Virtual Screening of Chemical Compounds for Discovery of Complement C3 Ligands

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Appendix S2. Explanation of pharmacophore feature abbreviations

Arginine -1
- Positive: a pharmacophore feature representing positive charge is placed on Arginine -1.

Cysteine 2
- HB->D (backbone): A pharmacophore feature representing the hydrogen bond donor capability of the backbone of Cysteine 2.

Valine 3
- Hydro: A pharmacophore feature representing the hydrophobic characteristic of Valine 3.

Tryptophan 4
- Arom (2): Both the six and five-member rings are defined as aromatic features.
- Hydro (2): Both the six and five-member rings are defined as hydrophobic features.
- Arom: Only the six-member ring is defined as an aromatic feature.
- Hydro: Only the six-member ring is defined as a hydrophobic feature.
- Arom+Hydro: The six-member ring defined as an aromatic feature and the five-member ring as a hydrophobic feature.
- Arom+ HB->D (NE1): The six-member ring defined as an aromatic feature and the five-member ring has a hydrogen bond donor feature at NE1.
- Hydro+ HB->D (NE1): The six-member ring defined as a hydrophobic feature and the five-member ring has a hydrogen bond donor feature at NE1.

Glutamine 5
- HB<-A (OE1): A hydrogen bond acceptor feature at OE1 of Glutamine 5.

Aspartic Acid 6
- HB->D (backbone): A pharmacophore feature representing the hydrogen bond donor capability of the backbone of Aspartic Acid 6.
- HB<-A (OD1 or OD2): A pharmacophore feature representing the hydrogen bond acceptor capabilities of OD1 or OD2 on Aspartic Acid 6.

Tryptophan 7
- Arom (2): Both the six and five-member rings are defined as aromatic features.
- Hydro (2): Both the six and five-member rings are defined as hydrophobic features.
- Arom: Only the six-member ring is defined as an aromatic feature.
- Hydro: Only the six-member ring is defined as a hydrophobic feature.
- Arom+Hydro: The six-member ring defined as an aromatic feature and the five-member ring as a hydrophobic feature.
- Arom+ HB->D (NE1): The six-member ring defined as an aromatic feature and the five-member ring has a hydrogen bond donor feature at NE1.
- Hydro+ HB->D (NE1): The six-member ring defined as a hydrophobic feature and the five-member ring has a hydrogen bond donor feature at NE1.

Alanine 9
- HB->D (backbone): A pharmacophore feature representing the hydrogen bond donor capability of the backbone of Alanine 9.

**Histidine 10**

- HB->D (backbone): A pharmacophore feature representing the hydrogen bond donor capability of the backbone of Histidine 10.
- HB->D (NE2): A hydrogen bond donor feature at NE1 of Histidine 10.
- HB->D (backbone + NE2): Combination of the above two features.
Appendix S3. Intermolecular interaction analysis

The binding site on C3 can be divided into 4 sectors based on residues in direct contact with compstatin (I: 344–349, II: 388–393, III: 454–462, and IV: 488–492)\(^1\)\(^-\)\(^3\). From analysis of the MD trajectory of C3c-bound compstatin analog with sequence Ac-RSI[CVQDWGAHRC]T-NH\(_2\), we identified persistent intermolecular interactions that confirm these contacts (Table 1). Occupancy analysis of hydrogen bonds in the MD trajectory demonstrate persistent (>60% occupancy) between compstatin and C3c. R-1, C2, W4, Q5, W7, A9, and H10 are some of the residues participating in these conserved hydrogen bonds that are also consistent with prior experimental data. Residues R-1 and D6 were identified as contributors to highly conserved salt bridges as well. These intermolecular interactions, corroborated by previous energetic analysis\(^4\), subsequently informed the choice of pharmacophore features and generation of the models.

