Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 4, 2024, 166
https://doi.org/10.33263/LIANBS134.166

Antimicrobial and Antifungal Evaluation of Some Novel Thiazolidin-4-one Scaffold Bearing Compounds

Swati Pawar [1], Ram Karan [1], Ravindra K. Rawal [2,*], Praveen Kumar Gupta [1,*]

[1] Department of Chemistry, Maharishi Markandeshwar (Deemed to be University), Mullana-133207, Ambala, Haryana, India
[2] Natural Products Chemistry Group, Chemical Sciences and Technology Division, CSIR-North East Institute of Science and Technology, Jorhat-785006, Assam, India
* Correspondence: praveenguptachem@gmail.com(P.K.G); rawal.ravindra@gmail.com (R.K.R);
Scopus Author ID 7004237035
Received: 20.09.2023; Accepted: 7.07.2024; Published: 25.08.2024

Abstract: A series of novel hybrid Thiazolidinone derivatives **4, 5 (a-e)** were designed and synthesized by combining more than one bioactive scaffold. All synthesized compounds were tested for their *in vitro* antimicrobial activity against gram-positive and gram-negative bacteria, including strains such as *S. aureus* (MTCC-737), *P. aeruginosa* ((MTCC-424), and *Salmonella typhi* (MTCC531). The antifungal activity was also screened for fungal strain *C. albicans* (MTCC-3378) against the reference drug Ciprofloxacin. Compounds **1**, **3**, **8**, and **9** were proved to show the highest activity against all bacterial and fungal strains at 500 µg/mL with Zone of Inhibition 15.22±0.08 - 19.93±0.09. The substitution with electron-donating groups on the phenyl ring decreases the antimicrobial activity, whereas the presence of the nitro group shows no antifungal activity. Chloro-substituted compounds **3** and **8** exhibited significant inhibition.

Keywords: thiazole; thiazolidinone; antimicrobial activity; bacterial strain; antifungal.

1. Introduction

Due to numerous diverse causes, such as increased awareness of good hygiene, environmental changes, and the absence of efficient therapies, infectious diseases are becoming more prevalent daily. Despite the availability of numerous antibiotics and chemotherapeutics over 20 years, treating contagious diseases remains a problematic therapeutic challenge because of the unabating emergence and dissemination of multidrug-resistant bacteria. New classes of antimicrobial drugs must be developed to reduce the rapid development of antibiotic resistance in harmful microorganisms [1,2]. Many innovative chemotherapeutic medicines have been synthesized recently due to the medicinal chemist's interest in the therapeutic properties of thiazolidinone compounds. Thiazolidinone medications offer a more comprehensive range of applications in treating different clinical ailments, such as anticancer [3,4], antimicrobial, antifungal [5-10], anti-inflammatory [11-13], anti-tuberculosis [14,15], antidiabetic [16,17] and anti-alzheimer [18-20] are some of the medicinal qualities of compounds containing thiazolidinone. The thiazole ring is the basic structural element of a broad class of synthetic and naturally occurring chemicals with diverse biological activities such as anticancer [21], antimicrobial [22], anti-inflammatory [23], and antiepileptic [24] properties. Researchers, however, are interested in the thiazolidinone core because of its varying degrees of pharmacological and therapeutic activity. The development of etozoline as an antihypertensive, ralitoline as a potent anti-convulsant, pioglitazone as a hypoglycemic agent, and thiazolidomycin as an antibacterial agent against Streptomyces sp. has successfully

established the promise of the thiazolidinone molecule due to the previously described factors, including the preceding outcomes [25,26] Encouraged by these findings, we have designed new heterocyclic thiazolidinone derivatives (Figure 1) using simple and conventional techniques to identify novel biologically active heterocyclic compounds and pursue more effective antimicrobial compounds [27-30].

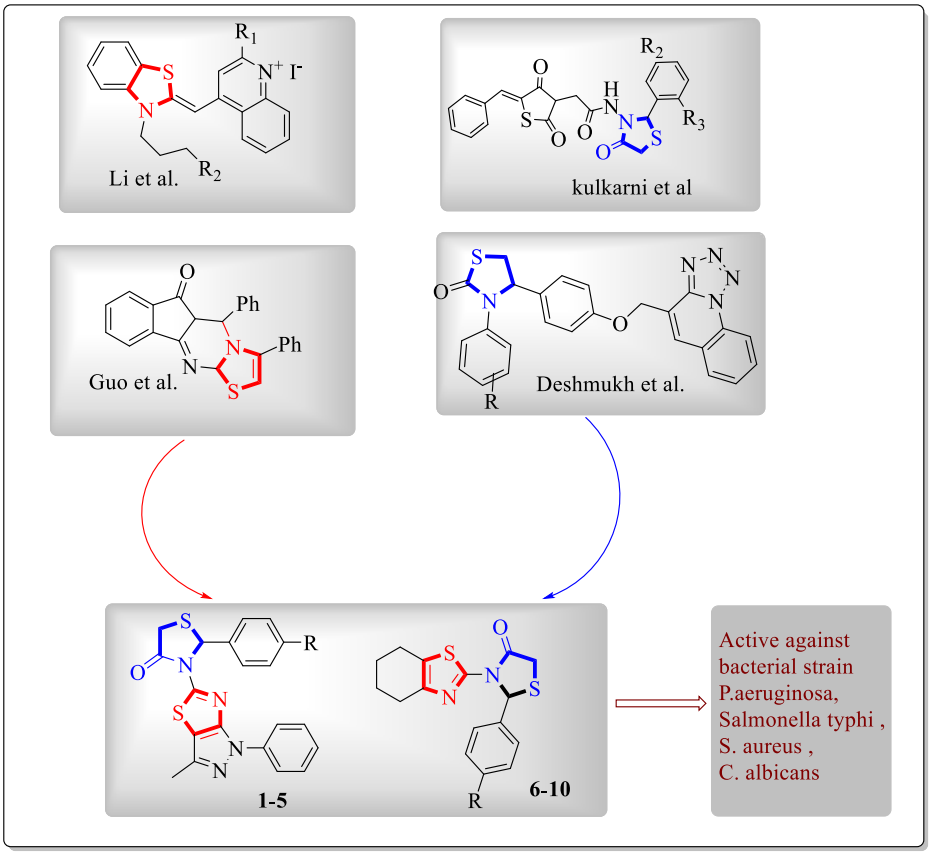


Figure 1. Design of the hybrid 2, 3-substituted thiazolidine-4-one hybrid molecule (1-10) containing biologically active unit.

2. Materials and Methods

The uncorrected melting points of all substances were determined in open capillary tubes. The FT-IR spectra were recorded on Shimadzu IR Spirit Fourier transform infrared spectrophotometer. Tetramethylsilane was used as an internal reference in a ^{1}H nuclear magnetic resonance (NMR) experiment using a Bruker DRX-400 MHz NMR. A XEVO G2-XS QTOF mass spectrometer was used to record the mass spectra. For the compounds, satisfactory C, H, N, and analysis were found within 0.4% of the theoretical values. The compounds' purity was examined on silica gel G plates using an iodine vapor and UV laser as a visualizing agent. All the reagents used in this experiment were of analytical grade and obtained from various chemical suppliers, including Sigma-Aldrich, Qualikems, Merck, and Lobachemie.

