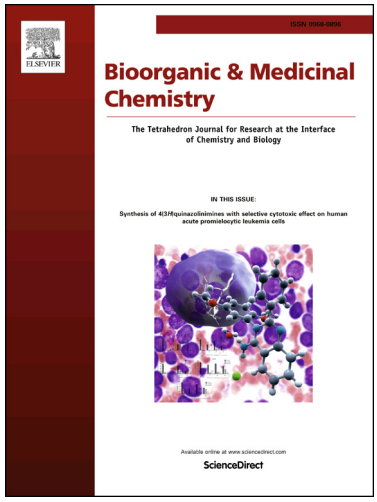
Accepted Manuscript



Identification of a novel fluoropyrrole derivative as a potassium-competitive acid blocker with long duration of action

Haruyuki Nishida, Yasuyoshi Arikawa, Keizo Hirase, Toshihiro Imaeda, Nobuhiro Inatomi, Yasunobu Hori, Jun Matsukawa, Yasushi Fujioka, Teruki Hamada, Motoo Iida, Mitsuyoshi Nishitani, Akio Imanishi, Hideo Fukui, Fumio Itoh, Masahiro Kajino

PII: S0968-0896(17)30497-2
DOI: http://dx.doi.org/10.1016/j.bmc.2017.04.014
Reference: BMC 13684

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 11 March 2017
Revised Date: 9 April 2017
Accepted Date: 11 April 2017

Please cite this article as: Nishida, H., Arikawa, Y., Hirase, K., Imaeda, T., Inatomi, N., Hori, Y., Matsukawa, J., Fujioka, Y., Hamada, T., Iida, M., Nishitani, M., Imanishi, A., Fukui, H., Itoh, F., Kajino, M., Identification of a novel fluoropyrrole derivative as a potassium-competitive acid blocker with long duration of action, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.04.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Identification of a novel fluoropyrrole derivative as a potassium-competitive acid blocker with long duration of action

Haruyuki Nishida*, Yasuyoshi Arikawa, Keizo Hirase, Toshihiro Imaeda, Nobuhiro Inatomi, Yasunobu Hori, Jun Matsukawa, Yasushi Fujioka, Teruki Hamada, Motoo Iida, Mitsuyoshi Nishitani, Akio Imanishi, Hideo Fukui, Fumio Itoh, and Masahiro Kajino

Pharmaceutical Research Division: Takeda Pharmaceutical Company, Ltd., 26-1, Muraokahigashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan

*To whom correspondence should be addressed. Phone: +81 466-32-1239. Fax: +81 466-29-4536. E-mail: haruyuki.nishida@takeda.com
Present address: 2-26-1, Muraokahigashi, Fujisawa, Kanagawa 251-8555, Japan

Keywords: H^+,K^+-ATPase; potassium-competitive acid blocker; fluoropyrrole; low lipophilicity; long duration of action

Abbreviations: absorption, distribution, metabolism, excretion, and toxicity (ADME-Tox), hepatic cytochrome P450 2C19 (CYP2C19), hepatic cytochrome P450 3A4 (CYP3A4), differential scanning calorimetry (DSC), Diisobutylaluminium hydride (DIBAL-H), 1,2-dimethoxyethane (DME), *N*,*N*-dimethylformamide (DMF), drug metabolism and pharmacokinetics (DMPK), half-maximal inhibitory concentration (IC_{50}), high-performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), human ether-a-go-go-related gene (hERG), intravenous injection (iv), ligand-lipophilicity efficiency (LLE), liquid chromatography with tandem mass spectrometry (LC/MS/MS), lithium diisopropylamide (LDA), melting point (mp), methoxy (MeO), molecular sieves 4 angstrom (MS4Å), *N*-chlorosuccinimide (NCS), *N*-methylmorpholine *N*-oxide (NMO), parallel artificial membrane permeability assay (PAMPA), per os (po), potassium-competitive acid blocker (P-CAB), proton pump inhibitor (PPI), pyridyl (Py), relative light units (RLU), room temperature (rt), structure-activity relationship (SAR), tetrahydrofuran (THF), thermogravimetry-differential thermal analysis (TG-DTA), thin-layer chromatography (TLC), p-toluenesulfonyl chloride (TsCl), tetrapropylammonium perruthenate (TPAP)

Abstract

With the aim to find a novel long-lasting potassium-competitive acid blocker (P-CAB) that would perfectly overcome the limitations of proton pump inhibitors (PPIs), we tried various approaches based on pyrrole derivative **1b** as a lead compound. As part of a comprehensive approach to

ACCEPTED MANUSCRIPT

identification of a new drug, we explored excellent compounds that have low lipophilicity by introducing a polar hetero-aromatic group at position 5 of the pyrrole ring. Among the compounds synthesized, fluoropyrrole derivative **37c**, which has a 2-F-3-Py group at the fifth position, lower pK_a, and much lower Clog P and log D values than **1b** dose, showed potent gastric-acid suppressive action resulting from gastric H^+,K^+-ATPase inhibition in animal models. Its maximum intragastric pH elevation effect was strong in rats, and its duration of action was much longer than that of either lansoprazole or lead compound **1b** in dogs. Therefore, compound **37c** can be considered a promising new P-CAB with long duration of action.

1. Introduction

Gastric H^+,K^+-ATPase is the key enzyme at the final step of gastric acid secretion. Proton pump inhibitors (PPIs) such as lansoprazole, omeprazole, rabeprazole, and pantoprazole inhibit gastric H^+,K^+-ATPase by covalently binding to its sulfhydryl group, resulting in the inhibition of gastric acid secretion.[1–5] PPIs are widely used in the treatment of acid-related diseases such as gastroesophageal reflux disease, and peptic ulcer disease,[6–8] and in combination with antibiotics to eradicate *Helicobacter pylori*.[9] Although PPIs are now the mainstay of therapy for acid-related diseases, there are several limitations in terms of acid lability, delayed onset of action, variation of efficacy among patients (largely because of CYP2C19 metabolism), and insufficient inhibition of nocturnal acid breakthrough.[10–14]

For improved intragastric pH control, several strategies other than PPIs have been explored. Potassium-competitive acid blocker (P-CAB)-type inhibitors of H^+,K^+-ATPase hold promise for better treatment of acid-related diseases. P-CABs, as the name suggests, inhibit H^+,K^+-ATPase activity in gastric parietal cells reversibly and in a potassium-competitive manner.[15]

Several pharmaceutical companies have attempted to develop P-CABs (Figure 1).

ACCEPTED MANUSCRIPT

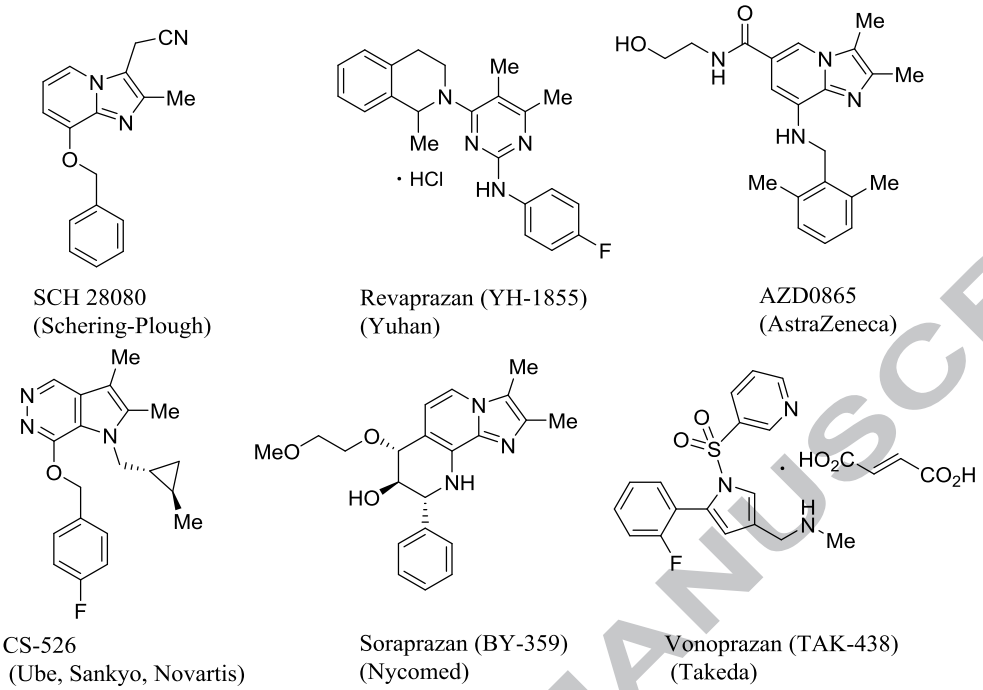


Figure 1. Structures of several reported potassium-competitive acid blockers.

These compounds were found to show a rapid onset of inhibition of acid secretion on the basis of rapid attainment of their peak plasma concentration in clinical studies. Moreover, the full effect was observed after the first dose. Among these compounds, revaprazan is used clinically only in South Korea and India.[16,17] Vonoprazan fumarate (TAK-438) was recently approved in Japan for the treatment of gastric ulcer, duodenal ulcer, erosive esophagitis, prevention of low-dose aspirin- or non-steroidal anti-inflammatory drug-induced ulcer recurrence, and as an adjunctive therapy for *Helicobacter pylori* eradication.[17,18] Other P-CABs are not used clinically because of their insufficient efficacy and/or hepatic toxicity.[19–24]

In our previous paper,[25] we have reported novel pyrrole compound **1b** and its derivatives as P-CABs (Figure 2), which showed not only potent and selective H^+,K^+-ATPase inhibitory activity in vitro but also potent inhibitory action on histamine-stimulated gastric acid secretion in rats and Heidenhain pouch dogs. Moreover, the duration of action of compound **1b** in Heidenhain pouch dog was obviously longer than that of lansoprazole.[25]

These results encouraged us to continue the optimization to identify a new type of P-CAB with longer duration of action in humans, while compound **1b** needed considerable improvement in various ADME-Tox parameters such as cytotoxicity and hERG-inhibitory activity.

Through the extensive data analysis of our pyrrole derivatives including the novel approach to evaluation of the structure–toxicity relations, a relatively strong correlation between the actual measured log D value at pH 7.4 and in vitro cytotoxicity was observed. In addition, a slight

ACCEPTED MANUSCRIPT

correlation between the measured log D value and hERG inhibition was estimated.

Consequently, we advanced a hypothesis that pyrroles with lowered log D have excellent ADME-Tox parameters. Moreover, such pyrroles may stay longer in the stomach in terms of the decline of membrane permeability in an acidic environment in addition to the physicochemical acid resistance.

Subsequently, we carried out further optimization of compound **1b**, which led to identification of TAK-438, which showed excellent activities in rats and Heidenhain pouch dogs.[26,27] In the study where TAK-438 was found, we initially focused on replacing the phenyl (Ph) group at the first position of the pyrrole ring through docking analysis in an H^+,K^+-ATPase homology model, and identified the 3-pyridyl (3-Py) group as the best substituent, which yielded potent activity, low lipophilicity, and preferable properties, mainly because of ligand-lipophilicity efficiency (LLE = pIC_{50} − log D).[28]

Apart from that optimization effort, we tried exploring highly safe compounds with longer duration of action by introducing a polar hetero-aromatic group at position 5 of the pyrrole ring based on the hypothesis about our pyrroles (Figure 2), and succeeded only in identifying a novel fluoropyrrole derivative after further optimizations.

Herein we report identification of a novel fluoropyrrole derivative as a P-CAB with long duration of action.

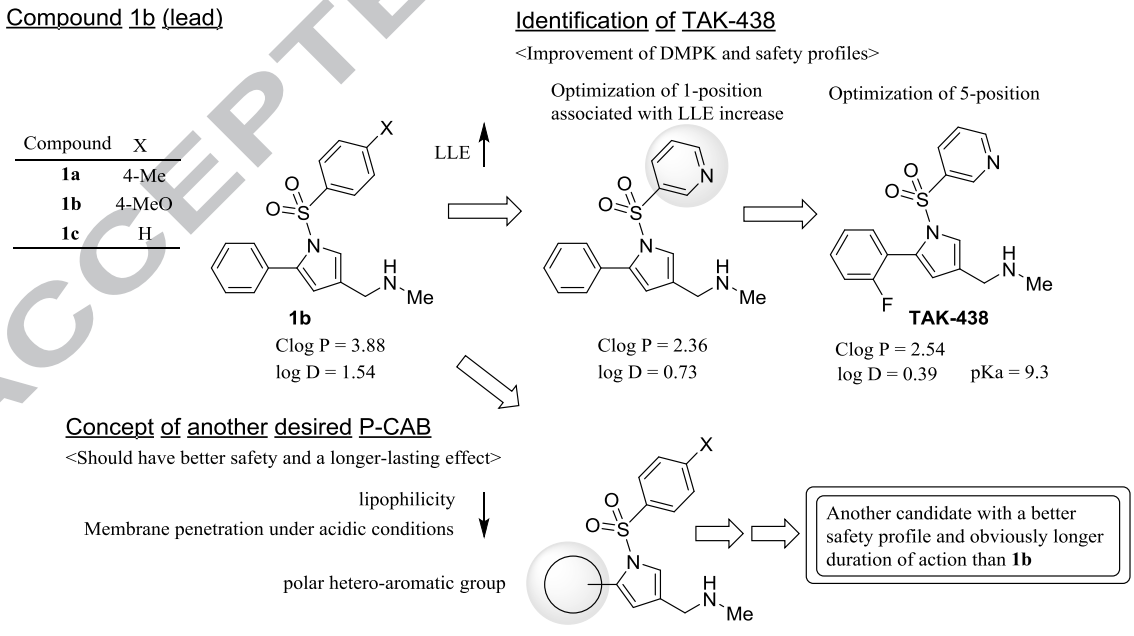
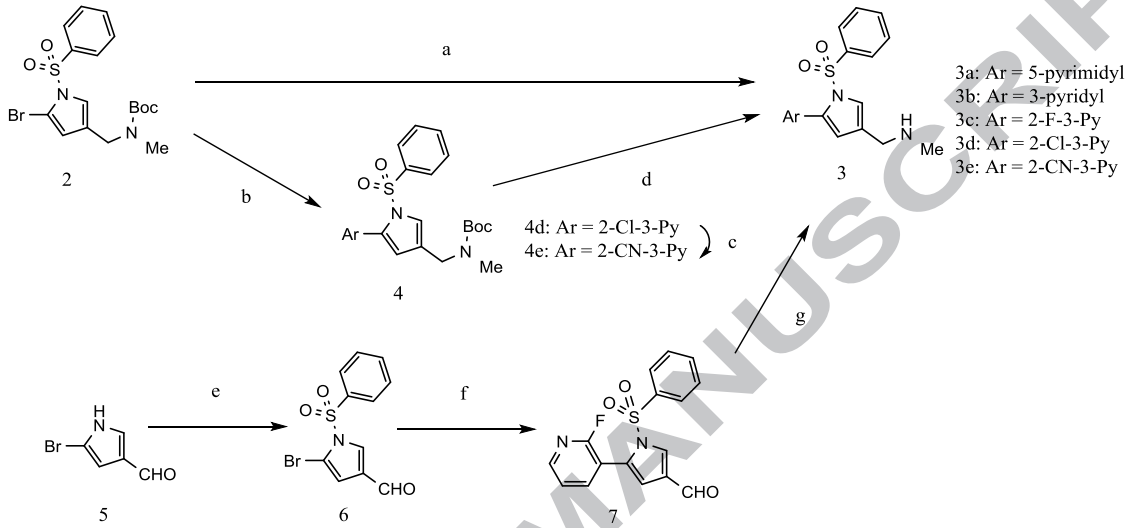


Figure 2. Another approach to identification of a novel pyrrole derivative as a P-CAB with better safety and a longer-lasting effect.

