Synthesis, Characterization, and Anti-Cancer Evaluation of Paclitaxel Analogs

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ABSTRACT

The number of reported cases of cancer is steadily increasing in both industrialized and developing countries. In spite of the fact that significant progress has been achieved in tumor biology, molecular genetics, and in the prevention, detection, and treatment of cancer over the last few years, adequate therapy remains elusive due to late diagnosis, inadequate strategies for addressing aggressive metastasis, and the lack of clinical procedures overcoming multidrug-resistant (MDR) cancer. Currently, as a low toxicity, high efficiency, and broad-spectrum natural anti-cancer drug, paclitaxel has been widely used against ovarian cancer, breast cancer, uterine cancer, and other cancers. The challenging and tedious task with the drug is its low oral bioavailability and permeability which laid down the drug under class IV of the biopharmaceutical classification system of the drug. In this study to overcome this challenge, we developed the derivative of paclitaxel with glucose conjugation which further was evaluated for their snit cancer action. The obtained result indicates that the conjugation with glucose moiety enhances the anti-cancer efficacy which indicates that analogs have better penetration in the cell as well as action.

Keywords: Paclitaxel, conjugation, glucose, anti-cancer

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INTRODUCTION:

A potent and reliable anti-tumor medication, paclitaxel has a broad range of therapeutic applications[1]. One of the most effective treatments for breast and ovarian cancer is paclitaxel (PTX). Because of its small concentration, treating patients with this medication necessitates a highly effective combination with a soluble pharmaceutical excipient in order to boost absorption and lessen the potential adverse effects [2]. This medication works by stabilizing cell microtubules by the suppression of denaturation of protein, which causes cell cycle arrest in the G2/M phase, which eventually results in cell death.[3] For women with

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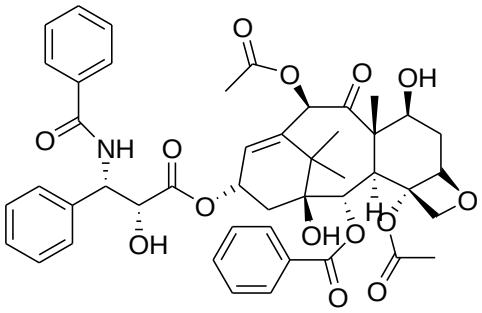
advanced epithelial ovarian cancer, combining treatment with 3-weekly paclitaxel plus carboplatin is the most popular first-line chemotherapeutic strategy[4].Small cell lung cancer (SCLC) and nonsmall cell lung cancer are the two main histologic categories of the heterogeneous group of illnesses known as lung cancer (NSCLC). In particular, NSCLC makes up about 85% of cases and is divided into three main histologic subtypes based on the tissue of origin: colon cancer (40%), nasopharyngeal carcinoma (25-30%), and large cell carcinoma (5-10%)[5].Paclitaxel (Taxol®), a component of the taxane family of anticancer medications, is derived from the bark of the California Yew Tree, Taxus brevifolia, and is frequently used to treat gynecological, breast, and non-small cell lung cancer, as well as AIDS-related Kaposi's sarcoma[6]. Paclitaxel is referred to by its chemical name, which is 5b,20- epoxy-1,2a,4,7b,10b,13a-hexahydroxytax-11-en-9-one-4,10-diacetate-2-benzoate 13 ester with (2R, 3S)-N-benzoyl-3-phenylisoserine (molecular formula C47H51NO14; molecular weight 853.9) [7]

Its chemical structure has been shown in Figure no. 1



Paclitaxel

Fig. 1 Chemical structure of Paclitaxel

Its 20-carbon component (C20) is a member of the natural chemical family known as diterpenes. Ring A, ring D (the oxetane ring), the C2 benzoyl group, and a few other components, including the C30 amide-acyl group and the OH group at C20, which binds on the side chain to C13, are known to have anticancer action. However, other groups, including the acetyl group on C10 and the carbonyl group on C9, have little impact on the therapeutic effectiveness of PTX. The acetyl group also contributes to the paclitaxel molecule's defined conformation [8-11].

