Inhibition of cardiac Kv4.3/KChIP2 channels by a sulfonylurea drug gliquidone
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Running title: gliquidone inhibits Kv4.3 currents

Number of pages: 18
Number of figures: 6
Number of suppl. figures: 1
Number of references: 43
Number of words: 6313

Abstract: 221
Introduction: 373 (with references)
Discussion: 872 (with references)

Keywords: Kv4.3; KChIP2; Ito; Gliquidone; Brugada syndrome

Abbreviations: BrS, Brugada syndrome; CAF, chronic atrial fibrillation; Ito, the transient
outward K⁺ current; K_ATP, ATP-sensitive potassium channels
Abstract

The Kv4.3 channel is featured of fast N-type inactivation and also undergoes a slow C-type inactivation. The gain-of-function mutations of Kv4.3 channels cause an inherited disease called Brugada syndrome characterized by a shortened duration of cardiac action potential repolarization and ventricular arrhythmia. The sulfonlurea drug glikudone, an ATP-dependent K⁺ channel antagonist, is widely used for the treatment of type 2 diabetes. Here, we report a novel role of glikudone in inhibiting Kv4.3 and Kv4.3/KChIP2 channels that encode the cardiac Ito currents responsible for the initial phase of action potential repolarization. Glikudone results in concentration-dependent inhibition of both Kv4.3 and Kv4.3/KChIP2 fast or steady-state inactivation currents with an IC₅₀ of approximately 8 μM. Glikudone also accelerates Kv4.3 channel inactivation and shifts the steady-state activation to a more depolarizing direction. Site-directed mutagenesis and molecular docking reveal that the residues S301 in the S4 and Y312A, L321A in the S4-S5 linker are critical for glikudone-mediated inhibition of Kv4.3 currents, as mutating those residues to alanine significantly reduces the potency for glikudone-mediated inhibition. Furthermore, glikudone also inhibits a gain-of-function Kv4.3 V392I mutant identified in BrS patients in voltage- and concentration-dependent manner. Taken together, our findings demonstrate that glikudone inhibits Kv4.3 channels by acting on the residues in the S4 and the S4-S5 linker. Therefore, glikudone may hold repurposing potential for the therapy of Brugada syndrome.

Significance Statement

We describe a novel role of glikudone in inhibiting cardiac Kv4.3 currents and the channel gain-of-function mutation identified from patients with Brugada syndrome, suggesting its repurposing potential for therapy for the heart disease.
Introduction

The transient outward K\(^+\) current (Ito) formed by the pore-forming \(\alpha\) subunit Kv4.3 and auxiliary subunit KChIP2 plays an essential role in the early phase of cardiac action potential repolarization (Abbott et al., 2007; Niwa and Nerbonne, 2010; Spitzer et al., 2006). Genetic studies demonstrate that gain-of-function mutations of Kv4.3 channels cause an inherited disease called Brugada syndrome (BrS) characterized by a shortened duration of action potential repolarization, ventricular arrhythmia and high incidence of sudden death (Antzelevitch, 2012; Giudicessi et al., 2011; Tomaselli, 2010). These observations suggest that the inhibition of overactive Ito currents may hold therapeutic potential for Brugada syndrome (Di Diego et al., 2020b; Kaufman, 2009; Minoura et al., 2013).

The gating of functional Kv4.3 channel current, encoded by \(KCND3\) gene, is characterized by fast activation at subthreshold membrane potentials and rapid inactivation (Zemel et al., 2018). Additionally, co-expression of auxiliary KChIP2 with Kv4.3 leads to a slow inactivation and an accelerated recovery from inactivation by sequestering both Kv4.3 N-terminal inactivation ball and the S6 helix (Ma et al., 2022; Wang et al., 2007), thus altering the channel pharmacology (Bett and Rasmusson, 2008).

Gliquidone, a second-generation sulfonylurea drug, is commonly used for the therapy of type 2 diabetes. Gliquidone promotes insulin release through inhibition of ATP-sensitive potassium (K\(_{ATP}\)) channels in the pancreatic islet \(\beta\) cells (Liu et al., 2015). In previous studies, several sulfonylurea receptor ligands, including nateglinide, glyburide and repaglinide were found to inhibit Kv channels including Kv1.5 and Kv4.3 or Kv4.3/KChIP3 in a concentration-dependent manner (Hu and Wang, 2001; Naranjo et al., 2016; Schaffer et al., 1999). It is notable that a sulfonylurea drug glyburide, the structural analog of gliquidone, was observed to inhibit the Ito current in human vascular and ventricular myocytes (Schaffer et al., 1999). Based on these literatures, we hypothesized that sulfonylurea drug gliquidone might also inhibit Kv4.3 channels.

