Research Article

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Design, Synthesis and Antimycobacterial Activity of 2-(Benzimidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl) propanenitrile Analogs

Oya UNSAL TAN*
ORCID: 0000-0002-4152-069X
Mustafa Mert SİRİM[1]
ORCID: 0000-0002-5511-0616
Vagolu Siva KRİSHNA[2]
ORCID: 0000-0003-1540-9995
Sriram DHARMARAJAN[2]
ORCID: 0000-0001-9970-2050

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

[2]Medicinal Chemistry & Antimycobacterial Research Laboratory, Pharmacy Group, Birla Institute of Technology & Science – Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, Andhra Pradesh, India

Corresponding author:
Oya UNSAL TAN
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Hacettepe, 06100, Ankara, Turkey,
phone&fax: +90-3123051872
e-mail: oyaunsal@hacettepe.edu.tr

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ABSTRACT

This paper reports the synthesis and evaluation of six new 2-(benzimidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl)propanenitrile derivatives (4a-f) for their in vitro antimycobacterial activities against Mycobacterium tuberculosis H37Rv. The target compounds (4a-f) were characterized using IR, 1H and 13C NMR, mass spectral data as well as elemental analysis. Among them, 4c (2-(1H-benzo[d]imidazol-2-yl)-3-(4-(4-(2-chlorophenyl)piperazin-1-yl)phenyl)propanenitrile) was found to be the most active compound with MIC of 1.56 µg/mL against M. tuberculosis. In addition, 4c showed 2.1 log reduction against nutrient starved M. tuberculosis which is more potent than first line drugs. Based on their predicted physicochemical values, the compounds obeyed the Lipinski's "rule of five," and TPSA/nROT limits. Molecular docking studies were performed to estimate the orientation of 4c at the active site of M. tuberculosis LAT enzyme. These results suggest that 4c may be a good candidate for drug development owing to potential against both active and dormant forms of M. tuberculosis.

Keywords: benzimidazole, propanenitrile, antimycobacterial activity, tuberculosis, nutrient starvation, lysine epsilon aminotransferase.

ÖZET

Bu makale, Mycobacterium tuberculosis H37Rv'ye karşı in vitro antimikobakteriyel aktiviteleri için altı yeni 2-(benzimidazol-2-yl)-3-(4-(4-substitüepiperazin-1-yl) fenil)propannitril türevlerinin sentezini ve aktivitelerinin değerlendirilmesini içermektedir. Hedef bileşiklerin (4a-f) yapıları, IR, 1H ve 13C NMR, kütle spektral verileri ve ayrıca element analizi kullanılarak kanıtlandı. Bileşikler arasında M. tuberculosis'e karşı 1.56 µg/mL MIC değeri ile en aktif bileşik 4c (2-(1H-benzo[d]imidazol-2-il)-3-(4-(4-(2-klorofenil)piperazin-1-il)fenil) propannitril) olarak bulundu. Ek olarak, 4c, besin açlık modeli testinde 2.1 log azalma sağlayarak birinci basamak ilaçlardan daha güçlü antimikobakteriyel aktivite göstermiştir. Hesaplanan fizikokimyasal değerlere dayanarak, bileşiklerin Lipinski'nin "beşler kuralına" ve TPSA/nROT limitlerine uyduğu tahmin edilmektedir. 4c'nin M. tuberculosis LAT enziminin aktif bölgesindeki oryantasyonunu tahmin etmek için moleküler yerleştirme çalışmaları yapıldı. Bu sonuçlar, M. tuberculosis'in hem aktif hem de latent formlarına karşı potansiyeli nedeniyle 4c'nin ilaç geliştirme için iyi bir aday olabileceğini düşündürmektedir.

Anahtar kelimeler: Benzimidazol, propannitril, antimikobakteriyel aktivite, tüberküloz, besin açlık modeli, lisin epsilon aminotransferaz

Introduction

Tuberculosis (TB), a chronic infectious disease usually caused by *Mycobacterium tuberculosis,* is one of the top ten causes of death worldwide [1]. According to the latest WHO report in 2019, 10 million people developed TB disease and among them 1.5 million people (1.2 million HIV-negative and 251.000 HIV-positive people) died from TB in 2018. There were about half a million new cases of drug-resistant TB such as rifampicin-resistant (RR), multidrug-resistant (MDR) or extensively drug-resistant (XDR) [2]. Effective therapy of TB consists of a combination of four first-line drugs administrated for at least 6 months. The treatment of drug resistant TB is longer, and requires drugs that are more expensive [3,4]drug-resistant tuberculosis (TB.

