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Antitumor Effect of 1-[(4-Chloro-Benzylidene)-Amino]-5-Phenyl-1H-Pyrrole-2-Thiol in Different Type of Cell Lines

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ABSTRACT

4-(amino)-5-phenyl-1-4H-1,2,4-triazole-3-thiol was prepared by reaction of potassium dithiocarbazine with hydrazine hydrate under reflux condition for (10 hours). The compound which has been synthesized successfully was subjected to addition reaction with p-chlorobenzaldehyde under reflux condition for (3hours) to synthesize Schiff bases. These compounds were characterized by using (FTIR), UV-visible and evaluated for their anticancer activity. The effect of (1,2,4-triazole derivative) on the activity of malignant cells was studied by using different types of cell line [Breast cancer, Lung cancer, Hepatic carcinoma, and human prostatic carcinoma]. And was used the Electron microscope to show the effect of the derivative on the cancer cells before and after 3 days of the injection time. It was found that the (schiff base of p -chlorobenzaldehyde) was effective in reducing both the size and density of malignant cells.

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INTRODUCTION

In the last few decades, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention owing to their synthetic and effective biological importance (Al-Soud, Y.A., 2003). For example, a large number of 1,2,4-triazole-containing ring systems have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, anti-anxiety, antimicrobial agents and antimycotic activity such as fluconazole, intraconazole, voriconazole. Also, there are known drugs containing the 1,2,4-triazole group e.g. triazolam, alprazolam. Among these heterocycles, the mercapto- and thione-substituted 1,2,4-triazole ring systems have been well studied and so far a variety of biological activities have been reported for a large number of their derivatives. Such as antibacterial, antifungal, antitubercular, anticancer properties (Maertens, J.A., 2004). 1,2,4-Triazole derivatives have received much attention due to their versatile biological properties including antibacterial. In addition to these important biological applications, mercapto-1,2,4-triazoles are also of great utility in preparative organic chemistry, for example, in the presence of various reagents, undergo different types of reaction to yield other (Wu, J., 2007).

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of

clinical importance. The heterocycles are enjoying their importance as being the center of activity. The nitrogen-containing heterocycles are found in abundance in most of the medicinal compounds. The success of imidazole as an important moiety of a number of medicinal agents led to the introduction of the triazoles. The triazoles are said to be the isosters of imidazole in which the carbon atom of imidazole is isosterically replaced by nitrogen. Triazoles are five-membered heterocyclic compounds with molecular formula $C_2H_3N_3$ containing three nitrogen and two carbon atoms. There are two types of triazole, the 1,2,3-triazoles and the 1,2,4-triazoles (Sztanke, K., 2008). 1,2,4-Triazole nucleus has been incorporated into a wide variety of therapeutically interesting molecules to transform them into better drugs (Bhat, K.S., 2009). Schiff bases of 1,2,4-triazoles have also been found to possess extensive biological activities. On the other hand, γ -substituted butenolide moiety represents a biologically important entity that is present in numerous biologically active natural products (Fram, R.J., 1989).

Synthesis:

Synthesis of benzoic acid hydrazide from methyl benzoate:

Methyl benzoate (1.36ml, 0.01M) in 25ml of ethanol is taken in a round bottom flask. To that hydrazine hydrate (0.07ml, 0.15M) is added and refluxed for 4 hours. The total volume of the solution

is reduced to half and it is cooled in ice water. The solid is precipitated out and recrystallized with ethanol. m.p (111-113) °C., Reported (112-114) °C yield 71 %, color: white crystal.

Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol:

A mixture of benzoic acid (1 g) (0.0072 mol) and (0.44 g) (0.008 mol) from potassium hydroxide, was dissolved in (10 ml) ethanol. After mixture was dissolved then (2 ml) (0.014 mol) from carbon disulfide and was added slowly. The reaction mixture was stirred for 10 hours. Dry ether (10 ml) was added and the yellow precipitate was filtered, washed with ether and dried. The yellow precipitate (potassium salt) was added to an excess of hydrazine hydride (20 ml), and was refluxed with stirring until the evaluation hydrogen sulfide; it was ceased by lead acetate paper. After cooling the reaction mixture was filtered, and then was acidified by Hydrochloric acid to yield the white precipitate. Yield (62%), m.p. (198-200)

Synthesis of Schiff base:

A mixture of 4[amino]-5-phenyl-4H-1,2,4-triazole-3-thiol (1.98g, 0.001mol), respective aromatic aldehyde (0.001mol) and 4-5 drops of concentrated glacial acetic acid in ethanol medium was refluxed for 3 hrs. The resulting solution was cooled to room temperature and the precipitated solid was filtered under suction, washed with cold ethanol and recrystallized with hot ethanol. m.p (213-215)°C. .yelid 68% . deep yellow (Bishop, J.M., 1987).

Biological Methods:

Determination of total antioxidant activity in vitro antioxidant activity

Free radical scavenging activity 2,2-diphenyl-picrylhydrazyl (DPPH Assay):

The antioxidant potential of any compound can be determined on the basis of its scavenging activity of the stable (DPPH) free radical as described by sadhu *et al* 25. DPPH is stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. The absorption maximum of a stable DPPH radical in methanol was at 517nm. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules and radical progresses, which results in the scavenging of the radical by hydrogen donation (Bayrak, H., 2009).

Preparation of standard solution:

Required quantity of ascorbic acid was dissolved in methanol to give the concentration of 0.005, 0.01, 0.05, 0.1, 0.5, and 1µg/ml

Preparation of test sample:

Stock solution of sample were prepared by dissolving 10mg of dried hydroalcoholic extract in 10ml of methanol to give concentration of 1mg/ml

.then prepared sample concentrations of 0.005, 0.01, 0.05, 0.1, 0.5, and 1µg/ml

Preparation of DPPH solution:

3.5mg of DPPH was dissolved in 3.0ml methanol, it was protected from light by covering the test tubes with aluminum foil.

Protocol for estimation of DPPH scavenging activity:

Antioxidant activity was measured by a decrease in absorbance at 517nm of a solution of colored DPPH in methanol. A stock solution of DPPH (1.3mg/ml in methanol) was prepared such that 75µl of it in 3ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of sample and standard at different concentrations was noted after 30 minutes. EC50 was calculated from % inhibition. A blank reading was taken using methanol instead of sample extract. Absorbance at 517nm is determined after 3 minutes using UV-visible spectrometer (Systronic double beam-UV2201) and IC50 (inhibition concentration to scavenge 50% free radicals) is also determined. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The capability to scavenge the DPPH radical was calculated using the following equation.

$$AA\% = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$$

where A control = absorbance of DPPH alone, A sample = absorbance of DPPH along with different concentrations of sample. IC₅₀ was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition (Clemons, M., 2004; Bhat, K.S., 2009).

Benzoic acid hydrazide were identified by FTIR spectroscopy. Figure (3.1) showed the FTIR spectrum of this derivatives using KBr disc which showed the following characteristic absorption bands: Stretching band appeared at 3298.0cm⁻¹, 3201.6cm⁻¹ for (NH₂ str), 3018.4cm⁻¹ for (Ar C-H str), and 1660.6 cm⁻¹ for (C=O str), as shown in figure (3.1).

Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (compound2):

4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol were identified by FTIR spectroscopy. Figure (3.2) showed the FTIR spectrum of this derivatives using KBr disc which showed the following characteristic absorption bands: Stretching band appeared at 943.1cm⁻¹ for (N-C-S str), 3296.1 and 3109.0 for (NH₂str), 684.7cm⁻¹ (C-S str), 2937.4 cm⁻¹ for (Ar C-H str), 1635.5cm⁻¹ (C=N str), 2756.1 cm⁻¹ for (S-H str) as shown in figure (3).

