TSRI MLSCN Probe Report for Inhibitors of ADAMTS-4: Piceatannol

Project Title: High Throughput Screening of Selective Inhibitors of ADAMTS-4
Grant Number: MH078948-01
Screening Center Name: The Scripps Research Institute Molecular Screening Center
Principal Investigator of Screening Center: Hugh Rosen, PhD
Assay Provider & Institution: Gregg B. Fields, Florida Atlantic University
Assay or Pathway Target: ADAMTS-4 (aggrecanase 1)
Probe PubChem Identifier (CID/SID): CID: 667639/ SID: 24278620.

The following screen has been published in: Lauer-Fields JL, Spicer TP, Chase PS, Cudic M, Burstein GD, Nagase H, Hodder P, Fields GB. Screening of potential a disintegrin and metalloproteinase with thrombospondin motifs-4 inhibitors using a collagen model fluorescence resonance energy transfer substrate. Anal Biochem. 2007 Sep 15; PMID: 17949675.

Assay Provider Information
Specific Aim: To identify selective exosite/active site inhibitors of A Disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS-4)

Significance:
Aggrecan breakdown leads to an increase in proteolytic susceptibility of articular collagen. Due to their role in aggrecan degradation and differing substrate specificity profiles, the pursuit of inhibitors for both aggrecanase 1 (a disintegrin and metalloproteinase with thrombospondin motifs-4 [ADAMTS-4]) and aggrecanase 2 (ADAMTS-5) is desirable. However, few inhibitors have been described to date for the aggrecanase members of the ADAMTS family [2-4].

Rationale: Selective inhibitors for ADAMTS-4 would allow a more definitive evaluation of this protease in osteoarthritis and also represent a potential next generation in metalloproteinase therapeutics. Previously described assays for aggrecanases are not particularly convenient for HTS because all require antibodies and most are discontinuous [5-8]. To develop an improved HTS assay for aggrecanases, we examined fluorescence resonance energy transfer (FRET) collagen model substrates recently described by the laboratory of the assay provider [1]. Importantly, these substrates can

interact with secondary binding sites (exosites) located outside the enzyme catalytic domain [1]. A potential application of ADAMTS exosite inhibitors is that they could be covalently linked to active site inhibitors to create high affinity and selective lead compounds. In addition, the use of collagen model aggrecanase substrates in HTS represents an efficient means to identify active site- and/or exosite-binding inhibitors. Finally, this system is convenient in that it is continuous and uses an increase in fluorescence to monitor substrate hydrolysis.

Screening Center information

Assay Implementation and Screening

PubChem Bioassay Name: N/A

List of PubChem bioassay identifiers generated for this screening project (AIDS): N/A

List of relevant AIDs that may be used as counterscreen information: PubChem BioAssays that take advantage of blue fluorescence can provide a means to identify fluorescence artifacts in the primary screen. These include β-lactamase (BLA) antagonist assays such as AID 467, and BLA agonist assays such as AID 843. Additionally, fluorescence-based BioAssays to identify MMP-13 inhibitors (AIDs 570, 734, 735, and 769) may be relevant counterscreens due to their fluorescence-based detection format and protease biochemistry. In the general scientific literature, the use of synthetic FRET substrates with Mca fluorophore and Dnp quencher has been established for HTS for MMPs [9-11].

Primary Assay Description as defined in PubChem: N/A.

Center Summary of the Primary and Confirmation Dose Response Screens:

ADAMTS-4 inhibition was assayed against a subset (n=960) of the Library of Pharmacologically Active Compounds^{1280} (LOPAC^{1280}) (catalog no. LO1280, Sigma), using a collagen model FRET substrate (fSSPa). Five compounds that inhibited ADAMTS-4 activity greater than the hit-cuttoff of 59.5% (calculated as the average % inhibition plus 3 times the standard deviation) at a concentration of 1 μM were identified (piceatannol; (R, R)-*cis*-diethyltetrahydro-2, 8-chrysenediol; (S)-(+)-camptothecin; IIK7; and 8-(*p*-sulfophenyl)theophylline. Secondary dose response studies and reversed-phase high-performance liquid chromatography (RP-HPLC) assays were performed to eliminate compounds that inhibit nonspecifically (e.g., interact with the substrate) or interfere with substrate fluorescence. Only piceatannol (CID: 667639/ SID: 24278620), a red wine polyphenolic compound and nonreceptor tyrosine kinase inhibitor, was confirmed as a novel inhibitor of ADAMTS-4, with an IC_{50} value of 1 μM. Because collagen model FRET substrates such as fSSPa have distinct conformational features that may interact with exosites, nonactive site-binding inhibitors can be identified via this approach.

