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Graphical Abstract

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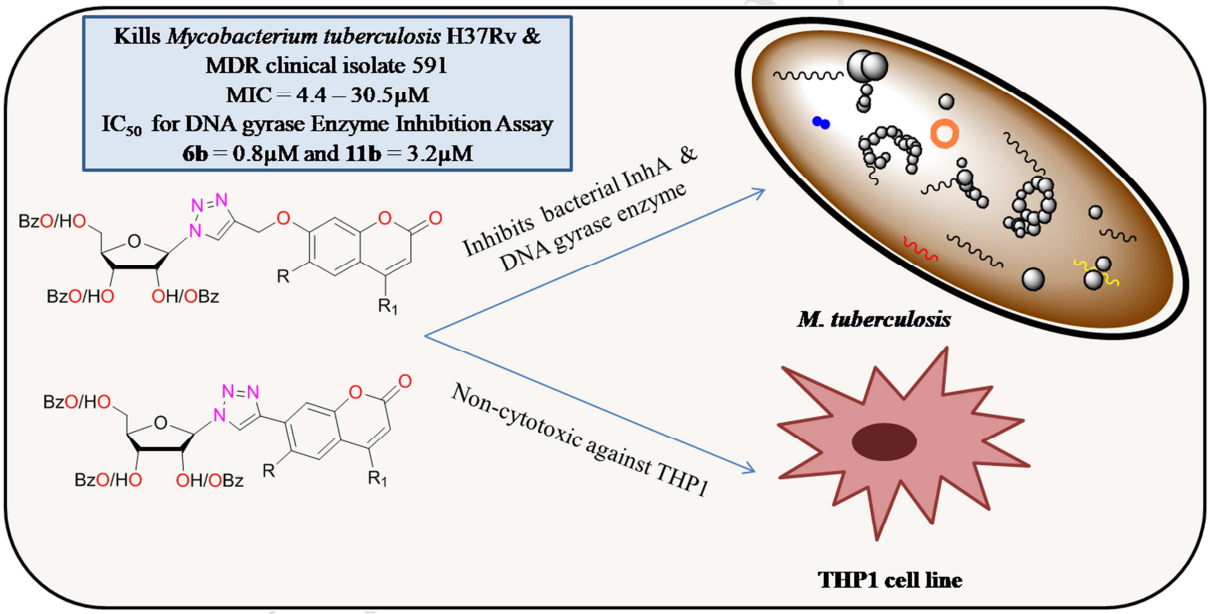
[#] First and second author contributed equally to this work and should be regarded as joint First authors.



Synthesis and Antimycobacterial Activity of 1-(β-D-Ribofuranosyl)-4-coumarinyloxymethyl- / -coumarinyl-1,2,3-triazole

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Abstract

A series of β-D-ribofuranosyl coumarinyl-1,2,3-triazoles have been synthesized by Cu-catalyzed cycloaddition reaction between azidosugar and 7-*O*- / 7-alkynylated coumarins in 62 to 70 % overall yields. The *in vitro* antimycobacterial activity evaluation of the synthesized triazolo-conjugates against *Mycobacterium tuberculosis* revealed that compounds were bactericidal in nature and some of them were found to be more active than one of the first line antimycobacterial drug ethambutol against sensitive reference strain H37Rv, and 7 to 420 times more active than all four first line antimycobacterial drugs (isoniazid, rifampicin, ethambutol and streptomycin) against multidrug resistant clinical isolate 591. Study of *in silico* pharmacokinetic profile indicated the drug like characters for the test molecules. Further, transmission electron microscopic experiments revealed that these compounds interfere with the constitution of bacterial cell wall possibly by targeting mycobacterial InhA and DNA gyrase enzymes. Study conducted on the activities of the test compounds on bacterial InhA and DNA gyrase revealed that the most bactericidal test compound, N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (**6b**) and its corresponding directly linked conjugate N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-yl)-1,2,3-triazole (**11b**) significantly inhibited the activity of both the enzymes. The results were further supported by molecular docking studies of the compound **6b** and **11b** with bacterial InhA and DNA gyrase B enzymes. Further, the cytotoxicity study of some of the better active compounds on THP-1 macrophage cell line using MTT assay showed that the synthesised compounds were non-cytotoxic.

Keywords: Antimycobacterial, Click chemistry, 1,2,3-Triazole, Cytotoxicity, *In silico* pharmacokinetics, Docking study.

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Introduction

There is a great need for the development of newer molecular entities to act as antimycobacterial agents due to the limitations of the existing drugs as well as the increasing resistance against causative bacteria, *Mycobacterium tuberculosis* (*M. tuberculosis*) [1-4]. In HIV-prevalent regions, infection by drug-resistant tuberculosis (TB) almost always has fatal consequences [5-6]. Drugs that are effective in reducing the length of treatments providing effective cure against multi-drug resistant TB (MDR), extremely drug resistant TB (XDR) and latent TB is the requirement of the time [2-3].

Carbohydrates are among the most ample raw materials provided by nature, which play a significant role at the interface of chemistry and biology [7-8]. On the other hand coumarins and their derivatives have shown their potential as antitubercular [1], anti-cancer [9-12], anti-HIV [13], anti-inflammatory [14-15], anti-fungal [16], anti-oxidant, [14-15] and anti-bacterial agents [17a]. Several coumarin derivatives, such as warfarin, carbochromen and acenocoumarol have been approved by FDA for their clinical therapeutic uses [17a-c]. Both, the sugar and the coumarin moieties are excellent biomolecules and have wide variety of applications as well as good biocompatibility [18-19]. There are few reports of synthesis of conjugates of sugar-coumarin or sugar-quinoline linked through a triazole ring that have shown interesting biological activities (Figure 1) [20-25]. We report herein the synthesis of small libraries of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazole and N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazole using Cu(I) catalyzed azide-alkyne 1,3-dipolar cycloaddition 'click' reaction (CuAAC) and study of their *in vitro* antimycobacterial activities against *M. tuberculosis* sensitive reference strain H37Rv and multidrug resistant clinical isolate 591.

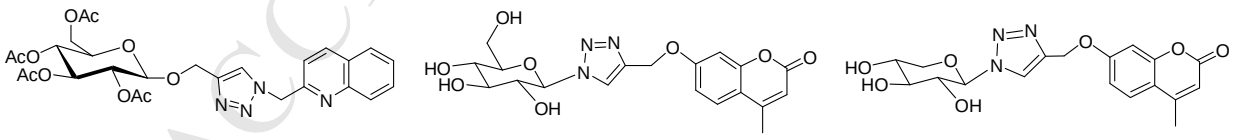
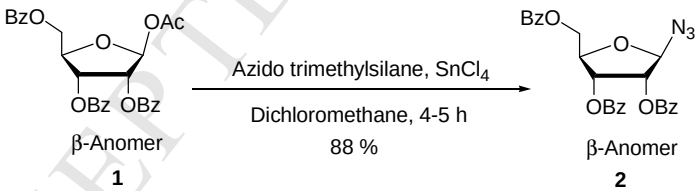


Figure 1: Biologically active sugar -quinoline / -coumarin triazoloconjugates

Results and Discussion

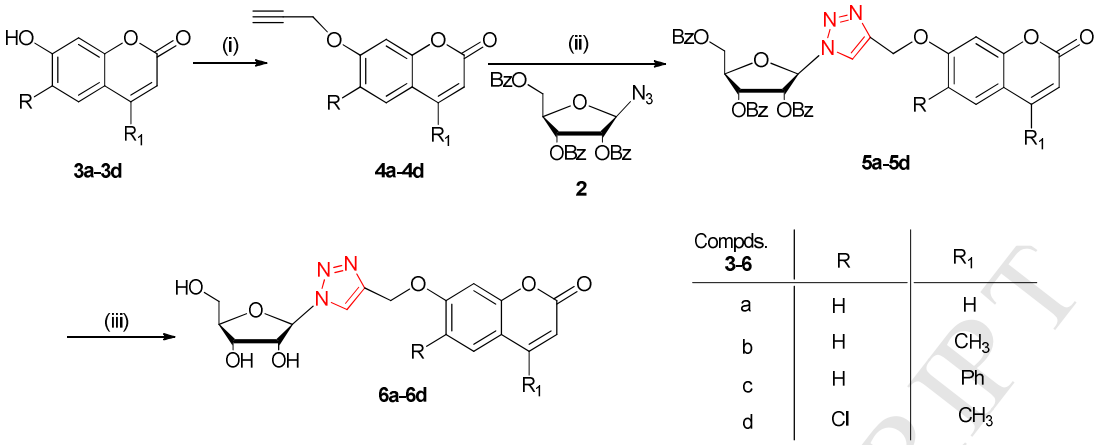
Chemistry

The synthesis of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles has been achieved by Cu(I) catalyzed Huisgen-Sharpless-Meldal [3+2] dipolar cycloaddition reaction of 1-azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) with 7-propargyloxycoumarins **4a**-**4d**, followed by debenzoylation of the resultant conjugates in 62 to 68 % overall yields. The azido-sugar **2** was synthesised by the azidation of commercially available 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**1**) with azido trimethylsilane in the presence of tin (IV) chloride in 88 % yield (**Scheme 1**) [26]. The coumarin precursors, 7-hydroxy-4-methylcoumarin (**3b**) & 7-hydroxy-4-phenylcoumarin **(3c**) were synthesised by the condensation of resorcinol with ethyl acetoacetate and ethyl benzoacetate in 78 and 80 % yields, respectively and 6-chloro-4-methyl-7-hydroxycoumarin (**3d**) was synthesized by condensation of 4-chloro-3-hydroxyphenol with ethyl acetoacetate in 75 % yield using Pechmann condensation protocol [27]. However, 7-hydoxycoumarin (umbelliferone, **3a**) was obtained commercially from Sigma Aldrich Chemical Company, USA. The 7-hydroxycoumarins **3a**-**3d** were propargylated using propargyl bromide in acetone in the presence of potassium carbonate as base to afford 7-propargyloxycoumarins (**4a**), 4-methyl-7-propargyloxycoumarins (**4b**), 4-phenyl-7-propargyloxycoumarins (**4c**) and 6-chloro-4-methyl-7-propargyloxycoumarin (**4d**) in 80-85 % yields (**Scheme 2**) [28-30].



