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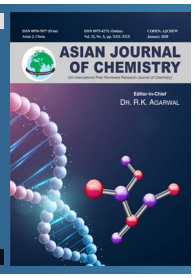

Design, Synthesis and Pharmacological Evaluation of Novel Imidazopyridine Analogues as Proton Pump Antagonist

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A series of novel imidazopyridine derivatives as proton pump inhibitors was designed with compounds of CID data base and explored considering AZD0865 as standard. Many compounds were identified and docked in proton pump ATPase pocket (PDB ID: 4ux2). Molecular docking studies revealed that many compounds showed good proton pump ATPase inhibitory activity. The docking poses revealed the interaction of ligands with amino acid. The standard drug AZD0865 had docking score of -7.112302 and displayed interactions with Asn138 and Asp137. A series of novel imidazopyridine derivatives as proton pump inhibitors were docked, synthesized and characterized by IR, NMR, CHN and MS spectral analysis. The target imidazopyridines were prepared from substituted 2-aminonicotinic acid and 2-bromo-1-substituted ethanone. *in vitro* Studies explained that few compounds exhibited moderate to good proton pump ATPase inhibitory activity in comparison with the reference drugs *i.e*. AZD0865. Compounds **11** and **12** shown higher activities with the IC_{50} 4.3. Compounds **1**, **4**, **6**, **7**, **8**, **10** and **13** showed weak anti-ulcer activity with its IC_{50} 5.2, 5.8, 5.5, 5.1, 4.9, 4.6 and 5.9 and positive control AZD0865 shown IC_{50} 2.0.

Keywords: Imidazopyridine, Proton pump ATPase, Antiulcer, Molecular docking.

INTRODUCTION

Gastric acid secretion is involved in the etiology of ulcer and gastroesophagal reflux disease with the erossion of the inner lining of the stomach. From many years, it is believed that acidic food, stress and infection by bacteria *Helicobacter pylori* cause ulcers. Other factors associated with recurrence of ulcer diseases includes cigarette smoking, chronic consumption of ulcerogenic drugs like NSAID, consumption of alcohol for prolonged periods, age, emotional stress and family history. The common symptom of ulcer is mild to moderate severe pain just below the breastbone may last for once or a few times daily typically after eating. Other symptoms include heartburn and nausea and vomiting [1-4].

Inhibition of H^+/K^+-ATPase, therefore, blocks the basal and stimulated acid secretion. Many benzimidazole sulfoxide pyridine classes as proton pump inhibitors (PPIs), significantly progressed in this field. Starting from 1974, timoprazole, picoprazole, omeprazole, pantoprazole, rabeprazole, *etc*. were discovered [5-7]. Extreme acid suppression also shown achlorohydria and that may produce enteric infections like typhoid, cholera and dysentery. Some time drug interactions leads to decreased absorption of some drugs like griseofulvin, ketoconazole, vitamin B_{12}, iron salts, *etc*. Unpredictable action shows hypergastrinemia, gastric polips and carcinoma [8-10].

The currently available PPIs requires long time to achieve maximum acid inhibition at therapeutic doses, primarily due to their chemical structural modification and irreversible inhibition of H^+/K^+-ATPase. Therapy failed to control sustained acid inhibition throughout the day and night, in spite of twice daily administration. Therefore, many novel strategies are used to solve the unmet needs of PPI therapy. Acid pump antagonists (APAs) could play a promising role, due to their faster onset and longer duration of action than irreversible PPIs by their ability to reversibly bind to the proton pump. The imidazopyridine based compound SCH28080 and AZD0865 (Fig. 1) was the prototype of this class. In comparison to omeprazole, SCH 28080 is a competitive inhibitor of high affinity luminal

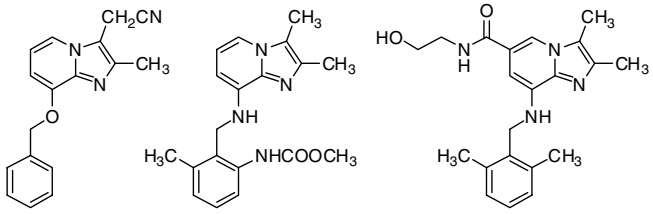


Fig. 1. Representative H^+/K^+-ATPase inhibitors

K^+ site of the gastric proton pump. In contrast to Na^+/K^+-ATPase, it is highly selective to H^+/K^+-ATPase activity. SCH 28080 is a protonable weak base, hence like omeprazole it accumulates in the acidic compartments of the parietal cells in its protonated form. SCH 28080 is chemically stable and after protonation, is itself active and does not need an acid-induced transformation, as required by PPIs [11-13].

Thus the development of another class of compounds targeting H^+/K^+-ATPase tries to combine the advantages of both PPIs and H2RAs and are active in absence of acid secretion. Designing such drug targeting H^+/K^+-ATPase may give rise to newer ideal drug to treat ulcer. Overcome the side effects of previous compounds and are active in absence of acid secretion [12-14]. The target compounds imidazopyridine were first selected from CID database and few active compounds with their derivatives were prepared from 2-aminonicotinic acid and 2-bromo-1-substituted ethanone to form 2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid. The same compound with amino group converted to form 3-amino-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid and later methyl-2-((8-((4-aminophenyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)acetate. In the present study, structures were docked in H^+/K^+-ATPase pocket and synthesized new imidazopyridine analogues **1-13** by the replacement of various substituent′s present on SCH 28080 and AZD0865 to achieve maximum biological activity.

