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SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL EVALUATION OF THIADIAZOLO[3,2-A]PYRIMIDINE-6-CARBOXYLATE DERIVATIVES CATALYZED BY ZIRCONIUM OXYCHLORIDE

Sridevi Khadgamala N.[1], N. Krishnarao[2*], Bhagavathula S. Diwakar[3*], V. Siddaiah[1], Chilukuri Sai Naga Satya Bharati[4]

[1]Department of chemistry, Andhra University, India
[2]Department of Organic Chemistry, PRISM PG&DG College, Visakhapatnam, India
[3]Department of Chemistry, SRKR Engineering College, Bhimavaram, India-534204
[4]Rangaraya Medical College, Kakinada, India-533003

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ABSTRACT. A new approach has been developed that is more efficient and economical than past efforts in the synthesis of several thiadiazolo[3,2-a] pyrimidine compounds. This study describes the synthesis of pyrimidine heterocycles containing the [1,3,4]-thiadiazole ring system via multicomponent reactions, thereby replacing classical synthetic methods. The compounds of the title [1,3,4] thiadiazolo[3,2-a] pyrimidine-6-carboxylates and derivatives have been generated by the condensation of substituted amino thiadiazole with substituted aromatic aldehydes and ethyl acetoacetate in the ratio of {1:1:1}. The reactions were carried out in ethanol as the solvent in the presence of the catalyst $ZrOCl_2 \cdot 8H_2O$. The approach used in this work and the compounds generated have substantial advantages over prior work: simplicity, straightforward reaction workup, higher yields of the target molecules, and much more manageable compounds. The compounds were generated by reaction, characterized by several advanced spectroscopic techniques, including ^1H and ^{13}C-NMR, and analyzed by LC-MS. Among the compounds generated, **5e** (3-chlorophenyl) showed potent antimicrobial activity against tested bacteria, and compound **5j** (4-nitrophenyl) against fungi, with ciprofloxacin and fluconazole used as reference standards for comparison. However, further mechanistic investigation is needed to better understand the microbial activity.

KEY WORDS: Multi-component reaction (MCRs), [1,3,4]Thiadiazolo [3,2-a] pyrimidines, $ZrOCl_28H_2O$, Antibacterial studies

INTRODUCTION

Thiadiazolo [3,2-a] pyrimidines are an important class of fused heterocyclic compounds that have garnered significant attention in medicinal and synthetic chemistry. These bicyclic scaffolds, which belong to the pseudo-purine class of compounds, combine a sulfur-containing five-membered thiadiazole ring with a six-membered pyrimidine nucleus. The resulting structure has emerged as a versatile platform for developing bioactive molecules with diverse pharmacological properties, including anticancer, antiviral, antibacterial, antitubercular, and antioxidant activities [1-3]. Several drug candidates and lead compounds based on the thiadiazolo [3,2-a] pyrimidine scaffold have been reported, including filanesib, which has demonstrated promising anticancer activity in preclinical studies, and sulfamethizole, which exhibits significant antibacterial activity [4-6]. The molecular planarity and heteroatom distribution confer interesting electronic properties, which, along with bioactivity, have driven synthetic innovation toward efficient, sustainable methods for assembling these frameworks [6-8]. Over the past decade, multiple synthetic strategies have been developed to access these compounds efficiently, ranging from traditional cyclization approaches to modern multicomponent reactions with environmentally benign catalysts. The various synthetic methods used to achieve these molecules are presented in Table 1. The synthesis of heterocyclic compounds through multi-component reactions (MCRs)

*Corresponding author. E-mail: diwakar.b@srkrec.edu.in; naallakrishnarao@gmail.com
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has emerged as a powerful tool for constructing molecular complexity in a single operational step with high atom economy, minimal waste, and short reaction times.

Especially 1,3,4-thiadiazoles which have been examined more with respect to its other properties, that have different pharmaceutical properties including antimicrobial agents [15,16], Antioxidant Activity [17], antitumor activity [18], cytotoxic evaluation [19], anti-inflammatory, analgesic [20], antidepressant and anxiolytic activity [21], anti-tubercular agents [22], anti-cholinesterase activity [23], insecticidal [24]. Literature reports show that acid catalyzes a sequential aldol/Knoevenagel condensation, Michael addition, intramolecular nucleophilic cyclization, and final dehydration, efficiently assembling highly substituted thiadiazolo[3,2-a]pyrimidines under mild, green conditions with high selectivity and excellent yields [25-28].

Table 1. Various types of chemical methods for the synthesis of thiadiazolo[3,2-a] pyrimidines.