In addition to active TB, treatment of latent TB is also critical to control and eliminate the disease [5]. Clearly, there is an urgent need to discover new therapeutic agents which should reduce treatment duration, have an acceptable tolerability profile, be active against drug resistant TB and be active against latent TB [6,7]

Among the heterocyclic pharmacophores, benzimidazole is frequently preferred in medicinal chemistry. It is not only found in the structure of naturally occurring compounds like vitamin B12 but also found in the structure of well-known drugs such as mebendazole, albendazole, astemizole, emedastine, omeprazole, lansoprazole, pantoprazole candesartan, telmisartan, bilastine, bendamustine and droperidol (**Figure 1**). It is therefore assumed that benzimidazole is easily recognized by various biological systems such as enzymes and receptors [8,9].

Many compounds containing benzimidazole moiety have been synthesized and evaluated for their antimycobacterial activities. Of these, especially 2-alkylsubstitutedbenzimidazoles have been found to exhibit remarkable antimycobacterial activity (**Figure 2**) [10–14]but the increasing resistance of mycobacterial species has heightened alarm, requiring the development of novel drugs in order to improve treatment outcomes. Here, as an effort to identify novel and effective antitubercular agents, we designed and synthesized a series of novel substituted benzimidazolallylidenehydrazinylmethylthiazole derivatives via a multi-component molecular hybridization approach with single molecular architecture. Our design strategy involved assembling the antitubercular pharmacophoric fragments benzimidazole, 2-aminothiazole and substituted α,β-unsaturated ketones via condensation reactions. All the newly synthesized compounds were fully characterized via NMR and mass spectral data and evaluated for in vitro biological activity against the H37Ra strain of Mycobacterium tuberculosis. From the biological evaluation data, we identified some effective compounds, of which 8g and 7e were the most active ones (both having MIC values of 2.5 μg mL−1.

Motivated by these findings, in the present study we focus on the design and synthesis of some novel benzimidazole derivatives with a propanitrile group at the C-2 position and the evaluation of their antimycobacterial activity against both active and dormant states of Mycobacterium tuberculosis.

Results and Discussion

Chemistry

The synthetic route for the target compounds 4a-f is outlined in Scheme 1. The starting compounds (2-(1*H*-benzo[d]imidazol-2-yl)acetonitrile 1 [15] and 4-substitutedbenzaldehydes 2a-f [16] were prepared by the method as previously reported. The reaction of **1** with appropriate aldehydes 2a-f in the presence of catalytic amount of piperidine gave 2-(1*H*-benzo[d]imidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl)acrylonitriles 3a-f. Reducing 3a-f by using $NaBH_4$ produced the desired compounds, 2-(benzo[*d*]imidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl)propanenitriles 4a-f.

The newly synthesized compounds were characterized using IR, ^1H and ^{13}C NMR, mass spectral data as well as elemental analysis. The details of spectral data are given in the experimental section. The IR spectra of the target compounds 4a-f showed characteristic absorption band at around 2250 cm^{-1} due to C≡N stretching. In the ^1H NMR spectra of the target compounds, signals observed at around 4.8 and 3.4 ppm were assigned to the protons on C_2 and C_3 of the propanenitrile respectively. The signals belonging to $H_{5,6}$, $H_{4,7}$ and N-H of benzimidazole were observed at around 7.2, 7.5 and 12.6 ppm respectively. In the ^{13}C NMR spectra, C_2 and C_3 carbons of propanenitrile moiety of 4a-f were seen at 33 and 36 ppm respectively. Furthermore, the structures of all target compounds were confirmed by the peaks belonging to $[M+Na]^+$ and $[M+H]^+$ seen in the ESI

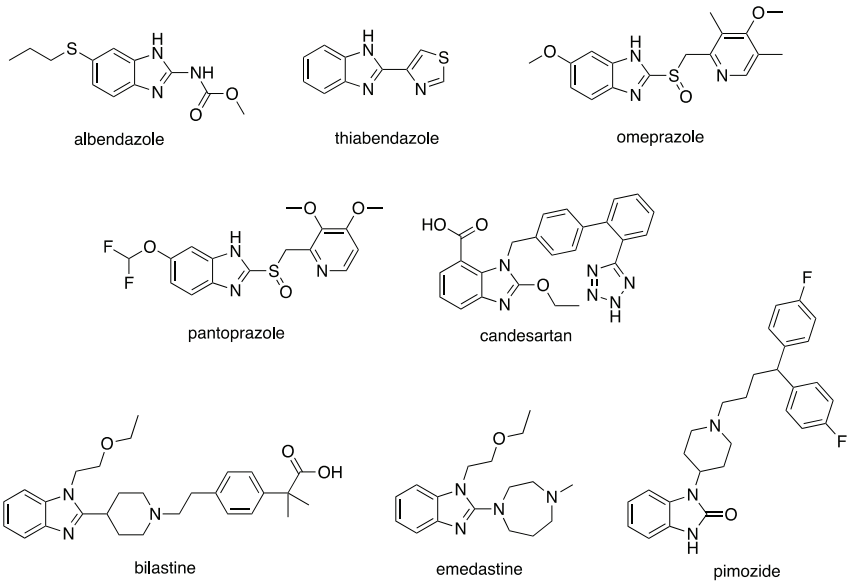

Figure 1. Chemical structures of some drugs containing benzimidazole moiety.

mass spectra. Elemental analysis agreed with the suggested chemical structures of the synthesized compounds.

