Synthesis, characterization and evaluation of potential of antimicrobial of triazolothiadiazines analogues

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ABSTRACT

A number of novel thiadizine derivatives with triazole nucleus using a solvent-free, environmentally friendly process because these heterocycles are significant in many ways for biology. To find strong physiologically active compounds, we characterised and screened the synthesised compounds for biological activity. It covers spectrum analysis and the synthesis of triazolo thiadiazine derivatives. A structurally diverse novel series of triazolothiadiazine derivative heterocycles with a medicinally preferred nucleus has been produced using simple, solvent-free, and ecologically safe methods. It was heated in a microwave without using any solvents and mixed with triazolo Thiol and β-ketoester, and a catalytic amount of hydrazine hydrate. DMF was used as an energy transfer agent to get a high yield of the product.

KEYWORDS: Antimicrobial, triazolothiadiazines, β-ketoester, Synthesis, NMR, IR

INTRODUCTION

The rising prevalence of antimicrobial resistance (AMR) has become a global health crisis, posing significant challenges to the treatment of infectious diseases (1). The capacity of microbes to adapt and create resistance mechanisms is causing traditional antibiotics, which were formerly effective against a wide range of infections, to lose their effectiveness (2). This has led to pressing demands for the identification and creation of new antibacterial substances

with distinct modes of action (3). In this context, developing and testing novel chemicals—particularly heterocyclic compounds—seems like a viable strategy to combat AMR (4). One class of these substances that has drawn a lot of interest is triazolothiadiazines, which have a variety of therapeutic applications, including the prevention of cancer, inflammation, and infection (5, 6). Triazolothiadiazines are a group of heterocyclic compounds that are fused together. They have a triazole ring and a thiadiazine ring, which are both known to have bioactive properties (7). The triazole ring is a five-membered heterocycle that is full of nitrogen and is found in many pharmaceutically active compounds. The thiadiazine ring, on the other hand, adds sulphur and nitrogen atoms that can make the bioactivity of the molecule (8-10). Some of the reasons triazolothiadiazines are so useful in drug design is because they can interact with many biological targets. This means that they could be used to make new antibiotics.In the past few years; a lot of research has been done on making triazolothiadiazine derivatives and looking into how to change their structures to make them more antimicrobial (11-13). These changes can have an effect on the compound's electronic, steric, and lipophilic properties, which can then have an effect on how well it binds to microbe targets (14). Furthermore, the incorporation of various substituents into the triazolothiadiazine framework can optimize its solubility, stability, and bioavailability, crucial factors for drug development (15). Characterization of triazolothiadiazine analogues involves a range of as spectroscopic methods techniques. These methods provide detailed information about the molecular structure, functional groups, and stereochemistry of the synthesized compounds (16). Once synthesized and characterized, the antimicrobial potential of triazolothiadiazine derivatives is evaluated through in vitro testing against a variety of bacterial and fungal strains. Some common tests are figuring out the minimum inhibitory concentration (MIC), doing agar diffusion tests, and time-kill studies (17-19). These help figure out how strong the compounds are, what kinds of activities they have, and how they work.Many times, triazolothiadiazine analogues' ability to kill microorganisms is because they can stop important biological processes in those organisms, like making cell walls, proteins, or DNA copies. Additionally, the compounds may work better when mixed with other antimicrobials, which can help get around mechanisms of resistance. Tribiazothiadiazines have been demonstrated to be effective against a variety of microorganisms, including fungi and both Gram-positive and Gram-negative bacteria (21-23). The development of novel antimicrobial drugs, such as triazolothiadiazine derivatives, is crucial given the growing threat posed by AMR and the dearth of therapeutically viable alternatives (24). This study aims to synthesize and characterize a series of triazolothiadiazine

analogues, evaluate their antimicrobial potential, and explore their mechanisms of action. The results of this study could help scientists find new antibiotics that work better against pathogens that are resistant to them.This offers hope for more effective treatments in the fight against infectious diseases (25). Because these heterocycles are biologically important in many ways, scientists made a bunch of new thiadizine derivatives with a triazole nucleus using a solvent-free method that is safe for the environment. The synthesized compounds underwent characterization and screened for their biological activities to explore potent biologically active molecules (26).

MATERIAL AND METHODS:

All of the synthetic compounds' uncorrected melting points were measured on an open aluminum block. We examined the purity using Merck silica gel G-60 and thin layer chromatography. We used the Shimadzu Affinity-1 FTIR spectrophotometer to record the infrared spectra in KBr. Using TMS as an internal standard; we recorded 1H NMR spectra using a spectrometer operating at 300 MHz. The mass spectra were recorded at 70 eV using the Jeol SX 102 spectrometer (27).

Synthesis of 4Amino triazole-3-thiol molecules:

The steps for making the substituted 4-amino-1, 2, 4-triazole-3-thiols needed to make triazolothiadiazine are shown below.

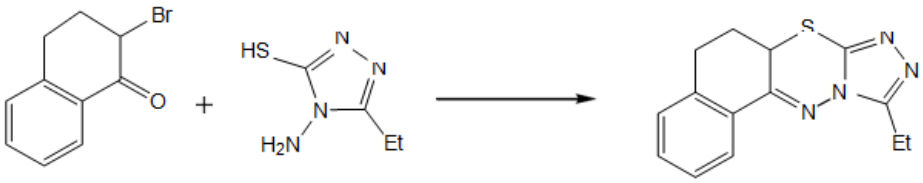


Figure 1: Synthesis of triazole thiols molecules

Synthesis of triazolo thiadiazines molecules

Under order to transfer energy, we heated Compound I (10 mmol), hydrazine hydrate (1 mmol) as a catalyst, and DMF (5 ml) under microwaves for 30 seconds. After 10 mmol of β-diketone/β-ketoester was added, the mixture was microwaved for three more minutes, with a break every 30 seconds. TLC cooled the reaction mixture and monitored its completion before transferring it to crushed ice. The separated material was filtered, washed with 50% ethanol, and then crystallised from ethanol to provide a pure product (figure 2) (28-30).

