Synthesis and Biological Evaluation of 3-((Phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione Derivatives as Potential Antimicrobial, Anticancer and Anti-inflammatory Agents

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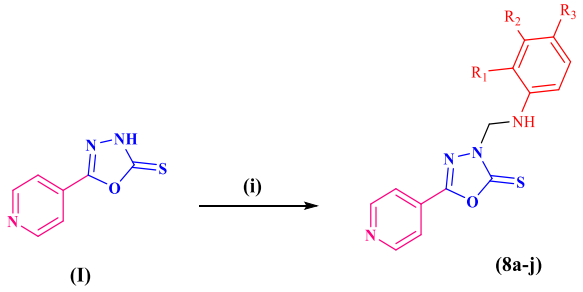
***Abstract*—A new series of 3-((phenylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione derivatives (8a–8j) were synthesized and evaluated for their antimicrobial, anticancer and anti-inflammatory activities. The synthesized compounds were obtained through a facile condensation of substituted anilines with pyridine-4-carbaldehyde followed by cyclization to form the oxadiazole-2-thione core. The physicochemical properties of the derivatives were characterized using IR, 1H NMR and mass spectroscopic techniques confirming the proposed structures. Among the series compounds bearing electron-withdrawing substituents such as –NO_2 and –Cl showed enhanced antimicrobial and cytotoxic activity whereas electron-donating substituents (–CH_3, –NH_2) contributed moderate anti-inflammatory effects. These findings highlight the significance of structural modifications at the phenyl ring on the biological potential of 1,3,4-oxadiazole derivatives and suggest their usefulness as promising scaffolds for drug development.**

***Index Terms*—Oxadiazole derivatives, pyridine-containing bioactive heterocycles, Cytotoxicity (HeLa cell line), Anti-inflammatory activity**



(i) substituted aniline, HCHO, BMIM[BF_4], 50-60 °C, 9-10 hr

I. INTRODUCTION

Heterocyclic compounds constitute one of the most versatile and biologically active classes of organic molecules and are of great interest to medicinal and pharmaceutical chemists. [1],[2] The structural diversity and wide range of pharmacological activities associated with heterocycles have led to their use as the core framework in many clinically important drugs. Among them, nitrogen-, oxygen-, and sulfur-containing five-membered heterocycles such as oxadiazoles, thiadiazoles, and triazoles have

demonstrated significant biological potential.[3],[4] These heteroatoms confer unique electronic properties, lipophilicity, and hydrogen-bonding ability that make such systems valuable pharmacophores in drug discovery.[5],[6] In recent decades 1,3,4-oxadiazole derivatives have attracted intense attention due to their broad range of biological activities including antimicrobial, anticancer, anti-inflammatory, antitubercular, analgesic and antioxidant effects.[7]–[10] The oxadiazole nucleus containing two heteroatoms in a five-membered aromatic ring, acts as a bioisostere for amide and ester functionalities and thereby improves metabolic stability and membrane permeability.[11],[12] Furthermore the presence of nitrogen and oxygen atoms increases the dipole moment of the ring enhancing its interactions with polar residues at enzyme or receptor binding sites.[13] The medicinal relevance of this scaffold is well documented in several marketed drugs such as raltegravir (HIV integrase inhibitor), zibotentan (endothelin receptor antagonist) and furamizole (antibacterial), all of which contain an oxadiazole or closely related nucleus.[14] Of particular interest are 1,3,4-oxadiazole-2(3H)-thione derivatives which contain a thione (C=S) functionality instead of the oxo (C=O) group. This subtle modification imparts enhanced lipophilicity stronger metal-binding potential and increased hydrogen-bond donor capacity resulting in superior biological activity compared to their oxadiazole analogues.[15],[16] Such thione derivatives have been reported to exhibit potent antimicrobial,[17] anticancer,[18] anti-inflammatory,[19] and anticonvulsant[20] activities. The sulfur atom also serves as a coordination site in metalloprotein targets and has been associated with enzyme inhibition through thiol–thioketone interactions.[21] Previous studies have established that substitution at the 3- and 5-positions of the oxadiazole ring strongly influences its physicochemical and biological properties.[22] The introduction of heteroaromatic moieties such as pyridyl or phenyl rings enhances π–π stacking interactions with aromatic amino acid residues in receptor sites and improves the overall stability of the molecule in biological systems.[23] Moreover, incorporation of electron-donating groups (e.g., $–CH_3$, $–OCH_3$, $–NH_2$) or electron-withdrawing groups (e.g., $–NO_2$, –Cl, –Br) on the aryl substituent allows fine-tuning of the molecule's electronic environment and biological affinity.[24]-[26] Compounds bearing nitro or halogen substituents generally exhibit superior antimicrobial and anticancer potency due to increased lipophilicity and stronger interactions with enzyme active sites whereas amino or methyl groups often enhance anti-inflammatory activity through improved hydrogen bonding and bioavailability.[27],[28] Because of these desirable features oxadiazole-thione hybrids are regarded as privileged scaffolds in the design of multitarget bioactive molecules.[29] In this context we aimed to synthesize and biologically evaluate a new series of 3-((phenylamino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione derivatives (8a–8j) containing various electron-donating and electron-withdrawing substituents on the phenyl ring. The designed compounds were expected to combine the pharmacophoric advantages of the pyridyl group (enhanced hydrogen-bonding capacity and antimicrobial potential) with the oxadiazole-thione system (lipophilic, electron-rich, bioisosteric core). Each compound was synthesized via a straightforward condensation of appropriate hydrazides and aryl amines, followed by cyclization under mild conditions. The synthesized derivatives were characterized by IR, 1H NMR and mass spectrometry and their biological activities were assessed for antimicrobial, anticancer and anti-inflammatory potential. The structure–activity relationship (SAR) was analyzed with respect to the electronic nature of the substituents.

