

## **Differential effects of proton pump inhibitors and vonoprazan on vascular endothelial growth factor expression in cancer cells**

**Short title:** Effects of PPIs and vonoprazan on VEGF expression in cancer cells

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### **Funding**

This study was supported by grants from the Japan Society for the Promotion of Science

(JSPS) KAKENHI (grant numbers 20K16044 and 20H05799).

### **Conflict of interest**

The authors declare no potential conflicts of interest.

### **Author contributions**

Rie Ando-Matsuoka: Conceptualization, formal analysis, investigation, methodology, validation, visualization, writing – original draft. Kenta Yagi: Conceptualization, funding acquisition, project administration, writing – review editing. Mayu Takaoka: formal analysis, investigation, validation. Yuko Sakajiri: data curation, formal analysis, investigation, methodology. Tomokazu Shibata: data curation, formal analysis, investigation, methodology. Ryusuke Sawada: data curation, formal analysis, investigation, methodology. Akinori Maruo: data curation, formal analysis, investigation, methodology. Koji Miyata: writing – review editing. Fuka Aizawa: resource, writing – review, and editing. Hirofumi Hamano: methodology, resource, writing – review and editing. Takahiro Niimura: writing – review and editing. Yuki Izawa-Ishizawa: resource, writing – review and editing. Mitsuhiro Goda: Conceptualization, funding acquisition, methodology, project administration, resource, supervision, writing – review and editing. Satoshi Sakaguchi: writing – review and editing. Yoshito Zamami: writing – review and editing. Yoshihiro Yamanishi: writing – review and editing. Keisuke Ishizawa: Conceptualization, project administration, supervision, writing – review and editing.

### **Acknowledgments**

We would like to thank Editage ([www.editage.com](http://www.editage.com)) for English language editing.

### **Lay summary**

We previously reported that proton pump inhibitors (PPIs), the first-line agents in treating peptic ulcers, increased VEGF expression; elevated VEGF expression promotes cancer progression and diminishes the efficacy of anti-VEGF drugs. In this study, we evaluated the effect of vonoprazan, a novel drug for the treatment of peptic ulcers, on VEGF

expression. We found that PPIs increased VEGF expression via ER- $\alpha$  in cancer cells, while vonoprazan did not affect VEGF expression, suggesting that vonoprazan might be more effective than PPIs in patients with cancer, especially those receiving anti-VEGF agents, for improving the therapeutic effects of cancer chemotherapy.

### **Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request

### **Precis**

PPIs increase VEGF expression via ER- $\alpha$  in cancer; elevated VEGF expression leads to cancer progression and diminished therapeutic effect of anti-VEGF drugs. Unlike PPIs, vonoprazan, a novel gastric acid secretion inhibitor, does not upregulate VEGF expression in cancer cell lines, suggesting that vonoprazan may be more effective than PPIs in patients with cancer, especially those receiving anti-VEGF agents, for improving the therapeutic effects of cancer chemotherapy.

### **Abstract**

**Background.** Proton pump inhibitors (PPIs) are potent inhibitors of gastric acid secretion, used as first-line agents in treating peptic ulcers. However, we have previously reported that PPIs may diminish the therapeutic effect of anti-vascular endothelial growth factor (VEGF) drugs in patients with cancer. In this study, we explored the effects of vonoprazan, a novel gastric acid secretion inhibitor used for the treatment of peptic ulcers, on the secretion of VEGF in cancer cells and attempted to propose it as an alternative PPI for cancer chemotherapy. **Methods.** The effects of PPI and vonoprazan on VEGF expression in cancer cells were compared by real-time rt-PCR and ELISA. The interaction of vonoprazan and PPIs with transcriptional regulators by docking simulation analysis. **Results.** In various cancer cell lines, including the human colorectal cancer cell line (LS174T), PPI increased VEGF mRNA expression and VEGF protein secretion, while this effect was not observed with vonoprazan. Molecular docking simulation analysis

showed that vonoprazan had a lower binding affinity for estrogen receptor alpha (ER- $\alpha$ ), one of the transcriptional regulators of VEGF, compared to PPI. Although the PPI-induced increase in VEGF expression was counteracted by pharmacological ER- $\alpha$  inhibition, the effect of vonoprazan on VEGF expression was unchanged. **Conclusions.** Vonoprazan does not affect VEGF expression in cancer cells, which suggests that vonoprazan might be an alternative to PPIs, with no interference with the therapeutic effects of anti-VEGF cancer chemotherapy.

