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Synthesis, Characterization and Pharmacological Screening of 1,3,4-Oxadiazoles

GANESH SONAWANE*, SHWETA SHARMA and RITU GILHOTRA

Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jaipur-302017, India

*Corresponding author: E-mail: gbsonawane8@gmail.com

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Two series of 1,3,4-oxadiazole scaffolds (**10a-j** & **12a-j**) were synthesized and comprehensively characterized using IR, NMR and mass spectra. Subsequently, the synthesized compounds underwent screening for anticancer activity against HepG2 and MCF-7 cell lines. All the 20 compounds exhibited substantial anticancer activity with compounds **10b** and **10e** demonstrating particularly significant activity surpassing that of the standard 5-fluorouracil. Significantly, compound **10b** displayed superior efficacy compared to compound **10e**, prompting its selection for further *in vivo* assessment against diethylnitrosamine-induced hepatocellular carcinoma. In *in vivo* evaluation, compound **10b** significantly reduced levels of AST, ALT, ALP and bilirubin in comparison to the positive control group. It also exhibited significant hepatoprotective effects, albeit marginally less potent than standard 5-fluorouracil. These findings underscore the considerable potential of compound **10b** as an anticancer agent within the series, suggesting its candidacy for further development and clinical exploration in cancer therapeutics.

Keywords: 1,3,4-Oxadiazoles, Cell lines, Diethylnitrosamine, Cancer, Hepatocellular carcinoma.

INTRODUCTION

Cancer, being the second leading cause of death globally, presents a significant challenge to public health. According to the World Health Organization (WHO), in 2022 alone, there were approximately 20 million new cancer cases and 9.7 million deaths attributed to the cancer [1,2]. The statistics underscore the urgent need for effective anticancer therapies. Despite notable advancements in treatment, the development of novel anticancer drugs remains a pressing endeavor for researchers worldwide [3-5].

A promising avenue in this quest lies in the exploration of heterocyclic compounds, particularly 1,3,4-oxadiazoles. These five-membered rings, containing two nitrogen atoms and one oxygen atom, have garnered significant attention due to their diverse pharmacological properties [6,7]. The incorporation of the 1,3,4-oxadiazole moiety into drug molecules has been shown to enhance metabolic stability, water solubility and reduce lipophilicity, thereby potentially improving pharmacokinetic profiles [8-10]. Moreover, the 1,3,4-oxadiazole ring serves as a crucial pharmacophore element, facilitating interactions with biological targets. Its structural versatility allows it to act as a flat aromatic linker, helping in the proper orientation of the molecule and enhancing its binding affinity [11,12]. This molecular architecture has been exploited in various therapeutic areas, including antibacterial, antimalarial, anti-inflammatory, antidepressive, antiviral and anticancer activities [13]. The rational design and synthesis of novel 1,3,4-oxadiazole derivatives offer a promising approach for the development of potent anticancer agents, as evidenced by existing literature [14,15].

EXPERIMENTAL

Design and *in silico* screening: To design possible 1,3,4-oxadiazoles in present investigation, a thorough literature review and database searches were carried out. Compounds with established anticancer activities were prioritized as conveniently accessible for the experimental validation. To achieve a thorough assessment, the selection method also took into account for the variety of 1,3,4-oxadiazoles. In the present study, twenty 1,3,4-oxadiazole derivatives (**10a-j** & **12a-j**) were synthesized. These derivatives were derived from two distinct intermediates *viz*. methylpiperazin-1-yl (**7**) and chloroquinoxalin-2-yl (**11**). From each intermediate, ten derivatives were synthesized, as illustrated in the accompanying schemes.

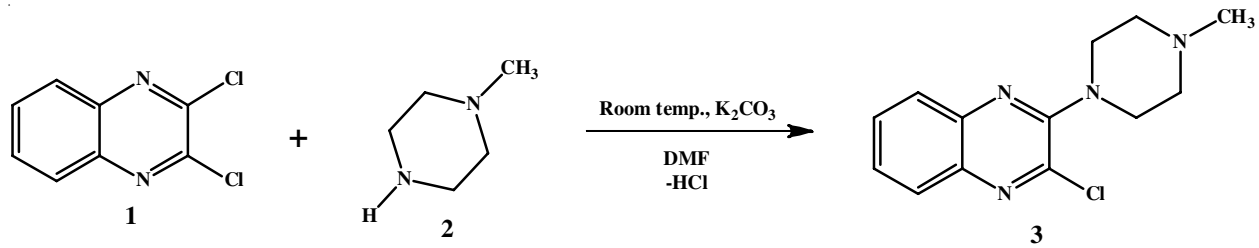
Previous work involved first docking studies to predict the binding modes with the BCL-2 protein, thereby evaluating the synthetic possibility of the titled compounds in the preliminary *in silico* screening [16]. The pharmacokinetic profiles (ADMET) of the compounds were also determined using the pkCSM server in the previous work [16]. These steps informed the selection of promising compounds for further experimental validation and synthesis, thereby streamlining the drug discovery process for cancer treatment.

Synthesis

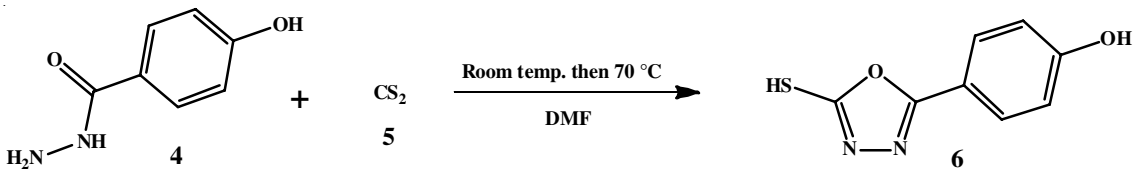
Synthesis of 2-chloro-3-(4-methylpiperazin-1-yl)quinoxaline (3): The synthesis of compound **3** involved the stirring of the reaction mixture containing 2,3-dichloroquinoxaline (**1**) (0.50 mmol), *N*-modified piperazine (**2**) (0.50 mmol) and K_2CO_3 (0.50 mmol) in DMF at room temperature [17]. The resulting solution was poured into water, partitioned with ethyl acetate, washed with brine, dried and concentrated (**Scheme-I**). The crude product was purified using silica gel column chromatography.Colour: yellow solid; m.p.: 155-157 ºC; m.f.: $C_{13}H_{15}ClN_4$; Relative molecular mass: 262.74; FTIR (KBr, ν_{max}, cm^{-1}): 1332 (C-N *str*. of the amide linkage), 754 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.96 (q, 2H, Ar-H), 7.92 (q, 2H, Ar-H), 7.72 (q, 2H, Ar-H), 7.68 (d, 2H, Ar-H), 3.63 (t, 2H, CH_2), 3.61 (t, 2H, CH_2), 2.38 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 46.5, 48.0, 48.2, 57.6, 57.9, 126.4, 128.6, 129.4, 130.0, 135.8, 136.5, 141.2, 170.6. MS *m/z*: 264 (M+1).

Synthesis of 4-(5-sulfanyl-1,3,4-oxadiazol-2-yl)phenol (6): Compound **6** was synthesized by stirring acid hydrazide (**4**) and CS_2 in DMF for 15 min followed by heating at 70 ºC [18]. TLC monitoring was employed to check the reaction progress. After cooling, the mixture was poured into ice-cold water, yielding a solid product, which was collected, washed and recrystallized to obtain the pure compound (**Scheme-II**). Colour: pale yellow solid; m.p.: 122-124 ºC; m.f.: $C_8H_6N_2O_2S$; Relative molecular mass: 194.21; FTIR (KBr, ν_{max}, cm^{-1}): 3340 (-OH br.), 2580 (S-H *str.*), 1475 (C=C *str.* aromatic ring); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 13.28 (s, 1H, SH), 9.67 (s, 1H, OH), 7.88 (s, 2H, Ar-H), 7.85 (s, 2H, Ar-H), 6.91 (s, 2H, Ar-H), 6.85 (s, 2H, Ar-H). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 116.8, 116.7, 116.4, 115.7, 118.8, 158.6, 166.2. MS *m/z*: 195 (M+1).