Primary Assay Description

A collagen model aggrecanase FRET substrate, fSSPa (C_6-(Gly-Pro-Hyp-Pro-Hyp-Gly)$_2$-Gly-Pro-Hyp-Gly-Thr-Lys(Mca)-Gly-Glu~Leu~Glu~Gly-Arg~Gly-Thr-Lys (Dnp)-Gly-Ile-Ser-(Gly-Pro-Hyp-Pro-Hyp-Gly)$_2$-Gly-Pro-Hyp-NH_2), was used for screening the library for ADAMTS-4 inhibitors. This substrate interacts with exosites, and incorporates the aggrecan 1480-1481 cleavage site with a fluorophore/quencher (Mca/Dnp) pair to monitor ADAMTS-4 activity. The screening campaign utilized a recombinant ADAMTS-4 variant, called ADAMTS-4-2, which is found during C-terminal processing of ADAMTS-4 [12, 13], and has reasonable activity against fSSPa [1]. ADAMTS-4-2 contains a typical reprolysin-type zinc–binding motif, a disintegrin domain, a single thrombospondin domain, and a Cys-rich domain. Inhibitor screening was performed in a 384-well plate format against a LOPAC library subset, which contains small organic compounds of known pharmacological activity, such as interaction with G protein-coupled receptors, and is designed to target various pathways of therapeutic interest, including cell signaling pathways, apoptotic pathways, and several different enzyme classes [14]. First, 20 µL per well of 40 nM ADAMTS-4 was added to test wells using a peristaltic bulk dispenser (Wellmate, Matrix Technologies), followed by the addition of 18 nL of compound or controls to the test wells via a 384-well pintool (Biomek FX, Beckman Coulter). Then 20 µL of 20 µM substrate was added to the appropriate wells. The assay was run in singlicate in a 40 µL total volume, resulting in a final test compound concentration of 1 µM. Plates were spun at 200*g* for 1 min and then read immediately on a SpectraMax M2 (Molecular Devices) at λ_{ex} = 324 nm and λ_{em} = 393 nm, followed by an 8 hour read. Data were analyzed via HTS database software (MDL Assay Explorer, Elsevier). Prior to normalization of screening data, the change in relative fluorescence units (ΔRFU) for each well was determined by the following equation:

$$\Delta RFU = (I_{393nm})_{8h} - (I_{393nm})_{0h},$$

where $(I_{393nm})_{xh}$ represents the measured fluorescence emission intensity at 393nm from the test well at the respective time point. The percentage inhibition for each well in the Primary screen was then calculated as follows:

$$\% \text{ Inhibition} = \left(1 - \frac{\Delta RFU \text{ compound} - \text{median } \Delta RFU \text{ high control}}{\text{median } \Delta RFU \text{ low control} - \text{median } \Delta RFU \text{ high control}}\right) \times 100$$

where the high control is defined as wells containing an IC_{100} of Matrix Metallo-Proteinase (MMP) inhibitor III (750 nM) and the low control is defined as wells containing dimethyl sulfoxide (DMSO) only.

A mathematical algorithm was used to determine nominally inhibitory compounds ("hits") in the primary screen. Two values were calculated: (i) the average percentage inhibition of all compounds tested and (ii) three times their standard deviation. The sum

of these two values was used as a cutoff parameter; that is, any compound that exhibited greater percentage inhibition than the cutoff parameter was declared active [15].

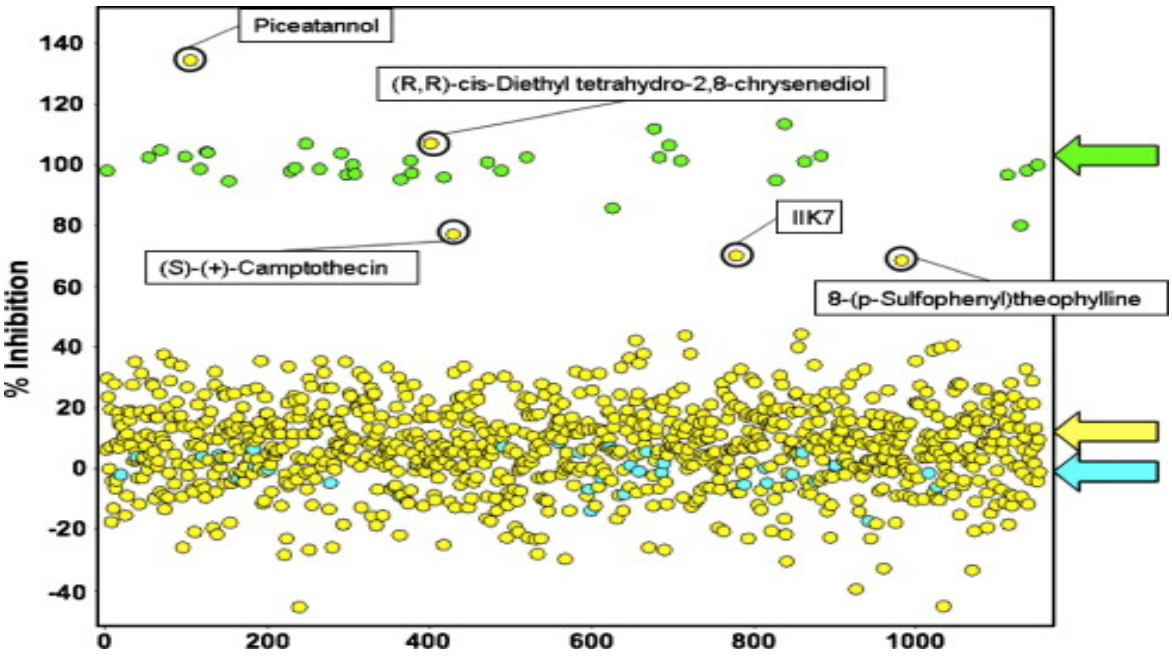


Figure 1. Results of the 384-well screen for ADAMTS-4–2 inhibitors. Displayed are the results of test compounds (n = 960, yellow arrow), positive controls (100% inhibition, green arrow) and negative controls (0% inhibition, blue arrow). Circled are the inhibition results for the five active compounds (hits) identified in the screen.

