

Identification of three antimicrobials activating serotonin receptor 4 in colon cells

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Materials. Serotonin hydrochloride (S0370) was purchased from TCI Chemicals. RS67333 hydrochloride (SML1882), tegaserod malate (SML1504), metoclopramide monohydrochloride (M0763), prucalopride (SML1371), cisapride (CDS021610), mosapride citrate (M2946) and zacopride hydrochloride hydrate (SML0081) were purchased from Sigma-Aldrich. Nano-Glo® Luciferase Assay System (N1120) was purchased from Promega. The natural products library (L1400) and the anti-infection library (L3100) were purchased from Selleck Chemicals. Breathe Easy Sealing Membrane was purchased from Electron microscope services. Luminescence white 96-well plates were purchased from Thermo.

Toxicity assessment. An overnight culture of PPY1808 in SD(HL⁻) was used adjusted to an OD of 1. In a 96 well plate, 190 μ L of pH=7 SD(HL⁻), 8 μ L of PPY1808, and 2 μ L of DMSO, ethracridine in DMSO, proflavine in water, or rezaprazan in DMSO (final concentrations 0, 10 μ M, 100 μ M) were added. The plate was incubated in a Tecan Infinite M200 Pro plate reader at 30 °C with shaking and OD₆₀₀ was measured at 2.5 hours.

Table S1. Table of Plasmids

Plasmid Number	Plasmid Name	Description	Citation
PPY111	pKM111	pESC-His3-pTEF1-tCYC1	Mukherjee et al. 2015
PPY1192	pTMC18	pESC-His3-pTEF1-HTR4	Ehrenworth et al. 2017
PPY1740	pEY15	pRS415-Leu2-pFIG1-NanoLuc	This study

Table S2. Table of Primers

Primer Name	Sequence
EY248	gctgttgaaataacaaagacattgg

Table S3. Table of Strains

Strain Number	Description	Citation
PPY140	<i>S. cerevisiae</i> W303 MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100 $\Delta far1$ $\Delta ste2$ $\Delta sst2$	Mukherjee et al. 2015
PPY1808	PPY140 carrying pTMC18 and pEY15	This study
PPY1809	PPY140 carrying pKM111 and pEY15	This study

Table S4. Table of chemical descriptors used in PCA analysis

Descriptors	Abbreviations	Descriptors	Abbreviations
Molecular Weight	MW	Ring system count	RSC
Carbon atom count	CAC	Size of largest ring	SLR
Hydrogen atom count	HAC	Van der Waals surface area	VSA
Oxygen atom count	OAC	Amine	-
Nitrogen atom count	NAC	Alcohol	-
Hydrogen-bond donors	HBD	Ether	-
Hydrogen-bond acceptor	HBA	Aldehyde	-
Rotatable bonds	RB	Ketone	-
Stereocenter count	SC	Carboxylic Acid	-
Topological polar surface area	TPSA	Ester	-
Number of rings	NOR	Amide	-
Aromatic ring count	ARC		

Table S5. Chemical space composition of the natural product library.

Chemical Space	PC1	PC2	PC3	Members	Population (%)	Chemical characteristics
1	-	-	-	128	16	Mix of aromatics and groups containing N and O atoms
2	+	-	-	88	11	Steric, heterocyclic with O-containing functional groups
3	-	-	+	177	22	small heteroaromatics (fused rings) with O-containing functional groups
4	+	-	+	90	11	Adjacent to CS 3, hence similar trend, except higher C-atom count
5	+	+	-	47	6	chiral with multiple functional groups (phosphate, sulfate, N and O-containing functional groups)
6	-	+	-	124	16	Two distinct groups: A) long C-tail containing only N or O-functional group. B) complex heterocyclic (individual benzene rings) having both N and O-containing functional groups
7	+	+	+	49	6	hydrocarbons and cyclic rings
8	-	+	+	98	12	Adjacent to CS 6, hence similar trend, except heteroaromatics are fused rings
			Total:	801	100	

