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Synthesis and in Vitro Antimicrobial Activity of N-[4-Chloro-6-(Morpholin-4-Yl)-1,3,5-Triazin-2-Yl]-2-Methylquinolin-8-Amine

KEYWORDS: 2-methylquinolin-8-amine, Morpholine, s-triazine, Antibacterial activity.

Anil Rathavi
Municipal Arts & Urban science college, Mahesana, Gujarat, India

M. K. Thakor
Municipal Arts & Urban science college, Mahesana, Gujarat, India

ABSTRACT *A variety of N-[4-chloro-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine,G were synthesized by using 2-methylquinolin-8-amine, morpholine and cyanuric chloride. And the structures of these compounds were confirmed by IR, NMR (1H & 13C) spectral analysis. The newly synthesized compounds were also evaluated for antimicrobial activity against variety of bacterial strains and some of these compounds have shown significant antibacterial and antifungal activities.*

Introduction:-

The extraordinary progress represented by the arrival of antibiotics has changed the medical prognosis of minor and major infections. Any bacterial species acquired resistance to the most common classes of antibiotics. Bacterial resistance continues to develop and pose a significant threat both in hospitals and more recently in the community. A relevant report on resistant antibacterial agents for human medicine is provided by World Health Organization. The panel agreed that the list of Critically Important antibacterial agents should be updated regularly as new information becomes available, including data on resistance patterns, new and emerging diseases and the development of new drugs. During the last few years the potential of s-triazine derivatives in agrochemical and medicinal properties have been subjcted to investigation. Literature survey reveals that amino substituted s-triazine derivatives are associated with number of pronounced antibacterial activities against gram positive (B. subtilis, B. sphaericus, S. aureus etc) and gram negative organism (E. coli, K. aerogenes, P. aeruginosa etc). The biological activity is a function of physicochemical properties of the targeted molecule and this assessment is made of the sorts of chemicals that might fit into an active site. To randomly explore the novel compounds. our idea was to combine, 2-methylquinolin-8-amine, Morpholine, and s-triazine nucleus using cyanuric chloride and various amines. Substituted-s-triazines, derivatives remain attractive, with their significant biological activities and further incorporation of these derivatives with commercial drug viz. isoniazid, could give access to a wide array of structures, which can be expected to show interesting antibacterial activities, thus, herein, we report the synthesis and antimicrobial activity of a variety of novel s-triazine derivatives.

Materials and Methods:-

All the melting points were taken in open capillaries tube. The purity of compounds was checked routinely by TLC (0.5 mm thickness) using silica gel – G coated Al – plates (Merck) and spots were visualized by exposing the dry plates in iodine vapours. IR spectra (nmax in cm-1) were recorded on Shimadzu FTIR spectrophotometer using KBr or Nujol technique.1H & 13C NMR spectra on a Bruker's WM 400 FT MHz NMR instrument using CDCl3 or DMSO-d6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The elemental analysis (C, H, N) of compounds was performed on Carlo Erba – 1108 elemental analyzer.

General Experimentation:-

(1) N-(4,6-dichloro-1,3,5-triazin-2-yl)-2-methylquinolin-8-amine (C)

To a stirred solution of cyanuric chloride (10 g, 0.054 mole) in anhydrous THF (50 ml) was added 2-methylquinolin-8-amine (8.53 g, 0.054 mole) drop wise at 0-5 degC. The resulting reaction mixture was stirred at this temperature for 3 hrs, then neutral the reaction mass by addition of 10% sodium bicarbonate($NaHCO_3$) solution stirring was continued for another 1 hr. The resulted reaction mixture was poured into crushed ice, and then filtered, dried and recrystalised from THF.

