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Molecular Hybridization Strategies for the Synthesis of Novel Fluorinated Pyrazole-1,3,4-Oxadiazole S-Alkyl Hybrids: Regioselective Construction and Evaluation as Potential Antimicrobial Leads

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Abstract: **The creation of new heterocyclic scaffolds with improved pharmacological profiles has become necessary due to the growing global problem of multi-drug resistant (MDR) bacterial strains. In this investigation, a number of unique 2-(5-(3,5-bis(trifluoromethyl)phenyl)The in vitro antibacterial potential of -1-(4-fluorophenyl)-1H-pyrazol-3-yl)-5-(alkylthio)-1,3,4-oxadiazole derivatives (6a–h) was carefully planned, produced, and assessed. The biologically powerful pyrazole and 1,3,4-oxadiazole nuclei were integrated into the molecular architecture of these hybrids, and fluorine-rich pharmacophores were then added to maximize lipophilicity and metabolic stability.The synthesis procedure produced a 1,3-diketoester intermediate by Claisen condensation of 3,5-bis(trifluoromethyl)acetophenone with diethyl oxalate. A combination of positional isomers (3a and 3b) was obtained via cyclization with 4-fluorophenyl hydrazine hydrochloride. The distinction of these isomers using ^{1}H NMR spectroscopy, where the magnetic anisotropy of the 4-fluorophenyl ring verified the shielding of the bis-trifluoromethyl phenyl protons in the major isomer 3a, was an important analytical discovery of this work. A carbohydrazide intermediate was used to convert 3a into the 1,3,4-oxadiazole-2-thiol scaffold. The final library was then obtained by nucleophilic S-alkylation with different alkyl halides.**

Several compounds have strong inhibitory activity, according to the antimicrobial screening against a panel of Gram-positive and Gram-negative microorganisms. With a Minimum Inhibitory Concentration (MIC) of 12.5 μg/mL against Pseudomonas aeruginosa—two times more effective than the reference standard, chloramphenicol (MIC 25 μg/mL)—the parent thio-oxadiazole intermediate 5 stood out as a particularly strong lead. Increasing the length of the S-alkyl side chain (such as n-butyl and allyl groups) is correlated with improved antibacterial activity, according to Structure-Activity Relationship (SAR) studies. These results highlight the trifluoromethyl-substituted heterocyclic hybrids' potential as viable options for the creation of antimicrobial agents of the future.

Keywords: **Pyrazole, 1,3,4-Oxadiazole, Trifluoromethyl, Antimicrobial Activity, MIC, Isomeric Shielding, S-Alkylation.**

1. INTRODUCTION

One of the biggest risks to international public health in the twenty-first century is the concerning increase of antimicrobial resistance (AMR) (Ferrer-Luque et al., 2023). Conventional antibiotics are often ineffective against multidrug-resistant (MDR) bacterial and fungal species, resulting in longer hospital stays and higher death rates (Sindhe et al., 2016). In addition to the difficulties in controlling infections, treating inflammatory diseases continues to be a fundamental aspect of

contemporary medicine. However, the systemic toxicity of non-steroidal anti-inflammatory medications (NSAIDs) frequently significantly limits their long-term clinical value. Due to their non-selective inhibition of cyclooxygenase (COX) enzymes, traditional NSAIDs are known to have serious side effects, such as hepatotoxicity, nephrotoxicity, and gastrointestinal ulcers (Paes Leme, 2021; StatPearls, 2024).

Therefore, one of the top priorities for medicinal chemists is the creation of new, multifunctional lead molecules that bridge the gap between reduced toxicity and antimicrobial activity. The development of Small Molecule High Affinity Ligands (SHALs), which emphasize precise binding and high affinity for biological targets to enhance therapeutic outcomes while reducing off-target effects, is one novel strategy (Zhang et al., 2023). The azole family, especially pyrazoles, has become a "privileged scaffold" among heterocyclic frameworks since it is found in many natural products and FDA-approved medications (Karrouchi et al., 2018). Pyrazoles are excellent candidates for the creation of novel therapeutic agents due to their wide range of pharmacological activities, which include strong antibacterial, antifungal, and anti-inflammatory qualities (Abd El-Karim et al., 2024; Zhang et al., 2023).

In order to increase bioactivity, two or more pharmacophores are merged into a single molecule using an effective drug discovery technique called structural hybridization (Othman et al., 2019). Particularly interesting is the incorporation of the thiadiazole and 1,3,4-oxadiazole moieties into a pyrazole core. These five-membered rings are known for their metabolic stability, capacity to interact with microbial targets through numerous hydrogen bonds, and ability to function as bioisosteres for amides and esters (Othman et al., 2019; Sindhe et al., 2016). According to reports in the literature, 2,5-disubstituted-1,3,4-oxadiazoles, which are frequently produced by cyclodehydrating acid hydrazides with phosphorus oxychloride ($POCl_3$), have improved membrane permeability and lipophilicity, both of which are essential for antimicrobial potency (Makwana & Naliapara, 2014; Othman et al., 2019).

