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SYNTHESIS AND CHARACTERISATION OF 7-(1H-BENZIMIDAZOL-2-YL)-5-(SUBSTITUTED PHENYL) PYRIDO [2, 3-D] PYRIMIDIN-4-AMINE FOR THEIR BIOLOGICAL ACTIVITY

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ABSTRACT

A new series of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine (6a-h) were synthesized by reacting substituted cyanopyridine derivatives with formamide, formic acid and dimethyl formamide under reflux. The title compounds have been characterized by IR, ^{1}H NMR and mass spectral data. The synthesized compounds were evaluated for their qualitative antibacterial and *in vitro* antioxidant activity by agar diffusion and DPPH methods, respectively. In general, the title compounds have shown mild to moderate antibacterial and antioxidant activity as well.

Key words: Benzimidazole, Characterization, Antibacterial, Antioxidant activity.

INTRODUCTION

Many naturally occurring and synthetic compounds containing the pyridopyrimidine scaffold posses interesting pharmacological properties. Among them pyridopyrimidine derivatives are well known for their versatile biological activities like antimicrobial[1], anti-inflammatory[2], anti-tumor[3] and antifungal[4] etc. Benzimidazole and their derivatives represent one of the most active classes of biologically active heterocyclic compounds. Some of the activities of these compounds include antibacterial[5], antifungal[6], antioxidant[7], anticancer[8], anthelmintic[9,10]. We have aimed at the synthesis of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine **(6a-h)** for antibacterial activity against two gram positive and two gram negative organisms using standard substance. The antioxidant activity was performed by DPPH$^{\cdot}$ method using ascorbic acid as standard. The

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title compounds **(6a-h)** were synthesized by reacting 2-amino-6-(1*H*-benzimidazol-2-yl)-4-(substituted phenyl) pyridine-3-carbonitrile **(5a-h)** with formamide, formic acid and dimethyl formamide. 2-amino-6-(1*H*-benzimidazol-2-yl)-4-(substituted phenyl) pyridine-3-carbonitrile **(5)** was prepared by reaction between benzimidazole chalcone **(4)** in malanonitrile and ammonium acetate in ethanol medium. Benzimidazole chalcone was prepared by reaction between 2-acetyl benzimidazole **(3)** in sodium hydroxide in aromatic aldehydes. 2-acetyl benzimidazole was prepared by reaction between 2-(α-hydroxyethyl) benzimidazole in potassium dichromate in sulphuric acid medium. 2-(α-hydroxyethyl) benzimidazole (**2**) was prepared by reaction between o-phenylenediamine (**1**) and lactic acid in sodium hydroxide medium[11-12] Fig. 1.