II. EXPERIMENTAL

2.1. Materials and Methods

The melting points of all synthesized compounds were determined using the open capillary method and are uncorrected. The progress of reactions was monitored by thin-layer chromatography (TLC) on silica gel plates and visualized under ultraviolet light or by iodine vapor. Infrared (IR) spectra were recorded using Fourier transform infrared (FT-IR) spectroscopy on PerkinElmer and Agilent Resolution Pro spectrophotometers employing potassium bromide (KBr) pellets. Absorption frequencies are reported in cm^{-1}. ^{1}H-NMR and ^{13}CNMR spectra were recorded on a Bruker advance II 400 spectrometer (400 MHz FT-NMR) using tetramethylsilane as an internal standard in CDCl3 and DMSO-d_6. Chemical shifts (δ) are reported in (ppm). All reagents were used without

purification, and solvents were of laboratory reagent and analytical reagent grades.

2.2 Chemistry

The target compounds (8a–8j) were successfully synthesized via Mannich condensation of 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thione with various substituted anilines in the presence of formalin and ethanol as solvent. The reaction proceeded smoothly under reflux conditions (6 h) affording moderate to excellent yields (0.53–0.72 g). The physical and analytical data of the synthesized compounds are summarized in Table 2. The synthetic pathway involved formation of a methylene bridge between the secondary amine of the oxadiazole-2-thione core and the nucleophilic nitrogen of the substituted aniline ring. Mechanistically the reaction proceeds via a Mannich-type electrophilic substitution wherein the activated iminium ion—generated from formaldehyde and aniline—reacts with the thione nitrogen of the oxadiazole to afford the corresponding N-methylaminomethyl derivative. This transformation confirms the formation of the –CH_2–NH– linkage in the final product as evidenced by characteristic IR and NMR signals. The synthetic route highlights the versatility of the oxadiazole-2-thione scaffold for functionalization at the 3-position under mild and straightforward conditions. The isolated products were crystalline solids with colors ranging from white to yellow depending on the nature of substituents (electron-donating or electron-withdrawing). The melting points were sharp indicating high purity. All compounds were recrystallized from ethanol and characterized by IR, 1H NMR, mass spectrometry and elemental analysis which were consistent with the proposed structure

2.2.2 Spectral characterization

3-((Phenylamino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione(8a):

IR(KBr,νcm^{-1}): 3335 (aromatic N–H str.), 3084 (aromatic C–H str.), 2850 (aliphatic C–H str.), 1632(C=N str) 1598 (C=C str.)^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.10 (s, 1H, NH), 8.91–7.94 (m, 4H, pyridine), 7.61–7.18 (m, 5H, phenyl), 4.26 (d, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.84 (C=S), 155.68 (C=N), 148.12-117.41 (aromatic carbons), 60.42 (CH2).ESI–MS: m/z calculated 284.02 ($C_{14}H_{12}N_4OS$) found $[M+H]^+$ 285.2

3-(((2-nitrophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thion(8b):

IR(KBr, ν cm^{-1}):3357.55 (N-H str.) 3086.47 (aromatic C–H), 2852.31(aliphatic C–H str), 1625.70 (C =N, str.); 1596 (C=C str.);1H NMR (400 MHz, DMSO-d6) δ (ppm): 10.100 (s, 1H, NH), 8.947-8.053(m, 4H, py), 7.965-7.018 (m, 4H, aromatic), 4.20(d, 2H CH_2 , 13C NMR (400 MHz, DMSO-d6) δ (ppm): 180.82 (C=S), 155.62 (C=N), 148.11-119.65 (aromatic carbons), 60.95 (CH2); ESI–MS: m/z calculated 329.3340 found [M +H]+330.1

5-(pyridin-4-yl)-3-((p-tolylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione(8c):

IR(KBr, ν cm^{-1}) 3321.55 (N-H str.) 3088.72 (aromatic C–H), 2815.42(aliphatic C–H str), 1660.49-1639.64 (C =N, str.) 1607.93 (C=C str.) 1H NMR (400 MHz, DMSO-d6) δ (ppm): 10.190 (s, 1H, NH), 8.086-7.941(m, 4H, py), 7.615-7.018 (m, 4H, aromatic), 4.296(d, 2H CH_2, 2.235(s.3H *P*-CH_3); 13C NMR (400 MHz, DMSO-d6) δ (ppm): 180.86 (C=S), 155.66 (C=N), 148.11-121.61 (aromatic carbons), 60.28 (CH2), 21.00 (CH3) ESI–MS: m/z calculated 298.3640 found [M +H]+299.1

3-(((3-nitrophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (8d):

