

SUPPLEMENTARY MATERIAL for

Identification of small molecules exhibiting oxacillin synergy through a novel assay for inhibition of *vraTSR* expression in methicillin resistant *Staphylococcus aureus*

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1. Supplementary Figures

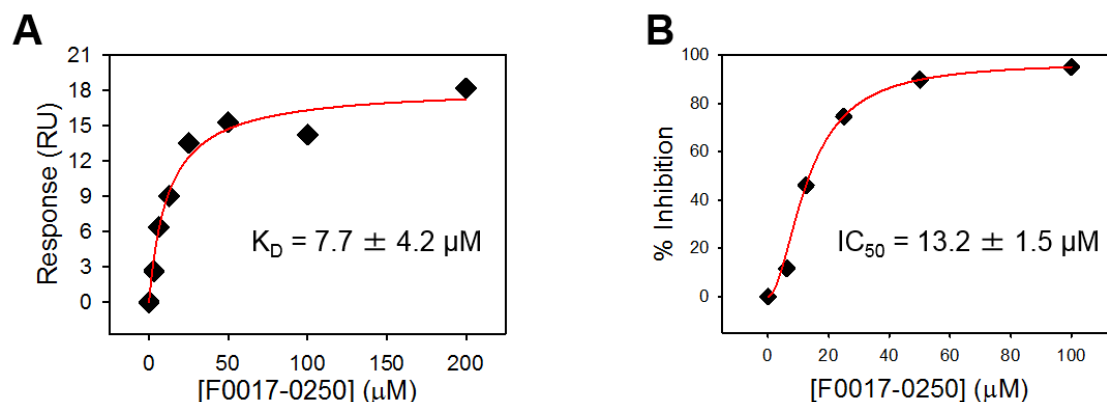


Figure S1. Hit validation assays. (A) Steady-state affinity fitting curve of F0017-0250 with VraS to determine binding affinity (K_D) by surface plasmon resonance (SPR). (B) The IC_{50} fitting curve of F0017-0250 using three parameter Hill equation. Inhibition of VraS kinase activity using the commercial ADP-Glo assay kit.

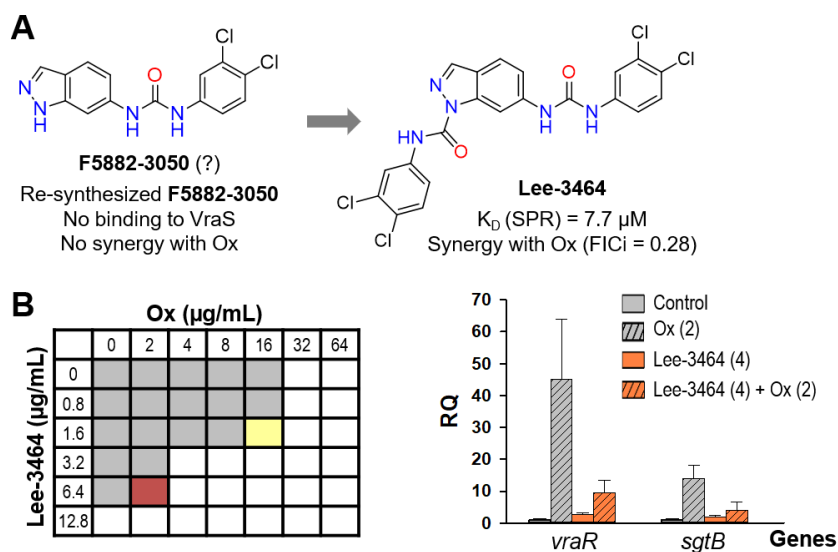
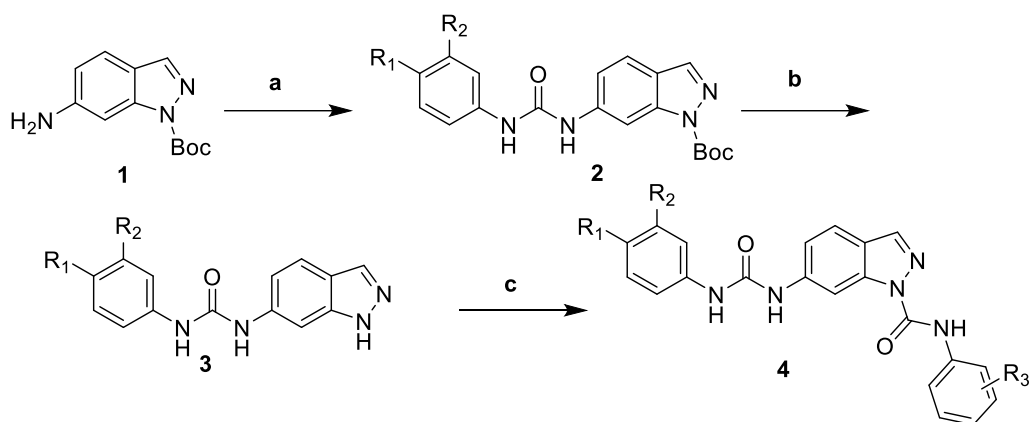


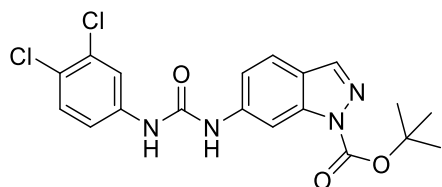
Figure S2. Re-synthesis of lead compounds. (A) Structure of true active compound in the commercial F5882-3050 sample. Quality control process revealed that activity came from a di-substituted urea compound (Lee-3464). (B) Checkerboard MIC (left panel) and qRT-PCR (right panel) results of Lee-3464. Numbers shown in parentheses are concentrations of Ox and tested compounds.

