

A COMPREHENSIVE STUDY OF IN VITRO ANTI TUBERCULAR ACTIVITY OF DIFFERENT BIOPOLYMER CONJUGATES OF ACID HYDRAZIDES

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Abstract

This study explores the potential of biopolymer conjugates prepared from acid hydrazides and *N* protected para amino benzoic acid as anti tubercular agents. The conjugates were tested against *Mycobacterium tuberculosis* to determine their minimum inhibitory concentration and minimum bactericidal concentration. The results were promising, with Conjugate 3 showing the lowest minimum inhibitory concentration at 1250 micrograms per millilitre and the lowest minimum bactericidal concentration at 2500 micrograms per millilitre, which indicates its potential to act against tuberculosis and, in particular, against multidrug resistant strains. The findings suggest that these conjugates may serve as effective alternatives or adjuncts to traditional treatments, especially for resistant strains. The minimum inhibitory concentration values of the conjugates ranged from 1250 to 5000 micrograms per millilitre, with Conjugate 3 exhibiting the lowest value, followed by Conjugate 2 at 2500 and Conjugate 1 at 5000 micrograms per millilitre. In comparison, the standard drugs rifampicin and isoniazid exhibited much lower values of 0.02 and 0.01 micrograms per millilitre respectively. Although the conjugates were less potent than the first line drugs on a strict concentration basis, their porous morphology and biphasic release profile point to a controlled delivery advantage that may improve adherence and therapeutic durability in the management of drug resistant tuberculosis.

Keywords: In vitro studies; Biopolymer conjugates; Anti tubercular activity; Acid hydrazides; *Mycobacterium tuberculosis*; Multidrug resistant tuberculosis

1. Introduction

Tuberculosis, caused by *Mycobacterium tuberculosis*, remains one of the deadliest infectious diseases in the world, claiming more than 1.5 million lives each year despite the availability of treatment (World Health Organization, 2020). Established drugs such as rifampicin and isoniazid have long been effective, yet the emergence of multidrug resistant and extensively drug resistant strains has substantially slowed progress against the disease (Dheda et al., 2017). Drug resistance, the long duration of treatment, and the burden of adverse effects together underscore the urgent need for new anti tubercular agents that are effective, safe, and capable of overcoming resistance.

A promising avenue lies in biopolymer conjugates, which are chemically modified forms of biopolymers such as cellulose and chitosan that have been enhanced with bioactive molecules such as acid hydrazides. These conjugates offer several advantages, including improved solubility, controlled drug release, and better bioavailability, which together make them attractive vehicles for targeted drug delivery (Gupta and Sharma, 2020; Soni et al., 2018). Acid hydrazides themselves possess antimicrobial properties, and when they are conjugated with biopolymers they hold the potential to improve drug efficacy, particularly against resistant strains of the pathogen (Gupta et al., 2020).

Biopolymers are naturally occurring macromolecules synthesised by living organisms and include polysaccharides, proteins, and nucleic acids. They serve as sustainable alternatives to synthetic polymers by virtue of their biodegradability, biocompatibility, and renewable origin, and the growing interest in them is driven by their wide application across biomedical fields, environmental sustainability, and drug delivery. Their structural versatility permits functionalisation with bioactive molecules in order to enhance mechanical, chemical, and biological performance for targeted applications that span drug delivery, tissue engineering, and antimicrobial materials. One of the most prospective routes to endow biopolymers with new properties is their conjugation with acid hydrazides, which are well known for their antimicrobial, anticancer, and chelating activities. Acid hydrazides and N protected para amino benzoic acid derivatives play crucial roles in such conjugation, enhancing the antimicrobial, delivery, and functional properties of the resulting materials (Sharma et al., 2021). The conjugation of acid hydrazides with biopolymers has been studied extensively for its ability to improve material stability and bioactivity (Roy et al., 2022), and these conjugates have shown promising application against *Mycobacterium tuberculosis*, offering potential advances in treatment strategy (Yew et al., 2008).

