

Screening of Natural Products and Approved Oncology Drug Libraries for Activity against *Clostridioides difficile*

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Table S1: Bacterial strains used in the study.

Bacterial strains / ID number	Source and comments
P2 /NR-32883	Toxin producing strain isolated from patient stool in western Pennsylvania, USA in 2001
P6/ NR-32886	Toxin producing strain isolated from the stool of a patient suffering from recurrent <i>C. difficile</i> infection in western Pennsylvania, USA in 2001
P7/ NR-32887	Toxin producing strain procured from patient stool in western Pennsylvania, USA in 2001
P8/ NR-32888	Toxin producing strain procured from patient stool in western Pennsylvania, USA in 2001
P9/ NR-32889	Toxigenic strain procured from fecal matter of a patient suffering from recurrent <i>C. difficile</i> infection in western Pennsylvania, USA in 2001
P19/ NR-32895	Toxigenic strain procured from fecal matter of a patient suffering from recurrent <i>C. difficile</i> infection in western Pennsylvania, USA in 2005
Isolate 1/ NR-13427	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 2/ NR-13428	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 4/ NR-13430	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 6/ NR-13432	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 9/ NR-13435	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 1/ NR-13436	Isolated from a patient diagnosed with CDI in the Mid-Atlantic region of the USA in 2008/2009
Isolate 20100502/ NR-49277	Isolated in 2010 from the fecal matter of an elderly male patient diagnosed with community-associated (CA) <i>C. difficile</i> infection in Colorado, USA
Isolate 20100207/ NR-49278	Isolated in 2010 from the stool of an elderly adult male patient diagnosed with healthcare-associated (HA) <i>C. difficile</i> infection in New York, USA
Isolate 20100211/ NR-49279	Isolated in 2010 from the stool sample of a pediatric female patient diagnosed with community-associated (CA) <i>C. difficile</i> infection in New York, USA
Isolate 20120016/ NR-49282	Isolated in 2011 from the stool sample of a pediatric female patient diagnosed with community-associated (CA) <i>C. difficile</i> infection in New York, USA

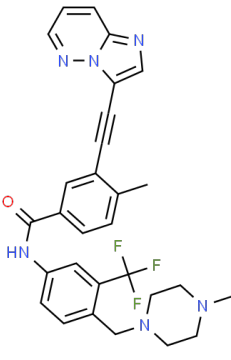
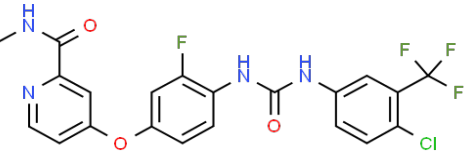
Isolate 20110999/NR-49286	Isolated in 2011 from the stool sample of an elderly female patient diagnosed with healthcare-associated (HA) <i>C. difficile</i> infection in western/midwestern, USA
Isolate 20110870/ NR-49288	Isolated in 2011 from the stool sample of a young adult female patient diagnosed with healthcare associated (HA) <i>C. difficile</i> infection in Tennessee, USA
Isolate 20120187/ NR-49290	Isolated in 2011 from the stool sample of an elderly adult male patient with healthcare-associated (HA) <i>C. difficile</i> infection in Tennessee, USA
ATCC BAA 1870	Presence of <i>cdtB</i> ^a , <i>tcdA</i> ^b , and <i>tcdB</i> ^c genes, classified as toxinotype IIIB, ribotype 027

