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Authors: Harun Patel, Rajshekhar Karpoormath, and Mahesh Palkar

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Exploring MDR-TB inhibitory potential of 4-amino quinazolines as *Mycobacterium tuberculosis* *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU ^{MTB}) inhibitors

Harun M. Patel [a,b], Mahesh Palkar [a], Rajshekhar Karpoormath [a]*

[a] *Department of Pharmaceutical Chemistry, University of KwaZulu-Natal (Westville Campus), Private Bag X54001, Durban- 4000, South Africa.*

[b] *Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra-425405, India*

Corresponding Author*

Tel: +91-8806621544, Fax: + 91-2563251808; Email: hpatel_38@yahoo.com

Tel: + 27-312607179; Fax: + 27-312607792; Email: karpoormath@ukzn.ac.za

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Abstract

Drug resistance tuberculosis is one of the challenging tasks that dictates the desperate need for the development of new anti-tubercular agents which operate *via* novel modes of action. Here, we are reporting the 4-amino quinazolines as *M. tuberculosis N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU MTB) inhibitors to overcome the problem of the MDR-TB. Amongst the synthesized compounds **HMP-05** and **HMP-15** was observed to be the effective compound of the series [IC_{50} = 6.4 µM (H37Rv), MIC = 25 µM (MDR-TB) and IC_{50} = 2.9 µM (H37Rv), MIC = 6.25 µM (MDR-TB) respectively].

Keywords: 4-amino quinazoline; *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU MTB); MDR-TB; Cytotoxicity

1. Introduction

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (MTB) which commonly affects the lungs and respiratory system. [1-5] The TB morbidity was reported decreasing, but it remains alarming due to an increase in incidence and prevalence of Multi-Drug Resistance-TB (MDR-TB). [6-10] Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS) as per the WHO 2018 reports. [1,11] Approximately 10.0 million people were infected with TB in 2018, which includes 57% male, 32% female, 11% children, and 8.6% HIV positive. [1] MDR phenotype is defined as resistance at least to isoniazid and rifampicin which are the most effective drugs recommended by the World Health Organization (WHO) and being used as the first-line treatment in TB patients. [12-13] Therefore, resistance towards these two drugs has become major problems in the treatment of TB patients. [14]

The development of compounds that target enzymes responsible for the biosynthesis and assembly of the mycobacterial cell wall is desperately needed for the development of effective anti-tubercular drugs. Examples include currently employed first and second-line TB drugs such as isoniazid, ethambutol and ethionamide. [15,16] These agents act by disrupting the biosynthesis or the incorporation of mycolic acid, a key lipid which constitutes a major component of the mycobacterial cell wall (**Figure 1**).

Another important component of the mycobacterial cell wall is the peptidoglycan layer which gives the bacterium structural strength and rigidity to withstand internal osmotic pressure.[16] Peptidoglycan biosynthesis interference has been an extremely successful way for the development of antibacterial agents against gram-positive and gram-negative bacteria. The enzymes responsible for the development of the peptidoglycan biosynthesis were not

thoroughly investigated as drug targets. [16] This is due to the broad-spectrum β-lactamase activity of MTB, which inactivates β-lactam antibiotics. Conversely, other enzymes that are responsible for the peptidoglycan biosynthesis remained vital targets for anti-tubercular drug development. One such novel target is *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU). [16-18] This bi-functional enzyme, which is found exclusively in bacteria, catalyzes the development of Uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc), an essential intermediate in peptidoglycan biosynthesis in MTB. [16-18] The crystal structure of the GlmU MTB has been recently resolved which provides further insight into the two active sites of the enzyme (N-terminal and C-terminal). *N*-terminal posses the uridyltransferase and C-terminal have acetyltransferase activities. The acetyltransferase domain, found in the C-terminus of the protein is responsible for the first step of the reaction, in which an acetyl group is transferred from Acetyl Co-A (AcCoA) to Glucosamine-1-phosphate (GlcN-1-P) forming N-acetyl glucosamine-1-phosphate (GlcNAc-1-P). [16] GlcNAc-1-P then serves as a substrate for the uridyltransferase active site in the *N*-terminus of the enzyme which forms Uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) (**Figure 1**). Computational kinetic modeling studies on GlmU MTB have shown uridyltransferase reaction to be the rate-limiting step and offer more flexibility to target for inhibition of GlmU MTB. [19]

Based on the above reviews in the current study, we are reporting the 4-aminoquinazolines as *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU) MTB inhibitors to overcome the problem of MDR-TB.

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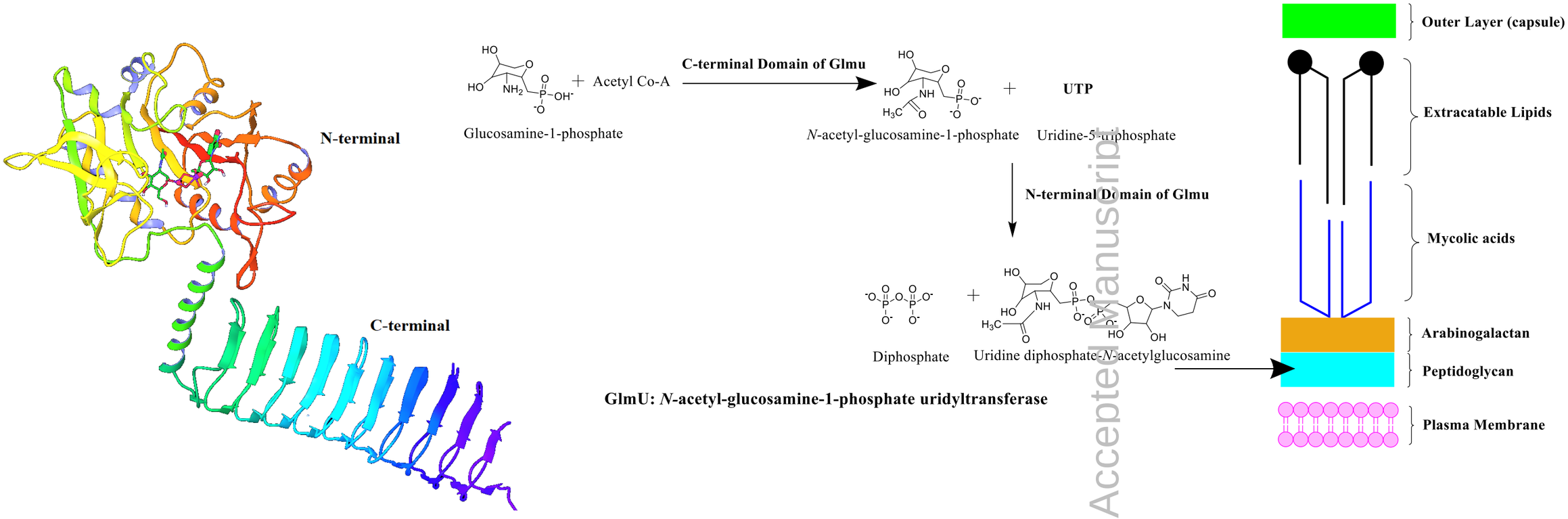

Figure 1. Role of *N*-acetyl-glucosamine uridyltransferase (GlmU) in the synthesis of peptidoglycan of *M. tuberculosis (Modified from Organic and Bio-molecular Chemistry with permission from Royal Society of Chemistry)*

