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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, molecular docking study, and anticancer activity of triaryl-1,2,4-oxadiazole

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Abstract This study describes synthesis of a new group of triaryl-1,2,4-oxadiazole derivatives and their anticancer activities. The target compounds were prepared from reaction of different imines and 4-substituted benzohydroxyiminoyl chlorides. All the synthesized compounds were screened for antiproliferative activities against MCF7 and K562 cell lines using MTT assay at 50-μM concentration. Four compounds that showed more than 50 % cytotoxicity were selected for determination of IC_{50}. Out of these, **6c**-**1y** showed remarkable inhibitory cytotoxicity activity against MCF7 and K562 cell lines with IC_{50} 6.50 and 21.66 μM, respectively. A molecular modeling study where **6c**-**1y** was docked in the binding site of COX-2 showed a 2.3-Å hydrogen bond forming via hydroxyl group of Ser516 residue and oxygen of central oxadiazole ring and triaryl moiety of **6c**-**1y** oriented toward the hydrophobic pockets of COX-2. Our data indicate that these derivatives may present promising chemotherapeutic agents, possibly targeting COX-2 pathway.

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Introduction

Over, the past few decades, the importance of chemotherapy has increased in the treatment of cancer. In this regards, drug resistance put forward as one the most reasons in failure chemotherapy. Therefore, there is a great interest to introduce new cytotoxic agents and also determine or predict their mechanisms of action.

The role of microtubules in cell proliferating has been recognized as a validated target in cancer chemotherapy (Aryapour *et al*., 2011). Three distinct binding sites were recognized on β-subunit of tubulin that has been targeted by many useful anticancer drugs (Alvarez *et al*., 2008). A 105972 and combretastatin are vicinal-diaryl stillben-like structure that binds to β-tubulin and strongly inhibit tubulin polymerization by binding to the colchicine site (Kumar *et al*., 2011). Combretastatin A-4, a *cis*-conformation stillben-like structure (Fig. 1a), was isolated from bark of the South African tree *Combretum caffrum*. Isomerization to the less active *trans*-form of combretastatin A-4 encouraged preparing di-aryl heterocyclic compounds (Fig. 1c) owing *cis*-restricted analogous (Odlo *et al*., 2008).

In the other hand, di-aryl heterocyclic compounds have been classically recognized as selective cyclooxygenase-2 (COX-2) inhibitors. Celecoxib (Fig. 1b), valdecoxib, and several related structures have been used for the treatment of inflammation-associated disorders in clinic. Previous studies have indicated that celecoxib is chemopreventive both in animal tumor models and in cancer patients (Steinbach *et al*., 2000; Reddy *et al*., 2000). In a clinical trial, celecoxib was found to reduce the number and size of

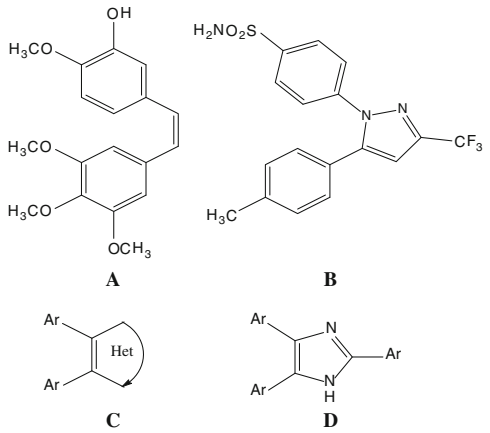


Fig. 1 Structure of combretastatin A-4, celecoxib and di- or triaryl heterocyclic compounds

polyps in-patients with familial adenomatous polyposis (FAP) (Steinbach *et al*., 2000). Recent studies have shown that celecoxib induces apoptosis in human chronic myeloid leukemia, K562 and breast cancer cell lines (Subhashini *et al*., 2005; Wang *et al*., 2012). The effects of celecoxib on the hematopoietic cancer cell line like Jukrat, HL60, and U937 revealed dose-dependent inhibition in growth of the cell with arrest of cell cycle at G0/G1 phase. Previous findings also suggested that COX-2 inhibition decreases cell growth and promotes chemotherapy-induced apoptosis in breast cancer cells (Wang *et al*., 2012). Collectively, these reports reveal that celecoxib could be a potential candidate for developing anticancer drug for the treatment of chronic myeloid leukemia and other types of cancer.

The cytotoxicity effects of several di- or triaryl heterocyclic rings such as pyrroles (Banwell *et al*., 2006), oxadiazole (Kiselyov *et al*., 2010), triazoles (Odlo *et al*., 2008) and imidazole (Schobert *et al*., 2010), and triazine (Krauth *et al*., 2010) have already been reported. From structure points of view, selective COX-2 inhibitors are divided into several classes. 1,2-di-aryl heterocyclic compounds, Coxibs, are one of the extensive groups that have a five-membered ring including furanone, pyrazole, oxazole, isoxazole, pyrole,… or six-membered ring such as pyranone, pyridine, and pyridazinone (Joo *et al*., 2004) in the center of the molecules that aryl groups is located as 1,2-substituted. Guathier showed that human recombinant COX-2 has been inhibited by 1,3-diaryl heterocyclic compounds (Fig. 1c). Molecular modeling findings also highlighted drug interaction with active site of both COX-1 and COX-2 suggested some modifications to enhance the selectivity of the compounds (Gauthier *et al*., 2006). Their model suggested that an additional aryl group adjoin to central heterocycle ring enhanced the interaction of the ligand in active site. Therefore, triaryl heterocycles have been proposed might better fit in COX-2 active site. Consequently, a series of triaryl imidazole derivatives (Fig. 1d) was introduced as selective COX-2 inhibitors with IC50 % ranging from 0.15 to 0.40 μM (Zarghi *et al*., 2012).

Keeping the above facts in mind, it was of our interest to synthesize a series of triaryl-1,2,4-oxadiazole and evaluated their anticancer activity against two different cancer cell lines with the MTT assay. Some active compounds were docked in the active site of murine COX-2 and their probable mechanisms of action have been discussed.

