Probe Report

Title: Placental Alkaline Phosphatase (PLAP) Luminescent HTS assay
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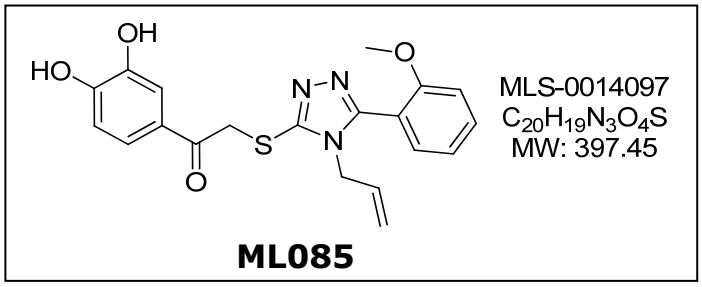
Assigned Assay Grant #: 1 R03 MH077602-01
Screening Center Name & PI: *Conrad Prebys* Center for Chemical Genomics (*formerly Burnham Center for Chemical Genomics*) & Dr. John C. Reed
Chemistry Center Name & PI: *Conrad Prebys* Center for Chemical Genomics (*formerly Burnham Center for Chemical Genomics)* & Dr. John C. Reed
Assay Submitter & Institution: Dr Jose Luis Millán & Sanford-Burnham Medical Research Institute (*formerly Burnham Institute for Medical Research*)

PubChem Summary Bioassay Identifier (AID): 1577

Probe Structure & Characteristics:



ML085

CID/ML	Target Name	IC50/EC50 (nM) [SID, AID]	Anti-target Name(s)	IC50/EC50 (μM) [SID, AID]	Select-ivity	Secondary Assay(s) Name: IC50/EC50 (nM) [SID, AID]
665093 ML085	PLAP	2,600 nM IC_{50} SID-56373725 AID-1512	TNAP	>100 uM IC_{50} SID-56373725 AID-518	> 42	
			IAP	>6.25 uM IC_{50} SID-56373725 AID-1017	>2.4	

Recommendations for the scientific use of this probe:

Placental alkaline phosphatase (PLAP) is highly expressed in primate placental tissue. Its biological function is still unknown (1,2). PLAP-like enzymes could be detected in serum of patients with primary testicular tumors, in particular seminoma (3) and other cancers (4). This PLAP-specific inhibitors with selectivity over tissue non-specific alkaline phosphatase (TNAP) and intestinal alkaline phosphatase (IAP) can be used as a tools to characterize the biological role of PLAP. The small molecule probe CID-665093 will be useful to elucidate the key biological functions and natural substrates of human placental alkaline phosphatase (PLAP)

1. Scientific Rationale for Project

Alkaline phosphatases (EC 3.1.3.1) (APs) catalyze the hydrolysis of phosphomonoesters, releasing phosphate and alcohol. APs are dimeric enzymes found in most organisms. In human, four isozymes of APs have been identified: three isozymes are tissue-specific and the fourth one is tissue-nonspecific. Placental alkaline phosphatase (PLAP) is highly expressed in primate placental tissue. Its biological function is still unknown. However, the identification of PLAP-specific inhibitors with selectivity over tissue non-specific alkaline phosphatase (TNAP) and intestinal alkaline phosphatase (IAP) will provide the necessary tools to characterize its biological role

2. Project Description

a. The original goal for probe characteristics.

This MLSCN carry-forward project was an early Cycle 2 assay proposal and a formal CPDP was not filed. It was derived on work with tissue non-specific alkaline phoshatase inhibitor and later activator work, that suggested that PLAP specific inhibitors would also useful tools for this class of isozyme with yet to be elucidated biological function.

b.

i. PubChem Bioassay Name(s), AID(s), Assay-Type (Primary, DR, Counterscreen, Secondary)

PubChem BioAssay Name	AIDs	Probe Type	Assay Type	Assay Format	Assay Detection & well format
Luminescent assay for HTS discovery of chemical inhibitors of placental alkaline phosphatase	690	Inhibitor	Primary	Biochemical	Luminescence (1536)
Luminescent assay for HTS discovery of chemical inhibitors of placental alkaline phosphatase confirmation	1512	Inhibitor	Confirmatory	Biochemical	Luminescence (384)
TNAP luminescent HTS assay [Confirmatory]	518	Inhibitor	Secondary Assay for specificity	biochemical	Luminescence (384)
Luminescent assay for identification of inhibitors of human intestinal alkaline phosphatase [Confirmatory]	1017	Inhibitor	Counterscreen Assay for specificity	biochemical	Luminescence (1536)
Luminescent assay for identification of inhibitors of bovine intestinal alkaline phosphatase [Primary Screening]	1019	Inhibitor	Counterscreen Assay for specificity	biochemical	Luminescence (1536)

Primary assay details are described below.
PLAP screening was developed and performed at the Burnham Center for Chemical Genomics (BCCG) within the Molecular Library Screening Center Network (MLSCN) as a selectivity screen for tissue nonspecific alkaline phosphatase (TNAP, AID 518). XO1 MH077602-01, Pharmacological inhibitors of tissue-nonspecific alkaline phosphatase (TNAP), Assay Provider Dr. Jose Luis Millán, Burnham Institute for Medical Research, La Jolla, CA.

Protocol:

PLAP assay materials:

1) PLAP protein was provided by Dr. Jose Luis Millán (Burnham Institute for Medical Research, San Diego, CA). The CDP-star was obtained from New England

Biolabs.
2) Assay Buffer: 250 mM DEA, pH 9.8, 2.5 mM $MgCl_2$, and 0.05 mM $ZnCl_2$.
3) PLAP working solution contained a 1/6400 dilution in assay buffer. The solution was prepared fresh prior to use.
4) CDP-star working solution contained 212.5 uM CDP-star in MQ water.
5) TCEP working solution - 5 mM in 10% DMSO.

PLAP HTS protocol:
1) 4 uL of 100 uM compounds in 10% DMSO were dispensed in columns 3-24 of Greiner 384-well white small volume plates (784075).
2) Using a Thermo wellmate dispenser 4 uL of the following solutions were added:
   a. TCEP working solution - column 1 (positive control).
   b. 10% DMSO - column 2 (negative control).
3) 8 uL of PLAP working solution was added to the whole plate using a WellMate bulk dispenser (Matrix).
4) 8 uL of CDP-star working solution was added to the whole plate using WellMate bulk reagent dispenser (Matrix).
5) Final concentrations of the components in the assay were as follows:
   a. 100 mM DEA, pH 9.8, 1.0 mM $MgCl_2$, 0.02 mM $ZnCl_2$ (columns 1-24)
   b. 1/16000 dilution PLAP (columns 1-24)
   c. 85 uM CDP-star (columns 1-24)
   d. 1 mM TCEP (columns 1)
   e. 2 % DMSO (columns 1-24)
   f. 20 uM compounds (columns 3-24)

6) Plates were incubated for 30 minutes at room temperature.
7) Luminescence was measured on an Envision plate reader (Perkin Elmer).
8) Data analysis was performed using CBIS software (ChemInnovations, Inc).

