Supporting Information:

Structure-Based Design, Synthesis and Biological Evaluation of Highly Selective and Potent G Protein-Coupled Receptor Kinase 2 Inhibitors

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Table S1. Crystal Refinement Statistics

Protein Complex	GRK2-Gβγ· CCG-215022	GRK2-Gβγ· CCG-224062	GRK2-Gβγ· CCG-224406	GRK2-Gβγ· CCG-224411
X-ray source	APS 21-ID-G	APS 21-ID-G	APS 21-ID-G	APS 21-ID-G
wavelength (Å)	0.97857	0.97857	0.97857	0.97857
D_{min} (Å)	30–2.6 (2.64–2.56)	30-3.2 (3.26-3.2)	30-2.8 (2.85-2.8)	30-2.8 (2.85-2.8)
space group	$C222_1$	$C222_1$	$C222_1$	$C222_1$
cell constants (Å)	*a*=60.6	*a*=62.0	*a*=60.7	*a*=60.0
	b=240 *c*=209	*b*=241 *c*=213	*b*=242 *c*=214	*b*=239 *c*=209
unique reflections	47002 (2068)	27203 (1277)	37227 (1655)	36395 (1800)
R_{sym} (%)	6.7 (39.1)	14.6 (0.0)	5.5 (76.0)	10.4 (93.6)
completeness (%)	97.0 (86.1)	96.7 (92.3)	94.0 (84.5)	93.3 (93.5)
$\langle I \rangle / \langle \sigma_I \rangle$	25.8 (2.6)	17.8 (1.5)	20.3 (1.6)	19.6 (1.9)
reduncancy	3.5 (2.5)	7.3 (6.9)	3.6 (2.7)	4.6 (4.5)
refinement resolution (Å)	30–2.6 (2.64–2.56)	30-3.2 (3.26-3.2)	30-2.8 (2.85-2.8)	30-2.8 (2.85-2.8)
total reflections used	44654	25783	35332	34492
RMSD bond lengths (Å)	0.013	0.0115	0.0115	0.012
RMSD bond angles (^{0})	1.65	1.57	1.55	1.57
est. coordinate error (Å)	0.25	0.37	0.31	0.29
Ramachandran plot:				
most favored, outliers (%)	92.3, 0.8	89.3, 1.3	91.3, 0.5	90.3, 1.2
R_{work}	0.205	0.172	0.202	0.198
R_{free}	0.264	0.250	0.277	0.257
protein atoms	8178	8171	8218	8183
water molecules	29	34	31	40
inhibitor atoms	37	41	41	40
average *B*-factor (Å^2)	71.3	100	70.6	75.5
Protein	71.5	101	71.0	75.6

Inhibitor	65.2	112	61.9	120
MolProbity score	2.32	2.39	2.14	2.59
MolProbity Cβ deviations	1	1	0	0
MolProbity bad backbone bonds	0	1	0	1
MolProbity bad backbone angles	1	1	1	0
PDB entry	5HE0	5HE1	5HE2	5HE3

Numbers in parentheses correspond to the highest resolution shell of data.

Table S2: Compound K_i, buried ASA, and number of hydrogen bonds with GRK2

Compound	K_i (μM)	Buried ASA (Å^2)	# Hydrogen Bonds
Balanol	0.55	420	8
Paroxetine	22	250	4
206584[43]	4.0	270	3
Takeda101	4.6	370	2
1	0.85	390	2
12h	2.4	380	5
12k	4.4	310	5
12n	2.05	400	5
12r	3.6	320	3
2	12	290	4

K_i values were calculated using the Cheng-Prussof equation. Buried ASA was calculated using the AREAIMOL tool in the CCP4 program suite.[44] See graphical representation as Figure 5 in text.

