Synthesis and antimicrobial screening of novel 1,3,4-thiadiazole derivatives bearing di chlorovinyl-substituted cyclopropane

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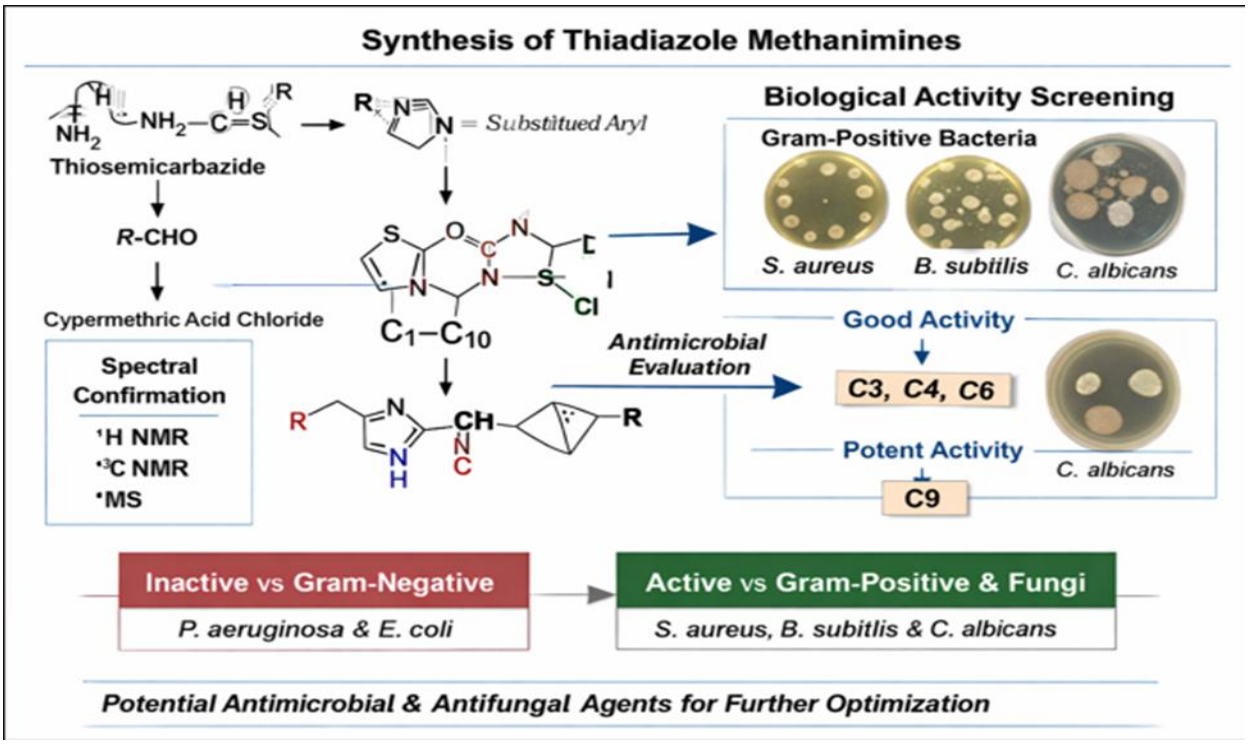
Abstract- A series of newly synthesized derivatives (C1–C10) were evaluated for their antibacterial and antifungal activities. The antimicrobial potential of the compounds was assessed by determining their (MICs) using the modified Kirby–Bauer disk diffusion method against selected bacterial and fungal strains. Ciprofloxacin and Fluconazole were used as reference standards for antibacterial and antifungal comparisons, respectively. Among the tested derivatives, compounds C3, C4, C6, and C8 exhibited notable antibacterial activity against *S. aureus*, showing zones of inhibition up to 14 mm at a concentration of 320 µg/mL. Compounds C4, C6, C8, and C9 also demonstrated appreciable inhibitory activity with zones of 11 mm at the same concentration, while the remaining derivatives displayed moderate to low activity. In antifungal evaluation, compound C9 showed remarkable potency against *C. albicans* with an MIC value of 12 µg/mL, comparable to the standard drug Fluconazole. Overall, the findings indicate that selected derivatives, particularly C9, possess promising antimicrobial potential and may serve as lead candidates for further structural optimization and pharmacological investigation.

