

Supplemental methods.

Immunoblotting.

Mitochondrial fractions were isolated using Mitochondria isolation kit for cultured cells (Thermo Fisher Scientific, catalog no. 89874). 30 μ g of total mitochondrial protein by BCA was separated on 8% SDS-PAGE gel and then transferred to PVDF membrane. Antibodies were used as follows: anti-NDUFV1 (Sigma-Aldrich, SAB2108612), anti-Pyruvate dehydrogenase E1-alpha subunit (Abcam, 110334) and custom anti-NDI1 (kind gift from Dr. Takao Yagi, Scripps Research Institute, La Jolla, CA). Membranes were visualized with IR-fluor labelled secondary antibodies on a LiCor scanner.

Fig. S1. (a-b) OCR measure in competition assay between PPV and complex I inhibitors piericidin A (a) and capsaicin (b). Values represent the means \pm SD. (c) OCR measurement of A549 cells after 5-hour treatment with PPV, atovaquone (ato) and metformin (met). Values represent the means \pm SD. (d) Drug washout experiment in A549 cells, the % OCR response to 3h of drug treatment (*magenta*) and removal 1 hour (*blue*) and 2 hours (*black*) prior to OCR measurement. 30 μ M atovaquone and 10 μ M PPV concentrations were used. Error bars represent standard deviation. (e-f) Trypan blue cell viability assessment (of $n = 3$) of A549 cells treated with 100 μ M papaverine or 1 μ M rotenone in normoxia (c) and hypoxia (d) (T = 72 h). Bar charts represent mean of viable cells in duplicates \pm standard deviation.

