G9a/GLP selective methyltransferase chemical probe, UNC0638

Release date: June 1, 2010

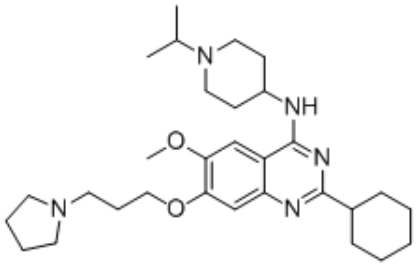
<table>
<tr><th>UNC0638</th><th colspan="4">Target Activity</th></tr>
<tr><td rowspan="2"> 
2-cyclohexyl-N-(1-isopropyl piperidin-4-yl)-6-methoxy-7-(3-(pyrrolidin-1-yl)propoxy) quinazolin-4-amine</td><td>Target</td><td>K_D / nM (ITC)</td><td>IC_{50} / nM (Activity)</td><td>Tm shift / °C[1]</td></tr>
<tr><td>G9a (EHMT2)
GLP (EHMT1)
SETD7
SETD8
PRMT3
SUV39H2
DOT1L
PRDM1
PRDM10
PRDM12
SMYD3
JMJD2E
HTATIP
DNMT1
MLL
EZH2
PRMT1
SUV39H1
SUV39H2
G9a</td><td></td><td><15 (Hill Slope 1.3)
19±1 (Hill Slope 0.8)
>10,000
>10,000
>10,000
>10,000
nt
nt
nt
nt
nt
4660 (Alphascreen)
nt
1287**
>10,000**
>10,000**
>10,000**
>10,000**
>10,000**
91**</td><td>4
8
nt
nd
nd
nt
nd
nd
nd
nd
nd
nt
nd
nt
nt
nt
nt
nt
nt
nt</td></tr>
<tr><td colspan="4">nt=not tested, nd=not detected, [1] singlicate @ 100 μM
**Tested at BPS Bioscience using different assay format</td></tr>
<tr><td>Selectivity
within target family</td><td colspan="4">See above, additional screening ongoing.</td></tr>
<tr><td>Selectivity
beyond target family</td><td colspan="4">• Ricerca Hit Profiling Screen of 29 targets, measuring inhibition of radioligand binding at 1 μM.
• <30% Inhibition for 26 receptors.
• >30% Inhibition for muscarinic M_2 (64%), adrenergic $alpha_{1A}$ (90%) and $alpha_{1B}$ (69%) receptors.</td></tr>
<tr><td rowspan="2">Physicochemical properties</td><td colspan="2">MW = 509.7</td><td colspan="2">cLogP 5.78</td></tr>
<tr><td colspan="4">Soluble in DMSO at least up to 10mM.</td></tr>
<tr><td>Storage</td><td colspan="4">Stable as solid in the dark at -20°C.</td></tr>
<tr><td>Cellular activity</td><td colspan="4">• Significant reduction in H3K9 dimethylation at 100nM in MDA-MB231 cells as measured by fluorescence immunostaining (Fig. 1 below).
• Good separation of functional and toxic effects (Fig. 2 below)</td></tr>
<tr><td>Co-crystal structures</td><td colspan="4">Structure solved PDB 3RJW</td></tr>
<tr><td>Primary reference</td><td colspan="4">Vedadi et al, Nat Chem Biol. 7:566-74 (2011).</td></tr>
<tr><td>Material availability</td><td colspan="4">Available through Sigma-Aldrich (Item U4885).</td></tr>
<tr><td>Notes</td><td colspan="4">An inactive structurally-related compound will be available shortly for use as a negative control.</td></tr>
<tr><td>Further information</td><td colspan="4">See probe website: http://www.thesgc.org/chemical_probes/UNC0638</td></tr>
<tr><td>Funding</td><td colspan="4">The work at SGC is supported by Ontario Research Fund Grant RE-03-003, and the work at The University of North Carolina CICBDD is supported by NIH grant Number RC1GM090732.</td></tr>
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Fig 1 (a) H3K9m2 immunostaining in MDA-MB231 cells with G9a and EHMT1 knockdown and cells treated with UNC638 and BIX-01294 inhibitors. BIX-01294 was identified as EHMT1 and 2 inhibitor, effective in mES cells at about 1uM (Kubicek et al, 2007 Molecular Cell 25, 473-481) (b) UNC0638 and BIX-01294 dose-response on H3K9 dimethylation levels in MDA-MB231 cells at 48h.