PLAP dose-response confirmation screening protocol:
1) Dose-response curves contained 10 concentrations of compounds obtained using 2-fold serial dilution. Compounds were serially diluted in 100% DMSO, and then diluted with water to 10% final DMSO concentration. 4 uL compounds in 10% DMSO were transferred into columns 3-22 of Greiner 384-well white small-volume plates (784075). Columns 1-2 and 23-24 contained 4 uL of TCEP working solution and 10% DMSO, respectively.
2) 8 uL of PLAP working solution was added to the whole plate using a WellMate bulk reagent dispenser (Matrix).
3) 8 uL of CDP-star working solution was added to the whole plate using a WellMate reagent bulk dispenser (Matrix).
4) Plates were incubated for 30 mins at room temperature.
5) Luminescence was measured on an Envision plate reader (Perkin Elmer).
6) Data analysis was performed using CBIS software (ChemInnovations, Inc) using a sigmoidal dose-response equation through non-linear regression

ii. Assay Rationale & Description.

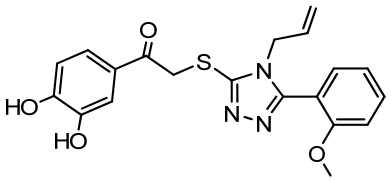
For the assay rationale, description and reagents protocols see section 2a above.

For this screen 95857 compounds were tested. The average Z' for the assay was 0.69, the average signal to background was 38.6, the average signal to noise was 122.7 and the average signal window was 7.8. Initially 192 compounds were identified as primary positives with >= 50% inhibition of activity in the assay. After performing dose-response experiments with liquid DMSO stocks of these compounds, 82 of the hits generated a dose-response curve.

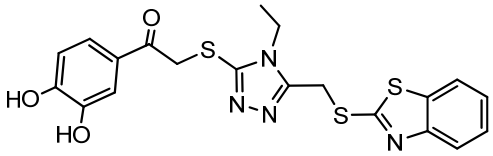
iii. Center Summary of Results

Primary hits from the PLAP HTS assay were confirmed and tested in parallel against TNAP for selectivity. A few selective hits were identified. Those hits were purchased along with 56 analogues (Table 1). There are many commercial analogues available and so Analogue-By-Catalogue (ABC) was performed in an iterative fashion. From the purchased compounds we were able to conclude that the dihydroxyl groups of the catechol are essential for PLAP activity. The SAR was completed and a probe molecule CID-665093 (MLS-0014097) was identified (see Table 1 below).

Medicinal chemistry focused on the two final probe candidates, CID-2102207 (MLS-0107074) and CID-665093 (MLS-0014097) (Figure 1) that were identified as low-micromolar inhibitors of PLAP through primary screening.



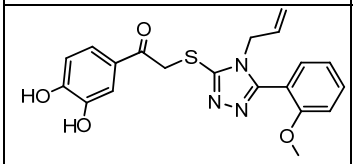
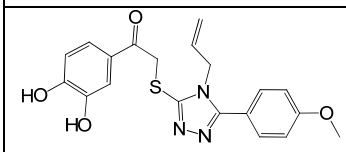
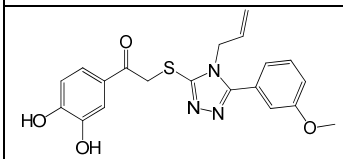
CID-665093 (MLS-0014097)

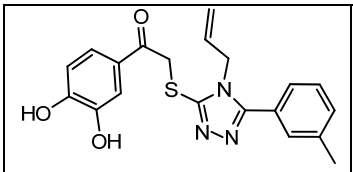
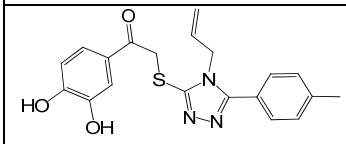
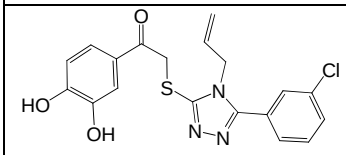
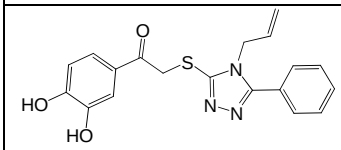
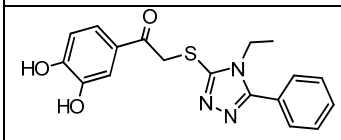
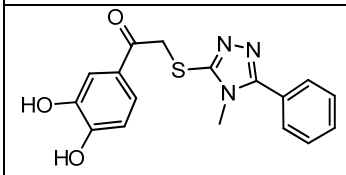
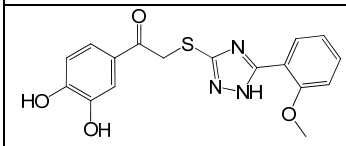
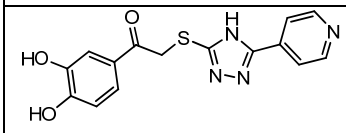
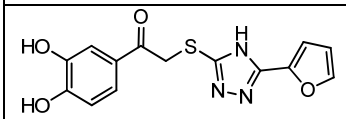
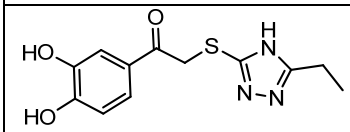
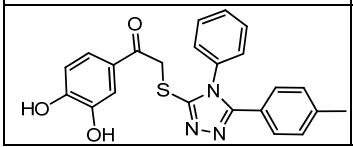


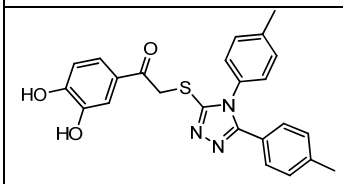
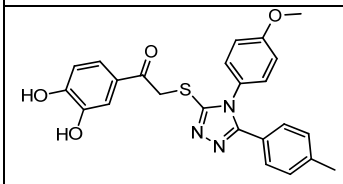
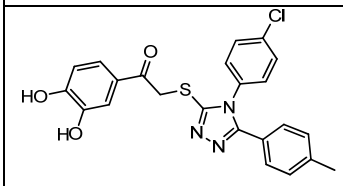
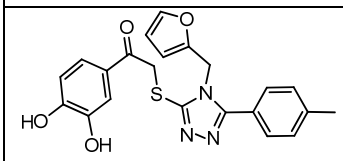
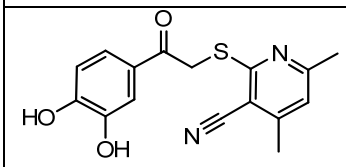
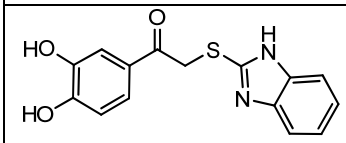
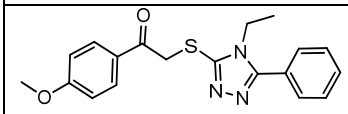
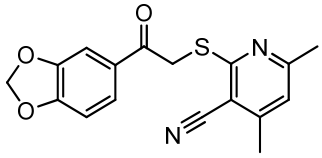
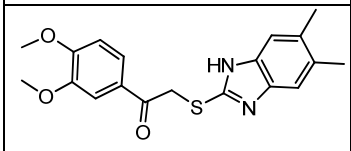
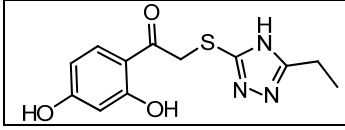
CID-2102207 (MLS-0107074)

Figure 1.

