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Research Article

Synthesis of *N*-(2,3-dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-benzamide and *N*-(2,3-dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-*N*-[^{14}C]methyl-benzamide as novel carbon-14 labelled CCK antagonists

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Summary

Two benzodiazepine CCK antagonists *N*-(2,3-dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1H 1,4-benzodiazepin-3-yl)-benzamide **2** and *N*-(2,3-dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-[^{14}C]methyl-benzamide **3** were synthesized in high yields through the reaction of *N*-(2,3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-benzamide **1** with [^{14}C] methyl iodide in different situations.

Key Words: cholecystokinin antagonists; benzodiazepine; carbon-14; methylations

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Introduction and discussion

Dealkylation and 3-hydroxylation are the most important metabolic routes for several 1,4-benzodiazepine derivatives.[1] Isotopic substitution at the site of metabolic transformation is one of the best methods for elucidating the mechanism of the metabolic process and several carbon-14 labelled benzodiazepine derivatives have been synthesized and their metabolic fates studied in human and animal models.[1–6] Since the isolation of the non-peptidal cholecystokinin (CCK) antagonist Asperlicin,[7] the 1,4-benzodiazepine ring system has served as a useful tool for delineating the pharmacologic action of CCK.

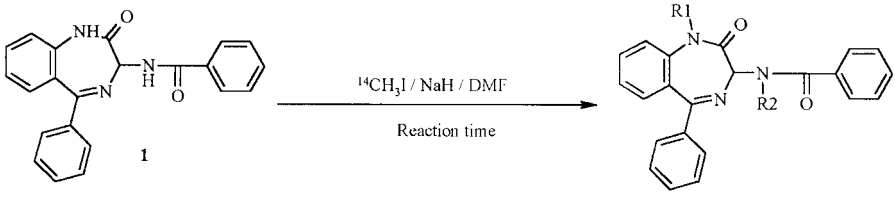
In order to study the rate of 1-dealkylation of benzodiazepine CCK antagonists and its influence on the pharmacologic profile of these compounds it became necessary to synthesize a CCK antagonist derivate labelled with carbon-14 at the N-1 position. Furthermore in order to determine the rate of chain *N*-methyl dealkylation, synthesis of a ligand, *N*-methylated both in N-1 and the amide chain appeared of interest.

The ^{14}C labelling step in synthesis of **2** and **3** consists of methylation of **1** with [^{14}C] methyl iodide, prepared by the known methods[8,9] from barium [^{14}C] carbonate via [^{14}C] methanol. The benzodiazepine CCK antagonist **1** was prepared through a benzotriazole-assisted synthetic route from benzamide and 2-aminobenzophenone.[10]

By utilization of molar equivalents of **1** and [^{14}C] methyl iodide and a short reaction time, the mono methylated product **2** in which only the N-1 of the benzodiazepine ring is methylated, is obtained. Using [^{14}C] methyl iodide in excess and extending the reaction time yields the dimethyl derivative **3**.

Both compounds **2** and **3** were synthesized for the first time and their structures were determined by IR, MS, ^{1}H NMR and ^{13}C NMR spectroscopy.

Biological studies on these compounds are currently under investigation.



Reaction Time: 5min. **2** $R_1=^{14}CH_3$, R_2=H
45min. **3** $R_1=R_2=^{14}CH_3$

Experimental

Radioactivity determinations were carried out with a liquid scintillation counter (Beckman LS 6500) using an internal standard method. Thin layer chromatography (TLC) analyses were carried out on silica gel coated foils (Schleicher & Schull) and the spots visualized under UV (254 nm). Melting points were determined on a Reichert hot plate apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Varian Unity Plus 400 spectrometer (399.866 MHz for ^{1}H NMR and 100.556 MHz for ^{13}C NMR). Mass spectra were obtained on a Finnigan TSQ-70 instrument. Infrared spectra were recorded on a Nicolet Magna IR 550 spectrometer.

[^{14}C] Methanol. According to a modification of the method of Cox *et al.*[8] [^{14}C] carbon dioxide generated from [^{14}C] barium carbonate (15.4 mCi, 0.197 g, 1 mmol) was introduced into a suspension of lithium aluminium hydride (0.16 g, 4.2 mmol) in tetrahydrofurfuryloxy-tetrahydropyran (7 ml) and the mixture stirred for 1 h at room temperature. Addition of tetrahydrofurfuryl alcohol (3.5 ml) and heating the system at 110°C with passing a slow stream of nitrogen into the reaction mixture gave [^{14}C] methanol (14 mCi, 29.2 mg, 0.91 mmol). The latter was stored in dry ice.

