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REVIEW ARTICLE

Carbohydrate-Isoxazole Hybrids: Design, Synthesis, and Therapeutic Applications in Drug Discovery

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ABSTRACT: Natural products have long served as a cornerstone for drug discovery due to their structural diversity and biological relevance. However, their clinical utility is often limited by poor solubility, low bioavailability, and metabolic instability. To overcome these challenges, structural modification through carbohydrate conjugation has emerged as a promising strategy to enhance physicochemical properties and pharmacological efficacy. Among these modifications, carbohydrate-isoxazole hybrids have garnered significant attention due to their broad-spectrum biological activities, including anti-tumor, anti-inflammatory, anti-fungal, anticoagulant, and anti-parasitic effects. These hybrids leverage the inherent bioactivity of the isoxazole ring—a five-membered heterocycle known for its role in drug design—while the carbohydrate moiety improves solubility and target specificity. Notable examples include compound **11**, a potent factor Xa (fXa) inhibitor with a remarkable binding affinity (Ki = 60 pM), highlighting its potential as a next-generation anticoagulant. Conversely, not all modifications yield enhanced activity; for instance, galactose-conjugated mofezolac derivatives showed no improvement in COX-1 inhibition, underscoring the need for rational design in hybrid optimization. Additionally, glycosylated isoxazole derivatives have demonstrated efficacy against *Mycobacterium tuberculosis* (MIC = 3.125 μg/mL) and *Trypanosoma cruzi*, the causative agent of Chagas disease, further expanding their therapeutic scope. This review comprehensively examines the medicinal chemistry of carbohydrate-isoxazole hybrids, focusing on their synthesis, structure-activity relationships (SAR), and mechanisms of action. We also discuss key challenges in clinical translation, such as metabolic stability and selective targeting, while highlighting future directions for optimizing these hybrids as multifunctional therapeutics.

Keywords: Carbohydrate-isoxazole hybrids, Drug design and optimization, Bioavailability enhancement, Heterocyclic medicinal chemistry, Targeted therapeutics, Structure-activity relationships (SAR)

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1. INTRODUCTION

Isoxazole is a five-membered heterocyclic compound containing three carbon atoms, one nitrogen atom, and one oxygen atom. The unique electronic arrangement of the isoxazole ring makes it a highly versatile scaffold in medicinal chemistry, where it plays a vital role in drug discovery and design. Isoxazole hybrids, which are molecules where the isoxazole ring is fused or linked to other biologically active fragments, have shown remarkable medicinal importance [1-3]. These hybrids often enhance the

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overall pharmacological profile by combining the intrinsic properties of isoxazole with other bioactive moieties. They exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, anticancer, antiviral, antioxidant, and analgesic effects. The presence of the isoxazole ring often improves the stability, bioavailability, and target selectivity of drug candidates. Isoxazole-containing drugs such as valdecoxib (a COX-2 inhibitor used for arthritis) and leflunomide (an immunomodulatory drug) are well-known examples that highlight the therapeutic significance of this ring system [4-6].

In recent years, the development of isoxazole hybrids has expanded rapidly, driven by the need for new treatments against drug-resistant infections, cancer, and chronic inflammatory diseases. Researchers have successfully designed isoxazole hybrids with dual or multiple biological targets, which can offer synergistic effects and reduce the likelihood of resistance [7-10]. For example, isoxazole-linked quinolines, triazoles, and sulfonamides have demonstrated potent anticancer and antibacterial activities, outperforming traditional agents in several studies. Additionally, the tunable electronic properties of the isoxazole ring allow medicinal chemists to fine-tune the molecular interactions with specific biological targets, leading to increased potency and reduced side effects. The hybridization strategy, therefore, opens new avenues for creating multifunctional drugs, making isoxazole a privileged structure in the future landscape of pharmaceutical research [11-13].

The field of medicinal chemistry has long been devoted to the discovery and development of new therapeutic agents that can effectively combat a wide array of diseases. One of the ongoing challenges in drug development is the need to improve the solubility, bioavailability, and selectivity of bioactive compounds [14-15]. Carbohydrate-isoxazole derivatives have emerged as a promising class of compounds that combine the beneficial properties of carbohydrates with the pharmacological versatility of isoxazole moieties. This hybridization offers a unique opportunity to address some of the key limitations that hinder the effectiveness of conventional drug candidates. By strategically incorporating carbohydrate structures into small molecule scaffolds, researchers aim to enhance drug solubility, improve pharmacokinetics, and modulate biological activity for a wide range of therapeutic targets [16-20].

Carbohydrates are natural biomolecules that play crucial roles in many biological processes, including cell signaling, immune response modulation, and molecular recognition. Their inclusion in drug design can significantly enhance the aqueous solubility and bioavailability of otherwise poorly soluble compounds. However, carbohydrates alone may lack sufficient bioactivity to target specific diseases or pathways [21]. This is where the isoxazole ring, a five-membered heterocyclic structure, comes into play. Isoxazole derivatives have demonstrated a wide array of biological activities, such as anti-inflammatory, antimicrobial, and anticancer properties, making them attractive candidates for drug development. The combination of carbohydrates and isoxazole moieties results in molecules that can capitalize on both the solubility benefits of carbohydrates and the bioactivity of isoxazole, creating compounds with enhanced therapeutic potential [22].

The therapeutic potential of carbohydrate-isoxazole hybrids has been explored in various disease areas, including cancer, infectious diseases, cardiovascular conditions, and autoimmune disorders. These hybrids have shown promise in inhibiting key enzymes, modulating immune responses, and interacting with specific receptors involved in disease progression. For example, some carbohydrate-isoxazole derivatives have demonstrated potent anti-tumor activity by targeting critical enzymes involved in cancer cell proliferation and metastasis. Others have been identified as effective inhibitors of pro-inflammatory pathways, such as cyclooxygenase (COX) enzymes, which are involved in pain and inflammation. Additionally, these compounds have been studied for their potential to inhibit microbial pathogens, including Mycobacterium tuberculosis (the causative agent of tuberculosis), and parasites such as *Trypanosoma cruzi* (which causes Chagas disease), showcasing their broad therapeutic applicability [23-24].

