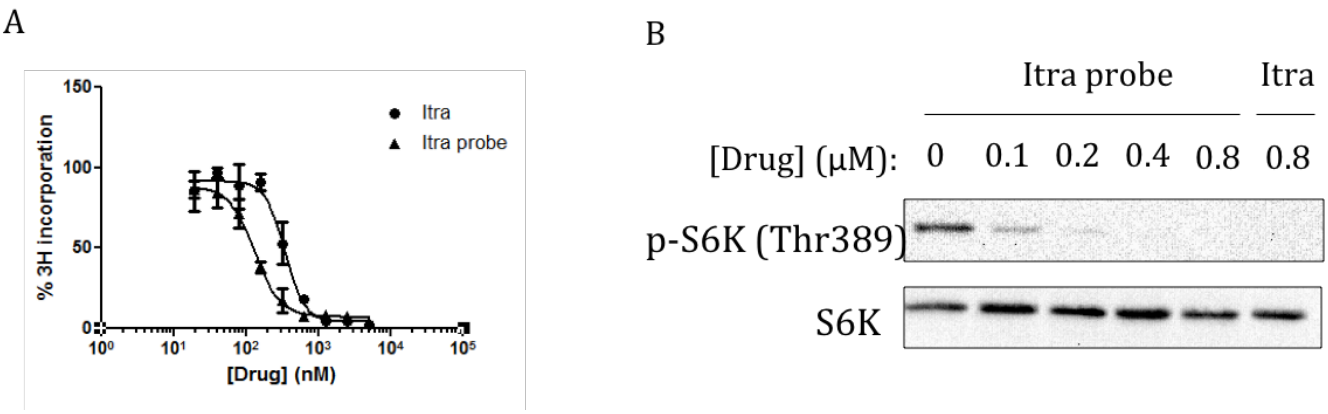
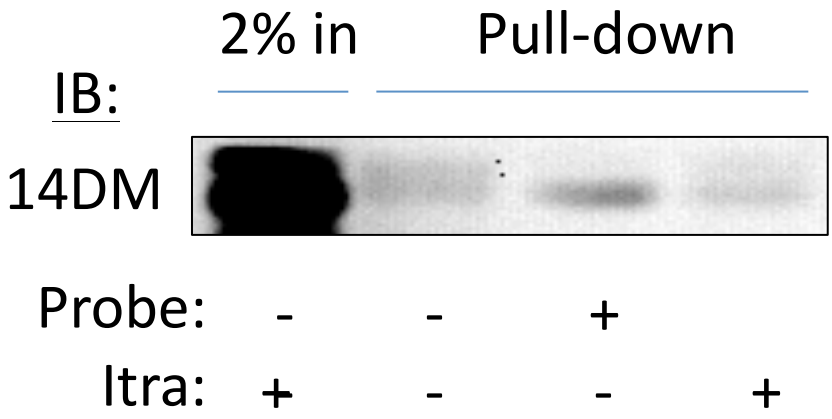
SI Appendix

Fig. S1. Itraconazole photoaffinity probe is active in HUVEC.



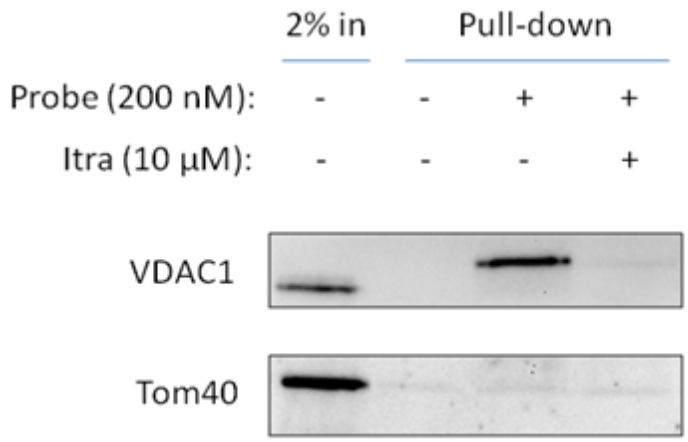
(a) The itraconazole probe was tested side-by-side with itraconazole for inhibition of HUVEC proliferation in the thymidine incorporation assay. The two compounds were found to have similar dose-response curves for proliferation inhibition. **(b)** HUVEC were treated with itraconazole or probe at the indicated concentrations for 24 hours. Both compounds were found to inhibit mTOR activity as measured by S6K phosphorylation.

Fig. S2. Itraconazole probe labels small amounts of 14-alpha demethylase.



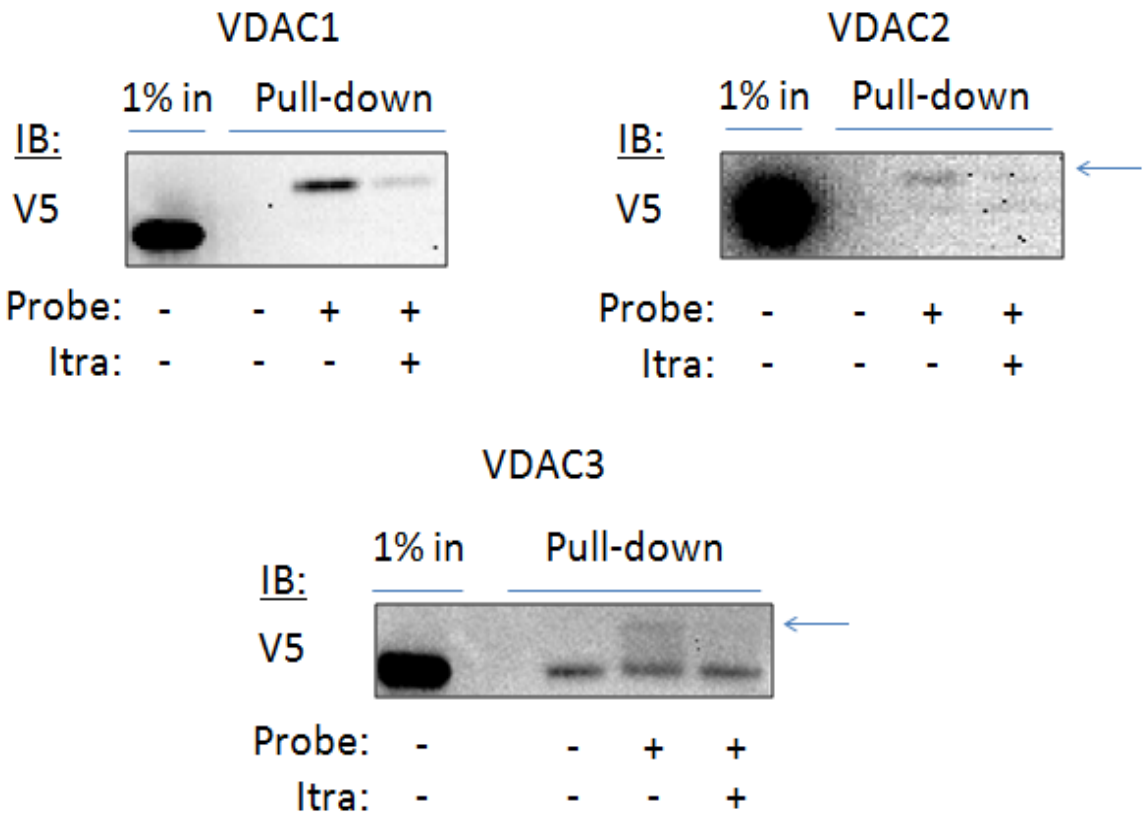
The itraconazole probe labels 14-alpha demethylase (14DM) in HUVEC, as detected after biotin pull-down and western blot with a 14DM antibody. However the signal in the probe sample is very weak compared with the input fraction, indicating that the amount of 14DM bound by the itraconazole probe is minimal.

