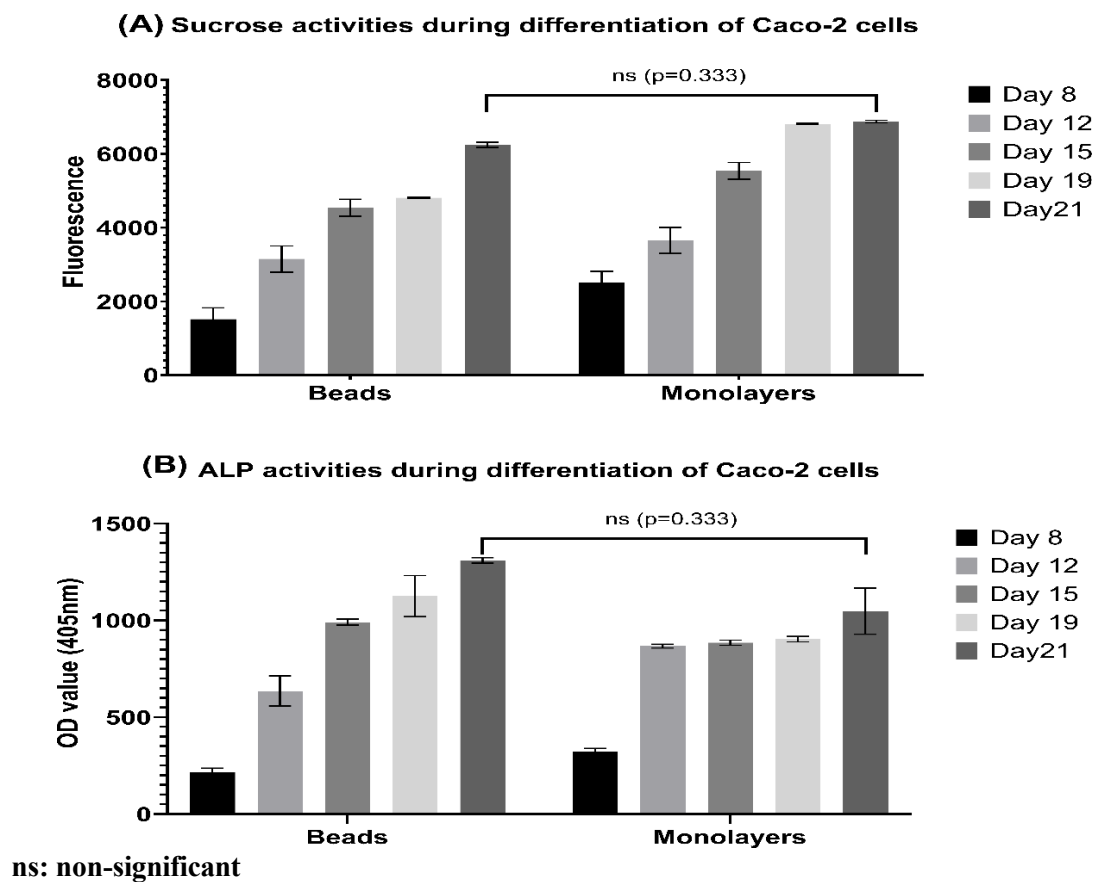
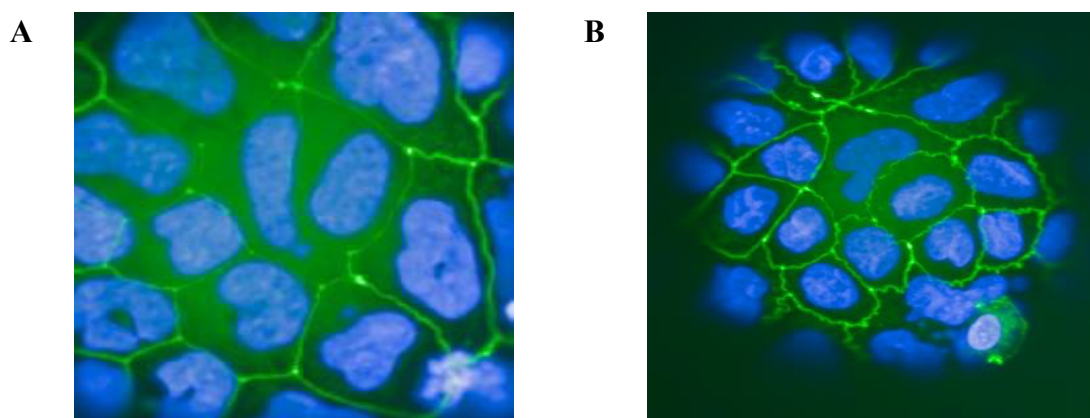


Supplementary figure 1. Comparison of ALP and Sucrose activity at various times after confluence between monolayer and 3-D caco-2 cell models