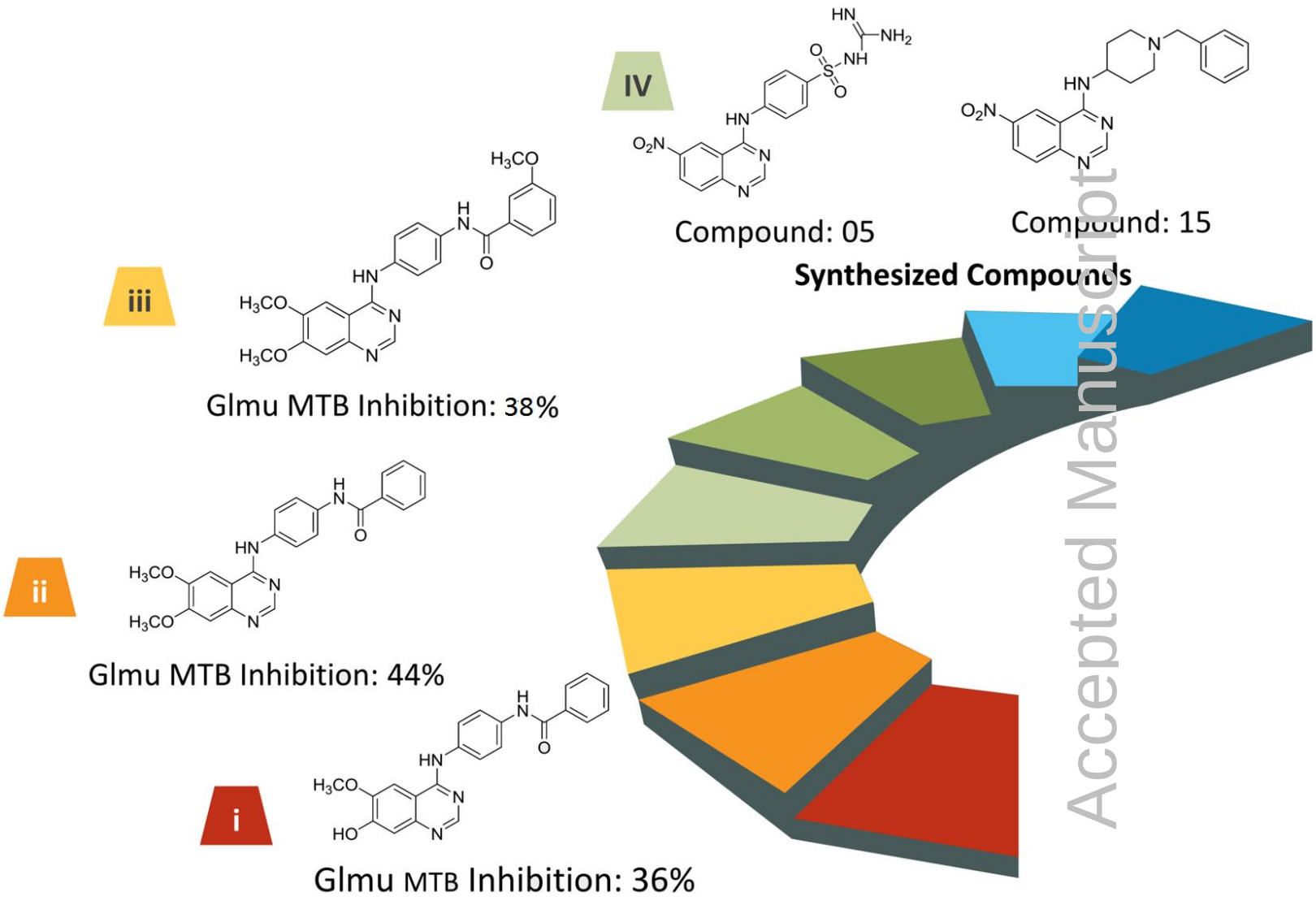

Figure 2. Reported and synthesized *N*-acetyl-glucosamine uridyltransferase (GlmU) MTB inhibitors

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2. Result and Discussion

2.1 Designing of the Compounds (Rationale)

Recently, Doig and co-workers from AstraZeneca reported the promising 4-aminoquinazoline GlmU MTB inhibitors against *M. tuberculosis* GlmU uridyltransferase (**Fig.2**). [20]

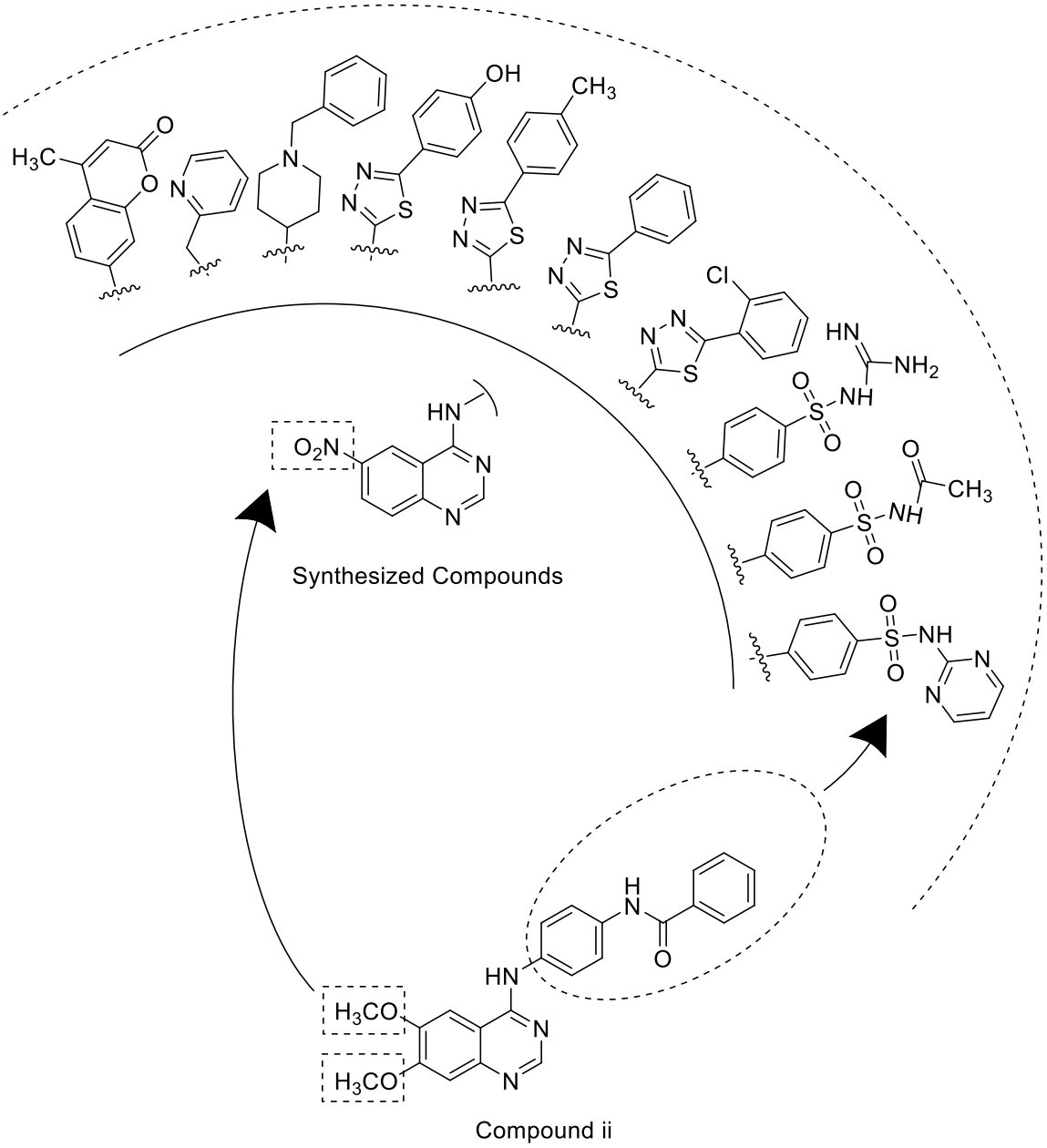


Figure 3. Proposed structural modification of Compound ii

Specifically, compound ii exhibited 44% inhibition of the uridyltransferase activity at 50 μM while compound i was slightly less potent with 36% inhibition at the same concentration. Further structural modification of the compound i and ii were made by the Tran et al that leads to the compound iii with 38% inhibition of the uridyltransferase. [16] Encouraged by the activity

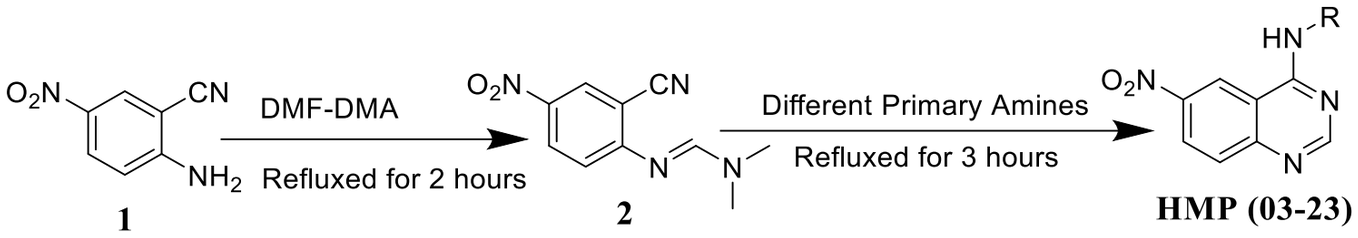
of the compound i, ii, iii and to explore further structural activity relationship (**Figure 2** and **3**), we replaced the hydrophobic group containing hydrogen bond acceptor and donor of **compound ii** at 4th position with different heterocyclic/homocyclic hydrophobic group with hydrogen bond acceptor and donor functionality. The substituted heterocyclic/homocyclic hydrophobic group at the 4th position of quinazoline ring includes; sulphonamides [21], piperidine [22], piperazine [23], thiazole [24], thiadiazole [25] and various aliphatic and aromatic amines. [26,27] These groups were introduced to evaluate the effect of incorporating hydrogen bond donors and acceptors as well as conformational flexibility at the 4th position of quinazoline on GlmU uridyltransferase inhibitory activity. We also substituted compounds with a nitro group at the 6th position of quinazolines since it has significant anti-mycobacterial potential as shown in **Figure 3**.[28]