2.1. Synthetic procedure of 2,3-substituted thiazolidin-4-ones.

2.1.1. Preparation of 3-methyl-1-phenyl-1*H*-pyrazol-5(*4H*)-one.

3-methyl-1-phenyl-1H-pyrazol-5(4H)-one was prepared by stirring ethyl acetoacetate and phenyl hydrazine at 120°C for 10-12 hours, followed by cooling overnight. The reaction mixture was heated for another 6-8 hours. The reaction completion was monitored on TLC

(2:8; ethyl acetate: hexane). On cooling, the solid appeared and was filtered, washed with diethyl ether thrice, and recrystallized with ethanol to obtain brown-colored crystals, M.p. 114-116°C [31].

2.1.2. Preparation of 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]thiazol-5-amine.

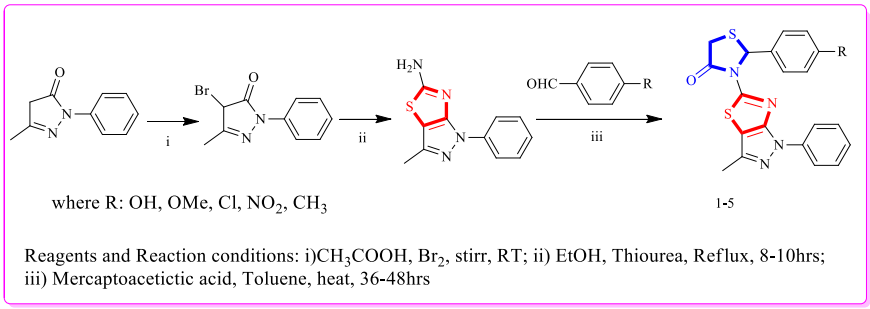
A solution of compound **1a** (0.25 mole) in 50 ml of glacial acetic acid and 0.25 mol of bromine in 50 ml of glacial acetic acid was added dropwise with occasional shaking at room temperature over the course of 1 hour. The reaction mixture was refluxed for half an hour in the water bath, cooled, and filtered. The solid obtained was washed with diethyl ether and recrystallized with glacial acetic acid, giving brown-colored crystals. The progress of the reaction was monitored by TLC (2:8; ethyl acetate: hexane). Thus, the solid obtained was cyclized without further purification with thiourea using ethanol as the solvent under reflux to afford the corresponding fused aminothiazole M.p. 120-122°C [32-33].

2.1.3. Preparation of 4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-amine.

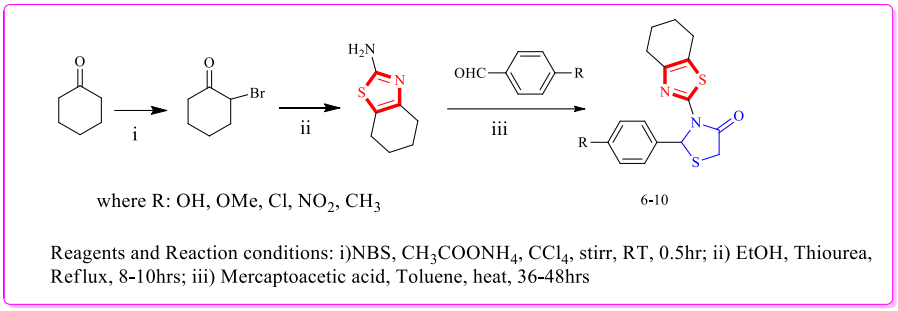
Bromination of cyclic ketones was done according to the procedure adopted in literature [34] in which cyclohexanone was reacted with 1.05 equivalent of n-bromosuccinimide and 10 mol% of ammonium acetate in CCl_4 at 25°C to give the corresponding α-mono brominated ketones in good yields. The purity of the product was checked by thin-layer chromatography using precoated aluminum plates. The α-brominated ketone (1 equiv.) thus obtained was cyclized in the presence of thiourea (1.5 equivalent) in ethanol by refluxing for 2 hours. After the reaction, yellow crystals were left behind and dissolved in water, and the pH was then raised to 8 by adding ammonia. The resulting precipitate was filtered, washed with water and diethyl ether, and recrystallized with glacial acetic acid, m.p. 96-99°C [33].

2.1.4. General method of preparation of thiazolidinone (1-10).

Under reflux conditions, toluene was agitated while the amines obtained in steps 2.1.2 and 2.1.3 (1.0 mmol) and substituted benzaldehydes (1.5 mmol) were added. After 12 hours, when all of the amine was consumed, the mercapto acid (2.0 mmol) was added to the reaction mixture, which continued to reflux for an additional 24 to 48 hours until all the amine was consumed. Under reduced pressure, the reaction mixture was concentrated to dryness, and the resulting residue was dissolved in ethyl acetate. The organic layer was then sequentially washed with water, 5% aqueous sodium hydrogen carbonate, 5% aqueous citric acid, and brine solution. The organic layer was dried over sodium sulfate to obtain a crude product, and the solvent was removed under reduced pressure. The product was then purified by column chromatography on silica gel with dichloromethane-methanol (7:3) (Scheme 1 and 2) [35].



Scheme 1. Synthesis of 2-(4-substituted phenyl)-3-(3-methyl-1-phenyl-1H-pyrazolo[3,4-d]thiazol-5-yl)thiazolidine-4-one.



Scheme 2. Synthesis of 2-(4-substituted phenyl)-3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)thiazolidine-4-one.

2.1.4.1. 2-(4-hydroxyphenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-d]thiazol-5-yl)thiazolidin-4-one (1).

Color cream white; M.p. 137-139°C; yield (66%); FTIR (cm^{-1}): 1706 (C=O), 656 (C-S-C), 1121 (C-N), 1538 (C=N), 1255 (N-N), 3457 (O-H); ^{1}H-NMR (δ, ppm) 7.11-7.28 (m, 9H, Ar-H); 4.15-4.28 (dd, 2H, CH_2); 4.96 (s, 1H, CH); 2.08(s, 3H, CH_3); 8.64 (s, Ar-OH); MS [M+H] (m/z): Calculated 409.07 Found 409.44, Elemental analysis for $C_{20}H_{16}N_4O_2S_2$ Calculated C, 58.80; H, 3.95; N, 13.72; O, 7.83; S, 15.70; Found C, 58.75; H, 3.89; N, 13.77; O, 7.77; S, 15.74.

2.1.4.2. 2-(4-methoxyphenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]thiazol-5-yl)thiazolidin-4-one **(2).**

Color white; M.p. 145-147°C; yield (61%); FTIR (cm^{-1}): 1722 (C=O), 655 (C-S-C), 1128 (C-N), 1547 (C=N), 1241 (N-N), 1254 (O-CH_3); ^{1}H-NMR (δ, ppm) 7.12-7.21 (m, 9H, Ar-H); 4.05-4.22 (dd, 2H, CH_2); 4.93 (s, 1H CH); 2.05(s, 3H, CH_3); 3.68 (s, OCH_3); MS [M+H] (m/z): Calculated 423.09; Found 423.48, Elemental analysis for $C_{21}H_{18}N_4O_2S_2$ Calculated C, 59.69; H, 4.29; N, 13.26; O, 7.57; S, 15.18; Found C, 59.61; H, 4.32; N, 13.18; O, 7.31; S, 15.11.

