Structurally Distinct Ligands Rescue Biogenesis Defects of the $K_{\text{ATP}}$ Channel Complex via a Converging Mechanism

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Running title: $K_{\text{ATP}}$ channel pharmacological chaperones

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Abbreviations: CBZ: carbamazepine; CFTR: cystic fibrosis transmembrane conductance regulator; GBC: glibenclamide; $K_{\text{ATP}}$: ATP-sensitive potassium channel; Kir6.2: inwardly rectifying potassium channel 6.2; NBD: nucleotide binding domain; SU: sulfonylureas; SUR1: sulfonylurea receptor 1; TMD: transmembrane domain.

Background: Carbamazepine and glibenclamide correct $K_{\text{ATP}}$ channel trafficking defects.

Result: Carbamazepine and glibenclamide share a binding pocket in the channel and enhance crosslinking of Kir6.2 to SUR1.

Conclusion: The two structurally distinct drugs correct $K_{\text{ATP}}$ channel biogenesis defects caused by mutations in SUR1 and Kir6.2 by promoting interactions between the two channel subunits.

Significance: Heteromeric subunit interface is an important target for pharmacological chaperones.

Summary

Small molecules that correct protein misfolding and misprocessing defects offer a potential therapy for numerous human diseases. However, mechanisms underlying pharmacological correction of such defects, especially in heteromeric complexes with structurally diverse constituent proteins, are not well understood. Here we investigate how two chemically distinct compounds, glibenclamide and carbamazepine, correct biogenesis defects in ATP-sensitive potassium (K$_{\text{ATP}}$) channels composed of sulfonylurea receptor 1 (SUR1) and Kir6.2. We present evidence that despite structural differences, carbamazepine and glibenclamide compete for binding to K$_{\text{ATP}}$ channels and both drugs share a binding pocket in SUR1 to exert their effects. Moreover, both compounds engage Kir6.2, in particular the distal N-terminus of Kir6.2 which is involved in normal channel biogenesis, for their chaperoning effects on SUR1 mutants. Conversely, both drugs can correct channel biogenesis defects caused by Kir6.2 mutations in a SUR1-dependent manner. Using an unnatural, photocrosslinkable amino acid, azido-phenylalanine, genetically encoded in Kir6.2, we demonstrate in living cells that both drugs promote interactions between the distal N-terminus of Kir6.2 and SUR1. These findings reveal a converging pharmacological chaperoning mechanism wherein glibenclamide and carbamazepine stabilize the heteromeric subunit interface critical for channel biogenesis to overcome defective biogenesis caused by mutations in individual subunits.
ATP-sensitive potassium (K$_{ATP}$) channels couple energy metabolism with membrane excitability, dysfunction of which underlies several human diseases (1,2). In pancreatic β-cells, the K$_{ATP}$ channel is formed by four sulfonylurea receptor 1 (SUR1) and four inwardly rectifying potassium channel Kir6.2 subunits (3,4). Loss-of-function channel mutations cause congenital hyperinsulinism (5), often due to reduced or lack of channel expression at the cell surface (6). Contrarily, gain-of-function mutations lead to neonatal diabetes (7); although these mutations frequently cause aberrant channel gating (1), they can also alter channel expression to affect presentation of the disease allele (8-10). SUR1 is an ATP-binding cassette (ABC) transporter with an ABC core structure of two transmembrane domains (TMD1, TMD2) and two nucleotide binding domains (NBD1 and NBD2) plus an additional N-terminal TMD0 with five transmembrane-helices followed by a large cytoplasmic loop called L0 (11,12). In comparison, the pore subunit Kir6.2 is small with two transmembrane helices and cytosolic N- and C-terminal domains (13). Mutations that disrupt channel biogenesis have been reported in both subunits (14).

Small molecules that can correct misfolding and mistargeting of mutant proteins offer a potential therapeutic solution for many human diseases caused by such defective proteins (15-18). Yet a lack of mechanistic understanding of how correctors rescue mutant proteins hinders further rational drug development for clinical applications. Many small molecules bind directly and specifically to the affected proteins thus acting as pharmacological chaperones. Pharmacological chaperones are generally thought to exert their effects via ligand-assisted protein folding and stabilization (16,17,19). While this concept is easy to understand in monomeric proteins or oligomeric proteins with similar subunits, it can have complex manifestations in hetero-multimeric proteins like the K$_{ATP}$ channel where not only folding of the mutant protein itself but also interactions with assembly partners need to be considered. Elucidating the structural mechanism of pharmacological chaperoning in these cases remains a challenge.

Sulfonylureas (SUs), K$_{ATP}$ channel antagonists commonly used to treat Type 2 diabetes, were the first K$_{ATP}$ channel pharmacological chaperones identified (20,21). Interestingly, even though trafficking mutations are found throughout SUR1, SUs only correct those in TMD0. Recently, we discovered that carbamazepine (CBZ), an anticonvulsant structurally unrelated to sulfonylureas (Fig. 1A), also corrects K$_{ATP}$ channel trafficking defects to restore channel surface expression (22,23). To our surprise, of the nineteen known trafficking mutations throughout SUR1 screened, only those in TMD0 responded to CBZ (22). Moreover, like SUs, CBZ inhibits K$_{ATP}$ channel activity and impairs channel response to MgADP (22,24). These findings led us to hypothesize that despite their structural differences, SUs and CBZ may correct K$_{ATP}$ channel trafficking defects by a converging mechanism.

Herein we present evidence that CBZ interacts directly with K$_{ATP}$ channels and competes for binding with the high affinity SU, glibenclamide (GBC). Mutations in SUR1 known to disrupt the binding and action of GBC prevent CBZ from exerting its effects on K$_{ATP}$ channels. In order to rescue SUR1 mutations, both drugs require co-expression of Kir6.2. Conversely, both drugs are capable of correcting channel biogenesis defects caused by Kir6.2 mutations in a SUR1-dependent manner. We identify the distal N-terminal 30 amino acids of Kir6.2 as critical for normal channel biogenesis and for the chaperoning effects of GBC and CBZ. Using a genetically encoded photocrosslinkable amino acid at the Kir6.2 N-terminus, we demonstrate directly in living cells that binding of both drugs increases the physical interaction between Kir6.2 N-terminus and SUR1. These findings reveal that CBZ and GBC overcome mutant channel biogenesis defects by altering the interface formed by Kir6.2 N-terminus and SUR1. The study establishes a pharmacological chaperoning mechanism in which small molecules stabilize the interaction between heteromeric constituent proteins in a complex to overcome biogenesis defects caused by mutations in either subunit.
MATERIALS AND METHODS

*Molecular Biology and cell culture--*FLAG-epitope tagged at the N-terminus of hamster SUR1 (f-SUR1) was in pECE and rat Kir6.2 was in pcDNAI. The FLAG tag has been shown to not interfere with channel biogenesis or function in prior studies (20,21,25). Point mutations of SUR1 were introduced into hamster SUR1 using the Quikchange site-directed mutagenesis kit (Stratagene) and confirmed by sequencing. Mutant clones from multiple PCR reactions were sequenced and used to avoid undesired mutations elsewhere introduced during PCR. Construction of adenoviruses carrying K<sub>ATP</sub> subunits cDNA was as described previously (8).

