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DISCOVERY OF SUBSTITUTED IMIDAZO [1, 2-c] PYRIDO [3, 2-e] PYRIMIDINE BASED DERIVATIVES AS NOVEL ANTI-MICROBIAL AGENTS

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ABSTRACT

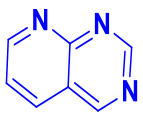
A Novel series of compounds were synthesised by cyclisation reaction of 2-amini nicotinic acid (1) with ammonium chloride and potassium cyanate to get pyrido [2,3-d] pyrimidine-2,4 (1H,3H) - dione (2) intermediate, which were further treated with $POCl_3$ in presence of N, N-di methylaniline to get 2, 4-dichloropyrido [2,3-d] pyrimidine (3) derivative. Which is reacted with aqueous ammonia, reflux for 16 hrs to get compound 2-chloropyrido [2,3-d] pyrimidin-4-amine (4), which was reacted with 4-substituted phenacyl bromides (5 a-f) to get bi cyclised compounds 5-chloro-2-p-Substituted imidazo [1,2-c] pyrido [3,2-e] pyrimidines (6a-6f), which were further reacted with 4-substituted phenols (7a-b) to get Target Compounds (8a-8 l). The structures of new compounds were confirmed by IR and ^{1}H NMR and ^{13}C NMR spectral data. Anti-bacterial and anti-fungal activities were evaluated and compared with the standard drugs, some compounds of the series exhibited promising anti-microbial and anti-fungal activity compared to standard drugs.

KEYWORDS: Pyrido [2, 3-d] Pyrimidines, Synthesis, Spectral data, anti-bacterial activity, antifungal activity.

INTRODUCTION

Hetero cyclic chemistry is a very important branch of organic chemistry accounting for nearly one-third of modern publications. In fact two thirds of organic compounds are Heterocyclic compounds. Hetero cyclic chemistry comprises at least half of all organic chemistry Research World Wide. Heterocyclic chemistry is the largest classical division of medicinal chemistry and display a broad range of industrial and pharmaceutical applications.

Pyridopyrimidine and its derivatives have been studied due to a variety of chemical and biological significance. The importance of pyridopyrimidines as biologically ac- tive compounds includes their use as antibacterial[1-3], antiallergic[4], antitumor[2, 3] antifolate[5], tyrosine kinase[6], antimicroibial[7], calcium channel antago- nists[8], antibacterial[9-12], anti-inflammatory, anal- gesic[13], antihypertensive[14], antileishmanial[15], tuber-culostatic[16], anticonvulsants[17], diuretic, po- tassiumsparing[18], and antiaggressive activities.[19] The need to reduce the amount of toxic waste and by- product arising from chemical processes requires increasing emphasis on the use of less toxic and environmentally compatible materials in the design of new synthetic methods. One of the most promising approaches is using water as the reaction media.[20-26] The Stucture of Pyrido [2,3 d] Pyrimidine as shown below.[Fig.1].



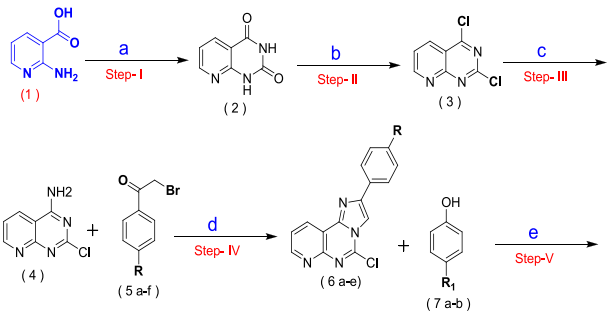
Structure of pyrido[2,3-*d*]pyrimidine

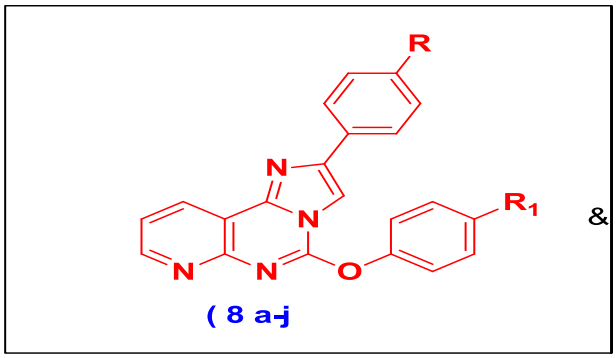
Fig.1 Structure of Pyrido [2, 3, d] Pyrimidine

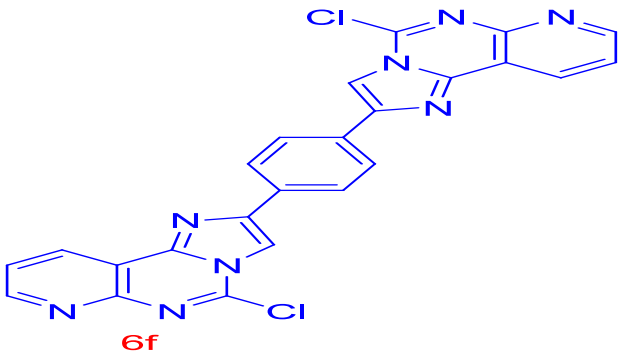
Encouraged by the diverse biological activities of pyrido [2, 3-d] pyrimidine Heterocyclic compounds, it was decided to prepare a new series of pyrido [2, 3-d] pyrimidine derivatives. Literature survey revealed that incorporation of Substituted phenoxy group in pyrido [2,3-d]pyrimidine Heterocyclic ring enhanced antibacterial and antifungal activity. In the present communication 2 , 4 di Chloro pyrido [2,3-d] pyrimidine (3) was reacted with ammonia in Ethanol at Room Temparature condition to form Compound 2-chloropyrido[2,3-d]pyrimidin-4-amine (4), which were further reacted with 4-Substituted phenacyl bromides (5 a-f) to get 5-chloro-2- Substituted imidazo [1,2-c] pyrido [3,2-e] Pyrimidines (6a-6f), which were further reacted with 4-Substituted Phenols (7 a-b) to get Target Compounds (8 a-l). The synthesis of the compounds as per the following **Scheme I** given below.

