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Green synthesis and antimicrobial evaluation ofpyrido[1,2-a]pyrimidine3-carbonitrile derivatives

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ABSTRACT

A simple, green one pot three component approach for the synthesis of pyrido[1,2-a]pyrimidine-3-carbonitrile derivatives directly from corresponding 2-aminopyridines, substituted aldehydes, and malononitrile by using Bleaching earth clay (pH 12.5) and PEG-400 a recyclable catalytic system have been developed. All the synthesized compounds were characterized by spectral analysis. Furthermore, synthesized compounds were screened for their antimicrobial activity. Most of the synthesized compounds showantimicrobial activity.

Keywords: Green synthesis,Bleaching earth clay pH (12.5), PEG-400, pyrido[1, 2-a]pyrimidine-3-carbonitrile, antimicrobial activity.

INTRODUCTION

There is an urgent requirement for a new class of antimicrobial agents having new and diverse structures from those of existing agents due to the development from conflict and unfavourable reactions toward existing antimicrobial drugs.Pyrido [1, 2-a] pyrimidine is an important privileged structure for the development of antimicrobial agents, as compounds containing this scaffold exhibits diversifiedbiological and pharmaceutical activities[1-2], includingantifungal[3-5],antibacterial[6-9], antiallergic[10],antiherpes[11], anticancer [12], anti-inflammatory[13] and xanthine oxidase inhibitors[14].There are a number of reports which show that natural and synthetic pyrido [1, 2-a] pyrimidine derivatives possess antimicrobial activity [15-17].

Over the years, numerous synthetic methods for the preparation of pyrido [1,2-a] pyrimidine-3-carbonitrile derivatives have been reported in the literature, including the one pot synthesis catalyzed by a number ofcatalyst, i.e.trifluoro methane sulfonic acid catalysis [18], polyphosphoric acid [19-20], phosphorus Oxy chloride [21], phenyl propellers ester [22] and piperidine [23]. However, some of the reported methods have several limitations such as elevated temperature, more time consuming, corrosive, costly ligands, non-green catalytic systems, partial conversion of starting materials, harmful reaction condition, dreary workup procedures with insufficient yields, and purification issues due to the formation of by-products.

Therefore, one step ahead growth ofheterogeneous catalysisrenovationsis a chief alarm from together economic and environmental points of view.Ease of handling,recyclability, isolation of catalyst, low corrosion, shorter reaction time, waste control, simple transport and catalyst dumping[24] are some of the leading applications of heterogeneous catalysis in organicsynthesis.Probably based on its distinctfeaturesnaturally occurring clay has unique physical and chemical properties such as shape selectivity, acidic/basic nature and thermal stability.

Currently, Bleaching earth clay(pH 12.5) has come out as a naturally occurring heterogeneous, easily separable, ecofriendly, cheap andsafe catalyst employed for various base-catalyzed organic transformationssuch as, synthesis

of pyrano-pyrazole derivatives[25], synthesis of 3-[2-(substituted-phenyl)-2-Oxo ethylidene]-1, 3-dihydro-indol-2-one[26],synthesis of α, β-unsaturated ketones[27], synthesis of 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives[28]. The small (5-micron) particle size of the clay renders a huge surface area compared with other solid-supported catalysts. Moreover, Bleaching earth clay (pH 12.5) is readily available in India and the USA at very low cost (0.10 USD per kg) compared with others, such as supportedZirconia, 12-tungstosilicicacid, etc. Bleaching earth clay (pH 12.5) is also used in refining of vegetable oil, fats and greases [29-30].

In this report the synthesis, characterization, and antimicrobial activities, of pyrido [1,2-a] pyrimidine-3-carbonitrile derivatives via one pot three component coupling of 2-aminopyridines, substituted aldehydes, and malononitrilecatalyzed by Bleaching earth clay (pH 12.5) in PEG-400 as a green reaction medium is described.