EXPERIMENTAL

The reagents and solvents were used as received from Sigma-Aldrich. Aluminum trichloride, tris-HCl were purchased from Sigma, Germany. $MgCl_2$, KCl, methanol and ATP were purchased from Loba Chem, India. Reactions were observed by TLC with silica gel plates. Silica gel (100-200 mesh) as fixed phase was used for column chromatography. MS spectra of the synthesized analogues were documented on Shimadzu QP-5050 spectrophotometer. ^{1}H NMR spectra were acquired on a Varian-300 (300 MHz NMR) spectrophotometer using $CDCl_3$ and DMSO-d_6 as solvent. The infrared spectra were retrieved using Perkin-Elmer Spectrum ES Version 10.5.3 Fourier transform infrared spectrometer. Elemental analysis was performed on FLASH EA 1112 CHN Elemental analyzer, Thermofinnigen, Italy.

Methyl 2-((8-((4-aminophenyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)acetate (1-4)

2-(Substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (1a-d): A mixture of 2-aminonicotinic acid (0.138 g, 1 mmol) and 2-bromo-1-substituted ethanone (1.2 mmol) refluxed at 60 ºC for 30 min. The mixture was cooled to room temperature and the residue was treated by $NaHCO_3$ upto pH 7 and then extracted with ethyl acetate. The final purification was performed by recrystallization from hot acetone to give 2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid as off-white solid.

Compound 1a: Yield: 0.20 g (87 %); m.p.: 275-280 ºC, IR (KBr, cm^{-1}): 3505 (OH), 3150 (C=C-H), 3055 (C-H), 1734 (COOH), 1565 (C=C), 1436 (C=N), 762, 712; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, CH_3), 7.22 (t, 1H, ArH), 7.26 (q, 1H, ArH), 7.32 (t, 1H, ArH), 7.42 (q, 1H, ArH), 7.20-7.60 (3H, complex ArH),7.88 (s, 1H, ArH), 11.14 (s, 1H, OH).

Compound 1b: Yield: 0.19 g (76 %); m.p.: 278-282 ºC, IR (KBr, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.91 (t, 3H, CH_3), 2.13 (q, 2H, CH_2), 7.20 (t, 1H, ArH), 7.22 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.85 (s, 1H, ArH), 11.10 (s, 1H, OH).

Compound 1c: Yield: 0.20 g (80 %); m.p.: 270-272 ºC. IR (KBr, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 6H, $2CH_3$), 7.22 (t, 1H, ArH), 7.76 (s, 1H, ArH), 7.26 (d, 2H, ArH), 7.28 (d, 1H, ArH), 7.34 (t, 1H, ArH), 7.44 (q, 1H, ArH), 11.22 (s, 1H, OH).

Compound 1d: Yield: 0.20 g (79 %); m.p.: 283-285°C, IR (KBr, cm^{-1}): 3465 (OH), 3150 (C=C-H), 3060 (CH), 3054 (C-H), 1730 (COOH), 1565 (C=C), 1434 (C=N), 1242 (C-O-C), 805, 765, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.74 (s, 3H, OCH_3), 7.21 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.60 (t, 1H, ArH), 7.85 (s, 1H, ArH), 11.10 (s, 1H, OH).

3-Amino-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2a-d): 2-Substituted imidazo[1,2-*a*]-pyridine-8-carboxylic acid (1.0 mmol) in acetic acid (25 mL) and sodium nitrite (0.10 g, 1.5 mmol) was added at room temperature under stirring for 5 h. The residue solid was treated with water, filtered and crystallized from acetonitrile nitroso compound. In order to reduce nitroso group, in HBr solution added tin (2 eq) in small fractions and stirred for 10 min. Then added product nitroso (1 equiv.) in small fractions and the reaction was stirred for 2 h at -10 ºC. The resulting solution was filtered and treated with $NaHCO_3$ at pH 7. The extraction was done with ethyl acetate and crystallized from acetonitrile to give compound 3-amino-2-substituted imidazo[1,2-*a*]-pyridine-8-carboxylic acid.

Compound 2a: Yield: 0.17 g (68 %), m.p.: 220-224 ºC, IR (KBr, cm^{-1}): 3500 (OH), 3400 (NH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, CH_3), 3.44 (s, 2H, NH_2), 7.21 (t, 1H, ArH), 7.25 (q, 1H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.20-7.60 (3H, complex ArH), 11.10 (s, 1H, OH).

Compound 2b: Yield: 0.17 g (65 %); m.p.: 228-230 ºC; IR (KBr, cm^{-1}): 1730 (COOH), 3500 (OH), 3420 (NH), 3152 (C=C-H), 3050 (C-H), 1563 (C=C), 1432 (C=N), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.91 (t, 3H, CH_3), 2.13 (q, 2H, CH_2), 3.46 (s, 2H, NH_2), 7.22 (d, 2H, ArH), 7.20 (t, 1H,

ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 11.10 (s, 1H, OH)

Compound 2c: Yield: 0.17 g (65 %); m.p.: 230-232 ºC; IR (KBr, cm^{-1}): 3505 (OH), 3422 (NH), 3150 (C=C-H), 3055 (C-H), 1732 (COOH), 1564 (C=C), 1430 (C=N), 762, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.92 (s, 6H, 2 × CH_3), 3.76 (s, 2H, NH_2), 7.22 (t, 1H, ArH), 7.23 (d, 1H, ArH), 7.26 (d, 1H, ArH), 7.30 (t, 1H, ArH), 7.38 (q, 1H, ArH), 11.26 (s, 1H, OH).

