

SIGNALCHEM COMPOUND PROFILING REPORT

EVALUATION OF COMPOUNDS AGAINST TARGET X

PREPARED FOR:

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X Pharmaceuticals



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1. Overview of SignalChem Profiling Service

The Cost-Effective Approach to Discovery in a Time Sensitive Manner.

SignalChem's compound profiling service utilizes a large and diverse panel of highly active protein kinase and phosphatase targets that are produced internally. SignalChem's active targets are subjected to rigorous quality control analyses and are extensively assayed against a panel of biologically relevant substrates ensuring that all reactions are performed under optimal assay conditions.

SignalChem's compound profiling service determines the respective profile (inhibition or activation) and the putative mechanism of action of a compound. All compounds are profiled against a panel of targets either using a single concentration or at multiple concentrations in order to allow in-depth IC₅₀ determinations. In addition, the protein kinase assays can be performed under varying ATP concentrations to evaluate competition with respect to ATP.

The compound profiling service offered by SignalChem is a very economical and convenient approach to the drug discovery continuum with a two-week turnaround of your specific profiling results. Compounds can be supplied by the client as DMSO stocks of known concentration, as solid material in vials, or in 96-well plates.

PROFILING BACKGROUND

The selectivity profile of any small molecule is of fundamental clinical importance as part of the drug development process. The information yielded from these studies will undoubtedly provide useful insight into the proposed mechanism of action of a given compound as well as leading to the identification of "off" target effects, thus leading to the selection of better lead candidates.

SIGNALCHEM TARGETS

SignalChem produces a diverse and ever-expanding range of targets that are actively being pursued from a drug development perspective, with a main focus on signaling proteins including active protein kinases and phosphatases.

SignalChem's highly purified active enzymes are generated from the full-length human genes and are mutation free. These targets do not harbor any activating mutations, as it is well established that these mutations do not occur *in vivo* in the disease state.

CONFIDENTIALITY

SignalChem maintains all information under strict confidentiality. All information and or materials supplied will be used as directed by the client. Upon completion of the project, all materials will be either returned to the client or disposed of accordingly. SignalChem is willing to execute confidentiality agreements with its clients.

2. Objectives

The client has requested that SignalChem perform the following service:

- Perform profiling of 4 compounds (listed in Table 1) against X target to determine the IC_{50} values. The 4 compounds should be profiled against X target at 5nM, 50nM, 100nM, 250nM, 500nM, 1 μ M, 2.5 μ M, 5 μ M, 10 μ M and 20 μ M final compound concentration.

Table 1. Panel of Compounds to be evaluated against X Target.

Compound No#	Compound ID
1	X1
2	X2
3	X3
4	X4

3. Materials

The X target to be employed in the compound profiling process was cloned, expressed and purified in-house at SignalChem using proprietary methods. Quality control testing is routinely performed on each of the SignalChem targets to ensure compliance to acceptable standards. Protein substrate employed in the compound profiling process was synthesized internally. ³³P-ATP was purchased from PerkinElmer. All other materials were of standard grade. The compounds were supplied by the client as a solid powder form. They were reconstituted in anhydrous DMSO to form a stock solution that was then diluted appropriately to give the desired concentrations.

4. Results

SignalChem evaluated the selectivity profile of 4 compounds (X1, X2, X3 and X4) against the X target. The selectivity profile of these compounds was evaluated employing the standardized assay methodology outlined in Appendix A. Ten compound concentrations (5nM, 50nM, 100nM, 250nM, 500nM, 1µM, 2.5µM, 5µM, 10µM, 20µM final compound concentration) were chosen for profiling the various compounds against the X target.

The results observed as % activity change compared to control are presented in Figure 2 while Appendix B contains all the raw data for the various compounds. The intra-assay variability was determined to be less than 10%. Inhibition of X target activity by the compound gives -ve value while activation of X target activity gives +ve value. SignalChem considers that values below 15% change in activity against a given target to be not highly significant.

