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Esters of 6-(4'-Fluorobenzylamino)-β-Carboline-3-Carboxylic Acid as Potential Benzodiazepine Imaging Agents for P.E.T.

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Summary

As potential P.E.T. imaging agents for the benzodiazepine receptor, two fluorine-18 labeled analogues of the β-carbolines were prepared *via* N-alkylation of the corresponding desbenzyl amine precursors with [^{18}F]fluorobenzyl iodide.

Key Words: β-Carbolines, P.E.T., [^{18}F]fluorobenzyl iodide, fluorine-18, benzodiazepine receptor.

Introduction

β-Carbolines represent a class of compounds which exert their pharmacological action through an interaction with the benzodiazepine (BZD) receptor.[1] Recent reports have shown that minor structural

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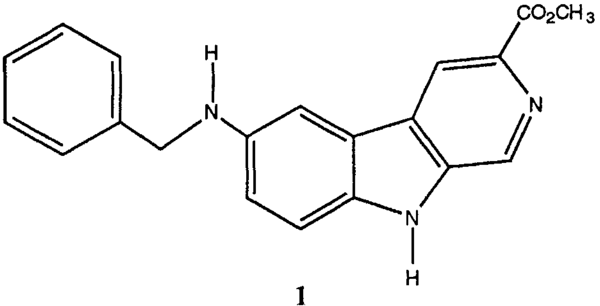
modifications on the β-carboline ring result in varied pharmacological action.[2] Hagen *et al.* have shown that 3,6-substituted β-carbolines such as methyl 6-(benzylamino)-β-carboline-3-carboxylate (**1**) exhibit many of the antagonistic characteristics of Ro15-1788 and has a high affinity for the BZD receptor.[3] As our interest lies in preparing antagonists for P.E.T. imaging of the BZD receptor, fluorobenzylamino analogues of **1** were prepared and labeled in the 6-position, by N-alkylation of the desbenzyl precursors, with [^{18}F]fluorobenzyl iodide.[4]



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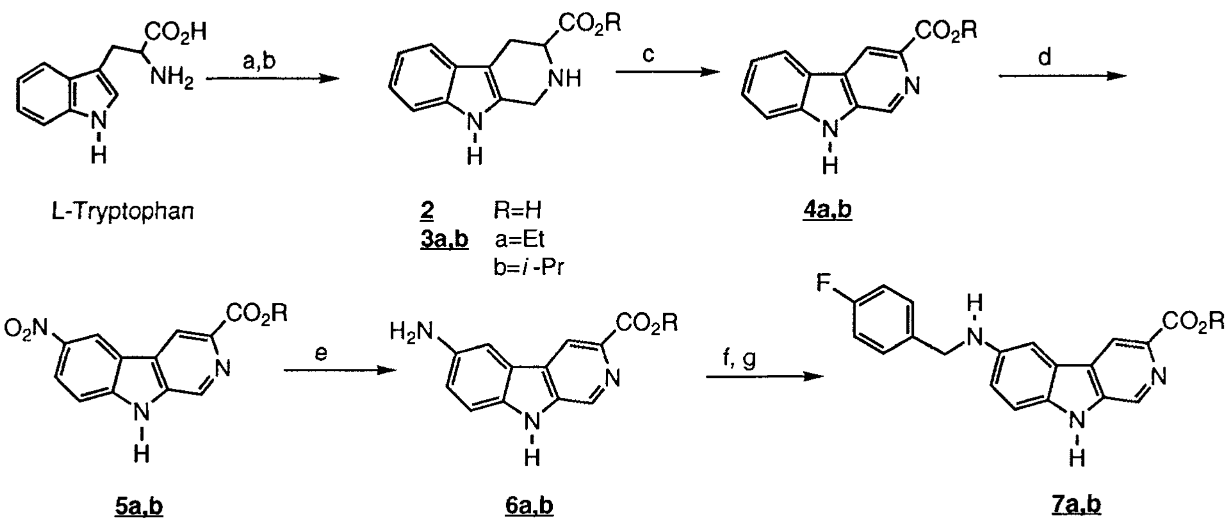
Results and Discussion

The desired labeling precursors, the 6-amino-β-carbolines (**6a, b**), were readily prepared in a 5 step reaction sequence, starting from L-tryptophan. The synthesis is shown in Scheme 1. Condensation of L-tryptophan with formaldehyde in either acidic or alkaline conditions gave the tetrahydrocarboline **2** (free acid of **3**, R=H). This insoluble compound was immediately esterified to give the ethyl or isopropyl esters **3a** and **3b**. Aromatization of **3a,b** with sulfur in refluxing xylene for 36 to 72 hr gave the β-carbolines **4a,b**. Nitration of **4a,b** with fuming HNO_3 at 0°C afforded the 6-nitro compounds **5a,b**. The amines **6a,b** were prepared through catalytic reduction of **5a,b** in a Parr hydrogenator. The fluorobenzyl compounds **7a,b** were prepared by reductive alkylation of **6a**, or **6b**, and fluorobenzaldehyde using $NaBH_4$ as the reducing agent.