INS-1 cells, a rat insulinoma cell line, were cultured in RPMI 1640 with 11.1 mM D-glucose (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, and 50 µM β-mercaptoethanol. COSm6 cells were cultured in DMEM with 11.1 mM D-glucose (Invitrogen) supplemented with 10% FBS, 100 units/ml penicillin, and 100 µg/ml streptomycin. For binding experiments, we used INS-1 cells infected with recombinant adenoviruses carrying K<sub>ATP</sub> channel subunits coding sequences. This expression system gives rise to high channel protein expression levels to yield higher signal to noise ratio in the binding assay. For immunoblotting and electrophysiology experiments, we used COSm6 cells transfected with K<sub>ATP</sub> channel subunits cDNAs and other plasmids as needed, since COSm6 cells do not express any endogenous K<sub>ATP</sub> channel subunits thus providing a clean background for analyzing channel properties.

**[3H]-GBC binding competition experiments--**[3H]-GBC (Perkin-Elmer) binding assays were carried out on membranes prepared from INS-1 cells virally expressing f-SUR1 and Kir6.2. Viral expression was done as previously described (8). Membranes were prepared 48 hours post-infection according to the following procedure: cells were harvested, washed three times with cold PBS then snap frozen; after thawing, cells were lysed in hypotonic buffer (5 mM Tris pH7.8, plus protease inhibitor cocktail) for 45 minutes with rotation at 4°C; cells were homogenized and centrifuged at 900xg for 20 min at 4°C. Supernatant was collected and membranes were pelleted by centrifugation at 100,000xg for 1 hour, 4°C. Membranes were resuspended in binding buffer (150 mM KCl/50 mM HEPES, pH7.5) and centrifuged at 2500xg for 15 min, 4°C, to remove any insoluble material. Membranes were used immediately for binding studies or aliquotted, snap frozen, and stored at -80°C. Membranes prepared from INS-1 cells infected with a control tTA virus served as negative controls for background [3H]-GBC binding due to low levels of endogenous K<sub>ATP</sub> channels and non-specific binding, which was ~6% of total binding observed in membranes prepared from INS-1 cells infected with K<sub>ATP</sub> channel viruses.

Binding assays were done in 100 µl reactions at room temperature. Each reaction contained 2.5 nM [3H]-GBC and 50-100 µg/ml protein in binding buffer, with varying concentrations of unlabeled inhibitor (final DMSO concentration was 5% to maintain solubility of CBZ and GBC). Binding reaction was initiated by addition of membranes and was allowed to equilibrate for 60 minutes. Bound ligand was separated from free ligand by rapid filtration over Whatman GF/B filters. Filters were washed three times with cold binding buffer and counted for presence of [3H] in 4 ml of scintillant (Scintiverse BD Cocktail, Fisher) on a Beckman LS6000 liquid scintillation counter. Nonspecific binding was done in the presence of 1 µM unlabeled GBC or 50 µM CBZ.

Binding data was analyzed with the use of the GraphPad Prism 5 statistical package. One-site competitive inhibition curves for specific binding were generated using the equation (1)

\[
y = NS + \frac{Total - NS}{1 + 10^{(logX - logIC_{50})}}
\]

where y is specific binding, NS is nonspecific binding, Total is total binding, and X is the concentration of unlabeled inhibitor. IC<sub>50</sub> values and their respective 95% confidence intervals were obtained through the regression analysis. For heterologous competition experiments, the inhibition constant, K<sub>i</sub>, was obtained according to the Cheng-Prusoff equation (2) (26):
\[
K_i = \frac{IC_{50}}{1 + \frac{L}{K_D}}
\]

with \(L\) denoting the concentration and \(K_D\) the equilibrium dissociation constant of the radioligand. \(K_D\) of 0.4 nM reported for \([3H]\)-GBC binding to \(K_{ATP}\) channels in Hambrock et al. (27) was used to calculate \(K_i\). Data are shown as means ± standard error of the mean. Each data point was run in duplicate, and each experiment was repeated at least three times.

**Immunoblotting**--COSm6 cells were transfected with \(f\)-SUR1 and Kir6.2 cDNA using FuGENE®6 according to the manufacturer’s instruction. Cells were lysed in lysis buffer containing 50 mM Tris·HCl, pH 7.0, 150 mM NaCl, and 1% TritonX 100, with Complete\(^\text{TR}^\text{TM}\) protease inhibitors (Roche) on ice for 30 min. Cell lysate was centrifuged at 16,000x \(g\) for 5 min at 4°C, and an aliquot of the supernatant was run on SDS-PAGE and transferred to nitrocellulose membrane. For detecting SUR1, the membrane was probed with a rabbit anti-SUR1 serum raised against a C-terminal peptide of SUR1 (KDSVFASFVRADK), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences), and visualized by enhanced chemiluminescence (Super Signal West Femto; Pierce) using FluorChem E equipped with a CCD-camera based detection system (Cell Biosciences). For detecting Kir6.2, the primary antibodies used were a commercial goat antibody directed against the N-terminal 18 amino acids of Kir6.2 (N-18; Santa Cruz Biotech). This antibody does not generate strong signals but shows less non-specific interactions and is therefore used for probing SUR1-Kir6.2 crosslinked bands. For all other immunoblotting experiments, a rabbit serum raised against the C-terminal region (amino acids 170-390) of Kir6.2 was used as it gives rise to stronger signals. The specificity of both antibodies has been previously demonstrated (8,21,28). Tubulin was also probed and served as a loading control.