Fig. S3. Binding of the itraconazole probe is specific to VDAC1 over Tom40.



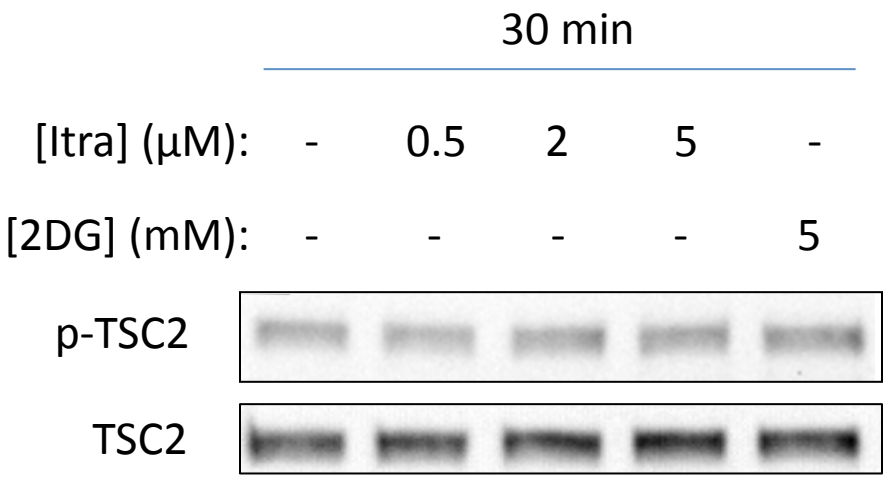
Biotin pull-down and western blot with antibodies against VDAC1 and another β-barrel protein of the outer mitochondrial membrane, Tom40, shows that the binding of the itraconazole probe is specific to VDAC1.

Fig. S4. Itraconazole probe predominantly labels VDAC1 over VDAC2 and 3.



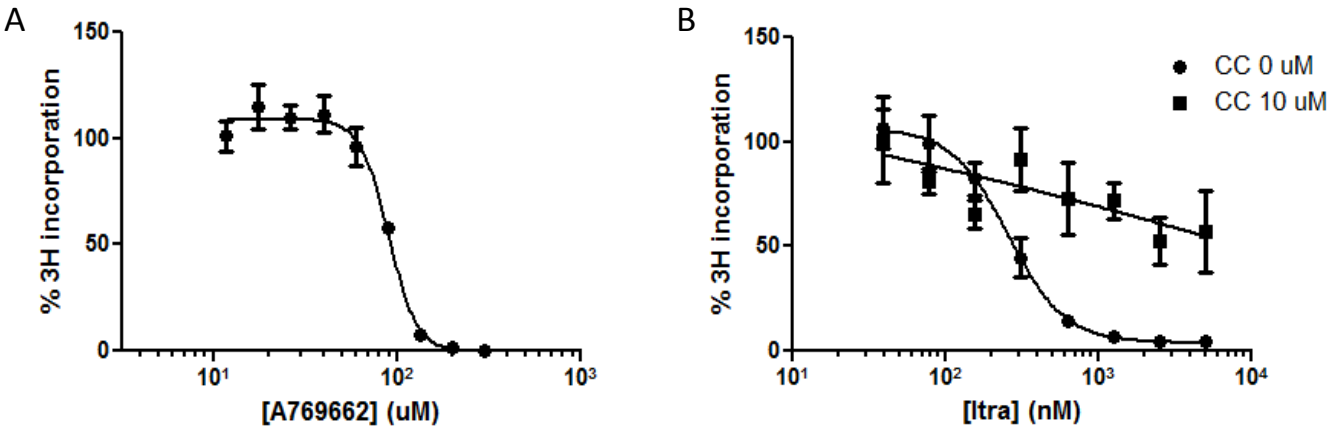
Each individual VDAC isoform a with C-terminal V5 tag was expressed in 293T cells before labeling with the itraconazole probe and biotin pull-down. Detection of the expressed protein by western blotting with a V5 antibody revealed clear labeling of VDAC1 and only very faint labeling of VDACs 2 and 3 (indicated by arrows).

Fig. S5. Itraconazole and 2DG do not increase TSC2 phosphorylation in HUVEC.



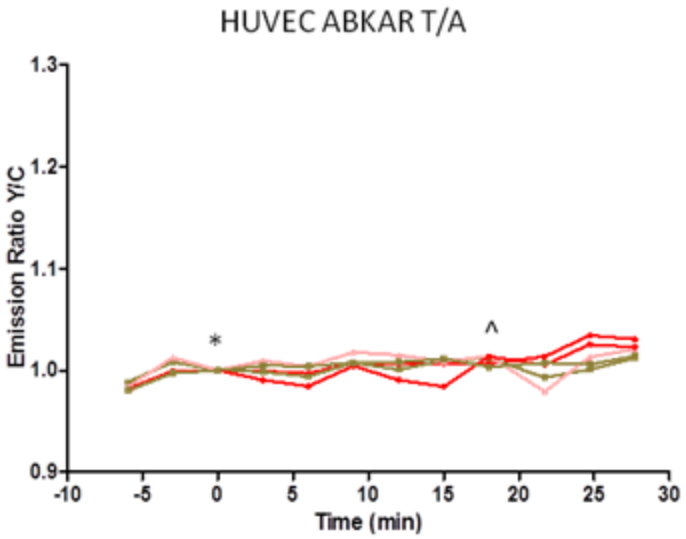
Phosphorylation of TSC2 at Ser1387 is not affected by 30 minute treatment of HUVEC with either itraconazole or 2DG at the indicated concentrations.

Fig. S6. AMPK activation causes proliferation inhibition in HUVEC.