One of the most exciting aspects of carbohydrate-isoxazole derivatives is their ability to enhance the delivery of active pharmaceutical ingredients to specific biological targets. By incorporating carbohydrate moieties, which are naturally recognized by various cell surface receptors, these derivatives can potentially improve the targeting and uptake of drugs, particularly in challenging environments such as the blood-brain barrier (BBB). This is particularly important in the context of neurological disorders, where delivering therapeutics to the brain in sufficient concentrations remains a significant obstacle. Moreover, the ability of carbohydrate-isoxazole hybrids to modulate immune responses and target inflammation pathways has significant implications for autoimmune diseases and chronic inflammatory conditions, offering a new avenue for developing safer, more effective treatments [25].

Carbohydrate-isoxazole hybrids represent an emerging area of medicinal chemistry, and while there are few commercially available drugs specifically based on carbohydrate-isoxazole hybrid structures, this combination has shown great promise in preclinical and early-phase development. However, there are a few classes of drugs where carbohydrate-based derivatives (or compounds with similar hybrid structures) have been explored, though they may not always feature the isoxazole ring specifically. Isosorbide dinitrate and isosorbide mononitrate, Enoxaparin (Lovenox), Glycosylated isoxazole compounds, Duloxetine (Cymbalta) and Sertraline (Zoloft), Carbohydrate-based cancer and anti-inflammatory drug candidates are some examples of drugs or drug candidates that involve carbohydrate modifications, or potentially similar hybrid structures, but are not necessarily full carbohydrate-isoxazole hybrids [26].

This review aims to provide a comprehensive overview of the medicinal chemistry behind carbohydrate-isoxazole hybrid compounds, exploring their biological activities, and

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therapeutic applications. It will delve into the mechanisms by which these hybrids exert their effects, including enzyme inhibition, receptor binding, and immune modulation. Furthermore, the chapter will highlight recent advancements in the development of carbohydrate-isoxazole derivatives as potential drug candidates and discuss the challenges that remain in their clinical translation. As this field continues to evolve, carbohydrate-isoxazole hybrids stand poised to offer novel therapeutic strategies for a wide range of diseases, bridging the gap between natural product chemistry and synthetic drug development.

2. THERAPEUTIC POTENTIAL OF CARBOHYDRATE-ISOXAZOLE DERIVATIVES

Neuroinflammation is increasingly recognized as a key contributor to the initiation and progression of a wide range of neurological and neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis. In the central nervous system, microglia—the resident immune cells—play a central role in mediating the inflammatory response. One of the major pro-inflammatory enzymes expressed by microglia is cyclooxygenase-1 (COX-1), which is constitutively active and contributes to the production of pro-inflammatory prostaglandins. Persistent activation of COX-1 can exacerbate neuroinflammatory conditions and accelerate neuronal damage. Therefore, selectively inhibiting COX-1 activity in the brain has emerged as a promising therapeutic strategy to mitigate inflammation and slow disease progression in the CNS.

To overcome the challenge of drug delivery to the brain, researchers have focused on enhancing the bioavailability and brain-targeting ability of COX-1 inhibitors. Mofezolac, a potent and selective COX-1 inhibitor, has been structurally modified to improve its ability to cross the blood–brain barrier (BBB). This has been achieved by chemically linking mofezolac to a galactose moiety via either ester or amide bonds, resulting in novel carbohydrate-based conjugates. These modified compounds not only retain the inhibitory activity of mofezolac but also exhibit improved brain permeability due to the presence of the galactose unit, which may facilitate transport across the BBB. By enabling effective delivery of the drug to the CNS, these galactose-conjugated derivatives of mofezolac offer a promising approach for targeting COX-1 in microglia, thereby modulating neuroinflammation and offering potential therapeutic benefits in the treatment of neurodegenerative diseases.

Compound **1** (Figure 1) has emerged as a highly selective cyclooxygenase-1 (COX-1) inhibitor with promising pharmacological characteristics. It demonstrates a COX-1 inhibitory concentration (IC_{50}) of 0.27 μM, compared to a COX-2 IC_{50} of 3.1 μM, yielding a selectivity index (SI) of 11.5, which indicates a strong preference for COX-1 over COX-2. Importantly, compound **1** exhibits both chemical and metabolic stability, enhancing its viability as a drug candidate. It has shown the capability to cross the Caco-2 cell monolayer—a well-established model that mimics the blood–brain barrier—suggesting its potential for central nervous system (CNS) penetration. This translocation across the cellular barrier is facilitated by the glucose transporter GLUT-1, implying a targeted delivery mechanism to the brain. In functional studies, compound **1** demonstrated superior inhibition of prostaglandin E2 (PGE2) release compared to mofezolac in LPS-stimulated BV2 mouse microglial cells, highlighting its potential as a more effective anti-inflammatory agent in neuroinflammatory conditions.

The synthesis of compound **1** involved a two-step chemical process. Initially, mofezolac was esterified with 1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (DIPG) using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) as coupling agents to form an intermediate. This intermediate was then subjected to deprotection with trifluoroacetic acid, resulting in the formation of the final product, compound **1**. On the other hand, compound **2** (Figure 1), which functions primarily as a COX-2 inhibitor (COX-2 IC_{50} = 0.27 μM; COX-1 IC_{50} = 0.40 μM), was synthesized through a similar synthetic strategy, employing 11-aminoundecanoic acid as a linker. Although both compounds share a common synthetic route, their distinct structural modifications result in differing selectivity profiles, with compound **1** showing clear preference for COX-1, while compound **2** exhibits more balanced inhibition across both COX isoforms [27].

