Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(3):25-32

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Synthesis and bioassay studies of substituted-*N*-(5-cyanopyrimidin-4yl)benzamides

P. Lavanya, K. Veena Vani, K. Vasu, M. Suresh, V. Jhansi Rani and C. Venkata Rao*

Department of Chemistry, S.V.University; Tirupati, India

ABSTRACT

4-Aminopyrimidine-5-carbonitrile (1) underwent facile condensation with various aromatic acid derivatives in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimidehydrochloride (EDC.HCl) and small catalytic amount of TEA afforded corresponding substituted-N-(5-cyanopyrimidin-4-yl)benzamide derivatives 3a-j. They were characterized by IR, ^{1}H, NMR and Mass spectral data. All the compounds were screened for anti-microbial, anti-oxidant activities.

Key words: Substituted-*N*-(5-cyanopyrimidin-4-yl)benzamide, spectral analysis, anti-microbial activity, anti oxidant.

INTRODUCTION

Heterocyclic chemistry is a very important branch of organic chemistry accounting for about 30% of modern publications. Of the wide variety of heterocyclic systems known today, the nitrogen heterocycles are of great importance as they are present in nucleic acids, vitamins, proteins and other biologically important molecular systems. Among different nitrogen heterocycles, the pyrimidine ring system is very important since several of its derivatives have been found to be medicinally useful. Recently there has been an increased demand for various types of pyrimidines and pyrimidine derivatives. Fused pyrimidinone derivatives have attracted the attention of numerous researchers over many years due to their important biological activities. It has been proved that some these heterocyclic compounds are effective as inhibitors of inflammatory mediators in intact cells[1], anti-tuberculosis[2] and human entero virus[3]. They also

show inhibitory activity towards both tubulin polymerization and cyclin-dependent kinase[4] prompted by these claims in continuing our synthetic studies on bioactive heterocycles[5]. 2-(phosphonomethoxy) alkyl derivatives of purine and pyrimidine bases-acyclic nucleoside phosphonates (ANPS)-Posses significant antiviral and cytostatic activity[6].

The nitrogen atom of the amide donates its lone pair electronics to C-N bond, both nucleophilicity of nitrogen and electrophilicity of carbonyl are hence decreased. There are several ways to activate the amide functional group in the literature. In the presence of a suitable nucleophile, a variety of compounds such as pipiridines[7], carboxylic acid derivatives[8] and pyrimidines[9] were thus constructed via this electrophilic activation of amide path way.

EXPERIMENTAL SECTION

The IR spectra were recorded in KBr discs (υ_{max} in cm^{-1}) on Perkin-Elmer FT-IR spectrophotometer. The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument. Mass spectra under electron impact conditions (EI) were recorded at 70 ev ionizing voltage with a VG Prospec instrument and the presented as m/z (% rel int.). Elemental analyses (C, N, H) results were found to be in good agreement with the calculated values. Melting points were determined with Thomas Hoover melting point apparatus and are uncorrected. TLC monitored all reactions and purity of the synthesized compounds.

General procedure for the preparation of Substituted-*N*-(5-cyanopyrimidin-4-yl)benzamide derivatives (3a-j):

A solution of respective aromatic substituted acids **2a-j** (1.1mmol) in DCM was added to a stirred solution of 4-Aminopyrimidine-5-carbonitrile (**1**) (1mmol) in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimidehydrochloride(EDC.HCl) (1.5mmol) and catalytic amount of dry TEA in a cool condition. After completion of the addition, stirring was continued for 4-6 h and the progress of the reaction was monitored by TLC. The reaction mixture was filtered, washed with $NaHCO_3$ solution and filtrated under reduced pressure to get the solid. The solid was recrystallization from ethanol.

