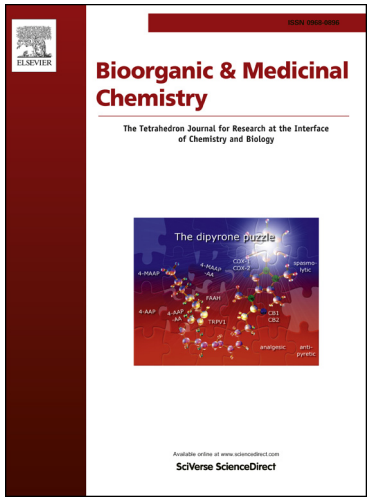
# Accepted Manuscript

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# Synthesis of novel benzohydrazone-oxadiazole hybrids as *β*-glucuronidase inhibitors and molecular modeling studies

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**Abstract:**

A series of compounds consisting of **25** novel oxadiazole-benzohydrazone hybrids (**6-30**) were synthesized through a five-step reaction sequence and evaluated for their *β*-glucuronidase inhibitory potential. The $IC_{50}$ values of compounds **6-30** were found to be in the range of 7.14 to 44.16 $\mu$M. Compounds **6**, **7**, **8**, **9**, **11**, **13**, **18**, and **25** were found to be more potent than *D*-saccharic acid 1,4-lactone (48.4 ± 1.25 $\mu$M). These compounds were further subjected for molecular docking studies to confirm the binding mode towards human *β-D*-glucuronidase active site. Docking study for compound **13** ($IC_{50}$ = 7.14 ± 0.30 $\mu$M) revealed that it adopts a binding mode that fits within the entire pocket of the binding site of *β-D*-glucuronidase. Compound **13** has the maximum number of hydrogens bonded to the residues of the active site as compared to the other compounds, *i.e.*, the *ortho*- hydroxyl group forms hydrogen bond with carboxyl side chain of Asp207 (2.1 Å) and with hydroxyl group of Tyr508 (2.6 Å). The other hydroxyl group forms hydrogen bond with His385 side chain (2.8 Å), side chain carboxyl oxygen of Glu540 (2.2 Å) and Asn450 side-chain's carboxamide NH (2.1 Å).

**Keywords:** Oxadiazole, Benzohydrazone, hybrids, *β*-Glucuronidase inhibition, Molecular docking

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## 1. Introduction

Heterocycles constitute among the largest division in medicinal chemistry and are of considerable importance biologically and industrially. Oxadiazole, which is a heterocyclic moiety, is also of immense importance in pharmaceutical industry due to its versatile biological actions. Compounds having oxadiazole nucleus are reported to possess unique antioedema and anti-inflammatory activities [1-4]. Besides that, compounds possessing oxadiazole ring have been reported for other activities such as analgesic [3-4], antitubercular [5], anti-hepatitis B viral activities [6], antimicrobial [7], and anticonvulsant [8]. The level of interest in oxadiazole for over the past few years is clearly indicated by the number of patent applications that have been filed. Plenty of compounds containing oxadiazole ring were being studied and are now in late stage of clinical trials such as zibotentan as an anticancer agent. Raltegravir, which also has an oxadiazole ring, was being studied as antiretroviral drug to treat HIV infection. Research has shown clearly that oxadiazoles impacted massively various drug discovery programs including cancer [9], obesity [10] infections [11], diabetes [12], inflammation [13], analgesic [14], antimitotic [15], anticonvulsive [16], antifungal [17] diuretic [18], and muscle relaxants [19]. Due to these potentials, oxadiazole moiety has played pivotal role in drug discovery and development as an essential or a part of the pharmacophore that could contribute to ligand binding [20]. Oxadiazole moieties have also been employed as an aromatic linker to place substituents in the right positions [21] as well as orientating molecular properties by locating them at the boundary of the molecules [22]. A recent study showed that oxadiazole regioisomers significantly changes thermodynamic properties by changing the water architecture within the active site of aldose reductase enzyme [23]. Oxadiazoles have also been used as replacements for carbonyl containing compounds such as carbamates, esters, hydroxamic esters and amides [24−26].

*β*-Glucuronidase is an enzyme that catalyzes cleavage of glucuronosyl-*O*-bonds [27]. This enzyme is involved in diseases such as inflammation related to joints, like rheumatoid arthritis [35, 36]. While in some cases, over-expression of this enzyme is reported to cause hepatic diseases and AIDS. Besides that, *β*-glucuronidase also takes place in diseases such as infection of the urinary tract [28-30], epilepsy [33], renal disease [31], rejection of transplantation [32], larynx and breast [34]. Various studies had been carried out to show that bacterial *β*-glucuronidase inhibitor decreases carcinogen induced colonic tumors [37].

Present work belongs to a simple and reliable synthetic protocol that has been developed for the synthesis of a series of benzohydrazone-oxadiazole hybrid pharmacophores in an effort towards developing more effective agents against *β*-glucuronidase enzyme. Evidence exists that hybridization

of two or more biologically active and new molecules with complementary pharmacophoric potentials or with different mechanisms of action generally render synergistic effects. This combined pharmacophore approach, in turn, may address the active site of different targets more effectively and could offer the possibility to overcome the issue of drug resistance as well as unwanted side effects [38]. The outcomes of this hybridization strategy have already been the subject of interest in finding novel antibacterial and antimalarial agents to overcome the issue of drug resistance [39].

Encouraged by these efforts, we decided to develop benzohydrazone-oxadiazole hybrids in order to reveal new perspectives in the biological potentials of newly synthesized hybrid molecules. *β*-Glucuronidase potential of benzohydrazones and oxadiazoles that have already been reported are being displayed in figure-1 [40-42]. However, the *β*-glucuronidase potential of benzohydrazone-oxadiazole hybrids has never been reported before. Here in, we are unveiling the synthesis of benzohydrazone-oxadiazole hybrids and their *β*-glucuronidase inhibitory potentials.

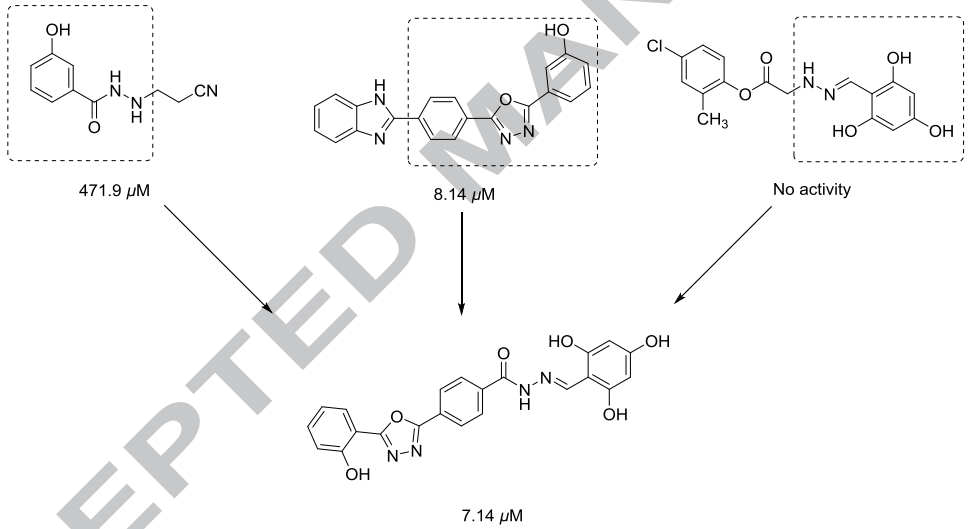
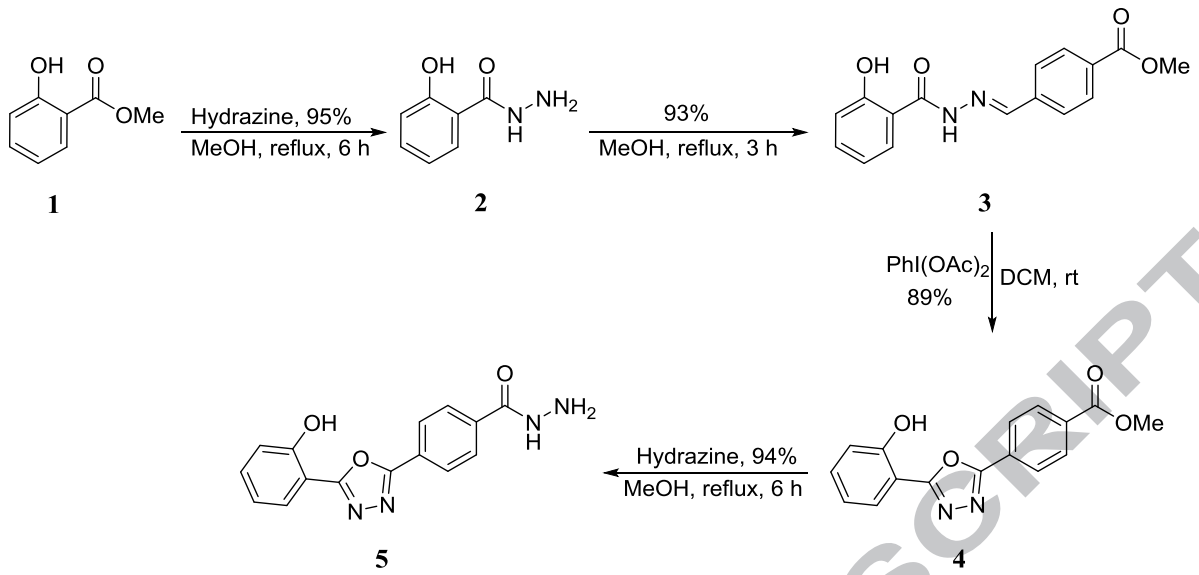



**Figure-1** Comparison between hydrazide, oxadiazole, hydrazone, and hybrid compound **13**.

## 2. Results and Discussion

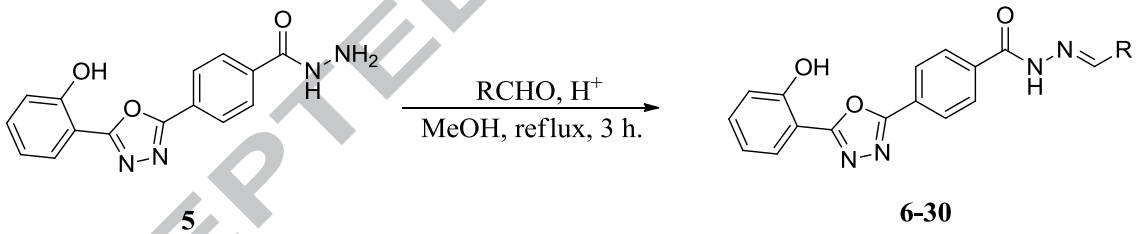
### 2.1 Chemistry

The synthetic route towards hybrids **6**-**30** is shown in Scheme **1** and **2**. Compound **2** was prepared by refluxing methyl 2-hydroxybenzoate **1** with the excess of hydrazine hydrate. The compound **2** was then reacted with methyl 4-formylbenzoate in methanol with catalytic amount of acetic acid to afford compound **3**. The resulting adduct **3** underwent oxidative cyclization using phenyliododiacetate ($PhI(OAc)_2$) in dry chloroform to form oxadiazole **4**. The ester functional group of compound **4** was further transformed into corresponding hydrazide **5** by treating with hydrazine hydrate.



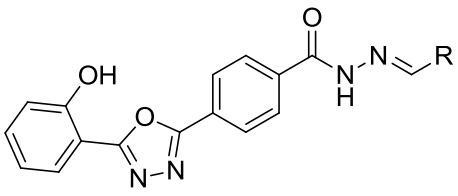

**Scheme-1.** Synthesis of benzohydrazide having oxadiazole ring compound **5**

In Scheme 2, the advanced intermediate hydrazide **5** was reacted with different aryl aldehydes in the presence of acetic acid to form the targeted oxadiazole benzohydrazone derivatives **6-30** (Table-1). The structures of oxadiazole benzohydrazone hybrids **6-30** were confirmed using spectroscopic techniques such as NMR, MS and were further confirmed using CHN analysis.

