SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL STUDIES OF CERTAIN TRIAZOLE CONTAINING S-TRIAZINE DERIVED COMPOUND.

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Abstract
Some new substituted 1,3,5 triazine with 1,2,4 triazole and substituted urea/thiourea were synthesized and evaluated for their *in vitro* antimicrobial activity against Gram positive and Gram negative strains using a microdilution procedure. Synthesized compounds **1a to 1i** prove to be effective with MIC (mg/ ml), among them **1c**, **1e**, **1g** showed excellent activity against a panel of microorganisms. The newly synthesized compounds were characterized using **IR**, ^{1}**H-NMR**.

Keywords: - 1, 2, 4 Triazole, Substituted urea/thiourea, Cyanuric chloride and Antimicrobial activity.

Introduction:-
S-Triazine derivatives represent an important class of compounds due to their potential to be biologically active. They are known to be anti-protozoals[1], anticancer agents[2], estrogen receptor modulators[3], antimalarials[4], cyclin-dependent kinase modulators[5], and antimicrobials[6]. These are valuable bases for estrogen receptor modulators [7] and also used as bridging agents to synthesize herbicides and in the production of drugs or polymers[8].
1,2,4 – Triazole have wide range of biological activities such as anti bacterial[9] , anti caner[10] , anti tubercular[11], anti HIV[12] and anti depressant activity, anti tumor [13] and anti viral[13] activity, anti hypertensive[14] activity , analgesic and anti inflammatory[15] activity.
Thiourea derivatives possess antibacterial [16], hypnotic antitubercular and possible anticonvulsant activities. It also represent a new class of human immuno deficiency virus type (HIV-1), non-nucleoside reverse transcriptase (NNRT) inhibitors [17], found as antagonist [18], and high density lipoprotein (HDL) elevating agents [19].
Urea derivatives are reported to possess antibacterial[20], antimicrobial antifungal, anticancer[21] and anticonvulsant[22] activities. Urea derivatives possess wide therapeutic activities such as antithyroidal, hypnotic and anesthetic, antibacterial, diuretic[23] and anthelmintics.
we wish to describe a simple and efficient protocol for the rapid preparation of ***1-(4-(3-(4-METHOXYPHENYL)THIOUREIDO)-6-(1H-1,2,4-TRIAZOL-1-YL)-1,3,5-TRIAZINE-2-YL)-3-PHENYLUREA*** at different temperature conditions. To the best of our knowledge, there are no reports on three-component coupling of triazole, substituted urea and thiourea to produce a title compound.We planned to undertake the synthesis and characterization of some triazine derivatives carrying the above biodynamic heterocyclic systems with the hope to achieve enhanced biological activity.

Experimental:-
General
All the melting points were taken in open capillaries tube and are uncorrected. 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 were recorded on FTIR spectrophotometer using KBr or Nujol technique. ^{1}H NMR spectra on a Varian 400 FT MHz NMR instrument at using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference.

Scheme:-
Step-1
Preparation Of 1-(4,6-Dichloro-1,3,5-Triazin-2-Yl)-3-(4-Methoxy Phenyl)Thiourea: (A)
To a stirred solution of cyanuric chloride (0.1 mole, 18.4 g.) in acetone (100 ml) at 0-5^{0}C, the solution of 1–(4-methoxyphenyl)thiourea (0.1 mole,17.3g) in acetone (45 ml) was added and pH being maintained neutral by the addition of 10% sodium bi-carbonate solution from time to time as per requirement of reaction condition. 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 crude product was purified by crystallization from absolute alcohol to get title compound.

Step-2

Preparation Of 1-(4-Chloro-6-(1 H-1,2,4-Triazol-1-Yl)-1,3,5-Triazine-2-Yl)-3-(4-Methoxyphenyl) Thiourea : (B)

To a stirred solution of (A) (0.1 mole, 33.0 g) in acetone (100 ml) was added, the solution of 1,2,4 triazole (0.1 mole, 6.9 g) in acetone (25 ml) was added drop wise maintaining the temperature at 40°C, the pH being maintained neutral by the addition of 10% sodium bi-carbonate solution from time to time as per requirement of reaction condition. The temperature was gradually raised to 45°C during three hours. After the completion of reaction, the resultant content was poured into ice-cold water. The solid product obtained was filtered and dried. The crude product was purified by crystallization from absolute alcohol to get the title compound.