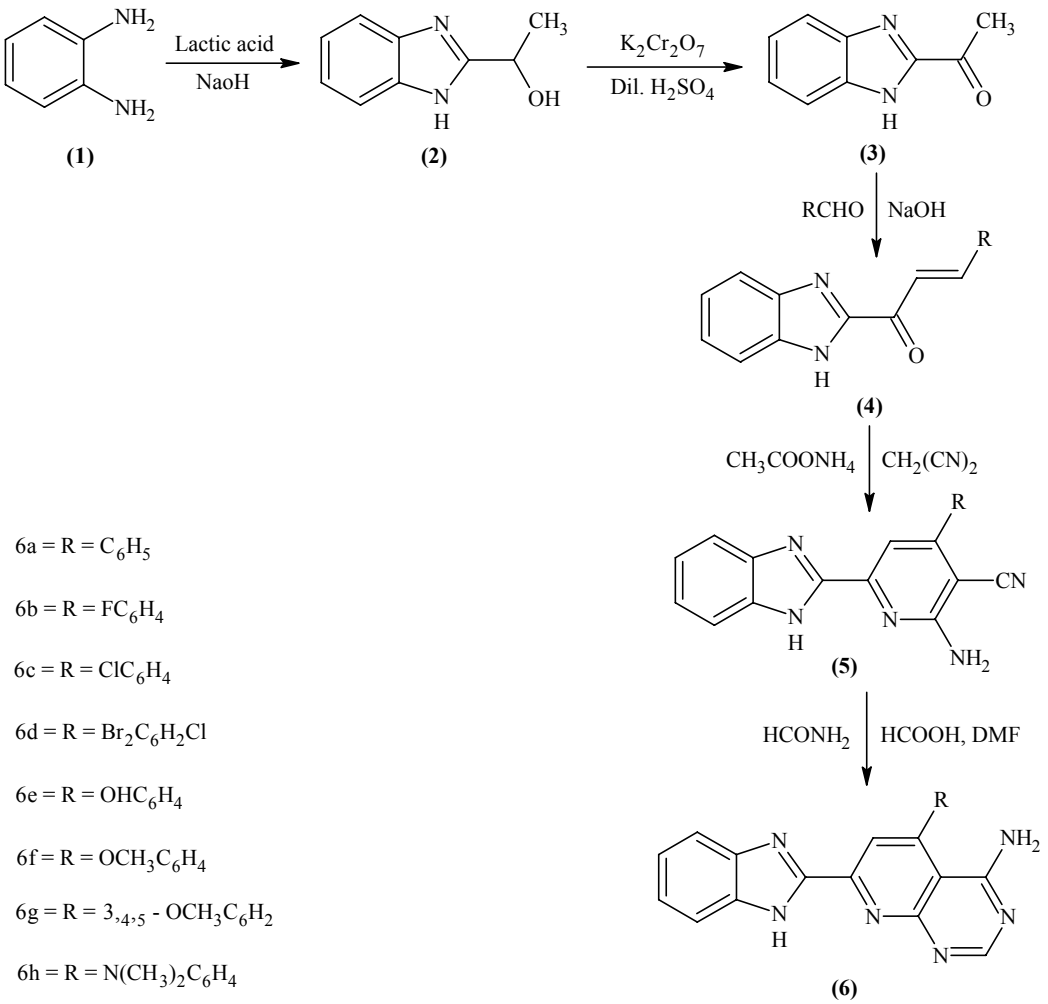


Fig. 1: Scheme of the reaction

The yields of the compounds **(6a-h)** were found to be in the range of 60.00-80.00%. The title compounds **(6a-h)** were confirmed by characteristic IR peak at 3200.72-34500.42 cm^{-1} (Ar-NH_2) for amino function. Compound **(6a-h)** showed NMR peaks. Compounds were also characterized by MS in which molecular ion peaks were in good agreement with the molecular weight of the title compounds. Partition coefficient of the title compounds have been determined by using ACD/labs software v11.0 and found to be in the range of 3.20-5.63.

EXPERIMENTAL

Melting points of the synthesized compounds were determined using Thiele's melting point apparatus and were found uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellets in the range 4000-400 cm^{-1} on a Fourier Transform IR Spectrophotometer (Model Shimadzu 8700, at Strides Arcolab Limited, Bangalore) and frequencies were recorded in wave numbers (cm^{-1}). The ^{1}H NMR spectra were recorded on Amx-400 liquid state PMR spectrometer (Indian Institute of Science, Bangalore). Chemical shifts (δ) are reported in parts per million (ppm) down field from internal reference tetramethylsilane (TMS). Mass spectrum was recorded by LC-MS (model-Shimadzu, Quest, Bangalore). Purity of the compounds was checked by thin layer chromatography. The physical constants of the title compounds are reported in Table 1.

General procedure for the preparation of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine

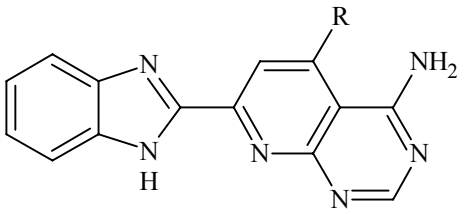
A mixture of 2-amino-6-(1*H*-benzimidazol-2-yl)-4-(substituted phenyl) pyridine-3-carbonitrile (0.01 mol), formamide (0.001 mol), formic acid (0.01 mol) and dimethyl formamide (0.001 mol) were taken in 100 mL round bottom flask, refluxed for 12 h at 100^{o}C. The reaction completion was monitored through TLC and reaction medium was cooled, the product obtained was filtered and recrystalized with ethanol. The formation of 7-(1h-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine **(6a-h)** is confirmed by the difference in M.P. and R_f value which are tabulated in Table 1.