M.P. 108 degC
M.W : 158.2 gm/mol

FT-IR (KBr): 3270 (N-H), 3060 (ArC-H), 2800-3000 (Alkane C-H), 1565, 1200, 850 (C-N, C3N3), 812 (s-triazine C-N str.);

(2) N-[4-chloro-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (E)

To a stirred solution of N-(4,6-dichloro-1,3,5-triazin-2-yl)-2-methylquinolin-8-amine (C) (8 g, 0.05 mole) in anhydrous THF (50 ml) was added morpholine (4.35 g, 0.05 mole) at 35-40 degC for 2 hr. Then neutral the reaction mass by addition of 10% sodium bicarbonate($NaHCO_3$) solution stirring was continued for another 3 hrs. Then reaction mass poured into crushed ice, filtered and dried and recrystalised from THF.

M.P. 119 degC
M.W : 356.8 gm/mol

FT-IR (KBr): 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 1250 cm^{-1} (C-O-C).

(3) General procedure for preparation of compounds (G).

To a solution of **E** (10.70gm 0.03 mole) in 1, 4-Dioxane (50 ml), added different substituted aniline derivatives and the reaction mixture was refluxed for 8 to 10 hrs. 10% Sodium bicarbonate was used for the neutralization of the reaction mixture. After the completion of the_reaction, it was treated with crushed ice,the precipitates obtained was

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filtered, dried and recrystalised from acetone to get final compound G.

(4)- Characterization of synthesized compounds (G).

(1) N-[4-aniline-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (G-1)

Yield: 73%; M.P : 120 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.);

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H23N7O: C, 66.81; H, 5.61; N, 23.71; O,3.87;

(2)N-[4-(2-methylaniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (G-2)

Yield: 70%; M.P : 117 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.);

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3); 1.0 (3H,s,-CH3 of Aniline);

Anal. Calcd. for C24H25N7O: C, 67.43; H, 5.89; N, 22.93; O,3.74;

(3)N-[4-(3-methyl aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (G-3)

Yield: 72%; M.P : 119 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.);

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3); 1.0 (3H,s,-CH3 of Aniline);

Anal. Calcd. for C24H25N7O: C, 67.43; H, 5.89; N, 22.93; O,3.74;

(4)N-[4-(4-methyl aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (G-4)

Yield: 68%; M.P : 116 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.);

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3); 1.0 (3H,s,-CH3 of Aniline);

Anal. Calcd. for C24H25N7O: C, 67.43; H, 5.89; N, 22.93; O,3.74;

(5)N-[4-(2-chloro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]- 2-methylquinolin-8-amine (G-5)

Yield: 62%; M.P : 128 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 650-850 (C-Cl str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N7OCl: C, 61.67; H, 4.95; N, 21.89; O,3.57; Cl, 7.92

(6)N-[4-(3-chloro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-2-methylquinolin-8-amine (G-6)

Yield: 64%; M.P : 126 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 650-850 (C-Cl str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N7OCl: C, 61.67; H, 4.95; N, 21.89; O,3.57; Cl, 7.92

(7)N-[4-(4-chloro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]- 2-methylquinolin-8-amine (G-7)

Yield: 65%; M.P : 129 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 650-850 (C-Cl str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N7OCl: C, 61.67; H, 4.95; N, 21.89; O,3.57; Cl, 7.92

(8)N-[4-(2-nitro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-]-2-methylquinolin-8-amine (G-8)

Yield: 71%; M.P : 131 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 1550, 1350 (-N=O str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N8O3: C, 60.25; H, 4.84; N, 24.44; O,10.47;

(9)N-[4-(3-nitro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-]-2-methylquinolin-8-amine (G-9)

Yield: 70%; M.P : 130 degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000

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(Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 1550, 1350 (-N=O str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N8O3: C, 60.25; H, 4.84; N, 24.44; O,10.47;

(10)N-[4-(2-nitro aniline)-6-(morpholin-4-yl)-1,3,5-triazin-2-yl]-]-2-methylquinolin-8-amine (G-10)

