Supplementary Figure 1. Synthetic scheme and purity of KCN1 3,4-dimethoxy-N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]-N-phenylbenzenesulfonamide. A) Starting from 4-hydroxybenzaldehyde (1), CuI mediated O-alkylation gave ether (2), which underwent Claisen rearrangement upon reflux in o-xylene. Then reductive amination converted aldehyde (3) to a secondary amine (4). Compound (4) reacted with 3,4-dimethoxy-benzenesulfonyl chloride in methylene chloride to give the final sulfonamide compound KCN1. KCN1 was purified and the structure was confirmed by UV, IR, MS and NMR spectroscopy. The purity of the compound was determined to be greater than 99%. B) High-performance liquid chromatography (HPLC) analysis of the KCN1 batch used in this study.

Supplementary Figure 2. KCN1 is chemically stable in cell culture medium under normoxic or hypoxic conditions. A) Analysis of KCN1 in cell culture medium by HPLC. KCN1 was diluted in cell culture medium (25 μM final concentration), added to LN229 cells, and incubated under normoxic or hypoxic (1% O₂) conditions at 37° C for indicated period of time. Medium (1 ml) was extracted with n-octanol (100 μl) and the organic phase was analyzed by HPLC. B) The peaks for KCN1 were used to establish standard curves and for quantitative analysis of samples. Quantification of the compound was achieved by determination of the peak area at the retention time of approximately 4.9 minutes.

Supplementary Figure 3. Effect of KCN1 in the NCI-60 Tumor Cell Line Screen (http://dtp.cancer.gov<http://dtp.cancer.gov/). Proliferation of the human tumor cell lines of the cancer screening panel in the presence of 10 μM KCN1 was performed by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute.
The growth percent column shows growth inhibition (%) of the untreated control (100%) for each cell line. Mean graph is generated by subtracting mean inhibition across the whole panel from inhibition of individual cell lines; bars to the right (negative values) indicate cell lines more sensitive to KCN1 than mean, bars to the left (positive values) indicate cell lines less sensitive to KCN1 than mean (Shoemaker R.H.: The NCI60 human tumor cell line anticancer drug screen. Nature Rev. Cancer, 813-823, 2006).

**Supplementary Figure 4. KCN1 inhibits the growth of pre-established human glioblastoma s.c. xenografts.** Nude mice (18 total) were implanted with LN229HRE-Luc/LacZ cells (s.c. in the flanks; 2 tumors/mouse). In week 3, when palpable tumors were formed, mice were randomly separated into the control and KCN1 group (9 mice each) and i.p. treatment started 5 days per week with 60 mg/kg KCN1 in 1:1 ethanol/cremophor (controls vehicle only). Average tumor size in each group (+S.E.) is shown. ** - p value <0.01

**Supplementary Figure 5. Effect of KCN1 treatment on mice weight.** KCN1 treatment was well tolerated and mice did not evidence any signs of extraneous toxicity. Average mouse body weights (g) in vehicle- and KCN1-treated groups corresponding to the experiment in Fig. 3B were measured over a period of 10 weeks. During the course of the experiment mice retained normal activity and bodily appearance and no behavioral abnormalities were observed.

**Supplementary Figure 6. Chronic systemic KCN1 administration shows little effect on major organs, except liver.** Organs from vehicle- and KCN1-treated mice of experiment in Fig. 3B were recovered at termination (week 10), formalin fixed, sectioned, and stained by H & E.
Pathological examination of main organs demonstrated no ultra-structural changes in brain, kidney, GI tract and lung. A treatment-related change was observed in the liver, where swelling was observed at autopsy and pathology shows tissue edema with bile duct stasis, yet without evidence of any hepatocyte death. The swelling was reversible within 2-3 weeks after treatment was discontinued (data not shown).

Supplementary Figure 7. Effect of KCN1 on the survival of mice with orthotopic (intracranial) brain tumor models.

A) Kaplan Meier survival analysis of mice with intracranial LN229 malignant gliomas with/without systemic KCN1 treatment. The survival curve shown is the combined result of three independent experiments using two different LN229 clones (CMV-Luc and HRE-Luc/LacZ). The first two experiments were conducted with 9 mice/group (CMV-Luc and HRE-Luc/LacZ) and the third (CMV-Luc) with 20 mice/group. All three experiments showed the same initial survival trend.

nu/nu athymic nude were intracranially inoculated with 1x10^6 LN229 cells (=day 0), and KCN1 i.p. treatment (60 mg/kg i.p. in cremophor:ethanol formulation; 5 days/week), started on day 10. The mice in the control groups were treated with vehicle alone. Logrank: p=0.1010.

B) Statistical analysis of the Kaplan-Meier survival data of experiment A)

The p value of differences in survival in the control and treated groups at days 10 to 70 are shown. Note the initial statistically significant survival in the KCN1 treated group between days 41 and 48. The logrank test was used to test the difference in the survival times of the control and treated groups. The significance levels were set at 0.05 for all tests. The SAS statistical
package V9.2 (SAS Institute, Inc., Cary, North Carolina) was used for data management and analyses.

C) Kaplan Meier survival analysis of mice with intracranial B16LS9 malignant melanoma with/without systemic KCN1 treatment. C57BL/6 mice (12 in the control group and 13 in the KCN1-treated group) were intracranially inoculated with 5x10^4 B16LS9 cells (=day 0) and treated with KCN1 as above starting on day 1. All mice were terminated at day 10 as they met the IACUC guidelines for euthanasia. Logrank: p=0.4707.

Supplementary Figure 8. Permeability coefficients (PC) of KCN1 calculated from the transfer across monolayers of brain endothelial cells. Transport of radiolabeled mannitol and KCN-1 across cell monolayers in the apical to basolateral direction was determined by counting the samples in a Beckman LS 6500 liquid scintillation counter (Beckman Instruments, Inc., Fullerton, CA). Apical-to-basolateral permeability across monolayer was expressed as a PC (centimeters per minute), calculated from the following equation:

PC= dQ/dt X 1/A X C_o where dQ/dt is the flux of tracer across the cell monolayer (dpm/min), A is the transport surface area (1.2 cm^2 for 12-well Transwell), and C_o is the original donor concentration (dpm/ml).