To test this hypothesis, our study investigated the effects of gliquidone on Kv4.3 channels both alone and co-expressed with auxiliary subunit KChIP2. Our findings demonstrate that gliquidone inhibits wild-type Kv4.3 currents as well as gain-of-function
Kv4.3 mutant currents by binding to three key residues in the S4 and the S4-S5 linker. These results suggest that gliquidone may hold promise for development potential for therapy of Brugada syndrome.

Materials and Methods
Reagents and compounds
Gliquidone (TargetMol, USA) was dissolved in dimethyl sulfoxide (DMSO) to generate a 100 mM stock solution that was stored at -20°C before use. Prior to electrophysiological recordings, dilution to its final concentrations in bath solution was performed with the highest concentration of DMSO containing no more than 0.3%.

Cell culture and transfection
Human embryonic kidney (HEK) 293T cells were cultivated in DMEM medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, PAIN, Australia) and maintained at 37°C in a humidified atmosphere with 5% CO₂. The hKv4.3 stable-expressing HEK293T cells were further cultured with DMEM medium supplemented with 0.3 μg/ml purinomycin (HanBio, China). HEK293T cells were trypsinized and plated onto glass coverslips 24 h before transfection. For transfection, HEK293T cells were co-transfected with 0.6 μg human Kv4.3 cDNA (gene accession number NM_172198.3) or its mutants plus 1.8 μg rattus norvegicus KChIP2 (gene accession number XM_039088730.1) with 0.3 μg GFP as an indicator using Lipofectamine™ 2000 transfection agent (Invitrogen, Hong Kong, China) at a 1:1 ratio. All cDNAs were subcloned into the pcDNA3.1 vector. Patch-clamp recordings were performed 18 h after transfection.

Electrophysiological recordings
For whole-cell patch clamp recordings in HEK293T cells, currents were recorded at room temperature using the EPC 10 amplifier with PatchMaster software (HEKA Electronics, Harvard, USA). Patch pipettes were pulled from borosilicate glass using a DMZ universal electrode puller (Zeitz-Instruments GmbH, Germany) and fire-polished to achieve resistance of approximately 2–8 MΩ. The sampling frequency was set to 10 kHz, and the current signals were subjected to low-pass filtering at 2.9 kHz using a four-pole Bessel filter before
digitization and storage. The bath solution contained the following: 135 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 1 mM MgCl₂, 1.5 mM CaCl₂, and 10 mM glucose with pH adjusted to 7.4 using 5 M NaOH; the pipette solution consisted of: 135 mM potassium gluconate, 10 mM KCl, 10 mM HEPES, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM EGTA with pH adjusted to 7.3 using 1M KOH.

Molecular docking

Molecular docking for interaction between gliquidone and Kv4.3 cryo-EM structure (PDB ID code: 7W3Y) was performed using Schrödinger Glide. The 3D structure of gliquidone was obtained from the PubChem database. After docking, the pose score with the lowest binding energy of -7.493 was chosen to visualize ligand–protein interactions using MOE 2020 program.

Gliquidone was confined to four binding pockets that were predicted based on the parameters of distance and hydrophobicity between amino acids and the compound using the SiteMap mode of Schrödinger. As a positive control, the docking of Kv4.3 inhibitor acacetin into the Kv4.3 cryo-EM structure was also performed, and the resulting docking score for acacetin was -6.042.

Statistical analysis

Statistical analyses were conducted using Origin2021 (Origin Lab, USA). The activation curve and steady-state inactivation curves were fitted using the Boltzmann function: \( y = \frac{1}{1 + \exp(V - V_{1/2})/k} \), where \( V_{1/2} \) is the voltage for half-maximal activation or inactivation, \( V \) is the test potential and \( k \) represents the slope of the curve. The time constants for recovery from inactivation were fitted to a single exponential function: \( y = A_1 \exp(-t/\tau) + y_0 \).