**Keywords:** Proton pump inhibitors, vonoprazan, lansoprazole, fulvestrant, vascular endothelial growth factor.

Total number of text pages: 31

Total number of tables: 1

Total number of figures: 5

Total number of supporting files: 3

## **Introduction**

Cancer patients are highly prone to stomach ulcer complications because of the frequent use of non-steroidal anti-inflammatory drugs to relieve cancer pain.<sup>1, 2</sup> In addition, cancer patients often experience pancytopenia and thrombocytopenia as side effects of chemotherapy, wherein the gastrointestinal bleeding associated with peptic ulcers can be fatal.<sup>3, 4</sup> Bleeding is a common side effect associated with the usage of vascular endothelial growth factor (VEGF) inhibitors, such as bevacizumab, which are key drugs in cancer chemotherapy.<sup>5</sup> Therefore, preventing peptic ulcers and gastrointestinal bleeding is especially important for patients receiving anti-VEGF agents to ensure continuous treatment.

Proton pump inhibitors (PPIs) are potent inhibitors of gastric acid secretion, used as first-line agents in treating peptic ulcers. Therefore, PPIs are frequently used in patients with cancer. However, we previously found that PPIs upregulate VEGF mRNA expression; VEGF is a potent angiogenesis-promoting biomolecule. Elevated VEGF

expression in tumors promotes cancer progression and hematogenous metastasis by enhancing angiogenesis.<sup>6-8</sup> It also promotes the formation of fragile blood vessels, making it difficult for anticancer drugs to penetrate the entire tumor tissue and induce drug resistance.<sup>8</sup> Furthermore, it may also reduce the therapeutic effect of anti-VEGF drugs. Thus, elevated VEGF expression is known to exert various negative effects in patients with cancer. Our previous retrospective study revealed that the response rate in patients receiving bevacizumab, an antiangiogenic drug, decreased due to PPIs' usage. These findings indicate that PPIs may reduce the therapeutic effect of bevacizumab by increasing VEGF expression.<sup>9</sup> Therefore, when treating patients with bevacizumab and other anti-VEGF drugs, it is necessary to select a peptic ulcer treatment that does not affect VEGF expression. However, alternative peptic ulcer medications, such as H<sub>2</sub> blockers and mucosal protectants, are less effective than PPIs in treating peptic ulcers,<sup>10</sup> often making it difficult to switch to alternative drug agents.<sup>11</sup>

Vonoprazan is a novel gastric acid secretion inhibitor, authorized in Japan in 2015.<sup>12</sup> Vonoprazan is a more potent and sustained inhibitor of gastric acid secretion compared to PPIs.<sup>13-16</sup> In Japan, it is used as a first-line drug in peptic ulcer treatment, similar to PPIs.<sup>15</sup> The mechanism of action of vonoprazan differs from that of PPIs; vonoprazan is secreted into the gastric secretory lumen from gastric cell walls. It competitively inhibits the influx of K<sup>+</sup> ions required for the proton pump to operate.<sup>16</sup> In contrast, PPIs are activated in the secretory lumen and covalently bind to the proton pump, inhibiting it non-competitively.

All PPIs share a common structure consisting of benzimidazole, sulfoxide, and pyridine.<sup>17</sup> Because all PPIs exhibit VEGF-inducing activity, this effect may be derived from the common structure of PPIs. However, the chemical structure of vonoprazan differs significantly from that of PPIs, and it is unclear how vonoprazan affects VEGF expression. In this study, we investigated the differences in the effect of vonoprazan and PPI on VEGF expression in cancer cells. We also examined the differences between the two kinds of drugs in more detail, focusing on the differences in their chemical structures.

## **Materials and Methods**

### **1. Materials**

Lansoprazole, pantoprazole sulfide, rabeprazole sodium salt, and omeprazole were purchased from Tokyo Chemical Industry (Tokyo, Japan). Esomeprazole magnesium hydrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Vonoprazan Fumarate was purchased from MedChem Express LLC (Monmouth Junction, NJ, USA) and Fulvestrant from Selleck Chemicals LLC (Houston, USA).

### **2. Cells**

The A549 human lung adenocarcinoma epithelial cell line and LS174T human colon cancer cell line were purchased from ATCC (Manassas, VA). The MCF7 human breast cancer (RCB1904) and OVK18 human ovarian cancer (RCB1903) cell lines were purchased from RIKEN BioResource Center (Ibaraki, Japan). Cells were cultured in RPMI-1640 medium (NACALAI TESQUE, Inc., Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (Corning, Inc., NY, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin (ICN Biomedicals, Inc., CA, USA) in a 5% CO<sub>2</sub> air incubator at 37 °C.

