

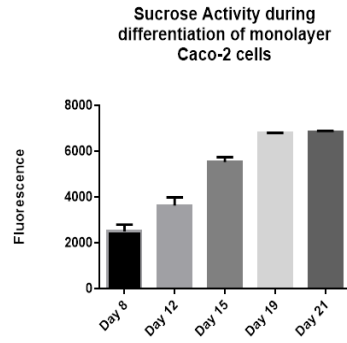
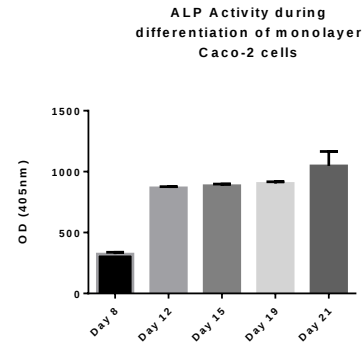
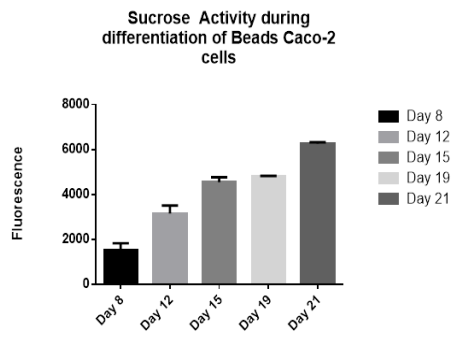
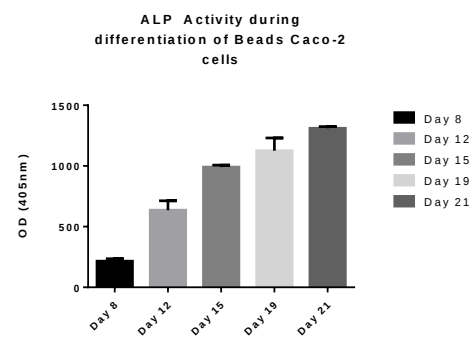
A**C****B****D**

Figure S1. Comparison of sucrose (A, B) and ALP (C, D) activity at various times after confluence between monolayer and 3-D caco-2 cell models.

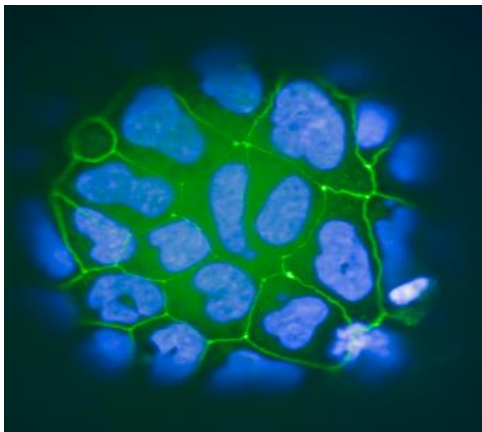
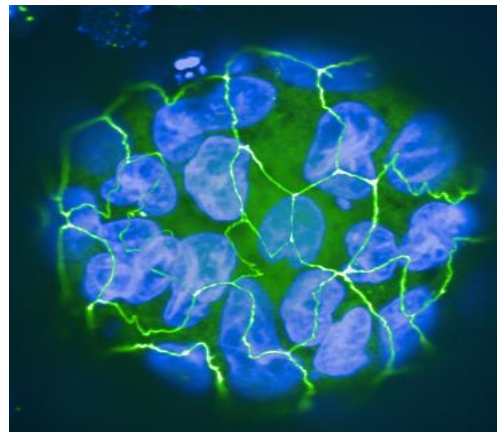
A**B**

Figure S2. The formation of tight-junctions in Caco-2 cells.

