Supplementary Information (SI) for RSC Medicinal Chemistry. This journal is © The Royal Society of Chemistry 2024

Supplementary data: Rational design and in-vitro testing of new urease inhibitors to prevent urinary catheter blockage, Heylen et al.

Supplementary Information

Series A



B2 B3 (R) B3 (S) **B**4 0 HS HS. HS. HS NH_2 NH_2 HS Ëн Ōн ŚН B7 (R,R) B6 (R) OH B6 (S) 0 ОН ö HS HS HS HS (R) (S) H Ш ОН Ν B7 (S,R) B7 (S,S) B8 (R) 0 B7 (R,S) HS ΟН ОН 0 OH NH₂ R) HS HS HS N ĒН ОН Ōн B11 (R) B10 (S) B9 (R) B9 (S) B10 (R) 0 0 0 HS,,, HS HS HS HS,,, `NH₂ NH₂ `NH₂ (R) (R) (S) `NH₂ (S) B11 (S) B12 B13 B15 B14 0 HS,,,, N⁻ 0 NH₂ (S) HS HS HS HS OH N H



OH

ŊН



Series B

B1

B5

Series C





Series D



Series E



Supplementary Figure 1. Chemical structures of all compounds assessed by the computational docking experiment. Series (A) based around thiourea, (B)&(C) 2-MA, (D) quercetin, and (E) quercetin and 2-MA. <u>Compounds B17 (R) and C2 (R), B17 (S) and C2 (S) are the same structure.</u> Compounds were drawn using ChemDraw 19.1.1.21 (PerkinElmer Informatics, Waltham, Massachusetts, US).

| Code | R groups | Docking | Manually | | | | | | |
|----------|-------------------------|------------------------------------|----------------|----------------|----------------|----------------|----------------|------------------|--------------------|
| | R ₁ | R ₂ | R ₃ | R ₄ | R ₅ | R ₆ | R ₇ | Score (LF dG) | number of contacts |
| Series A | Ą | | | | | | | | |
| A9 | СООН | CH ₂ (pyridi n-3-yl) | - | - | - | - | - | -9.652 | 9 |
| A3 | СООН | Н | - | - | - | - | - | -9.007 | 6 |
| A4 | CSSH | Н | - | - | - | - | - | -8.714 | 8 |
| A12 | benzene | CH₂(pyridi n-3-yl) | - | - | - | - | - | -8.209 | 4 |
| A11 | CH₂(pyridi n-3-yl) | CH ₂ (pyridi n-3-yl) | - | - | - | - | - | -7.213 | 6 |
| A7 | pyridin-3- yl | Н | - | - | - | - | - | -7.017 | 7 |
| A13 | isopropyl | CH₂(pyridi n-3-yl) | - | - | - | - | - | -6.945 | 5 |
| A6 | CH₂(benz ene) | Н | - | - | - | - | - | -6.581 | 3 |
| A10 | н | CH₂(pyridi n-3-yl) | - | - | - | - | - | -6.374 | 9 |
| A5 | benzene | Н | - | - | - | - | - | -6.102 | 3 |
| A16 | COCH3 3-methyl | Н | - | - | - | - | - | -5.925 | 5 |
| A8 | pyridin-4- vl | Н | - | - | - | - | - | -5.759 | 5 |
| A15 | isopropyl | Н | - | - | - | - | - | -5.671 | 4 |
| A14 | COCH ₃ | CH₂(pyridi n-3-vl) | - | - | - | - | - | -5.56 | 8 |
| A1 | Me | H | - | - | - | - | - | -5.419 | 4 |
| A2 | ОН | Н | - | - | - | - | - | -5.05 | 5 |
| Thio- | ы | ы | | | | | | 4 711 | 4 |
| urea | 11 | 11 | - | - | - | - | - | -4.711 | 4 |
| Series E | 3 | | | | | | | | |
| B17 | pyridin-4- | pyridin-4- | _ | _ | _ | _ | _ | -9 321 | 6 |
| (R) | yl | yl | | | | | | 5.521 | 0 |
| B11 | pyridin-4- | н | - | - | _ | _ | _ | -8 852 | 7 |
| (S) | yl | | | | | | | 0.002 | , |
| B18 | 2 x pyridin-4- vl | pyridin-4- yl | - | - | - | - | - | -8.737 | 4 |
| B9 (R) | , pyridin-2- vl | н | - | - | - | - | - | -8.501 | 7 |
| B17 | , pyridin-4- | pyridin-4- | - | - | - | - | - | -8.092 | 5 |

