Crystal and cryo-EM structures of the gastric proton pump bound with potassium-competitive acid blockers

Acid-related gastric diseases are associated with disorders of the digestive tract, such as peptic ulcers or gastroesophageal reflux disease (GERD). Gastric mucosal injury induced by the continuous use of non-steroidal anti-inflammatory drugs (NSAIDs) or gastrin-producing tumors may also cause peptic ulcers [1]. Current therapies to treat these conditions either prevent the stimulation of parietal cells or inhibit the final step in acid production. The former is accomplished by antagonizing histamine H2 receptors, and the latter by targeting the gastric proton pump, H^+,K^+-ATPase. The suppression of gastric acid secretion in combination with antimicrobial agents has also been used for the eradication of *Helicobacter pylori*, which is recognized as the main cause of gastric ulcers and gastric cancers [2]. Proton pump inhibitors (PPIs) such as omeprazole have been utilized for acid suppression. The PPI drug itself is a prodrug, requiring acid activation in the secretory canaliculus to inhibit H^+,K^+-ATPase. Once activated, the PPI forms a covalent bond with Cys813 of H^+,K^+-ATPase and irreversibly inhibits acid secretion. However, given its relatively short plasma half-life and the need for an acidic pH to convert the prodrug to the active compound, considerable effort has been expended to develop different types of H^+,K^+-ATPase inhibitor [3]. K^+-competitive acid blockers (P-CABs) differ from the PPIs in that they do not depend on acid activation, are relatively stable in the acidic canaliculus, and bind directly to the proton pump, thereby providing a more rapid onset and better inhibition of acid secretion. They are currently in clinical use in some Asian countries. However, as these compounds have been developed by phenotypic screening, their detailed binding poses and interactions are unknown, except for two previously reported crystal structures of H^+,K^+-ATPase [4] bound to SCH28080 (a prototypical P-CAB) or vonoprazan (approved for the clinical treatment in Japan).

We determined new crystal (at SPring-8 **BL41XU** and **BL45XU**) and cryo-EM (**EM01CT**) structures of H^+,K^+-ATPase in complexes with four different P-CABs, tegoprazan, soraprazan, PF-03716556 and revaprazan, at resolutions reaching 2.8 Å [5]. The structures reveal molecular details of their interactions (Figs. 1 and 2) and are supported by the results of functional analyses of tailored mutations. As expected, the three SCH28080-related P-CABs show similar but distinguishable binding modes to the prototypic compound. On the other hand, revaprazan has a

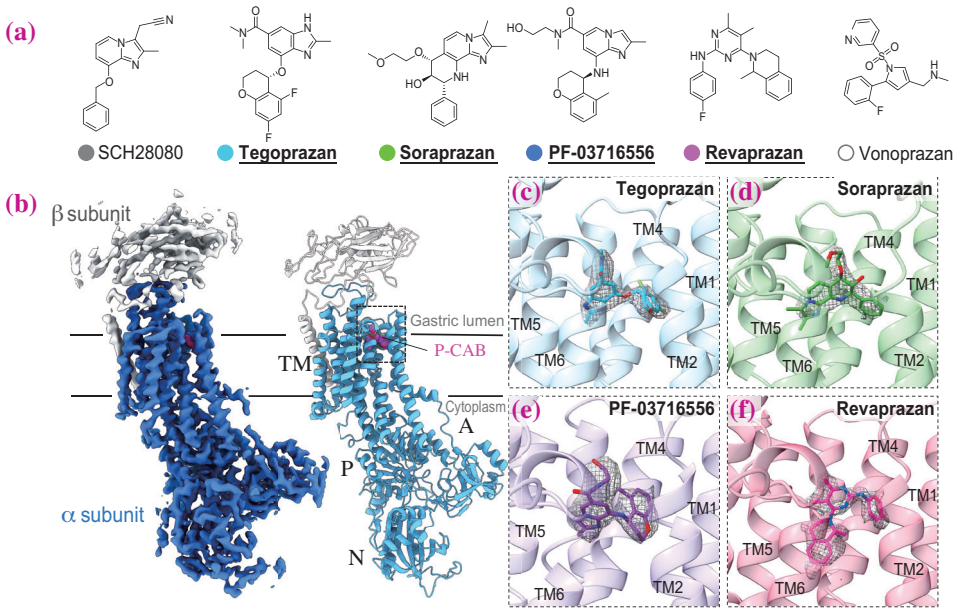

Fig. 1. Structure of P-CABs bound to H^+,K^+-ATPase. **(a)** Chemical structures of P-CABs. The four P-CABs used for structural determination are highlighted. **(b)** Overall structure of revaprazan-bound form of H^+,K^+-ATPase composed of α- and β-subunits. Three cytoplasmic domains (A, P and N domains) and TM helices are indicated. **(c–f)** Close-up view of P-CAB binding site (indicated by dotted box in **b**) for tegoprazan (**c**, blue), soraprazan (**d**, green), PF-03716556 (**e**, purple) and revaprazan (**f**, magenta), with their electron or EM density maps (mesh). Figures are viewed along the membrane plane with luminal-side up.