Compound 2d: Yield: 0.29 g (65 %); m.p.: 238-240 ºC; IR (KBr, cm^{-1}): 3460 (OH), 3455 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 810, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.72 (s, 1H, NH_2), 3.74 (s, 3H, OCH_3), 7.21 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.60 (t, 1H, ArH), 11.10 (s, 1H, OH).

3-((2-Methoxy-2-oxoethyl)amino)-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (3a-d): To a solution of compound 3-amino-2-substituted imidazo[1,2-*a*]-pyridine-8-carboxylic acid (1.0 mmol) in THF (25 mL) and K_2CO_3 (0.41 g, 3.0 mmol) was added dropwise followed by the addition of methyl 2-bromoacetate (0.152 mL, 1.0 mmol) at room temperature under constant stirring. The reaction mixture was heated at 90 ºC for 4 h and then evaporated under reduced pressure. The residue was treated with aq. HCl and recrystallized from ethanol to obtained 3-((2-methoxy-2-oxoethyl)-amino)-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid.

Compound 3a: Yield: 0.26 g (82 %); m.p.: 220-222 ºC; IR (KBr, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1740 (C-O-R), 1730 (COOH), 1563 (C=C), 1432 (C=N), 1230 (C-O-C), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, CH_3), 2.44 (s, 2H, CH_2), 3.54 (s, 1H, NH), 3.77 (s, 3H, OCH_3), 7.21 (t, 1H, ArH), 7.25 (q, 1H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.20-7.60 (3H, complex ArH), 11.10 (s, 1H, OH).

Compound 3b: Yield: 0.24 g (86 %); m.p.: 234-236 ºC; IR (KBr, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1735 (COOCH3), 1730 (COOH), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.91 (t, 3H, CH_3), 2.13 (q, 2H, CH_2), 2.48 (s, 2H, CH_2), 3.39 (s, 3H, OCH_3), 3.46 (s, 2H, NH), 7.20 (t, 1H, ArH), 7.22 (2H, d, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 11.10 (s,1H,OH).

Compound 3c: Yield: 0.30 g (88 %); m.p.: 243-245 ºC; IR (KBr, cm^{-1}): 3154 (C=C-H), 3050 (C-H), 3500 (OH), 1738 (COOCH3), 1734 (COOH), 1566 (C=C), 1434 (C=N), 1244 (C-O-C), 764; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.90 (s, 6H, 2 × CH_3), 2.48 (s, 2H, CH_2), 3.39 (s, 3H, OCH_3), 4.05 (s, 2H, NH), 7.20 (t, 1H, ArH), 7.22 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 11.24 (s, 1H, OH).

Compound 3d: Yield: 0.27 g (78 %); m.p.: 218-220 ºC; IR (KBr, cm^{-1}): 3460 (OH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1730 (COOH), 1685 ($COOCH_3$), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 810, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.45 (s, 2H, CH_2), 3.72 (s, 1H, NH), 3.74 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 7.21 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 1H, ArH), 7.40 (q, 1H, ArH), 7.60 (t, 1H, ArH), 11.10 (s, 1H, OH).

Methyl 2-((8-((3-(aminomethyl)benzyl)carbamoyl)-2-(sustituted)imidazo[1,2-*a*]pyridine-3-yl)amino)acetate (4a-d): To a solution of 3-((2-methoxy-2-oxoethyl)amino-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid (1.0 mmol) in DCM (30 mL) and *tert*-butyl-3-(aminophenyl)carbamate (0.20 g, 1.0 mmol), triethylamine (TEA) (0.21 mL, 2.1 mmol), 4-dimethylaminopyridine (DMAP) (0.01 g, 0.11 mmol) and (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.21 g, 1.1 mmol) were added. The reaction mixture was stirred at room temperature for 5-10 h after analyzing TLC. Trifluroacetic acid (TFA) (0.22 mL, 2 mmol) was added to the reaction mixture dropwise and stirred at room temperature for again 5 h. The mixture was neutralized by $NaHCO_3$ extracted with ethyl acetate. The crude product was purified by column chromatography to obtained pure methyl-2-((8-((4-aminophenyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)-acetate.

Compound 1: Yield: 0.25 g (68 %); m.p.: 218-220 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3150 (C=C-H), 1680 (CONH), 1660 (Ar-CONH), 1560 (C=C), 1430 (C=N), 1230 (C-O-C), 840, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.12 (s, 3H, CH_3), 2.42 (s, 2H, CH_2), 3.43 (s, 2H, NH_2), 3.72 (s, 3H, OCH_3), 4.44 (s, 1H, NH), 4.48 (s, 1H, NH), 7.10-8.00 (complex ArH). MS: *m/z* 398 $[M+H]^+$. Elemental analysis % calcd. (found): C, 67.12 (67.16); H, 5.40 (5.46); N, 16.31 (16.38); O, 11.18 (11.24).