Method	Reactants	Solvent	Temperature	Time (min)	Yield (%)
Multicomponent Reaction (MCR) with V_2O_5/Fap [9]	2-amino-1,3,4-thiadiazole, aldehyde, active methylene	Absolute ethanol	Room temperature	25-30 min	90-97
Phosphotungstic acid ($H_3PW_{12}O_{40}$) catalysis [10]	Dimedone, thiadiazolo-pyrimidine-dione	Acetonitrile	Reflux	Moderate	70-90
Molecular iodine-catalysed synthesis [11]	2-phenyl-dihydropyrimidine, aldehyde, active methylene	Organic solvent	Mild conditions	Variable	Good to excellent
PPA-Based intramolecular cyclization [12]	Phenacylthio-pyrimidine hydro bromide	PPA medium	~100Â°C	120 min	Good to excellent
Polyphosphate ester (PPE) method [13]	Thiosemicarbazide, carboxylic acid	Below 85Â°C	< 85Â°C	Variable	Good
PCl_5/$POCl_3$ solid-phase synthesis [14]	Thiosemicarbazide, carboxylic acid	PCl_5/$POCl_3$	Room temp to reflux	Variable	91-94

The pyrimidine core scaffold represents a privileged heterocyclic framework amenable to multifaceted chemical modifications that significantly modulate biological activity in Figure 1. Strategic substitutions at pyrimidine positions C-5, C-6, and C-7 have been demonstrated to enhance anticancer efficacy through various mechanistic pathways. Thiadiazole ring modifications, particularly 2-amino and 5-aryl substitutions, confer anticancer activity by targeting cellular proliferation pathways [29-31].

Fusion of additional heterocyclic rings onto the pyrimidine backbone generates diverse polycyclic architectures with enhanced pharmacological profiles. Pyrazole fusion generates tricyclic derivatives exhibiting potent anticancer activity against MCF-7 breast cancer cells with IC_{50} values ranging from 5.69-7.53 μM, demonstrating superior cytotoxicity compared to unmodified analogs. Pyran fusion, particularly when coupled with benzoyl substitution, yields compounds with exceptional anticancer efficacy through enhanced cellular interactions.

Aminoazole and pyridine fusions facilitate superior enzyme inhibition mechanisms, while naphthalene-substituted derivatives incorporating naphoxyalkyl chains exhibit dual functionality through both PARP1 inhibition and topoisomerase targeting. Alkoxy and aryloxy substituents, including chloro and methoxy groups, provide antioxidant activity that synergizes with cytotoxic effects, potentially reducing off-target toxicity.

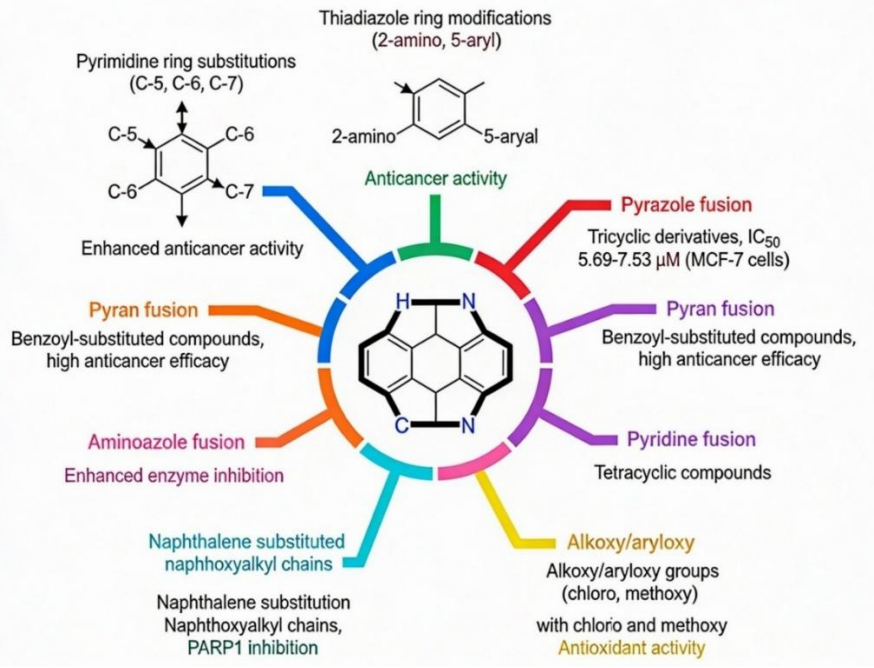


Figure 1. Structural modifications of [1, 3, 4]thiadiazolo [3,2-a]pyrimidines and corresponding biological activities.

