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Synthesis, Characterization, and Anti-Inflammatory Evaluation of 1-(2-(1-Benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-substituted-1,3,4-oxadiazol-3(2H)-yl)ethanone Derivatives: In Vitro and In Silico Studies

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Abstract

The study focuses on the synthesis and evaluation of 1-(2-(1-benzoyl-3-(thiophen-2-yl)- 1H-pyrazol-4-yl)-5-substituted-1,3,4-oxadiazol-3(2H)-yl)ethanone derivatives (8a-g) for their anti-inflammatory properties and drug-like behavior. Spectral studies confirmed the structural integrity of the compounds through characteristic IR, 1H NMR, and 13C NMR signals. The HRBC membrane stabilization assay revealed significant anti-inflammatory activity, particularly for compounds 8g (IC50 107.54 ± 0.06 µg/ml) and 8f (IC50 145.75 ± 0.05 µg/ml), comparable to the standard drug Diclofenac Sodium (DFS). Docking studies showed strong binding affinities to COX-1 and COX-2 enzymes, with compound 8f displaying the best binding affinity for COX-1 (-8.5) and compound 8e for COX-2 (-7.4). ADME analysis indicated that all compounds adhered to Lipinski's Rule of Five, with favorable pharmacokinetic parameters, including appropriate lipophilicity, water solubility, and cell permeability. These findings suggest that the synthesized 1,3,4-oxadiazole derivatives exhibit potential as effective anti-inflammatory agents with suitable drug-like properties, warranting further development and clinical exploration.

1.1 Introduction

1,3,4-Oxadiazole derivatives have emerged as a vital class of heterocyclic compounds in medicinal chemistry due to their diverse pharmacological properties. Among their various biological activities, the anti-inflammatory potential of these compounds stands out prominently [1]. The 1,3,4-oxadiazole core is a five-membered ring containing three heteroatoms, which imparts unique electronic properties and facilitates interactions with various biological targets [2]. These derivatives exert their anti-inflammatory effects by inhibiting key enzymes and pathways involved in the inflammatory response, such as cyclooxygenase (COX) and

lipoxygenase (LOX) pathways [3, 4]. Docking studies have demonstrated the potential of 1,3,4 -oxadiazole derivatives to bind effectively to these enzyme active sites, indicating their role as promising anti-inflammatory agents [5, 6]. Additionally, these compounds have been shown to modulate the production of pro-inflammatory cytokines, further contributing to their anti-inflammatory efficacy [7].

The ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of 1,3,4-oxadiazole derivatives have been favorable, indicating good bioavailability, metabolic stability, and low toxicity, which are crucial for drug development [8, 9]. For instance, studies have shown that certain 1,3,4-oxadiazole derivatives possess high permeability and low risk of metabolic interactions, making them suitable candidates for further development [10]. The combination of computational docking studies and experimental validation underscores the therapeutic potential of 1,3,4-oxadiazole derivatives in treating inflammatory diseases [11]. Detailed evaluations have highlighted their capability to interact with various inflammatory targets, providing a robust foundation for future research and development in this area [12, 13].

Further investigations into the structure-activity relationships of these compounds have revealed that specific substitutions on the oxadiazole ring can significantly enhance anti-inflammatory activity [14]. This insight has led to the design and synthesis of novel 1,3,4-oxadiazole derivatives with improved efficacy and safety profiles [15]. Overall, the promising results from various studies suggest that 1,3,4-oxadiazole derivatives hold great potential as therapeutic agents for managing inflammatory conditions.

1.2 Materials & Methods

1.2.1 Chemistry

Substrates, reagents, and solvents were bought from Merck and used for purposes of synthesis, purification, and analysis. The progress of the reaction was tracked by thin layer chromatographic plates (Merck) and analyzed by visualizing under Iodine chamber and melting points were determined in open capillary tubes using digital MP490 melting point system. The spectra of infrared (IR) were recorded on FT-IR, and the corresponding maximum absorption of Shimadzu-8400S was stated in wave numbers (νmax, cm^{-1}). The spectra of 1H NMR were

measured in DMSO on the Bruker 400MHz instrument and their corresponding chemical shift values were stated in parts per million (ppm) at the δ scale.