Re-synthesis of these targets was undertaken and these compounds were subjected to the BCCG target independent ADME assay panel (see also Table 1 below)

Structure	Compound _ID	PubChem _SID	PubChem_ CID	IC50(uM)	
				PLAP	TNAP
	MLS-0014097	56373725	665093	2.6	17.8
	MLS-0390850	57287596	1400698	13.6	>100
	MLS-0390843	57287589	1409759	3.97	>100

	MLS-0390851	57287597	1400246	6.02	>100
	MLS-0390858	57287605	1394536	9.4	>100
	MLS-0390857	57287604	1398345	7.45	>100
	MLS-0390841	57287587	1416608	2.7	12.1
	MLS-0108835	56373724	1399636	2.53	40.3
	MLS-0315681	56373578	838842	2.79	93.4
	MLS-0315688	56373639	1314069	7.29	2.78
	MLS-0315684	56373634	715398	6.95	6.53
	MLS-0315686	56373636	715363	12.2	21.7
	MLS-0315685	56373635	715330	1.78	10.9
	MLS-0390847	57287593	1400320	>100	>100

	MLS-0390852	57287598	1399934	8.73	>100
	MLS-0390854	57287600	1401217	5.59	>100
	MLS-0390846	57287592	1383304	13.0	>100
	MLS-0390848	57287594	4283152	4.94	19.2
	MLS-0315680	56373577	720372	1.56	6.71
	MLS-0315887	56373703	4206177	4.79	5.44
	MLS-0315884	56373700	996625	>100	>100
	MLS-0315888	56373704	698547	>100	>100
	MLS-0315883	56373696	1223257	>100	>100
	MLS-0315889	56373705	715329	>100	>100

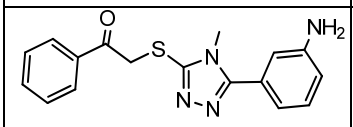
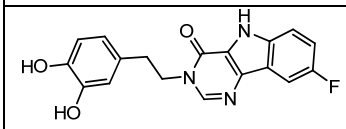
	MLS-0080017	56373623	881297	>100	>50
	MLS-0390860	57287607	932151	1.04	0.25

Table 1 SAR results generated using analogue-by-catalogue (ABC).

c. Probe Optimization

Describe SAR & chemistry strategy (including structure and data) that led to the probe.

SAR was developed primarily by the ABC approach for this MLSCN project due to the availability of numerous commercial analogues. Analysis of the SAR identified some key structural features, including: (1) Reposition or derivatization of the hydroxyl groups leads to inactive compounds (SIDs -56373705, -56373700 and -56373696). (2) Selectivity for PLAP apparently resides in the N-substituted triazole group (Figure 1). Unsubstituted triazoles also potently inhibit PLAP but do not display selectivity vs. TNAP and IAP. Though 58 analogues were purchased to explore R1 and R2 (see Figure 2, and attached triazole list at end), no significant improvement in either selectivity or potency was achieved. For example, analogue SID-57287606 is in the submicromolar range for PLAP, but it is not as selective as the proposed probe molecule. The objective of chemistry was to develop a tractable route for the synthesis of the probe candidates, prepare authentic samples, confirm structure and purity, and provide enough material for submission to MLSMR.

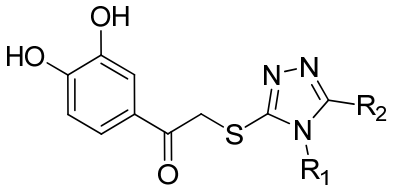


Figure 2. Probe analogs R1 & R2 variations

3. Probe

a. Chemical name

1-(3,4-dihydroxyphenyl)-2-[[5-(2-methoxyphenyl)-4-prop-2-enyl-1,2,4-triazol-3-yl]sulfanyl]ethanone **[ML085]**

