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Research Article

Synthesis of carbon-14 analogue of N-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-benzamide-[carboxyl-^{14}C] as CCK-A antagonist

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Summary

N-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-benzamide-[carboxyl-^{14}C] has been synthesized from benzonitrile-[cyano-^{14}C]. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: 3-substituted 1,4-benzodiazepine; cholecystokinin antagonist; carbon-14

Introduction

The gastrointestinal peptide hormone cholecystokinin (CCK) plays a key role in a number of physiological processes including pancreatic and bilary secretion, gall bladder contraction, and gut motility. CCK is also a putative neuromodulator involved in dopaminergic transmission, satiety and analgesia.[1] Since the isolation and identification of the nonpeptidal CCK antagonist asperlicin, the 1,4-benzodiazepine ring system has served as a useful tool for delineating the pharmacological actions of CCK.[2] Therefore, various 3-substituted benzodiazeines have been designed as agonists and/or antagonists of the peripheral (CCK-A) and/or central (CCK-B) receptor subtypes.[3] The development of devazepide FK480, and others as CCK-A antagonists[4] and CCK-A agonists[5] such as 3-(1H-indazol-3-ylmethyl)-1,5-benzodiazepines (for instance GW5823) have demonstrated the significance of these investigations. For studies of pharmacokinetics and drug metabolism of these compounds, versions with a metabolically suitable carbon-14 label were required.[6] This paper reports the synthesis of *N*-(1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)-benzamide-[carboxyl-^{14}C] **1** as CCK-A antagonist.

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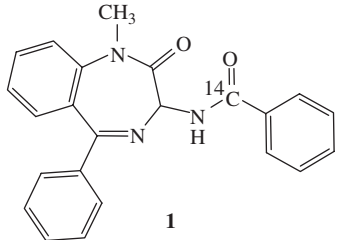

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Discussion

In this approach , according to the synthetic pathway shown in Scheme 1, the aryl nitrile[cyano-^{14}C] **4**[7] was derived from addition of cuprous iodide **2** and potassium [^{14}C]cyanide to aryl iodide **3** with good yield.[8]

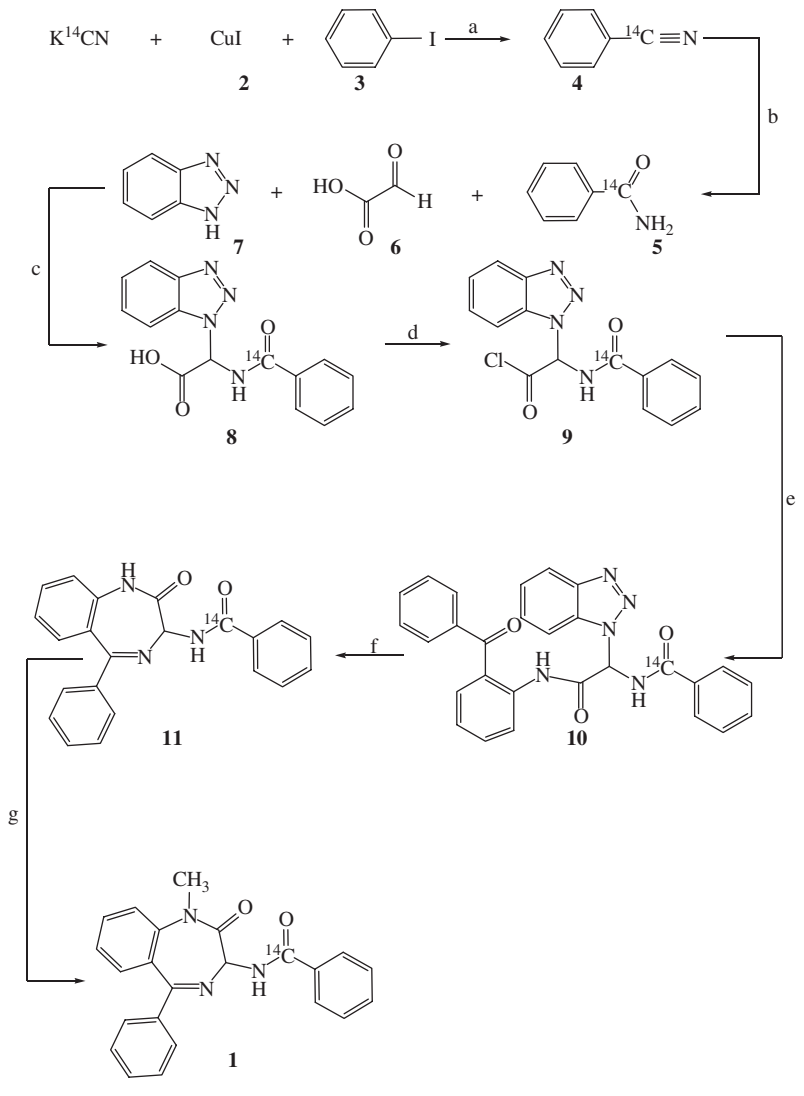
In the next step, after conversion of the aryl nitrile[cyano-^{14}C] **4** to the amide[carboxyl-^{14}C] **5** by using basic hydrogen peroxide in DMSO,[9] α-(benzotriazol-1-yl)-*N*-benzoyl[carbonyl-^{14}C] glycine **8** was prepared by condensation of the amide[carboxyl-^{14}C] **5** and glyoxylic acid **6** and 1H-benzotriazole **7** with azeotropic removal of water.[10] After conversion of the compound **8** to acyl chloride **9** in the presence of oxalyl chloride in DMF, acyl coupling of **9**[2] with 2-aminobenzophenone and subsequent displacement of the benzotriazole moiety with ammonia followed by cyclization of the resulting amino-ketone intermediate **10**, gave the 1,4-benzodiazepine derivative **11**.[11] In the final step, the desired product **1** was achieved from addition of methyl iodide to the 1,4 –benzodiazepine derivative **11** in the presence of sodium hydride.[12] The compound **1** was synthesised for the first time and biological studies on this compound are currently under investigation.