**Construction, expression, and UV-induced photocrosslinking of Kir6.2 with genetically encoded p-Azido-L-phenylalanine**--COSm6 cells were co-transfected with plasmids coding for azidophenylalanine tRNA, azidophenylalanine tRNA synthetase, WT \(f\)-SUR1 and Kir6.2 containing stop codons at position 12, 18 & 52 using FuGENE®6 as described by Grunbeck et al. (29). Growth media was supplemented with 0.5-1 mM azidophenylalanine 12 hours after transfection and cells were grown for 36-48 hours with or without GBC or CBZ. Cells were harvested in cold PBS and incubated with drugs for 10 min at 37°C and exposed to UV for 15 min with occasional shaking. Cells were pelleted and lysed in lysis buffer (20 mM HEPES, pH 7.2, 125 mM NaCl, 4 mM EDTA, 1 mM EGTA, 1% TritonX-100) with complete protease inhibitors for 30 min, centrifuged for 15 min in a table top centrifuge, and supernatant was collected and incubated with anti-FLAG agarose beads overnight, washed 3X with 1 ml of a buffer containing 20 mM HEPES, pH 7.2, 150 mM NaCl, 4 mM EDTA, 1 mM EGTA, 1% Igepal, 0.1% SDS, and 0.04% deoxycholic acid, and finally bound proteins were eluted with 1% SDS, run on 3-8% SDS Tris-Acetate gels and subjected to immunoblotting analysis as described above. The crosslinked band intensity as a percentage of the total SUR1 signal intensity was quantified by densitometry using ImageJ. To avoid potential problems with signal saturation, quantification was performed using images obtained from three different exposure times that gave consistent values. The average of these technical repeats was then used for calculating the mean of the biological repeats shown in the bar graphs.

**Patch-clamp recording**--COSm6 cells were transfected with \(f\)-SUR1 and Kir6.2 cDNA, along with cDNA encoding the green fluorescent protein to identify transfected cells. Cells were replated onto coverslips 24 hours post-transfection and used for inside-out patch voltage-clamp recording the following day. Micropipettes were pulled from non-heparinized Kimble glass (Fisher) on a horizontal puller (Sutter Instrument, Novato, CA) with resistance typically ~1–2 megaohms. The bath (intracellular) and pipette (extracellular) solutions were K-INT: 140 mM KCl, 10 mM K-HEPES, pH 7.3. ATP was added as the potassium salt (Sigma). Recording was performed at room temperature and currents were measured at a membrane potential of -
50 mV and inward currents shown as upward deflections. Quantification of the effects of 
GBC and CBZ on $K_{\text{ATP}}$ channel activity was performed as described in detail in the figure 
legends. To estimate the IC$_{50}$ of carbamazepine 
inhibition, dose-response data were fitted by the 
Hill equation (Residual current (%) = 
$1/\{1 + [(\text{CBZ})/\text{IC}_{50}]^n\}$), similar to that described 
previously for estimating the IC$_{50}$ of ATP 
inhibition (30).

$\text{Kir6.2 homology model}$--A homology model 
of Kir6.2 tetramer marking the positions of the 
mutations studied was made with Modeller and 
Chimera using a chicken Kir2.2 channel crystal 
structure (PDB ID: 3JYC) (31) as the template. 
Amino acids 30-352 of Kir6.2 which correspond 
to amino acids 42-369 of chicken Kir2.2 based 
on sequence alignment were modeled.

$\text{Statistical analysis}$--Data are shown as mean 
± standard error of the mean (s.e.m.). Statistical 
analysis was performed using Student’s $t$-test 
when comparing two groups. When comparing 
three or more groups one-way ANOVA was 
used with Tukey’s post-hoc test.

$\text{RESULTS}$

$\text{CBZ inhibits}$ $[^3\text{H}]-\text{GBC}$ $\text{binding to}$ $K_{\text{ATP}}$ $\text{channels}$--To test the hypothesis that CBZ and 
GBC rescue $K_{\text{ATP}}$ channel trafficking mutants by 
similar mechanisms despite structural variations, 
we first determined whether CBZ competes with 
GBC for binding to the channel. Equilibrium 
binding of $[^3\text{H}]-\text{GBC}$ to channels was assessed 
in the presence of unlabeled GBC or CBZ. 
Unlabeled GBC inhibited $[^3\text{H}]-\text{GBC}$ binding 
with a half inhibition concentration (IC$_{50}$) of 
4.35 ± 1.13 nM. CBZ also inhibited $[^3\text{H}]-\text{GBC}$ 
binding, with an IC$_{50}$ of 184.50 ± 17.80 nM (Fig. 
1B). Using equation 2 (see Materials and 
Methods) the Ki value calculated for GBC is 
0.60 nM, in agreement with values reported in 
the literature (32-34); and the Ki for CBZ is 
25.45 nM.

The estimated CBZ affinity for the channel 
from competition binding assays is significantly 
higher than CBZ concentrations we recently 
reported for rescuing surface expression of 
SUR1-TMD0 trafficking mutants (0.2-50 µM) 
(22), likely because the cell-based chaperoning 
experiments require CBZ to reach the cell 
interior. In addition to correcting $K_{\text{ATP}}$ channel 
trafficking defects, our recent studies have 
shown that CBZ imparts an inhibitory effect on 
channels in inside-out patch-clamp recording at 
a concentration used for trafficking rescue (10 
µM) (22,24). The inside-out patch-clamp 
recording experimental setup is more 
comparable to the binding assay to better assess 
the affinity between channel-carbamazepine 
interactions. Using this experimental setup, we 
found that channel activity was indeed inhibited 
by >50% at 50 nM CBZ (Fig. 1C and 1D), with 
an estimated IC$_{50}$ of ~23 nM (by fitting data in 
Fig.1D to the Hill equation as described in 
Materials and Methods), which is close to the Ki 
value of 25.45 nM calculated from the 
competition binding study (Fig.1B). These 
results confirm the high affinity interaction 
between CBZ and the channel. As a comparison, 
the IC$_{50}$ for GBC inhibition of $K_{\text{ATP}}$ channel 
activity reported in the literature and from our 
own studies is 1-3 nM (35-37), and the Ki 
calculated for GBC from the competition 
binding assays shown in Fig.1B is 0.6 nM.

$\text{CBZ and GBC share binding sites in}$ SUR1--
GBC binding to SUR1 has been proposed to 
involve two sites referred to as site A and B 
(38), located in TMD2 and L0, respectively (Fig. 
2A). Site A interacts with the short-chain 
sulfonylurea moiety like tolbutamide, whereas 
site B binds to the non-sulfonylurea, benzamido 
moiety of GBC. Point mutations in the proposed 
site A (S1238Y) or site B (Y230A) (Fig. 2B) 
render channels less sensitive to GBC block, and 
combining both mutations completely abolishes 
channel inhibition by GBC (39-41). The same 
set of mutations (referred to as GBC binding 
mutations hereinafter) also disrupt GBC rescue 
of SUR1-TMD0 trafficking mutations without 
affecting channel biogenesis or gating 
themselves as reported in our previous study 
(41).

