**SYNTHESIS, CHARACTERIZATION AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY OF SOME CYANOPYRIDINE DERIVATIVES**

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**Abstract**

The synthesis of Substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-yl amino)phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile (7a-h) by the condensation of substituted (E)-1-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-3 (phenyl) prop-2-en-1-one(6a-h) chalcones with Malono Nitrile and Ammonium acetate in DMF. All the synthesized compounds were evaluated for their anti-fungal and anti-bacterial activity. Most of the compound showed potent activity.

**Keywords:** Cyanuric Chloride, Malono Nitrile, Ammonium Acetate, s-Triazine, Cyanopyridine Antimicrobial.

**I. Introduction**

The s-triazine primarily based totally chalcones and their derivatives show various biological activities and in well-known were studied substantially due to their extensive variety of biological activity [1–13]. They are found to be powerful as local anaesthetic [1], antibacterial [2, 3], antimalarial [4–6], antiprotozoal [7,8] antitubercular [9], anticancer [10,11] and antifungal agents [12,13]. These various properties of chalcones have forced us to synthesize them which will observe their biological activities.

Cyanopyridine derivatives [14] have attracted sizable interest in view in their exceptional significance as anticonvulsant [15], antifungal [16], antibacterial [17], herbicidal [18]. Antihypertensive [19], antiepileptic [20], antitubercular[21], analgesic [22], insecticidal[23-24], antisoriasis[25] , antiallergic[26], antiinflamatory[27], properties. Therefore synthesis of cyanopyridines is of interest because of their widespread prevalence in biologically active derivatives. Hence, sizable interest has been centered at efficient and pharmaceutical important cyanopyridines derivatives.

In view of the above and continuation of our work [28-29] .we have got synthesized new series of cyanopyridine derivatives. From these observations and in order to in addition discover the pharmacological profile of this class of compounds; the existing consists of synthesis of novel 3-cyanopyridines.

**II. Materials and methods**

**Experimental**

All melting points have been carried out in an open capillary and are uncorrected.IR spectra have been recorded the usage of Perkin –Elmer spectrometer.1H NMR spectra have been recorded on Brucker Advance II 400 spectrometer in DMSO via way of means of the usage of TMS as internal standard. Thin layer chromatography done with E. Merk pre lined TLC plates, silica gel 60F254 with thickness of 0.25mm and spots have been visualized with UV (254 nm) or iodine to test the purity of the synthesized compounds.



**Scheme 1:** Synthesis of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile. (7a-7h)

**General procedure for the synthesis of 1-(4-(4,6-dichloro-1,3,5-triazin-2-yl amino) phenyl)ethanone (3)**

4-amine acetophenone (0.01 M) was added slowly to cyanuric chloride (0.01 M) in acetone (30 ml) with constant stirring over a period of 4 h at 0°C to 50°C. Then, sodium carbonate (0.005 M) dissolved in water (10 ml) and added drop wise to neutralize HCl evolved during the reaction. Finally, the contents were poured into crushed ice. The solid was separated out by filtration and washed with water. The product is dried, recrystallized from alcohol to give the product (3).

**General procedure for the synthesis 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 added slowly to sodium ethoxide (0.02 M) with constant stirring in DMF: H2O (9:1 ml) over a period of 4 h at room temperature and refluxed for 4 h at 80°C.The contents were poured onto ice cold water and filtered. The product 4 was obtained and recrystallized from DMF.

**General procedure for the synthesis of substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl amino)phenyl)-3-phenylprop-2-en-1-one (Chalcone) (6a-6h)**

Compound 4 (0.01 M) was dissolved in DMF (25 ml) and substituted benzaldehyde (5a-h) (0.01 M) was added with constant stirring at room temperature for 30 min, then sodium hydroxide (40% w/v) was added to the reaction mixture which was again stirred at RT for 24 h. The progress of reaction was monitored by TLC. After completion of the reaction, crushed ice was added in the reaction mixture and neutralized with HCl. The product separated was filtered, washed with water, dried and recrystallized from DMF to get pure product (Chalcone) (6a-6h).