Dose-response curves were fitted with a Hill equation: \( y = \text{START} + (\text{END} - \text{START}) \times x^n / (k^n + x^n) \). Statistical significance was determined using a paired Student’s \( t \)-test. For multiple comparisons between groups, data were analyzed using two-way ANOVA followed by Tukey test or one-way ANOVA with the Dunnett follow-up test with a threshold value of \( p < 0.05 \) to be considered statistically significant. All data are expressed as the mean ± standard deviation (SD).
Results

Concentration-dependent inhibition of Kv4.3 and Kv4.3/KChIP2 channels by gliquidone

We first investigated the effect of gliquidone at different concentrations on Kv4.3 channels stably expressed in HEK293T cells using the whole-cell patch clamp technique. Fig. 1A illustrate the Kv4.3 currents recorded in a representative cell in the absence or presence of various concentrations of gliquidone using a 500-ms voltage step to +50 mV from a holding potential of -80 mV. Gliquidone at 1-100 µM resulted in concentration-dependent inhibition of Kv4.3 peak currents measured at +50 mV (Fig. 1A, left panel). Further analysis for the dose-dependent inhibition of the area under curve (AUC) of Kv4.3 currents gave rise to an IC$_{50}$ value of 8.4 ± 1.5 µM and a hill coefficient at 1.7 ± 0.3 (Fig. 1A, middle panel). We also analyzed the fast inactivation kinetic of Kv4.3 currents elicited by a depolarizing potential at +50 mV, and gliquidone at 30 µM significantly accelerated the fast inactivation time about 5-fold to 9.6 ms from 42.8 ms (Fig. 1A, right panel and Table 1).

Since binding of auxiliary subunit KChIP2 changes Kv4.3 gating kinetics and pharmacological modulations. We then further tested the effect of gliquidone on Kv4.3/KChIP2 channel complexes. Similarly, gliquidone concentration-dependently inhibited Kv4.3/KChIP2 channel currents with an IC$_{50}$ value of 7.7 ± 1.2 µM and a hill coefficient at 2.2 ± 0.6. (Fig. 1B, left and middle panel). Gliquidone also accelerated the fast inactivation about 8-fold to 12.5 ms from 99.6 ms (Fig. 1B, right panel and Table 1).

We next examined if gliquidone-mediated inhibition of Kv4.3 currents is dependent on voltage. The current-voltage relationships of Kv4.3 and Kv4.3/KChIP2 channels were evaluated in the absence and presence of 30 µM gliquidone. Kv4.3 alone and Kv4.3/KChIP2 currents were elicited by a series of test potentials ranging from -80 to +50 mV from a holding potential at -80 mV (Fig. 2A and 2B, left panels). Gliquidone at 30 µM inhibits Kv4.3 (Fig. 2A, middle and right panels) and Kv4.3/KChIP2 peak currents (Fig. 2B, middle and right panels). The open channel blocking properties of Kv4.3 and Kv4.3/KChIP2 by gliquidone were also further analyzed by determining the time to peak and the current inactivation time constant in different depolarizing potentials. As shown in Fig. 2C and 2D,
gliquidone obviously reduced the time to peak and accelerated the channels inactivation.

**Rightward shift steady-state activation of Kv4.3 by gliquidone**

We further investigated the voltage dependence of the steady-state activation and inactivation of Kv4.3 and Kv4.3/KChIP2 channels by gliquidone. The normalized tail current amplitudes were plotted against depolarizing pulse potentials using the Boltzmann equation. As shown in Fig. 3A and 3B and Table 1, perfusion of 30 μM gliquidone caused a significant rightward shift of Kv4.3 and Kv4.3/KChIP2 activation to more depolarized membrane potentials at 10.6 ± 1.3 mV from -15.8 ± 0.9 mV (Kv4.3) and at 20.9 ± 14.1 mV from -5.7 ± 1.6 mV (Kv4.3/KChIP2). However, gliquidone had little shift on half-inactivation voltage (V_{1/2}) of Kv4.3 (-44.8 ±1.8 mV) and Kv4.3/KChIP2 channels (-35.3 ± 1.9 mV) from their V_{1/2} of -42.5 ± 1.8 mV and -32.9 ± 0.4 mV, respectively (Fig. 3C and 3D, Table 1). These results suggest that gliquidone may markedly altered the voltage-dependent activation of Kv4.3 channels.

