SUPPLEMENTARY MATERIAL

Punigratane, a novel pyrrolidine alkaloid from *Punica granatum* rind with putative efflux inhibition activity.

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Abstract

A new pyrrolidine alkaloid named Punigratane was isolated from the rind of *Punica granatum*. This is the first report of a pyrrolidine like structure from the rind. The activity of this compound was tested in a representative MDR *Klebsiella pneumoniae* strain which exhibited high efflux pump activity. At a concentration of 6 mg, this compound Punigratane was found to have efflux inhibition activity.

**Keywords:** Pyrrolidine alkaloid, Punigratane, *Punica granatum*, pomegranate rind, RND efflux pump inhibitor, multidrug resistance, *Klebsiella pneumoniae*.

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Figure S1: Mass Spectra of Punigratane
Figure S2: Infrared Spectra of Punigratane
Figure S3: $^{13}$C NMR of Punigratane
Figure S4: $^1$H NMR of Punigratane
Figure S5: $^1$H NMR of Punigratane – Expansion

Figure S6: Chemdraw Simulated $^1$H NMR of Punigratane
ChemNMR $^1$H Estimation

Estimation quality is indicated by color: good, medium, rough

### Protocol of the $^1$H NMR Prediction:

| Node | Shift Base + Inc. | Comment (ppm rel. to TMS) |
|------|------------------|---------------------------|
| CH   | 2.54             | pyrroldine                 |
|      | -0.01            | 1 beta - C from methine   |
|      | -0.05            | 1 unknown substituent(s)  |
| CH   | 2.64             | pyrroldine                 |
|      | -0.01            | 1 beta - C from methine   |
|      | -0.05            | 1 unknown substituent(s)  |
| CHZ 1.68 1.425000 | 2.02               | pyrroldine                 |
|      | -0.20            | 1 c - C from H-CN        |
| CHZ 1.68 1.426000 | 1.69               | pyrroldine                 |
|      | -0.12            | 1 unknown substituent(s)  |
| CHZ 2.26             | 0.92               | methyl                     |
|      | -0.04            | 1 beta - C from methylene |
|      | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.38             | 2.52               | methyl                     |
|      | 0.04             | 1 alpha - H(SC)           |
| CHZ 1.38             | -0.04            | 1 beta - C                 |
| CHZ 1.38             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.38             | 1.97               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.38             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.25             | 1.77               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.25             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.25             | 1.33               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.25             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.29             | 1.37               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.29             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.29             | 1.37               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.29             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.29             | 1.37               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.29             | -0.04            | 1 unknown substituent(s)  |
| CHZ 1.31             | 1.37               | methyl                     |
|      | -0.04            | 1 beta - C                 |
| CHZ 1.31             | -0.04            | 1 unknown substituent(s)  |
| CHZ 0.88             | 1.37               | methyl                     |
|      | 0.02             | 1 beta - C                 |
| CHZ 0.88             | 0.02             | 1 unknown substituent(s)  |
| CHZ 0.88             | 0.02             | 1 methyl                   |
|      | 0.02             | 1 unknown substituent(s)  |

### IH NMR Coupling Constant Prediction

**shift**  **atom index**  **coupling partner, constant and vector**

| 2.54 | 2 | H-C=CH-H | 7.0 |
| 2.54 | 3 | H-C=CH-H | 7.0 |
| 2.54 | 4 | H-C=CH-H | 7.0 |
| 1.95 | 5 | 4 diastereotopic | 11.4 |
| 1.95 | 6 | H-C=CH=H | 7.1 |
| 1.95 | 7 | 4 diastereotopic | 11.4 |
| 2.28 | 8 | 7.0 |
| 1.95 | 9 | H-C=CH=H | 7.1 |
| 1.25 | 10 | 7.1 |
| 1.25 | 11 | H-C=CH=H | 7.1 |
| 1.25 | 12 | 7.1 |
| 1.25 | 13 | H-C=CH=H | 7.1 |
| 1.25 | 14 | 7.1 |
| 1.25 | 15 | H-C=CH=H | 7.1 |
| 1.25 | 16 | 7.1 |
| 1.25 | 17 | H-C=CH=H | 7.1 |
| 1.25 | 18 | 7.1 |
| 1.25 | 19 | H-C=CH=H | 7.1 |
| 1.25 | 20 | 7.1 |
| 1.25 | 21 | H-C=CH=H | 7.1 |
| 1.25 | 22 | 7.1 |
| 1.25 | 23 | H-C=CH=H | 7.1 |
| 1.25 | 24 | 7.1 |
| 1.25 | 25 | H-C=CH=H | 7.1 |
| 1.25 | 26 | 7.1 |
| 1.25 | 27 | H-C=CH=H | 7.1 |