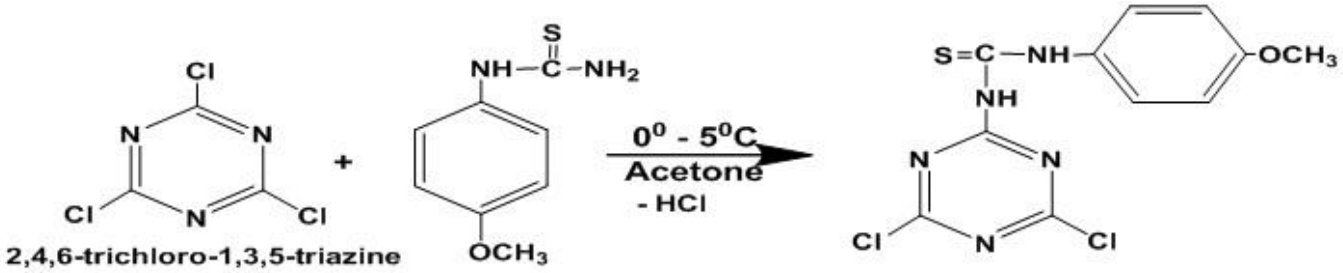
Step-3

Preparation Of Final Compound:-

A mixture of (B) (0.01 mole, 3.62 g) and aryl urea (0.01 mole) in DMF (20ml) was refluxed in oil bath. The temperature was gradually raised to 80-100^{0}C during four hours, the pH being maintained neutral by the addition of 10% sodium bi-carbonate solution from time to time as per requirement of reaction condition. After the completion of reaction, add little charcoal in R.B.F. and then filter it into cold water. The solid product obtained was filtered and dried. The crude product was purified by recrystallization from absolute alcohol. Prepare all derivatives by this method. Analytical data are given below.

ROUTE OF SYNTHESIS :-

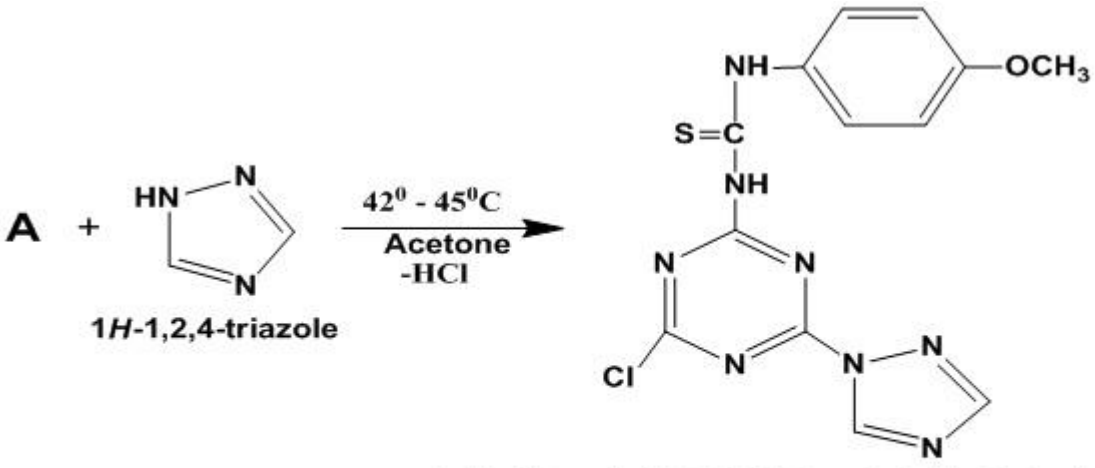
STEP-1



2,4,6-trichloro-1,3,5-triazine — 1-(4-methoxyphenyl)thiourea — 1-(4,6-dichloro-1,3,5-triazin-2-yl)-3-(4-methoxyphenyl)thiourea

[A]