**Gliquidone delaying the recovery from inactivation of Kv4.3/KChIP2 channels**

To examine the effect of gliquidone on Kv4.3 channels inactivation recovery, the cells were depolarized to +40 mV for 500 ms and then repolarized for a variable period before applying the second depolarizing pulse (Fig. 4A, insert panel). The time constants for recovery from inactivation of Kv4.3 currents and Kv4.3/KChIP2 currents were well defined by a single exponential equation. Gliquidone both increased the inactivation recovery time of Kv4.3 channel from 90.1 ± 3.2 ms to 147.5 ± 11.8 ms (Fig. 4A and Table 1), and Kv4.3/KChIP2 channel from 67.1 ± 4.7 ms to 105.3 ± 9.6 ms. (Fig. 4B and Table 1). These results indicate that gliquidone delayed the recovery from inactivation of Kv4.3 and Kv4.3/KChIP2.

**Concentration-dependent inhibition of gain-of-functional Kv4.3-V392I mutant by gliquidone**

The gain-of-function variant of Kv4.3 encoded by KCND3-V392I gene was known to cause sudden death in patients with BrS (Giudicessi et al., 2012). We also tested the inhibitory effect of gliquidone on this mutant channel. Gliquidone concentration-dependently inhibited
Kv4.3-V392I currents with an IC$_{50}$ value of 17.8±1.3 μM and a hill coefficient at 1.1 ± 0.1. (Fig. 5A). Similar with the effect on wildtype Kv4.3 channel, 30 μM gliquidone inhibited Kv4.3-V392I mutant channel currents, reduced the time to peak and accelerated inactivation at various voltages (Fig. 5B and 5C).

**Identification of residues in S4 and S4-S5 linker critical for Kv4.3 channel blockade by gliquidone**

To further identify residues critical for gliquidone binding to Kv4.3, we carried out the molecular docking of gliquidone onto the cryo-EM structure of human KCND3 (PDB: 7W3Y) using the Glide model of Schrödinger (Ma et al., 2022). The docking analysis predicts that gliquidone is confined to the pocket near the S4 and S5 segments, with binding score of -7.493 (Fig. 6A). Upon its binding, gliquidone is recognized by residues S301, Y312, L321, through several van der Waals forces (Fig. 6A, right panel, orange dotted line). As a positive control, we also performed the docking of acacetin into the channel structure. Acacetin is recently shown to inhibit the WT Kv4.3 and Kv4.3 gain-of-function V392I mutant (Ye et al., 2022). Acacetin is docked into the pocket formed by five residues T366, T367, V392, I395, and V399 in which two key residues T366, T367 are critical for interacting with acacetin (Supplemental Fig. S1), which is consistent with the recent observation (Wu et al., 2013).

To further confirm the three residues critical for the gliquidone binding obtained from the above docking, we made alanine mutations of S301A, Y312A and L321A. Whole-cell patch-clamp recordings confirmed that mutating these three residues resulted in a marked reduction of gliquidone-induced inhibition of Kv4.3 currents in concentration dependent manner with IC$_{50}$ values of 97.5 ±19.9 μM for S301A, 69.4 ± 8.5 μM for Y312A, and 168.8 ± 56.7 μM for L321A (Fig. 6B). Additionally, mutating those residues also attenuated gliquidone-induced acceleration on channel inactivation as compared with WT (Fig. 6C). All together, these results demonstrate that the three residues 301, 312 and 321 in the S4 and S4-S5 linker are critical for forming noncovalent interactions with gliquidone and mediating the channel inhibition by gliquidone.
Discussion

Pharmacological blockade of Kv4.3 channel prolongs ventricular action potential duration, which is considered a promising strategy for treatment of cardiovascular diseases, such as chronic atrial fibrillation (CAF) (de Haan et al., 2006; Gómez et al., 2008; Wu et al., 2013), Brugada syndrome (BrS) (Li et al., 2020; Ye et al., 2022; You et al., 2015), and early repolarization syndrome (ERS) (Ye et al., 2022).

