SYNTHESIS OF 1-(2,3-DIHYDROXYPROPYL)-2-NITRO-1H-IMIDAZOLE-2-^{14}C AND N-(2-HYDROXYETHYL)-2-(2-NITRO-1H-IMIDAZOL-1-YL-2-^{14}C) ACETAMIDE

Mary T. Fong and Morris A. Leaffer
Life Sciences Division, Bio-Organic Chemistry Laboratory
SRI International, Menlo Park, California, 94025

SUMMARY

We have prepared the ^{14}C-labeled analogs of NSC 261036, 1-(2,3-dihydroxypropyl)-2-nitro-1H-imidazole-2-^{14}C, and NSC 301467, N-(2-hydroxyethyl)-2-(2-nitro-1H-imidazol-1-yl-2-^{14}C) acetamide, for pharmacological, drug distribution, and mechanisms of action studies. The latter is an analog designed for lower toxicity and improved properties. The former is a metabolite of, and appears to be less toxic than, misonidazole.

Key Words: ^{14}C-radiosensitizer , 1-(2,3-dihydroxypropyl)-2-nitro-1H-imidazole-2-^{14}C and N-(2-hydroxyethyl)-2-(2-nitro-1H-imidazol-1-yl-2-^{14}C) acetamide

INTRODUCTION

The lack of oxygen in some tumor cells (hypoxic cells) makes them resistant to irradiation. This is believed to be a major cause of failure in cancer radiotherapy. Recently attention has been focused on chemical sensitizers designed to mimic oxygen and to selectively sensitize hypoxic neoplastic cells to radiation.[1] The rationale is that these radiosensitizers are not rapidly metabolized (as oxygen is) and can diffuse from capillaries to the hypoxic cells in tumor. Some mechanisms of action have been proposed.

A series of nitrobenzenes[2,3] and nitrofurans have been found to possess good radiosensitization properties *in vitro* but not *in vivo*.[1] Nitropyrroles have also been studied.[4] Metronidazole, a 5-nitroimidazole derivative, has been reported to sensitize known hypoxic cells both *in vitro* and *in vivo*.[5,6] Misonidazole, a derivative of 2-nitroimidazole, has been found to be a very good radiosensitizing agent and is effective in at least 16 different animal tumors.[7,8] It is currently under clinical trials.[9] However misonidazole causes peripheral neuropathies.[9,10] In order to seek a less toxic analog, a series of 2-nitroimidazole derivatives were synthesized[13,14] and the relationship between

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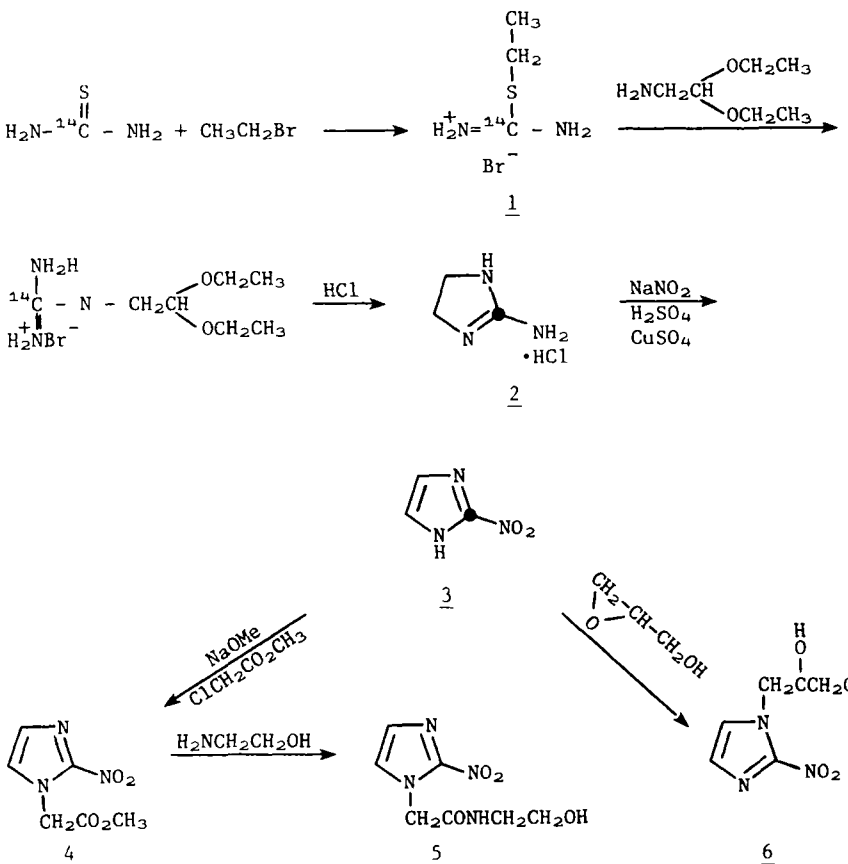
structure and biological activity was studied. Compound NSC 301467 appeared to be less toxic and possess other improved properties over misonidazole. Therefore NSC 301467 was chosen for preclinical pharmacology studies by the National Cancer Institute. Carbon-14 labeled misonidazole has been synthesized[15,16] for studying the mechanism of biological activity. We have synthesized 1-(2,3-dihydroxypropyl)-2-nitro-1H-imidazole-2-^{14}C, NSC 261036, which is the less toxic metabolite of misonidazole and N-(2-hydroxyethyl)-2-(2-nitro-1H-imidazol-1-yl-2-^{14}C) acetamide, NSC 301467.