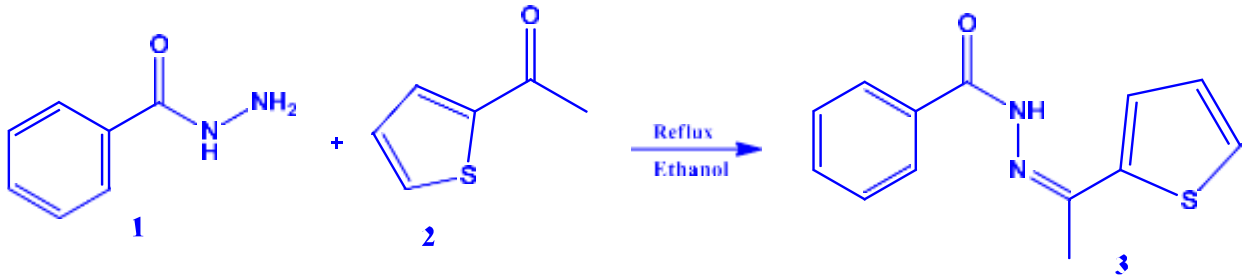
1.2.2 Synthesis of ZnO-TiS nanomaterial

Preparation and characterization of ZnO-TiS nanocomposite material were followed by using Manivarman et al. i.e Na_2S was dissolved in 100 mL of deionized water. Under vigorous stirring Tetra isopropyl orthotitanate solution was added into the TiS solution at room temperature respectively. The pH solution was adjusted to about 9 by addition of ammonia solution for the complete precipitation of TiS. Then the precipitate was collected and dried in an oven at 100 0C for 12 h. The TiS suspension was added to 100 Ml deionized water then Zinc nitrate hexahydrate solution and stirred for 3 hrs and conc. Nitric acid and 5mL deionized water was added then solution was taken in sonication bath 30 min and make certain complete precipitate was formed respectively the precipitate was filtered and washed with distilled water and ethanol until alkali phases were removed from precipitation. Then the precipitate was collected and dried in an oven at 100 ^{0}C for 12 h. The resulting powder was finally calcined at 500 0C for 3 h. SEM images shows that ZnO-TiS nanocomposite material have clear that has uniform flakes morphology structure and this size of particle agglomerated due to calcinations and ventilation process of nanomaterial. EDX analysis was confirming the distribution Zn, Ti, S and O in the surface of nanocomposite material**.** TEM image particle posses mostly spherical structure otherwise round edges for no sign may be crystal defects was Obtained. ZnO-TiS nanocomposite material shows increased absorption both UV and visible region which is that of ZnO-TiS material can be used as a catalytic nanocomposite material.

As expected, the catalytic system is influenced by various reaction parameters, such as amount of the catalyst employed, effect of catalyst and solvent system. 1-benzoyl-3-(thiophen-2-yl)-2,3-dihydro-1H-pyrazole-4-carbaldehyde and thiophene-2-carbohydrazide in ethanol were selected as model substrates for carrying out the optimization studies for synthesis of hydrazone.

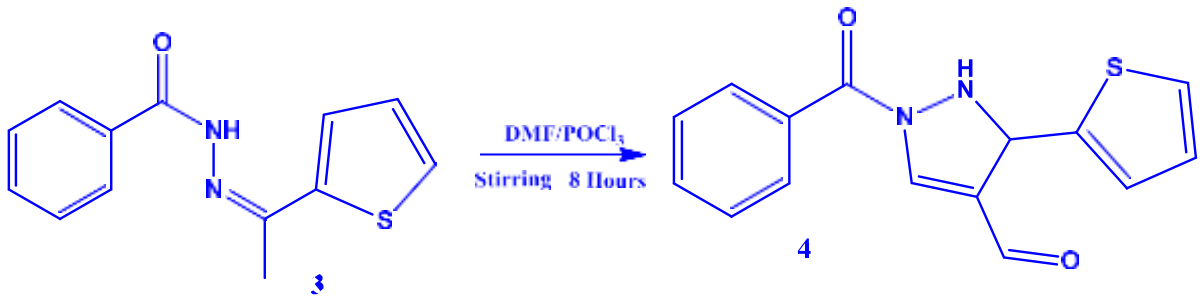
1.2.3 General procedure for the synthesis (Z)-N'-(1-(thiophen-2-yl)ethylidene)benzohydrazide

The ethanolic solution of benzohydrazide (0.01 mol) and 5 -acetylthiophen-3-ylium (0.01 mol) was refluxed for 2-3 h. The precipitates obtained were filtered off, washed, and recrystallized from ethanol[16].



1.2.4 **General procedure for the synthesis of 1-benzoyl-3-(thiophen-2-yl)-2,3-dihydro-1H-pyrazole-4-carbaldehyde**

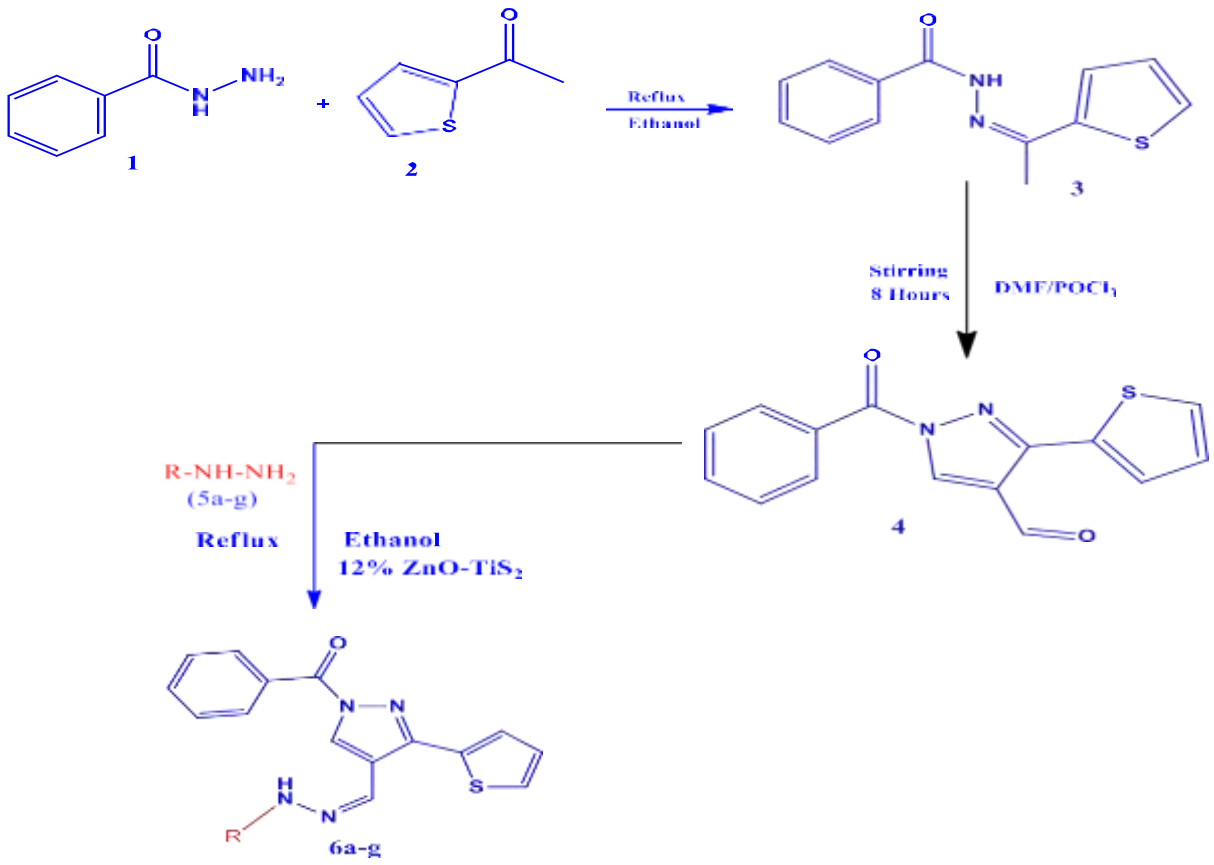
To the Vilsmeier-Haack reagent preparedfrom DMF (10 mL) and $POCl_3$ (1.1 mL), (Z)-N'-(1-(thiophen-2-yl)ethylidene)benzohydrazide (0.9 g, 3 mmol) was added, the reaction mixture was stirred at 60-65 ºC for 2.5 h and then it was poured into ice cold water. The solid that separated on neutralization $NaHCO_3$ was filtered, washed with water and was purified by column chromatography



