Original Research Article

Synthesis and characterization and antimicrobial activities of Pyrido [2, 3-d: 6, 5-d′] dipyrimidines

ABSTRACT

Fused pyrimidines,2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-aryl-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidines, were prepared in good yield through a simple, clean and three-component one-pot cyclo-condensation reaction of a barbituric acid, aromatic aldehydes and 4-ethylaniline under reflux in a nitrogen atmosphere. The synthesized products were characterized by IR, NMR spectroscopy and elemental analyses are in good agreement with their expected molecular structure. All the compounds have been screened for their antimicrobial activity with four human pathogenic bacteria: *Bacillus cereus*, *Salmonella typhi*, *Shigella dysenteria*, *Listeria monocytogenes* and three phytopathogenic fungi: *Macrophomina phaseolina*, *Fusarium equiseti*, *Colletotrichum corchori* and *Alternaria alternata*. The precursor barbituric acid was compared with the activity exhibited for the synthesized fused pyridopyrimidines, which show the increased inhibition against the bacterial growth after cyclization. The enhanced ability to inhibition may be due to the stability and aromaticity of heterocyclic synthesized in this study.

Keywords: Pyridopyrimidines, antimicrobial activity, barbituric acid, inhibition, disc diffusion method.

1. INTRODUCTION

Fused pyrimidines show the importance to find the common source for the development of new potential therapeutic agents[1]. In due attention, among them, pyridopyrimidine moiety and their oxo and thioxo derivatives is a well-known pharmacophore in drug design and it is associated with a wide range of biological activities [2-4]. Pyrido[3,2-d]pyrimidines have been described as tyrosine kinase inhibitors and as dihydrofolate reductase inhibitors [5-6]. They also possess dihydrofolate reductase inhibiting and antitumor activity [7-8] and used as organocatalyzed annulation reaction[9].

A series of novel 7-alkyl/arylsulfonyl-4-pyrrol-1-yl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]-thieno[2,3-d]pyrimidine were synthesized for evaluation of their antibacterial and antifungal activity [10]. Bcr-abl tyrosine kinase inhibitor of Pyrido [2,3-d]pyrimidine class (PD166326) was screened and described with substantial activity against STI-resistant mutant Bcr-abl proteins [11]. Their findings show that TK drug resistance is a structure-specific phenomenon and can be overcome by TK inhibitors of other structural classes, suggesting new approaches for future anticancer drug development. PD166326 is a prototype of a new generation of anti-Bcr-abl compounds with picomolar potency and substantial activity against STI571-resistant mutants. A structure activity relationship study was conducted for 2-anilino-6-phenylpyrido [2,3-d]pyrimidin-7(8H)-ones and found 10-100 fold more potential as inhibitors of the non-receptor kinase c-Src than the G2/M checkpoint kinase Wee1. Variation of substituents on the 6-phenyl ring did not markedly alter this preference where as solubilizing substituent's off the 2-anilino ring in many cases increased Wee1 activity, thus lowering this preference to about 10-fold. A two-step process can be followed to prepare pyridopyrimidines from the acyclic precursors. They use sodium cyanamide as the base to catalyze malononitrile to the enone and then drive the cyclization to yield the aromatic pyrido [2,3-d]pyrimidines, which completes a flexible and straightforward approach to this interesting kind of compounds[12]. An efficient method for the syntheses of pyrido[2,3-*d*]pyridmidines from the precursors using the catalytic amount of palladium acetate was reported[13].

In this study, we have synthesized pyrido[2,3-d:6,5-d′]pyrimidines through the reaction of a barbituric acid, 4-ethylaniline and aromatic aldehydes, such as benzaldehyde, *p*-methoxy-benzaldehyde, *p*-hydroxybenzaldehyde, *o*-chlorobenzaldehyde, *p*-nitrobenzaldehyde, 2,4-dimethoxybenzaldehyde and *p*-tolualdehyde respectively. Several synthetic approaches to prepare such type of compounds have been described in the literature, but the title compounds have not synthesized so far. The structure of the prepared compounds was confirmed by IR and NMR spectroscopy and elemental analysis. The antibacterial and antifungal screening was performed with commonly used bacterial pathogens and fungal strains.

