

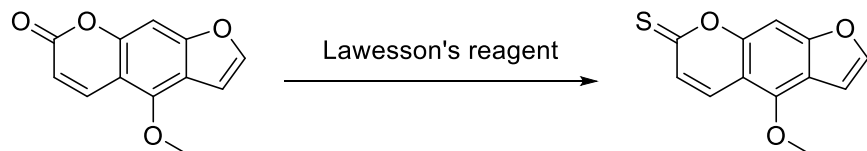
## SUPPLEMENTAL METHODS AND RESULTS

### METHODS

#### Chemistry

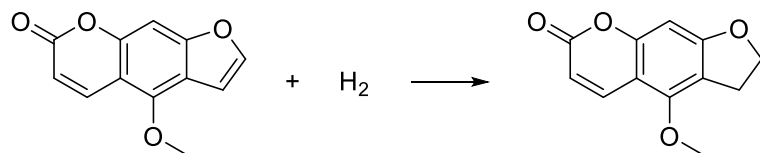
Compounds **1-5**, **7-9**, **11-15**, **17-20**, and **26** were commercially available. Compounds **16**, **23**, and **32** were synthesized as previously published<sup>1</sup>.

**6**



**5** (bergapten; 54mg, 0.25 mmol) was dissolved in 5 mL of anhydrous toluene and Lawesson's reagent (202 mg, 0.5 mmol) was added. The resulting solution was refluxed overnight. The solution was concentrated in vacuo. Purification of the crude product by flash chromatography (Hexanes /ethyl acetate) gave compound **6**.

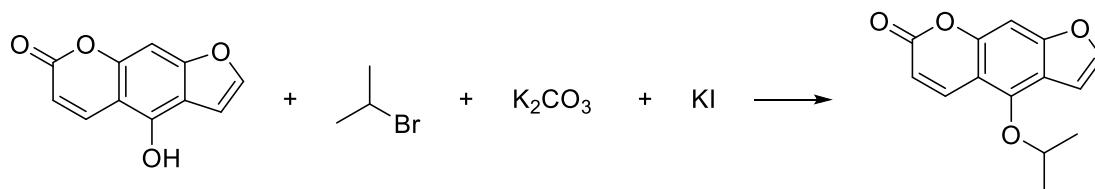
**10**



**5** (42 mg, 0.2 mmol) was dissolved in acetone (5 mL) at 40 C. Pd/C (10%, 84 mg), formic acid (75 uL, 2.0 mmol) and triethylamine (334 uL, 2.4 mmol) were added to the solution. After 10 minutes, the reaction was complete. The solution was filtered through Celite and the solvent was removed under reduced pressure. The solid was dissolved in acetone and pre-absorbed onto silica. Purification by column chromatography furnished the desired compound after elution with ethyl acetate–hexane (3:7) increasing

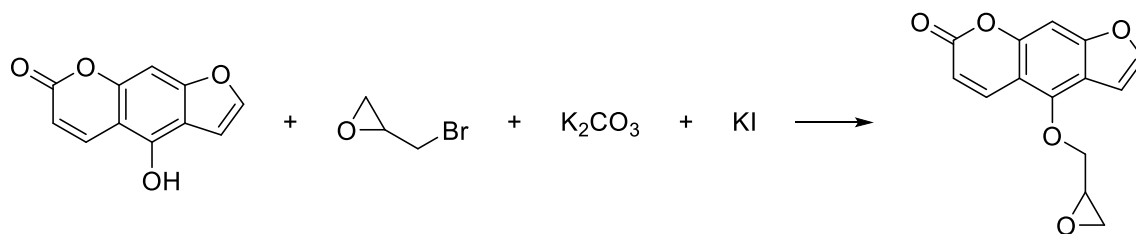
to ethyl acetate (100%). The relevant fractions were combined and the solvent was removed under reduced pressure, yielding an off-white solid compound **10**.

