

Supplementary Information:

The *Clostridium difficile* drug pipeline: challenges in discovery and development of new agents

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1 Method

1.1 Chemoinformatic analysis of compounds with antimicrobial activity against *C. difficile*

Compounds with reported antimicrobial activity against *C. difficile* (Target ID: ChEMBL614965) were extracted from ChEMBL (Version 17) database into an Excel spreadsheet. The data was then manually separated into known, marketed antibiotics and research compounds based on the assigned chemical name (MOL_PREF_NAME). The activity data was standardized into $\mu\text{g/mL}$ and molar units, from which pMIC were calculated. PipelinePilot, Accelrys (Version 8.5.0.200) was used to further analyse the data extracted from ChEMBL. Briefly, Pipeline Pilot was used to generate the molecular structure from SMILES. The data was then merged based on the ChEMBL parent ID and pivoted on Standard_type with pivot value= Standard_Value . Compounds were clustered by structural similarity using tanimoto distances between FCFP_4 and ECFP_6 fingerprints and using the inbuilt partitioning method. The clustering was optimized by visual inspection of the clusters, resulting in a maximum of 14 clusters, and by recentering the clusters twice. The members of each clusters were exported into an Excel worksheet for further analysis, which permitted further adjustment of the clusters. The cluster groups were manually changed for structural outliers, such as if a β -lactam clustered with glycopeptides. The number of compounds in each distinct chemical class was then plotted in order to visualize the chemical space explored for *C. difficile* antimicrobials in the scientific literature.

2 Supplementary Table

Supplementary Table 1: Summary of single drug candidates tested against *C. difficile*.

	No. strains	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Year	Reference
Fluoroquinolones						
QA-241	24	12.5-25	25	25	1989	1
BMV40062	17	2-8	2	8	1989	2
Sitafloxacin (DU-6859a)	1	0.12			1997	3
CFC-222	13	0.39->50	3.13	25	1997	4
Y-688	12	≤0.008 to 1	0.5	0.5	1998	5
Sitafloxacin (DU-6859a)	143	0.03-1	0.12	0.12	2000	6
PGE 9262932	9	0.06	0.5	-	2001	7
PGE 9509924	9	1	1	-	2001	7
PGE 4175997	9	0.5-1	0.5	-	2001	7
WCK 771	12	0.5-4	0.5	1	2001	8
Delafloxacin (ABT-492)	12	≤0.015	≤0.015	≤0.015	2003	9
DX-619	17	0.25-32	2	2	2005	10
DX-619	1	0.125	-	-	2006	11
DC-159a	15	0.25-64	2	4	2007	12
ACH-702 (isothiazoloquinolone)	10	0.25-4	0.25	4	2011	13
Compound 1 (isothiazolone)	10	0.25-4	0.25	2	2007	14
ABT-719 (pyridone)	10	0.12	0.12	0.12	1995	15
Carbapenems						
BMS-181139	25	1-32	16	32	1995	16
BO-2727 (lenapenem),	11	0.25-4	1	2	1995	17
BO-3482	25	0.2-0.39	0.39	0.39	1997	18
J-111,347	21	0.5-1	1	1	2000	19
Cephalosporins						
RU 29 246	12	0.13-8	0.13	8	1992	20
FK041	1	12.5	-	-	1999	21
BMS-247243	2	32	-	-	2002	22
PPI-0903M (TAK-599)	10	0.06-8	2	4	2005	23
Clindamycin						
Pirlimycin	5	2-500	-	-	1984	24
Oxazolidinone						
Ranbezolid	10	0.03	0.03	0.03	2003	25
DA-7867	15	≤0.06 to 0.25	0.12	0.25	2004	26
B-lactam						
GV104326 (Sanfetrinem)	10	1-4	2	4	1996	27
BMS-180680 (SQ 84,100)	12	>128	>128	>128	1997	28
Tetracycline						
TBG-MINO	10	0.12-1	0.5	1	1999	29
DMG-MINO	10	≤0.06-0.5	0.25	0.5	1999	29
DMG-DMDOT	10	≤0.06-2	0.5	2	1999	29
Ketolide						
HMR 3647 (RU 66647)	10	0.06- >64	0.25	>64	1997	30
RU 64004	11	0.06- >64	0.12	>64	1997	31
HMR 3004 (RU 64004)	10	0.06->64	0.125	>64	1997	30

	No. strains	MIC range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Year	Reference
Telithromycin (HMR 3647; RU 66647)	14	0.06- >32	0.25	>32	1999	32
ABT-773	14	≤0.06->32	0.125	>32	2001	33
RBx 14255	28	0.125-8	0.5	4	2012	34

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