Primary Assay Results

The results of the Primary screen are shown in **Figure 1**. Primary actives for ADAMTS-4 were selected based on a standard "average plus 3 standard deviations" inhibition cutoff algorithm. This resulted in a cutoff of greater than 59.5% inhibition. The five compounds with circled datapoints in Figure 1 passed these selection criteria: piceatannol (100% inhibition); (*R,R*)-*cis*-diethyl tetrahydro-2,8-chrysenediol (100% inhibition); (*S*)-(+)-camptothecin (77% inhibition); IIK7 (70% inhibition); and (8-(*p*-sulfophenyl) theophylline (69% inhibition). The structures of these compounds are shown in **Figure 2.**

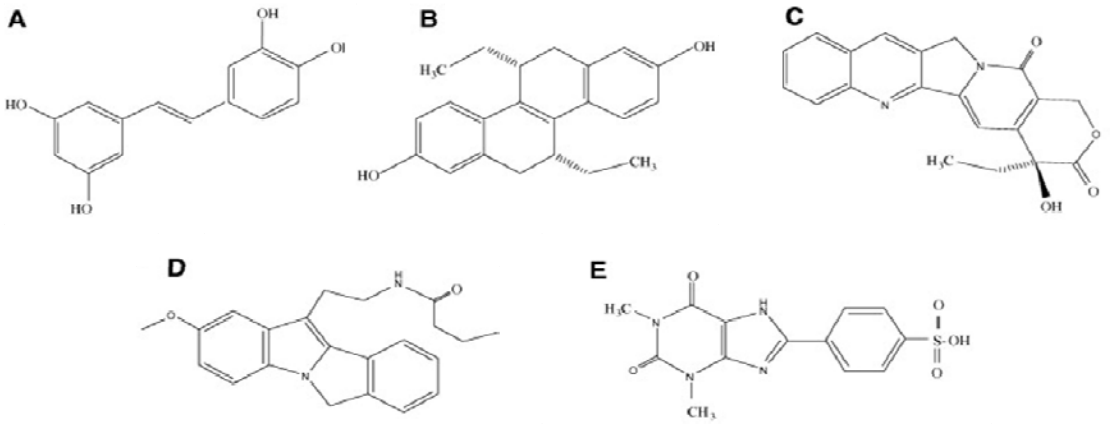


Figure 2. Structures of piceatannol ((E)-4-[2-(3,5-dihydroxyphenyl)ethenyl]1,2-benzenediol; trans-3,3′,4,5′-tetrahydroxystilbene) (A); (R,R)-cis-diethyltetrahydro-2,8-chrysenediol (B); (S)-(+)-camptothecin (4-ethyl-4-hydroxy-1H-pyrano[3′,4′:6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)dione) (C); IIK7 (N-butanoyl-2-(2-methoxy-6H-isoindolo[2,1-a]indole-11-yl)ethanamine) (D); and 8-(p-sulfophenyl)theophylline (E).

Dose Response Assay Description

Following the primary screen, a secondary screen to determine compound potency was initiated. Stock solutions of fSSPa were prepared at 100 μM concentration in Tris salt buffer (TSB: 50 mM Tris HCl[pH 7.5], 100 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij 35, and 0.02% NaN_3). Inhibitors were prepared as 2 mM solutions in DMSO and then further diluted with TSB. ADAMTS-4 assays were conducted by incubating 10 μM substrate with 10 nM enzyme for 24 hours in the presence and absence of inhibitors. Final inhibitor concentrations were 0.1, 1.0, and 10 μM. Fluorescence readings (λ_{ex} = 324 nm and λ_{em} = 393 nm) were obtained at 0, 5, and 24 h. The assay was performed in a 384-well plate format. Dose response curves were prepared using ΔRFU values calculated as follows:

$$\Delta RFU = RFU\ (I_{393nm})_{24h} - (I_{393nm})_{0h},$$

where $(I_{393nm})_{xh}$ represents the measured fluorescence emission intensity at 393nm from the test well at the respective time point.

Dose Response Assay Results

The results of the dose response assay are shown in **Figure 3**. Piceatannol and (*S*)-(+)-camptothecin were found to inhibit ADAMTS-4-2, with IC_{50} values of 1.0 and 4.0 μM, respectively**.** These findings confirmed the behavior of piceatannol and (*S*)-(+)-camptothecin in the primary screen, where the former compound was found to be more active than the latter compound.

