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Synthesis characterization and in vitro antimicrobial activity of [4-(5,6-dihydro-4H-thieno[2,3-B]pyridin-7-yl)-6-morpholin-4-yl-[1,3,5]triazin-2-yl]-phenyl-amine derivatives

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ABSTRACT

A variety of [4-(5,6-dihydro-4h-thieno[2,3-b]pyridin-7-yl)-6-morpholin-4-yl-[1,3,5]triazin-2-yl]-phenyl-amine derivatives were synthesized by using 4,5,6,7-Tetrahydro-thieno[2,3-b]pyridine, morpholine and cyanuric chloride. And the structures of these compounds were confirmed by Mass, IR and ^{1}H NMR 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 antimicrobial activities.

Keywords: 4,5,6,7-Tetrahydro-thieno[2,3-b]pyridine, Morpholine, s-triazine, Antimicrobial activity.

1. INTRODUCTION

The increasing incidence of infection caused by the rapid development of bacterial resistance to most of the known antibiotics is serious health problem. While many factors may be responsible mutations in microbial genomes, it has been widely demonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes. As multidrug-resistant bacterial strains proliferate, the necessity for effective therapy has stimulated research into the design and synthesis of novel antimicrobial molecules. The chemistry of [1,3,5] triazine compounds has been studied intensively and is the subject of many reviews. The triazine scaffold has provided the basis for the design of biologically relevant molecules with broad biomedical value as therapeutics. For example, triazine compounds possess potent antiprotozoal, antimalarial, and antiviral activity. Also, it was reported that some of these compounds possess potent antimicrobial activity. The starting material for these compounds is cyanuric chloride. This is an inexpensive commercially available reagent which makes its use more attractive. Increased interest in this scaffold lies in the different reactivities of the substituent chlorine atoms, which are controlled by temperature. This allows sequential introduction of various substituents into the [1,3,5] triazine ring. As part an effort towards the discovery of novel therapeutics for the treatment of infectious diseases, a search was initiated to discover small molecules that possess significant antimicrobial activity. thus, herein, we report the synthesis and antimicrobial activity of a variety of novel s-triazine derivatives.

2. 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 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.

2.1. General experimentation

2.1.2. (1) Synthesis of 7-(4,6-Dichloro-[1,3,5]triazin-2-yl)-4,5,6,7-tetrahydro-thieno [2,3-b]pyridine) (C)

To a stirred solution of cyanuric chloride (0.1 mol) **(1)** in THF (100 ml) at 0-5°C, the solution of 4,5,6,7-Tetrahydro-thieno[2,3-b]pyridine **(B)** (0.1 mol) in THF (100 ml) was added drop-wise and pH was maintained neutral

by the addition of 10% $NaHCO_3$ solution. The stirring was continued at 0-5°C for 2 hours. After the completion of reaction the stirring was stopped and the solution was treated with crushed ice. The solid product obtained was filtered and dried. The progress of reaction was monitored by TLC using ethyl acetate: hexane (6:4) as eluent. The crude product was purified by crystallization from absolute alcohol.

M.P. 111-113 degC; M.W: 287.16 gm/mol; FT-IR (KBr): 3050 (ArC-H), 2800-3000 (Alkane C-H), 1560, 1210, 855 (C-N, C3N3), 815 (s-triazine C-N str.);

2.1.3. (2) Synthesis of 7-(4,6-dichloro-1,3,5-triazin-2-yl)-4,5,6,7-tetrahydrothieno [2,3-*b*]pyridine (E)

The solution of Morpholine (0.1 mol) in THF (100 ml) was added drop-wise to well stirred suspension of 7-(4,6-Dichloro-[1,3,5]triazin-2-yl)-4,5,6,7-tetrahydro-thieno[2,3-b]pyridine **(C)** (0.1 mol) in THF (100 ml) maintaining the temp 40°C the pH was kept neutral by the addition of 10 % $NaHCO_3$ solution . The temperature was gradually raised to 45°C during 2 hours and further maintained for 2 hours. After the completion of reaction the solution was poured in ice cold water. The solid product was filtered and dried. The crude was purified by recrystalization from absolute alcohol.

M.P.200-203 degC; M.W : 337.82 gm/mol; FT-IR (KBr): 3070 (ArC-H), 2800-3000 (Alkane C-H), 1568, 1290, 857 (C-N, C3N3), 813 (s-triazine C-N str.); 1255 cm^{-1} (C-O-C).

