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# Modification and In Vitro Biological Evaluation of Ceftriaxone-Imine Derivatives: Possible Anticancer and Antimicrobial

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***Abstract****. This research investigates the modification of ceftriaxone to synthesized imine derivatives (A-C), assessing their chemical and biological efficacy. This study underscores the notable antibacterial effectiveness of synthesized derivatives against Staphylococcus aureus and Escherichia coli, exhibiting a determined dose correlation. The synthesis procedure reacts ceftriaxone with different aldehydes, such as 2-Hydroxy-4-Methoxybenzaldehyde and 4-ethoxy-2-hydroxy-benzaldehyde, and 5-Chloro-2-hydroxybenzaldehyde yielding imines analyzed by various methods, such as FT-IR and 1H-NMR. The results indicate that imines may have implications in medical therapies, especially in addressing multidrug-resistant bacteria and a human breast cancer cell line MCF-7. Imine derivative C exhibits significant antibacterial efficacy, measuring 33 mm at 0.1 ppm against Staphylococcus aureus, while 30 mm at 0.1 as anti-Escherichia coli more activity from other synthesized derivatives. The derivative C recorded a value of 29.2441 after 24 hours as an inhibition MCF-7 cell line.*

***Keywords:*** *Antibacterial; Imine, Ceftriaxone, Medical treatment, MCF.*

## 1. INTRODUCTION

Ceftriaxone, a third-generation cephalosporin, is frequently utilized to manage serious infections attributed to Enterobacteriaceae, such as *Escherichia coli* (Shah et al., 2024). The escalating global incidence of antimicrobial resistance (AMR) in Enterobacteriaceae results in elevated patient morbidity and mortality rates, heightened healthcare costs, and greater dependence on last-resort medications (Begum, 2024). The World Health Organization has recognized ceftriaxone-resistant bacteria as a significant priority pathogen. Extended-spectrum β-lactamases are the principal mechanisms conferring ceftriaxone resistance in Enterobacteriaceae. The genes that encode these enzymes can be horizontally transferred across bacteria, promoting the spread of resistance (Flynn & Guarner, 2023; Tebano et al., 2024).

Antimicrobial resistance, mostly induced by microorganisms, constitutes a significant global public health issue (Salam et al., 2023). The escalating antibiotic resistance in bacteria that are critical infections in humans and the transfer of resistance from hospitals to broader communities presents a substantial danger to the public's safety (Kareem et al., 2024). Ceftriaxone is expected to demonstrate effectiveness against a wide spectrum of both gram-negative and gram-positive bacteria (Mubaraka et al., 2023).

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Various microorganisms, including bacteria, fungi, and actinomycetes, synthesize chemical substances called antibiotics, also termed antibacterial or chemotherapeutic agents. These substances can inhibit or eliminate other bacteria (Pancu et al., 2021). Antibacterial drugs are synthetically produced that have not been physiologically sourced despite their designation as antibiotics (Amaning Danquah et al., 2022). A more suitable term would be antibacterial/chemotherapeutic agents (Sadiq, Haddad, et al., 2024).

The study aims to synthesize imine derivatives (A-C) and assess their biochemical properties, namely their antibacterial and anticancer attributes. The research examines imine properties using FT-IR and 1H-NMR. Furthermore, it evaluates the cytotoxicity of synthesized derivative C on MCF-7 cells and its antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli*.