**General procedure for the synthesis of substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile. (7a-7h)**

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 (0.01 mole) in 25 ml of DMF was refluxed for 10 Hrs. After completion of reaction (checked by TLC), the reaction mixture was cooled and poured into ice cold water. The product separated was filtered, washed with water, dried and recrystallized from DMF to get pure product (7a-h).

**III. Results and discussion**

The synthesis of compounds substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino)phenyl)-3-phenylprop-2-en-1-one (Chalcone) (6a-6h) was accomplished by reacting 1-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)ethanone(4) with substituted benzaldehyde (5a-5h) in DMF. The chalcones undergoes Ring formation reaction via condensation with Malono Nitrile and Ammonium acetate to give substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl) pyridine-3-carbonitrile. The synthesis of Title compound is described in scheme- 1.

The structure of all synthesized compounds was confirmed by elemental analysis and spectral data (IR,H1NMR,and Mass spectroscopy) The IR spectrum of compounds chalcones(6a-6h) in KBr shows the characteristic band in the region of 1650cm-1 which indicate the presence of -C=O group. The IR spectral of (7a-7h) shows characteristic band in region the of 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) there is no Band at 1650 cm-1 to 1700 cm-1 which confirmed formation of (7a-7h).

Further their 1H NMR (DMSOd6) spectrum appearance signals at δ8.11-8.00 (s ,1H ,-CH, pyridine) ,7.95-7.12 (m, 8H, Ar‐H) confirmed the presence of cyanopyridine ring The synthetic pathway followed for the synthesis of the title compounds is described in Scheme-1.

**IV. Spectral data of synthesized compounds (7a-7h)**

(7a) 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-ptolylpyridine-3-carbonitrile

IR (KBr pellets cm‐1): 3330.70 (N-H), 3200.69 (Ar-H), 2936.69 Ali(C-H), 2185.68 (C≡N), 1506.59 (C=N), 1397.51 (C-N). 1H NMR (DMSO-d6, 400 MHz), δ10.73-10.37 (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, -CH2-CH3) , 3.20-3.15(s ,3H ,Ali-CH3) 3.07-2.85 (t,6H, CH3-CH2-) MS: m/z 467 (M+1).

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

IR (KBr pellets cm‐1): 3330.72 (N-H), 3200.71 (Ar-H), 2936.72 Ali(C-H), 2185.73(C≡N), 1506.61 (C=N), 1397.54 (C-N).1H NMR (DMSO-d6, 400 MHz), δ10.75-10.39 (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, -CH2-CH3) , 3.22-3.17(s ,3H ,OCH3) 3.09-2.87 (t,6H, CH3-CH2-) MS: m/z 483 (M+1).

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

IR (KBr pellets cm‐1): 3330.74 (N-H), 3200.73 (Ar-H), 2936.75 Ali(C-H), 2185.76(C≡N), 1506.63 (C=N), 1397.56 (C-N). 1H NMR (DMSO-d6, 400 MHz), δ10.77-10.42 (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, -CH2-CH3) , 3.25-3.19(s ,9H ,OCH3) 3.11-2.88 (t,6H, CH3-CH2-) MS: m/z 543 (M+1).

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

IR (KBr pellets cm‐1): 3330.76 (N-H), 3200.75 (Ar-H), 2936.77 Ali(C-H), 2185.79 (C≡N), 1506.65 (C=N), 1397.58 (C-N). 1H NMR (DMSO-d6, 400 MHz), δ10.77-10.42 (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, -CH2-CH3) , 3.25-3.19(s ,9H ,OCH3) 3.11-2.88 (t,6H, CH3-CH2-) MS: m/z 543 (M+1).

