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Synthesis and screening of (*E*)-1-(β-D-galactopyranosyl)-4-(aryl)but-3-ene-2-one against *Mycobacterium tuberculosis* ☆

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ABSTRACT

A series of galactose-derived aryl enones were synthesised and screened against *Mycobacterium tuberculosis* $H_{37}Rv$. Preliminary results were promising with MIC values in the range 1.56–12.5 μg/mL.

Introduction: According to the WHO estimate approximately 1.7 million lives were wiped off by TB in 2009 alone. Even though there is a marginal decrease in the TB death rate, there were 9.4 million new cases in 2009, including the 1.1 million cases of co-infection with HIV. The more alarming fact is that the extensively drug resistant cases (XDR-TB) have been reported from 58 countries and are reportedly resistant to a majority of drugs in use against TB.[2] In order to tackle the issue of resistance there is an urgent need for new drugs with novel modes of action and hence less susceptible to resistance.

C-Glycosides are stable surrogates of *O*-glycosides and are often used as investigative tool in chemical biology and medicinal chemistry, in particular *C*-glycoside analogs are often used as substrate mimics for different enzymes involved in the carbohydrate metabolism.[3,4] Carbohydrate-rich cell wall architecture of *Mycobacterium tuberculosis* (*M.tb*) makes it susceptible to different sugar derivatives targeting different enzymatic processes involved in the cell wall biosynthesis.

Chalcones are well known for their anti-infective properties,[5] in particular they are reported to have anti-tubercular activity.[6,7] Lopez et al. reported a series of anti-fungal chalcones acting against the cell wall biosynthesis by inhibiting the β-(1,3)-glucan synthase and/or chitin synthase.[8] Following the Lubineau's method for the synthesis of β-*C*-glycosidic ketones,[9] *C*-glycosides have been increasingly used for the synthesis of bioactive compounds. Alkanoyl glycosides derived from β-*C*-glycosidic ketones were shown to posses α-glucosidase, glucose-6-phosphatase and glycogen phosphorylase inhibition properties.[10] Likewise, enones derived from *C*-furyl glycosides were shown to have anti-microbial effect[11] (Fig. 1). Since the anti-microbial activity and the safety issues of the enone-containing chalcones have been estabilsed in the literature we decided to make anaolgs of uridine 5c-diphosphate galactopyranose (UDP-Gal*p*), by replacing the phosphate bridge with an enone-linker and the uridine component with variously substituted aromatic moeities (Fig. 2), which can potentially act as cell wall biosynthesis inhibitor in mycobacteria, particularly the mutase enzyme which converts the galactopyranose to a galactofuranose residue (which is essential for the cell wall biosynthesis, and hence the viability of the microbe). Absence of Gal*f*, and hence Gal*p*-mutase in mammals, makes Gal*f* metabolism an attractive and potential target for antimycobacterial chemotherapy.[12]

In order to validate this hypothesis a small library of sugar-derived enones bearing different aromatic moieties (potential mimic of the uridine part) were synthesized and screened against *M.tb.* $H_{37}Rv$.

The *C*-glycoside of D-galactose was prepared by the reaction of reducing sugar with acetylacetone. Out of the different procedures reported for this process, the reaction condition reported by Bragnier and Schermann[13] was found to give the desired 1-*C*-(β-D-galactopyranosyl)-propan-2-one (**5**, Scheme 1) in high yields. The ^1H NMR spectrum of the product typically showed a signal for the methyl (singlet at δ 2.20 ppm) and methylene (double doublet

☆ See Ref. 1.

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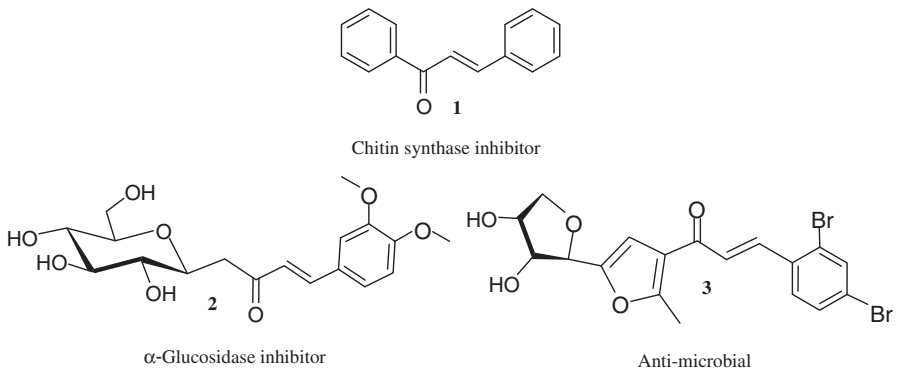


Figure 1. Bioactive chalcones and sugar-derived enones.

