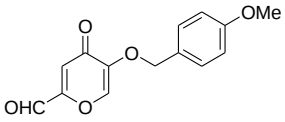
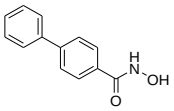
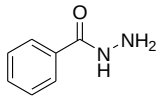
Miscellanoeus (6 compounds)			
			
			

Table S2. IC_{50} of aurone representatives (in μM).

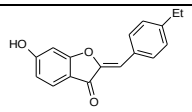
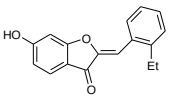
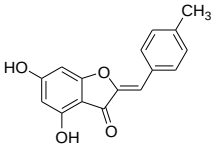
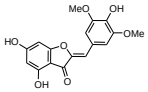
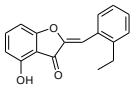
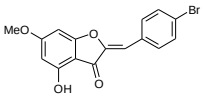
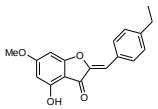
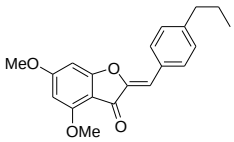
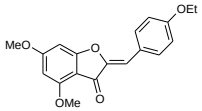
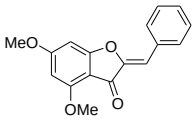
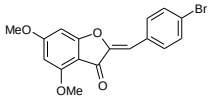
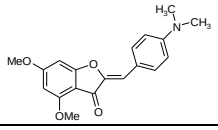
Structure	MES-SA	Dx5	MES-SA/Dx5
	56.5 ± 5.7	43.7 ± 6.6	1.3
	104.2 ± 20.6	91.0 ± 14.0	1.1
	88.9 ± 15.6	56.4 ± 10.0	1.6
	50.1 ± 10.8	109.2 ± 19.0	0.46
	99.0 ± 18.1	53.4 ± 5.0	1.9
	116.4 ± 24.1	44.7 ± 4.1	2.6
	63.0 ± 1.5	32.1 ± 6.1	2.0
	111.6 ± 30.3	38.4 ± 4.8	2.9
	29.6 ± 1.5	21.4 ± 2.8	1.4
	44.2 ± 0.8	18.2 ± 5.3	2.4
	93.2 ± 6.4	33.0 ± 2.8	2.8
	60.1 ± 3.9	15.0 ± 0.1	4.0

Table S3. Effects of azaaurone **4** in various P-gp-expressing cell lines.

Cell lines		SR
A431	**A431-B1**	
44.6 ± 6.9	27.0 ± 3.0	1.7
KB-3-1	**KB-V1**	**SR**
29.4 ± 0.4	20.9 ± 0.9	1.4
MDCK II	**MDCK II-B1**	**SR**
20.4 ± 4.5	16.8 ± 1.7	1.2

Values are represented as IC_{50} values ± SD (μM). SR is the abbreviation of selectivity ratio: the IC_{50} value against the parental cell line divided by the IC_{50} value against the P-gp-expressing derivative. KB-3-1 is a HeLa derived endocervical adenocarcinoma cell line. KB-V1 is its vinblastine-selected P-gp-expressing variant. MDCK II is a spontaneously immortalized canine kidney cell line. MDCK II-B1 cell line stably expressing the human wild-type ABCB1 was created by the Sleeping Beauty transposon-based gene delivery system, using the 100 × hyperactive SB transposase.

Table S4. Contribution of P-gp to the selective toxicity.

Azaaurone	**Mes-Sa**	**Dx5**	**SR**	**Mes-Sa(TQ)**	**Dx5(TQ)**	**SR(TQ)**
4	17.8 ± 4.1	3.7 ± 0.6	4.8**	3.2 ± 0.7	0.8 ± 0.2	4.0*
5	17.1 ± 1.7	5.8 ± 1.3	2.9*	4.3 ± 0.4	1.1 ± 0.3	3.9*
6	11.9 ± 1.6	3.4 ± 0.2	3.8**	2.5 ± 0.3	0.6 ± 0.01	4.2*
7	61.9 ± 10.0	13.1 ± 5.6	2.7**	16.9 ± 0.3	4.9 ± 0.4	3.4**
10	9.2 ± 2.3	19.5 ± 3.5	0.5*	9.6 ± 1.0	6.7 ± 0.7	1.4

Table S5. Physicochemical characterization of azaaurones (compounds 1-13) synthesized according to Scheme 1.

