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Synthesis and evaluation of anti-tubercular activity of new dithiocarbamate sugar derivatives

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ABSTRACT

The present study was undertaken to optimize the anti-tubercular activity of 2-acetamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (OCT313, Glc-NAc-DMDC), a lead compound previously reported by us. Structural modifications of OCT313 included the replacements of the DMDC group at C-1 by pyrrolidine dithiocarbamate (PDTC) and the acetyl group at C-2 by either propyl, butyl, benzyl or oleic acid groups. The antimycobacterial activities of these derivatives were evaluated against *Mycobacterium tuberculosis* (MTB). Glc-NAc-pyrrolidine dithiocarbamate (OCT313HK, Glc-NAc-PDTC) exhibited the most potent anti-tubercular activity with the minimal inhibitory concentration (MIC) of 6.25–12.5 μg/ml. The antibacterial activity of OCT313HK was highly specific to MTB and *Mycobacterium bovis* BCG, but not against *Mycobacterium avium*, *Mycobacterium smegmatis*, *Staphylococcus aureus* or *Escherichia coli*. Importantly, OCT313HK was also effective against MTB clinical isolates, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Interestingly, OCT313HK was exerted the primary bactericidal activity, and it was also exhibited the bacteriolytic activity at high concentrations. We next investigated whether the mycobacterial monooxygenase EthA, a common activator of thiocarbamide-containing anti-tubercular drugs, also activated OCT313HK. Contrary to our expectations, the anti-tubercular activity of dithiocarbamate sugar derivatives and dithiocarbamates were not dependent on *ethA* expression, in contrast to thiocarbamide-containing drugs. Overall, this study presents OCT313HK as a novel and potent compound against MTB, particularly promising to overcome drug resistance.

More than 9.4 million people develop tuberculosis (TB) annually, and 1.7 million die each year.[1] New case of TB is still increasing all over the world, especially in low-income countries, and TB infection including both multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) is a leading cause of death worldwide. The spread of both MDR-TB and XDR-TB, due to poor compliance of anti-TB drugs, becomes a global health problem. Forty years have passed since the last development of anti-TB drug and the development of novel and innovative compounds is urgently needed.[2] We have recently reported that 2-acetamido-2-deoxy-β-D-glucopyranosyl *N,N*-dimethyldithiocarbamate (Glc-NAc-DMDC), named as OCT313, exhibited the potent antimycobacterial activity.[3]

Studies on the structure–activity relationships (SAR) at C-1, C-4 and C-6 positions of OCT313 established that the DMDC group at C-1 position was critical to the bactericidal activity. In this study, in order to improve the antimycobacterial activity of OCT313, we synthesized the derivative of dithiocarbamate group at the C-1 position and its antimycobacterial activity was evaluated. We first examined whether the elongation of alkyl side chain of dimethyldithiocarbamate, for example, diethyl and dibutyl, improved the antimycobacterial activity against *Mycobacterium tuberculosis* (MTB) H_{37}Rv. The elongation of carbon chain resulted in decreasing of anti-tubercular activity (Table 1). Previously, the antimycobacterial activity of pyrrolidine dithiocarbamate (PDTC) and dialkyldithiocarbamate derivatives have been demonstrated.[4–6] Next, we investigated whether dithiocarbamates containing heterocyclic ring, for example, 4-imidazodithiocarboxylic acid (IMTC) and PDTC were effective against MTB. As a result, PDTC was the most potent compound in our experiments, which was similar to first-line drugs in vitro.

Based on these findings, we synthesized C-1 derivative of OCT313, 2-acetamido-2-deoxy-β-D-glucopyranosyl pyrrolidine-1-

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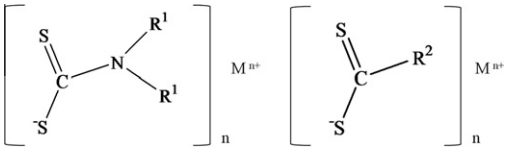
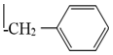
Table 1
Antimycobacterial activity of dithiocarbamate derivatives



Agent	*n*	M	R^1	R^2	MIC for (MIC, μg/ml)		
					M. tuberculosis $H_{37}Rv$	*M. bovis* BCG str. Tokyo 172	*M. smegmatis* JATA 64-01
Carbon chain							
DMDC	1	Na	$-CH_3$	—	1.56	1.56	>100
DDC	1	Na	$-CH_3CH_3$	—	3.13	3.13	>100
DDC	2	Zn	$-CH_2CH_3$	—	1.56	1.56	>100
DBuDC	2	Zn	$-CH_2CH_2CH_2CH_3$	—	12.5	12 5	>I00
Aromatic ring							
DBzDC	2	Zn			25	25	>100
Heterocyclic ring							
IMTC	1	Na			6.25	12 5	>I00
PDTC	1	NH_3			0.2	0.4	>I00

