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A Click Synthesis, Molecular Docking, Cytotoxicity on Breast Cancer (MDA-MB 231) and Anti-HIV Activities of New 1,4-Disubstituted-1,2,3-Triazole Thymine Derivatives

Faeza Abdul Kareem Almashal[a], Hamsa Hussein Al-Hujaj[b], Ahmed Majeed Jassem[a, 1], and Najim Aboud Al-Masoudi[c]

[a] *College of Education for Pure Sciences, Department of Chemistry, Basrah University, Basrah, Iraq*

[b] *College of Pharmacy, University of Basrah, Basrah, Iraq*

[c] *College of Science, Department of Chemistry, Basrah University, Basrah, Iraq*

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Abstract—A new series of 1,4-disubstituted-1,2,3-triazolethymine derivatives (**VIa**–**e**) were synthesized and characterized by spectroscopic studies. The in vitro cytotoxic activities of selected compounds against human cancer cell line (MDA-MB 231) were evaluated by MTT assay. 4-Azido-*N*-substituted-benzenesulfonamides (**Vc**–**e**) and 4,4'-(4,4'-((5-methyl-2,4-dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))-bis(*N*-(4-methyl pyrimidin-2-yl)benzenesulfonamide) (**VIc**) displayed a significant cytotoxic activity with IC_{50} values of 1.61, 1.41, 1.61 and 1.81 μM, respectively. Molecular docking study of 4-azido-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**Vd**) and 4,4'-(4,4'-((5-methyl-2,4-dioxopyrimidine-1,3(2*H*,4*H*)-diyl)bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))-bis (*N*-(4-methyl pyrimidin-2-yl)benzenesulfonamide) (**VIc**) showed hydrogen bonding with the amino acid residues of the receptors 1X7R and 1A53, respectively. These derivatives are useful as starting points for further study of new anticancer drugs and to confirm the potential of triazole-sulfonamide analogues as lead compounds in anticancer drug discovery. In addition, 1,4-disubstituted-1,2,3-triazolethymine derivatives (**VIa**–**e**) were evaluated in vitro for antiviral activity against the replication of HIV-1 and HIV-2 in MT-4 cells. The results showed that 1,4-disubstituted-1,2,3-triazolethymine derivatives (**VIc**–**e**) possess a potent activity against HIV-1 replication with IC_{50} values of 11.42, ≥15.25,and 14.36 μM, SI > 4, ≤6, >9, respectively.

Keywords: breast cancer cell line (MDA-MB 231), anti-HIV activity, molecular docking, click reaction, 1,2,3-triazole thymine derivatives

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INTRODUCTION

Breast cancer or metastatic breast cancer is diagnosed as an incurable disease by current-cure strategies. The breast cancer is common as a malignant disease in United States, women, accounting for > 400 deaths each year [1, 2]. Mortality from this type of cancer remains high because the current cures are limited by the emergence of the treatment-resistant cancer cells [3]. Breast cancer's treatment normally involves healing strategies such as immunotherapy, surgery, radiotherapy, and chemotherapy [4]. The developed resistance toward the chemotherapeutic agents and combined with side effects are a major obstacle to restrict a chemotherapeutic treatment of breast cancer [5]. Therefore, the synthesis of effective anti-breast cancer compounds is the top way to overcome this obstacle and lead to an obvious clinical benefit with development of effective therapies [6].

Triazole derivatives have attracted a considerable attention for the past few decades due to their chemotherapeutic values such as antimalarial [7–9], antituberculosis agents [10], anticancer [11–20], antiviral [21–23], analgesic [24], fungicidal [25–27], antimicrobial [28], anticonvulsant activity [29], Src kinase [30], neuraminidase inhibitors [31], and protein tyrosine phosphatase inhibitors [32]. The synthesis of several inhibitors for the treatment of breast cancer (e.g., MCF-7 cells) has since become an emerging field. Compound **A** (Fig. 1), a 1,2,3-triazole analogue as a potent antitumor agent, exhibited an IC_{50} values of 46 nM against MCF-7 cancer cell line [33]. A 1,2,3-triazol-dithiocarbamate-urea hybrid (compound **B**), showed IC_{50} vlaues of 1.62 and 1.86 μM against MGC-803 and MCF-7 cell line, respectively [34]. Jin et al. [35] have prepared novel derivatives of phenyl-substituted berberine triazolyls with a notable anticancer activity against MCF-7 cells compared with berberine, meanwhile, Bethala et al. [36] have demonstrated that methyl oleate with-CH_2OH as 1,2,3-tri-

[1] Corresponding author: e-mail: ahmed.majedd@uobasrah.edu.iq.

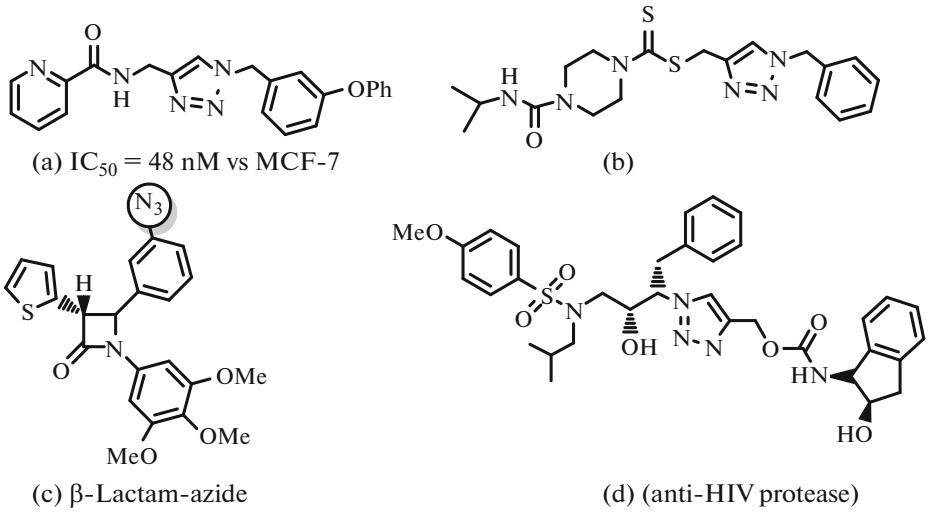


Fig. 1. Azide and 1,2,3-triazole derivatives with anticancer activity.

azole side chain exhibited a good anticancer activity against DU-145, HeLa, MCF-7, and A549 human cancer cell lines. The study of new hybrid systems from the combination of 1,2,3-triazole moiety and pyrimidine comprises an unexplored field of research. Liu et al. [37] have reported that hybridization of 1,2,3-triazole with pyridimine moietyexhibited about 7 fold more potent than 5-Fu against EC-109. Additionally, some azide derivatives have demonstrated a significant antitumor and antimitotic activity, such as β-lactam azide analogue (compound **C**) exhibited a potent antiproliferative activity against MGC-803 cells with an IC_{50} value of 0.106 μM via an induction of G2/M and an inhibition of the epithelial to mesenchyml transition [38]. On the other hand, 1,2,3-triazole derivatives have also showed a significant anti-HIV activity. Examples of these analogues which have been identified as potent HIV-1 inhibitors [39], triazole derivative (compound **D**) showed a potent HIV-1 protease inhibition with an IC_{50} value of 0.01 M [40] (Fig. 1).

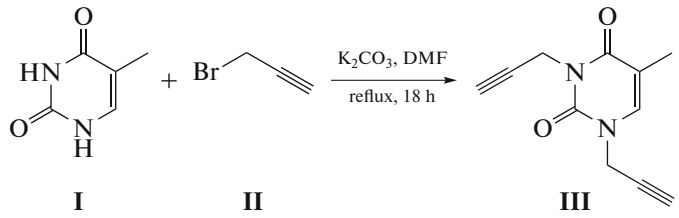
A lot of methods for the synthesis of 1,2,3-triazole derivatives have been reported [41–45]. Among of these methods, a click chemistry which involves a 1,3-dipolar cycloaddition reaction between alkyl (triple bonds) and aryl azides to provide a versatile and selective chemical reaction in organic chemistry [46, 47].