Compound 2: Yield: 0.29 g (65 %); m.p.: 228-230 ºC; IR (KBr, cm^{-1}): 3412 (NH), 3150 (C=C-H), 3056 (C-H), 1710 ($COOCH_3$), 1684 (C-O-NH), 1564 (C=C), 1434 (C=N), 1242 (C-O-C), 844, 762, 715; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (t, 3H, CH_3), 2.14 (q, 2H, CH_2), 2.46 (s, 2H, CH_2), 3.40 (s, 3H, OCH_3), 3.76 (s, 2H, NH_2), 4.16 (s, 2H, NH), 4.11 (s, 1H, NH), 7.44 (q, 1H, ArH), 7.34 (t, 1H, ArH), 7.24 (t, 1H, ArH), 7.20 (d, 2H, ArH), 7.18 (d, 2H, ArH). MS: *m/z* 414 $[M+H]^+$. Elemental analysis % calcd. (found): C, 67.70 (67.77); H, 5.68 (5.70); N, 15.79 (15.85); O, 10.82 (10.84).

Compound 3: Yield: 0.25 g (75 %); m.p.: 214-216 ºC; IR (KBr, cm^{-1}): 3152 (C=C-H), 3040 (C-H), 3418 (NH), 1708 (COOCH3), 1688 (CONH), 1562 (C=C), 1434 (C=N), 1248 (C-O-C), 760, 712, 846; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 6H, 2×CH_3), 2.50 (s, 2H, CH_2), 3.40 (s, 3H, OCH_3), 3.74 (s, 2H, NH_2), 4.10 (s, 2H, NH), 4.14 (s, 1H, NH), 7.22 (t, 1H, ArH), 7.24 (d, 2H, ArH), 7.30 (d, 2H, ArH), 7.32 (t, 1H, ArH), 7.42 (q, 1H, ArH). MS: *m/z* 412 $[M+H]^+$. Elemental analysis % calcd. (found): C, 67.70 (67.76); H, 5.68 (5.75); N, 15.79 (15.86); O, 10.82 (10.86).

Compound 4: Yield: 0.30 g (76 %); m.p.: 210-212 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3154 (C=C-H), 3062 (CH), 3052 (C-H), 1692 (CONH), 1686 ($COOCH_3$), 1564 (C=C), 1434 (C=N), 1240 (C-O-C), 810, 760, 710. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.37 (s, 2H, CH_2), 3.40 (s, 1H, NH), 3.68 (s, 2H, NH_2), 3.72 (s, 1H, NH), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 7.22 (d, 2H, ArH), 7.26 (d, 2H, ArH), 7.32-7.70 (7H, complex ArH). MS: *m/z* 414 $[M+H]^+$. Elemental analysis % calcd. (found): C, 64.71 (64.76); H, 5.20 (5.25); N, 15.72 (15.76); O, 14.37 (14.42).

Methyl 2-((8-((4-aminomethyl)benzyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)acetate (5-9): To a solution of 3-((2-methoxy-2-oxoethyl)amino-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid (1.0 mmol) in

DCM (30 mL) and *tert*-butyl-4-(aminomethyl)benzylcarbamate (0.236 g, 1.0 mmol), triethylamine (0.21 mL, 2.1 mmol), 4-dimethylaminopyridine (0.01 g, 0.11 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.21 g, 1.1 mmol) were added. The reaction mixture was stirred at room temperature for 5-10 h after analyzing TLC. Trifluroacetic acid (0.22 mL, 2 mmol) was added to the reaction mixture dropwise and stirred at room temperature again for 5 h. The mixture was neutralized by $NaHCO_3$ extracted with ethyl acetate. The crude was purified by column chromatography to obtained pure methyl-2-((8-((4-aminomethyl)benzyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)acetate.

Compound 5: Yield: 0.25 g (68 %); m.p.: 18-220 ºC; IR (KBr, cm^{-1}): 3412 (NH_2), 3154 (C=C-H), 3056 (C-H), 1692 ($COOCH_3$), 1688 (CONH), 1564 (C=C), 1434 (C=N), 1244 (C-O-C), 762, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 3H, CH_3), 2.36 (s, 2H, CH_2), 2.54 (s, 2H, CH_2), 2.64 (s, 2H, CH_2), 3.48 (s, 3H, OCH_3), 3.66 (s, 1H, NH), 3.74 (s, 2H, NH_2), 4.72 (s, 1H, NH), 7.20 (t, 1H, ArH), 7.22 (d, 6H, ArH), 7.24 (d, 2H, ArH), 7.32 (t, 1H, ArH), 7.40 (q, 1H, ArH). MS: *m/z* 426 $[M+H]^+$. Elemental analysis % calcd. (found): C,68.25 (68.30); H, 5.95 (5.98); N, 15.31 (15.39); O, 10.49 (10.56).

Compound 6: Yield: 0.29 g (65 %), m.p.: 228-230 ºC; IR (KBr, cm^{-1}): 3410 (NH), 3152 (C=C-H), 3050 (C-H), 1690 ($COOCH_3$), 1685 (CONH), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.92 (t, 3H, CH_3), 1.26 (q, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.44 (s, 2H, CH_2), 2.46 (s, 2H, CH_2), 3.62 (s, 3H, OCH_3), 3.66 (s, 1H, NH), 3.70 (s, 1H, NH), 3.72 (s, 2H, NH_2), 7.20 (d, 2H, ArH), 7.28 (d, 2H, ArH), 7.30 (t, 4H, ArH), 7.42 (q, 2H, ArH). MS: *m/z* 442 $[M+H]^+$. Elemental analysis % calcd. (found): C, 68.77 (68.82); H, 6.20 (6.26); N, 14.85 (14.90); O, 10.18 (10.26).