Experimental

Barium [^{14}C]carbonate was purchased from Amersham Pharmacia Biotech UK limited (Amersham Place, Little Chalfont, Buckinghamshire, England HP7 9NA) and converted to potassium [^{14}C]cyanide according to the standard procedure.[13] IR spectra were recorded on a Bruker FT-IR, Vector 22 instrument and the ^{1}H-NMR spectra were recorded on a Varian unity plus 400 spectrometer (400 MHz). Radioactivity was determined using a Beckman LS6500 liquid scintillation spectrometer.

Benzonitrile-[cyano-^{14}C] **4**

Cuprous iodide **2** (815 mg) was ground finely and dried in the reaction flask under high vacuum by heating with a hot air gun and cooled under nitrogen. Potassium [^{14}C]cyanide (18.97 mCi, 228 mg) was added slurried in dry DMF (10 ml), followed by iodobenzene **3** (1600 mg). The mixture was heated at reflux, under nitrogen, for 10 h. On cooling a solution of potassium cyanide



Scheme 1. (a) DMF; (b) H_2O_2 30%, K_2CO_3, DMSO; (c) toluene; (d) oxalyl chloride/DMF; (e) 2-amino benzophenone, N-methyl-morpholine; (f) NH_3(g)/ NH_4OAc, HOAc; (g) methyl iodide/NaH/DMF

(1.2 g) in water (50 ml) and ethyl acetate (20 ml) were added and the mixture stirred vigorously for 15 min. The layers were separated, the aqueous thoroughly extracted with ethyl acetate, the combined organics washed three times with water, dried over anhydrous $MgSO_4$, filtered and the solvent carefully evaporated. The crude product was purified by silica gel chromatography using ethyl acetate: hexane (1:9) as eluant to give the title compound benzonitrile[cyano-^{14}C] **4** (16.21 mCi, 310 mg). IR(KBr): 3092, 2280, 1600, 1490, 1450 cm^{-1}.

Benzoamide[carboxyl-^{14}C] **5**

To a stirred solution of benzonitrile[cyano-^{14}C] **4** (16.21 mCi, 310 mg) in DMSO (5.25 ml) cooled in an ice bath was added H_2O_2 (30%, 2.1 ml) and potassium carbonate (350 mg).The mixture was then allowed to warm up to room temperature. After 1 h, distilled water (90 ml) and ethyl acetate (100 ml) were added to the mixture and the organic phase was separated, dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure. The product was purified by silica gel chromatography using chloroform:methanol (9:1) as eluant to give the title compound **5** (15.50 mCi, 349 mg).