Antimycobacterial activity

The target compounds were evaluated for their *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv strain by microplate alamar blue assay (MABA). Isoniazid, rifampicin and ethambutol were used as reference compounds. The antimycobacterial test results were summarized in Table 1. According to the results, 4c having Cl atom at *ortho*-position of the phenyl, displayed the highest antimycobacterial activity with MIC of 1.56 µg/mL. It was found to be equipotent to the reference compound ethambutol. Among other derivatives, compound 4e with OCH_3 moiety at *ortho*-position of the phenyl exhibited MIC 25 µg/mL. 4a, 4b, 4d and 4f (substituted with 4-Cl, 3-Cl, 3-OCH_3 and 4-F respectively) were not active at an MIC of 25 µg/mL. The results showed that the substitution at the *ortho* position of the phenyl ring resulted in the compounds with significant antimycobacterial activity than substitution at *para* or *meta*.

Nutrient starvation model of *M. tuberculosis*

M. tuberculosis is able to survive for extended periods in a non-growing state in conditions of reduced oxygen and nutrient deprivation. It is one of the major obstacles in the treatment of TB because the current first line drugs are not effective against this form (latent) of TB. In this study, the most active compound, 4c have been screened against nutrient starved dormant TB model. According to the results (Figure 3), 4c displayed 2.1 log fold reduction in *M. tuberculosis* count which is more potent than first line drugs isoniazid, ciprofloxacin, rifampicin and moxifloxacin (1.5–2.0 log fold). As 4c inhibited the growth of *M. tuberculosis* in the nutrient starvation model, it is suggested that 4c may be effective against active and dormant forms of MTB.

Molecular modeling

*Lipinski'*s rule of five is widely used as a filter for drug-like properties. According to this rule, drug-like molecules have a log P ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and number of hydrogen bond donor ≤5 [17]. In addition to *Lipinski's* rule of five, number of rotatable bond (nROTB) and topological polar surface area (TPSA) are also considered as important predictors of good oral bioavailability. It is accepted that violation of the limits (TPSA ≤ 120 $Å^2$; nROT ≤10) decreases oral bioavailability of drugs [18,19]. All the target compounds in this study fulfilled of the *Lipinski'*s rule of five as well as TPSA and nROT limits (Table 1).

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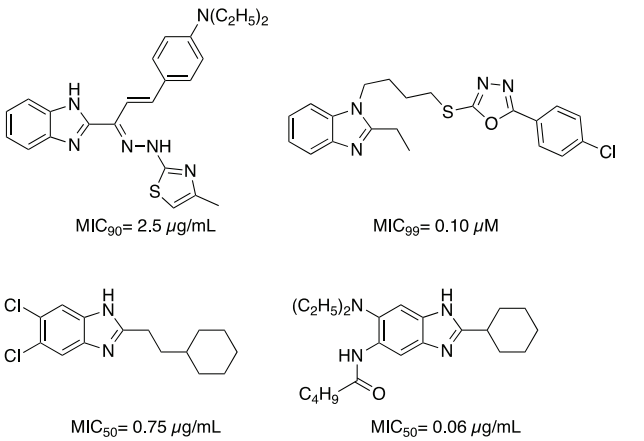
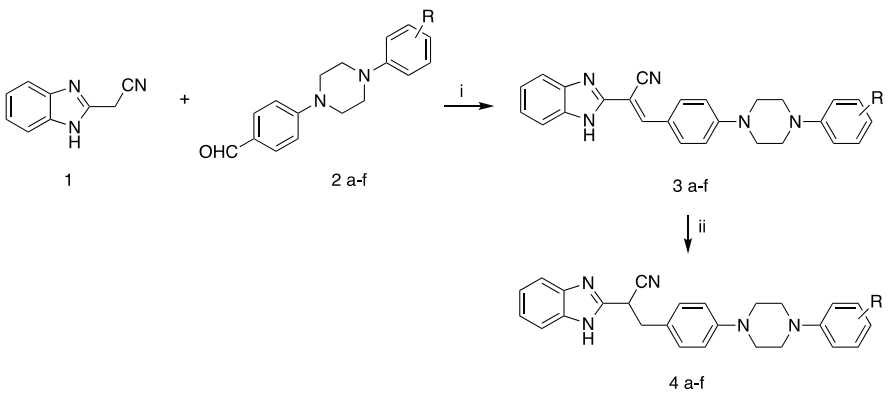


Figure 2. 2-Alkylbenzimidazoles and their antimycobacterial activities.



