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Z-(-,-)-[^{76}Br]BrQNP: A High Affinity PET Radiotracer for Central and Cardiac Muscarinic Receptors

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Summary

Racemic E-1-azabicyclo[2.2.2]oct-3-yl α-(1-bromo-1-1-propen-3-yl)-α-hydroxy-α-phenylacetate (BrQNP) was prepared and evaluated *in vivo* as a potential candidate for imaging muscarinic acetylcholinergic receptors by Positron Emission Tomography. Initial *in vivo* blocking studies utilizing Z-(-,-)-[^{125}I]IQNP as a radiolabelled muscarinic probe demonstrated that a preinjection of cold E-BrQNP effectively blocks the uptake of the radiolabelled probe in the brain and heart, by 71% and 86% respectively.

Z-(-,-)-[^{76}Br]BrQNP was prepared by electrophilic substitution from a tributylstannyl precursor. Peracetic acid and chloramine T were evaluated as oxidizing agents. After purification by SPE and RP-HPLC, radiolabelling yields of 85% and 95% were obtained with peracetic acid and chloramine T, respectively. The final radiochemical yield was 70% for both oxidizing agents.

Rat biodistribution studies of Z-(-,-)-[^{76}Br]BrQNP showed high uptake in organs with high concentrations of muscarinic receptors (heart and brain).

Key words: Z-(-,-)-[^{76}Br]BrQNP, muscarinic receptors, IQNP, QNB, Positron Emission Tomography

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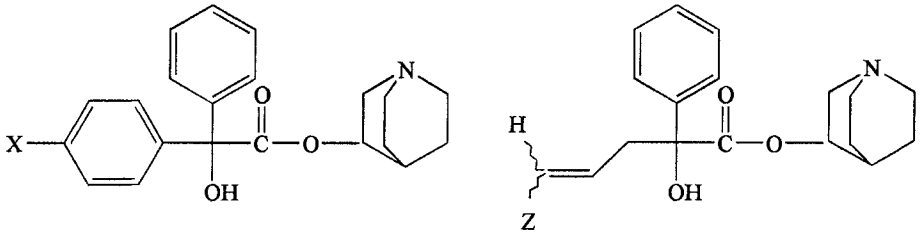
Introduction

On the basis of recent molecular biological studies five different muscarinic acetylcholine receptor (m-AChR) subtypes have been cloned (1-4). Each subtype has been found to be localized in the highest concentration in discrete brain regions or peripheral tissues (1-3) and has distinct pharmacological (5) and biochemical (6) profiles. Recent findings have demonstrated a selective diminution of the M_2 subtype in neurological disorders such as Alzheimer's disease (7-10). When assayed *in vitro* with non subtype-selective radioligands such as [^{3}H]QNB, the density of m-AChR in the cortex from Alzheimer patients has been reported to be unchanged compared to aged-matched controls (11). However, when these studies were repeated using radioligands which are selective for the M_2 subtype such as [^{3}H]AF-DX116, it becomes clear that the M_2 receptor concentration is selectively decreased by 61% (7,8,12). This finding has increased the importance of developing M_2 selective muscarinic radioligands that could be useful for *in vivo* quantification of M_2 receptors with Single Photon Emission Computed Tomography (SPECT) or Positron Emission Tomography (PET).

3-Quinuclidinyl benzilate (QNB,**a**) is a high affinity mAChR antagonist that has been labelled with radioiodine (4-IQNB,**b**) (13-15) and ^{11}C (16). Iodine-123-labeled 4-IQNB has been used in SPECT studies in healthy individuals and patients with dementias to image mAChR (17-22). McPherson *et al* have recently reported a novel analogue of QNB in which a phenyl group has been replaced by a vinyl iodide moiety (IQNP,**c**) (23). This modification allows the ligand to retain a high *in vitro* binding affinity for mAChR, to readily pass the blood-brain-barrier (BBB) and to display high specificity for mAChR *in vivo*. Evaluation of the stereoisomers of IQNP has shown that the affinity and mAChR subtype selectivity is influenced by the absolute configuration at the two chiral centers and the vinyl iodide stereochemistry (24-28). While radioiodinated E-(-,+)-IQNP cleared rapidly from the brain and the heart, E-(-,-)-, Z-(-,-)-, and Z-(-,+)-IQNP have high uptake and retention in mAChR rich areas of the brain.