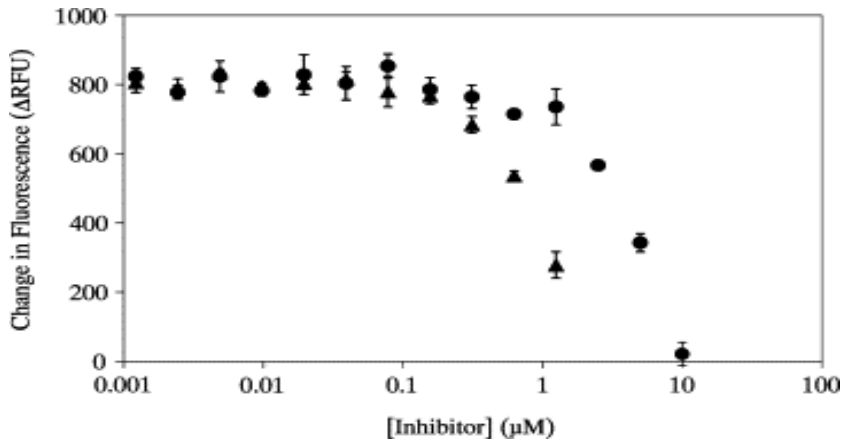


Figure 3. Inhibition of ADAMTS-4-2 by piceatannol (closed triangles) or (S)-(+)-camptothecin (closed circles). The ΔRFU for 10 nM ADAMTS-4-2 hydrolysis of 10 μM fSSPa was monitored over an inhibitor concentration range of 1 nM to 10 μM. IC50 = 1.0 and 4.0 μM for piceatannol and (S)-(+)-camptothecin, respectively. Assays were performed in triplicate. Bars indicate standard deviations.

Secondary RP-HPLC Screen Description

After the final RFU readings at 24 h were collected for the dose response curve, the reaction solution was analyzed by RP–HPLC for the five putative ADAMTS-4 inhibitors. Analytical RP–HPLC was performed on a Hewlett–Packard 1100 Liquid Chromatograph equipped with a Vydac 208TP5415 protein and peptide C_8 column (10–15 μm particle size, 300 Å pore size, 150 × 4.1 mm). Eluants were 0.1% trifluoroacetic acid (TFA) in water (A) and 0.1% TFA in acetonitrile (B). The elution gradient was 0 to 50% B in 20 min with a flow of 1 ml/min. Detection was at $\lambda = 220$, 324, and 363 nm. Reaction yields in the presence of inhibitors were evaluated by the integration of the HPLC peaks formed compared with the enzyme reaction without inhibitor present. Integrations were averaged from two injections. Product identification was achieved by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) on an ABD DE-STR Voyager mass spectrometer using α-cyano-4-hydroxycinnamic acid matrix.

Secondary RP-HPLC Screen Results

The results of the secondary screening obtained by the fluorescence and RP-HPLC methodologies for piceatannol were compared. Piceatannol showed inhibition of ADAMTS-4 by both methods **(Figures 4 and 5)**. However, the other four compounds did not inhibit ADAMTS-4 activity when product formation was analyzed by RP–HPLC **(Figure 6)**, suggesting that they are likely fluorescence artifacts.

Figure 4. RP–HPLC elution profiles of ADAMTS-4-2 hydrolysis of fSSPa in the absence (blue) and presence (magenta) of piceatannol. ADAMTS-4–2 (10 nM) hydrolysis of 10 µM fSSPa was examined 24 h after the addition of 0 or 10 µM piceatannol. The intact substrate eluted at 14.667 min, whereas cleavage products were observed at 12.069, 12.361, and 13.410 min. The identities of the cleavage products were described previously [1].

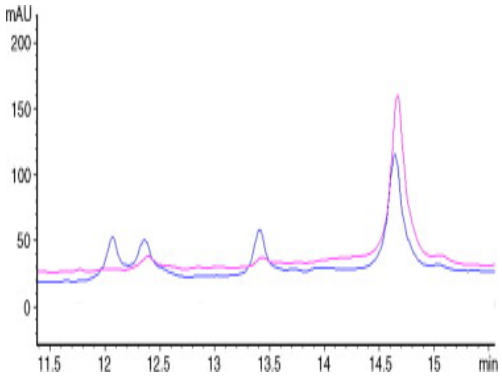


Figure 5. Inhibition of ADAMTS-4–2 by piceatannol, as monitored by RP–HPLC and fluorescence. The change in RP–HPLC peak areas (closed circles) or the ΔRFU (closed triangles) for 10 nM ADAMTS-4–2 hydrolysis of 10 µM fSSPa was monitored over a piceatannol concentration range of 0.1 to 10 µM. Assays were performed in duplicate.

