*Supplementary Materials*

# Evaluation of Cyclic Peptide Inhibitors of the Grb7 Breast Cancer Target: Small Change in Cargo Results in Large Change in Cellular Activity

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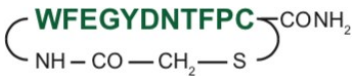
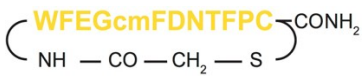
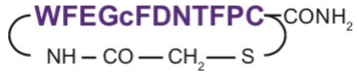
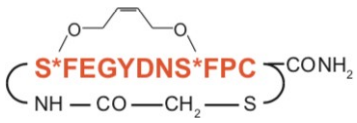
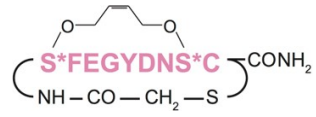
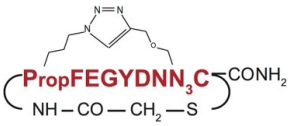
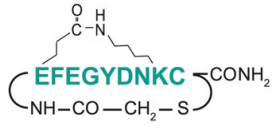
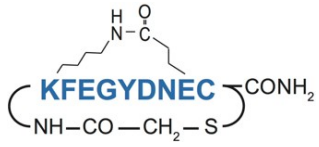
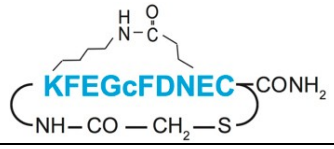
**Supporting Information Table S1**

**Affinity of G7-peptides for Grb7-SH2 domain**

Provided is a summary of G7-peptides previously reported. The schematics show the single letter amino acid nomenclature in coloured font and chemical structures are represented in black.

| Peptide Name | $K_D$ (μM) | Buffer | Reference |
|---|---|---|---|
| G7-18NATE   | 18.1 | 1 mM $Na_3PO_4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [1] |
| G7-M1   | 5.7 | 1 mM $Na_3PO_4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [1] |
| G7-M2   | 2.1 | 1 mM $Na_3PO_4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [1] |
| G7-B1   | 1.5 | 50 mM $Na_3PO4$, 150 mM NaCl, 1 mM DTT (pH 7.4) | [2] |
| G7-B4   | 0.83 | 50 mM $Na_3PO4$, 150 mM NaCl, 1 mM DTT (pH 7.4) | [2] |
| G7-B5   | 7.8 | 1 mM $Na_3PO4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [3] |
| G7-B6   | 15.7 | 1 mM $Na_3PO4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [3] |

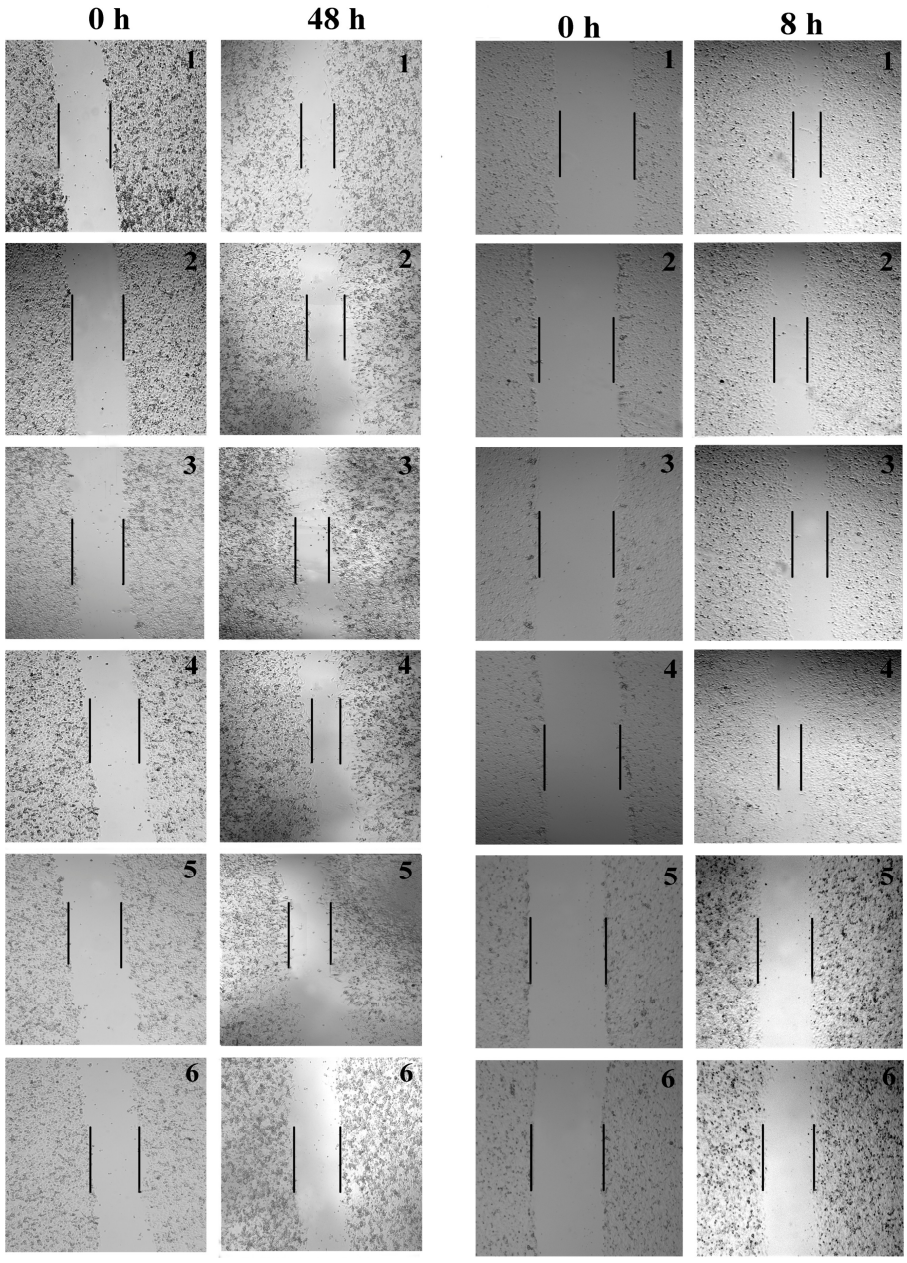
| | | | |
|---|---|---|---|
| G7-B7   | 1.1 | 1 mM $Na_3PO4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [3] |
| G7-B7M1   | 0.22 | 1 mM $Na_3PO4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [3] |
| G7-B7M2   | 0.13 | 1 mM $Na_3PO4$, 20 mM Tris, 150 mM NaCl, 1 mM DTT (pH 7.4) | [3] |

