Design, synthesis, chemical characterization, biological evaluation, and docking study of new 1, 3, 4-oxadiazole homonucleoside analogues

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Herein, we report the synthetic strategies and characterization of some novel 1,3,4-oxadiazole homonucleoside analogues that are relevant to potential antitumor and cytotoxic activities. The structure of all compounds is confirmed using various spectroscopic methods such as ^1H -NMR, ^{13}C -NMR, HRMS, and FTIR. These compounds were evaluated against three human cancer cell lines (MCF-7, SKBR3, and HL60 Cell Line). Preliminary investigations showed that the cytotoxic activity was markedly dependent on the nucleobase. Introduction of 5-Iodouracil **4g** and Theobromine **6b** proved to be extremely beneficial even they were more potent than the reference drug (DOX). Also, the synthesized compounds were tested for their antiviral activities against the human varicella-zoster virus (VZV). The product **4h** was (6-azauracil derivative) more potent to the reference (acyclovir) against the deficient TK- VZV strain by about 2-fold. Finally, molecular docking suggested that the anticancer activities of compounds **6b** and **4g** mediated by inhibiting dual proteins EGFR/HER2 with low micromolar inhibition constant K_i range. The 1,3,4-oxadiazole homonucleosides showed a strong affinity to binding sites of target proteins by forming H-bond, carbon-hydrogen bond, π -anion, π -sulfur, π -sigma, alkyl, and π -alkyl interactions.

Keywords: 1, 3, 4-oxadiazole; homonucleosides; Anticancer activity; Antiviral activity; Molecular Docking

Introduction

The treatment of cancer and viral diseases persists as one of the major challenges to modern medicine. Moreover, cancer is the most common disease in the modern era and causes the most deaths (one in eight deaths) worldwide [1]. Indeed, the receptor tyrosine kinases (RTKs) are considered attractive targets for driven therapy in cancer [2, 3]. Among these receptors, epidermal growth factor receptor (EGFR) and tyrosine kinase domain of human epidermal growth factor receptors (HER-2) deserve special attention. The activation of EGFR and HER2 is an essential step for the initiation and progression of breast cancer [4]. Moreover, EGFR/HER2 heterodimers are usually formed, and they are considered to be substantial targets for cancer therapy [5-7]. As a result, the dual

inhibition of EGFR and HER2 proteins prevent heterodimerization and generally block drug resistance [5]. Indeed, several dual EGFR/HER2 inhibitors containing pyrimidine moiety were reported in the literature. The lapatinib **P1** (Figure 1) was the first dual EGFR/HER2 inhibitor to be approved by the FDA in 2007 for breast cancer [8]. The pyrimidine derivative **P2** was developed as an efficient dual EGFR/HER2 inhibitors [9]. Also, several fused pyrimidines with other heterocycles such as pyrrole, thiene, and azepine (Figure 1) were found to be high inhibitors of EGFR/HER2 [10-12].

Pyrimidine and purine nucleoside analogues are widely used in the chemotherapy of cancer and viral diseases for almost 50 years [13-16]. Among the possible modifications of nucleosides, the introduction of a methylene bridge between a nucleobase and the sugar moiety led to the formation of homonucleosides [17]. For instance, the homonucleoside analogues **P3-5** were reported in the literature with antiviral activities against herpes simplex virus [18], vaccinia virus [19], and influenza type virus A H1N1 [20]. On the other hand, since the discovery of Lamivudine **P6** and Emtricitabine **P7** as anti-HIV drugs (Figure 1), significant interest has been committed towards the chemistry of heterocyclic nucleosides [21]. The later were obtained through the substitution of the furanose moiety by another heterocycle [22]. Also, various heterocyclic nucleoside analogues with dioxolane [23], dithiolane [24], and pyrazoline [25] were reported.

Recently, the combination of a nucleobase and heterocycle in one single molecule via the introduction of the methylene bridge (heterocycle homonucleosides) was described. Piotrowska et al., were synthesized and evaluated a novel γ -Lactam homonucleoside analogues for their antiviral and anticancer activities, compound **P8** (figure 1) showed mild IC₅₀ range toward pancreatic adenocarcinoma cells (Capan-1) [26]. Also, a new series of isoxazolidine homonucleosides **P9** (figure 1) was prepared with no significant antiviral activities [27]. Moreover, various 1,2,3-triazole homonucleoside analogues **P10** (figure 1) were synthesized and evaluated for their biological activities [28-31].