ACCEPTED MANUSCRIPT

2. Chemistry

The synthesis of initial-stage key compounds containing polar hetero-aromatic groups at position 5, e.g., **3a–e** was mainly accomplished by starting from two previously reported bromo-pyrrole intermediates **2** and **5** as shown in Scheme 1.[25,28]



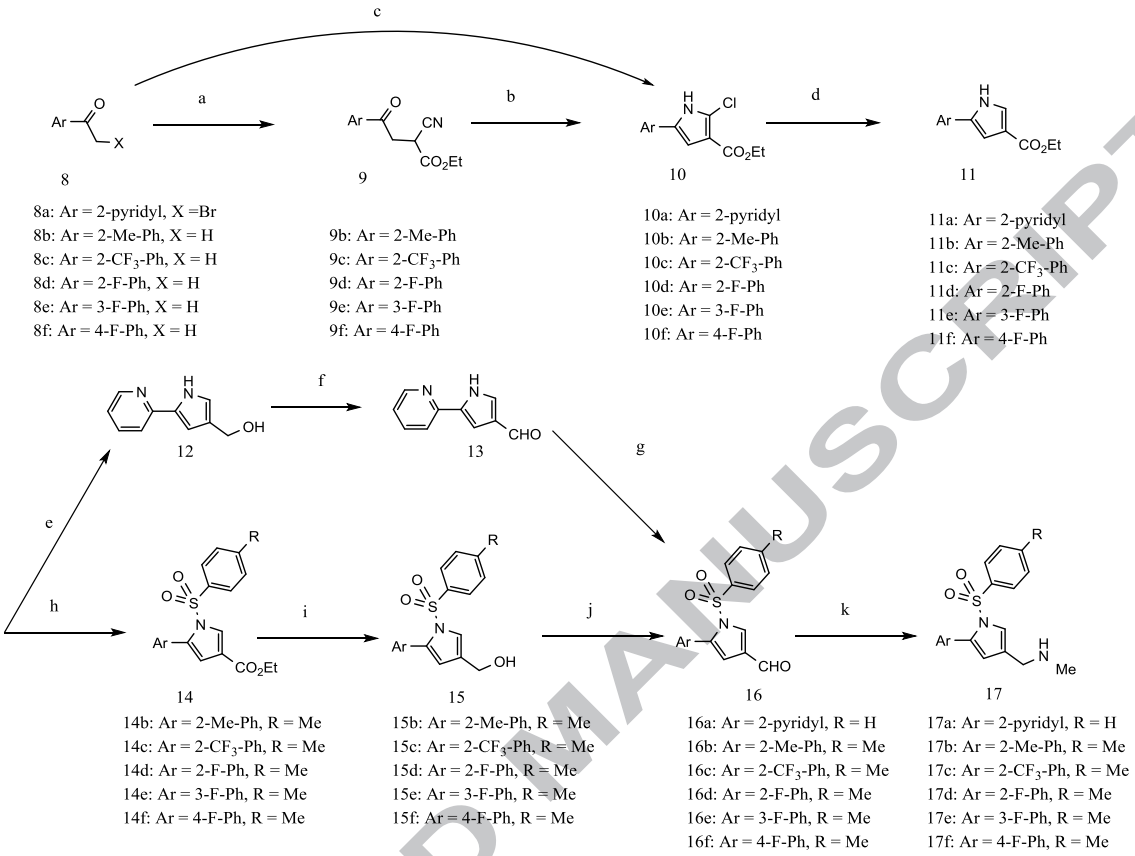
Scheme 1. Reagents and conditions: (a) (1) Ar-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 90 °C; (2) 4 mol/L HCl/EtOAc, MeOH, 70 °C; (b) Ar-$B(O\text{-}iPr)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 105 °C; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C; (d) 4 mol/L HCl/EtOAc, EtOH, room temperature (rt), or 4 mol/L HCl/EtOAc, MeOH, EtOAc, rt; (e) NaH, Ph-SO_2Cl, THF, rt; (f) 2-F-3-Py-$B(OH)_3$, $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, 80 °C; (g) (1) 40% $MeNH_2$ in MeOH, MeOH, rt; (2) $NaBH_4$, rt; (3) fumaric acid, EtOH.

Compounds **3a** and **3b** were synthesized from **2** via the Suzuki-Miyaura coupling reaction using corresponding boronic acid followed by treatment with a strong acid. Sulfonylation of **5** under basic conditions yielded sulfonyl pyrrole **6**; then, we used the Suzuki-Miyaura coupling reaction with 2-F-3-Py boronic acid, which afforded compound **7**, which was then converted by reductive amination to compound **3c** containing an *N*-methyl methanamine moiety.

2-Cl-3-Py derivative **4d**, which was isolated before deprotection, was transformed into 2-CN-3-Py derivative **4e** by Pd-catalyzed cyanation. Thus obtained compounds **4d** and **4e** were finally deprotected by treatment with hydrogen chloride to produce compounds **3d** and **3e**, respectively, as hydrochlorides (Scheme 1).

The synthesis of hetero-aryl and aryl derivatives at the fifth position, e.g., 2-Py compound **17a** and substituted phenyl compounds **17b-f** was conducted as shown in Scheme 2.

ACCEPTED MANUSCRIPT

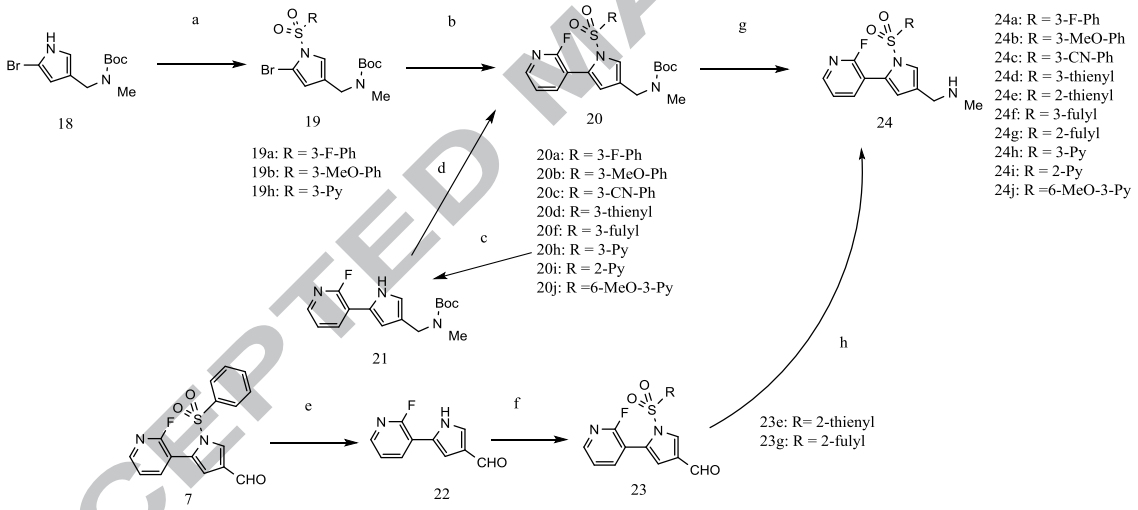


Scheme 2. Reagents and conditions: (a) (1) Br_2, Et_2O, rt, or Br_2, Et_2O, $CHCl_3$, rt, or $CuBr_2$, AcOEt, refluxed temperature; (2) ethyl cyanoacetate, K_2CO_3, 40–45 °C, then rt, acetone; (b) HCl(g), THF, rt, or 4 mol/L HCl/EtOAc, rt; (c) (1) ethyl cyanoacetate, K_2CO_3, acetone, 45 °C; (2) 4 mol/L HCl/EtOAc, 60 °C; (3) 4 mol/L HCl/EtOAc, EtOAc; (d) H_2, 10% Pd–C, EtOH, 50 °C or rt; (e) 1.5 mol/L DIBAL-H in toluene, THF, −50 °C; (f) TPAP, NMO, MS4Å, MeCN, rt; (g) NaH, THF, 15-crown-5, Ph-SO_2Cl, rt; (h) NaH, DMF, TsCl, rt; (i) 1.5 mol/L DIBAL-H in toluene, THF, −78 °C; (j) TPAP, MNO, MS4Å, MeCN, rt; (k) 40% $MeNH_2$ in MeOH, MeOH, $NaBH_4$, rt, or methylamine hydrochloride, $NaBH_3CN$, THF, rt.

Each pyrrole-3-carboxylic acid ester **11** was synthesized from the corresponding α-bromo derivatives, which are commercially available **8a** or were prepared via bromination of acetophenone derivatives **8b–f**. Condensation of α-bromo derivatives of **8b–f** with ethyl cyanoacetate yielded corresponding intermediates **9b–f**, which were cyclized under acidic conditions to obtain **10b–f**, followed by dehalogenation to produce pyrrole-3-carboxylic acid esters **11b–f**. As for compound **8a**, it was also converted to cyclized compound **10a** without intermediate isolation by a similar method, followed by dehalogenation by a similar method to obtain **11a**.

ACCEPTED MANUSCRIPT

Reduction of the ester group of **11a** with diisobutylaluminum hydride (DIBAL-H) gave alcohol **12**, which was followed by oxidation with tetra-*n*-propylammonium perruthenate and *N*-methylmorpholine-*N*-oxide to obtain formyl pyrrole **13**. The latter was then sulfonylated by means of benzene sulfonyl chloride in the presence of a base to produce sulfonyl derivative **16a**. Sulfonylation of **11b–f** was performed in a manner similar to the above-mentioned method to obtain the corresponding sulfonyl derivatives **14**, which were converted to formyl compounds **16** via alcohol **15** using a procedure similar to the method for transformation of **11a** into **13**. Subsequent transformation of formyl derivatives **16** into methylaminomethyl derivatives **17** was also conducted in a manner similar to the above-mentioned procedure (Scheme 2). Conversion of the aryl sulfonyl group at the first position including the hetero-aromatic group was achieved via 5-(2-fluoropyridin-3-yl) -1*H*-pyrrole intermediates **20** starting from compound **18**, which was reported in our previous paper,[28] or via another similar intermediate **23** starting from compound **7**. Their synthesis methods are shown in Scheme 3.



Scheme 3. Reagents and conditions: (a) NaH, 15-crown-5, R-SO_2Cl, THF, rt; (b) 2-F-3-Py-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, DME, H_2O, 105 °C; (c) 8 mol/L NaOH, THF, MeOH, rt; (d) NaH, 15-crown-5, R-SO_2Cl, THF, 0 °C, then rt; (e) 8 mol/L NaOH, THF, MeOH, rt; (f) NaH, THF, 15-crown-5, R-SO_2Cl, rt; (g) 4 mol/L HCl/EtOAc, EtOH, rt or (1) 4 mol/L HCl/EtOAc, MeOH, EtOAc, rt; (2) $NaHCO_3$, H_2O; (3) fumaric acid, MeOH or EtOH, EtOAc. (h) (1) 40% $MeNH_2$ in MeOH, MeOH, rt; (2) $NaBH_4$, rt; (3) fumaric acid, MeOH, EtOAc.

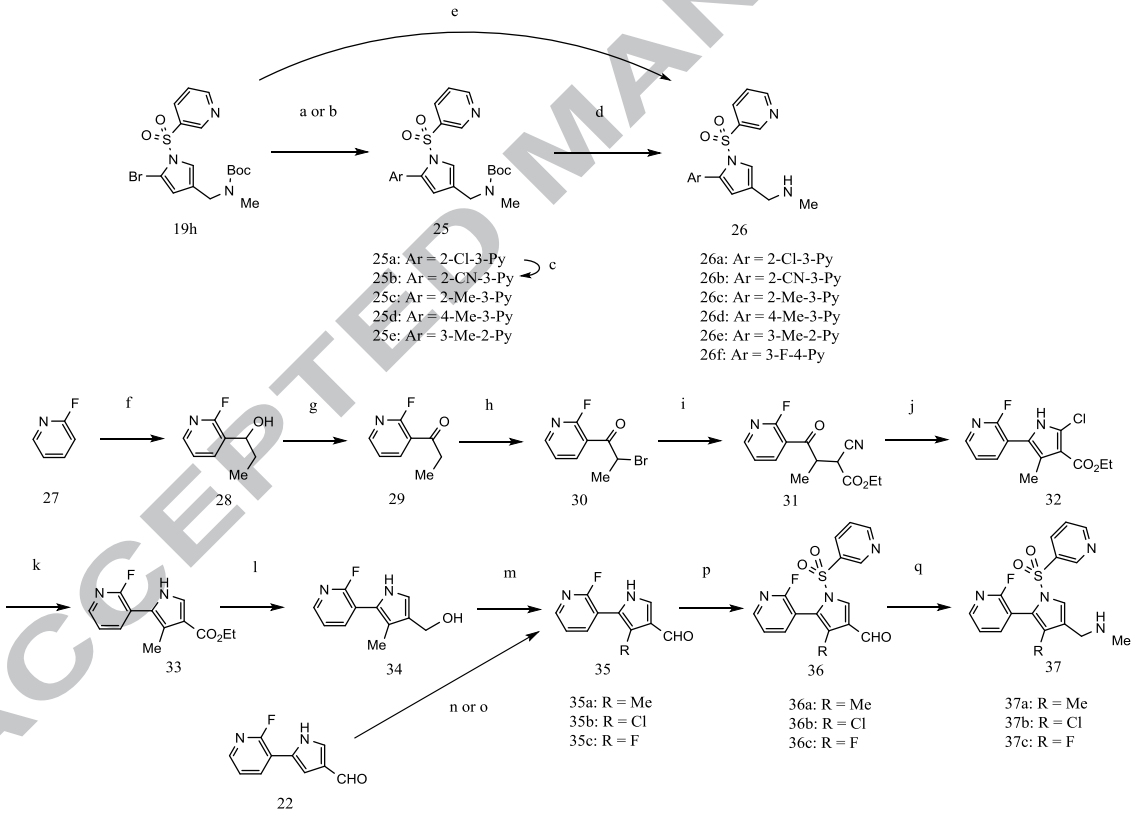
As for sulfonylation of **18**, **21**, and **22**, addition of 15-crown-5 under basic conditions was essential or effective for this reaction in many cases. In particular, the reaction did not proceed without 15-crown-5 in the case of a highly polar sulfonyl chloride with low reactivity and/or low stability in

ACCEPTED MANUSCRIPT

a reaction mixture. It is presumed that 15-crown-5 largely enhanced the reactivity of the pyrrole anion by keeping the sodium ion in the molecule and by reducing the influence on the pyrrole anion. The reaction of coupling of **19** with 2-F-3-Py boronic acid and subsequent N-Boc deprotection of **20** were conducted using an approach similar to the above-mentioned method.

Desulfonylation reaction of compound **7** with a strong alkali afforded key intermediate **22**, which was sulfonylated in a manner similar to the procedure mentioned before to obtain **23**, followed by introduction of an *N*-methyl methanamine moiety by a method similar to the procedure for transformation of **7** into **3** to produce target compounds **24** (Scheme 3).

The synthesis of various pyridyl derivatives substituted at position 5 and introduction of a methyl and halogen group at position 4 were achieved by starting from intermediate **19h**, commercially available **27**, and intermediate **22**, respectively. Their synthesis methods are shown in Scheme 4.