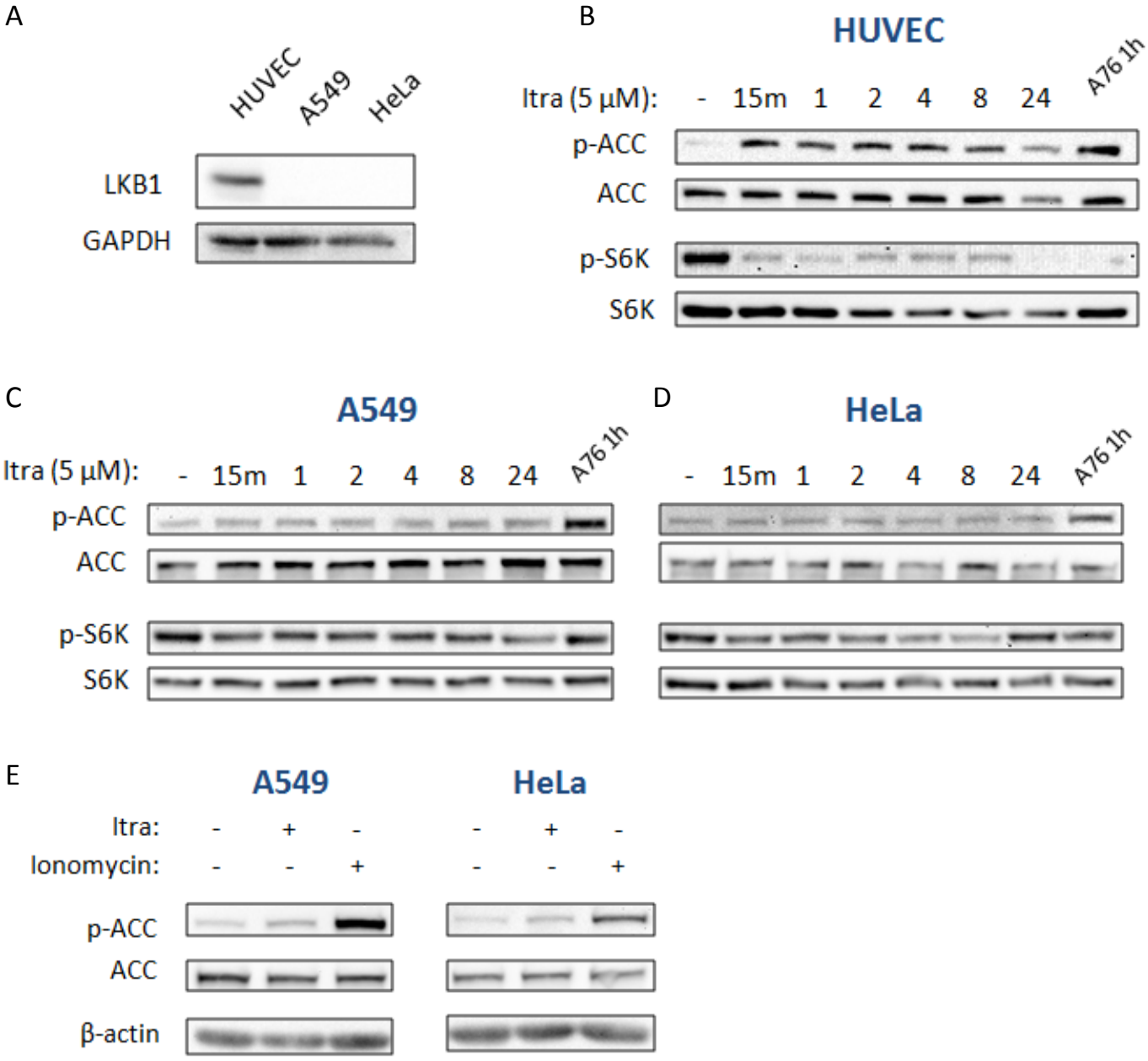
(a) HUVEC were treated with varying concentrations of A769662 for 24 hours before being pulsed for 6 hours with ^{3}H-thymidine. The average IC_{50} from 3 independent experiments was 73 μM +/- 8.34 (SEM). **(b)** HUVEC were pretreated with 10 μM Compound C for 30 minutes before being treated with varying concentrations of itraconazole for 24 hours. Cells were then pulsed for 6 hours with ^{3}H-thymidine. Results shown are averaged from 3 independent experiments, and error bars represent SEM.

Fig. S7. FRET-based reporter of AMPK activity and T/A mutant



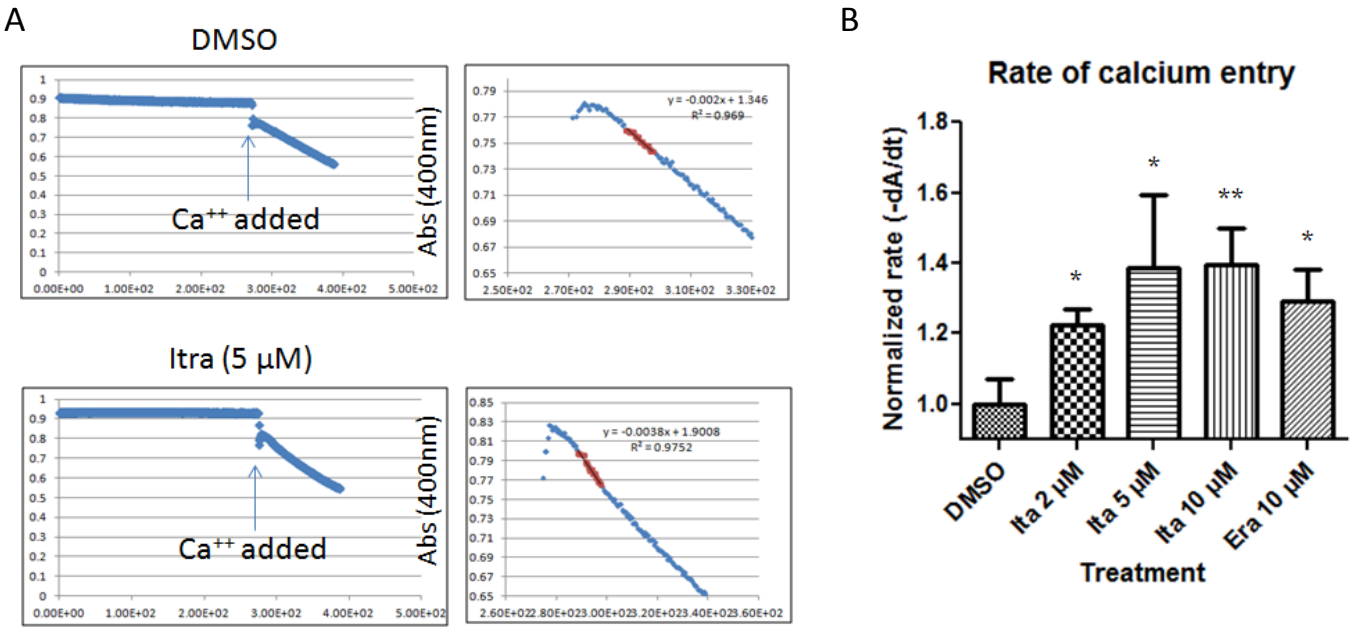
HUVEC expressing the ABKAR reporter with a T/A mutation in the AMPK substrate motif, which cannot be phosphorylated by AMPK, show no response to itraconazole (*) or 2DG (^) treatment.

Fig. S8. LKB1-deficient cells are insensitive to itraconazole.



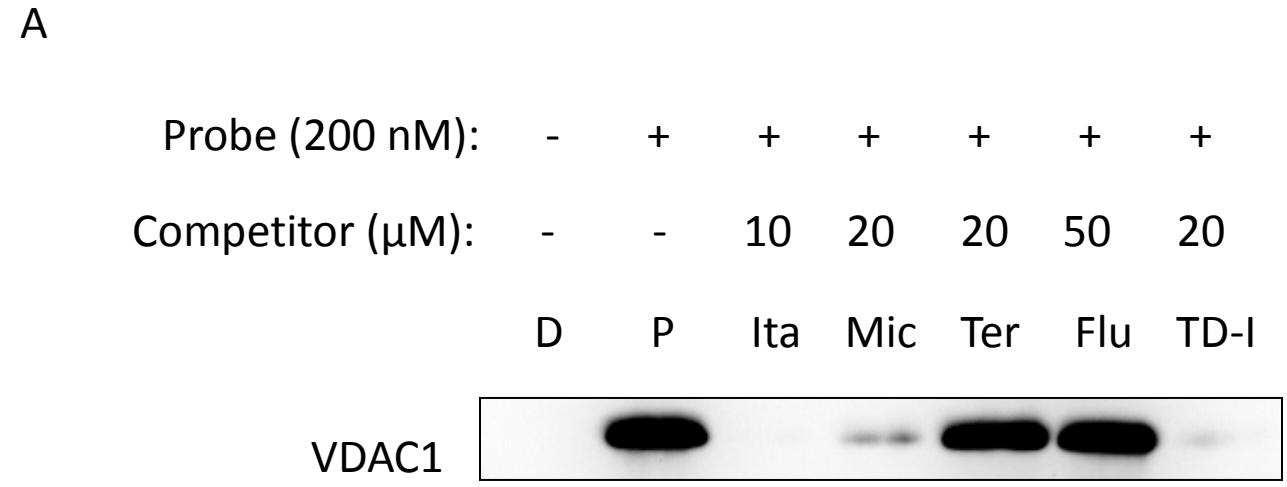
(a) A549 and HeLa, two commonly used cell lines which are reported to be LKB1-deficient, do not express LKB1 as measured by western blot. **(b, c, d)** HUVEC, A549, and HeLa cells were treated with 5 µM itraconazole for the indicated times (in hours except where indicated) or 100 µM A769662 for 1 hour. **(e)** A549 and HeLa cells were treated with either 5 µM itraconazole or 3 µM ionomycin for 15 minutes. Ionomycin activates AMPK in these cell lines while itraconazole does not.