The current work builds on these discoveries by designing and synthesizing a novel class of pyrazole-linked 1,3,4-oxadiazole and thiadiazole derivatives. Our goal is to create new SHALs that circumvent typical pathogens' resistance mechanisms by adding these heterocycles to substituted pyrazole-4-carboxylic acids. In order to establish preliminary structure-activity relationships (SAR), this work describes the synthesis process, the thorough characterisation of novel compounds using spectroscopic techniques (IR, NMR, and mass spectrometry), and an in vitro assessment of their antibacterial and antifungal properties.

2. MATERIAL AND METHODS

Chemical and reagent selection -

Sigma-Aldrich, CDH, Rankem, Spectrochem, and Alfa Aesar are just a few of the well-known commercial suppliers from whom the basic reagents and several chemical precursors used in this experimental synthesis were carefully purchased. Because all of the liquid reagents and solvents used in the multi-step chemical transformations were of the highest analytical grade, there was very little interference from contaminants. These solvents were used exactly as they were purchased; no additional distillation or purification was required. Thin Layer Chromatography (TLC) was carried out utilizing premium silica gel-G coated plates, visible under UV light and iodine chambers, to guarantee the accuracy of the synthetic process and the complete purity of the final heterocyclic compounds.

Analytical instrumentation and characterization -

Thermal Analysis: A sophisticated VEEGO-VMP-DS melting point equipment was used to determine the melting point of each synthesized derivative using the open capillary method. The recorded values are provided as uncorrected.

Nuclear Magnetic Resonance (NMR): Using ^1H NMR spectroscopy recorded on Bruker DPX 400 and 300 MHz instruments, structural elucidation was carried out. Tetramethylsilane (TMS) was used as the internal reference standard when preparing samples in $CDCl_3$ or DMSO-d_6 . Spin-spin coupling constants (J) are measured in Hertz (Hz), while chemical shift values (delta) are reported in parts per million (ppm).

Microanalysis: Using an Elementar Vario EL III elemental analyzer that provided the percentage (%) standard for carbon, hydrogen, and nitrogen, elemental analysis was carried out to validate the empirical formula of the new scaffolds.

Advanced synthetic methodologies for the Pyrazole core -

The determination of the most effective strategy for medication design, the production of the core pyrazole nucleus was examined using many complex synthetic pathways:

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Route 1 (Diketone Cyclization): This process produces a very high 95% ratio of the primary isomer by regioselectively cyclizing substituted 1,3-diketones with phenylhydrazine hydrochloride in a DMAc medium at room temperature for a whole day (Fig. no 1).

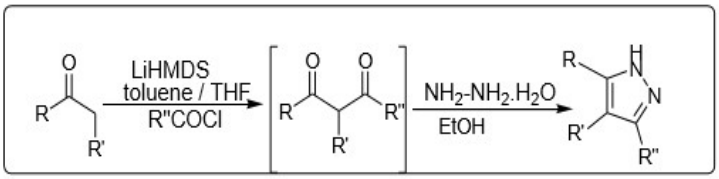

Fig. No. - 1 Regioselective synthesis of pyrazoles via the cyclization of 1,3-diketones

Route 2 (Palladium Catalysis): This contemporary method uses $PdCl_2(PPh_3)$ as a catalyst for the multicomponent coupling of phenylhydrazine, aryl iodides, and phenylacetylene in a THF/H_2O binary solvent system at ambient pressure in a carbon monoxide (CO) environment (Fig. no. 2).

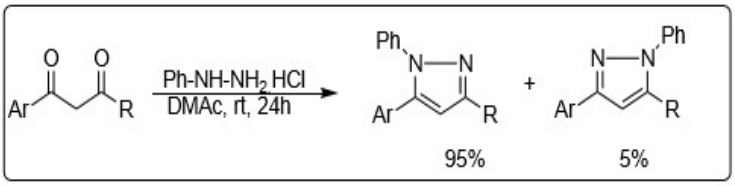

Fig. No. - 2 Palladium-catalyzed multicomponent synthesis of pyrazoles

Route 3 (Oxidation/Aromatization): Dihydro-pyrazoles were converted to completely aromatic pyrazoles through catalytic oxidative cyclization using molecular oxygen (O_2) and activated carbon in glacial acetic acid at a temperature of 120°C (Fig. no 3).

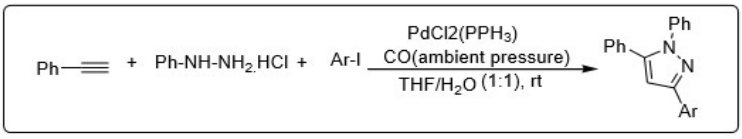

Fig. No. – 3 Catalytic oxidative cyclization of pyrazolines to pyrazoles

Route 4 (Organometallic Lithiation): A strict low-temperature process in which aryl halides were treated with Bu-Li in anhydrous THF at -78°C to produce reactive intermediates, then reacted with 1,3-diketones and cyclized using 4M HCl in a dioxane/THF mixture at 80°C (Fig. no 4).Base has been used to promot Cyclization.