1.2.5 Synthesis of (Z)-N'-((1-benzoyl-3-(thiophen-2-yl)-2,3-dihydro-1H-pyrazol-4-yl)methylene)substituted hydrazide

A solution of 1-benzoyl-3-(thiophen-2-yl)-2,3-dihydro-1H-pyrazole-4-carbaldehyde (1mmol) and substituted hydrazide (1mmol), and 10 ml of ethanol in 12% ZnO-TiS_2 nanocatalyst were refluxed occasionally for 1.5 hour. After the completion of the reaction, the

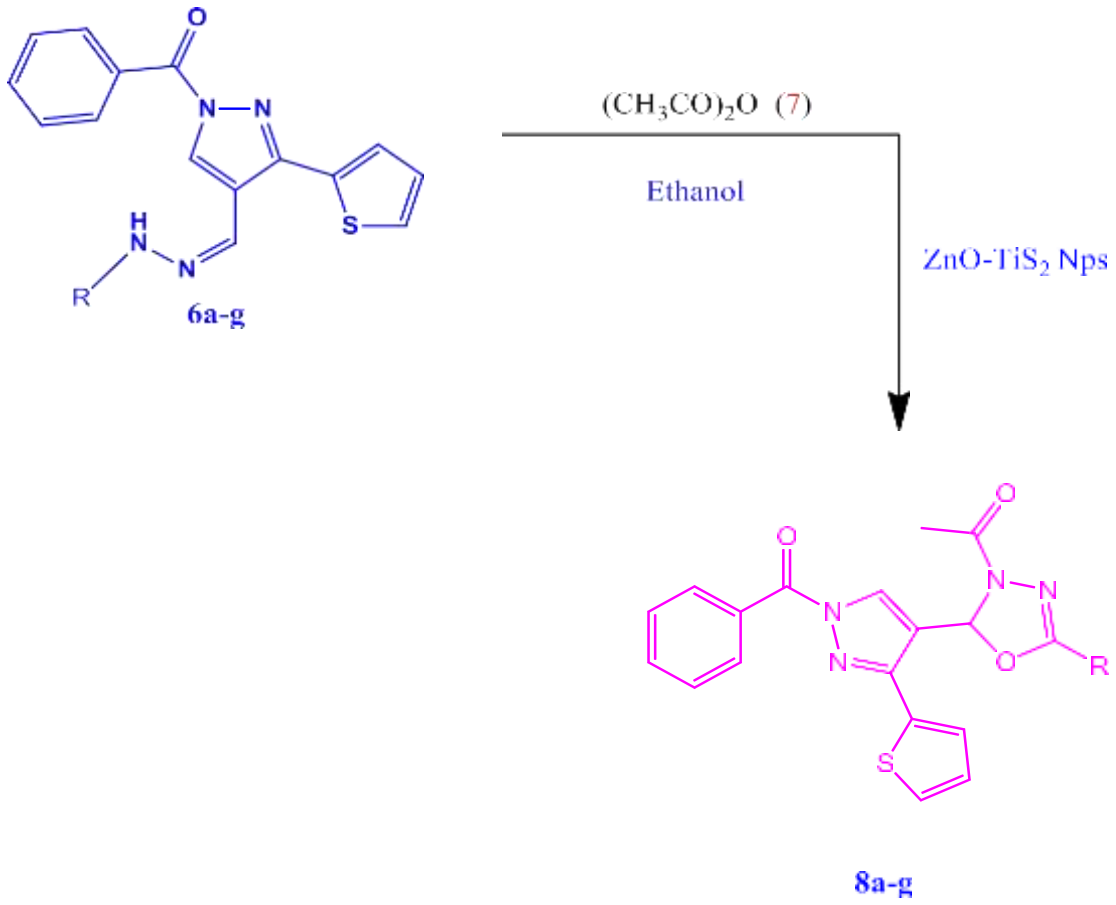
mixture was cooled at room temperature. The resulting precipitate was filtered and washed with cold water. The product appeared as pale yellow solid. Then this was recrystallised using ethanol to obtain pale yellow glittering solid.



