NITROGEN RICH IMIDAZOLE-TRIAZOLOPYRIMIDINE HYBRIDS: CATALYST FREE SYNTHESIS, ANTIMICROBIAL AND ANTITUBERCULAR STUDIES

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Herein we have reported a simple, rapid and efficient synthesis of 7-(substituted phenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine derivatives. In the present study, PEG-400 was used as an alternative and green reaction solvent in the first step and n-butanol in the second step. The protocol proposed in this work offers advantages, such as common starting materials, simple work-up, shorter reaction time, high yield and absence of the catalyst. The structural elucidation of these compounds is based on mass, IR, ^{1}H NMR and ^{13}C NMR spectral data. All the synthesized compounds have been evaluated for their *in vitro* antimicrobial and antitubercular activity.

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INTRODUCTION

The synthesis of high nitrogen-containing heterocyclic systems has been attracting increasing interest over the past decade because of their utility in various applications. In recent years, the chemistry of triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance. For the preparation of complex molecules, great efforts have been directed towards the synthetic manipulation of triazolopyrimidine derivatives.

Recently, triazolopyrimidine derivatives were synthesized by using different catalysts such as triethylamine(Et_3N),[1] thiamine hydrochloride,[2] sodium methoxide,[3-4] piperidine,[5] sodium acetate[6] in different organic solvents at elevated temperature. Some researchers have been carried out a rapid, efficient, clean and environmentally benign exclusive synthesis of 1,2,4-triazolo[4,3-*a*]pyrimidines in excellent yields using microwaves or ultrasonic waves.[7-9]

The 1,2,4-triazolopyrimidines have attracted growing interest due to their important pharmacological activities, such as antitumor potency,[10-13] antimalarial,[14] antimicrobial,[15-19] anti-inflammatory,[20] inhibition of kinase insert domain-containing receptor (KDR kinase),[21] antifungal,[22] antitubercular,[23] antibacterial agents,[24] and calcium channel modulators.[25] Besides triazolo[4,3-*a*] pyrimidine derivatives were reported to be useful as cardiovascular agents,[26] aromatase inhibition activities[27] and anticancer agents.[28]

Considerable interest has been focused on the triazole structure and chalcones, which has been known to possess a broad spectrum of biological activities. So, we have combined both active moieties in one structure to result in the fused heteroaromatic systems i.e., triazolo[4,3-*a*] pyrimidine derivatives. Our main aim is to achieve the product without catalyst and evaluation of antimicrobial and antitubercular activities of synthesized compounds.

EXPERIMENTAL

All chemicals were purchased and used without any further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel-G plates of 0.5 mm thickness, visualizing with ultraviolet light. Melting points were recorded in open capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu FT-IR-8400 instrument using DRS (diffusive reflectance system) method and are expressed in cm^{-1} (KBr). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II spectrometer at 400 and 101.1 MHz, respectively, using DMSO-d_6 as a solvent; the chemical shifts are referenced to tetramethylsilane(TMS). Mass spectra were recorded on the Shimadzu GC-MS-QP-2010 model using a direct inlet probe technique.

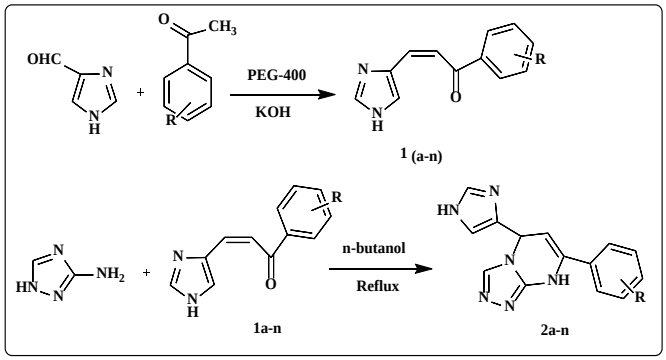
General procedure for the synthesis of 1-(Substituted phenyl)-3-(1*H*-imidazole-4-yl)prop-2-en-1-ones (1a-n)

An equimolar mixture of 4-formyl imidazole (1 mmol), substituted acetophenones (1 mmol) and KOH (2 mmol) was stirred in PEG-400 (12 mL) at 50 ºC for 2 hrs. On completion of the reaction monitored by TLC, the crude mixture was worked up in ice-cold water (100 mL). The product which separated was filtered. The filtrate was evaporated to remove water leaving PEG-400 behind. The same PEG-400 was utilized to synthesize further chalcones.