Although the structures of CBZ and GBC 
appear quite different (Fig. 1A), given that CBZ 
inhibits $[^3\text{H}]-\text{GBC}$ binding to the channel we 
worried whether the two drugs have 
overlapping binding sites. First, we asked 
whether CBZ rescue of TMD0 trafficking 
mutants is disrupted by GBC binding mutations, 
Y230A, S1238Y or both (Y230A/S1238Y). The 
F27S SUR1-TMD0 mutation previously shown
to respond to GBC and CBZ rescue (22) was used as an example. Cells were treated with 0.1% DMSO as vehicle control, 5 µM GBC, or 10 µM CBZ overnight (16 hours) prior to Western blot analysis; these conditions have been shown in our previous study to result in optimal rescue effects (22).

In Western blots, two SUR1 bands were detected for WT channels: the core-glycosylated lower band representing the immature form in the ER and the complex-glycosylated upper band corresponding to mature SUR1 that has exited the ER and traversed the Golgi (42). By contrast, only the immature band was observed in cells co-expressing F27S-SUR1 and Kir6.2, a defect that was efficiently corrected by GBC and CBZ. As expected, the rescue effect of GBC was attenuated by Y230A or S1238Y and abolished by Y230A/S1238Y. Remarkably, both single binding mutations alone as well as the double binding mutation also rendered CBZ ineffective in rescuing F27S (Fig. 2C). Results from experiments described above provide compelling evidence that CBZ corrects trafficking defects of TMD0-SUR1 mutants by binding to the channel and acting as a pharmacological chaperone. Moreover, the sites involved in CBZ binding are similar to or overlap with those involved in GBC binding. We note that the SUR1 upper band was used as a readout for pharmacological correction of mutant channel trafficking defects in this and subsequent experiments as our previous studies have established that the intensity of the SUR1 upper band in the COS cell expression system correlates well with the level of channel expression at the cell surface assessed by surface staining, surface biotinylation, a quantitative immune-based surface chemiluminescence assay, and functional assays (21,22,24,43).

Next, we tested whether the effects of CBZ on channel gating are also dependent on sites required for GBC’s actions. As shown in Fig. 2D, Y230A rendered channels less sensitive to CBZ inhibition. The S1338Y mutation, while it did not affect the extent of inhibition by CBZ, made the inhibition highly reversible. Combining Y230A and S1238Y completely prevented CBZ from inhibiting channel activity. The gating profiles of these mutants in response to CBZ mirrored those in response to GBC. The remarkable similarities in how GBC binding mutations negatively impact channel response to CBZ and GBC indicate that like GBC, CBZ interacts directly with the channel, at least with SUR1, to exert its effects.

Involvement of Kir6.2 in the chaperoning effects of GBC and CBZ--GBC or CBZ could correct SUR1 mutations associated with K\textsubscript{ATP} channel biogenesis defects by correcting the folding defect of mutant SUR1 per se or by a process that engages both channel subunits. These possibilities can be distinguished using a SUR1 variant in which the ER retention signal RKR is mutated to AAA (SUR1\textsubscript{RKR→AAA}), which allows SUR1 to traffic to the cell surface without Kir6.2 (42). Using this approach, we found that the chaperoning effect of CBZ is dependent on Kir6.2. For this set of experiments, SUR1-TMD0 trafficking mutations A116P and V187D which we have shown previously to respond to GBC and CBZ rescue (20,22,23,41,44) were used as examples. In SUR1\textsubscript{RKR→AAA} bearing A116P or V187D expressed without Kir6.2, both exhibited only the core-glycosylated lower band, in contrast to WT-SUR1\textsubscript{RKR→AAA} which showed both lower and upper bands; treatment with CBZ failed to correct the mutant SUR1 processing defects (Fig. 3A). Upon co-expression with Kir6.2, however, CBZ was able to induce the mature upper band of both mutants. These findings are reminiscent of those previously reported for GBC (41), leading us to conclude that Kir6.2 plays a requisite role in the chaperoning effects of CBZ and GBC. Interestingly, we noted that CBZ treatment significantly enhanced the core-glycosylated A116P- and V187D-SUR1\textsubscript{RKR→AAA} band intensity even in the absence of Kir6.2. A likely explanation is that CBZ protects the misfolded SUR1 proteins against ER-associated degradation, which would be consistent with our previous metabolic pulse-chase study showing that GBC also slows down the degradation rate of A116P-SUR1 expressed alone without Kir6.2 (20).

The above results prompted us to ask the converse question, i.e. whether GBC and CBZ can also correct trafficking defects caused by Kir6.2 mutations in a SUR1-dependent manner. A number of Kir6.2 mutations associated with either congenital hyperinsulinism or neonatal
diabetes have been reported to impair channel biogenesis (8,45,46). We tested the effects of GBC and CBZ on several such mutations, including Q52R, V59G, R201H and I296L associated with neonatal diabetes, and W91R, H259R, and R301H associated with congenital hyperinsulinism. Correction of channel biogenesis efficiency was assessed by Western blots of SUR1 co-expressed with mutant Kir6.2. GBC and CBZ increased the SUR1 upper band signal in some mutants to varying degrees (Fig. 3B and C) but not W91R and H259R (not shown). Among these, the I296L showed the greatest response to both compounds as compared to the DMSO vehicle-treated control. We analyzed further whether the rescue effect is dependent on the intact binding sites for these drugs in SUR1. The Y230A/S1238Y double mutation in SUR1, which abolished the ability of both drugs to rescue the trafficking defects of TMD0-SUR1 mutations also rendered the drugs unable to correct the processing defect of SUR1 co-expressed with I296L-Kir6.2 (Fig. 3D). The results lend further support to the notion that both channel subunits are involved in the chaperoning effects of GBC and CBZ.

An essential role of the Kir6.2 N-terminus in channel biogenesis and pharmacological correction--Although SUR1 is the primary high-affinity binding subunit for SU's, several studies have indicated that Kir6.2 also contributes to binding. Deletion of the distal N-terminus of Kir6.2 (ranging from 10-30 amino acids) reduces GBC binding affinity, photolabeling of Kir6.2 co-expressed with SUR1 by [125I]azido-GBC, and channel sensitivity to GBC inhibition (34,47-49). This raises the question of whether the distal N-terminus of Kir6.2 plays a role in mediating the chaperoning effect of GBC and CBZ. To address this, we examined whether deletion of the N-terminus of Kir6.2 affects the ability of GBC or CBZ to rescue channel trafficking defects. Using F27S-SUR1, we found that while processing of the mutant SUR1 in cells co-expressing WT-Kir6.2 was fully normalized by both drugs as evidenced by the abundant upper band, it was not the case when F27S-SUR1 was co-expressed with ΔN30-Kir6.2 where the upper band in the drug-treated cells was barely detectable (Fig. 4A). Deletion of the distal C-terminus of Kir6.2 (ΔC25), on the other hand, did not affect the rescue by either drug. Control experiments comparing WT-SUR1 co-expressed with WT, ΔN30, or ΔC25 Kir6.2 showed that deletion of the N-terminus also severely compromised the maturation efficiency of WT-SUR1 (Fig. 4B), even though ΔN30-Kir6.2 was expressed at levels similar to WT-Kir6.2 (Fig. 4C). By contrast, deletion of the Kir6.2 C-terminus had little impact on SUR1 maturation, in agreement with previous studies (50). These experiments reveal that the Kir6.2 N-terminus is a critical determinant not only for WT channel biogenesis but also for pharmacological chaperone rescue of mutant channels.