Scheme 4. Reagents and conditions: (a) $ArB(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, DME, H_2O, 105 °C; (b) 3-Me-2-($SnBu_3$)Py, $Pd(PPh_3)_4$, toluene, 120 °C; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C; (d) (1) 4 mol/L HCl/EtOAc, MeOH, EtOAc, rt or 70 °C; (2) $NaHCO_3$, H_2O; (3) fumaric acid, MeOH or EtOH, EtOAc; (e) (1) 3-F-4-Py-$B(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, 80 °C; (2) 4 mol/L HCl/EtOAc, MeOH, 70 °C; (3) $NaHCO_3$, H_2O; (4) fumaric acid, MeOH, EtOAc; (f) (1) LDA, THF, –78 °C; (2) 2-F-Py, THF, –78 °C; (3) propionaldehyde, THF, –78 °C; (g) SO_3-Py, Et_3N, DMSO, rt; (h) Br_2, 25%

ACCEPTED MANUSCRIPT

HBr, AcOH, rt; (i) ethyl cyanoacetate, iPr_2NEt, THF, rt; (j) 4 mol/L HCl/EtOAc, EtOAc, rt; (k) H_2, 10% Pd–C, Et_3N, EtOH, 60 °C; (l) 1.5 mol/L DIBAL-H in toluene, THF, –78 °C, then 0 °C; (m) TPAP, NMO, MS4Å, MeCN, rt; (n) 1-fluoro-2,6-dichloropyridinium triflate, THF, MeCN, rt (R = F); (o) NCS, DMF, 80 °C (R = Cl); (p) NaH, THF, 15-crown-5, 3-Py-SO_2Cl, rt; (q) (1) 40% $MeNH_2$ in MeOH, MeOH, THF, rt; (2) $NaBH_4$, rt; (3) fumaric acid, EtOH, EtOAc; or (1) methylamine hydrochloride, $NaBH(OAc)_3$, MeOH, rt; (2) fumaric acid, MeOH, EtOAc, or fumaric acid, EtOH, EtOAc.

Conversion of the bromine group of **19h** to various pyridyl groups via the Suzuki-Miyaura coupling reaction or Migita-Kosugi-Stille coupling reaction gave **25**, which was then subjected to a deprotection reaction to obtain compounds **26** in a manner similar to the above-mentioned procedure.

Reaction of **27** with LDA followed by a reaction of nucleophilic addition to propionaldehyde yielded secondary alcohol **28**, which was converted via an oxidation reaction with a pyridine-sulfur trioxide complex to ketone derivative **29** followed by bromination to produce α-bromo derivative **30**. Condensation of compound **30** with ethyl cyanoacetate in the presence of *N*, *N*-diisopropylethylamine afforded intermediate **31**, which was cyclized under acidic conditions to obtain **32** followed by dehalogenation to produce pyrrole-3-carboxylic acid ester **33**. Subsequent reduction of **33** and oxidation of **34** to obtain **35a** were conducted by a method similar to the procedure described above.

Chlorination of **22** with NCS gave **35b**, and fluorination of **22** was performed by means of 1-fluoro-2,6-dichloropyridinium triflate to obtain **35c**.

Subsequent sulfonylation of **35** in the presence of 15-crown-5 and reductive amination of **36** to produce compounds **37** were performed in a manner similar to the method described above (Scheme 4).

Intermediates **2**, **5**, and **18** were prepared by a method similar to a previously reported procedure.[25,28] As for compounds **1a–c** described above, they were prepared in a manner similar to a previously reported method.[25]

3. Results and discussion

The compounds synthesized were evaluated for their Clog P values, log D values at pH 7.4, and H^+,K^+-ATPase-inhibitory activities at pH 6.5 according to their IC_{50} values (in vitro), and some of the compounds were analyzed for their inhibitory effects on histamine-induced gastric acid secretion in anesthetized rats (in vivo), effects on ATP content (cytotoxicity) and hERG-inhibitory activity. We also calculated their values of LLE (= pIC_{50} − log D), which is a parameter for estimating their drug-likeness based on their linking potency and lipophilicity,[29] to facilitate the selection of better

ACCEPTED MANUSCRIPT

compounds.

An in vivo assay was carried out by intravenous administration of the compound at 1 mg/kg, and the total acid output for 3 h after histamine injection was compared to that obtained after administration of vehicle. The log D values were measured at pH 7.4 with relative retention time to standard compounds in HPLC analysis.[30] The results are shown in Tables 1, 3 and 4.

First, we examined the effects of replacement of the phenyl group with highly polar heteroaromatic groups at the fifth position (R^1) of the pyrrole ring to assess the possibility of favorable drug-like profiles, and the results are summarized in Table 1.

5-Pyrimidyl compound **3a**, 3-pyridyl compound **3b** and 2-pyridyl compound **17a** all showed a more dramatic reduction in log D values than expected from their Clog P values, although their in vitro activities were considerably reduced as compared to original phenyl compound **1c**. As we expected, their ADME-Tox parameters in vitro were noticeably improved. Especially, 3-pyridyl compound **3b** had a relatively large LLE value (6.81), and actually showed good ADME-Tox parameters such as metabolic stability, solubility, CYP inhibition, cytotoxicity, and hERG inhibition (Table 1, some data not shown). These results prompted us to continue further modification of 3-pyridyl compound **3b**, although the basicity of pyridine at position 5 was assumed to be not beneficial for relevant activities judging by the results of both previous docking analysis in an H^+,K^+-ATPase model and actual in vivo activity on this compound (4% inhibition at 1mg/kg, iv).

To estimate the priority of further modifications of **3b**, the influence of some substituent groups on activities and ADME-Tox parameters was studied on the basis of compound **1a**. 2-Me-Ph derivative **17b**, 2-CF_3-Ph derivative **17c**, and 2-F-Ph derivative **17d**, all of which have some kind of substituent at the *ortho*-position of the phenyl group at position 5 of the pyrrole ring, all showed enhanced in vivo activities: inhibition by 92%, 86% and 91% at 1mg/kg, iv, respectively. Although ADME-Tox parameters of 2-Me-Ph derivative **17b** and 2-CF_3-Ph derivative **17c** were worse, e.g., in terms of CYP3A4 inhibition and cytotoxicity, 2-F-Ph derivative **17d** showed relatively good parameters (Table 1, some data not shown). Moreover, its log D value (1.56) was much lower than that of original Ph compound **1a** (log D = 1.84) contrary to our expectations. On the other hand, 3-F-Ph derivative **17e** (log D = 1.98) and 4-F-Ph derivative **17f** (log D = 2.05) had greater log D values than **1a** did, reflecting the increased Clog P values caused by lipophilicity of the fluorine atom. pK_a values of **17d**, **17e**, **17f**, and **1a** were 9.48, 9.31, 9.40, and 9.49, respectively, and there were no significant differences among these four compounds. Although we cannot offer a conclusive explanation for such unique behavior of the log D value at present, it may be caused by the presence of an oxygen atom close to a fluorine.[31] Steric conformations among these pyrrole compounds were almost comparable in single-crystal structures including non-fluorinated compound **1a** (Figure 3), but the shortest distance between oxygen atoms and the fluorine atom in each single-crystal structure was 4.34 Å in *ortho* compound **17d**, 5.53 Å in *meta* compound **17e**, and 5.80 Å in *para* compound

ACCEPTED MANUSCRIPT

17f. In addition, the shortest distance between a fluorine and the center of benzene ring of the tosyl group was 4.02 Å in *ortho* compound **17d**, 6.06 Å in *meta* compound **17e**, and 7.26 Å in *para* compound **17f**.

To determine the behavior in an aqueous solution, several NMR analyses in D_2O and DMSO-d_6 were conducted (Table 2). Because in D_2O, there were few significant differences in chemical shifts in the benzene ring of the tosyl group and methylaminomethyl group among the compounds, it was presumed that they indicated a high potential for almost the same conformation even in an aqueous solution regardless of whether fluorine is actually presemt at any position. Nevertheless, the chemical shifts of ^{13}C-NMR in the tosyl group were almost comparable among four compounds, but the chemical shifts of ^{1}H-NMR in the tosyl group of *ortho* compound **17d** were slightly shifted downfield as compared to the other compounds (Table 2). This downfield shift of the proton signals seems to be caused by the influence of the interaction with the fluorine group of **17d** because the distance between the fluorine atom and each benzene ring proton of the tosyl group was significantly shorter than that in **17e** and **17f**. Judging by this measurable fluorine effect, it is possible that such a fluorine group polarizes the neighboring oxygen atoms, and this change leads to stronger hydrogen bonds between the oxygen atoms and neighboring water molecules in addition to increasing overall polarity of the molecule.

Moreover, *ortho* compound **17d** showed a much smaller difference in the chemical shift during ^{19}F-NMR analysis between the DMSO-d_6 solution and D_2O solution as compared to the other compounds (Table 2). This result suggests that a stronger intramolecular interaction of fluorine may form in the case of *ortho* compound **17d**.

In terms of their overall inhibitory activities in vivo including DMPK properties, *ortho* compound **17d** (IC_{50} = 34 nM, 91% inhibition at 1mg/kg, iv) showed excellent inhibition as compared to *meta* compound **17e** (IC_{50} = 19 nM, 77% inhibition at 1mg/kg, iv) or *para* compound **17f** (IC_{50} = 90 nM, 86% inhibition at 1mg/kg, iv).

With these observations in mind, we designed several 2-(electron-withdrawing substituent)-3-Py derivatives including a hybrid compound with the 2-F-3-Py group. Exceeding our expectations, each of the three synthesized compounds (**3c**, **3d**, and **3e**) had both a low log D value and strong gastric-acid suppressive activity in rats. Their in vivo potency was even stronger than that of lead compound **1b**. 2-CN-3-Py derivative **3e** had relatively weak in vitro activity (IC_{50} = 120 nM) but showed strong in vivo action and an excellent profiles of ADME-Tox parameters.

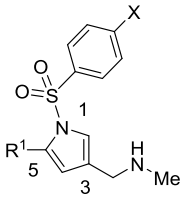
In contrast, 2-F-3-Py derivative **3c** and 2-Cl-3-Py derivative **3d** showed strong activities not only in vivo but also in vitro (IC_{50}=26 and 43 nM, respectively). Nevertheless, their hERG-inhibitory activities were slightly out of the acceptable range for their preferable ADME-Tox profiles.

It seems to be difficult to discuss the relative superiority of the three compounds including their actual potency at this stage of the lead optimization study, but the direction of compound design

ACCEPTED MANUSCRIPT

based on our hypothesis was assumed to be basically appropriate because their LLE values increased. Among the three compounds that showed stronger in vivo action than **1a** did (95% inhibition), 2-F-3-Py derivative **3c** had the highest LLE value (7.68), accompanied by high potency (IC_{50} = 26 nM) and low log D value (–0.09).

Table 1 Effects of substituents at position 5 (R^1) on activities and properties of pyrrole compounds



Compound.	R^1	X	Clog P	log D	In vitro H^+,K^+-ATPase inhibitory activities (IC_{50}, nM)	LLE	In vivo Acid secretion in rats (1 mg/kg, iv, % inhibition)	ATP content at 100 μM (%control)	hERG % inhibition at 10 μM FCS (-)
1a	Ph	Me	4.21	1.83	46	5.51	66	(38.6)[a]	NT[b]
1b	Ph	MeO	3.88	1.54	30	5.98	95	(22.1)[a]	89.1
1c	Ph	H	3.71	1.44	9.4	6.59	96	(53.8)[a]	87.5
3a	5-Pyrimidyl	H	1.42	–0.4	410	6.79	NT[b]	97.5	24.2
3b	3-Py	H	2.36	0.08	130	6.81	4	95	43.3
17a	2-Py	H	2.57	0.28	120	6.64	NT[b]	91.2	48.0
17b	2-Me-Ph	Me	4.41	2.12	62	5.09	92	(0.3)[a]	NT[b]
17c	2-CF_3-Ph	Me	5.15	1.94	88	5.12	86	(0.5)[a]	NT[b]
17d	2-F-Ph	Me	4.39	1.56	34	5.91	91	(8)[a]	NT[b]
17e	3-F-Ph	Me	4.39	1.98	19	5.74	77	(0.6)[a]	NT[b]
17f	4-F-Ph	Me	4.39	2.05	90	5.00	86	(0.4)[a]	NT[b]
3c	2-F-3-Py	H	2.56	–0.09	26	7.68	99	59.9	57.0
3d	2-Cl-3-Py	H	2.88	–0.1	43	7.47	99	76.3	40.9
3e	2-CN-3-Py	H	2.17	–0.32	120	7.24	99	82.1	32.9

[a] ATP content at 30 μM (% control)

[b] Not tested

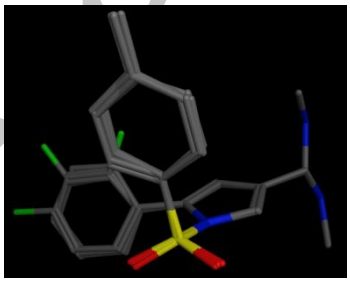
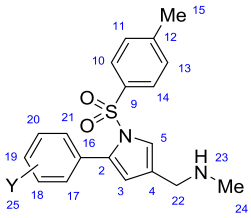


Figure 3. Superposition of single-crystal structures obtained by X-ray structural analysis for **1a**, 2-F-Ph (*ortho*) compound **17d**, 3-F-Ph (*meta*) compound **17e**, and 4-F-Ph (*para*) compound **17f**.

ACCEPTED MANUSCRIPT

Table 2 NMR analysis of pyrrole compounds regarding the effect of fluorine substitution at position 5



Compound	17d		17e		17f		1a	
Y	2-F		3-F		4-F		H	
	^{1}H-NMR (600MHz, D_2O) δ (ppm)	^{13}C-NMR (151MHz, D_2O) δ (ppm)	^{1}H-NMR (600MHz, D_2O) δ (ppm):	^{13}C-NMR (151MHz, D_2O) δ (ppm)	^{1}H-NMR (600MHz, D_2O) δ (ppm)	^{13}C-NMR (151MHz, D_2O) δ (ppm)	^{1}H-NMR (600MHz, D_2O) δ (ppm)	^{13}C-NMR (151MHz, D_2O) δ (ppm)
2		132.22		138.42		138.74		139.92
3	6.446	119.89	6.397	119.08	6.353	118.72	6.362	118.69
4		119.99		119.87		119.73		119.96
5	7.775	127.68	7.736	127.99	7.722	127.56	7.722	127.68
9		135.90		136.00		136.10		136.14
10, 14	7.321	129.65	7.305	129.65	7.280	129.62	7.284	129.58
11, 13	7.318	132.80	7.290	132.68	7.280	132.66	7.284	132.65
12		149.80		149.74		149.65		149.58
15	2.388	23.49	2.371	23.47	2.371	23.47	2.371	23.46
16		120.71		134.81		128.99		133.00
17		163.33	6.943	120.22	7.170	135.52	7.201	133.43
18	7.152	117.87		164.40	7.090	117.34	7.380	130.53
19	7.522	134.62	7.208	118.53		165.74	7.475	131.79
20	7.185	126.58	7.361	132.24	7.090	117.34	7.380	130.53
21	7.075	135.88	7.011	129.45	7.170	135.52	7.201	133.43
22	4.131	47.00	4.118	46.98	4.115	47.04	4.119	47.02
23								
24	2.686	34.35	2.672	34.31	2.677	34.32	2.675	34.31
	^{19}F-NMR (376MHz, D_2O) δ (ppm)	^{19}F-NMR Difference[a] (ppm)	^{19}F-NMR (376MHz, D_2O) δ (ppm)	^{19}F-NMR Difference[a] (ppm)	^{19}F-NMR (376MHz, D_2O) δ (ppm)	^{19}F-NMR Difference[a] (ppm)	^{19}F-NMR (376MHz, D_2O) δ (ppm)	^{19}F-NMR Difference[a] (ppm)
25	–113.970	0.289	–115.577	1.309	–114.016	1.887		

[a] Difference in the chemical shift between D_2O and DMSO-d_6 solutions

Second, we evaluated the effects of position 1 (R^2) with regard to a series of 2-F-3-Py derivatives at position 5 (R^1) and the results are shown in Table 3.

