

## Supporting Information

### Repurposing studies of FDA-approved sulfonamide carbonic anhydrase inhibitors for treatment of *Neisseria gonorrhoeae*

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**Table S1.** *N. gonorrhoeae* strains used in the study

| <i>N. gonorrhoeae</i> strains | Description  |
|-------------------------------|--|
| CDC 165                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC 166                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC 167                       | Resistant to azithromycin  |
| CDC 168                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC 169                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC173                        | Resistant to tetracycline, ciprofloxacin and penicillin  |
| CDC 178                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC 179                       | Resistant to azithromycin  |
| CDC 181                       | Resistant to azithromycin and tetracycline   |
| CDC 182                       | Resistant to tetracycline, ciprofloxacin and penicillin  |
| CDC 183                       | Resistant to tetracycline, ciprofloxacin and penicillin  |
| CDC 184                       | Resistant to tetracycline, ciprofloxacin and penicillin  |
| CDC 186                       | Resistant to tetracycline, ciprofloxacin and penicillin  |
| CDC 187                       | Resistant to penicillin  |
| CDC 197                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| CDC 202                       | Resistant to azithromycin  |
| CDC 211                       | Resistant to tetracycline, penicillin, and ciprofloxacin   |
| ATCC 700825                   | Isolated from male patient with disseminated gonococcal infection in 1983<br>Resistant to streptomycin   |
| WHO-V                         | Isolated from a urethritis patient, Sweden, 2012   |
| WHO-W                         | Isolated in Hong Kong, 2007<br>Resistant to tetracycline, ciprofloxacin and penicillin   |
| WHO-X                         | Isolated in Japan, 2009<br>Resistant to tetracycline, ciprofloxacin and penicillin<br>Reduced susceptibility to ceftriaxone and cefixime   |
| WHO-Z                         | Isolated from a female patient with genital infection. Australia, 2013<br><br>Penicillin, tetracycline and ciprofloxacin-resistant<br><br>Reduced susceptibility to ceftriaxone and cefixime |

**Table S2.** MICs ( $\mu\text{g/mL}$ ) against clinical *Neisseria gonorrhoeae* isolates in  $\text{CO}_2$  and ambient Non- $\text{CO}_2$  conditions.

| <i>N. gonorrhoeae</i> strains | Ethoxzolamide |                    | Acetazolamide |                    | Azithromycin  |                    | Ceftriaxone   |                    |
|-------------------------------|---------------|--------------------|---------------|--------------------|---------------|--------------------|---------------|--------------------|
|                               | $\text{CO}_2$ | Non- $\text{CO}_2$ | $\text{CO}_2$ | Non- $\text{CO}_2$ | $\text{CO}_2$ | Non- $\text{CO}_2$ | $\text{CO}_2$ | Non- $\text{CO}_2$ |
| CDC 165                       | >64           | 0.25               | >64           | 4                  | 2             | 1                  | 0.06          | 0.06               |
| CDC 166                       | >64           | 0.125              | >64           | 4                  | 1             | 1                  | 0.125         | 0.125              |
| CDC 167                       | 64            | 0.25               | >64           | 2                  | 8             | 4                  | 0.015         | 0.015              |
| CDC 168                       | 64            | 0.125              | 64            | 4                  | 1             | 1                  | 0.125         | 0.125              |
| CDC 169                       | >64           | 0.125              | >64           | 4                  | 1             | 1                  | 0.125         | 0.125              |
| CDC 173                       | 64            | 0.06               | >64           | 1                  | 1             | 1                  | 0.125         | 0.125              |
| CDC 178                       | >64           | 0.125              | >64           | 2                  | 1             | 1                  | 0.03          | 0.03               |
| CDC 179                       | >64           | 0.125              | >64           | 4                  | 8             | 4                  | 0.008         | 0.008              |
| CDC 181                       | 64            | 0.25               | >64           | 2                  | >64           | >64                | 0.015         | 0.015              |
| CDC 182                       | 64            | 0.125              | 32            | 4                  | 1             | 1                  | 0.03          | 0.03               |
| CDC 183                       | 32            | 0.06               | 32            | 2                  | 1             | 1                  | 0.06          | 0.03               |
| CDC 184                       | 32            | 0.06               | 64            | 2                  | 1             | 0.5                | 0.06          | 0.06               |
| CDC 186                       | 64            | 0.125              | 64            | 2                  | 0.5           | 0.5                | 0.06          | 0.03               |
| CDC 187                       | 64            | 0.125              | 64            | 2                  | 2             | 2                  | 0.015         | 0.015              |
| CDC 197                       | >64           | 0.25               | 64            | 4                  | 2             | 2                  | 0.015         | 0.015              |
| CDC 202                       | >64           | 0.25               | >64           | 4                  | 16            | 16                 | 0.008         | 0.008              |
| CDC 211                       | 64            | 0.25               | 64            | 1                  | 2             | 2                  | 0.03          | 0.03               |
| ATCC 700825                   | >64           | 0.125              | 64            | 0.5                | 0.125         | 0.125              | 0.008         | 0.008              |
| WHO-V                         | 32            | 0.015              | >64           | 0.5                | 16            | 16                 | 0.008         | 0.008              |
| WHO-W                         | >64           | 0.25               | >64           | 2                  | 0.25          | 0.125              | 0.06          | 0.06               |
| WHO-X                         | >64           | 0.25               | >64           | 1                  | 0.25          | 0.25               | 0.5           | 0.5                |
| WHO-Z                         | >64           | 0.5                | >64           | 4                  | 2             | 2                  | 0.5           | 0.5                |
| <b>MIC<sub>50</sub></b>       | 64            | 0.125              | >64           | 2                  | 1             | 1                  | 0.03          | 0.03               |
| <b>MIC<sub>90</sub></b>       | >64           | 0.25               | >64           | 4                  | 16            | 16                 | 0.125         | 0.125              |

MIC<sub>50</sub>: minimum inhibitory concentration at which the compound/drug inhibited 50% of the tested strains. MIC<sub>90</sub>: minimum inhibitory concentration at which the compound/drug inhibited 90% of the tested strains.

**Table S3.** Drug accumulation values in *N. gonorrhoeae* ATCC700825

|                     | <b>Accumulation (nmol/10<sup>9</sup> CFUs)</b> |               |               |                |
|---------------------|--|---------------|---------------|----------------|
|                     | <b>10 min</b>                                  | <b>30 min</b> | <b>60 min</b> | <b>120 min</b> |
| <b>Tetracycline</b> | 12.5 ± 3.8                                     | 7.9 ± 1.2     | 5.0 ± 0.8     | 5.5 ± 0.3      |
| <b>AZM</b>          | 4.3 ± 0.3                                      | 5.8 ± 0.1     | 7.5 ± 0.4     | 7.7 ± 3.0      |
| <b>EZM</b>          | 17.3 ± 2.9                                     | 21.1 ± 2.2    | 20.3 ± 4.7    | 19.7 ± 4.0     |

Values represent average from two biological replicates ± S.D. Each sample contained a dose of 50 nmol/10<sup>9</sup> CFUs for each compound.