Building Blocks

Robust Solutions for Critical Issues in Medicinal Chemistry

Magic Methyl Group in Medicinal Chemistry

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The methyl group is one of the most commonly occurring carbon fragments in small-molecule drugs. This simple alkyl fragment appears in more than 60% of the top-selling drugs, highlighting the importance of the simple methyl group as a very useful structural modification in the rational design of bioactive compounds and drugs. One reason the methyl group is so popular in drug discovery is the magic methyl effect: a rare but welcome phenomenon where installation of a methyl group can increase potency, improve selectivity, increase solubility, increase permeability, decrease metabolism or address toxicity issues. [1-2] In **Figure 63**, several drug molecules and clinical candidate molecules which contain methyl group are listed. For each case, methyl group(s) were incorporated into molecules with different purposes which will be introduced below. [3-10]

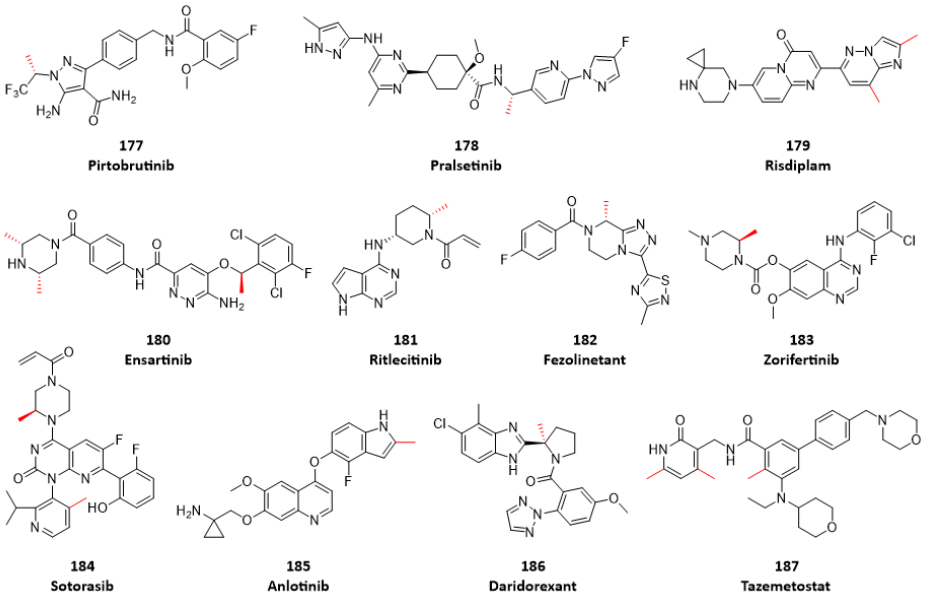

Figure 63. Representative drug and clinical candidate molecules containing methyl group

Compound **188** was identified as a potent and selective JAK3 covalent inhibitor. However, it suffers

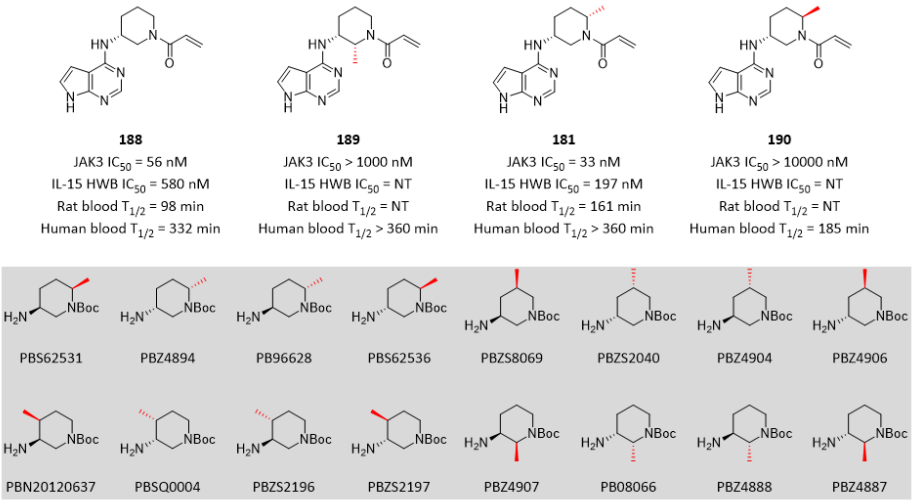

Figure 64. Methyl groups on piperidine ring modulated whole blood stability.

