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Synthesis of Novel Isoxazoline Derivatives Containing s-Triazine via Chalcones and their Anti-Microbial Studies

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ABSTRACT

In this series different Isoxazoline were prepared i.e., 4,6-diethoxy-N-(4-(4,5-dihydro-5-phenylisoxazol-3yl)phenyl)-1,3,5-triazin-2-amine 7(a-h) via cyclisation of chalcone intermediate. The structures of prepared compounds were confirmed by spectral analysis. The antibacterial and antifungal activities of the final products against various bacteria and fungi have also been reported. Most of the compound showed good to moderate activity.

Keywords: Cyanuric chloride, 2-chloro-4,6-diethoxy-1,3,5-triazine, Chalcone, Isoxazoline, Anti-bacterial activity

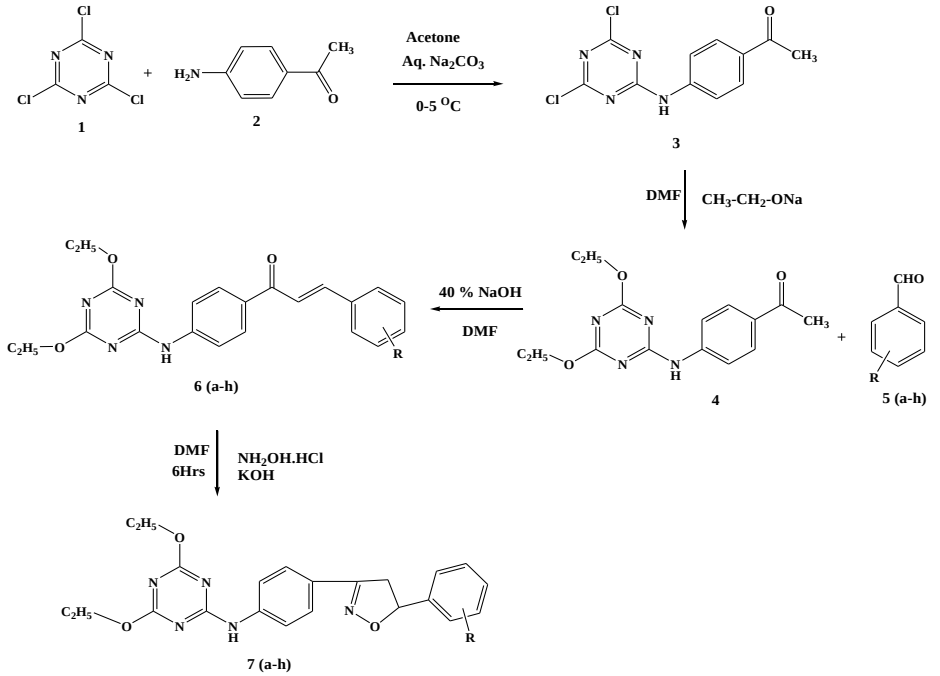
INTRODUCTION

A heterocyclic compound is one which possesses a cyclic structure with hetero atoms such as nitrogen and oxygen atom in the ring in addition with carbon atom. Nitrogen and oxygen containing heterocyclic compounds have received considerable attention due to their wide range of pharmacological activities. Five membered heterocyclic compounds are much more important in organic chemistry [1] Isoxazoline is five membered heterocyclic compounds and it has more application in agro chemistry [2] and Pharmaceutical sciences.

A literature survey indicated that heterocyclic compounds containing Isoxazoline, Containing s-triazine were found to exhibit much more application as pharmaceutical and Agrochemical agents. The synthesis and study of Isoxazoline derivatives containing s-triazine have been of interest because of facile synthesis and broad spectrum of biological and Pharmacological activities [3]. Many s-triazine containing Isoxazoline derivatives have recently craned great interest in chemotherapy. These derivatives have been reported to possess antifungal [4,5], antibacterial [6], Anticonvulsant [7] antioxidant, cytotoxicity [8] and anti-inflammatory [9] activity.

As our interest is to synthesize heterocyclic templates capable of bearing some potential pharmacophore which can enhance the inherent biological activity, therefore systematic propagation of heterocyclic rings in Chalcones with the installation of biological active heterocyclic units such as isoxazoline containing s-triazine ring.