MATERIALS AND METHODES

Melting points were determined in an open capillary tube and are uncorrected. The chemical solvents used were purified prior to practice. Bleaching earth clay (pH 12.5) was a gift from Supreme Silicones, Pune. Completion of the reaction was monitored by thin layer chromatography on a precoated sheet of silica gel-G using iodine vaporsfor detection. IR spectra were recorded in the Perkin Elmer spectrometer ^{1}H-NMR spectra were recorded in dimethyl sulphoxide (DMSO)-d_6 using an Avance spectrometer at a frequency of 400 MHz using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an EI-Shimadzu QP2010PLUS GC–MS.

General procedure for the preparation of substituted pyrido[1,2-a]pyrimidine -3-carbonitrile*derivatives*
A mixture of 2-aminopyridine (1 mmol), substituted aldehydes (1 mmol), malononitrile (1 mmol) and Bleaching earth clay (pH 12.5) (10 weight %) were stirred in PEG-400 (15 ml) at 70-80°C (as indicated in **Table-3**). After completion of the reaction (monitored by TLC), the solid catalyst in the resulting reaction mixture was recovered by filtration. The reaction mix was poured into ice-cold water, and the solid separates out; the separated solid was filtered and purified by crystallization **(4a-j).** The experimental data, reaction time, yield and melting points of compounds are presented in **Table 3**.

Spectra of selected compounds
4-Amino-2-(4-chlorophenyl)-2H-pyrido[1, 2-a]pyrimidine-3-carbonitrile **(4a);**
Yellow solid, ($CHCl_3$), mp 165-167°C; IR (KBr, cm^{-1})υ_{max}; 3385 (NH_2), 2222 (C≡N), 1590 (C=N), 1542 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.00 (s, 2H, NH_2), 7.86-7.66 (m, 8H,Ar-H), 4.00 (s, 1H, methine); ^{13}C NMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 165.6, 161.0, 135.1, 130.2, 128.8,122.5, 117.2, 115.2, 114.8, 83.2, 54.7 ; ESMS; 282.90 $[M]^+$ chemical formula : $C_{15}H_{11}ClN_4$.

4-Amino-2-(4-nitro-phenyl) -2H-pyrido[1,2-a]pyrimidine-3-carbonitrile **(4b);**
Yellow solid, ($CHCl_3$),mp 168-170°C; IR (KBr, cm^{-1}) υ_{max}; 3330 (NH_2), 2210 (C≡N), 1585 (C=N), 1530 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.31 (s, 2H, NH_2), 7.85-7.63 (m, 8H,Ar-H), 3.90 (s,1H, methine); ^{13}C NMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 164.6, 161.0, 134.1, 130.2, 130.8, 129.8, 128.8, 130.6, 115.2, 126.7, 114.8, 83.2, 55.7; ESMS; 293.08 $[M]^+$ chemical formula: $C_{15}H_{11}N_5O_2$.

4-Amino-2-(4-fluoro-phenyl)-2H-pyrido[1, 2-a]pyrimidine-3-carbonitrile **(4c);**
Pale Yellow solid, ($CHCl_3$),mp 176-178°C; IR (KBr, cm^{-1}) υ_{max}; 3354 (NH_2), 2223 (C≡N), 1639 (C=N) 1563 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.20 (s, 2H, NH_2), 7.64-7.41 (m, 8H,Ar-H), 4.10 (s,1H, methine); ^{13}C NMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 163.6, 160.0,134.1, 131.2, 126.8, 116.2, 114.8, 83.2, 54.7; ESMS; 268.27 [M +2]chemical formula : $C_{15}H_{11}FN_4$.

4-Amino-2-(3-nitro-phenyl)-2H-pyrido[1,2-a]pyrimidine-3-carbonitrile **(4d);**
Brown solid, ($CHCl_3$), mp 160-162°C; IR (KBr, cm^{-1}) υ_{max}; 3345 (NH_2), 2192 (C≡N), 1586 (C=N), 1546 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.90 (s, 2H, NH_2), 8.66-7.52 (m, 8H ,Ar-H), 3.92 (s, 1H, methine); ^{13}C NMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 164.7, 161.0, 134.1, 130.2, 129.8,122.4, 120.4, 117.2, 115.2, 114.7, 83.1, 55.7; ESMS; 293.08 $[M]^+$ chemical formula $C_{15}H_{11}N_5O_2$.