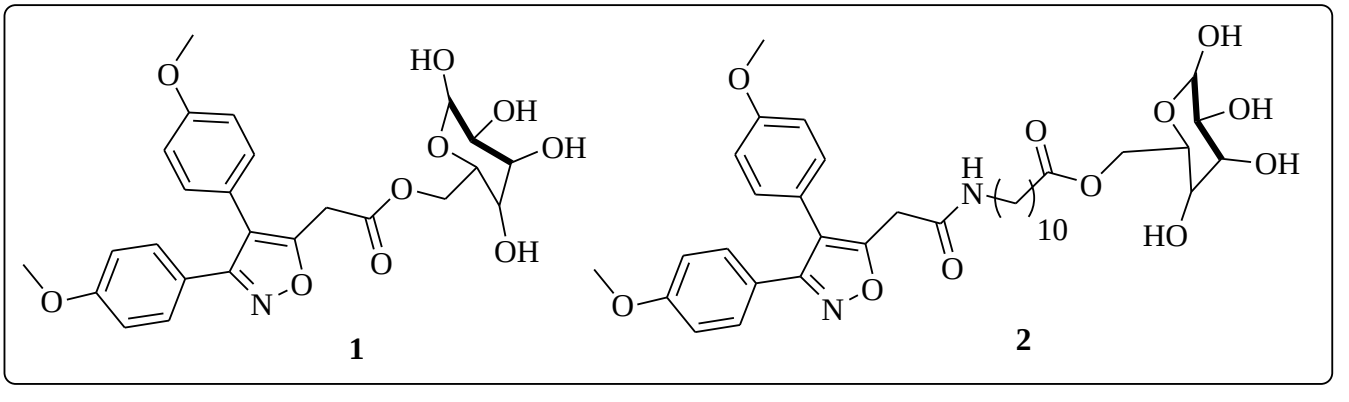


Fig. 1. Carbohydrate-isoxazole derivatives **1-2**.

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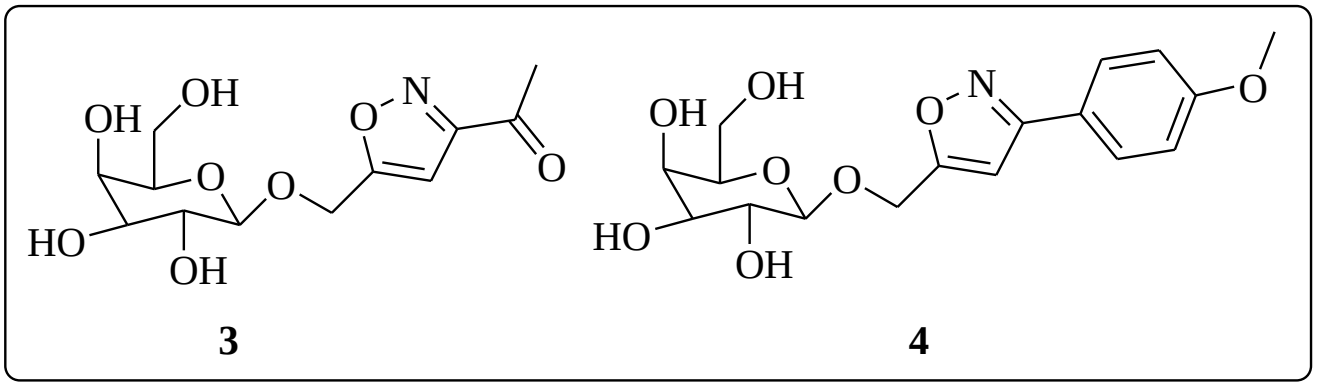


Fig. 2. Carbohydrate-isoxazole derivatives **3-4.**

Giguère *et al.* [28] conducted a detailed investigation into the synthesis of galactoside derivatives containing an isoxazole moiety, aiming to explore their potential as inhibitors of galectins—specifically galectin-1 and galectin-3. Galectins are a family of β-galactoside-binding proteins that play crucial roles in various biological processes, including cell-cell adhesion, immune response modulation, inflammation, and cancer progression. Given their biological significance, galectins have emerged as important therapeutic targets in the context of inflammatory diseases and cancer. By incorporating the isoxazole ring into galactoside scaffolds, the researchers sought to enhance binding affinity and specificity toward galectin proteins, while also exploring new structural frameworks for inhibitor design.

Among the synthesized compounds, two in particular—compound **3** and compound **4** (Figure 2), exhibited measurable inhibitory activity against galectin-1. Compound **3** demonstrated an inhibitory concentration (IC) of 2.5 mM, while compound **4** exhibited a stronger inhibitory effect, with an IC of 1.25 mM. Although these values indicate moderate potency, the findings were significant as they represented some of the earliest examples of galactoside-isoxazole hybrids acting as galectin-1 inhibitors. These compounds laid the groundwork for further optimization in the development of more potent and selective galectin inhibitors. The study by Giguère *et al.* thus contributed to a growing interest in the design of carbohydrate-based small molecules targeting lectins, offering valuable insights into structure–activity relationships and the therapeutic potential of such hybrid molecules.

Zhang *et al.* undertook an extensive medicinal chemistry study focused on modifying 23-hydroxybetulinic acid (HBA), a naturally occurring pentacyclic triterpenoid known for its broad-spectrum biological activities, particularly anti-tumor properties [29]. In their work, the researchers synthesized a series of HBA derivatives by introducing an isoxazole moiety to the parent structure, aiming to enhance its pharmacological potential. Isoxazole, a five-membered heterocyclic ring known for its presence in various bioactive compounds, was strategically chosen to improve the anti-cancer efficacy of HBA through structural diversification. The introduction of this moiety was expected to modulate key interactions with biological targets involved in cancer cell proliferation and survival.

Among the synthesized derivatives, one compound in particular—compound **5** (Figure 3), a glycosylated isoxazole-substituted HBA—stood out for its potent anti-tumor activity. This compound exhibited strong cytotoxic effects against a panel of cancer cell lines, including HL-60 (human promyelocytic leukemia), BEL-7402 (human liver cancer), SF-763 (human glioblastoma), HeLa (human cervical cancer), and B16 (mouse melanoma). The IC_{50} values for compound **5** ranged from 15.10 to 26.71 μM, indicating effective tumor cell inhibition across various cancer types. The glycosylation of the molecule likely contributed to improved water solubility and bioavailability, enhancing its therapeutic potential. These findings underscore the value of combining natural product scaffolds with heterocyclic and carbohydrate moieties in the rational design of new anti-cancer agents [29].

