Supplemental Material

Metabolism and Disposition of Verinurad, a Uric Acid Reabsorption Inhibitor, in Humans

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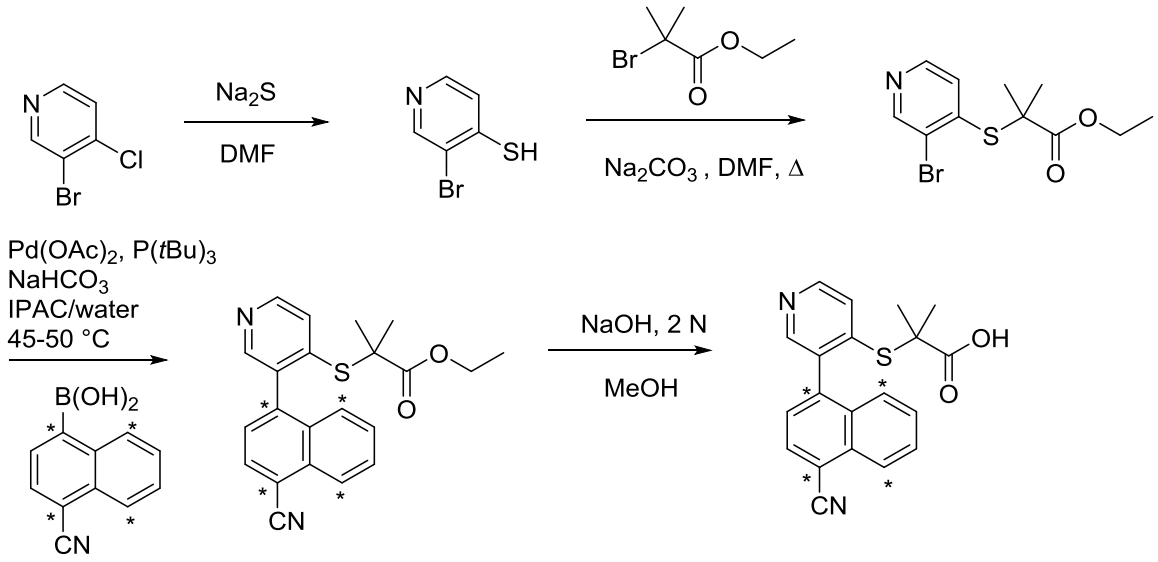
Synthetic Methodology for 14Cverinurad, M1, M4 and M8

To a suspension of anhydrous sodium sulfide (6.08 g, 78 mmol) in DMF (100 ml) at 130 °C with rigorous stirring was added drop-wise a solution of 3-bromo-4-chloropyridine (10.0 g, 52 mmol) in DMF over a period of 1 hr. The DMF was removed under reduced pressure. Water (100 mL) was added to the dry residue and the resulting solution was cooled to -5 ^{o}C. Aqueous HCl (6 N, 26 mL) was added dropwise with rigorously stirring. The resulting yellow paste (pH ~6-7) was filtered and washed with water. The yellow solid collected was dried under reduced pressure to give 3-bromopyridine-4-thiol as a yellow dry solid (9.5 g, 96%). A mixture of 3-bromopyridine-4-thiol (4.75g 25 mmol), ethyl 2-Bromoisobutyrate (7.31 g, 37.5 mmol), and sodium carbonate (5.3 g, 50 mmol) in DMF (50 mL) was heated to 60 °C with stirring for 1 hour. The reaction mixture was partitioned between water and ethyl acetate. The organic layer was separated and washed with water and saturated sodium chloride. The organic layer was dried over sodium sulfate, concentrated, and purified by normal phase chromatography (a gradient of 0-25% ethyl acetate in hexane) to yield ethyl 2-(3-bromopyridin-4-ylthio)-2-methylpropanoate as a pale yellow oil (6.9 g, 23 mmol, 92%). A mixture of ethyl 2-(3-bromopyridin-4-ylthio)-2-methylpropanoate (140 mg, 0.46 mmol), 4-cyano-1,4,5,8-^{14}C- naphthyl boronic acid (95 mg, 0.48 mmol), sodium bicarbonate (155 mg, 1.8 mmol), palladium acetate (4.1 mg, 0.018 mmol) and tritertbutylphosphine 10% in hexane (168 mg, 0.08 mmol) in isopropyl acetate (0.42 mL)