b. Probe chemical structure

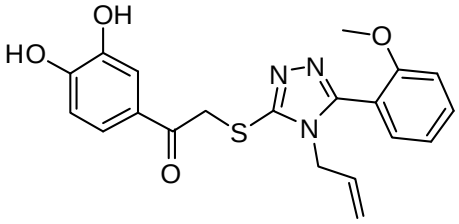


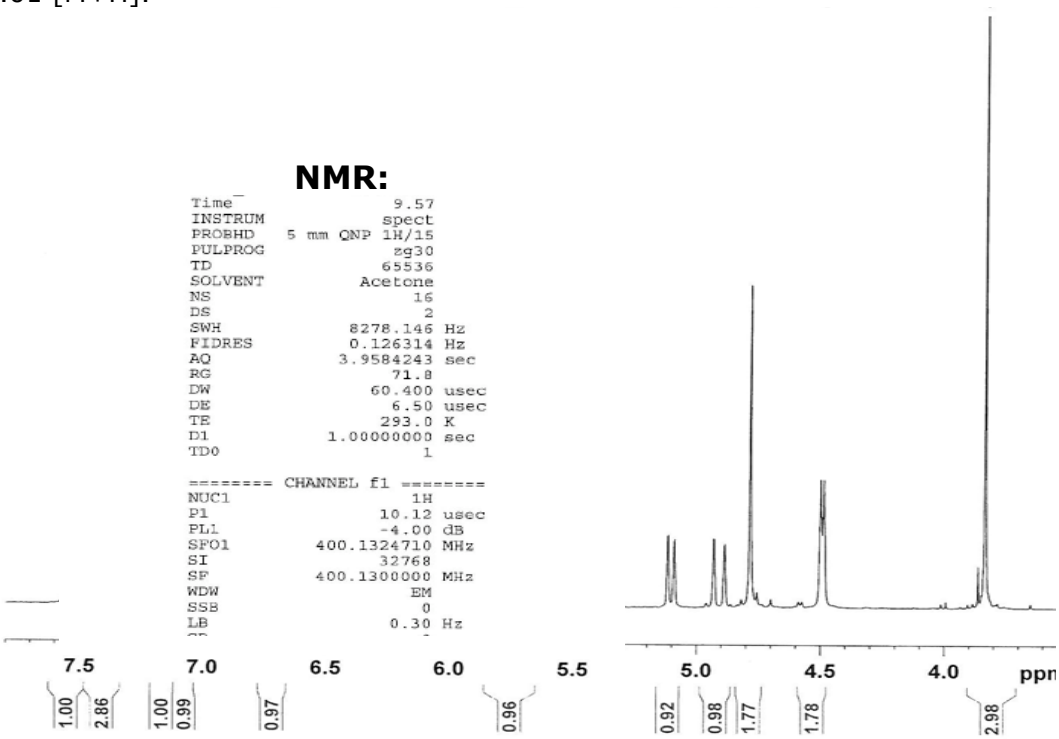
Figure 3. Probe compound structure

c. Structural Verification Information of probe SID

SID-56373725

Spectral data supporting proposed structure: ^{1}H NMR (400 MHz, Acetone-D6) δ 7.54 (ddd, *J* = 8.4, 7.5, 1.8, 1H), 7.46 – 7.33 (m, 3H), 7.17 (d, *J* = 8.1, 1H), 7.07 (dt, *J* = 7.5, 0.9, 1H), 6.72 (d, *J* = 8.2, 1H), 5.89 – 5.60 (m, 1H), 5.10 (dd, *J* = 10.4, 1.1, 1H), 4.91 (dd, *J* = 17.2, 1.1, 1H), 4.78 (s, 2H), 4.49 (dd, *J* = 3.7, 1.6, 2H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, Acetone-D6) δ 191.32, 159.49, 158.26, 154.41, 151.21, 148.89, 133.16, 132.97, 132.87, 124.45, 123.57, 121.53, 117.97, 117.73, 115.86, 113.80, 112.18, 55.92, 47.44, 42.01. MS (ESI) calculated $C_{20}H_{19}N_3O_4S$ *m/z* = 397.11, found *m/z* = 398.01 [M+H].

NMR:



Purity: 94% (HPLC-MS)

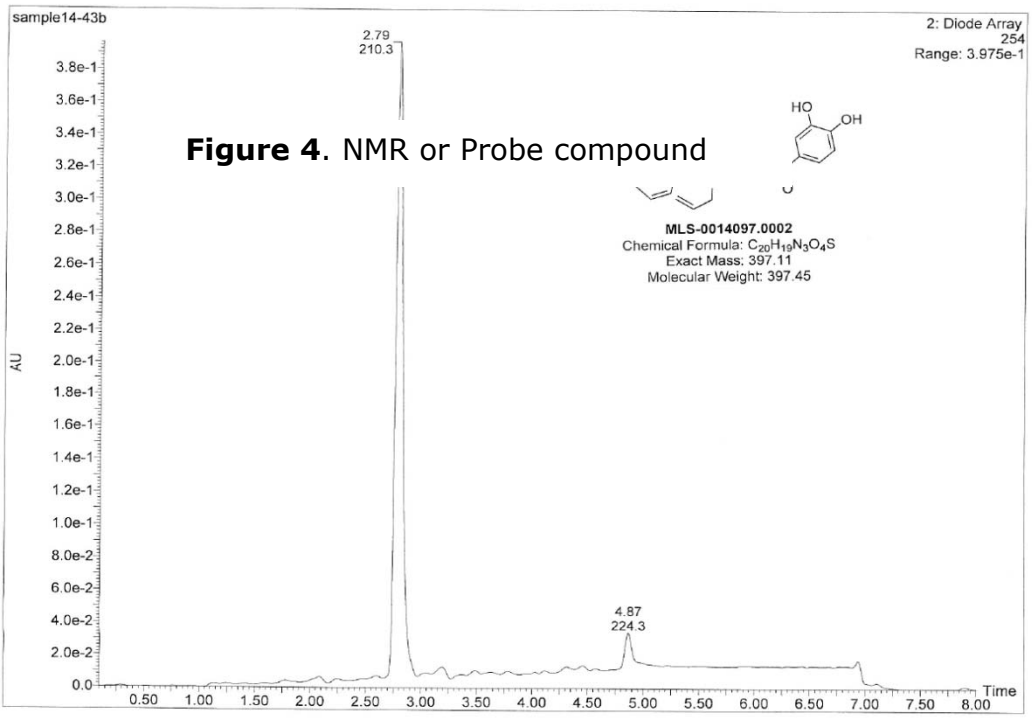


Figure 4. NMR or Probe compound

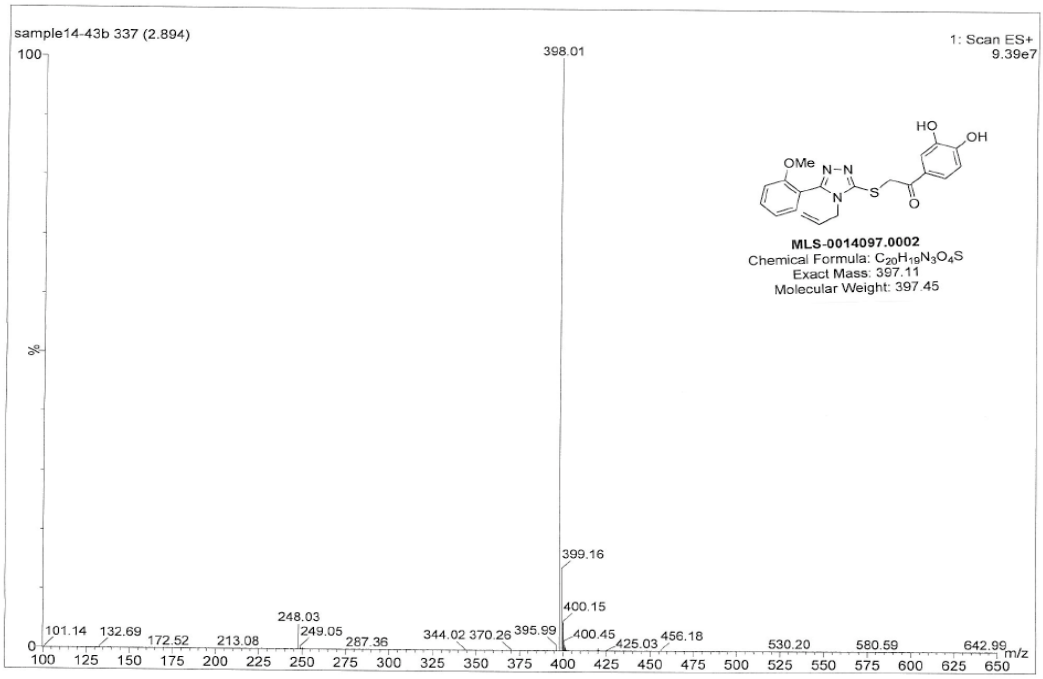


Figure 5. LC-MS traces for probe purity

d. PubChem CID (corresponding to the SID)
665093

e. Availability from a vendor.
The probe is commercially available from InterBioScreen (ID # STOCK4S-90308).