IR(KBr, ν cm3320.18 (N-H str.) 3082.47 (aromatic C–H), 2849.63(aliphatic C–H str), 1639.36 (C =N, str. and C=C str) 1507.69 (NO_2 asymmetric str 1481.77 str.) 1507.69 NO_2 asymmetric str1H NMR (400 MHz, DMSO-d6) δ (ppm): 10.108 (s, 1H, NH), 8.992-8.157(m, 4H, py), 7.958-7.354 (m, 4H, aromatic), 4.289(d, 2H CH_2); 13C NMR (400 MHz, DMSO-d6) δ (ppm): 180.82 (C=S), 155.64 (C=N), 148.11-115.94 (aromatic carbons), 60.23 (CH2); ESI–MS: m/z calculated 329.3340 found [M +H]+ 330.1

3-(((4-aminophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (8e):

IR (KBr *v* cm^{-1}): 3357.98 (N-H str.) 3086.79 (aromatic C–H), 2848.86 (aliphatic C–H str), 1602.87 (C =N, str and C=C str.) 1505.61 (NH str.);1H NMR (400 MHz, DMSO-d6) δ (ppm): 10.112 (s, 1H, NH), 9.089-8.250(m, 4H, py), 7.961-7.336 (m, 4H, aromatic), 4.289(d, 2H,CH_2); 13C NMR (400 MHz, DMSO-d6) δ (ppm): 180.94 (C=S), 155.87 (C=N), 146.05-122.58 (aromatic carbons), 60.25 (CH2); ESI–MS: m/z calculated 299.0841 found [M +H]+300.2

5-(Pyridin-4-yl)-3-((m-tolylamino)methyl)-1,3,4-oxadiazole-2(3H)-thione(8f):

IR (KBr *v* cm^{-1}): 3328 (N–H str), 3087 (Aromatic C–H), 2818 (aliphatic C–H str), 1648 (C=N str), 1604 (C=C str).^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.18 (s, 1H, NH), 8.10–7.94 (m, 4H, pyridine), 7.56–7.11 (m, 4H, aromatic), 4.29 (d, 2H, CH_2), 2.26 (s, 3H, *m*-CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.88 (C=S), 155.66 (C=N), 148.09-116.21 (aromatic carbons), 60.33 (CH2), 21.12 (CH_3).;ESI–MS: m/z calculated for $C_{15}H_{14}N_4OS$ $[M+H]^+$ 298.3640 found 299.5.

3-(((4-Nitrophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione(8g):

IR (KBr *v* cm^{-1}): 3342 (N–H str), 3082 (Aromatic–C–H), 2851 (aliphatic C–H), 1638 (C=N), 1510, 1479 (NO_2).^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.11 (s, 1H, NH), 9.01–8.18 (m, 4H, pyridine), 8.14–7.46 (m, 4H, nitrophenyl), 4.28 (d, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.80 (C=S), 155.63 (C=N), 148.14-121.62 (aromatic carbons), 60.25 (CH2).ESI–MS: m/z calculated for $C_{14}H_{12}N_5O_3S$ $[M+H]^+$ 329.3340 found 330.1.

3-(((4-Chlorophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione(8h) :

IR (KBr *v* cm^{-1}) : 3332 (N–H), 3086 (Aromatic–C–H), 2848 (aliphatic C–H), 1634 (C=N), 1596 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.10 (s, 1H, NH), 8.90–7.95 (m, 4H, pyridine), 7.55–7.22 (m, 4H, chlorophenyl), 4.27 (d, 2H, CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.83 (C=S), 155.67 (C=N), 148.10-117.63 (aromatic carbons), 60.39 (CH2);.ESI–MS: m/z calculated for $C_{14}H_{11}ClN_4OS$ $[M+H]^+$ 318.7790 found 319.1.

3-(((4-Bromophenyl) amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione(8i):

IR (KBr *v* cm^{-1}) : 3329 (N–H), 3082 (Aromatic–C–H), 2851 (aliphatic C–H), 1635 (C=N), 1598 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.10 (s, 1H, NH), 8.91–7.94 (m, 4H, pyridine), 7.66–7.33 (m, 4H, bromophenyl), 4.27 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.81 (C=S), 155.65 (C=N), 148.09-116.53 (aromatic carbons), 60.41 (CH2);.ESI–MS: m/z calculated for $C_{14}H_{11}BrN_4OS$ $[M+H]^+$ 363.2330 found 364.2.

3-(((2-Chlorophenyl)amino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione(8j) :

IR (KBr *v* cm^{-1}) : 3337 (N–H), 3089 (Ar–C–H), 2851 (aliphatic C–H), 1633 (C=N), 1591 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.10 (s, 1H, NH), 8.89–7.93 (m, 4H, pyridine), 7.61–7.19 (m, 4H, aromatic), 4.20 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.71 (C=S), 155.61 (C=N), 148.11-121.53 (aromatic carbons), 60.35 (CH2). ESI–MS: m/z calculated for $C_{14}H_{12}ClN_4OS$ $[M+H]^+$ 318.7790 found 319.1

