IRJSE International Research Journal of Science & Engineering *Int. Res. J. of Science & Engineering,* 2020; Special Issue A7:127-132 SJIF Impact Factor 6.68
ISSN: 2322-0015
RESEARCH ARTICLE **OPEN ACCESS**

Design, Synthesis, Characterization and Antimicrobial Activity of 1,6-dihydro-2-methyl-4-(substituted)-6-oxopyrimidine-5-carbonitrile Derivatives.

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Manuscript Details
Available online on http://www.irjse.in
ISSN: 2322-0015

Cite this article as:
Kalyankar BD, Shewate VT, Yadao SB and Wasekar CP. Design, Synthesis, Characterization and Antimicrobial Activity of 1,6-dihydro-2-methyl-4-(substituted)-6-oxopyrimidine-5 carbonitrile Derivatives, *Int. Res. Journal of Science & Engineering,* February 2020, Special Issue A7: 127-132.

ABSTRACT

Synthesis of novel heterocyclic 1,6-dihydro-2-methyl-4-(methylthio)-6-oxopyrimidine-5-carbonitrile (3) was prepared by condensing acetamidine hydrochloride (1) with ethyl-2-cyano-3,3-bis(methylthio)acrylate (2) in DMF and potassium carbonate as catalyst. Compound (3) has methylthio group at fourth position, which is replaced by different nucleophiles such as substituted anilines, phenols, hetryl amines and compounds containing active methylene group (4a-4p) to afford 1,6-dihydro-2-methyl-4-(substituted)-6-oxopyrimidine-5 carbonitrile derivatives (5a-5p). The newly synthesized compounds were characterized by IR, ^{1}H-NMR, Mass spectral analysis. Furthermore, these synthesized compounds were tested for antioxidant and antimicrobial activity.

Keywords: Acetamidine hydrochloride, ethyl-2-cyano-3,3-bis(methylthio)acrylate, DMF and potassium carbonate.

INTRODUCTION

Pyrimidines are interesting class of nitrogen containing compound which are basically found in bio-organic and medicinal chemistry. The pyrimidine heterocyclic core is an important subunit because of its widespread abundance in the basic structure of numerous natural products [1].

The presence of pyrimidine base in thymine, cytosine and uracil which are important building blocks of nucleic acid is one possible reason for their widespread therapeutic application. It is also found in many synthetic compounds such as barbiturates and HIV drugs zidovudine. In addition to this various analogues of pyrimdines have been found to posses antibacterial [2], antifungal [3], antidiabetic [4], anti-inflammatory [5], antiallergic [6], analgesic [7], anticonvulsant [8], antipyretic [9], antiviral [10], CNS-depressant [11] herbicidal [12], anticancer activities [13].

By survey of literature it is found that number of synthetic methods are available for the preparation of pyrimidines [14-21]. Acetamidine hydrochloride is a di-nucleophilic in nature and efficient precursor which have been extensively utilised in heterocyclic synthesis. Ram Vishnu and co-workers reported the synthesis of 4-amino-2-methyl-6-(methylthio) pyrimidine-5-carbonitrile from acetamidine hydrochloride and bis(methylthio)methylene malononitrile [22].

Keeping in view of divers antimicrobial activities of pyrimidines, it was thought to construct a novel system which may combine with bioactive rings together in same framework by different methodology. Hence as a part of our ongoing program to develop efficient and robust method for the preparation of biologically relevant substituted pyrimidines and its different derivatives. Pyrimidine was synthesized by condensation of acetamidine hydrochloride and ethyl-2-cyano-3,3-bis(methylthio)acrylate. Further these compounds were treated with different substituted nucleophiles such as aryl amines, phenols, heteryl amines and active methylene compounds to obtain 4-substituted derivatives of pyrimidines.

METHODOLOGY

Electrothermal IA 9000 SERIES digital melting point apparatus was used to determine the melting points of synthesized compounds and were uncorrected. Homogeneity of all the compounds were routinely checked on 0.2 mm silica gel-C plates using ethyl acetate:hexane (3:7) as irrigant, the spots were examined under UV light chamber. Infrared spectra were recorded in Nujol or as potassium bromide pallets on infrared spectrophotometer, nuclear magnetic resonance spectra were obtained on Brukner advance spectrophotometer 400 MHz in DMSO-d6 using tetramethylsilane (TMS) as internal reference, Mass spectra were recorded on FT-VC-7070 H Mass spectrometer using the EI technique at 70 eV. All the reactions were carried out under ambient atmosphere.

General procedure:

Synthesis of 1,6-dihydro-2-methyl-4-(methylthio)-6-oxopyrimidine-5-carbonitrile (3).

