Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 5s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Antimicrobial Evaluation of Rifamycin, 8-(Morpholinomethyl)- For the Treatment of Mycobacterium Tuberculosis

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Cite this paper as: Syed Safiullah Ghori, Rahemeen Bano, Mohammed Muaaz Ahmed, Abdul Arafath Raza k, Fatima Tabassum, Ayesha Soghra, Rashed Ali, Irbaz Ahmed, Shaik Waseem Akram, Mohammed Abdul Basith, (2025) Antimicrobial Evaluation of Rifamycin, 8-(Morpholinomethyl)- For the Treatment of Mycobacterium Tuberculosis. *Journal of Neonatal Surgery*, 14 (5s), 917-922.

ABSTRACT

Background: Tuberculosis is an ancient scourge. It has plagued humankind throughout known history and human pre-history. Mycobacterium tuberculosis have killed more persons than any other microbial pathogen [1]. Although the thorax is most frequently involved, tuberculosis may involve any of a number of organ systems like the respiratory, cardiac, central nervous system, musculoskeletal, gastrointestinal, and genitourinary systems and timely diagnosis of the disease is paramount, since delayed treatment is associated with severe morbidity [2].Thus the antibacterial properties of Rifamycin, 8-(morpholinomethyl)-, a molecule with the chemical formula C42H56N2O13 and a molecular weight of 796.9 g/mol, are investigated in this work.

Methodology: Rifamycin, 8-(morpholinomethyl)- was obtained in solid powder form and chemically synthesized. It was then examined using the Hexa disc method for Mycobacterium tuberculosis. When the pathogen was inoculated over sterilized agar plates, different doses of Rifamycin, 8-(morpholinomethyl)- were administered. The inhibitory zones that evolved around the wells were measured in diameter. The outcome was contrasted with a positive control [3].

Results and Discussion: The study found that Rifamycin, 8-(morpholinomethyl)- had substantial antibiotic action against Mycobacterium , with inhibitory zones growing in size with increasing concentrations. The findings suggest that the chemical compound can be used in the treatment of tuberculosis.

Conclusion: This study highlights Rifamycin, 8-(morpholinomethyl)-'s potent antibacterial qualities, which make it a viable option for treating tuberculosis. The compound's metabolites outperform its positive control thus serve a significant part in their efficacy. This study creates opportunities for further research and antitubercular application development.

Keywords: *Tuberculosis,Genitourinary system, Rifamycin,8-(morpholinomethyl), Antitubercular application.*

1. INTRODUCTION

Tuberculosis is an infectious disease caused by bacterium called Mycobacterium Tuberculosis. Tuberculosis is one of the most ancient diseases of mankind and has co-evolved with humans for many thousands of years or perhaps for several million years. [6]. Tuberculosis wasn't recognized as a distinct illness until the 1820s, and J. L. Schonlein finally called it "tuberculosis" in 1839.Tuberculosis is an infectious disease that primarily affects the lungs. When tuberculosis patients cough, sneeze, or spit, it spreads via the air.If tuberculosis spreads to another part of the body such as glands (lymph nodes), bones or brain, other symptoms, including: swollen glands,body aches and pains,tummy or pelvic pain,constipation,a headache,feeling confused etc are also noticed. [7]. Mycobacterium tuberculosis was discovered by Robert Koch; and for this discovery, he was awarded Nobel prize in physiology or medicine in 1905[8]. Tuberculosis is one of the top three infectious killing diseases in the world: HIV/AIDS kills 3 million people each year, Tuberculosis kills 2 million and malaria kills 1 million[9]. A WHO fact sheet dated March 2010 on tuberculosis stated that overall one third of the world's population