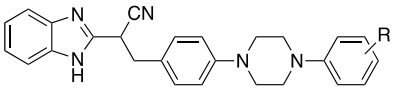
R= 4-Cl, 3-Cl, 2-Cl, 3-OCH_3, 2-OCH_3, 4-F
Scheme 1. Synthesis of the target compounds. Reagents and conditions: **i.** 1-2 drop piperidine, ethanol **ii.** $NaBH_4$, ethanol.

Finding specific targets to the latent stage of *M. tuberculosis* is a crucial step in the treatment of latent tuberculosis. Lysine-ε-aminotransferase (LAT) can be potential target to the latent stage of *M. tuberculosis* because it is highly upregulated under non-replicative conditions in *M. Tuberculosis* [20,21]. In this study, molecular docking studies were performed with the most active derivative (4c) and *M. tuberculosis* LAT enzyme to find a clue for the *in vitro* results. The docking result of 4c showed that benzimidazole moiety established π-H interactions with Val 273. Additionally, nitrile moiety made a hydrogen bonding interaction with important amino acid, Arg 170 (Figure 4A). It can be seen clearly from 2D representation (Figure 4B), there is no space available for the substituted phenyl ring in the active site. It might be assumed that the substituents on *ortho* position could be tolerated only.

Conclusion

In summary, the present study describes the synthesis and evaluation of 2-(benzimidazol-2-yl)-3-(4-substitutedphenyl)propanenitrile derivatives against *M. tuberculosis* H37Rv. Among the derivatives examined, 4c, the most active derivative of the series, was found to be as effective as the standard drug ethambutol with an MIC value of 1.56 µg / mL. Moreover, 4c showed 2.1 log reduction in the bacterial count of dormant forms of mycobacterium which is more potent than first line drugs isoniazid, ciprofloxacin, rifampicin and moxifloxacin. On the other hand, it was encouraging to note that all the compounds were in agreement with the *Lipinski's* "rule of five," and TPSA /nROT limits. To gain insights into the latent TB activity, binding mode of 4c into *M. tuberculosis* LAT enzyme was predicted using docking studies. In the light of these findings, it can be considered

Table 1. Antimycobacterial activities and physicochemical parameters of the target compounds.



Compounds	R	MIC (µg/mL)	LogP[a]	H bond acc./don.	MW	Rot	TPSA[a]
4a	4-Cl	>25	4.49	3/2	441	6	59.0
4b	3-Cl	>25	4.53	3/2	441	6	59.0
4c	2-Cl	1.56	4.49	3/2	441	6	59.0
4d	3-OCH_3	>25	3.89	4/2	437	7	68.2
4e	2-OCH_3	25	3.85	4/2	437	7	68.2
4f	4-F	>25	4.05	3/2	425	6	59.0
Isoniazid		0.05					
Rifampisin		0.1					
Ethambutol		1.56					

a TPSA and LogP calculated using Molecular Operating Environment, Chemical Computing Group (MOE) 2018.0101.

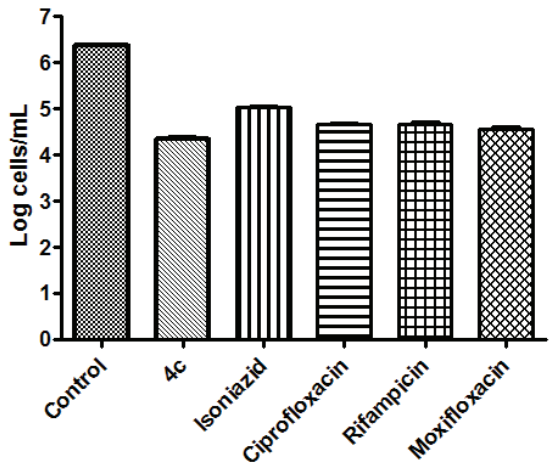


Figure 3. Biological activities of **4c** against *M. tuberculosis* in the nutrient starvation model. Bacterial count estimation (Mean ± S.D., n = 3) for control and treated groups conducted by using the MPN (most probable number) assay. **4c** gave significant inhibition of growth of *M. tuberculosis* in this model as compared to the control ($p < 0.0001$, two way ANOVA using GraphPad Prism Software.

that 4c is promising compound to combat active and dormant forms of *M. tuberculosis*.

Materials and Method

Chemistry

Melting points were determined with a Thomas-Hoover Capillary Melting Point Apparatus (Philadelphia, PA, USA); the results are uncorrected. ATR-FTIR spectra were obtained using the MIRacle ATR accessory (Pike technologies) in conjunction with a Spectrum BX FTIR spectrometer (Perkin Elmer) and were reported in cm^{-1}. The ^{1}H and ^{13}C NMR spectra (DMSOd_6) were recorded on a Varian Mercury 400 FT NMR spectrophotometer using TMS as an internal reference (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR; Chemical shift represented in δ ppm). The ESI-MS spectra were measured on a micromass ZQ-4000 single quadruple mass spectrometer. Elemental analyses (C, H and N) were performed on a Leco CHNS 932 analyzer.