2.1.4.3. 2-(4-chlorophenyl)-3-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]thiazol-5-yl)thiazolidin-4-one (3).

Color pale yellow; M.p. 149-151°C; yield (65%); FTIR (cm^{-1}): 1716 (C=O), 652 (C-S-C), 1207 (C-N), 1596 (C=N), 1262 (N-N), 839 (O-Cl); ^{1}H-NMR (δ, ppm) 7.13-7.25 (m, 9H, Ar-H); 4.17-4.23 (dd, 2H, CH_2); 4.98 (s, 1H CH); 2.03(s, 3H, CH_3); MS [M+H] (m/z): Calculated 427.06; Found 427.86, Elemental analysis for $C_{20}H_{15}ClN_4OS_2$ Calculated C, 56.26; H, 3.54; Cl, 8.30; N, 13.12; O, 3.75; S, 15.02; Found C, 56.21; H, 3.49; Cl, 8.34; N, 13.22; O, 3.68; S, 15.11.

2.1.4.4. 3-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]thiazol-5-yl)-2-(4-nitrophenyl)thiazolidin-4-one (4).

Color yellow; M.p. 132-134°C; yield (72%); FTIR (cm^{-1}): 1712 (C=O), 659 (C-S-C), 1224 (C-N), 1597 (C=N), 1268 (N-N), 1534 (NO_2); ^{1}H-NMR (δ, ppm) 7.13-8.12 (m, 9H, Ar-H); 4.14-4.21 (dd, 2H, CH_2); 4.96 (s, 1H CH); 2.05(s, 3H, CH_3); MS [M+H] (m/z): Calculated 438.06; Found 438.34, Elemental analysis for $C_{20}H_{15}ClN_5O_3S_2$ Calculated C, 54.91; H, 3.46; N, 16.01; O, 10.97; S, 14.66; Found C, 54.87; H, 3.51; N, 16.15; O, 10.85; S, 14.61.

2.1.4.5. 3-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]thiazol-5-yl)-2-p-tolylthiazolidin-4-one (5).

Color cream white; M.p. 1462-148°C; yield (75%); FTIR (cm^{-1}): 1706 (C=O), 653 (C-S-C), 1119 (C-N), 1583 (C=N), 1277 (N-N); ^{1}H-NMR (δ, ppm) 7.13-8.13 (m, 9H, Ar-H); 4.14-4.23 (dd, 2H, CH_2); 4.91 (s, 1H CH); 2.05(s, 3H, CH_3); 2.84(s, 3H, CH_3; MS [M+H] (m/z): Calculated 407.10; Found 407.21, Elemental analysis for $C_{21}H_{18}ClN_4OS_2$ Calculated C, 62.04; H, 4.46; N, 13.78; O, 3.94; S, 15.78; Found C, 62.12; H, 4.51; N, 13.71; O, 3.88; S, 15.69.

2.1.4.6. 2-(4-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (6).

Color yellow; M.p. 152-154°C; yield (68%); FTIR (cm^{-1}): 1719 (C=O), 662 (C-S-C), 1134 (C-N), 1588 (C=N), 3454 (O-H); ^{1}H-NMR (δ, ppm) 7.16-7.71 (m, 4H, Ar-H); 1.14 (s, 2H, fused ring-H); 1.48 (s, 2H, fused ring-H); 3.49-3.68 (m, 4H, fused ring-H); 4.29-4.36 (dd, 2H, CH_2); 7.19(s, 1H, CH); 8.68 (s, Ar-OH); MS [M+H] (m/z): Calculated 333.07; Found 333.16, Elemental analysis for $C_{16}H_{16}N_2S_2O_2$ Calculated C, 57.81; H, 4.85; N, 8.43; O, 9.63; S, 19.29; Found C, 57.77; H, 4.79; N, 8.55; O, 9.71; S, 19.34.

2.1.4.7. 2-(4-methoxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (7).

Color cream white; M.p. 156-158°C; yield (65%); FTIR (cm^{-1}): 1724 (C=O), 668 (C-S-C), 1219 (C-N), 1594 (C=N), 1247 (O-CH_3); ^{1}H-NMR (δ, ppm) 7.16-7.72 (m, 4H, Ar-H); 1.18 (d, 2H, fused ring-H); 1.45 (d, 2H, fused ring-H); 3.46-3.62 (m, 4H, fused ring-H); 4.32-4.38 (dd, 2H, CH_2); 7.21(s, 1H, CH); 3.66 (s, OCH_3); MS [M+H] (m/z): Calculated 347.08; Found 347.15, Elemental analysis for $C_{17}H_{18}N_2S_2O_2$ Calculated; C, 58.93; H, 5.24; N, 8.09; O, 9.24; S, 18.51; Found C, 58.85; H, 5.27; N, 8.14; O, 9.35; S, 18.44.

2.1.4.8. 2-(4-chlorophenyl)-3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (8).

Color brown; M.p. 157-159°C; yield (70%); FTIR (cm^{-1}): 1731 (C=O), 668 (C-S-C), 1202 (C-N), 1537 (C=N), 852 (O-Cl); ^{1}H-NMR (δ, ppm) 7.16-7.30 (m, 4H, Ar-H); 1.16 (d, 2H, fused ring-H); 1.44 (d, 2H, fused ring-H); 3.46-3.62 (m, 4H, fused ring-H); 4.34-4.38 (dd, 2H, CH_2); 4.96-4.98(d, 1H, CH); MS [M+H] (m/z): Calculated 354.03; Found 351.84, Elemental analysis for $C_{22}H_{16}N_2S_2O_2$ Calculated C, 54.77; H, 4.31; Cl, 10.10; N, 7.98; O, 4.56; S, 18.28; Found C, 54.67; H, 4.27; Cl, 10.15; N, 7.88; O, 4.51; S, 18.23.

2.1.4.9. 2-(4-nitrophenyl)-3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)thiazolidin-4-one (9).

Color yellow; M.p. 154-156°C; yield (79%); FTIR (cm^{-1}): 1733 (C=O), 655 (C-S-C), 1214 (C-N), 1585 (C=N), 1267, 1549 (NO_2); ^{1}H-NMR (δ, ppm) 7.16-7.71 (m, 4H, Ar-H); 1.19 (s, 2H, fused ring-H); 1.54 (s, 2H, fused ring-H); 3.46-3.92 (m, 4H, fused ring-H); 4.30-4.36 (dd, 2H, CH_2); 7.19(s, 1H, CH); MS [M+H] (m/z): Calculated 362.06 Found 362.28, Elemental analysis for $C_{16}H_{15}N_3O_3S_2$ Calculated: C, 53.17; H, 4.18; N, 11.63; O, 13.28; S, 17.74; Found C, 53.22; H, 4.11; N, 11.70; O, 13.31; S, 17.83.

2.1.4.10. 3-(4,5,6,7-tetrahydrobenzo[*d*]thiazol-2-yl)-2-*p*-tolylthiazolidin-4-one (10).