Results and discussion

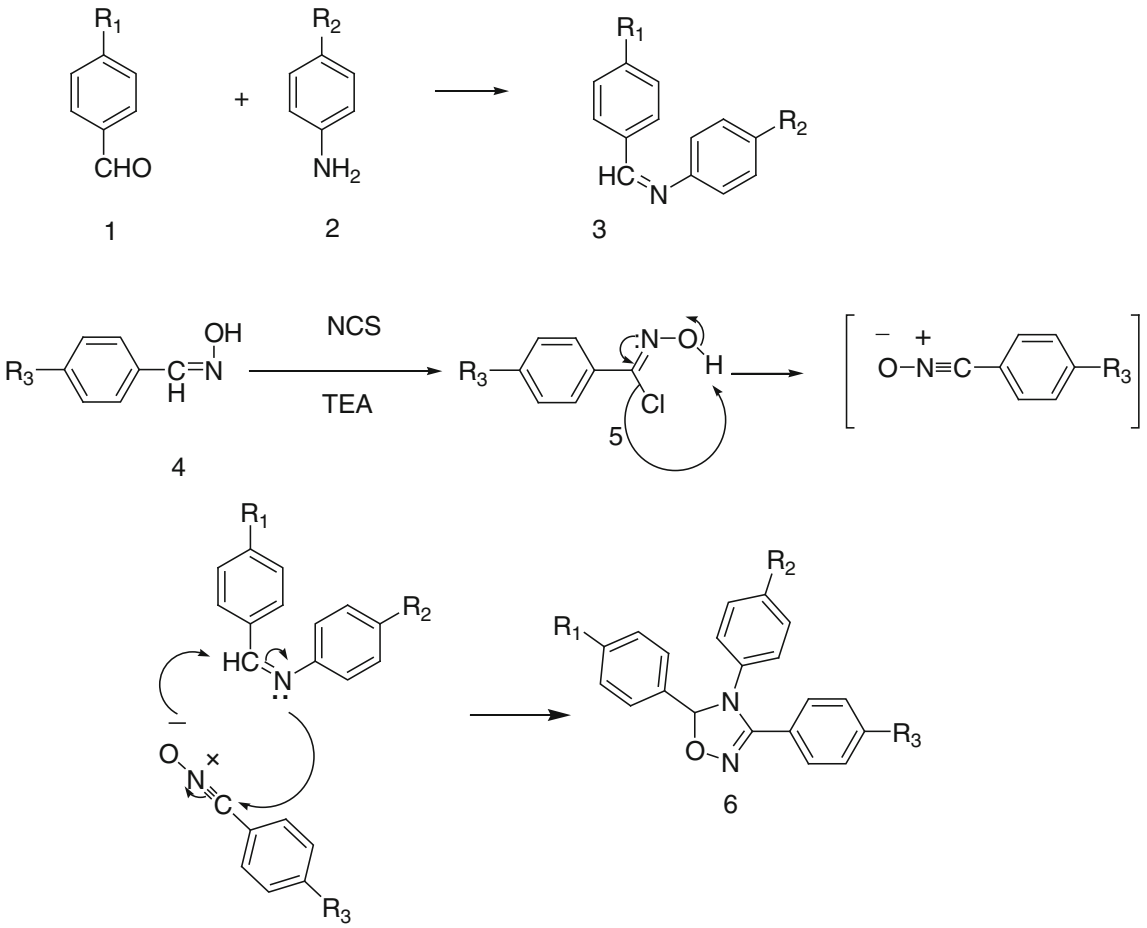
Chemistry

Synthesis of 1,2,4-oxadiazoles via the 1,3-dipolar cycloaddition of benzonitrile oxide and imines were reported (Alcaide *et al*., 1987). Benzonitrile oxide tends to dimerize within a few minutes to several days related to nature of substituted group in aryl ring (Liu *et al*., 1980). In this study, an easy method for preparation of new triaryl oxadiazole derivatives (**6**) from condensation of 4-substituted benzohydroxyiminoyl chlorides (**5**) and imines (**3**) in diethyl ether was developed. In Scheme 1, the preparation of compound **6** has been illustrated. 4-Substituted benzohydroxyiminoyl chlorides that are stable and storable were prepared from arylaldehyde oximes and *N*-chlorosuccinimide in DMF as reported procedure (Liu *et al*., 1980). All final compounds were pure and stable. The compounds were characterized by ^{1}H and ^{13}C- nuclear magnetic resonance and CHN analysis. ^{1}HNMR spectra of the triaryl-1,2,4-oxadiazole derivatives (**6**) show a typical proton signal for the H_5-oxadiazole ring at 6.45–6.52-ppm range. In ^{13}CNMR spectra of compound **6**, the signals at ~123 and 100 ppm are attributed to C_3 and C_5 of oxadiazole ring, respectively.

Biological study

*Inhibitory effects of compound (**6**) on viability of breast cancer and leukemia cells*

To evaluate the effect of synthetic compound (**6**) on human breast adenocarcinoma (MCF-7) and human erythroleukemia K562 cells, cytotoxicity was measured using the MTT assay. For this purpose, cultured cells were incubated in the absence and in the presence of 50 μM of different compounds for 24 h. A considerable difference in percent of cytotoxicity of compounds was evident in



Scheme 1 Synthesis of 1,2,4-oxadiazole derivatives

MCF7- and K562-treated cells. Based on the desirable cytotoxic effects of compounds **6e-3y**, **6a-1y**, **6c-1y**, and **6e-1y** (more than 50 % of cell cytotoxicity at 50 μM), we examined the effect of these compounds on the proliferation of MCF-7 and K562 cells to obtain IC_{50} values. The dose–response curves for four tested compounds are shown in Figs. 2 and 3. The results revealed that all compounds caused a dose-dependent inhibition of cell proliferation. It is also noteworthy that for both tested cell lines, compounds **6c-1y** and **6e-1y** showed a better cytotoxic activity than two clinically established chemotherapeutics; doxorubicin and paclitaxel which depend on the cell type (Table 1). As it shown in Table 1, a more potent antiproliferative activity was documented for compounds **6c-1y** and **6e-1y** in MCF-7 cells compared with doxorubicin. It is interesting to suggest that MCF-7 cells reveal some resistance against doxorubicin, whereas these two compounds decreased the cell viability significantly (IC_{50} value ~10 μM). Moreover, comparison of cytotoxic effects of compounds **6c-1y** and **6e-1y** with reference drug in K562 cells demonstrated that these compounds are more potent than paclitaxel in this cell line. On the other hand, the leukemia-resistant cell line (K562) against this chemotherapeutic (higher IC_{50}) showed a remarkable chemosensitivity to the two tested compounds. Considering all data, compounds **6c-1y** and **6e-1y** were found to exhibit the highest cytotoxic activity in comparison with other derivatives against two human cancer cells studied here and the order of inhibition against both cell lines are as follows: **6c-1y** > **6e-1y** > **6e-3y** > **6a-1y**, which might be attributed

to the presence of F or Cl as R_1 and R_3. With respect to H substituent at R_2 position, replacement of the hydrogen substituent as R_1 side chain (**6a**-**1y**) with fluoro and chloro (**6c**-**1y**, **6e**-**1y**) resulted in a remarkable increase in cytotoxic activity. Moreover, 5-fluoro derivative is more potent than chloro analog (**6c**-**1y** vs **6e**-**1y**). It is worth noting that both of the analogs (IC_{50}: 6.5–10.1 μM) are more potent than two reference drugs which depend on the cell type, MCF-7 or K562. Another interesting point is the presence of chlorine atom in R_3 for all the active compounds. The substitution of F instead of Cl at R3 (compounds **6**(**a**–**e**)-**3x**) caused to decrease the activity.

Molecular modeling

All four active compounds (**6e**-**3y**, **6a**-**1y**, **6c**-**1y**, and **6e**-**1y**) were docked in a similar manner in the active site of COX-2. As exemplified by **6c**-**1y** and could be seen in Fig. 4 a 2.3-Å hydrogen bond formed via –OH moiety of Ser516 residue and oxygen of central oxadiazole ring. The chlorophenyl moiety of **6c**-**1y** oriented toward a hydrophobic pocket constructed by lipophilic side chains of Val335, Leu345, Val102, Tyr341, and Leu517. In the middle, the phenyl lies in the vicinity of Ser339, Tyr341, Val509, Phe504, and Leu338 residues. The fluorophenyl group well fitted in another hydrophobic side pocket of COX-2 containing Leu338, Phe504, Trp375, Met508, Leu370, and Tyr 371 lipophilic side chains.

A summary of HYDE reported free energy of binding of compounds with COX-2 could be found in Table 2. Docking of these compounds was also accomplished on COX-1 but neither of them docked on it which may indicate the predicted selectivity toward COX-2 for these structures.

