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## Design, Synthesis, Cytotoxicity and Molecular Docking Studies of Novel Baylis-Hillman Derived 1,2,3-Triazole Derivatives

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### ABSTRACT

A series of new 1,4-disubstituted 1,2,3-triazole derivatives are synthesized from Baylis-Hillman derived azides in a clean, efficient and simple method. These compounds are assessed for *in vitro* anticancer activity against six cancer cell lines and some of the compounds exhibited very good activity against different cell lines. Especially, three compounds 8h, 8i and 8j showed potent anticancer activity against prostate adenocarcinoma cancer cell line. Furthermore, molecular docking studies were carried out on DNA and eight different proteins involved in cancer progression to predict the possible molecular target for the synthesized molecule. As per docking results the molecules showed good binding values with respect to Heat Shock Protein and Epidermal Growth Factor Receptor, followed by Tubulin and MEK protein and is least for DNA binding.

**Keywords:** Baylis Hillman azides, 1,3-dipolar cycloaddition, 1,4-disubstituted 1,2,3-triazoles, Anticancer activity, Docking studies.

### INTRODUCTION

Cancer has become a major health problem throughout the world wide. Among different types of cancer, Prostate cancer, a malignant disease without curative therapy was the most commonly diagnosed cancer for aged men in advanced countries [1-3]. In the past years many novel target molecules including small molecular inhibitors of heat shock protein have been developed for the treatment of prostate cancer [4-6]. As we know that always heterocyclic compounds have attracted special attention of organic chemist due to their broad range of biological activities [7]. 1,2,3-Triazole is an important biological active heterocyclic scaffold due to its simple synthetic approach *via* click chemistry [8]. The copper catalysed 1,3-dipolar cycloaddition of substituted azides and alkynes afford regioselective 1,4-disubstituted 1,2,3-triazole with high yields. Triazole nucleus holds high stability and capability to bind the target bio molecules through hydrogen bonding. These scaffolds have shown interesting biological properties such as antidiabetic [9], antitubercular [10,11], anti-inflammatory [12], antibacterial [13,14], antifungal [15-17], and antiviral [18,19]. Triazoles also present in herbicides [20] and dyes [21]. Many drugs like Anastrozole and Letrozole contain 1,2,3-triazole moiety in their structure are available in the market. Moreover, Carboxyamidotriazole (CAI) used as calcium channel blocker and preclinical studies demonstrates anti-tumour activity (Figure 1) [22].

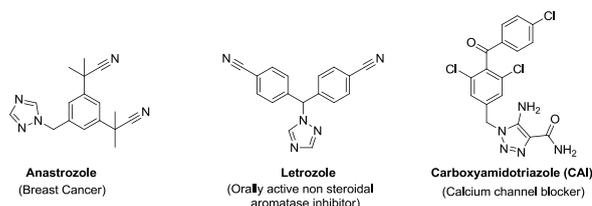


Figure 1: Triazole containing drugs in the market

In recent years, Baylis-Hillman reaction [23-27] has been recognized as a useful and emerging reaction having enormous synthetic potential with various biological activities. The above investigation encouraged us to incorporate triazole ring in the Baylis-Hillman matrix to increase its potency for the synthesis of anticancer agents. In continuation of our research on the synthesis of heterocyclic compounds and their bioevaluation [28-31], herein, we describe the preparation of a series of new triazole derivatives from Baylis-Hillman allyl azides in an efficient and straightforward manner. Furthermore, these compounds are evaluated for their *in vitro* anticancer activity against six cancer cell lines. Four compounds exhibited potent anticancer activity out of 10 synthesized compounds. Biological evaluation and computational studies of these compounds are clearly discussed elsewhere.

## MATERIALS AND METHODS

### Experimental

#### General

All reactions involving air-sensitive reagents were performed under nitrogen atmosphere. Solvents were freshly dried and purified by conventional methods prior to use. The progress of all the reactions were monitored by TLC, using TLC aluminium sheets precoated with silica gel 60 F254 to a thickness of 0.25 mm (Merck). Flash column chromatography was done using silica gel (Merck, 60-120 mesh). Melting points were determined on a MEL-TEMP II melting point apparatus and were uncorrected. IR spectra were recorded on a Perkin-Elmer FTIR spectrophotometer. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were recorded on a Varian Gemini 200 MHz, Bruker Avance 300 MHz spectrometer; TMS was used as an internal standard and CDCl<sub>3</sub>/DMSO-d<sub>6</sub> are used as solvents. Mass spectra were recorded on VG Micromass 7070 H (EI), QSTAR XL High resolution mass spectrometer (HRMS), Thermofinnigan ESI ion trap Mass spectrometer.

**General procedure for the 1,3-dipolar cycloaddition reactions, 8a-j:** A mixture of alkyne 7 (1 eq.), BH azide (1 eq.), sodium ascorbate (0.2 eq), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.1 eq) in 2: 1 *t*-BuOH/H<sub>2</sub>O (10 ml/1 mmol) was vigorously stirred at room temperature for 20 h (monitored by TLC). After disappearance of alkyne, the mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with water, dried under Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo. The crude residue was recrystallized with methanol to afford the corresponding pure triazoles in yields 80-87%. All the products are well characterized by spectroscopic data.

