Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

# BIOACTIVITY OF 1, 4-DISUBSTITUTED 1, 2, 3-TRIAZOLES AS CYTOTOXIC AGENTS AGAINST THE VARIOUS HUMAN CELL LINES

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**ABSTRACT**

In view of the potentiality of the triazole nucleus in eliciting various pharmacological properties in particular anticancer potency and the lipophilicity of the substituent groups contributing to influence the bioactivity, has been proposed to synthesize the series of 1, 4-disubstituted 1,2,3-triazoles by utilizing click chemistry, in a one pot synthesis and evaluate them for anticancer activity. A series of 1, 4-disubstituted 1,2,3-triazole analogues of phenyls have been prepared. The triazoles 4-(Phenyl-1-(1- phenyl-ethyl)-1H-(1, 2,3) triazole (compound-RK) displayed potent cytotoxic activity against several cancer cell lines with IC50 values in the microomolar range. Phenyl substituent at fourth position and α-methyl benzyl group at first position exhibited the highest activity against HL 60 cells with $IC_{50}$ values of 1.15 µM.

**Keywords:** 1, 4-Disubstituted 1, 2, 3-triazoles; Cytotoxic agents; Bioactivity; Human Cell lines; Anticancer.

## INTRODUCTION

Search for novel medicinally active compounds and optimization of fast and efficient approaches to synthesize them with desired function has drawn considerable attention in recent years. Cu (I) catalyzed ligation of organic azides and terminal alkynes, which belong to a group of reactions, referred to as click chemistry has enjoyed much use since its discovery. Triazoles are known to possess remarkable biological properties such as, anti-allergic[1], anti-bacteria[2], anti-HIV[3], anti-viral, anti-epileptic activities and applications in drug discovery, bioconjugates, medicinal chemistry as well as materials chemistry[4].

In view of the potentiality of the triazole nucleus in eliciting various pharmacological properties in particular anticancer potency and the lipophilicity of the substituent groups contributing to influence the bioactivity, has been proposed to synthesize the series of 1,4-disubstituted 1,2,3-triazoles by utilizing click chemistry[5], in a one pot synthesis and evaluate them for anticancer activity. Filippo Minutolo, *et al*[6] reported the Synthesis of a Resveratrol analogue with High Ceramide-mediated proapoptotic activity on human breast cancer cells. All Resveratrol analogues display antiproliferative effect in MDA-MB-231 cancer cells (Figure 1).

Francesca Pagliai, et al[7] reported the rapid synthesis of triazole-modified Resveratrol analogues via click chemistry. They decided to replace the double bond with a triazole ring using the archetypical click reaction: the Huisgen [3 + 2] cycloaddition. Seventy-two triazole derivatives were synthesized via a parallel combinatorial approach. Preliminary data suggest that this procedure can lead to the synthesis of compounds that display some, but not all, of resveratrol's actions with increased potency (Figure 2).

The present synthesis was effected by using appropriately substituted acetylenes, $TMSN_3$ and appropriately substituted secondary alcohols as shown in scheme I.

## METHODS

### Cell lines, media, chemicals and reagents

Cell lines used in this study, Hep G2 (human, liver), Hela, HL-60 (human promyelocytic leukemia). These cell lines were obtained from National center for Cell science (NCCS), Pune, India. DMEM (Dulbeccos Modified Eagles Medium), RPMI-1640, MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide],Trypsin, EDTA were purchased from Sigma Chemicals Co (st.Louis, MO), Fetal bovine serum were purchased from Arrow labs,96 well flat bottom tissue culture plates were purchased from Tarson.

### Maintenance of cell lines

The HL-60 cell line was grown in suspension in RPMI-1640medium supplemented with 10% fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml streptomycin, 2mM L-glutamine. Hep-G2 cell line was grown as adherent in DMEM media supplemented with 10% fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml streptomycin, 2mM L-glutamine, 0.1 mM non essential amino acids, 1 mM sodium pyruvate. Hela cell line was grown as adherent in DMEM media supplemented with 10% fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml streptomycin, 2mM L-glutamine all the above three cultures were maintained in a humidified atmosphere with 5% $CO_2$.

### Preparation of samples for cytotoxicity

20 mM stock solutions were prepared in DMSO. This stock solutions was further diluted with DMSO before addition to the cell culture medium at a final DMSO concentration of 1%.

### MTT assay

### For adherent cell lines

Hep-G2 and Hela cell lines were seeded at a density of 2x $10^4$ cells (cell number was determined by Trypan blue exclusion dyemethod) per each well in 200µl of DMEM supplemented with 10% FBS. 12 hrs after seeding, above media was replaced with fresh DMEM supplemented with 10% FBS then various concentrations of compounds (200µm, 100µM, 50µM, 10µM and 1µM were added to each well in triplicates) as a final concentration and 1% DMSO was added in triplicates as control. The above cells were incubated for 48 hrs at at 37$^0$C with 5% $CO_2$. After 48 hrs incubation the above media was replaced with 100 µl of fresh DMEM without FBS and to this 10 µl of MTT (5mg dissolved in 1ml of PBS) was added and incubated for 4 hrs at 37$^0$C with 5% $CO_2$. After 4 hrs incubation the above media was removed with multichannel pipette the 200 µl of DMSO was added to each well and the incubated at 37$^0$C for 15min. Finally the plate was readed at 560 nm using spectrophotometer (Spectra Max, Molecular devices).