2. METHODS AND EXPERIMENTAL

2.1. General method for the preparation of 2,4,6,8-Tetrahydro-5-(4′-ethyl phenyl)-10-aryl-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidines

All seven pyrido[2,3-d:6,5-d′]dipyrimidine [**1-7**] **(Fig. 1)** have been synthesized according to the developed method reported in the literature [14]. Equimolar amount of Aryl aldehydes and 4-ethylaniline (Sigma-Aldrich, for synthesis) were added to annealing substrate barbituric acid (0.26g, 2.0 mM) (Sigma-Aldrich, *Reagent Plus*®, 99%) in a round-bottom flask equipped with a reflux condenser, a magnetic stirrer and an inlet and outlet for N_2 gas using solvent 20 mL of methanol (Sigma-Aldrich, AR grade 98%) to produce target compound, pyrido[2,3-d:6,5-d′]dipyrimidines [1-7]. After two hours, the color of the reaction mixture suddenly changes into a green from colorless. Reaction progress was monitored by thin layer chromatography, using aluminum sheets coated with silica gel with the solvent mixture of n-hexane and ethylacetate (1:1, v/v) as the mobile phase. The mixture was then kept overnight at room temperature. To allow better precipitation, the reaction mixture was cooled in the refrigerator for another 24 h. The separated solid was filtered off and crystallized from $CHCl_3$-MeOH (1:3) to the product pyrido [2,3-d:6,5-d′]dipyrimidine as crystalline solids. All the aldehydes were purchased from the chemical companies, and used without purification: Benzaldehyde (Sigma-Aldrich, Reagent Plus, ≥ 99%), *p*-methoxybenzaldehyde (Merck, for synthesis), *p*-hydroxybenzaldehyde (Sigma-Aldrich, ≥ 97%), 2-chlorobenzaldehyde (Sigma-Aldrich, 99%), *p*-nitrobenzaldehyde (Sigma-Aldrich, 98%), 2,4-dimethoxybenzaldehyde (Merck, for synthesis), *p*-tolualdehyde /4-methylbenzaldehyde (Merck, for synthesis). The physical states and melting temperature of the synthesized heterocycles were placed in Table 1. Melting points were recorded on an Electrothermal 9100 melting point apparatus and left uncorrected. Evaporation of solvents was performed under reduced pressure on a Buchi rotary evaporator. Thin layer chromatography was performed on keiselgel GF25 and visualization was accomplished by Ionic Flask or UV lamp. Column chromatography was carried out with silica gel G60 (100-200 mesh).