In the present work, 4,6-diethoxy-N-(4-(5-(substituted-phenyl)-4,5-dihydroisoxazol-3yl)phenyl)-1,3,5-triazin-2-amine(7a-7h) have been synthesized by the treatment of 1-(4-(4,6 diethoxy-1,3,5-triazin-2-ylamino)phenyl)-3-substitutedphenylprop-2-en-1-one (Chalcone) [10-16] (6 a-6h) with Hydroxylamine Hydrochloride and KOH in DMF (Scheme 1). The structure of all prepared isoxazoline derivatives were given on the basis of IR, Mass, 1 H NMR, elemental analysis and evaluated their antimicrobial activity and obtained good results.



Scheme 1: 4,6-diethoxy-N-(4-(5-(substituted-phenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

MATERIALS AND METHODS

EXPERIMENTAL

All the melting points were determined in open capillary and are incorrect. The IR spectra of the synthesized compounds were recorded on Perkin Elmerspectrometer 1H NMR spectra were recorded on Bruker spectrophotometer (400 MHz) in DMSO-d6 solvent using Tetra Methyl Silane (TMS) as an internal standard. Mass spectra were recorded by LC MS method. To monitor reaction and to identify purity of reactant and product thin layer chromatography was performed on micro-slide coated with silica gel- G using suitable solvent system and the spots were visualized by exposure of iodine vapors.

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 0ºC to 5ºC. 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) [11].

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 80ºC. 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) [10].

General procedure for the synthesis of substituted N-(4-(5-(Substituted phenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)-4,6-diethoxy-1,3,5-triazin-2-amine (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-6h), Hydroxyamine Hydrochloride (0.01M) and KOH (0.01M) in 25 ml DMF was refluxed for 6 h after completion of the reaction the reaction mixture was cooled and poured into ice cold water. The resultant solid product (7a-7h) was filtered, washed with sufficient cold water, dried and purified by recrystallization from DMF.

RESULTS AND DISCUSSION

The prepared compounds i.e., 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 (6a-6h) undergoes ring closure reaction with hydroxylamine hydrochloride and KOH to give substituted 4,6-diethoxy-N-(4-(5-(phenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine (7a-7h).

From literature data the possible mechanism for the reaction is realized in two steps in first step nucleophilic attach of carbonyl group by NH_2 moiety takes place then oxime formation takes place and then intermolecular cyclisation leads to five membered ring product (Scheme 1) [12,13].

The assigned structure and molecular formula of newly prepared compounds were confirmed and supported by IR, Mass,^{1}H-NMR, and elemental analysis which is fully agreement with proposed structure. The IR spectrum of (7a-h) exhibited a band due to N-H str. (3185-3070 Cm^{-1}), Ar-H str. (3070-3000 cm^{-1}) C=C str. (1662-1385 Cm^{-1}), C=N (ring) (1650-1580Cm^{-1}) stretching vibration band which indicates the presence of the isoxazoline ring. Further, in their ^{1}H NMR (DMSO-d6) spectrum, the appearance of a signal at δ 5.25-4.20 (dd, 1H, Hx isoxazoline), 2.53-2.50 (dd, 1H, H_B isoxazoline) and 2.57-2.52 (dd, 1H, H_A isoxazoline) confirms the presence of the isoxazoline ring.

Spectral data of synthesized compounds (7a-7h)

(7a) 4,6-diethoxy-N-(4-(4,5-dihydro-5-p-tolylisoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

Yield 76%; M.P. 178ºC: Elemental analysis Calcd for($C_{23}H_{25}N_5O_3$); C, 65.85; H, 6.01; N, 16.70 ; found: C, 65.76; H,6.07; N, 16.65%; IR (KBr pellets cm^{-1}): 3185 (N-H), 3070 (Ar-H), 2991 Ali(C-H), 1511 (C=N), 1386 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.90-6.95 (m, 8H, Ar-H), 4.80-4.22 (dd, 1H, H_x Isoxazoline), 3.42-3.38 (s, 1H, N-H), 2.91-2.77. (q, 4H, CH3-CH2), 2.57-2.52 (dd,1H_A,- CH2-CH),2.53-2.50(dd,1H_B,CH2-CH),2.36-2.30(s,3H-CH3) 2.20-2.13 (t, 6H,CH3-CH2), MS: m/z 419 (M+1).

