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Radioiodinated α_1–Adrenergic Receptor Ligands

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Summary

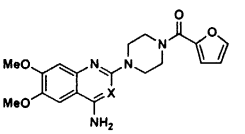
The synthesis and preliminary characterization of three radioiodinated α_1-adrenergic receptor ligands [^{125}I]**5a-c** are described. These tracers are analogs of L-760,478, **1b**, itself an analog of prazosin, **1a**. The highest affinity tracer, [^{125}I]**5a**, has six fold higher affinity for the α_1 receptor subtypes than prazosin. These ligands could be useful for autoradiographic studies of the α_1 receptor subtypes.

Keywords: Alpha-1 adrenergic receptor, radioiodination.

Introduction

The α_1 receptor represents a primary target for symptomatic relief in benign prostatic hyperplasia (BPH)(1). Nonselective α_1 receptor antagonists, such as prazosin, **1a**, have unwanted side effects such as postural hypotension (2), limiting their clinical dose. These side effects presumably arise by the nonselective interaction with the α_1 receptor subtypes α_{1a}, α_{1b} and α_{1d} (3). Antagonism of the α_{1a} receptor subtype is believed to be necessary for relief of BPH (4).

Figure 1.



1a, X=N, prazosin
1b, X=C, L-760,478

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Selective radioligands for receptor subtypes can be an aid in assessing the physiologic role of receptors by demonstrating the presence of the subtype in a particular tissue, either through tissue homogenate studies, or more selectively, *via* autoradiography. Since autoradiographic studies with tritiated ligands require long exposure times compared to radioiodinated ligands, we were interested in developing iodine-125 labelled α_1 receptor ligands for use in autoradiographic studies to determine the tissue distribution of α_1 receptor subtypes. Additionally, because of the higher specific activity achievable with iodine-125, these radioligands would be useful for determining receptor subtype concentrations in tissues with a low concentration of receptor. Work was begun to develop both α_{1a} selective (5) and α_1 subtype nonselective ligands. The work described here is the development of the subtype nonselective ligands.

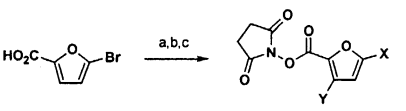
The known ligand 2-[β-(4-hydroxy-3-[^{125}I]]iodophenyl)ethylamino-methyl]tetralone (6), (HEAT or BE 2254) is an α_1 subtype nonselective ligand that has been used extensively in autoradiographic studies of the α_1 adrenergic receptor (7), but with [^{125}I]HEAT, we found unacceptably high levels of nonspecific binding in autoradiographic studies in a variety of tissues. Our target was radioiodinated tracers with high affinity (IC_{50}<1 nM) for each of the α_1 receptor subtypes and suitable log P (<3) to minimize nonspecific binding.

Prazosin, **1a**, and L-760,478, **1b**, are both potent, nonselective α_1 antagonists, but **1b** is less lipophilic than **1a** (Table 1). Because simply adding iodine to an aromatic ring of a compound could raise the log P of the resulting compound by more than one log unit (8), the lower lipophilicity of **1b** compared with **1a**, made iodinated analogs of **1b** more attractive.

Discussion

Analogs of **1b**, containing iodinated furan, pyridine and phenyl rings, were investigated. Scheme 1 shows the synthesis of the N-hydroxysuccinimidyl esters **3a** and **3b**, and scheme 2 shows the coupling reactions between piperazine **4** (9) and acyl compounds **3a-d** to give the desired compounds **5a-d**. Halogen-metal exchange of 5-bromofuroic acid, **2**, using t-butyllithium in tetrahydrofuran, followed by quenching with a solution of iodine in tetrahydrofuran, gave a mixture of the desired 5-iodo-2-furoic acid as the major product, along with 3-iodo-2-furoic acid (10). The isomers were separated at this point (11), and each iodinated isomer was converted to its corresponding N-hydroxysuccinimidyl ester **3a** and **3b**. These two esters, along with N-hydroxysuccinimidyl-5-iodopyridine-3-carboxylate, **3c**, (12) and 4-iodobenzoyl chloride, **3d**, were coupled with piperazine **4** to give the desired compounds **5a-5d**.

Scheme 1[a].

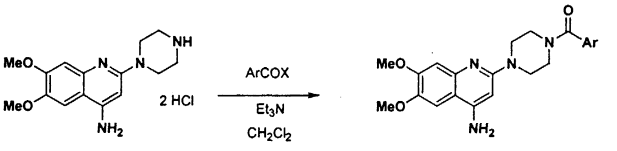


2

3a, X=I, Y=H
3b, X=H, Y=I

[a]Key (a) tBuLi, THF, -78°C (b) I_2, THF, -78°C to RT (c) NHS, DCC, CH_2Cl_2.