The obtained results were matching with cytotoxicity assay findings. It is evident that the compounds **6c**-**1y**, which was predicted *in silico* to be active compounds, has been observed the most potent. As seen in Fig. 4, compound **6c**-**1y** was having strong H bonding via –OH moiety of Ser516 residue and oxygen of central oxadiazole ring.

Although the MTT screening protocol did not conclude of any possible mechanisms for the observed anticancer activity of the test compounds, activity of **6c**-**1y**, **6e**-**1y** may be attributed to apoptosis pathway due to the inhibition of COX-2 route that require to the further studies.

In summary, our work provides new analogous of COX-2 inhibitors with a 1,2,4-oxadiazole in center of the molecules as the heterocycle moiety, showing effective anticancer activity and it present a promising lead for the generation of new chemotherapeutic agents in selected cancer cells which needs further research.

Materials and methods

General

Melting points were determined using a Thomas Hoover melting point apparatus. A Bruker FT-500 MHz instrument

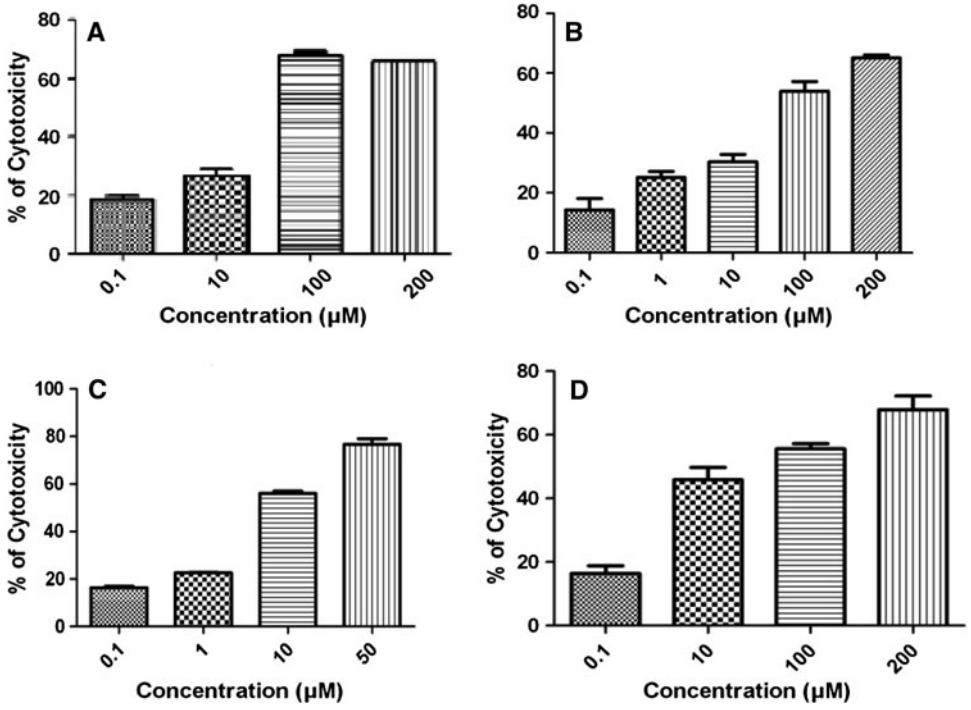


Fig. 2 Percentage of cytotoxicity of MCF7 cells after treatment with compounds **6e**-**3y** (**a**), **6a**-**1y** (**b**), **6c**-**1y** (**c**), and **6e**-**1y** (**d**) for 24 h. Data represent the means of experiments performed in triplicate

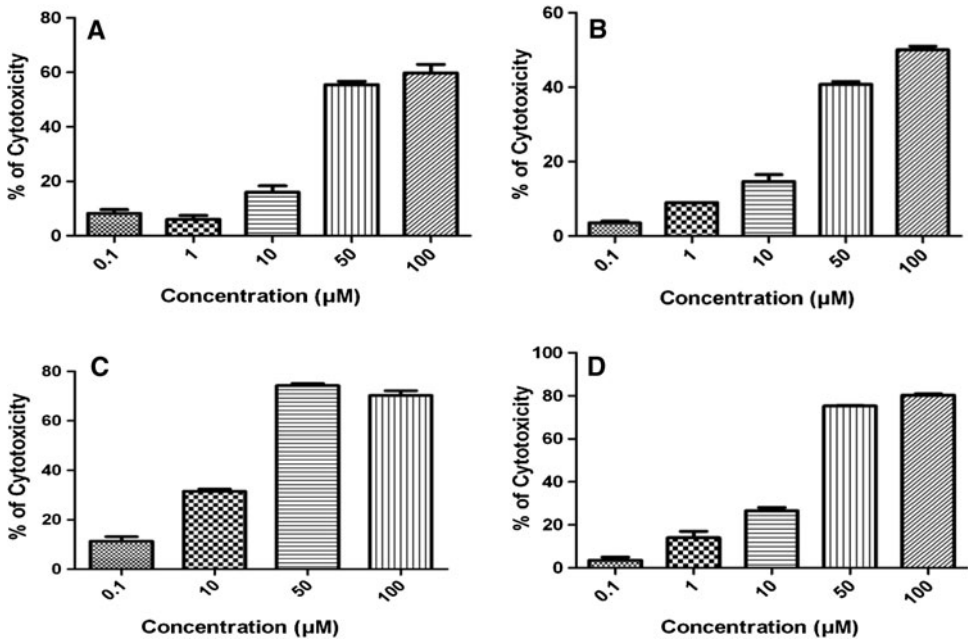
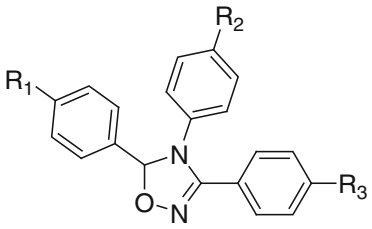


Fig. 3 Percentage of cytotoxicity of K562 cells after treatment with compounds **6e-3y** (**a**), **6a-1y** (**b**), **6c-1y** (**c**), and **6e-1y** (**d**) for 24 h. Data represent the means of experiments performed in triplicate

Table 1 IC_{50} values (μM) for antiproliferative activity of compounds **6e-3y**, **6a-1y**, **6c-1y** and **6e-1y** toward MCF7 and K562 cells



Compounds	R_1	R_2	R_3	IC50 (μM)	
				MCF-7	K562
6e-3y	Cl	Cl	Cl	22.60 ± 1.25	51.24 ± 1.11
6a-1y	H	H	Cl	23.70 ± 1.27	97.01 ± 1.11
6c-1y	F	H	Cl	6.50 ± 1.09	21.66 ± 1.17
6e-1y	Cl	H	Cl	10.10 ± 1.20	22.23 ± 1.09
Doxorubicin				>100	4.49 ± 1.39
Paclitaxel				1.86 ± 1.40	>100

Values are presented as mean ± SE of three independent experiments, performed in triplicate

was used to acquire ^{1}HNMR spectra. The instrument was set at 125 MHz for acquiring ^{13}CNMR spectra. Coupling constant (*J*) values are presented in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triple), and m (multiple). Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus. The results of the elemental analysis (C, H, N) were within ±0.4 % of the calculated amounts.

Chemistry

General procedure for synthesis of 3,4,5-triaryl-4,5-dihydro-1,2,4-oxadiazole

To a solution of *N*-benzylideneaniline derivative (Alcaide *et al*., 1987) (1 eq) in ether was added *N*-hydroxybenzimidoyl chloride (1 eq) and triethylamine (1.2 eq) successively.