DMDC. Na, sodium dimethyldithiocarbamate; DDC. Na, sodium diethyldithiocarbamate; DDC. Zn, zinc bis (diethyldithiocarbamate); DBuDC. Zn, zinc bis(dibutyldithiocarbamate); DBzDC. Zn, zinc bis(dibenzyldithiocarbamate); IMTC. Na, sodium 4-imidazodithiocarboxylic acid; PDTC. NH_3, ammonium 1-pyrrolidine dithiocarbamate.

carbodithioate (OCT313HK, Glc-NAc-PDTC),[14,15] which is the substitution of the DMDC group at C-1 position of OCT313 to the PDTC group and was determined the antibacterial activity (Table 2). OCT313HK exhibited the potent antimycobacterial activity against both MTB and *Mycobacterium bovis* BCG Tokyo with MICs of 6.25 μg/ml and 12.5 μg/ml, respectively (Table 2). However, OCT313HK failed to inhibit the growth of *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Escherichia coli, Enterococcus faecalis, Enterococcus faecium* and *Pseudomonas aeruginosa*. (Table 2 and Supplementary Table 1). Meanwhile, PDTC exhibited the anti-tubercular activity (MIC = 0.2 μg/ml) and antibacterial activities against *S. aureus* (MIC = 8–12.5 μg/ml), *E. faecalis* (MIC = 32 μg/ml), *E. faecium* (MIC = 32 μg/ml), but not *P. aeruginosa* (Table 2 and Supplementary Table 1). These data indicate that the antibacterial spectrum of OCT313HK is narrow compared to PDTC and exhibit 2 to 4-fold higher anti-tubercular activity than OCT313 (MIC = 25 μg/ml, Table 2).

We next evaluated the antimycobacterial activities of OCT313HK and PDTC against 40 MTB clinical isolates, including drug-sensitive and drug-resistant strains. As shown in Table 3, OCT313HK exhibited the anti-tubercular activity with the MIC of 6.25–12.5 μg/ml, whereas the MIC value of PDTC was 0.2–0.4 μg/ml. Importantly, no cross resistance to almost currently used anti-TB drugs was demonstrated, that OCT313HK and PDTC exhibited comparable activities against all these strains, including five

Table 2
Antibacterial activity of OCT313HK in vitro (MIC, μg/ml)[a]

Compound	Organisms							
	M tuberculosis $H_{37}RV$	*M. bovis* BCG str. Tokyo 172	*M. avium* subsp. hominissuis 104	*M. avium* subsp. avium ATCC2529I	*M. smegmatis* JATA 64-01	*S. aureus*	MRSA 873	*E. coli* DH5α
Synthetic derivative								
OCT313HK	6.25	12.5	100	50	>100	>100	>100	>100
OCT313	25	31.3	>100	>100	>100	>100	>100	>100
Raw material								
Glc-NAc free	>100	>100	>100	>100	>100	>100	>100	>100
DMDC. Na	0.78	1.56	>100	>100	>100	>100	>100	>100
PDTC. NHi	0.2	0.4	3.13	3 13	100	12.5	12.5	12.5
anti-IB drug								
INH	0.04	0.04	1.56	3 13	6.25	>100	>100	>100
RFP	0.004	0.004	0.25	<0.05	1.56	0.002	0.004	50
SM	0.39	0.2	1.56	3,13	0.39	50	>100	50
EB	2.5	1.5	1.6	1.6	12.5	>100	>100	>100
KM	1.56	0.3	3.13	3.13	3.13	12.5	>100	12.5
CPFX	0.39	0.1	0.39	1.56	0.39	0.2	ne	0.2
β-Lactam antibiotics								
PCG	500	500	ne	ne	ne	31.3	>500	25
ABPC	12.5	12.5	ne	ne	ne	50	>100	>100
IPM	3.13	3.13	ne	ne	ne	0.1	0.1	0.1

[a] Broth dilution methods using MiddleBrook 7H9 broth containing albumin, dextrose, and catalase for derivatives (ne, not examined). For *Staphylococcus aureus*, we used the LB broth. OCT313HK, Glc-NAc-PDTC; OCT313, Glc-NAc-DMDC, Glc-NAc free, *N*-acetyl glucosamine; DMDC. Na, sodium dimethyldithiocarbamate; PDTC. NH_3, ammonium 1-pyrrolidine dithiocarbamate; INH, isoniazid; RFP, rifampicin, SM, streptomycin; EB, ethambutol; KM, kanamycin, PAS, para-aminosalicylic acid; CPFX, ciprofloxacin; PCG, penicillin G; ABPC, aminobenzyl penicillin; IPM, imipenem.