Synthesis of Schiff base _ of parachloro-benzaldehyde:

FTIR spectrum for synthesis of Schiff base _ of para chloro-benzaldehyde were identified by FTIR spectroscopy. Figure (3.1.3) showed the FTIR

spectrum of this derivatives using KBr disc which showed the following characteristic absorption bands:

Stretching band appeared 3097cm⁻¹ for (Ar C-H str), 1604cm⁻¹ (C=N str), 2756.1 cm⁻¹ for (S-H str) 684.7cm⁻¹ (C-S str) as shown

Ultra violet spectrum Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (compound 2):

4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol were identified by Ultra violet spectrum appeared one band , at λ_{max} = 260 nm due to electronic transition ($\pi - \pi^*$) state

Ultra violet spectrum Synthesis of schif base-of para chloro benzaldehyde:

schif base-of para chloro benzaldehyde were identified by Ultra violet spectrum appeared two band , due to electronic transition ($\pi - \pi^*$) state and $n - \pi^*$ respectively 234nm , 236nm .

Cytotoxic activity of Schiff base:

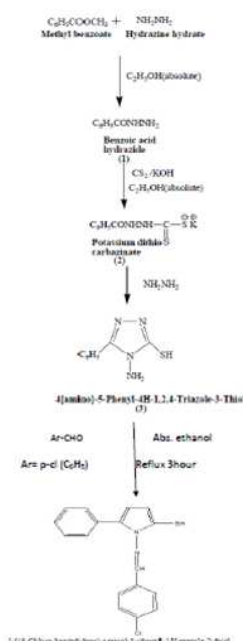
The Schiff base of 1,2,4-triazole in concentration 0.05 and 0.1 showed the activity for breast cancer cell line(MCF₇) IC₅₀ =1.57, prostate cancer cell line (DU₁₄₅) IC₅₀=3.7, lung cancer cell line (A₅₄₉) IC₅₀=0.2 and no activity for hepatic carcinoma cell line (HepG₂) IC₅₀=7.9. And in the concentration 0.5 and 1 and 5 showed the activity for breast cancer cell line(MCF₇) IC₅₀ =1.57, prostate cancer cell line (DU₁₄₅) IC₅₀=3.7, lung cancer cell line(A₅₄₉) IC₅₀=0.2, and hepatic carcinoma cell line(HepG₂) IC₅₀=7.9 and observed the less activity in(HepG₂) IC₅₀=7.9, And the IC₅₀ for A₅₄₉=0.2 ,and DU₁₄₅=3.7 ,HepG₂=7.9 ,MCF₇=1.57 this indicate when the IC₅₀ decrease the activity of cell line increase.

Antioxidants:

DPPH Free radical scavenging activity:

In free radical scavenging activity, DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm, which is induced by different antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. 1,2,4-triazole exhibited a comparable antioxidant activity with that of standard ascorbic acid at varying concentration tested (0.005, 0.01, 0.05, 0.1, 0.5, 1) μ g/ml. there was a dose dependent increase in the percentage antioxidant activity for all concentrations tested (Figure 3.6).

The sample at a concentration of 1mg/ml showed a higher percentage inhibition of 89% and lower percentage for 0.005mg/ml it was 10%. Ascorbic acid was used as standard for the determination of the antioxidant activity by DPPH method. The concentration of ascorbic acid varied in the same concentrations of Schiff 1,2,4-Triazole(0.005, 0.01, 0.05, 0.1, 0.5,1) μ g/ml. Ascorbic acid at a concentration of 1 μ g/ml exhibited a higher percentage inhibition of 92% and for 0.005 μ g/ml exhibited a lower percentage it was 15% (Figure 1). A graded increase in percentage of inhibition was observed for the increase. All determinations were done in duplicate and mean values were determined. Hence DPPH is usually used as a substance to evaluate the antioxidant activity (Mavrova, A.T., 2009).



Scheme (1): synthesis Schiff base of 1, 2, 4-Triazole.

