SYNTHESIS OF ^{3}H- AND ^{14}C-KETANSERIN

Cor G.M. Janssen*, Herman A.C. Lenoir, Jos B.A. Thijssen, Alfons G.Knaeps,

Willy L.M. Verluyten and Jozef J.P. Heykants

Department of Drug Metabolism and Pharmacokinetics, Janssen Research

Foundation, B-2340 Beerse, Belgium

SUMMARY

Ketanserin, 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione is a new serotonin S_2-receptor blocking agent used in hypertension and related diseases. Pharmacokinetic studies required the synthesis of monolabelled ^{3}H- and ^{14}C-ketanserin tartrate. The tritium compound had the label placed at the fluorobenzoyl moiety, whereas for ^{14}C-ketanserin the heterocyclic part of the quinazolinedione system was chosen. Receptor binding studies and application in radioimmunoassay necessitated the synthesis of a molecule at higher specific activity, hence a dilabelled molecule was constructed with tritium atoms placed at opposite positions in the molecule.
The title compounds were obtained at specific activities of 169.5, 5.66 and 34900 mCi/mmol, respectively and with HPLC purities of > 98%.

KEY WORDS: ^{3}H-ketanserin, ^{14}C-ketanserin, serotonin S_2-receptor blocking agent, antihypertensive

INTRODUCTION

Ketanserin, the first specific S_2-serotonergic blocking agent devoid of partial agonistic properties, is used to lower blood pressure in hypertensive patients.[1,2]

Pharmacokinetic studies necessitated the synthesis of specifically ^{3}H- and ^{14}C-labelled ketanserin whereas receptor binding studies[3] and radioimmunoassay

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*To whom correspondence should be addressed.

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(RIA) required the material at high specific activities. For these reasons we chose to synthesize tritiated ketanserin VIIa and carbon-14 labelled ketanserin XII (both isolated as their tartrates) and di-tritiated ketanserin VIIb (Figure 1).

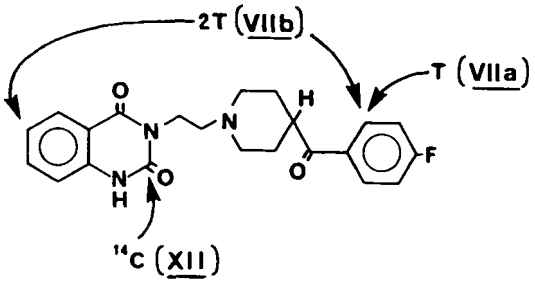
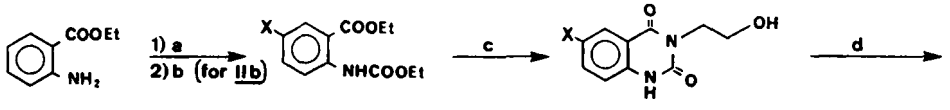


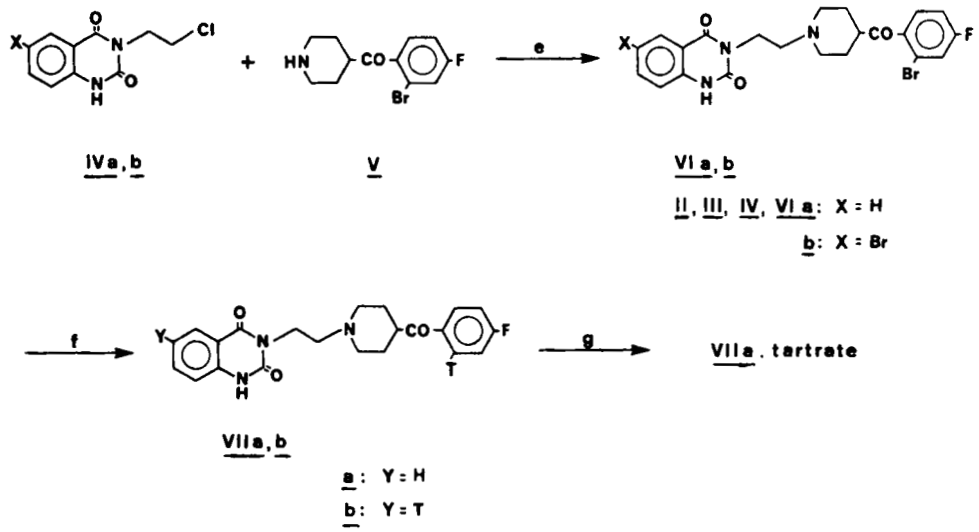
Figure 1: ^{3}H-; di-^{3}Hand ^{14}C-labelled ketanserin