Pharmacological chaperones increase photocrosslinking of Kir6.2 N-terminus with SUR1--Our evidence so far indicates that CBZ and GBC rescue channel biogenesis defects by engaging both channel subunits. This led us to ask whether and how GBC and CBZ binding to the channel cause structural changes to overcome the defects. Because the N-terminal 30 amino acids of Kir6.2 are important for channel biogenesis and pharmacological rescue we focused our attention to this region, with the hypothesis that GBC and CBZ may alter interactions between Kir6.2 and SUR1 to correct channel biogenesis defects caused by mutations in either subunits. We employed a site-directed crosslinking approach to probe potential structural changes that may be brought on by drug binding. To achieve this, we engineered genetically encoded photocrosslinkable unnatural amino acid, p-azido-L-phenylalanine (AzF), at specific positions in Kir6.2. In this scheme, the nonsense amber stop codon, TAG, replaces the WT amino acid codon at a designated position. Suppression of the stop codon is achieved by co-transfecting cells with plasmids for an engineered orthogonal pair of tRNA_CUG and aminoacyl tRNA synthetase specific for AzF, and growing cells in the presence of AzF (51). Importantly, this approach allows us to monitor pharmacological chaperone-dependent changes in SUR1-Kir6.2 interactions in living cells where the chaperoning actions occur. Moreover, it allows us to interrogate the structural regions involved by placing AzF at specified positions (Fig. 5A). We chose to incorporate AzF into Kir6.2
position 12 and 18 which harbor a tyrosine and alanine respectively in the WT protein. As a positive control, we also placed the stop codon at position 52 of Kir6.2 based on our previous finding using cysteine-crosslinking that this position is in close proximity to amino acid 203 in SUR1 (52).

To first confirm incorporation of AzF in the three Kir6.2 TAG mutants, we co-transfected cells with cDNAs for WT f-SUR1, Kir6.2-TAG, the aminoacyl tRNA synthetase for AzF and tRNA_{CUG} and then incubated cells in the absence or presence of AzF. Western blots showed that for all three Kir6.2-TAG constructs no Kir6.2 was detected in the absence of AzF and the corresponding SUR1 only showed the lower band; however, in the presence of AzF, a Kir6.2 band was detected indicating suppression of the stop codon, with the corresponding SUR1 showing both the lower and upper band (Fig. 5B). Note in cells transfected with the mutant Kir6.2-TAG and WT SUR1 plasmids, the mutant Kir6.2 and SUR1 signals were significantly weaker compared to signals seen in cells transfected with WT Kir6.2 and WT SUR1 (Fig. 5B). This is due to the lower expression efficiency and transfection efficiency inherent to the mammalian cell amber-stop codon suppression experimental system (53). Patch-clamp recording further confirmed that the Kir6.2-TAG constructs formed functional ATP-sensitive channels with SUR1 (Fig. 5C).

Next, we tested whether Kir6.2 can be crosslinked to SUR1 upon UV exposure and whether the crosslinking is modulated by pharmacological chaperones. In cells expressing the Kir6.2-Y12AzF channel and treated with DMSO, UV exposure yielded a faint band above the SUR1 upper band and below the 250 kD marker detected by the anti-SUR1 antibody (Fig. 6A). The size of the band suggests it could be SUR1 crosslinked to Kir6.2. To verify this, the blots were also probed with antibodies against the N-terminus (see Materials and Methods) of Kir6.2. The anti-Kir6.2 N-terminus antibody recognized the band that was detected by anti-SUR1 antibody, confirming that the band corresponds to SUR1 crosslinked to Kir6.2. Additional bands were also observed above the SUR1-Kir6.2 crosslinked band and could be Kir6.2 crosslinked to other Kir6.2 or other non-K_{ATP} channel proteins. The intensity of the crosslinked band was substantially stronger in cells incubated overnight with GBC or CBZ. Quantification of this band from four independent experiments (see Materials and Methods) revealed a statistically significant increase in the signal in cells treated with GBC or CBZ compared to control (2.00 ± 0.33 and 1.76 ± 0.31-fold increase for GBC and CBZ, respectively; Fig. 6B).

Similar results were obtained for Kir6.2-A18AzF, although for this position little crosslinking was detected in DMSO vehicle-treated control samples and only a weak crosslinked SUR1-Kir6.2 band was observed in GBC- or CBZ-treated samples (Fig. 6C; n = 3). We then tested whether pharmacological chaperones will induce crosslinking of Kir6.2 to the TMD0-SUR1 trafficking mutant F27S. In DMSO-treated cells, no crosslinked SUR1-Kir6.2 band was detected upon UV exposure; however, in GBC-treated cells a crosslinked band was clearly visible (Fig. 6D; n =3). These results show that pharmacological chaperones increase physical interactions between the Kir6.2 distal N-terminus and SUR1 and that this effect underlies, at least in part, the ability of GBC and CBZ to correct channel biogenesis defects.

Finally, we tested the positive control, Kir6.2-Q52AzF, which is expected to crosslink to SUR1 independent of chaperone treatment. Indeed, crosslinking between SUR1 and Kir6.2 was observed in cells treated with DMSO, GBC, or CBZ (Fig. 7A). Quantification from three independent experiments showed that the extent of crosslinking in drug-treated samples did not differ significantly from that in DMSO vehicle-treated samples (Fig. 7B).

