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Synthesis and biological evaluation of triazine derivatives

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Abstract

*Some new triazine derivatives (**4a-4l**) have been synthesized. The newly synthesized compounds were characterized on the basis of elemental analysis, IR and [1]H-NMR spectra. All the synthesized compounds were tested for their antibacterial activity against 20 strains of Gram positive and Gram negative bacteria. Among the compounds tested, the compounds **4a** & **4l** showed good antimicrobial activity in comparison to standard sulphamethoxazole. Compound **4l** was found to be most active in the series against H. pylori with MIC 25 µg/mL.*

Keywords: 1,2,4 Triazine, Antimicrobial activity, MIC value, isatin.

INTRODUCTION

Antibacterial diseases are very common in all over the world. Currently used antimicrobial agents are not effective due to the resistance developed by the microbes. And therefore, it is an ongoing effort to synthesize new antimicrobial agents [1]. In these days, a number of drugs containing simple heterocyclic or a combination of different moieties have been in use [2].

1,2,4-Triazines and their derivatives have been widely studied in terms of their synthetic methodologies and reactivity since some of these derivatives were reported to have promising biological activities like A1 Adenosine receptor antagonists[3], Age-related muscular degeneration[4], Analgesic-antiinflammatory activity[5], Anticancer Activity[6,7], anticonvulsant activity[8], antimicrobial activity[9,10], antinociceptive activity[11], Antiproliferative activity[12], anxioselective activity[13], Kinase inhibitor activity[14], Muscle Relaxant activity[15]. In the present investigation twelve 1,2,4 triazine derivatives (4a-4l) were prepared. The synthesized 1,2,4 triazine derivatives (4a-4l) were tested for their antimicrobial activity.

RESULTS AND DISCUSSION

1, 2, 4-triazine heterocyclic entity are very interesting components in terms of their biological properties, such as antifungal, antibacterial and herbicidal. Synthesized compounds **4a-4l** were tested against a panel of microorganisms including Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli ATCC-25922, Escherichia coli ATCC-35218, Morganella morganii, Prot.mirabilis, Providencia rettgeri, Salmonella paratyphi, Shigella sonnei, Shigella boyelli, Vibrio cholerae, Prot.vulgaris, E.faecalis ATCC-2921, Pseudo.aeruginosa ATCC-27853, Shigella flexneri, Klebsiella oxytoca, Kleb. pneumoniae, Salmonella enteritidis, Salmonella typhi MTCC 2316, H. pylori, Salmonella typhi,*) was using conventional agar-dilution method. The MIC values for these compounds (4a-4l) were determined by comparison to sulphamethoxazole as reference drug.

Sensitivity testing was performed for all compounds. Which showed that some synthetic compounds (**4c, 4e, 4g, 4i, 4j, 4k,**) were not sensitive against all bacteria and rest compounds were sensitive against all bacteria.

Compounds **4a-4l** were not active against *Shigella boyedli, Proteus vulgaris, Salmonella typhi MTCC 3216, Salmonella enteritidis, Kleb. pneumoniae.* Further some compounds were not active against specific bacteria like **4a** (*Shigella sonnei),* **4a, 4b and 4l,** (*Providencia rettgeri),* **4a, 4f and 4l,** (*E.coli ATCC35218),* **4a, 4b, 4d and 4l** (*Proteus mirabilis),* **4d and 4h** (*Morganella morganii),* **4a** *(Salmonella paratyphi),* **4a, 4b, 4d and 4h,** (*Vibrio cholerae*), **4a, 4b and 4h,** (*Pseudo.aeruginosa ATCC27853),* **4b** (*Shigella flexinerii),* **4b**, **4d, 4f, 4h and 4l** (*Klebsiella oxytoca),* **4b and 4h** (*H. pylori),* **4a, 4b and 4f** (*Salmonella typhi),* **4a** (*Staphylococcus aureus).*

The MIC values of synthesized compounds were tested against organism displayed a significant activity with wide degree of variation (**Table III**). On gram positive bacteria, Compounds **4a-4l** were found to be 12.5 to 25 times more active than standard drug. On gram negative bacteria, Compounds **4a-4l** were found to be 2.5 to 100 times more active than standard drug.

