TSRI MLSCN Probe Report for Inhibitors of MMP-8:

- **2-[(4-phenylphenyl)sulfonylamino]pentanedioic acid ("compound C")**

Additional data not published in PubChem but presented in this probe report 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-8
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-8, matrix metalloproteinase-8
Probe PubChem Compound Identifier (SID/CID):
Compound C (4) = 842343/ 644601

1. Assay Provider Information

Specific Aim: To identify selective chemical inhibitors of MMP-8

Significance: Although essential during embryonic development [3], increased production and activity of matrix metalloproteinases (MMPs) can be pathogenic. Initial clinical trials with MMP inhibitors targeting active sites were disappointing, due to a lack of selectivity [4]. The key role of MMP-8 (also known as neutrophil collagenase or collagenase-2) in diseases such as cancers [5, 6], osteoarthritis [7], inflammation [8], and heart disease [9], combined with the challenge of identifying truly selective MMP inhibitors, makes the identification of selective MMP-8 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 [10]. 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 [11, 12]. 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-8 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 [11, 13, 14]. The use of these FRET THP (fTHP) technologies in HTS will improve the identification of selective MMP-8 inhibitors.

2. Screening Center Information

2A. Assay Implementation and Screening

PubChem bioassay names and identifiers: Probe development initiated with AID 570 (inhibitors of MMP-13). Out of this initial screen, several compounds were assayed for selectivity against other MMPs and related proteases (references 1 and 2).

List of Relevant AIDs that may be used as counterscreen information: The primary HTS campaign used a substrate labeled with the fluorophore 7-methoxycoumarin. Published AIDs such as 734, 735, and 769 that use this detection reagent may be helpful in identifying fluorescent artifacts, monitoring assay performance, and identifying nonselective MMP-8 inhibitors.

***Primary Screen Summary (AID 570*)**: The MMP-8 probe was originally identified in this primary screen for MMP-13 inhibitors. Specific methods and details can be found in PubChem at http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=570. The results of this 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 0.071% hit rate.

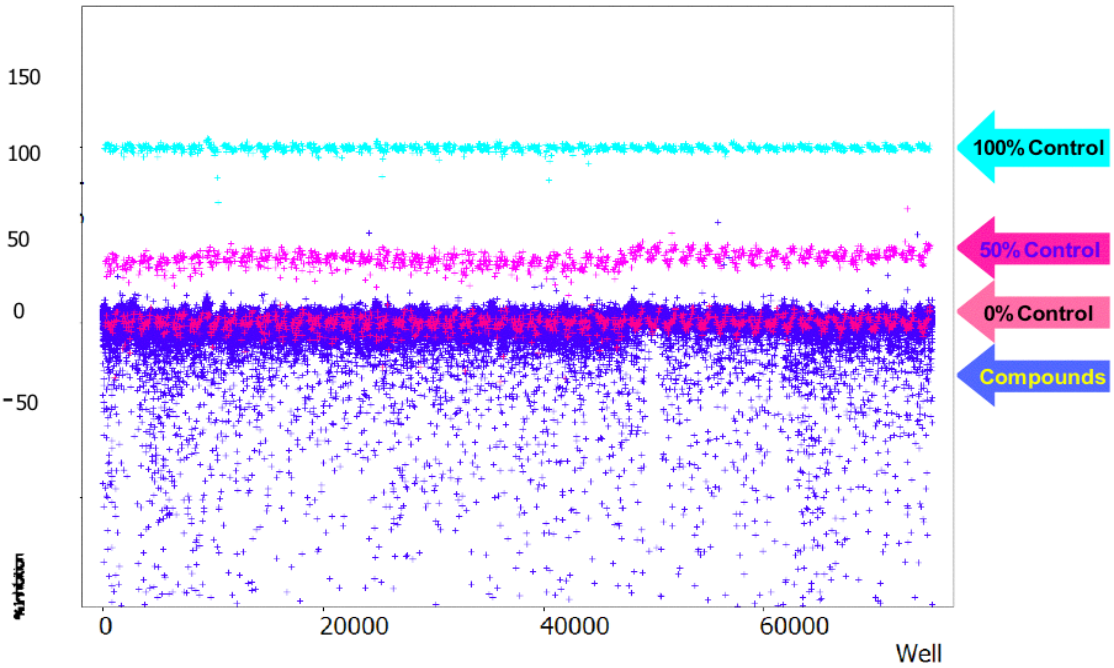


Figure 1. Scattergram of MMP-13 primary screen results. Percent MMP-13 inhibition values are plotted against well number. Negative inhibition is attributed to overlap of the UV absorbance of a test compound with the substrate fluorophore (7-methoxycoumarin) during excitation. "Compounds" indicates wells 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). Refer to AID 570 for details.