These structure activity relationships demonstrate that rational design strategies combining multiple heterocyclic functionalities generate multitarget anticancer agents with enhanced potency and selectivity, establishing a foundation for advanced drug development initiatives in medicinal chemistry.

Nowadays, the conventional method has been highly utilized as a very efficient non-conventional technique to enhance several organic transformations. The main benefits of this method are low reaction time, higher selectivity, higher yields, higher product purity, minimized quantities of by-products, and milder and environmentally friendly reaction conditions. In this context, ethyl 7-methyl-2,5-diphenyl-5H-thiadiazolo[3,2-a]pyrimidine synthesis suffers from prolonged reaction times requiring harsh acidic catalysts (PTSA, p-TsOH). Mechanistic understanding of regioselectivity at C-2/C-5/C-7 positions remains incomplete. Competing geometric/regio-isomers and their thermodynamic/kinetic control lack systematic study. ADME/bioavailability data for the diphenyl-substituted scaffold are insufficient for drug development. Green catalytic platforms (ionic liquids, heterogeneous catalysts) have not been adapted to this specific polycyclic system. The present research work fills the gap and tries to develop an efficient method for the preparation of [1, 3, 4]-thiadiazolo [3, 2-a] pyrimidine-6-carboxylates promoted by the Lewis acid catalyst at 75 ℃. This methodology is a conventional method of procedure that synthesis can be obtained by the thidiazoleamine (1equiv.), ethylacatoacetate, (1equiv.), and aromatic aldehyde (1equiv.) and also evaluated the biological study like antmicrobialcal activity against bacterial as well as fungal strains.

EXPERIMENTAL

The chemicals, solvents, and synthetic grade reagents necessary for the reactions are obtained commercially from Sigma Aldrich and utilized without additional purification. The overall completion of the reactions was identified using TLC (n-hexane: EtOAc-7:3). The melting points of the target compounds were measured using an Agrawal melting point apparatus. The ^{1}H and ^{13}C-NMR spectra of the newly synthesized compounds were recorded on Bruker 400-MHz and 100-MHz spectrometers, employing $CDCl_3$ as the solvent and TMS as the internal standard, respectively.

*Synthesis of [1, 3, 4]-thiadiazolo [3,2-a] pyrimidine-6-carboxylates (**5a-5j**).* A mixture of 5-amino-1,3,4-thiadiazole (1 equiv.), ethyl acetoacetate (1 equiv.), and aromatic aldehyde (1 equiv.) was combined with the Lewis acid catalyst zirconium oxychloride octahydrate ($ZrOCl_2 \cdot 8H_2O$, 25 mmol%). The mixture was slowly dissolved in 30 mL of ethanol in a 50 mL round-bottom flask. Then, it was placed on a magnetic stirrer, and the reaction was heated to 75 °C for 2 hours. The progress of the reaction was monitored by using thin-layer chromatography (TLC; n-hexane: ethyl acetate in a 7:3 ratio). Once the reaction was complete, the crude mixture was carefully poured into 100 mL of ice-cold water and neutralized with a 30 mL solution of sodium bicarbonate. The product was extracted with ethyl acetate, the organic layer was separated, and then the aqueous layer was washed twice with distilled water (2 × 100 mL). Finally, the desired product was obtained after recrystallization from absolute ethanol. The spectroscopic analysis data of the selected compounds were presented in the supporting information file.

*Ethyl7-methyl-2,5-diphenyl-5H-[1,3,4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate (**5a**).* Pale tallow solid, yield 70%; m.p: 181-183 °C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm : 1.148(t, J =7.2 Hz, CH_3), 2.187 (s, CH_3),3.917- 4.016 (q, J=:8.0 Hz,CH_2), 5.128 (s, CH), 7.447 (d, J = 7.9 Hz, 3H, Ar-H),7.573 (t, J = 8.0 Hz, 1H), 7.882 (d, J = 7.7 Hz, 1H), 7.945(d, J = 7.8 Hz, 1H), 8.220 (d, J = 9.4 Hz, 1H), 8.324 (d, J = 10.2 Hz, 2H), 8.450 (s, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 14.32, 22.55, 60.44, 62.74, 102.78, 121.92, 122.85, 124.07, 127.78, 128.19, 129.06, 131.98, 134.75, 142.77, 148.85, 150.48, 156.70, 164.05, 167.66. MS (ESI) m/z: 377.45(M^+H); Molecular formulae: $C_{21}H_{19}N_3O_2S$.