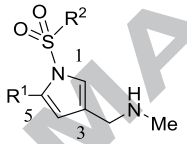
Replacement of the phenyl group with the 3-F-Ph group (compound **24a**) yielded similar activities, but in the case of replacement with 3-MeO-Ph (compound **24b**), a slight reduction of in vivo activity was observed. On the other hand, 3-CN-Ph derivative **24c** retained strong in vivo activity in spite of the reduced in vitro activity. Thus, 3-(polar substituent)-Ph derivatives almost maintained strong activities. Nonetheless, obvious signs of improvement of hERG-inhibitory activities were not observed. 3-Thienyl derivative **24d** and 2-thienyl derivative **24e** also retained almost equal in vitro

ACCEPTED MANUSCRIPT

and in vivo activities as compared to **3c** in spite of a big reduction in the log D value. Their LLE values were high, more than 8, but their hERG inhibition was not improved. Both 3-furyl derivative **24f** and 2-furyl derivative **24g** showed a greater reduction in log D values than did thienyl derivatives, but their in vivo activities were reduced to 82% and 74%, respectively, and the hERG-inhibitory activities were not improved either.

Both 3-pyridyl derivative **24h** and 2-pyridyl derivative **24i** showed remarkably reduced in vitro activities, but 3-Py derivative **24h** exerted strong inhibitory action in vivo (96% inhibition), despite its quite low log D value, and showed excellent ADME-Tox parameters including hERG-inhibitory activity in association with its high LLE value (7.53). Therefore, we selected compound **24h** as a lead compound for further optimization (Table 3).

Table 3 Effects of substituents at the first position (R^2) on activities and properties of pyrrole compounds



Compound.	R^1	R^2	Clog P	log D	In vitro H^+,K^+-ATPase inhibitory activities (IC_{50}, nM)	LLE	In vivo Acid secretion in rats (1 mg/kg, iv, % inhibition)	ATP content at 100 μM (%control)	hERG % inhibition at 10 μM FCS (-)
1a	Ph	4-Me-Ph	4.21	1.83	55	5.43	66	(38.6)[a]	NT[b]
1b	Ph	4-MeO-Ph	3.88	1.54	30	5.98	95	(22.1)[a]	89.1
1c	Ph	Ph	3.71	1.44	9.4	6.59	96	(53.8)[a]	87.5
3c	2-F-3-Py	Ph	2.56	–0.09	26	7.68	99	59.9	57.0
24a	2-F-3-Py	3-F-Ph	2.71	0.23	33	7.25	99	65.9	56.2
24b	2-F-3-Py	3-MeO-Ph	2.72	0.10	28	7.45	92	30.6	72.8
24c	2-F-3-Py	3-CN-Ph	2.00	–0.36	89	7.41	99	46.7	46.7
24d	2-F-3-Py	3-thienyl	2.28	–0.83	32	8.32	98	64.0	60.0
24e	2-F-3-Py	2-thienyl	2.28	–0.69	32	8.18	96	74.9	63.0
24f	2-F-3-Py	3-furyl	1.74	–1.31	92	8.35	82	77.4	59.3
24g	2-F-3-Py	2-furyl	1.74	–1.15	59	8.38	74	68.7	49.1
24h	2-F-3-Py	3-Py	1.21	–0.85	210	7.53	96	85.7	4.4
24i	2-F-3-Py	2-Py	1.21	0.05	120	6.97	81	78.6	NT[b]

[a] ATP content at 30 μM (% control)

[b] Not tested

Lastly, we analyzed several analogs of compound **24h** in detail to understand not only the SAR but also the relation of structure with ADME-Tox parameters, and the results are summarized in Table 4.

Introduction of a methoxy group at position 6 of the 3-pyridyl group in the R^2 moiety yielded ~5-fold more potent in vitro activity (compound **24j**, IC_{50} = 40 nM) as compared to original compound **24h**. Compound **24j** also had a 97% inhibitory activity in vivo, but its cytotoxicity (effect

ACCEPTED MANUSCRIPT

on ATP content) was assumed to be slightly out of range for the preferable ADME-Tox profiles for an ideal long-lasting P-CAB.

Replacement of the 2-F-3-Py group at position 5 (R^1) of the pyrrole ring by a 2-Cl-3-Py group showed enhanced in vitro activity (compound **26a**, IC_{50} = 120 nM). In addition, this replacement maintained excellent ADME-Tox parameters, while the in vivo activity decreased slightly to 92%.

In the case of replacement with the 2-CN-3-Py group (compound **26b**), it yieded a lower log D value and greatly reduced activities not only in vivo but also in vitro.

To determine the influence of basicity at position 5, two *ortho*-methylated 3-pyridyl derivatives, **26c** and **26d**, were studied next. Consequently, in vitro activity of each compound was almost identical to that of **24h**, but in vivo activity of each compound was greatly reduced. Basicity of pyridine at the fifth position of the pyrrole ring seemed to be unfavorable for in vivo activities judging by the data on a comparison of 2-Me-Ph compound **17b** (IC_{50} = 62 nM, 92% inhibition at 1mg/kg, iv) with 2-F-Ph compound **17d** (IC_{50} = 34 nM, 91% inhibition at 1mg/kg, iv). Replacement with other types of *ortho*-substituted pyridyl groups, such as 3-Me-2-Py (compound **26e**) or 3-F-4-Py (compound **26f**), did not produce sufficient enhancement of activities.

Compound **37a** with a methyl group at position 4 of the pyrrole ring (R^3) retained in vitro activity (IC_{50} = 220 nM) almost equal to that of **24h**, but showed slightly decreased in vivo activity (93% inhibition at 1mg/kg, iv).

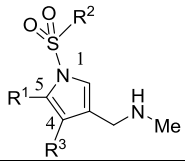
Compound **37b** with a chlorine atom at position 4 of the pyrrole ring (R^3) possessed increased in vitro activity (IC_{50} = 99 nM), and retained almost equal in vivo activity (95% inhibition at 1mg/kg, iv) as compared to **24h**.

By contrast, compound **37c** having a fluorine atom at position 4 of the pyrrole ring (R^3) exerted more potent activities both in vitro and in vivo (IC_{50} = 49 nM, 98% inhibition at 1mg/kg, iv). Moreover, compound **37c** showed an excellent ADME-Tox profile, especially low cytotoxicity (100.2% ATP content at 100 μM).

Compounds with a substituent at the second position of the pyrrole ring were not studied because an obvious reduction of in vivo activity was observed in our previous study.[25]

ACCEPTED MANUSCRIPT

Table 4 Effects of substituents (R^1, R^2, and R^3) on activities and properties of pyrrole compounds



Compound	R^1	R^2	R^3	Clog P	log D	In vitro H^+,K^+-ATPase inhibition (IC_{50}, nM)	LLE	In vivo Acid secretion in rats (1 mg/kg, iv, % inhibition)	ATP content at 100 μM (%control)	hERG % inhibition at 10 μM FCS (-)
1b	Ph	4-MeO-Ph	H	3.88	1.54	30	5.98	95	(22.1)[a]	89.1
24h	2-F-3-Py	3-Py	H	1.21	–0.85	210	7.53	96	85.7	4.4
24j	2-F-3-Py	6-MeO-3-Py	H	1.97	0.24	40	7.16	97	47.6	33.1
26a	2-Cl-3-Py	3-Py	H	1.53	–0.5	120	7.42	92	82.9	10.6
26b	2-CN-3-Py	3-Py	H	0.81	–1.29	530	7.57	48	85.3	1.9
26c	2-Me-3-Py	3-Py	H	1.18	0.06	250	6.54	–41	107.2	NT[b]
26d	4-Me-3-Py	3-Py	H	1.18	–0.51	230	7.15	–54	86.0	8.3
26e	3-Me-2-Py	3-Py	H	1.39	0.53	1300	5.36	5	88.7	NT[b]
26f	3-F-4-Py	3-Py	H	1.21	–0.34	290	6.88	48	78.8	18.8
37a	2-F-3-Py	3-Py	Me	1.36	–0.4	220	7.07	93	47.1	30.3
37b	2-F-3-Py	3-Py	Cl	1.77	0.48	99	6.52	95	64.5	41.3
37c	2-F-3-Py	3-Py	F	1.45	0.04	49	7.27	98	100.2	39.8

[a] ATP content at 30 μM (% control)

[b] Not tested

Because compounds **3e**, **24h** and **37c** showed superior ADME-Tox properties in addition to promising activities, we studied the effects of compounds **3e**, **24h**, and **37c** and lansoprazole on the pH of a gastric perfusate during histamine stimulation in anesthetized rats (Figure 4).

The pH value of saline under these experimental conditions was 6.0 to 6.3. Intravenous infusion of histamine 2HCl at 8 mg/kg/h stimulated gastric acid secretion and decreased pH of the gastric perfusate to ~2. Lansoprazole, which is one of typical PPIs, resulted in a rapid increase of perfusate pH but not up to 5 after administration of a 3 mg/kg dose, and the perfusate pH gradually decreased thereafter even at such a high dose (Figure 4A).

On the other hand, intravenous administration of compounds **3e**, **24h**, and **37c** clearly increased pH of the gastric perfusate at a low dose of 1 mg/kg or less.

Compound **3e** increased perfusate pH value (>5) within 2 h after the administration at 1 mg/kg, but the perfusate pH gradually decreased thereafter (Figure 4B).

Compound **24h** also increased pH of the gastric perfusate, and pH reached ~5 at a dose of 1 mg/kg. Moreover it took 150–180 min for the pH of the perfusate to reach its peak value after administration of this compound (Figure 4C).

In contrast, intravenous administration of **37c** at 0.7 mg/kg caused a relatively rapid increase in pH of the gastric perfusate, to approximately 6, and the effect was sustained for more than 5 h after administration (Figure 4D).

According to these results, compound **37c**, which showed the longest duration of action in rats, was

ACCEPTED MANUSCRIPT

selected for further analysis.

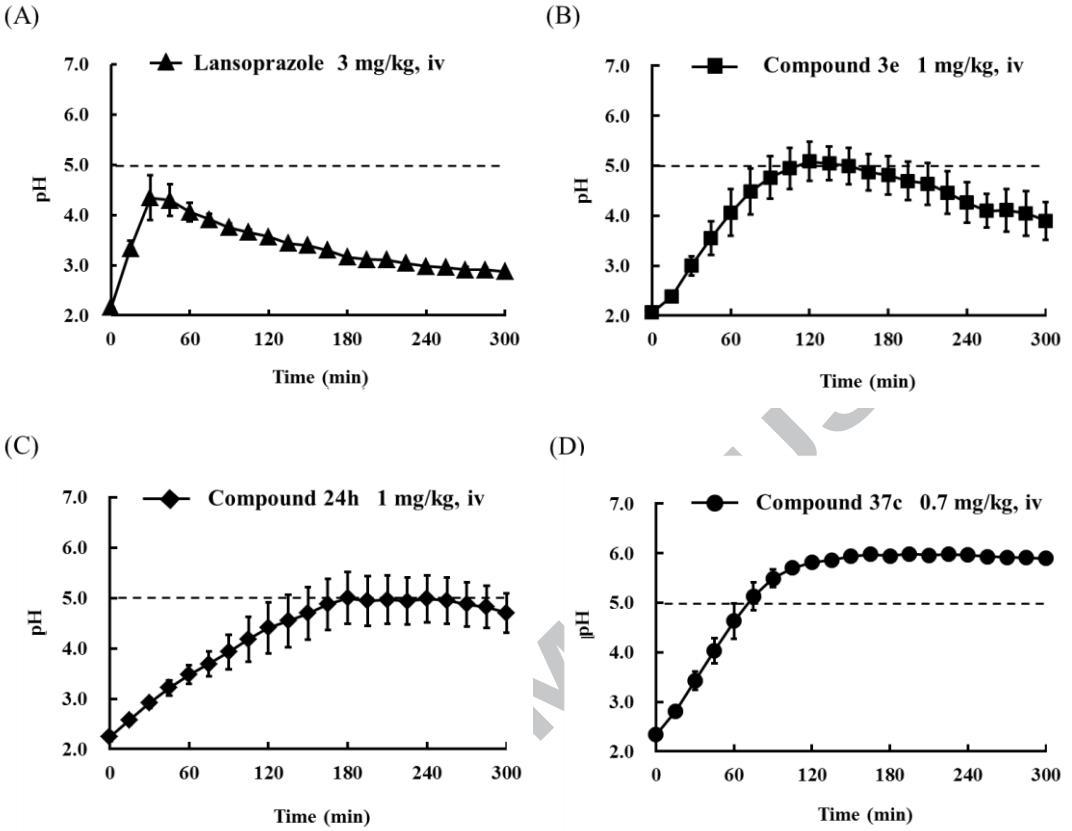


Figure 4. Effects of intravenous administration of lansoprazole (A) and compounds **3e** (B), **24h** (C), and **37c** (D) on pH of a gastric perfusate under conditions of histamine stimulation in anesthetized rats. Each data point represents mean ± SE from three or four rats.

We evaluated the effect of oral administration of compound **37c** in rats and dogs (Figures 5 and 6). As shown in Figure 5, compound **37c** attenuated histamine-stimulated acid secretion in anesthetized rats in a dose-dependent manner. In addition, it caused complete inhibition at a dose of 4 mg/kg, probably because acid activation is not required for compound **37c** to exert its acid-suppressive effect in contrast to PPIs.

ACCEPTED MANUSCRIPT

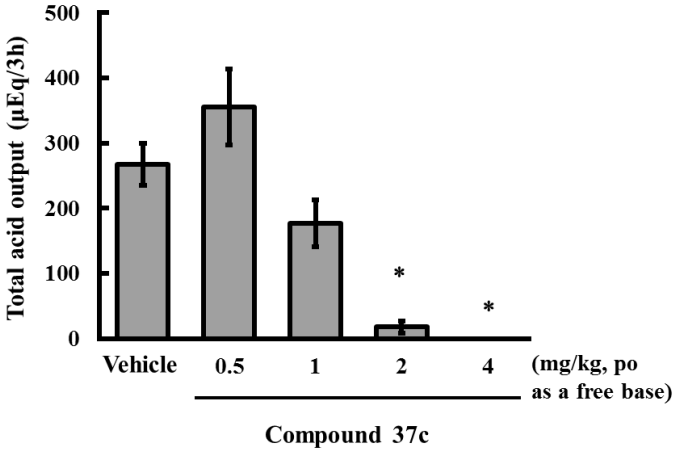


Figure 5. Effects of oral administration of compound **37c** on histamine-stimulated acid secretion in anesthetized rats. Each column represents the mean ± SE from six rats. Statistical significance of the difference was determined by the one-tailed Shirley-Williams test; *$p < 0.025$ compared to vehicle.

Compound **37c** completely inhibited histamine-stimulated gastric acid secretion in Heidenhain pouch dogs after oral administration at a dose of 0.8 mg/kg. Its duration of action was much longer than that of lansoprazole at 3 mg/kg, per os (po). Moreover, its effect was clearly longer as compared to that of lead compound **1b** at 1 mg/kg, po, which was reported in our previous paper,[25] and well-pronounced suppression of acid secretion was observed even 48 h after the administration (Figure 6).