Compound	Characterization
1	**(Z)-2-(4-Chlorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 80%; yellow powder; mp 146-148 °C; **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.4 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H3′, H5′), 7.3 (dd, 2H, J_1 = 8.5 Hz, J_2 = 0.4 Hz, H2′, H6′), 6.6 (s, 1H, =CH-), 6.1 (d, 1H, J = 1.6 Hz, H7), 5.85 (d, 1H, J = 1.6 Hz, H5), 3.85 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3); **^{13}C NMR** ($CDCl_3$, 50 MHz) δ 182.33, 168.89, 160.61, 156.64, 136.64, 133.87, 133.68, 130.54, 129.44, 108.36, 105.07, 91.56, 88.69, 56.06, 56.03, 27.09. **HRMS** (ESI/LTQ Orbitrap) calcd for $C_{17}H_{15}ClNO_3$ ($M+H^+$) 316.0662, found 316.0650. **Anal.** ($C_{17}H_{14}ClNO_3$) C, H, N.
2	**(Z)-2-(4-Ethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 70%; yellow powder; mp 215-217 °C; **^{1}H NMR** (200 MHz, $CDCl_3$) δ 7.45 (dd, 2H, J_1 = 8.2 Hz, J_2 = 0.4 Hz, H2′, H6′), 7.25 (dd, 2H, J_1 = 8.2 Hz, J_2 = 0.4 Hz, H3′, H5′), 7.1 (bs, 1H), 6.7 (s, 1H), 6.1 (d, 1H, J = 1.8 Hz, H7), 5.9 (d, J = 1.8 Hz, 1H, H5), 3.9 (s, 3H), 3.8 (s, 3H), 2.7 (q, 2H, J = 6 Hz, -CH_2), 1.3 (t, J = 6 Hz, 3H, CH_3); **^{13}C NMR** (CD_3COCD_3, 50 MHz) δ 182.89, 169.88, 161.74, 158.71, 145.59, 137.21, 134.12, 130.91, 129.89, 108.70, 105.62, 92.23, 90.13, 56.77, 56.60, 16.56. **HRMS** (ESI/LTQ Orbitrap) calcd for $C_{19}H_{20}NO_3$ $[M+H]^+$. 310.1365, found 310.1365. **Anal.** ($C_{19}H_{19}NO_3$) C, H, N.
3	**(Z)-2-(2-Ethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 74%; yellow powder; mp 197-198 °C; **^{1}H NMR** (400MHz, $CDCl_3$) δ 7.51-7.49 (m, H6), 7.25 (bs, 3H, H3′, H4′, H5′), 6.90 (s, 1H, =CH-), 6.78 (bs, 1H, NH), 6.00 (s, 1H, H7), 5.92 (s, 1H, H5), 3.94 (s, 3H, OCH_3), 2.79-2.73 (q, J = 7.28 Hz, 2H, CH_2), 1.22-1.18 (t, J = 7.52 Hz, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 183.58, 170.14, 162.06, 158.04, 145.81, 138.58, 134.67, 130.76, 129.95, 129.83, 127.77, 109.11, 106.66, 92.53, 89.58, 57.38, 57.34, 28.22, 16.04. **HRMS** (ESI/LTQ Orbitrap) calcd for $C_{19}H_{20}NO_3$ $[M+H]^+$. 310.1028, found 310.1109. **Anal.** ($C_{19}H_{19}NO_3$) C, H, N.
4	**(Z)-2-(4-Buthylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 59%; yellow powder; mp 90-92 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.2 Hz, 2H, H2′, H6′), 7.27 (d, J = 8.2 Hz, 2H, H3′, H5′), 6.8 (bs, 1H, NH), 6.75 (s, 1H, =CH-), 6.07 (d, J = 1.69 Hz, 1H, H7), 5.93 (d, J = 1.72 Hz, 1H, H5), 3.92 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 2.63 (t, J = 7.5 Hz, 2H, $\underline{CH_2}$-Ph), 1.63 (bs, 4H, CH_2-CH_2), 0.94 (t, J = 7.5 Hz, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 170.02, 161.91, 158.5, 144.86, 137.37, 133.89, 130.85, 111.56, 106.62, 92.69, 89.85, 57.33, 37.06, 35.01, 90.3, 23.91, 15.51. **HRMS** calcd for $C_{21}H_{23}NO_3$: 337.42, found 338.22 $[M+H]^+$. **Anal.** ($C_{21}H_{23}NO_3$) C, H, N.
