
# TSRI MLSCN Probe Report for Inhibitors of MMP-13:

- **2-[(4-chlorophenyl)methylsulfanyl]-1,5,6,7-tetrahydrocyclopenta[e]pyrimidin-4-one ("compound Q")**

- **2-[(4-methoxyphenyl)methylsulfanyl]-6-methyl-1H-pyrimidin-4-one ("compound W")**

Data for this report not published in PubChem may be found in the following publications:

*[1] Ranking the selectivity of PubChem screening hits by activity-based protein profiling: MMP13 as a case study*. Ryuichiro Nakai, Cleo M. Salisbury, Hugh Rosen†, and Benjamin F. Cravatt. Bioorganic & Medicinal Chemistry (submitted), and

*[2] High throughput screening of potentially selective MMP-13 exosite inhibitors utilizing a triple-helical FRET substrate.* Janelle L. Lauer-Fields, Dmitriy Minond, Peter S. Chase, Pierre E. Baillargeon, Peter Hodder, and Gregg B. Fields (Bioorganic & Medicinal Chemistry, submitted)

**Project Title:** High Throughput Screening for Selective Inhibitors of MMP-13
**Grant Number:** 1 X01 MH078948-01
**Screening Center Name:** The Scripps Research Institute Molecular Screening Center
**Principal Investigator of Screening Center:** Hugh Rosen
**Assay Provider & Institution:** Gregg Fields, Florida Atlantic University
**Assay or Pathway Target:** MMP-13, matrix metalloproteinase-13
**Probe PubChem Compound Identifier (SID/CID)**:
Compound Q (1) = 4257091 / 2047223
Compound W (2) = 7974872 / 716696

# 1. Assay Provider Information

***Specific Aim***: To identify selective chemical inhibitors of MMP-13

***Significance***: Osteoarthritis (OA) is an age-related debilitating disease affecting more than 80% of people over the age of 75, caused by the destruction of articular cartilage [3]. The major components of the cartilage extracellular matrix (ECM) are type II collagen and the chondroitin sulfate proteoglycan, aggrecan [4]. MMP-13 is believed to be a prominent collagenase in OA [5, 6]. Initial clinical trials with MMP inhibitors targeting active site were disappointing, due to a lack of selectivity [7]. The key role of MMP-13 in diseases such as cancer, heart disease, and osteoarthritis, combined with the challenge of identifying truly selective MMP inhibitors, makes the identification of selective MMP-13 inhibitors necessary.

***Rationale***: Although many MMP inhibitors have been developed, most have failed in clinical trials due to their ability to inhibit other MMP family members, leading to off-target effects. The discovery of exosites (secondary substrate binding sites) presents unique opportunities for the design of selective inhibitors [8]. In addition, collagen-model conformationally-constrained fluorescence resonance energy transfer (FRET) substrates can aid in the determination of kinetic parameters and activation energies for collagenolytic MMPs, such as MMP-13 [9, 10]. Because the collagen-model FRET substrates have distinct conformational features that interact with exosites, non-active-site binding inhibitors can be identified that bind to MMP. Thus, combining our knowledge of exosites with FRET technology is likely an efficient model for identifying unique probes for MMP inhibition. A continuous assay method, which utilizes an increase in fluorescence upon hydrolysis, allows for rapid and convenient kinetic evaluation of proteases, both in solution and cell surface bound. For the specific application of collagenolytic MMPs, triple-helical peptides (THPs) have been developed as substrates to measure MMP activities. These THPs utilize FRET/ intramolecular fluorescence energy transfer via incorporation of a fluorophore group and a quencher group within the same peptide chain [9, 11, 12]. The use of these FRET THP (fTHP) technologies in HTS will improve the identification of selective MMP-13 inhibitors.

# 2. Screening Center Information

## 2A. Assay Implementation and Screening

***PubChem bioassay names and identifiers:*** Probe development followed two paths (Table 1). One path focused exclusively on the selection and profiling of compounds that did not appear to be fluorescent at the HTS assay excitation wavelengths (AIDs 570, 735, and Nakai et al.). The other included the profiling of compounds that appeared to be fluorescent at HTS excitation wavelengths (AIDs 734, 769, Lauer-Fields et al.). The probes presented here do not appear to be fluorescent.

***List of relevant AIDs that may be used as counterscreen information:*** Compounds with large negative inhibition values in AID 570 that are not active in AID 734 are possibly autofluorescent. Additionally, PubChem AIDs that use the fluorophore 7-methoxycoumarin as a detection reagent may be helpful in identifying other fluorescent artifact.

**Table 1. Summary of MMP-13 PubChem BioAssays.**

| PubChem AID | BioAssay Title | Assay Type | Number of Compounds Tested | Number of Actives | Hit Rate (%) |
|---|---|---|---|---|---|
| 570 | Primary biochemical high-throughput screening assay for inhibitors of Matrix Metalloproteinase 13 (MMP13) activity | Primary | 64,925 | 46 | 0.071 |
| 734 | Assay to identify inhibitors among the possible fluorescent artifacts from the primary HTS inhibition assay of Matrix Metalloproteinase 13 (MMP13) activity | Single Concentration, Triplicate results | 5,149 | 8 | 0.156 |
| 735 | Dose-response biochemical assay for inhibitors of Matrix Metalloproteinase 13 (MMP13) activity | Dose response | 42 | 15 | 35.7 |
| 769 | Dose response biochemical assay for autofluorescent inhibitors of Matrix Metalloproteinase 13 (MMP13) activity | Dose Response | 8 | 4 | 50 |