*Ethyl7-methyl-5-(4-nitrophenyl)-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carboxylate (**5b**).* Dark brown solid, Yield-87%; m.p: 197-199 °C; ^{1}H-NMR (400MHz, $CDCl_3$) δ ppm: 1.147 (t, J = 7.2Hz, CH_3), 2.354 (s, CH_3), 3.917-4.028 (q, J=7.6 Hz, CH_2), 5.324 (s, 1H, CH), 7.321-7.645 (m, 5H, Ar-H), 8.148 (d, J= 8.8 Hz,2H, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm: 14.45, 22.56, 61.82, 62.72, 102.17, 123.57, 126.64, 128.04, 128.68, 129.78, 132.04, 147.35, 149.41, 152.86, 155.74, 162.81, 166.79. MS (ESI) m/z: 423.58(M+H); Molecular formulae: $C_{21}H_{18}N_4O_4S$.

*Ethyl7-methyl-5-(3-nitrophenyl)-2-phenyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carboxylate (**5c**).* Pale Yellow solid, Yield-88%; m.p- 187-189 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.156 (t, J=8.0Hz, CH_3), 2.365 (s, CH_3), 3.889-3.957 (q, J= 6.8 Hz, CH_2), 4.892 (s, CH), 7.198-7.557 (m, 5H, Ar-H), 7.784 (d, J=8.9 Hz,1H, Ar-H), 8.077 (d, J=8.8 Hz, 1H, Ar-H), 8.256 (s,1H, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 13.88, 24.06, 59.66, 62.07, 101.79, 120.95, 124.04, 126.44, 127.86, 128.21, 128.78, 129.22, 129.77, 131.07, 133.34, 145.07, 148.53, 152.76, 157.55, 162.71, 167.78. MS (ESI) m/z: 423.29(M+H); Molecular formulae: $C_{21}H_{18}N_4O_4S$.

*Ethyl 5-(4-chlorophenyl)-7-methyl-2-phenyl-5H-[1,3,4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate (**5d**).* Yellow solid, Yield-87%; m.p- 217-219 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.174 (t, J= 7.2 Hz, CH_3), 2.417 (s, CH_3), 3.894-4.047 (q, J= 7.6 Hz, CH_2), 5.124 (s, CH), 7. 184

(s, 1H, Ar-H), 7.427-7.624 (m, 3H, Ar-H), 7.760 (d, J=7.9 Hz, 2H, Ar-H), 7.880 (d, J=8.4 Hz, 1H, Ar-H), 8.074 (d, J=7.6 Hz, 2H, Ar-H).^{13}C-NMR (100MHz, $CDCl_3$) δppm: 13.59, 23.02, 58.05, 62.75, 102.81, 121.77,126.72, 128.10, 128.54, 128.97, 129.36, 129.77, 131.07, 132.46, 154.79, 156.76, 164.72, 167.77. MS (ESI) m/z: 411.38(M^+),413.18(M+2); Molecular formulae: $C_{21}H_{18}ClN_3O_2S$.

*Ethyl5-(3-chlorophenyl)-7-methyl-2-phenyl-5H-[1,3,4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate (**5e**).* Yellow powder, Yield-89 %; m.p. 224-226 ℃; ^{1}H-NMR(400 MHz, $CDCl_3$) δ ppm: 1.054 (t, J=7.6 Hz, CH_3), 2.514 (s, CH_3), 3.954-4.035 (q, J=6.8 Hz, CH_2),5.257 (s, CH), 7.558 (d, J=8.5 Hz, 2H, Ar-H), 7.760(d, J= 8.0 Hz, 2H, Ar-H), 7.857(s,1H), 8.057(d, J= 8.8 Hz, 2H, Ar-H),8.178 (d, J=7.2 Hz, 2H, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$) δppm: 13.45, 23.50, 54.89, 58.54, 62.26, 102.47, 114.40, 126.42, 127.49, 128.79, 129.45, 133.42, 137.72, 146.51, 149.42, 152.52, 154.72, 159.02, 163.71, 167.53 MS (ESI) m/z: 441.59(M^+), 443.50(M+2); Molecular formulae: $C_{21}H_{18}ClN_3O_2S$.