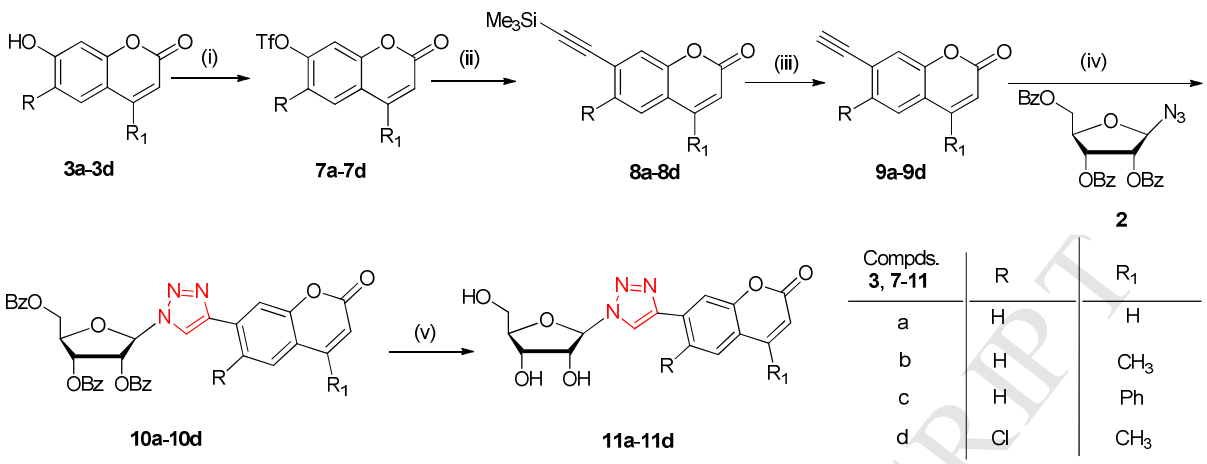
Scheme 1: Synthesis of 1-azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose

The Cu(I) catalyzed cycloaddition reaction of 1-azido-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**2**) with 7-propargyloxycoumarins **4a**-**4d** in the presence of sodium ascorbate-$CuSO_4$ in THF:*tert*-BuOH:H_2O (1:1:1, v/v/v) resulted in the formation of N^1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles **5a**-**5d** in 75-80 % yields. The debenzoylation of the resulted triazole derivatives **5a**-**5d** with sodium methoxide in methanol led to the formation of targeted compounds, N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles **6a**-**6d** in 80-85 % yields (**Scheme 2**).



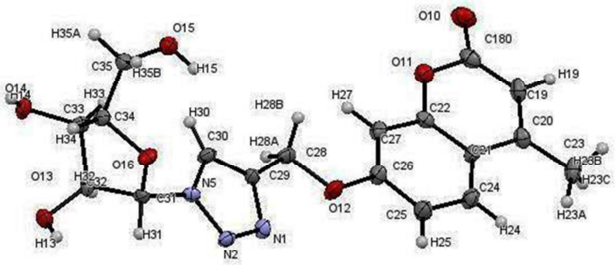
Scheme 2: Synthesis of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles. *Reagents and conditions*: (i) propargyl bromide, K_2CO_3, acetone, 8-10 h, reflux, 80-85 %; (ii) sodium ascorbate, $CuSO_4$, THF:*tert*-BuOH:H_2O (1:1:1), 10-15 h, rt, 75-80 %; (iii) NaOMe, MeOH, 5-6 h, rt, 80-85 %.

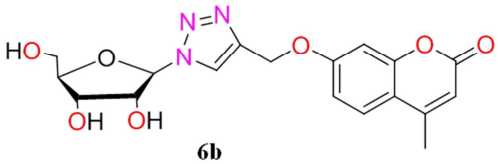
Similarly, synthesis of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles has been achieved by Cu(I) catalyzed [3+2] cycloaddition reaction of azidosugar **2** with 7-acetynylcoumarins **9a-9d** followed by debenzoylation of the resulted N^1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles **10a-10d** with sodium methoxide in methanol in an overall yields of 62 to 70 % (**Scheme 3**). In turn, the synthesis of 7-acetynylcoumarins **9a-9d** was achieved in three steps starting with 7-hydroxycoumarins **3a-3d**. Thus the hydroxycoumarins **3a-3d** were converted into their corresponding triflates **7a-7d** by treatment with trifluoromethane sulfonic anhydride (triflic anhydride) in dichloromethane-pyridine at 0 ^oC; alkynylation of the triflated coumarins **7a-7d** with trimethylsilylacetylene using Sonogashira coupling reaction followed by removal of the trimethylsilyl protection from the resulted 7-trimethylsilylacetynylcoumarins **8a-8d** led to the formation of 7-acetynylcoumarins **9a-9d** in an overall yields of 42 to 51 % (**Scheme 3**) [31-33].



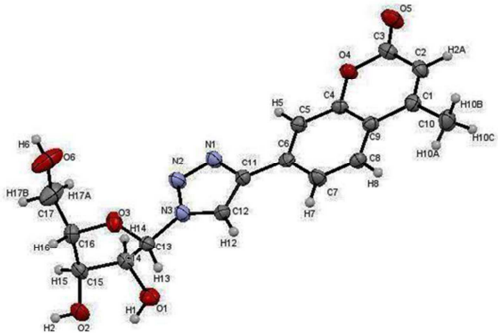
Scheme 3: Synthesis of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles. *Reagents and conditions*: (i) triflic anhydride, pyridine, 1-2 h, rt, 80-85 %; (ii) Et_3N, $Pd(PPh_3)_4$, CuI, acetonitrile, trimethylsilylacetylene, 5-6 h, 70-75 %; (iii) K_2CO_3, MeOH, 1-2 h, rt, 75-80 %; (iv) $CuSO_4$, sodium ascorbate, THF:*tert*-BuOH:H_2O (1:1:1), 12-15 h, rt, 77-82 %; (v) NaOMe-MeOH, 5-6 h, rt, 80-85 %.

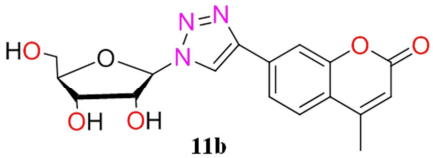
The structures of all the synthesized compounds **2**, **4a-11a**, **3b-11b**, **3c-11c** and **3d-11d** were unambiguously established on the basis of their spectral (IR, ^{1}H-, ^{13}C-, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR NMR and HRMS) data analysis. Three hydroxyl groups present in the sugar moiety of the targeted compounds **6a-6d** and **11a-11d** was confirmed by ^{1}H NMR spectra using D_2O exchange experiments. The structures of known compounds **2**, **3b-3d**, **4a-4d**, **7a-7d**, **8a**, **8b**, **8d**, **9a**, **9b** and **9d** was further confirmed by comparing their physical and spectral data with those reported in the literature [26-33]. The structures, stereochemistry and connectivities of the sugar and the coumarin moiety with triazole ring in N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles **6a-6d** and N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles **11a-11d** were unambiguously confirmed by X-ray diffraction studies on the single crystals of N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (**6b**) and N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-yl)-1,2,3-triazole (**11b**). The ORTEP diagrams of crystal structures of compounds **6b** and **11b** have been given in Figure 2. The detailed crystallographic data of compounds **6b** and **11b** was deposited in the Cambridge Crystallographic Data Centre with CCDC no. **1535099** and **1419805**, respectively.





6b





11b

Figure 2: ORTEP diagram of compounds **6b** and **11b** drawn in 50 % thermal probability ellipsoids showing atomic numbering scheme.

Antimycobacterial Activity and Structure-Activity Relationship

All the synthesized benzoyl and hydroxy conjugates, *i.e.* N^1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles **5a-5d** and N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles **6a-6d**, respectively with oxymethylene linker between triazole and coumarin, and N^1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles **10a-10d** and N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles **11a-11d** with direct triazole-coumarin linkage were screened for their activity against *M. tuberculosis* sensitive reference strain H37Rv and multidrug resistant clinical isolate 591 obtained from Department of Microbiology, V. P. Chest Institute, University of Delhi, India. The minimum inhibitory concentration (MIC) values of above mentioned sixteen test compounds and reference compounds, *i.e.* isoniazid, rifampicin, ethambutol and streptomycin were determined using the micro-plate alamar blue assay (MABA) (Table 1) [3, 34, 35]. The results revealed that the antimycobacterial activity of conjugates with oxymethylene linker between triazole and coumarin moieties, *i.e.* **5a-5d** and **6a-6d** had higher activity than the conjugates with direct triazole-coumarin linkage, *i.e.* **10a-10d** and **11a-11d**. The synthesized candidate drugs were active against both the sensitive reference

strain as well as MDR clinical isolate 591. However, the antimycobacterial activity of almost all synthesized compounds against MDR clinical isolates 591 was many times higher than any of the four screened first line antitubercular drugs. The MIC of all sixteen tested compounds against sensitive reference strain H37Rv and multidrug resistant clinical isolate 591 ranged from 4.4-30.5 µM. The compounds **5c**, **6b** and **6c** were most active against both sensitive reference strain H37Rv and multidrug resistant clinical isolate 591 having MIC values 5.2, 5.1, 4.4 µM and 5.2, 10.3, 8.9 µM, respectively. The activity of these three compounds against reference strain was found to be better than the activity of one of the first line antitubercular drug, ethambutol. Importantly, the activity of compounds **5c**, **6b** and **6c** against MDR clinical isolate 591 was many folds higher (7-420 times) than the activity of first line antitubercular drugs isoniazid, rifampicin, ethambutol and streptomycin, respectively (Table 1).

It was envisioned that respectively high level of activity of conjugates with -OCH_2- bridge between triazole and coumarin moieties may be due to the provision of greater flexibility to the molecule to bind to the required target. The minimum bactericidal concentration (MBC) of the synthesized conjugates and reference drug isoniazid was also determined which revealed that hydroxyl conjugate **6b** has the lowest MBC, *i.e.* 6.4 µM against reference strain and 11.5 µM against MDR clinical isolate, respectively (Table 1). A compound is considered to be bactericidal if the ratio of MBC/MIC is ≤ 4 [3, 34, 35]. The calculated MBC/MIC ratio was 1.25 for reference strain and 1.13 for MDR clinical isolate for compounds **6b** showing its bactericidal nature. The MBC/MIC ratios of all the sixteen test compounds clearly revealed their bactericidal nature (Table 1).

Serial No.	First line drug and synthesized compound	MIC against H37Rv sensitive strain (µM)	MIC against multidrug resistant clinical isolate 591 (µM)	MBC (µM)		MBC/MIC ratio	
				H37Rv	591	H37Rv	591
1	**Isoniazid**	0.2	2187.5	0.3	-	1.6	-
2	**Rifampicin**	0.02	151.9	-	-	-	-
3	**Ethambutol**	9.7	73.4	-	-	-	-
4	**Streptomycin**	0.43	69	-	-	-	-
5	**5a**	11.6	14.5	26.2	-	2.25	-
6	**5b**	8.5	11.4	17.1	-	2	-

5c	5.2	5.2	10.5	-	2	-
5d	13.5	20.4	27.2	-	2	-
6a	10.6	15.9	26.6	-	2.5	-
6b	5.1	10.3	6.4	11.5	1.25	1.13
6c	4.4	8.9	17.7	-	4	-
6d	18.9	18.9	37.7	-	2	-
10a	15.2	30.4	30.4	-	2	-
10b	11.9	29.8	26.8	-	2.25	-
10c	10.9	13.6	24.5	-	2.25	-
10d	14.2	14.2	25.5	-	1.8	-
11a	28.9	28.9	46.3	-	1.6	-
11b	16.6	22.2	16.7	22.2	1	1
11c	14.2	18.9	28.5	-	2	-
11d	30.5	25.4	-	-	-	-

Table 1: MIC, MBC and (MBC/MIC ratio) results of test compounds and first line drugs against *M. tuberculosis* sensitive reference strain H37Rv and multidrug resistant clinical isolate 591.
- Not assessed.
[a] Values are the results of three independent experiments.

