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SYNTHESIS OF ^{3}H- AND ^{14}C-LABELLED CP-88,059: A POTENT ATYPICAL ANTIPSYCHOTIC AGENT

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SUMMARY

The syntheses of ^{3}H- and ^{14}C-labelled CP-88,059 [i.e., 5-(2-(4-(1,2-benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1, 3-dihydro-2H-indol-2-one] are described. CP-88,059 (**5b**) is a combined $D_2/5\text{-}HT_2$ antagonist currently undergoing clinical evaluation as an antipsychotic agent with reduced potential for induction of EPS in schizophrenic patients. Displacement of bromine from the 7-position of the benzisothiazole moiety, by reductive dehydrogenation with tritium gas and $Pd/BaSO_4$ catalysis, provided ^{3}H-CP-88,059 (**5c**). Incorporation of ^{14}C into the ethylene portion of the molecule was achieved via the Friedel-Crafts acylation of 6-chlorooxindole with [2-^{14}C]-chloroacetyl chloride, followed by triethylsilane reduction of the aryl carbonyl and coupling with N-(1,2-benzisothiazol-3-yl)piperazine in refluxing aqueous Na_2CO_3.

Keywords: ^{3}H- and ^{14}C-labelled CP-88,059, antipsychotic, $D_2/5\text{-}HT_2$ antagonist

INTRODUCTION

In the search for antipsychotic treatments for the control of the positive symptoms (e.g., auditory hallucinations) of schizophrenia, several new drugs have recently been discovered which also appear to address the negative aspects (e.g., lack of affect) which often emerge.[1] At Pfizer, we

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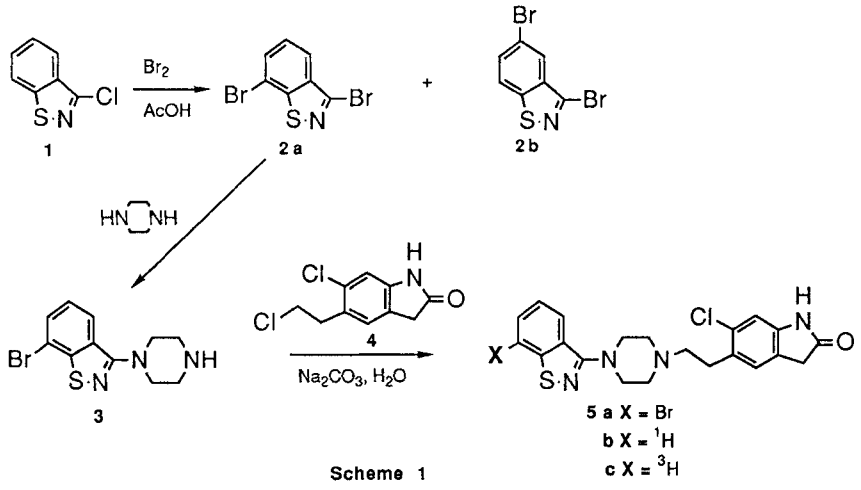
have developed such an agent in CP-88,059, a potent dopamine D_2 and serotonin 5-HT_2 antagonist which has demonstrated a preclinical profile predictive of atypical antipsychotic activity.[2] Furthermore, early clinical experience with CP-88,059 has confirmed its safety and efficacy in man, without the apparent induction of extrapyramidal side effects (EPS) which have been associated with chronic D_2 receptor blockade in striatal regions of the brain.[3]

In order to determine the metabolism and tissue distribution of this compound, we prepared both the ^3H- and ^{14}C-labelled congeners with the isotope incorporated into metabolically stable sites on the molecule. Thus, displacement of an aryl bromide using tritium gas was envisioned as a viable means of obtaining the tritiated ligand, whereas a modification of the cold synthesis of CP-88,059, using ^{14}C-chloroacetyl chloride in the acylation step, was designed to provide the latter compound with the least number of manipulations.