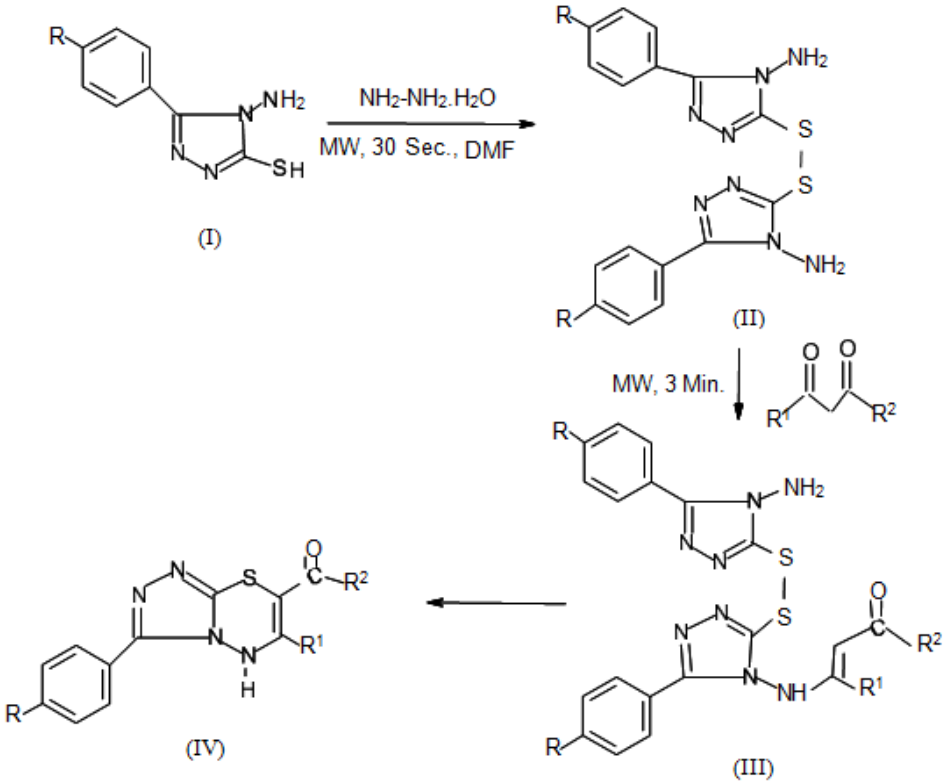
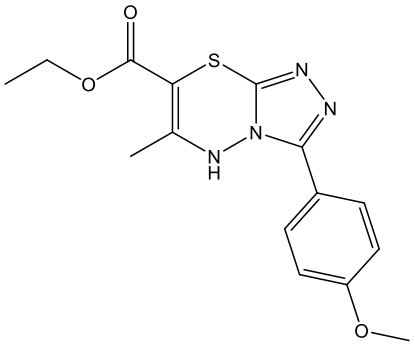


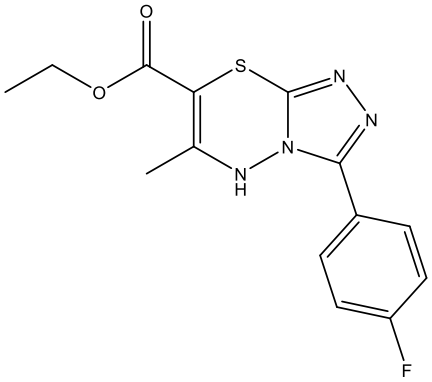
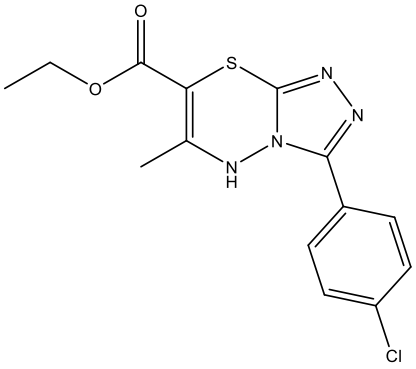
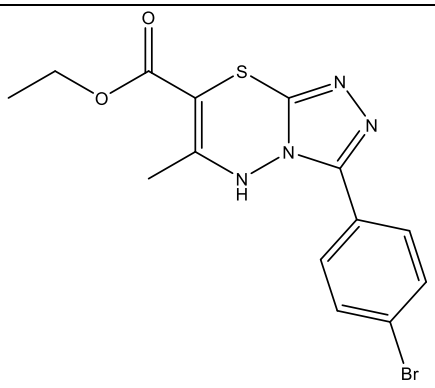
Figure 2: Synthesis of triazolo thiadiazines derivatives

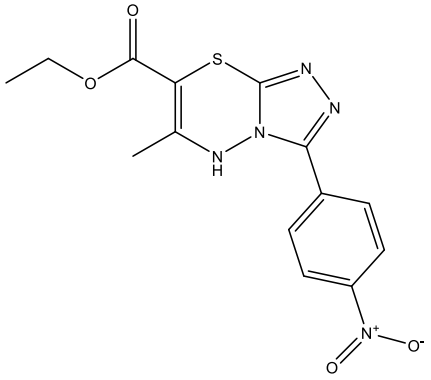
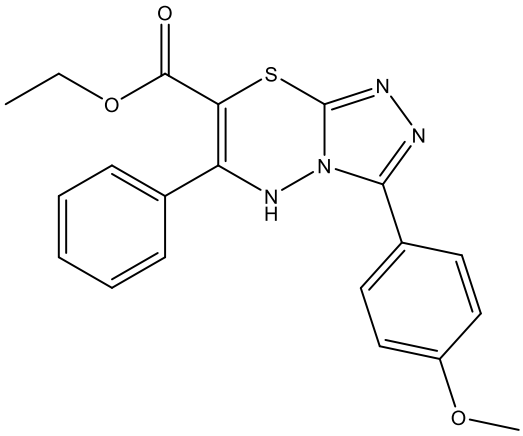
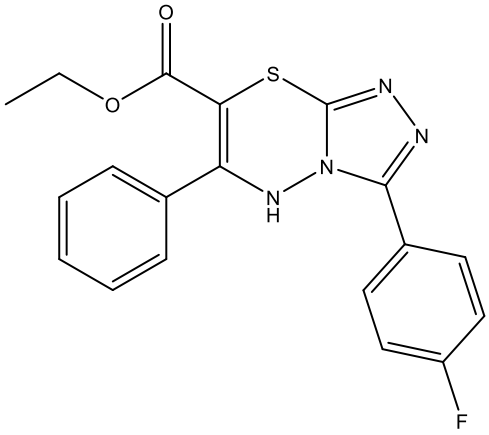
RESULT AND DISCUSSION:

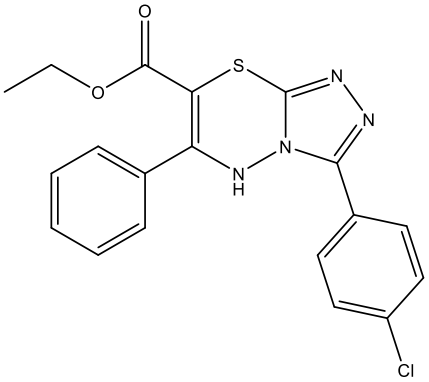
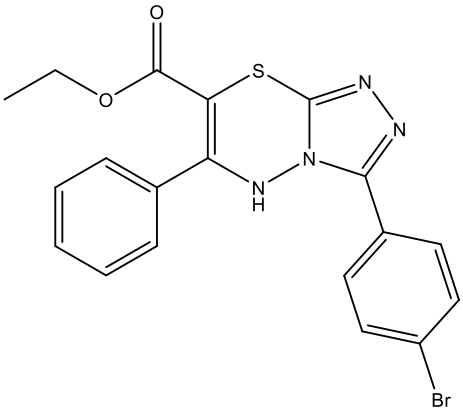
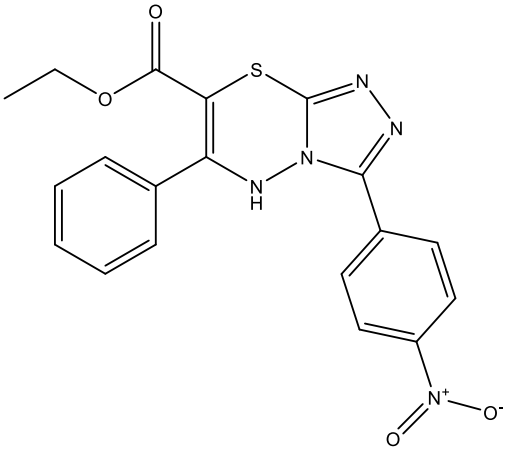
In present investigation following triazolo thiadiazines have been synthesized:

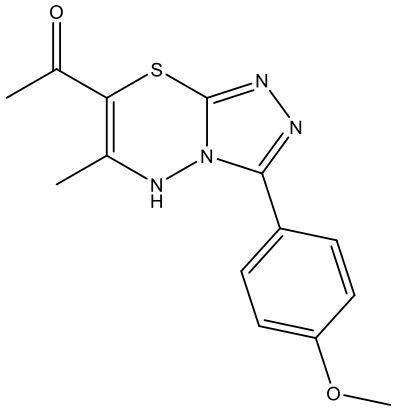
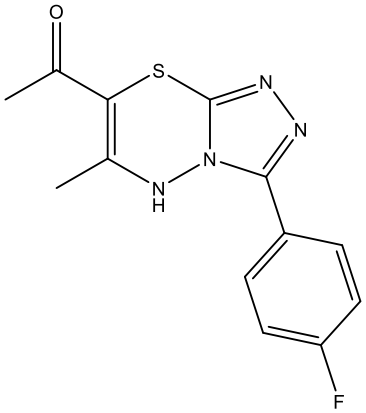
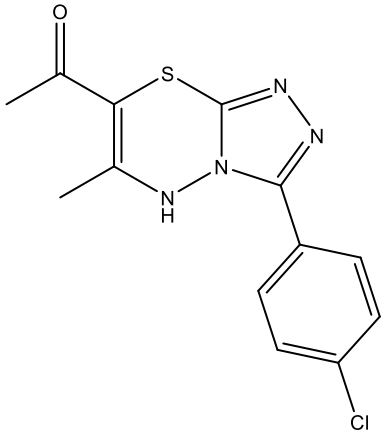
Table 1: Physical data of the synthesized compounds

Sr. NO	Name and structure	Molecular Formula	Yield	M.P
1	 ethyl 3-(4-methoxyphenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{15}H_{16}N_4O_3S$	65%	135ºC

2	 ethyl 3-(4-fluorophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{14}H_{13}FN_4O_2S$	60%,	132ºC
3	 ethyl 3-(4-chlorophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{14}H_{13}ClN_4O_2S$	66%	129ºC
4	 ethyl 3-(4-bromophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{14}H_{13}BrN_4O_2S$	70%	139ºC

5	 ethyl 6-methyl-3-(4-nitrophenyl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{14}H_{13}N_5O_4S$	63%	124ºC
6	 ethyl 3-(4-methoxyphenyl)-6-phenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{20}H_{18}N_4O_3S$	65%	141ºC
7	 ethyl 3-(4-fluorophenyl)-6-phenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{19}H_{15}FN_4O_2S$	62%	138ºC

