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Design and Synthesis of Novel Imidazopyridine Analogues and Evaluation as H^+/K^+-ATPase Antagonist

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CID data base were explored considering AZD0865 as standard and docked in proton pump ATPase pocket (PDB ID: 4ux2) to find out novel imidazopyridine derivatives as proton pump inhibitors. A number of compounds showed good proton pump ATPase inhibitory activity as per the molecular docking study as compared to standard compound AZD0865. The compound AZD0865showed a docking score of -7.11 and revealed the interactions with amino acids Asn 138 and Asp 137. 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 the intermediate substituted 2-aminonicotinic acid and 2-bromo-1-substituted ethanone. *in vitro* pharmacological studies explained that some compounds exhibited moderate to good proton pump ATPase inhibitory activity in comparison with the reference drugs *i.e.* AZD0865. Compound *N*-(3-(aminomethyl)benzyl)-3-(benzylamino)-2-(*o*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxamide and *N*-(3-(aminomethyl)benzyl)-3-(benzylamino)-2-(4-ethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxamide showed higher activities with the IC_{50} 6.2 and 6.0 µg. Many compounds showed IC_{50} as weak antiulcer activity as compared to positive control AZD0865.

Keywords: Acid Pump Antagonist, Imidazopyridines, Antiulcer activity, Docking study.

INTRODUCTION

The common traits of ulcer is mild to moderate-severe pain just below the breastbone may last for once or a few times daily typically after eating. Etiology of gastric ulcer and gastroesophagal reflux disease reveals with the erosion of the inner lining of the stomach due to acidic food, stress and infection by bacteria *Helicobacter pylori*. There are some contributory factors for ulcer diseases includes cigarette smoking, chronic consumption of ulcerogenic drugs like non-steroidal anti-inflammatory drugs, consumption of alcohol for prolonged periods, age, emotional stress and hereditory [1-4].

Large number of benzimidazole sulfoxide pyridine classes as proton pump inhibitors (PPIs), significantly progressed in this field with the interruption of H^+/K^+-ATPase [5-7]. Extreme acid suppression also leads to achlorohydria and leads to enteric infections like typhoid, cholera and dysentery. Some time drug interplay leads to reduced absorption of some drugs like griseofulvin, ketoconazole, vitamin B_{12}, iron salts, *etc*. Unpredictable action shows hypergastrinemia, gastricpolyps and carcinoma [8-10]. The main drawback of recent available proton pump inhibitors (PPIs) requires a long time to achieve almost acid inhibition at therapeutic doses. Primarily may be a chemical structural modification and irreversible inhibition of H^+/K^+-ATPase. Therapy is not reliable to control sustained acid inhibition throughout the twenty-four hours even dosing the drug in twice daily. Therefore, many novel strategies are used to solve the unmet needs of PPI therapy.

Acid pump antagonists (APAs) could play a significant role, due to their faster onset and longer duration of action than irreversible PPIs by their ability to reversibly bind to the proton pump. Many researchers worked to find out novel APAs but currently none is marketed. The imidazopyridine based compounds SCH28080 and AZD0865 (Fig. 1) are the prototype of this class. In comparison to omeprazole, SCH 28080 is a competitive inhibitor of the high affinity luminal K^+ site of the gastric proton pump. In contrast to Na^+/K^+-ATPase, it is highly selective to Na^+/K^+-ATPase activity. Compound SCH 28080 is a proton-

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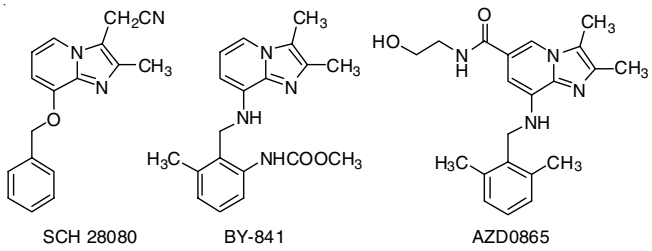


Fig. 1. Symbolic H^+/K^+-ATPase antagonist

able 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 proton pump inhibitors (PPIs) [11-13].

Thus, there is a need to develop another class of compounds targeting the H^+/K^+-ATPase, which may combine the advantages of both PPIs and H2RAs and act actively in the absence of acid secretion. Designing and synthesizing such H^+/K^+-ATPase antagonist may give rise to the newer ideal drug to treat ulcer and may surpress the side effects of the reported compounds [12-14].

CID database of imidazopyridines were selected and few active compounds with their derivatives were synthesized 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-((2-methoxy-2-oxoethyl)amino)-2-(substituted phenyl)-imidazo[1,2-*a*]pyridine-8-carboxylic acid and later to give *N*-(3-(aminomethyl)benzyl)-3-(benzylamino)-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxamide. In this work, the structures were docked in H^+/K^+-ATPase pocket and synthesized new imidazopyridine analogues **1-24** by the replacement of various substituent′s present on SCH 28080 and AZD0865 to get potent biological activity.

EXPERIMENTAL

The laboratory grade reagents and solvents were procured from the various sources. The completion of the reactions were observed by TLC using silica gel plates. Silica gel (100-200 mesh) as a fixed phase was used for column chromatography. The melting points were determined in the open capillaries and not corrected. The 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 solvents. The infrared (IR) 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).

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 $NaHCO_3$ up to pH 7 and finally 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 **(1a-d)**.

2-(*o*-Tolyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (1a): Yield: 0.20 g: m.p.: 275-280 ºC. IR (KBr, ν_{max}, cm^{-1}): 3505 (OH), 3150 (C=C-H), 3055 (C-H), 1734 (COOH), 1565 (C=C), 1436 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, C<u>H</u>$_3$), 7.22 (t, 1H, Ar<u>H</u>), 7.26 (q, 1H, Ar<u>H</u>), 7.32 (t, 1H, Ar<u>H</u>), 7.42 (q, 1H, Ar<u>H</u>), 7.20-7.60 (3H, complex, Ar<u>H</u>), 7.88 (s, 1H, Ar<u>H</u>), 11.14 (s, 1H, O<u>H</u>).