(7b) 4,6-diethoxy-N-(4-(4,5-dihydro-5-(4-methoxyphenyl)isoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

Yield 82%; M.P. 183ºC: Elemental analysis Calcd for ($C_{23}H_{25}N_5O_4$) C, 63.44; H, 5.79; N, 16.08; C, 63.40; H, 5.70; N, 16.10; 16.65%; IR (KBr pellets cm^{-1}): 3183 (N-H), 3072 (Ar-H), 2993 Ali(C-H), 1513 (C=N), 1386 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.91-6.94 (m, 8H, Ar-H), 4.81-4.23 (dd, 1H, H_x Isoxazoline), 3.76-3.75(s,3H,OCH3)3.40-3.36 (s, 1H, N-H), 2.89-2.75. (q, 4H, CH3-CH2), 2.55-2.50 (dd,1H_A,- CH2-CH),2.51-2.48(dd,1H_B,CH2-CH), 2.18-2.11 (t, 6H,CH3-CH2), MS: m/z 436 (M+1).

(7c) 4,6-diethoxy-N-(4-(4,5-dihydro-5-(2,3,4-trimethoxyphenyl)isoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

Yield 80%; M.P. 176ºC: Elemental analysis Calcd for $C_{25}H_{29}N_5O_6$ (C, 60.60; H, 5.90; N, 14.13; C, 60.58; H, 5.85; N, 14.10;)IR (KBr pellets cm^{-1}): 3186 (N-H), 3071 (Ar-H), 2992 Ali(C-H), 1512 (C=N), 1387 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.91-6.96 (m, 6H, Ar-H), 4.81-4.23 (dd, 1H, H_x Isoxazoline), 3.74-3.71(s,9H,OCH3),3.43-3.39 (s, 1H, N-H), 2.92-2.78. (q, 4H, CH3-CH2), 2.58-2.53 (dd,1H_A,- CH2-CH),2.54-2.51(dd,1H_B,CH2-CH), 2.21-2.14 (t, 6H,CH3-CH2), MS: m/z 494 (M+1).

(7d) 4,6-diethoxy-N-(4-(4,5-dihydro-5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

Yield 80%; M.P. 176ºC: Elemental analysis Calcd for $C_{25}H_{29}N_5O_6$ (C, 60.60; H, 5.90; N, 14.13; C, 60.58; H, 5.85; N, 14.10; IR (KBr pellets cm^{-1}): 3188 (N-H), 3073 (Ar-H), 2994 Ali(C-H), 1514 (C=N), 1388 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.93-6.98 (m, 6H, Ar-H), 4.83-4.23 (dd, 1H, H_x Isoxazoline), 3.78-3.73(s,9H,OCH3),3.45-3.39 (s, 1H, N-H), 2.94-2.79. (q, 4H, CH3-CH2), 2.59-2.55 (dd,1H_A,- CH2-CH),2.56-2.53(dd,1H_B,CH2-CH), 2.23-2.15 (t, 6H,CH3-CH2), MS: m/z 494 (M+1).

(7e) 4,6-diethoxy-N-(4-(5-(4-fluorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-1,3,5-triazin-2-amine

Yield 70%; M.P. 190ºC: Elemental analysis Calcd for $C_{22}H_{22}FN_5O_3$ (C, 62.40; H, 5.24; N, 16.5 Found C, 62.38; H, 5.21; N, 16.40)IR (KBr pellets cm^{-1}): 3184 (N-H), 3069 (Ar-H), 2990 Ali(C-H), 1510 (C=N), 1385 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.89-6.94 (m, 8H, Ar-H), 4.79-4.21 (dd, 1H, H_x Isoxazoline),3.41-3.37 (s, 1H, N-H), 2.90-2.76. (q, 4H, CH3-CH2), 2.56-2.51 (dd,1H_A,- CH2-CH),2.52-2.49(dd,1H_B,CH2-CH), 2.21-2.12 (t, 6H,CH3-CH2), MS: m/z 423 (M+1).

(7f) N-(4-(5-(2-chlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,6-diethoxy-1,3,5-triazin-2-amine

Yield 76%; M.P. 185ºC: Elemental analysis Calcd for $C_{22}H_{22}ClN_5O_3$ (C, 60.07; H, 5.04; N, 15.92; Found C, 60.01; H, 5.00; N, 15.90; IR (KBr pellets cm^{-1}): 3186 (N-H), 3071 (Ar-H), 2992 Ali(C-H), 1512 (C=N), 1387 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.91-6.96 (m, 8H, Ar-H), 4.81-4.23 (dd, 1H, H_x Isoxazoline),3.43-3.39 (s, 1H, N-H), 2.92-2.78. (q, 4H, CH3-CH2), 2.58-2.53 (dd, 1 H_A,- CH2-CH-), 2.54-2.51(dd, 1H_B, CH2-CH), 2.21-2.14 (t, 6H,CH3-CH2), MS: m/z 439 (M+1).