*Ethyl7-methyl-2-(4-nitrophenyl)-5-phenyl-5H-[1,3,4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate (**5f**).* Yellow solid, Yield-82%; m.p: 174-176 ℃; ^{1}H-NMR (400 MHz, $CDCl_3$) *δ* ppm: 1.125 (t, J=8.0Hz, CH_3), 2.148 (s,3H, CH_3),3.981- 4.126 (q, J:6.4 Hz,CH_2), 4.981 (s,1H, CH), 7.051-7.389 (m, 5H, Ar-H), 7.560 (t, J=8.8 Hz, 1H, Ar-H), 7.714 (d, J:7.6 Hz, 1H, Ar-H), 7.845 (d, J=8.4 Hz, 1H, Ar-H), 8.143 (s,1H, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm: 13.51, 23.73, 58.61, 62.59, 103.47, 121.20, 125.45, 127.04, 128.36, 128.67, 130.13, 130.89, 132.08, 142.37, 146.05, 149.09, 154.28, 161.74, and 167.21. MS (ESI) m/z : 423.87(M+H) ; Molecular formulae: $C_{21}H_{18}N_4O_4S$.

*Ethyl5-(3-chlorophenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4] thiadiazolo [3,2-a] pyrimidine-6-carboxylate (**5g**).* Dark yellow solid, Yield- 84%; m.p: 219-221℃; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.154 (t, J=7.2 Hz, CH_3), 2.225 (s, CH_3), 4.021-4.227 (q, J= 7.6 Hz,CH_2), 5.118 (s, CH), 7.342-7.687 (m, 5H, Ar-H), 7.914 (d, J=7.6 Hz, 1H, Ar-H), 8.238 (s, 1H, Ar-H). ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 13.77, 23.06, 58.52, 62.74, 101.98, 121.86, 124.07, 127.64, 128.26, 128.84, 129.38, 132.64, 138.48, 144.09, 147.94, 151.03, 157.83, 161.49, and 166.47. MS (ESI) m/z: 456.08(M^+), 458.37(M+2); Molecular formulae: $C_{21}H_{17}ClN_4O_4S$.

*Ethyl5-(4-chlorophenyl)-7-methyl-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-6-carboxylate (**5h**).* Yellow powder, Yield 85%; m.p.: 195-197 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.321 (t, J=7.2 Hz, CH_3), 2.205 (s, CH_3), 3.957- 4.110 (q, J=7.6 Hz, CH_2), 5.211 (s, CH), 7.238-7.565 (m, 5H, Ar-H), 7.820 (d, J=8.8 Hz, 2H, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm: 12.97, 21.85, 61.42, 63.04, 101.77, 125.71, 127.44, 127.09, 128.14, 129.54, 136.98, 145.47, 147.08, 153.02, 157.52, 161.89, 164.78. MS (ESI) m/z :456.44(M^+), 458.45(M+2); Molecular formulae:C_{21}-$H_{17}ClN_4O_4S$.

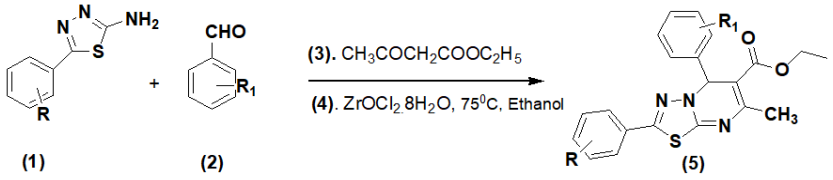
*Ethyl7-methyl-5-(3-nitrophenyl)-2-(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-6-carboxylate (**5i**).* Yellow solid, Yield 69%; m.p: 180-183 ℃. ^{1}H-NMR (400 MHz, CDCl3) δ ppm: 1.184 (t, J= 6.8 Hz, CH3), 2.210(s, CH_3), 3.915-4.023 (q, J =7.6 Hz,CH_2), 4.882 (s,1H, CH), 7.441 (t, J=:8.8 Hz, 1H, Ar-H), 7.604(t, J=8.0 Hz, 1H, Ar-H), 7.754 (d, J=6.8 Hz, 1H, Ar-H),7.891 (d, J= 8.8 Hz, 1H, Ar-H), 8.116 (d, J=7.2 Hz, 1H,Ar-H), 8.210-8.314 (m, 2H, Ar-H), 8.384 (s, 1H, Ar-H).^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 13.54, 21.21, 61.54, 63.44, 101.98, 120.55 122.05, 124.13, 126.74, 128.88, 129.54, 130.35, 131.00, 134.25, 142.57, 146.35, 149.46, 150.81, 155.33, 163.05, 168.14. MS (ESI) m/z: 468.45(M+H); Molecular formulae: $C_{21}H_{17}N_5O_6S$.