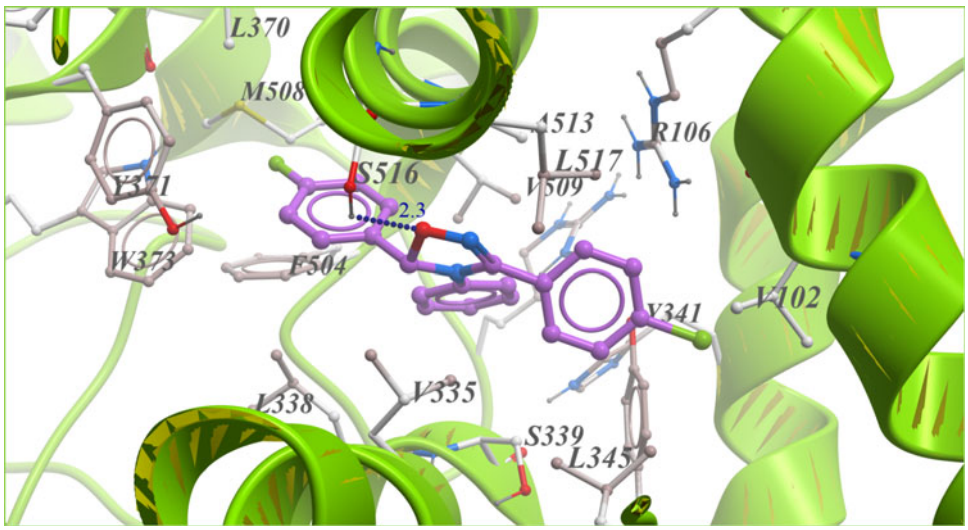


Fig. 4 Orientation of **6c-1y** (carbons in *purple*) in the active site of COX-2. As could be seen a 2-Å hydrogen bond formed via hydroxyl group of Ser516 and oxygen of oxadiazole ring (The pictures were prepared by the Molsoft ICM-Browser.) (Color figure online)

Table 2 Hyde scoring reported affinities of ligands toward COX-2

Compounds	HYDE reported $\Delta G_{intramolecular}$(kJ/mol)
6e-3y	−59
6a-1y	−52
6c-1y	−57
6e-1y	−55

The reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure. The residue was crystallized in acetic acid–water to yield 3,4,5-triaryl-4,5-dihydro-1,2,4-oxadiazoles.

*3,4-Bis(4-chlorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (**6a-3y**)* Yield: 88 %. Mp: 143–144 °C. ^{1}HNMR ($CDCl_3$) δ: 6.49 (s, 1H, H_5-oxadiazole), 6.73 (d, J = 7.45 Hz, 2H, $H_{2,6}$-NAr), 7.16 (d, J = 7. 45 Hz, 2H, $H_{3,5}$-NAr), 7.34 (d, J = 7.50 Hz, 2H, $H_{3,5}$-Ar_3), 7.46 (m, 3H, Ar_5), 7.53 (d, J = 7.50 Hz, 2H, $H_{2,6}$-Ar_3), 7.57 (m, 2H, Ar_5). ^{13}CNMR ($CDCl_3$) δ: 154.10 (C_1-NAr), 139.45 [C_1-Ar_3 (C_1-Ar_5)], 138.37 [C_1-Ar_5 (C_1-Ar_3)], 136.84 (C_4-Ar_3), 131.54 (C_4-NAr), 130.06 (C_4-Ar_5), 129.51 ($C_{2,6}$-Ar_5), 129.23 ($C_{3,5}$-Ar_5), 129.16 ($C_{3,5}$-Ar_3), 128.96 ($C_{2,6}$-Ar_3), 127.16 ($C_{3,5}$-NAr), 125.53 ($C_{2,6}$-NAr), 123.55 (C3-oxadiazole), 100.53 (C5-oxadiazole). Anal. Calcd. For $C_{20}H_{14}Cl_2N_2O$: C, 65.06; H, 3.82; N, 7.59. Found: C, 65.26; H, 3.61; N, 7.44.

*3,4-Bis(4-chlorophenyl)-5-(p-tolyl)-4,5-dihydro-1,2,4-oxadiazole (**6b-3y**)* Yield: 86 %. Mp: 120–122 °C. ^{1}HNMR ($CDCl_3$) δ: 2.41 (s, 3H, CH_3), 6.46 (s, 1H, H_5-oxadiazole), 6.72 (d, J = 8.45 Hz, 2H, $H_{2,6}$-NAr), 7.16 (d, J = 8.45 Hz, 2H, $H_{3,5}$-NAr), 7.26 (d, J = 7.7 Hz, 2H, $H_{3,5}$-Ar_5), 7.34 (d, J = 8.30 Hz, 2H, $H_{3,5}$-Ar_3), 7.45 (d, J = 7.7 Hz, 2H, $H_{2,6}$-Ar_5), 7.52 (d, J = 8.30 Hz, 2H, $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 154.06 (C_1-NAr), 140.12 (C_4-NAr), 139.41 (C_1-Ar_3), 136.76 (C_1-Ar_5), 135.31 (C_4-Ar_3), 131.42 (C_4-NAr), 129.63 [$C_{2,6}$-Ar_5 ($C_{3,5}$-Ar_5)], 129.45 [$C_{3,5}$-Ar_5 ($C_{2,6}$-Ar_5)], 129.22 [$C_{3,5}$-Ar_3 ($C_{2,6}$-Ar_3)], 129.13 [$C_{2,6}$-Ar_3 ($C_{2,6}$-Ar_3)], 127.16 ($C_{3,5}$-NAr), 125.52 ($C_{2,6}$-NAr), 123.65 (C_3-oxadiazole), 100.53 (C5-oxadiazole), 21.37 (CH_3). Anal. Calcd. For $C_{21}H_{16}Cl_2N_2O$: C, 65.81; H, 4.21; N, 7.31. Found: C, 65.66; H, 4.09; N, 7.25.

*3,4-Bis(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6c-3y**)* Yield: 73 %. Mp: 110–112 °C. ^{1}HNMR ($CDCl_3$) δ: 6.47 (s, 1H, H_5-oxadiazole), 6.72 (d, J = 8.35 Hz, 2H, $H_{2,6}$-NAr), 7.13–7.20 (m, 4H, $H_{3,5}$-NAr and $H_{3,5}$-Ar_5), 7.34 (d, J = 8.15 Hz, 2H, $H_{3,5}$-Ar_3), 7.51 (d, J = 8.15 Hz, 2H, $H_{2,6}$-Ar_3), 7.55 (t, J = 7.50 Hz, 2H, $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 161.71 (d, J_{CF} = 247.5 Hz, C_4-Ar_5), 154.20 (C_1-NAr), 139.28 (C_1-Ar_3), 136.93 [C_4-Ar_3 (C_1-Ar_5)], 134.17 [C_1-Ar_5 (C_4-Ar_3)], 131.85 (C_4-NAr), 129.62 ($C_{3,5}$-Ar_3), 129.19 [$C_{2,6}$-Ar_5 and $C_{3,5}$-NAr(d, J_{CF} = 7.30 Hz)], 125.74 ($C_{2,6}$-NAr), 123.38 (C_3-oxadiazole), 116.00 (d, J_{CF} = 21.25 Hz, $C_{3,5}$-Ar_5), 99.95 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{13}Cl_2FN_2O$: C, 62.03; H, 3.38; N, 7.23. Found: C, 62.28; H, 3.51; N, 7.41.