It contains ovarian, lung, and breast cancer-fighting properties that have been clinically verified. This compound's low solubility, re-crystallization after dilution, and cosolvent-induced toxicity make use challenging. In these situations, the use of nanotechnology and nanoparticles offers several benefits over traditional drug delivery methods, including lengthened medication half-lives, reduced toxicity, and targeted and selective distribution [12]. Nano drugs have the capacity to accumulate in the tissue, which may be related to increased permeability and retention as well as greater antitumor effects while having low toxicity in normal tissues [13]. The taxine alkaloids accidentally ingested from the plant cause cardiac cells' sodium and calcium channels to become blocked, raising the calcium levels in the cytoplasm. Bradycardia, hypotension, and arrhythmias are the results of its cardiotoxicity [14, 15].Varied functional peptides have different effects on paclitaxel's

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solubility in water, tumor permeability, accumulation in tumor tissues, and anticancer activity. These effects can all be increased by conjugates. Additionally, partial conjugates can restore paclitaxel's therapeutic effectiveness against resistant cancers as a result of the altered cell entrance mechanism [16-20].

MATERIAL AND METHOD:

Material: Paclitaxel 100 mg was gifted by United Biotech IndiaPvt. Ltd. Dichloro methane and Glucose monohydrate were obtained from Ozone international Mumbai(India), potassium di chromate and sodium iodide were obtained from qualigens fine chemicals Mumbai, Thionyl chloride were obtained from central drug house Delhi.

Instrument Used

Different Instruments were used for an additional specified purpose in between or after the synthesis reaction. Mettler Toledo SF-400C was operated for weighing the content amount [21]. In addition, the following instruments were performed for the characterization of synthesized compounds..

- IR Spectral was obtained from Bruker and Perkin Elmer Spectrum 65 FT-IR Spectrometer using Potassium bromide (KBr, IR grade)
- 1H NMR spectral was evaluated in CDRI Lab on Bruker AvIII HD-300 [FT NMR] Instrument using Dimethyl sulfoxide (DMSO, NMR grade D6) and DMSO as solvents.
- 13C NMR spectral was evaluated in CDRI Lab on Bruker AvIII HD-300 [FT NMR] Instrument using Dimethyl sulfoxide (DMSO, NMR grade D6) and DMSO as solvents [22-24].

Software and database

All synthesized compounds and schemes defining the experimental work performed were drawn, and theoretical, and analytical calculations were completed with the help of, ChemDraw Pro 8.0 (ChemDrow Pro 2004)

EXPERIMENTAL WORK:

SYNTHESIS OF PACLITAXEL DERIVATIVE

Procedure: $K_2Cr_2O_7$ was triturated to a fine powder. 10, 10 mg of paclitaxel and $K_2Cr_2O_7$were taken and dissolved in dichloromethane. The mixture was heated to 100◦c for 2 hourson a magnetic stirrer.After cooling the reaction mixture was filtered.TLC was employed for the indication of completion of the reaction.

The reaction mixture was taken and dissolved in 5 ml of thionyl chloride,The mixture was stirred at room temperature for 3 hoursreaction mixture was filtered TLC was employed for the indication of completion of the reaction.

The reaction mixture was reacted with sodiumiodide 1.3 mol. And glucose monohydrate was added to it The mixture was stirred at room temperature for 3 hoursReaction mixture was filteredTLC was employed for the indication of completion of the reaction. The product was obtained and collected.