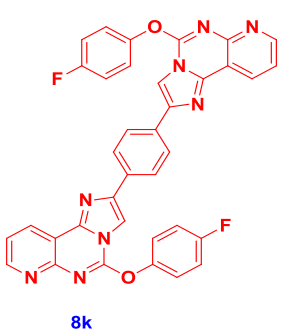
The synthetic route was depicted in scheme **I.** The structures of all synthesized compounds were assigned on the basis of IR, Mass, ^{1}H NMR spectral data analysis. Further these compounds were subjected for antifungal and antibacterial activity.

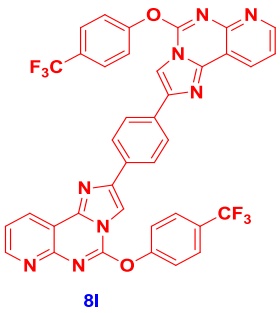
SYNTHETIC SCHEME











R = -H, -CH_3, -OCH_3, -Cl, -Br, -CO-CH_2-Br R_1 = -F, -CF_3

Reagents and Reaction conditions: ***(a)*** ammonium chloride, potassium cyanate (KNCO),Water, 200^0C ***(b)*** *$POCl_3$, N,N-dimethylaniline ,Reflux* ***(c)*** *25% Aqueous Ammonia, Reflux (d) Ethanol, Sodium bicarbonate, Reflux (e) Sodium hydride, DMF, 100^0C.*

Compound	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8i**	**8j**	**8k**	**8l**
R	-H	-H	$-CH_3$	$-CH_3$	$-OCH_3$	$-OCH_3$	-Cl	-Cl	-Br	-Br	----	----
R_1	-F	$-CF_3$	-F	$-CF_3$	-F	$-CF_3$	-F	$-CF_3$	-F	$-CF_3$	-F	$-CF_3$

MATERIALS AND METHODS

General Methods

Fresh solvents were used without purification. Melting points were obtained in open capillary tubes by using a MEL-Temp II melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1600 series Fourier Transform instrument with the samples as KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL 400 MHz spectrometer at ambient temperature using tetramethylsilane as an internal reference. The antimicrobial tests were carried out at the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Sri Krishnadevaraya University. ChemDrawUltra-12.0 has been used for the nomenclature of the prepared compounds.

All chemical were purchased from Sigma Aldrich with purity not less than 99.9%. Analytical Thin Layer Chromatography (TLC) was carried out by using silica gel 60 F254 pre-coated plates. Visualization was accomplished with UV lamp and P-Anisaldehyde stain. All products were characterized by their NMR and Mass spectra. purity has been checked by thin layer chromatography and melting point. Conventional method has been used for synthesis of pyrido [2,3-d]pyrimidine derivatives. Stirring and reflux method were used for synthesis of pyrido derivatives 8 (a-l) respectively.

The synthetic route was depicted in scheme **I.**

The title compounds 8(a-l) were synthesised in five sequential steps using different reagents and reaction conditions, the 8(a-l) were obtained in moderate yields. The structure were established by spectral (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data.

EXPERIMENTAL SECTION

All reactions were carried out under argon in oven-dried glassware with magnetic stirring. Unless otherwise noted, all materials were obtained from commercial suppliers and were

used without further purification. All solvents were reagent grade. THF was distilled from sodium benzophenone ketyl and degassed thoroughly with dry argon directly before use. Unless otherwise noted, organic extracts were dried with anhydrous Na_2SO_4, filtered through a fitted glass funnel, and concentrated with a rotary evaporator (20–30 Torr). Flash chromatography was performed with silica gel (200–300 mesh) by using the mobile phase indicated. The NMR spectra were measured with a 400 MHz Bruker Avance spectrometer at 400.1 and 100.6 MHz, for ^{1}H for ^{13}C, respectively, in $CDCl_3$ solution with tetra methyl silane as internal standard. Chemical shifts are given in ppm (δ) and are referenced to the residual proton resonances of the solvents. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded using tetramethylsilane (TMS) in the solvent of $CDCl_3$-d or DMSO-d_6 as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm ,DMSO at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.16 ppm, DMSO at 40.00 ppm).

Synthesis of pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (Compound 2)

A mixture of 2-amino nicotinic acid (1) (**1 m.mol**), ammonium chloride (**10 m.mol**) and potassium cyanate (**7 m.mol**) in water (10 ml) was heated to 80° C. and maintained at this temperature with stirring for 30 minutes before being heated to 200° C. The mixture was stirred for 2 hours at this elevated temperature and then left to cool. Water (20 ml) was then added and the resultant mixture filtered. The solid was washed with hot water (50 ml) and then with cold water (50 ml) to give a yellow solid which corresponded to the title compound (90 % Yield.)

^{1}H NMR (DMSO-d_6, 400 MHz): 8.7 (m, 1 H), 8.22 (d, J = 6.4 Hz, 1 H), 8. 53 (m, 1 H).
^{13}C NMR (DMSO-d_6, 100 MHz): 155,120,150,165, 115,150, 151
IR (KBr, cm^{-1}): O-H (3510, sharp), Ar stretch C-H (3130.34), C-O (1060), C=N (1608.69), C=C (1344.43) m/z (LC-MS Shows 95% purity.): 164 $[M+H]^{+}$, RT=2.18 mins.

