SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF 1,3,5-TRIAZIN-2YL} OXY) BENZONITRILE DERIVATIVES

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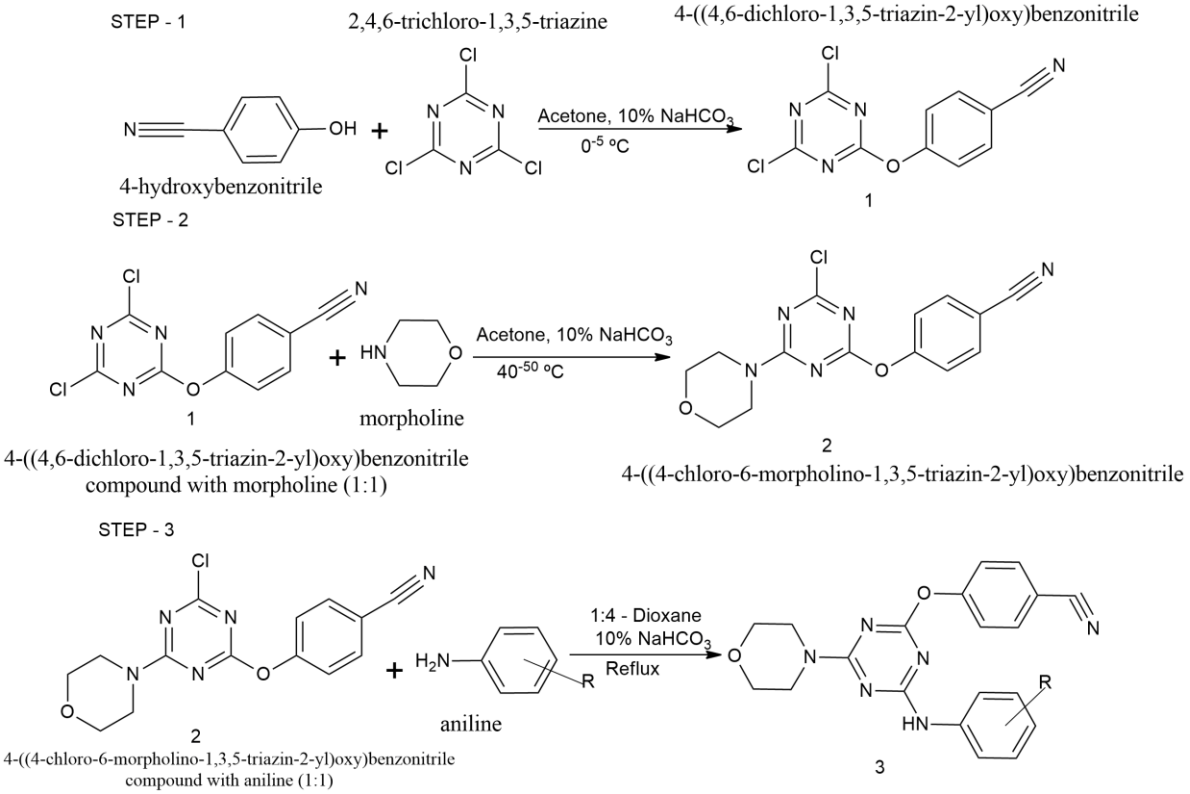
ABSTRACT :
The present study describes the synthesis, characterisation, and biological evaluation of a new series of 1,3,5-triazin-2-yl)oxy)benzonitrile derivatives and related N-(4-aryl amine)-2-{[4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl}acetamides. The synthetic pathway involved a sequential approach beginning with pyridine-4-carboxylic acid, which was converted into the corresponding ester and subsequently treated with hydrazine hydrate to obtain pyridine-4-carbohydrazide. Condensation with phenyl thioisocyanate afforded the intermediate semicarbazide, which underwent cyclization to generate 4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol. In parallel, a series of chloroacetamide intermediates wassynthesised by reacting substituted amines with chloroacetyl chloride. Coupling the triazole thiol with these intermediates yielded the target acetamide derivatives (Scheme 1).
In an alternative route (Scheme 2), 1-{4-[(3-chloroprop-1-en-2-yl)amino]phenyl}ethanone was reacted with the triazole thiol, followed by condensation with various aldehydes to furnish chalcone-based derivatives. All synthesised compounds were purified and structurally confirmed using standard spectroscopic techniques, ensuring their identity and chemical integrity.
The derivatives were evaluated for antimicrobial efficacy against Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), Escherichia coli, Candida albicans, and Microsporum gypseum. Among the three final compounds—two from Scheme 1 and one from Scheme 2-compound 3 demonstrated the most pronounced antimicrobial activity. Its enhanced potency, particularly against MRSA and fungal pathogens, surpassed that of compounds 1 and 2.
Overall, the study underscores the potential of triazole-based acetamide and chalcone derivatives as promising antimicrobial scaffolds. These findings contribute to the development of new therapeutic agents capable of combating resistant microbial strains and addressing an urgent global healthcare challenge.
Keywords: Antimicrobial activity, Pyridine-4-carbohydrazide

