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Novel pyrimidines as acid pump antagonists (APAs)

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ABSTRACT

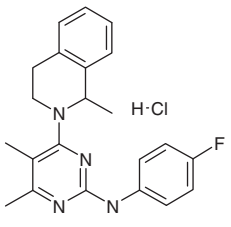
A series of pyrimidine derivatives as acid pump antagonists (APAs) was synthesized and the inhibitory activities against H^+/K^+ ATPase isolated from hog gastric mucosa were determined. After elaborating on substituents at C2 and C4 position of the pyrimidine scaffold, we have observed that the compound **7h** is a potent APA with H^+/K^+ ATPase, IC_{50} = 52 nM.

Gastroesophageal reflux disease (GERD) is a common acid-related disorder with a prevalence estimated to range between 10% and 20% in Western countries and about 5% in Asia when it is defined as at least weekly heartburn and/or acid regurgitation.[1] The severity of the symptoms and esophageal mucosal damage were correlated well with the exposure of the esophagus to gastric acid.[2] Therefore, acid suppression is considered as the first-line therapy for GERD, and many drugs that suppress the acid secretion are now available including histamine 2 receptor antagonists (H_2RAs) and proton pump inhibitors (PPIs). Presently, PPIs are recognized as the 'treatment of choice' in most countries.[3] However, PPIs still continue to have their set of limitations. The currently available PPIs require around 3–5 days to achieve maximum acid inhibition at existent therapeutic doses, primarily due to their chemical structures and irreversible inhibition of H^+/K^+ ATPase.[4] Failure to demonstrate a sustained acid inhibition throughout the day and night, in spite of twice daily administration and nocturnal acid breakthrough (NAB) are found to be common in patients taking PPIs.[5] Therefore, many novel strategies to address the unmet needs of existent PPI therapy have been investigated, and acid pump antagonists (APAs) could play a promising role, as they provide faster onset and longer duration of action than conventional PPIs by virtue of their ability to reversibly bind to the proton pump.[6]

APAs are lipophilic and weak bases that have diverse structures such as imidazopyridines, pyrimidines, imidazonaphthyridines, or quinolines, etc.[6] Revaprazan (**1**), the representative agent based on pyrimidine scaffold, was shown to inhibit gastric acid secretion by reversibly binding to H^+/K^+ ATPase, and it also displayed excellent antisecretory properties both in animals and human beings (Fig. 1).[7–9] Revaprazan (**1**) was launched in Korea in 2007 for the treatment of duodenal ulcer, gastric ulcer and gastritis. It is also undergoing phase III clinical studies for the treatment of GERD.

In this paper, we described our extended work to the corresponding pyrimidines related to revaprazan (**1**). Pyrimidine derivatives (**2**) that have substituent at C2 other than aniline group were prepared and their inhibitory activities against H^+/K^+ ATPase were evaluated (Fig. 2). We were able to identify pyrimidine APAs that have inhibitory activity superior to that of revaprazan (**1**).

Treating the commercially available 4-fluorophenylacetonitrile **3a** with iodomethane using potassium *t*-butoxide and 18-crown-6 at −78 °C afforded **3b** in good yield (92%). Also the reaction of **3a** with 1-bromo-2-chloroethane using *N,N,N*-trimethylbenzylammonium chloride as a phase transfer catalyst led to **3c** (42%). Subsequently, amidine derivatives of structure **4** can be readily obtained by following the procedure as described in the literature.[10] Nitrile derivatives **3** were added to the mixture of trimethylaluminum and ammonium chloride in toluene at 0 °C, and the solution was heated to 80 °C for 22 h to afford **4** in good yield



Revaprazan(1)

Figure 1. Structure of pyrimidine APA, revaprazan (**1**).