Compound **4a** exhibited significant activity against *E.coli* ATCC 25922, *H. pylori* and *Shigella flexinerii* with MIC value 50µg/mL. Other then these bacteria, compound **4a** showed moderate activity. Compounds **4l** showed greater activity against *H. pylori* with MIC value 25µg/mL. Other then this bacteria, this compound was showed moderate activity. Synthesized compounds **4a, 4b, 4d, 4f, 4h** and **4l** exhibited moderate activity against all bacteria.

Thiol group containing compounds (C=S) like (**4b, 4d, 4f, 4h and 4l**) were found to be more active than keto group containing compounds (C=O) (**4a**). Nitro group containing compounds (NO_2) (**4l**) was found to be active against some bacteria than other group containing compounds (Br). It was found to be 5 to 12.5 times more active than standard drug.

On the basis of MIC values, synthesized compounds were divided into three parts
1-Weak active (300-500) - **4d**
2-Moderate active (50-300µg/mL) - **4d, 4f** & **4h**
3-Most active (12.5-50µg/mL) - **4a** & **4l**
Antibacterial screening revealed that synthetic compounds exhibited moderate activity as compared to standard.

Table III: *In vitro* antibacterial activities of compounds 4a-l against selected strains (MICs in µg /mL)

S.No.	Name of bacteria	4a	4b	4d	4f	4h	4l	Sulph.
1	*Staphylococcus aureus*	300	---	200	300	200	400	5000
2	*E.coli* ATCC25922	50	400	300	300	150	400	1250
3	*E.coli* ATCC35218	---	500	200	---	400	---	1250
4	*E.faecalis* ATCC29212	50	500	400	200	300	250	5000
5	*H. pylori*	50	---	100	400	---	25	2500
6	*Klebsiella oxytoca*	150	---	---	---	---	---	5000
7	*Kleb. Pneumoniae*	---	---	---	---	---	---	2500
8	*Morganella morganii*	300	500	---	300	---	500	2500
9	*Proteus vulgaris*	---	---	---	---	---	---	2500
10	*Pseudo.aeruginosa* ATCC27853	---	---	300	500	---	250	5000
11	*Providencia rettgeri*	---	---	400	500	250	---	2500
12	*Proteus mirabilis*	---	---	---	500	300	---	2500
13	*Shigella sonnei*	---	500	500	400	400	200	2500
14	*Shigella boyedli*	---	---	---	---	---	---	2500
15	*Salmonella paratyphi*	---	500	400	500	300	200	2500
16	*Shigella flexinerii*	50	---	250	400	200	500	2500
17	*Salmonella enteritidis*	---	---	---	---	---	---	2500
18	*Salmonella typhi* MTCC 3216	---	---	---	---	---	---	2500
19	*Salmonella typhi*	---	---	300	---	300	500	2500
20	*Vibrio cholera*	---	---	---	300	---	250	5000

Sulphamethoxazole as standard drug; ---=Insensitive (inactive)