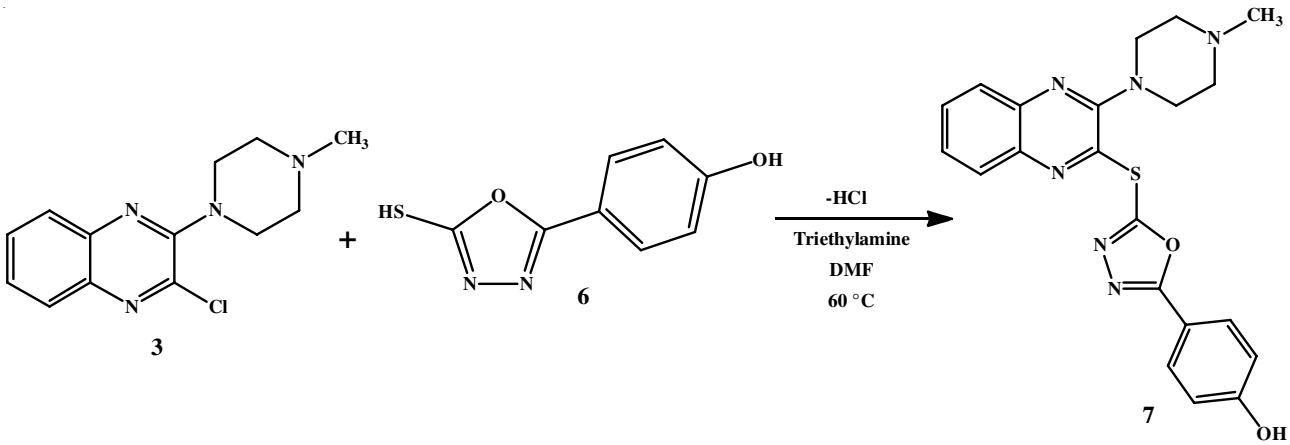
Synthesis of 4-(5-{[3-(4-methylpiperazin-1-yl)quinoxalin-2-yl]sulfanyl}-1,3,4-oxadiazol-2-yl)phenol (7): Compound **7** was synthesized by stirring compound **3** (0.21 mmol) and compound **6** (0.42 mmol), with triethylamine (Et_3N, 0.42 mmol) in DMF at 60 ºC overnight. The solution was then poured into water, partitioned with EtOAc, washed with brine, dried and concentrated. The residue was purified using silica gel column chromatography (**Scheme-III**). Yellow solid; m.p.: 166-168 ºC; m.f.: $C_{21}H_{20}N_6O_2S$; Relative molecular mass: 420.49; FTIR



Scheme-I: Synthesis of 2-chloro-3-(4-methylpiperazin-1-yl)quino-xaline (**3**)



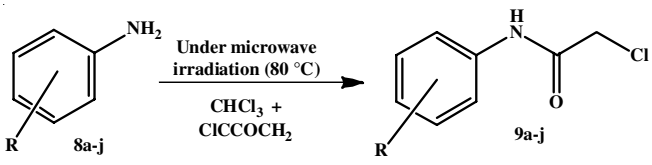
Scheme-II: Synthesis of 4-(5-sulfanyl-1,3,4-oxadiazol-2-yl)phenol (**6**)



Scheme-III: Synthesis of 4-(5-{[3-(4-methylpiperazin-1-yl)quinoxa-lin-2-yl]sulfanyl}-1,3,4-oxadiazol-2-yl)phenol (**7**)

(KBr, ν_{max}, cm^{-1}): 3348 (-OH br), 2920 (N-CH_3 *str.*), 1578 (C=N *str.* of the oxadiazole linkage); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.02 (s, 1H, OH), 7.96 (d, 2H, Ar-H), 7.92 (d, 2H, Ar-H), 7.84 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H), 7.72 (t, 2H, Ar-H), 7.67 (t, 2H, Ar-H), 6.94 (d, 2H, Ar-H), 6.87 (d, 2H, Ar-H), 3.57 (d, 2H, CH_2), 3.48 (d, 2H, CH_2), 2.62 (d, 2H, CH_2), 2.54 (d, 2H, CH_2), 2.34 (s, 1H, CH_3); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 167.2, 162.4, 157.8, 152.6, 148.8, 138.6, 138.9, 129.6, 128.2, 128.0, 127.4, 127.2, 117.4, 115.6, 56.2, 48.3, 44.6. MS *m/z*: 195 (M+1).

Synthesis of 2-chloro-*N*-phenylacetamide derivatives (9a-j): Compounds **9a-j** were synthesized by adding chloroacetyl chloride dropwise to a solution of substituted anilines (**8a-j**) (3.21 g, 30 mmol) in chloroform. The reaction mixture was microwaved at 80 ºC for 5 min. After separation of the organic layer, excess solvent was removed by slow evaporation and the residue was filtered and dried to obtain the desired compounds [19] (**Scheme-IV**).



Scheme-IV: Synthesis of 2-chloro-*N*-phenylacetamide derivatives (**9a-j**)

Synthesis of 1-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)furan-2-yl)phenoxy)-3-phenylpropan-2-one derivatives (10a-j): The synthesis of titled compounds (**10a-j**) was accomplished by stirring the reaction mixture consisting of compound **7** (0.12 mmol), compounds (**9a-j**) (0.24 mmol) and K_2CO_3 (0.36 mmol) in DMF at 50 ºC. The resulting mixture was then concentrated, washed and dried (**Scheme-V**). Subsequently, the residue was subjected to purification using silica gel column chromatography.

***N*-(4-Chlorophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)-quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10a):** Pale yellow solid; m.p.: 126-128 ºC; m.f.: $C_{29}H_{26}ClN_7O_3S$; Relative molecular mass: 164.99; FTIR (KBr, ν_{max}, cm^{-1}): 1748 (C=O *str.* of the amide linkage), 1275 (C-N *str.* of amide linkage), 769 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.56 (s, 1H, NH), 8.11 (d, 2H, Ar-H), 7.95 (q, 2H, Ar-H), 7.70 (q, 2H, Ar-H), 7.68 (d, 2H, Ar-H), 7.37 (d, 2H, Ar-H), 7.07 (d, 2H, Ar-H), 4.68 (s, 1H, CH_2), 3.63 (t, 2H, CH_2), 2.49 (t, 2H, CH_2), 2.36 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 40.3, 40.4, 43.9, 49.0, 57.8, 60.5, 115.1, 115.6, 116.0, 121.5, 122.0, 122.1, 126.0, 126.1, 128.3, 130.0, 132.0, 132.2, 134.6, 135.4, 140.5, 155.6, 157.7, 159.6, 162.0. MS *m/z*: 589 (M+1).

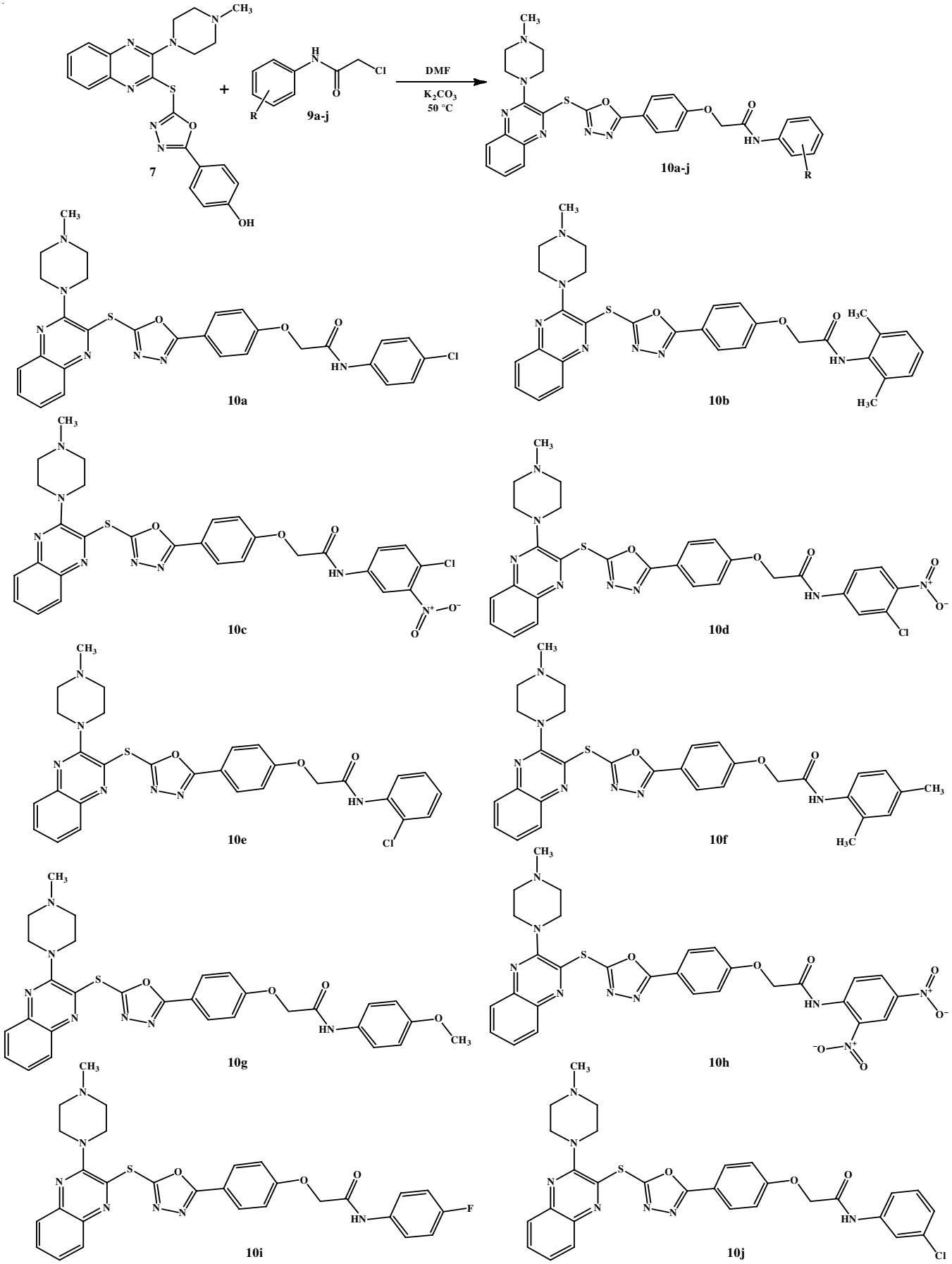
***N*-(2,6-Dimethylphenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10b):** Yellow solid; m.p.: 96-98 ºC; m.f.: $C_{31}H_{31}N_7O_3S$; Relative molecular mass: 169.91; FTIR (KBr, ν_{max}, cm^{-1}): 1640 (C=O *str.* of the amide linkage), 1279 (C-N *str.* of amide linkage), 2927 (N-H *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.19 (s, 1H, NH), 8.11 (d, 2H, Ar-H), 7.81 (q, 2H, Ar-H), 7.65 (q, 2H, Ar-H), 7.11 (d, 3H, Ar-H), 7.09 (d, 2H, Ar-H), 4.05 (s, 1H, CH_2), 3.50 (t, 2H, CH_2), 2.36 (t, 2H, CH_2), 2.19 (s, 1H, CH_3), 2.13 (s, 2H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 18.1, 18.4, 46.0, 48.9, 60.2, 62.3, 125.5, 125.7, 126.9, 127.3, 127.9, 128.3, 128.7, 131.3, 131.3, 132.2, 132.3, 133.4, 135.2, 138.4, 139.6, 140.5, 141.2, 145.0, 151.4, 162.4. MS *m/z*: 582 (M+1).