(over 2 billion) is currently infected with the Tuberculi bacillus. According to it, every second, someone in the world is newly infected with Tuberculi bacilli and 1 in every 10 of these newly infected people become sick or infectious later in life. Since concurrent infection with HIV weakens the immune system, people with co-infection of HIV and TB are much more likely to develop TB; it is a leading cause of death among HIV-positive people.[10]. Mycobacterium tuberculosis infects approximately 8,000,000 people annually, and about 2–3 million die from the disease. It is estimated that 33% of the absolute people are infected with latent TB, with 40% coming from India. With a yearly rate of 2,000,000 new cases, more than 40% of India's population is infected with latent Tuberculosis.[11]. Having passed through so many generations, Tuberculosis remains a major infectious disease among the world's population, as up to one quarter (~25%) is still infected [12,13].Tuberculosis cases have decreased since the Bacillus Calmette–Guérin (BCG) vaccine was made available in 1921 and antimicrobial drugs such as streptomycin, isoniazid, and rifampicin were made available by prescription in 1943, 1952, and 1963.New anti-TB drugs include:isonicotinic acid (isoniazid-INH) – discovered in 1951, pyrazinamide (PZA) and cycloserine – 1952, ethionamide – 1956, rifampicin (RIF) – 1957, and ethambutol – 1962.[14,15,16,17].Current anti-TB therapy protocols comprise of a six-month combination course of four drugs: INH, RIF, PZA and ethambutol. The first two months are known as the intensive phase. During this time all four drugs are taken. In the last four months (the continuation phase) only RIF and INH are taken.[18,19].The length of treatment is crucial for the effective and complete eradication of the different sub-populations of the Tubercule bacilli. Side effects and adverse drug reactions (ADRs) of current anti-TB drugs coupled with combination drug regimens and lengthy treatment durations often complicate the therapy.[20] Antibiotics that are used for the treatment of Tuberculosis leave some major side effects on the patients treated, such as :Streptomycin sometimes leads to renal damage and vestibular and auditory nerve damage,Isoniazid may cause hepatitis,Rifampicin causes thrombocytopenia, pain, vomit ing, nausea, and hepatitis,Pyrazinamide leads to arthralgia,Ethambutol may cause neuritis and color blindness,Cycloserine results in convulsions, dizziness, depression,Kanamycin may cause vertigo, damage to the auditory nerve, nephrotoxicity, skin rash from thioacetazone, and exfoliative dermatitis.[21]The global number of deaths officially classified as caused by Tuberculosis (1.3 million) in 2020 was almost double the number caused by HIV/AIDS (0.68 million).In recent times, Tuberculosis progress has been reversed by more than a decade due to Covid-19 pandemic, according to the WHO report of 2021. As a result of this, Tuberculosis mortality has increased for the first time in over a decade.[22].There are several tests to treat the Tuberculosis such as an X-ray, ultrasound, echocardiogram or CT scan of your chest or the part of your body that may be affected, biopsy,Tuberculin skin test . The tuberculin skin test (TST) has been used to diagnose Tuberculosis for > 100 years, but it fails to distinguish patients with LTBI from those with a Tuberculosis. To overcome the limitations of TST, several new skin tests and interferon-gamma release assays have been developed, such as the Diaskintest, C-Tb skin test, EC-Test, and T-cell spot of the TB assay, QuantiFERON-TB Gold In-Tube, Quantiferon-TB Gold-Plus, Liaison Quantiferon -TB Gold Plus test, and Lioferon TB/LTBI.[23]. Traditionally, medicinal plants were used for treating TB, such as smoke from the burnt leaves of Artemisia afra, the whole plant of Myrothamnus flabellifolius, leaves of Carica papaya, Zanthoxylum capense (roots), and seeds of Combretum hereroense, which was were inhaled 3–4 times every day. Leaves of Artemisia afra and Lippia javanica were infused into a hot water sink and patients inhaled the steam by wrapping a blanket over their heads. Leaves of crushed Citrus lemon, Artemisia afra, and Mentha sp. were burned in a paper wrapper 2 or 3 times daily. Such ayurvedic treatments are administered for around two weeks up to a month, subject to the patient's reaction to the formulation and tolerance to the medicine and administration.[24,25]. TB case detection rates were compromised due to a shift of manpower and other resources in COVID-19 management, but it has also given us hope for speedy vaccine development for various infectious diseases including TB. The director general of WHO also highlighted the recent WHO-commissioned study that estimates that a vaccine with 50% efficacy can avert up to 76 million new TB cases and 8·5 million deaths, in the next 25 years. However, it will require a budget of US$41·five billion. If the new vaccine is aimed to be 75% efficacious, it can avert up to 110 million new TB cases and 12·3 million deaths, but much more funding shall be required.[26].

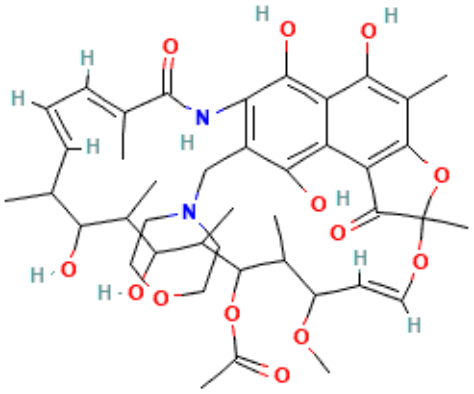
Compounds Used for the study[4]

Compound Name: Rifamycin, 8-(morpholinomethyl)-

Molecular Formula: $C_{42}H_{56}N_2O_{13}$

Molecular Weight: 796.9 g/mol

Chemical Structure:



IUPAC Name

[(9*E*,19*E*,21*E*)-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-26-(morpholin-4-ylmethyl)-6,23-dioxo-8,30-dioxa-24-azatetracyclo[23.3.1.1^{4,7}.0^{5,28}]triaconta-1(29),2,4,9,19,21,25,27-octaen-13-yl] acetate

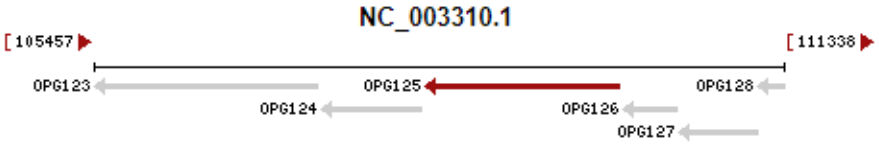
CAS

4075-43-8

Gene:

OPG125

Gene ID: 929047



2. MATERIALS AND METHODS [3]

Chemical Compound:

The Rifamycin, 8-(morpholinomethyl)- chemical in solid powder form was procured from GMPL, Hyderabad.

