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Synthesis, characterization and biological activities of new star-shaped cyclodextrin-triazole hybrids and glycoside analogues via click chemistry approach

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Abstract

The growing demand for innovative and effective anticancer agents has steered research toward the development of multifunctional bioactive compounds. Herein, we describe the synthesis of a novel pyridine/cyclodextrin derivative through a copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) click reaction. Initially, a pyridine derivative bearing a terminal alkyne group was synthesized and subsequently reacted with an azido-functionalized β-cyclodextrin. Structural characterization of the resulting conjugate was conducted using FTIR, NMR, and SEM analyses, confirming the successful formation and morphological features of the hybrid compound. The biological evaluation demonstrated remarkable multifunctional activity of the synthesized compound, including potent antioxidant capacity (DPPH radical scavenging activity reaching 93%), strong antibacterial efficacy (inhibition zones of 2.6–3.7 cm), notable antifungal effect (inhibition zone of 1.3 cm), and pronounced anticancer activity (IC_{50} = 2.42 µg/mL, equivalent to 4.3 µM). These findings underscore its potential as a promising candidate for advanced biomedical applications.

Keywords: Cyclodextrin; pyridine derivatives; 1,2,3-Triazole; SEM; NMR; antibacterial; anticancer.

1. Introduction

Cancer remains a leading global health challenge, responsible for approximately 10 million fatalities in 2020, as reported by the World Health Organization [1]. Despite significant advances in diagnostics and treatment modalities, conventional chemotherapeutic agents often suffer from major drawbacks such as systemic toxicity, low selectivity, and the emergence of multidrug resistance [2-4]. Therefore, the development of novel therapeutic agents with improved efficacy and minimized adverse effects remains a high priority in medicinal chemistry.

In recent years, cyclodextrins (CDs) have emerged as promising carriers and functional entities in pharmaceutical and biomedical applications due to their unique molecular architecture [5-8]. CDs are cyclic oligosaccharides composed of α-(1,4)-linked glucopyranose units, forming a hydrophobic inner cavity and a hydrophilic outer surface. This amphiphilic structure allows them to form host–guest inclusion complexes with various molecules, thereby enhancing drug solubility, bioavailability, and stability [9-14]. Beyond their role as excipients, chemically modified CDs have shown intrinsic biological activities, including antioxidant, antimicrobial, and even anticancer properties [15-20]. Azido-functionalized CDs, in particular, serve as versatile intermediates for "click" conjugation, enabling precise and efficient attachment of bioactive moieties [21-24].

On the other hand, pyridine derivatives are well-known scaffolds in drug discovery research owing to their broad range of biological activities [25-30]. Pyridine rings are often present in FDA-approved drugs and pharmacologically active compounds, playing a pivotal role in binding interactions with biological targets [31,32]. Substituted pyridines have demonstrated notable anticancer, antibacterial, antiviral, and anti-inflammatory properties [33-38]. Their heteroaromatic structure is amenable to functionalization, allowing the rational design of new derivatives with improved pharmacokinetics and pharmacodynamics.

The conjugation of pyridine-based pharmacophores with cyclodextrin frameworks via click chemistry represents an effective approach for the development of novel hybrid molecules with enhanced biological potential. Pyridine moieties are well recognized for their broad spectrum of pharmacological activities, whereas cyclodextrins contribute to improved solubility, stability, and bioavailability. The resulting hybrids thus integrate intrinsic bioactivity with favorable delivery characteristics, providing a distinct advantage over conventional molecular scaffolds [39-41]. Click chemistry is widely appreciated due to its high effectiveness, regioselectivity, and mild reaction conditions, making it particularly attractive for the synthesis of

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multifunctional bio-conjugates [42–46]. Importantly, the CuAAC reaction generates a 1,2,3-triazole ring, a five-membered heterocycle known for its remarkable chemical stability and wide range of biological properties, such as anticancer, antioxidant, antimicrobial, and anti-inflammatory activities [46–50]. This triazole core has the ability to donate or accept hydrogen bonds and often participates in π–π stacking or metal coordination, further enhancing the pharmacodynamic potential of the conjugate. Several studies have reported successful conjugation of biologically active compounds onto cyclodextrin platforms using this approach, yielding materials with augmented therapeutic and diagnostic properties [51,55].
In this study, we designed and synthesized a new pyridine/cyclodextrin derivative by first preparing an alkyne-functionalized pyridine derivative and an azido-functionalized β-cyclodextrin. These precursors were then coupled via CuAAC click reaction to yield the target conjugate. The structure and morphology of the synthesized compound were characterized by structural and morphological characterization was performed using FTIR, NMR, and SEM analyses. The resulting hybrid exhibited excellent antioxidant, antibacterial, and anticancer activities, suggesting strong potential for further development as a multifunctional therapeutic agent.

2. Materials and Methods

2.1 Chemistry

All chemicals, solvents, and reagents investigated in this study were of analytical grade and purchased from commercial suppliers such as Sigma-Aldrich, Fluka, or Merck. Unless otherwise stated, no further purification was carried out.

2.1.1. Synthesis of substituted azidoalkyl-pyridine derivatives

4-(4-Fluorophenyl)-6-oxo-1,6-dihydro-[2,3'-bipyridine]-5-carbonitrile (100,1B)

In a 250 mL round-bottom, ammonium acetate (8.7 mmol, 6.74 g) was solubilized in ethanol (125 mL) then ethyl cyanoacetate (2.5 mmol, 2.77 g) was added. 3-Acetylpyridine (24.7 mmol, 2.99 g) and 4-flourobenzaldehyde (25.0 mmol, 3.1 g) were then added, and for 4h, the reaction mixture was refluxed. The resulted precipitated solid was then filtered and dried. White powder, Yield 37.7%; M.p. 218-221 ºC.