The profiling data for compound X1 shows robust inhibition of X activity. An IC_{50} value of 1.54 µM was generated (derived from the best-fit line graph where the R value was 0.99) for this compound against X target. The X target was inhibited by 88% at 20 µM of X1.

Two of the compounds (X2 and X3) both showed a very weak mode of inhibition of X. At the highest tested concentration (20 µM) these compounds gave only between 19-31% inhibition of X activity and therefore, an IC_{50} value of >30 µM was generated for these compounds. The profile of compound X4 towards the X target was determined to be of insignificant value; for further information, refer to the data in Table 2 & 3 and in Appendix B.

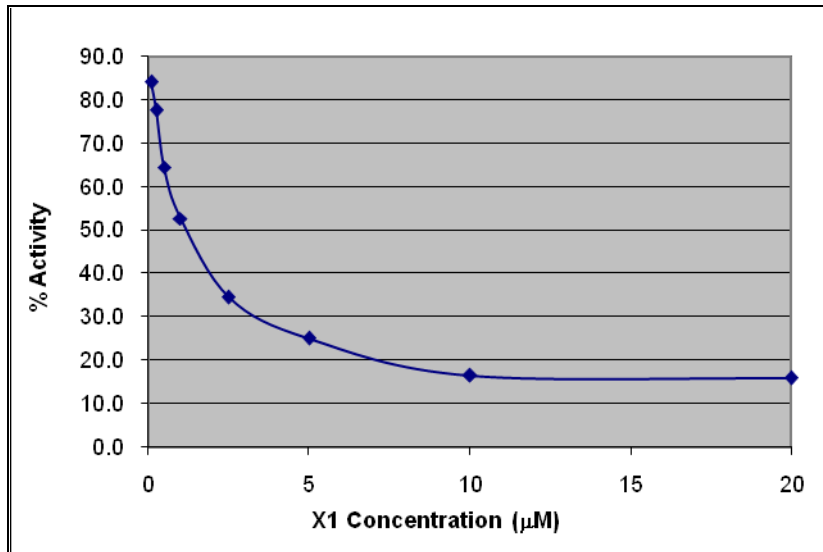
Table 2. % Activity change of X evaluated against various compounds.

Compound ID	% Activity change (5 nM)	% Activity change (50 nM)	% Activity change (100 nM)	% Activity change (250 nM)	% Activity change (500 nM)	% Activity change (1 µM)	% Activity change (2.5 µM)	% Activity change (5 µM)	% Activity change (10 µM)	% Activity change (20 µM)
X1	-5	-10	-8	-15	-26	-38	-61	-75	-81	-88
X2	1	0	-1	0	-4	-1	0	-2	-11	-19
X3	1	1	2	3	2	2	4	0	-12	-31
X4	1	2	1	0	3	3	1	-3	-1	-1

IC₅₀ Determination for X1

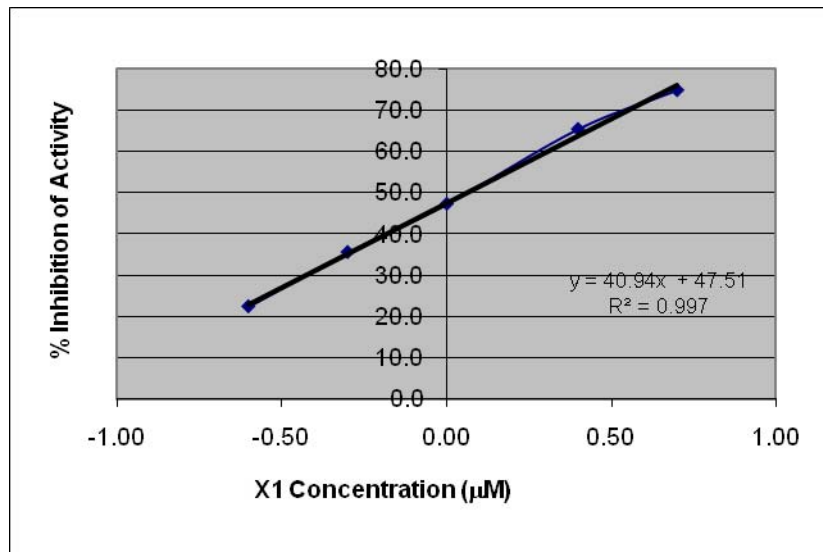
A graph of X1 concentration (μM) was plotted against % activity of X (Figure 1). A curve graph was generated which showed decrease in X activity with increasing X1 concentration. Based on this graph, an IC₅₀ value for X1 against X was calculated to be approximately 2 μM.