Preparation of Rifamycin, 8-(morpholinomethyl)-:

To a mixture of 7-acetylamino-2-chloro-4-morpholinomethyl-1,8-naphthyridine derivative (X) (2.0 g, 7.82 mmole) and 4-methoxybenzylamine (3.55 ml, 27 mmole) was added pyridine (35 ml) under N2. The mixture was heated to reflux for 48 h and then cooled to room temperature, the pyridine was removed and the compound (1) was obtained by followed by recrystallization

Test organisms:

Test organisms used for the screening of Rifamycin, 8-(morpholinomethyl)- against cell lines of Mycobacterium tuberculosis (MTB) bacteria such was procured from GMPL, Hyderabad.

Determination of antitubercular activity by Hexa disc method [3].

100 µl of a new culture of pathogens was swabbed on each sterile petriplate after 20 ml of sterilized agar has solidified. A sterile 5 mm cork borer was used to punch the wells over the agar plates, and 50 µl of aqueous plant extract was then added. After ten to fifteen minutes to dissipate, the plates were incubated at 350°C for 24 hours. The diameter of the inhibitory zones that appeared around each well after incubation were recorded in millimeters. The readings were taken in three distinct fixed directions for each replicate, and the average value was recorded.

After pouring 20 ml of sterilized MHA into sterile petriplates and solidifying, 100 µl of fresh pathogen culture was swabbed on each plate. A sterile 5 mm cork borer were employed to punch the wells over the agar plates. Each plant extract was then introduced to the wells at different concentrations, ranging from 20 mg/ml to 40, 60, 80, and 100 mg/ml. The plates were incubated at 370°C for 24 hours. The diameter of the inhibitory zones that appeared around each well after incubation was recorded in millimetres. All three replicates' readings were obtained in three distinct fixed directions, and the average value was noted.

Table no : 1

Test	**Diameter of Zone of inhibition (in mm)**					
	20µg	**40µg**	**60µg**	**80µg**	**100µg**	**Positive Control**
Rifamycin, 8-(morpholinomethyl)-	11±2.15	13.8±2.19	16.2±2.33	18.8±2.12	21.4±2.58	19.8±3.15
Mean±SEM	19.3±2.2	21.5±3.17	25.3±2.37	26.9±2.24	27.4±3.55	29.56±3.78

3. RESULTS AND DISCUSSION

The results of the experiment are listed in table 1.Tuberculosis is a disease that may be prevented and is typically cured. However, in 2023, tuberculosis is likely to reclaim its position as the world's top cause of mortality from a single infectious agent, having been replaced by coronavirus disease (COVID-19) for three years and accounting for nearly twice as many fatalities as HIV/AIDS.[27]Effective treatment of tuberculosis is relied on many bactericidal and sterilising medications provided in combination for a suitable length, to ensure antimicrobial efficacy while preventing selection of drug-resistant mutants and achieve permanent cure.[29]However, it is difficult to establish an obvious benefit of a new anti-TB agent over pre-existing medications, as clinical studies entail multidrug combination therapy employing highly effective regular anti-TB drugs[28]finally,due to the microbial evaluation of RIFAMYCIN, 8-(MORPHOLINOMETHYL) compound we came across the inhibition of tuberculosis causing microbes through the disc diffusion method. where the minimum inhibition zone observed during the experiment ranges from 19.3±2.2 to 27.4±3.55. The main goal was to assess level of the 8 -morpholinomethyl compound against the mycobacterium eventhough the rifamycin does show Prolonged anti-TB therapy increases the risk of major side events such as liver damage, cardiac arrhythmias, gastrointestinal difficulties, ototoxicity, neurotoxicity, and mental disorders. [30]. According to scientific research, L. javanica possesses a broad spectrum of pharmacological actions, such as pesticidal, anticancer, antiamoebic, antidiabetic, antimalarial, antimicrobial, antioxidant, and antiplasmodial properties.[31]However, the most effective anti-tuberculosis drugs are first-line essentials, which are a must for any short-term treatment plan. The medications in this group include streptomycin, pyrazinamide, ethambutol, isoniazid, and rifampin. Second-line anti-tuberculosis medications are clinically far less effective than first-line agents and are more likely to cause serious responses.[32] Thus we conclude that rifamycin is considered as one of anti tubercular drug which can help in treatment of tuberculosis.

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