and water (0.56 mL) was stirred at 45-50 °C until reaction completion. The mixture was cooled and diluted with additional isopropyl acetate, the organic layer was separated and washed with brine and concentrated under reduced pressure. Methanol was added, then evaporated twice to chase other organic solvents, then added again and heated to 40-50 °C and cooled to allow crystallization of ethyl 2-(3-(4-cyanonaphthalen-(1,4,5,8-^{14}C)-1-yl)pyridin-4-ylthio)-2-methylpropanoate which was isolated by filtration and dried (170 mg, 75%). To ethyl 2-(3-(4-cyanonaphthalen-(1,4,5,8-^{14}C)-1-yl)pyridin-4-ylthio)-2-methylpropanoate (100 mg, 0.3 mmol) was added methanol (1.6 mL), and sodium hydroxide (2 M aqueous, 1.6 mL). The resulting mixture was stirred at ambient temperature for 2 hours then concentrated under reduced pressure. To the residue was added HCl (6 N aqueous) with stirring until pH reached 6, resulting in the formation of a white precipitate, which was isolated by filtration. The solid was washed with water, and dried under vacuum overnight to yield ^{14}C verinurad as a white powder (56 mg, 64%) which was further purified by HPLC. The radiochemical purity was 99.3% and the specific activity of the isolated compound was 57.1 mCi/mmol.



M1 Synthesis

Metabolite M1 was synthesized starting from verinurad, and condensing 1-bromo- *O*-acyl-protected glucuronic acid methyl ester in a mixture of pyridine and toluene in the presence of cesium carbonate. After workup, the oil obtained was purified by silica gel chromatography. The residue was then subjected to enzymatic deprotection, followed by HPLC purification to lead to M1 as a white solid. Purity was 87.6%. ^{1}H NMR DMSO d_6 + D_2O (ppm); 8.48 (m, 1H), 8.33 (s, 1H), 8.19 (m, 2H), 7.81 (t, 1H), 7.62 (m, 1H), 7.55-7.35 (m, 3H), 5.42 (d, 1H), 3.76 (m, 1H), 3.36-3.20 (m, 3H), 1.40-1.31 (m, 6H)

M4 and M8 Synthesis

A mixture of 3-Bromo-4-chloropyridine, 4-cyanonaphthyl boronic acid, sodium bicarbonate, palladium acetate and tritertbutylphosphine in isopropyl acetate and water was stirred at 60 ^{o}C until reaction completion. The 4-(4-chloropyridin-3-yl)-1-naphthonitrile product was isolated by precipitation, then reacted with hydrogen peroxide in acetic acid to yield 4-chloro-3-(4-cyanonaphthalen-1-yl)pyridine 1-oxide which was isolated by precipitation after addition of water. 4-Chloro-3-(4-cyanonaphthalen-1-yl)pyridine 1-oxide was reacted with thiourea in the presence of methanol and hydrochloric acid at 70 ^{o}C, then the solvents were removed under reduced pressure and a 2 N solution of aqueous sodium hydroxide was added to form 3-(4-cyanonaphthalen-1-yl)-4-mercaptopyridine 1-oxide in solution. The water was evaporated, and DMF and ethyl-2-bromoisobutyrate were added to form 3-(4-cyanonaphthalen-1-yl)-4-((1-ethoxy-2-methyl-1-oxopropan-2-yl)thio)pyridine 1-oxide which was extracted in the organic layer after addition of ethyl acetate and water. The product was purified by silica gel chromatography, then reacted with aqueous 1 N sodium hydroxide to saponify the ethyl ester.