In silico Pharmacokinetic Study

The benzoylated β-D-ribofuranosyl coumarinyl-1,2,3-triazoles **5a-5d** & **10a-10d** and β-D-ribofuranosyl coumarinyl-1,2,3-triazoles **6a-6d** & **11a-11d** were further subjected to *in silico* pharmacokinetic studies. Isoniazid (INH) was considered as the reference standard to compare certain important pharmacokinetic parameters, such as molecular weight, lipophilicity, hydrogen-bonding capabilities, etc. These properties were predicted using molecular property prediction tool, Molinspiration and the results were analyzed on the basis of Lipinski rule of five [36-38]. According to Lipinski's rule an orally active drug should not violate more than one of the following criteria-number of hydrogen bond donor and acceptor that mostly determine membrane permeability should be ≤ 5 and ≤ 10, respectively; molecular mass should be ≤ 500, Log P and Log S values corresponding to lipophilicity and solubility and helpful in predicting the intestinal absorption and consequent efficiency of the trans-cellular transport of the drug should be ≤ 5 [36]. All the eight hydroxylated β-D-ribofuranosyl coumarinyl-1,2,3-triazoles **6a-6d** and **11a-11d** exhibited good drug likeness properties and did not violated any of the pharmacokinetic parameters as proposed in Lipinski's rule (Table 2). None of the benzoylated conjugates passed all four criteria of

Lipinski's filter possibly due to the presence of benzoyl groups instead of hydroxyls, which make them too lipophilic to behave like a drug.

Compound No.	Molecular weight	#Log P	¥Log S	*HBA	+HBD	Drug Likeness	No. of Violations
5a	687.65	5.69	-9.51	12	0	-0.49	3
5b	701.67	6.04	-9.61	12	0	-0.44	3
5c	763.74	7.37	-10.75	12	0	-0.50	3
5d	736.12	6.64	-10.25	12	0	-0.30	3
6a	375. 33	-0.74	-3.28	9	3	-0.37	0
6b	389.36	-0.39	-3.38	9	3	-0.44	0
6c	451.43	0.93	-4.52	9	3	-0.37	0
6d	423.80	0.20	-4.02	9	3	-0.04	0
10a	657.62	5.22	-9.93	11	0	-0.69	3
10b	671.65	6.27	-10.03	11	0	-0.61	3
10c	733.72	7.59	-11.57	11	0	-0.67	3
10d	706.09	6.87	-10.40	11	0	-0.42	3
11a	345.30	-0.52	-3.69	8	3	-0.57	0
11b	359.33	-0.17	-3.80	8	3	-0.62	0
11c	421.40	1.16	-4.94	8	3	-0.62	0
11d	393.77	0.11	0.43	8	3	-0.24	0
INH	137.14	0.82	-0.86	4	1	0.67	0

Table 2: Some important pharmacokinetic parameters of test compounds; INH reference anti-TB drug, #log P octanol/water partition coefficient (log P ≤ 5), ¥log S water solubility (log S ≤ 5), *HBA hydrogen bond acceptor (HBA ≤ 10), +HBD hydrogen bond donor (HBD ≤ 5).

Transmission Electron Microscopic Study

On the basis of MIC, MBC and *in silico* pharmacokinetic study results, one of the most active compound **6b** and its directly linked conjugate **11b** was selected for transmission electron microscopy. The untreated and treated cells of *M. tuberculosis* sensitive reference strain H37Rv with test compounds **6b** at 1.38 µM, **11b** at 4.15 µM and first line antitubercular drug isoniazid at 0.05 µM concentrations ($1/4^{th}$ concentration of their MICs) were subjected to transmission electron microscopy (TEM). The TEM images of the treated cells with **6b**, **11b** and isoniazid revealed cell-wall disintegrating nature of the test compounds as well as the drug (**Figure 3**). It has been observed that the untreated / control cells retained their shape with a clearly visible cell wall, however cell-wall disruption at quite a few places was observed in the treated cells of the bacilli (**Figure 3a-d**) [3, 39]. The cell-wall in some treated cells was found to be completely diminished with cell remnants being seen while some of the disintegrating cells showed vacuole formation (**Figure 3c-f**).

The TEM study indicated the change in cell morphology of the treated H37Rv strain of *M. tuberculosis*, which encouraged us to explore the possibility of finding drug targets present in the cell wall of *M. tuberculosis.* The other reason for the investigation of the targets in the cell wall is the presence of coumarin moiety in the synthesized candidate drugs which has been previously reported to target InhA and DNA gyrase bacterial enzymes [3, 40, 41]. Thus, the interaction of the synthesized drug candidates with both the bacterial enzymes, *i.e.* InhA and DNA gyrase was studied.

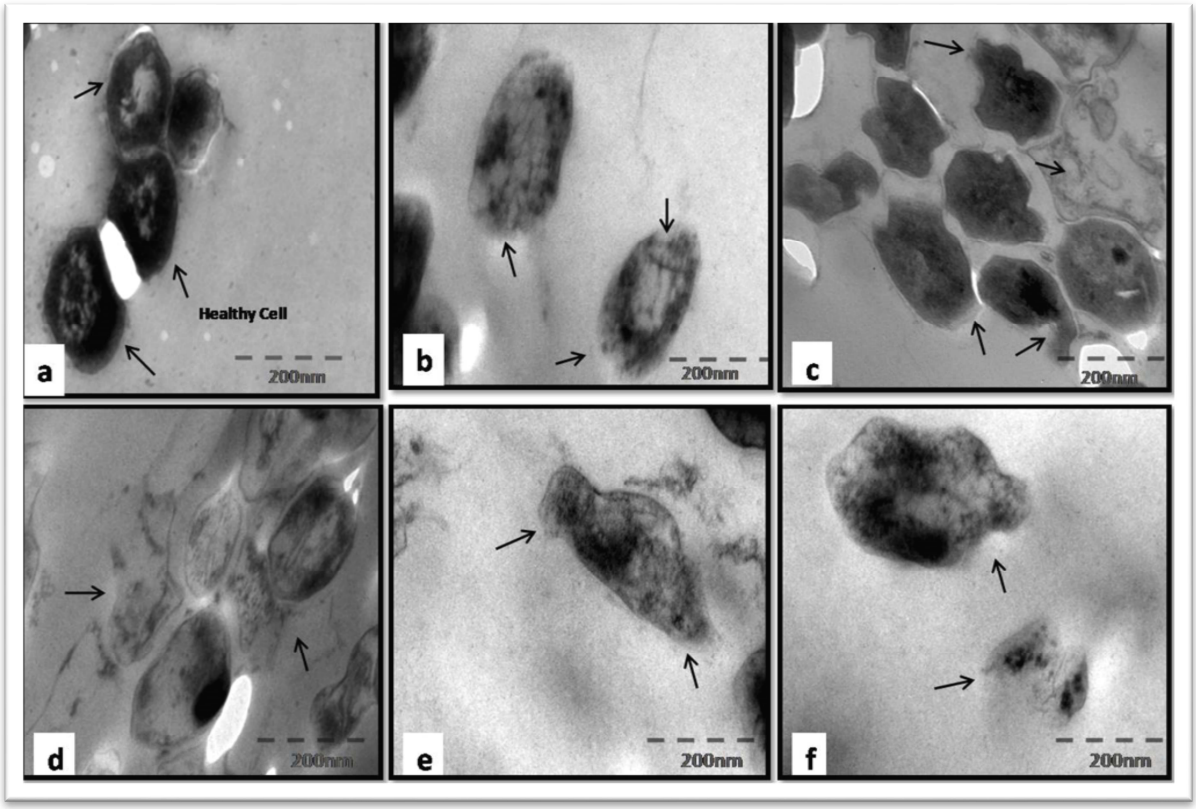


Figure 3: Transmission electron micrographs of untreated and compound-treated *M. tuberculosis* H37Rv cells. (a) untreated cells; (b) Isoniazid treated; (c) & (d) **6b** treated and (e) & (f) **11b** treated. Arrows indicate changes in cell wall morphology, ranging from initiation of cell wall disappearance to complete disintegration and show empty cell-like appearance.

Assay for InhA Enzyme Inhibition

InhA, the enoyl acyl carrier protein reductase (EACPR) from *M. tuberculosis* is one of the essential enzymes involved in the mycobacterial fatty acid elongation cycle and is important for cell survival [41-44]. It is an effective antimicrobial drug target [44]. InhA–drug interaction experiment was carried out to validate InhA as drug target for our test compounds as indicated by TEM studies [41]. To identify whether the test compounds **6b** and **11b** inhibit InhA activity of *M. tuberculosis*; the enzyme activity was observed in the presence and the absence of compound **6b** and **11b** at single concentration**,** i.e. 5 µM (**Figure 4**). In the

experiment, a visible decrease in the absorbance at 340 nm was reported in the reaction mixture containing enzyme InhA, cofactor NADH and substrate crotonoyl-CoA. However, on addition of the test compounds **6b** and **11b** to the reaction mixture, a slight decrease in absorbance was observed initially and there after it remained constant. This indicated the inhibition of InhA enzyme activity in the presence of compounds **6b** and **11b** (**Figure 4**). The results were statistically found to be significant by one way ANOVA test showing p value ≤ 0.0019.

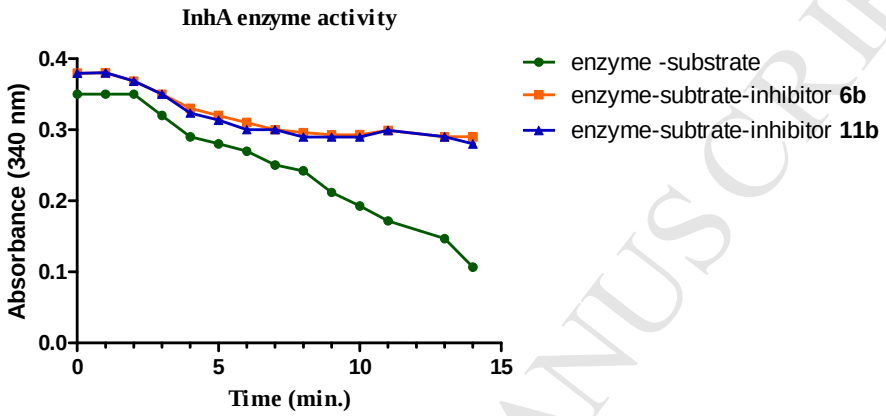
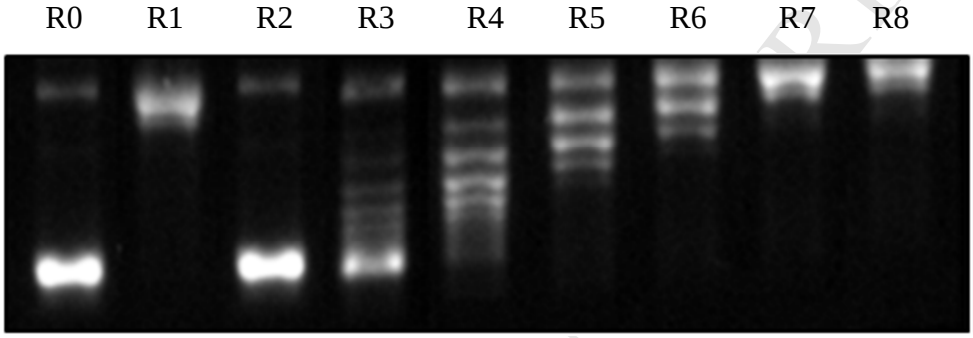


Figure 4: Assay for the activity of bacterial enzyme InhA in presence of test compounds **6b** and **11b**. Values are the results of three independent experiments.