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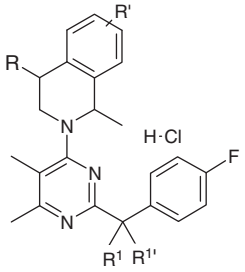


2

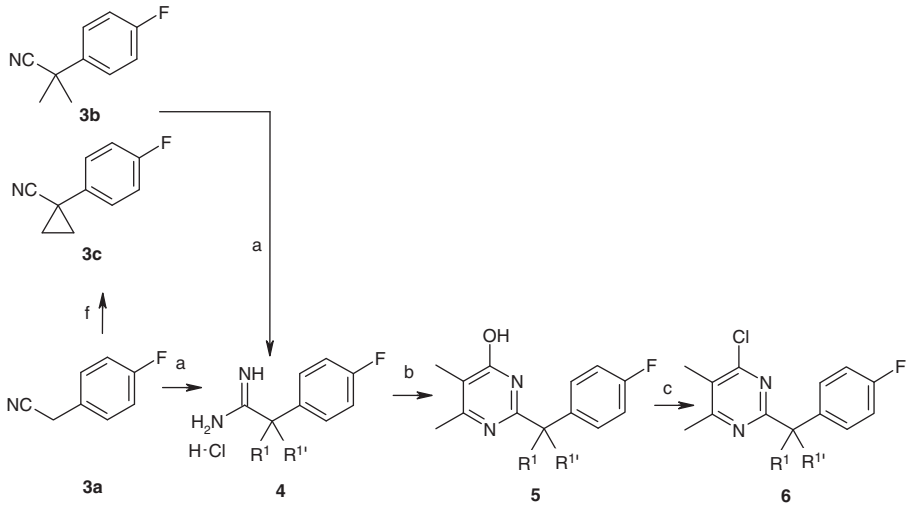
R = H, CH_3
R' = H, F, Cl, CH_3, OCH_3
$R^1 = R^{1'}$ = H
$R^1 = R^{1'} = CH_3$
R^1 = H, $R^{1'}$ = F, OH
R^1-$R^{1'}$: cyclopropyl, =O

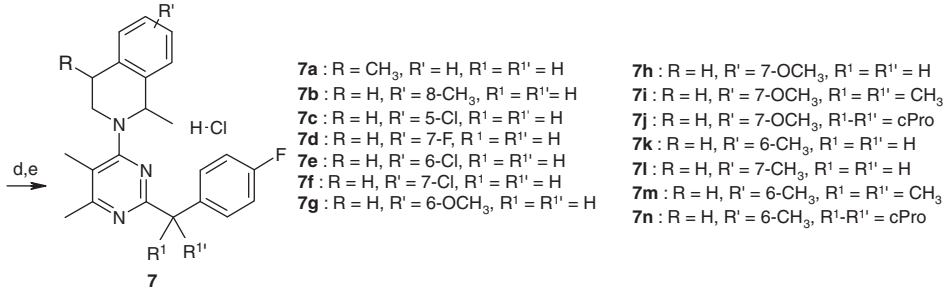
Figure 2. Structure of pyrimidine APAs (**2**).

(65–69%). Condensation of amidine derivatives of **4** with ethyl 2-methylacetoacetate in methanol using sodium methoxide provided 4-hydroxy pyrimidine derivatives **5** in satisfactory yield (51–65%). After chlorination of **5** with phosphorus oxychloride (**6**), substitution with respective 1,2,3,4-tetrahydroisoquinoline derivatives[11] and subsequent crystallization of the intermediate from a solution of hydrochloric acid in ethyl acetate afforded corresponding pyrimidine derivatives **7** in good yield (46–86%, Scheme 1).