Key words 1,3,4-Thiadiazole; antimicrobial activity & antifungal activity.



Graphical abstract

I. INTRODUCTION

Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. It acts as "hydrogen binding domain" and "two electron donor system" with a constrained pharmacophore [1-5]. Many drugs containing thiadiazole nucleus are available in the market such as acetazolamide, methazolamide, sulfamethazole, etc. Thiadiazole can act as the bio-isosteric replacement of thiazole moiety [6-11], so it acts like third and fourth generation cephaelosporins. Hence can be used in antibiotic preparations. Thiadiazole is 5-membered ring system containing two nitrogen and one Sulphur atom. They occur in nature in four compounds are called 1, 3, 4-thiadiazole (two nitrogen and one other heteroatom in a five-membered ring). They occur in nature in four isomeric forms as. 1,2,3-thiadiazole; 1,2,5- thiadiazole; 1,2,4-thiadiazole and 1,3,5-thiadiazole. 1, 3, 5-thiadiazole are important because of their versatile biological actions [12-20]. In particular, compounds bearing the 1, 3, 5-thiadiazole nucleus is known to have unique antibacterial and anti-inflammatory activities [21-25]. Differently substituted thiadiazole moieties have also been found to have other interesting activities such as analgesic, antimicrobial, anti-tubercular, anticonvulsant and antihepatitis B viral activities [26-30]. The present work deals with Thiadiazole fused Cypermethric acid chloride and their analogs with synthesis, characterization and biological evaluation of 1-(substituted aryls)-*N*-(5- {[3-(2, 2-dichloroethenyl)-2, 2-dimethyl cyclopropyl]}-1, 3, 4-thiadiazol-2-yi) methanimines (C_1-C_{10}).

II. EXPERIMENTAL

2.1. Materials and Methods

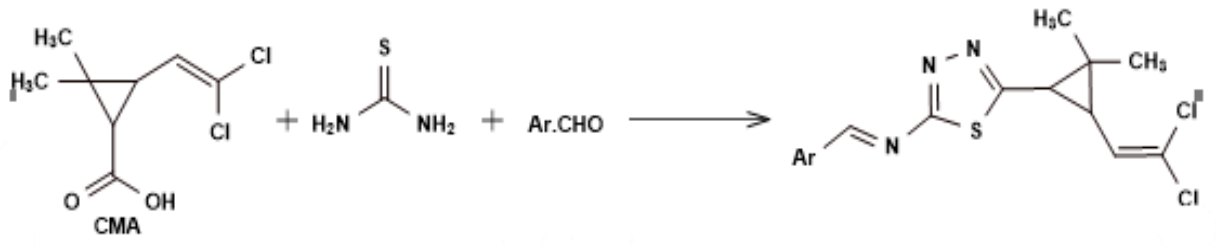
The melting points of all synthesized compounds were measured using the open capillary method and are uncorrected. Reactions were monitored by thin-layer chromatography on silica gel plates, which were visualized under ultraviolet light or with iodine. Infrared spectra were obtained using Fourier-transform infrared spectroscopy with a PerkinElmer Infrared Spectrophotometer Model RZX and an Agilent Resolution Pro FT-IR spectrometer, employing potassium bromide pellets. The frequencies are reported in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker advance II 400 spectrometer (400 MHz FT-NMR) using tetramethyl silane as an internal standard in $CDCl_3$ and dimethyl sulfoxide-d_6. Chemical shifts are reported in δ (ppm). All reagents were used without purification, and solvents were of laboratory reagent and analytical reagent grades.