Synthesis of 2, 4-di chloropyrido [2,3-d]Pyrimidine (Compound 3)

pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (2) (**0.3 m.mol**) was added to a stirred solution of $POCl_3$ (**1 m.mol**) at room temperature, and then N,N-dimethyl aniline (**1.5 m. mol**) was added drop wise to the mixture. The reaction mixture was heated to $90^{0}C$ for 6 h. The mixture was concentrated under reduced pressure. The residue was poured into ice water ,stirred at room temperature for 1 hr and separated by filtration to give title compound 3 as a off White solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 9.34 (1H, m), 8.66 (1H, m), 7.76 (1H, m);

^{13}C NMR (DMSO-d_6, 100 MHz): 153**,** 121,130,155, 120, 160, 162

IR (KBr, cm^{-1}): Ar stretch C-H (3120), C=N (1618), C=C (1340), C-Cl (757.49).

EI-MS (m/z): 200 $[M+H]^+$

Synthesis of 2-chloropyrido [2,3-d]pyrimidin-4-amine (Compound 4)

A suspension of 2, 4-dichloropyrido [2, 3-d] pyrimidine (**5 m.mol**) in 25% ammonia (30 ml) was heated under reflux for 2 hrs. The resulting precipitate was filtered off and washed with water (60ml). The corresponding intermediate pyrido [2, 3-d] pyrimidine was used without purification.

^{1}H NMR ($CDCl_3$-d_1, 400 MHz): 8.43 (d, 1 H), 7.38 (t, 1 H), 8. 50 (m, 1 H), 7.8 (2H, bs).

^{13}C NMR (DMSO-d_6, 100 MHz): 155,111,128,158, 154, 105, 155.

IR (KBr, cm^{-1}): N-H (3330 & 3450 Two bands) Ar stretch C-H (3110), C=N (1628), C=C (1360), C-Cl (767.49).

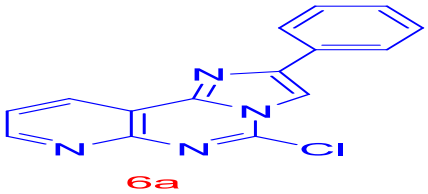
MS Shows 181 $[M+H]^+$

Synthesis of 5-chloro-2-phenylimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6a), 5-chloro-2-p-tolylimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6b), 5-chloro-2-(4-methoxyphenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6c), 5-chloro-2-(4-chlorophenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6d), 2-(4-bromophenyl)-5-chloroimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6e), 1,4-bis(5-chloroimidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (6f): A mixture of 4-Substitutedphenacyl bromides (5 a – 5e) (**1.1 m.mol**) , 2-chloropyrido[2,3-d] pyrimidin-4-amine (Compound 4 **1 m.mol**), and(**4 m.mol**) of sodium hydrogencarbonate was refluxed in 8 ml of ethanol for 5 hours. After completion of the reaction, the resulting crystal was filtered off, washed with wat er and acetone (9:1), and dried under reduced pressure to obtained compounds (6a-6e). (yield: 80-85 %). In case of 6f preparation 5f (**1.1 m.mol**), compound 4 (**2 m. mol**), $NaHCO_3$ (**10 m.mol**).

Table 1 Yields & Melting Points of Corresponding Compounds (6 a-6f)

S.NO	Yield (%)	Melting Point (^{o}C)	Physical Appearence
6a	80	102-104	White Solid
6b	82	214-215	Off white Solid
6c	80	198-199	White Solid
6d	81	219-221	Pale brown Solid
6e	82.3	220-222	Pale brown solid
6f	83.2	233-234	Pale yellow solid

5-chloro-2-phenylimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6a)



6a

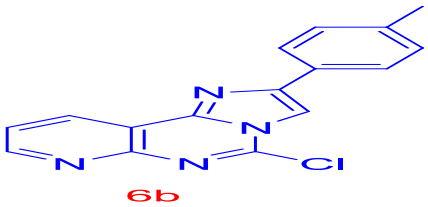
IR (KBr, cm^{-1}): Ar stretch C-H (3006.29), C=N (1526.15), C=C (1318.38), C-Cl (821.21)

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.6 (1H,d, Pyridine ring proton), 8.9 (1H,S), 7.4-8.2(5H, m, Ar-H).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-160.5 (13 aromatic carbons)

Mass: 280 (100%), 282 (32%) So it indicates molecule contains one –Cl atom.

5-chloro-2-p-tolylimidazo [1,2-c]pyrido[3,2-e]Pyrimidine (6b)



6b

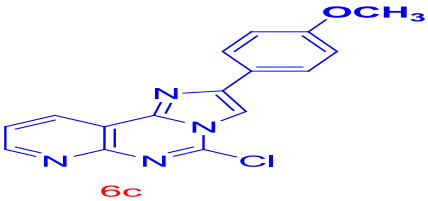
IR (KBr, cm^{-1}): Ar stretch C-H (3026.29), aliphatic C-H (2903.83), C=N (1526.15), C=C (1318.38), C-Cl (821).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.6 (1H,d, Pyridine ring proton), 8.9 (1H,S), 7.7(2H, d, Ar-H), 7.3 (2H,d, Ar-H), 2.3 (3H,S, -C**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-160.5 (13 aromatic carbons), 23.6 (aromatic methyl carbon).

Mass: 294 (100%), 296 (32%)So it indicates molecule contains one –Cl atom).

5-chloro-2-(4-methoxyphenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6c)



6c

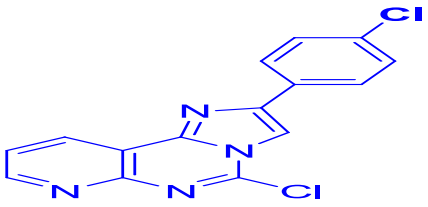
IR (KBr, cm^{-1}): Ar stretch C-H (3050), aliphatic C-H (2915), C=N (1536.15), C=C (1328.38), C-Cl (831), C-O-C (1150).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.6 (1H,d, Pyridine ring proton), 8.9 (1H,S), 7.1(2H, d, Ar-H), 7.9 (2H,d, Ar-H), 3.8 (3H,S, -OC**H**$_3$) .

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-160.5 (13 aromatic carbons), 56 (aromatic methoxy carbon).