#### Characterization data for 1,4-disubstituted triazoles (8a-j)

**(E)-methyl 2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(*o*-tolyl)acrylate (8a):** Cream solid (80%), M. p. 128 °C-130 °C, IR (film): 3393, 3264, 2951, 2923, 1711, 1566, 1435, 1368, 1338, 1291, 1258, 1220, 807, 792, 751 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 8.12 (s, 1H), 7.61 (s, 1H), 7.52-7.51 (d, 1H, *J*=6.5 Hz), 7.29-7.24 (m, 3H), 6.35 (s, 1H), 5.54 (bs, 1H), 5.21 (s, 2H), 4.72 (s, 2H), 3.79 (s, 3H), 2.29 (s, 9H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.4, 166.7, 161.8, 145.0, 136.9, 132.9, 130.2, 129.6, 126.7, 126.1, 122.8, 110.1, 52.4, 46.6, 37.0, 29.6, 23.8, 19.9. ESI-MS: *m/z*: 393 (M+H)<sup>+</sup>. Anal. calcd for C<sub>21</sub>H<sub>24</sub>N<sub>6</sub>O<sub>2</sub>: C 64.27; H 6.16; N 21.41; found C 64.27; H 6.20; N 21.45.

**(E)-methyl 3-(4-chlorophenyl)-2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)acrylate (8b):** Cream solid (83%), M. p. 115 °C-117 °C, IR (film): 3266, 2925, 1703, 1566, 1490, 1311, 1175, 1013, 794 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 8.28-8.25 (d, 2H, *J*=6.3 Hz), 8.05 (s, 1H), 7.75 (s, 1H), 7.70-7.65 (t, 1H, *J*=7.9 Hz), 7.26 (s, 2H), 6.35 (s, 1H), 5.54 (bs, 1H), 4.75-4.73 (d, 2H, *J*=6.1 Hz), 3.84 (s, 3H), 2.30 (s, 6H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=161.5, 166.3, 161.7, 145.9, 144.1, 136.1, 132.0, 131.1, 129.2, 125.9, 123.1, 110.1, 61.6, 46.5, 37.1, 29.7, 23.8, 14.1. ESI-MS: *m/z*: 411 (M+H)<sup>+</sup>. Anal. calcd for C<sub>20</sub>H<sub>21</sub>ClN<sub>6</sub>O<sub>2</sub>: C 58.18; H 5.13; N 20.35; found C 58.21; H 5.20; N 20.39.

**(E)-ethyl 3-(3-chlorophenyl)-2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)acrylate (8c):** Cream solid (82%), M. p. 141 °C-143 °C, IR (film): 3400, 2925, 1707, 1585, 1567, 1250, 1214, 1044, 1022, 792, 711, 685 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm)=7.96 (s, 1H), 7.70 (s, 1H), 7.64-7.62 (m, 1H), 7.59 (s, 1H), 7.39-7.38 (m, 2H), 6.33 (s, 1H), 6.08 (bs, 1H), 5.29 (s, 2H), 4.75-4.74 (d, 2H, *J*=6.0 Hz), 4.27-4.23 (q, 2H, *J*=7.1, 14.2 Hz), 2.29 (s, 6H), 1.29-1.26 (t, 3H, *J*=7.1 Hz); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.4, 166.1, 161.6, 145.9, 143.6, 135.4, 134.8, 129.7, 129.5, 127.8, 127.5, 126.8, 126.2, 124.7, 123.1, 110.1, 61.6, 46.5, 37.0, 29.6, 29.3, 23.7, 14.1. ESI-MS: *m/z*: 427 (M+H)<sup>+</sup>. Anal. calcd for C<sub>21</sub>H<sub>23</sub>ClN<sub>6</sub>O<sub>2</sub>: C 59.08; H 5.43; N 19.69; found C 59.10; H 5.47; N 19.75.

**(E)-ethyl 3-(2-chlorophenyl)-2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)acrylate (8d):** Cream solid (87%), M. p. 140 °C-142 °C, IR (film): 3258, 1709, 1568, 1438, 1367, 1338, 1297, 1285, 1095, 766 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm)=8.12 (s, 1H), 7.84-7.82 (q, 1H, *J*=3.7, 5.5 Hz), 7.67 (s, 1H), 7.45-7.44 (q, 1H, *J*=3.5, 5.6 Hz), 7.36-7.34 (q, 2H, *J*=3.5, 5.7 Hz), 6.35 (s, 1H), 5.38 (bs, 1H), 5.21 (s, 2H), 4.73-4.72 (d, 2H, *J*=5.3 Hz), 4.27-4.23 (q, 2H, *J*=7.1, 14.3 Hz), 2.29 (s, 6H), 1.29-1.27 (t, 3H, *J*=7.1 Hz); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.4, 165.9, 161.9, 142.2, 134.1, 132.3, 130.8, 129.6, 127.5, 127.2, 123.1, 110.2, 61.6, 46.7, 37.1, 23.9, 14.1. ESI-MS: *m/z*: 427 (M+H)<sup>+</sup>. Anal. calcd for C<sub>21</sub>H<sub>23</sub>ClN<sub>6</sub>O<sub>2</sub>: C 59.08; H 5.43; N 19.71; found C 59.10; H 5.45; N 19.80.