4-(4-fluorophenyl)-6-(prop-2-yn-1-yloxy)-[2,3'-bipyridine]-5-carbonitrile (106)

the substituted pyridone compound 100 (12 mmol) was dissolved in DMF (60 mL) then the reaction flask was provided with anhydrous K2CO3 (12 mmol) followed by stirring for 50-60 min. To the latter mixture was then added gradually (over a period of 10 min) propargyl bromide (14 mmol) while stirring is continued in an ice bath. After stirring of the reaction content for 8 h at rt, it was added portion wise to an ice-cold water mixture with continues stirring for 30 min after which precipitate was formed. The obtained precipitate was isolated through filtration, thoroughly washed with water, dried, and subsequently recrystallized in ethanol to afford the acetylenic product 106.
Dark brown powder, Yield 92%; M.p. 244-249 ºC ; IR (KBr) ύ (cm-1): 3300 (≡CH), 3100 (=CH-Ar), 2075 (-CH), 2195 (C≡CH), 2226 (C≡N), 1609 (C=N); 1H NMR (400 MHz, CDCl3) δ 9.32 (d, J = 2.4 Hz, 1H, Pry-H), 8.73 (dd, J = 5.0, 1.8 Hz, 1H, Pry-H), 8.40 (dt, J = 8.1, 2.1 Hz, 1H, Pry-H), 8.02 (s, 1H, Pry-H), 7.71 – 7.63 (m, 2H, HAr), 7.53 (s, 1H, Pry-H), 7.46 (dd, J = 8.0, 4.8 Hz, 2H, HAr), 5.23 (d, J = 2.4 Hz, 2H, CH2), 2.54 (t, J = 2.4 Hz, 1H, Acetylene-H); 13C NMR (101 MHz, CDCl3) δ (Pry-C) 162.54, 156.29, 151.36, 114.18, 92.5, (Pry-C) 148.74, 148.7, 134.74, 132.59, 123.75, (Ar-C) 132.59, 130.51, 130.42, 116.50, 116.28, (C≡N) 114.18, (C≡C) 76.70, 77.97, (CH2-O) 54.96. Analysis calcd. for C20H12FN3O, 329.334: C, 72.94; H, 3.67; F, 5.77; N, 12.76; O, 4.86.

2.1.2. Synthesis of substituted β-cyclodextrin derivatives.

(3R,4R,5S,6S)-6-(iodomethyl)-5-methoxy-2-methyltetrahydro-2H-pyran-3,4-diol (112)

In a 250 mL round-bottom flask equipped with a condenser and maintained under a nitrogen atmosphere, triphenylphosphine (PPh_3, 23.1 g, 88 mmol) was dissolved in 90 mL of anhydrous DMF. Elemental iodine (I_2, 22.3 g, 88 mmol) was then added gradually while the mixture was heated at 50 °C. Subsequently, β-cyclodextrin (5 g, 4.4 mmol) was introduced into the resulting dark brown solution, and the reaction was allowed to stir for 18 h at 70 °C. Upon completion, the solvent (DMF) was removed under reduced pressure to concentrate the reaction mixture, which was then cooled to room temperature. A methanolic solution of sodium methoxide (5.6 g in 80 mL), pre-cooled in an ice bath under nitrogen, was carefully added to the mixture while maintaining cooling conditions. After a few minutes of stirring, the mixture precipitated in 600 mL of methanol.

White powder, Yield: 90% - 1H NMR (101 MHz, DMSO) δ 6.03 (d, 7H, OH2), 5.93 (d, 7H, OH3), 4.98 (d, 7H, H1), 3.10 - 3.90 (m, 42H, H2, H3, H4, H5, H6);13C NMR (100 MHz, DMSO) δ 102.14 (C1), 85.96 (C4), 72.18 (C5), 71.93 (C3), 70.98 (C2), 9.57 (C6).

(3R,4R,5S,6R)-6-(azidomethyl)-5-methoxy-2-methyltetrahydro-2H-pyran-3,4-diol, (113)

Per-(6-deoxy-6-iodo)-β-cyclodextrin 113 (4 g, 2.1 mmol) was dissolved in 70 mL of DMF in a 250 mL round-bottom flask under nitrogen. Sodium azide (1.4 g, 21 mmol) was added, and the mixture was stirred at 60 °C for 20 h. The solvent was then

removed under reduced pressure. 500 milliliters of water were used to precipitate the mixture. A significant amount of water was used to filter and wash the white, insoluble product. The obtained solid was vacuum-dried for overnight at 80°C.

White powder, Yield: 90% - 1H NMR (101 MHz, DMSO) δ 5.92 (d, 7H, OH2) 5.76 (d, 7H, OH3), 4.91 (d, 7H, H1), 3.20 - 3.82 (m, 42H, H2, H3, H4, H5, H6); 13C NMR (100 MHz, DMSO) δ 102.16 (C1), 83.30 (C4), 72.71 (C2), 72.10 (C3), 70.44 (C5), 51.44 (CH2-N3), ESIMS (MeOH): Calcd for (M+Na+)+ C42H63O28N21Na: 1332.4; Found: 1332.8 uma.

per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin (114)

For the synthesis of the protected per-azido β-CD, we dissolve 2 g of per-(6-deoxy-6-azido)-β-cyclodextrin in 35 mL of dry pyridine, 25 mL of acetic anhydride and a catalytic quantity of 4 dimethylaminopyridinet were added to a 250 mL flask. Under N2, the solution was agitated for 24 hours at 70 °C. Following dissolution in 150 mL of CH_2Cl_2, the resultant solution was rinsed with HCl 1M (3x100 mL), saturated NaHCO3 (3x100 mL), and water (3x100 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated at a lower pressure in order to remove all of the dichloromethane. The item was vacuum-dried at 80°C for the overnight.

