SGC

UNC Collaboration

Testing the Binding of 53 compounds against WDR41 by SPR

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Molecular Biophysics

SGC Toronto
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UNICAMP

Summary

- **Binding of 53 compounds to WDR41 (30-459aa) were tested in singlet (summary slides 2-3):**
  - **16 compounds (highlighted in Black) showed binding with the regular binding signals (RU, >20; slides 4-5).**
  - **11 compounds (highlighted in Gray) showed binding with the lower signals (around 11 RU) that are higher than the negative control with around 5 RU (slides 6-7).**
  - **9 compounds (highlighted in Yellow) showing the linear signals up to 100 µM (slide 8).**
  - **17 compounds (highlighted in Red, NB: Not binding) showed low binding signals (<10) that are similar to the negative control (slide 9).**
  - **K_D values were generated from the steady state fittings.**
- **RBBP4 was used as a negative control protein.**

SPR Conditions:

WDR41 (**30-459**aa) was immobilized on the flow cell of a CM5 sensor chip in 1x HBS-EP buffer, yielding 8000 RU. RBBP4 (1-425aa) was immobilized on another flow cell of CM5 chip, yielding 6000 RU as a negative control. Using the same buffer with **2%** DMSO and single cycle kinetic with 60s contact time and a dissociation time of 120s at a flow rate of 40 µL/min. All compounds were tested at 100 µM as the highest concentration, with dilution factor of 0.25 to yield 5 concentrations.

Summary

Note
Hits:
Black: regular signals (RU>20).

Possible hits:
Gray: Low signal (likely the K_D values are not accurate).

False positives:
Red: No binding (NB).
Yellow: Linear up to 100 µM.

Compound			K_D (µM)
ChemiReg ID	Toronto ID	Cat#	
XS125008b	XSSU125008b	CSSS00026120636	1.3
XS127764b	XSSU127764b	CSSS00026108053	2.5
MR042879a	MRSU042879a	CSSS00028033776	3.2
MR042843a	MRSU042843a	CSSS00027743374	4.5
MR042865a	MRSU042865a	CSSS00026109023	5.4
MR042860a	MRSU042860a	CSSS00026190317	7.5
XS171346b	XSSU171346b	CSSS00026107916	8
MR042872a	MRSU042872a	CSSS00026476575	8
MR042834a	MRSU042834a	CSSS00026476522	9
MR042849a	MRSU042849a	CSSS00027958535	9
MR042859a	MRSU042859a	CSSS00026166444	12
MR042848a	MRSU042848a	CSSS00026187536	14
MR042853a	MRSU042853a	CSSS00133078949	15
MR042871a	MRSU042871a	CSSS00026476108	16
MR042873a	MRSU042873a	CSSS00028333170	18
MR042864a	MRSU042864a	CSSS00027963090	25
MR042838a	MRSU042838a	CSSS00027743321	27
MR042854a	MRSU042854a	CSSS00138549331	27
MR042850a	MRSU042850a	CSSS00103934117	44
XS138274b	XSSU138274b	CSSS00026190245	50
MR042877a	MRSU042877a	CSSS00028033818	52
MR042867a	MRSU042867a	CSSS00027743331	58
XS171510b	XSSU171510b	CSSS00027738113	76
MR042847a	MRSU042847a	CSSS00028033815	106
MR042856a	MRSU042856a	CSSS00026190247	>100
XS127442b	XSSU127442b	CSSS00026476824	>100
MR042875a	MRSU042875a	CSSS00026476817	>100

Compound			K_D (µM)
ChemiReg ID	Toronto ID	Cat#	
MR042846a	MRSU042846a	CSSS00026106898	>100
MR042845a	MRSU042845a	CSSS00026476391	Linear
MR042835a	MRSU042835a	CSSS00026190395	Linear
MR042836a	MRSU042836a	CSSS00138548301	Linear
MR042839a	MRSU042839a	CSSS00028229619	Linear
MR042851a	MRSU042851a	CSSS00138550542	Linear
MR042852a	MRSU042852a	CSSS00138533454	Linear
MR042866a	MRSU042866a	CSSS00026120644	Linear
MR042876a	MRSU042876a	CSSS00028034086	Linear
MR042844a	MRSU042844a	CSSS00026120601	NB
MR042837a	MRSU042837a	CSSS00026111267	NB
MR042840a	MRSU042840a	CSSS00026119822	NB
MR042841a	MRSU042841a	CSSS00026475421	NB
MR042842a	MRSU042842a	CSSS00028033840	NB
MR042855a	MRSU042855a	CSSS00138538427	NB
XS137714b	XSSU137714b	CSSS00026160571	NB
MR042857a	MRSU042857a	CSSS00027779705	NB
MR042858a	MRSU042858a	CSSS00026120592	NB
MR042868a	MRSU042868a	CSSS00027955419	NB
MR042869a	MRSU042869a	CSSS00028033762	NB
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MR042861a	MRSU042861a	CSSS00000737961	NB
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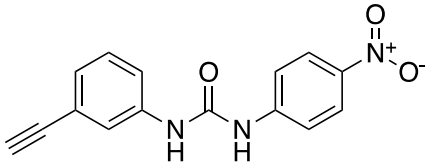
MR042878a	MRSU042878a	CSSS00026475530	NB

Structures of 16 hits



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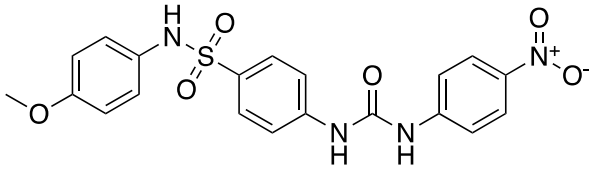

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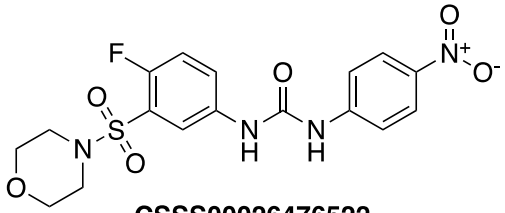

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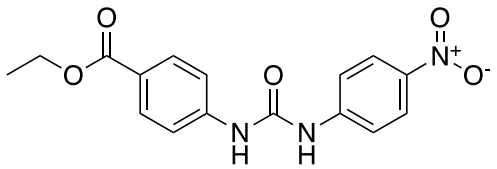

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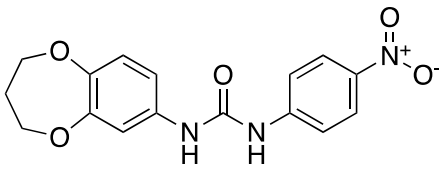

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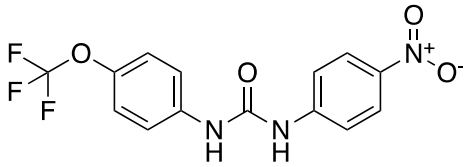

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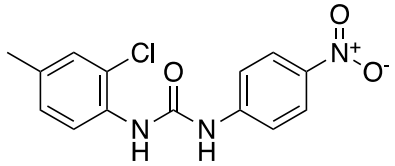

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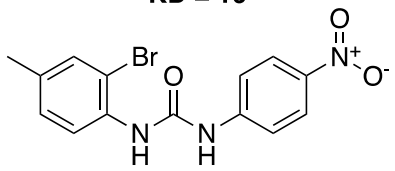

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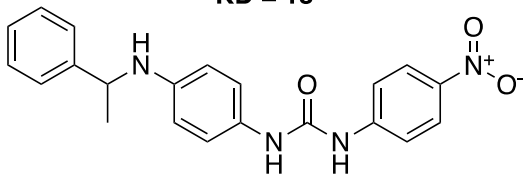

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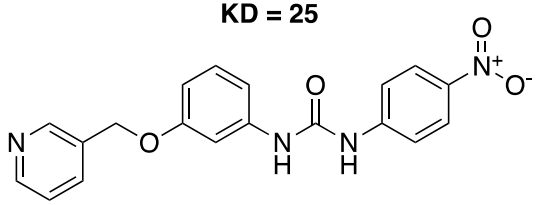

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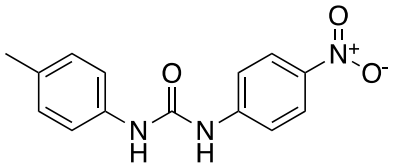

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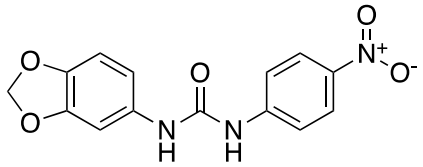

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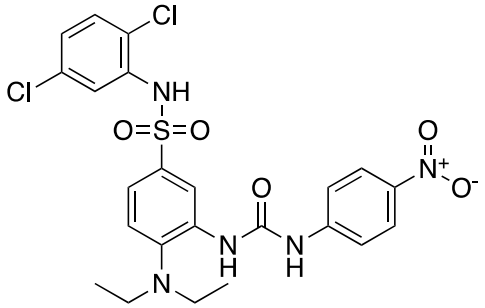

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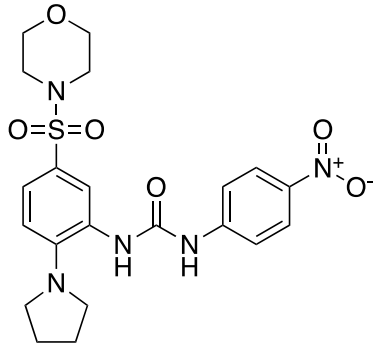

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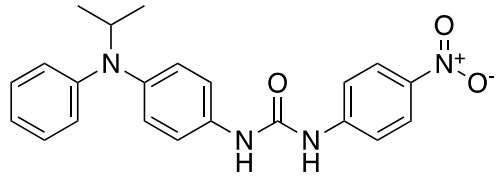

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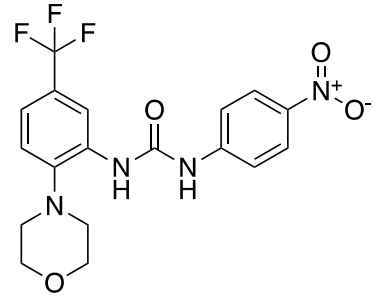

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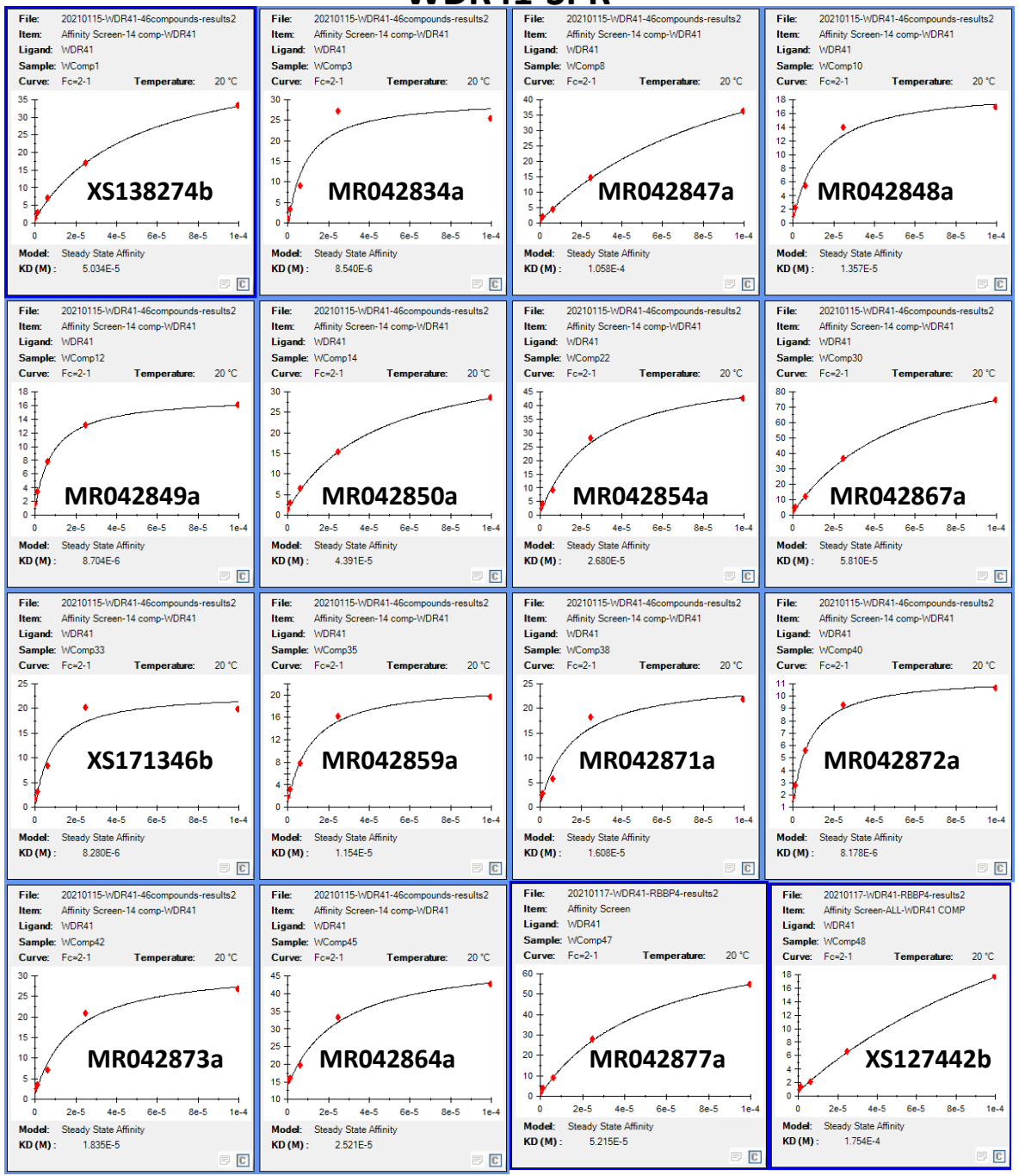
16 Compounds with Regular Signals -Steady State Responses (expected RU_{max} 50-90)