**(E)-methyl 3-(2,6-dichlorophenyl)-2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)acrylate (8e):** Cream solid (86%), M. p. 130 °C-132 °C, IR (film): 3264, 2952, 1721, 1584, 1568, 1430, 1338, 1211, 1218, 1044, 775 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm)=7.79 (s, 1H), 7.78-7.70 (m, 1H), 7.59-7.52 (m, 2H), 7.39-7.31 (t, 1H), 6.39 (s, 1H), 5.32 (s, 2H), 4.69-4.67 (d, 2H, *J*=6.1 Hz), 3.81 (s, 3H), 2.31 (s, 6H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.3, 165.4, 161.7, 145.9, 139.4, 133.6, 131.5, 130.6, 130.4, 128.1, 121.9, 109.9, 52.6, 46.7, 36.8, 23.7. ESI-MS: *m/z*: 447 (M+H)<sup>+</sup>. Anal. calcd for C<sub>20</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>6</sub>O<sub>2</sub>: C 53.70; H 4.51; N 18.79; found C 53.72; H 4.54; N 18.80.

**(E)-methyl 3-(3-bromophenyl)-2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)acrylate (8f):** Cream solid (81%), M. p. 182 °C-184 °C; IR (film): 3267, 2923, 1712, 1585, 1568, 1466, 1211, 1216, 1045, 771, 668 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm)=7.91 (s, 1H), 7.81-7.70 (m, 3H), 7.59-7.51 (d, 1H, *J*=8.9 Hz), 7.39-7.32 (t, 1H, *J*=7.8 Hz), 6.38 (s, 1H), 5.32 (s, 2H), 4.76-4.73 (d, 2H, *J*=6.1 Hz), 3.81 (s, 3H), 2.31 (s, 6H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.2, 166.7, 166.6, 161.0, 139.6, 137.9, 136.1, 132.1, 130.8, 130.7, 128.8, 127.5, 123.0, 122.9, 120.1, 52.3, 49.6, 42.3, 24.2, 24.1. ESI-MS: *m/z*: 457 (M+H)<sup>+</sup>. Anal. calcd for C<sub>20</sub>H<sub>21</sub>BrN<sub>6</sub>O<sub>2</sub>: C 51.48; H 4.32; N 18.96; found C 51.52; H 4.54; N 18.80.

**(E)-methyl 2-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(3-nitrophenyl)acrylate (8g):** Cream solid (83%), M. p. 183 °C-185 °C, IR (film): 3267, 2923, 1712, 1585, 1568, 1466, 1211, 1216, 1045, 771, 668 cm<sup>-1</sup>; <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ (ppm)=8.41 (s, 1H), 8.28-8.26 (d, 2H, *J*=6.3 Hz), 8.05 (s, 1H), 7.75 (s, 1H), 7.70-7.65 (t, 1H, *J*=7.9 Hz), 6.35 (s, 1H), 5.39 (s, 1H), 5.27 (s, 2H), 4.75-4.73 (d, 2H, *J*=6.1 Hz), 3.84 (s, 3H), 2.30 (s, 6H); <sup>13</sup>C-NMR (75 MHz, CDCl<sub>3</sub>) δ (ppm)=167.5, 166.3, 161.8, 148.4, 142.6, 135.2, 130.2, 128.0, 124.5, 124.3, 123.4, 110.2, 52.7, 46.3, 37.1, 23.9. ESI-MS: *m/z*: 424 (M+H)<sup>+</sup>. Anal. calcd for C<sub>20</sub>H<sub>21</sub>N<sub>7</sub>O<sub>4</sub>: C 56.73; H 5.00; N 23.16; found C 56.80; H 5.09; N 23.19.

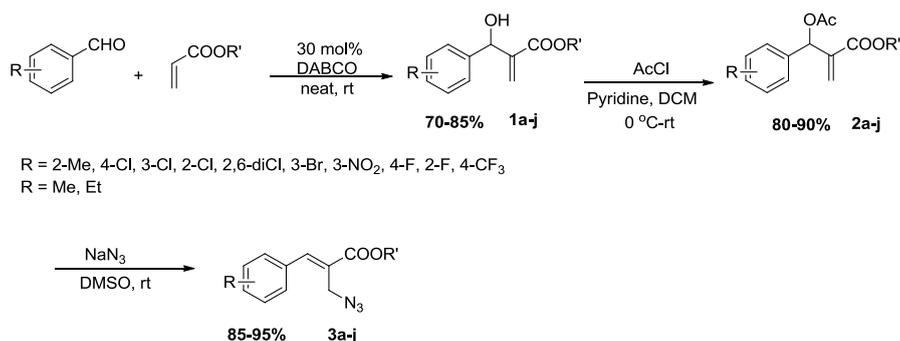
**(E)-methyl 2-((4-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(4-fluorophenyl)acrylate (8h):** Pale greenish solid (80%), M. p. 147 C-149 C, IR (film): 3264, 2911, 1708, 1567, 1509, 1436, 1297, 1161, 1045, 793, 770  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm)=7.96 (s, 1H), 7.74-7.67 (m, 3H), 7.55-7.53 (d, 1H,  $J=8.9$  Hz), 7.32-7.31 (t, 1H,  $J=7.9$  Hz), 6.34 (s, 1H), 5.82 (bs, 1H), 5.29 (s, 2H), 4.75-4.74 (d, 2H,  $J=5.9$  Hz), 3.81 (s, 3H), 2.30 (s, 6H);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm)=167.4, 166.9, 165.2, 161.9, 144.6, 132.0, 131.9, 129.7, 129.6, 116.2, 115.9, 52.5, 46.6, 37.0. ESI-MS:  $m/z$ : 397 (M+H) $^+$ . Anal. calcd for  $\text{C}_{20}\text{H}_{21}\text{FN}_6\text{O}_2$ : C 60.60; H 5.34; N 21.20; found C 60.52; H 5.52; N 21.50.