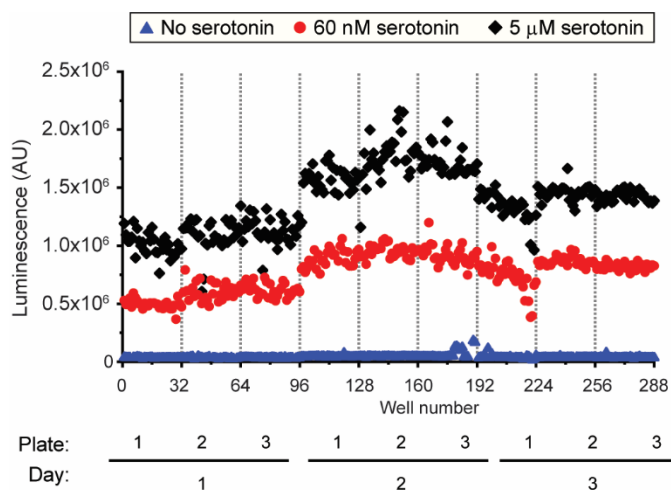


Fig. S1. Three-day 96 well-plate validation assay. Luminescence data with no, medium and high levels of serotonin. No obvious intra-plate edge effects or drift observed. The signal activation levels did fluctuate from day to day, but all raw midpoint CVs were below 20%.