DNA Gyrase Inhibition Assay

DNA gyrase-drug interaction experiment was carried out by DNA gyrase inhibition assay to validate DNA gyrase as drug target [40, 45-47]. Bacterial DNA gyrase, a type II DNA topoisomerase found exclusively in all bacteria and is essential for cell survival. It is a proven target for antibacterial chemotherapy and is a common target of many drugs which includes coumarin and quinolones derived drugs such as ciprofloxacin, novobiocin, chlorobiocin etc [40]. The DNA gyrase B protein from *E. coli* and mycobacterium species such as *M. tuberculosis* and *Mycobacterium smegmatis* (*M. smegmatis)* share ~ 80 % amino acid sequence identity (supporting information **S23 Figure 1**). Hence, the two active test compounds **6b** and **11b** were studied for their effect on *E. coli* DNA gyrase enzyme at single concentration of 0.66 μM and 100 μM (**Figure 5**). The inhibition of DNA gyrase activity was checked by agarose gel electrophoresis and visualized in GELDOC. Results showed inhibition of DNA gyrase supercoiling activity in the presence of ciprofloxacin (**Figure 5**, **Lane R5** and **R8** taken as positive control), test compounds **6b** (**Figure 5**, **Lane R4** and **R7**) and **11b** (**Figure 5**, **Lane R3** and **R6**) where as supercoiling was observed in negative control

(relaxed DNA and DNA gyrase enzyme only) (**Figure 5**, **Lane R2**). Result shows ciprofloxacin and the test compounds **6b** and **11b** significantly inhibited the bacterial DNA gyrase activity at 0.66 µM and showed complete inhibition at 100 µM concentration. Preliminary assay results showed inhibition of DNA gyrase activity by our test compounds **6b** and **11b** having IC_{50} 0.8 µM and 3.2 µM respectively (supporting information **S25 Figure 3** and **S26 Figure 4**). The DNA gyrase enzyme inhibition assay results at single point concentration as well and IC_{50} determination for the DNA gyrase enzyme inhibition indicated DNA gyrase as one of the probable drug target for our test compounds.



	Lane R0	Lane R1	Lane R2	Lane R3	Lane R4	Lane R5	Lane R6	Lane R7	Lane R8
Supercoiled DNA	+	-	-	-	-	-	-	-	-
Relaxed DNA	-	+	+	+	+	-	+	+	+
DNA Gyrase	-	-	+	+	+	-	+	+	+
DMSO (µl)	-	-	-	0.2	0.2	-	0.2	0.2	-
Ciprofloxacin	-	-	-	-	-	0.66µM	-	-	100 µM
6b	-	-	-	-	0.66µM	-	-	100µM	-
11b	-	-	-	0.66µM	-	-	100µM	-	-

Figure 5: **6b**, **11b** and Ciprofloxacin **(**0.66 µM and 100 µM) showed inhibition of supercoiling activity of DNA gyrase enzyme; Lane R0: Supercoiled Plasmid control, Lane R1: Linear relaxed plasmid, Lane R2: Super coiling of linear plasmid in presence of DNA-Gyrase, Lane R3: **11b** (0.66 µM), Lane R4: **6b** (0.66 µM)**,** Lane R5: ciprofloxacin (0.66 µM), Lane R6: **11b** (100 µM), Lane R7: **6b** (100 µM), Lane R8: Ciprofloxacin (100 µM).

Molecular Docking

The molecular docking studies were performed to understand the interaction of active β-D-ribofuranosyl coumarinyl-1,2,3-triazoles **6b** & its directly linked conjugate **11b** and the docking protocol was validated using the prediction for the interaction of co-crystallized ligands (carboximde, chlorobiocin and aminopyrazinamide derivatives) with the binding site

of *M. tuberculosis* InhA , *E.coli* DNA gyrase B and *M. smegmatis* DNA gyrase B, and the results of the docked conformations of best scored compounds are given in Table 3.

Compounds	**Docking results with *M. tuberculosis* InhA**		**Docking results with *E. coli* DNA gyrase B**		**Docking results with *M. smegmatis* DNA gyrase B**	
	XP GScore	**Glide Energy**	**XP GScore**	**Glide Energy**	**XP GScore**	**Glide Energy**
Compound 6b	-9.88	-66.18	-9.21	-60.34	-8.28	-59.30
Compound 11b	-9.34	-63.45	-9.96	-73.05	-8.22	-54.33
(3*S*)-1-Cyclohexyl -n-(3,5-dichloro phenyl)- 5-oxo pyrrolidine-3-carboxamide	-9.98	-73.10	-	-	-	-
Chlorobiocin	-	-	-10.32	-76.28	-	-
6-(3,4-Dimethyl phenyl)-3-[[4-[3-(4-methylpiperazin-1-yl) propoxy] phenyl] amino] pyrazine-2-carb oxamide	-	-	-	-	-9.72	-64.59

Table 3: Glide XP docking scores (kcal mol^{-1}) and docking energies of best bioactive molecules along with the reference inhibitors: pyrrolidine carboxamide, chlorobiocin and aminopyrazinamide bound to *M. tuberculosis* InhA, *E. coli* DNA gyrase and *M. smegmatis* DNA gyrase respectively.

These docking results clearly indicated that compounds **6b** and **11b** exhibited significant binding affinities towards the *M. tuberculosis* InhA protein (Glide energy range -66.19 $kcalmol^{-1}$ to -52.14 $kcalmol^{-1}$ and Glide score in range -9.88 to -7.11 $kcalmol^{-1}$). The predicted binding pose of compounds **6b** and **11b** with hydrogen bonding and hydrophobic interactions in the binding site of *M. tuberculosis* InhA protein has been shown in supporting material (**S27 Figure 5**). The lowest binding energy pose (-66.19 $kcalmol^{-1}$) and high Glide XP score (-9.88 $kcalmol^{-1}$) of compound **6b**, when docked in the ligand binding site of *M. tuberculosis* InhA has been shown in **S27 Figure 5A**. Compound **6b** binds deep in the InhA binding site forming hydrogen bond between nitrogen of triazole ring of the compound and side chain OH of Tyr158 (**S27 Figure 5A**). Further, a π-π interaction between aromatic ring of Tyr158 and the triazole ring of the compound was observed. These binding patterns are similar to that observed for the co-crystallized pyrrolidine carboxamide in *M. tuberculosis* InhA protein [44]. However, the binding pose for compound **11b** having Glide energy: -

63.45 kcalmol^{-1} and Glide XP score: -9.34 kcalmol^{-1} is predicted to form hydrogen bond interactions between the OH group of hydroxymethyl substituent at tetrahydrofuran moiety of the compound and main chain carbonyl group of Met98 residue of *M. tuberculosis* InhA (**S27 Figure 5B**).

One of the frontline drugs for treating TB is isonicotinic acid hydrazide (INH), which is an inhibitor of NADH-dependent enoyl-ACP reductase (InhA). In fact, INH is a prodrug which is first activated to acyl-radical by another enzyme, catalase-peroxidase (KatG). NADH bound as a co-factor in InhA forms a covalent adducts with acyl-radical of INH, *i.e.* INH acyl radical-NADH-InhA. Mutations associated with KatG accounts for 50 % of the INH-resistant TB clinical isolates. Therefore, efforts were made to develop potent InhA inhibitors that are capable of directly binding to InhA active site, avoiding activation by KatG. So far, none of the direct inhibitors of bacterial InhA has been approved for the treatment of TB. This signifies the importance of presently synthesized candidate drugs. Similarly, the interaction of test compounds **6b** and **11b** with the active site of *E.coli* DNA gyrase B (**S28 Figure 6**) and *M. smegmatis* DNA gyrase B (**S29 Figure 7**) was studied using molecular docking to understand hydrogen bonding and hydrophobic interactions of the candidates [45-47]. The interaction of compounds **6b** and **11b** was studied with the DNA gyrase B protein from *E. coli* (**S28 Figure 6**). Both the active test compounds showed H-bond and hydrophobic interactions similar to that of the co-crystallized ligand chlorobiocin in the active site of *E. coli* DNA gyrase B protein [40(d)]. Compound **6b** with Glide energy -60.34 kcal mol^{-1} and Glide XPscore -9.21 kcal mol^{-1} showed H-bond interaction between the carbonyl oxygen atom of pyran ring and side chain nitrogen atoms of Arg136 residue. Another H-bond interaction between the two OH groups of furano sugar moiety and side chain oxygen atom of Asp73 is observed in supporting information **S28 Figure 6A**. Compound **11b** with Glide energy -73.05 kcal mol^{-1} and Glide XPscore -9.96 kcal mol^{-1} shows H-bond interaction between heteroatom oxygen of pyran ring and side chain nitrogen atom of Arg136 and another H-bond interaction between the two OH groups of furano sugar moiety and side chain oxygen atom of Asp73 residue. Further, a cation-π interaction is observed between side chain of Arg76 residue of the protein and pyran ring of the compound **11b** shown in supporting information **S28 Figure 6B**. Such interaction is evident in the binding of chlorobiocin (a coumarin analogue) in the active site of DNA gyrase B crystal structure. Further the interaction of compounds **6b** and **11b** was predicted in the binding site of *M. smegmatis* DNA gyrase B active site. The binding pose of **6b** having the best binding scores

with Glide energy -59.30 kcal mol^{-1} and Glide XPscore -8.28 kcal mol^{-1} indicates hydrogen bonding interaction between one of the secondary OH group of furano-sugar moiety of the compound and oxygen atom of Asp79 side chain in supporting information **S29 Figure 7A**. The interaction pattern of compound **11b** with the Glide energy -54.33 kcal mol^{-1} and Glide XPscore -8.22 kcal mol^{-1} is shown in supporting information **S29 Figure 7B**. In the case of compound **11b**, the OH group of hydroxymethyl substituent at tetrahydrofuran of **11b** forms hydrogen bond with oxygen atom of Asp79 side chain. Further, two hydrogen bonds were formed with the side chain NH atoms of the conserved Arg141 and ethereal oxygen & carbonyl oxygen of coumarin ring. Such interaction is evident in the binding of aminopyrazinamide analogue in the active site of DNA gyrase B crystal structure [47].

