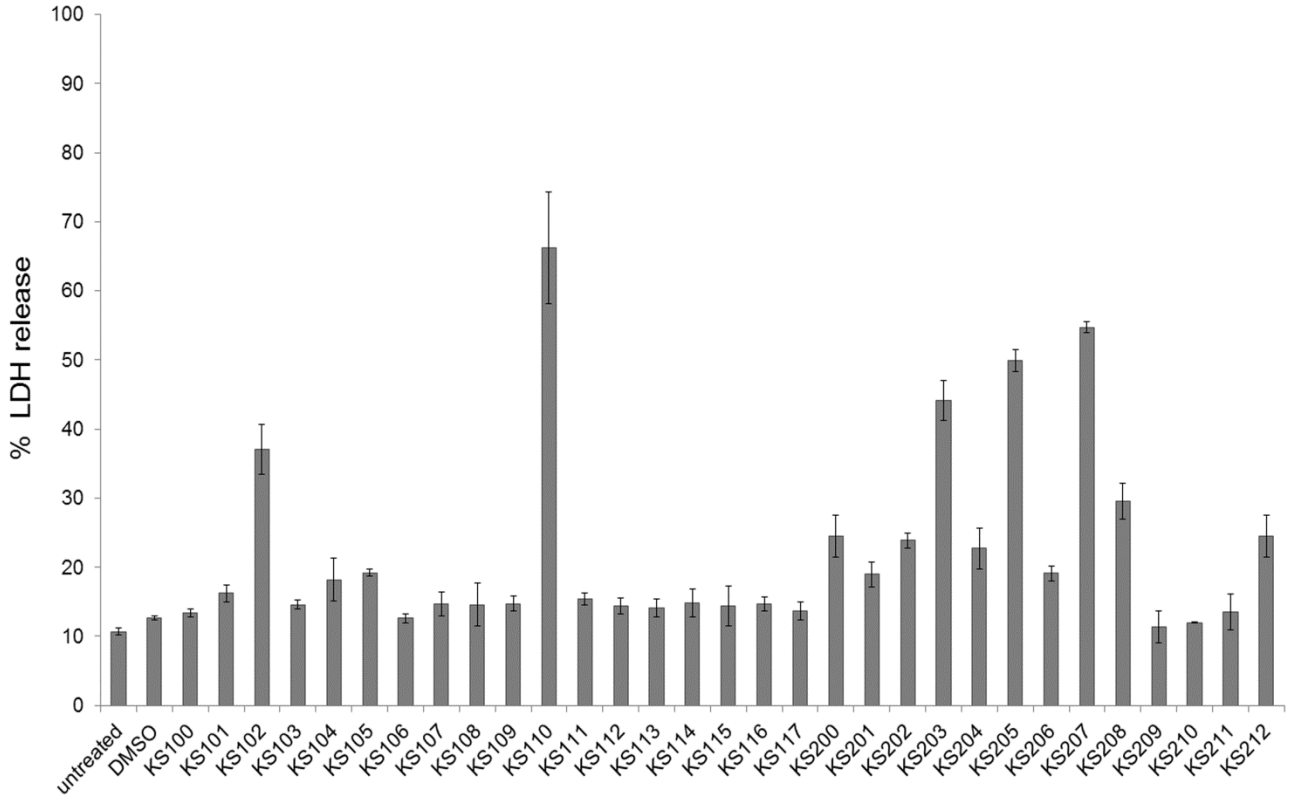


Fig. S1. Cytotoxicity of the compounds towards J774 macrophages. J774 cells were incubated with 0.5 % DMSO or compounds at 50 µM for 24 h. Data are presented as means ± SD of three wells from one representative experiment of three.