After labelling with ^{125}I, Z-(-,-)-IQNP demonstrates a high uptake and a relatively slow clearance in areas of the brain rich in M_2 mAChR subtype (26). This led to the evaluation of a brominated analogue of IQNP (BrQNP,**d**) to be used as a potential probe for M_2 receptor density by PET. Since bromine behaves in an analogous manner to iodine, similar labeling reactions can be used. In addition, a carbon-bromine bond (67.5 kcal/mol) is stronger than a carbon-iodine bond (50.5 kcal/mol) which may reduce *in vivo* dehalogenation. To take advantage of the higher resolution and more accurate quantification of PET, we have prepared Z-(-,-)-[^{76}Br]BrQNP and evaluated its pharmacological properties as a potential candidate for imaging mAChR.



a) QNB (X = H) c) IQNP (Z = I)

b) 4IQNB (X = I) d) BrQNP (Z = Br)

Materials and methods

General

E-Ethyl α-hydroxy-α-phenyl-α-(1-tributylstannyl-1-propen-3-yl)acetate (**E-1**) and Z-(-)-1-azabicyclo[2.2.2]oct-3-yl (-)-α-hydroxy-α-phenyl-α-(1-tributylstannyl-1-propen-3-yl)acetate (**Z-1**) were prepared as described previously (26). All other chemicals were reagent grade and used without further purification. Thin layer chromatographic analysis (TLC) was performed with 250 μm layers of silica gel coated on glass (Alltech). Flash column chromatography was performed

with silica gel 200-400 mesh (Aldrich). Proton (^{1}H) and carbon 13 (^{13}C) were recorded on a Varian Gemini 200 instrument. Proton spectra are reported using tetramethylsilane (0.0 ppm) as an internal standard and carbon spectra are reported using chloroform (77.0 ppm) as the internal standard. Compound (**E-3**) was examined by thermal desorption electron impact ionization Fourier transform mass spectrometry using perfluorotributylamine (PFTBA) as an internal calibrant. Bromine-76 was produced by irradiation of natural arsenic (1.7 g) with a beam of 30 MeV [^{3}He] ions. After decay of [^{75}Br] ($t_{1/2}$= 1.6 h), the target was dissolved in 40 ml concentrated sulfuric acid at 180°C. After cooling to 50°C, 4 g chromic acid in 12 ml water were added. The radioactive bromine was formed in a nitrogen stream and trapped as ammonium bromide in 2 ml 1M ammonia and evaporated to dryness ($t_{1/2}$ [^{76}Br] = 16.2 h).

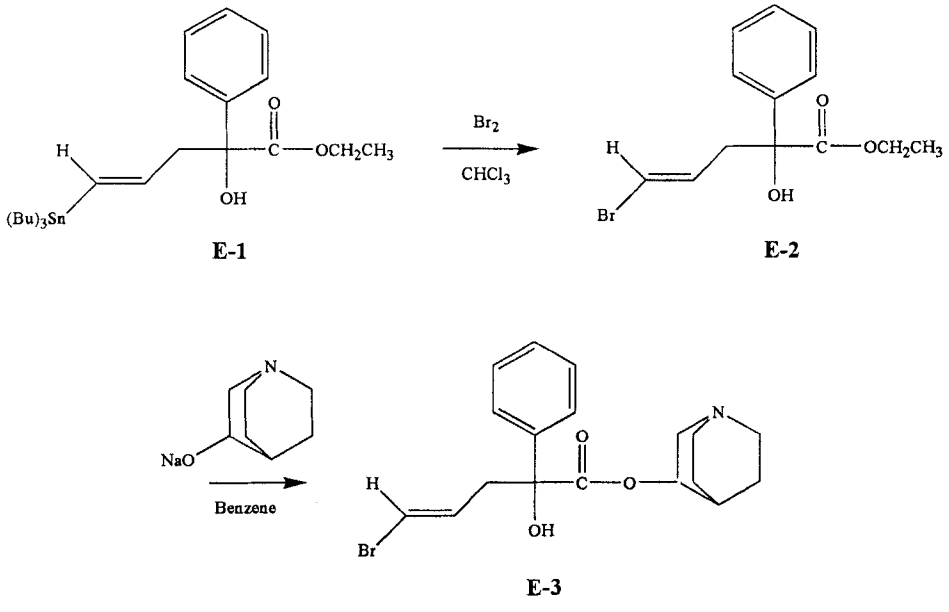
E-Ethyl α-(1-bromo-1-propen-3-yl)-α-hydroxy-α-phenylacetate (E-2)