80 **Table 1: Analysis result of the synthesized pyridodipyrimidines [1-7]**

Comp	M.F. (MW)	Spectroscopic Analyses		Elemental analysis	
		^{1}H-NMR	^{13}C-NMR	Found	Calculated
1	$C_{23}H_{19}N_5O_4$ (429)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 4.40 (s, 1H, 5-CH), 5.0 (s, 1H, -OH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 6.61-6.89 (m, 4H, Ph), 10.0 (s, 2H, 2×-NH).	16.1 (CH_3), 25.7 (C-5), 28.6 (CH_2), 79.7 (C-4a, 5a), 115.0-115.6 (C-2,4, N-Ph, C-3,5,Ph), 128.7 (C-3,5, N-Ph), 130.3 (C-1`), 130.6 (C-2,6, ph), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6).	C 64.82 H 4.31 N 15.97	C 64.34 H 4.43 N 16.32
2	$C_{24}H_{21}N_5O_5$ (459)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 4.43 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 7.00-7.15 (m, 3H, Ph), 10.0 (s, 2H, 2×-NH)	16.1 (-CH_3), 25.7 (C-5), 28.6 (=CH_2), 79.7 (C-4a, 5a), 115.0 (C-2,4, N-Ph), 126.5, 128.7, 128.8 (C-3,5, N-Ph), 130.6, 134.5 (C-2,6, Ph), 138.1 (C-1`), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6)	C 62.08 H 3.67 N 14.79	C 62.74 H 3.92 N 15.25
3	$C_{23}H_{19}N_5O_5$ (445)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 4.40 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 7.32-8.07 (m, 4H, Ph), 10.0 (s, 2H, 2×-NH)	16.1 (CH_3), 25.7 (C-5), 28.6 (CH_2), 79.7 (C-4a, 5a), 115.0 (C-2,4, N-Ph), 123.5 (C-3,5,Ph), 128.7 (C-3,5, N-Ph), 130.1 (C-2,6, Ph), 143.8 (C-1`), 145.4 (C-4`Ph), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6)	C 61.86 H 4.34 N 18.12	C 62.02 H 4.04 N 18.88
4	$C_{23}H_{18}N_5O_4Cl$ (464)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 4.43 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 7.00-7.15 (m, 3H, Ph), 10.0 (s, 2H, 2×-NH)	16.1 (-CH_3), 25.7 (C-5), 28.6 (=CH_2), 79.7 (C-4a, 5a), 115.0 (C-2,4, N-Ph), 126.5, 128.7, 128.8 (C-3,5, N-Ph), 130.6, 134.5 (C-2,6, Ph), 138.1 (C-1`), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6)	C 59.09 H 3.52 N 14.85	C 59.48 H 3.88 N 15.09
5	$C_{23}H_{18}N_6O_6$ (474)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 4.40 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 7.32-8.07 (m, 4H, Ph), 10.0 (s, 2H, 2×-NH)	16.1 (CH_3), 25.7 (C-5), 28.6 (CH_2), 79.7 (C-4a, 5a), 115.0 (C-2,4, N-Ph), 123.5 (C-3,5,Ph), 128.7 (C-3,5, N-Ph), 130.1 (C-2,6, Ph), 143.8 (C-1`), 145.4 (C-4`Ph), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6)	C 57.73 H 4.20 N 17.93	C 58.23 H 3.80 N 17.72
6	$C_{25}H_{23}N_5O_6$ (489)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 3.73 (s, 6H, 2×-OCH_3), 4.43 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.16-6.87 (m, 7H, 2×Ph), 10.0 (s, 2H, 2×-NH).	16.1 (CH_3), 25.7 (C-5), 28.6 (CH_2), 56.0 (C-OMe), 79.7 (C-4a, 5a), 114.0-115.0 (C-2,4, N-Ph, C-3,5,Ph), 128.7 (C-3,5, N-Ph), 130.0 (C-1`), 130.2 (C-2,6, Ph), 143.9 (C-1`), 151.2-5 (m, C-2,8,9a,10a), 164.4 (C-4,6)	C 60.85 H 5.01 N 14.781	C 61.35 H 4.70 N 14.31
7	$C_{24}H_{21}N_5O_4$ (443)	1.24 (t, 3H, CH_3), 2.59 (q, 2H, CH_2), 2.35 (s, 3H, CH_3), 4.51 (s, 1H, 5-CH), 6.0 (s, 2H, 2×-NH), 6.41-6.87 (m, 4H, Ph), 6.94 (m, 4H, Ph), 10.0 (s, 2H, 2×-	16.1 (-CH_3), 20.9 (s, 3H, CH_3), 25.7 (C-5), 28.6 (CH_2), 79.7 (C-4a, 5a), 115.0 (C-2,4, N-Ph), 128.7 (C-3,5, N-Ph), 129.1 (m, 4H, Ph), 130.2 (C-4`), 134.7 (C-1,4, Ph), 143.9 (C-1`), 151.2-5	C 64.79 H 4.52 N 16.54	C 65.01 H 4.74 N 15.80

NH)	(m, C-2,8,9a,10a), 164.4 (C-4,6).