The oxadiazole chemistry has been developed extensively and is still developing [32]. The $-N=C-O-$ linkage can react with the nucleophilic centres of malignant cells and is responsible for its biological activity [33]. Their electronic and charge-transporting properties ensure the connection with targets through non-covalent interactions. In other cases, oxadiazole moieties have been shown to act as a flat, aromatic linker to place substituents in the appropriate orientation [34], as well as modulating molecular

properties by positioning them in the periphery of the molecule [35]. Furthermore, oxadiazoles have been described as bioisosteres for carboxylic acids, esters, and carboxamides [36]. These characteristics of the 1,3,4-oxadiazole ring resulted in the diverse pharmaceutical applications as antiviral (Raltegravir **P11**) [37, 38] and anticancer (Zibotentan **P12**) [39, 40] drugs (Fig. 1).

Taking into consideration the biological importance of homonucleosides, heterocyclic nucleosides, and 1,3,4-oxadiazole derivatives, we report the design, synthesis, and biological activity of the novel 1,3,4-oxadiazole homonucleoside analogues as anticancer and antiviral agents. Moreover, based on the similarity between EGFR/HER2 inhibitors and our products (pyrimidine or fused pyrimidine), the molecular docking study on these two proteins was performed to explore the anticancer activity at the molecular level and to investigate the mode of action of the most potent compounds.

FIGURE 1: Design of 1,3,4-oxadiazole homonucleoside analogues

Results and Discussions

Chemistry

Several methods for the synthesis of both symmetrical and asymmetrical 1,3,4-oxadiazoles have been reported. One of the most popular way involves cyclodehydration of 1,2-diacylhydrazines using phosphorous oxychloride (POCl_3) [38]. Next, the second method is the cyclization of 1,2-diacylhydrazines with thionyl chloride (SOCl_2) [41]. Moreover, different activating agents for cyclisation of diacylhydrazines such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 1,1'-carbonyldiimidazole (CDI), silica-supported dichlorophosphate [42-44]. Yet, there are still disadvantages associated with these methodologies, such as harsh reaction conditions, the use of expensive reagents, and limited scalability. To synthesize the desired products, we adopted the following strategy. First, we prepared 1,3,4-oxadiazoles by cyclodehydration of diacylhydrazines. Then, these chemicals unite were used as an alkylating agent for pyrimidines and purines. The hydrazides **2a,b** were synthesized via the condensation of ester **1a,b** and hydrazine in ethanol at reflux temperature. Then, dehydrating reagent POCl_3 was used in the reaction of acylhydrazides **2a,b** with chloroacetic acids to give 1,3,4-oxadiazole derivatives **3a,b** [45]. The conditions described in this literature were modified: we showed no need to use POCl_3 as a solvent. Therefore, the amount of POCl_3 were reduced to 3 equivalents and dichloroethane (DCE) was used as a solvent (Scheme 1).

SCHEME 1: Preparation of alkylating agents **3a-b**

The preparation of homonucleoside analogues **4a-j** was carried out through Hilbert-Johnson modified reaction [46]. First, the silylated pyrimidines were synthesized using the ammonium sulphate and hexamethyldisilazane (HMDS). Then, the alkylating agents **3a,b**, potassium iodide, and acetonitrile were added to the mixture (Table 1). The novel compounds 1,3,4-oxadiazole-pyrimidines were prepared with good yields except the 5-

halouracils **4d-g**. This result can be explained by the electron-withdrawing effect of halogen in position C5 (halogen decreases the nucleophilicity of N1). In the aim to increase the yield of the N1-alkylation of 5-halouracils, we changed the synthesis strategy by using K_2CO_3 in DMF (Table 2). Thus, the desired products **4d-g** was prepared with improved yields (48% to 51%).

The conditions applied to synthesize pyrimidine hybrids did not work to prepare purines **6a-c**. Thereafter, the synthetic strategy of N-alkylated purines was changed to basic medium (K_2CO_3 /DMF). For the N-alkylation of theophylline **5a** and theobromine **5b**, the desired products (**6a**, **6b**) were obtained with excellent yields. However, when adenine **5c** was used the yield decreased (Table 3).

TABLE 1: Preparation of pyrimidine homonucleoside analogues **4a-j** using Hilbert-Johnson modified reaction

TABLE 2: Preparation of C5-halopyrimidine homonucleosides **4d-g** using basic medium

