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SYNTHESIS AND EVALUATION OF ANTITUBERCULOSIS ACTIVITY OF 1-ADAMANTYL HYDRAZIDE HYDRAZONES SUPPORTED WITH MOLECULAR DOCKING

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ABSTRACT:

Tuberculosis is a significant cause of illness and death worldwide. More importantly, the multidrug resistance cases are increasing. This requires a strong need to develop novel compounds possessing antimycobacterial properties and to enhance current therapies. In view of this, novel 1-adamantyl derivatives were designed, synthesized and tested for its antituberculosis effectiveness against a susceptible strain of *Mycobacterium tuberculosis* (H37Rv). The samples were assessed using a classic growth based method, which involved the L.J. MIC method (Lowenstein and Jensen method). The results indicated that the synthesized 1-adamantyl hydrazide hydrazones (5-BVAC & 5-NVAC) showed significant antituberculosis activity when compared with known antituberculosis drug Isoniazid with minimum inhibitory concentration MIC of 0.9 ppm and 1.25 ppm.

The mode of interaction was studied by carrying molecular docking study with MmpL3 from *M. smegmatis* which has the similar genus as *Mycobacterium tuberculosis* (H37Rv) with similarity in cell structure and genetic organisation. The binding affinity score of best binding conformation of 5-BVAC & 5-NVAC was −8.7 Kcal/mol

The results show that 5-BVAC & 5-NVAC to be a new inhibitor of MmpL3 with the binding mode similar to that of other antituberculars that target MmpL3. Hence the new scaffolds containing an adamantane and hydrazide hydrazone moiety may emerge as the potential anti TB agent, which can have the capability to enhance TB treatment in combination with other standard therapies.

KEYWORDS: 1-adamantyl hydrazide hydrazone, L.J. MIC method, *Mycobacterium tuberculosis* H37Rv, *M. smegmatis*.

INTRODUCTION:

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, one of the leading infectious diseases in humans, which lead to 1.5 million deaths in the year 2020 [1]. The current treatment for drug-sensitive TB is a combination of isoniazid, ethambuthol, rifampicin and pyrazinamide, but requires months under observation. Even after many efforts to end the epidemic, TB prevails and antibiotic-resistant strains of *Mycobacterium tuberculosis* keeps rising. Hence, it is required to develop new molecules with antimycobacterial activities [2,3].

The WHO recommendations for the cure of drug-resistant and multi-drug resistant (MDR) TB include fluoroquinolones, linezolid and bedaquiline [4,5]. Bedaquiline was a new drug that was accepted in 2012 for the treatment of MDR TB.

Other anti-TB medications employed for second-line regimens, are para-aminosalicylic acid, fluoroquinolones, Clofazimine, and Cycloserine and betalactams. These medicines can be used in different combinations. The reappearance of MDR TB due to COVID-19 pandemic makes it more necessary to recognize new molecules [6].

Recently, a key area of research focus is the advancement of novel adamantane based drug molecules with enhanced pharmacokinetic and pharmacodynamic properties [7]. The adamantyl moiety is well established as a crucial pharmacophore in biologically active compounds. Incorporating the adamantyl core into molecules can significantly influence their lipophilicity, pharmacological, and biological properties [8]. Therefore, adamantane can effectively modify the therapeutic index of parent structures, making it widely utilized for diverse therapeutic applications. Adamantane derivatives have been shown to interact with various enzymes and exhibit a range of therapeutic activities such as anti-viral e.g. Tromatadine [9] and anti-proliferative activities [10]. Adamantane derivatives, amantadine, has been found to have antiviral activity [11]. Adamantyl ureas such as AU1235 [12] and SQ109 [13] were previously identified as a group of compounds active against M.tuberculosis as potential inhibitors of MmpL3. SQ109 has completed phase 2 clinical trials. [14, 15].

MmpL3 is a essential transmembrane protein that depends on the proton motive force (PMF) for the transport of mycolic acids in the form of trehalose monomycolates (TMMs) across the cell membrane. Many MmpL3 inhibitors with varied chemical scaffolds have been described such as a 1,2-diamine, SQ109 [13] , the pyrrole derivative BM212[16], the adamantyl urea AU1235 [12] etc. These molecules specifically bind to MmpL3 and thus block its activity [17]. The crystal structure of *M. smegmatis* MmpL3 has been determined and it has been observed that inhibitors with various chemical scaffolds bind to the same pocket in the proton translocation channel [18].