I. INTRODUCTION

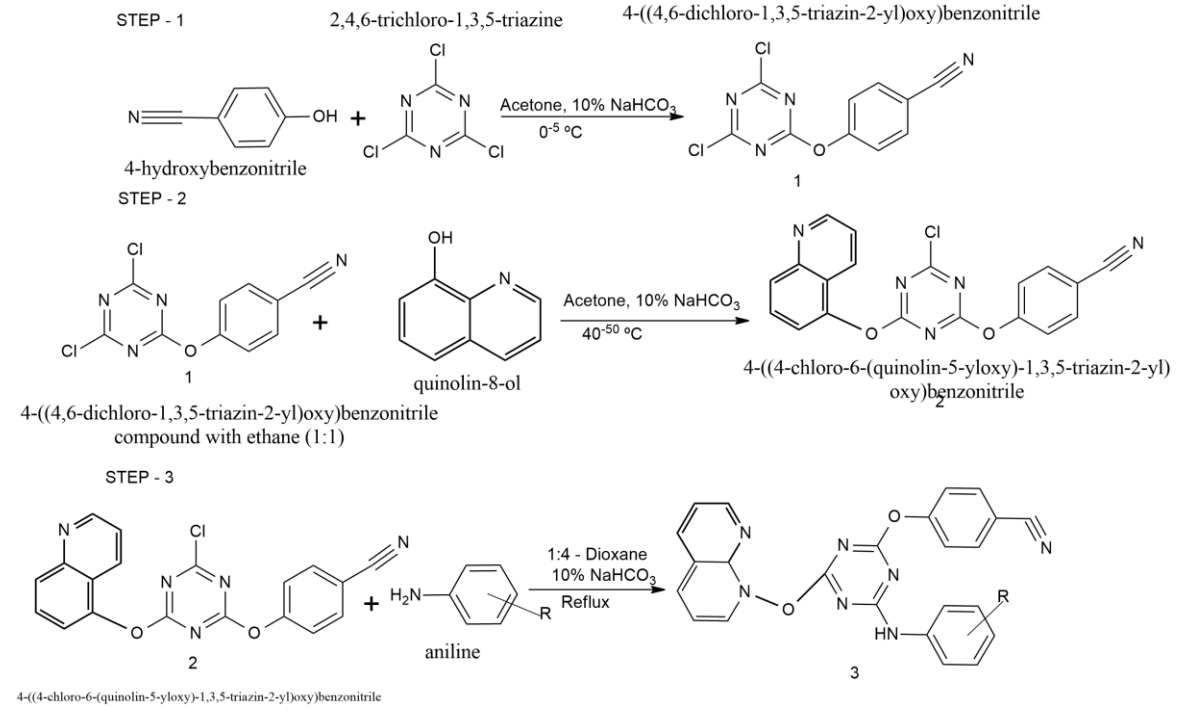
The majority of organic substrates are heterocyclic compounds, which include at least two distinct kinds of atoms in their ring structure. Inorganic heterocyclics are rings that do not include any carbon atoms, whereas organic heterocyclics are rings that contain carbon atoms or other heteroatoms (N, O, or S). Heterocyclic compounds are the most abundant kind of organic compound, accounting for more than half of all known chemical compounds. The physical and chemical characteristics of heterocyclic compounds are greatly enhanced by the presence of heteroatoms. Vitamins, hormones, antibiotics, and pigments are just a few examples of the numerous natural compounds that include heterocycles as structural components. Heterocycles are plentiful in nature and play an important role in human existence.2,3 Therefore, these compounds have garnered a lot of interest in the field of designing molecules with biological activity.4 Models for some naturally occurring substances with physiological activity that are synthetically problematic are the nitrogen-containing heterocyclics.5- 7 Drugs, insecticides, colors, plastics, cosmetics, data storage, solvents, antioxidants, and vulcanization accelerators are just a few of the numerous ways in which modern civilization relies on synthetic heterocycles.8-11 These substrates are not only abundant in natural products, but they are also essential building blocks of biological molecules, including nucleotides, the most fundamental macromolecules in the universe. Among the many medicinal applications of synthetic heterocycles are their actions against bacteria, fungi, mycobacteria, trypanocidal, HIV, leishmanial, genotoxic, tuberculosis, malaria, herbicidal, inflammation, pain, convulsions, muscles, cancer, lipid peroxidation inhibitor, hypnotics, antidepressants, tumors, helminths, and insects.12-18

