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SYNTHESIS AND EVALUATION OF ANTI-CANCER POTENTIAL OF NOVEL DERIVATIVES OF 1,4-DIOXANE

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ABSTRACT

Cancer refers a disease that develops when a few cells grow-out and spread into other organs-internal. Cancer can manifest almost anywhere in body. The present research was based on the synthesis and evaluation of anti-cancer potential of novel derivatives of 1,4-dioxane. 1,4-Dioxane was first identified in 1863. It started to be used commercially in the 1930s as a solvent in the production of plastics and cellulose acetate. Starting in the 1950s, 1,4-dioxane was utilized as a stabilizer that were chlorinated. Since 1,4-Dioxane is a contaminant, there are further worries regarding the amount of this substance in shampoos, detergents, and cosmetics. To novel derivatives based on physicochemical properties i.e., melting point, Rf value, FTIR, NMR and Mass spectroscopy techniques and evaluated for in-vitro anticancer potential of synthesized novel derivatives on following cell lines i.e., PC3, MCF-7. For the 1,4-dioxane derivatives, the boiling point was determined as 128ºC ºC, 146ºC, 164ºC, 144ºC, 158ºC and 172ºC for compounds C1, to C6, respectively. Significant findings were also made regarding molecular weight in the produced 1,4-dioxane derivatives. Compounds C1 to C6 were determined to have molecular weights of 155.5, 247, 135.1, 137.1, 200, and 151.1, respectively. At concentration (100µg/ml), 1,4-dioxane derivatives demonstrated the % cytotoxicity as 73.53%, 74.23%, 72.24%, 75.48%, 74.62%, and 76.16%, in the C1, C2, C3, C4, C5 and C6., respectively. Thus, all the derivatives were shown the significant anti-cancer effect when observed in the PC3 cells. Thus, it might be said that effects of 1,4-dioxane

derivatives were almost similar in both the models i.e., PC3 cell and MCF-7 cells. It concluded that the novel derivatives of 1,4-dioxane have a potential anti-cancer activity when observed in-vitro. All the derivatives exhibited anti-cancer response in the both the models i.e., PC3 cells and MCF-7 cells with % cytotoxicity of 70-80%, approximately at the concentration of 100µg/ml.

KEYWORDS: 1,4-Dioxane, synthesis, anti-cancer, PC3, MCF-7.

INTRODUCTION

Cancer refers a disease that develops when a few cells grow-out and spread into other organs-internal. Cancer can manifest almost anywhere in body. When the body requires regeneration, human cells frequently divide (cell proliferation & multiplication). Tumour can move across different parts of body to produce new tumour or invading in neighbouring tissues. Malignant tumour is another form of tumour. Blood cancer i.e., leukaemia and other cancer types.[1]

1,4-Dioxane

1,4-Dioxane was first identified in 1863. It started to be used commercially in the 1930s as a solvent in the production of plastics and cellulose acetate. Starting in the 1950s, 1,4-dioxane was utilized as a stabilizer that were chlorinated. Since 1,4-Dioxane is a contaminant, there are further worries regarding the amount of this substance in shampoos, detergents, and cosmetics.[2] The heterocyclic chemical molecule 1,4-dioxane is categorized as an ether. It is a colorless liquid with a subtle sweetness to it, akin to diethyl ether. Since the additional dioxane isomers (1,2- and 1,3-) are uncommon, the molecule is frequently referred to as just dioxane. In addition to being employed in the laboratory, dioxane finds utility as a stabilizer for the transportation of chlorinated hydrocarbons in aluminum containers and as a solvent in many real-world applications.[3] Because the dioxane molecule is centrosymmetric, it takes on the chair conformation that is characteristic of cyclohexane cousins. However, the molecule exhibits structural flexibility, making it simple to assume the boat shape, as shown in the chelation of metal cations, for example. Dioxane has just two ethyleneoxyl units, making it similar to a smaller crown ether.[4]

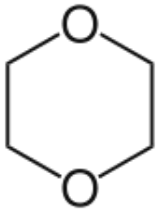


Fig. 4: Structure of 1,4-dioxane.

Molecular formula: C4–H8–O2

MATERIALS AND METHODS

Experimental Requirements

1. 2-Substitutedoxirane, carbon di-oxide, Sodium bi-carbonate, distilled water, paraffin, and ethanol.
2. Weighing balance, RBM, condenser, thermometer, and pH meter.

4.2 Synthesis of novel derivatives of 1,4-dioxane

All the 6 novel synthesized derivatives will be synthesized through following scheme. These will be evaluated for physiochemical parameters- spectroscopy analysis and pharmacological (anti-cancer) activity as follows.

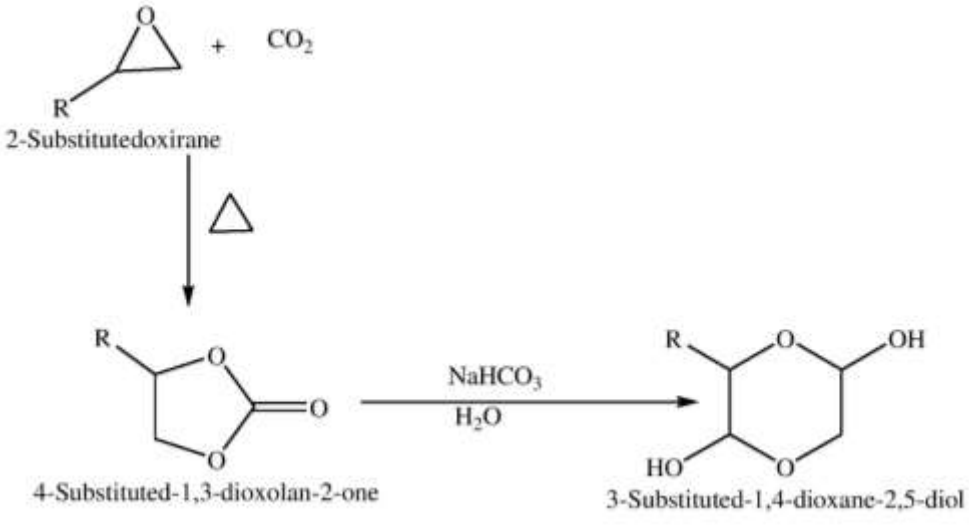
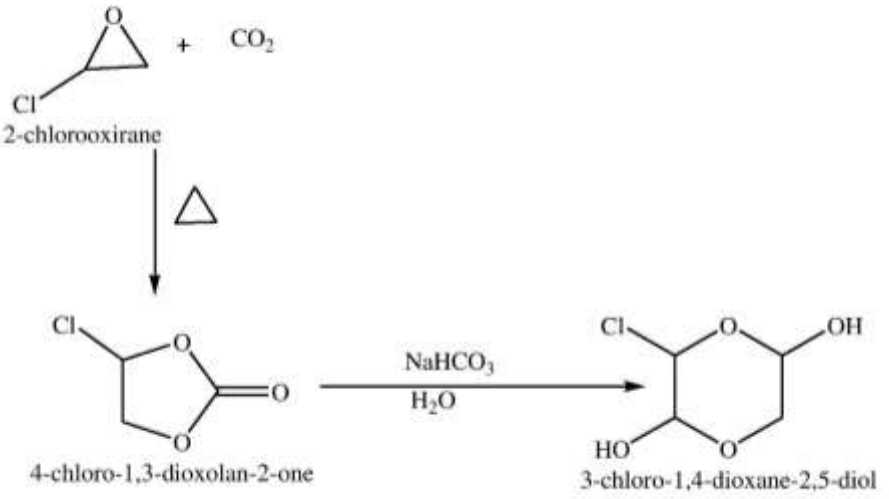
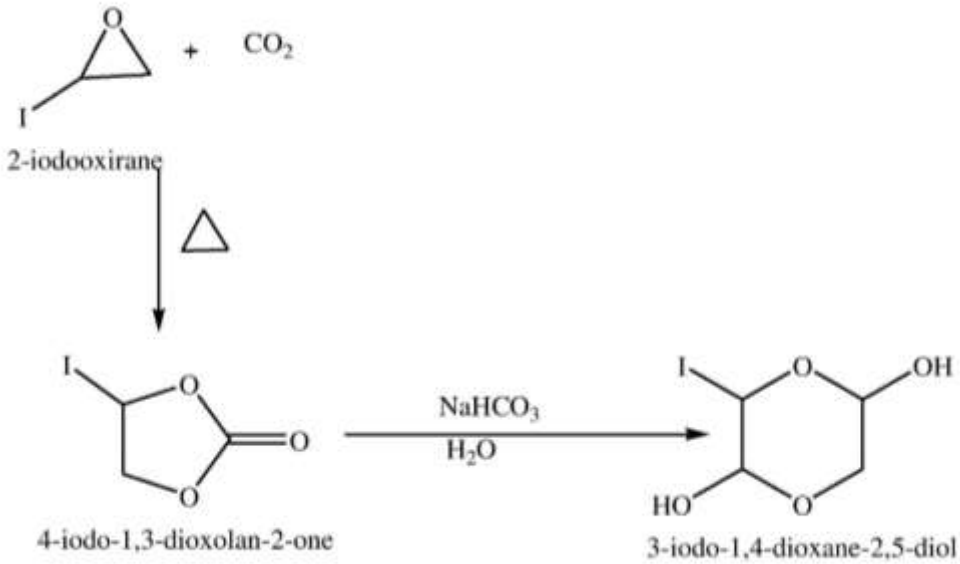


Fig. 3. Scheme for synthesis of 1,4-dioxane derivatives.