2.2. Chemistry

General synthesis of 1-(substituted aryls)-*N*-(5- {[3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclo propyl]}-1, 3, 4-thiadiazol-2-yl) methanimines (C_1-C_{10})

A mixture of Cyper methric acid (0.01 mol), thiosemicarbazide (0.012mol), and aromatic aldehyde (0.02 mol) in 30 ml of ethanol (95%) was refluxed in water bath at 90°c temp. for 4-5 hr. The completion of reaction was confirmed by TLC at every 1.5 hr. and after completion of reaction, the reaction mixture was allowed to attain room temp. Then reaction mixture was cooled and poured on crushed ice, cooled to 10°c temp. The solid separated was washed with water and crystallized from DMF to obtain the product.

Scheme-1



2.2.2 Spectral characterization

(1*E*)-*N*-(5- {[3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropyl] methyl}-1, 3, 4-thiadiazolyl)-2-phenylethanimine (C_1): IR (KBr) *v* cm^{-1}: 3062.64-2918.57 (aromatic C-H), 1597.65 (N-H aromatic), 1353.17 (C-N), 743.83 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.58(s, 1H, C-N=H), 7.21 – 7.83 (m, 5H, aromatic), 2.46-2.52(s, 6H CH_3), 1.14- 1.17 (cyclopropane ring) 1.87-1.89 (C-CH_2) 5.97-5.99 (s, H), 3.60, (C-CH) ^{13}C NMR (100MHz, $CDCl_3$) δ(ppm): 170.86-156.99 (C-N=H), 139.46-125.02 (aromatic), 129.79 (CH_2-CH_2), 119.13, 38.97, 38.77 (CH_3-CH_3), 33.45-28.59 (CH_2-CH_2), 21.65-14.22 (cyclopropane). ESI-MS: $[M+H]^+$ 380.29.

2-{(2*E*)-2- [(5- {[3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropyl] methyl}-1, 3, 4-thiadiazol-2-yl) imino] ethyl} phenol (C_2): IR (KBr) *v* cm^{-1}: 3452.07 (O-H), 3062.13-2918.57(aromatic C-H), 1594.65 (N-H aromatic), 1345.17 (C-N), 743.83 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):8.02(s, 1H, C-N=H), 6.00-7.99 (aromatic), 2.49-2.51(6H CH_3), 1.15- 1.52 (cyclopropane ring) 2.51-3.38, (CH_2-CH_2) 5.37 (s, 1 H), 3.60, (CH-H), 8.26(-OH). ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 178.14-170.85(C-N=H), 149.87-125.16 (aromatic carbon), 129.79 (CH_2-CH_2), 119.01, 38.97, 38.77 (CH_3-CH_3), 33.56-19.86 (cyclopropane carbon), 33.41(CH_2-CH_2). ESI-MS: $[M+H]^+$ 396.29.

(1*E*)-*N*-[5-({3-[(*E*)-2-chloroethenyl]-2, 2-dimethylcyclopropyl} methyl)-1, 3, 4-thiadiazol-2-yl]-2-(2, 3-dichlorophenyl) ethanimine (C_5): IR (KBr) *v* cm^{-1}: 3062.21-2918.54 (aromatic C-H), 1591.52 (N-H aromatic), 1345.12 (C-N), 743.81 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.08(s, 1H, C-N=H), 6.01-7.95 (aromatic), 2.44-2.49 (6H CH_3), 1.20- 1.52 (cyclopropane ring) 2.50-3.41, (CH2-CH2) 5.34 (s, 1 H), 3.57, (CH-H). ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 178.10-170.79 (C-N=H), 149.83-125.12 (aromatic carbon), 129.70 (CH_2-CH_2), 119.01, 38.88, 38.79 (CH_3-CH_3), 33.53-19.85 (cyclopropane carbon), 33.42 CH_2-CH_2). ESI-MS: $[M+H]^+$ 449.18