Yellowish-white powder, Yield: 72% - 1H NMR (101 MHz, DMSO) δ 5.29 (t, 7H, H3), 5.07 (d, 7H, H1), 4.81 (dd, 7H, H2), 4.10 – 3.90 (m, 7H, H5), 3.50 – 3.82 (m, 21H, H6, H4), 2.04 (d, 42H, COCH3). 13C NMR (100 MHz, DMSO) δ 170.61 (COCH3), 169.57 (COCH3), 96.67 (C1), 77.07 (C4), 70.96 (C5), 70.64 (C2), 70.42 (C3), 51.32 (CH2-N3), 20.83 (COCH3).

3.2.3 Synthesis of substituted 1,2,3-triazole-βCD derivatives via click chemistry.

Dry DMF (10 mL) was used to dissolve the substituted pyridone compound 106 (2 mmol). Then, to the solution, per-(2,3-di-O-acetyl)-(6-deoxy-6-azido)-β-cyclodextrin (500 mg, 0.26 mmol) and hydrated CuSO4 (500 mg, 2 mmol) were added. A freshly prepared aqueous solution of sodium ascorbate (850 mg, 4 mmol, 3 M) was introduced dropwise, and the resulting mixture was stirred for 48 h. Following solvent evaporation, the residue was dissolved in 300 mL of CH2Cl2 and washed with 3x200 mL of ammonium hydroxide 5N. After being dried over MgSO4, the organic layer was filtered and allowed to evaporate at a lower pressure. The product was vacuum-dried for an entire night at 50°C to obtain the compound 115.

2.2. Characterization techniques

Measurements of melting points were performed on a Stuart SMP30 apparatus. IR spectra were obtained using Agilent Technologies at Qassim University's College of Science. Proton and carbon NMR spectra were collected at 400 MHz and 100 MHz, respectively, on a Bruker Avance III spectrometer at the same institution, using DMSO-d6 as a solvent. Chemical shifts (δ) were reported in ppm relative to TMS. The DMSO-d6 solvent signals were at δH 2.55 and δC 40. Surface morphology of the synthesized compounds was examined by scanning electron microscopy (SEM) using a JEOL JSM-IT200 microscope under high vacuum mode after gold sputtering to improve conductive properties.

4. Results and discussion

3.1. Chemistry

The synthesis of the target 1,2,3-triazole-linked pyridine/β-cyclodextrin derivatives involved the preparation of two key components: a terminal alkyne-functionalized pyridine derivative and a per-azido-β-cyclodextrin.
The pyridine intermediate was synthesized via a Knoevenagel condensation between 4-fluorobenzaldehyde and 3-acetylpyridine in the presence of ethyl cyanoacetate and ammonium acetate in ethanol under reflux at 70 °C for 4 hours. This reaction led to the formation of compound 100, a yellowish-white solid (m.p. 218–221 °C, yield: 37.7%), confirmed as 4-(4-fluorophenyl)-6-oxo-1,6-dihydro-[2,3′-bipyridine]-5-carbonitrile.
Subsequently, compound 106, the terminal alkyne derivative, was synthesized via a Williamson ether synthesis, in which compound 100 was reacted with propargyl bromide with K_2CO_3 as a catalyst in DMF. This SN2 substitution yielded a dark brown precipitate with a melting point of 244–249 °C and an excellent yield of 92%. The ^{1}H NMR spectrum displayed characteristic signals including a triplet at 2.53–2.55 ppm (acetylenic proton), a doublet at 5.23–5.55 ppm (CH_2–O group), and aromatic signals at 8.02 and 9.32–9.33 ppm. The ^{13}C NMR spectrum validated the occurrence of triazole precursor functionalities with signals for acetylenic carbons (76.70–77.97 ppm), ether carbon (54.96 ppm), aromatic carbons, and a nitrile carbon (114.18 ppm).