Purification of the product was carried out using diethyl ether to afford analytically pure products.

General procedure for the synthesis of 7-(substituted phenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro[1,2,4]triazo-lo[4,3-*a*]pyrimidines (2a-n)

To a stirred solution of substituted chalcone (10 mmol) in n-butanol, 1*H*-1,2,4-triazol-3-amine (10 mmol) was added. The mixture was stirred to reflux for 8 hrs. On completion of the reaction monitored by TLC, the separated solid was filtered and washed with diethyl ether to obtain the solid crude product. Crystallization of the synthesized products was carried out in an appropriate solvent.



Scheme 1. Synthetic route for the preparation of title compounds **(2a-n)** R= 4-Cl (a), 4-Br (b), 4-Me (c), 4-OH (d), 4-MeO (e), 3-MeO (f), 2-MeO (g), 2-OH (h), 4-F (i), 3-Cl (j), 3-NO_2 (k), -H (l), 2,4-$(MeO)_2$ (m), 4-NO_2 (n)

The optimization in reaction conditions, in terms of time, solvent, catalyst and yields, are reported in **Table 1**.

Table 1. Conditions for the synthesis of triazolo[4,3-*a*]pyrimidines under reflux in each solvent

Catalyst	Solvent	Time, h	Yield, %
HCl	Ethanol 95%	10	56
Piperidine	Ethanol 95%	9	60
-	-	01	45
-	Methanol	10	51
-	Ethanol 95%	10	65
-	n-Propanol	11	58
-	n-Butanol	8	85
-	DMF	12	54
-	Iso-propanol	11	55
-	1,4-Dioxane	11	65

We carried out the synthesis of 1,2,4-triazolo[4,3-*a*] pyrimidines with acidic catalyst, i.e., HCl, and basic catalyst, i.e., piperidine at reflux temperature. But there was not much satisfaction with the yield and purity of obtained products. Again the same reaction was carried out without any solvent or catalyst in fused condition. We obtained a very much messy reaction due to the fused condition. So from the all above reaction condition, we came to conclude that solvent is necessary for the reaction and either we have to change the catalyst or carried out the reaction with other suitable solvents which reflux at a higher temperature. So from the above conclusion, we have chosen alcoholic solvents such as methanol, n-propanol, isopropanol, n-butanol and polar solvent like dimethyl formamide (DMF) and 1,4-dioxane. After doing various optimizations in the experimental conditions, it was concluded that to our best knowledge, when the reaction was carried out in n-butanol solvent showed better yield in less reaction time (Entry 7).

7-(4-Chlorophenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine (2a)

Yield: 84 %, m.p. 115-117 ^oC, IR (cm^{-1}): 3077 (C-H stretching in aromatic), 1685 (C=N of the pyrimidine system), 1560 (aromatic ring skeleton), 1288 (C-N stretching in aromatic ring), 1140 (C-H bending aromatic), 815 (*p*-disubstituted aromatic ring), 742 (C-Cl stretching), ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (s, 1H, -NH of imidazole), 9.84 (s, 1H, -NH of pyrimidine), 7.75 (s, 1H, ArH), 7.67-7.58 (d, 2H, ArH, *J* = 8.35 Hz), 7.55-7.57 (d, 2H, ArH, *J* = 3.46 Hz), 7.51 (s, 1H, ArH), 7.07 (s, 1H, ArH), 6.16-6.13 (d, 1H, ArH, *J* = 2.35 Hz), 5.20-5.18 (d, 1H, chiral, *J* = 3.46 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm:150.15, 141.67, 136.24, 134.45, 132.53, 130.41, 128.24, 122.36, 114.21, 98.57, 55.10, MS *m/z*: 298 (M^+).