WORKING SCHEME

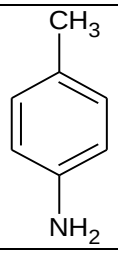
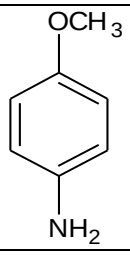
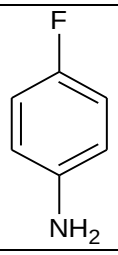
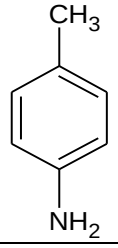
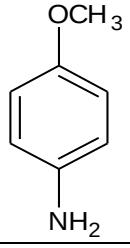
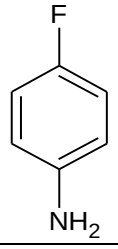
SCHEME 1



SCHEME 2



Compound	IUPAC Name	Amines

SCHEME 1		
TB1	4-({4-[(4-methylphenyl) amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl} oxy) benzonitrile	
TB2	4-({4-[(4-methoxyphenyl) amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl} oxy) benzonitrile	
TB3	4-({4-[(4-fluorophenyl) amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl} oxy) benzonitrile	
SCHEME 2		
TB4	4-({4- [(4-methyl phenyl) amino]-6-(quinolin-8-yl-oxy)-1,3,5-triazin-2-yl} oxy) benzonitrile	
TB5	4-({4- [(4-methoxy phenyl) amino]-6-(quinolin-8-yl-oxy)-1,3,5-triazin-2-yl} oxy) benzonitrile	
TB6	4-({4-[(4-fluorophenyl) amino]-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl} oxy) benzonitrile	

II. EXPERIMENTAL WORK

Experimental

Only chemicals and reagents of analytical quality were used. The melting points were calculated using an electrothermal instrument with open capillaries and have not been taken into account for correction. The experiment included the use of thin-layer chromatography on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was conducted using UV light at wavelengths of 254 and 365nm, or alternatively, using iodine vapor. The infrared (IR) spectra were acquired using a Bruker-Fourier transform infra-red (FTIR)-8400 Spectrophotometer instrument equipped with KBrdisc. Bruker DPX-400 MHz spectrometer was used to record 1H

NMR spectra. The expression of chemical shifts is denoted in µ ppm downfield from TMS, which serves as the internal reference. The SHIMADZU-MS was used to get the mass spectrum data.