ACCEPTED MANUSCRIPT

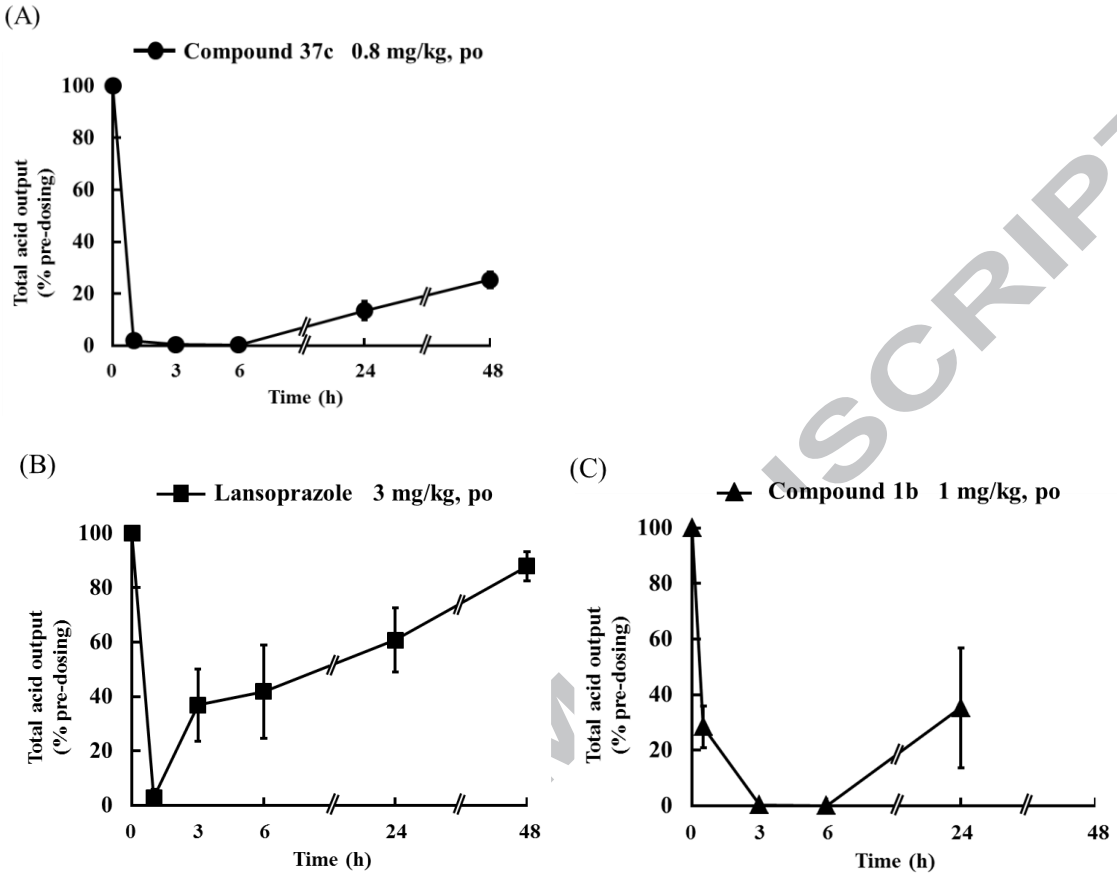


Figure 6. Effects of oral administration of compound **37c** (A), lansoprazole (B), or lead compound **1b** (C)[25] on histamine-stimulated acid secretion in Heidenhain pouch dogs. Each data point represents mean ± SE from three or four dogs.

Compound **37c** was designed to have moderate basicity in addition to weak lipophilic properties (Clog P = 1.45); the log D value was 0.04 at pH 7.4, and pK_a of the side chain amino group portion was 8.54. Therefore, compound **37c** mostly dissociates in vivo, while showing excellent membrane penetration at pH 7.4, which is similar to blood pH. The lowered pK_a value of compound **37c** caused by introduction of the fluorine atom at position 4 of the pyrrole ring may have contributed to the excellent membrane penetration at pH 7.4 [256 Pe (nm/sec) in parallel artificial membrane permeability assay (PAMPA)] (Table 5).

In contrast, a pronounced decline in membrane penetration was observed at lower pH, even in a slightly acidic environment, e.g., at pH 5.0, because of a further increase in the proportion of its dissociated molecules, accompanied by a decrease in lipophilicity [47 Pe (nm/sec) at pH 5.0 in PAMPA] (Table 5). Based on these physicochemical properties, compound **37c** is considered to have rapid onset and long duration of action after single administration because of moving rapidly into the acidic secretory canaliculi and remaining there for a long period. Therefore, compound **37c** was

ACCEPTED MANUSCRIPT

assumed to exert stronger and longer-lasting action than lead compound **1b** dose.

Table 5 Physicochemical properties and biological activities of hit compounds **1b** and **37c**

Compound	Clog P	log D at pH 7.4	pK_a	In vitro H^+,K^+-ATPase inhibition (IC_{50}, nM)	In vivo Acid secretion in rats (1 mg/kg, iv, % inhibition)	PAMPA[a] pH 7.4 Pe (nm/sec)	PAMPA[a] pH 5.0 Pe (nm/sec)
1b	3.88	1.54	9.48	30	95	246	198
37c	1.45	0.04[a]	8.54	49	98	256	47

[a] Parallel artificial membrane permeability assay

These results indicate that the pharmacological effect of **37c** is superior to that of lansoprazole. This compound also showed good acid resistance and limited susceptibility to CYP polymorphism (data not shown) unlike lansoprazole. Furthermore, the overall DMPK and safety profiles of **37c** were much better than those of the lead compound **1b**. Additionally, compound **37c** was expected to have longer duration of action in humans as compared to TAK-438 according to its physicochemical properties.

On the basis of these findings, compound **37c**, which has physicochemical properties different from those of TAK-438, e.g., in terms of lipophilicity and basicity, may be a promising alternative as a P-CAB, if the need arises.

4. Conclusion

We synthesized novel pyrrole derivatives with low lipophilicity in accordance with our hypothesis on the basis of lead compound **1b** and evaluated their potency as P-CABs and their safety profiles. Our initial aim to greatly reduce log D values implied introduction of polar hetero-aromatic groups at the fifth position of the pyrrole ring. We found that substituted 3-pyridyl groups with lower basicity possess favorable properties. Eventually, our experiments revealed that the combination of the replacement with a 2-F-3-Py group at position 5, replacement with a 3-Py group at positions 1, and introduction of a fluorine atom at position 4 of the pyrrole ring is the most suitable for both potent activities with longer duration of action and low lipophilicity with effective improvement of ADME-Tox parameters.

Thus, the identified compound **37c** shows potent H^+,K^+-ATPase-inhibitory activity in vitro, and potent and long-lasting inhibitory activities on histamine-induced gastric acid secretion in rats and Heidenhain pouch dogs.

We succeeded in identifying a new P-CAB with long duration of action as a possible alternative to TAK-438, if the need arises.

ACCEPTED MANUSCRIPT

5. Experimental section

Experimental procedures and characterization data for all synthetic intermediates are reported in Supplementary data.

5.1. General

Commercial reagents and solvents were used without additional purification. The pK_a values were measured by pH-metric assays using a Sirius T3 system (Sirius Analytical Ltd., UK). The assay measures the concentration of H^+ ions in solution between pH 2 and 12 using a pH electrode (Ag/AgCl). Melting points were determined on a Yanagimoto micro melting point apparatus or Büche B-545 or by differential scanning calorimetry (DSC) or TG-DTA analyses. DSC analyses were performed using a DSC1 system (Mettler Toledo, Switzerland). The thermograms were obtained at a temperature of 25–300 °C and a heating rate of 5 °C/min under nitrogen gas at a flow rate of 40 mL/min. The powders (~1 mg) were weighed in an aluminum pan, crimped, and then placed in the thermal analysis chamber. TG-DTA analyses were conducted by from Sumika Chemical Analysis Service, Ltd. Nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR) spectra were recorded on a Varian Gemini-200, a Varian Mercury-300, a Bruker AV-300M, a Bruker AV-400 or a Bruker AV- 600 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane or sodium 3-(trimethylsilyl)-1-propane-1,1,2,2,3,3-d_6-sulfonate as an internal standard for ^{1}H and ^{13}C-NMR, with sodium trifluoroacetate as the internal standard for ^{19}F-NMR (–76.53 ppm). Coupling constants are reported in hertz (Hz). Spectral splitting patterns were designated as follows: s, singlet; br, broad; d, doublet; t, triplet; q, quartet; and m, multiplet. High-resolution mass spectrometry (HRMS) experiments were carried out by Sumika Chemical Analysis Service, Ltd. All mass spectrometry (MS) experiments were conducted using electrospray ionization (ESI) in positive or negative ion mode. Elemental analyses were performed by Takeda Analytical Laboratories, Ltd., or Sumika Chemical Analysis Service, Ltd. Thin-layer chromatography (TLC) analyses were carried out on Merck Kieselgel 60 F_{254} plates or Fuji Silysia Chemical, Ltd., Chromatorex NH-TLC plates. Silica gel column chromatography was run by means of Merck 0.063–0.200 mm silica gel 60, Fuji Silysia Chemical Ltd. 100–200 mesh Chromatorex NH silica DM1020 or Purif-Pack (SI 60 μM or NH 60 μM, Fuji Silysia Chemical Ltd.).

*5.2. N-Methyl-1-[1-(phenylsulfonyl)-5-(pyrimidin-5-yl)-1H-pyrrol-3-yl]methanamine hydrochloride (**3a**)*

A mixture of *tert*-butyl {[5-bromo-1-(phenylsulfonyl)-1*H*-pyrrol-3-yl]methyl}methylcarbamate **2** (170 mg, 0.40 mmol), pyrimidin-5-ylboronic acid (123 mg, 0.99 mmol), Na_2CO_3 (147 mg, 1.39 mmol) and tetrakis(triphenylphosphine)palladium (46 mg, 0.040 mmol) in DME (10 mL) and H_2O (5 mL) was stirred at 90 °C for 3 h under Ar atmosphere. After cooling to room temperature, the

ACCEPTED MANUSCRIPT

mixture was poured into H_2O, and extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (n-hexane/EtOAc = 4/1–1/3), and the resulting oil was dissolved in MeOH (20 mL), and then 4 mol/L HCl/EtOAc (2 mL) was added. The mixture was stirred at 70 °C for 30 min, and concentrated under reduced pressure. The residue was suspended in EtOAc and collected by filtration to obtain **3a** (42.0 mg, 29%) as a colorless solid: mp 179 °C; ^{1}H-NMR (DMSO-d_6) δ 2.50 (3H, m), 4.00 (2H, t, *J* = 5.8 Hz), 6.71 (1H, d, *J* = 1.8 Hz), 7.44–7.47 (2H, m), 7.55–7.60 (2H, m), 7.73–7.78 (1H, m), 7.89 (1H, d, *J* = 1.8 Hz), 8.62 (2H, s), 9.18 (2H, br), 9.23 (1H, s); HRMS (ESI) calcd for $C_{16}H_{16}N_4O_2S$ $(M+H)^+$ *m/z* 329.1067, found *m/z* 329.1026.

*5.3. N-Methyl-1-[1-(phenylsulfonyl)-5-(pyridin-3-yl)-1H-pyrrol-3-yl]methanamine dihydrochloride (**3b**)*

Compound **3b** was prepared from **2** in a manner similar to that described for compound **3a**. A colorless solid (49%): mp 187 °C; ^{1}H-NMR (DMSO-d_6) δ 2.47 (3H, t, *J* = 5.5 Hz), 3.98 (2H, t, *J* = 5.5 Hz), 6.72 (1H, d, *J* = 1.8 Hz), 7.45–7.58 (4H, m), 7.70–7.76 (2H, m), 7.88 (1H, d, *J* = 1.3 Hz), 7.95–7.98 (1H, m), 8.53 (1H, d, *J* = 1.8 Hz), 8.76 (1H, dd, J=1.3, 5.3 Hz), 9.34 (2H, br), 1H not detected; HRMS (ESI) calcd for $C_{17}H_{17}N_3O_2S$ $(M+H)^+$ *m/z* 328.1114, found *m/z* 328.1085.

*5.4. 1-[5-(2-Fluoropyridin-3-yl)-1-(phenylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**3c**)*

To a stirred mixture of 5-(2-Fluoropyridin-3-yl)-1-(phenylsulfonyl)-1*H*-pyrrole-3-carbaldehyde **7** (160 mg, 0.48 mmol) and 40% methanol solution of methylamine (188 mg, 2.42 mmol) in MeOH (16 mL) was added sodium borohydride (55 mg, 1.45 mmol) at room temperature under N_2 atmosphere, and the mixture was stirred at room temperature for 30 min, quenched with a solution of $NaHCO_3$, and then extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 99/1), and then crystalized from a solution of fumaric acid (57 mg, 0.4911 mmol) in EtOH (5 mL). The obtained crystals were collected by filtration and rinsed with EtOH to produce **3c** (92 mg, 41%) as colorless crystals: mp 191–192 °C; ^{1}H-NMR (DMSO-d_6) δ 2.39 (3H, s), 3.79 (2H, s), 6.48 (2H, s), 6.51 (1H, d, *J* = 1.5 Hz), 7.38–7.42 (1H, m), 7.46–7.49 (2H, m), 7.54–7.60 (2H, m), 7.67–7.77 (3H, m), 8.30–8.33 (1H, m), 3H not detected; Anal. Calcd for $C_{21}H_{20}FN_3O_6S$: C, 54.66; H, 4.37; N, 9.11. Found: C, 54.57; H, 4.31; N, 9.02.

*5.5. 1-[5-(2-Chloropyridin-3-yl)-1-(phenylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine hydrochloride (**3d**)*

To a solution of compound **4d** (259 mg, 0.56 mmol) in EtOH (2 mL) was added 4 mol/L HCl/EtOAc

ACCEPTED MANUSCRIPT

(2 mL), and the mixture was stirred at room temperature for 2 h, concentrated under reduced pressure, and then recrystallized from EtOH to produce compound **3d** (124 mg, 56%) as colorless crystals: mp 215–216 °C; ^{1}H-NMR (DMSO-d_6) δ 2.51 (3H, s), 4.00 (2H, s), 6.57–6.61 (1H, m), 7.46–7.52 (3H, m), 7.57–7.62 (3H, m), 7.74–7.83 (2H, m), 8.49–8.51 (1H, m), 9.04–9.23 (2H, m); Anal. Calcd for $C_{17}H_{17}Cl_2N_3O_2S$: C, 51.26; H, 4.30; N, 10.55. Found: C, 51.28; H, 4.23; N, 10.56.

*5.6. 3-{4-[(Methylamino)methyl]-1-(phenylsulfonyl)-1H-pyrrol-2-yl}pyridine-2-carbonitrile hydrochloride (**3e**)*

To a solution of compound **4e** (250 mg, 0.55 mmol) in EtOAc (5mL) and MeOH (3 mL) was added 4 mol/L HCl/EtOAc (3 mL), and the mixture was stirred at room temperature for 4h, concentrated under reduced pressure, and then recrystallized from EtOH to produce compound **3e** (127 mg, 59%) as colorless crystals: mp 240–250 °C (decomposition); ^{1}H-NMR (DMSO-d_6) δ 2.49 (3H, s), 4.03 (2H, s), 6.80 (1H, d, J = 1.8 Hz), 7.45–7.48 (2H, m), 7.56–7.61 (2H, m), 7.75–7.94 (4H, m), 8.81–8.83 (1H, m), 9.21 (2H, brs); Anal. Calcd for $C_{18}H_{17}ClN_4O_2S$: C, 55.59; H, 4.41; N, 14.41. Found: C, 55.46; H, 4.31; N, 14.32.

*5.7. N-Methyl-1-[1-(phenylsulfonyl)-5-(pyridin-2-yl)-1H-pyrrol-3-yl]methanamine oxalate (**17a**)*

To a solution of **16a** (78 mg, 0.25 mmol) in MeOH (10 mL) was added 40% methylamine methanol solution (100 mg, 1.28 mmol) at room temperature. After stirred for 10 min, sodium borohydride (29 mg, 0.77 mmol) was added at room temperature, and the reaction mixture was stirred for 1 h and then quenched with 1 mol/L HCl (20 mL). After stirred for 10 min, the mixture was basified with a solution of $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 1/0–7/3) and dissolved in EtOAc (10 mL). Oxalic acid (50 mg) was added, and the mixture was stirred for 15 min. The resulting crystals were collected by filtration to obtain **17a** (47 mg, 45%) as colorless crystals: mp 146–148 °C; ^{1}H-NMR (DMSO-d_6) δ 2.55 (3H, s), 4.02 (2H, s), 6.70 (1H, d, J = 1.8 Hz), 7.33–7.38 (1H, m), 7.51–7.54 (1H, m), 7.63–7.68 (2H, m), 7.74–7.91 (5H, m), 8.44–8.46 (1H, m), 3H not detected; HRMS (ESI) calcd for $C_{17}H_{17}N_3O_2S$ $(M+H)^+$ m/z 328.1114, found m/z 328.1085.