2.1.4. General procedure for preparation of compounds (G)

A mixture of 7-(4-Chloro-6-morpholin-4-yl-[1,3,5]triazin-2-yl)-4,5,6,7-tetrahydro-thieno[2,3-b]pyridine **(E)** (0.1 mol) and substituted aryl amine (0.1 mol) in dioxane (50 ml) was refluxed on heating mental with stirring at 100-110°C for 5 hours. The pH was adjusted to neutral by addition of 10 % $NaHCO_3$ solution. After the completion of reaction the content was added to ice-cold water. The product was filtered and dried the progress of reaction was monitored by TLC using ethyl acetate: hexane (4:6) eluent. Purification of all the synthesized compounds was achieved by recrystalization and purity of each compound was monitored by TLC.

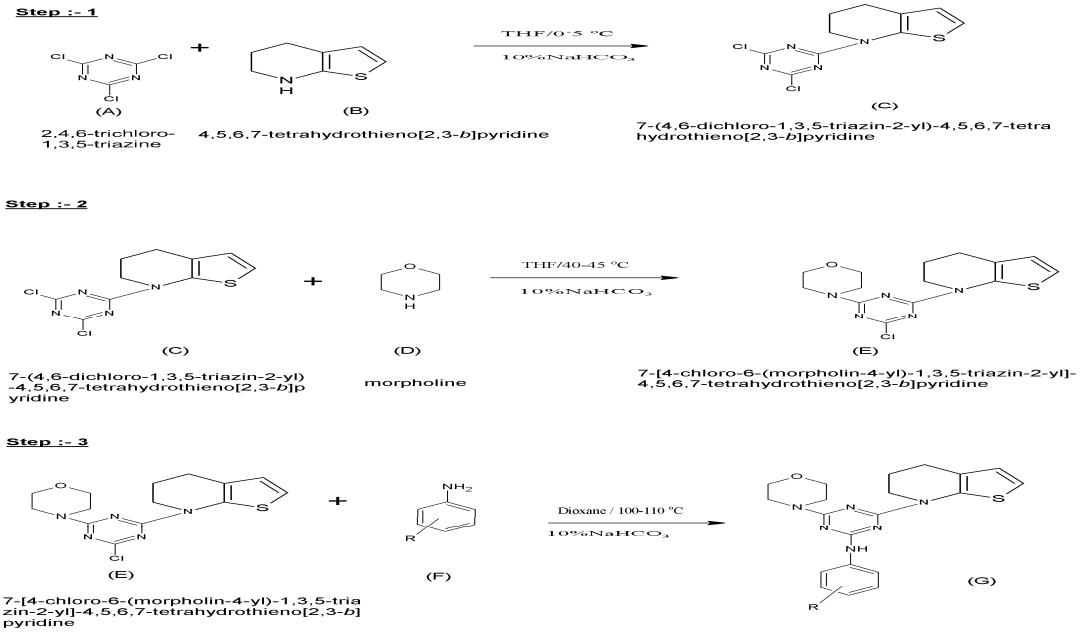
3. RESULTS AND DISCUSSION

3.2. Characterization of synthesized compounds (G)

3.2.1. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-phenyl-amine. (G-1)

Yield: 71%; M.P : 125-127 degC; **IR (KBr,cm^{-1}) :** 840 (C=N in s-Triazine), 3335(-NH in Amine),1159 (C-O in Morpholine) 1116,(C-N in Morpholine),1150 (C-N in Thieno-pyridine), 3030, 1500 (Aromatic C-H), 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6,* δ ppm) 3.43-3.64 (m, 8H, Morpholine), 6.65-7.38 (m, 8H, Thieno pyridine), 7.69-7.85(m, 5H, Ar-H), 9.70(s, 1H, NH).

Anal. Calcd. for $C_{20}H_{22}N_6OS$: C, 60.91; H, 5.58; N, 21.32;



Reaction Scheme

3.2.2. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(2-methyl phenyl)-amine (G-2)

Yield: 70%; M.P: 123-125 degC; **IR (KBr,cm^{-1}) :** 828 (C=N in s-Triazine), 3345 (-NH in Amine) 1172 (C-O in Morpholine), 1107 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 2950 (C-CH_3 in aromatic), 3030, 1500 (Aromatic C-H), 1600 (stretching). **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.35-3.52 (m, 8H, Morpholine), 6.76-7.35 (m, 8H, Thieno pyridine), 7.57-8.24(m, 4H, Ar-H), 9.82 (s, 1H, NH), 2.25 (s, 3H, CH_3).