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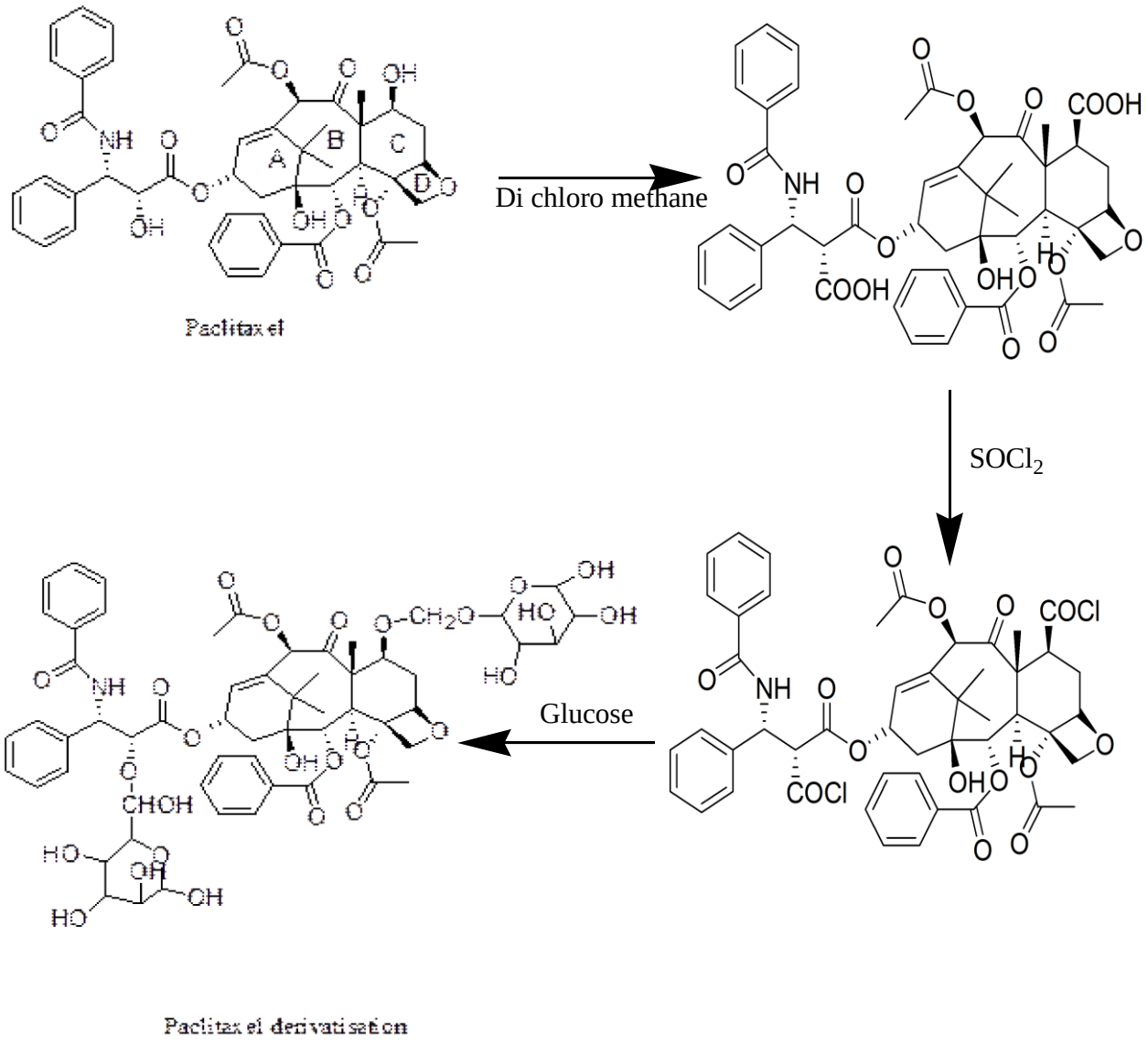

Fig 2. Scheme for synthesis of Paclitaxel Analogs

DETERMINATION OF ANTICANCER ACTIVITY OF PACLITAXEL AND ITS DERIVATIVE (IN VITRO METHOD)

MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] is a paleyellow substrate that is cleaved by living cells to yield a dark blue formazan product. This process requires active mitochondria, and even freshly dead cells do not cleave a significant amount of MTT. Thus the amount of MTT cleaved is directly proportional to the number of viable cells present, which is quantified by colorimetric methods. This assay was performed at Deshpande Laboratories, Bhopal using the standard operating procedures. Briefly, the compounds were dissolved in DMSO and serially diluted with a complete medium to get the concentrations a range of test concentrations. DMSO concentration was kept < 0.1% in all the samples HT29 cells maintained in appropriate conditions were seeded in 96 well plates and treated with different concentrations of the test samples and incubated at 37 °C, 5% CO2 for 96 hours. MTT reagent was added to the wells and incubated

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for 4 hours; the dark blue formazan product formed by the cells was dissolved in DMSO under a safety cabinet and read at 550nm. Percentage inhibitions were calculated and plotted with the concentrations used to calculate the IC50 values [25-27].