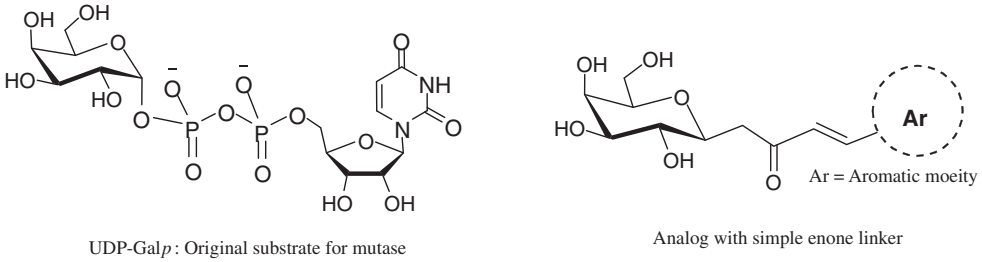
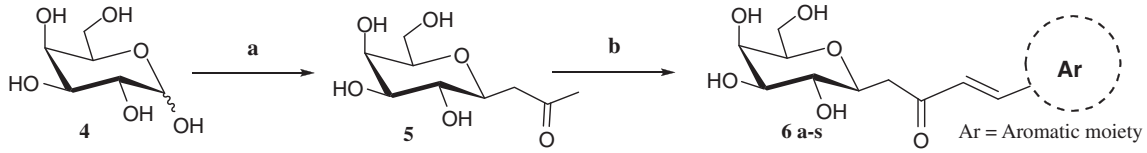


Figure 2. Comparison of UDP-Gal*p* and its potential mimic with enone linker.

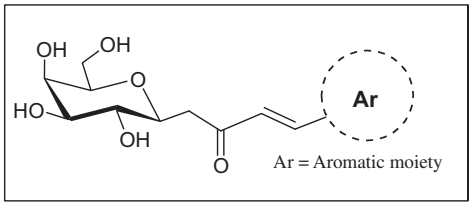


Scheme 1. Synthesis of D-galactose-derived enones. Reagents and conditions: (a) 2,4-Pentanedione, $NaHCO_3$, H_2O–THF (2:1), 90 °C, 24 h, 70%; (b) ArCHO, $LiOH{\cdot}H_2O$, MeOH, 30–60 min, 60–70%.

at δ 2.64 ppm) of the propan-2-one moiety as expected. It was further supported by the matching physical constants (mp 169–171 °C, literature 175–176 °C[13]; $[\alpha]_D$ +19.5 (*c* 1, H_2O), literature $[\alpha]_D$ +20 (*c* 1, H_2O)[13]).