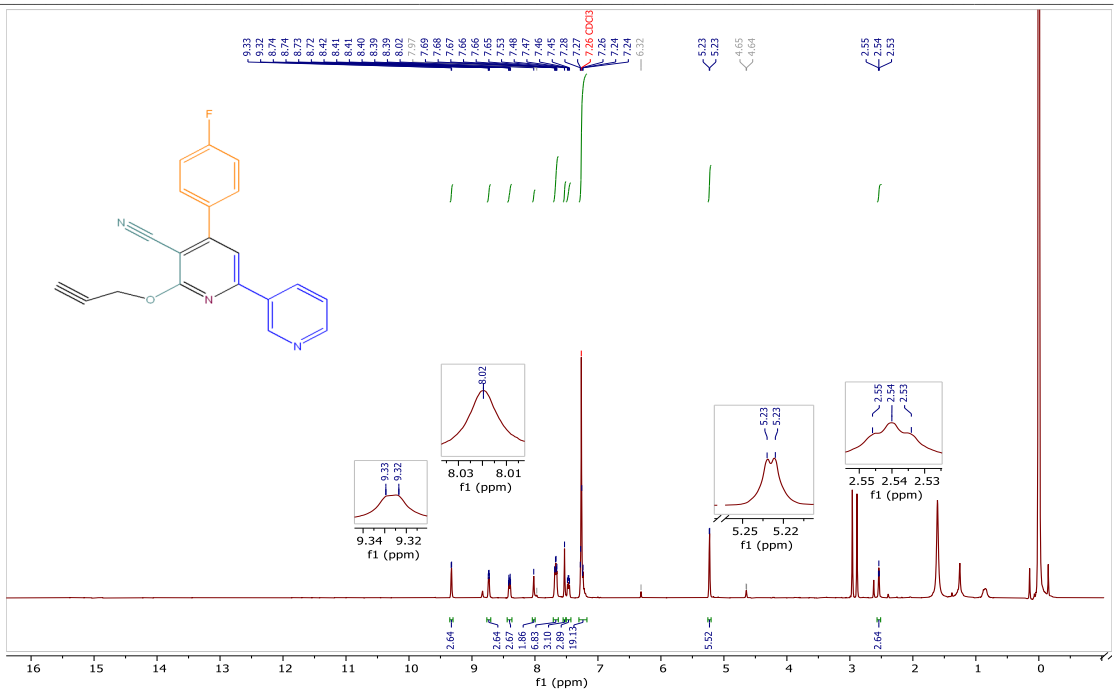


Figure 1. 1H NMR spectrum for the synthesized compound 106

In parallel, the azido-functionalized β-cyclodextrin was prepared through a two-step process. Initially, per-iodinated β-cyclodextrin (compound 112) was synthesized by treating β-CD with iodine and triphenylphosphine (PPh_3) in DMF under reflux for 15 hours. Sodium methoxide and methanol were added post-reaction to quench excess iodine and eliminate byproducts. The appearance of a new signal around 10 ppm in the ^{13}C NMR spectrum confirmed CH_2–I substitution at the C6 position.

This was followed by a nucleophilic substitution using sodium azide (NaN_3) in DMF, yielding compound 113, the per-6-azido-β-cyclodextrin (Figure 2). The substitution was verified by the disappearance of the iodine signal (~10 ppm) and the appearance of a new signal at ~51 ppm (CH_2–N_3) in the ^{13}C NMR spectrum (Figure 3).

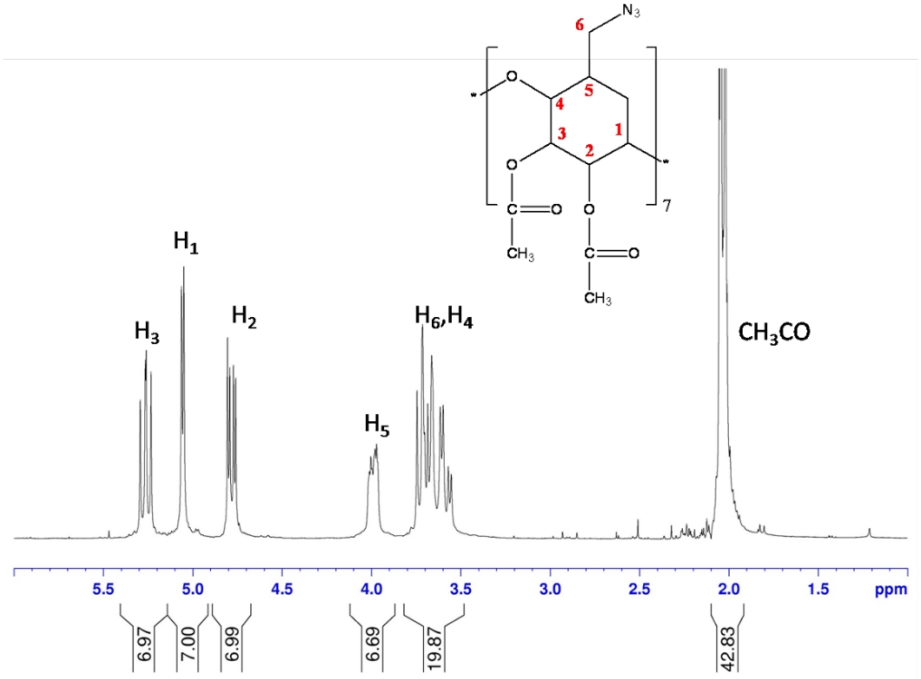


Figure 2. ^1H NMR specter of the peracetylated azido-β-CD derivative (compound 114)