## 21



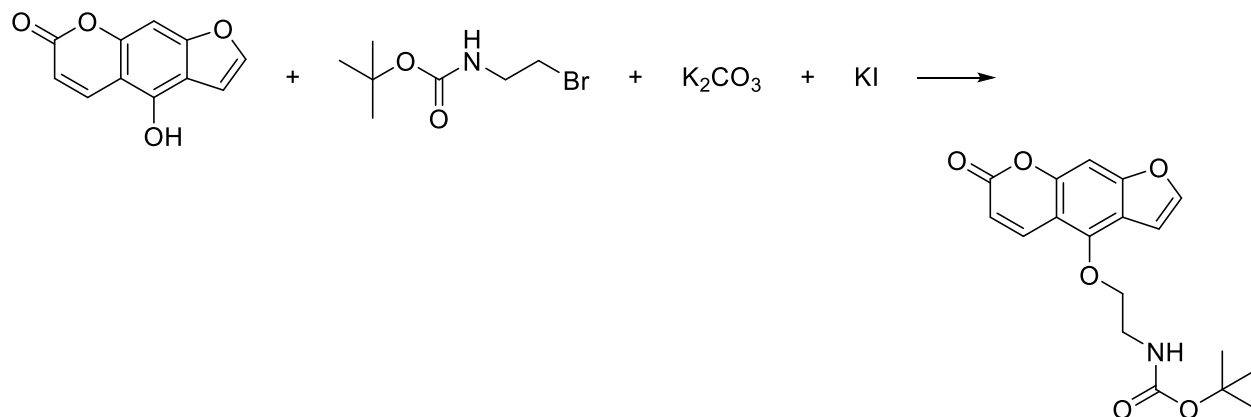
Under a  $N_2$  atmosphere, **16** (4-hydroxypsoralen; 45 mg, 0.225 mmol) was dissolved in anhydrous DMF (1 mL/mmol), and treated with  $K_2CO_3$  (47mg, 0.3375 mmol) and KI (cat.). 2-bromopropane (21  $\mu$ L, 0.225 mmol) was added and the reaction was stirred at 50  $^{\circ}C$  for 8 h. The reaction mixture was diluted with EtOAc and poured onto 1N HCl. The aqueous phase was then extracted three times with EtOAc. The combined organic layers were washed with  $H_2O$  and brine, dried with  $MgSO_4$ , and concentrated. The residue was purified via column chromatography eluting with EtOAc/Hexanes to obtain the product.

## 22



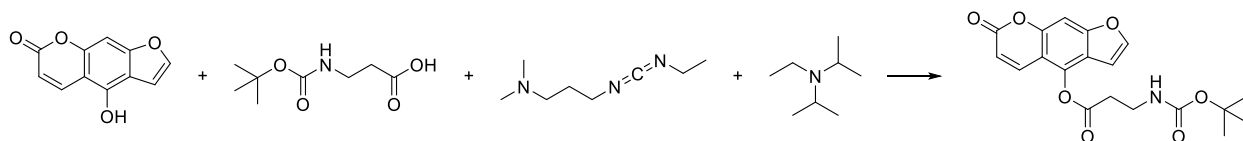
Methods as described for **21**.

24



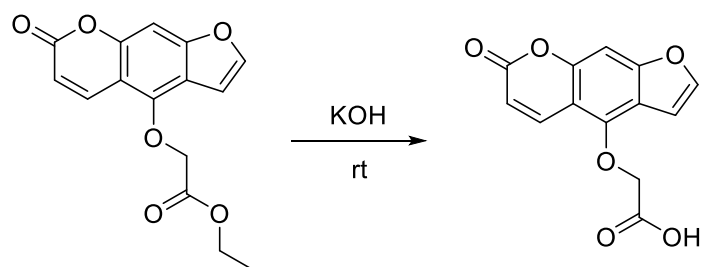
Methods as described for **21**.

25



EDCI (92mg, 0.48 mmol) was added to a solution of **16** (4-hydroxypsoralen; 80 mg, 0.4 mmol) and 3-((*tert*-butoxycarbonyl)amino)propanoic acid (90mg, 0.48 mmol) in DMF (2 mL). The mixture was stirred for five minutes. DIPEA (105 uL, 0.6 mmol) and DMAP (cat.) were added successively. The reaction mixture was stirred overnight at room temperature. After the completion of the reaction as shown by TLC, water was added to dilute the reaction mixture. The organic layer was extracted with DCM. The combined organic layer was washed with saturated brine, dried over anhydrous magnesium sulfate and concentrated under vacuum. The crude product was purified by flash chromatography (EtOAc/Hexane).