***Primary screen summary (AID 570)***. Specific methods and details of this screen can be found in PubChem at http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=570. The results of the screen are shown in **Figure 1**. A mathematical algorithm was used to determine nominally inhibitory compounds in the primary screen. Two values were calculated: (1) the average percent inhibition of all wells in the sample field of control plate devoid of test or control compounds, and (2) three times their standard deviation. The sum of these two values was used as a cutoff parameter, i.e. any compound that exhibited greater %inhibition than the cutoff parameter was declared active. A total of 46 compounds were identified as active using these selection criteria, which yielded a

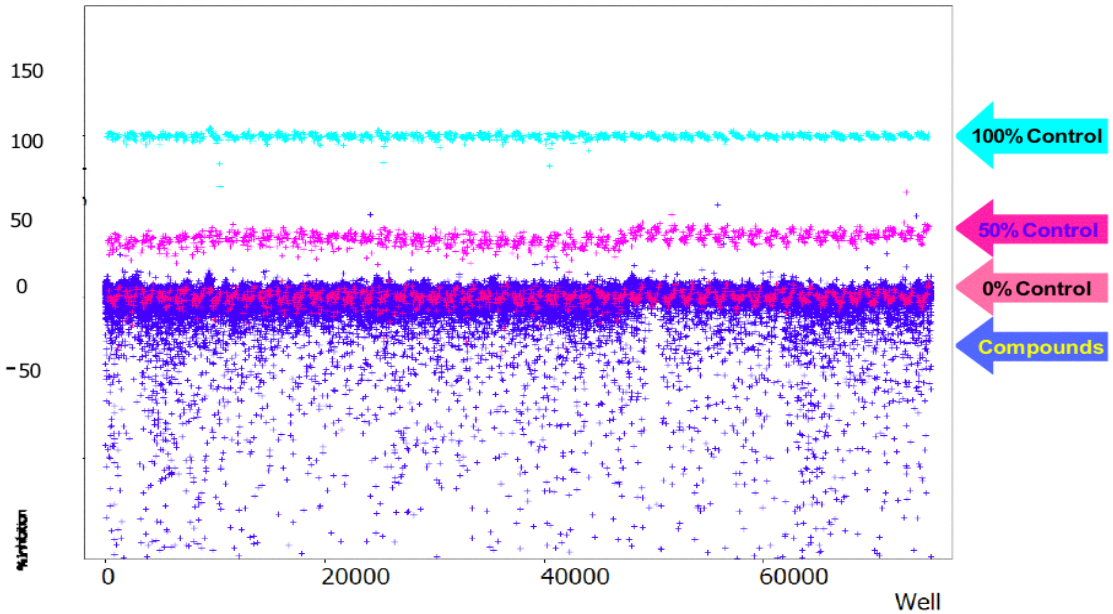



**Figure 1.** Scattergram of MMP-13 primary screen results. Percent MMP-13 inhibition values are plotted against well number. Negative inhibition is attributed to overlap of the UV absorbance of a test compound with the substrate fluorophore (7-methoxycoumarin) during excitation. "Compounds" indicates wells containing 4 µM test compound. The % inhibition by each control is also shown. These controls are 0% inhibition (wells containing 0.3% DMSO), 50% inhibition (wells containing 80 nM MMP-13 inhibitor), and 100% inhibition (wells containing 8 µM MMP-13 inhibitor).

0.071% hit rate. Using cutoff criteria similar to that used to identify inhibitors, fluorescent artifacts were also identified. Specifically, wells that had an inhibition value of lower than -10.66% indicated the possible presence of a fluorescent test compound in the well. Triage of these compounds is discussed in detail in Appendix A.

***Dose-response assay to determine the $IC_{50}$ of non-fluorescent compounds for MMP-13 inhibition (AID 735)***. Specific methods and details of this screen can be found in PubChem at http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=735. Forty-two available non-fluorescent compounds out of the 46 active in the primary screen were assessed in dose response experiments. Compounds with $IC_{50}$ values greater than 10 µM were considered inactive; compounds with $IC_{50}$ values equal to or less than 10 µM were considered active. **Table 2** provides $IC_{50}$ results from this screen (cf. column 5) and vendor information (cf. column 3). Thirty-four compounds produced pharmacological dose-response curves, and 15 were reported as active in PubChem.

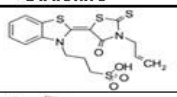
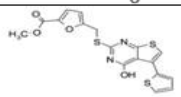
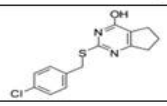
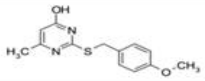
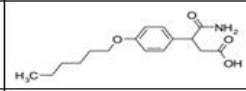
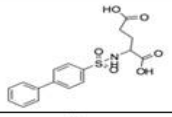
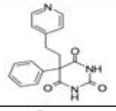
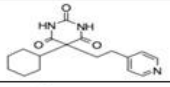
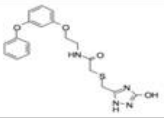
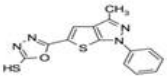
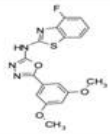
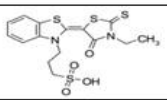
**Table 2. Results of AID 735 and substrate assays from references [1] and [2].** MMP13 probes are highlighted in yellow. *Indicates average (95% CI) of three independent experiments). **Compound 1 (Q) shows partial inhibition (maximal inhibition ~70%).

