RESEARCH PAPER

1,2,4-oxadiazole derivatives: Design, synthesis, and evaluation of anti-cancer activity

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ABSTRACT

Cancer is a life-threatening disease characterized by the uncontrolled proliferation of cells and their potential to invade surrounding tissues or metastasize to distant sites. Heterocyclic compounds have played a crucial role in the development of novel anticancer agents due to their diverse biological activities. In this study, we designed and synthesized a novel series of 1,2,4-oxadiazole derivatives and evaluated their potential anticancer activity. All the synthesized derivatives were structurally characterized and assessed for *In-vitro* cytotoxicity against MCF-7 and MDA-MB-231 human breast cancer cell lines. Among them, derivative11o exhibited the most potent anticancer activity. Structure–activity relationships (SARs) analysis indicated that the presence of a nitro-substituted phenyl ring at the 5-position of the 1,2,4-oxadiazole scaffold significantly enhanced anticancer activity compared to chloro, methoxy, methyl, and bromo substituents. Furthermore, *In-silico* ADMET profiling was performed for all synthesized derivatives. The biological evaluation suggests that substitution at the 5-position of the 1,2,4-oxadiazole core plays a critical role in modulating anticancer activity.

Keywords: 1,2,4-oxadiazole, anticancer activity, heterocyclic compounds, breast cancer, SARs

Abbreviations: MDAMB231, Human breast cancer cell line (isolated at M D Anderson from a pleural effusion of a patient with invasive ductal carcinoma); ADMET, Absorption Distribution Metabolism Excretion Toxicity; FDA, Food and Drug Administration; LDA, Lithium di-isopropylamide; THF, Tetrahydrofuran; DMP, Dess-Martin periodinane; SRB, sulforhodamine B; TCA, Trichloroacetic acid; BBB, Blood–brain barrier

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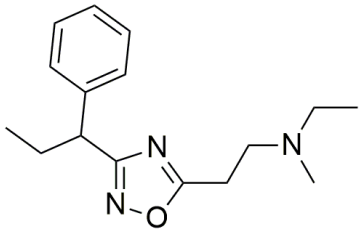
Conflict of interest: None

1. INTRODUCTION

Cancer is one of the most prevalent and life-threatening diseases, primarily caused by the uncontrolled growth of cells, leading to the formation of tumors. Various factors such as exposure to radiation, aging, environmental pollution, tobacco use, and stress have been identified as major contributors to cancer initiation and progression [1–3]. Cancer is a second leading cause of death world-wide following cardiovascular diseases in both developed and developing countries, due to its high potential to metastasize to other parts of the body. Cancer treatment typically involves three primary modalities: radiation therapy, surgery, and chemotherapy. Among these, chemotherapy plays a vital role, employing chemotherapeutic agents to target and kill cancer cells. Over the years, numerous chemotherapeutic drugs have been developed and extensively studied for their efficacy and mechanisms of action [4–8].

Similarly, 1,2,4-oxadiazoles represent a significant class of five-membered heterocyclic scaffold that have attracted considerable interest among medicinal chemists due to their broad spectrum of pharmacological activities. This heterocyclic scaffold is found in various bioactive molecules, functional materials, and pharmaceutical products [9–12]. It has been associated with a wide range of therapeutic applications [13] and are considered as bio-isosteres of esters and amides which making them valuable in drug designing [14]. Derivatives of 1,2,4-oxadiazoles have demonstrated diverse biological activities, including antitumor [15,16], immunosuppressive [17], analgesic [18], insecticidal [19], antimicrobial [20], antihelmintic [21], muscarinic receptor modulation [22], antiparasitic [23],

anti-inflammatory [24], antidiabetic [25], and histamine H3 receptor antagonism [26]. Notably, the FDA-approved analgesic and muscle relaxant drug proxazole contains 1,2,4-oxadiazole scaffold as an essential structural component for the pharmacological activity [27].



Proxazole

In light of the biological significance of 1,2,4-oxadiazole scaffold and ongoing efforts to discover novel anticancer agents, a series of derivatives titled "2-(3-(4-((5-aryl-1,2,4-oxadiazol-3-yl)methoxy)phenyl)isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4 amines" (**11a to 11o**) were designed and synthesized. The chemical structures of all the synthesized derivatives were confirmed using FT-IR, ^{1}HNMR, mass spectrometry, and CHN elemental analysis. Further, the anticancer potential of these derivatives was evaluated *In-vitro* against the MCF-7 and MDA-MB-231 human breast cancer cell line. We also performed *In-silico* ADMET predictions to assess their pharmacokinetic properties.

2. RESULT AND DISCUSSION

2.1. Chemistry

In this study, fifteen derivatives of 1,2,4-oxadiazole have been synthesized as showed in **Scheme 1**. The chemical structure of all the compounds and synthesized derivatives were confirmed by IR, ^{1}HNMR, ^{13}CNMR, mass spectrometry, and CHN elemental analysis. The physicochemical properties of all the compounds and synthesized derivatives are depicted in

Table 1. Physiochemical properties of compounds and 1,2,4-oxadiazoles derivatives

S. No.	Comp. No.	R (11a-11o)	Molecular Formula	Molecular Weight
1.	**Compound 3**	**-**	$C_{13}H_{12}BrNO_3S$	342.21
2.	**Compound 4**	**-**	$C_{13}H_{10}BrNO_3S$	340.19
3.	**Compound 5**	**-**	$C_{13}H_9BrN_2O_2S$	337.19
4.	**Compound 7**	**-**	$C_{22}H_{21}N_3O_2S$	391.49
5.	**Compound 8**	**-**	$C_{21}H_{19}N_3O_2S$	377.46
6.	**Compound 10**	**-**	$C_{23}H_{20}N_4O_2S$	416.50
7.	**11a**	2-Chloro	$C_{30}H_{24}ClN_5O_3S$	570.06
8.	**11b**	2,4-dichloro	$C_{30}H_{23}Cl_2N_5O_3S$	604.51
9.	**11c**	2-Methoxy	$C_{31}H_{27}N_5O_4S$	565.64
10.	**11d**	3-Methoxy	$C_{31}H_{27}N_5O_4S$	565.64
11.	**11e**	3,4-dimethoxy	$C_{32}H_{29}N_5O_5S$	595.67
12.	**11f**	2-Bromo	$C_{30}H_{24}BrN_5O_3S$	614.51
13.	**11g**	2,4-dibromo	$C_{30}H_{23}Br_2N_5O_3S$	693.41
14.	**11h**	2-Nitro	$C_{30}H_{24}N_6O_5S$	580.61
15.	**11i**	4-Nitro	$C_{30}H_{24}N_6O_5S$	580.61

16.	**11j**	2,4-dinitro	$C_{30}H_{23}N_7O_7S$	625.61
17.	**11k**	2,3-dinitro	$C_{30}H_{23}N_7O_7S$	625.61
18.	**11l**	2-Methyl	$C_{31}H_{27}N_5O_3S$	549.64
19.	**11m**	2,4-dimethyl	$C_{32}H_{29}N_5O_3S$	563.67
20.	**11n**	2-bromo-3,5-dinitro	$C_{30}H_{22}BrN_7O_7S$	704.51
21.	**11o**	2-chloro-3,5-dinitro	$C_{30}H_{22}ClN_7O_7S$	660.06

IR spectral data of compound **3** characteristic peak at υ cm^{-1} 934, 1280, 1360¸ 1580, and 1710 due to C-Br, C-O, C-N-C, C=C, C=O functional group respectively. IR spectra of compound **3** also showed aromatic C-H peak at 3120 and O-H peak at 3540 cm^{-1}. Compound**5** showed IR peak at υ cm^{-1} 1310, 1520, 1720 due to presence of C-N-C, C=N, C-O-N respectively. Compound **7** showed IR peak at υ cm^{-1} at 2920 and 3340 due to C-H of methyl group and N-H group. In compound **8** peak due to C-O group was disappeared. Compound**10** showed IR peak at υ cm^{-1} peak at 3444 due to presence of N-H group and peak due to O-H functional group was disappeared.