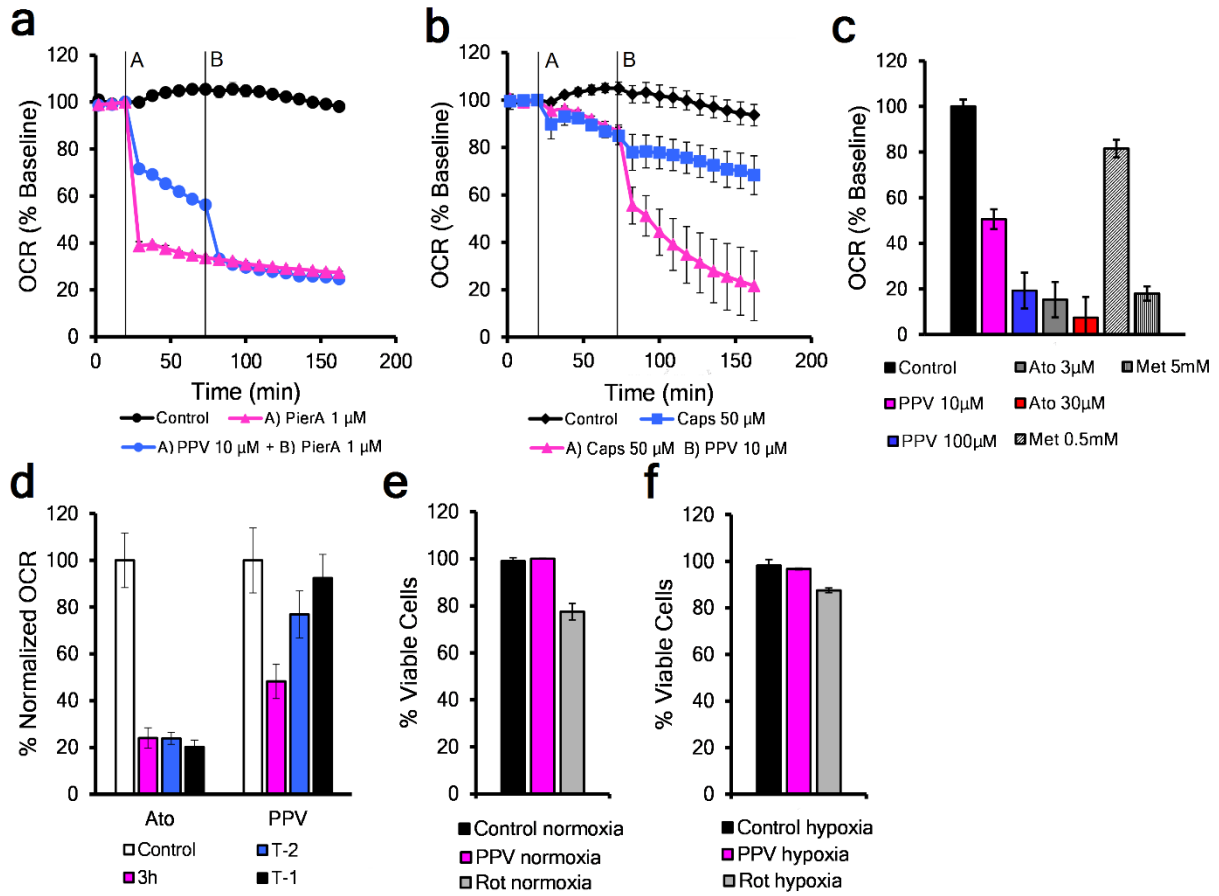
Fig. S2. (a-b) In vitro radiation survival assessment of A549 cell line treated with 10 μ M PPV in normoxia (a) and anoxia (b). Normoxic cells were irradiated in triplicates on a cell culture dish, anoxic cells were irradiated in high density suspension after sealing with mineral oil 45 minutes before radiation. Values represent averaged colony counts \pm standard deviation. (c) Quantification of tumor growth delay in orthotopic EO771 tumors grown in C57Bl6 mice receiving either 5 Gy XRT (*magenta*) or 2 mg/kg PPV 35 minutes prior to 5 Gy XRT (*blue*) ($n = 3-4$). Values are mean tumor volumes \pm SEM. (d) Quantification of tumor growth delay of heterotopic EO771 flank tumors in nude mice receiving either 5 Gy XRT (*magenta*) or 2 mg/kg PPV 35 minutes after (*blue*) or prior to 5 Gy XRT (*red*) ($n = 4$). Values are mean tumor volumes \pm SEM. (e-f) Related to Fig 2g tumor growth delay. When tumors reached 4-fold volume increase, groups receiving no treatment or PPV only (e); and XRT only or PPV followed by XRT (f), tumor weights were compared. Values are mean tumor weights \pm SEM ($n = 8$). P values were calculated with two-tailed two-sample t test. (g) Related to Fig 2g tumor growth delay. Representative images of orthotopic EO771 tumors harvested on day 11 after treatment with XRT (top) or 2 mg/kg PPV followed by XRT (bottom). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; n.s., not significant.

Fig. S3. (a-b) Uncropped western blot membrane used for figure 3a showing NDUFV1 and PDH E1 α levels (a) before probing for NDI1 (b). (c) Quantification of baseline OCR in parent A549 and NDUFV1 KO \pm NDI1 cells. Values represent mean OCR from at least 5 replicates \pm SD. (d) In vitro radiation survival assessment of A549 NDUFV1 KO cells \pm NDI1 treated with 10 μ M PPV. Curves represent averaged colony counts \pm SD. (e) Tumor growth delay of heterotopic NDUFV1 KO NDI1 (different NDUFV1 KO clone) flank xenografts in nude mice after receiving either 8 Gy dose of XRT (*magenta*) or 2 mg per kg body weight PPV 35 minutes prior