Hydrazide hydrazone groups have an azomethine linkage bonded to an amide group (-CH=N-NH-CO-), which has a crucial role in pharmacological activities. They have been found to have biological activity, such as antimicrobial [19,20], antituberculosis [21,22], and anticancer [23] drugs. Thus, combining two groups, adamantane and hydrazones has the potential to form novel molecules with excellent biological activities.

On the light of the above points, we have developed and created two adamantane hydrazide hydrazone derivatives namely N'-(5-Bromo-4-hydroxy-3-methoxybenzylidene) adamantane-1-carbohydrazide (5-BVAC) and N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC) that targets MmpL3.

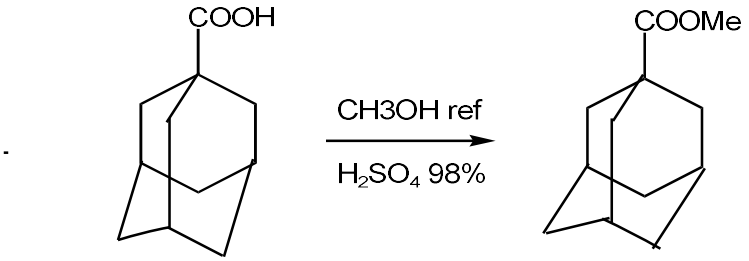
METHODOLOGY:

Chemicals were sourced from SBL, Loba Chemie, and Ottokemi. The structural verification of adamantane derivative was performed using FTIR, NMR spectroscopy & elemental analysis. FTIR was performed using the KBr pellet technique on a Bruker 3000 Hyperion Microscope with a Vertex 80 FTIR system (Germany). ^{1}H NMR spectra were acquired in deuterated dimethyl sulfoxide (DMSO-d6) at 600 MHz with a JEOL ECZR Series 600 mega Hertz NMR Spectrometer (Japan), using TMS as an internal standard, and chemical shifts are recorded in δ ppm. Elemental analysis was carried with ThermoFisher Scientific Flash smart V CHNS/O analyzer. Anti-tuberculosis activity was assessed using a classic growth based method, which incorporated the L.J. MIC method (Lowenstein and Jensen) against a susceptible strain of *Mycobacterium tuberculosis* (H37Rv) [24]. *M. tuberculosis* sensitive strain (H37Rv) was obtained from National Institute for Research in Tuberculosis in Chennai, Tamil Nadu.

Molecular Docking was performed with pyrx tool using Vina wizard. The synthesised compounds 5-BVAC and 5-NVAC were drawn using Kings draw software and converted into 3D using Discovery

studio Analyser. The protein molecule MmpL3 was energy minimised in Discovery studio and UCSF Chimera.

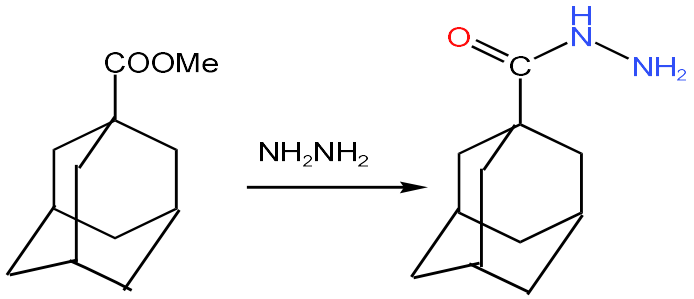
(a)Synthesis of Methyl Adamantane-1-Carboxylate: 5 grams (27 mmol) of adamantane-1-carboxylic acid was reacted with 50 cm^3 of methanol (1235 mmol) and 9.2 grams of 98 percent sulphuric acid (5.11 cm^3). This mixture was stirred and heated with reflux for 4 hours. After this, the mixture was neutralized to pH 7–8 using a 10% aqueous sodium bicarbonate ($NaHCO_3$) solution. The solution was kept at room temperature. Following this, 200 cm^3 of cold water was mixed, and then subjected to recrystallization with absolute ethanol yielding 4.92 grams of white, needle-shaped crystals of Methyl Adamantane-1-Carboxylate [25,26], with an 88.2% yield. The melting point observed was 37 °C. It was identified by comparing its m.p. with the published value [27].