Compound **3** showed the ^{1}HNMR spectral peak at δ 7.02 and 8.02 which indicate the presence of aromatic proton, peak at 5.12 indicate the presence of OH group. This OH group peak was disappeared in the compound **4**. In compound **5** the ^{1}HNMR peak of CH_2 group was disappear due to cyclization which confirm the presence of oxazole ring. Compound **7** showed the ^{1}HNMR peak at δ 2.16 which indicate the presence of alkyl (CH_3) group in aromatic ring. Compound **8** showed the ^{1}HNMR peak at δ 6.31 due to presence of phenolic OH group.

The ^{13}CNMR spectra of compound **11a** showed various peak peak at 161 ppm and 170.5 ppm due to C-3 and C-5 carbons of 1,2,4-oxadiazole ring. C-3 and C-5 carbons of isoxazole showed peak at 150.4 ppm and 104.7 ppm. Carbons in thiazole are aromatic showed peak at 122.4 and 140.4 ppm. The methylene (–OCH_2–) carbon showed peak 72.4 ppm. The three methyl carbons on the 3,4,5-trimethylphenyl showed peak at 17.5 and 23.6 ppm. The aromatic carbons of this tri-methyl phenyl ring showed peak at 128.4 and 132.5 ppm. The carbons ortho to chlorine are deshielded, shifting further downfield and showed peak 129.2 ppm and meta carbon showed peak at 129.7 ppm. 161.3 peak due tooxazole carbon attached to phenyl ring and at 170.5 due to 1,3,5-oxadiazole carbon which are attached to phenyl ring. Peak of C-Cl was get at 131.2 ppm. The MASS spectral data of compound **3** showed the base peak at m/z 233 and molecular ion peak at m/z 341. The compound **4** and **5** displayed the molecular ion peak at 340, 337 and base peak at m/z 135, 319 respectively. Compounds **7**, **8** and **10** showed molecular ion peak at m/z 392, 378 and 417 respectively. The chemical structure of the entire compound was also confirmed by the MASS spectral data.

The synthesized derivatives **11a**, **11b** showed IR peak at υ cm^{-1} 721, 825, due to presence of C-Cl group. Compound **11c**, **11d** and **11e**showed IR peak at υ cm^{-1} 1250, 1260 and 1088 respectively, which indicate the presence of OCH_3 group. Derivative **11f** and **11g** showed IR peak at υ cm^{-1} 610 and 690 due to presence of bromo group.

Derivative **11h, 11i, 11j** and **11k** displayed IR peak at υ cm^{-1} 1520, 1579, 1577, 1577 due to presence of NO_2 functional group. Derivative **11n** showed IR peak at υ cm^{-1} 622 due to presence of bromo group and at 1544 due to presence of NO_2 group. Derivative **11o** showed IR peak at υ cm^{-1} 720 due to presence of Cl group and at 1409 due to presence of NO_2 group.

2.2. Biological Evaluation

Sulforhodamine B (SRB) assay

The anti-proliferative activities of all synthesized 1,2,4-oxadiazole derivatives were evaluated against MCF-7 and MDA-MB-231 human breast cancer cell lines using the Sulforhodamine B (SRB) assay. The cytotoxic efficacy of each derivative was quantified in terms of GI_{50} values (μM) which are shown in **Table 2**.

Table 2. The *In-vitro* anti-proliferative activity (GI_{50}), CC_{50} and SI index of synthesized derivatives of 1,2,4 oxadiazole against MCF-7 and MDA-MB-231 human breast cancer cell line

Derivatives No.	GI_{50} (μM)		CC_{50} (μM) Vero cell lines	SI	
	MCF-7	MDA-MB-231		MCF-7	MDA-MB-231
11a	0.097 ± 0.21	0.072 ± 0.27	0.41	4.23	5.69
11b	0.071 ± 0.27	0.064 ± 1.49	0.18	2.54	2.81
11c	0.089 ± 0.24	0.082 ± 0.51	0.52	5.84	6.34
11d	0.110 ± 0.42	0.090 ± 0.34	1.08	9.82	12.00

11e	0.119 ± 0.24	0.097 ± 0.28	0.62	5.21	6.39
11f	92.16± 0.22	NA	87.09	0.94	NA
11g	82.10± 0.22	NA	95.71	1.17	NA
11h	0.072± 0.12	0.060 ± 0.35	0.23	3.19	3.83
11i	0.060± 0.25	0.056 ± 0.68	0.42	7.00	7.50
11j	0.054± 0.22	0.048 ± 0.27	0.51	9.44	10.63
11k	0.058± 0.20	0.052 ± 0.21	0.57	9.83	10.96
11l	78.5 ± 0.10	70.5 ± 0.45	23.15	0.29	0.33
11m	72.0 ± 0.73	65.0 ± 0.73	108.08	1.50	1.66
11n	0.128± 0.32	0.109 ± 0.24	1.01	7.89	9.27
11o	0.030± 0.22	0.038 ± 0.75	0.42	14.00	11.05
[a]ADR	0.005± 0.10	0.006 ± 0.12	ND	ND	ND

GI_{50}=The required concentration of derivatives for inhibition of cell proliferation by 50%.
CC_{50} =The concentration atwhich 50% cells survive
NA: No activity.
ND: Not Determined.
[a]ADR = Adriamycin, positive control compound

Among the tested 1,2,4-oxadiazole derivatives, derivative **11o** exhibited the most potent anti-cancer activity against both MCF-7 and MDA-MB-231 cancer cell lines, with a GI_{50} value of 0.030 ± 0.22 and 0.038 ± 0.75 µM respectively. Structural analysis revealed that 1,2,4-oxadiazole, scaffolds are essential for anticancer activity against both cancer cell lines. Substitution of bulkier group (isoxazole and thiazole) at 3rd position of 1,2,4-oxadiazole and less bulky group (substituted phenyl) at 5th position of 1,2,4-oxadiazole are favourable for anticancer activity. Substitution of a 2-chloro-3,5-dinitrophenyl group at the R-position of the 1,2,4-oxadiazole ring significantly enhanced anti-proliferative activity, in comparison to derivatives bearing 2,4-dinitrophenyl or 2,3-dinitrophenyl groups. Conversely, derivatives containing 2-bromophenyl or 2,4-dibromophenyl substituents at the 5th position of the oxadiazole ring exhibited no detectable activity against the MDA-MB-231 cell line, suggesting the critical influence of substitution pattern and electronic properties on cytotoxic potential. The selectivity index (SI) was calculated by dividing CC_{50} of VERO cell line by GI_{50} of both MCF-7 and MDA-MB-231 cell line SI= (CG_{50}) / (GI_{50}).