*3,4-Bis(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1,2,4-oxadiazole (**6d-3y**)* Yield: 66 %. Mp: 80–82 °C. ^{1}HNMR ($CDCl_3$) δ: 3.85 (s, 3H, OCH_3), 6.45 (s, 1H, H_5-oxadiazole), 6.71 (d, J = 9.00 Hz, 2H, $H_{2,6}$-NAr), 6.96 (d, J = 9.00 Hz, 2H, $H_{3,5}$-Ar_5), 7.15 (d, J = 9.00 Hz, 2H, $H_{3,5}$-NAr), 7.34 (d, J = 9.00 Hz, 2H, $H_{2,6}$-Ar_3), 7.48–7.51 (m, 4H, $H_{2,6}$-Ar_3 and $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 160.97 (C_4-Ar_5), 154.12 (C_1-NAr), 139.29 (C_1-Ar_3), 136.74 (C_4-Ar_3), 131.48 (C_4-NAr), 130.21 (C_1-Ar_5), 129.47,129.20, 129.13, 128.75 [$C_{2,6}$-Ar_3 ($C_{3,5}$-Ar_3, $C_{3,5}$-Ar_5, $C_{3,5}$-NAr), 123.67 (C3-oxadiazole), 55.38 (OCH_3). Anal. Calcd. For $C_{21}H_{16}Cl_2N_2O_2$: C, 63.17; H, 4.04; N, 7.02. Found: C, 63.36; H, 3.91; N, 7.29.

*3,4,5-Tris(4-chlorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6e-3y**)* Yield: 79 %. Mp: 97–99 °C.^{1}HNMR ($CDCl_3$) δ: 6.50 (s, 1H, H_5-oxadiazole), 6.80 (d, $J = 7.50$ Hz, 2H, $H_{2,6}$-NAr), 7.21 (d, $J = 7.50$ Hz, 2H, $H_{3,5}$-NAr), 7.32 [d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_3 ($H_{3,5}$-Ar_5)], 7.43 [d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_5 ($H_{3,5}$-Ar_3)], 7.52 (m, 4H, $H_{2,6}$-Ar_3 and $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 154.20 (C_1-NAr), 139.28 (C_4-Ar_5), 136.98 [C_4-Ar_3(C_1-Ar_3)], 136.82 [C_1-Ar_3(C_4-Ar_3)], 136.04 (C_1-Ar_5), 131.90 (C_4-NAr), 129.66 ($C_{2,6}$-Ar_3 and $C_{2,6}$-Ar_5), 129.21 ($C_{3,5}$-Ar_3 and $C_{3,5}$-Ar_5), 128.57 ($C_{3,5}$-NAr), 125.71 ($C_{2,6}$-NAr), 123.30 (C_3-oxadiazole), 99.86 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{13}Cl_3N_2O$: C, 59.50; H, 3.25; N, 6.94. Found: C, 59.81; H, 3.21; N, 7.12.

*4,5-Diphenyl-3-(4-chlorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6a-1y**)* Yield: 73 %. Mp: 133–135 °C. ^{1}HNMR ($CDCl_3$) δ: 6.53 (s, 1H, H_5-oxadiazole), 6.81 (d, $J = 7.5$ Hz, 2H, $H_{2,6}$-NAr), 7.10–7.14 (m, 1H, H_4-NAr), 7.19–7.22 (m, 2H, $H_{3,5}$-NAr), 7.32 (d, $J = 8.20$ Hz, 2H, $H_{3,5}$-Ar_3), 7.45 (m, 3H, $H_{3,4,5}$-Ar_5), 7.55 (d, $J = 8.20$ Hz, 2H, $H_{2,6}$-Ar_3), 7.59 (m, 2H, $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 154.36 (C_1-NAr), 141.04 (C_1-Ar_3), 138.78 (C_4-Ar_3), 136.59 (C_1-Ar_5), 129.87 (C_4-Ar_5), 129.36, 129.29, 129.03, 128.88 [$C_{2,6}$-Ar_5 ($C_{3,5}$-Ar_5, $C_{3,5}$-Ar_3, $C_{3,5}$-NAr), 127.16 ($C_{2,6}$-Ar_3), 125.91 (C_4-NAr), 124.32 ($C_{2,6}$-NAr), 123.95 (C_3-oxadiazole), 100.59 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{15}ClN_2O$: C, 71.75; H, 4.52; N, 10.59. Found: C, 71.54; H, 4.63; N, 10.47.

*4-Phenyl-3-(4-chlorophenyl)-5-(p-tolyl)-4,5-dihydro-1,2,4-oxadiazole (**6b-1y**)* Yield: 77 %. Mp: 116–118 °C. ^{1}HNMR ($CDCl_3$) δ: 2.41 (s, 3H, CH_3), 6.50 (s, 1H, H_5-oxadiazole), 6.80 (d, $J = 7.60$ Hz, 2H, $H_{2,6}$-NAr), 7.16 (t, $J = 7.20$ Hz, 1H, H_4-NAr), 7.18 (t, $J = 7.20$ Hz, 2H, $H_{3,5}$-NAr), 7.25 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.32 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_3), 7.48 (d, $J = 7.6$ Hz, 2H, $H_{2,6}$-Ar_5), 7.55 (d, $J = 8.00$, 2H, $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 154.30 (C_1-NAr), 140.99 (C_4-Ar_5), 139.96 (C_1-Ar_3), 136.51 [C_1-Ar_5 (C_4-Ar_3)], 135.95 [C_4-Ar_3 (C_1-Ar_5)], 129.54, 129.31, 129.25, 128.90, 128.44 [$C_{3,5}$-Ar_5 ($C_{2,6}$-Ar_3, $C_{3,5}$-Ar_3, $C_{2,6}$-Ar_5, $C_{3,5}$-NAr)], 126.44 (C_4-NAr), 125.71 ($C_{2,6}$-NAr), 124.05 (C_3-oxadiazole), 100.50 (C_5-oxadiazole), 21.36 (CH_3). Anal. Calcd. For $C_{21}H_{17}ClN_2O$: C, 72.31; H, 4.91; N, 8.03. Found: C, 72.45; H, 4.69; N, 8.23.

*4-Phenyl-3-(4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6c-1y**)* Yield: 62 %. Mp: 106–108 °C. ^{1}HNMR ($CDCl_3$) δ: 6.51 (s, 1H, H_5-oxadiazole), 6.79 (d, $J = 7.50$ Hz, 2H, $H_{2,6}$-NAr), 7.13–7.16 (m, 3H, $H_{3,4,5}$-NAr), 7.21 (t, $J = 7.50$ Hz, 2H, $H_{3,5}$-Ar_5), 7.31 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.53 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-Ar_3), 7.57 (t, $J = 7.50$ Hz, $H_{2,6}$-Ar_5). 13CNMR ($CDCl_3$) δ: 160.00 (d, $J_{CF} = 247.5$ Hz, C_4-Ar_5), 154.45 (C_1-NAr), 140.80 (C_1-Ar_3), 136.68 (C_4-Ar_3), 134.67 (C_1-Ar_5), 129.45, 129.24, 129.04 [$C_{2,6}$-Ar_3, ($C_{3,5}$-NAr, $C_{3,5}$-Ar_3)], 129.15 (d, $J_{CF} = 8.75$ Hz, $C_{3,5}$-Ar_5), 126.17 (C_4-NAr), 124.52 ($C_{2,6}$-NAr), 123.77 (C_3-oxadiazole), 115.89 (d, $J_{CF} = 22.5$ Hz, $C_{2,6}$-Ar_5), 100.00 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{14}ClFN_2O$: C, 68.09; H, 4.00; N, 7.94. Found: C, 68.21; H, 3.82; N, 7.69.