Thiamethoxazole (HYM) is a broad-spectrum fungicide known for its effectiveness in controlling various fungal pathogens. However, when HYM is absorbed by plants, it undergoes rapid metabolism, converting into two glucoside metabolites. These metabolites exhibit significantly weaker anti-fungal activity compared to the parent compound, which limits the overall efficacy of HYM in planta.

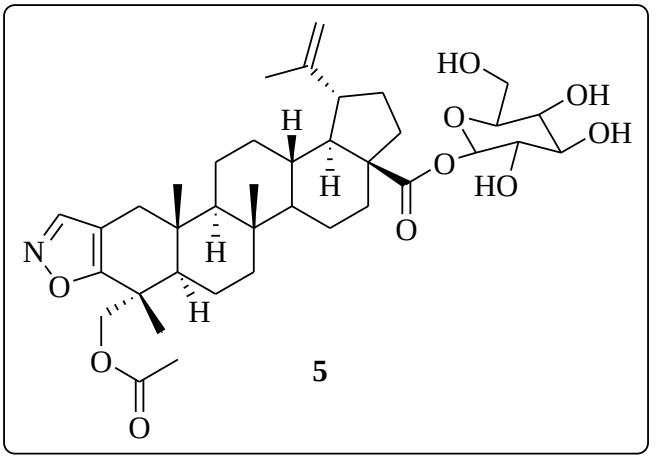


Fig. 3. Carbohydrate-isoxazole derivative **5.**

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To address this issue and enhance its antifungal potency, researchers explored the potential of glycosylation, particularly with amino sugars, to simulate the natural glycosylation processes that occur in plants. This strategy aimed to maintain the strong anti-fungal activity of HYM both *in vitro* and *in vivo*, mimicking the plant's own metabolic mechanisms for enhancing the fungicidal properties of the compound.

Among the glycosylated derivatives synthesized, glycosides **6** and **7** (Figure 4) demonstrated the highest anti-fungal activity. Specifically, the use of N-acetylglucosamine as a glycosylating agent showed a significant synergistic effect with HYM, further boosting its anti-fungal properties. Glycoside **7**, in particular, not only exhibited potent antifungal activity but also had an added benefit of promoting plant growth. It stimulated an increase in plant defense enzyme activity, contributing to enhanced plant resilience against fungal infections.

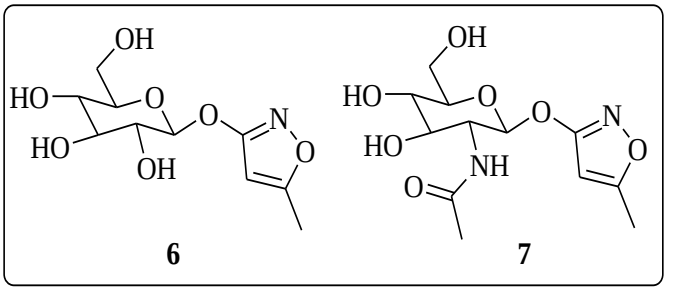


Fig. 4. Carbohydrate-isoxazole derivative **6-7.**

Further analysis using electron microscopy and proteomics revealed that glycoside **7** operates through a unique anti-fungal mechanism, distinguishing it from the action of HYM itself. These findings, as reported by Gao *et al*, demonstrate the potential of glycosylated derivatives as more effective, plant-friendly fungicides with additional agronomic benefits, offering an innovative approach to combating fungal pathogens in crops [30].

Gueron *et al.* conducted a comprehensive study focused on the synthesis and evaluation of D-nucleoside furanoside derivatives containing isoxazole moieties, aiming to assess their anti-tumor potential. The researchers synthesized a series of these derivatives, hoping to identify compounds with significant growth-inhibitory effects against cancer cells. Among the derivatives, compound **8** (Figure 5) stood out for its ability to inhibit the growth of PC3 prostate cancer cells, with an inhibition rate of 34.14% at a concentration of 10 μM. This compound was tested for its cytotoxic activity through a series of assays, with a particular focus on its impact on the cell cycle and its mechanism of action in tumor cell growth inhibition.

Further investigation of the mechanism by which compound **8** exerts its anti-tumor effects revealed interesting findings related to the cell cycle. When PC3 cells were treated with compound **8** for 24 hours, a notable shift in the cell cycle distribution occurred. A significant proportion of the treated cells (54.5%) were arrested in the G0/G1 phase, suggesting that compound **8** primarily exerts its inhibitory effects by blocking cells at an early stage of the cell cycle. The increase in G0/G1 phase cells was largely at the expense of the S phase population, indicating that compound **8** may interfere with DNA synthesis or replication processes. Interestingly, the decrease in the population of cells in the G2/M phase was minimal, suggesting that the compound's activity does not significantly affect the mitotic process. These findings highlight the potential of compound **8** as a promising anti-tumor agent that operates through cell cycle regulation, particularly in preventing cells from progressing beyond the G0/G1 phase, thereby inhibiting their proliferation [31].

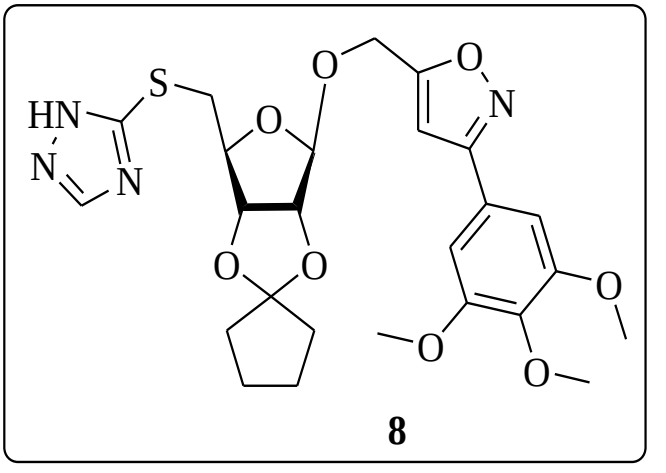


Fig. 5. Carbohydrate-isoxazole derivative **8.**

Lopopolo *et al.* reported the design and synthesis of a series of potent serine protease factor Xa (fXa) inhibitors, incorporating O-glucoside moieties to enhance their pharmacological activity. Factor Xa is a crucial enzyme in the coagulation cascade, and its inhibition has been widely explored for the treatment of thromboembolic diseases, such as deep vein thrombosis and pulmonary embolism. The research team focused on creating compounds that could effectively inhibit fXa activity, and their work demonstrated that the inclusion of O-glucoside groups in the inhibitor structure significantly improved the potency and specificity of the compounds. These inhibitors were tested for their anticoagulant activity *in vitro*, and the results were promising, showcasing strong potential for further development as therapeutic agents.

