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# N-Glycosyl-N′-[p-(isoamyloxy)phenyl]-thiourea Derivatives: Potential Anti-TB Therapeutic Agents

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# *N*-Glycosyl-*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 an anti-tuberculosis agent. In an effort to improve the therapeutic value of THC, several *N*-glycosyl-*N*′-[*p*-(isoamyloxy)phenyl]-thiourea derivatives were synthesized by coupling an aniline derivative and glycosyl isothiocyanates. The minimum inhibitory concentration (MIC) values of the new products against *M. tuberculosis* were determined.

**Keywords:** Isoxyl, *Mycobacterium tuberculosis*, thiocarlide, thiourea

## INTRODUCTION

The human pathogen *Mycobacterium tuberculosis* (*M.tb*) causes tuberculosis and is responsible for the most deaths of people by any single infectious agent.[1] Nearly 33% of the world's population is infected with *M.tb*, and 10% are predicted to develop the disease during their lifetimes.[2] One of the therapeutic agents 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.[3] The minimum inhibitory concentration (MIC) of THC against most clinical isolates of *M.tb*, including multidrug-resistant ones, was determined to be

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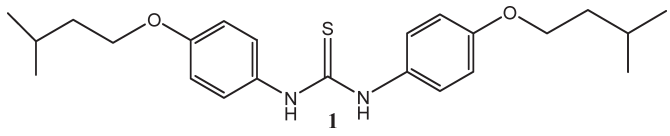


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*Figure 1.* Structure of THC.



2 μg/ml.[4] In spite of this promising result, the clinical use of THC was discontinued, apparently because of the poor bioavailability of the highly nonpolar product.[5]

In an effort to synthesize THC analogs with better hydrophilic character, we have examined a novel approach, consisting of the substitution of one of the isoamyloxyphenyl moieties in **1** by a carbohydrate. The synthesis of di-*N*,*N*′-glycosyl derivatives of thiourea (both symmetrical and nonsymmetrical) has already been described.[6–8] Syntheses of mono-*N*-glycosyl-*N*′-alkyl and even *N*-glycosyl-*N*′-aryl-thiourea derivatives have also been reported.[7,9–11] However, the synthesis of *N*-glycosyl-*N*′-aryl thiourea derivatives in which the aryl moiety is *p*-isoamyloxyphenyl (as in **1**) has not been described yet. In this article, we report the synthesis of this new type of thiourea derivatives (Fig. 2).

The products described in this article were obtained by the coupling of a suitably protected glycosyl isothiocyanate with *p*-isoamyloxyphenyl aniline (**4**) following the general schemes described in the literature,[9,10,12] but in several cases more efficient reagents were utilized.

The synthesis of the key intermediate **4** was accomplished by alkylation of *p*-nitrophenol (**2**) with *p*-isoamyloxy bromide in the presence of a crown ether, potassium carbonate in acetone to give **3**,[13] and subsequent reduction of **3**. The reduction of the nitro function could be achieved by using tin chloride in ethanol,[14] but a product with a higher degree of purity (95%, as judged by $^1$H NMR and thin-layer chromatography, TLC) was obtained when Pd/C 10% catalyst and ammonium formate in ethanol were employed (Scheme 1).[15] The yields in this two-step synthesis were high (87% and 85% respectively).