General Experiments

SCHEME 1

Step -1

The addition of a solution of 4-hydroxy Benzonitrile (0.1mole) in acetone (90ml) was performed dropwise over a period of two hours to a stirred solution of cyanuric chloride (0.1mol) in acetone (100ml) at a temperature range of 0-5ºC. To preserve the neutrality of the reaction mixture, 10% NaHCO3 was added throughout the reaction.
The reaction process was monitored using thin-layer chromatography (TLC) with acetone: toluene (2:8) as the eluent. Once the reaction had concluded, the stirring process was stopped, and the solution underwent treatment with crushed ice. The resultant product underwent filtration and subsequent drying. Purification and recrystallization of the crude product (1) were performed using alcohol.

Step -2

The solution of morpholine in acetone was gradually added to a stirred solution of 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]benzonitrile (1) in acetone at room temperature over a period of 2 hours. Over a period of two hours, the temperature was elevated to 45 ºC.To ensure the neutrality of the reaction mixture, a 10% solution of NaHCO3 was introduced and allowed to remain in solution for a duration of 2 hours. TLC was used to monitor the development of the reaction using a mixture of Acetone and Toulene at a ratio of 2:8. The solution was then put into ice-cold water after the end of the reaction. The solid product (2), obtained by filtering, was then dried and recrystallized using absolute alcohol.

Step -3

The reaction mixture was subjected to reflux for a duration of 6-10 hours after the addition of various substituted aryl amine derivatives to a solution of 4-[4-chloro-6-(morpholin-4-yl)-1,3,5-triazin-2-yl] oxybenzonitrile (2) (0.01 mol in 20 ml of 1,4-Dioxane). A 10% solution of NaHCO3 was used to neutralize the reaction mixture. Following the end of the reaction, the mixture was subjected to treatment with crushed ice and then neutralized with dil. The resulting precipitate was dried and recrystallized from 100% alcohol using HCl.

SCHEME 2

Step -1

The addition of a solution of 4-hydroxy Benzonitrile (0.1mole) in acetone (90ml) was performed dropwise over a period of two hours to a stirred solution of cyanuri chloride (0.1mol) in acetone (100ml) at a temperature range of 0-5^{0}C. To preserve the neutrality of the reaction mixture, 10% NaHCO3 was added throughout the reaction. The reaction process was monitored using thin-layer chromatography (TLC) using actone: toluene (2:8) as the eluent. Once the reaction had concluded, the stirring process was stopped, and the solution underwent treatment with crushed ice. The resultant product underwent filtration and subsequent drying. The first product underwent purification and subsequent recrystallization using alcohol.

Step -2

The addition of a solution of 8-hydroxy quinoline in acetone was gradually introduced over a period of 2 hours to a stirred solution of 4-[(4,6-dichloro-1,3,5-triazin-2-yl)oxy]benzonitrile (1) in acetone at ambient temperature. Over a period of two hours, the temperature was elevated to 45°C.A 10% NaHCO3 solution was added to the reaction mixture to ensure its neutrality, which was then maintained for a duration of 2 hours. The reaction progress was monitored using thin-layer chromatography (TLC) using a mixture of Acetone and Toluene at a ratio of 2:1. After the reaction was finished, the solution was put into ice-cold water. The solid product produced after filtering was then dried and recrystallized using absolute alcohol, resulting in the formation of the chemical mentioned in the title.

Step- 3

The reaction mixture was subjected to reflux for a duration of 6-10 hours after the addition of various substituted aryl amine derivatives to a solution of 4-[4-chloro-6-(quinolin-8-yloxy)-1,3,5-triazin-2-yl] oxybenzonitrile (0.01 mol in 20 ml of 1,4-Dioxane). A 10% solution of NaHCO3 was used to neutralize the reaction mixture. Following the end of the reaction, the mixture was subjected to treatment with crushed ice and then neutralized with dil. The resulting precipitate was dried and recrystallized from 100% alcohol using HCl.