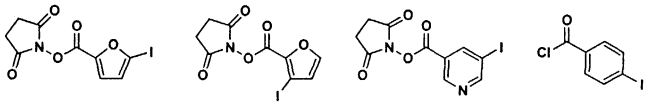
Scheme 2. Synthesis of iodoaryl analogs 5a-5d



4

5a, Ar = 5-Iodo-2-furyl
5b, Ar = 3-Iodo-2-furyl
5c, Ar = 5-Iodo-3-pyridyl
5d, Ar = 4-Iodophenyl

ArCOX =

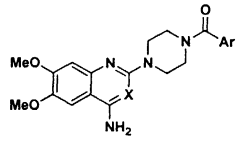


3a 3b 3c 3d

The partition coefficients and K_i values of **5a-d** were determined and compared to prazosin, **1a**, and L-760,478, **1b** (Table 1). All of the iodinated analogs had the desired log P value of <3, and iodofurans **5a** and **5b** maintained the desired subnanomolar affinities for each of the receptor subtypes. Compound **5c** was also of interest because it was somewhat selective for the α_{1b} subtype. Compounds **5a-c**, were radioiodinated for further characterization.

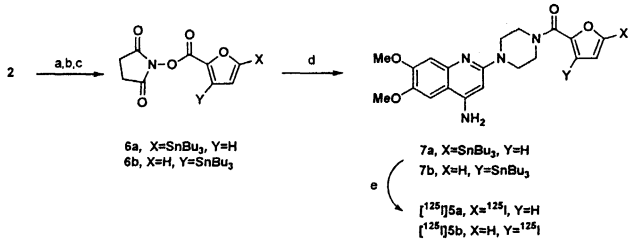
The required stannanes could have been synthesized from the final iodides, but in order to conserve **5a-c**, the stannanes were synthesized from **2** and **8** (Scheme 3 and 4) following the procedure used to synthesize **5a-c**. As seen in the synthesis of **5a-b**, halogen-metal exchange of **2** (Scheme 3) followed by quenching with tributylstannyl chloride gave the 5- and 3-tributylstannyl isomers (13). Rather than separating the isomers at this stage, it was more convenient to convert them to N-hydroxysuccinimidyl esters **6a** and **6b**, and then separate them. Coupling of these isomeric esters **6a** and **6b** with piperazine **4** gave the required stannanes **7a** and **7b**. The synthesis of stannane **9** was carried out in a similar fashion using stannylpyridine **8** (14), synthesized from 3-bromonicotinic acid. After exploring various iodination conditions, the radioiodinated compounds [^{125}I]**5a-c** were synthesized using Na^{125}I/N-chlorosuccinimide in methanol with acetic acid. With these iodinated tracers in hand, their binding to human expressed alpha-1 receptor subtypes was measured and compared to [^{3}H]prazosin (Table 2).

Table 1. [^{125}I]HEAT Binding to Human Expressed α_1 Receptor Subtypes.



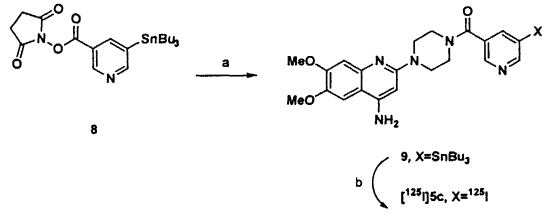
	X	Ar	Log P		K_i, nM	
				α_{1a}	α_{1b}	α_{1d}
1a	N	2-Furyl	2.03	0.52	0.19	0.23
1b	C	2-Furyl	0.99	0.35	0.13	0.2
5a	C	5-Iodo-2-furyl	2.18	0.15	0.06	0.06
5b	C	3-Iodo-2-furyl	2.02	0.80	0.08	0.19
5c	C	5-Iodo-3-pyridyl	1.61	7.7	0.14	0.78
5d	C	4-Iodophenyl	2.75	1.03	0.18	0.40

Scheme 3[a]. The synthesis of [^{125}I]5a-b.



[a]Key (a) tBuLi, THF, -78°C (b) nBu_3SnCl, -78°C to RT (c) NHS, DCC, CH_2Cl_2 (d) **4**, Et_3N, CH_2Cl_2 (e) $Na^{125}I$, N-chlorosuccinimide, 30% HOAc/MeOH, Dulbecco's PBS buffer.

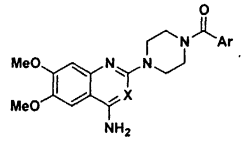
Scheme 4[a]. The synthesis of [^{125}I]5c.