**DISCUSSION**

In this study, we investigated how two chemically distinct small molecules, GBC and CBZ, correct processing defects of heteromeric K_{ATP} channels. This mechanistic study follows up on our previous work showing that CBZ and GBC both correct K_{ATP} channel trafficking defects in a similar subset of mutations and both inhibit channel activity (22,24). We show that...
despite the structural difference, both drugs require the same residues in SUR1 and the N-terminus of Kir6.2 for their actions, implicating shared binding sites and chaperoning mechanisms. Furthermore, both drugs enhance the interaction between the Kir6.2 N-terminus and SUR1. These results led us to propose that CBZ and GBC, rather than correcting the folding defect of the mutant subunit itself to allow complex assembly, bind to the mutant SUR1-Kir6.2 complex and stabilize SUR1-Kir6.2 interface to allow mutant channels to adopt a correctly folded state that can pass the ER quality control (Fig. 8A). The findings presented here reveal a converging mechanism whereby pharmacological chaperones correct biogenesis defects caused by mutations in individual subunits of large heteromeric KATP channel protein complexes by targeting the interface of structurally diverse constituent proteins. The study has important implications for channel pharmacology and biogenesis mechanism as discussed below.

CBZ has been shown or implicated to have salutary effects on several conformational diseases including cystic fibrosis caused by the cystic fibrosis transmembrane conductance regulator (CFTR) mutation ΔF508 (54), hepatic fibrosis caused by misfolding and aggregation of α1-antitrypsin (55), and some neurodegenerative diseases (56, 57). In the latter two cases, CBZ is thought to alleviate disease phenotypes by activating the autophagy pathway to clear aggregated mutant proteins rather than acting as pharmacological chaperones. For ΔF508 CFTR, although CBZ was found to improve the processing of the mutant protein the underlying mechanism remains unknown. In our recently published work, we have shown that CBZ corrects KATP channel trafficking defects by a mechanism independent of autophagy (22). Our results in this study provide compelling evidence that CBZ interacts directly with the KATP channel and acts as a bona fide pharmacological chaperone. Moreover, our results suggest that CBZ and GBC share similar binding sites in the KATP channel complex in the currently proposed binding site model for GBC. At first sight, this notion may seem surprising given that the two chemicals appear to have quite different structures and that both drugs have been characterized extensively. GBC is ~17Å long in its extended conformation with eleven rotatable bonds, endowing the molecule with considerable conformational freedom. In contrast, CBZ, which is structurally related to tricyclic antidepressants, is relatively compact and rigid. Computational studies have demonstrated that GBC can acquire a folded U shape conformation in vacuo due to preference of hydrophobic interactions, while in the presence of aqueous solvent it prefers extended conformation due to preference for solvation interactions with water (58) (Fig. 8B). Given the environmental sensitivity of the GBC conformation, we speculate that GBC may attain a considerably folded state upon binding to a rigid binding pocket in SUR1 and loss of entropy is probably compensated by optimal positioning of hydrophobic groups in the binding pocket. Alternatively, there remains a possibility that CBZ and GBC may bind to different sites in the channel complex to allosterically impact a common Kir6.2-SUR1 interface. Future work defining the precise physical binding sites of glibenclamide and carbamazepine in the channel complex is needed to address this issue.

SUR1 belongs to the ABC transporter family, which comprises a large number of proteins involved in movement of structurally diverse endogenous and xenobiotic compounds across biological membranes (59). Unlike other members in the family, however, SUR has uniquely evolved to regulate the activity of an ion channel without being a transporter or ion channel itself (60). Despite functional divergence, all ABC transporters share some structural similarities. It is worth noting that both GBC and CBZ have been reported to bind or are substrates of ABC transporters (59). Bessadok et al. (61) recently showed that the multidrug resistance transporter P-glycoprotein (ABCB1) recognizes several SUR1 ligands including GBC and diazoxide. Moreover, CBZ has been shown to correct the trafficking defect of AF508 CFTR (54), another ABC transporter. Although it remains unknown whether CBZ binds directly to CFTR, these observations nevertheless raise the interesting possibility that there may be common structural features in ABC transporters that endow them the ability to interact with diverse compounds, albeit with
different affinities. Examination of additional ABC transporter substrates may uncover new pharmacological chaperones or modulators for K\textsubscript{ATP} channels and other ABC transporters.

Our results show that the distal N-terminus of Kir6.2, whose sequence is unique among Kir6 subfamily members, is an important structural conduit for channel assembly and chaperone action. Deletion of Kir6.2 N-terminal 30 amino acids greatly impairs SUR1 maturation and precludes the rescue effect of pharmacological chaperones on TMD0 trafficking mutants. This is unlikely due to severe misfolding of Kir6.2\DeltaN30 as this construct has been shown to form functional channels, although with altered gating properties (62-65). Interestingly, using Kir2.1/Kir6.2 chimeras co-expressed with SUR proteins in Xenopus oocytes, it has been shown that the distal N-terminus of Kir6.2 is important for specific interactions with SUR and for surface expression of K\textsubscript{ATP} channels (66). Moreover, 	extsuperscript{125}I-azido-GBC co-photolabeling of truncated Kir6.2 containing all or part of the N-terminus and the first transmembrane helix when co-expressed with SUR1 suggests physical proximity of the Kir6.2 N-terminus to GBC bound SUR1 (49). Our results that GBC and CBZ promote crosslinking of the unnatural amino acid AzF engineered in Kir6.2 amino acid position 12 and 18 to SUR1 are consistent with previous studies, and importantly, directly capture in living cells the critical role of this region in interacting with SUR1 to mediate channel biogenesis during pharmacological rescue.

An intriguing feature shared by GBC and CBZ is that all SUR1 mutations responsive to rescue by the two drugs are located in TMD0. Our observation that in the absence of pharmacological chaperones, F27S-SUR1 fails to confer crosslinking suggests F27S disrupts SUR1-Kir6.2 interactions, perhaps due to misfolding of TMD0. A SUR1 region that has been proposed to interact with the distal N-terminus of Kir6.2 is the L0 linker that connects TMD0 to the ABC core structure. Evidence suggests L0 of SUR1 and N-terminus of Kir6.2 form site B of the GBC binding pocket. We speculate that in the case of TMD0 mutants, stabilization of interactions between L0 of SUR1 and Kir6.2 N-terminus by CBZ or GBC is key to stabilizing the mutant channel complex to allow mutant TMD0 to eventually adopt the correct conformation instead of being directed to ER-associated degradation (Fig.8A). This would explain why GBC and CBZ do not rescue the trafficking defects of SUR1\textsubscript{RKR→AAA} harboring TMD0 mutations in the absence of Kir6.2 even though SUR1 alone can still bind GBC with high affinity (32,34). It is worth noting that we have recently found that substitution of glutamine at the N-terminal amino acid position 52 of Kir6.2 by glutamate or aspartate suppresses the processing defect caused by F27S or A116P mutations in the TMD0 of SUR1 (67), consistent with a model of coupled conformational maturation between SUR1 and Kir6.2.