Among the synthesized compounds, compounds **9**, **10**, and **11** (Figure 6) exhibited remarkable inhibitory potency against fXa, with Ki values of 37 nM, 0.2 nM, and 0.06 nM, respectively. These values indicated that compound **11**, in particular, was highly potent, showing exceptional inhibitory activity against fXa at nanomolar concentrations. The strong *in vitro* anti-coagulant effects of these compounds suggest their potential for use in clinical settings, where precise modulation of the coagulation process is critical.

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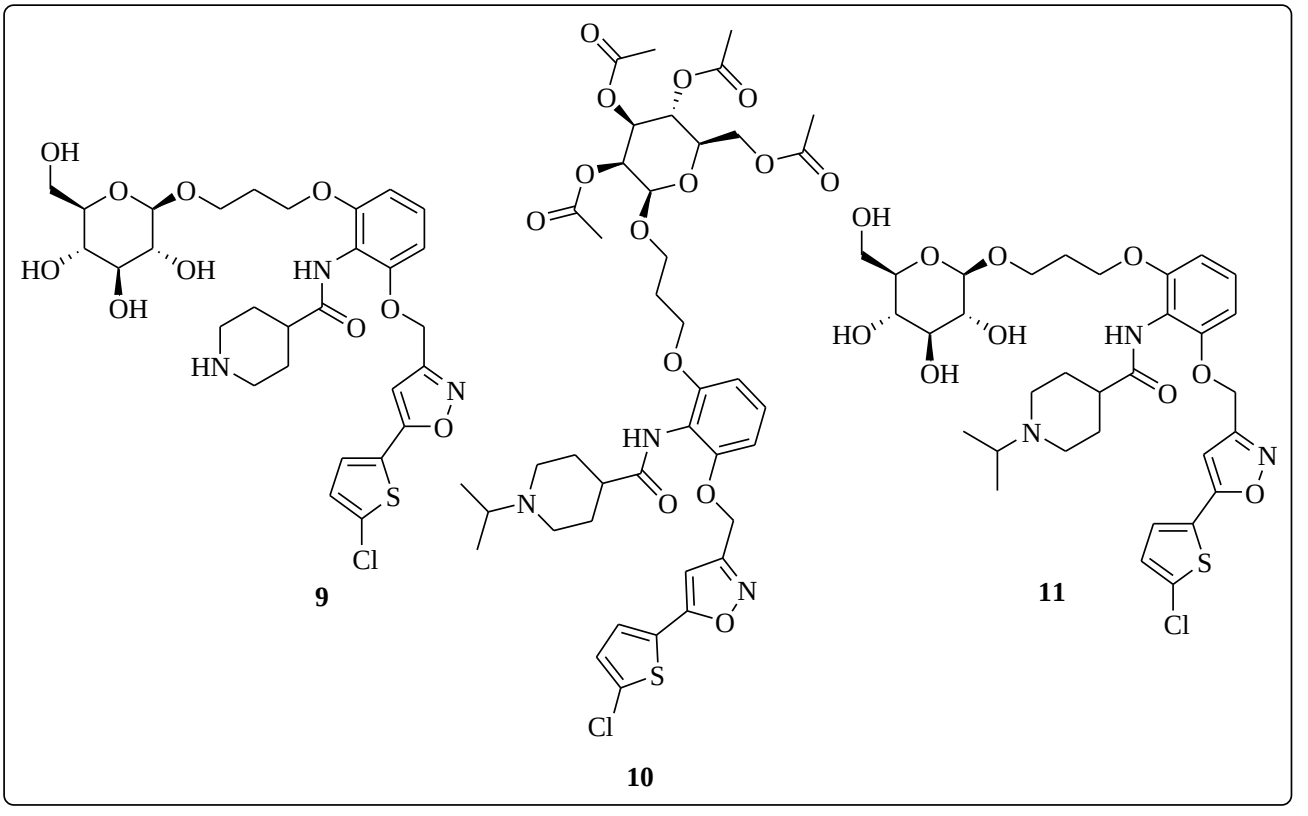


Fig. 6. Carbohydrate-isoxazole derivative **9-11.**

The study by Lopopolo *et al.* highlights the promise of O-glucoside-modified fXa inhibitors as potential candidates for anticoagulant drug development, with these compounds demonstrating the necessary potency and selectivity required for effective thromboembolic disease management [32].

Marchiori *et al.* as reported by da Rosa *et al*., made significant advancements in the development of novel inhibitors for the treatment of *Trypanosoma cruzi* infection, the causative agent of Chagas disease. The research team synthesized a series of O-3-triazole-linked galactosyl arylsulfonamides with the aim of identifying compounds that could inhibit the invasion of host cells by *T. cruzi*. The design of these compounds was based on their ability to interact with galectin-3, a carbohydrate-binding protein that plays a crucial role in the invasion of host cells by *T. cruzi*. By linking galactosyl groups to arylsulfonamides through a triazole moiety, the researchers sought to enhance the affinity of the compounds for galectin-3, thus potentially interfering with the parasite's ability to invade cells.

Among the compounds tested, compound **12** (Figure 7) stood out due to its significant ability to reduce the infection index of *T. cruzi*, with a remarkable reduction of approximately 20-fold in cell invasion. Additionally, when tested using Corning Epic label-free assays, compound **12** displayed a high binding affinity to galectin-3, with an EC_{50} value of 17.2 µM, indicating that it effectively interacts with the galectin-3 protein. The ability of compound **12** to inhibit *T. cruzi* invasion, coupled with its high binding affinity for galectin-3, positions it as a promising candidate for further development as an anti-parasitic agent. These findings suggest that O-3-triazole-linked galactosyl arylsulfonamides could be a novel class of inhibitors with potential therapeutic applications in the treatment of Chagas disease and possibly other infections where galectin-3 plays a critical role in host cell invasion [33].