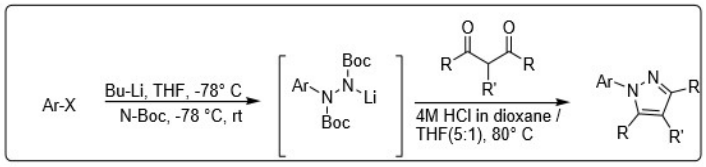

Fig. No. – 4 Synthesis of pyrazoles by reaction with 1,3-diketones

Route 5: This method enhances the interaction between ketones and acid chlorides by using LiHMDS in a toluene/THF environment. This is followed by a final cyclization step with hydrazine hydrate in boiling ethanol (Fig. no 5).

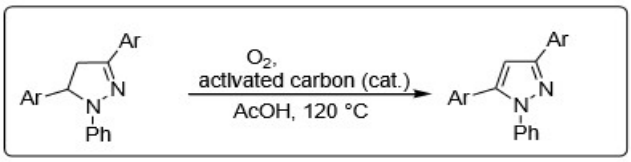

Fig. No. -5 Synthesis of pyrazoles via the reaction of ketones

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Route 6 (Enaminone Route): Highly substituted pyrazole-4-carboxylate derivatives were directly synthesized by condensing specific enaminones with aryl hydrazines in ethanol at room temperature (Fig. no 6).

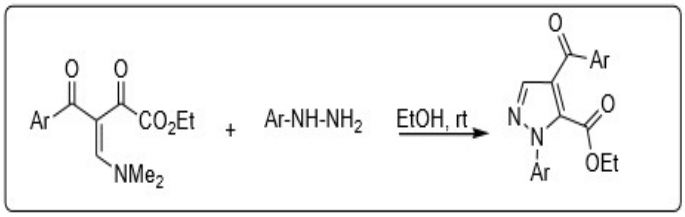

Fig. No. – 6 Synthesis of pyrazole-4-carboxylate derivatives

Innovative straergies for 1,3,4-Oxadizole Construction -

The 1,3,4-oxadiazole ring was created utilizing a number of different dehydrative and oxidative techniques in order to promote the "clubbing" of the two powerful nuclei:

Method A (Dehydrative Cyclization): To create the oxadiazole ring, the diacylhydrazine intermediates were cyclodehydrated employing a range of dehydrating agents (X), such as $POCl_3$, $SOCl_2$, P_2O_5, H_2SO_4, or triflic anhydride (Fig. no 7).

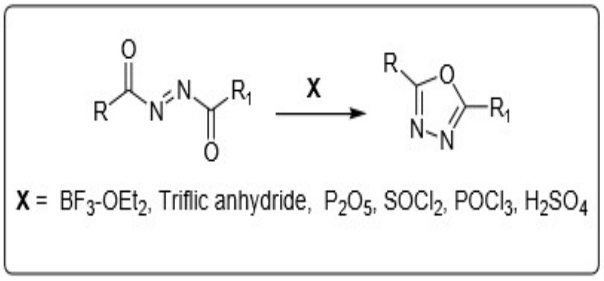

Fig. No. – 7 General dehydrative cyclization of diacylhydrazines into 1,3,4-oxadiazoles

Method B (Direct Condensation): An effective coupling technique that involves acid hydrazides reacting directly under regulated conditions with either carbonyl halides or carboxylic acids (X = H, OH) (Fig. no 8).

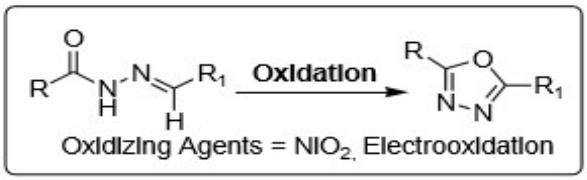

Fig. No. – 8 Direct coupling methodology for the synthesis of 1,3,4-oxadiazoles

Method C (Hydrazone Oxidation): This strategy used electro oxidation or the application of strong oxidizing chemicals like NiO_2 to oxidatively cyclize acylhydrazones (Fig. no 9).

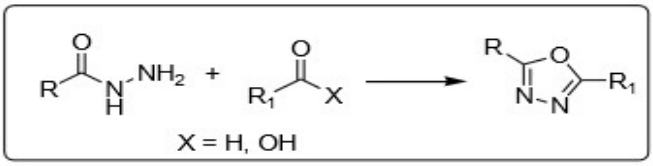

Fig. No. – 9 Oxidative cyclization of acylhydrazones into 1,3,4-oxadiazoles

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Rigorous Biological Protocol -

The therapeutic potential of the final molecular scaffolds produced by the fusion of pyrazole and oxadiazole moieties against pathogenic strains of Escherichia coli and Staphylococcus aureus was assessed.

Culture Standardization: To provide a consistent inoculum of roughly 1.5×10^{8}CFU/mL, the microbial strains were carefully subcultured at 28° C for an entire night, and their turbidity was standardized to a 0.5 McFarland standard.

Experimental Assay: Using Mueller-Hinton and Sabouraud Dextrose broth as the growing medium, the serial plate dilution technique was used to measure the antibacterial effectiveness.

MIC Determination and Comparison: To assess a range from 100 to 6.25ug/mL, test compounds were produced in a stock solution of 200ug/mL in DMF and successively diluted. The lowest dose at which no discernible growth may be seen with the unaided eye is known as the Minimum Inhibitory dose (MIC). For every antimicrobial test, ciprofloxacin served as the baseline reference standard.

