chemistry proceedings

MDPI

Proceeding Paper

Development and Biological Evaluation of Novel 1,3,4-Thiadiazole Compounds Targeting TNF-α in Cancer Treatment †

Yogita B. Thombare[1], Santosh R. Tambe[1] and Pritam N. Dube [2,*]

[1] MGVs Pharmacy College, Panchavati, Nashik 422003, Maharashtra, India; email1@email.com (S.R.T.)
[2] Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik 422213, Maharashtra, India; email2@email.com (P.N.D.)
* Correspondence: pritamdube@gmail.com
† Presented at the 29th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-29); Available online: https://sciforum.net/event/ecsoc-29.

Abstract

In the pursuit of novel anticancer agents, a new series of 1,3,4-thiadiazole derivatives was designed and synthesized, aiming to inhibit tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine implicated in cancer progression. The synthesis involved the initial condensation of substituted anilines with chloroacetic acid to yield 2-(substituted phenylamino)acetic acids, which were then esterified and converted to hydrazides. Cyclization with carbon disulfide and further functionalization produced oxadiazole, thiadiazole, and triazole intermediates. Final thiadiazole-based derivatives (compounds **7a–7d**) were obtained by alkylation with substituted phenacyl bromides. These compounds were biologically evaluated for anticancer potential with specific focus on TNF-α inhibition, a critical target in inflammatory and tumorigenic signaling pathways. Molecular docking studies suggested strong binding affinities of the synthesized molecules to the TNF-α active site, indicating their possible role in downregulating pro-inflammatory responses associated with tumor development. Biological screening demonstrated promising cytotoxicity profiles in preliminary in vitro cancer models. Structure-activity relationship (SAR) analysis revealed that electron-withdrawing groups (Cl and F) on the thiadiazole scaffold significantly enhanced TNF-α targeting and anticancer activity. These findings support the potential of these thiadiazole derivatives as promising anticancer agents targeting TNF-α.

Keywords: 1,3,4-thiadiazole; TNF-α inhibition; anticancer agents; synthesis; MCF-7

Academic Editor(s): Name

Published: date

Citation: Thombare, Y.B.; Tambe, S.R.; Dube, P.N. Development and Biological Evaluation of Novel 1,3,4-Thiadiazole Compounds Targeting TNF-α in Cancer Treatment. *Chem. Proc.* **2025**, *volume number*, x. https://doi.org/10.3390/xxxxx

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, driven not only by uncontrolled cell proliferation but also by chronic inflammation and dysregulation of signaling pathways [1,2]. Among various inflammatory mediators, tumor necrosis factor-alpha (TNF-α) has been identified as a crucial cytokine involved in cancer progression, angiogenesis, and metastasis [3–5]. Persistent overexpression of TNF-α promotes tumor survival through activation of NF-κB and other downstream oncogenic signals. Hence, inhibition of TNF-α offers a promising therapeutic approach to suppress both inflammatory and tumorigenic processes [6,7].

Heterocyclic scaffolds, particularly 1,3,4-thiadiazoles, have attracted significant attention in medicinal chemistry due to their broad pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer properties [8]. Structural modifications of the thiadiazole nucleus can enhance biological activity and selectivity toward specific molecular targets. In recent years, computational and experimental studies have suggested that thiadiazole derivatives can modulate cytokine signaling, making them attractive candidates for anticancer drug development [9].