Table S1 - Electrostatic analysis of the MD trajectory of C3c:RSI-compstatin\(^*\).

| Hydrogen Bonds        | Compstatin | C3c   |
|-----------------------|------------|-------|
| S0-O                  | N390-ND2   |       |
| W4-O                  | R456-NE    |       |
| Q5-OE1                | M457-N     |       |
| R(-1)-NE              | E372-OE2   |       |
| R(-1)-NH1             | E372-OE1   |       |
| C2-N                  | N390-OD1   |       |
| W4-N                  | G345-O     |       |
| W7-NE1                | M457-O     |       |
| A9-N                  | D491-OD2   |       |
| H10-N                 | D491-OD1   |       |
| H10-ND1               | D491-OD1   |       |

| Ionic Interactions (< 8 Å) | Compstatin | C3c   |
|---------------------------|------------|-------|
| R(-1)-CZ                  | E372-CD    |       |
| D6-CG                     | R459-CZ    |       |

\(^*\)Hydrogen bonds and ionic interactions are those with at least 60% occupancy in the trajectory. A hydrogen bond is identified according to Baker-Hubbard criteria as implemented in MDTraj. An ionic interaction is identified using a range of up to 8 Å.
Appendix S6. Two-dimensional structures of experimentally tested chemical compounds

ZINC12000754.pdbqt
ZINC12079160.pdbqt

ZINC12093978.pdbqt
ZINC12194353.pdbqt
Figure S1 - Two-dimensional structures of experimentally tested chemical compounds
Appendix S8. Pharmacophore models utilized in Stages 1 and 2 of the alternative approach

**Table S2.** The eight C3c amino acids in the compstatin binding site that were targeted and the pharmacophore features used to target them. The first round of pharmacophore models from the alternative approach consists of a combination of four features. The combination of the four features target each of the four sectors that have been identified as being important to compstatin binding to human C3c<sup>1-3</sup>. All pharmacophore features have a radius of 1.5 Å.

| Sector | C3c Target | Compstatin | Feature | Additional Feature |
|--------|------------|------------|---------|--------------------|
| I      | M346 - Hydrophobic sidechain | V3 - Sidechain | Hydrophobic | |
| I      | L347 - Hydrophobic sidechain | V3 - Sidechain | Hydrophobic | |
| II     | N390 - Polar sidechain | C2 - Backbone | Hydrogen bond acceptor | |
| II     | H392 - Aromatic/polar sidechain | W4 - Sidechain | Aromatic | Hydrogen bond donor |
| III    | R459 - Charged sidechain | D6 - Backbone | Hydrogen bond acceptor | Negative ion |
| III    | R456 - Charged sidechain | W4 - Backbone | Hydrogen bond acceptor | Negative ion |
| IV     | D491 - Charged sidechain | Q5 - Sidechain | Hydrogen bond donor | Positive ion |
| IV     | L492 - Hydrophobic sidechain | H10-Sidechain | Hydrophobic | |

**Table S3.** The five additional pharmacophore models that were developed based on the five molecules with the lowest average binding energy identified from the first round of the alternative approach. From the simulations of the five molecules in complex with human C3c, the lowest binding energy snapshot was extracted. Based on the five extracted simulation snapshots, these three pharmacophore models were generated. The pharmacophore features in this round have a radius of 1.5 Å and were required to target amino acids within at least three of the four sectors.

| Additional model | C3c Target | Feature | C3c Target | Feature | C3c Target | Feature | C3c Target | Feature |
|-----------------|------------|---------|------------|---------|------------|---------|------------|---------|
| 1               | M346 - Hydrophobic sidechain | Hydrophobic | R456 - Charged sidechain | Aromatic | R459 - Charged sidechain | Hydrogen bond acceptor | D491 - Charged sidechain | Hydrogen bond donor |
| 2               | M346 - Hydrophobic sidechain | Hydrophobic | R456 - Charged sidechain | Aromatic | R459 - Charged sidechain | Hydrogen bond acceptor | D491 - Charged sidechain | Hydrogen bond donor |
| 3               | M346 - Hydrophobic sidechain | Hydrophobic | N390 – Polar sidechain | Hydrogen bond acceptor | R456 - Charged sidechain | Hydrogen bond acceptor | D491 - Charged sidechain | Hydrogen bond donor |
| 4               | M346 - Hydrophobic sidechain | Hydrophobic | R456 - Charged sidechain | Hydrogen bond acceptor | D491 - Charged sidechain | Hydrogen bond donor | D491 - Charged sidechain | Hydrogen bond donor |
| 5               | M346 - Hydrophobic sidechain | Hydrophobic | R456 - Charged sidechain | Hydrogen bond acceptor | D491 - Charged sidechain | Hydrogen bond donor | D491 - Charged sidechain | Hydrogen bond donor |
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