5	**(Z)-2-(4-Isopropylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 53%; yellow powder; mp 252 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.47 (d, 2H, J = 4 Hz, H2′, H6′), 7.31 (d, 1H, J = 4 Hz, H3′, H5′), 6.86 (bs, 1H, NH), 6.77 (s, 1H, =CH-), 6.07 (d, 1H, J = 1.6 Hz, H5), 5.94 (d, 1H, J = 2 Hz, H7), 3.94 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.01 (m, 1H, $\underline{CH}(CH_3)_2$), 1.28 (d, 6H, J = 6.8 Hz, $(CH_3)_2$); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 179.94, 166.27, 158.10, 154.23, 147.01, 133.50, 130.23, 127.22, 124.97, 107.99, 102.78, 88.95, 86.07, 53.88, 53.62, 31.69, 21.58, 21.48. **HRMS** calcd for $C_{20}H_{21}NO_3$: 323.5857, found 323.6541. **Anal.** ($C_{20}H_{21}NO_3$) C, H, N.
6	**(Z)-2-(4-tert-Buthylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 51%; brown powder; mp 196-197 °C; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ 7.46 (bs, 2H, H2′, H6′), 7.25 (bs, 2H, H3′, H5′), 6.84 (bs, 1H, NH), 6.77 (s, 1H, =CH-), 6.06 (d, J = 1.69 Hz, 1H, H7), 5.94 (d, J = 1.67 Hz, 1H, H5), 3.94 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 1.37 (bs, 9H, -$C(CH_3)_3$); **^{13}C NMR** ($CDCl_3$, 400 MHz) δ 184.07, 170.04, 161.92, 158.12, 152.94, 137.50, 133.69, 130.70, 127.57, 111.34, 106.59, 92.67, 89.88, 57.32, 36.30, 32.73. **HRMS** calcd for $C_{21}H_{23}NO_3$: 337.42, found 336.22 $[M-H]^+$. **Anal.** ($C_{21}H_{23}NO_3$) C, H, N.
7	**(Z)-2-(4-Cyanobenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 82%; yellow powder; mp 135 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.46 (d, 2H, J = 8.4 Hz, H3′, H5′), 7.39 (d, 2H, J = 8.4 Hz, H2′, H6′), 6.80 (bs, 1H, NH), 6.62 (s, 1H, =CH-), 6.01 (d, 1H, J = 2 Hz, H7), 5.88 (d, 1H, J = 1.6 Hz, H5), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.11 (s, 1H, CH); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 182.19, 168.75, 140.47, 156.50, 136.50, 133.73, 133.54, 130.40, 129.31, 108.21, 104.93, 91.52, 88.55, 77.27, 55.92, 55.88, 26.95. **SM** (EI): m/z 305 $[M]^+$. **HRMS** (ESI/LTQ Orbitrap) calcd for $C_{18}H_{14}N_2O_3$ 306.1004, found 307.0990 $[M+H]^+$. **Anal.** ($C_{18}H_{14}N_2O_3$) C, H, N.

