Section: Research Paper

Inhibitory Effects of Substituted P-Acetamidobenzene Sulfonyl 1, 2, 3 -Triazoles on Colon Cancer

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ABSTRACT

The series of synthesised various-1,2,3-triazole 3a-3f & 5a-5i target compounds described here are designed as analogues for the anti-cancer drug Combretastatin A-4 with varied side substituents. The crystal violet cytotoxicity assay was used to test the anticancer activity of the synthesised compounds against the colon cancer cell line SW480. The findings revealed that all of the synthesised chemicals have a growth inhibiting impact on cancer cells at varying levels of inhibition. Compounds 3a and 5a were found to be the most active, inhibiting cell growth by 77.4% and having a 10 M IC50 value. They were also shown to have relatively minimal cytotoxicity when tested against MDCK (Madin-Darby canine kidney) normal cells.

Keywords: Triazole, Anticancer, SW480, MDCK.

INTRODUCTION

The pharmacologically active nitrogenous compounds, particularly 1, 2, 3-triazoles and their derivatives attracted considerable attention for the past few decades due to their chemotherapeutical value and parcel of the bimolecular diversity. Cancer is considered as the

second lethal disease worldwide [1]. Colon cancer is the third most common type of cancer around the world and one of the leading reasons of cancer-related deaths [2]. The design and synthesis of anticancer agents is an interesting research area in which scientists are endeavouring to develop synthetic or natural compounds that can contribute in the treatment of cancer and help patients to be cured of this life-threatening disease[3]. There is a very broad spectrum of natural compounds which are considered as anticancer agents[4] among them combretastatin A 4 (CA-4) which is natural product isolated from the South African tree; Combretum caffrum Kuntze (Combretaceae)[5]. The anticancer activity of CA-4 is attributed to the inhibition of tubulin polymerization by binding to the colchicines binding site [6]. The simplicity of the structure of CA-4 and its remarkable anticancer efficacy make its structure a fundamental part of wide range of synthetic anticancer agents which are considered as combretastatin A-4 analogues [7-9]. Based on the structure-activity relationship (SAR) studies, it is reported the CA-4 analogues must have fundamental structural requirements for the optimal activity, the molecule must have 3,4,5-trimethoxyphenyl and 4-methoxyphenyl groups attached to two adjacent as bridge[10-11].

A great attention has been devoted to the 1,2,3-triazol derivatives because of their biological activities, particularly oxygen-containing ones found to have a broad-spectrum of herbicidal[12],anticancer[13],antidepressant[14],antimicrobial[15],antifungal[16],analgesic/ anti-inflammatory[17], antiviral[18], and antioxidant[19]. Many studies have shown that the pharmacological activities of 1,2,4-triazole and their derivatives due to existence of designed to meet the structural requirements of the highest expected anticancer activity of CA-4 analogues to be tested as anticancer agents against colon cancer cell line SW480.

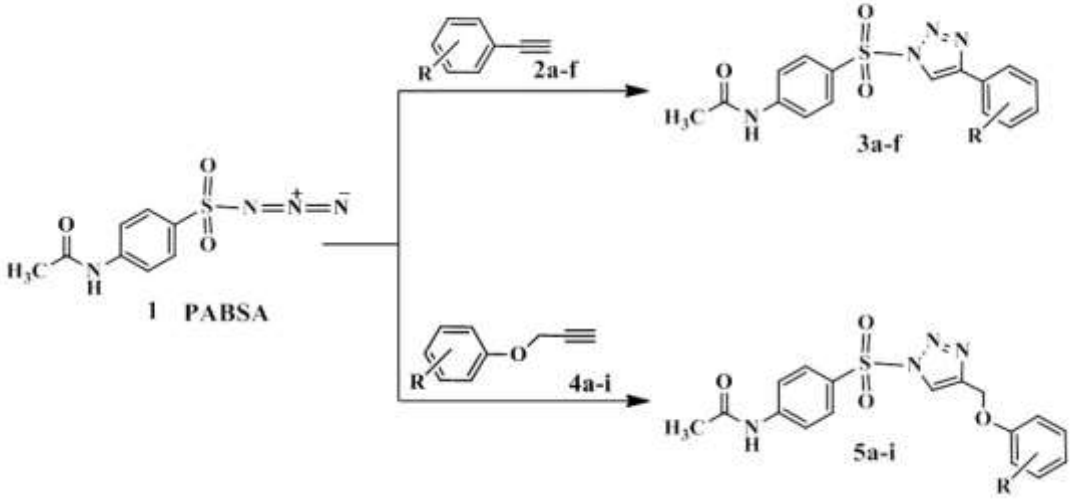

Fig 1: Newly synthesized substituted 1, 2, 3-triazoles [20]