MATERIALS AND METHODS

Experimental work

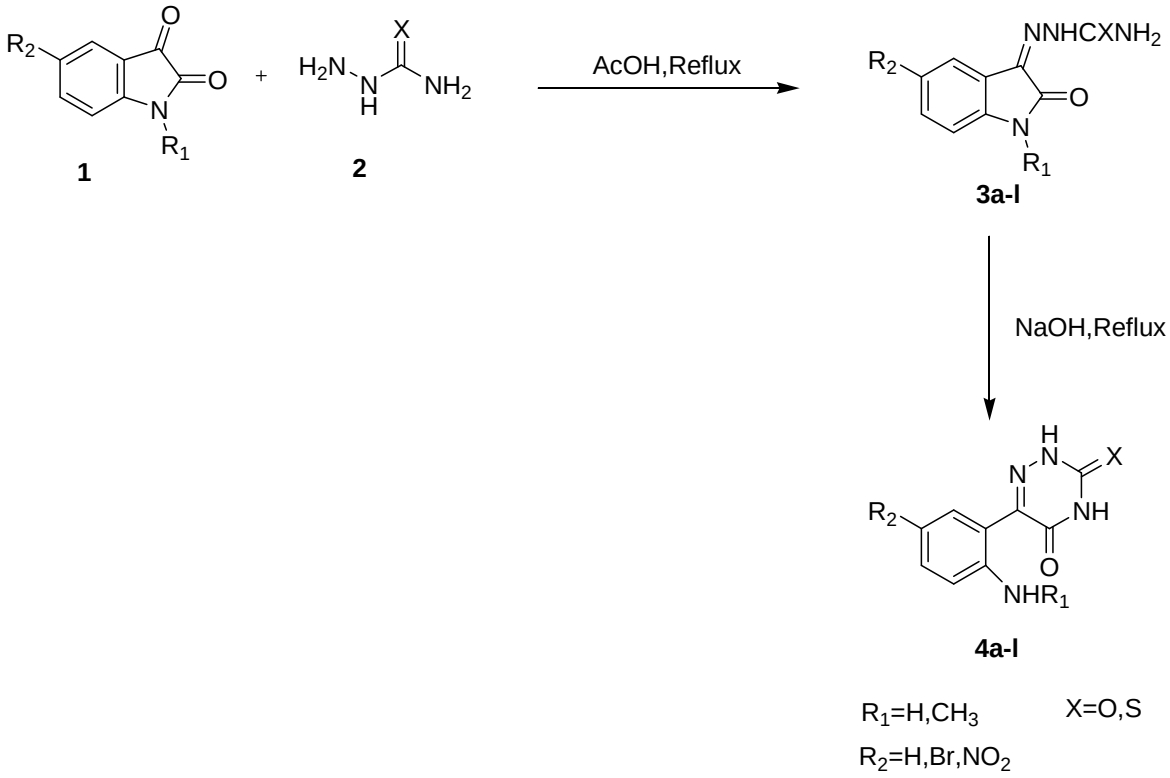
General Procedures. Melting point was determined using open capillary tubes on a Thiel's melting point apparatus and uncorrected. The purity of the synthesized compounds were checked by TLC (solvent system -Ethyl acetate: n-hexane = 5: 5, Visualising agent- iodine vapour) and column chromatography. FT-IR was recorded on a Perkin Elmer FT-IR spectrophotometer, ^{1}H NMR spectra were recorded at 300 MHz on a Bruker DRX-300 FT-NMR spectrophotometer (CDRI, Lucknow). The elemental analysis was obtained on a Elementar Vario EL III instrument (CDRI, Lucknow).

N-Methylindoline-2, 3-dione (N-Methyl isatin)

Isatin (5.00 g) & dimethyl sulphate (0.033mL) were added in dil. sodium hydroxide (1N) in round bottom flack. This mixture was refluxed for approx. 50 minute on oil bath. After this the reaction mixture was poured in beaker & evaporated under reduced pressure. Then solid compound was obtained.

m.p. = 136 ^{0} C, R_f = 0.10, & yield = 83.94%

Scheme 1: Synthesis of 1, 2, 4-trizine derivatives



General procedure for bromination of isatin and its derivatives

Bromine (1.00 mL) was added in acetic acid (4 mL) in 100 ml beaker in ice cold condition with stirring. Isatin derivative (1.00 g) was dissolved in acetic acid in 100 mL beaker with stirring. Bromine solution (0.35 mL) was mixed in isatin derivative solution in ice cold condition with stirring. Then mixture was placed in freezer for 24 hours. The compound get solidified which was dried it at room temp.

5-Bromoindoline-2, 3-dione (5-Bromo isatin)

m.p. = 184^0 C, R_f = 0.70 & yield= 25%

5-Bromo-1-methylindoline-2,3-dione (5-Bromo N-methyl isatin)

m.p. = 46 ^{0} C, R_f =0.87, & Percentage yield=27%

General procedure for nitration of isatin and its derivatives

Isatin derivative (1.00 g) was dissolved in acetic acid in 100 mL beaker. Then sulphuric acid (1.8 mL) was added drop wise in ice cold condition with stirring. The nitric acid (0.6 mL) was added in mixture, stirred for some time. Then mixture was placed in freezer for 24 hours. The solid precipited was formed, dried it.

5-Nitroindoline-2, 3-dione (5-Nitro isatin)

m.p. = 230 ^{0} C, R_f = 0.79 & Percentage yield= 67%

1-Methyl-5-nitroindoline-2, 3-dione (N-methyl 5-nitro isatin)

m.p. = 255 ^{0} C, R_f = 0.72, & yield= 27%

General procedure for synthesis of thio or semicarbazones of isatin derivatives (3a-l)
Isatin derivative (1 mole) was dissolved in boiling acetic acid (50 mL) in 100 mL beaker. Thio or semicarbazide (1 mole) was dissolved in distil water (10 mL) in another 100 mL beaker. Then this solution was added in boiling isatin solution. The mixture was boiled for approx. 20 min. with stirring (for **3a, 3c, 3e, 3g, 3i, and 3k**) or refluxed for about 10 hours (for **3b, 3d, 3f, 3h, 3j, 3l**). After cooling the solid formed was filtered off, washed with acetic acid followed with water. The dried product was recrystallized from acetic acid affording yellow crystals.