2-(4-Ethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (1b): Yield: 0.19 g, m.p.: 278-282 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.91 (t, 3H, C<u>H</u>$_3$), 2.13 (q, 2H, C<u>H</u>$_2$), 7.20 (t, 1H, Ar<u>H</u>), 7.22 (d, 2H, Ar<u>H</u>), 7.25 (d, 2H, Ar<u>H</u>), 7.31 (t, 1H, Ar<u>H</u>), 7.40 (q, 1H, Ar<u>H</u>), 7.85 (s, 1H, Ar<u>H</u>), 11.10 (s, 1H, O<u>H</u>).

2-(3,5-Dimethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (1c): Yield: 0.20 g, m.p.: 270-272 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 6H, 2×C<u>H</u>$_3$), 7.22 (t, 1H, Ar<u>H</u>), 7.76 (s, 1H, Ar<u>H</u>), 7.26 (d, 2H, Ar<u>H</u>), 7.28 (d, 1H, Ar<u>H</u>), 7.34 (t, 1H, Ar<u>H</u>), 7.44 (q, 1H, Ar<u>H</u>), 11.22 (s, 1H, O<u>H</u>).

2-(4-Methoxyphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (1d): Yield: 0.20 g, m.p.: 283-285 ºC. IR (KBr, ν_{max}, 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). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.74 (s, 3H, OC<u>H</u>$_3$), 7.21 (d, 2H, Ar<u>H</u>), 7.25 (d, 2H, Ar<u>H</u>), 7.31 (t, 1H, Ar<u>H</u>), 7.40 (q, 1H, Ar<u>H</u>), 7.60 (t, 1H, Ar<u>H</u>), 7.85 (s, 1H, Ar<u>H</u>), 11.10 (s, 1H, O<u>H</u>).

3-Amino-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2a-d): To a solution of 2-substituted imidazo-[1,2-*a*]pyridine-8-carboxylic acid (1 mmol) in acetic acid (25 mL), sodium nitrite (0.1 g, 1.5 mmol) was added at room temperature under stirring for 5 h. The residual solid was treated with water, filtered and crystallized from acetonitrile nitroso compound.

A reduction of nitroso group done by following method: In a solution containing HBr at -10 ºC was added in small fractions of tin (2 equiv.) allowing stirred for 10 min. The product nitroso (1 equiv.) was added in small fraction and the reaction was stirred for 2 h at -10 ºC. After 24 h at room temperature, the resulting solution was filtered and treated with $NaHCO_3$ up to pH 7. The extraction was done with ethylacetate and crystallized from acetonitrile to give compound 3-amino-2-substituted imidazo[1,2-*a*]pyridine-8-carboxylic acid (**2a-d**).

3-Amino-2-(*o*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2a): Yield: 0.17 g, m.p.: 220-224 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3400 (NH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, C<u>H</u>$_3$), 3.44 (s, 2H, N<u>H</u>$_2$), 7.21 (t, 1H, Ar<u>H</u>), 7.25 (q, 1H, Ar<u>H</u>), 7.31 (t, 1H, Ar<u>H</u>), 7.40 (q, 1H, Ar<u>H</u>), 7.20-7.60 (3H, complex, Ar<u>H</u>),11.10 (s,1H,O<u>H</u>).

3-Amino-2-(4-ethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2b): Yield: 0.17 g, m.p.: 228-230 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3420 (NH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz,

DMSO-d_6) δ ppm: 0.91 (t, 3H, C$\underline{H}_3$), 2.13 (q, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 7.22 (d, 2H, Ar$\underline{H}$), 7.20 (t, 1H, Ar$\underline{H}$), 7.25 (d, 2H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 11.10 (s, 1H, O$\underline{H}$).

3-Amino-2-(3,5-dimethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2c): Yield: 0.17 g, m.p.: 230-232 ºC. IR (KBr, ν_{max}, cm^{-1}): 3505 (OH), 3422 (NH), 3150 (C=C-H), 3055 (C-H), 1732 (COOH), 1564 (C=C), 1430 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.92 (s, 6H, 2×C$\underline{H}_3$), 3.76 (s, 2H, N$\underline{H}_2$), 7.22 (t, 1H, Ar$\underline{H}$), 7.23 (d, 1H, Ar$\underline{H}$), 7.26 (d, 1H, Ar$\underline{H}$), 7.30 (t, 1H, Ar$\underline{H}$), 7.38 (q, 1H, Ar$\underline{H}$), 11.26 (s, 1H, O$\underline{H}$).

3-Amino-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (2d): Yield: 0.29 g, m.p.: 238-240 ºC. IR (KBr, ν_{max}, 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). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.72 (s, 1H, N$\underline{H}_2$), 3.74 (s, 3H, OC$\underline{H}_3$), 7.21 (d, 2H, Ar$\underline{H}$), 7.25 (d, 2H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 7.60 (t, 1H, Ar$\underline{H}$), 11.10 (s, 1H, O$\underline{H}$).

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 phenyl) imidazo-[1,2-*a*]pyridine-8-carboxylic acid (1 mmol) in THF (25 ml) and K_2CO_3 (0.41 g, 3 mmol), bromomethyl (benzene) (0.170 mL, 1 mmol) was added dropwise at room temperature under 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 obtain 3-(benzylamino)-2-(substitutedphenyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid **(3a-d)**.