2-(1*H*-Benzo[d]imidazol-2-yl)acetonitrile 1

Ethyl cyanoacetate and 1,2-phenylenediamine in a 2:1 ratio was refluxed at 150 °C to obtain **1** as mentioned in literature [15].

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General procedure for the preparation of 4-substitutedbenzaldehydes 2a-f

4-Fluorobenzaldehyde and various piperazine derivatives were reacted by using potassium carbonate as catalyst in DMSO as mentioned in the literature [16].

General procedure for the preparation of 2-(1*H*-benzo[d]imidazol-2-yl)-3-(4-(4-substitutedpiperazin-1-yl)phenyl) acrylonitrile 3a-f

The mixture of equimolar amounts of 2-(1*H*-benzo[d]imidazol-2-yl)acetonitrile 1 and appropriate aldehyde 2a-f was stirred in 20 mL absolute ethanol in the presence of 1-2 drop piperidine. Upon completion of the reaction (monitored by TLC), the precipitate was collected by filtration and crystallized from ethyl acetate.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(4-chlorophenyl)piperazin-1-yl)phenyl) acrylonitrile (3a)

m.p. 250-2 °C (lit. [15] 250-2°C).

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(3-chlorophenyl)piperazin-1-yl)phenyl) acrylonitrile (3b)

m.p. 282 °C (lit. [15] 280-1°C).

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(2-chlorophenyl)piperazin-1-yl)phenyl) acrylonitrile (3c)

Yield 77%; m.p. 271-2 °C. IR; 2970, 2837, 2217, 1607, 1577, 1548, 1525, 1512, 1479, 1441, 1229, 1193, 1156, 1029, 747 cm^{-1}. ^{1}H-NMR (DMSOd_6); **δ** 3.09-3.12 (4H; m; piperazine), 3.53-3.56 (4H; m; piperazine), 7.04-7.08 (1H; td; Ar-H *J=7.6 and 0.8 Hz*), 7.14-7.24 (5H; m; Ar-H and benzimidazole H $_{5,6}$), 7.30 (1H; t; Ar-H), 7.42 (1H; d; Ar-H *J=7.2 Hz*), 7.49 (1H; d; benzimidazole H $_{4 \text{ or } 7}$ *J=7.6 Hz*), 7.62 (1H; d; benzimidazole H $_{4 \text{ or } 7}$ *J=7.6 Hz*), 7.91-7.93 (2H; d; Ar-H *J=8.8 Hz*), 8.15 (1H; s; CH), 12.81 (1H; s; NH) ppm. ESI-MS (m/z); 462.17 $[M+Na]^+$, 442.20 $[M+H+2]^+$, 440.19 $[M+H]^+$ (100%). Anal. Calcd. For $C_{26}H_{22}ClN_5$: C, 70.98; H, 5.04; N, 15.92. Found: C, 70.69; H, 5.17; N, 15.63.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(3-methoxyphenyl)piperazin-1-yl)phenyl) acrylonitrile (3d)

m.p. 254-5 °C (lit. [15] 255-6°C).

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(2-methoxyphenyl)piperazin-1-yl)phenyl) acrylonitrile (3e)

Yield 69%; m.p. 279-80 °C. IR; 2823, 2252, 1614, 1594, 1517, 1499, 1430, 1235, 1149, 1118, 1040, 740

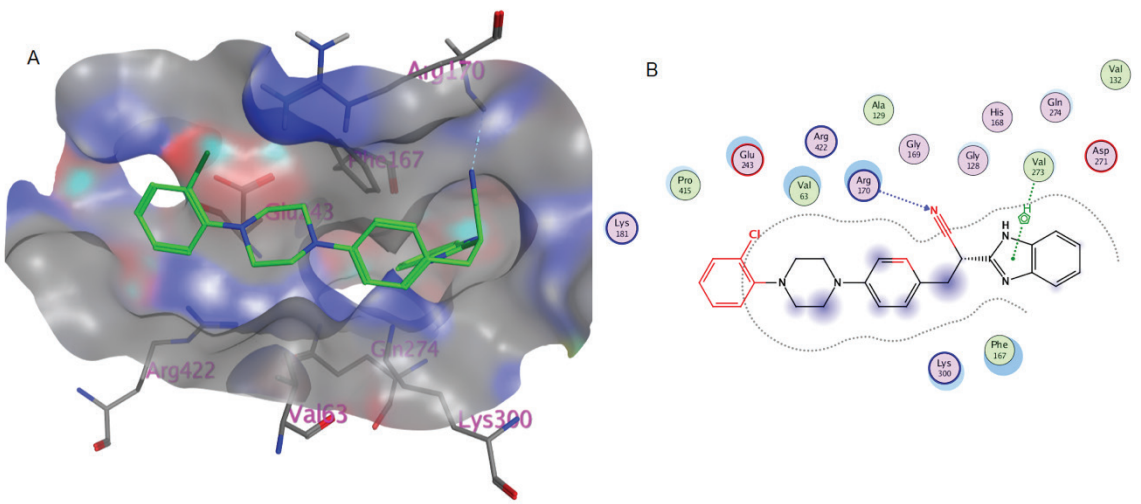

Figure 4. (A) Representation of the binding mode of 4c (green) in the *M. tuberculosis* LAT active site (B) 2D interaction between **4c** and residues of the *M. tuberculosis* LAT active site.