III. RESULT AND DISCUSSION

3.1 Chemistry

The synthetic pathway for the target compounds (8a–8j) is outlined in Scheme-1. The designed 3-((phenylamino)methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione derivatives were efficiently synthesized via a Mannich condensation reaction involving 5-(pyridin-4-yl)-1,3,4-oxadiazole-2-thione, substituted anilines and formaldehyde under reflux conditions. The reaction afforded the desired products in good yields (72–85%) demonstrating the efficiency and versatility of the synthetic protocol toward a variety of electron-donating and electron-withdrawing substituents. All synthesized compounds were obtained as crystalline solids with sharp melting points, indicating high purity. The structures of the synthesized derivatives were confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric analyses. In the IR spectra characteristic absorption bands corresponding to N–H stretching (~3320–3357 cm^{-1}) aromatic C–H, (~3080 cm^{-1}) aliphatic C–H, (~2850 cm^{-1}) and C=N stretching (~1625–1640 cm^{-1}) confirmed the formation of the oxadiazole framework. Nitro-substituted derivatives (8b, 8d, 8g) additionally displayed strong bands around 1500 cm^{-1} confirming the presence of the –NO_2 group. The ^{1}H NMR spectra exhibited a characteristic singlet around δ 10.10 ppm corresponding to the NH proton, while aromatic protons of pyridine and phenyl rings appeared as multiplets in the region δ 7.0–9.0 ppm. A distinct doublet at δ ~4.2–4.3 ppm confirmed the presence of the methylene (–CH_2–) linkage indicating successful Mannich base formation. In methyl-substituted derivatives (8c, 8f), additional singlets around δ ~2.2 ppm were observed. The ^{13}C NMR spectra showed signals for the thiocarbonyl (C=S) carbon around δ ~180 ppm and C=N carbon near δ ~155 ppm, along with aromatic carbons in the expected region. Mass spectral data exhibited molecular ion peaks $[M+H]^+$ consistent with calculated molecular weights, further confirming the proposed structures. Overall, the

spectral data strongly support the successful synthesis of the target oxadiazole derivatives.

3.2 Biology

3.2.1 Anticancer Activity

The in vitro cytotoxicity of the synthesized compounds was evaluated using the MTT assay on the HeLa (human cervical cancer) cell line [30]-[32]. Cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 atmosphere. Cells (1×10^4 cells/well) were seeded in a 96-well plate and treated with different concentrations (1–100 μM) of the test compounds for 24 hours. After incubation, 20 μL of MTT reagent (5 mg/mL) was added to each well and incubated for 4 hours. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. The IC50 values were calculated with compound N24c(3-CH_3) showing the most potent activity. The target compounds (N24A–N24J) were successfully synthesized through the condensation of 3-(((substituted phenyl) amino) methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione (VIII a-j) in Methanol (Scheme 2). The reaction mixture was refluxed for 10-12 hours. The reaction mixture was then poured into crushed ice**,** resulting in the formation of a solid precipitate. The solid product was filtered, washed thoroughly with cold methanol and distilled water to remove unreacted materials, and dried under vacuum. The crude product was purified by recrystallization from methanol (or ethanol) to afford the corresponding in good to excellent yields. Upon completion as monitored by TLC the reaction mass was cooled and the solid product was filtered, washed thoroughly with cold ethanol and recrystallized to afford analytically pure Oxadiazole and substituted aniline based manich derivatives. The yields were generally high (68–72%) confirming the efficiency of the condensation process under these optimized conditions. Concentrated HCl was employed as an acid catalyst to activate formaldehyde and promote iminium ion formation, thereby facilitating Mannich condensation. Methanol was used as a polar protic solvent due to its good solvating ability for all reactants, stabilization of ionic intermediates, and ease of product isolation The physical properties such as melting point, colour and appearance were consistent with formation of new conjugated systems. All compounds displayed sharp melting points, indicating purity. The general synthetic procedure demonstrated excellent reproducibility and adaptability to a wide range of aromatic amine. These findings establish an efficient and environmentally gentle route for constructing novel 1,3,4-oxadiazole-2-thione manich bases suitable for further biological evaluation. Concentrated HCl was employed as an acid catalyst to activate formaldehyde and promote iminium ion formation, thereby facilitating Mannich condensation. Methanol was used as a polar protic solvent due to its good solvating ability for all reactants, stabilization of ionic intermediates, and ease of product isolation.

3.2.2 Antimicrobial Activity

Antimicrobial activity was evaluated by the disc diffusion method against selected bacterial and fungal strains:

- Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*
- Gram-positive bacteria: *Staphylococcus aureus*, *Bacillus cereus*
- Fungal strain: *Candida albicans*

Antimicrobial screening of the synthesized compounds was performed using the disc diffusion method to evaluate their efficacy against representative bacterial and fungal strains [33]-[35]. A saline suspension of bacterial culture was prepared from overnight incubated agar plate (As per 0.5 McFarland turbidity standard. MHA plates were prepared and inoculated with test organisms using a sterile swab. Sterile empty discs were placed on inoculated plates and 30 microlitre of compounds with concentrations from 20mg to 1.25mg/ml were loaded on the discs. Three positive controls ampicillin, tetracycline and gentamicin, solvent control DMSO and water as negative control were also placed. Then plates were incubated at 37°C for 24 -48 hrs. After incubation the zone was measured with the help of zone measuring scale. Among all tested derivatives, compounds N24B (2-NO_2) and N24c (4-CH_3) exhibited the most significant antibacterial and antifungal activities respectively which indicating that the presence of nitro and methyl substituents enhances the biological affinity. The results highlight the potential of these oxadiazole–manich base hybrids as

promising antimicrobial leads for further optimization and development.

