



SYNTHESIS, CHARACTERIZATION, AND EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF SOME CYANOPYRIDINE DERIVATIVES

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ABSTRACT

A new series of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino)phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile derivatives had been prepared. All the compounds (7a-h) were prepared by conventional methods and characterized by IR, ¹HNMR, and Mass spectra. The compounds were evaluated for their anti-bacterial against species like *E. coli*, *Salmonella typhi*, and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum*. Compounds having methoxy and chloro group substitution show better activity than other compounds.

Keywords: Trichlorotriazine, Malono Nitrile, Ammonium Acetate, s-Triazine, Cyanopyridine Antimicrobial.

1. INTRODUCTION

1,3,5-Triazine based chalcones and their derivatives show biological activities hence these derivatives studied extensively for their biological activity [1-13]. Due to the various properties of chalcones we have synthesized s-triazine based chalcones. Bremner et al. have prepared 2',4',6'-trihydroxychalcone and evaluated the microbial activity of the known compounds [2]. Nielsen et al. performed a study and showed that the capability of changing the proteolytic properties of the 4-hydroxy group rises acidity and replacing the hydroxy group with carboxylic acids or a bioisoster of this group [3]. Li et al. showed that a new class of chemical types, chalcone (1,3-diphenyl-2-propen-1-one) and its derivatives, as possible novel antimalarials that are active against chloroquine-resistant strains of *P. falciparum* [4]. Liu et al. studied hydroxylated chalcones that have been prepared and estimated for *in vitro* antimalarial activity [5]. Go et al. investigated that chalcones have antiplasmodial activities [6, 13]. Antileishmanicidal activity of chalcones were studied by zhai et al. [7]. Farma'cia, et al. reported promising leishmanicidal and trypanocidal activities [8]. Lin et al presented the anti-tuberculosis activity by chalcone-like compounds, flavones, and flavanones [9]. Modzelewska et al. studied the inhibition of breast cancer cell lines [10]. Rao et al prepared chalcones which showed important aspects of chalcone pharmacology and

toxicology [11]. Svetaz et al. reported the separation and identification of antifungal compounds [12]. Krivokolysko et al. prepared cyano derivatives [14]. Pavia et al reported that 6-Alkoxy-N,N-disubstituted-2-pyridinamine as anticonvulsant agents [15]. Cyanopyridine derivatives [14] have received notable interest in view of their exceptional significance as anticonvulsant, [15] antifungal, antibacterial, herbicidal, antihypertensive, antiepileptic, antitubercular, analgesic, insecticidal, anti-psoriasis, anti allergic, anti-inflammatory properties. Therefore preparation of cyanopyridines is of interest because of their widespread prevalence in biologically active derivatives. Hence, sizable interest has been centered on efficient and pharmaceutical important cyanopyridine derivatives. So much importance and as a part of our research work in the field of synthesis containing s-triazine moiety, we have synthesized a series of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino)phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile derivatives and carried their characterization, antibacterial and antifungal study [16-18].