Color greenish white; M.p. 141-143°C; yield (77%); Colour yellow; M.p. 154-156°C; yield (79%); %); FTIR (cm^{-1}): 1728 (C=O), 665 (C-S-C), 1226 (C-N), 1568 (C=N); ^{1}H-NMR (δ, ppm) 7.16-7.25 (m, 4H, Ar-H); 1.12 (d, 2H, fused ring-H); 1.41 (d, 2H, fused ring-H); 3.46-3.77 (m, 4H, fused ring-H); 4.28-4.46 (dd, 2H, CH_2); 7.15(s, 1H, CH); 2.87(s, 3H CH_3) MS

[M+H] (m/z): Calculated 331.09; Found 331.22, Elemental analysis for $C_{17}H_{18}N_2OS_2$ Calculated: C, 61.79; H, 5.49; N, 8.48; O, 4.84; S, 19.41; Found C, 61.71; H, 5.37; N, 8.39; O, 4.91; S, 19.37.

2.2. Biological studies.

2.2.1. Antimicrobial activity assay.

Each compound's antibacterial effect was examined against gram-positive and gram-negative bacterial strains and Candida albicans (MTCC-3378) as a fungus strain. The entire strain of microorganisms was incubated in a nutrient broth medium at 37°C for 24 hours, and the fungus was incubated in Sabouraud dextrose broth for five days at 22–25°C (around 105 c.f.u. of culture/ml). *C. albicans* was identified by microscope after isolating on corn agar with tween-80 media. The Gram-negative bacteria were examined using the Maconkey agar test, the Eosin methylene blue test, the Indole test using Kovac's reagent, the VP test, the Motility test, the TSI test with the production of hydrogen sulfide and the fermentation of sugars, the growth in Simmons citrate, and the Gram staining. The Gram-positive bacteria were isolated on blood agar, milk agar, and Baird Parker agar medium and examined under a microscope for their morphological characteristics and the crucial step of colony-level Gram staining for identification.

2.2.2. Antimicrobial activity.

The agar well diffusion method [36–37] and MIC based on zones obtained at four distinct concentrations, namely 50 g/ml, 100 g/ml, 250 g/ml, and 500 g/ml, were used to assess the antibacterial activity of synthetic compounds. The inoculum was combined with 500 microliters of nutrient agar (pH 7.2) for bacterial culture and Sabouraud dextrose agar for fungal culture in the autoclaved medium. After properly combining the media, it was placed onto Petri dishes, solidified, 8.0 mm-diameter wells were made in plates, and the sample was immediately diluted. The plates were incubated at 37°C for 18–24 hours for bacteria and 22–25°C for five days for fungus after being left at room temperature for 30 min to allow sample diffusion. After the incubation period, inhibition zones around the wells in Petri dishes were measured, and positive and negative controls were performed using samples against the antibacterial Ciprofloxacin and fluconazole (10 g/ml for each). The investigated samples' inhibition zones revealed bactericidal chemical activity. DMSO was used to prepare each of the compounds under evaluation, and it was also loaded as a negative control. Bactericidal action is characterized by the visibly cleansed areas surrounding discs, which indicates that it effectively destroys bacteria. The absence of inhibition zones in the negative control tests indicates that DMSO has no inhibitory effect on bacterial or fungal strains.

3. Results and Discussion

3.1. General preparation of 2, 3-substituted Thiazolidin-4-ones.

The novel thiazolidinone hybrid derivatives **1-10** containing bioactive moiety were synthesized as depicted in Scheme 1 and 2. 2-amino thiazoles were synthesized starting from the bromination of active methyl ketones followed by cyclization with thiourea. 2-aminothiazole thus obtained was refluxed with different 4-substituted aldehydes and mercaptoacetic acid via a one-pot reaction in toluene for 48 hours to afford the final target

compounds **1-10** in 61-77% yield (Table 1) followed by flash column chromatography purification on silica gel (230–400 mesh size) in moderate to excellent yields. The starting material 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one was prepared by stirring ethyl acetoacetate and phenyl hydrazine at 120°C for 10-12 hours, followed by cooling overnight. The physical data, including the compounds' antibacterial activity data, are reported in Tables 1 and 2. The FTIR, ^{1}H NMR, and Mass spectra of most active compounds are depicted in Figure 2-7. The FTIR spectrum of the synthesized compounds exhibits new absorption bands due to C=O, C-S-C, C-N, C=N of thiazolidinone moiety in the range 1706-133, 652-666, 1121-1226, and 1537-1597 respectively. ^{1}H spectra of compound **3** displayed signals of two protons of thiazolidinone at δ 4.17-4.23 ppm and signal of one proton at δ 4.98 ppm. Finally, structure **3** was further supported by the presence of [M+H] ion peak at m/z 427.86 for $C_{20}H_{15}ClN_4OS_2$ in mass spectrometry.

Table 1. Physical data of compounds (1-10).

Compound No.	R	Molecular formula	% yield	Color	Melting point in °C
1	OH	$C_{21}H_{14}N_2S_2O_4$	66	Cream white	137-139
2	Ome	$C_{22}H_{16}N_2S_2O_4$	61	White	145-147
3	Cl	$C_{21}H_{13}ClN_2S_2O_3$	65	Pale yellow	149-151
4	NO_2	$C_{21}H_{13}N_3S_2O_5$	72	Yellow	132-134
5	CH_3	$C_{23}H_{18}N_2S_2O_4$	75	Cream white	146-147
6	OH	$C_{22}H_{16}N_2S_2O_2$	68	Yellow	152-154
7	Ome	$C_{23}H_{18}N_2S_2O_2$	65	Cream white	156-158
8	Cl	$C_{22}H_{15}ClN_2S_2O$	70	Brown	157-159
9	NO_2	$C_{22}H_{15}N_3S_2O_3$	77	Yellowish	154-156
10	CH_3	$C_{17}H_{18}N_2OS_2$	77	Greenish white	141-143

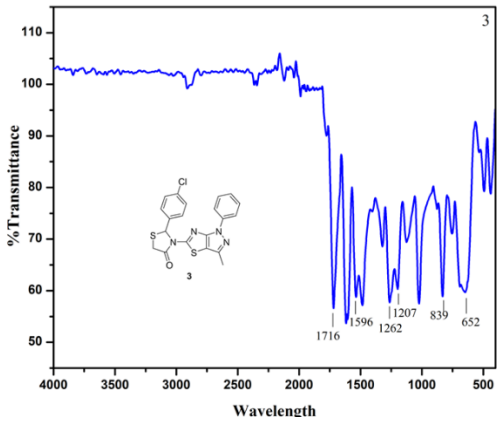


Figure 2. FTIR spectra of compound 3.

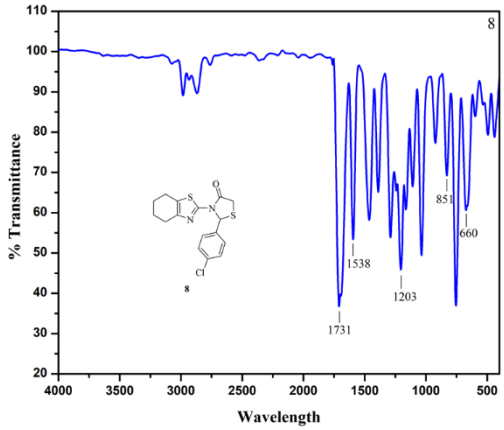


Figure 3. FTIR spectra of compound 8.

Similarly, compound **8** exhibits signals of two protons of thiazolidinone at δ 4.34-4.38 ppm and doublet of one proton at δ 4.96-4.98 ppm. The [M+H] ion peak at m/z 351.84 was observed for $C_{22}H_{16}N_2S_2O_2$ in **8,** which confirmed the structure of the compound. Similar spectral information was obtained for the other compounds of the series, which supported the structure of derivatives.