**(E)-ethyl 2-((4-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(2-fluorophenyl)acrylate (8i):** Pale greenish solid (87%), M. p. 138 C-140 C, IR (film): 3261, 3019, 1708, 1568, 1411, 1215, 768, 752, 667  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 (s, 1H), 7.83-7.82 (t, 1H,  $J=5.5$  Hz), 7.67 (s, 1H), 7.45-7.43 (m, 1H), 7.35-7.34 (m, 2H), 6.35 (s, 1H), 5.39 (s, 1H), 5.21 (s, 2H), 4.73-4.72 (d, 2H,  $J=7.1$ , 14.3 Hz), 4.27-4.23 (q, 2H,  $J=7.1$ , 14.3 Hz), 2.29 (s, 6H), 1.29-1.27 (t, 3H);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  167.4, 166.0, 162.1, 161.9, 158.8, 138.0, 137.9, 131.7, 131.6, 130.8, 127.6, 124.7, 124.6, 121.8, 121.6, 115.8, 110.1, 61.6, 46.9, 37.1, 23.8, 14.1. ESI-MS:  $m/z$ : 411 (M+H) $^+$ . Anal. calcd for  $\text{C}_{21}\text{H}_{23}\text{FN}_6\text{O}_2$ : C 61.45; H 5.65; N 20.48; found C 61.50; H 5.70; N 20.50.

**(E)-methyl 2-((4-(((4,6-dimethylpyrimidin-2-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)methyl)-3-(4-(trifluoromethyl)phenyl)acrylate (8j):** Cream solid (86%), M. p. 140 C-142 C, IR (film): 3019, 1712, 1587, 1324, 1214, 1133, 1068, 744, 667  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm)=8.04 (s, 1H), 7.81-7.79 (d, 1H,  $J=8.4$  Hz), 7.73-7.72 (d, 2H,  $J=3.5$  Hz), 7.69 (s, 1H), 6.35 (s, 1H), 5.46 (s, 1H), 5.27 (s, 2H), 4.75-4.73 (d, 2H,  $J=5.8$  Hz), 3.82 (s, 3H), 2.29 (s, 6H);  $^{13}\text{C-NMR}$  (75 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm)=167.4, 166.5, 161.9, 146.1, 143.8, 137.0, 131.2, 129.8, 127.2, 125.8, 125.7, 125.5, 123.2, 121.9, 110.1, 52.6, 46.4, 37.0, 23.8. ESI-MS:  $m/z$ : 447 (M+H) $^+$ . Anal. calcd for  $\text{C}_{21}\text{H}_{21}\text{F}_3\text{N}_6\text{O}_2$ : C 56.50; H 4.74; N 18.83; found C 56.53; H 4.67; N 18.89.

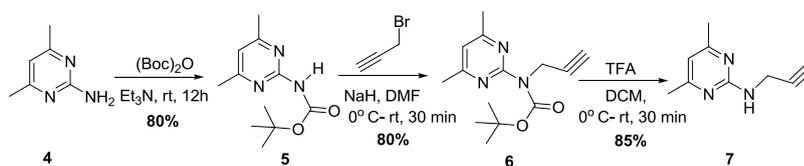
## RESULTS AND DISCUSSION

The target compounds 1,4-disubstituted triazoles are attained from allyl azides of Baylis-Hillman (BH) adducts. For this, various Baylis-Hillman allyl azides are required to accomplish the target work. In this regard, several BH adducts were synthesized by treating various aldehydes with acrylates in presence of 30 mol% DABCO as a catalyst under solvent free conditions. Thus synthesized Baylis-Hillman adducts (1a-j) data were in good agreement with the reported data [32,33]. The obtained BH adducts were subjected to acetylation using acetyl chloride and pyridine at 0°C to give corresponding BH acetates 2a-j [34]. The BH acetates were then treated with  $\text{NaN}_3$  in DMSO to produce corresponding azides (3a-j) in good yields [35]. The synthetic strategy was as shown in Scheme 1.



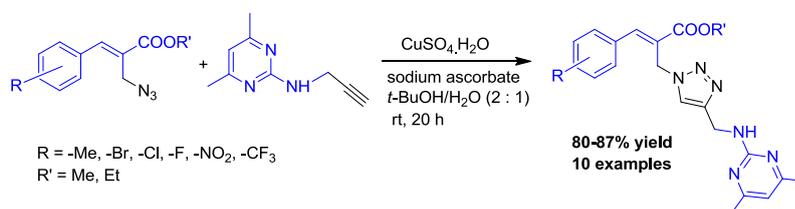
Scheme 1: Synthesis of Baylis-Hillman derived allyl azides

On the other hand, 4,6-dimethyl-2-amino pyrimidine (4) was taken as starting material which was subjected to Boc protection followed by treatment with propargyl bromide in presence of sodium hydride and finally on Boc deprotection using TFA to produce 2-alkynylamino derivative, 7 outlined in the Scheme 2.