Adamantane-1-carboxylic acid Methyl Adamantane-1-Carboxylate

Scheme1: Synthesis of Methyl Adamantane-1-Carboxylate

(b)Synthesis of Adamantane-1-Carbohydrazide: 4 grams (20 mmol) of Methyl Adamantane-1-Carboxylate and 25 cm^3 (412 mmol) of 80 percent hydrazine hydrate solution in 18 cm^3 of ethanol was refluxed for 15 hours. Upon completion, 200 cm^3 of ice-cold water was added in the reaction. The formed precipitate was then filtered and given washings with ice water, and dried to yield 3.82 grams of an opalescent, scaly solid identified as Adamantane-1-Carbohydrazide, with an 88.99% yield. The melting point observed was 148 °C. FTIR υ_{max} (cm^{-1}): 3332.47, 3278.23 (N-H), 2912.48, 2894.13, 2849.49 (C-H), 1613.79 (C=O), 1523.69 (N-H), 1452.70, 1368.80 (C-H) [19, 21]. ^{1}H NMR (600 mHz, DMSO-d_6, δ-ppm): 1.93 (3H, adamantane), 1.74 (6H, adamantane), 1.63 (6H, adamantane), 4.12 (2H, -NH_2), 8.68 (H, NH-C) [26].

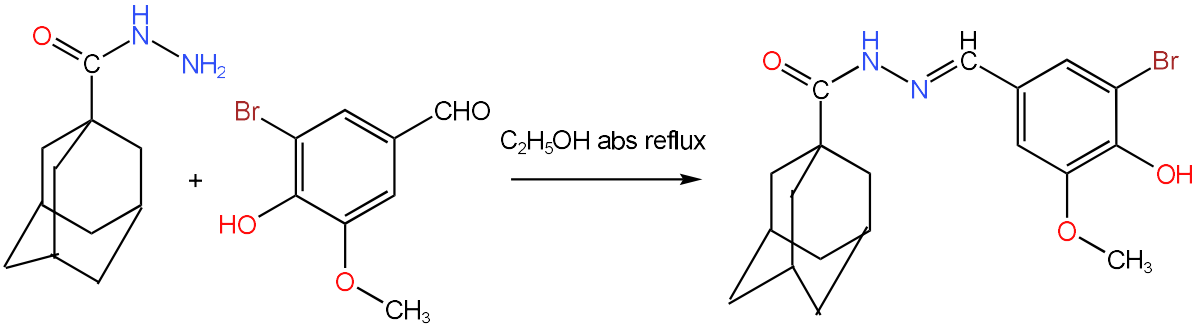


Methyl Adamantane-1-Carboxylate Adamantane-1-Carbohydrazide

Scheme 2: Synthesis of Adamantane-1-Carbohydrazide

(c)Synthesis of 5-Bromo Vanillin Adamantane Carbohydrazide (5BVAC): A combination of 2 millimol of adamantane-1-carbohydrazide and 2 millimol of 5-Bromovanillin in 15 cm^3 of ethanol was kept on stirring and refluxing for 4 hours. After completion of reaction, the solvent was evaporated further allowed to crystallize at 0–5°C. The resulting crystals were filtered and given washings with ethanol, and air-dried, yielding N'-(5-Bromo-4-hydroxy-3-methoxybenzylidene) adamantane-1-carbohydrazide (5-BVAC) with an 83.98% yield. The melting point was determined to be 230 °C. Elemental analysis CHN: Found (Calculated): C,56.170 (56.05);H,5.905(5.65),N,6.683 (6.88). The Molecular formula was confirmed to be $C_{19}H_{23}O_3N_2Br$, Molecular wt =407.09 g/mol. FTIR υ_{max} cm^{-1}: 3505.81 (O-H), 3255.98 (N-