(7g) N-(4-(5-(4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,6-diethoxy-1,3,5-triazin-2-amine

Yield 72%; M.P. 180ºC: Elemental analysis Calcd for $C_{22}H_{22}ClN_5O_3$ (C, 60.07; H, 5.04; N, 15.92;Found C, 60.00; H, 5.01;, N, 15.91;)IR (KBr pellets cm^{-1}): 3187 (N-H), 3072 (Ar-H), 2993 Ali(C-H), 1513 (C=N), 1388 (C-N): ^{1}H NMR (DMSO-d6, 400 MHz), δ 7.92-6.97 (m, 8H, Ar-H), 4.82-4.24 (dd, 1H, H_x Isoxazoline),3.44-3.40 (s, 1H, N-H), 2.93-2.80. (q, 4H, CH3-CH2), 2.59-2.55 (dd,1H_A,- CH2-CH),2.53-2.50(dd,1H_B,CH2-CH), 2.22-2.15 (t, 6H,CH3-CH2), MS: m/z 439 (M+1).

(7h) N-(4-(5-(2,4-dichlorophenyl)-4,5-dihydroisoxazol-3-yl)phenyl)-4,6-diethoxy-1,3,5-triazin-2-amine

Yield 83%; M.P. 181C: Elemental analysis Calcd for;($C_{22}H_{21}Cl_2N_5O_3$ C, 55.71; H, 4.46; N 14.76 Found C, 55.68; H, 4.42; N, 14.70;);)IR (KBr pellets cm^{-1}): 3182 (N-H), 3067 (Ar-H), 2988 Ali(C-H), 1509 (C=N), 1383 (C-N): 1H NMR (DMSO-d6, 400 MHz), δ 7.87-6.92 (m, 8H, Ar-H), 4.77-4.20 (dd, 1H, H_x Isoxazoline),3.39-3.35 (s, 1H, N-H), 2.88-2.74. (q, 4H, CH3-CH2), 2.54-2.49 (dd,1H_A,- CH2-CH),2.50-2.47(dd,1H_B,CH2-CH), 2.17-2.10 (t, 6H,CH3-CH2), MS: m/z 473(M+1).

BIOLOGICAL ACTIVITY

Antimicrobial activity

All newly synthesized compounds were screened for anti-bacterial and anti-fungal activities by using paper disc diffusion method [14,15] using Penicillin (100 µb/disc) as reference standard for their anti-bacterial activity. Selected bacteria are *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis* and for anti-fungal activities by using by poison plate method using Griseofulvin (100 µb/disc) as reference standard and DMSO as control solvent. Selected fungal species are *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum* and *Fusurium moneliforme.* The investigation of antibacterial screening shows that some of the compounds are significant property while few of them has moderately active. The investigation of antifungal activity data revealed that some compounds have promising and some showed no antifungal activity. The observed Minimal Inhibitory Concentrations (MIC) values are shown in Tables 1 and 2.

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

S. No.	Compounds	*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Bacillus subtilis*
1	7a	14	12	28	16
2	7b	16	15	30	19
3	7c	18	18	34	17
4	7d	18	23	35	18
5	7e	15	23	28	16
6	7f	17	19	29	18
7	7g	16	15	30	20
8	7h	16	22	28	21
9	Penicillin	22	25	38	25
10	DMSO	-ve	-ve	-ve	-ve

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

S. No.	Compounds	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium chrysogenum*	*Fusurium moneliforme*
1	7a	-ve	RG	-ve	-ve
2	7b	+ve	+ve	+ve	+ve
3	7c	+ve	+ve	+ve	+ve
4	7d	RG	+ve	+ve	+ve
5	7e	-ve	+ve	-ve	-ve
6	7f	+ve	-ve	-ve	-ve
7	7g	+ve	+ve	RG	-ve
8	7h	-ve	-ve	+ve	+ve
9	Griseofulvin	-ve	-ve	-ve	-ve
10	DMSO	+ve	+ve	+ve	+ve
-ve No growth Antifungal activity present +ve Growth Antifungal activity absent RG Reduced growth					

CONCLUSION

All the prepared compounds were screened for anti-bacterial and anti-fungal activities. Majority of the prepared compounds were found potentially active against Gram positive, Gram negative bacteria and selected fungus. Compounds containing chloro,fluoro and electron releasing group such as methoxy shows good activity. These results shows that Chalcones and Isoxazoline derivatives have an opportunity to behave as broad spectrum antimicrobial agents and have excellent scope for further development as commercial antimicrobial agents.

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