7-(1*H*-benzimidazol-2-yl)-5-phenylpyrido [2, 3-*d*] pyrimidin-4-amine (6a)

IR (KBr) (υ, cm^{-1}), 2360 (NH_2), 1969 (ArC-H), 1331 (C=N), 1209 (ArC=C).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 5.12 (s, 2H, -NH_2), 4.82 (s, 1H, -NH), 7.32-7.48 (s, 5H, Ar-H), 7.26-6.70 (s, 4H, Ar-H), 7.82 (m, 1H, -CH), 8.02 (s, 1H, Ar-H), LCMS m/z: 339 (M^+). Elemental analysis Calcd: C (70.99%), H (4.17%), N (14.84%), Found: C (71.05%), H (4.25%), N (14.70%).

Table 1: Physical properties of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine (6a-h)



Compd.	R	M.F.	MW	Yield (%)	M.P. (^oC)	Rf[a]	Clog P[b]
6a	C_6H_5	$C_{20}H_{13}N_5$	339	72.6	193	0.79	3.79
6b	FC_6H_4	$C_{20}H_{12}N_5F$	356	79.2	188	0.73	3.93
6c	ClC_6H_5	$C_{20}H_{12}N_5Cl$	372	64.1	201	0.75	4.50
6d	$Br_2C_6H_3Cl$	$C_{20}H_{10}N_5Br_2Cl$	530	68.3	216	0.61	5.63
6e	OHC_6H_4	$C_{20}H_{15}N_5O$	355	77.6	176	0.73	3.31
6f	$OCH_3C_6H_4$	$C_{21}H_{15}N_5O$	369	64.8	171	0.80	3.80
6g	3,4,5-$OCH_3C_6H_2$	$C_{23}H_{19}N_5O_3$	428	66.2	191	0.86	3.20
6h	$N(CH_3)_2C_6H_4$	$C_{22}H_{18}N_6$	381	75.7	163	0.81	4.06

[a]Benzene: Ethanol (8 : 2)
[b]Clog P was calculated using ACD/labs software v11.0

7-(1*H*-benzimidazol-2-yl)-5-(4-fluorophenyl) pyrido [2, 3-*d*] pyrimidin-4-amine (6b)

IR (KBr) (υ, cm^{-1}), 2290 (NH_2), 2947 (ArC-H), 1701 (C=N), 1527 (Ar C=C), 521 (C-F).

^1H NMR ($CDCl_3$, 400 MHz) δ (ppm), 4.02 (m, 2H, -NH_2), 6.62 (s, 1H, -NH), 7.02-7.48 (s, 4H, Ar-H), 8.41-9.70 (m, 4H, Ar-H), 7.62 (s, 1H, -CH), 8.41 (s, 1H, Ar-CH), LCMS m/z: 355 (M^+). Elemental analysis Calcd: C (67.41%), H (3.68%), F (5.33%), N (13.58%), Found: C(66.92%), H(3.46%), F(5.91%), N(13.71%).

7-(1*H*-benzimidazol-2-yl)-5-(4-chlorophenyl) pyrido [2,3-*d*]pyrimidin-4-amine (6c)

IR (KBr) (υ, cm^{-1}), 3310 (NH_2), 3907 (ArC-H), 1671 (C=N), 1507 (ArC=C), 681 (C-Cl).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 3.92 (s, 2H, -NH_2), 4.91 (s, 1H, -NH), 7.42-8.48 (s,4H, Ar-H), 7.61-8.91 (m, 4H, Ar-H), 7.02 (s, 1H, -CH), 8.71 (s, 1H, Ar-CH); LCMS m/z: 372 (M^+). Elemental analysis Calcd: C (64.43%), H (3.51%), Cl (6.51%), N (12.54%), Found: C (65.32%), H (3.46%), Cl (6.91%), N (11.31%).