*Ethyl7-methyl-2,5-bis(4-nitrophenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-6-carboxylate (**5j**).* Dark Yellow solid, Yield: 86%; m.p.: 186-188 ℃. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.198

(t, J= 6.8 Hz, CH_3), 2.218 (s, CH_3), 3.984-4.021 (q, J=8.0 Hz, CH_2), 5.012 (s,1H, CH), 7.274-7.541 (m, 3H, Ar-H), 7.622 (d, J=7.6 Hz, 1H, Ar-H), 7.857 (d, J=7.8Hz, 2H, Ar-H), 8.123 (d, J=8.0 Hz, 1H, Ar-H), 8.388 (s, 1H, Ar-H). ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm: 14.55, 22.35, 59.44, 62.53, 103.78, 120.71, 124.21, 126.78 128.55, 129.44, 130.66, 132.58, 145.50, 146.61, 149.06, 151.58, 157.19, 164.26, 167.76. MS (ESI) m/z :468.45(M+H); Molecular formulae: $C_{21}H_{17}N_5O_6S$.

RESULTS AND DISCUSSION

In this investigation, a pilot reaction was attempted using substituted thiadiazoloamine (1), various aromatic aldehydes (2), ethyl acetoacetate (3), and a catalyst (4) in the presence of ethanol (Scheme I).



Scheme 1. Synthesis of thiadiazolo [3,2-a]pyrimidines.

The target thiadiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (5a-5j) were synthesized via a three-step multicomponent process. The synthesis began with the preparation of substituted 5-amino-1,3,4-thiadiazoles (1) by reacting appropriate benzoic acids with methanesulfonic acid-activated thiosemicarbazide at room temperature [32]. Next, the substituted 5-amino-1,3,4-thiadiazoles (1) underwent a one-pot multicomponent reaction with different aromatic aldehydes (2) and ethyl acetoacetate (3), using zirconium oxychloride octahydrate ($ZrOCl_2 \cdot 8H_2O$) as a Lewis acid catalyst at 75 °C in ethanol.

To study the reaction conditions, the analogous derivatives (**5a-5j**) were specifically prepared for this search. The optimal reaction conditions, the nature of the catalyst, the amount of catalyst used in this reaction, and the suitability of the solvents were observed as shown in Tables 2, 3, and 4. Catalysts in protic media were used to enhance the reaction, such as $FeCl_3$, $ZnCl_2$, $TiCl_4$, and $ZrOCl_2 \cdot 8H_2O$. Increasing the catalyst loading progressively improved the outcome, and 25 mmol% of $ZrOCl_2 \cdot 8H_2O$ was found to be optimal, since lower loadings gave significantly reduced yields, while no further improvement was observed beyond this amount. The generation of the designed derivatives, as well as reduced reaction time, is considered most significant for the synthesized compound, as shown in Table 2.

Table 2: The effect of the catalyst the preparation of derivative (**5e**)[a].

Entry	Catalyst	Time (min)	Yield (%)[b]
1	$FeCl_3$	120	72
2	$ZnCl_2$	120	63
3	$TiCl_4$	120	68
4	$ZrOCl_2$	80	89

[a] Reaction conditions: substituted 5-amino-1,3,4-thiadiazoles (10 mmol), ethylacatoacetate (10 mmol) with aryl aldehyde (10 mmol) and $ZrOCl_2.8H_2O$ (25 mmol%) in C_2H_5OH (30 mL) were refluxed at 75 °C. [b] Isolated yields.

The catalyst is most responsible for the product yield, which showed high reaction benefits. The Lewis acid catalyst was applied, and the product percentage and the effect of time were measured. As shown in Table 2, $ZrOCl_2.8H_2O$ has the major impact of all the Lewis acid moieties when compared to other $FeCl_3$, $ZnCl_2$, and $TiCl_4$ compositions. The key role of this reaction was the high catalyst loading used (Scheme 1). As reported, 25 mmol% of $ZrOCl_2 \cdot 8H_2O$ is required for an effective catalyst in the reaction to obtain the desired product. It is essential to note that a sufficient amount of catalyst was required to complete the reaction. The reaction could not proceed in the absence of the catalyst, and when 25 mmol% of catalyst was used, the yield of the desired product gradually increased. In the overall reaction, 25 mmol% of catalyst was sufficient to obtain the maximum product yield. Subsequently, exceeding the amount of catalyst applied during the reaction would not yield a saturated product, as shown in Table 3 given below.

Table 3. The effect of amount of catalyst for the synthesis of derivatives (5e)[a].

Entry	Amount of catalyst (mmol%)	Time (min)	Yield (%) [b]
1	10	80	26
2	15	80	56
3	20	80	74
4	25	80	89

[a] Reaction conditions: substituted 5-amino-1,3,4-thiadiazoles (10 mmol), ethylacetoacetate (10 mmol), and aryl aldehyde (10 mmol) in and $ZrOCl_28H_2O$ in C_2H_5OH (30 mL) were refluxed at 75 °C. [b] Isolated yields.