Scheme 2: Synthesis of 2-alkynylamino dimethyl pyrimidine

Having alkyne and azide partners in hand, we investigated the click assembling reaction. According to the standard procedure, we performed 1,3-dipolar cycloaddition with a slight excess of azido reactant, hydrated  $\text{CuSO}_4$  as copper source and sodium ascorbate as reducing agent. Thus, as expected the Cu (I) catalyzed version of Huisgen reaction, these conditions produced 1,4-disubstituted triazoles 8a-j as single products (Scheme 3).



Scheme 3: Synthesis of 1,4-disubstituted triazole derivatives

We also found out that *t*-BuOH/H<sub>2</sub>O as the best solvent system and most of the click reactions were completed within 20 h at room temperature with very good yields (Table 1).

**Table 1: Synthesis of 1,4-disubstituted triazole derivatives (8a-j)**

S. No.	R	R'	Yield (%)	M. P. (°C)
8a	2-Me	Me	80	128-130
8b	4-Cl	Me	83	115-117
8c	3-Cl	Et	82	141-143
8d	2-Cl	Et	87	140-142
8e	2,6-diCl	Me	86	130-132
8f	3-Br	Me	81	182-184
8g	3-NO <sub>2</sub>	Me	83	183-185
8h	4-F	Me	80	147-149
8i	2-F	Et	87	138-140
8j	4-CF <sub>3</sub>	Me	86	140-142

The origin of the cell lines used in the current study and the culture media are fully detailed in references [36]. The *in vitro* cytotoxicity of triazole derivatives were determined in selected human cancer cell lines of MCF-7 (Human ER+/Pr+/Her2 breast cancer cell line), MDA-MB-231 (Human ER+/Pr+/Her2 breast cancer cell line), and HepG2 (Human hepatocellular carcinoma cell line), Mia-PaCa-2 (Human pancreatic carcinoma cell line), HeLa (Human cervical cancer cell line), DU-145 (Human prostate adenocarcinoma cell line) cells as well as control cells-HEK293 (Human embryonic kidney cell line) were seeded on to 96 well plates at a cell density of  $5 \times 10^3$  cells/well in 100  $\mu$ l of complete medium and incubated at 37°C for 24 h. Cells were treated with compounds at increasing concentrations (0.01, 0.1, 1, 10 and 100  $\mu$ m) for 48 h followed by fixing with 40  $\mu$ l of 20% TCA and incubated at 4°C for 1 h. Subsequently, plates were washed with deionized water for five times, air dried for 24 h and stained with 0.4% of SRB (40  $\mu$ l) prepared in 1% acetic acid solution followed by incubation at room temperature in dark for 20 min. Then cells were washed with 1% acetic acid solution thrice after removing SRB and plates were dried for 4 h. Tris base (100  $\mu$ l) was added to each well to solubilise the bound SRB and absorbance was measured at 510 nm using multimode plate reader. Doxorubicin was used as a positive control. Percent inhibition was calculated for all the compounds followed by IC<sub>50</sub> analysis using Graph Pad Prism software 6.0.

The IC<sub>50</sub> Values were listed in Table 2 and the wellknown anticancer drug Doxorubicin was used as positive control. Based on the screening results, It was observed that compound 8h, 8i and 8j with IC<sub>50</sub> values 0.0948  $\mu$ m, 0.0372  $\mu$ m and 0.248  $\mu$ m are proved to be most potent to anticancer activity against DU145 compared to standard drug Doxorubicin (IC<sub>50</sub> 0.48  $\mu$ m). Compound 8j also exhibited good anticancer activity against HeLa cell line. Similarly compound 8i and 8j showed moderate to good cytotoxic activity against HeLa, HepG2, MCF7, and MiaPaCa-2 cell lines. Compound 8g and 8h showed moderate activity against MDA-MB-231 and HepG2. In compounds 8h, 8i and 8j by introducing fluoro group and CF<sub>3</sub> group on phenyl ring had increased a profound influence on anticancer activity. Compounds 8a-8f showed no substantial cytotoxicity against all tested cell lines. From the above analysis, it is suggested that the introduction of substituted group on phenyl ring may play a key role in determining activity. Introducing groups like Cl, Br, NO<sub>2</sub>, and CH<sub>3</sub> on phenyl ring resulted in a dramatic drop of potency.