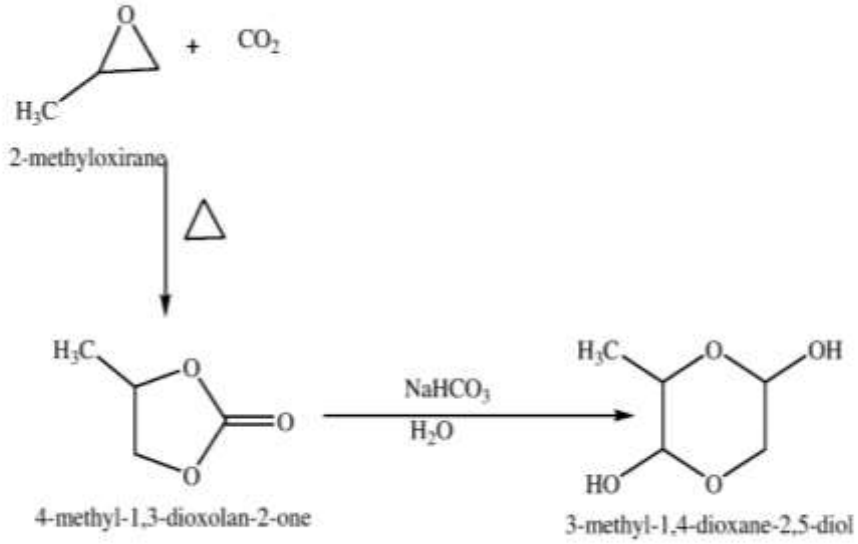
Procedure of Synthesis (C1)



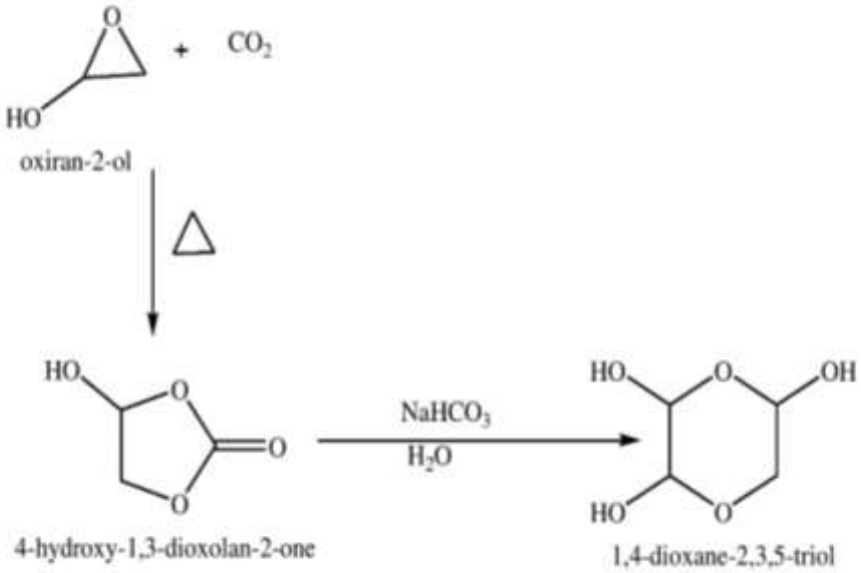
Procedure of Synthesis (C2)



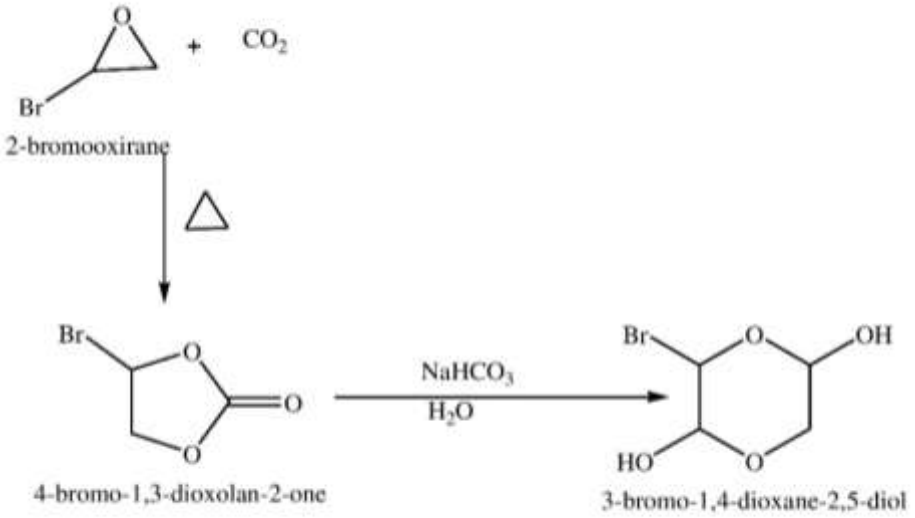
Procedure of Synthesis (C3)



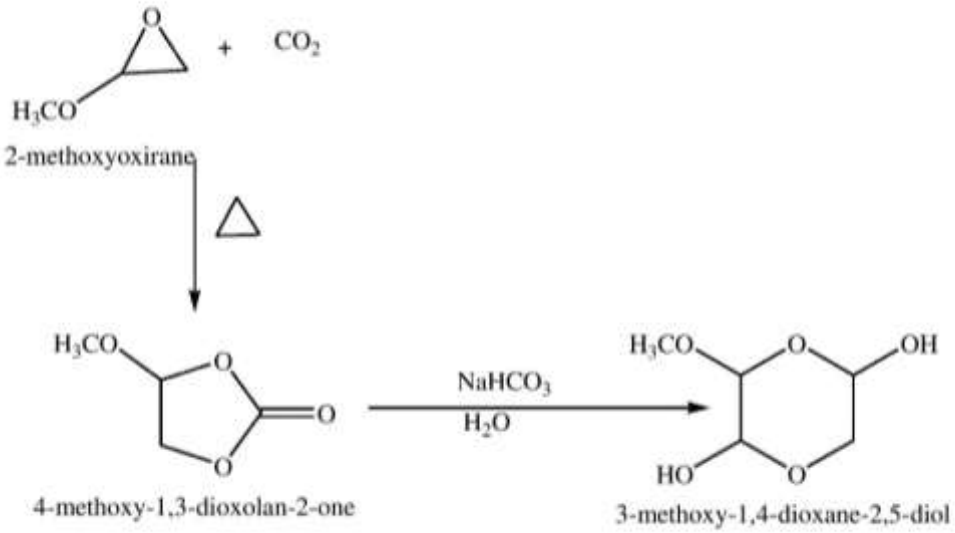
Procedure of Synthesis (C4)



Procedure of Synthesis (C5)



Procedure of Synthesis (C6)



A. Identification of physicochemical properties

- **Melting point**

The melting point of an organic compound was ascertained using Thiel's melting point tube. Finding a compound's melting point is the most crucial and direct way to differentiate one from another.[5]

- **Rf value**

Thin layer chromatography, or TLC for short, is a technique in synthetic chemistry that uses a compound's variable Rf value to deduce the molecule's synthesis. It also helps to validate the reaction's advancement.[6]

- **Infrared Spectroscopy**

One classifies the infrared spectrum as a vibrational-rotational spectrum. For solid compounds, the KBr pellet technique is utilized; for liquid compounds, the Nujol mull method is employed. It is a very useful document that provides details about the functional groups found in organic molecules. When electromagnetic radiation with a wavelength spanning from 500 cm-1 to 4000 cm-1 passes through a sample, the mechanism of bond stretching and bending occurs.[7]

- **NMR Spectroscopy**

Proton NMR is the most widely utilized NMR method due to its high sensitivity and extensive characteristic information. The chemical shift (δ) range is 0–14 ppm. The test unknown compound's chemical shift was compared to TMS protons, which had an attribution of 0 ppm. However, the shift extends to the component.[8] for the organic compound range δ 0 – 14.