1-(2-Oxoindolin-3-ylidene) semicarbazide **(3a)**
m.p. = 240 ^{0}C, yield= 55%, **IR (KBr) in cm^{-1}:** 3406 (2^{0}NH), 3315 (1^{0}NH), 1725 (C= O), 1621 (amide C= O), 1660 (C= N). **^{1}H-NMR (DMSO) δ ppm:** 7.23- 8.01 (m, 4H, Ar-H), 7.21 (s, 1H, NH), 6.49 (s, 2H, NH_2), 8.39 (s, 1H, indole-NH).

1-(2-Oxoindolin-3-ylidene) thiosemicarbazide **(3b)**
m.p. = 220 ^{0}C, yield= 64%, **IR (KBr) in cm^{-1}:** 3456 (2° NH), 3388 (1^0 NH), 1678 (C= O), 1624 (C= N), 1019 (C= S). **^{1}H- NMR (DMSO) δ ppm:** 7.33-7.81 (m, 4H, Ar-H), 7.36 (s, 1H, NH), 2.22 (s, 2H, NH_2), 8.25 (s, 1H, indole-NH).

1-(1-Methyl-2-oxoindolin-3-ylidene) semicarbazide **(3c)**
m.p. = 255 ^{0}C, yield= 51%, **IR (KBr) in cm^{-1}:** 3425 (2° NH), 3318 (NH), 2922 (-CH_3), 1655 (C= O), 1672 (amide C= O), 1690 (C= N). **^{1}H- NMR (DMSO) δ ppm:** 7.39-7.98 (m, 4H, Ar-H), 7.17 (s, 1H, NH), 6.55 (s, 2H, NH_2), 3.19 (s, 3H, CH_3).

1-(1-Methyl-2-oxoindolin-3-ylidene) thiosemicarbazide **(3d)**
m.p. = 190 ^{0}C, yield= 57%, **IR (KBr) in cm^{-1}:** 3443 (2° NH), 3344 (1^0 NH), 2909 (-CH_3), 1674 (C= O), 1613 (C= N), 1023 (C= S). **^{1}H- NMR (DMSO) δ ppm:** 7.23-7.91(m, 4H, Ar-H), 7.38 (s, 1H, NH), 2.49 (s, 2H, NH_2), 3.09 (s, 3H, CH_3).

1-(5-Bromo-2-oxoindolin-3-ylidene) semicarbazide **(3e)**
m.p. = 175 ^{0}C, yield= 48%, **IR (KBr) in cm^{-1}:** 3442 (2^0 NH), 3223 (1^0 NH), 1732 (C= O), 1665 (amide C= O), 1615 (C= N), 544 (C-Br). **^{1}H- NMR (DMSO) δ ppm:** 7.57-8.01 (m, 3H, Ar-H), 7.38 (s, 1H, NH), 6.28 (s, 2H, NH_2), 8.25 (s, 1H, indole-NH).

1-(5-Bromo-2-oxoindolin-3-ylidene) thiosemicarbazide **(3f)**
m.p. = 145 ^{0}C, yield= 77%, **IR (KBr) in cm^{-1}:** 3448 (2° NH), 3378 (1^0 NH), 2935 (-CH3), 1683 (C= O) 1604 (C= N), 1090 (C= S), 534 (C-Br). **^{1}H- NMR (DMSO) δ ppm:** 7.41-7.96 (m, 3H, Ar-H), 7.38 (s, 1H, NH), 2.49 (s, 2H, NH_2), 8.41 (s, 1H, indole-NH).

1-(5-Bromo-1-methyl-2-oxoindolin-3-ylidene) semicarbazide **(3g)**
m.p. = 195 ^{0}C, yield= 16%, **IR (KBr) in cm^{-1}:** 3475 (2° NH), 3363 (NH), 2937(-CH_3), 1645 (C= O), 1626 (amide C= O), 1679 (C= N), 546 (C-Br). **^{1}H-NMR (DMSO) δ ppm:** 7.69- 8.07 (m, 3H, Ar-H), 2.99 (s, 3H, CH_3), 7.53 (s, 1H, NH), 6.67 (s, 2H, NH_2).