***N*-(4-Chloro-3-nitrophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)-phenoxy)acetamide (10c):** Yellow solid; m.p.: 102-104 ºC; m.f.: $C_{29}H_{25}ClN_8O_5S$; Relative molecular mass: 173.81; FTIR (KBr, ν_{max}, cm^{-1}): 1676 (C=O *str.* of the amide linkage), 1278 (C-N *str.* of amide linkage), 2925 (N-H *str.*), 1556 (N-O *str.* of nitro group), 767 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.81 (s, 1H, NH), 8.14 (s, 1H, Ar-H), 8.11 (d, 1H, Ar-H), 7.80 (q, 2H, Ar-H), 7.78 (q, 1H, Ar-H), 7.67 (q, 2H, Ar-H), 7.64 (d, 2H, Ar-H), 4.66 (s, 1H, CH_2), 3.79 (t, 2H, CH_2), 2.50 (t, 2H, CH_2), 2.36 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ: 40.3, 40.4, 41.9, 43.9, 49.0, 57.8, 60.5, 62.1, 115.1, 115.6, 118.2, 118.6, 119.2, 119.7, 126.0, 128.3, 130.0, 130.9, 131.0, 132.2, 133.5, 133.5, 139.8, 140.5, 150.7, 152.2, 155.6. MS, *m/z*: 634 (M+1).

***N*-(3-Chloro-4-nitrophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)-phenoxy)acetamide (10d):** Yellow solid; m.p.: 169-171 ºC; m.f.: $C_{29}H_{25}ClN_8O_5S$; Relative molecular mass: 173.81; FTIR (KBr, ν_{max}, cm^{-1}): 1697 (C=O *str.* of the amide linkage), 1333 (C-N *str.* of amide linkage), 2924 (N-H *str.*), 1504 (N-O *str.* of nitro group), 752 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 11.10 (s, 1H, NH), 8.26 (d, 1H, Ar-H), 8.14 (s, 1H, Ar-H), 8.08 (d, 2H, Ar-H), 7.93 (q, 2H, Ar-H), 7.95 (q, 1H, Ar-H), 7.93 (d, 2H, Ar-H), 7.08 (d, 2H, Ar-H), 4.68 (s, 1H, CH_2), 3.65 (t, 2H, CH_2), 2.52 (t, 2H, CH_2), 2.50 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 39.9, 40.4, 40.4, 57.8, 60.5, 114.0, 115.1, 115.6, 115.9, 123.4, 123.4, 125.5, 127.0, 128.3, 130.1, 140.1, 140.5, 145.0, 145.0, 155.6, 163.4, 163.4. MS, *m/z*: 634 (M+1).

***N*-(2-Chlorophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10e):** Brown solid; m.f.: $C_{29}H_{26}ClN_7O_3S$; m.p.: 195-197 ºC; Relative molecular mass: 164.99; FTIR (KBr, ν_{max}, cm^{-1}): 1695 (C=O *str.* of the amide linkage), 1331 (C-N *str.* of amide linkage), 2923 (N-H *str.*), 754 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.76 (s, 1H, NH), 8.10 (d, 1H, Ar-H), 8.09 (s, 2H, Ar-H), 7.82 (q, 2H, Ar-H), 7.67 (q, 1H, Ar-H), 7.57 (d, 1H, Ar-H), 7.41 (t, 1H, Ar-H), 7.39 (t, 1H, Ar-H), 7.32 (d, 2H, Ar-H), 4.77 (s, 1H, CH_2), 3.53 (t, 2H, CH_2), 2.50 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 39.4, 39.6, 40.1, 40.2, 42.8, 43.4, 49.0, 60.3, 62.2, 126.6, 127.3, 127.6, 128.3, 128.7, 130.0, 130.2, 131.3, 132.2, 133.8, 134.6, 138.4, 139.5, 141.2, 151.4, 162.8, 165.6. MS, *m/z*: 589 (M+1).

***N*-(2,4-Dimethylphenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10f):** Yellow solid; m.p.: 97-99 ºC; m.f.: $C_{31}H_{31}N_7O_3S$;



Scheme-V: Synthesis of 1-(4-(5-((3-(4-methylpiperazin-1-yl)quino-xalin-2-yl)thio)furan-2-yl)phenoxy)-3-phenylpropan-2-one derivatives (**10a-j**)

Relative molecular mass: 169.91; FTIR (KBr, ν_{max}, cm^{-1}): 1667 (C=O *str.* of the amide linkage), 1232 (C-N *str.* of amide linkage), 2924 (N-H *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.52 (s, 1H, NH), 8.01 (d, 2H, Ar-H), 7.81 (q, 2H, Ar-H), 7.68 (q, 2H, Ar-H), 7.24 (d, 1H, Ar-H), 7.06 (d, 2H, Ar-H), 7.02 (t, 1H, Ar-H), 7.02 (d, 1H, Ar-H), 4.76 (s, 1H, CH_2), 3.52 (t, 2H, CH_2), 2.26 (t, 2H, CH_2), 2.24 (d, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 18.1, 20.9, 43.5, 46.0, 48.9, 60.2, 62.3, 125.5, 125.7, 126.9, 127.3, 127.9, 128.3, 128.7, 131.3, 131.3, 132.2, 132.3, 133.4, 135.2, 135.8, 138.4, 139.6, 140.5, 141.2, 145.0, 151.4, 162.4. MS, *m/z*: 582 (M+1).

***N*-(4-Methoxyphenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10g):** Dark brown solid; m.f.: $C_{30}H_{29}N_7O_4S$; m.p.: 76-78 ºC; Relative molecular mass: 166.47; FTIR (KBr, ν_{max}, cm^{-1}): 1672 (C=O *str.* of the amide linkage), 1343 (C-N *str.* of amide linkage), 2924 (N-H *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.24 (s, 1H, NH), 8.09 (d, 2H, Ar-H), 7.95 (q, 2H, Ar-H), 7.58 (q, 2H, Ar-H), 7.47 (d, 2H, Ar-H), 7.09 (d, 2H, Ar-H), 6.86 (d, 2H, Ar-H), 4.62 (s, 1H, CH_2), 3.81 (s, 1H, CH_3), 3.63 (t, 2H, CH_2), 2.36 (t, 2H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 42.8, 43.4, 49.0, 60.3, 62.2, 126.6, 127.3, 127.6, 128.0, 128.0, 128.7, 130.0, 130.2, 131.3, 132.2, 133.8, 134.6, 138.4, 139.5, 141.2, 151.4, 162.8, 165.6. MS, *m/z*: 584 (M+1).

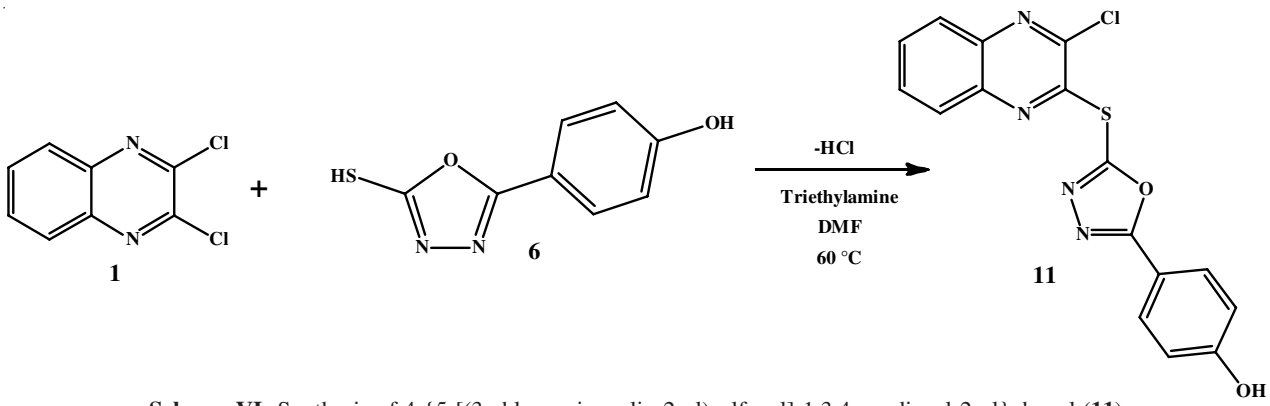
***N*-(2,4-Dinitrophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10h):** Dark yellow solid; m.p.: 144-146 ºC; m.f.: $C_{29}H_{25}N_9O_7S$; Relative molecular mass: 177.62; FTIR (KBr, ν_{max}, cm^{-1}): 1596 (C=O *str.* of the amide linkage), 1233 (C-N *str.* of amide linkage), 2928 (N-H *str.*), 1542 (N-O *str.* of nitro group); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.02 (s, 1H, NH), 8.80 (s, 1H, Ar-H), 8.39 (d, 1H, Ar-H), 8.18 (d, 1H, Ar-H), 8.16 (d, 2H, Ar-H), 7.82 (q, 2H, Ar-H), 7.72 (q, 2H, Ar-H), 7.11 (d, 2H, Ar-H), 4.66 (s, 1H, CH_2), 3.74 (t, 2H, CH_2), 2.36 (t, 2H, CH_2), 2.27 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 40.5, 41.9, 48.7, 57.8, 60.5, 114.0, 115.6, 115.9, 123.4, 123.4, 123.8, 126.0, 126.1, 130.1, 140.1, 140.5, 145.0, 145.0, 150.8, 151.8, 152.2, 155.6, 163.4. MS, *m/z*: 644 (M+1).