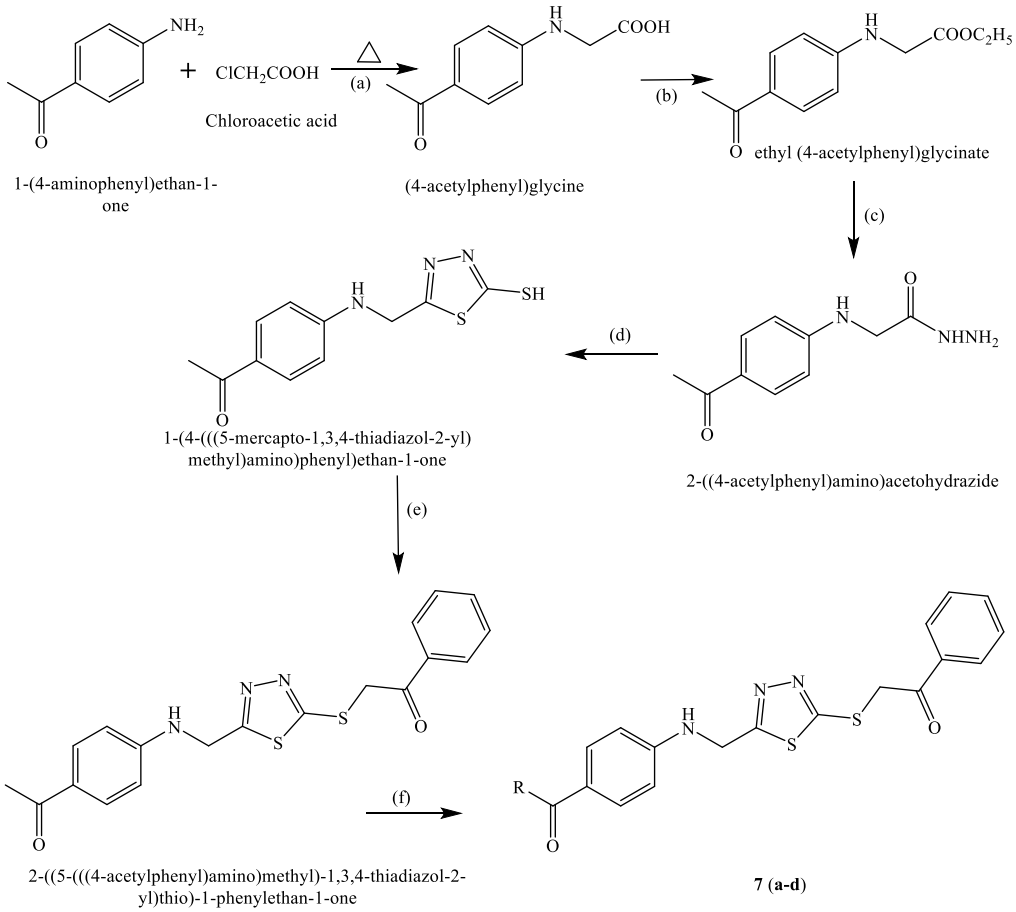
The present work reports the design, synthesis, and biological evaluation of a novel series of 1,3,4-thiadiazole derivatives aimed at targeting TNF-α [10]. The study combines synthetic chemistry, molecular docking, and preliminary biological screening to establish a correlation between structure and activity, thereby providing insights into the development of potent TNF-α inhibitors with anticancer potential.

2. Materials and Methods

2.1. Chemicals and Reagents

All starting materials, including benzaldehyde derivatives, 1-(4-aminophenyl)ethan-1-one, chloroacetic acid, carbon disulfide, and phenacyl bromides, were procured from certified suppliers (Sigma-Aldrich, Merck). Analytical grade solvents were used without further purification. Reaction progress was monitored by thin-layer chromatography (TLC) on silica gel plates, visualized under UV light.

2.2. Scheme for Synthesis of 1,3,4-Thiadiazole Derivatives



Scheme 1. Reagents: (a) Ethanol, 6 h, 90 °C; (b) Conc. Sulphuric acid, Dry ethanol, 8 h, 90 °C; (c) Hydrazine hydrate, Ethanol, 5 h, 85 °C; (d) Carbon disulphide, Conc. SulphuricAcid, 5 h, 85 °C; (e) phenacylbromide, 1 h, 90 °C; (f) Benzaldehyde derivatives (**a**–**d**), ethanol, stirring.