WDR41-SPR

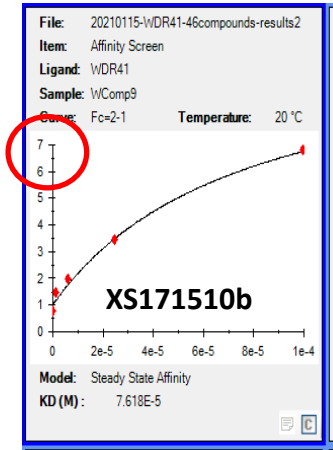


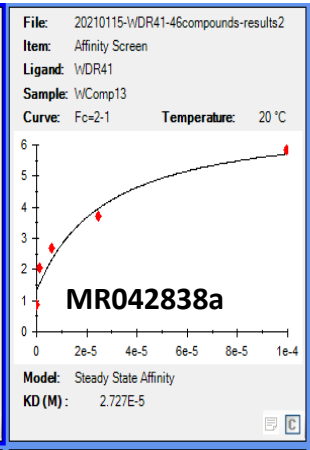
Negative control_RBBP4

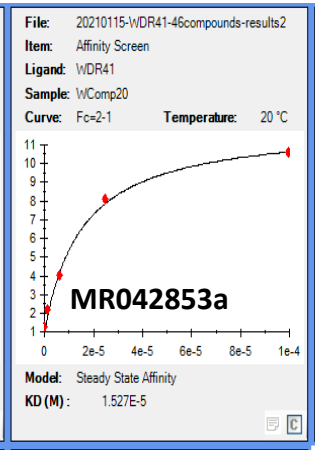
WDR41 SPR: 16 Compounds SPR-Sensorgrams with Regular Signals (expected RU_{max} 50-90)

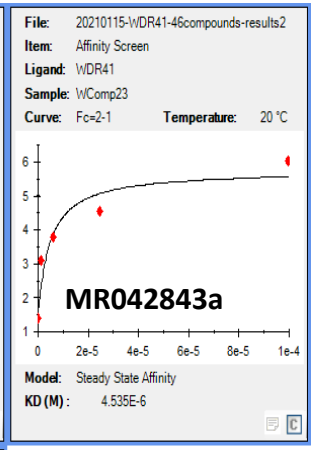
11Compounds with Lower binding Signals (around 11RU, expected RU_{max} 50-90)

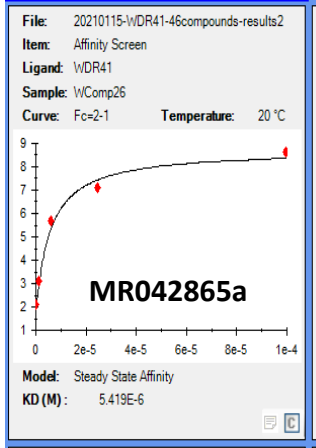
WDR41-SPR

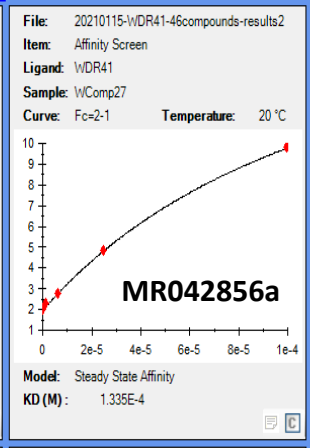


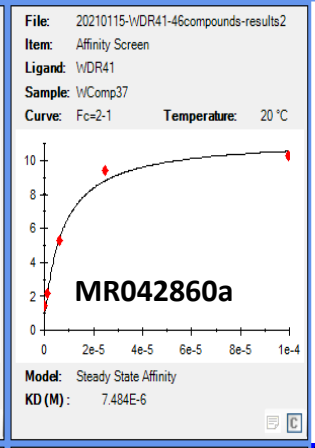


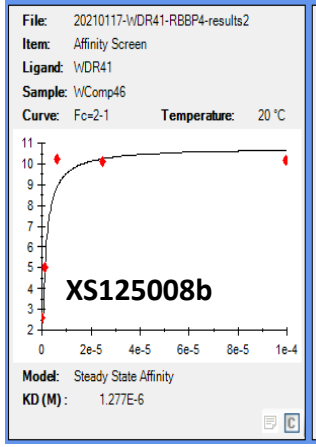


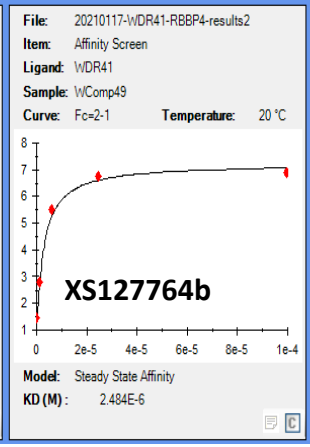


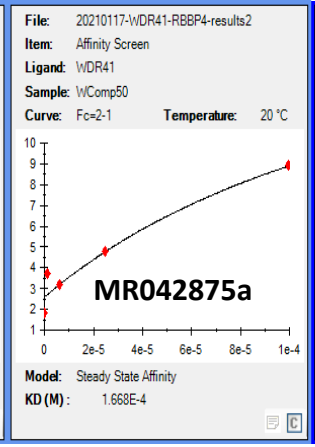


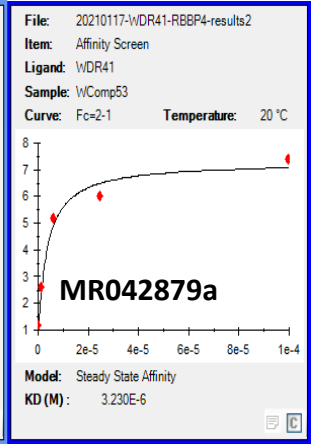




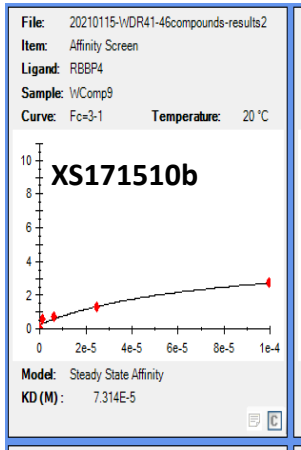


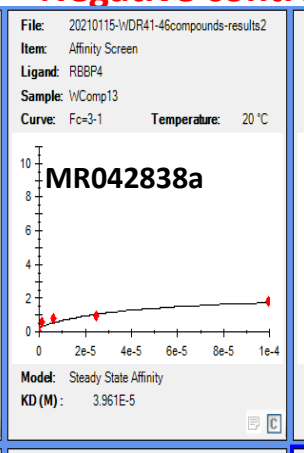


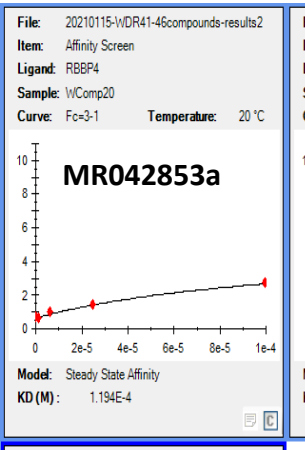


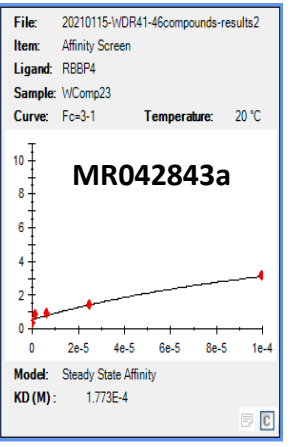


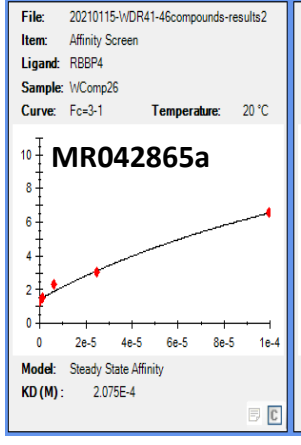
Negative control-RBBP4-SPR

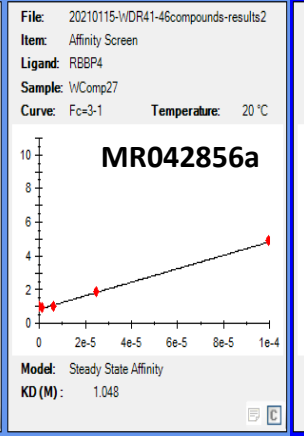


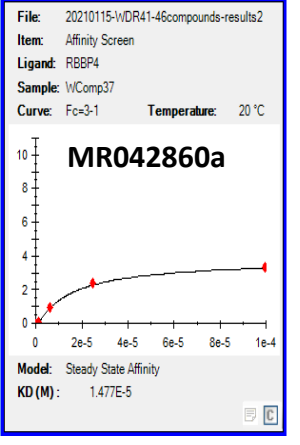


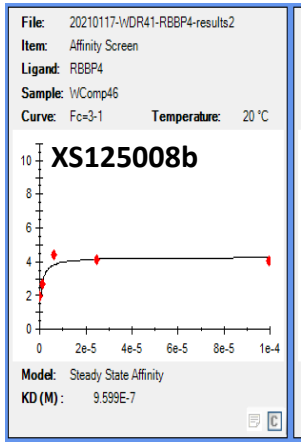


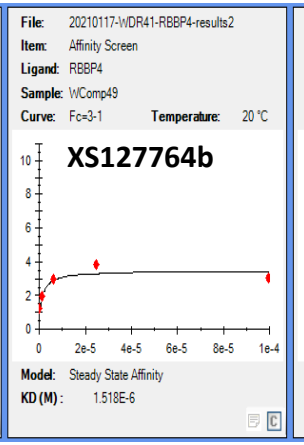


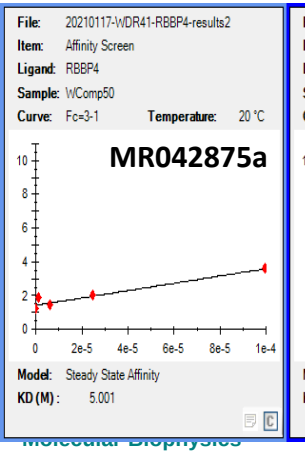


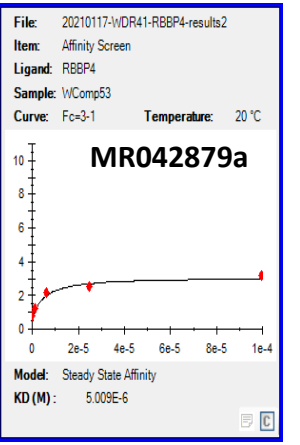




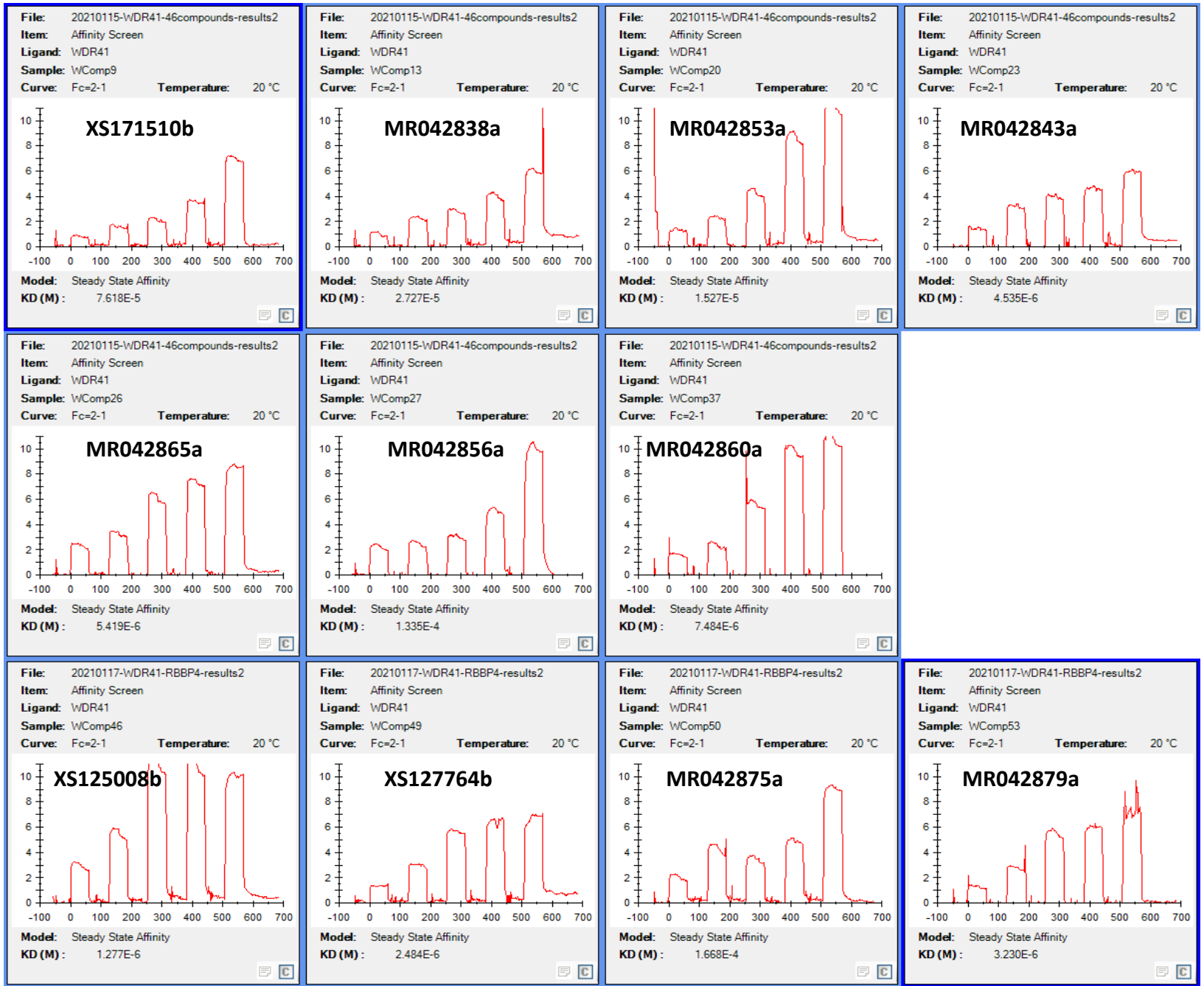






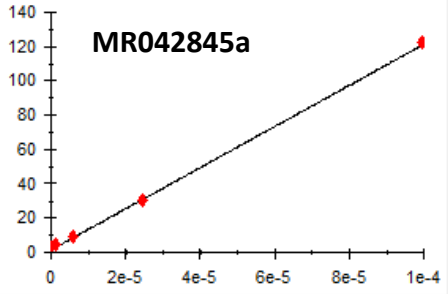


11 Compound SPR-Sensorgrams with Lower binding Signals (around 11RU)



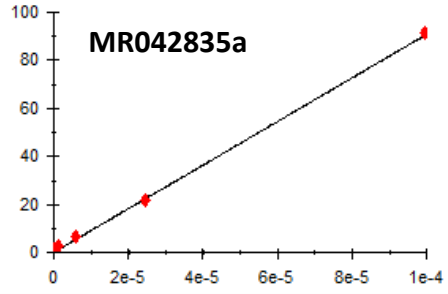
9 Compounds Linear up to 100uM (expected RUmax 50-90)