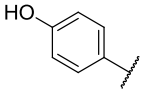
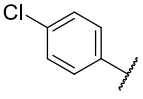


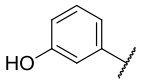
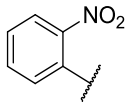
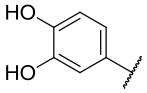
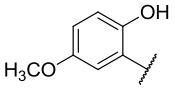
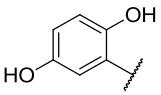
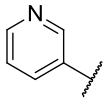
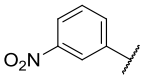
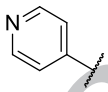
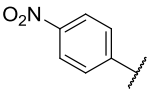
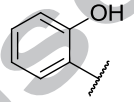
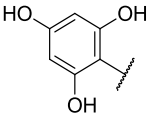
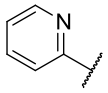
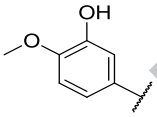
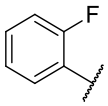
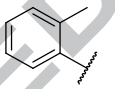
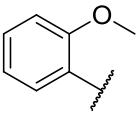
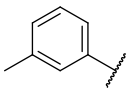
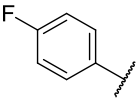
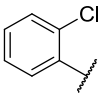
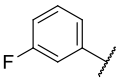
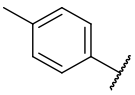
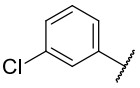
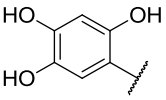

**Scheme-2.** Synthesis of novel oxadiazole benzohydrazones **6-30**

**Table-1.** Synthesis of oxadiazole benzohydrazones **6-30**




**6-30**

| Comp. no. | R | Comp. no. | R |
|---|---|---|---|
| **6** |  | **19** |  |

| | | | |
|---|---|---|---|
| **7** |  | **20** |  |
| **8** |  | **21** |  |
| **9** |  | **22** |  |
| **10** |  | **23** |  |
| **11** |  | **24** |  |
| **12** |  | **25** |  |
| **13** |  | **26** |  |
| **14** |  | **27** |  |
| **15** |  | **28** |  |
| **16** |  | **29** |  |
| **17** |  | **30** |  |
| **18** |  | | |

## 2.2 *β*-glucuronidase inhibition

In continuation of our search on new enzyme inhibitor [43], we have evaluated (25) derivatives **6-30** of oxadiazole bearing Schiff base linkage for their *β*–glucuronidase inhibition potential. The $IC_{50}$

values for potent compounds were in the range of 7.14 to 44.16 $\mu$M (Table-2). Compound **13** that is a trihydroxy substituted derivative displayed exceptionally potent single-digit activity with an $IC_{50}$ value of 7.14 ± 0.30 $\mu$M as compared to D-Saccharic acid 1,4-lactone. Compounds **6** ($IC_{50}$ = 18.46 ± 0.65 $\mu$M), **7** ($IC_{50}$ = 34.46 ± 0.85 $\mu$M), **8** ($IC_{50}$ = 29.15 ± 0.75 $\mu$M), **9** ($IC_{50}$ = 15.14 ± 0.55 $\mu$M), **11** ($IC_{50}$ = 44.16 ± 1.20 $\mu$M, **18** ($IC_{50}$ = 21.14 ± 1.05 $\mu$M) and **25** ($IC_{50}$ = 42.26 ± 1.16 $\mu$M) also showed good inhibitory potentials better than standard. It was observed that presence of hydroxyl group on aromatic side chain enhanced the inhibitory potential of certain compounds against $\beta$-glucuronidase when compared with the compounds without having the hydroxyl substituents. These findings corroborated our previous results that presence of hydroxyl groups at particular sites are important and decisive factors for compounds to be active against $\beta$-glucuronidase [41]. However, in current series, it is observed that presence of an *ortho*- hydroxyl group on benzene ring attached to oxadiazole moiety is not involved in the inhibitory potential of these compounds. For example, if we look at compounds **14-17**, **20**, **22**, **23**, and **27-30**, these all compounds lack hydroxyl group on benzene ring that is directly attached with hydrazone part while all these compounds have an *ortho*- hydroxyl group on benzene ring attached to oxadiazole part and were found all inactive. This observation suggests that presence of *ortho*- hydroxyl group on aromatic ring attached with oxadiazole moiety is not imperative for these compounds to be active. One could imagine the unavailability of this hydroxyl group since it may be involved in hydrogen bonding with nearby oxygen atom of oxadiazole part.

To better understand the inhibition mechanism of active compounds and their binding modes with enzyme active site, molecular modeling studies were performed. The compounds were docked into the binding pocket of $\beta$-glucuronidase to further confirm the inhibition potential and also the docked Goldscore is reported for newly synthesized **6-30** compounds (Table-2).

**Table-2.** In vitro $\beta$-glucuronidase inhibition activity of oxadiazole derivatives 6-30 and their docking studies Goldscore results

| Compound | Goldscore | External H-bond energy | External van der waals energy | $IC_{50}$ ($\mu$M ± SEM[a]) |
|---|---|---|---|---|
| **6** | **64.78** | **11.10** | 46.02 | 18.46 ± 0.65 |
| **7** | **69.07** | **11.04** | 48.25 | 34.46 ± 0.85 |
| **8** | **73.77** | **11.07** | 49.80 | 29.15 ± 0.75 |
| **9** | **68.78** | **11.13** | 45.29 | 15.14 ± 0.55 |
| **10** | 68.97 | 7.89 | 50.28 | 64.12 ± 1.62 |
| **11** | **66.02** | **9.84** | 49.11 | 44.16 ± 1.20 |
| **12** | 71.99 | 6.90 | 49.98 | 82.16 ± 2.25 |
| **13** | **72.89** | **13.74** | 45.93 | 7.14 ± 0.30 |

| | | | | |
|---|---|---|---|---|
| **14** | 64.72 | 6.74 | 43.87 | N.A |
| **15** | 67.49 | 5.37 | 47.37 | N.A |
| **16** | 66.57 | 0.00 | 49.25 | N.A |
| **17** | 66.33 | 2.32 | 48.36 | N.A |
| **18** | **70.05** | **11.09** | 46.09 | 21.14 ± 1.05 |
| **19** | 65.23 | 6.03 | 47.14 | 86.12 ± 2.15 |
| **20** | 66.98 | 5.07 | 45.22 | N.A. |
| **21** | 71.17 | 6.01 | 47.56 | 88.14 ± 2.38 |
| **22** | 66.16 | 2.54 | 50.42 | N.A. |
| **23** | 63.46 | 1.49 | 47.17 | N.A. |
| **24** | 70.83 | 5.98 | 46.66 | 91.4 ± 2.45 |
| **25** | **63.43** | **10.97** | 45.08 | 42.26 ± 1.16 |
| **26** | 64.26 | 5.54 | 47.23 | 108.14 ± 3.14 |
| **27** | 67.59 | 3.87 | 44.90 | N. A. |
| **28** | 63.34 | 2.83 | 45.51 | N. A. |
| **29** | 63.54 | 3.51 | 46.16 | N. A. |
| **30** | 67.39 | 2.87 | 49.08 | N. A. |
| **D-Saccharic acid 1,4-lactone** | 59.80 | 5.83 | 50.06 | 48.4 ± 1.25 |

The most active compounds Goldscore and external h-bond energy are highlighted in bold.

## 2.3 Docking study

Molecular docking studies were carried out on crystallographic structure of human *β*-glucuronidase (PDB code: 1BHG). Synthetic compounds **6-30** were docked into active site of human *β*-glucuronidase to reveal the binding mode of each compound. The binding mode of known substrate *p*-nitrophenyl *β*-glucuronide on *β*-glucuronidase active site reveals that glycoside bond of *p*-nitrophenyl *β*-glucuronide was perfectly oriented towards the catalytic residues Glu540 and Glu451 and acts as the acid/base catalyst where Glu540 functions as the nucleophilic residue. Docked binding mode showed *p*-nitrophenyl β-glucuronide perfectly fitting into the active site of *β-D*-glucuronidase forming hydrogen bonding with residues Glu451, His385 and Lys606 that could significantly interact with the enzyme, which is also shown in our previous study [44].

*β-D*-Glucuronidase structure was employed in this docking study to reveal the favorable binding mode of newly synthesized derivatives **6-30**. Predicted binding modes for biologically active and inactive compounds and their docked Goldscore are reported in **Table-2**.

The overall Goldscore (Mainly consist of external h-bonding energy and external van der waals energy) for active molecules were expected to be high when compared to the least active and inactive compounds. But by considering the reported Goldscore in this study as in **Table-2**, significant relationship cannot be established between Goldscore and inhibitory activity. Our previous studies showed that hydrophilic (h-bond) characteristic feature of *β-D*-Glucuronidase inhibitors are crucial for the inhibitory activity. Therefore, the overall activity of these compounds mainly depends on h-bonding and hence the activities of these compounds mainly depend on external h-bonding energy, while the external van der waals energy (**Table-2**) may not significantly influence the activity profile. From **Table-2** it could be noted that, as the external h-bond energy value increases the inhibitory activity profile of compounds also increases. For the most active compound **13**, compound **9**, compound **6** and compound **18**, the external h-bonding energy values were 13.74, 11.13, 11.10 and 11.09, respectively. For the inactive molecules these values were considerably low. Consequently, a significant relationship could be established for all compounds in **Table-2** based on external h-bond energy value and inhibitory activity.

Docking analysis of the binding mode for the most active compound **13** ($IC_{50}$ = 7.14 ± 0.30 $\mu$M) indicated that compound **13** adopts a binding mode that fits well the entire furrow of the binding site of *β-D*-glucuronidase. Analysis of the best ranked binding pose of compound **13** discovered that it has the maximum number of hydrogen bonded to the active site residues as compared to the other compounds and thus making compound **13** most potent. The benzenetriol group at *ortho* position forms hydrogen bond with side chain carboxyl oxygen of Asp207 (2.1 Å) and Tyr508 side chain hydroxyl group (2.6Å). While the other hydroxyl group at the *ortho* position forms hydrogen bonding with His385 side chain imidazole nitrogen (2.8 Å), Glu540 side chain carboxyl oxygen (2.2 Å) and Asn450 side-chain's carboxamide NH at a distance of 2.1 Å. Similarly, the benzohydrazide nitrogen forms hydrogen bond with Tyr504 side chain hydroxyl group (2.4Å), while the other NH forms hydrogen bond with carboxyl oxygen (2.2 Å) of Glu540. Benzohydrazide oxygen forms hydrogen bond with Tyr508 side chain hydroxyl group (2.6 Å). Predicted binding mode of compound **13** is shown in Fig.2a. Additionally, the phenyl group of the Tyr504, Tyr508 and the side chain of Asn484 forms hydrophobic interaction with the compound **13** that stabilize the binding mode in the *β-D*-glucuronidase active site.

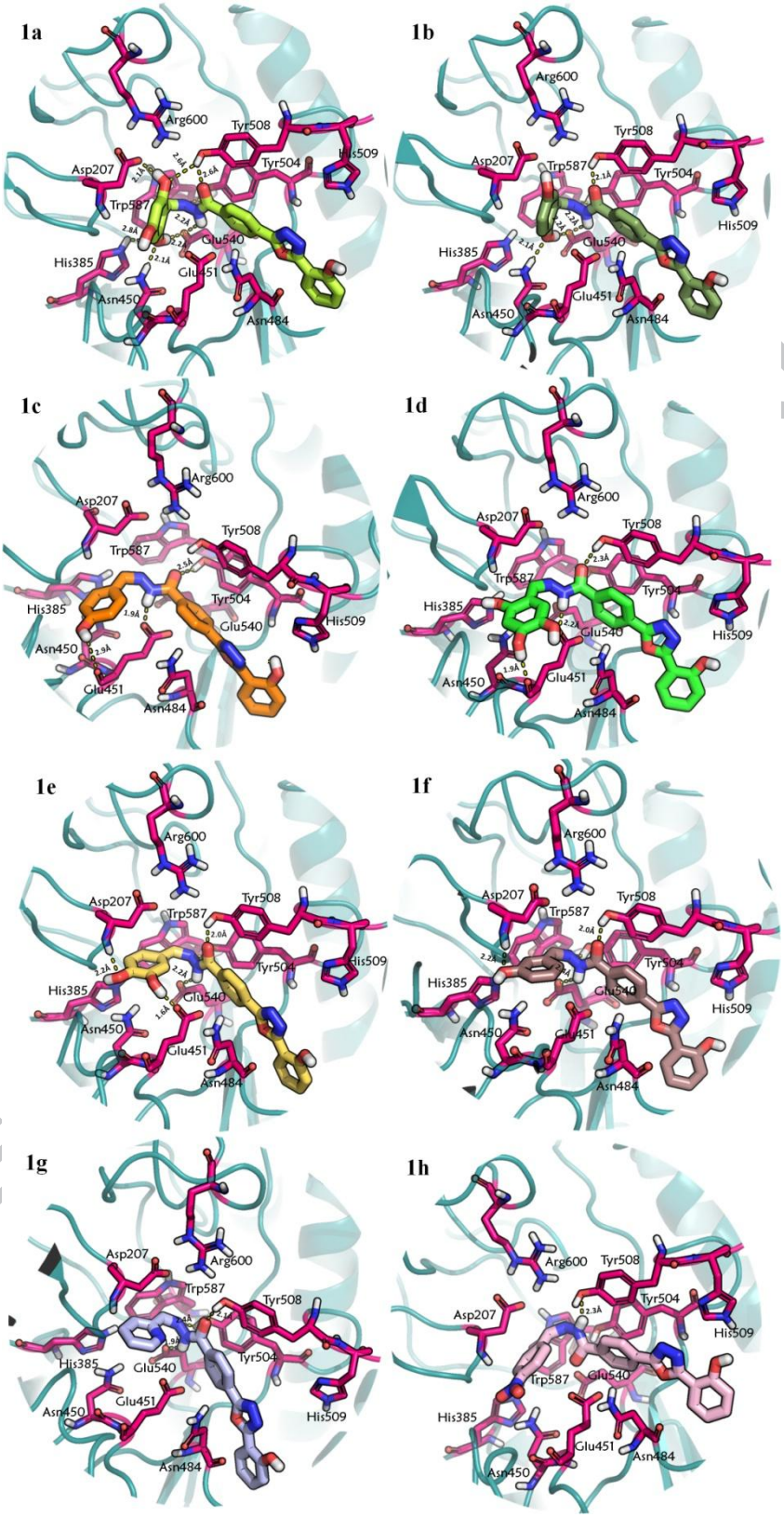



**Figure-2.** Shows the putative binding mode of the potent compounds among the synthesized derivatives in the active site of *β*-glucuronidase. The key interacting residues are shown in magenta color line and compound in stick representation, while the hydrogen bonds are shown in yellow dashed line. Compound **13** in split pea color **(1a)**, Compound **9** in dark green color **(1b)**, Compound **6** in orange color **(1c)**, Compound **18** in fluorescent color **(1d)**, Compound **8** in pale yellow **(1e)**,

Compound **7** in dirty violet color **(1f)**, Compound **25** in blue white **(1g)** and Compound **11** in light pink color **(1h)**.