2.3. Physicochemical and *In-silico* ADMET properties prediction

As per shown in **Table 3**, all synthesized 1,2,4-oxadiazole derivatives exhibited molecular weights ranging from 549 to 704 g/mol, suggesting favourable molecular sizes for potential drug transport and absorption. Analysis of hydrogen bonding characteristics revealed that all derivatives possessed <5 hydrogen bond donors, and 11 derivatives had <10 hydrogen bond acceptors, aligning well with standard drug-likeness criteria. The logP values of the title derivatives were found to range between 4.55 and 7.33, indicating moderate to high lipophilicity. Notably, none of the derivatives demonstrated blood–brain barrier (BBB) permeability, which may be advantageous for targeting non-CNS tumors such as breast cancer.

Table 3. Data obtained from predicted absorption, distribution, metabolism, excretion and toxicity parameters

Comp. No.	No. of HBA	No. of HBD	TPSA	Consensus LogP	GIT Absorption	ESOL class	BBB permeation	BA score	Lipinski violations	LD_{50} (mg/kg)
11a	7	1	127.34	6.69	Low	Poorly soluble	No	0.55	1	2050
11b	7	1	127.34	7.17	Low	Poorly soluble	No	0.17	2	2050
11c	8	1	136.57	6.17	Low	Poorly soluble	No	0.55	1	1000

11d	8	1	136.57	6.17	Low	Poorly soluble	No	0.55	1	3000
11e	9	1	145.8	6.1	Low	Poorly soluble	No	0.55	1	6200
11f	7	1	127.34	6.75	Low	Poorly soluble	No	0.55	1	1000
11g	7	1	127.34	7.33	Low	Poorly soluble	No	0.17	2	1000
11h	9	1	173.16	5.41	Low	Poorly soluble	No	0.17	2	3000
11i	9	1	173.16	5.49	Low	Poorly soluble	No	0.17	2	1000
11j	11	1	218.98	4.64	Low	Poorly soluble	No	0.17	2	3000
11k	11	1	218.98	4.55	Low	Poorly soluble	No	0.17	2	3000
11l	7	1	127.34	6.47	Low	Poorly soluble	No	0.55	1	1000
11m	7	1	127.34	6.83	Low	Poorly soluble	No	0.55	1	1000
11n	11	1	218.98	5.2	Low	Poorly soluble	No	0.17	2	3000
11o	11	1	218.98	5.07	Low	Poorly soluble	No	0.17	2	3000

BA score: Bioavailability score; BBB: Blood–brain barrier; ESOL class: Solubility class in LogS scale; ESOL LogS: ESOL model logarithm of molar solubility in water; HBA: Hydrogen bond acceptor; HBD: Hydrogen bond donor; LogP: Logarithm of the partition coefficient; MS: Moderately soluble; MW: Molecular weight; PS: Poorly soluble; TPSA: Topological polar surface area.

Importantly, *In-silico* ADME profiling using SwissADME confirmed that none of the derivatives violated more than two of Lipinski's rules, supporting their classification as 'drug-like' molecules. Derivatives **11b, 11g, 11h, 11i, 11j, 11k, 11n** and **11o** violate two Lipinski rule and remaining derivatives violet only one Lipinski rule. The Bioavailability Score (BA score) represents the probability that a compound will have at least 10% oral bioavailability in rats. A BA score of 0.55, as observed in most derivatives, indicates moderate bioavailability, suggesting favourable oral absorption. The ESOL model categorizes compounds based on water solubility. All the derivatives fell under "poorly soluble" classes, likely due to the presence of hydrophobic groups. The LD_{50} values, predicted using the ProTox-II tool, are expressed in mg/kg body weight, indicating the median lethal dose required to cause death in 50% of a test population. Out of the 15 synthesized compounds, 7 compounds were found to violate only one of Lipinski's rules of five. These findings suggest that the synthesized derivatives possess favourable pharmacokinetic properties and could serve as promising candidates for further optimization and lead development in anticancer drug discovery.