***N*-(4-Fluorophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10i):** Dark brown solid; m.f.: $C_{29}H_{26}FN_7O_3S$; m.p.: 89-91 ºC; Relative molecular mass: 159.94; FTIR (KBr, ν_{max}, cm^{-1}): 1594 (C=O *str.* of the amide linkage), 1236 (C-N *str.* of amide linkage), 2927 (N-H *str.*), 1025 (C-F *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.67 (s, 1H, NH), 8.09 (d, 2H, Ar-H), 7.96 (q, 2H, Ar-H), 7.72 (q, 2H, Ar-H), 7.64 (q, 2H, Ar-H), 7.20 (t, 2H, Ar-H), 7.17 (d, 2H, Ar-H), 4.70 (s, 1H, CH_2), 3.66 (t, 2H, CH_2), 2.73 (t, 2H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 40.4, 43.9, 49.0, 57.8, 60.5, 115.1, 115.6, 115.7, 115.9, 116.0, 121.5, 121.6, 126.0, 126.1, 128.3, 130.0, 132.0, 132.2, 134.6, 135.4, 140.5, 145.0, 155.6, 157.7, 159.6, 161.5, 162.0. MS, *m/z*: 572 (M+1).

***N*-(3-Chlorophenyl)-2-(4-(5-((3-(4-methylpiperazin-1-yl)quinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-acetamide (10j):** Dark brown solid; m.f.: $C_{29}H_{26}ClN_7O_3S$; m.p.: 91-93 ºC; Relative molecular mass: 164.99; FTIR (KBr, ν_{max}, cm^{-1}): 1681 (C=O *str.* of the amide linkage), 1232 (C-N *str.* of amide linkage), 2925 (N-H *str.*), 766 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.00 (s, 1H, NH), 8.09 (d, 2H, Ar-H), 7.84 (q, 2H, Ar-H), 7.84 (q, 1H, Ar-H), 7.54 (d, 2H, Ar-H), 7.49 (t, 1H, Ar-H), 7.34 (t, 1H, Ar-H), 7.27 (d, 1H, Ar-H), 7.21 (d, 2H, Ar-H), 4.78 (s, 1H, CH_2), 3.66 (t, 2H, CH_2), 2.73 (t, 2H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 40.3, 40.4, 41.9, 43.9, 49.0, 57.8, 62.1, 115.1, 115.6, 118.2, 118.6, 119.2, 119.7, 123.4, 125.8, 126.0, 126.1, 128.3, 130.0, 130.9, 131.0, 131.7, 139.8, 140.5, 150.7, 155.6, 165.5. MS *m/z*: 589 (M+1).

Synthesis of 4-{5-[(3-chloroquinoxalin-2-yl)sulfanyl]-1,3,4-oxadiazol-2-yl}phenol (11): The synthesis of compound **11** was performed by stirring 2,3-dichloroquinoxaline (**1**) (0.50 mmol), compound **6** (0.42 mmol) and Et_3N (0.50 mmol) in DMF (6 mL) at 60 ºC (**Scheme-VI**) [17]. The resulting mixture was concen-trated under vacuum and subsequently purified using silica gel column chromatography. Colour: yellow solid; m.p.: 156-158 ºC; m.f.: $C_{16}H_{10}N_4O_2SCl$; Relative molecular mass: 356.78; FTIR (KBr, nmax, cm^{-1}): 3356 (-OH br), 758 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.62 (s, 1H, OH), 7.94 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 7.63 (t, 2H, Ar-H), 7.58 (d, 2H, Ar-H), 7.52 (t, 2H, Ar-H), 7.48 (d, 2H, Ar-H); ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 167.6, 161.2, 155.6, 152.6, 143.7, 142.5, 140.6, 134.1, 133.6, 128.9, 128.2, 128.4, 116.4, 114.0. MS *m/z*: 357 (M+1).

Synthesis of 1-(4-(5-((3-chloroquinoxalin-2-yl)thio)-furan-2-yl)phenoxy)-3-phenylpropan-2-one derivatives (12a-j): The synthesis of titled compounds **12a-j** were achieved by stirring of compounds (**9a-j**) (0.24 mmol), compound **11**

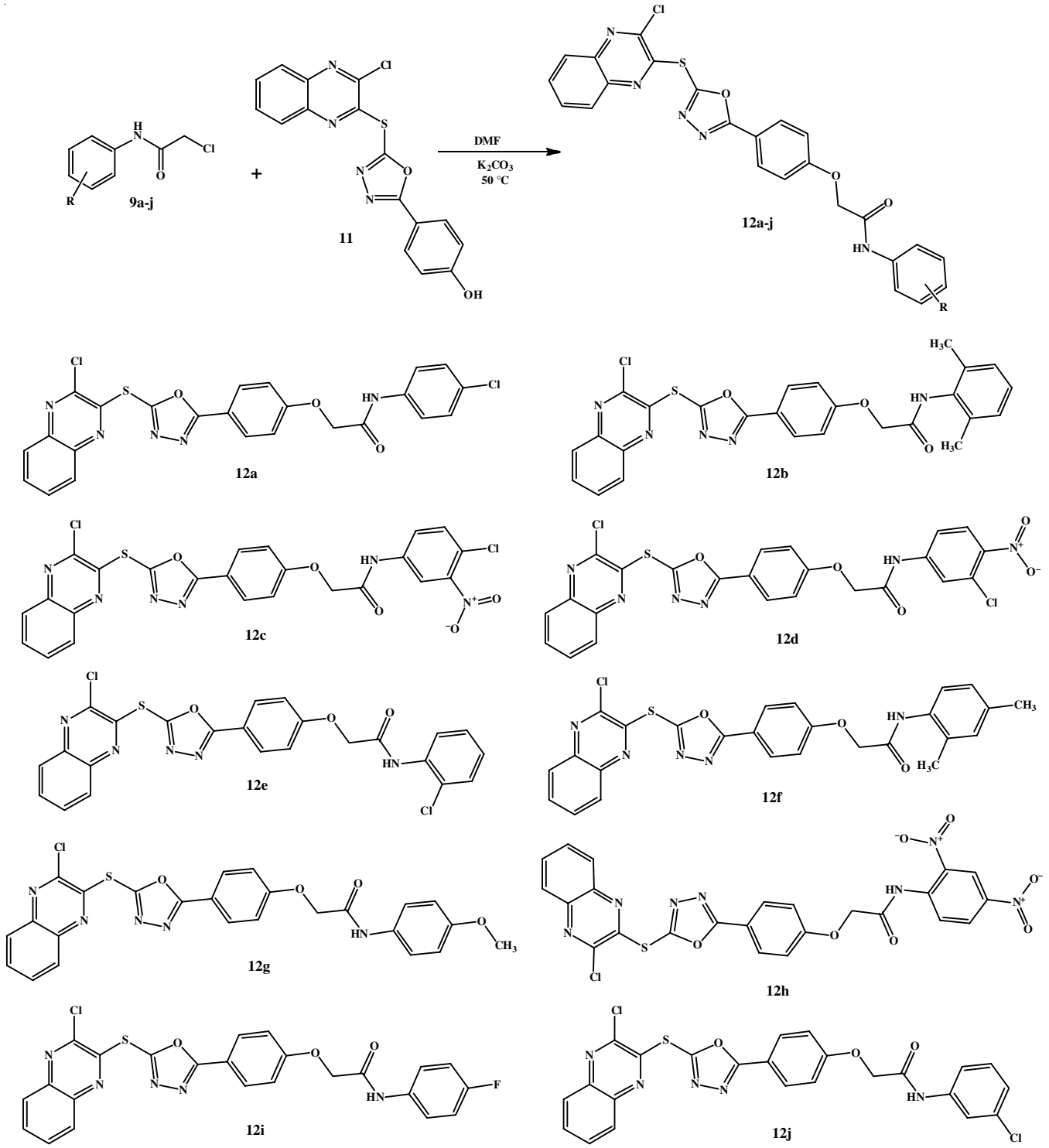


Scheme-VI: Synthesis of 4-{5-[(3-chloroquinoxalin-2-yl)sulfanyl]-1,3,4-oxadiazol-2-yl}phenol (**11**)

(0.12 mmol) and K_2CO_3 (0.36 mmol) in DMF (1.5 mL) at 50 ºC. The resulting solution was transferred to water, partitioned with ethyl acetate, washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The residue obtained was then purified using silica gel column chromatography (**Scheme-VII**).

