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N-D-Aldopentofuranosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea derivatives: Potential anti-TB therapeutic agents

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Abstract—Thiocarlide (THC; *N*,*N*′-bis[*p*-(isoamyloxy)phenyl]-thiourea; also known as isoxyl) has been used in the past as anti-tuberculosis agent. In an effort to improve the therapeutic value of THC several *N*-pentofuranosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea derivatives were synthesized by coupling of an aniline derivative and pentofuranosyl isothiocyanates. The MIC values of the new products against *M.tb* indicate that this new approach to the synthesis of potential anti-TB therapeutic agents was successful.

The human pathogen *Mycobacterium tuberculosis* (*M. tb*) causes tuberculosis and is responsible for the deaths of millions of people, the most by any single infectious agent.[1]

One of the therapeutic agents that were used in the clinical treatment of tuberculosis in the 1960s was a derivative of thiourea known as thiocarlide (THC; *N*,*N*′-bis[*p*-(isoamyloxy)phenyl]-thiourea; **1**, Fig. 1; also known as Isoxyl®). This powerful compound was first chemically synthesized in 1951.[2] The minimum inhibitory concentration (MIC) value of THC against most clinical isolates of *M. tb*, including multi-drug resistant ones, was determined to be 2 μg/ml.[3] Nevertheless, the clinical use of THC was discontinued, apparently because of the poor bioavailability of the highly non-polar product.[4]

In an effort to improve the therapeutic value of THC, we have synthesized several *N*-glycosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea derivatives and determined their MIC values against *M.tb*.[5] The arabinfuranosyl product (**2**, Fig. 2), the only pentosyl derivative in the previous work,[5] turned out to be the most promising one, exhibiting an MIC value of 2.5 μg/ml. This encouraging result prompted us to focus our attention on the synthesis and testing of the remaining D-aldopentofuranosyl derivatives. These compounds were obtained by the coupling of protected pentofuranosyl isothiocyanate with *p*-isoamyloxy aniline (Scheme 1), as previously described by us[5] and others.[6–8] However, we have used acetates as the protecting groups instead of the TBDMS groups used previously[5] as resynthesis of the arabino product showed an increase in the yield of the final product because of the milder deprotecting conditions employed.

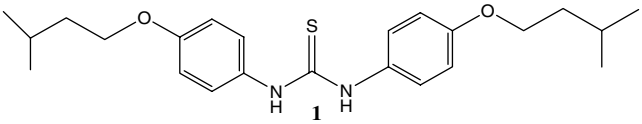

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Fig. 1. The structure of THC.

The isothiocyanates were synthesized from the corresponding tetraacetates.[9] All of the fully acetylated D-aldopentofuranoses are already described.[9] The general scheme consists of conversion of the pentose into the corresponding methyl pentofuranoside, and subsequent acetylation.[10] We then subjected these intermediates to acetolysis, using a mixture of glacial acetic acid, acetic anhydride, and a 5% solution of sulfuric acid in acetic acid. Under these conditions the tetraacetates were obtained in high yields (90–95%) and no purification of the products was necessary. The ^{1}H NMR data of the tetraacetates are in agreement with the reported values,[9] thus confirming that the products exist in the furanose form. Conversion of the acetates into the bromides was carried out by treatment with trimethylsilyl bromide in

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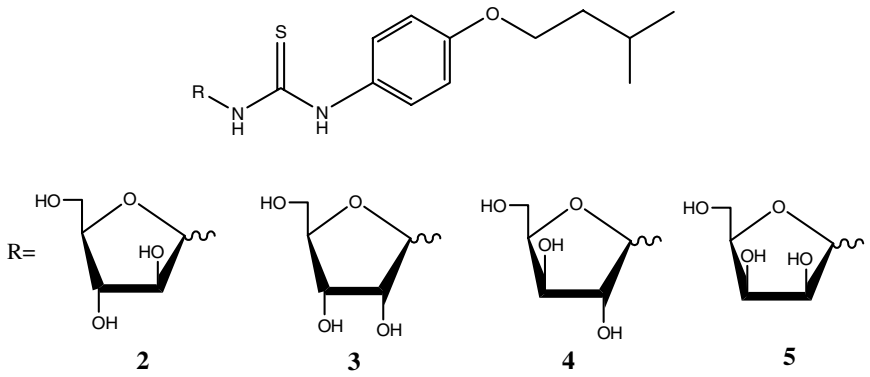
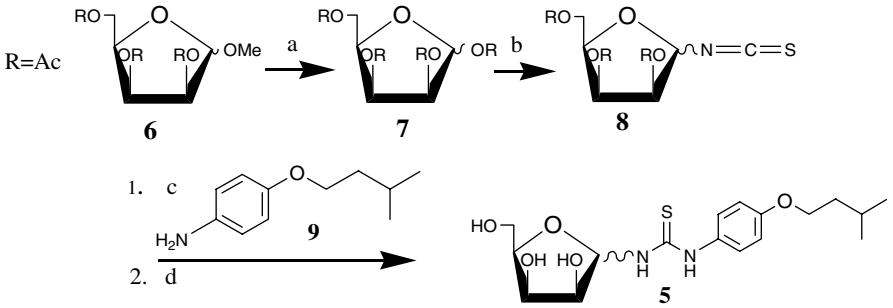


Fig. 2. The structures of the *N*-D-pentofuranosyl-*N*′-*p*-(isoamyloxy) phenyl-thiourea products.