2.2. Chemistry

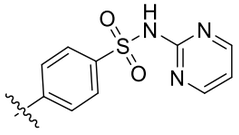
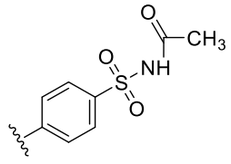
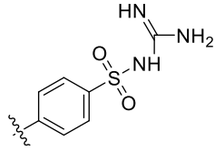
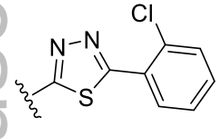
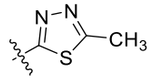
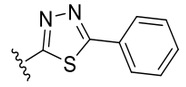
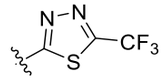
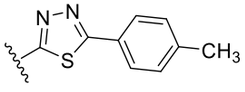
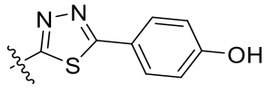
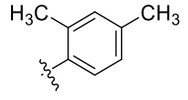
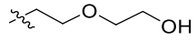
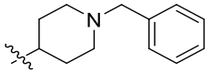
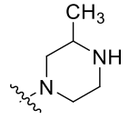
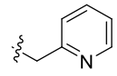
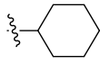
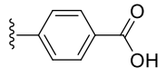
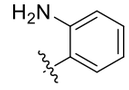
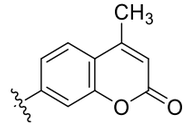
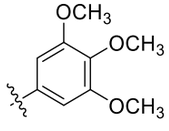
The synthesis of final compounds **HMP** (**3-23**) was carried out according to **Scheme 1**, where *N'*-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformamide (**2**) was synthesized by reacting 2-amino-5-nitrobenzonitrile (**1)** with dimethylformamide dimethyl acetal to give **2** (**Scheme 1**). Cyclization of the intermediate **2** was performed by different primary amino groups containing aromatic, aliphatic and heterocyclic moieties to afford the desired final compounds **HMP** (**3-23**). All the newly synthesized compounds showed acceptable analysis of their anticipated structures, which are outlined in the experimental section.

In general, the IR spectrum of final synthesized compounds revealed typical absorption bands around 1350 cm^{-1} and 1550 cm $^{-1}$ for NO_2 group, and NH stretching around 3200 cm^{-1}. This was further substantiated from the ^{1}H-NMR spectrum, which showed the presence of a singlet signal around 10 to 11 ppm for the N–H proton, confirming the cyclization of final compounds. Further ^{13}C NMR spectra and Mass data are in full agreement with their delineated structures. LogP value which denotes the lipophilicity of a drug, plays a vital role in the infiltration of a compound into bacterial cells. LogP values (the log of the octanol/water partition coefficient) were calculated for all the synthesized compounds using ChemBioDraw Ultra 14, which decides the hydrophobicity of the substituents as given in **Table 1**.

Scheme 1



Where R =

Compound Code	R	Compound Code	R	Compound Code	R	Compound Code	R
HMP-03		HMP-04		HMP-05		HMP-06	
HMP-07		HMP-08		HMP-09		HMP-10	
HMP-11		HMP-12		HMP-13		HMP-14	
HMP-15		HMP-16		HMP-17		HMP-18	
HMP-19		HMP-20		HMP-21		HMP-22	
HMP-23							

2.3. Bio-evaluation and in-silico study

In vitro assessment of the anti-mycobacterial activity of the newly synthesized compounds, HMP (**3-23**) was completed at the National Institute of Allergy and Infectious Diseases (NIAID) (Bethesda, MD, USA). The MIC was established against H37Rv MTB strain grown under aerobic conditions, utilizing a dual readout (OD 590 and fluorescence) assay. [29,30] The MDR-TB inhibitory activity was evaluated against the phenotypic MDR-TB strain at the Micro Care Laboratory and Tuberculosis Research Center Surat, India.

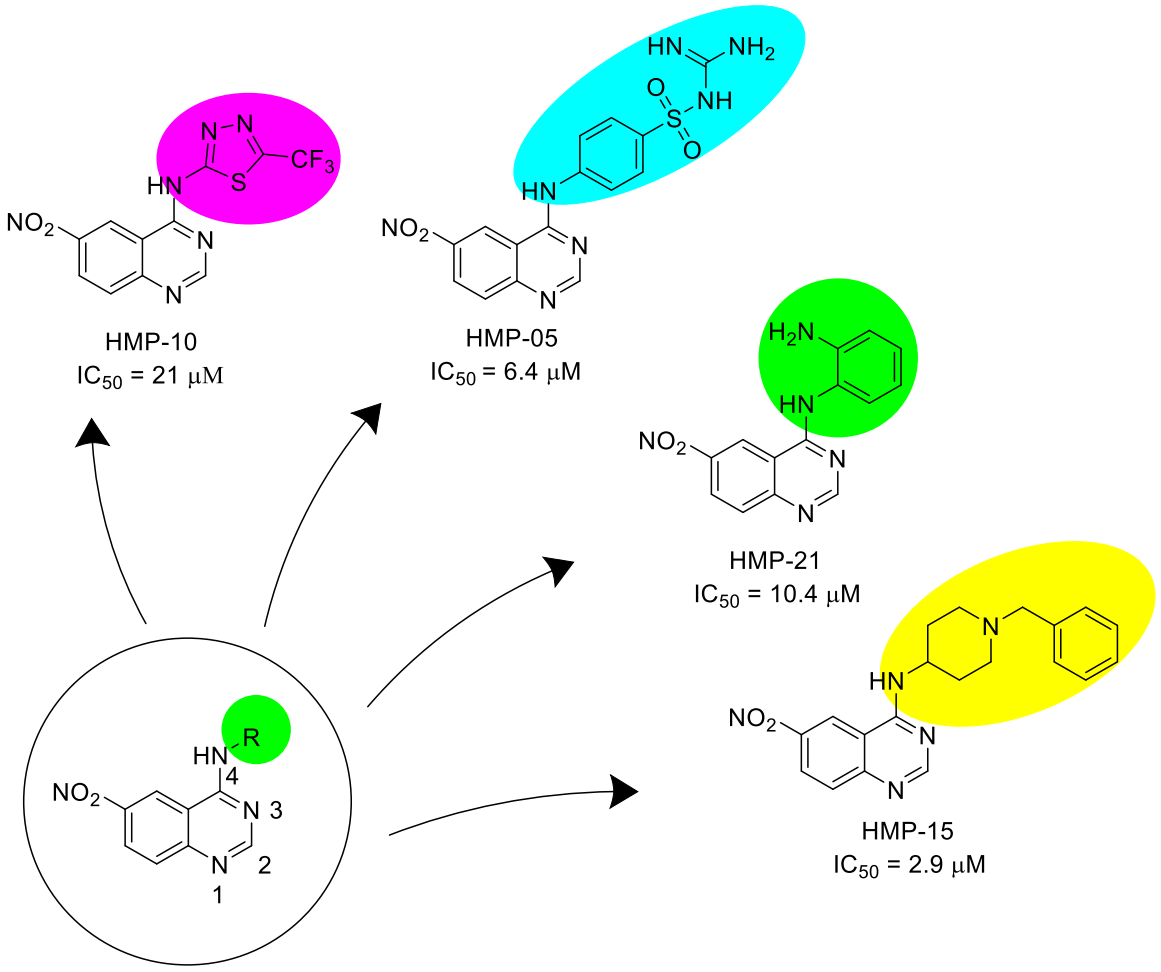


Figure 4. Structure-activity correlationship of quinazolines

All the targeted synthesized compounds demonstrated an interesting anti-mycobacterial activity profile, with IC_{50} values from 2.9 to 200 µM against the mycobacterial H37Rv strain and MIC 6.25 µM to 200 µM against the MDR-TB strain. We additionally considered the impacts of various