3. RESULT AND DISCUSSION

To investigate the synergistic antibacterial properties of these heterocyclic scaffolds, pyrazole and 1,3,4-oxadiazole nuclei were strategically hybridized. In order to guarantee good regioselectivity and yield, the synthetic process started with the optimization of the pyrazole core using a variety of techniques documented in the literature (Fig. no 1–6). A series of steps including the conversion of pyrazole-carbohydrazides into their respective thio-oxadiazole derivatives were used to produce the 1,3,4-oxadiazole ring (Fig. no 7–9). Ultimately, a number of new S-alkylated hybrids (6a–h) were created using a methodical process (Fig. no-11), and sophisticated spectroscopic investigation (Fig. no-11-15) thoroughly verified their structural integrity.

Synthetic chemistry and isomeric differentiation -

The molecular hybridization of a highly lipophilic pyrazole core with a functionalized 1,3,4-oxadiazole moiety was the main focus of the target molecules' architectural design. The Claisen condensation of 3,5-(bis-trifluoromethyl) acetophenone 1 and diethyl oxalate under the influence of sodium hydride (NaH) in toluene marked the beginning of the synthetic process (Fig. No-10). The important intermediate, ethyl 4-(3,5-bis(trifluoromethyl)phenyl)-2,4-dioxobutanoate 2, was effectively produced by this process.

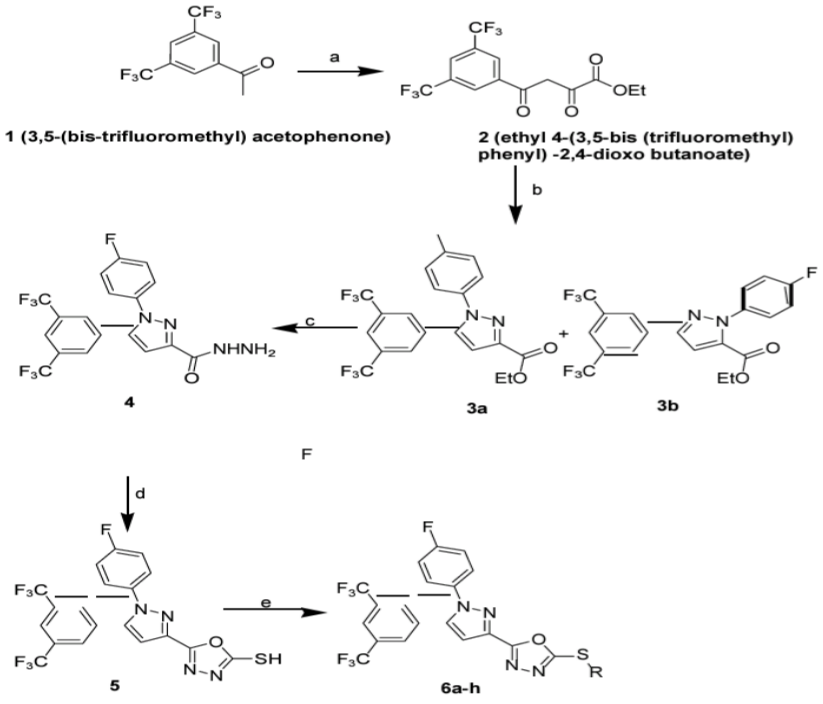

Figure No. – 10 Complete synthetic sequence for the target 2-(5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazol-3-yl)-5-(alkylthio)-1,3,4-oxadiazole derivatives (6a-h)