*4-Phenyl-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1,2,4-oxadiazole (**6d-1y**)* Yield: 72 %. Mp: 119–122 °C. ^{1}HNMR ($CDCl_3$) δ: 3.85 (s, 3H, OCH_3), 6.49 (s, 1H, H_5-oxadiazole), 6.79 (d, $J = 7.50$ Hz, 2H, $H_{2,6}$-NAr), 6.96 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.13 (t, $J = 7.50$ Hz, 1H, H_4-NAr), 7.19 (t, $J = 7.50$ Hz, 2H, $H_{3,5}$-NAr), 7.31 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_3), 7.51 (d, $J = 8.00$ Hz, 2H, $H_{2,6}$-Ar_5), 7.54 (d, $J = 8.00$ Hz, 2H, $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 160.83 (C_4-Ar_5), 154.36 (C_1-NAr), 140.86 (C_1-Ar_3), 136.49 (C_4-Ar_3), 130.80 (C_1-Ar_5), 129.30, 129.23, 128.97, 128.70 [$C_{3,5}$-Ar_3, ($C_{3,5}$-Ar_5, $C_{2,6}$-Ar_3, $C_{3,5}$-NAr)] 125.86 (C_4-NAr), 124.44 ($C_{2,6}$-NAr), 124.06 (C_3-oxadiazole), 114.21 ($C_{2,6}$-Ar_5), 100.52 (C_5-oxadiazole), 55.35 (OCH_3). Anal. Calcd. For $C_{21}H_{17}ClN_2O_2$: C, 69.14; H, 4.70; N, 7.68. Found: C, 69.26; H, 4.55; N, 7.54.

*4-Phenyl-3,5-bis(4-chlorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6e-1y**)* Yield: 88 %. Mp: 124–126 °C. ^{1}HNMR ($CDCl_3$) δ: 6.50 (s, 1H, H_5-oxadiazole), 6.80 (d, $J = 7.50$ Hz, 2H, $H_{2,6}$-NAr), 7.16 (t, $J = 7.00$ Hz, 1H, H_4-NAr), 7.22 (t, $J = 8.00$ Hz, 2H, $H_{3,5}$-NAr), 7.32 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_5), 7.42 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.53 (d, $J = 8.50$ Hz, 4H, $H_{2,6}$-Ar3 and $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 154.45 (C_1-NAr), 140.80 (C_1-Ar_3), 137.29, 136.72 [C_4-Ar_5 (C_4-Ar_3)], 135.01 (C_1-Ar_5), 129.98, 129.25, 129.12, 129.05, 128.57 [$C_{2,6}$-Ar_5 ($C_{2,6}$-Ar_3, $C_{3,5}$-Ar_5, $C_{3,5}$-Ar_3, $C_{3,5}$-NAr)], 126.22 (C_4-NAr), 123.67 (C_3-oxadiazole), 99.96 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{14}Cl_2N_2O$: C, 65.06; H, 3.82; N, 7.59. Found: C, 65.23; H, 3.97; N, 7.50.

*4-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (**6a-3x**)* Yield: 68 %. Mp: 112–115 °C. ^{1}HNMR ($CDCl_3$) δ: 6.50 (s, 1H, H_5-oxadiazole), 6.73 (d, $J = 9.00$ Hz, 2H, $H_{2,6}$-NAr), 7.06 (t, $J = 7.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.16 (d, $J = 9.00$ Hz, 2H, $H_{2,6}$-NAr), 7.46–7.48 (m, 3H, $H_{3,4,5}$-Ar_5), 7.56–7.61 (m, 4H, $H_{2,6}$-Ar_5 and $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 164.01 (d, $J_{CF} = 250.00$ Hz, C_4-Ar_3), 154.08 (C_1-NAr), 139.51 (C_1-Ar_3), 138.35 (C_1-Ar_5), 131.45 (C_4-NAr), 130.12 (C_4-Ar_5),130.04 (d, $J_{CF} = 6.25$ Hz, $C_{2,6}$-Ar_3), 129.46 ($C_{2,6}$-Ar_5), 128.95 ($C_{3,5}$-Ar_5), 127.15 ($C_{3,5}$-NAr),125.52 ($C_{2,6}$-NAr), 121.19 (C_3-oxadiazole), 116.08 (d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_3),

100.38 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{14}ClFN_2O$: C, 68.09; H, 4.00; N, 7.94. Found: C, 68.32; H, 4.12; N, 7.78.

*4-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(p-tolyl)-4,5-dihydro-1,2,4-oxadiazole (**6b-3x**)* Yield: 86 %. Mp: 80–82 °C. ^{1}HNMR ($CDCl_3$) δ: 2.43 (s, 3H, CH_3), 6.46 (s, 1H, H_5-oxadiazole), 6.72 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-NAr), 7.05 (t, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_3), 7.15 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-NAr), 7.27 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_5), 7.46 (d, $J = 8.00$ Hz, 2H, $H_{2,6}$-Ar_5), 7.56–7.60 (m, 2H, $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 163.50 (d, $J_{CF} = 252.0$ Hz, C_4-Ar_3), 154.04 (C_1-NAr), 140.07 (C_4-Ar_5), 139.46 (C_1-Ar_3), 135.39 (C_4-NAr), 131.31 (C_1-Ar_5), 130.8 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_3), 129.62, 129.41 [$C_{3,5}$-NAr ($C_{2,6}$-Ar_5)], 127.16 (C3,5-Ar5), 125.50 ($C_{2,6}$-NAr),121.27 (C_3-oxadiazole), 116.08 (d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_3), 100.37 (C_5-oxadiazole). Anal. Calcd. For $C_{21}H_{16}ClFN_2O$: C, 68.76; H, 4.40; N, 7.64. Found: C, 68.39; H, 3.99; N, 7.78.

*4-(4-Chlorophenyl)-3,5-bis(4-fluorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6c-3x**)* Yield: 55 %. Mp: 92–94 °C. ^{1}HNMR ($CDCl_3$) δ: 6.47 (s, 1H, H_5-oxadiazole), 6.72 (d, $J = 9.00$, Hz, 2H, $H_{2,6}$-NAr), 7.06 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-NAr), 7.12–7.20 (m, 4H, $H_{3,5}$-Ar_5 and $H_{3,5}$-Ar_3), 7.54–7.59 (m, 4H, $H_{2,6}$-Ar_3 and $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 164.04 [d, $J_{CF} = 251.25$ Hz, C_4-Ar_5 (C_4-Ar_3)], 163.71 [d, $J_{CF} = 247.50$ Hz, C_4-Ar_3 (C_4-Ar_5)], 154.18 (C_1-N–Ar), 139.33 (C_1-Ar), 134.27 (C_4-Ar_4), 131.75 (C_1-Ar_5), 130.06 [d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_3 ($C_{2,6}$-Ar_5)], 129.57 (C_4-NAr), 129.16 [d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_5 ($C_{2,6}$-Ar_3)], 125.73 (C_1-NAr), 121.02 (C_3-oxadiazole), 116.12 [d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_3 ($C_{3,5}$-Ar_5)], 115.91 [d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_5 ($C_{3,5}$-Ar_3)], 99.79 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{13}ClF_2N_2O$: C, 64.79; H, 3.53; N, 7.56. Found: C, 64.41; H, 3.82; N, 7.68.