BIOLOGICAL EVALUATION

Antimicrobial activity

Antimicrobial screening was conducted using the cup-plate method[19] at a concentration of 100µg/mL. All the compounds were assessed for their in vitro antimicrobial activity against different strains of bacteria, such as Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 43300, Staphylococcus aureus, resistant to methicillin ATCC 43300 (MRSA), and fungi such as Microsporum gypseum, and Candida albicans. Solvent DMSO was used as a solvent control, Mueller-Hinton agar (MHA) broth was used as the medium for the antibacterial test and Sabouraud Dextrose Agar (SDA) broth was used for the antifungal test. Standard drugs like gentamicin and ketoconazole were used for comparison purposes. After incubation at 37°C for 24h for bacteria and 48h for C. albicans and 7 days for dermatophytes, the zones of inhibition were measured. The bioactivity data of the screening test of synthesized compounds are given in Table 3. The minimum inhibitory concentration (MIC) values of the active compounds screened from Table 1 were determined following the microdilution method described in the National Committee for Clinical Laboratory Standards[20]. The concentrations of the tested substance were prepared within the range from 1024 to 0.125 µg/ml. Put 2 mL of the diluted solutions into small wells of the plate. To the diluted solution on the 96-well plates, 1-2 µL of test organism suspension (104 CFU/ml) was added. Incubate in an incubator at 37 °C for 24h for bacteria and in 48h for C. albicans, and 7 days for Dermatophytes. The MIC was the lowest concentrations of test compound that was able to inhibit visible growth of the bacteria and was determined in triplicate.The MIC results were displayed in Table-4.

III. RESULTS AND DISCUSSION

Physical data of1,3,5-Triazin-2yl} Oxy) Benzonitrile Derivatives

Sr. No.	Comp.Code	Molecular Formula	M.W.	M.P. (0C)	Rf Value	% Yield
1	TB1	$C_{21}H_{20}N_6O_2$	388	140-142	0.56	87
2	TB2	$C_{21}H_{20}N_6O_3$	404	99-101	0.63	75
3	TB3	$C_{20}H_{17}FN_6O_2$	392	84-86	0.51	85
4	TB4	$C_{266}H_{18}N_6O_2$	446	145-150	0.58	77
5	TB5	$C_{26}H_{28}N_6O_3$	462	130-133	0.55	69
6	TB6	$C_{25}H_{15}FN_6O_2$	450	112-117	0.51	65

SPECTROSCOPY ANALYSIS

IR spectra of 4-((4-morpholino-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)benzonitrile.(TB1)

880 cm^{-1}	-C=N- Stretching in S-triazine
1624-1446cm^{-1}	-C=C- and –C=N- Stretching
1162cm^{-1}	-C-O- Stretching in Morpholine
1033cm^{-1}	-C-N- Stretching in Morpholine
2349cm^{-1}	-C=N Stretching
2915cm^{-1}	-CH Stretching in Methyl
3367cm^{-1}	-NH Stretching in Secondary amine
1224 cm^{-1}	-C-O- Stretching

NMR spectra 4-((4-morpholino-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)benzonitrile

No.	Chemical shift (δ ppm)	Multiplicity	Proton Assignment	No. of Protons
1	9.062	Singlet	Ar-NH	1
2	2.13	Singlet	Ar-CH3	3
3	3.17-3.12	Multiplate	Morpholine Proton	8
4	7.63-7.50	Multiplate	Ar-H	4

5	6.98-6.87	Multiplate	Ar-H	4

Mass spectra of 4-((4-morpholino-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)benzonitrile

Mol.Wt.	Mol.Formula	Mass(M/Z)
388.42	$C_{21}H_{20}N_6O_2$	390.9(M+2)

IR spectra of 4-({4-[(4-methoxyphenyl) amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile(TB 2)

872 cm^{-1}	-C=N- Stretching in S-triazine
1621-1455 cm^{-1}	-C=C- and –C=N- Stretching
1164cm^{-1}	-C-O- Stretching in Morpholine
1012cm^{-1}	-C-N- Stretching in Morpholine
2221cm^{-1}	-C=N Stretching
3094 cm^{-1}	-CH Stretching in Methyl
3365 cm^{-1}	-NH Stretching in Secondary amine
1154cm^{-1}	-C-O- Stretching

NMR spectra of 4-({4-[(4-methoxyphenyl) amino]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}oxy)benzonitrile