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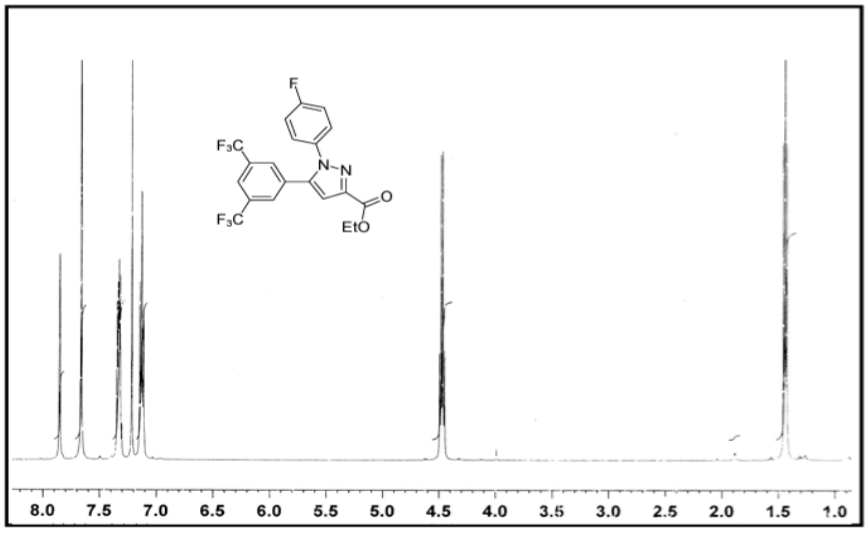
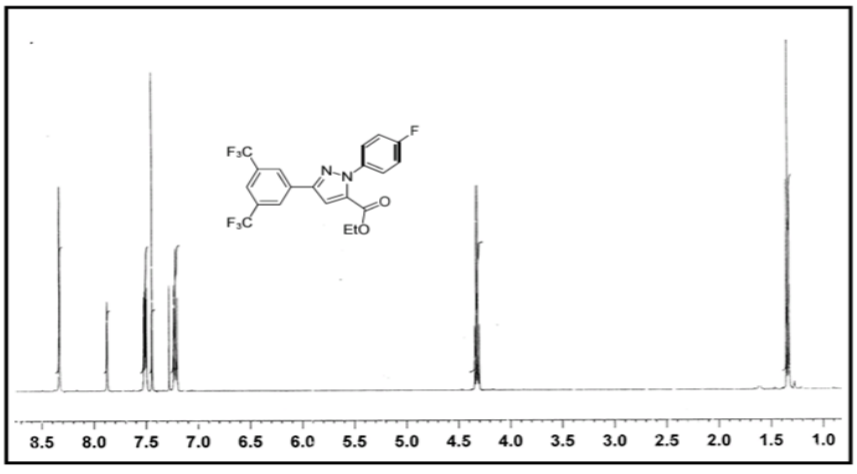
Compound 2's structural integrity was confirmed by ^{1}H NMR, which showed the characteristic ethyl ester protons as a quartet at δ 4.36 and a triplet at δ 1.44. The trisubstituted phenyl ring with two trifluoromethyl groups was represented by two different singlets in the aromatic region at δ 8.5 and δ 8.2.The reaction of 1,3-diketoester 2 with 4-fluorophenyl hydrazine hydrochloride was a crucial stage in the synthesis. This reaction produced two positional isomers, the main isomer 3a (75% yield) and the minor isomer 3b (25% yield), as reported in the literature for such unsymmetrical 1,3-diketones (Fig. no -10). Silica gel column chromatography (9:1 Hexane:Ethyl Acetate) was used to carefully separate these isomers.

There was a significant scientific difference between 3a and 3b. The bis-trifluoromethyl phenyl protons were detected at δ 7.89 and δ 7.70 in the ^{1}H NMR spectra of 3a (Fig. no.-11). In contrast to 3b (Fig. No.-12), where the singlet for two protons seemed deshielded at δ 8.33, these were interestingly protected. The magnetic anisotropy of the 4-fluorophenyl ring directly causes this shielding effect in 3a, confirming the main isomer's spatial orientation. This was corroborated by IR spectroscopy, which showed ester carbonyl stretching frequencies of 1748 cm^{-1} for 3a and 1734 cm^{-1} for 3b.



Spectrum 1: ^{1}H NMR of compound 3a

Figure No. – 11 Representative ^{1}H NMR for the structural elucidation and isomeric differentiation of compounds 3a



Spectrum 2: ^{1}H NMR of compound 3b

Figure No. – 12 Representative ^{1}H NMR for the structural elucidation and isomeric differentiation of compounds 3b

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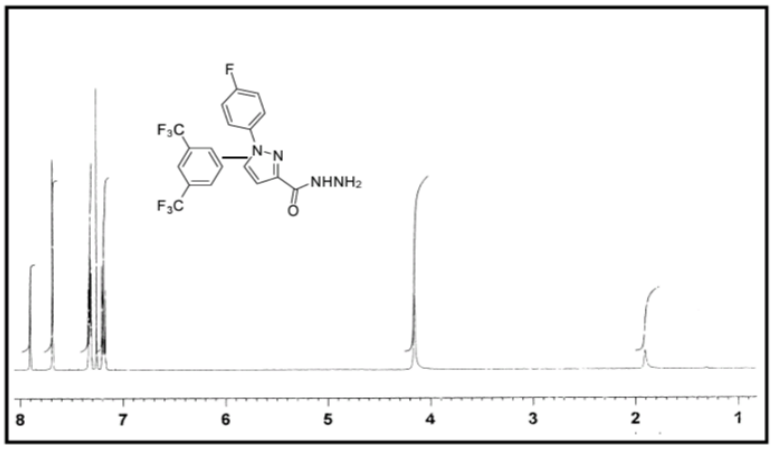
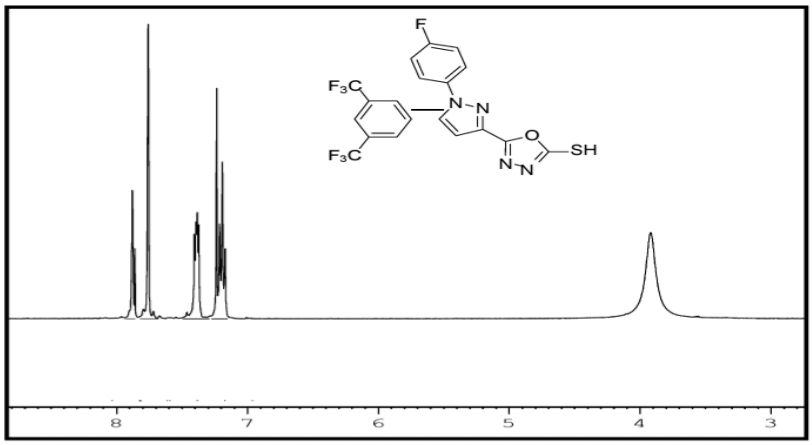
Functional Group Transformation and Oxadiazole Cyclization -