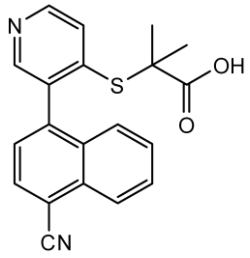
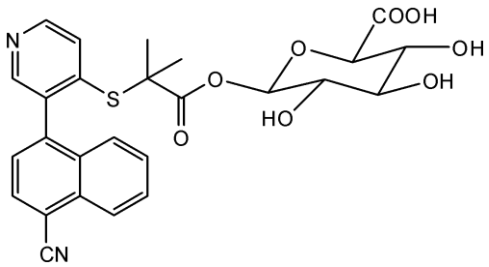
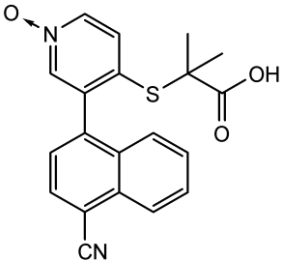
The product 4-((2-carboxypropan-2-yl)thio)-3-(4-cyanonaphthalen-1-yl)pyridine 1-oxide (M4) was isolated by precipitation after addition of hydrochloric acid to the solution. Purity was 99.3%. ^{1}H NMR DMSO d_6 (ppm); 8.4-8.3 (m, 3H), 8.09 (d, 1H), 7.8 (m, 2H), 7.7-7.6 (m, 3H), 1.42-1.38 (m, 6H).

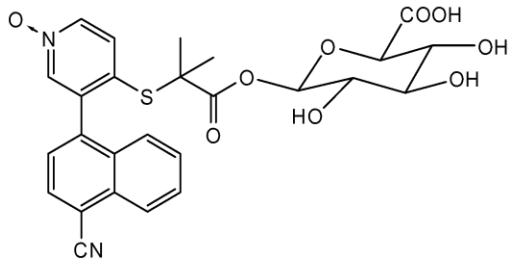
M4 was reacted with 1-bromo- *O*-acyl-protected glucuronic acid methyl ester in a mixture of pyridine and toluene in the presence of cesium carbonate. After workup, the oil obtained was purified by silica gel chromatography. The residue was then subjected to enzymatic deprotection, followed by HPLC purification to lead to M8 as a white solid. Purity was 99%. ^{1}H NMR CD_3OD (ppm); 8.34-8.29 (m, 3H), 8.12 (d, 1H), 7.85-7.79 (m, 2H), 7.69 (m, 1H), 7.61-7.58 (m, 2H), 5.54 (d, 1H), 3.96-3.93 (m, 1H), 3.6-3.41 (m, 3H), 1.41-1.35 (m, 6H)

Supplementary Table

TABLE 1

Chemical Names and Structures of Verinurad and its Metabolites Observed in Healthy Adult Male Subjects

Name of Chemicals	Chemical Structure and Name
VERINURAD Chemical Formula: $C_{20}H_{16}N_2O_2S$ Exact Mass: 348.09325 Molecular Weight: 348.41824	 2-((3-(4-cyanonaphthalen-1-yl)pyridin-4-yl)thio)-2-methylpropanoic acid
M1 Chemical Formula: $C_{26}H_{24}N_2O_8S$ Exact Mass: 524.12534 Molecular Weight: 524.54236	 (2R,3R,4R,5S,6R)-6-((2-((3-(4-cyanonaphthalen-1-yl)pyridin-4-yl)thio)-2-methylpropanoyl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid
M4 Chemical Formula: $C_{20}H_{16}N_2O_3S$ Exact Mass: 364.08816 Molecular Weight: 364.41764	 4-((2-carboxypropan-2-yl)thio)-3-(4-cyanonaphthalen-1-yl)pyridine 1-oxide

M8 Chemical Formula: $C_{26}H_{24}N_2O_9S$ Exact Mass: 540.12025 Molecular Weight: 540.54176	 4-((1(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-methyl-1-oxopropan-2-yl)thio)-3-(4-cyanonaphthalen-1-yl)pyridine 1-oxide