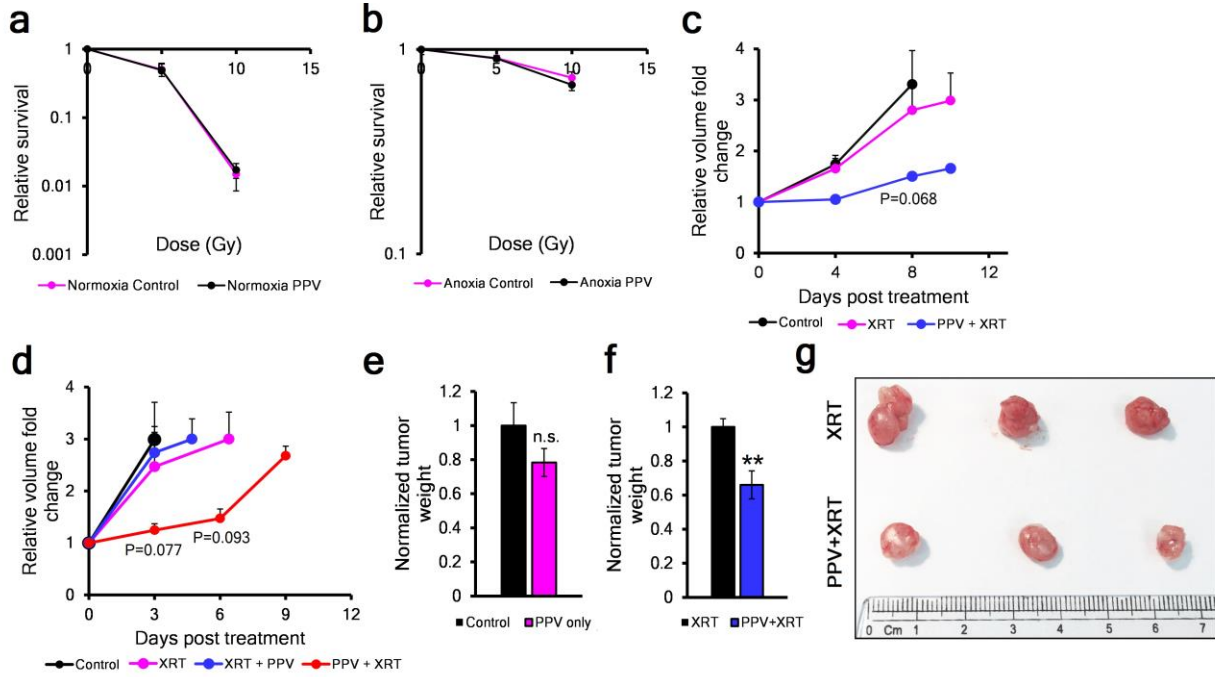
to 8 Gy XRT (*blue*) ($n = 4$). Values are mean tumor volumes \pm SEM. P values were calculated against XRT with two-tailed two-sample t test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; n.s., not significant.

Fig. S4. (a) Seahorse OCR data showing no effect of increasing concentrations of phosphodiesterase 5 inhibitor sildenafil citrate (0, 5, 10, 20 μ M) in Panc1 cell line. Values are means \pm SD. (b) Seahorse OCR analysis showing no effect of treatment with increasing concentrations of synthetic cAMP analog 8-Bromo-cAMP (0, 10, 20 μ M) in Panc1 cells. Values are means \pm SD. (c) Quantification of OCR and PDE10 inhibitory activities of 41 novel PPV derivatives normalized to PPV evaluated by Seahorse (OCR inhibition at 10 μ M concentration, 1 μ M for rotenone) and PDE10A enzymatic assay (PDE10A inhibition at 1 μ M). Bars represent mean OCR and PDE10 inhibitory activities from at least 5 (OCR) or 3 (PDE) replicates. All experiments were repeated at least twice. (d) Competition assay with SMV-32 and rotenone in A549 cells. (e) Cellular toxicity of PPV derivatives in vitro measured by trypan blue after 72 h treatment at 10 μ M. Values are mean \pm SEM.

