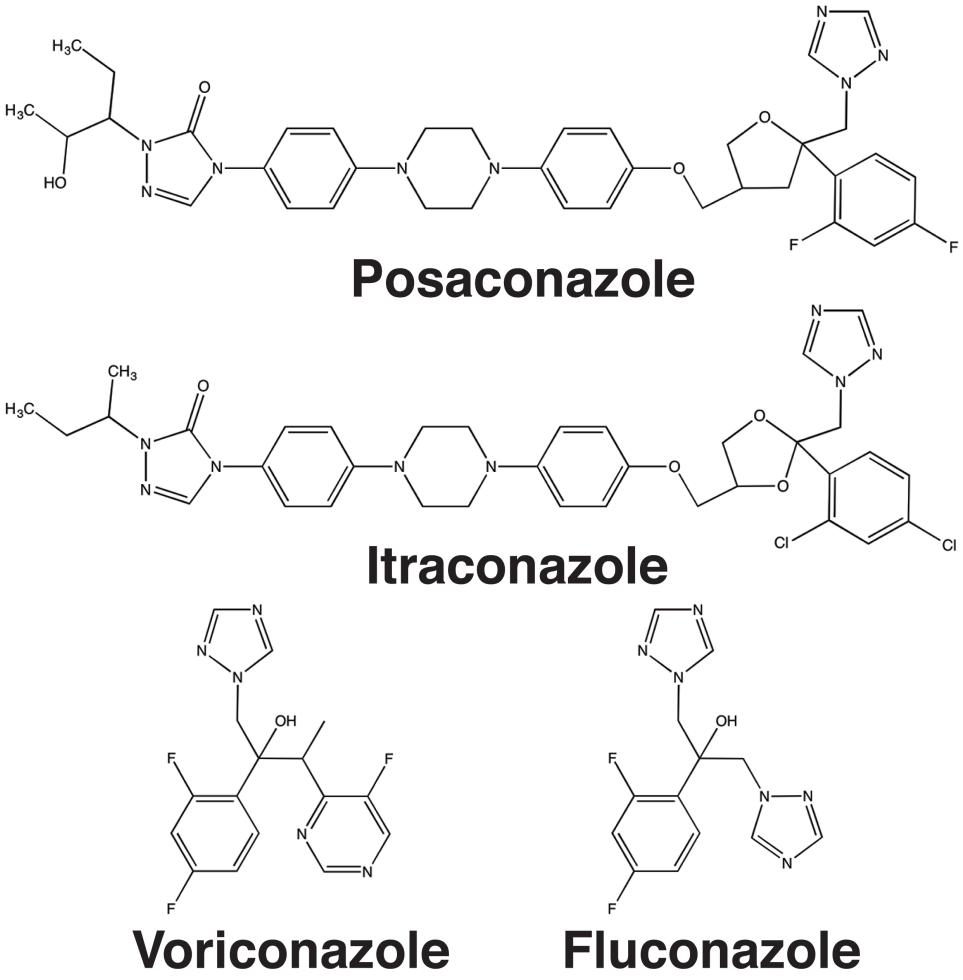
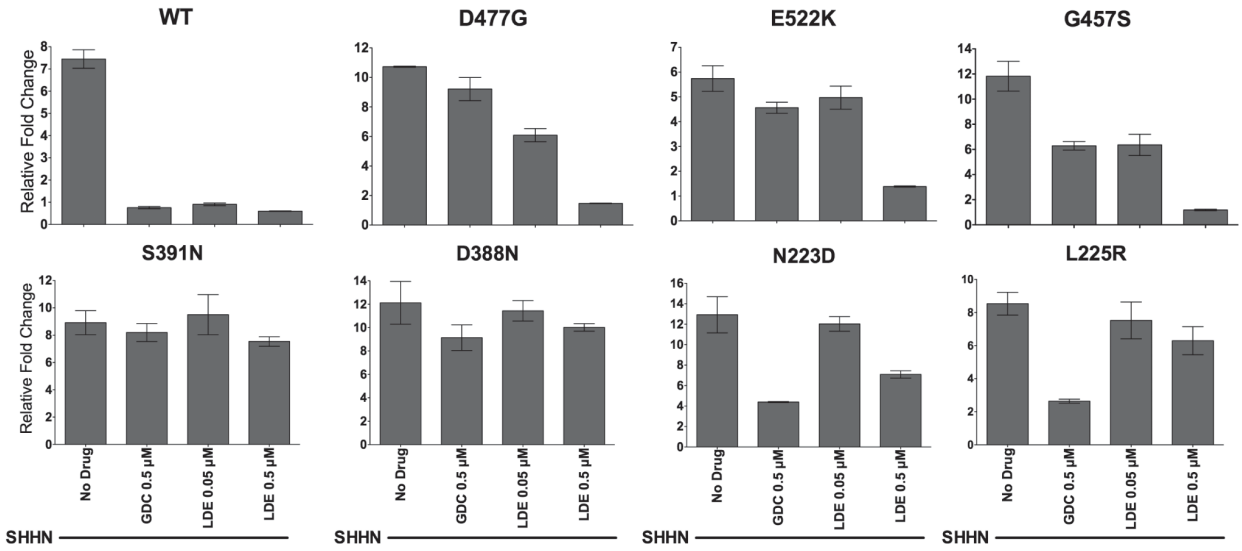
SUPPLEMENTAL INFORMATION