RESULTS AND DISCUSSION

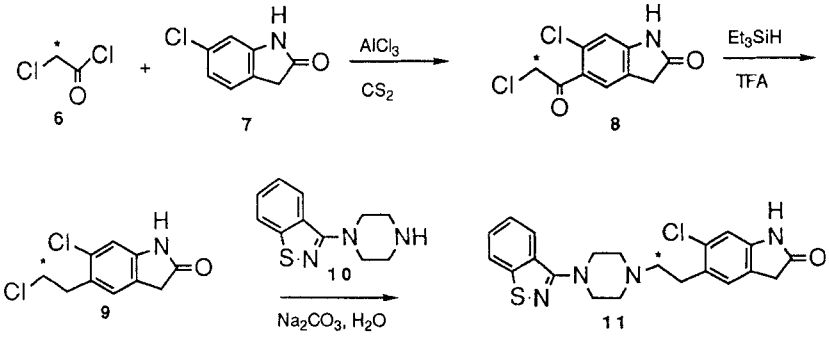
Examination of the structure of CP-88,059 reveals a number of possible sites into which a radioisotope might be incorporated. In practice, the introduction of a tritium atom into CP-88,059 (**5b**) was achieved most efficiently by selective replacement of the bromine atom at the 7-position (of the precursor **5a**) in the presence of the chlorine atom on the indolinone fragment (Scheme 1). The synthesis of **5a** began with the bromination of **1** using bromine in acetic acid with $FeCl_3$ catalysis. This generated two isomers, 3,7-dibromo-1,2-benzisothiazole (**2a**) and the 3,5-dibromo analog (**2b**) which were separable using flash chromatography. Reaction of **2a** with piperazine in refluxing diglyme provided a 71% yield of **3** and the location of the bromine atom was conclusively established through NOE experiments with this intermediate. Condensation of **3** with the indolinone fragment **4** in aqueous Na_2CO_3 at 115 °C for 18 hr provided the key intermediate **5a** in 89% yield (97% purity).



Scheme 1

While Pd/C (5% or 10%) in THF at 25 °C showed little evidence for the conversion of **5a** to **5b**, a 53% conversion was realized using 10% Pd on $BaSO_4$[4] in THF for 6 hr at 45-50 psi. Further exposure to these conditions showed evidence of dechlorination of the indolinone portion of the molecule. There was no visible separation in a variety tlc systems between **5a** and **5b**, but they were readily separable by reverse phase HPLC using a C18 column (R_t 45-50 min vs R_t 9 min, respectively). When **5a** was treated with tritium gas under the same conditions, **5c** was obtained. This material was purified by preparative HPLC to provide drug with high specific activity and radiochemical purity (10.3 Ci/mmol, ≥ 98%).

Preparation of the ^{14}C-labelled drug was accomplished in a manner analogous to that employed for the "cold" synthesis[5] as shown in Scheme 2. When indolinone **7** was treated with [2-^{14}C]-chloroacetyl chloride (ChemSyn Science Laboratories) a tan solid (**8**) was obtained (spec. act. 48 mCi/mmol). This was converted to **9** using triethylsilane (Et_3SiH) in TFA at 25°C for 20 hr, combined with 3 weight equivalents of **4** (unlabelled **9**) and recrystallized from THF. Reaction of this intermediate with the substituted piperazine **10**, as previously described, gave the free base. Stirring the crude **11** in 3N HCl at 65-70°C for 18 hr formed the HCl salt of **11** with a specific activity of 9.6 mCi/mmol and a radiochemical purity of >98%.



Scheme 2

In conclusion, we have prepared the atypical antipsychotic agent CP-88,059 with ^{3}H and ^{14}C (**5c** and **11**, resp.) located at potential metabolically-stable sites on the molecule. Their use in the studies of metabolism and tissue distribution in man will be documented in forthcoming communications from our group.

EXPERIMENTAL SECTION

Melting points were determined with Pyrex capillary tubes on a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H-nmr spectra were recorded using Bruker WM-250 or AM-250 spectrometers; shifts are presented in parts per million (ppm, δ) with reference to the deuterium lock signal of the solvent. Mass spectral data were obtained using a Finnigan 4510 instrument for low resolution (LRMS) or an AEI MS-30 instrument for high resolution (HRMS) determinations. Analytical thin-layer chromatography (tlc) was conducted on 0.25 mm thick silica gel 60 F-254 plates (E. Merck & Co.) or 250 micron silica gel GHLF 10 X 20 cm Uniplates (Analtech, Inc.); plates were visualized with UV light or phosphomolybdate (PMA) reagent Flash chromatography was performed using Baker silica gel (40μm). Elemental analyses were performed by the Pfizer Analytical Department and values agree to within ±0.4% of the calculated values. Commercial reagents were used without purification.