As a part of our interest with a click chemistry and the view of varied pharmacological activities of azide and 1,2,3-triazole derivatives, we report the synthesis of some new 4-azido-*N*-substituted-benzenesulfonamides (**Va–e**) and their 1,2,3-triazole analogues (**VIa–e**), as well as their cytotoxicity on MDA-MB 231 breast cancer cells, anti-HIV activities, and an in silico molecular docking study.

RESULTS AND DISCUSSION

Chemistry

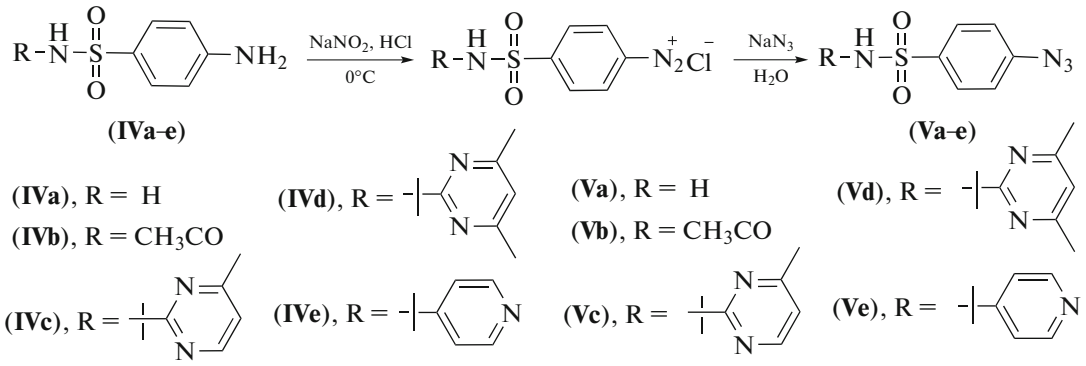
The dipropargyl thymine (**III**) was synthesized from thymine (**I**) and propargyl bromide (**II**) in the presence of K_2CO_3 by refluxing the mixture in DMF for 18 h. The formation of the dipropargyl thymine (**III**) was indicated by TLC (CH_2Cl_2 : MeOH, 95 : 5) for the crude reaction mixture (Scheme 1).



I **II** **III**

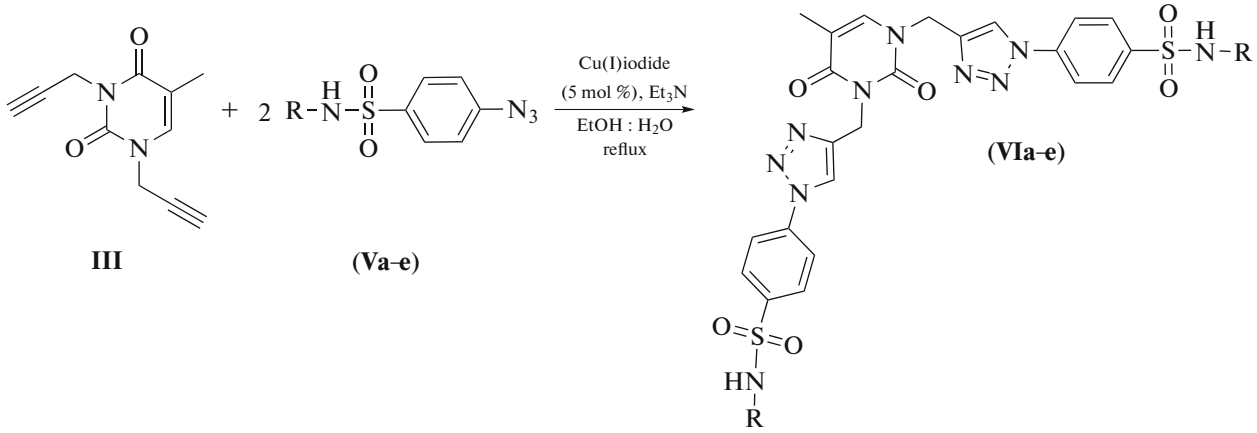
Scheme 1. Dialkylation of thymine (**I**) with propargyl bromide (**II**).

Next, an initial treatment of various amino-sulfonamide derivatives (**IVa–e**) with con. HCl and $NaNO_2$ at 0–5°C afforded the solutions of diazonium salts that were directly used for the next step without purification. Thus, an addition of an aq. solution of NaN_3 to diazonium salts solutions furnished the 4-azido-*N*-substituted-benzenesulfonamides (**Va–e**) (Scheme 2).



Scheme 2. Synthetic route of 4-azido-*N*-substituted-benzenesulfonamides(**Va**–**e**).

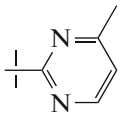
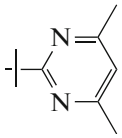
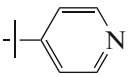
The final step includes the synthesis of 1,4-disubstituted-1,2,3-triazole thymine derivatives (**VIa**–**e**) through a 1,3-cycloaddition reaction (Huisgen reaction). A copper(I) iodide catalyzed click reaction was utilized for the synthesis of novel 1,4-disubstituted-1,2,3-triazolethymine derivatives (**VIa**–**e**). To improve and optimize the reactivity of a catalytic amount, the reaction was carried out under different catalytic concentrations. It was found that the reaction was affected by the amount of catalyst and reaction time. Initially, the reaction was tested by mixing dipropargyl thymine (**III**), aryl sulfonamide-azide **(Va)**, and a catalytic amount of Cu(I) iodide (5 mol %) in the presence of triethylamine (Et_3N) and refluxing for an appropriate time. When the amount of Cu(I) iodide was dropped from 5 to 2.5 mol %, there was a low in the yield. When the amount of Cu(I) iodide was increased from 5 to 10 mol %, the desired product yield was not changed. It was observed that the reaction time was significantly influenced by the substituent on the aryl sulfonamide-azides. As results, 5 mol % of catalyst Cu(I) iodide and EtOH : H_2O (1 : 1, v/v) were chosen as the most appropriate conditions for the click synthesis of novel 1,4-disubstituted-1,2,3-triazole thymine derivatives (**VIa**–**e**) (Scheme 3 and Table 1).



Scheme 3. Synthetic route of novel 1,4-disubstituted-1,2,3-triazole thymine derivatives (**VIa**–**e**).

The structures of compounds (**VIa**–**e**) were assigned by their IR and ^1H NMR, and mass spectra. In the IR spectra, the frequencies at the regions 3080–3105 cm^{-1} assigned to C–H stretching of triazole ring, while the strong absorption bands at the regions 1633–1666 cm^{-1} were attributed to the carbonyl amide groups (C=O). In the ^1H NMR spectra, compounds (**VIa**–**e**) showed singlets at the regions δ 1.87–1.85 ppm attributed to the methyl group at C-5 of pyrimidine backbone, whereas the doublets that appeared at the regions δ 5.10–5.16 were assigned to methylene protons ($2CH_2$, 7 + 7') (*J* ~3.0–2.8 Hz). Singlets or broad singlet at the regions δ 7.51–7.80 ppm were attributed to CH-6 of pyrimidine moiety, except for those of compound **VIe** which appeared as multiplet together with protons of pyridine ring at δ 7.70–7.80 ppm. CH-(e + e') of triazole residue resonated as two singlets at the regions δ 8.10–8.93 ppm. Additionally, the methyl group of (COMe) of compound **VIb** appeared as a singlet at δ 1.95 ppm, while methyl residues of C-6a and C-6b of compound **VIc** were appeared as a singlet at δ 2.29 ppm. The other protons of the aromatic rings and the substituents were fully analysed (see experimental section). Moreover,

Table 1. A click reaction catalyzed byCu(I) iodide

Compd.	R	Reaction time, h	M.p, °C	Yield, %
VIa	H	5	dec > 292	60
VIb	CH_3CO	48	193–196	60
VIc		8	dec > 198	30
VId		30	dec > 276	40
VIe		10	163–165	55

mass spectra data of compounds (**VIa**–**e**) were in consistent with the expected structures.