***N*-(4-Chlorophenyl)-2-(4-(5-((3-chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)acetamide (12a):** Pale yellow solid; m.p.: 139-141 ºC; m.f.: $C_{24}H_{15}Cl_2N_5O_3S$; Relative molecular mass: 133.74; FTIR (KBr, ν_{max}, cm^{-1}): 1544 (C-N *str.* of the amide group), 1240 (C-N *str.* of the oxadiazole ring), 1710 (C=O *str.* of the amide group); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.46 (s, 1H, NH), 8.11 (d, 2H, Ar-H), 7.96 (q, 2H, Ar-H), 7.64 (q, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 7.40 (d, 2H, Ar-H), 7.38 (d, 2H, Ar-H), 4.26 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.9, 121.3, 127.8, 128.3, 129.2, 132.2, 137.9, 140.5, 145.1, 165.2. MS, *m/z*: 525 (M+1).

2-(4-(5-((3-Chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-*N*-(2,6-dimethylphenyl)acetamide (12b):



Scheme-VII: Synthesis of 1-(4-(5-((3-chloroquinoxalin-2-yl)thio)-furan-2-yl)phenoxy)-3-phenylpropan-2-one derivatives (**12a-j**)

Pale yellow solid; m.p.: 136-138 ºC; m.f.: $C_{26}H_{20}ClN_5O_3S$; Relative molecular mass: 138.66; FTIR (KBr, ν_{max}, cm^{-1}): 1177 (C-N *str.* of the oxadiazole ring), 1524 (C-N *str.* of the amide group), 1638 (C=C *str.* of the aromatic ring)), 658 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.62 (s, 1H, NH), 8.11 (d, 2H, Ar-H), 7.96 (q, 2H, Ar-H), 7.76 (q, 2H, Ar-H), 7.11 (d, 3H, Ar-H), 7.06 (s, 2H, Ar-H), 4.66 (s, 1H, CH_2), 2.13 (s, 2H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 18.0, 43.5, 115.5, 123.4, 125.5, 126.0, 127.0, 128.3, 131.3, 132.2, 132.3, 133.4, 135.2, 140.5, 145.1, 162.7, 165.2. MS, *m/z*: 518 (M+1).

***N*-(4-Chloro-3-nitrophenyl)-2-(4-(5-((3-chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)acetamide (12c):** Dark yellow solid; m.p.: 137-139 ºC; m.f.: $C_{24}H_{14}Cl_2N_6O_5S$; Relative molecular mass: 142.56; FTIR (KBr, ν_{max}, cm^{-1}):1680 (C=O *str.* of the amide linkage), 1651 (C=C *str.* of bonds in the aromatic rings), 1332 (C-N *str.* of amide linkage); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.74 (s, 1H, NH), 8.49 (d, 1H, Ar-H), 8.11 (d, 1H, Ar-H), 8.08 (d, 2H, Ar-H), 7.82 (q, 2H, Ar-H), 7.80 (q, 1H, Ar-H), 7.63 (q, 2H, Ar-H), 7.08 (d, 2H, Ar-H), 4.38 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.5, 115.5, 123.4, 126.0, 126.4, 127.1, 127.3, 128.0, 128.3, 130.0, 132.2, 134.6, 140.5, 145.0, 165.6. MS *m/z*: 570 (M+1).

***N*-(3-Chloro-4-nitrophenyl)-2-(4-(5-((3-chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)acetamide (12d):** Yellow solid; m.p.: 111-113 ºC; m.f.: $C_{24}H_{14}Cl_2N_6O_5S$; Relative molecular mass: 142.56; FTIR (KBr, ν_{max}, cm^{-1}): 1676 (C=O) *str.* of the amide linkage), 1329 (C-N *str.* of amide linkage), 1508 (N-O *str.* of nitro group), 759 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.19 (s, 1H, NH), 8.22 (d, 1H, Ar-H), 8.10 (d, 1H, Ar-H), 8.08 (d, 2H, Ar-H), 7.97 (q, 2H, Ar-H), 7.94 (q, 1H, Ar-H), 7.93 (q, 2H, Ar-H), 7.09 (d, 2H, Ar-H), 4.51 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 59.9, 114.0, 115.5, 115.9, 123.4, 123.6, 124.4, 125.1, 125.4, 128.3, 132.2, 136.3, 140.5, 140.9, 144.1, 145.0, 151.8, 155.6, 166.3. MS *m/z*: 570 (M+1).

***N*-(2-Chlorophenyl)-2-(4-(5-((3-chloroquinoxalin-2-yl)-thio)-1,3,4-oxadiazol-2-yl)phenoxy)acetamide (12e):** White solid; m.p.: 79-81 ºC; m.f.: $C_{24}H_{15}Cl_2N_5O_3S$; Relative molecular mass: 133.74; FTIR (KBr, ν_{max}, cm^{-1}):3257 (N-H *str.*), 758 (C-Cl *str.*), 1667 (C=O *str.* of the amide); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.88 (s, 1H, NH), 8.10 (d, 1H, Ar-H), 8.08 (d, 2H, Ar-H), 7.97 (q, 2H, Ar-H), 7.94 (q, 2H, Ar-H), 7.74 (d, 1H, Ar-H), 7.51 (t, 1H, Ar-H), 7.37 (t, 1H, Ar-H), 7.36 (d, 2H, Ar-H), 4.39 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.5, 115.5, 123.4, 126.0, 126.4, 127.1, 127.3, 128.0, 128.3, 130.0, 132.2, 134.6, 140.5, 145.0, 165.6. MS *m/z*: 525 (M+1).

2-(4-(5-((3-Chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-N-(2,4-dimethylphenyl)acetamide (12f): White solid; m.p.: 138-140 ºC; m.f.: $C_{26}H_{20}ClN_5O_3S$; Relative molecular mass: 138.66; FTIR (KBr, ν_{max}, cm^{-1}): 1658 (C=O), 3009 (C-H *str.*), 1257 (C-O *str.*), 1095 (C-N *str.*), 758 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 9.59 (s, 1H, NH), 8.14 (d, 2H, Ar-H), 7.97 (q, 2H, Ar-H), 7.94 (q, 2H, Ar-H), 7.24 (d, 1H, Ar-H), 7.03 (d, 2H, Ar-H), 6.98 (d, 1H, Ar-H), 4.28 (s, 1H, CH_2), 2.25 (s, 1H, CH_3), 2.15 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 18.0, 20.9, 43.5, 115.5, 123.4, 125.5, 126.0, 127.0, 128.3, 131.3, 132.2, 132.3, 133.4, 135.2, 140.5, 145.1, 162.7, 165.2. MS *m/z*: 518 (M+1).

2-(4-(5-((3-Chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-N-(4-methoxyphenyl)acetamide (12g): Black solid; m.p.: 122-124 ºC; m.f.: $C_{25}H_{18}ClN_5O_4S$; Relative molecular mass: 135.22; FTIR (KBr, ν_{max}, cm^{-1}): 1660 (C=O *str.* of the amide linkage), 1336 (C-N *str.* of amide linkage), 774 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.16 (s, 1H, NH), 8.08 (d, 2H, Ar-H), 7.94 (q, 2H, Ar-H), 7.50 (q, 2H, Ar-H), 7.49 (d, 2H, Ar-H), 7.12 (d, 2H, Ar-H), 6.89 (d, 2H, Ar-H), 4.36 (s, 1H, CH_2), 3.86 (s, 1H, CH_3). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.7, 59.9, 114.0, 115.5, 115.9, 123.4, 123.6, 126.0, 126.0, 128.3, 132.2, 136.3, 140.5, 140.9, 144.1, 145.0, 151.8, 155.6, 166.3. MS *m/z*: 520 (M+1).

2-(4-(5-((3-Chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-*N*-(2,4-dinitrophenyl)acetamide (12h): Yellow solid; m.p.: 174-176 ºC; m.f.: $C_{24}H_{14}ClN_7O_7S$; Relative molecular mass: 146.37; FTIR (KBr, ν_{max}, cm^{-1}): 1670 (C=O *str.* of the amide linkage), 1255 (C-N *str.* of amide linkage), 1548 (N-O *str.* of nitro group), 760 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.02 (s, 1H, NH), 8.80 (s, 1H, Ar-H), 8.38 (d, 1H, Ar-H), 8.12 (d, 1H, Ar-H), 8.08 (d, 2H, Ar-H), 7.97 (q, 2H, Ar-H), 7.94 (q, 2H, Ar-H), 7.11 (d, 2H, Ar-H), 3.35 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.5, 115.5, 123.4, 126.0, 126.4, 127.1, 127.3, 128.0, 128.3, 130.0, 132.2, 134.6, 140.5, 145.0, 165.6. MS *m/z*: 580 (M+1).