8	**(Z)-2-(2,4-Dimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 45%; brown powder; mp 235 °C; **^{1}H NMR** (200 MHz, CD_3COCD_3) δ 7.5 (m, 1H, H2′), 7.06 (m, 2H, H4′, H6′), 6.6 (s, 1H, =CH-), 6.2 (d, *J* = 1.8 Hz, 1H, H7), 5.99 (d, *J* = 1.78 Hz, 1H, H5), 3.67 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3), 2.28 (s, 3H, CH_3); **^{13}C NMR** (CD_3COCD_3, 50 MHz) δ 182.07, 169.26, 161.19, 158.18, 138.42, 138.36, 137.22, 132.19, 131.81, 129.26, 127.79, 105.56, 105.11, 91.47, 89.39, 56.18, 56.02, 21.28, 20.13. **HRMS** calcd for $C_{19}H_{19}NO_3$: 309.37, found 310.23 $[M+H]^+$. **Anal.** ($C_{19}H_{19}NO_3$) C, H, N.
9	**(Z)-2-(2,4,5-Trimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 10%; yellow powder; mp 229 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.27 (s, 1H, H6′), 7.03 (s, 1H, H3′) 6.84 (bs, 2H, NH, =CH-), 6.04-6.03 (d, *J* = 1.61 Hz, 1H, H7), 5.91 (d, *J* = 1.58 Hz, 1H, H5), 3.92 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 183.56, 169.95, 162.03, 157.89, 138.46, 137.83, 132.29, 135.87, 13.86, 132.73, 130.62, 109.71, 106.84, 92.56, 89.55, 57.41-57.34, 21.13. **HRMS** calcd for $C_{20}H_{21}NO_3$: 323.40, found 324.19 $[M+H]^+$. **Anal.** ($C_{20}H_{21}NO_3$) C, H, N.
10	**(Z)-2-(2,3,5,6-Tetramethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 18%; yellow powder; mp 260 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.97 (s, 1H, H4′), 6.85 (s, 1H, =CH-), 5.89 (d, *J* = 1.66 Hz, 1H, H7), 5.87 (d, *J* = 1.69 Hz, 1H, H5), 3.93 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.25 (s, 6H, 2 CH_3), 2.15 (s, 6H, 2 CH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 182.79, 170.18, 162.10, 157.57, 139.32, 135.63, 134.55, 134.02, 132.65, 111.82, 92.24, 89.08, 57.43, 57.29, 21.64, 18.33. **HRMS** calcd for $C_{21}H_{23}O_3N$: 337.41, found 338.23 $[M+H]^+$. **Anal.** ($C_{21}H_{23}NO_3$) C, H, N.
11	**(Z)-2-(2,4-Dimethoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 46%; orange powder; mp 214-216 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.42-7.39 (d, 1H, *J* = 8.42, H6′), 6.82 (s, 1H, H3′), 6.56 (d, 1H, *J* = 8.37 Hz, H5′), 6.51 (s, 1H, =CH-), 6.01 (s, 1H, H7), 5.89 (s, H, H5), 3.92 (s, 6H, 2 OCH_3), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 183.56, 169.95, 162.03, 157.89, 138.46, 137.83, 132.29, 135.87, 13.86, 132.73, 130.62, 109.71, 106.84, 92.56, 89.55, 57.41, 57.34, 21.13. **HRMS** calcd for $C_{19}H_{19}NO_5$: 341,369, found 342.42 $[M+H]^+$. **Anal.** ($C_{19}H_{19}NO_5$) C, H, N.
12	**(Z)-2-(2,4,6-Trimethoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 60%; yellow powder; mp 109-111 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.29 (bs, 1H, NH), 6.98 (s, 1H, =CH-), 6.19 (bs, 2H, H3′, H5′), 5.97 (d, 1H, *J* = 1.51 Hz, H7), 5.86 (d, 1H, *J* = 1.47 Hz, 1H, H5), 3.91 (s, 3H, OCH_3), 3.89 (s, 6H, 2 OCH_3), 3.85 (s, 6H, 2 OCH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 182.34, 168.20, 162.09, 160.48, 159.32, 155.44, 135.60, 106.88, 105.31, 102.19, 92.04, 90.52, 87.86, 56.61, 56.00, 55.83, 55.64. **HRMS** calcd $C_{20}H_{21}NO_6$: 371.396, found 372.06 $[M+H]^+$. **Anal.** ($C_{20}H_{21}NO_6$) C, H, N.
13	**(Z)-2-(2,6-Dimethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1*H*-indol-3-one:** Yield: 25%; yellow powder; mp 217-218 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31-7.03 (m, *J* = 7.62 Hz, 4H, H3′, H4′, H5′, NH), 6.84 (s, 1H, =CH-), 6.03 (s, 1H, H7), 5.92 (s, 1H, H5), 3.93 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3), 2.35 (s, 3H, CH_3); **^{13}C NMR** ($CDCl_3$, 100 MHz) δ 183.49, 170.05, 162.09, 157.90, 138.21, 137.24, 136.81, 135.27, 132.35, 130.49, 130.05, 109.52, 106.79, 92.53, 89.56, 57.47, 57.35, 22.69, 21.19. **HRMS** calcd for $C_{19}H_{19}NO_3$: 309.37, found 310.23 $[M+H]^+$. **Anal.** ($C_{19}H_{19}NO_3$) C, H, N.