### Non – adherent cell line

HL-60 cell line were seeded at a density of 5x $10^4$ cells (cell number was determined by Trypan blue exclusion dyemethod)per each well in 200µl of RPMI supplemented with 10% FBS. 12 hrs after seeding, above media was replaced with fresh RPMI supplemented with 10% FBS after centrifugation of plate at 1500 rpm for 5 minutes, then various concentrations of compounds (200µm, 100µM, 50µM, 10µM and 1µM were added to each well in triplicates) as a final concentration and 1% DMSO was added in triplicates as control. The above cells were incubated for 48 hrs at at 37$^0$C with 5% CO2. After 48 hrs incubation the above media was replaced with 100 µl of fresh

RPMI with out FBS after centrifugation of plate at 1500 rpm for 5 minutes, and to this 10 µl of MTT (5mg dissolved in 1ml of PBS) was added and incubated for 4 hrs at 37$^0$C with 5% CO2. After 4 hrs incubation, 100µl of 0.1N HCl in isopropanol was added to each well and incubated for 30 minutes at room temperature. Finally the plate was readed at 560 nm using spectrophotometer (Spectra Max, Molecular devices).

| Compound | Hela cell line IC50 µM | HepG2 cell line IC50 µM | HL 60 cell line IC50 µM |
|---|---|---|---|
| A | >200 | 228.55 | >200 |
| B | 126.31 | 224.58 | 201.2 |
| C | >200 | >200 | >200 |
| D | >200 | >200 | 242.29 |
| E | 84.55 | >200 | >200 |
| F | >200 | >200 | >200 |
| G | 148.52 | >200 | 9.16 |
| H | >200 | no activity | 219.29 |
| I | 148.12 | no activity | 201.10 |
| RK | 41.40 | 278.75 | 1.15 |

A=4-(4-Pentyl-phenyl)-1-(1-phenyl-ethyl)-1H-[1,2,3]triazole
B=1-(1-Naphthalen-2-yl-ethyl)-4-phenyl-1H-(1,2,3)triazole
C=4-(6-Methoxy-naphthalen-2-yl)-1-(1-phenyl-ethyl)-1H-[1,2,3]triazole
D=1-(1-Phenyl-ethyl)-4-thiophen-3-yl-1H-[1,2,3]triazole
E=1-(1-(4-Bromo-phenyl)-ethyl)4-phenyl-1H-(1,2,3) triazole
F=4-Phenyl-1-(1-phenyl-propyl)-1H-[1,2,3]triazole
G=1-(1-Phenyl-ethyl)-4-p-tolyl-1H-[1,2,3]triazole
H=4-(4-Methoxy-phenyl)-1-(1-phenyl-ethyl)-1H-[1,2,3]triazole
I=1-Benzhydryl-4-phenyl-1H-[1,2,3]triazole
RK=4-(Phenyl-1-(1- phenyl-ethyl)-1H-(1,2,3)triazole

**RESULTS AND DISCUSSION**

Cytotoxicity of various synthetic compounds with codes A, B, C, D, E, F, G, H, I, & RK were performed in three different cell lines (Hep G2, Hela, HL 60 cell lines) with protocol as follows.

Among all the tested compounds phenyl substituent at 4$^{th}$ position and α-methyl benzyl group at 1$^{st}$ position exhibited the highest activity against HL 60 cells with IC 50 values of 1.15 µM. Compound I also showed the greatest potency among all against Hela cells with IC50 values of 41.40 µM .Next in potency , the compound VII with P-tolyl substitutent at 4$^{th}$ position and α- methyl substitutent benzyl group at 1st position exhibited with IC50 values of 9.16 against HL-60 cell and 148.52 µM against Hela cells. The remaining eight compounds were found to be least potent and showed IC50 values above 200 µM against HL60 cells. Whereas compound II and III with β-naphthyl-1-ethyl and 4-bromophenyl substituent at position-1 and simple phenyl substituted at position-4 exhibited moderate potency against Hela cells ( IC50 values 126.30 and 84.5 µM ). Compound VI with diphenylmethyl group at 1$^{st}$ position showed equipotency to that of compound VII against the Hela cells. However none of the compounds tested showed significant inhibition of Hep G2 cells.

These result indicate that the methyl substitution at p-position of the 4-phenyl group significant decrease in the potency of the compound against HL60 cell lines. Similar decrease in potency was observed against Hela cell lines.

**Declaration of interest**

The authors are thankful to Management, MakroCare Clinical Research Ltd for providing the research grant. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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