Our findings reveal a previously unknown role of gliquidone in the inhibition of Kv4.3 and Kv4.3/KChIP2 channels by functional interaction with the voltage sensor of the channel. The inhibition of Kv4.3 channel by gliquidone is featured with the following: 1) gliquidone inhibits both Kv4.3 and Kv4.3/KChIP2 current in a concentration- and voltage-dependent manner; 2) gliquidone accelerates both Kv4.3 and Kv4.3/KChIP2 channel inactivation, which is common for some Kv4 channel blockers, such as quinidine (Wang et al., 1995; Zhang et al., 2019), flecanide (Wang et al., 1995), nicardipine (Hatano et al., 2003) and diltiazem (Caballero et al., 2004); 3) gliquidone shifts the steady-state activation curve of Kv4.3 and Kv4.3/KChIP2 currents in a depolarizing direction, which is similar to several other Kv4.3 inhibitors such as acacetin and neferine that were reported to show potential therapeutic for J wave syndromes and BrS (Di Diego et al., 2020a; Wang et al., 2015). A right-shift in the steady-state activation of Kv4.3 currents causes a delayed reactivation of the channel upon gliquidone binding, which means the channel is more difficult to open in the presence of gliquidone (Wang et al., 2015); 4) Gliquidone has no significant effect on both Kv4.3 and Kv4.3/KChIP2 steady-state inactivation with about 2 mV leftward shift, which is also similar to many Kv4.3 inhibitors, including acacetin ($V_{1/2}$: 3 mV left-shift) (Wu et al., 2013), nicardipine ($V_{1/2}$: 5 mV left-shift) (Hatano et al., 2003) and neferine ($V_{1/2}$: 7 mV left-shift) (Wang et al., 2015); 5) gliquidone also prolongs the recovery from inactivation of both Kv4.3 and Kv4.3/KChIP2 channels, which is consistent with most of Kv4.3 channel blockers.

It is known that the auxiliary subunit KChIP2 increases cardiac Kv4.3 current density and modulates the channel gating by interacting with Kv4.3 N-terminal inactivation ball and C-terminal helix for slowing down inactivation and speeding up recovery from inactivation (Birnbaum et al., 2004; Kise et al., 2021; Ma et al., 2022). In this study, gliquidone inhibits
both Kv4.3 channel alone and the Kv4.3/KChIP2 channel complex with no obvious different kinetics, suggesting gliquidone mainly affects the α-subunit Kv4.3 but not β-subunit KChIP2. The site-directed mutagenesis confirms that the three residues S301 in the S4 and Y312, L321 in the channel S4-S5 linker are important for gliquidone-mediated Kv4.3 inhibition. These findings are consistent with the observations that the four-helical bundle voltage sensing S1-S4 domains (VSDs) and the S4-S5 linker of voltage gated channels are important pharmacological targets for their binding with small molecules and peptide toxins (Abbott, 2020; Ahuja et al., 2015; Dehong et al., 2022; DeSimone et al., 2009; Montero-Domínguez et al., 2022; Xu et al., 2019).

It is well known that the Ito current contributes to the initial phase of rapid repolarization of cardiac action potentials in human and canine hearts (Akar et al., 2004; Dixon et al., 1996). Arrhythmic Brugada syndrome, characterized by loss of the action potential dome at the right ventricular subepicardium, is linked to gain-of-function mutations that result in an overactive Ito current (Brugada et al., 2018; Corrado et al., 2018). This indicates that blocking overactive Ito can suppress the electrocardiographic and arrhythmic manifestations in BrS (Di Diego et al., 2020a). Studies have demonstrated that Ito inhibitors 4-AP, quinidine can restore the epicardial action potential dome, decrease transmural and epicardial dispersion of repolarization, normalize ST segments, and prevent phase 2 reentry and ventricular tachycardia/fibrillation in experimental canine models of Brugada syndrome (Di Diego et al., 2020b; Yan and Antzelevitch, 1999). These findings suggest that Ito blockade holds promise as a therapeutic strategy for managing ventricular arrhythmias associated with Brugada syndrome. While gliquidone is primarily recognized as a second-generation hypoglycemic sulfonylurea that promotes insulin release from pancreatic β-cells and effectively lowers blood glucose levels, its potential impact on cardiac benefit has not been explored. Nevertheless, gliquidone's potential as a therapeutic agent for Brugada syndrome is intriguing based on our in vitro electrophysiological findings. It's important to note that further comprehensive evaluation is essential before considering application of gliquidone in clinical settings.