Radio-tlc scans were obtained on a Model LB242K Varian Aerograph /Berthold Radio scanner or a BIOSCAN BID 200 radiochromatogram scanner.

Scintillation determinations were made with a Beckman LS 3801 liquid scintillation counter using Fisher Liquid ScintiVerse scintillation cocktail, correcting for counting efficiency using an internal standard technique. Chemical and radiochemical purities were determined on a Varian 5040-1095 instrument with a Valco manual injector (50 μL loop) and a Radioamatic HP 30 FLO ONE radiodetector, or a Varian 5560 HPLC instrument with a Rheodyne 7125 injector (200 μL loop) and a Waters Associates 3.9x150 mm Nova-Pak C18 column, monitored with a 229 nm UV detector and a Berthold Model LB504 Radioactivity monitor; elution was performed with 0.02M potassium phosphate / CH_3CN / CH_3OH (70:20:10), pH 3.0, at a flow rate of 1.5 mL/min.

3,7-Dibromo-1,2-benzisothiazole (2a). A mixture of pure 3-chloro-1,2-benzisothiazole **1** (8.62 g, 50.8 mmol)[6] and 125 mL of AcOH was treated with $FeCl_3 \cdot 6H_2O$ (0.57 g, 3.5 mmol). Bromine (11 mL, 214 mmol) was added dropwise, the mixture was heated to reflux and after 20 hr was cooled to room temperature and evaporated *in vacuo* to dryness. The crude solid was dissolved in CH_2Cl_2 and washed with dilute aqueous $NaHCO_3$ and NaCl. After drying over $MgSO_4$, the solvent was removed *in vacuo* to produce a yellow solid which was chromatographed with hexanes. The major product fraction, **2a**, was isolated as a white solid, 2.62 g (18%), m.p. 67-69 °C; ^{1}H-nmr ($CDCl_3$, δ) 7.41 (*t*, 1H), 7.72 (*d*, J=7.5 Hz, 1H), 7.95 (*d*, J=7.5 Hz, 1H); ^{13}C-nmr ($CDCl_3$,ppm) 113.0, 123.6, 127.3, 131.5, 135.6, 135.8; LRMS: *m/e* 295 (60%, M^{+4}), 293 (100%, M^{+2}), 291 (65%, M^{+}), 214, 212, 133; Anal. calcd for $C_7H_3Br_2NS$: C 28.69, H 1.03, N 4.78; found: C 28.60, H 1.01, N 4.70.

An additional 2.15 g of **2a**, contaminated with a small amount of the isomer **2b**, was also isolated.

7-Bromo-3-piperazinyl-1,2-benzisothiazole (3). A mixture of **2a** (2.62 g, 8.94 mmol) and piperazine (14.91 g, 173 mmol) in 33 mL of diglyme was refluxed for 20 hr, cooled to 25 °C, diluted with H_2O and stirred for another 90 min. The mixture was then extracted with CH_2Cl_2

(3x100 mL) and the combined organics were washed with saturated aqueous NaCl, dried ($MgSO_4$) and concentrated to a yellow oil. Flash chromatography (conc. NH_4OH : CH_3OH : CH_2Cl_2 - 1:5:94) gave pure **3** as a pale yellow oil which slowly solidified , 1.80 g (68 %), m.p. 117-120 °C.
^{1}H-nmr ($CDCl_3$, δ) 1.80 (*s*, 1H), 3.08 (*m*, 4H), 3.48 (*m*, 4H), 7.24 (*m*, 1H), 7.58 (*d*, J=7.5 Hz, 1H), 7.85 (*d*, J=7.5 Hz, 1H); ^{13}C-nmr ($CDCl_3$, ppm) 45.9, 51.4, 114.1, 122.8, 125.5, 129.3, 130.3, 154.5, 164.6; LRMS: *m/e* 299 (20%, M^{+2}), 297 (20%, M^+), 257, 255 (68%), 231, 229, 215, 133, 56 (100%); Anal. calcd for $C_{11}H_{12}BrN_3S$: C 44.31, H 4.05, N 14.09; found: C 44.20, H 3.88, N 13.83.