2-(4-(5-((3-Chloroquinoxalin-2-yl)thio)-1,3,4-oxadiazol-2-yl)phenoxy)-*N*-(4-fluorophenyl)acetamide (12i): Dark brown solid; m.p.: 127-129 ºC; m.f.: $C_{24}H_{15}ClFN_5O_3S$; Relative molecular mass: 128.69; FTIR (KBr, ν_{max}, cm^{-1}): 1670 (C=O *str.* of the amide linkage), 1335 (C-N *str.* of amide linkage), 828 (C-Cl *str.*), 1218 (C-F *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.37 (s, 1H, NH), 8.09 (d, 2H, Ar-H), 7.96 (q, 2H, Ar-H), 7.63 (q, 2H, Ar-H), 7.60 (q, 2H, Ar-H), 7.18 (t, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 4.25 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.9, 115.8, 115.9, 121.6, 121.6, 128.3, 132.2, 135.3, 135.3, 140.5, 145.0, 157.8, 159.7, 165.0. MS *m/z*: 508 (M+1).

***N*-(3-Chlorophenyl)-2-(4-(5-((3-chloroquinoxalin-2-yl)-thio)-1,3,4-oxadiazol-2-yl)phenoxy)acetamide (12j):** Yellow solid; m.p.: 83-85 ºC; m.f.: $C_{24}H_{15}Cl_2N_5O_3S$; Relative molecular mass: 133.74; FTIR (KBr, ν_{max}, cm^{-1}): 1687 (C=O *str.* of the amide linkage), 1338 (C-N *str.* of amide linkage), 772 (C-Cl *str.*); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 10.52 (s, 1H, NH), 8.12 (d, 2H, Ar-H), 7.96 (q, 2H, Ar-H), 7.79 (q, 2H, Ar-H), 7.47 (q, 2H, Ar-H), 7.16 (t, 2H, Ar-H), 7.12 (d, 2H, Ar-H), 4.27 (s, 1H, CH_2). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm: 43.5, 115.5, 123.4, 125.5, 126.0, 127.0, 128.3, 131.3, 132.2, 132.3, 133.4, 135.2, 140.5, 145.1, 162.7, 165.2. MS *m/z*: 525 (M+1).

***In vitro* study:** The cytotoxicity of synthesized derivatives (**10a-j** and **12a-j**) was assessed *in vitro* using the MTT assay against HepG2 and MCF-7 cancer cell lines using 5-fluorouracil as standard [19].

In vivo study

Experimental animals: Adult male albino Wistar rats (200-250 g) were grouped into six rats per group. They were

housed individually in polypropylene cages with paddy husk bedding and provided *ad libitum* access to water and standard laboratory feed before dietary manipulation [20,21]. Experimental procedures were ethically reviewed and approved by the Institutional Animal Ethics Committee (Reg. No. 1566/Po/Re/S/11/CPCSEA).

Preparation of test solutions: Compound **10b** demonstrated the superior binding affinity and cytotoxic activity compared to other synthesized compounds. Therefore, it was selected for *in vivo* study. A solution of compound **10b** was prepared using DMSO as solvent for oral administration to the experimental animals. DMSO was selected for its ability to effectively solubilize organic compounds and its low toxicity [22]. Prior to administration, compound **10b** was carefully weighed and added to DMSO to achieve the desired concentration. This ensured uniform dispersion and accurate dosing in the study, contributing to reliable results.

Dose selection: The dose selection for compound **10b** followed a systematic approach; starting with a conservative dose of 2000 mg/kg body weight p.o. subsequent doses were chosen using a dose escalation strategy, with a lower dose of 200 mg/kg, representing 1/10th of the initial dose, for further investigation. This allowed for the evaluation of both efficacy and safety while minimizing risks. Animals were closely monitored for signs of toxicity and physiological changes throughout the study to ensure reliability and ethical conduct.

Experimental protocol: The experimental protocol involved acclimatizing adult albino male Wistar rats, individually housed with unrestricted access to feed and water [23]. They were randomly assigned to different groups (Table-1): a positive control group received with diethylnitrosamine (G1), a standard group with 5-fluorouracil (G2) and a test group with compound **10b** (G3). Rats were administered a single intraperitoneal dose of diethylnitrosamine at a concentration of 200 mg/kg body weight. This was followed by weekly subcutaneous injections of carbon tetrachloride (CCl_4) at a dose of 200 mg/kg body weight once a week for the period of 3 weeks. Combining CCl_4 and N-nitrosodiethylamine (DEN) in experimental models has been shown to produce the synergistic effects in inducing hepatocellular carcinoma. The use of CCl_4 in combination with DEN in rats offers a well-characterized, reproducible and clinically relevant model for studying hepatocellular carcinogenesis. These treatments were employed to induce hepatocellular carcinoma in the experimental animals. Then for next 4 weeks, 5-fluorouracil as standard and compound **10b** as test were administered orally to G2 & G3, respectively.

TABLE-1
EXPERIMENTAL DESIGN FOR *in vivo* STUDY

Group	Name	Treatment	Number of animals
G1	Positive control	Diethylnitrosamine	6
G2	Standard	5-Fluorouracil	6
G3	Test	1,3,4-Oxadiazole derivative	6

Physiological parameters assessment: Physiological parameters, including initial and final body weights, food and water consumption, were rigorously evaluated across experimental groups. Body weights were recorded before and after the treatment period, with any deviations subjected to statistical analysis [24]. Daily assessments of food and water intake aided in detecting changes in appetite or hydration. These observations provided valuable insights into the physiological responses to the experimental interventions.

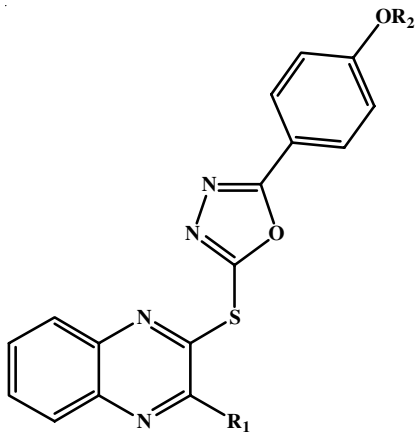
Oxidative and enzymatic parameters: Oxidative parameters, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA), were assessed in tissue samples collected from experimental groups. Samples were homogenized and assays were performed using commercial kits following established protocols [25]. Absorbance or fluorescence signals were measured with spectrophotometry or fluorescence microplate readers. Statistical analysis was conducted to identify significant differences between groups.

Blood collection and biochemical analysis: Blood samples were collected for analysis of serum liver enzymes (AST, ALT, ALP), total protein levels (albumin, globulin) and bilirubin levels. These assessments provide insights into liver function and health, aiding in understanding the impact of treatments on liver parameters in the experimental model [26,27].

Statistical analysis: A one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was utilized for statistical analysis, considering a sample size of 6, with data presented as Mean ± SEM.

RESULTS AND DISCUSSION

Design and preliminary *in silico* screening: The 1,3,4-oxadiazole scaffold provides opportunities for structural modifications at various positions (Fig. 1), enabling the synthesis of a diverse array of derivatives. Substituents can be introduced to tailor the compounds for specific biological targets or to enhance pharmacokinetic properties. Design of the 1,3,4-oxadiazole derivatives were done with established anticancer properties and pharmacophoric features. To achieve a thorough assessment, the selection method also took into account for the variety of 1,3,4-oxadiazoles. Modifications is done as replacement of



R_1= Chlorine and methyl piperazine
R_2= N-phenyl acetamide

Fig. 1. Structural modification opportunities in 1,3,4-oxadiazole derivatives for enhanced anticancer activity

alkyl aromatic linker (R_2) at phenoxy group of 1,3,4-oxadiazole by *N*-phenyl acetamide to check affinity towards the protein. From the designed molecules, 20 molecules were (**10a-j** & **12a-j**) selected followed by *in silico* analysis.

The synthesis of compounds **10a-j** & **12a-j** was conducted according to the procedures outlined in **Schemes I-VII**, followed by characterization using IR, NMR and mass spectroscopic techniques. In the IR spectra, the presence of characteristic peaks corresponding to C=O stretching of amide linkage (1680-1630 cm^{-1}), C-N stretching of amide linkage (1350-1200 cm^{-1}) and N-H stretching (~2925 cm^{-1}) confirmed the synthesis of compounds **10a-j**. Similarly, presence of characteristic peaks corresponding to C=O stretching of the amide linkage (1680-1630 cm^{-1}), C-N stretching of amide linkage (1350-1200 cm^{-1}) and N-H stretching (~2925 cm^{-1}) and C-Cl stretching (850-550 cm^{-1}) confirmed the synthesis of compounds **12a-j**. The structures of the synthesized compounds **10a-j** & **12a-j** were further confirmed by ^{1}H NMR, ^{13}C NMR and mass spectra analysis. The ^{1}H NMR spectra of the synthesized derivatives revealed the characteristic peaks indicative of certain structural elements. A singlet peak at δ 10 ppm suggests the presence of an amide -NH group attached to a nitrogen atom in all derivatives. Aromatic protons are observed at δ 8.11, 7.81, 7.65, 7.11 and 7.09 ppm, with doublet, quartet and doublet patterns indicating *para*-, *meta*- and *ortho*-substituted aromatic rings, respectively. This pattern suggests the presence of multiple aromatic rings in all synthesized derivatives. Methylene (-CH_2-) groups were identified at δ 4.05 and 3.50 ppm, with singlet and triplet patterns, indicating their presence in an alkyl chain or connected to a heteroatom in all derivatives. Singlet peaks at δ 2.19 and 2.13 ppm indicate the presence of methyl (-CH_3) groups in all derivatives. Collectively, these findings confirm the presence of aromatic protons consistent with specified substituents such as the chlorophenyl group and the quinoxaline ring in all synthesized derivatives, providing robust evidence for the proposed molecular structures. Further, the ^{13}C NMR spectra also revealed the consistent features: distinct methyl (-CH_3) groups at δ 18.1 and 18.4 ppm, methylene (-CH_2-) signals at δ 46.0 and 48.9 ppm and potential methylene (-CH_2-) or methine (-CH-) groups at δ 60.2 and 62.3 ppm. Aromatic carbon peaks between δ 125.5 to 162.4 ppm indicate diverse substitution patterns, while peaks from δ 145.0 to 162.4 ppm suggest carbonyl-containing functional groups. In the mass spectra, all synthesized compounds displayed M+1 peaks consistent with their respective chemical formulas.