**Table 2: IC<sub>50</sub> values ( $\mu$ M) of synthesised compounds against various cell lines**

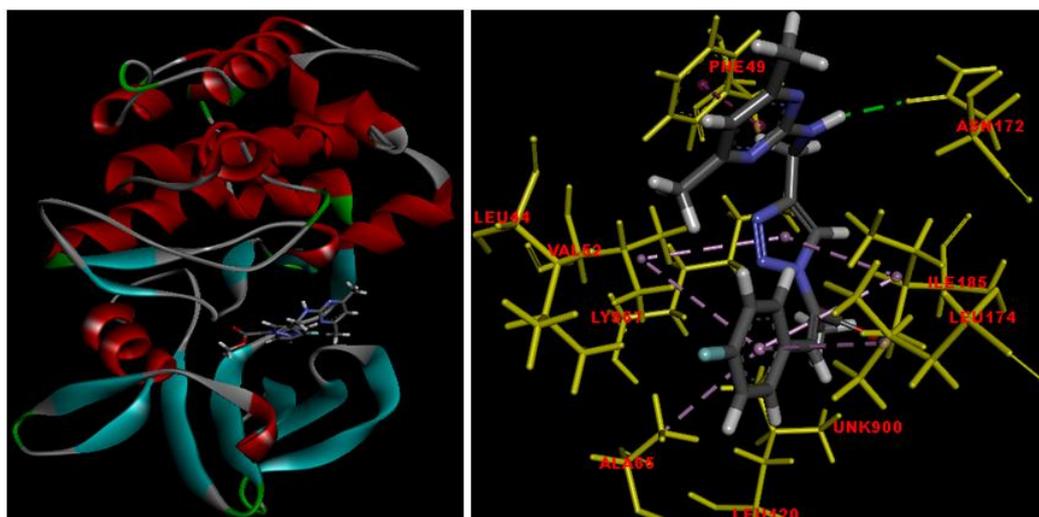
Compounds	HEK293	DU145	HeLa	HepG2	MCF7	MiaPaCa-2	MDA-MB-231
	(control cells)						
8a	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
8b	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100
8c	NI	≥ 100	≥ 100	≥ 100	≥ 100	≥ 100	NI
8d	≥ 100	≥ 100	≥ 100	55.33	NI	≥ 100	NI
8e	≥ 100	≥ 100	≥ 100	≥ 100	NI	≥ 100	NI
8f	NI	≥ 100	≥ 100	NI	NI	≥ 100	NI
8g	≥ 100	≥ 100	≥ 100	NI	NI	≥ 100	29.07
8h	≥ 100	0.0948	≥ 100	≥ 100	NI	79.41	≥ 100
8i	8.559	0.03716	3.607	61.23	40.94	11.62	NI
8j	2.719	0.248	1.546	9.25	19.24	25.35	NI
Doxorubicin	34.5	0.48	0.9	3.3	2.4	0.7273	1.1

Molecular docking studies of synthesized triazole molecules were initially carried out on DNA, to determine their binding affinity towards DNA intercalation as compared to doxorubicin [37]. All the molecules showed very low binding affinity towards DNA, indicating poor interaction with DNA base pairs. Subsequently, proteins known to be involved in cancer progression were selected for molecular docking studies. VEGFR-2 and EGFR are known targets for anticancer agents that are extensively studied [38], these were downloaded from protein data bank. Along with VEGFR-2 and EGFR few other proteins reported to be involved in prostate cancer progression were also selected, like HSP90, PIM-1 Kinase, Serine protease Hepsin, androgen receptor, 17 $\beta$ -hydroxysteroid dehydrogenase type 5 (AKR1C3), and cytochrome P450 17A1 [39-43]. Anticancer studies on the synthesized molecules showed some favorable results in terms of DU145 cell line, wherein molecules 8h, 8i and 8j

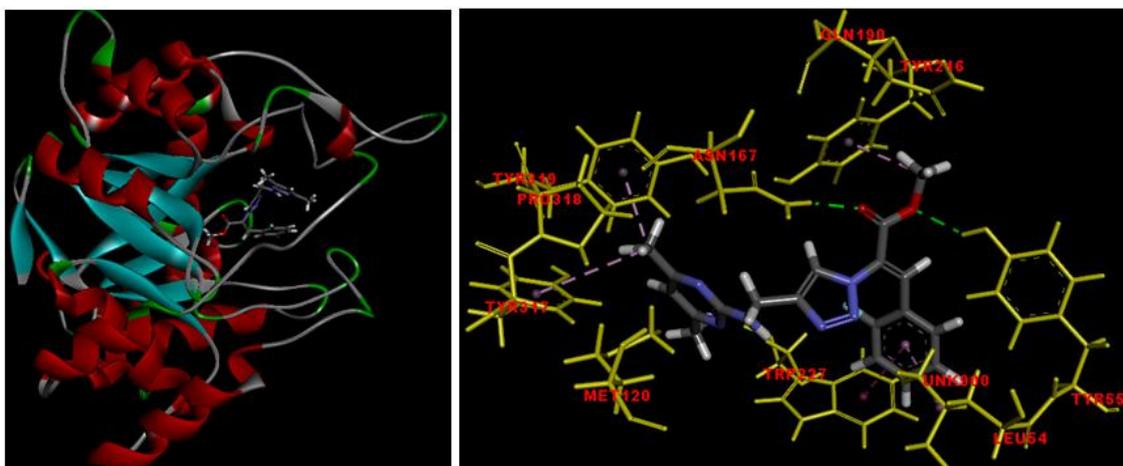
showed better cytotoxicity than the standard doxorubicin. Docking results also showed affinity of these molecules towards proteins involved in prostate cancer progression, in particular to Heat Shock Protein 90, 17  $\beta$ -hydroxysteroid dehydrogenase type 5 (AKR1C3) and PIM1 kinase (Dock score are provided in Table 3). Molecule 8h showed highest dock score of -9.568 kcal/mol with PIM-1 kinase and, molecule 8i showed highest dock score of -9.205 kcal/mol with AKR1C3. In case of docking studies on HSP90 all molecules showed dock score above -8.0 kcal/mol. Figures 2-4 represent the dock pose and molecular interaction of molecule 8h, 8i and 8j with PIM-1 kinase, AKR1C3 and HSP90 respectively.