Similarly, compound **9** which is the second most active compound ($IC_{50}$ = 15.14 ± 0.30 $\mu$M) in the series revealed that the hydroxyl group at ortho position of dihydroxybenzylidene is involved in the hydrogen bond interaction with Asn450 side-chain's carboxamide NH at a distance of 2.2 Å and with Glu540 side chain carboxyl oxygen (2.2 Å). The benzohydrazide nitrogen forms hydrogen bonding with Tyr504 side chain hydroxyl group (2.4 Å), whereas oxygen of benzohydrazide forms hydrogen bond with Tyr508 side chain hydroxyl group (2.1Å). Benzohydrazide NH forms hydrogen bond with carboxyl oxygen of Glu540 (2.2 Å). Additionally, Tyr504 phenyl ring, Tyr508 side chain phenyl ring and Asp207 side chain methyl group forms hydrophobic interaction with the compound **9** that stabilize the binding mode in the *β-D*-glucuronidase active site. Lack of hydroxyl group at the other *ortho* position of compound **9** hinders the formation of hydrogen bonding with Asp207, hence showing reduction in activity profiling (Fig.2b).

The third most active compound in the series is compound **6** ($IC_{50}$ = 18.46 ± 0.65 $\mu$M), in which the hydroxyl group at the *para* position of the hydroxybenzylidene forms hydrogen bond with backbone carbonyl oxygen group of Glu451 at a distance of 2.9 Å. The side chain carboxyl oxygen of Glu451 forms hydrogen bonding with the benzohydrazide nitrogen at a distance of 1.9 Å. Benzohydrazide oxygen also forms hydrogen bonding with Tyr504 side chain hydroxyl group (2.5 Å). The phenyl ring of Tyr504 and Tyr508 forms hydrophobic interaction with benzene ring of compound **6** (Fig.2c). The next active molecule in the list is compound **18** ($IC_{50}$ = 21.14 ± 1.05 $\mu$M). Even though the benzenetriol moiety for compound **18** has hydroxyl group at *ortho, meta* and *para* position, the hydroxyl group at the *ortho* and *meta* position does not interact with any key residue. Hydroxyl group at *para* position forms hydrogen bond with backbone carbonyl oxygen of Glu451 at a distance of 1.9 Å, while the oxygen of side chain carboxyl for residue Glu451 forms hydrogen bond with the benzohydrazide nitrogen at a distance of 2.2 Å. Additionally, the benzohydrazide oxygen forms hydrogen bond with Tyr508 side chain hydroxyl group (2.3 Å). On the other hand phenyl ring of Tyr504 and Tyr508 form hydrophobic interaction with benzene of compound **18** (Fig.2d). As for compound 3 which is the fifth active compound among the list ($IC_{50}$ = 29.15 ± 0.75 $\mu$M), oxygen of *para* hydroxyl group forms hydrogen bond with the backbone NH of Asp207 at a distance of 2.2 Å, while the *meta* hydroxyl group forms hydrogen bond with carboxyl oxygen of Glu451 side chain (1.6 Å). Similarly, the benzohydrazide nitrogen forms hydrogen bond with Glu540 side chain carboxyl oxygen (2.2 Å) and benzohydrazide oxygen hydrogen bond with Tyr508 side chain hydroxyl group (2.0 Å). For compound **8**, phenyl group of the Tyr504, Tyr508 and Tyr504 forms hydrophobic interaction with the oxygen of benzohydrazide and the other 2 hydroxyl groups to stabilize the binding mode (Fig.2e).

Compound **7** with activity ($IC_{50}$ = 34.46 ± 0.85 $\mu$M) shows that the benzohydrazide nitrogen forms hydrogen bonding with Glu540 side chain carboxyl oxygen (2.4 Å) and benzohydrazide oxygen forms hydrogen bonding with Tyr508 side chain hydroxyl group (2.0 Å). While the hydroxyl benzylidene *meta* positioned hydroxyl oxygen hydrogen bond with the backbone NH of Asp207 at a distance of 2.2 Å. Aromatic ring of Tyr504 and Tyr508 forms hydrophobic interaction with benzene ring of compound **7** (Fig.2f).

The binding mode observed of compound **25** ($IC_{50}$ = 42.26 ± 1.16 $\mu$M) suggests that the benzohydrazide NH forms hydrogen bonding with Glu540 side chain carboxyl oxygen (1.9 Å), while the other nitrogen forms hydrogen bond with the Tyr504 side chain hydroxyl group (2.4 Å). It was also observed that the benzohydrazide oxygen forms hydrogen bond with Tyr508 side chain hydroxyl group (2.1 Å). The presence of pyridine ring did not play any key role in the activity profile, since there were no key residues to interact with in the binding mode of compound **25**. Hydrophobic interaction was established by the aromatic ring of Tyr504 and Tyr508 with the benzene ring of compound **25** (Fig.2g). Compound **11** being the last compound among the considerable activity series with activity ($IC_{50}$ = 42.26 ± 1.16 $\mu$M), shows that the benzohydrazide nitrogen forms hydrogen bond with Tyr508 side chain hydroxyl group (2.3 Å).

In contrast, the presence of nitrobenzene moiety could not influence the activity profile since there were no key residues to interact with nitro group in the binding orientation of compound **11** (Fig.2h). Furthermore, there were some compounds with marginal activity profile, like compound **10** ($IC_{50}$ = 64.12 ± 1.62 $\mu$M), compound **12** ($IC_{50}$ = 82.16 ± 2.25 $\mu$M), compound **19** ($IC_{50}$= 86.12 ± 2.15 $\mu$M), compound **21** ($IC_{50}$ = 88.14 ± 2.38 $\mu$M), compound **24** ($IC_{50}$ = 91.4 ±2.45 $\mu$M), compound **25** ($IC_{50}$ = 108.14 ±3.14 $\mu$M). Altogether these compounds could only partially stabilize the binding interaction within the key residues of human $\beta$-D-glucuronidase, which ultimately results in low inhibitory profile.

Among the 25 compounds, the order of inhibitory potency tends to increase with tri-substituted position rather than di and mono substitution at benzylidene ring. Presence of the hydrogen bond donor group at *ortho, meta* and *para* position gives higher inhibitory activity. In contrast, hydrophobic group substituted at these positions show less inhibitory activity and thus elucidates the significance of chemical moiety in chemical space for activity profiling. Alternatively, the top ranked binding poses of inactive compounds i.e. compounds **14**, **15**, **16**, **17**, **20**, **22**, **23**, **27**, **28**, **29**, and **30** are shown in Fig.3a. The binding mode instability clearly elucidates the inactive profiling of these compounds. As shown in Fig.3a, these compounds do not make significant hydrogen bonding interaction with the crucial active site residues. Binding mode analysis suggested that inactivity of these compounds is dependent upon hydrophobic substituted group, while the active site residues are of hydrophilic in nature. The top ranked binding poses of active compounds are shown in Fig.3b.

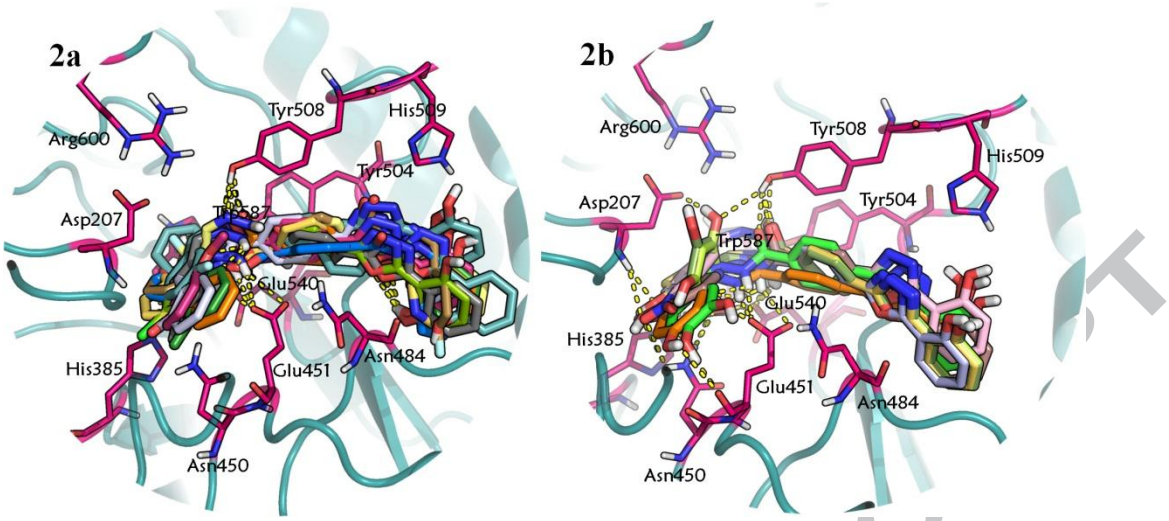



**Figure-3.** Shows the binding mode of the synthesized derivatives in the active site of β-glucuronidase. **(2a)** inactive compounds binding modes and **(2b)** active compounds binding modes.

The binding mode of the potent compounds illuminates the activity profile, where the hydrophilic nature of these compounds form stable hydrogen bond network with the key hydrophilic residues in the active site. Consistently, the activity profile of the compounds directly depends on magnitude of hydrogen bonding.

**Conclusions**

In conclusion, the molecular docking studies have evidently demonstrated the interaction pattern of the synthetic compounds in correspondence with biological inhibitory assay. Analysis of the binding mode clearly demonstrated that the presence of hydrophilic group, such as hydroxyl group on benzene ring next to hydrazone part plays a key role in the activity profile. However, *ortho*- hydroxyl group of benzene ring next to oxadiazole moiety did not seem to involve in inhibitory potentials.

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## 3. Experimental

### 3.1 General

NMR experiments were performed on UltraShield Bruker FT NMR 500 MHz, CHN analysis was performed on a Carlo Erba Strumentazion-Mod-1106, Italy. Electron impact mass spectra (EI-MS) were recorded on a Finnegan MAT-311A, Germany. Thin layer chromatography (TLC) was performed

on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by UV at 254 and 365 nm.

### 3.2 *β*-glucuronidase inhibition activity

*β*-glucuronidase (E.C. 3.2.1.31 from bovine liver, G-0251) inhibition activity of the compounds had been evaluated by using method as reported in [41]. In this study, D-saccharic acid 1,4-lactone has been used as a standard inhibitor [45].

### 3.3 Molecular Docking studies

In order to computationally demonstrate our experimental findings, molecular docking studies were performed for all synthesized compounds to reveal their binding mode in human *β*-D-glucuronidase active site. The human *β*-D-glucuronidase crystal structure was first retrieved from the protein data bank (PDB code: 1BHG) [46] and the structure was optimized by removing the hetero atoms, B-chain and co-factors. Hydrogen atoms, missing atoms, bonds and charges were computed using chimera [47]. Similarly, the 3D chemical structures for all 25 synthesized compounds were built and optimized using Marvin Sketch [48].