aromatic/ heteroaromatic substituents at the 4th position of the quinazoline ring. The structure anti-mycobacterial relationship at 5th position of 1,3,4- thiadiazole substituted at the 4th position of quinazoline uncovers that direct attachment of electron-withdrawing group to the 5th position on 1,3,4-thiadiazole is more active (**HMP-10**) (H37Rv IC_{50} = 21 µM and MDR-TB MIC = 50 µM) than any other substitution (**HMP-06**, **HMP-08**, **HMP-09**, **HMP-11**, **HMP-12**). Among the sulphonamides; sulphaguanidine substituted quinazoline derivatives **HMP-05** (H37Rv IC_{50} = 6.4 µM and MDR-TB MIC = 25 µM) was found to be the active, followed by sulphacetamide **HMP-04** (H37Rv IC_{50} = 28 µM and MDR-TB MIC = 50 µM) **(Figure 4**). *N*-substituted piperidine containing quinazoline derivative **HMP-15** was standout amongst the most effective compound of the series (H37Rv IC_{50} = 2.9 µM and MDR-TB MIC = 6.25 µM). Likewise, piperazine substitution at the 4th position of the quinazoline (**HMP-16**) indicated moderate anti-mycobacterial activity (H37Rv IC_{50} = 194 µM). Similar to **HMP-15**; **HMP-21** (H37Rv IC_{50} = 10.4 µM and MDR-TB MIC = 25 µM) showed significant anti-mycobacterial activity. Further we have correlated the LogP value with the IC_{50} value (H37Rv *Mycobacterium* strain) and we observed that the logP value higher than 3.3 seemed to be not favorable for antimycobacterial activity as shown in **Figure 5**. The synthesized potent compounds of the series were further screened for cytotoxicity (IC_{50}) in a mammalian Vero cell line (**Table 1**). All the tested derivatives showed lower toxicity with IC_{50} values >238 µM and none of the synthesized compounds displayed significant activity against the mammalian Vero cell line at concentrations <100 µM.

The crystal structure of GlmU MTB (PDB: 3ST8) was processed for the addition of hydrogens, bond order correction etc. [31] Water molecules interacting with UDP-GlcNAc and those present around 5 Å of these substrates were retained to maintain the thermodynamic stability. In the case of UDP-GlcNAc, the diphosphate tail was found to be stabilized by polar contacts with Asn 239, Arg 19 and one coordinate covalent bonds with Mg 496. Uridyl moiety of UDP-GlcNAc is showing hydrogen

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bonding interaction with Gly 15, Ala 14, Gln 83, and Gly 88. Glucosamine of UDP-GlcNAc is showing polar interaction with Thr 89, Glu166, Asn181, Gly151 and with water molecules as shown in (**Figure 6a**). Potent **compound 5** of the series with sulphaguanidine moiety at 4th position of quinazoline forms a polar hydrogen bond with Glu 166 and Gln205 *via* guanidine moiety. Lys 26 forms pi-pi stacking with phenyl ring of sulphaguanidine and hydrogen bonding with a nitro functional group at 6th position of quinazoline ring (**Figure 6b**). Similarly, nitro functional group of **compound 15** shows hydrogen bond interaction with Gln 83 and Gly88 (**Figure 6c**).

These outcomes are vital, as compounds with increased cytoliability are appealing in the development of new chemical entities for the treatment of tuberculosis. This is primarily because the management of tuberculosis requires an extensive course of drug treatment, leading to a number of side-effects with a high margin of safety.

Table 1. Physical and Anti-mycobacterial data of the synthesized compounds against the H37Rv and MDR-TB strains

Compound Code	Melting Point	Molecular Formula	Molecular Weight	Anti-mycobacterial Activity against H37Rv (μM) Under Aerobic Condition			Log P	Cytotoxicity IC_{50} (μM) [d]	MDR-TB MIC (μM) [e]
				IC_{50} [a]	IC_{90} [b]	MIC [c]			
HMP-03	291	$C_{18}H_{13}N_7O_4S$	423.4053	26	50	200	2.15	---	---
HMP-04	311	$C_{18}H_{13}N_7O_4S$	423.4053	28	43	45	1.61	238	50
HMP-05	297	$C_{18}H_{13}N_7O_4S$	423.4053	6.4	8.3	12	1.12	246	25
HMP-06	>300	$C_{16}H_9ClN_6O_2S$	384.7997	>200	>200	>200	4.19	---	---
HMP-07	271	$C_{11}H_7N_5O_2S$	273.2706	16	18	25	2.23	242	50
HMP-08	>300	$C_{11}H_8N_6O_2S$	288.2852	>200	>200	>200	1.08	---	---
HMP-09	>300	$C_{16}H_{10}N_6O_2S$	350.3546	>200	>200	>200	4.01	---	---
HMP-10	200	$C_{11}H_5F_3N_6O_2S$	342.2566	21	38	40	2.60	278	50
HMP-11	>300	$C_{17}H_{12}N_6O_2S$	364.3812	>200	>200	>200	4.47	---	---
HMP-12	>300	$C_{16}H_{10}N_6O_3S$	366.3540	>200	>200	>200	3.65	---	---
HMP-13	180	$C_{16}H_{14}N_4O_2$	294.3080	52	110	119	3.79	241	200
HMP-14	190	$C_{12}H_{14}N_4O_4$	278.2640	8.7	14.2	20	0.37	289	25
HMP-15	232	$C_{20}H_{21}N_5O_2$	363.4130	2.9	4.4	5.4	3.30	287	6.25
HMP-16	256	$C_{13}H_{16}N_6O_2$	288.3051	194	>200	>200	0.84	---	---
HMP-17	195	$C_{11}H_{10}N_4O_2$	230.2227	121	198	200	1.86	---	---
HMP-18	212	$C_{14}H_{11}N_5O_2$	281.2694	23	141	181	1.53	---	---
HMP-19	170	$C_{14}H_{16}N_4O_2$	272.3024	141	>200	>200	3.55	---	---
HMP-20	338	$C_{15}H_{10}N_4O_4$	310.2643	42	108	178	2.98	---	---
HMP-21	194	$C_{14}H_{11}N_5O_2$	281.2694	10.4	18	23	1.91	287	25
HMP-22	>300	$C_{18}H_{12}N_4O$	348.3123	>200	>200	>200	3.42	---	---
HMP-23	250	$C_{17}H_{16}N_4O_5$	356.3327	21	98	200	2.81	---	---
Isoniazid	--	--	--	---	---	---	---	---	> 200
Rifampicin	--	--	--	0.0063	0.0034	0.0059	---	---	---

LogP value is calculated using the ChemBioDraw Ultra 14 [32]

[a] IC_{50}= concentration at which growth is inhibited by 50%; [b] IC_{90} value = concentration at which growth is inhibited by 90%; [c] MIC = minimum inhibitory concentration at which *M. tuberculosis* H37Rv growth was completely inhibited; [d] Cytotoxicity activity was determined on mammalian Vero cell line; --- = not determined; [e] Resistant phenotypic MDR-TB strain