Supplementary Table 1. Description of the compounds with docking scores and manually predicted contacts. Compounds are separated by series and ranked according to docking score.

| (S) B10 (R) | yl pyridin-3- yl | yl H | - | - | - | - | - | -7.949 | 8 |
|----------------------|------------------------|------------------|---------------------------|---|---|---|---|---------|----|
| B6 (R) | н | CH(OH)M e | - | - | - | - | - | -7.73 | 8 |
| B11 (R) | pyridin-4- yl | Н | - | - | - | - | - | -7.675 | 8 |
| B16 | н | pyridin-4- yl | - | - | - | - | - | -7.395 | 8 |
| B7 (S <i>,</i> R) | ОН | CH₂OH | - | - | - | - | - | -7.35 | 12 |
| B10 (S) | pyridin-3- yl | н | - | - | - | - | - | -7.237 | 9 |
| B8 (S) | , benzene | Н | - | - | - | - | - | -7.229 | 5 |
| B2 | SH | Н | - | - | - | - | - | -7.194 | 6 |
| B7 (R,S) | ОН | CH₂OH | - | - | - | - | - | -7.152 | 9 |
| B7 (R <i>,</i> R) | ОН | CH₂OH | - | - | - | - | - | -7.128 | 12 |
| B6 (S) | Н | CH(OH)M e | - | - | - | - | - | -6.959 | 13 |
| B7 (S,S) | ОН | CH₂OH | - | - | - | - | - | -6.937 | 15 |
| B9 (S) | pyridin-2- yl | Н | - | - | - | - | - | -6.867 | 11 |
| B13 | H | benzene | - | - | - | - | - | -6.483 | 4 |
| B5 | Н | Et | - | - | - | - | - | -6.298 | 6 |
| B12 | Н | CH₂OH | - | - | - | - | - | -6.288 | 10 |
| B15 | н | pyridin-3- yl | - | - | - | - | - | -6.279 | 11 |
| B4 | Н | Me | - | - | - | - | - | -5.871 | 6 |
| B3 (S) | ОН | Н | - | - | - | - | - | -5.765 | 6 |
| B3 (R) | ОН | Н | - | - | - | - | - | -5.755 | 8 |
| B1 (2- MA) | Н | Н | - | - | - | - | - | -5.611 | 8 |
| B14 | н | pyridin-2- yl | - | - | - | - | - | -5.316 | 8 |
| B8 (R) | benzene | Н | - | - | - | - | - | -4.702 | 5 |
| C10 | CH(OH) ₂ | pyridin-4- | pyridin-4- | - | - | - | - | -10.195 | 11 |
| (R) | 0.1(0.1)2 | yl | yl | | | | | | |
| Series C | a substant of | 4 منامنات م | a substance of the second | | | | | | |
| C7 (R) | yl | yl | yl | - | - | - | - | -9.761 | 8 |
| C7 (S) | pyridin-4- yl | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -9.617 | 6 |
| C2 (R) | н | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -9.288 | 5 |
| C9 (S) | CH₂OH | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -9.209 | 9 |
| C6 (S) | isopropyl | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -9.158 | 7 |
| C8 (R) | ОН | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -9.088 | 7 |
| C9 (R) | CH₂OH | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -8.955 | 7 |
| C6 (R) | isopropyl | pyridin-4- yl | pyridin-4- yl | - | - | - | - | -8.942 | 5 |
| C8 (S) | ОН | pyridin-4- | pyridin-4- | - | - | - | - | -8.888 | 9 |