8	 ethyl 3-(4-chlorophenyl)-6-phenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{19}H_{15}ClN_4O_2S$	67%	134ºC
9	 ethyl 3-(4-bromophenyl)-6-phenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{19}H_{15}BrN_4O_2S$	69%	145°C
10	 ethyl 3-(4-nitrophenyl)-6-phenyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine-7-carboxylate	$C_{19}H_{15}N_5O_4S$	63%	151ºC

11	 1-(3-(4-methoxyphenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)ethan-1-one	$C_{14}H_{14}N_4O_2S$	64%	122ºC
12	 1-(3-(4-fluorophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)ethan-1-one	$C_{13}H11FN_4OS$	62%	114ºC
13	 1-(3-(4-chlorophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)ethan-1-one	$C_{13}H_{11}ClN_4OS$	65%	119ºC

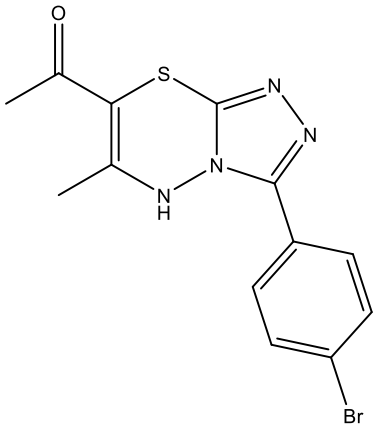
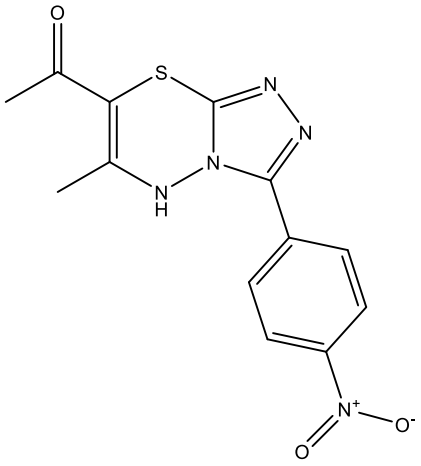
14	 1-(3-(4-bromophenyl)-6-methyl-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)ethan-1-one	$C_{13}H_{11}BrN_4OS$	6%	135°C
15	 1-(6-methyl-3-(4-nitrophenyl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazin-7-yl)ethan-1-one	$C_{13}H_{11}N_5O_3S$	67%	128°C

Table 2: Spectral data of the synthesized compounds

Sr. NO	**IR**	**NMR**	**Mass**
1	IR (KBr, λmax, cm^{-1}): 3380 (N-H), 1225, 1030 (C-O-C), 2990 (C-H aliphatic), 3070 (C-H aromatic), 1597 (C=N), 1680 (>C=O), 1058 (N-N)	^{1}HNMR($CDCl_3$) δ: 7.2-7.9 (4H, m, Ar-H), 8.7 (1H, s, N-H), 2.20 (3H, s, CH_3 at C_6), 3.8 (3H, s, OCH_3 at 4'), 4.10 (2H, q, CH_2 at C_7), 1.20 (3H, t, CH_3 at C_7);	MS (m/z): 332 [M^+], 331 [M^+-H], 303 [M^+-C_2H_5], 304 [M^+-C_2H_4], 287 [M^+-OC_2H_5], 285 [M^+-C_2H_5OH+CO], 225 [M^+-$C_6H_4OCH_3$] Anal. calculated (%). Found: C, 53.95; H, 4.76; N, 16.79.
2	IR (KBr, λmax, cm^{-1}): 3385 (N-H), 1230, 1035 (C-O-C), 2995	1HNMR ($CDCl_3$) δ: 7.8-8.5 (4H, m, Ar-H), 8.75 (1H, s, N-	MS (m/z): 320 [M^+], 319 [M^+-H], 291 [M^+-C_2H_5], 292 [M^+-C_2H_4], 275