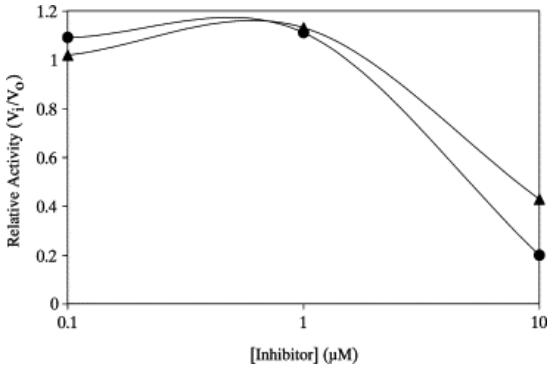
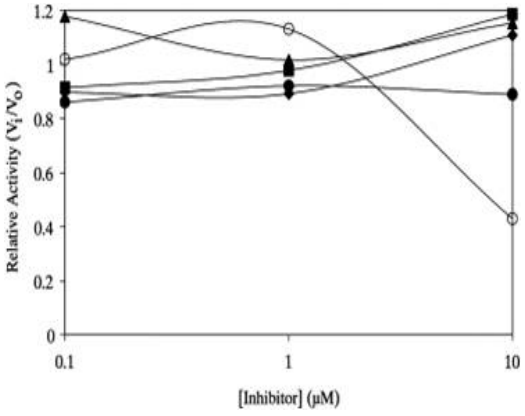


Figure 6. Inhibition of ADAMTS-4–2 by piceatannol (open circles), (*R,R*)-*cis*-diethyltetrahydro-2,8-chrysenediol (closed squares), (*S*)-(+)-camptothecin (closed diamonds), IIK7 (closed circles), and 8-(*p*-sulfophenyl)theophylline (closed triangles), as monitored by RP–HPLC. The change in RP–HPLC peak areas for 10 nM ADAMTS-4-3 hydrolysis of 10 µM fSSPa was monitored over an inhibitor concentration range of 0.1 to 10 µM. Assays were performed in duplicate.



Probe Optimization

Chemical name of probe compound
piceatannol, (E)-4-[2-(3, 5-dihydroxphenyl)ethynyl]1, 2-benzenediol

Draw probe chemical structure and show stereochemistry if known
see Figure 2a.

Comparative data on probe, similar compound structures and information on existing probes available to the public

Piceatannol has structural similarities to the green tea antioxidant (–)-epigallocatechin gallate (EGCG), which inhibits ADAMTS-4 and ADAMTS-5 with IC_{50} values of 100 to 150 nM, and inhibits ADAMTS-1 with an IC_{50} value of 200 to 250 nM [4]. EGCG inhibition of aggrecanases is not due to zinc chelation [4]. A previous report has demonstrated that a related compound, trans-resveratrol, decreased secretion of MMP-2 in cultured human liver myofibroblasts [16], suggesting that red wine and green tea antioxidants with structures similar to piceatannol merit further study.

Mode of action for biological activity of probe

The precise mode of action of piceatannol has not been investigated, although many studies have identified cellular targets. Piceatannol is a red wine polyphenolic, nonreceptor Tyr kinase inhibitor [17]. Unlike most aggrecanase inhibitors [2, 3], piceatannol does not possess a zinc-chelating hydroxamic acid. It is reported that piceatannol can inhibit the lymphoid cell lineage-specific protein tyrosine kinase p56lck [18, 19], which would make piceatannol a promising tool for modulating lymphocyte proliferation and immune function. Piceatannol can reduce lipopolysaccharide (LPS)-induced septic shock, tissue damage, inflammatory cascade, and coagulation pathway in human U373 astrocytoma cells and mice [20]. Future experiments will evaluate the mode of ADAMTS-4 inhibition by piceatannol and determine the key functional groups. It should be noted that piceatannol itself has a wide range of activities, including down-regulation of the transcription factor STAT-3 [21], which has been associated with cytotoxic effects primarily in tumor cells [22] but potentially other cell types as well. Piceatannol also stimulates osteogenesis through effects on bone morphogenetic protein-2 production [23] and up-regulates endothelial heme oxygenase-1 expression [24].

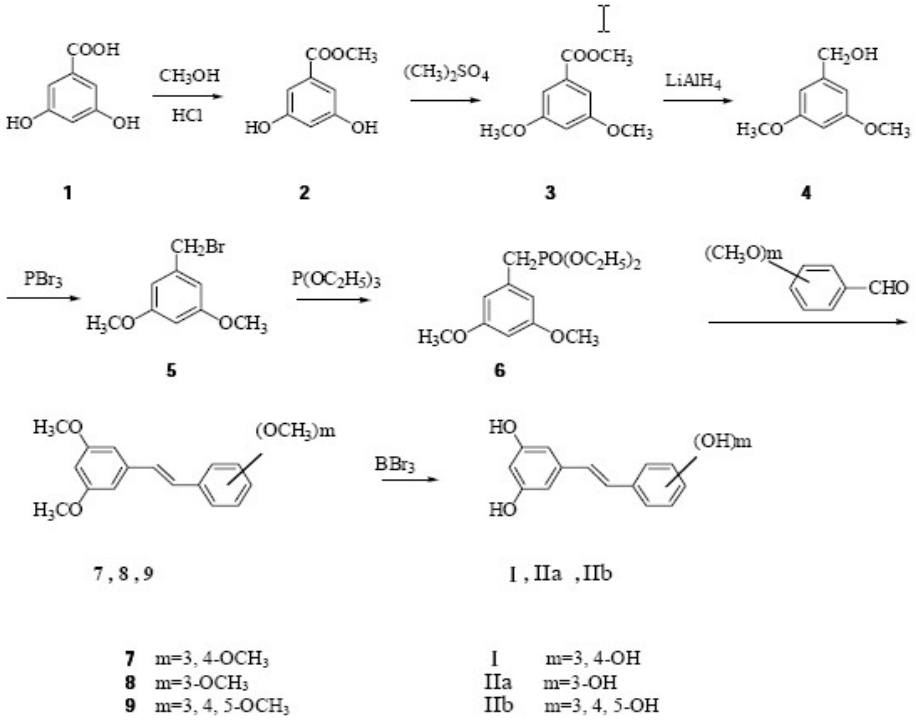
Has this compound been provided to the MLSMR
The compound is available from the MLSMR as CID667639/ SID 24278620.