Scheme 1. Synthesis of *N*-D-lyxofuranosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea. Reagents and conditions: (a) AcOH, Ac_2OH, 5% H_2SO_4; rt, 2.5H. (b1) BrTMS, CH_2Cl_2; rt, 1 h. (b2) KSCN, tetrabutylammonium hydrogen sulfate, 4A molecular sieves, acetonitrile; rt, 2 h. (b3) The products from (b1) and (b2), acetonitrile, 65°, 2 h; (c) isoamyloxy aniline, pyridine; rt, 70 min. (d) *M* $NaOCH_3$, MeOH.

methylene chloride.[11] The isothiocyanates were prepared from the corresponding bromides by treatment with potassium thiocyanate and tetrabutyl ammonium hydrogen sulfate. The pentofuranosyl isothiocyantes were found to be unstable, and their purification by column chromatography on silica gel could not be achieved. After extraction of the crude isothiocyanates with a petroleum ether–ethyl acetate 3:1 mixture, they were coupled to *p*-(isoamyloxy) aniline (**9**, Scheme 1) as described before.[5] Using this procedure (Scheme 1) we first prepared the D-xylo and D-lyxo products (**4** and **5**, respectively). The analogous D-ribo product (**3**) could be obtained by the same procedure, but a cleaner product was obtained when the commercially available 2,3,5-tri-*O*-benzoyl-D-ribofuranose was used as the starting material. The synthesis of the arabinose product (**2**) has already been described by us.[5] However, in that synthesis TBDMS groups were used, and the removal of these groups (by treatment with ammonium fluoride in methanolic ammonium hydroxide at 65°) in the final step was not as efficient as the O-deacetylation described in this report. Accordingly, we repeated the synthesis of **2**, using tetra-*O*-acetyl-D-arabinofuranose as a starting material.

The structures of the new products were confirmed by mass spectrometry and ^1H NMR spectroscopy. In the positive electro spray mode the products exhibited the 393 ion which corresponds to M+Na. The ^1H NMR spectra showed that the D-xylo and D-ribo products were anomerically pure while the other products existed as a mixture of the two anomers that could not be separated by chromatography. The signals of the anomeric protons appeared at low field, as expected (δ 5.88–5.45). In all cases, these signals were broad and the determination of the coupling constants was not feasible. The ring carbohydrate protons usually appeared as complex multiplets.The signals of the isoamyloxyphenyl moiety appeared as a pair of doublets in the aromatic region (J = 9.0 Hz; except the spectrum of the D-lyxo product which showed two pairs of doublets, one for each of the two anomers), a two proton-triplet at around δ 4.0 ppm, a one-proton multiplet at δ 1.96–1.82 ppm, a two proton-doublet of doublets at δ 1.7 ppm, and a pair of three protons—singlets at δ 1.03 ppm and δ 1.01 ppm.

The new products were tested as growth inhibitors against *M. tuberculosis* H37Rv, using the microplate alamar blue dye assay.[12] Re-testing of the arabino analog (**2**) showed it to be more potent than THC itself (which was tested under the same conditions) while the ribo analog (**3**) displayed an MIC value in the same range as THC (Table 1).[13] The D-xylo analog (**4**) was somewhat less active and the D-lyxo product (**5**) showed activity only at a concentration of 50 μg/mL. The sensitivity of the products to the stereochemical configuration suggests that the carbohydrate group not only adds needed hydrophilicity to the molecule but also adds specificity.

Further testing of the best product (**2**) in mice, including the bioavailability assay, is now in progress. The bioavailability assay estimates the drug levels in mice at specific time points post oral dosing, using the growth

Table 1. MIC values of THC and products **2**–**5** against *M. tuberculosis* H37

Product	MIC values (μg/mL)
THC	2.5–5.0
2	1.56–3.125
3	3.125–6.25
4	6.25–12.5
5	50

The MIC values were determined by the microplate alamar blue dye assay. INH (isoniazid) was used as a standard at a concentration of 0.35 μg/mL.

of *M. tuberculosis* H37Rv as an indicator for drug activity.[14] Also, the *c*Log *P* (log of the octanol/water partition coefficient) value of the new products, **2** and **3**, is 1.56, which is significantly lower than that of THC (6.22), and this decrease in *c*Log *P* may result in an increase in bioavailability.