Compound 7: Yield: 0.25 g (75 %); m.p.: 214-216 ºC; IR (KBr, cm^{-1}): 3410 (NH), 3152 (C=C-H), 3055 (C-H), 1694 ($COOCH_3$), 1686 (CONH), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 760,710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.92 (t, 6H, 2 × CH_3), 2.38 (s, 2H, CH_2), 2.42 (s, 2H, CH_2), 2.44 (s, 2H, CH_2), 3.62 (s, 1H, NH), 3.65 (s, 1H, NH), 3.70 (s, 2H, NH_2), 3.72 (s, 3H, OCH_3), 7.22 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31 (t, 4H, ArH), 7.40 (q, 2H, ArH). MS: *m/z* 440 $[M+H]^+$. Elemental analysis % calcd. (found): C, 68.77 (68.82); H, 6.20 (6.28); N,14.85 (14.94); O, 10.18 (10.26).

Compound 8: Yield: 0.30 g (76 %); m.p.: 210-212 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H),1690 (CONH), 1685 ($COOCH_3$), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 810, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.35 (s, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.42 (s, 1H, NH), 3.70 (s, 2H, NH_2), 3.72 (s, 1H, NH), 3.74 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 7.21 (d, 2H, ArH), 7.25 (d, 2H, ArH), 7.31-7.70 (7H, complex ArH). MS: *m/z* 443 $[M+H]^+$. Elemental analysis % calcd. (found): C, 65.95 (65.98); H, 5.75 (5.83); N, 14.79 (14.86); O, 13.52 (13.58).

Compound 9: Yield: 0.24 g (66 %); m.p.: 238-240 ºC; IR (KBr, cm^{-1}): 3450 (NH_2), 3150 (C=C-H), 1684 (CONH), 1660 (ArCONH), 1566 (C=C), 1434 (C=N), 1232 (C-O-C), 764; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.10 (s, 3H, CH_3), 2.10 (s, 2H, CH_2-NH_2), 2.32 (s, 2H, CH_2-NH), 2.42 (s, 2H, CH_2), 3.44 (s, 2H, NH_2), 3.72 (s, 3H, OCH_3), 4.20 (s, 1H, NH), 4.40 (s, 1H, NH), 7.10-8.00 (complex ArH). MS: *m/z* 429 $[M+H]^+$. Elemental analysis % calcd. (found): C, 68.25 (68.32); H, 5.95 (5.98); N, 15.31 (15.37); O, 10.49 (10.58).

Ethyl-2-((8-((3-(aminomethyl)benzyl)carbamoyl-2-substituted imidazo[1,2-*a*]pyridine-3-yl)amino)acetate (10-13): To a solution of 3-((2-ethoxy-2-oxoethyl)amino-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid (1.0 mmol) in DCM (30 mL), *tert*-butyl-4-(aminomethyl)benzylcarbamate (0.236 g, 1.0 mmol), triethylamine (TEA) (0.21 mL, 2.1 mmol), 4-dimethylaminopyridine (0.01 g, 0.11 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.21 g, 1.1 mmol) were added. The reaction mixture was stirred at room temperature for 5-10 h after analyzing TLC. Trifluroacetic acid (0.22 mL, 2 mmol) was added to the reaction mixture dropwise and stirred at room temperature for again 5 h. The mixture was neutralized by $NaHCO_3$ extracted with ethyl acetate. The crude was purified by column chromatography to obtained pure ethyl-2-((8-((4-(aminomethyl)benzyl)carbamoyl)-2-substituted imidazo[1,2-*a*]pyridin-3-yl)amino)acetate.

Compound 10: Yield: 0.27 g (62 %); m.p.: 243-245 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3150 (C=C-H), 3062 (CH), 3052 (C-H), 1690 (CONH), 1686 ($COOCH_3$), 1563 (C=C), 1430 (C=N), 1242 (C-O-C), 815, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.35 (s, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.42 (s, 1H, NH), 3.70 (s, 2H, NH_2), 3.72 (s, 1H, NH), 3.74 (s, 3H, OCH_3), 7.21 (t, 3H, ArH), 7.25 (d, 2H, ArH), 7.31-7.70 (7H, complex ArH), 7.20 (t, 1H, ArH), 7.22 (d, 6H, ArH),7.24 (d, 2H, ArH), 7.32 (t, 1H, ArH), 7.40 (q, 1H, ArH). MS: *m/z* 412 $[M+H]^+$. Elemental analysis % calcd. (found) C, 67.70 (67.78); H, 5.68 (5.75); N, 15.79 (15.88); O, 10.82 (10.90).

Compound 11: Yield: 0.28 g (66 %); m.p.: 240-242 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COOCH_3$), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 810, 760,710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.93 (t, 3H, CH_3), 2.35 (s, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.43 (q, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.42 (s, 1H, NH), 3.70 (s, 2H, NH_2), 3.72 (s, 1H, NH), 3.74 (s, 3H, OCH_3), 7.21 (d, 2H, ArH), 7.25 (t, 1H, ArH), 7.31-7.70 (7H, complex ArH). MS: *m/z* 442 $[M+H]^+$. Elemental analysis % calcd. (found): C, 68.77 (68.85); H, 6.20 (6.26); N, 14.85 (14.90); O, 10.18 (10.24).