	(C-H aliphatic), 3050 (C-H aromatic), 1588 (C=N), 1675 (>C=O), 1055 (N-N);	H), 2.2 (3H, s, CH_3 at C_6), 4.05 (2H, q, CH_2 at C_7), 1.15 (3H, t, CH_3 at C_7);	$[M^{+-}OC_2H_5]$, 247 $[M^{+-}C_2H_5OH+CO]$, 225 $[M^{+-}C_6H_4F]$. Anal. Calculated (%) for C14H13FN4O2S: C, 52.5; H, 4.1; N, 17.5. Found: C, 52.1; H, 3.95; N, 17.31.
3	IR (KBr, λmax, cm^{-1}): 3380 (N-H), 1220, 1030 (C-O-C), 2990 (C-H aliphatic), 3060 (C-H aromatic), 1590 (C=N), 1675 (>C=O), 740 (C-Cl), 1043 (N-N);	1HNMR ($CDCl_3$) δ: 7.6-8.1 (4H, m, Ar-H), 8.7 (1H, s, N-H), 2.25 (3H, s, CH_3 at C_6), 4.0 (2H, q, CH_2 at C_7), 1.25 (3H, t, CH_3 at C_7);	MS (m/z): 336 $[M^+]$ 335 $[M^-H]$, 307 $[M^-C_2H_5]$, 308 $[M^-C_2H_4]$, 291 $[M^-OC_2H_5]$, 262 $[M^-C_2H_5OH+CO]$, 35 [Cl], 255 $[M^-C_6H_4Cl]$. Anal. Calculated (%) for C14H13ClN4O2S: C, 49.9; H, 3.8; N, 16.6. Found: C, 50.52; H, 3.5; N, 16.2.
4	IR (KBr, λmax, cm^{-1}): 3340 (N-H), 1230, 1035 (C-O-C), 2995 (C-H aliphatic), 3070 (C-H aromatic), 1593 (C=N), 1665 (>C=O), 1060 (N-N),	1HNMR ($CDCl_3$) δ: 7.3-7.9 (4H, m, Ar-H), 8.65 (1H, s, N-H), 2.15 (3H, s, CH_3 at C_6), 4.15 (2H, q, CH_2 at C_7), 1.25 (3H, t, CH_3 at C_7);	Anal. Calculated (%) for $C_{14}H_{13}BrN_4O_2S$: C, 44.0; H, 3.4; N, 14.6. Found: C, 43.84; H, 3.21; N, 14.35.
5	IR (KBr, λmax, cm^{-1}): 3370 (N-H), 1230, 1040 (C-O-C), 3070 (C-H aromatic), 2990 (C-H aliphatic), 1590 (C=N), 1680 (>C=O), 1060 (N-N);	^{1}HNMR ($CDCl_3$) δ: 7.45-8.25 (4H, m, Ar-H), 8.5 (1H, s, N-H), 2.20 (3H, s, CH_3 at C_6), 4.05 (2H, q, CH_2 at C_7), 1.15 (3H, t, CH_3 at C_7);	Anal. Calculated (%) for $C_{14}H_{13}N_5O_4S$: C, 48.41; H, 3.74; N, 20.17. Found: C, 48.34; H, 3.71; N, 20.14.
6	IR (KBr, λmax, cm^{-1}): 3385 (N-H), 1235, 1040 (C-O-C), 2998 (C-H aliphatic), 3090 (C-H aromatic), 1595 (C=N), 1670 (>C=O), 1065 (N-N);	1HNMR ($CDCl_3$) δ: 7.8-8.2 (10H, m, Ar-H), 8.8 (1H, s, N-H), 3.75 (4H, s, OCH_3 at 4'), 4.1 (2H, q, CH_2 at C7), 1.18 (3H, t, CH_3 at C7);	MS (m/z): 394 $[M^+]$, 393 $[M^+ - H]$, 365 $[M^+ - C_2H_5]$, 366 $[M^+ - C_2H_4]$, 349 $[M^+ - OC_2H_5]$, 320 $[M^+ - C_2H_5OH + CO]$, 317 $[M^+ - C_6H_5]$, 287 $[M^+ - C_6H_4OCH_3]$. Anal. Calculated (%) for C20H18N4O3S: C, 60.91; H, 4.56; N, 14.21. Found: C, 60.78; H, 4.52; N, 14.16.
7	IR (KBr, λmax, cm^{-1}): 3385 (N-H), 1240, 1045 (C-O-C), 3090 (C-H aromatic), 2985 (C-H aliphatic), 1610 (C=N), 1685 (>C=O), 1075 (N-N);	^{1}HNMR ($CDCl_3$) δ: 8.0-8.7 (9H, m, Ar-H), 8.75 (1H, s, N-H), 4.10 (2H, q, CH_2 at C7), 1.20 (3H, t, CH_3 at C7);	MS (m/z): 382 $[M^+]$, 381 $[M^-H]$, 353 $[M^-C_2H_5]$, 354 $[M^-C_2H_4]$, 337 $[M^-OC_2H_5]$, 308 $[M^-C_2H_5OH]$, 305 $[M^-C_6H_5]$, 287 $[M^-C_6H_4F]$. Anal. Calculated (%) for C19H15FN4O2S: C, 59.5; H, 3.9; N, 14.6. Found: C, 59.32; H, 3.78; N, 14.49.

