SUPPLEMENTARY DATA

Supplementary Table S1. Summary of Pharmacological Parameters from Automated Planar Patch Clamp, Tl^+ Flux, and [^{3}H]Dofetilide Binding Assays

	[^{3}H]Dofetilide	Tl^+ Flux Assays		APPC Assays			APPC Over (Fold)	
Drugs	(^{3}H) K_i (μM)	EC_{50} (μM)	Hill Slope	EC_{50} (μM)	Emax (%)	Hill Slope	^{3}H	Tl^+
Amantadine	10.47	NI	NA	41.69	55.4 ± 8.0	1	0.3	NA
Amiodarone	0.59	2.75	1	1.07	~100.0	0.80 ± 0.07	0.6	2.6
Amitriptyline	8.91	28.18	1.39 ± 0.17	1.35	~100.0	1.11 ± 0.08	6.6	20.9
Aripiprazole	1.02	8.13	1.72 ± 0.18	0.24	84.0 ± 15.3	1	4.3	33.9
Asetmiozole	0.003	2.09	2.21 ± 0.13	0.02	95.3 ± 5.7	1	0.2	97.7
Bepridil	0.51	4.37	2.61 ± 0.41	0.39	~100.0	1	1.3	11.2
Chlorpheniramine	5.50	14.79	1.42 ± 0.17	2.14	98.4 ± 5.0	1	2.6	6.9
Chlorpromazine	2.09	6.61	1.70 ± 0.14	2.14	~100.0	1	1.0	3.1
Ciclopirox	16.98	93.33[a]	1	263	~100.0	1	0.1	0.4
Cisapride	0.039	0.34	1	0.02	~100.0	1	1.9	16.6
Clomipramine	3.02	14.13	2.14 ± 0.16	1.00	~100.0	1	3.0	14.1
Clozapine	3.47	11.75	1.19 ± 0.10	0.81	~100.0	1	4.3	14.5
Clozapine-N-oxide	9.33	NI	NA	NI	NA	NA	NA	NA
Dofetilide	0.004	0.23	1	0.02	~100.0	1.00	0.2	12.9
Doxepin	8.32	21.38	1.22 ± 0.12	2.14	96.1 ± 5.8	1.00	3.9	10.0
Droperidol	0.29	0.47	1	0.05	95.4 ± 5.9	1	6.3	10.2
E-4031	0.022	0.19	1.42 ± 0.13	0.03	~100.0	1.80 ± 0.41	0.8	7.1
Fluoxetine	4.27	7.14	1.55 ± 0.17	0.36	~100.0	1	11.8	20.0
Fluvoxamine	7.59	19.05	1.52 ± 0.18	3.16	~100.0	1	2.4	6.0
Grepafloxacin	16.63	NI	NA	NI	NA	NA	NA	NA
Haloperidol	0.10	0.27	0.86 ± 0.03	0.03	98.9 ± 3.9	1	3.3	8.5
Imipramine	3.47	31.62	1.53 ± 0.16	0.51	~100.0	0.64 ± 0.12	6.8	61.7
LAAM	3.24	5.75	1.45 ± 0.11	0.72	98.4 ± 7.7	1	4.5	7.9
Lidoflazine	0.060	3.02	2.11 ± 0.30	0.10	~100.0	1	0.6	30.9
Loratadine	5.50	21.88	2.21 ± 0.31	1.51	~100.0	0.71 ± 0.17	3.6	14.5
Maprotilene	4.37	27.54	1.86 ± 0.23	5.13	~100.0	1	0.9	5.4
Mefloquine	4.79	25.12	2.17 ± 0.26	2.19	~100.0	1	2.2	11.5

(*Continued*)

Supplementary Table S1. (Continued)

