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Synthesis, characterization and cytotoxicity studies of 1,2,3-triazoles and 1,2,4-triazolo [1,5-*a*] pyrimidines in human breast cancer cells

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ABSTRACT

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) is essential for physiological functions of tissues and neovasculature. VEGFR signaling is associated with the progression of pathological angiogenesis in various types of malignancies, making it an attractive therapeutic target in cancer treatment. In the present work, we report the synthesis of 1,4-disubstituted 1,2,3-triazoles and 1,2,4-triazolo[1, 5-*a*]pyrimidine derivatives *via* copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction and screened for their anticancer activity against MCF7 cells. We identified 1-(2′-ethoxy-4′-fluoro-[1,1′-biphenyl]-4-yl)-4-phenyl-1H-1,2,3-triazole (EFT) as lead cytotoxic agent against MCF7 cell lines with an IC_{50} value of 1.69 μM. Further evaluation revealed that EFT induces cytotoxicity on Ishikawa, MDA-MB-231 and BT474 cells with IC_{50} values of 1.97, 4.81 and 4.08 μM respectively. However, EFT did not induce cytotoxicity in normal lung epithelial (BEAS-2B) cells. Previous reports suggested that 1,2,3-triazoles are the inhibitors of VEGFR1 and therefore, we evaluated the effect of EFT on the expression of VEGFR1. The results demonstrated that EFT downregulates the expression of VEGFR1 in MCF7 cells. In summary, we identified a potent cytotoxic agent that imparts its antiproliferative activity by targeting VEGFR1 in breast cancer cells. The novel compound could serve as a lead structure in developing VEGFR1 inhibitors.

Angiogenesis is the process of formation of a new blood vessel from the pre-existing vasculature and vascular endothelial cells. Angiogenesis is essential for nutrient supply, disposal of metabolic wastes, growth, and development.[1] Angiogenesis is a key player in several pathological conditions including cancer, wound healing, inflammation, rheumatoid arthritis, ischemic cardiovascular diseases and age-related macular degeneration.[2] However, aberrant angiogenesis is one of the fundamental processes responsible for the transformation of the dormant tumor to malignancy.[3] Initially, Judah Folkman postulated that angiogenesis is essential for tumor proliferation and tumor releases proangiogenic factors called tumor angiogenesis factors (TAF) which are essential for neovasculature.[4] Further studies on angiogenesis revealed that vascular endothelial growth factor (VEGF) is the pivotal proangiogenic mitogen that stimulates the growth of new blood vessels by interacting with vascular endothelial growth factor receptors (VEGFR).[5] VEGFR belongs to receptor tyrosine kinase superfamily with three major subtypes namely, VEGFR1, VEGFR2, and VEGFR3. The binding of VEGF to VEGFR results in the receptor dimerization and activation of an intracellular signaling cascade that leads to the expression of genes responsible for cell survival, proliferation, migration, angiogenesis, and vascular permeability.[6] Even though VEGFR2 has relatively major role than VEGFR1 in VEGF-mediated signaling, it is noteworthy that VEGFR1 mediates the survival of endothelial cells and contributes to the progression of cancer.[7,8] VEGF has been reported to act as an intracrine survival factor in breast cancer cells through its binding to VEGFR1 and the same study also revealed that knockdown of

Abbreviations: EFT, 1-(2′-ethoxy-4′-fluoro-[1,1′-biphenyl]-4-yl)-4-phenyl-1H-1,2,3-triazole