***In vitro* study:** The study evaluated the effects of all the synthesized derivatives on the viability of HepG2 and MCF-7 cell lines, which are commonly, used models for liver and breast cancer [28], respectively (Table-2). Among the compounds tested, **10b** and **10e** showed the significant activity. Compound **10b** displayed IC_{50} values of 4.87 µM against HepG2 cells and 6.90 µM against MCF-7 cells. Similarly, compound **10e** exhibited IC_{50} values of 5.59 µM against HepG2 cells and 7.82 µM against MCF-7 cells. These results suggest that both compounds **10b** & **10e** have potential cytotoxic effects on liver and breast cancer cells, as indicated by their ability to inhibit cell viability at relatively low concentrations. This implies that compounds **10b** and **10e** may hold promise as potential anticancer agents.

TABLE-2
CYTOTOXIC ACTIVITY OF TESTED COMPOUNDS (**10a-j** AND **12a-j**) ON CANCER CELL LINES

Compd.	IC_{50} (µM)		Compd.	IC_{50} (µM)	
	HepG2 cell line	MCF-7 cell line		HepG2 cell line	MCF-7 cell line
10a	28.04	34.82	**12a**	16.78	18.28
10b	4.87	6.90	**12b**	31.24	39.96
10c	48.3	53.30	**12c**	44.58	45.77
10d	49.53	52.48	**12d**	13.12	17.79
10e	5.59	7.82	**12e**	11.67	17.37
10f	15.06	16.32	**12f**	8.12	8.68
10g	53.55	57.68	**12g**	46.42	50.51
10h	54.87	56.46	**12h**	47.67	52.46
10i	20.39	20.70	**12i**	15.19	17.91
10j	15.9	16.99	**12j**	43.92	44.25
5-FU	5.17	5.75	FU = Fluorouracil		

Structure-activity relationship (SAR): Based on the findings from both *in vitro* assays and molecular docking studies, it is evident that the 1,3,4-oxadiazole ring plays a crucial role in conferring anticancer activity. Substitutions at the specific positions within this ring (Fig. 2) further enhance this activity. Introduction of a thio-ether linked with a quinoxaline moiety at the 2nd position of the oxadiazole ring, along with additional substitution with methyl piperazine, significantly improves the compound's anticancer potency. Moreover, at the 5th position of the oxadiazole ring, the presence of a substituted phenyl

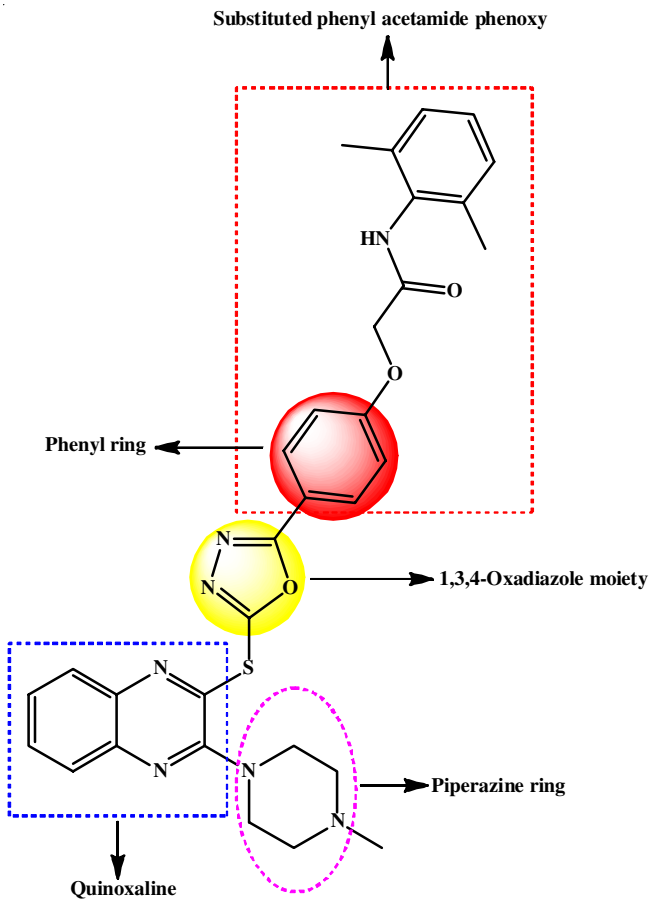


Fig. 2. Structure of **10b** showing substitutions at specific positions

acetamide phenoxy linker is associated with remarkable anticancer effects. Compounds bearing this substitution pattern, such as comounds **10b** and **10e**, demonstrated promising activity against cancer cells. Compound **10b** exhibits superior anticancer activity, with an IC_{50} value of 4.87 µM, outperforming the standard compound with an IC_{50} value of 5.17 µM. On the other hand, compound **10e** shows significant anticancer efficacy against the HepG2 cell line, with an IC_{50} value of 5.59 µM. These observations underscore the importance of structural modifications, particularly at the 2nd and 5th positions of the oxadiazole ring, in optimizing the anticancer activity.

In vivo study

Physiological parameters: The physiological parameters, including initial and final body weights, food consumption and water consumption (Table-3), were assessed across the experi-mental groups (G1, G2 and G3) subjected to distinct treatments (control, standard and test). The initial body weights were comparable, with G1 starting at 250.00 ± 5.00 g, G2 at 248.00 ± 4.00 g and G3 at 247.50 ± 3.50 g. Following the treatment period, the final body weights exhibited a slight decrease, with G1 recording 245.00 ± 5.00 g, G2 at 242.00 ± 4.00 g and G3 at 240.00 ± 3.00 g.

Oxidative and enzymatic parameters: Table-4 illustrates the levels of oxidative parameters, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA), across different experimental groups. When compared with G1-treated rats, G2-treated rats exhibited a significant increase ($p < 0.001$) in hepatic levels of GSH, SOD and Catalase, alongside a significant decrease ($p < 0.001$) in MDA levels. On the other hand, G2-treated rats demonstrated a significant increase in hepatic levels of GSH ($p < 0.01$), SOD ($p < 0.01$) and catalase ($p < 0.01$), along with a significant decrease ($p < 0.01$) in MDA levels compared to G1-treated rats. These results imply that compound **10b** (G3) may possess antioxidant properties.

Hepatoprotective and anticancer activity of compound 10b: The intent of the study was to assess the hepatoprotective properties of compound **10b** in comparison to the usual treatment, 5-fluorouracil and a positive control group exposed to diethylnitrosamine (Table-5, Fig. 3). In comparison to G1-treated rats, those treated with G2 demonstrated a significant decrease ($p < 0.01$) in AST and ALT levels, as well as a notable decrease ($p < 0.001$) in ALP and bilirubin levels. Similarly, G3-treated rats exhibited significant reductions ($p < 0.001$) in AST, ALT and ALP levels compared to G1-treated rats, with a significant decrease ($p < 0.01$) in bilirubin levels. The adminis-

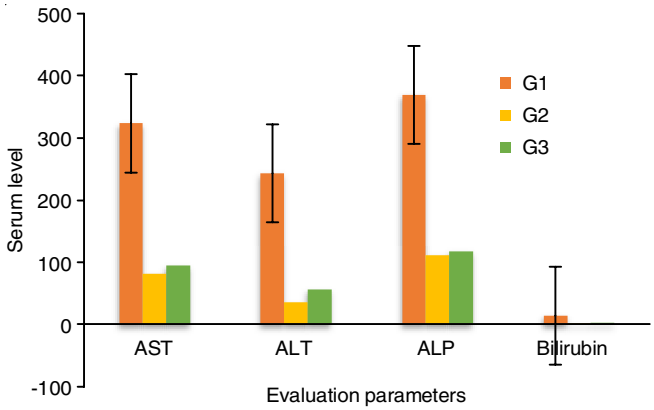


Fig. 3. Hepatoprotective and anticancer activity of compound **10b**

TABLE-3
ASSESSMENT OF PHYSIOLOGICAL PARAMETERS

Group	Name	Initial weight (g)	Final weight (g)	Food consumption (g/day)	Water consumption (mL/day)
G1	Positive control	250.00 ± 5.00	245.00 ± 5.00	20.00 ± 1.00	50.00 ± 2.00
G2	Standard	248.00 ± 4.00	242.00 ± 4.00	19.50 ± 1.50	49.00 ± 2.50
G3	Test	247.50 ± 3.50	240.00 ± 3.00	19.75 ± 1.25	48.75 ± 2.25

Results are presented as mean ± SEM, (n = 6). ANOVA followed by Dunnett test.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ when compared with G1 group.

