Supporting Information

Characterization of Chemical Libraries for Luciferase Inhibitory Activity

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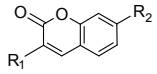
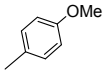
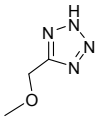
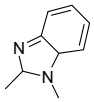
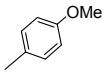
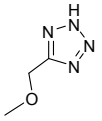
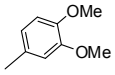
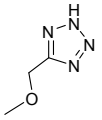


Table S1. Characterization of selected coumarin compounds

Analogue #	R_1	R_2	IC_{50}	IC_{50Luc}	IC_{50PE}	$IC_{50Promega}$	$IC_{50SteadyGlo}$	$IC_{50BrightGlo}$	$IC_{50Renilla}$
18			0.38± 0.03	0.24± 0.11	0.14± 0.05	16± 3.7	0.12± 0.08	0.4± 0.4	5.3± 3.8
19			5.4± 0.05	1.1± 0.4	3.2± 0.4	Inactive	10± 5.8	13.3± 12.2	n.d.
			luciferase qHTS data						
18			0.04μM, Class 1a (100% inhibition)						
20			1μM, Class 1b (28% inhibition)						
19			3μM, Class 1a (100% inhibition)						
21			25μM, Class 3						

PubChem CIDs: 18) 94381, 19) 667285, 20) 100335, 21) 666789. Activity of compounds was determined via measurement of luminescence in the indicated format. IC50, activity in primary assay (Lonza, PK-Light); IC_{50Luc} activity against purified *P. Pyralis* luciferase; IC_{50PE}, activity using EasyLite; $IC_{50Promega}$, activity using KinaseGlo reagent ; $IC_{50SteadyGlo}$, activity in luciferase reporter gene detection mix; $IC_{50BrightGlo}$, activity in luciferase reporter gene detection mix; $IC_{50Renilla}$, activity against *R. reniformas*. Potencies in μM. Data shown is the mean ± SD for at least four replications.

Table S1

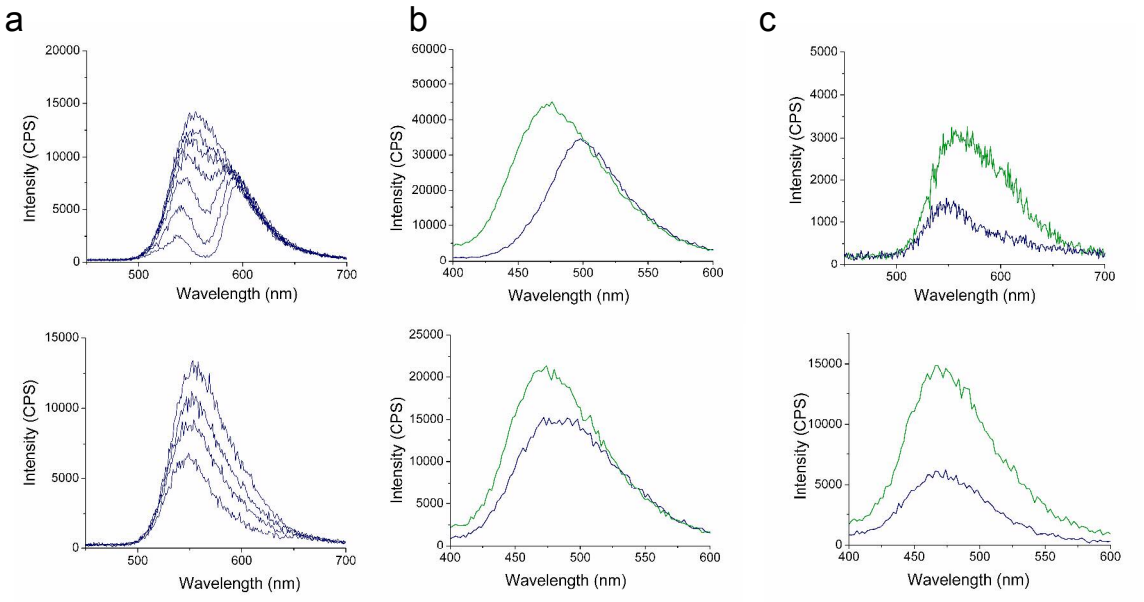


Figure S1. **Effect of various colored compounds on *P. pyralis* and *R. reniforimas* luciferase luminescence**. a) *P. pyralis* luciferase luminescence in the presence of lissamine (top left ; lines from bottom are 50 μM, 25 μM, 12.5 μM, 6.25 μM and 3.1 μM. 1.5 μM and 0 μM lissamine), napthol blue/black (bottom left; lines from bottom are 100 μM, 50 μM, 25 μM, 0 μM). b) *R. reniforimas* luminescence in the absence (green line) or presence (blue line) of 100 μM tartrazine (top) or the absence (green line) or presence (blue line) of 100 μM mGluR5 antagonist **16** (bottom). No quenching of *P. pyralis* luciferase luminescence was observed for compound **16** or tartrazine. c) *P. pyralis* luciferase luminescence (top) and *R. reniforimas* luciferase luminescence (bottom) in the in the absence (green line) or presence (blue line) of 20 μM compound Evans Blue, (compound **15**).

Figure S1