BIOLOGICAL ACTIVITY

Cytotoxicity on Breast Cancer Cells

The synthesized azidecompounds (**Vb**–**e**) and 1,2,3-triazole analogues (**VIa**–**e,** except (**VIb**) were selected for the evaluation of their cytotoxic activity in vitro against breast cancer cell line (MDA-MB231), using MTT assay [48] after 48 h of the treatment with concentrations (0–500 μM) of these compounds.

GraphPad Prism 8.1 was used to estimate the IC_{50} values for the selected compounds, and the results are summarized in Table 2. In particular, compounds (**Vc**–**e**) and (**VIc**) displayed a significant cytotoxic activity with IC_{50} values of 1.61, 1.41, 1.61 and 1.81 μM, respectively, meanwhile compounds(**Vb**), (**VIa**), (**VId**) and (**VIe**) exhibited a moderate cytotoxic activity against MDA-MB231 cancer cells with IC_{50} values of 2.32, 3.20, 2.95, and 4.77 μM, respectively. It is clear from these data (Fig. 2), the azide residue hybrids with pyridine-sulfonamide moieties enhance the cytotoxic effect of these compounds on the breast cancer cells, while the nature of the combination of the triazole moiety with a selective pyrimidine-sulfonamide scaffold (e.g. compound (**VIc**)) influences the relative cytotoxicity. This can be attributed to its disparity in either protein binding properties or bioavailability.

Table 2. Cytotoxic activity of some azido-benzenesulfonamides and their 1,2,3-triazole analogues

Compd.	IC_{50}, μM
(**Va**)	nd
(**Vb**)	2.32
(**Vc**)	1.61
(**Vd**)	1.41
(**Ve**)	1.61
(**VIa**)	3.20
(**VIb**)	nd
(**VIc**)	1.81
(**VId**)	2.95
(**VIe**)	4.77
Dox.	1.03 ± 0.3

Dox, doxorubicin (reference antineoplasticdrug); nd: not determined.

The IC_{50} values of the screened compounds being closer to those obtained from doxorubicin against MDA-MB231cell lines (1.03 ± 0.3 μM), meaning, these compounds are promising as anticancer agents.

Molecular Docking Study

Estrogen plays a major role in the progression of breast cancers [49]. Estrogen promotes breast cancer proliferation through a number of established pathways [50]. In many breast cancers, ERα activation by estrogens is considered as a responsible factor for enhancing cancer proliferation, whereas ERβ activation exerts an anti-proliferative effect [51]. Theoretically, breast cancer patients with estrogen-responsive disease should respond positively to the treatment with ERα-antagonists and/or ERβ-agonists. Estrogen receptors (ERα and ERβ) are nuclear transcription factors that are involved in the regulation of many complex physiological processes in humans.

The human estrogen receptors (ERα) have been exploited as main therapeutic targets for breast PDB (receptors 1X7R and 1A53). Estrogen receptor (ERα) with genistein was taken as an important model for the generation of the best inhibitor for gene activation (breast cancer). The three-dimensional structures of human (ERα) were obtained from the Brookhaven Protein Data Bank (PDB ID: 1X7R and 1A53 (http:/www.rcsb.org). The crystal structures were refined by removing water molecules, cofactor, and phosphate ion. Hydrogen atoms were added and electronic charges were assigned to the protein atoms using the kollman united atoms force field (AutoDock Vina 1.1.2–4, 2011) [52].

In our search for new lead compounds as human ERα inhibitors, we have selected compounds (**Vd**) and (**VIc**) for the docking modelling study. The calculated binding energy score for compounds (**Vd**) and (**VIc**) are –7.6 and –8.3 kcal mol^{-1} which indicate a good selectivity and potency for binding to an active site of the protein receptors pocket (1X7R and 1A53), respectively. As visualized in Fig. 3, both N-1 and N-2 atoms of the azide group of the compound (**Vd**) bind firmly at the target site of 1X7R with two conventional hydrogen bonds (Glu352) and one C-H interaction between C-3 of aromatic ring and Leu346. Additionally, pyrimidine-2,4-dione backbone of compound

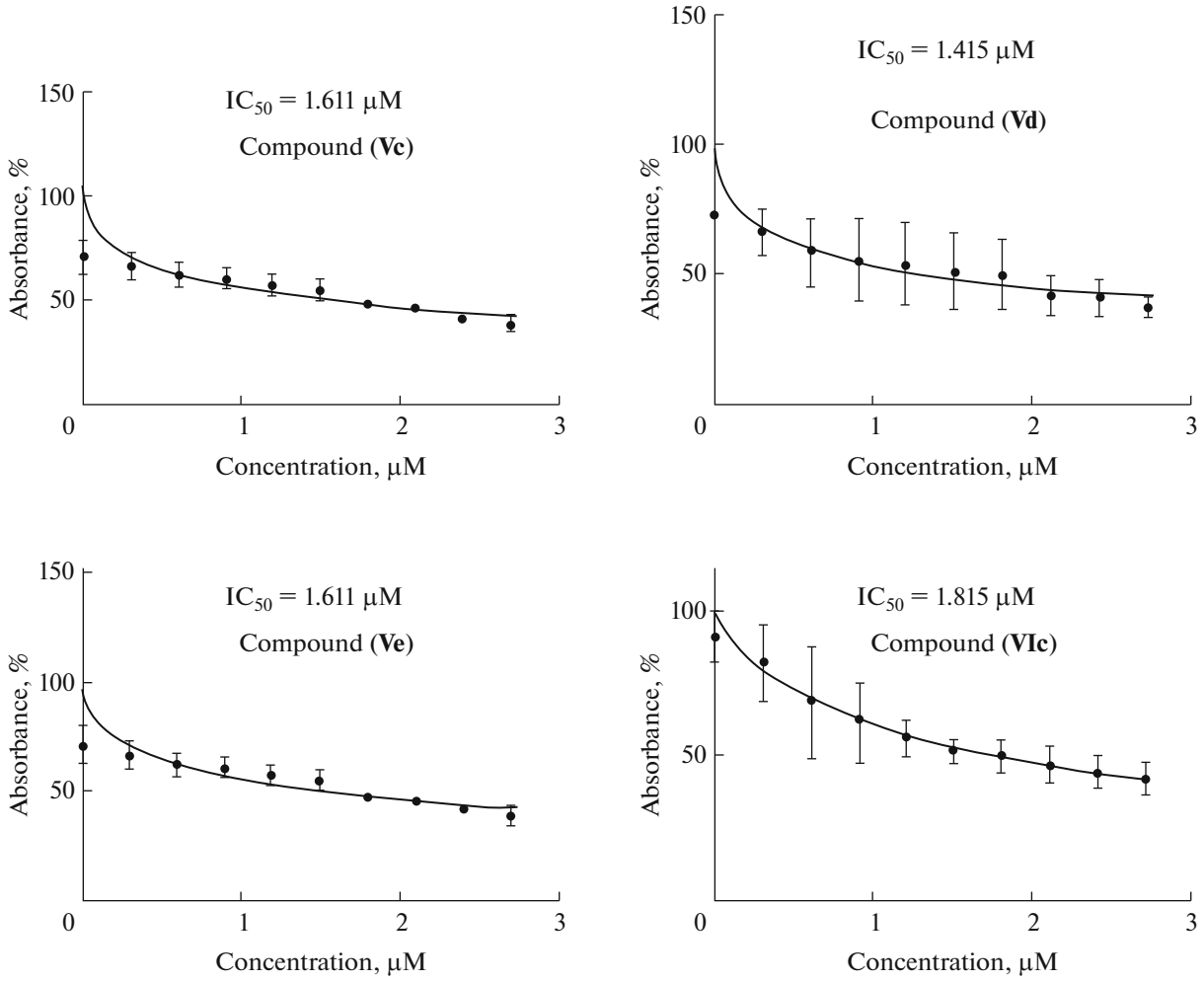


Fig. 2. GraphPad Prism 8.1 for estimation of IC_{50} values of compounds (**Vc**–**e**), and (**VIc**), the values of $^*p < 0.05$ were considered as a statistical significant.