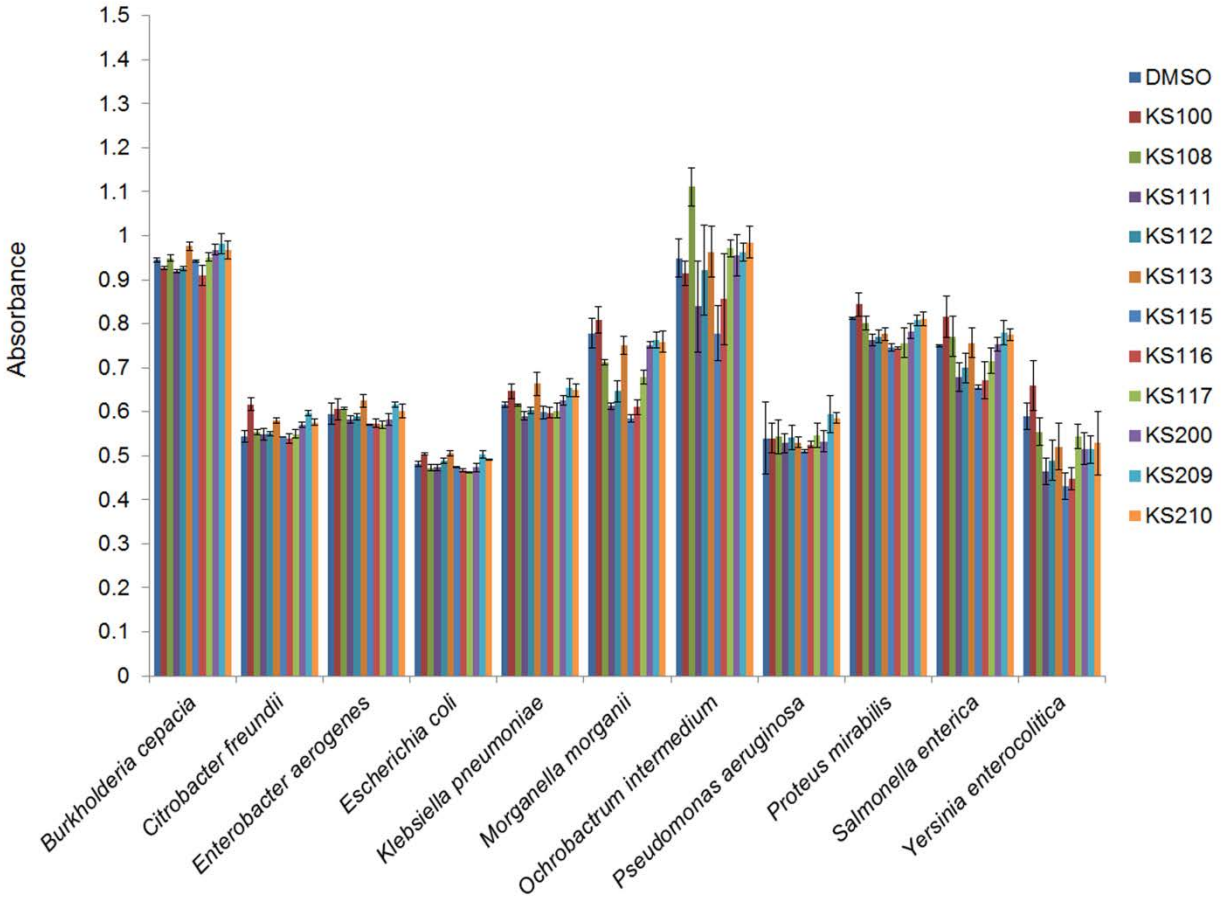


Fig. S2. Effects of the compounds on the growth of strains of selected Gram-negative species. Bacterial strains were incubated with 0.5 % DMSO or compounds at 50 µM for 18 h. Experiments were carried out in triplicates, and the means ± SD from one representative experiment of three are shown. The addition of compounds did in no case significantly affect the growth of the bacterial strains in comparison to cultures with DMSO only ($P > 0.05$).