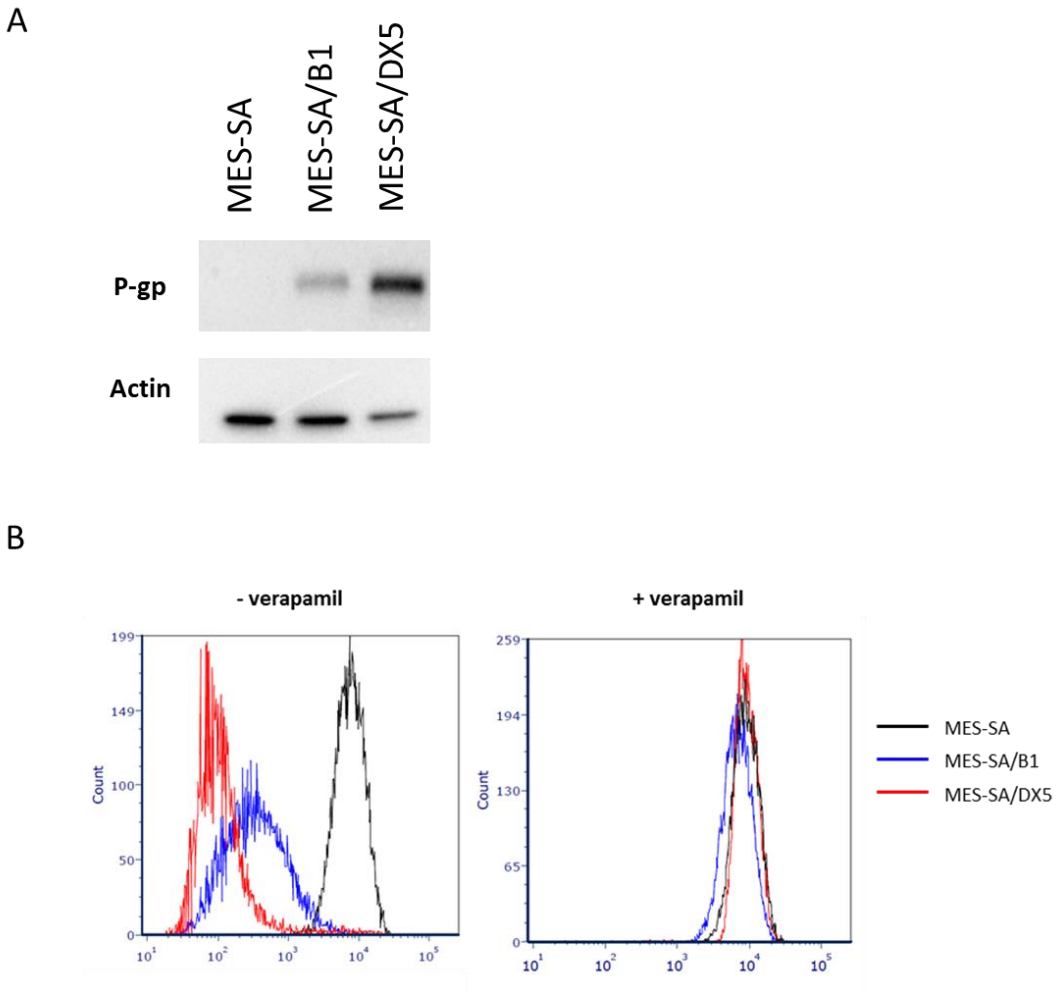


Figure S1. Characterization of P-glycoprotein expression and activity in MES-SA/Dx5 cells. **(A)** P-gp (upper lane) was visualized by Western blotting using the C219 antibody; Beta-actin is shown as loading control (lower lane). **(B)** Characterization of P-gp activity with the calcein assay. Calcein-AM is extruded by P-gp from MES-SA/Dx5 cell lines, preventing the accumulation of fluorescent calcein in the cells (left). Inhibition of the transporter by verapamil (10 μM) restores fluorescence to the levels observed in parental MES-SA cells (right). MES-SA/B1 cells, expressing P-gp by viral transduction, are shown as control (Cserepes *et al.*, 2019).