27

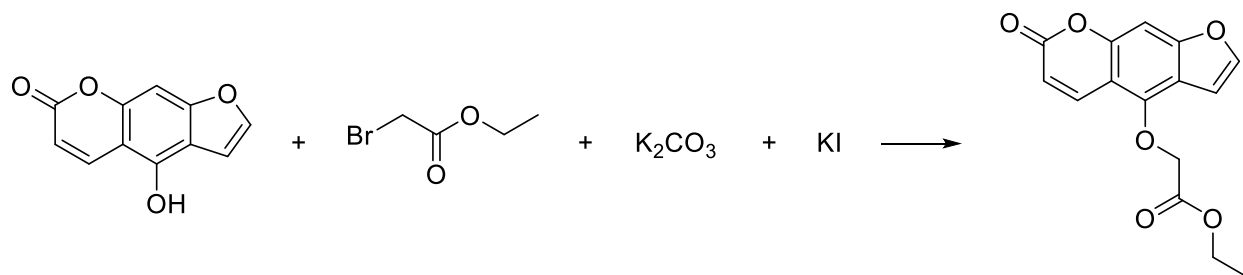


99% yield

**28** was dissolved in ethanol (20 ml). A solution of potassium hydroxide (1M, 5 ml) was added and stirred at room temperature for 1.5 h. The mixture was poured into hydrochloric acid (1 mol/L). The product was formed as white precipitate and filtered out.

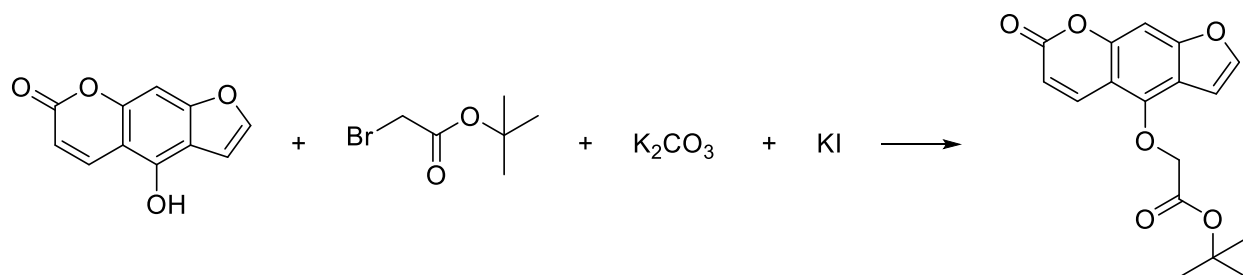
<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.32 (d, *J* = 10.0 Hz, 1H), 8.04 (s, 1H), 7.41 (s, 1H), 7.22 (2, 1H), 6.38 (d, *J* = 10.0 Hz, 1H), 5.12 (s, 2H).

**28**



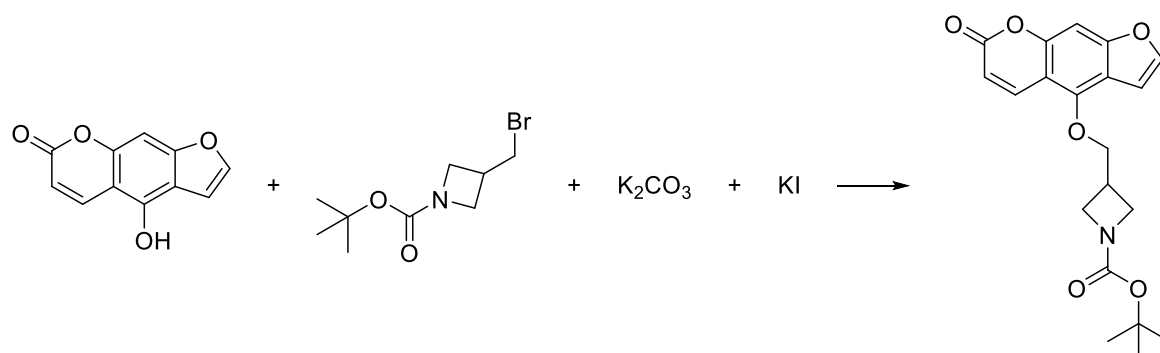
Methods as described for **21**. 70% yield. <sup>1</sup>H NMR (500 MHz, Chloroform-*d*)  $\delta$  8.37 (d, *J* = 9.8 Hz, 1H), 7.63 (d, *J* = 2.3 Hz, 1H), 7.22 (s, 1H), 6.87 (d, *J* = 2.3 Hz, 1H), 6.34 (d, *J* = 9.8 Hz, 1H), 4.99 (s, 2H), 4.28 (q, *J* = 7.1 Hz, 2H), 1.33 – 1.28 (t, *J* = 7.1 Hz, 3H).