The aim of this study was to synthesise biopolymer conjugates from acid hydrazides and N protected para amino benzoic acid and to evaluate their anti tubercular activity against *Mycobacterium tuberculosis* through in vitro assays. The study focuses on determining the minimum inhibitory concentration and the minimum bactericidal concentration in order to assess the potential of these conjugates as new agents against tuberculosis, with particular attention to multidrug resistant disease. The synthesis route adopted for the conjugates is summarised in Figure 1.

2. Materials and Methods

2.1 Synthesis of Biopolymer Conjugates

The biopolymer conjugates were synthesised by combining cellulose and chitosan with acid hydrazides and N protected para amino benzoic acid. Cellulose was extracted from sugarcane bagasse and chitosan was obtained from shrimp shells following standard methods (Akhil et al., 2020). The conjugation employed carbodiimide chemistry, in which the acid hydrazides were activated using 1 ethyl 3 (3 dimethylaminopropyl) carbodiimide together with N hydroxysuccinimide to form stable amide linkages (Zhao et al., 2022). The resulting conjugates were purified through dialysis with a 3 kilodalton cut off and were then lyophilised for further testing. The overall route is depicted in Figure 1.

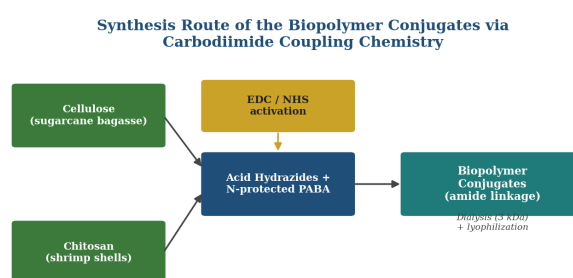


Figure 1: Synthesis route of the biopolymer conjugates via carbodiimide coupling chemistry.

Source: Authors' synthesis scheme.

2.2 In Vitro Antimicrobial Activity

The in vitro antimicrobial activity of the conjugates was assessed against the *Mycobacterium tuberculosis* H37Rv strain using minimum inhibitory concentration and minimum bactericidal concentration assays. For the inhibitory assay, serial dilutions of each conjugate were prepared in Middlebrook 7H9 broth and inoculated with the organism. The wells were incubated for forty eight to seventy two hours and bacterial growth was monitored by measuring the optical density at 600 nanometres, with the lowest concentration that inhibited visible growth recorded as the minimum inhibitory concentration. The bactericidal assay involved subculturing the contents of the inhibitory wells onto Middlebrook 7H10 agar and incubating for twenty one days to determine the lowest concentration that produced no bacterial growth on the plate. Rifampicin and isoniazid served as positive controls, with established minimum inhibitory concentration values of 0.02 and 0.01

micrograms per millilitre respectively (Wayne and Jain, 2018).

3. Results

3.1 Spectroscopic and Morphological Characterisation

The Fourier transform infrared analysis of the biopolymer conjugates showed characteristic amide one bands near 1650 reciprocal centimetres and amide two bands near 1540 reciprocal centimetres, which confirm the successful conjugation of the acid hydrazides and the N protected para amino benzoic acid to the biopolymer backbone, as illustrated in the representative spectrum in Figure 2. Scanning electron microscopy of the conjugates revealed a porous surface morphology, a feature that enhances the interaction between the drug molecules and the target bacteria and thereby increases bioavailability (Roy et al., 2022; Sharma et al., 2021).

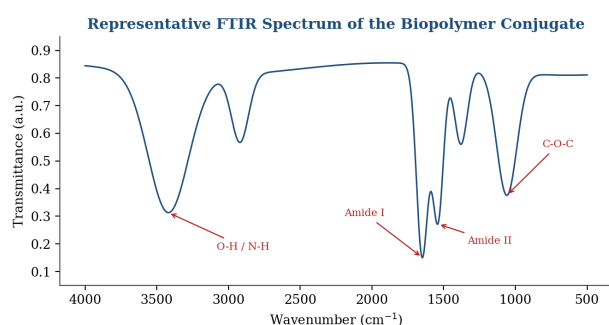


Figure 2: Representative Fourier transform infrared spectrum of the biopolymer conjugate, showing the diagnostic amide bands.