Figure 1. Graph of X1 Concentration against % Activity of X



A graph of log X1 concentration (μM) was plotted against % inhibition of X activity (Figure 2). A best-fit line graph was generated and the slope of the line used to calculate the IC₅₀ value. A IC₅₀ value for X1 against X was determined to be 1.54 μM.

Figure 2. Graph of X1 Concentration against % Inhibition of Activity



5. Discussion and Conclusion

Compound profiling activity was undertaken, using the X target produced in-house at SignalChem, against various concentrations of 4 compounds (X1, X2, X3 AND X4) in the presence of 50 μM ATP.

Out of all the compounds, only X1 showed the most potent effects on the X target while the effects of the other compounds were not highly potent. However, X2 and X3 did show weak effects and displayed 19-31% inhibition of X activity at the highest tested concentration (20 μM). SignalChem considers that values below 15% of change in activity of a given target to be not highly significant.

Thus, overall compound X1 was observed to be a fairly potent inhibitor of X activity *in vitro*.

Table 3. IC₅₀ Values for various compounds Against X.

Compound ID	IC ₅₀
X1	1.54 μM
X2	>20 μM
X3	>20 μM
X4	>20 μM

6. Signatures

Signature _____

Lead Investigator Dr. Jasbinder Sanghera

Date of Completion 01-10-2007

Appendix A

The assay condition for X was optimized to yield acceptable enzymatic activity. In addition, the assay was optimized to give high signal-to-noise ratio (>50:1).

ASK1 Assay

SignalChem uses a radioisotope assay format for compound profiling evaluation against protein kinase targets and all assays are performed in a designated radioactive working area. X assays (in triplicate) were performed at 30 °C for 15 min in a final volume of 25µl according to the following assay reaction recipe:

- Component 1. 5µl of diluted active X
- Component 2. 5µl of stock solution of substrate or assay buffer
- Component 3. 5µl of assay buffer
- Component 4. 5µl of compound or 2% DMSO
- Component 5. 5µl of ³³P-ATP (250µM stock solution, 0.8 µCi)

The assay was initiated by the addition of ³³P-ATP and the reaction mixture incubated at 30°C for 15 minutes. After the 15 minute incubation period, the assay was terminated by spotting 20µl of the reaction mixture onto phosphocellulose P81 plate. The phosphocellulose P81 plate was washed 3 times for approximately 15 minutes each in a 1% phosphoric acid solution. The radioactivity on the P81 plate was counted in the presence of scintillation fluid in a scintillation counter.

Blank control, which included all the assay components except the addition of the X substrate (replace with equal volume of assay dilution buffer), was set up. The corrected activity for X was determined by removing the blank control value.

Appendix B

Assay Results

1 : Compound X1

Assay Condition	Assay Results (cpm)	Average Value (cpm)	Corrected Values (cpm)	% Activity	% Change
Blank (- substrate)	4140	4540	-	-	-
	4940				
Control (- compound)	62532	63667	59127	100	-
	64578				
	63890				
+ 5 nM compound	63375	60434	55894	95	-5
	59037				
	58891				
+ 50 nM compound	57719	57461	52921	90	-10
	58069				
	56595				
+ 100 nM compound	59334	59195	54655	92	-8
	56375				
	61877				
+ 250 nM compound	54067	54966	50426	85	-15
	53574				
	57257				
+ 500 nM compound	48459	48524	43984	74	-26
	50446				
	46668				
+ 1 μ M compound	40993	41343	36803	62	-38
	41387				
	41650				
+ 2.5 μ M compound	28146	27550	23010	39	-61
	29317				
	25186				
+ 5 μ M compound	19333	19196	14656	25	-75
	21192				
	17063				
+ 10 μ M compound	17600	15981	11441	19	-81
	16134				
	14209				
+ 20 μ M compound	11759	11341	6801	12	-88
	11710				
	10555				

