For the interaction residues of vdW anchors V1 (K151), V2 (H57 and F156), and V3 (F156), the mutation~~,~~ K151A~~,~~ exhibited less than 50% detectable inhibitory efficacy when ~~treating~~treated with 150 μM NSC351204 (IC_{50} ~~>~~ ≥150 μM~~,~~; Fig. 5C) because of the loss of not only ~~the~~its electrostatic force/H-bond ~~interaction~~interactions with negatively charged inhibitor moieties ~~of inhibitors~~ but also ~~partially~~the partial loss of the hydrophobic force in anchor V1. The residue H57 often forms hydrogen bonds with the phosphate group of IPP, facilitating IPP binding and catalysis. Furthermore, our model ~~suggested~~suggests that residues H57 and F156 play ~~the~~a critical ~~roles of~~role by forming hydrophobic environments in anchors V2 and V3 (Figs. 1E and 1F), which are preferred ~~to the~~by inhibitors with large hydrophobic moieties. As expected, the mutation H57A showed only 25% ~~relative~~of wild type enzymatic activity and caused ~~more than five~~a >5-fold increase in the IC_{50} value ~~compared to the wild type~~ (IC_{50} ~~>~~ ≥150 μM~~,~~; Figs. 5E and 6B). When F156 was replaced with alanine (A156), this mutation exhibited a 48% inhibitory effect at a concentration of 100 μM NSC451204 (IC_{50}>~~–~~100 μM~~,~~; Fig. 5C). However, F156A retained ~88% ~~relative~~of its catalytic activity ~~due to~~because F156 is not ~~direct~~directly associated with the catalytic reaction (Fig. 6B). These site-directed mutagenesis studies revealed that these interacting residues of SiMMap ~~participated~~participate in biological functions and inhibitor binding in the inhibitory site of hGGPPS.

Identification of key residues for ~~specifically inhibiting~~ hGGPPS-specific inhibition

In order to explore the ~~specific~~ binding mechanisms ~~of~~ specific to hGGPPS, we attempted to find the ~~diversities~~differences between hGGPPS and ~~yeast GGPPS (~~yGGPPS~~),~~, which ~~share~~ are similar in their sequence (44% shared identity and 60% similarity) and structure (RMSD is 1.06~~) similarities.~~). The compound zoledronate is potent for the prenyltransferase family (~~*i.e.,*~~ IC_{50} for yGGPPS ~~is~~: 0.66 μM) ~~except~~with the exception of hGGPPS (IC_{50} for hGGPPS ~~is~~: 100 μM). To address this issue, we performed structure-based studies and site-~~direct~~directed mutagenesis experiments on the proteins of the prenyltransferase family. According to the structure-based study, the superimposition of three crystal structures, including hGGPPS-GGPP (PDB code: 2Q80), yGGPPS-zoledronate (PDB codes: 2E91), and yGGPPS- GGPPS (PDB code: 2E8V), showed that most residues were well ~~alignment~~aligned except for the residue K228 (yGGPPS~~)~~), which corresponds to

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K202 (~~in~~ hGGPPS~~)~~ (Fig. 6A). Among 22 crystal structures of yGGPPS in PDB, we found that the residue K228 is visible in two structures (2E8V and 2E93~~) is visible and the result implies~~), implying that K228 is not stable. Conversely, K202 is stable in hGGPPS and its side-chain orientation is significantly different from ~~the one~~that of K228 in yGGPPS (Fig. 6A). These observations ~~suggested~~suggest that K202 can be a key residue ~~to cause~~behind the different inhibitory ~~effect~~effects between hGGPPS and yGGPPS. To verify this hypothesis, K202 was replaced with Gly202 to mimic the structural environment, and tested ~~by~~using zoledronate. Indeed, the inhibition against hGGPPS of zoledronate regained 29-fold ~~compared to~~more activity than in wild type (IC_{50} for K202G is 3.4 μM~~,~~; Fig. 6C). Interestingly, the catalytic activity of K202G was ~~raised as~~increased the same as for K212A (not shown).