4-Amino-2-(2-chloro-phenyl)-2H-pyrido [1,2-a]pyrimidine-3-carbonitrile (**4e);**
Yellow solid, ($CHCl_3$), mp 166-169°C; IR (KBr,cm^{-1}) υ_{max}; 3340 (NH_2), 2200 (C≡N), 1580 (C=N), 1560 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.20 (s, 2H, NH_2), 7.62-7.40 (m, 8H,Ar-H), 4.00 (s, 1H, methine); ^{13}C NMR (DMSO-d_6,100 MHz, 25°C) δ ppm; 165.6, 162.0, 135.1, 130.2, 129.8,123.2, 120.2, 118.1, 115.2, 114.8, 83.2, 55.7; ESMS; 282.90 $[M]^+$ chemical formula: $C_{15}H_{11}ClN_4$.

4-Amino-2-(2-hydroxy-phenyl)-2H-pyrido [1,2-a]pyrimidine-3-carbonitrile (**4f);**
Yellow solid, ($CHCl_3$), mp 153-155°C; IR (KBr, cm^{-1}) υ_{max}; 3340 (NH_2), 3400 (OH), 2210 (C≡N), 1580 (C=N), 1578(C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 11.80 (s, OH), 8.21 (s, 2H, NH_2), 7.95-7.77 (m, 8HAr-H), 4.12 (s, 1H, methine); ^{13}C NMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 164.4, 161.2, 140.3, 137.5, 134.1, 127.2, 125.1,122.1, 120.2, 116.1, 85.2, 55.7; ESMS; 264.10 $[M]^+$ chemical formula $C_{15}H_{12}N_4O$.

4-Amino-2-phenyl-2H-pyrido [1,2-a]pyrimidine-3-carbonitrile(**4g**)**;**
Yellow solid, ($CHCl_3$),mp 172-175°C; IR (KBr, cm^{-1}) υ_{max}; 3340 (NH_2), 2230 (C≡N),1580 (C=N), 1555 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.22 (s, 2H, NH_2), 7.54-7.62 (m,9H,Ar-H), 4.15 (s,1H, methine); ^{13}C NMR (DMSO-d_6,100 MHz, 25°C) δ ppm; 164.4, 160.2, 141.3,137.5, 134.1, 127.2, 125.1,122.1, 117.2, 116.1, 84.2, 54.7; ESMS; 248.11 $[M]^+$ chemical formula: $C_{15}H_{12}N_4$.

4-Amino-2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-2H-pyrido[1,2-a]Pyrimidine-3-carbonitrile (**4h);**
Brown solid, ($CHCl_3$),mp 173-175°C; IR (KBr, cm^{-1}) υ_{max}; 3027 (NH_2), 2229 (C≡N), 1596 (C=N), 1512 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 7.70 (s,2H,NH_2), 7.40-7.07 (m, 7H,Ar-H), 4.53 (s, 1H, methine), 2.33 (s, 3H, CH_3); ^{13}CNMR (DMSO-d_6,100 MHz, 25°C) δ ppm; 165.6,161.0,143.1, 135.1, 130.2, 128.8, 124.4, 123.1, 119.1, 117.2, 115.2, 83.2, 54.7, 21.1;ESMS;348.09 $[M]^+$ chemical formula : $C_{19}H_{13}ClN_6$.

4-Amino-2-(furan-2-yl)-2H-pyrido[1,2-a]pyrimidine-3-carbonitrile(**4i);**
Yellow solid, ($CHCl_3$),mp132-135°C; IR (KBr,cm^{-1}) υ_{max}; 3340(NH_2), 2200 (C≡N), 1580 (C=N), 1576 (C=C) ; ^{1}H NMR (DMSO-d_6 400 MHz 25°C) δ ppm; 8.40 (s, 2H, NH_2),), 7.70-7.40 (m, 7H, Ar-H), 4.00 (s, 1H, methine); ^{13}CNMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 165.7, 161.0, 135.6,130.9, 128.8, 123.9, 120.1, 119.1, 117.3, 115.2, 106.9, 83.2, 54.7; ESMS; 238.09$[M]^+$chemical formula: $C_{13}H_{10}N_4$.