7-(4-Bromophenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine (2b)

Yield: 85 %, m.p. 240-242 ^oC, IR (cm^{-1}): 3088 (C-H stretching in aromatic), 1653 (C=N of the pyrimidine system), 1556 (aromatic ring skeleton), 1344 (C-N stretching in aromatic ring), 1139 (C-H bending aromatic), 817 (*p*-disubstituted aromatic ring), 636 (C-Br stretching); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.02 (s, 1H, -NH of imidazole), 9.87 (s, 1H, -NH of pyrimidine), 7.74 (s, 1H, ArH), 7.63-7.60 (d, 2H, ArH, *J* = 8.40 Hz), 7.57-7.56 (d, 2H, ArH, *J* = 3.60 Hz), 7.55 (s, 1H, ArH), 7.08 (s, 1H, ArH), 6.15-6.14 (d, 1H, ArH, *J* = 2.80 Hz), 5.19-5.18 (d, 1H, chiral, *J* = 3.20 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm:149.14, 140.79, 135.33, 133.62, 131.76, 129.69, 127.84, 121.82, 113.58, 97.52, 54.08, MS *m/z*: 343 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(*p*-tolyl)-5,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine (2c)

Yield: 81 %, m.p. 113-114 ^oC, IR (cm^{-1}): 2978 (C-H stretching in aromatic), 1658 (C=N of the pyrimidine system), 1585 (aromatic ring skeleton), 1238 (C-N stretching in aromatic ring), 1138 (C-H bending aromatic), 871 (*p*-disubstituted aromatic ring); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (s, 1H, -NH of imidazole), 9.78 (s, 1H, -NH of pyrimidine), 7.92 (s, 1H, ArH), 7.50-7.48 (d, 2H, ArH, *J* = 7.32 Hz), 7.41 (s, 1H, ArH), 7.35-7.33 (d, 2H, ArH, *J* = 7.56 Hz), 7.06 (s, 1H, ArH), 6.14 (s, 1H, ArH), 5.10 (s, 1H, chiral), 2.32 (s, 3H, -CH_3), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 170.95, 150.66, 149.28, 141.08,138.33, 135.27, 131.58, 129.32, 127.74, 125.54, 113.45, 96.09, 54.11, 21.04, MS *m/z*: 278 (M^+).

4-(5-(1*H*-Imidazol-4-yl)-5,8-[1,2,4]triazolo[4,3-*a*]pyrimidin-7-yl)phenol (2d)

Yield: 65 %, m.p. 201-203 ^oC, IR (cm^{-1}): 3521 (-OH stretching), 3022 (C-H stretching in aromatic), 1644 (C=N

of the pyrimidine system),1555 (aromatic ring skeleton), 1325 (-OH bending), 1257 (C-N stretching in aromatic ring), 1141 (C-H bending aromatic), 854 (*p*-disubstituted aromatic ring), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.05 (s, 1H, -NH of imidazole), 9.85 (s, 1H, -NH of pyrimidine), 7.75 (s, 1H, ArH), 7.62-7.59 (d, 2H, ArH, *J* = 8.46 Hz), 7.55-7.53 (d, 2H, ArH, *J* = 3.40 Hz), 7.52 (s, 1H, ArH), 7.10 (s, 1H, ArH), 6.16-6.15 (d, 1H, ArH, *J* = 2.76 Hz), 5.31 (s, 1H, -OH), 5.21-5.19 (d, 1H, chiral, *J* = 3.26 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 155.23, 150.25, 142.31, 140.59, 136.21, 132.27, 130.42, 128.64, 127.21, 120.47, 112.24, 96.87, 53.21, MS *m/z*: 280 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(4-methoxyphenyl)-5,8-dihydro[1,2,4]-triazolo[4,3-*a*]pyrimidine (2e)