**Table 3: Dock score of molecules in Heat Shock Protein 90, 17  $\beta$ -hydroxysteroid dehydrogenase type 5 (AKR1C3) and PIM-1 kinase**

Molecule	Dock score in kcal/mol		
	HSP90 (PDB id: 5CF0,)	PIM-1 Kinase (PDB id: 5V82,)	AKR1C3 (PDB id: 4DBS)
8a	-9.836	-4.623	-8.552
8b	-8.094	-8.952	-8.877
8c	-9.102	-7.412	-8.634
8d	-9.659	-9.311	-8.487
8e	-8.587	-4.483	-8.32
8f	-9.797	-9.211	-8.521
8g	-8.732	-8.734	-7.791
8h	-9.11	-9.568	-8.159
8i	-9.49	-9.109	-9.205
8j	-9.71	-8.052	-8.747
Doxorubicin	-7.841	-12.82	-12.246



**Figure 2: [a] Molecule 8h docked at active site of PIM-1 kinase [b] Molecular interaction of molecule AS8 showing hydrogen bond interaction with Asn172,  $\pi$ - $\pi$  interaction with Phe49 and hydrophobic interaction Val52, Ala65, Leu174, Ile185**



**Figure 3: [a] Molecule 8i docked at active site of AKR1C3 [b] Molecular interaction of molecule AS9 showing hydrogen bond interaction with Asn167 and Tyr55,  $\pi$ - $\pi$  interaction with Trp227 and hydrophobic interaction Leu54, Tyr317, Tyr319 and Tyr216**

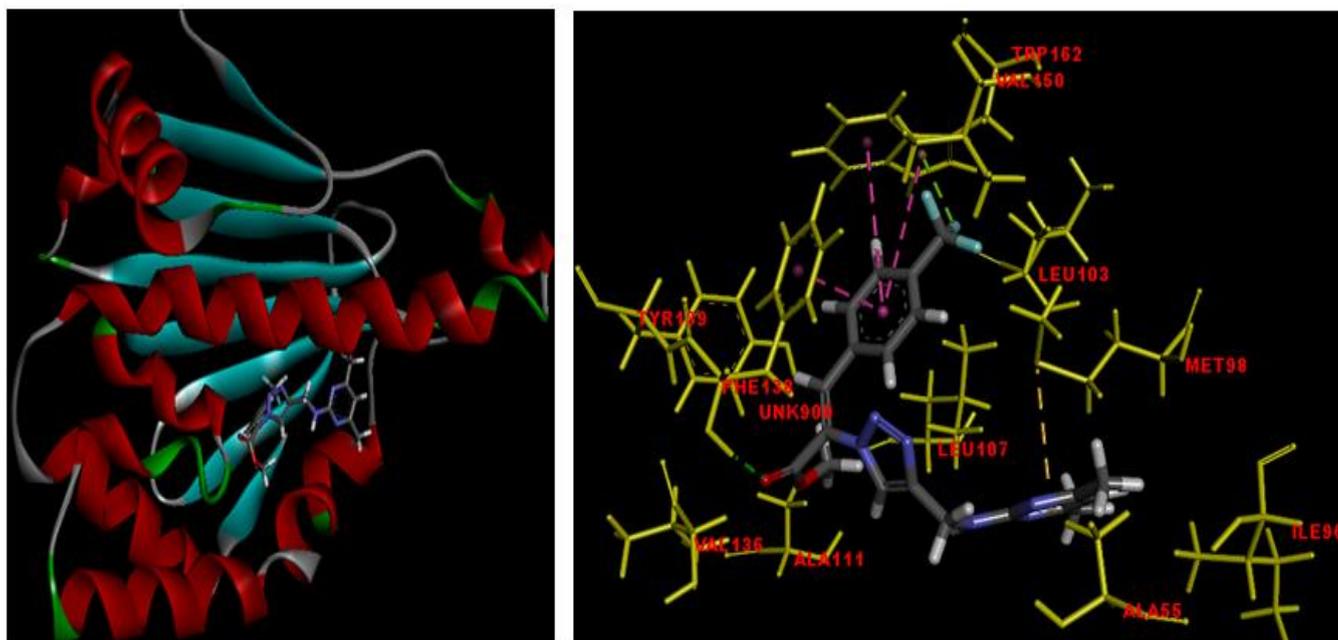


Figure 4: [a] Molecule 8j docked at active site of HSP90 [b] Molecular interaction of molecule AS10 showing hydrogen bond interaction with Phe138,  $\pi$ - $\pi$  interaction with Trp162 and Phe138, and electrostatic interaction with Met98

#### ADME properties

The ADME properties (required pharmacokinetic properties of viable drug compounds) were calculated by Qikprop and analyzed by applying Lipinski's rule of five (Table 4) [44]. Molecular weight less than 650, partition coefficient between octanol and water ( $\log P_{o/w}$ ) between  $-1$  and  $6.5$  and solubility ( $\log S$ ) greater than  $-7$ . Pmdck greater than  $5$  and  $\log BB$  greater than  $-3$ , this parameter tells us about the ability of the drug to pass through blood brain barrier. Majority of the molecules have the properties in range, partition coefficient of molecules ranged within the acceptable limits, solubility were in range except for molecule 8j that had values less than  $-7.0$ . Cell permeability and Blood Brain Barrier permeability for all molecules is in permissible range. Water solubility and oral absorption for half of the molecules is 100%. The values invariably imply that these molecules are potential drug molecules, which show better drug like property than standard doxorubicin and can be further optimized.