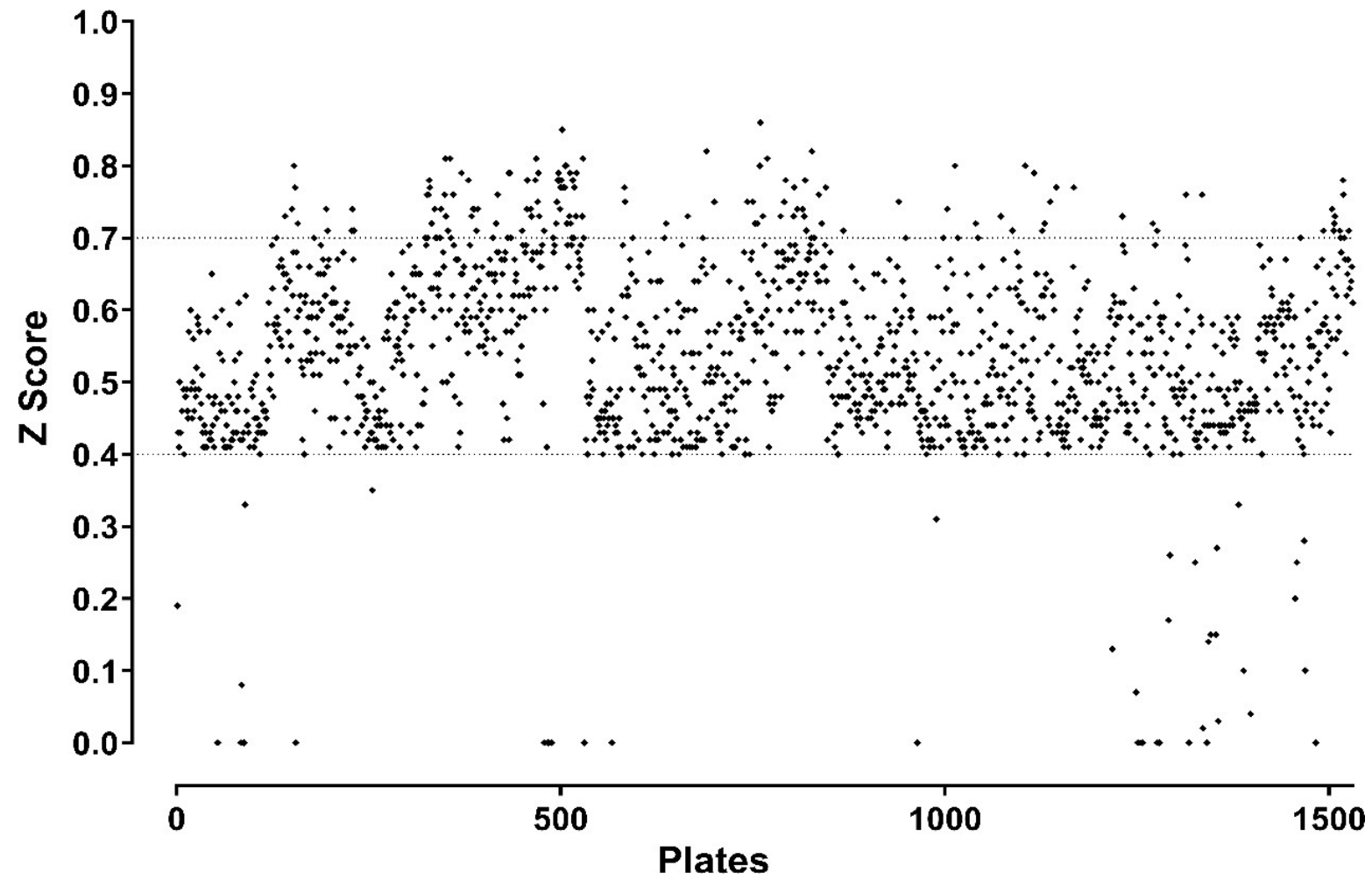
Supplementary figure 2. The formation of tight-junctions in Caco-2 cells.

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



Supplementary figure 3: 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 1,521 plates is shown here.



Supplementary table 1. Optimal experimental 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

Supplementary table 2. *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

Supplementary table 3. Bacterial strains, cell lines and plasmids

Strain/Cell Line/Plasmid	Phenotype	Source
Bacterial 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 lines		
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 exressed under control of Tac promoter	Invitrogen
	Kanamycin resistance	

Supplementary table 4. Intra- and inter-assay on IC50 values (μM)

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

Supplementary table 5: Equations for determining assay performance or sensitivity

Parameters	Equations
Coefficient of Variation	$\%CV = \frac{\sigma}{\mu} \times 100$
Z' factor	$Z' = 1 - \frac{(3\sigma_{\max} + 3\sigma_{\min})}{ \mu_{\max} - \mu_{\min} }$
Signal to background	$S:B = \frac{\mu_{\max}}{\mu_{\min}}$
Inhibited rate	$\%Inhibition = (1 - \frac{\text{Compound signal} - \text{min signal}}{\text{max signal} - \text{min signal}}) \times 100$
pIC50 value	$pIC50 = -\log_{10}(IC50)$
Note: σ , s.d. of the assay signal; μ , mean of the assay signal; max signal, signal with no inhibition; min signal, signal with 100% inhibition.	

Supplementary table 6. IC50 comparison between monolayer-based and bead-based assays

Moxifloxacin IC50 (μM)		
	Monolayers	Beads
	0.0236	0.0361
	0.0234	0.0327
	0.0228	0.0226
Average	0.0232	0.0305
<i>Mann Whitney</i> test		
P value	0.7	
Exact or approximate P value?	Exact	
P value summary	ns	
Significantly different (P < 0.05)?	No	

Supplementary table 7. Validation set output

Profile		Compound A	Compound B	Compound C	Compound D
In vitro biology	Intra (Caco2) IC50(uM)	5.03	6.13	6.85	36.31
	Extra IC50 (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 IC50 (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

Supplementary table 8. Toxicity profiles

Compound	Cluster	Caco2 pIC50	HepG2 pIC50	Selectivity (log) Caco pIC50 vs Cytotox	Structure Alert
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