Yield: 85 %, m.p. 185-187 °C, IR (cm^{-1}): 3055 (C-H stretching in aromatic), 1633 (C=N of the pyrimidine system), 1544 (aromatic ring skeleton), 1244 (C-N stretching in aromatic ring), 1158 (C-H bending aromatic), 1055 (C-O stretching), 850 (*p*-disubstituted aromatic ring), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.05 (s, 1H, -NH of imidazole), 9.89 (s, 1H, -NH of pyrimidine),7.75 (s, 1H, ArH) 7.64-7.61 (d, 2H, ArH, *J* = 8.36 Hz), 7.58-7.57 (d, 2H, ArH, *J* = 3.62 Hz),7.54 (s, 1H, ArH), 7.10 (s, 1H, ArH), 6.17-6.15 (d, 1H, ArH, *J* = 2.76 Hz), 5.18-5.17 (d, 1H, chiral, *J* = 3.18 Hz), 3.81 (s, 3H, -OCH_3), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 159.47, 150.21, 141.12, 135.31, 131.54, 128.41, 124.36, 120.28, 111.63, 112.42, 93.74, 54.21, 56.47, MS *m/z*: 294 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(3-methoxyphenyl)-5,8-dihydro[1,2,4]-triazolo[4,3-*a*]pyrimidine (2f)

Yield: 78 %, m.p. 197-199 °C, IR (cm^{-1}): 3056 (C-H stretching in aromatic), 1656 (C=N of the pyrimidine system), 1578 (aromatic ring skeleton), 1256 (C-N stretching in aromatic ring), 1147 (C-H bending aromatic), 1012 (C-O stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.07 (s, 1H, -NH of imidazole), 9.87 (s, 1H, -NH of pyrimidine),7.71 (s, 1H, ArH), 7.62-7.60 (dd, 1H, ArH, *J* = 8.36 Hz, 8.36 Hz), 7.57 (s, 1H, ArH), 7.56-7.55 (d, 1H, ArH, *J* = 3.26 Hz), 7.54-7.53 (d, 1H, ArH, *J* = 3.46 Hz), 7.52 (s, 1H, ArH), 7.12 (s, 1H, ArH), 6.14-6.13 (d, 1H, ArH, *J* = 2.86 Hz), 5.19-5.18 (d, 1H, chiral, *J* = 3.26 Hz), 3.80 (s, 3H, -OCH_3), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 160.52, 149.25, 142.10, 136.33, 132.45, 129.70, 123.60, 121.27, 112.58, 110.21, 93.25, 54.15, 56.54, MS *m/z*: 294 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(2-methoxyphenyl)-5,8-dihydro[1,2,4]-triazolo[4,3-*a*]pyrimidine (2g)

Yield: 80 %, m.p. 212-215 °C, IR (cm^{-1}): 3085 (C-H stretching in aromatic), 1614 (C=N of the pyrimidine system), 1566 (aromatic ring skeleton), 1253 (C-N stretching in aromatic ring), 1151 (C-H bending aromatic), 1036 (C-O stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.09 (s, 1H, -NH of imidazole), 9.86 (s, 1H, -NH of pyrimidine),7.72 (s, 1H, ArH), 7.63-7.52 (m, 4H, ArH), 7.51 (s, 1H, ArH), 7.10 (s, 1H, ArH), 6.13-6.11 (d, 1H, ArH, *J* = 2.36 Hz), 5.17-5.16 (d, 1H, chiral, *J* = 3.36 Hz), 3.81 (s, 3H, -OCH_3), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 161.03, 148.78, 142.24, 137.12, 132.43, 128.87, 122.67, 121.54, 111.07, 110.58, 93.21, 53.85, 56.41, MS *m/z*: 294 (M^+).

