SYNTHESIS OF TRITIUM LABELED 1-AMIDINO-3-(P-NITROPHENYL)UREA : A POTENTIAL ANTIMALARIAL AGENT

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SUMMARY

Five steps were required to synthesize the title compound. This involved a catalytic exchange reaction, nitration, hydrolysis, and condensation. The overall radiochemical yield was 28.87% and the specific activity was 12.4 mCi/mmol, with a radiochemical purity of more than 95.7%

Key Words: Antimalarial agent, nitroguanil-[^{3}H], catalytic exchange reaction, nitration, hydrolysis, condensation, quality control.

INTRODUCTION

Nitroguanil is a derivative of Chloroguanide. It was reported under the original Polish Academy code No.T-27 (1,2), and its chemical name is 1-amidino-3-(p-nitrophenyl)urea. Nitroguanil was introduced because of its low toxicity relative to its antimalarial

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action, compared to Chlorguanide,1-(p-chlorophenyl)-5-isopropyl-amidinourea. It has been reported to possess an antimalarial activity against both *plasmodium gallinnaceum* and *p. berghei* in experimental animals (3,4,5,6,7,8). Recently, Nitroguail was also reported to possess an antileishmanicidal action for a viscerotropic infection of *Leishmania infectum* LV9 in mice (9,10). The parasite originated from a human case of kala-azar in Ethiopia. This agent was also tested against *L. major* LV39 and *L. Mexicana amazonensis* LV78 both in tissue culture and in animals (11). Furthermore, an arylamidino urea derivative 1-amidino-3-(2,4-dichlorophenyl)urea was found to have gastrointestinal spasmolytic, antimotility, antidiarrheal and antisecretory effects (12), and, the 1-amidino-3-(2,4,6-trimethylphenyl)urea derivative is being investigated for use as a livestock growth promoting agent through utilization of its antimotility, and increased absorption and digestion-promoting properties (13,14,15).

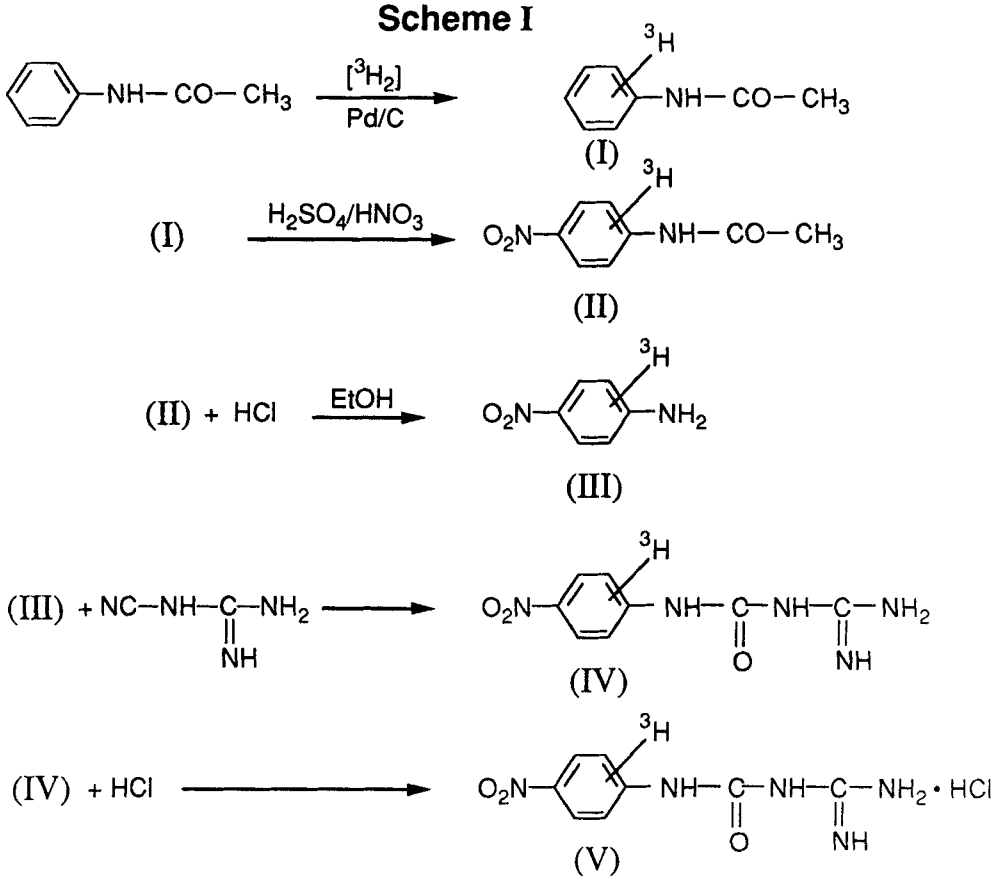
Tritium labeled Nitroguanil was prepared in order to better understand its pharmacokinetic and pharmacodynamic pathways and therefore its mode of action.