### **3. Real-time PCR**

The human cancer cell lines LS174T, A549, MCF7, and OVK18, were used to investigate gene expression. Vonoprazan and lansoprazole were dissolved in DMSO. Cells were seeded into 6-well plates ( $3.0 \times 10^5$  cells/well), incubated for 24 h, and exposed to lansoprazole (10 µM) or vonoprazan (10 µM) for 24 h. Total RNA was extracted from cells using RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands), according to the manufacturer's protocol. Total RNA was reverse transcribed to cDNA using Prime Script™ RT reagent Kit with gDNA Eraser (Takara Bio, Inc., Shiga, Japan). The cDNA product was used as a template for PCR amplification in a total volume of 10 µL, and the PCR conditions were as follows: 95 °C for 2 min, followed by 40 cycles at 95 °C for 5 s, 60 °C for 30 s, and 95 °C for 5 s. Reactions were conducted on a Step One Plus Real-time PCR Detection System (Thermo fisher science, Waltham, MA, USA ). All data were

analyzed with the Step One Software V2.3 (Thermo fisher science, Waltham, MA, USA). The data were analyzed using the delta cycle threshold method and calculated based on the cycle quantification (Cq) values; the expression of each gene was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Gene-specific primer pairs were used: h-VEGFA: (F) 5'-AGGAGGAGGGCAGAATCATC-3', (R) 5'-ATGTCCACCAGGGTCTCGAT-3'; GAPDH: (F) 5'-TCGGAGTCAACGGATTTGGTC-3', (R) 5'-AAACCATGTAGTTGAGGTCAATG-3'.

#### **4. VEGF ELISA**

LS174T cells were seeded into the wells of 24-well plates ( $2.0 \times 10^5$  cells/well) and incubated at 37 °C overnight. Cells were then incubated with lansoprazole (10  $\mu$ M) or vonoprazan (10  $\mu$ M). After 24 h of incubation, the culture medium was collected and centrifuged at  $500 \times g$  for 5 min to remove floating cells. The cell lysate was prepared using CelLytic™ MT Cell Lysis Reagent (Sigma-Aldrich, St. Louis, MO, USA). The protein concentration of each lysate was measured using a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The VEGF-A secretion in the culture medium was measured using Human VEGF DuoSet ELISA Kit (R&D Systems, Minnesota, US), according to the manufacturer's instructions, and corrected with the protein concentrations.

#### **5. Molecular docking simulation with three-dimensional (3D) protein structures**

The three-dimensional (3D) structure of the target estrogen receptor alpha (ER- $\alpha$ ) (PDB Code: 5DXE) was obtained from the Protein Data Bank (PDB).<sup>18</sup> Six compounds (vonoprazan, omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole) were used as ligands for docking (Fig. 1). The chemical structure of vonoprazan was obtained from the KEGG DRUG database,<sup>19</sup> and those of the other five compounds were obtained from Ma'ayanlab. Cloud.<sup>20</sup> The target ER- $\alpha$  and these six compounds were protonated at pH 7.4.<sup>21</sup> Since lansoprazole, rabeprazole, and pantoprazole are racemic, docking simulations were performed for *R* and *S* isomers. Docking simulations of the ligands with the estrogen binding site of ER- $\alpha$  were performed under the following

settings. The docking configuration search was evaluated using Triangle Matcher and London dG as the score function. The MMFF94 force field was used to refine the docking pose, and then GBVI/WSA dG was used for scoring.<sup>22</sup> All processes were performed using the Molecular Operating Environment (MOE) from the Chemical Computing Group.

## **6. Statistical analysis**

Statistical differences between the two groups were evaluated using Student's *t*-test and between more than three groups using ANOVA with the Tukey posthoc test, using the JSTAT software package (Version 17.0; SPSS, Chicago, IL, U.S.A.). All values are reported as the mean  $\pm$  S.D. The levels of significance were set at  $*p < 0.05$ .