Commercial vendor information if available for probe or analogs that have been purchased
(E)-4-[2-(3,5-dihydroxyphenyl)ethenyl]1,2-benzenediol (piceatannol): Sigma Chemical (catalog # P0453).

Reduced-to-practice detailed synthetic pathway for making probe

Piceatannol is naturally synthesized in grapes during the ripening process or during fungal infection, via the action of the stilbene synthase gene [25, 26]. It was first isolated in 1984 from the bark of the spruce *Euphorbia lagascae* [26]. A protocol for piceatannol synthesis from 3,5-dihydroxyphenylacetic acid and protocatechualdehyde can be found at http://www.rsc.org/ejarchive/JR/1963/JR9630002875.pdf. Additionally, **Scheme I** below, taken directly from [27], describes piceatannol synthesis:

Scheme 1 Synthetic route of piceatannol and its analogs



7 m=3, 4-OCH_3
8 m=3-OCH_3
9 m=3, 4, 5-OCH_3

I m=3, 4-OH
IIa m=3-OH
IIb m=3, 4, 5-OH

Known Probe Properties as listed in PubChem

Molecular Weight: 244.24268 g/mol
Molecular Formula: C14H12O4
Hydrogen Bond Donor Count: 4
Hydrogen Bond Acceptor Count: 4
Rotatable Bond Count: 2
Tautomer Count: 80
Topological Polar Surface Area: 80.9
XLogP: 1.8
Exact Mass: 244.073559
MonoIsotopic Mass: 244.073559
Heavy Atom Count: 18
Charge: 0
Complexity: 282
Isotope Atom Count: 0
Defined Atom StereoCenter Count: 0
Undefined Atom StereoCenter Count: 0
Defined Bond StereoCenter Count: 1
Undefined Bond StereoCenter Count: 0
Covalently-Bonded Unit Count: 1

Probe Solubility

The reported solubility data on piceatannol powder, provided by Sigma, (catalog # P0453) are: H_2O: 0.5 mg/mL, DMSO: 10 mg/mL, Ethanol: 10 mg/mL

Probe Absorbance/Fluorescence

Four false positives were observed in the screening protocol. One problem with FRET-based assays is that compounds being screened may have absorption maxima that coincide with the emission wavelength of the fluorophore. However, the RP-HPLC study demonstrated that the inhibitory effect observed for piceatannol is real, i.e. not due to an absorbance or fluorescence issue.

Probe Reactivity

No data available

Probe Toxicity

The ChemIDPlus database at the Toxicology Data Network (TOXNET, found at http://toxnet.nlm.nih.gov/cgi-bin/sis/search) lists a normalized intraperitoneal LD_{50} of piceatannol in mice as 217 mg/kg activity [28]. This information is found by following the NLM Toxicology links to ChemIDPlus from the PubChem compound site for piceatannol (CID 667639.)

Previous reports reveal that piceatannol is cytotoxic in acute leukemia and lymphoma cells and that it has antiproliferative activity in colorectal cancer cell lines [29-31]. An additional study showed that piceatannol induces apoptosis in melanoma cells [32]. Additional study is needed to identify the mechanism of action of piceatannol and its cytotoxicity profile in normal (unchallenged) cells.

Recommendations for the scientific use of probe as a research tool

Piceatannol was identified and validated as a potent inhibitor of ADAMTS-4 in the present campaign. Subsequent (unpublished) experiments have examined the individual components of piceatannol, and found that some regions of the molecule participate in competitive inhibition while other regions are non-competitive inhibitors. Available toxicity data suggest that piceatannol can be cytotoxic in cell and animal systems, so the intact compound may not be suitable for human use. Further assays will identify the selectivity of piceatannol components for other ADAMTS family members and evaluate the cytotoxicity of these components.