1-(5-Bromo-1-methyl-2-oxoindolin-3-ylidene) thiosemicarbazide **(3h)**
m.p. = 150 ^{0}C, yield= 29%, **IR (KBr) in cm^{-1}:** 3465 (2° NH), 3381 (NH), 2960 (-CH_3), 1740 (C= O), 1617 (C= N), 1064 (C= S), 534 (C-Br). **^{1}H- NMR (DMSO) δ ppm:** 7.69-8.10 (m, 3H, Ar-H), 7.42 (s, 1H, NH), 2.49 (s, 2H, NH_2), 3.39 (s, 3H, CH_3).

1-(5-Nitro-2-oxoindolin-3-ylidene) semicarbazide **(3i)**
m.p. = 265 ^{0}C, yield= 36%, **IR (KBr) in cm^{-1}:** 3436 (2° NH), 3319 (NH), 1742 (C= O), 1643 (amide C= O), 1651 (C= N), 1342 (C-NO_2). **^{1}H- NMR (DMSO) δ ppm:** 8.23- 8.67 (m, 3H, Ar-H), 7.47 (s, 1H, NH), 6.81 (s, 2H, NH_2), 8.89 (s, 1H, indole-NH).

1-(5-Nitro-2-oxoindolin-3-ylidene) thiosemicarbazide **(3j)**
m.p. = 220 ^{0}C, yield= 51%, **IR (KBr) in cm^{-1}**: 3434 (2° NH), 3323 (1^0 NH), 1734 (C= O), 1664 (amide C= O), 1688 (C= N), 1545, 1323 (C-NO_2), 1087 (C= S). **^{1}H- NMR (DMSO) δ ppm:** 8.47-8.78 (m, 3H, Ar-H), 7.85 (s, 1H, NH), 2.69 (s, 2H, NH_2), 8.37 (s, 1H, indole-NH).

1-(1-Methyl-5-nitro-2-oxoindolin-3-ylidene) semicarbazide **(3k)**
m.p. = 250 ^{0}C, yield= 42%, **IR (KBr) in cm^{-1}:** 3447 (2° NH), 3335 (1^0 NH), 2949 (-CH_3), 1765 (C= O), 1634 (amide C= O), 1674 (C= N), 1335 (C-NO_2). **^{1}H- NMR (DMSO) δ ppm:** 8.13-8.79, (m, 3H, Ar-H), 7.19 (s, 1H, NH), 6.23 (s, 2H, NH_2), 2.99 (s, 3H, CH_3).

1-(1-Methyl-5-nitro-2-oxoindolin-3-ylidene) thiosemicarbazide **(3l)**
m.p. = 230 ^{0}C, yield= 27%, **IR (KBr) in cm^{-1}:** 3431 (2° NH), 3321 (NH), 2930 (-CH_3), 1740 (C= O), 1666 (amide C= O), 1650 (C= N), 1344 (C-NO_2), 1023 (C= S). **^{1}H- NMR (DMSO) δ ppm:** 7.99-8.61(m, 3H, Ar-H), 7.46 (s, 1H, NH), 2.39 (s, 2H, NH_2), 2.88 (s, 3H, CH_3).

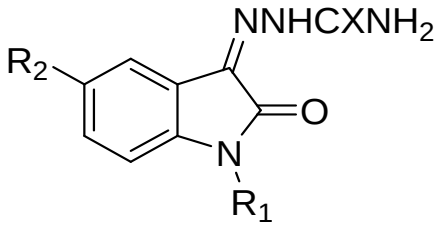
General procedure for synthesis of triazine derivatives in presence of NaOH (4a-l)
Thio or semicarbazone derivative (2.00 g) was dissolved in a boiling solution of sodium hydroxide (1M, 100 mL) in 250 mL conical flask. This mixture was refluxed for approx 3.00 hours. After cooling, the mixture was acidified with acetic acid. The solid formed was immediately filtered off, washed with water and dried affording the yellow solid.

6-(2-Aminophenyl)-1, 2, 4-triazine-3, 5(2H,4H)-dione **(4a)**
m.p. = 245-260 ^{0}C, yield= 72%, **IR (KBr) in cm^{-1}:** 3489 (NHstr, $ArNH_2$), 3435 (NHstr, cyclic CONH), 1660 (C= O, cyclic CONH), 1654 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 6.66-7.77 (m, 4H, Ar-H), 4.47 (s, 2H, NH_2), 7.55 (s, 1H, cyclic NNHCO), 10.47 (s, 1H, cyclic CONH).