8	IR (KBr, λmax, cm^{-1}): 3390 (N-H), 1245, 1035 (C-O-C), 3045 (C-H aromatic), 2980 (C-H aliphatic), 1605 (C=N), 1685 (>C=O), 1060 (N-N), 765 (C-Cl);	1HNMR ($CDCl_3$) δ: 7.7-8.2 (9H, m, Ar-H), 8.70 (1H, s, N-H), 4.05 (2H, q, CH_2 at C7), 1.15 (3H, t, CH_3 at C7);	MS (m/z): 398 [M^+], 397 [M^-H], 369 [$M^-C_2H_5$], 370 [$M^-C_2H_5$], 353 [$M^-OC_2H_5$], 324 [$M^-C_2H_5OH+CO$], 321 [$M^-C_6H_5$], 287 [$M^-C_6H_4Cl$]. Anal. Calculated (%) for C19H15ClN4O2S: C, 57.5; H, 3.7; N, 14.0. Found: C, 56.98; H, 3.6; N, 13.89
9	IR (KBr, λmax, cm^{-1}): 3390 (N-H), 1240, 1035 (C-O-C), 3080 (C-H aromatic), 2985 (C-H aliphatic), 1598 (C=N), 1665 (>C=O), 1068 (N-N);	^{1}HNMR ($CDCl_3$) δ: 7.4-6.98 (9H, m, Ar H), 8.75 (1H, s, N-H), 4.10 (2H, q, CH_2 at C7), 1.15 (3H, t, CH_3 at C7);	Anal. Calculated (%) for $C_{19}H_{15}BrN_4O_2S$: C, 51.4; H, 3.3; N, 12.6. Found: C, 51.05; H, 3.12; N, 12.48.
10	IR (KBr, λmax, cm^{-1}): 3360 (N-H), 1240, 1035 (C-O-C), 3085 (C-H aromatic), 2985 (C-H aliphatic), 1585 (C=N), 1675 (>C=O), 1065 (N-N);	^{1}HNMR ($CDCl_3$) δ: 7.5-8.2 (9H, m, Ar-H), 8.65 (1H, s, N-H), 4.0 (2H, q, CH_2 at C7), 1.20 (3H, t, CH_3 at C7);	Anal. Calculated (%) for $C_{19}H_{15}N_5O_4S$: C, 55.74; H, 3.66; N, 17.11. Found: C, 55.68; 3.64; N, 17.04.
11	IR (KBr, λmax, cm^{-1}): 3390(N-H), 2970(C-H aliphatic), 3080(C-H aromatic), 1590(C=N), 1660(>C=O), 1060(N-N);	^{1}HNMR ($CDCl_3$) δ: 7.8-8.5(4H, m, Ar-H), 8.7 (1H, s, N-H), 2.15 (3H, s, CH_3 at C_6), 3.8(3H, s, OCH_3 at 4), 3.5(3H, s, $COCH_3$ at C_7);	MS (m/z): 302[M^+], 301[M^-H], 287[M^-CH_3], 259[M^-COCH_3], 242[M^-CH_3OH+CO], 195[$M^-C_6H_4OCH_3$]. Anal. Calculated (%) for C14H14N4O2S: C, 55.6; H, 4.63; N, 18.54. Found: C, 55.54; H, 4.59; N, 18.48.
12	IR (KBr, λmax, cm^{-1}): 3380 (N-H), 2995 (C-H aliphatic), 3065 (C-H aromatic), 1590 (C=N), 1650 (>C=O), 1070 (N-N);	^{1}HNMR ($CDCl_3$) δ: 7.9-7.5 (4H, m, Ar-H), 8.7 (1H, s, N-H), 2.20 (3H, s, CH_3 at C_6), 3.6 (3H, s, $COCH_3$ at C_7);	MS (m/z): 290 [M^+], 289 [M^+ - H], 275 [M^+ - CH_3], 247 [M^+ - $COCH_3$], 230 [M^+ - CH_3OH + CO], 195 [M^+ - C_6H_4F]. Anal. Calculated (%) for C13H11FN4OS: C, 53.7; H, 3.7; N, 19.3. Found (%): C, 53.59; H, 3.62; N, 19.12.
13	IR (KBr, λmax, cm^{-1}): 3370 (N-H), 2985 (C-H aliphatic), 3065 (C-H aromatic), 1595 (C=N), 1660 (>C=O), 1065 (N-N);	^{1}HNMR ($CDCl_3$) δ: 7.7-8.3 (4H, m, Ar-H), 8.8 (1H, s, N-H), 2.15 (3H, s, CH_3 at C_6), 3.45 (3H, s, $COCH_3$ at C_7);	Anal. Calculated (%) for $C_{13}H_{11}ClN_4OS$: C, 50.8; H, 3.5; N, 18.2. Found: C, 50.68; H, 3.43; N, 18.13.
14	IR (KBr, λmax, cm^{-1}): 3385 (N-H), 2985 (C-H aliphatic), 3075 (C-H aromatic), 1598 (C=N), 1655 (>C=O), 1065 (N-N);	^{1}HNMR (CDCl) δ: 7.6-8.3 (4H, m, Ar-H), 8.7 (1H, s, N-H), 2.25 (3H, s, CH_3 at C_6), 3.4 (3H, s, $COCH_3$ at C_7);	MS (m/z): 351 [M^+], 350 [M^-H], 336 [M^-CH_3], 308 [M^-COCH_3], 291 [M^-CH_3OH+CO], 195 [$M^-C_6H_4Br$], 272 [M^-Br]. Anal.

			Calculated (%) for C13H11BrN4OS: C, 44.4; H, 3.1; N, 15.9. Found: C, 44.15; H, 2.98; N, 15.81.
15	IR (KBr, λmax, cm^{-1}): 3370 (N-H), 3070 (C-H aromatic), 2990 (C-H aliphatic), 1580 (C=N), 1665 (>C=O), 1050 (N-N);	1HNMR ($CDCl_3$) δ: 7.60-8.30 (4H, m, Ar-H), 8.5 (1H, s, N-H), 2.20 (3H, s, CH_3 at C_6), 3.45 (3H, s, $COCH_3$ at C_7);	MS (m/z) 317 [M^+], 316 [M^-H], 302 [M^-CH_3], 274 [M^-COCH_3], 257 [M^-CH_3OH+CO], 195 [$M^-C_6H_4NO_2$], 271 [M^-NO_2], 287 [M^-NO]; Anal. Calculated (%) for $C_{13}H_{11}N_5O_3S$: C, 49.21; H, 3.47; N, 22.08. Found: C, 49.12; H, 3.44; N, 21.02.

Characterization/ Spectral analysis:

With the help of IR, NMR, and MS spectral data and correct elemental analysis, the structures of the synthesised compounds were confirmed. All of the synthesized triazolothiadiazines had an infrared spectrum with a C=O stretching absorption peak in the 1685–1650 cm^{-3} range and an N-H stretching absorption peak in the 3390–3340 cm^{-3} range. Compounds in the 1240–1220 cm^{-1} and 1045–1030 cm^{-1} regions, respectively, have 1–10 bands due to C-O–C symmetric and asymmetric vibrations. The role of functional groups in the cyclisation of triazole to triazolothiadiazines was shown by the fact that the synthesized compounds had NH_2 and SH peaks in their aminotriazole thiols in their 1H NMR spectra. All of the synthesized compounds have a broad singlet peak in the 8.5-8.8 δ area for the N-H proton, while aromatic ring protons are responsible for the multiplets in the 6.3-7.8 δ region. Methoxy derivatives have a singlet peak at around 3.7 δ because of the OCH_3 group at 4'. The observed molecular ion peak (M^+) in the mass spectra of each synthesized triazolothiadiazine conforms to the specified molecular formulas of the corresponding compounds. In accordance with the given structure, other peaks such as M+-C_2H_4, M+-C_2H_5, M+-COC_2H_5, M+-OC_2H_5, M+-$COCH_3$, M+-$C_6H_4NO_2$, and M+-C_6H_4Br were also detected in the mass spectra of related compounds.

Evaluation of Antimicrobial Activity:

Using the agar-plate diffusion technique 18, all of the synthesized thiadiazine derivatives were assessed for antibacterial activity in vitro by measuring the inhibition zone in

millimetres. A 40-µg/ml concentration of amoxicillin and ciprofloxacin was used to test the antibacterial activity against *Pseudomonas aeruginosa (P. aeruginosa)*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus (S. aureus)*, and *Bacillus megaterium* (*B. megaterium*). Following the incubation period, the diameter in millimetres of the zone of inhibition of growth was assessed (Table 1). Griseofulvin was used as a standard drug to test the antifungal activity against *Candida albicans (C. albicans)* and *Aspergillusniger (A. niger)* at a concentration of 40 µg/ml of samples. Following the conclusion of the incubation period, the diameter in millimetres of the zone of inhibition of growth was assessed (Table 1).