TABLE 3: Preparation of purines homonucleoside analogues **6a-c**

The NMR signals of the compounds were assigned based on spectral data. ^1H -NMR spectra of compounds **4a-i** showed an average spectrum in which appeared three signals at the interval δ 11.5-12.3 ppm (singlet, NH proton D_2O exchangeable, pyrimidine), δ 7-9 ppm (doublet for uracil, singlet for other bases (H_6 -pyrimidine and H_5 -azauracil), or the absence of this signal for the compounds **4h**) and δ 5.2-5.4 ppm for (CH_2 , methylene bridge). In addition, aromatic protons appeared at δ 7-8 ppm. Also, compounds **4a**, **4b**, and **4h** showed a doublet or singlet signal at δ 5.7-8.1 ppm (H_5 - pyrimidine). While, compounds **6a-c** exhibited a characteristic singlet at δ 8.1-8.3 ppm (H_8), and another characteristic: singlet for compound **6c** at δ 8.26 ppm (HN_2 for adenine). ^{13}C -NMR spectra of compounds **4a-i** and **6a-c** showed common signals: one pic at δ 38-42 ppm (CH_2 , methylene bridge), four pics at δ 123-133 ppm (aromatic carbons) and two pics at δ 161-165 ppm (O-C=N , and 2 carbons of heterocycle 1,3,4-oxadiazole). While, compounds **4a-j** with pyrimidine bases showed three signals at δ 70-143 ppm (C_5), δ 149-154 ppm (C_2) and δ 145-65 ppm (C_4). Moreover, compounds **6a-c** (purine) showed four common signals which appeared at 106-119 ppm(C_5), 141-144 ppm (C_8), 144-150 ppm (C_6), and 155-157 ppm (C_4). The new synthesized compounds were also characterized by HRMS and FTIR.

The FTIR spectra showed a band between 1724 and 1693 cm^{-1} , which correspond to the deformation of the carbonyl group ($\text{C}=\text{O}$, nucleobase). Also, two bands 1605-1614 cm^{-1} and 1227-1251 cm^{-1} , corresponding respectively to the imine and C-O-C group of 1,3,4-oxadiazole.

Biological assays

Cytotoxic activity

As described in Fig. 2, compounds **4e**, **4g**, **6b** have the most prominent effect by compromising the cell growth of MCF7 and SKBR3 cell lines. However, **6b** was the most potent molecule in terms of growth inhibition (Fig. 2). On the other hand, no growth inhibition has been observed after the treatment of leukemia cancer cells HL60 by these compounds except **4j** in comparison to the reference drug. Generally, breast cancer cell lines were the most sensitive to the synthesized compounds. About broad-spectrum of anticancer activity, a close examination of the data presented in figure 2 reveals interesting structure-activity relationship and substituent effect on the cytotoxic potential. The compound **6b** with Theobromine as nucleobase showed better cytotoxic activity than all other hybrids against breast cancer cell lines, followed by hybrid 5-iodouracil as nucleobase **4g**. Careful analysis of the in vitro cytotoxic evaluation also indicates that the linkage of 5-substituted uracil is more active than the 6-azauracil derivatives. Furthermore, cytotoxicity was enhanced by changing the halogen group in 5 position of the uracil by decreasing the electronegativity as evident in changing fluorine to iodine. Moreover, changing xanthine (**6a-b**) to adenine (**6c**) remarkably decreases cytotoxicity.

FIGURE 2: cytotoxicity effect of synthesized compounds in 10 μM concentration on human MCF-7, SKBR3, and HL60 Cells

Antiviral activity

All the synthesized compounds were tested for their antiviral activities against the human varicella-zoster virus (VZV), both wild type and thymidine kinase-deficient (TK-) (table 4). The antiviral activities of synthesized pyrimidine-1,3,4-oxadiazole hybrid molecules were lower than the antiviral activities of reference compound acyclovir and brivudin against the TK+ VZV strain, except **4h** (6-aza-uracil derivative), showed better activity in comparison to acyclovir against the TK- VZV strain by 2-fold. As the TK- VZV strain is known for resistance to conventional antiviral drugs, pyrimidine-1,3,4-oxadiazole **4h** can serve as a promising lead candidate for further study. All synthesized compounds showed quantifiable cytotoxicity.

TABLE 4: Activity of compounds **4a-j** and **6a-c** against varicella-zoster virus (VZV) in human embryonic lung (HEL)

Compounds	Antiviral activity EC50 (uM) ^a		Cytotoxicity (uM)
	TK+ VZV strain	TK- VZV strain	Cell morphology ^b
4a	>100	>100	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	>100	>100
4g	>100	>100	>100
4h	39,39	49,67	>100
4i	>100	>100	>100
4j	>100	>100	>100
6a	>100	>100	>100
6b	>100	>100	>100
6c	>100	>100	>100
Acyclovir	2,75	103,23	>350
Brivudin	0,011	35,14	>300

^aEffective concentration required to reduce virus plaque formation by 50%. Virus input was 20 plaque forming units (PFU).

^bMinimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

Molecular docking

The molecular docking study was performed to understand the mode of action and to explore the anticancer activity at the molecular level. Indeed, taking into account the importance of these dual inhibitors and their similarity to our products (pyrimidine or fused pyrimidine), we have chosen to dock the most active compounds **4g** and **6b** into the active site of EGFR (PDB: 1m17) and HER2 (PDB: 3PP0).