Table S3: Minimum inhibitory concentrations of inhibitors tested in mouse cardiomyocytes

	Control (DMSO)	Paroxetine 10 µM	2 1 µM	12h 0.5 µM	12d 0.5 µM	12m 1 µM	12n 0.5 µM
Baseline before isoproterenol							
max contraction amplitude (% cell length)	5.7 ± 0.4	4.7 ± 0.2	6.0 ± 0.7	5.9 ± 0.2	4.6 ± 0.9	5.5 ± 0.7	3.9 ± 0.5
After isoproterenol							
max contraction amplitude (% cell length)	12 ± 0.5	16.5 ± 0.6*	17.5 ±0.8*	16 ± 1*	17 ± 1*	15 ± 0.6*	15 ± 1*
% increase in contraction amplitude	110 ± 10	260 ± 30*	200 ± 30*	175 ± 20*	340 ± 70*	210 ± 40*	340 ± 65*

Values represent the mean ± SEM for 6-10 cardiomyocytes. *,$p<0.05$ vs Control

Table S4: Cardiomyocyte contractility results for compound 12h from 0.1 to 10 µM

12h	Control (DMSO)	0.1 µM	0.5 µM	1 µM	10 µM
Baseline before isoproterenol					
max contraction amplitude (% cell length)	5.7 ± 0.4	5.6 ± 0.5	5.9 ± 0.2	5.9 ± 0.55	4.6 ± 0.5
After isoproterenol					
max contraction amplitude (% cell length)	12 ± 0.5	12 ± 1	16 ± 1*	16.5 ± 1*	13 ± 1
% increase in contraction amplitude	110 ± 10	115 ± 20	175 ± 20*	170 ± 20*	220 ± 40*

Values represent the mean ± SEM for 8-10 cardiomyocytes. *,$p<0.05$ vs Control

Table S5: Cardiomyocyte contractility results for compound 12d from 0.1 to 10 µM

12d	Control (DMSO)	0.1 µM	0.5 µM	1 µM	10 µM
Baseline before isoproterenol					
max contraction amplitude (% cell length)	5.7 ± 0.4	6.0 ± 2	4.6 ± 0.9	5.6 ± 0.6	5.7 ± 0.2
After isoproterenol					
max contraction amplitude (% cell length)	12 ± 0.5	12 ± 2	17 ± 1*	16 ± 0.6*	15 ± 0.6*
% increase in contraction amplitude	110 ± 10	110 ± 30	340 ± 70*	200 ± 30*	175 ± 20*

Values represent the mean ± SEM for 8-10 cardiomyocytes. *,$p<0.05$ vs Control

Table S6: Cardiomyocyte contractility results for compound 12m from 0.1 to 10 μ M

12m	Control (DMSO)	0.1 µM	0.5 µM	1 µM	10 µM
Baseline before isoproterenol					
max contraction amplitude (% cell length)	5.7 ± 0.4	5.2 ± 0.4	5.05 ± 0.5	5.5 ± 0.7	5.1 ± 1
After isoproterenol					
max contraction amplitude (% cell length)	12 ± 0.5	13 ± 0.8	13 ± 0.6	15 ± 0.6*	17 ± 1*
% increase in contraction amplitude	110 ± 10	150 ± 15	175 ± 30*	210 ± 40*	275 ± 50*

Values represent the mean ± SEM for 8-10 cardiomyocytes. *,$p<0.05$ vs Control

Table S7: Cardiomyocyte contractility results for compound 12n from 0.1 to 10 µM

12n	Control (DMSO)	0.1 µM	0.5 µM	1 µM	10 µM
Baseline before isoproterenol					
max contraction amplitude (% cell length)	4.5 ± 0.3	4.5 ± 0.5	3.9 ± 0.5	4.4 ± 0.7	4.8 ± 0.4
After isoproterenol					
max contraction amplitude (% cell length)	11.5 ± 1	12 ± 1	15 ± 1*	18 ± 0.7*	16 ± 0.6*
% increase in contraction amplitude	160 ± 20	170 ± 30	340 ± 65*	350 ± 70*	240 ± 30*

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

Table S8: Cardiomyocyte contractility results for compound 2 at 0.5 and 1 µM

GSK180736A (2)	Control (DMSO)	0.5 µM	1.0 µM
Baseline before isoproterenol			
max contraction amplitude (% cell length)	5.7 ± 0.4	5.2 ± 0.6	6.0 ± 0.7
After isoproterenol			
max contraction amplitude (% cell length)	12 ± 0.5	15.5 ± 2	17.5 ± 0.8*
% increase in contraction amplitude	110 ± 10	180 ± 40	201 ± 30*

Values represent the mean ± SEM for 8-10 cardiomyocytes. *,$p<0.05$ vs Control