- **Mass Spectroscopy**

An essential physico-chemical tool for determining the structures of chemicals found in natural goods, such as medicinal herbs, is mass spectrometry. The application of various physical techniques for sample ionization and ion generation based on mass to charge ratio (m/z) is the fundamental idea of mass spectrometry. Electrospray ionization, air pressure chemical ionization, electron ionization, chemical ionization, rapid atom bombardment, and matrix analysis laser desorption ionization are among the ionization techniques that are accessible. Compared to NMR, which has a sensitivity limit of the nanogram range and above, mass spectrometry has a high sensitivity with a detection limit of the fentogram. MS is a versatile analytical tool because to its sensitivity and versatility for hypenation with other chromatographic techniques.[9]

B. Evaluation of anticancer potential

➢ Cytotoxicity in PC3 cells

PC3 cells (1×104) were cultured for 24 hours (37°C) and 5% CO2 in humidified air in each well of a 96-well culture plate using 100 µL of complete media. According to the experimental design, 1,4-dioxane derivatives were dissolved in a minimal amount of DMSO that was non-toxic to cells, diluted to the necessary concentrations (100µg/ml) in the medium, and then added in triplicate to the wells containing exponentially growing cells. Following the 21-hour treatment period, 10µL of MTT (5 mg/ml of medium free of serum and phenol red) was added. The plate was then incubated for a further 3 hrs (37°C). Following the removal of the supernatant, 100 µL of DMSO was added to each well and allowed to sit at 37 °C for 10 minutes. Using the wells, a microplate Elisa reader measured the absorbance at 540 nm. After adding the DMSO, all of the plates were typically read in one hour.[10]

Formula:

$$[\%\ \text{cytotoxicity} = \{1 - (A_T/A_C)\} \times 100].$$

Where AT is the treatment absorbance value and AC is the control absorbance value.

➢ Antiproliferative MTT assay using MCF-7 cells

The MCF-7 was used to test the 1,4-dioxane derivatives for potential in vitro anticancer activities. In order to assess the impact of artificial derivatives on the viability of MCF-7, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was utilized. The procedure was followed as it was previously explained.[11] 96 well culture plates were seeded with 1×104cells per well and left to adhere throughout the entire night. The cells were cultured for 72 hours after being stimulated with various doses of poplar bud extracts (100µg/ml) on the second day. Following the 72-hour incubation period, 10µL of the 5mg/ml solution from the Sigma-Aldrich MTT kit was added to the cells, and they were left to incubate for a further three hours. The formazan crystals that were obtained were disintegrated in 100µl of the lysis solution that came with the MTT kit. At 570 nm, the absorbance was measured using a microplate reader.

RESULTS AND DISCUSSION

Synthesized derivatives

Newer derivatives of 1,4-dioxane were developed. The procedure was used for the 1,4-dioxane synthesis as mentioned under materials and methods chapter. 1,4-dioxane was

observed as a clear, colourless liquid with a faint, pleasant odor that is denser than water and heavier than air.

5.2 Identification of physicochemical properties

Boiling point determination

For the 1,4-dioxane derivatives, the boiling point was determined as 128ºC ºC, 146ºC, 164ºC, 144ºC, 158ºC and 172ºC for compounds C1, to C6, respectively.

Rf value in TLC

Synthetic chemistry uses thin layer chromatography to verify the synthesis of a molecule based on its Rf value, which changes according to the substance. The Rf values of the C1, C2, C3, C4, C5, and C6 derivatives of 1,4-dioxane were found to be 0.68, 0.73, 0.74, 0.71, 0.72, and 0.67, respectively.

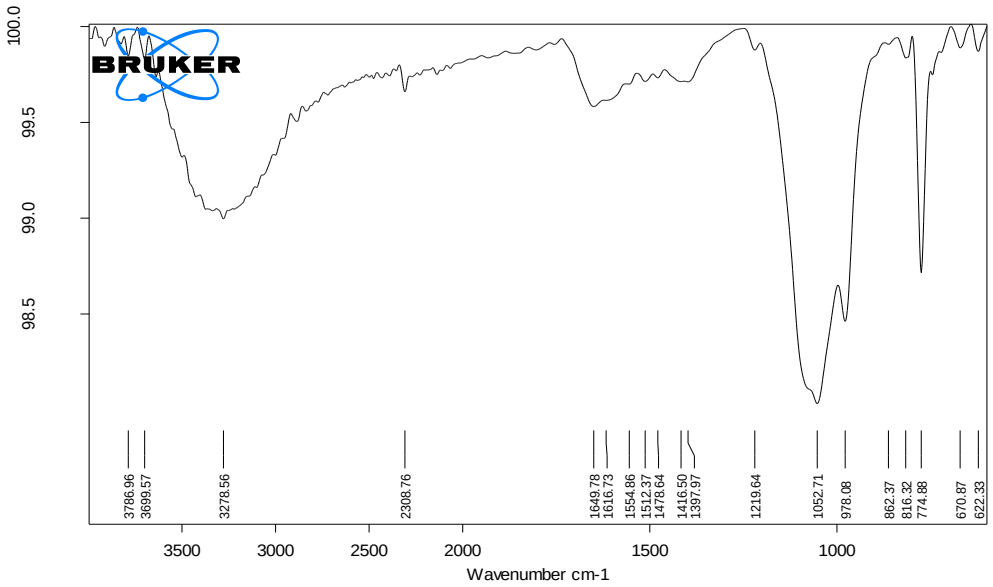
The physicochemical characteristics of each synthesized 1,4-dioxane derivative, such as molecular weight, boiling point, and % yield, were examined. The highest percentage yields, 66.34% and 67.12%, were shown for C2 and C5. C6 had the lowest yield percentage, 64.32%. Significant findings were also made regarding molecular weight in the produced 1,4-dioxane derivatives. Compounds C1 to C6 were determined to have molecular weights of 155.5, 247, 135.1, 137.1, 200, and 151.1, respectively. The physicochemical characteristics of each compound were compiled into the following table.

Higher boiling point confirms for its greater density and water and heavier than air. Thus, it might be utilized in different pharmaceutical aids and other commercial applications.

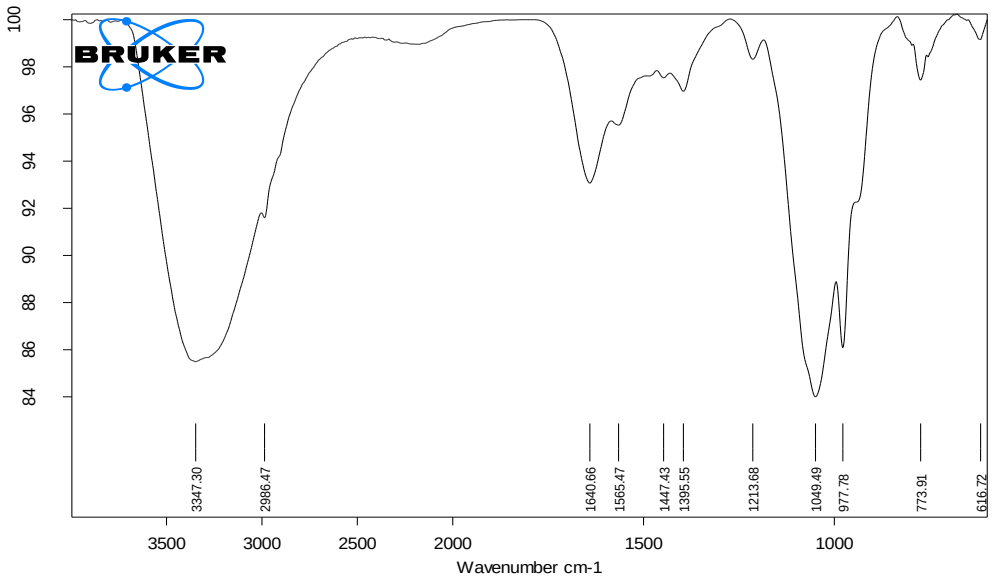
Table 1. Physicochemical properties of 1,4-dioxane derivatives.

Compound	Yield (%)	Rf Value	Boiling point	Molecular weight
C1	62.25	0.68	128ºC	155.5
C2	66.34	0.73	146ºC	247
C3	63.72	0.74	164ºC	135.1
C4	64.24	0.71	144ºC	137.1
C5	67.12	0.72	158ºC	200
C6	65.35	0.67	172ºC	151.1