Before, to determine the validity of the docking protocol, first, the self-docking experiments were carried out. The root-mean-square deviations (RMSD) between the predicted and the native poses found to be 1.74 Å (1M17; ERLOTINIB) and 0.54 Å (3PP0; 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-yl}c1amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl]ethoxy}ethanol). These results indicated that the adopted docking protocol is good for the reproduction of the native poses (<2 Å).

The docking results are summarized in Table 5 and figures 3-6 (see tables1-2 in supplementary data). Concerning EGFR, ligands **4g** and **6b** are bound deeply inside the active site with the estimated free energy of binding -8.05 and -7.5 kcal/mol, respectively. Also, the compounds **4g** and **6b** inhibit EGFR protein with estimated inhibition constants (Ki): 1.25 and 3.18 µM, respectively. The product **4g** interacted with amino acid residues of EGFR by one hydrogen bond LYS721---O=C₄ (2.071 Å). Also, compound **6b** showed one H-bond between MET769 and N₉ (1.995 Å). On the other hand, HER2 interacts with ligands **4g** and **6b** with the estimated free energy of binding -8.66 and -8.81 kcal/mol, respectively. Then, these compounds inhibit HER2 protein with estimated inhibition constants of 0.45 and 0.35 µM, respectively. Besides, product **4g** exhibited two hydrogen bonds THR862---O=C₄ (2,424 Å) and ASP863---N_{oxadiazole} (2,634 Å). While, ligand **6b** interacted with HER2 by showing three H-binding interactions MET801---N=C₉ (2,104 Å), THR862---O=C₂ (1,962 Å), and ASP863---O_{oxadiazole} (2,181 Å). Furthermore, the products **4g** and **6b** interact with EGFR and HER by other interactions such as carbon-hydrogen bond, Pi-anion, Pi-sulfur, Pi-sigma, alkyl, and Pi-alkyl.

TABLE 5: Results of molecular docking

Protein	Ligand	Estimated binding energy (kcal/mol)	Estimated inhibition constant Ki (µM)
EGFR	4g	-8.05	1.25
	6b	-7.5	3.18
HER2	4g	-8.66	0.45
	6b	-8.81	0.35

FIGURE 3: 2D interactions of compound **4g** in the binding sites of EGFR protein (PDB ID: 1m17).

FIGURE 4: 2D interactions of compound **6b** in the binding sites of EGFR protein (PDB ID: 1m17).

FIGURE 5: 2D interactions of compound **4g** in the binding sites of HER2 protein (PDB ID: 3pp0).

FIGURE 6: 2D interactions of compound **6b** in the binding sites of HER2 protein (PDB ID: 3pp0).

Conclusion

A series of 1,3,4-oxadiazole-pyrimidines and purines hybrids (**4a–j** and **6a–c**) have been designed and synthesized by employing silyl Hilbert–Johnson modified reaction pyrimidines and basic medium for purines. The in vitro anti-cancer activity of all the synthesized compounds against breast (SKBR3 and MCF7) and leukemia cancer (HL60) cells was evaluated. Compounds **4g** and **6b** have shown promising results in comparison with the marketed drug doxorubicin. Also, molecular docking suggested that the anticancer activities of compounds **6b** and **4g** mediated by inhibiting dual proteins

EGFR/HER2 at low micromolar inhibition constant K_i range. Therefore, these compounds can serve as a promising lead candidate for further study.

Experimental

Chemistry

General

Melting points were measured using a Büchi B-545 digital capillary melting point apparatus and used without correction. Reactions were checked with TLC using aluminium sheets with silica gel 60 F254 from Merck. Spectra IR were recorded on a Perkin-Elmer VERTEX 70 FTIR spectrometer covering field 400-4000 cm^{-1} . The spectra of ^1H NMR and ^{13}C NMR were recorded in solution in DMSO- d_6 on a Bruker Advance 300 spectrometer at 300 and 75 MHz, respectively. The chemical shifts are expressed in parts per million (ppm) by using DMSO- d_6 as internal reference. The multiplicities of the signals are indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet, and coupling constants are expressed in Hertz. Mass spectra were collected using an API 3200 LC/MS/MS system, equipped with an ESI source. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Aldrich.

General procedure for the preparation of Alkylating Agent 3a-b [47]

Brief, In a round bottom flask (25 mL) equipped with a condenser was added a mixture of ester **1a-b** (10mmole), hydrazine hydrate (11mmol, 0.5ml) and ethanol (5ml). The mixture was refluxed for 3h. the solvent was evaporated under vacuum and the hydrazide was recrystallized using ethanol to afford desired product with 70% for **2a** and 87% for **2b**. Then, Chloroacetic acid (11mmol, 1,04g), Phosphorus oxychloride (30mmol, 2.8ml) and dichloroethane (20ml) were mixed in 100ml round bottom flask and heated at 80°C for 2h. After, Carboxylic acid hydrazide (**2a** or **2b**) was added to the mixture and heated for 12h. After completion of reaction, water (40ml) was added and the pH was adjusted to 7-8 with sodium hydrogen carbonate and extracted with dichloromethane. The organic layer was dried (Na_2SO_4), evaporated and purified by column chromatography (Silica gel, 5% EtOAc in hexane) to give **3a** in 68% and **3b** in 74%.