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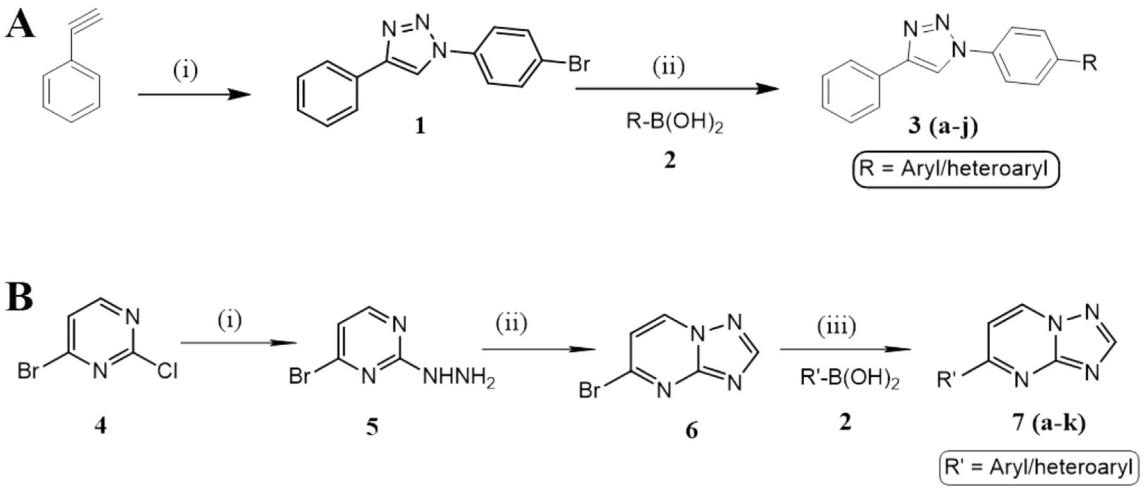

Fig. 1. A. Scheme 1. Synthesis of 1,4 disubstituted 1,2,3 triazole derivatives. Reagents and condition: i) 4-Bromophenylazide, (+)-Sodium L-ascorbate, $CuSO_4{\cdot}5H_2O$, H_2O:*t*-BuOH, RT, 18 h; ii) Ŕ-B(OH)2, Dikis, EtOH: H_2O: Dioxan (1:1:5), 120° C, 40 min. B. Scheme 2. Synthesis of [1, 2, 4]triazolo[1,5-*a*]pyrimidine derivatives. Reagents and condition: i) $NH_2NH_2{\cdot}H_2O$, EtOH, Δ, 1.5 h; ii) CH_2O, EtOH, RT, 2 h; and $IPh(OAc)_2$, CH_2Cl2, RT, 15 h; iii) Ŕ-B(OH)2, Dikis, EtOH: H_2O:Dioxan (1:1:5), 120° C, 40 min.

VEGFR2 had no effect on the survival of tested cancer cells.[9] In another study, Dales et al. investigated the prognostic value of VEGFRs and reported that expression of VEGFR1 is positively correlated with the high risk of metastasis and relapse.[10] They concluded that VEGFR1 may further be considered as a potential tool to evaluate the aggressiveness of breast cancer.[10] Taken together, these reports support the intimate relationship between VEGFR1 and cancer progression and encourage the development of VEGFR1 inhibitors as anticancer therapeutics.

Triazole-based heterocycles were identified as biologically important pharmacophores and were reported to possess good anticancer potential targeting in multiple types of malignancies.[11] Several studies have suggested that triazoles target VEGFR to induce their anticancer activity. Specifically, (1,2,3-triazol-4-yl)benzenamines were identified as potent inhibitors of VEGFR1 and VEGFR2, and the activity was comparable to Vatalanib in both homogenous time-resolved fluorescence (HTRF) enzymatic and cellular assays.[12] In another study, 1,2,4-triazol-3-yl-anilines were described as potent ATP-competitive inhibitors of VEGFR1 and 2.[13] In continuation of our efforts in exploring the anticancer potential of various heterocycles[14–20], we prepared the series of novel 1,2,3- and 1,2,4-triazoles and investigated the effect of newly synthesized compounds on breast cancer (MCF7) cells. The lead compounds 1-(2′-ethoxy-4′-fluoro-[1,1′-biphenyl]-4-yl)-4-phenyl-1H-1,2,3-triazole (EFT, **3a**) and 1-(4-(benzo [b]thiophen-2-yl)phenyl)-4-phenyl-1H-1,2,3-triazole (**3j**) were chosen to investigate the possible VEGFR inhibitory activity in breast cancer cells.