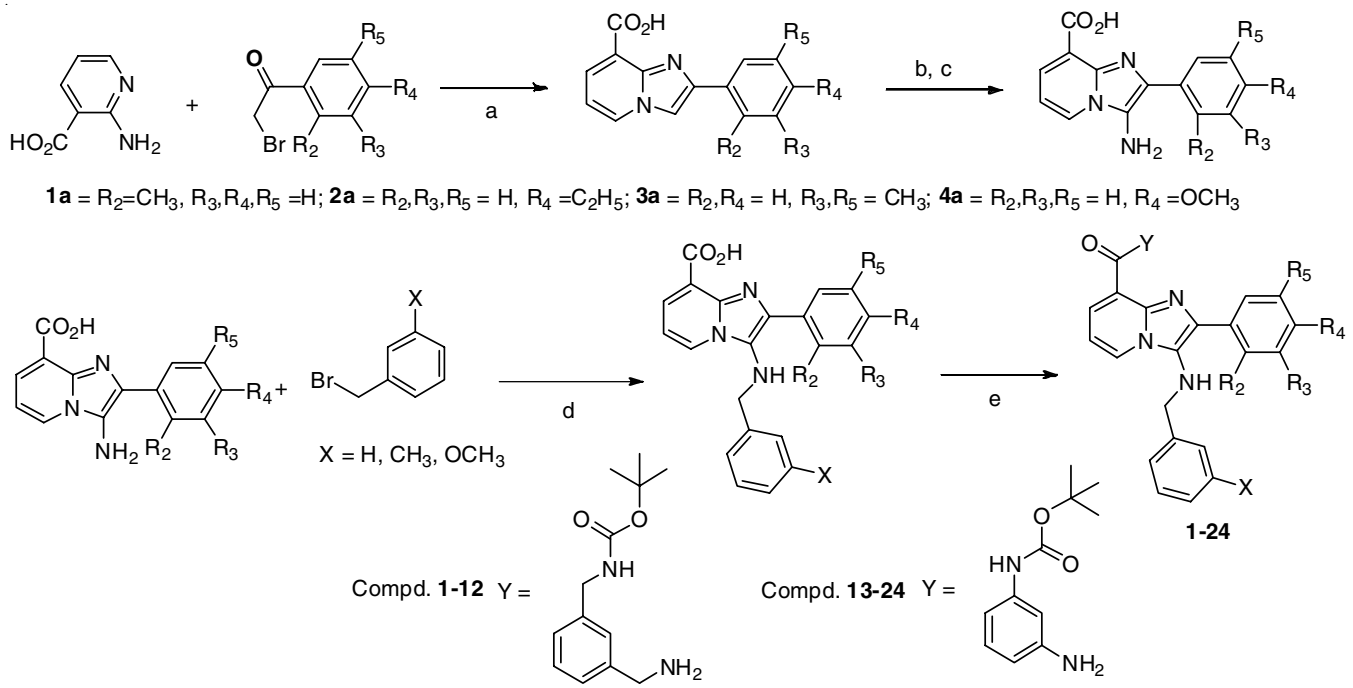
3-(Benzylamino)-2-(*o*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxylic acid (3a): Yield: 0.26 g, m.p.: 231-232 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.22 (s, 3H, C$\underline{H}_3$), 2.44 (s, 2H, CH_2), 3.54 (s, 1H, N$\underline{H}$), 7.21 (t, 1H, Ar$\underline{H}$), 7.25 (q, 1H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 7.20-7.60 (3H, complex, Ar$\underline{H}$), 11.12 (s, 1H, O$\underline{H}$).

3-(Benzylamino)-2-(4-ethylphenyl)imidazo[1,2-*a*]-pyridine-8-carboxylic acid (3b): Yield: 0.27 g, m.p.: 234-236 ºC. IR (KBr, ν_{max}, cm^{-1}): 3510 (OH), 3152 (C=C-H), 3050 (C-H), 1730 (COOH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.12 (t, 3H, C$\underline{H}_3$), 1.28 (q, 2H, CH_2), 2.44 (s, 2H, CH_2), 3.54 (s, 1H, N$\underline{H}$), 7.21 (t, 1H, Ar$\underline{H}$), 7.25 (q, 1H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 7.20-7.60 (3H, complex, Ar$\underline{H}$), 11.14 (s, 1H, O$\underline{H}$).

3-(Benzylamino)-2-(3,5-dimethylphenyl)imidazo[1,2-*a*]-pyridine-8-carboxylic acid (3c): Yield: 0.28 g, m.p.: 231-233 ºC. IR (KBr, ν_{max}, cm^{-1}): 3505 (OH), 3155 (C=C-H), 3050 (C-H), 1730 (COOH), 1566 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.14 (s, 6H, 2×C$\underline{H}_3$), 2.46 (s, 2H, CH_2), 3.56 (s, 1H, N$\underline{H}$), 7.21 (t, 1H, Ar$\underline{H}$), 7.25 (q, 1H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 7.20-7.60 (3H, complex, Ar$\underline{H}$), 11.14 (s, 1H, O$\underline{H}$).

3-(Benzylamino)-2-(4-methoxyphenyl)imidazo[1,2-*a*]-pyridine-8-carboxylic acid (3d): Yield: 0.27 g, m.p.: 244-246 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500 (OH), 3158 (C=C-H), 3050 (C-H), 1732 (COOH), 1564 (C=C), 1432 (C=N), 1250 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.82 (s, 3H,OC$\underline{H}_3$), 2.44 (s,2H,CH_2), 3.56 (s, 1H, N$\underline{H}$), 7.21 (t, 1H, Ar$\underline{H}$), 7.25 (q, 1H, Ar$\underline{H}$), 7.31 (t, 1H, Ar$\underline{H}$), 7.40 (q, 1H, Ar$\underline{H}$), 7.20-7.60 (3H, complex, Ar$\underline{H}$), 11.16 (s, 1H, O$\underline{H}$).

***N*-(3-(Aminomethyl)benzyl)-3-(benzylamino)-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxamide:**



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: Chemicals and state: (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

To a solution of 3-(benzylamino)-2-(substituted phenyl)-imidazo[1,2-*a*]pyridine-8-carboxylic acid (1 mmol) in DCM (30 mL), *tert*-butyl-4-(aminomethyl)benzyl carbamate (0.236 g, 1 mmol), triethylamine (0.21 mL, 2.1 mmol), 4-dimethyl amino-pyridine (0.01 g, 0.11 mmol) and EDC·HCl (0.21 g, 1.1 mmol) were added. The reaction mixture was stirred at room temperature for 5-10 h after analyzing TLC. Trifluoroacetic 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 $NaHCO_3$ and extracted with ethyl acetate. The crude was purified by column chromatography to obtain pure *N*-(4-(aminomethyl)benzyl)-3-(benzylamino)-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxamide (**Scheme-I**).