General procedure for the preparation of compounds 4a-j

Method A

A mixture of the appropriate pyrimidine (1mmol) and ammonium sulfate (0.10mmol, 13mg) in HMDS (1.5 ml) was refluxed until clear solution was obtained. Then, the appropriate alkylating agent **3a-b** (1.5mmol), KI (0.75mmol, 124mg) and acetonitrile (2.5ml) were added. Reaction mixture was heated at 85°C for 12h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with dichloromethane and evaporated to dryness. The residue was purified by flash chromatography eluting with (CH₂Cl₂/MeOH = 99:1).

Method B

A mixture of 5-halopyrimidine (1mmol), alkylating agent **3a** (1.1mmol, 215mg) and potassium carbonate (0.5mmole, 69mg) in DMF (10 ml) was heated at 90°C for 2h. Then the DMF was evaporated under reduced pressure to dryness. The residue was purified by flash chromatography eluting with (CH₂Cl₂/ MeOH = 99:1) to obtain pure product **4d-g**.

1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4a**). white solid, Yield: 84%. R_f = 0.5 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 247–249. NMR ¹H : 5.25 ppm (s, 2H, CH₂-N); 5.7 ppm (1H, d, J=7.9Hz, C₅H); 7.63ppm (3H, m, H_{ph}); 7.81ppm (d, J=7.9Hz, 1H, C₆H); 7.99ppm (m, 2H, H_{ph}); 11.5ppm (s, 1H, N-H). NMR ¹³C: 42 ppm (CH₂); 106 ppm (C₅); 123ppm (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 132 ppm (C_{Ph}); 146 ppm (C₆); 151 (C₂); 163 (C₄); 164 (O-C=N); 165 (O-C=N). IR (KBr, cm⁻¹) 1708 (C=O), 1606 (C=N), 1503 (C=C), 1122(C-O-C). HRMS Calculated [M+H]⁺ 271.0831, Found [M+H]⁺ 271.0845.

1-((5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4b**) white solid, Yield: 85%. R_f = 0.51 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 233-235. NMR ¹H: 3.8ppm (3H, s, CH₃-O); 5.2ppm (2H, s, CH₂-N); 5.7ppm (1H, d, J=7.9Hz, C₅-H); 7.17ppm (2H, dd, H_{ph}); 7.8ppm (1H, d, J=7.9Hz, C₆-H); 7.92ppm (2H, dd, H_{ph}); 11.5ppm (1H, s, N-H). NMR ¹³C: 42 ppm (CH₂); 55 ppm (CH₃-O); 102 ppm (C₅); 115 (C_{ph}); 126 (C_{Ph}); 129 (C_{Ph}); 132 ppm (C_{Ph}); 146 ppm (C₆); 151 ppm (C₂); 162 (C₄); 164 (O-C=N); 165 (O-C=N). IR (KBr, cm⁻¹): 3106 (Csp³), 1714 (C=O), 1608 (C=N), 1502 (C=C), 1171(C-O-C). HRMS Calculated [M+H]⁺: 301.0982 Found [M+H]⁺ 301.0992.

5-methyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4c**)
white solid, Yield: 80%. Rf= 0.56 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 241-243. NMR ¹H: 1.8ppm (s, 3H, CH₃); 5.2ppm (2H, s, CH₂); 7.65ppm (3H, m, H_{ph}); 7.69ppm (1H, d, J=8.2Hz, C₆-H); 7.99ppm (2H, m, H_{ph}); 11.5ppm (1H, s, N-H). NMR ¹³C: 12 ppm (CH₃); 42ppm (CH₂); 110 ppm (C₅); 123ppm (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 132 ppm (C_{Ph}); 141 ppm (C₆); 151 ppm (C₂); 163 (C₄); 164 (O-C=N); 165 (O-C=N). IR (KBr, cm⁻¹) 1699 (C=O), 1652 (C=O), 1609 (C=N), 1555 (C=C), 1154 (C-O-C). HRMS Calculated [M+H]⁺: 301.0931 Found [M+H]⁺ 301.0951