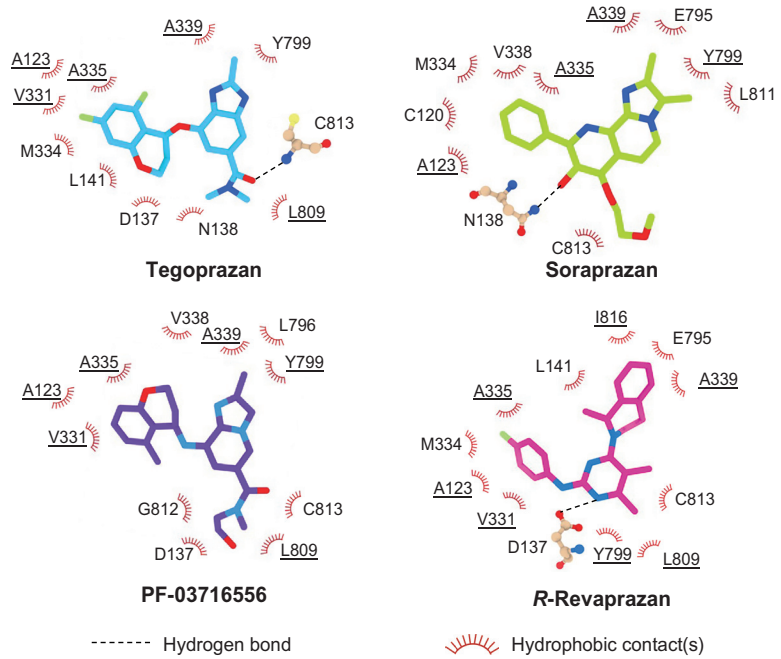

Fig. 2. Schematic representation of P-CAB binding. Schematics of the molecular interactions between H^+,K^+-ATPase and each P-CABs. The hydrophobic residues located within 3.9 Å from bound P-CAB are shown. Expected hydrogen bonds within 3.3 Å are shown as dotted lines. The mutant residues evaluated in Ref. 5 are highlighted with an underbar.

novel binding mode, in which its tetrahydroisoquinoline moiety binds deep in the pocket; such biding is thus clearly distinct from the binding of SCH28080-related compounds. To our knowledge, vonoprazan shows the most potent binding affinity for H^+,K^+-ATPase *in vitro* ($IC_{50,\text{vonoprazan}}$ = 0.015 μM), and this compound is exceptional among P-CABs, because it binds deep in the binding pocket. Our structural analysis reveals that revaprazan directs its tetrahydroisoquinoline moiety toward the cation binding site similar to the vonoprazan binding pose (Fig. 3). According to a previous report, compounds with methoxy substitution in the tetrahydroisoquinoline group of revaprazan, which is facing the cation binding site, show a significant improvement in binding affinity. Our structural and mutagenesis studies suggest that the binding state of revaprazan is somewhat loose, but further optimization of the tetrahydroisoquinoline moiety, especially on the side that faces the cation binding site, may further improve the binding affinity of this type of P-CAB.

The mechanism of action of these P-CABs can now be evaluated at the molecular level, which will facilitate the rational development and improvement of currently available P-CABs, leading to a better treatment of acid-related gastrointestinal diseases and a possible eradication of their main cause, namely, *H. pylori*.

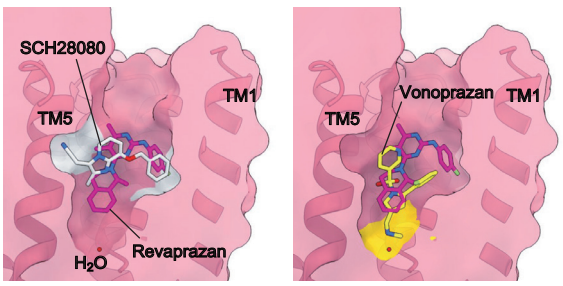

Fig. 3. Binding modes of revaprazan and other representative P-CABs. Cross section of the TM helices at the P-ACB binding site of revaprazan-bound H^+,K^+-ATPase (surface), with superimposed SCH28080 (left, gray) and vonoprazan (right, yellow), viewed approximately parallel to the membrane plane. A red sphere indicates a likely water molecule bound at the cation binding site. Binding surfaces of SCH28080 and vonoprazan are indicated in gray and yellow, respectively.

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