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp4
Curve: Fc=2-1 Temperature: 20 °C



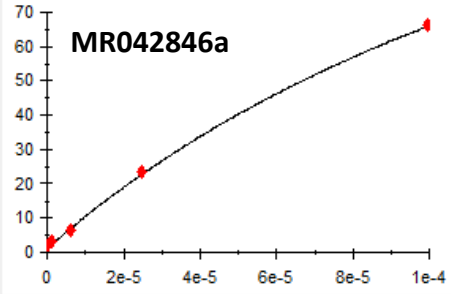
Model: Steady State Affinity
KD (M): 2.521

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp5
Curve: Fc=2-1 Temperature: 20 °C



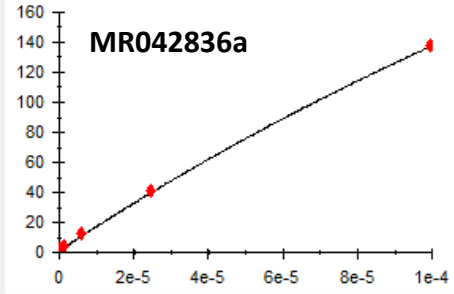
Model: Steady State Affinity
KD (M): 5.205

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp6
Curve: Fc=2-1 Temperature: 20 °C



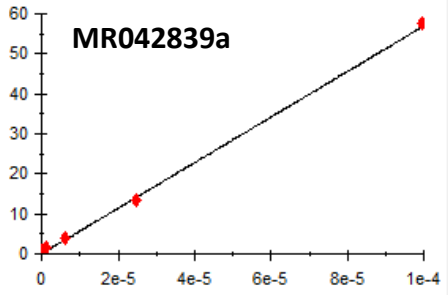
Model: Steady State Affinity
KD (M): 1.946E-4

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp7
Curve: Fc=2-1 Temperature: 20 °C



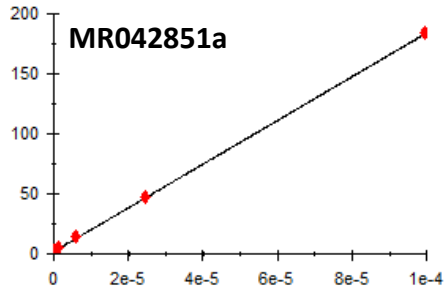
Model: Steady State Affinity
KD (M): 4.774E-4

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp15
Curve: Fc=2-1 Temperature: 20 °C



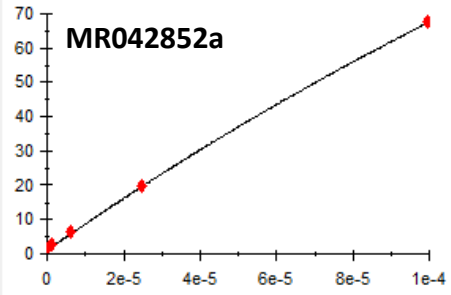
Model: Steady State Affinity
KD (M): 5.286

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp16
Curve: Fc=2-1 Temperature: 20 °C



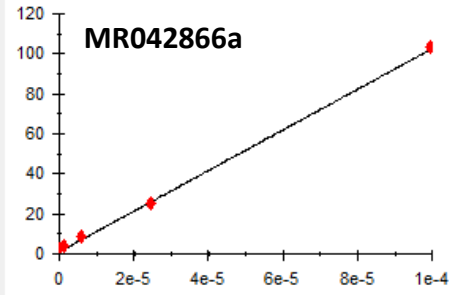
Model: Steady State Affinity
KD (M): 0.2712

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp18
Curve: Fc=2-1 Temperature: 20 °C



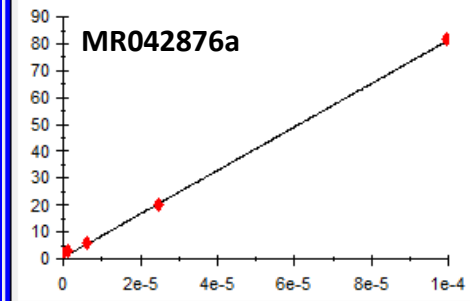
Model: Steady State Affinity
KD (M): 5.652E-4

File: 20210115-WDR41-46compounds-results2
Item: Affinity Screen 2
Ligand: WDR41
Sample: WComp28
Curve: Fc=2-1 Temperature: 20 °C



Model: Steady State Affinity
KD (M): 5.253

File: 20210117-WDR41-RBBP4-results2
Item: Affinity Screen
Ligand: WDR41
Sample: WComp52
Curve: Fc=2-1 Temperature: 20 °C



Model: Steady State Affinity
KD (M): 5.213

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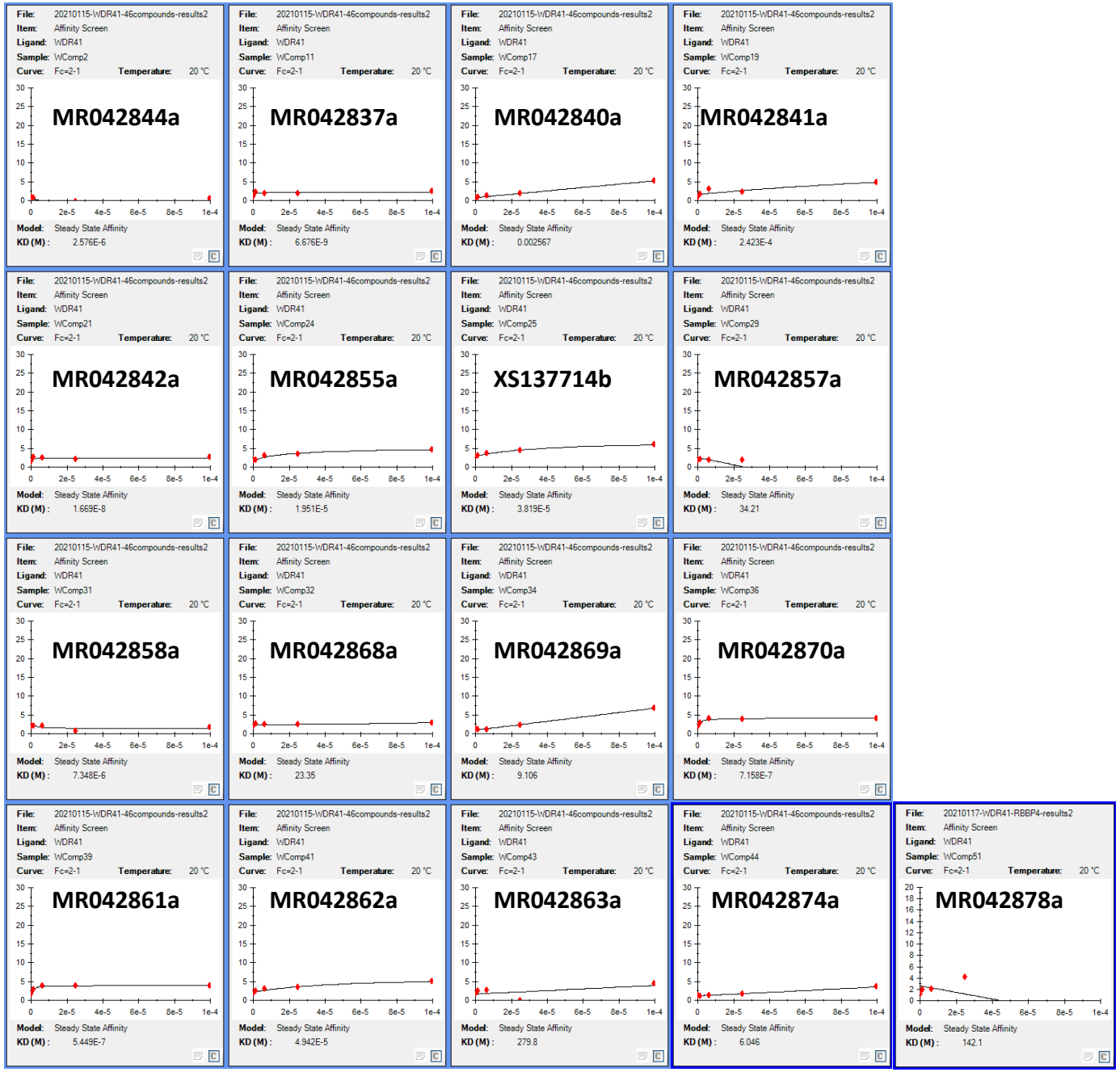
17 Compounds No Significant binding to WDR41 (expected RU_{max} 50-90)



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SGC Toronto

Masoud Vedadi
Principal Investigator, Molecular Biophysics

Protein was purified by:
Elisa Gibson

Compound Management:
Albina Bolotokova

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UNC collaboration

Testing UNC 423 compounds library against WDR41 by DSF

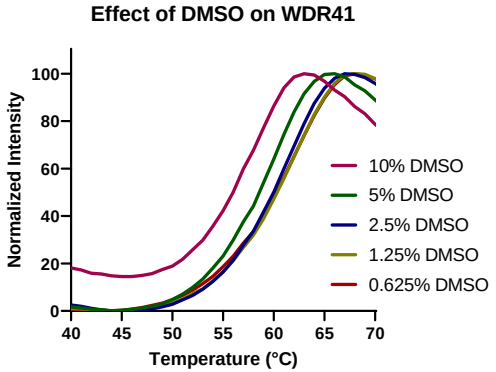
Albina Bolotokova
Molecular Biophysics

SGC Toronto

27 October 2020

Testing UNC 423 compound library against WDR41 by DSF

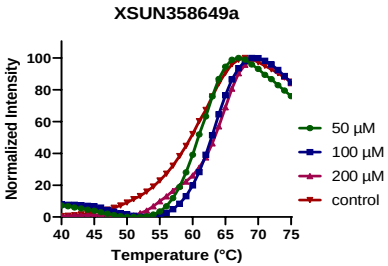
- A library of 423 close analogs was screened by DSF against WDR41 in 100 mM Hepes, 150 mM NaCl and pH 7.5 buffer at 0.1mg/ml protein at two DMSO concentrations.
- Initially the compounds were prepared for screening at 50 µM in 0.25% DMSO. Most compounds showed visible precipitation in this buffer. The plate was sonicated which increased compound resuspension, and compounds were tested. The original hit (XSOI050073c) was used as a control.
- More than 140 compounds showed ΔT_m of more than 2°C. However, due to heavy compound precipitation, we decided to re-screen the compounds at higher DMSO concentrations.
- Our assessment indicated that WDR41 can tolerate up to 2.5% DMSO with no significant effect on stability of the protein.
- Compounds were re-screened at 50, 100 and 200 µM in 2.5% DMSO in original buffer.
- The original hit (XSOI050073c) was used as a control.
- 9 compounds were identified as hits at all three concentrations.