| Compound ID in Ref 2 (Ref 1) | MLS # | Vendor | Vendor Cat # | Compound $IC_5$ (µM) in MMP-13 Assays | | | Compound $IC_{50}$ (µM) in MMP-8 Substrate Assays (Ref 1) | Compound $IC_{50}$ (µM) in MMP-9 Substrate Assays (Ref 1) |
|---|---|---|---|---|---|---|---|---|
| | | | | AID 735 | ABPP Assay (Ref 1)* | Substrate Assay (Ref 1)** | | |
| A | MLS000075845 | Asinex | BAS 08769335 | 13.3 | | | | |
| B | MLS000035239 | Asinex | ASN 05830127 | 4.7 | | | | |
| C (4) | MLS000075919 | Asinex | BAS 07869980 | 2.1 | 13 (10-18) | 0.28 (0.25-0.32) | 0.024 (0.023-0.025) | 14 (11-17) |
| D | MLS000035419 | Asinex | BAS 06754276 | 9.9 | | | | |
| E | MLS000073086 | Asinex | BAS 04834866 | 2.5 | | | | |
| F | MLS000068249 | Asinex | ASN 04363458 | 9.4 | | | | |
| G | MLS000027771 | Asinex | BAS 09533241 | 15.3 | | | | |
| H | MLS000031372 | Asinex | BAS 00506290 | 10.9 | | | | |
| I | MLS000071970 | Asinex | BAS 00506281 | 24.7 | | | | |
| N | MLS000051260 | ChemBridge | 7925560 | 6.8 | | | | |
| P | MLS000111675 | ChemBridge | 6370266 | >40 | | | | |
| J | MLS000105542 | ChemBridge | 5161874 | 12.6 | | | | |
| O | MLS000108799 | ChemBridge | 5927508 | 7.7 | | | | |
| M | MLS000049833 | ChemBridge | 7894782 | 16.5 | | | | |
| L | MLS000096979 | ChemBridge | 7685300 | >40 | | | | |
| R | MLS000062315 | ChemBridge | 6624994 | 3.6 | | | | |
| Q (1) | MLS000062185 | ChemBridge | 6512965 | 3.4 | 3.8 (2.8-5.2) | 0.92 (0.72-1.2) | >100 | >100 |
| K | MLS000104229 | ChemBridge | 5215570 | 10.3 | | | | |
| C' | MLS000047713 | ChemDiv | 2324-0448 | 12.0 | | | | |
| B' | MLS000092741 | ChemDiv | C656-0067 | 23.3 | | | | |
| W (2) | MLS000109390 | Deltagen | 4065-0146 | 4.3 | 20 (13-33) | 1.6 (1.2-2.0) | >100 | >100 |
| V (3) | MLS000073581 | Deltagen | K408-0544 | 4.8 | 27 (19-40) | 1.3 (1.2-1.5) | 0.67 (0.63-0.70) | 0.16 (0.14-0.19) |
| X | MLS000057191 | EnAmine | T5347014 | 8.4 | | | | |
| Y | MLS000057778 | EnAmine | T0511-0376 | 13.3 | | | | |
| Z | MLS000055414 | EnAmine | T0507-2864 | 8.1 | | | | |
| U | MLS000069631 | Sigma | C1386 | 10.3 | | | | |
| T | MLS000043319 | ChemBridge | 7461374 | 0.9 (AID 769) | | | | |
| S | MLS000106248 | ChemBridge | 5344221 | 1.7 (AID 769) | | | | |
| A' | MLS000045466 | ChemDiv | C505-0274 | 3.5 (AID 769) | | | | |
| D' | MLS000041467 | ChemDiv | 6917-0116 | 4.0 (AID 769) | | | | |

**Secondary RP-HPLC screen to confirm compound activity remove assay artifact:** This low-throughput assay validated 25 (out of 30 available) compounds as MMP-13 inhibitors and eliminated compounds that inhibit non-specifically (e.g., interact with substrate) or interfere with fluorescence of the Mca-containing peptide fragment. Refer to [2] for details.

**Probe RP-HPLC & ABPP Selectivity Profiling Assays:** Twelve of the compounds identified in the RP-HPLC screen were selected for singlicate counter screening at 100 µM against 6 representative MMP family members [2]. Four compounds (H, X, Q, C') were selective for MMP-13 in this assay (Table 3). Four compounds (Q, W, V, and C) were assayed in triplicate using affinity-based protein profiling (ABPP) and found to be MMP-13 inhibitors (Figure 2, Table 2) [1].

**Table 3. Results of RP-HPLC Selectivity Profiling Assays.** Reported is the % MMP activity in the presence of compound (reference 2).

| Compound | Structure | MMP-1 | MMP-2 | MMP-3 | MMP-8 | MMP-9 | MMP-12 | MMP-13 |
|---|---|---|---|---|---|---|---|---|
| H |  | 96 | 100 | 100 | 100 | 100 | 100 | 36 |
| X |  | 93 | 100 | 92 | 94 | 100 | 92 | 42 |
| Q |  | 94 | 100 | 96 | 80 | 99 | 100 | 62 |
| W |  | 98 | 47 | 97 | 80 | 66 | 72 | 25 |
| V |  | 91 | 0 | 43 | 10 | 0 | 0 | 15 |
| C |  | 25 | 7 | 63 | 2 | 14 | 2 | 4 |
| R |  | 27 | 17 | 87 | 8 | 3 | 1 | 14 |
| E |  | 47 | 49 | 96 | 50 | 27 | 21 | 35 |
| M |  | 85 | 19 | 96 | 34 | 27 | 33 | 35 |
| T |  | 68 | 100 | 75 | 32 | 100 | 75 | 45 |
| A' |  | 96 | 73 | 95 | 36 | 75 | 64 | 35 |
| C' |  | 88 | 80 | 90 | 100 | 81 | 94 | 44 |

Additionally, substrate assay $IC_{50}$ data against MMP-13 and MMPs-8 and -9 (**Table 2**) revealed that compounds Q and W are selective MMP-13 inhibitors [1]. These assays also demonstrate that compound C (4) is selective against MMP-8**.** This compound will be the focus of a separate probe report. Refer to reference [1] for details.