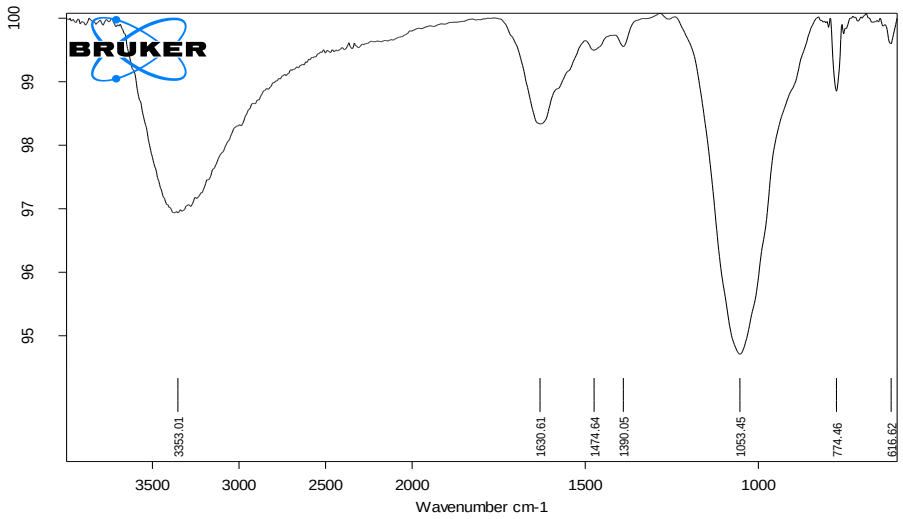
FTIR Spectroscopy



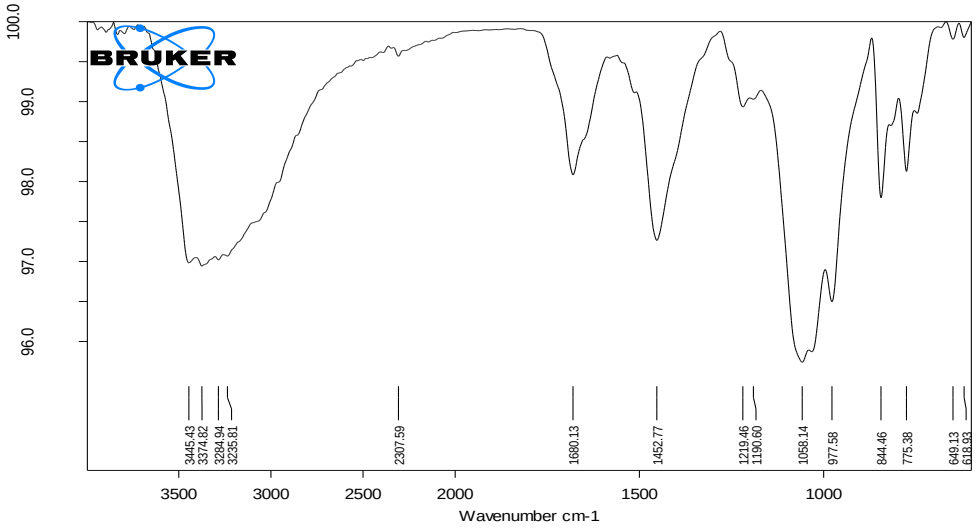
FTIR Spectrum of C1.



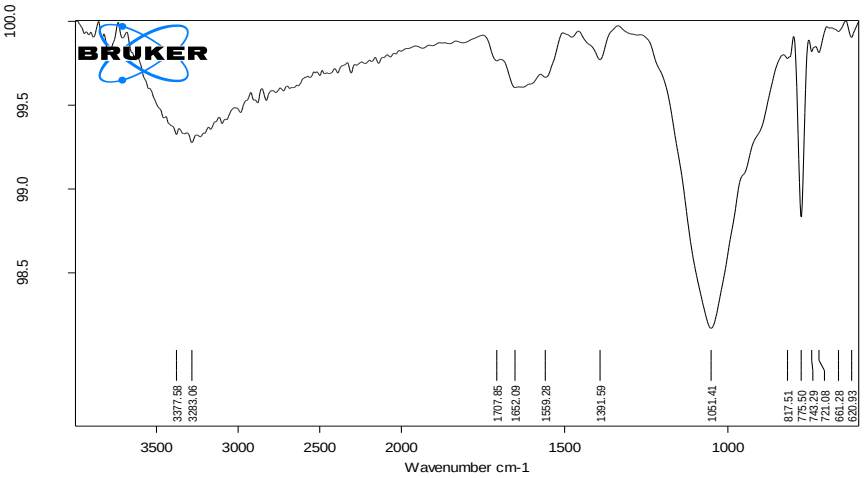
FTIR Spectrum of C2.



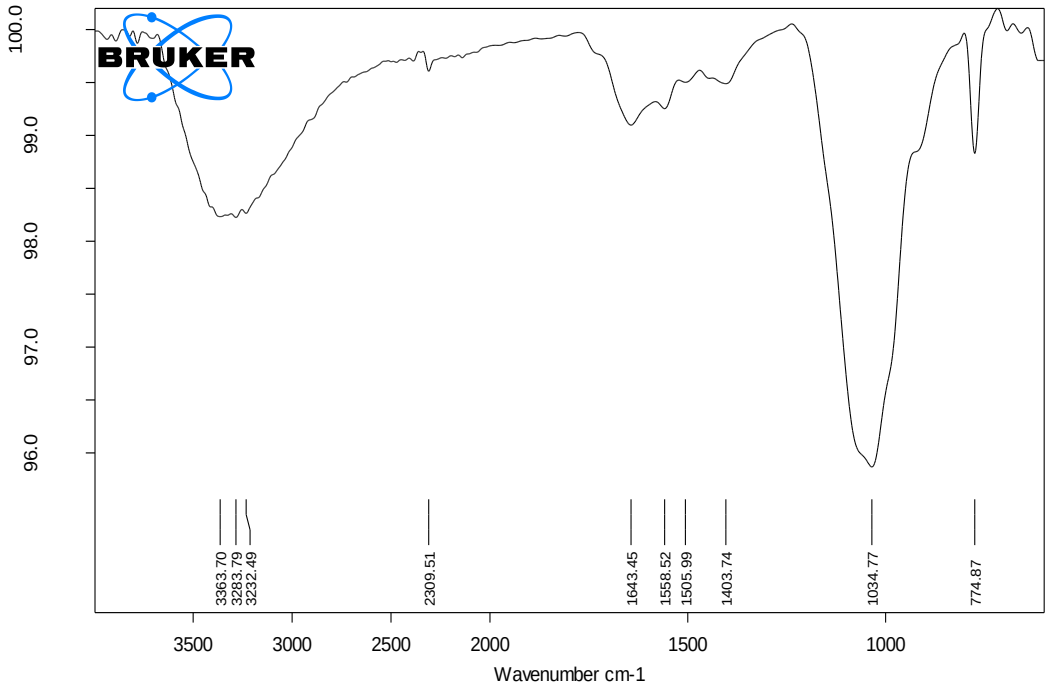
FTIR Spectrum of C3.



FTIR Spectrum of C4.

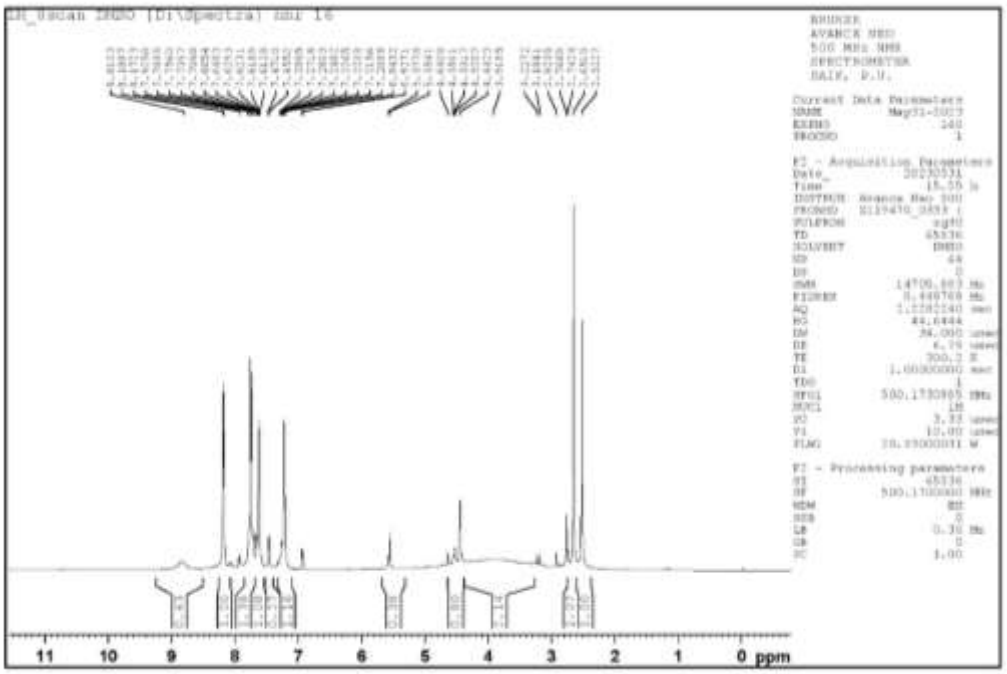


FTIR Spectrum of C5.