2-{(2*E*)-2-[(5-{[3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropyl] methyl}-1,3,4-thiadiazol-2-yl) imino] ethyl} aniline (C_6): IR (KBr) *v* cm^{-1}: 3353.07 (N-H), 3023.64-2918.22 (aromatic C-H), 1597.32 (N-H aromatic), 1345.17 (C-N), 743.83(C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.05(s, 1H, C-N=H), 6.02-7.98 (aromatic), 2.42-2.51 (6H CH_3), 1.22- 1.54 (cyclopropane ring) 2.49-3.43, (CH_2-CH_2) 5.36 (s, 1 H), 3.58, (CH-H), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 178.15-170.81 (C-N=H), 149.81-125.10 (aromatic carbon), 129.69 (CH_2-CH_2), 119.02, 33.43 (CH_2-CH_2), 19.80-33.52 (cyclopropane carbon), 38.80, 38.89 (CH_3-CH_3). ESI-MS: $[M+H]^+$ 395.30

(*E*)-5-((3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropyl) methyl)-*N*-(2-(2-fluoro phenyl) ethylidene)-1, 3, 4-thiadizol-2-amine. (C_{10}): IR (KBr) *v* cm^{-1}: 3022.54-2911.67 (aromatic C-H), 1597.32 (N-H aromatic), 1345.34 (C-N), 1031.83 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.04 (s, 1H, C-N=H), 6.02-8.06 (aromatic), 2.48-2.50 (6H CH_3), 1.04- 1.49 (cyclopropane ring) 2.52-3.45, (CH2-CH2) 5.38 (s, 1 H), 3.55, (CH-H), ^{13}C NMR (100MHz, $CDCl_3$) δ (ppm): 178.79-170.85 (C-N=H), 149.83-125.12 (aromatic carbon), 129.79 (CH_2-CH_2), 119.10 (CH_2=CCl_2), 38.90, 38.88 (CH_3-CH_3),33.46-19.85 (cyclopropane carbon), 33.37 (CH_2-CH_2), ESI-MS: $[M+H]^+$ 398.28

III. RESULT & DISCUSSION

3.1 Chemistry

The synthetic pathway for the target compounds (C1–C10) is illustrated in Scheme–I. Initially, thiosemicarbazide was condensed with substituted aromatic aldehydes under reflux conditions to afford the corresponding 1,3,4-thiadiazole derivatives. The obtained intermediate was subsequently treated with cypermethric acid chloride, a key intermediate, to yield the desired (Z)-1-(substituted aryls)-N-(5-{[3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropyl]}-1,3,4-thiadiazol-2-yl) methanimines in good yields. The structures of all synthesized compounds were confirmed by spectral analysis (^{1}H NMR, ^{13}C NMR, and Mass spectroscopy). In the ^{1}H NMR spectra ($CDCl_3$), a characteristic singlet at δ 6.58 ppm was attributed to the azomethine proton (–CH=N–). The aromatic protons appeared as multiplets in the region δ 7.21–7.83 ppm. A singlet corresponding to six protons of the gem-dimethyl group was observed at δ 2.46–2.52 ppm. Signals corresponding to the cyclopropane ring protons appeared in the region δ 1.87–1.89 ppm, while additional singlets at δ 5.97–5.99 ppm and δ 3.60 ppm were assigned to vinylic and methine protons, respectively. The ^{13}C NMR spectra showed characteristic signals for the azomethine carbon in the range δ 170.86–156.99 ppm and aromatic carbons between δ 139.46–125.02 ppm. The

dichlorovinyl carbon (CH_2=CCl_2) resonated at δ 119.13 ppm, while signals corresponding to methyl and cyclopropane carbons were observed in the expected aliphatic region. Furthermore, mass spectral data of all compounds exhibited molecular ion peaks consistent with their calculated molecular weights, thereby confirming the proposed structures.Overall, the spectral data strongly support the successful synthesis of the targeted thiadiazole-based methanimine derivatives. (Figure-1)