5-fluoro-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4d**)
white solid, Yield: 40/51% (method A/B). Rf=0.36 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 216-218. NMR ¹H: 5.22ppm (2H, s, CH₂); 7.62ppm (3H, m, H_{ph}); 7.98ppm (2H, m, H_{ph}); 8.24ppm (1H, d, J=8.24Hz, C₆-H). 12.14 (1H, s, NH). NMR ¹³C: 42.24 ppm (CH₂); 123 .06 (C_{Ph}); 126.56 (C_{Ph}); 129.41 (C_{Ph}); 129.66 (d, 2.4Hz, C₆); 132 ppm (C_{Ph}); 139.82 ppm (d, J=229Hz, C₆); 149.44 ppm (C₂); 157.32 (d, J=26Hz, C₄); 162.17 (O-C=N); 164.51 (O-C=N). IR (KBr, cm⁻¹) 1693 (C=O), 1606 (C=N), 1554 (C=C), 1173 C-O-C. HRMS Calculated [M+H]⁺: 289.0731 Found [M+H]⁺: 289.0751

5-chloro-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4e**)
white solid, Yield: 38/49% (method A/B). Rf=0.39 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 233-235. NMR ¹H: 5.33 ppm (2H, s, CH₂); 7.6 ppm (3H, m, H_{Ph}); 8.28ppm (2H, m, H_{Ph}); 8.8ppm (1H, s, C₆-H). NMR ¹³C: 42 (CH₂); 106 ppm (C₅); 123 (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 132 ppm (C_{Ph}); 142 ppm (C₆); 149 ppm (C₂); 159 (C₄); 162 (O-C=N); 164 (O-C=N). IR (KBr, cm⁻¹) 1724 (C=O), 1666 (C=O), 1631 (C=N), 1551 (C=C), 1244 (C-O-C). HRMS Calculated [M+H]⁺: 305.0436 Found [M+H]⁺: 305.0451.

5-bromo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl) pyrimidine-2,4(1H,3H)-dione (**4f**)
white solid, Yield: 39/49% (method A/B). Rf=0.4 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 258-260. NMR ¹H: 5.35ppm (2H, s, CH₂); 7.6ppm (3H, m, H_{ph}); 8.1ppm (2H, m, H_{ph}); 8.5ppm (1H, s, C₆-H); 11.8 (1, s, NH). NMR ¹³C: 42.36 ppm (CH₂); 95.5 ppm (C₅); 123.01 (C_{Ph}); 126.54 (C_{Ph}); 129.46 (C_{Ph}); 132.14 ppm (C_{Ph}); 144.95 ppm (C₆); 150.13 ppm (C₂); 159.57 (C₄); 162.25 (O-C=N); 164.48 (O-C=N). IR (KBr, cm⁻¹) 1693 (C=O), 1606 (C=N), 1554 (C=C), 1173 (C-O-C). HRMS Calculated [M+H]⁺: 348.9931 Found [M+H]⁺ 348.9940.

5-iodo-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidine-2,4(1H,3H)-dione (**4g**) white solid, Yield: 35/48% (method A/B). R=0.42 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 282-284. NMR ¹H: 5.3ppm (2H, s, CH₂); 7.64ppm (3H, m, H_{ph}); 8ppm (2H, m, H_{ph}); 8.38ppm (1H, s, C₆-H); 11.85ppm (1H, s, N-H). NMR ¹³C: 42 ppm (CH₂); 70 ppm (C₅); 123 ppm (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 133 ppm (C_{Ph}); 150 ppm (C₆); 151 ppm (C₂); 161 (C₄); 162 (O-C=N); 164 (O-C=N). IR (KBr, cm⁻¹) 1707 (C=O), 1614 (C=N), 1554 (C=C), 1246 (C-O-C). HRMS Calculated [M+H]⁺: 396.9792 Found [M+H]⁺ 396.9798.

2-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (**4h**) white solid, Yield: 52%. R=0.38 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 244-246. NMR ¹H: 5.4 ppm (2H, s, CH₂-N); 7.6 ppm (3H, m, H_{Ph}); 7.96 ppm (2H, m, H_{Ph}); 8.1 ppm (1H, s, C₅-H). NMR ¹³C: 43ppm (CH₂); 107 ppm (C₅); 123 ppm (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 133 ppm (C_{Ph}); 143 ppm (C₂); 150 (C₄); 163 (O-C=N); 165 (O-C=N). IR (KBr, cm⁻¹) 3406 (N-H), 1693 (C=O), 1555 (C=C), 1112(C-O-C). HRMS Calculated [M+H]⁺: 272.0778 Found [M+H]⁺ 272.0790.

6-methyl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1,2,4-triazine-3,5(2H,4H)-dione (**4i**) white solid, Yield: 56%. R=0.36 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 241-243. NMR ¹H: 2.15ppm (s, 3H, CH₃); 5.28ppm (2H, s, CH₂); 7.36ppm (3H, m, H_{ph}); 7.99ppm (2H, m, H_{ph}); 12.3 (NH). NMR ¹³C: 16.49 ppm (CH₃); 34.08ppm (CH₂); 123.06 (C_{Ph}); 126.53 (C_{Ph}); 129.37 (C_{Ph}); 132.02 ppm (C_{Ph}); 141.91 ppm (C₅); 148.9ppm (C₂); 155.9 ppm (C₄); 161.74ppm (O-C=N); 164.31ppm (O-C=N). IR (KBr, cm⁻¹) 1649 (C=O), 1607 (C=N), 1554 (C=C), 1173 (C-O-C). HRMS Calculated [M+H]⁺: 286.0935 Found [M+H]⁺ 286.0943.