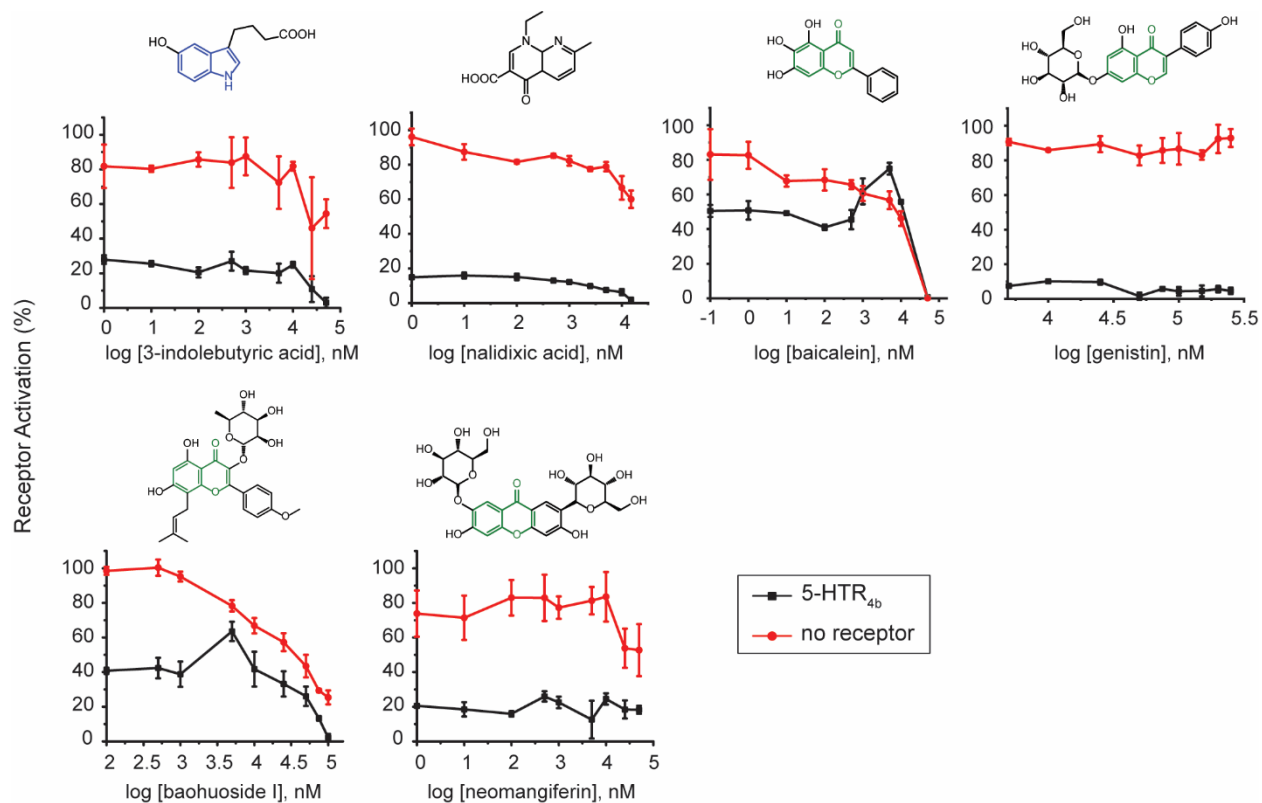


Fig. S2. Dose response curves of the 6 natural product hits that could not be validated

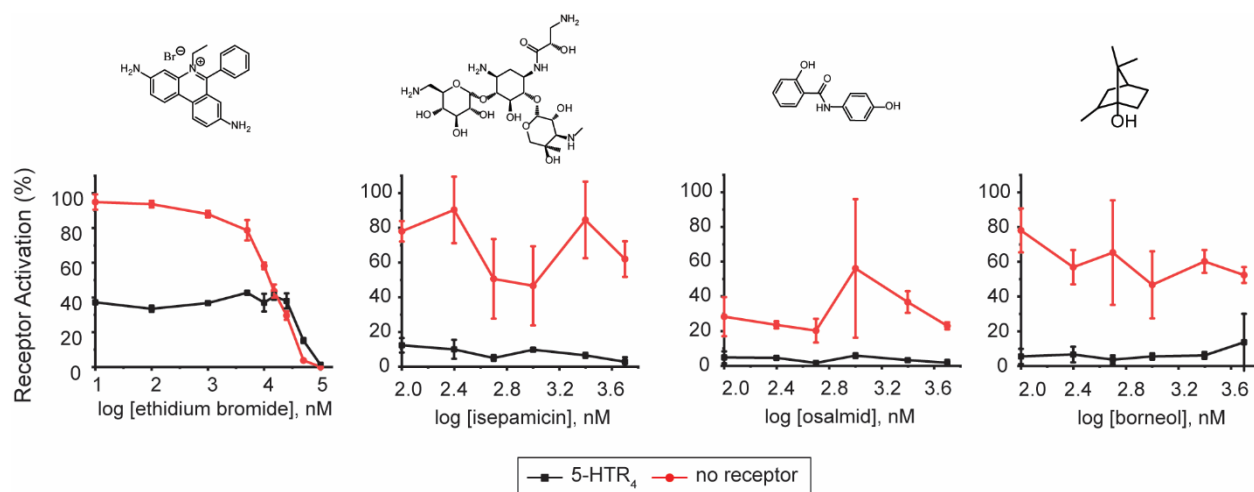


Fig. S3. Dose response curves of the four anti-infection hits that could not be validated