Simple chalcones can be conveniently prepared by a base-catalysed condensation reaction. For carbohydrate-derived enone formation, organic bases like pyrrolidine[14] and a combination of L-proline and triethylamine[15] have been reported. These reactions catalysed by organic bases were found to be slow, low yielding and prone to the isomerisation of the glycosidic linkage. Base-catalysed epimerization of the *C*-glycoside bearing a keto group has been studied earlier,[16,17] and has been shown to attain an equilibrium. The epimerization (anomerisation) was observed even when the peracetylated *C*-glycoside was subjected to deacetylation using sodium methoxide.[18] $LiOH{\cdot}H_2O$ is a known catalyst for chalcone formation,[19] and when the enone formation was attempted in the presence of this catalyst; a quicker reaction was observed, but again it was found to be accompanied by anomerisation though to a smaller extent. Thus, sugar-derived enones **6a–6s** were prepared in the presence of $LiOH{\cdot}H_2O$[20] (Scheme 1) and were subjected to chromatographic purification to get the respective pure β-isomers (**6a–6s**). The *trans* double bond in the enone was established by the large coupling constant (J = 8.0 Hz) between the two olefinic protons.[21] Thus, a small library of sugar-derived enones were successfully synthesized using different aromatic aldehydes and were screened against *M.tb* $H_{37}Rv$.[22] Assay results are promising with MIC in the range 1.56–12.5 μg/mL. Out of the 19 compounds screened five molecules (**6a**, **6e**, **6f**, **6j**, and **6k**, Table 1) were found to be promising with MIC of 1.56 μg/mL which is equal to that of one of the first line drugs ethambutol (one of the positive controls used) under the test condition. Also four other compounds (**6b**, **6m**, **6o**, and **6p**) showed a good MIC value of 3.13 μg/mL (Table 1). Apart from the distant structural similarity of this analog to the original substrate, the electron withdrawing and donating property of the substituents and the hydrogen bonding ability of the heteroatom of the substituent on the aromatic ring, appeared to have a possible influence on the activity. Compound **6a** containing the furan moiety turned out to be one of the most active molecules, possibly due to its structural similarity to the ribofuranosyl moiety of the uridine and its hydrogen bonding properties. In compound **6b**, the electron donating and the hydrogen bond accepting methoxy substituent in the *para* position of the phenyl ring appeared to play a determining role in the activity, as possibly evident from the fact that absence of such a group and presence of an electron withdrawing nitro group in the *ortho* position of the phenyl ring made the compounds **6c** and **6d** almost inactive. The positive role of an electron donating, hydrogen bond accepting substituent in the *para* position of the phenyl ring can also be observed in compounds **6e**, **6f**, and **6k** all the three compounds are most active with MIC 1.56 μg/mL. Also in compounds **6g**, **6h**, and **6l** this effect was evident, though to a lesser extent. In the case of compound **6i**, though it possessed an electron donating and hydrogen bond accepting

Table 1
In vitro assay results against *M.tb* $H_{37}Rv$



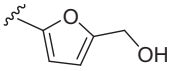
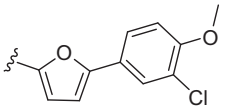
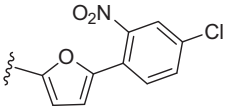
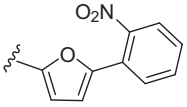
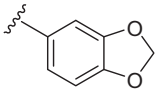
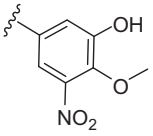
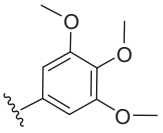
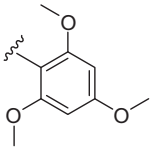
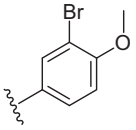
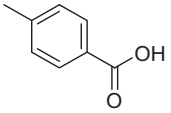
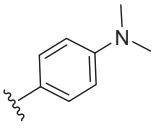
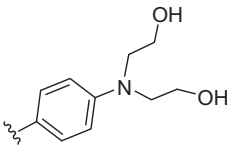
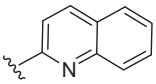
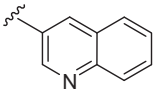
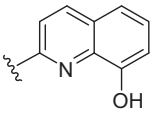
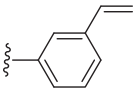
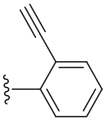
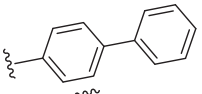
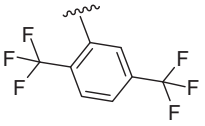
Molecule	Ar moiety	MIC (μg/mL)
6a		1.56
6b		3.13
6c		>12.5
6d		>12.5
6e		1.56
6f		1.56
6g		6.25
6h		6.25
6i		>12.5
6j		1.56
6k		1.56
6l		6.25

Table 1 (*continued*)

Molecule	Ar moiety	MIC (μg/mL)
6m		3.13
6n		6.25
6o		3.13
6p		3.13
6q		>12.5
6r		6.25
6s		12.5
Ethambutol		1.56
Isoniazid		0.05
Rifampicin		0.10

methoxy substituent in the *para* position of the phenyl ring, the bromine present in the *meta* position was found to be detrimental. One of the most active compounds **6j** possessing an electron withdrawing carboxyl group, even though did not fit in the observed trend of increased activity with electron donating substituent in the *para* position as to be expected from the foregoing observations, fit in to the hydrogen bond accepting category very well. Among the compounds containing a quinoline residue, the linkage-position appeared to influence the activity. Thus, compound **6m** linked through C-2 of the quinoline residue was more active than compound **6n** that is linked through C-3 of the same moiety; and the presence of a –OH group on C-8 of the quinoline seemed to play no role in determining the activity, as compounds **6m** and **6o** gave the same activity. Compounds containing lipophilic substituents performed poor in general with the exception of **6p** bearing a vinyl substituent at the *meta* position of the phenyl ring.