2.2.3. Anti-inflammatory Activity

The anti-inflammatory potential of the synthesized compounds were assessed by the egg albumin denaturation assay which is a well-established in vitro model for evaluating the ability of compounds to prevent protein denaturation, a key process involved in inflammatory responses [36]-[38]. Test samples at concentrations ranging from 100 to 500 µg/mL were mixed with 0.2 mL of freshly prepared egg albumin and phosphate-buffered saline (pH 6.4). The mixtures were incubated at 37 °C for 20 minutes, followed by heating at 70 °C for 5 minutes to induce controlled denaturation. After cooling to room temperature, absorbance was recorded at 660 nm, and the percentage inhibition of protein denaturation was calculated relative to a control sample containing only buffer and albumin. Diclofenac sodium served as the reference anti-inflammatory agent, ensuring accurate comparative analysis. Among all derivatives, compound N24a (-H) exhibited the highest inhibition percentage, indicating potent anti-inflammatory activity likely due to enhanced lipophilicity and effective stabilization of protein structures under stress conditions. The results suggest that substitution with halogen atoms significantly contributes to anti-inflammatory efficacy in oxadiazole–manich base hybrids. The synthesized compounds were screened for anticancer, antimicrobial, and anti-inflammatory activities using standard in vitro assays. Results are presented in Table 3.

IV. CONCLUSION

A new series of 3-(((substituted phenyl) amino) methyl)-5-(pyridin-4-yl)-1,3,4-oxadiazole-2(3H)-thione derivatives (VIIIa–j) was successfully synthesized via a Mannich-type reaction employing mild and efficient conditions. The adopted synthetic methodology was straightforward, reproducible and compatible with a wide range of electron-donating and electron-withdrawing substituents on the phenyl ring leading to the formation of the desired products in good yields. All the synthesized compounds were unambiguously characterized using FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometric techniques. The obtained spectral data were in close agreement with the proposed chemical structures confirming the successful construction of the oxadiazole–thione framework. Differences observed in melting points and physical characteristics among the derivatives further reflected the influence of various substituents on the molecular framework. In summary the present work demonstrates an efficient approach for the synthesis of structurally diverse oxadiazole-based derivatives providing a useful scaffold for further exploration. These compounds may serve as promising candidates for subsequent biological evaluation and structure–activity relationship studies thereby contributing to the development of new heterocyclic molecules of potential pharmacological interest.

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Tables & Figures:

Table 1: Substitution pattern of aniline for compounds 8a-j

Comp No.	R_2	R_3	R_4
8a	-H	-H	-H
8b	$-NO_2$	-H	-H
8c	-H	-H	$-CH_3$
8d	-H	$-NO_2$	-H
8e	-H	-H	$-NH_2$
8f	-H	$-CH_3$	-H
8g	-H	-H	$-NO_2$
8h	-H	-H	-Cl
8i	-H	-H	-Br
8j	-Cl	-H	-H

Table 2. Physical and analytical data of synthesized compounds (8a– 8j)

Compound No.	X/R	Colour	Crystallization Solvents	M.P. (_C)	Yield (%)	Mol. Formula (Mol. Wt.)
8a	-H	Yellow	Ethanol	189ºC	80	$C_{14}H_{12}N_4OS$(284.3370)
8b	2-NO_2	Yellow	Ethanol	187ºC	81	$C_{14}H_{11}N_5O_3S$(329.3340)
8c	4-CH_3	Pale yellow	Ethanol	138ºC	79	$C_{14}H_{11}N_5O_3S$(329.3340)
8d	3- NO_2	Dark Yellow	Ethanol	178ºC	85	$C_{14}H_{11}N_5O_3S$(329.3340)
8e	4-NH_2	Pale yellow	Ethanol	179ºC	73	$C_{15}H_{14}N_4OS$(298.3640)
8f	3-CH_3	White	Ethanol	122ºC	77	$C_{15}H_{14}N_4OS$(298.3640)
8g	4-NO_2	Yellow	Ethanol	179ºC	83	$C_{14}H_{11}ClN_4OS$(318.7790)
8h	4-Cl	Pale yellow	Ethanol	165ºC	76	$C_{14}H_{11}ClN_4OS$(318.7790)
8i	4-Br	Pale yellow	Ethanol	168ºC	74	$C_{14}H_{11}BrN_4OS$(363.2330)
8j	2-Cl	White	Ethanol	159ºC	72	$C_{14}H_{11}BrN_4OS$(363.2330)

Table 3. Biological activity data of synthesized compounds

Compound	IC50 (µM, HeLa) µg/ml	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)	% Inhibition (Anti-inflammatory)
		E. coli	*P. aeruginosa*	*B. cereus*	*S. aureus*	*C. albicans*	
N24A	197.50	----	----	----	11	10	-72.7
N24B	413.86	10	10	----	11	10	83.1
N24C	151.18	----	----	9	17	11	13.46
N24D	171.08	----	----	11	23	13	-51.0
N24E	361.80	10	9	----	23	13	13.46
N24F	240.50	----	----	----	----	----	100.0
N24G		----	----	----	----	----	----
N24H		----	----	----	----	----	----
N24I		----	----	----	----	----	----
N24J		----	----	----	----	----	----
Ampicillin (10 mcg)	----	----	----	10	34	26	----
Tetracycline (10 mcg)	----	20	----	14	29	19	----
Gentamicin (10 mcg)	----	28	20	24	22	16	----

