Novel 3-methylindoline inhibitors of EZH2: Design, synthesis and SAR

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Abstract

EZH2 (enhancer of zeste homologue 2) is the catalytic subunit of the polycomb repressive complex 2 (PRC2) that catalyzes the methylation of lysine 27 of histone H3 (H3K27). Dysregulation of EZH2 activity is associated with several human cancers and therefore EZH2 inhibition has emerged as a promising therapeutic target. Several small molecule EZH2 inhibitors with different chemotypes have been reported in the literature, many of which use a bicyclic heteroaryl core. Herein, we report the design and synthesis of EZH2 inhibitors containing an indoline core. Partial saturation of an indole to an indoline provided lead compounds with nanomolar activity against EZH2, while also improving solubility and oxidative metabolic stability.

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Covalent modifications of histones play pivotal roles in the organization of the chromatin structure and the regulation of gene expression. A large number of enzymes are known to catalyze the addition or removal of covalent groups from specific amino acids in histone proteins including histone methyltransferases (HMTs). Enhancer of Zeste Homologue 2 (EZH2) is the catalytic subunit of Polycomb repressive complex 2 (PRC2), which catalyzes the methylation of lysine 27 of histone 3 (H3K27). Tri-methylation of the lysine-27 of histone 3 (H3K27) is associated with the silencing of specific genes, including many tumor suppressor genes. EZH2 has been found to be significantly overexpressed in a variety of human cancers and its expression level correlates with cancer progression and poor prognosis. In addition, point mutations of residues, such as Y641, A677 and A687, within the catalytic domain of EZH2 have been found in follicular lymphomas and diffuse large cell B lymphomas (DLBCL). Several studies have revealed that EZH2 knockdown in tumor cell lines can cause decreased cell proliferation, migration, angiogenesis and increased apoptosis. Collectively, this evidence supports EZH2 as an important target for cancer therapy and its inhibitors to be potential anticancer agents. Therefore significant efforts have been made on the discovery and optimization of small molecule EZH2 inhibitors. Through these efforts various small molecule EZH2 inhibitors have been reported with demonstrated efficacy both in vitro and in vivo.

An analysis of EZH2 inhibitors reported in the literature led to the recognition that a substituted pyridone-methyl amide fragment constituted a highly optimized moiety for binding to EZH2. Examples of published molecules which have the pyridone group are shown in Fig. 1. Of these, EPZ6438 (7) and GSK126 (2) are currently being evaluated in human clinical trials. We noted that EPZ6438 and EPZ11989 are exceptions in the pyridone containing EZH2 inhibitors; the pyridone-methyl amide is attached to a phenyl core in place of a bicyclic heteroaryl core. It has been demonstrated that increased fraction of sp3 carbons (Fsp3 = number of sp3 hybridized carbons/total carbon count) correlates with improved solubility, an experimental physical property important to success in drug discovery. These observations prompted us to use a bicyclic non-planar indoline core for making novel EZH2 inhibitors, where the core consists of a phenyl residue fused with a saturated 5-membered ring. Our design is drawn in Fig. 2, where we show that the indoline can be derived from the EPZ6438 (7) core by C7-C8 cyclization or by saturation of indole core at C-2 and C-3 present in Ei1 (1) and GSK126 (2). We envisaged that the new molecules with a non-planar indoline core might have improved solubility and pharmacokinetic properties due to higher degree of saturation and non-planarity of the core.

We started our lead generation effort by preparing compounds incorporating an indoline core (Scheme 1). Key

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intermediates \(12a\) and \(12c\) were synthesized by a reductive amination of various ketones with \(11\), which was obtained by reduction of commercially available indole \(10\). Intermediates \(12a\) and \(12b\) were then converted to \(15a\) and \(15b\) using trimethylaluminium as the coupling agent. Cyanation of \(15a\), \(15b\) and \(15c\) afforded \(16\), \(17\) and \(18\) respectively. Alternatively, intermediate \(12c\) was transformed to \(19\) via Suzuki coupling followed by hydrolysis and amide bond formation.

Compounds were tested in vitro for their ability to inhibit the enzymatic activity of EZH2, mutant EZH2 (Y641F) and the homolog EZH1. The enzymatic activity was determined using the indicated HMT complexes incubated with \(^3\)H-SAM (tritiated S-adenosyl L-methionine) and histone H3 and IC\(_{50}\) curves were determined for each compound (see Supplemental information). The IC\(_{50}\) results for inhibition of EZH2 are shown in Table 1.