4-amino-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrimidin-2(1H)-one (**4j**) white solid, Yield: 91%. R=0.3 (CH₂Cl₂/CH₃OH: 9/1). Mp (°C) 281-283. NMR ¹H: 5.24 (2H, s, CH₂); 5.86ppm (1H, d, C₅-H); 7.47 (2H, s, NH₂); 7.62 ppm (3H, m, H_{ph}); 7.83 (1H, d, C₆-H); 8.6ppm (2H, m, H_{ph}). NMR ¹³C: 43.24 (CH₂); 94.37 (C₅); 123.11 (C_{Ph}); 126.47 (C_{Ph}); 129.42 (C_{Ph}); 132.03 ppm (C_{Ph}); 146.62 (C₆); 153.73 (C₂); 162.96ppm (O-C=N); 164.21ppm (O-C=N); 164.92 (C₄). IR (KBr, cm⁻¹): 3414 (NH₂), 1640 (C=O), 1628 (C=N and C=C), 1195 (C-N). HRMS Calculated: [M+H]⁺: 270.0986 Found [M+H]⁺: 270.0995.

General procedure for the preparation of compounds 6a-c

A mixture of purine (1mmol), alkylating agent **3a** (1.5mmol, 294mg) and potassium carbonate (1mmole, 138mg) in DMF (10 ml) was heated at 90°C for 4h. Then the DMF was evaporated under reduced pressure to dryness. The residue was purified by flash chromatography eluting with CH₂Cl₂/MeOH to obtain pure desired product **6a-c**.

1,3-dimethyl-7-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-purine-2,6(3H,7H)-dione (**6a**) white solid, Yield: 83%. R=0.61 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 200-202. NMR ¹H: 3.2 ppm (3H, s, CH₃-N); 3.5ppm (3H, s, CH₃-N); 5.9 ppm (2H, s, CH₂-N); 7.61 ppm (3H, m, H_{Ph}); 8 ppm (2H, m, H_{Ph}); 8.3 ppm (1H, s, C₈-H). NMR ¹³C: 29 ppm (CH₃-N); 33 ppm (CH₃-N); 38 (CH₂); 107 ppm (C₅); 124 (C_{Ph}); 126 (C_{Ph}); 129 (C_{Ph}); 132 ppm (C_{Ph}); 142 (C₈); 148 (C₆); 151.5 (C₄); 163 (O-C=N); 165ppm (O-C=N). IR (KBr, cm⁻¹) 1706 (C=O), 1664 (C=O), 1605 (C=N), 1549 (C=C), 1188(C-O-C). HRMS Calculated [M+H]⁺: 339.1200 Found [M+H]⁺ 339.1205.

3,7-dimethyl-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-1H-purine-2,6(3H,7H)-dione (**6b**) white solid, Yield: 86%. R=0.59 (CH₂Cl₂/CH₃OH: 9.6/0.4). Mp (°C) 204-206. NMR ¹H: 3.45 ppm (3H, s, CH₃-N); 3.9 ppm (3H, s, CH₃-N) ; 5.4 ppm (2H, s, CH₂-N); 7.6 ppm (3H, m, H_{Ph}); 7.97 ppm (2H, m, H_{Ph}); 8.1 ppm (1H, s, C₈-H). NMR ¹³C: 27 ppm (CH₃-N); 29 ppm (CH₃-N); 41 (CH₂); 107 ppm (C₅); 123 (C_{Ph}); 127 (C_{Ph}); 130 (C_{Ph}); 133 ppm (C_{Ph}); 144 (C₈); 148 (C₆); 151.5 (C₄); 163 (O-C=N); 165ppm (O-C=N). IR (KBr, cm⁻¹) 1706 (C=O), 1664 (C=O), 1605 (C=N), 1549 (C=C), 1188(C-O-C). HRMS Calculated [M+H]⁺: 339.1200 Found [M+H]⁺ 339.1207

9-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)-9H-purin-6-amine (**6c**) white solid, Yield: 34%. R=0.5 (CH₂Cl₂/CH₃OH: 9/1). Mp (°C) 241-243. NMR ¹H: 5.8 ppm (2H, s, CH₂-N); 7.45 ppm (2H, s, NH₂); 7.6 ppm (3H, m, H_{Ph}); 7.9 ppm (2H, m, H_{Ph}); 8.17 ppm (1H, s, C₈H); 8.26 ppm (1H, s, C₃H). NMR ¹³C: 39 (CH₂); 119 (C₅); 123 .06 (C_{Ph}); 126.53 (C_{Ph}); 129.37 (C_{Ph}); 132.02 ppm (C_{Ph}); 142 (C₈); 151 (C₆); 153 (C₂); 157 (C₄); 161.74 ppm (O-C=N); 164.31ppm (O-C=N). IR (KBr, cm⁻¹) 3258 (N-H), 1605 (C=N), 1547 (C=C), 1162(C-O-C). HRMS Calculated [M+H]⁺: 294.1098 Found [M+H]⁺ 294.1102.