TABLE-4
THE LEVEL OF OXIDATIVE AND ENZYMATIC PARAMETERS

Group	Superoxide dismutase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPx)	Malondialdehyde (MDA)
G1	10.25 ± 1.32	8.92 ± 1.15	14.57 ± 2.08	12.43 ± 1.72
G2	22.47 ± 2.15***	15.78 ± 1.98***	26.84 ± 2.45***	6.89 ± 0.98***
G3	17.89 ± 1.94**	12.36 ± 1.67**	20.19 ± 1.86**	9.75 ± 1.21**

Results are presented as mean ± SEM, (n = 6). ANOVA followed by Dunnett test.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ when compared with G1 group.

TABLE-5
HEPATOPROTECTIVE AND ANTICANCER ACTIVITY OF COMPOUND **10b**

Group	Name	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)
G1	Positive control	Diethylnitrosamine	323.28±2.67	243.31±1.5	369.55±0.24	3.94±1.20
G2	Standard	5-fluorouracil	81.41±1.64**	35.15±2.09**	110.95±0.75***	1.44±1.80***
G3	Test	Compound **10b**	95.29±2.44***	56.11±1.76***	117.78±2.98***	2.06±1.2**

Results are presented as mean ± SEM, (n = 6). ANOVA followed by Dunnett test.
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ when compared with G1 group.

tration of G2, representing standard treatment with 5-fluorouracil, resulted in consistent and considerable declines in AST, ALT, ALP and bilirubin levels compared to the positive control group (G1). While G3 (compound **10b**) also showed significant hepatoprotective effects, albeit slightly less potent than 5-fluorouracil (G2), suggesting promising therapeutic potential in alleviating diethylnitrosamine-induced liver damage.

Conclusion

In conclusion, the successful synthesis of a series of novel 1,3,4-oxadiazoles (**10a-j** & **12a-j**) was achieved through diverse synthetic routes. The *in vitro* evaluation of the synthesized compounds demonstrated considerable cytotoxicity against the HepG2 and MCF-7 cell lines. Remarkably, compounds **10b** and **10e** displayed significant potency with IC_{50} values of 4.87 µM and 6.90 µM against HepG2 cells and 5.59 µM and 7.82 µM against MCF-7 cells, respectively. These findings underscore the promising anticancer potential of these compounds against liver and breast cancer cells. *In vivo* studies further substantiated the anticancer activity of compound **10b**. Moreover, administration of compound **10b** demonstrated significant hepatoprotective effects, albeit slightly less potent than the standard treatment with 5-fluorouracil. This suggests a potential therapeutic role in alleviating diethylnitrosamine-induced liver damage. Overall, these results contribute valuable insights into the potential of 1,3,4-oxadiazoles as promising candidates for the development of novel anticancer agents with potential hepatoprotective properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Bray, M. Laversanne, H. Sung, J. Ferlay, R.L. Siegel, I. Soerjomataram and A. Jemal, *CA: Cancer J. Clin.*, **74**, 229 (2024); https://doi.org/10.3322/caac.21834
2. G. Ioele, M. Chieffallo, M.A. Occhiuzzi, M. De Luca, A. Garofalo, G. Ragno and F. Grande, *Molecules*, **27**, 5436 (2022); https://doi.org/10.3390/molecules27175436
3. H.E. Marei, C. Cenciarelli and A. Hasan, *Cancer Cell Int.*, **22**, 255 (2022); https://doi.org/10.1186/s12935-022-02679-8
4. Y. Xia, M. Sun, H. Huang and W.-L. Jin, *Signal Transduct. Target Ther.*, **9**, 92 (2024); https://doi.org/10.1038/s41392-024-01808-1
5. F. Wang, D.-Y. Ruan and R.-H. Xu, *Cell*, **187**, 1578 (2024); https://doi.org/10.1016/j.cell.2024.02.040
6. J.-J. Wang, W. Sun, W.-D. Jia, M. Bian and L.-J. Yu, *J. Enzyme Inhib. Med. Chem.*, **37**, 2304 (2022); https://doi.org/10.1080/14756366.2022.2115036
7. S. Nayak, S.L. Gaonkar, E.A. Musad, A. Mohammed and A.L. Dawsar, *J. Saudi Chem. Soc.*, **25**, 101284 (2021); https://doi.org/10.1016/j.jscs.2021.101284
8. T. Glomb, K. Szymankiewicz and P. Swiatek, *Molecules*, **23**, 3361 (2018); https://doi.org/10.3390/molecules23123361
9. S. Bajaj, M.S. Kumar, H. Tinwala and M. Yc, *Bioorg. Chem.*, **111**, 104873 (2021); https://doi.org/10.1016/j.bioorg.2021.104873
10. M.J. Ahsan, *Mini Rev. Med. Chem.*, **22**, 164 (2022); 10.2174/1389557521666210226145837
11. D. Kumar, N. Aggarwal, A. Deep, H. Kumar, H. Chopra, R.K. Marwaha and S. Cavalu, *Pharmaceuticals*, **16**, 254 (2023); https://doi.org/10.3390/ph16020254
12. E.M. Goldblatt and W.H. Lee, Am. J. Transl. Res., 2, 1 (2010).
13. B. Victoir, C. Croix, F. Gouilleux and G. Prié, *Cancers*, **16**, 461 (2024); https://doi.org/10.3390/cancers16020461
14. L. Yurttas, B.K. Çavusoglu, G.A. Çiftçi and H.E. Temel, *Anticancer Agents Med. Chem.*, **18**, 914 (2018); https://doi.org/10.2174/1871520618666180322123327
15. A.C. Kumar, J. Rangaswamy, Madalambika, P.M. BharathKumar, P.R. Patil, M. Salavadi and N. Naik, *J. Mol. Struct.*, 138749 (2024); https://doi.org/10.1016/j.molstruc.2024.138749
16. G. Sonawane, S. Sharma and R. Gilhotra, *Asian J. Chem.*, **36**, 1072 (2024); https://doi.org/10.14233/ajchem.2024.31342
17. Y. Ono, M. Ninomiya, D. Kaneko, A.D. Sonawane, T. Udagawa, K. Tanaka, A. Nishina and M. Koketsu, *Bioorg. Chem.*, **104**, 104245 (2020); https://doi.org/10.1016/j.bioorg.2020.104245
18. M. Soleiman-Beigi, R. Aryan, M. Yousofizadeh and S. Khosravi, *J. Chem.* **2013**, 476358 (2013); https://doi.org/10.1155/2013/476358
19. T. Glomb, K. Szymankiewicz and P. Swiatek, *Molecules*, **23**, 3361 (2018); https://doi.org/10.3390/molecules23123361
20. D. Mukherjee and R. Ahmad, *Interdiscip. Toxicol.*, **8**, 1 (2015); https://doi.org/10.1515/intox-2015-0001
21. K.K. Dar, S. Ali, M. Ejaz, S. Nasreen, N. Ashraf, S.F. Gillani, N. Shafi, S. Safeer, M.A. Khan, S. Andleeb and T.A. Mughal, *Braz. J. Biol.*, **79**, 629 (2019); https://doi.org/10.1590/1519-6984.186565
22. S. Sundaresan and P. Subramanian, *Pol. J. Pharmacol.*, **55**, 37 (2003).
23. R. Kartik, C.V. Rao, P. Pushpangadan, S.P. Trivedi and G.D. Reddy, *Iranian J. Pharm. Sci.*, **6**, 99 (2010).
24. E.S. Mohammed, N.M. El-Beih, E.A. El-Hussieny, E. El-Ahwany, M. Hassan and M. Zoheiry, *Arch. Med. Sci.*, **17**, 218 (2021); https://doi.org/10.5114/aoms.2020.93739
25. S. Mahmoud, S. Hussein, H. Rashed, E.M.A. Abdelghany and A.I. Ali, *Curr. Mol. Pharmacol.*, **15**, 434 (2022); https://doi.org/10.2174/1874467214666210531164546
26. R.H. Hussein and F.K. Khalifa, *Saudi J. Biol. Sci.*, **21**, 589 (2014); https://doi.org/10.1016/j.sjbs.2014.03.004
27. K. Pansare, C. Upasani, A. Upaganlwar, G. Sonawane and C. Patil, *Vidyabharati Int. Interdisciplinary Res. J.*, 320 (2021).
28. G.M. Safwat, K.M.A. Hassanin, E.T. Mohammed, E.K. Ahmed, M.R. Abdel Rheim, M.A. Ameen, M. Abdel-Aziz, A.M. Gouda, I. Peluso, R. Almeer, M.M. Abdel-Daim and A. Abdel-Wahab, *Oxid. Med. Cell. Longev.*, **2021**, 4759821 (2021); https://doi.org/10.1155/2021/4759821