3. EXPERIMENTAL

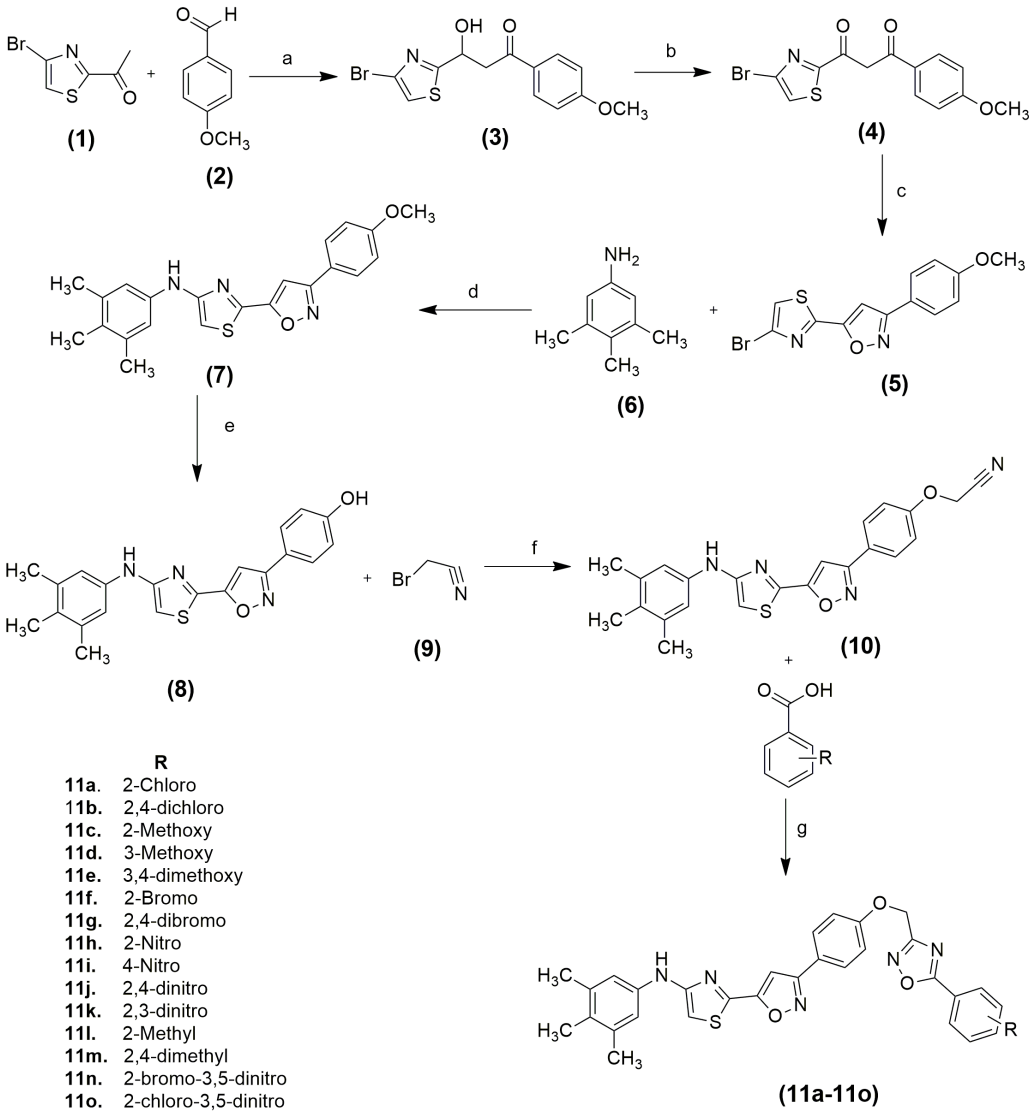
3.1. Materials and methods

All chemicals, reagents, catalysts, and solvents used in the study were of analytical grade and procured from Sigma-Aldrich, S.D. Fine-Chem Ltd., and E. Merck India Ltd. The synthesis of all derivatives was carried out under anhydrous conditions. The progress of the reactions was monitored by thin-layer chromatography (TLC) using a mobile phase of ethyl acetate and chloroform. TLC spots were visualized under a UV light cabinet. The melting points (M.P.) of the synthesized derivatives were determined using open glass capillaries with a standard melting point apparatus and are

reported in degrees Celsius (°C). The derivatives were purified by recrystallization using ethanol. FT-IR spectra were recorded in the range of 4000–400 cm^{-1} on a PerkinElmer RX1 Fourier Transform Infrared (FTIR) spectrophotometer using KBr pellet method. ^{1}HNMR spectra were recorded on a Bruker 500 MHz spectrometer using $CDCl_3$ as a solvent, with tetramethylsilane (TMS) as the internal standard. Molecular weights of the synthesized derivatives were confirmed by mass spectrometry (MASS) using methanol as the solvent.

3.2. Chemistry and synthetic scheme

In this present work we have synthesized fifteen derivatives of 1,2,4-oxadiazole, the synthesis process is shown in the **Scheme 1** (Table **1**).



Scheme 1: (a) LDA, THF, -78ºC to rt; (b) DMP, DCM, rt; (c) $NH_2OH.HCl$, EtOH, rt; (d) n-butanol, Et_3N, Reflux; (e) BBr_3, DCM, rt; (f) Acetone, K_2CO_3, reflux; (g) $NH_2OH.HCl$, 4-(dimethylamino) pyridinium acetate, 100ºC

Synthesis of 3-(4-Bromothiazol-2-yl)-3-hydroxy-1-(4-methoxyphenyl) propan-1-one (3)

To a solution of lithium diisopropylamide (LDA) (0.1365 mmol) in anhydrous tetrahydrofuran (THF) (150 mL), ketone (**1**) (0.1050 mmol) was added in dropwise manner at –78 °C under stirring. The mixture was stirred for 1 hour at the same temperature. A mixture of dry ice and methanol was used to maintain –78°C temperature. Subsequently, 4-methoxybenzaldehyde (**2**) (0.0954 mmol) was added to the reaction mixture, and the temperature was gradually raised to 25 °C. The progress of the reaction was monitored by TLC. After the completion of reaction, the organic layer was separated and dried over anhydrous magnesium sulfate ($MgSO_4$). Product was filtered and concentrated under reduced pressure. The crude product was purified by recrystallization to yield pure compound **3**.

Synthesis of 1-(4-Bromothiazol-2-yl)-3-(4-methoxyphenyl)propane-1,3-dione (4)

Compound **3** (0.04970 mol) was dissolved in alcohol, and anhydrous dichloromethane (100 mL) was added. To this solution, Dess–Martin periodinane (DMP) (0.07456 mol) was added gradually with stirring. The reaction mixture was stirred at 37 °C for 1 hour. After completion, the reaction mixture was extracted with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization to afford pure compound **4**.

Synthesis of 5-(4-Bromothiazol-2-yl)-3-(4-methoxyphenyl) isoxazole (5)

Compound **4** (0.03911 mmol) was dissolved in ethanol, and hydroxylamine (50.07823 mmol) was added to this solution. The reaction mixture was stirred at 37 °C for 72 hours, and the progress of the reaction was monitored by TLC. After completion of the reaction, mixture was treated with distilled water and extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization to get pure compound **5**.

Synthesis of 2-(3-(4-Methoxyphenyl)isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine (7)

Compound **5** (0.03264 mmol) was dissolved in anhydrous n-butanol, and 3,4,5-trimethylaniline hydrochloride (**6**) (0.04896 mmol) was added. Triethylamine (0.09792 mmol) was added dropwise to the reaction mixture and reflux the reaction mixture for 8 hours with stirring. Upon completion of the reaction, the mixture was cooled and water was added to it. The aqueous layer was extracted with ethyl acetate, and the combined organic layers were dried over anhydrous magnesium sulfate ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by recrystallization to get pure compound **7**.

Synthesis of 4-(5-(4-(3,4,5-Trimethylphenylamino) thiazol-2-yl)isoxazol-3-yl)phenol (8)

An anhydrous dichloromethane solution of boron tribromide (0.06887 mmol) was added drop wise to a stirred solution of compound **7** (0.02295 mmol) in dichloromethane (40 mL) and maintain temperature 0 °C. The reaction mixture was then stirred at 37 °C for 6 hours. After completion, the reaction mixture was carefully quenched with water in an ice-water bath, followed by extraction with ethyl acetate. The organic layer was sequentially washed with aqueous sodium hydrogen carbonate solution and saturated sodium chloride solution, then dried over anhydrous magnesium sulfate ($MgSO_4$), and concentrated under reduced pressure. The resulting crude product was purified by recrystallization to afford pure compound **8**.

Synthesis of 2-(4-(5-(4-(3,4,5-Trimethylphenylamino) thiazol-2-yl) isoxazol-3-yl) phenoxy) acetonitrile (10)

Compound **8** (0.0185 mmol) was dissolved in acetone (100 mL). To this solution, 2-bromoacetonitrile (**9**) (0.0185 mmol) and potassium carbonate (0.037 mmol) were added. The reaction mixture was heated under reflux for 12 hours. Upon completion of the reaction, the mixture was cooled to room temperature, and the insoluble potassium carbonate was removed by filtration. The filtrate was concentrated under reduced pressure to remove the solvent. The resulting crude product was purified by recrystallization and pure compound **10**was obtained.