*4-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1,2,4-oxadiazole (**6d-3x**)* Yield: 71 %. Mp: 80–83 °C. ^{1}HNMR (CDCl3) δ: 3.88 (s, 3H, OCH_3), 6.45 (s, 1H, H_5-oxadiazole), 6.71 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-NAr), 6.97 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-NAr), 7.05 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-Ar_3), 7.17 (d, $J = 9.00$ Hz, 2H, $H_{2,6}$-Ar_5), 7.49 (d, $J = 9.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.55 (t, $J = 7.5$ Hz, 2H, $H_{2,6}$-Ar_3). ^{13}CNMR ($CDCl_3$) δ: 163.01 (d, $J_{CF} = 251.0$ Hz, H_4-Ar_3), 161.2 (C_4-Ar_5), 154.04 (C_1-NAr), 139.46 (C_1-Ar_3), 131.31, 130.04 [C_1-Ar_5 (C_4-NAr)], 130.11 (d, $J_{CF} = 8.70$ Hz, $C_{2,6}$-Ar_3), 129.62, 128.75 [$C_{2,6}$-Ar_5 ($C_{3,5}$-N–Ar)], 125.51 ($C_{2,6}$-NAr), 121.37 (C_3-oxadiazole), 115.55 (d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-Ar_3), 114.05 ($C_{3,5}$-Ar_5), 100.37 (C_5-oxadiazole), 54.90 (OCH_3). Anal. Calcd. For $C_{21}H_{16}ClFN_2O_2$: C, 65.89; H, 4.21; N, 7.32. Found: C, 65.73; H, 3.98; N, 7.40.

*4,5-Bis(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6e-3x**)* Yield: 64 %. Mp: 80–82 °C. ^{1}HNMR (CDCl3) δ: 6.46 (s, 1H, H_5-oxadiazole), 6.73 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-NAr), 7.06 (t, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.18 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-NAr), 7.43 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.52 (d, $J = 8.0$ Hz, 2H, $H_{2,6}$-Ar_3), 7.55–7.59 (dd, $J = 7.00$ Hz and $J = 3.5$ Hz, 2H, $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 164.03 (d, $J_{CF} = 250.0$ Hz, C_4-Ar_3), 154.18 (C_1-NAr), 139.33 (C_1-Ar_3), 136.90,135.99 [C_4-NAr (C_4-Ar_5)], 131.79 (C_1-Ar_5), 130.08 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_3), 129.61, 129.21, 128.56 [$C_{2,6}$-Ar_5 ($C_{3,5}$-Ar_5, $C_{3,5}$-NAr)], 125.70 ($C_{2,6}$-NAr), 120.92 (C_3,-oxadiazole), 116.15 (d, $J_{CF} = 21.75$ Hz, $C_{3,5}$-Ar_3), 99.71 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{13}Cl_2FN_2O$: C, 62.03; H, 3.38; N, 7.23. Found: C, 62.41; H, 3.50; N, 7.47.

*3-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-phenyl-4,5-dihydro-1,2,4-oxadiazole (**6a-2y**)* Yield: 78 %. Mp: 143–145 °C. ^{1}HNMR (CDCl3) δ: 6.45 (s, 1H, H_5-oxadiazole), 6.78–6.82 (m, 2H, $H_{2,6}$-NAr), 6.90 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-NAr), 7.32 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.45–7.48 (m, 3H, $H_{3,4,5}$-Ar_5), 7.52 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-Ar_3), 7.55–7.60 (m, 2H, $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 160.70 (d, $J_{CF} = 251.0$ Hz, C_4-NAr), 154.60 (C_1-NAr), 138.30 (C_1-Ar_5), 136.89, 136.69 [C_1-Ar_3 (C_4-Ar_3)], 130.00 (C_4-Ar_5), 129.24 ($C_{2,6}$-Ar_5), 129.05 ($C_{3,5}$-Ar_5), 128.89 ($C_{3,5}$-Ar3), 127.29 ($C_{2,6}$-Ar_3), 126.80 (d, $J_{CF} = 7.55$ Hz, $C_{2,6}$-NAr),, 123.61 (C_3-oxadiazole), 116.36 (d, $J_{CF} = 22.5$Hz, $C_{3,5}$-NAr), 100.99 (C_5-oxadiazole). Anal. Calcd. For $C_{20}H_{14}ClFN_2O$: C, 68.09; H, 4.00; N, 7.94. Found: C, 68.31; H, 3.91; N, 7.67.

*3-(4-Chlorophenyl)-4-(4-fluorophenyl)-5-(p-tolyl)-4,5-dihydro-1,2,4-oxadiazole (**6b-2y**)* Yield: 65 %.^{1}HNMR ($CDCl_3$) δ: 2.46 (s, 3H, CH_3), 6.42 (s, 1H, H_5-oxadiazole), 6.79 (m, 2H, $H_{2,6}$-NAr), 6.89 (t, $J = 7.50$ Hz, 2H, $H_{3,5}$-NAr), 7.27 (d, $J = 8.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.32 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.45 (d, $J = 8.00$ Hz, 2H, $H_{2,6}$-Ar_5), 7.51 (d, $J = 8.50$ Hz, 2H, $H_{2,6}$-Ar_3).^{13}CNMR ($CDCl_3$) δ; 160.67 (d, $J_{CF} = 246.0$ Hz, C_4-NAr), 154.57 (C_1-NAr), 140.06 (C_1-Ar_3), 136.88, 136.62 [C_4-Ar_5 (C_4-Ar3)], 135.34 (C_1-Ar_5), 129.57, 129.23, 129.03 [$C_{2,6}$-Ar_5 ($C_{3,5}$-Ar_3, $C_{3,5}$-Ar_5)], 127.30 ($C_{2,6}$-Ar_3), 126.79 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-NAr), 123.72 (C_3-oxadiazole), 116.30 (d, $J_{CF} = 22.50$ Hz, $C_{3,5}$-NAr), 100.99 (C_5, oxadiazole), 21.37 (CH_3). Anal. Calcd. For $C_{21}H_{16}ClFN_2O$: C, 68.76; H, 4.40; N, 7.64. Found: C, 68.46; H, 4.19; N, 7.39.

*3-(4-Chlorophenyl)-4,5-bis(4-fluorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6c-2y**)* Yield: 70 %. Mp: 113–115 °C. ^{1}HNMR ($CDCl_3$) δ: 6.42 (s, 1H, H_5-oxadiazole), 6.78–6.80 (m, 2H, $H_{2,6}$-NAr), 6.92 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-NAr), 7.15 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-Ar_5), 7.32 (d,

$J = 9.00$ Hz, 2H, $H_{3,5}$-Ar_3), 7.49 (d, $J = 9.00$ Hz, 2H, $H_{2,6}$-Ar_3), 7.53–7.55 (m, 2H, $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 163.72 [d, $J_{CF} = 248.5$ Hz, C_4-NAr (C_4-Ar_5)], 160.84 [d, $J_{CF} = 245.0$ Hz, C_4-Ar_5 (C_4-NAr)], 154.69 (C_1-NAr), 136.79, 136.55 [C_4-Ar_3 (C_1-Ar_3)],134.22 (C_1-Ar_5), 129.27 [d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-Ar_5 ($C_{2,6}$-NAr)], 129.21, 129.08 [$C_{2,6}$-Ar_3 ($C_{3,5}$-Ar_3)], 126.92 [d, $J_{CF} = 7.5$ Hz, $C_{2,6}$-NAr ($C_{2,6}$-Ar_5)], 123.44 (C_3-oxadiazole), 116.48 [d, $J_{CF} = 22.50$ Hz, $C_{3,5}$-Ar_5 ($C_{3,5}$-NAr)], 115.94 [d, $J_{CF} = 21.25$ Hz, $C_{3,5}$-NAr ($C_{3,5}$-Ar_5)], 100.39 (C5-oxadiazole). Anal. Calcd. For $C_{20}H_{13}ClF_2N_2O$: C, 64.79; H, 3.53; N, 7.56. Found: C, 64.78; H, 3.72; N, 7.66.