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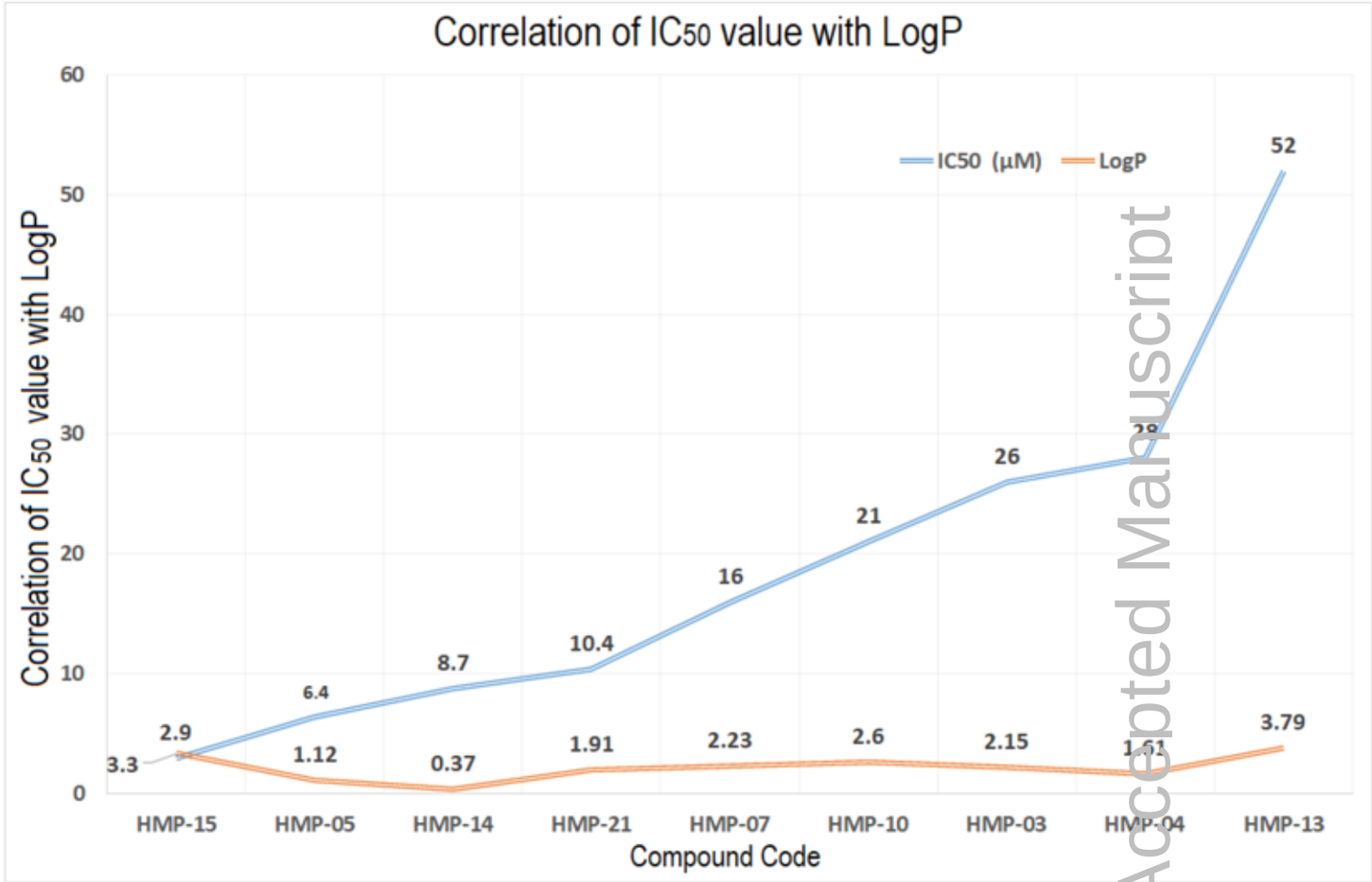

Figure 5. Correlation of LogP value and IC_{50} value of the potent compounds against the H37Rv *Mycobacterium* strain

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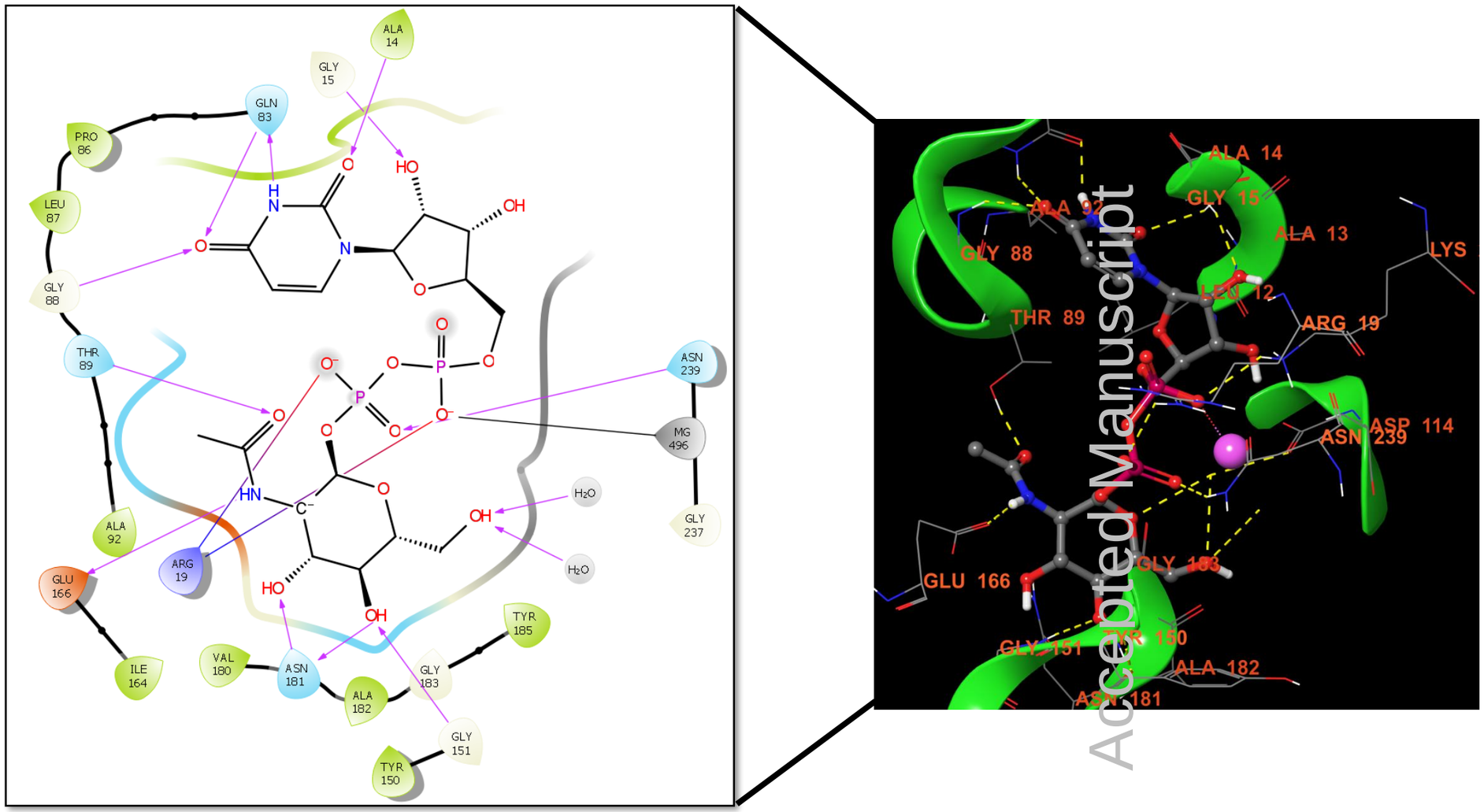

Figure 6a. Binding of *N*-acetyl-glucosamine UDP in *N*-acetyl-glucosamine uridyltransferase

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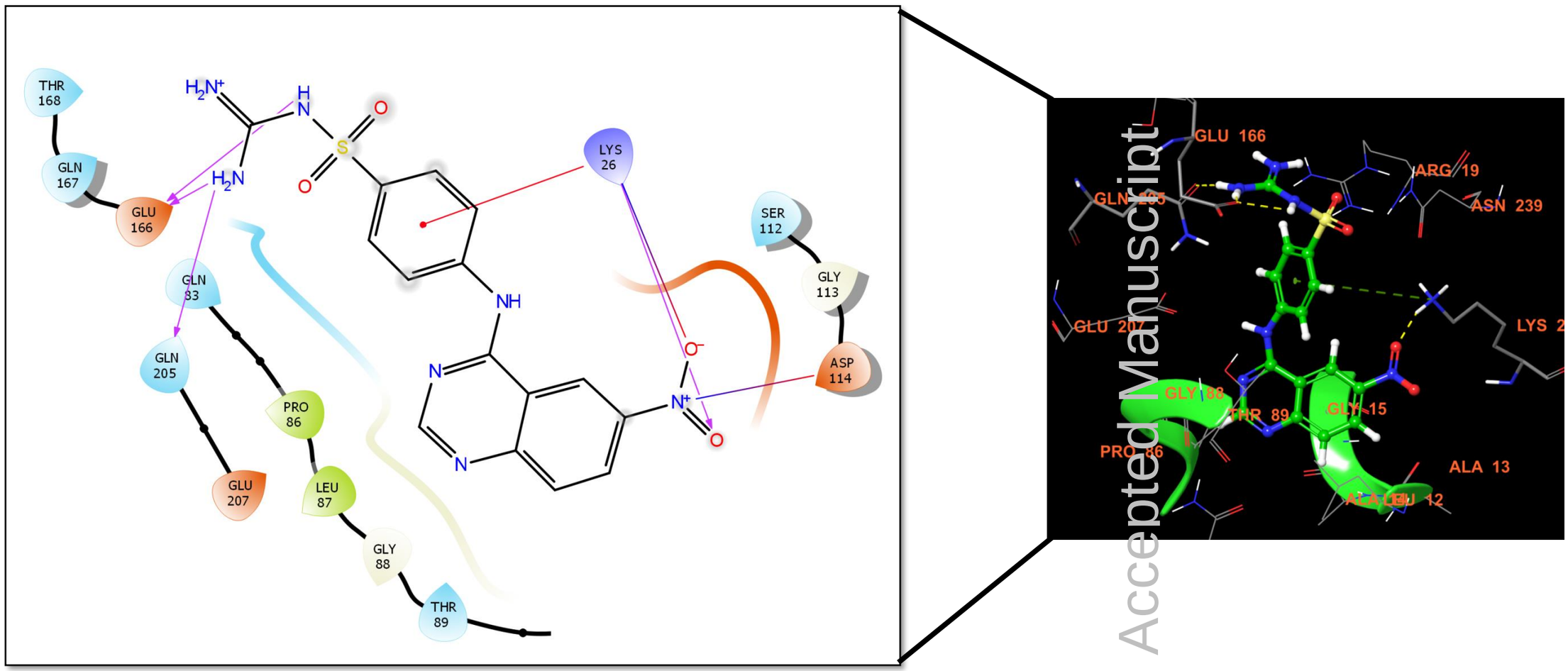