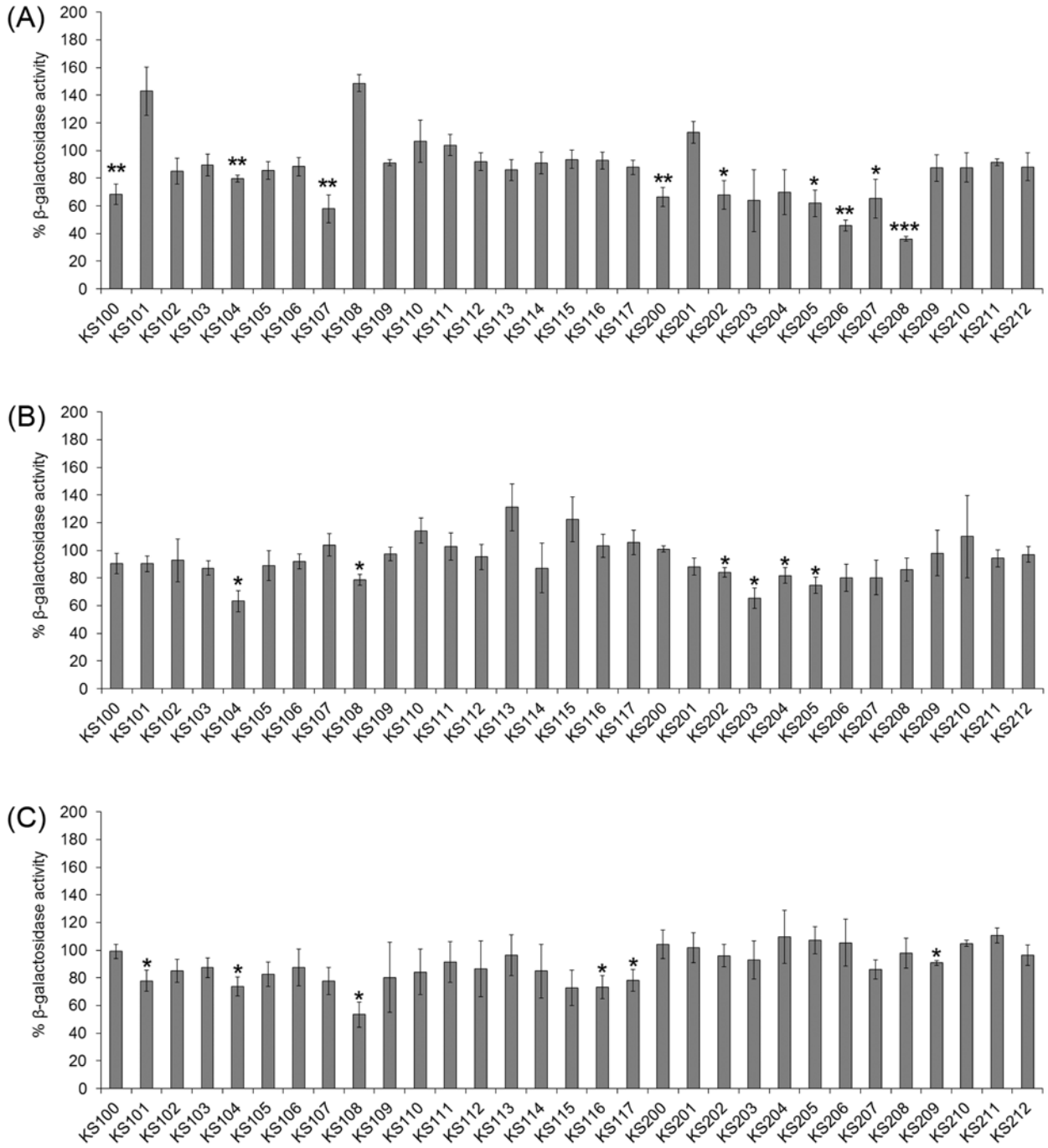


Fig. S3. KS100 and KS200 and the analogues inhibitory effects on the Bacterial-2-hybrid interactions between VipA-VipB (A), AaiA-AaiB (B), or HsiB2-HsiC2 (C) were tested by assessing the β-galactosidase activity and expressed as percentages relative to the control with DMSO only. Data are presented as means ± SD of three wells from one representative experiment of three. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Table S1. Strains and plasmids used in this study.