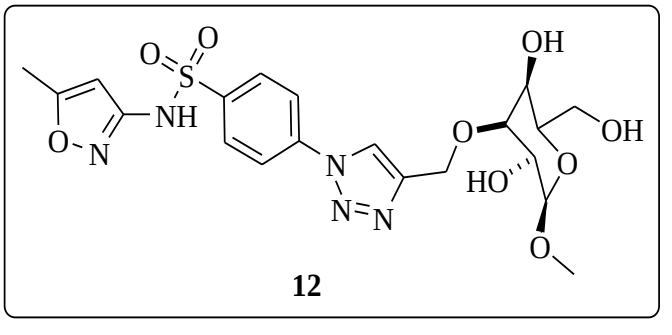


Fig. 7. Carbohydrate-isoxazole derivative **12.**

Pérez-Balderas *et al.* carried out a detailed study focused on the design and synthesis of a series of isoxazole-glycoconjugates and evaluated their potential to interact with concanavalin A, a well-known lectin that binds to specific carbohydrate structures. Lectins like concanavalin A are important tools for studying carbohydrate-protein interactions, and their inhibition can have significant

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implications for modulating various biological processes. The research aimed to explore the inhibitory effects of isoxazole-glycoconjugates on the binding of concanavalin A, with the hypothesis that the isoxazole ring could enhance the affinity of these conjugates for the lectin. To assess this, the team utilized an enzyme-linked lectin assay (ELLA), which is a highly sensitive method for detecting and quantifying lectin binding to carbohydrate ligands.

Among the synthesized isoxazole-glycoconjugates, compounds **13** through **14** (Figure 8) exhibited notable inhibitory effects on the binding of horseradish peroxidase (HRP)-labeled concanavalin A. The inhibitory concentrations (IC_{50}) for these conjugates ranged from 0.067 to 0.96 mM, indicating varying degrees of potency in blocking the interaction between concanavalin A and its carbohydrate targets. These findings suggest that the isoxazole-glycoconjugates can effectively interfere with concanavalin A's ability to bind to carbohydrates, providing valuable insights into the design of carbohydrate-based inhibitors. The results also open up possibilities for using these conjugates in the development of new therapeutic agents that target lectin-carbohydrate interactions in various pathological conditions, including cancer and immune modulation [34].

Hajlaoui *et al.* made significant strides in the design and synthesis of novel glycosubstituted isoxazole derivatives, with the aim of developing effective antibacterial agents against *Mycobacterium tuberculosis* (Mtb), the causative agent of tuberculosis (TB). Tuberculosis remains a major global health threat, with the emergence of drug-resistant strains complicating treatment strategies. In response to this challenge, the researchers focused on creating compounds that could inhibit the growth of Mtb by incorporating isoxazole and glycosylated groups into the molecular structure, hoping to enhance the antibacterial properties of the compounds. These glycosubstituted isoxazole derivatives were screened for their activity against the standard Mtb H37Rv strain, providing a basis for evaluating their potential as anti-tuberculosis agents.

Among the synthesized compounds, compounds **15** through **16** (Figure 9) exhibited potent anti-tuberculosis activity, with a minimum inhibitory concentration (MIC) of 3.125 µg/mL, indicating strong bactericidal effects against Mtb. This level of potency suggests that these compounds have the potential to serve as effective candidates for further development into therapeutic agents for tuberculosis treatment. On the other hand, compounds **17** through **18** (Figure 9) showed moderate inhibitory activity, with MIC values of 12.5 µg/mL. While these compounds demonstrated less potency compared to the previous group, their activity still positions them as promising leads for optimization. The results of this study highlight the potential of glycosubstituted isoxazole derivatives as new candidates in the fight against tuberculosis, underscoring the importance of continued research into novel antibacterial agents [23].

Chen *et al.* reported the synthesis of compound **19** (Figure 10), a novel molecule designed to inhibit the binding of the α-Gal epitope to human anti-Gal antibodies. The αGal epitope is a carbohydrate structure found on the surface of various mammalian cells, and its recognition by anti-Gal antibodies has important implications in xenotransplantation and immune responses [35].