MATERIAL AND METHODS

Culturing of SW480 and MDCK Cell Lines

The stock human colon cancer (SW480) and Madin-Darby Canine Kidney (MDCK) cell lines were obtained and Cell Culture at CCMB Hyderabad. The cells were grown as monolayer and maintained as an exponentially growth phase in Dulbecco's modified Eagle's medium

(DMEM) supplemented with 5% heat inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (GIBCO®) in tissue culture flask and incubated at 37oC in humidified atmosphere containing 5% CO2. The cells were maintained by the replacement of fresh medium.

Anticancer activity

The test compounds were dissolved in acetone at concentration of 10 mM and diluted with DMEM to get the desired concentrations for the treatments of cell lines. Mixture of acetone: DMEM was added to the negative control samples, the added volumes of acetone have no any effect on the cell growth when compared with the positive control samples, and accordingly a normalized control was depended in the study. Overnight cultures of the cells grown in 25 cm2 tissue culture flask were examined under inverted microscope. Cells were detached using trypsin, washed twice by adding 5 ml of PBS and centrifugation at 1500 rpm for 5 minutes. These exponentially growing cells were seeded in 96-well plates (5000 cells/well) and incubated at 37°C for 24 h for attachment. Then, the media was removed and 200 µl of fresh medium containing the test compound at different concentrations were added to each well in four replicates and incubated at 37°C for 48 hours. The wells were then washed by adding 200 µl of PBS for each well, left for 5 min and removed by sterile pasture pipet. This process was repeated twice. The wells were treated with 100 µl of crystal violate solution for 10 minutes. The dye was discarded and the plates were washed with distilled water three times. The plates were left to dry at room temperature, then the absorbance was measured at 570 nm by using plate reader (Human).

The inhibition percentage was calculated using the following formula:

$$\%\text{inhibition}=[(\text{Abs control}-\text{Abs samples})/(\text{Abs control})]*100$$