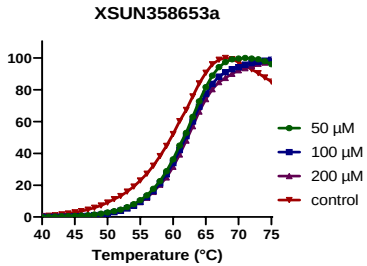


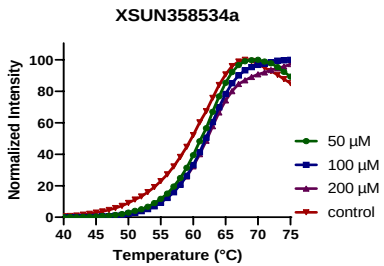
DMSO %	T_m (°C)
10	56.7
5	58.6
2.5	60.0
1.25	60.4
0.625	60.3

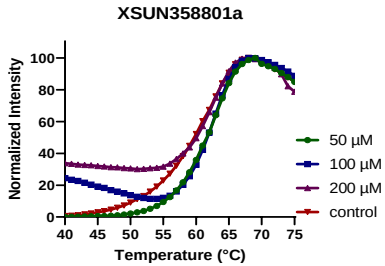
Summary

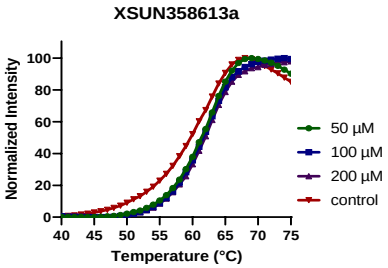
Compound			Screen #1	Screen #2		
			ΔTm (°C) @ Conc. (µM)			
ChemiReg ID	Catalog Number	Toronto Local ID	50	50	100	200
XS358649a	CSC027288209	XSUN358649a	2	**1.2**	**3.2**	**3.7**
XS358653a	CSC027743159	XSUN358653a	2.2	**2.1**	**2.1**	**2.4**
XS358534a	CSC052076151	XSUN358534a	1.5	**1.7**	**2.2**	**2.2**
XS358801a	CSC027743325	XSUN358801a	1.5	**1.9**	**2.4**	**2.2**
XS358613a	CSC000743789	XSUN358613a	3.7	**1.8**	**1.8**	**2**
XS358603a	CSC026190132	XSUN358603a	2.6	**1.7**	**1.6**	**1.9**
XS358570a	CSC026182648	XSUN358570a	1.3	1.3	1.6	NA (HF)
XS358561a	CSC026476555	XSUN358561a	3.2	2	NA	NA
XS358631a	CSC028079896	XSUN358631a	2.3	2.6	NA	NA
	control	XSOI050073c	5.6	**2.4**	**3.7**	**7.7**

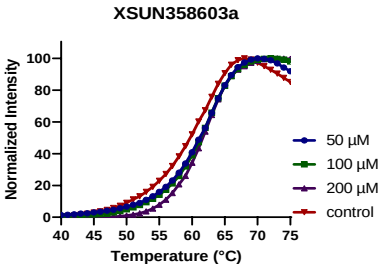


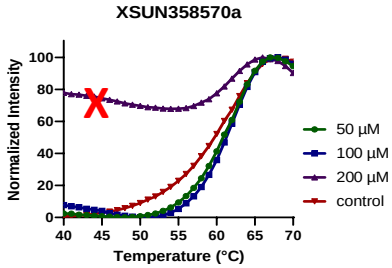












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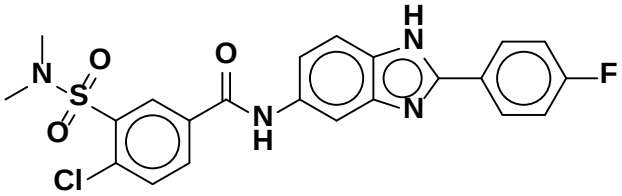

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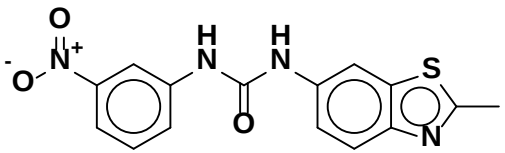

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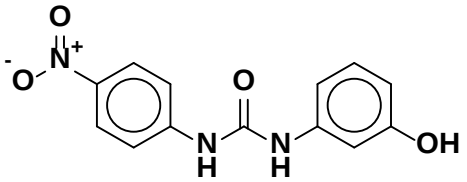

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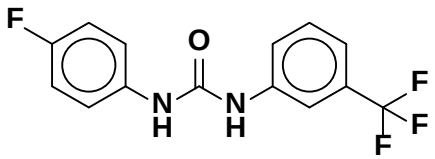

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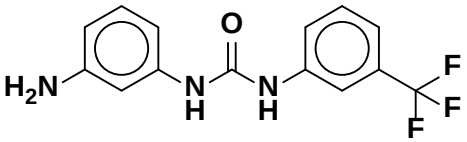

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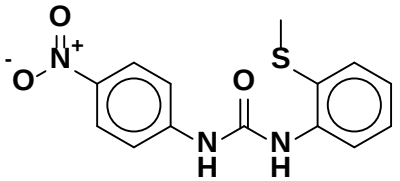

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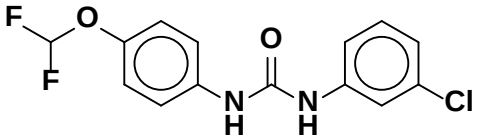

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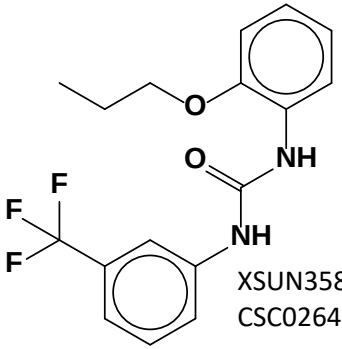

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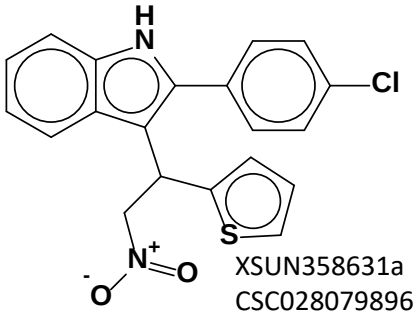

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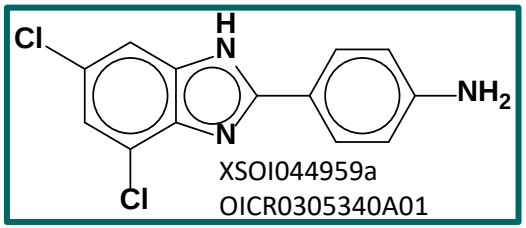

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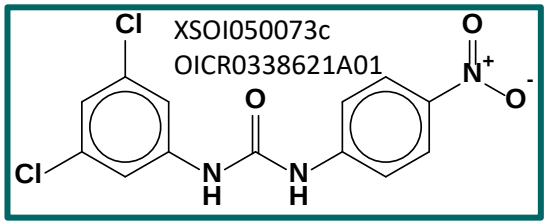

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OICR0338621A01

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Masoud Vedadi
Principal Investigator, Molecular Biophysics

Protein was purified by:
Elisa Gibson

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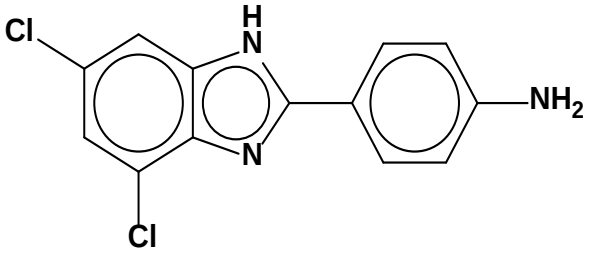
Reproducible WDR41 DSF Screening hits for further Hit Expansion and Chemistry

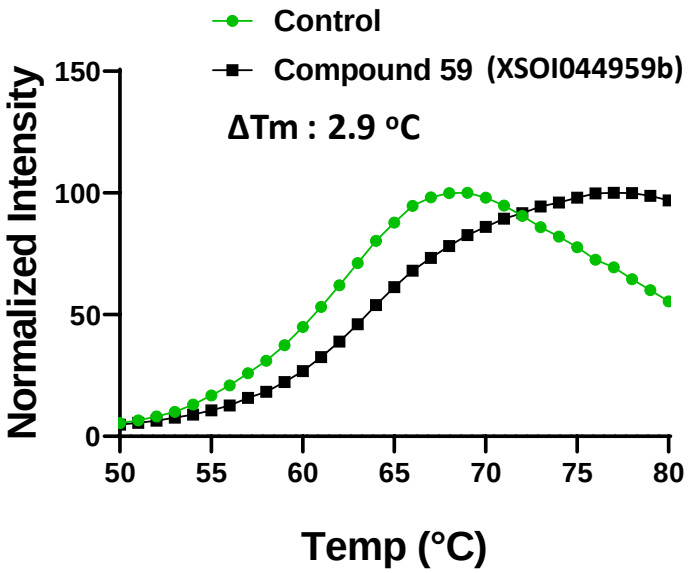
Aliakbar Khalili Yazdi, Ling Yan Gao, Albina Bolotokova and Masoud Vedadi

Molecular Biophysics, SGC Toronto.

June 8th 2020

Compounds to consider

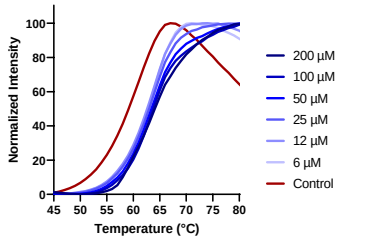


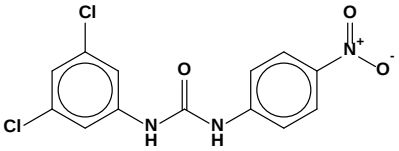


Compounds to consider

4.8 (°C)

XSOI050073c

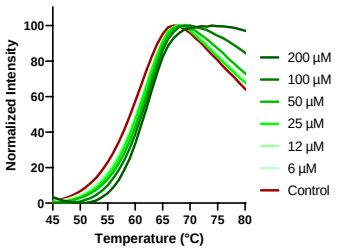


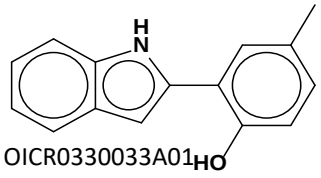


OICR0338621A01
XSOI050073a

2.6 (°C)

XSOI048925c

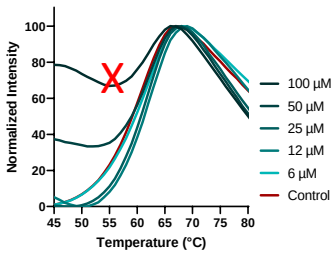


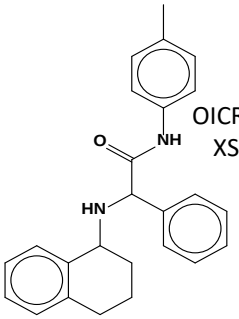


OICR0330033A01
XSOI048925a

2.9 (°C)

XSOI052110e

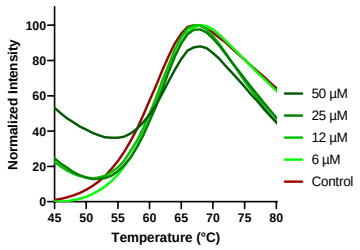


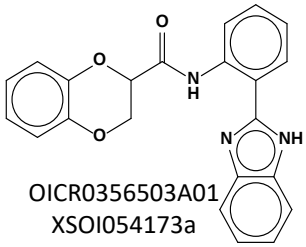


OICR0361454L01
XSOI052110a

3.1 °C

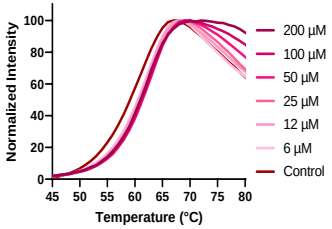
XSOI054173c

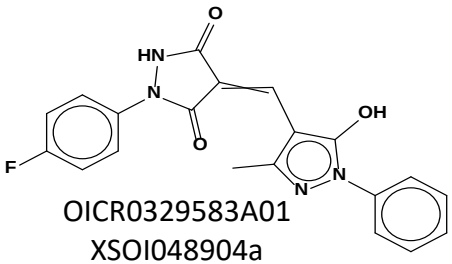




OICR0356503A01
XSOI054173a

XSOI048904d 2.4 (°C)





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2.4 °C

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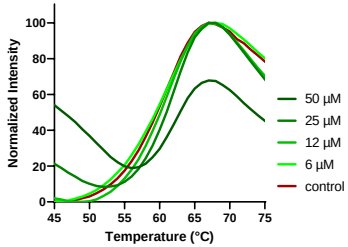

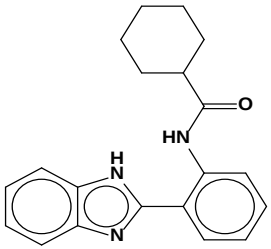

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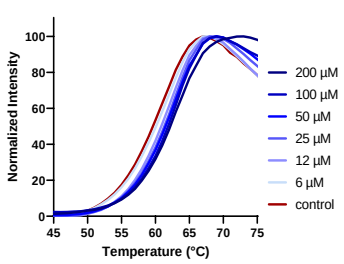
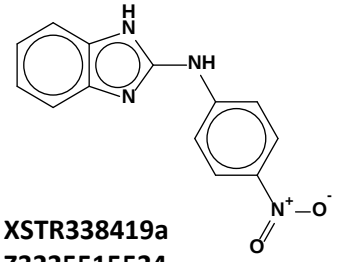
2.7 °C

XSTR338419a





XSTR338419a
Z3225515524

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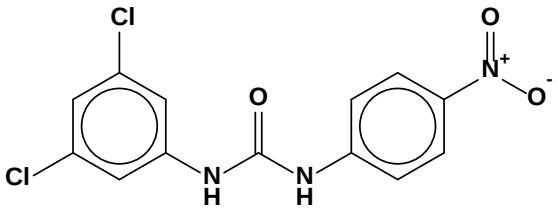
Compounds to consider

Compound Name	Catalog Number	Internal ID	K_D (µM)_SPR	Rmax	Notes
OICR0338621A01	7821679	XSOI050073c	8	11	Precipitation at high Conc.
OICR0356503A01	Z30822756	XSOI054173c	65	43	Confirmed by DSF
OICR0330033A01	6356628	XSOI048925c	95	17	Confirmed by DSF
Z1263529568	Z1263529568	XSOI327455a	145*	61	Analogue of XSOI044959b
Z50131928	Z50131928	XSOI327456a	160*	50	Analogue of XSOI044959b
Z2465619968	Z2465619968	XSOI327457a	> 250*	67	Analogue of XSOI044959b



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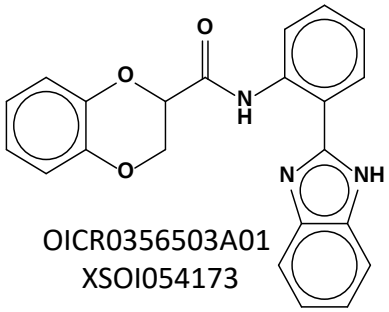

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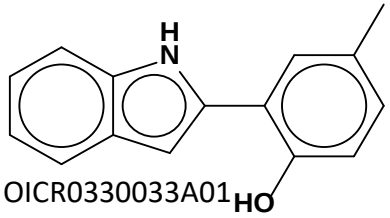

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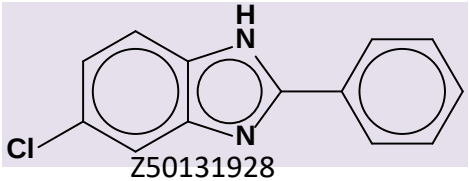

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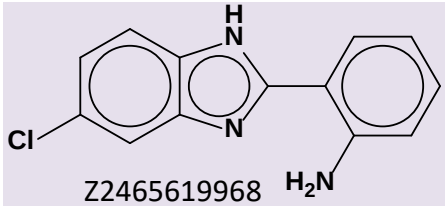

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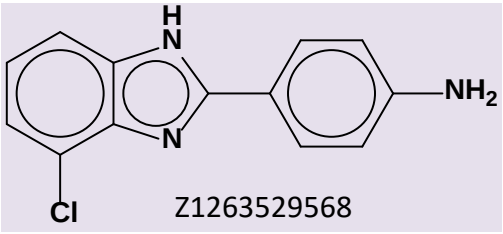

Z1263529568
XSOI327455a

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Acknowledgements

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Masoud Vedadi
Principal Investigator, Molecular Biophysics

Screening: Ling Yan Gao and Albina Bolotokova

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Albina Bolotokova

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Aliakbar Khalili Yazdi (PDF)

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Internal Update

Testing a 20K library against WDR41 by DSF

Albina and Masoud

30/05/ 2020

- ~19840 compounds from the OICR_20K library were screened by DSF at 50 µM in 100 mM Hepes pH 7.5, 150 mM NaCl buffer, 0.1 mg/ml **WDR41:PBC016-F05**

- 52 compounds were found to have a ΔTm of ≥2°C and were cherry picked for confirmation

- 9 compounds from the reconfirmed hit list and 3 analogs of XSOI044959a were ordered and tested by titration. (Note: synthesis of XSOI044959a was not successful, therefore the compound was not received)

- Further, 5 compounds (XSOI054173c, XSOI052110e, XSOI050073c, XSOI048925c and XSOI048904d) were verified by titration for binding.