***tert*-Butyl-3-((3-(benzylamino)-2-(*o*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxamido)methyl)benzyl carbamate (1):** Yield: 0.28 g, m.p.: 251-253 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_3$: C, 75.76 (75.82); H, 6.15 (6.24); N, 14.73 (14.78); O, 3.36 (3.44). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH, CH_3), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, C<u>H</u>$_3$), 2.35 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.45 (s, 2H, C<u>H</u>$_2$), 3.42 (s, 1H, N<u>H</u>), 3.70 (s, 2H, N<u>H</u>$_2$), 3.72 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 475 *m/s* 484 (M^+) (49%), 369 (67%), 340 (52%).

***tert*-Butyl-3-((3-(benzylamino)-2-(4-ethylphenyl)-imidazo[1,2-*a*]pyridine-8-carboxamido)methyl)benzyl-carbamate (2):** Yield: 0.30 g, m.p.: 244-246 ºC. Elemental analysis calcd. (found) % for $C_{36}H_{39}N_5O_3$: C, 76.05 (76.12); H, 6.38 (6.44); N, 14.30 (14.38); O, 3.27 (3.36). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH, CH_3), 3050 (CH), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (t, 3H, C<u>H</u>$_3$), 1.22 (q, 2H, C<u>H</u>$_2$), 2.35 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.45 (s, 2H, C<u>H</u>$_2$), 3.42 (s, 1H, N<u>H</u>), 3.70 (s, 2H, N<u>H</u>$_2$), 3.72 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 489 *m/s* 460 (M^+) (49%), 428 (63%), 383 (55%), 354 (41%).

***tert*-Butyl-3-((3-(benzylamino)-2-(3,5-dimethylphenyl)-imidazo[1,2-*a*]pyridine-8-carboxamido) methyl)benzyl-carbamate (3):** Yield: 0.28 g, m.p.: 236-238 ºC. Elemental analysis calcd. (found) % C,76.05 (76.10); H, 6.38 (6.46); N, 14.30 (14.36); O, 3.27 (3.36). IR (KBr, ν_{max}, cm^{-1}): 3452 (NH), 3154 (C=C-H), 3060 (CH, CH_3), 3050 (C-H), 1690 (CONH), 1564 (C=C), 1434 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 6H, 2×C<u>H</u>$_3$), 2.36 (s, 2H, C<u>H</u>$_2$), 2.45 (s, 2H, C<u>H</u>$_2$), 2.48 (s, 2H, C<u>H</u>$_2$), 3.76 (s, 2H, N<u>H</u>$_2$), 3.70 (s, 1H, N<u>H</u>), 3.44 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 489 *m/s* 460 (M^+) (53%), 428 (65%), 383 (51%), 354 (41%).

tert-Butyl-3-((3-(benzylamino)-2-(4-methoxyphenyl)-imidazo[1,2-*a*]pyridine-8-carboxamido)methyl)benzyl-carbamate (4): Yield (0.31 g, m.p.: 228-230 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_4$: C, 73.30 (73.38); H, 5.95 (6.08); N, 14.25 (14.34); O, 6.51 (6.59). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH, CH_3), 3050 (CH), 1690 (CONH), 1563 (C=C), 1432 (C=N), 1245 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.38 (s, 2H, C<u>H</u>$_2$), 2.42 (s, 2H, C<u>H</u>$_2$), 2.46 (s, 2H, C<u>H</u>$_2$), 3.44 (s, 1H, N<u>H</u>), 3.72 (s, 2H, N<u>H</u>$_2$), 3.74 (s, 1H, N<u>H</u>), 3.86 (s, 3H, OC<u>H</u>$_3$), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 491 m/s 460 (M^+) (52%), 430 (61%), 385 (58%), 356 (45%).

***N*-(3-(Aminomethyl)benzyl)-3-((3-methylbenzyl)amino-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxamide (5):** Yield (0.32 g, m.p.: 220-222 ºC. Elemental analysis calcd. (found) % for $C_{36}H_{39}N_5O_3$: C, 76.05 (76.12); H, 6.38 (6.44); N, 14.30 (14.35); O, 3.27 (3.35). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3154 (C=C-H), 3066 (CH-CH_3), 3050 (C-H), 1690 (CONH), 1565 (C=C), 1430 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, C<u>H</u>$_3$), 0.98 (s, 3H, C<u>H</u>$_3$), 2.35 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.45 (s, 2H, C<u>H</u>$_2$), 3.46 (s, 1H, N<u>H</u>), 3.70 (s, 2H, N<u>H</u>$_2$), 3.72 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 489 *m/s* 460 (M^+) (58%), 428 (60%), 383 (56%), 354 (43%).

***tert*-Butyl-3-((2-(4-ethylphenyl)-3-((3-methylbenzene)-amino)imidazo[1,2-*a*]pyridine-8-carboxamido)methyl)-benzylcarbamate (6):** Yield: 0.33 g, m.p.: 215-217 ºC. Elemental analysis calcd. (found) % for $C_{37}H_{41}N_5O_4$: C, 76.31 (76.33); H, 6.60 (6.64); N, 13.91 (13.95); O, 3.18 (3.22). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH, CH_3), 3050 (C-H), 1690 (CONH),1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (t, 3H, C<u>H</u>$_3$), 0.98 (s, 3H, C<u>H</u>$_3$), 1.21 (q, 2H, CH_2), 2.35 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.46 (s, 2H, C<u>H</u>$_2$), 3.42 (s, 1H, N<u>H</u>), 3.70 (s, 2H, N<u>H</u>$_2$), 3.75 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 503 *m/s* 474 (M^+), 53%, 442 (65%), 401 (59%), 368 (51%).