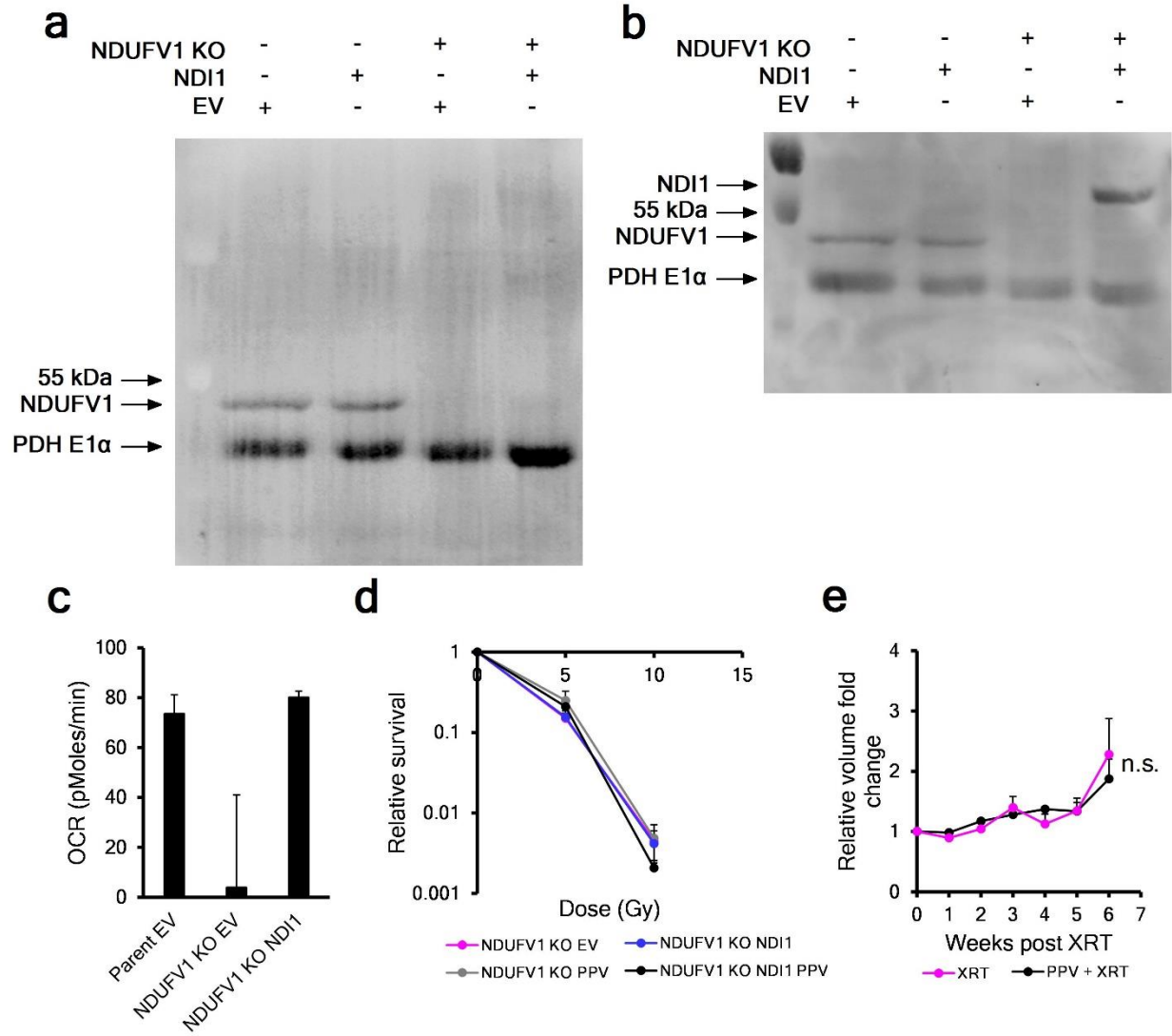
Benej et al Figure S1



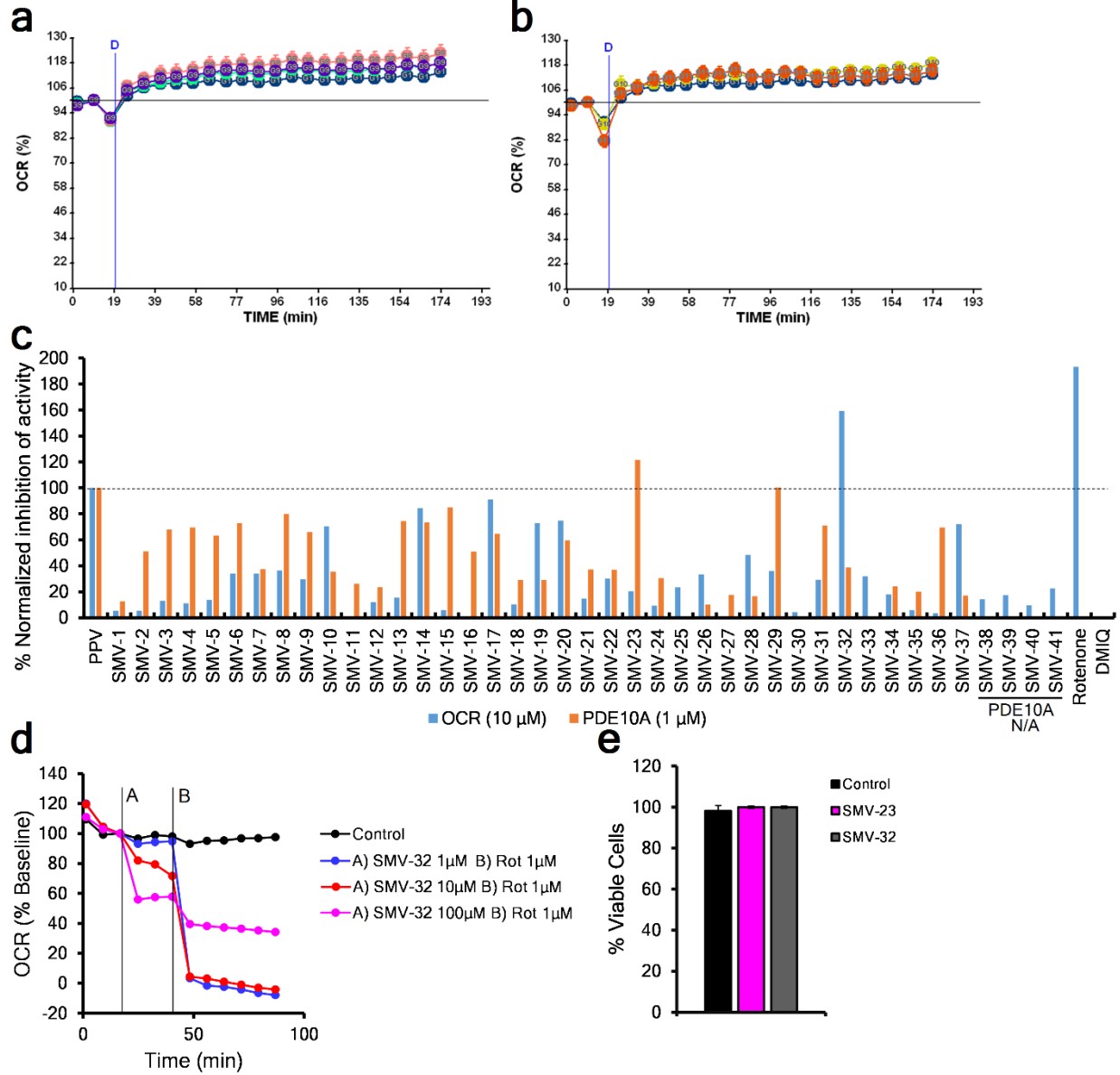
Benej et al Figure S2



Benej et al Figure S3



Benej et al Figure S4



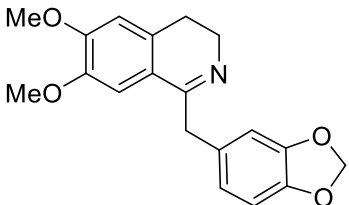
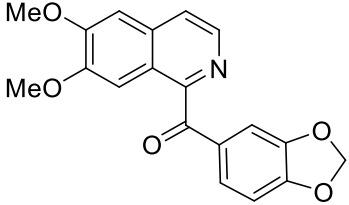
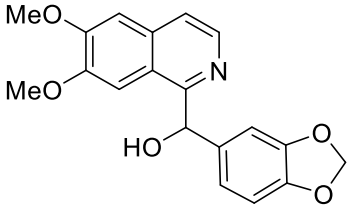
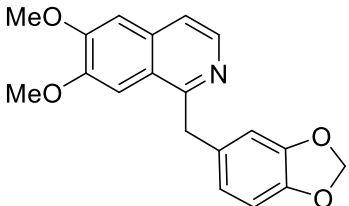
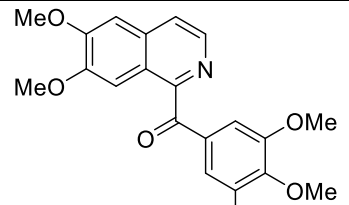
Benej et al Table S1