2-(5-(1*H*-Imidazol-4-yl)-5,8-[1,2,4]triazolo[4,3-*a*]pyrimidin -7-yl)phenol (2h)

Yield: 72 %, m.p. 217-219 °C, IR (cm^{-1}): 3551 (-OH stretching), 3016 (C-H stretching in aromatic), 1674 (C=N of the pyrimidine system), 1562 (Aromatic ring skeleton), 1337 (-OH bending), 1263 (C-N stretching in aromatic ring), 1136 (C-H bending aromatic), ^{1}H NMR (400 MHz, DMSO-d_6) δppm: 12.10 (s, 1H, -NH of imidazole), 9.78 (s, 1H, -NH of pyrimidine), 7.74 (s, 1H, ArH), 7.65-7.50 (m, 4H, ArH), 7.53 (s, 1H, ArH), 7.12 (s, 1H, ArH), 6.15-6.13 (d, 1H, ArH, *J* = 3.26 Hz), 5.33 (s, 1H, -OH), 5.20-5.18 (d, 1H, Chiral, *J* = 3.36 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δppm: 156.41, 151.05, 141.76, 139.86, 135.22, 133.25, 130.36, 127.48, 126.84, 120.21, 113.12, 97.08, 53.56, MS *m/z*: 280 (M^+).

7-(4-Fluorophenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine (2i)

Yield: 83 %, m.p.: 178-180 °C, IR (cm^{-1}): 3068 (C-H stretching in aromatic), 1663 (C=N of the pyrimidine system), 1556 (aromatic ring skeleton), 1278 (C-N stretching in aromatic ring), 1252 (C-F stretching), 1156 (C-H bending aromatic), 820 (*p*-disubstituted aromatic ring), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.01 (s, 1H, -NH of imidazole), 9.80 (s, 1H, -NH of pyrimidine), 7.75 (s, 1H, ArH), 7.62-7.59 (d, 2H, ArH, *J* = 8.36 Hz), 7.56-7.55 (d, 2H, ArH, *J* = 3.40 Hz), 7.54 (s, 1H, ArH), 7.18 (s, 1H, ArH), 6.10-6.09 (d, 1H, ArH, *J* = 2.86 Hz), 5.20-5.19 (d, 1H, Chiral, *J* = 3.36 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δppm:161.21, 149.23, 140.21, 136.23, 133.45, 131.41, 130.24, 127.28, 120.14, 112.78, 95.41, 54.07, MS *m/z*: 282 (M^+).

7-(3-Chlorophenyl)-5-(1*H*-imidazol-4-yl)-5,8-dihydro[1,2,4]tri-azolo[4,3-*a*]pyrimidine (2j)

Yield: 78 %, m.p. 189-192 °C, IR (cm^{-1}): 3065 (C-H stretching in aromatic), 1663 (C=N of the pyrimidine system), 1563 (aromatic ring skeleton), 1265 (C-N stretching in aromatic ring), 1136 (C-H bending aromatic), 735 (C-Cl stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.03 (s, 1H, -NH of imidazole), 9.88 (s, 1H, -NH of pyrimidine), 7.76 (s, 1H, ArH), 7.63-7.61 (dd, 1H, ArH, *J* = 3.86 Hz, 3.46 Hz), 7.58 (s, 1H, ArH), 7.56-7.54 (d, 1H, ArH, *J* = 3.86 Hz), 7.53-7.51 (d, 1H, ArH, *J* = 3.26 Hz), 7.50 (s, 1H, ArH), 7.05 (s, 1H, ArH), 6.15-6.13 (d, 1H, ArH, *J* = 3.26 Hz), 5.21-5.19 (d, 1H, chiral, *J* = 3.50 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm:151.65, 140.47, 135.14, 133.68, 131.41, 130.78, 129.11, 121.87, 115.42, 97.47, 56.23, MS *m/z*: 298 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(3-nitrophenyl)-5,8-dihydro[1,2,4]tria-zolo[4,3-*a*]pyrimidine (2k)

Yield: 66 %, m.p. 241-243 °C, IR (cm^{-1}): 3076 (C-H stretching in aromatic), 1654 (C=N of the pyrimidine system), 1552 (aromatic ring skeleton), 1344 (aromatic C-NO_2), 1207 (C-N stretching in aromatic ring), 1139 (C-H bending aromatic), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm:

