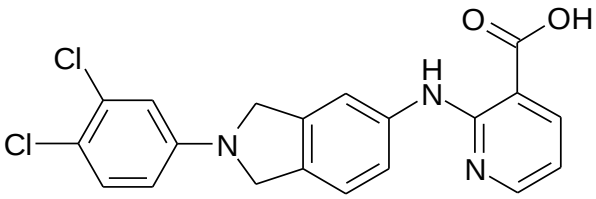
SUPPLEMENTAL DATA

1. Confirmation of PD307243 structure

Chemical shifts were reported in δ units part per million (ppm) relative to the signal at DMSO-d_6 (δ 2.5) for ^{1}H-NMR, and relative to the central DMSO-d_6 resonance (δ 39.5) for ^{13}C-NMR. ^{1}H NMR (DMSO-d_6) δ 4.60 (d, J = 12, 4H); 6.64 (dd, J = 2.6, 6.8 Hz, 1H); 6.86 (m, 2H); 7.37 (m, 2H); 7.54 (dd, J = 2.2, 8.2 Hz, 1H); 7.85 (m, 1H); 8.26 (dd, J = 1.8, 7.8 Hz, 1H); 8.41 (dd, J = 2.2, 4.8 Hz, 1H) 10.54 (s, br, 1H). ^{13}C NMR (DMSO-d_6) δ 53.18, 53.66, 107.63, 112.00, 112.66, 113.89, 113.99, 116.90, 119.42, 122.85, 130.65, 130.77, 131.59, 137.80, 139.14, 140.58, 146.84, 152.70, 155.61, 169.12. ESI m/z 398 (M – H); HRMS (EI) calculated for $C_{20}H_{15}N_3O_2Cl_2$ 399.054135, found 399.0545. The analysis confirmed the chemical structure of PD307243 as **2-[2-(3,4-Dichloro-phenyl)-2,3-dihydro-1H-isoindol-5-ylamino]-nicotinic acid:**



2. Time control of the hERG current amplitude recorded using the static bath conditions

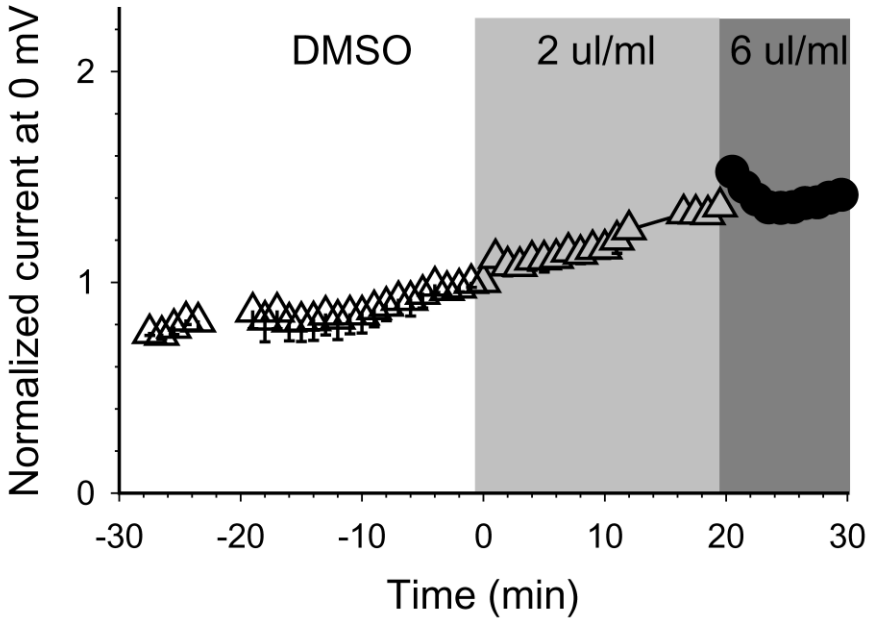


Fig. S1 Shown is time course of changes in hERG current amplitude (measured as time-dependent current during 1-s step to 0 mV) before and after adding DMSO 2 and 6 ul to a non-flow bath of 1 ml volume. The amounts of DMSO were equivalent to those added when studying PD 20 uM and NS 60 uM. Data were averaged from 3 oocytes (where SE bars are 'missing', they are smaller than the symbol size).