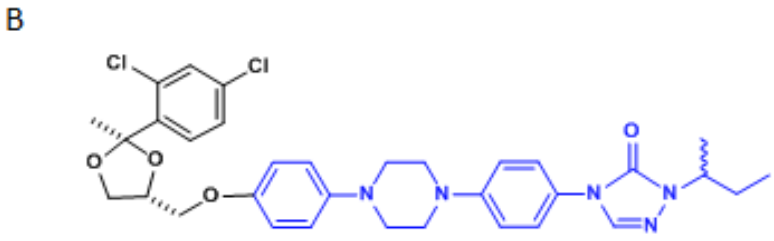
Fig. S9. Itraconazole and erastin increase the rate of calcium–induced mitochondrial swelling.

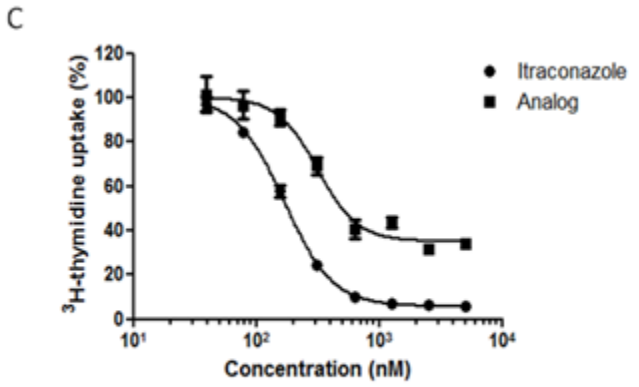


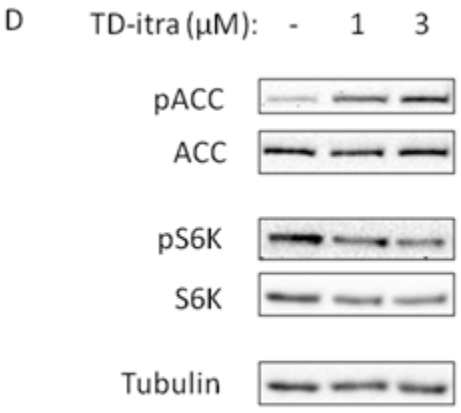
(a) Purified rat liver mitochondria were preincubated with itraconazole or erastin at the concentrations shown for 10 minutes before the addition of calcium, and mitochondrial swelling was monitored at 400 nm. The initial rate of swelling was calculated by linear fitting. A representative plot of absorbance vs. time is shown for DMSO treated and 5 µM itraconazole-treated mitochondria. **(b)** Itraconazole and erastin significantly increase the rate of calcium-induced mitochondrial swelling as compared to DMSO treatment. Results shown are representative of three experiments. Error bars represent SEM of 4-5 experimental replicates. Statistically significant increase in swelling rate was calculated by an unpaired, one-tailed t-test. * $p < 0.05$, ** $p < 0.01$.

Fig. S10. Inactive azoles do not bind VDAC1, but non-azole analog does

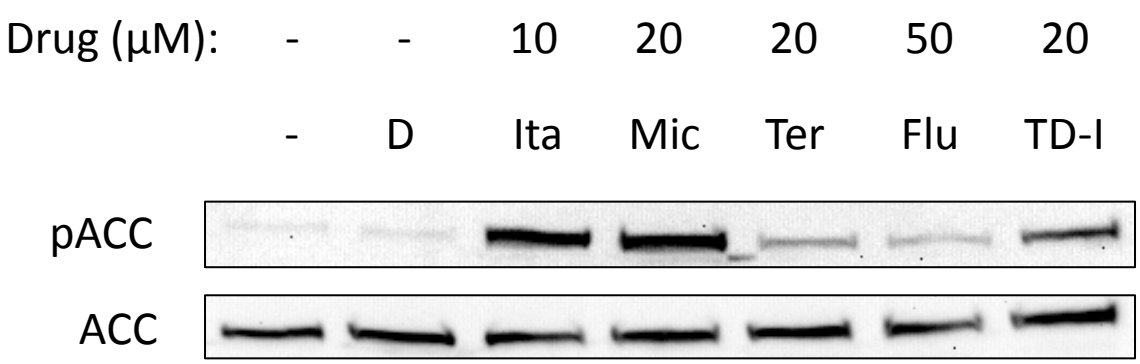








E



(a) The ability of itraconazole-like molecules to bind to VDAC1 was assessed by their ability to compete binding of the itraconazole probe to VDAC1. Cells were treated with DMSO alone (D), probe alone (P), or probe plus itraconazole (Ita), miconazole (Mic), terconazole (Ter), Fluconazole (Flu), or analog TD-itra (shown in panel b) at the indicated concentrations. **(b)** Chemical structure of TD-itra. **(c)** Dose-response curves of itraconazole and TD-itra against HUVEC proliferation. **(d)** TD-itra is able to activate AMPK and inhibit mTOR in HUVEC after a 15 minute treatment. **(e)** Compounds which bind to VDAC1 also activate AMPK.

Table S1. MS hits from DMSO control sample (background)