## 2. METHODOLOGY

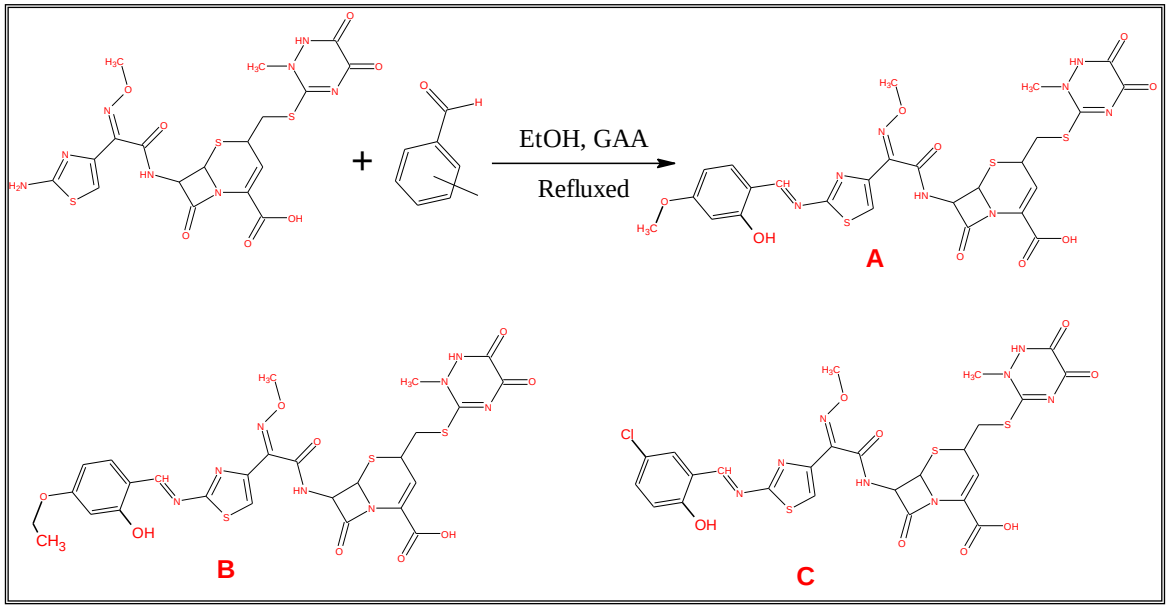
### Materials

Some chemicals used, such as 2-hydroxy-4-methylbenzaldehyde, 4-ethoxy-2-hydroxybenzaldehyde, and 5-chloro-2-hydroxybenzaldehyde, were of higher purity and obtained from the Sigma Aldrich company, while ceftriaxone was obtained from the Samaraa company for drug synthesis. Absolute ethanol (99,9%) was obtained from the BDH company.

### Methods

### Synthesis of ceftriaxone-imine derivatives (A-C)

To modify ceftriaxone into imine derivatives, dissolve 2-Hydroxy-4-Methoxybenzaldehyde (1.5 g, 0.01 mmol), 4-ethoxy-2-hydroxy-benzaldehyde (1.6 g, 0.01 mol), and 5-Chloro-2-hydroxybenzaldehyde (1.5 g, 0.01 mmol) in 25 ml of absolute ethanol, respectively. Subsequently, 3 drops of glacial acetic acid and ceftriaxone (5.4 g, 0.01 mmol) were incorporated into these solutions and refluxed for 2 hours at 65 °C with continuous stirring. After four hours, the solutions were allowed to cool to 20 °C. The yellow precipitates were filtered, rinsed with ethanol, and dried (Sadiq, Mohamed, et al., 2024). The structure of these derivatives is seen in Scheme 1.




Scheme 1: Structures of Schiff bases (A and B).

**Antibacterial Effectiveness**

The antibacterial activity of imine derivatives (A, B, and C) was assessed in *Staphylococcus aureus* and *Escherichia coli* isolates of bacteria using the diffusion-based technique (Gendy et al., 2022). Bacterial infection adjusted to 0.5 McFarland turbidity standards was applied to Muller-Hinton, and wells were formed. Imine derivatives (A, B, and C) were introduced at concentrations (0.1, 0.001, and 0.00001 ppm). Following incubation, antibacterial efficacy was evaluated by measuring the reducing zone surrounding every hole (Al-Zaqri et al., 2022; Sabouri et al., 2021).

## 3. RESULTS AND DISCUSSION

In FTIR spectroscopy, after ceftriaxone reacts with different aldehydes and imine derivatives (A-C) synthesized, the primary amine group of ceftriaxone and carbonyl with C-H of aldehydes disappears in FTIR and appears imine group (Alnoman et al., 2024). In the $^{1}H$ NMR spectra of imine derivatives characteristics, the imine proton of the azomethine group is the most distinctive signal, which manifests as a singlet within the δ 8–9 ppm, dependent upon the electrical environment for protons vibrations (Kolcu et al., 2024). This alteration is affected by electron-withdrawing or electron-donating groups in the structure.

Ceftriaxone-imine (A): The spectrum of FTIR ($cm^{-1}$), as shown in Figure 1, Broad band of -OH shows at 3477, C-H aromatic at 3012, C-H aliphatic appeared at 2932 and 2885, and

the carbonyl group of carboxylic acid at 1709, respectively. The azomethine group shows at 1629 and C-C of the aromatic ring at 1592 (Raauf et al., 2019).