2-(4-Fluorobenzoyl) substituted pyrimidine derivatives were obtained starting from commercially available 2,4-dihydroxy-5,6-dimethylpyrimidine **8** (Scheme 2). Chlorination of **8** with phosphorus oxychloride using *N,N*-dimethylaniline (DMA) as a catalyst yielded 2,4-dichloro-5,6-dimethylpyrimidine **9** (85%). Substitution by respective 1,2,3,4-tetrahydroisoquinoline derivatives occurred mainly at C4 position of pyrimidine ring with C2 substituted one as a by-product. Reaction of **9** and respective 1,2,3,4-tetrahydroisoquinoline derivatives with triethylamine (TEA) in DMF at room temperature led to **10** in 44–56% yield. 2-Stannylpyrimidine derivatives of structure **11** were prepared by stannyl anion substitution in 2-chloropyrimidines **10** using lithium diisopropylamide (LDA) and tributyltin hydride (15–42%).[12] Stille coupling of **11** with 4-fluorobenzoyl chloride and subsequent crystallization of the intermediate from a solution of hydrochloric acid in ethyl acetate afforded corresponding 2-(4-fluorobenzoyl) substituted pyrimidine derivatives **12** (44–92%, over two-steps).[12] Also reduction of 2-(4-fluorobenzoyl) substituted pyrimidine derivatives **12** with sodium borohydride and subsequent transformation with (diethylamino)sulfur trifluoride (DAST) provided **13** and **14** in good yield (88% and 73%, respectively).

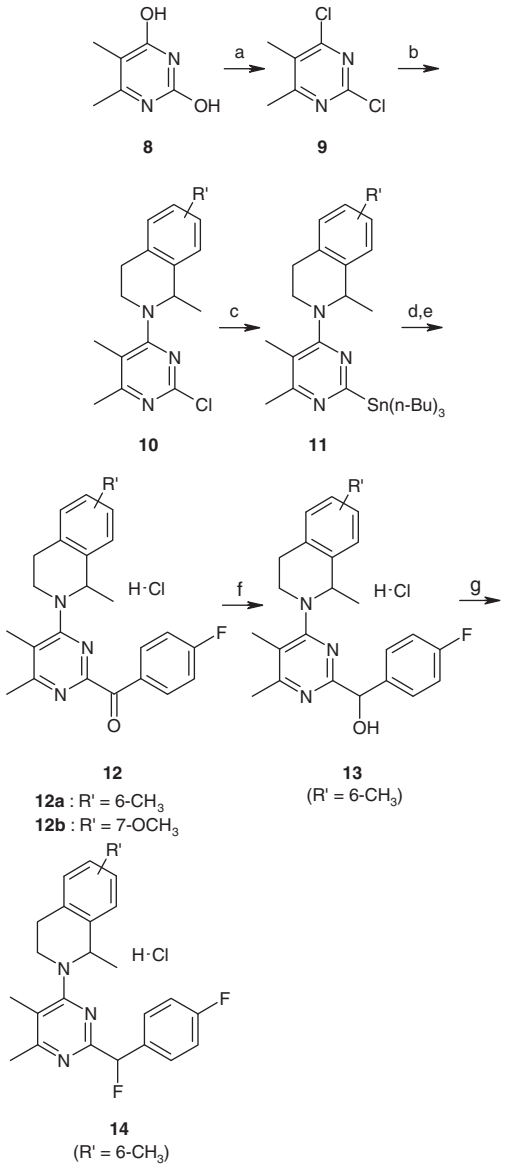
Table 1 enlists the inhibitory activities of pyrimidine derivatives **7**, **12**, **13** and **14** against H^+/K^+ ATPase isolated from hog gastric mucosa.[13] The inhibitory activity against H^+/K^+ ATPase of 2-(4-fluorobenzyl) substituted pyrimidines with various 1-methyl-1,2,3,4-tetrahydroisoquinoline substituents at C4 was evaluated. It was noteworthy that the electron-donating substituents, methyl (R = CH_3, R′ = H or R = H, R′ = CH_3) and methoxy groups (R = H, R′ = OCH_3), at 1-methyl-1,2,3,4-tetrahydroisoquinoline were more favorable than the substituents with electron-withdrawing properties (R = H, R′ = F, Cl); **7g** (R = H, R′ = 6-OCH_3), **7h** (R = H, R′ = 7-OCH_3), **7k** (R = H, R′ = 6-CH_3), and **7l** (R = H, R′ = 7-CH_3) were more