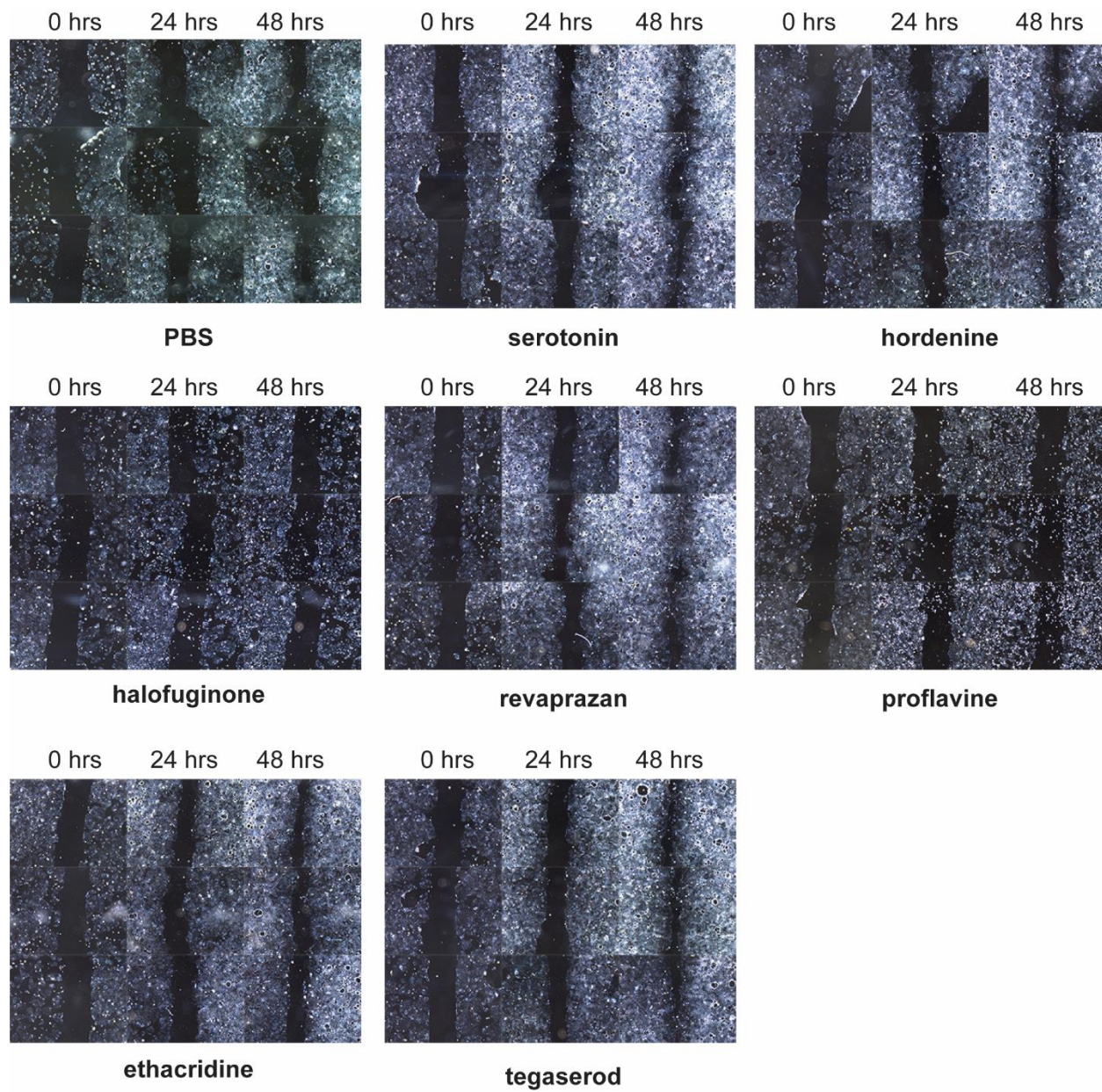


Fig. S4. Photomicrographs of colon cell migration assay after 0, 24, and 48 hours.