5-(2-(4-(7-Bromo-1,2-benzisothiazol-3-yl)piperazinyl)-ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one (5a). A mixture of **3** (1.70 g, 5.7 mmol), 6-chloro-5-(2-chloroethyl)-1,3-dihydro-2H-indol-2-one (**4**)[6] and anhydrous Na_2CO_3 (1.35 g, 12.74 mmol) in 11 mL H_2O was heated in a 35 mL flask over an oil bath at 115 °C for 20 hr. After cooling to room temperature, the reaction mixture was partitioned between H_2O and CH_2Cl_2 and the insoluble crude material was collected by filtration. It was washed with additional H_2O and isopropanol until the filtrate appeared colorless and dried to a pale tan solid, 2.5 g (89 %), m.p. 262-265 °C;
^{1}H-nmr (DMSO-d_6, δ) 2.52 (*m*, 2H), 2.67 (*m*, 4H), 2.83 (*m*, 2H), 3.45 (*m*, 6H), 6.80 (*s*, 1H), 7.22 (*s*, 1H), 7.41 (*t*, 1H), 7.82 (*d*, J=7.3 Hz, 1H), 8.13 (*d*, J=8.0 Hz, 1H), 10.42 (*s*, 1H); LRMS (FAB): *m/e* 495 (34%, M^{+4}), 493 (100%, M^{+2}), 491 (85%, M^+), 235, 157 (100%); Anal. calcd for $C_{21}H_{20}BrClN_4OS$: C 51.28, H 4.09, N 11.39; found: C 51.07, H 4.04, N 11.30.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one (5b). A solution of **5a** (9.3 mg, 0.02 mmol) in 2 mL of anhydrous THF was hydrogenated in the presence of 10 mg of 10% Pd/$BaSO_4$ (Fluka Chemical Co.) at atmospheric pressure. After 6 hr at 25 °C the catalyst was removed by filtration through diatomaceous earth (d.e.), the d.e. cake was rinsed with another 10 mL of THF and the organics were combined and concentrated *in vacuo* to a solid (10

mg). HPLC analysis showed this solid to be a mixture (43:50) of **5a** and **5b**, with retention times of 46.9 min and 9.2 min, resp., and compound **5b** to be identical to authentic CP-88,059.

Repeating this reaction, but replacing hydrogen gas with deuterium, a mixture of **5a** and ^{2}H-**5b** (52:48) was isolated.

[7-^{3}H]-5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)-ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one (5c). A suspension of **5a** (9.3 mg, 0.018 mmol) in 2 mL of anhydrous tetrahydrofuran (THF) was transferred to a 3-mL reaction vial containing 10% Pd / $BaSO_4$ (33 mg). The suspension was degassed (X 3) *in vacuo* and stirred under 12.5 Ci (0.21 mmol) of tritium gas at room temperature for 16 hr. Catalyst was removed by filtration through d.e. and washed with 15 mL of THF. Solvent was removed under reduced pressure and labile tritium was removed by evaporation with 3 X 1 mL of methanol. The resulting crude product (113 mCi) was approximately 51% radiochemically pure by TLC. Partial purification was achieved by filtration of a THF suspension of crude product through a membrane syringe filter to remove the marginally soluble, unreacted **5a**. Prep-TLC (Whatman LK6F, 5 X 20 cm; $CHCl_3$ / CH_3OH 95:5) on four analytical plates, using multiple developments, gave 22 mCi of product with a radiochemical purity of 98% by TLC (96% by HPLC). Final purification was achieved by reverse phase HPLC (Whatman Partisil 5 ODS 3, 4.6 X 250 mm) using an isocratic solvent system of 55% A (0.1 M NH_4OAc, pH 7.2 in CH_3CN 70:30), 45% CH_3CN. The product was recovered by extraction of the concentrated mobile phase with THF / hexane (1:1) giving 23.1 mCi **5c** ([^{3}H]-CP-88,059) with a specific activity of 10.3 Ci / mmol and a radiochemical purity ≥ 98%.

5-([2-^{14}C]chloroacetyl)-6-chloro-1,3-dihydro-2H-indol-2-one (8). Chloroacetyl[2-^{14}C]chloride was prepared by heating a mixture of freshly distilled chloro[2-^{14}C]acetic acid (92 mCi, 48 mCi/mmol) and phthaloyl dichloride (2.5 mL) at 80 °C for 3 hr, followed by transfer of the

product to a cold trap under vacuum. Yield: 75 mCi, 81%. The purity was estimated to be >95% from the proton NMR spectrum.