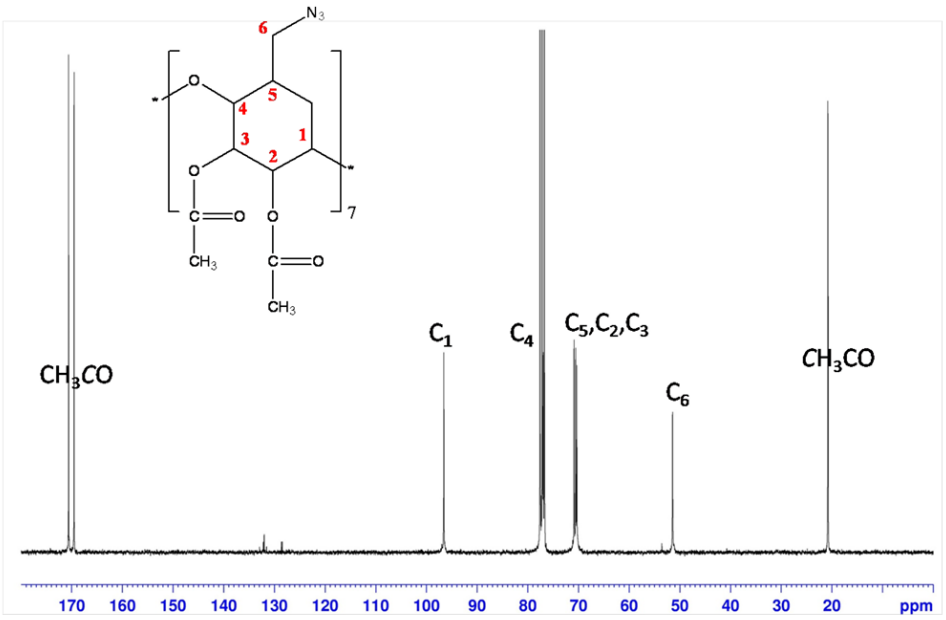
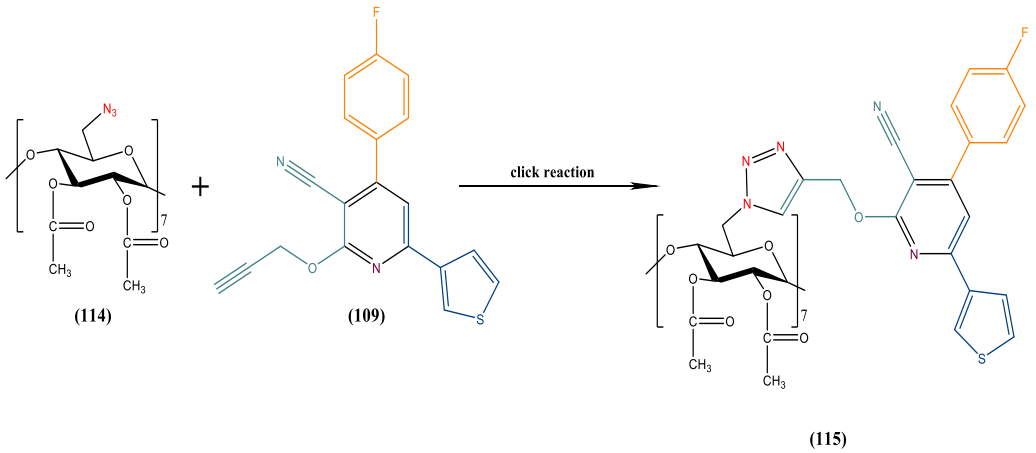


Figure 3. ^{13}C NMR specter of the peracetylated azido-β-CD derivative (compound 114)

To prevent side reactions during click conjugation, the secondary hydroxyl groups at positions 2 and 3 of compound 113 were protected via acetylation, using acetic anhydride in pyridine at 70 °C. The resulting peracetylated azido-β-CD derivative (compound 114) was obtained after work-up with CH_2Cl_2 and aqueous HCl washes.

The final click conjugation step was carried out between compound 114 and alkyne-bearing pyridine derivatives (compounds 106). The reactions were performed in a DMSO/n-butanol mixture, using copper(II) sulfate and sodium ascorbate as the catalytic system. Stirring at room temperature for 48 hours afforded compounds 115 and 116 in high yields (>90%). The formation of the triazole ring was confirmed by a singlet at ~8.01 ppm in the ^1H NMR spectrum, attributed to the triazole proton, and a signal at ~5.02 ppm corresponding to the anomeric proton (C1) of β-CD.
A final deacetylation step was performed to yield the deprotected cyclodextrin conjugate, compound 116, suitable for biological evaluation.



Scheme 1. The Route of synthesis of compound 115.

3.2. SEM characterization

SEM Characterization and Morphological Analysis

The surface morphology of native β-cyclodextrin (β-CD) and its click-modified derivative (compounds 116) was investigated using scanning electron microscopy (SEM). Native β-CD in Figure 4 displayed a compact, crystalline structure with smooth, low-porosity surfaces and minimal microstructural features. In contrast, the modified compound (Figure 5) exhibited markedly rougher, more porous morphologies with visible cavities and irregular textures, indicating a disruption of the native crystalline order. These changes are attributed to the successful incorporation of triazole-linked moieties via click chemistry, which introduced polar functionalities and induced micro- and mesoporous features. The increased surface roughness and porosity are consistent with enhanced hydrophilicity and accessibility, which likely contribute to the superior antioxidant, antibacterial, and anticancer activities observed. Thus, SEM analysis confirms that structural modifications not only alter the physical properties of β-CD derivatives but also play a critical role in enhancing their bioactivity through improved surface interaction and molecular accessibility.

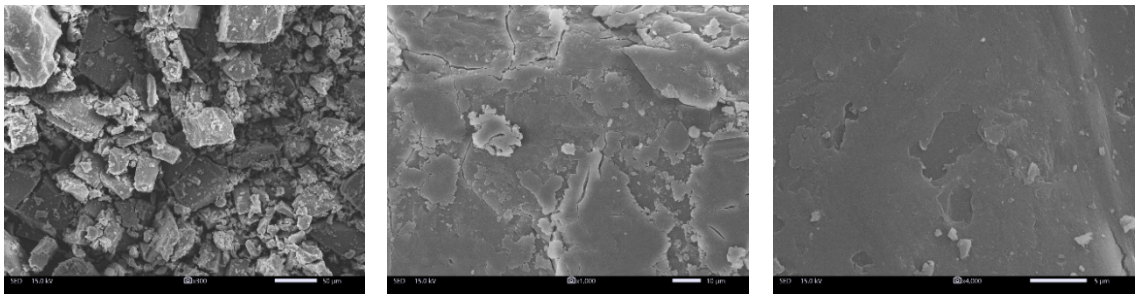


Figure 4. SEM photomicrographs of the native β-CD macromolecule at different magnifications.