Strain or plasmid	Relevant genotype or phenotype	Reference
Strain		
Escherichia coli		
KDZif1ΔZ	*araD(gpt-lac)5, rpsL (Str^R), ΔspoS3::cat (Cm^R) [F' lacI^q (Z321[-61] lacZYA*) Km^R]*	(1)
CCUG 10979	clinical isolate	http://www.ccug.se
536	UPEC isolate (O6:K15:H31), Sm^R	(2)
363	clinical isolate, Km^R	this study
Top10	F-*mcrA*, Δ(*mrr-hsdRMS-mcrBC*), ϕ80*lacZ*ΔM15, Δ*lacX*74, *recA1*, *deoR*, *araD*139, Δ(*ara-leu*)7679, *galU*, *galK*, *rpsL* (Str^R), *endA1*, *nupG*	Invitrogen
DH5α F´IQ	F-φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (rk-, mk+) *pho*A *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1/F´ *pro*AB+ *lac*IqZΔM15 zzf::Tn5 [Km^R].	Invitrogen
Vibrio cholerae		
V52	O37 serotype	(3)
Burkholderia cepacia		
CCUG 13226	clinical isolate	http://www.ccug.se
Citrobacter freundii		
CCUG 418	clinical isolate	http://www.ccug.se

Enterobacter aerogenes		
CCUG 1429	clinical isolate	http://www.ccug.se
Klebsiella pneumonia		
CCUG 225	clinical isolate	http://www.ccug.se
Morganella morganii		
CCUG 6328	clinical isolate	http://www.ccug.se
Ochrobactrum intermedium		
CCUG 39736	clinical isolate	http://www.ccug.se
Proteus mirabilis		
CCUG 26767	clinical isolate	http://www.ccug.se
Pseudomonas aeruginosa		
1824	clinical isolate	(4)
PAK	wild type	(5)
Salmonella enterica		
CCUG 19369	clinical isolate	http://www.ccug.se
Yersinia enterocolitica		
CCUG 8233	clinical isolate	http://www.ccug.se

Plasmid		
pCR®4-TOPO®	TA cloning vector, Km^R, Cb^R	Invitrogen
pACTR-AP-Zif	Zif/ω Bacterial 2-Hybrid reporter vector, Tet^R	(1)
pJEB794	pACTR-AP-Zif encoding VipA, Tet^R	(6)

pACTR-MglA-Zif	pACTR-AP-Zif encoding MglA, Tet^R	(7)
pMOL135	pACTR-AP-Zif encoding IglA, Tet^R	(8)
pMOL139	pACTR-AP-Zif encoding IglB, Tet^R	(8)
pSK201	pACTR-AP-Zif encoding ECP0238, Tet^R	this study
pSK202	pACTR-AP-Zif encoding PA1657, Tet^R	this study
pBRGPω	Zif/ω Bacterial 2-Hybrid reporter vector, Cb^R	(1)
pJEB799	pBRGPω encoding VipB, Cb^R	(6)
pBRSspA-ω	pBRGPω encoding SspA, Cb^R	(7)
pMOL133	pBRGPω encoding IglA, Cb^R	(8)
pMOL134	pBRGPω encoding IglB, Cb^R	(8)
pSK203	pBRGPω encoding ECP0237, Cb^R	this study
pSK204	pBRGPω encoding PA1658m, Cb^R	this study