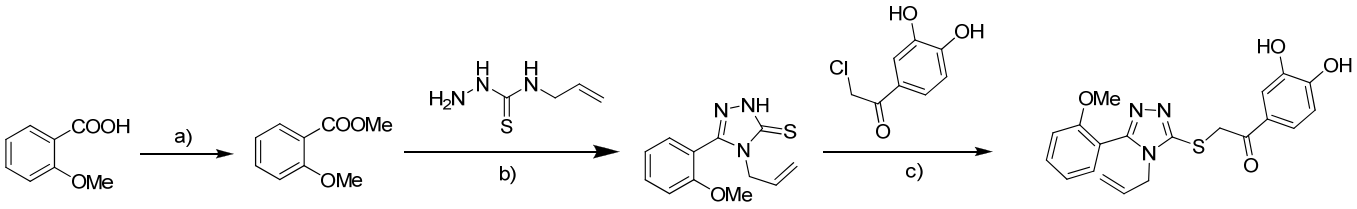
f. MLS# that verifies the submission of probe molecule and five related samples that were submitted to the SMR collection:

Table 2 Submission information on Probe and analogs

Probe /Analog	MLS-# (BCCG#)	CID	SID	Source (vendor or BCCG syn)	Amt (mg)	Date ordered/ submitted
Probe	0014097	665093	56373725	InterBioScreen	20	4/15/09
Analog 1	0390843	1409759	57287589	InterBioScreen	20	4/15/09
Analog 2	0390850	1400698	57287596	InterBioScreen	20	4/15/09
Analog 3	0390851	1400246	57287597	InterBioScreen	20	4/15/09
Analog 4	0390857	1398345	57287604	InterBioScreen	20	4/15/09
Analog 5	0390858	1394536	57287605	InterBioScreen	20	4/15/09

g. Mode of action for biological activity of probe
The probe is a biochemical inhibitor of PLAP. The mode of action for the biological activity of this probe has not yet been elucidated.

h. Detailed synthetic pathway for making probe



Reagents: a) c. H_2SO_4, MeOH; b) NaOMe, MeOH; c) Cs_2CO_3, MeCN.

Solid 2-methoxybenzoic acid (5.0g, 32.9mmol) was transferred to a 250ml round-bottom flask and dissolved in 50 mL dry MeOH. 8 drops of c.H_2SO_4 were added to the reaction mixture. The reaction mixture was heated at reflux for 12h. After 12 h the reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude reaction mixture was dissolved in 50 mL diethylether and washed with saturated $NaHCO_3$ (2 × 50mL) and brine solution (2 × 50mL). The organic layer was collected and the solvent was removed under reduced pressure to yield the methyl ester as a white solid (5.4 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (dd, *J* = 7.9, 1.8, 1H), 7.49 (ddd, *J* = 8.5, 7.4, 1.8, 1H), 7.00 (dtd, *J* = 4.7, 3.4, 1.0, 2H), 3.93 (s, 3H), 3.91 (s, 3H).

Methyl anisate (50.0mg, 0.3 mmol) was transferred to a glass vial and dissolved in 5 mL dry MeOH. 4-Allyl thio-semicarbazide (59.2 mg, 0.45 mmol) was added to the reaction mixture. This was followed by addition of 0.2 mL of 25%(wt) NaOMe solution. The resultant reaction mixture was heated overnight at 85^{o}C in a sealed vial. After the reaction was complete, the solvents were evaporated under reduced pressure. The reaction mixture was cooled to 0^{o}C and 20 mL deionized water was added to it. The pH of the reaction mixture was lowered to 5 using 10% AcOH (dropwise addition). The resultant white precipitate (4-allyl-3-(2-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione) was filtered, dried and weighed (55mg, 74.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.47 (m, 1H), 7.35 (dd, *J* = 7.5, 1.6, 1H), 7.08 (ddd, *J* = 19.5, 13.1, 4.6, 2H), 5.75 (ddd, *J* = 16.0, 11.4, 5.7, 1H), 5.10 (d, *J* = 10.3, 1H), 4.91 (d, *J* = 17.1, 1H), 4.60 (d, *J* = 5.8, 2H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.79, 157.60, 150.56, 133.03, 131.79, 130.63, 120.98, 118.47, 114.67, 111.15, 55.58, 46.99.

Solid 4-allyl-3-(2-methoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (25.0 mg, 0.1mmol) was dissolved in 5 mL dry acetonitrile. 2-Chloro-3',4'-dihydroxyacetophenone (18.8mg, 0.1mmol) was added to the reaction mixture followed by addition of Cs_2CO_3(33mg, 0.1mmol). the resultant reaction mixture was stirred at room temperature for 8 hrs. The solid was filtered from the reaction mixture and the filtrate was concentrated to yield the final product 2-(4-allyl-5-(2-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio)-1-(3,4-dihydroxyphenyl)ethanone (25mg, 62.5%). The product was further purified by crystallization using acetone. ^{1}H NMR (400 MHz, Acetone-D6) δ 7.54 (ddd, *J* = 8.4, 7.5, 1.8, 1H), 7.46 – 7.33 (m, 3H), 7.17 (d, *J* = 8.1, 1H), 7.07 (dt, *J* = 7.5, 0.9, 1H), 6.72 (d, *J* = 8.2, 1H), 5.89 – 5.60 (m, 1H), 5.10 (dd, *J* = 10.4, 1.1, 1H), 4.91 (dd, *J* = 17.2, 1.1, 1H), 4.78 (s, 2H), 4.49 (dd, *J* = 3.7, 1.6, 2H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, Acetone-D6) δ 191.32, 159.49, 158.26, 154.41, 151.21, 148.89, 133.16, 132.97, 132.87, 124.45, 123.57, 121.53, 117.97, 117.73, 115.86, 113.80, 112.18, 55.92, 47.44, 42.01. MS (ESI) calculated $C_{20}H_{19}N_3O_4S$ *m/z* = 397.11, found *m/z* = 398.01 [M+H].

i. Center summary of probe properties (solubility, absorbance/fluorescence, reactivity, toxicity, etc.)

Our internal counter screen efforts has showed CID-665093 to be inactive in two assays TNAP inhibition (AID 518) and G6DPH (AID-1020), and 10-fold less active vs. IAP (AID-1017), a very close family member of PLAP.

In Vitro Pharmacology:

Compounds CID-665093 (MLS-0014097) and CID-2102207 (MLS-0107074) were subjected to a battery of *in vitro* pharmacology assays to assess aqueous solubility, cellular permeability, plasma protein binding (PPB), plasma stability, and microsome stability. The results of these assays are presented in Table 2.

Table 3: Pharmacologic Properties of MLS-01014097, MLS-0107074.