| | | yl | yl | | | | | | |
|--------------|-----------------------|------------------|------------------|----|---|---|---|---------|----|
| (3 (B) | F† | pyridin-4- | pyridin-4- | - | _ | _ | _ | -8 8/11 | 7 |
| CJ (II) | L1 | yl | yl | | | | | 0.041 | 7 |
| (2(5)) | н | pyridin-4- | pyridin-4- | - | - | _ | _ | -8 692 | 3 |
| 02 (0) | | yl | yl | | | | | 0.052 | 5 |
| C1 (R) | Me | pyridin-4- | pyridin-4- | - | - | - | - | -8.594 | 8 |
| - () | | yl | yl | | | | | | |
| C16 | pyridin-4- | Н | pyridin-4- | - | - | - | - | -8.579 | 3 |
| 6 2 4 | yı | | yl | | | | | | |
| (24 | CH(OH)₂ | pyridin-4- | pyridin-4- | - | - | - | - | -8.526 | 14 |
| (S)* | CII /honz | yı puridin 4 | yı puridin 4 | | | | | | |
| C5 (S) | | pynain-4- | pynain-4- | - | - | - | - | -8.436 | 9 |
| C23 | pyridin_4- | yı pyridin_4- | yı pyridin-4- | | | | | | |
| (R)* | ynum-4- | vi | vi | - | - | - | - | -8.311 | 7 |
| (11) | y. | pyridin-4- | pyridin-4- | | | | | | |
| C4 (S) | benzene | vl | vl | - | - | - | - | -8.04 | 8 |
| C23 | pyridin-4- | pyridin-4- | pyridin-4- | | | | | | |
| (S)* | yl | yl | yl | - | - | - | - | -7.994 | 8 |
| C10 | , (11(011) | , pyridin-4- | , pyridin-4- | | | | | 7.074 | |
| (S) | CH(OH) ₂ | yl | yl | - | - | - | - | -7.974 | 11 |
| C24 | | pyridin-4- | pyridin-4- | | | | | 7.07 | 10 |
| (R)* | | yl | yl | - | - | - | - | -7.97 | 13 |
| (2)(5) | F+ | pyridin-4- | pyridin-4- | _ | _ | _ | _ | -7 627 | 10 |
| C3 (3) | L1 | yl | yl | - | - | - | - | -7.027 | 10 |
| C5 (R) | CH₂(benz | pyridin-4- | pyridin-4- | - | - | _ | - | -7 54 | 8 |
| 65 (11) | ene) | yl | yl | | | | | 7.51 | 0 |
| C14 | CH ₂ (benz | н | pyridin-4- | - | - | - | - | -7.497 | 4 |
| | ene) | | yl | | | | | | |
| C13 | benzene | Н | pyridin-4- | - | - | - | - | -7.47 | 2 |
| | | | yı | | | | | | |
| C20 | CH ₂ (benz | Н | Н | - | - | - | - | -7.371 | 5 |
| | ene) | pyridin 4 | pyridin 4 | | | | | | |
| C1 (S) | Me | yriain-4- | yriun-4- | - | - | - | - | -7.19 | 8 |
| | | yı pyridin-4- | yı pyridin-4- | | | | | | |
| C4 (R) | benzene | vl | vl | - | - | - | - | -7.077 | 5 |
| | | y. | pyridin-4- | | | | | | |
| C11 | Me | Н | vl | - | - | - | - | -7.014 | 9 |
| C22 | CH ₂ (benz | pyridin-4- | pvridin-4- | | | | | | _ |
| (R)* | ene) | yl | yl | - | - | - | - | -6.981 | 7 |
| C19 | benzene | Ĥ | Ĥ | - | - | - | - | -6.92 | 6 |
| C1F | iconronul | | pyridin-4- | | | | | C 910 | 0 |
| C15 | ізоргоруі | п | yl | - | - | - | - | -0.819 | 8 |
| C12 | C+ | ы | pyridin-4- | | | | | 6 696 | E |
| CIZ | EL | п | yl | - | - | - | - | -0.000 | 5 |
| C22 | CH ₂ (benz | pyridin-4- | pyridin-4- | - | _ | _ | _ | -6 409 | 7 |
| (S)* | ene) | yl | yl | | | | | 0.405 | , |
| C21* | benzene | pyridin-4- | pyridin-4- | - | - | - | - | -6.235 | 6 |
| 011 | 001120110 | yl | yl | | | | | 0.200 | C |
| C18 | Et | Н | Н | - | - | - | - | -6.213 | 8 |
| C17 | Me | н | н | - | - | - | - | -5.901 | 6 |
| 2-MA | Н | Н | Н | - | - | - | - | -5./58 | / |
| Series D | <u>^</u> | <u> </u> | <u> </u> | N. | | | | 44 474 | 12 |
| DIII2 | L | L | C C | N | - | - | - | -11.1/1 | 12 |
| Diii3 | C | N | С | C | - | - | - | -10.631 | 16 |
| Diii1 | С | С | Ν | С | - | - | - | -10.189 | 14 |
| Diii4 | N | N | С | С | - | - | - | -9.865 | 14 |