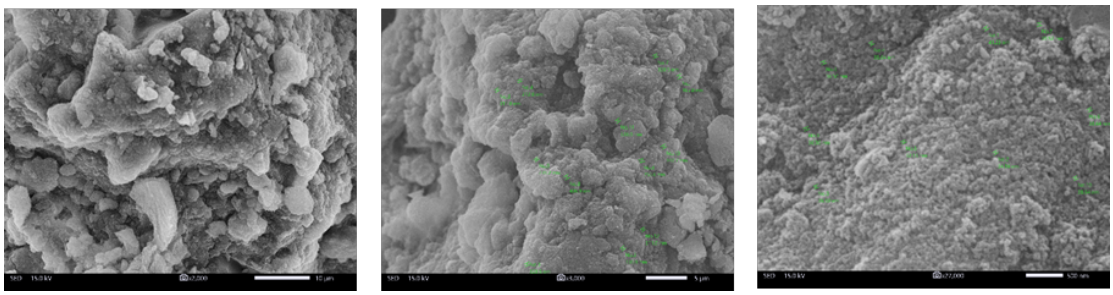


Figure 5. SEM photomicrographs of the β-CD-triazole derivative 116 at different magnifications.

3.2. Cytotoxicity

3.2.1. Microbiological assessments

3.2.1.1. Antibacterial activity

The microbiological assays were performed by measuring the inhibition zone appearing around samples, which serves as an indicator of the antimicrobial activity of the compounds, using the Mueller-Hinton agar disk diffusion method. [56]. The antibacterial evaluation of the β -CD macromolecule and the two new cyclodextrins based triazole derivatives were conducted against tow bacteria types; Staphylococcus aureus ATCC 25923 (Sa) as gram positive bacteria and Escherichia coli ATCC 35218 (Ec) as gram negative bacteria strain. The antifungal assays were performed on a pathogenic reference strain of yeast Candida albicans ATCC 90028 (Ac).

As shown in Figure 6, the native β-CD shows an activity against the gram-positive bacteria Sa and a low activity against the Ec gram-negative bacteria and no fungal activity against Ac fungal strain. This is a line with the majority of reported studies stating that β-CD generally showed stronger effects on gram-positive bacteria than on gram-negative bacteria [57,58].

The tow synthesized β-CD based triazole compounds showed a higher inhibitory activity toward the two bacterial strains. The greater effect was against the Staphylococcus aureus bacteria with inhibition zone of 3.8 for the derivative compound 116.

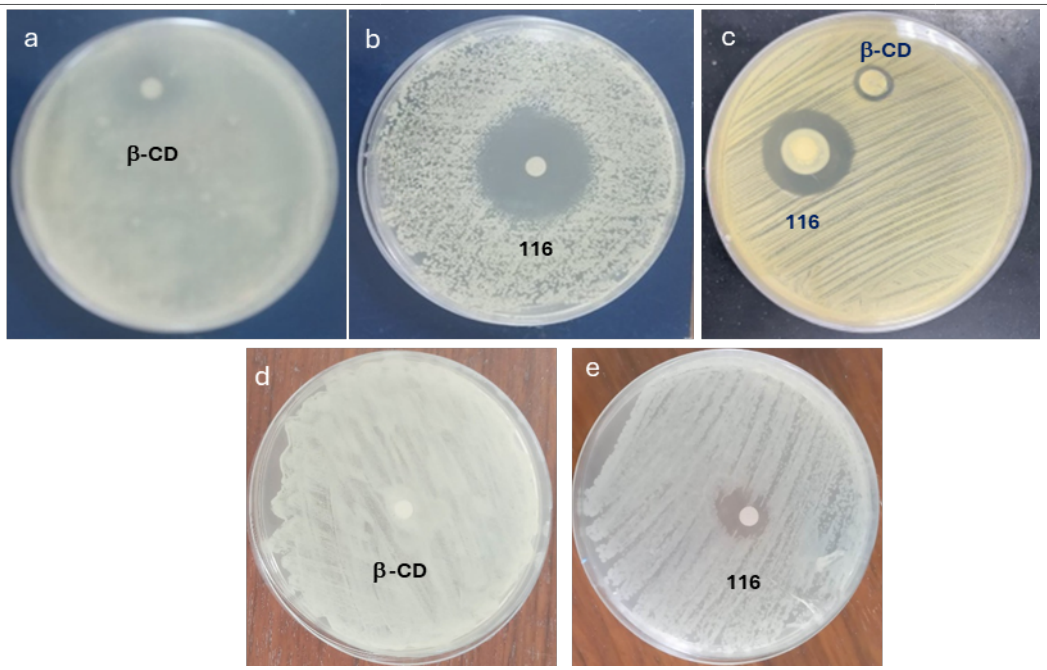


Figure 6. Antimicrobial and antifungal screens of the native β-CD and the β -CD-triazole derivative against (a, b) Staphylococcus aureus, Escherichia coli (c) and Candida albicans (d, e) microbial strains.