[a]Key (a) **4**, Et_3N, CH_2Cl_2 (b) $Na^{125}I$, N-chlorosuccinimide, 30% HOAc/MeOH, Dulbecco's PBS buffer.

When the K_d values of these radioiodinated tracers were determined (Table 2), their affinities agreed in general with their K_i values determined *via* competitive binding assay. [^{125}I]**5a** exhibits the most balanced affinity profile of these ligands, similar to that of [^{3}H]prazosin, but with affinities 6-fold higher for the respective α_1 receptor subtypes. [^{125}I]**5c**, while not as balanced as [^{125}I]**5a** or [^{125}I]**5b**, may be useful as an α_{1b} selective ligand. The combination of high affinity, reasonable lipophilicity and a balanced affinity profile suggests that radiotracers [^{125}I]**5a** and [^{125}I]**5b** should be ideal for the study of α_1 receptors in tissues in which the concentration of receptor is low, or in which autoradiographic characterization is desirable.

Table 2. Radiotracer Binding to Human Expressed α_1 Receptor Subtypes.



	X	Ar		K_d, nM	
			α_{1a}	α_{1b}	α_{1d}
[^{3}H]1a	N	5-[^{3}H]-3-Furyl	0.132	0.056	0.080
[^{125}I]5a	C	5-[^{125}I]-2-Furyl	0.021	0.0082	0.014
[^{125}I]5b	C	3-[^{125}I]-2-Furyl	0.157	0.022	0.090
[^{125}I]5c	C	5-[^{125}I]-3-Pyridyl	1.6	0.039	0.23

Experimental

^{1}H NMR were recorded using a Varian Infinity-300 spectrometer operating at 300 MHz. Mass spectral analyses were carried out using a VG 7070E. Melting points were taken using a Thomas Hoover capillary melting point apparatus or a Fisher Scientific hot stage melting point apparatus. Analytical and preparative HPLC was carried out using a Waters 600E Powerline Multi Solvent Delivery System with 100 μL heads with a Rheodyne 7125 injector and a Waters 990 Photodiode Array Detector with a Gilson FC203 Microfraction collector. The acetonitrile used for the HPLC analyses was Fisher Optima grade. The HPLC radiodetector used was a Beckman 171 Radioisotope detector with a Beckman 110B solvent delivery system and Beckman Ready Flow III scintillation cocktail. A Vydac C-18 column, 4.6 x 250 mm (The Nest Group) was used for analytical and preparative HPLC.

Solutions of radioactivity were concentrated using a Jouan vacuum centrifuge. Calibration curves and chemical concentrations were determined using a Hewlett Packard Model 8452A UV/Vis Diode Array Spectrophotometer. Sample radioactivities were determined in an LKB Wallac 1410 liquid scintillation counter. The identity of labelled compounds was determined by HPLC coelution with authentic compounds. Reagents were purchased from Aldrich Chemical Co., the $Na^{125}I$ was purchased from Amersham Pharmacia Biotech and the Dulbecco's phosphate-buffered saline (PBS) buffer was purchased from GIBCO Laboratories.

N-Hydroxysuccinimidyl-5-iodo-2-furoate, 3a: A clear brown solution of **2** (100 mg, 0.52 mmol) in THF (10 mL) was cooled to -78°C and treated with t-butyllithium (1.7M in pentane, 1.5 mL, 2.5 mmol). After stirring the mixture at -78°C for thirty minutes, a solution of I_2 (300 mg, 1.04 mmol) in THF (3 mL) was added *via* syringe over several minutes giving an opaque purple mixture. The reaction was stirred as the cold bath warmed to ambient temperature and the reaction was stirred overnight giving a clear purple solution. The reaction was quenched with aqueous saturated NH_4Cl, diluted with H_2O and placed in a separatory funnel. The aqueous layer was acidified to pH~2 and extracted with ethyl acetate. The organic layers were combined and washed with 5% $Na_2S_2O_3$. The ethyl acetate layer was dried over magnesium sulfate, filtered and concentrated *in vacuo* to give 122 mg of a yellow solid. Preparative HPLC (Waters C-18 μBondapak radial compression cartridge, 10 x 25 cm, 20% MeCN:H_2O(0.1% TFA), 10 mL/min, 254 nm) gave 22 mg (18%) of 3-iodofuroic acid (retention time 21 min) as a white solid and 65 mg (53%) of 5-iodofuroic acid (retention time 27 min) as a white solid:
5-Iodo-2-furoic acid: mp 196-197°C; ^1H NMR (δ, $CDCl_3$): 7.18 (1H, d, J=3.4 Hz), 6.73 (1H, d, J= 3.4 Hz); MS m/z (relative intensity) for $C_5H_3IO_3$: 239 (M+1, 75%), 203 (100%).
3-Iodo-2-furoic acid: ^1H NMR (δ, $CDCl_3$): 7.57 (1H, d, J=1.5 Hz), 6.75 (1H, d, J=1.5 Hz).
A room temperature solution of 5-iodo-2-furoic acid (62 mg, 0.26 mmol) in methylene chloride (10 mL) was treated with N-hydroxysuccinimide (46 mg, 0.4 mmol) and dicyclohexylcarbodiimide (78 mg, 0.39 mmol). After stirring overnight at ambient temperature, the resulting opaque mixture was filtered through celite, rinsed with methylene chloride and concentrated *in vacuo* to give 137 mg of a white solid. The product was purified using rotary chromatography (30% to 50% ethyl acetate:hexane) to give 95 mg of a white semi-solid still contaminated with dicyclohexylurea. This material was dissolved in hot ethyl acetate, cooled and the solid removed by filtration. The filtrate was concentrated to give 66 mg (76%) of **3a** as a tan solid: mp