7a : R = CH_3, R' = H, $R^1 = R^{1'}$ = H
7b : R = H, R' = 8-CH_3, $R^1 = R^{1'}$= H
7c : R = H, R' = 5-Cl, $R^1 = R^{1'}$ = H
7d : R = H, R' = 7-F, $R^1 = R^{1'}$ = H
7e : R = H, R' = 6-Cl, $R^1 = R^{1'}$ = H
7f : R = H, R' = 7-Cl, $R^1 = R^{1'}$ = H
7g : R = H, R' = 6-OCH_3, $R^1 = R^{1'}$ = H
7h : R = H, R' = 7-OCH_3, $R^1 = R^{1'}$ = H
7i : R = H, R' = 7-OCH_3, $R^1 = R^{1'} = CH_3$
7j : R = H, R' = 7-OCH_3, R^1-$R^{1'}$ = cPro
7k : R = H, R' = 6-CH_3, $R^1 = R^{1'}$ = H
7l : R = H, R' = 7-CH_3, $R^1 = R^{1'}$ = H
7m : R = H, R' = 6-CH_3, $R^1 = R^{1'} = CH_3$
7n : R = H, R' = 6-CH_3, R^1-$R^{1'}$ = cPro

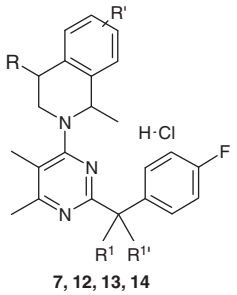
Scheme 1. Reagents and conditions: (a) NH_4Cl, Al $(CH_3)_3$, toluene, 80 °C, 22 h, 65–69%; (b) ethyl 2-methylacetoacetate, NaOMe, MeOH, reflux, 51–65%; (c) $POCl_3$, reflux, 2 h, 62–97%; (d) 1,2,3,4-tetrahydroisoquinoline derivatives, TEA, DMF, reflux, 7 h; (e) HCl(g), EtOAc, 0 °C, 46–86% over two-steps; (f) iodomethane (**3b**), *t*-BuOK, 18-crown-6, THF, −78 °C, 2 h, 92%; *N,N,N*-trimethylbenzylammonium chloride, 1-bromo-2-chloroethane (**3c**), 50% NaOH, 45 °C, 6 h, 42%.



Scheme 2. Reagents and conditions: (a) $POCl_3$, DMA, 130 °C, 2 h, 85%; (b) 1,2,3,4-tetrahydroisoquinoline derivatives, TEA, DMF, rt, 1 h, 44–56%; (c) LDA, Bu_3SnH, THF, 0 °C, 3 h, 15–42%; (d) 4-fluorobenzoyl chloride, THF, −78 °C, 3 h; (e) HCl(g), EtOAc, 0 °C, 44–92%(over two-steps); (f) $NaBH_4$, IPA, reflux, 14 h; HCl(g), EtOAc, 0 °C, 88%; (g) DAST, MC, −78 °C, 1 h; HCl(g), EtOAc, 0 °C, 73%.

than three-fold potent compared to the compounds **7c** (R = H, R′ = 5-Cl), **7d** (R = H, R′ = 7-F), **7e** (R = H, R′ = 6-Cl), and **7f** (R = H, R′ = 7-Cl). And **7g** (R = H, R′ = 6-OCH_3), **7h** (R = H, R′ = 7-OCH_3), **7k** (R = H, R′ = 6-CH_3), and **7l** (R = H, R′ = 7-CH_3) were even more potent than revaprazan (**1**). Especially, **7h** (R = H, R′ = 7-OCH_3) was shown to have nanomolar inhibitory concentration (IC_{50} = 52 nM) in a dose-dependent manner (Fig. 3). Although **7a** (R = CH_3, R′ = H) and **7b** (R = H, R′ = 8-CH_3) were more potent than the compounds that have electron-withdrawing substituents at 1-methyl-1,2,3,4-tetrahydroisoquinoline (**7c**, **7d**, **7e**, and **7f**), they were less favored than those with methyl substituent at the phenyl ring of 1-methyl-1,2,3,4-tetrahydroisoquinoline (**7k** and **7l**). Among the compounds with electron-withdrawing substituents at 1-methyl-1,2,3,4-tetrahydroisoquinoline, **7c** (R = H, R′ = 5-Cl) was shown to have lowest inhibitory activity against H^+/K^+ ATPase. Therefore, it was evident that 1-methyl-1,2,3,4-tetrahydroisoquinoline derivatives substituted by electron-donating groups at C6 or C7 are favored at C4 of pyrimidines.