Anal. Calcd. for $C_{21}H_{24}N_6OS$: C, 61.76; H, 5.88; N, 22.93;

3.2.3. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(3-methyl phenyl)-amine (G-3)

Yield: 72%; M.P: 122-124 degC; **IR (KBr,cm^{-1}) :** 835 (C=N in s-Triazine), 3325(NH in Amine), 1167(C-O in Morpholine), 1127(C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 2950 (C-CH_3 in aromatic), 3030, 1500 (Aromatic C-H), 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6* ,δppm) 3.41-3.60 (m, 8H, Morpholine), 6.63-7.36 (m, 8H, Thieno pyridine), 7.47-8.18 (m, 4H, Ar-H), 9.87 (s, 1H, NH), 2.25 (s, 3H, CH_3).

Anal. Calcd. for $C_{21}H_{24}N_6OS$: C, 61.76; H, 5.88; N, 20.58;

3.2.4. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(4-methyl phenyl)-amine (G-4)

Yield: 73%; M.P : 126-128 degC; **IR (KBr,cm^{-1}) :** 817 (C=N in s-Triazine), 3320 (NH in Amine), 1143 (C-O in Morpholine), 1124 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 2950 (C-CH_3 in aromatic), 3030, 1500 (Aromatic C-H) 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.36-3.54(m, 8H, Morpholine), 6.68-7.32 (m, 8H, Thieno pyridine), 7.37-8.19 (m, 4H, Ar-H), 9.49 (s, 1H, NH), 2.25 (s, 3H, CH_3).

Anal. Calcd. for $C_{21}H_{24}N_6OS$: C, 61.76; H, 5.88; N, 20.58;

3.2.5. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(2-Chloro phenyl)-amine (G-5)

Yield: 69%; M.P : 123-125 degC; **IR (KBr,cm^{-1}) :** 823 (C=N in s-Triazine), 3315 (NH in Amine), 1141(C-O in Morpholine), 1132 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 3030, 1500 (Aromatic C-H), 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.34-3.51(m, 8H, Morpholine), 6.73-7.23(m, 8H, Thieno pyridine), 7.30-8.17 (m, 4H, Ar-H), 9.84 (s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_6OSCl$: C, 56.00; H, 4.90; N, 19.60;

3.2.6. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(3-Chloro phenyl)-amine (G-6)

Yield: 72%; M.P : 126-128 degC; **IR (KBr,cm^{-1}) :** 828 (C=N in s-Triazine), 3347 (NH in Amine), 1169(C-O in Morpholine), 1098 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 3030, 1500 (Aromatic C-H), 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.38-3.59 (m, 8H, Morpholine), 6.78-7.50 (m, 8H, Thieno pyridine), 7.56-8.27 (m, 4H, Ar-H), 9.59(s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_6OSCl$: C, 56.00; H, 4.90; N, 19.60;

3.2.7. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin- 4-yl-[1,3,5]triazin-2-yl]-(4-Chloro phenyl)-amine (G-7)

Yield: 73%; M.P : 127-130 degC; **IR (KBr,cm^{-1}) :** 856 (C=N in s-Triazine),3337 (NH in Amine), 1148 (C-O in Morpholine), 1130 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 3030, 1500 (Aromatic C-H), 1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.31-3.49 (m, 8H, Morpholine), 6.68-7.43(m, 8H, Thieno pyridine), 7.49-8.18(m, 4H, Ar-H), 9.86 (s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_6OSCl$: C, 56.00; H, 4.90; N, 19.60;

3.2.8. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin-4-yl-[1,3,5]triazin-2-yl]-(2-nitro phenyl)-amine (G-8)

Yield: 74%; M.P: 128-130 degC; **IR (KBr,cm^{-1}) :** 856 (C=N in s-Triazine), 3337 (NH in Amine), 1148 (C-O in Morpholine), 1130 (C-N in Morpholine), 1150 (C-N in Thieno-pyridine), 1573 (N-O of Nitro), 3030, 1500 (Aromatic C-H) 1600 (stretching);**1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.31-3.49 (m, 8H, Morpholine), 6.68-7.43 (m, 8H, Thieno pyridine), 7.49-8.18 (m, 4H, Ar-H), 9.86 (s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_7O_3S$: C, 54.67; H, 4.78; N, 22.32;