註解 [Editor3]:

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According to our model, enzymatic ~~-~~activity ~~assay~~assays, and structure-based studies, K212 in hGGPPS is a significant interacting residue for inhibitor binding and it differs from corresponding residues in yGGPPS and *E. coli* OPPS. For example, the side ~~-~~chains of K238 in yGGPPS were often not consistent with K212 in hGGPPS (Fig. 6A). The enzymatic activity ~~was obtained~~showed the reverse results when replacing K212 with A212 in hGGPPS and K235 with L235 in *E. coli* OPPS. These results also ~~suggested~~suggest that K212 may play an important role ~~for~~in selective inhibition between hGGPPS and ~~the~~ other ~~prenyltransferase.~~prenyltransferases. To confirm ~~the~~this idea, K212A was also tested by zoledronate. The IC_{50} of zoledronate on the hGGPPS with the mutation K212A ~~is~~was 2.4 μM (Fig. 6D) and mutating K212 in hGGPPS successfully ~~regains~~induced a 42-fold relative inhibitory efficacy of zoledronate against hGGPPS, which ~~demonstrated~~demonstrates that K212 ~~could cause~~causes the selectivity between hGGPPS and ~~the~~ other prenyltransferases.

To further ~~prove~~support our hypothesis, we tested two ~~identified~~ non-bisphosphonate inhibitors, NSC351204 and NSC45174, ~~based on~~identified from our computational strategies ~~were tested for~~against K212A and yGGPPS. From our predicted binding models ~~of~~for these two compounds, NSC351204 interacts with K212 (Fig. 5B) but NSC45174 does not interact with K202 ~~and~~or K212 (Fig. 5C). Moreover, site-direct mutagenesis and catalytic activity assays ~~are~~were consistent with our predicted model and binding modes (Fig. 5E and 6B). The results show that NSC351204 can exhibit 85% detectable inhibitory effect against hGGPPS (WT) but has low inhibition against yGGPPS and K212A when ~~treating the~~used at a

concentration of 100 μM. However, NSC45174 can ~~both~~ efficiently inhibit both yGGPPS and K212A (Figs. 6E and 6F). These results ~~provided the evidences for~~lend support to our hypotheses and suggest that K202 and K212 in hGGPPS are key residues for ~~specifically inhibiting~~ hGGPPS-specific inhibition.

Tumor cell growth inhibition by non-bisphosphonate hGGPPS inhibitors

The potential of bisphosphonates as antitumor agents has been suggested by several *in vitro* and *in vivo* preclinical studies [26]. For example, BPH-675 has good activity against GGPPS and three tumor cell lines: MCF-7 (breast cancer), NCI-H460 (~~nonsmall~~non-small cell lung cancer), and SF-268 (human glioblastoma) [21]. ~~Zoledronate~~In one study, zoledronate (+endocrine) therapy was found to significantly ~~reduced~~reduce disease ~~progression~~progression in premenopausal breast cancer patients [27]. ~~Moreover, we~~We tested ~~for~~ the activity of NSC351204 (Fig. 5A~~),~~) —our non-bisphosphonate inhibitor with the best inhibitory effect for hGGPPS~~,~~ —in ~~tumor cell~~ killing tumor cells, using MCF-7 (breast cancer) and MDA-MB-231 (breast adenocarcinoma~~).~~) cell lines. The results of cell growth inhibition clearly show that NSC351204 ~~is active for inducing~~induced tumor cell death ~~though~~, and that its EC_{50} values in these two breast cell lines ~~are~~were more potent than those of zoledronate and pamidronate ~~(Figs. 7A and 7B) (~~: in MCF-7~~:~~ cells, the EC_{50} of NSC351204 is ~400 μM and the EC_{50} of zoledronate and pamidronate are ~24 μM and ~151 μM, respectively; in MDA-MB-231~~:~~ cells, the EC_{50} of NSC351204 is ~300 μM and the EC_{50} of zoledronate and pamidronate are ~20 μM and ~100 μM, respectively (Figs. 7A and 7B). According to our model, in anchor V3 (Fig. 1E and Table 2), the sulfate and nitro moieties in NSC351204 might