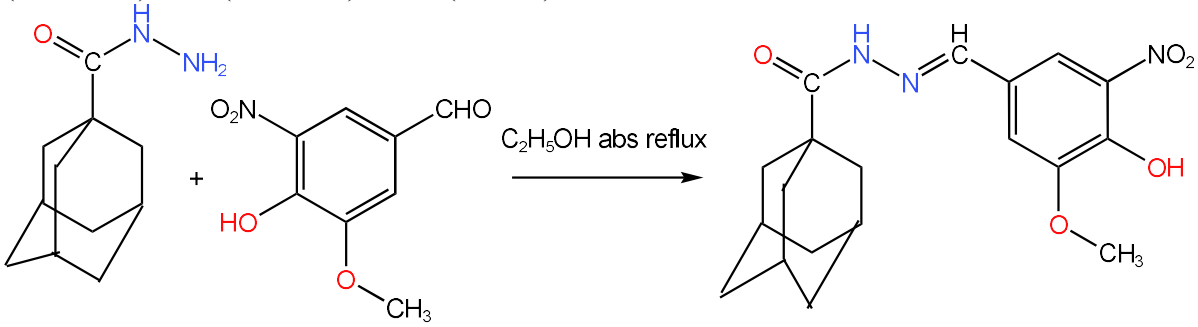
H), 3073.22 (C-H aromatic), 2904.34, 2848.77 (C-H aliphatic), 1650.14 (C=O), 1597.11 (C=N), 1547.02 (N-H), 1498, 1451.57, 1416.19, 1386.69 (C-H). ^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): 1.95 (3H, adamantane), 1.82 (6H, adamantane), 1.65 (6H, adamantane), 3.82 (3H, OCH_3), 7.28, 7.21 (2H, Ar-H), 8.19 (H, -N=CH), 9.91 (H, -NH-C), 10.72 (H, -OH).



Adamantane-1-Carbohydrazide | 5-Bromo Vanillin | N'-(5-Bromo-4-hydroxy-3-methoxy benzylidene) adamantane-1-carbohydrazide

Scheme 3: Synthesis of N'-(5-Bromo-4-hydroxy-3-methoxy benzylidene) adamantane-1-carbohydrazide (5-BVAC)

(d)Synthesis of 5-Nitro Vanillin Adamantane Carbohydrazide (5NVAC): A mixture of 2 millimol of adamantane-1-carbohydrazide (3) and 2 millimol of 5-nitrovanillin in 15 cm^3 of ethanol was kept on stirring and refluxing for 4 hrs. Upon completion of the reaction, ethanol was evaporated. The resulting mixture was allowed to crystallize at 0–5°C. The resulting crystals were filtered and given washings with ethanol, and air-dried, yielding N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC) with a 92.98% yield. The melting point was determined to be 190 °C. Elemental analysis CHN: Found (Calculated): C, 61.331(61.15); H, 5.947(6.16); N, 10.971(11.25). The Molecular formula was confirmed to be $C_{19}H_{23}O_5N_3$, Molecular wt =373.19 g/mol. FTIR υ_{max} cm^{-1}: 3243.02 (N-H), 3084.15 (C-H aromatic), 2904.07, 2850.52 (C-H aliphatic), 1654.60 (C=O), 1617 (C=N), 1531.91 (N-H), 1453.53, 1422.95, 1369.42 (C-H). ^{1}H NMR (600 MHz, DMSO-d_6, δ ppm): 2.00 (3H, adamantane), 1.86 (6H, adamantane), 1.69 (6H, adamantane), 3.92 (3H, OCH_3), 7.67, 7.53 (2H, Ar-H), 8.32 (H, -N=CH), 9.80 (H, -NH-C), 10.89 (H, -OH).



Adamantane-1-Carbohydrazide | 5-Nitro Vanillin | N'-(4-hydroxy-3-methoxy-5- nitro benzylidene) adamantane-1-carbohydrazide

Scheme 4: Synthesis of N'-(4-hydroxy-3-methoxy-5-nitro benzylidene) adamantane-1-carbohydrazide (5-NVAC)

Antituberculosis Activity:

In vitro testing of samples 5-BVAC & 5-NVAC against a susceptible strain of *Mycobacterium tuberculosis* (H37Rv) was performed. The samples were assessed using a classic growth based method, which incorporated the L.J. MIC method (Lowenstein and Jensen). During the experiment, 2% Malachite green solution, homogenized eggs solution, and mineral salt solutions were added to Lowenstein Jensen

(LJ) medium. Inoculated with a mycobacterium suspension strain whose concentration was equivalent to the McFarland standard, the medium containing various concentrations of samples (100, 50, 25, 12.5, 6.25, 3.125, 10, 5, 2.5, 1.25, 8, 4, 2, 1, 0.5, 0.25 µg/mL) was then maintained at 37°C with regular monitoring. The *M. tuberculosis* H37Rv was studied with Isoniazid, a well-known drug giving Minimum Inhibitory Concentration MIC of 0.2 µg /cm^3. The MIC for synthesized compounds 5-BVAC & 5-NVAC was observed to be 0.9 µg/cm^3 and 1.2 µg/cm^3.