rank	prot_acc	prot_desc (Tax_Id=9606 Gene_Symbol)	prot_score	prot_mass
1	IPI00291006	MDH2 Malate dehydrogenase, mitochondrial	752	35481
2	IPI00219217	LDHB L-lactate dehydrogenase B chain	700	36615
3	IPI00022434	ALB Putative uncharacterized protein ALB	227	71658
4	IPI00027462	S100A9 Protein S100-A9	226	13234
5	IPI00013933	DSP Isoform DPI of Desmoplakin	223	331569
6	IPI00027547	DCD Dermcidin	218	11277
7	IPI00398625	HRNR Hornerin	178	282228
8	IPI00008530	RPLP0 60S acidic ribosomal protein P0	167	34252
9	IPI00396378	HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	159	37407
10	IPI00789324	JUP cDNA FLJ60424, highly similar to Junction plakoglobin	156	62577
11	IPI00021700	PCNA Proliferating cell nuclear antigen	156	28750
12	IPI00219221	LGALS7;LGALS7B Galectin-7	148	15066
13	IPI00397801	FLG2 Filaggrin-2	148	247928
14	IPI00025753	DSG1 Desmoglein-1	138	113644
15	IPI00217966	LDHA Isoform 1 of L-lactate dehydrogenase A chain	127	36665
16	IPI00007047	S100A8 Protein S100-A8	123	10828
17	IPI00025447	EEF1A1 Elongation factor 1-alpha	122	47839
18	IPI00021439	ACTB Actin, cytoplasmic 1	117	41710
19	IPI00219018	GAPDH Glyceraldehyde-3-phosphate dehydrogenase	86	36030
20	IPI00790298	- 20 kDa protein	86	19534
21	IPI00915869	MDH1 Malate dehydrogenase	80	23024
22	IPI00005969	CAPZA1 F-actin-capping protein subunit alpha-1	78	32902
23	IPI00654755	HBB Hemoglobin subunit beta	76	15988
24	IPI00014587	CLTA Isoform Brain of Clathrin light chain A	76	27060
25	IPI00019038	LYZ Lysozyme C	75	16526
26	IPI00022974	PIP Prolactin-inducible protein	69	16562
27	IPI00032325	CSTA Cystatin-A	67	11000
28	IPI00166729	AZGP1 alpha-2-glycoprotein 1, zinc precursor	65	34237
29	IPI00329332	STX12 Syntaxin-12	63	31622
30	IPI00216099	DSC1 Isoform 1A of Desmocollin-1	60	99924
31	IPI00554711	JUP Junction plakoglobin	57	81693
32	IPI00013885	CASP14 Caspase-14	54	27662
33	IPI00219806	S100A7 Protein S100-A7	51	11464
34	IPI00387020	MYOZ2 Myozenin-2	51	29879
35	IPI00215911	APEX1 DNA-(apurinic or apyrimidinic site) lyase	50	35532
36	IPI00418169	ANXA2 Isoform 2 of Annexin A2	47	40386

Table S2. MS hits from itraconazole probe sample

rank	prot_acc	prot_desc (Tax_Id=9606 Gene_Symbol)	prot_score	prot_mass
1	IPI00013933	DSP Isoform DPI of Desmoplakin	847	331569
2	IPI00216308	VDAC1 Voltage-dependent anion-selective channel protein 1	749	30754
3	IPI00027462	S100A9 Protein S100-A9	673	13234
4	IPI00745872	ALB Isoform 1 of Serum albumin	649	69321
5	IPI00219217	LDHB L-lactate dehydrogenase B chain	560	36615
6	IPI00291006	MDH2 Malate dehydrogenase, mitochondrial	495	35481
7	IPI00789324	JUP cDNA FLJ60424, highly similar to Junction plakoglobin	398	62577
8	IPI00025753	DSG1 Desmoglein-1	341	113644
9	IPI00218918	ANXA1 Annexin A1	300	38690
10	IPI00397801	FLG2 Filaggrin-2	272	247928
11	IPI00398625	HRNR Hornerin	262	282228
12	IPI00554711	JUP Junction plakoglobin	236	81693
13	IPI00021439	ACTB Actin, cytoplasmic 1	222	41710
14	IPI00219806	S100A7 Protein S100-A7	209	11464
15	IPI00011229	CTSD Cathepsin D	192	44524
16	IPI00007047	S100A8 Protein S100-A8	190	10828
17	IPI00019038	LYZ Lysozyme C	189	16526
18	IPI00179330	UBC;RPS27A;UBB ubiquitin and ribosomal protein S27a precursor	169	17953
19	IPI00060800	ZG16B Zymogen granule protein 16 homolog B	169	22725
20	IPI00032325	CSTA Cystatin-A	167	11000
21	IPI00790298	- 20 kDa protein	163	19534
22	IPI00022204	SERPINB3 Isoform 1 of Serpin B3	155	44537
23	IPI00219221	LGALS7;LGALS7B Galectin-7	152	15066
24	IPI00027547	DCD Dermcidin	145	11277
25	IPI00418169	ANXA2 Isoform 2 of Annexin A2	131	40386
26	IPI00300376	TGM3 Protein-glutamine gamma-glutamyltransferase E	128	76584
27	IPI00219018	GAPDH Glyceraldehyde-3-phosphate dehydrogenase	124	36030
28	IPI00216298	TXN Thioredoxin	112	11730
29	IPI00007797	FABP5;FABP5L2;FABP5L7;FABP5L9 Fatty acid-binding protein, epidermal	107	15155
30	IPI00947285	SBSN suprabasin isoform 1 precursor	106	60505
31	IPI00008530	RPLP0 60S acidic ribosomal protein P0	104	34252
32	IPI00216099	DSC1 Isoform 1A of Desmocollin-1	103	99924
33	IPI00154742	IGL@;IGLV1-40;IGLC1;IGLV3-21;IGLC3;LOC100293277;IGLV2-14;LOC100293440;IGLV1	100	24777
34	IPI00021536	CALML5 Calmodulin-like protein 5	97	15883
35	IPI00022974	PIP Prolactin-inducible protein	96	16562
36	IPI00166729	AZGP1 alpha-2-glycoprotein 1, zinc precursor	95	34237
37	IPI00010303	SERPINB4 Serpin B4	93	44825
38	IPI00217966	LDHA Isoform 1 of L-lactate dehydrogenase A chain	91	36665
39	IPI00025447	EEF1A1 Elongation factor 1-alpha	89	47839

40	IPI00396378	HNRNPA2B1 Isoform B1 of Heterogeneous nuclear ribonucleoproteins A2/B1	88	37407
41	IPI00024145	VDAC2 Isoform 2 of Voltage-dependent anion-selective channel protein 2	87	30393
42	IPI00013885	CASP14 Caspase-14	85	27662
43	IPI00013877	HNRNPH3 Isoform 1 of Heterogeneous nuclear ribonucleoprotein H3	82	36903
44	IPI00103242	POF1B Isoform 1 of Protein POF1B	80	68653
45	IPI00015614	PRSS3 Isoform A of Trypsin-3	76	32508
46	IPI00384938	LOC100290320;IGHG1;IGHV4-31;LOC100294459 Putative uncharacterized protein D	73	52819
47	IPI00031564	GGCT Isoform 1 of Gamma-glutamylcyclotransferase	70	20994
48	IPI00910602	NEFH Isoform 1 of Neurofilament heavy polypeptide	67	112411
49	IPI00005969	CAPZA1 F-actin-capping protein subunit alpha-1	63	32902
50	IPI00033583	SERPINB12 Serpin B12	58	46247
51	IPI00019502	MYH9 Isoform 1 of Myosin-9	58	226392
52	IPI00152881	SHROOM3 shroom family member 3 protein	56	216724
53	IPI00003348	GNB2 Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2	55	37307
54	IPI00291560	ARG1 Isoform 1 of Arginase-1	52	34713
55	IPI00915869	MDH1 Malate dehydrogenase	32	23024

SI METHODS

Western Blot. Cells (100,000 per well in 2 mL medium) were seeded in six-well plates 24 h before drug treatment. Drugs then were added to each well from 200× DMSO stock solutions (final DMSO concentration of 0.5%) and were incubated for the indicated times. Plates then were placed on ice and washed once with ice-cold PBS before the addition of 2× Laemmli sample buffer directly to the cells. Lysates were collected after 10 min and were incubated for 5 min at 95 °C before being subjected to SDS/PAGE. Proteins were then transferred to nitrocellulose membranes (Bio-Rad) and stained with Ponceau to confirm the quality of protein transfer and equal loading of samples. Membranes then were incubated in 5% (wt/vol) blotto (Santa Cruz Biotechnologies) dissolved in TBS containing 0.05% Tween-20 (TBST) for 1 h at room temperature before overnight incubation with primary antibodies at 4 °C. Membranes were washed three times for 5 min each with TBST and then were incubated with HRP-conjugated secondary antibodies (GE Healthcare) diluted in 5% blotto/TBST for 1 h at room temperature. Membranes were washed another three times for 5 min with TBST before the immune complexes were detected with Chemiluminescent ECL substrate (Millipore) on a Kodak Image Station (440CF). Band intensity was quantified using ImageJ.