Table 1
H^+/K^+ ATPase inhibition assay results for **7**, **12**, **13** and **14**



7, 12, 13, 14

Compds	R	R′	R^1	$R^{1\prime}$	H^+/K^+ ATPase IC_{50} (μM)
1					0.35
7a	CH_3	H	H	H	0.61
7b	H	8-CH_3	H	H	0.70
7c	H	5-Cl	H	H	1.99
7d	H	7-F	H	H	1.24
7e	H	6-Cl	H	H	0.94
7f	H	7-Cl	H	H	1.63
7g	H	6-OCH_3	H	H	0.17
7h	H	7-OCH_3	H	H	0.052
7i	H	7-OCH_3	CH_3	CH_3	34.2%[a]
7j	H	7-OCH_3	cPro		1.4%[a]
7k	H	6-CH_3	H	H	0.23
7l	H	7-CH_3	H	H	0.30
7m	H	6-CH_3	CH_3	CH_3	1.76
7n	H	6-CH_3	cPro		1.29
12a	H	6-CH_3	=O		1.91
12b	H	7-OCH_3	=O		1.12
13	H	6-CH_3	H	OH	1.51
14	H	6-CH_3	H	F	1.13

[a] Inhibition percentage at 4 μM.

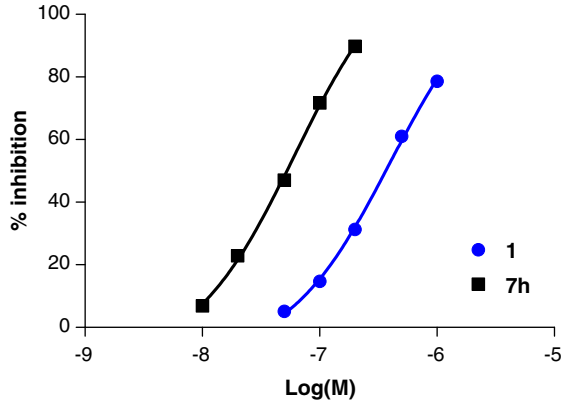


Figure 3. Dose-dependent inhibitions of H^+/K^+ ATPase by pyrimidine APA **1** and **7h**.

After exploring the substituents at C4 of pyrimidines to improve inhibitory activity against H^+/K^+ ATPase, we chose to more extend C2 substituents with 7-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (R = H, R′ = 7-OCH_3, as seen in **7h**) or 1,6-dimethyl-1,2,3,4-tetrahydroisoquinoline (R = H, R′ = 6-CH_3 as seen in **7k**) at C4 fixed. Replacement of 4-fluorobenzyl group with 4-fluorophenylcyclopropyl (**7j** and **7n**) or 2-methyl-2-(4-fluorophenyl)ethyl (**7i** and **7m**) led to the reduction in the inhibitory activity against H^+/K^+ ATPase compared to the 2-(4-fluorobenzyl) group substituted ones (**7h** and **7k**). Same trend was also observed in 2-(4-fluorobenzoyl) substituted pyrimidines **12a** and **12b**, and in hydroxy and fluoro group substituted pyrimidines (**13** and **14**) instead of carbonyl group at C2 indicating the 4-fluorobenzyl group at C2 of pyrimidines is the most favored for inhibiting H^+/K^+ ATPase.