2.2. IR, NMR and elemental analysis

The formation and purity of the synthesized compounds was confirmed using infrared (IR) spectroscopy (Shimadzu 8400S), nuclear magnetic resonance (^{1}H-NMR) spectroscopy (Bruker Avance, 400 MHz) and CHNS analysis (Leco 932). For NMR, (5 to 10) mg of sample was dissolved 0.7 cm^3 of deuterated dimethylsulfoxide solvent (d-DMSO). The observed peaks are abbreviated as s (singlet), d (doublet), t (triplet) and m (multiplet). Chemical shifts were reported in δ unit (ppm) with reference to TMS as an internal standard. The carbon, hydrogen, nitrogen and sulfur percentages in ILs were analyzed according to the approved method ASTM D-5291 by employing Leco-CHNS-932 analyzer. The analyzer was calibrated using standard calibration sample with known chemical composition provided by supplier before each measurement. All the samples were analyzed triplicate and the average values were reported.

2.3. Anti-microbial study

All the synthesized compounds (**1-7**) were assayed for their antibacterial and antifungal activity against four bacterial isolates and four fungal spores obtained from the Department of Microbiology, University of Chittagong,Chittagong, Bangladesh. These were: human pathogenic bacteria gram-positive *Bacillus cereus* BTCC 19, *Listeria monocytogenes* LO28, gram-negative *Salmonella enterica* AE 14612, *Shigella dysenteriae* AE 14396 (**Tables 2**) and the antifungal strain *Macrophomina phaseolina* (Tassi) Goid, *Fusarium equiseti* (Corda) Saccardo 1886, *Colletotrichum corchori* Ikata & Yoshida and *Alternaria alternata* (Fr.) Kessler (**Table 2**). These tests were conducted at the Department of Microbiology, University of Chittagong, Bangladesh, using the disc-diffusion method [15] for bacteria and, the poisoned-food technique [16] for fungus using the solvent dimethylsulfoxide (DMSO). The tested compounds were studied in two different concentrations 1% and 10% (w/v) in DMSO. Commercial antibacterial and antifungal brands, respectively Ampicillin and Nystatin were also tested under similar conditions for comparison. Nutrient agar (NA) and potato dextrose agar (PDA) were used as basal media to test bacteria and fungi respectively.

2.3.1. Bacterial media (Nutrient Agar, NA) preparation and test protocol

A suspension of agar powder (15.0 g), beef extract (3.0 g), peptone (5.0 g) and sodium chloride (0.5 g) in distilled water (1000 mL) in a beaker was boiled gently with constant stirring until agar powder and the other material were completely dissolved. The medium was then transferred to a conical flask with stopper and autoclaved for half an hour at 120°C and 15 psi to destroy the unexpected organisms. After autoclaving, the sterilized medium becomes ready for test. Stock culture of test organisms were maintained on NA slants and preserved at 10°C. Occasional sub-culture (3/4 weeks intervals) system was also maintained to keep the culture inactive condition with character unimpaired. Just before the use of test organism, suspension in sterile distilled water was prepared with 48 hour-old cultures. Bacterial concentration was determined through UV spectroscopy and maintained optical density (OD) for each experiment at 1-5$\times10^5$ CFU/mL. Sensitivity spectrum analysis was done by Disc diffusion method (Bauer et al., 1966) with little modification. Sterile paper disc of 4 mm in diameter and Petri-dishes of 90 mm in diameter were used throughout the experiment. Before use, paper discs were dried at 100°C in an oven. The autoclaved NA media, cooled to 45°C, was poured into sterilized Petri dishes to a depth of 3 to 4 mm and allowed to cool for solidification. The plates were inoculated with 1 ml of standard bacterial suspensions (as of McFarland 0.5 standard) by the help of sterilized glass hockey stick and allowed to dry for three to five minutes. Dried and sterilized filter paper discs were socked separately with 20 μL (250 μg/disc) of tested chemicals at concentrations 1% and 10% (w/v) in DMSO, dried in air under aseptic condition and were placed at equidistance in a circle on the seeded plate. A negative control examination with 20 μL of DMSO and positive control with 20 μL of standard antibiotic ampicillin (20 μg/disc) (BEXIMCO Pharma, Bangladesh) solution in water, were also performed for each organism studied. The plates were then incubated at 37°C, for 24 h or until visible growth was established, and the diameter of the inhibition-cleared zone around each disc was determined. The screening results were compared with a standard antibacterial antibiotic ampicillin.