N-(5-Cyanopyrimidin-4-yl)benzamide **3a:** Yield was found to be 88%, mp 165-166°C. IR (KBr) cm^{-1}: 3312 (NH), 2226 (CN), 1679 (C=O), 1574 (C=N). ^{1}H NMR ($CDCl_3$), δ, ppm: 7.5-8.0 (m, 5H Ar-H), 8.61 (s,1H, NH), 9.0 (s, 1H), 9.17 (s, 1H). Mass spectrum, m/z: 225 $(M+1)^+$. *Anal.* Calculated. For $C_{12}H_8N_4O$: C, 64.28; H, 3.60; N, 24.99. Found, %: C, 64.22; H, 3.56; N, 24.95.

N-(5-Cyanopyrimidin-4-yl)-2-methylbenzamide **3b:** Yield was found to be 83%, mp 203-204°C. IR (KBr) cm^{-1}: 3320(NH), 2228 (CN), 1680 (C=O), 1578(C=N). ^{1}H NMR ($CDCl_3$), δ, ppm: 2.6(s, 3H, CH_3) 7.3-7.68 (m, 4H, Ar-H), 8.30(s, 1H, NH), 8.98(s, 1H), 9.11(s,1H). Mass spectrum, m/z: 239 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found, %: C, 65.510H, 4.18; N, 23.48.

N-(5-Cyanopyrimidin-4-yl)-4-methylbenzamide **3c***:* Yield was found to be 84%, mp 251-252°C. IR (KBr) cm^{-1}: 3292 (NH), 2237 (CN), 1675 (C=O), 1581 (C=N) ^{1}H NMR ($CDCl_3$), δ, ppm: 2.25 (s, 3H, CH_3), 7.37-7.4 (d, 2H), 7.81-7.90 (d, 2H), 8.58 (s,1H, NH), 8.99 (s, 1H, NH), 9.16

(s, 1H). Mass spectrum, m/z: 239 (M+1). *Anal.* Calculated. For $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found, %: C, 65.50; H, 4.19; N, 23.48.

N-(5-Cyanopyrimidin-4-yl)-4-nitrobenzamide **3d:** Yield was found to be 79%, mp 225-226°C. IR (KBr) cm^{-1}: 3279 (NH), 2234 (CN), 1678 (C=O), 1582 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 8.19-8.21(d, 2H), 8.41-8.50 (d, 2H), 8.77(s, 1H, NH), 9.12(s, 1H), 9.2(s, 1H). Mass spectrum, m/z: 270 (M+1). *Anal.* Calculated. For $C_{12}H_7N_5O_3$: C, 53.54; H, 2.62; N, 26.01. Found, %: C, 53.50; H, 2.57; N, 25.98.

4-Chloro-N-(5-cyanopyrimidin-4-yl)benzamide **3e:** Yield was found to be 90%, mp 238-239°C. IR (KBr) cm^{-1}: 3298 (NH), 2229 (CN), 1676 (C=O), 1580 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 7.5-7.6 (d, 2H), 7.85-7.99 (d, 2H), 8.6 (s, 1H, NH), 9.0 (s, 1H), 9.15 (s, 1H). Mass spectrum, m/z: 259 $(M+1)^+$. *Anal.* Calculated. For $C_{12}H_7ClN_4O$: C, 55.72; H, 2.73; N, 21.66. Found, %: C, 55.69; H, 2.70; N, 21.61.

4-Bromo-N-(5-cyanopyrimidin-4-yl)benzamide **3f:** Yield was found to be 81%, mp 224-225°C. IR (KBr) cm^{-1}: 3316 (NH), 2235 (CN), 1676 (C=O), 1579 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 7.38-7.44 (d, 2H), 7.76-7.82 (d, 2H), 8.62 (s, 1H, NH), 8.95 (s, 1H), 9.10 (s, 1H). Mass spectrum, m/z: 304 $(M+1)^+$. *Anal.* Calculated. For $C_{12}H_7BrN_4O$: C, 47.55; H, 2.33; N, 18.48. Found, %: C, 47.53; H, 2.35; N, 18.44.