**29**



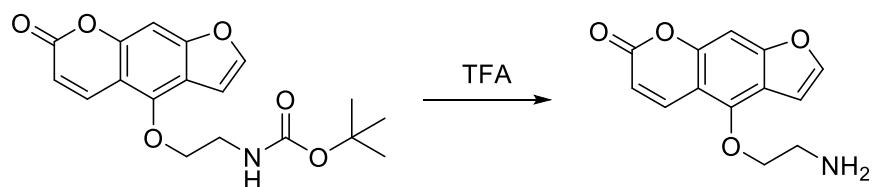
Methods as described for **21**. 83% yield.  $^1\text{H}$  NMR (500 MHz, Chloroform- $d$ )  $\delta$  8.43 (d,  $J$  = 9.8 Hz, 1H), 7.69 (d,  $J$  = 2.3 Hz, 1H), 7.26 (s, 1H), 6.95 (d,  $J$  = 2.4 Hz, 1H), 6.39 (d,  $J$  = 9.8, 1H), 4.97 (s, 2H), 1.56 (s, 9H).

**32**



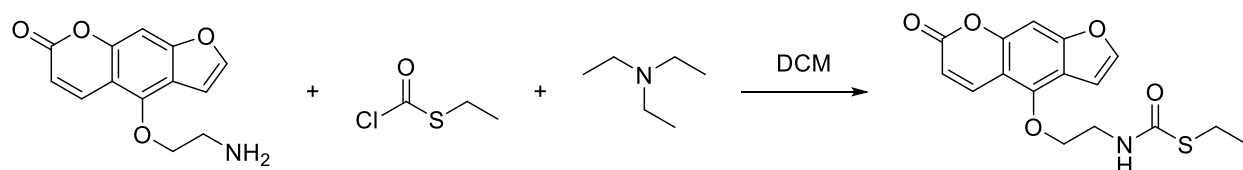
Methods as described for **21**.

**33 and 35**



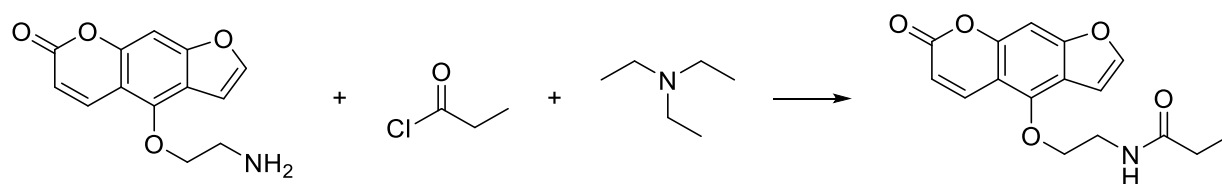
TFA (4 mL) was added to a stirred solution of **24** in DCM (4 mL). The mixture was allowed to stir for 5 h, after which time the reaction was diluted EtOAc. The aqueous was extracted three times with EtOAc (3 x 10 mL) and the combined organic layers were washed with  $\text{NaHCO}_3$  (15 mL), brine (15 mL),  $\text{H}_2\text{O}$  (15 mL), dried with  $\text{MgSO}_4$  and concentrated. The residue was purified via column chromatography eluting

with EtOAc/ Hexanes to obtain **35**.  $^1\text{H}$  NMR (500 MHz, Methanol- $d_4$ )  $\delta$  8.38 (s, 1H), 7.79 (d,  $J$  = 2.5 Hz, 1H), 7.17 (d,  $J$  = 17.2 Hz, 2H), 6.27 (dd,  $J$  = 9.9, 2.3 Hz, 1H), 4.73 (d,  $J$  = 6.2 Hz, 2H), 3.53 (d,  $J$  = 7.3 Hz, 2H).



Under a N<sub>2</sub> atmosphere, **35** was dissolved in anhydrous DCM (1 mL/mmol), and treated with triethylamine (10 eq). The S-ethyl chloridithiocarbonate (10 eq) was added and the reaction stirred at 0 °C for 4-8 h. After this time the reaction was diluted with EtOAc and poured onto 1N HCl. The aqueous phase was then extracted three times with EtOAc. The combined organic layers were washed with brine, H<sub>2</sub>O, dried with MgSO<sub>4</sub> and concentrated. The residue was purified via column chromatography eluting with EtOAc/ Hexanes to obtain the product.  $^1\text{H}$  NMR (500 MHz, Chloroform- $d$ )  $\delta$  8.08 (d,  $J$  = 9.8 Hz, 1H), 7.56 (d,  $J$  = 2.3 Hz, 1H), 7.02 (s, 1H), 6.90 (d,  $J$  = 2.4 Hz, 1H), 6.21 (d,  $J$  = 9.8 Hz, 2H), 4.49 (t,  $J$  = 5.2 Hz, 2H), 3.78 (d,  $J$  = 5.5 Hz, 2H), 2.93 (q,  $J$  = 7.4 Hz, 2H), 1.29 (t,  $J$  = 7.4 Hz, 3H).