(A) Monolayer of Caco-2 cells; (B) Caco-2 cells on cytodex³ bead

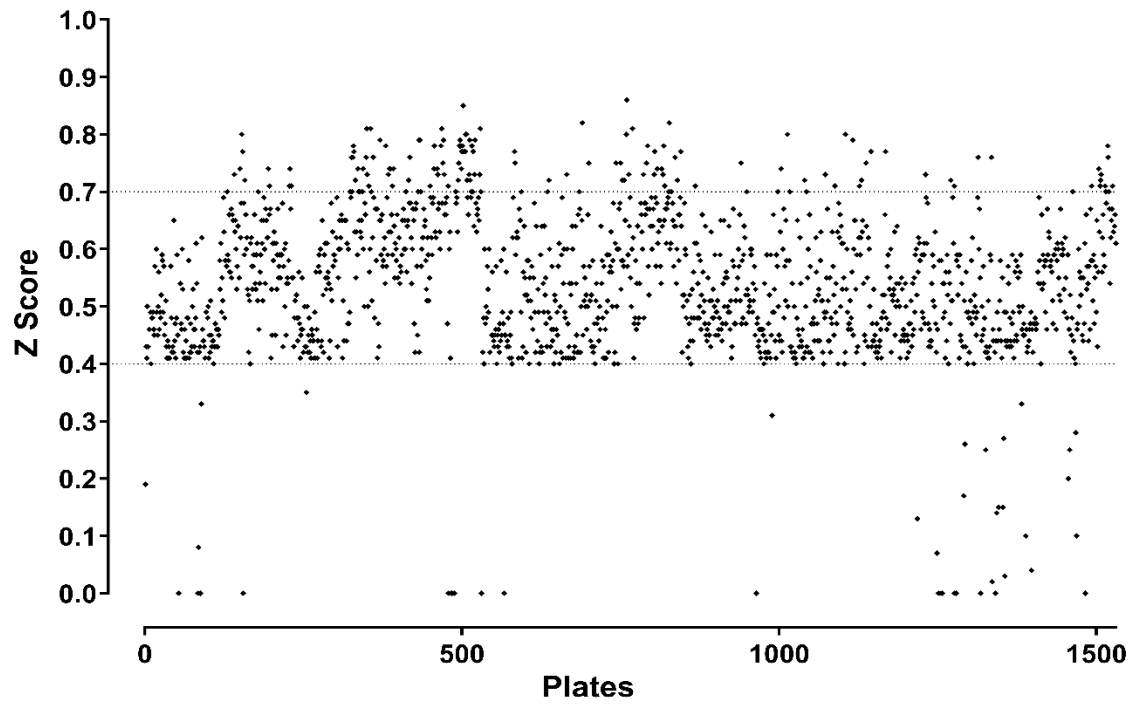


Figure S3: Primary screening result. Using a z-score cutoff of 0.4, 45 plates were defined as fail and were re-run. The distribution of the z-score for the 1521 plates is shown here.

Table S1: Strains, Cell Line and Plasmids used in this study.

Strain/Cell Line/Plasmid	Phenotype	Source
STRAINS		
<i>Shigella flexneri</i> 2a 2457T <i>Castellani and Chalmers</i> (ATCC® 700930™)	Wild type	American Type Culture Collection
<i>Shigella flexneri</i> SF_nanoluc	<i>S. flexneri</i> ATCC 700930 derivative containing plasmid pMK-RQ_Tac+Nanoluc	
<i>Shigella flexneri</i> 2b 12022 <i>Castellani and Chalmers</i> (ATCC® 12022GFP™)	<i>S. flexneri</i> 12022 derivative containing a multicopy vector encoding green florescent protein GFPmut3	American Type Culture Collection
	Ampicilin resistance	
CELL LINE		
Caco-2 cell (ATCC® HTB-37™)	Ethipelial cell	American Type Culture Collection
PLASMID		
pMK-RQ_Tac+Nanoluc	pMK-RQ derivative carrying nanoluciferase gene which is expressed under control of Tac promoter	Invitrogen
	Kanamycin resistance	

Table S2: Optimal conditions

	Invasive time (Hours)										Bead number (Beads/ml)		
	4H		6H		8H		10H		Over 16H		1000	2000	4000
MOI	%Cover	Z´	% Cover	Z´	% Cover	Z´	% Cover	Z´	% Cover	Z´	Z'	Z'	Z'
5	18.75	< 0	28.13	< 0	59.38	< 0	62.50	< 0	46.88	< 0	ND	ND	ND
	15.63	< 0	34.38	< 0	50.00	< 0	84.38	< 0	76.19	< 0	ND	ND	ND
	12.50	< 0	28.13	< 0	62.50	< 0	90.63	< 0	56.25	< 0	ND	ND	ND
10	15.63	< 0	46.88	< 0	81.25	< 0	59.38	< 0	37.50	< 0	ND	ND	ND
	12.50	< 0	34.38	< 0	90.63	< 0	81.25	< 0	40.63	< 0	ND	ND	ND
	21.88	< 0	40.63	< 0	87.50	< 0	84.38	< 0	0	< 0	ND	ND	ND
100	96.88	0.15	84.38	< 0	96.88	0.06	ND	ND	ND	ND	ND	ND	ND
	93.75	0.16	100	0.51	100	0.40	ND	ND	ND	ND	ND	ND	ND
	87.50	< 0	96.88	0.35	100	0.46	ND	ND	ND	ND	ND	ND	ND
150	100	0.62	100	0.52	96.88	0.31	ND	ND	ND	ND	< 0	0.22	0.61
	96.88	0.31	100	0.58	93.75	0.23	ND	ND	ND	ND	0.06	0.37	0.61
	100	0.59	100	0.61	100	0.37	ND	ND	ND	ND	< 0	0.34	0.50