4-amino-2-(4-methoxyphenyl)-2H-pyrido[1,2-a]pyrimidine-3-carbonitrile(**4j);**
Yellow solid, ($CHCl_3$),mp 152-155°C; IR (KBr, cm^{-1}) υ_{max}; 3330 (NH_2), 2240 (C≡N), 1595 (C=N), 1540 (C=C); ^{1}H NMR (DMSO-d_6, 400 MHz, 25°C) δ ppm; 8.20 (s, 2H, NH_2), 7.60-7.40 (m, 9H,Ar-H), 4.00 (s, 1H, methine), 3.81 (s, 3H, OCH_3); ^{13}CNMR (DMSO-d_6, 100 MHz, 25°C) δ ppm; 165.6, 161.08, 135.18, 130.27, 128.81, 123.15, 119.10, 115.27, 106.86, 83.22, 54.76, 38.3; ESMS; 278.12 $[M]^+$ chemical formula $C_{16}H_{14}N_4O$.

Biology:
Antibacterial activity
The antimicrobial activities of the synthesized compounds **(4 a-j)** were determined by the agar diffusion method [31], the compounds were evaluated for antibacterial activity against *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), the antifungal activity was evaluated against *Aspergillusniger* (MTCC 282), *Aspergillus flavus* (MTCC 3008), *Candida albicans* (MTCC 227). The antibiotic Ampicillin (25µg/ml) was used for reference for antibacterial activity and Nystatin (25µg/mL) used as standard drugantifungal activities. The culture strains of the bacteria were maintained on nutrientagar slants at 37 ± 0.5 °C for 24 h. The antibacterial activity was evaluated using nutrient agar plates seeded with 0.1 ml of the respective bacterial culture strain suspension prepared in sterile saline (0.85 %) at 10^5 CFU/mL dilutions. The stock solutions were made by diluting compounds in DMSO to final concentrations ranging from 25 to 100µg/mL.The wells, of 6 mm diameter, were filled with 0.1 ml of the compound solution separately for each bacterial strain. All the plates were incubated at 37 ± 0.5 °C for 24 h. Zones of inhibition of compounds in mm and MIC were noted.The results of antibacterial activity given in **Table 4**.

For antifungal activity, all the culture strains of fungi were maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 °C for 24–48 h until sporulation. Spores of strains were transferred into 5 mL of sterile distilled water containing 1 % Tween-80 (to properly suspend the spore). The spores were counted by a hemocytometer(10^6CFU/mL). Sterile PDA plates were prepared containing 2 % agar, and 0.1 ml of each fungal spore suspension was spread on each plate and incubated at 27 ± 0.2 °C for 12 h. After incubation, wells were prepared using a sterile cork borer and each agar well was filled with 0.1 ml of the compound solution at the fixed concentration of 10 µg/mL. The plates were kept in a refrigerator for 20 min for diffusion and then incubated at 27 ± 0.2 °C for 24–48 h. After incubation, zones of inhibition of compounds were measured in mm, along with the standard. The result of antifungal activity is given in **Table 5**.

RESULTS AND DISCUSSION

Keep in mind above perspectives and limitation of existing methodologies and as part of the continuation of our studies on the development of efficient and environmentally benign synthetic methodologies [32-35], herein, we report a facile synthesis of pyrido [1,2-a] pyrimidine-3-carbonitrile derivatives in good yields. In a single-pot

procedure, compounds **4a-j** were obtained via three component coupling of substituted aldehydes **1a-j**, 2-amino pyridines **2** and malononitrile**3** using a catalytic amount of Bleaching earth clay (pH 12.5) at 70 -80°C for 30-40 mins in PEG-400.