(7e): 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(4-fluorophenyl)pyridine -3-carbonitrile

IR (KBr pellets cm‐1): 3330.68 (N-H), 3200.67 (Ar-H), 2936.68 Ali(C-H), 2185.66 (C≡N), 1506.57 (C=N), 1397 (C-N), 836.75 (C‐F). 1H NMR (DMSO-d6, 400 MHz), δ10.71-10.35 (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, -CH2-CH3) , 3.06-2.82 (t,6H, CH3-CH2-) MS: m/z 471 (M+1).

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

IR (KBr pellets cm‐1): 3330.74 (N-H), 3200.73 (Ar-H), 2936.76 Ali(C-H), 2185.74 (C≡N), 1506.66 (C=N), 1397.04 (C-N), 836.81 (C‐Cl). 1H NMR (DMSO-d6, 400 MHz), δ10.76-10.39 (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, -CH2-CH3) , 3.09-2.86 (t,6H, CH3-CH2-)MS: m/z 487 (M+1).

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

IR (KBr pellets cm‐1): 3330.72 (N-H), 3200.71 (Ar-H), 2936.73 Ali(C-H), 2185.70 (C≡N), 1506.61 (C=N), 1397.02 (C-N), 836.79 (C‐Cl). 1H NMR (DMSO-d6, 400 MHz), δ10.73-10.37 (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).

(7h) 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-(2,4-dichlorophenyl) pyridine-3-carbonitrile 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), δ10.78-10.42 (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).

**V. Biological activity:**

**Antimicrobial hobby**

Newly synthesized all compounds have been examined for anti-bacterial activity the using species E. coli, Salmonella typhi and Staphylococcus aureus via way of means of disc diffusion method [30-31]. Using Penicilline as a standard drug and antifungal using of species like Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum via way of means of poison plate method [32] using Griseofulvin as reference standard and DMSO as a control solvent. Some of compounds display significant property of anti-bacterial and a number of the compounds display moderate activity. Study of anti-fungal activity suggests that a number of compounds are promisingly active at the same time as others aren't so much active. The results are shown in Table 1 and 2 respectively.

**Table 1-Antibacterial screening results of the compounds 7a-h**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sr. No. | Compounds | E. coli | Salmonella  typhi | Staphylococcus  aureus |
| 1 | 7a | 10 | 13 | 15 |
| 2 | 7b | 15 | 17 | 16 |
| 3 | 7c | 17 | 19 | 28 |
| 4 | 7d | 20 | 22 | 25 |
| 5 | 7e | 12 | 14 | 18 |
| 6 | 7f | 16 | 19 | 17 |
| 7 | 7g | 17 | 18 | 21 |
| 8 | 7h | 17 | 20 | 19 |
| 9 | Penicillin | 22 | 25 | 35 |
| 10 | DMSO | -ve | -ve | -ve |

**Table 2:Antifungal screening results of the compounds 7a-7h.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sr. No. | Compounds | E.coli | Salmonella typhi | Staphylococcus  aureus |
| 1 | 7a | +ve | RG | +ve |
| 2 | 7b | -ve | +ve | +ve |
| 3 | 7c | -ve | -ve | -ve |
| 4 | 7d | -ve | -ve | -ve |
| 5 | 7e | +ve | +ve | +ve |
| 6 | 7f | RG | -ve | -ve |
| 7 | 7g | -ve | +ve | +ve |
| 8 | 7h | -ve | -ve | RG |
| 9 | Greseofulvin | -ve | -ve | -ve |
| 10 | DMSO | +ve | +ve | +ve |
| -ve: No growth, Antifungal activity present; +ve: Growth, Antifungal activity absent;  RG: Reduced growth | | | | |

**VI. Conclusion**

From the results of Anti-Bacterial and Anti-Fungal Activity; it could be concluded that compounds having chloro and Methoxy groups indicates significant activity than different compounds They confirmed precise antibacterial and anti-fungal activity. Therefore it is able to taken into consideration as a further design and improvement of new chemical entities.

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