No.	Chemical shift (δ ppm)	Multiplicity	Proton Assignment	No. of Protons
1	9.55	Singlet	Ar-NH	1
2	3.70	Singlet	Ar-OCH3	3
3	3.59	Multiples	Morpholine Proton	8
4	7.92-7.50	Multiples	Ar-H	4
5	6.87-6.71	Multiples	Ar-H	4

Mass spectra of 4-({4-[(4-methoxyphenyl) amino]-6-(morphin-4-yl)-1,3,5-triazine-2-yl}oxy)benzonitrile

Mol.Wt.	Mol.Formula	Mass(M/Z)
404.42	$C_{21}H_{20}N_6O_3$	406.4(M+2)

IR Spectra of 4-((4-(quinolin-8-yloxy)-6-(p-tolylamino)-1,3,5-triazin-2-yl)oxy)benzonitrile(TB 3)

878 cm^{-1}	-C=N- Stretching in S-triazine
1602-1448cm^{-1}	-C=C- and –C=N- Stretching
1255cm^{-1}	-C-O-C Stretching
1107cm^{-1}	-C-N- Stretching
2312cm^{-1}	-C=N Stretching
2889cm^{-1}	-CH Stretching in Methyl
3395cm^{-1}	-NH Stretching in Secondary amine
1167cm^{-1}	-C-O- Stretching

NMR spectra of 4-({4- [(4-methyl phenyl) amino]-6-(quinolin-8yloxy)-1,3,5-triazin-2-yl}oxy)benzonitrile

No.	Chemical shift (δ ppm)	Multiplicity	Proton Assignment	No. of Protons
1	10.80	Singlet	Ar-NH	1
2	2.50	Singlet	Ar-CH3	3
3	8.91-7.90	Doublet	Ar-H (quinoline)	1
4	7.70-7.64	Multiplates	Ar-H	2
5	7.62-7.50	Multiplates	Ar-H	3
6	7.49-7.29	Multiplates	Ar-H	4

7	6.99-6.87	Multiplates	Ar-H	4

Mass spectra of 4-({4- [(4-methyl phenyl) amino]-6-(quinolin-8yloxy)-1,3,5-triazin-2-yl}oxy)benzonitrile

Mol.Wt.	**Mol.Formula**	**Mass(M/Z)**
446.46	$C_{26}H_{18}N_6O_2$	447.1(M+1)

IR spectra of 4-({4- [(4-methoxy phenyl) amino]-6-(quinolin-8yloxy)-1,3,5-triazin-2-yl}oxy)benzonitrile(TB 4)

893 cm^{-1}	-C=N- Stretching in S-triazine
1621-1443cm_{-1}	-C=C- and –C=N- Stretching
1241cm^{-1}	-C-O- C Stretching
1035cm^{-1}	-C-N- Stretching
2231cm^{-1}	-C=N Stretching
2885cm^{-1}	-CH Stretching in Methyl
3387cm^{-1}	-NH Stretching in Secondary amine
1161cm^{-1}	-C-O- Stretching

NMR spectra of 4-({4- [(4-methoxy phenyl) amino]-6-(quinolin-8yloxy)-1,3,5-triazin-2-yl}oxy)benzonitrile

No.	**Chemical shift (δ ppm)**	**Multiplicity**	**Proton Assignment**	**No. of Protons**
1	10.72	Singlet	Ar-NH	1
2	3.70	Singlet	Ar-CH3	3
3	8.01-7.95	Doublet	Ar-H (quinoline)	1
4	7.91-7.66	Multiplates	Ar-H	2
5	7.64-7.51	Multiplates	Ar-H	3
6	7.43-7.27	Multiplates	Ar-H	4
7	6.92-6.74	Multiplates	Ar-H	4

Mass spectra of 4-({4--[(4-methoxy phenyl) amino 6-(quinolin-8yloxy)-1,3,5-triazin-2- yl} oxy)benzonitrile

Mol.Wt.	**Mol.Formula**	**Mass(M/Z)**
462.45	$C_{26}H_{18}N_6O_3$	463.1(M+1)