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. Thirty-four compounds produced pharmacological dose-response curves, and 15 were reported as active in PubChem. **Table 1** shows a subset of IC_{50} results from this screen (cf. column 5) and vendor information (cf. column 3) for compounds Q, W, V, and C.

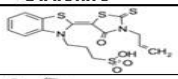
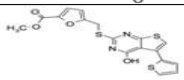
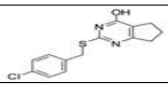
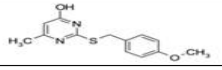
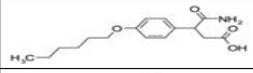
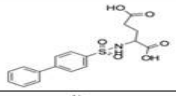
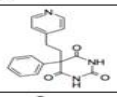
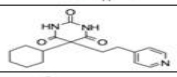
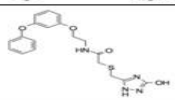
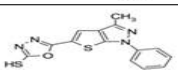
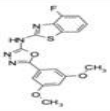
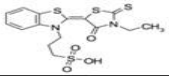
Table 1. Dose response data of inhibition of MMP-8, -9, and -13 by compounds as measured by substrate or ABPP assays. *Indicates average (95% CI) of three independent experiments. Refer to [1] for details.

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)*		
Q (1)	MLS000062185	ChemBridge	6512965	3.4	3.8 (2.8-5.2)	0.92 (0.72-1.2)	>100	>100
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)
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)

Secondary RP-HPLC screen to confirm compound activity and remove assay artifacts: 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 Substrate & 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 **(Table 2)** [2]. Several compounds (V, C, R, E, M, T, A') inhibited MMP-8 in this assay (**Table 2, column 6)**. To further define their selectivity for MMP-8, these compounds were screened in titration assays against a panel of metalloproteases using ABPP and substrate assays [1] **(Table 1).** The IC_{50} data against MMPs -8, -9, and -13 (**Table 2)** and representative gel images for all 27 metalloprotease substrates **(Figure 2)** are shown. Of the four, compound C (listed as "compound 1" in Nakai et al, reference 1) was the most potent inhibitor of probe labeling of MMP-8 with IC_{50} value of 0.024 μM**.** Together these data support compound C (4) as a selective inhibitor of MMP-8 [1].

Table 2. Results of RP-HPLC Selectivity Profiling Assays. Reported is the % MMP activity in the presence of 100 μM 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