Preparation of 1,4 disubstituted 1,2,3 triazoles 3(a-j): The synthesis of the title compounds was carried out as illustrated in Scheme 1 (Fig. 1A). In the present work, 1-(4-bromophenyl)-4-phenyl-1,2,3-triazole (**1**) was synthesized *via* copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) between 4-bromophenylazide and phenylacetylene in the presence of sodium ascorbate and $CuSO_4{\cdot}5H_2O$ in *tert*-butanol and water mixture as the solvent system. In the next step, 1-(4-bromophenyl)-4-phenyl-1,2,3-triazole was used for Suzuki-Miyaura cross-coupling reactions. We treated 1-(4-bromophenyl)-4-phenyl-1,2,3-triazole with various aryl /heteroaryl boronic acids (**2**) in presence of K_2CO_3 and Pd(II) catalyst under a nitrogen atmosphere to yield a novel series of 1,4 disubstituted 1,2,3 triazoles **3(a–j)** (Scheme 1) with a yield of 70–92% as summarised in Table 1. The structure of all the synthesized compounds **3(a–j)** were confirmed by nuclear magnetic resonance (NMR) and high-resolution mass spectral (HRMS) analysis. All the final compounds **3(a–j)** showed a characteristic singlet around 8.2 δ corresponding to a proton on the triazole ring. Spectral analysis data is provided as Supplementary Information.

Preparation of [1,2,4] triazolo [1,5-*a*] pyrimidine derivatives **7(a–k)**.[21]: The synthesis of the title compounds was carried out as illustrated in Scheme 2 (Fig. 1B). Initially, starting from the transition metal free coupling reaction between 2-chloro-5-bromopyrimidine (**4**) with hydrazine hydrate to produce the corresponding hydrazine (**5**) which was condensed with formaldehyde to afford hydrazones. Further, hydrazone intermediate was *in situ* cyclized in the presence of IPh $(OAc)_2$ to yield 5-bromo-[1,2,4]-triazolo[1,5-*a*]pyrimidine (**6**). Similarly, scaffold (**6**) was crossed-coupled with various aryl/heteroaryl boronic acids under inert (nitrogen) atmosphere in the presence of K_2CO_3 and palladium(II) catalyst to obtain novel series of [1,2,4]triazolo[1,5-*a*]pyrimidines **7(a–k)** in 65–90% yield as shown in Table 2.

1,2,3-Triazoles elicit growth inhibitory effect on breast cancer cells: Initially, we investigated the growth inhibitory effect of 1,2,3-triazoles against MCF7 cells and 1,2,4-triazolo-pyrimidines against MCF7 and MDA-MB-231 cells using the AlamarBlue assay.[22,23] The detailed methodology is provided in the Supplementary Information. Among the tested compounds, 1-(2′-ethoxy-4′-fluoro-[1,1′-biphenyl]-4-yl)-4-phenyl-1H-1,2,3-triazole (EFT, **3a**) and 1-(4-(benzo[*b*]thiophen-2-yl) phenyl)-4-phenyl-1H-1,2,3-triazole (**3j**) were identified as lead anticancer compounds with an IC_{50} values of 1.69 and 14.4 μM for MCF7 cells respectively. Further, we evaluated the effect of EFT on Ishikawa, MDA-MB-231 and BT474 cells and IC_{50} values were found to be 1.97, 4.81 and 4.08 μM respectively. In addition, we also evaluated the effect of new compounds on the viability of MCF7 cells at different time points (24, 48 and 72 h) at 25 μM. The results revealed that EFT significantly decreases the cell viability of MCF7 in a time-dependent manner (Fig. 2A). However, 1,2,4-triazolo-pyrimidines did not induce significant growth inhibition in the tested cells at 25 μM for 72 h. Further, we tested the effect of EFT on normal lung epithelial (BEAS-2B) cells at 100 μM for 72 h using MTT assay as described earlier.[24,25] The lead compound, EFT, did not induce significant cytotoxicity against the tested non-diseased cells (Fig. 2B).

EFT reduces the expression of VEGFR1 in breast cancer cells: Kiselyov and co-workers described triazole derivatives as potent and ATP