Drugs	[^{3}H]Dofetilide	Tl^+ Flux Assays		APPC Assays			APPC Over (Fold)	
	(^{3}H) K_i (μM)	EC_{50} (μM)	Hill Slope	EC_{50} (μM)	Emax (%)	Hill Slope	^{3}H	Tl^+
Mibefradil	0.95	6.76	2.48 ± 0.29	0.93	99.4 ± 7.5	1	1.0	7.2
Nortriptyline	4.79	29.51	1.57 ± 0.11	1.51	85.9 ± 11.3	1	3.2	19.5
Olanzapine	6.31	52.48	1	2.34	~100.0	0.57 ± 0.09	2.7	22.4
Ouabain	17.78	NI	NA	NI	NA	NA	NA	NA
PD-118057	12.30	52.48[a]	1	Activator	NA	1	NA	NA
Perphenazine	1.05	6.17	1.41 ± 0.17	1.15	~100.0	1	0.9	5.4
Phenytoin	18.20	NI	NA	0.21	59.8 ± 13.0	0.47 ± 0.16	85.1	NA
Pimozide	0.023	1.62	1.40 ± 0.20	0.07	~100.0	1	0.3	23.4
Propafenone	1.82	3.80	1.92 ± 0.25	0.09	~100.0	1	21.4	44.7
Pyrilamine	5.89	8.91	1	1.95	~100.0	1	3.0	4.6
Quetiapine	4.47	11.48	1	2.51	~100.0	1	1.8	4.6
Quinidine	2.75	6.31	1	0.26	~100.0	0.51 ± 0.16	10.5	24.0
Risperidone	1.00	1.66	1	0.28	~100.0	1.33 ± 0.23	3.6	5.9
Sertindole	0.13	3.02	1.74 ± 0.17	0.41	~100.0	1.30 ± 0.25	0.3	7.4
Tamoxifen	0.95	22.39	1	6.76	~100.0	1	0.1	3.3
Terfenadine	0.13	5.01	1	0.13	~100.0	0.59 ± 0.18	1.0	37.2
Terodiline	2.14	4.27	1.60 ± 0.11	0.32	~100.0	0.60 ± 0.17	6.6	13.2
Thioridazin	1.20	11.22	2.92 ± 0.70	0.51	~100.0	1	2.3	21.9
Trifluoperazine	0.98	17.38	2.45 ± 0.17	1.55	99.2 ± 6.9	1	0.6	11.2
Venlafaxine	16.60	NI	NA	21.38	95.9 ± 4.2		0.8	NA
Verapamil	1.55	2.82	1.78 ± 0.17	0.58	~100.0	1	2.7	4.9
Way 161503	8.13	44.67	1	3.55	97.2 ± 7.3	1	2.3	12.6

Values represent average binding affinity (K_i from binding assays) and potency (EC_{50} from Tl^+ or APPC assays) taken directly from curve-fittings in Prism. Multiple assays ($n \geq 2$) were normalized to percentage inhibition and pooled for analysis. All [^{3}H]dofetilide competition binding curves favored ($P < 0.05$) four-parameter logistic model with Hill slope of 1. For Tl^+ flux and APPC assays, if a curve favored ($P < 0.05$) variable slope model, variable slope and corresponding potency values were reported; otherwise, Hill slope of 1 and corresponding potency value were reported. The maximal inhibition in competition binding and Tl^+ flux assays were 100% and not listed in the table (see Results for PD-110857 and ciclopirox). The last two columns list potency difference (in fold) between APPC over [^{3}H]dofetilide binding assay (^{3}H) or Tl^+ flux assay. In average, the APPC assay is 16.7-fold more potent than Tl^+ assay and 5-fold more potent than the [^{3}H]-dofetilide binding assay. Those drug names in bold are the 10 withdrawn drugs from the market since 1990s.

[a]Indicated potencies for hERG activator activity in the Tl^+ flux or APPC assay.

APPC, automated planar patch clamp; NI, no inhibition at up to 100 μM; NA, not applicable; hERG, human ether-a-go-go related gene.

Supplementary Table S2. Chronic Effect of Ouabain and Pentamidine on Human Ether-a-go-go–Related Gene Activity as Determined in Chronic Tl^+ Flux Assays

Time (Min)	Maximum Inhibition (%)	Average EC_{50} (μM)	Hill Slope
Ouabain			
15	29.3 ± 2.1	1.17	1
30	35.8 ± 2.2	1.08	1.87 ± 0.81
45	55.9 ± 1.6	0.60	1.46 ± 0.20
60	70.7 ± 1.3	0.53	1.28 ± 0.11
75	88.4 ± 1.9	0.46	1.19 ± 0.12
90	99.2 ± 1.9	0.36	1.64 ± 0.20
120	~100.0	0.16	4.16 ± 0.43
180	~100.0	0.11	4.66 ± 2.10
240	~100.0	0.050	3.43 ± 0.27
960 (16 h)	~100.0	0.018	5.72 ± 0.48
Pentamidine			
240	78.7 ± 10.3	46.79	1
480	~100.0	29.22	1
960	~100.0	11.17	1.26 ± 0.06

Cells were first incubated with ouabain or pentamidine for desired time (minutes to hours) at 37°C in cell incubator, washed once with assay buffer, and loaded with Tl^+ flux dye solution for 90 min at room temperature in the dark, and remaining total hERG activity was then determined. Initial slope of the curves was normalized to percentages with buffer control as 0% inhibition and complete inhibition (initial slope of around 2 with 10 μM cisapride) as 100% and pooled for analysis in the Prism with built-in four-parameter logistic function. Figures are presented in *Figure 3*. Potency, Hill slope, and maximum inhibition were reported directly from Prism. While curve-fitting, bottom was constrained to 0%, top was constrained to < 100%, and Hill slope was determine by the built-in comparison function in the Prism. Intracellular (if they entered cells) and residual drugs might be still working after removal of drug solution and during 90 min incubation with Tl^+ flux dye.