12.05 (s, 1H, -NH of imidazole), 9.89 (s, 1H, -NH of pyrimidine), 7.73 (s, 1H, ArH), 7.65-7.63 (dd, 1H, ArH, *J* = 3.86 Hz, 3.86 Hz), 7.60 (s, 1H, ArH), 7.59-7.57 (d, 1H, ArH, *J* = 3.46 Hz), 7.56-7.54 (d, 1H, ArH, *J* = 3.28 Hz), 7.53 (s, 1H, ArH), 7.09 (s, 1H, ArH), 6.13-6.11 (d, 1H, ArH, *J* = 2.86 Hz), 5.18-5.16 (d, 1H, chiral, *J* = 3.36 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 161.21, 151.22, 140.87, 137.47, 135.22, 131.54, 130.47, 129.04, 122.21, 114.14, 97.47, 53.01, MS *m/z*: 309 (M^+).

5-(1*H*-Imidazol-4-yl)-7-phenyl-5,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine (2l)

Yield: 77 %, m.p. 169-172 ºC, IR (cm^{-1}): 3060 (C-H stretching in aromatic), 1662 (C=N of the pyrimidine system), 1587 (aromatic ring skeleton), 1238 (C-N stretching in aromatic ring), 1161 (C-H bending aromatic), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.08 (s, 1H, -NH of imidazole), 9.72 (s, 1H, -NH of pyrimidine), 7.76 (s, 1H, ArH), 7.61-7.56 (m, 5H, ArH), 7.54 (s, 1H, ArH), 7.19 (s, 1H, ArH), 6.12-6.10 (d, 1H, ArH, *J* = 3.86 Hz), 5.22-5.20 (d, 1H, chiral, *J* = 2.86 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm:158.23, 150.54, 141.74, 137.21, 134.47, 132.31, 131.87, 128.24, 121.54, 112.45, 96.12, 53.45, MS *m/z*: 264 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(2,4-dimethoxyphenyl)-5,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine (2m)

Yield: 76 %, m.p. 172173 ºC, IR (cm^{-1}): 3081 (C-H stretching in aromatic), 1687 (C=N of the pyrimidine system) 1536 (aromatic ring skeleton), 1270 (C-N stretching in aromatic ring), 1156 (C-H bending aromatic), 1025 (C-O stretching), ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.10 (s, 1H, -NH of imidazole), 9.86 (s, 1H, -NH of pyrimidine), 7.76 (s, 1H, ArH) 7.63-7.61 (d, 1H, ArH, *J*=3.26 Hz), 7.59-7.57 (d, 1H, ArH, *J* = 3.46 Hz), 7.55 (s, 1H, ArH), 7.52 (s, 1H, ArH), 7.11 (s, 1H, ArH), 6.14-6.12 (d, 1H, ArH, *J* = 3.76 Hz), 5.16-5.15 (d, 1H, chiral, *J* = 3.26 Hz), 3.82 (s, 6H, -OCH_3), ^{13}C NMR (101 MHz, DMSO-d_6) δppm: 160.22, 150.56, 140.97, 136.33, 132.46, 129.54, 125.23, 121.24, 111.21, 112.14, 92.78, 53.89, 56.51, MS *m/z*: 324 (M^+).

5-(1*H*-Imidazol-4-yl)-7-(4-nitrophenyl)-5,8-dihydro[1,2,4]triazolo[4,3-*a*]pyrimidine (2n)

Yield: 67 %, m.p. 158-160 ºC, IR (cm^{-1}): 3066 (C-H stretching in aromatic), 1636 (C=N of the pyrimidine system), 1555 (aromatic ring skeleton), 1325 (aromatic C-NO_2), 1209 (C-N stretching in aromatic ring), 1152 (C-H bending aromatic), 821 (*p*-disubstituted aromatic ring), ^{1}H NMR (400 MHz, DMSO-d_6) δppm: 12.02 (s, 1H, -NH of imidazole), 9.87 (s, 1H, -NH of pyrimidine), 7.74 (s, 1H, ArH), 7.63-7.60 (d, 2H, ArH, *J* = 8.40 Hz), 7.57-7.56 (d, 2H, ArH, *J* = 3.60 Hz), 7.55 (s, 1H, ArH), 7.08 (s, 1H, ArH), 6.15-6.14 (d, 1H, ArH, *J* = 2.80 Hz), 5.19-5.18 (d, 1H, chiral, *J* = 3.20 Hz), ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 162.32, 150.21, 141.41, 136.21, 134.23, 132.64, 130.21, 128.41, 122.81, 114.31, 97.53, 53.08, MS *m/z*: 309 (M^+).