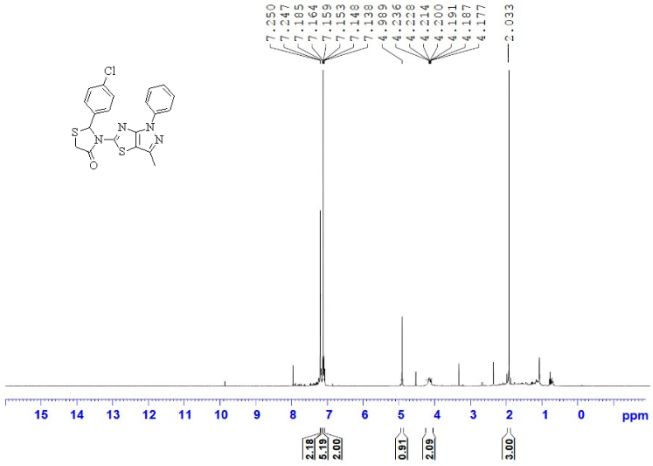


Figure 4. ^{1}H NMR spectra of compound 3.

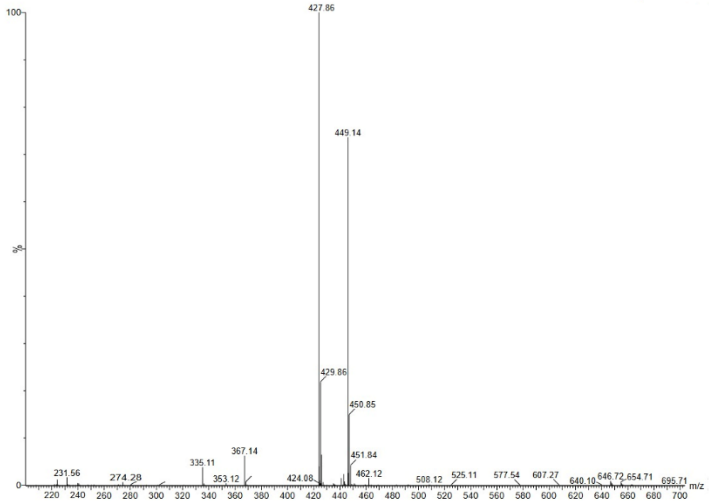


Figure 5. Mass spectra of compound 3.

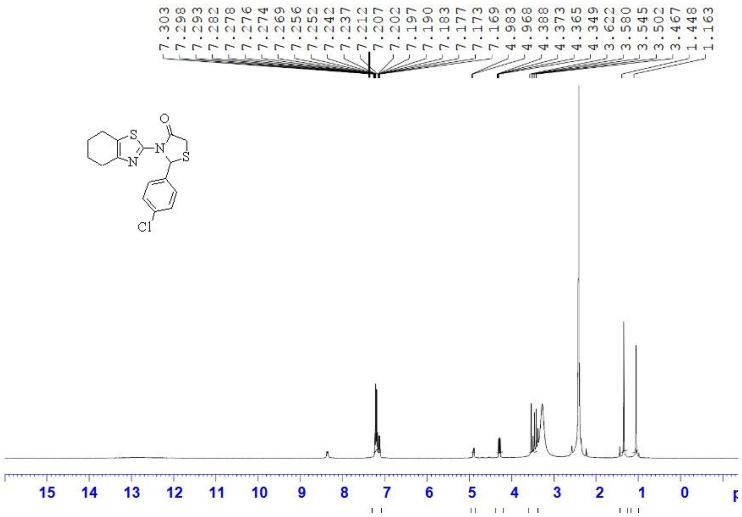


Figure 6. ^{1}H NMR spectra of compound 8.

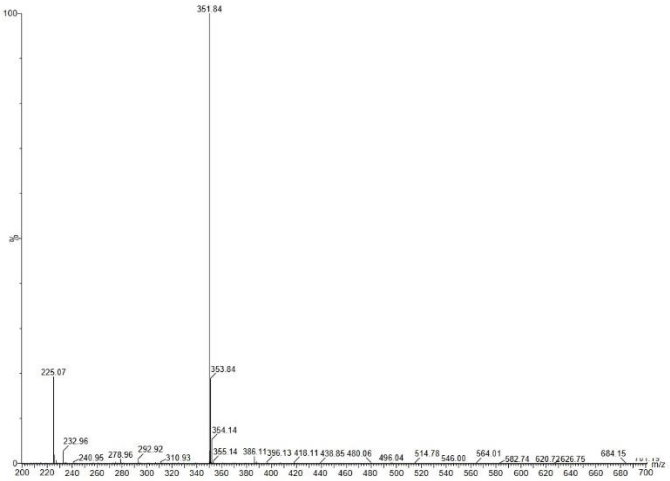


Figure 7. Mass spectra of compound 8.

3.2. Biological evaluation

All the synthesized derivatives **(1-10)** were screened for their in vitro antibacterial activity against the gram-negative and gram-positive bacterial strains such as *P.aeruginosa* (MTCC-424), *Salmonella typhi* (MTCC531), *S.aureus (*MTCC-737) and fungal strain *Candida albicans* (MTCC-3378) by agar well diffusion method. DMSO was used as a negative control and to dissolve the compounds. Ciprofloxacin was used as a reference drug to test against *P.aeruginosa*, *Salmonella typhi* (gram-negative strain), and *S. aureus* (gram-positive) bacterial strain. Fluconazole was used as a reference against fungal strains (*Candida albicans*). The four compounds (**1**, **3**, **8**, **9**) were proved to be most active against all bacterial and fungal strains at 500 µg/mL concentration with a zone of inhibition as shown in Table 2 and Figure 6. It is to be noted that all compounds (except **4** and **6**) were proven potent against gram-negative strain *P. aeruginosa*. The substitution of electron donating groups such as methyl and methoxy group (**2**, **5**, **7**, **10**) at the phenyl ring decreases the activity against both gram-negative bacterial strains with MIC ranges from 9.01±0.13 - 10.01±0.23 and proved inactive against gram-positive bacterial strain *S. aureus* (MTCC-737). However, substituting nitro group (**4**) at the phenyl ring showed no activity against all strains. The hydroxy (MIC 18.44±0.10) and nitro (MIC 18.88±0.14) derivatives are found to be more potent antifungal agents than standard medication (MIC 16.33±0.13). The chloro group at the phenyl ring of compounds **3** and **8** showed moderate to maximum growth inhibition against all tested strains. Furthermore, the response of compound **4-7** with nitro, methyl, hydroxy, and methoxy substitution was nil against gram-positive bacterial strain *S. aureus* (MTCC-737) and fungal strain *C. albicans* (MTCC-3378).

Table 2. In vitro antimicrobial activity of substituted thiazolidin-4-one (1-10) with minimum zone of inhibition at 500 µg/ml.