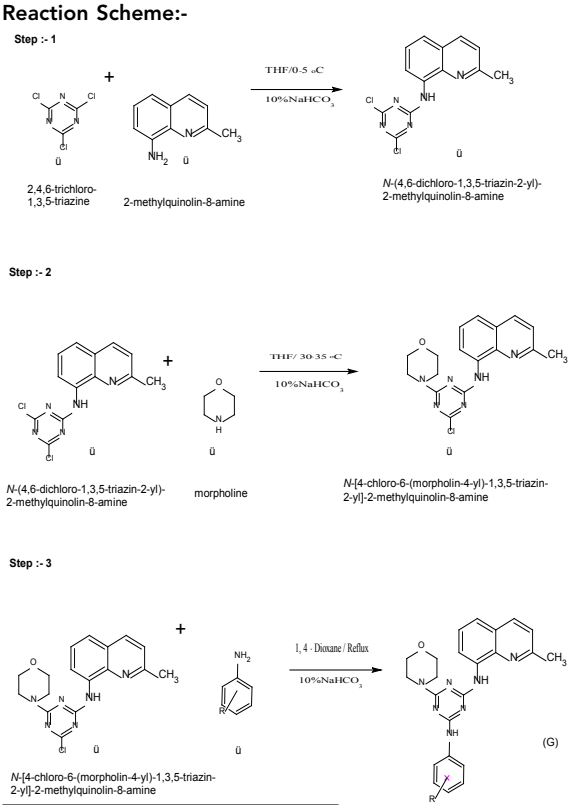
Yield: 68%; M.P : 132degC.

IR (KBr,cm^{-1}) : 3150-3350 (N-H), 3070 (ArC-H), 2800-3000 (Alkane C-H), 1255 (C-O-C), 1568, 1300, 847 (C-N, C3N3), 813 (s-triazine C-N str.); 1550, 1350 (-N=O str.)

1H NMR (400 MHz, DMSO- d6) δ 7.1-7.3 (5H, s, -ArH), 7.3-7.6 (5H, m, -quinoline), 0.7-5 (2H, s,-NH linkage), 1.37 (8H, m,Morpholine); 0.9 (3H,s,-CH3);

Anal. Calcd. for C23H22N8O3: C, 60.25; H, 4.84; N, 24.44; O,10.47;

Reaction Scheme:-



Compounds	R	Melting point (degC)	% yield	Analytically calculated (Found)		
				%Carbon	%Hydrogen	%Nitrogen
G-1	H	120	73	66.81	5.61	23.71
G-2	2-CH3	117	70	67.43	5.89	22.93
G-3	3-CH3	119	72	67.43	5.89	22.93
G-4	4-CH3	116	68	67.43	5.89	22.93
G-5	2-Cl	128	62	61.67	4.95	21.89
G-6	3-Cl	126	64	61.67	4.95	21.89
G-7	4-Cl	129	65	61.67	4.95	21.89
G-8	2-NO2	131	71	60.25	4.84	24.44
G-9	3-NO2	130	70	60.25	4.84	24.44
G-10	4-NO2	132	68	60.25	4.84	24.44

Antibacterial activity:-

In vitro antibacterial screening of all the compounds were evaluated against selected (Table 1) Gram-positive organisms viz. Bacillus subtilis(MTCC 441), Bacillus sphaericus(MTCC 11), Staphylococcus aureus(MTCC 96) and Gram-negative organisms viz. Chromobacterium violaceum (MTCC 2656), Klebseilla aerogenes (MTCC 39), Pseudomonas aeruginosa(MTCC 741), Salomonella paratyphi A(MTCC 735) and Escherichia coli(MTCC 443) by broth dilution method recommended by National Committee for Clinical Laboratory (NCCL) standards21. Standard antibacterial agent like Benzyl Penicillin and Streptomycin were also screened under identical conditions for comparison.

Antifungal activity:-

A. awamori, A. niger and C. albicans was employed for testing antifungal activity using the cup–plate method21. The culture was maintained on Sabouraud's agar slants.

Fifteen milliliters of sterilized Sabouraud's agar medium was spread in a Petri dish (13 cm in diameter) and allowed to set for 30 min. Five milliliters of sterilized Sabouraud's agar medium was inoculated with 72 h old 0.2 ml suspension of fungal spores in a test-tube and spread over the previously settled layer of Sabouraud's agar medium in the Petri dish. The cups (8 mm in diameter) were punched in the Petri dish and lled with 0.05 ml (40 µg) of a solution of the sample in DMF. The plates were incubated at 30 °C for 48 h. After the completion of the incubation period, the zones of inhibition of growth in millimeter were measured. Along with the test solutions in each Petri dish one cup was filled up with solvent, which acts as the control. Standard antifungal agent like Griseofulvin was also screened under identical conditions for comparison. The zones of inhibition are recorded in Table 3.