Docking studies were done using GOLD (Genetic Optimization for Ligand Docking) version 5.1 for all the 25 synthesized compounds targeting the active site of human *β*-D-glucuronidase [49]. GOLD uses genetic algorithm method for protein–ligand docking and it is well-known for its performance and accuracy specifically for the protein targets with buried active site. [50]. GOLD software has four scoring functions namely ChemPLP, Goldscore, Chemscore, and ASP (the Astex Statistical Potential) which takes into account the hydrogen bonding, van der Waal and intramolecular energies. In our previous GOLD docking study on human *β*-D-glucuronidase, Goldscore scoring function outperformed well over the other scoring function [47]. Therefore, in the present study we chose Goldscore scoring function. Docking was carried out with settings of population size (100); selection-pressure (1.1); number of operations (10,000); number of islands (1); niche size (2); operator weights for migrate (0), mutate (100), and cross-over (100). The active site was defined with a cavity 12 A˚ radius with specified centroid (x, y, z coordinates: 80.43, 84.41, 90.48) and with 100 GA run. Results divergent by less than 0.75 Å in ligand-all atom RMSDs were clustered together. Top ranked score and best cluster poses were saved and analyzed by PyMOL [51].

Prior to the docking study of the 25 synthetic compounds, our previous study docked bound state conformation of the substrate *p*-nitrophenyl *β*-glucuronide on *β*-glucuronidase was considered as reference in the present study [46].

### 3.4 Synthesis of 2-hydroxybenzohydrazide (2)

Methyl 2-hydroxybenzoate (**1**) (7.60 g, 53 mmol) and 20 ml of hydrazine hydrate were mixed in methanol (50 mL). The mixture was refluxed for 6 hours. Methanol was then evaporated and the product formed was being rinsed with plenty of water to remove excess hydrazine hydrate. The product formed was left to dry at room temperature and yielded 7.21 g (94.9 %). White solid, m.p.149-150 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 4.10 (s, 2H, $NH_2$); 6.96 (dd, $J$ = 7.0, 2.0 Hz, 1H), 7.25 (t, $J$ = 7.0 Hz, 1H), 7.53 (t, $J$ = 7.0 Hz, 1H), 7.82 (dd, $J$ = 7.0, 2.0 Hz, 1H,), 9.98 (s, 1H, NH); $^{13}$C-NMR (150 MHz, DMSO-$d_6$): $\delta$ 167.8, 159.1, 133.6, 129.3, 119.7, 118.2, 115.7; Anal. Calcd for $C_7H_8N_2O_2$, C = 55.26, H = 5.30, N = 18.41, found C = 55.28, H = 5.28, N = 18.42; EI MS *m/z* (% rel. abund.): 152 ($M^+$, 47), 121 (100), 93 (31), 76 (28).

### 3.5 Synthesis of (*E*)-methyl 4-((2-(2-hydroxybenzoyl)hydrazono)methyl)benzoate (3)

A mixture of compound **2** (7.00 g, 46 mmol), methyl 4-formylbenzoate (7.56 g, 46 mmol) and catalytic amount of acetic acid in methanol (50 mL) was refluxed for 3 hours. The solvent was evaporated and the residue (**3**) was washed with diethyl ether, filtered, dried, and then crystallized from ethanol and gives white solid, (12.8 g, 93%). m.p. 275-277 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.88 (s, 3H, $CH_3$), 6.97 (d, $J$ = 8.0 Hz. 1H)7.01 (t,1H, $J$ = 8.0 Hz), 7.47 (t, $J$ = 7.5 Hz, 1H), 7.90 (d, $J$ = 8.0 Hz, 3H) 8.06 (d, $J$ = 8.0 Hz, 2H), 8.52 (s, 1H) 11.97 (s, 1H, NH), 11.73 (s, 1H, OH); $^{13}$C-NMR (150 MHz, DMSO-d6): $\delta$ 167.5, 161.6, 160.6, 149.5, 137.1, 134.5, 134.5, 129.8, 128.7, 128.7, 127.8, 127.8, 119.0, 118.8, 114.2, 52.0; Anal. Calcd for $C_{16}H_{14}N_2O_4$, C = 64.42, H = 4.73, N = 9.39, found C = 64.43, H = 4.74, N = 9.37; EI MS *m/z* (% rel. abund.): 298 ($M^+$, 50.), 267 (30), 239 (40), 121 (100), 93 (20).

### 3.6 Synthesis of methyl 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzoate (4)

A mixture of compound **3** (11.0 g, 37 mmol) and equivalent amount of $PhI(OAc)_2$ was stirred in dichloromethane (100 ml) at room temperature overnight [52]. The solvent was evaporated and the residue (**4**) was washed with diethyl ether, filtered, dried, and then crystallized from ethanol to gives white solid, (9.8 g, 89 %). m.p. 274-276 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.91 (s, 3H, $CH_3$), 7.54 (t, $J$ = 7.5 Hz, 1H), 7.60 (t, $J$ = 8.0 Hz, 1H), 8.14 (d, $J$ = 8.5Hz, 1H), 8.16 (d, $J$ = 8.0 Hz, 2H), 8.28 (d, $J$ = 8.5 Hz, 2H,) 10.13 (s, 1H); $^{13}$C-NMR (150 MHz, DMSO-$d_6$): $\delta$ 167.8, 165.3, 158.7, 155.1, 131.0, 130.5, 129.7, 129.7, 129.1, 128.7, 125.3, 125.3, 119.8, 118.0, 111.4, 52.3; Anal. Calcd for $C_{16}H_{12}N_2O_4$, C = 64.86, H = 4.08, N = 9.46, found C = 64.87, H = 4.10, N = 9.48; EI MS *m/z* (% rel. abund.): 296 ($M^+$, 100), 265 (38), 237 (50), 161 (30), 93 (60).

### 3.7 Synthesis of 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (5)

Compound **4** (9.00 g, 30 mmol) and 15 ml of hydrazine hydrate were mixed in methanol (50 mL). The mixture was refluxed for 6 hours. Methanol was then evaporated and the product formed was being rinsed with plenty of water to remove excess hydrazine hydrate. The product formed (**5**) was left to dry at room temperature and yielded 8.50 g (94 %). m.p. 291-292 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 4.59 (br. s, 2H, $NH_2$); 7.64 (dt, $J$ = 2.0 Hz, 6.0 Hz, 1H), 7.71 (dt, $J$ = 2.0 Hz, 6.0 Hz, 1H), 7.77 (d, $J$ = 7.5 Hz, 1H), 8.06 (d, $J$ = 8.5 Hz, 2H), 8.18 (dd, $J$ = 2.0 Hz, 7.0 Hz, 1H), 8.20 (d, $J$ = 8.5 Hz, 2H), 10.02 (s, 1H, NH); $^{13}$C-NMR (150 MHz, DMSO-$d_6$): $\delta$ 167.7, 165.4, 158.2, 155.6, 132.5, 132.5, 131.2, 130.1, 127.1, 127.1, 126.3, 126.3, 119.6, 118.8, 111.1; Anal. Calcd for $C_{15}H_{12}N_4O_3$, C = 60.81, H = 4.08, N = 18.91, found C = 60.82, H = 4.09, N = 18.89; EI MS *m/z* (% rel. abund.): 296 ($M^+$, 30), 265 (100), 237 (30), 161 (20), 93 (40).

#### 3.7.1 General procedure for synthesis of oxadiazole benzohydrazones (6-30)

Equimolar quantities (1 mmol) of compound **5** and substituted benzaldehydes (1 mmol) in methanol (25 mL) were refluxed for 3 h, in the presence of catalytic amount of glacial acetic acid. The resulting solid was filtered and recrystallized from methanol in good yields.

#### 3.7.1.1. *N*'-(4-hydroxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (6)

Brown solid. Yield: 84.1%. m.p.: 274-275 °C. IR (cm$^{-1}$, KBr): 3233.82 (N-H), 1646.10 (C=N). $^{1}$H NMR (500 MHz, DMSO-$d_6$): δ 6.85 (d, $J$ = 8.5 Hz, 2H), 7.06 (t, $J$ = 7.5 Hz, 1H) 7.12 (d, $J$ = 8.5 Hz, 1H), 7.50 (t, $J$ = 7.0 Hz, 1H), 7.59 (d, $J$ = 8.5 Hz, 2H), 7.96 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.13 (d, $J$ = 8.5 Hz, 2H), 8.24 (d, $J$ = 8.0 Hz, 2H), 8.39 (s, 1H), 11.90 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.3, 158.4, 158.2, 155.1, 149.3, 133.1, 132.0, 131.7, 130.1, 129.2, 129.2, 128.3, 128.3, 126.2, 126.0, 126.0, 119.8, 118.8, 115.6, 115.6, 111.0; Anal. Calcd for $C_{22}H_{16}N_4O_4$, C = 66.00, H = 4.03, N = 13.99, Found C = 66.02, H = 4.01, N = 13.97; EI MS m/z (% rel. abund.): 400 ($M^+$, 41), 280 (10), 265 (100), 161 (15), 93 (20).

#### 3.7.1.2. *N*'-(3-hydroxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (7)

Yellowish white solid. Yield: 72.9%. m.p.: 254-255 °C. IR (cm$^{-1}$, KBr): 3233.92 (N-H), 1656.93 (C=N); $^{1}$H NMR (500 MHz, DMSO-$d_6$): δ 6.85 (d, $J$ = 7.5 Hz, 1H); 7.07 (t, $J$ = 7.5 Hz, 1H), 7.12-7.15 (m, 2H), 7.23 (s, 1H), 7.28 (t, $J$ = 8.0 Hz, 1H), 7.50 (t, $J$ = 8.0 Hz, 1H), 7.96 (d, $J$ = 7.5 Hz, 1H), 8.14 (d, $J$ = 8.0 Hz, 2H), 8.25 (d, $J$ = 8.0 Hz, 2H), 8.40 (s, 1H), 9.67 (s, 1H), 12.01 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.6, 164.5, 158.8, 156.8, 155.5, 148.6, 137.3, 133.7, 132.0, 131.5, 130.9, 130.4,

128.38, 128.38, 126.7, 126.7, 120.0, 119.5, 119.4, 118.3, 114.6, 111.8; Anal. Calcd for $C_{22}H_{16}N_4O_4$, C = 66.00, H = 4.03, N = 13.99, Found C = 65.98, H = 4.02, N = 14.01; EI MS m/z (% rel. abund.): 400 ($M^+$, 26), 280 (15), 265 (100), 161 (21), 93 (17).

**3.7.1.3. *N*'-(3,4-dihydroxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzo-hydrazide (8)**

Yellow solid. Yield: 80.9%. m.p.: 258-259 °C. IR ($cm^{-1}$, KBr): 3241.92 (N-H), 1593.98 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 6.96 (d, *J* = 6.5 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.12 (d, *J* = 6.0 Hz, 1H), 7.27 (s, 1H), 7.51 (t, *J* = 7.0 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 8.14 (d, *J* = 8.0 Hz, 2H), 8.25 (d, *J* = 8.0 Hz, 2H), 8.30 (s, 1H), 9.27 (s, 1H), 9.41 (s, 2H), 11.78 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.7, 164.3, 158.4, 155.5, 148.6, 148.3, 145.6, 133.7, 132.1, 131.6, 130.4, 128.3, 128.3, 127.9, 126.0, 126.0, 121.02, 119.8, 118.4, 116.3, 115.7, 111.2; Anal. Calcd for $C_{22}H_{16}N_4O_5$, C = 63.46, H = 3.87, N = 13.46, Found C = 63.48, H = 3.89, N = 13.44; EI MS m/z (% rel. abund.): 416 ($M^+$, 15), 398 (17), 265 (100), 161 (22), 93 (19).

**3.7.1.4.*N*'-(2,5-dihydroxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (9)**

Pale yellow solid. Yield: 74.7%. m.p.: 268-269 °C. IR ($cm^{-1}$, KBr): 3236.03 (N-H), 1654.27 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 6.73-6.77 (m, 2H), 7.00 (s, 1H), 7.05 (t, *J* = 8.0 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.50 (t, *J* = 7.5 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 2H), 8.24 (d, *J* = 8.0 Hz, 2H), 8.61 (s, 1H) 8.91 (s, 2H), 10.31 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.4, 164.8, 158.7, 155.2, 152.6, 151.7, 149.8, 133.2, 132.8, 131.1, 130.5, 128.8, 128.8, 126.5, 126.5, 122.7, 121.4, 119.6, 118.4, 118.4, 116.7, 111.5; Anal. Calcd for $C_{22}H_{16}N_4O_5$, C = 63.46, H = 3.87, N = 13.46, Found C = 63.49, H = 3.85, N = 13.47; EI MS m/z (% rel. abund.): 416 ($M^+$, 16), 398 (20), 265 (100), 161 (20), 93 (15).