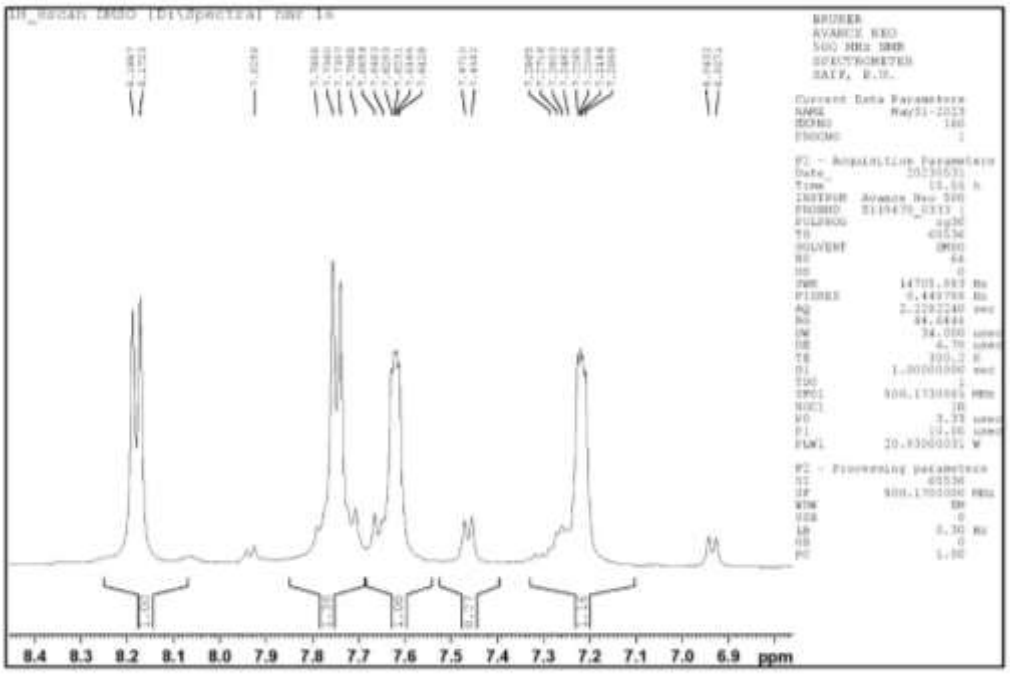


FTIR Spectrum of C6.

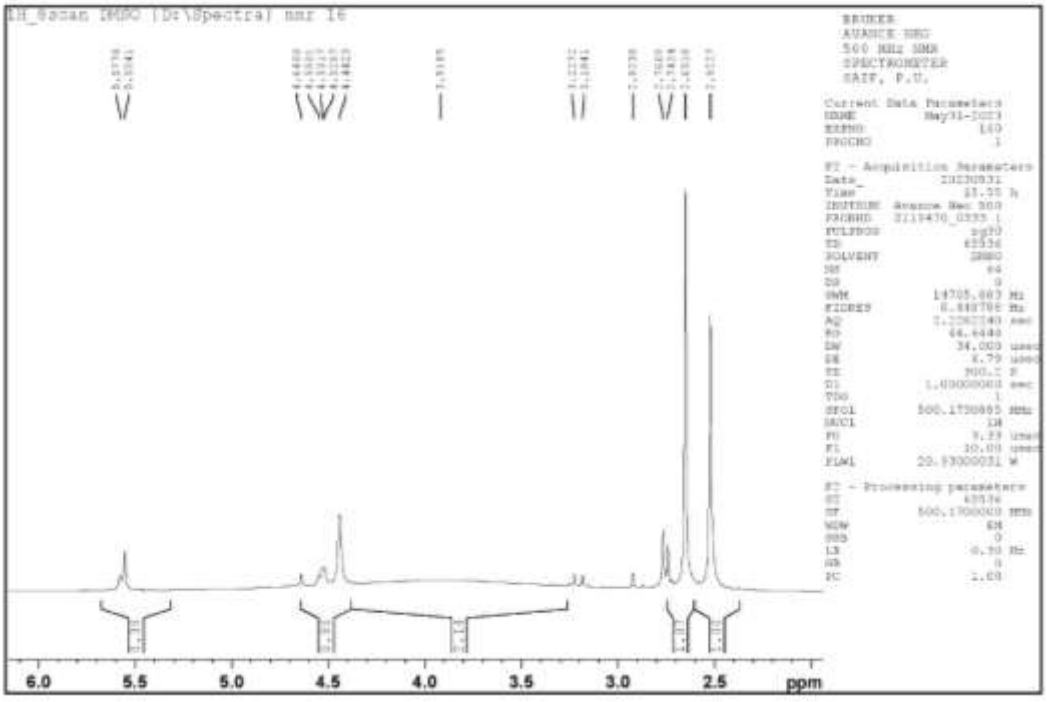
NMR spectroscopy



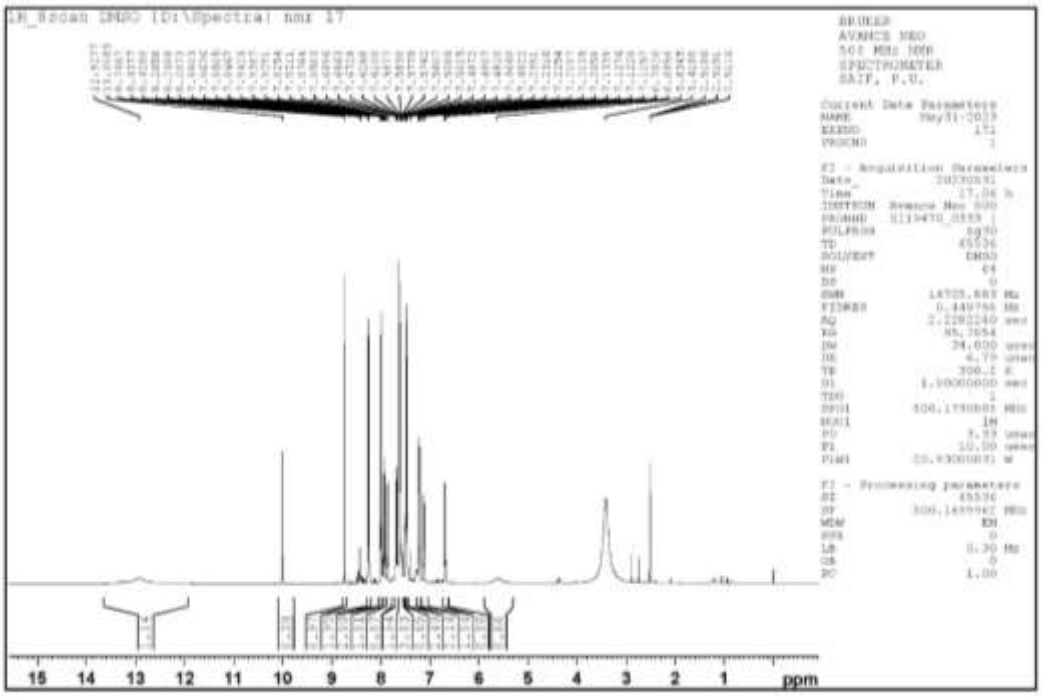
NMR Spectrum of C1.



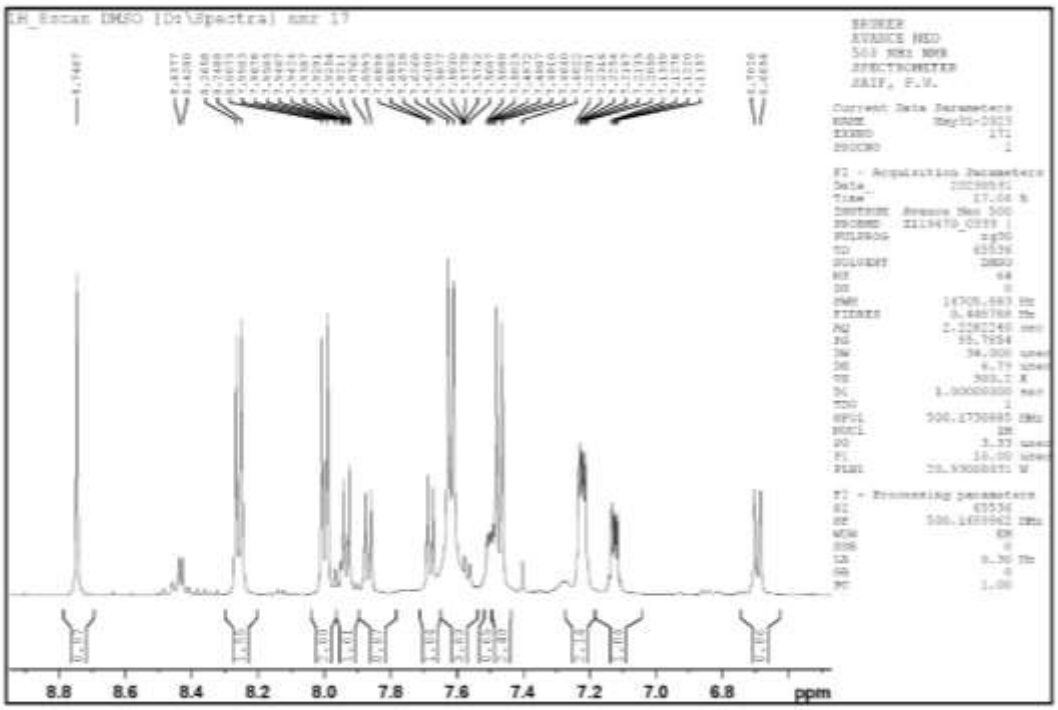
NMR Spectrum of C2.