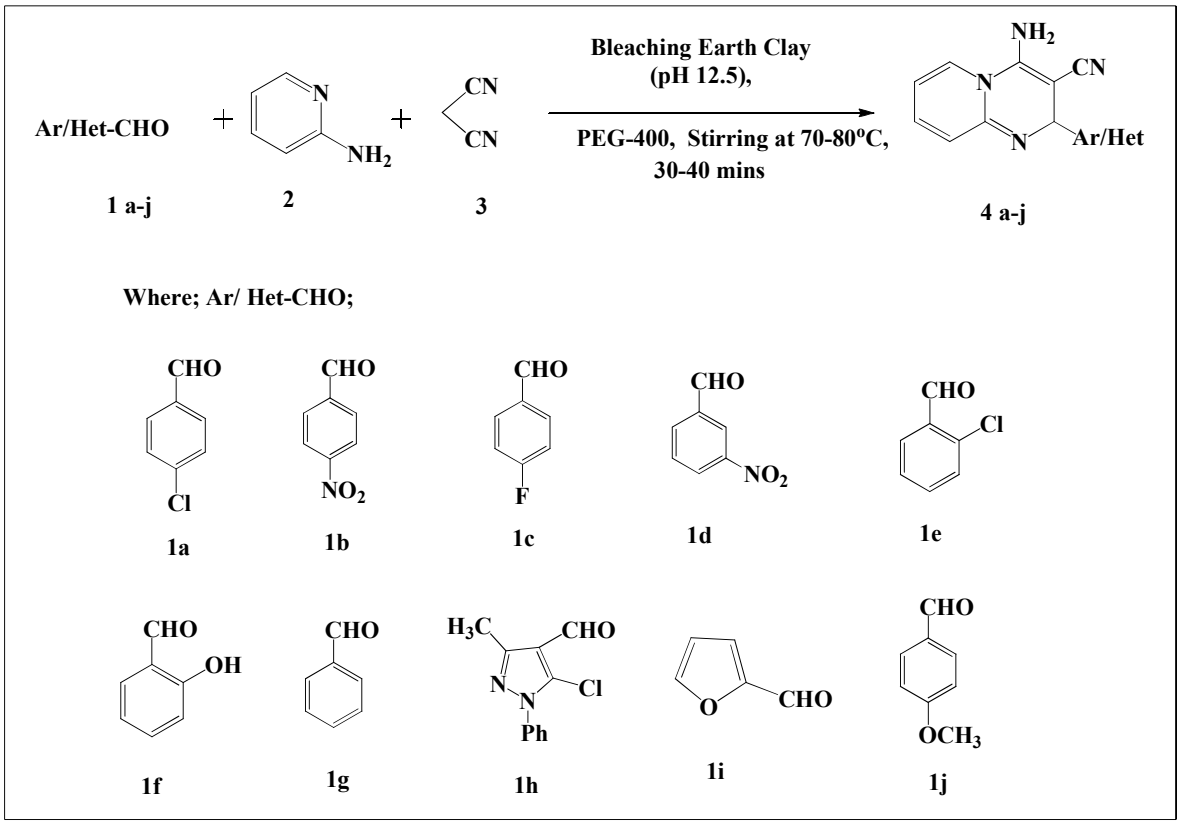


Figure 1.Green Synthesis of pyrido [1, 2-a] pyrimidine - 3-carbonitrilederivatives 4a-j

Initially, condensation reaction carried out between substituted aldehydes **1a-j**, 2-amino pyridines**2** and malononitrile**3** in PEG-400 at room temperature for 24 hours in the absence of the catalyst, but it led to very poor yields of the products (entry 1, Table 1). To enhance the yields of the desired products the temperature of the reaction was increased up to70^{o}C, with no substantial improvement in the product yield observed (entry 2, Table 1).