The above results indicate that the Michael-accepting property of the enones perhaps does not play a prominent role in determining the activity (e.g., compound **6j** containing an electron withdrawing carboxyl group and compound **6k** containing an electron donating dimethylamino group gave similar results (MIC, 1.56 μg/mL, Table 1). To ascertain the mechanism of action, including the role of the enone moiety investigations are in progress currently using analogous molecules with different types of linkers in the place of the enones described here. This followed by the enzyme inhibition and the toxicity studies would shed more light on the nature of the action of these potentially important molecules. Finally, evidence for the stability of the enones under the test conditions was obtained from blank experiments conducted, using two of the active compounds **6e** and **6k** as representative examples, in which the samples at the end

of 28 days when subjected to extraction and LC–MS analysis were found to be intact.

Conclusion: Though the exact target is currently unknown, galactose-derived enones were found to be highly effective as antimycobacterial agents against *M.tb* $H_{37}Rv$ as expected. SAR of the screened compounds suggests that antimycobacterial activity is not because of the Micheal-acceptor ability of these compounds. Detailed enzyme inhibition studies related to the cell wall biosynthesis to ascertain its likely inhibition and toxicity studies are currently underway and will be published in due course.

Acknowledgment

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20. *Representative procedure for synthesis of* ***6a***: To a solution of 1-C-(β-D-galactopyranosyl)-propan-2-one (1 mmol, 220 mg) in anhydrous methanol (5 mL) was added $LiOH{\cdot}H_2O$ (0.1 mmol) and was stirred for 10 min followed by the addition of the desired piperonal (1 mmol, 150 mg). The reaction mixture was stirred at room temperature for 45 min. After the completion of the reaction, it was quenched with Dowex 50WX8-200 (H^+) resin and the resin was filtered off. The filtrate was concentrated under reduced pressure and was subjected to chromatographic purification to get the pure product (230 mg, 65% β-isomer).
21. *Representative physical and NMR data for* ***6a***: yield 65%; brownish solid; mp 169.5–170.2; $[\alpha]_D$ −113.9 (*c* 0.05, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (d, *J* = 16 Hz, 1H, H-1), 7.42 (s, 1H, Ar-H), 7.13, 6.85 (2×d, *J* = 8 Hz, 2H, Ar-H), 6.76 (d, *J* = 16 Hz, 1H, H-2), 6.01 (s, 2H, –O–CH_2–O–), 3.87–3.69 (m, 1H, H-4′), 3.72–3.62 (m, 3H, H-6′a, b and H-1′), 3.50–3.44 (m, 3H, H-5′, H-3′ and H-2′), 3.09–3.04 (dd, *J* = 16 Hz, 1H, H-4a), 2.95–2.89 (dd, *J* = 9.2 Hz, *J* = 16 Hz, 1H, H-4b); ^{13}C NMR (100 M Hz, CD_3OD) δ 199.77, 149.77, 148.57, 143.51, 129.00, 125.01, 124.17, 108.10, 106.19, 101.69, 78.75, 76.70, 74.89, 70.89, 69.45, 61.14, 42.91; IR (KBr) ν_{max} 3441, 1660, 1626, 1660, 1597, 1506, 1451 cm^{-1}; MALDI-TOF MS: *m*/*z* calculated for $C_{17}H_{20}O_8$: 352.34. Found: 375.74 $[M+Na]^+$, 391.75 $[M+K]^+$.
22. *Agar dilution method*[23]: Ten-fold serial dilutions of each test compound/drug were incorporated into Middlebrook 7H11 agar medium with OADC Growth Supplement (Drug concentration from 12.5 to 0.78 μg mL^{-1}). Inoculum of *M.tb* $H_{37}Rv$ was prepared from fresh Middlebrook 7H11 agar slants with OADC Growth Supplement was adjusted to 1 mg mL^{-1} (wet weight) in Tween 80 (0.05%) saline diluted to 10^{-2} to give a concentration of approximately 10^7 cfu/mL. A 5 μL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 28 days. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of the compound required to give the complete inhibition of bacterial growth.
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