Cpd-ID	**Aqueous Solubility (µg/mL)** pH 5.0/6.2/7.4 (AID1615)	**Permeability (×10-6 cm/s)** pH 5.0/6.3/7.4 (AID1557)	**Plasma Protein Binding (% Bound)** (AID1617)		**Plasma Stability (%Remaining)** Human/Mouse (AIDs -1591/-1592)	**Microsomal Stability (%Remaining)** Human/Mouse (AIDs -1555/pending)
			Human (10µM/1µM)	**Mouse** (10µM/1µM)		
CID-665093 MLS-0014097	8.4/8.4/18.0	90/98/58	98.6/98.7	87.9/89.5	100.2/83.7	47.0/41.8
CID-2102207 MLS-0107074	0.3/0.3/0.9	98/91/75*	99.9/99.1	99.7/99.3	82.4/77.5	17.0/1.8

CID-665093 (MLS-0014097) exhibits modest solubility in aqueous buffer and poor cell permeability. On the other hand, since PLAP is an extracellular enzyme, poor permeability could provide an added advantage to the probe, helping to ensure selectivity for its biological function. The compound is most soluble in aqueous buffer at pH 7.4, indicating that the compound will be sufficiently soluble for use in cellular assays conducted under normal culture conditions. CID-665093 (MLS-0014097) is extensively bound to plasma proteins in human plasma. In mouse plasma, the percent compound bound is slightly less than in human plasma. The amount of free compound available to bind the target (PLAP) is therefore greater in mice suggesting that the efficacy of this compound might be greater in mice than in humans. The compound also exhibits robust metabolic stability when exposed to both mouse and human microsome preparations.

CID-2102207 (MLS-0107074) exhibits poor solubility in aqueous buffer at all pH levels tested. In order to assess the cellular permeability, it was required that a cosolvent (20% ACN) be used in the assay to facilitate solubility. Despite the use of ACN, cellular permeability remained poor. The compound is extensively bound to plasma proteins in human and mouse plasma, and exhibits moderate stability in human and mouse plasma. CID-2102207 (MLS-0107074) was significantly metabolized in human and mouse hepatic microsomes. This suggests that the compound will be subjected to extensive first pass metabolism, limiting the use of the compound *in vivo*. Taken together these attributes indicate that this probe is best suited for biochemical experiments, but may also be used in cell based experiments when formulated with an appropriate co-solvent.

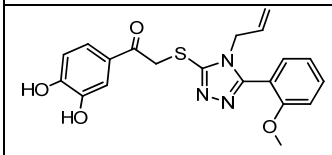
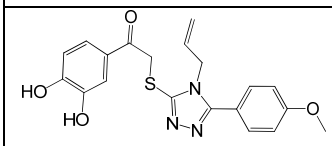
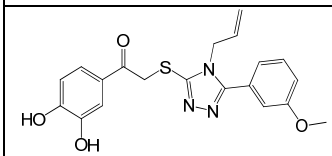
j. Properties Computed from Structure

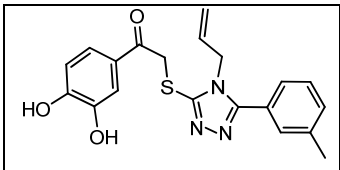
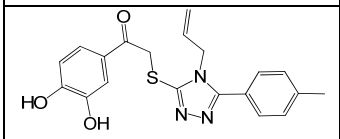
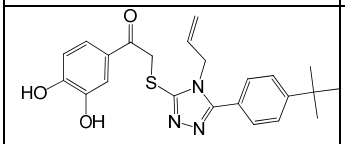
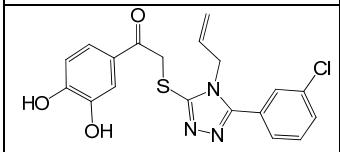
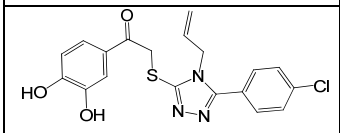
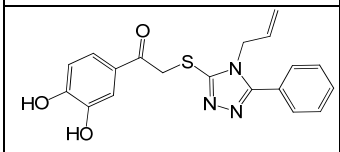
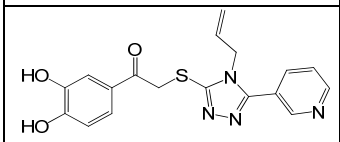
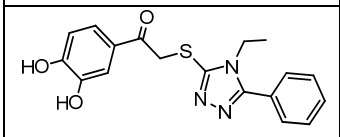
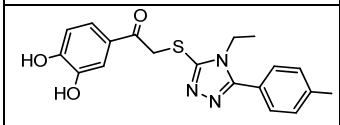
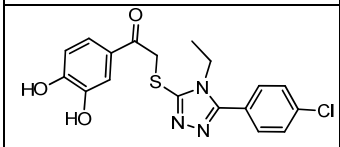
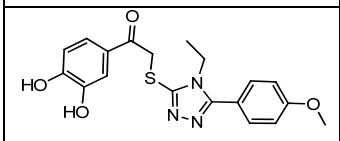
Property	Value
Molecular Weight	397.44756 [g/mol]
Molecular Formula	$C_{20}H_{19}N_3O_4S$
XLogP3-AA	3.3
H-Bond Donor	2
H-Bond Acceptor	6
Rotatable Bond Count	8
Tautomer Count	22
Exact Mass	397.109627
MonoIsotopic Mass	397.109627
Topological Polar Surface Area	123
Heavy Atom Count	28
Formal Charge	0
Complexity	538
Isotope Atom Count	0
Defined Atom StereoCenter Count	0
Undefined Atom StereoCenter Count	0
Defined Bond StereoCenter Count	0
Undefined Bond StereoCenter Count	0
Covalently-Bonded Unit Count	1

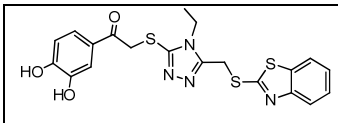
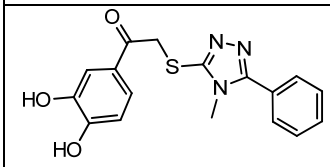
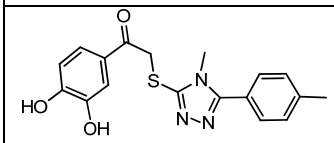
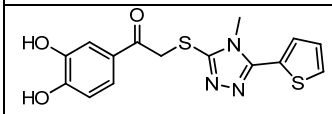
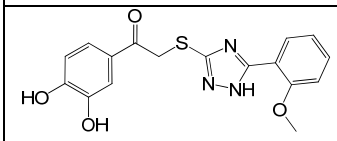
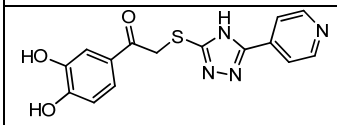
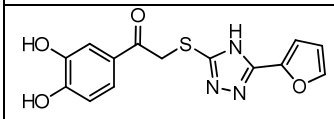
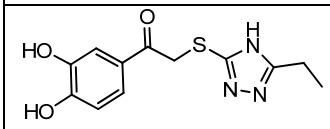
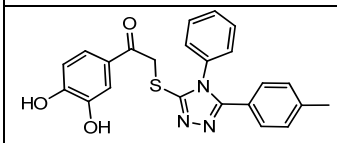
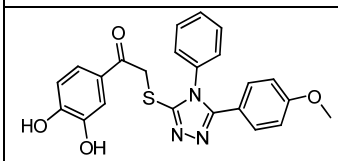
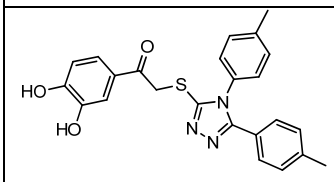
4. Appendices