General procedure for synthesis of compound 11a-11o

In a 500 mL round-bottom flask, compound **10** (7.19 mmol), hydroxylamine hydrochloride (14.38 mmol), and different derivatives of benzoic acid (**11a–11o**) (7.19 mmol) were added along with 4-(dimethylamino)pyridinium acetate (35.95 mmol). The reaction mixture was heated to 100 °C and stirred for 24 hours. After completion, the temperature was reduced to 37 °C, followed by the addition of ethanol, and stirring was continued for an additional 30 minutes. Water was added to the reaction mixture to dissolve the ionic liquid. The resulting solid was collected by filtration and washed with water three times. The crude products were purified by recrystallization to afford the pure derivatives**11a–11o**.

3.3. Structural analysis of the compounds and synthesized derivatives

3-(4-bromo-1,3-thiazol-2-yl)-3-hydroxy-1-(4-methoxyphenyl)propan-1-one (3)

Solid, Yield: 77%; M.P.: 272-274ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 934 (C-Br), 1280 (C-O of OCH_3), 1360 (C-N-C), 1580 (Ar C=C), 1710 (C=O), 3120 (Ar C-H), 3190 (C-H thiazole), 3540 (O-H); ^{1}HNMR (500 MHz, DMSO-d6): δ 2.66 (d, 2H, J = 7 Hz), 3.79 (s, 3H), 5.12 (s, 1H), 6.72 (s, 1H), 7.02 (d, 2H, J = 8 Hz), 8.02 (d, 2H, J = 8 Hz); MS (ESI) (m/z, %): 341 [M^+], 233.9 (100).

1-(4-bromo-1,3-thiazol-2-yl)-3-(4-methoxyphenyl) propane-1,3-dione (4)

Solid, Yield: 87%; M.P.:278-280ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 940 (C-Br), 1220 (C-O of OCH_3), 1310 (C-N-C), 1530 (Ar C=C), 1740 (C=O), 3140 (Ar C-H), 3170 (C-H thiazole); ^{1}HNMR (500 MHz, DMSO-d6): δ 2.66 (d, 2H, J = 7 Hz), 3.78 (s, 3H), 6.62 (s, 1H), 7.02 (d, 2H, J = 8 Hz), 8.02 (d, 2H, J = 8 Hz); MS (ESI) (m/z, %): 340.0 [M^+], 135.0 (100).

5-(4-bromo-1,3-thiazol-2-yl)-3-(4-methoxyphenyl)-1,2-oxazole (5)

Solid, Yield: 70%; M.P.: 288-290ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 912 (C-Br), 1240 (C-O of OCH_3), 1310 (C-N-C), 1520 (C = N), 1720 (C-O-N), 1810 (Ar C=C), 3010 (Ar C-H), 3220 (C-H thiazole). ^{1}HNMR (500 MHz, DMSO-d6): δ 3.72 (s, 3H), 7.12 (d, 1H, J = 8 Hz), 7.52 (d, 2H, J = 9 Hz), 7.71 (d, 2H J = 8 Hz); MS (ESI) (m/z, %): 337.0[M^+],319.9 (100).

2-[3-(4-methoxyphenyl)-1,2-oxazol-5-yl]-N-(3,4,5-trimethylphenyl)-1,3-thiazol-4-amine (7)

Solid, Yield: 67%; M.P.: 233-235ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1242 (C-O of OCH_3), 1352 (C-N-C), 1680 (C = N), 1790 (C-O-N), 1910 (Ar C=C), 2920 (C-H Methyl group), 3010 (Ar C-H), 3220 (C-H thiazole), 3340 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.16 (s, 3H), 2.32 (s, 6H), 3.72 (s, 3H), 6.31 (d, 2H, J = 7 Hz), 6.92 (d, 2H, J = 8 Hz), 7.40 (s, 1H), 7.52 (s, 1H), 7.73 (d, 2H, J = 8 Hz); MS (ESI) (m/z, %): 392.1[M^+],376.1 (100).

4-[5-[4-(3,4,5-trimethylanilino)-1,3-thiazol-2-yl]-1,2-oxazol-3-yl]phenol (8)

Solid, Yield: 75%; M.P.: 243-245ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1252 (C-N-C), 1660 (C = N), 1770 (C-O-N), 1910 (Ar C=C), 2910 (C-H Methyl group), 3010 (Ar C-H), 3220 (C-H thiazole), 3340 (N-H), 3420 (O-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.16 (s, 3H), 2.32 (s, 6H), 6.31 (d, 2H, J = 7 Hz), 7.40 (s, 1H), 6.92 (d, 2H, J = 8 Hz), 7.34 (s, 1H), 7.73 (d, 2H, J = 8 Hz); MS (ESI) (m/z, %): 378.4[M^+],348.0 (100).

2-[4-[5-[4-(3,4,5-trimethylanilino)-1,3-thiazol-2-yl]-1,2-oxazol-3-yl]phenoxy]acetonitrile (10)

Solid, Yield: 77%; M.P.: 253-255ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1472 (C-N-C), 1520 (Ar C=C), 1629 (C = N), 1720 (C-O-N), 1940 (Ar C=C), 2910 (C-H Methyl group), 3097 (Ar C-H), 3180 (C-H thiazole), 3218 (C-H isoxazole), 3444 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.12 (s, 3H), 2.32 (s, 6H), 4.84 (s, 2H), 6.32 (d, 2H, J = 7 Hz), 7.12 (d, 2H, J = 8 Hz), 7.40 (s, 1H), 7.63 (d, 2H, J = 8 Hz); MS (ESI) (m/z, %): 417.0[M^+],374.1 (100).

2-(3-(4-((5-(2-chlorophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11a)

Solid; yield 54%; M.P. 263-265ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 721 (C-Cl), 1449 (C-N-C), 1485 (Ar C=C), 1629 (C = N), 1700 (C-O-N), 3097 (Ar C-H), 3180 (C-H thiazole), 3215 (C-H isoxazole), 3444 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.45 (s, 3H), 2.63 (s, 6H), 5.05 (s, 2H), 7.18 (d, 2H, J = 9.0 Hz), 7.20 (s, 2H), 7.45 (s, 1H), 7.71 (d, 2H, J = 8.00 Hz), 7.82 (d, 2H, J = 8.00 Hz), 7.99 (d, 2H, J = 8 Hz), 8.10 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 17.5, 23.6, 72.4, 104.7, 115.7, 117.8, 122.4, 127.5, 128.4, 129.2, 129.7, 131.2, 131.7, 132.5, 138.5, 140.4, 143.4, 149.6, 150.4, 156.2, 158.7, 161.3, 170.5; MS (ESI) (m/z, %): 570.1 [M^+],138.2(100). Anal. Calc. for $C_{30}H_{24}ClN_5O_3S$: C, 63.21; H, 4.24; Cl, 6.22; N, 12.29; O, 8.42; S, 5.62. Found: C, 63.24; H, 4.21; Cl, 6.19; N, 12.31; O, 8.44; S, 5.61.