A 0.25M solution of bromine in chloroform was added slowly to a solution of **E-1** (2.0 g, 3.9 mmol) in chloroform (25 ml) until the color of bromine persisted. The solution was stirred at room temperature for 2.5 h and then evaporated to dryness. The residue was dissolved with ethyl acetate (50 ml) and the solution was washed with saturated aqueous sodium thiosulfate solution (50 ml), water (50 ml), dried over magnesium sulfate, and finally evaporated to dryness. The product was purified by flash column chromatography (hexane:ethyl acetate [9.5:0.5]) to afford **E-2** as a pale oil (1.05 g, 3.5 mmol, 89%). TLC (silica gel, hexane:ethyl acetate [8:2]) Rf = 0.62. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.60 (m, 2H), 7.35 (m, 3H), 6.20 (m, 2H), 4.26 (m, 2H), 3.90 (s, 1H), 2.80 (m, 2H), and 1.30 (t, 3H). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.82 (CO), 140.76 (C), 131.66 (CH), 128.21 (CH), 127.85 (CH), 125.16 (CH), 108.21 (CHBr), 77.49 (C), 62.66 (CH_2), 43.01 (CH_2), and 14.16 (CH_3).

E-1-Azabicyclo[2.2.2]oct-3-yl α-(1-bromo-1-propen-3-yl)-α-hydroxy-α-phenylacetate (E-3)

A solution of 3-quinuclidinol (1.68 g, 13.2 mmol) in benzene (120 ml) was refluxed for 1 h under an argon atmosphere utilizing a Dean-Stark trap to remove moisture. Freshly cut pieces of sodium metal (ca. 0.62 g) were then added and the solution refluxed for 1 h. The solution was allowed to cool slightly and transferred, via a double tipped needle technique to remove unreacted sodium

metal, to a flask containing **E-2** (1.05 g, 3.5 mmol) in benzene (120 ml) which had been refluxed for 1 h under argon utilizing a Dean-Stark trap to remove moisture. The solution was then refluxed for 5 h under argon, cooled to room temperature, evaporated to dryness and diluted with water (100 ml). After extraction with ethyl acetate (100 ml), the organic phase was washed with water (2 x 100 ml), dried over magnesium sulfate, and evaporated to dryness. The product was purified by flash column chromatography (chloroform:methanol:ammonium hydroxide [9.4:0.5:0.1]) to afford **E-3** (1.0 g, 2.6 mmol, 74%). TLC (chloroform:methanol [8.5:1.5]) Rf = 0.43. ^{1}H NMR ($CDCl_3$, δ, ppm): 7.56 (m, 2H), 7.30 (m, 3H), 6.18 (m, 2H), 4.85 (m, 1H), 4.30 (s, 1H), 3.40-2.35 (m, 8H), 2.02 (m, 1H), and 1.92-1.21 (m, 4H). ^{13}C NMR ($CDCl_3$, δ, ppm): 173.72 (CO), 140.91 (C), 131.70 (CH), 128.24 (CH), 127.93 (CH), 125.42 (CH), 108.23 (CHBr), 77.41 (C), 74.02 (CH), 55.02 (CH_2), 47.21 (CH_2), 46.24 (CH_2), 42.97 (CH_2), 25.24 (CH), 24.37 (CH_2), and 19.54 (CH_2). High resolution mass analysis of $(m+H)^+$: Calc. for $C_{18}H_{22}BrNO_3$: 380.0856; found 380.0845.