2.3. Anticancer Screening

In Vitro Screening

In vitro testing is performed using SRB assay protocols, each drug is tested at four dose levels (1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M, and 1×10^{-4} M, or 10, 20, 40, and 80 μg/mL). Appropriate positive controls are run in each experiment, and each experiment is repeated thrice. Results are given in terms of GI_{50}, TGI, and LC_{50} values. The compounds were tested for their cytotoxic assay using MCF-7 and MDA-MB-231 cancer cell lines.

2.4. Results and Discussion

Chemistry

The synthetic strategy successfully yielded a new series of thiadiazole derivatives (**7a–7d**) with good to moderate yields (68–85%). The structures of the synthesized compounds were confirmed by spectral data, including IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The melting points of all compounds were recorded and are uncorrected. The presence of electron-withdrawing substituents (Cl, F) on the phenyl ring enhanced the stability and polarity of the molecules, thereby contributing to favorable interactions with potential biological targets.

2.5. Procedure for Preparation of (4-Acetylphenyl)glycine

1-(4-Aminophenyl)ethan-1-one (1.34 g, 10 mmol) was dissolved in 20 mL ethanol–water (1:1), and chloroacetic acid (1.00 g, 12 mmol) was added followed by sodium carbonate (1.27 g, 12 mmol). The mixture was refluxed for 5 h, cooled, and acidified with dilute HCl to pH 2–3. The precipitated solid was filtered, washed with cold water, recrystallized from ethanol–water, and dried to obtain (4-acetylphenyl)glycine.

2.6. Procedure for Preparation of Derivatives of 2-((5-(((4-Benzoylphenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one (7)

These obtained 2-((5-(((4-acetylphenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one compound (0.005 mole) were treated with equimolar quantity of p-substituted bezaldehyde (0.005 mole in the presence of ethanol (50 mL). Stirred for 24 h on magnestic stirrer to give the different derivatives of 1,3,4-thiadiazole respectively.

2-((5-(((4-benzoylphenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one (**7a**): Chemical Formula: $C_{24}H_{19}N_3O_2S_2$, Yield 70%; M.p. 244–246; IR (KBr,υ,cm^{-1}): 3375.92 (NH); 2992.12 (aromatic C-H); 1722.85 (C=O); 747.60 (C-Cl); ^{1}H NMR (DMSO-d_6, δppm): 7.91 (s, 1H, Ar-H); 7.71–7.40 (m, 6H, Ar-H); 6.84 (s, 1H, Ar-H); 4.16 (s, 1H, NH); 3.59 (d, 2H, -S-CH_2-CO); 2.44 (d, 2H, CH_2).

2-((5-(((4-(4-chlorobenzoyl)phenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one (**7b**): Chemical Formula: $C_{24}H_{18}ClN_3O_2S_2$, Yield 62%; M.p. 228–229 °C; IR (KBr, υ, cm^{-1}): 3348.94 (NH); 3037.57 (aromatic C-H); 1738.68 (C=O); 714.27 (C-Cl); ^{1}HNMR (DMSO-d_6, δppm): 7.91–7.40 (m,7H, Ar-H); 4.16 (s, 1H, NH); 3.54 (s, 2H, -S-CH_2-CO); 2.07 (d, 2H, CH_2); MS (ESI-QqTOF) m/z: 395.0 $[M+H]^+$.

2-((5-(((4-(4-fluorobenzoyl)phenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one (**7c**): Chemical Formula: $C_{24}H_{18}FN_3O_2S_2$, Yield 45%; M.p. 240–241 °C; IR (KBr, υ, cm^{-1}): 3375.53 (NH); 3048.22 (aromatic C-H); 1723.23 (C=O); 725.44 (C-F); ^{1}HNMR (DMSO-d_6, δppm): 8.61 (d, 2H, Ar-H); 7.77–7.52 (m, 4H, Ar-H); 3.99 (s, 1H, NH); 3.01 (s, 2H, S-CH_2-CO); 2.62 (d, 2H, CH_2); MS (ESI-QqTOF) m/z: 478.1 $[M+H]^+$.