2-(3-(4-((5-(2,4-dichlorophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11b)

Solid; Yield 82%; M.P. 272-274ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 825 (C-Cl), 1450 (C-N-C), 1500 (Ar C=C), 1610 (C = N), 1720 (C-O-N), 3020 Aliphatic C-H stretch (Methyl), 3120 (Ar C-H), 3270 (C-H isoxazole), 3502 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.45 (s, 3H), 2.63 (s, 6H), 5.05 (s, 2H), 7.18 (d, 2H, J = 9.0 Hz), 7.20 (s, 1H), 7.45 (s, 1H), 7.71 (d, 2H, J = 8.00 Hz), 7.82 (d, 2H, J = 8.00 Hz), 7.99 (d, 2H, J = 8 Hz), 8.10 (s, 1H), 8.25 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 17.5, 23.6, 57.6, 61.4, 72.5, 104.5, 106.7, 115.3, 117.8, 122.4, 126.8, 127.5, 128.6, 129.4, 138.5, 140.3, 143.8, 149.7, 150.6, 153.4, 156.8, 158.7, 163.7, 170.2; MS (ESI) (m/z, %): 604.0[M^+],416.1 (100). Anal. Calc. for $C_{30}H_{23}Cl_2N_5O_3S$: C, 59.61; H, 3.83; Cl, 11.73; N, 11.59; O, 7.94; S, 5.30. Found: C, 59.55; H, 3.89; Cl, 11.78; N, 11.54; O, 7.90; S, 5.34.

2-(3-(4-((5-(2-methoxyphenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine (11c)

Solid, Yield 78%; M.P.: 206-208ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1250 (C-O of OCH_3), 1320 (C-N-C), 1510 (Ar C=C), 1620 (C = N), 1735 (C-O-N), 2860 (C-H of OCH_3), 3070 (Ar C-H), 3170 (C-H thiazole), 3220 (C-H isoxazole), 3475 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 3.91 (s, 3H), 5.02 (s, 2H), 7.17 (d, 2H, J = 8 Hz), 7.20 (s, 2H), 7.32 (d, 2H, J = 7 Hz), 7.44 (s, 1H), 7.69 (d, 2H, J = 7 Hz), 7.98 (d, 2H, J = 8 Hz), 8.10 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ17.5, 23.6, 57.8, 72.6, 104.6, 104.8, 105.3, 115.6, 117.4, 122.3, 127.8, 128.4, 129.8, 132.4, 138.5, 140.8, 143.4, 149.7, 150.3, 156.5, 158.4, 163.2, 166.8, 170.8; MS (ESI) (m/z, %): 566.1 [M^+],134.0 (100). Anal. Calc. for $C_{31}H_{27}N_5O_4S$: C, 65.82; H, 4.81; N, 12.38; O, 11.31; S, 5.67. Found: C, 65.78; H, 4.85; N, 12.40; O, 11.27; S, 5.70.

2-(3-(4-((5-(3-methoxyphenyl)-1,2,4-oxadiazol-3 yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine (11d)

Solid, Yield 76%; M.P.: 215-217ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1260 (C-O of OCH_3), 1340 (C-N-C), 1515 (Ar C=C), 1677 (C = N), 1745 (C-O-N), 2940 (C-H of OCH_3), 2980 Aliphatic C-H (Methyl), 3115 (Ar C-H), 3237 (C-H isoxazole), 3420 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 3.91 (s, 3H), 5.02 (s, 2H), 7.17 (d, 2H, J = 8 Hz), 7.20 (s, 2H), 7.32 (d, 2H, J = 7 Hz), 7.44 (s, 1H), 7.69 (d, 2H, J = 7.9 Hz), 7.98 (d, 2H, J = 8.9 Hz), 8.10 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ17.5, 23.6, 57.9, 72.5, 104.8, 115.6, 117.6, 118.2, 118,7, 122.3, 127.1, 127.6, 128.6, 129.7, 138.6, 140.7, 143.4, 149.8, 150.7, 156.2, 160.7, 161.8, 163.8, 170.6; MS (ESI) (m/z, %): 566.1 [M^+],135.0 (100). Anal. Calc. for.$C_{31}H_{27}N_5O_4S$: C, 65.82; H, 4.81; N, 12.38; O, 11.31; S, 5.68. Found: C, 65.88; H, 4.73; N, 12.43; O, 11.25; S, 5.71.

2-(3-(4-((5-(3,4-dimethoxyphenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11e)

Solid, Yield 78%; M.P.: 266-268ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1088 (C-O of OCH_3), 1372 (C-N-C), 1592 (Ar C=C), 1610 (C = N), 1695 (C-O-N), 2830 (C-H of OCH_3), 3015 Aliphatic C-H (Methyl), 3120 (C-H thiazole), 3218 (Ar C-H), 3480 (N-H). ^{1}HNMR (500 MHz,DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 3.91 (s, 3H), 5.02 (s, 2H), 7.17 (d, 2H, J = 8 Hz), 7.20 (s, 4H), 7.32 (d, 2H, J = 7 Hz), 7.44 (s, 1H), 7.69 (d, 2H, J = 7 Hz), 7.98 (d, 2H, J = 8 Hz), 8.10 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ17.5, 23.6, 72.8, 104.7, 115.3, 117.8, 122.4, 126.8, 127.5, 127.9, 129.6, 132.5, 133.6, 138.6, 140.4, 143.4, 149.6, 150.2, 156.5, 160.8, 163.4, 170.6; MS (ESI) (m/z, %): 596.1 [M^+],594.1 (100). Anal. Calc. for $C_{32}H_{29}N_5O_5S$: C, 64.52; H, 4.91; N, 11.76; O, 13.43; S, 5.38. Found: C, 64.48; H, 4.89; N, 11.80; O, 13.44; S, 5.39.

2-(3-(4-((5-(2-bromophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11f)

Solid, Yield 80%; M.P.: 251-253ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 610 (C-Br), 1240 (C-O of OCH_3), 1280 (C-N-C), 1622 (Ar C=C), 1690 (C = N), 1780 (C-O-N), 2885 Aliphatic C-H (Methyl), 2960 (C-H of OCH_3), 3030 (Ar C-H), 3210 (C-H thiazole), 3320 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ

2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.18 (d, 2H, J = 9.0 Hz), 7.20 (s, 2H), 7.44 (s, 1H), 7.73 (d, 2H, J = 8. Hz), 7.84 (d, 2H, J = 8 Hz), 7.99 (d, 2H, J = 9.0 Hz), 8.11 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ17.5, 23.6, 72.8, 104.7, 115.6, 117.8, 122.4, 127.4, 128.6, 129.3, 130.5, 131.4, 131.9, 134.7, 138.6, 143.7, 149.8, 150.3, 156.3, 160.9, 163.8, 170.7; MS (ESI) (m/z, %): 612 [M^+],182.9 (100). Anal. Calc. for. $C_{30}H_{24}BrN_5O_3S$: C, 58.64; H, 3.94; Br, 13.00; N, 11.40; O, 7.81; S, 5.22. Found: C, 58.60; H, 3.97; Br, 13.02; N, 11.46; O, 7.78; S, 5.17.