Mass: 310 (100%), 312 (32%) So it indicates molecule contains one –Cl atom).

5-chloro-2-(4-chlorophenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6d)



6d

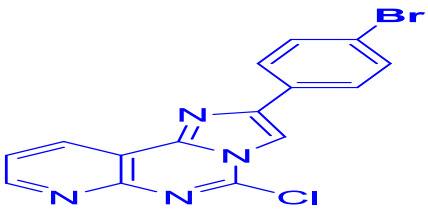
IR (KBr, cm^{-1}): Ar stretch C-H (3050), aliphatic C-H (2915), C=N (1536.15), C=C (1328.38), C-Cl (831).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.6 (1H,d, Pyridine ring proton), 8.8 (1H,S), 7.5(2H, d, Ar-H), 7.93 (2H,d, Ar-H), 3.8 (3H,S, -OC**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-160.3 (13 aromatic carbons)

Mass: 314 (100%), 316 (32%) So it indicates molecule contains one –Cl atom).

2-(4-bromophenyl)-5-chloroimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (6e)



6e

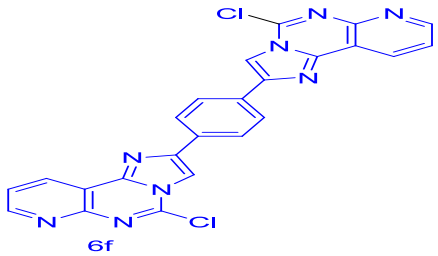
IR (KBr, cm^{-1}): Ar stretch C-H (3050), aliphatic C-H (2915), C=N (1536.15), C=C (1328.38), C-Cl (841), C-Br (550).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.52 (1H,d, Pyridine ring proton), 8.9 (1H,S), 7.8(2H, d, Ar-H), 7.7 (2H,d, Ar-H).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-162 (13 aromatic carbons)

Mass: 358 (100%), 360 (98%) So it indicates molecule contains one –Br atom).

1,4-bis(5-chloroimidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (6f)



6f

IR (KBr, cm^{-1}): Ar stretch C-H (3060), C=N (1536.15), C=C (1328.38), C-Cl (825).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.6 (1H,d, Pyridine ring proton), 8.8 (1H,S), 7.5(2H, d, Ar-H), 7.93 (2H,d, Ar-H), 3.8 (3H,S, -OC**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 110-160.3 (13 aromatic carbons)

Mass: 314 (100%), 316 (32%) So it indicates molecule contains one –Cl atom).

Synthesis of 5-(4-fluorophenoxy)-2-phenylimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8a), 2-phenyl-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8b), 5-(4-fluorophenoxy)-2-p-tolylimidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8c), 2-p-tolyl-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8d), 5-(4-fluorophenoxy)-2-(4-methoxyphenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8e), 2-(4-methoxyphenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e] Pyrimidine (8f), 2-(4-chlorophenyl)-5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8g), 2-(4-chlorophenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8h), 2-(4-bromophenyl)-5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-

e]Pyrimidine (8i), 2-(4-bromophenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8j), 1,4-bis(5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (8k), 1,4-bis(5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (8l)

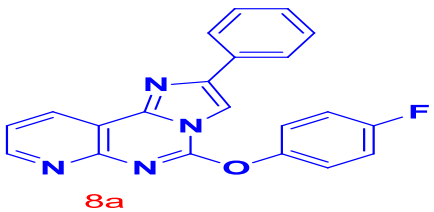
Sodium hydride (60% in oil, **1.1 m.mol**) was added to a 0° C. solution of 4-Substituted Phenols (7a - 7 b) (**1.1 m.mol**) in DMF (5 ml). After stirring for 20 min at 0° C., the mixture was allowed to warm to RT and a solution of 2-chloropyrimidine substituted derivatives (6a-6f) (**1.1 m.mol**) in DMF (8 ml) was added. The resulting mixture was heated to 100°C for 18 hrs. the residue was dissolved in ethyl acetate, washed with water and brine, dried (Na_2SO4), and concentrated to provide 70-75 percent yield of the title compounds 8a-8l.

In case of Compounds 8k, 8l preparations 4 substituted phenols (**2.2 m.mol**) were used.

Table 2 Yields & Melting Points of Corresponding Compounds (8 a-8l)

S.NO	Yield (%)	Melting Point (°C)	Physical Appearence
8a	73.8	220-223	Off White Solid
8b	74	214-215	white Solid
8c	75	198-199	White crystalline Solid
8d	71	219-221	Pale yellow Solid
8e	71.3	220-222	Pale brown solid
8f	75.2	233-234	Pale yellow solid
8g	75	134-136	Buffy crystals
8h	71	245-247	Buffy crystals
8i	70	131-134	Yellow crystals
8j	82	150-153	Brown crystals
8k	73	152-155	Buff crystals
8l	74.3	235-237	Buff crystals

5-(4-fluorophenoxy)-2-phenylimidazo [1,2-c]pyrido[3,2-e]Pyrimidine (8a)



8a

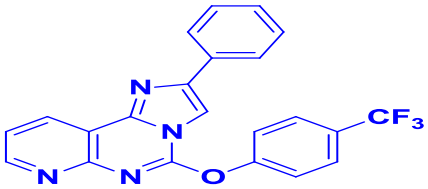
IR (KBr, cm^{-1}): Ar stretch C-H (3090), C-N (1326.15), C=C (1356.38), C-F (1125).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.5 (1H,d, Pyridine ring proton), 8.9 (1H,S,Imidazole ring Proton), 7.35(2H, d, Ar-H), 7.1 (2H,d, Ar-H), 7.4-8.2 (5H, Ar-H, m).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 135 (Ar C), 126-130 (5 Ar CH), 173 (Ar C, N-C-O), 149 (Ar C, O-C), 124 (Ar CH), 117 (Ar CH), 160 (Ar C, C-F).