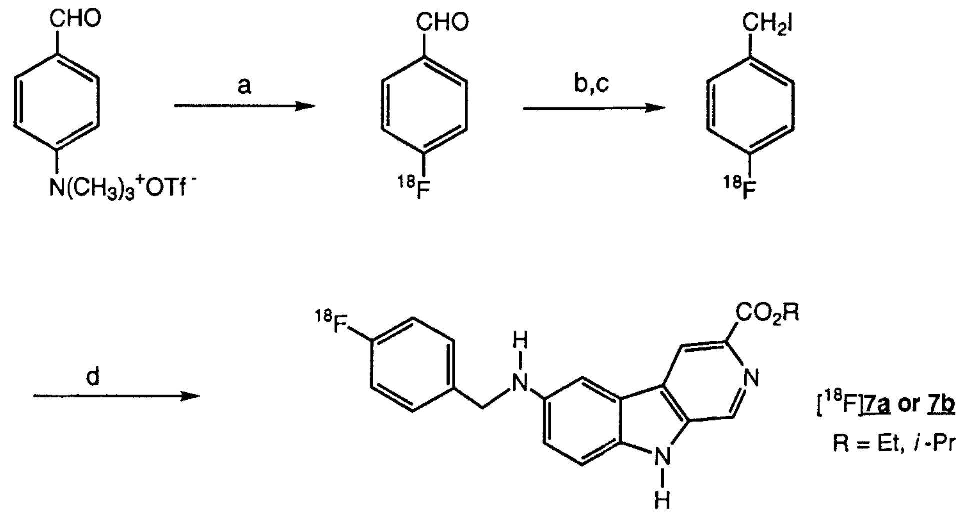
Scheme 1. Synthesis of ethyl and isopropyl esters of 6-(4'-fluorobenzylamino)-β-carboline-3-carboxylate.



Reagents: a: NaOH, CH_2O; b: ROH, HCl; c: sulfur, xylene, Δ; d: 90% HNO_3, 5°C; e: H_2, PtO_2; f: 4-fluorobenzaldehyde, benzene, reflux; g: $NaBH_4$.

Fluorine-18 radiolabeled **7a,b** were synthesized through N-alkylation of the desbenzyl precursors **6a,b** with [^{18}F]4-fluorobenzyl iodide as is shown in Scheme 2. The desbenzyl precursor (**6a** or **6b**) in DMF was added to [^{18}F]FBI and the mixture was heated at 90°C for 10 min. The final product was purified by C-18 reversed-phase HPLC. The overall yield of the 4-step sequence was approximately 15% from starting [^{18}F]fluoride and required a total synthesis time of 115 min. Specific activities of 50-80 mCi/μmol, decay corrected to E.O.B., were obtained. The low specific activities observed are directly a result of irradiation conditions (10 μA/10 min.), and the resulting low levels of [^{18}F]fluoride ion produced, and not due to the introduction of large amounts of unwanted carrier fluoride. *In vivo* biodistribution and regional brain uptake studies of fluorine-18 radiolabeled **7a,b** are underway.[5]

Scheme 2. Synthesis of the ^{18}F-labeled analogues of **7a** and **7b**.



Reagents: a: [^{18}F]CsF, DMSO$_{aq}$, 120°C; b: LiAlH$_4$, THF, pentane; c: 47% HI$_{aq}$, 90°C
d: 6-amino-3-carboalkoxycarbonyl-β-carboline, DMF, 90°C.

Experimental

Melting points were determined on a Meltemp melting point apparatus and are uncorrected. Infrared spectra (IR) were taken as KBr pellets on a Perkin Elmer 1600 Series FT-IR spectrophotometer. Proton NMR spectra were determined on a Varian EM 360L NMR spectrometer. Elemental analysis were performed at Atlantic Microlabs, Norcross, GA. Compounds **2**, **3a**, **3b**, **4a**, **4b**, **5a**, and **5b** were prepared as previously described.[3,6]

Ethyl 6-amino-β-carboline-3-carboxylate (**6a**). The nitro compound **5a** (1.0 g, 3.2 mmol) and 10% Pd/C (0.5g) were suspended in EtOH (200 mL) and placed in a Parr hydrogenator for 24 hr under 50 psig of hydrogen. The solution was then filtered to remove the catalyst and the solvent was removed under reduced pressure to yield a dark brown solid. The product was isolated as the HCl salt by dissolving the solid in a small amount of absolute EtOH and bubbling HCl gas through the solution. The product was collected by filtration to yield a orange solid (0.51 g, 56.3%). mp 215-217°C (free amine); IR (KBr)1730, 1639, 1600, 1504, 1328, 1280, 1107 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (t, 3H), 4.2 (s, 2H), 4.3 (q, 2H), 6.9-7.2 (m, 3H), 8.8 (s, 1H), 8.9 (s, 1H). Analysis calculated for $C_{14}H_{13}N_3O_2\cdot 0.25\ H_2O$: C, 64.74; H, 5.20. Found: C, 64.64; H, 5.35.

