Synthesis of Aldose Reductase Inhibitor, 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2^{14}C benzothiazolyl]methyl]-1-phthalazineacetic acid

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Summary

Synthesis of the title compound, C^{14} labeled CP-73,850 (zopolrestat), was accomplished by condensation of 1-phthalazineacetic acid, 3-^{14}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester with 3-amino-4-mercapto-benzotrifluoride, followed by base hydrolysis of the resultant product, 1-phthalazineacetic acid, 3,4-dihydro-4-oxo-3-[[5-trifluoromethyl)-2-benzothiazolyl]^{14}C methyl]-3,4-dihydro-4-oxo-, ethyl ester.

Keywords:

CP-73,850 (zopolrestat), 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2^{14}C benzothiazolyl]methyl-1-phthalazineacetic acid, aldose reductase inhibitor.

Introduction

Long term diabetes leads to complications which include neuropathy, retinopathy and cataracts. The precise biochemical events which trigger the pathological changes in the affected tissues are not clearly understood. It has been known for sometime that glucose is metabolized to sorbitol by the NADPH dependent enzyme, aldose reductase (1). This pathway is called sorbitol or polyol pathway. Tissues susceptible to diabetic complications do not require insulin for glucose transport and contain aldose reductase. It is hypothesized that the increased glucose flux in the diabetic state with attendant intracellular accumulation of sorbitol, mediated by aldose reductase, may play a role in development of complications. This hypothesis is gaining momentum and strong support through significant progress made in design/synthesis, pharmacological and clinical testing of aldose reductase inhibitors (2-5). Spurred by this, several pharmaceutical companies are continuing their

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efforts to develop safe and potent inhibitors for clinical evaluation. In our program, 3,4-dihydro-4-oxo-3-[[5-(trifluoromethyl)-2-benzothiazolyl]methyl]-1-phthalazineacetic acid (zopolrestat) has been identified as a potent *in vivo* aldose reductase inhibitor with favorable pharmacokinetics in humans (6,7). In this report, we describe the synthesis of the title compound for use in metabolism and pharmacokinetic studies.