Mass: 357 (M+H)

2-phenyl-5-(4-(trifluoromethyl) phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8b)



8b

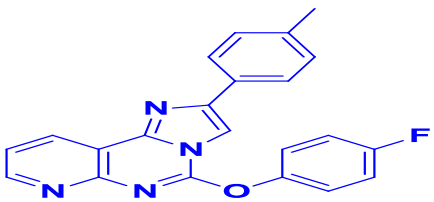
IR (KBr, cm^{-1}): Ar stretch C-H (3100), C-N (1346.15), C=C (1356.38), C-O-C (1075), C-F (1345).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.4 (1H,d,Pyridine ring proton), 7.4(1H,t, Pyridine ring proton), 8.5 (1H,d, Pyridine ring proton), 8.9 (1H,S,Imidazole ring Proton), 7.55(2H, d, Ortho to –CF_3, Ar-H), 7.2 (2H,d, Ortho to Phenoxy, Ar-H), 7.4-8.2 (5H, Ar-H, m).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 108 (Ar CH), 130 (Ar C), 135 (Ar C), 126-130 (5 Ar CH), 173 (Ar C, N-**C**-O), 159 (Ar C, O-**C**), 126 (Ar CH), 128 (Ar CH), 127 (Ar C), 125 (-**C**F_3 carbon).

Mass: 407 (M+H).

5-(4-fluorophenoxy)-2-p-tolylimidazo [1,2-c]pyrido[3,2-e]Pyrimidine (8c)



8c

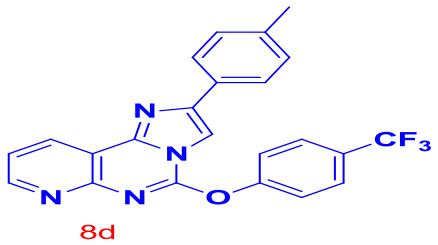
IR (KBr, cm^{-1}): Ar stretch C-H (3080), Aliphatic C-H (2870), C-O-C (1265), C-N (1346.15), C=C (1356.38), C-F (1295) .

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.7 (1H,S,Imidazole ring Proton), 7.05(2H, d, Ortho to –F, Ar-H), 7.3 (2H,d, Ortho to Phenoxy, Ar-H), 7.8 (2H,d), 7.3 (2H,d), 2.35 (3H, S, -Ar-C**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 135 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 131 (1 Ar C), 23 (Ar-CH_3), 173 (Ar C, N-C-O), 150 (Ar C, O-C), 124 (Ar CH), 117 (Ar CH), 160 (Ar C, C-F).

Mass: 371 (M+H).

2-p-tolyl-5-(4-(trifluoromethyl) phenoxy) imidazo [1,2-c]pyrido[3,2-e]Pyrimidine (8d).



8d

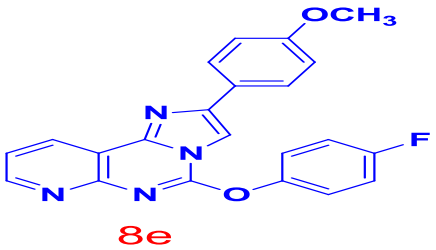
IR (KBr, cm^{-1}): Ar stretch C-H (3120), Aliphatic C-H (2870), C-O-C (1255), C-N (1346.15), C=C (1356.38), C-F (1265).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.7 (1H,S,Imidazole ring Proton), 7.65(2H, d, Ortho to –CF_3, Ar-H), 7.2 (2H,d, Ortho to Phenoxy, Ar-H), 7.8 (2H,d), 7.3 (2H,d), 2.35 (3H, S, -Ar-C**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 132 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 132 (1 Ar C), 23 (Ar-CH_3), 173 (Ar C, N-C-O), 157 (Ar C, O-C), 126 (2 Ar CH), 129 (2 Ar CH), 130 (Ar C, **C**-CF_3), 125 (-**C**F_3 carbon).

Mass: 421 (M+H).

5-(4-fluorophenoxy)-2-(4-methoxyphenyl)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8e)



8e

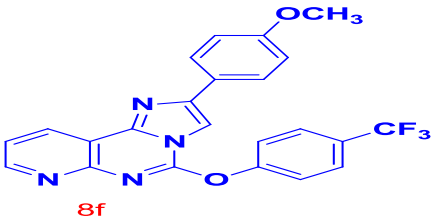
IR (KBr, cm^{-1}): Ar stretch C-H (3120), Aliphatic C-H (2870), C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1295).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.7 (1H,S,Imidazole ring Proton), 7.09(2H, d, Ortho to –F, Ar-H), 7.28 (2H,d, Ortho to Phenoxy, Ar-H), 7.9 (2H,d), 7.1 (2H,d), 3.85 (3H, S, -Ar-OC**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 135 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 131 (1 Ar C), 56.5 (Ar-O-**C**H_3), 173 (Ar C, N-C-O), 150 (Ar C, O-C), 124 (Ar CH), 117 (Ar CH), 160 (Ar C, C-F).

Mass: 387 (M+H).

2-(4-methoxyphenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e] Pyrimidine (8f)



8f

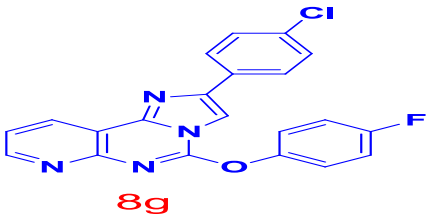
IR (KBr, cm^{-1}): Ar stretch C-H (3120), Aliphatic C-H (2870), C-O-C (1055), C-N (1346.15), C=C (1356.38), C-F (1265) .

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.7 (1H,S,Imidazole ring Proton), 7.65(2H, d, Ortho to –CF_3, Ar-H), 7.2 (2H,d, Ortho to Phenoxy, Ar-H), 7.8 (2H,d), 7.3 (2H,d), 3.85 (3H, S, -Ar-O-C**H**$_3$).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 132 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 132 (1 Ar C), 55 (Ar-O-**C**H_3), 173 (Ar C, N-C-O), 157 (Ar C, O-C), 126 (2 Ar CH), 129 (2 Ar CH), 130 (Ar C, **C**-CF_3), 125 (-**C**F_3 carbon).