2-(3-(4-((5-(2,4-dibromophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11g)

Solid, Yield 82%; M.P.: 261-263ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 690 (C-Br), 1255 (C-O of OCH_3), 1310 (C-N-C), 1630 (C = N), 1710 (C-O-N), 1800 (Ar C=C), 2910 Aliphatic C-H (Methyl), 2990 (C-H of OCH_3), 3045 (Ar C-H), 3220 (C-H thiazole), 3370 (N-H). ^{1}H NMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.18 (d, 2H, J = 9 Hz), 7.20 (s, 1H), 7.44 (s, 1H), 7.73 (d, 2H, J = 8. Hz), 7.84 (d, 2H, J = 8 Hz), 7.99 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.24 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d6): δ 17.5, 23.6, 72.9, 104.8, 115.6, 117.8, 122.6, 125.8, 127.8, 128.6, 129.5, 136.4, 138.9, 140.7, 143.4, 147.6, 149.7, 150.5, 156.8, 160.3, 163.8, 170.6; MS (ESI) (m/z, %): 691.9 [M^+],415.1 (100). Anal. Calc. for. $C_{30}H_{23}Br_2N_5O_3S$: C, 51.96; H, 3.34; Br, 23.05; N, 10.10; O, 6.92; S, 4.62. Found: C, 51.93; H, 3.37; Br, 23.08; N, 10.08; O, 6.94; S, 4.60.

2-(3-(4-((5-(2-nitrophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11h)

Solid, Yield 77%; M.P.: 266-268ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1277 (C-O of OCH_3), 1335 (C-N-C), 1520 (C-NO_2 Asym), 1677 (C = N), 1755 (C-O-N), 1820 (Ar C=C), 2940 Aliphatic C-H (Methyl), 3065 (Ar C-H), 3240 (C-H thiazole), 3360 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.15 (s, 4H), 7.45 (s, 1H), 7.76 (d, 2H, J = 8 Hz), 7.87 (d, 2H, J = 8 Hz), 8.01 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 581.1 [M^+],123 (100). Anal. Calc. for: $C_{30}H_{24}N_6O_5S$: C, 62.06; H, 4.17; N, 14.47; O, 13.78; S, 5.52. Found: C, 62.10; H, 4.13; N, 14.44; O, 13.80; S, 5.53.

2-(3-(4-((5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11i)

Solid, Yield 80%; M.P.: 272-274ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1244 (C-O of OCH_3), 1366 (C-N-C), 1579 (C-NO_2 Asym), 1667 (C = N), 1719 (C-O-N), 1880 (Ar C=C), 2943 Aliphatic C-H (Methyl), 3035 (Ar C-H), 3210 (C-H thiazole), 3360 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.15-7.28 (m, 4H), 7.45 (s, 1H), 7.76 (d, 2H, J = 8 Hz), 7.87 (d, 2H, J = 8 Hz), 8.01 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 581.1 [M^+],148.0 (100). Anal. Calc. for: $C_{30}H_{24}N_6O_5S$: C, 62.06; H, 4.17; N, 14.47; O, 13.78; S, 5.52. Found: C, 62.02; H, 4.20; N, 14.48; O, 13.80; S, 5.50.

2-(3-(4-((5-(2,4-dinitrophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine (11j)

Solid, Yield 69%; M.P.: 260-262ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1265 (C-O of OCH_3), 1322 (C-N-C), 1577 (C-NO_2 Asym), 1634 (C = N), 1735 (C-O-N), 1847 (Ar C=C), 2994 Aliphatic C-H (Methyl), 3088 (Ar C-H), 3243 (C-H thiazole), 3328 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.15-7.28 (m, 3H), 7.45 (s, 1H), 7.76 (d, 2H, J = 8 Hz), 7.87 (d, 2H, J = 8 Hz), 8.01 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 626.1 [M^+],166.0 (100). Anal. Calc. for: $C_{30}H_{23}N_7O_7S$: C, 57.60; H, 3.71; N, 15.67; O, 17.90; S, 5.13. Found: C, 57.62; H, 3.69; N, 15.68; O, 17.87; S, 5.14.

2-(3-(4-((5-(2,3-dinitrophenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11k)

Solid, Yield 71%; M.P.: 282-284ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1274 (C-O of OCH_3), 1325 (C-N-C), 1577 (C-NO_2 Asym), 1620 (C = N), 1735 (C-O-N), 1847 (Ar C=C), 2980 Aliphatic C-H (Methyl), 3088 (Ar C-H), 3285 (C-H thiazole), 3328 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.06 (s, 2H), 7.15-7.28 (m, 3H), 7.45 (s, 1H), 7.76 (d, 2H, J = 8 Hz), 7.87 (d, 2H, J = 8 Hz), 8.01 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 626.1 [M+H]$^+$[M^+],178.0 (100).Anal. Calc. for: $C_{30}H_{23}N_7O_7S$: C, 57.60; H, 3.71; N, 15.67; O, 17.90; S, 5.13. Found: C, 57.62; H, 3.69; N, 15.69 O, 17.89; S, 5.11.

2-(3-(4-((5-(2-methylphenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11l)

Solid, Yield 82%; M.P.: 212-214ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1249 (C-O of OCH_3), 1307 (C-N-C), 1605 (C = N), 1794 (C-O-N), 1835 (Ar C=C), 2933 Aliphatic C-H (Methyl), 3037 (Ar C-H), 3268 (C-H thiazole), 3385 (N-H). ^{1}HNMR (500 MHz,DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.03 (s, 5H), 7.17 (d, 2H, J = 8 Hz), 7.20 (s, 2H), 7.36 (d, 2H, J = 7 Hz), 7.44 (s, 1H), 7.51 (d, 2H, J = 7 Hz), 7.98 (d, 2H, J = 8 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 550.1 [M^+],91.0 (100). Anal. Calc. for: $C_{31}H_{27}N_5O_3S$: C, 67.74; H, 4.95; N, 12.74; O, 8.73; S, 5.83. Found: C, 67.71; H, 4.97; N, 12.76; O, 8.70; S, 5.86.

2-(3-(4-((5-(2,4-dimethylphenyl)-1,2,4-oxadiazol-3-yl) methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5-trimethyl phenyl)thiazol-4-amine (11m)

Solid, Yield 73%; M.P.: 222-224ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 1207 (C-N-C), 1282 (C-O of OCH_3), 1628 (C = N), 1737 (C-O-N), 1890 (Ar C=C), 2908 Aliphatic C-H (Methyl), 3044 (Ar C-H), 3275 (C-H thiazole), 3355 (N-H). ^{1}HNMR (500 MHz,DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 2.69 (s, 3H), 5.03 (s, 4H), 7.17 (d, 2H, J = 8 Hz), 7.20 (s, 2H), 7.36 (d, 2H, J = 7 Hz), 7.44 (s, 1H), 7.51 (d, 2H, J = 7 Hz), 7.98 (d, 2H, J = 8 Hz), 8.11 (s, 1H), 8.25 (s, 1H); MS (ESI) (m/z, %): 564.2 [M^+],117.0 (100). Anal. Calc. for: $C_{32}H_{29}N_5O_3S$: C, 68.19; H, 5.19; N, 12.42; O, 8.52; S, 5.69. Found: C, 68.16; H, 5.21; N, 12.44; O, 8.49; S, 5.70.