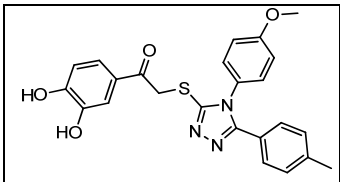
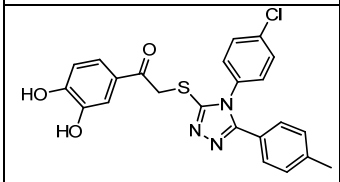
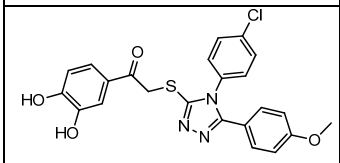
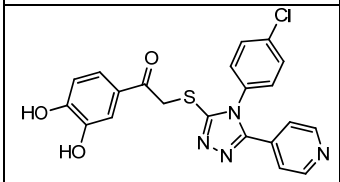
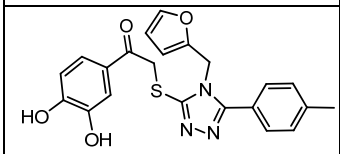
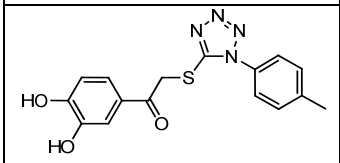
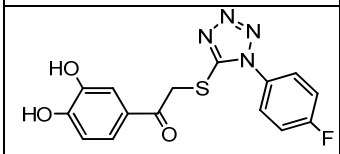
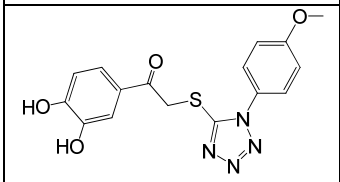
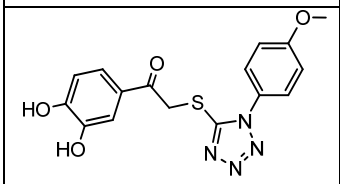
a. Comparative data on (1) probe, (2) similar compound structures (establishing SAR) and (3) prior probes

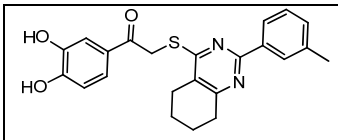
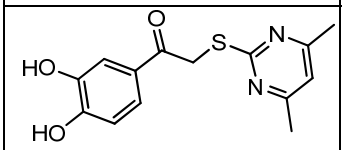
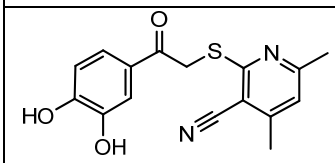
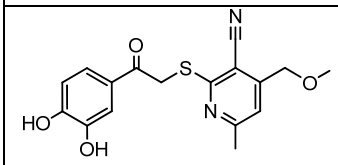
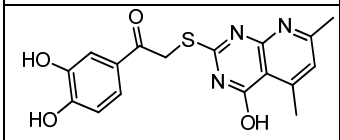
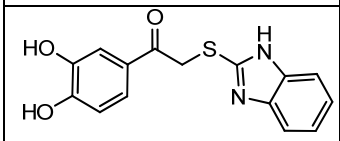
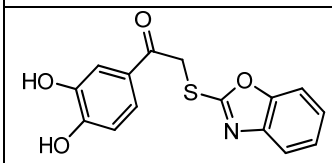
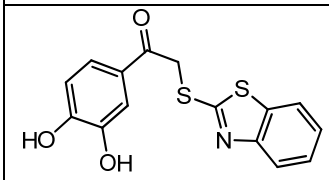
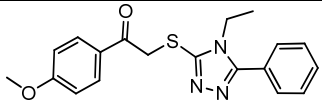
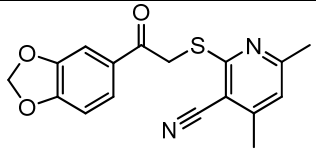
Table 5. All Purchased Analogs.

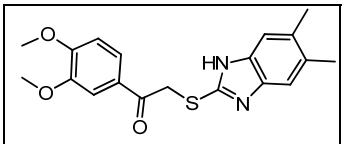
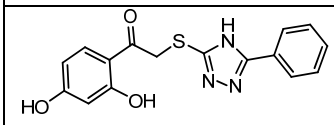
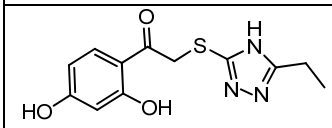
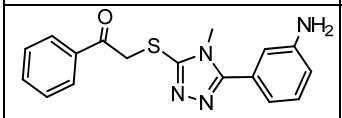
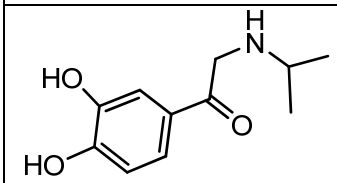
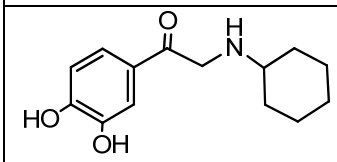
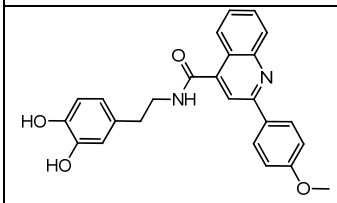
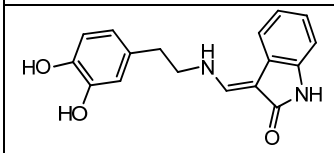
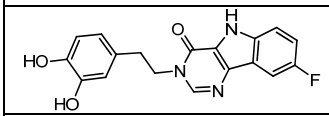
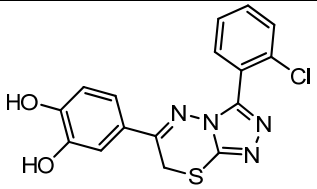
Structure	Cmpd ID	IC50(uM)		SID	CID	Vendor	VendorID
		PLAP	TNAP				
	MLS-0014097	2.6	>100	56373725	665093	InterBio-Screening	STOCK4S-90308
	MLS-0390850	13.6	>100	57287596	1400698	InterBio-Screening	STOCK4S-91062
	MLS-0390843	3.97	>100	57287589	1409759	InterBio-Screening	STOCK4S-87186