The improved antibacterial properties of the β-CD triazole derivative compound as shown in Figure 7 can be assigned to the synergistic effects of both β-CD and triazole and the propargylic derivative. Indeed, the β-cyclodextrin can interact with the cell membranes of bacteria by encapsulating hydrophobic molecules, which may disrupt the bacterial membrane structure or alter the function of membrane-associated proteins, leading to the inhibition of bacterial growth. Another mechanism is allowed to the interaction of the β-CD with Lipids and Membranes, some studies suggest that β-CD can bind to the lipids in the bacterial cell membrane, which may disrupt the membrane's integrity, thereby increasing the bacterial cell's susceptibility to antimicrobial agents [59,60].

The native β-CD exhibits no fungal activity against the tested microbial strain. However, the modified β-CD derivative revealed a significant antifungal activity with an inhibition area of 1.2 for the synthesized β-CD derivative.

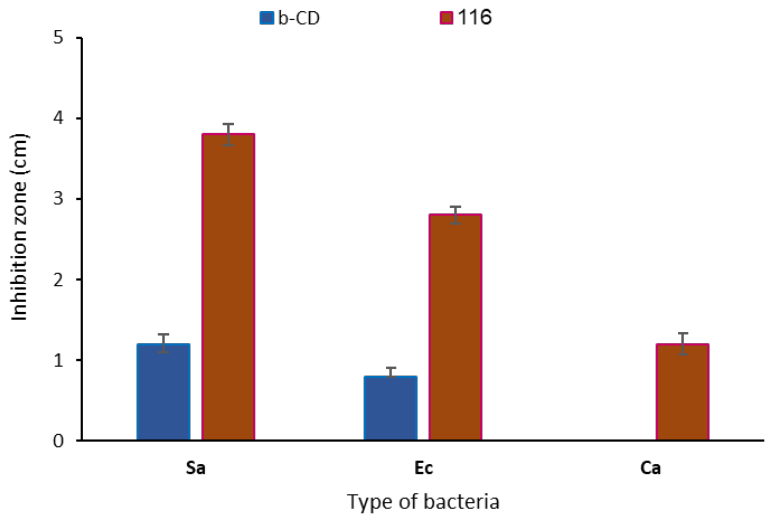


Figure 7. Antibacterial and antifungal activities of the native β-CD and the β -CD-triazole derivative against the different microbial strains.

3.2.1.2 Antioxidant activity

For human health, oxidative stress represents a major concern and is often considered an important indicator of cancer as well as chronic illnesses. Antioxidant compounds help protect against the progression of these conditions. The DPPH assay is a quick and simple method to assess antioxidant activity, making it a popular choice for evaluating antioxidants derived from natural as well as synthetic origins. As shown in Figure 8, the synthesized β-CD derivative compound under investigation demonstrated high antioxidant potential. Interestingly, the DPPH radical scavenging effect extended to a high activity of 93%. The native synthesized β-CD exhibited a notably low antioxidant effect of 16% inhibition of DPPH radicals.

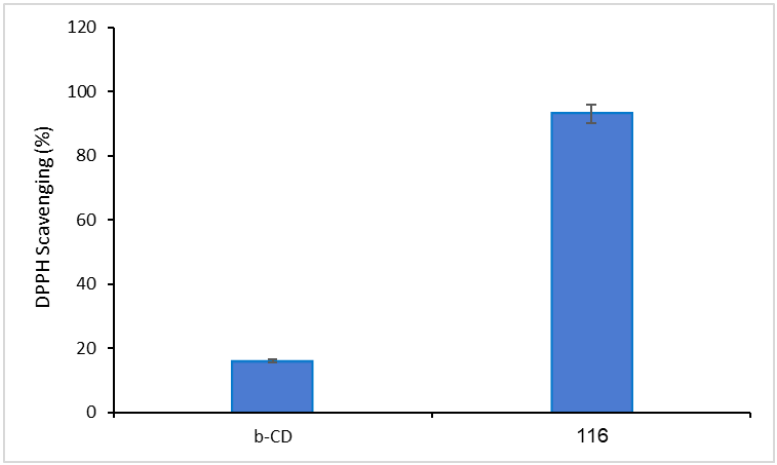


Figure 8. Antioxidant potency of the native β-CD and the synthesized β-CD derivative.

3.3. Assessment of Cell Viability and Antitumor Activity

The biocompatibility and anticancer characteristics of the native and synthesized β-CD derivative were evaluated through in vitro cell analysis. MCF-10A, a non-cancerous human mammary epithelial cell line, alongside MCF-7, a breast cancer cell line, were used to evaluate the biocompatibility and anticancer activity of β-CD and the synthesized triazole-based β-CD. The tests were conducted using different macromolecule concentrations during the MTT assay. The native β-CD did not exhibit any cytotoxicity when in contact with normal epithelial cells, but moreover from a concentration of 50 µg/mL it promotes cell growth, as indicated by the results in Figure 9. This finding aligns with multiple published studies confirming the excellent biocompatibility of native β-CD [61,62]. The non-cancerous breast cells (MCF10A) exhibited excellent cytocompatibility with both of the synthesized polymeric cyclodextrin derivative across various doses. This represents a significant improvement, as the success of anticancer treatments largely relies on their ability to interact effectively with healthy cells, which are the intended targets of cancer therapies. Furthermore, the improvement recorded in cell growth from a low concentration of 5 µg/ml confirms the high cytocompatibility of the synthesized β-CD derivative and strongly supports its employment as efficient candidate as part of drug delivery system. Based on the anticancer assessment shown in Figure 10, the native β-CD alone could not kill cancer cells even at high concentrations.