[^{14}C] Methyl iodide. A solution of [^{14}C] methanol (14 mCi, 29.2 mg, 0.91 mmol) in 55% hydrogen iodide (4 ml) was heated at 40–90°C for 30 min. Distillation of the reaction mixture by passing a slow nitrogen stream and redistillation of the distillate on a vacuum line gave [^{14}C] methyl iodide (13.8 mCi, 28.8 mg, 0.9 mmol).

N-(2,3-Dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1-H1,4-Benzodiazepin-3-yl)-benzamide 2. Fresh sodium hydride (60% dispersion in mineral oil, 40 mg, 1 mmol) was added to a solution of **1** (355 mg, 1 mmol) in anhydrous DMF (4 ml) at 0°C under a nitrogen atmosphere. After 5 min [^{14}C] methyl iodide (15.3 mCi, 31.9 mg, 1 mmol) was added via a micropipette and the reaction mixture stirred for 5 min. The reaction mixture was then added to a vigorously stirred solution of water (25 ml) containing aqueous sodium hydrogen sulfate (0.5 ml, 1 N).

The reaction slurry was filtered after 5 min and washed with water, ether and cold methanol and dried under high vacuum. Recrystalization from ethylacetate/*n*-hexan (6 : 4) gave pure **2** (12 mCi, 295 mg, 0.8 mmol), m.p. 242°C, ^{1}H NMR (δ, $CDCl_3$):3.51 (s, 3H, methyl), 5.71 (d, 1H, $J = 8$ Hz, CH), 7.38–7.63 (m, 12H, aromatics), 7.95 (d, 2H, $J = 8$ Hz, aromatics), 8.03 (d, 1H, $J = 8$ Hz, amide NH) ppm; ^{13}C NMR

(δ, $CDCl_3$): 35.37, 67.58, 121.51, 124.55, 127.36, 128.22, 128.52, 129.13, 129.83, 130.67, 130.77, 131.76, 131.87, 134.00, 138.09, 142.80, 166.93, 167.41, 167.83; IR (KBr): $v = 691.9$, 778.9, 1516.4, 1644.4, 1690.5, 3052.6, 3277.9; mass: $m/z = 369$.

N-(2,3-dihydro-1-[^{14}C]methyl-2-oxo-5-phenyl-1-H1,4-Benzodiazepin-3-yl)-N-[^{14}C]methyl-benzamide 3. Fresh sodium hydride (60% dispersion in mineral oil, 80 mg, 2 mmol) was added to a solution of **1** (355 mg, 1 mmol) in anhydrous DMF (4 ml) at 0°C under a nitrogen atmosphere. After 45 min [^{14}C] methyl iodide (30.6 mCi, 63.8 mg, 2 mmol) was added via a micropipette and the reaction mixture stirred for 45 min. The reaction mixture was then added to a vigorously stirred solution of water (25 ml) containing aqueous sodium hydrogen sulfate (0.5 ml, 1 N).

The reaction slurry was filtered after 5 min and washed with water, ether and cold methanol and dried under high vacuum. Recrystallization from ethylacetate/*n*-hexan (1 : 9) gave pure **3** (21.4 mCi, 268 mg, 0.7 mmol), m.p. 179°C, ^{1}H NMR (δ, $CDCl_3$): 3.45 (s, 3H, methyl), 3.47(s, 3H, methyl), 6.18 (s, 1H, CH), 7.42-7.60 (m, 12H, aromatics), 7.70 (d, 2H, $J = 8$ Hz, aromatics) ppm; ^{13}C NMR (δ, $CDCl_3$): 35.03, 35.35, 72.14, 121.73, 124.05, 127.21, 127.30, 128.24, 128.96, 129.59, 129.75, 130.22, 130.64, 131.83, 136.58, 138.26, 143.26, 166.61, 168.69, 172.56; IR (KBr): $v = 687.4$, 1081.1, 1357.4, 1450.3, 1624.8, 1685.5, 2921.2, 3267.9; mass: $m/z = 383$.

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