- Due to difficulty buying XSOI044959a, a list of analogs of XSOI044959a was created (Peter and Santha). 21 compounds were chosen and purchased for testing.

- Out of 21 analogs, 2 compounds (XSTR338417a and XSTR338419a) showed good binding to WDR41.

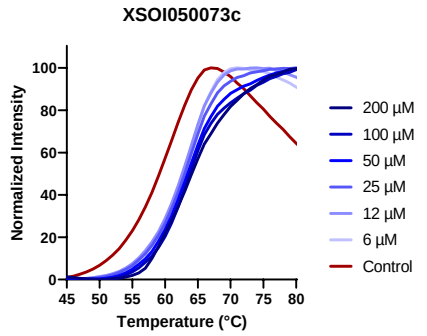
Testing hits from powders

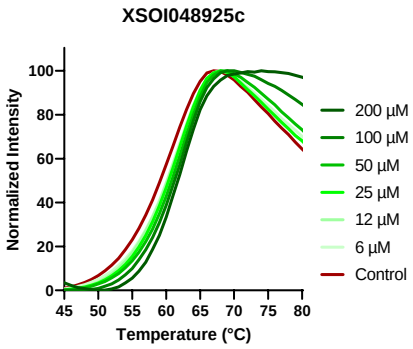
10 compounds from the original hit list and 3 analogs of XSOI044959a were ordered and tested for confirmation by titration.

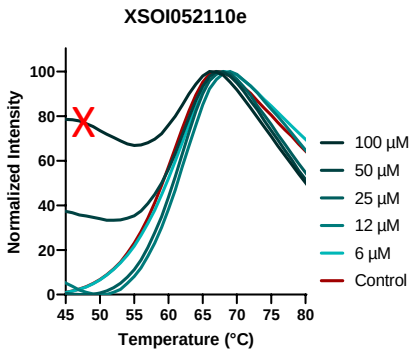
11 compounds from the list (two more are still to receive) were titrated from 6 µM to 200 µM in duplicate

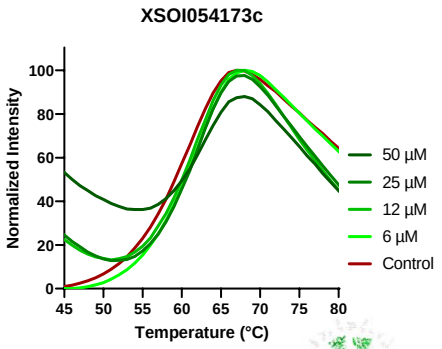
	200 µM	100 µM	50 µM	25 µM	12 µM	6 µM
XSOI327457a	0.3	0.9	1.5	1.3	0.6	0.8
XSOI327456a	-0.1	0.3	0.4	0.2	0.0	0.7
XSOI327455a	1.2	0.7	0.7	0.4	0.1	0.7
XSOI054173c	NA	NA	3.1	2.1	1.7	1.5
XSOI052110e	NA	2.9	2.4	1.6	2.2	0.7
XSOI051337e	NA	1.3	1.9	2.1	3.2	1.3
XSOI050073c	4.8	4.5	4.1	3.8	3.5	3.6
XSOI049568e	0.4	0.5	1.0	1.3	1.3	1.0
XSOI048925c	2.6	2.1	1.6	1.2	1.1	1.0
XSOI048904d	1.9	2.1	2.4	2.2	1.7	1.3
XSOI035428c	-0.1	0.4	0.6	0.9	1.5	1.3

Compound Name	Catalog Number	Internal ID	Comment
Z2465619968	Z2465619968	XSOI327457a	Analogue of XSOI044959b
Z50131928	Z50131928	XSOI327456a	Analogue of XSOI044959b
Z1263529568	Z1263529568	XSOI327455a	Analogue of XSOI044959b
OICR0356503A01	Z30822756	XSOI054173c	
OICR0361454L01	Z44519772	XSOI052110e	
OICR0415607A01	Z243534060	XSOI051337e	
OICR0338621A01	7821679	XSOI050073c	
OICR0332395A01	6883111	XSOI049568e	
OICR0330033A01	6356628	XSOI048925c	
OICR0329583A01	6211414	XSOI048904d	
OICR0388449A01	Z48867750	XSOI035428c	
OICR0305340A01	Z3220179707	XSOI044959b	to be tested
OICR0305364A01	EN300-235704	XSOI044938b	to be tested









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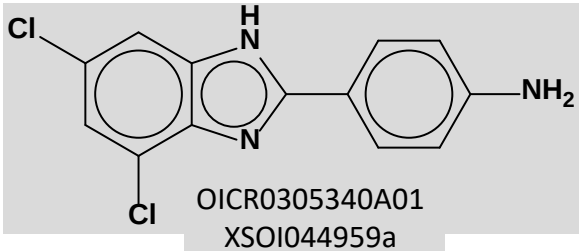
Testing an expansion list

- 21 compounds were selected as an expansion to the original confirmed hit (XSOI044959a) and tested in duplicate from 6 µM to 200 µM in 100 mM Hepes pH 7.5, 150 mM NaCl buffer, 0.1 mg/ml **WDR41:PBC016-F05**

Catalog Number	Internal ID	200 µM	100 µM	50 µM	25 µM	12 µM	6 µM
EN300-235704	XSOI044938d (original hit)	-0.3	NA	1.4	1.4	1.8	1.1
Z3225841132	XSTR338420a	NA	0.8	0.1	-0.3	0.5	0.5
Z3225515524	XSTR338419a	2.7	2.2	1.9	1.7	1.2	0.5
Z220533898	FMTR000532b	-0.2	-0.3	-0.2	-0.1	0.8	0.5
Z30822308	XSTR338417a	NA	NA	2.4	1.6	0.6	0.0
Z57044173	XSTR338416a	1.2	1.0	0.7	0.6	0.5	0.0
Z28141409	XSTR338415a	0.6	0.6	0.8	0.5	1.0	0.8
Z30181385	XSTR338414a	0.4	0.0	0.1	-0.1	0.7	0.8
Z30822225	XSTR338413a	0.0	0.2	0.4	0.3	1.1	1.2
Z44592077	XSTR338412a	1.6	1.4	2.2	0.6	2.1	2.2
Z68085277	XSTR338411a	0.0	0.1	0.3	0.3	0.6	0.9
Z44583833	XSTR338410a	0.4	0.5	0.4	0.4	0.3	0.0
Z234893453	XSTR338409a	0.3	0.0	0.0	-0.1	-0.2	0.0
Z235344383	XSTR338408a	0.6	0.7	0.5	0.2	-0.2	-0.3
Z1262324631	XSTR338407a	-0.2	0.0	0.2	0.5	0.6	1.1
5109730	XSTR338406a	-2.1	-1.3	-0.8	-0.3	0.1	0.9
7953084	XSTR338405a	1.7	1.8	1.3	0.7	0.3	0.0
Z56756575	XSTR210323c	0.0	0.4	0.4	0.5	0.6	1.1
7682431	XSTR047713c	-0.4	-0.2	0.1	0.6	0.8	1.1
7909359	XSTR033363b	1.3	1.4	1.2	0.7	0.3	0.0
Z57115067	XSTR016487j	-0.2	-0.2	-0.1	0.1	0.1	0.8
7700857	XSTR003232d	0.4	0.4	0.5	0.9	1.2	1.4



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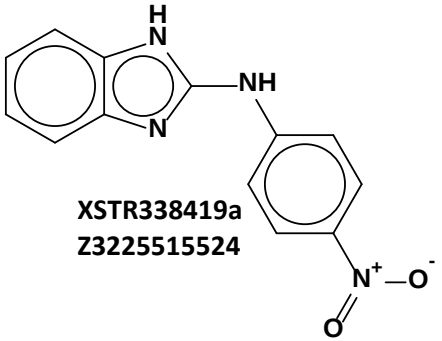
***structures of inactive compounds are on the next slide**



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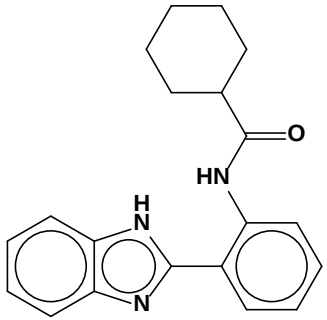

XSTR338417a
Z30822308

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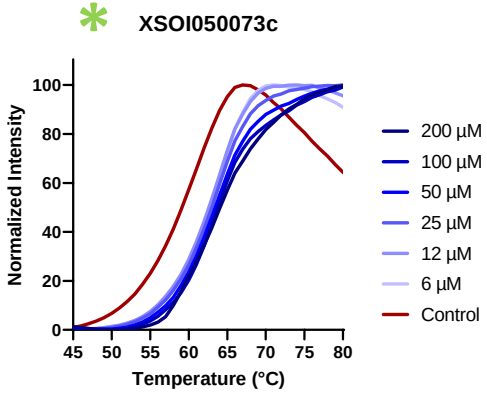
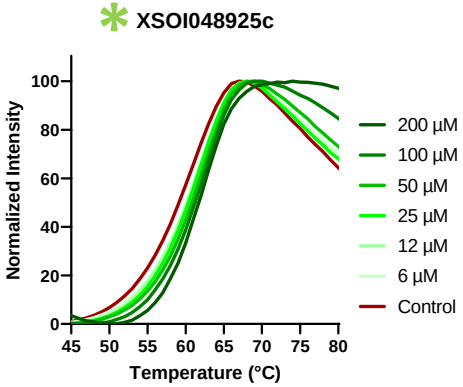
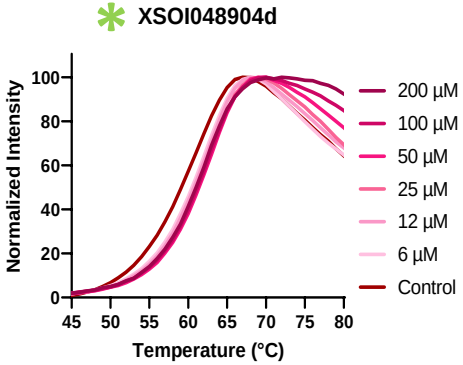
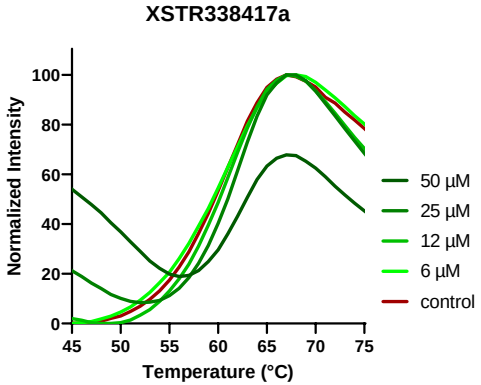
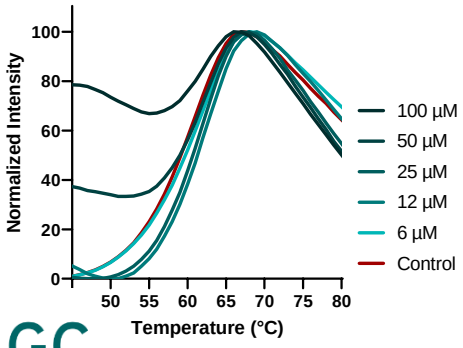
Compound	Concentration (µM)					
	200	100	50	25	12	6
XSOI054173c	NA	NA	3.1	2.1	1.7	1.5
XSOI052110e	NA	2.9	2.4	1.6	2.2	0.7
XSOI050073c	4.8	4.5	4.1	3.8	3.5	3.6
XSOI048925c	2.6	2.1	1.6	1.2	1.1	1
XSOI048904d	1.9	2.1	2.4	2.2	1.7	1.3
XSTR338419a	2.7	2.2	1.9	1.7	1.2	0.5
XSTR338417a	NA	NA	2.4	1.6	0.6	0