Mono(VIIa) and ditritiated ketanserin (VIIb) were synthetized according to Scheme I. N-Carbethoxylation of ethyl 2-aminobenzoate I afforded IIa, part of which was brominated to IIb. Both compounds were reacted with 2-aminoethanol to IIIa, b.[4] Chlorination of the alcohol with thionyl chloride provided IVa, b[4] which upon coupling with (2-bromo-4-fluorophenyl) (4-piperidinyl)methanone (V)[5] gave VIa, b. Reductive dehalogenation[6] under tritium atmosphere led to the desired mono- and ditritiated ketanserin VIIa, b of which VIIa was isolated as the tartrate salt.

Scheme I: Synthesis of mono- and ditritiated ketanserin[a]



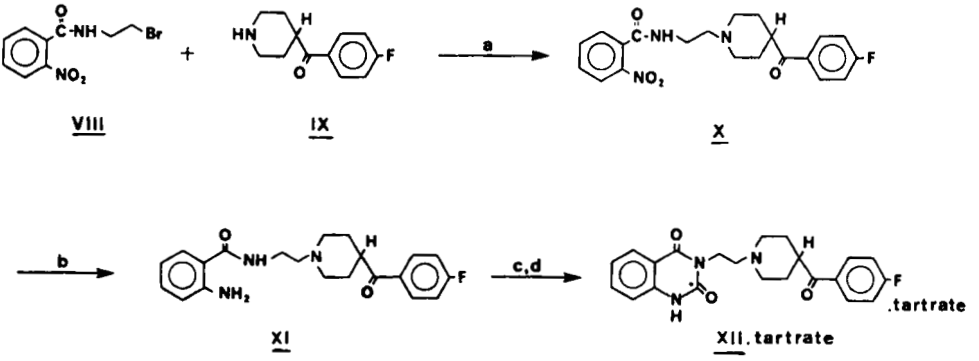
I IIa,b IIIa,b



[a](a) ClCOOEt, K_2CO_3, toluene; (b) Br_2, $CHCl_3$; (c) $NH_2CH_2CH_2OH$; (d) $SOCl_2$, $CHCl_3$; (e) Na_2CO_3, MIK; (f) T_2, Pd (10%)-C, CaO, thiophene, tetrahydrofuran; (g) tartaric acid.

Carbon-14 labelled XII.tartrate (Scheme II) was prepared by reaction of N-(2-bromoethyl)-2-nitro-benzamide VIII[7] with (4-fluorophenyl) (4-piperidinyl)-methanone IX[8] to X. Reduction of the nitro function afforded XI which was ringclosed with ^{14}C-urea[9] to yield ^{14}C-labelled ketanserin XII; the material was isolated as its tartrate salt.

Scheme II: Synthesis of ^{14}C-labelled ketanserin[a]



[a](a)Na_2CO_3, MIK; (b) H_2, Pt (5%)-C, thiophene, methanol; (c) ^{14}C-urea, xylene; (d) tartaric acid.