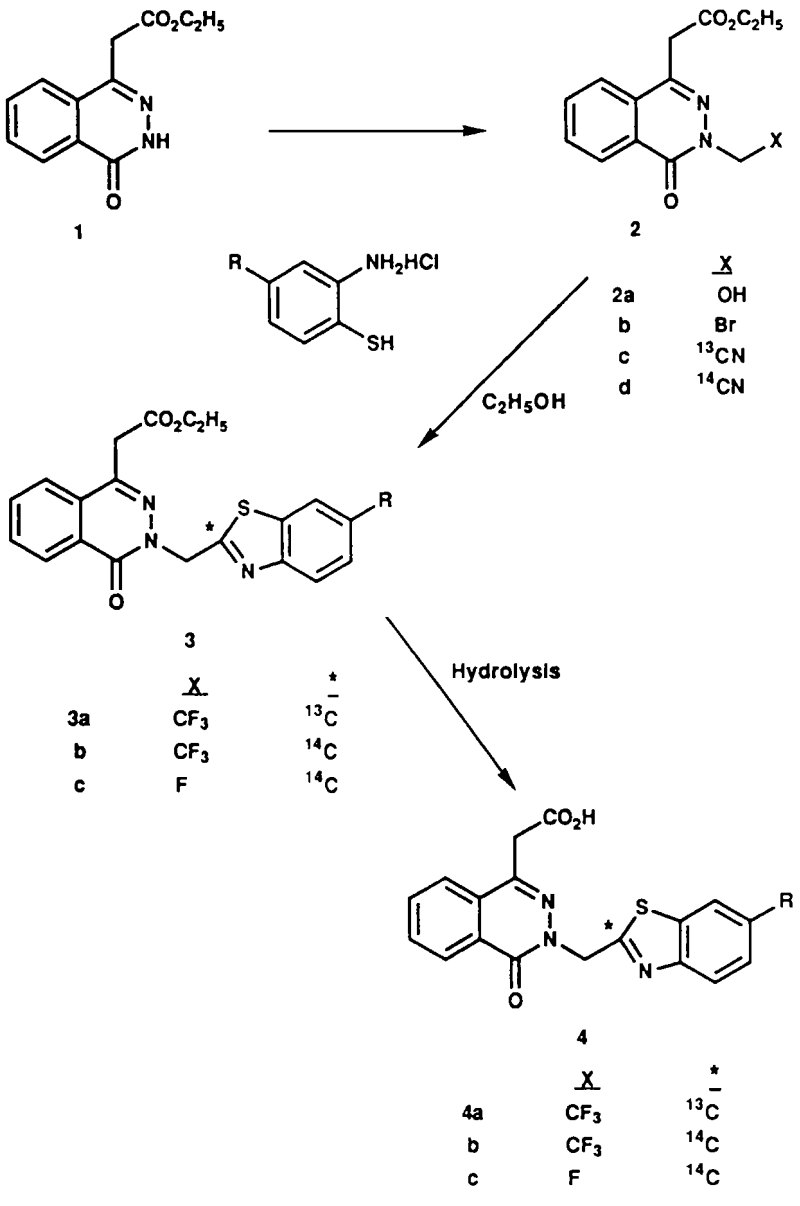
Results and Discussion

The synthetic strategy employed is outlined in Scheme 1. Exposure of 1-phthalazineacetic acid, 3,4-dihydro-4-oxo-, ethyl ester **1** (8) to aqueous formaldehyde in ethanol gave 1-phthalazineacetic acid, 3-hydroxymethyl-3,4-dihydro-4-oxo-, ethyl ester in 72% yield. Treatment of this hydroxymethyl derivative **2a** with phosphorous tribromide in methylene chloride effected smooth transformation to the expected bromomethyl compound **2b** in nearly quantitative yield. Since the bromomethyl compound showed propensity to revert to **1** under nucleophilic displacement conditions, we had to develop optimum conditions to incorporate labeled cyanide. We first examined the introduction of unlabeled cyanide and later as a better marker, the relatively inexpensive ^{13}CN. In both instances, we found that under carefully controlled conditions using aqueous acetone as a solvent in the presence of a catalytic amount of potassium iodide we could obtain a reproducible yield of the desired cyanomethyl compound **2c** in 75% yield. Following success in coupling ^{13}C labeled **2c** with 3-amino-4-mercapto-benzotrifluoride (9) to obtain the precursor benzothiazole ester **3a**, we repeated the reaction sequence using $K^{14}CN$. As in the case of cold runs, we obtained the desired ^{14}C labeled ester **2d** without any evidence of side reactions involving condensation of the ester group with the amino mercapto benzotrifluoride. Upon chromatography, ester **3b** was obtained as a pure crystalline solid. The hydrolysis of the ester **3b** to the target acid **4b** was uneventful giving a combined 75% yield (for the two steps) of ^{14}C labeled zopolrestat. The most attractive feature of this synthesis scheme is the simplicity and versatility in building the complex target, using the relatively inexpensive labeled cyanide, in good yield and excellent radiochemical purity with high specific activity. As an example of the versatility of the method, we have also prepared 3,4-dihydro-4-oxo-3-[[5-(fluorophenyl)-2-benzothiazolyl]methyl-1-phthalazineacetic acid, another potent aldose reductase inhibitor, **4c** starting from **2d** and 2-amino-4-fluoro-benzenethiol (10,11).

Experimental

$K^{14}CN$ was purchased from New England Nuclear (specific activity, 10.0 mCi/mmol). All solvents were of analytical reagent quality. The reactions were routinely monitored using thin layer

SCHEME I



chromatography and the plates used for monitoring reactions were Analtech silica gel GF. Radioactivity was measured using a Beckman LS9000 liquid scintillation counter. All high pressure liquid chromatography was carried out on Spectra Physics instrumentation. Radiopurity was determined by HPLC coupled to a RAM2 detector. Weighings were carried out on a Sartorius 200 balance and Mettler Microanalytical M5A5 balance. Melting points are uncorrected and were determined on a Thomas Hoover capillary melting point apparatus.

1-Phthalazineacetic acid, 3-^{13}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester, 2c. To a solution of 1-phthalazineacetic acid, 3-bromomethyl-3,4-dihydro-4-oxo, ethyl ester (7) (2.43 g) in acetone (35 ml) maintained at 0°C was added a solution of K^{13}CN (0.49 g) and a catalytic amount of KI in water (3.5 ml). The resulting solution was stirred for 2 hours while gradually warming to room temperature. The reaction mixture was poured onto ice-water (200 ml) containing hydrochloric acid (10 ml, 6N). The precipitated crude solid was collected and then purified by flash chromatography over silica gel, eluting with a mixture 95% methylene chloride and 5% ethyl acetate (yield 1.54 g; mp, 125°C, ^{13}CN resonance, 115.7968 ppm).

1-Phthalazineacetic acid, 3-^{14}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester, 2d. To an ice-cold solution of 1-phthalazineacetic acid, 3-bromomethyl-3,4-dihydro-4-oxo-, ethyl ester (976 mg) in acetone (30 ml) was added K^{14}CN (195.3 mg, 10 mCi/mmol) and a catalytic amount of KI dissolved in water (1.5 ml). The rest of the procedure was according to that described above (yield: 0.51 g; mp 115°C). The radiochemical purity was 95.1% as measured by high pressure liquid chromatography (retention time, 9.63 min.).

1-Phthalazineacetic acid, 3,4-dihydro-4-oxo-3-[[5-trifluoromethyl)-2^{13}C benzothiazolyl]methyl]-, ethyl ester, 3a. A mixture of 1-phthalazineacetic acid, 3-^{13}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester (1.35 g), 3-amino-4-mercapto-benzotrifluoride hydrochloride (1.15 g) and ethanol (20 ml) was gently refluxed for 20 hours. Upon cooling a heavy white precipitate was obtained which was filtered and the collected solid was wash with ice-cold ethanol (5 ml). The yield of the crystallized product (from ethanol) was 1.74 g (mp, 135-136°C; ^{13}C resonance for the benzothiazole 2-carbon, 169.9124 ppm).