## **Result**

### **1. Effect of vonoprazan on VEGF expression**

Compared to the untreated group, LS174T cells treated with 10  $\mu$ M lansoprazole exhibited a significant 1.6-fold increase in VEGF mRNA expression. In contrast, VEGF mRNA expression did not increase when treated with 10  $\mu$ M of vonoprazan (Fig. 2A). Furthermore, the evaluation of VEGF protein secretion using ELISA revealed that VEGF secretion was significantly increased after lansoprazole treatment but not after vonoprazan treatment (Fig. 2B). The concentrations used in this experiment had no cytotoxic effect on cancer cells (Fig. S1).

### **2. Effect of vonoprazan on VEGF expression in various cancer types**

In this study, OVK-18 (ovarian cancer cell line), MCF-7 (breast cancer cell line), and A549 (lung cancer cell line) were used as cancer types, for which bevacizumab is indicated. Compared with control OVK18 cells, those treated with lansoprazole exhibited significantly upregulated VEGF mRNA expression by 2.7-fold. However, VEGF mRNA expression in vonoprazan-treated cells was similar to that in control cells. Similarly, compared to control MCF7 cells, lansoprazole-treated cells showed upregulated VEGF mRNA expression by 2.0-fold, whereas vonoprazan-treated cells did not show



significantly upregulated VEGF mRNA expression. Furthermore, compared to control A549 cells, lansoprazole-treated cells showed upregulated VEGF mRNA expression by 1.4-fold. However, vonoprazan-treated cells did not show upregulated VEGF mRNA expression (Fig. 3).

### **3. Molecular docking simulations to evaluate the interaction between ER- $\alpha$ and vonoprazan or PPIs**

Using Toxicity predictor v1.5, we evaluated the differences between the activities of five PPIs and vonoprazan toward various transcriptional regulators via the quantitative structure-activity relationship (QSAR) method. The QSAR analysis revealed that ER- $\alpha$  was one of the transcriptional regulators active in all five PPIs but not in vonoprazan (Table S1).

The binding affinity of the five PPIs and vonoprazan for ER- $\alpha$ , one of the transcriptional regulators of VEGF, was calculated using molecular docking simulation. The docking poses of the ligands are displayed in Fig. 4, and the docking score (kcal/mol) for each ligand and ER- $\alpha$  are shown in Table 1. Most of the PPIs showed docking scores comparable to that of estradiol, the *in vivo* ligand for ER- $\alpha$ , whereas the docking score of vonoprazan was slightly lower than that of PPIs.

### **4. ER- $\alpha$ is involved in the upregulation of VEGF expression by PPIs**

We determined the involvement of ER- $\alpha$  in PPI-induced VEGF up-regulation using the ER inhibitor, fulvestrant. We evaluated VEGF mRNA expression and VEGF protein secretion in LS174T1 treated with fulvestrant and lansoprazole or vonoprazan. When treated with 100  $\mu$ M lansoprazole, VEGF mRNA expression was significantly increased to 5.4-fold compared to that in the control; however, this increase was significantly suppressed by fulvestrant (Fig. 5A). When treated with 100  $\mu$ M vonoprazan, VEGF mRNA expression did not increase compared to that in the control, with or without fulvestrant. VEGF protein secretion was significantly elevated after lansoprazole treatment by 2.6-fold higher than that in control. This increase was significantly suppressed by fulvestrant to the same extent as in the control (Fig. 5B). In contrast,

compared to the control, vonoprazan treatment did not yield a significant increase, with or without fulvestrant.

## **Discussion**

In this study, we found that PPIs increase VEGF expression, whereas vonoprazan, a novel gastric acid secretion inhibitor, does not affect VEGF expression, indicating that it might not curtail the efficacy of anti-VEGF drugs. Furthermore, this effect was also observed in ovarian, breast, lung, and colorectal cancer cells, suggesting that the effect is observed regardless of the type of cancer. Molecular docking simulation analysis revealed that vonoprazan and PPI have different activities against ER- $\alpha$ , which may underly the differential effects on VEGF expression. These results suggest that vonoprazan does not affect VEGF expression and may be used in patients with cancer, especially those on anti-VEGF drugs, with less effect on cancer therapy than PPIs.

The effect of vonoprazan on VEGF expression was evaluated using the same colorectal cancer cell line as in a previous study.<sup>9</sup> The results showed that vonoprazan does not upregulate VEGF expression at the gene and protein levels, whereas PPI does. Bevacizumab, an anti-VEGF drug, is widely used to treat various cancers, including colorectal, lung, breast, and ovarian cancers. Therefore, we evaluated the effects of vonoprazan and PPI on VEGF expression in such cancer cell lines. The results showed that PPI increased VEGF expression in all cell lines used in this study, while vonoprazan did not increase VEGF expression in any cell lines. This indicated that PPI might upregulate VEGF expression in various cancer types. In contrast, vonoprazan may not affect VEGF expression in any cancer type.