Table 1: Optimization of the reaction condition for product(4a-j)

Entry	Catalyst	Time (min/hr)[a]	Temp °C	Yields %[b]
1	Without catalyst	24 (hr)	RT	-
2	Without catalyst	12(hr)	70	-
3	Triethyl Amine	>60(min)	70	45
4	Piperidine	>60(min)	70	55
5	Cat (10wt %)	30 (min)	70	90
6	Cat (20wt %)	40 (min)	70	70

[a]*Reaction progress monitored by TLC;* [b]*Yields refer to isolated yield.*

Accordingly, after examining the issue of conventional catalysts viz. TEA, piperidine in PEG-400 for their conversion, but, none of the conventional catalysts afford a high yield of products. Hence, we attempted the synthesis of desired product using the Bleaching earth clay (pH 12.5) in PEG-400 as a green reaction medium . To our delight result shows an unexpected improvement in the yield of products within shorter reaction time. All reactions were completed within 30-40 mins. At the moment we repeated the reaction in the presence of Bleaching earth clay (pH 12.5) and evaluated the amount of catalyst required for this transformation using 1 wt %, 3 wt %, 5 wt % and 10 wt % of catalyst, and it observed that the increase in amount of catalyst increases the yield 30%, 70%, 80%, 90% respectively. Maximum yield (90%) was obtained when the reaction was carried out with 10 wt % of the catalyst. Any further addition of catalyst loading does not touch on the yield (entry, Table 2).

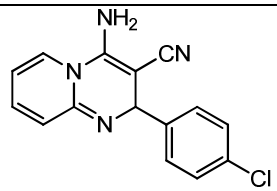
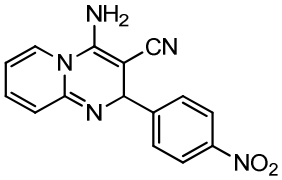
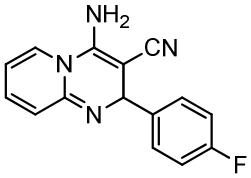
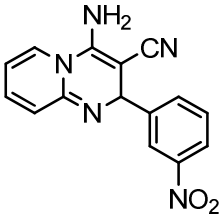
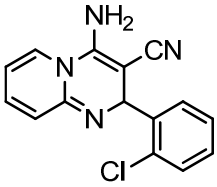
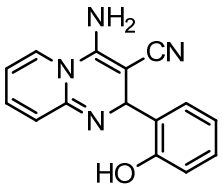
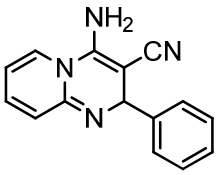
The used catalyst can be reused for the next reaction without any refining. The catalytic activity of Bleaching earth clay (pH 12.5) was studied by using the catalyst for 4-5 cycles and it has been observed that catalyst shown good catalytic power up to five cycles with insignificant loss of its activity (Table-2).

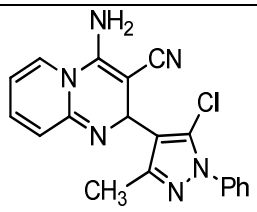
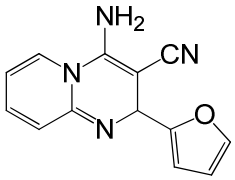
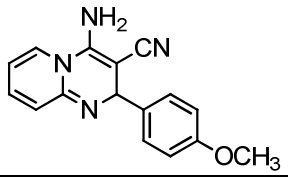
Table 2: Catalytic activity of Bleaching earth clay

Cycle	1st	2nd	3rd	4th	5th
Time in Min	30	35	40	45	60
Yield (%)	90	88	84	82	80

The utility of reaction were auxiliary examined by using a wide variety of aromatic aldehydes with various substitutes, and it revealed that the aromatic aldehydes with ortho, meta and para-substitution underwent evenly with 2-aminopyridine and malononitrile to afford the desired products **4 a-j** (Scheme 1 and Table 3).

Table 3: Synthesis of pyrido [1, 2-a] pyrimidine-3-carbonitrile derivatives (4 a-j)

Entry	**Products (4a-j)**	**Time (Min)[b]**	**Yield(%)**	**Temp °C**
4a		30	90	70
4b		35	85	75
4c		35	80	75
4d		40	80	75
4e		30	84	80
4f		35	80	80
4g		40	87	77

4h		40	80	80
4i		40	85	78
4j		35	84	75

[b] *Reaction progress monitored by TLC*

Furthermore, electronic effects of aryl substituent were examined and it was observed that the electronic effect was recessive over the yield of the product. Reaction profile is really trustworthy and no side products are organized.