STEP-2

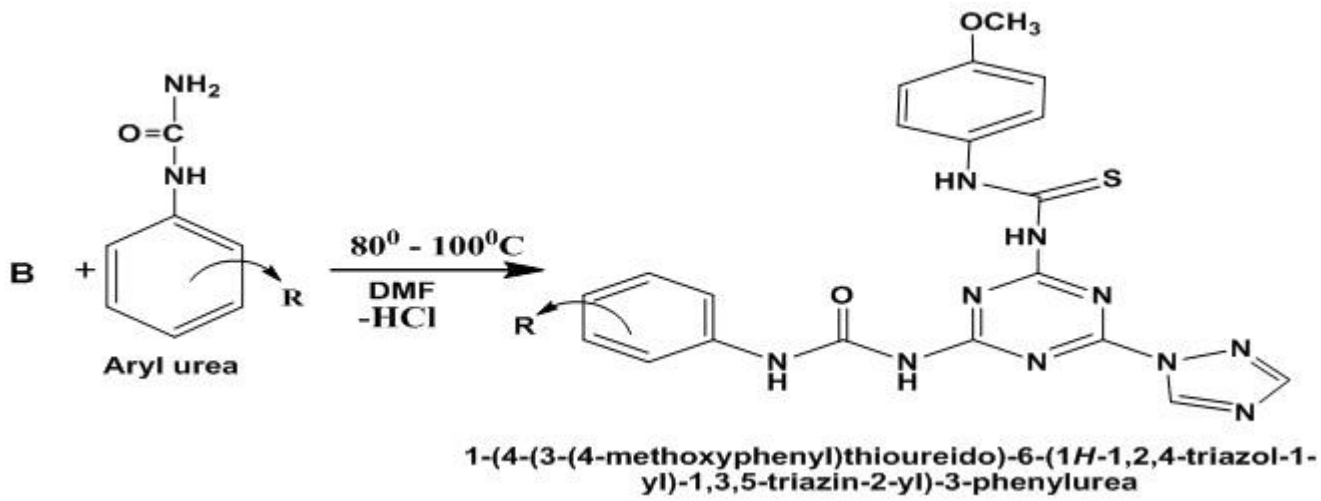


1*H*-1,2,4-triazole

1-(4-chloro-6-(1*H*-1,2,4-triazol-1-yl)-1,3,5-triazin-2-yl)-3-(4-methoxyphenyl)thiourea

[B]

STEP-3



1-(4-(3-(4-methoxyphenyl)thioureido)-6-(1*H*-1,2,4-triazol-1-yl)-1,3,5-triazin-2-yl)-3-phenylurea

Where **R** = given in below table.

Table 1 Physical data of synthesized compounds:-

Sr. No.	R	Mol. Formula	Mol. Weight	M.P. ^{O}C	Yield %
1a	H	$C_{20}H_{18}N_{10}O_2S$	462.49	120	60
1b	2-OCH_3	$C_{21}H_{20}N_{10}O_3S$	492.51	122	61
1c	4-CH_3	$C_{21}H_{20}N_{10}O_2S$	476.51	190	59
1d	4-Cl	$C_{20}H_{17}ClN_{10}O_2S$	496.93	220	74
1e	4-OCH_3	$C_{21}H_{20}N_{10}O_3S$	492.51	140	59
1f	2-CH_3	$C_{21}H_{20}N_{10}O_2S$	476.51	140	58
1g	2-Cl	$C_{20}H_{17}ClN_{10}O_2S$	496.93	135	62
1h	4-Br	$C_{20}H_{17}BrN_{10}O_2S$	541.38	180	60
1i	4-F	$C_{20}H_{17}FN_{10}O_2S$	480.48	175	65