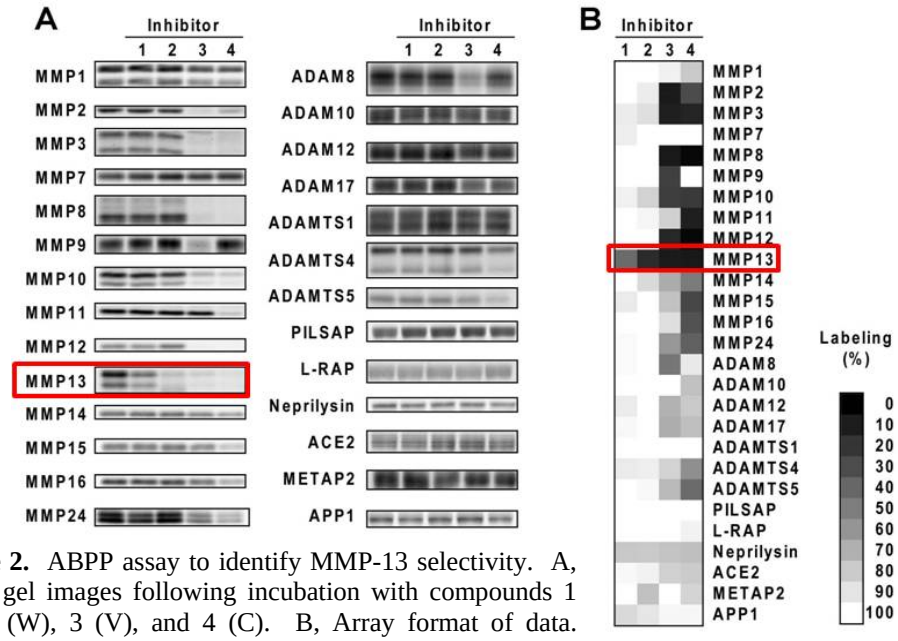
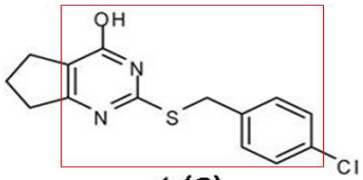


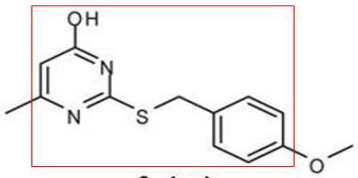

**Figure 2.** ABPP assay to identify MMP-13 selectivity. A, ABPP gel images following incubation with compounds 1 (Q), 2 (W), 3 (V), and 4 (C). B, Array format of data. MMP-13 is highlighted.

**Comparative data on probe, similar compound structures and information on existing probes available to the public**. Upon query, PubChem lists 23 links to "similar compounds" for compound Q, and 51 for compound W. Selective compounds Q and W are similar to the Warner-Lambert pyrimidinediones, which have been characterized as allosteric MMP-13 selective inhibitors [13]. Compound Q is perhaps the most interesting. In addition to being selective for MMP-13, it may be mechanistically distinct from the other inhibitors identified here, as it was more effective against MMP-13 triple-helical peptidase activity compared with MMP-13 single-stranded peptidase activity and thus may interact with an MMP-13 collagen-binding exosite.




1 (Q)

**MLS000062185**
**23 similar compounds (CID 2047223)**
**70 similar substances (SID 4257091)**
2-[(4-chlorophenyl)methylsulfanyl]-1,5,6,7-tetrahydrocyclopenta[e]pyrimidin-4-one
AID 735 $IC_{50}$ (μM): 3.4
ABPP $IC_{50}$ (μM): 3.8 (2.8-5.2)
Substrate $IC_{50}$ (μM): 0.92 (0.72-1.2)




2 (W)

**MLS000109390**
**51 similar compounds (CID 716696)**
**183 similar substances (SID 7974872)**
2-[(4-methoxyphenyl)methylsulfanyl]-6-methyl-1H-pyrimidin-4-one
AID 735 $IC_{50}$ (μM): 4.3
ABPP IC50 (μM): 20 (13-33)
Substrate $IC_{50}$ (μM): 1.6 (1.2-2.0)

**Figure 3.** Chemical probes identified and data summary of the MMP-13 inhibitor campaign. The box outlines the core pyrimidinedione structure shared by the two compounds. The IUPAC name is listed, with red text highlighting different substituents.

**Center summary of probe properties (solubility, absorbance/fluorescence, reactivity, toxicity, etc.)**: There is no toxicity data available for compounds Q and W in PubMed, ChemIDplus, and ToxNet. Other calculated probe properties are indicated in Table 4. These data were prepared using ChemInfo.