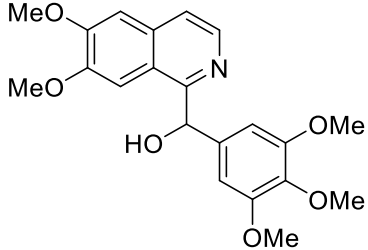
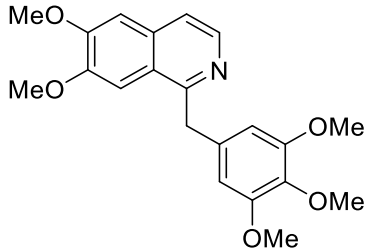
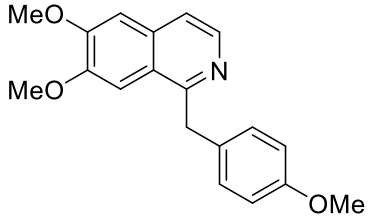
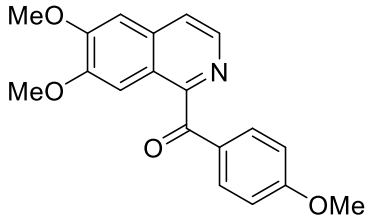
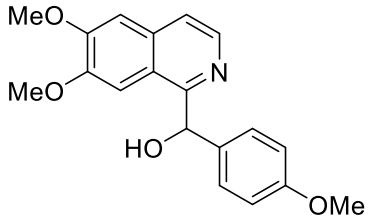
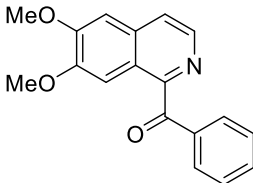
List of cell lines with sensitivity to PPV in the low micromolar range

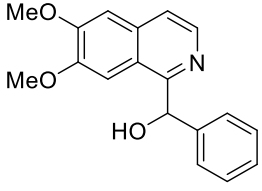
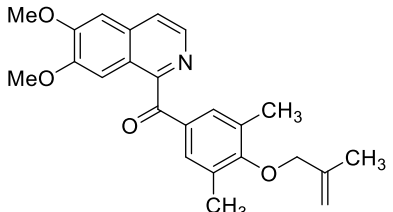
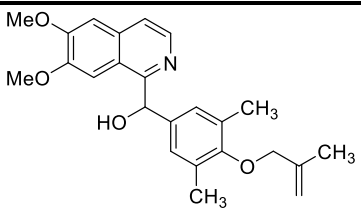
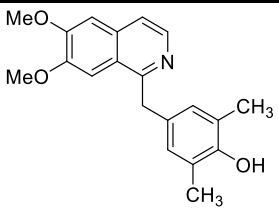
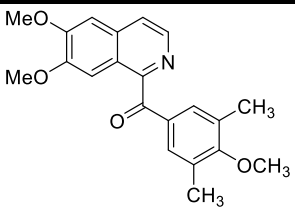
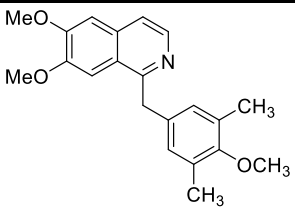
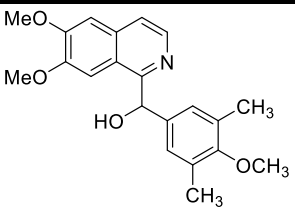
Cell line	Origin	Species
EO771	Breast	Murine
HCT116	Colon	Human
RKO	Colon	Human
U937	Leukemia	Human
MOLM13	Leukemia	Human
TIB-190	Leukemia	Human
THB-1	Leukemia	Human
K-562	Leukemia	Human
A549	Lung	Human
HCC-827	Lung	Human
H460	Lung	Human
H23	Lung	Human
H441	Lung	Human
H727	Lung	Human
H1568	Lung	Human
Bone marrow naïve	Normal	Murine
HCF	Normal	Human
HEK293T	Normal	Human
OSA16	Osteosarcoma	Canine
OSA18	Osteosarcoma	Canine
BxPC3	Pancreas	Human
Panc1	Pancreas	Human
MiaPaca2	Pancreas	Human
SU.86	Pancreas	Human
PSN-1	Pancreas	Human
KPC	Pancreas	Murine
FaDu	Pharynx	Human
Cal27	Tongue	Human