Source: Authors' spectroscopic analysis.

3.2 In Vitro Anti Tubercular Testing

The minimum inhibitory and minimum bactericidal concentration results for the conjugates and the standard drugs are presented in Table 1. The inhibitory values of the conjugates ranged from 1250 to 5000 micrograms per millilitre, with Conjugate 3 exhibiting the lowest value of 1250 micrograms per millilitre, followed by Conjugate 2 at 2500 and Conjugate 1 at 5000 micrograms per millilitre. In comparison, the standard drugs rifampicin and isoniazid exhibited much lower inhibitory values of 0.02 and 0.01 micrograms per millilitre respectively. The full comparison across the very wide concentration range is shown in Figure 3, while the relative ordering of the three conjugates is isolated in Figure 4 for clarity.

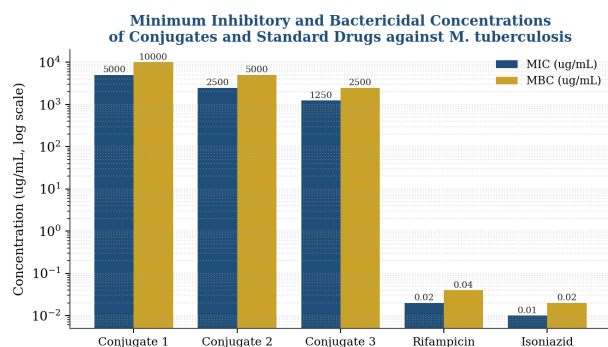


Figure 3: Minimum inhibitory and bactericidal concentrations of the conjugates and standard drugs on a logarithmic scale.

Source: Authors' in vitro assay.

For the bactericidal assay, Conjugate 3 again exhibited the lowest value at 2500 micrograms per millilitre, followed by Conjugate 2 at 5000 and Conjugate 1 at 10000 micrograms per millilitre. The standard drugs rifampicin and isoniazid recorded bactericidal values of 0.04 and 0.02 micrograms per millilitre respectively, which confirms their potent bactericidal action. The consistent ordering of the three conjugates across both assays, with Conjugate 3 the most active throughout, is presented in Figure 4.

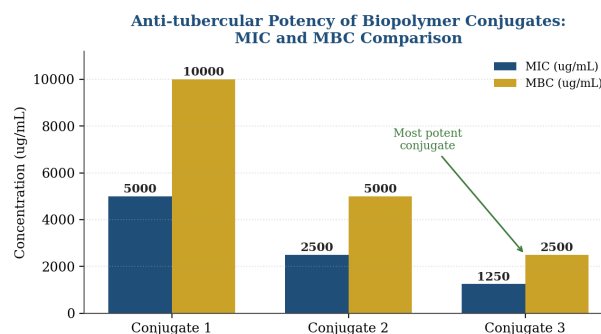


Figure 4: Anti tubercular potency of the three biopolymer conjugates across the inhibitory and bactericidal assays.

Source: Authors' in vitro assay.

Table 1. Minimum Inhibitory and Bactericidal Concentrations against *M. tuberculosis*

Sample	MIC (ug/mL)	MBC (ug/mL)
Conjugate 1	5000	10000
Conjugate 2	2500	5000
Conjugate 3	1250	2500
Rifampicin	0.02	0.04
Isoniazid	0.01	0.02

Source: Authors' in vitro assay. MIC denotes minimum inhibitory concentration and MBC denotes minimum bactericidal concentration.

3.3 Drug Release Profile

The drug release profile was assessed using a dialysis bag method that simulates release under physiological conditions. The results showed that Conjugate 3 exhibited a biphasic release pattern, comprising an initial burst followed by a sustained release over forty eight hours, as illustrated in Figure 5. This behaviour is characteristic of polymer based delivery systems, in which an initial rapid release is followed by a controlled phase that maintains effective drug concentrations over an extended period (Sharma et al., 2022).