Acknowledgment

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10. General procedure for the synthesis of *N*-*D*-aldopentofuranosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea derivatives: To a cold (ice bath) solution of the methyl 2,3,5-tri-*O*-acetyl-pentofuranoside (610 mg) in acetic acid (1.6 mL) and acetic anhydride (1.6 mL) was added a 5% solution of sulfuric acid in acetic acid (0.8 mL). The ice bath was removed and the mixture was stirred at room temperature for 3 h. It was neutralized with solid sodium bicarbonate and the product was extracted with ethyl acetate. The organic solution was washed with water and dried to give 656 mg (98%) of the tetraacetate. A part of the product (290 mg, 0.92 mmol) was dissolved in methylene chloride (0.6 mL) and trimethylsilyl bromide (0.6 mL) was added. The mixture was stirred at room temperature for 2 h and dried. In a separate flask a mixture of potassium thiocyanate (250 mg), tetrabutylammonium hydrogen sulfate (205 mg) and molecular sieves (4A, 1 g) in acetonitrile (10 mL) was stirred at room temperature for 2 h and then added to the crude bromide. The reaction mixture was stirred at 65° for 2 h. It was cooled and filtered through celite. The filtrate was dried and petroleum ether–ethyl acetate 3:1 was added to the residue. The organic extract was filtered and the filtrate was dried to give the crude isothiocyanate that was not purified. The crude isothiocyanate was then treated with *p*-isoamyloxy aniline[5] (100 mg, 0.6 mmol) in pyridine (2 mL) at room temperature for 70 min. The mixture was dried and the residue was chromatographed on silica gel (Sigma, mesh 70–230). Elution with ethyl acetate–petroleum ether 1:1 removed an un-identified by-product. Continued elution with the same solvent system gave the coupled product. After re-chromatography of mixed fractions a combined sample of 165 mg (54%) was obtained. Part of the product (115 mg) was treated with *M* sodium methoxide solution (0.15 mL) in methanol (1.5 mL) at room temperature for 50 min. The mixture was neutralized with Dowex 50 (H^+) and the resin was filtered off and washed with methanol. The filtrate was dried and the residue was chromatographed on silica gel. Elution with methylene chloride–methanol 9:1 removed fast moving impurities. Continued elution with methylene chloride–methanol 7:1 gave the product (52 mg, 60%).
Compound **2**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.26 (d, 2H, J = 9.0 Hz), 6.97 (d, 2H, J = 9.0 Hz), 5.88 (bs, 0.4H, H-1 α), 5.58 (bd, 0.6H, H-1 β), 4.05 (t, 2H, J = 6.6 Hz), 3.96-3.90 (m, 2H), 3.82–3.58 (m, 2H), 3.64 (dd, 1H, J = 3.4 Hz, J = 12.1 Hz), 1.96–1.82 (m, 1H), 1.71 (dd, 2H, J = 6,6 Hz, J = 13.2 Hz), 1.03 (s, 3H), 1.01(s, 3H); MS ESI$^+$ *m*/*z* 393.141 $(M+Na)^+$.
Compound **3**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.27 (2H, J = 9.0 Hz), 6.98(d, 2H, J = 9.0 Hz), 5.84 (bs, 1H), 4.05 (t, 2H, J = 6.6 Hz), 3.94 (bs, 1H), 3.78 (t, 1H, J = 3.0 Hz), 3.76–3.58 (m, 2H), 3.52 (dd, 1H, J = 3.6 Hz, J = 12.8 Hz), 1.96–1.82 (m, 1H), 1.71 (dd, 2H, J = 6,6 Hz, J = 13.2 Hz), 1.03 (s, 3H), 1.01 (s, 3H); MS ESI$^+$ *m*/*z* 393.136 $(M+Na)^+$.
Compound **4**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.28 (2H, J = 9.0 Hz), 6.95 (d, 2H, J = 9.0 Hz), 5.45 (bs, 1H), 4.05 (t, 2H, J = 6.6 Hz), 4.02 (t, 1H, partially obscured by the 4.05 signal, J = 6.6 Hz), 3.95 (t, 1H, J = 6.6 Hz), 1.96–1.92 (m, 1H), 1.76 (dd, 2H, J = 6,6 Hz, J = 13.2 Hz), 1.03 (s, 3H), 1.01 (s, 3H); MS ESI$^+$ *m*/*z* 393.145 $(M+Na)^+$.
Compound **5**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.29, 7.26 (2d, 2H, J = 9.0 Hz), 6.98, 6.96 (2d, 2H, J = 9.0 Hz), 5.82 (bs, 0.5H, H-1 α), 5.72 (bd, 0.5H, H-1 β), 4.06–4.02 (m, 1H, obscured by the 4.04 signal), 4.04 (t, 2H, J = 6.6 Hz), 3.97 (dd, 1H, J = 2.1 Hz, J = 4.8 Hz), 3.86 (dd, 1H, J = 2.1 Hz, J = 11.5 Hz), 3.80–3.65 (m, 2H), 1.98–1.84 (m, 1H), 1.71 (dd, 2H, J = 6,6 Hz, J = 13.2 Hz), 1.03 (s, 3H), 1.01 (s, 3H); MS ESI$^+$ *m*/*z* 393.139 $(M+Na)^+$.
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