Cytotoxicity Study against THP-1 Macrophage Cell Line using MTT Assay

Out of sixteen tested compounds, N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles (**5a-5d** & **6a-6d**) and N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles (**10a-10d** & **11a-11d**); the four active compounds **5c**, **6a**, **6b** and **6c** having MIC ≤ 10 µM were screened for their cytotoxicity on THP-1 macrophage cell line using MTT assay [3,47]. The cytotoxicity study was also carried out for the corresponding directly linked hydroxyl conjugates **11b** and **11c** of the most active compounds **6b** and **6c** (Table 4). The stock solutions of the test compounds were prepared with maximum concentration 150 µg/mL and were used in serial double dilution for MTT assay. Cells were incubated with different concentration of test compounds (4.68, 9.36, 18.72, 37.48, 75.00 and 150 µg/mL) and cell viability was checked after 24 and 48 h (supporting information, **S30**, **Table 1**). All the six tested compounds were found to be non-toxic to THP-1 cells even after the incubation of 48 h at 150 µg/mL concentration; compounds **6a**, **6b**, **6c**, **11b** and **11c** were almost as safe as isoniazid and the other screened standard compound streptomycin under the same condition. Among six tested compounds, THP-1 cells viability was found to be more than 90 % for compounds **6b**, **6c** and **11b** after 24 h of incubation and up to the concentration 48.1 µM, 41.5 µM and 52.1 µM, respectively (37.48 µg/mL). The two most active compounds, **6b** (IC_{50} >385 µM) and **6c** (IC_{50} >332 µM) were found to be safer than streptomycin at the highest studied concentration *i.e.*, 385 µM (150 µg/mL) and 332 µM (150 µg/mL), respectively even after 48 h of incubation (supporting information, **S30**, **Table 1**). Antimycobacterial activity is considered to be specific when the selectivity index is >10. The selectivity index indicates the range of concentration of the compound on which it is active against mycobacteria but not

toxic towards host cells [3]. Although the therapeutic index of isoniazid is very high, it is important to consider the significance of this study with respect to the developing resistance among microorganisms against available antibiotics. In the current work all the compounds exhibited good selectivity index *i.e.* SI >10, indicating their potential as an antimycobacterial agent, and can be explored further for drug development.

Compound No.	IC_{50} (μM)	Selectivity Index (SI) (IC_{50}/MIC)
Isoniazid	547	2735
Streptomycin	>258	600
5c	>196	37.6
6a	>400	37.7
6b	>385	75.4
6c	>332	75.4
11b	>417	25
11c	>355	25

Table 4: IC_{50} , Selectivity index and Cell viability (%) of human THP1 cell line after 48 h treatment with test compounds and first line drug isoniazid and streptomycin at different concentrations.

Conclusion

Sixteen novel β-D-ribofuranosyl coumarinyl 1,2,3-triazole conjugates have been synthesized using Cu-catalyzed 1,3-dipolar cycloaddition reaction of azidosugar with 7-*O*- / 7-alkynylated coumarins in good yields. These compounds possess moderate to high antimycobacterial activity against *M. tuberculosis* sensitive reference strain H37Rv as well as multidrug resistant clinical isolate 591. Compounds **5c**, **6b** and **6c** were most active having MICs ≤ 5.2 μM against sensitive reference strain H37Rv and MICs ≤ 10.3 μM in case of multidrug resistant clinical isolate 591. Notably, the activity of compounds **5c**, **6b** and **6c** against MDR clinical isolate 591 was many folds higher than the activity of first line antitubercular drugs. The most bactericidal compound N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (**6b**) and its directly linked conjugate N^1-(β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-yl)-1,2,3-triazole (**11b**) were direct inhibitors of

bacterial InhA and DNA gyrase B bacterial enzymes and interferes with the constitution of call wall to exhibit its antimycobacterial activity. Isonicotinic acid hydrazide (INH) is the first line drug for the treatment of TB, which acts as an inhibitor of InhA. However, INH acts as prodrug and has to be activated by another enzyme KatG to act as an active inhibitor of InhA. This signifies the importance of development of direct inhibitors of InhA as compounds **6b** and **11b**. The cytotoxicity study revealed that the most active synthesised compounds **5c**, **6a**, **6b** and **6c**, as well as the compounds **11b** and **11c**, which are directly linked conjugates of compounds **6b** and **6c**, respectively are safe against human THP1 cell line.

Experimental Section

General

Reactions were conducted under nitrogen atmosphere, when anhydrous solvents were used. Melting points were determined on Buchi M-560 instrument and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrometer by making KBr disc for solid samples and thin film for oils. The ^{1}H- and ^{13}C NMR spectra were recorded on a Jeol alpha-400 spectrometer at 400 and 100.6 MHz, respectively, using TMS as internal standard. The chemical shift values are on δ scale and the coupling constants (*J*) are in Hz. Signals from OH groups in ^{1}H NMR spectra were verified by removing them by shaking the NMR solution with D_2O. Mass spectra were recorded on Agilent-G6530AA high-resolution mass spectrometer in positive ion mode. HRMS-ESI-TOF analyses were carried out on a microTOF instrument from Bruker Daltonics, Bremen on ESI positive mode. Analytical TLCs were performed on precoated Merck silica-gel $60F_{254}$ plates; the spots were detected under UV light and carbohydrate compounds were detected by charring using 4 % H_2SO_4 in ethanol solution. Silica gel (100-200 mesh) was used for column chromatography. Chemicals were obtained from commercial suppliers and were used without any further purification unless otherwise noted. The single crystal X-ray diffraction data was collected on an Oxford Diffraction X'Calibur single crystal X-ray instrument having CCD camera [Cu Kα radiation (λ = 0.71073)] at USIC, University of Delhi, Delhi.

General procedure for the synthesis of *N*^{1}-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*C*^{4}-(methylenoxy-coumarin-7-yl)-1,2,3-triazoles (5a-5d)

To a stirred solution of 7-propargyloxycoumarin (**4a-4d**, 0.20 g, 0.06 mmol) and 1-azido sugar (**2**, 0.066 mmol) in a solvent mixture of THF:*tert*-BuOH:Water (1:1:1, 30 mL) were added

copper sulphate (0.024 mmol) and sodium ascorbate (0.048 mmol). Reaction mixture was stirred for 10-15 h at room temperature. On completion of reaction (checked by analytical TLC), the reaction mixture was concentrated on rotavapour and coevaporated with toluene (2 x 20 mL) to remove water completely from reaction mixture. The crude thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazole (5a)

It was obtained as off-white solid (0.53 g, 78 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 120-122 ^oC; IR (KBr) ν_{max}: 1719, 1610, 1261, 1118, 1022, 835 and 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.04-7.94 (6H, m), 7.88 (1H, s), 7.64-7.36 (11H, m), 6.87 (2H, s), 6.45 (1H, d, *J* = 4.0 Hz), 6.28 (1H, s), 6.26 (1H, t, *J* = 4.0 Hz), 6.14 (1H, t, *J* = 5.2 Hz), 5.15 (2H, q, *J* = 10.0 Hz), 4.92-4.82 (2H, m) and 4.60 (1H, dd, *J* = 12.0, 4.0 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.05, 165.12, 165.04, 161.13, 161.09, 155.66, 143.51, 143.31, 133.93, 133.75, 133.46, 129.86, 129.78, 129.72, 128.89, 128.60, 128.58, 128.52, 128.33, 122.27, 113.50, 113.01, 112.63, 102.01, 90.43, 81.19, 75.23, 71.41, 63.46 and 61.98. HRMS: *m/z* calculated for [$C_{38}H_{29}N_3O_{10}$+H^+] 688.1931, observed 688.1924.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (5b)

It was obtained as white solid (0.52 g, 80 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 230-234 ^oC; IR (KBr) ν_{max}: 1723, 1612, 1267, 1119, 753 and 710 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.04-7.94 (6H, m), 7.90 (1H, s), 7.60-7.36 (10H, m), 6.90-6.86 (2H, m), 6.46 (1H, d, *J* = 2.8 Hz), 6.27 (1H, t, *J* = 8.0 Hz), 6.16-6.13 (2H, m), 5.14 (2H, q, *J* = 8.8 Hz), 4.91-4.82 (2H, m), 4.59 (1H, dd, *J* = 12.0, 4.4 Hz) and 2.39 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 165.99, 165.07, 164.97, 161.14, 160.90, 154.97, 152.45, 133.85, 133.69, 133.38, 129.78, 129.70, 129.65, 129.11, 128.53, 128.49, 128.45, 128.28, 125.64, 122.42, 114.00, 112.24, 112.18, 101.96, 90.37, 81.08, 75.16, 71.37, 63.41, 61.85 and 18.60. HRMS: *m/z* calculated for [$C_{39}H_{31}N_3O_{10}$+H^+] 702.2088, observed 702.2084.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(4-phenylcoumarin-7-oxymethyl)-1,2,3-triazole (5c)

It was obtained as white fluffy solid (0.41 g, 75 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 151-153 ^oC; IR (KBr) ν_{max}: 1721, 1607, 1265, 1150, 1119 and 755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.04-7.89 (7H, m), 7.61-7.36 (15H, m), 6.95 (1H, s), 6.83 (1H, dd, *J* = 9.2, 2.0 Hz), 6.46 (1H, d, *J* = 3.2 Hz), 6.27-6.24 (2H, m), 6.14 (1H, t, *J* = 5.2 Hz), 5.16 (2H, dd, *J* = 22.0, 12.0 Hz), 4.92-4.83 (2H, m) and 4.60 (1H, dd, *J* = 12.4, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.04, 165.11, 165.03, 161.10, 155.77, 155.69, 143.50, 135.42, 133.92, 133.74, 133.45, 129.86, 129.78, 129.71, 129.61, 128.83, 128.56, 128.51, 128.36, 128.14, 122.29, 113.02, 112.32, 112.20, 102.27, 90.44, 81.19, 75.24, 71.42, 63.45 and 61.96. HRMS: *m/z* calculated for [$C_{44}H_{33}N_3O_{10}+H^+$] 764.2244, observed 764.2223.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(6-chloro-4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (5d)

It was obtained as light yellow fluffy solid (0.45 g, 77 % yield); R_f = 0.5 (10 % methanol in chloroform). M. Pt.: 155-157 ^oC; IR (KBr) ν_{max}: 1720, 1606, 1259, 1158, 1098, 754 and 709 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.00-7.88 (6H, m), 7.89 (1H, s), 7.57-7.33 (10H, m), 6.98 (1H, s), 6.44 (1H, d, *J* = 2.8 Hz), 6.26 (1H, t, *J* = 8 Hz), 6.15 (1H, s), 6.13 (1H, t, *J* = 5.2 Hz), 5.17 (2H, q, *J* = 12.4 Hz), 4.89-4.79 (2H, m), 4.57 (1H, dd, *J* = 12.0, 4.8 Hz) and 2.34 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.02, 165.08, 165.00, 160.49, 156.02, 153.33, 151.47, 143.02, 133.87, 133.70, 133.38, 129.83, 129.75, 129.69, 129.13, 128.56, 128.48, 128.33, 125.49, 122.56, 119.22, 114.29, 113.17, 101.69, 90.38, 81.17, 75.18, 71.40, 63.42, 63.00 and 18.61. HRMS: *m/z* calculated for [$C_{39}H_{30}{}^{35}ClN_3O_{10}+H^+$] 736.1698, observed 736.1687.