Figure 6b. Interaction of **Compound 5** in *N*-acetyl-glucosamine uridyltransferase

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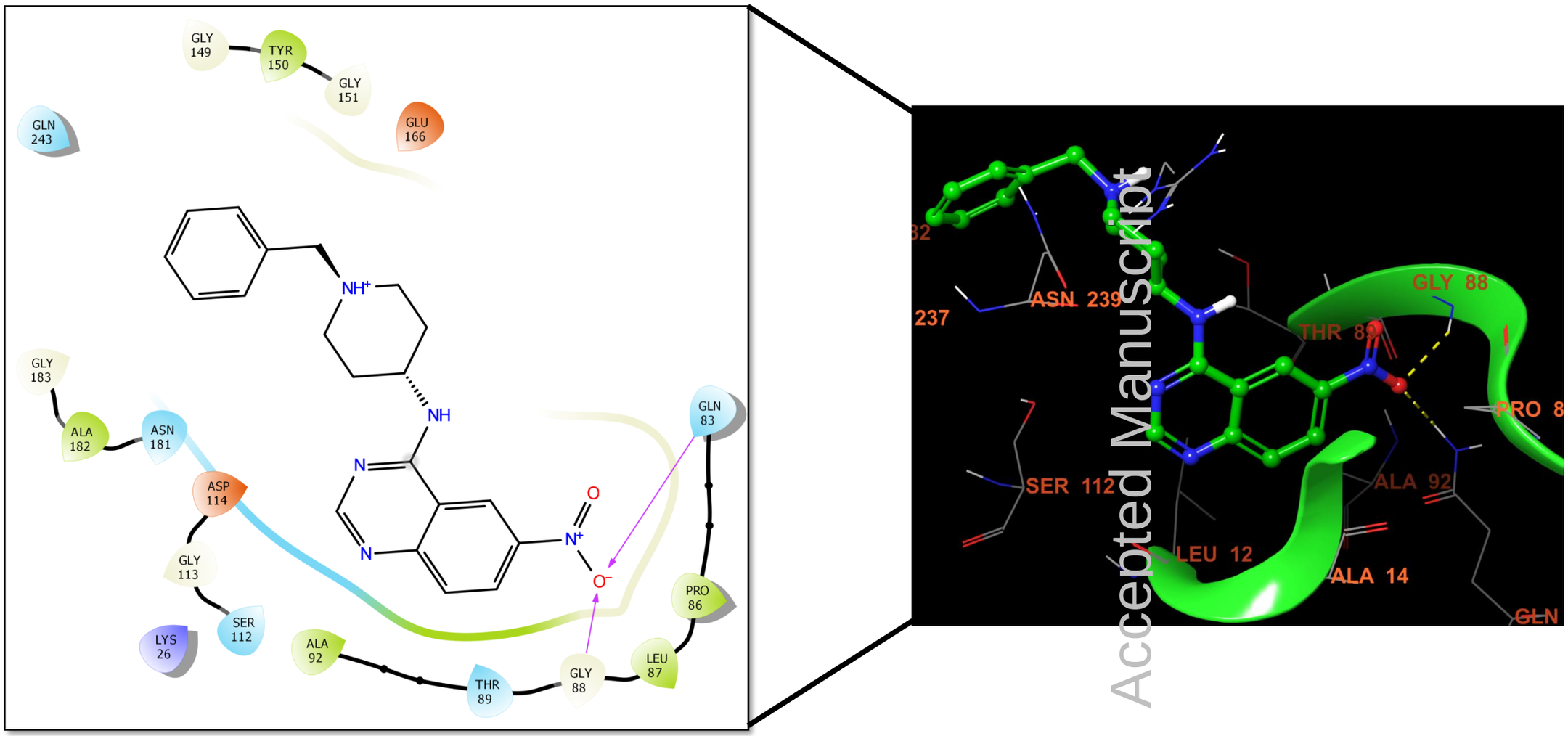

Figure 6c. Interaction of **Compound 15** in *N*-acetyl-glucosamine uridyltransferase

3. Conclusion

To overcome the problem of drug resistance tuberculosis there is a desperate need of new anti-tubercular agents that operate *via* novel modes of action. The rigidity to the MTB cell wall is attributed by the peptidoglycan layer. UDP-GlcNAc serves as the starting material in the biosynthesis of this peptidoglycan layers. This UDP-GlcNAc is synthesized by *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU MTB). Conclusively in the current study, we have reported the synthesis and *in vitro* anti-mycobacterial activity of twenty two, 4 -substituted quinazoline analogues (**HMP 3-23)** as *M.tuberculosis N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU MTB) inhibitors. Among the tested compounds, *N*-substituted piperidine containing quinazoline derivative, **HMP-15** was observed to be the most effective compound of the series against the H37Rv and MDR-TB strain [IC_{50} = 2.9 µM against H37Rv and MIC = 6.25 µM against the MDR-TB]. Similar to the compounds **HMP-15,** compound **HMP-05** was observed to be the other effective compound of the series [IC_{50} = 6.4 µM (H37Rv), MIC = 25 µM (MDR-TB)]. The structure activity-relationship study uncovered that the *N*-substituted piperidine and sulphaguanidine ring at the 4^{th} position of quinazoline ring is decisive for the activity. The synthesized compounds, which demonstrated significant anti-mycobacterial action, additionally evaluated for their cytotoxicity (IC_{50}) against the mammalian Vero cell line, utilizing the MTT assay. The outcomes demonstrated that these compounds showcase anti-mycobacterial activity at non-cytotoxic level. The docking study has been carried out to get the further insight of GlmU MTB inhibition. Potent **compound 5** of the series with sulphaguanidine moiety at 4^{th} position of quinazoline forms polar hydrogen bond with Glu 166 and Gln 205 *via* guanidine moiety. Lys 26 forms pi-pi stacking with phenyl ring of sulphaguanidine and hydrogen bonding with nitro functional group at 6^{th} position of quinazoline ring.

4. Experimental

All the solvents and chemicals have been provided by, Spectrochem and Sigma-Aldrich. The reactions have been supervised with the aid of pre-coated silica gel TLC aluminium sheets. Melting points were established using an Analab Scientific Melting point apparatus. FTIR spectrum was documented utilizing FTIR-8400S Shimadzu spectrometer. ^{1}HNMR (DMSO/$CDCl_3$) spectra of the compounds have been determined by Bruker Avance-II spectrometer at 400 MHz (Punjab University-Chandigarh). Chemical shift have been assessed relative to the internal standard TMS and are reported in δ ppm. Mass spectra of the compounds were determined at Oxygen Heath Care Pvt.Ltd.at Ahmadabad, Gujarat.

4.1 N'-(2-cyano-4-nitrophenyl)-N,N-dimethylformimidamide (2)

It is prepared as per the procedure reported by Samar Mowafy et al. [33]

4.2 6-nitro-N-substituted quinazolin-4-amine HMP (3-23)

A mixture of *N*'-(2-cyano-4-nitrophenyl)-*N*,*N*-dimethylformamide (**2**) (13.7 mmol) and appropriate aniline (15.1 mmol) in glacial acetic acid (30 mL) was stirred and refluxed for 3 h, reaction was monitored by TLC. After completing the reaction, mixture was filtered to get the final compound, which was further recrystallized from ethanol.

