Aryl pyrazoles as potent inhibitors of protein arginine methyltransferases: identification of the first PRMT6 tool compound

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Background

PRMT6 is a member of the protein arginine methyltransferase (RMT) family which comprises 45 enzymes, 9 of which are known to catalyze protein arginine N-methylation reactions1. These post-translational modifications regulate RNA processing, transcription, signal transduction and other cellular processes3. A nuclear-localized RMT, PRMT6 creates omega-(G)-monomethylarginine and asymmetric omega-(G),G-(G)-dimethylarginine derivatives on histone and other protein substrates containing a GAR motif4. It is the only RMT known to mediate the H3R2 mark5. This mark can act in opposition to the activating H3K4me3 mark, effectively behaving as a transcriptional repressor6.

PRMT6 has been reported to play a role in a variety of cellular processes including maintenance of stem cell pluripotency7, regulation of cell cycle8, DNA repair8, regulation of cell death9, and other processes10. Overexpression of PRMT6 has been reported in several cancer types including melanoma11 and bladder, lung12, and prostate13 carcinoma, suggesting that PRMT6 inhibition may have therapeutic utility. Until now, no small molecule PRMT6 inhibitor has been available for use as tool compound for in vitro or in vivo target validation studies.

Results

Aryl pyrazole EPZ020411 is a potent inhibitor of PRMT6

<table>
<thead>
<tr>
<th>Pyrazole Compounds</th>
<th>IC50 PRMT6 (µM)</th>
<th>IC50 PRMT8 (µM)</th>
<th>IC50 PRMT1 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.051</td>
<td>0.867</td>
<td>0.558</td>
</tr>
<tr>
<td>2</td>
<td>0.069</td>
<td>1.5</td>
<td>0.487</td>
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<tr>
<td>3</td>
<td>0.091</td>
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<td>0.106</td>
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<tr>
<td>4</td>
<td>0.217</td>
<td>0.240</td>
<td>0.050</td>
</tr>
<tr>
<td>5</td>
<td>0.065</td>
<td>0.040</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Analoging efforts showed that PRMT6 activity could be decreased with di-methyl substitution of the aryl ring as seen with compound 7.

Crystal structure of EPZ020411 bound to PRMT6

PRMT6 activity is inhibited by EPZ020411 (IC50 = 0.010 µM) in a biochemical assay measuring transfer of the benzyl acetal group from 6-hydroxy-2-methylpyridine for 45 min. EPZ020411 inhibits PRMT6 and PRMT8 activity with IC50 = 0.010 and 0.025 µM respectively.

EPZ020411 has 10-fold selectivity for PRMT6 over PRMT1 and PRMT8

EPZ020411 inhibits methylation of PRMT6 substrates in cells

Overexpression of PRMT6 induces methylation of the canonical PRMT6 substrate H3R2.

Crystal structures of complexes of PRMT6 and SAH with 1 (2.4Å) and EPZ020411 (2.1Å) were obtained (PDB IDs 4VY4 and 4VY5). The diamine side-chain occupies the putative site of the substrate arginine side-chain. The majority of the interactions between the two ligands and the protein occur via the diamine side-chain and the pyrazole.

Conclusions

The arylypyrazole EPZ020411 is a potent and selective small molecule inhibitor of PRMT6 with a biochemical IC50 of 10nM

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References

1. Keene JD, Spithill TW, de la Peña Á, Cote L, Reisinger S, Fatouros A, Chappell JFL, Chambon P, Altieri D. Crystal structures of complexes of PRMT6 and SAH with 1 (2.4Å) and EPZ020411 (2.1Å) were obtained (PDB IDs 4VY4 and 4VY5). The diamine side-chain occupies the putative site of the substrate arginine side-chain. The majority of the interactions between the two ligands and the protein occur via the diamine side-chain and the pyrazole.

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