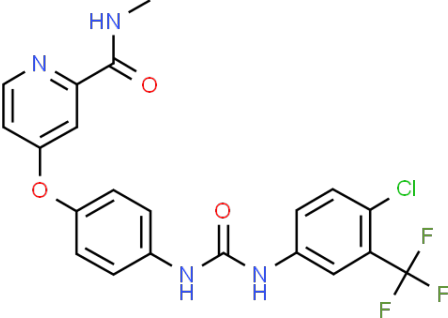
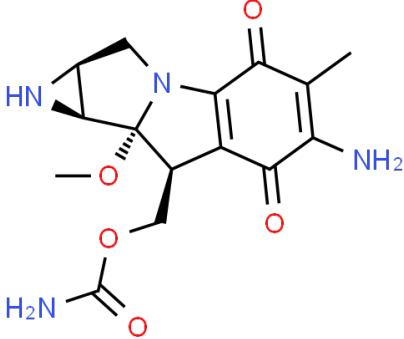
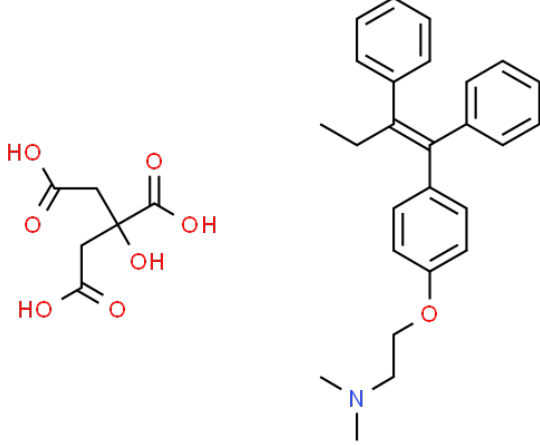
^a *cdtB*= *C. difficile* binary toxin

^b *tcdA*= *C. difficile* toxin A gene

^c *tcdB*= *C. difficile* toxin B gene

Table S2: Initial screening data, chemical structure, and description of the hits for the approved oncology drugs set V library against *C. difficile* ATCC BAA 1807.

	Compound name	Chemical structure	MIC (μM)	Description and use
1	Ponatinib		16	Tyrosine kinase receptor inhibitor that used in the therapy of refractory chronic myelogenous leukemia (CML).
2	Regorafenib		8	Oral multi-kinase inhibitor used in the therapy of refractory metastatic colorectal cancer, hepatocellular carcinoma and gastrointestinal stromal tumor.

3	Sorafenib	 <p>The chemical structure of Sorafenib consists of a central benzene ring. At the 1-position, there is a methanesulfonyl group (-SO₂CH₃). At the 4-position, there is a pyridine ring connected via an oxygen atom (-O-pyridin-2-yl). At the 5-position, there is a bis(4-chloro-2,2-difluorophenyl)amino group (-NH-CO-NH-2,2-difluoro-4-chlorophenyl).</p>	8	Protein kinase inhibitor used in the therapy of advanced renal cell, liver, and thyroid cancer.
4	Mitomycin C	 <p>The chemical structure of Mitomycin C features a central 1,4-dihydroquinone core. It has a methyl group at position 2, an amino group (-NH₂) at position 3, and a methyl group at position 4. At position 5, there is a side chain containing a bicyclic nitrogen-containing ring system (a decalin derivative) and a methyl ester group (-COOCH₃).</p>	0.5	Antineoplastic antibiotic used in stomach and pancreatic cancers.
5	Tamoxifen citrate	 <p>The image shows two chemical structures. On the left is the citrate salt of Tamoxifen, represented as a central carbon atom bonded to a hydroxyl group (-OH), a carboxylic acid group (-COOH), and two carboxylate groups (-COO⁻). On the right is the Tamoxifen molecule, which consists of a central carbon-carbon double bond. One carbon of the double bond is bonded to an ethyl group (-CH₂CH₃) and a phenyl ring. The other carbon is bonded to another phenyl ring and a 4-(dimethylaminoethoxy)phenyl group (-O-CH₂-CH₂-N(CH₃)₂).</p>	16	Selective estrogen receptor modulator used in malignant glioma and other cancers overexpressing protein kinase C expression.