from moderate to poor PK in rat despite very reasonable oxidative stability as judged by liver microsomes. It was speculated that glutathione-S-transferase (GST) mediated glutathione (GSH)

addition to the acrylamide is accounted for this discrepancy. Stability of compound **188** in rat blood is only 98 min, which explained the observed poor PK profile in rat. In order to decrease GSH addition, the team attempted to inhibit chemical reactivity by adding substituents to the acrylamide, as steric hinder around the electrophile should reduce the ability of GST to catalyze GSH addition. As depicted in **Figure 64**, methyl groups were introduced on piperidine ring adjacent to acrylamide in compound **189**, **181** and **190**. Compound **189** lost potency completely due to a potential steric clash between the methyl group with protein side chain residue Leu956 in the ATP pocket. The hypothesis was realized in compound **181** which displayed comparable enzymatic potency and 3-fold higher HWB potency which was consistent with higher stability in HWB assay. It was interesting to found that chirality of the methyl group impacted potency significantly, as seen in compound **190** where the methyl group had an opposite chirality, resulting in potency loss completely. [9-10]

In the course of discovery of a potent, oral and CNS-penetrant EGFR inhibitor, compound **191** was identified as a promising lead with high potency, idea efflux ratio and excellent BBB-penetration. Metabolite identification of compound **191** suggested that the piperazine oxidation was the main metabolic pathway. With this in mind, the team incorporated methyl groups into molecules to block metabolic sites on piperazine ring in compound **192**, **193**, **194** and **195**. Potency of compound **192** and **193** was decreased by 3-4 fold, and PK profile was worse than compound **191**. Although efflux ratio of compound **192** and **193** was decreased, plasma-to-brain ratio was not affected. Both compound **194** and **195** kept comparable potency, while compound **194** had improved both blood exposure and brain exposure comparing to compound **191** and **195** (**Figure 65**). [6] The promising data package of compound **194** (**AZD3759**, **Zorifertinib**) which has excellent central nervous system penetration, strongly supported its selection as a clinical candidate for development for the treatment of brain metastases. Methyl piperazine building blocks (**Figure 66**) played crucial roles in quick access of designed molecules and systematic SAR and SPR studies.

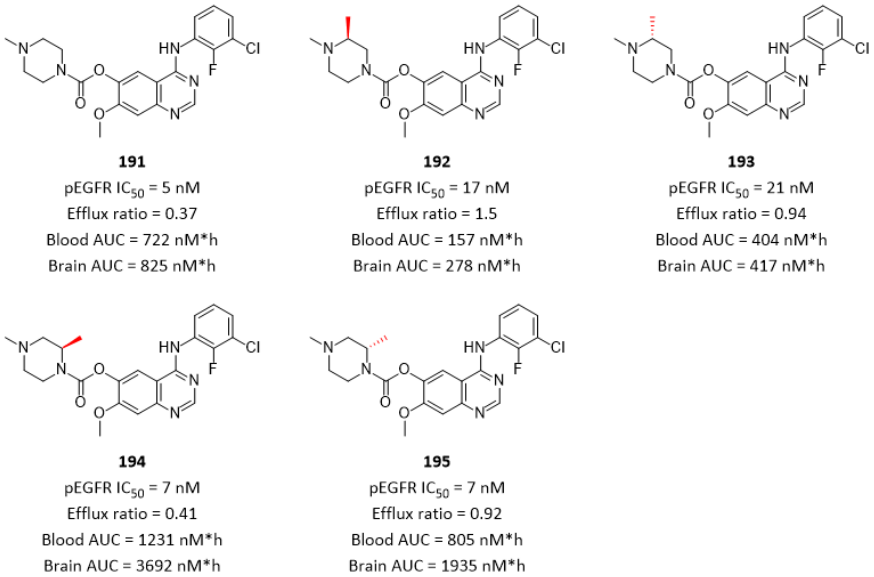

Figure 65. Methyl groups on piperazine modulated potency and PK profile of EGFR inhibitor.