Antibacterial Activity

All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method with two gram-positive bacteria, two gram-negative bacteria and two fungal strains. The MIC values of the test compounds are recorded in the following **Table 2.** The biological activities of the synthesized compounds have been compared with standard drugs.

Table 2. Antimicrobial data of synthesized compounds **2a-n**

Sr. No.	Minimum inhibitory concentration µg mL^{-1}						
	Gram +ve bacteria		Gram -ve bacteria		Antifungal activity		
	A	B	C	D	E	F	G
2a	250	500	500	250	500	1000	1000
2b	500	250	200	500	1000	500	500
2c	250	200	250	500	100	200	200
2d	250	500	250	500	500	500	1000
2e	200	62.5	100	125	1000	500	500
2f	500	100	250	200	1000	1000	500
2g	500	250	200	500	1000	1000	1000
2h	250	500	500	250	1000	500	500
2i	500	250	250	250	500	500	500
2j	500	500	250	250	500	1000	1000
2k	250	250	500	250	500	1000	500
2l	250	250	500	500	1000	500	1000
2m	100	100	62.5	100	1000	1000	1000
2n	500	500	500	250	1000	250	500
Am.	250	100	100	100	-	-	-
Cl.	50	50	50	50	-	-	-
Cp.	50	50	25	25	-	-	-
Ny.	-	-	-	-	100	100	100
Gr.	-	-	-	-	500	100	100

A: *S.aureus*, **B:** *S.Pyogenus*, **C:** *E. Coli*, **D:** *P. aeruginosa*, **E:** *C.Albicans*, **F:** *A. Niger*, **G:** *A.clavatus*, **Am.**: Ampicillin, **Cl.**: Chloramphenicol, **Cp.**: Ciprofloxacin, **Ny.**: Nystatin, **Gr.** : Griseofulvin

Table 3. The antitubercular activity of compounds **2a-n**

Sr. No.	% average inhibition dormant stage μg mL^{-1} concentration				% average inhibition active stage μg mL^{-1} concentration			
	100	**50**	**25**	**12.5**	**100**	**50**	**25**	**12.5**
2a	90.25	89.62	80.25	25.33	92.32	88.23	65.23	55.23
2b	80.23	75.25	60.23	12.05	90.25	80.14	63.24	52.03
2c	50.21	44.85	36.52	30.51	42.10	10.25	8.25	5.23
2d	22.21	15.23	NA	NA	10.52	20.55	NA	NA
2e	30.23	16.54	12.36	5.62	10.25	9.52	5.24	2.30
2f	23.21	13.65	9.66	4.12	10.23	5.58	NA	NA
2g	36.22	10.54	NA	NA	14.85	5.28	NA	NA
2h	10.25	8.56	NA	NA	10.84	4.25	NA	NA
2i	89.23	80.78	75.26	54.37	82.54	80.54	41.21	36.37
2j	90.34	82.54	80.23	60.27	91.58	85.47	70.23	44.78
2k	23.21	17.34	10.77	5.56	NA	NA	NA	NA
2l	36.35	25.54	17.58	11.87	NA	NA	NA	NA
2m	33.76	29.54	14.21	10.98	NA	NA	NA	NA
2n	20.55	15.66	10.22	6.33	NA	NA	NA	NA

NA= Not active

Antitubercular Activity

All the synthesized compounds were evaluated for antitubercular activity against *M. tuberculosis H$_{37}$Rv* in dormant and active stage at μg mL^{-1} concentration using XTT Reduction Menadione (XRMA) method. The results of the antitubercular activity are shown in **Table 3**.