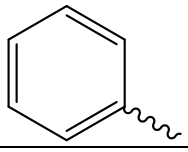
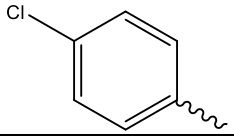
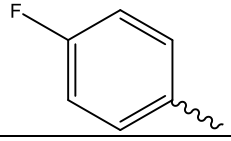
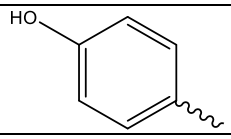
2-((5-(((4-(4-hydroxybenzoyl)phenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)thio)-1-phenylethan-1-one (**7d**): Chemical Formula: $C_{24}H_{19}N_3O_3S_2$, Yield 54%; M.p. 240–241 °C; IR (KBr, υ, cm^{-1}): 3348.94 (NH); 3214.44 (O-H); 3037.57 (aromatic C-H); 1738.68 (C=O); 717.14 (C-F); ^{1}HNMR (DMSO-d_6, δppm): 7.83–7.02 (m, 4H, Ar-H); 6.60 (d, 2H, Ar-H); 4.09 (s, 1H,

NH); 3.83 (s, 2H, -S-CH_2-CO); 2.41(d, 2H, CH_2); 2.02 (s, 1H, CH_3); MS (ESI-QqTOF) m/z: 413.01 $[M+H]^+$.

2.6.1. Biological Activity

In vitro cytotoxicity assays demonstrated that the thiadiazole derivatives exhibited moderate to strong anticancer activity, with LC_{50} values ranging between 12–30 μg/mL. Compounds containing chloro- and fluoro-substituents (**7b** and **7c**) displayed the highest cytotoxicity, correlating well with docking predictions. These findings indicate that electron-withdrawing groups significantly enhance TNF-*α* binding and anticancer effects.

Table 1. In-vitro anticancer activity (μg/mL) of synthesized compounds.

Tested Compounds	R	MCF-7			MDA-MB-231		
		LC_{50} [a]	TGI [b]	GI_{50} [c]	LC_{50} [a]	TGI [b]	GI_{50} [c]
Control	-	94.8	91.1	88.6	>100	72.4	86.4
7a		65.4	44.8	<10	51.1	21.9	54.8
7b		**12.6**	**13.8**	<10	34.4	24.6	<0.1
7c		52.7	14.4	<10	>80	53.6	<0.1
7d		28.8	14.2	<10	68.2	36.4	32.2
TAM	-	26.4	10.2	<10	60.4	35.8	<0.1

Most potent compounds shown by bold text as compare to standard TAM (tamoxifen). [a] Compound concentration that produces 50% cytocidal effect. [b] Compound concentration that produces total growth inhibition. [c] Compound concentration that produces 50% growth inhibition.

2.6.2. Structure-Activity Relationship (SAR)

The structure–activity relationship (SAR) studies revealed that electron-withdrawing substituents enhanced TNF-*α* inhibition, while lipophilic substituents improved cell membrane permeability, thereby facilitating better biological activity. Furthermore, substitutions at the para-position on the phenyl ring were found to favor stronger activity compared to other positions. Overall, these findings validate the design strategy and emphasize the potential of thiadiazole derivatives as promising leads for TNF-*α*-targeted anticancer therapy.

3. Conclusions

The present study reports the successful synthesis and biological evaluation of novel 1,3,4-thiadiazole derivatives targeting TNF-*α*. Molecular docking studies confirmed strong binding affinities, while in vitro cytotoxicity assays demonstrated promising anticancer potential, particularly for derivatives bearing electron-withdrawing substituents. These findings suggest that structural modifications of the thiadiazole scaffold can yield potent TNF-*α* inhibitors, supporting further optimization and preclinical evaluation.