The key point of the parameter in the reaction was the role of various solvents at a constant reaction temperature in scaffolding fruitful compounds (**5a-5j**), and it was recognized that changes in reaction time yield different product yields. This can be attributed to the various solvents, such as polar solvents (C_2H_5OH, CH_3OH) and non-polar solvents (CH_2Cl_2, CH_3CN, $C_6H_5CH_3$). Hence, this is the conventional method of synthesis that yielded the highest yield in the shortest reaction time in ethanol, as shown in Table 4 below.

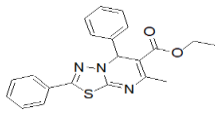
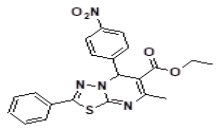
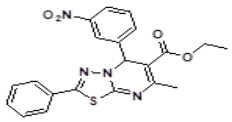
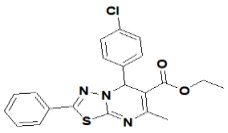
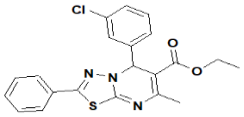
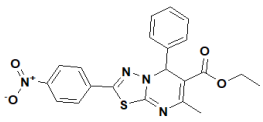
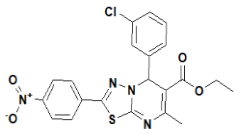
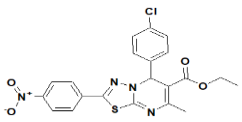
Table 4. Optimization of solvent for the preparation of compound (5e)[a].

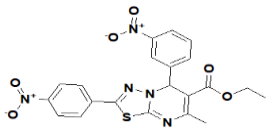
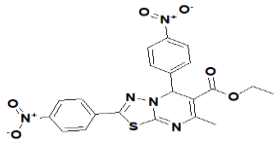
S.NO	Solvent	Time (min)	Yield (%)[b]
1	CH_2Cl_2	80	49
2	$C_6H_5CH_3$	80	68
3	CH_3CN	80	76
4	CH_3OH	80	73
5	C_2H_5OH	80	89

[a] Reaction conditions: substituted 5-amino-1,3,4-thiadiazoles (10 mmol), ethylacatoacetate (10 mmol) with aryl aldehyde (10 mmol) and $ZrOCl_28H_2O$ in C_2H_5OH (30 mL) were refluxed at 75 °C. [b] Isolated yields.

The efficiency of the synthesis depended significantly on the electronic properties of the substituents on both the thiadiazole and aldehyde components. The results are presented in Table 5. The products with electron-withdrawing groups (3-NO_2, 4-NO_2, 3-Cl, 4-Cl) on the aldehyde generally produced higher yields (82-89%) with moderate reaction times (76-83 minutes), as shown by compounds **5e**, **5f**, **5g**, and **5h**. In contrast, the unsubstituted analog (**5a**) and meta-nitro derivative (**5i**) had lower yields (69-70%), likely due to steric hindrance. Melting points ranged from 174 to 226 °C, indicating the thermal stability of the synthesized heterocycles.

Table 5. Details of reaction time and yields of substituted thiadiazolo [3,2-a] pyrimidines.

S.No	Code	Product	Time[a] (min)	Yield (%)[b]	M.P (°C)[c]
1.	**5a**		80	80	181-183
2.	**5b**		73	87	197-199
3.	**5c**		79	88	187-189
4.	**5d**		78	77	217-219
5.	**5e**		80	89	224-226
6.	**5f**		77	82	174-176
7.	**5g**		83	89	219-221
8.	**5h**		76	85	195-197

9.	**5i**		79	69	180-183
10.	**5j**		72	89	224-226

[a] Reaction time monitored by TLC; [b] Isolated yields; [c] uncorrected melting points

The scope and benefits of Lewis acid catalysts, which are most relevant in terms of abundance, low toxicity, lower cost, eco-friendliness, sustainability, and adaptability, have been brought to attention in organic transformations. In organic synthetic chemistry, Lewis acid catalysts have found different advantages and are also normally employed in organic synthesis. Now, the enhancement of the synthesis that is most significant to biology, including nitrogen and sulfur heterocycles.