Starting from thiourea-^{14}C, S-ethylisothiouronium bromide (1) is obtained and this was reacted with aminoacetaldehyde diethylacetal as described by Storey, et al.[17] to give the 2-aminoimidazolium-2-^{14}C chloride (2). Diazotization of this intermediate and subsequent reaction with sodium nitrite in the presence of $CuSO_4$ and H_2SO_4[13] gave 2-nitroimidazole-2-^{14}C (3). To prepare 5 and 6 the procedures of Beaman, et al.[14,18] were followed with minor modifications.

EXPERIMENTAL

S-Ethylisothiouronium-^{14}C Bromide (1)--Thiourea-^{14}C [(Cal. Bionuclear Corporation), 0.52 g (179 mCi) and thiourea 0.52 g; total 13.7 mmol] and 8 ml of ethyl bromide were heated at 55-60°C in 10 ml of ethanol for 2 h. An additional 8 ml of ethyl bromide was added, and the reaction was kept overnight at 55-60°C. The solution was cooled to room temperature and 500 ml of ethyl ether was added. The oily residue that formed solidified when scratched. The reaction mixture was cooled in an ice bath, and the precipitate was collected by filtration and washed with two small portions of cold ether. Another crop was obtained by concentrating the mother liquor and precipitating with ether. A total of 2.41 g (95%) mp 26-28°C of white crystals was obtained.

2-Aminoimidazolium 2-^{14}C Chloride (2)--Compound 1 (2.41 g, 13 mmol) and 2-aminoacetaldehyde diethyl acetal (Aldrich Chemical Co., 2.0 ml, 13.6 mmol) in 10 ml of H_2O were heated at 90°C for 1.5 h. The reaction mixture was evaporated under reduced pressure and evaporated once more with a small portion of water to give an oil. This was refluxed for 15 min with 5.5 ml of HCl, evaporated *in vacuo*, then evaporated with a small portion of water to give 4.3 g of a brown oil 2 (100%).

2-Nitroimidazole-2-^{14}C (3)--To a 500-ml round-bottom flask were added 16N H_2SO_4 (130 ml), an aqueous solution of 3.3 g $CuSO_4 \cdot 5H_2O$ in 30 ml of water, and a 35 ml aqueous solution of 4.3 g of 2, with the temperature kept at 20°C or less. The reaction mixture was chilled to -20 to -25°C in a Dry Ice-acetone bath, while an aqueous solution of 17.9 g of $NaNO_2$ in 60 ml of water was added dropwise with the funnel tip in the reaction mixture. When the addition was completed, the mixture was allowed to reach room temperature and stand for 48h. The bath temperature was adjusted to -10°C, while NH_4OH was added slowly to bring the pH to 1. The reaction mixture was filtered and the solid washed with 2 × 10 ml water and dried *in vacuo* to give 0.272 g of yellow product. The mother liquor was extracted with ethyl acetate (5 × 30 ml). The ethyl acetate solution as washed twice with 20 ml of water and evaporated under reduced pressure. The residue was triturated with 4 ml of ethanol, and allowed to cool in a refrigerator. The precipitate was collected by centrifugation and decantation, washed