The equivalent carbohydrazide 4 was obtained by treating the main pyrazole ester 3a with hydrazine hydrate in ethanol. The elimination of the distinctive ethyl ester signals in ^{1}H NMR (Fig. no - 13) and the introduction of new signals for the hydrazide (-CONHNH$_2$) group as singlets at δ 4.15 and δ 1.90 clearly demonstrated this change. By reacting hydrazide 4 with carbon disulfide (CS_2) in the presence of ethanolic KOH, the 1,3,4-oxadiazole ring was constructed (Fig. no- 7). The 1,3,4-oxadiazole-2-thiol intermediate 5 was the outcome. The creation was verified by ^{1}H NMR (Fig. no - 14), which revealed the aromatic protons of the pyrazole and phenyl rings at their anticipated chemical shifts (δ 7.17–7.87) and a wide singlet at δ 3.92, indicative of the thiol (-SH) proton.



Spectrum 3: ^{1}H NMR of compound 4

Figure no. – 13 Representative ^{1}H NMR for the structural elucidation and isomeric differentiation of compounds 3c



Spectrum 4: ^{1}H NMR of compound 5

Figure no. – 14 Representative ^{1}H NMR for the structural elucidation and isomeric differentiation of compounds 3d

S-Alkylation and Final Lead Molecule Optimization -

In the last stage, the thiol group in 5 was nucleophilically substituted with different alkyl halides while triethylamine (TEA) was present as a base (Fig.no- 8). As a result, a number of new S-alkylated compounds, 6a–h, were produced. High-resolution NMR and LC-MS studies were used to validate the final library's structure. For example, a methyl singlet appeared at delta 2.71 in the spectrum of 6a (Fig. no-14), suggesting effective thio-ether synthesis. Accordingly, the methyl carbon at delta 14.62 was identified by ^{13}C NMR (Fig. no-15). While the allyl derivative 6f displayed intricate multiplet patterns in the 3.94–6.02 range, signifying the unsaturated allyl system, the spectrum for the ethyl derivative 6b displayed a clear quartet-triplet pattern for the ethyl group.

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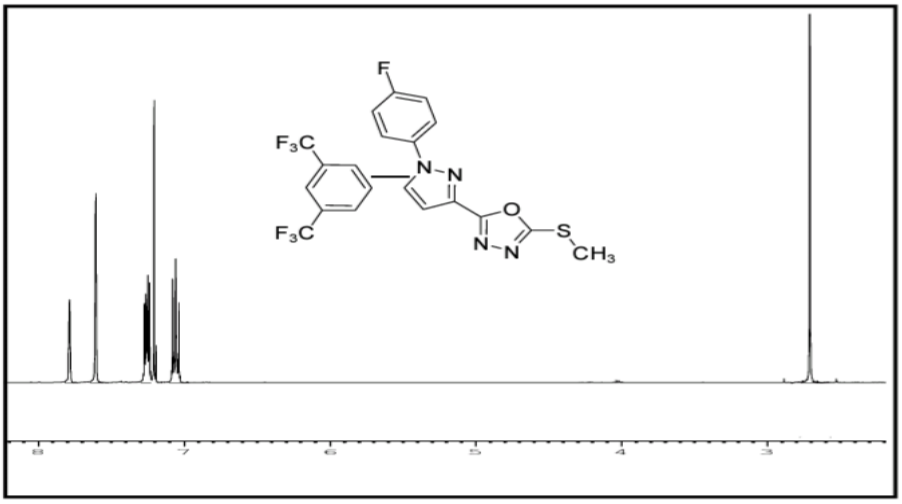
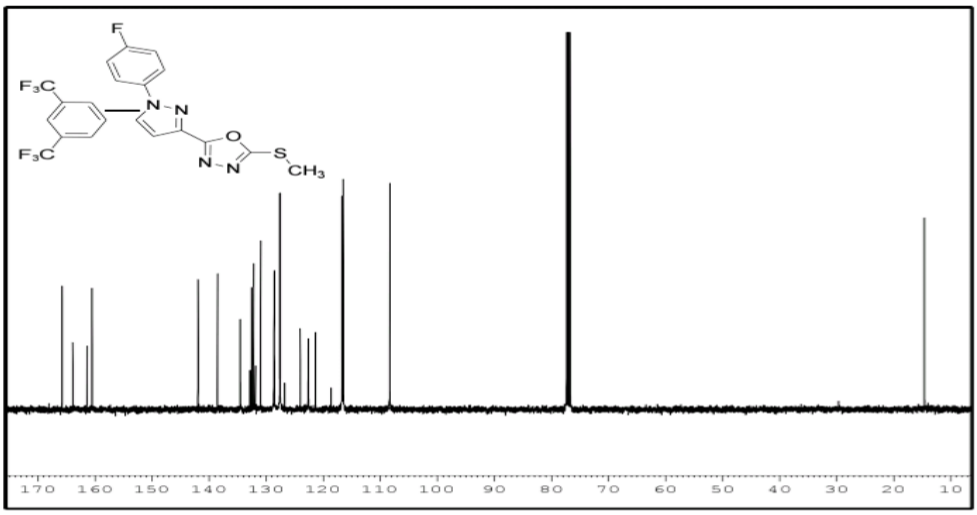