General procedure for the synthesis of N^1-(β-D-ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazoles (6a-6d)

A solution of compound **5a-5d** (0.2 g, 0.05 mmol) was taken in a round bottom flask in methanol solvent (20 mL) and sodium methoxide (0.1mmol) was added into it, reaction mixture was stirred at room temperature upto the completion of the reaction, checked on analytical TLC. After the completion of reaction, it was neutralized using seralite (H^+) resin and filtered and solvent was evaporated under vaccum. The crude thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system.

N^1-(β-D-Ribofuranosyl)-C^4-(coumarin-7-oxymethyl)-1,2,3-triazole (6a)

It was obtained as off white solid (0.089 g, 82 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 162-164 ^{o}C; IR (KBr) ν_{max}: 3302, 2922, 1729, 1605, 1399, 1032, and 826 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.51 (1H, s), 7.98 (1H, d, *J* = 9.2 Hz), 7.63 (1H, d, *J* = 8.0 Hz), 7.15 (1H, s), 7.03 (1H, dd, *J* = 4.4, 2.0 Hz), 6.31 (1H, d, *J* = 9.6 Hz), 5.96 (1H, d, *J* = 4.4 Hz), 5.60 (1H, d, *J* = 6.0 Hz), 5.28 (1H, s), 5.26 (2H, d, *J* = 5.2 Hz), 5.00 (1H, t, *J* = 6.0 Hz), 4.39 (1H, q, *J* = 6.0 Hz), 4.13 (1H, q, *J* = 5.2 Hz), 3.98 (1H, q, *J* = 4.4 Hz) and 3.64-3.48 (2H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 161.10, 160.11, 155.31, 144.30, 142.30, 129.54, 123.55, 112.85, 112.73, 112.64, 101.57, 92.09, 85.94, 75.15, 70.39, 61.62 and 61.30. HRMS: *m/z* calculated for [$C_{17}H_{17}N_3O_7$+H^+] 376.1145, observed 376.1135.

N^1-(β-D-Ribofuranosyl)-C^4-(4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (6b)

It was obtained as white solid (0.094 g, 85 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 160-162 ^{o}C; IR (KBr) ν_{max}: 3409, 1699, 1613, 1395, 1280, 1113, 1023 and 826 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.51 (1H, s), 7.68 (1H, d, *J* = 8.8 Hz), 7.14 (1H, s), 7.03 (1H, d, *J* = 9.2 Hz), 6.21 (1H, s), 5.95 (1H, d, *J* = 3.6 Hz), 5.62 (1H, d, *J* = 3.6 Hz), 5.27 (3H, s), 5.00 (1H, t, *J* = 5.2 Hz), 4.38-3.96 (3H, m), 3.61-3.48 (2H, m) and 2.38 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 161.02, 160.00, 154.68, 153.46, 142.34, 126.57, 123.55, 113.44, 112.58, 111.34, 101.60, 92.08, 85.95, 75.16, 70.40, 61.61, 61.31 and 18.19. HRMS: *m/z* calculated for [$C_{18}H_{19}N_3O_7$+H^+] 390.1296, observed 390.1287.

N^1-(β-D-Ribofuranosyl)-C^4-(4-phenylcoumarin-7-oxymethyl)-1,2,3-triazole (6c)

It was obtained as off white solid (0.10 g, 85 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 191-192 ^{o}C; IR (KBr) ν_{max}: 3238, 1716, 1605, 1378, 1027 and 813 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.52 (1H, s), 7.55-7.51 (5H, m), 7.35 (1H, d, *J* = 9.2 Hz), 7.27 (1H, d, *J* = 2.0 Hz), 7.00 (1H, dd, *J* = 9.2, 2.4 Hz), 6.25 (1H, s), 5.95 (1H, d, *J* = 4.4 Hz), 5.63 (1H, s), 5.29 (3H, s), 5.01 (1H, s), 4.37 (1H, t, *J* = 4.4 Hz), 4.11 (1H, s), 3.96 (1H, d, *J* = 4.0 Hz) and 3.61-3.48 (2H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 161.12, 159.98, 155.36, 154.93, 142.21, 134.88, 129.64, 128.82, 128.39, 127.82, 123.60, 112.89, 112.13, 111.57, 102.03, 92.05, 85.88, 75.10, 70.36, 61.65 and 61.26. HRMS: *m/z* calculated for [$C_{23}H_{21}N_3O_7$+Na^+] 474.1277, observed 474.1267.

N^1-(β-D-Ribofuranosyl)-C^4-(6-chloro-4-methylcoumarin-7-oxymethyl)-1,2,3-triazole (6d)

It was obtained as white solid (0.09 g, 80 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 114-116 ^oC; IR (KBr) ν_{max}: 3358, 2930, 1724, 1609, 1387, 1280, 1207, 1044 and 848 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.54 (1H, s), 7.83 (1H, s), 7.48 (1H, s), 6.29 (1H, s), 5.97 (1H, d, *J* = 4.8 Hz), 5.61 (1H, d, *J* = 6.4 Hz), 5.37 (2H, s), 5.25 (1H, d, *J* = 5.2 Hz), 4.98 (1H, t, *J* = 5.2 Hz), 4.39 (1H, q, *J* = 4.4 Hz), 4.11 (1H, q, *J* = 4.4 Hz), 3.97 (1H, q, *J* = 4.8 Hz), 3.63-3.42 (2H, m) and 2.39 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 159.75, 155.80, 153.24, 152.72, 141.82, 126.01, 123.65, 117.73, 114.03, 112.22, 102.16, 92.09, 85.96, 75.12, 70.39, 62.57, 61.22 and 18.24. HRMS: *m/z* calculated for $[C_{18}H_{18}{}^{35}ClN_3O_7+Na^+]$ 446.0731, observed 446.0739.

4-Phenyl-7-trimethylsilylethynylcoumarin (8c)

4-Phenyl-7-triflouromethanesulfonyloxycoumarin (**7c**, 0.8 g, 0.13 mmol), copper iodide (0.02 g, 0.013 mmol) and tetrakis triphenylphosphine palladium (0) (0.03 g, 0.006 mmol) were taken in round bottom flask under argon atmosphere. Triethyl amine (0.29 mL, 0.26 mmol) and acetonitrile was added into the reaction mixture at 0 ^oC and the reaction mixture was stirred at room temperature for 5-6 h. Reaction mixture was concentrated under reduced pressure and the crude thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system. It was obtained as light yellow solid (0.48 g, 70 % yield); R_f = 0.5 (15 % EtOAc in Pet. ether); M.Pt. = 167-169 ^oC; IR (cm^{-1}, KBr) ν_{max}: 3058, 2954, 1725, 1606 and 842cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.51-7.37 (8H, m), 6.34 (1H, s) and 0.25 (9H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 160.66, 155.29, 153.99, 135.09, 130.04, 129.17, 128.61, 127.86, 127.05, 126.97, 120.55, 119.17, 115.64, 103.37 and 98.87; HRMS: *m/z* calculated for $[C_{20}H_{18}O_2Si+H]^+$ 319.1154, observed 319.1164.

7-Ethynyl-4-phenylcoumarins (9c)

4-Phenyl-7-trimethylsilylethynylcoumarin (**8c**, 0.4 g, 0.1 mmol) was dissolved in methanol (15 ml) and potassium carbonate (0.08 g, 0.44 mmol) was added to the reaction. After 1 hr stirring at rt, the completion of the reaction was checked by TLC. Reaction mixture was filtered and washed with methanol 2-3 times and the solvent obtained was removed on rotavapour. The crude thus obtained was purified by silica gel column chromatography using ethyl acetate in petroleum ether as gradient solvent system. It was obtained as light orange solid (0.23 g, 75 % yield); R_f = 0.4 (20 % EtOAc in Pet. ether); M.Pt. = 148-150 ^oC; IR (cm^{-1}, KBr) ν_{max}: 1724, 1607, 1491 and 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.55-7.30 (8H, m), 6.39 (1H, s) and 3.28 (1H, s); ^{13}C

NMR (100.6 MHz, $CDCl_3$): δ 160.19, 155.28, 153.65, 134.57, 129.86, 128.97, 128.37, 127.74, 126.92, 125.74, 120.65, 119.22, 115.64, 82.18 and 80.69; HRMS: *m/z* calculated for $[C_{17}H_{10}O_2+H]^+$ 247.0749, observed 247.0754.

General procedure for the synthesis of *N*^{1}-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-*C*^{4}-(coumarin-7-yl)-1,2,3-triazoles (10a-10d)

To a stirred solution of 7-ethynylcoumarin (**9a-9d**, 0.2 g, 0.06 mmol) and 1-azido sugar (**2**, 0.066 mmol) in a solvent mixture of THF:*tert*-BuOH:Water (1:1:1, 30 mL) were added copper sulphate (0.024 mmol) and sodium ascorbate (0.048 mmol). Reaction mixture was stirred for 10-15 h at room temperature. On completion, reaction mixture was concentrated on rotavapour and coevaporated with toluene (2 x 20 mL) to remove water completely from reaction mixture. The crude thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system.

***N*^{1}-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-*C*^{4}-(coumarin-7-yl)-1,2,3-triazole (10a)**

It was obtained as white solid (0.59 g, 77 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 124-126 ^oC; IR (KBr) ν_{max}: 1728, 1618, 1267, 1108, 985, 757 and 711 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09-7.97 (7H, m), 7.71-7.39 (13H, m), 6.54 (1H, d, *J* = 3.6 Hz), 6.43 (1H, d, *J* = 9.6 Hz), 6.29 (1H, d, *J* = 5.2 Hz), 6.16 (1H, t, *J* = 5.6 Hz), 4.96-4.92 (2H, m) and 4.62 (1H, dd, *J* = 14.8, 4.4 Hz) ; ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.14, 165.15, 165.05, 160.61, 154.32, 146.60, 142.96, 133.93, 133.77, 133.66, 133.54, 129.86, 129.79, 129.65, 129.08, 128.75, 128.59, 128.54, 128.29, 121.74, 119.57, 118.59, 116.61, 113.52, 90.48, 81.40, 75.23, 71.48 and 63.34. HRMS: *m/z* calculated for $[C_{37}H_{27}N_3O_9+Na^+]$ 680.1645, observed 680.1623.