*5.8. N-Methyl-1-{5-(2-methylphenyl)-1-[(4-methylphenyl)sulfonyl]-1H-pyrrol-3-yl}methanamine hydrochloride (**17b**)*

To a solution of **16b** (0.46 g, 1.36 mmol) in THF was added methylamine hydrochloride (1.11 g, 13.6 mmol) and sodium cyano borohydride (0.26 g, 4.14 mmol), and the mixture was stirred at room temperature for 20 h and then concentrated under reduced pressure. A solution of $NaHCO_3$ was added, and then the mixture was extracted with EtOAc. The extract was washed with brine, dried

ACCEPTED MANUSCRIPT

over anhydrous $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/MeOH = 1/0–5/1) and dissolved in EtOAc (5 mL). 4 mol/L HCl/EtOAc (2 mL) was added, and the mixture was concentrated in vacuo. The residue was crystalized from n-hexane/EtOAc to produce **17b** (0.37 g, 70%) as colorless cryatals: mp 192–193 °C; ^{1}H-NMR (DMSO-d_6) δ 1.79 (3H, s), 2.38 (3H, s), 3.32 (3H, s), 4.00 (2H, s), 6.34 (1H, d, J = 1.8 Hz), 6.84 (1H, d, J = 6.2 Hz), 7.11–7.21 (2H, m), 7.25–7.36 (6H, m), 7.72 (1H, s), 9.02 (1H, br); Anal. Calcd for $C_{20}H_{23}ClN_2O_2S$: C, 61.45; H, 5.93; N, 7.17. Found: C, 61.23; H, 5.96; N, 7.22.

5.9.

*N-Methyl-1-{1-[(4-methylphenyl)sulfonyl]-5-[2-(trifluoromethyl)phenyl]-1H-pyrrol-3-yl}methanamine hydrochloride (**17c**)*

Compound **17c** was prepared from compound **16c** using a similar procedure as for the preparation of compound **17b**. White crystals (35%): mp 195–196 °C; ^{1}H-NMR (DMSO-d_6) δ 2.39 (3H, s), 2.50 (3H, s), 3.32 (2H, s), 6.43 (1H, s), 7.12 (1H, d, J = 6.8 Hz), 7.37 (4H, s), 7.63–7.79 (4H, m), 8.92 (2H, br); Anal. Calcd for $C_{20}H_{20}ClF_3N_2O_2S$: C, 53.99; H, 4.53; N, 6.30. Found: C, 53.91; H, 4.55; N, 6.24.

*5.10. 1-{5-(2-fluorophenyl)-1-[(4-methylphenyl)sulfonyl]-1H-pyrrol-3-yl}-N-methylmethanamine hydrochloride (**17d**)*

Compound **17d** was prepared from compound **16d** using a similar procedure as for the preparation of compound **17b**. White crystals (76%): mp 172 °C; ^{1}H-NMR (DMSO-d_6) δ 2.37 (3H, s), 3.32 (3H, s), 3.97 (2H, s), 6.48 (1H, d, J = 1.8 Hz), 7.02–7.08 (1H, m), 7.18–7.34 (6H, m), 7.47–7.55 (1H, m), 7.74 (1H, d, J = 1.8 Hz), 9.01 (2H, brs); Anal. Calcd for $C_{19}H_{20}ClFN_2O_2S$: C, 57.79; H, 5.10; N, 7.09. Found: C, 57.57; H, 5.21; N, 6.79.

*5.11. 1-{5-(3-Fluorophenyl)-1-[(4-methylphenyl)sulfonyl]-1H-pyrrol-3-yl}-N-methylmethanamine hydrochloride (**17e**)*

Compound **17e** was prepared from compound **16e** using a similar procedure as for the preparation of compound **17b**. White crystals (69%): mp 164–165 °C; ^{1}H-NMR (DMSO-d_6) δ 2.36 (3H, s), 3.32 (3H, s), 3.98 (2H, s), 6.48 (1H, d, J = 1.8 Hz), 6.94–7.00 (2H, m), 7.25–7.45 (6H, m), 7.73 (1H, d, J = 1.8 Hz), 8.94 (2H, brs); Anal. Calcd for $C_{19}H_{20}ClFN_2O_2S$: C, 57.79; H, 5.10; N, 7.09. Found: C, 57.82; H, 5.20; N, 6.87.

*5.12. 1-{5-(4-Fluorophenyl)-1-[(4-methylphenyl)sulfonyl]-1H-pyrrol-3-yl}-N-methylmethanamine hydrochloride (**17f**)*

Compound **17f** was prepared from compound **16f** using a similar procedure as for the preparation of

ACCEPTED MANUSCRIPT

compound **17b**. White crystals (71%): mp 224 °C; ^{1}H-NMR (DMSO-d_6) δ 2.36 (3H, s), 2.51 (3H, s), 3.97 (2H, s), 6.43 (1H, d, *J* = 1.8 Hz), 7.16–7.36 (8H, m), 7.71 (1H,s), 9.05 (1H, brs), 1H not detected; Anal. Calcd for $C_{19}H_{20}ClFN_2O_2S$: C, 57.79; H, 5.10; N, 7.09. Found: C, 57.57; H, 5.09; N, 6.97.

5.13.

*1-{1-[(3-Fluorophenyl)sulfonyl]-5-(2-fluoropyridin-3-yl)-1H-pyrrol-3-yl}-N-methylmethanamine hydrochloride (**24a**)*

Compound **24a** was prepared from **20a** using a similar procedure as for the preparation of compound **3d** from **4d**. Colorless crystals (54%): mp 211–212 °C; ^{1}H-NMR (DMSO-d_6) δ 2.51 (3H, s), 3.99 (2H, s), 6.67 (1H, d, *J* = 1.8 Hz), 7.33–7.36 (2H, m), 7.41–7.46 (1H, m), 7.65–7.76 (3H, m), 7.87 (1H, d, *J* = 1.8 Hz), 8.34–8.36 (1H, m), 9.18 (2H, brs); Anal. Calcd for $C_{17}H_{16}ClF_2N_3O_2S$: C, 51.07; H, 4.03; N, 10.51. Found: C, 51.01; H, 4.04; N, 10.46.

5.14.

*1-{5-(2-Fluoropyridin-3-yl)-1-[(3-methoxyphenyl)sulfonyl]-1H-pyrrol-3-yl}-N-methylmethanamine fumarate (**24b**)*

To a solution of compound **20b** (303 mg, 0.637 mmol) in EtOAc (1 mL) and MeOH (1 mL) was added dropwise 4 mol/L HCl/EtOAc (3 mL), and the mixture was stirred at room temperature for 2 h. The reaction was quenched by a solution of $NaHCO_3$, and the resulting mixture was extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (n-hexane/EtOAc = 1/1–1/9), and then dissolved in EtOAc (2 mL). A solution of fumaric acid (46 mg) in MeOH (2 mL) was added to the solution, and then the mixture was concentrated under reduced pressure. The residue was recrystallized from EtOH/H_2O (9/1) to produce **24b** (138 mg, 44%) as colorless crystals: mp 177 °C; ^{1}H-NMR (DMSO-d_6) δ 2.40 (3H, s), 3.75 (3H, s), 3.82 (2H, s), 6.47 (2H, s), 6.53 (1H, d, *J* = 1.5 Hz), 6.86–6.88 (1H, m), 7.05–7.08 (1H, m), 7.27–7.31 (1H, m), 7.38–7.51 (2H, m), 7.69–7.75 (2H, m), 8.31–8.32 (1H, m), 3H not detected; Anal. Calcd for $C_{22}H_{22}FN_3O_7S$: C, 53.76; H, 4.51; N, 8.55. Found: C, 53.61; H, 4.53; N, 8.58.

*5.15. 3-({2-(2-Fluoropyridin-3-yl)-4-[(methylamino)methyl]-1H-pyrrol-1-yl}sulfonyl)benzonitrile fumarate (**24c**)*

Compound **24c** was prepared from **20c** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (44%): mp 149 °C; ^{1}H-NMR (DMSO-d_6) δ 2.40 (3H, s), 3.82 (2H, s), 6.47 (2H, s), 6.57 (1H, d, *J* = 1.8 Hz), 7.39–7.44 (1H, m), 7.71–7.81 (4H, m), 7.95–7.96 (1H, m), 8.21–8.24 (1H, m), 8.32–8.34 (1H, m), 3H not detected; Anal. Calcd for $C_{22}H_{19}FN_4O_6S$: C, 54.32; H,

ACCEPTED MANUSCRIPT

3.94; N, 11.52. Found: C, 54.25; H, 4.01; N, 11.52.

*5.16. 1-[5-(2-Fluoropyridin-3-yl)-1-(thiophen-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**24d**)*

Compound **24d** was prepared from **20d** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (63%): mp 173–175 °C; ^{1}H-NMR (DMSO-d_6) δ 2.41 (3H, s), 3.81 (2H, s), 6.48 (2H, s), 6.53 (1H, d, *J* = 1.8 Hz), 7.08–7.10 (1H, m), 7.38–7.42 (1H, m), 7.64–7.79 (3H, m), 8.08–8.10 (1H, m), 8.30–8.32 (1H, m), 3H not detected; Anal. Calcd for $C_{19}H_{18}FN_3O_6S_2$: C, 48.81; H, 3.88; N, 8.99. Found: C, 48.87; H, 3.90; N, 8.95.

*5.17. 1-[5-(2-Fluoropyridin-3-yl)-1-(thiophen-2-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**24e**)*

Compound **24e** was prepared from **23e** using a similar procedure as for the preparation of compound **3c** from **7**. Colorless crystals (75%): mp 204 °C; ^{1}H-NMR (DMSO-d_6) δ 2.40 (3H, s), 3.80 (2H, s), 6.48 (2H, s), 6.55 (1H, d, *J* = 1.5 Hz), 7.18 (1H, dd, *J* = 4.9 Hz, 4.2 Hz), 7.40–7.45 (1H, m), 7.47 (1H, dd, *J* = 4.0 Hz, 1.3 Hz), 7.62 (1H, d, *J* = 1.5 Hz), 7.75–7.82 (1H, m), 8.11 (1H, dd, *J* = 5.1 Hz, 1.3 Hz), 8.31–8.33 (1H, m), 3H not detected; Anal. Calcd for $C_{19}H_{18}FN_3O_6S_2$: C, 48.81; H, 3.88; N, 8.99. Found: C, 48.88; H, 4.09; N, 9.06.

*5.18. 1-[5-(2-Fluoropyridin-3-yl)-1-(furan-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine hydrochloride (**24f**)*

To a solution of compound **20f** (253 mg, 0.581 mmol) in EtOAc (3 mL) and iPrOH (2 mL) was added dropwise 4 mol/L HCl/EtOAc (6 mL), and the mixture was stirred at room temperature for 2.5 h, and then concentrated in vacuo. The residue was recrystallized from EtOAc/EtOH (1/1) to obtain **24f** (134 mg, 62%) as colorless crystals: mp 205 °C; ^{1}H-NMR (DMSO-d_6) δ 2.53 (3H, s), 4.00 (2H, s), 6.63–6.67 (2H, m), 7.43 (1H, ddd, *J*=7.1 Hz, 5.0 Hz, 1.9 Hz), 7.75 (1H, d, *J* = 1.9 Hz), 7.80 (1H, ddd, *J* = 9.6 Hz, 7.5 Hz, 1.9 Hz), 7.94 (1H, t, *J* = 1.9 Hz), 8.27–8.30 (1H, m), 8.31–8.37 (1H, m), 9.03 (2H, brs); Anal. Calcd for $C_{15}H_{15}ClFN_3O_3S$: C, 48.45; H, 4.07; N, 11.30. Found: C, 48.48; H, 4.04; N, 11.34.

*5.19. 1-[5-(2-Fluoropyridin-3-yl)-1-(furan-2-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**24g**)*

Compound **24g** was prepared from **23g** using a similar procedure as for the preparation of compound **3c** from **7**. Colorless crystals (66%): mp 197 °C; ^{1}H-NMR (DMSO-d_6) δ 2.41 (3H, s), 3.81 (2H, s), 6.48 (2H, s), 6.58 (1H, d, *J* = 1.9 Hz), 6.72 (1H, dd, *J* = 3.7 Hz, 1.8 Hz), 7.10 (1H, dd, *J* = 3.7 Hz, 0.8 Hz), 7.40–7.44 (1H, m), 7.57 (1H, d, *J* = 1.8 Hz), 7.78–7.84 (1H, m), 8.07 (1H, dd, *J* = 1.8 Hz,

ACCEPTED MANUSCRIPT

0.8 Hz), 8.30–8.33 (1H, m), 3H not detected; Anal. Calcd for $C_{19}H_{18}FN_3O_7S$: C, 50.55; H, 4.02; N, 9.31. Found: C, 50.65; H, 4.15; N, 9.48.

*5.20. 1-[5-(2-Fluoropyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**24h**)*

Compound **24h** was prepared from **20h** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (29%): mp 183–184 °C; ^{1}H-NMR (DMSO-d_6) δ 2.39 (3H, s), 3.78 (2H, s), 6.48 (2H, s), 6.56 (1H, d, *J* = 1.8 Hz), 7.40–7.44 (1H, m), 7.61–7.65 (1H, m), 7.72–7.79 (2H, m), 7.89–7.93 (1H, m), 8.32–8.34 (1H, m), 8.62 (1H, d, *J* = 1.8 Hz), 8.88–8.90 (1H, m), 3H not detected; Anal. Calcd for $C_{20}H_{19}FN_4O_6S$: C, 51.94; H, 4.14; N, 12.12. Found: C, 51.92; H, 4.23; N, 12.04.

*5.21. 1-[5-(2-Fluoropyridin-3-yl)-1-(pyridin-2-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine hydrochloride (**24i**)*

Compound **24i** was prepared from **20i** using a similar procedure as for the preparation of compound **3d** from **4d**. Colorless crystals (15%): mp 198 °C; ^{1}H-NMR (DMSO-d_6) δ 2.53 (3H, s), 3.34 (2H, s), 6.64 (1H, d, *J* = 1.3 Hz), 7.38 (1H, ddd, *J* = 7.2 Hz, 5.0 Hz, 1.9 Hz), 7.61–7.88 (4H, m), 8.10 (1H, dt, *J* = 7.8 Hz, 1.7 Hz), 8.24–8.38 (1H, m), 8.71 (1H, dt, *J* = 3.9 Hz, 0.8 Hz), 8.93 (2H, brs); HRMS (ESI) calcd for $C_{16}H_{15}FN_4O_2S$ $(M+H)^+$ *m/z* 347.0973, found *m/z* 347.0936.

*5.22. 1-{5-(2-fluoropyridin-3-yl)-1-[(6-methoxypyridin-3-yl)sulfonyl]-1H-pyrrol-3-yl}-N-methylmethanamine hydrochloride (**24j**)*

Compound **24j** was prepared from **20j** using a similar procedure as for the preparation of compound **3d** from **4d**. Colorless crystals (29%): mp 223–225 °C; ^{1}H-NMR (DMSO-d_6) δ 2.52 (3H, s), 3.94 (3H, s), 3.99 (2H, s), 6.65 (1H, d, *J* = 1.8 Hz), 6.99–7.02 (1H, m), 7.42–7.47 (1H, m), 7.73–7.80 (2H, m), 7.84 (1H, d, *J* = 1.8 Hz), 8.27–8.28 (1H, m), 8.34–8.36 (1H, m), 9.10 (2H, brs). Anal. Calcd for $C_{17}H_{18}ClFN_4O_3S$: C, 49.45; H, 4.39; N, 13.57. Found: C, 49.16; H, 4.42; N, 13.44.