3.2 Biology

The synthesized compounds (C1–C10) were evaluated for their antibacterial and antifungal activities by determining their minimum inhibitory concentrations (MICs) using the modified Kirby–Bauer disk diffusion method. The antimicrobial efficacy was assessed against selected bacterial and fungal strains and compared with standard drugs, Ciprofloxacin (antibacterial) and Fluconazole (antifungal). Among the tested derivatives, compounds C3, C4, C6, and C8 exhibited significant antibacterial activity against *S. aureus*, showing zones of inhibition up to 14 mm at a concentration of 320 µg/mL. Compounds C4, C6, C8, and C9 also demonstrated appreciable inhibitory activity with zone diameters of 11 mm at the same concentration. The remaining derivatives displayed moderate to weak antibacterial activity, indicating that substitution patterns on the aryl moiety influence antimicrobial potency.In antifungal screening, compound C9 showed remarkable activity against *C. albicans*, exhibiting a low MIC value of 12 µg/mL, comparable to the reference drug Fluconazole. Other compounds demonstrated higher MIC values and comparatively lower antifungal potency. These findings suggest that structural variation within the thiadiazole-based methanimine framework significantly affects biological activity, with compound C9 emerging as a promising lead candidate for further optimization. (Table-1 & Table-2)

3.3. Structure activity relationship.

The antimicrobial data reveal a distinct trend in activity among the synthesized thiadiazole-based methanimine derivatives (C1–C10). All compounds exhibited negligible activity against Gram-negative bacteria (*P. aeruginosa* and *E. coli*), with MIC values >320 µg/mL. The uniform inactivity suggests that the presence of the bulky dichlorovinyl cyclopropane moiety and substituted aryl groups may limit penetration through the outer membrane of Gram-negative organisms, which is known to act as an effective permeability barrier. In contrast, moderate antibacterial activity was observed against Gram-positive strains (*S. aureus* and *B. subtilis*). Compounds C3, C4, and C6 demonstrated comparatively better activity, producing larger zones of inhibition (14–15 mm for *S. aureus* and up to 14 mm for *B. subtilis*). This indicates that certain aryl substitutions enhance interaction with Gram-positive bacterial targets, possibly due to improved lipophilicity or electronic effects favoring cell wall penetration. For antifungal activity, compound C9 showed relatively improved inhibition against *C. albicans* at higher concentration (12 mm at 320 µg), suggesting that specific substitution on the aryl ring may enhance antifungal interaction. Overall, the results indicate that antimicrobial activity is influenced by the nature of aryl substitution, with selective efficacy toward Gram-positive bacteria, while Gram-negative resistance may be attributed to structural and permeability limitations.

IV. CONCLUSION

A novel series of 1,3,4-thiadiazole-based methanimine derivatives (C1–C10) incorporating a dichlorovinyl-substituted cyclopropane moiety were successfully synthesized and structurally confirmed by spectral analyses (^{1}H NMR, ^{13}C NMR, and Mass spectroscopy). The antimicrobial evaluation revealed that the synthesized compounds exhibited selective activity against Gram-positive bacteria, while showing negligible activity against Gram-negative strains (Pseudomonas aeruginosa and Escherichia coli), with MIC values >320 µg/mL. Among the tested derivatives, compounds C3, C4, and C6 demonstrated comparatively better antibacterial activity against Staphylococcus aureus and Bacillus subtilis. In antifungal screening, compound C9 showed relatively improved activity against Candida albicans at higher concentration. Overall, the study indicates that structural modifications on the aryl moiety significantly influence antimicrobial activity, with enhanced efficacy observed primarily against Gram-positive organisms. These findings suggest that thiadiazole-based methanimine derivatives may serve as potential lead structures for further structural

optimization to improve potency and broaden antimicrobial spectrum.