E-1 **E-2**

E-3

Scheme 1

Bromine-76-radiolabelling of Z(-,-)-QNP

Two different oxidizing agents were evaluated for the preparation of Z-(-,-)-[^{76}Br]BrQNP (**Z-2**) by electrophilic substitution of the tributylstannyl precursor (**Z-1**) (50 μg) (Scheme 2): peracetic acid 1% in 100 μl acetic acid, 50 μl hydrogen peroxide or chloramine T (10^{-3}M) in 0.1N HCl. The reactions were conducted at room temperature.

The purification of the radio-labelled product from the reaction mixture was carried out using a C18 cartridge (Sep-Pak, Waters). Polar by-products and unreacted [^{76}Br]BrNH$_4$ were washed from the cartridge with 5 ml of water. The product was further eluted with 3 ml of methanol and isolated by HPLC using a μ-Bondapak C18 column (300 x 3.9 mm, Waters) with a mixture of acetonitrile and 0.1M ammonium acetate (50/50) as the mobile phase at a flow rate of 1 ml/min. The effluent was monitored with an UV detector at 254 nm (M440, Waters) and a Geiger-Müller radioactivity detector. The radioactive peak eluted at the retention time of authentic BrQNP. The product was collected and the solvent evaporated. The residue was dissolved in sterile saline. The time course of the radiolabelling yield and the radiochemical purity were assessed by radio-TLC using a silica gel plate with a chloroform-methanol mixture (85:15) and a RP-plate with acetonitrile-0.1M ammonium acetate (60:40). The chemical purity of Z-(-,-)-[^{76}Br]BrQNP (**Z-2**) was checked by RP-HPLC using similar conditions to those described above.

*E-BrQNP (**E-3**) in vivo competition study in rats*

In vivo blocking studies were performed using female Fisher VAF rats (125 g). The animal care and use procedures were in accordance with the Guide for Care and Use of Laboratory Animals and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use committee. The muscarinic radiolabelled probe, Z-(-,-)-[^{125}I]IQNP, was prepared as previously described (26).

Groups of rats (n=5) were injected with E-BrQNP (**E-3**) (3 mg/kg) via the lateral tail vein one hour prior to the injection of Z-(-,-)-[^{125}I]IQNP (1.2 μCi, 0.04 MBq)). Another group of rats was injected only with Z-(-,-)-[^{125}I]-IQNP (1.2 μCi, 0.04 MBq) as a control. At three hours post-injection of the radioiodinated ligand the animals were sacrificed. The heart and brain were

removed (29), rinsed with saline, blotted dry and weighed. A blood sample was obtained from the chest cavity after removal of the heart. The activity of these samples were measured in a Packard minaxi 5000 sodium iodide auto γ-counter.

Biodistribution of Z(-,-)-[^{76}Br]BrQNP

The regional uptake of the bromine-76-labeled compound (**Z-2**) was followed for 6 h in Wistar male rats which were injected in the tail vein with 10 μCi (0.37 MBq) of Z-(-,-)-[^{76}Br]BrQNP (**Z-2**). The rats were sacrificed and the brain regions, liver, lung, heart, kidney, muscle and blood were removed and the radioactivity of aliquots were measured in a well γ-counter. The radioactivity concentrations were expressed as percent of injected dose per gram of tissue (% ID/g) and were plotted versus time.