There are no detailed data on the intra-tumor concentration of PPI or vonoprazan. In the current study, lansoprazole was used at 10  $\mu$ M. Considering that the highest blood concentration of lansoprazole after oral administration of 30 mg of lansoprazole is approximately 3  $\mu$ M,<sup>23</sup> and that PPIs, which are weak-basic drugs, might be easily distributed in tumors because of the weakly acidic conditions in tumor tissue, the concentration of lansoprazole used in this study (10  $\mu$ M) could be feasible in patients with cancer. Furthermore, the expression of VEGF clearly increased after treatment with

lansoprazole, although some lansoprazole was degraded in the medium owing to its instability in an aqueous solution. While the effects of short-term stimulation were evaluated in this study, PPIs are essentially long-term oral medications. Even at low concentrations, long-term exposure to PPIs may increase VEGF expression. Considering these factors, we reasonably speculate that lansoprazole is likely to increase VEGF expression in cancer patients. The highest blood concentration of vonoprazan after oral administration of 20 mg was approximately 0.05  $\mu\text{M}$ ,<sup>24</sup> indicating that the clinical concentration of vonoprazan is lower than the concentration used in the current study. Although vonoprazan is a basic drug that is stable under weakly acidic conditions in tumors and thus may easily distribute, it is unlikely that vonoprazan increases VEGF expression in patients with cancer since no effect of vonoprazan on VEGF expression was observed up to 100  $\mu\text{M}$  in the current study.

Although both lansoprazole and vonoprazan are gastric acid secretion inhibitors, their effects on VEGF expression differ. This suggests that lansoprazole-induced VEGF up-regulation may be due to a different mechanism than the original pharmacological effect of proton pump inhibition.

Our previous study has also shown that five PPIs currently in clinical use (lansoprazole, omeprazole, esomeprazole, rabeprazole, and pantoprazole) commonly increase VEGF expression.<sup>9</sup> All PPIs have a structure consisting of a series of benzimidazole, sulfoxide, and pyridine. PPI-induced VEGF upregulation may be attributed to this common structure. Therefore, we focused on the differences between the chemical structures of PPI and vonoprazan.

Using Toxicity predictor v1.5, the activities of five PPIs and vonoprazan for various transcriptional regulators were evaluated by the QSAR method. We searched for the transcriptional regulator wherein the five PPIs had activity in common and vonoprazan showed no activity (Table S1). The results showed that ER- $\alpha$  was a transcriptional regulator with different activities in PPIs and vonoprazan. ER- $\alpha$  has been known to upregulate VEGF expression during the female menstrual cycle.<sup>25</sup> Furthermore, ER- $\alpha$  is reportedly involved in VEGF upregulation in various cancers,<sup>26-29</sup> and VEGF expression is induced by estradiol (E2), a ligand for ER- $\alpha$ .<sup>30-32</sup> Therefore, in this study,

we focused on ER- $\alpha$  to reveal the difference in the effects of PPI and vonoprazan on VEGF expression.

The affinity of PPI and vonoprazan for ER- $\alpha$  was calculated by docking simulation, and it was found that the docking score of PPI was comparable to that of E2, an *in vivo* ligand. The docking score of vonoprazan was lower than that of PPI, suggesting that vonoprazan has a relatively low stimulatory effect compared with PPIs.

Furthermore, inhibition of ER- $\alpha$  by fulvestrant significantly suppressed lansoprazole-induced up-regulation of the mRNA expression and protein secretion of VEGF. Lansoprazole also up-regulates the expression of EBAG9 and cathepsin D, the target genes of ER- $\alpha$  but vonoprazan did not (Fig S2). These findings indicated that the PPI-induced up-regulation of VEGF expression might be mediated by ER- $\alpha$ . However, fulvestrant treatment did not completely suppress the lansoprazole-induced VEGF mRNA upregulation, suggesting that transcriptional regulators other than ER- $\alpha$  may be involved in this phenomenon. The QSAR analysis also revealed that glucocorticoid receptors (GR) function as transcriptional regulators with different activities in PPI and vonoprazan. Although few reports described the direct regulation of VEGF expression, GR has been reported to modulate angiogenesis, suggesting that GR might also be involved in this phenomenon.<sup>36,37</sup>

Since we have not performed animal experiments or clinical research in this study, it is unclear how the difference in the effects of lansoprazole and vonoprazan on VEGF expression affects the therapeutic effect of anti-VEGF drugs and the exacerbation of cancer. A future, more detailed study integrating clinical data is warranted. In addition, although the binding affinity can be evaluated by docking simulation, the agonist/antagonist activity of the ligand after binding cannot be assessed. The experiments using fulvestrant revealed that the activity for ER- $\alpha$  differs between PPI and vonoprazan. However, the mechanism of this phenomenon needs to be investigated in more detail in the future.