2.3.2. Potato Dextrose Agar (PDA) media preparation and Antifungal Mycelial Growth Inhibition test protocol:

The *in the vitro* antifungal activity of the synthesized pyridopyrimidines was determined by the poisoned food technique. PDA medium was prepared from boiled potato infusion and dextrose in deionised water and used for the culture of fungi. A required amount of PDA was taken in conical flasks separately and was sterilized by autoclave (121°C, 15 psi) for 15 minutes. The autoclaved PDA was cooled to 45°C and poured at the rate of 15-20 mL in each Petri dish. After solidification of the medium, the small portions of mycelium of each fungus were placed carefully at the center of each plate with the help of sterilized needles. 100 μL of the test chemicals of concentrations 1% and 10%

(w/v) in DMSO were thoroughly mixed with 15-20 mL sterilized melted PDA and poured into separate glass Petri dishes. After solidification, 5 days old fungal mycelial block (4 mm in diameter) was placed at the center of each Petri dish in an inverted position and incubated at 27°C. A control set was also maintained in each experiment. Linear mycelial growth of fungus was measured after 3-5 days of incubation in triplicate. The average of two measurements was taken as mycelial colony diameter of the fungus in mm. All the antifungal results were compared with the standard antifungal antibiotic Nystatin (100 µg/mL PDA). DMSO was used as negative control. The percentage inhibition of radial mycelial growth of the test fungus was calculated as follows:

$$I = \frac{C - T}{C} \times 100 \qquad \text{Eq. (1)}$$

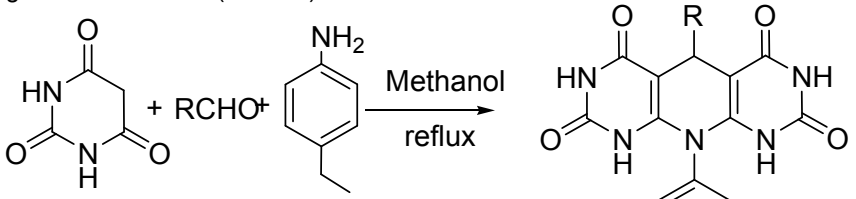
Where, I = percentage inhibition, C = diameter of the fungal colony in DMSO (control), T = diameter of the fungal colony in treatment.

3. RESULTS AND DISCUSSION

3.1. Chemical structures of synthesized pyrido dipyrimidines [1-7]

The reactions employed in this study are encapsulated in the scheme shown in **Fig. 2.** Seven pyrido[2,3-d:6,5-d′]dipyrimidines were prepared: 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-phenyl-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d: 6,5-d′]-dipyrimidine [**1**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(4′-methoxyphenyl)-2,4,6,8-tetra-oxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimid-ine [**2**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(4′-hydroxyphenyl)-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidine [**3**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(2′-chlorophen-yl)-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidine [**4**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(4′-nitrophenyl)-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidine [**5**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(2′,4′-dimethoxyphenyl)-2,4,6,8-tetraoxo-5H,10H-pyri-do[2,3-d:6,5-d′]dipyrimidine [**6**], 2,4,6,8-Tetrahydro-5-(4′-ethylphenyl)-10-(4′-methylphenyl)-2,4,6,8-tetraoxo-5H,10H-pyrido[2,3-d:6,5-d′]dipyrimidine [**7**] were synthesized and characterized. The product yields ranged from 56 to 85 % as shown in Table 1. The structures of the compounds were characterized according to their IR, ^{1}H & ^{13}C-NMR and elemental analysis data.