Supplementary Table S3. Protocol Table for the [^{3}H]Dofetilide Binding Assay (96-Well Format)

Step	Parameter	Value	Description
1	Fresh binding buffer	pH 7.40	Make fresh binding buffer, 25 mL per plate
2	[^{3}H]dofetilide	50 μL	Working stock at 5× final of 1.5nM
3	Samples, Dofetilide as positive control	50 μL	Working stock at 5×, 11 concentrations
4	Total binding	50 μL	Binding buffer in the column 1
5	Membrane homogenates	150 μL	Made from hERG HEK 293 cells
6	Incubation	1 h	At room temperature in the dark
7	Filtration and wash	3 times	With ice cold buffer
8	Dry filter paper	1 min	Remove water
9	Apply scintillation cocktail	1 min	Melt a sheet of cocktail on a hot plate
10	Count radioactivity	5 min	1450 MicroBeta Trilux plate counter

Step Notes

1. (mM) 135 NaCl, 5 KCl, 0.8 $MgCl_2$, 10 HEPES, 10 Glucose, 1 EGTA, 0.01% BSA, pH 7.40.
2. Prepare and aliquot 50 μL of 7.5 nM [^{3}H]dofetilide to all wells.
3. Row A and B are for positive control with dofetilide; row C to E for one test compound; row F to H for another test compound. Low to high concentrations in columns 2–12, correspondingly.
4. Column 1 contains no drug for total binding with buffer.
5. Homogenize membrane pellets by forcing through a 26-gauge needle several times.
7. Washing buffer: 50 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM EDTA, pH 7.40, 4°C.
9. Meltilex scintillant (PerkinElmer Life and Analytic Sciences) was applied to the dried filter on a hot plate.
10. Radio activity was counted for 5 min/well on PerkinElmer 1450 MicroBeta Trilux plate counter.

Supplementary Table S4. Protocol Table for the Tl^+ Flux Assay (384-Well Format)

Step	Parameter	Value	Description
1	Coat 384-well plates with PLL	20 min	30 μL/well PLL at 50 μg/mL
2	Plate cells, hERG HEK 293 cells	40 μL	25,000 cells per well
3a	Load FluxOR reagent for 90 min	20 μL	Only for acute Tl^+ flux assays
4a	Incubate with drug, go to Step 6	25 μL	Only for acute Tl^+ flux assays
3b	Incubate with drug	Overnight	Only for chronic Tl^+ flux assays
4b	Load FluxOR reagent for 90 min	20 μL	Only for chronic Tl^+ flux assays
5b	Remove drugs and add assay buffer	25 μL	Only for chronic Tl + flux assays
6	Add stimulation buffer with FLIPR	6.25 μL	For both acute & chronic Tl^+ assays
7	Assay readout	490/525 nm	Fluorescence was read with FLIPR

Step Notes

1. Assay buffer: 1 × Hank's balanced salt solution, 20 mM HEPES, 2.5 mM Probenecid, pH 7.40
2. FluxOR™ Thallium potassium ion channel assay kit F10017 from Invitrogen

1. Coat black clear-bottom 384-well plate with Poly-L-Lys for minimum of 20 min, and wash with H_2O.
2. Cells were plated in Dulbecco's modified Eagle's medium + 5% fetal bovine serum without G418 for overnight. Cryopreserved cells could also be used for acute Tl^+ flux assays at about 5 h after plating.

3a and 4b. Cells were incubated with FluxOR reagent at room temperature for 90 min in the dark.
To make fresh FluxOR reagent working solution, dilute the FluxOR reagent stock (Component A) at 1:1,000 and PowerLoad (Component C) at 1:100 with assay buffer.

4a. Drugs were tested at 10 μM in quadruplicate set for primary screening and 16-points for dose–response assays. Positive control Cisapride was included. Drug working solutions were prepared in assay buffer. Cells were incubated with drugs for 15 min.

3b. Drugs were tested at 10 μM in quadruplicate set for primary screening and 16-points for dose–response. Positive control ouabain was included. Drug working solutions
were prepared in assay buffer. Cells were incubated with drugs overnight or variable incubation times.