2. Supplementary Schemes

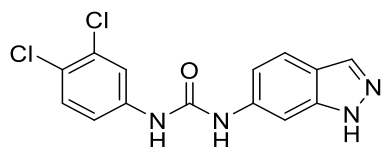


Scheme S1. Reagents and conditions: (a) **1**, aryl isocyanate, CH₂Cl₂, 0°C-rt, 16 h, 90%; (b) **2**, trifluoroacetic acid, CH₂Cl₂, rt, 3-5 h, 96%; (c) **3**, aryl isocyanate, THF, 0°C-rt, 16 h, 76%.

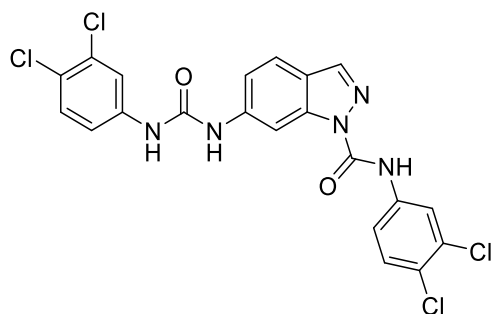
3. Procedures and Characterization Data



tert-butyl 6-(3-(3,4-dichlorophenyl)ureido)-1*H*-indazole-1-carboxylate. To *tert*-butyl 6-amino-1*H*-indazole-1-carboxylate (3.0 mmol, 1.0 eq.) in anhydrous DCM (15.0 mL, 0.20 M) at 0°C under an atmosphere of nitrogen was added 3,4-dichlorophenyl isocyanate (3.0 mmol, 1.0 eq.). The reaction mixture was stirred while warming to room temperature and after 6 h was filtered. The precipitate was washed with DCM to give the title compound as a white solid (1.14 g, 90%). TLC *R*_f 0.5 (5% MeOH/DCM). LC-MS (ESI) *m/z*: 421 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.24 (s, 1H), 9.07 (s, 1H), 8.57 (s, 1H), 8.29 (s, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.37 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.27 (dd, *J* = 8.7, 1.8 Hz, 1H), 1.67 (s, 9H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 152.67, 149.16, 140.91, 140.50, 140.22, 140.08, 131.52, 131.08, 123.82, 122.26, 121.20, 119.93, 118.99, 116.55, 102.88, 84.64, 28.22.



1-(3,4-dichlorophenyl)-3-(1*H*-indazol-6-yl)urea. To a suspension of *tert*-butyl 6-(3-(3,4-dichlorophenyl)ureido)-1*H*-indazole-1-carboxylate (1.0 mmol, 1.0 eq.) in DCM (4.0 mL, 0.25 M) in a nitrogen atmosphere at room temperature was added TFA (50.0 mmol, 50 eq.). The reaction mixture was stirred at room temperature for 5 h, then concentrated *in vacuo* to obtain an oil which was triturated with ether. The precipitate was filtered to obtain the title compound as a white solid (0.31 g, 96%). TLC R_f 0.4 (5% MeOH/DCM). LC-MS (ESI) *m/z*: 323 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.86 (br s, 1H), 9.03 (s, 1H), 8.98 (s, 1H), 7.95 (s, 1H), 7.93 (s, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.65 (d, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.35 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.95 (dd, *J* = 8.6, 1.7 Hz, 1H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 152.86, 141.09, 140.41, 137.96, 133.71, 131.50, 131.05, 123.58, 121.20, 119.76, 119.09, 118.84, 114.35, 98.18.



N-(3,4-dichlorophenyl)-6-(3-(3,4-dichlorophenyl)ureido)-1*H*-indazole-1-carboxamide. A mixture of 1-(3,4-dichlorophenyl)-3-(1*H*-indazol-6-yl)urea (0.15 mmol, 1.0 eq.) and 3,4-dichlorophenyl isocyanate (0.15 mmol, 1.0 eq.) was stirred in THF (1.5 mL, 0.10 M) in a nitrogen atmosphere at 0°C while warming to room temperature over 16 h. The reaction mixture was concentrated *in vacuo*, then recrystallized from ethanol and filtered to obtain the title compound as a white solid (0.058 g, 76%). M.p. 326.8-329.4°C. LC-MS (ESI) *m/z*: 510 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.65 (s, 1H), 9.31 (s, 1H), 9.09 (s, 1H), 8.72 (s, 1H), 8.43 (s, 1H), 8.20 (d, *J* = 2.3 Hz, 1H), 7.94 (d, *J* = 2.4 Hz, 1H), 7.86 (dd, *J* = 8.9, 2.4 Hz, 1H), 7.82 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.55 (d, *J* = 8.8 Hz, 1H), 7.37 (ddd, *J* = 13.9, 8.7, 2.1 Hz, 2H). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 152.67, 149.62, 141.13, 140.27, 140.22, 139.07, 138.79, 131.53, 131.37, 131.06, 130.99, 125.80, 123.82, 122.44, 122.19, 121.37, 121.03, 119.94, 119.02, 116.76, 102.84.