*5.23. 1-[5-(2-Chloropyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**26a**)*

To a solution of compound **25a** (280 mg, 0.605 mmol) in MeOH (10 mL) was added dropwise 4 mol/L HCl/EtOAc (2 mL), and the mixture was stirred at 70 °C for 30 min, and then concentrated in vacuo. A solution of $NaHCO_3$ was added to the residue and the mixture was extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL), and a solution of fumaric acid (116 mg, 1.0

ACCEPTED MANUSCRIPT

mmol) in MeOH (3 mL) was added dropwise to the solution. The resulting crystals were collected by filtration and rinsed with EtOAc to obtain **26a** (197 mg, 68%) as colorless crystals: mp 170–174 °C; ^{1}H-NMR (DMSO-d_6) δ 2.40 (3H, s), 3.81 (2H, s), 6.49 (2H, s), 6.52 (1H, d, *J* = 1.9 Hz), 7.47–7.52 (1H, m), 7.61–7.73 (3H, m), 7.90–7.94 (1H, m), 8.50 (1H, dd, *J* = 4.9 Hz, 1.9 Hz), 8.63–8.64 (1H, m), 8.90 (1H, dd, *J* = 4.5 Hz, 1.5 Hz), 3H not detected. Anal. Calcd for $C_{20}H_{19}ClN_4O_6S$: C, 50.16; H, 4.00; N, 11.70. Found: C, 49.98; H, 4.06; N, 11.63.

*5.24. 3-{4-[(Methylamino)methyl]-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-2-yl}pyridine-2-carbonitrile fumarate (**26b**)*

Compound **26b** was prepared from **25b** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (39%): mp 204–205 °C; ^{1}H-NMR (DMSO-d_6) δ 2.39 (3H, s), 3.83 (2H, s), 6.48 (2H, s), 6.74 (1H, d, *J* = 1.8 Hz), 7.60–7.65 (1H, m), 7.78–7.83 (2H, m), 7.88–7.95 (2H, m), 8.58–8.59 (1H, m), 8.80–8.82 (1H, m), 8.89–8.91 (1H, m), 3H not detected. Anal. Calcd for $C_{21}H_{19}N_5O_6S$: C, 53.73; H, 4.08; N, 14.92. Found: C, 53.54; H, 4.03; N, 14.92.

*5.25. N-Methyl-1-[5-(2-methylpyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]methanamine fumarate (**26c**)*

Compound **26c** was prepared from **25c** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (32%): mp 203–204 °C; ^{1}H-NMR (DMSO-d_6) δ 2.00 (3H, s), 2.43 (3H, s), 3.83 (2H, s), 6.42 (1H, s), 6.47 (2H, s), 7.20–7.24 (1H, m), 7.28–7.31 (1H, m), 7.59–7.63 (1H, m), 7.70 (1H, s), 7.80–7.84 (1H, m), 8.49–8.51 (2H, m), 8.88–8.90 (1H, m), 3H not detected. Anal. Calcd for $C_{21}H_{22}N_4O_6S$: C, 55.01; H, 4.84; N, 12.22. Found: C, 54.94; H, 4.90; N, 12.25.

*5.26. N-Methyl-1-[5-(4-methylpyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]methanamine fumarate (**26d**)*

Compound **26d** was prepared from **25d** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (48%): mp 184 °C; ^{1}H-NMR (DMSO-d_6) δ 1.89 (3H, s), 2.43 (3H, s), 3.84 (2H, s), 6.45 (1H, d, *J* = 1.9 Hz), 6.48 (2H, s), 7.29 (1H, d, *J* = 4.9 Hz), 7.60–7.65 (1H, m), 7.73 (1H, d, *J* = 1.9 Hz), 7.81–7.85 (1H, m), 7.98 (1H, s), 8.47 (1H, d, *J* = 4.9 Hz), 8.51 (1H, d, *J* = 1.9 Hz), 8.90 (1H, dd, *J* = 4.9 Hz, 1.5 Hz), 3H not detected. Anal. Calcd for $C_{21}H_{22}N_4O_6S$: C, 55.01; H, 4.84; N, 12.22. Found: C, 54.73; H, 4.79; N, 12.16.

*5.27. N-Methyl-1-[5-(3-methylpyridin-2-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]methanamine fumarate (**26e**)*

Compound **26e** was prepared from **25e** using a similar procedure as for the preparation of compound **24b** from **20b**. Colorless crystals (53%): mp 185–186 °C; ^{1}H-NMR (DMSO-d_6) δ 2.18 (3H, s), 2.44

ACCEPTED MANUSCRIPT

(3H, s), 3.86 (2H, s), 6.46 (2H, s), 6.52 (1H, d, *J* = 1.8 Hz), 7.32–7.36 (1H, m), 7.66–7.74 (3H, m), 8.17–8.21 (1H, m), 8.28–8.30 (1H, m), 8.87–8.90 (2H, m), 3H not detected; Anal. Calcd for $C_{21}H_{22}N_4O_6S$: C, 55.01; H, 4.84; N, 12.22. Found: C, 54.95; H, 4.82; N, 12.24.

*5.28. 1-[5-(3-Fluoropyridin-4-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**26f**)*

A degassed mixture of compound **19h** (215 mg, 0.50 mmol), (3-fluoropyridin-4-yl)boronic acid (120 mg, 0.76 mmol), tetrakis(triphenylphosphine)palladium (87 mg, 0.075 mmol) and $NaHCO_3$ (126 mg, 1.50 mmol) in DME (8 mL) and water (2 mL) was stirred at 80 °C for 6 h. After cooling to room temperature, the reaction mixture was diluted with a solution of $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (n-hexane/EtOAc = 1/1), and then the obtained a pale yellow oil (60 mg) was dissolved in MeOH (5 mL). 4 mol/L HCl/EtOAc (1.5 mL) was added to the solution, and the mixture was stirred at 70 °C for 30 min, and then concentrated in vacuo. A solution of $NaHCO_3$ was added to the residue and the mixture was extracted with EtOAc. The extract was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL), and a solution of fumaric acid (19 mg, 0.164 mmol) in MeOH (1 mL) was added to the solution. The resulting crystals were collected by filtration and rinsed with EtOAc to obtain **26f** (45 mg, 19%) as colorless crystals: mp 201 °C; ^{1}H-NMR (DMSO-d_6) δ 2.37 (3H, s), 3.78 (2H, s), 6.49 (2H, s), 6.64 (1H, d, *J* = 1.5 Hz), 7.30–7.33 (1H, m), 7.62–7.66 (1H, m), 7.77 (1H, d, *J* = 1.5 Hz), 7.94–7.98 (1H, m), 8.49–8.51 (1H, m), 8.64 (1H, d, *J* = 1.5 Hz), 8.69 (1H, d, *J* = 2.3 Hz), 8.90 (1H, dd, *J* = 4.9 Hz, 1.5 Hz), 3H not detected. Anal. Calcd for $C_{20}H_{19}FN_4O_6S$: C, 51.94; H, 4.14; N, 12.12. Found: C, 51.73; H, 4.13; N, 12.15.

*5.29. 1-[5-(2-Fluoropyridin-3-yl)-4-methyl-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**37a**)*

Compound **37a** was prepared from **36a** using a similar procedure as for the preparation of compound **3c** from **7**. Colorless crystals (32%): mp 173–175 °C; ^{1}H-NMR (DMSO-d_6) δ 1.76 (3H, s), 2.42 (3H, s), 3.75 (2H, s), 6.50 (2H, s), 7.42–7.46 (1H, m), 7.59–7.75 (3H, m), 7.84–7.88 (1H, m), 8.33–8.35 (1H, m), 8.56–8.57 (1H, m), 8.86–8.88 (1H, m), 3H not detected; Anal. Calcd for $C_{21}H_{21}FN_4O_6S$: C, 52.94; H, 4.44; N, 11.76. Found: C, 52.66; H, 4.42; N, 11.76.

5.30.

*1-[4-chloro-5-(2-fluoropyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine fumarate (**37b**)*

ACCEPTED MANUSCRIPT

To a solution of methylamine hydrochloride (591 mg, 8.75 mmol) in MeOH (mL) was added compound **36b** (320 mg, 0.875 mmol) at room temperature, and the mixture was stirred for 30 min. $NaBH(OAc)_3$ (557 mg, 2.63 mmol) was added to the mixture, which was stirred at room temperature for 3 h and then evaporated under reduced pressure. The residue was partitioned between EtOAc and a solution of $NaHCO_3$. The separated organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (EtOAc/MeOH = 99/1–19/1), and then dissolved in EtOAc (8 mL). A solution of fumaric acid (102 mg, 0.879 mmol) in MeOH (2 mL) was added to the solution, and then the mixture was concentrated under reduced pressure. The residue was recrystallized from EtOAc/MeOH to produce **37b** (52mg, 12%) as colorless crystals: mp 156 °C; ^{1}H-NMR (DMSO-d_6) δ 2.39 (3H, s), 3.70 (2H, s), 6.65 (2H, s), 7.48–7.53 (1H, m), 7.63–7.68 (1H, m), 7.81 (1H, s), 7.84–7.96 (2H, m), 8.40–8.42 (1H, m), 8.65 (1H, d, *J* = 1.9 Hz), 8.93 (1H, dd, *J* = 4.9 Hz, 1.5 Hz), 3H not detected. Anal. Calcd for $C_{20}H_{18}ClFN_4O_6S$: C, 48.34; H, 3.65; N, 11.28. Found: C, 48.20; H, 3.74; N, 11.29.

5.31.

*Bis{1-[4-fluoro-5-(2-fluoropyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine}fumarate (**37c**)*

To a solution of methylamine hydrochloride (911 mg, 13.5 mmol) in MeOH (20 mL) was added a solution of compound **36c** (277 mg, 0.792 mmol) in MeOH (4 mL) at room temperature. After stirring for 5 min, $NaBH(OAc)_3$ (953 mg, 4.50 mmol) was added in one portion at room temperature and the mixture was stirred for 2 h. The reaction was quenched by H_2O, and the mixture was concentrated under reduced pressure. The resulting residue was extracted with EtOAc, and the extract was successively washed with a solution of $NaHCO_3$, H_2O and brine, dried over anhydrous Na_2SO_4, filtered and then concentrated under reduced pressure. The residue was purified by basic silica gel column chromatography (EtOAc), and then dissolved in EtOAc (2 mL). The resulting solution was added to a solution of fumaric acid (38.2 mg, 0.329 mmol) in EtOH (2 mL) at room temperature and the mixture was concentrated under reduced pressure. The resulted solid was recrystallized from EtOH/H_2O. The crystals were collected by filtration and dried in vacuo to obtain compound **37c** (165mg, 49%) as colorless crystals: mp 185–187 °C; ^{1}H-NMR (DMSO-d_6) δ 2.32 (3H, s), 3.64 (2H, s), 6.50 (1H, s), 7.47–7.51 (1H, m), 7.61–7.66 (2H, m), 7.88–7.94 (2H, m), 8.36–8.38 (1H, m), 8.62 (1H, d, *J* = 2.4 Hz), 8.90 (1H, d, *J* = 4.8 Hz), 2H not detected. Anal. Calcd for $C_{18}H_{16}F_2N_4O_4S$: C, 51.18; H, 3.82; N, 13.26. Found: C, 51.13; H, 3.77; N, 13.27.

In case of the crystallization by adding slightly excess of fumaric acid in MeOH to EtOAc solution of free base, 1-[4-Fluoro-5-(2-fluoropyridin-3-yl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamin

ACCEPTED MANUSCRIPT

e fumarate (monofumarate) was yielded as colorless crystals: mp 146–148 °C; ^{1}H-NMR (DMSO-d_6) δ 2.35 (3H, s), 3.69 (2H, s), 6.55 (2H, s), 7.47–7.52 (1H, m), 7.62–7.69 (2H, m), 7.88–7.94 (2H, m), 8.37–8.39 (1H, m), 8.63 (1H, d, *J* = 2.3 Hz), 8.92 (1H, dd, *J* = 4.9 Hz, 1.5 Hz), 3H not detected; Anal. Calcd for $C_{20}H_{18}F_2N_4O_6S$: C, 50.00; H, 3.78; N, 11.66. Found: C, 49.82; H, 3.71; N, 11.67.

5.32. Measurement of H^+, K^+-ATPase activity

This procedure was performed using the method described previously.[25]

5.33. An assay of inhibition of acid secretion in anesthetized rats by intravenous administration

This assay was performed by the method described elsewhere.[25]

5.34. An assay of inhibition of acid secretion in anesthetized rats by oral administration

A test compound at doses of 0.5, 1, 2, or 4 mg/kg (as the free base) or vehicle was administered orally 1 h before pylorus ligation and histamine 2HCl (30 mg/kg, subcutaneous) administration. Gastric contents were collected 3 h after histamine administration, and total acid output was calculated.

5.35. Measurement of pH of a gastric perfusate during histamine stimulation in anesthetized rats

Rats were anesthetized with urethane (1.2 g/kg, intraperitoneal injection). The abdomen was opened, and the stomach was exposed. Cannulas were introduced into the stomach from the duodenum and also from the forestomach, and the esophagus was ligated. The stomach was perfused with saline at a rate of 0.5 mL/min, and the pH of the perfusate was continuously measured with a glass electrode (6961-15C and 2461A-15T; Horiba, Kyoto, Japan). Histamine 2 HCl (8 mg/kg/h) was infused intravenously via the cervical vein. When pH stabilized, a test compound or vehicle was administered intravenously. The pH level of the perfusate was measured for 5 h after administration of the drug or vehicle.

5.36. An assay of inhibition of acid secretion in Heidenhain pouch dogs

This assay was performed using an approach similar to a previously reported method.[25] Compounds or vehicle were given orally (0.2 mL/kg) to the dogs in a blinded manner. Histamine 2 HCl (30 μg/kg) was injected subcutaneously 1 day before and 1, 3, 6, 24 and 48 h after administration of a drug or vehicle. Gastric juice from the pouch was collected continuously for three consecutive 30-min periods after each dosing with histamine 2 HCl. The volume of gastric juice was measured and the acid concentration was determined by automatic titration to pH 7.0 with 0.1 mol/L NaOH solution (COM-555SC; Hiranuma Sangyo Co., Ltd., Japan). The total acid output during the 90 min period (μEq/90 min) at each time point was calculated and expressed as a percentage of the

ACCEPTED MANUSCRIPT

pre-dosing value measured 1 day before the administration.

5.37. A cytotoxicity assay

HepG2 cells were seeded at 2×10^4 cells/well in a 96-well white plate, and cultured in DMEM supplemented with 0.5% fetal bovine serum and a test compound for 1 day. The cell viability was determined by cellular ATP content. The latter was measured by means of ATPLite™-M (PerkinElmer). ATP content was calculated to the following. ATP content (% of control) = (RLU of test compound ÷ RLU of 1% DMSO) × 100.

5.38. Whole-cell patch-clamp for an hERG inhibition assay

hERG/HEK cells stably expressing hERG potassium channel were established by Takeda Pharmaceutical Company.[32] The cells were cultured at 37°C and 5% CO_2 in the minimum Eagle's medium (MEM) supplemented with 10% of fetal bovine serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 0.2 mg/mL Geneticin (Invitrogen Corp., Carlsbad, CA). Whole-cell voltage clamp recordings of hERG currents were performed on hERG/HEK cells. Borosilicate glass pipettes (Harvard Apparatus, Kent, U.K.) were pulled and firepolished to attain final resistances of 2.0–3.5 MΩ. The pipette solution consisted of 130 mM KCl, 7 mM NaCl, 1 mM $MgCl_2$, 5 mM ATP-2Na, 5 mM EGTA, and 5 mM HEPES; pH 7.2. Series resistances values were less than 6 MΩ and were compensated by 60–85%. Cells were perfused with Tyrode's solution consisting of 137 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 11 mM glucose and 10 mM HEPES pH 7.4. Whole-cell currents were recorded using an Axopatch 200B amplifier and Clampex software (Molecular Devices Corp., Sunnyvale, CA). Membrane currents were low-pass-filtered at 1 kHz and sampled at 2.5 kHz with a Digidata 1320 data acquisition system (Molecular Devices Corp.). The membrane potential was held at –75 mV, and depolarization pulses set to 10 mV for 0.5 sec were applied. Tail currents were measured at –40 mV. The protocol was repeated every 5 sec or 10 sec, allowing for complete recovery of the current between test pulses. The experiments were conducted at rt. A amplitudes of currents were measured after a steady-state level of drug blockade was reached at each concentration. The percentage of hERG inhibition was calculated from the peak amplitudes of the tail current before and after the drug applications.