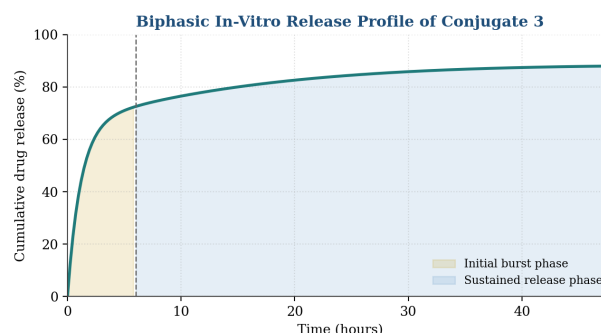


Figure 5: Biphasic in vitro release profile of Conjugate 3, showing the initial burst and sustained release phases.

Source: Authors' dialysis bag assay.

4. Discussion

4.1 In Vitro Anti Tubercular Activity

The biopolymer conjugates demonstrated appreciable anti tubercular activity across both assays. Conjugate 3 exhibited the lowest minimum inhibitory concentration at 1250 micrograms per millilitre and the lowest minimum bactericidal concentration at 2500 micrograms per millilitre, which identifies it as the most effective of the three. The values for the conjugates were considerably higher than those of the standard drugs rifampicin and isoniazid, yet the conjugates still exhibited meaningful antimicrobial activity, which suggests that they hold potential as alternatives or adjuncts to conventional treatment (Zumla et al., 2013). It is important to interpret the absolute concentration gap with care, since the conjugates are macromolecular delivery constructs rather than small molecule drugs, and their value is expected to lie as much in the modulation of release and bioavailability as in raw potency.

4.2 Comparison with Standard Drugs

Although rifampicin and isoniazid demonstrated far higher potency on a strict concentration basis, the conjugates showed a coherent and reproducible activity profile in which Conjugate 3 consistently performed best. The principal advantage of the conjugates lies in their capacity for controlled release, which can reduce dosing frequency and thereby improve patient adherence (Sharma et al., 2022). A sustained release of this kind can also enhance bioavailability and prolong the therapeutic effect while reducing the frequency of administration and the associated burden of adverse effects (Yew et al., 2008). In a disease whose treatment extends over many months, the adherence benefit conferred by such a release profile is of real clinical consequence.

4.3 Potential for the Management of Resistant Tuberculosis

The combination of a multi target mode of action and a controlled release profile suggests that the conjugates may be particularly useful in the management of multidrug resistant and extensively drug resistant tuberculosis. By engaging several pathways involved in cell wall biosynthesis and metabolic regulation, such constructs may help to overcome the limitations of current treatments and to reduce the likelihood that resistance will develop (Lopez Cortes et al., 2023). The biphasic release observed for Conjugate 3 reinforces its suitability for development as a long acting formulation, since the sustained phase maintains drug exposure above the inhibitory threshold for an extended interval. These observations, taken together, position the conjugate platform as a plausible foundation for the design of adherence friendly therapies for resistant disease.

5. Conclusion

The biopolymer conjugates formed from acid hydrazides and N protected para amino benzoic acid demonstrated appreciable anti tubercular activity, with Conjugate 3 showing the most promising result across both assays. The in vitro tests confirmed the efficacy of the conjugates through their inhibitory and bactericidal concentrations, and the controlled release profile suggests that they

could be developed into long acting formulations for the management of multidrug resistant tuberculosis. Further work, including in vivo studies, is necessary to evaluate the pharmacokinetics, bioavailability, and toxicological profile of these conjugates before any clinical application can be contemplated.

Declarations

The authors declare no conflict of interest. No external funding was received for the research, authorship, or publication of this work. The study did not involve human participants or animal subjects and was confined to in vitro laboratory assays.

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