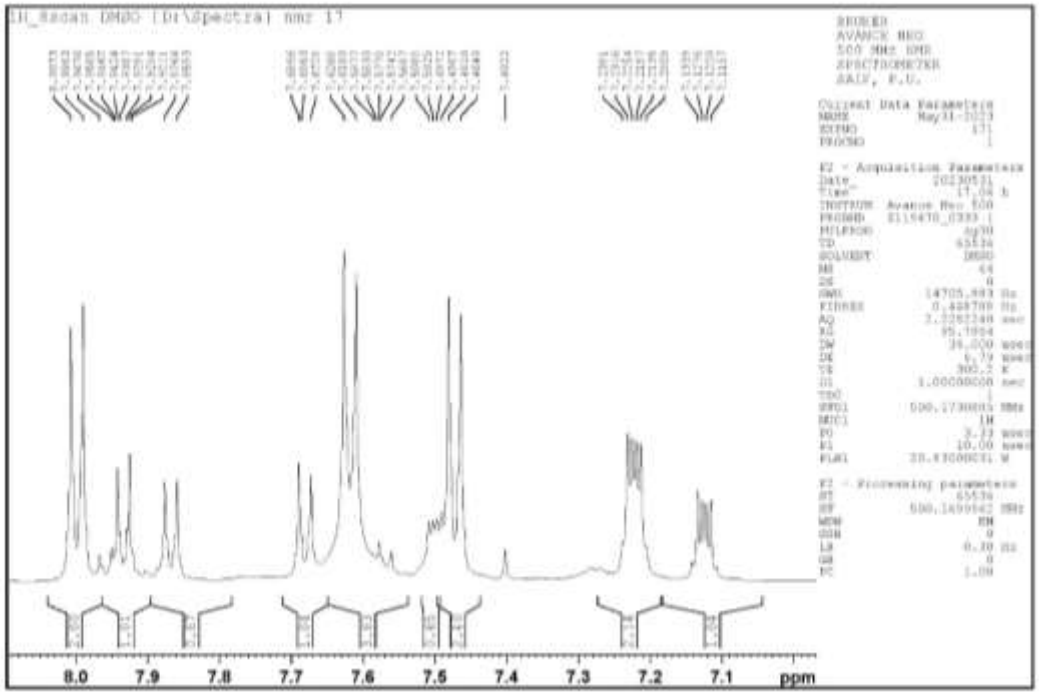
NMR Spectrum of C3.



NMR Spectrum of C4.

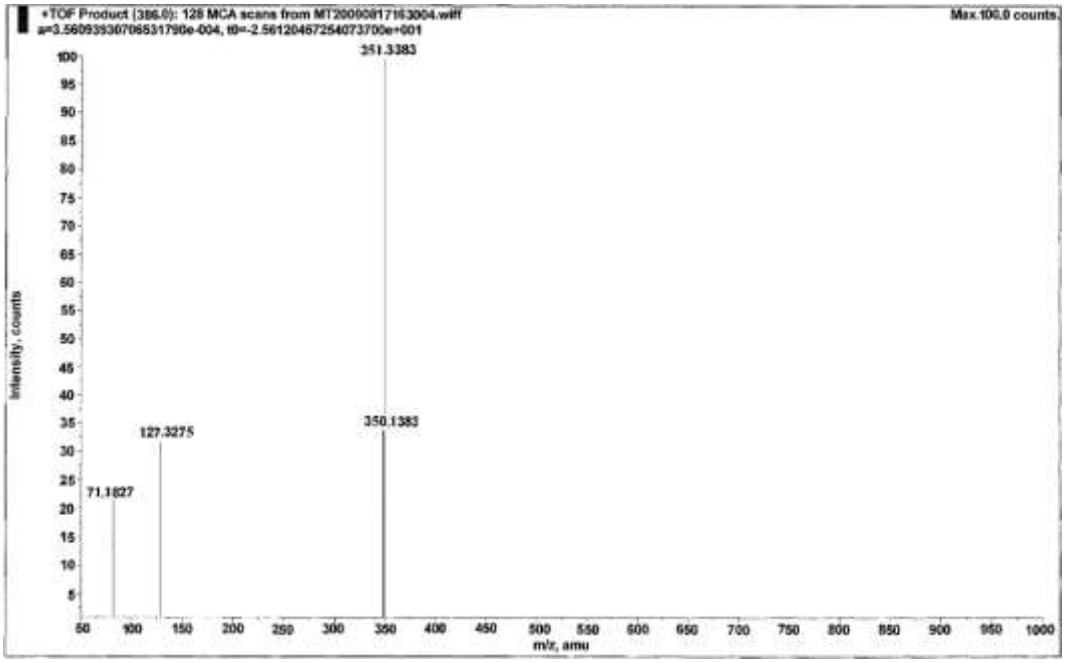


NMR Spectrum of C5.

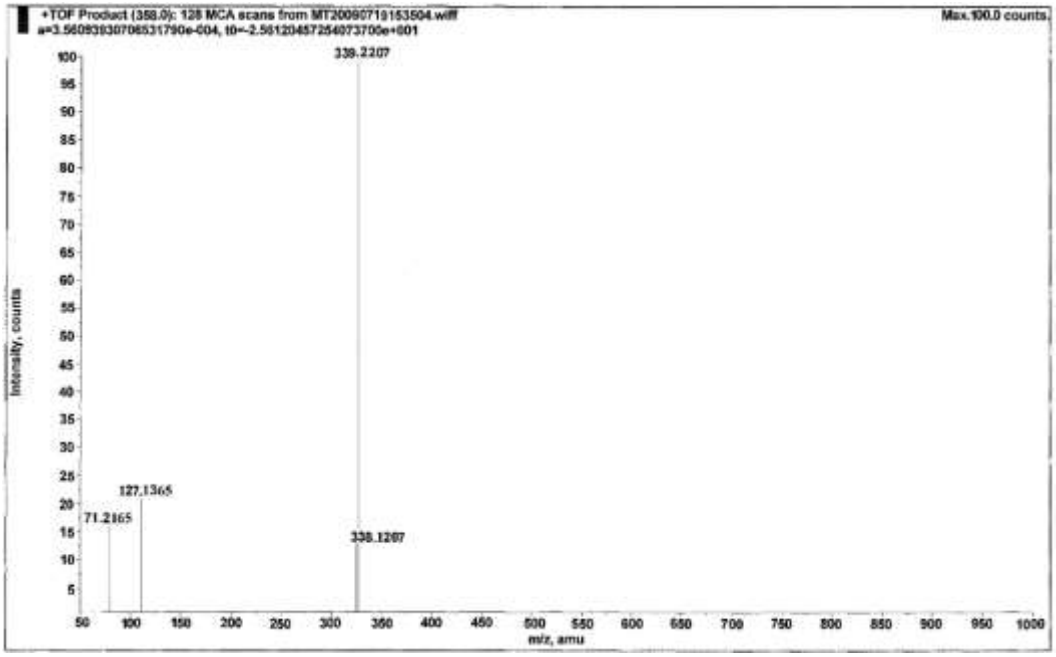


NMR Spectrum of C6.