5.39. PAMPA

Donor wells were filled with 200 μL of PRISMA HT buffer (pH 7.4, pION inc.) containing 10 μmol/L test compound. The filter on the bottom of each acceptor well was coated with 4 μL of a GIT-0 Lipid Solution (pION Inc.) and filled with 200 μL of Acceptor Sink Buffer (pION Inc.). The acceptor filter plate was placed on the donor plate and incubated for 3 h at room temperature. After

ACCEPTED MANUSCRIPT

that, the amount of the test compound in both the donor and acceptor wells was measured by LC/MS/MS.

5.40. X-ray structure analysis

All analyses were conducted on a Rigaku R-AXIS RAPID-191R diffractometer using graphite monochromated Cu-K α radiation. The structure was solved by direct methods in SIR2008 and was refined using full-matrix least-squares on F^2 with SHELXL-2013/4.[33] All non-H atoms were refined with anisotropic displacement parameters.

Crystal data for compound **1a**: $C_{19}H_{21}N_2O_2S^+ \cdot Cl^-$, MW = 376.90; crystal size, 0.16 × 0.10 × 0.05 mm; colorless, block; monoclinic, space group $P2_1/n$, a = 7.61861(17) Å, b = 30.9046(8) Å, c = 8.12703(19) Å, $\alpha = \gamma = 90°$, β = 103.043(7)°, V = 1864.14(9) Å^3, Z = 4, Dx = 1.343 g/cm^3, T = 100 K, μ = 2.980 mm^{-1}, λ = 1.54187 Å, R_1 = 0.055, wR_2 = 0.130.

Crystal data for compound **17d**: $C_{19}H_{20}FN_2O_2\ S^+ \cdot Cl^-$, MW = 394.89; crystal size, 0.15 × 0.15 × 0.06 mm; colorless, platelet; monoclinic, space group $P2_1/n$, a = 7.6032(7) Å, b = 30.876(3) Å, c = 8.2198(8) Å, $\alpha = \gamma = 90°$, β = 102.381(7)°, V = 1884.8(3) Å^3, Z = 4, Dx = 1.392 g/cm^3, T = 100 K, μ = 3.053 mm^{-1}, λ = 1.54187 Å, R_1 = 0.076, wR_2 = 0.149.

Crystal data for compound **17e**: $C_{19}H_{20}FN_2O_2\ S^+ \cdot Cl^-$, MW = 394.89; crystal size, 0.33 × 0.13 × 0.11 mm; colorless, block; monoclinic, space group $P2_1/n$, a = 7.6633(5) Å, b = 31.0682(19) Å, c = 8.1413(6) Å, $\alpha = \gamma = 90°$, β = 102.340(7)°, V = 1893.5(2) Å^3, Z = 4, Dx = 1.385 g/cm^3, T = 100 K, μ = 3.039 mm^{-1}, λ = 1.54187 Å, R_1 = 0.052, wR_2 = 0.136.

Crystal data for compound **17f**: $C_{19}H_{20}FN_2O_2\ S^+ \cdot Cl^-$, MW = 394.89; crystal size, 0.27 × 0.20 × 0.05 mm; colorless, chip; triclinic, space group P-1, a = 7.5691(9) Å, b = 9.5845(10) Å, c = 14.1450(14) Å, α = 104.394(7)°, β = 97.005(7)°, γ = 101.481(7)°, V = 958.04(18) Å^3, Z = 2, Dx = 1.369 g/cm^3, T = 100 K, μ = 3.003 mm^{-1}, λ = 1.54187 Å, R_1 = 0.059, wR_2 = 0.132.

CCDC 1526437 for compound **1a**, CCDC 1526434 for compound **17d**, CCDC 1526435 for compound **17e**, and CCDC 1526436 for compound **17f** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Acknowledgments

We thank Dr. Koji Ono, Mr. Osamu Kurasawa, Mr. Ikuo Fujimori, Dr. Kazuo Nakai, Dr. Yukihiro Ikeda, Dr. Sumie Yoshitomi, Ms. Yasumi Kumagai, and Mr. Youichi Nagano for helpful discussions, and Ms. Miyuki Yokomizo for X-ray crystallographic analysis. We are also grateful to Drs. Takashi Ichikawa and Toshimasa Tanaka for proofreading this manuscript.

A. Supplementary data

ACCEPTED MANUSCRIPT

Supplementary data associated with this article can be found, in the online version.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure

The authors declare that they have no conflicts of interest.

References

1) Sachs G, Carlsson E, Lindberg P, Wallmark B. Gastric H,K-ATPase as therapeutic target. *Ann Rev Pharmacal Taxical*. 1988;28:269–284.
2) Nagaya H, Satoh H, Kubo K, Maki Y. Possible mechanism for the inhibition of gastric (H^+ + K^+)-adenosine triphosphatase by the proton pump inhibitor AG-1749. *J Pharmacol Exp Ther*. 1989;248:799–805.
3) Wolfe MM, Sachs G. Acid suppression: optimizing therapy for gastroduodenal ulcer healing, gastroesophageal reflux disease, and stress-related erosive syndrome. *Gastroenterology*. 2000;118(2):S9–S31.
4) Shin JM, Cho YM, Sachs G. Chemistry of covalent inhibition of the gastric (H^+, K^+)-ATPase by proton pump inhibitors. *J Am Chem Soc*. 2004;126:7800–7811.
5) Shi S, Klotz U. Proton pump inhibitors: An update of their clinical use and pharmacokinetics. *Eur J Clin Pharmacol*. 2008;64:935–951.
6) Graham DY, Agrawal NM, Campbell DR, Haber MM, Collis C, Lukasik NL, Huang B. Ulcer prevention in long-term users of nonsteroidal anti-inflammatory drugs: results of a double-blind, randomized, multicenter, active- and placebo-controlled study of misoprostol vs lansoprazole. *Arch Intern Med*. 2002;162:169–175.
7) Frazzoni M, De Micheli E, Grisendi A, Savarino V. Effective intra-oesophageal acid suppression in patients with gastro-oesophageal reflux disease: Lansoprazole vs. pantoprazole. *Aliment Pharmacol Ther*. 2003;17:235–241.
8) Robinson M. Proton pump inhibitors: Update on their role in acid-related gastrointestinal diseases. *Int J Clin Pract*. 2005;59(6):709–715.
9) Malfertheiner P, Mössner J, Fischbach W, Layer P, Leodolter A, Stolte M, Demleitner K, Fuchs W. Helicobacter pylori eradication is beneficial in the treatment of functional dyspepsia. *Aliment Pharmacol Ther*. 2003;18:615–625.
10) Fass R, Shapiro M, Dekel R, Sewell J. Systematic review: Proton-pump inhibitor failure in gastro-oesophageal reflux disease–where next? *Aliment Pharmacol Ther*. 2005;22:79–94.

ACCEPTED MANUSCRIPT

11) Dammann HG, Burkhardt F. Pantoprazole versus omeprazole: influence on meal-stimulated gastric acid secretion. *Eur J Gastroenterol Hepatol*. 1999;11(11): 1277–1282.

12) Katz PO, Hatlebakk JG, Castell DO. Gastric acidity and acid breakthrough with twice-daily omeprazole or lansoprazole. *Aliment Pharmacol Ther*. 2000;14:709–714.

13) Ang TL, Fock KM. Nocturnal acid breakthrough: Clinical significance and management. *J Gastroenterol Hepatol*. 2006;21:S125–S128.

14) Furuta T, Shirai N, Sugimoto M, Nakamura A, Hishida A, Ishizaki T. Influence of CYP2C19 pharmacogenetic polymorphism on proton pump inhibitor-based therapies. *Drug Metab Pharmacokinet*. 2005;20(3):153–167.

15) Andersson K, Carlsson E. Potassium-competitive acid blockade: A new therapeutic strategy in acid-related diseases. *Pharmacol Ther*. 2005;108:294–307.

16) Yoon YA, Park CS, Cha MH, Choi H, Sim JY, Kim JG. Novel pyrimidines as acid pump antagonists (APAs). *Bioorganic Med Chem Lett*. 2010;20:5735–5738.

17) Inatomi N, Matsukawa J, Sakurai Y, Otake K. Potassium-competitive acid blockers: Advanced therapeutic option for acid-related diseases. *Pharmacol Ther*. 2016;168:12–22.

18) Garnock-Jones KP. Vonoprazan: First global approval. *Drugs*. 2015;75:439–443.

19) Kaminski JJ, Bristol JA, Puchalski C, Lovey RG, Elliott AJ, Guzik H, Solomon DM, Conn DJ, Domalski MS, Wong SC, Gold EH, Long JF, Chiu PJS, Steinberg M, McPhail AT. Antiulecer agents. 1. Gastric antisecretory and cytoprotective properties of substituted imidazo[1,2-a]pyridines. *J Med Chem*. 1985;28:876–892.

20) Gedda K, Briving C, Svensson K, Maxvall I, Andersson K. Mechanism of action of AZD0865, a K^+-competitive inhibitor of gastric H^+,K^+-ATPase. *Biochem Pharmacol*. 2007;73:198–205.

21) Kahrilas PJ, Dent J, Lauritsen K, Malfertheiner P, Denison H, Franzen S, Hasselgren G. A randomized, comparative study of three doses of AZD0865 and esomeprazole for healing of reflux esophagitis. *Clin Gastroenterol Hepatol*. 2007;5(12):1385–1391.

22) Ito K, Kinoshita K, Tomizawa A, Inaba F, Morikawa-Inomata Y, Makino M, Tabata K, Shibakawa N. Pharmacological profile of novel acid pump antagonist 7-(4-fluorobenzyloxy)-2,3-dimethyl-1-{[(1S,2S)-2-methyl cyclopropyl]methyl}-1H-pyrrolo[2,3-d]pyridazine (CS-526). *J Pharmacol Exp Ther*. 2007;323:308–317.

23) Simon WA, Herrmann M, Klein T, Shin JM, Huber R, Senn-Bilfinger J, Postius S. Soraprazan : Setting new standards in inhibition of gastric acid secretion. *J Pharmacol Exp Ther*. 2007;321:866–874.

24) Parsons ME, Keeling DJ. Novel approaches to the pharmacological blockade of gastric acid secretion. *Expert Opin Investig Drugs*. 2005;14(4):411–421.

25) Nishida H, Hasuoka A, Arikawa Y, Kurasawa O, Hirase K, Inatomi N, Hori Y, Sato F, Tarui N,

ACCEPTED MANUSCRIPT

Imanishi A, Kondo M, Takagi T, Kajino M. Discovery, synthesis, and biological evaluation of novel pyrrole derivatives as highly selective potassium-competitive acid blockers. *Bioorganic Med Chem*. 2012;20:3925–3938.

26) Hori Y, Imanishi A, Matsukawa J, Tsukimi Y, Nishida H, Arikawa Y, Hirase K, Kajino M, Inatomi N. 1-[5-(2-Fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamine Monofumarate (TAK-438), a novel and potent potassium-competitive acid blocker for the treatment of acid-related diseases. *J Pharmacol Exp Ther*. 2010;335:231–238.
27) Hori Y, Matsukawa J, Takeuchi T, Nishida H, Kajino M, Inatomi N. A study comparing the antisecretory effect of TAK-438, a novel potassium-competitive acid blocker, with lansoprazole in animals. *J Pharmacol Exp Ther*. 2011;337:797–804.
28) Arikawa Y, Nishida H, Kurasawa O, Hasuoka A, Hirase K, Inatomi N, Hori Y, Matsukawa J, Imanishi A, Kondo M, Tarui N, Hamada T, Takagi T, Takeuchi T, Kajino M. Discovery of a novel pyrrole derivative 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1*H*-pyrrol-3-yl]-*N*-methylmethanamine fumarate (TAK-438) as a potassium-competitive acid blocker (P- CAB). *J Med Chem*. 2012;55:4446–4456.
29) Leeson PD, Springthorpe B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat Rev Drug Discov*. 2007;6:881–890.
30) Yamamoto K, Ikeda Y. Kinetic solubility and lipophilicity evaluation connecting formulation technology strategy perspective. *J Drug Deliv Sci Technol*. 2016;33:13–18.
31) Böhm HJ, Banner D, Bendels S, Kansy M, Kuhn B, Müller K, Obst-Sander U, Stahl M. Fluorine in medicinal chemistry. *Chembiochem*. 2004;5:637–643.
32) Imai YN, Ryu S, Oiki S. Docking model of drug binding to the human ether-à-go-go potassium channel guided by tandem dimer mutant patch-clamp data: A synergic approach. *J Med Chem.* 2009;52:1630–1638.
33) Sheldrick GM. A short history of SHELX. *Acta Crystallogr A*. 2008;64:112–122.

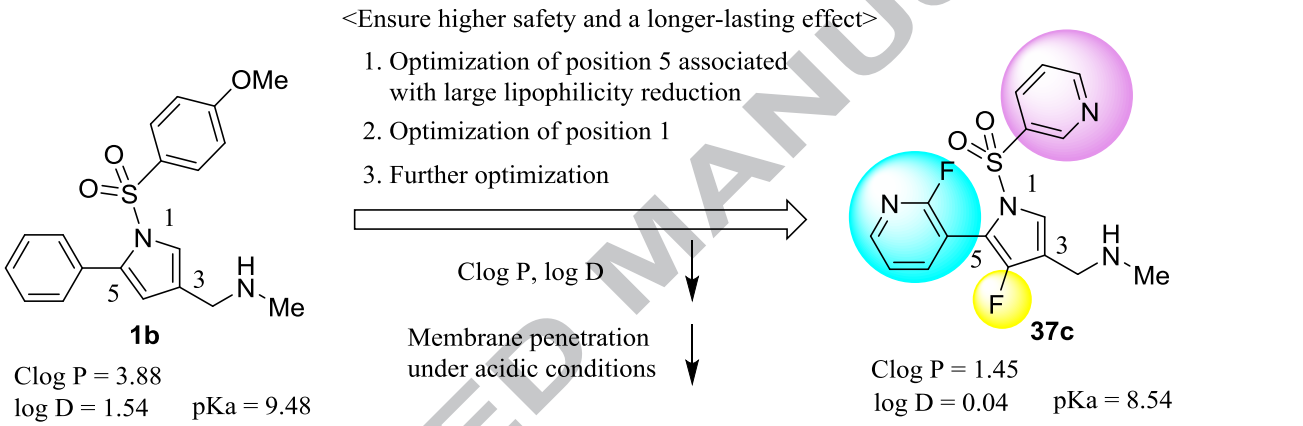
ACCEPTED MANUSCRIPT

Graphical Abstract

Identification of a novel fluoropyrrole derivative as a potassium-competitive acid blocker with long duration of action

Haruyuki Nishida, Yasuyoshi Arikawa, Keizo Hirase, Toshihiro Imaeda, Nobuhiro Inatomi, Yasunobu Hori, Jun Matsukawa, Yasushi Fujioka, Teruki Hamada, Motoo Iida, Mitsuyoshi Nishitani, Akio Imanishi, Hideo Fukui, Fumio Itoh, and Masahiro Kajino

Pharmaceutical Research Division: Takeda Pharmaceutical Company, Ltd., 26-1, Muraokahigashi 2-chome, Fujisawa, Kanagawa 251-8555, Japan



ACCEPTED MANUSCRIPT