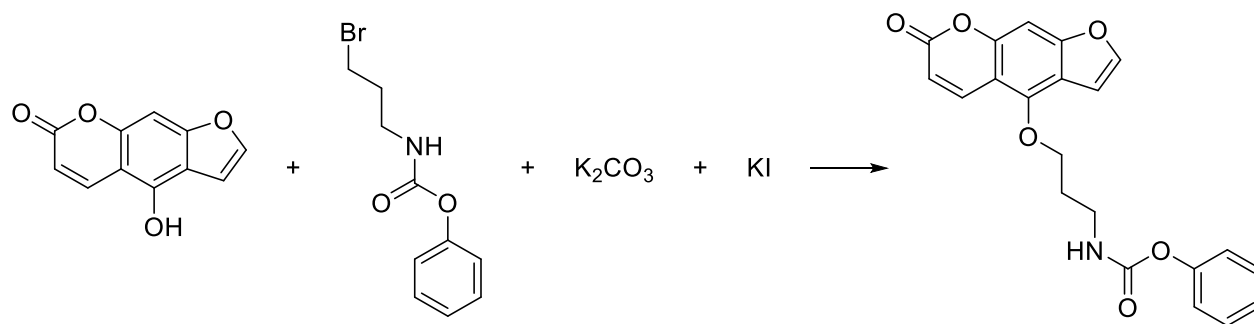
### 36



Under a N<sub>2</sub> atmosphere, **35** was dissolved in anhydrous DCM (1 mL/mmol), and treated with triethylamine (10 eq). Propionyl chloride (10 eq) was added and the reaction stirred at 0 °C for 4-8 h. After this time the reaction was diluted with EtOAc and poured onto 1N HCl. The aqueous phase was then extracted three times with EtOAc. The combined organic layers were washed with brine, H<sub>2</sub>O, dried with MgSO<sub>4</sub> and concentrated. The residue was purified via column chromatography eluting with

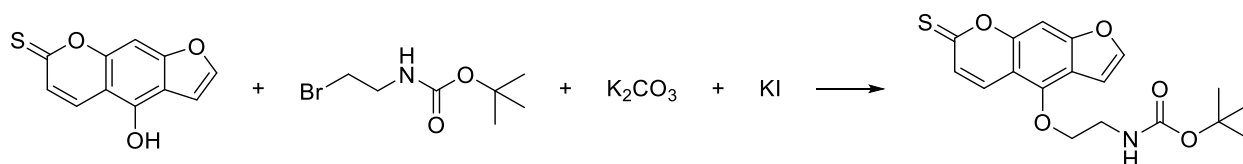
EtOAc/Hexanes to obtain the product.  $^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  8.08 (d,  $J$  = 9.8 Hz, 1H), 7.59 – 7.54 (m, 1H), 6.93 (d,  $J$  = 2.4 Hz, 1H), 6.39 (d,  $J$  = 24.9 Hz, 1H), 6.18 (d,  $J$  = 9.8 Hz, 1H), 4.51 (t,  $J$  = 5.3 Hz, 2H), 3.74 (t,  $J$  = 5.3 Hz, 2H), 2.29 (q, 2H), 1.17 (t,  $J$  = 7.6, 3H).

**37**



Methods as described for **21**.