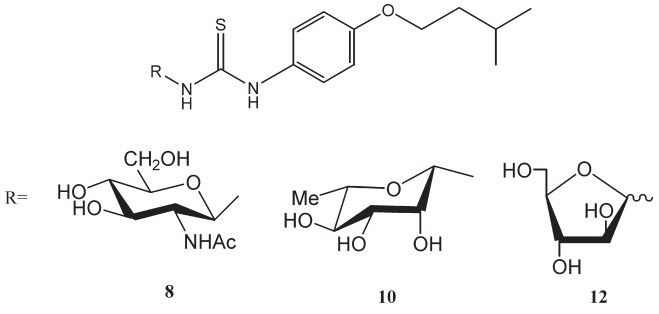


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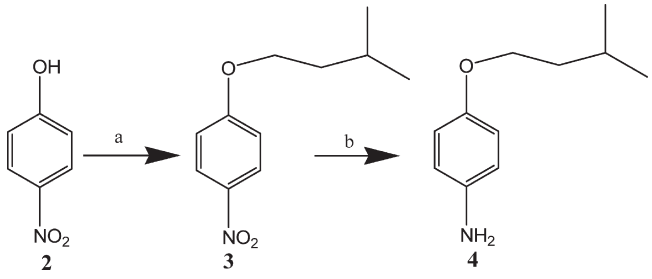
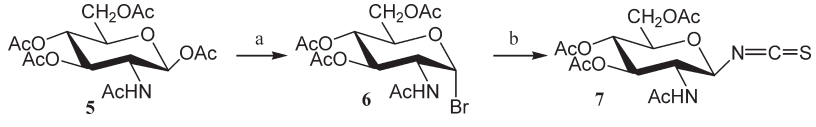
*Figure 2.* Structures of the *N*-glycosyl-*N*′-(isoamyloxyphenyl)-thiourea derivatives.




***Scheme 1.*** Synthesis of *p*-isoamyloxy-aniline: (a) isoamyloxy bromide, anhydrous potassium carbonate, 18-crown-6 ether, boiling acetone, 15 h; (b) Pd/C 10%, ammonium formate, ethyl alcohol, 3 h, rt.

This approach has been utilized in the past,[16] but the reduction step was significantly inconvenient (a mixture of sodium sulfide and sulfur in refluxing methanol for 15 h). Alternatively, product **4** has been obtained in the past by treatment of *p*-isoamyloxy acetamidophenol with 50% aqueous sodium hydroxide solution under reflux.[17] The structure of **4** was confirmed by $^1$H NMR: a new signal, corresponding to the amino group protons appeared ($\delta$ 3.459), and the signals of the aromatic protons shifted to a higher field, as expected (from $\delta$ 8.161 and 6.938 to $\delta$ 6.810 and 6.678).

Conversion of 2-acetamido-2-deoxy-$\beta$-D-glucopyranose tetraacetate (**5**) into the $\alpha$-bromide **6** and subsequent treatment with potassium thiocyanate (Scheme 2) gave the corresponding isothiocyanate (**7**), which was found to have the $\beta$-configuration. In support of the structure of **7**, there was a significant shift of the H-1 signal in its $^1$H NMR spectrum: from $\delta$ 6.291 (in **6**) to 5.305. Finally, coupling of the isothiocyanate **7** with the amine **4** in pyridine[10] and subsequent O-deacetylation yielded the *N*-glucosyl thiourea derivative **8**. The analytical data for **8** are in agreement with the structure and with the $\beta$-D-configuration. In a similar manner, L-rhamnose was converted into the $\alpha$-L-*N*-rhamnosyl thiourea derivative **10** (via the corresponding isothiocyanate **9**, not shown). The synthesis of the analogous D-arabino product (**12**) proceeded by the same scheme, using 2,3,5-tri-O-tert butyldimethylsilyl (TBDMS)-$\alpha$-D-arabinofuranosyl bromide[18] as the starting material. The use of TBDMS groups for protection in the case of the arabino product is necessary to ensure that the product will exist in the furanose configuration.




***Scheme 2.*** Synthesis of the *N*-acetylglucoseamine-isothiocyanate derivative: (a) 30% HBr/AcOH, DCM, 2 h, rt; (b1) KSCN, tetrabutylammonium hydrogen sulfate, 4A molecular sieves, acetonitrile, 2 h, rt; (b2) mixture of the thiocyanate and 6, 2 h, 65 °C.

Removal of the TBDMS groups was achieved by treatment with ammonium fluoride as described before.[18] In this case, the final product was isolated as a mixture of the $\alpha$ and $\beta$ anomers (as indicated by $^1$H NMR).

The final products, **8**, **10**, and **12**, were fully characterized by $^1$H NMR, mass spectroscopy, elemental analysis, and optical rotation. The $^1$H NMR data for the gluco (**7**) and the rhamno (**9**) intermediates appear in the Experimental section. Attempts to obtain an analytical sample of the arabino-isothiocyanate intermediate (**11**, not shown) failed because of the instability of the product.

The new products were tested as growth inhibitors against *M.tb* H37Rv, using the microplate alamar blue dye assay.[19] Encouragingly, one product (**12**) was found to have an MIC value of 2.5 μg/mL, which is almost as good as that of the parent THC product (Table 1). Further testing of this product will be conducted.