6-(2-Aminophenyl)-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one **(4b)**
m.p. = 260 ^{0} C, yield=22%, **IR (KBr) in cm^{-1}:** 3457 (NHstr, $ArNH_2$), 3317 (NHstr, cyclic CONH), 1065 (C=Sstr), 1659 (cyclic C=N). **^{1}H-NMR (DMSO) δ ppm:** 6.76-7.53 (m, 4H, Ar-H), 4.61(s, 2H, NH_2), 7.44 (s, 1H, cyclic NNHCS), 8.43 (s, 1H, cyclic CONH).

6-(2-(Methylamino) phenyl)-1, 2, 4-triazine-3,5(2H,4H)-dione **(4c)**
m.p. = 270 ^{0}C, yield= 43%, **IR (KBr) in cm^{-1}:** 3436 (NHstr, ArNH), 3342 (NHstr, cyclic CONH), 2884 (CHstr, CH_3), 1664 (C= O, cyclic CONH), 1428 (CH_{bend}, CH_3), 1623 (cyclic C=N). **^{1}H- NMR (DMSO) δ ppm:** 6.63-7.78 (s, 4H, Ar-H), 2.92 (s, 3H, CH_3), 4.30 (s, 1H, NH), 7.1 (s, 1H, cyclic NNHCO), 10.54 (s, 1H, cyclic CONH).

Table 1: Structures and physicochemical data of compounds 3a-l



Code No.	R_1	R_2	X	Mol. For.	Mol. Wt	% yield	M.P. (^{0}C)	R_f	Log *p**	Elemental analysis Cal.(found)(%)
3a	H	H	O	$C_9H_8N_4O_2$	204.18	55	240	0.53	-0.3	C52.94(52.97), H3.95(3.90), N27.44(27.48)
3b	H	H	S	$C_9H_8N_4OS$	220.25	64	220	0.39	0.26	C49.08(49.05), H3.66(3.69), N25.44(25.47)
3c	CH_3	H	O	$C_{10}H_{10}N_4O_2$	218.21	51	255	0.84	-0.07	C55.04(55.09), H4.62(4.67), N25.68(25.73)
3d	CH_3	H	S	$C_{10}H_{10}N_4O_S$	234.27	57	190	0.91	0.49	C51.27(51.31), H4.30(4.26), N23.91(23.94)
3e	H	Br	O	$C_9H_7BrN_4O_2$	283.08	48	175	0.62	0.53	C38.19(38.23), H2.49(2.52), N19.79(19.75)
3f	H	Br	S	$C_9H_7BrN_4OS$	299.14	77	145	0.47	1.09	C36.13(36.17), H2.36(2.39), N18.73(18.75)
3g	CH_3	Br	O	$C_{10}H_9BrN_4O_2$	297.10	16	195	0.83	0.76	C40.43(40.46), H3.05(3.01), N18.86(18.90)
3h	CH_3	Br	S	$C_{10}H_9BrN_4OS$	313.17	29	150	0.87	1.32	C38.35(38.39), H2.90 (2.88), N17.89(17.94)
3i	H	NO_2	O	$C_9H_7N_5O_4$	249.18	36	265	0.36	-0.86	C43.38(43.41), H2.83(2.87), N28.11(28.15)
3j	H	NO_2	S	$C_9H_7N_5O_3S$	265.24	51	220	0.86	-0.36	C40.75(40.79),H2.66(2.69), N26.40(26.44)
3k	CH_3	NO_2	O	$C_{10}H_9N_5O_4$	263.20	42	250	0.62	-1.14	C45.63(45.67), H3.45(3.48), N26.61(26.65)
3l	CH_3	NO_2	S	$C_{10}H_9N5O_3S$	279.27	27	230	0.90	-0.64	C43.01 (43.05), H3.25 (3.27), N25.08%)

Log p value calculated by Chem office 2004 software.
TLC solvent was ethyl acetate: n-hexane=5:5

***6-(2-Amino-5-bromophenyl)-3, 4-dihydro-3-thioxo-1, 2, 4-triazin-5(2H)-one (*4f)**
m.p. = 220 ^{0}C, yield= 82%, **IR (KBr) in cm^{-1}:** 3363 (NHstr, ArNH$_2$), 3327 (NHstr, cyclic CONH), 1695 (C= O), 1075 (C= Sstr), 580 (C-Br)**,** 1663 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 6.51-7.94 (m, 3H, Ar-H), 4.43 (s, 2H, NH$_2$), 7.66(s, 1H, cyclic NNHCS), 8.37 (s, 1H, cyclic CONH).