2 : Compound X2

Assay Condition	Assay Results (cpm)	Average Value (cpm)	Corrected Values (cpm)	% Activity	% Change
Blank (- substrate)	4750	4984	-	-	-
	5217				
Control (- compound)	58634	60667	55683	100	-
	60421				
	62946				
+ 5 nM compound	62749	61223	56239	101	1
	62053				
	58868				
+ 50 nM compound	61357	60788	55804	100	0
	62547				
	58461				
+ 100 nM compound	58765	60143	55159	99	-1
	62240				
	59423				
+ 250 nM compound	61941	60449	55465	100	0
	60890				
	58517				
+ 500 nM compound	59242	58519	53535	96	-4
	58789				
	57525				
+ 1 μ M compound	61760	59923	54939	99	-1
	57872				
	60138				
+ 2.5 μ M compound	62406	60720	55736	100	0
	62047				
	57708				
+ 5 μ M compound	60784	59356	54372	98	-2
	58452				
	58832				
+ 10 μ M compound	54822	54728	49744	89	-11
	54234				
	55128				
+ 20 μ M compound	49288	50277	45293	81	-19
	51781				
	49762				

3 : Compound X3

Assay Condition	Assay Results (cpm)	Average Value (cpm)	Corrected Values (cpm)	% Activity	% Change
Blank (- substrate)	4248	4702	-	-	-
	5156				
Control (- compound)	63967	64176	59474	100	-
	64050				
	64510				
+ 5 nM compound	67381	64652	59950	101	1
	64312				
	62262				
+ 50 nM compound	62339	64926	60224	101	1
	65245				
	67194				
+ 100 nM compound	66459	65069	60367	102	2
	66990				
	61758				
+ 250 nM compound	62642	66040	61338	103	3
	66965				
	68514				
+ 500 nM compound	68422	65282	60580	102	2
	65032				
	62392				
+ 1 μ M compound	65645	65653	60951	102	2
	65221				
	66094				
+ 2.5 μ M compound	66866	66310	61608	104	4
	67643				
	64422				
+ 5 μ M compound	66598	64441	59739	100	0
	61762				
	64963				
+ 10 μ M compound	60713	57224	52522	88	-12
	54645				
	56313				
+ 20 μ M compound	45850	45900	41198	69	-31
	45133				
	46717				

4 : Compound X4

Assay Condition	Assay Results (cpm)	Average Value (cpm)	Corrected Values (cpm)	% Activity	% Change
Blank (- substrate)	4574	4830	-	-	-
	5086				
Control (- compound)	62480	61145	56315	100	-
	59300				
	61654				
+ 5 nM compound	61918	61793	56963	101	1
	62522				
	60939				
+ 50 nM compound	61241	62371	57541	102	2
	61910				
	63963				
+ 100 nM compound	60003	61522	56692	101	1
	63019				
	61544				
+ 250 nM compound	60973	61310	56480	100	0
	59566				
	63390				
+ 500 nM compound	63280	62701	57871	103	3
	61499				
	63325				
+ 1 μ M compound	63585	62605	57775	103	3
	63220				
	61011				
+ 2.5 μ M compound	61139	61642	56812	101	1
	62288				
	61498				
+ 5 μ M compound	59777	59669	54839	97	-3
	58100				
	61130				
+ 10 μ M compound	59034	60656	55826	99	-1
	61022				
	61912				
+ 20 μ M compound	61667	60652	55822	99	-1
	58639				
	61650				