## EXPERIMENTAL

### *p*-Isoamyloxy Aniline (4)

A mixture of *p*-nitrophenol (270 mg, 1.94 mmol), isoamyl bromide (0.6 mL, 4.76 mmol), anhydrous potassium carbonate (290 mg, 2.10 mmol), and 18-crown-6 ether (74 mg) in acetone (6 mL) was stirred at 64 °C under nitrogen for 16 h. The mixture was cooled and diluted with acetone, and the insoluble solid material was filtered off and washed with acetone. The filtrate was dried, and the residue was chromatographed on silica gel (Sigma, 70–230 mesh). Elution with ethyl acetate–petroleum ether 1:1 gave the chromatographically pure product (**3**; 355 mg, 87% yield). The homogeneity of the product was established by $^1$H NMR as well. Part of **3** (200 mg, 0.95 mmol) was treated with Pd/C 10% catalyst (120 mg) and ammonium formate (500 mg, 7.93 mmole) in ethanol (5 mL) at rt for 3 h. The mixture was filtered through Celite,® and the filtrate was dried to give the product **4**, which was found by $^1$H NMR and TLC to be 95% pure (145 mg, 85% yield).

***Table 1.*** MIC values of products **8**, **10**, and **12** against *M. tuberculosis* H37Rv

| Product | MIC values (mg/mL) |
|---|---|
| **8** | 50–100 |
| **10** | 8 |
| **12** | 2.5 |

*Note*: 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.

### 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl isothiocyanate (7)

A solution of hydrogen bromide in acetic acid (30%; 0.4 mL, 1.48 mmol) was added to a cold (ice bath) suspension of 2-acetamido-2-deoxy-β-D-glucopyranose-1, 3,4,6-tetraacetate (220 mg, 0.56 mmol) in methylene chloride (2.5 mL). The mixture was then stirred at room temperature for 2 h. The excess of the hydrogen bromide was removed by evaporation under nitrogen, and the residue was diluted with ethyl acetate. The organic solution was washed with a saturated sodium bicarbonate solution and dried to give the corresponding bromide. In a separate flask, potassium thiocyanate (133 mg, 1 mmol) was treated with tetrabutylammonium hydrogen sulfate (160 mg, 0.47 mmol) and molecular sieves (4A, 750 mg) in acetonitrile (10 mL) at room temperature for 3 h. This mixture was then added to the crude bromide, and the new mixture was stirred at 65 °C for 1.5 h. The mixture was cooled, and the solid material was filtered off and washed with acetone. The filtrate was dried, and the residue was chromatographed on silica gel. Elution with ethyl acetate gave the chromatographically homogeneous product (116 mg; 52%). The homogeneity of the product was established by $^1$H NMR as well.

### *N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl]-*N*′-(*p*-isoamyloxyphenyl)thiourea (9)

*p*-Isoamyloxyaniline (**4**, 62 mg, 0.34 mmole) was dissolved in pyridine (1 mL), and the solution was added to the isothiocyanate intermediate **7**. The mixture was stirred at room temperature for 2 h and dried. The residue was chromatographed on silica gel, and the chromatographically homogenous product was eluted with methylene chloride–methanol 9:1. Yield 159 mg (94%). Part of the product (28 mg) was dissolved in methanol (1 mL), and *M* sodium methoxide solution in methanol (0.2 mL) was added. The mixture was stirred at room temperature for 2 h and neutralized with Dowex 50 ($H^+$). The resin was filtered off, and the filtrate was dried. The residue was chromatographed on silica gel. Elution with methylene chloride–methanol 5:1 gave the product (16 mg, 76%). The homogeneity of the product was established by $^1$H NMR, mass spectrometry, and elemental analysis (CHN). The product was also characterized by optical rotation.

## ANALYTICAL DATA

### Compound 3

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.161 (d, $J = 9.0$ Hz, 2H, aromatic), 6.938 (d, $J = 9.0$ Hz, 2H, aromatic), 4.084 (t, $J = 6.6$ Hz, 2H, $CH_2$), 1.920–1.784

(m, 1H, CH), 1.611 (dd, $J = 6.9$, 13.5 Hz, 2H, $CH_2$), 0.995, 0.973 (2s, 6H, $2CH_3$).

### Compound 4

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 6.810 (d, $J = 9.0$ Hz, 2H, aromatic), 6.678 (d, $J = 9.0$ Hz, 2H, aromatic), 3.976 (t, $J = 6.6$ Hz, 2H, $CH_2$), 3.459 (s, 2H, $NH_2$), 1.980–1.740 (m, 1H, CH), 1.715 (dd, $J = 6.6$, 13.2 Hz, 2H, $CH_2$), 1.041, 1.019 (2s, 6H, $2CH_3$).