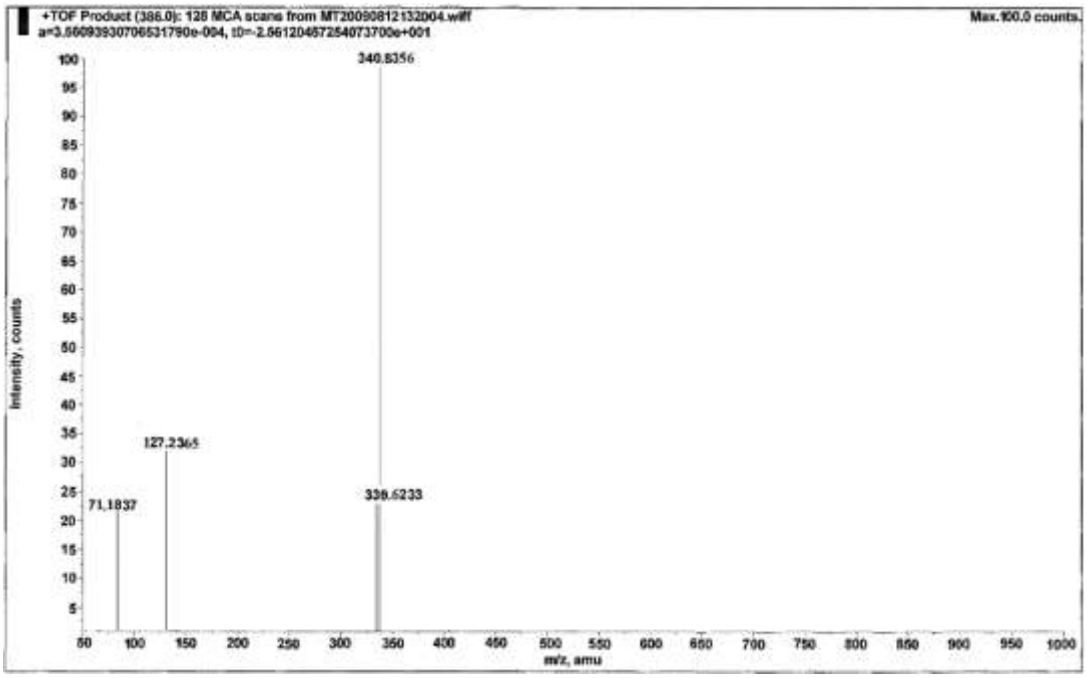
Mass Spectroscopy



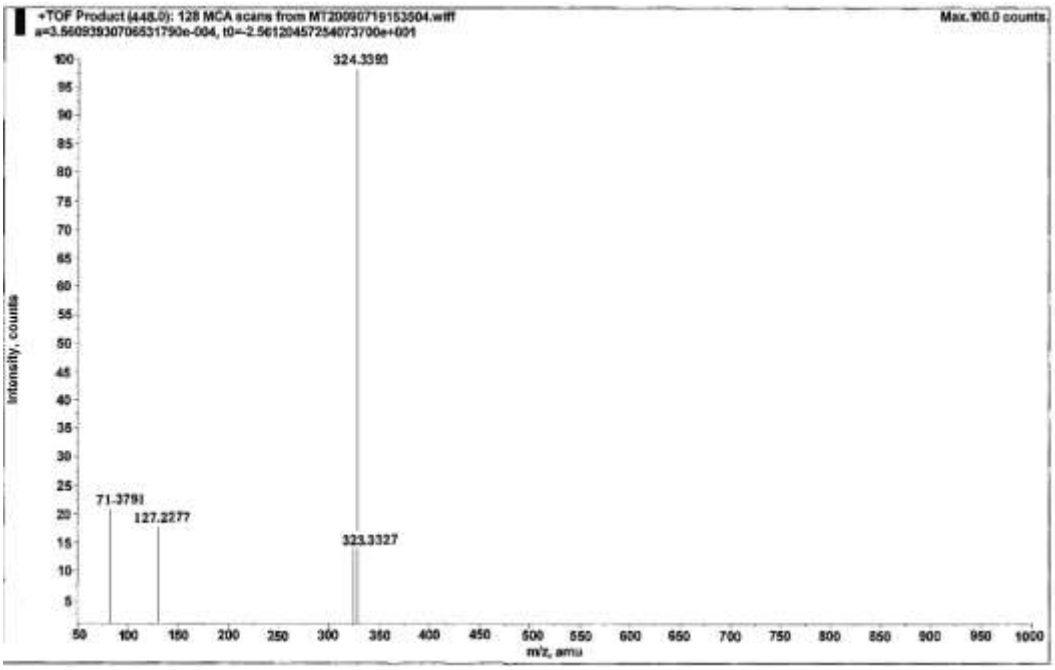
Mass Spectrum of C1.



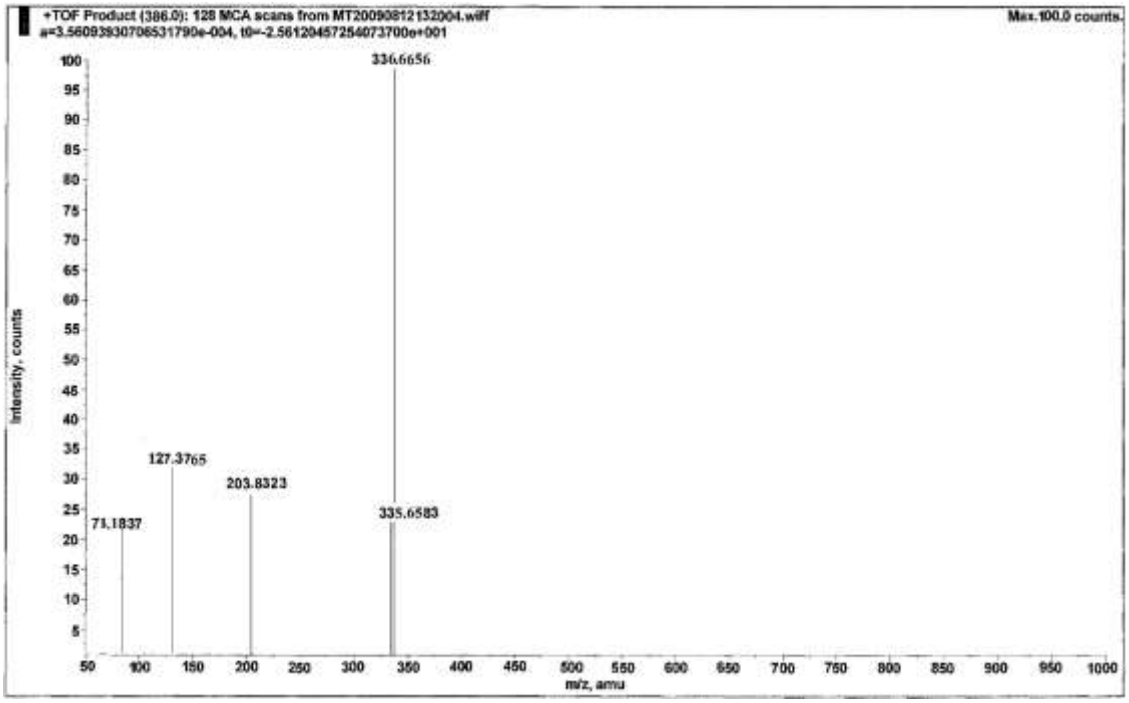
Mass Spectrum of C2.



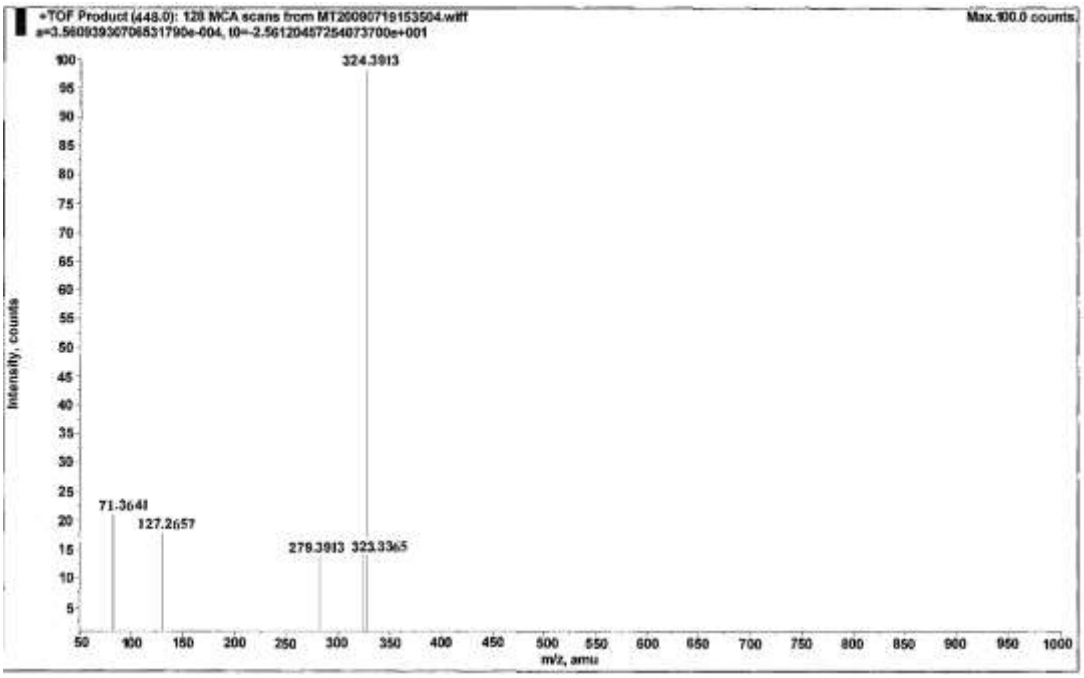
Mass Spectrum of C3.



Mass Spectrum of C4.



Mass Spectrum of C5.



Mass Spectrum of C6

5.3 Evaluation of in-vitro anti-cancer potential

- **Effect of 1,4-dioxane derivatives for cytotoxicity in PC3 cells**

Prostate carcinoma PC3 cell line was used to test the anticancer impact of 1,4-dioxane derivatives using the MTT assay for cytotoxicity. It was discovered that the cells' morphological forms were significantly altered in a dose-dependent way. Photomicrographs made it abundantly evident that in the treatment groups, cells were becoming rounded and separating from the surface. Significant cell death was also seen, and at greater concentrations, this was indicated by surface detachment, cellular shrinkage, and cell body deformation. Based on the obtained sigmoidal curve, the 1,4-dioxane derivatives' IC50 value was determined to be 79.5µg/ml. Additional photomicrographs show the specifics of nuclear apoptosis caused by MECD that were detected using fluorescent DAPI labeling.

Apoptotic cells were defined as those having contracted and fragmented nuclei. The graph illustrates how 1,4-dioxane derivatives significantly triggered nuclear condensation in PC3 cells, demonstrating the induction of apoptosis in a dose-dependent manner. Fluorescent photomicrographs of DCFH-DA-stained PC3 cells effectively illustrate the impact of intracellular ROS activity level caused by MECD. It suggests that elevated ROS activity in PC3 cells contributes to apoptotic induction via a number of different mechanisms.