**3.7.1.5. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(3-nitrobenzylidene)benzohydrazide (10)**

Dark brown solid. Yield: 78.2%. m.p.: 272-273 °C. IR (cm-1, KBr): 3236.24 (N-H), 1654.53 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): *δ* 6.87 (dd, *J* = 6.5, 2.0 Hz, 1H ), 7.14 (d, *J* = 7.0 Hz, 1H), 7.17 (t, *J* = 7.0 Hz, 1H), 7.24 (s, 1H), 7.11 (d, J = 8.5 Hz, 1H), 7.28 (t, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.33 (dt, *J* = 7.5,2.0 Hz, 1H), 8.02 (dd, *J* = 7.0, 2.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 2H), 8.25 (d, *J* = 8.0 Hz, 2H); 8.41 (s, 1H) 9.66 (s, 1H, OH); 12..01 (s, 1H, NH); $^{13}$C NMR (150 MHz, DMSO-$d_6$): *δ* 165.6, 164.3, 158.2, 155.2, 148.1, 147.7, 137.6, 133.8, 132.2, 132.2, 131.2, 130.5, 129.4, 128.3, 128.3, 126.7, 126.7, 124.8, 122.2, 119.5, 118.7, 111.3; Anal. Calcd for $C_{22}H_{15}N_5O_5$, C = 61.54, H = 3.52, N = 16.31,

Found C = 61.55, H = 3.57, N = 16.32; EI MS m/z (% rel. abund.): 429 ($M^+$, 20), 383 (18), 265 (100), 161 (14), 93 (19).

**3.7.1.6. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(4-nitrobenzylidene)benzohydrazide (11)**

White solid. Yield: 74.4%. m.p.: 306-307 °C. IR ($cm^{-1}$, KBr): 3236.30 (N-H), 1660.79 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 1H), 7.49-7.53 (t, $J$ = 7.0 Hz, 1H), 7.96 (dd, $J$ = 1.5 Hz, 7.5 Hz, 1H), 8.03-8.06 (m, 2H), 8.17 (d, $J$ = 8.0 Hz, 2H), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.32 (d, $J$ = 8.0 Hz, 2H), 8.59 (s, 1H), 10.39 (s, 1H), 12.33 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.3, 164.1, 158.8, 155.1, 149.4, 148.8, 139.1, 133.4, 132.4, 131.7, 130.1, 128.4, 128.4, 127.5, 127.5, 126.3, 126.3, 124.8, 124.8, 119.1, 118.6, 111.0; Anal. Calcd for $C_{22}H_{15}N_5O_5$, C = 61.54, H = 3.52, N = 16.31, Found C = 61.56, H = 3.53, N = 16.29; EI MS m/z (% rel. abund.): 429 ($M^+$, 25), 383 (40), 265 (100), 161 (19), 93 (22).

**3.7.1.7.4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(2,4,6-trihydroxybenzylidene)benzohydrazide (12)**

Yellowish solid. Yield: 72.1%. m.p.: 314-315 °C. IR ($cm^{-1}$, KBr): 3229.75 (N-H), 1637.93 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): δ 5.86 (s, 2H), 7.07 (t, $J$ = 8.0 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.51 (t, $J$ = 8.0 Hz, 1H), 7.96 (d, $J$ = 7.5 Hz, 1H), 8.16 (d, $J$ = 8.5 Hz, 2H), 8.25 (d, $J$ = 8.0 Hz, 2H), 8.85 (s, 1H), 9.86 (s, 1H), 10.35 (s, 1H), 11.10 (s, 1H), 12.08 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): δ 165.3, 164.8, 163.1, 161.3, 161.7, 158.5, 155.3, 144.5, 133.9, 132.4, 131.2, 130.9, 128.6, 128.6, 126.1, 126.1, 119.8, 118.7, 111.2, 106.4, 95.4, 95.4; Anal. Calcd for $C_{22}H_{16}N_4O_6$, C = 61.11, H = 3.73, N = 12.96, Found C = 61.13, H = 3.71, N = 12.94; EI MS m/z (% rel. abund.): 432 ($M^+$, 5), 414 (20), 265 (100), 161 (22), 93 (19).

**3.7.1.8. *N*'-(3-hydroxy-4-methoxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (13)**

Yield: 75.5%. m.p.: 244-245 °C. IR ($cm^{-1}$, KBr): 3242.90 (N-H), 1655.22 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.83 (s, 3H), 6.99 (d, $J$ = 8.0 Hz, 1H); 7.06 (d, $J$ = 8.0 Hz, 1H), 7.10 (d, $J$ = 6.0 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.31 (s, 1H), 7.51 (t, $J$ = 7.0 Hz, 1H), 7.97 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.14 (d, $J$ = 8.0 Hz, 2H), 8.24 (d, $J$ = 8.0 Hz, 2H), 8.35 (s, 1H), 9.32 (s, 1H), 10.34 (s, 1H), 11.87 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.1, 164.7, 158.3, 155.1, 149.9, 148.3, 146.7, 133.4, 132.2, 131.7, 130.1, 129.4, 128.3, 128.3, 126.5, 126.5, 120.2, 119.7, 118.1, 115.6, 115.6, 111.4, 56.7; Anal. Calcd for $C_{23}H_{18}N_4O_5$, C = 64.18, H = 4.22, N = 13.02, Found C = 64.19, H = 4.24, N = 13.01; EI MS m/z (% rel. abund.): 430 ($M^+$, 15), 399 (18), 265 (100), 161 (20), 93 (22).

### 3.7.1.9. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(2-methylbenzylidene)benzohydrazide (14)

White solid. Yield: 82.2%. m.p.: 228-229 °C. IR ($cm^{-1}$, KBr): 3239.07 (N-H), 1656.16 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 2.38 (s, 3H), 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.28 (d, $J$ = 7.5 Hz, 1H), 7.37 (t, $J$ = 8.0 Hz, 1H), 7.52 (t, $J$ = 7.0 Hz, 1H), 7.54 (d, $J$ = 7.5 Hz, 1H), 7.60 (s, 1H), 7.96 (dd, $J$ = 1.5 Hz, 7.5 Hz, 1H), 8.16 (d, $J$ = 8.5 Hz, 2H), 8.25 (d, $J$ = 8.5 Hz, 2H), 8.46 (s, 1H), 10.34 (s, 1H), 12.03 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.4, 164.3, 158.4, 155.1, 145.8, 136.3, 133.4, 132.8, 132.2, 131.7, 130.9, 130.1, 128.3, 128.2, 126.9, 126.9, 126.0, 119.6, 118.8, 111.7, 20.5; Anal. Calcd for $C_{23}H_{18}N_4O_3$, C = 69.34, H = 4.55, N = 14.06, Found C = 69.36, H = 4.56, N = 14.08; EI MS m/z (% rel. abund.): 398 ($M^+$, 36), 384 (6), 265 (100), 161 (22), 93 (21), 91 (22).

### 3.7.1.10. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(3-methylbenzylidene)benzohydrazide (15)

White solid. Yield: 68.9%. m.p.: 248-249 °C. IR (cm-1, KBr): 3236.93 (N-H), 1650.89 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 2.48 (s, 3H), 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.28-7.32 (m, 2H), 7.35 (d, $J$ = 7.5 Hz, 1H), 7.52 (t, $J$ = 8.0 Hz, 1H), 7.87 (d, $J$ = 7.5 Hz, 1H), 7.97 (d, $J$ = 7.5 Hz, 1H), 8.17 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.0 Hz, 2H), 8.79 (s, 1H), 10.35 (s, 1H), 12.02 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.4, 164.3, 158.4, 155.8, 145.8, 136.1, 133.8, 133.5, 132.1, 131.5, 130.5, 130.1, 128.2, 128.2, 128.0, 126.7, 126.7 126.3, 125.4, 119.8, 118.1, 111.4, 20.2; Anal. Calcd for $C_{23}H_{18}N_4O_3$, C = 69.34, H = 4.55, N = 14.06, Found C = 69.33, H = 4.53, N = 14.04; EI MS m/z (% rel. abund.): 398 ($M^+$, 32), 384 (5), 265 (100), 161 (20), 93 (20), 91 (18).

### 3.7.1.11. *N*'-(2-chlorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (16)

White solid. Yield: 61.2%. m.p.: 251-252 °C. IR ($cm^{-1}$, KBr): 3216.59 (N-H), 1650.86 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.47-7.54 (m, 3H), 7.56 (d, $J$ = 7.0 Hz, 1H), 7.97 (dd, $J$ = 1.5 Hz, 6.5 Hz, 1H), 8.06 (d, $J$ = 7.5 Hz, 1H), 8.18 (d, $J$ = 8.0 Hz, 2H), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.92 (s, 1H), 10.35 (s, 1H), 12.27 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.6, 158.2, 155.8, 149.4, 133.4, 132.4, 132.3, 131.7, 131.1, 130.5, 130.1, 128.8, 128.8, 128.6, 127.8, 127.2, 126.6, 126.6, 119.3, 118.4, 111.6; Anal. Calcd for $C_{22}H_{15}ClN_4O_3$, C = 63.09, H = 3.61, N = 13.38, Found C = 63.08, H = 3.59, N = 13.37; EI MS m/z (% rel. abund.): 420 ($M^+$+2, 10), 418 ($M^+$, 20), 383 (12) 265 (100), 161 (19), 93 (18).

**3.7.1.12. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(4-methylbenzylidene)benzohydrazide (17)**

White solid. Yield: 77.3%. m.p.: 270-271 °C. IR ($cm^{-1}$, KBr): 3233.75 (N-H), 1655.19 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): δ 2.37 (s, 3H), 7.07 (t, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 7.5 Hz, 2H), 7.97 (d, *J* = 7.0 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 2H), 8.25 (d, *J* = 8.0 Hz, 2H), 8.46 (s, 1H), 10.35 (s, 1H), 11.98 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): δ 165.8, 164.7, 158.2, 155.6, 149.1, 138.0, 133.4, 132.9, 131.9, 131.7, 130.1, 129.5, 129.5, 128.2 128.2, 127.5, 127.5, 126.3, 126.3, 119.8, 118.1, 111.4, 21.2; Anal. Calcd for $C_{23}H_{18}N_4O_3$, C = 69.34, H = 4.55, N = 14.06, Found C = 69.35, H = 4.57, N = 14.07; EI MS m/z (% rel. abund.): 398 ($M^+$, 30), 384 (8), 265 (100), 161 (18), 93 (24), 91 (25).

**3.7.1.13.4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(2,4,5-trihydroxybenzylidene)benzohydrazide (18)**

Yellow solid. Yield: 89.7%. m.p.: 296-297 °C. IR ($cm^{-1}$, KBr): 3233.51 (N-H), 1626.54 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): *δ* 6.36 (s, 1H), 6.93 (s, 1H), 7.07 (t, *J* = 7.0 Hz, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.97 (d, *J* = 6.5 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 2H), 8.25 (d, *J* = 8.0 Hz, 2H), 8.51 (s, 1H), 8.57 (s, 1H), 9.56, (s, 1H), 10.35 (s, 1H), 10.53 (s, 1H), 11.98 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): *δ* 165.5, 164.2, 158.6, 155.8, 153.3, 150.5, 149.2, 140.7, 133.1, 132.5, 131.6, 130.8, 128.6, 128.6, 126.5, 126.5, 119.7, 118.4, 117.4, 112.2, 111.1, 102.5; Anal. Calcd for $C_{22}H_{16}N_4O_6$, C = 61.11, H = 3.73, N = 12.96, Found C = C = 61.10, H = 3.75, N = 12.97; EI MS m/z (% rel. abund.): 432 ($M^+$, 10), 414 (30), 265 (100), 161 (20), 93 (15).

**3.7.1.14. *N*'-(4-chlorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (19)**

White solid. Yield: 81.6%. m.p.: 278-279 °C. IR ($cm^{-1}$, KBr): 3233.34 (N-H), 1655.06 (C=N); $^{1}H$ NMR (500 MHz, DMSO-$d_6$): *δ* 7.06 (t, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 7.50 (t, J = 8.5 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.79 (d, *J* = 8.0 Hz, 2H), 7.96 (dd, *J* = 1.5 Hz, 7.5 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 2H), 8.26 (d, *J* = 8.0 Hz, 2H), 8.49 (s, 1H), 10.34 (s, 1H), 12.12 (s, 1H); $^{13}C$ NMR (150 MHz, DMSO-$d_6$): *δ* 165.5, 164.7, 158.1, 155.3, 149.6, 135.2, 134.7, 133.1, 132.8, 131.6, 130.1, 129.8, 129.8, 129.5, 129.5, 128.4, 128.4, 126.7, 126.7, 119.6, 118.0, 111.0; Anal. Calcd for $C_{22}H_{15}ClN_4O_3$, C = 63.09, H = 3.61, N = 13.38, Found C = 63.11, H = 3.63, N = 13.39; EI MS m/z (% rel. abund.): 420 ($M^+$+2, 8), 418 ($M^+$, 16), 383 (10) 265 (100), 161 (17), 93 (14).