### Notes:

- **Shifts** are given in ppm relative to TMS.
- **Coupling Constants** are given in Hz.
- **Vector** indicates the direction of the coupling interaction.
- **Diastereotopic** refers to chemical shifts that are not superimposable due to spatial orientation.

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Each chemical shift and coupling constant is accompanied by a detailed comment indicating the nature of the substituent(s) involved.
Figure S7: Simulated $^1$H NMR of Punigratane- nmrdb.org

| AtomID | From  | To    |
|--------|-------|-------|
| 54     | 0.866 | 0.866 |
| 38     | 0.866 | 0.866 |
| 40     | 0.866 | 0.866 |
| 39     | 0.866 | 0.866 |
| 55     | 0.866 | 0.866 |
| 53     | 0.866 | 0.866 |
| 47     | 1.233 | 1.233 |
| 48     | 1.233 | 1.233 |
| 32     | 1.233 | 1.233 |
| 33     | 1.233 | 1.233 |
| 50     | 1.24  | 1.24  |
| 34     | 1.24  | 1.24  |
| 49     | 1.24  | 1.24  |
| 35     | 1.24  | 1.24  |
| 45     | 1.251 | 1.251 |
| 31     | 1.251 | 1.251 |
| 30     | 1.251 | 1.251 |
| 45     | 1.251 | 1.251 |
| 29     | 1.255 | 1.255 |
| 44     | 1.255 | 1.255 |
| 29     | 1.255 | 1.255 |
| 43     | 1.255 | 1.255 |
| 51     | 1.278 | 1.278 |
| 52     | 1.278 | 1.278 |
| 36     | 1.278 | 1.278 |
| 37     | 1.278 | 1.278 |
| 25     | 1.33  | 1.33  |
| 27     | 1.33  | 1.33  |
| 42     | 1.33  | 1.33  |
| 41     | 1.33  | 1.33  |
| 24     | 1.7435| 1.7435|
| 29     | 1.7435| 1.7435|
| 25     | 1.7435| 1.7435|
| 21     | 1.7435| 1.7435|
| 58     | 2.364 | 2.364 |
| 57     | 2.364 | 2.364 |
| 56     | 2.364 | 2.364 |
| 22     | 2.818 | 2.818 |
| 23     | 2.818 | 2.818 |
Figure S8: Simulated $^{13}$C NMR of Punigratane
### Protocol of the C-13 NMR Prediction:

| Node | Shift | Base | ppm rel. to TMS | Comment |
|------|-------|------|----------------|---------|
| CH   | 69.3  | -9.1 | pyrroldine     | good    |
| CH   | 69.3  | -9.1 | pyrroldine     | general corrections |
| CH2  | 27.0  | -13.3| pyrroldine     | general corrections |
| CH2  | 27.0  | -13.3| pyrroldine     | general corrections |
| CH2  | 27.3  | -13.3| pyrroldine     | general corrections |
| CH2  | 27.3  | -13.3| pyrroldine     | general corrections |
| CH2  | 27.6  | -13.3| pyrroldine     | general corrections |

### Estimation quality is indicated by color:
- good
- medium
- rough

### Diagram:

[Chemical structure diagram]

### Notes:
- The chemical structure diagram shows the connectivity of carbon atoms with their respective shifts.
- The estimation quality is indicated by color:
  - good
  - medium
  - rough
Figure S9: Simulated $^{13}$C NMR of Punigratane – nmrdb.org