***tert*-Butyl-3-((2-(3,5-dimethylphenyl)-3-((3-methyl-benzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamido)-methyl)benzylcarbamate (7):** Yield: 0.34 g, m.p.: 247-249 ºC. Elemental analysis calcd. (found) % for $C_{37}H_{41}N_5O_4$: C, 76.31 (76.33); H, 6.60 (6.65); N, 13.91 (13.96); O, 3.18 (3.25). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH, CH_3), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 6H, 2 × C<u>H</u>$_3$), 0.96 (s, 3H, C<u>H</u>$_3$), 2.48 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.35 (s, 2H, C<u>H</u>$_2$),3.42 (s, 1H, N<u>H</u>), 3.70 (s, 2H, N<u>H</u>$_2$), 3.72 (s, 1H, N<u>H</u>), 7.11-7.80 (16H, complex, Ar<u>H</u>). Mass: 504 *m/s* 429 (M^+) (60%), 413 (69%), 384 (52%), 355 (48%).

***tert*-Butyl-3-((2-(4-methoxyphenyl)-3-((3-methyl-benzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamido)-methyl)benzylcarbamate (8):** Yield: 0.34 g, m.p.: 241-243 ºC. Elemental analysis calcd. (found) % for $C_{36}H_{39}N_5O_4$: C, 73.64 (73.66); H, 6.18 (6.23); N, 13.85 (13.90); O, 6.33 (6.36). IR (KBr, ν_{max}, cm^{-1}): 3452 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1698 (CONH), 1563 (C=C), 1432 (C=N), 1240 (C-O-C), 1220 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 3H, C<u>H</u>$_3$), 2.35 (s, 2H, C<u>H</u>$_2$), 2.40 (s, 2H, C<u>H</u>$_2$), 2.45 (s, 2H, C<u>H</u>$_2$), 3.42 (s, 1H, N<u>H</u>), 3.70 (s, 3H, OC<u>H</u>$_3$), 3.74 (s, 2H, N<u>H</u>$_2$), 3.76 (s, 1H, N<u>H</u>), 7.11-7.80 (15H, complex, Ar<u>H</u>). Mass: 505 *m/s* 414 (M^+) 51%, 474 (64%), 399 (52%), 370 (34%).

***N*-(3-(Aminomethyl)benzyl)-3-((3-methoxybenzyl)-amino-2-(substituted phenyl)imidazo[1,2-*a*]pyridine-8-carboxamide (9):** Yield: 0.38 g, m.p.: 232-234 ºC. Elemental analysis calcd. (found) % for $C_{36}H_{39}N_5O_4$: C, 73.64 (73.67); H, 6.18 (6.21); N, 13.85 (13.87); O, 6.33 (6.38). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COCH_3$), 1563 (C=C), 1432 (C=N), 1220 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H,

C$\underline{H}_3$), 2.44 (s, 2H, C$\underline{H}_2$), 2.46 (s, 2H, C$\underline{H}_2$), 2.48 (s, 2H, C$\underline{H}_2$), 3.42 (s, 1H, N$\underline{H}$), 3.70 (s, 2H, N$\underline{H}_2$), 3.74 (s, 3H, OC$\underline{H}_3$), 3.76 (s, 1H, N$\underline{H}$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: 505 *m/s* 414 (M^+) (61%), 399 (69%), 370 (53%).

***tert*-Butyl-3-((2-((4-ethylphenyl)-3-((3-methoxybenzyl)-amino)imidazo[1,2-*a*]pyridine-8-carboxamido)methyl)-benzylcarbamate (10):** Yield: 0.40 g, m.p.: 231-233 ºC. Elemental analysis calcd. (found) % for $C_{37}H_{41}N_5O_4$: C, 73.96 (73.99); H, 6.40 (6.46); N, 13.48 (13.54); O, 6.16 (6.21). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COCH_3$), 1563 (C=C), 1432 (C=N), 1220 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 3H, C$\underline{H}_3$), 2.35 (s, 2H, C$\underline{H}_2$), 2.40 (s, 2H, C$\underline{H}_2$), 2.45 (s, 2H, C$\underline{H}_2$), 2.48 (s, 2H, C$\underline{H}_2$), 3.42 (s, 1H, N$\underline{H}$), 3.70 (s, 2H, N$\underline{H}_2$), 3.72 (s, 3H, OC$\underline{H}_3$), 3.74 (s, 1H, N$\underline{H}$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: 519 *m/s* 490 (M^+), 488 (43%), 428 (60%), 413 (51%), 384 (34%).

***tert*-Butyl-3-((2-((3,5-dimethylphenyl)-3-((3-methoxybenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamido)-methyl)benzylcarbamate (11):** Yield (0.41 g, m.p.: 220-222 ºC. Elemental analysis calcd. (found) % for $C_{37}H_{41}N_5O_4$: C, 73.96 (73.98); H, 6.40 (6.47); N, 13.48 (13.53); O, 6.16 (6.22). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COCH_3$),1563 (C=C), 1432 (C=N), 1220 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.98 (s, 6H, 2C$\underline{H}_3$), 2.35 (s, 2H, C$\underline{H}_2$), 2.40 (s, 2H, C$\underline{H}_2$), 2.45 (s, 2H, C$\underline{H}_2$), 3.42 (s, 1H, N$\underline{H}$), 3.70 (s, 2H, N$\underline{H}_2$), 3.72 (s, 1H, N$\underline{H}$), 3.74 (s, 3H, OC$\underline{H}_3$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: 519 *m/s* 488 (M^+), 428 (61%), 413 (51%), 384 (44%).

***tert*-Butyl-3-((3-((3,5-methoxybenzyl)amino)-2-((4-methoxyphenyl)imidazo[1,2-*a*]pyridine-8-carboxamido)-methyl)benzylcarbamate (12):** Yield (0.43 g, m.p.: 228-230 ºC. Elemental analysis calcd. (found) % for $C_{36}H_{39}N_5O_5$: C, 71.38 (71.43); H, 5.99 (6.04); N, 13.43 (13.47); O, 9.20 (9.23). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1685 ($COCH_3$),1563 (C=C), 1432 (C=N), 1240 (C-O-C), 1220 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.36 (s, 2H, C$\underline{H}_2$), 2.44 (s, 2H, C$\underline{H}_2$), 2.48 (s, 2H, C$\underline{H}_2$), 3.42 (s, 1H, N$\underline{H}$), 3.70 (s, 3H, OC$\underline{H}_3$), 3.72 (s, 1H, N$\underline{H}$), 3.76 (s, 2H, N$\underline{H}_2$), 3.78 (s, 3H, OC$\underline{H}_3$), 7.11-7.84 (15H, complex, Ar$\underline{H}$). Mass: 521 *m/s* 490 (M^+), 430 (53%), 415 (65%), 386 (53%).