A mixture of freshly distilled carbon disulfide (4 mL), aluminum chloride (313 mg, 2.35 mmol), 6-chloro-1,3-dihydro-2H-indol-2-one (**7**, 78 mg, 0.47 mmol) and freshly prepared chloroacetyl[2-^{14}C]chloride (25 mCi, 0.52 mmol) was heated to reflux in a 10 mL flask under argon atmosphere for 9 hr, then cooled and filtered. The filter cake was rinsed with cold CS_2 and then taken up in 5 mL of distilled water and allowed to stir at room temperature for 24 hr to decompose the $AlCl_3$-product complex. The product was collected by filtration and dried *in vacuo* to yield 16 mCi (83.9 mg, 0.34 mmol) of **8** as a pale tan solid (73%).

5-([2-^{14}C]-2-chloroethyl)-6-chloro-1,3-dihydro-2H-indol-2-one (9). A mixture of **8** (83 mg, 0.338 mmol) in 1.0 mL of trifluoroacetic acid (TFA) was treated with triethylsilane (0.13 mL, 0.78 mmol) at 25° C. After stirring for 20 hr, a tlc radio-chromatogram (1:1, EtOAc:Hexane) indicated complete reduction of the starting material **8**. Water was added and stirring was then continued for another 24 hr at which point the crude solid was filtered, washed with fresh H_2O and air dried to 58.5 mg (75 %) of tan solid. This material was combined with 180 mg of **4** and recrystallized from 10 mL of THF, washed after filtration with petroleum ether and dried to constant weight. The crystalline product, 238 mg, appeared as a single spot by tlc radiochromatogram analysis (100 % EtOAc, R_f =0.56), identical to authentic **4**.

5-(2-(4-(1,2-Benzisothiazol-3-yl)piperazinyl)-[2-^{14}C]-ethyl)-6-chloro-1,3-dihydro-2H-indol-2-one hydrochloride hydrate (11). A mixture of **9** (158.5 mg, 0.683 mmol), **10** (149.6 mg, 0.683 mmol) and anhydrous Na_2CO_3 (159.3 mg, 1.50 mmol) in 2.3 mL of H_2O was stirred and heated over a 2 hr period to 110 ± 5 °C (oil bath temperature) and maintained at this temperature for another 23 hr. A tlc (100% EtOAc) at this point showed a major product spot with an R_f identical to that of **5b**

(CP-88,059). After cooling, the solid mass was filtered, carefully ground up while washing liberally with H_2O, then isopropanol, to remove unreacted **9** and **10**. After drying at 25 °C for another 24 hr, the crude free base (242 mg) was suspended in 5.0 mL of 3N HCl in a 25 mL flask and the mixture was heated over an oil bath at 65-70 °C for 24 hr. The insoluble HCl salt was filtered, washed with fresh isopropanol and dried over $CaCl_2$ under vacuum for 24 hr, providing the title product as a pale tan solid, 235 mg (87 %). HPLC analysis showed this material to be >98% radiochemically pure (72% chemically pure). The specific activity of compound **11** was calculated to be 9.6 mCi/mmol.

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REFERENCES

1.a. New, J.S, Takaki, K.S. -- *Annu. Rep. Med. Chem.*, **24**:1(1989).

b. Abou-Gharbia, M., Moyer, J.A. -- *ibid*, **25**:1(1990).

c. Wise, L.D., Heffner, T.G. --*ibid*, **26**:53(1991).

2. Seeger, T.S. *et al* -- unpublished results.

3. Heym, J., Seeger, T, Seymour, P., Howard, H., Smolarek, T., Tremaine, L. Wilner, K., Gerber, N., Wong, D., and Etienne, P. *Abstract 31st Ann. Mtg. Amer. Coll. Neuropsychopharmacology,* San Juan, PR, Dec.14-18, 1992.

4. Brundish, D.E., Kane, P.D. -- *J. Label. Comp. and Radiopharm.* , **25**:1361(1988).

5. Bowles, P. -- U.S. Patent 5,206,366 (April 27, 1993).

6. Lowe, J.A.,III, Nagel, A.A. --U.S. Patent 4,831,031 (May 16, 1989).