(**VIc**) was located in middle of the binding pocket, and the most of its substituents anchored in a favourable position of hydrogen bonding for the amino acid residues. Thus, four hydrogen bonds were observed including: NH groups of two sulphonamide groups with Met421 and Glu323, as well as the lone pair of an oxygenatom of the sulphonamide moiety with Met357, while the fourth hydrogen bond appeared between N-1 of 2-amino-4-methylpyrimidine scaffold and Lys449. Besides these binding, non-bonding amino acid residues were surrounded by compounds (**Vd**) and (**VIc**), which further would enhance their inhibitory potency.

In Vitro Anti-HIV Activity

Compounds (**VIa**–**e**) were screened for their in vitro anti-HIV-1 (strain III_B) and HIV-2 (strain ROD) activities in human T-lymphocyte (MT-4) cells based on an MTT assay [53]. The results are summarized in Table 3. The data for nevirapine (BOE/BIRG587) [54] and azidothymidine (DDN/AZT) [55] are included for comparison.

Compounds-induced cytotoxicity were also measured in MT-4 cells parallel with antiviral activity. All synthesized compounds are inactive except compounds (**VIc**–**e**) which showed IC_{50} values of 11.42 μM (CC_{50} = >50.00 μM), ≥15.25 μM (CC_{50} = 89.69 μM) and 14.36 μM (CC_{50} = >125.00 μM) against HIV-1(strain III_B), resulting selectivity index (SI) values of >4, ≤6, >9, respectively.

With respect to structure-activity relationship studies, Camarasa et al. [56] reported that 1,2,3-triazole nucleosides trigger significant activities as HIV-1 inhibitors. As regards with the electronic nature of the substituents of the synthesized compounds, both pyridine and pyrimidinesulfonamido-1,2,3-triazole groups revealed good activities. Accordingly, the combined four portions (pyrimidine, pyridine, benzensulfonamide residues, and 1,2,3-triazole moieties) of compounds (**VIc**) and (**VIe**) are considered as the

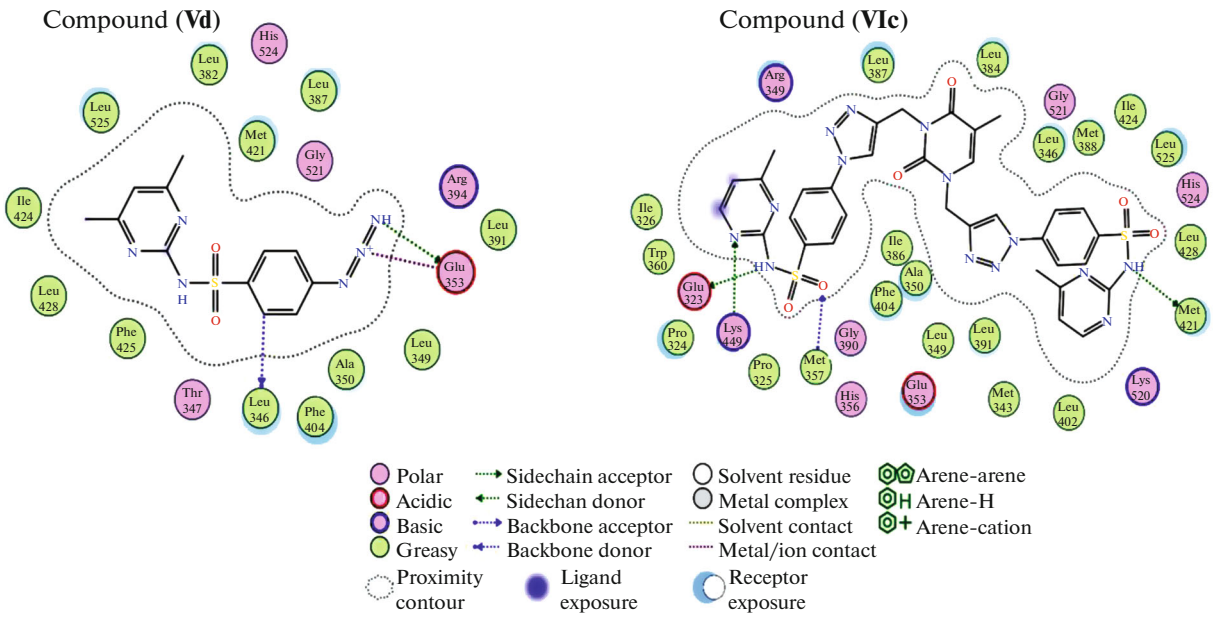


Fig. 3. Computer models of compound (**Vd**) with human ERα (pdb id 1X7R), and compound (**VIc**) with human Erα (pdb id 1A53).

optimal substituents that would offer a rise to the optimal activity.

Our results show that compounds (**VIc**) and (**VIe**) were found to be potent agents against HIV-1(strain III_B) and identified as new candidates to act as a NNRTI. Thus, these compounds might be considered as promising agents for further pharmacological evaluation.

In conclusion, a new series of 1,4-disubstituted 1,2,3-triazole hybrids with thymine were synthesized via a convenient Cu(I) iodide catalyzed 1,3-dipolar cycloaddition (click reaction). Selected azido and

Table 3. In vitro anti-HIV-1 and HIV-2 activities of 1,4-disubstituted-1,2,3-triazolethymine derivatives (**VIa–e**)

Compd.	V. strain (III_B[a] and ROD[b])	av. IC_{50}, μM[c]	av. CC_{50}, μM[d]	SI[e]
(VIa)	III_B	>125.00	>125.00	X1
	ROD	>125.00	>125.00	X1
(VIb)	III_B	>125.00	>125.00	X1
	ROD	>125.00	>125.00	X1
(VIc)	III_B	11.42	>50.00	>4
	ROD	>50.00	>50.00	X1
(VId)	III_B	≥15.25	89.69	≤6
	ROD	>89.69	89.69	X1
(VIe)	III_B	14.36	>125.00	>9
	III_B	>125.00	>125.00	X1
Nevirapine	III_B	0.05	>4.00	>80
	ROD	>4.00	>4.00	<1
AZT	III_B	0.0019	>25	>13144
	ROD	0.0018	>25	>14245

[a]Anti-HIV-1 activity measured against strain III_B; [b]anti-HIV-2 activity measured against strain ROD; [c]Average IC_{50}: compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 and 2-induced cytopathogenic effect; [d]Average CC_{50}: compound concentration that reduces the viability of mock-infected MT-4 cells by 50%; [e]SI: selectivity index (CC_{50}/IC_{50}). All data represent the mean values that were obtained from three separate experiments.