*ND: Not done

Table S3. Shigella invasive efficiency

Invasive time	2h	4h	6h
Invasive rate (%)	0.003	0.032	0.083
Shigella per cell (CFU/cell)	0.010	0.044	0.069
Shigella per bead (CFU/bead)	1.748	7.750	15.077

Table S4. Intra- and inter-assay on inhibition rate and IC₅₀ values

Drugs	1 st Batch		2 nd Batch		IC50 value CV (%)		Source
	T1	T2	T3	T4	Intra-	Inter-	
Meropenem	0.3486	0.3654	0.4044	0.3993	1.49	6.13	M2574, Sigma-Aldrich
Tetracycline	1.6104	1.5576	1.1877	1.1806	0.98	14.51	T7660, Sigma-Aldrich
Faropenem	1.2991	1.2299	1.4312	1.444	1.59	6.67	F8182, Sigma-Aldrich
Ceftriaxone	0.0151	0.0151	0.0196	0.0196	0.00	12.97	C5793, Sigma-Aldrich
Azithromycin	0.569	0.5105	0.6244	0.6242	2.72	8.09	75199, Sigma-Aldrich
Furazolidone	1.0673	1.0674	1.3558	1.3678	0.22	12.13	F9505, Sigma-Aldrich
Moxifloxacin	0.0252	0.0221	0.0235	0.0235	3.28	4.66	SML1581, Sigma-Aldrich
Ciprofloxacin	0.0119	0.0119	0.0156	0.0157	0.16	13.61	17850, Sigma-Aldrich
Gentamicin	1.5172	1.2734	1.1708	1.2406	5.82	10.03	G1264, Sigma-Aldrich
CHIR-090	0.1895	0.1955	0.2593	0.2593	0.78	14.82	SML3092, Sigma-Aldrich
PF5081090	0.0957	0.0919	0.0937	0.0938	1.04	1.43	PZ0194, Sigma-Aldrich

Table S5. Validation set output

Profile		Compound A	Compound B	Compound C	Compound D
In vitro biology	Intra (Caco2) IC ₅₀ (uM)	5.03	6.13	6.85	36.31
	Extra IC ₅₀ (Res,uM)	8.9	>100	8.7	>100
Physch-invitro DMPK	MW	350.3	470.5	410.3	463.4
	ChromLogD ^{7.4} / PFI	(1.05 th /4.05 th) 2.46 / 5.46	(3.47 th /7.47 th) 3.18/ 7.18	(4.6 th /7.6 th) 5.69 / 8.69	(4.05 th /7.05 th) 4.63 / 7.63
	CAD_Sol (uM)/Fassif	122	5	13	49
	AMP(nm/s)	Low th / nd	Low th / < 3	High th /350	High th /nd
Safety	HepG2 IC ₅₀ (uM)	Not done	>100	100	Not done
	<i>In silico</i> eXP	No alerts	BSEP, MAOA	BSEP, 5HT2C	PLD, HT2Cantg, BSEP, hERG, 5HT2Aantg
	<i>In silico</i> Tox Alerts	Furan toxicity Aromatic amine (hepatotoxicity)	Imidazole tox Aromatic amine (mutagenicity)	Aniline (mutagenicity) Sulphonamide (CYP)	Pyridine tox

Table S6: Toxicity profiles

Compound	Cluster	Caco2 pIC50	HepG2 pIC50	Selectivity (log)	Structure Alert
				Caco pIC50 vs Cytotox	
1	Series 1	4.63	4.71	0	Aromatic nitro; Aromatic amine; Pyridine tox
2	Series 1	4.69	4.64	0	Aromatic nitro; Thiophene tox
3	Series 2	4.86	nd	nd	Aromatic nitro; Aromatic amine; Secondary sulfonamides
4	Series 2	5.06	<4	>1.06	Aromatic amine; Secondary sulfonamides
5	Singleton 1	4.72	<4	>0.72	No alerts
6	Singleton 2	5.04	<4	>1.04	No alerts
7	Singleton 3	5.49	<4	>1.49	Aromatic amine
8	Singleton 4	4.64	4.26	0.38	Aromatic amine
9	Singleton 5	4.7	4.12	0.58	No alerts
10	Singleton 6	5.07	<4	1.07	Thiazole toxicity
11	Singleton 7	5.24	4.63	0.61	Aromatic nitro
12	Singleton 8	5.80	<4	1.80	No alerts
13	Singleton 9	4.61	<4	>0.61	No alerts
14	Singleton 10	4.61	<4	>0.61	Aromatic amine; Furan toxicity
15	Singleton 11	4.76	<4	>0.76	Aromatic amine; Secondary sulfonamides