***N*-(3-Aminophenyl)-3-(benzylamino)-2-(*m*-tolyl)-imidazo[1,2-*a*]pyridine-8-carboxamide (13):** Yield: 0.34 g, m.p.: 230-232 ºC. Elemental analysis calcd. (found) % for $C_{33}H_{33}N_5O_3$: C, 75.15 (75.22); H, 5.63 (5.69); N, 15.65 (15.70); O, 3.58 (3.65). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.92 (s, 3H, C$\underline{H}_3$), 2.45 (s, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 3.70 (s, 1H, N$\underline{H}$), 3.72 (s, 1H, N$\underline{H}$), 7.11-7.80 (16H, complex, Ar$\underline{H}$). Mass: 447 *m/s* 356 (M^+), 341 (59%), 340 (60%), 312 (53%).

***N*-(3-Aminophenyl)-3-((3-methylbenzyl)amino-2-(*m*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxamide (14):** Yield: 0.37 g, m.p.: 234-236 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_3$: C, 75.46 (75.48); H, 5.90 (5.93); N, 15.17 (15.20); O, 3.47 (3.51). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 6H, 2×C$\underline{H}_3$), 2.45 (s, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 3.70 (s, 1H, N$\underline{H}$), 3.72 (s, 1H, N$\underline{H}$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: 461 *m/s* 370 (M^+), 355 (61%), 354 (58%), 326 (38%).

***N*-(4-Aminophenyl)-3-(benzylamino)-2-(4-ethylphenyl)-imidazo[1,2-a]pyridine-8-carboxamide (15):** Yield: 0.38 g, m.p.: 230-232 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_3$: C, 75.46 (75.51); H, 5.90 (5.94); N, 15.17 (15.23); O, 3.47 (3.54). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.96 (s, 3H, C$\underline{H}_3$), 2.46 (s, 2H, C$\underline{H}_2$), 2.48 (s, 2H, C$\underline{H}_2$), 3.52 (s, 2H, N$\underline{H}_2$), 3.70 (s, 1H, N$\underline{H}$), 3.76 (s, 1H, N$\underline{H}$), 7.12-7.84 (16H, complex, Ar$\underline{H}$). Mass: 461 *m/s* 432 (M^+), 370 (56%), 355 (60%), 354 (55%), 326 (49%).

***N*-(3-Aminophenyl)-3-((3-methoxybenzyl)amino-2-(*m*-tolyl)imidazo[1,2-*a*]pyridine-8-carboxamide (16):** Yield: 0.38 g, m.p.: 210-212 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_4$: C, 72.94 (72.98); H, 5.70 (5.73); N, 14.66 (14.69); O, 6.70 (6.74). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3065 (CH), 3052 (C-H), 1692 (CONH), 1565 (C=C), 1432 (C=N), 1252 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, C$\underline{H}_3$), 2.45 (s, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 3.68 (s, 3H, OC$\underline{H}_3$), 3.70 (s, 1H, N$\underline{H}$), 3.72 (s, 1H, N$\underline{H}$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: *m/z* 446 (M^+), 386 (63%), 371 (57%), 370 (44%), 342 (28%).

***N*-(4-Aminophenyl)-3-(benzylamino)-2-(4-methoxyphenyl)imidazo[1,2-*a*]pyridine-8-carboxamide (17):** Yield: 0.39 g, m.p.: 236-238 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_3$: C, 72.55 (72.60); H, 5.44 (5.48); N, 15.11 (15.17); O, 6.90 (6.92). IR (KBr, ν_{max}, cm^{-1}): 3452 (NH), 3150 (C=C-H), 3062(CH), 3050 (C-H), 1692 (CONH), 1561 (C=C), 1430 (C=N), 1242 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.45 (s, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 3.70 (s, 1H, N$\underline{H}$), 3.73 (s, 3H, OC$\underline{H}_3$), 3.76 (s, 1H, N$\underline{H}$), 7.11-7.80 (16H, complex, Ar$\underline{H}$). Mass: 463 *m/s* 432 (M^+), 370 (58%), 355 (63%), 354 (54%), 326 (48%).

***N*-(4-Aminophenyl)-3-(benzylamino)-2-(3,5-dimethylphenyl)imidazo[1,2-*a*]pyridine-8-carboxamide (18):** Yield: Yield: 0.32 g, m.p.: 233-235 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_3$: C, 75.46 (75.50); H, 5.90 (5.98); N, 15.17 (15.21); O, 3.47 (3.55). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563(C=C), 1432 (C=N). ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 0.98 (s, 6H, 2C$\underline{H}_3$), 2.45 (s, 2H, C$\underline{H}_2$), 3.46 (s, 2H, N$\underline{H}_2$), 3.70 (s, 1H, N$\underline{H}$), 3.72 (s, 1H, N$\underline{H}$), 7.11-7.80 (15H, complex, Ar$\underline{H}$). Mass: 461 *m/s* 370 (M^+), 355 (63%), 354 (55%), 326 (43%).

***N*-(4-Aminophenyl)-2-(4-ethylphenyl)-3-((3-methylbenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamide (19):** Yield: 0.40 g, m.p.: 236-238 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_3$: C, 73.76 (73.81); H, 6.15 (6.21); N, 14.73 (14.75); O, 3.36 (3.40). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, C$\underline{H}_3$), 0.97 (t, 3H, C$\underline{H}_3$), 2.38 (q, 2H, C$\underline{H}_2$), 2.42 (s, 2H, C$\underline{H}_2$), 3.48 (s, 2H, N$\underline{H}_2$), 3.68 (s, 1H, N$\underline{H}$), 3.72 (s, 1H,

NH), 7.14-7.84 (15H, complex, ArH). Mass: 475 *m/s* 446 (M^+), 384 (58%), 369 (50%), 368 (49%), 340 (38%).