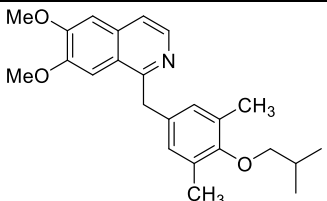
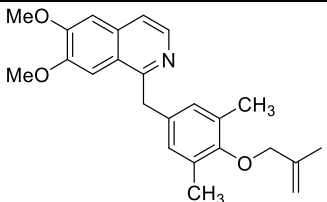
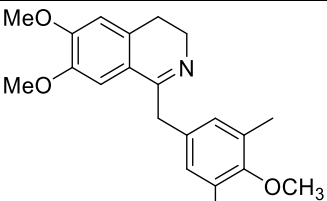
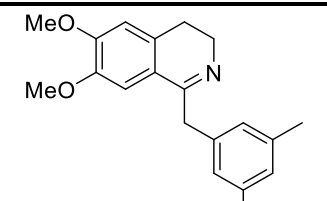
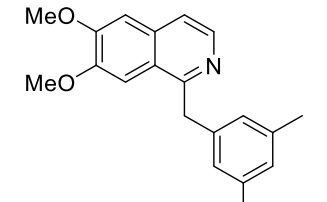
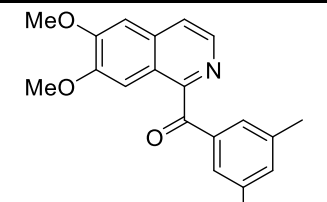
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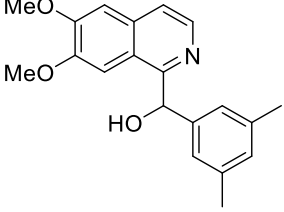
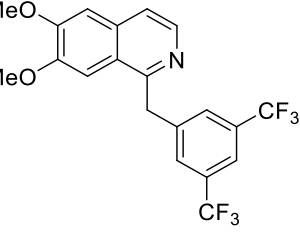
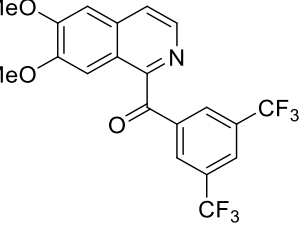
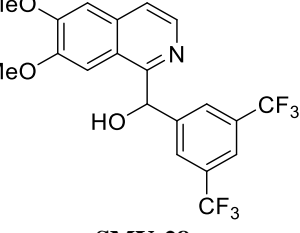
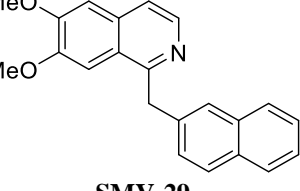
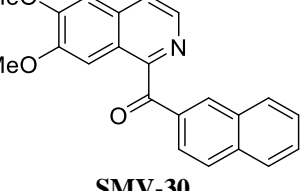
Chemical structures of the papaverine derivatives and their activity.