Compound (1a): Yield: 60%; m.p. 120^{0}C (dec.); **IR (KBr,cm^{-1}) :** 798 cm^{-1} (-C=N- s-triazine) 818.47 cm^{-1}(1,4 Di sub. in benzene) 1416.15 cm^{-1}(>N-,3^0 amine) 1548.50 cm^{-1}(-NH-def)1656.15 cm^{-1} (-C=O-) 3290.15 cm^{-1}(-NH-str) 2800.50 cm^{-1}(–OCH_3 str) 1170.64 cm^{-1}(-C=S-)1023.14 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.90(s,2H,-CONH), 10.30(s,2H,-CSNH),3.64(s,3H, -OCH_3), 7.20-7.98 (m,11H, Ar-H).
Compound (1b): Yield: 61%; m.p. 122^{0}C (dec.); **IR (KBr,cm^{-1}) :** 801 cm^{-1} (-C=N- s-triazine) 819.25 cm^{-1}(1,4 Di sub. in benzene) 1410.98 cm^{-1}(>N-,3^0 amine)1562.70cm^{-1} (-NH-def)1643.16 cm^{-1} (-C=O-) 3311.16 cm^{-1}(-NH-str)2916.48 cm^{-1} (–OCH_3 str)1177.34cm^{-1}(-C=S-)1033.34 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.64(s,2H,-CONH) 10.22(s,2H,-CSNH),3.69(s,6H, -OCH_3)7.40-7.78 (m,10H, Ar-H).
Compound (1c): Yield: 59%; m.p. 190^{0}C (dec.); **IR (KBr,cm^{-1}) :** 783.07 cm^{-1} (-C=N- s-triazine) 821.07 cm^{-1}(1,4 Di sub. in benzene)1410.98 cm^{-1}(>N-,3^0 amine) 1596.6 cm^{-1}(-NH-def) 1643.16cm^{-1} (-C=O-) 3311.16 cm^{-1}(-NH-str) 2916.48 cm^{-1} (–OCH_3 str) 1177.34 cm^{-1}(-C=S-)1308.20 cm^{-1} (-C-CH_3 str) 1033.34 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.55(s,2H,-CONH) 10.10(s,2H,-CSNH),3.71(s,3H, -OCH_3)3.74(s,3H,C-CH_3) 7.32-7.66 (m,10H, Ar-H).
Compound (1d): Yield: 74%; m.p. 220^{0}C (dec.); **IR (KBr,cm^{-1}) :** 795 cm^{-1}

Sr. No & Comp no.		R	Minimum Inhibitory Concentration (μg/ml)				
			Gram positive bacteria		Gram negative bacteria		Fungus
			S. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans
1.	1a	H	125	250	125	125	125
2.	1b	2-OCH_3	125	125	125	250	125
3.	1c	4-CH_3	125	**31.25**	125	**31.25**	**31.25**
4.	1d	4-Cl	125	250	125	125	125
5.	1e	4-OCH_3	**62.5**	125	125	125	125
6.	1f	2-CH_3	125	125	125	125	125
7.	1g	2-Cl	**62.5**	125	125	125	250
8.	1h	4-Br	125	125	125	125	250
9.	1i	4-F	125	125	125	125	125
Ampicillin		------	250	100	100	100	------
Nystatin		------	---	---	---	---	100

(-C=N- s-triazine) 816.34 cm^{-1}(1,4 Di sub. in benzene) 1418.09 cm^{-1}(>N-,3^0 amine)1559.90 cm^{-1}(-NH-def)1638.80 cm^{-1} (-C=O-) 3330.30 cm^{-1}(-NH-str)2890.50 cm^{-1} (–OCH_3 str)1180.80cm^{-1}(-C=S-)707 cm^{-1}(-C-Cl-str)1029.34 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.64(s,2H,-CONH) 10.22(s,2H,-CSNH),3.69(s,3H, -OCH_3)7.40-7.78 (m,10H, Ar-H).

Compound (1e): Yield: 59%; m.p. 140^{0}C (dec.); **IR (KBr,cm^{-1}) :** 804 cm^{-1}
(-C=N- s-triazine) 812.40 cm^{-1}(1,4 Di sub. in benzene) 1416.30 cm^{-1}(>N-,3^0 amine)1569.70 cm^{-1}(-NH-def)1651.60 cm^{-1} (-C=O-) 3334.11 cm^{-1}(-NH-str)2930.30 cm^{-1} (–OCH_3 str)1169.64cm^{-1}(-C=S-)1028.71 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.90(s,2H,-CONH) 10.90(s,2H,-CSNH),3.80(s,6H, -OCH_3)7.50-8.18 (m,10H, Ar-H).