Results

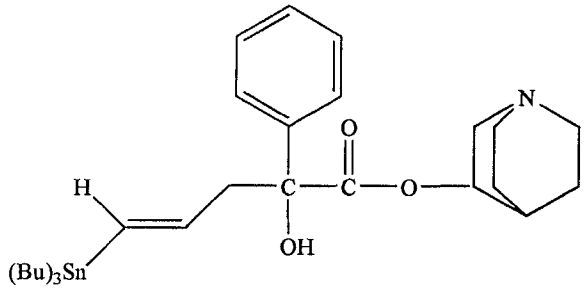
Chemistry

Racemic E-1-azabicyclo[2.2.2]oct-3-yl α-(1-bromo-1-propen-3-yl)-α-hydroxy-α-phenylacetate (BrQNP, **E-3**) was prepared as shown in Scheme 1 for the initial *in vivo* evaluation. Racemic E-ethyl α-hydroxy-α-phenyl-α-(1-tributylstannyl-1-propen-3-yl) acetate (**E-1**) was treated with bromine in chloroform to afford E-ethyl α-(1-bromo-1-propen-3-yl)-α-hydroxy-α-phenylacetate (**E-2**). Transesterification of **E-2** with racemic 3-quinuclidinol afforded racemic E-BrQNP (**E-3**).

Radiolabelling

Z-(-,-)-[^{76}Br]BrQNP (**Z-2**) was prepared by electrophilic substitution from the tributylstannyl precursor (**Z-1**) with no-carrier-added [^{76}Br]$BrNH_4$ (Scheme 2). Two different oxidizing agents were evaluated: peracetic acid and chloramine T. The exchange between ^{76}Br and the leaving group occurred in 20 min at room temperature in the presence of peracetic acid 1% (Figure 1) while it occurred in 10 min at the same temperature with chloramine T. Radiolabelling yields of 85% and 95% were obtained for the peracetic and chloramine T conditions, respectively.

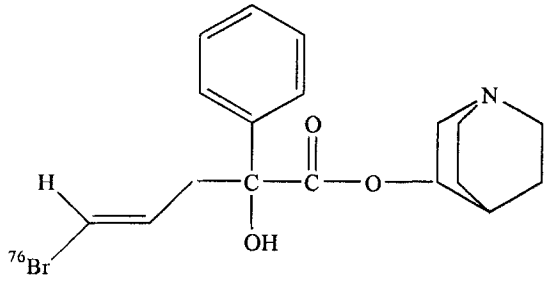
Unreacted [^{76}Br]bromide and radiolabelled polar by-products were eliminated using solid phase extraction. 95% of the radiolabelled compound was eluted by methanol. By using an analytical reverse phase column for HPLC (Figure 2), the radiotracer eluted at the retention time of authentic racemic BrQNP (t_R = 12 min) and before the unlabelled precursor, avoiding any risk of contamination of the radiopharmaceutical by the tributylstannyl substrate. The final radiochemical yield was approximately 70% for either oxidizing agent.



Z-1

1) MeOH
2) [^{76}Br]$BrNH_4$
3) HCl 0.1M
4) Chloramine T 10^{-3}M
5) RT (10 min)
6) Sep-Pak C 18 purification
7) HPLC purification

1) [^{76}Br]$BrNH_4$
2) Peracetic acid (1%)
3) H_2O_2
4) RT (20 min)
5) Evaporation to dryness
6) Sep-Pak C18 purification
7) HPLC purification



Z-2

Scheme 2

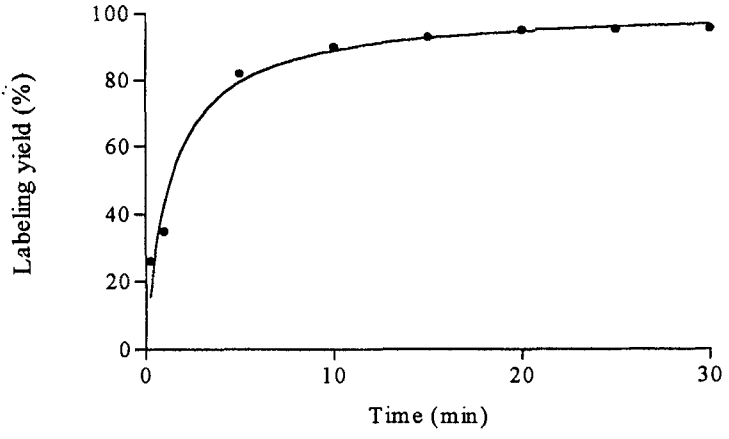


Figure 1: Labeling yield time-course of the tributylstannyl-BrQNP precursor with [^{76}Br]bromine and peracetic acid