3.2.9. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin-4-yl-[1,3,5]triazin-2-yl]-(3-nitro phenyl)-amine (G-9)

Yield: 75%; M.P : 130-133 degC; **IR (KBr,cm^{-1}) :** 836 9C=N in s-Triazine),3327 (NH in Amine),1169(C-O in Morpholine), 1103 (C-N in Morpholine),1150 (C-N in Thieno-pyridine),1573 (N-O of Nitro),3030, 1500 (Aromatic C-H),1600 (stretching); **1H NMR:** (400 MHz, DMSO- *d6*, δppm) 3.45-3.65(m, 8H, Morpholine), 6.67-7.38(m, 8H, Thieno pyridine), 7.44-8.05(m, 4H, Ar-H), 9.59 (s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_7O_3S$: C, 54.67; H, 4.78; N, 22.32;

3.2.10. [4-(5,6-Dihydro-4H-thieno[2,3-b]pyridin-7-yl)-6-morpholin-4-yl-[1,3,5]triazin-2-yl]-(4-nitro phenyl)-amine (G-10)

Yield: 73%; M.P : 129-132 degC; **IR (KBr,cm^{-1}) :** 849 (C=N in s-Triazine),3318 (NH in Amine),1168(C-O in Morpholine), 1132(C-N in Morpholine),1150(C-N in Thieno-pyridine),1573(N-O of Nitro) 3030, 1500(Aromatic C-H),1600(stretching); **1H NMR** (400 MHz, DMSO- *d6*, δppm) 3.42-3.63(m, 8H, Morpholine),6.64-7.43(m, 8H, Thieno pyridine),7.49-8.16 (m, 4H, Ar-H),9.81(s, 1H, NH).

Anal. Calcd. for $C_{20}H_{21}N_7O_3S$: C, 54.67; H, 4.78; N, 22.32.

Table - 1: Physical data of all compounds

Compounds	R	Melting point(degC)	% yield	Analytically calculated (Found)		
				% Carbon	% Hydrogen	% Nitrogen
G-1	H	125-127	71	60.91	5.58	21.32
G-2	2-CH3	123-125	70	61.76	5.88	22.93
G-3	3-CH3	122-124	72	61.76	5.88	22.93
G-4	4-CH3	126-128	73	61.76	5.88	22.93
G-5	2-Cl	123-125	69	56.00	4.90	19.60
G-6	3-Cl	126-128	72	56.00	4.90	19.60
G-7	4-Cl	127-130	73	56.00	4.90	19.60
G-8	2-NO2	128-130	74	54.67	4.78	22.32
G-9	3-NO2	130-133	75	54.67	4.78	22.32
G-10	4-NO2	129-132	73	54.67	4.78	22.32

Table -2 : Data of Antimicrobial activity of novel triazine derivatives

Compound Number	Diameter of zone of inhibition			
	S.aureus	*B. Subtilis*	*E.Coli*	*P.aeruginosa*
G-1	50	100	250	100
G-2	100	50	50	50
G-3	50	50	100	50
G-4	100	200	100	200
G-5	50	100	25	100
G-6	50	250	100	300
G-7	100	200	50	100
G-8	100	50	250	150
G-9	100	200	50	200
G-10	50	25	50	150
Ampicillin	250	100	100	100
Chloramphenicol	50	50	50	50

3.3. Antimicrobial activity

The compounds tested for antimicrobial activity are listed show size of zone of inhibition of bacterial growth procedure by test compounds for broad range of antimicrobial activity inhibiting growth of Gram-positive bacterial strains *B.Subtillis* and *S.Aureus* and Gram-negative bacterial strains *E.Coli* and *Ps. Aeruginosa*.

Comparison of antimicrobial activity of produced compounds with that of standard antimicrobial drugs reveals that the produce compounds shows moderate to good activity against all four bacterial strains.

In vitro antibacterial activity data of s-triazine derivatives **(Table 2)** against tested organisms displayed significant activity with a wide degree of variation. It is found that compound **G-4, G-6 and G-8** displayed substantial activity and remaining compounds are significantly active.

This 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 antimicrobial activity.

4. CONCLUSION

Trisubsituted s-triazine derivatives, Compound **G** was synthesized and characterized for their structure elucidation. antimicrobial 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,4,5,6,7-Tetrahydro-thieno[2,3-b]pyridine, and morpholine.

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