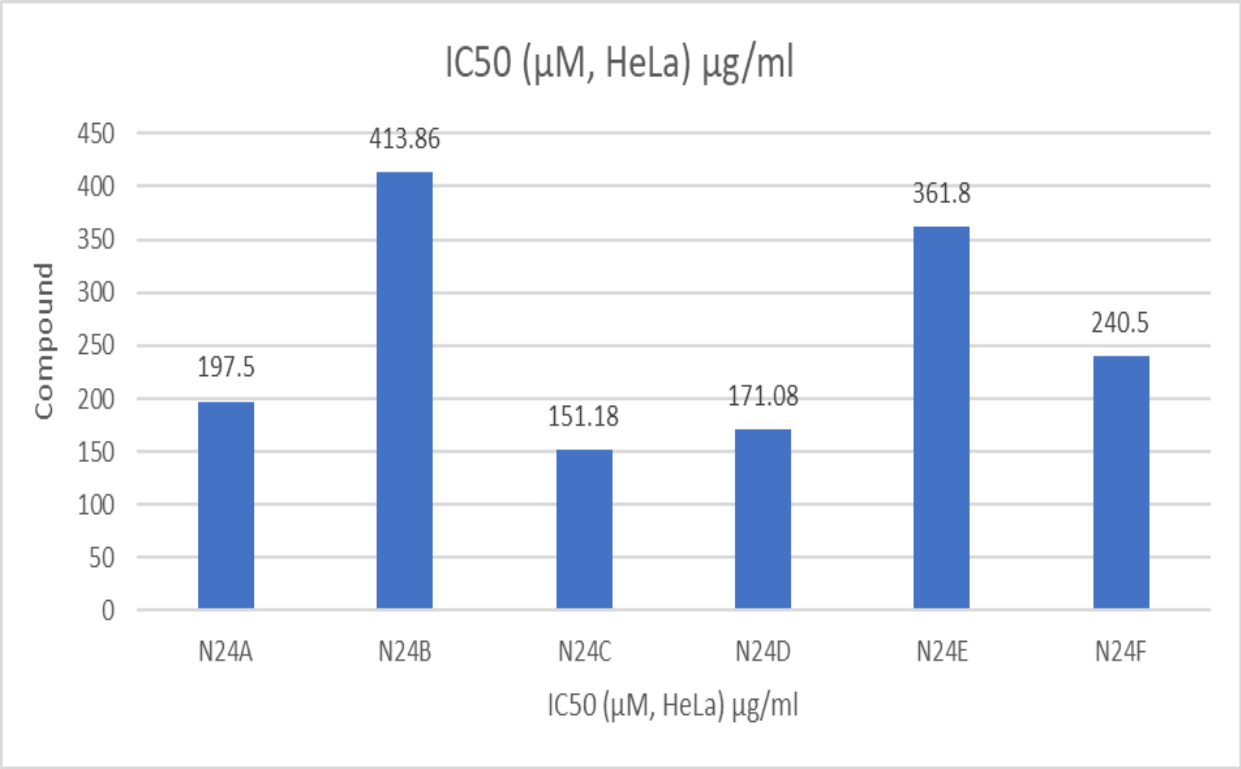

Fig. 1. In vitro anticancer activity of newly synthesized compounds.