In summary, the study presented here unveils similarities between the mechanism of action of GBC and CBZ on K\textsubscript{ATP} channels. It offers a striking demonstration of how stabilization of hetero-subunit interface by pharmacological chaperones can profoundly impact the biogenesis efficiency of the mutant complex. Our findings underscore the importance of subunit interface as drug targets to modulate protein complex stability and function (68-71), and provide the impetus for designing new mechanism-based K\textsubscript{ATP} channel pharmacological chaperones and gating modulators. Furthermore, the genetically encoded crosslinkable unnatural amino acid employed here demonstrates the drug-dependent propensity of interactions between the Kir6.2 N-terminus and SUR1 and is a promising approach for detecting conformational changes associated with physiological and pharmacological functional state transitions in K\textsubscript{ATP} channels as well as other proteins. Finally, as CBZ is a FDA-approved drug for treating certain human diseases, our studies suggest that the drug may also have applications for K\textsubscript{ATP} channelopathies.
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Figure Legends

Figure 1 CBZ inhibits binding of [3H]-GBC to the KATP channel complex. (A) Chemical structures of GBC and CBZ. (B) Inhibition of [3H]-GBC binding to KATP channels by GBC and CBZ using membranes prepared from INS-1 cells infected with recombinant adenoviruses carrying KATP channel subunits coding sequences as described in Materials and Methods. Data are means ± s.e.m. of 3-5 experiments and are expressed as percent specific binding. Nonspecific binding determined in the presence of 1 µM unlabeled GBC or 50 µM CBZ as described in Materials and Methods was less than 5% of total binding. Curves represent least square fits of the data to a one-site competition model. (C) Dose-dependent inhibition of KATP channel activity by CBZ. Representative inside-out patch voltage-clamp recordings from COSm6 cells transfected with cDNAs for SUR1, Kir6.2 and GFP as described in Materials and Methods. Membrane patches were excised into K-INT solution (see Materials and Methods) and briefly exposed to 1mM ATP to confirm the identity of the current. The patch was subsequently returned to K-INT and exposed to 0.1% DMSO (vehicle control), 50 nM, 100 nM, or 500 nM CBZ as indicated by the arrows. The dotted lines indicate where current inhibition had reached a near steady state. (D) Averaged residual currents at near steady state inhibition expressed as percent of currents prior to CBZ exposure. Each bar represents the mean ± s.e.m. of 4 patches.

Figure 2. Point mutations in SUR1 known to interfere with GBC binding disrupt the effects of both GBC and CBZ on KATP channel biogenesis and gating. (A) Pharmacophore model of GBC proposed by Grell et al. (38). (B) Topology model of SUR1 showing the location of GBC binding site A mutation S1238Y and site B mutation Y230A. TMD0 trafficking mutations F27S, A116P and V187D used in the study as well as the ER retention motif RKR are also shown. (C) Trafficking defective SUR1 mutant containing TMD0 mutation F27S in combination with GBC binding mutation Y230A (left), or S1238Y (middle), or double binding mutation Y230A/S1238Y (right) was co-expressed with Kir6.2 and subjected to drug treatment (V: 0.1% DMSO vehicle; G: 5 µM GBC; C: 10 µM CBZ) overnight (16 hours) as indicated. For comparison, WT control (WT SUR1 + WT Kir6.2) was included in each blot. Tubulin blots served as loading controls. The filled and open circles in this and subsequent blots indicate the complex- and core-glycosylated SUR1 proteins, respectively. (D) Representative current traces of mutant channels obtained by inside-out patch voltage-clamp recording. COSm6 cells were transfected with Kir6.2 and WT-SUR1, Y230A-SUR1, S1238Y-SUR1 or Y230A/S1238Y-SUR1. Membranes were excised into K-INT solution and briefly exposed to 1mM ATP to confirm the identity of the current. The patch was subsequently exposed to 10 µM CBZ (top row) or 0.1 µM GBC (bottom row). The initial jump of currents in each trace was due to channel opening upon patch-excision into ATP-free K-INT solution. Currents in 10 µM CBZ or 0.1 µM GBC after inhibition has reached near steady-state were quantified and expressed as percent of currents observed in K-INT at the beginning of the recording (bar graph on the right; each bar represents the mean ± s.e.m. of 3-4 patches). Note the inhibition was not reversible for WT and the Y230A mutant but was reversible for the S1238Y mutant.

Figure 3. Role of Kir6.2 in the chaperoning effects of GBC and CBZ. (A) Western blot of WT or mutant (A116P or V187D) SUR1RKR→AAA (WTAAA, A116PAAA, V187DAAA, respectively) expressed alone or with Kir6.2 and treated with 0.1% DMSO (V) or 10 µM CBZ (C) overnight as indicated. Note the complex-glycosylated SUR1RKR→AAA band (indicated by the grey circle) runs slower than the corresponding band of WT SUR1 co-expressed with Kir6.2, as reported in previous studies (42). (B) GBC and CBZ also rescue SUR1 processing defects caused by mutations in Kir6.2. Top: SUR1 blots from cells co-expressing WT-SUR1 and WT or mutant Kir6.2 treated overnight with 0.1% DMSO (V), 5 µM GBC (G) or 10 µM CBZ (C). (C) Location of the Kir6.2 trafficking mutations shown in (B) in a homology model of Kir6.2. Red space fills are PNDM/DEND mutations and the cyan space fill is a CHI mutation. (D) Pharmacological rescue of the I296L-Kir6.2 trafficking mutation is dependent on the Y230 and S1238 sites in SUR1.
**Figure 4.** The N-terminus of Kir6.2 is critical for channel biogenesis. (A) Effects of Kir6.2 N- and C-terminal deletions on pharmacological rescue of trafficking defective SUR1-TMD0 mutant F27S. SUR1 blots of cells expressing F27S-SUR1 along with WT-, ΔN30, or ΔC25-Kir6.2 and treated overnight with V: DMSO (0.1%), G: 5 µM GBC, or C: 10 µM CBZ. (B) SUR1 blots of cells expressing WT-SUR1 with WT-, ΔN30, or ΔC25-Kir6.2 and treated overnight with DMSO, 5 µM GBC, or 10 µM CBZ. (C) Kir6.2 blots from cells co-expressing WT- or F27S-SUR1 and WT- or ΔN30-Kir6.2 and treated with DMSO, 5 µM GBC, or 10 µM CBZ. Note ΔN30-Kir6.2 migrated faster than WT-Kir6.2 due to the deletion.