| Div7 | С | С | С | Ν | Н | ОН | Н | -9.716 | 7 |
|------------|--------|----------------------|--------------|------------------------------|----------------------|-----------------|----|---------|----|
| Dv2 | у | - | - | - | - | - | - | -9.554 | 10 |
| Dii2 | NH_2 | - | - | - | - | - | - | -9.197 | 10 |
| Div6 | С | С | С | Ν | Н | Н | ОН | -9.163 | 10 |
| Di2 | OH | ОН | | | | | | -9.15 | 11 |
| Div8 | С | С | С | Ν | ОН | Н | Н | -9.11 | 9 |
| Dv1 | х | - | - | - | - | - | - | -9.103 | 6 |
| Di1 | OH | Н | - | - | - | - | - | -8.897 | 10 |
| Dv3 | Z | - | - | - | - | - | - | -8.836 | 8 |
| Dii1 | OH | - | - | - | - | - | - | -8.722 | 15 |
| Div9 | С | С | С | Ν | Н | NH ₂ | Н | -8.678 | 9 |
| Div3 | С | Ν | С | С | Н | - | Н | -8.486 | 7 |
| Div5 | С | С | С | Ν | Н | Н | Н | -8.313 | 7 |
| Div4 | С | С | Ν | С | Н | Н | - | -8.113 | 8 |
| Div2 | Ν | С | С | С | - | Н | Н | -8.006 | 8 |
| Div1 | С | С | С | С | Н | Н | Н | -7.88 | 6 |
| Series E | | | | | | | | | |
| E5 (S) | ОН | Н | ОН | OCOCSH(pyridin-4- yl) | ОН | - | - | -12.902 | 11 |
| E5 (R) | ОН | н | ОН | OCOCSH(pyridin-4- yl) | ОН | - | - | -11.943 | 11 |
| E10 (S) | ОН | OCOCH₃ | ОН | OCOCOH(pyridin-4- yl) | ОН | - | - | -11.71 | 11 |
| E3 | ОН | Н | ОН | OCOCH₂S H | ОН | - | - | -11.215 | 11 |
| E2 | ОН | Н | OCOCH₂S H | Н | ОН | - | - | -11.095 | 9 |
| E14 | ОН | Н | ОН | CH ₂ CONH 2 | ОН | - | - | -10.252 | 10 |
| E7 | ОН | Н | ОН | OCOCH₂O H | ОН | - | - | -10.237 | 10 |
| E10 (R) | ОН | OCOCH₃ | ОН | OCOCOH(pyridin-4- yl) | ОН | - | - | -10.145 | 11 |
| E1 | ОН | Н | ОН | Н | OCOCH₂S H | - | - | -9.648 | 7 |
| E17 | ОН | Н | ОН | CH ₂ CHO | ОН | - | - | -9.611 | 10 |
| E8 | ОН | Н | ОН | OCOCH ₃ | ОН | - | - | -9.549 | 10 |
| E13 | ОН | Н | ОН | н | CH ₂ CONH | - | - | -9.157 | 10 |
| E6 | ОН | Н | OCOCH₂O H | Н | ОН | - | - | -9.097 | 11 |
| E11 | ОН | OCOCH₂O H | ОН | н | ОН | - | - | -8.754 | 8 |
| E12 | Н | H | ОН | Н | OH | - | - | -8.601 | 11 |
| E18 | Н | CH ₂ CONH | ОН | Н | ОН | - | - | -8.6 | 11 |
| E9 | ОН | OCOCH₂O H | ОН | Н | ОН | - | - | -8.491 | 11 |
| E16 | ОН | CH ₂ CONH | ОН | Н | ОН | - | - | -8.403 | 9 |
| E4 | ОН | OCOCH₂S H | ОН | н | ОН | - | - | -7.836 | 9 |