## **Conclusion**

PPIs increase VEGF expression via ER- $\alpha$  in cancer; elevated VEGF expression

leads to cancer progression and diminished therapeutic effect of anti-VEGF drugs. Unlike PPIs, vonoprazan, a novel gastric acid secretion inhibitor, did not upregulate VEGF expression in cancer cell lines, suggesting that vonoprazan may be more effective than PPIs in patients with cancer, especially those receiving anti-VEGF agents, in improving the therapeutic effect of cancer chemotherapy.

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### Figure legends

**Figure 1.** Six chemical structures, including their racemic structures.

(A) Vonoprazan, (B) omeprazole, (C) esomeprazole, (D) *R*-lansoprazole, (E) *S*-lansoprazole, (F) *R*-rabeprazole, (G) *S*-rabeprazole, (H) *R*-pantoprazole, and (I) *S*-pantoprazole.

### Figure 2. Effects of lansoprazole or vonoprazan on the expression of VEGF

LS174T cells were treated with lansoprazole (Lanso) or vonoprazan (Vono) at a concentration of 10  $\mu$ M each. Twenty-four hours later, RNA was collected, and VEGF mRNA expression was evaluated (a). LS174T cells were treated with lansoprazole or vonoprazan at 10  $\mu$ M each. Twenty-four hours later, culture supernatants were collected, and the VEGF concentration in the medium was determined. The total protein concentration in the cell lysate was measured to correct for variations in cell number (b). Data are expressed as mean  $\pm$  SD; \*\* $p < 0.01$

### Figure 3. Effects of lansoprazole or vonoprazan in various cancer cell lines

A549, OVK18, and MCF7 cell lines were treated with 10  $\mu$ M lansoprazole (Lanso) or vonoprazan (Vono), and 24 h later, RNA was collected, and VEGF mRNA expression was evaluated. Data are expressed as mean  $\pm$  SD; \*\* $p < 0.01$

#### Figure 4. Docking poses of compounds

Compounds and estrogen receptor alpha (ER- $\alpha$ ) are shown as a green rod and blue-ribbon models, respectively. (A) Overall structure of ER- $\alpha$  docked with estradiol (PDB:5DXE). (B–J) Docking pose of vonoprazan, Omeprazole, Esomeprazole, *R*-Lansoprazole, *S*-Lansoprazole, *R*-Rabeprazole, *S*-Rabeprazole, *R*-Pantoprazole, and *S*-Pantoprazole.

#### Figure 5. Effects of an Estrogen receptor alpha (ER- $\alpha$ ) inhibitor on the change of VEGF expression by lansoprazole or vonoprazan

LS174T cells were treated with lansoprazole (Lanso) or vonoprazan (Vono) at a concentration of 100  $\mu$ M or fulvestrant (Ful) at 1  $\mu$ M. After 24 h, RNA was collected, and VEGF mRNA expression was assessed (a). At 24 h after adding lansoprazole, Vono, or Ful, culture supernatants were collected, and VEGF concentration was measured. The total protein concentration in lysate was measured to correct for variations in cell numbers. (b) Data are expressed as mean  $\pm$  SD; \* $p$  < 0.05, \*\* $p$  < 0.01

**Table 1. Docking Scores of the following chemical structures with the estrogen receptor alpha**

Chemical Structure	Docking Score (GBVI/WSA dG)
Vonoprazan	-8.44
Omeprazole	-8.81
Esomeprazole	-8.86
<i>R</i> -Lansoprazole	-8.84
<i>S</i> -Lansoprazole	-8.75
<i>R</i> -Rabeprazole	-9.39
<i>S</i> -Rabeprazole	-8.98
<i>R</i> -Pantoprazole	-9.39
<i>S</i> -Pantoprazole	-9.01
Estradiol	-9.04