**3.7.1.15. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(2-nitrobenzylidene)benzohydrazide (20)**

White solid. Yield: 83.1%. m.p.: 256-257 °C. IR (cm-1, KBr): 3191.20 (N-H); 1649.84 (C=N) 1H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 1H), 7.49 (t, $J$ = 8.5 Hz, 1H), 7.71 (t, $J$ = 7.5 Hz, 1H), 7.85 (t, $J$ = 7.5 Hz, 1H), 7.96 (d, $J$ = 1.5 Hz, 1H), 8.10 (d, $J$ = 8.5 Hz, 1H), 8.18-8.20 (m, 3H), 8.26 (d, $J$ = 8.5 Hz, 2H), 8.93 (s, 1H), 10.35 (s, 1H), 12.36 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.2, 158.5, 155.9, 147.1, 142.4, 133.8, 133.5, 132.1, 131.3, 130.7, 130.1, 129.0, 128.6, 128.6, 128.0, 126.5, 126.5, 125.9, 119.3, 118.6, 111.7; Anal. Calcd for $C_{22}H_{15}N_5O_5$, C = 61.54, H = 3.52, N = 16.31, Found C = 61.56, H = 3.53, N = 16.29; EI MS m/z (% rel. abund.): ($M^+$, 10), 383 (26), 265 (100), 161 (20), 93 (25).

**3.7.1.16. *N*'-(2-hydroxy-5-methoxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (21)**

White solid. Yield: 77.2%. m.p.: 282-283 °C. IR ($cm^{-1}$, KBr): 3226.13 (N-H), 1649.59 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.75 (s, 3H), 6.88 (d, $J$ = 9.0 Hz, 1H), 6.93 (dd, $J$ = 3.0 Hz, 9.0 Hz, 1H), 7.06 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.17 (d, $J$ = 3.0 Hz, 1H), 7.52 (t, $J$ = 8.5 Hz, 1H), 7.97 (dd, $J$ = 1.5 Hz, 7.5 Hz, 1H), 8.17 (d, $J$ = 8.5 Hz, 2H ), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.69 (s, 1H), 10.34 (s, 1H), 10.61 (s, 1H), 12.26 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.6, 164.2, 158.7, 155.5, 154.4, 152.2, 149.6, 133.4, 132.9, 131.9, 130.1, 128.5, 128.5, 126.3, 126.3, 121.0, 119.8, 118.7, 116.4, 116.1, 112.4, 111.5, 56.0; Anal. Calcd for $C_{23}H_{18}N_4O_5$, C = 64.18, H = 4.22, N = 13.02, Found C = 64.20, H = 4.20, N = 13.03; EI MS m/z (% rel. abund.): 430 ($M^+$, 20), 399 (21), 265 (100), 161 (22), 93 (19).

**3.7.1.17. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(pyridin-3-ylmethylene)benzohydrazide (22)**

Brown solid. Yield: 84.7%. m.p.: 241-242 °C. IR ($cm^{-1}$, KBr): 3414.14 (N-H), 1655.04 (C=N); 1H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 6.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.52 (t, $J$ = 8.5 Hz, 1H), 7.97 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.17-8.19 (m, 3H), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.56 (s, 1H), 8.64 (d, $J$ = 4.0 Hz, 1H), 8.90 (s, 1H), 10.34 (s, 1H), 12.22 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.6, 164.3, 158.7, 155.6, 149.5, 146.1, 145.7, 135.5, 133.1, 133.0, 132.9, 131.9, 130.1, 128.6, 128.6, 126.9, 126.9, 124.1, 119.6, 118.0, 111.7; Anal. Calcd for $C_{21}H_{15}N_5O_3$, C = 65.45, H = 3.92, N = 18.17, Found C = 65.47, H = 3.93, N = 18.15; EI MS m/z (% rel. abund.): 385 ($M^+$, 29), 265 (100), 161 (20), 93 (18), 77 (17).

**3.7.1.18. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(pyridin-4-ylmethylene)benzohydrazide (23)**

Yield: 80.1%. m.p.: 261-262 °C. IR ($cm^{-1}$, KBr): 3229.73 (N-H), 1656.60 (C=N); $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 8.0 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.52 (t, $J$ = 8.5 Hz, 1H), 7.70 (d, $J$ = 4.0 Hz, 2H), 7.97 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.17 (d, $J$ = 7.5 Hz, 2H), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.50 (s, 1H), 8.68 (d, $J$ = 4.0 Hz, 2H), 10.34 (s, 1H), 12.32 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.4, 164.3, 158.4, 155.7, 150.4, 150.4, 149.1, 140.5, 133.8, 132.1, 131.4, 130.7, 128.5, 128.5, 126.7, 126.7, 122.5, 122.5, 119.8, 118.0, 111.4; Anal. Calcd for $C_{21}H_{15}N_5O_3$, C = 65.45, H = 3.92, N = 18.17, Found C = 65.46, H = 3.94, N = 18.18; EI MS m/z (% rel. abund.): 385 ($M^+$, 31), 265 (100), 161 (21), 93 (16), 77 (21).

**3.7.1.19 *N*'-(2-hydroxybenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (24)**

White solid. Yield: 46.8%. m.p.: 284-285 °C. IR (cm-1, KBr): 3228.83 (N-H); 1625.63 (C=N) 1H NMR (500 MHz, DMSO-$d_6$): $\delta$ 6.93-6.97 (m, 2H), 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.31 (t, $J$ = 7.0 Hz, 1H), 7.50-7.54 (t, J = 8.5 Hz, 1H), 7.59 (d, $J$ = 6.5 Hz, 1H), 7.97 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.18 (d, $J$ = 8.0 Hz, 2H), 8.27 (d, $J$ = 8.5 Hz, 2H), 8.70 (s, 1H), 10.34 (s, 1H), 11.21 (s, 1H), 12.32 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.3, 164.6, 158.9, 158.2, 155.4, 151.3, 133.8, 132.7, 131.6, 130.5, 129.7, 128.4, 128.4, 128.4, 126.7, 126.7, 121.9, 120.3, 119.8, 118.0, 117.3, 111.2; Anal. Calcd for $C_{22}H_{16}N_4O_4$, C = 66.00, H = 4.03, N = 13.99, Found C = 66.01, H = 4.04, N = 14.02; EI MS m/z (% rel. abund.): 400 ($M^+$, 30), 280 (22), 265 (100), 161 (25), 93 (21).

**3.7.1.20.4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(pyridin-2-ylmethylene)benzohydrazide (25)**

Brown solid. Yield: 84.7%. m.p.: 232-233 °C. IR ($cm^{-1}$, KBr): 3213.98 (N-H), 1649.98 (C=N); $^{1}$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 1H), 7.45 (t, $J$ = 6.0 Hz, 1H), 7.49 (t, $J$ = 7.0 Hz, 1H), 7.91 (t, $J$ = 7.5 Hz, 1H), 7.96 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.01 (d, $J$ = 8.0 Hz, 1H), 8.17 (d, $J$ = 8.0 Hz, 2H), 8.27 (d, $J$ = 8.0 Hz, 2H), 8.53 (s, 1H), 8.64 (d, $J$ = 4.0 Hz, 1H), 10.35 (s, 1H), 12.25 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.3, 158.3, 155.4, 154.9, 148.6, 146.1, 137.4, 133.2, 132.8, 131.9, 130.1, 128.3, 128.3, 126.4, 126.4, 123.0, 119.8, 119.8, 118.1, 111.0; Anal. Calcd for $C_{21}H_{15}N_5O_3$, C = 65.45, H = 3.92, N = 18.17, Found C = 65.43, H = 3.90, N = 18.16; EI MS m/z (% rel. abund.): 385 ($M^+$, 22), 265 (100), 161 (18), 93 (21), 77 (16).

**3.7.1.21.*N*'-(2-fluorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (26)**

White solid. Yield: 69.2%. m.p.: 267-268 °C. IR ($cm^{-1}$, KBr): 3235.10 (N-H); 1655.10 (C=N) 1H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.0 Hz, 1H), 7.31 (t, $J$ = 7.5 Hz, 1H), 7.49-7.62 (m, 4H), 7.96 (dd, $J$ = 1.5 Hz, 7.5 Hz, 1H), 8.16 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.0 Hz, 2H), 8.51 (s, 1H), 10.35 (s, 1H), 12.17 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.4, 164.6, 160.5 (d, $J$ = 262.0 Hz), 158.4, 155.5, 149.6, 149.1, 133.9, 132.0, 131.7, 130.7, 130.2, 130.1, 129.8, 129.0, 128.3, 128.3, 126.0, 126.0, 125.3, 125.3, 124.6, 124.5, 119.1, 118.7, 117.2, 117.0, 111.70; Anal. Calcd for $C_{22}H_{15}FN_4O_3$, C = 65.67, H = 3.76, N = 13.92, Found C = 65.69, H = 3.75, N = 13.90; EI MS m/z (% rel. abund.): 402 ($M^+$, 27), 383 (18) 265 (100), 161 (23), 93 (20), 76 (11).

**3.7.1.22. 4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-*N*'-(2-methoxybenzylidene)benzohydrazide (27)**

Yield: 78.6%. m.p.: 222-223 °C. IR ($cm^{-1}$, KBr): 3233.17 (N-H), 1655.39 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 3.84 (s, 3H), 7.05 (t, $J$ = 8.5 Hz, 2H), 7.12 (d, $J$ = 8.0 Hz, 1H), 7.31 (d, $J$ = 7.0 Hz, 2H), 7.40 (t, $J$ = 8.0 Hz, 1H), 7.51 (t, $J$ =8.0 Hz, 1H), 7.96 (d, $J$ = 8.0 Hz, 1H), 8.15 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.0 Hz, 2H), 8.48 (s, 1H), 10.39 (s, 1H), 12.07 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.4, 159.8, 158.6, 155.3, 150.1, 133.7, 132.4, 131.3, 130.6, 128.5, 128.5, 128.3, 127.9, 126.0, 126.0, 125.1, 121.5, 119.6, 118.8, 113.6, 111.0, 56.9; Anal. Calcd for $C_{23}H_{18}N_4O_4$, C = 66.66, H = 4.38, N = 13.52, Found C = 66.68, H = 4.36, N = 13.54; EI MS m/z (% rel. abund.): 414 ($M^+$, 22), 383 (15) 265 (100), 161 (18), 93 (19), 76 (8).

**3.7.1.23. *N*'-(4-fluorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (28)**

White solid. Yield: 82.3%. m.p.: 225-226 °C. IR ($cm^{-1}$, KBr): 3228.43 (N-H), 1651.10 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.33 (t, $J$ = 9.0 Hz, 2H), 7.51 (t, $J$ = 8.5 Hz, 1H), 7.83 (t, $J$ = 8.5 Hz, 2H), 7.96 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.15 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.5 Hz, 2H), 8.50 (s, 1H), 10.36 (s, 1H), 12.07 (s, 1H); $^{13}$C NMR (150 MHz, DMSO-$d_6$): 165.4, 164.3, 161.2 (d, $J$ = 262.0 Hz), 158.4, 155.8, 149.5, 133.2, 132.9, 131.6, 130.8, 130.3, 130.3, 130.1, 128.3, 128.3, 126.0, 126.0, 119.6, 118.0, 115.7, 115.7, 111.4; Anal. Calcd for $C_{22}H_{15}FN_4O_3$, C = 65.67, H = 3.76, N = 13.92, Found C = 65.68, H = 3.73, N = 13.94; EI MS m/z (% rel. abund.): 402 ($M^+$, 40), 383 (22) 265 (100), 161 (21), 93 (19), 76 (20).

### 3.7.1.24. *N*'-(3-fluorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (29)

White solid. Yield: 61.8%. m.p.: 254-255 °C. IR ($cm^{-1}$, KBr): 3219.38 (N-H), 1651.54 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 8.0 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.31-7.35 (m, 2H), 7.49-7.53 (m, 2H), 7.96-8.00 (dd, J = 1.5 Hz, 8.0 Hz, 2H), 8.17 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.0 Hz, 2H), 8.76 (s, 1H), 10.35 (s, 1H), 12.17 (s, 1H); 13C-NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.7, 164.3, 162.7 (d, $J$ = 265.0 Hz), 158.4, 155.1, 148.8, 138.5, 133.4, 132.9, 131.7, 130.1, 130.0, 128.7, 128.7, 126.5, 126.5, 121.4, 119.8, 118.0, 116.3, 114.1, 111.0; Anal. Calcd for $C_{22}H_{15}FN_4O_3$, C = 65.67, H = 3.76, N = 13.92, Found C = 65.65, H = 3.77, N = 13.93; EI MS m/z (% rel. abund.): 402 ($M^+$, 25), 383 (15) 265 (100), 161 (20), 93 (18), 76 (8).

