**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 prepared compounds had been assessed for their anti-fungal and anti-bacterial activity. Maximum of the compound confirmed mighty activity.

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

**I. Introduction**

The s-triazine primarily based on 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 initiate 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 paying attention 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.

Understanding of the above and extension of our work [28-29] .we have got prepared new series of cyanopyridine derivatives. From these observations and in order to discover the pharmacological profile of this class of compounds; the existing consists of preparation of novel 3-cyanopyridines.

**II. Materials and methods**

**Experimental**

All melting points were performed 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 prepared 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-7h)

**General procedure for the 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 cyanuric chloride (0.01 M) in acetone (30 ml) with customary blending over a length of four hr at 0°C to 5°C. Then, sodium carbonate (0.5 M) broke down in water (10 ml) and conveyed drop reasonable to neutralize HCl created sooner or later of the response. At last, the items had been filled beaten ice. The strong isolated out through method of method for filtration and washed with water. The item is dried, recrystallized from liquor to offer the item (3).

**General procedure for the 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) became conveyed gradually to sodium ethoxide (0.02 M) with consistent blending in DMF: H2O (9:1 ml) over a length of four hr at room temperature and refluxed for four h at 80°C.The items have been poured on super cold water and sifted. The item four became gotten and recrystallized from DMF.

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

Yet again compound 4 (0.01 M) became broken up in DMF (25 ml) and subbed benzaldehyde (5a-h) (0.01 M) became conveyed with consistent blending at room temperature for 30 min, then, at that point, sodium hydroxide (40% w/v) became conveyed to the reaction total which became mixed at RT for 24 hrs. The advancement of reaction became observed through TLC. Subsequent to completing mark of the response, squashed ice was included the response combination and neutralized with HCl. The item isolated became separated, washed with water, dried and recrystallized from DMF to get unadulterated item (Chalcone) (6a-6h).

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

A blend of subbed 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 acetic acid derivation (0.01 mole) in 25 ml of DMF changed into refluxed for 10Hrs.Afterward fruition of the response (checked through method of method for TLC), the rough item cooled and filled super cold water. The item isolated out sifted, washed with water, dried and recrystallized from DMF to get item (7a-h).

**III. Results and discussion**

The preparation 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 prepared 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 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[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 prepared all compounds have been observed 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 | Aspergillus  niger | Aspergillus  flavus | Penicillium  chrysogenum |
| 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 specifies noteworthy activity than different compounds they confirmed precise antibacterial and anti-fungal activity. Therefore it is able to taken into consideration as a more design and improvement of new chemical entities.

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