In conclusion, our findings demonstrate that the sulfonylurea drug gliquidone exerts
inhibitory effects on Kv4.3 and Kv4.3/KChIP2 currents expressed in HEK293T cells. This inhibition is attributed to its interaction with specific residues: S301 in the S4 and Y312 and L321 within the S4-S5 linker of Kv4.3 channel. Gliquidone also effectively inhibits the current of gain-of-function Kv4.3-V392I mutant identified from patients with Brugada syndrome. The identification of gliquidone as a novel Kv4.3 inhibitor underscores its repurposing potential for new treatment of Brugada syndrome.

**Acknowledgements:** This study was supported by grants from the National Natural Science Foundation of China (81973299 for K. W. and 82104149 for Y. L.), and the National Natural Science Foundation of Shandong Province (ZR2020QH100 for Y.L., and ZR2022MC049 for F.H.)

**Data availability statement:** The authors declare that all the data supporting the findings of this study are contained within the paper and its Supplemental Data.

**Conflict interest statement:** No author has an actual or perceived conflict of interest with the contents of this article.

**Authorship contribution:**
Participated in research design: Wang KW, Liu YN and Hu F.
Conducted experiments: Yang CX and Li QQ.
Performed data analysis: Yang CX, Li QQ, and Liu YN.
Wrote the writing of the manuscript: Yang CX, and Liu YN.
Finalized the manuscript: Wang KW.
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Legends for Figures

Figure 1. Concentration-dependent inhibition of Kv4.3 and Kv4.3/KChIP2 currents by gliquidone.

(A) Left panel, a concentration-dependent inhibition of Kv4.3 peak currents stably expressed in HEK293T cells in response to a 500-ms test pulse from -80 to +50 mV (inset) in the absence or presence of different concentrations (1, 3, 10, 30 and 100 μM) of gliquidone in whole-cell patch clamp recordings. Middle panel, a concentration-dependent inhibition for the area of under curve (AUC) of whole-cell Kv4.3 currents with an IC$_{50}$ value of 8.4 μM obtained from curve fitting to the Hill equation (n=6-15). Right panel, a comparison for Kv4.3 current inactivation time constants recorded at +50 mV in the absence or presence of 30 μM gliquidone (***$p<0.05$, vs control, by paired student’s $t$ test).

(B) Left panel, a representative whole-cell recording of Kv4.3/KChIP2 channel complex currents elicited by a 1000-ms depolarizing pulse +50 mV from a holding potential of -80 mV in the absence or presence of gliquidone at different concentrations. Middle panel, a concentration-dependent inhibition of Kv4.3 currents with an IC$_{50}$ value of 7.7 μM (n=4-8). Right panel, comparison for inactivation time constants of Kv4.3/KChIP2 currents before and after 30 μM gliquidone (***$p<0.05$, vs control, by paired student’s $t$ test). All data were expressed as the means ± SD.

Figure 2. Inhibitory properties of Kv4.3 and Kv4.3/KChIP2 currents by gliquidone.

(A) Representative current traces recorded in HEK293T cells stably expressing Kv4.3 channels in response to a family of pulses from -80 mV to +50 mV with an increase of 10 mV (inset) before (left panel) and after (middle panel) gliquidone at 30 μM. Right panel, comparison of peak currents before (black) and after (red) gliquidone (n=5, ***$p<0.001$, **$p<0.01$, *$p<0.05$ vs. control, by two-way ANVOA).

(B) Representative traces of whole-cell Kv4.3/KChIP2 currents before (left panel) and after (middle panel) of gliquidone. Right panel, comparison of peak currents before and after gliquidone (n=6, ***$p<0.001$, **$p<0.01$, *$p<0.05$ vs. control, by two-way ANVOA).
(C) Left panel, expanded current traces of Kv4.3 inhibition at +10 mV before and after 30 μM gliquidone. Summary for the time to peak (middle panel) and inactivation time constants (right panel) of Kv4.3 currents at different voltages ranging from 0 mV to +50 mV before and after gliquidone (n = 5, ***p < 0.001, * p < 0.05 vs. control, by two-way ANOVA).

(D) Left panel, an expanded traces of Kv4.3/KChIP2 currents at +10 mV before and after 30 μM gliquidone. Summary for Kv4.3/KChIP2 inhibition time to peak (left panel) and inactivation time constants (right panel) at different voltages ranging from 0 mV to +50 mV before and after gliquidone (n = 5, ***p < 0.001, * p < 0.05 vs. control, the statistical significance analyzed by two-way ANOVA). All data were expressed as the means ± SD.