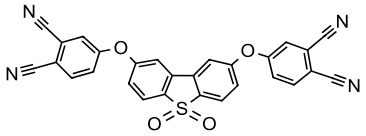
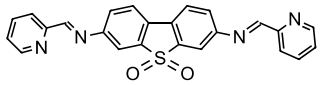
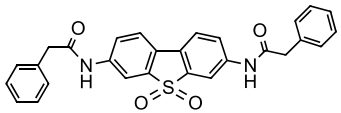
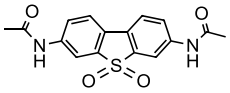
Table S2. Oligonucleotides used in this study

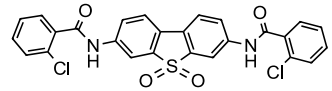
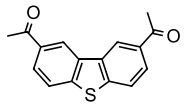
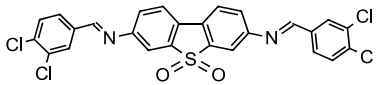
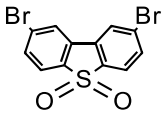
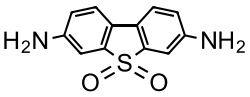
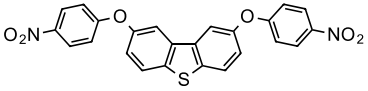
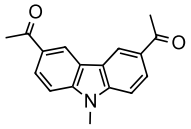
Insert	Oligonucleotide pairs
ECP0237	ECP_0237_F: 5´-*CAT ATG* TCA GTA AAG GAA GAA ATT GC-3´ (*Nde*I) and ECP_0237_NotI_R: 5´-*GCG GCC GC*T TCC TTA TCC AGT CGT CC3´ (*Not*I)
ECP0238	ECP_0238_F: 5´-*CAT ATG* ATA GCA GTA AAA GAT ATA ACT GAT-3´ (*Nde*I) and ECP_0238_NotI_R: 5´- *GCG GCC GC*T TTC TGA ACG GCG ATA CC-3´ (*Not*I)
PA1657	PA1657_F: 5´-*CAT ATG* ATG GCC AAA GAA GGC TCG GTA-3´ (*Nde*I) and PA1657_NotI_R: 5´- *GCG GCC GC*G GCG TCC TGG GAG GGG-3´ (*Not*I)
PA1658m	PA1658_F: 5´-*CAT ATG* ATG AGC ACC AGT GCC GCA CAG-3´ (*Nde*I) and PA1658_NotIm_R: 5´- CTT CG GA CCG GCG GC**T** GCC ACG-3´ PA1658_NotIm_F5´- GCG CCA TTC GTG GC**A** GCC GCC-3´ and PA1658_NotI_R: 5´- *GCG GCC GC*C TCT TTG TCC AGC TTG CCG A-3´ (*Not*I)

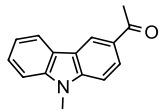
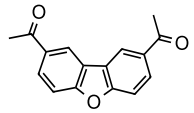
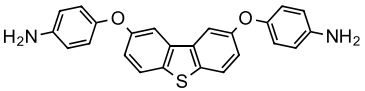
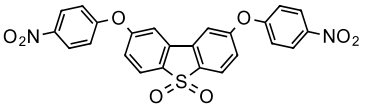
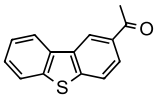
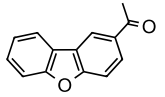
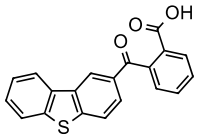
The oligonucleotide sequences in italics represent the incorporated *Nde*I and *Not*I restriction sites used for cloning of the PCR amplified DNA fragments.