***N*-(4-Aminophenyl)-2-(3,5-dimethylphenyl)-3-((3-methylbenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamide (20):** Yield: 0.39 g, m.p.: 229-231 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_4$: C, 75.76 (75.80); H, 6.15 (6.21); N, 14.73 (14.76); O, 3.36 (3.40). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.94 (s, 3H, CH_3), 0.96 (s, 6H, $2CH_3$), 2.46 (s, 2H, CH_2), 3.46 (s, 2H, NH_2), 3.68 (s, 1H, NH), 3.72 (s, 1H, NH), 7.11-7.84 (14H, complex, ArH). Mass: 475 *m/s* 384 (M^+), 369 (51%), 368 (45%), 340 (28%).

***N*-(4-Aminophenyl)-2-(4-methoxyphenyl)-3-((3-methylbenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamide (21):** Yield: 0.39 g, m.p.: 239-241 ºC. Elemental analysis calcd. (found) % for $C_{33}H_{33}N_5O_3$: C, 72.94 (72.99); H, 5.70 (5.75); N, 14.66 (14.70); O, 6.70 (6.76). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N), 1250 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, CH_3), 2.45 (s, 2H, CH_2), 3.46 (s, 2H, NH_2), 3.68 (s, 3H, OCH_3), 3.70 (s, 1H, NH), 3.72 (s, 1H, NH), 7.11-7.80 (15H, complex, ArH). Mass: 477 *m/s* 446 (M^+), 386 (47%), 371 (41%), 370 (33%), 342 (22%).

***N*-(4-Aminophenyl)-2-(4-ethylphenyl)-3-((3-methoxybenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamide (22):** Yield: 0.32 g, m.p.: 232-234 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_4$: C, 73.30 (73.34); H, 5.95 (5.97); N, 14.25 (14.30); O, 6.51 (6.55). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N), 1250 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 3H, CH_3), 2.45 (s, 2H, CH_2), 2.48 (s, 2H, CH_2), 3.46 (s, 2H, NH_2), 3.68 (s, 3H, OCH_3), 3.70 (s, 1H, NH), 3.72 (s, 1H, NH), 7.12-7.88 (15H, complex, ArH). Mass: 491 *m/s* 462 (M^+), 460 (66%), 385 (58%), 384 (42%), 356 (38%).

***N*-(4-Aminophenyl)-2-(3, 5-dimethylphenyl)-3-((3-methoxybenzyl)amino)imidazo[1,2-*a*]pyridine-8-carboxamide (23):** Yield: 0.42 g, m.p.: 230-232 ºC. Elemental analysis calcd. (found) % for $C_{35}H_{37}N_5O_4$: C, 73.30 (73.36); H, 5.95 (5.98); N, 14.25 (14.31); O, 6.51 (6.56). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N), 1250 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 0.95 (s, 6H, $2CH_3$), 2.45 (s, 2H, CH_2), 3.46 (s, 2H, NH_2), 3.68 (s, 3H, OCH_3), 3.70 (s, 1H, NH), 3.72 (s, 1H, NH), 7.11-7.80 (14H, complex, ArH). Mass: 491 *m/s* 460 (M^+), 385 (66%), 384 (52%),356 (40%).

***N*-(4-Aminophenyl)-3-((3-methoxybenzyl)amino)-2-(4-methoxyphenyl)imidazo[1,2-a]pyridine-8-carboxamide (24):** Yield: 0.38 g, m.p.: 236-238 ºC. Elemental analysis calcd. (found) % for $C_{34}H_{35}N_5O_5$: C, 70.57 (70.61); H, 5.51 (5.57); N, 14.19 (14.24); O, 9.72 (9.76). IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3152 (C=C-H), 3060 (CH), 3050 (C-H), 1690 (CONH), 1563 (C=C), 1432 (C=N), 1250 (C-O-C), 1230 (C-O-C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.45 (s, 2H, CH_2), 3.46 (s, 2H, NH_2), 3.68 (s, 3H, OCH_3), 3.70 (s, 1H, NH), 3.72 (s, 3H, OCH_3), 3.74 (s, 1H, NH), 7.11-7.80 (15H, complex, ArH). Mass: 493 *m/s* 462 (M^+), 387 (68%),386 (51%), 358 (44%).

Biological assay

Preparation of parietal cells: Animals were cared for in compliance with the guidelines approved by the Institutional Animal Ethical Committee of R.C. Patel Institute of Pharmaceutical Education & Research, Shirpur, India (Lett. No. IAEC/CPCSEA/RCPIPER/2017-2022).The parietal cell suspension was prepared from mucosal scrapings of the goat stomach obtained from the slaughter house and then homogenized in 200 mM tris-HCl buffer, pH 7.4 and centrifuged for 10 min at 5000x g. The resulting supernatant was subsequently centrifuged at 5000x 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 concentrations of the test compound for 30 min, to this 2 mM $MgCl_2$ and 2 mM KCl was added. Then, the 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 addi-tion of 10% trichloroacetic acid followed by centrifugation at 2000x 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 compounds **(1-24)** potently reduced the hydrolysis of ATP by the goat gastric ATPase with IC_{50} = 6-10.2 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 the preparation wizard' in Maestro 8.0 in two preparation and refinement steps. Preparation of Grids were produced by centering on co-crystalized ligand. The ligands were generated using a 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 ligands were chosen and docked into the grid generated from protein structures by standard precision docking mode.

RESULTS AND DISCUSSION

The design for the series of imidazopyridines to be considered for study was performed using virtual screening protocol [15-17]. Considering the pharmacophoric requirements and standard compound AZD0865, the CID database was explored. The *in silico* checked compounds were then observed for the

Lipinski's 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 has very good inhibitory potential and must be explored for H^+/K^+-ATPase inhibitory activity. Hence, imidazopyridine analogues for H^+/K^+-ATPase inhibition were synthesized.