***6-(5-Bromo-2-(Methylamino) phenyl)-1, 2, 4-triazine-3, 5(2H, 4H)-dione* (4g)**
m.p. = 240 ^{0}C, yield= 85%, **IR (KBr) in cm^{-1}:** 3445 (NHstr, ArNH), 3336 (NHstr, cyclic CONH), 2869 (CHstr, CH$_3$), 1630 (C= O, cyclic CONH), 1412 (CH$_{bend}$, CH$_3$), 1377, 1323 (NOstr, NO$_2$), 770 (CSstr)**,** 1683 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 6.38-7.99(m, 3H, Ar-H), 3.10 (s, 3H, CH$_3$), 4.27 (s, 1H, NH), 7.74 (s, 1H, cyclic NNHCO), 10.49 (s, 1H, cyclic CONH).

***6-(5-Bromo-2-(Methylamino) phenyl)-3, 4-dihydro-3-thioxo-1, 2, 4-triazin-5 (2H)-one* (4h)**
m.p. = 230 ^{0}C, yield= 34%, **IR (KBr) in cm^{-1}:** 3463 (NHstr, ArNH), 3318 (NHstr, cyclic CONH), 2855 (CHstr, CH$_3$), 1432 (CH$_{bend}$, CH$_3$), 578 (C-Br), 1085 (C=Sstr), 1639 (cyclic C=N). **^{1}H- NMR (DMSO) δ ppm:** 6.39-7.58 (m, 3H, Ar-H), 3.02 (s, 3H, CH$_3$), 4.76 (s, 1H, NH), 7.47 (s, 1H, cyclic NNHCS), 8.37 (s, 1H, cyclic CONH).

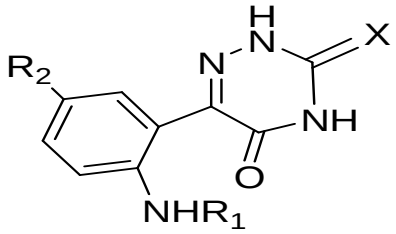
***6-(2-Amino-5-nitrophenyl)-1, 2, 4-triazine-3, 5 (2H, 4H)-dione* (4i)**
m.p. = 295 ^{0}C, yield= 67%, **IR (KBr) in cm^{-1}:** 3439 (NHstr, ArNH$_2$), 3328 (NHstr, cyclic CONH), 1678 (C= O, cyclic CONH), 1384, 1317 (NOstr, NO$_2$), 1681 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 6.98-8.79 (m, 3H, Ar-H), 4.46 (s, 2H, NH$_2$), 7.41(s, 1H, cyclic NNHCO), 10.39 (s, 1H, cyclic CONH).

***6-(2-Amino-5-nitrophenyl)-3, 4-dihydro-3-thioxo-1, 2, 4-triazin-5(2H)-one* (4j)**
m.p. = 290 ^{0}C, yield= 85%, **IR (KBr) in cm^{-1}:** 3467 (NHstr, ArNH$_2$), 3346 (NHstr, cyclic CONH), 1364, 1312 (NOstr, NO$_2$), 1059 (C= Sstr)**,** 1685 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 6.91-8.89 (m, 3H, Ar-H), 4.76 (s, 2H, NH$_2$), 7.77 (s, 1H, cyclic NNHCS), 10.61 (s, 1H, cyclic CONH).

***6-(2-(Methylamino)-5-nitrophenyl)-1, 2, 4-triazine-3, 5(2H, 4H)-dione* (4k)**
m.p. = 280 ^{0} C, yield= 67%, **IR (KBr) in cm^{-1}:** 3433 (NHstr, ArNH), 2854 (CHstr, CH$_3$), 1605 (C= O, cyclic CONH), 1459 (CH$_{bend}$, CH$_3$), 1377, 1323 (NOstr, NO$_2$)**,** 1605 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 7.03-8.69 (m, 3H, Ar-H), 3.11 (s, 3H, CH$_3$), 4.15 (s, 1H, NH), 7.61 (s, 1H, cyclic NNHCO), 10.26 (s, 1H, cyclic CONH).