Thymidine Incorporation Assay. HUVEC or MEFs were seeded in 96-well plates (Costar) at a density of 2,000 cells per well and were allowed to attach overnight. Drugs were serially diluted in DMSO at 200× final concentrations (0.5% DMSO) and then were diluted in medium before being added to the cells. Cells were incubated with drugs for 24 h before the addition of 1 μCi of [3H]-labeled thymidine (American Radiolabeled Chemicals) for a further 6 h. Cells then were harvested onto glass-fiber filters (PerkinElmer) using a Mach III M Harvester 96 (Tomtec, Inc.). Filters were dried overnight before being sealed in sample bags with 4 mL of Betaplate Scint scintillation fluid (PerkinElmer), and then

scintillation was counted using a 1450 Microbeta apparatus (PerkinElmer). The counts per minute of drug-treated cells were normalized to control cells treated with DMSO alone. Drug dose–response curves and IC50 values were generated using GraphPad Prism 5 software.

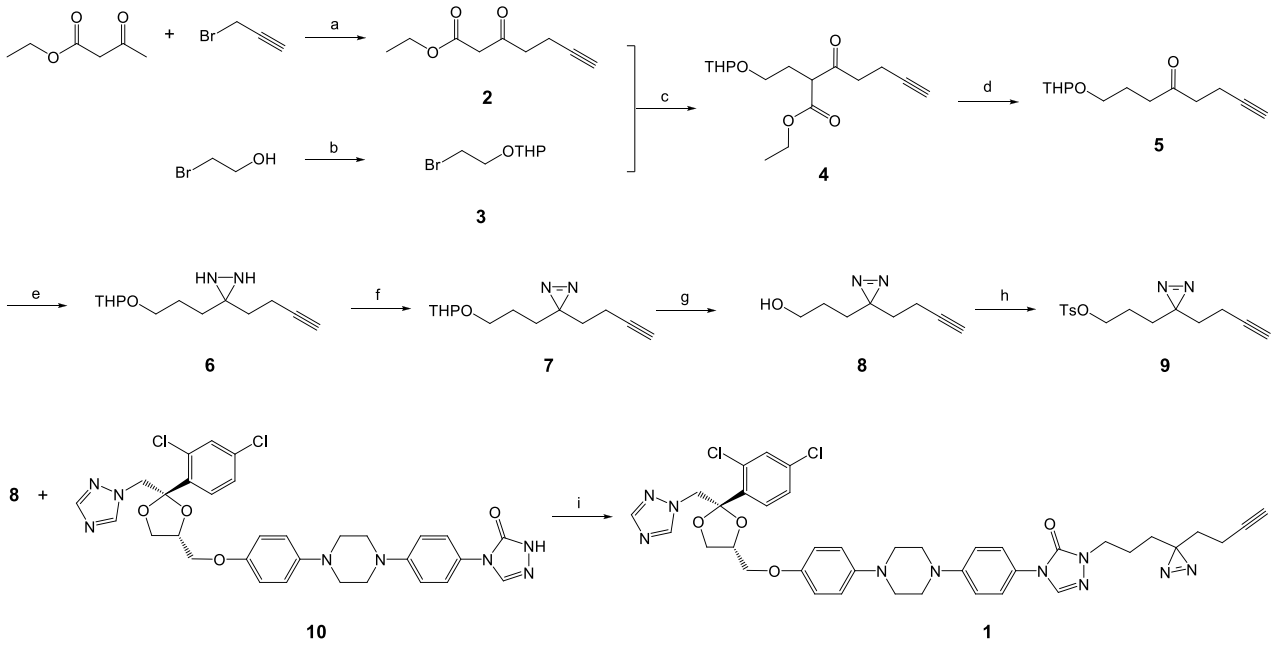
Chemistry. Reactions were carried out in oven-dried glassware. All reagents were purchased from commercial sources and were used without further purification unless noted. Unless stated otherwise, all reactions were carried out under a argon atmosphere and monitored by Merck precoated silica gel 60F-254 plates and visualized using 254 nm UV light. Column chromatography was performed on silica gel (200–400 mesh, Merck). The ratio between silica gel and crude product ranged from 100 to 50:1 (w/w). NMR data were collected on a Varian Unity-400 (400 MHz ^{1}H, 100 MHz ^{13}C) machine in the Department of Pharmacology and Molecular Sciences at the Johns Hopkins School of Medicine. ^{1}H NMR spectra were obtained in deuteriochloroform ($CDCl_3$) with either tetramethylsilane (TMS, $\delta = 0.00$ for ^{1}H) or chloroform ($CHCl_3$, $\delta = 7.27$ for ^{1}H) as an internal reference. ^{13}C NMR spectra were proton decoupled and were in $CDCl_3$ with either TMS ($\delta = 0.0$ for ^{13}C) or $CHCl_3$ ($\delta = 77.0$ for ^{13}C) as an internal reference. Chemical shifts are reported in ppm (δ). Data are presented in the form: chemical shift (multiplicity, coupling constants, and integration). ^{1}H data are reported as though they were first order. The errors between the coupling constants for two coupled protons were less than 0.5 Hz, and the average number was reported. Low-resolution mass spectra were obtained on a API 150EX™ single quadrupole LC/ESI-MS system in the Department of Pharmacology and Molecular Sciences or on a Voyager DE-STR, MALDI-TOF instrument at the AB Mass Spectrometry/Proteomics Facility at the Johns Hopkins University. The reported purity values were obtained with a JASCO PU-2089S Plus quaternary pump system, using an MD-2010 Plus PDA detector at the wavelength of 256 nm and a

Varian Microsorb-MV 100_5 C18 column. The eluant consisted of acetonitrile and 0.125% diethylamine in water, the ratio and flow rate of which depends on the compound.

1. Synthesis and Characterization of itraconazole probe

The synthesis of the probe started with 2-bromoethanol and ethyl acetoacetate (**Scheme S1**). Ethyl 3-oxohept-6-ynoate (**2**) was synthesized from ethyl acetoacetate based on the previously reported procedure.[1] The THP (3,4-dihydro-2*H*-pyran) protection of 2-bromoethanol yielded **3**, which was coupled with **2**, followed by thermal decarboxylation under strong basic conditions, to give compound **5** in moderate yield over two steps. This reaction sequence allows flexible control of the chain length by replacing 2-bromoethanol with other bromo-substituted alcohols if needed. Next, by following the literature-reported procedure (1) the ketone group was converted to the diazirine moiety. The yield could be significantly improved if pure ammonia had been used instead of the ammonia solution in methanol (2). Compound **9** was prepared in high yield from **7** by the deprotection of THP, followed by the tosylation of the free hydroxyl group. Subsequently, the S_N2 displacement of the tosylate group by the triazolone compound **10** (3) afforded the probe **1**.

Scheme S1[a]



[a] Reagents and conditions: (a) LDA, THF, 0 ºC, 74%; (b) THP, PTSA, CH_2Cl_2, rt, 91%; (c) K_2CO_3, KI, DMF/Acetone, 80 ^oC; (d) 10% aq. KOH, MeOH, 48% over 2 steps; (e) 7N ammonia in MeOH, NH_2SO_4H; (f) I_2, Et_3N, CH_2Cl_2, 17% over 2 steps; (g) CSA, MeOH, rt, 86%; (h) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 93%; (i) K_2CO_3, 18-crown-6, CH_3CN, 40 ^oC, 52%.