Supplementary Figure 2. Calibration curves of AHA (A) and N,N'-Bis(3-pyridinylmethyl)thiourea (A11) (B), determined using UV-vis spectroscopy for quantifying release through the Biomodics catheter's balloon.



Supplementary Figure 3. A. molecular docking of urea, **B.** AHA, into the active site of *S. pasteruii* urease, the top compound is the crystallized AHA and the bottom is the docked ligand AHA, RMSD = 0.977 Å. Pink asterixis indicate Ni ions in the center of the urease, green dotted lines show the distance between the compound and the amino acids within the active site. Molecules docked with Cresset, Flare v. 4.0.2. Images generated using Flare[™] from Cresset[®].



Supplementary Figure 4. Minimum inhibition growth curves. **A.** *P. mirabilis* in the grown in varying concentrations of AHA. **B.** *E. coli* with AHA. **C.** *P. mirabilis* with Bis-TU (A11). **D.** *E. coli* with Bis-TU (A11). The highest concentration of all compounds contained 2.5% DMSO which was diluted further during compound dilutions. The experiment was conducted with three biological repeats, error bars represent standard deviation. Graphs were drawn using GraphPad Prism v.9.4.1.

| Bacterial species | [AHA]/ mM | [Bis-TU]/mM |
|-------------------|-------------|-------------|
| P. mirabilis B4 | 3.13 – 25.0 | 1.25 - 5.00 |
| E. coli NSM59 | 12.5 – 25.0 | 1.25 - 5.00 |

Supplementary Table 2. MIC ranges for AHA and Bis-TU (A11) in *P. mirabilis* and *E. coli*.

Supplementary Table 3. IC50 fitting data

Data fitted to:

% Urease activity =
$$\frac{A1 + (A2 - A1)}{1 + 10^{(logXo - X)}.P}$$

| Model | Dose Resp | | | | | | | | | |
|--------------|---|------------------|------------|------------|------------|------|--|--|--|--|
| Equation | y = A1 + (A2-A1)/(1 + 10^((LOGx0-x)*p)) | | | | | | | | | |
| | | | | | | | | | | |
| Plot | AHA | A5 | Quercetin | A6 | A11 | 2-MA | | | | |
| A1 | 1.73858 ± | -2833.55254 ± | -6.87402 ± | 5.56987 ± | -6.89555 ± | | | | | |
| | 5.56086 | 571357.59569 | 9.90633 | 6.7131 | 4.6294 | | | | | |
| A2 | 91.30088 ± | 141.84726 ± | 68.52898 ± | 78.71291 ± | 85.90046 ± | | | | | |
| | 2.97347 | 812.39328 | 7.38724 | 4.0369 | 7.52903 | | | | | |
| LOGx0 | -4.43621 ± | -5.015 ± 0.11323 | -6.06228 ± | -4.64408 ± | -5.85143 ± | | | | | |
| | 0.18293 | | 0.30034 | 0.17038 | 0.25846 | | | | | |
| Reduced Chi- | 2.92888 | 3.347 | 2.64774 | 88.59753 | 1.32268 | | | | | |
| Sqr | | | | | | | | | | |
| Adj. R- | 0.97678 | 0.986 | 0.9453 | 0.97726 | 0.98853 | | | | | |
| Square | | | | | | | | | | |
| Model | Dose Resp | | | | | | | | | |

a. Proteus mirabilis whole cell