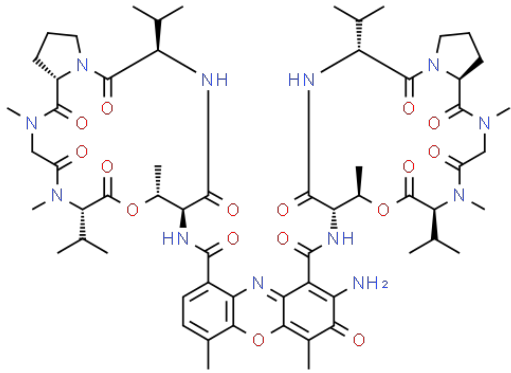
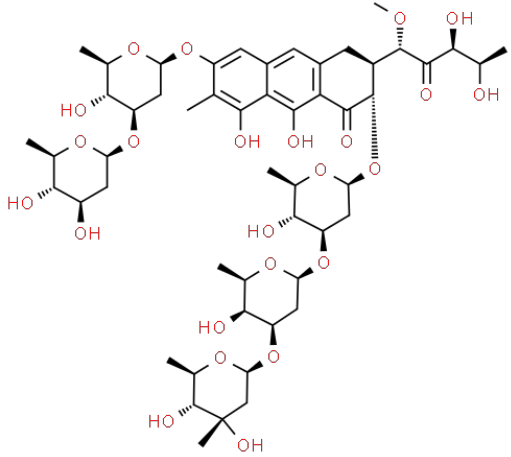
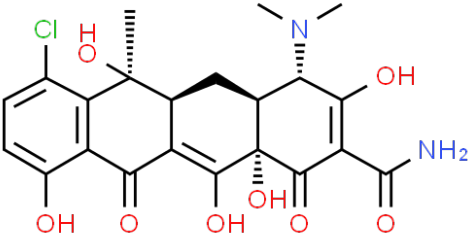
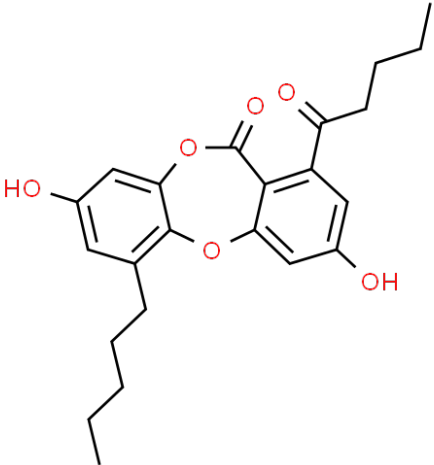
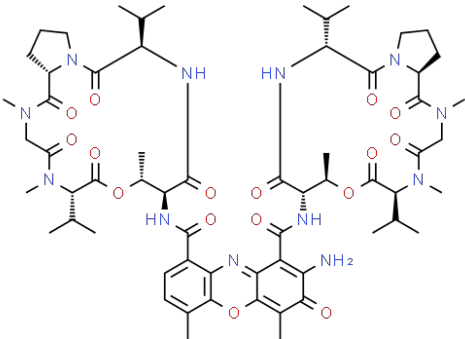
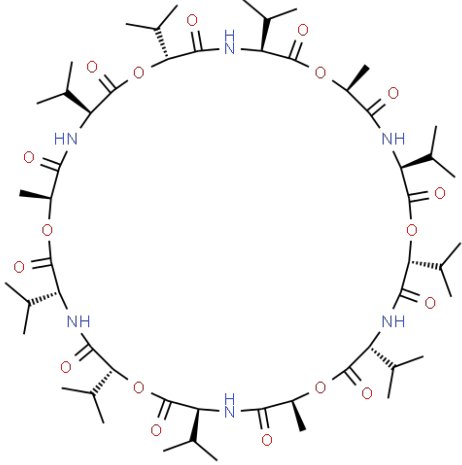
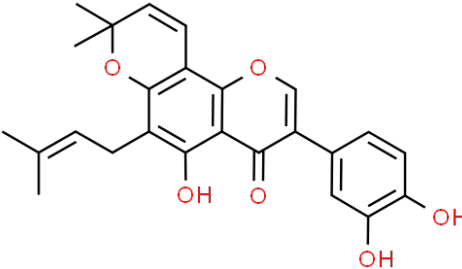
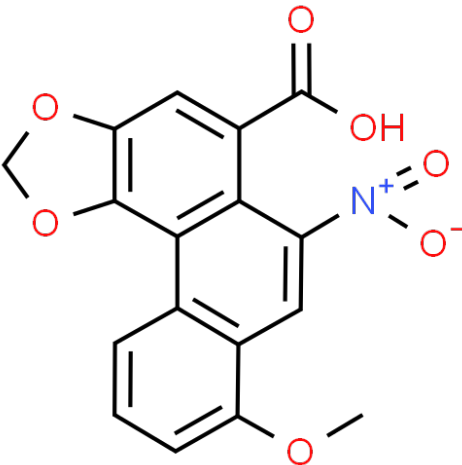
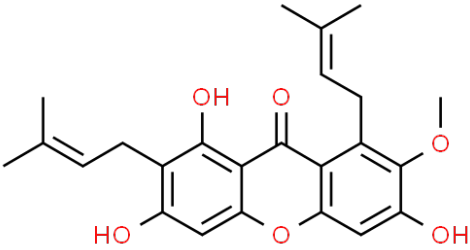
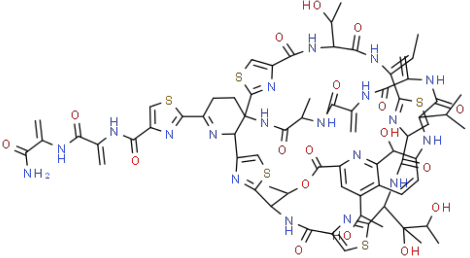
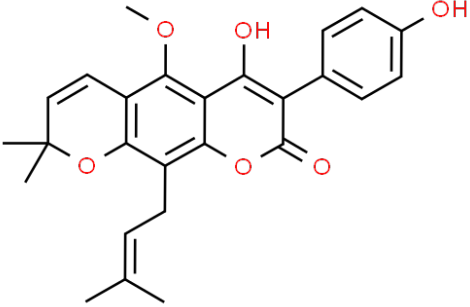
6	Actinomycin D		32	Antineoplastic antibiotic used in treating solid tumors in children and choriocarcinoma in adult women.
7	Mithramycin A/ Plicamycin		≤0.25	Antineoplastic antibiotic used in the treatment of testicular cancer.