1-Phthalazineacetic acid, 3,4-dihydro-4-oxo-3-[[5-trifluoromethyl)-2^{14}C benzothiazolyl]methyl]-, ethyl ester, 3b. The above experiment was repeated using 1-phthalazineacetic acid, 3^{14}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester (339 mg), 3-amino-4-mercapto-benzotrifluoride hydrochloride (287 mg) and ethanol (12 ml). The product was used directly in the next step without further purification. The NMR of the crude product was identical to the authentic unlabeled product [^{H}NMR ($CDCl_3$, 60 MHz), 1.38 (t,J = 8.0 Hz, 3H), 4.1 (s,2H), 4.25 (q,J = 8 Hz, 2H), 5.84 (s,2H), 7.0-7.3 (m,1H), 7.7-8.0 (m,5H), 8.4-8.5 (m,1H)].

3,4-Dihydro-4-oxo-[[5-(trifluoromethyl)-2^{14}C benzothiazolyl]methyl]-1-phthalazineacetic acid, 4b. To the ester product **3a** from the above reaction dissolved in a mixture of ethanol/tetrahydrofuran

(8 + 17 ml) was added an aqueous solution potassium hydroxide (5 ml, 5%) and allowed to stir at room temperature for 1 hour. The solution was then concentrated under reduced pressure and was added water (25 ml) to the concentrate. The basic aqueous solution was extracted with ether (25 ml) and the aqueous layer was collected and then acidified with hydrochloric acid (6N) to adjust the pH to ~2. The precipitated solid was collected, air-dried and then slurried in ethyl acetate (5 ml) for 30 min. The solid was collected and dried in vacuum to obtain the title product [yield: 390 mg; mp, 196°C; ^{1}H NMR (DMSO; 300 MHz), 4.08 (s,2H), 5.81 (s,2H), 7.8 (d,J = 8.5 Hz, 1H), 7.9-8.1 (m,3H), 8.3-8.5 (m,3H)]. The radiochemical purity as determined by high pressure liquid chromatography was 99.3% and the specific activity was 22.5 μCi/mg.

High Pressure Liquid Chromatography was carried out using the following parameters: Eluent – 6.5% 0.01M pH 7.0 phosphate butter, 25% methanol and 10% tetrahydrofuran. Flow rate – 1 ml/min. Detector – UV 254 nM. Temperature – 23°C. Column – Waters Associates analytical C-18. Retention Time – 14.98 min.

3,4-Dihydro-4-oxo-3-[(5-fluoro-2^{14}C benzothiazolyl)-methyl]-1-phthalazineacetic acid, 4c. A mixture of 1-phthalazineacetic acid, 3-^{14}C cyanomethyl-3,4-dihydro-4-oxo-, ethyl ester (136 mg) and 2-amino-5-fluorobenzenethiol hydrochloride (90 mg) and ethanol (10 ml) was gently refluxed for 48 hours. Upon cooling a heavy precipitate, **3c**, was obtained, which was filtered, collected and immediately used in the next hydrolysis step. Following the procedure described for the trifluoromethyl congener, the title product was obtained [yield: 106 mg; mp, 218-219°C; ^{1}H NMR (DMSO, 300 MHz), 4.05 (s,2H), 5.78 (s,2H), 7.3-7.45 (m,1H), 7.8-8.2 (m, 5H), 8.45 (d, J = 8.5 Hz, 1H). The radiochemical purity by high pressure liquid chromatography was 97.9% (retention time, 5.36 min.) and the specific activity was 25.1 μCi/mg.

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References

1. Hayman, S., Kinoshita, J. H.-*J. Biol. Chem.* 240, 877 (1965).

2. Kador, P. F.-*Med. Res. Rev.* 8, 325 (1988).

3. Humber, L. G.-*Prog. Med. Chem.* 24, 299 (1987).

4. Larson, E. R., Lipinski, C. A., and Sarges, R.-*Med. Res. Rev.* 8, 159 (1988).

5. Harrison, H. E., Stribling, D., Armstrong, F. M., and Perkins, C. M.-*J. Diabetic Complications* 3, 6 and 70 (1989).

6. Inskeep, P. B., *et al.*, Manuscript in preparation.

7. Mylari, B. L., *et al.*, manuscript submitted to *J. Med. Chem.*

8. Foldeak, S., Puodziunas, A., Kost, A. N., and Gribauskas, P.-*Khim.-Farm. Zh.* 4, 22 (1970).

9. Dannley, R. L., and Zazaris, D. A.-*Can. J. Chem.* 43, 2610 (1965).

10. Mital, R. L., and Taunk, P. C.-*Monatsh. Chemie* 102, 760 (1971).

11. EP222576; All new compounds in the unlabeled series gave satisfactory analytical data.