**38**



Under a  $\text{N}_2$  atmosphere, 4-hydroxy-7-thione-7H-benzofuro[2,3-b]pyran-2-one was dissolved in anhydrous DMF (1 mL/mmol), and treated with  $\text{K}_2\text{CO}_3$  (2 eq) and KI (cat.). The 2-(Boc-amino)ethyl bromide (1.2 eq) was added and the reaction stirred at 50 °C for 8 h. the reaction mixture was diluted with EtOAc and poured onto 1N HCl. The aqueous phase was then extracted three times with EtOAc. The combined organic layers were washed with  $\text{H}_2\text{O}$ , brine, dried with  $\text{MgSO}_4$  and concentrated. The residue was purified via column chromatography eluting with EtOAc/Hexanes to obtain the product. 50% yield,  $^1\text{H}$  NMR (500 MHz, Chloroform-*d*)  $\delta$  7.91 (d,  $J$  = 9.3 Hz, 1H), 7.64 (d,  $J$  = 2.4 Hz, 1H), 7.32 (s, 1H), 7.12 (d,  $J$  = 9.5 Hz, 1H), 6.99 – 6.95 (m, 1H), 4.93 (s, 1H), 4.51 (t,  $J$  = 4.8 Hz, 2H), 3.64 (t,  $J$  = 4.6 Hz, 2H), 1.46 (s, 9H).

## TABLE

**Supplemental Table 1. Potency of structural analogs of 1 in cultured HL-1 cardiomyocytes.**

Compound	EC <sub>50</sub> in Fish	EC <sub>50</sub> in HL-1 Cells
24	3 nM	> 50 µM
23	7 nM	> 50 µM
32	10 nM	> 50 µM
37	33 nM	> 50 µM
22	52 nM	> 50 µM
6	105 nM	5 µM
20	190 nM	2 µM
7	363 nM	1 µM
17	382 nM	5 µM
5	401 nM	3 µM
4	587 nM	20 µM
19	648 nM	10 µM
13	940 nM	7 µM
15	984 nM	10 µM
1	1 µM	10 µM
18	1 µM	10 µM
12	1 µM	25 µM
11	2 µM	7 µM
20	3 µM	6 µM
14	3 µM	> 30 µM

34	6 $\mu$ M	> 50 $\mu$ M
25	> 10 $\mu$ M	> 50 $\mu$ M
16	> 10 $\mu$ M	> 50 $\mu$ M
10	> 10 $\mu$ M	10 $\mu$ M
8	> 10 $\mu$ M	15 $\mu$ M
3	> 10 $\mu$ M	> 50 $\mu$ M
9	> 10 $\mu$ M	> 50 $\mu$ M
2	> 10 $\mu$ M	> 50 $\mu$ M

Select analogs were tested in HL-1 cultured cardiomyocytes to assess for efficacy in preventing DOX-induced apoptosis. Results are ordered by potency in zebrafish.

## FIGURE LEGENDS

**Supplemental Figure 1. *In vivo* Dose-Response Curves for Analogs of 1.** Fish were treated with DOX and assessed for the development of the cardiomyopathy phenotype (decreased cardiac contraction, pericardial edema, and decreased tail blood flow). Zebrafish were considered to be rescued if all three features of the cardiomyopathy phenotype were absent upon co-treatment with a visnagin derivative. Data are depicted as mean  $\pm$  standard error of the mean ( $n = 6$  fish per dose; each experiment was performed in triplicate). Data for concentrations at or above the  $TD_{50}$  were excluded from regression analysis for each compound. Those compounds lacking *in vivo* activity at doses below  $10 \mu\text{M}$  were also excluded from the analysis.

**Supplemental Figure 2. Structure (A) and dose-response curve (B) for compound 38.** Incorporation of a thiocarbonyl group did not improve potency compared to 24.

**Supplemental Figure 3. RNA levels of topoisomerase 2 $\beta$  (*top2b*) and the mitochondrial iron transporters solute carrier family 25 member 28 (*slc25a28*) and ATP-binding cassette sub-family B (MDR/TAP) member 8 (*abcb8*).** Compounds 1 and 23 did not modulate expression of genes previously implicated in doxorubicin cardiotoxicity. Expression levels were normalized to beta-actin and to control fish treated with DMSO. Data are depicted as mean  $\pm$  the standard error of the mean and represent 12 fish treated per condition per experiment. Each experiment was repeated three times.

## **DATA FILE LEGEND**

**Supplemental Data File 1.** Excel file showing full results of zebrafish proteomics analysis.

## REFERENCES

1. Liu Y, Asnani A, Zou L, Bentley VL, Yu M, Wang Y, Dellaire G, Sarkar KS, Dai M, Chen HH, Sosnovik DE, Shin JT, Haber DA, Berman JN, Chao W, Peterson RT. Visnagin protects against doxorubicin-induced cardiomyopathy through modulation of mitochondrial malate dehydrogenase. *Science translational medicine*. 2014;6:266ra170