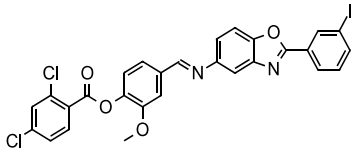
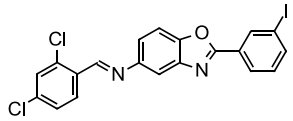
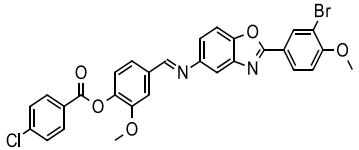
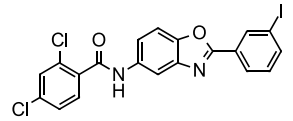
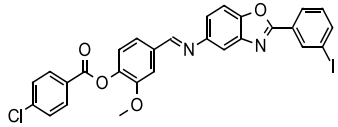
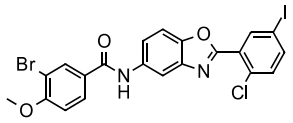
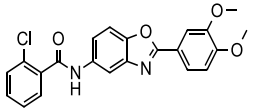
Underlined sequences indicate the complementary sequences in the overlap PCR primers. In the primers that were used to generate amino acid substitutions, the nucleotides substituted are indicated in boldface.

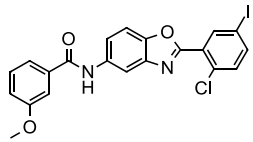
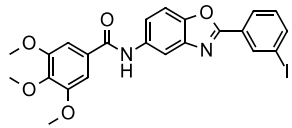
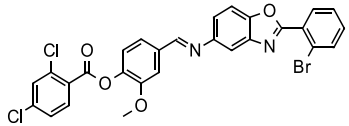
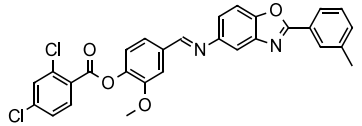
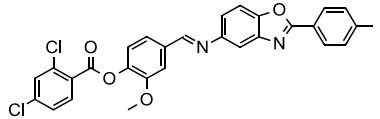
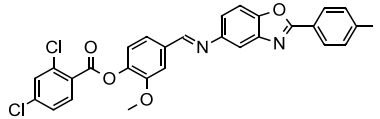
Table S3. Structures, *in silico* calculations, effects on VipA-VipB interaction, Hcp secretion, phospholipase activity and cytotoxicity.

ID	logP[a]	logS[b]	Caco-2[c]	MDCK[d]	SASA[e]	B2H (%)[f]	Hcp secretion[g]	PLA_1(%)[h]	LDH release(%)[i]	Cytotoxicity[j]	Structure
KS100	1.4	-7.0	10	0	750	68.4±7.4** [k]	4	42.5±3.4	13.4±0.6	1	
KS101	3.7	-5.8	430	200	758	142.9±17.3*	3	96.9±1.8	16.2±1.2**	3	
KS102	4.1	-6.8	230	100	821	85.0±9.3	4	80.2±5.9	37±3.6***	5	
KS103	0.3	-3.2	120	50	603	89.6±7.9	5	42.2±5.0	14.6±0.7*	4	

KS104	4.1	-7.2	230	370	798	79.6±2.4**	2	86.1±1.2	18.2±3.1*	4	
KS105	2.7	-3.7	1000	890	518	85.6±6.6	5	57.1±2.9	19.2±0.5***	4	
KS106	7.0	-9.6	1100	10000	858	88.4±6.8	4	52.6±3.1	12.6±0.6	4	
KS107	2.7	-5.9	1500	5300	472	57.9±10.2**	2	48.4±3.3	14.7±1.7	4	
KS108	0.3	-2.7	100	40	445	148.5±6.1***	5	88.4±2.2	14.6±3.1	1	
KS109	5.7	-8.9	140	110	726	91.1±2.3	3	37.6±4.0	14.7±1.1*	3	
KS110	4.1	-3.9	980	490	535	106.7±15.2	4	98.0±0.4	66.2±8.1***	5	