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Tables & Figures:

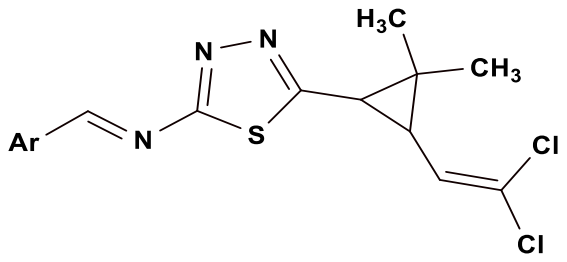

Figure-1

Table-1

Comp.	Time (h.)	M.P. °C	Yield %
C_1	5.0	185-187	80
C_2	4.5	225-227	65
C_3	4.0	215-216	75
C_4	5.0	180-182	66
C_5	4.0	210-212	81

C_6	4.0	270-272	69
C_7	5.0	220-222	70
C_8	4.0	188-190	73
C_9	4.5	225-227	62
C_{10}	5.0	175-177	69

Table-2 Antimicrobial activity of compound $C_{(1-10)}$.

	Pseudomonas aeruginosa							
Gram Negative Bacteria	Comp.	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	MIC
	C_1	10	10	10	10	10	10	>320
	C_2	10	10	10	10	10	10	>320
	C_3	10	10	10	10	10	10	>320
	C_4	10	10	10	10	10	10	>320
	C_5	10	10	10	10	10	10	>320
	C_6	10	10	10	10	10	10	>320
	C_7	10	10	10	10	10	10	>320
	C_8	10	10	10	10	10	10	>320
	C_9	10	10	10	10	10	10	>320
	C_{10}	10	10	10	10	10	10	>320
	Escherichia coli							
	Comp.	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	MIC
	C_1	10	10	10	10	10	10	>320
	C_2	10	10	10	10	10	10	>320
	C_3	10	10	10	10	10	10	>320
	C_4	10	10	10	10	10	10	>320
	C_5	10	10	10	10	10	10	>320
	C_6	10	10	10	10	10	10	>320
	C_7	10	10	10	10	10	10	>320
	C_8	10	10	10	10	10	10	>320
	C_9	10	10	10	10	10	10	>320
	C_{10}	10	10	10	10	10	10	>320
	Staphylococcus aureus							
Gram Positive Bacteria	Comp.	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	MIC µg
	C_1	10	10	10	10	10	10	>320
	C_2	10	10	10	10	10	10	>320
	C_3	14	14	14	15	15	15	>320
	C_4	14	14	14	14	14	14	>320
	C_5	10	10	10	10	10	10	>320
	C_6	15	15	15	15	15	15	>320
	C_7	10	10	10	10	10	10	>320
	C_8	10	10	10	10	10	12	>320
	C_9	10	10	10	10	10	10	>320
	C_{10}	10	10	10	10	11	13	>320
	Bacillus subtilis							
	Comp.	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	MIC µg
	C_1	10	10	10	10	10	10	>320
	C_2	10	10	10	10	10	10	>320

	C_3	12	12	12	13	13	13	>320
	C_4	12	12	12	12	12	12	>320
	C_5	10	10	10	10	10	10	>320
	C_6	13	13	13	13	14	14	>320
	C_7	10	10	10	10	10	10	>320
	C_8	10	10	10	10	10	11	>320
	C_9	10	10	11	12	13	15	>320
	C_{10}	10	10	10	10	10	10	>320
Candida albicans								
Fungi	Comp.	10µg	20 µg	40 µg	80 µg	160 µg	320 µg	MIC µg
	C_1	10	10	10	10	10	10	>320
	C_2	10	10	10	10	10	10	>320
	C_3	10	10	10	10	10	10	>320
	C_4	10	10	10	10	10	10	>320
	C_5	10	10	10	10	10	10	>320
	C_6	10	10	10	10	10	10	>320
	C_7	10	10	10	10	10	10	>320
	C_8	10	10	10	10	10	10	>320
	C_9	10	10	10	10	10	12	>320
	C_{10}	10	10	10	10	10	10	>320