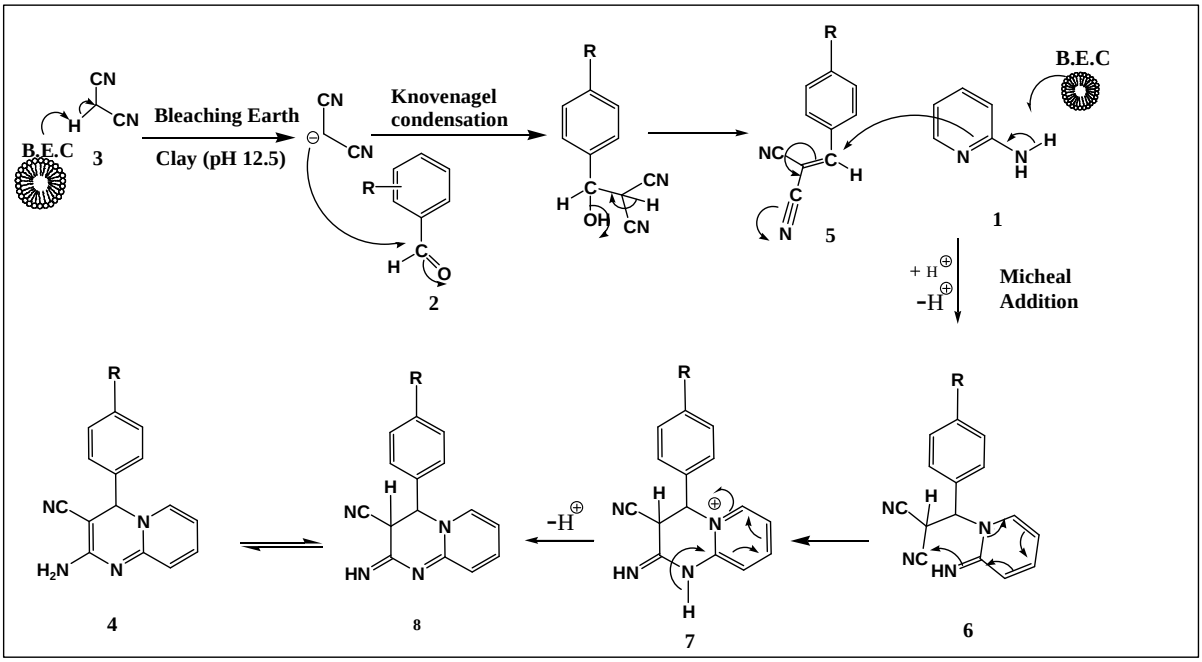


Fig 2: Proposed Mechanism for synthesis of pyrido [1, 2-a] pyrimidine -3-carbonitrile derivatives

The structures of all the synthesized compounds were established on the basis of analytical and chemical data. The structure **4a** was assigned on the basis of IR spectrum 3385 cm-[1] and 2222 cm-[1] show presence of amino and cyanofunctional groups. ^{1}H NMR spectra revealed a singlet at δ 8.04 that integrated for two protons this was ascribed to an amino functional group. The downfield shift of this amino function could be justified by the anisotropic effect of the ring nitrogen. The ^{1}H NMR spectra revealed singlet at 4.00 is methine proton. The aromatic protons resonate at multiplate at expected regions. Mass analysis study of compounds **(4a)** exhibited molecular ion peak at m/z =282.7 which is corresponds to its molecular formula $C_{15}H_{11}N_4Cl$. Further this compound is confirmed on the basis ^{13}C-NMR.

Pyrido[1,2-a] pyrimidine-3-carbonitriles were confirmed chemically by the condensation of substituted aldehydes with the active methylene compound to yield the corresponding β-arylacrylonitrile derivative followed by the exocyclic amino function in 2-amino pyridine to the activated double bond system to form a Michael adduct.