### 3.7.1.25. *N*'-(3-chlorobenzylidene)-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)benzohydrazide (30)

White solid. Yield: 82.6%; m.p.: 243-244 °C, IR ($cm^{-1}$, KBr): 3240.01 (N-H), 1657.34 (C=N); $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.07 (t, $J$ = 7.5 Hz, 1H), 7.13 (d, $J$ = 8.5 Hz, 1H), 7.50-7.53 (m, 3H), 7.73 (s, 1H), 7.82 (s, 1H), 7.96 (dd, $J$ = 1.5 Hz, 8.0 Hz, 1H), 8.16 (d, $J$ = 8.0 Hz, 2H), 8.26 (d, $J$ = 8.0 Hz, 2H), 8.48 (s, 1H), 10.35 (s, 1H), 12.20 (s, 1H); 13C-NMR (150 MHz, DMSO-$d_6$): $\delta$ 165.4, 164.3, 158.4, 155.8, 148.8, 135.3, 134.0, 133.4, 132.0, 131.7, 130.1, 130.1, 128.7, 128.7, 128.2, 127.6, 126.7, 126.7, 125.3, 119.6, 118.0, 111.5; Anal. Calcd for $C_{22}H_{15}ClN_4O_3$, C = 63.09, H = 3.61, N = 13.38, Found C = 63.07, H = 3.62, N = 13.40 EI MS m/z (% rel. abund.): 420 ($M^+$+2, 6), 418 ($M^+$, 17), 383 (10) 265 (100), 161 (16), 93 (18).

## References


1. A. Husain, M. Ajmal, Synthesis of novel 1,3,4-oxadiazole derivatives and their biological properties, *Acta Pharm*. *59* (**2009**) 223-233.
2. E. Palaska, G. Sahin, P. Kelicen, N. T. Durlu, G. Altinok, Synthesis and anti-inflammatory activity of 1-acylthiosemicarbazides, 1,3,4- oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazole-3-thiones, II *Farmaco 57* (**2002**) 101-107.
3. B. Narayana, K.K. Vijayaraj, B.V. Ashlatha, N.S. Kumari, Synthesis of some new 2-(6-methoxy-2-naphthyl)-5-aryl-1,3,4-oxadiazoles as possible non-steroidal anti-inflammatory and analgesic agents, *Arch Pharm. 338* (**2005**) 373-377.
4. M. Amir, S. Kumar, Synthesis and evaluation of anti-inflammatory, analgesic and lipid peroxidation properties of ibuprofen derivatives, Acta Pharm. 57 (**2007**) 31-45.

5. M.A. Ali, M.S. Yar, Oxadiazole Mannich bases: Synthesis and antimycobacterial activity, *Bioorg. Med. Chem. Lett. 17* (**2007**) 3314-3316.

6. T.M. Tan, Y. Chen, K.H. Kong, J. Bai, Y. Li, S.G. Lim, T.H. Ang, Synthesis and the biological evaluation of 2-benzenesulfonylalkyl-5-substituted-sulfanyl-(1,3,4)-oxadiazoles as potential anti-hepatitis B virus agents, *Antiviral Res. 71* (**2006**) 7-14.

7. S.L. Gaonkar, K.M. Rai, B. Prabhuswamy, Synthesis and antimicrobial studies of a new series of 2-4-[2-(5-ethylpyridin-2-yl)ethoxy] phenyl_-5-substituted-1,3,4-oxadiazoles, *Eur J Med Chem. 41* (**2006**) 841-846.

8. A. Zarghi, S.A. Tabatabai, M. Faizi, A. Ahadian, P. Navabi, V. Zanganeh, A. Shafiee, Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1,3,4-oxadiazole, *Bioorg. Med. Chem. Lett. 15* (**2005**) 1863-1865.

9. H.Z. Zhang, S. Kasibhatla, J. Kuemmerle, W. Kemnitzer, M.K. Ollis, L. Qiu, C, Crogan-Grundy, B. Tseng, J. Drewe, S.X. Cai. Discovery and structure−activity relationship of 3-aryl-5-aryl-1,2,4-oxadiazoles as a new series of apoptosis inducers and potential anticancer agents. *J. Med. Chem. 48* (**2005**) 5215-5223.

10. S.H. Lee, H.J. Seo, S.H. Lee, M.E. Jung, J.H. Park, J. Yoo, H. Yun, J. Na, S.Y. Kang, K.S. Song, M.A. Kim. Biarylpyrazolyl oxadiazole as potent, selective, orally bioavailable cannabinoid-1 receptor antagonists for the treatment of obesity. *J. Med. Chem. 51* (**2008**) 7216-7233.

11. D.M. Cottrell, J. Capers, M.M. Salem, K. DeLuca-Fradley, S.L. Croft, K.A. Werbovetz, Antikinetoplastid activity of 3-aryl-5-thiocyanatomethyl-1,2,4-oxadiazoles. *Bioorg. Med. Chem. 12* (**2004**) 2815-2824.

12. R.M. Jones, J.N. Leonard, D.J. Buzard, J. Lehmann, GPR119 agonists for the treatment of type 2 diabetes. *Expert Opin Ther. Pat. 19* (**2009**) 1339-1359.

13. P.B. Unangst, G.P. Shrum, D.T. Connor, R.D. Dyer, D.J. Schrier, Novel 1,2,4-oxadiazoles and 1,2,4-thiadiazoles as dual 5-lipoxygenase and cyclooxygenase inhibitors. *J. Med. Chem. 35* (**1992**) 3691-3698.

14. S.V. Bhandari, K.G. Bothara, M.K. Raut, A.A. Patil, A.P. Sarkate, V. J. Mokale, Design, synthesis and evaluation of antiinflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1, 3, 4-oxadiazole-2-thiol and Schiff bases of diclofenac acid as nonulcerogenic derivatives. *Bioorg. Med. Chem. 16* (**2008**) 1822-1831.

15. X. Ouyang, E.L. Piatnitski, V. Pattaropong, X. Chen, H.Y. He, A.S. Kiselyov, A. Velankar, J. Kawakami, M. Labelle, L. Smith, J. Lohman, S.P. Lee, A. Malikzay, J. Fleming, J. Gerlak, Y.

Wang, R.L. Rosler, K. Zhou, S. Mitelman, M. Camara, D. Surguladze, J.F. Doody, M.C. Tuma, Oxadiazole derivatives as a novel class of antimitotic agents: Synthesis, inhibition of tubulin polymerization, and activity in tumor cell lines. *Bioorg. Med. Chem. Lett. 16* (**2006**) 1191-1196.

16. A. Almasirad, S.A. Tabatabai, M. Faizi, A. Kebriaeezadeh, N. Mehrabi, A. Dalvandi, A. Shafiee, Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy) phenyl]-1, 3, 4-oxadiazoles and 1, 2, 4-triazoles. *Bioorg. Med. Chem. Lett. 14* (**2004**) 6057-6059.

17. P. Puthiyapurayil, B. Poojary, C. Chikkanna, S.K. Buridipad, Design, synthesis and biological evaluation of a novel series of 1,3,4-oxadiazole bearing *N*-methyl-4-(trifluoromethyl)phenyl pyrazole moiety as cytotoxic agents. *Eur. J. Med. Chem. 53* (**2012**) 203-210.

18. J.W. Watthey, M. Desai, R. Rutledge, R. Dotson, Synthesis and diuretic profile of 3-(3-amino-1, 2, 4-oxadiazol-5-yl)-5-chloro-2, 6-pyrazinediamine, an amiloride-type diuretic. *J. Med. Chem. 23* (**1980**) 690-692.

19. H.L. Yale, K. Losee, 2-Amino-5-substituted 1,3,4-oxadiazoles and 5-imino-2-substituted-1,3,4-oxadiazolines. A group of novel muscle relaxants *J. Med. Chem. 9* (**1966**) 478-483.

20. K. Ohmoto, T. Yamamoto, T. Horiuchi, H. Imanishi, Y. Odagaki, K. Kawabata, T. Sekioka, Y. Hirota, S. Matsuoka, H. Nakai, M. Toda, J.C. Cheronis, L.W. Spruce, A. Gyorkos, M. Wieczorek, Design and synthesis of new orally active nonpeptidic inhibitors of human neutrophil elastase. *J Med. Chem. 43* (**2000**) 4927-4929.

21. M. Ono, M. Haratake, H. Saji, M. Nakayama, Development of novel β-amyloid probes based on 3,5-diphenyl-1,2,4-oxadiazole. *Bioorg. Med. Chem. 16* (**2008**) 6867-6872.

22. B.S. Orlek, F.E. Blaney, F. Brown, M.S.G. Clark, M.S. Hadley, J. Hatcher, G.J. Riley, H.E. Rosenberg, H.J. Wadsworth, P. Wyman, Comparison of azabicyclic esters and oxadiazoles as ligands for the muscarinic receptor. *J. Med.Chem. 34* (**1991**) 2726-2735.

23. J.E. Ladbury, G. Klebe, E. Freire, Adding calorimetric data to decision making in lead discovery: a hot tip. *Nat. Rev. Drug Discovery 9* (**2010**) 23-27.

24. G. A. Patani, E.J. LaVoie, Bioisosterism: A rational approach in drug design. *Chem. Rev. 96* (**1996**) 3147-3176.

25. J.S. Warmus, C. Flamme, L.Y. Zhang, S. Barrett, A. Bridges, M. Kaufman, H. Tecle, R. Gowan, J. Sebolt-Leopold, W. Leopold, R. Merriman, S. Przybranowski, H. Valik, J. Chen, J. Ohren, A. Pavlovsky, C. Whithead, E. Ahang, 2-Alkylamino- and alkoxysubstituted 2-amino-1,3,4-oxadiazoles O-Alkyl benzohydroxamate esters replacements retain the desired inhibition and selectivity against MEK (MAP ERK kinase). *Bioorg. Med. Chem. Lett. 18* (**2008**) 6171-6174.

*26.* M.D. McBriar, J.W. Clader, I. Chu, R.A. Del Vecchio, L. Favreau, W.J. Greenlee, L.A. Hyde, A.A. Nomeir, E.M. Parker, D.A. Pissarnitski, L. Song, L. Zhang, Z. Zhao Discovery of amide and heteroaryl isosteres as carbamate replacements in a series of orally active γ-secretase inhibitors. *Bioorg. Med. Chem Lett. 18* (**2008**) 215-219.

27. B. Sperker, J.T. Backman, K. Kromer, The role of beta-glucuronidase in drug disposition and drug targeting in humans. *Clin. Pharm. 33* (**1997**) 18-31

28. E. Boyland, J.E. Gasson, D.C. Williams, Enzyme activity in relation to cancer, the urinary beta-glucuronidase activity of patients suffering from malignant disease. *Brit. J. Cancer. 11* (**1957**) 120-129.

29. J.C. Caygill, D.A. Pitkeathly, A study of beta-acetylglucosaminase and acid phosphatase in pathological joint fluids. *Ann. Rheum. Dis. 25* (**1966**) 137-144.

30. N. Bank, S.H. Bailine, Urinary Beta-Glucuronidase Activity in Patients with Urinary-Tract Infection. *New. Engl. J. Med. 272* (**1965**) 70-75.

31. A.P. Roberts, J. Frampton, S.M. Karim, R.W. Beard, Estimation of beta-glucoronidase activity in urinary-tract infection. *New. Engl. J. Med. 276* (**1967**) 1468-1470.

32. A.R. Ronald, F. Silverblatt, H. Clark, R.E. Cutler, M. Turck, Failure of urinary beta-glucuronidase activity to localize the site of urinary tract infection. *App Microbiol. 21* (**1971**) 990-992

33. A. Schapiro, W. Paul, H.J. Gonick. Urinary beta-glucuronidase in urologic diseases of the kidneys. *Urol. 100* (**1968**) 146-157.

34. H.A. Kallet, L.J. Lapco, Urine beta glucuronidase activity in urinary tract disease. *Urol. 97* (**1967**) 3523-356.

35. H.C. Gonick, H.J. Kramer, A.E. Schapiro. Urinary -glucuronidase activity in renal disease. *Arch. Int Med 132* (**1973**) 63-69.