Ceftriaxone-imine (A): $^{1}$H-NMR (500 MHz, DMSO-d6) as shown in Figure 4, δ 11.65 (s, 1H) of hydroxyl group, δ 9.29 (s, 1H) of secondary amine group in cyclic ring, 8.73 (s, 1H) proton of imine group, δ 7.41 -6.60 (m, 10H) protons of aromatic protons, δ 3.95 (s, 3H) protons of methoxy that linked -N=C, δ 3.82 (s, 3H) protons of methoxy group that linked to aromatic cyclic, δ 3.72 (d, 2H) protons of S-CH2-, δ 3.46 (s, 3H) protons of methyl group.

Ceftriaxone-imine (B): The spectrum of FTIR ($cm^{-1}$), as shown in Figure 2, broad band of -OH shows at 3477, C-H aromatic at 3012, C-H aliphatic appeared at 2932 and 2885, and the carbonyl group of carboxylic acid at 1708, respectively. The azomethine group shows at 1642 and C-C of the aromatic ring 1603 (Thejeel).

Ceftriaxone-imine (B): $^{1}$H-NMR (500 MHz, DMSO-d6) as shown in Figure 5, δ 11.52 (s, 1H) of hydroxyl group, δ 9.62 (s, 1H) of secondary amine group in cyclic ring, 8.60 (s, 1H) proton of imine group, δ 7.87-7.21 (m, 10H) protons of aromatic protons, δ 3.95 (s, 3H) protons of methoxy that linked -N=C, δ 3.74 (s, 3H) protons of methoxy group that linked to aromatic cyclic, δ 3.61 (d, H) protons of S-CH2-, δ 1.86 (s, 2H) protons of -$OCH_2$- group, δ 4.05 (t, 3H) methyl that linked to -O-$CH_2$- (Bikas et al., 2022).

Ceftriaxone-imine (C): The spectrum of FTIR ($cm^{-1}$), as shown in Figure 3, Broad band of -OH shows at 3482, C-H aromatic at 3067, C-H aliphatic appeared at 2993, and the carbonyl group of carboxylic acid at 1696, respectively. The azomethine group shows at 1646 and C-C of the aromatic ring at 1600 (Ali et al., 2023).

Ceftriaxone-imine (C): $^{1}$H-NMR (500 MHz, DMSO-d6) as shown in Figure 6, δ 11.47 (s, 1H) of hydroxyl group, δ 9.52 (s, 1H) of secondary amine group in cyclic ring, 8.50 (s, 1H) proton of imine group, δ 7.87-7.25 (m, 10H) protons of aromatic protons, δ 3.17 (s, 3H) protons of methyl that linked N-cyclic ring, δ 3.27 (d, H) protons of S-CH2-, δ 3.86 (s, 2H) protons of methoxy that linked -N=C (Kroonen et al., 2024).

**Biological activity:** Ceftriaxone is a competitive inhibitor of the microbial enzyme dihydropteroate synthase (Maashi, 2023). Research results indicate all evaluated substances, such as ceftriaxone, are linked to concentration antibacterial activity against *Staphylococcus aureus* and *E. Coli*, with more extensive zones of inhibition noted at elevated concentrations (0.1) relative to diminished ones (0.001 and 0.00001). The derivative C had the most potent action, with a maximal zone of reducing 33 mm at a dose of 0.1, surpassing ceftriaxone, which recorded 28 mm at the identical concentration. Derivatives B and A demonstrated modest action, with inhibition zones somewhat smaller than those of derivative C, however similar to

one another. Ceftriaxone, a conventional antibiotic, demonstrated efficacy; however, it was slightly weaker than derivative C across all evaluated doses, indicating the possibility of derivative C as a more effective antibacterial agent. The diminished efficiency of all drugs at lower concentrations underscores the necessity of sustaining sufficient doses to guarantee effectiveness against Staphylococcus aureus, highlighting the relevance of additional research to assess resistance, protection, and medical applicability (Dalhoff, 2021), as shown in Figure 7.

The research results demonstrate a dosage-dependent antimicrobial action of the investigated chemicals and Ceftriaxone against *E. coli*, exhibiting greater reduction regions at 0.1 and diminished activity at lower concentrations. Derivative C showed the greatest zone of reduction (30 mm) at a concentration of 0.1, succeeded by derivative B (27 mm), derivative A (26 mm), and Ceftriaxone (27 mm). At a dosage of 0.001, derivative C (23 mm) surpassed the other compounds; however, at a concentration of 0.00001, all derivatives had little action, with inhibition zones between 7- and 10-mm. Derivative C had more uniform antimicrobial action at various dosages, as shown in Figure 8.