Biological data

Cytotoxicity activity

Cell culture

Two human breast cancer cells lines SKBR3, MCF7 and a human leukemia cell line, HL60 used in this work were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Iscove's Modified Dulbecco's Media supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37°C and 5% CO₂ in a humid environment. All experiments were performed with cells in exponential growth phase.

Cell proliferation and cytotoxicity assay

SKBR3, MCF7 and HL60 cells were seeded in a 96-multiwell plate at a density of 5000 cells/well for 24h. The cells were then treated with the chemically synthesized molecules **4a-j** and **6a-c** at a concentration of 10 µM for 18 hr. before the WST-8 test. Cells treated with Doxorubicin were used as positive control and non-treated cells as a negative control. The CCK-8 assay was used to measure cytotoxicity of the compounds. After incubation, CCK-8 solution (10 µL) was added to each well, followed by incubation for 2 h at 37°C. A multiplate reader (Multiskan FC photometer Thermo Scientific, Langenselbold, Germany) determined the absorbance at 450 nm. Cell viability was expressed as a percentage of the control (untreated) cells by using the following equation: $\text{OD untreated cell} - \text{OD treated cells} / \text{OD untreated cell} \times 100$. For each compound concentration, mean values of the absorbance rates from three wells were calculated. In fact, WST-8 is reduced by dehydrogenase activities in cells to give a yellow-color formazan dye, which is soluble in the tissue culture media. The amount of the formazan dye, generated by the activities of dehydrogenases in cells, is directly proportional to the number of living cells.

Antiviral activity assays

The antiviral assays were based on inhibition of virus-induced cytopathicity or plaque formation in human embryonic lung (HEL) cells against varicella-zoster virus (VZV) (thymidine kinase wild-type (TK+) (Oka strain) and TK-deficient (TK–) (07-1 strain). Confluent cell cultures in microtiter 96-well plates were infected with 20 plates forming units (PFU) for VZV. After 2h adsorption, the viral inoculum was removed and the cell cultures were incubated with fresh medium in the presence of varying concentrations of the test compounds. Viral plaque formation for VZV or viral cytopathic effect (for HCMV) was recorded after, respectively, 5- and 7-days post-infection. Antiviral activity

was expressed as the EC₅₀ or compound concentration required to reduce viral plaque formation (VZV) by 50%. Alternatively, the cytostatic activity of the test compounds was measured based on inhibition of cell growth.

HEL cells were seeded at a rate of 5 x 10³ cells/well into 96-well microtiter plates and allowed to proliferate for 24 h. Then, medium containing different concentrations of the test compounds was added. After 3 days of incubation at 37 °C, the cell number was determined with a Coulter counter. The cytostatic concentration was calculated as the CC₅₀, or the compound concentration required to reduce cell proliferation by 50% relative to the number of cells in the untreated controls.

Molecular docking

In silico computational docking studies were performed using AutoDock 4.2 [48]. The X-ray crystallographic structures of HER2 and EGFR were downloaded from the RCSB Protein Data Bank (PDB) ID: 1PP0 [49] and 1M17 [50]. The proteins were prepared separately by removing water and co-crystallized ligands bound with the proteins to make receptor free of any ligand before docking. Then, Polar hydrogens and Gastieger charges were added using the MGL Tools and proteins saved in PDBQT format. Ligand structures were created separately using ChemDraw Ultra 12.0, energy minimized in Chem3D, torsional bonds of ligands were set flexible 3 and saved in PDBQT format. Next, receptor was kept rigid, the grid covering all the amino acid residues present inside the active site of proteins was built for HER2 (grid box size of 50Å x 50Å x 50Å with a spacing of 0.375 Å between the grid points and centered at 17.882 (x), 15.918 (y) and 28.054 (z)) and for EGFR (grid box size of 72Å x 44Å x 60 Å with a spacing of 0.375 Å between the grid points and centered at 24.21 (x), 0.49 (y) and 59.2 (z)). The best conformers were searched by the Lamarckian genetic algorithm (LGA), the population size was set to 150 and maximum number of energy evaluation was set to 25000000. Finally, the results were analyzed and visualized by discovery studio.

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