Table 1: viability inhibition of triazole derivative for different tumors cell line Breast cancer cell line (Mcf7), Hepatic carcinoma cell line (HepG7) ,Prostate cancer cell line (Du145), Lung cancer cell line (A549) conc. Of this derivative.

Cell line\Conc. Mg\ml	MCF ₇	HepG ₂	Du ₁₄₅	A ₅₄₉
0.05	25.6	0	7.6	34.7
0.1	37.2	0	16.2	44
0.5	48.5	4.2	29.8	52.2
1	60.3	17.8	43.8	78.7
5	70.1	30.7	56.4	88.4

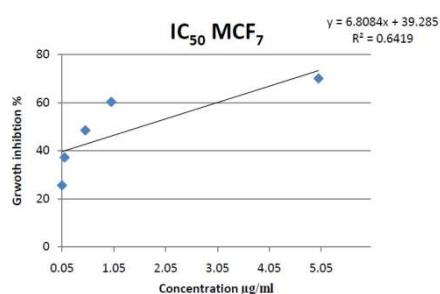


Fig. 3.6: show the IC₅₀ for Breast cancer cell line (Mcf7).

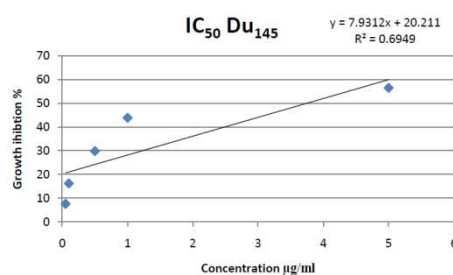


Fig. 3.7: show the IC₅₀ for Prostate cancer cell line (Du145).

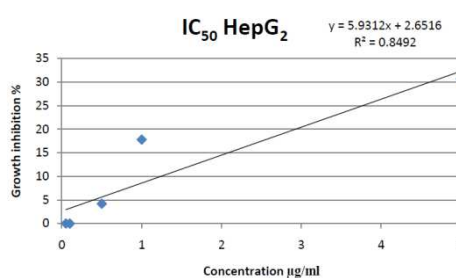


Fig. 3.8: show the IC₅₀ Hepatic carcinoma cell line (HepG7).

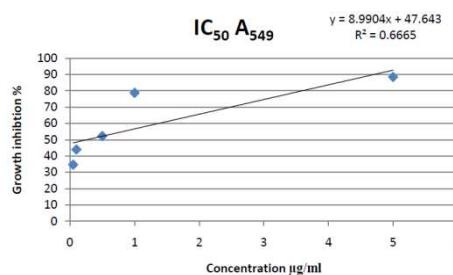


Fig. 3.9: Show the IC₅₀ for Lung Cancer Cell Line (A₅₄₉).

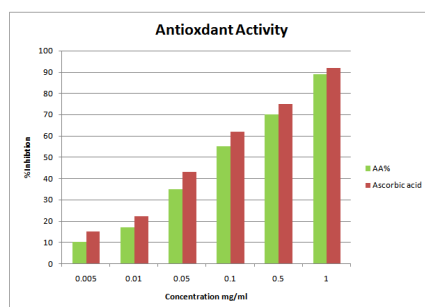


Fig. (3.6): antioxidant activity of 1,2,4-Triazole Schiff base at different concentration AA%=the percentage inhibition of sample.

Conclusion:

Schiff base *p*-chloro benzaldehyde possesses anticancer activity as described in percentage inhibition of tumor cell lines. And the best concentration for high anticancer activity at 5 µg/ml. 1,2,4-triazole derivative was found to be active against the type of tumor used in this research. 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol(2) possesses potent antioxidant activity as described in percentage inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) production, and the best concentration for high antioxidant inhibition at 1 µg/ml. Currently, there has been an increased interest globally to identify antioxidant compounds (Triazole derivative) which are pharmacologically potent and have low or no side effects for use in protective medicine.

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