Appendix: Bibliography

1. Lauer-Fields, J.L., D. Minond, T. Sritharan, M. Kashiwagi, H. Nagase, and G.B. Fields, *Substrate conformation modulates aggrecanase (ADAMTS-4) affinity and sequence specificity. Suggestion of a common topological specificity for functionally diverse proteases.* J Biol Chem, 2007. **282**(1): p. 142-50.
2. Yao, W., Z.R. Wasserman, M. Chao, G. Reddy, E. Shi, R. Liu, M.B. Covington, E.C. Arner, M.A. Pratta, M. Tortorella, R.L. Magolda, R. Newton, M. Qian, M.D. Ribadeneira, D. Christ, R.R. Wexler, and C.P. Decicco, *Design and Synthesis of a Series of (2R)-N4-Hydroxy-2-(3-hydroxybenzyl)-N1- [(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden- 1-yl]butanediamide Derivatives as Potent, Selective, and Orally Bioavailable Aggrecanase Inhibitors.* J. Med. Chem., 2001. **44**(21): p. 3347-3350.
3. Yao, W., M. Chao, Z.R. Wasserman, R.Q. Liu, M.B. Covington, R. Newton, D. Christ, R.R. Wexler, and C.P. Decicco, *Potent P1' biphenylmethyl substituted aggrecanase inhibitors.* Bioorg Med Chem Lett, 2002. **12**(1): p. 101-4.
4. Vankemmelbeke, M.N., G.C. Jones, C. Fowles, M.Z. Ilic, C.J. Handley, A.J. Day, C.G. Knight, J.S. Mort, and D.J. Buttle, *Selective inhibition of ADAMTS-1, -4 and -5 by catechin gallate esters.* Eur J Biochem, 2003. **270**(11): p. 2394-403.
5. Miller, J.A., R.Q. Liu, G.L. Davis, M.A. Pratta, J.M. Trzaskos, and R.A. Copeland, *A microplate assay specific for the enzyme aggrecanase.* Anal Biochem, 2003. **314**(2): p. 260-5.
6. Peppard, J., F. Glickman, Y. He, S.I. Hu, J. Doughty, and R. Goldberg, *Development of a high-throughput screening assay for inhibitors of aggrecan cleavage using luminescent oxygen channeling (AlphaScreen).* J Biomol Screen, 2003. **8**(2): p. 149-56.
7. Will, H., M. Dettloff, P. Bendzko, and P. Sveshnikov, *A quantitative assay for aggrecanase activity.* J Biomol Tech, 2005. **16**(4): p. 459-72.
8. Thomas, M., M. Sabatini, F. Bensaude, B. Mignard, J.C. Ortuno, I. Caron, J.A. Boutin, and G. Ferry, *A microplate assay for the screening of ADAMTS-4 inhibitors.* Matrix Biol, 2006. **25**(4): p. 261-7.
9. Schullek, J.R., J.H. Butler, Z.J. Ni, D. Chen, and Z. Yuan, *A high-density screening format for encoded combinatorial libraries: assay miniaturization and its application to enzymatic reactions.* Anal Biochem, 1997. **246**(1): p. 20-9.
10. Szardenings, A.K., V. Antonenko, D.A. Campbell, N. DeFrancisco, S. Ida, L. Shi, N. Sharkov, D. Tien, Y. Wang, and M. Navre, *Identification of highly selective inhibitors of collagenase-1 from combinatorial libraries of diketopiperazines.* J Med Chem, 1999. **42**(8): p. 1348-57.
11. Vassiliou, S., A. Mucha, P. Cuniasse, D. Georgiadis, K. Lucet-Levannier, F. Beau, R. Kannan, G. Murphy, V. Knauper, M.C. Rio, P. Basset, A. Yiotakis, and V. Dive, *Phosphinic pseudo-tripeptides as potent inhibitors of matrix metalloproteinases: a structure-activity study.* J Med Chem, 1999. **42**(14): p. 2610-20.