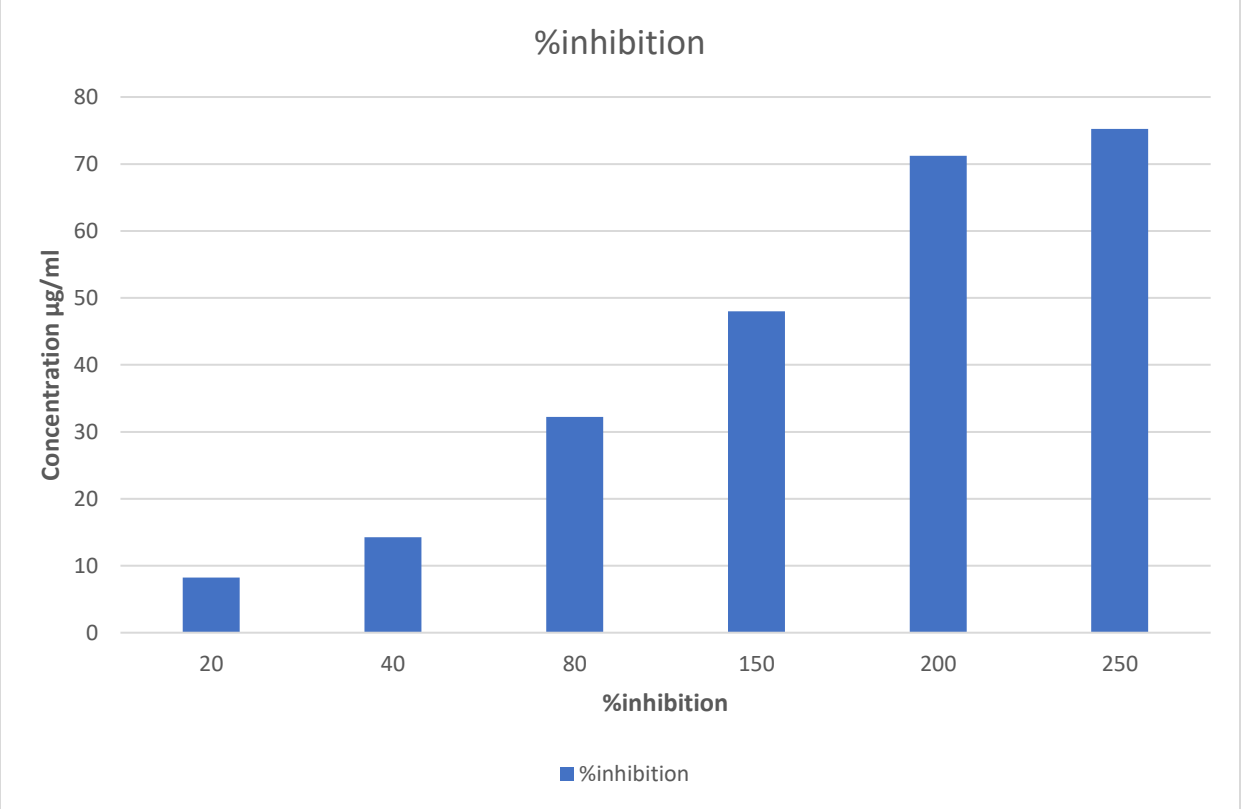

Fig. 2. In vitro anticancer activity against HeLa cell line for compound N24C with different concentration

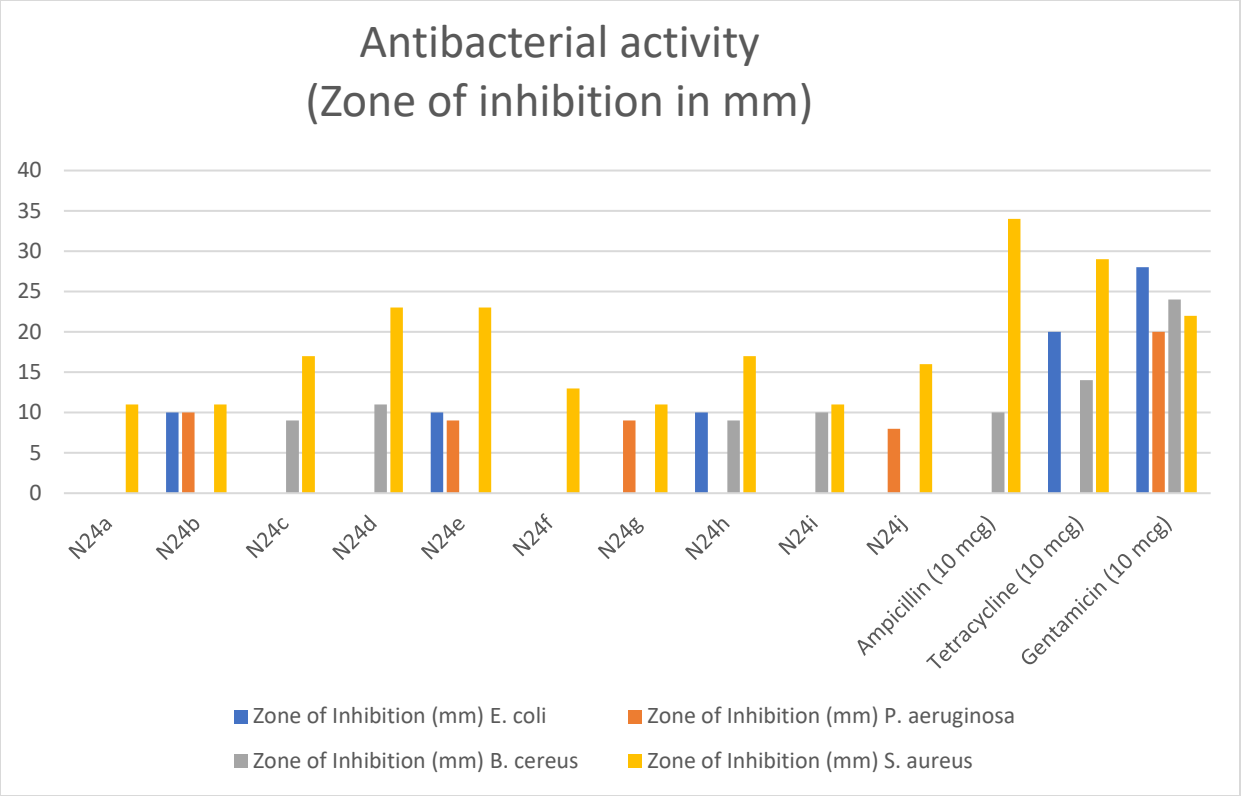

Fig. 3. In vitro antibacterial activity of newly synthesized compounds.