RESULT

Chemistry

Percentage yield : **Melting point** 216◦C **IR (KBr)** 3321(OH), 1265(C=N), 1604(CO), 1438(C=C); **^{1}H NMR** (DMSO-d6); **δppm** = 10.6 (s, 1H, COOH), 8.35 (d, 2H, Ar- H), 7.53 (t, 3H, Ar-H), 6.16 (d, 1H, CH), 3.41 (d, 2H, -CH); **^{13}C NMR** (DMSO d6)**δppm** =172.78 (C=O) 157.54-148.94 (3C C-O)129.96 (1C C=CH2) 109.35 (1C1=C-) 81.11(1C,C=C)76.27(1C,C=C)66.83- 43.86 (12C,alkane)

In vitro anticancer potency

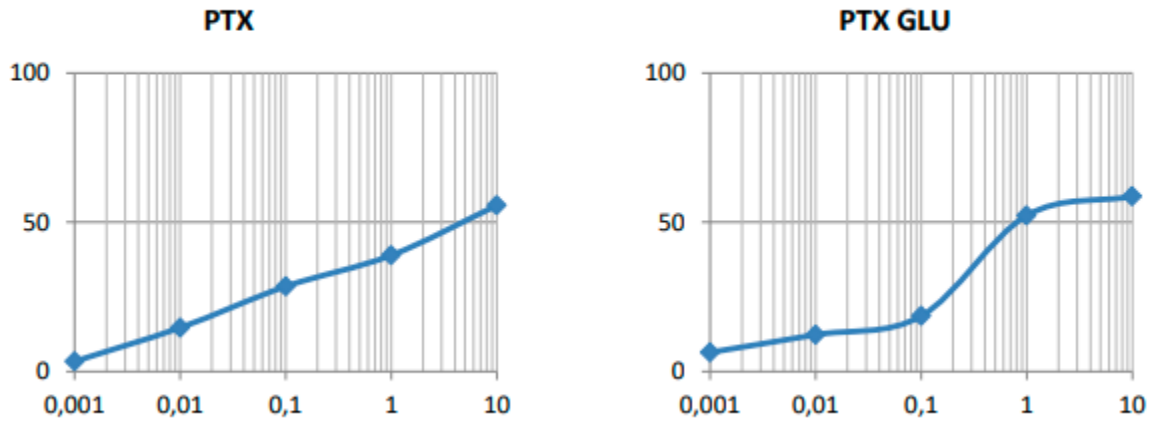

Fig 3: Result of *in vitro* Anticancer activity of tested compounds

	HT29	
Concentration	PTX	PTX GLU
10	55.61	58.61
1	38.91	52.26
0.1	28.46	18.55
0.01	14.64	12.28
0.001	3.35	6.37
IC50 VALUE ug/ml	4	0.8

Fig 4: Anticancer activity of tested compounds

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CONCLUSION

Novel paclitaxel derivatives with anticancer action are reported to have been synthesised in the study titled "Derivatization,Characterisation, Evaluation of anticancer activity of paclitaxel analogues." Results from both assays indicated that the synthesised paclitaxel derivative has moderate to powerful anticancer activity. The molecule showed substantial activity in an MTT experiment, with an IC50 value of 0.8 g/mL compared to the IC50 value of 4.0 for the reference medication.

By shedding light on how to improve upon Paclitaxel, our study will help scientists throughout the globe create more effective cancer treatments with fewer negative side effects.

CONFLICT OF INTEREST

The author confirms that this article's content has no conflict of interest.

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