Figure 3. Rightward shift of voltage-dependent activation of Kv4.3 and Kv4.3/KChIP2 currents by gliquidone.

(A) Representative traces of Kv4.3 currents elicited by voltage steps between -80 and 60 mV in 10 mV increments from a holding potential at -80 mV in the absence (left panel) and presence of 30 μM gliquidone (middle panel). Right panel, comparison for voltage-dependent activation of normalized tail currents at -50 mV before (black) and after (red) gliquidone after fitting with Boltzmann function (n=4-6).

(B) Representative traces of Kv4.3/KChIP2 currents in the absence (left panel) and presence of 30 μM gliquidone (middle panel). Right panel, comparison for voltage-dependent activation of normalized tail currents at -50 mV before (blue) and after (red) gliquidone after fitting to the Boltzmann function (n = 4-6).

(C) Representative traces of Kv4.3 currents in response to pre-pulses at different voltages between -110 and +20 mV before the pulse at +40 mV in the absence (control, left panels) and presence of 30 μM gliquidone (middle panels). Right panel, comparison for voltage-dependent inactivation of the normalized currents (I/Imax) before and after 30 μM gliquidone after fitting to the Boltzmann function (n=6).

(D) Representative traces of Kv4.3/KChIP2 currents in response to prepulses at different voltages before the pulse at +40 mV in the absence (control, left panels) and presence of 30 μM gliquidone (middle panels). Right panel, comparison for voltage-dependent inactivation
of the normalized post-pulse currents (I/I_{max}) before and after 30 μM gliquidone after fitting to the Boltzmann function (n=6). All data are expressed as the means ± SD.

**Figure 4. Prolongation of time recovery from inactivation of Kv4.3 and Kv4.3/KChIP2 currents by gliquidone.**

(A) Representative traces of Kv4.3 currents recorded using double-pulse protocols consisting of two depolarizing pulses to +40 mV lasting 500 ms for the first pulse and 300 ms for the second pulse at a variable duration (5-10340 ms) between the two depolarizing steps at −80 mV for recovery, in the absence (left panel) and presence of gliquidone (middle panel). Right panel, time recovery from inactivation curves were fitted with a single exponential function by normalizing the current elicited from the second test pulse to the first conditioning pulse (n=3-5).

(B) Representative traces of Kv4.3/KChIP2 currents recorded using double-pulse protocols consisting of two depolarizing pulses to +40 mV lasting 500 ms for first pulse and 300 ms for second pulse, and a variable duration step between two depolarizing steps at −80 mV (5-10340 ms) for recovery in the absence (left panel) and presence of gliquidone (middle panel). Right panel, time recovery from inactivation was determined by normalizing the current elicited from the second test pulse to the first conditioning pulse and was fitted with a single exponential function (n=3-5). All data are expressed as the means ± SD.

**Figure 5. Inhibition of gain-of-function Kv4.3-V392I mutant currents by gliquidone.**

(A) Left panel, a representative whole-cell recording of Kv4.3-V392 channel currents elicited by a 500-ms depolarizing pulse +50 mV from a holding potential of -80 mV in the absence or presence of gliquidone at different concentrations. Right panel, a concentration-dependent inhibition of Kv4.3-V392I currents at +50 mV with an IC_{50} value of 17.8 μM obtained from the curve fitting to the Hill equation (n=9). (B) Representative whole-cell Kv4.3-V392I current traces recorded from HEK293T cells in response to a family of pulses (inset) before (left panel) and after (middle panel) gliquidone. Right panel, current-voltage curves from left and middle panels for comparison of peak currents before (green) and after (red) gliquidone.
(n=6, ***p< 0.001, **p< 0.01, vs. control, by two-way ANOVA). (C) Summary for the time to peak (left panel) and inactivation time constants (right panel) of Kv4.3 currents from 0 mV to +50 mV before and after gliquidone (n = 5, ***p< 0.001, *p< 0.05 vs. control, the statistical significance analyzed by two-way ANOVA). All data are expressed as the means ± SD.

Figure 6. Identification of residues critical for gliquidone binding to Kv4.3 channel

(A) Representative conformations of gliquidone confined to the pocket consisting of F298, S301 in the voltage sensor domain S4 and Y312 and L321 in the S4-S5 linker in side view (left panel) and top-down view (middle panel). Gliquidone is shown in green. Right panel, side view of gliquidone (green) and Kv4.3 subunit interaction.