3. **Positions on the outer vestibule of the hERG channel where side chain substitution by cysteine or modification of the engineered cysteine side chain impaired the agonist effect of NS1643**

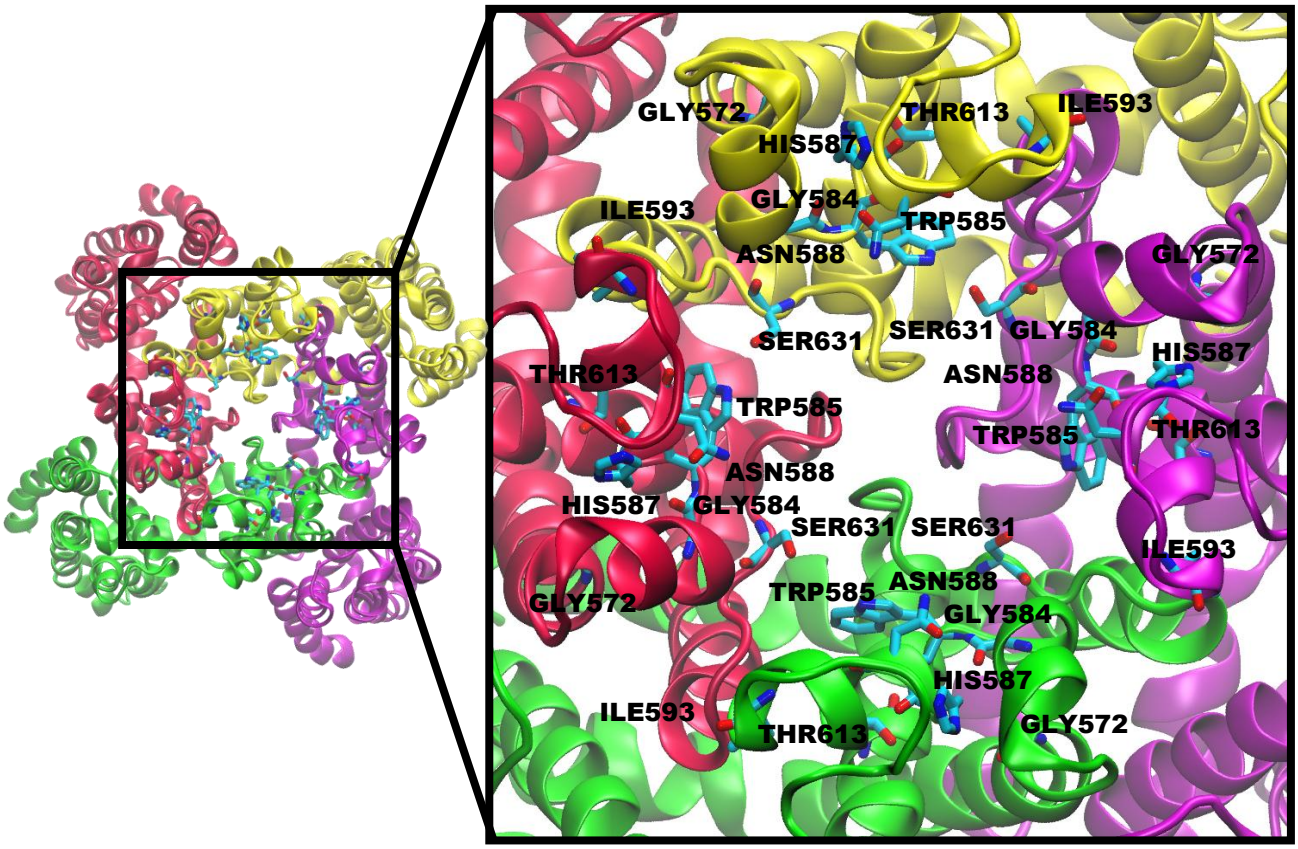


Fig. S2 Shown is the hERG homology model described by Tseng *et al.* (2007). The left panel shows a top view of the hERG channel model as C_α ribbons. The four subunits are color coded red, green, pink and yellow. A close-up view of the boxed region is shown on the right. Positions studied here (listed in Fig. 6A) are marked. Note that this model was created based on the 'peptide toxin foot printing' experiments using BeKm-1, and likely represents the hERG outer vestibule in resting and activated states. Previous studies suggested that there are dynamic conformational changes in this region during hERG inactivation, possibly bringing the S5-P helices close to each other (to allow intersubunit disulfide bond formation between cysteine side chains engineered into equivalent positions). Therefore, some of the positions may be closer to each other in the inactivated state (that we hypothesize to be perturbed by NS1643 binding) than suggested by this model.