**Table 4.** Calculated Probe Properties

| Compound ID | Q | W |
|---|---|---|
| PubChem SID | 4257091 | 7974872 |
| PubChem CID | 2047223 | 716696 |
| MF | C14H13ClN2OS | C13H14N2O2S |
| MW | 292.78381 | 262.32745 |
| Formal Charge | 0 | 0 |
| H Acceptor | 4 | 5 |
| H Donor | 1 | 1 |
| Atom Count | 19 | 18 |
| Rotatable Bonds | 3 | 4 |
| Rings | 3 | 2 |
| Stereoatoms | 0 | 0 |
| AlogP | 4.723 | 3.26 |
| logD | 4.723 | 3.26 |
| Polar surface area | 71.31 | 80.54 |
| Aqueous solubility[a] | -4.8689 | -3.65233 |
| ADMET BBB[b] | 0.62 | 0.027 |
| ADMET BBB level[c] | 1 | 1 |
| ADMET absorption level[d] | 0 | 0 |
| ADMET solubility[e] | -5.267 | -3.686 |
| ADMETT solubility level[f] | 2 | 3 |
| Vendor | ChemBridge | ChemBridge |
| Vendor Catalog Number | 6512965 | 5942399 |

[a]Aqueous solubility is expressed as logS, where S is the solubility in mol/L. The method used to estimate the solubility is the multiple linear regression model based on Electrotopological State indices published in [16].
[b]ADMET_BBB: Log of Brain/Blood partition coefficient (LogBB).
[c]ADMET_BBB_Level: Ranking of the LogBB values into one of the following levels:
0: Very High 1: High 2: Medium 3: Low 4: Undefined (molecule is outside the confidence area of the regression model used to calculate LogBB).
[d]ADMET Passive Intestinal Absorption properties. A ranking of the molecule into one of the following levels: 0: Good 1: Moderate 2: Poor 3: Very Poor
[e]ADMET_Solubility: Log of the water solubility at 25 degrees, LogSw, in mol/L.
[f]ADMET_Solubility_Level: Ranking of the aqueous solubility values into the following classes:
0: Extremely Low 1: Very Low 2: Low 3: Good 4: Optimal 5: Very Soluble

A PubChem query was performed to determine the activity of Compounds Q and W in other assays. Please see Appendices C & D for details. The results of this query show that they are active in PubChem only in AIDs 570 and 735 (MMP-13 primary and dose response inhibitor screens). Interestingly, neither compound induced cell death (toxicity) or proliferation effects against lung tumor cell lines (AID 598), colon cancer cells (AID 818), breast cancer cells (AID 847), HT1080 cells (AID 620), endothelial cells (AID

648), lung fibroblasts (AID 719), HPDE-C7 cells (AID 430), or yeast (AID804). Additionally, neither compound was active in screens for inhibitors of important signal transduction pathways such as JNK3 (AID 746), STAT3 (AID 862), HER kinase (AID 645), Ras GTPases (AID 758), NFκB (AID 465), PKA (AID 524), or MT1-MMP (AID 618), nor did they modify CYP2C19 (AIDs 777 and 778). These data suggest that compounds Q and W work by an as yet defined mechanism to inhibit MMP-13.

**Recommendations for the scientific use of probe as research tool**

The 2 compounds described herein are selective, potent inhibitors of MMP-13. These can be used as research tools for MMP-13.

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13. Breuer, E., J. Frant, and R. Reich, *Recent non-hydroxamate matrix metalloproteinase inhibitors.* Expert Opin. Ther. Patents, 2005. **15**: p. 253-269.

# Appendix A.
# Additional Assays executed for the MMP-13 HTS campaign.

This appendix details additional assays that were uploaded to PubChem but not used to identify the probes presented in this report.

**Screen to identify fluorescent MMP inhibitors (AID 734):** Specific methods and details of this screen can be found in PubChem at http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=734. This screen tested 5,149 compounds from the primary screen that demonstrated potential activity, but were fluorescent. To avoid the contribution of these compounds' intrinsic fluorescence to the measured assay signal, two readings were performed over time. This screen identified 8 active compounds, four of which (A', S, D', and T) passed activity selection criteria in the subsequent fluorescence dose response screen (AID 769). N.B., the bottom of Table 2 shows $IC_{50}$ values for fluorescent compounds T, S, A', and D' using the fTHP substrate (PubChem AID 769, see below). These compounds will be discussed in future probe reports.

**Dose response biochemical assay for autofluorescent inhibitors of Matrix Metalloproteinase 13 (MMP13) activity (AID 769):** Specific methods and details of this screen can be found in PubChem at http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=769**.** The 8 compounds that were scored as nominally active in the assay for autofluorescent MMP-13 inhibitors (AID 734) were tested in triplicate using 10 point 1:3 serial dilutions starting at a nominal concentration of 40 µM. Four compounds (T, S, A', and D') produced pharmacological dose response curves (**Table 2)**. However, selectivity profiling assays revealed that T and A' are not MMP-13 selective, with no selectivity data available for S and D'.