As shown in **Figure 63**, there was also a chiral methyl group on piperazine ring in compound **184** (**AMG510**, **Sotorasib**). In the course of discovery of **Sotorasib**, compound **196** was identified as one of promising hit compounds with high potency. However, PK profile was poor (data not shown). Employing similar strategy in the discovery of compound **194** (**AZD3759**, **Zorifertinib**) described above, a chiral methyl group was incorporated on piperazine ring in compound **197** which had increased cellular potency by 3-fold while oral bioavailability was improved (F = 12%). Comparing

compound **198** and **199**, the same trend was observed with cellular potency and oral bioavailability both increased, especially oral bioavailability, in compound **199** bearing a chiral methyl group on piperazine ring (**Figure 66**). [8]

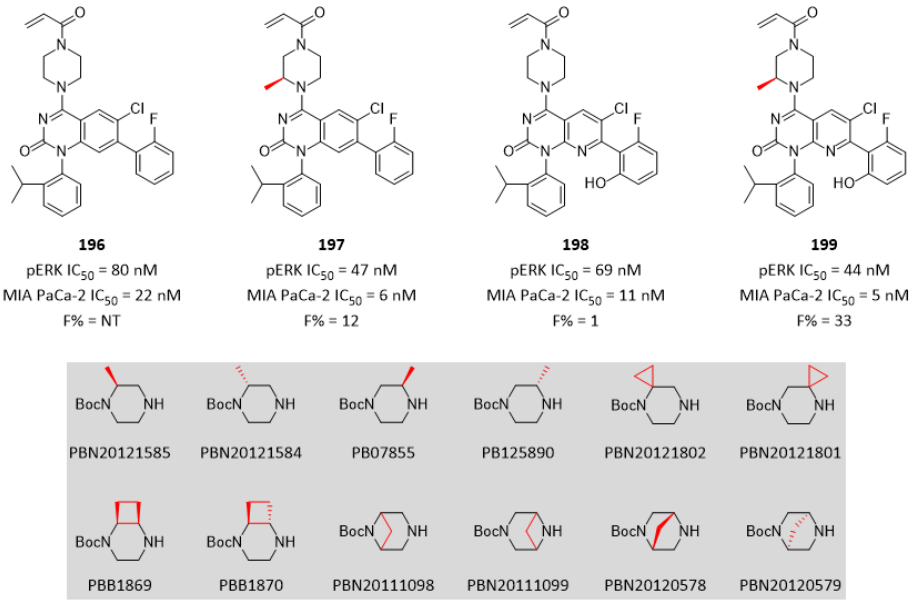

Figure 66. Methyl groups on piperazine ring modulated potency and PK profile of KARS G12C inhibitors.

In the course of discovery of a potent and reversible dual orexin receptor antagonist, compound 200 was identified as a promising lead. As shown in **Figure 66**, both trans-methyl groups in compound **201** and **202** increased potency significantly by 96-fold for OX1R, 145-fold for OX2R and

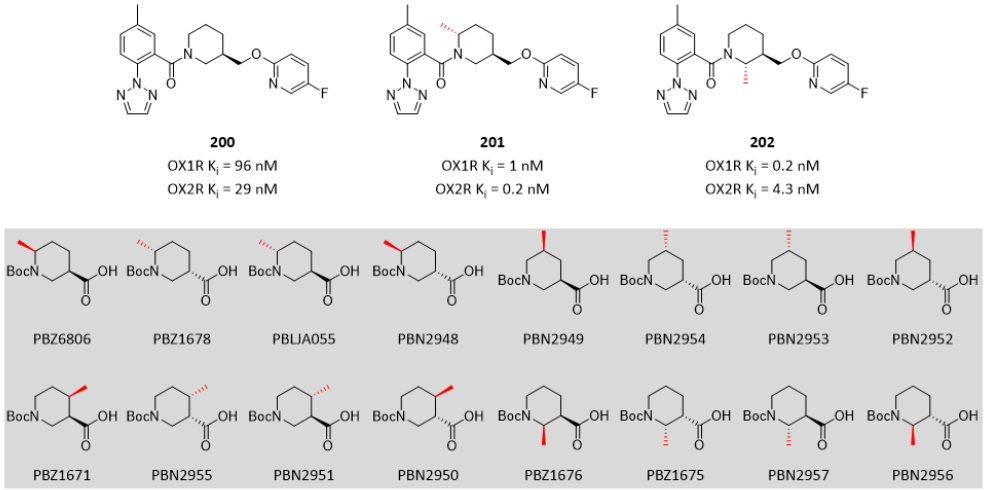

Figure 67. Methyl groups on piperidine ring increased potency.

by 505-fold for OX1R, 6-fold for OX2R respectively. This observation is consistent with structural hypothesis that alpha-methylation favors the trans-diaxially substituted piperidine with axial orientation for the 3-CH_2OAr group (**Figure 67**). [11]

Metabolic studies confirmed that the pyrrolidine moiety in compound **203** was exceptionally metabolically active. Therefore, the initial structural modification was to introduce substituents onto the pyrrolidine to directly block its metabolism. Considering the profile that small substituents on pyrrolidine were preferred for potency against PI3Kdelta along with the availability of starting material, a small (S)-methyl group was introduced onto the pyrrolidine ring in compound **204**.