cm^{-1}. ^{1}H-NMR (DMSOd_6); **δ** 3.09-3.11 (4H; m; piperazine), 3.53-3.55 (4H; m; piperazine), 3.82 (3H; s; OCH_3), 6.88-7.02 (4H; m; Ar-H), 7.15 (2H; d; Ar-H *J=9.2 Hz*), 7.19-7.26 (2H; m; benzimidazole $H_{5,6}$), 7.52 (1H; d; benzimidazole $H_{4 \text{ or } 7}$ *J=7.2 Hz), 7.65 (*1H; d; benzimidazole $H_{4 \text{ or } 7}$ *J=7.2 Hz*), 7.93 (2H; d; Ar-H *J=8.8 Hz*), 8.17 (1H; s; CH), 12.86 (1H; brs; NH) ppm. ESI-MS (m/z); 458.24 $[M+Na]^+$ (100%), 436.26 $[M+H]^+$. Anal. Calcd. For $C_{27}H_{25}N_5O$: C, 74.46; H, 5.79; N, 16.08. Found: C, 74.20; H, 6.08; N, 15.96.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)phenyl) acrylonitrile (3f)

m.p. 280-1 °C (lit. [15] 280°C).

General procedure for the preparation of 2-(1*H*-benzo[d]imidazol-2-yl)-3-(4-(4-substtutedpiperazin-1-yl)phenyl) propanenitrile 4a-f

The solution of $NaBH_4$ (4 mmol) in absolute ethanol was added dropwise to the solution of appropriate acrylonitrile derivates 3a-f (2 mmol) in absolute ethanol at 0 °C. Then, the mixture was stirred at room temperature. After monitoring by thin-layer chromatography, the obtained solid was filtered and crystallized from ethanol.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(4-chlorophenyl)piperazin-1-yl)phenyl) propanenitrile (4a)

Yield 59 %; mp. 239-40 °C. IR; 2828, 2254, 1613, 1595, 1517, 1495, 1433, 1386, 1325, 1229, 1157, 1141, 743 cm^{-1}. ^{1}H -NMR (DMSOd_6); δ 3.23–3.38 (10H; m; piperazine, CH_2 and DMSO-H_2O), 4.77–4.80 (1H; q; CH; *J* = 6.4 Hz), 6.90 (2H; d; ArH *J* = 8.4), 6.97 (2H; d; ArH J = 8.8), 7.12-7.24 (6H; m; ArH and benzimidazole $H_{5,6}$), 7.55 (2H; br; benzimidazole $H_{4,7}$), 12.64 (1H; s; NH) ppm. ^{13}C NMR (DMSOd_6); δ 33.70 (propanenitrile C_2), 36.42 (propanenitrile C_3), 47.98, 48.03 (piperazine), 115.45, 117.00, 118.59, 122.53, 126.79, 128.56, 129.59, 148.14, 149.64, 149.79 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 464.14 $[M+Na]^+$ (100%), 444.18 $[M+H+2]^+$, 442.19 $[M+H]^+$. Anal. Calcd. For $C_{26}H_{24}N_5Cl$: C, 70.66; H, 5.47; N, 15.85. Found: C, 70.35; H, 5.57; N, 15.48.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(3-chlorophenyl)piperazin-1-yl)phenyl) propanenitrile (4b)

Yield 44 %; mp. 252-3 °C. IR; 2829, 2256, 1593, 1516, 1430, 1325, 1272, 1231, 1157, 804, 763, 681, 740 cm^{-1}. ^{1}H -NMR (DMSOd_6); δ 3.18–3.28 (10H; m; piperazine and CH_2), 4.78–4.82 (1H; q; CH; *J* = 6.4 Hz), 6.78 (1H; d; ArH *J* = 8 Hz), 6.89-6.98 (4H; m; ArH), 7.13-7.23 (5H; m; ArH and benzimidazole $H_{5,6}$), 7.50 (1H; d; benzimidazole $H_{4 \text{ or } 7}$ *J* = 7.2 Hz), 7.61 (1H; d; benzimidazole $H_{4 \text{ or } 7}$ *J* = 8.8 Hz), 12.69 (1H; s; NH) ppm. ^{13}C NMR (DMSOd_6); δ 33.80 (propanenitrile C_2), 36.48 (propanenitrile C_3), 47.69, 48.00 (piperazine), 111.50, 113.88, 114.77, 115.53, 118.31, 118.86, 121.62, 122.64, 126.87, 129.68, 130.43, 133.83, 134.40, 142.62, 148.22, 149.83, 152.13 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 464.18 $[M+Na]^+$ (100%), 444.18 $[M+H+2]^+$, 442.20 $[M+H]^+$. Anal. Calcd. For $C_{26}H_{24}N_5Cl$: C, 70.66; H, 5.47; N, 15.85. Found: C, 70.11; H, 5.68; N, 15.57.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(2-chlorophenyl)piperazin-1-yl)phenyl) propanenitrile (4c)