**Synthesized ceftriaxone-imine (C) cytotoxicity**

The information in Table 1 supplements the figure by presenting the mean cell viability % and standard deviations of MCF-7 cells receiving derivative C at several levels throughout a 24-hour period. Both Figure 9 and Table 1 demonstrate a distinct dose-dependent reduction in cell viability as the dosage of derivative C escalates. At 20 ppm, the average cell viability declines to 74.45% (SD ± 1.02), with additional reductions noted at elevated concentrations: 40 ppm (69.34%), 80 ppm (57.31%), 160 ppm (44.02%), and the minimum at 320 ppm (29.24%, SD ± 3.33). The IC50 value of 135.5 ppm corresponds with the significant decrease in cell viability around the 160-ppm dosage, validating the substance's efficacy in diminishing MCF-7 cell viability. The rising standard deviations at elevated doses indicate variability in the response, which may be attributable to cellular heterogeneity or laboratory variables. The table and graph collectively substantiate the cytotoxic efficacy of derivative C against the MCF-7 cell line.

## 4. CONCLUSION

This research safely synthesized imine derivatives (A-C) by modification of ceftriaxone. The generated imines demonstrated considerable antimicrobial effectiveness against *Staphylococcus aureus* and *Escherichia coli*, exhibiting a dependent on-dosage association. The cytotoxicity of derivative C on MCF-7 cells was assessed, demonstrating a gradually

reduced cell viability, suggesting significant medical applications. Analysis methods, including FT-IR and $^{1}$H-NMR. Results indicate that synthesized derivatives have considerable potential for use in antibacterial and medicinal domains.

**Tables and Figures**

Table 1: Effectiveness levels in imine (C) generated MCF-7 cells.

| Dosage (PPM) | 24 h | |
|---|---|---|
| | Mean | SD |
| 0 | 100 | 2.65783 |
| 20 | 74.4519 | 1.02371 |
| 40 | 69.3373 | 1.91693 |
| 80 | 57.3052 | 1.14772 |
| 160 | 44.0183 | 2.86610 |
| 320 | 29.2441 | 3.33197 |

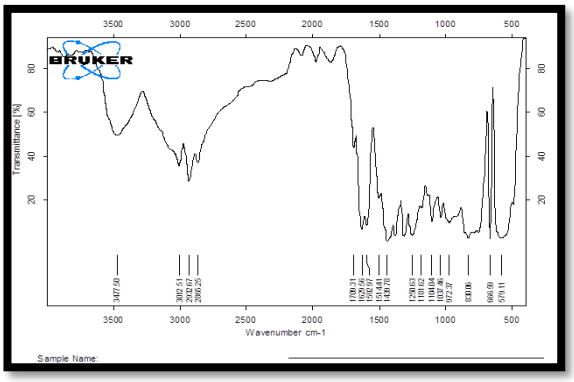



Figure 1. FTIR spectrum of derivative A.

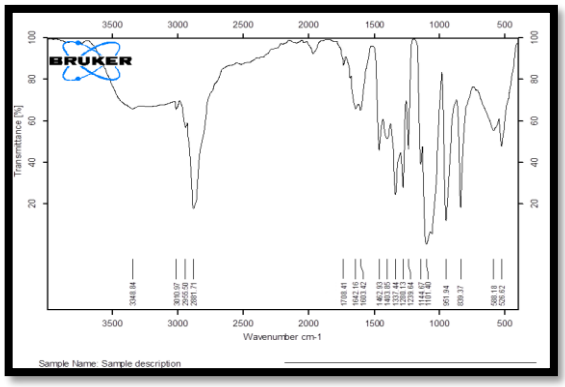



Figure 2. FTIR spectrum of derivative B.

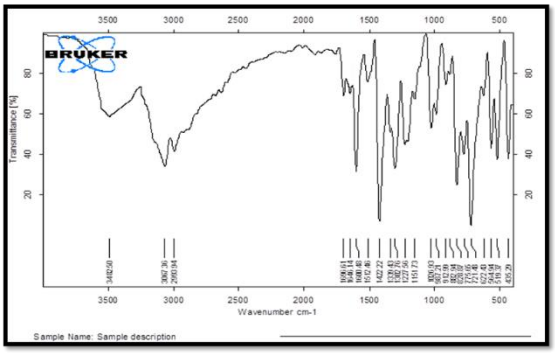



Figure 3. FTIR spectrum of derivative C.