Compound 12: Yield: 0.31 g (68 %); m.p.: 233-235 ºC; IR (KBr, cm^{-1}): 3450 (NH), 3152(C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COOCH_3$), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 810, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 6H, 2 × CH_3), 2.38 (s, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.46 (s, 2H, CH_2), 3.44 (s, 1H, NH), 3.70 (s, 1H, NH), 3.72 (s, 2H, NH_2), 3.75 (s, 3H, OCH_3), 7.22 (d, 2H, ArH), 7.26 (t, 1H, ArH), 7.32-7.70 (7H, complex ArH). MS: *m/z* 440 $[M+H]^+$. Elemental analysis % calcd. (found): C, 68.77 (68.86); H, 6.20 (6.28); N, 14.85 (14.92); O, 10.18 (10.25).

Compound 13: Yield: 0.31 g (66 %); m.p.: 228-230 ºC; IR (KBr, cm^{-1}): 3452 (NH), 3150 (C=C-H), 3062 (CH), 3050 (C-H), 1685 (CONH), 1680 ($COOCH_3$), 1563 (C=C), 1430 (C=N), 1240 (C-O-C), 810, 760, 710; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.35 (s, 2H, CH_2), 2.40 (s, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.42 (s, 1H, NH), 3.70 (s, 2H, NH_2), 3.74 (s, 3H, OCH_3), 3.72 (s, 1H, NH), 3.79 (s, 3H, OCH_3), 7.21 (d, 2H, ArH),

7.25 (t, 1H, ArH), 7.31-7.70 (7H, complex ArH). MS: *m/z* 443 $[M+H]^+$. Elemental analysis % calcd. (found) C, 65.95 (66.08); H, 5.75 (5.83); N, 14.79 (14.86); O, 13.52 (13.58).

Biological assays

Preparation of parietal cells: Parietal cell suspension was prepared from mucosal scrapings of goat stomach obtained from slaughter house and then homogenized in 200 mM tris-HCl buffer, pH 7.4, centrifuged for 10 min at 5000 × g. The resulting supernatant was subsequently centrifuged at 5000 × g for 20 min. The protein concentration in the supernatant was determined with bovine serum albumin as standard. The parietal cell extract was then employed to determine H^+/K^+-ATPase activity.

Determination of H+K⁺-ATPase: The H^+/K^+-ATPase activity in the presence of different concentrations of test compounds (10-60 µg/mL) and AZD0865 (Linaprazan) (10-60 µg/mL) was assayed by the method of Reyes-Chilpa *et al*. [23]. The enzyme source was pre-incubated with different concentration of the test compound for 30 min, to this 2 mM $MgCl_2$ and 2 mM KCl was added. Then reaction was started with the addition of 2 mM adenosine-5′-triphosphate (ATP) and incubated for 30 min at 30 ºC and terminated by the addition of 10 % trichloroacetic acid followed by centrifugation at 2000 × g. The amount of inorganic phosphorous released from adenosine-5′-triphosphate (ATP) was determined spectrophotometrically at 640 nm. The enzyme source was also treated similarly with the standard drug AZD0865 (Linaprazan) and the enzyme activity was measured.

Assay of H^+/K^+-ATPase activity: The inhibitory activity was concentration dependent and the results were comparable to standard drug AZD0865 (Linaprazan). The test compound (**1-13**) potently reduced the hydrolysis of ATP by the goat gastric ATPase with IC_{50} = 4-10.2. Standard compound used as positive control reduced H^+/K^+-ATPase activity with an IC_{50} = 2. The H^+/K^+-ATPase activity was measured with 10-60 µg/mL of the test and standard compound. Experiments were performed in triplicates.

Molecular docking studies: *in silico* Study was done by GLIDE into the H^+/K^+-ATPase pouch with the crystal of protein PDB ID: 4ux2. The protein structure was prepared by using preparation wizard' in Maestro 8.0 software in two preparation and refinement steps. Preparations of Grids were produced by centering onco-crystalized ligand. The molecules were generated using maestro build panel and built by Ligprep 2.2 module that produces the low energy structure of ligands using OPLS 2005 force field. The low energy structures of the compounds were chosen and docked into the grid generated from protein structures by with standard precision docking mode.

RESULTS AND DISCUSSION

The design for the series of compounds to be considered for study was performed using virtual screening protocol [15-17]. Considering the pharmacophoric requirements and the standard compound AZD0865, the CID database was explored. The *in silico* checked compounds were then observed for Lipinsky rule of five to evaluate different parameters. The novelty of compounds in terms of H^+/K^+-ATPase inhibitory activity was checked over SciFinder. These virtually screened hits were synthesized along with its derivatives and evaluated for their inhibitory potential. It was found that imidazopyridine have very good inhibitory potential and must be explored for H^+/K^+-ATPase inhibitory activity. Hence, we studied imidazopyridine motif for H^+/K^+-ATPase inhibition. A series of compound were synthesized as imidazopyridine analogues.

