**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) 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 evaluated for their anti-fungal and anti-bacterial activity. Most of the compound confirmed mighty 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 were executed in an open capillary and are 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 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)**

4-amine acetophenone (0.01 M) was added slowly to cyanuric chloride (0.01 M) in acetone (30 ml) with regular stirring over a length of four h at 0°C to 50°C. Then, sodium carbonate (0.half M) dissolved in water (10 ml) and delivered drop sensible to neutralize HCl developed at some point of the reaction. Finally, the contents had been poured into beaten ice. The solid separated out via way of means of filtration and washed with water. The product is dried, recrystallized from alcohol to offer 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) became delivered slowly to sodium ethoxide (0.02 M) with consistent stirring in DMF: H2O (9:1 ml) over a length of four h at room temperature and refluxed for four h at 80°C.The contents have been poured onto ice cold water and filtered. The product four became received 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) became dissolved in DMF (25 ml) and substituted benzaldehyde (5a-h) (0.01 M) became delivered with steady stirring at room temperature for 30 min, then sodium hydroxide (40% w/v) became delivered to the response aggregate which became once more stirred at RT for 24 hrs. The development of response became monitored through TLC. After completion of the reaction, crushed ice was added in the reaction mixture and neutralized with HCl. The product separated became 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 combination 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 changed into refluxed for 10Hrs.After completion of the reaction (checked via way of means of TLC), the crude product cooled and poured into ice cold water. The product separated out filtered, washed with water, dried and recrystallized from DMF to get natural 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) become 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 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. The synthesis of Title compound is described in scheme- 1.

The structure of all synthesized compounds were confirmed through elemental analysis and spectral data (IR,H1NMR,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).

Further their 1H NMR (DMSOd6) spectrum signal at δ8.11-8.00 (s, 1H,-CH, pyridine) ,7.95-7.12 (m, 8H, Ar‐H) confirm thepresence 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[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), 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 [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 entities.

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