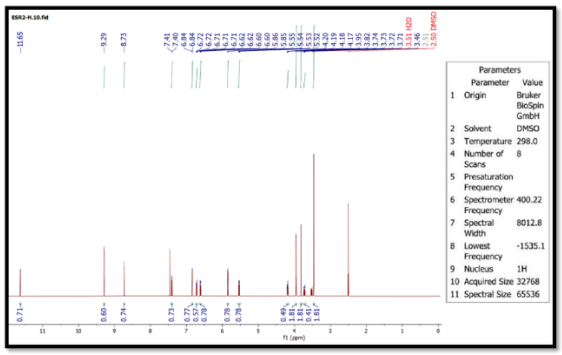



Figure 4. $^{1}$HNMR spectrum of derivative A.

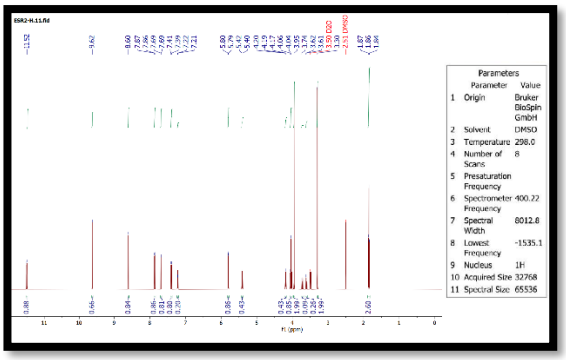


Figure 5. [1]HNMR spectrum of derivative B.

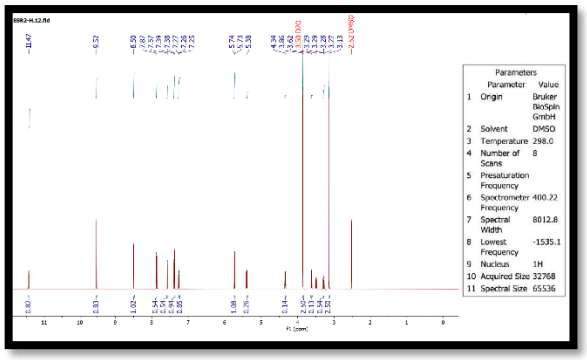


Figure 6. [1]HNMR spectrum of derivative C.

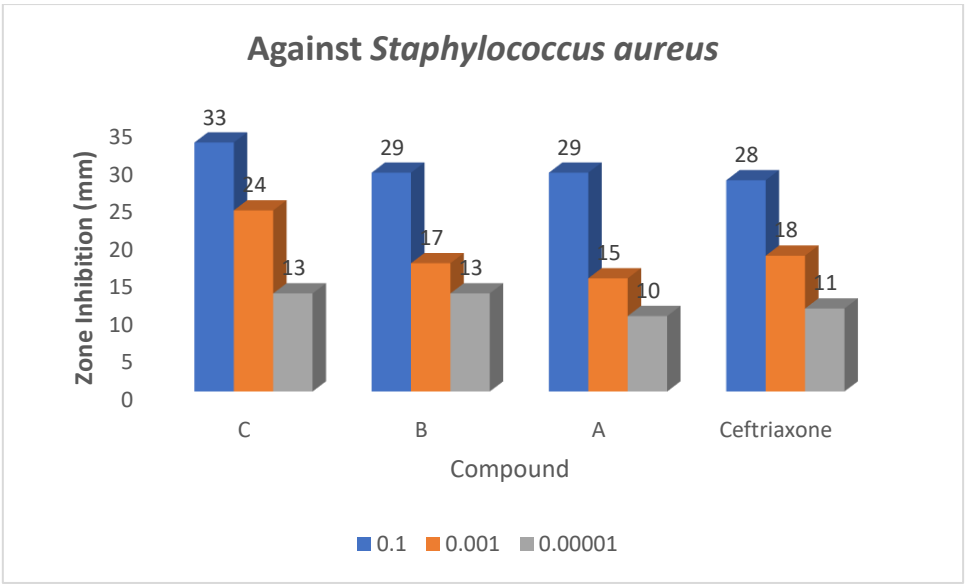



Figure 7. Ceftriaxone and its derivatives biological activity.