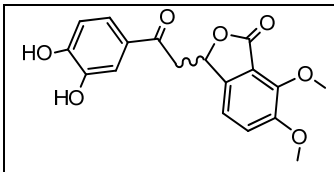
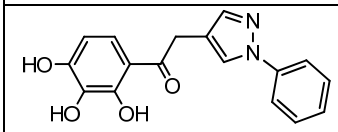
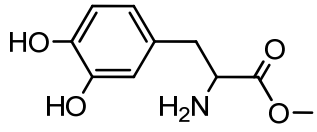
	MLS-0390851	6.02	>100	57287597	1400246	InterBio-Screening	STOCK4S-92693
	MLS-0390858	9.4	>100	57287605	1394536	InterBio-Screening	STOCK5S-03688
	MLS-0390855	>100	>100	57287601	1409691	InterBio-Screening	STOCK4S-97188
	MLS-0390857	7.45	>100	57287604	1398345	InterBio-Screening	STOCK4S-99075
	MLS-0390849	11.8	>100	57287595	1400269	InterBio-Screening	STOCK4S-90841
	MLS-0390841	2.7	12.1	57287587	1416608	InterBio-Screening	STOCK4S-84785
	MLS-0297802	2.92	>100	57287584	1400947	InterBio-Screening	STOCK4S-15744
	MLS-0108835	2.53/	40.3	56373724	1399636	InterBio-Screening	STOCK4S-90782
	MLS-0101351	11.8	>100	57287602	3878415	InterBio-Screening	STOCK4S-97273
	MLS-0390844	8.76	>100	57287590	1398070	InterBio-Screening	STOCK4S-88147
	MLS-0390842	8.31	>100	57287588	1402438	InterBio-Screening	STOCK4S-86311

	MLS-0107074	3.87	>100	57309171	2102207	BCCG	PMK-14-59
	MLS-0315681	2.79	93.4	56373578	838842	Chem-Bridge	5672011
	MLS-0390856	11.7	>100	57287603	1400150	InterBio-Screening	STOCK4S-97686
	MLS-0297827	4.9	>100	57287583	3735808	InterBio-Screening	STOCK3S-98364
	MLS-0315688	7.29	2.78	56373639	1314069	Chem-Bridge	7587885
	MLS-0315684	6.95	6.53	56373634	715398	Chem-Bridge	6593203
	MLS-0315686	12.2	21.7	56373636	715363	Chem-Bridge	6617586
	MLS-0315685	1.78	10.9	56373635	715330	Chem-Bridge	6607811
	MLS-0390847	>100	>100	57287593	1400320	InterBio-Screening	STOCK4S-90030
	MLS-0390853	7.22	>100	57287599	1400627	InterBio-Screening	STOCK4S-94912
	MLS-0390852	8.73	>100	57287598	1399934	InterBio-Screening	STOCK4S-94867

	MLS-0390854	5.59	>100	57287600	1401217	InterBio-Screening	STOCK4S-95328
	MLS-0390846	13	>100	57287592	1383304	InterBio-Screening	STOCK4S-89768
	MLS-0390839	25.0	>100	57287585	1973575	InterBio-Screening	STOCK4S-16633
	MLS-0390840	6.63	40.7	57287586	1984977	InterBio-Screening	STOCK4S-20993
	MLS-0390848	4.94	19.2	57287594	4283152	InterBio-Screening	STOCK4S-90562
	MLS-0315690	1.58	8.58	56373641	946537	Chem-Bridge	7746693
	MLS-0238024	1.89	5.98	56373642	713665	Chem-Bridge	7764114
	MLS-0008112	1.42	5.93	56373572	1515366	Chem-Bridge	7764254
	MLS-0255212	1.76	3.48	56373643	2010274	Chem-Bridge	7775407

	MLS-0315689	>100	78.5	56373640	1305489	Chem-Bridge	7642884
	MLS-0315687	1.85	7.19	56373637	715454	Chem-Bridge	6622859
	MLS-0315680	1.56	6.71	56373577	720372	Chem-Bridge	5484218
	MLS-0051931	2.16	4.82	56373536	586278	ChemDiv	3294-0014
	MLS-0315683	8.77	>100	56373633	5350662	Chem-Bridge	6588132
	MLS-0315887	4.79	5.44	56373703	4206177	Specs	AI-204/31718048
	MLS-0315886	3.06	4.15	56373702	4450383	Specs	AI-204/31685046
	MLS-0315885	3.42	3.03	56373701	4066568	Specs	AI-204/31680049
	MLS-0315884	>100	>100	56373700	996625	Specs	AG-690/40754100
	MLS-0315888	>100	>100	56373704	698547	Specs	AM-807/12427007

	MLS-0315883	>100	>100	56373696	1223257	Specs	AE-848/36959517
	MLS-0315682	>100	3.71	56373579	2877377	Chem-Bridge	5933089
	MLS-0315889	>100	>100	56373705	715329	Specs	AP-048/15613011
	MLS-0080017	>100	>50	56373623	881297	Chem-Bridge	6385545
	MLS-0315678	39.3	>100	56373922	2830496	Chem-Bridge	5147768
	MLS-0315679	18.8	>100	56373923	2841226	Chem-Bridge	5317936
	MLS-0390862	>100	>100	57287609	16415295	InterBio-Screening	STOCK6S-42849
	MLS-0390859	0.374	3.14	57287606	5922288	InterBio-Screening	STOCK1S-39868
	MLS-0390860	0.93	0.25	57287607	932151	InterBio-Screening	STOCK2S-62329
	MLS-0390861	15	7.38	57287608	16000021	InterBio-Screening	STOCK4S-15750

	MLS-0077534	2.95	29.2	56373574	2940433	Chem-Bridge	7471251
	MLS-0068603	>100	>100	56373638	805519	Chem-Bridge	6680720
	MLS-0073729	8.43	>100	56373913	9551841	InterBio-Screening	STOCK2S-13283

b. Comparative data showing probe specificity for target

Table 4: SAR and Selectivity.

Compound ID	Assay Format	Assay Target	No of Runs	IC_{50} Value (µM)
MLS-0014097	Luminescent	TNAP	4	>100
MLS-0014097	Luminescent	PLAP	~ 6	2.34
MLS-0014097	Luminescent	IAP	1	20.7
MLS-0014097	Colorimetric	GAPDH	2	>100
MLS-0014097	FP	BCL-b/TR3	1	>100
MLS-0014097	FP	Bfl-1	1	>100

5. Bibliography

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4. WH Fishman (1987) Clin Biochem 20, 387-92. "Clinical and biological significance of an isozyme tumor marker—PLAP."