Based on reported compounds and published SAR,\(^\text{13}\) we maintained the 4,6-dimethylpyridone methyl amide as a constant. Our first analogs scoped out the effect of substitution at the 1- and 6-positions of the indoline. The isopropyl substituted \(16\) was inactive (EZH2 IC\(_{50}\) = 26.6 nM) while the cyclopentyl substituted \(17\) had a 47-fold improvement in potency (EZH2 IC\(_{50}\) = 571 nM).

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R^1)</th>
<th>(R^2)</th>
<th>EZH2 IC(_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td></td>
<td>-C=C=N</td>
<td>26,600</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>-C=C=N</td>
<td>571</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>-C=C=N</td>
<td>15,700</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td>237</td>
</tr>
</tbody>
</table>

\(^*\) Experimental details are in the Supplemental information.
However, a tetrahydropropyran (THP) substitution led to a drastic loss of potency (18 EZH2 IC\textsubscript{50} = 15.7 \mu M). We also made compound 19 with a large phenyl-methyl morpholine at the 6-position, and surprisingly, while this compound has a THP group at the 1-position, its activity improved to EZH2 IC\textsubscript{50} = 237 nM. With some uncertainty about our SAR conclusions at this point, we turned our attention to substitution at the 3-position.

For compounds containing a 3-position methyl substituent, we synthesized several analogs using different R\textsubscript{i} and R\textsubscript{2} groups, including those reported in the literature\textsuperscript{13} for molecules having an indole core. R\textsubscript{2} groups included nitrile, phenyl-methyl morpholine, piperazino-pyridine, piperazino-pyrimidine and piperazino-isoquinoline. R\textsubscript{i} groups included isopropyl, cyclopentyl, cyclohexyl, piperazino-pyridine, piperazino-pyrimidine and piperazino-isoquinoline. An indole core. R\textsubscript{2} groups included nitrile, phenyl-methyl morpholine, piperazino-pyridine, piperazino-pyrimidine and piperazino-isoquinoline. As a result, we tested our SAR conclusions at this point, we turned our attention to substitution at the 3-position.

Scheme 2 describes the synthesis of compounds 27, 28, 30, 31, 33 and 34. Briefly, intermediate 24a and 24b were synthesized from 10 by alkylation followed by formylation and reduction while 24c and 24d were synthesized by reductive amination of 23 which was obtained from 10 via 22. Cyanation of 24a and 24b resulted in 25a and 25b which after hydrolysis followed by amide bond formation with 3-(aminomethyl)-4,6-dimethylpyridin-2(1H)-one gave 27 and 28, respectively. Compound 29a, 29b and 29c were obtained by amide bond formation of 3-(aminomethyl)-4,6-dimethylpyridin-2(1H)-one with 24a, 24d and 24c respectively, using trimethylaluminium as coupling agent. Suzuki coupling of 29a and 29b with aryloboronic ester yielded 30 and 31 respectively. Suzuki coupling of 24b and 24c respectively gave 32a and 32b which after amidation afforded 33 and 34 respectively.

Scheme 3 describes the synthesis of compounds 36, 39, 40, 41 and 45. Boc-piperazine-isoquinoline boronic ester (synthesized by a method described in Supplemental information) was coupled with 29c to give 35 which after Boc-deprotection afforded 36. Compound 24a, 24b and 24c were coupled with commercially available Boc-piperazine-pyridine boronic ester to afford 37a, 37b and 37c which after amide bond formation followed by Boc-deprotection afforded 39, 40 and 41 respectively. Suzuki coupling of Boc-piperazine-pyridine boronic ester with 24c resulted 42 which was converted to 45 via 43 and 44.

With this set of methyl-substituted compounds in hand, we tested for activity against EZH2; the active analogs were additionally tested for activity against EZH1 and the Y641F EZH2 mutant. The Y641 residue is frequently mutated in certain lymphomas. Moreover, lymphomas with this mutation have increased H3K27 methylation.\textsuperscript{15} The SAR is illustrated in Table 2.