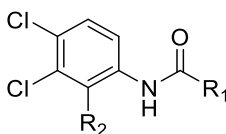
4. Supplementary tables.

Table S1. Summary of Checkerboard data of compounds vs Ox among a panel of clinical isolates

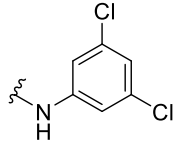
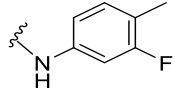
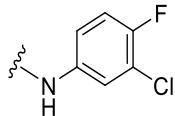
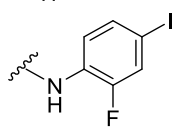
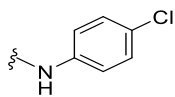
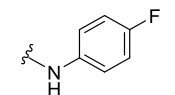
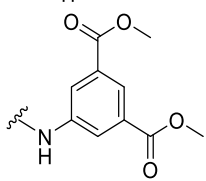
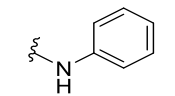
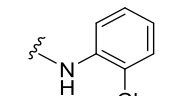
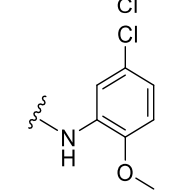
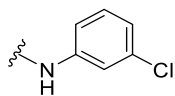
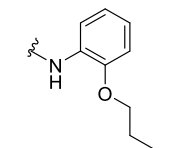
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Lee-3464 /F5882-3050 N = 105	ave	6.8	0.40	1.5	22.0
	min	3.2	0.19	0.8	2
	MX	12.8	1.00	6.4	256
	MEDIAN	6.4	0.38	1.6	8
F0017-0250 N = 82	ave	3.5	0.52	1.34	33.9
	min	0.8	0.08	0.8	2
	MX	12.8	1.00	6.4	1024
	MEDIAN	3.2	0.53	1.6	4
F2619-0556 N=104	ave	9.1	0.50	2.7	48.0
	min	6.4	0.19	0.4	2
	MX	25.6	1.00	25.6	512
	MEDIAN	6.4	0.50	1.6	16
F1374-0037 N = 104	ave	0.43	0.63	0.22	32.3
	min	0.1	0.07	0.1	2
	MX	3.2	1.00	2.0	512
	MEDIAN	0.4	0.56	0.2	8
F1374-0033 N= 12	ave	1.0	0.60	0.6	78.7
	min	0.8	0.50	0.4	2
	MX	2.0	0.75	0.8	256
	MEDIAN	0.8	0.63	0.60	12.0
F1374-2739 N=32	ave	0.4	0.60	0.2	47.0
	min	0.1	0.16	0.1	1
	MX	1.6	1.00	0.4	512
	MEDIAN	0.3	0.53	0.2	6
F2518-0327 N=79	ave	0.2	0.50	0.1	24.7
	min	0.05	0.13	0.05	1
	MX	0.8	1.00	0.2	512
	MEDIAN	0.2	0.53	0.1	2

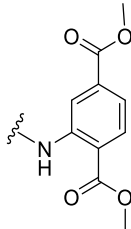
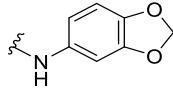
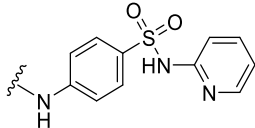
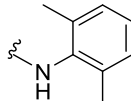
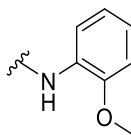
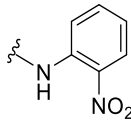
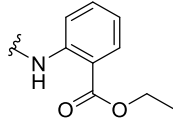
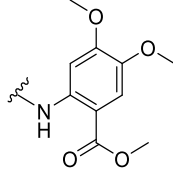
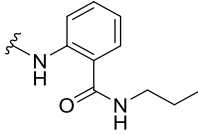
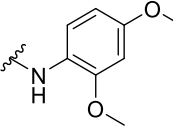
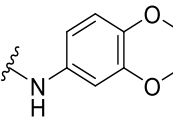
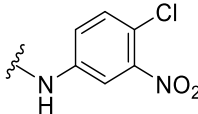
Summary of Checkerboard data of compounds vs Ox among a panel of clinical isolates: Each compound (com) was tested in varying concentrations with varying Oxacillin (Ox) concentrations. Data show the average (ave), minimum (min), maximum (max) and median values among the indicated number (N) isolates. MIC com (MIC of compound), FICI (ideal FIC index in the checkerboard); Low com MIC (Concentration of compound in the reported FIC index), Low Ox MIC (lowest concentration of Ox in the checkerboard).