2-*(1H-benzotriazol-1-yl)*-**2**-*(benzoyl[cabonyl-^{14}C]amino)acetic acid* **8**

The benzoamide[carboxyl-^{14}C] **5** (13.41 mCi, 302 mg), glyoxylic acid monohydrate **6** (230 mg), and 1H-benzotriazole **7** (298 mg) in toluene (12.5 ml) were refluxed in a Dean-Stark apparatus for 3 h. The title product **8** precipitated upon cooling and was purified by washing with ether, crystallization from methanol/ether, and drying under high vacuum to give the title compound **8** (10.85 mCi, 598 mg) (yield: 81%). ^{1}H-NMR (DMSO-d_6, TMS) : δ7.42 (t, 1H, $J = 7.6$ Hz); δ7.47–7.52 (m, 3H); δ7.56–7.60 (m, 2H); δ7.91–7.94 (m, 2H); δ8.05–8.12 (m, 2H); δ10.17 (d, 1H, $J = 7.6$ Hz); IR (KBr): 1150, 1277, 1523, 1659, 1722, 3323 cm^{-1}.

*N-(***2***-oxo-***5***-phenyl-***2***,***3***-dihydro-1H-*1,**4**-*benzo[e][1,4]diazepin-***3***-yl)-benzamide [carboxyl-^{14}C]* **11**

Oxalyl chloride (0.17 ml, 2 mmol) and anhydrous DMF (0.02 ml) were added to a solution of 2-(1H-benzotriazol-1-yl)-2-(benzoyl[cabonyl-^{14}C] amino)acetic acid **8** (10.74 mCi, 592 mg) in anhydrous THF (8 ml) under N_2 at 0–5°C, and the mixture was stirred below 5°C for 2 h. A solution of 2-aminobenzophenone (394 mg, 2 mmol) and anhydrous *N*-methyl morpholine (0.42 ml) in anhydrous THF (3 ml) was added to the stirred mixture at 5°C over 30 min, and the reaction mixture allowed to reach room temperature. The reaction slurry was filtered, and the mother liquor was saturated with ammonia gas, diluted with methanol (16 ml), and again saturated with ammonia gas for approximately 30 min. After evaporation of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (10 ml), washed successively with 1 N aqueous NaOH solution and brine, dried over anhydrous sodium sulfate and concentrated under reduced pressure. Following the treatment of the resulting crude amino ketone intermediate **10** with a solution of ammonium acetate (660 mg) in glacial acetic acid (8 ml), the reaction solution was stirred under N_2 overnight. The solvent was evaporated under reduced pressure, the residue was suspended in ethyl acetate

(2 ml) and diethyl ether(10 ml), and the pH of the mixture adjusted to approximately 8.5 by addition of 1 N aqueous NaOH solution. The crude product, which precipitated upon cooling the suspension, was crystallized from ethyl acetate/diethyl ether to give the pure title compound **11** (7.18 mCi, 475 mg) (yield: 67%)

^{1}H-NMR (DMSO-d_6, TMS) : δ5.21 (d, 1H, $J = 6.8$ Hz); δ7.03–7.25 (m, 3H); δ7.43–7.58 (m, 9H); δ7.99 (d, 2H, $J = 6.8$ Hz); δ9.27(d, 1H, $J = 6.8$ Hz); IR (KBr): 523, 770, 1260, 1383, 1527, 1660, 1705, 2889, 3319, 3462 cm^{-1}.

*N-(1-methyl-**2**-oxo-**5**-phenyl-2,**3**-dihydro-1H-benzo[e][1,**4**]diazepin-**3**-yl)-benzamide-[carboxyl-^{14}C]***1***

Fresh sodium hydride (60% dispersion in mineral oil, 40 mg, 1 mmol) was added to a solution of *N*-(2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzo[e][1,4]-diazepin-3-yl)-benzamide [carboxyl-^{14}C] **11** (5.37 mCi, 355 mg) in anhydrous DMF (4 ml) at 0°C under a nitrogen atmosphere. After 5 min methyl iodide (142 mg) 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. Recrystallization from ethylacetate/*n*-hexane(6:4) gave the pure title compound **1** (4.29 mCi, 295 mg).

^{1}H-NMR ($CDCl_3$, TMS) : δ3.51 (s, 3H, methyl); δ5.71 (d, 1H, $J = 8$ Hz, CH); δ7.38–7.63 (m, 12H); δ7.95 (d, 2H, $J = 8$ Hz, aromatics); δ8.03 (d, 1H, $J = 8$ Hz, amide NH); IR (KBr): 692, 779, 1516, 1644, 1690, 3052, 3278 cm^{-1}.

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