Compound No.	R	*P. aeruginosa* (MTCC-424)	*Salmonella typhi* (MTCC531)	*S. aureus* (MTCC-737)	*C. albicans* (MTCC-3378)
1	OH	22.39±0.09	19.11±0.08	19.93±0.09	18.44±0.10
2	Ome	9.01±0.13	9.61±0.18	*	*
3	Cl	18.22±0.11	19.46±0.09	16.12±0.12	15.22±0.08
4	NO_2	*	*	*	*
5	CH_3	10.01±0.23	9.33±0.19	*	*
6	OH	*	9.81±0.10	*	*
7	Ome	9.22±0.12	*	*	*
8	Cl	17.22±0.09	19.22±0.11	18.46±0.22	17.41±0.14
9	NO_2	19.11±0.17	16.99±0.17	18.29±0.14	18.88±0.14

Compound No.	R	*P. aeruginosa* (MTCC-424)	*Salmonella typhi* (MTCC531)	*S. aureus* (MTCC-737)	*C. albicans* (MTCC-3378)
10	CH_3	9.55±0.89	*	*	8.22±0.07
Ciprofloxacin		19.64 ±0.11	19.15±0.12	18.01±0.15	NT
Fluconazole		NT	NT	NT	16.33±0.13

*= No activity; NT = Not tested

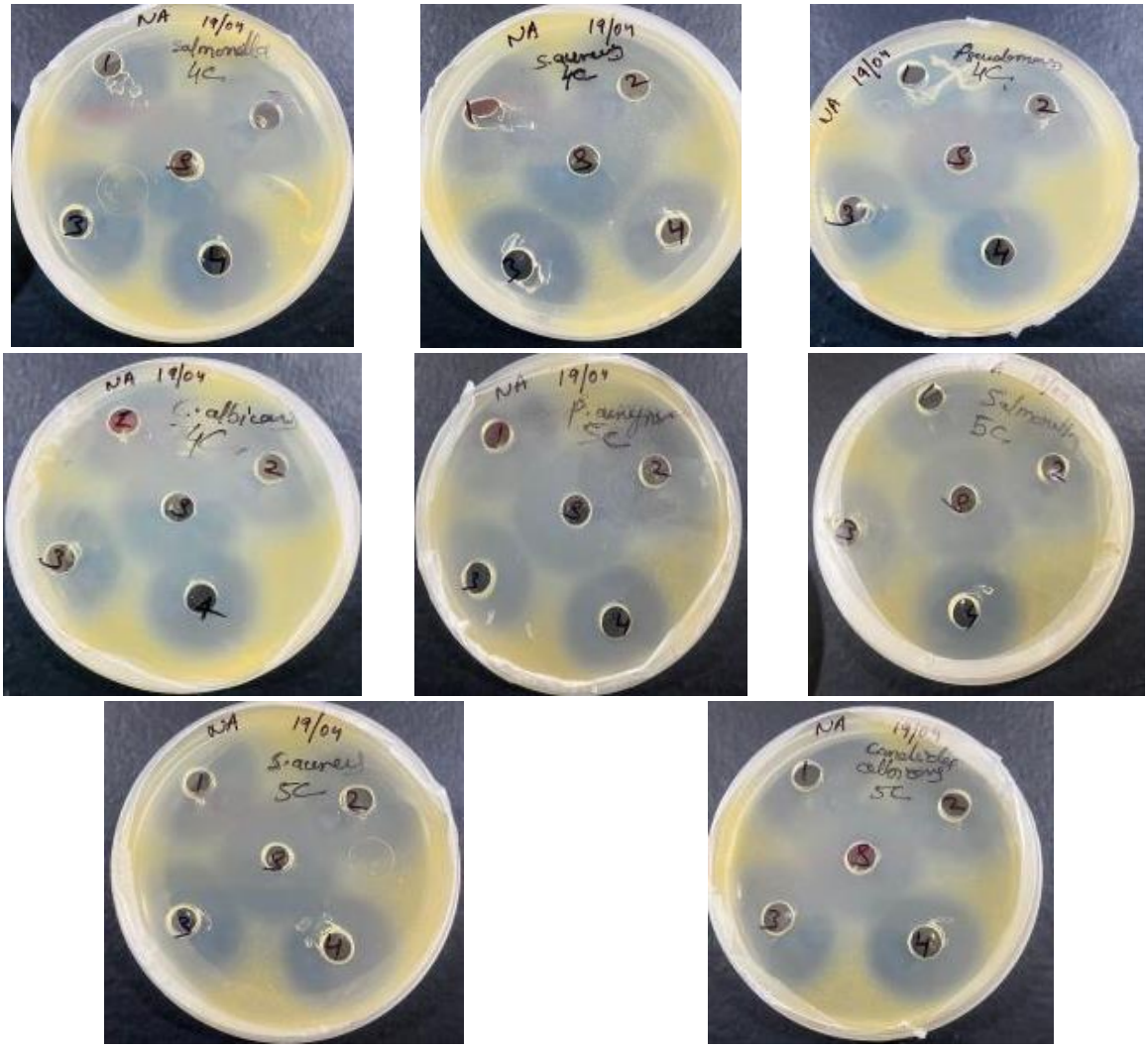


Figure 8. Zone of inhibition of compounds 4c and 5c for gram-positive [*S. aureus* (MTCC-737)] and gram-negative bacterial [*Ps. aeruginosa* (MTCC-424)], [*Salmonella typhi* (MTCC531)] and fungal strain [(*Candida albicans* (MTCC-3378)].

4. Conclusions

A new series of 2,3-substituted thiazolidine-4-one derivatives (1-10) have been designed and synthesized. FTIR, ^{1}H NMR, and mass spectral analyses were used to characterize each of the synthesized compounds. Furthermore, the agar well diffusion method tested the synthesized compounds for in vitro antibacterial activity. Compounds 1, 3, 8, and 9 were found to be the most effective among the synthesized compounds against all tested strains, with zones of inhibition (ZOI) between 15.22±0.08 and 19.93±0.09 at 500 µg/ml. Electron-donating groups reduced activity against gram-negative bacteria and were inactive against S. aureus. Nitro-substituted compound 4 was inactive against all strains. Hydroxy and nitro derivatives were more potent antifungals than fluconazole. Chloro-substituted compounds 3 and 8 showed significant inhibition, while compounds 4-7 were ineffective against S. aureus and C. albicans. The exploration of these compounds with different substituents in various

positions and additional design considerations is needed. These identified compounds could serve as prospective starting points for developing new antibacterial and antifungal drugs**.**

Funding

This research received no external funding.

Acknowledgments

The authors are thankful to the Maharishi Markandeshwar (Deemed to be University), Mullana, Haryana, India.

Conflicts of Interest

There are no conflicts of interest among authors.