Compound (1f): Yield: 58%; m.p. 140^{0}C (dec.); **IR (KBr,cm^{-1}) :** 795.65 cm^{-1}

(-C=N- s-triazine) 819.40 cm^{-1}(1,4 Di sub. in benzene)1418.30 cm^{-1}(>N-,3^0 amine) 1584.20 cm^{-1}(-NH-def) 1637.60cm^{-1} (-C=O-) 3320.65 cm^{-1}(-NH-str) 2898.28 cm^{-1} (–OCH_3 str) 1169.70 cm^{-1}(-C=S-)1316.60 cm^{-1} (-C-CH_3 str) 1040.85 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.60(s,2H,-CONH) 9.95(s,2H,-CSNH),3.66(s,3H, -OCH_3)3.60(s,3H,C-CH_3) 7.22-7.90 (m,10H, Ar-H).

Compound (1g): Yield: 62%; m.p. 135^{0}C (dec.); **IR (KBr,cm^{-1}) :** 789.30 cm^{-1}
(-C=N- s-triazine) 825.30 cm^{-1}(1,4 Di sub. in benzene) 1430.10 cm^{-1}(>N-,3^0 amine)1565.60 cm^{-1}(-NH-def)1650.70 cm^{-1} (-C=O-) 3310.80 cm^{-1}(-NH-str)2900.50 cm^{-1} (–OCH_3 str)1170.60cm^{-1}(-C=S-)717 cm^{-1}(-C-Cl-str)1036.74 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.70(s,2H,-CONH) 10.29(s,2H,-CSNH),3.75(s,3H, -OCH_3)7.50-7.84 (m,10H, Ar-H).

Compound (1h): Yield: 60%; m.p. 180^{0}C (dec.); **IR (KBr,cm^{-1}) :** 797.40 cm^{-1} (-C=N- s-triazine) 829.90 cm^{-1}(1,4 Di sub. in benzene) 1420.10 cm^{-1}(>N-,3^0 amine)1560.50cm^{-1} (-NH-def)1654.90 cm^{-1} (-C=O-) 33170.55 cm^{-1}(-NH-str)2920.45 cm^{-1} (–OCH_3 str) 1162.70cm^{-1}(-C=S-)692 cm^{-1}(-C-Br-str) 1028.47 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.67(s,2H,-CONH) 10.75(s,2H,-CSNH),3.66(s,3H,-OCH_3)7.42-7.74(m,10H, Ar-H).

Compound (1i): Yield: 65%; m.p. 175^{0}C (dec.); **IR (KBr,cm^{-1}) :** 790.30 cm^{-1}
(-C=N- s-triazine) 820.40 cm^{-1}(1,4 Di sub. in benzene) 1435.10 cm^{-1}(>N-,3^0 amine)1561.50 cm^{-1}(-NH-def)1642.60 cm^{-1} (-C=O-) 3300.75 cm^{-1}(-NH-str)2914.50 cm^{-1}
(–OCH_3 str)1160.60cm^{-1}(-C=S-)1055 cm^{-1}(-C-F-str) 1040.10 cm^{-1} (-N-N-str) **^{1}H-NMR:δ** 8.75(s,2H,-CONH) 10.40(s,2H,-CSNH),3.85(s,3H, -OCH_3)7.70-7.94 (m,10H, Ar-H).

Antimicrobial Activity

For the testing antimicrobial activity various microorganism were used for the study. The **broth dilution** method was used for this study. Following general procedure is adopted[24].The antimicrobial activity of all the compounds was studies at 1000 ppm concentration *in vitro*. The

different types of microorganism used were some gram negative bacteria [*Escherichia coli, Pseudomonas aeruginosa*], gram positive bacteria [*Bacillus subtilis, Staphylococcus aureus*] and fungus [*Candida albicans*].

80% DMSO are used as solvent to dissolve compound 1a to 1i to 10**(µg/ml).**

Conclusions:-

A series of cyanuric chloride derivatives were prepared and tested for their *in vitro* antibacterial activity against the four strains of bacteria (gram +ve, gram –ve).Three compounds of the obtained series showed high *in vitro* antimicrobial activity. Compound (**1c, 1e, 1g**) showed excellent activity against *Staphylococcus aureus.* Whereas compound 1c has excellent activity against *B. subtilis*, *P. aeruginosa, C. albicans.*

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