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A convenient method for 14C-labeling of N-(7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)thiophene-2-carboxamide as CCK-A antagonist

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N-(7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)thiophene-2 carboxamide-[^{14}C-carboxy] was prepared as part of a 6-step sequence from thiophene-2-carbonitrile -[cyano-^{14}C] as a key synthetic intermediate which has been synthesized from 2-iodothiophene and zinc [^{14}C]-cyanide in the presence of tetrakis (triphenylphosphine) palladium.

Keywords: thiophene-2-carbonitrile; Cholecystokinin antagonist; Carbon-14; benzodiazepin

1. 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 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 the 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, etc as CCK-A antagonists [4] and CCK-A agonists [5] such as GW5823 have demonstrated the significance of these investigations. A novel series of N-(2,3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-2-carboxamides were introduced by Khalaj et al. [6]. For studies of pharmacokinetics and drug metabolism of these compounds, the version with a metabolically suitable carbon-14 label were required[7-8]. This paper reports a convenient method for ^{14}C-labelling of N-(7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)thiophene-2-carboxamide**1** as CCK-A antagonist.

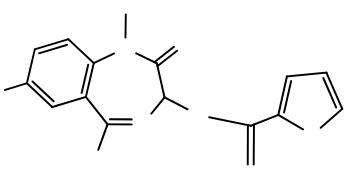


Fig.1

2. EXPERIMENTAL

Barium [^{14}C]- carbonate was converted to potassium [^{14}C]–cyanide according to the standard procedure[9-10]. 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 400spectrometer(400MHz).Radioactivity was determined using a Beckman LS6500 liquid scintillation spectrometer. Mass spectra were obtained on a Finnigan TSQ-70 instrument.

2.1. Zinc [^{14}C]-cyanide 3

To a solution of zinc chloride (380 mg) in water (1.1 ml) was added drop wise an aqueous solution of potassium [^{14}C]-cyanide **2** (242mg, 440 kBq) in water (2.2 ml) at room temperature. Precipitation of the white zinc [^{14}C]-cyanide **3** started immediately. The reaction mixture was stirred at room temperature for 5min and then centrifuged. The supernatant was removed and remaining solid was washed with water (4× 1ml) and diethyl ether (2× 1ml). The obtained white crystals were dried overnight using high vacuum affording the desired product **3** in 95.4% yield (210mg,420kBq).

2.2. Thiophene-2-carbonitrile-[cyano-^{14}C] 5

2-Iodothiophene **4** (747mg) , zinc [^{14}C]-cyanide **3** (200mg, 400kBq) and tetrakis(triphenyl - phosphine)palladium (196mg) were weighed in a dry flask charged with argon. Freshly deoxygenated DMF (10ml) was added and yellow slurry was heated to 80^{0}C under argon until HPLC shows no starting material remaining (1-2h). The mixture was cooled to room temperature, diluted with ethyl acetate(100ml), and washed twice with 2N ammonium hydroxide (20ml). The ethyl acetate solution was then washed with brine (10ml) . The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo and the residue was purified by silica gel chromatography using ethyl acetate as eluant to give the title compound **5** (339mg, 360kBq) in 90% yield.IR(KBr): 3120, 2221, 1520, 1045cm^{-1}. ^{1}H-NMR($CDCl_3$, TMS) : δ 7.95(d, 1H, J=4Hz); δ 7.50(d, 1H, J=4Hz); δ 7.20(t, 1H, J=4Hz) ; MS (70eV): m/z = 111[M^{+}].

2.3. Thiophene-2-carboxamide-[carboxy-^{14}C] 6

To a stirred solution of thiophene-2-carbonitrile-[cyano-^{14}C] **5** (358kBq, 337mg) in DMSO (5.25ml) cooled in an ice bath was added H_2O_2 (30%,2.1ml) and potassium carbonate (350mg).The mixture was then allowed to warm up to room temperature. After one hour, distilled water (90ml) and ethyl acetate (100ml) were added to the mixture and the organic phase was separated, dried over anhydrous sodium sulfate, filtered and the solvent was removed under reduced pressure. The product was purified by silica gel chromatography using chloroform: methanol (9:1) as eluant to give the title compound **6** (327kBq, 360mg). IR(KBr): 3467, 3425 3125, 1667, 1520, 1050cm^{-1}. MS (70eV): m/z = 129[M^{+}].