Molecular Docking of MmpL3 with 5-BVAC & 5-NVAC:

Molecular docking [28] was carried out using MmpL3 from M.smegmatis (PDBID:6AJH) obtained from the Protein data bank. It has the similar genus as *Mycobacterium tuberculosis* (H37Rv) with similarity in cell structure and genetic organisation. M.smegmatis is often used as a model organism for M. Tuberculosis.

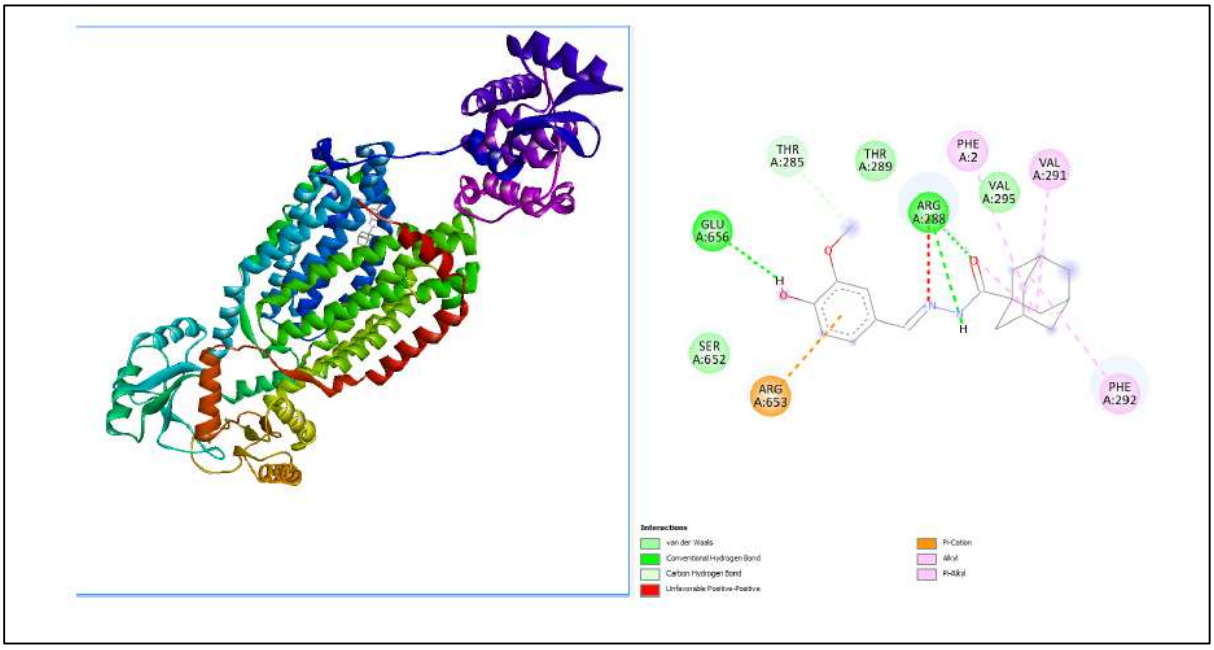


Fig 1: Molecular docking of MmpL3 with 5-BVAC

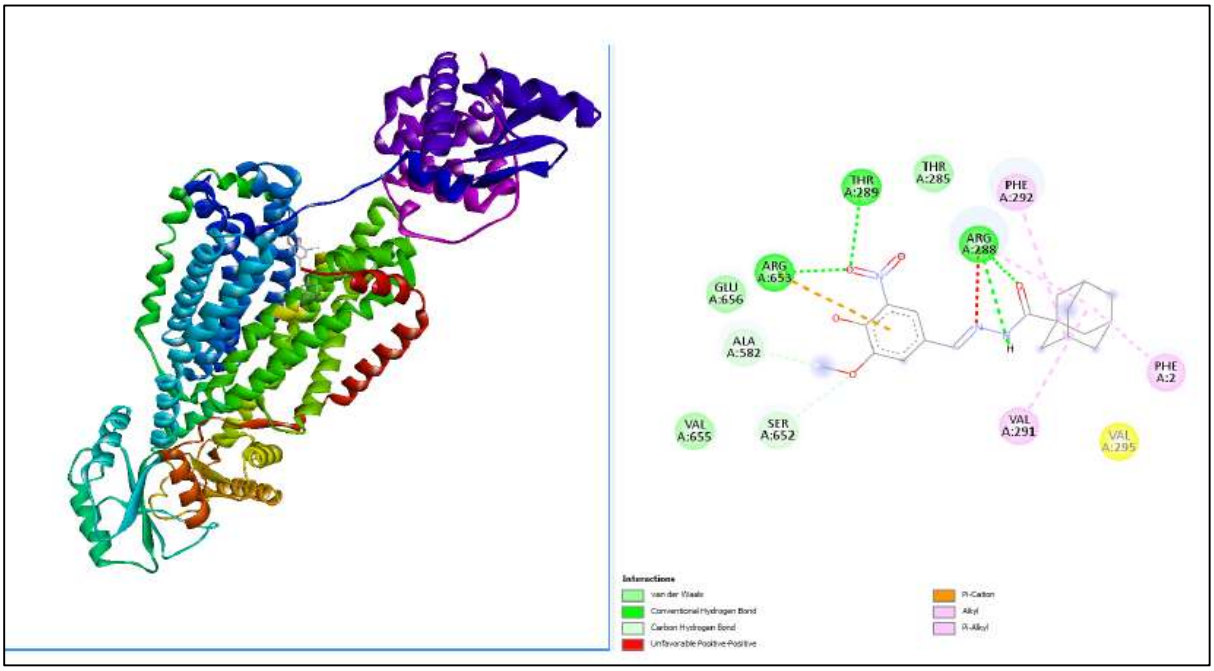


Fig 2: Molecular docking of MmpL3 with 5-NVAC

The binding affinity score of the best binding conformation of both 5-BVAC & 5-NVAC was observed to be −8.7 kcal/mol. The binding site with MmpL3 is expected to be similar to that of other antituberculars SQ109 and AU1235 that target MmpL3 in the same pocket inside the proton-translocating channel, in the transmembrane region.

RESULTS:

Structure of 5-BVAC & 5-NVAC were recognised using elemental analysis, FTIR and ^{1}H NMR. The establishment of hydrazone structure was proved with IR studies; following the detection of hydrazide C=O and azomethine –CH=N- peak in IR spectra.

^{1}H NMR also proved the establishment of hydrazide structure in 5-BVAC & 5-NVAC. ^{1}H NMR signals from the adamantane moiety appeared at δ 1.65-2.00 ppm. Aromatic protons appeared as expected at δ 7.21-7.67 ppm. The signal from the methyne proton (CH=N) appeared as a singlet at