Novel diketoquinolines **1-24** were synthesized 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 $NaHCO_3$. In a condensation reaction, cyclization of amino group leads to form a new ringm which is confirmed by PMR and IR spectral analysis. In the second step, introduction of amino group by replacing the acidic proton of five-membered imidazole ring takes in the presence of acetic acid, sodium nitrite, HBr and addition of tin in a 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 the fourth step, introduced a new aromatic ring by cyclization of COOH and NH_2 in presence of DCM, triethylamine and substituted carbamates. In this step, NH_2 of *m*-nitroaniline is protected and removed after reaction completion.

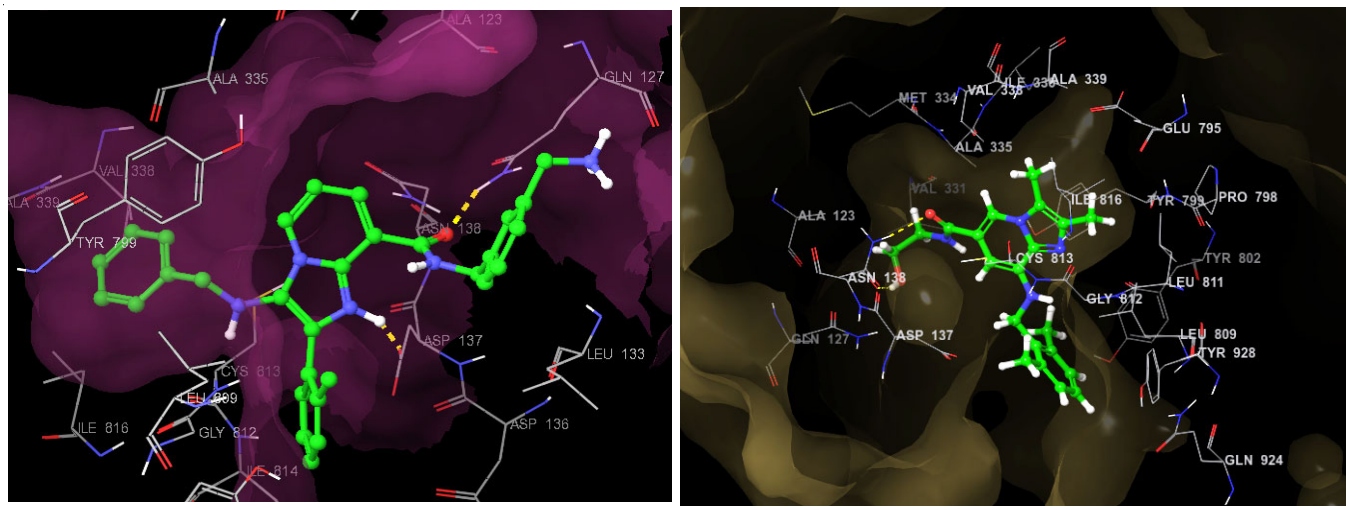
Biological activity: Imidazopyridine analogues **1-24** were evaluated *in vitro* for their proton pump inhibitory activity using an H^+/K^+-ATPase inhibition assay [22-25]. It is observed that acetate and carboxamide derivatives are more potent with a high selectivity against H^+/K^+-ATPase. The replacement of the methyl group from C_2 and C_3 of AZD0865 (linaprazan) by substituted phenyl group and further alkyl phenyl group did not increase the activity. Substitution at imidazopyridine C_2 by methyl phenyl, ethyl phenyl and C_3 by amino methyl phenyl (**1** and **2**) showed IC_{50} of 6.2 and 6.0 µg against H^+/K^+-ATPase enzyme (Table-1). This suggests that the substitutions at C_2 and C_3 by alkyl and amino phenyl moiety affects the ability of the inhibitors to bind with H^+/K^+-ATPase enzyme. Other substitution does not make any significant interaction with the H^+/K^+-ATPase enzyme.

TABLE-1
INHIBITION OF H^+/K^+-ATPase BY COMPOUNDS **1-24**

Compd. No.	-log IC_{50}	Compd. No.	-log IC_{50}
1	6.2	**14**	8.2
2	6.0	**15**	7.3
3	7.5	**16**	9.4
4	7.8	**17**	9.4
5	7.6	**18**	8.4
6	7.1	**19**	8.2
7	7.0	**20**	8.2
8	7.8	**21**	9.6
9	9.1	**22**	9.8
10	8.9	**23**	9.3
11	8.3	**24**	10.2
12	9.4	AZD0865	2.0
13	7.6	–	–

Molecular docking: The binding mechanisms of synthesized compounds were investigated using molecular docking studies. The docked effect for the designed and synthesized molecules was performed using Schrodinger Suite. For docking study, GLIDE was used into the H^+/K^+-ATPasepouch. The crystal moiety of H^+/K^+-ATPase was downloaded from the protein data bank, PDB ID: 4ux2. The protein structure was prepared by the wizard method in Maestro 8.0. Grids were produced by aiming at co-crystallized ligand. The structures were developed using a maestro build panel and prepared by the Ligprep 2.2 module that produces the low energy conformer of ligands using OPLS 2005 force field. The low energy structures of the ligands were identified and docked into the grid.

The docking poses revealed the interaction of a few ligands with desired amino acids. The standard drug AZD0865 had a docking score of -7.11 and displayed interactions with Asn138



CID_24077526 (compound **1**) AZD0865

Fig. 2. Docked view for imidazopyridine compounds

and Asp137. When AZD0865were docked in the same active site, a comparable docking scores and interaction patterns were displaced (Fig. 2). Compounds **1** and **2** had maximum potency with IC_{50} 6.2 and 6.0 µg an enzyme inhibition assay.

Conclusion

A series of imidazopyridine were synthesized having different moities on the 2nd, 3rd and 6th-position by hydrophobic benzyl moiety, carboxamide and amino phenyl group, instead of alkyl and toluene, which are present on standard compound AZD 0865. A replacement of a 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 a moderate activity. Compounds **1** and **2** showed IC_{50} value 6.2 and 6.0 µg against proton pump ATPase. This result suggested that substitution on AZD 0865 by phenyl alkyl and amino-phenyl moiety at C_2 and C_3 showed effect on the ability of the inhibitors to bind with ATPase enzyme. Other substitutions did not show any significant interaction with the 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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