1.1. 2-(2-Bromoethoxy)tetrahydro-2*H*-pyran (3): 3,4-dihydro-2*H*-pyran (THP) (0.77 g, 9.24 mmol) and a catalytic amount of *p*-toluenesulfonic acid (PTSA) (8.8 mg, 0.046 mmol) was added to a solution of 2-bromoethanol (1.15 g, 9.24 mmol) in CH_2Cl_2 (15 mL) and the mixture was allowed to stir at room temperature for 3 h. The reaction mixture was washed with aqueous Na_2CO_3 solution. The resulting solution was extracted with more CH_2Cl_2, dried over Na_2SO_4, filtered, and concentrated to yield the crude product, which was purified by column chromatography (neat Hexanes → 50:1 Hexanes–Ethyl Acetate) to afford **3** (1.76 g, 91%) as a colorless thin oil: ^1H NMR (400 MHz, $CDCl_3$, δ_H) 4.66 (t, J = 4.0 Hz, 1H), 4.05–3.95 (m, 1H), 3.92–3.85 (m, 1H), 3.79–3.71 (m, 1H), 3.55–3.45 (m, 3H), 1.90–1.47 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 98.89, 67.51, 62.24, 30.90, 30.42, 25.36, 19.25.

1.2. 1-(Tetrahydro-2*H*-pyran-2-yloxy)oct-7-yn-4-one (5): 2-(2-Bromoethoxy)tetrahydro-*2H*-pyran (**3**) (1.76 g, 8.42 mmol), ethyl 3-oxohept-6-ynoate (**2**) (1.84 g, 10.95 mmol), and K_2CO_3 (3.03 g, 21.9 mmo1) were mixed in acetone (40 mL) and DMF (4 mL), and refluxed for 24 h. Most of the acetone was removed under reduced pressure and the residue was worked up with water and extracted with diethyl ether. The solvent was removed again and the crude residue was purified by a quick column to remove most of the dialkylated by-product. The collected ethyl 3-oxo-2-(2-(tetrahydro-*2H*-pyran-2-yloxy)ethyl)hept-6-ynoate (**3**) (1.57 g) was refluxed for 2 h in a mixture of 10% aqueous KOH (6 mL, 10.7 mmol) and methanol (6 mL). The mixture was diluted with water and extracted with diethyl ether. The resulting solution was dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (50:1 → 10:1 Hexanes–Ethyl Acetate) to afford **5** (0.91 g, 48%) as a colorless thin oil: ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.55 (t, J = 4.0 Hz, 1H), 3.88–3.80 (m, 1H), 3.78–3.70 (m, 1H), 3.54–3.45 (m, 1H), 3.45–3.36 (m, 1H), 2.69 (t, J = 7.3 Hz, 2H), 2.62–2.52 (m, 2H), 2.50–2.42 (m, 2H), 1.98–1.66 (m, 5H), 1.65–1.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 208.28, 98.92, 83.19, 68.66, 66.50, 62.49, 41.28, 39.66, 30.68, 25.43, 23.89, 19.70, 12.93.

1.3. 3-(But-3-ynyl)-3-(3-(tetrahydro-2*H*-pyran-2-yloxy)propyl)-3H-diazirine (7): To a 100 mL thick wall pressure vessel containing 1-(Tetrahydro-*2H*-pyran-2-yloxy)oct-7-yn-4-one (**5**) (0.91 g, 4.06 mmol) in an ice bath was added 7N ammonia in MeOH (4 mL, 28 mmol). After the vessel was sealed, the reaction mixture was stirred at 0 ^{o}C for 4 h. Hydroxylamine O-Sulfonic acid (0.53 g, 4.67 mmol) was dissolved in methanol and then added dropwise into the reaction mixture. After stirring overnight, most ammonia was removed by gently blowing air through the suspension using a glass pipette, and then the white precipitate was filtered off. After solvents were removed under vacuum, the residue was redissolved in CH_2Cl_2 followed by the addition of triethylamine (0.62 g, 6.09 mmol). Subsequently, the solution was cooled to 0 ^{o}C, and iodine was slowly added until the color of iodine persists for 1 min.

After 2 h, CH_2Cl_2 was evaporated and the reaction mixture was extracted with ether and dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (50:1 → 4:1 Hexanes–Ethyl Acetate) to afford **7** (163.1 mg, 17%) as a colorless thin oil: ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 4.54 (t, J = 3.5 Hz, 1H), 3.87–3.77 (m, 1H), 3.72–3.62 (m, 1H), 3.55–3.44 (m, 1H), 3.38–3.28 (m, 1H), 2.10–1.95 (m, 3H), 1.85–1.35 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 98.83, 82.80, 69.08, 66.42, 62.33, 32.27, 30.65, 29.50, 28.05, 25.43, 24.11, 19.58, 13.33.

1.4. 3-(3-(But-3-ynyl)-3*H*-diazirin-3-yl)propan-1-ol (8): To a solution of compound **7** (163.1 mg, 0.69 mmol) in methanol (3 mL) was added (+)-camphorsulfonic acid (CSA) (16 mg, 0.069 mmol). The resulting mixture was stirred at room temperature and monitored by TLC. Once the reaction was over, the solution was neutralized with saturated aqueous Na_2CO_3 and extracted with ether. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (50:1 → 2:1 Hexanes–Ethyl Acetate) to afford **8** (90.3 mg, 86%) as a colorless thin oil: ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 3.60 (t, J = 6.4 Hz, 2H), 2.05–1.97 (m, 3H), 1.69–1.60 (m, 3H), 1.57–1.50 (m, 2H), 1.42–1.33 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 82.80, 69.16, 61.85, 32.27, 28.96, 28.03, 26.76, 13.32.

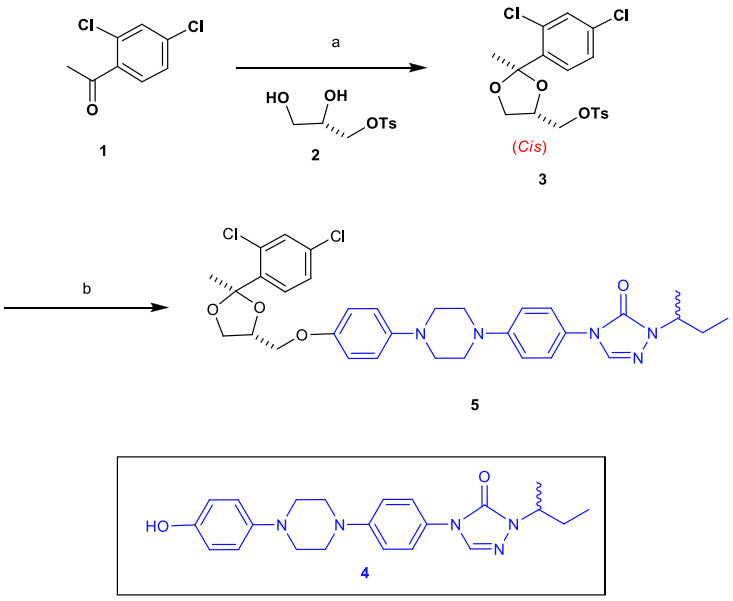
1.5. 3-(3-(But-3-ynyl)-3*H*-diazirin-3-yl)propan-1-ol (9): To a solution of **8** (90.3 mg, 0.59 mmol), Et_3N (90.0 mg, 0.89 mmol), and *N*,*N*-dimethylamino-pyridine (DMAP) (70.0 mg, 0.59 mmol) in CH_2Cl_2 was slowly added a solution of *p*-toluenesulfonic chloride (TsCl) (146.2 mg, 0.77 mmol) in CH_2Cl_2 at 0 ^{o}C. The reaction mixture was stirred at 0 ^{o}C for 1 h, and then warmed to room temperature for another 3 h. After diluted with water, the reaction mixture was extracted with more CH_2Cl_2. The organic layer was combined, dried (Na_2SO_4), filtered, and concentrated to yield the crude product, which was purified by column chromatography (50:1 → 10:1 Hexanes–Ethyl Acetate) to afford **9** (90.3 mg, 86%) as a