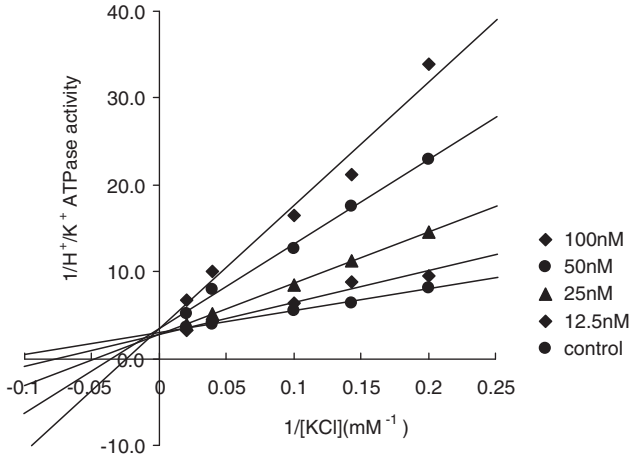


Figure 4. Lineweaver–Burk plot showing H^+/K^+ ATPase activity versus K^+ concentration for various concentrations of **7h**.

The mechanism of inhibition by **7h** was determined in relation to the activation of H^+/K^+ ATPase activity by K^+. Compound **7h** inhibited H^+/K^+ ATPase activity in a K^+-competitive manner with K_i = 16.5 nM (Fig. 4).[14] The Lineweaver–Burk plot showed H^+/K^+ ATPase activity versus K^+ concentration for various concentrations of **7h**, and demonstrated a common intercept with the Y-axis, which is characteristic of competitive inhibition.

In summary, we have prepared a series of novel pyrimidines as APAs. Optimization of substituents at C2 and C4 led to some potent pyrimidine APAs. Especially, compound **7h** was shown to have excellent inhibitory activity against H^+/K^+ ATPase (IC_{50} = 52 nM). Therefore, compound **7h** is a promising lead for further development as APAs, and this series of pyrimidine derivatives would be explored for further optimization.

Acknowledgements

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13. Ion-leaky membrane vesicle enriched in gastric H^+/K^+-ATPase was derived from pig stomach as per the method described by Saccomani et al. with suitable modifications (Saccomani, G.; Stewart, H. B.; Show, D.; Lewin, M.; Sachs, G. *Biochem. Biophy. Acta* **1977**, *465*, 311). The inhibitory effects of the K^+ specific H^+/K^+-ATPase activity was calculated based on the difference between the activity of H^+/K^+-ATPase with and without K^+ ion. The lyophilized vesicle in 5 mM Pipes/Tris buffer (pH 6.1) was pre-incubated in the presence of various concentrations of compounds. After 5 min preincubation, negative and positive buffers were, respectively, added to the previous reaction mixture. As the substrate ATP was added to the reaction buffer, and incubated for 30 min at 37 °C. Enzymatic activity was stopped by adding colorimetric reagent and the amount of mono phosphate (P_i) in the reaction was measured at 620 nm using the microplate reader. The difference between P_i production with and without K^+ was taken as K^+ stimulated H^+/K^+-ATPase activity. The IC_{50}s of test compounds were calculated from each % inhibition value of compounds using the method described by Litchfield–Wilcoxon (Litchfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *95*).
14. The inhibition kinetics were determined in relation to the activation of H^+/K^+-ATPase activity by K^+. The lyophilized vesicle in 5 mM Pipes/Tris buffer (pH 6.1) was pre-incubated in the presence of various concentrations of compounds. Compounds were examined for its ability to inhibit the generation of inorganic phosphate induced by various concentrations of KCl. The difference between the Pi production with K^+ and without K^+ is taken as K^+ stimulated H^+/K^+-ATPase activity. The K^+-stimulated H^+/K^+-ATPase activities were analyzed by Lineweaver–Burk plot.