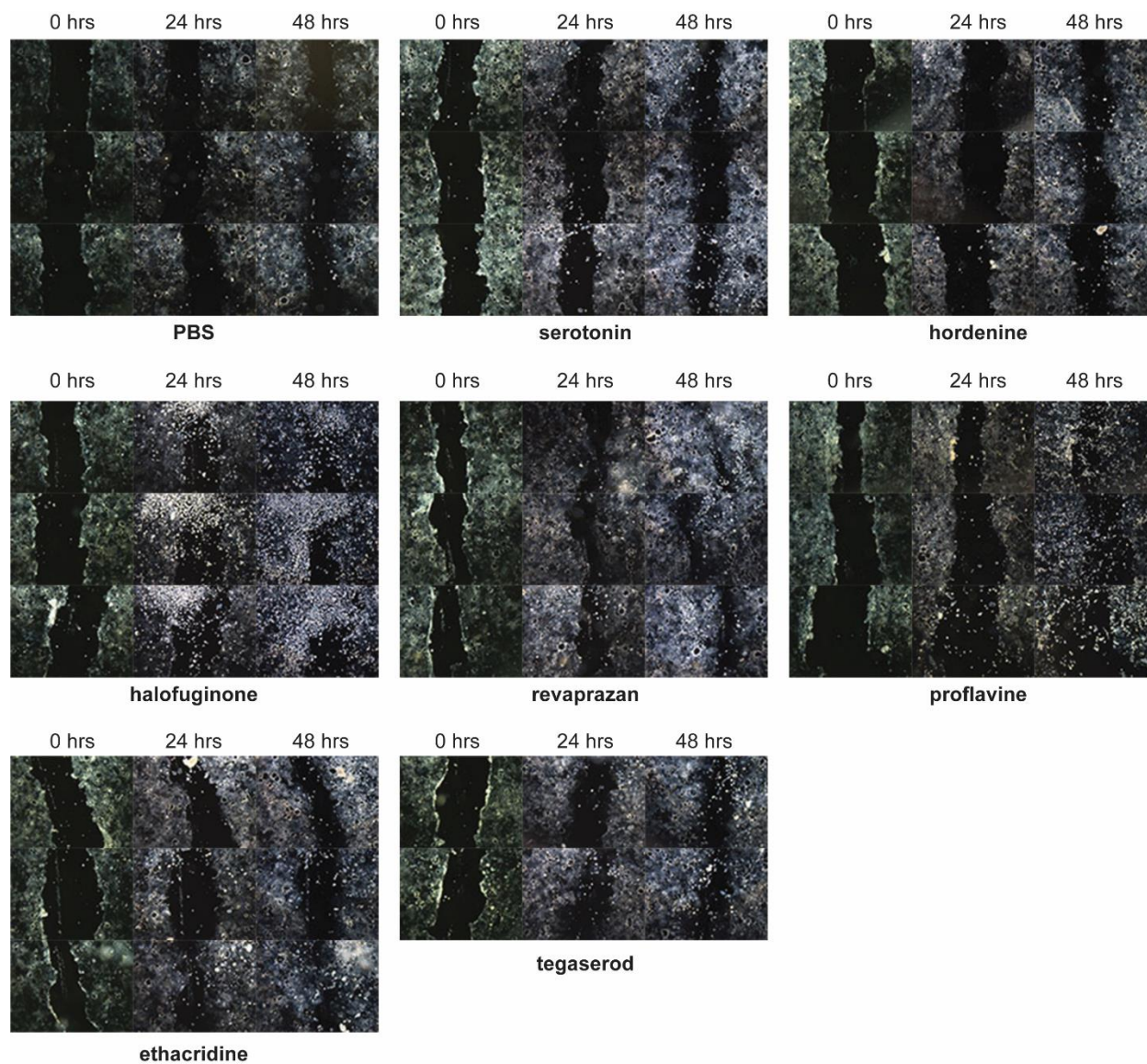


Fig. S5. Photomicrographs of colon cell scratch wound healing assay after 0, 24, and 48 hours.

Sequences

Yeast optimized Nanoluc sequence

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ATGGTCTTCACCTTGGAAGATTTTCGTCGGTGATTGGAGACAAACCGCTGGTTACAACCTTGG
ATCAAGTCTTAGAGCAGGGTGGAGTCTCCTCCTTGTTTCAAACCTTGGGAGTCTCCGTCAC
CCCAATTCAAAGAATTGTTTTGTCCGGTGAAAATGGTTTGAAAATTGATATTCATGTCATTAT
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ACCCAGTTGATGATCATCATTTTTAAAGTCATTTTGCATTACGGTACCTTGGTCATTGATGGT
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TGTGAAAGAATTTTGGCTTAA

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