Yield 39 %; mp. 256-8 °C. IR; 3058, 2820, 2257, 1614, 1516, 1426, 1385, 1325, 1230, 1033, 740 cm^{-1}. ^{1}H -NMR (DMSOd_6); δ 3.07–3.09 (4H; m; piperazine), 3.24–3.32 (6H; m; piperazine and CH_2), 4.73 (1H; br; CH), 6.90 (2H; d; ArH *J* = 8.4), 7.04 (1H; td; ArH J = 6.8 and 1.2 Hz), 7.11–7.19 (5H; m; ArH and benzimidazole $H_{5,6}$), 7.30 (1H; td; ArH *J* = 8.8 and 2.4 Hz), 7.41 (1H; dd; ArH J = 8 and 1.6 Hz), 7.49–7.53 (2H; m; benzimidazole $H_{4,7}$) ppm. ^{13}C NMR (DMSOd_6); δ 34.14 (propanenitrile C_2), 36.64 (propanenitrile C_3), 48.31, 50.74 (piperazine), 115.13, 115.29, 119.04, 120.79, 121.24, 123.95, 126.98, 127.53, 128.02, 129.57, 130.25, 139.63, 148.74, 149.47, 149.83 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 464.18 $[M+Na]^+$, 444.14 $[M+H+2]^+$, 442.15 $[M+H]^+$ (100%). Anal. Calcd. For $C_{26}H_{24}N_5Cl$: C, 70.66; H, 5.47; N, 15.85. Found: C, 70.57; H, 5.39; N, 15.57.

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2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(3-methoxyphenyl)piperazin-1-yl)phenyl) propanenitrile (4d)

Yield 45 %; mp. 271-2 °C. IR; 3364, 2912, 2819, 2258, 1601, 1516, 1457, 1429 1323, 1269, 1208, 1165, 841, 762, 683 cm^{-1}. ^1H -NMR (DMSOd_6); δ 3.25–3.38 (10H; m; piperazine and CH_2), 3.70 (3H; s; OCH_3), 4.78–4.82 (1H; q; CH; *J* = 6.4 Hz), 6.36-6.39 (1H; dd; ArH *J* = 8.2 and 2 Hz), 6.48 (1H; t; ArH), 6.54-6.57 (1H; dd; ArH J = 8 and 2 Hz), 6.90 (2H; d; ArH *J* = 8.8 Hz), 7.19–7.21 (5H; m; ArH and benzimidazole H $_{5,6}$), 7.55–7.57 (2H; m; benzimidazole H $_{4,7}$) ppm. ^{13}C NMR (DMSOd_6); δ 33.79 (propanenitrile C_2), 36.47 (propanenitrile C_3), 48.16, 48.27 (piperazine), 54.87 (OCH_3), 101.72, 104.50, 108.26, 115.51, 118.69, 122.16, 126.81, 129.65, 129.67, 148.22, 149.91, 152.27 160.20 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 460.26 $[M+Na]^+$ (100%), 438.27 $[M+H]^+$. Anal. Calcd. For $C_{27}H_{27}N_5O$: C, 74.12; H, 6.22; N, 16.01. Found: C, 74.20; H, 6.57; N, 15.79.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(2-methoxyphenyl)piperazin-1-yl)phenyl) propanenitrile (4e)

Yield 42 %; mp. 268 °C. IR; 2851, 2250, 1618, 1512, 1434, 1349, 1226, 1163, 1041, 833 cm^{-1}. ^1H -NMR (DMSOd_6); δ 3.05–3.38 (10H; m; piperazine, CH_2 and DMSO H_2O), 3.77 (3H; s; OCH_3) 4.78–4.82 (1H; q; CH *J* = 6.4 Hz), 6.85-6.98 (6H; m; ArH), 7.12-7.23 (4H; m; ArH and benzimidazole H $_{5,6}$), 7.50 (1H; d; benzimidazole H $_{4 \text{ or } 7}$ *J* = 7.2 Hz), 7.61 (1H; d; benzimidazole H $_{4 \text{ or } 7}$ *J* = 8.8 Hz), 12.69 (1H; s; NH) ppm. ^{13}C NMR (DMSOd_6); δ 33.73 (propanenitrile C_2), 36.41 (propanenitrile C_3), 48.30, 49.98 (piperazine), 55.26 (OCH_3), 111.41, 111.83, 115.20, 117.90, 118.64, 118.77, 120.76, 121.52, 122.52, 126.49, 129.56, 134.32, 140.91, 142.55, 148.16, 150.00, 151.90 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 460.25 $[M+Na]^+$ (100%), 438.23 $[M+H]^+$. Anal. Calcd. For $C_{27}H_{27}N_5O$: C, 74.12; H, 6.22; N, 16.01. Found: C, 73.90; H, 6.58; N, 15.89.