KS111	3.4	-3.7	3100	1700	473	103.9±7.8	4	90.1±1.6	15.4±0.8*	1	
KS112	2.1	-3.1	1000	500	495	72.0±6.2	4	85.8±0.1	14.4±1.2	1	
KS113	5.0	-7.3	670	570	678	85.9±7.8	4	16.9±4.7	14.1±1.3	1	
KS114	3.2	-5.7	20	10	743	90.9±7.8	4	40.3±3.6	14.8±2.0	4	
KS115	3.4	-3.7	3200	3100	456	93.6±6.5	4	84.2±1.4	14.4±2.9	1	
KS116	2.7	-3.2	3200	1700	432	72.7±6.1	4	79.6±0.6	14.7±1.0*	1	
KS117	4.2	-5.5	140	180	575	87.8±5.0	4	98.9±1.7	13.7±1.3	1	

KS200	7.6	-11.5	2700	10000	896	66.4±6.8**	2	94.1±0.7	24.5±3.0**	2	
KS201	6.6	.8.1	5600	10000	684	113.2±8.0	1	41.2±6.1	19.0±1.8**	2	
KS202	7.2	-10.0	2600	8900	929	67.7±10.4*	3	87.7±0.9	23.9±1.1***	5	
KS203	5.6	-9.1	2600	10000	682	63.7±22.3	3	59.9±4.4	44.1±2.9***	5	
KS204	7.2	-10.7	2600	9800	893	69.8±16.1	3	53.9±4.0	22.7±2.9**	4	
KS205	5.8	-10.3	2400	10000	732	61.9±9.6*	2	55.8±6.8	49.9±1.6***	5	
KS206	4.6	-6.3	2600	2700	698	45.7±3.8**	1	48.9±3.1	19.1±1.1***	4	

KS207	5.2	-7.3	2400	7700	702	65.2±13.9*	3	74.3±3.3	54.7±0.8***	5	
KS208	5.1	-7.2	2500	3800	763	36.1±1.9***	2	45.3±4.2	29.5±2.6***	5	
KS209	7.4	-9.5	2500	10000	882	87.3±9.8	3	56.1±2.9	11.4±2.3	1	
KS210	7.2	-9.1	2700	6800	893	87.8±10.7	3	40.8±4.1	12±0.1**	1	
KS211	7.6	-9.9	2700	10000	896	91.4±2.6	2	63.7±0.3	13.5±2.6	3	
KS212	6.1	-9.1	5600	10000	682	88.2±10.0	4	71.0±4.0	24.5±3.0**	5	

[a] The octanol/water partition coefficient. A high value indicates a more hydrophobic compound.

[b] The water solubility. A high value indicates a water soluble compound.

[c] Caco-2 cell membrane permeability. A high value indicates a permeability similar to that of permeable molecules out of 126 drugs (9, 10) (<25 low, >500 marked).

[d] MDCK-cell membrane permeability. A high value indicates s permeability similar to that of permeable molecules out of 52 drugs (11) (<25 low, >500 marked).

[e] The solvent accessible surface area.

[f] Effects on the VipA-VipB interaction. The values of β-galactosidase activity were shown as percentage vs. the control with DMSO only. Means ± SD values are shown. A low value indicates a strong inhibition.

[g] Hcp secretion. A high value indicates a strong inhibition (*i.e.*, no band could be detected in the Western blot analysis).

[h] Inhibition of phospholipase A_1 activity. The values of inhibitory effect on phospholipase activity were shown as the control with DMSO only. Means ± SD values are shown. A high value indicates a strong inhibition.

[i] LDH release. The values of LDH release were shown as percentage *vs*. the control with DMSO only. Means ± SD values are shown. A high value indicates poor cell membrane integrity. The values of samples treated with DMSO or not treated were 12.6 ± 0.3 % and 10.7±0.5 %, respectively.

[j] Cell morphology. A high value indicates that a high proportion of cells showed abnormal morphology (*i.e*. rounding up of the cells and detachment).

[k] Significance of β-galactosidase activity and LDH release was determined using a two-tailed t-test ($^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

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