RESULTS AND DISCUSSION

In the currently developed method, 1,2,4-triazolo[4,3-*a*]pyrimidines were synthesized in n-butanol solvent. The advantages of this currently developed method over other methods are reduced milder conditions, higher yields, low cost and easy work-up. We have confirmed the structure based on spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

Molecular ion peak was observed in agreement with the molecular weight of the respective compounds. The IR spectrum of synthesized compounds exhibited a characteristic absorption band at 3076 cm^{-1} for the aromatic C=C stretching band. Characteristic absorption band observed at 1280 cm^{-1} for C-N stretching in imidazole ring and at 1658.78 for C=N of the pyrimidine ring system. ^{1}H NMR spectra of the compounds showed characteristic singlet signals at 12.03 δppm due to the proton of imidazole -NH and at 9.97 δppm due to the proton of fused pyrimidine -NH. Chiral carbon proton was observed doublet at 5.19-5.18 δppm with 3.20 Hz coupling constant value. The aromatic ring protons and *J* value were found to be per the substitution pattern on the phenyl ring. In ^{13}C NMR spectroscopy, compound **2b** showing a signal at 54.11 δppm for the chiral carbon while at 96.09 δppm for the pyrimidine ring C=C carbon.

Antibacterial activity

The examination of antimicrobial activity data revealed that some compounds had shown good antibacterial activity when compared with ampicillin and chloramphenicol. Against Gram-positive bacterium, *S. aureus*, compound **2m** (100 μg mL^{-1}) has shown better activity upon comparison with standard drug ampicillin while compound **2e** (62.5 μg mL^{-1}) has shown excellent activity as compare to ampicillin against *S. pyogenus.*

Antifungal activity

The synthesized compounds were not much active towards the fungal strains that we had considered for studies in comparison to the standard drugs. However, compound **2c** (100 μg mL^{-1}) has shown activity equal to the standard drug, nystatin and excellent to the standard drug, griseofulvin, against *C.albicans.* Compounds **2a, 2d, 2i, 2j** and **2k** (500 μg mL^{-1}) have shown comparative activity against *C. albicans* to standard drug griseofulvin. From the results of antimicrobial activity, we concluded that compound **2m** (with 2,4-dimethoxy substituted aromatic ring) had shown excellent activity against Gram-positive as well as Gram-negative bacterial strains.

Antitubercular activity

The results of antitubercular activity data reveal that all the synthesized compounds have shown moderate to excellent activity against a dormant and active stage of *Mycobacterium tuberculosis H$_{37}$Rv* at μg mL^{-1} concentration level. **2a, 2b, 2i,** and **2j** are showing a good percentage of average inhibition in the dormant and active stage of tuberculosis. Compound **2a**, is most active and showing 90.25 and 92.32 % inhibition at 100 μg mL^{-1} concentration at the dormant and active stage, respectively. Compound **2b** showing 80.14 % inhibition at 50 μg mL^{-1} concentration at the active stage. Compound **2j** is active and showing 80.23 and 70.23 % inhibition at 25 μg mL^{-1} concentrations at the dormant and active stage, respectively.

Thus, compounds with aromatic ring substituted with 4-chloro, 4-bromo and 4-fluoro have shown good activity against the dormant and active stage of *Mycobacterium tuberculosis H$_{37}$Rv* at μg mL^{-1} concentration level.

CONCLUSION

We have developed facile, coherent and catalyst-free reactions for the synthesis of 1,2,4-triazolo[4,3-*a*] pyrimidines using n-butanol as a solvent. We have confirmed the structure based on the spectroscopic technique. The protons and carbons belonging to the triazolopyrimidine ring, imidazole ring and phenyl substituents were observed at expected chemical shift and integral values. Considering the results obtained from antibacterial and antifungal tests together, we conclude that [1,2,4]triazolo[4,3-*a*]pyrimidines are good antibacterial agents rather than antifungal agents. From the results of antitubercular activity, we concluded, the synthesized compounds have shown better activity against the dormant stage as compared to the active stage of *Mycobacterium tuberculosis H37Rv* at µg/ml concentration level.

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