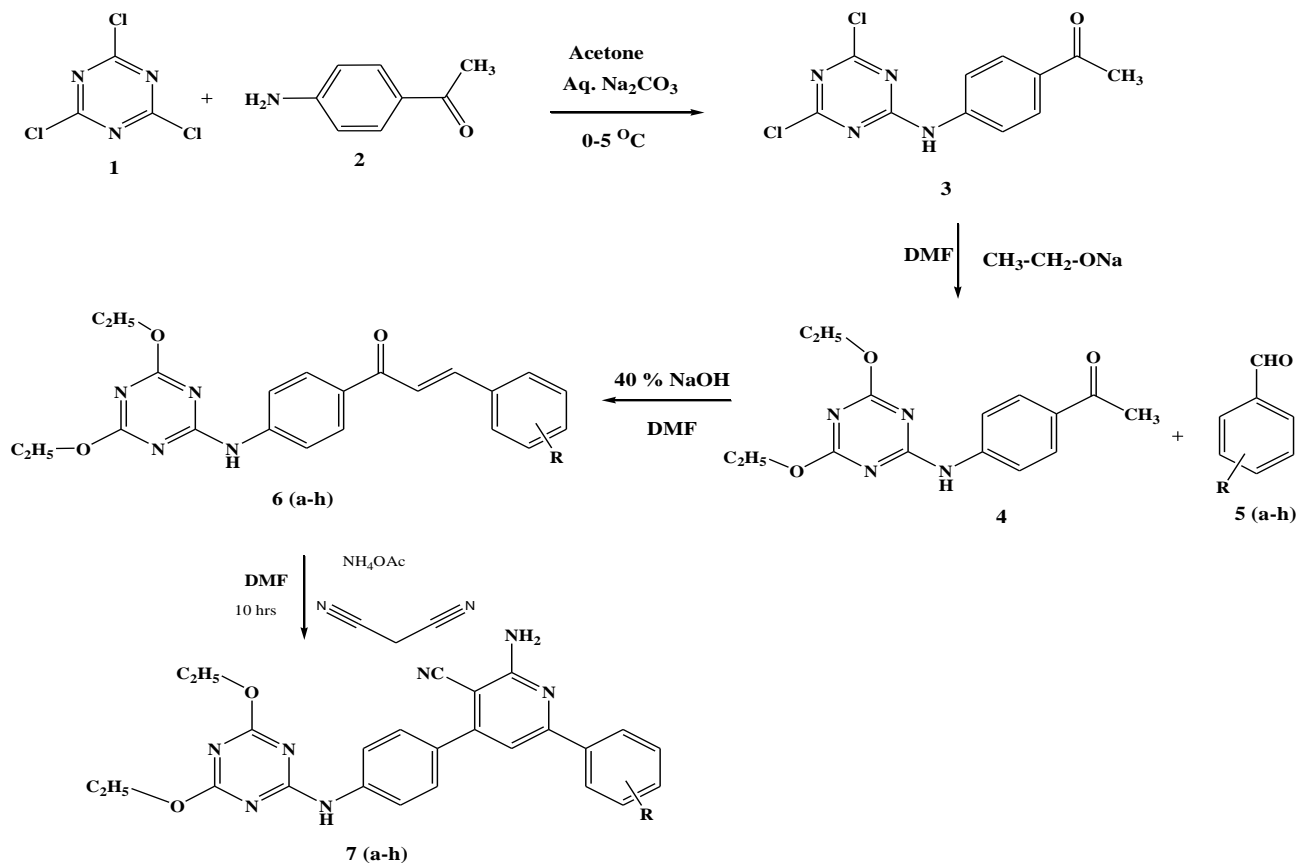
2. MATERIAL AND METHODS

2.1. Experimental

Melting points of all compounds were taken in open capillary tubes hence may be incorrect. IR Spectra were

recorded on Perkin-Elmer spectrometer. ^1H NMR spectra were recorded by the use of Bruker Advance II 400 spectrometer in DMSO and TMS as inner standard.

Thin layer chromatography was performed by using a TLC plate of silica gel spots that were visualized by an Iodine chamber to identify the purity of compounds.



Scheme 1: Preparation of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile (7a-h) [16, 18].

2.2. General Procedure

2.2.1. Preparation of 1-(4-(4,6-dichloro-1,3,5-triazin-2-yl amino) phenyl)ethanone (3)

4-amine acetophenone (0.01 M) was added bit by bit to cyanuril chloride (0.01 M) in acetone (30 ml) with continued stirring over a length of four hr at 0°C to 5°C . Sodium carbonate (0.5 M) was dissolved in water (10 ml) and conveyed a drop reasonable to neutralize HCl created sooner or later in the response. At last, the product was poured into crushed ice. Then the mixture was separated, filtered, and washed with water. Then it was dried and recrystallized from alcohol to get (3) [18].

2.2.2. Preparation of 1-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)ethanone (4)

1-(4-(4,6-dichloro-1,3,5-triazin-2-yl amino) phenyl)ethanone (3) (0.01 M) was gradually added to sodium

ethoxide (0.02 M) with consistent stirring in DMF: H_2O (9:1 ml) over a length of four hours at RT and refluxed for four hour at 80°C . The items were poured into super-cold water and sifted. The product was recrystallized from DMF [18].

2.2.3. Preparation of substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl amino)phenyl)-3-phenylprop-2-en-1-one (Chalcones) (6a-h)

Compound no. 4 (0.01 M) was dissolved in DMF (25 ml) and substituted benzaldehyde (5a-h) (0.01 M) with stirred continuously at room temperature for 30 min, then, at that point, sodium hydroxide (40% w/v) was added at RT for 24 hrs. The advancement of reaction was observed through TLC. Crushed ice was added and neutralized with HCl. The product was isolated, washed with water, dried, and recrystallized from DMF to get the product (Chalcone) (6a-h) [16, 18].

2.2.4. Preparation of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile (7a-h)

A mixture of substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino)phenyl)- 3-phenylprop-2-en-1-one (Chalcone) (6a-h) (0.01 mole), malono nitrile (0.01mole) and ammonium acetate in DMF (0.01 mole) was refluxed for 10Hrs. Afterward, this reaction mixture was cooled and poured into ice-cold water. Solid product was filtered and recrystallized from DMF (7a-h) [16].