RESULTS

The synthesis of 1-amidino-3-(p-nitrophenyl)urea-[^{3}H], monohydrochloride; Nitroguanil-[^{3}H] monohydrochloride (V) consists first of a catalytic exchange reaction of acetanilide with tritium gas to form acetanilide-[^{3}H] (I), which in turn is reacted with H_2SO_4/HNO_3 to yield p-nitroacetanilide-[^{3}H] (II), which underwent acid hydrolysis to yield p-nitroaniline-[3H] (III). Compound III was reacted with dicyanodiamide to produce 1-amidino-3-(p-nitrophenyl)urea-[^{3}H] (IV), which was converted into a monohydrochloride salt by treatment with concentrated HCl to form the final compound, 1-amidino-(p-nitrophenyl)urea-[^{3}H];

(Nitroguanil-[^{3}H]) The total yield from compound (I) to (V) was 28.87%. The specific activity of the final compound (V) was 212.4 mCi/mmol. The synthetic scheme is outlined in figure 1.

Scheme I



EXPERIMENTAL

Melting points are obtained on a Fisher-Johns hot stage and are corrected. IR spectra recorded on a Perkin-Elmer 337 grating ir spectrophotometer. Type QIF silica gel plates from Quantum Industries are used for tlc development. Radiochromatograms are recorded on a 4TT Tracer-Lab Scanner using tlc plates. Radioassay are performed using a Packard-Carb Liquid Scintillation Spectrometer, Model 3320.

Acetanilide-[^{3}H] (I) A catalytic exchange on 81 mg (0.60 mmol) acetanilide using 20 Ci of tritium gas yield of a mixture of acetanilide-[^{3}H] and reduction products. The crude product was purified by preparative thick layer chromatography on silica gel GF plates developed with ethyl acetate-chloroform (1:1). Two separations were required to produce a high purity of (I). The purified product was diluted with non-labeled acetanilide to yield 1,237 mCi in 810 mg (6.0 mmol), at a specific activity of 206.17 mCi/mmol.

p-Nitroacetanilide-[^{3}H] (II) Compound (I) 810 mg, (6.0 mmol; 1,237 mCi) was dissolved in 1.13 mL of sulfuric acid with stirring at room temperature. The mixture was cooled in an ice bath and 0.57 mL of nitric acid was added. The reaction temperature was held below 20°C. The mixture was stirred at room temperature for 20 min, and then poured into 12 mL of water. The precipitate was filtered, and washed with water until no longer acidic. The product was then dissolved in ethanol, treated with charcoal, and crystallized and recrystallized from ethanol-water. The yield was 840 mg (78%), at 4.66 mmol (964.86 mCi) with a melting point of 215.5-216.5°C.

p-Nitroaniline-[^{3}H] (III) The compound (II), 840 mg, (4.66 mmol) was refluxed with 23.7 mL of 20% HCl and 4.86 mL of ethanol overnight at 110°C, to cause complete hydrolysis. The solvent was evaporated *in vacuo,* the residue was dissolved in hot water, and 30 mL of 10% NaOH solution was added. (III) was precipitated out to yield 633 mg, (98.5%, 4.58 mmol, 950.39 mCi). The melting point of (III) was 149-150.5°C.

1-Amidino-3-(p-nitrophenyl)urea-[^{3}H] (IV) Nitroguanil-[^{3}H] To a mixture of (III), 608.6 mg; 4.4 mmol (913 mCi), in 0.73 mL of

HCl and 0.47 mL of water, dicyanodiamide, 424 mg (5.04 mmol) was added slowly, and the mixture was kept at 50°C until a yellow solid precipitated from the resulting yellowish black solution. The mixture was then refluxed at 45 min and the solid was filtered. The product was washed with 22% HCl, then with ethanol, and air dried. The product was dissolved in water and treated with 10% NaOH. The precipitate was crystallized and recrystallized from DMF/water, and vacuum dried at 100°C. The yield was 458 mg, (47.8%; 2.05 mmol, 436.41 mCi) which melted at 224-229°C.

1-Amidino-3-(p-Nitrophenyl)urea-[^{3}H] monohydrochloride (V) Nitroguanil-[^{3}H] monohydrochloride Compound (IV) was suspended in hot water and a small amount of HCl was added. The suspension changed from a yellow solid to white fibers. The product was filtered and dried *in vacuo* to yield 486.8 mg. The product was purified by crystallization from DMF-ether, and dried *in vacuo* to yield 466 mg (87.6%,1.8 mmol, 382.3 mCi) with a specific activity of 212.4 mCi/mmol. The product was identified by paper chromatography in comparison with authentic material supplied by Walter Reed U.S. Army Medical Research and Development Command in the following solvent systems: n-butanol:ethanol:water (4:1:1,v/v/v/) and n-propanol:ammonia:water (6:3:1,v/v/v). The compound was also identified by comparing mp, and IR spectra with those of authentic sample.

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