***3, 4-Dihydro-6-(2-(methylamino)-5-nitrophenyl)-3-thioxo-1, 2, 4-triazin-5(2H)-one* (4l)**
m.p. = 295 ^{0}C, yield= 78 %, **IR (KBr) in cm^{-1}:** 3445 (NHstr, ArNH), 2580 (CHstr, CH$_3$), 1412 (CH$_{bend}$, CH$_3$), 1102 (C= Sstr)**,** 1630 (cyclic C= N). **^{1}H- NMR (DMSO) δ ppm:** 7.04-8.89 (m, 3H, Ar-H), 3.06 (s, 3H, CH$_3$), 4.56 (s, 1H, NH), 7.71(s, 1H, cyclic NNHCS), 8.47 (s, 1H, cyclic CONH).

Table 2: Structures and physicochemical data of compounds 4a-l.



Code No.	R_1	R_2	X	Mol. For.	Mol.wt.	% yield	M.P.(^{0}C)	R_f	Log *p**	Elemental analysis Cal.(found)(%)
4a	H	H	O	$C_9H_8O_2N_4$	204.19	72	250	0.51	-0.07	C52.94(53.01), H3.95(3.91), N27.44(27.47)
4b	H	H	S	$C_9H_8ON_4S$	220.25	22	260	0.27	0.48	C49.08(49.12), H3.66(3.69), N25.44(25.45)
4c	CH_3	H	O	$C_{10}H_{10}O_2N_4$	218.21	43	270	0.51	0.23	C55.04(55.07), H4.62(4.58), N25.68(25.72)
4d	CH_3	H	S	$C_{10}H_{10}ON_4S$	234.28	36	210	0.63	0.78	C51.27(51.31), H4.30(4.27), N23.91(23.95)
4e	H	Br	O	$C_9H_7BrN_4O_2$	283.08	67	275	0.28	0.76	C38.19(38.23), H2.49(2.53), N19.79(19.76)
4f	H	Br	S	$C_9H_7BrN_4OS$	299.15	82	220	0.78	1.31	C36.13(36.17), H2.36(2.40), N18.73(18.79)
4g	CH_3	Br	O	$C_{10}H_9BrN_4O_2$	297.11	85	240	0.35	1.06	C40.43(40.40),H3.059(3.09), N18.86(18.89)
4h	CH_3	Br	S	$C_{10}H_9BrN_4OS$	313.17	34	230	0.83	1.61	C38.35(38.38), H2.90(2.93), N17.89(17.92)
4i	H	NO_2	O	$C_9H_7N_5O_4$	249.18	67	295	0.60	-0.61	C43.38(43.36), H2.83(2.85), N28.11(28.07)
4j	H	NO_2	S	$C_9H_7N_5O_3S$	265.25	85	290	0.70	-0.10	C40.75(40.77), H2.66(2.60), N26.40(26.37)
4k	CH_3	NO_2	O	$C_{10}H_7N_5O_3$	263.21	67	280	0.55	0.12	C45.63(45.60), H3.45(3.48), N26.61(26.58)
4l	CH_3	NO_2	S	$C_{10}H_9N_5O_3S$	279.28	78	295	0.45	0.63	C43.01(43.05), H3.25(3.22), N25.08(25.11)

**Log p value calculated by Chem Draw 2004 software.; TLC solvent was ethyl acetate: n-hexane=5:5*

Biological evaluation

***In vitro* antibacterial activity**

Synthesized compounds were evaluated for their *in-vitro* antibacterial activity against pathogenic bacteria by the agar dilution method. MIC values were considered to be the lowest concentration that was completely inhibited growth on agar plates. Sulphamethoxazole was used as the standard in all antibacterial screening studies.

CONCLUSION

Antimicrobial studies revealed that the most active compounds are **4a** (6-(2-Aminophenyl)-1, 2, 4-triazine-3, 5 (2H,4H)-dione) with MIC value 50 µg/ml against *E.coli* ATCC25922, **4h** (6- (5-Bromo-2- (methylamino) phenyl)-3, 4-dihydro-3-thioxo-1, 2, 4-triazin-5 (2H)-one) with MIC value 150 µg/ml against *E.coli* ATCC25922, and **4l** (3, 4-Dihydro-6-(2-(methylamino)-5-nitrophenyl)-3-thioxo-1, 2, 4-triazin-5 (2H)-one) with MIC value 25 µg/ml against *H. pylori*. Based on the above studies, the most active compounds can be evaluated for *in vivo* antimicrobial studies as a future perspective.

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