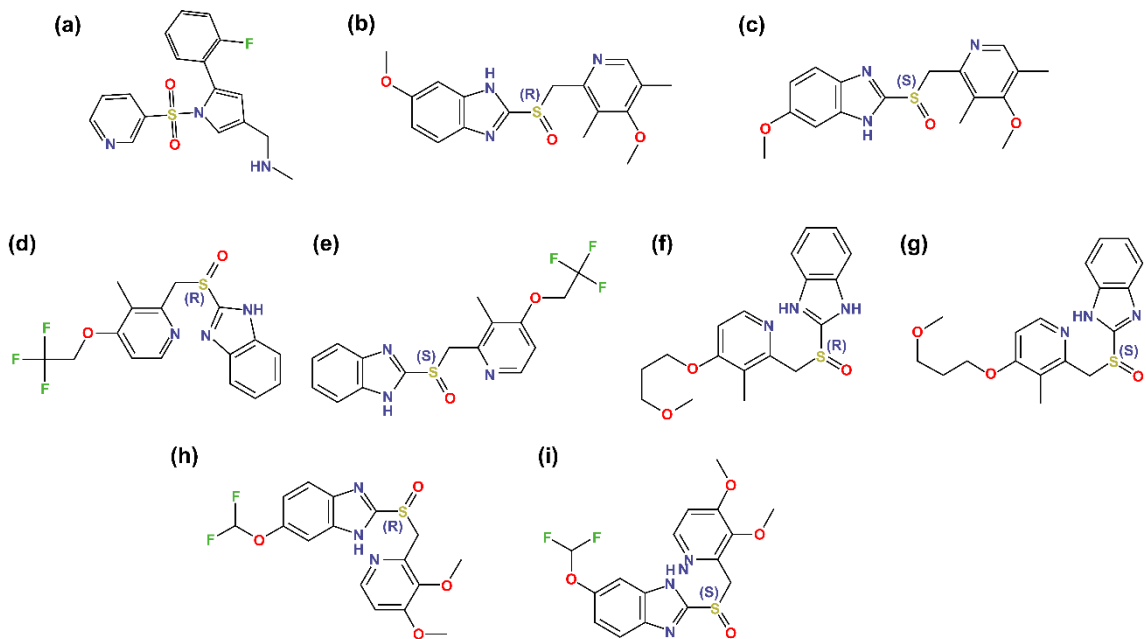
## Supporting information list

Figure S1. *In vitro* cytotoxicity of proton pump inhibitors (PPIs) and vonoprazan in cancer cells.

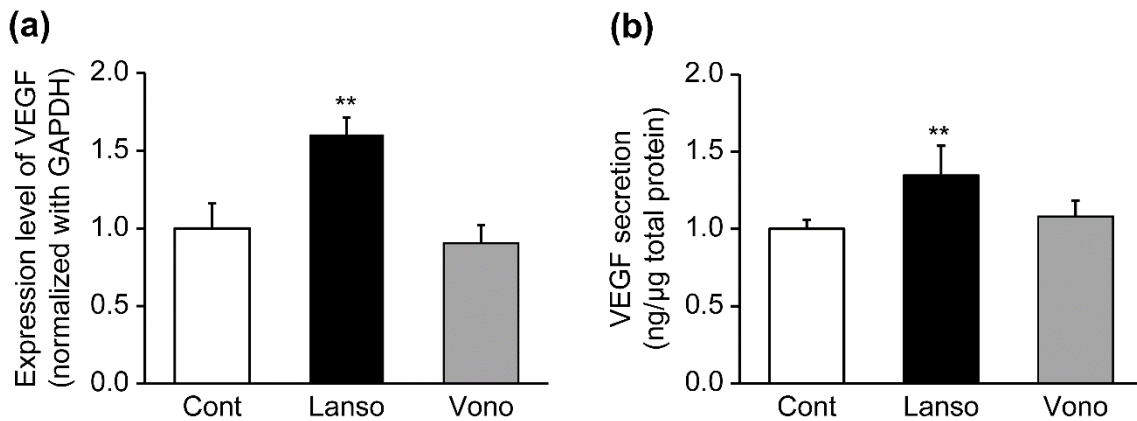
Figure S2. Effect of lansoprazole or vonoprazan on the expression of ER- $\alpha$  target genes

Table S1. Toxicity predictor analysis.