**References**


1. Watson, G.M.; Gunzburg, M.J.; Ambaye, N.D.; Lucas, W.A.; Traore, D.A.; Kulkarni, K.; Cergol, K.M.; Payne, R.J.; Panjikar, S.; Pero, S.C., et al. Cyclic peptides incorporating phosphotyrosine mimetics as potent and specific inhibitors of the Grb7 breast cancer target. *J. Med. Chem.* **2015**, *58*, 7707-7718.
2. Gunzburg, M.J.; Kulkarni, K.; Watson, G.M.; Ambaye, N.D.; Del Borgo, M.P.; Brandt, R.; Pero, S.C.; Perlmutter, P.; Wilce, M.C.; Wilce, J.A. Unexpected involvement of staple leads to redesign of selective bicyclic peptide inhibitor of Grb7. *Sci. Rep.* **2016**, *6*, 27060.
3. Watson, G.M.; Kulkarni, K.; Sang, J.; Ma, X.; Gunzburg, M.J.; Perlmutter, P.; Wilce, M.C.J.; Wilce, J.A. Discovery, Development, and Cellular Delivery of Potent and Selective Bicyclic Peptide Inhibitors of Grb7 Cancer Target. *J Med Chem* **2017**, *60*, 9349-9359.

**Supporting Information Figure S1**

Representative monolayer wound healing assays conducted for SKBR-3 (left) and MDA-MB-231 cells (right) treated with 1: control; 2: Pen; 3: G7-B7-Pen; 4: G7-B7M2-Pen; 5: G7-M2-Pen; 6: G7-18NATE-Pen. Results of triplicate experiments form the basis of Figure 4 of the main manuscript.



**Supporting Information Table S2**

Peak areas for quantifier transitions were normalized against internal standard using MultiQuant v3.0 (SCIEX) software. Peptide concentration in each sample was determined via comparison of normalized signal peak areas with external calibration curves made in sample-equivalent matrices on the same day as the cell assay. The experiment was performed in triplicate and repeated on two independent days.

| Analyte | Transition | Q1 (*m*/*z*) | Q3 (*m*/*z*) | CE[a] (V) | DP[b] (V) | RT (mins) |
|---|---|---|---|---|---|---|
| G7-18NATE | Quantifier | 720.3 | 1077.2 | 31 | 130 | 2.44 |
| " | Qualifier | 720.3 | 1264.3 | 40 | 130 | 2.44 |
| " | Qualifier | 720.3 | 1094.3 | 41 | 130 | 2.44 |
| G7-18NATE-Pen | Quantifier | 911.9 | 907.9 | 43 | 130 | 1.91 |
| " | Qualifier | 1215.6 | 1210.1 | 66 | 130 | 1.91 |
| " | Qualifier | 911.9 | 1095.1 | 51 | 130 | 1.91 |
| G7-B7M2-Pen | Quantifier | 677.1 | 798.1 | 31 | 130 | 1.66 |
| " | Qualifier | 846.1 | 1079.0 | 46 | 130 | 1.66 |
| " | Qualifier | 846.1 | 1035.6 | 47 | 130 | 1.66 |

[a] Collision energy; [b] declustering potential.

**Reference**

Anderson, L., and Hunter, C. L. (2006) Quantitative Mass Spectrometric Multiple Reaction Monitoring Assays for Major Plasma Proteins. Mol. Cell. Proteom. 5, 573-588.

**Supporting Information Figure S2**

MRM plots for G7-18NATE (panel A), G7-18NATE-Pen (panel B), and G7-B7M2-Pen (panel C), illustrating coelution and relative intensities of their transitions. MRM quantifier shown with blue trace and (blue) and qualifiers in (green and red).