2-(1*H*-Benzo[*d*]imidazol-2-yl)-3-(4-(4-(4-fluorophenyl)piperazin-1-yl)phenyl) propanenitrile (4f)

Yield 54 %; mp. 259-60 °C. IR; 2826, 2254, 1611, 1508, 1432, 1324, 1272, 1228, 1155, 814 cm^{-1}. ^1H -NMR (DMSOd_6); δ 3.16–3.38 (10H; m; piperazine, CH_2 and DMSO-H_2O), 4.78–4.82 (1H; q; CH; *J* = 6.4 Hz), 6.91 (2H; d; ArH *J* = 9.2), 6.96-7.19 (8H; m; ArH and benzimidazole H $_{5,6}$), 7.55 (2H; br; benzimidazole H $_{4,7}$), 12.68 (1H; s; NH) ppm. ^{13}C NMR (DMSOd_6); δ 33.79 (propanenitrile C_2), 36.47 (propanenitrile C_3), 48.18, 49.10 (piperazine), 111.49, 115.17, 115.39, 115.49, 117.39, 117.46, 118.10, 118.69, 118.77, 121.61, 122.63, 126.81, 129.66, 147.79, 147.81, 148.22, 149.89, 154.98, 157.33 (aromatic carbons and propanenitrile C_1) ppm. ESI-MS (*m*/*z*); 448.21 $[M+Na]^+$ (100%), 426.22 $[M+H]^+$. Anal. Calcd. For $C_{26}H_{24}N_5F$: C, 73.39; H, 5.69; N, 16.46. Found: C, 73.66; H, 5.92; N, 16.42.

In-vitro M. tuberculosis MABA assay

The minimum inhibitory concentrations (MIC) of the tested compounds were determined against *M. tuberculosis* H37Rv strain using the reported protocol [22]. The inoculum was prepared from fresh LJ medium. Stock solutions of test compounds were made as 10 mM DMSO solution and further diluted in Middlebrook 7H9 broth at four-fold the final highest concentration tested was 100 µM. Serial dilution was performed for each compound in sterile 96-well microtiter plates using 100 µl of Middlebrook 7H9 broth. Assay was performed in duplicate for each sample and the readings were compared to standard TB drugs (isoniazid, ethambutol and rifampicin). Sterile water was added to all perimeter wells to maintain the humidity during the incubation period of 7 days at 37 °C. After the incubation period, Alamar blue solution (30 µL) was added to each well, and the plate was re-incubated overnight. Bacterial growth was observed by color change from blue (oxidized state) to pink (reduced). The MIC was described as the lowest concentration of compound that didn't change the color.

Nutrient starvation model

A culture of *M. tuberculosis* H37Rv (O.D. of 0.8–1.0) grown in Middlebrook 7H9 medium supplemented with OADC was pelleted and washed twice with PBS. The pellet was resuspended in PBS in sealed bottles and incubated at 37 °C for 6 weeks. Aliquots of these cultures were then treated with standard drugs (isoniazid, ciprofloxacin, rifampicin and moxifloxacin) and 4c for 7 days at a concentration of

10 µg/ml. The frequency of persistors was enumerated by MPN (most probable number) assay [23,24].

Molecular modeling studies

The *in silico* physicochemical parameters and docking studies were performed using MOE Version 2018.0101 software, available from Chemical Computing Group Inc., 1010 Sherbrooke St. West, Suite 910, Montreal, Canada H3A 2R7, http://www.chemcomp.com.

The crystal structure of *M. tuberculosis* LAT (PDB: 2CJH) was used as target. All the non bonded residues and water molecules were removed from the enzymes. The errors in the enzymes were corrected by the "Structure Preparation" application. The ligands were constructed using the MOE builder tool and energy was minimized using the Merck Molecular Force Field (MMFF94x, gradient: 0.05 kcal mol^{-1} $Å^{-1}$). Docking studies were performed using the Triangle Matcher method. The results were ranked with the London dG scoring function and rescored with the GBVI/WSA dG scoring function. The pose with the lowest S score was selected for the enzyme.

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Conflict Of Interest

The authors declare that there is no conflict of interest.

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