Isopropyl 6-amino-β-carboline-3-carboxylate (**6b**). Yield: 32.7%. mp 165-170°C (HCl salt); IR (KBr) 3362, 3230, 2980, 1708, 1508, 1353, 1299, 1255, 1097 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (d, 6H), 3.5 (s, 2H), 5.3 (hep, 1H), 6.7-7.3 (m, 3H), 8.6 (s, 1H) 8.9 (s, 1H). Analysis calculated for $C_{15}H_{15}N_3O_2\cdot 0.25\ H_2O$: C, 68.57; H, 5.03. Found: C, 68.46; H, 4.93.

Ethyl 6-(4'-fluorobenzylamino)-β-carboline-3-carboxylate (**7a**). The amine **6a** (0.5 g, 1.6 mmol) and 4-fluorobenzaldehyde were refluxed in benzene (125 mL) for 4 hr. The solvent was then removed to yield an orange oil. The oil was taken up in EtOH (100 mL) and $NaBH_4$ was added. The solution was stirred for 24 hr and the solvent was again removed. EtOH/HCl was added and the solution was refluxed for 2 hr. After removal of the solvent, H_2O was added followed by NH_4OH_{aq} until alkaline, and the solution was then extracted with CH_2Cl_2 (2 x 50 mL). The combined organic layers were dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product was chromatographed on silica gel (CH_2Cl_2) to yield a yellow solid (0.52 g, 78.1%). mp 251-253°C; IR (KBr) 3328, 1706, 1508, 1471, 1367, 1305, 1233, 1101, 1020 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (t, 3H) 4.3 (s, 2H), 4.4 (q, 2H), 6.8-7.6 (m, 7H), 8.6 (s, 1H), 8.7 (1H). Analysis calculated for $C_{21}H_{18}N_3O_2 \cdot 0.25\ H_2O$: C, 65.81; H, 5.67. Found: C, 65.34; H, 5.78.

Isopropyl 6-(4'-fluorobenzylamino)-β-carboline-3-carboxylate (7b): Yield: 61.6%. mp 225-228°C; IR (KBr) 3425, 3231, 2971, 1703, 1510, 1480, 1313, 1233, 1102 cm^{-1}; NMR ($CDCl_3$) δ 1.4 (d, 6H, CH_3), 4.3 (s, 1H, CH), 6.8-7.6 (m, 7H, ar), 8.6 (s, 1H, ar), 8.8 (1H, ar). Analysis calculated for $C_{22}H_{20}N_3O_2 \cdot 0.5\ H_2O$: C, 68.39; H, 5.44. Found: C, 68.22; H, 5.31.

Ethyl and Isopropyl [^{18}F](6-fluorobenzylamino)-β-carboline-3-carboxylate ([^{18}F]7a and [^{18}F]7b). Fluorine-18 labeled 7a and 7b were prepared by alkylation of the appropriate desbenzyl precursor with [^{18}F]FBI.[4] The amine 6a (2 mg, 7.8 μmol) or 6b (2 mg, 7.4 μmol) was dissolved in DMF (500 μL) and added to the reaction vial containing [^{18}F]fluorobenzyl iodide (15-20 mCi). The reaction mixture was heated for 15 min then cooled. Following addition of HPLC mobile phase (1.5 mL) to the vial, the reaction mixture was purified by semi-preparative HPLC (C_{18} reversed-phase, MeOH:0.1N HCO_2NH_4 (75:25, v:v). Radiochemical yields ranged from 50-80% and final product had a specific activity of 50-80 mCi/μmol, corrected to E.O.B. Analytical HPLC showed the products to be >99% radiochemically pure.

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References

1. Lippke K.P., Schunack W.G., Wenning W., and Müller W.E. - J. Med. Chem. 26: 499 (1983)
2. Borea P.A., Pietrogrande M.C., and Biagi G.L. - Biochem. Pharm. 37: 3953 (1988)
3. Hagen T., Skolnick, P., and Cook, J. - J. Med. Chem. 30: 750 (1987)
4. Mach R.H., Elder S.T., Morton T.E., Nowak P.A., Evora P.H., Scripko J.G., Luedtke R.R., Unsworth C.D., Filtz T., Rao A.V., Molinoff P.B., and Ehrenkaufer R.L. - Nucl. Med. Biol. 20: 777 (1993)
5. Mach R.H., Elder S.T., Ehrenkaufer R.L., Moroney D.A., and Reivich M. - J. Nucl. Med. 32: 1094 (1991)
6. Neef G., Eder U., Huth A., Rahtz D. - Heterocycles. 20: 1295 (1986)