### Compound 7

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 6.063 (d, $J = 8.7$ Hz, 1H, NH), 5.366 (dd, $J = 9.3$, 10.5 Hz, 1H, H-3), 5.305 (d, $J = 9.3$ Hz, H-1), 5.124 (t, $J = 9.6$ Hz, 1H, H-4), 4.276 (dd, $J = 4.8$, 12.6 Hz, 1H, H-6a), 4.179 (dd, $J = 2.4$, 12.6 Hz, 1H, H-6b), 4.091–4.056 (m, 1H, H-2), 3.850–3.808 (m, 1H, H-5) 2.108, 2.079, 2.076, (3s, 9H), 1.975 (s, 3H), 10950–1.801 (m, 1H), 1.705 (dd, $J = 6.6$, 13.2 Hz, 2H), 1.015, 0.994 (2s, 6H).

### Compound 8

$^1$H NMR (300 MHz, methanol-*d*): $\delta$ 7.198 (d, $J = 9.0$ Hz, 2H), 6.97 (d, $J = 9.0$ Hz, 2H), 5.558 (bd, $J = 8.1$ Hz, 1H), 4.065 (t, $J = 6.6$ Hz, 2H), 3.876 (m, 2H), 3.727 (dd, $J = 4.5$, 11.7 Hz, 1H), 3.536 (dd, $J = 8.4$, 9.9 Hz, 1H), 3.368 (m, 2H, partially obscured by the solvent signal), 2.03 (s, 3H), 1.898 (m, 1H), 1.719 (dd, $J = 6.6$, 13.2 Hz, 2H), 1.029, 1.023 (2s, 6H); mass spectrometry (electrospray positive ion): 442.4 (M + 1); optical rotation: $[\alpha]_D$ + 22.5° (*c* 1.1, methanol). Calc. for $C_{20}H_{31}N_3O_6S$: C, 54.40; H, 7.07; N, 9.51. Found: C, 54.64; H, 7.31; N, 9.66.

### Compound 9

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 5.376 (d, $J = 1.5$, 1H, H-1), 5.164–5.102 (m, 2H), 4.925 (t, $J = 9.6$ Hz, 1H, H-4), 3.922–3.823 (m, 1H, H-5), 2.036. 1.949, 1.873 (3s, 9H, $COCH_3$), 1.133 (d, $J = 6.3$ Hz, C-5-$CH_3$).

### Compound 10

$^1$H NMR (300 MHz, methanol *d*): $\delta$7.329 (d, $J = 9.0$ Hz, 2H), 6.946 (d, $J = 9.0$ Hz, 2H), 5.880 (bs, 1H), 4.044 (t, $J = 6.6$ Hz, 2H), 3.971 (dd, $J = 2.4$, 3.0 Hz, 1H), 3.623 (dd, $J = 3.0$, 8.4 Hz, 1H), 3.585–3.465

(m, 2H), 1.95–1.82 (m, 1H), 1.712 (dd, $J = 6.6$, 13.2 Hz, 2H), 1.359 (d, $J = 6.0$ Hz, 3H), 1.035, 1.014 (2s, 6H). Mass spectrometry (electrospray positive ion): 385.1 (M + 1); optical rotation: $[\alpha]_D$ −52° (*c* 1.3, methanol). Calc. for $C_{18}H_{28}N_2O_5S$: C, 56.23; H, 7.34; N, 7.28. Found: C, 56.00; H, 7.20; N, 7.38.

### Compound 12

$^1$H NMR (400 MHz, methanol *d*): $\delta$ 7.1949 (d, $J = 8.8$ Hz, 2H), 6.904 (d, $J = 8.8$ Hz, 2H), 4.05–3.85 (m, 3H), 3.78–3.45 (m, 3H), 3.28–3.27 (m, obscured by the solvent signal, 2H), 1.88–1.78 (m, 1H), 1.68–1.60 (m, 2H), 0.94 (d, $J = 6.8$ Hz, 6H). Mass spectrometry (FAB): 371.2 (M + 1); calc. for $C_{17}H_{26}N_2O_5S$: C, 55.12; H, 7.07; N, 7.56. Found: C, 54.85; H, 7.00; N, 7.38.

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