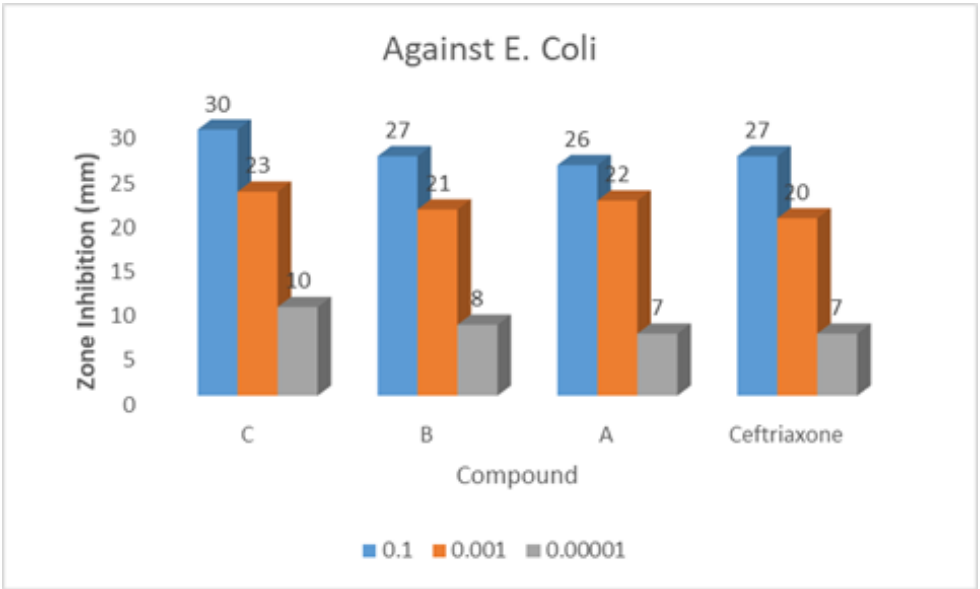



Figure 8. Biological activity of ceftriaxone and its derivatives.

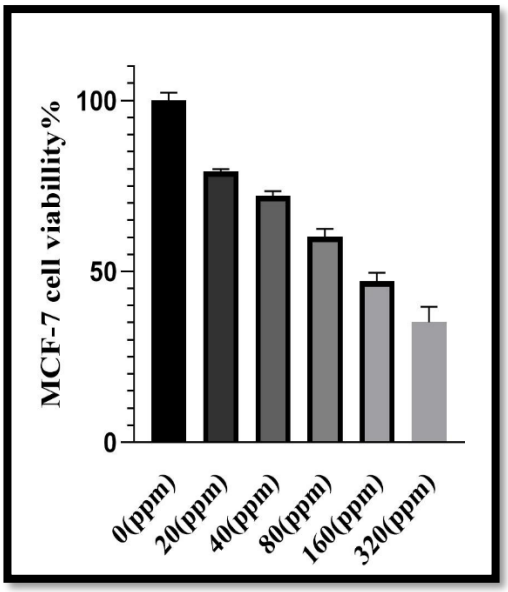



Figure 9. Cytotoxicity of derivative C against MCF-7 cell line.

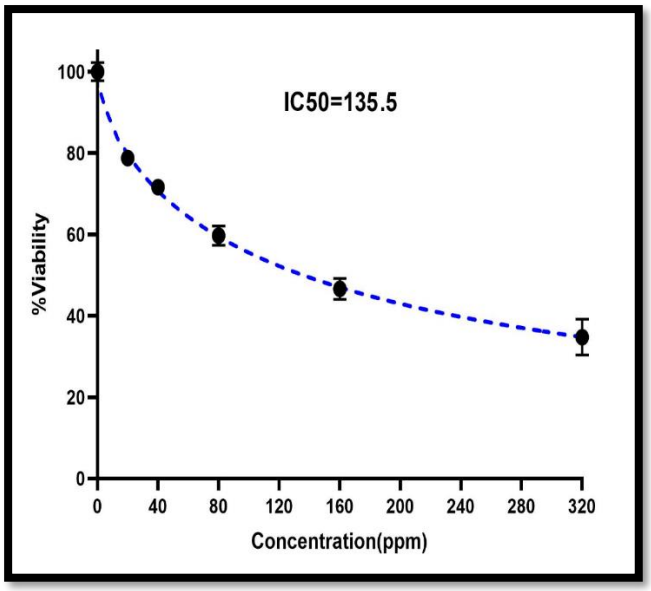



Figure 10. IC50 of derivative C against the MCF-7 cell line.

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