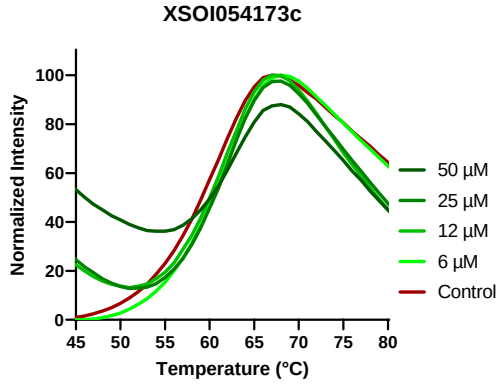


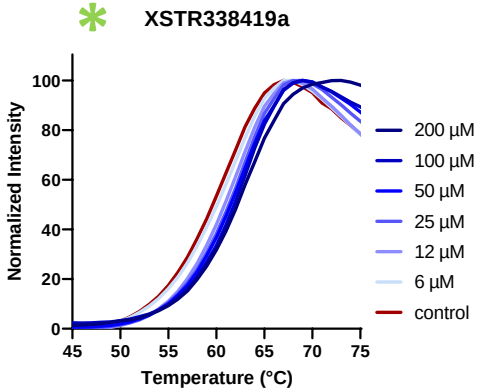












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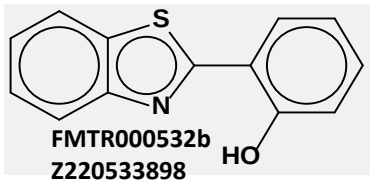
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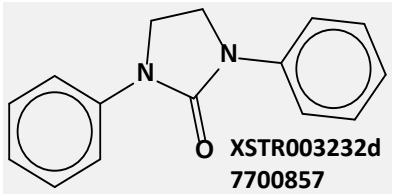
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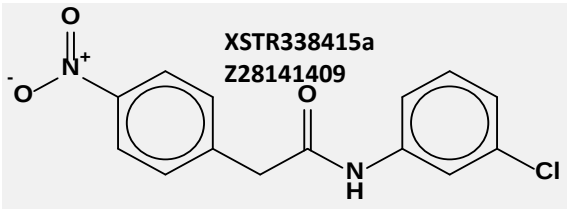
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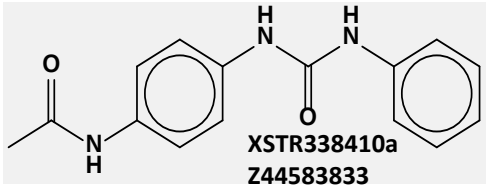
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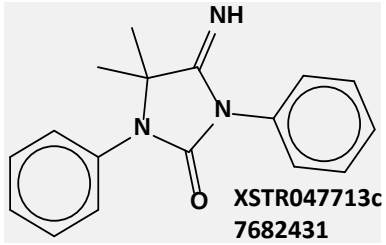
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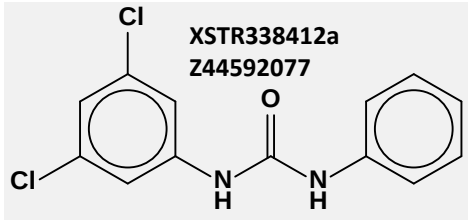
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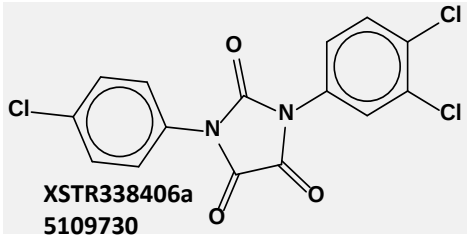
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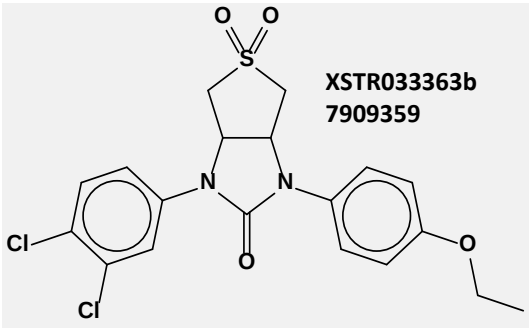
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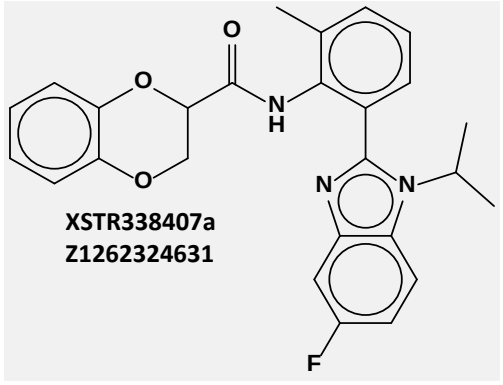
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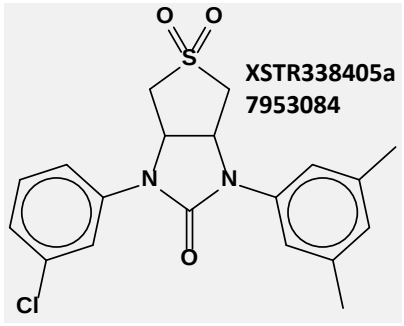
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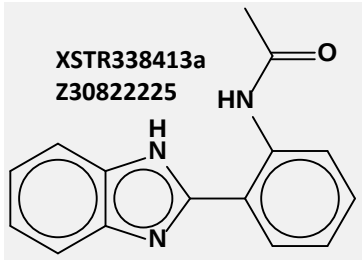
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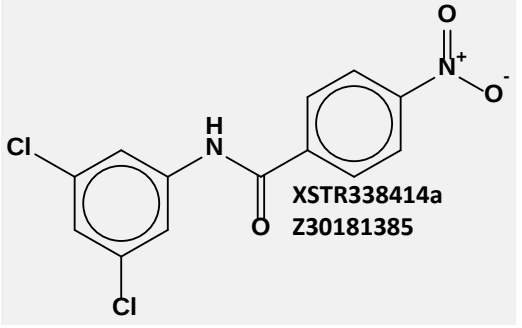
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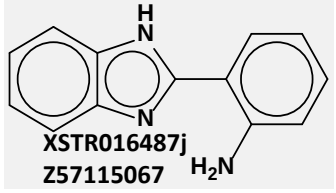
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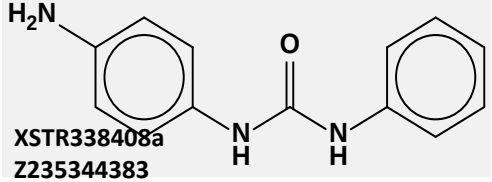
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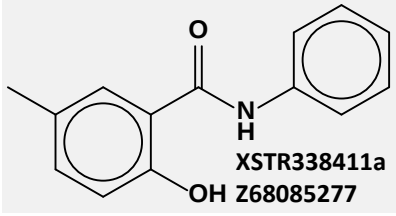
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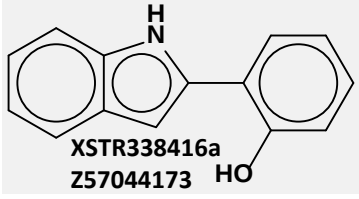
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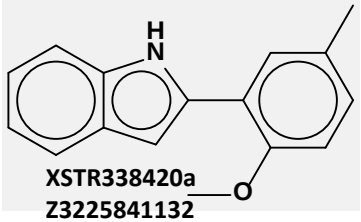
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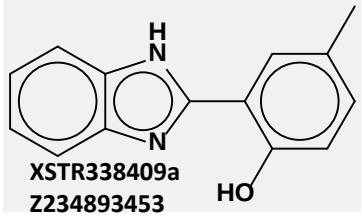
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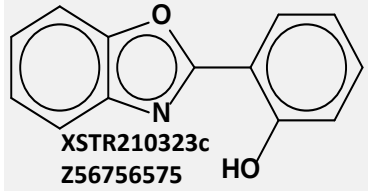
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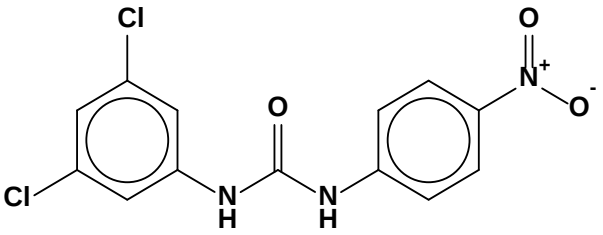

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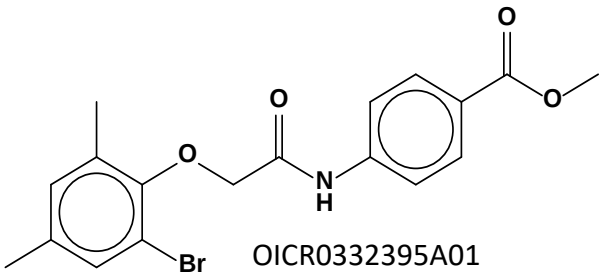

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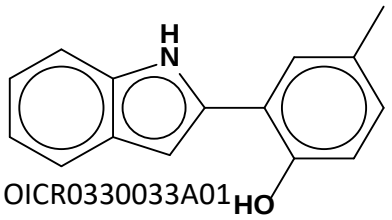

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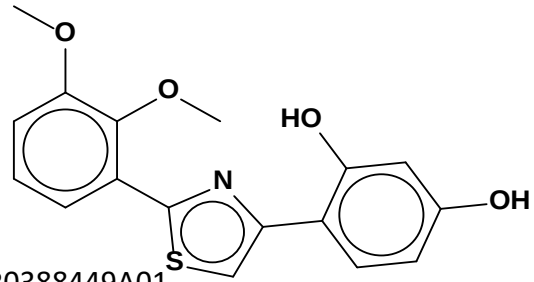

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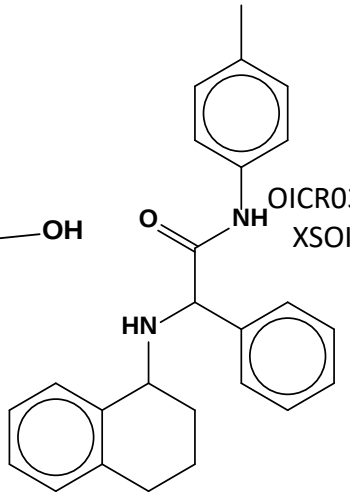

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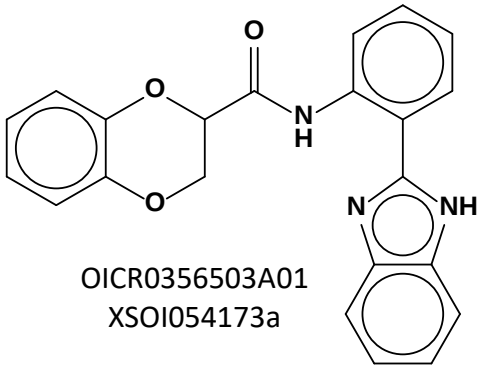

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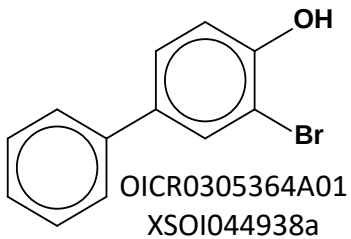

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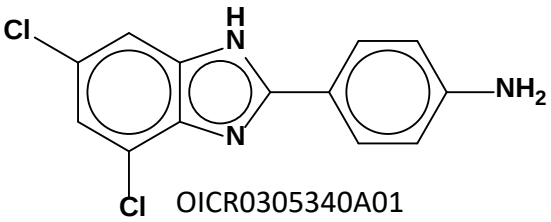

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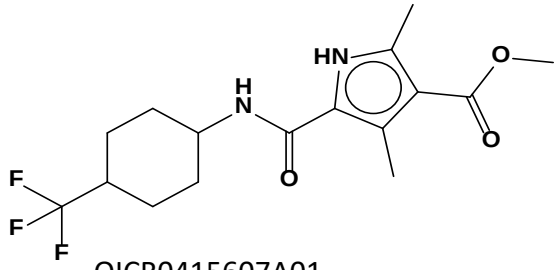

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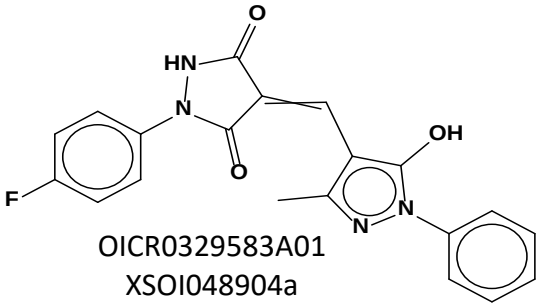

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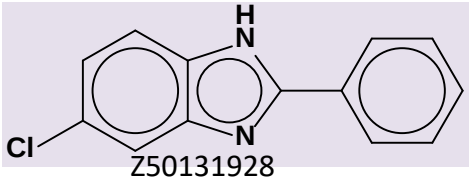

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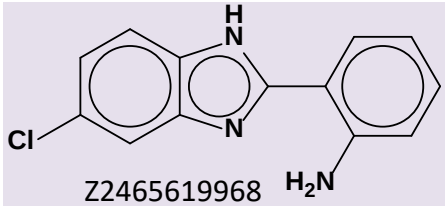

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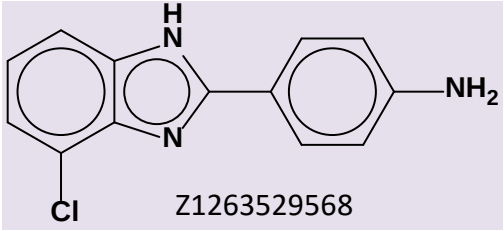

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K_D Determination for 11 WDR41 Compounds Using SPR

Aliakbar Khalili Yazdi, Ling Yan Gao and Albina Bolotokova

Molecular Biophysics, SGC Toronto.