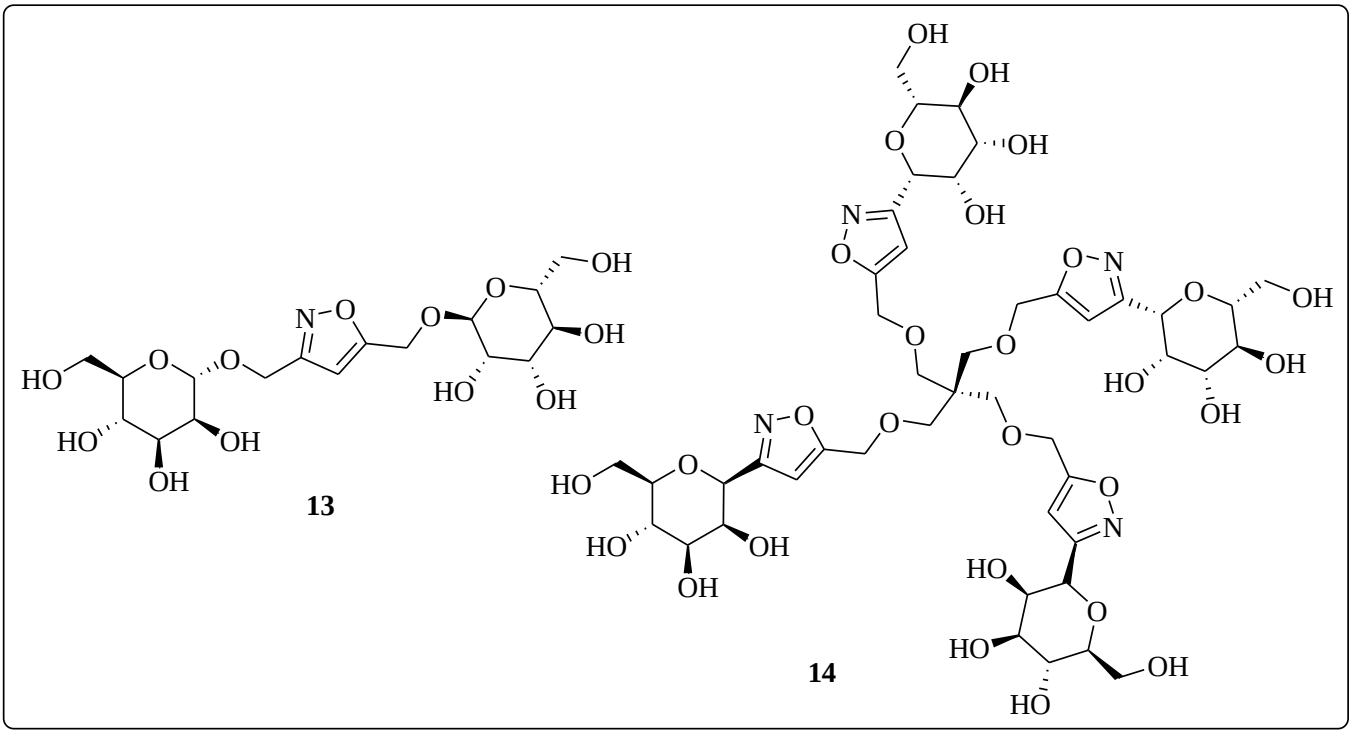


Fig. 8. Carbohydrate-isoxazole derivative **13-14.**

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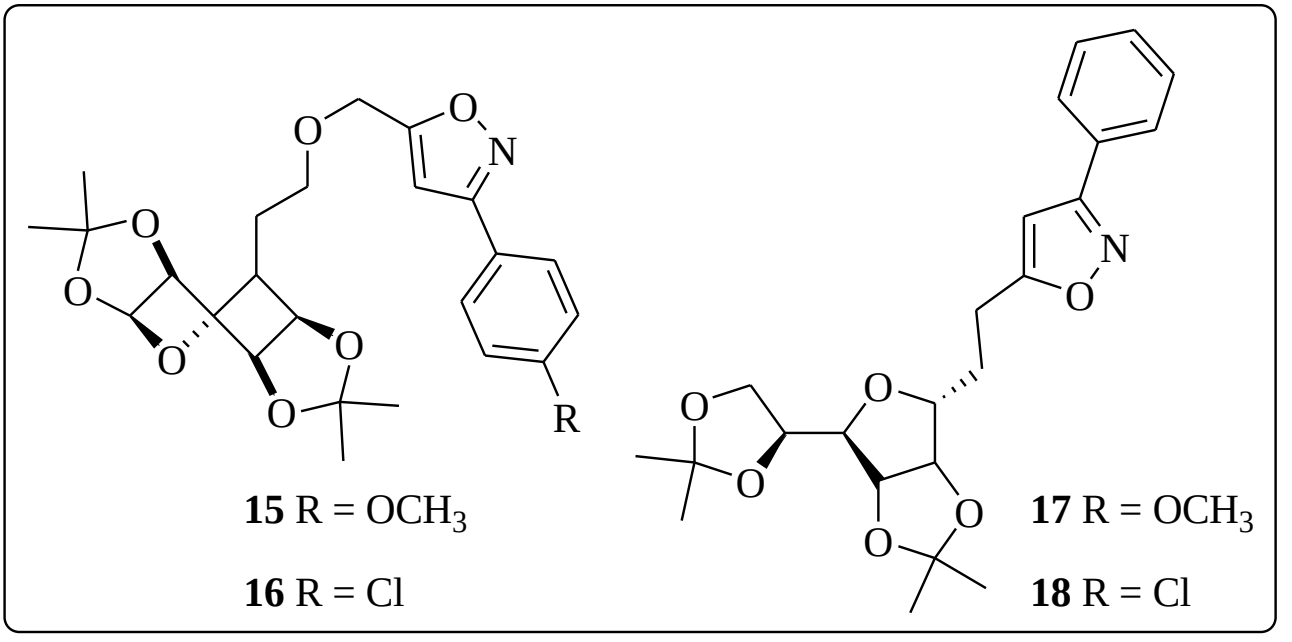