METHODS AND MATERIALS

Preparative thin-layer chromatography (TLC) was performed on silica gel plates (Merck 60F254) with the eluate composition described where appropriate. The apparatus used for purification by means of HPLC consisted of a Gilson model 303 pump, a pumphead 25S and a Varian U.V. detector. The samples were injected by a Rheodyne 7125 injector. Purification of VIIa by preparative reversed-phase high-performance liquid chromatography (HPLC) was conducted on Lichrosorb RP8 (10 μm) bonded phase in a narrow-bore stainless steel column (4.6 x 300 mm) with a flow rate of 2 ml/min and U.V. detection at 230 nm. Compound VIIb was purified identically on Hypersil ODS (5 μm) bonded phase. The apparatus used for radioactivity measurements and for analytical HPLC have been described earlier.[10] Compounds VIIa and XII were analyzed on Lichrosorb RP8 (10 μm) and compound VIIb was analyzed on Hypersil ODS (5 μm).

The specific activity for compounds VIIa and b was determined by measuring the U.V. absorbance (on HPLC) relative to the absorbance of known amounts of injected unlabelled standards and the radioactivity contents in the HPLC eluate by means of liquid scintillation counting. The specific activity of VIIa tartrate and XII was measured by counting a known amount of the material.

Gas-chromatography (GC) was performed on a Varian 3700 gas chromatograph using a 1 m OV-17 carbowax column with a linear temperature gradient run of 26 minutes from 50°C to 310°C. Melting points were taken on a Fisher-Johns block and are uncorrected. All labelled products were HPLC identical to authentic unlabelled material.

KETANSERIN VIIa.tartrate and VIIb

Ethyl 2-[(ethoxycarbonyl)amino]benzoate (IIa)

To a stirred mixture of ethyl 2-aminobenzoate (20.0 g, 121 mmol) and potassium carbonate (30.0 g) in toluene (100 ml) was dropped ethyl chloroformate (20.0 g, 133 mmol). The reaction mixture was stirred for 24 hours at 100°C, then it was cooled to room temperature and washed with water (100 ml) and brine (100 ml). Drying on magnesium sulphate and evaporation at aspirator pressure afforded IIa (28.5 g, 99.3%) with a GC purity of 99.7%; mp. 40-41°C. ^{1}H-NMR ($CDCl_3$) δ 1.33 and 1.41 (2t, 6, $2CH_3$); 4.23 and 4.37 (2q, 4, $2CH_2$); 7.03-8.45 (m, 4, ArH), 10.53 (br s, 1, NH). Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.71; H, 6.28; N, 5.83%.

Ethyl 2[(ethoxycarbonyl)amino]-5-bromo-benzoate (IIb)

To a stirred solution of IIa (23.7 g, 100 mmol) in chloroform (150 ml) was dropped bromine (16 g, 100 mmol). After 3 hours the discoloured solution was washed with a saturated sodium bisulphite solution (100 ml) and water (100 ml), dried over magnesium sulphate and evaporated to afford IIb (30.0 g, 95%) with

a 98.3% GC purity; mp. 95-97°C. ^{1}H-NMR ($CDCl_3$) δ 1.33 and 1.43 (2t, 6, $2CH_3$); 4.23 and 4.38 (2q, 4, $2CH_2$); 7.60-8.37 (m, 3, ArH); 10.45 (br s, 1, NH). Anal. Calcd. for $C_{12}H_{14}BrNO_4$: C, 45.59; H, 4.46; N, 4.43. Found: C, 45.86; H, 4.41; N, 4.44%.

3-(2-Hydroxyethyl)-6-bromo-2,4(1H,3H)-quinazolinedione (IIIb)

A mixture of IIb (15.8 g, 50 mmol) and 2-aminoethanol (4.25 ml, 70 mmol) was warmed to 160°C under continuous removal of ethanol. The mixture turned solid within 30 minutes. It was triturated with 2-propanol (50 ml), filtered and washed with diisopropyl ether to supply IIIb (13.0 g, 91.2%). The product was TLC-pure [R_f = 0.39; eluate: chloroform-methanol (9-1; v/v)] and had a mp. of 264-265°C. ^{1}H-NMR (DMSO-d_6) δ 3.57 (t, 2, CH_2O); 3.98 (t, 2, NCH_2); 4.80 (br, 1, OH); 7.12-7.97 (m, 3, ArH), low field (br, 1, NH). Anal. Calcd. for $C_{10}H_9BrN_2O_3$: C, 42.13; H, 3.18; N, 9.83. Found: C, 42.58; H, 3.17; N, 9.65%.