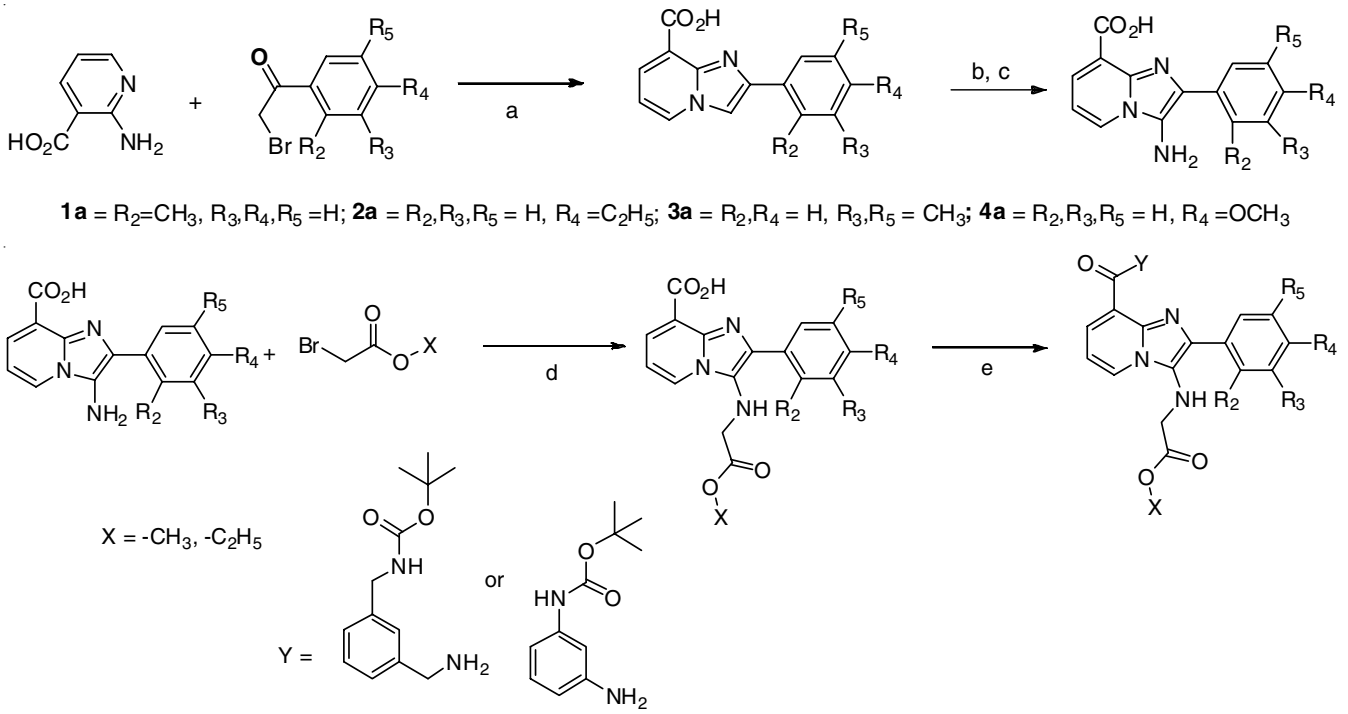
The target diketoquinolines were prepared from the commercially procured intermediates such as 2-aminonicotinic acid, 2-bromo-1-substituted ethanone, substituted 2-bromoacetate, *tert*-butyl-3-(aminophenyl)carbamate, *tert*-butyl-4-(aminomethyl)benzylcarbamate, bromomethyl (substituted benzene) [18-21]. In the first step, 2-aminonicotinic acid and 2-bromo-1-substituted ethanone to form 2-substituted imidazo[1,2-*a*]-pyridine-8-carboxylic acid in presence of $NaHCO_3$ takes place. In condensation reaction, cyclization of amino group leads to form new ring and confirmed by PMR and IR spectral analysis. In second step, introduction of amino group by replacing the acidic proton of five member imidazole ring took in presence of acetic acid, sodium nitrite, HBr and addition of tin in fraction. In third step, ester linkage at nitrogen atom in alkaline medium takes place in presence of substituted bromoacetate with K_2CO_3 and THF. In fourth step, introduced new aromatic ring by cyclization of COOH and NH_2 in presence of DCM, triethylamine and substituted carbamates. In this NH_2 of *m*-nitro aniline was protected and removed after reaction completion. All the steps were confirmed by spectral analysis to yield the target compounds. The synthetic route is outlined in **Scheme-I**.

Biological activity: Imidazopyridine analogues **1-13** were experienced *in vitro* for their proton pump inhibitory activity using a H^+/K^+-ATPase inhibition assay [8,22-24]. The IC_{50} values were produced from triplicate experiments in Table-1. It was observed for imidazopyridine compounds that acetate and carboxamide series derivatives were more potent with a high selectivity against H^+/K^+-ATPase. The replacement of methyl group from C_2 and C_3 of AZD0865 (Linaprazan) by substituted phenyl group and further alkyl phenyl group did not increased activity. Substitution at imidazopyridine C_2 by dimethyl phenyl, ethyl phenyl and C_3 by acetate (**11**, **12**) showed IC_{50} of 4.3 µg against H^+/K^+-ATPase enzyme. This suggests that substitution of imidazopyridine at C2 and C3 by alkyl and amino acetate moiety affect the ability of the inhibitors to bind with H+/K+-ATPase enzyme. Other substitution does not make any significant interaction with H^+/K^+-ATPase enzyme.