A mixture acetamidine hydrochloride (1) (0.01mol) and ethyl-2-cyano-3,3-bis(methylthio)acrylate (2) (0.01mol) in 10 ml of DMF and anhydrous potassium carbonate (10mg) was refluxed for 8 hours. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of reaction, the reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from DMF-ethanol mixture to give pure compound (3).

Synthesis of 1,6-dihydro-2-methyl-4-(substituted)-6-oxopyrimidine-5-carbonitrile derivatives (5a-5p)

A mixture of compound (3) (0.001mol) refluxed independently with substituted anilines/ phenols/ hetryl amines/ compound containing active methylene groups (4a-4p) (0.001mol) in 10 ml of DMF and anhydrous potassium carbonate(10mg) for 6 hours. The reaction progress was checked by TLC. The reaction mixture was cooled to room temperature and poured in to ice cold water. The separated solid product was filtered, washed with water and recrystalized from DMF-ethanol (2:8) mixture to give pure compounds (5a-5p).

Spectral Analysis:

1,6-dihydro-2-methyl-4-(methylthio)-6-oxopyrimidine-5-carbonitrile (3)

Brown powder, Yield 75%, m.p. 144°C. IR (KBr/cm^{-1}) 2252 (CN), 3284 (-NH stretch): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.36 (s, 3H, CH_3), 2.58 (s, 3H, SCH_3), 7.8 (s, 1H, NH): ^{13}C NMR (DMSO-d_6): δ = 13.64

(SCH_3), 24.18 (CH_3), 82.29 (C-CN), 115.64 (CN), 164.15 (C=N), 166.04 (C=O), 173.22 (C-SCH_3) EI-MS(m/z: RA%): $C_7H_7N_3OS$, 181 (M^+).

1,6-dihydro-2-methyl-4-(*p*-tolylamino)-6-oxopyrimidine-5-carbonitrile (5a)

Yellow powder, Yield 58.39%, m.p. 208 °C. IR (KBr/cm^{-1}) 2237 (CN), 3351 (-NH stretch): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.11 (s, 3H, Ar-CH_3), 2.31 (s, 3H, Ar-CH_3), 6.5-6.9(m, 4H, Ar-H), 7.10 (s, 1H, NH), 8.4 (s, 1H, NH): EI-MS(m/z: RA%): $C_{13}H_{12}N_4O$, 240 (M^+).

1,6-dihydro-2-methyl-4-(*p*-tolyloxy)-6-oxopyrimidine -5-carbonitrile (5e)

Pale Yellow powder, Yield 53.01%, m.p. 166°C. IR (KBr/cm^{-1}) 2264 (CN), 3228 (-NH stretch): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.18 (s, 3H, Ar-CH_3), 2.27 (s, 3H, Ar-CH_3), 6.7-7.2 (m, 4H, Ar-H), 7.9 (s, 1H, NH): EI-MS (m/z: RA%): $C_{13}H_{11}N_3O_2$, 241 (M^+).

1,6-dihydro-2-methyl-4-(dicyanomethyl)-6-oxopyrimidine-5-carbonitrile (5i)

Brown powder, Yield 62.24%, m.p. 187°C. IR (KBr/cm^{-1}) 2263 (CN), 3296 (NH_2): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 2.29 (s, 3H, CH_3), 3.38 (s, 1H, CH), 7.7 (s, 1H, NH): EI-MS (m/z: RA%): $C_9H_5N_5O$, 199 (M^+).

1,6-dihydro-2-methyl-4-(pyrrolidine-1-yl)-6-oxopyrimidine-5-carbonitrile (5m)

Brown powder, Yield 58.16%, m.p. 211°C. IR (KBr/cm^{-1}) 2214 (CN), 3378 (NH_2): ^{1}HNMR (400 MHz,DMSO-d_6): δ = 1.2 (t, 4H, CH_2), 2.6 (t, 4H, CH_2), 2.3 (s, 3H, CH_3), 8.10 (s, 1H, NH): EI-MS (m/z: RA%): $C_{10}H_{12}N_4O$, 204 (M^+).

RESULTS AND DISCUSSION

In present communication we wish to report new, simple and chief method for synthesis of 1,6-dihydro-2-methyl-4-(methylthio)-6-oxopyrimidine-5-carbonitrile and its 4-substituted derivatives. In our first scheme we condensed acetamidine hydrochloride (1) and ethyl-2-cyano-3,3-bis(methylthio)acrylate (2) in DMF and catalytic amount of anhydrous K_2CO_3 to afford (3) **Scheme-1**.