2.4. 2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-(thiophene-2-carboxamido-[carboxy-^{14}C])acetic acid9

The thiophene-2-carboxamide-[carboxy-^{14}C] **6** (288kBq, 317mg), glyoxylic acid monohydrate **6** (230mg), and 1H-Benzotriazole **7** (298mg) in toluene (12.5ml) were refluxed in Dean-Stark apparatus for 3h. The title product **9** precipitated upon cooling were purified by washing with ether, crystallization from methanol/ether, and drying under high vacuum to give the title compound **9** (243kBq, 638mg).(yield: 84.4%). ^{1}H-NMR (DMSO-d_6 , TMS) :δ 7.20 (t, 1H, J=4Hz) ; δ 7.44(t, 1H, J=7.6Hz) ; δ 7.56(d, 1H, J=4Hz) ; δ 7.60(t, 1H, J=7.6Hz) ; δ 7.87(d, 1H, J=8Hz) ; δ 8.05(d, 1H, J=7.6Hz) ; δ 8.07(d, 1H, J=4Hz) ; δ 8.09(d, 1H, J=7.6Hz) ; δ 10.31 (d, 1H, J=8Hz). IR (KBr): 1156, 1156, 1402, 1549, 1622, 1759, 3218 cm^{-1}; MS(70eV): m/z=304(M^{+}).

2.5. N-(7-chloro-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)thiophene-2-carboxamide-[^{14}C-carboxy] 12

Oxalyl chloride (0.15ml) and anhydrous DMF (0.02mL) were added to a solution of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-(thiophene-2-carboxamido-[carboxy-^{14}C])acetic acid **9** (208kBq, 546mg) in anhydrous THF (7mL) under N_2 at 0-5^{o}C, and the mixture was stirred below 5^{o}C for 2h.A solution of 2-amino-5-chlorobenzophenone (402mg) and anhydrous N-methylmorpholine (0.37mL) in anhydrous THF (2.6mL) was added to the stirred mixture at 5^{o}C over 30min., thereafter, the reaction mixture was allowed to reach room temperature the reaction slurry was filtered, and the mother liquor was saturated with ammonia gas, diluted with methanol (14mL), and again saturated with ammonia gas for approximately 30min. After evaporation of the solvent under reduced pressure, the residue was dissolved in ethyl acetate (10mL), washed successively with 1N 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 **11** with a solution of ammonium acetate (573mg) in glacial acetic acid (7mL), the reaction solution was stirred under N_2 overnight. The solvent was evaporated under reduced pressure, the residue was suspended in ethyl acetate (2ml) and diethyl ether(10ml), and the pH of the mixture adjusted to approximately 8.5 by addition of 1N aqueous NaOH solution. The crude product, which precipitated upon cooling the suspension, was crystallized from ethyl acetate/diethyl ether and further purification was achieved by using chromatography (silicagel, dichloromethane: hexane: ethanol, 5:5:1) to give the pure the title compound **12** (146kBq, 502mg).(yield: 70%). ^{1}H-NMR (DMSO-d_6, TMS) : δ 5.69 (d, 1H, J=7.6 Hz) ; δ 7.11-7.14 (m, 2H); δ 7.35-7.54(m, 8H); δ 7.73 (d, 1H, J=3.6 Hz); δ 7.78(d, 1H, J=7.6 Hz); IR (KBr):532, 705, 1281, 1490, 1537, 1644, 1706, 2356, 2930, 3416 cm^{-1}; MS(70eV): m/z=397(M^+).

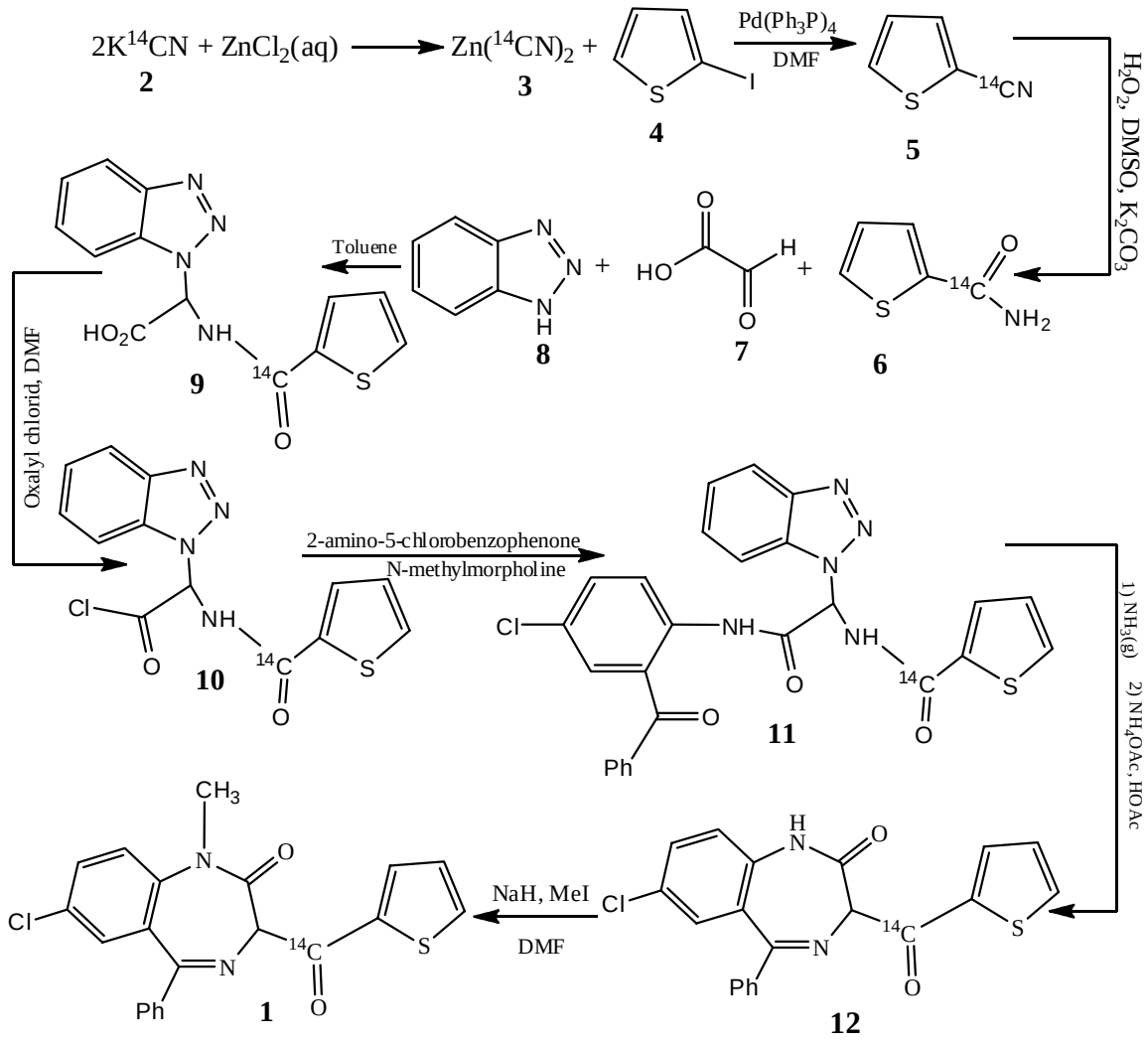
2.6. N-(7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl) thiophene-2-carboxamide-[^{14}C-carboxy] 1