1.2.6 Synthesis of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-substituted-1,3,4-oxadiazol-3(2H)-yl)ethanone

To synthesize 1,3,4-oxadiazole from acetic anhydride and hydrazone, first dissolve the hydrazone in ethanol and add ZnO-TiS nanoparticles as a catalyst. Slowly add acetic anhydride to the mixture while stirring continuously. Heat the reaction mixture to reflux for 5 hours, monitoring the reaction progress. After completion, cool the reaction mixture to room temperature and filter to remove the catalyst. Wash the catalyst with dichloromethane (DCM)

and combine the filtrate and washings. Dry the combined organic layers over anhydrous sodium sulfate and filter. Evaporate the solvent under reduced pressure to obtain the crude product. Purify the product by recrystallization from methanol to obtain pure 1,3,4-oxadiazole[17].



1.2.7 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-phenyl-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{24}H_{18}N_4O_3S$; M.p : 120^0C; M.wt: 442; Colour: yellowish white; Yield : 75%; IR (KBr), v max: 1626 [C=O], 1597 [C=N], 1163 [C-O-C], 2929 [C-H, Aliphatic], 3070 [C-H, Aromatic]; 1H NMR (CDCl3, 400 MHz, ppm): δ 2.56 (s, 1H, -COCH3 [Anhydride]), 6.72 (s, 1H, CH (oxadiazole)), 6.82 – 8.11 (m, 1 H [Aromatic]) ; 13C NMR (100 MHz, CDCl3, ppm): δ 175.3 (C=O)[hydrazide], 187.9 (C=O)[anhydride] 89.26 (CH)[Oxadiazole], 118.9 – 147.6 (Aromatic carbons).

1.2.8 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(thiophen-2-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{22}H_{16}N_4O_3S_2$; M.p : 120^0C; M.wt: 448; Colour: yellowish white; Yield : 60%; IR (KBr), v max: 1687 [C=O], 1649 [C=N], 1180 [C-O-C], 2926 [C-H, Aliphatic], 3066 [C-H, Aromatic]; 1H NMR (CDCl3, 400 MHz, ppm): δ 2.99 (s, 1H, -COCH3 [Anhydride]), 6.84 (s, 1H, CH (oxadiazole)), 6.88 – 8.69 (m, 1 H [Aromatic]) ; 13C NMR (100 MHz, CDCl3, ppm): δ 167.2 (C=O)[hydrazide], 168.6 (C=O)[anhydride] 89.07 (CH)[Oxadiazole], 121.8 – 145.5 (Aromatic carbons).

1.2.9 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{25}H_{20}N_4O_3S$; M.p : 120^0C; M.wt: 456; Colour: yellowish white; Yield : 75%; IR (KBr), v max: 1687 [C=O], 1649[C=N], 1236 [C-O-C], 2926 [C-H, Aliphatic], 3068 [C-H, Aromatic]; 1H NMR (CDCl3, 400 MHz, ppm): δ 2.38 (s, 1H, -COCH3 [Anhydride]), 6.83 (s, 1H, CH (oxadiazole)), 6.85 – 8.63 (m, 1 H [Aromatic]) ; 13C NMR (100 MHz, CDCl3, ppm): δ 165.8 (C=O)[hydrazide], 172.1 (C=O)[anhydride] 88.2 (CH)[Oxadiazole], 122.6 – 143.7 (Aromatic carbons).

1.2.10 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(4-bromophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{24}H_{17}BrN_4O_3S$; M.p : 120^0C; M.wt: 521; Colour: Pale yellow; Yield : 70%; IR (KBr), v max: 1687 [C=O], 1649 [C=N], 1230 [C-O-C], 2989 [C-H, Aliphatic], 3066 [C-H, Aromatic]; 1H NMR (CDCl3, 400 MHz, ppm): δ 2.92 (s, 1H, -COCH3 [Anhydride]), 6.85 (s, 1H, CH (oxadiazole)), 6.86 – 8.72 (m, 1 H [Aromatic]) ; 13C NMR (100 MHz, CDCl3, ppm): δ 175.6 (C=O)[hydrazide], 189.5 (C=O)[anhydride] 89.5 (CH)[Oxadiazole], 119.1 – 147.9 (Aromatic carbons).

1.2.11 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{23}H_{17}N_5O_3S$; M.p : 120^0C; M.wt: 443; Colour: dirty white; Yield : 75%; IR (KBr), v max: 1674 [C=O], 1627 [C=N], 1190 [C-O-C], 2922 [C-H, Aliphatic], 3059 [C-H, Aromatic];

1H NMR (CDCl3, 400 MHz, ppm): δ 2.98 (s, 1H, -COCH3 [Anhydride]), 6.83 (s, 1H, CH (oxadiazole)), 6.85 – 8.66 (m, 1 H [Aromatic]) ; 13C NMR (100 MHz, $CDCl_3$, ppm): δ 154.8 (C=O)[hydrazide], 174.8 (C=O)[anhydride] 89.6 (CH)[Oxadiazole], 118.8 – 144.7 (Aromatic carbons).

1.2.12 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{23}H_{17}N_5O_3S$; M.p : 120^0C; M.wt: 443; Colour: Pale yellow; Yield : 65%; IR (KBr), v max: 1687 [C=O], 1591 [C=N], 1182 [C-O-C], 2920 [C-H, Aliphatic], 3066 [C-H, Aromatic]; 1H NMR ($CDCl_3$, 400 MHz, ppm): δ 2.29 (s, 1H, -$COCH_3$ [Anhydride]), 7.19 (s, 1H, CH (oxadiazole)), 7.26 – 8.38 (m, 1 H [Aromatic]) ; ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 161.0 (C=O)[hydrazide], 175.2 (C=O)[anhydride] 85.3 (CH)[Oxadiazole], 115.5 – 146.7 (Aromatic carbons).