References

1. Mohanty, P.; Behera, S.; Behura, R.; Shubhadarshinee, L.; Mohapatra, P.; Barick, A.K.; Jali, B.R. Antibacterial Activity of Thiazole and its Derivatives: A Review. *Biointerface Res. Appl. Chem.* **2022**, *12*, 2171–2195, https://doi.org/10.33263/BRIAC122.21712195.
2. Mohi El-Deen, E.M.; Abd El-Meguid, E.A.; Hasabelnaby, S.; Karam, E.A.; Nossier, E.S. Synthesis, Docking Studies, and In Vitro Evaluation of Some Novel Thienopyridines and Fused Thienopyridine–Quinolines as Antibacterial Agents and DNA Gyrase Inhibitors. *Molecules* **2019**, *24*, 3650, https://doi.org/10.3390/molecules24203650.
3. Pawar, S.; Kumar, K.; Gupta, M.K.; Rawal, R.K. Synthetic and Medicinal Perspective of Fused-Thiazoles as Anticancer Agents. *Anti-Cancer Agents Med. Chem.* **2021**, *21*, 1379-1402, https://doi.org/10.2174/1871520620666200728133017.
4. Othman, I.M.M.; Alamshany, Z.M.; Tashkandi, N.Y.; Gad-Elkareem, M.A.M.; Abd El-Karim, S.S.; Nossier, E.S. Synthesis and biological evaluation of new derivatives of thieno-thiazole and dihydrothiazolo-thiazole scaffolds integrated with a pyrazoline nucleus as anticancer and multi-targeting kinase inhibitors. *RSC Adv.* **2022**, *12*, 561-577, https://doi.org/10.1039/D1RA08055E.
5. Nagaraju, P.; Reddy, P.N.; Padmaja, P.; Ugale, V.G. Microwave-Assisted Synthesis of Thiazole/Benzothiazole Fused Pyranopyrimidine Derivatives and Evaluation of their Biological Activity. *Lett. Org. Chem.* **2021**, *18*, 49-57, https://doi.org/10.2174/1570178617999200517130138.
6. Abbas, S.Y.; Abd El-Aziz, M.M.; Awad, S.M.; Mohamed, M.S. Synthesis and evaluation of antipyrine derivatives bearing a thiazole moiety as antibacterial and antifungal agents, *Synth. Commun.* **2023**, *53*, 1812-1822, https://doi.org/10.1080/00397911.2023.2248306.
7. Petrou, A.; Geronikaki, A.; Kartsev, V.; Kousaxidis, A.; Papadimitriou-Tsantarliotou, A.; Kostic, M.; Ivanov, M.; Sokovic, M.; Nicolaou, I.; Vizirianakis, I.S. *N*-Derivatives of (Z)-Methyl 3-(4-Oxo-2-thioxothiazolidin-5-ylidene)methyl)-1*H*-indole-2-carboxylates as Antimicrobial Agents—In Silico and In Vitro Evaluation. *Pharmaceuticals* **2023**, *16*, 131, https://doi.org/10.3390/ph16010131.
8. Nandurkar, Y.; Shinde, A.; Bhoye, M.R.; Jagadale, S.; Mhaske, P.C. Synthesis and Biological Screening of New 2-(5-Aryl-1-phenyl-1*H*-pyrazol-3-yl)-4-aryl Thiazole Derivatives as Potential Antimicrobial Agents. *ACS Omega* **2023**, *8*, 8743-8754, https://doi.org/10.1021/acsomega.2c08137.
9. Wang, J.; Long, S.; Liu, Z.; Rakesh, K.P.; Verma, R.; Verma, S.K.; Sharath Kumar, K.S. Structure-activity relationship studies of thiazole agents with potential anti methicillin-resistance *Staphylococcus aureus* (MRSA) activity. *Process Biochem.* **2023**, *132*, 13-29, https://doi.org/10.1016/j.procbio.2023.06.013.
10. Tratrat, C.; Petrou, A.; Geronikaki, A.; Ivanov, M.; Kostić, M.; Soković, M.; Vizirianakis, I.S.; Theodoroula, N.F.; Haroun, M. Thiazolidin-4-Ones as Potential Antimicrobial Agents: Experimental and In Silico Evaluation. *Molecules* **2022**, *27*, 1930, https://doi.org/10.3390/molecules27061930.
11. Saliyeva, L.; Holota, S.; Grozav, A.; Yakovychuk, N.; Litvinchuk, M.; Slyvka, N.; Vovk, M. Synthesis and Evaluation of Antimicrobial and Anti-inflammatory Activity of 6-aryliden-2-methyl-2, 3-dihydroimidazo [2, 1-*b*][1, 3] thiazoles. *Biointerface Res. Appl. Chem.* **2022**, *12*, 292–303, https://doi.org/10.33263/BRIAC121.292303.

12. Bhatnagar, A.; Pemawat, G. An overview on synthetic routes of anti-inflammatory active scaffolds including thiazole and thiazolidine cores. *Phosphorus Sulfur Silicon Relat. Elem.* **2023**, *198*, 554-565, https://doi.org/10.1080/10426507.2023.2189253.
13. Gupta, K.; Sirbaiya, A.K.; Kumar, V.; Rahman, M.A. Current Perspective of Synthesis of Medicinally Relevant Benzothiazole based Molecules: Potential for Antimicrobial and Anti-Inflammatory Activities. *Mini Rev. Med. Chem.* **2022**, *22*, 1895-1935, https://doi.org/10.2174/1389557522666220217101805.
14. Hemeda, L.R.; El Hassab, M.A.; Abdelgawad, M.A.; Khaleel, E.F.; Abdel-Aziz, M.M.; Binjubair, F.A.; Al-Rashood, S.T.; Eldehna, W.M.; El-Ashrey, M.K. Discovery of pyrimidine-tethered benzothiazole derivatives as novel anti-tubercular agents towards multi- and extensively drug resistant *Mycobacterium tuberculosis*. *J. Enzyme Inhib. Med. Chem.* **2023**, *38*, 2250575, https://doi.org/10.1080/14756366.2023.2250575.
15. Moulishankar, A.; Thirugnanasambandam, S. Quantitative structure activity relationship (QSAR) modeling study of some novel thiazolidine 4-one derivatives as potent anti-tubercular agents. *J. Recept. Signal Transduct.* **2023**, *43*, 83-92, https://doi.org/10.1080/10799893.2023.2281671.
16. Khan, S.A.; Ali, M.; Latif, A.; Ahmad, M.; Khan, A.; Al-Harrasi, A. Mercaptobenzimidazole-Based 1,3-Thaizolidin-4-ones as Antidiabetic Agents: Synthesis, In Vitro α-Glucosidase Inhibition Activity, and Molecular Docking Studies. *ACS Omega* **2022**, *7*, 28041-28051, https://doi.org/10.1021/acsomega.2c01969.
17. Ramkumar, S.; Ramarajan, R. Design, synthesis, spectral characterization, multiple-biological activities, docking and in silico ADMET studies of thiazolidinones. *Res. Chem.* **2023**, *5*, 100861, https://doi.org/10.1016/j.rechem.2023.100861.
18. Aggarwal, N.; Jain, S.; Chopra, N. Hybrids of Thiazolidin-4-Ones and 1,3,4-Thiadiazole: Synthesis and Biological Screening of A Potential New Class of Acetylcholinesterae Inhibitors. *Biointerface Res. Appl. Chem.* **2022**, *12*, 2800-2812, https://doi.org/10.33263/BRIAC123.28002812.
19. Hemaida, A.Y.; Hassan, G.S.; Maarouf, A.R.; Joubert, J.; El-Emam, A.A. Synthesis and Biological Evaluation of Thiazole-Based Derivatives as Potential Acetylcholinesterase Inhibitors. *ACS Omega* **2021**, *6*, 19202-19211, https://doi.org/10.1021/acsomega.1c02549.
20. Kumar, A.; Virendra, S.A.; Shome, A.; Chawla, P.A. In Silico Design and Evaluation of Triazine Based 4-Thiazolidinone (TBT) Analogues as Anti Alzheimer's Agents through BACE 1 Inhibition. *Biomed. J. Sci. Technol. Res.* **2022**, *46*, 37433-37441, https://doi.org/10.26717/BJSTR.2022.46.007352.
21. Mahmoud, H.K.; Abdelhady, H.A.; Elaasser, M.M.; Hassain, D.Z.H.; Gomha, S.M. Microwave-Assisted One-Pot Three Component Synthesis of Some Thiazolyl(Hydrazonoethyl)Thiazoles as Potential Anti-Breast Cancer Agents. *Polycyclic Aromat. Compd.* **2022**, *42*, 7232-7246, https://doi.org/10.1080/10406638.2021.1998146.
22. Muhammed Aziz, D.; Hassan, S.A.; Amin, A.A.M.; Abdullah, M.N.; Qurbani, K.; Aziz, S.B. A synergistic investigation of azo-thiazole derivatives incorporating thiazole moieties: a comprehensive exploration of their synthesis, characterization, computational insights, solvatochromism, and multimodal biological activity assessment. *RSC Adv.* **2023**, *13*, 34534-34555, https://doi.org/10.1039/D3RA06469G.
23. Zahra, F.T.; Saeed, A.; Ahmed, A.; Ismail, H.; Ijaz, M.U.; Albericio, F. Synthesis of amantadine clubbed *N*-aryl amino thiazoles as potent urease, α-amylase & α-glucosidase inhibitors, kinetic and molecular docking studies. *RSC Adv.* **2023**, *13*, 24988-25001, https://doi.org/10.1039/D3RA05330J.
24. Chauhan, B.; Kumar, R.; Salahuddin; Singh, H.; Afzal, O.; Altamimi, A.S.A.; Abdullah, M.M.; Yar, M.S.; Ahsan, M.J.; Kumar, N.; Yadav, S.K. Design, Synthesis, *In Vivo*, and *In Silico* Evaluation of Benzothiazoles Bearing a 1,3,4-Oxadiazole Moiety as New Antiepileptic Agents. *ACS Omega* **2023**, *8*, 2520–2530, https://pubs.acs.org/doi/10.1021/acsomega.2c06967.
25. Cheerala, V.S.K.; Akhir, A.; Saxena, D.; Maitra, R.; Chopra, S.; Neelakantan, S.C. Discovery of benzoxazole–thiazolidinone hybrids as promising antibacterial agents against *Staphylococcus aureus* and *Enterococcus species*. *RSC Med. Chem.* **2023**, *14*, 1712-1721, https://doi.org/10.1039/D3MD00290J.
26. Chawla, P.A.; Wahan, S.K.; Negi, M.; Faruk, A.; Chawla, V. Synthetic strategies and medicinal perspectives of 4-thiazolidinones: Recent developments and structure–activity relationship studies. *J. Heterocycl. Chem.* **2023**, *60*, 1248-1286, https://doi.org/10.1002/jhet.4596.
27. Li, Y.; Sun, N.; Ser, H.-L.; Long, W.; Li, Y.; Chen, C.; Zheng, B.; Huang, X.; Liu, Z.; Lu, Y.-J. Antibacterial activity evaluation and mode of action study of novel thiazole-quinolinium derivatives. *RSC Adv.* **2020**, *10*, 15000-15014, https://doi.org/10.1039/d0ra00691b.
28. Yan Guo, F.; Ji Zheng, C.; Wang, M.; Ai, J.; Ying Han, L.; Yang, L.; Fang Lu, Y.; Xuan Yang, Y.; Guan Piao, M.; Piao, H.-R.; Jin, C.-M.; Jin, C.H. Front Cover: Synthesis and Antimicrobial Activity Evaluation