3-(2-Chloroethyl)-6-bromo-2,4(1H,3H)-quinazolinedione (IVb)

To a slurry of IIIb (2.85 g, 10 mmol) in chloroform (30 ml) was added thionyl chloride (2.19 ml, 30 mmol) and the whole was refluxed for 4 hours. The solid was filtered off and washed with chloroform and low-boiling petroleum ether to give IVb (2.8 g, 92%). The material was TLC pure [R_f = 0.56; eluate: chloroform-methanol (9-1; v/v)]; G.C. 99.9% pure and had a mp. of 245-248°C. ^{1}H-NMR (DMSO-d_6) δ 3.78 (t, 2, CH_2Cl); 4.22 (t, 2, NCH_2); 7.15-7.98 (m, 3, ArH), 11.67 (s, 1, NH). Anal. Calcd. for $C_{10}H_8BrClN_2O_2$: C, 39.57; H, 2.66; N, 9.23. Found: C, 39.78; H, 2.67; N, 9.05%.

6-Bromo-3-[2-[4-(2-bromo-4-fluorobenzoyl)piperidinyl]ethyl]-2,4(1H,3H)quinazolinedione (VIb, dibromoketanserin)

A mixture of IVb (303 mg, 1 mmol), (2-bromo-4-fluorophenyl) (4-piperidinyl) methanone[5] (V, 323 mg, 1 mmol) and sodium carbonate (212 mg, 2 mmol) in methyl isobutyl ketone (MIK, 6 ml) was stirred and refluxed for 20 hours. Water was

added and a precipitate formed under vigorous stirring. The solid was filtered, washed with water (20 ml), ice-cold 2-propanol (3 ml), ice-cold diisopropyl ether (3 ml) and dried to the air to furnish dibromoketanserin VIb (84 mg, 15%). The solid was TLC-pure [R_f = 0.53; eluate: chloroform-methanol (9-1; v/v)] and had a mp. of 189-191°C. ^{1}H-NMR (DMSO-d_6) δ 1.48 (m, 2, piper-NCCH$_2$; ax); 1.72 (m, 2, piper-NCCH$_2$; eq); 2.03 (m, 2, piper-NCH$_2$; ax); 2.50 (t, 2, NCCH$_2$N-piper); 2.92 (m, 2, piper-NCH$_2$; eq); 3.02 (m, 1, CHCO); 4.00 (t, 2, CONCH$_2$); 7.13-7.99 (m, 6, ArH); 11.55 (br, 1, NH). Anal. Calcd. for $C_{22}H_{20}Br_2FN_3O_3$: C, 47.76; H, 3.64; N, 7.60. Found: C, 47.75; H, 3.54; N, 7.39%.

3-[2-[4-(2-Bromo-4-fluorobenzoyl)piperidinyl]ethyl]-2,4(1H,3H)quinazolinedione (VIa, monobromoketanserin)

Identical to the synthesis of VIb, yield 27%. The product was TLC-pure [R_f = 0.48; eluate: chloroform-methanol (9-1; v/v)] and had a mp. of 178-179°C. ^{1}H-NMR (DMSO-d_6) δ 1.50 (m, 2, piper-NCCH$_2$; ax); 1.71 (m, 2, piper-NCCH$_2$; eq); 2.03 (m, 2, piper-NCH$_2$; ax); 2.48 (t, 2, NCCH$_2$N-piper); 2.94 (m, 2, piper-NCH$_2$; eq); 3.03 (m, 1, CHCO); 4.02 (t, 2, CONCH$_2$); 7.09-8.03 (m, 7, ArH); 11.60 (br, 1, NH). Anal. Calcd. for $C_{22}H_{21}BrFN_3O_3$: C, 55.71; H, 4.46; N, 8.86. Found: C, 55.46; H, 4.41; N, 8.75%.