EXPERIMENTAL SECTION

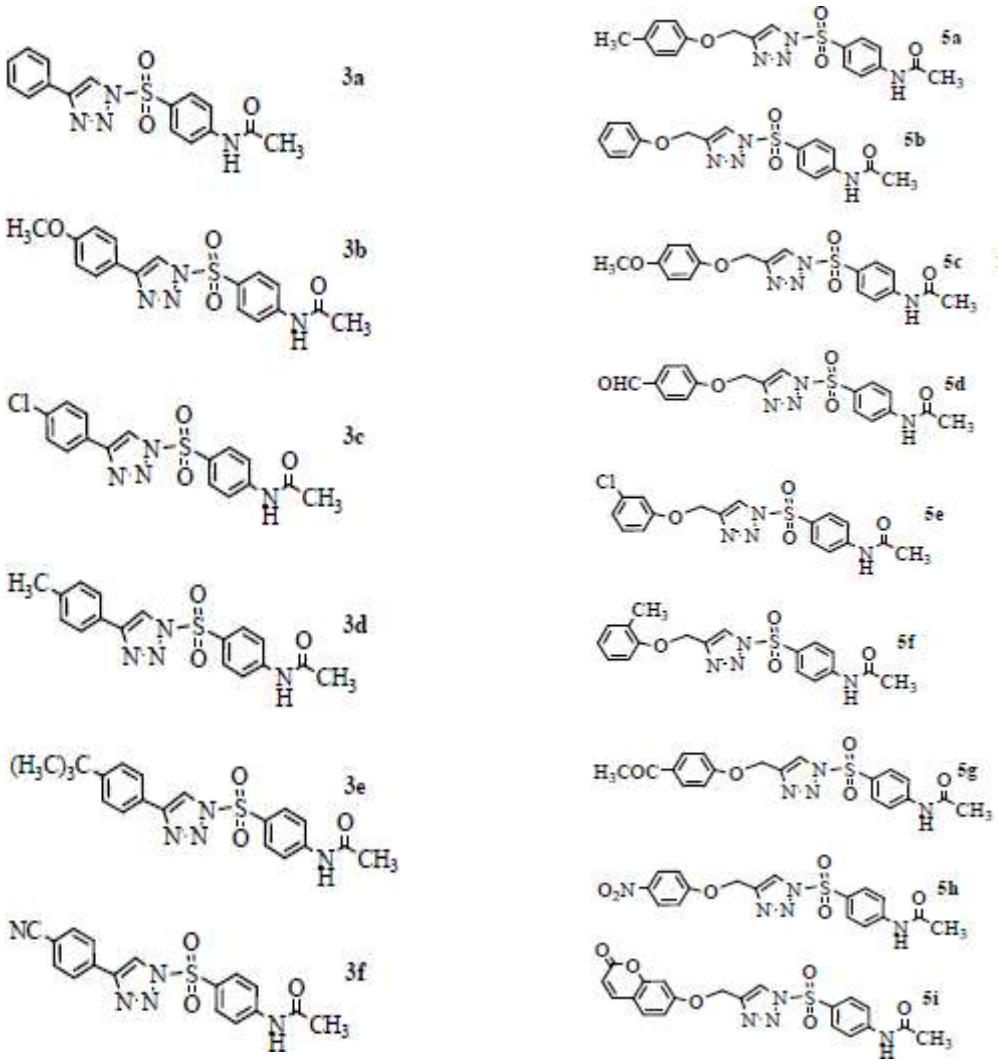

Fig 2: Structures of various substituted 1, 2, 3- triazoles of 3a-3f & 5a-5i

Determination of IC50 Value

The IC50 of compounds were determined by treating the cancer cell line (SW480) with different concentrations of 5a to find out the concentration that results a 50% cell growth inhibition. The IC50 value was calculated by using Sigma Plot version 12 software.

RESULTS AND DISCUSSION

Anticancer Activity

To evaluate the anticancer activity of compound 4 and study the effect of structure modification on the activity of compounds 5a-j, human colon cancer cell lines (SW480) were treated with two different concentrations (10 μM and 50 μM) of compounds 4, 5a-j for 48 h

the cell growth inhibition was determined using crystal violet cytotoxicity assay, Fig. 2 shows the effect of the compound on the cells viability. The cell growth inhibition was calculated, Table 1 shows the obtained results, treatment of the cancer cell lines (SW480) with groups caused 6 and 10% cell growth inhibition at 10μM and 50μM respectively. Substitution of the side chain caused a significant increase in the cytotoxic activity where the values of cell growth inhibition were found to be 50.4 and 77.4% at 10μM and 50μM respectively, compounds 3b, 3c, 3d, 5b, 5c and 5d respectively caused moderate improvement in the cytotoxic activity, within the results related to compounds.

The results indicated that 3a, 5a is the most potent cytotoxic agent in the series, accordingly the next step was to evaluate the IC50 of compound 5a against the same cell line, and the results showed that 10μM is the required concentration to cause 50% cancer cell lines (SW480) growth inhibition after incubation for 48 hours. Furthermore, to test the cytotoxicity of 5a against normal cells, MDCK normal cell lines were treated with 5a at 10 μM concentration (IC50) for 48 h only 20% cell growth inhibition was observed, see Fig. 2 and Table 1.

Table 1: Cytotoxicity effect of 3a-3f & 5a-5i against cancer and normal cells at different concentrations for 48 hours

Entry	Percentage of cell growth inhibition for SW480		IC_{50}(μM)	Percentage of cell growth inhibition for MDCK normal cells at IC_{50}
	50 μM	10 μM		
3a	74	50.4	10	20
3b	51.2	24.2	--	--
3c	34.5	18	--	--
3d	13	16	--	--
3e	22	3	--	--
3f	11	6	--	--
5a	77.4	48.4	10	20
5b	60.1	26.2	--	--
5c	51.2	17.5	--	--
5d	34.5	16	--	--
5e	13	3	--	--
5f	22	6	--	--
5g	11	2	--	--
5h	25	3.7	--	--
5i	13	3.4	--	--

CONCLUSION

Series of various-1,2,3-triazole derivatives3a-3f & 5a-5i have been designed as combretastatin analogues with different substituents attached to side chain. The cytotoxicity of compounds were evaluated against colon cancer line (SW480), compound 5a was found to be the most potent one and showed a kind of selectivity to cancer cells when subjected to evaluating its cytotoxicity against MDCK normal cell lines at 10μM concentration (IC50). The results revealed most of the compounds showed promising anticancer activity shown in Fig-1,2 & Table-1.

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