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**Scheme 2.** Synthesis of 27, 28, 30, 31, 33 and 34. Reagents and conditions: (a) NaH, alkyl bromide, DMF, 0-RT, 16 h; (b) i) POCl\textsubscript{3}, DMF, 2.5 h (ii) 2 N NaOH; (c) i) ZnI\textsubscript{2}, THF, 0-RT, 16 h; (d) ArOH, aldehyde/ketone, Na\textsubscript{2}OAc\textsubscript{2}, C\textsubscript{17}H\textsubscript{3}N\textsubscript{2}, DCE, RT, 16 h; (e) CuBr\textsubscript{2}, PPh\textsubscript{2}, dppbf, DMA, 135 °C, 18 h; (f) LiOH, THF, MeOH, Water, RT, 6 h; (g) 3-(Aminomethyl)-4,6-dimethylpyridin-2(1H)-one, PyBOP, TEA, DMSO, RT, 16 h; (h) 3-(Aminomethyl)-4,6-dimethylpyridin-2(1H)-one, 2 M AlMe\textsubscript{3} in toluene, THF, 0-80 °C, 18 h; (i) Boronic ester, Na\textsubscript{2}CO\textsubscript{3}, Pd(PPh\textsubscript{3})\textsubscript{4}, dioxane, water, 90 °C, 15 h. Further details are in the Supplemental information.
The methyl substituent had a dramatic effect on potency in the series. As an illustrative example, 16 has EZH2 IC\textsubscript{50} of 26.6 \textmu M, while the methyl substituted active enantiomer 27\textsuperscript{e1} has EZH2 IC\textsubscript{50} of 88 nM - a 302 fold improvement. The inactive enantiomer 27\textsuperscript{e2} has no measurable activity against EZH2. This interesting phenomenon is an example of the "magic methyl" effect that has been described in the literature.\textsuperscript{18} During the prosecution of this program, a patent application describing indoline core containing EZH2 inhibitors was published.\textsuperscript{19} While the broad claims included multiple 3- substituted indolines and some compounds containing 3,3-dimethyl substituted indolines were described, no compounds containing 3-monomethyl substituted indolines were exemplified.

We found that for all R\textsuperscript{1} and R\textsuperscript{2} groups, the 2 enantiomers of a pair had very disparate activity. This difference in activity in an enantiomeric pair was more pronounced in the case of a small R\textsuperscript{2} substituent (-CN, in 27\textsuperscript{e1}, 27\textsuperscript{e2}, 28\textsuperscript{e1} and 28\textsuperscript{e2}). In these examples, the difference in activity between enantiomers is >100 fold, with the less potent enantiomer (27\textsuperscript{e2} and 28\textsuperscript{e2}) having no measurable IC\textsubscript{50} against EZH2 up to 100 \textmu M. With the larger R\textsuperscript{2} substituents shown in Table 2, the less active enantiomer had measurable activity, albeit in the micromolar range. This indicates that in the 3-methyl substituted indoline series, there appears to be some cross talk between the 3- and 6-position substituents. In an effort to understand the "magic methyl" effect, we used the published crystal structure of EZH2\textsuperscript{20} to dock both enantiomers of compound 34 (see Supplemental information). This pair of enantiomers presented the dimethyl pyridine moiety to the protein in a manner identical to the published bound inhibitor, forming 2 H-bonds with the backbone of W624. The active enantiomer (34\textsuperscript{e1}, predicted to be the R enantiomer based on docking) indeed has the methyl group sitting in a groove lined by T678, F665, and the face of R685, possibly displacing an isolated water molecule. The inactive enantiomer (34\textsuperscript{e2}) is seen to rotate the indoline group by 180\degree, while still anchored to the protein through the pyridone. This likely happens in an attempt to avoid a steric clash with the protein. In this state, R685 and F665 interactions with the ligand are lost, and a high entropy stranded water is likely interacting with R685, thus explaining the potency loss.

Next, we decided to study effect of substituent at C-6 position of indoline on activity (Table 2). We used different substituents at this position and pyridine-2-yl-piperizine residue was found to...
be the best substituent at this position as far as the biological activity against WT EZH2 inhibition is concerned (Compounds 39–41, Table 2).

The pyrimidine analog of 41e1/C1 (45e1) was found to be 9-fold less potent than 41e1. We also explored a 3-ethyl substituted analog of 41e1 and found it to be 13-fold less potent (EZH2 IC₅₀ = 145 nM, data not shown).

While these compounds maintained relatively good potency for EZH2, they did not display appreciable inhibitory activity against EZH1 (IC₅₀ > 1.35 µM), indicating their relative selectivity for EZH2 versus the homolog EZH1. Regarding Y641F EZH2 activity, overall, this series suffered a 7–40-fold loss in potency relative to WT EZH2. Compound 28e1 with a cyano group at the R₂ position had the best potency against Y641F EZH2.

We tested EPZ6438 (7, EZH2 IC₅₀ = 2 nM in our assay) and two of our lead compounds with the indoline core (34e1 and 41e1) in solubility and microsomal stability assays; the data are shown in Table 3. These two compounds demonstrated superior kinetic solubility in PBS (pH 7.5) and improved oxidative stability in human and mouse liver microsomes (HLM and MLM) compared to EPZ6438 (7).

In conclusion, we describe the indoline as a novel core for making EZH2 inhibitors. These findings provide a foundation for future analoging and SAR; lead compounds will be tested in cell assays, in vivo for optimization of pharmacokinetic properties and evaluation in pharmacology models.
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**A. Supplementary data**

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.11.080.

**References**