*4-(4-Fluorophenyl)-3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1,2,4-oxadiazole (**6d-2y**)* Yield: 76 %. Mp: 98–102 °C. ^{1}HNMR ($CDCl_3$) δ: 3.88 (s, 3H, OCH_3), 6.40 (s, 1H, H_5-oxadiazole), 6.76–6.79 (m, 2H, $H_{2,6}$-NAr), 6.89 (t, $J = 9.00$ Hz, 2H, $H_{3,5}$-NAr), 6.97 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_5), 7.32 (d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3), 7.49 (d, $J = 8.00$ Hz, 4H, $H_{2,6}$-Ar_3 and $H_{3,5}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 160.95 (C_4-Ar_5), 160.69 (d, $J_{CF} = 245.0$ Hz, C_4-NAr), 154.64 (C_1-NAr), 136.77, 136.40 [C_4-Ar3 (C_1-Ar3)], 130.25 (C_1-Ar_5), 129.21, 129.02 [$C_{2,6}$-Ar_3 ($C_{3,5}$-Ar_3)], 128.87 ($C_{2,6}$-Ar_5), 126.92 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-NAr), 123.75 (C_3-oxadiazole), 116.32 (d, $J_{CF} = 22.50$ Hz, $C_{3,5}$-NAr), 114.24 ($C_{3,5}$-Ar_5), 100.94 (C_5-oxadiazole), 55.36 (OCH_3). Anal. Calcd. For $C_{21}H_{16}ClFN_2O_2$: C, 65.89; H, 4.21; N, 7.32. Found: C, 65.53; H, 4.11; N, 7.46.

*4-(4-Fluorophenyl)-3,5-bis(4-chlorophenyl)-4,5-dihydro-1,2,4-oxadiazole (**6e-2y**)* Yield: 70 %. Mp: 107–109 °C. ^{1}HNMR ($CDCl_3$) δ: 6.50 (s, 1H, H_5-oxadiazole), 6.79–6.81 (m, 2H, $H_{2,6}$-NAr), 6.92 (t, $J = 8.50$ Hz, 2H, $H_{3,5}$-NAr), 7.32 [d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_3 ($H_{3,5}$-Ar_5)], 7.43 [d, $J = 8.50$ Hz, 2H, $H_{3,5}$-Ar_5 ($H_{3,5}$-Ar_3)], 7.33–7.52 (m, 4H, $H_{2,6}$-Ar_3 and $H_{2,6}$-Ar_5). ^{13}CNMR ($CDCl_3$) δ: 160.87 (d, $J_{CF} = 245.0$ Hz, C_4-NAr), 154.70 (C_1-NAr), 136.77, 136.60 [C_4-Ar_3 (C_1-Ar_3)], 129.21, 129.02 [$C_{2,6}$-Ar_3 ($C_{3,5}$-Ar_3)], 128.69 ($C_{2,6}$-Ar_5), 126.95 (d, $J_{CF} = 8.75$ Hz, $C_{2,6}$-NAr), 123.75 (C_3-oxadiazole), 116.32 (d, $J_{CF} = 22.75$ Hz, $C_{3,5}$-NAr), 114.24 ($C_{3,5}$-Ar_5),100.94 (C5-oxadiazole), 55.36 (OCH_3). Anal. Calcd. For $C_{20}H_{13}Cl_2FN_2O$: C, 62.03; H, 3.38; N, 7.23. Found: C, 62.19; H, 3.50; N, 7.46.

Docking study

The molecular geometry of compounds **6e-3y**, **6a-1y**, **6c-1y**, and **6e-1y** which have most prominent biological results was fully minimized by MMFF94 force field using ChemBio3D Ultra 12.0 (Cambridgesoft), setting the terminal condition as the RMS of potential energy smaller than 0.0001 kcal $Å^{-1}$ mol^{-1}. The crystal structure of cyclo-oxygenase-2 in complex with celecoxib (entry code 3LN1) was retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The FlexX program interfaced with LeadIT 2.1.2 (BioSolveIT GmbH, Sankt Augustin, Germany) was used to dock all compounds. FlexX is an automated docking program, which considers ligand conformational flexibility by an incremental fragment placing method (Rarey *et al*., 1996). The active site for docking was determined as all atoms within 7-Å radius of the cocrystallized ligand. Validation of the docking procedure was accomplished by energy minimization of celecoxib structure by the above-mentioned method and the optimized geometry structure was redocked in the active site of COX-2 which resulted in predicted docking pose with RMSD 0.723 within the best scored poses.

For evaluation of the ligands affinity toward docked receptor, the Hyde assessment facility of LeadIT software was implemented to report the free energy of binding (ΔG) and ligand efficiency of some of the best dock scored poses. HYDE is an empirical scoring function, which assesses protein–ligand complex by considering hydrogen bond interactions and also hydrophobic and desolvation effects and provides estimation for the binding affinity (Reulecke *et al*., 2008; Schneider *et al*., 2011).

Biological study

Chemicals and reagent

Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Gibco-BRL (Rockville, IN, USA). Fetal bovine serum (FBS) was supplied by Gibco-BRL. All other chemicals were from Merck (Darmstadt, Germany) and Sigma-Aldrich (St Louis, MO, USA).

Cell culture and in vitro cytotoxicity assay

Human breast adenocarcinoma (MCF-7) and human erythromyeloblastoid leukemia (K562) cell lines were obtained from the cell bank of Pasture Institute of Iran (NCBI), and routinely cultured in DMEM medium supplemented with 10 % FBS, 100-U/mL penicillin and 100-μg/mL streptomycin under the conditions of 5 % CO_2 at 37 °C. Cell cytotoxicity was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MCF-7 (7×10^3) and K562 (2×10^4) cells/well were cultured in 96-well plates. Then, cells were treated with various concentrations of each compound (0.1–200 μM) and incubated for 24 h. Afterward, 20 μL of MTT (5 mg/mL in PBS) was added to each well and the cells were incubated for another 4 h at 37 °C. The supernatants were then aspirated carefully and 200 μL of dimethyl sulfoxide (DMSO) was added to each well. The plates were shaken

for an additional 15 min and the absorbance values were read by the Microplate Reader (Star Fax-2100, ST. Louis, USA) at 545 nm. Solvent control (DMSO) was included to check that the DMSO had no effect at the concentration used. The cytotoxicity of the complex was measured from the spectrophotometric data by means of this equation: % cell cytotoxicity $= [1 - \mathrm{Abs_{drug}}/\mathrm{Abs_{control}}] \times 100$.

The IC_{50} value was measured by plotting the percentage cytotoxicity versus concentration on a logarithmic graph. To compare the cytotoxic effect of compounds reported here with that of the reference drugs, we also examined the cytotoxicity of doxorubicin and paclitaxel on MCF-7 and K562 cells under similar conditions.

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