Table 3: Antimicrobial activities of triazolo thiadiazines derivatives

Comp.	**Zoneof Inhibition in mm**					
	Antibacterial Activity				**Antifungal Activity**	
	E. coli	***P. aeruginosa***	***S. aureus***	***B.megaterium***	***A. niger***	***C. albicans***
1.	10.00	12.25	13.00	11.50	9.50	9.00
2.	16.00	17.25	18.00	16.25	14.50	16.50
3.	13.50	12.50	13.00	12.00	11.50	11.00
4.	12.50	13.00	12.50	11.75	10.00	10.75
5.	15.00	15.50	16.00	15.00	13.00	14.75
6.	11.20	10.75	11.75	10.50	9.00	9.25
7.	17.00	18.00	19.00	17.75	15.75	16.00
8.	13.00	14.25	15.25	14.75	12.00	11.75
9.	11.50	12.75	12.00	12.25	11.00	11.25
10.	16.50	16.00	16.75	15.50	13.00	12.75
11.	10.75	10.75	11.50	11.25	9.75	10.00
12.	18.00	18.00	19.00	18.25	16.25	16.00
13.	14.25	14.25	14.00	13.75	10.50	11.25
14.	12.50	13.00	13.25	13.50	10.75	10.25
15.	15.75	15.50	16.00	17.00	14.00	13.75

CONCLUSION:

All synthesized triazolothiadiazines exhibited an infrared spectrum with C=O stretching absorption peaking at 1685-1650 cm^{-3} and N-H stretching absorption peaking at 3390-3340 cm^{-3}. Compounds in the 1240-1220 cm^{-1} and 1045-1030 cm^{-1} areas exhibit 1-10 bands from C-O–C symmetric and asymmetric vibrations. To demonstrate the importance of functional

groups in the cyclisation of triazole to triazolothiadiazines, the synthesized compounds contained NH_2 and SH peaks in their 1H NMR spectra for aminotriazole thiols. All synthesized compounds have a broad N-H proton peak at 8.5-8.8 δ, while aromatic ring protons cause multiplets at 6.3-7.8 δ. Methoxy derivatives have a singlet peak at 3.7 δ due to the 4' OCH_3 group. The observed molecular ion peak (M^+) in mass spectra of synthesised triazolothiadiazine compounds matches their molecular formulae. All synthesised thiadiazine derivatives were tested for antibacterial activity in vitro by measuring the inhibition zone in millimetres using the agar-plate diffusion technique 18. The antibacterial activity of amoxicillin and ciprofloxacin at 40 µg/ml was tested against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus,* and *Bacillus megaterium.* Griseofulvin, a conventional medication, was tested for antifungal activity against Candida albicans and Aspergillus niger at a dosage of 40 µg/ml. After incubation, the growth inhibition zone diameter was measured in millimetres.

DISCLOSURE STATEMENT

There is no conflict of interest financial or otherwise.

ADDITIONAL INFORMATION

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REFERENCES:

1. Tiwari, G., Gupta, M., Devhare, L. D., & Tiwari, R. (2024). Therapeutic and phytochemical properties of thymoquinone derived from Nigella sativa. *Current Drug Research Reviews Formerly: Current Drug Abuse Reviews*, *16*(2), 145-156.

2. Mostafa, M. S., Radini, I. A. M., El-Rahman, N. M. A., & Khidre, R. E. (2024). Synthetic Methods and Pharmacological Potentials of Triazolothiadiazines: A Review. *Molecules*, *29*(6), 1326.

3. Tiwari, R., Khatri, C., Tyagi, L. K., & Tiwari, G. (2024). Expanded Therapeutic Applications of Holarrhena Antidysenterica: A Review. *Combinatorial Chemistry & High Throughput Screening*, *27*(9), 1257-1275.

4. Dincel, E. D., & Güzeldemirci, N. U. (2019). Discovery, Synthesis and Activity Evaluation of Novel Compounds Bearing 1, 2, 4-triazolo [3, 4-b][1, 3, 4] thiadiazine Moiety: A Review. *Sağlık Bilimlerinde İleri Araştırmalar Dergisi*, *2*(2), 60-70.

5. Tiwari, G., Tiwari, R., & Kaur, A. (2023). Pharmaceutical Considerations of Translabial Formulations for Treatment of Parkinson's Disease: A Concept of Drug

Delivery for Unconscious Patients. *Current Drug Delivery*, *20*(8), 1163-1175.

6. Tiwari, R., Tiwari, G., & Parashar, P. (2023). Theranostics Applications of Functionalized Magnetic Nanoparticles. In *Multifunctional And Targeted Theranostic Nanomedicines: Formulation, Design And Applications* (pp. 361-382). Singapore: Springer Nature Singapore.

7. Tiwari, R., Tiwari, G., Mishra, S., & Ramachandran, V. (2023). Preventive and therapeutic aspects of migraine for patient care: An insight. *Current Molecular Pharmacology*, *16*(2), 147-160.

8. Boraei, A. T., Ghabbour, H. A., Gomaa, M. S., El Ashry, E. S. H., & Barakat, A. (2019). Synthesis and anti-proliferative assessment of triazolo-thiadiazepine and triazolo-thiadiazine scaffolds. *Molecules*, *24*(24), 4471.

9. Tiwari, R., & Pathak, K. (2023). Local drug delivery strategies towards wound healing. *Pharmaceutics*, *15*(2), 634.

10. Tiwari, R., Tiwari, G., Sharma, S., & Ramachandran, V. (2023). An Exploration of herbal extracts loaded phyto-phospholipid complexes (Phytosomes) against polycystic ovarian syndrome: Formulation considerations. *Pharmaceutical Nanotechnology*, *11*(1), 44-55.