Author Contributions:

Funding:

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Conflicts of Interest:

References

1. Aggarwal, B.B.; Gupta, S.C.; Kim, J.H. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. *Blood* **2012**, *119*, 651–665.
2. Sahu, N.K.; Balbhadra, S.S.; Choudhary, J.; Kohli, D.V. Exploring pharmacological significance of chalcone scaffold: A review. *Curr. Med. Chem.* **2012**, *19*, 209–225.
3. Kumar, D.; Bhat, Z.A.; Kumar, V. 1,3,4-Thiadiazole: A biologically active scaffold. *Eur. J. Med. Chem.* **2013**, *63*, 805–833.
4. Trott, O.; Olson, A.J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function. *J. Comput. Chem.* **2010**, *31*, 455–461.
5. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674.
6. Indelicato, S.; Bongiorno, D.; Mauro, M.; Cascioferro, S. Recent Developments of 1,3,4-Thiadiazole Compounds as Anticancer Agents. *Pharmaceuticals* **2025**, *18*, 580. https://doi.org/10.3390/ph18040580.
7. Janowska, S.; Paneth, A.; Wujec, M. Cytotoxic Properties of 1,3,4-Thiadiazole Derivatives—A Review. *Molecules* **2020**, *25*, 4309. https://doi.org/10.3390/molecules25184309.
8. Kumar, D.; Kumar, H.; Kumar, V.; Deep, A.; Sharma, A.; Marwaha, M.G.; Marwaha, R.K. Mechanism-Based Approaches of 1,3,4 Thiadiazole Scaffolds as Potent Enzyme Inhibitors for Cytotoxicity and Antiviral Activity. *Med. Drug Discov.* **2023**, *17*, 100150. https://doi.org/10.1016/j.medidd.2022.100150.
9. El-Masry, R.M.; Essa, B.M.; Selim, A.A.; El-Emam, S.Z.; Mohamed, K.O.; Sakr, T.M.; Kadry, H.H.; Taher, A.T.; Abou-Seri, S.M. New 5-Aryl-1,3,4-Thiadiazole-Based Anticancer Agents: Design, Synthesis, In Vitro Biological Evaluation and In Vivo Radioactive Tracing Studies. *Pharmaceuticals* **2022**, *15*, 1476. https://doi.org/10.3390/ph15121476.
10. Abouzied, A.S.; Al-Humaidi, J.Y.; Bazaid, A.S.; Qanash, H.; Binsaleh, N.K.; Alamri, A.; Ibrahim, S.M.; Gomha, S.M. Synthesis, Molecular Docking Study, and Cytotoxicity Evaluation of Some Novel 1,3,4-Thiadiazole as Well as 1,3-Thiazole Derivatives Bearing a Pyridine Moiety. *Molecules* **2022**, 27, 6368. https://doi.org/10.3390/molecules27196368.
11. Alsehli, M.; Aljuhani, A.; Ihmaid, S.K.; El-Messery, S.M.; Othman, D.I.A.; El-Sayed, A.-A.A.A.; Ahmed, H.E.A.; Rezki, N.; Aouad, M.R. Design and Synthesis of Benzene Homologues Tethered with 1,2,4-Triazole and 1,3,4-Thiadiazole Motifs Revealing Dual MCF-7/HepG2 Cytotoxic Activity with Prominent Selectivity via Histone Demethylase LSD1 Inhibitory Effect. *Int. J. Mol. Sci.* **2022**, *23*, 8796. https://doi.org/10.3390/ijms23158796.
12. Serag, M.I.; Tawfik, S.S.; Eisa, H.M.; Badr, S.M.I. Design, Synthesis, Biological Evaluation and Molecular Docking Study of New 1,3,4-Thiadiazole-Based Compounds as EGFR Inhibitors. *Drug Dev. Res.* **2025**, *86*, e70035. https://doi.org/10.1002/ddr.70035.