Supplementary Methods

cAMP assay

For testing, growth media was aspirated and cells were incubated with 100 µL per well of compound-containing assay buffer for 15 minutes at room temperature. Following incubation, assay buffer was removed and cells were lysed by addition of 100 µL/well 0.1 M HCl for 20 minutes. Intracellular cAMP in the lysate was quantified using a commercially available cAMP ELISA kit (Cayman Chemical, 581002) following the manufacturer's protocol. Specifically, cell lysate was diluted 1:5 in ELISA buffer and transferred to a 96-well ELISA plate, and cAMP tracer and antiserum were added per kit protocol. A cAMP standard curve and appropriate buffer-only controls were included on each plate. After overnight incubation, plates were washed and developed with Ellman's reagent for 30 minutes, then absorbance was measured at 410 nm using a Biotek Synergy HTX plate reader. Raw absorbance values were converted to cAMP concentrations by back-calculating from the standard curve fitted to the cAMP standards (logit-linear fit), per kit protocol. Computed cAMP concentrations were normalized to the per-plate buffer-only controls as appropriate.

Calcium flux assay

For testing, growth media was aspirated and cells were washed 2 times with 150 µL/well PBS to remove serum-containing media. Next, 50 µL of dye loading solution containing 3 µM Fluo-4 AM (Ion Biosciences, 1041F), 2.5 mM probenecid, and 1× pluronic F-127 (a detergent that helps disperse the calcium dye; Ion Biosciences, 7601A) was added to the cells, which were incubated for 1 hour at 37°C. After this, the dye loading solution was removed and replaced with 50 µL of compound-containing buffer. The plate was immediately read on a Biotek Synergy HTX plate reader using the following settings: kinetic read at 5 second intervals over 5 minutes, excitation at 485/20 nm and emission at 528/20 nm, read from bottom of the plate. In order to minimize the sampling interval of the kinetic read, the cell plate was processed one column at a time.

To quantify the normalized fluorescence response for each sample, a bi-exponential rise-decay curve was fit to the raw fluorescence traces for each sample replicate:

$$F(t) = b + a \cdot (e^{-t/\tau 2} - e^{-t/\tau 1})$$

- t = time in seconds
- F(t) = fluorescence at time t (in relative fluorescence units, RFUs)
- b = baseline fluorescence
- a = scaling factor
- τ_1 = time constant for the rising phase of the calcium transient
- τ_2 = time constant for the decay phase

The curve was fitted using the Levenberg–Marquardt algorithm as implemented in the scipy.optimize.curve_fit function. The normalized fluorescence response for each sample was computed from the fitted curve as:

$$\Delta F/F0 = (Fpeak - F0) / F0$$

- F_{peak} = maximum of the fitted curve (computed by setting $dF(t)/dt = 0$ and solving for t, then evaluating $F(t_{peak})$)
- F_0 = mean fluorescence across all time points of the control (buffer-only) condition

Concentration response curves

All concentration-response data were fit to a four-parameter logistic equation:

$$y = a + (d - a) / (1 + 10(c - \log[drug]) \cdot b)$$

- a = bottom asymptote
- b = Hill slope
- c = $\log(EC_{50})$
- d = top asymptote

Parameters were estimated using the Levenberg–Marquardt algorithm as implemented in the scipy.optimize.curve_fit function. Potency metrics IC/EC_{50} and I_{max}/E_{max} were derived directly from the fitted parameters. 95% confidence intervals for IC/EC_{50} were computed from the asymptotic covariance matrix of the fitted parameters returned by the optimizer.

The statistical significance of the curve fit was computed using an extra sum-of-squares F test:

$$F = ((SS_{null} - SS_{4PL}) / (dfnull - df4PL)) / (SS4PL / df4PL)$$

where SS_{null} and SS_{4PL} are the residual sums of squares for the null (flat-line) and 4PL models, respectively, $df_{null} = n - 1$, and $df_{4PL} = n - 4$.