11. Tiwari, G., Chauhan, A., Sharma, P., & Tiwari, R. (2022). Nutritional Values and Therapeutic Uses of Capra hircus Milk. *International Journal of Pharmaceutical Investigation*, *12*(4).

12. Kaushik, D., Sardana, S., & Mishra, D. N. (2009). 5-fluorouracil loaded guar gum microspheres for colon delivery: preparation, characterization and in vitro release. *Yao xue xue bao= Acta pharmaceutica Sinica*, *44*(11), 1278-1284.

13. Deep, A., Kaur Bhatia, R., Kaur, R., Kumar, S., Kumar Jain, U., Singh, H., ... & Kishore Deb, P. (2017). Imidazo [1, 2-a] pyridine scaffold as prospective therapeutic agents. *Current topics in medicinal chemistry*, *17*(2), 238-250.

14. Dincel, E. D., Akdağ, Ç., Kayra, T., Coşar, E. D., Aksoy, M. O., Akalın-Çiftçi, G., & Ulusoy-Güzeldemirci, N. (2022). Design, synthesis, characterization, molecular docking studies and anticancer activity evaluation of novel hydrazinecarbothioamide, 1, 2, 4-triazole-3-thione, 4-thiazolidinone and 1, 3, 4-oxadiazole derivatives. *Journal of Molecular Structure*, *1268*, 133710.

15. Jyoti, K., Pandey, R. S., Kush, P., Kaushik, D., Jain, U. K., & Madan, J. (2017). Inhalable bioresponsive chitosan microspheres of doxorubicin and soluble curcumin augmented drug delivery in lung cancer cells. *International journal of biological*

macromolecules, *98*, 50-58.

16. Kaushik, D., Sardana, S., & Mishra, D. N. (2009). In vitro cytotoxicity analysis of 5-fluorouracil loaded guar gum microspheres on HT-29 colon cancer cell line. *Int J Pharm Sci Drug Res*, *1*(2), 83-4.

17. Indora, N., & Kaushik, D. (2015). Design, development and evaluation of ethosomal gel of fluconazole for topical fungal infection. *International journal of engineering science invention research & development*, *1*(8), 280-306.

18. Kaushik, D., Kumar, P., & Sardana, S. (2015). Design development and evaluation of nanosuspension of azithromycin. *International Journal of Pharmaceutical Sciences and Drug Research*, *7*(5), 384-394.

19. Kaushik, D., Malik, J., & Sardana, S. Formulation and Evaluation of Self Nanoemulsifying Drug Delivery System of Nifedipine.

20. Kaushik, D., Sharma, K., & Sardana, S. (2016). Colon targeting guar gum microspheres of 5-aminosalicylic acid: evaluation of various process variables, characterization and in-vitro drug release. *Cell*, *91*, 130-2221072.

21. Atri, V., Kaushik, D., & Bhushan, B. (2023). Design Development and Evaluation of Tenoxicam Microsponge as Gel. *Int. J. Pharm. Res. Appl*, *8*, 1988-2011.

22. Sharma, P., Pasha, T. Y., Aparna, T. N., Singaram, G., Shirsat, M. K., Kumar, V., & Tiwari, G. (2025). Inulin: A Versatile Polymer for Drug Delivery System. In Inulin for Pharmaceutical Applications: A Versatile Biopolymer (pp. 53-72). Singapore: Springer Nature Singapore.

23. Balekundri, A., Ahire, E. D., Surana, K. R., Keservani, R. K., & Kshirsagar, S. J. (2025). Role of Physical Exercise in Overall Metabolic Health and Body Recomposition. In *Body Recomposition* (pp. 417-426). CRC Press.

24. Sharma, S., & Kaushik, D. (2023). Cefuroxime axetil: An oral prodrug of cefuroxime sodium. *Asian Journal of Pharmacy and Technology*, *13*(4), 281-284.

25. Ahire, E. D., Jadhav, R. L., Patil, M. V., & Keservani, R. K. (2025). The Role of Functional Foods to Combat Weight Gain and Obesity. In *Nutraceuticals in Obesity Management and Control* (pp. 413-424). Apple Academic Press.

26. Yeola, C. A., Sonawane, V. N., Sonawane, V. N., Surana, K. R., Patil, D. M., & Sonawane, D. D. (2023). Development and Validation of Simple UV-Spectrophotometric Method for Estimation of Diclofenac Sodium. *Asian Journal of Pharmaceutical Analysis*, *13*(3), 183-189.

27. Sonawane, V. N., Yeola, C. A., Sonawane, V. N., Surana, K. R., Patil, D. M., &

Sonawane, D. D. (2023). Estimation of Paracetamol in various brands of Paracetamol Tablets and their Comparative Study. *Asian Journal of Pharmaceutical Analysis*, *13*(3), 155-161.

28. Rane, B. R., Amkar, A. J., Pawar, S. S., Gadekar, P. S., Keservani, R. K., & Jain, A. S. (2025). Follicular Delivery of Nanocarriers to Improve Skin Disease Treatment. In *Novel Nanocarriers for Skin Diseases* (pp. 209-237). Apple Academic Press.

29. Singh, A., Diwaker, M., Thakur, A., Surana, K., Chopra, M., Kumar, H., & Sharma, S. (2023). Regioselective Pd-catalyzed decarboxylative C-6 acylation of 7-O-carbamate coumarins and their anti-inflammatory evaluation. *Tetrahedron*, *134*, 133295.

30. Balekundri, A., Satya, S., Ahire, E. D., & Keservani, R. K. (2025). Plant Metabolites and Vegetables for Kidney Disease Prevention and Treatment. In *Plant Metabolites and Vegetables as Nutraceuticals* (pp. 361-380). Apple Academic Press.