Spectrum 5: ^{1}H NMR of compound 6a

Figure no. – 15 Representative ^{1}H NMR for the structural elucidation and isomeric differentiation of compounds 6a



Spectrum 6: ^{13}C NMR of compound 6a

Figure no. – 15 Representative ^{13}C NMR for the structural elucidation and isomeric differentiation of compounds 6a

Biological Potential and Structure Activity Relationship -

The "clubbing" of the pyrazole and oxadiazole nuclei resulted in molecules with greater therapeutic potential than conventional medicines, according to the antibacterial evaluation.

Antibiotic Lead Identification: The parent thiol-oxadiazole 5 was shown to be quite effective, particularly against P. aeruginosa, where its MIC value (12.5 ug/mL) was much lower than that of the conventional Chloramphenicol (25 ug/mL).

S-Alkyl Chain Length's Effect: The SAR analysis showed a distinct pattern: the antibacterial activity improved as the aliphatic chain length rose. In comparison to their methyl (6a) and ethyl (6b) equivalents, compounds 6d (n-butyl), 6e (iso-butyl), and 6f (allyl) had better zones of inhibition. This implies that improved penetration through the lipid bilayer of the bacterial cell wall is made possible by higher lipophilicity in the S-alkyl region (Table no.-1 & 2).

Fluorine Influence: The compounds' metabolic stability and binding affinity as Small Molecule High Affinity Ligands (SHALs) were both improved by the addition of the 3,5-bis(trifluoromethyl) and 4-fluorophenyl groups.

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Table 1: Antimicrobial potential of synthesized compound (Zone of inhibition for compounds (5, 6a-h)

Compounds (100µg/mL)	Name of the test organisms					
	B.subtilis	*P.aeruginosa*	*K.terrigena*	*E.coli*	*S.aureus*	*S.pyogenes*
	Zone of inhibition in mm					
Chloram-phenicol	21.05	22.7	23.1	22.2	22.95	23.4
5	14.1	13.54	13.78	12.4	12.7	13.9
6a	16.1	16.32	16.25	15.9	15.85	16.22
6b	17.65	16.5	16.1	16.15	16.46	15.92
6c	17.82	17.1	17.5	16.82	16.9	17.2
6d	18.25	17.62	18.80	18.9	18.40	18.30
6e	18.85	18.9	19.26	19.5	19.46	18.70
6f	19.3	18.54	18.28	18.78	18.64	17.06
6g	18.92	18.3	19.12	17.8	18.6	17.8
6h	18.6	17.98	18.85	17.6	18.2	18.1

Table 2 Anti-bacterial activity of Compounds 5 and 6 (a-h) (MIC and MBC)

Compounds	*E.coli*		*P.aeruginosa*		*K.terrigena*		*S.aureus*		*B.subtilis*	
(100 µg /mL)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Chloram Phenicol	6.25	200	25	100	0.78	200	3.125	200	6.25	3.12
5	50	200	12.5	12.5	-	-	50	50	25	100
6a			-	-	12.5	-	50	-	-	-
6b	50	200	25	-	-	-	-	-	-	-
6c	50	-	-	-	25	200	-	-	-	-
6d	-	200	25	25	-	-	-	50	-	-
6e	-	-	-	25	-	200	-	-	-	-
6f	50	200	-	-	-	-	-	-	-	-
6g	50	200	-	-	25	200	-	-	-	-
6h	100		50	100	-	-	50	50	-	-

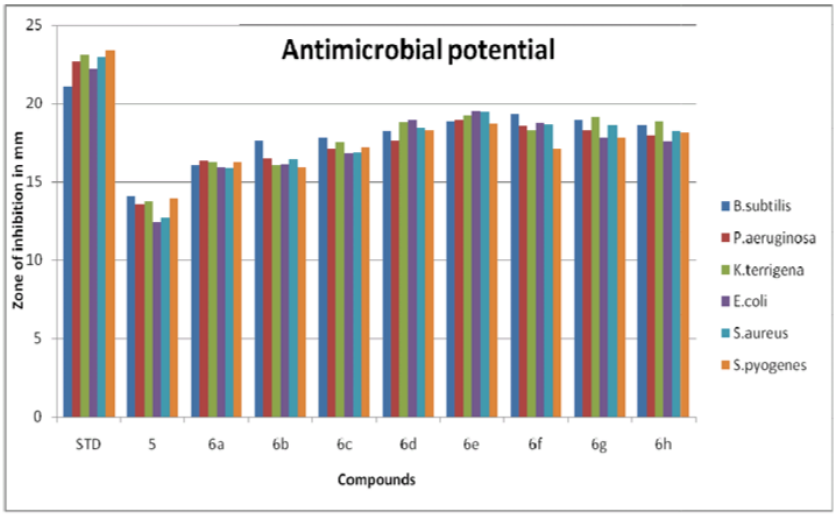

Fig: Antimicrobial potential of synthesized compound

Synthesis of ethyl 4-(3,5-bis(trifluoromethyl)phenyl)-2,4-dioxobutanoate (2) –

The crucial 1,3-diketoester intermediate 2 was created by a methodical Claisen condensation. Diethyl oxalate (1.5 mol) was added dropwise in an inert environment to a vigorously agitated solution of 3,5-bis(trifluoromethyl)acetophenone (1.0 mol) in anhydrous toluene (500 mL). The enolization was then catalyzed by adding sodium hydride (2.0 mol, 60% dispersion in mineral oil) piece by portion. For eighteen hours, the reaction mixture was continuously stirred magnetically while being kept at room temperature.