4b. Remove drug/media and quick wash with assay buffer, then load cells with Tl^+ dye for 90 min at room temperature in the dark.

5b. Another positive control cisapride was added at this step; incubate for 15 min.

6. Stimulation buffer (5 mL total): 2.5 mL deionized water + 1 mL FluxOR chloride-free buffer (5 ×, Component E) + 1 mL K_2SO_4 stock (125 mM, Component F) + 0.5 mL Tl_2SO_4 (50 mM, Component G). The potassium ion concentration is 50 mM and the Tl^+ ion concentration is 10 mM in the stimulation buffer.
7. FLIPR recorded 10 readings (one reading per second) before addition and continued to read for another 90 s. Initial slope of curve (initial 15 s at the rising phase of fluorescence intensity readings) was exported for data processing.

Supplementary Table S5. Protocol Table for the Automated Planar Patch Clamp Assay			
Step	**Parameter**	**Value**	**Description**
1	Subculture hERG HEK 293 cells		2 days in advance
2	Prepare External & Internal solutions	60 min	Osmolarity: 28–300 mmol/kg
3	Prime PatchXpress system	17 min	With fresh solutions
4	Prepare cells for PatchXpress	35 min	2.5 mL/10-cm dish Accutase, recovery
5	Prepare drug solutions (360 μL/well)	15 min	In 96-well plate
6	Load SealChip and Start procedure	7 min	Before the system asking for cells
7	Pellet cells for PatchXpress	3 min	250 × *g* at room temperature
8	Load cells to the PatchXpress	150 μL	About 1 million cells in External buffer
9	Aliquot cells	3.5 μL	20,000 cells in 3.5 μL/chamber
10	Detect cells	2 min	Cells descending to holes
11	Seals and whole-cell configuration	5 min	Giga seals and 2nd seals
12	Start voltage protocol from HP − 80 mV	0.1 Hz	− 50 mV (50 ms), + 20 mV (5 s), − 50 mV (1.7 s)
13	Stabilize	8 min	Terminated for seals with < 0.2 nA
14	Obtain peak tail current		With buffer, as a control
15	Add drug at 25 μL/s	3 × 50 μL	11°s apart between addition, 7 points
16	Washout	7 min	With External buffer to reveal recovery

Step Notes

1. hERG HEK 293 cells were subcultured in growth media without G418 for 2 days before use, should not overgrow > 80%.
2. Solutions were vacuum filtered to remove bubbles and small particles before use. External buffer: (mM) 137 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH 7.4. Internal buffer: (mM) 15 NaCl, 70 KF, 60 KCl, 1 $MgCl_2$, 5 HEPES, 5 EGTA, 4 ATP, 0.4 GTP pH 7.2
3. While the PatchXpress system is being primed, start Step 4.
4. Detach cells with Accutase (2.5 mL/10-cm dish for 4 min) at room temperature, transfer into a 50 mL tube in total 20 mL media, and sit the tube in incubator for 30 min with loose cap.
5. Prepare drug working solutions with External buffer while cells were incubated (Step 4).
6. At the end of 30-min incubation (Step 4), load a SealChip and start procedure.
7. While the SealChip is being prepared for recording (Step), pellet 1 million cells in a new 50 mL tube by centrifugation at 250 × *g* for 2.5 min at room temperature
8. When the SealChip is ready from Step 6, the system asks for cells.
9. The PatchXpress takes over from here and execute procedures automatically according to user-defined patch clamping protocols. Robot pipette aliquots 3.5 μL cell suspension into each chamber of the SealChip.
10. Chambers with low seal resistance (<100 MΩ) were terminated; chambers with ≥ 200 MΩ would enter Step 11. User can choose to terminate troubled chambers.
11. Form seals and obtain whole-cell configuration, Giga seals for seal resistance (Rseal) > 1GΩ and 2nd seals for Rseal > 200 MΩ.
12. Voltage protocol: holding potential at −80 mV, depolarization to −50 mV for 50 ms for leaking current, depolarization + 20 mV for 5 s to activate hERG channels, repolarization to −50 mV for 1.7 s to remove inactivation and elicit hERG tail current, repolarization back to holding potential −80 mV. The protocol was executed continuously every 10 s until the end of Step 16.
13. After obtaining whole-cell configuration, cells were maintained for 5 min to reach stable state. At the end of this stage, cells with <0.2nA peak tail current were terminated.
14. Apply control (buffer) and obtain peak tail current as 100% hERG activity.
15. Apply 7-point drug dose–response, triple addition (3 × 50 μL) for each concentration point, 11 s apart between each addition, 5 min maximal between two concentrations.