1.2.13 Spectral studies of 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-(furan-2-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone

M.F: $C_{22}H_{16}N_4O_4S$; M.p : 120^0C; M.wt: 432; Colour: Pale yellow; Yield : 65%; IR (KBr), v max:1691 [C=O], 1622 [C=N], 1207 [C-O-C], 2937 [C-H, Aliphatic], 3068 [C-H, Aromatic]; 1H NMR ($CDCl_3$, 400 MHz, ppm): δ 2.99 (s, 1H, -$COCH_3$ [Anhydride]), 6.83 (s, 1H, CH (oxadiazole)), 6.85 – 8.31 (m, 1 H [Aromatic]) ; ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 169.1 (C=O)[hydrazide], 174.8 (C=O)[anhydride] 88.8 (CH)[Oxadiazole], 118.8 – 144.7 (Aromatic carbons).

1.3 Results and discussion

The study demonstrated the impact of different solvents, catalysts, catalytic loadings, and the recyclability of ZnO-TiS nanoparticles on the synthesis of 1,3,4-oxadiazole derivatives. Initially, various organic solvents were tested to optimize the reaction conditions using ZnO-TiS nanoparticles as a catalyst. The findings indicated that polar solvents outperformed non-polar ones, with ethanol providing the best results (Table 1), yielding 75% of the desired product within 5 hours. In contrast, non-polar solvents like DCM only produced trace amounts.

Table 1. The model reaction in different solvents			
Entry	Solvent	Time (h)	Yield (%)
1	Methanol	6	64
2	Ethanol	5	75
3	Acetonitrile	10	50
4	$CHCl_3$	6	55
5	DCM	5	Trace

This emphasized the importance of solvent polarity in enhancing reaction efficiency. Subsequent experiments compared the efficacy of ZnO-TiS nanoparticles with other metal oxide catalysts. Without any catalyst, the reaction yielded 60% of the product in 8 hours. When using bare ZnO, commercial ZnO, bulk ZnO, and bare TiS2 nanoparticles, the yield remained moderate (60-65%)

with a longer reaction time of 3 hours. However, ZnO-TiS2 nanoparticles significantly reduced the reaction time to 1.5 hours and increased the yield to 75%, highlighting their superior catalytic performance (Table 2). The effect of catalytic loading was also examined, revealing that 10 mol% of ZnO-TiS nanoparticles was optimal. Lower catalyst loadings (3-7 mol%) resulted in longer reaction times and reduced yields, while increasing the catalyst load beyond 10 mol% did not significantly enhance the reaction rate or yield, likely due to catalyst coagulation reducing the effective surface area(Table 3). Therefore, maintaining an appropriate catalyst concentration is crucial for maximizing efficiency

. **Table 2**. Effect of various catalysts in the model reaction			
Entry	Catalyst	Time (h)	Yield (%)
1	Nil catalyst	8	60
2	Bare ZnO	3	60
3	Commercial ZnO	3	65
4	Bulk ZnO	3	60
5	Bare TiS_2	3	60
6	ZnO-TiS_2	1.5	75

Lastly, the recyclability of the ZnO-TiS catalyst was tested over five cycles. The catalyst was regenerated through a straightforward process involving filtration, washing with

dichloromethane and methanol, and drying. The results showed consistent catalytic activity, with yields remaining at 75% for the first three cycles and slightly decreasing to 72% in the fourth and fifth cycles(Table 4).

Table 3. Effect of quantity of ZnO- TiS NPs on the model reaction

Entry	Amount (mol %)	Time (h)	Yield (%)
1	3	6	60
2	5	6	60
3	7	6	60
4	10	5	75
5	12	5	70

This demonstrates the practical applicability and economic advantage of using ZnO-TiS nanoparticles, as they can be effectively reused multiple times without significant loss of activity.

Table 4. Recycled of ZnO- TiS in the synthesis of 1,3,4-oxadiazole derivatives

Catalyst	**Runs**				
	1	**2**	**3**	**4**	**5**
Product (%)	75	75	75	72	72

Overall, these findings underscore the potential of ZnO-TiS nanoparticles as a highly efficient and reusable catalyst for the synthesis of 1,3,4-oxadiazole derivatives.

The spectral studies of various 1-(2-(1-benzoyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)-5-substituted-1,3,4-oxadiazol-3(2H)-yl)ethanone derivatives provide valuable insights into their structural and electronic properties. The IR spectra of these compounds consistently show characteristic absorption bands, such as the C=O stretching at around 1626-1691 cm^{-1}, indicating the presence of carbonyl groups. The C=N stretching vibrations appear in the range of 1591 - 1649 cm^{-1}, confirming the oxadiazole ring's presence. Additionally, the C-O-C stretching bands observed between 1163-1236 cm^{-1} further support the formation of the oxadiazole ring. The aliphatic and aromatic C-H stretching bands are also evident, typically appearing at around 2920-2989 cm^{-1} and 3059-3070 cm^{-1}, respectively.