***N*^{1}-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-*C*^{4}-(4-methylcoumarin-7-yl)-1,2,3-triazole (10b)**

It was obtained as white fluffy solid (0.59 g, 82 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 170-172 ^oC; IR (KBr) ν_{max}: 1716, 1620, 1257, 1092, 1026, 859 and 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.08-7.96 (7H, m), 7.69-7.39 (12H, m), 6.54 (1H, d, *J* = 3.6 Hz), 6.31-6.29 (2H, m), 6.17 (1H, t, *J* = 5.6 Hz), 4.95-4.90 (2H, m), 4.62 (1H, dd, *J* = 12.0, 5.6 Hz) and 2.45 (3H, s); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.05, 165.14, 165.05, 160.65, 153.77, 151.99, 146.56, 133.91, 133.75, 133.64, 133.44, 129.86, 129.78, 129.65,

129.09, 128.72, 128.58, 128.53, 128.31, 125.08, 121.49, 119.72, 119.62, 115.05, 113.65, 90.47, 81.38, 75.23, 71.52, 63.37 and 18.60. HRMS: *m/z* calculated for [$C_{38}H_{29}N_3O_9$+Na^+] 694.1796, observed 694.1790.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(4-phenylcoumarin-7-yl)-1,2,3-triazole (10c)

It was obtained as yellow fluffy solid (0.47 g, 80 % yield); R_f = 0.5 (10 % methanol in chloroform). M.Pt.: 150-152 ^oC; IR (KBr) ν_{max}: 1722, 1260, 1093, 1026, 804 and 754 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.08-7.97 (7H, m), 7.65-7.38 (17H, m), 6.54 (1H, d, *J* = 3.2 Hz), 6.39 (1H, s), 6.29 (1H, q, *J* = 3.6 Hz), 6.17 (1H, t, *J* = 5.2 Hz), 4.95-4.91 (2H, m) and 4.62 (1H, dd, *J* = 13.2, 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.16, 166.05, 165.20, 165.13, 165.05, 160.66, 155.27, 154.43, 146.44, 133.90, 133.75, 133.70, 133.63, 133.56, 133.24, 129.85, 129.77, 129.68, 129.65, 129.42, 129.08, 128.89, 128.71, 128.57, 128.52, 128.40, 128.29, 127.46, 121.45, 119.80, 118.66, 114.99, 113.87, 93.24, 90.46, 81.36, 79.78, 75.23, 71.47, 71.40, 63.70 and 63.35. HRMS: *m/z* calculated for [$C_{43}H_{31}N_3O_9$+H^+] 734.2139, observed 734.2140.

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-C^4-(6-chloro-4-methylcoumarin-7-yl)-1,2,3-triazole (10d)

It was obtained as yellow solid (0.5 g, 78% yield); R_f = 0.5 (10 % Methanol in Chloroform). M.Pt.: 187-189 ^oC; IR (KBr) ν_{max}: 1727, 1612, 1452, 1267, 1098, 809 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 8.44 (1H, s), 8.13 (1H, s), 7.98-7.90 (6H, m), 7.53-7.32 (10H, m), 6.47 (1H, d, *J* = 3.6 Hz), 6.26 (2H, s), 6.14 (1H, t, *J* = 5.2 Hz), 4.87-4.79 (2H, m), 4.55 (1H, dd, *J* = 12.0, 3.6 Hz) and 2.32 (3H, s) ; ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.11, 165.15, 165.10, 160.14, 151.90, 150.71, 143.10, 133.93, 133.75, 133.44, 131.69, 129.87, 129.80, 129.72, 129.06, 128.54, 128.34, 127.71, 126.44, 125.87, 123.02, 120.53, 117.61, 116.31, 90.39, 81.41, 75.23, 71.58, 63.47 and 18.44. HRMS: *m/z* calculated for [$C_{38}H_{28}{}^{35}ClN_3O_9$+$Na^+$] 728.1412, observed 728.1440.

General procedure for the synthesis of N^1-(β-D-Ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazoles (11a-11d)

A solution of compound **10a-10d** (0.2 g, 0.05mmol) in methanol was taken in a round bottom flask, and sodium methoxide (0.1mmol), was added into it, reaction mixture was stirred at rt

upto the completion of the reaction, checked on analytical TLC. The solution was neutralized using seralite (H^+) resin and filtered and solvent was evaporated under vaccum. The crude thus obtained was purified by silica gel column chromatography using methanol in chloroform as gradient solvent system.

N^1-(β-D-Ribofuranosyl)-C^4-(coumarin-7-yl)-1,2,3-triazole (11a)

It was obtained as white solid (0.08 g, 80 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 220-222 ^oC; IR (KBr) ν_{max}: 3410, 2924, 1690, 1616, 1110 and 852 cm^{-1}.; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.96 (1H, s), 8.08 (1H, d, *J* = 10.0 Hz), 7.88-7.79 (3H, m), 6.49 (1H, d, *J* = 9.2 Hz), 5.97 (1H, d, *J* = 4.4 Hz), 5.68 (1H, d, *J* = 5.6 Hz), 5.30 (1H, d, *J* = 5.6 Hz), 5.01 (1H, t, *J* = 5.2 Hz), 4.42 (1H, q, *J* = 5.6 Hz), 4.15 (1H, q, *J* = 4.8 Hz), 3.99 (1H, q, *J* = 3.6 Hz) and 3.65-3.50 (2H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 159.65, 145.01, 144.30, 129.09, 121.72, 118.51, 116.03, 112.23, 92.15, 86.14, 74.56, 69.98 and 61.22. HRMS: *m/z* calculated for [$C_{16}H_{15}N_3O_6$+H^+] 346.1034, observed 346.1035.

N^1-(β-D-Ribofuranosyl)-C^4-(4-methylcoumarin-7-yl)-1,2,3-triazole (11b)

It was obtained as white solid (0.09 g, 85 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 213-215 ^oC; IR (KBr) ν_{max}: 3427, 2939, 1669, 1617, 1057, 1014 and 852 cm^{-1}.; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.96 (1H, s), 7.87-7.80 (3H, m), 6.38 (1H, d, *J* = 1.6 Hz), 5.98 (1H, d, *J* = 4.4 Hz), 5.69 (1H, d, *J* = 3.7 Hz), 5.31 (1H, d, *J* = 5.2 Hz), 5.00 (1H, t, *J* = 4.8 Hz), 4.43 (1H, q, *J* = 8.0, 4.4 Hz), 4.16 (1H, q, *J* = 12.0, 5.2 Hz), 4.00 (1H, q, *J* = 8.8, 4.4 Hz), 3.65-3.48 (2H, m) and 2.43 (3H, s); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 159.79, 153.53, 153.07, 145.25, 133.98, 126.30, 121.40, 121.06, 119.25, 114.54, 112.46, 92.36, 86.01, 75.15, 70.33, 61.36 and 18.11. HRMS: *m/z* calculated for [$C_{17}H_{17}N_3O_6$+H^+] 360.1191, observed 360.1190.

N^1-(β-D-Ribofuranosyl)-C^4-(4-phenylcoumarin-7-yl)-1,2,3-triazole (11c)

It was obtained as light yellow solid (0.09 g, 82 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 160-162 ^oC; IR (KBr) ν_{max}: 3281, 2926, 1681, 1622, 1387, 1134 and 839 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 8.99 (1H, s), 7.93 (1H, s), 7.86 (1H, d, *J* = 8.4 Hz), 7.58-7.53 (6H, m), 6.43 (1H, s), 5.98 (1H, d, *J* = 4.4 Hz), 5.70 (1H, d, *J* = 5.2 Hz), 5.35 (1H, dd, *J* = 14.8, 3.6 Hz), 5.03 (1H, brs), 4.43 (1H, q, *J* = 4.4 Hz), 4.15 (1H, q, *J* = 4.4 Hz), 3.99

(1H, q, *J* = 4.4 Hz), 3.67-3.52 (2H, m); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 159.88, 154.28, 145.01, 134.35, 129.58, 128.76, 121.93, 121.38, 114.14, 113.05, 94.75, 92.02, 85.70, 84.66, 75.24, 74.82, 70.03, 62.12 and 60.99. HRMS: *m/z* calculated for [$C_{22}H_{19}N_3O_6$+H^+] 422.1352, observed 422.1336.

N^1-(β-D-Ribofuranosyl)-C^4-(6-chloro-4-methylcoumarin-7-yl)-1,2,3-triazole (11d)

It was obtained as light yellow solid (0.09 g, 80 % yield); R_f = 0.3 (10 % methanol in chloroform). M.Pt.: 138-140 ^{o}C; IR (KBr) ν_{max}: 3484, 2914, 2116, 1665, 1606, 1388, 1244, 1056 and 753 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.14 (1H, s), 8.00 (2H, d, *J* = 5.4 Hz), 6.49 (1H, s), 6.08 (1H, d, *J* = 4.0 Hz), 5.74 (1H, d, *J* = 5.6 Hz), 5.31 (1H, d, *J* = 5.6 Hz), 5.15 (1H, t, *J* = 5.6 Hz), 4.45 (1H, q, *J* = 5.2 Hz), 4.21 (1H, q, *J* = 5.2 Hz), 4.03 (1H, q, *J* = 4.0 Hz), 3.72-3.54 (2H, m) and 2.46 (3H, s); ^{13}C NMR (DMSO, 100.6 MHz): δ 159.55, 152.14, 151.95, 142.22, 132.10, 127.58, 125.44, 123.89, 120.77, 116.39, 115.71, 93.05, 85.99, 75.06, 69.73, 61.45, 17.74. HRMS: *m/z* calculated for [$C_{17}H_{16}{}^{35}ClN_3O_6$+$H^+$] 394.0806, observed 394.0808.

Compound code	**6b**	**11b**
Empirical formula	C_{18} H_{19} N_3 O_7	C_{17} H_{17} N_3 O_6
Formula weight	389.36	359.33
Temperature	293(2) K	293(2) K
Wavelength	0.71073 A°	0.71073 A°
Crystal system	Monoclinic	Orthorhombic
Space group	P 21	P 21 21 21
Unit cell dimensions	a = 7.107(5) A° alpha = 90 deg b = 15.119(5) A° beta = 90 deg. c = 16.055(5) A° gamma = 90 deg.	a = 6.7824(5) A° alpha = 90 deg. b = 8.0573(5) A° beta = 90 deg. c = 29.495(2) A° gamma = 90 deg.
Volume	1716.8(14) A°3	1611.86(19) A°3
Z	4	4
Calculated density	1.506 Mg/m^3	1.427 Mg/m^3
Absorption coefficient	0.120 mm^{-1}	0.111 mm^{-1}
F(000)	704	724
Theta range for data collection	2.88 to 25.00 deg.	2.88 to 25.00 deg.
Limiting indices	- 8<=h<=8, -17<=k<=17, -19<=l<=19	-8<=h<=8, -9<=k<=9, -35<=l<=35
Reflections collected / unique	20161/6024 [R(int) = 0.1248]	18672 / 2844 [R(int) = 0.0777]
Completeness to theta	= 25.00 99.8 %	= 25.00 99.8 %
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	6024/1/505	2844 /0/235
Goodness-of-fit on F^2	0.908	0.806
Final R indices [I>2sigma (I)]	R1 = 0.0836, wR2 = 0.2001	R1 = 0.0473, wR2 = 0.1344
R indices (all data)	R1 = 0.1858, wR2 = 0.2863	R1 = 0.0523, wR2 = 0.1394
Absolute structure parameter	3(3)	0.7(15)
Largest diff. peak and hole	0.308 and -0.300 e.A°$^{-3}$	0.343 and -0.354 e.A°$^{-3}$
CCDC No.	**1535099**	**1419805**