**Figure 5.** Incorporation of the photocrosslinkable unnatural amino acid azidophenylalanine (AzF) in Kir6.2. (A) Cartoon depicting the amino acid positions in Kir6.2 where the TAG stop codon is introduced: 12 (12TAG), 18 (18TAG) or 52 (52TAG). (B) Western blots of SUR1 (top) and Kir6.2 (bottom) from COSm6 cells co-transfected with SUR1 and Kir6.2-12 TAG, Kir6.2-18 TAG or Kir6.2-52 TAG, as well as the tRNA and tRNA synthetase necessary for read-through of the stop codon in the absence or presence of azidophenylalanine (AzF). Suppression of the amber stop codon in cells grown in medium containing AzF is evident by the presence of full-length Kir6.2 protein and the upper band of SUR1. Note the Kir6.2 mutant and SUR1 signals are much weaker than WT (see text for explanation). To avoid signal saturation, the WT control lanes were shown separately with a shorter exposure time. Numbers on the left of the blots indicate molecular mass markers in kD. (C) Functional analysis of channels formed by SUR1 and Kir6.2 with azidophenylalanine incorporated at amino acid position 12, 18 and 52 (Kir6.2Y12AzF, Kir6.2A18AzF, Kir6.2Q52AzF). Shown are representative inside-out patch-clamp recordings. All three mutant channels are functional as evidenced by currents that were inhibited by 1 mM ATP. A current trace of WT channels (WT SUR1 plus WT Kir6.2) is included for comparison. For WT and each of the mutants, 3-4 cells were recorded with similar results.

**Figure 6.** Pharmacological chaperone-dependent photocrosslinking of the Kir6.2 N-terminus with SUR1 in living cells. (A) Photocrosslinking of Kir6.2 amino acid 12 to SUR1 is enhanced by GBC and CBZ. Cells expressing WT channels (WT SUR1+WT Kir6.2) served as a negative control. Cells expressing WT SUR1 plus Kir6.2Y12AzF but treated with 0.1% DMSO overnight prior to cross linking served as a vehicle control for the effects of drugs. The crosslinked SUR1-Kir6.2 band detected by anti-SUR1 antibody (SUR1 Ab) is marked by the asterisk in this and subsequent blots (top blot). This crosslinked band was also recognized by an antibody against the first 18 amino acids of Kir6.2 (Kir6.2 N-ter Ab; bottom blot). Note some signals above the crosslinked SUR1-Kir6.2 band are present in the blot probed with Kir6.2 antibody and may represent Kir6.2 crosslinked to other unknown proteins that were co-purified with the KATP channel complex. (B) Bar graph showing quantification of averaged crosslinked SUR1-Kir6.2 band intensity as a fraction of total SUR1 intensity in DMSO, GBC, or CBZ treated cells (see Materials and Methods for quantification procedures for this figure and Fig.7). Each bar is the mean ± s.e.m. of four independent experiments. *p <0.001 by one-way ANOVA with Tukey’s post-test. (C) GBC and CBZ also enhanced photocrosslinking of Kir6.2A18AzF to SUR1 seen as the band recognized by both the anti-SUR1 antibody (top blot) and the anti-Kir6.2 N-ter antibody (bottom blot). (D) Kir6.2Y12AzF crosslinks to F27S-SUR1 only in cells treated overnight with 5 µM GBC to rescue the trafficking defect caused by F27S. Note no quantification of crosslinking was performed to compare the DMSO-treated versus drug-treated samples since little or no crosslinking occurred in DMSO-treated samples (n = 3).

**Figure 7.** Crosslinking of Kir6.2Q52AzF to SUR1 is not sensitive to GBC or CBZ. (A) Representative blot showing that photocrosslinking of Kir6.2Q52AzF to SUR1 occurred in DMSO treated cells as predicted, and GBC and CBZ treatment did not further enhance the extent of crosslinking. (B) Quantification of averaged crosslinked SUR1-Kir6.2 band intensity as a fraction of total SUR1 intensity in DMSO, GBC, or CBZ treated cells (mean ± s.e.m. of three independent experiments). There is no statistically significant difference between the groups. p > 0.05 by one-way ANOVA with Tukey’s post-test.
Figure 8. (A) Proposed mechanism of pharmacological chaperone rescue of $K_{ATP}$ channels. WT-SUR1 and Kir6.2 subunits co-assemble to form pre-$K_{ATP}$ channel complex (in brackets) which undergoes structural changes possibly with help from cellular chaperones resulting in coupling of SUR1 and Kir6.2 to form mature $K_{ATP}$ channels. Mutations in either SUR1 TMD0 or Kir6.2 result in a perturbed interface between SUR1 and Kir6.2 in pre-$K_{ATP}$ channel complex (small red patches), which expands to global misfolding/misassembly and results in ER-associated degradation (ERAD). Binding of pharmacological chaperones to mutant pre-$K_{ATP}$ channel complex lowers the energy barrier for structural rearrangements and promote channel maturation. (B) 3D structure of glibenclamide. Adapted from Yuriev et al. (58) with permission from the publisher. Hydrogens are omitted for clarity. Color coding: C, grey; O, red; N, blue; S, yellow; Cl, green. Left: Crystal structure. Middle: Lowest energy conformation resulting from the systematic conformational search in vacuo. Right: 3D conformer of carbamazepine, adapted from PubChem.
Figure 1
Figure 2
Figure 3

A

|     | WT | WT<sub>AAA</sub> | A116P<sub>AAA</sub> | V187D<sub>AAA</sub> |
|-----|----|-----------------|---------------------|--------------------|
| Kir6.2  |    |                 |                     |                    |
| SUR1    | +  | -               | -                   | -                  |
| Tubulin | V  | C               | C                   | C                  |

B

|     | WT | WT<sub>Q52R</sub> | WT<sub>V59G</sub> |
|-----|----|------------------|-------------------|
| Kir6.2  |    |                  |                   |
| SUR1    |    |                  |                   |
| Tubulin | V  | G                | C                 |

C

D

|     | WT | Y230A/S1238Y |
|-----|----|--------------|
| Kir6.2  |    |              |
| SUR1    | V  | G            | C                |
| Tubulin |     |              |                  |
Figure 4
Figure 5
Figure 6
Figure 7

A

|       | DMSO | GBC | CBZ |
|-------|------|-----|-----|
| UV    | -    | +   | -   | +   | -   | +   |
| WT    |      |     |     |     |     |     |
| Kir6.2Q52AzF |      |     |     |     |     |     |

SUR1 Ab

B

% Crosslinking

DMSO  GBC  CBZ

Figure 7
Figure 8
Structurally Distinct Ligands Rescue Biogenesis Defects of the K\textsubscript{ATP} Channel Complex via a Converging Mechanism
Prasanna K. Devaraneni, Gregory M. Martin, Erik M. Olson, Qing Zhou and Show-Ling Shyng

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