The structures of the desired derivatives (**5a-5j**) were evidenced by ^{1}H-NMR, ^{13}C-NMR, and mass spectra data. ^{1}H-NMR spectra of the newly prepared compounds showed that different aromatic protons appeared at δ 8.452 to 7.012 ppm. The methoxy protons showed at 3.745, and the methyl protons appeared at 1.524- 1.124 ppm. Mass spectra of the derivatives bearing a halogen atom exhibited a molecular ion peak at (m/z) (M+2).

In order to examine the generality of the method of the process, several diversified aldehydes were used in the present method for the synthesis of thiadiazolo [3,2-a] pyrimidines. The reaction of substituted aromatic aldehyde (1), thidiazoleamine (2), and ethyl acetoacetate (3) with various aromatic aldehydes having electron-withdrawing groups (such as nitro, halide) was carried out in the presence of a catalyst. The yields of the synthesized products were excellent without formation of any by-products such as thiadiazolo [3, 2-a] pyrimidines, which are commonly observed under the influence of Lewis acid catalysts. During the reaction, the aromatic aldehydes containing electron-withdrawing groups reacted at a much faster rate compared with aromatic aldehydes having electron-donating groups.

The method demonstrates excellent tolerance for different functional groups and is simple to operate, making $ZrOCl_2 \cdot 8H_2O$ an effective and economical catalyst for this reaction. This approach offers a scalable way to produce structurally diverse thiadiazolo [3,2-a]pyrimidine compounds that are suitable for further biological evaluation.

Biological assay

Anti-microbial activity

The titled derivatives of newly obtained compounds were evaluated for *in vitro antibacterial activity against* Gram-positive *(*Gr(+Ve) bacteria *Bacillus subtilis, Staphylococcus aureus, and* Gram-negative (Gr(-Ve) bacteria *Escherichia coli, Pseudomonas aeruginosa, and two fungi, Aspergillus Niger, Candida albicans, by using the* disc diffusion procedure at 10 µg/disc. The cultural media used for bacteria were nutrient agar medium and potato-dextrose-agar medium. The reference drugs were used as solutions of Ciprofloxacin and Fluconazole for antibacterial and antifungal drugs, respectively.

We screened in vitro antibacterial potency using nutrient agar medium, and the cup-plate method is a simple way to determine *the* inhibition zone of microorganisms. Hence, this procedure was used for antibacterial examination of the test derivatives. The medium was prepared from nutrient agar 2%, peptone 1%, beef extract 1%, sodium chloride 0.5%, and distilled water, made up to 100 mL. All the ingredients were calculated and poured into water. The solution was heated in a water bath for an appropriate period of time until it became clear. The autoclave was used to sterilize the nutrient medium.

The *in vitro* antifungal activity was evaluated using Sabouraud agar medium, prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in 100 mL of ionized water, with the pH adjusted to 5.7. Normal saline was used to prepare a spore suspension of the fungal strain for lawn inoculation. A loopful of the selected fungal strain was transferred to 3 mL of saline to obtain a uniform suspension. Each Petri dish was filled with agar medium, and the excess suspension was poured onto it, as shown in Table 6.

Table 6. The evaluation of Antimicrobial Activity (**5a**-**5j**) with zone of inhibition in mm.

Entry	Gr(+Ve) bacteria		Gr(-Ve) bacteria		Fungal Strains	
	B subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. Niger*	*C. albicans*
5a	08	08	06	09	05	07
5b	14	16	16	14	15	15
5c	13	15	12	12	17	18
5d	19	20	21	20	12	13
5e	24	24	23	24	17	15
5f	10	12	08	11	20	19
5g	12	14	10	12	17	17
5h	14	10	09	10	18	12
5i	08	10	09	11	20	19
5j	10	12	09	10	21	20
Ciprofloxacin	27	27	30	30		
Fluconazole					25	25

The results in the table above showed that the newly prepared derivatives, such as the analogous derivative **5e**, had better antibacterial activity. The derivatives bearing an electron-withdrawing group exhibited appreciable antifungal activity against fungal strains, and derivative **5j** showed maximum antifungal activity against fungal strains. The remaining derivatives showed moderate to good activity.

CONCLUSION

In summary, the present study provides a straightforward way to prepare thiadiazolo[3,2-a]pyrimidine-6-carboxylate derivatives (**5a**-**5j**) via a $ZrOCl_2 \cdot 8H_2O$-catalyzed one-pot reaction in ethanol. This method is easy to use, works quickly, and gives good to excellent yields with many different aromatic aldehydes. The new heterocycles were identified using spectroscopic techniques and exhibited strong antimicrobial activity, underscoring their value for synthesis and biology. Overall, this approach offers a practical way to quickly create thiadiazolo[3,2-a]pyrimidines that could be useful in future medicinal chemistry work.

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