Compound **204** gave improved clearance compared to compound **203**, which demonstrated the rationality of modification strategy. Moreover, the results of this comparison are reminiscent of the methyl effect in the molecular modification strategy, also known as the magic methyl effect, where the addition of a methyl group in place of hydrogen leads to a dramatic improvement in metabolic stability due to an additional labile group for CYP oxidation (**Figure 68**). [12]

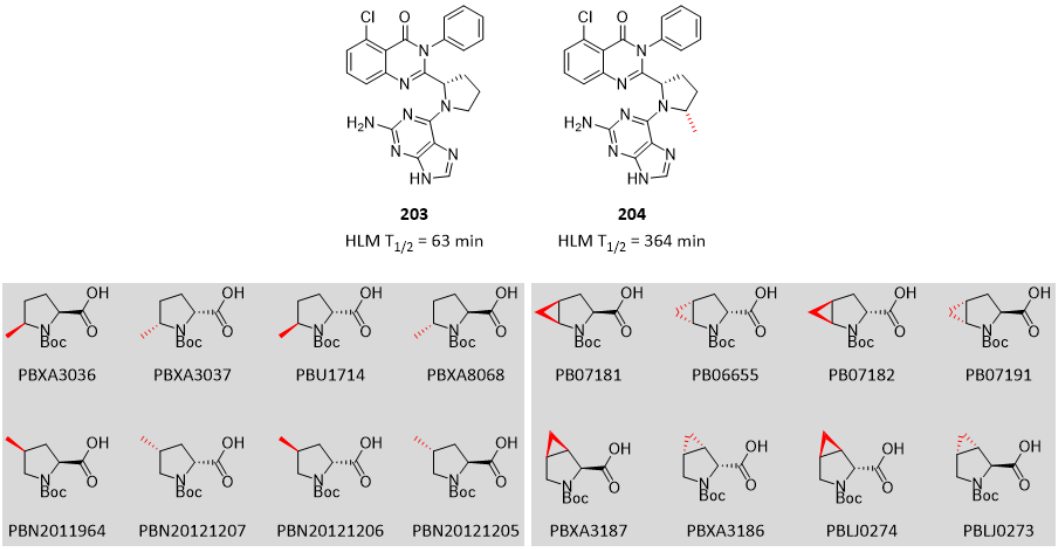

Figure 68. A methyl group increased metabolic stability significantly.

Modifying the initial lead compound **205** to compound **207** resulted in 0.6 log improvement in bioactivity due to the (R)-Me substitution on piperazine ring. Moreover, a stereochemical SAR on piperazine ring methyl substitution was evident with the order of antagonist bioactivity as follows: (R)-Me (compound **207**) > des-Me (compound **205**) > (S)-Me (compound **206**), with compound **207** 10-fold more potent than compound **206** (**Figure 69**). [4-5]

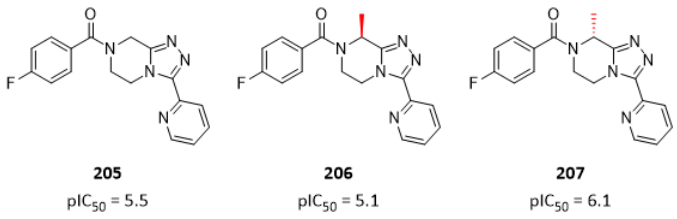

Figure 69. Methyl groups with opposite chirality impacted potency in different way.