4.2.1 4-(6-nitroquinazolin-4-ylamino)-N-(pyrimidin-2-yl)benzenesulfonamide (HMP-03)

Solid, 81% yield; mp 291-293 °C; IR (KBr, vmax, cm^{-1}): 3212 (NH), 2945 (CH), 1345 and 1543 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 11.29 (s, 1H, SO_2NH), 8.82 (s, 1H, NH), 6.38-8.71 (m, 11H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 171.12, 168.34, 159.78, 156.34, 151.45, 145.78, 143.34, 130.78, 129.23, 127.12, 126.34, 120.23, 115.46, 114.89, 114.23, 112.68; EI-MS: m/z 423.1 [M+] and 425.1 [M+2]

4.2.2. N-(4-(6-nitroquinazolin-4-ylamino)phenylsulfonyl)acetamide (HMP- 04)

Solid, 62% yield; mp > 300 °C; IR (KBr, vmax, cm^{-1}): 3211 (NH), 3012 (CH), 1664.43 (C=O), 1347.90 and 1548.54 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 11.13 (s, 1H, SO_2NH), 8.56 (s, 1H,

NH), 7.73-8.78 (m, 8H, Ar-H) 2.90 (s, 3H,CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 171.78, 170.23, 159.45, 151.56, 145.67, 143.67, 130.67, 129.78, 128.34, 126.45, 115.34, 114.56, 112.67, 21.34; EI-MS: m/z 387.1 [M+] and 389.1 [M+2]

4.2.3 N-carbamimidoyl-4-(6-nitroquinazolin-4-ylamino)benzenesulfonamide (HMP-05)

Solid, 58% yield; mp 296-298 °C; IR (KBr, vmax, cm^{-1}): 3249 and 3235 (NH_2), 3072 (CH), 1338 and 1556 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 10.79 (s, 1H, C=NH), 10.75 (s, 1H, NH), 7.79-8.77 (m, 8H, Ar-H), 6.73 (s, 2H, NH) 3.31 (s, 1H, NH) ; ^{13}C NMR (DMSO-d6) δ ppm: 172.12, 158.61, 158.08, 157.39, 152.97, 144.60, 141, 139.79, 129.54, 126.73, 126.19, 122.02, 120.83; EI-MS: m/z 387.0 [M+] and 389.0 [M+2]

4.2.4 5-(2-chlorophenyl)-N-(6-nitroquinazolin-4-yl)-1,3,4-thiadiazol-2-amine (HMP- 06)

Solid, 78% yield; mp > 300 °C; IR (KBr, vmax, cm^{-1}): 3220 (NH), 2923 (CH), 1345 and 1551 (NO_2), 728.67 (C-Cl); ^{1}H NMR (DMSO-d6) δ ppm: 8.53 (s, 1H, NH), 7.60-8.79 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 175.23, 173.45, 162.46, 154.67, 152.78, 148.23, 138.89, 133.34, 131.23, 130.56, 129.34, 128.45, 127.56, 126.78, 117.57, 114.34; EI-MS: m/z 384.0 [M+] and 386.0 [M+2]

4.2.5 N-(6-nitroquinazolin-4-yl)thiazol-2-amine (HMP-07)

Solid, 82% yield; mp 272-274 °C; IR (KBr, vmax, cm^{-1}): 3224 (NH), 2913 (CH), 1350 and 1552 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 8.56 (s, 1H, NH), 6.70-8.79 (m, 6H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 173.56, 162.34, 158.23, 153.23, 147.56, 138.12, 132.56, 128.88, 117.56, 116.23, 113.24; EI-MS: m/z 273.2 [M+] and 275.2 [M+2]

4.2.6 5-methyl-N-(6-nitroquinazolin-4-yl)-1,3,4-thiadiazol-2-amine (HMP-08)

Solid, 84% yield; mp > 300 °C; IR (KBr, vmax, cm^{-1}): 3242 (NH), 2920 (CH), 1341 and 1552 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 8.57 (s, 1H, NH), 7.62-8.80 (m, 4H, Ar-H), 2.89 (s, 3H, CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 173.34, 162.46, 153.12, 151.78, 147.68, 143.69, 131.34, 128.23, 117.68, 114.34, 20.23; EI-MS: m/z 288.2 [M+]

4.2.7 N-(6-nitroquinazolin-4-yl)-5-phenyl-1,3,4-thiadiazol-2-amine (HMP-09)

Solid, 79% yield; mp > 300 °C; IR (KBr, νmax, cm^{-1}): 3226 (NH), 2921 (CH), 1358 and 1544 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 8.40 (s, 1H, NH), 6.78-8.87 (m, 9H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 175.45, 173.46, 159.23, 153.89, 151.23, 147.67, 134.89, 131.67, 130.23, 129.12, 127.89, 126.34, 117.23, 114.34; EI-MS: m/z 350.0 [M+] and 352.0 [M+2]

4.2.8 N-(6-nitroquinazolin-4-yl)-5-(trifluoromethyl)-1,3,4-thiadiazol-2-amine (HMP- 10)

Solid, 63% yield; mp 200-202 °C; IR (KBr, νmax, cm^{-1}): 3260 (NH), 2927 (CH), 1356 and 1534 (NO_2), 1098 (C-F); ^{1}H NMR (DMSO-d6) δ ppm: 8.48 (s, 1H, NH), 6.82-7.87 (m, 4H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 174.45, 162.45, 158.76, 153.89, 151.89, 147.98, 131.34, 126.56, 118.78, 117.56, 114.34; EI-MS: m/z 342.4 [M+] and 344.2 [M+2]

4.2.9 N-(6-nitroquinazolin-4-yl)-5-p-tolyl-1,3,4-thiadiazol-2-amine (HMP-11)

Solid, 54% yield; mp >300 °C; IR (KBr, νmax, cm^{-1}): 3262 (NH), 2918 (CH), 1345 and 1573 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 8.97 (s, 1H, NH), 6.65-8.89 (m, 8H, Ar-H), 2.89 (s, 3H, CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 175.45, 173.34, 162.78, 154.78, 153.78, 147.34, 132.56, 130.67, 128.34, 127.68, 126.23, 120.67, 117.68, 1154.56, 22.23; EI-MS: m/z 364.0 [M+] and 366.0 [M+2]

4.2.10 4-(5-(6-nitroquinazolin-4-ylamino)-1,3,4-thiadiazol-2-yl)phenol (HMP-12)

Solid, 58% yield; mp >300 °C; IR (KBr, νmax, cm^{-1}): 3380 (OH), 3253 (NH), 2920 (CH), 1374 and 1557 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.75 (s, 1H, OH), 8.70 (s, 1H, NH), 6.67-8.82 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 175.45, 171.78, 162.34, 159.89, 154.34, 152.34, 147.68, 131.34, 129.23, 128.56, 126.24, 117.78, 116.34, 114.45; EI-MS: m/z 366.0 [M+] and 368.0 [M+2]

4.2.11 N-(2,4-dimethylphenyl)-6-nitroquinazolin-4-amine (HMP-13)

Solid, 65% yield; mp 180-182 °C; IR (KBr, νmax, cm^{-1}): 3271 (NH), 2918 (CH), 1376 and 1574 (NO_2), ^{1}H NMR (DMSO-d6) δ ppm: 8.28 (s, 1H, NH), 6.72-8.80 (m, 7H, Ar-H), 2.30 (s, 3H, CH_3), 2.16 (s, 3H, CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 173.45, 162.89, 153.34, 147.89,

140.34, 138.45, 132.34, 130.36, 128.48, 127.34, 125.57, 118.34, 116.78, 114.89, 22.34, 19.14; EI-MS: m/z 294.2 [M+]

4.2.12 2-(2-(6-nitroquinazolin-4-ylamino)ethoxy)ethanol (HMP-14)

Solid, 58% yield; mp 190-192 °C; IR (KBr, vmax, cm^{-1}): 3263 (NH), 2920 (CH), 1342 and 1549 (NO_2), 1176.43 (C=O); ^{1}H NMR (DMSO-d6) δ ppm: 9.97 (s, 1H, NH), 6.75-8.88 (m, 4H, Ar-H), 5.45 (s, 1H, OH), 3.20-3.50 (m, 8H, CH_2CH_2-O-CH_2CH_2); ^{13}C NMR (DMSO-d6) δ ppm: 163.46, 161.68, 153.34, 147.22, 132.56, 128.58, 117.34, 114.12, 71.46, 69.58, 63.12, 45.10; EI-MS: m/z 278.2 [M+]