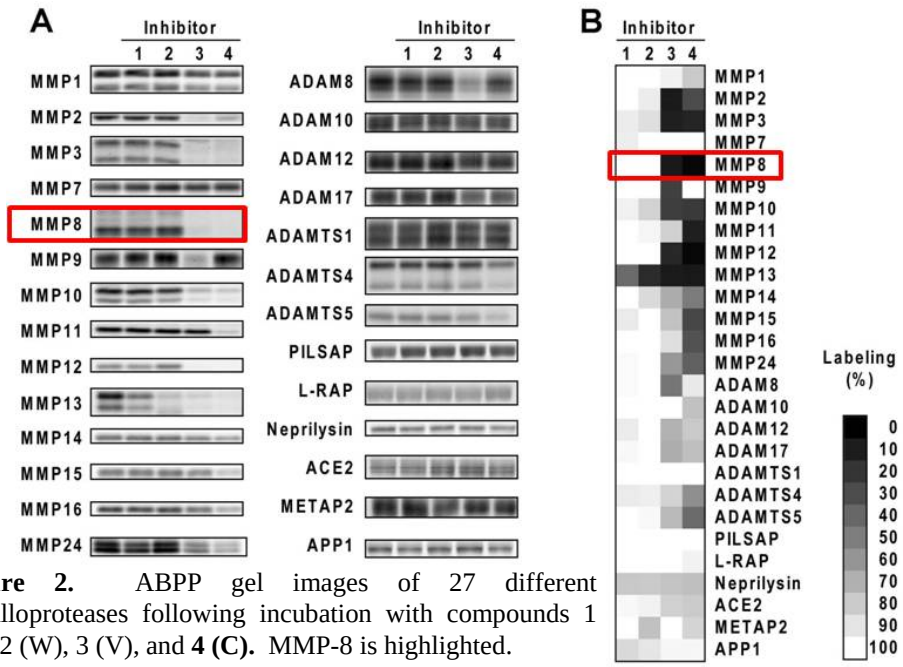


Figure 2. ABPP gel images of 27 different metalloproteases following incubation with compounds 1 (Q), 2 (W), 3 (V), and **4 (C).** MMP-8 is highlighted.

Comparative data on probe, similar compound structures and information on existing probes available to the public: A PubChem query lists 14 links for "similar compounds" and 26 links for "similar substances" for compound C. Compound C is active in only 2 PubChem AIDs (AIDs 570 & 735, MMP-13 inhibitor screens, see appendix for a list of results from all assays queried). It was not reported active in cytotoxicity screens against normal and tumor cells (AIDs 598, 818, 847, 620, 648, 719, 430, 804). This compound was not active in screens for modulators of various 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), suggesting that compound C works by an as yet defined mechanism to inhibit MMP-8. **Figure 3** shows the 2-member cluster of compounds assayed in AID 570 to which compound C belongs. Although they share structural similarity, no data are available for the effect of compound SID 4265914 on other MMPs.

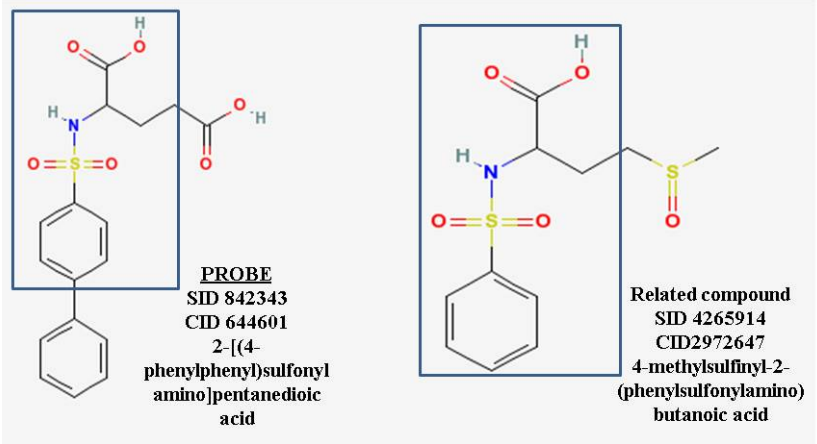
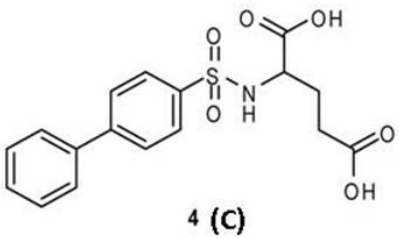


Figure 3. Two-member cluster of compounds containing the MMP-8 probe compound C (left) and a related compound (right) from AID 570. The box outlines the core structural similarity. The IUPAC name is listed. SID 4265914 was inactive in AID 570.