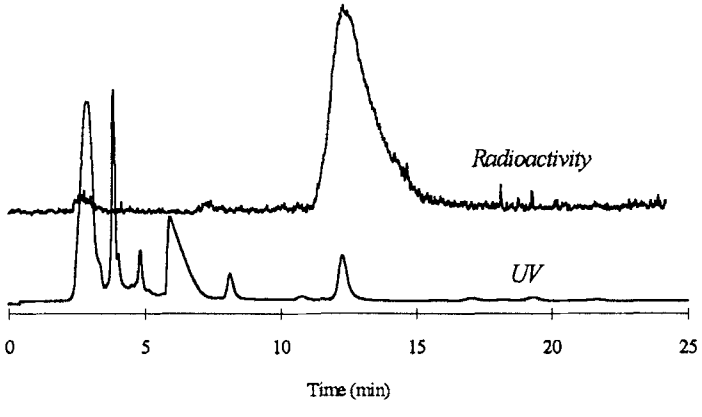


Figure 2. HPLC radiochromatogram of Z-(-,-)-[^{76}Br]BrQNP purification

*E-BrQNP (**E-3**) in vivo competition study in rats*

The ability of **E-3** to pass the BBB and block the uptake of Z-(-,-)-[^{125}I]IQNP was tested in female rats. Z-(-,-)-[^{125}I]IQNP was chosen as the radiolabelled probe due to its demonstrated high cerebral binding in M_1 and M_2 mAChR subtypes *in vivo* (24-26). A dose of 3 mg/kg of **E-3** was

chosen since in a previous study a similar dose of QNB blocked more than 80% of the radioactivity uptake in receptor rich tissue while exhibiting no adverse effects in the animals (23). In our study, the uptake of the radioactivity in the heart and brain was reduced by 86% and 71%, respectively, while the level of activity remained practically unchanged in the blood (Table 1). This study demonstrates that the replacement of the iodide of IQNP with bromine does not markedly affect the ligand's ability to cross the BBB and to bind to mAChR receptors.

Organ	Z(-,-)-[^{125}I]IQNP (%ID/g) 3h p.i. of E-BrQNP	
	Control	BrQNP Loading
Bood	0.18 ± 0.02	0.14 ± 0.02
Heart	2.25 ± 0.19	0.30 ± 0.08
Brain	1.07 ± 0.09	0.31 ± 0.07

Table 1: BrQNP (E-3) in vivo competition study in rats (mean ± SD, n=5)

Biodistribution of Z(-,-)-[^{76}Br]BrQNP

The rat biodistribution study demonstrated high uptake in the cortex and in the striatum. Uptake in areas of poor m-AChR receptor concentration of the brain (cerebellum) was low. At 6 h p.i., the radioactivity concentration in the cortex and in the striatum was 2.0 and 1.4% ID/g, respectively. At this time, the cortex to cerebellum and the striatum to cerebellum radioactivity concentration ratios were still 4.3 and 3.0. In the heart, the uptake was 2.7% ID/g 30 min p.i. and decreased to 0.6% 6 h p.i. The heart to lung radioactivity ratio was about 1.5 and remained relatively constant for 6 h.

Conclusion

1-Azabicyclo[2.2.2]oct-3-yl α-(2-bromo-1-propen-1-yl)-α-hydroxy-α-phenylacetate (BrQNP) has been evaluated for its uptake in m-AChR areas. Initial *in vivo* competition studies demonstrate that BrQNP blocks, in the brain and the heart of rats, the uptake of the radiolabelled probe Z-(-,-)-[^{125}I]IQNP.

Z-(-,-)-[^{76}Br]BrQNP was prepared by electrophilic substitution from the tributylstannyl precursor with no-carrier-added [^{76}Br]$BrNH_4$. The radiolabelling and the purification steps resulted in radiochemically and chemically pure products. Preliminary pharmacological results obtained *in vivo* indicate that Z-(-,-)-[^{76}Br]BrQNP is a good candidate for further imaging of m-AChR receptors in the brain and in the heart, using PET.

Acknowledgments

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