Mass: 387(M+H).

2-(4-chlorophenyl)-5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8g)



8g

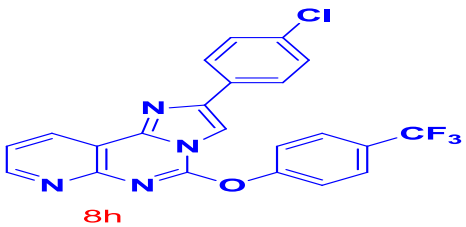
IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-Cl (750) C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1295).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.8 (1H,S,Imidazole ring Proton), 7.09(2H, d, Ortho to –F, Ar-H), 7.32 (2H,d, Ortho to Phenoxy, Ar-H), 7.95 (2H,d), 7.57 (2H,d).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 135 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 135 (1 Ar C), 173 (Ar C, N-C-O), 150 (Ar C, O-C), 124 (Ar CH), 117 (Ar CH), 160 (Ar C, C-F).

Mass: 391(M^+, 100%), 316 (M+2, 32%) So it indicates molecule contains one –Cl atom).

2-(4-chlorophenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8h)



8h

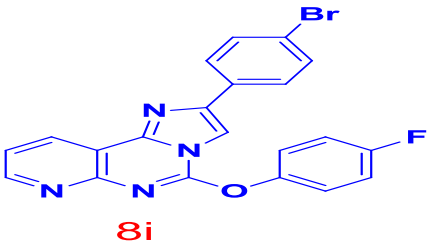
IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-Cl (780) C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1275).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.7 (1H,S,Imidazole ring Proton), 7.65(2H, d, Ortho to $–CF_3$, Ar-H), 7.2(2H,d, Ortho to Phenoxy, Ar-H), 7.8 (2H,d), 7.5 (2H,d).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 132 (Ar C), 126 (2 Ar CH), 130 (2 Ar CH), 132 (1 Ar C), 173 (Ar C, N-C-O), 157 (Ar C, O-C), 126 (2 Ar CH), 129 (2 Ar CH), 130 (Ar C, **C**-CF_3), 125 (-**C**F_3 carbon).

Mass: 441 (M^+, 100%), 443 (M+2, 32%) So it indicates molecule contains one –Cl atom).

2-(4-bromophenyl)-5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8i)



8i

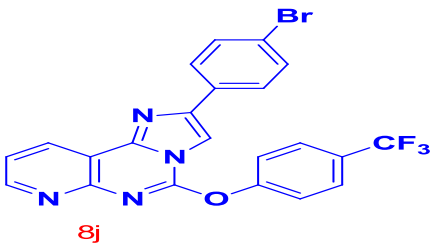
IR (KBr, cm^{-1}): Ar stretch C-H (3100), C-Br (570), C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1275).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (1H,d,Pyridine ring proton), 7.45(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.9 (1H,S,Imidazole ring Proton), 7.05(2H, d, Ortho to –Fluorine, Ar-H), 7.3(2H,d, Ortho to Phenoxy, Ar-H), 7.6 (2H,d), 7.8 (2H,d).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 132 (Ar C), 129 (2 Ar CH), 132 (2 Ar CH), 125 (1 Ar C), 173 (Ar C, N-**C**-O), 150 (Ar C, O-C), 126 (2 Ar CH), 116 (2 Ar CH), 160 (Ar C, **C**-F).

Mass: 435(M^+, 100%), 437 (M+2, 100%) So it indicates molecule contains one –Br atom).

2-(4-bromophenyl)-5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]Pyrimidine (8j)



8j

IR (KBr, cm^{-1}): Ar stretch C-H (3120), C-Br (550), C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1355).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.5 (1H,d,Pyridine ring proton), 7.5(1H,t, Pyridine ring proton), 8.55 (1H,d, Pyridine ring proton), 8.8 (1H,S,Imidazole ring Proton), 7.65(2H, d, Ortho to $–CF_3$, Ar-H), 7.2(2H,d, Ortho to Phenoxy, Ar-H), 7.8 (2H,d), 7.7 (2H,d).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring Ar CH), 121 (pyridine ring Ar CH), 131 (pyridine ring Ar CH), 116 (Ar C), 160 (Ar C), 143(Ar C), 107 (Ar CH), 130 (Ar C), 132 (Ar C), 129 (2 Ar CH), 132 (2 Ar CH), 123 (1 Ar C), 173 (Ar C, N-C-O), 157 (Ar C, O-C), 126 (2 Ar CH), 129 (2 Ar CH), 127 (Ar C, **C**-CF_3), 125 (-**C**F_3 carbon).

Mass: 485(M^+, 100%), 487 (M+2, 98%) So it indicates molecule contains one –Br atom).

1,4-bis(5-(4-fluorophenoxy)imidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (8k)

8k

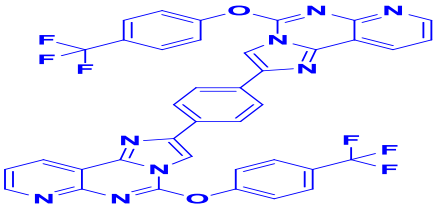
IR (KBr, cm^{-1}): Ar stretch C-H (3100), C-O-C (1075), C-N (1346.15), C=C (1356.38), C-F (1275).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (2H,d,Pyridine ring proton), 7.45(2H,t, Pyridine ring proton), 8.55 (2H,d, Pyridine ring proton), 8.9 (2H,S,Imidazole ring Proton), 7.1(4H, d, Ortho to –Fluorine, Ar-H), 7.23(4H,d, Ortho to Phenoxy, Ar-H), 8.3(4H,S).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring 2 Ar CH), 121 (pyridine ring 2 Ar CH), 131 (pyridine ring 2 Ar CH), 116 (2 Ar C), 160 (2 Ar C), 143(2 Ar C), 107 (2 Ar CH), 130 (2 Ar C), 132 (2 Ar C), 129 (4 Ar CH), 125 (2 Ar C), 173 (2 Ar C, N-**C**-O), 150 (2 Ar C, O-C), 126 (4 Ar CH), 118 (4 Ar CH), 160 (2 Ar C, **C**-F).