1,2,3-triazole analogues were assayed in vitro for anti-breast cancer cell line (MDA-MB231). The results exhibited that hybrid molecules have a significant cytotoxicity against breast cancer (MDA-MB231) cells. Docking studies of azido derivative (**Vd**) and 1,2,3-triazole analogue (**VIc**) were performed with the human estrogen (Erα) homology models (PDB: 1X7R and 1A53), respectively. The docking studies suggest that azido derivatives (**Vc**–**e**) and 1,2,3-triazole analogue (**VIc**) could be promising agents as anti-breast cancer (MDA-MB231) due to their potent cytotoxic activity. In addition, the new synthesized 1,2,3-triazole analogues (**VIa**–**e**)were evaluated against HIV-1 (strain III_B) and HIV-2(strain ROD), and the results showed that 1,2,3-triazole analogues (**VIc**–**e**) have a potent activity against HIV-1 replication.

EXPERIMENTAL

Chemicals and solvents were purchased from Sigma-Aldrich. 4-Aminobenzenesulfonamide (**IVa**), *N*-((4-aminophenyl)sulfonyl)acetamide (**IVb**), 4-amino-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (**IVc**), 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (**IVd**), and 4-amino-*N*-(pyridin-4-yl)benzenesulfonamide (**IVe**) were obtained from MerCK. All chemicals were at least of ACS grade, and solvents were obtained in analytical grade. Thin layer chromatography (TLC) analysis of reaction mixture was performed by using aluminum plates coated with silica gel (60 F254) sheet layer (Merck). All synthesized compounds were visualized by UV light at 254 nm. Merck silica gel (60–120 mesh) was used for column chromatography and the purification of synthesized compounds was performed with purity >95%. Melting points were obtained from a Gallenkamp melting point apparatus in capillary tubes. ^{1}H NMR spectra were recorded on the Bruker 250 MHz spectrometer using deuterated DMSO-d_6 (^{1}H NMR: DMSO-d_6: δ 2.50 ppm)as solvent and tetramethylsilane (TMS) as an internal standard (Iran).Chemical shifts are given in ppm (δ scale) and coupling constant (*J*) values are evaluated in hertz (Hz). The splitting pattern is abbreviated as follows: (s, singlet), (d, doublet) (dd, doublet doublet) and (m, multiplet). Fourier transform infrared (FT-IR) spectra were recorded on Shimadzu FTIR-8300 spectrophotometer (Iraq) by using 1% KBr discs and the absorbance were taken between 4000–500 cm^{-1}. Accurate mass was recorded on a Micro Mass LCT operating in electro-spray ionization-mass spectrum mode (ESI-MS) (Iran).

Synthesis of 5-methyl-1,3-di-(prop-2-yn-1-yl)pyrimidine-2,4-dione (III). To a stirred solution of thymine (**I**) (126 mg, 1.0 mmol) in DMF (20 mL) containing K_2CO_3 (138 mg, 1.0 mmol), propagyl bromide (**II**) (350 mg, 3.0 mmol) was added. The reaction mixture was refluxed for 18 h and the resulting mixture was monitored by TLC until the starting materials were consumed. The crude product was partitioned between $CHCl_3$ (3 × 15 mL) and water (10 mL). The combined organic layers were collected, dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by a flash column chromatography (CH_2Cl_2 : MeOH 9 : 1, R_f = 0.3) as an eluent to give compound (**III**). Yield 182 mg (90%), a colorless solid, mp 102–104°C, all the physical data was identical for those prepared previously, [Lit. [57] mp 101°C].

General method for synthesis of 4-azido-*N*-substituted-benzenesulfonamides (Va–e). To a solution of substituted amino-sulfonamide derivatives (**IVa**–**e**) (1.0 mmol), conc. HCl (0.35 mL) and water (10 mL), $NaNO_2$ (1.0 mmol) dissolved in water (5 mL) was added drop wise at 0°C. After the mixture was stirred for 15 min, water (10 mL) was added. To above solution, NaN_3 (65 mg, 1.0 mmol) dissolved in water (5 mL) was slowly added and the resulting mixture was stirred at 0°C till a white solid was appeared. The collected precipitate was filtered, dried, and re-crystallized from $CHCl_3$ to give a color less needle crystal (**Va**–**e**).

4-Azidobenzenesulfonamide (Va). Yield (85%), a color less solid, mp 116–117°C, FT-IR (ν, cm^{-1}): 1159, 1332 (SO_2), 1284 (C–N), 1589 (C=C), 2133 (N_3), 3020 (C–H_{arom}), 3142–3248 (NH_2). ^{1}H NMR (DMSO-d_6): δ 7.28 (d, 2H, *J* = 8.6 Hz, H_{arom}), 7.36 (s, 2H, NH_2), 7.82 (d, 2H, *J* = 8.7 Hz, H_{arom}). ESI-MS: *m/z* 198 $[M]^+$ observed for $C_6H_6N_4O_2S$.

***N*-((4-Azidophenyl)sulfonyl) acetamide (Vb).** Yield (90%), a colorless solid, mp 142–143°C, FT-IR (ν, cm^{-1}): 1181, 1338 (SO_2), 1274 (C–N), 1589 (C=C), 1728 (C=O), 2133 (N_3), 2856 (C–H_{alph}), 3020 (C–H_{arom}), 3307 (NH). ^{1}H NMR (DMSO-d_6): δ 1.89(s, 3H, Me), 7.31 (d, 2H, *J* = 7.2 Hz, H_{arom}), 7.88 (d, 2H, *J* = 7.2 Hz, H_{arom}), 12.06 (s, 1H, NH). ESI-MS: *m/z* 240 $[M]^+$ observed for $C_8H_8N_4O_3S$.

4-Amino-*N*-(4-methylpyrimidin-2-yl) benzenesulfonami (Vc). Yield (80%), a colorless solid, mp 197–198°C, FT-IR (ν, cm^{-1}): 1163, 1334 (SO_2), 1290 (C–N), 1585 (C=C), 2127 (N_3), 2866 (C–H_{alph}), 3037 (C–H_{arom}), 3076 (C–H_{arom}). ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3H, Me), 6.88 (d, 1H, *J* = 5.1 Hz, H_{arom}), 7.27 (d, 2H, *J* = 2.1 Hz, H_{arom}), 7.94 (d, 2H, *J* = 2.1 Hz, H_{arom}), 8.29 (d, 1H, *J* = 5.1 Hz, H_{arom}), 11.79 (s, 1H, NH). ESI-MS: *m/z* 290 $[M]^+$ observed for $C_{11}H_{10}N_6O_2S$.

4-Azido-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide (Vd). Yield (60%), a colorless solid, mp 140–142°C, FT-IR (ν, cm^{-1}): 1178, 1350 (SO_2), 1286 (C–N), 1585 (C=C), 2135 (N_3), 2993 (C–H_{alph}), 3050 (C–H_{arom}). ^{1}H NMR (DMSO-d_6): δ2.31 (s, 6H, 2Me), 6.81 (s, 1H, H_{arom}), 7.32 (d, 2H, *J* = 8.5 Hz, H_{arom}), 8.41 (d, 2H, *J* = 8.5 Hz, H_{arom}), 9.80 (s, 1H,

NH). ESI-MS: *m/z* 305 $[M]^+$ observed for $C_{12}H_{12}N_6O_2S$.

4-Azido-*N*-(pyridin-4-yl)benzenesulfonamide (Ve). Yield (85%), a colorless solid, mp 188–190°C, FT-IR (ν, cm^{-1}): 1181, 1351 (SO_2), 1281(C–N), 1608 (C=C), 2133 (N_3), 2933 (C–H_{alph}), 3055 (C–H_{arom}). ^1H NMR (DMSO-d_6): δ 6.82 (d, 2H, J = 6.27 Hz, H_{arom}), 7.22 (d, 2H, J = 8.3 Hz, H_{arom}), 7.84 (d, 2H, J = 8.3 Hz, H_{arom}), 7.96 (d, 2H, J = 4.7 Hz, H_{arom}), 12.08 (s, 1H, NH). ESI-MS: *m/z* 275 $[M]^+$ observed for $C_{11}H_9N_5O_2S$.