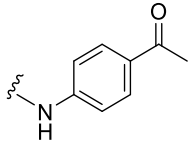
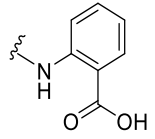
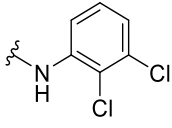
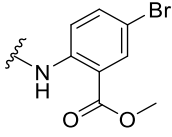
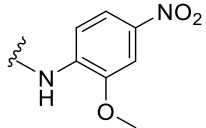
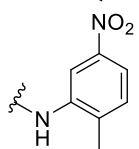
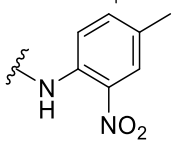
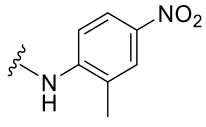
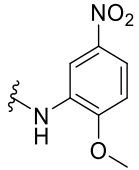
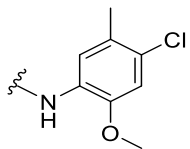
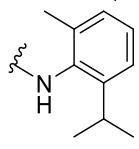
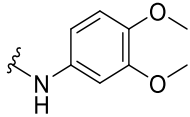
Table S2. SAR of urea compounds.

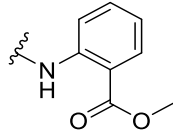
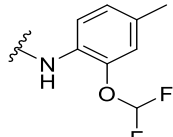
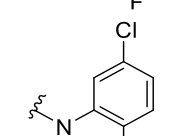
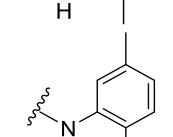
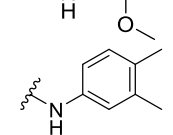
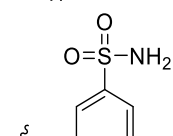
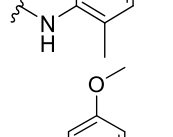
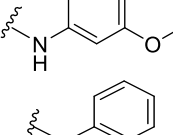
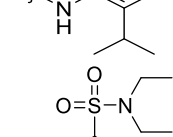
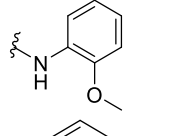
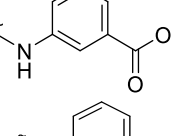
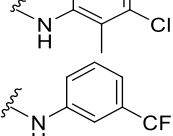


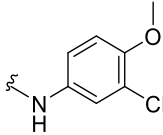
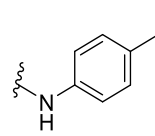
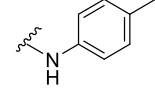
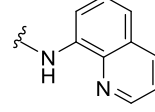
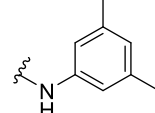
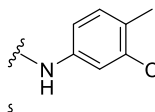
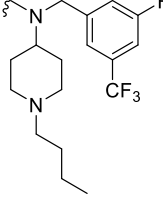
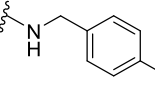
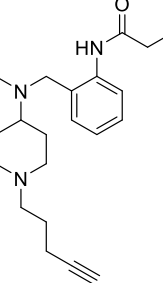
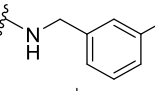
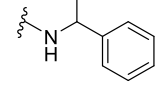
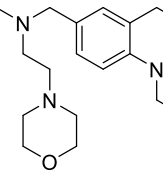
Compd.	R ₁	R ₂	MIC (μg/mL)	FICI ^a
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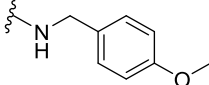
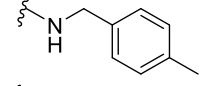
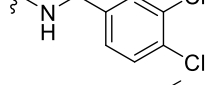
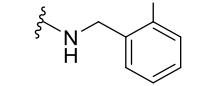
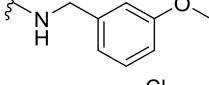
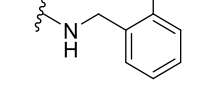
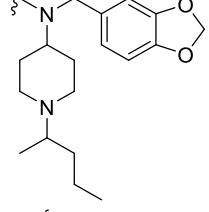
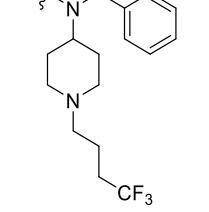
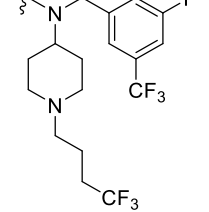
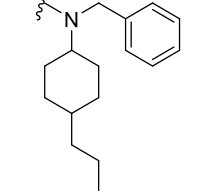
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SJ000146666-1		H	0.8	0.52
SJ000146671-1		H	0.8	0.52
SJ00046663-2		H	1.6-3.2	0.52
SJ000361152-1		H	6.4	0.52
SJ000181139-1		H	3.2-6.4	0.57
SJ000140961-1		H	3.2	0.58
SJ000146304-3		H	6.5	0.58
SJ000512507-2		H	1.6	0.63
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SJ000181347-1		H	6.4	0.75