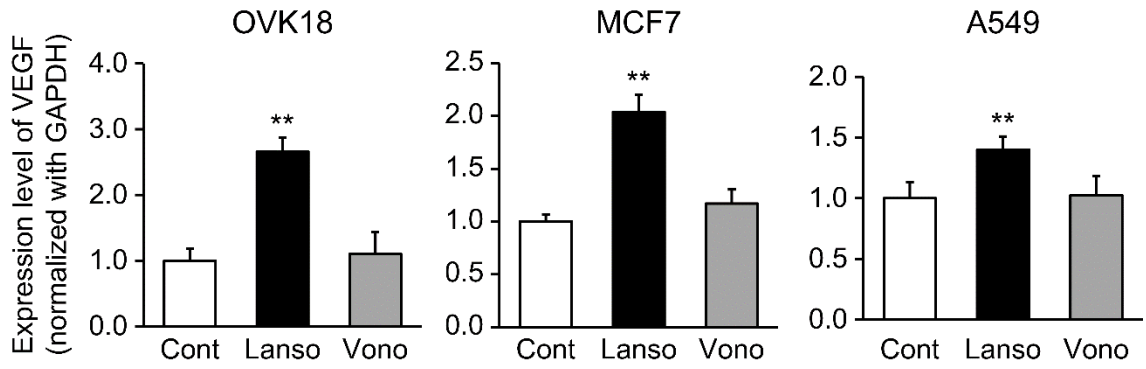
### Figure 1



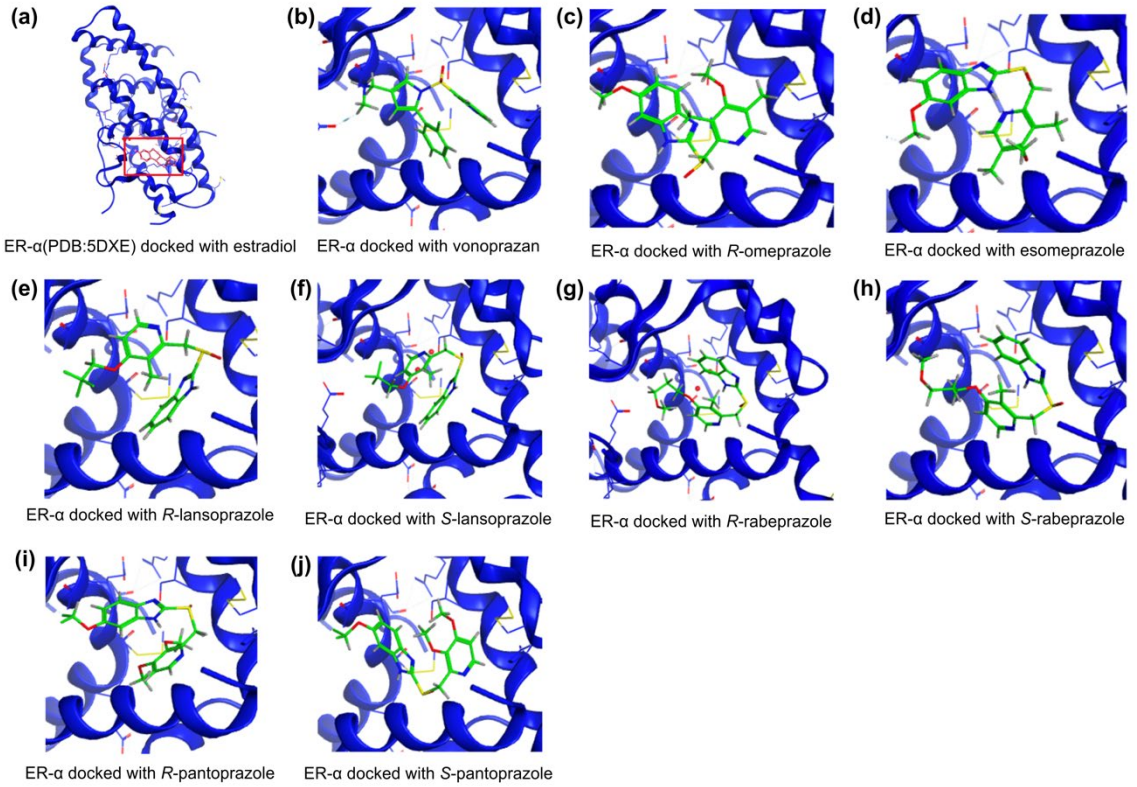
### Figure 2



**Figure 3**



**Figure 4**



**Figure 5**