20th January 2020

Analysis of the Interaction of 11 Compounds with WDR41 Using SPR

Compound Name	Catalog Number	Internal ID	K_D (µM)_SPR	Rmax	Notes
OICR0338621A01	7821679	XSOI050073c	8	11	Precipitation at high Conc.
OICR0388449A01	Z48867750	XSOI035428c	29	16	Not re-confirmed by DSF
OICR0356503A01	Z30822756	XSOI054173c	65	43	Confirmed by DSF
OICR0330033A01	6356628	XSOI048925c	95	17	Confirmed by DSF
Z1263529568	Z1263529568	XSOI327455a	145*	61	Analogue of XSOI044959b
Z50131928	Z50131928	XSOI327456a	160*	50	Analogue of XSOI044959b
Z2465619968	Z2465619968	XSOI327457a	> 250*	67	Analogue of XSOI044959b
OICR0329583A01	6211414	XSOI048904d	PNSB	>1000	High Rmax
OICR0361454L01	Z44519772	XSOI052110e	PNSB	>1000	High Rmax
OICR0332395A01	6883111	XSOI049568e	NB	2	Low Rmax
OICR0415607A01	Z243534060	XSOI051337e	NB	2	Low Rmax

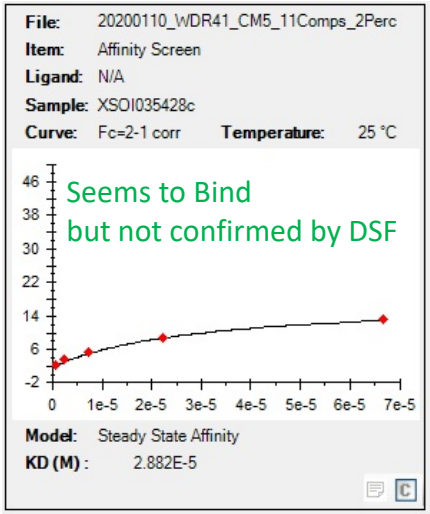
Expected Rmax for small compounds is between 45-60; **NB:** No Binding; **PSNB** = Possible Non-specific binding; *Not expected to be easily detected by DSF.

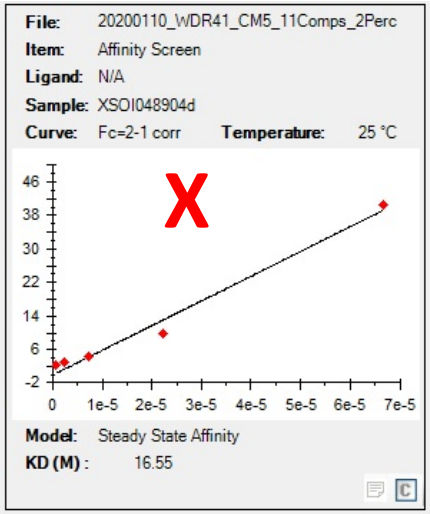
- 8 WDR41 hits and 3 analogues of compound 59 were received and tested by SPR.
- WDR41 was coupled to the CM5 chip by amine coupling.
- 2 compounds (XSOI048904d and XSOI052110e) showed signs of possible non-specific binding (PNSB).
- For 2 compounds (showed with NB), the observed Rmax was much lower than the expected Rmax.
- The rest of the compounds showed measurable K_D values.
- Compound XSOI050073c showed visible sign of precipitation in SPR buffer (specially the 2 highest concentrations).

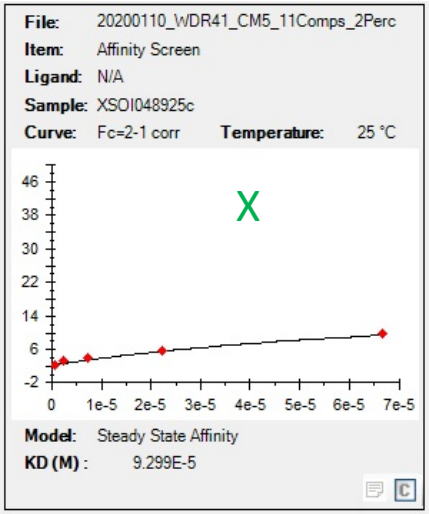
SPR Conditions:

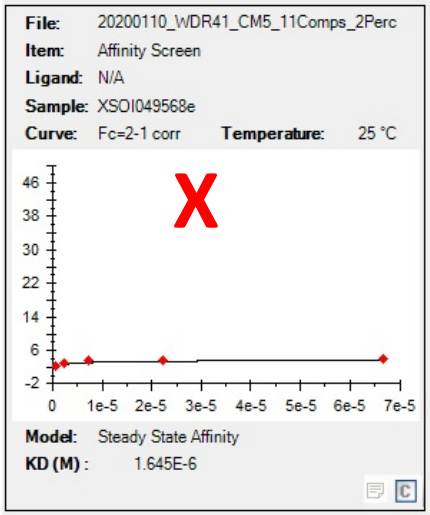
- HBS-EP Buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 2 mM EDTA, 0.005% Tween 20, and 2% DMSO) was used for SPR, using a Biacore T200 instrument.
- His-tagged-WDR41, was coupled on a CM5 SPR Sensor chip by amine coupling (GE healthcare). Compounds were injected into the sensitized chip at 5 concentrations (0.82, 2.47, 7.40, 22.22, and 66.66 µM) plus 2% DMSO at 40 µL/min. Contact time was 60 (S), and disassociation time was 60 (S). Buffer only (plus 2 % DMSO) was used for blank injections; and buffers containing 1 to 3 % DMSO were used for solvent corrections.

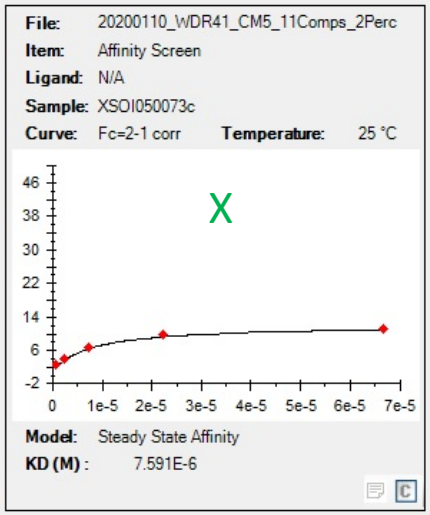
K_D determination by SPR for 11 Compounds

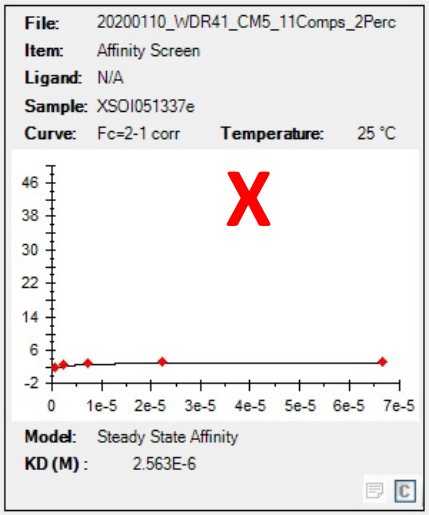


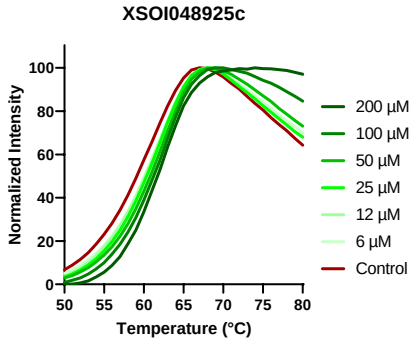


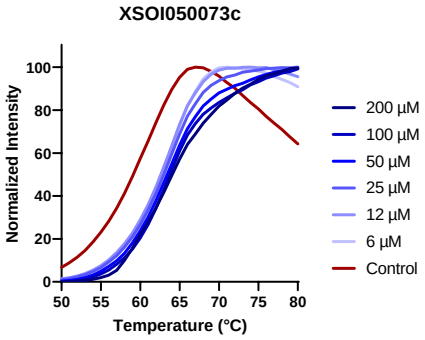












- **<u>Expected Rmax for small compounds is between 45-60</u>.**
- For easier comparison between samples, the Y-axis of plots are set to the same value.

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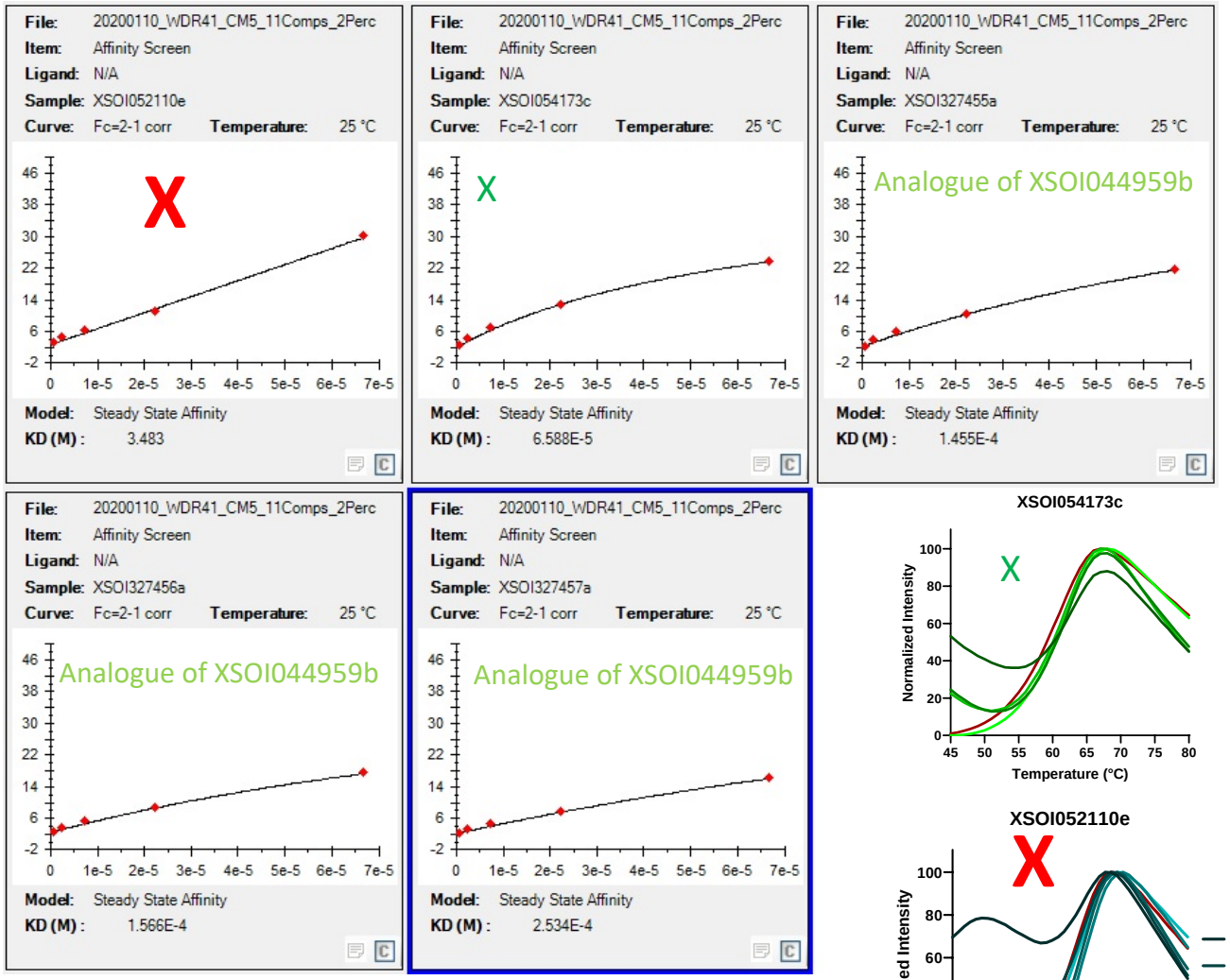
K_D determination by SPR for 11 Compounds



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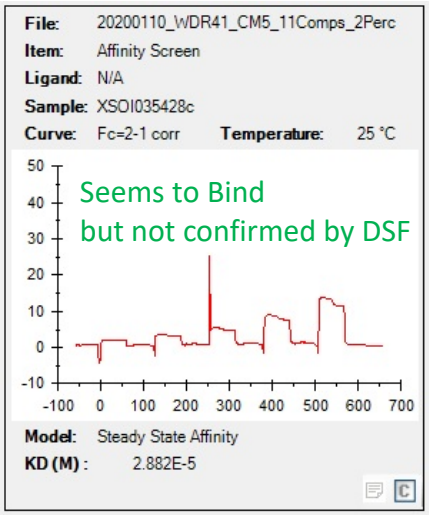