(B) A comparison for the concentration-dependent inhibition (AUC) of WT and mutant Kv4.3 currents by gliquidone after fitting with Hill equation.

(C) Summary for normalized inactivation time constants (τGliquidone/τControl) of WT and mutant Kv4.3 at +50 mV after 30 μM gliquidone (n=5, *p < 0.05, ***p < 0.001 vs. WT, the statistical significance analyzed by one-way ANOVA with the Dunnett follow-up test).

Table 1. Effect of Gliquidone on Channel Gating

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<th></th>
<th>Kv4.3</th>
<th>Kv4.3/KChIP2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Gliquidone*</td>
</tr>
<tr>
<td>Time to peak (ms)#</td>
<td>2.4±0.1</td>
<td>1.6±0.1</td>
</tr>
<tr>
<td></td>
<td>n =5</td>
<td>n =5</td>
</tr>
<tr>
<td>Inactivation τ (ms)#</td>
<td>42.8±1.2</td>
<td>9.6±6.0</td>
</tr>
<tr>
<td></td>
<td>n =5</td>
<td>n =5</td>
</tr>
<tr>
<td>Voltage-dependent activation $V_{1/2}$ (mV)</td>
<td>-15.8±0.9</td>
<td>10.6±1.3</td>
</tr>
<tr>
<td></td>
<td>n=4-6</td>
<td>n=4-6</td>
</tr>
<tr>
<td></td>
<td>Voltage-dependent inactivation</td>
<td>Recovery from inactivation</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td>( V_{1/2} ) (mV)</td>
<td>( \tau ) (ms)</td>
</tr>
<tr>
<td></td>
<td>(-42.8\pm1.8)</td>
<td>(90.1\pm3.6)</td>
</tr>
<tr>
<td></td>
<td>(-44.8\pm1.8)</td>
<td>(147.5\pm11.8)</td>
</tr>
<tr>
<td></td>
<td>(-32.9\pm0.4)</td>
<td>(67.1\pm4.7)</td>
</tr>
<tr>
<td></td>
<td>(-35.3\pm1.9)</td>
<td>(105.3\pm9.6)</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=3-5)</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=3-5)</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=4)</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=4)</td>
</tr>
</tbody>
</table>

#: test potential at +50 mV; Gliquidone*: gliquidone in 30 μM; Values are the means ± SD.
Figure 3

A

Kv4.3

Control

30 μM Gliquidone

1 nA

10 ms

+60 mV

-80 mV

-50 mV

30 μM Gliquidone, \( V_{1/2} = 10.6 \pm 1.3 \text{ mV} \)

-100 -80 -60 -40 -20 0 20 40 60 80

Voltage (mV)

Normalized Current

B

Kv4.3/KChIP2

Control

30 μM Gliquidone

1 nA

10 ms

-100 -80 -60 -40 -20 0 20 40 60 80

Voltage (mV)

Normalized Current

C

Kv4.3

Control

30 μM Gliquidone

0.5 nA

250 ms

-80 mV

500 ms

1000 ms

+40 mV

-110 mV

30 μM Gliquidone, \( V_{1/2} = 44.8 \pm 1.8 \text{ mV} \)

-120 -100 -80 -60 -40 -20 0 20

Voltage (mV)

Normalized Current

D

Kv4.3/KChIP2

Control

30 μM Gliquidone

0.5 nA

250 ms

30 μM Gliquidone, \( V_{1/2} = 35.3 \pm 1.9 \text{ mV} \)

-120 -100 -80 -60 -40 -20 0 20

Voltage (mV)

Normalized Current
Supplemental figure 1 and legend

Inhibition of cardiac Kv4.3/KChIP2 channels by a sulfonylurea drug gliclazide
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Journal title: Molecular Pharmacology
Manuscript number: MOLPHARM-AR-2023-000787
Supplemental Fig. S1. Molecular docking of acacetin to the Kv4.3 channel
(A) A representative bound conformations of acacetin confined to its binding pocket consisting of two key residues, T366 and T367 from the P-loop and A396 in S6 in side view (left panel) and top-down view (right panel) with acacetin shown in red.
(B) A side view for acacetin (red) and a Kv4.3 subunit interaction within the binding pocket formed by the P-loop and the S6.