Tablet-3. Antimicrobial activity of Synthesized Compounds (diameter of zone of inhibition in mm)

Compounds	**zone of inhibition in mm**				
	Bacteria			Fungi	
	S.aureus	MRSA	E. coli	C.albicans	M.gypseum
TB1	16	-	14	14	30
TB2	15	-	11	20	28
TB3	11	-	10	19	25
TB4	10	-	10	9	26
TB5	10	-	8	9	26
TB6	12	-	8	7	25
Gentamicin	27	-	22	-	-
Ketoconazole	-	-	-	16	-

(-): inactive at concentration 100µg/Ml

Tablet-4. Antimicrobial activity of synthetic compounds (minimum inhibitory concentration in µg/mL)

Compounds	MICs on tested Bacteria (µg/mL)	MICs on tested Fungi (µg/mL)

	S. aureus	MRSA	E. coli	C. albicans	M. gypseum
TB1	32	-	64	64	16
TB2	64	-	128	32	32
TB3	128	-	128	32	64
TB4	128	-	128	64	64
TB5	128	-	256	64	64
TB6	64	-	256	128	64
Gentamicin	4	-	8	–	–
Ketoconazole	–	-	–	8	–

(-): inactive at concentration 64 µg/mL; ND: not determined

Antimicrobial and Antifungal screening for the synthesized compounds showed that compound TB2 was found highly active towards both bacteria and fungi. While compounds 2 and compound 3 showed comparatively moderate activity towards this series of bacteria. Synthesized compounds of shows MBC concentration at 1000 to 500µg/ml, which indicates poor activity compared to standard drugs.

Antimicrobial activity of synthesized compounds against the gram-positive bacteria Escherichia coliwas not found that interesting compare to Staphylococcus aureus.

Compound 3 was found good active against both gram-negative bacteria E. Coli. This compound was showing activity at the Minimal Bactericidal Concentration of 500µg/ml.

Activity against the gram-negative bacteria Escherichia Coli by most of the synthesized compounds was found to be poor.

Antifungal activities of this series of synthesized compounds were not found that good as compared to standard drug used.

CONCLUSION

In the present study, a new series of 1,3,5-triazin-2-yl oxy benzonitrile derivatives, including N-(4-aryl amine)-2-{[4-phenyl-5-(pyridin-4-yl)-4H-1,2,4-triazol-3-yl]sulfanyl} acetamidesand chalcone-based analogs, were successfully synthesized and characterized using appropriate spectroscopic techniques such as IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The multistep synthetic approach employing pyridine-4-carboxylic acid as the starting material provided structurally diverse triazole derivatives with potential biological significance. The antimicrobial screening revealed that all compounds exhibited moderate to significant activity against tested bacterial and fungal strains, with compound 3 demonstrating superior potency, particularly against *Staphylococcus aureus*, MRSA, and fungal pathogens. These results suggest that triazole-based acetamide and chalcone frameworks represent promisinglead structures for the development of novel antimicrobial agents. Further structural optimization and detailed mechanistic studies may enhance their therapeutic potential against resistant microorganisms.

Overall, the results suggest that structural modification of these thiophene derivatives can enhance antimicrobial potential, with TB1 standing out as the most promising scaffold for further optimization in developing new antimicrobial agents.

The synthesized thiophene-based derivatives (TB1–TB6) demonstrated moderate antibacterial and antifungal activities compared to the standard drugs. Among the tested compounds, TB1 exhibited the most potent broad-spectrum activity, showing significant inhibition zones (16 mm against S. aureus, 14 mm against E. coli, 14 mm against C. albicans, and 30 mm against M. gypseum) and the lowest MIC values (32–64 µg/mL for bacteria and fungi, with 16 µg/mL for M. gypseum). Compounds TB2 and TB3 also showed relatively better activity, particularly against C. albicans and M. gypseum.

However, the antimicrobial activity of the synthesized derivatives was weaker than the reference drugs—Gentamicin for bacteria and Ketoconazole for fungi—both of which showed significantly lower MICs (4–8 µg/mL) and larger inhibition zones.

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