In the course of discovery of DNA-PK inhibitors, compound **208** was identified as a promise lead. Ortho- methyl group was added into compound **209** and compound **210**, which confirmed a significant boost in potency for compound **210**, but not compound **209**. Comparing compound **211** and compound **212**, the same trend was observed. The ortho- methyl group on the aniline group gave at least 10-fold increase in biochemical potency while maintaining LogD, thus could be described as “magic methyl”. This striking increase in potency suggests that, in addition to making an effective lipophilic interaction in the hydrophobic pocket consisting of Tyr3791, Leu3806 and Ile3940, the methyl group may also confer a beneficial conformational effect, by favoring a bioactive conformation where there is a twist between the aromatic amine and the purinone core (**Figure 70**). [13]

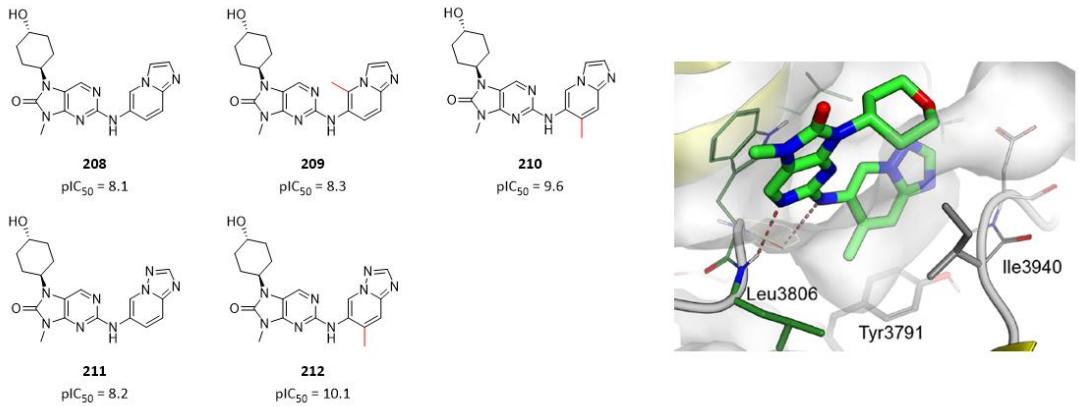

Figure 70. Methyl groups on aniline increased potency by at least 10-fold. (PDB code: 6T3C)

In the course of discovery of **Tazemetostat**, "magic methyl" effect was observed as shown in **Figure 71**. Comparing compound **213** and compound **214**, lacking a methyl group for compound **213**, the activity is significantly decreased by 190-fold. The "magic methyl" effect was magnified in comparison of compound **215** and compound **216**, with a significant 1400-fold difference observed. The steric-directing effect of the methyl group is not limited to the amide moiety, but also has a profound effect on the adjacent aniline substituent. This optimized substitution pattern led to a significant potency breakthrough allowing the team to drive potency to the threshold of single-digit nanomolar levels for the first time (**Figure 71**). Further optimization of compound 216 led to discovery of **Tazemetostat** which was approved as a first EZH2 inhibitor. [7]

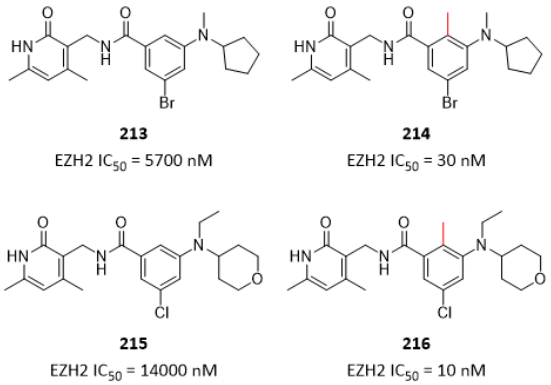

Figure 71. Methyl groups on phenyl ring increased potency significantly.

It is extremely supportive if medicinal chemists have convenient access of diverse building blocks containing methyl groups which could potentially exert "magic methyl" effect to impact positively activity, selectivity or properties of molecules (**Figure 72**). As mentioned in section "**Dihedral Angle in Medicinal Chemistry**", modulating dihedral angle through steric hinder between methyl group and adjacent moieties can influence a lot of properties of molecules.