4.2.13 N-(1-benzylpiperidin-4-yl)-6-nitroquinazolin-4-amine (HMP-15)

Solid, 55% yield; mp 232-234 °C; IR (KBr, vmax, cm^{-1}): 3235 (NH), 2920 (CH), 1372 and 1584 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.12 (s, 1H, NH), 6.68-8.90 (m, 9H, Ar-H), 1.50-2.60 (m, 9H, CH_2 Piperidine), 3.12 (s, 2H, CH_2); ^{13}C NMR (DMSO-d6) δ ppm: 162.46, 159.12, 154.78, 147.34, 137.24, 132.58, 130.34, 129.56, 127.24, 126.12, 117.56, 114.88, 65.46, 57.89, 52.34, 32.23; EI-MS: m/z 363.2 [M+]

4.2.14 N-(3-methylpiperazin-1-yl)-6-nitroquinazolin-4-amine (HMP-16)

Solid, 58% yield; mp 256-258 °C; IR (KBr, vmax, cm^{-1}): 3272 (NH), 2936 (CH), 1336 and 1544 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.85 (s, 1H, NH), 6.72-8.88 (m, 4H, Ar-H), 1.56-2.85 (m, 6H, CH_2 piperazine), 1.89 (s, 1H, NH piperazine), 1.10 (s, 3H, CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 173.35, 161.12, 153.78, 147.34, 132.24, 128.46, 117.24, 114.46, 69.24, 63.12, 52.34, 40.58, 15.12; EI-MS: m/z 288.3 [M+]

4.2.15 N-cyclopropyl-6-nitroquinazolin-4-amine (HMP-17)

Solid, 59% yield; mp 195-197 °C; IR (KBr, vmax, cm^{-1}): 3263 (NH), 2974 (CH), 1371 and 1541 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.74 (s, 1H, NH), 8.20-8.80 (m, 4H, Ar-H), 0.50-2.87 (m, 5H, CH_2); ^{13}C NMR (DMSO-d6) δ ppm: 162.24, 159.12, 153.11, 147.34, 131.45, 128.12, 117.56, 116.56, 27.22, 08.78; EI-MS: m/z 230.2 [M+]

4.2.16 6-nitro-N-(pyridin-2-ylmethyl)quinazolin-4-amine (HMP- 18)

Solid, 52% yield; mp 212-214 °C; IR (KBr, νmax, cm^{-1}): 3290.13 (NH), 2964.42 (CH),1350 and 1504 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.96 (s, 1H, NH), 6.78-8.79 (m, 8H, Ar-H), 4.68 (s, 2H, CH_2); ^{13}C NMR (DMSO-d6) δ ppm: 162.24, 159.24, 157.89, 153.34, 148.78, 145.34, 140.24, 132.68, 129.24, 122.57, 119.34, 117.34, 114.24, 49.89; EI-MS: m/z 281.1 [M+]

4.2.17 N-cyclohexyl-6-nitroquinazolin-4-amine (HMP-19)

Solid, 56% yield; mp 170-172 °C; IR (KBr, νmax, cm^{-1}): 3278 (NH), 3026 (CH), 1317 and 1571 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.09 (s, 1H, NH), 8.35-8.80 (m, 4H, Ar-H), 1.10-1.75 (m, 10H, CH_2 Cyclohexyl); ^{13}C NMR (DMSO-d6) δ ppm: 162.34, 159.12, 153.46, 147.89, 131.34, 126.89, 117.24, 116.80, 56.23, 33.34, 26.68, 25.24; EI-MS: m/z 272.3 [M+]

4.2.18 4-(6-nitroquinazolin-4-ylamino)benzoic acid (HMP-20)

Solid, 64% yield; mp > 300 °C; IR (KBr, νmax, cm^{-1}): 3294 (NH), 3486 (OH) 2961 (CH), 1708 (C=O), 1345 and 1530 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 12.10 (s, 1H, OH), 8.89 (s, 1H, NH), 7.45-7.83 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 173.34, 168.24, 159.46, 153.58, 147.24, 132.12, 130.38, 129.24, 126.28, 121.12, 117.38, 114.12, 112.58; EI-MS: m/z 310.1 [M+]

4.2.19 N1-(6-nitroquinazolin-4-yl)benzene-1,2-diamine (HMP-21)

Solid, 73% yield; mp 194- 96 °C; IR (KBr, νmax, cm^{-1}): 3476 (NH), 2958 (CH), 1371 and 1544 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 9.54 (s, 2H, NH), 3.91 (s, 1H, NH_2), 6.78-7.80 (m, 8H, Ar-H); ^{13}C NMR (DMSO-d6) δ ppm: 173.46, 159.24, 153.12, 147.34, 143.58, 137.24, 133.80, 131.34, 130.12, 128.34, 127.12, 120.34, 117.54, 114.34; EI-MS: m/z 281.2 [M+]

4.2.20 4-methyl-7-(6-nitroquinazolin-4-ylamino)-2H-chromen-2-one (HMP-22)

Solid, 61% yield; mp >300 °C; IR (KBr, νmax, cm^{-1}): 3045 (NH), 2933 (CH), 1374 and 1508 (NO_2), 1711 (C=O); ^{1}H NMR (DMSO-d6) δ ppm: 8.92 (s, 1H, NH), 6.20-7.78 (m, 8H, Ar-H), 2.45 (s, 3H, CH_3); ^{13}C NMR (DMSO-d6) δ ppm: 173.56, 161.34, 159.90, 154.45, 153.56,

151.34, 147.58, 143.89, 131.34, 128.34, 125.89, 117.68, 116.68, 113.48, 110.48, 109.89, 107.46, 20.34; EI-MS: m/z 348.2 [M+]

4.2.21 6-nitro-N-(3,4,5-trimethoxyphenyl)quinazolin-4-amine (HMP- 23)

Solid, 65% yield; mp 250 °C; IR (KBr, νmax, cm^{-1}): 3273 (NH), 2986 (CH) 1087 (C=O), 1340 and 1574 (NO_2); ^{1}H NMR (DMSO-d6) δ ppm: 8.45 (s, 1H, NH), 6.48-8.79 (m, 6H, Ar-H), 3.83 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d6) δ ppm: 173.56, 162.78, 155.68, 153.48, 147.46, 137.87, 131.34, 128.47, 126.38, 117.38, 114.58, 97.93, 62.85, 54.83; EI-MS: m/z 356.2 [M+]

4.3. Antimycobacterial activity

In vitro assessment of the anti-mycobacterial activity of the newly synthesized compounds **HMP** (**3-23**) was completed at the National Institute of Allergy and Infectious Diseases (NIAID) (Bethesda, MD, USA). The MIC was established against *M. tuberculosis* strain H37Rv grown under aerobic conditions, utilizing a dual readout (OD_{590} and fluorescence) assay. [34]

4.4. MDR-Inhibitory Activity

MDR-TB inhibitory activity has been performed at the Micro Care Laboratory and Tuberculosis Research Center Surat, India screening program utilizing by Löwenstein-Jensen method against the clinically isolated phenotype MDR strain as per the procedure reported by Soni *et al* . [35]

4.5. Cytotoxicity

The selected set of compounds which showed potent activity against MTB (H37Rv) strains were also evaluated for their cytotoxicity on VERO cell Lines by MTT assay as per the procedure reported by Falzari et al. [36]

4.6 Molecular Docking

The crystal structure of GlmU MTB (PDB: 3ST8) containing substrates, *N*-acetyl glucosamine-1-phosphate (NAcGlc-1-P) and uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) was

processed using Protein Preparation Wizard module of Schrödinger 9.3. [31] This involved some parameters such as assigning bond orders as the protein is large and contain two substrates, addition of hydrogens as pdb file does not contain hydrogens, adjusting bond orders and formal charges for any cofactors if needed, correcting mislabeled elements, adjusting the ionization and tautomerization states of both protein and substrates etc. The crystallographic water molecules at distance above 5 Å were removed. However, the water molecules interacting with the active sites were retained. In the next approach of protein preparation, minimization of the protein was performed using OPLS_2005 force field up to maximum deviation of 0.8 Å before it was processed for generating grid. [31] To soften the potential for non-polar parts of the receptor, all atoms were scaled for van der Waal radii of 1.0 Å with partial atomic charges less than the cut-off value of 0.25 electron unit. The binding region (grid) was defined corresponding to X, Y, and Z coordinates, over the co-crystallized ligands uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) present in the active site respectively. [31]

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Authors Contribution

Harun M. Patel and Rajshekhar Karpoormath have performed the experiments, analyzed the data and wrote the paper. Mahesh Palkar analyzed the data.

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