The 1H NMR spectra reveal significant signals corresponding to different protons in the molecule. The singlet signals around δ 2.29-2.99 ppm are attributed to the -COCH3 (anhydride) group, while the singlet near δ 6.72-7.19 ppm corresponds to the CH proton of the oxadiazole ring. The aromatic protons exhibit multiplet signals between δ 6.82-8.72 ppm, indicative of the complex aromatic environment within the molecules. The 13C NMR spectra provide further confirmation of the structural features, with carbonyl carbon signals observed at δ 154.8-189.5 ppm, corresponding to the hydrazide and anhydride groups. The oxadiazole CH carbon appears at around δ 85.3-89.6 ppm, while the aromatic carbons are spread across δ 115.5-147.9 ppm. Overall, these spectral studies confirm the successful synthesis of the targeted 1,3,4-oxadiazole derivatives, demonstrating their structural integrity and consistency. The characteristic IR, 1H NMR, and 13C NMR signals align well with the expected functional groups and molecular framework, validating the chemical structures of these novel compounds. The substitution on the oxadiazole ring influences the spectral features slightly, reflecting the electronic and steric effects of different substituents, thereby contributing to the understanding of their potential pharmacological properties[18].

The IC50 values of compounds 8a-g, calculated using the HRBC (Human Red Blood Cell) membrane stabilization method, provide a clear indication of their anti-inflammatory potential. The HRBC membrane stabilization assay is a well-established method to evaluate the anti-inflammatory activity of compounds by assessing their ability to prevent lysis of red blood cells. The results show that compound 8g exhibited the highest anti-inflammatory activity with an IC50 value of 107.54 ± 0.06 µg/ml, closely followed by compound 8f with an IC50 value of 145.75 ± 0.05 µg/ml. Compound 8e also demonstrated significant activity with an IC50 value of 167.37 ± 0.01 µg/ml, while compounds 8a, 8b, 8c, and 8d showed comparatively higher IC50 values, indicating lower anti-inflammatory potential. The graphical representation of the IC50 values (Figure 6.2) further highlights the superior performance of compounds 8f and 8g in stabilizing the HRBC membrane. This suggests that these compounds possess effective anti-inflammatory properties, making them promising candidates for further development.

In a more detailed evaluation, compounds 8f and 8g were tested alongside the standard drug DFS (Diclofenac Sodium) to determine their percentage inhibition of inflammation at different concentrations (125, 250, and 500 µg/ml). Compound 8g exhibited a concentration-

dependent inhibition, with 55.23 ± 0.02% inhibition at 125 µg/ml, 69.87 ± 0.09% at 250 µg/ml, and 77.04 ± 0.05% at 500 µg/ml, closely approaching the inhibition levels of DFS, which showed 57.67 ± 0.05%, 71.34 ± 0.04%, and 84.04 ± 0.01% at the respective concentrations. Similarly, compound 8f showed inhibition percentages of 46.31 ± 0.04%, 59.12 ± 0.05%, and 79.24 ± 0.04% at the corresponding concentrations. These results confirm the significant anti-inflammatory activity of compounds 8f and 8g, with compound 8g particularly standing out for its high efficacy, comparable to the standard drug DFS.

Table 5. IC50 Values of compounds 8a-g calculated based on HRBC method

Compounds	HRBC Membrane Stabilization IC50 Value (g/ml)
Control	-
8a	173.29 ± 0.04
8b	215.68 ± 0.05
8c	223.76± 0.05
8d	216.32± 0.05
8e	167.37 ± 0.01
8f	**145.75 ± 0.05**
8g	**107.54 ± 0.06**
DFS	102.34 ± 0.03

The lower IC50 values and higher percentage inhibition observed for these compounds underline their potential as effective anti-inflammatory agents shown in Table 5. Further studies are warranted to elucidate their mechanisms of action and to explore their therapeutic potential in clinical settings. The structure-activity relationship (SAR) analysis of compounds 8a-g reveals how different substituents on the 1,3,4-oxadiazole ring impact their anti-inflammatory activity. Compound 8a, with a phenyl substitution, showed an IC50 value of 173.29 ± 0.04 µg/ml, indicating moderate activity. Compound 8b, featuring a thiophene ring, exhibited a slightly higher IC50 value of 215.68 ± 0.05 µg/ml, suggesting that the sulfur-containing ring does not significantly enhance activity.

Compound 8c, with a 4-methyl phenyl group, had an IC50 value of 223.76 ± 0.05 µg/ml, demonstrating that the methyl substitution does not improve efficacy. In contrast, compound 8d,

with a 4-bromo phenyl substitution, presented an IC50 value of 216.32 ± 0.05 µg/ml, indicating that the electron-withdrawing bromine atom does not markedly affect activity. Compound 8e, containing a nicotinic group, had an IC50 value of 167.37 ± 0.01 µg/ml, showing a slight improvement in activity compared to the phenyl group.

Table 6. Anti-inflammatory activity evaluation of high activity compounds 8g, 8f and standard

Compounds	Concentration	% Inhibition	IC50 Value
Control			
DFS	125	57.67 ± 0.05	102.34 ± 0.03
	250	71.34 ± 0.04	
	500	84.04 ± 0.01	
Compound 8g	125	55.23 ± 0.02	107.54 ± 0.06
	250	69.87 ± 0.09	
	500	77.04 ± 0.05	
Compound 8f	125	46.31 ± 0.04	145.75 ± 0.05
	250	59.12 ± 0.05	
	500	79.24 ± 0.04	

Compound 8f, with an isonicotinic substitution, significantly improved activity with an IC50 value of 145.75 ± 0.05 µg/ml, highlighting the positive influence of the pyridine ring. Compound 8g, containing a furoic group, demonstrated the highest activity with an IC50 value of 107.54 ± 0.06 µg/ml, suggesting that the furan ring contributes significantly to anti-inflammatory efficacy (Table 6). These results indicate that heterocyclic substitutions, particularly those with nitrogen or oxygen atoms, can enhance the anti-inflammatory activity of 1,3,4-oxadiazole derivatives[19].