3-[2-[4-(4-Fluoro-2-tritiobenzoyl)piperidinyl]ethyl]-2,4(1H,3H)quinazolinedione (VIIa, monotritioketanserin)

Monobromoketanserin VIa (200 mg, 0.42 mmol) was dissolved in dry tetrahydrofuran (10 ml). To the solution was added Pd (10%) on charcoal (200 mg), powdered calcium oxide (400 mg) and a 4% thiophene solution in diisopropyl ether (30 µl). Tritiation was performed with approximately 50 Ci of tritium gas for 18 hours at room temperature. The excess of tritium was absorbed on active charcoal, the catalyst was removed by Millipore filtration (Millex-SR) and the filtrate was lyophilized twice with methanol to remove labile tritium. The residue was purified with preparative TLC using acetate buffer (pH 4.8)-methan-

ol-chloroform-ethyl acetate (5-18-23-54; v/v) as an eluate. The radioactive zone corresponding to authentic ketanserin was scraped off and eluted with ethanol. The product, stored in ethanol (90 ml) proved to be 93% HPLC-pure ketanserin VIIa (810 mCi). Part of it (15 mCi) was purified on Lichrosorb RP8 by means of HPLC using acetonitrile-water-diisopropylamine (30-70-0.2; v/v) as an eluate to afford 98% pure ketanserin (2.4 mCi) with a specific activity of 15.5 Ci/mmol.

Monotritioketanserin.tartrate (VIIa.tartrate)

Isotopical dilution of the 93% HPLC-pure VIIa (510 mCi), performed in two steps with unlabelled ketanserin (500 mg and 250 mg respectively) and crystallization of the combined solids from 2-propanol-dimethyl formamide (12.0 ml; 1-1 v/v) at 65°C afforded VIIa (595 mg, 255 mCi). The material was dissolved in acetone-water (9.0 ml; 85-15 v/v), 1 equivalent of L-(+)-tartaric acid (226 mg) was added and the mixture was warmed at 60°C until complete solution. The solution was allowed to cool to room temperature, the crystalline material was filtered and recrystallized from acetone (10 ml) to yield VIIa.tartrate (695 mg, 216 mCi, 169.5 mCi/mmol) which was 98% HPLC-pure.

3-[2-[4-(4-Fluoro-2-tritiobenzoyl)piperidinyl]ethyl-2,4(1H,3H)-6-tritio-quinazolinedione (VIIb, ditritioketanserin)

Dibromoketanserin VIa (50 mg, 0.09 mmol) in dry THF (5 ml) containing Pd (10%) on charcoal (100 mg), powdered calcium oxide (100 mg) and a 4% thiophene solution in diisopropyl ether (20 µl) was tritiated with approximately 30 Ci of tritium gas for 18 hours at room temperature. Work-up was performed as for VIIa. From the residue after lyophilization (2.1 Ci; 50% HPLC-pure) a part (210 mCi) was purified on Hypersil ODS (5 µm) by means of HPLC with acetonitrile-water-diisopropylamine (29-71-0.2; v/v) as an eluate to afford 99.7% pure ditritioketanserin (80.2 mCi) with a specific activity of 34.9 Ci/mmol.