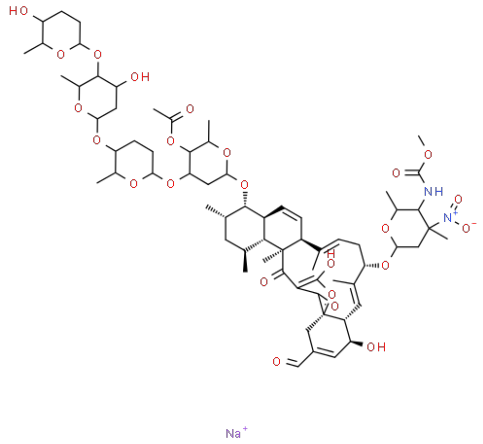
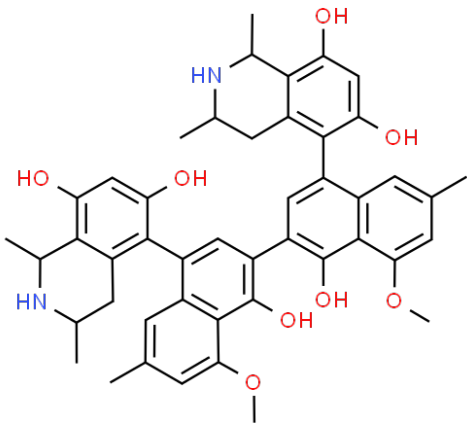
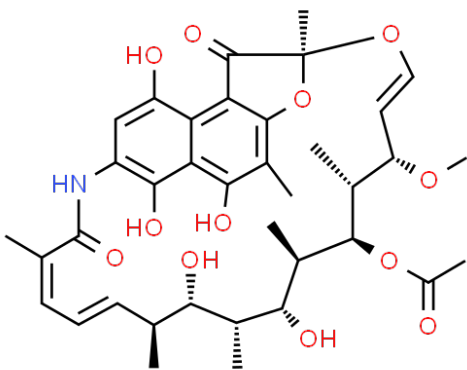
Table S3: Initial screening data, chemical structure, and description of the hits for the natural product set III library against *C. difficile* ATCC BAA 1807:

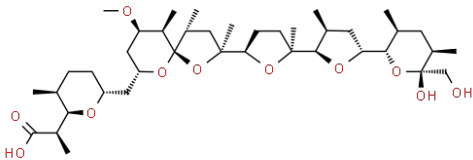
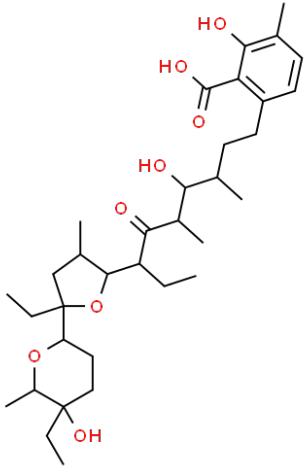
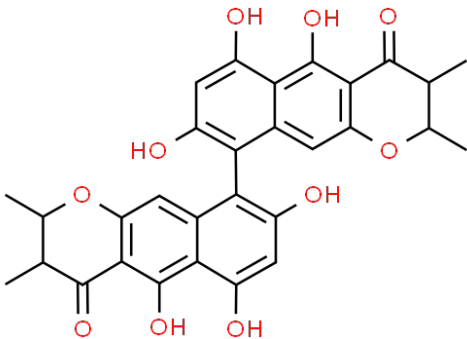
	Compound name	Chemical structure	MIC (μM)	Description and use
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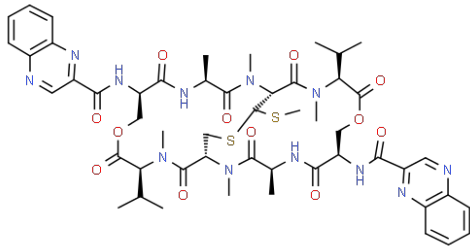
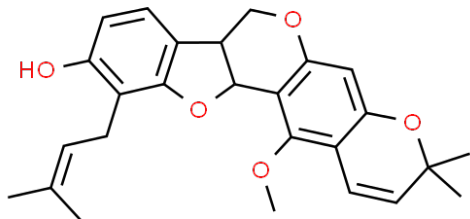
1	Aureomycin	 <p>The chemical structure of Aureomycin is a complex polycyclic molecule. It features a central benzene ring fused to a six-membered ring containing a nitrogen atom with two methyl groups. This is further fused to a five-membered ring with a carbonyl group and a hydroxyl group. The structure is highly substituted with various functional groups including a chlorine atom, multiple hydroxyl groups, and an amide group.</p>	0.5	An antiprotozoal and antibacterial drug.
2	Norlobaric acid	 <p>The chemical structure of Norlobaric acid is a dimeric molecule consisting of two substituted benzene rings linked by two ether bridges. Each benzene ring has a hydroxyl group and a long alkyl chain. The structure is highly substituted with various functional groups including hydroxyl groups and a long alkyl chain.</p>	32	--
3	Actinomycin D	 <p>The chemical structure of Actinomycin D is a complex polycyclic molecule. It features a central benzene ring fused to a six-membered ring containing a nitrogen atom with two methyl groups. This is further fused to a five-membered ring with a carbonyl group and a hydroxyl group. The structure is highly substituted with various functional groups including a chlorine atom, multiple hydroxyl groups, and an amide group.</p>	32	Antineoplastic antibiotic

<p>4</p>	<p>Valinomycin</p>		<p>32</p>	<p>Antibiotic</p>
<p>5</p>	<p>Pomiferin</p>		<p>32</p>	<p>A prenylated isoflavone found in <i>Maclura pomifera</i></p>
<p>6</p>	<p>Aristolochin</p>		<p>32</p>	<p>Monocarboxylic acid found in <i>Aristolochia</i> sp.</p>

7	Mangostin		16	Antineoplastic antibiotic isolated from the stems of <i>Cratoxylum cochinchinense</i> ,
8	Siomycin A		≤ 0.25	Antibiotic, antineoplastic
9	Lonchocarpic acid		32	Isoflavonoid

10	Tetrocarcin A, sodium salt	 <p style="text-align: center;">Na⁺</p>	0.5	Antitumor antibiotics
11	Michellamine B		16	Novel plant alkaloid inhibiting HIV virus
12	Rifamycin		≤0.25	Natural antibiotic

13	Nigericin		≤0.25	Polyether antibiotic
14	Antibiotic X-536A		1	--
15	Chaetochromin		0.5	Small molecule obtained from <i>Chaetomium gracile</i> fungi.

16	Levomycin		≤0.25	Polypeptide antibiotic
17	Gangetin		8	Hexane extract from the root of the plant <i>Desmodium gangeticum</i>