Fig. 9. Carbohydrate-isoxazole derivative **15-18.**

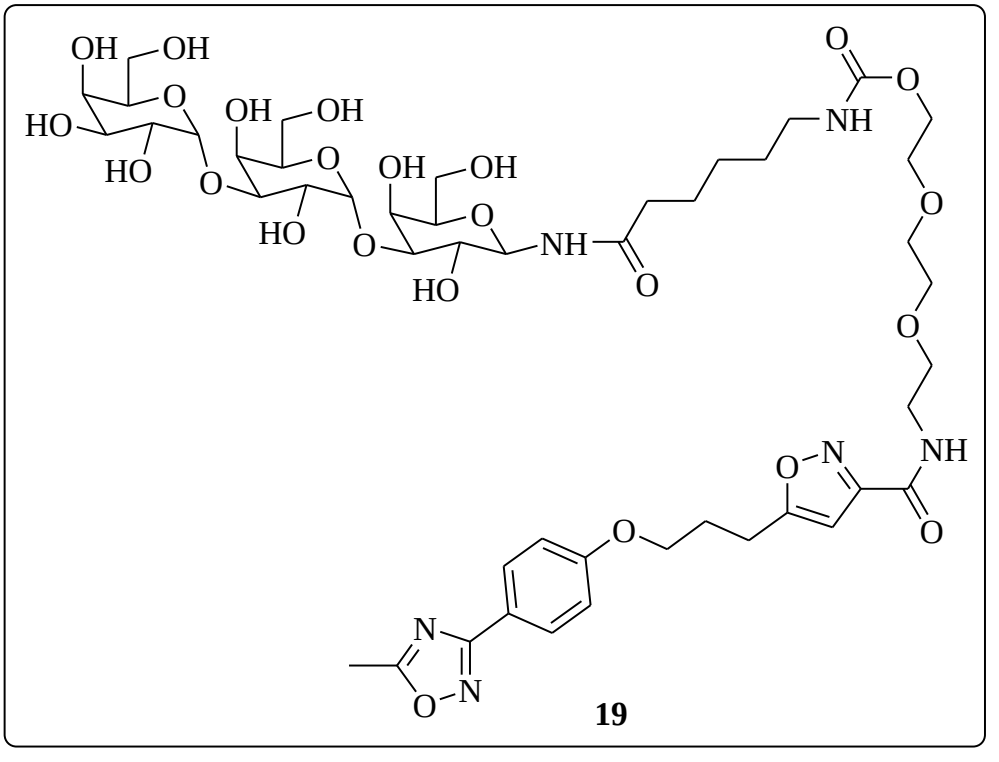


Fig. 10. Carbohydrate-isoxazole derivative **19.**

The researchers sought to develop compounds that could block the interaction between αGal and anti-Gal antibodies, a critical factor in the immune rejection of transplanted tissues from non-human primates or other species. In their study, they compared the binding affinity of compound **19** to that of two known αGal trisaccharide standards: Galα1-3Galβ1-4GlcNAcβ-Allyl (IC_{50} = 0.03 mM) and Galα1-3Galβ1-4Glcβ-NHAc (IC_{50} = 0.07 mM), both of which are known to bind to anti-Gal antibodies.

The results revealed that compound **19** exhibited an anti-Gal binding affinity that was comparable to the two αGal standards. Specifically, compound **19** demonstrated an IC_{50}

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value of 0.08 mM, indicating strong inhibition of the αGal-anti-Gal interaction. This finding suggested that compound **19** could serve as an effective inhibitor of anti-Gal binding, with potential applications in reducing immune rejection in xenotransplantation. The comparative analysis with the αGal trisaccharide standards provided further evidence of compound **19**'s efficacy, positioning it as a promising lead for the development of therapeutic strategies aimed at modulating immune responses against xenografts. These results underscore the potential of carbohydrate-based inhibitors in the design of new immunomodulatory agents for transplantation and other immune-related conditions [35].

3. FUTURE DIRECTIONS

The field of carbohydrate-isoxazole hybrids holds immense potential for future drug development, yet several key challenges must be addressed to fully realize their clinical applicability. One major area of focus should be the rational design of hybrids with improved metabolic stability. Many glycosylated compounds undergo rapid enzymatic degradation in vivo, limiting their therapeutic efficacy. Strategies such as the incorporation of non-natural sugars (e.g., C-glycosides or thio-sugars) or the use of prodrug approaches could mitigate this issue, enhancing systemic exposure and prolonging drug action.

Another critical direction involves optimizing blood-brain barrier (BBB) penetration for neurotherapeutic applications. While some carbohydrate-isoxazole hybrids, such as galactose-conjugated mofezolac derivatives, have shown enhanced BBB permeability via GLUT-1 transporters, further structural refinements are needed to maximize CNS delivery. Computational modeling and fragment-based drug design could aid in identifying optimal linker chemistries and carbohydrate configurations to improve brain uptake while minimizing off-target effects.

Additionally, expanding the antimicrobial and anticancer repertoire of these hybrids is essential. The rise of multidrug-resistant pathogens necessitates novel agents with unique mechanisms of action. For instance, glycosylated isoxazole derivatives could be engineered to target bacterial cell wall biosynthesis or fungal ergosterol pathways, leveraging the carbohydrate moiety for selective microbial uptake. Similarly, in oncology, conjugating cytotoxic isoxazoles with tumor-targeting sugars (e.g., glucose or galactose analogs) could enhance selectivity toward cancer cells overexpressing glucose transporters (GLUTs).

Advancements in synthetic methodologies will also play a pivotal role. Click chemistry, enzymatic glycosylation, and one-pot multicomponent reactions could streamline the production of diverse hybrid libraries, enabling high-throughput screening against emerging biological targets. Furthermore, nanocarrier-assisted delivery systems (e.g., liposomes or polymeric nanoparticles) could improve the pharmacokinetics of poorly soluble hybrids, particularly for intravenous or topical administration.

Lastly, translational studies are imperative to bridge the gap between preclinical promise and clinical reality. Robust in vivo toxicity profiling, formulation optimization, and biomarker-driven patient stratification will be crucial for advancing lead candidates into clinical trials. Collaborative efforts between chemists, biologists, and clinicians will be essential to unlock the full therapeutic potential of carbohydrate-isoxazole hybrids in treating complex diseases.

4. CONCLUSION

Carbohydrate-isoxazole hybrids represent a versatile and pharmacologically promising class of compounds, combining the bioactivity of isoxazole heterocycles with the solubility-enhancing properties of carbohydrates. Their broad therapeutic potential spans anticoagulation (e.g., compound **11**, Ki = 60 pM), neuroinflammation modulation (e.g., COX-1-targeting galactose-mofezolac conjugates), and antimicrobial therapy (e.g., anti-tubercular glycosides with MICs as low as 3.125 μg/mL). However, the success of these hybrids hinges on strategic structural optimization, as evidenced by cases where carbohydrate conjugation did not improve activity (e.g., mofezolac derivatives). Key challenges remain, including metabolic instability, selective tissue targeting, and scalable synthesis. Future research should prioritize the development of metabolically robust analogs through C-glycosylation or prodrug strategies, as well as BBB-penetrant designs for CNS disorders. Computational tools and AI-driven drug discovery could accelerate the identification of optimal hybrid configurations, while nanotechnology may address delivery hurdles. The translational potential of carbohydrate-isoxazole hybrids is underscored by their multifunctionality—capable of acting as enzyme inhibitors, immune modulators, and antimicrobial agents. Collaborative interdisciplinary efforts will be critical to advance these compounds into clinical trials, ensuring they meet the stringent demands of efficacy, safety, and manufacturability. As the field evolves, these hybrids may redefine therapeutic strategies for diseases with high unmet medical need, bridging the gap between natural product inspiration and synthetic innovation.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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