KETANSERIN.TARTRATE (XII.tartrate)

2-Nitro-N-[2-[4-(4-fluorobenzoyl)-piperidinyl]ethyl]benzamide (X)

A mixture of N-(2-bromoethyl)-2-nitro-benzamide VIII[7] (18 g, 66 mmol), (4-fluorophenyl) (4-piperidinyl)methanone.HCl IX[8] (14.6 g, 60 mmol) and sodium carbonate (21 g, 200 mmol) in methyl isobutyl ketone (MIK) was stirred and refluxed for 18 hours. The mixture was extracted with water, dried on magnesium sulphate and evaporated at aspirator pressure. The crude oil was filtered over a short pad of silica (200 g) with chloroform-methanol (9-1; v/v) as an eluate to afford a residue (18 g). Crystallization from 2-propanol gave pure X (15.3 g, 58%); mp. 149-150°C. ^{1}H-NMR ($CDCl_3$) δ 1.65-2.05 (m, 4, $(CCH_2)_2$; eq and ax), 2.20 (td, 2, $N(CH_2C)_2$; ax), 2.67 (t, 2, CH_2N-piper), 3.07 (m, 2, $N(CH_2C)_2$; eq), 3.14 (tt, 1, $N(CC_2)CHCO$), 3.60 (dt, 2, CH_2CN-piper), 6.57 (br, 1, CONH), 7.11-8.15 (m, 8, ArH). Anal. Calcd. for $C_{21}H_{22}FN_3O_4$: C, 63.15; H, 5.55; N, 10.52. Found: C, 62.89; H, 5.72; N, 10.30%.

2-Amino-N-[2-[4-(4-fluorobenzoyl)piperidinyl]ethyl]benzamide (XI)

A 1000 ml Parr hydrogenation bottle was charged with a solution of X (10 g, 25 mmol) in methanol (250 ml) containing a 4% thiophene solution in diisopropylether (2 ml). Then platinum 5% on charcoal (2 g) was cautiously added and the reaction mixture was shaken under a 50 psi atmosphere of hydrogen gas until 3 equivalents of H_2 were absorbed. The catalyst was removed by filtration through dicalite and the filtrate was stripped to leave a solid residue. Crystallization from 2-propanol (80 ml) afforded XI (7.5 g, 81%); mp. 144-145°C. ^{1}H-NMR ($CDCl_3$) δ 1.73-1.94 (m, 4, $N(CCH_2)_2$; eq and ax), 2.19 (td, 2, $N(CH_2C)_2$; ax), 2.61 (t, 2, CH_2N-piper), 3.03 (m, 2, $N(CH_2C)_2$; eq), 3.23 (tt, 1, $N(CC_2)CHCO$), 3.51 (dt, 2, CH_2CN-piper), 5.53 (br, 2, NH_2), 6.64-8.02 (m, 8, ArH), 6.68 (br, 1, CONH). Anal. Calcd. for $C_{21}H_{24}FN_3O_2$: C, 68.27; H, 6.55; N, 11.38. Found: C, 68.03; H, 6.48; N, 11.28%.

3-[2-[4-(4-Fluorobenzoyl)piperidinyl]ethyl]-2,4(1H,3H)-[2-^{14}C]quinazolinedione. tartrate (XII.tartrate)

A mixture of ^{14}C-urea[9] (5.64 mg, 0.091 mmol containing 5 mCi of radioactivity) and XI (37 mg, 0.1 mmol) in xylene (0.5 ml) was kept for 40 hours at 135°C. The solvent was evaporated under a gentle stream of dry nitrogen and the residue was purified with preparative TLC using chloroform-methanol (the latter saturated with ammonia; 95:5 v/v) as an eluate. The radioactive zone, corresponding to authentic ketanserin was scraped off and eluted with methanol. The solvent was removed *in vacuo* and the residue was diluted with unlabelled ketanserin (150 mg, 0.38 mmol). Crystallization from 2-propanol-dimethyl formamide (3.0 ml; 1:1 v/v) afforded [^{14}C]-ketanserin [151 mg, total radioactivity 2.71 mCi (54%)] with a 100% HPLC purity. The material was dissolved in acetone-water (1.9 and 0.2 ml respectively) and 1 equivalent of L-(+)-tartaric acid (57.0 mg) was added. Crystallization as for VIIa.tartrate yielded XII.tartrate (163 mg, 1.69 mCi, 5.66 mCi/mmol) which was 99.5% HPLC-pure.

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