The IR spectra of these compounds confirm the formation of the products. The appearance of aromatic stretching at ~1660 cm^{-1} and the shifting of the peaks regarding carbonyl and secondary amine groups at ~1705 and ~3265 cm^{-1} respectively showed good agreement of the structure. The alkyl groups (methyl, methylene), aromatic NH and CH proton and hydroxyl structures (from vacuum-lactim tautomerism) detected via ^{1}H-NMR are consistent with the IR data. The NMR spectra showed a good characteristic δ value (in ppm) for the confirmation of the compounds. One carbon 5-CH proton in pyridine ring appears always in an up-field position at about δ 4.40 compare to other alkyl group's position. Two multiplet's position at around δ 6.41-6.87 and δ 7.06-7.14 for two phenyl group and broad peaks with the agreement of the NH protons achieved the formation of the target compounds. ^{13}C-NMR spectrum also showed clear understanding about the number and position of carbons corresponding to their molecular formula, which was in confirm with the structure assigned to them [1-7]. The calculated carbon, hydrogen, and nitrogen percentages are in good agreement with the corresponding observed values (Table 1).



1. R = -C_6H_5
2. R = -C_6H_4(OMe) (*p*)
3. R = -C_6H_4(OH) (*p*)
4. R = -C_6H_4(Cl) (*o*)
5. R = -$C_6H_4(NO_2)$ (p)
6. R = -$C_6H_3(OMe)_2$ (o&p)
7. R = -C_6H_4(Me) (p)

Figure 1: One pot cyclocondensation reaction scheme

The possible pathway for the formation of pyrido [2,3-d:6,5-d′]dipyrimidines [1-7] is shown here in Fig. 1. The reaction is believed to proceed via the formation of a bisproduct through the Michael addition of barbituric acid to the 5-arylidene barbituric acid A, which on further reaction with appropriate amine gave the final product. This was confirmed by the isolation of one of the dimers B by the reaction of the barbituric acid with aryl aldehyde in methanol. Its further reaction with aromatic amine afforded the required pyrido [2,3-d:6,5-d′]dipyrimidine [1-7].