36. C.M. Plum, Beta-glucuronidase activity in serum, cerebrospinal fluid and urine in normal subjects and in neurological and mental patients. *Enzymol Biol Clin. 8* (**196**7) 97-112.

37. G. Weissmann, R.B. Zurier, P.J. Spieler, I.M. Goldstein, Mechanisms of lysosomal enzyme release from leukocytes exposed to immune complexes and other particles. *J Exp Med. 134* (**1971**) 149-165.

38. a) C. Hu, V. R. Solomon, G. Ulibarri, H. Lee, The efficacy and selectivity of tumor cell killing by Akt inhibitors are substantially increased by chloroquine, *Bioorg. Med. Chem*. **2008**, *16*, 7888-7893; b) C. Hu, V. R. Solomon, P. Cano, H. Lee, A 4-aminoquinoline derivative that markedly

sensitizes tumor cell killing by Akt inhibitors with a minimum cytotoxicity to non-cancer cells, *Eur. J. Med. Chem*. **2010**, *45*, 705-709; c) C. T. Keith, A. A. Borisy, B. R. Stockwell, Multicomponent therapeutics for networked systems, *Nature Reviews Drug Discovery*, **2005**, *4*, 71-78; d) Y. C. Mayur, G. J. Peters, V. V. S. R. Prasad, C. Lemos, N. K. Sathish, Design of New Drug Molecules to be Used in Reversing Multidrug Resistance in Cancer Cells, *Current Cancer Drug Targets*, **2009**, *9*, 298-306.

39. a) V. R. Solomon, C. Hu, H. Lee, Hybrid pharmacophore design and synthesis of isatin–benzothiazole analogs for their anti-breast cancer activity, *Bioorg. Med. Chem.*, **2009**, *17*, 7585-7592; b) B. Meunier, Hybrid Molecules with a Dual Mode of Action: Dream or Reality?, *Acc. Chem. Res.*, **2008**, *41*, 69-77; c) C. Hubschwerlen, J. L. Specklin, D. K. Baeschlin, Y. Borer, S. Haefeli, C. Sigwalt, S. Schroeder, H. H. Locher, *Bioorg. Med. Chem. Lett.*, **2003**, *13*. 4229-4233; d) S.K. Bhanot, M. Singh, N. R. Chatterjee, The Chemical and Biological Aspects of Fluoroquinolones Reality and Dreams, *Curr. Pharm. Des*., **2001**, *7*, 311-335; e) J. Adamec, R. Beckert, D. Weiss, V. Klimesova, K. Waisser, U. Mollmann, J. Kaustova, V. Buchta, Hybrid molecules of estrone: New compounds with potential antibacterial, antifungal, and antiproliferative activities, *Bioorg. Med. Chem.*, **2007**, *15*, 2898-2906; f) V. V. Kouznetsov, A. Gomez.-Barrio, Recent developments in the design and synthesis of hybrid molecules basedon aminoquinoline ring and their antiplasmodial evaluation, *Eur. J. Med. Chem.*, **2009**, *44*, 3091-3113.

40. Khan, K. M., Shujaat, S., Rahat, S., Hayat, S., & Choudhary, M. I.. Beta-N-cyanoethyl acyl hydrazide derivatives: a new class of beta-glucuronidase inhibitors. *Chemical & pharmaceutical bulletin 50* (**2002**) 1443-6.

41. W. Jamil, S. Perveen, S.A.A. Shah, M. Taha, N.H. Ismail, N. Ambreen, K.M. Khan, M.I. Choudhary, Phenoxyacetohydrazide Schiff bases: *β*-Glucuronidase inhibitors. *Molecules 19* (**2014**) 8788-8802.

42. Zawawi, N. K. N. A., Taha, M., Ahmat, N., Wadood A., Ismail N. H., Rahim F., Ali M., Abdullah N, Khan, K. M. (2015). Novel 2, 5-disubtituted-1, 3, 4-oxadiazoles with benzimidazole backbone: A new class of *β*-glucuronidase inhibitors and in silico studies. *Bioorg. Med. Chem*. 23 (2015) 3119-25.

43. a) Z.S. Saify, A. Kamil, S. Akhtar, M. Taha, A. Khan, K.M. Khan, S. Jahan, F. Rahim, S. Perveen, M.I.Choudhary, 2-(2'-Pyridyl) benzimidazole Derivatives and their Urease Inhibitory Activity. *Med. Chem. Res. 23*, (**2014)**, 4447-4454; b) K.M. Khan, F. Rahim, A. Wadood, M. Taha, M. Khan, S. Naureen, N. Ambreen, S. Hussain, S. Perveen, M.I. Choudhary. Evaluation of *Bis*indole

as Potent *β*-Glucuronidase Inhibitors: Synthesis and *In Silico* Based Studies. *Bioorg. Med. Chem. Lett. 24* (**2014**) 1825-1829; c) K.M. Khan, F. Naz, M. Taha, A. Khan, S. Perveen, M.I. Choudhary, W. Voelter. Synthesis and *In Vitro* Urease Inhibitory Activity of *N,N'*-Disubsituted Thioureas. *Eur. J. Med. Chem. 74* (**2014**) 314-323; d) K.M. Khan, F. Rahim, A. Wadood, N. Kosar, M. Taha, S. Lalani A. Khan, M.I. Fakhri, M. Junaid, W. Rehman, M. Khan, S. Perveen, M. Sajid, M.I. Choudhary. Synthesis and Molecular Docking Studies of Potentn *α*-Glucosidase Inhibitors Based on Biscoumarin Skeleton. *Eur J Med Chem. 81* (**2014**) 245-252; e) F. Rahim, K. Ullah, H. Ullah, A. Wadood, M. Taha, A. U. Rehman, M. Ashraf, A. Shaukat, W. Rehman, S. Hussain, K. M. Khan *Bioorg. Chem. 58* (**2015**) 81-87; f) M. Taha, N. H. Ismail, S. Lalani, M.Q. Fatmi, Atia-tul-Wahab, S. Siddiqui, K.M. Khan, S. Imran, M.I. Choudhary, Synthesis of Novel Inhibitors of *α*-Glucosidase Based on the Benzothiazole Skeleton Containing Benzohydrazide Moiety and their Molecular Docking Studies. *Eur J. Med. Chem. 92* (**2015**) 387-400; g) M. Taha N.H. Ismail, W. Jamil , H. Rashwan, S.M. Kashif, A.A. Sain, M.I. Adenan, E.H. Anouar, M. Ali, F. Rahim, K.M. Khan. Synthesis of novel derivatives of 4-methylbenzimidazole and evaluation of their biological activities. *Eur J Med Chem. 84* (**2014**) 731-738; h) K.M. Khan, Z. Shah, V.U. Ahmad, N. Ambreen, M. Khan, M. Taha, F. Rahim, S. Noreen, S. Perveen, M.I. Choudhary, W. Voelter, 6-Nitrobenzimidazole derivatives: potential phosphodiesterase inhibitors: synthesis and structure-activity relationship. *Bioorg. Med. Chem. 20* (**2012**) 1521-1526.

44. K.M. Khan, F. Rahim S.A. Halim, M. Taha, M. Khan, S. Perveen, Zaheer-Ul-Haq, M.A. Mesaik, M.I. Choudhary, Synthesis of novel inhibitors of β-glucuronidase based on benzothiazole skeleton and study of their binding affinity by molecular docking. *Bioorg Med Chem. 19*, (**2011**), 4286-4294

45. A) O. Lauren, Court, M.H. Effect of the β-glucuronidase inhibitor saccharolactone on glucuronidation by human tissue microsomes and recombinant UDP-glucuronosyltransferases. *J. Pharm. Pharmaco. 60* (**2008**) 1175-1182 b) K.M. Khan, S.M. Saad, N.N. Shaikh, S, Hussain, M.I. Fakhri, S. Perveen, M.Taha, M.I. Choudhary. Synthesis and *β*-glucuronidase inhibitory activity of 2-arylquinazolin-4(3H)-ones. *Bioorg. Med. Chem. 22* (**2014**) 3449-3454; c) K.M. Khan, N. Ambreen, M. Taha, S.A. Halim, Zaheer-ul-Haq, S. Naureen, S. Rasheed, S. Perveen, S. Ali, M.I. Choudhary. Structure-Based Design, Synthesis and Biological Evaluation of ß-Glucuronidase Inhibitors. *J. Comput. Aided Mol. Des. 28* (**2014**) 577-585; d) N.K.N.Z., Abdullah, M. Taha, N. Ahmat, A. Wadood, N.H. Ismail, F. Rahim, M. Ali, N. Abdullah, K.M. Khan, Novel 2,5-Disubtituted-1,3,4-Oxadiazoles with Benzimidazole Backbone: A New Class of β-Glucuronidase Inhibitors and In Silico Studies *Bioorg. Med. Chem.* (**2015**) In press.

46. S. Jain, W.B. Drendel, Z.W. Chen, F.S. Mathews, W.S. Sly, J.H. Grubb. *Nat. Struct. Biol. 3* (**1996**) 375-381.

47. E. F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin, UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput Chem. 25* (**2004**) 1605-1612

48. Product version: MarvinSketch V 5.2.6, copyright 1998-2009 ChemAxon Ltd, http://www.chemaxon.com

49. G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol. 267* (**1997**) 727-748.

50. G.T. Costakes, Overview of current docking programs. *MMG 445 Basic Biotechnology e Journal* 1 (**2006**).

51. Schrödinger LLC (**2010**), The PyMOL Molecular Graphics System. 1.3 ed.

52. M. Taha, N.H. Ismail, S. Imran, K.M. Khan, 4-[5-(2-Methoxyphenyl)-1,3,4-oxadiazol-2-yl]benzohydrazide. *Molbank* M826 (**2014**).

**Captions of figures and tables**

**Figure-1** Comparison between hydrazide, oxadiazole, hydrazone, and hybrid compound **13**.

**Figure-2** Shows the putative binding mode of the potent compounds among the synthesized derivatives in the active site of *β*-glucuronidase. The key interacting residues are shown in magenta color line and compound in stick representation, while the hydrogen bonds are shown in yellow dashed line. Compound **13** in split pea color **(1a)**, Compound **9** in dark green color **(1b)**, Compound **6** in orange color **(1c)**, Compound **18** in fluorescent color **(1d)**, Compound **8** in pale yellow **(1e)**, Compound **7** in dirty violet color **(1f)**, Compound **25** in blue white **(1g)** and Compound **11** in light pink color **(1h)**.

**Figure-3** Shows the binding mode of the synthesized derivatives in the active site of β-glucuronidase. **(2a)** inactive compounds binding modes and **(2b)** active compounds binding modes.

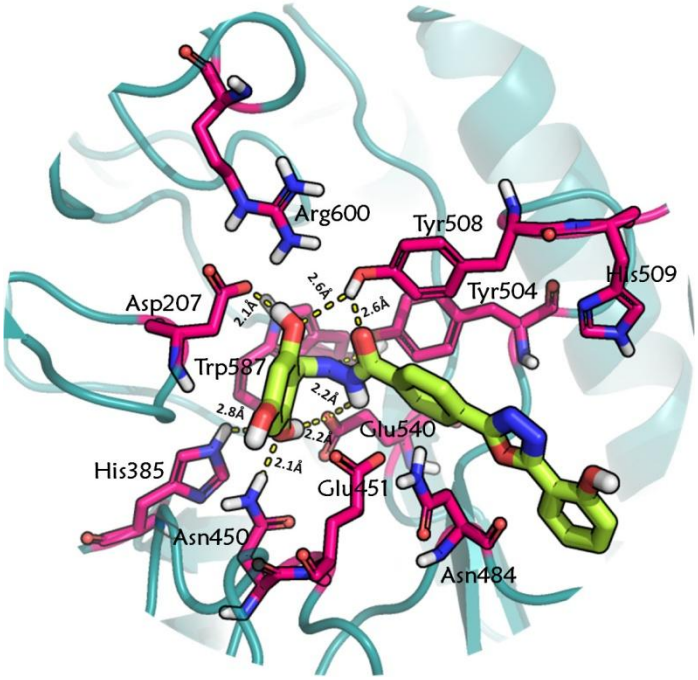
**Scheme-1.** Synthesis of benzohydrazide having oxadiazole ring compound **5**

**Scheme-2.** Synthesis of novel oxadiazole benzohydrazones **6-30**

**Table-1.** Structure of oxadiazole benzohydrazones **6-30**

**Table-2.** *In vitro β*-glucuronidase inhibition activity of oxadiazole derivatives **6-30** and their docking studies Goldscore results.

**Graphical Abstract**




A series consisting of 25 novel oxadiazole-benzohydrazone hybrids have been synthesized and evaluated for their $\beta$-glucuronidase inhibition activity.