N-(5-Cyanopyrimidin-4-yl)-4-methoxybenzamide **3g:** Yield was found to be 83%, mp 202-203°C. IR (KBr) cm^{-1}: 3302 (NH), 2236 (CN), 1679 (C=O), 1584 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 4.05 (s, 3H), 7.40-7.44 (d, 2H), 7.9-7.94 (d, 2H), 8.6 (s, 1H, NH), 9.0 (s, 1H), 9.15 (s, 1H). Mass spectrum, m/z: 255 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found, %: C, 61.37; H, 3.89; N, 22.09.

4-Chloro-N-(5-cyanopyrimidin-4-yl)-2-methylbenzamide **3h:** Yield was found to be 85%, mp 235-236°C. IR (KBr) cm^{-1}: 3312 (NH), 2234 (CN), 1686 (C=O), 1589 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 2.65 (s, 3H), 7.42-7.68 (m, 3H), 8.70 (s, 1H, NH), 9.10 (s, 1H), 9.21 (s, 1H). Mass spectrum, m/z: 273 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_9ClN_4O$: C, 57.26; H, 3.33; N, 20.55. Found, %: C, 57.29; H, 3.30; N, 20.51.

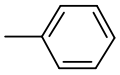
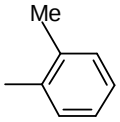
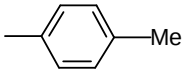
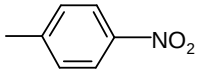
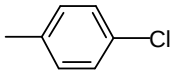
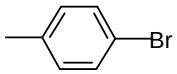
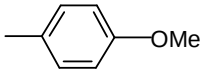
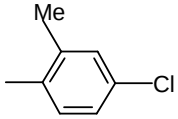
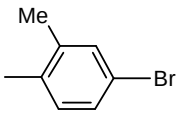
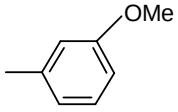
4-Bromo-N-(5-cyanopyrimidin-4-yl)-2-methylbenzamide **3i:** Yield was found to be 86%, mp 252-253°C. IR (KBr) cm^{-1}: 3326 (NH), 2215 (CN), 1676 (C=O), 1582 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 2.55 (s, 3H), 7.34-7.59 (m, 3H), 8.62 (s, 1H, NH), 9.05 (s, 1H), 9.12 (s, 1H). Mass spectrum, m/z: 318 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_9BrN_4O$: C, 49.23; H, 2.86; N, 17.67. Found, %: C, 49.25; H, 2.79; N, 17.69.

N-(5-Cyanopyrimidin-4-yl)-3-methoxybenzamide **3j:** Yield was found to be 89%, mp 244-245°C. IR (KBr) cm^{-1}: 3319 (NH), 2235 (CN), 1678 (C=O), 1581 (C=N). ^1H NMR ($CDCl_3$), δ, ppm: 3.98 (s, 3H), 7.35-7.65 (m, 4H), 8.45 (s, 1H, NH), 8.85 (s, 1H), 9.05 (s, 1H). Mass spectrum, m/z: 255 $(M+1)^+$. *Anal.* Calculated. For $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found, %: C, 61.38; H, 3.98; N, 22.12.

SCHEME

1 **2 a-j** **3 a-j**

Synthetic Scheme for 3a-j

Compound	R
3a	
3b	
3c	
3d	
3e	
3f	
3g	
3h	
3i	
3j	

Antimicrobial Testing

The compound 3**a-j** was tested for in vitro antimicrobial activity at two different concentrations 100 and 200/µg per disc. The antibacterial activity was screened against *Staphylococcus aureus,*

Bacillus subtilis (Gram-positive bacteria) and *Proteus vulgaris, Klebsiella pneumoniae* (Gram-nagative bacteria) on nutrient agar plates at 37 ^{0}C for 24 hrs using chloramphenicol as reference. The compounds were also evaluated for their antifungal activity against *Aspergillus niger* and *Pencillium chrysogenium* using fluconazole as standard drug. The fungi cultures were grown on potato dextrose agar (PDA) medium at 25 ^{0}C. The spore suspension was adjusted to 10^6 pores ml^{-1} at an mg ml^{-1} concentration by the Vincent and Vincent method.