Fresh sodium hydride (60% dispersion in mineral oil, 40mg, 1mmol) was added to a solution of 7-chloro-5-phenyl-3-(thiophene-2-carbonyl-[carbonyl-^{14}C])-1H-benzo[e][1,4]diazepin-2(3H)-one **12** (115kBq, 395mg) in anhydrous DMF (4ml) at 0^{0}C under a nitrogen atmosphere. After 5min methyl iodide (142mg) was added via a micropipette and the reaction mixture stirred for 5min. the reaction mixture was then added to a vigorously stirred solution of water (2.5ml) containing aqueous sodium hydrogen sulfate (0.5ml, 1N). The reaction slurry was filtered after 5min and washed with water, ether and cold methanol and dried under high vacuum. Recrystallization from ethylacetate:hexane(6:4) gave the pure title compound **1** (91kBq, 324mg). ^{1}H-NMR ($CDCl_3$, TMS) : δ 3.52 (s, 3H, methyl) ; δ 5.69 (d, 1H, J=7.6 Hz) ; δ 7.11-7.14 (m, 2H); δ 7.35-7.54(m, 8H); δ 7.73 (d, 1H, J=3.6 Hz); δ 7.78(d, 1H, J=7.6 Hz); IR (KBr):532, 705, 1281, 1490, 1537, 1644, 1706, 2356, 2930, 3416 cm^{-1}; MS(70eV): m/z=411(M^+).

3. DISCUSSION

In this approach , according to the synthetic pathway shown in scheme 1 , after conversion of potassium [^{14}C]-cyanide 2 to zinc [^{14}C]-cyanide 3 in the presence of an aqueous solution of zinc chloride, thiophene-2-carbonitrile-[cyano-^{14}C] 5 was derived from reaction of 2-iodothiophene 4 with zinc [^{14}C]-cyanide 3 and tetrakis(triphenylphosphine)palladium in dry DMF[11-12]. In the next step, after coversion of the nitrile-[cyano-^{14}C] 5 to thiophene-2-carboxamide -[carboxy-^{14}C] 6 by using basic hydrogen peroxid in DMSO[13], 2-(1H-benzo[d][1,2,3]triazol-1-yl)-2-(thiophene-2-carboxamido-[carboxy-^{14}C])acetic acid **9** was prepared by condensation of the amide-[carboxy-^{14}C] 6 and glyoxylic acid 7 and 1H-Benzotriazole 8 with azeotropic removal of water [14]. After

conversion of the compound **9** to acyl chloride **10** in the presence of oxalyl chloride in DMF, acyl coupling of **10** [15] with 2-amino-5-chlorobenzophenone and subsequent displacement of the benzotriazole moiety with ammonia followed by cyclization of the resulting amino-ketone intermediate **11**, gave the 1,4-benzodiazepine derivative **12** [16]. In the final step, the desired product **1** was achieved from addition of methyl iodide to the 1,4-benzodiazepine derivative **12** in the presence of sodium hydride. The ^{14}C-labelled compound **1** was synthesized for the first time and biological studies on this compound are currently under investigation.



Scheme I

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