3. RESULTS AND DISCUSSION

3.1. Spectral Data (7a-7h)

3.1.1. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-ptolyipyridine-3-carbonitrile(7a)

IR (KBr pellets cm^{-1}): 3330.70 (N-H), 3200.69 (Ar-H), 2936.69 Ali(C-H), 2185.68 ($\text{C}\equiv\text{N}$), 1506.59 ($\text{C}=\text{N}$), 1397.51 (C-N). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.73-10.37 (s, 2H, N-H) 9.36-9.31 (s 1H, N-H) 8.13-8.04 (s, 1H, -CH, pyridine), 7.97-7.13 (m, 8H, Ar-H) 3.54-3.29 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.20-3.15 (s, 3H, Ali- CH_3) 3.07-2.85 (t, 6H, CH_3-CH_2-) MS: m/z 467 (M+1).

3.1.2. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(4-methoxyphenyl)pyridine-3-carbonitrile (7b)

IR (KBr pellets cm^{-1}): 3330.72 (N-H), 3200.71 (Ar-H), 2936.72 Ali(C-H), 2185.73($\text{C}\equiv\text{N}$), 1506.61 ($\text{C}=\text{N}$), 1397.54 (C-N). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.75-10.39 (s, 2H, N-H) 9.38-9.33 (s 1H, N-H) 8.15-8.06 (s, 1H, -CH, pyridine), 7.99-7.15 (m, 8H, Ar-H) 3.56-3.31 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.22-3.17(s, 3H, OCH_3) 3.09-2.87 (t, 6H, CH_3-CH_2-) MS: m/z 483 (M+1).

3.1.3. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(2,3,4-trimethoxy phenyl)pyridine-3-carbonitrile (7c)

IR (KBr pellets cm^{-1}): 3330.74 (N-H), 3200.73 (Ar-H), 2936.75 Ali(C-H), 2185.76($\text{C}\equiv\text{N}$), 1506.63 ($\text{C}=\text{N}$), 1397.56 (C-N). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.77-10.42 (s, 2H, N-H) 9.41-9.35 (s 1H, N-H) 8.17-8.08 (s, 1H, -CH, pyridine), 8.00-7.17 (m, 6H, Ar-H) 3.58-3.33 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.25-

3.19(s, 9H, OCH_3) 3.11-2.88 (t, 6H, CH_3-CH_2-) MS: m/z 543 (M+1).

3.1.4. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(3,4,5-trimethoxy phenyl)pyridine-3-carbonitrile (7d)

IR (KBr pellets cm^{-1}): 3330.76 (N-H), 3200.75 (Ar-H), 2936.77 Ali(C-H), 2185.79 ($\text{C}\equiv\text{N}$), 1506.65 ($\text{C}=\text{N}$), 1397.58 (C-N). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.77-10.42 (s, 2H, N-H) 9.41-9.35 (s 1H, N-H) 8.17-8.08 (s, 1H, -CH, pyridine), 8.00-7.17 (m, 6H, Ar-H) 3.58-3.33 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.25-3.19(s, 9H, OCH_3) 3.11-2.88 (t, 6H, CH_3-CH_2-) MS: m/z 543 (M+1).

3.1.5. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(4-fluorophenyl)pyridine-3-carbonitrile(7e)

IR (KBr pellets cm^{-1}): 3330.68 (N-H), 3200.67 (Ar-H), 2936.68 Ali(C-H), 2185.66 ($\text{C}\equiv\text{N}$), 1506.57 ($\text{C}=\text{N}$), 1397 (C-N), 836.75 (C-F). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.71-10.35 (s, 2H, N-H) 9.34-9.30 (s 1H, N-H) 8.11-8.00 (s, 1H, -CH, pyridine), 7.95-7.12 (m, 8H, Ar-H) 3.52-3.27 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.06-2.82 (t, 6H, CH_3-CH_2-) MS: m/z 471 (M+1).

3.1.6. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(2-chlorophenyl) pyridine-3-carbonitrile(7f)

IR (KBr pellets cm^{-1}): 3330.74 (N-H), 3200.73 (Ar-H), 2936.76 Ali(C-H), 2185.74 ($\text{C}\equiv\text{N}$), 1506.66 ($\text{C}=\text{N}$), 1397.04 (C-N), 836.81 (C-Cl). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.76-10.39 (s, 2H, N-H) 9.40-9.37 (s 1H, N-H) 8.15-8.04 (s, 1H, -CH, pyridine), 7.98-7.16 (m, 8H, Ar-H) 3.56-3.32 (q, 4H, $-\text{CH}_2-\text{CH}_3$), 3.09-2.86 (t, 6H, CH_3-CH_2-) MS: m/z 487 (M+1).

