Supporting Information:

Structure-Based Design of Highly Selective and Potent G Protein-Coupled Receptor Kinase 2 Inhibitors Based on Paroxetine

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Supplementary Table 1. Crystal Refinement Statistics.

Protein Complex	GRK2-Gβγ·**14ak**	GRK2-Gβγ·**14bd**	GRK2-Gβγ·**14as**
X-ray source	APS 21-ID-G	APS 21-ID-G	APS 21-ID-D
wavelength (Å)	0.9785	0.9786	0.9785
D_{min} (Å)	50.00-2.60 (2.64-2.60)	50.00-2.15 (2.19-2.15)	30-3.0 (3.04-3.03)
space group	*P*2	*C*2	*C*2
unit cell constants (Å)	*a*=113.1, *b*=62.4, *c*=102.0	*a*=194.3, *b*=71.4, *c*=111.3	*a*=189.0, *b*=74.2, *c*=123.2
(˚)	*β*=92.8	*β*=110.5	*β*=115.5
unique reflections	39963 (1987)	77337 (3805)	29942 (4657)
R_{sym} (%)	9.8	16.1	12.9
completeness (%)	85.8	99.0	98.5
$\langle I \rangle / \langle \sigma_I \rangle$	9.2 (0.8)	24.9 (1.4)	12.8 (2.0)
redundancy	2.6 (2.5)	18.3 (11.2)	6.8 (6.8)
refinement resolution (Å)	30.00-2.60 (2.69-2.60)	30.00-2.15 (2.23-2.15)	30.00-3.03 (3.14-3.03)
total reflections used	35989	73370	207836
RMSD bond lengths (Å)	0.011	0.018	0.012
RMSD bond angles (˚)	1.53	1.90	1.56
est. coordinate error (Å)	0.360	0.142	0.404
Ramachandran Plot:			
most favored, allowed, outliers (%)	92.5, 5.6, 1.9	95.3, 3.8, 0.9	93.2, 5.3, 1.8
R_{work}	0.2207	0.1821	0.1971
R_{free}	0.2807	0.2275	0.2516
protein atoms	8089	8261	8192
water molecules	43	388	23
inhibitor atoms	34	35	33
average *B*-factor (Å^2)	80.2	55.2	105.0
protein	80.3	55.6	105.0
inhibitor	70.2	69.3	123.3
MolProbity score	2.01	1.70	1.74
MolProbity % C*β* deviations	0	0.52	0
MolProbity % bad backbone bonds	0	0.04	0.01
MolProbity % bad backbone angles	0	0.05	0.01
PDB entry	---	---	---

*Entries in parentheses indicate data in the highest resolution shell

Supplementary Table 2: Mouse cardiomyocyte contractility in response to paroxetine, 2, 14ak, and 14as.

	Paroxetine				**2**				**14ak**			**14as**			
Concentration (μM)	0	0.5	1	10	0	0.1	0.5	1	0	0.5	1	0	0.1	0.5	1
Baseline before isoproterenol															
MCA (% cell length)	5.0 ± 0.5	4.0 ± 0.3	4.3 ± 0.8	4.2 ± 0.5	4.8 ± 0.5	3.6 ± 0.4	4.2 ± 0.3	4.4 ± 0.5	5.1 ± 1.1	5.1 ± 1.1	5.2 ± 0.6	4.4 ± 0.3	3.3 ± 0.2	3.5 ± 0.4	3.5 ± 0.2
After isoproterenol															
MCA (% cell length)	12 ± 0.6	14 ± 1	13 ± 0.8	16 ± 0.5*	13 ± 0.5	12 ± 0.7	16 ± 1*	17 ± 1.3*	12 ± 1.3	12 ± 1.1	13 ± 0.9	11 ± 0.9	14 ± 0.7*	18 ± 1.3*	15 ± 1.2*
% increase	146 ± 16	248 ± 26	262 ± 60	296 ± 45*	178 ± 24	252 ± 27	277 ± 28*	293 ± 35*	198 ± 66	166 ± 38	173 ± 41	161 ± 20	341 ± 33*	433 ± 90*	342 ± 65*

Values represent the mean ± SEM for 6-8 cardiomyocytes. *,$p<0.05$ vs DMSO Control. MCA, maximum contraction amplitude.

Supplementary Table 3: Biochemical Data for previously reported compounds **15, 16,** and **17**.

Compound	GRK2 IC_{50} (μM)	GRK1 IC_{50} (μM)	GRK5 IC_{50} (μM)	PKA IC_{50} (μM)	ROCK1 IC_{50} (μM)
15	0.13±0.03	> 100	> 100	> 100	6.7±8.2
16	0.07±0.01	>100	63±32	>100	5.8±5.5
17	0.15±0.07	3.9±1.0	0.38±0.06	>100	0.01±0.01