12. Kashiwagi, M., J.J. Enghild, C. Gendron, C. Hughes, B. Caterson, Y. Itoh, and H. Nagase, *Altered proteolytic activities of ADAMTS-4 expressed by C-terminal processing.* J Biol Chem, 2004. **279**(11): p. 10109-19.
13. Gao, G., J. Westling, V.P. Thompson, T.D. Howell, P.E. Gottschall, and J.D. Sandy, *Activation of the proteolytic activity of ADAMTS4 (aggrecanase-1) by C-terminal truncation.* J Biol Chem, 2002. **277**(13): p. 11034-41.
14. F. Darvas, G.D., T. Karancsi, T. Nagy and I. Bágyi,, *Estimation of stability and shelf life for compounds, libraries, and repositories in combination with systematic discovery of new rearrangment pathways.*, in *Handbook of Combinatorial Chemistry*, R.H.a.W.H. K.C. Nicolaou, Editor. 2002, Wiley–VCH: Weinheim, Germany. p. 806–828.
15. Hodder, P., J. Cassaday, R. Peltier, K. Berry, J. Inglese, B. Feuston, C. Culberson, L. Bleicher, N.D. Cosford, C. Bayly, C. Suto, M. Varney, and B. Strulovici, *Identification of metabotropic glutamate receptor antagonists using an automated high-throughput screening system.* Anal Biochem, 2003. **313**(2): p. 246-54.
16. Godichaud, S., S. Krisa, B. Couronne, L. Dubuisson, J.M. Merillon, A. Desmouliere, and J. Rosenbaum, *Deactivation of cultured human liver myofibroblasts by trans-resveratrol, a grapevine-derived polyphenol.* Hepatology, 2000. **31**(4): p. 922-31.
17. Ashikawa, K., S. Majumdar, S. Banerjee, A.C. Bharti, S. Shishodia, and B.B. Aggarwal, *Piceatannol inhibits TNF-induced NF-kappaB activation and NF-kappaB-mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation.* J Immunol, 2002. **169**(11): p. 6490-7.
18. Thakkar, K., R.L. Geahlen, and M. Cushman, *Synthesis and protein-tyrosine kinase inhibitory activity of polyhydroxylated stilbene analogues of piceatannol.* J Med Chem, 1993. **36**(20): p. 2950-5.
19. Geahlen, R.L. and J.L. McLaughlin, *Piceatannol (3,4,3',5'-tetrahydroxy-trans-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor.* Biochem Biophys Res Commun, 1989. **165**(1): p. 241-5.
20. Dang, O., L. Navarro, and M. David, *Inhibition of lipopolysaccharide-induced interferon regulatory factor 3 activation and protection from septic shock by hydroxystilbenes.* Shock, 2004. **21**(5): p. 470-5.
21. Kumari, A.L., A.M. Ali, S. Das, B.V. Pardhasaradhi, C. Varalakshmi, and A. Khar, *Role of STAT3 and NFkappaB signaling in the serum factor-induced apoptosis in AK-5 cells.* Biochem Biophys Res Commun, 2005. **336**(3): p. 860-7.
22. Chowdhury, S.A., K. Kishino, R. Satoh, K. Hashimoto, H. Kikuchi, H. Nishikawa, Y. Shirataki, and H. Sakagami, *Tumor-specificity and apoptosis-inducing activity of stilbenes and flavonoids.* Anticancer Res, 2005. **25**(3B): p. 2055-63.
23. Chang, J.K., Y.L. Hsu, I.C. Teng, and P.L. Kuo, *Piceatannol stimulates osteoblast differentiation that may be mediated by increased bone morphogenetic protein-2 production.* Eur J Pharmacol, 2006. **551**(1-3): p. 1-9.
24. Wung, B.S., M.C. Hsu, C.C. Wu, and C.W. Hsieh, *Piceatannol upregulates endothelial heme oxygenase-1 expression via novel protein kinase C and tyrosine kinase pathways.* Pharmacol Res, 2006. **53**(2): p. 113-22.

25. Bavaresco, L., C. Fregoni, E. Cantu, and M. Trevisan, *Stilbene compounds: from the grapevine to wine.* Drugs Exp Clin Res, 1999. **25**(2-3): p. 57-63.
26. Bavaresco, L., S. Vezzulli, P. Battilani, P. Giorni, A. Pietri, and T. Bertuzzi, *Effect of ochratoxin A-producing Aspergilli on stilbenic phytoalexin synthesis in grapes.* J Agric Food Chem, 2003. **51**(21): p. 6151-7.
27. Piao ZS, W.L., Feng YB, Zhao ZZ, *Studies on the Synthesis of a Natural Product-Piceatannol and its Analogs.* Chinese Chemical Letters, 2002. **13**(6): p. 521 – 524.
28. Inamori, Y., Y. Kato, M. Kubo, M. Yasuda, K. Baba, and M. Kozawa, *Physiological activities of 3,3',4,5'-tetrahydroxystilbene isolated from the heartwood of Cassia garrettiana CRAIB.* Chem Pharm Bull (Tokyo), 1984. **32**(1): p. 213-8.
29. Wieder, T., A. Prokop, B. Bagci, F. Essmann, D. Bernicke, K. Schulze-Osthoff, B. Dorken, H.G. Schmalz, P.T. Daniel, and G. Henze, *Piceatannol, a hydroxylated analog of the chemopreventive agent resveratrol, is a potent inducer of apoptosis in the lymphoma cell line BJAB and in primary, leukemic lymphoblasts.* Leukemia, 2001. **15**(11): p. 1735-42.
30. Tolomeo, M., S. Grimaudo, A. Di Cristina, M. Roberti, D. Pizzirani, M. Meli, L. Dusonchet, N. Gebbia, V. Abbadessa, L. Crosta, R. Barucchello, G. Grisolia, F. Invidiata, and D. Simoni, *Pterostilbene and 3'-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells.* Int J Biochem Cell Biol, 2005. **37**(8): p. 1709-26.
31. Wolter, F., A. Clausnitzer, B. Akoglu, and J. Stein, *Piceatannol, a natural analog of resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines.* J Nutr, 2002. **132**(2): p. 298-302.
32. Larrosa, M., F.A. Tomas-Barberan, and J.C. Espin, *The grape and wine polyphenol piceatannol is a potent inducer of apoptosis in human SK-Mel-28 melanoma cells.* Eur J Nutr, 2004. **43**(5): p. 275-84.