7-(1*H*-benzimidazol-2-yl)-5-(3,5-dibromo-4-chlorophenyl) pyrido [2,3-*d*]pyrimidin-4-amine (6d)

IR (KBr) (υ, cm^{-1}), 3410 (NH_2), 3107 (ArC-H), 1711 (C=N), 1587 (ArC=C), 681 (C-Cl), 596 (C-Br).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 4.04 (s, 2H, -NH_2), 5.02 (s, 1H, -NH), 7.62-8.42 (s, 2H, Ar-H), 7.04-8.85 (m, 4H, Ar-H), 8.04 (s, 1H, -CH), 6.76 (m, 1H, Ar-CH), LCMS m/z: 530 (M^+). Elemental analysis Calcd: C (45.27%), H (2.09%), Br (30.12%), Cl (5.68%), N (15.84%), Found: C (45.32%), H (2.46%), Br (32.01%), Cl (5.91%), N (13.11%).

4-[4-amino-7-(1*H*-benzimidazol-2-yl) pyrido [2,3-*d*]pyrimidin-5-yl]phenol (6e)

IR (KBr) (υ, cm^{-1}), 3210 (NH_2), 2947 (ArC-H), 1711 (C=C), 1647 (-OH) 1587 (ArC=C).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 3.84 (s, 2H, -NH_2), 4.75 (s, 1H, -NH), 5.72 (s, 1H, Ar-OH),6.81-7.97 (s ,4H, Ar-H), 7.67-8.35 (s, 4H, Ar-H), 7.76 (s, 1H, Ar-CH), 8.94 (s, 1H, -CH, pyrimidine), LCMS m/z: 354 (M^+). Elemental analysis Calcd: C (67.79%), H (3.98%), N (13.72%), O (4.51%), Found: C (66.84%), H (3.46%), N (13.11%), O (4.51%).

7-(1*H*-benzimidazol-2-yl)-5-(4-methoxyphenyl) pyrido [2,3-*d*]pyrimidin-4-amine (6f)

IR (KBr) (υ, cm^{-1}), 3423 (NH_2), 3047 (ArC-H), 1701 (C=N), 1587 (ArC=C), 1243 (C-O).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 3.59 (s, 3H, -OCH_3), 3.62 (s, 2H, -NH_2), 4.87 (s, 1H, -NH), 6.94-8.12 (s ,4H, Ar-H), 7.47-8.85 (s, 4H, Ar-H), 7.83 (s, 1H, Ar-CH), 8.84 (s, 1H, -C), LCMS m/z: 368 (M^+). Elemental analysis Calcd: C(68.47%), H(4.38%), N(22.81%), O (4.34%), Found: C(67.51%), H(4.46%), N(23.11%), O(4.92%).

7-(1*H*-benzimidazol-2-yl)-5-(3, 4, 5-methoxyphenyl) pyrido [2, 3-*d*] pyrimidin-4-amine (6g)

IR (KBr) (υ, cm^{-1}), 3403 (NH_2), 2997 (ArC-H), 1691 (C=N), 1607 (ArC=C), 1193 (C-O).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 3.91 (m, 9H, -OCH_3),4.19 (m, 2H, -NH_2), 4.76 (s, 1H, -NH),7.15 (s ,2H, Ar-H), 7.87-8.75 (s, 4H, Ar-H), 7.73 (s, 1H, Ar-CH), 8.76 (m, 1H,

-CH), LCMS m/z: 428 (M^+). Elemental analysis Calcd: C (64.48%), H (4.71%), N (11.62%), O (11.20%), Found: C (66.24%), H (4.76%), N (11.01%), O (9.99%).