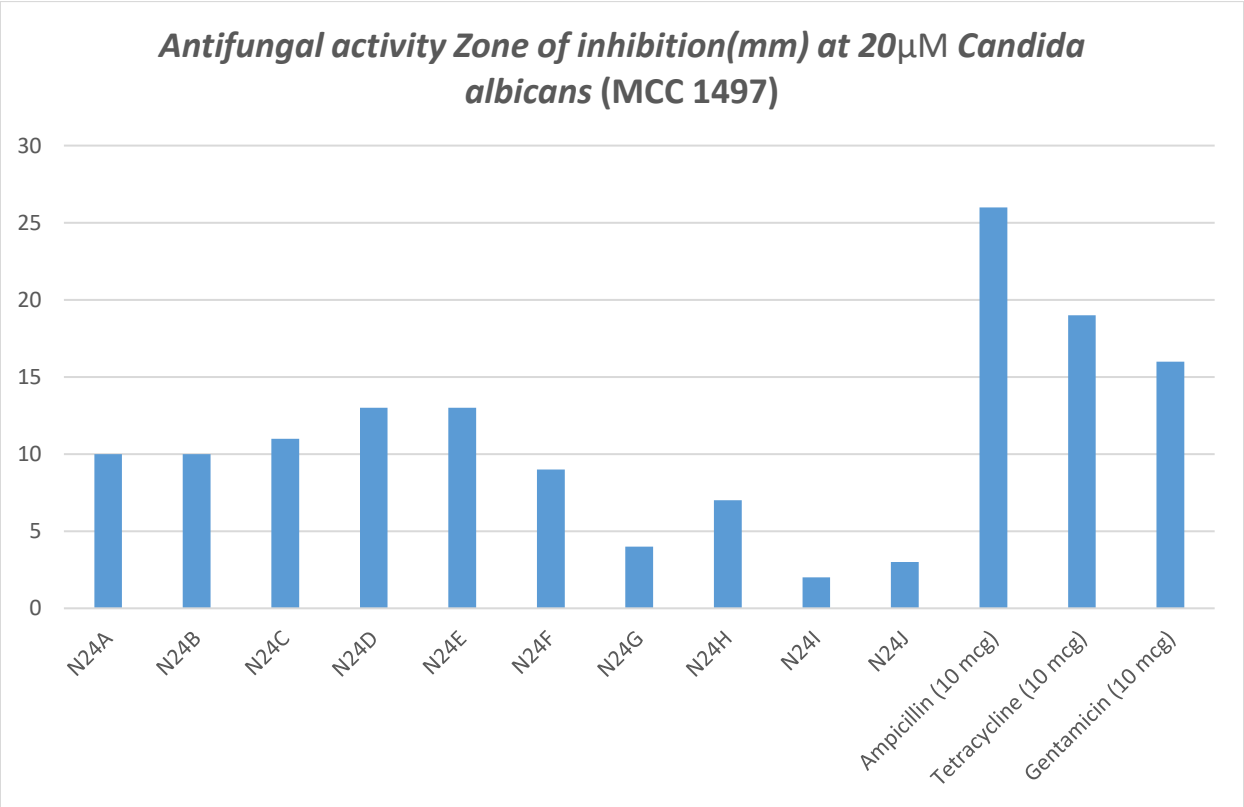

Fig. 4. In vitro antifungal activity of newly synthesized compounds

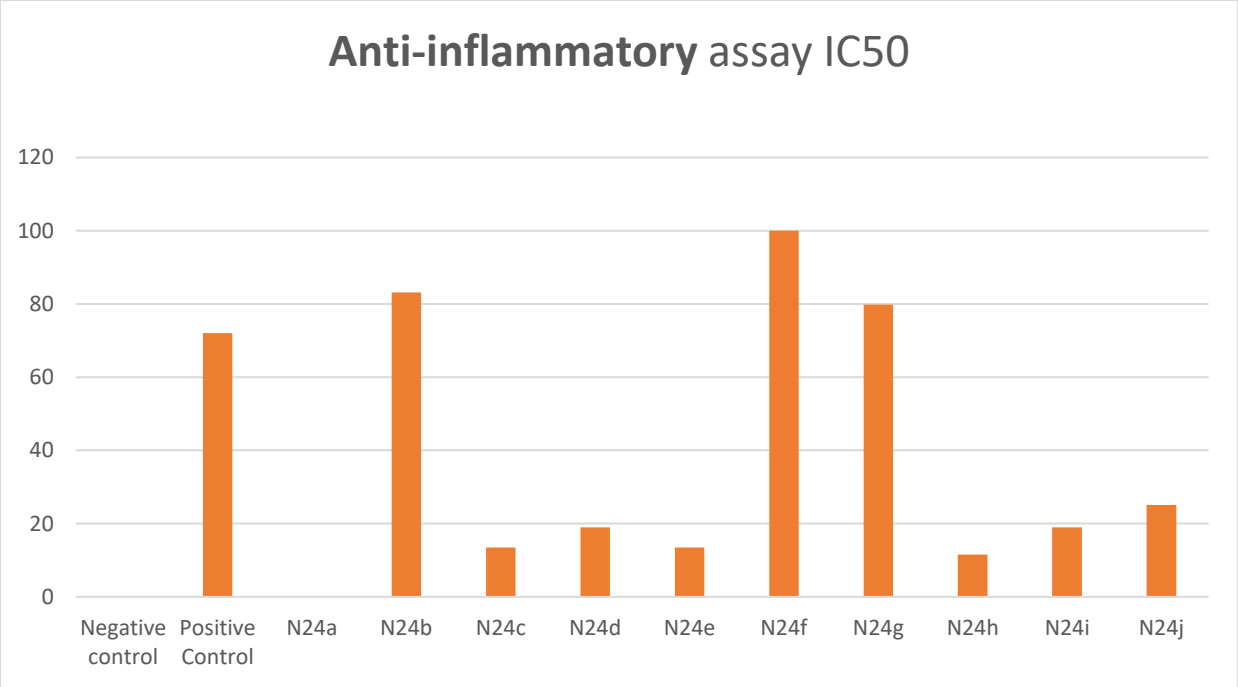

Fig. 5. In vitro Anti-inflammatory activity of newly synthesized compounds