2-(3-(4-((5-(2-Bromo-3,5-dinitrophenyl)-1,2,4-oxadiazol -3-yl)methoxy)phenyl) isoxazol-5-yl)-N-(3,4,5 trimethyl phenyl)thiazol-4-amine (11n)

Solid, Yield: 77%; M.P.: 288-290ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 622 (C-Br), 1237 (C-N-C), 1284 (C-O of OCH_3), 1544 (C-NO_2 Asym), 1656 (C = N), 1767 (C-O-N), 1894 (Ar

C=C), 2942 Aliphatic C-H (Methyl), 3005 (Ar C-H), 3290 (C-H thiazole), 3350 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.11 (s, 2H), 7.17-7.30 (m, 4H), 7.44 (s, 1H), 8.05 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H), 8.69 (s, 2H); MS (ESI) (m/z, %): 704.0[M^+],268.9 (100). Anal. Calc. for: $C_{30}H_{22}BrN_7O_7S$: C, 51.15; H, 3.15; Br, 11.34; N, 13.92; O, 15.90; S, 4.55. Found: C, 51.13; H, 3.17; Br, 11.32; N, 13.94; O, 15.88; S, 4.56.

2-(3-(4-((5-(2-chloro-3,5-dinitrophenyl)-1,2,4-oxadiazol-3-yl)methoxy)phenyl)isoxazol-5-yl)-N-(3,4,5-trimethylphenyl) thiazol-4-amine (11o)

Solid, Yield: 72%; M.P.: 290-292ºC, FTIR ($CHCl_3$, υ/cm^{-1}): 720.7 (C-Cl), 1222 (C-N-C), 1290 (C-O of OCH_3), 1371 (C-NO_2 Sym), 1409 (C-NO_2 Asym),1624 (C = N), 1729 (C-O-N), 1868 (Ar C=C), 2939Aliphatic C-H (Methyl), 2988 (C-H of OCH_3), 3097 (Ar C-H), 3250 (C-H thiazole), 3349 (N-H). ^{1}HNMR (500 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.63 (s, 6H), 5.11 (s, 2H), 7.17-7.30 (m, 4H), 7.44 (s, 1H), 8.05 (d, 2H, J = 9 Hz), 8.11 (s, 1H), 8.25 (s, 1H), 8.69 (s, 2H); MS (ESI) (m/z, %): 660.2[M^+],224.9 (100). Anal. Calc. for: $C_{30}H_{22}ClN_7O_7S$: C, 54.59; H, 3.36; Cl, 5.37; N, 14.85; O, 16.97; S, 4.86. Found: C, 54.61; H, 3.34; Cl, 5.35; N, 14.88; O, 16.95; S, 4.87.

3.4. *In-vitro* cytotoxicity assay

All synthesized 1,2,4-oxadiazole derivatives were evaluated for their anticancer activity using the Sulforhodamine B (SRB) assay against the human breast cancer cell lines MFC-7 and MDA-MB-231, adriamycin serving as a reference drug. The cytotoxicity assay was conducted according to National Cancer Institute (NCI) protocols. For cell culture, RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine was used. Cells were seeded in 96-well microtiter plates and incubated at 37 °C in a humidified atmosphere with 5% CO_2 and 95% air for 24 hours to allow cells to attach.Following incubation, each synthesized compound was added in 10 µL volumes to wells containing 90 µL of medium, to achieve a series of test concentrations (typically starting from 10^{-2} M, with appropriate dilutions). The plates were then incubated for an additional 48 hours. To terminate the assay, cold trichloroacetic acid (TCA) was added, and the plates were incubated at 4°C for 1 hour to fix the cell monolayers. The supernatant was discarded, and the plates were washed thoroughly with tap water and allowed to air dry.

After drying, 50 µL of 0.4% (w/v) SRB solution in 1% acetic acid was added to each well to stain the cellular proteins. The plates were incubated at room temperature for 20 minutes. Unbound dye was removed by washing the plates multiple times with 1% (v/v) acetic acid, followed by air drying. The protein-bound dye was solubilized in 10 mM Trizma base, and the absorbance was measured using an ELISA plate reader at wavelength of 540 nm with a reference wavelength of 690 nm. Cytotoxicity results were expressed as GI_{50}values (concentration required to inhibit 50% of cell growth), and the data are summarized in **Table 2** [28, 29].

The percentage of growth for test wells relative to control wells was calculated on a plate-by-plate basis.Percentage of growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells multiplied by 100. The percentage of growth inhibition (GI_{50}) was calculated from [(Ti-Tz)/(CTz)] ×100=50, where Tz represents for time zero, C for control growth, and Ti for test growth in the presence of drug at the four concentration levels. CC_{50} value of VERO cell line and selectivity index also calculated and presented in **Table 2**. The Cytotoxic Concentration (CC_{50}), represents the concentration of a substance that causes 50% of a cell population to die or become non-viable.

3.5. *In-silico* prediction of ADMET properties

The pharmacokinetic properties such as absorption (A), distribution (D), metabolism (M), and excretion (E) all synthesized 1,2,4-oxadiazole derivatives were calculated using the SWISSADME online tool. The evaluation included assessment of compliance with Lipinski's Rule of Five, a well-established guideline for predicting oral bioavailability. Derivatives violating one or more of Lipinski's criteria may exhibit unfavourable pharmacokinetic profiles and reduced oral efficacy [30].

Additionally, ProTox (https://tox.charite.de/protox3/) was employed to predict the toxicological profiles of the synthesized derivatives, including the oral rat LD_{50} values (mg/kg) [31]. Structures of all the derivatives were prepared by using chemdraw for the prediction of physicochemical properties.

4. CONCLUSION

In this study, a novel series of 2-(3-(4-((5-aryl-1,2,4-oxadiazol-3-yl)methoxy)phenyl)isoxazol-5-yl)-N-(3,4,5-trimethylphenyl)thiazol-4-amine derivatives (**11a to 11o**) was designed and successfully synthesized. The structures of all synthesized derivatives were confirmed through FT-IR, ^{1}HNMR, ^{13}CNMR, MASS spectroscopy, and elemental analysis.

The anticancer potential of these derivatives was evaluated *In-vitro* against MCF-7 and MDA-MB-231 human breast cancer cell lines using the Sulforhodamine B (SRB) assay, with adriamycin employed as the standard reference drug. All final derivatives exhibited good to moderate cytotoxicity against MCF- cancer cell line and except two derivatives (**11f** and **11g**) all showed activity against MDA-MB-231 cancer cell line.

Pharmacokinetic predictions indicated that derivatives had low gastrointestinal absorption and no blood–brain barrier (BBB) permeability, which may be advantageous for peripheral anticancer activity. Among the series, compound **11o** demonstrated the most potent anticancer effect, making it a promising candidate for further development as a lead compound.

5. REFERENCES

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