7-(1*H*-benzimidazol-2-yl)-5-[4-(dimethylamino) phenyl] pyrido [2,3-*d*]pyrimidin-4-amine (6h)

IR (KBr) (υ, cm^{-1}), 3393 (NH_2), 3275 (ArC-H), 1721 (C=C), 1685 (ArC=C).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm), 2.64 (m, 6H, -CH_3),4.36 (s, 2H, -NH_2), 5.01 (s, 1H, -NH),6.97-7.84 (s ,4H, Ar-H), 7.76-8.55 (s, 4H, Ar-H), 8.31 (s, 1H, Ar-CH), 8.84 (m, 1H, -CH), LCMS m/z: 381 (M^+). Elemental analysis Calcd: C(69.27%), H(5.02%), N(15.70%), Found: C(71.41%), H(4.84%), N(13.75%).

Antibacterial activity[13]

The synthesized compounds were screened for their antibacterial activity against two gram positive organisms such as *Staphylococcus aureus, Bacillus stearothermophilus,* and two gram negative organisms such as *Escherichia coli* and *Salmonella typhi*. The technique used was agar diffusion method using 100 µg/100 mL of Penicillin and Streptomycin as standard for Gram positive and Gram negative respectively.

Antioxidant Activity[14]

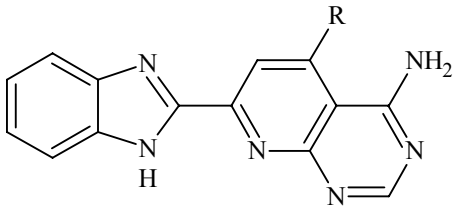
The antioxidant potential of all the compounds were screened by *in-vitro* free radical scavenging activity using DPPH (2, 2-diphenyl-1picryl hydrazyl) reduction method. Ascorbic acid was taken as the standard. Significant effect is not to be seen as most of the compounds had IC_{50} value near 10.

RESULTS AND DISCUSSION

Antibacterial activity of the synthesized compounds was expressed as zone of inhibition in mm Table 2. Antibacterial activity of the synthesized compounds **(6a-h)** revealed that moderate activity is shown by compounds **(6a-h)** against *S. aureus*. Similarly in the case of *Bacillus stearothermophilus* almost all compounds showed mild antibacterial activity. Medium activity is shown by almost all compounds within the series against *E. coli* and low antibacterial activity was shown by all the compounds against *S. typhi.* However, none of the compounds showed activity more than the standard.

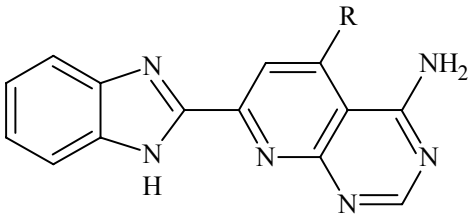
However, compared to other compounds **6a** and **6b** showed good antioxidant activity with IC_{50} value of 10µg/mL compared to the standard Table 3.

Table 2: Antibacterial study of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine (6a-h)



Compd. code	R	Antibacterial activity (Zone of inhibition in mm)			
		S. aureus	***E. coli***	***B. Stearothermophilus***	***S. typhi***
6a	C_6H_5	13	12	14	14
6b	FC_6H_4	16	11	14	17
6c	ClC_6H_4	18	16	11	16
6d	$Br_2C_6H_2Cl$	15	16	18	16
6e	OHC_6H_4	14	14	12	14
6f	$OCH_3C_6H_4$	16	15	11	12
6g	3,4,5-$OCH_3C_6H_2$	19	16	18	16
6h	$N(CH_3)_2C_6H_4$	21	12	18	16
Standard	Penicillin	-	22	-	24
	Streptomycin	22	-	23	-

Table 3. Antioxidant activity of 7-(1H-benzimidazol-2-yl)-5-(substituted phenyl) pyrido [2, 3-d] pyrimidin-4-amine (6a-h)



Compound code	R	Antioxidant activity (IC_{50} in µg/mL)
6a	C_6H_5	21
6b	FC_6H_4	25
6c	ClC_6H_4	39
6d	$Br_2C_6H_2Cl$	41
6e	OHC_6H_4	31
6f	$OCH_3C_6H_4$	36
6g	3,4,5-$OCH_3C_6H_2$	28
6h	$N(CH_3)_2C_6H_4$	32
Standard	Ascorbic acid	10.72

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