# Appendix B.
# PubChem *In silico* Profiling Results for Compound W.

This appendix shows the results of a PubChem query executed on 19DEC2007. As can be seen below, in the 147 assays queried, it was found to be active only in AID 570 & 735.

| Name | Assay ID | Active | Inactive | Discrepant | Tested | Outcome Method |
|---|---|---|---|---|---|---|
| 1 | 735 | 1 | | | 1 | Confirmatory |
| 2 | 570 | 1 | | | 1 | Screening |
| 3 | 902 | | 1 | | 1 | Confirmatory |
| 4 | 901 | | | | 1 | Confirmatory |
| 5 | 900 | | 1 | | 1 | Confirmatory |
| 6 | 898 | | 1 | | 1 | Screening |
| 7 | 893 | | 1 | | 1 | Confirmatory |
| 8 | 892 | | 1 | | 1 | Confirmatory |
| 9 | 889 | | 1 | | 1 | Confirmatory |
| 10 | 887 | | 1 | | 1 | Confirmatory |
| 11 | 886 | | 1 | | 1 | Confirmatory |
| 12 | 881 | | 1 | | 1 | Confirmatory |
| 13 | 878 | | 1 | | 1 | Screening |
| 14 | 875 | | 1 | | 1 | Confirmatory |
| 15 | 871 | | 1 | | 1 | Screening |
| 16 | 868 | | 1 | | 1 | Screening |
| 17 | 862 | | 1 | | 1 | Screening |
| 18 | 861 | | 1 | | 1 | Screening |
| 19 | 853 | | 1 | | 1 | Screening |
| 20 | 848 | | 1 | | 1 | Other |
| 21 | 845 | | 1 | | 1 | Other |
| 22 | 841 | | 1 | | 1 | Screening |
| 23 | 836 | | 1 | | 1 | Screening |
| 24 | 834 | | 1 | | 1 | Screening |
| 25 | 828 | | 1 | | 1 | Screening |
| 26 | 827 | | 1 | | 1 | Screening |
| 27 | 819 | | 1 | | 1 | Screening |
| 28 | 818 | | 1 | | 1 | Screening |
| 29 | 817 | | 1 | | 1 | Other |
| 30 | 813 | | 1 | | 1 | Screening |
| 31 | 808 | | 1 | | 1 | Screening |
| 32 | 804 | | 1 | | 1 | Screening |
| 33 | 803 | | 1 | | 1 | Screening |
| 34 | 802 | | 1 | | 1 | Screening |
| 35 | 800 | | 1 | | 1 | Screening |

| | | | | |
|---|---|---|---|---|
| 36 | 799 | 1 | 1 | Screening |
| 37 | 798 | 1 | 1 | Screening |
| 38 | 797 | 1 | 1 | Screening |
| 39 | 796 | 1 | 1 | Screening |
| 40 | 793 | 1 | 1 | Screening |
| 41 | 782 | 1 | 1 | Screening |
| 42 | 781 | 1 | 1 | Screening |
| 43 | 778 | 1 | 1 | Screening |
| 44 | 777 | 1 | 1 | Screening |
| 45 | 775 | 1 | 1 | Screening |
| 46 | 774 | 1 | 1 | Other |
| 47 | 764 | 1 | 1 | Screening |
| 48 | 761 | 1 | 1 | Screening |
| 49 | 760 | 1 | 1 | Screening |
| 50 | 759 | 1 | 1 | Screening |
| 51 | 758 | 1 | 1 | Screening |
| 52 | 757 | 1 | 1 | Screening |
| 53 | 750 | 1 | 1 | Screening |
| 54 | 748 | 1 | 1 | Other |
| 55 | 746 | 1 | 1 | Screening |
| 56 | 740 | 1 | 1 | Screening |
| 57 | 739 | 1 | 1 | Screening |
| 58 | 738 | 1 | 1 | Screening |
| 59 | 736 | 1 | 1 | Screening |
| 60 | 731 | 1 | 1 | Screening |
| 61 | 729 | 1 | 1 | Screening |
| 62 | 727 | 1 | 1 | Screening |
| 63 | 720 | 1 | 1 | Screening |
| 64 | 719 | 1 | 1 | Screening |
| 65 | 717 | 1 | 1 | Screening |
| 66 | 710 | 1 | 1 | Screening |
| 67 | 709 | 1 | 1 | Screening |
| 68 | 708 | 1 | 1 | Screening |
| 69 | 707 | 1 | 1 | Screening |
| 70 | 704 | 1 | 1 | Screening |
| 71 | 697 | 1 | 1 | Screening |
| 72 | 696 | 1 | 1 | Screening |
| 73 | 693 | 1 | 1 | Screening |
| 74 | 690 | 1 | 1 | Screening |
| 75 | 689 | 1 | 1 | Confirmatory |
| 76 | 684 | 1 | 1 | Screening |
| 77 | 680 | 1 | 1 | Screening |
| 78 | 662 | 1 | 1 | Confirmatory |

| | | | | |
|---|---|---|---|---|
| 79 | 648 | 1 | 1 | Screening |
| 80 | 645 | 1 | 1 | Screening |
| 81 | 641 | 1 | 1 | Screening |
| 82 | 640 | 1 | 1 | Screening |
| 83 | 639 | 1 | 1 | Screening |
| 84 | 633 | 1 | 1 | Screening |
| 85 | 631 | 1 | 1 | Screening |
| 86 | 630 | 1 | 1 | Screening |
| 87 | 629 | 1 | 1 | Screening |
| 88 | 628 | 1 | 1 | Screening |
| 89 | 626 | 1 | 1 | Screening |
| 90 | 620 | 1 | 1 | Other |
| 91 | 619 | 1 | 1 | Screening |
| 92 | 618 | 1 | 1 | Screening |
| 93 | 612 | 1 | 1 | Screening |
| 94 | 606 | 1 | 1 | Confirmatory |
| 95 | 605 | 1 | 1 | Confirmatory |
| 96 | 604 | 1 | 1 | Screening |
| 97 | 603 | 1 | 1 | Confirmatory |
| 98 | 602 | 1 | 1 | Screening |
| 99 | 598 | 1 | 1 | Screening |
| 100 | 597 | 1 | 1 | Confirmatory |
| 101 | 596 | 1 | 1 | Confirmatory |
| 102 | 595 | 1 | 1 | Confirmatory |
| 103 | 585 | 1 | 1 | Confirmatory |
| 104 | 584 | 1 | 1 | Confirmatory |
| 105 | 583 | 1 | 1 | Other |
| 106 | 581 | 1 | 1 | Screening |
| 107 | 577 | 1 | 1 | Screening |
| 108 | 574 | 1 | 1 | Screening |
| 109 | 573 | 1 | 1 | Screening |
| 110 | 571 | 1 | 1 | Screening |
| 111 | 568 | 1 | 1 | Confirmatory |
| 112 | 567 | 1 | 1 | Screening |
| 113 | 565 | 1 | 1 | Screening |
| 114 | 561 | 1 | 1 | Screening |
| 115 | 560 | 1 | 1 | Screening |
| 116 | 559 | 1 | 1 | Screening |
| 117 | 556 | 1 | 1 | Screening |
| 118 | 555 | 1 | 1 | Screening |
| 119 | 539 | 1 | 1 | Screening |
| 120 | 538 | 1 | 1 | Screening |
| 121 | 526 | 1 | 1 | Confirmatory |