The compound (3) posseses replaceable active methyl-thio group which is activated by nitrogen atom, electron withdrawing cyano group. When compound (3) (1mole) was condensed independently with substituted anilines/ phenols/ hetryl amines/ compound containing active methylene groups in DMF and catalytic amount of anhydrous K_2CO_3 to afford 1,6-dihydro-2-methyl-4-(Substituted)-6-oxopyrimidine -5-carbonitrile derivatives (5a-5p) **Scheme-2.**

The structure of newly synthesized compounds were assigned on the basis of IR, ^{1}HNMR, Mass spectral data.

IR Specrums: In IR spectrum of compounds absorption bands appear in the region 2270-2210 and 3400-3200 $cm^{-1}$for -CN, -NH stretch respectively.

^{1}HNMR spectrum: ^{1}HNMR spectra of compounds shows singlet peaks in the region of 2.1-2.4 and 7.1-8.1 ppm due to -CH_3, -NH protons respectively .

^{13}CNMR spectrum: ^{13}CNMR spectrum of parent compound (3) shows peaks at 13.64, 24.18, 115.64 , 166.04 and 173.22 for –SCH_3, -CH_3, -CN, -C=O and -C-SCH_3 carbons respectively.

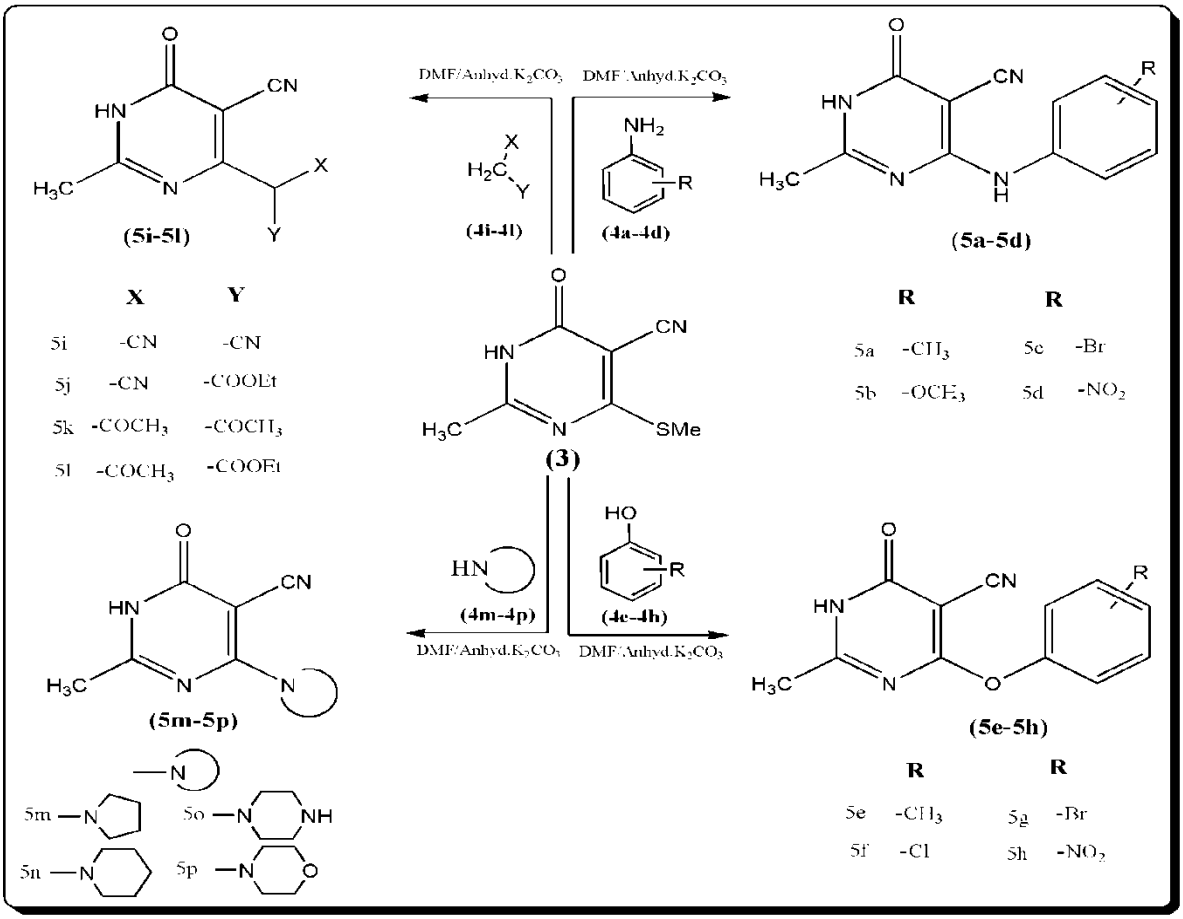
MASS spectrum: MASS spectra shows molecular ion peak which is corresponds to the molecular weight of respective compounds.