δ 8.19 & 8.32 ppm respectively, whereas that of the amide proton recorded a singlet at δ 9.91 & 9.80 ppm respectively. The –OH proton was recorded at δ 10.72 ppm, whereas the –OCH_3 protons was seen at δ 3.82 & 3.92 ppm respectively. All spectra were in full agreement with the proposed structure.

Molecular docking indicates that 5-BVAC & 5-NVAC binds to the same centre as that of other antituberculars that target MmpL3.

CONCLUSION:

The increase in drug-resistant TB presents a significant health challenge. Reducing the disease will necessitate new molecules capable of enhancing treatment. Here, we identified compound 5 BVAC & 5-NVAC as probable inhibitor of MmpL3 with a new scaffold containing an adamantane group and a hydrazide hydrazone group. Compound 5-BVAC & 5-NVAC demonstrated anti-mycobacterial activity against *M. tuberculosis* H37Rv. The anti-TB activity was evaluated using L.J. MIC method (Lowenstein and Jensen) method. The MIC for 5 BVAC & 5-NVAC was observed to be 0.9 µg/ml & 1.2 µg/ml.

Number of studies have suggested that certain inhibitors indirectly target MmpL3 and distrupt the membrane potential [29,30]. Future works with other compounds showing similarity with synthesised compound may provide useful information. Based on our results, we consider 5-BVAC & 5-NVAC to have promising therapeutic potential in future.

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