General method for synthesis of 1,4-disubstituted-1,2,3-triazole thymine derivatives (VIa–e). To a solution of propargylthymine (**III**) (200 mg, 1.0 mmol), Et_3N (1.0 mmol), and Cu(I) iodide (50 mg, 1.0 mmol) in water–EtOH (1 : 1) (10 mL), the appropriateazido benzenesulfonamide derivatives (**Va–e**) (2.0 mmol) was slowly added. The reaction mixture was refluxed for an appropriate time as shown in Table 1. The resulting mixture was monitored by TLC until the starting materials were consumed. The solvents were evaporated, and the crude product was purified by a flash column of chromatography (THF : hexane 4 : 1) as an eluent to afford 1,4-disubstituted-1,2,3-triazole thymine derivatives (**VIa–e**).

4,4'-(4,4'-((5-Methyl-2,4-dioxopyrimidine-1,3-(2*H*,4*H*)-diyl)bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))dibenzenesulfonamide (VIa). Yield 359 mg (60%), a yellow solid, mp dec >292°C, FT-IR (ν, cm^{-1}): 1161, 1332 (SO_2), 1247 (C–N), 1465 (C=C), 1497 (N=N), 1504 (C=N), 1637, 1664 (C=O), 2956 (C–H_{alph}), 3020 (C–H_{arom}), 3090 (C–$H_{triazole}$), 3143–3248 (NH_2). ^1H NMR (DMSO-d_6): δ1.87 (s, 3H, C-5, pyrimid.-Me), 5.15 (d, 4H, J = 3.0 Hz, CH_2-7 + CH_2-7'), 7.51 (br s, 1H, C-6, pyrimid.-H), 8.02 (dd, 4H, J = 2.0, 7.7 Hz, H_{arom}-3' + H_{arom}-5' + H_{arom}-3" + H_{arom}-5"), 8.10 (dd, 4H, J = 2.1, 7.7 Hz, H_{arom}-2' + H_{arom}-6' + H_{arom}-2" + H_{arom}-6"), 8.75, 8.90 (2 s, 2H, e + e', triazole). ESI-MS: *m/z* 598 $[M]^+$ observed for $C_{23}H_{22}N_{10}O_6S_2$.

***N*,*N*'-(4,4'-(4,4'-((5-Methyl-2,4-dioxopyrimidine-1,3(2*H*,4*H*)-diyl)-bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))-bis(4,1-phenylenesulfonyl))diacetamide (VIb).** Yield 409 mg (60%), a colorless solid, mp 193–196°C, FT-IR (ν, cm^{-1}): 1163, 1228 (SO_2), 1274 (C–N), 1467 (N=N), 1506 (C=N), 1597 (C=C), 1635, 1664 (C=O), 2860 (C–H_{alph}), 3034 (C–H_{arom}), 3082 (C–$H_{triazole}$), 3456 (N–H). ^1H NMR (DMSO-d_6): δ 1.87 (s, 3H, C-5, pyrimid.-Me), 1.95 (s, 6H, 2COMe), 5.16 (d, 4H, J = 3.0 Hz, CH_2-7 + CH_2-7'), 7.82 (s, 1H, C-6, pyrimid.-H), 8.03 (dd, 4H, J = 1.8, 7.6 Hz, H_{arom}-3' + H_{arom}-5' + H_{arom}-3" + H_{arom}-5"), 8.16 (dd, 4H, J = 1.9, 7.7 Hz, H_{arom}-2' + H_{arom}-6' + H_{arom}-2" + H_{arom}-6"), 8.75, 8.90 (2 s, 2H, NH), 8.78, 8.93 (2 s, 2H, e + e', triazole). ESI-MS: *m/z* 682 $[M]^+$ observed for $C_{27}H_{26}N_{10}O_8S_2$.

4,4'-(4,4'-((5-Methyl-2,4-dioxopyrimidine-1,3-(2*H*,4*H*)-diyl)bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))-bis(*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide) (VIc). Yield 235 mg (30%), a green solid, mp dec198°C, FT-IR (ν, cm^{-1}): 1163, 1338 (SO_2), 1250 (C–N), 1465 (N=N), 1506 (C=N), 1595 (C=C), 1633, 1664 (C=O), 2926 (C–H_{alpha}), 3073 (C–H_{arom}), 3105 (C–$H_{triazole}$), 3101 (N–H). ^1H NMR (DMSO-d_6): δ 1.83 (s, 3H, C-5, pyrimid.-Me), 2.29 (s, 6H, C-6a + 6b, pyrimid.-2Me), 5.11 (d, 4H, J = 2.8 Hz, CH_2-7 + CH_2-7'), 6.88 (d, 2H, J = 1.9 Hz, 5a + 5b), 7.78 (s, 1H, C-6, pyrimid.-H), 8.09–8.10 (m, 8H, H_{arom}), 8.30 (d, 2H, J = 2.1 Hz, 4a + 4b), 8.71, 8.86 (2 s, 2H, e + e', triazole). ESI-MS: *m/z* 782 $[M]^+$ observed for $C_{33}H_{30}N_{14}O_6S_2$.

4,4'-(4,4'-((5-Methyl-2,4-dioxopyrimidine-1,3-(2*H*,4*H*)-diyl)-bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))-bis(*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide) (VId). Yield 329 mg (40%), as a yellow solid, mp dec >276°C, FT-IR (ν, cm^{-1}): 1163, 1350 (SO_2), 1270 (C–N), 1438 (N=N), 1504 (C=N), 1597 (C=C), 1647, 1666 (C=O), 2960 (C–H_{alph}), 3045 (C–H_{arom}), 3080 (C–$H_{triazole}$), 3253 (N–H). ^1H NMR (DMSO-d_6): δ 1.85 (s, 3H, C-5, pyrimid.-Me), 2.25 (s, 12H, C-4a + 4b, C-6a + 6b, pyrimid.-4Me), 5.10 (d, 4H, J = 2.8 Hz, CH_2-7 + CH_2-7'), 6.74 (br s, 2H, 2NH), 7.77 (s, 2H, C-5a + 5b), 7.82 (s, 1H, C-6, pyrimid.-H), 8.09–8.10 (m, 8H, H_{arom}), 8.73, 8.89 (2 s, 2H, e + e', triazole). ESI-MS: *m/z* 810 $[M]^+$ observed for $C_{35}H_{34}N_{14}O_6S_2$.

4,4'-(4,4'-((5-Methyl-2,4-dioxopyrimidine-1,3-(2*H*,4*H*)-diyl)bis(methylene))-bis(1*H*-1,2,3-triazole-4,1-diyl))bis(*N*-(pyridin-4-yl)benzenesulfonamide (VIe). Yield 414 mg (55%), as a green solid, mp 163–165°C, FT-IR (ν, cm^{-1}): 1163, 1350 (SO_2), 1270 (C–N), 1438 (N=N), 1504 (C=N), 1597 (C=C), 1647, 1666 (C=O), 2960 (C–H_{alph}), 3046 (C–H_{arom}), 3080 (C–$H_{triazole}$), 3253 (N–H). ^1H NMR (DMSO-d_6): δ 1.85 (s, 3H, C-5, pyrimid.-Me), 5.12 (d, 4H, J = 2.9 Hz, CH_2-7 + CH_2-7'), 6.86 (m, 2H, 5a + 5b), 7.22 (m, 2H, 3a + 3b), 7.74–7.77 (m, 4H, 2a + 2b, 6a + 6b), 7.80 (s, 1H, C-6, pyrimid.-H), 8.03–8.05 ((m, 8H, H_{arom}), 8.72, 8.87 (2 s, 2H, e + e', triazole). ESI-MS: *m/z* 752 $[M]^+$ observed for $C_{33}H_{28}N_{12}O_6S_2$.