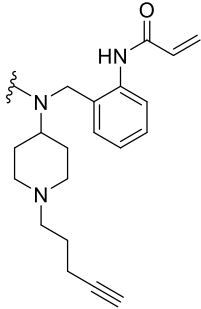
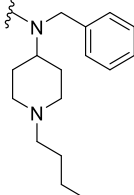
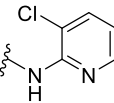
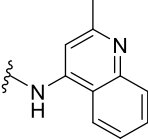
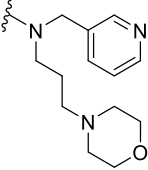
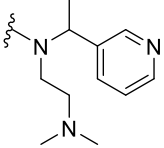
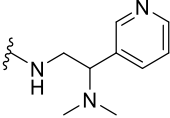
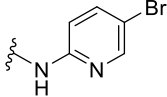
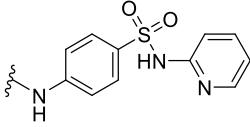
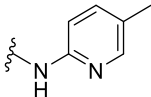
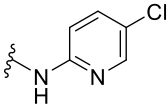
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SJ000136896-1		H	>12.8	N.D.
SJ000146096-1		H	>12.8	N.D.
SJ000146099-1		H	>12.8	N.D.
SJ000141138-1		H	>12.8	N.D.
SJ000141523-1		H	>12.8	N.D.
SJ000166939-1		H	>12.8	N.D.
SJ000167678-1		H	>12.8	N.D.
SJ000154934-1		H	>12.8	N.D.
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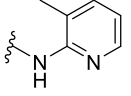
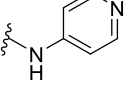
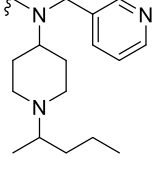
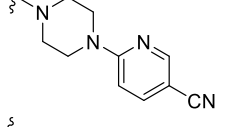
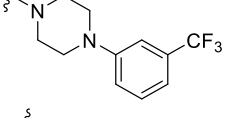
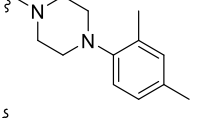
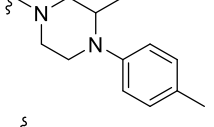
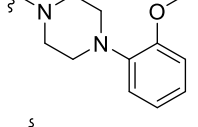
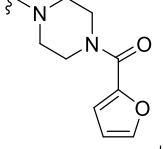
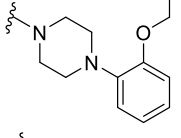
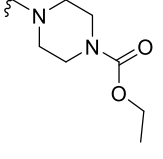
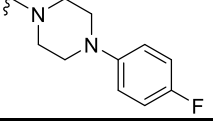
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SJ000175353-1		H	>12.8	N.D.
SJ000197666-1		H	>12.8	N.D.
SJ000197667-1		H	>12.8	N.D.
SJ000197668-1		H	>12.8	N.D.
SJ000197669-1		H	>12.8	N.D.
SJ000197670-1		H	>12.8	N.D.
SJ000197671-1		H	>12.8	N.D.
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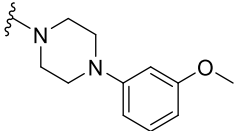
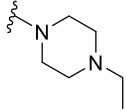
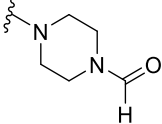
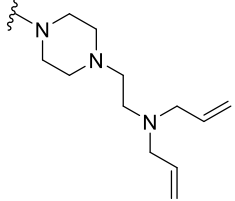
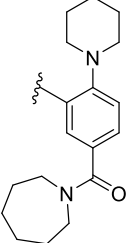
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SJ000182650-1		H	>12.8	N.D.
SJ000182625-1		H	>12.8	N.D.
SJ000182287-1		H	>12.8	N.D.
SJ000203781-1		H	>12.8	N.D.
SJ000182106-1		H	>12.8	N.D.
SJ000197674-1		H	>12.8	N.D.
SJ000197715-1		H	>12.8	N.D.
SJ000280405-1		H	>12.8	N.D.
SJ000141158-5		H	>12.8	N.D.

SJ000197672-2		H	>12.8	N.D.
SJ000361143-1		H	>12.8	N.D.
SJ000048157-2		H	>12.8	N.D.
SJ000361149-1		H	>12.8	N.D.
SJ000361151-1		H	>12.8	N.D.
SJ000512493-2		H	>12.8	N.D.
SJ000866730-1		H	12.8	0.51
SJ000181101-1		H	12.8	0.63
SJ000862646-1		H	6.4	0.69
SJ000220706-1		H	3.2	0.75
SJ000146691-1		H	6.4	N.D.
SJ000119862-1		H	>12.8	N.D.