Mass: 635 (M^+, 100%), 636 (M+1, 38%).

1,4-bis(5-(4-(trifluoromethyl)phenoxy)imidazo[1,2-c]pyrido[3,2-e]pyrimidin-2-yl)benzene (8l)



8l

IR (KBr, cm^{-1}): Ar stretch C-H (3100), C-O-C (1055), C-N (1346.15), C=C (1356.38), C-F (1280).

^{1}H NMR (δ ppm, 400 MHZ, $CDCl_3$): 8.45 (2H,d,Pyridine ring proton), 7.45(2H,t, Pyridine ring proton), 8.55 (2H,d, Pyridine ring proton), 8.9 (2H,S,Imidazole ring Proton), 7.5(4H, d, Ortho to $–CF_3$, Ar-H), 7.15(4H,d, Ortho to Phenoxy, Ar-H), 8.4(4H, S).

^{13}C NMR ($CDCl_3$-d_1, 100 MHz): 155 (pyridine ring 2 Ar CH), 121 (pyridine ring 2 Ar CH), 132(pyridine ring 2 Ar CH), 116 (2 Ar C), 160 (2 Ar C), 143(2 Ar C), 107 (2 Ar CH), 130 (2 Ar C), 132 (2 Ar C), 129 (4 Ar CH), 173 (2 Ar C, N-**C**-O), 150 (2 Ar C, O-C), 126 (4 Ar CH), 129 (4 Ar CH), 127(2 Ar C), 125 (2 **C**F_3).

Mass: 735 (M^+, 100%), 736 (M+1, 38%).

Biological Activity

Antibacterial activity: Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (Bacillus subtilis MTCC 121, Staphylococcus aureus MTCC 7443) and Gram-negative bacteria (Xanthomonas campestris MTCC 7908 and Escherichia coli MTCC 7410) in DMF by disc diffusion method on nutrient agar medium (Andrews, 2001). The sterile medium (nutrient agar medium, 15 ml) in each Petri plate was uniformly smeared with cultures of Gram-positive and -negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was placed in the Petri plates, to which 50 µl (1 mg/ml, i.e. 50 µg/disc)

of the different synthesized compounds were added. The treatments also included 50 µl of DMF as negative, bacteriomycin and gentamycin as positive control for comparison. For each treatment, three replicates were maintained. The plates were incubated at 37± 2^{o}C for 24 h and the zone of inhibition was determined.

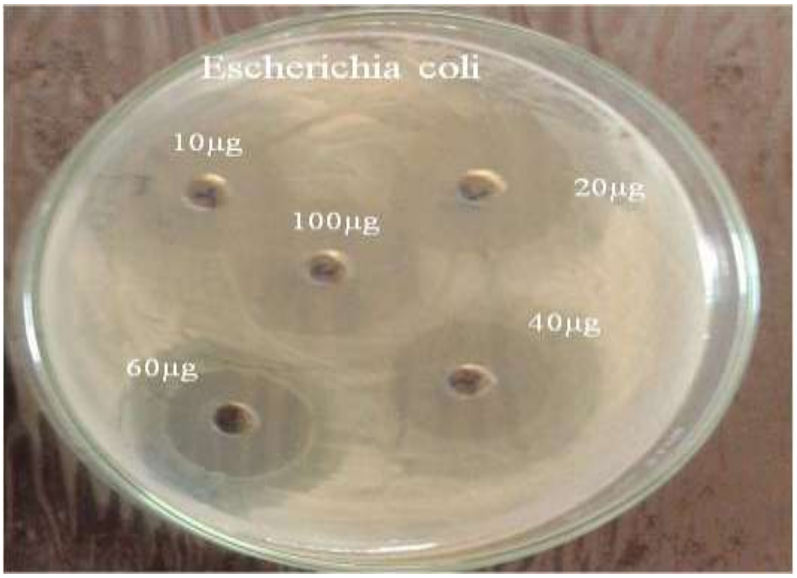
Antifungal activity: The synthesized compounds were screened for their antifungal activity against Fusarium oxysporum MTCC 2480 in DMF by poisoned food technique (Satish et al., 2007). Potato dextrose agar (PDA) media was prepared and about 15 ml of PDA was poured into each Petri plate and allowed to solidify. 5 mm disc of 7 days old culture of the test fungi was placed at the center of the Petri plate and incubated at 26^{o}C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard. All the synthesized compounds were tested (at the dosage of 500 µl of the new compounds/Petri plate, where concentration was 0.1 mg/ml) by poisoned food technique.

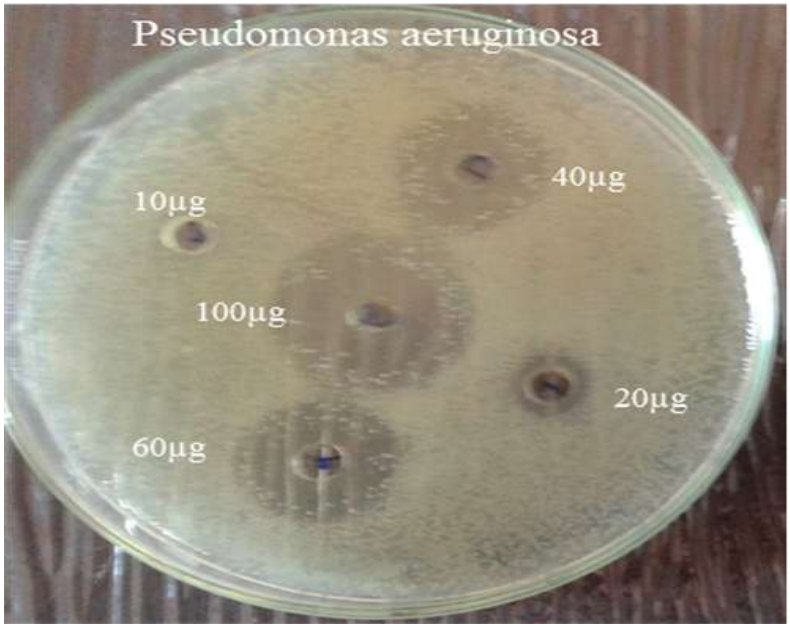
The Novel imidazo[1,2-c] pyrido[3,2-e] pyrimidine derivates containing $-CF_3$ (8l) and flouro (8k) showed more activity than other substituent's