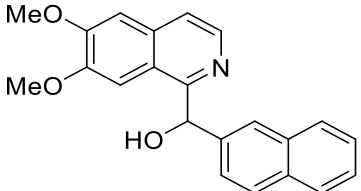
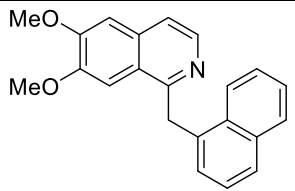
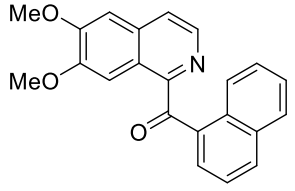
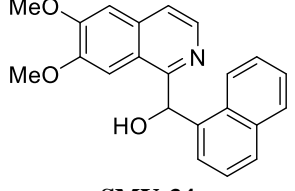
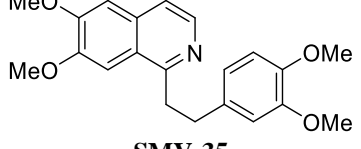
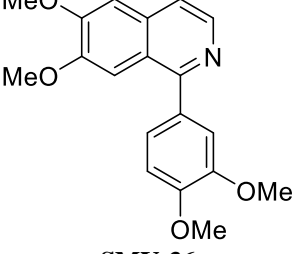
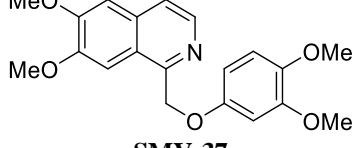
Compound No.	MW	Inhibitory activity	
	(g/mol)	OCR % of PPV	PDE10A % of PPV
 SMV-1	325.36	5.55	12.77
 SMV-2	337.33	5.55	51.12
 SMV-3	339.35	12.96	67.90
 SMV-4	323.35	11.11	69.54
 SMV-5	383.40	13.63	63.39

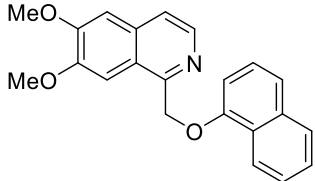
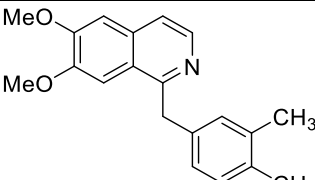
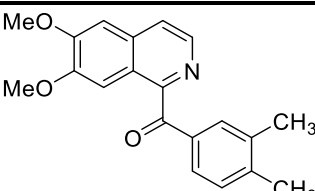
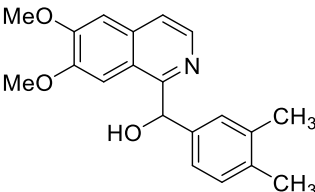
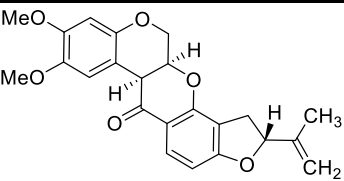
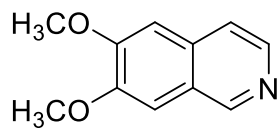
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 <p style="text-align: center;">SMV-12</p>	295.34	12.07	23.60
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 <p style="text-align: center;">SMV-17</p>	337.42	91.18	64.65
	353.42	10.35	29.18

SMV-18			
 SMV-19	379.50	72.88	29.13
 SMV-20	377.48	74.81	59.69
 SMV-21	339.44	14.81	37.27
 SMV-22	309.41	30.09	40.00
 SMV-23	307.39	20.37	121.49
 SMV-24	321.38	9.26	30.52

 <p>SMV-25</p>	323.39	23.40	0
 <p>SMV-26</p>	415.34	33.4	10.28
 <p>SMV-27</p>	429.32	0	17.69
 <p>SMV-28</p>	431.33	48.27	16.58
 <p>SMV-29</p>	329.40	35.99	100
 <p>SMV-30</p>	343.38	4.34	0

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 <p style="text-align: center;">SMV-41</p>	323.39	22.69	N/A
 <p style="text-align: center;">Rotenone</p>	394.41	193	0
 <p>6,7-dimethoxyisoquinoline DMIQ</p>	189.21	0	0