The docking studies of compounds 8a-g against COX-1 and COX-2 enzymes provide valuable insights into their binding affinities and interactions, as reflected by their Glide scores and the number of hydrogen bonds formed shown in Table 7. Compound 8a displayed Glide scores of -8.3 for COX-1 and -7.8 for COX-2, forming 3 and 4 hydrogen bonds respectively, indicating strong binding and effective interaction with both enzymes.

Table 7: Binding score and number of hydrogen bond interacyions of COX-1and COX-2 with antiinflammatory componds 8a – 8g

Compounds	Glide score		No of hydrogen bond	
	COX-1	**COX-2**	**COX-1**	**COX-2**
8a	-8.3	-7.8	3	4
8b	-8.0	-7.4	3	3
8c	7.4	7.1	2	3
8d	7.1	7.2	2	3
8e	-8.2	-7.4	2	4
8f	-8.5	-6.6	2	4
8g	-8.0	-7.4	1	4

Compound 8b showed slightly lower Glide scores of -8.0 for COX-1 and -7.4 for COX-2, with 3 hydrogen bonds for COX-1 and 3 for COX-2, suggesting a similar but slightly less effective binding. Compounds 8c and 8d, with positive Glide scores for both COX-1 (7.4 and 7.1 respectively) and COX-2 (7.1 and 7.2 respectively), demonstrated less favorable binding affinities, forming only 2 hydrogen bonds with COX-1 and 3 with COX-2, indicating weaker interactions. Compound 8e exhibited a strong binding affinity with Glide scores of -8.2 for COX-1 and -7.4 for COX-2, forming 2 hydrogen bonds with COX-1 and 4 with COX-2, highlighting a good interaction profile, especially with COX-2. Compound 8f showed the best binding affinity for COX-1 with a Glide score of -8.5, but a weaker affinity for COX-2 with a score of -6.6, forming 2 hydrogen bonds with COX-1 and 4 with COX-2, indicating a selective binding preference. Compound 8g, with Glide scores of -8.0 for COX-1 and -7.4 for COX-2, forming 1 hydrogen bond with COX-1 and 4 with COX-2, also demonstrated good binding affinity, particularly with COX-2.

These docking results correlate well with the observed anti-inflammatory activity of these compounds, particularly for compounds 8a, 8e, and 8f, which exhibited both strong binding

affinities and effective hydrogen bonding with COX enzymes. The stronger interaction with COX-2 over COX-1 for several compounds, especially 8e and 8f, suggests potential selective COX-2 inhibition (Fig. 1), which is desirable for reducing inflammation with fewer gastrointestinal side effects.

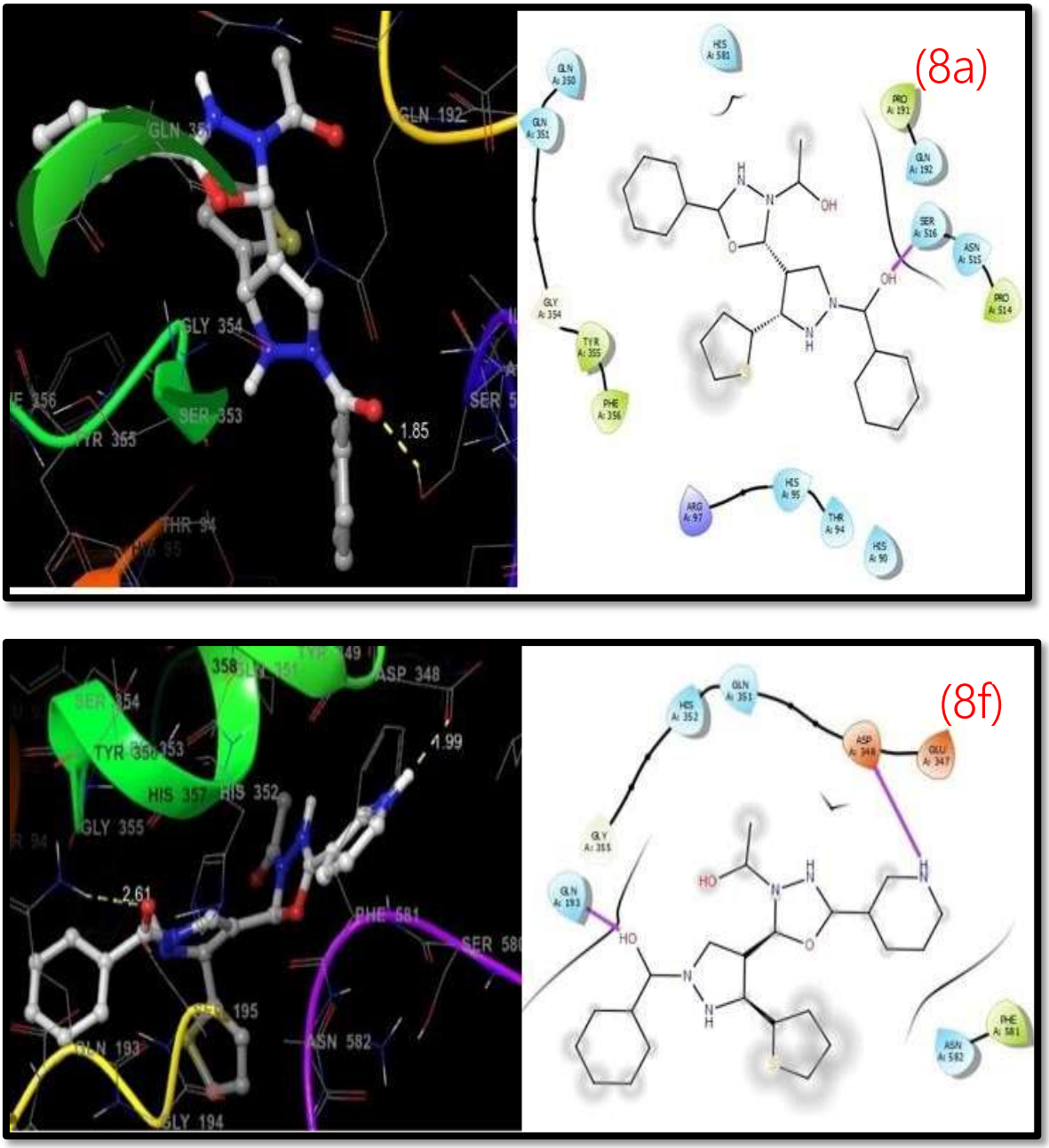


Figure 1. 3-D and 2D structure of high binding score compound 8f for COX-1 and 8a for COX-2