Using an n-hexane:ethyl acetate (8:2) solvent combination, Thin Layer Chromatography (TLC) was used to track the condensation's progress and the initial acetophenone's disappearance (Image 1). After finishing, the extra toluene was contained under low pressure (in vacuo). The resultant viscous residue was neutralized to pH 3–4 using cooled diluted hydrochloric acid (2N) and quenched by pouring onto crushed ice. To obtain pure 1,3-diketoester 2, the precipitated crude yellow-white material was filtered, extensively cleaned with cold water to eliminate inorganic ions, and vacuum-dried.

Regioselective Synthesis of Pyrazole Isomers (3a and 3b)-

The 1,3-diketoester 2 was cyclocondensed with an aryl hydrazine to create the pyrazole scaffold. Compound 2 (1.0 mol) and 4-fluorophenyl hydrazine hydrochloride (1.1 mol) were combined and dissolved in a binary solvent solution consisting of ethanol and glacial acetic acid (2:1 v/v). The reaction media was kept under reflux for six hours after being heated to 100 ^{o}C. TLC was used to monitor the reaction's progress and showed the production of two distinct spots that corresponded to the positional isomers 3a and 3b. A crude solid precipitated when the reaction mixture was quenched into one liter of ice-cold water after the allotted time.

The crude material was submitted to Silica Gel Column Chromatography (60–120 mesh) using a gradient elution of n-hexane and ethyl acetate (9:1) in order to isolate the primary regioisomer. After recrystallization from pure ethanol, the main isomer, ethyl 1-(4-fluorophenyl)-5-(3,5-bis(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxylate (3a), was produced as a white crystalline solid.

Synthesis of 2,5-disubstituted-1,3,4-oxadiazole S-alkyl derivatives (6a–h) –

The thiol-functionalized oxadiazole intermediate 5 was nucleophilically substituted to create the final library of S-alkylated hybrids. Typically, 5-(5-(3,5-bis(trifluoromethyl)phenyl)-1-(4-fluorophenyl)-1H-pyrazol-3-yl1,3,4-oxadiazole 50 milliliters of pure ethanol were used to dissolve -2-thiol (5, 1.0 mol). Triethylamine (TEA, 0.5 mol), an organic base, was added to this solution as an acid scavenger before the corresponding alkyl halide (1.2 mol) was added stoichiometrically. After heating the reaction mixture to 50 ^{o}C, it was refluxed for eight to ten hours while being continuously stirred. The disappearance of the starting material on TLC was used to track the change from thiol to thio-ether (Image 8). The solvent was evaporated in vacuo once the reaction was finished. After that, the residue was filtered, dried, and triturated with cold water. The finished goods (6a–h) were refined. To guarantee analytical purity for biological screening, the final products (6a–h) were purified using either flash column chromatography or recrystallization from an appropriate solvent (Ethanol/Methanol).

4. CONCLUSION

The design, synthesis, and biological assessment of a novel series of pyrazole-1,3,4-oxadiazole hybrids with 3,5-bis(trifluoromethyl)phenyl and 4-fluorophenyl pharmacophores are successfully demonstrated in this study. A streamlined path involving Claisen condensation, regioselective cyclization, and thio-alkylation was eventually followed after the synthetic strategy was strategically improved employing a variety of techniques. The effective isolation and structural characterisation of pyrazole regioisomers 3a and 3b was a major feature of our investigation. It was determined through sophisticated ^{1}H NMR analysis that the magnetic anisotropy of the 4-fluorophenyl ring offers a clear shielding effect, enabling accurate identification of the primary isomer. The consistency of the ensuing S-alkylation processes and the ensuing biological efficacy of the final derivatives 6a–h depended heavily on this isomeric purity.

The produced hybrids showed moderate to excellent antibacterial properties from a pharmacological standpoint. With a minimum inhibitory concentration (MIC) of 12.5 ug/mL against P. aeruginosa, the parent thio-oxadiazole 5 outperforms the reference standard chloramphenicol, according to the Structure-Activity Relationship (SAR) study. Additionally, it was

shown that there was a positive correlation between antimicrobial inhibition and the systematic lengthening of the S-alkyl chain (from methyl to n-butyl and allyl groups), most likely as a result of increased lipophilicity and better cellular membrane penetration.

In conclusion, a dual-heterocyclic framework that incorporates trifluoromethyl and fluoro-substituted aryl groups offers a viable scaffold for the creation of powerful antimicrobial medicines. These results point to compounds 5, 6d, and 6f as promising lead candidates for additional medicinal chemistry optimization and in vivo toxicity investigations to tackle the increasing problem of bacterial resistance.

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