TABLE-1
INHIBITION OF H^+/K^+-ATPase BY COMPOUNDS **1-13**

Comp. No.	-log IC_{50}	Comp. No.	-log IC_{50}
1	5.2	**8**	4.9
2	6.5	**9**	7.0
3	6.0	**10**	4.6
4	5.8	**11**	4.3
5	6.3	**12**	4.3
6	5.5	**13**	5.9
7	5.1	AZD0865	2.0

Molecular docking: The binding mechanisms of synthesized compounds were investigated using molecular docking studies. The docked effect for the designed and synthesized



1a = R_2=CH_3, R_3,R_4,R_5 =H; **2a** = R_2,R_3,R_5 = H, R_4 =C_2H_5; **3a** = R_2,R_4 = H, R_3,R_5 = CH_3; **4a** = R_2,R_3,R_5 = H, R_4 =OCH_3

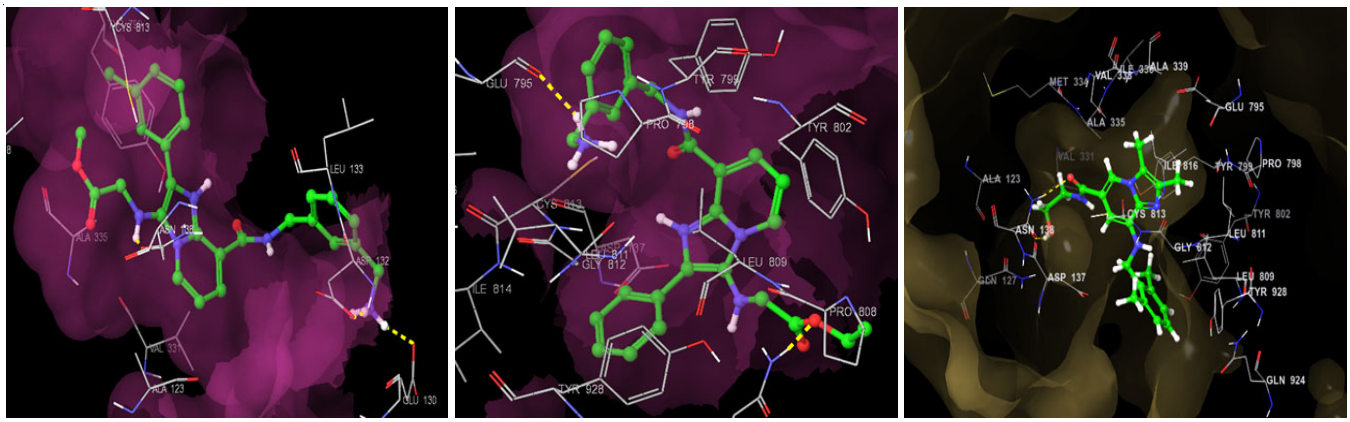
Scheme-I: Reagents and conditions: (a) refluxed 30 min, $NaHCO_3$, ethyl acetate; (b) acetic acid, sodium nitrite, stirred 5 h. (c) HBr, tin, stirred 10 min, 2 h at -10 °C, $NaHCO_3$, ethylacetate (d) K_2CO_3, THF, RT stirred, ref. 90 °C for 4 h (e) DCM, TEA, DMAP, EDC. HCl, stirred RT 5-10 h

molecules were performed using Schrodinger Suite. For docking studies, GLIDE was used into the H^+/K^+-ATPase pouch [25-27]. The crystal moiety of H^+/K^+-ATPase was downloaded from the protein data bank, PDB ID: 4ux2. The protein structure was prepared by wizard method in Maestro 8.0. Grids were produced by aiming on co-crystallized ligand. The structures were developed using maestro build panel and prepared by Ligprep 2.2 module that produces the low energy conformer of ligands using OPLS 2005 force field. The low energy structures of the ligands was identified and docked into the grid.

The docking poses revealed the interaction of few ligands with desired amino acids. The standard drug AZD0865 had docking score of -7.112302 and displayed interactions with Asn138 and Asp137. When AZD0865 were docked in the same active site, they displayed comparable docking scores and interaction patterns. Compounds **11** and **12** had maximum potency with IC_{50} 4.3 µg in enzyme inhibition assay. Fig. 2 represents the docked view.

Conclusion

Many derivatives of imidazopyridine series of compounds were synthesized with different substitution on the 2, 3 and 6-position by hydrophobic benzyl moiety, acetate and amino phenyl group, instead of alkyl and toluene, which were present on standard compound AZD 0865. All compounds were observed for their enzymatic inhibitory activity. The replacement of small alkyl group from C_2 and C_3 of AZD 0865 does not lead to significant improvement in proton pump ATPase inhibition. Substitution at C_6 by amino phenyl and amino alkyl phenyl instead of methyl toluene showed moderate activity. Compounds **11** and **12** showed IC_{50} value 4.3 µg against proton pump ATPase. This result suggests that substitution on AZD 0865 by phenyl



CID_23880300 (Compound **1**) CID_23763056 (Compound **10**) AZD0865

Fig. 2. Docked view for imidazopyridine compounds

alkyl and acetate moiety at C_2 and C_3 affect on the ability of inhibitors to bind with ATPase enzyme. Other substitution does not make any significant interaction with ATPase enzyme.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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