3.1.7. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(4-chlorophenyl) pyridine-3-carbonitrile (7g)

IR (KBr pellets cm^{-1}): 3330.72 (N-H), 3200.71 (Ar-H), 2936.73 Ali(C-H), 2185.70 ($\text{C}\equiv\text{N}$), 1506.61 ($\text{C}=\text{N}$), 1397.02 (C-N), 836.79 (C-Cl). ^1H NMR (DMSO- d_6 , 400 MHz), δ 10.73-10.37 (s, 2H, N-H) 9.36-9.33 (s 1H, N-H) 8.13-8.01 (s, 1H, -CH,

pyridine), 7.96-7.14 (m, 8H, Ar-H) 3.54-3.29 (q, 4H, -CH₂-CH₃), 3.08-2.84 (t, 6H, CH₃-CH₂-) MS: m/z 487 (M+1).

3.1.8. Spectral data of 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(2,4-dichlorophenyl)pyridine-3-carbonitrile (7h)

IR (KBr pellets cm⁻¹): 3330.76 (N-H), 3200.76 (Ar-H), 2936.77 Ali(C-H), 2185.76 (C≡N), 1506.66 (C=N), 1397.08 (C-N), 836.85 (C-Cl). ¹H NMR (DMSO-d₆, 400 MHz), δ 10.78-10.42 (s, 2H, N-H) 9.41-9.37 (s, 1H, N-H) 8.18-8.06 (s, 1H, -CH, pyridine), 7.99-7.18 (m, 7H, Ar-H) 3.57-3.33 (q, 4H, -CH₂-CH₃), 3.12-2.87 (t, 6H, CH₃-CH₂-) MS: m/z 522 (M+1) [16-19].

3.2. Antibacterial and antifungal activity

All compounds were evaluated for anti-bacterial activity using species *E. coli*, *Salmonella typhi*, and *Staphylococcus aureus* via disc diffusion method [20-22] using Penicillin as a standard drug and antifungal activity using species like *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* via poison plate method [23] using Griseofulvin as standard and DMSO as a control solvent. Some of the compounds show significant property of anti-bacterial and a number of the compounds showed moderate activity. A study of anti-fungal activity proposes that several compounds are promisingly active at the same time as others aren't so much active. The results are shown in Tables 1 and 2 respectively [16, 18].

Preparation of substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino)phenyl)-3-phenylprop-2-en-1-one (6a-h) compounds was completed through reacting 1-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl) ethanone (4) with substituted benzaldehyde (5a-5h) in DMF. The chalcones undergo Ring formation through condensation with malono nitrile and ammonium acetate to form substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl) pyridine-3-carbonitrile (7a-7h).

The structure of prepared compounds was confirmed by elemental analysis and spectral data (IR, ¹H NMR, and Mass spectra). The IR spectra of chalcones (6a-h) in KBr indicates the characteristic band in the region of 1650cm⁻¹ which suggest the presence of -C=O group. The IR spectral of (7a-7h) shows a characteristic band at 3330.68 (N-H), 3200.67 (Ar-H), 2936.68 Ali(C-H), 2185.66 (C≡N), 1506.57 (C=N), 1397 (C-N). But in

(7a-7h) but there was no band at 1650 cm⁻¹ to 1700 cm⁻¹ which showed the formation of (7a-h).

Table 1: Antibacterial activity of the compounds 7a-h [18]

Compounds	<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>
7a	11	14	16
b	16	18	17
c	18	20	29
d	21	23	26
e	13	15	19
f	17	20	18
g	18	19	22
7h	18	21	20
Penicillin	22	25	35
DMSO	-ve	-ve	-ve

Table 2: Antifungal activity of the compounds 7a-h [18]

Compounds	<i>A. niger</i>	<i>A. flavus</i>	<i>P. chrysogenum</i>
7a	+ve	LG	+ve
b	-ve	+ve	+ve
c	-ve	-ve	-ve
d	+ve	-ve	+ve
e	+ve	+ve	+ve
f	LG	-ve	-ve
g	-ve	+ve	+ve
h	-ve	-ve	LG
Griseofulvin	-ve	+ve	-ve
DMSO	+ve	+ve	+ve

-ve: No growth of organism, Antifungal activity present; +ve: Growth of organism, Antifungal activity absent; LG: Less growth

¹H NMR (DMSO-d₆) spectrum signal at δ 8.11-8.00 (s, 1H, -CH, pyridine), 7.95-7.12 (m, 8H, Ar-H) confirm the existence of cyanopyridine ring the synthetic path followed for the synthesis of the title compounds is described in Scheme-1.

The above data shows that the replacement of hydrogen from the targeted compound (7a-h) from the Para position by electron-withdrawing groups like chloro, fluoro, and methoxy increases microbial activity against microorganisms [16,18].

4. CONCLUSION

From the microbial activity results; it could be concluded that compounds having chloro and methoxy groups specify remarkable activity than different compounds they confirmed precise antibacterial and anti-fungal activity. Therefore it can be taken into consideration as a more design and improvement of new chemical entities.

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Conflict of interest

There is no conflict of interest.

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