Table–2 :- Antibacterial activity (Zone of inhibition in mm)

Compound	B.s	B.sph	S.a	K.a	C.v	P.a	E.c	S.p
G-1	15	16	17	14	15	17	16	17
G-2	14	15	15	16	21	16		18
G-3	18	17	17	15	15	16	21	17
G-4	17	16	16	18	17	17	22	20
G-5	23	21	19	18	18	17	19	15
G-6	15	14	15	16	16	14	18	16
G-7	18	16	16	17	18	18	16	15
G-8	20	23	18	17	18	15	15	17
G-9	19	22	18	18	17	15	16	20

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G-10	19	18	22	17	20	17	18	16
Benzyl Penicillin	27	29	30	30	28	29	31	29
Streptomycin	33	31	30	31	29	32	33	30
Gram +ve Organisms B.s: : Bacillus subtilis (MTCC 121), B.sph.: Bacillus sphaericus (MTCC 11) S.a. : Staphylococcus aureus (MTCC 96)			Gram –ve Organisms K.a. : Klebseilla aerogenes (MTCC 39), C.v. : Chromobacterium violaceum (MTCC 2656), P.a. :Pseudomonas aeruginosa (MTCC 791), E.c. : Escherichia coli (MTCC 443) S.p. : Salomonella paratyphi A (MTCC 735)					

*** Negative Control :- Acetone**

Table–3 :- Antifungal activity (Zone of inhibition in mm)

Compound.	A. awamori	A. niger	C. albicans
G-1	10	11	12
G-2	13	12	10
G-3	14	14	9
G-4	15	10	8
G-5	12	13	11
G-6	14	15	12
G-7	15	14	12
G-8	11	14	18
G-9	10	15	19
G-10	13	18	16
Griseofulvin	23	25	24

Results and Discussion:-

In vitro antibacterial activity data of s-triazine derivatives (Table 1) against tested organisms displayed significant activity with a wide degree of variation. It is found that

compound G-5 displayed substantial activity against B. subtilis and remaining compounds are significantly active. Also G-5, G-8 and G-9 are equipotent against B. sphaericus compared to reference compound. Rest of the compounds has exhibited significant to substantial activity against the same strain. Substantial activity is achieved in case of compounds G-10 against S. aureus and the remaining compounds are significantly active against the same species. All the s-triazine derivatives have exhibited significant to moderate activity against Gram-negative bacteria. Derivatives G-2 and G-10 have exhibited substantial activity against C. violaceum. Against Salomonella paratyphi A, compounds G-4 and G-9 has been found to possess significant activity, comparatively weak activity has been reported by remaining compounds. E. coli was found to be more susceptible than rest of the other strains of bacteria, among them compounds G-3 and G-4 were showing significant activity for the same strain. All s-triazine derivatives in this communication are inactive towards P.aeruginosa, also decreased activity is observed in case of K. aerogenes with all the s-triazines. From in vitro antifungal activity (Table 2), data reveals that all the newly synthesized compounds displayed moderate to significant activity in comparison to standards. Thus, it is obvious from the structure-activity profile of substituted s-triazines; a small structural variation may induce an effect on antibacterial activity.

Conclusion:-

Trisubsituted s-triazine derivatives, Compound **G** was synthesized and characterized for their structure elucidation. Antibacterial and antifungal studies of these compounds indicated that compounds were found to be showing comparable activity against some bacteria compared to standard antibiotic drugs. The produced compounds have good microbial toxicity due to presence of three pharmacologically active nucleus viz. s-triazine, 2-methylquinolin-8-amine and morpholine. so such compounds may give good comparable anti-Tuberculosis effect, which will be studied in details hereafter.

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