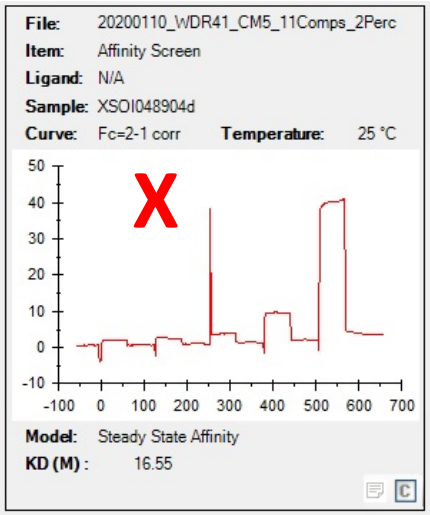

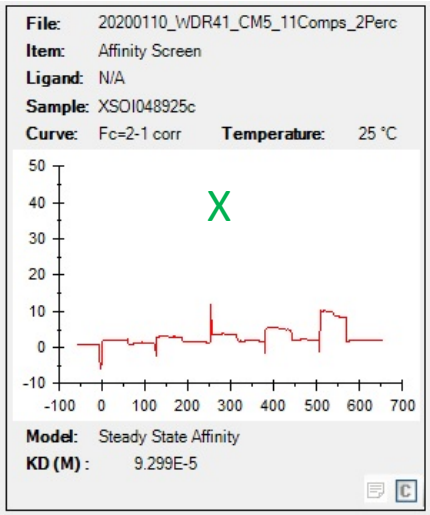

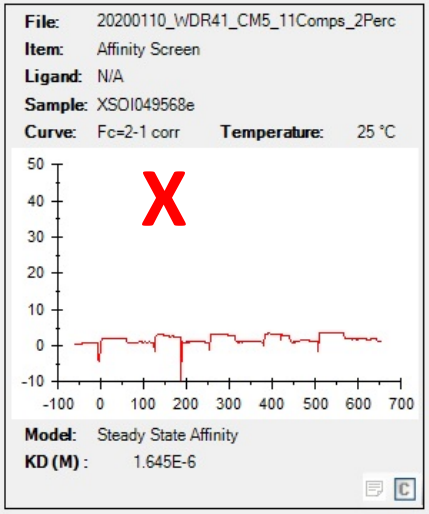

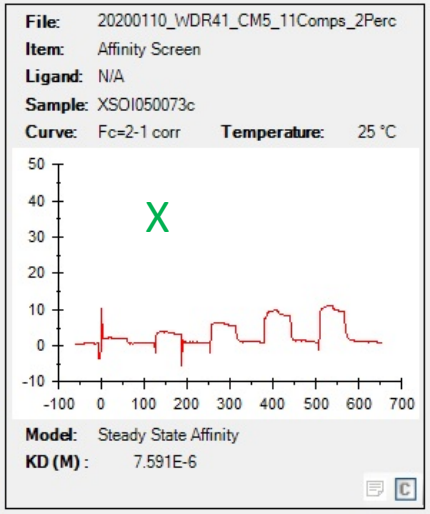

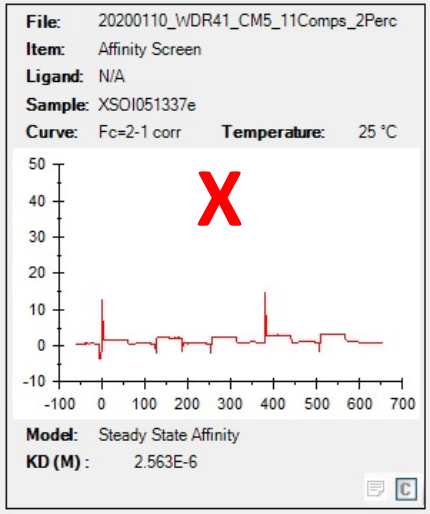

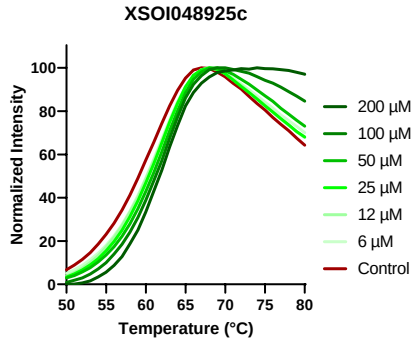

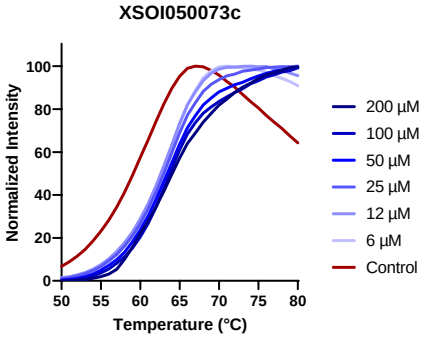

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K_D determination by SPR for 11 Compounds

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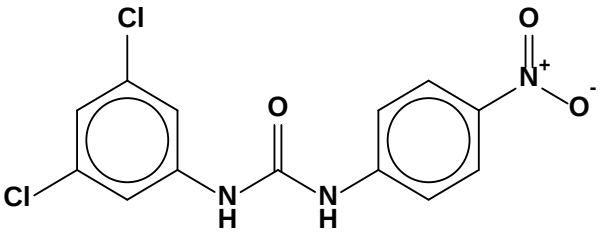

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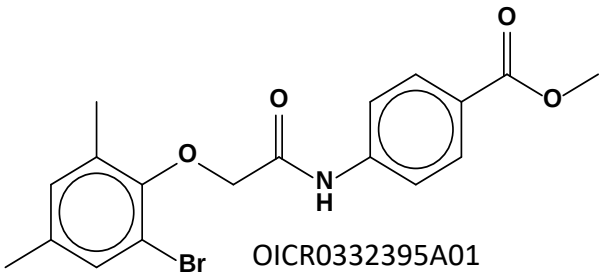

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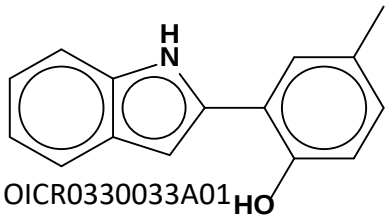

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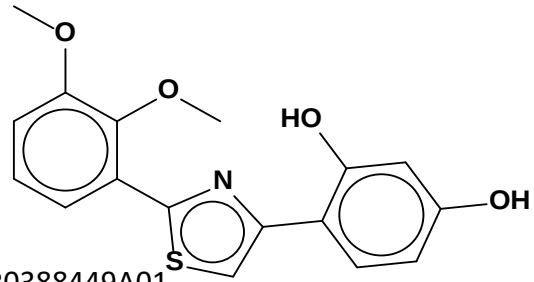

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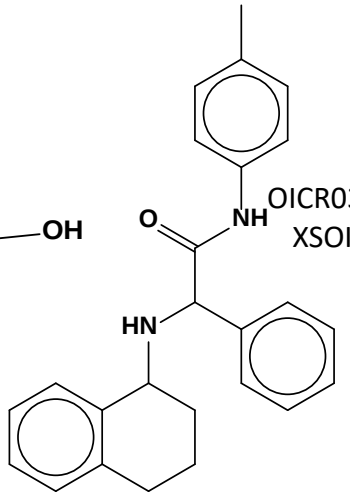

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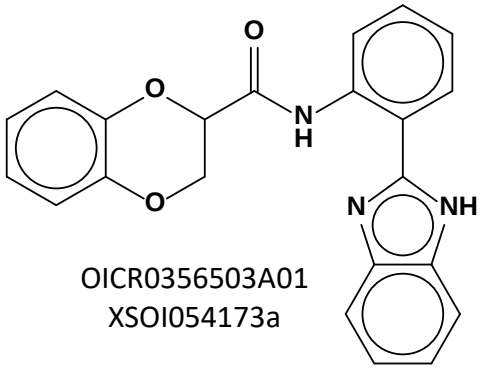

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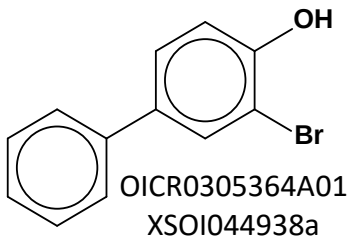

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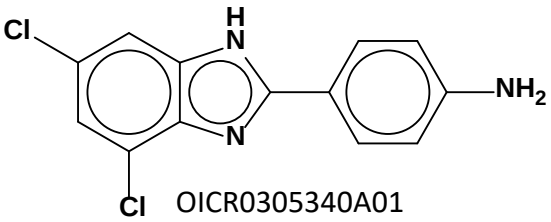

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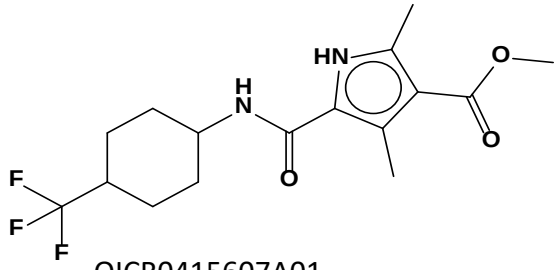

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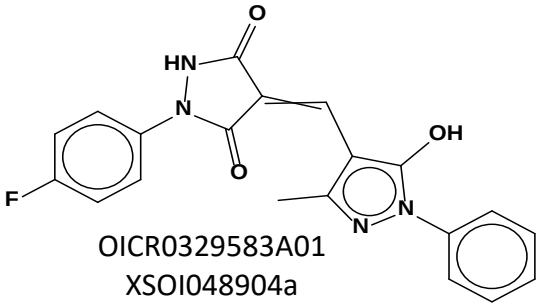

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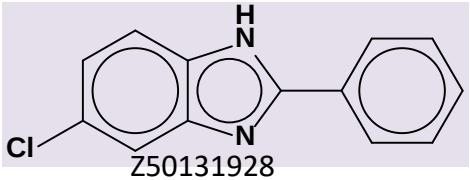

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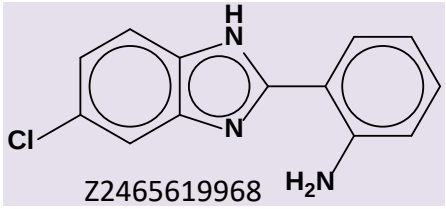

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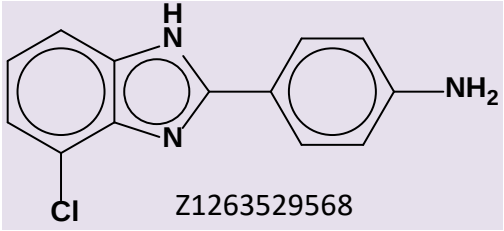

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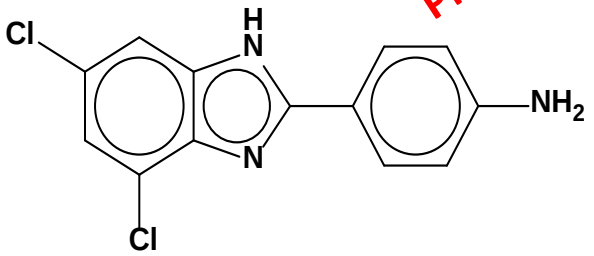
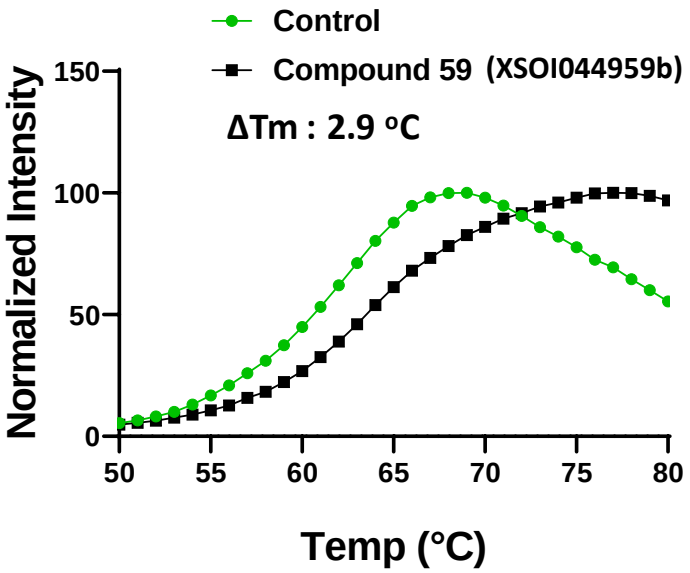
WDR41 is amenable to screening by DSF

- WDR41 is amenable to screening by differential scanning fluorimetry (DSF).
- WDR41 was screened against a library of 20K diverse compounds at 50 µM:
  - 52 compounds were identified as hits with ΔT_m of ≥2°C. The compounds were cherry picked from the stock solutions for confirmation.
  - Compounds were titrated from 3 µM to 100 µM.
  - The data for 10 compounds were reproducible from the stock solutions.
- Hits have been ordered and compounds will be tested from solid by DSF and orthogonal methods.

Previously Reported





We haven't received XSOI044959 yet.

Acknowledgements

Molecular Biophysics
SGC Toronto

Masoud Vedadi
Principal Investigator, Molecular Biophysics

Compound Management and DSF by:
Albina Bolotokova

SPR:
Aliakbar Khalili Yazdi (PDF)

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Screening WDR41 against a library of 20K diverse compounds by DSF

Masoud

20/ 12/ 2019

WDR41 is amenable to screening by DSF

- WDR41 is amenable to screening by differential scanning fluorimetry (DSF).

- WDR41 was screened against a library of 20K diverse compounds at 50 µM:
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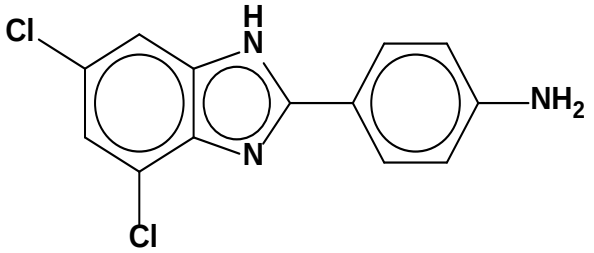

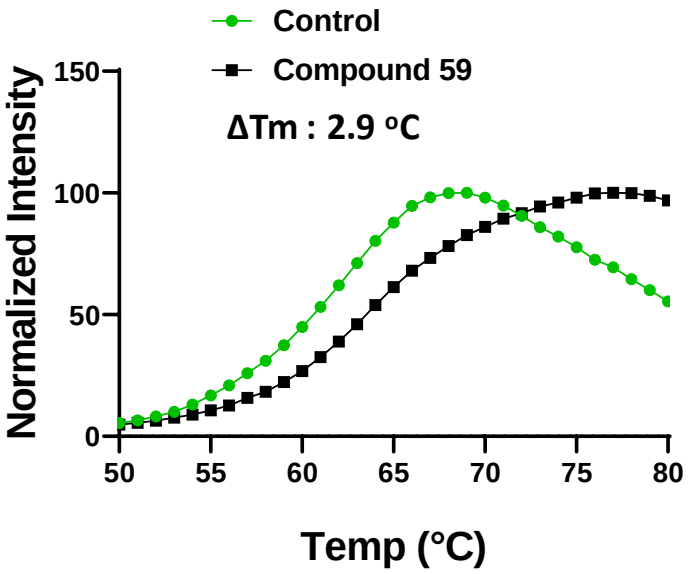

Acknowledgements

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Screened by:
Ling Yan Cao (Pharmacology Student)
Albina Bolotokova

Compound Management:
Albina Bolotokova

Protein was purified by:
Levon Halabelian

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