Table 1. Antibacterial Activity of the Target Compounds 3a-j

Compound	Concentration	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
	(µg)	*S.aureus*	*B.subtilis*	*P.vulgaris*	*K.pneumoniae*
3a	100	19	21	17	18
	200	26	26	21	23
3b	100	29	32	31	33
	200	36	39	41	38
3c	100	24	22	21	23
	200	31	25	24	29
3d	100	12	11	14	11
	200	13	14	19	17
3e	100	31	27	24	27
	200	36	31	28	27
3f	100	25	26	19	22
	200	31	32	26	25
3g	100	11	13	13	14
	200	15	11	16	18
3h	100	11	12	11	11
	200	15	18	12	14
3i	100	28	31	22	21
	200	33	34	25	23
3j	100	23	23	20	22
	200	33	29	23	27
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

** c = 100 µg / ml; * c = 200 µg / ml*

Table 2. Antifungal Activity* of the Target Compounds 3a-j

Compound	Concentration	Zone of Inhibition (mm)	
	(µg/ml)	*A.niger*	*P.chrysogenium*
3a	100	25	20
	200	26	26
3b	100	12	14
	200	20	17
3c	100	27	25
	200	32	30
3d	100	32	15
	200	38	19
3e	100	12	26
	200	19	29
3f	100	25	30
	200	27	32

3g	100	30	26
	200	34	28
3h	100	14	16
	200	21	18
3i	100	30	28
	200	38	34
3j	100	32	34
	200	36	36
Fluconazole	100	38	42
	200	42	44

** c = 100 μg / ml; * c = 200 μg / ml*

Antioxidant Testing: The compounds **3a-j** is tested for antioxidant property by nitric oxide and DPPH methods.

Assay for Nitric Oxide (NO) Scavenging Activity

Sodium nitroprusside (5μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in a suitable solvent (methanol) and tubes were incubated at 25^0C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals 0.5 ml of incubation solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at 546 nm.

TABLE 3: Antioxidant Activity* of the Target Compounds 3a-j

Compound	% Inhibition at 100 μM	
	Nitric oxide method	DPPH method
3a	80.15	84.74
3b	30.25	38.12
3c	89.14	93.65
3d	24.21	27.75
3e	72.23	70.25
3f	79.10	76.85
3g	22.25	35.22
3h	25.78	24.15
3i	72.14	73.05
3j	94.20	92.35

** c = 100 μM*

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical (DPPH Method)

The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100 μM) were added to DPPH (100 μM) in ethanol. The tubes were kept at an

ambient temperature for 25 minutes and the absorbance was measured at 517 nm. The difference between the test and the control experiments was taken and expressed as the percentage scavenging of the DPPH radical.

RESULTS AND DISCUSSION

The results of the compounds of preliminary antimicrobial testing are shown in Tables-1 and 2. The results revealed that the inhibitory activity against Gram-positive bacteria was higher than Gram-negative bacteria. The pyrimidine derivatives 3**d, 3g,** and **3h** were displayed least activity. The compounds **3a, 3b, 3c, 3e, 3f, 3i**, and **3j** showed excellent activity against Gram-positive bacteria (inhibitory zone>25mm) and good activity against Gram negative bacteria (inhibitory zone>20mm). All the test compounds **3a, 3c, 3d, 3f, 3g, 3i** and **3j** excellent activity and compounds **3b, 3e** and 3**h** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Aspergillus niger*. Compounds **3a, 3c, 3e, 3f, 3g, 3i and 3j** excellent activity and compounds **3b, 3d** and **3h** exhibited moderate activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Pencillium chrysogenium* (Table 1 & 2). The compounds **3a, 3c, 3e, 3f, 3i,** and **3j** exhibited high antioxidant property and compounds **3b, 3d, 3g, 3h** exhibited moderate activity in both **Nitric Oxide** and **DPPH** methods at 100 µM concentration (Table 3).