CELL CULTURE

Human breast cancer cells (MDA-MB231) were maintained in plate (10 cm) containing DMEM and supplemented with FBS (10%), penicillin (100 units/mL) and streptomycin (100 μg/mL) at 37°C with a humid atmosphere (CO_2, 5%).

CYTOTOXICITY AND DOSE RESPONSE

MDA-MB231 cells were growth in 96 well plate for 48 h and treated with each compound (0–500 μM) for 48 h. Cell viability was measured at 570 nm in a microplate reader (Thermo Scientific) and the all experiments were repeated in triplicate.

STATISTICS ANALYSIS

Results were expressed as mean ± SEM, GraphPad Prism 8.1 and the values of $*p < 0.05$ were considered as a statistical significant.

IN VITRO ANTI-HIV ASSAY

In vitro anti-HIV assay, the evaluation of antiviral activity of 1,2,3-triazole derivatives (**VIa–e**) against HIV-1 (strain III_B) and HIV-2 (strain ROD) in MT-4 cells was performed using an MTT assay as described previously [53]. In brief, stock solutions (10 times final concentration) of tested compounds in volumes (50 μL) were added to two series of triplicate wells in order to allow a simultaneous evaluation of their effects on mock and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of tested compounds were prepared directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments). Untreated control, HIV-infected, and mock-infected cell samples were included for each sample. HIV-1 (III_B) [(58] and HIV-2 (ROD) [59] stock (50 μL) at 100–300 $CCID_{50}$ (50% cell culture infectious dose) or culture medium [10% heat-inactivated Fetal Calf Serum (FCS), 2 mM-glutamine, 0.1% sodium bicarbonate, and 20 μg/mL gentamicin] were added to either of the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of tested compounds on uninfected cells in order to assess the cytotoxicity of the tested compounds. Exponentially growing MT-4 cells [60] were centrifuged for 5 min at 1000 rpm (Minifuge T, rotor 2250; Heraeus, Germany), and the supernatant was discarded. The MT-4 cells were re-suspended at 6×10^5 cells per mL. Thus, volumes (50 μL) were transferred to the microtiter tray wells. Five days after infection, the viability of the mock-infected and HIV infected cells were determined by spectrophotometric method.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human participants performed by any of the authors and does not contain any studies involving animals performedby any of the authors.

Conflict of Interests

The authors declare that they have no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available for this article at https://doi.org/10.1134/S1068162020030024 and are accessible for authorized users.

REFERENCES

1. Al-Hajj, M., Wicha, M.S., Benito-Hernandez, A., Morrison, S.J., and Clarke, M.F., *Proc. Natl. Acad. Sci. U. S. A.*, 2003, vol. 100, pp. 3983–3986.
2. Siegel, R., Ward, E., Brawley, O., and Jemal, A., *Cancer Stat.*, 2011, vol. 61, pp. 212–236.
3. Stockler, M., Wilcken, N.R.C., Ghersi, D., and Simes, R.J., *Cancer. Treat. Rev.*, 2000, vol. 26, pp. 151–168.
4. Siegel, R., Ma, J., Zou, Z., and Jemal, A., *Cancer Stat.*, 2014, vol. 64, pp. 9–29.
5. Raguz, S. and Yague, E., Br. J. Cancer, 2008, vol. 99, pp. 387–391.
6. Senwar, K.R., Sharma, P., Reddy, T.S., Jeengar, M.K., Nayak, V.L., Naidu, V.G.M., Kamal, A., and Shankaraiah, N., *Eur J. Med. Chem.,* 2015, vol. 102, pp. 413–424.
7. Gujar, R., Marwaha, A., White, J., Creason, S., Shackleford, D.M., Baldwan, J., Charman, W.M., Buckner, F.S., Rathod, P.K., and Phillip, M.A., *J. Med. Chem.*, 2009, vol. 52, pp. 1864–1872.
8. Chu, X.M., Wang, C., Wang, W.L., Liang, L.L., Liu, W., Gong, K.K., and Sun, K.L., *Eur. J. Med. Chem.*, 2019, vol. 166, pp. 206–223.
9. D'hooghe, M., Vandekerckhove, S., Mollet, K., Vervisch, K., Dekeukeleire, S., Lehoucq, L., Lategan, C., Smith, P.J., Chibale, K., and De Kimpe, N., *Beils. J. Org. Chem.*, 2011, vol. 7, pp. 1745–1752.
10. Labadie, G.R., de la Iglesia, A., and Morbidoni, H.R., *Mol. Div.*, 2011, vol. 15, pp. 1017–1024.
11. Chinthala, Y., Thaku, S., Tirunagari, S., Chinde, S., Domatti, A.K., Arigari, N.K., Srinivas, K.S., Alam, S., Jonnala, K.K., Khan, F., Tiwari, A., and Grover, P., *Eur. J. Med. Chem.*, 2015, vol. 93, pp. 564–573.
12. Holla, B.S., Poojary, K.N., Rao, B.S., and Shivananda, M.K., *Eur. J. Med. Chem.*, 2002, vol. 37, pp. 511–51.
13. Prachayasittikul, V., Pingaew, R., Anuwongcharoen, N., Worachartcheewan, N., Nantasenamat, C., Prachaya-

sittikul, S., Ruchirawat, S., and Prachayasittikul, V., *Springer Plus*, 2015, vol. 4, pp. 571–593.