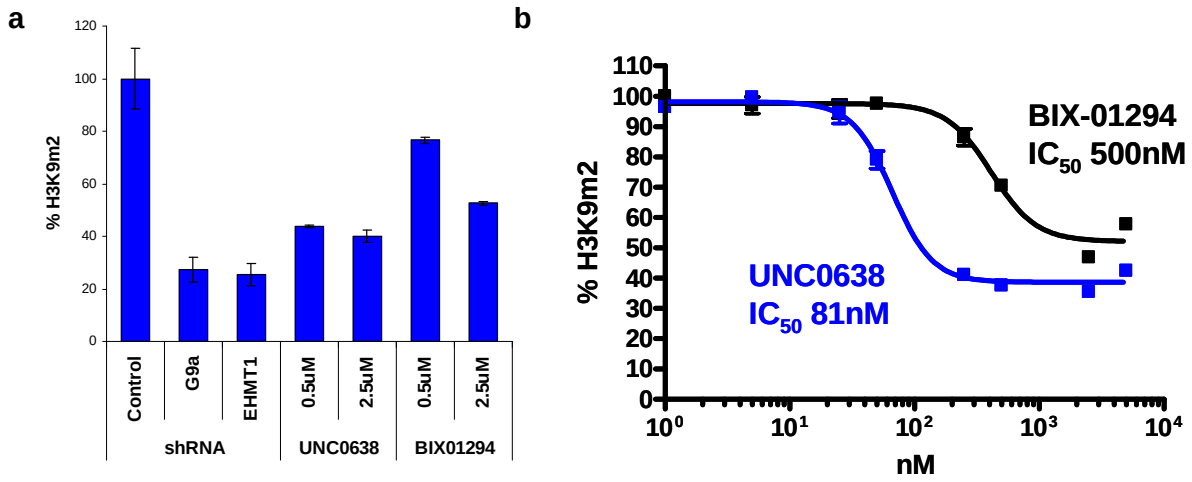
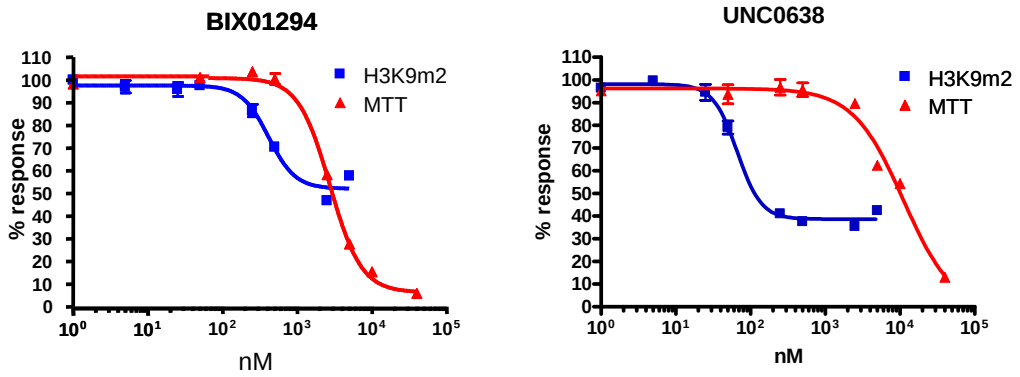


Fig 2 Effect of UNC0638 and BIX-01294 on H3K9 dimethylation levels and cell toxicity measured by MTT assay at 48h in MDA-MB231 cells.



Poor separation of functional and toxic effects

Good separation of functional and toxic effects

	In Vitro G9a IC_{50}(nM)	H3K9Me2 48h IC_{50}(nM)	Cell Tox 48h EC_{50}(nM)	Tox/Func Ratio
BIX-01294	133 ± 15	500 ± 43	2805	5.6
UNC0638	<15	81 ± 9	11190	138