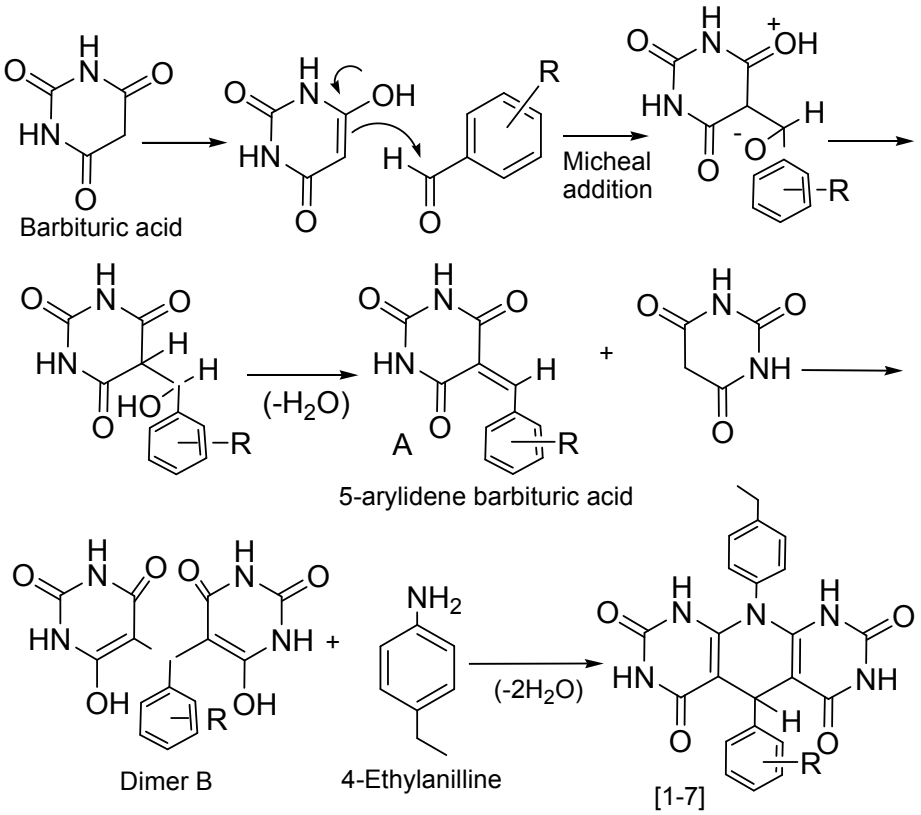


Figure 2: The proposed reaction pathway for pyridopyrimidines synthesis

3.2 Antimicrobial assay

The anti-microbial activities of the synthesized pyridopyrimidines were established by evaluating the inhibition zones on the agar plates. This zone is defined as the area on the agar plate where the growth of a microbe is prevented by the test compound. The results obtained are summarized in Table 2. The chemicals concentration of 1% (w/v) doesn't appear to show any anti-bacterial activity, which can give information to play with a higher concentration for the test. Disc diffusion method for the antibacterial screening and Poisoned-food technique for antifungal screening were assigned for the tested chemicals at the concentration of 1% (w/v) using negative control DMSO [15]. DMSO alone had no inhibition of all the strain studied. The test conducted with Barbituric acid (BA) to compare the effects of the cyclisation, which is, in most cases, more effective than the free ligand (BA).

Table 2 Inhibition zone (in mm) exerted by the tested compounds against bacterial and fungal strains: (Ampicillin and Nystatin used as reference), '-' denotes no inhibition

	BA	1	2	3	4	5	6	7	Ampicillin	Nystatin
Bacterial strain										
Bacillus cereus	9	10	-	-	11	-	14	10	21	
Listeria monocytogenes	11	15	20	17	15	12	21	12	28	
Salmonella enterica	-	13	21	-	11	13	-	12	24	
Shigella dysenteriae	10	15	11	13	17	-	24	21	30	
Fungal Strain										
Macrophomina phaseolina	20	51	25	32	26	23	53	43		72

Fusarium equiseti	15	-	25	23	-	34	36	33		45
Colletotrichum corchori	17	25	31	25	21	22	36	33		41
Alternaria alternata	19	35	25	35	45	24	43	45		52

It was found that Compound 5 and 6 were more effective than that of other chemicals for all bacterial strain. Bacterial stain *Bacillus cereus* had lower inhibition of all chemicals studied. Other compounds were either inactive or moderately to fairly active against the tested bacterial strain. As far as the fungal activity concerned, all the compounds showed good to excellent activity against all the fungi. Compound 5 and 6 show stronger activity against *colletotrichum corcori* and *Macrophomina phaseolina*. From the observation of inhibitions of the tested compounds, it was ascertained that generally pyridopyrimidine derivatives 5 and 6 exhibited higher activities against human pathogenic bacteria and phytopathogenic fungi.

4.0 CONCLUSION

Pyridopyrimidines with aromatic side chain were synthesized in good yields and characterized. The characteristic spectroscopy and elemental analysis confirmed the structure of those compounds. Antimicrobial screening showed a dependable knowledge base to use them as broad antibiotic agents and pesticides against common human pathogenic bacteria and phytopathogenic fungi respectively. Further studies with other biological application should be needed to develop a database for those pyridopyrimidines.

5.0 REFERENCE

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