Spectral studies of all compounds shows that compounds were stable and do not exhibit any tautomerism. All the compounds were screened for their antibacterial activities. Investigation of antimicrobial activity, it was found that 1,6-dihydro-2-methyl-4-(Substituted)-6-oxopyrimidine-5-carbonitrile derivatives (5b), (5h) and (5k) showed higher activity against all the micro-organisms employed for antimicrobial screening.

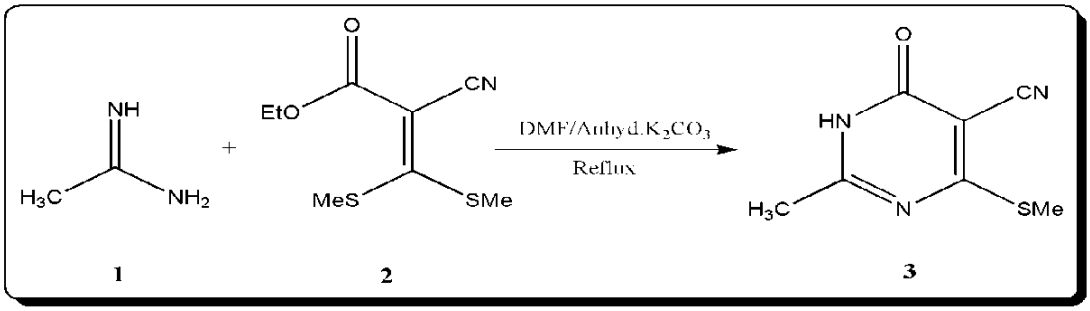
In summary, most of our synthesized compounds showed high and moderate activity against *Bacillus subtilis, Staphylococcus aureus,* and *Salmonella typhi*.

Table 1.Antimicrobial activity of compound (5a-5p)

Compounds	Gram positive		Gram negative	
	B.subtilis	*S.aureus*	*S.typhi*	*E.Coli*
5a	11	07	10	--
5b	14	19	22	15
5c	10	13	12	08
5d	06	12	10	14
5e	13	16	09	10
5f	12	08	15	12
5g	--	07	10	--
5h	19	24	21	16
5i	08	14	09	10
5j	15	12	10	08
5k	20	15	21	23
5l	13	10	12	--
5m	--	12	08	06
5n	10	13	18	12
5o	07	12	10	08
5p	08	13	16	12
Streptomycin	22	--	--	19
Penicillin	--	24	20	--



Scheme-2. Formation of 1,6-dihydro-2-methyl-4-(Substituted)-6-oxopyrimidine-5-carbo nitrile derivatives (5a-5p)



Scheme-2. Formation of 1,6-dihydro-2-methyl-4-(methylthio)-6-oxopyrimidine-5-carbonitrile (3)

ANTIMICROBIAL ACTIVITY

All synthesized compounds were evaluated for their antimicrobial screening against different pathogenic micro-organisms (gram +ve and gram -ve) such as *Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Escherichia coli.* The technique used in this experiment was paper disk diffusion method. To studying the activities of these compounds streptomycin and penicillin were used as standard drugs. All the compounds were dissolved in dimethyl sulphoxide (100µg/ml in DMSO). For bacterial growth incubation period was 24 hours at temperature 37°c. Activity of compounds was determined by measuring the diameter of zone of inhibition, values obtained was compared with the values produced from standard drugs like streptomycin and penicillin. From all synthesized compounds (5b), (5h) and (5k) shows comparative activity with standard drugs (streptomycin and penicillin). The newly synthesized compounds show zone of inhibition 5-24 mm in diameter where as standard streptomycin exhibit zone of inhibition 19-24 mm in diameter.

CONCLUSION

This work describe proficient and absolute method for the synthesis of novel heterocyclic compounds such as 1,6-dihydro-2-methyl-4-(substituted)-6-oxopyrimidine-5-carbonitrile derivatives by simple and efficient route with good product yield. This protocol includes some important advantages such as mild reaction condition, easy work-up, product purity and short reaction time.

Acknowledgement: The authors are grateful to Principal, Yeshwant Mahavidyalaya, Nanded, for providing laboratory facilities, School of Life Science, SRTMU, Nanded, for providing antioxidant activity and the Director, Panjab University, Chandigarh for providing spectra.

Conflicts of interest: The authors stated that no conflicts of interest.

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