| | | | | |
|---|---|---|---|---|
| 122 | 525 | 1 | 1 | Screening |
| 123 | 524 | 1 | 1 | Screening |
| 124 | 522 | 1 | 1 | Screening |
| 125 | 521 | 1 | 1 | Other |
| 126 | 518 | 1 | 1 | Screening |
| 127 | 501 | 1 | 1 | Screening |
| 128 | 488 | 1 | 1 | Screening |
| 129 | 485 | 1 | 1 | Screening |
| 130 | 465 | 1 | 1 | Screening |
| 131 | 463 | 1 | 1 | Screening |
| 132 | 460 | 1 | 1 | Screening |
| 133 | 453 | 1 | 1 | Screening |
| 134 | 449 | 1 | 1 | Screening |
| 135 | 447 | | 1 | Confirmatory |
| 136 | 445 | 1 | 1 | Confirmatory |
| 137 | 436 | 1 | 1 | Other |
| 138 | 432 | 1 | 1 | Confirmatory |
| 139 | 431 | 1 | 1 | Screening |
| 140 | 430 | 1 | 1 | Screening |
| 141 | 429 | 1 | 1 | Screening |
| 142 | 425 | 1 | 1 | Other |
| 143 | 422 | 1 | 1 | Screening |
| 144 | 374 | 1 | 1 | Screening |
| 145 | 373 | 1 | 1 | Screening |
| 146 | 372 | 1 | 1 | Screening |
| 147 | 368 | 1 | 1 | Screening |

# Appendix C.
# PubChem *In silico* Profiling Results for Compound Q.

This appendix shows the results of a PubChem query executed on 19DEC2007. As can be seen below, in the 136 assays queried, it was found to be active only in AID 570 & 735.

| AID | Active | Inactive | Discrepant | Tested | Outcome Method | Name | |
|---|---|---|---|---|---|---|---|
| 1 | | 735 | 1 | | | 1 | Confirmatory |
| 2 | | 570 | 1 | | | 1 | Screening |
| 3 | | 902 | | 1 | | 1 | Confirmatory |
| 4 | | 901 | | 1 | | 1 | Confirmatory |
| 5 | | 900 | | 1 | | 1 | Confirmatory |
| 6 | | 898 | | 1 | | 1 | Screening |
| 7 | | 893 | | 1 | | 1 | Confirmatory |
| 8 | | 892 | | 1 | | 1 | Confirmatory |
| 9 | | 889 | | 1 | | 1 | Confirmatory |
| 10 | | 887 | | 1 | | 1 | Confirmatory |
| 11 | | 886 | | 1 | | 1 | Confirmatory |
| 12 | | 881 | | 1 | | 1 | Confirmatory |
| 13 | | 878 | | 1 | | 1 | Screening |
| 14 | | 875 | | 1 | | 1 | Confirmatory |
| 15 | | 871 | | 1 | | 1 | Screening |
| 16 | | 868 | | 1 | | 1 | Screening |
| 17 | | 862 | | 1 | | 1 | Screening |
| 18 | | 861 | | 1 | | 1 | Screening |
| 19 | | 847 | | 1 | | 1 | Screening |
| 20 | | 841 | | 1 | | 1 | Screening |
| 21 | | 834 | | 1 | | 1 | Screening |
| 22 | | 828 | | 1 | | 1 | Screening |
| 23 | | 827 | | 1 | | 1 | Screening |
| 24 | | 818 | | 1 | | 1 | Screening |
| 25 | | 817 | | 1 | | 1 | Other |
| 26 | | 813 | | 1 | | 1 | Screening |
| 27 | | 804 | | 1 | | 1 | Screening |
| 28 | | 803 | | 1 | | 1 | Screening |
| 29 | | 800 | | 1 | | 1 | Screening |
| 30 | | 799 | | 1 | | 1 | Screening |
| 31 | | 798 | | 1 | | 1 | Screening |
| 32 | | 797 | | 1 | | 1 | Screening |
| 33 | | 793 | | 1 | | 1 | Screening |
| 34 | | 782 | | 1 | | 1 | Screening |
| 35 | | 781 | | 1 | | 1 | Screening |
| 36 | | 778 | | 1 | | 1 | Screening |