Table 4: Calculated ADME properties of synthesized molecules

Molecule	QP $\log P_{o/w}$ <sup>a</sup>	QPlogS <sup>b</sup>	QPlogBB <sup>c</sup>	QP <sub>PMDCk</sub> <sup>d</sup>	Percent Human
					Oral Absorption <sup>e</sup>
8a	4.042	-6.043	-1.104	413.472	100
8b	4.262	-6.658	-1.093	806.742	100
8c	4.232	-6.25	-0.97	839.299	100
8d	4.13	-6.197	-1.036	655.329	100
8e	4.576	-6.784	-0.877	1465.139	100
8f	4.247	-6.08	-0.922	856.135	100
8g	3.174	-5.238	-1.759	99.734	74.747
8h	4.004	-6.282	-1.138	591.231	100
8i	3.904	-6.031	-1.149	460.105	100
8j	4.743	-7.333	-1.005	1426.621	100
Doxorubicin	-0.533	-1.947	-2.889	1.06	0

<sup>a</sup>Predicted octanol/water partition coefficient  $\log P$  (acceptable range: 2.0-6.5); <sup>b</sup>Predicted aqueous solubility in mol/l (acceptable range: 6.5-0.5); <sup>c</sup>Predicted blood brain barrier permeability (acceptable range: 3-1.2); <sup>d</sup>Predicted apparent MDCK cell permeability in nm/s (acceptable range in nm/s (acceptable range: <25 is poor and >500 is great); <sup>e</sup>Percentage of human oral absorption (acceptable range: <25 is poor and >80% is high)

#### Molecular modeling studies

To understand the mode of binding and molecular interactions of 1,2,3-triazole derivatives, molecular docking studies were performed (Table 5). Crystal Structure of proteins were downloaded from protein data bank (PDB id: 1N37, 4AG8, 1WKQ, 5CE1, 5CF0, 5V82, 2YLP, 3SWZ, 4DBS) [45-51]. Interactions of the molecules with the proteins were analyzed to identify their hypothetical binding mode and binding affinity. All the molecular modeling calculations were performed using Schrödinger Suite 2010 on Linux platform. The proteins were prepared using protein preparation module applying the default parameters, hydrogen atoms were added and unwanted water molecules were removed from the protein structure followed by hydrogen bond optimization and energy minimization. Grid was generated around the active site (ligand binding

site) defined by the co-crystallized ligand. Receptor Vander Waals scaling for the nonpolar atoms was set to 0.9 [52]. Molecules were built using Maestro build panel and prepared by LigPrep module using OPLS\_2005 force field, GLIDE 5.6 was used for molecular docking. Low energy conformation of the ligands was selected and docked into the grid using extra precision (XP) docking mode. Further the absorption, distribution, metabolism and excretion (ADME) properties were calculated using Qikprop module in Schrodinger Suite.

**Table 5: Dock score of molecules in all the protein used in docking studies**

S. No.	Dock score in kcal/mol										
	1N37	4AG8	4WKQ	4ALW	5CE1	5CF0	5V82	2YLP	3SWZ	4DBS	5IRQ
1	-3.831	-7.804	-5.63	-6.26	-6.518	-9.836	-4.624	-3.966	-6.672	-8.553	-6.275
2	-5.008	-7.575	-6.419	-5.76	-6.514	-8.094	-8.952	-3.867	-6.4	-8.878	-6.944
3	-4.396	-7.871	-5.494	-5.898	-6.453	-9.103	-7.413	-5.373	-7.47	-8.635	-6.792
4	-3.946	-7.829	-4.972	-4.942	-6.477	-9.659	-9.312	-4.14	-6.724	-8.488	-6.494
5	-3.326	-6.616	-5.396	-4.724	-5.763	-8.587	-4.483	-3.22	-7.431	-8.32	-6.549
6	-2.941	-8.092	-6.651	-5.726	-7.249	-9.798	-9.212	-4.263	-8.532	-8.522	-6.83
7	-4.329	-6.298	-5.183	-5.411	-5.982	-8.732	-8.735	-2.617	-6.163	-7.792	-5.075
8	-4.612	-6.958	-5.622	-6.136	-5.958	-9.11	-9.568	-4.952	-5.495	-8.16	-6.313
9	-4.211	-7.81	-6.721	-5.66	-5.719	-9.491	-9.11	-6.108	-7.495	-9.206	-6.322
10	-5.075	-7.902	-6.151	-8.872	-5.565	-9.71	-8.053	-3.492	-6.414	-8.748	-4.648
DOX	-11.258	-12.456	-14.423	-10.315	-7.396	-7.842	-12.82	-11.34	----	-12.246	-7.719

## CONCLUSION

In conclusion, we demonstrated an efficient synthesis of a series of some novel Baylis-Hillman derived 1,2,3-triazole derivatives. The structures of newly synthesized compounds were characterized by IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and mass spectral analysis and also were screened for *in vitro* anticancer activity against six cancer cell lines and promising results are obtained. Based on the screening results, it was observed that three compounds 8h, 8i and 8j are proved to be most potent to anticancer activity against prostate cancer cell line (DU145) compared with reference to standard drug Doxorubicin. Additionally, molecular docking studies were carried out on DNA to predict the possible molecular target for the synthesised molecule and the values invariably imply that these molecules are potential drug candidates. Synthesis of nitrile containing Baylis Hillman target compounds and its library with further biological activity of these compounds is being studied and will be reported in due course.

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