Table 5. Single crystal X-ray diffraction data of compound **6b** and **11b**

Experimental: Biology

Antimycobacterial Activity (MIC & MBC study)

The strains and isolates used for antimycobacterial activity study comprised of the reference strain *M. tuberculosis* H37Rv and the MDR clinical isolate 591. They were obtained from the Department of Microbiology, V. P. Chest Institute, Delhi, India. The cultures were maintained on Middlebrook 7H9 medium (Difco Laboratories, MI, USA). The MICs of the series of test compounds and the first line drugs isoniazid, rifampicin, ethambutol and streptomycin, taken as reference standards, were determined using the micro-plate alamar blue assay (MABA) [3,34,35]. Sterile water (200 mL) was added to all the boundary wells of a 96-well U-bottom plate to minimize evaporation and maintain humidity. A single-cell suspension was prepared from a log-phase culture of *M. tuberculosis* by passing it through an 8 µm filter (Millipore). The Optical Density at 600 nm was measured using a Cary UV spectrophotometer and the cell density was then calculated using McFarland standards. The required amount of 7H9 medium, the calculated amount of the drug and $1x10^6$ cells were added to each well, so as to make up the volume to 200 mL. Positive (medium+inoculum) and negative (medium only) controls were also included. The plates were then sealed with parafilm and incubated aerobically at 37 °C. After 10 days of incubation, 30 mL of 0.02 % resazurin solution was added to each well and the plates were again incubated overnight at 37 °C before being observed for colour change. A colour change from blue to pink was considered as growth and the MIC value was recorded as the lowest drug concentration that prevented visible growth/colour change. A similar procedure was also followed for MDR clinical isolate 591 and the dye was added on the fifteenth day of incubation. Different range of drug concentrations was checked to determine the exact MIC and the experiments were done in triplicate to check the result reproducibility. MBCs were determined using the method adapted from a previously reported protocol [3]. MBC study was carried out directly from the 96-well plates used for MIC determination. Observations were taken on days 15, 21 and 28. The MBC was recorded as the minimum concentration of the drug that led to 99 % inhibition of growth at the end of 4 weeks of incubation. A compound is considered to be bactericidal if the ratio of MBC/MIC is ≤ 4 and if the MBC/MIC is > 4 it is considered to be bacteriostatic in nature. MBC/MIC ratios were also calculated to determine whether the compounds are bacteriostatic or bactericidal in nature.

In silico Pharmacokinetic Property Predictions

The active series of compounds were further subjected to *in silico* pharmacokinetic prediction to gather information about certain important pharmacokinetic parameters, such as lipophilicity, solubility, hydrogen bond accepters and hydrogen bond donors. Isoniazid was taken as the reference standard for comparing the compound series. The said properties were predicted considering parameters of Lipinski filter using online drug likeness predicting software Molinspiration. This software uses certain mathematical models to quantitatively predict properties by a set of rules/keys that specify threshold pharmacokinetic characteristics for the chemical structure of the molecules, based on the available drug information. Lipophilicity is estimated from log P values. The model is based on a genetic partial least squares method on a training set of 784 compounds with experimentally measured solubilities. This property explorer software enables the prediction of drug-relevant properties for any valid structure [36-38]. Structures can be drawn online using this software and the results can be seen immediately. The prediction results as obtained are valued.

Transmission Electron Microscopy Studies

Transmission electron microscopy of the untreated as well as the drug-treated bacterial samples was carried out in order to examine the changes in cell morphology. Sensitive reference strain H37Rv was grown in the presence and absence of sub-lethal doses ($1/4^{th}$ MIC) of the chosen test compounds **6b**, **11b** and first-line drug INH. The initial inoculum was fixed at 10^6 cells / mL. After 30 days of incubation, cells were harvested and washed thrice with 0.1 M phosphate buffer (PB). The cells were then fixed in 2.5 % glutaraldehyde at 48 °C for 18–24 h. The fixative was then removed from the cell pellet by washing with 0.1 M phosphate buffer thrice [3, 39] The cell pellet was then resuspended in 0.1 M phosphate buffer and the samples were submitted to the Sophisticated Analytical Instrumentation Facility (SAIF), Department of Anatomy, All India Institute of Medical Sciences, New Delhi for further analysis.

Assay for InhA Enzyme Activity

Cloning, expression and purification of InhA (*Rv1484*) was done to study the effect of test molecules on the activity of the enzyme. InhA (*Rv1484*) was cloned in *E. coli* and purified

InhA protein. Protein estimation was done by Bradford's method. InhA-drug interaction experiment was carried out to validate the drug target proposed through docking experiment. Assay was performed in 96 well plates with 200 µL reaction volume in each well, reactions were performed in triplicate. 2 µg purified enoyl-ACP reductase (InhA) was incubated with test compounds (**6b** and **11b** having concentration 5 µM from 100 µM stock solution respectively, dissolved in DMSO such that the final concentration of this co-solvent was constant at ≤ 1 % v/v) in 100 mM sodium phosphate buffer pH 7.5 for 30 minutes. Enzyme activity was carried out using a plate reader spectrophotometer (model: Infinite f200 pro; Tecan) by monitoring NADH absorbance at 340 nm (A340). Decrease in absorbance over time indicates the enzyme activity which facilitates the conversion of NADH to NAD^+ as the reaction proceeds [41]. The assay was started by addition of 100 µM NADH and 100 µM crotonoyl-CoA. The plate was incubated at room temperature and OD at 340 nm was recorded over a time period of 15 minutes using spectrophotometer (model: Infinite f200 pro; Tecan) [41-44].The chemicals and reagents, NADH and Crotonoyl CoA were purchased from Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA). The results were statistically checked for significance by one way ANOVA test using Graph Pad Prism software.

DNA gyrase Inhibition Assay

DNA gyrase-drug interaction experiment was performed by DNA gyrase inhibition assay using protocol as recommended in manufacturer's manual provided with New England Bio Labs (NEB), *E. coli* DNA gyrase (Catalogue No. M0306S). Assays were performed with relaxed pUC19 plasmid DNA. The reaction was carried in 30 µL volume containing DNA gyrase assay buffer 1X, Relaxed pUC19 DNA 0.3 µg, 1 unit DNA gyrase enzyme. To this reaction mix, 0.2 µL ciprofloxacin (taken as positive control from 100 µM stock), DMSO (solvent control) and 0.2 µL of the test compounds **6b** and **11b** (from 100 µM stock) were added respectively. The reaction mixtures were incubated at 37 ºC for one hour. The reactions were terminated by addition of 50 % glycerol and 0.25 % bromophenol blue. The samples were subjected to electrophoresis in 1 % agarose gel in 0.5X Tris-borate EDTA (TBE) buffer pH 8.3, for 16 hours at 30 mA. The gel was stained in ethidium bromide (0.7µg / mL) and visualized in UV light. The plasmid and gyrase enzyme was purchased from New England Bio Labs, USA (Lot no.0021609).

Molecular Docking

In order to study the molecular interactions of derivatives of β-D-ribofuranosyl coumarinyl 1,2,3-triazoles with InhA and DNA gyrase B, the 2D structures of all the compounds were generated by drawing on ChemBioDraw Ultra 12.0 (www.cambridgesoft.com). Ligprep module implemented in Schrödinger was used to generate energy minimized 3D structures. Partial atomic charges were computed using the OPLS_2005 force field. The correct Lewis structure, tautomers and ionization states (pH 7.0±2.0) for each of these ligands were generated and optimized with default settings (Ligprep 2.5, Schrödinger, LLC, New York, NY, 2012).The 3D crystal structure of InhA protein co-crystallized with pyrrolidine carboxamide derivative from *M. tuberculosis* (PDB ID:4TZK; resolution 1.62 Å) was retrieved from protein data bank (www.rcsb.org) [44]. Keeping in view the fact that the DNA gyrase B ATPase from *E. coli* and mycobacterial species share significant amount of similarity in structure [40] (**S23**, **Figure 1**) and amino acid sequence and is inhibited by several class of inhibitors, the X-ray crystal structure of *E coli* DNA gyrase B ATP binding domain bound to chlorobiocin (PDB ID: 1KZN; resolution 2.3 Å) [40(d)] and *Mycobacterium smegmatis (M. smegmatis)* DNA gyrase B ATP binding domain, co-crystallized with aminopyrazinamide derivative (PDB ID: 4B6C; resolution 2.2 Å) was utilized for the present study [47]. The proteins were prepared for docking using Protein Preparation Wizard (Maestro 10.0 Schrödinger, LLC, New York, NY, 2012). Water molecules within 5 Å of the protein structures was considered. Bond order and formal charges were assigned and hydrogen atoms were added to the crystal structure. In order refine the structure OPLS-2005 force field parameter was used to alleviate steric clashes and the minimization was terminated when RMSD reached maximum cutoff value of 0.30 Å. The location of co-crystallized ligands in both the protein structures were used to choose the center and size of the receptor grid, which was generated using Glide 5.8 (Schrödinger, LLC, New York, NY, 2012) with default settings for all parameters. The grid size was chosen sufficiently large to include all active site residues involved in substrate binding. The cofactor, NADH in the InhA was also considered as part of the receptor proteins. All ligand conformers were docked to each of the receptor grid files (InhA and gyrase B structures) using Glide extra precision (XP) mode. Default settings were used for the refinement and scoring.

Cytotoxicity Assay

Human THP-1 cell line was used for MTT assay and was procured from National Centre for Cell Science, Pune (India). THP-1 cells were seeded at 10^4 cells per well into 96-well plates. Phorbol myristate acetate (20 ng / mL) was used for cell adhesion and proliferation. After adhesion, the cells were charged with fresh medium (RPMI supplemented with 10 % foetal calf serum and 2 mM L-glutamine). The required concentration of the drug was added into each well. Controls with and without DMSO were also maintained. The plates were then incubated for different time intervals (24 and 48 h) at 37 °C in a 5 % CO_2 incubator. Then, 20 mL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and the plates were further incubated for 4 h. The medium was then gently removed and 100 mL of DMSO was added to dissolve the formazan crystals that had formed due to reduction of MTT by viable cells. The absorbance of the plates was then read at 540 nm and the percentage survival of THP-1 cells was calculated [3, 48]. Chemicals and reagents and RPMI media (RPMI-1640) were purchased from Sigma-Aldrich Chemical Company (St. Louis, Missouri, USA). The therapeutic index was calculated by dividing the 50% growth inhibition concentration (IC_{50}) for cell lineTHP1 by the MIC for in vitro activity against *M. tuberculosis* sensitive reference strain H37Rv [3].

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Highlights:

- ✓ A series of novel ribofuranosyl coumarinyl-1,2,3-triazoles have been synthesized.
- ✓ Activity against *M. tuberculosis* H37Rv and MDR isolate 591 was observed between 4.4-30.5 µM
- ✓ Synthesized compounds were non-cytotoxic to THP1 cells.
- ✓ TEM study showed cell wall attacking nature of the compounds.
- ✓ Enzymatic assay showed InhA and DNA gyrase inhibition.