of Imidazole-Fused Imidazo[2,1-*b*][1,3,4]thiadiazole Analogues (15/2021). *ChemMedChem* **2021**, *16*, 2287, https://doi.org/10.1002/cmdc.202100501.

29. Kulkarni, P.S.; Karale, S.N.; Khandebharad, A.U.; Agrawal, B.R.; Sarda, S.R. Design, Synthesis, and Biological Evaluation of Newer Arylidene Incorporated 4-Thiazolidinones Derivatives as Potential Antimicrobial Agents. *Polycyclic Aromat. Compd.* **2022**, *42*, 2031-2040, https://doi.org/10.1080/10406638.2020.1823861.
30. Deshmukh, A.R.; Dhumal, S.T.; Nawale, L.U.; Khedkar, V.M.; Sarkar, D.; Mane, R.A. Dicationic liquid mediated synthesis of tetrazoloquinolinyl methoxy phenyl 4-thiazolidinones and their antibacterial and antitubercular evaluation. *Synth. Commun.* **2019**, *49*, 587-601, https://doi.org/10.1080/00397911.2018.1564928.
31. Ramshini, A.; Nezhad, S.M.; Pourmousavi, S.A.; Nazarzadeh Zare, E.; Pourjafar, M.; Sharifi, E. Synthesis, and Anticancer Evaluation of 4-[(Indol-3-yl)-arylmethyl]-1-phenyl-3-methyl-5-pyrazolone Derivatives via a Magnetic Aminated Starch Biocatalyst. *Catalysts* **2023**, *13*, 908, https://doi.org/10.3390/catal13050908.
32. Brindha, J.; Fen Reji, T.F.A. Synthesis and Molecular Docking Studies of Coumarinyl Thiazole as Cell Division Protein Kinase 2 Inhibitor. *Asian J. Chem.* **2019**, *31*, 2453-2456, https://doi.org/10.14233/ajchem.2019.22106.
33. Abedi-Jazini, Z.; Safari, J.; Zarnegar Z.; Sadeghi M. A Simple and Efficient Method for the Synthesis of 2-Aminothiazoles Under Mild Conditions. *Polycyclic Aromat. Compd.* **2018**, *38*, 231-235, https://doi.org/10.1080/10406638.2016.1200104.
34. Tanemura, K.; Suzuki, T.; Nishida, Y.; Satsumabayashi, K.; Horaguchi, T. A mild and efficient procedure for α-bromination of ketones using *N*-bromosuccinimide catalysed by ammonium acetate. *Chem. Commun.* **2004**, 470-471, https://doi.org/10.1039/B315340A.
35. Rawal, R.K.; Tripathi, R.; Katti, S.B.; Pannecouque, C.; De Clercq, E. Synthesis and evaluation of 2-(2,6-dihalophenyl)-3-pyrimidinyl-1,3-thiazolidin-4-one analogues as anti-HIV-1 agents. *Bioorg. Med. Chem.* **2007**, *15*, 3134-3142, https://doi.org/10.1016/j.bmc.2007.02.044.
36. Abdel-Wahab, B.F.; Awad, G.E.A.; Badria, F.A. Synthesis, antimicrobial, antioxidant, anti-hemolytic and cytotoxic evaluation of new imidazole-based heterocycles. *Eur. J. Med. Chem.* **2011**, *46*, 1505-1511, https://doi.org/10.1016/j.ejmech.2011.01.062.
37. Puthran, D.; Poojary, B.; Purushotham, N.; Harikrishna, N.; Nayak, S.G.; Kamat, V. Synthesis of novel Schiff bases using 2-Amino-5-(3-fluoro-4-methoxyphenyl)thiophene-3-carbonitrile and 1,3-Disubstituted pyrazole-4-carboxaldehydes derivatives and their antimicrobial activity. *Heliyon* **2019**, *5*, e02233, https://doi.org/10.1016/j.heliyon.2019.e02233.