Center summary of probe properties (solubility, absorbance/fluorescence, reactivity, toxicity, etc.): Various properties of compound C are listed below. There is no toxicity data available in PubMed, ChemIDplus, and ToxNet, however the cytotoxicity data from assays in PubChem (see above) suggest the compound is not cytotoxic. However, the vendor Asinex provides the following data for this compound on their website http://chemnet.asinex.ru/results.aspx:
The Predicted Logarithm of the Aqueous Solubility (mol/l): -3.3070
Solubility in Water (mg/l): 179.2134
The Predicted Apparent Caco-2 Cell Permeability in nm/sec: 5.25



4 (C)

MLS000075919
14 similar compounds (CID 644601)
26 similar substances (SID 842343)
2-[(4-phenylphenyl)sulfonylamino]pentanedioic acid

Compound C (4) Probe Properties
CID 644601 / SID 842343 / MLS000075919
SR-01000365325
Vendor = Asinex (catalog # BAS 07869980)
Molecular Weight: 363.38498 g/mol
Molecular Formula: $C_{17}H_{17}NO_6S$ **XLogP:** 0
Hydrogen Bond Donor Count: 3
Hydrogen Bond Acceptor Count: 7
Rotatable Bond Count: 8 **Exact Mass:** 363.077658
MonoIsotopic Mass: 363.077658
Topological Polar Surface Area: 121
Heavy Atom Count: 25 **Charge:** 0 **Complexity:** 556
Isotope Atom Count: 0
Defined Atom StereoCenter Count: 0
Undefined Atom StereoCenter Count: 1
Defined Bond StereoCenter Count: 0
Undefined Bond StereoCenter Count: 0
Covalently-Bonded Unit Count: 1

Recommendations for the scientific use of probe as research tool

The compound described herein is a selective, potent inhibitor of MMP-8. It can be used as a research tool for MMP-8.

Bibliography

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Appendix
PubChem *In silico* Profiling Results for Compound C (CID 644601/SID 842343/ MLS 000075919).

This appendix shows the results of a PubChem query executed on 19DEC2007. As can be seen below, in the 153 assays queried, this compound was found to be active only in AID 570 and 735.

#	AID	Active	Inactive	Discrepant	Tested	Outcome Method
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	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	740		1		1	Screening
56	739		1		1	Screening
57	738		1		1	Screening
58	736		1		1	Screening
59	731		1		1	Screening
60	729		1		1	Screening
61	727		1		1	Screening
62	720		1		1	Screening
63	719		1		1	Screening
64	717		1		1	Screening
65	710		1		1	Screening
66	709		1		1	Screening
67	708		1		1	Screening
68	707		1		1	Screening
69	704		1		1	Screening
70	701		1		1	Screening

71	697		1		1	Screening
72	696		1		1	Screening
73	693		1		1	Screening
74	690		1		1	Screening
75	687		1		1	Screening
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	603		1		1	Confirmatory
97	602		1		1	Screening
98	598		1		1	Screening
99	597		1		1	Confirmatory
100	596		1		1	Confirmatory
101	595		1		1	Confirmatory
102	594		1		1	Confirmatory
103	593		1		1	Other
104	592		1		1	Other
105	591		1		1	Other
106	590		1		1	Other
107	589		1		1	Other
108	588		1		1	Other
109	587		1		1	Other

110	585		1		1	Confirmatory
111	584		1		1	Confirmatory
112	583		1		1	Other
113	581		1		1	Screening
114	577		1		1	Screening
115	574		1		1	Screening
116	573		1		1	Screening
117	571		1		1	Screening
118	568		1		1	Confirmatory
119	567		1		1	Screening
120	565		1		1	Screening
121	561		1		1	Screening
122	560		1		1	Screening
123	559		1		1	Screening
124	556		1		1	Screening
125	555		1		1	Screening
126	539		1		1	Screening
127	538		1		1	Screening
128	526		1		1	Confirmatory
129	525		1		1	Screening
130	524		1		1	Screening
131	522		1		1	Screening
132	521		1		1	Other
133	518		1		1	Screening
134	501		1		1	Screening
135	488		1		1	Screening
136	465		1		1	Screening
137	460		1		1	Screening
138	453		1		1	Screening
139	448		1		1	Confirmatory
140	447				1	Confirmatory
141	445		1		1	Confirmatory
142	436		1		1	Other
143	432		1		1	Confirmatory
144	431		1		1	Screening
145	430		1		1	Screening
146	429		1		1	Screening
147	425		1		1	Other
148	422		1		1	Screening

149	411		1		1	Confirmatory
150	374		1		1	Screening
151	368		1		1	Screening
152	361		1		1	Confirmatory
153	360		1		1	Confirmatory