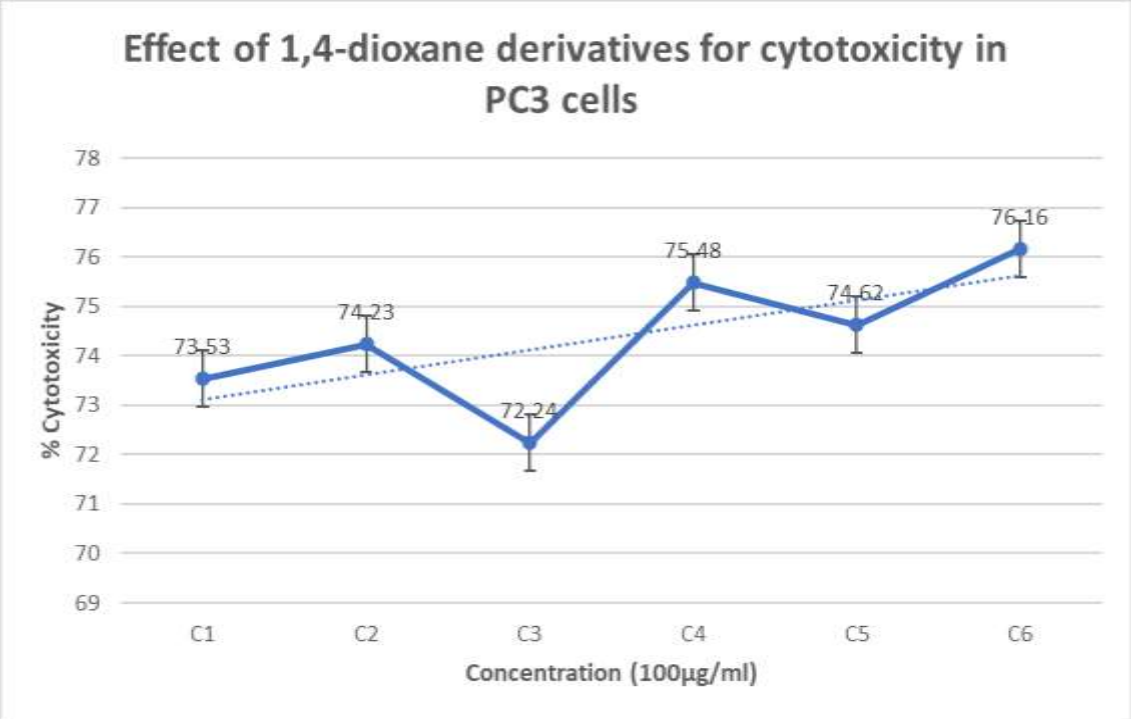


Fig. Effect of 1,4-dioxane derivatives for cytotoxicity in PC3 cells.

All the derivatives (C1-C6) were tested for % cytotoxicity in PC3 cells. At concentration (100µg/ml), 1,4-dioxane derivatives demonstrated the % cytotoxicity as 73.53%, 74.23%, 72.24%, 75.48%, 74.62%, and 76.16%, in the C1, C2, C3, C4, C5 and C6., respectively. Thus, all the derivatives were shown the significant anti-cancer effect when observed in the PC3 cells.

- **Effect of 1,4-dioxane derivatives for viability in MCF-7 cells**

Using MCF-7, the impact of 1,4-dioxane derivatives was evaluated and contrasted with the Control group. The screened samples on cancer cells were demonstrated using MCF-10A breast epithelial cells, a non-tumorigenic cell line. It showed how 1,4-dioxane derivatives affected MCF-7 human breast cancer cells over a 72-hour stimulation period. After 72 hours of stimulation, the IC50 value of 1,4-dioxane derivatives on MCF- was 68.24µg/ml. Tumor cell viability decreased in dose dependent way after treatment with 1,4-dioxane derivatives.

The effect of the 1,4-dioxane derivatives on the % viability was determined in the MCF-7 cells. Lowest % viability was observed in C5 and highest viability in C6 derivative. It refers the percentage of cells that are viable (living).

Thus, it might be said that effects of 1,4-dioxane derivatives were almost similar in both the models i.e., PC3 cell and MCF-7 cells.

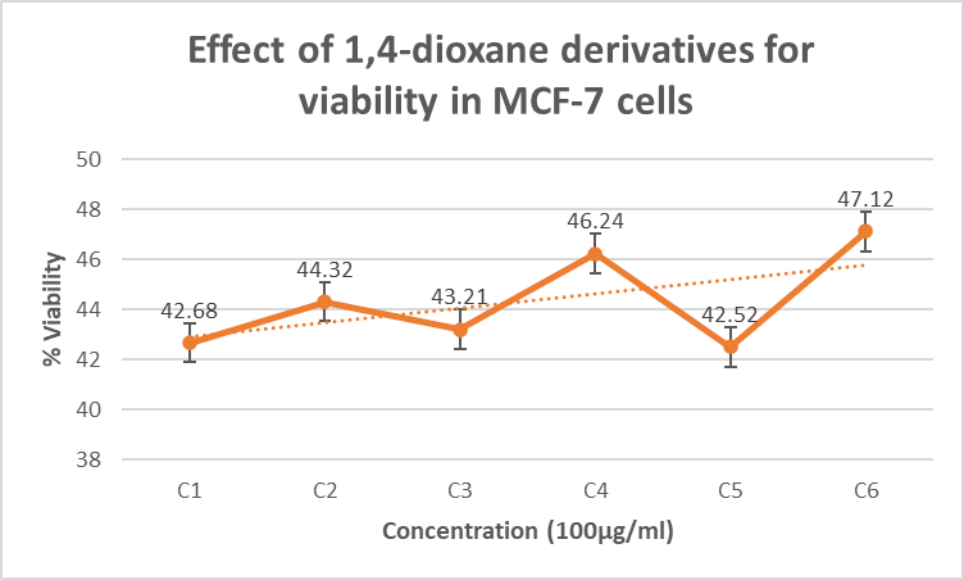


Fig. Effect of 1,4-dioxane derivatives for viability in MCF-7 cells.

Its progression may be due to a malfunction in the apoptotic machinery.[12,13] Therefore, drugs that can influence PC3 cell death may be useful in the treatment of prostate cancer. These days, phytochemicals are used extensively to treat a wide range of illnesses and have shown great promise as side-effect-free anticancer treatment agents.[14] Additionally, although MECD revealed antioxidant role in the current investigation and phytochemicals showed substantial potential as side-effect-free anticancer treatment agents, it was not investigated for toxicity on normal cell lines. It is commonly known that phenolics and flavonoids induce apoptosis and exhibit anticancer action on a variety of cancer cell lines.[15,16]

Prostate carcinoma, PC3 was used to test the anticancer activity of extract MECD using the MTT assay for cytotoxicity. Significant cell death was also seen, and at greater concentrations. Based on the sigmoidal curve, the herbal extract's IC50 value was determined to be 74.5µg/ml. Fluorescent DAPI labeling was used to observe the nuclear apoptosis that it caused. Additionally, it significantly promoted dose-dependent nuclear condensation, which showed that PC3 cells were being induced to undergo apoptosis. When PC3 cells treated with MECD were compared to controls.[17] The MTT assay results indicated that MECD therapy considerably and dose-dependently increased the cytotoxicity of cancer cell lines.

This study's primary goal was to assess the possibility of using Pg extract against the human breast cancer cell line MCF-7 as a proapoptotic and antiproliferative agent. As far as we are aware, this method has never been used previously. Furthermore, statistics indicate that the tested extracts target tumor cells only. While information about the antitumoral activity of black poplar buds on various cell lines is lacking, a growing body of research has shown that the buds of Populus nigra L. are a rich source of phytocompounds that may have anticancer properties. Sukardiman et al. assessed pinostrobin, one of these substances. The research team evaluated the antiproliferative capability of 10, 50, and 100 µg/mL of pinostrobin against the T47D with checkpoints at 24, 48, and 72 hours. The findings showed that this substance increases the proportion of apoptotic cells and has anticancer effect.[18,19,20]

CONCLUSION

Heterocyclic systems' exceptional physicochemical potencies, versatility, and natural inventiveness have allowed them to establish themselves as genuine pillars of medicinal chemistry. The main heterocyclic systems are found in most medications and natural products that are now prescribed. Among them, nitrogen heterocycles are unique because they make up over 60% of the FDA-approved medications.

It concluded that the novel derivatives of 1,4-dioxane have a potential anti-cancer activity when observed in-vitro. All the derivatives exhibited anti-cancer response in the both the models i.e., PC3 cells and MCF-7 cells with % cytotoxicity of 70-80%, approximately at the concentration of 100µg/ml.

It might be prostate cancer or breast cancer which are commonest types of cancer nowadays. It would be reasonable in price with easy availability among society due to easy method of synthesis.

Suggestions are to determine the actual mechanism of action that how 1,4-dioxane derivatives treat the cancer subtypes whether prostate or breast cancer.

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