14. Salmon, A.J., Williams, M.L., Wu, Q.K., Morizzi, J., Gregg, D., Charman, S.A., Vullo, D., Supuran, C.T., and Poulsen, S.-A., *J. Med. Chem.*, 2012, vol. 55, pp. 5506–5517.
15. Senwar, K.R., Sharma, P., Reddy, T.S., Jeengar, M.K., Nayak, V.L., Naidu, V.G.M., Kamal, A., and Shankaraiah, N., *Eur. J. Med. Chem.*, 2015, vol. 102, pp. 413–424.
16. Wei, G., Luan, W., Wang, S., Cui, Li.F., Liu, Y., Ya, Liu., and Cheng, M., *Org. Biomol. Chem.*, 2015, vol. 13, pp. 1507–1514.
17. Stefely, J.A., Palchaudhuri, R., Miller, P.A., Peterson, R.J., Moraski, G.C., Hergenrother, P.J., and Miller, M.J., *J. Med. Chem.*, 2010, vol. 538, pp. 3389–3395.
18. Yadav, P., Lal, K., Kumar, A., Guru, S.K., Jaglan, S., and Bhushan, S., *Eur. J. Med. Chem.*, 2017, vol. 126, pp. 944–953.
19. Salmon, A.J., Williams, M.L., Wu, Q.K., Morizzi, J., Gregg, D., Charman, S.A., Vullo, D., Supuran, C.T., and Poulsen, S.-A., *J. Med. Chem.*, 2012, vol. 55, pp. 5506–5517.
20. Doiron, J., Soultan, A.H., Richard, R., Touré, M.M., Picot, N., Richard, R., Čuperlović-Culf, M., Robichaud, G.A., and Touaibia, M., *Eur. J. Med. Chem.*, 2011, vol. 46, pp. 4010–4024.
21. Cheng, H., Wan, J., Lin, M.-I., Liu, Y., Lu, X., Liu, J., Xu, Y., Chen, J., Tu, Z., and Cheng, Y.-S.E., *J. Med. Chem.*, 2012, vol. 55, pp. 2144–2153.
22. Jordao, A.K., Ferreira, V.F., Souza, T.M.L., de Souza, FariaG.G., Machado, V., Abrantes, J.L., De Souza, M.B.C.V., and Cunha, A.C., *Bioorg. Med. Chem.*, 2011, vol. 19, pp. 1860–1865.
23. Ferreira, M.G., Pinheiro, L.C.S., Santos-Filho, O.S., Pecanha, M.D.S., Sacramento, C.Q., Machado, V., Ferreira, V.F., Souza, T.M.L., and Bia Boechat, N., *Med. Chem. Res.*, 2014, vol. 23, pp. 501–1511.
24. Wuest, F., Tang, X., Kniess, T., Pietzsch, J., and Suresh, M., *Bioorg. Med. Chem.*, 2009, vol. 17, pp. 1146–1151.
25. Aher, N.G., Pore, V.S., Mishra, N.N., Kumar, A., Shukla, P.K., Sharma, A., and Bhat, M.K., *Bioorg. Med. Chem. Lett.*, 2009, vol. 19, pp. 759–763.
26. Bakunov, S.A., Bakunova, S.M., Wenzler, T., Ghebru, M., Werbovetz, K.A., Brun, R., and Tidwell, R.R., *J. Med. Chem.*, 2010, vol. 53, pp. 254–272.
27. Dai, Z.-C., Chen, Y.-F., Zhang, M., Li, S.-K., Yang, T.-T., Shen, L., Wang, J.-X., Qian, S.S., Zhu, H.-L., and Ye, Y.-H., *Org. Biomol. Chem.*, 2015, vol. 13, pp. 477–486.
28. Sumangala, V., Poojary, B., Chidananda, N., Fernandes, J., and Kumar, N.S., *Arch. Pharm. Res.*, 2010, vol. 33, pp. 1911–1918.
29. Song, M.-X. and Deng, X.-Q., *J. Enzym. Inhib. Med. Chem.*, 2018, vol. 33, pp. 453–478.
30. Lebeau, A., Abrioux, C., Benimelis, D., Benfodda, Z., and Meffre, P., *Med. Chem.*, 2017, vol. 13, pp. 40–48.
31. Das A., Adak, A.K., Ponnapalli, K., Lin, C.-H., Hsu, K.-C., Yang, J.-M., Hsu, T.-A.,and Lin, C.-C., *Eur. J. Med. Chem.*, 2016, vol. 123, pp. 397–406.
32. Thirumurugan, P., Matosiuk, D., and Jozwiak, K., *Chem. Rev.*, 2013, vol. 113, pp. 4905–4979.
33. Ohmoto, K., Yamamoto, T., Horiuchi, T., Imanishi, H., Odagaki, Y., Kawabata, K., Sekioka, T., and Hirota, Y., *J. Med. Chem.*, 2000, vol. 43, pp. 4927–4929.
34. Duan, Y.-C., Zheng, Y.-C., Li, X.-C., Wang, M.-M., Ye, X.-W., Guan, Y.-Y., Liu, G.-Z., Zheng, J.-X., and Liu, H.-M., *Eur. J. Med. Chem.*, 2013, vol. 64, pp. 99–110.
35. Jin, X., Yan, T.H., Yan, L., Li, Q., Wang, R.L., Hu, Z.L., Jiang, Y.Y., Sun, Q.Y., and Cao, Y.B., *Clin. Cancer Res.*, 2014, vol. 8, pp. 1047–1059.
36. Manneganti, V., Lakshmi, Anu., Prabhavathi, Devi., Bethala, L.D., Rachapudi, B.P., Singh, A., and Ummanni, R., *Int. J. Pharm. Sci. Res.*, 2017, vol. 38, pp. 1635–1649.
37. Ma, L.-Y., Pang, L.-P., Wang, B., Zhang, M., Hu, B., Xue, D.-Q., Shao, K.-P., Zhang, B.-L., Lui, Y., Zhang, E., and Hong-Min Liu, *Eur. J. Med. Chem.*, 2014, vol. 86, pp. 368–380.
38. Fu, D.-J., Fu, L., Liu, Y.-C., Wang, J.-W., Wang, Y.-Q., Han, B.-K., and Li, Z.-R., *Sci. Rep.*, 2017, vol. 7, pp. 1–12.
39. Mohammed, I., Kummetha, I.R., Singh, G., Sharova, N., Lichinchi, G., Dang, J., Stevenson, M., and Rana, T.M., *J. Med. Chem.*, 2016, vol. 59, pp. 7677–7682.
40. Whiting, M., Muldoon, J., Lin, Y.C., Silverman, S.M., Lindstrom, W., Olson, A.J., Kolb, H.C., Finn, M., Sharpless, K.B., and Elder, J.H., *Angew. Chem. Inter. Ed.*, 2006, vol. 45, pp. 1435–1439.
41. Zhao, Y.-L., Dichtel, W.R., Trabolsi, A., Saha, S., Aprahamian, I., and Stoddart, J.F., *J. Am. Chem. Soc.*, 2008, vol. 130, pp. 11294–11296.
42. Fournier, D., Hoogenboom, R., and Schubert, U.S., *Chem. Soc. Rev.*, 2007, vol. 36, pp. 1369–1380.
43. Lutz, J.F., *Angew. Chem. Int. Ed.*, 2007, vol. 46, pp. 1018–1025.
44. Angell, Y.L. and Burgess, K., *Chem. Soc. Rev.*, 2007, vol. 36, pp. 1674–1689.
45. Moses, J.E. and Moorhouse, A.D., *Chem. Soc. Rev.*, 2007, vol. 36, pp. 1249–1262.
46. Zhou, Y., Zhao, Y., O'Boyle, K.M., and Murphy, P.V., *Bioorg. Med. Chem. Lett.*, 2008, vol. 18, pp. 954–958.
47. Joshi, M.C., *Arkivoc*, 2011, vol. 10, pp. 139–147.
48. Mosmann, T.J., *Immunol. Methods*, 1993, vol. 65, pp. 5–63.
49. Harris, J.R., Lippman, M.E., Veronesi, U., and Willet, W., *New Eng. J. Med.*, 1992, vol. 337, pp. 390–395.
50. Yager, J.D. and Davidson, N.E., *New Eng. J. Med.*, 2006, vol. 354, pp. 270–282.
51. Chang, E.C., Frasor, J., Komm, B., and Katzenellenbogen, B.S., *Endocrinology*, 2006, vol. 147, pp. 483–4842.

52. Trott, O. and Olson, A.J., *J. Comput. Chem*., 2010, vol. 31, pp. 455–461.

53. Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., and De Clercq, E., *J. Virol. Methods*, 1988, vol. 20, pp. 309–321.

54. Hargrave, K.D., Proudfoot, J.R., Grozinger, K.G., Cullen, E., Kapadia, S.R., Patel, U.R., Fuchs, V.U., and Mauldin, S.C., *J. Med. Chem*., 1991, vol. 34, pp. 2231–2241.

55. Mitsuya, H., Weinhold, K.J., Furman, P.A., Clair, M.H., Lehrmann, S.N., Gallo, R., Bolognesi, D., Barry, D.W., and Broder, S., *Proc. Natl. Acad. Sci. U. S. A*., 1985, vol. 82, pp. 7096–7100.

56. Alvarez, R., Velazquez, S., San-Felix, A., Aquaro, S., De Clercq, E., Perno, C.F., Karlsson, A., Balzarini, J., and Camarasa, M.J., *J. Med. Chem*., 1994, vol. 37, pp. 4185–4194.

57. Krim, J., Sillahi, B., Taourirte, M., Rakib, E.M., and Engels, J.W., *Arkivoc*, 2009, vol. 13, pp. 142–152.

58. Popovic, M., Sarngadharan, M.G., Read, E., and Gallo, R.C., *Science*, 1984, vol. 224, pp. 497–500.

59. Barré -Sinoussi, F., Chermann, J.C., Rey, F., Nugeyre, M.T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W., and Montagnier, L., *Science*, 1993, vol. 220, pp. 868–871.

60. Miyoshi, I., Taguchi, H., Kobonishi, I., Yoshimoto, S., Ohtsuki, Y., Shiraishi, Y., and Akagi, T., *Gann. Monogr. Cancer. Res*., 1982, vol. 28, pp. 219–228.