Posaconazole, a second-generation triazole antifungal drug, inhibits the Hedgehog signaling pathway and progression of basal cell carcinoma.

Baozhi Chen, Vinh Trang, Alex Lee, Noelle S. Williams, Alexandra N. Wilson, Ervin H. Epstein Jr., Jean Y. Tang, and James Kim



Supplementary Figure S1. Structures of Triazole Antifungal Drugs



Supplementary Figure S2. Verification of Drug-resistance of SMO Mutants. Signaling assays of *Smo*$^{-/-}$ fibroblasts transfected with 8x-Gli luciferase reporter and either wild-type or mutant Smo constructs resistant to vismodegib (GDC-0449) or sonidegib (LDE225) and stimulated with SHHN CM. Vismodegib (GDC) or sonidegib (LDE) had no inhibitory effect on their respective drug-resistant SMO variants. Vismodegib-resistant SMO are represented by D477G and E522K. All other SMO mutants are resistant to sonidegib. 'WT' = Wild type SMO. Data represents mean of triplicates +/- S.D.

Supplementary Table S1. Murine Serum Pharmacokinetic Parameters

Parameter	Posa 30 mg/kg	Posa 60 mg/kg
$t_{1/2}$	17.7 hr	32.0 hr
C_{max}	16.1 μg/ml	15.9 μg/ml
AUC_{0-24h}	244.7 μg*hr/ml	288.5 μg*hr/ml

$t_{1/2}$: Half life; C_{max}: Maximum drug concentration; AUC_{0-24h}: Area under the curve from 0 to 24 hours

SUPPLEMENTARY METHODS

In Vivo Pharmacokinetic Study

All studies were approved by and conformed to the policies and regulations of Institutional Animal Care and Use Committees (IACUC) at UTSW. Mice were maintained in a standard facility and conditions of a 12 h light/dark cycle, temperature and humidity at University of Texas Southwestern (UTSW). CD-1 mice (6 weeks old) were given 1 dose of posaconazole (Noxafil, Merck) 30 mg/kg or 60 mg/kg by oral gavage. Posaconazole was diluted with sterile water to the appropriate concentrations. Drinking water was acidified (pH 2.5) starting 24 hours prior to the first dose as human data suggested this improved posaconazole absorption (1). Mice were sacrificed at various time points and whole blood isolated with acidified citrate dextrose as an anticoagulant. Plasma was isolated by centrifugation, treated with methanol containing 0.15% formic acid and 150 ng/ml tolbutamide internal standard in a 1:2 ratio (plasma:methanol), and subsequent supernatant used for posaconazole measurement. Standard curves were prepared by addition of posaconazole to commercial mouse plasma (Bioreclamation, Westbury, NY). A value of 3x above the signal obtained in blank plasma was designated the limit of detection (LOD). The limit of quantitation (LOQ) was defined as the lowest concentration at which back calculation yielded a concentration within 20% of the theoretical value and above the LOD signal. The LOQ value for posconazole was 1 ng/ml. An AB Sciex (Framingham, MA) 3200 Qtrap mass spectrometer coupled to a Shimadzu (Columbia, MD) Prominence LC was used. Posaconazole was detected in multiple reaction monitoring mode by following the precursor to fragment ion transition 701.5 to 127.2. A 271.2 to 91.2 transition was followed for the IS. An Agilent (Santa Clara, CA) XDB C18 chromatography column (50 X 4.6 mm, 5 micron packing) was used as follows: Buffer A: dH20 + 0.1% formic acid, Buffer B: methanol + 0.1% formic acid, 0 – 1. 0 min 3% B, 1.0 – 1.5 min gradient to 100% B, 1.5 – 3.0 min 100% B, 3.0 to 3.1 min gradient to 3% B, 3.1 to 4.1 min 3% B. Pharmacokinetic parameters were calculated using the noncompartmental analysis tool in Phoenix WinNonlin (Certara Corp, Princeton, NJ.)

SUPPLEMENTARY REFERENCES

1. Krishna G, Moton A, Ma L, Medlock MM, McLeod J. Pharmacokinetics and absorption of posaconazole oral suspension under various gastric conditions in healthy volunteers. Antimicrob Agents Chemother. 2009;53:958-66.