A suggested mechanism for the reaction is outlined in **Fig 2**. The reaction initiated by catalyst to form the most stable carbanion which condenses with substituted aldehydes and its considered to be a Knoevenagel condensation between aryl aldehydes and malononitrile, promoted by Bleaching earth clay (pH 12.5) to form β-arylacrylonitrile derivatives. The Michael addition reaction of β-arylacrylonitrile derivatives with 2 amino-pyridine followed by rearrangement and cyclization give rise pyrido [1, 2-a] pyrimidine -3-carbonitrile in good to excellent yield.

Biology

Antibacterial activity

The newly synthesized pyrido [1, 2-a] pyrimidine-3-carbonitrile derivatives **(4 a-j)** exhibited a varying pattern of antibacterial and antifungal activities, results shown in **Table 4** and **5**respectively, reveals that most of the synthesized compounds **(4 a-j)** showed antibacterial and antifungal activity. A cursory look at the results of *in vitro* antibacterial activity **(Table 4)**revealed that some of the synthesized compounds exhibited equipotent activity in comparison with standard drug , compounds **4a,4b,4c**,**4d**, and **4h, 4i, 4j** shown good zone of inhibition against *E.coli*, *B.subtilis*, and *S.aureus*. The compounds **4e,4f,4g,**shown moderate zone of inhibition against *E.coli* compound **4e** is inactive against *B.subtilis*, compound **4f** is inactive against *S.aureus*.Ampicillin is used as standard for antibacterial activity.

Table 4: Activity of synthesized compounds (4 a-j)

Product	*Escherichia coli (MTCC443)*	*Bacillus subtilis (MTCC441)*	*Staphylococcus aureus (MTCC96)*
4a	20(25)	14(25)	17(25)
4b	21(25)	15(25)	18(25)
4c	19(25)	12(25)	15(25)
4d	18(25)	16(25)	21(25)
4e	16(50)	-	14(50)
4f	12(50)	16(50)	-
4g	17(50)	14(50)	14(50)
4h	24(25)	21(25)	18(25)
4i	17(25)	19(50)	20(25)
4j	21(25)	17(50)	20(50)
Ampicillin	20(25)	18(25)	24(25)

Zones of inhibition measured in mm; MIC values (µg/ml) are given in parentheses

Antifungal activity

Antifungal data in **Table 5** revealed that compounds **4a,4b,4c**,**4d**and **4h,4i,4j** showed good activity against *Aspergillus niger* compounds**4e,4f** show poor activity against *Aspergillus flavus and Candida albicans* respectively. All other compounds show moderate activity against *Aspergillus flavus* and *Candida albicans* Nystatin is used as standard for antifungal activity.

Table 5: Antifungal activity of synthesized compounds (4a-j)

Product	*Aspergillusniger (MTCC 282)*	*Aspergillusflavus (MTCC3008)*	*Candida albicans (MTCC227)*
4a	20(25)	14(25)	17(50)
4b	26(50)	17(25)	18(25)
4c	22(25)	12(25)	21(50)
4d	18(25)	10(25)	15 (50)
4e	16(25)	-	14(50)
4f	12(50)	16(50)	-
4g	17(50)	14(50)	15(50)
4h	24(50)	21(25)	18(25)
4i	18(25)	19(50)	20(25)
4j	21(25)	17(50)	20(50)
Nystatin	20(25)	18(25)	22(25)

Zones of inhibition measured in mm; MIC values (µg/ml) are given in parentheses

CONCLUSION

In summary, we reported a facile, one-pot three component method for the synthesis of pyrido [1,2-a] pyrimidine-3-carbonitrile derivatives catalyzed by Bleaching earth clay (pH 12.5) in PEG-400 as a green reaction medium in good yields. The Bleaching earth clay (pH 12.5)found to be good heterogeneous catalyst imparting high yields of the product. The compounds **4a**, **4b,4c,4c**,**4d** and **4h,4i**,**4j**were exhibited siginificantantimicrobial activity. The higher activity may be due to the presence of substitution of halo group in para positions, Nitro substitution at para and meta positions, $–OCH_3$at para position in the phenyl ring contributes for antimicrobial activity. Hence, it is concluded that there is enough scope for further study in the developing these as a good lead molecule.

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