SJ000168468-1		H	>12.8	N.D.
SJ000181562-1		H	>12.8	N.D.
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SJ000866735-1		H	>12.8	N.D.
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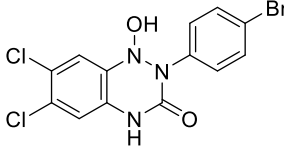
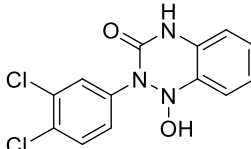
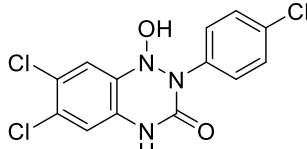
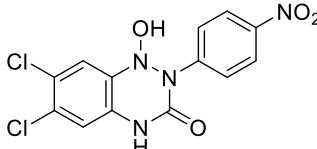
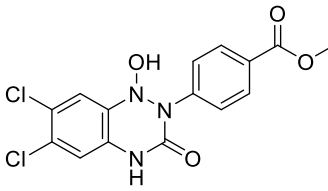
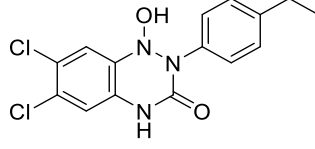
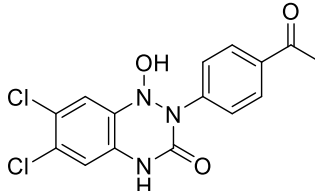
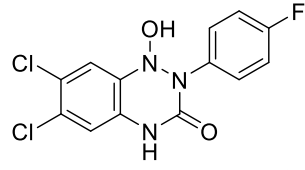
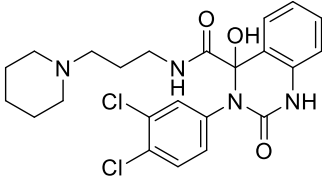
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SJ000151775-1		H	6.4	0.54
SJ000311686-1		H	1.6	0.64
SJ000119617-1		H	>12.8	N.D.
SJ000119763-1		H	>12.8	N.D.
SJ000115533-1		H	>12.8	N.D.
SJ000150426-1		H	>12.8	N.D.
SJ000136896-1		H	>12.8	N.D.
SJ000158183-1		H	>12.8	N.D.
SJ000230659-1		H	>12.8	N.D.

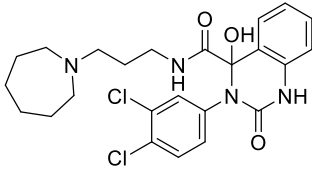
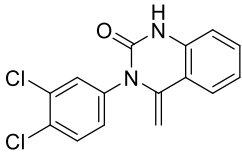
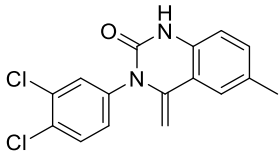
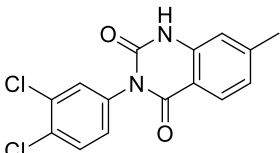
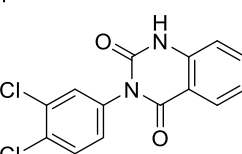
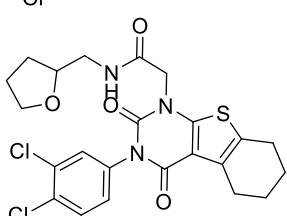
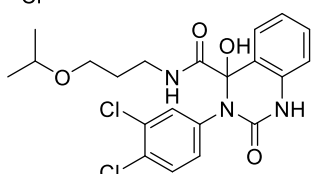
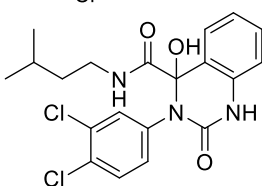
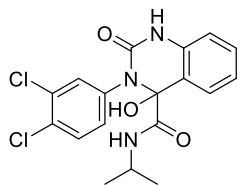
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SJ000860545-1		H	>12.8	N.D.
SJ000862376-1		H	>12.8	N.D.
SJ000165350-1		H	25.6	0.38
SJ000220773-1		H	6.4-12.8	0.5
SJ000220782-1		H	6.4	N.D.
SJ000159890-1		H	>12.8	N.D.
SJ000150474-1		H	>12.8	N.D.
SJ000151720-1		H	>12.8	N.D.
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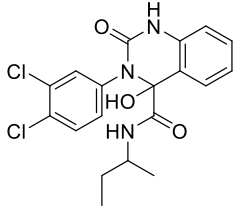
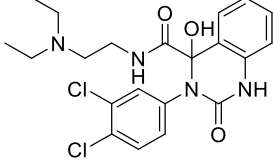
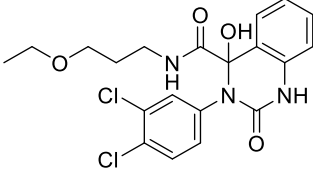
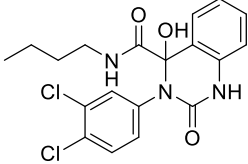
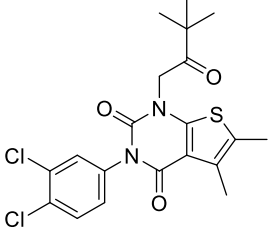
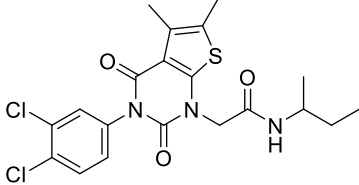
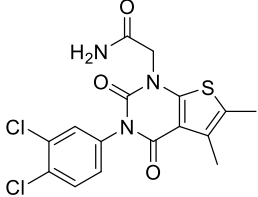
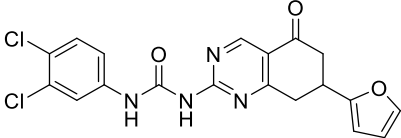
SJ000188470-1		H	>12.8	N.D.
SJ000201235-1		H	>12.8	N.D.
SJ000222996-1		H	>12.8	N.D.
SJ000222962-1		H	>12.8	N.D.
SJ000119622-1		H	>12.8	N.D.

