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

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**Abstract**

The preparation of Substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-yl amino)phenyl)-2-amino-6-(phenyl)pyridine-3-carbonitrile (7a-h) via way of means of 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 had been assessed for their anti-fungal and anti-bacterial activity. Most of the compound confirmed mighty activity.

**Keywords:** Trichlorotriazine, 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 prepare 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 preparation 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 continuance of our work [28-29] .we have got prepared 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**

melting points were taken in an open capillary and may be uncorrected. IR spectra were recorded the use of Perkin –Elmer spectrometer.1H NMR spectra were recorded on Brucker Advance II 400 spectrometer in DMSO through manner of way of the use of TMS as an inner standard. Thin layer chromatography performed with E. Merk pre coated TLC plates, silica gel 60F254 with thickness of 0.25mm and spots were visualized with UV (254 nm) or iodine to check 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)**

A 4-amine acetophenone (0.01 M) was added to cyanuric chloride (0.01 M) in acetone (30 ml) with continuous stirring for four hrs. at 0°C to 50°C. Sodium carbonate dissolved in 10 ml water and added in above reaction mixture till neutralizes HCl which is end point of reaction. Then the reaction mixture is poured on crushed ice, separated solid is separated and washed with water. Obtained product is dried, recrystallized from alcohol to obtain the product (3).

**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 consistent stirring in DMF: H2O (9:1 ml) for about four hrs. at room temperature and then it was refluxed for four hrs. at 80°C. Then reaction contents have been poured onto crushed ice cold and then it was filtered and recrystallized from DMF.

**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 then substituted benzaldehyde (5a-h) (0.01 M) was added with constant stirring at room temperature for 30 min, then sodium hydroxide (40% w/v) became added to the response aggregate which became homogeneous again stirred at RT for 24 hrs. Completion of reaction is monitored through TLC. After completion of the reaction, product is poured on crushed ice and neutralized with HCl. Obtained product was separated by filtration, washed with water, dried and recrystallized from DMF. (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 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 DMF 25 ml and refluxed for 10Hrs. Completion of the reaction is checked means of TLC, the crude product cooled and poured into ice cold water. The obtained product filtered and washed with water, it was dried and recrystallized from DMF to (7a-h).

**III. Results and discussion**

The synthesis targeted compounds substituted 1-(4-(4,6-diethoxy-1,3,5-triazin-2-yl-amino) phenyl)-3-phenylprop-2-en-1-one (Chalcone) (6a-6h) become completed by reacting 1-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)ethanone (4) with substituted benzaldehyde (5a-5h) in DMF. The obtained chalcones undergoes Ring formation through condensation with Malono Nitrile and Ammonium acetate to offer substituted 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino) phenyl)-2-amino-6-(phenyl) pyridine-3-carbonitrile. It is described in scheme- 1.

Structure of synthesized compounds were confirmed through elemental analysis and spectral data (IR, 1HNMR, and Mass spectroscopy) The IR spectrum of compounds chalcones(6a-6h) 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 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 may be no Band at 1650 cm-1 to 1700 cm-1 which showed formation of (7a-7h).

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

**IV. Spectral data (7a-7h)**

**(7a) 4-(4-(4,6-diethoxy-1,3,5-triazin-2-ylamino)phenyl)-2-amino-6-ptolylpyridine-3-carbonitrile [30]**

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), 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, -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:**

**Anti-microbial hobby**

Novel synthesized 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 [31-32]. 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 [33] 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 motifs.

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