The order of activity was **8l> 8k> 8h> 8j> 8i>8g > 8f >8d>8b>8e> 8c>8a**.

Table 3 In vitro antibacterial and antifungal activities of the synthesized compounds (8a-8l)

Compounds	Zone of inhibition in diameter (mm)				% inhibition
	B. subtilis	S. Aureus	P.aeruginosa	E. Coli	F. oxysporum
8a	11	12	11	10	49
8b	16	18	16	15	55.2
8c	14	15	13	12	51
8d	17	19	18	19	57.9
8e	15	16	14	14	52.6
8f	22	20	21	20	62.4
8g	22	21	22	21	63.0
8h	26	24	24	26	67.8
8i	23	22	21	22	63.4
8j	24	23	21	23	64.5
8k	27	25	25	27	74.0
8l	28	27	29	28	86.6
Bacteriomycin	-	-	34	-	-
Gentamycin	35	30	-	35	-
Nystatin	--	-	-	-	100





RESULTS AND DISCUSSIONS

Chemistry: The Title Compounds Novel substituted imidazo[1,2-c] pyrido[3,2-e] pyrimidine based derivatives Derivatives were synthesized in good yields (scheme-I). All these compounds were tested for Anti-microbial activity showed considerable activity when compared to the standard drug.

In the present communication pyrido[2,3-d]pyrimidine-2,4(1H,3H)-dione (2) was synthesised from 2-amino nicotinic acid According to the reported procedure.[27] 2 , 4 di Chloro pyrido[2,3-d]Pyrimidine was synthesised from compound (2) reflux in $POCl_3$ According to the reported procedure.[28] 2 , 4 di Chloro pyrido[2,3-d]pyrimidine (3) was reacted with

ammonia in water at Refiux Temparature to form Compound 2-chloropyrido[2,3-d]pyrimidin-4-amine (4) According to the reported procedure[29], which were further reacted with 4-Substituted phenacyl bromides (5 a-f) to get 5-chloro-2- Substituted imidazo[1,2-c] pyrido[3,2-e] Pyrimidines (6a-6f) According to the reported procedure[30], which were further reacted with 4-Substituted Phenols (7 a-b) to get Target Compounds (8 a-l) According to the reported procedure.[31]

Structures of Compounds 8a-8l were confirmed by IR, ^{1}H & ^{13}C NMR, mass Spectroscopic Techniques. All of the imidazo[1,2-c] pyrido[3,2-e] Pyrimidines possesses Similar basic skeletal structure.

Characterization: The FT-IR spectra of 8a–8l were recorded using KBr pellets in the range of 4,000–400 cm-^{1}.The IR spectrum of the title Compounds 8(a-l) has given stretching vibration 3420 cm^{-1} due to the stretching vibration corresponding to N-H Stretching vibrations. 3100cm^{-1}, due to the stretching vibration corresponding to Ar-H Stretching vibrations. The absorption peak at 2930 cm^{-1} is due to The stretching vibration corresponding to the SP^{3} C-H (methyl gp).The strong Intensity absorption at 1150 cm^{-1} is due to The stretching vibration of -C-O-C Stretching, 1360 cm^{-1} is due to The stretching vibration of C-F bond. 760 cm^{-1} is due to The stretching vibration of C-Cl bond. 560 cm^{-1} is due to The stretching vibration of C-Br bond. The weak Intensity absorption at 1620 cm^{-1} corresponds to a C=N Stretching vibration.

It has been observed from chemical structure of compounds 8(a-l) that different pair of protons. The protons of Methyl group which is attached to benzene ring appeared as a singlet at δ =2.3 ppm, The protons of Methoxy group appeared as a Singlet at δ =3.85 ppm,. The protons attached pyridine ring appeared between δ =7.4-8.5 ppm respectively.

The chemical shifts of the final compound carbon vary from δ = 165 to 23 ppm. The carbon nucleus under the influence of a strong electronegative environment appeared down field, The carbon chemical shift of the methyl group at δ= 23 ppm. The carbon chemical shift of the Methoxy group at δ= 58 ppm.

Readily available starting materials and Simple Synthesizing procedures make this method very attractive and convenient for the synthesis of Fused Pyrimidine triazole derivatives. Formation of products was confirmed by recording their ^{1}H NMR, ^{13}C, FT-IR, mass spectra.

The Elemental analysis data showed good agreement between the experimentally determined values and the theoretically calculated values with in ±0.4%.

Anti -microbial screening: The results of Anti -microbial studies of newly synthesized compounds reveal that the compounds possess significant Anti -microbial activities. The results of these studies are given in **Table 3**. From Anti -Microbial screening results, it has been observed that compounds 8l, 8k possess good activity.

CONCLUSION

In conclusion, a series of novel imidazo [1,2-c] pyrido[3,2-e] Pyrimidine derivatives 8 (a-l) were synthesised in good yield, characterised by different spectral studies and their anti-microbial activity have been evaluated. Among the synthesised compounds 8l, 8k, and 8h showed more anti-microbial activity when compared to other compounds in the series.

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