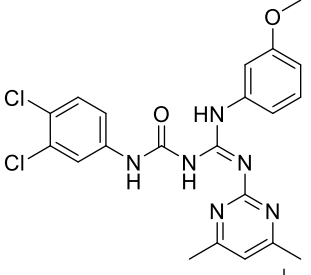
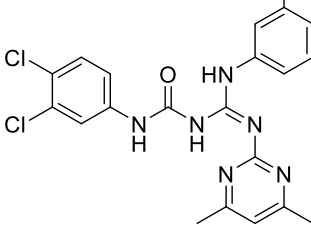
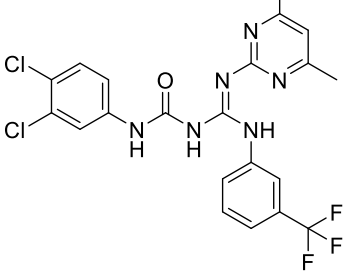
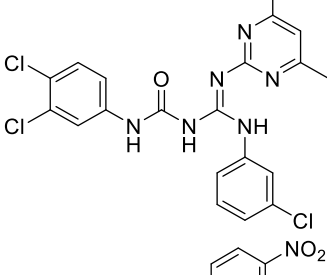
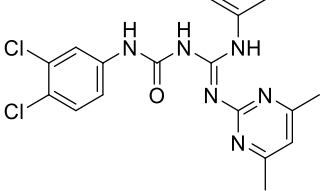
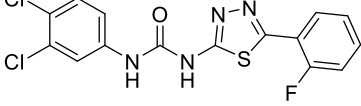
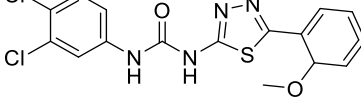
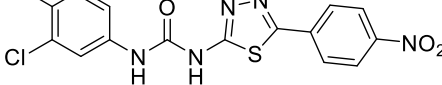
^aN.D. not determined

Table S3. Additional inactive urea compounds.

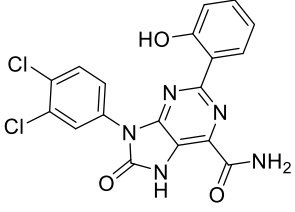
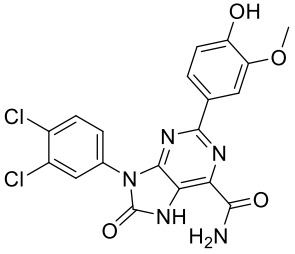
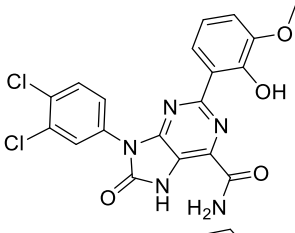
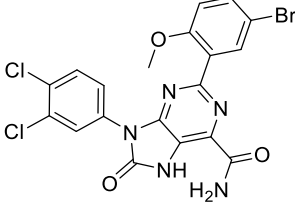
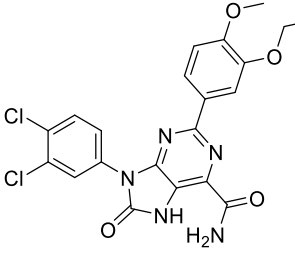
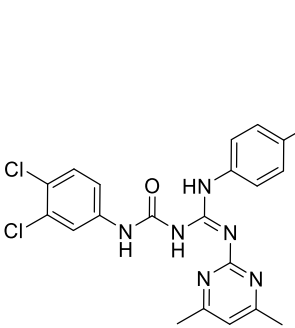
Compd.	Structure	MIC (µg/mL)	FIC _i ^a
SJ000014232-1		>12.8	N.D.
SJ000014217-1		>12.8	N.D.
SJ000014233-1		>12.8	N.D.
SJ000014234-1		>12.8	N.D.
SJ000014235-1		>12.8	N.D.
SJ000014236-1		>12.8	N.D.
SJ000014237-1		>12.8	N.D.
SJ000014231-1		>12.8	N.D.
SJ000114389-1		>12.8	N.D.