The new synthesized β-CD derivative exhibited significant cancer cell-killing effect in a dose-dependent manner. Consistent with its antibacterial performance (IC50 = 2.42 µg/mL, equivalent to 4.3 µM). The modification of β-CD markedly decreased the viability of MCF-7 breast cancer cells to 9.2 ± 0.6% after 72 hours of treatment at 200 µg/mL. In contrast, both native β-CD and its derivative demonstrated good cytocompatibility toward non-cancerous epithelial cells (Figure 6). Furthermore, the in vitro anticancer studies demonstrated that synthesized β-CD derivative had potent anticancer effects, which were significantly amplified by increasing the used dose. For comparison, commercial chemotherapeutic agents such as doxorubicin under similar in vitro conditions (25 µM, 24 h) reduce MCF-7 cell viability to nearly 16 ± 6% of control, while cisplatin at high concentrations (75-80 µM for 48 h) can reduce viability to approximately 6%. In contrast, our β-cyclodextrin modified compound reduces viability to 9.2 ± 0.6% within the same cell line and over comparable treatment durations. Such performance places our compound among the more potent candidates, underscoring its promise for further preclinical development.

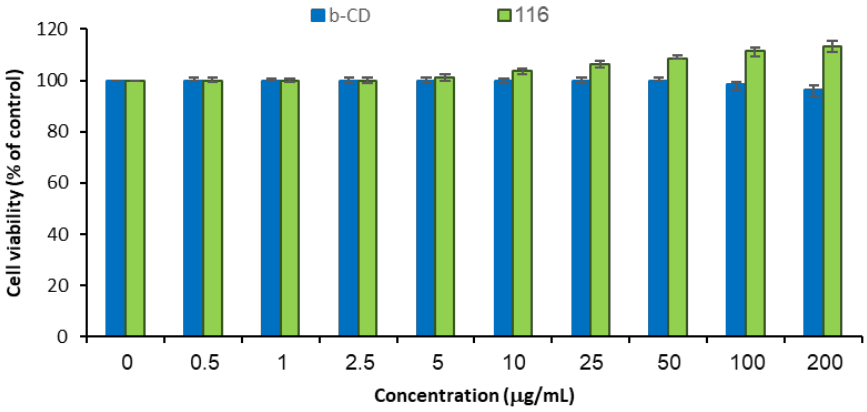


Figure 9. Assessment of cytocompatibility in normal breast cells and anticancer activity on MCF-7 cells after 72 h exposure to native and modified β-CD at varying doses.

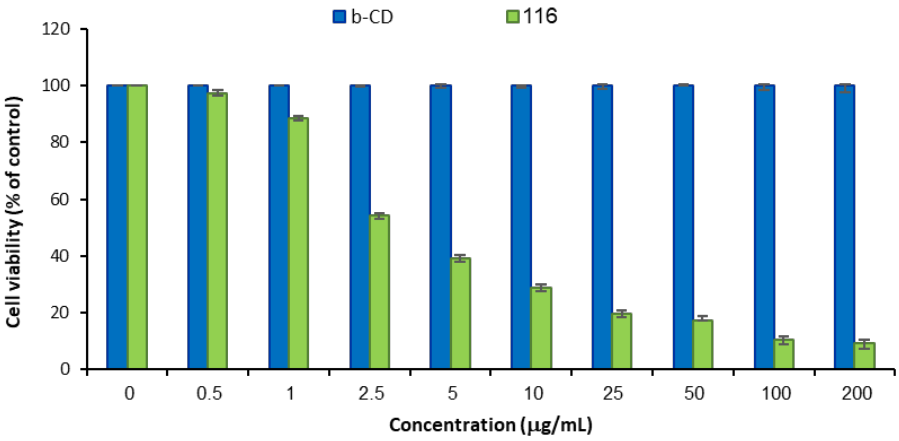


Figure 10. Anticancer potential in MCF-10A cells after 72 h exposure to native and modified β-CD at varying concentrations, measured via cell growth inhibition.

4. Conclusion

In this study, a novel pyridine/β-cyclodextrin macromolecular derivative was successfully synthesized through a click reaction between an alkyne-functionalized pyridine and an azido-modified β-cyclodextrin. Comprehensive spectroscopic and morphological analyses (FT-IR, ^{1}H and ^{13}C NMR, SEM) confirmed the successful formation of the triazole-linked conjugate and revealed marked structural modifications, including increased surface roughness and porosity. These features enhanced the material's hydrophilicity and molecular accessibility, directly contributing to its superior biological performance.

The click-modified β-cyclodextrin derivative exhibited strong multifunctional bioactivity, including outstanding antioxidant capacity (DPPH inhibition 89.4%), pronounced antibacterial and antifungal effects against both Gram-positive and Gram-negative strains, and significant anticancer efficacy, reducing MCF-7 cell viability to 9.2 ± 0.6%. Notably, this cytotoxic effect is comparable to established chemotherapeutic agents such as doxorubicin and cisplatin, underscoring the potency of the designed conjugate. The incorporation of bioactive pyridine-triazole moieties within the β-cyclodextrin framework thus demonstrates a synergistic enhancement in biofunctionality, positioning this compound as a promising candidate for therapeutic and drug delivery applications.

While these findings validate the design strategy and highlight the strong translational potential of click-based cyclodextrin systems, further investigations are warranted. Future work will focus on improving the compound's solubility and long-term stability, exploring controlled drug-loading and release properties, and evaluating its pharmacokinetics, in vivo toxicity, and therapeutic efficacy in relevant biological models. Addressing these aspects will help overcome current limitations and advance this class of macromolecular conjugates toward practical biomedical applications.

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Conflicts of Interest: The authors declare no conflict of interest.

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