| | | | | |
|---|---|---|---|---|
| 37 | 777 | 1 | 1 | Screening |
| 38 | 775 | 1 | 1 | Screening |
| 39 | 774 | 1 | 1 | Other |
| 40 | 764 | 1 | 1 | Screening |
| 41 | 761 | 1 | 1 | Screening |
| 42 | 760 | 1 | 1 | Screening |
| 43 | 759 | 1 | 1 | Screening |
| 44 | 758 | 1 | 1 | Screening |
| 45 | 757 | 1 | 1 | Screening |
| 46 | 750 | 1 | 1 | Screening |
| 47 | 748 | 1 | 1 | Other |
| 48 | 746 | 1 | 1 | Screening |
| 49 | 739 | 1 | 1 | Screening |
| 50 | 738 | 1 | 1 | Screening |
| 51 | 719 | 1 | 1 | Screening |
| 52 | 710 | 1 | 1 | Screening |
| 53 | 709 | 1 | 1 | Screening |
| 54 | 708 | 1 | 1 | Screening |
| 55 | 696 | 1 | 1 | Screening |
| 56 | 690 | 1 | 1 | Screening |
| 57 | 684 | 1 | 1 | Screening |
| 58 | 680 | 1 | 1 | Screening |
| 59 | 662 | 1 | 1 | Confirmatory |
| 60 | 648 | 1 | 1 | Screening |
| 61 | 645 | 1 | 1 | Screening |
| 62 | 641 | 1 | 1 | Screening |
| 63 | 639 | 1 | 1 | Screening |
| 64 | 633 | 1 | 1 | Screening |
| 65 | 630 | 1 | 1 | Screening |
| 66 | 629 | 1 | 1 | Screening |
| 67 | 628 | 1 | 1 | Screening |
| 68 | 626 | 1 | 1 | Screening |
| 69 | 620 | 1 | 1 | Other |
| 70 | 618 | 1 | 1 | Screening |
| 71 | 612 | 1 | 1 | Screening |
| 72 | 606 | 1 | 1 | Confirmatory |
| 73 | 605 | 1 | 1 | Confirmatory |
| 74 | 604 | 1 | 1 | Screening |
| 75 | 603 | 1 | 1 | Confirmatory |
| 76 | 602 | 1 | 1 | Screening |
| 77 | 598 | 1 | 1 | Screening |
| 78 | 597 | 1 | 1 | Confirmatory |
| 79 | 596 | 1 | 1 | Confirmatory |

| | | | | |
|---|---|---|---|---|
| 80 | 595 | 1 | 1 | Confirmatory |
| 81 | 594 | 1 | 1 | Confirmatory |
| 82 | 593 | 1 | 1 | Other |
| 83 | 592 | 1 | 1 | Other |
| 84 | 591 | 1 | 1 | Other |
| 85 | 590 | 1 | 1 | Other |
| 86 | 589 | 1 | 1 | Other |
| 87 | 588 | 1 | 1 | Other |
| 88 | 587 | 1 | 1 | Other |
| 89 | 585 | 1 | 1 | Confirmatory |
| 90 | 584 | 1 | 1 | Confirmatory |
| 91 | 583 | 1 | 1 | Other |
| 92 | 581 | 1 | 1 | Screening |
| 93 | 577 | 1 | 1 | Screening |
| 94 | 574 | 1 | 1 | Screening |
| 95 | 573 | 1 | 1 | Screening |
| 96 | 571 | 1 | 1 | Screening |
| 97 | 568 | 1 | 1 | Confirmatory |
| 98 | 567 | 1 | 1 | Screening |
| 99 | 565 | 1 | 1 | Screening |
| 100 | 561 | 1 | 1 | Screening |
| 101 | 560 | 1 | 1 | Screening |
| 102 | 559 | 1 | 1 | Screening |
| 103 | 556 | 1 | 1 | Screening |
| 104 | 555 | 1 | 1 | Screening |
| 105 | 552 | 1 | 1 | Screening |
| 106 | 539 | 1 | 1 | Screening |
| 107 | 538 | 1 | 1 | Screening |
| 108 | 526 | 1 | 1 | Confirmatory |
| 109 | 525 | 1 | 1 | Screening |
| 110 | 524 | 1 | 1 | Screening |
| 111 | 522 | 1 | 1 | Screening |
| 112 | 521 | 1 | 1 | Other |
| 113 | 518 | 1 | 1 | Screening |
| 114 | 501 | 1 | 1 | Screening |
| 115 | 488 | 1 | 1 | Screening |
| 116 | 485 | 1 | 1 | Screening |
| 117 | 465 | 1 | 1 | Screening |
| 118 | 463 | 1 | 1 | Screening |
| 119 | 460 | 1 | 1 | Screening |
| 120 | 453 | 1 | 1 | Screening |
| 121 | 448 | 1 | 1 | Confirmatory |
| 122 | 447 | | 1 | Confirmatory |

| | | | | |
|---|---|---|---|---|
| 123 | 445 | 1 | 1 | Confirmatory |
| 124 | 436 | 1 | 1 | Other |
| 125 | 432 | 1 | 1 | Confirmatory |
| 126 | 431 | 1 | 1 | Screening |
| 127 | 430 | 1 | 1 | Screening |
| 128 | 429 | 1 | 1 | Screening |
| 129 | 425 | 1 | 1 | Other |
| 130 | 422 | 1 | 1 | Screening |
| 131 | 411 | 1 | 1 | Confirmatory |
| 132 | 374 | 1 | 1 | Screening |
| 133 | 373 | 1 | 1 | Screening |
| 134 | 368 | 1 | 1 | Screening |
| 135 | 361 | 1 | 1 | Confirmatory |
| 136 | 360 | 1 | 1 | Confirmatory |