REFERENCES

[1] J.M. Quintela, C. Peinador, L. Gonzalez, I. Devesa, M.L. Ferrandiz, M.J. Alcaraz, R. Riguera, *Bioorg. Med. Chem*., **2003**, 11, 863–868.
[2] O. Moukha-Chafiq, M.L. Taha, H.B. Lazrek, C. Pannecouque, M. Witvrouw, E. De Clercq, J.L. Barascut, J.L. Imbach, Nucleos. Nucleot. *Nucleic Acids*, **2001**, 20**,** 1797– 1810.
[3] J.H. Chern, K.S. Shia, T.A. Hsu, C.L. Tai, C.C. Lee, Y.C. Lee, C.S. Chang, S.N. Tseng, S.R. Shih, *Bioorg. Med. Chem. Lett*., **2004**, 14**,** 2519–2525.
[4] (a) V. Krystof, D. Moravcova, M. Paprskarova, P. Barbier, V. Peyrot, A. Hlobilkova, L. Havlicek, M. Strnad, *Eur. J. Med. Chem*., **2006**, 41, 1405–1411. (b) J.A. Markwalder, M.R. Arnone, P.A. Benfield, M. Boisclair, C.R. Burton, C.H. Chang, S.S. Cox, P.M. Czerniak, C.L. Dean, D. Doleniak, R. Grafstrom, B.A. Harrison, R.F. Kaltenbach, D.A. Nugiel, K.A. Rossi, S.R. Sherk, L.M. Sisk, P. Stouten, G.L. Trainor, P. Worland, S.P. Seitz, *J. Med. Chem*., **2004**, 47**,** 5894– 5911. (c) D.C. Kim, Y.R. Lee, B.S. Yang, K.J. Shin, D.J. Kim, B.Y. Chung, K.H. Yoo, Eur. *J. Med. Chem*., **2003**, 38, 525–532.
[5] (a) M. Bakavoli, M. Nikpour, M. Rahimizadeh, *J. Heterocycl. Chem*., **2006**, 43**,** 1327–1329. (b) M. Rahimizadeh, M. Nikpour, M. Bakavoli, *J. Heterocycl. Chem*., **2007,** 44**,** 463–465. (c) M. Bakavoli, E. Mollashahi, S.M. Seyedi, M. Rahimizadeh, *Heterocyclic. Commun.*, **2007,** 13**,** 283–288. (d) M. Bakavoli, H. Raisi, J. Tajabadi, M. Karimi, A. Davoodnia, M. Nikpour, *J. Sulfur Chem*., **2008**, 29**,** 25–30. (e) M. Bakavoli, M. Nikpour, M. Rahimizadeh, M.R. Saberi, H. Sadeghian, *Bioorg. Med. Chem*., **2007**, 15**,** 2120–2126. (f) M. Bakavoli, H. Sadeghian, Z. Tabatabaei, E. Rezaei, M. Rahimizadeh, M. Nikpour, *J. Mol. Mod*., **2008**, 14, 471–478.
[6] (a) Holy, A. In Advances in Antiviral Drug Design; De Clercq, E., Ed.; JAI: *Greenwich, CT*., **1994**, pp 179–232. (b) Holy, A. In Recent Advances in Nucleosides: *Chemistry and Chemotherapy*; Chu, C. K., Ed.; *Elsevier*, **2002**, pp 167–238.

[7] Charette, A. B.; Grenon, M.; Lemire, A.; Pourashraf, M.; Martel, J. *J. Am. Chem. Soc*., 2001**,** 123, 11829.
[8] Charette, A. B.; Grenon, M. *Can. J. Chem*., **2001**, 79, 1694.
[9] Movassaghi, M.; Hill, M. D. *J. Am. Chem. Soc*., **2006**, 128, 14254.