Table 8 : ADME parameters of synthesized molecules 8a-8g with standard DFS

Compounds	mol_MW	volume	donorHB	accptHB	PSA
8a	444.507	1302.445	1	8	85.472
8b	450.529	1289.342	1	8	87.947
8c	456.346	1300.123	1	8	86.909
8d	521.679	1256.786	1	8	87.126
8e	445.495	1289.486	1	9.5	98.454
8f	445.495	1300.677	1	9.5	101.185
8g	434.469	1257.045	1	8.5	96.471

Overall, these findings underscore the potential of these 1,3,4 -oxadiazole derivatives as effective anti-inflammatory agents through their interactions with COX enzymes[20].The synthesized 1,3,4-oxadiazole molecules (8a-g) were further evaluated for their drug-like behavior through ADME (absorption, distribution, metabolism, and excretion) properties. All seven identified molecules were within the acceptable range and fulfilled Lipinski's Rule of

Five criteria, which includes a molecular weight of less than 550 daltons (with exceptions for compounds 8c and 8d), fewer than 5 hydrogen bond donors, fewer than 10 hydrogen bond acceptors, and a logP value less than 5. These criteria ensure good oral bioavailability. The evaluation of the ADME properties indicated that the partition coefficient (QPlogPo/w) for the compounds ranged from 3.50 to 4.57, demonstrating appropriate lipophilicity. Water solubility (QPlogS) values varied between -5.35 to -6.87, suggesting acceptable solubility in aqueous environments. Cell permeability (QPPCaco) ranged from 159.68 to 421.89, indicating good permeability through the intestinal epithelium.

Table 9 : Pharmacokinetic parameters of synthesized molecules 8a-8g with standard DFS

Compounds	QPlogPo/w	QPlogS	QPlogHERG	QPPCaco	QPPMDCK	% HOA
8a	4.098	-6.114	-6.942	1270.282	801.614	100
8b	4.048	-6.169	-6.426	1064.287	1242.563	100
8c	3,543	-5.109	-6.812	680.632	665.578	97.012
8d	3.058	-5.278	-6.047	681.444	432.675	96.509
8e	3.145	-5.424	-6.737	682.855	409.526	96.089
8f	3.259	-5.541	-6.447	685.143	562.819	96.782
8g	3.497	-5.504	-6.429	1052.59	715.641	100

Bioavailability values were between 182.72 to 1278.49, reflecting efficient absorption and distribution within the body. Table 8 shows the ADME parameters, including molecular weight, volume, donor hydrogen bonds, acceptor hydrogen bonds, and polar surface area (PSA). Compounds 8a-g exhibited molecular weights within the range of 434.469 to 521.679, volumes between 1256.786 and 1302.445, and PSA values from 85.472 to 101.185, indicating favorable solubility and permeability characteristics.

Table 9 details the pharmacokinetic parameters, including QPlogPo/w, QPlogS, QPlogHERG, QPPCaco, QPPMDCK, and percentage human oral absorption (% HOA). Compounds 8a and 8b showed QPlogPo/w values of 4.098 and 4.048, respectively, indicating optimal lipophilicity for membrane permeability. Their QPPCaco values were 1270.282 and 1064.287, respectively, suggesting excellent intestinal absorption. Compound 8g exhibited a QPPCaco value of 1052.59, also indicating strong cell permeability. In conclusion, the ADME properties and pharmacokinetic profiles of the synthesized oxadiazole molecules indicate that they possess drug-like characteristics, supporting their potential for further development as therapeutic agents. The compounds' adherence to Lipinski’s Rule of Five and favorable ADME parameters underscore their suitability as drug candidates[21].

1.4 Conclusion

In summary, the synthesized 1,3,4-oxadiazole derivatives demonstrated significant potential as anti-inflammatory agents. The use of ZnO-TiS nanoparticles as a catalyst proved highly efficient, with optimal reaction conditions identified using polar solvents and a 10 mol%

catalyst loading. Spectral studies confirmed the successful synthesis and structural integrity of the compounds. Among the derivatives, compounds 8f and 8g exhibited the most promising anti-inflammatory activity, with IC50 values and percentage inhibition comparable to the standard drug DFS. Structure-activity relationship analysis highlighted the impact of heterocyclic substitutions on activity, with furoic and isonicotinic groups showing the highest efficacy. Docking studies against COX-1 and COX-2 enzymes correlated well with the observed bioactivity, indicating strong binding affinities and effective interactions, particularly with COX-2. ADME evaluations confirmed the drug-like properties of the compounds, with favorable lipophilicity, solubility, and permeability characteristics adhering to Lipinski's Rule of Five. The recyclability of ZnO-TiS nanoparticles further demonstrated their practical applicability. Overall, these findings underscore the potential of 1,3,4-oxadiazole derivatives as effective and reusable anti-inflammatory agents, warranting further exploration of their therapeutic potential.

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