once with 1 ml cold EtOH and dried in vacuo to give 0.15 g of yellow solid mp 283-285°C (lit.[13] mp = 288°C) UV in 0.1N NaOH ϵ_{max374} = 12,600 (ref. sample ϵ_{max374} = 12,690). A total of 0.42 g (28.5%) of yellow product was obtained.

1-(2,3-Dihydroxypropyl)-2-nitro-1H-imidazole-2-^{14}C (6)--To a 20-ml round-bottom flask equipped with a reflux condenser were added 2-nitroimidazole-2-^{14}C (3, 0.33 g, 2.9 mmol), K_2CO_3 (0.04 g), glycidol (Aldrich Chemical Co., 0.4 ml, 6.0 mmol), and ethanol (3 ml). The reaction mixture was immersed in a preheated oil bath at 95 to 100°C and stirred for 80 min. Then the solvent was removed. The residue was purified by passing through a short column of silica gel (GS 90-200 mesh, 1 × 10 cm) with 60 ml of 15% CH_3OH in acetone and 50 ml of acetone. The eluent was evaporated and the residue was recrystallized from ethanol to give 235 mg of yellow solid. A second crop gave another 30 mg of yellow crystals. The two batches were combined and recrystallized again in absolute ethanol to give 0.2 g of yellow solid mp 109-111°C, (lit.[13] 110-112°C). UV in EtOH ϵ_{max316} = 6980, (lit.[13] ϵ_{max316} = 6600). This was analyzed by TLC, radioautography and HPLC and found to be 98% pure. A total of 13.2 μCi of (6) was synthesized with a specific activity of 12.4 mCi/mmole. (6% Yield based on thiourea-^{14}C.)

Methyl 2-(2-nitro-1H-imidazol-1-yl-2-^{14}C acetate (4)--To a slurry of 0.428 g of 2-nitroimidazole-2-^{14}C (3) in 5 ml of DMF in a 50-ml round-bottom flask was added a drop of phenolphthalein. A solution of 4N sodium methoxide in CH_3OH was added dropwise until a pink color persisted. About 1 mg of 2-nitroimidazole was added to make the pink color just disappear. The solution was heated at 152-155°C to remove the CH_3OH. When the temperature had cooled to 115°C, 0.5 ml of methyl chloroacetate was added. Stirring was continued at 110-115°C for 15 min and the solvent was removed in vacuo at 65-70°C. The residue was dissolved in acetone, and the precipitated salt was removed by filtration. The filtrate was evaporated to dryness. The residue was triturated with ethanol and evaporated in vacuo to give 0.56 g a tan solid (4) (80%), mp 93-95°C (lit.[13] mp 94-95°C).

N-(2-Hydroxyethyl)-2-(2-nitro-1H-imidazol-1-yl-2-^{14}C) acetamide (5)--To a slurry of 0.56 g (3 mmol) compound 4 in 4 ml of CH_3OH was added 0.8 ml of 2-aminoethanol. The reaction flask was shaken until the solid dissolved and then

was allowed to stand overnight at room temperature. After cooling at 5°C the precipitate that formed was collected. It was then dissolved in 50 ml of acetone and passed through a short silica gel column (E. Merck 70-230 mesh) in a pipet. Recrystallization from absolute ethanol gave 0.53 g (83%) of 5 mp 161-164°C (lit.[18] mp 162-163°C). UV in CH_3OH ϵ_{max315} = 7570 (ref. sample ϵ_{max315} = 7670). Analysis by UV and TLC radioautography showed the compound to be 98% pure. A total of 32.3 mCi of 5 was obtained with a specific activity of 13.06 mCi/mmole. (18% yield based on thiourea-^{14}C.)

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