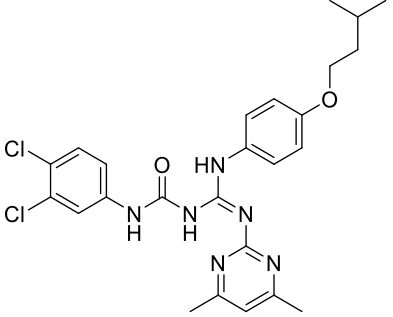
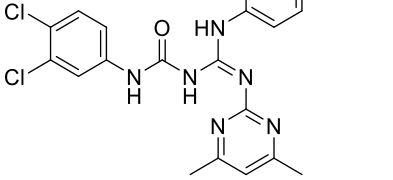
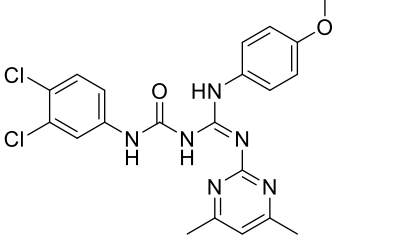
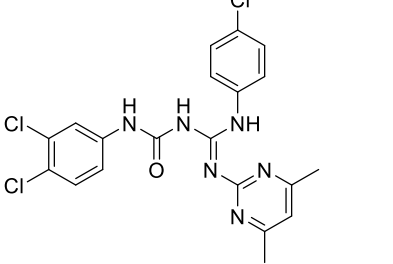
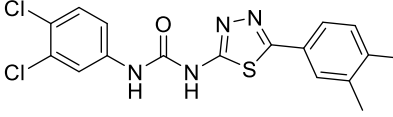
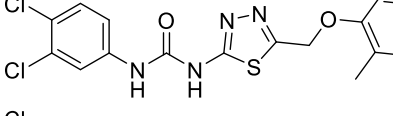
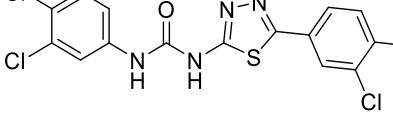
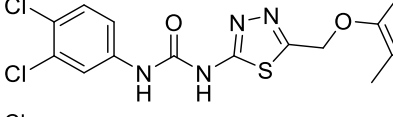
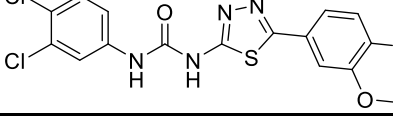
SJ000114390-1		>12.8	N.D.
SJ000122344-1		>12.8	N.D.
SJ000122345-1		>12.8	N.D.
SJ000122347-1		>12.8	N.D.
SJ000122348-1		>12.8	N.D.
SJ000114802-1		>12.8	N.D.
SJ000058817-1		>12.8	N.D.
SJ000058802-1		>12.8	N.D.
SJ000058818-1		>12.8	N.D.

SJ000058828-1		>12.8	N.D.
SJ000058797-1		>12.8	N.D.
SJ000058814-1		>12.8	N.D.
SJ000058823-1		>12.8	N.D.
SJ000063674-1		>12.8	N.D.
SJ000063682-1		>12.8	N.D.
SJ000063675-1		>12.8	N.D.
SJ000130406-1		>12.8	N.D.

SJ000028723-1		>12.8	N.D.
SJ000028724-1		>12.8	N.D.
SJ000028725-1		>12.8	N.D.
SJ000028741-1		>12.8	N.D.
SJ000028726-1		>12.8	N.D.
SJ000158763-1		>12.8	N.D.
SJ000158779-1		>12.8	N.D.
SJ000158787-1		>12.8	N.D.

SJ000158803-1		>12.8	N.D.
SJ000158733-1		>12.8	N.D.
SJ000158748-1		>12.8	N.D.
SJ000158884-1		>12.8	N.D.
SJ000158893-1		>12.8	N.D.
SJ000158672-1		>12.8	N.D.
SJ000158665-1		>12.8	N.D.
SJ000158666-1		>12.8	N.D.
SJ000158659-1		>12.8	N.D.
SJ000158675-1		>12.8	N.D.
SJ000158685-1		>12.8	N.D.
SJ000158843-1		>12.8	N.D.
SJ000158828-1		>12.8	N.D.
SJ000158879-1		>12.8	N.D.

SJ000241363-1		>12.8	N.D.
SJ000314326-1		>12.8	N.D.
SJ000314334-1		>12.8	N.D.
SJ000314342-1		>12.8	N.D.
SJ000314350-1		>12.8	N.D.
SJ000496489-1		>12.8	N.D.

			
SJ000496491-1		>12.8	N.D.
SJ000497351-1		>12.8	N.D.
SJ000497352-1		>12.8	N.D.
SJ000507933-1		>12.8	N.D.
SJ000507934-1		>12.8	N.D.
SJ000507935-1		>12.8	N.D.
SJ000507381-1		>12.8	N.D.
SJ000507749-1		>12.8	N.D.

^aN.D. not determined