colorless thin oil: ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.78 (d, *J* = 8.1 Hz, 2H), 7.78 (d, *J* = 8.1 Hz, 2H), 3.98 (t, *J* = 5.9 Hz, 2H), 2.46 (s, 3H), 2.00–1.91 (m, 3H), 1.60–1.52 (m, 2H), 1.52–1.39 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 144.96, 132.88, 129.93, 127.89, 82.55, 69.32 (2C), 32.12, 28.73, 27.48, 23.30, 21.68, 13.23.

1.6. *Cis*-4-(4-(4-(4-(((2S,4R)-2-((1*H*-1,2,4-triazol-1-yl)methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)phenyl)-1-(3-(3-(but-3-ynyl)-3H-diazirin-3-yl)propyl)-1*H*-1,2,4-triazol-5(4*H*)-one (1): To a slurry of **10** (25.3 mg, 0.039 mmol) in acetonitrile was added **9** (17.9 mg, 0.058 mmol), K_2CO_3 (10.8 mg, 0.078 mmol) and 18-Crown-6 (10.3 mg, 0.039 mmol). After the mixture was stirred at 40–50 °C under argon for 16 h, the solvent was removed under vacuum to yield the crude product, which was purified by column chromatography (neat CH_2Cl_2 → 40:1 CH_2Cl_2–CH_3OH) to afford **1** (15.8 mg, 52%) as a white wax-like solid: ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 8.23 (s, 1H), 7.92 (s, 1H), 7.64–7.56 (m, 2H), 7.52–7.38 (m, 3H), 7.30–7.24 (m, 1H), 7.08–6.91 (m, 4H), 6.82 (d, *J* = 8.5 Hz, 2H), 4.83 (q, *J* = 14.4 Hz, 2H), 4.42–4.31 (m, 1H), 3.98–3.75 (m, 5H), 3.53–3.45 (m, 1H), 3.42–3.32 (m, 4H), 3.30–3.19 (m, 4H), 2.07–1.95 (m, 3H), 1.70–1.52 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 152.63, 152.11, 151.49, 150.67, 145.98, 136.08, 134.23, 134.06, 133.14, 131.46, 129.63, 127.28, 125.65, 123.63, 118.51, 116.68, 115.27, 107.63, 82.72, 74.71, 74.55, 69.23, 67.63, 67.46, 53.62, 50.61, 49.17, 44.81, 32.19, 29.90, 27.81, 23.15, 13.33. ESI-MS: 783.2 ($M+H^+$), 755.2 ($M+H^+–N_2$). Purity: 93%, t_R = 10.2 min.

2. Synthesis and Characterization of Triazole Deleted-Itra (TD-Itra).

Scheme S2[a]:



[a] Reagents and conditions: (a) **2**, TfOH, toluene, rt, 60 h, 60%; (b) **4**, NaH, DMSO, 80 ^{o}C, 12 h, 73%.

((2R,4S)-2-(2,4-dichlorophenyl)-2-methyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (3):

TfOH (4 eq) was added to the solution of 2′,4′-Dichloroacetophenone (**1**) (1 eq) and (*S*)-1-tosyloxy 2,3-propanediol (**2**) (2 eq) in toluene under argon atmosphere. Then the reaction was stirred at room temperature for 60h. The reaction was quenched by adding saturated aqueous $NaHCO_3$, then extracted with ethylacetate, washed with brine and dried over Na_2SO_4. The solvent was removed under the reduced pressure and the crude product was purified by column chromatography to afford the *cis* dioxalane product **3** as colorless oil in 60% yield.

^{1}H NMR (500 MHz, $CDCl_3$, δ_H): 7.73 (dd, J = 10.5, 2.0 Hz, 2H), 7.41 (d, J = 8.5 Hz, 1H), 7.28 (d, J = 2.5 Hz, 3H), 7.12 (d, J = 3.0 Hz, 1H), 4.11-4.07 (m, 1H), 4.02 (dd, J = 10.2, 5.2 Hz, 1H), 3.96 (dd, J = 10.0, 6.0 Hz, 1H), 3.78 (dd, J = 9.0, 4.0 Hz, 1H), 3.63 (dd, J = 9.0, 7.0 Hz, 1H), 2.37 (s, 3H), 1.61 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 145.2, 137.6, 132.6, 131.2, 130.0, 128.0, 126.8, 109.4, 77.4, 77.1, 76.9, 72.8, 69.4, 66.2, 25.6, 21.7.

1-sec-butyl-4-(4-(4-(4-(((2R,4R)-2-(2,4-dichlorophenyl)-2-methyl-1,3-dioxolan-4 yl)methoxy)phenyl)piperazin-1-yl)phenyl)-1H-1,2,4-triazol-5(4H)-one (4).

To a solution of tosylate **3** (1 eq) in dry DMF was added sodium hydride (NaH, 60% dispersion in mineral oil, (1.5 eq)) under argon atmosphere. After the reaction mixture was stirred at 50 ^{o}C for 1 hour. A solution of **4** (1.2 eq) in DMF was adder slowly at the same temperature. After the addition the temperature was increased to 90 ^{o}C and stirred for another 3 hours. The reaction mixture was quenched by the saturated sodium chloride, and the resulting mixture was extracted twice with dichloromethane. The organic fractions were dried over Na_2SO_4, filtered and concentrated under vacuum to yield the crude product which was purified by column chromatography to afford the desired product **5** in 73% yield.

^{1}H NMR (500 MHz, $CDCl_3$, δ_H): 7.62 (s, 1H), 7.61 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 3.0 Hz, 1H), 7.23 (dd, J = 8.5, 2.0 Hz, 1H), 7.03 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 9.0 Hz, 2H), 4.34-4.27 (m, 2H), 4.12 (dd, J = 9.5, 5.5 Hz, 1H), 4.01 (dd, J = 8.5, 4.5 Hz, 1H), 3.96 (dd, J = 9.2, 6.2 Hz, 1H), 3.84 (dd, J = 7.5, 6.7 Hz, 1H), 3.37 (bs, 4H), 3.24 (bs, 4H), 1.90 – 1.84 (m, 1H), 1.82 (s, 3H), 1.75 – 1.69 (m, 1H), 1.39 (d, J = 6.5 Hz, 3H), 0.91 (t, J = 7.2 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$, δ_C): 138.2, 134.6, 133.9, 132.8, 131.2, 128.9, 126.8, 123.6, 118.5, 116.7, 115.5, 109.1, 73.9, 69.3, 67.0, 52.7, 50.7, 49.3, 28.5, 25.7, 19.3, 10.8.

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