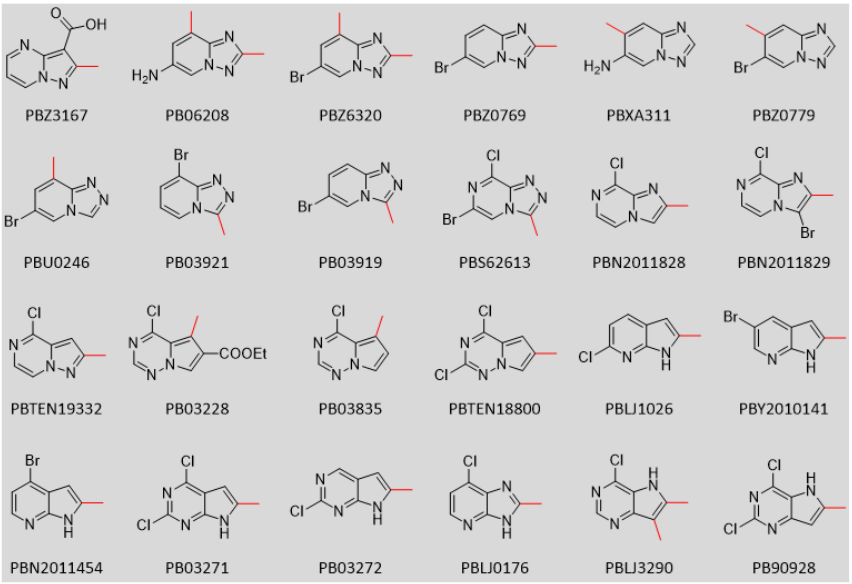

Figure 72. Scaffold building blocks containing a methyl group with potential "magic methyl" effect

With lead compound **217** in hand, the team turned their attention to modification of the benzylic linker, based on hypothesis that the incorporation of an appropriate substituent on the alpha-position of the benzylic amine liker could constrain the linker to adopt the bioactive conformation and, therefore reduce the entropic cost of ligand binding. Moreover, methyl substitution could also block the metabolism at the alpha-position of then benzylic group. Based on modeling, the methyl-substituted linker with (S) stereochemistry is more conformationally constrained, and the bioactive conformation is still near an energy minimum. This extra constraint on the linker conformation would be expected to modestly improve the potency. By contrast, the (R) enantiomer is expected to be substantially less potent, because it has an energy minimum that does not resemble the bioactive conformation observed in the crystal structure. For the (R) enantiomer to adopt the bioactive conformation, there would be a steric clash between the methyl group and the carbonyl oxygen of the quinolinone. As X-ray structure of analogue suggests that the protein can only accommodate a small group in the vicinity of the benzylic group linker, the team selected and investigated small methyl group in compound **218** and compound **219**. As depicted in **Figure 73**, the (S)-methyl group in compound **219** was favored, whereas the (R)-methyl group in compound **218** lost most of its activity. Compound 219 also demonstrated excellent metabolic stability in human microsome and improved solubility. Taken together, the data showed that an (S)-methyl-substituted benzylic linker in compound **219** was the optimal choice, not only providing very potent inhibitory activity against the R132H and R132C mutants but also conferring excellent metabolic stability and enhanced solubility (**Figure 73**). [14] In this context, amine building blocks containing chiral methyl groups at alpha-position of benzylic linker played critical roles in quick synthesis of designed molecules.

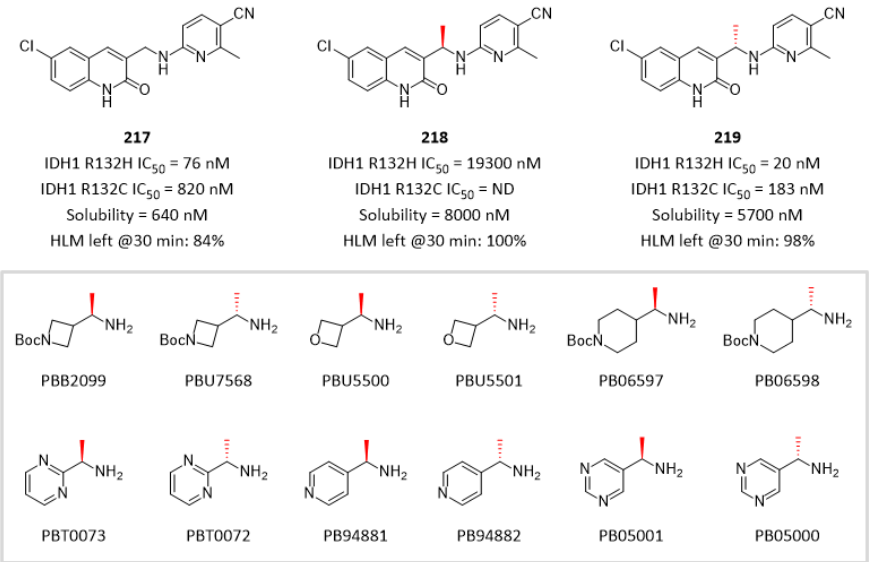

Figure 73. Methyl group at alpha-benzylic position impacted activity and properties.

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