127-128°C; ^{1}H NMR (δ, $CDCl_3$): 7.34 (1H, d, J=3.6 Hz), 6.79 (1H, d, J=3.4 Hz), 2.91 (4H, s); MS m/z (relative intensity) for $C_9H_6INO_5$: 358 (M+Na, 100%).

N-Hydroxysuccinimidyl-3-iodo-2-furoate, 3b: Prepared in the same manner as **3a** using 19 mg (0.08 mmol) of 3-iodo-2-furoic acid as prepared above to give 16 mg (60%) of **3b** as a tan solid: mp 161-163°C, ^{1}H NMR (δ, $CDCl_3$): 7.63 (1H, d, J=1.5 Hz), 6.82 (1H, d, J=1.5 Hz), 2.91 (4H, s); MS m/z (relative intensity) for $C_9H_6INO_5$: 358 (M+Na, 100%).

4-Amino-6,7-dimethoxy-2-[4-(5-iodo-2-furoyl)piperazinyl]quinoline dihydrochloride, 5a: A mixture of **4** (16 mg, 0.044 mmol) in methylene chloride (1 mL) was treated with **3a** (31 mg, 0.093 mmol) and triethylamine (15 μL, 0.11 mmol) and stirred overnight at ambient temperature. The reaction was concentrated to dryness, absorbed onto silica gel and purified by column chromatography (10% MeOH/NH_3 saturated $CHCl_3$) followed by radial chromatography (1% to 2% MeOH/NH_3 saturated $CHCl_3$) to give 14 mg (63%) of **5a** as a yellow oil. This was dissolved in methanol, cooled to 0°C and HCl(g) was bubbled in. Removal of the solvent left 14.6 mg of the hydrochloride salt as an off white solid: ^{1}H NMR (δ, MeOH-d_4): 7.52 (1H, s), 7.24 (1H, s), 7.03 (1H, d, J=3.66Hz), 6.81 (1H, d, J=3.66Hz), 6.09 (1H, s), 4.1-3.7 (8H, m), 3.98 (3H, s), 3.94 (3H, s); MS m/z (relative intensity) for $C_{20}H_{21}IN_4O_4$: 509 (M+1, 100%).

4-Amino-6,7-dimethoxy-2-[4-(3-iodo-2-furoyl)piperazinyl]quinoline dihydrochloride, 5b: Prepared in the same manner and on the same scale as **5a** except 20 mg (0.06 mmol) of **3b** was used to give 18 mg (59%) of **5b** as a pale yellow oil. Conversion to the hydrochloride salt as above gave 19.3 mg of an off white solid: ^{1}H NMR (δ, MeOH-d_4): 7.69 (1H, d, J=1.96Hz), 7.52 (1H, s), 7.22 (1H, s), 6.77 (1H, d, J=1.95Hz), 6.09 (1H, s), 3.98 (3H, s), 3.94 (3H, s), 3.9-3.7 (8H, m); MS m/z (relative intensity) for $C_{20}H_{21}IN_4O_4$: 509 (M+1, 100%).

4-Amino-6,7-dimethoxy-2-[4-(5-iodonicotinoyl)piperazinyl]quinoline, 5c: Prepared as **5a** starting with 30 mg (0.083 mmol) of **4** and 29 mg (0.083 mmol) of **3c** to give 15 mg (35%) of **5c** as a tan solid: mp 212-213°C; ^{1}H NMR (δ, MeOH-d_4): 8.92 (1H, d, J=1.9Hz), 8.63 (1H, d, J=1.7Hz), 8.31 (1H, m), 7.27 (1H, s), 7.02 (1H, s), 6.14 (1H, s), 3.94 (6H, s), 3.6 (8H, m); MS m/z (relative intensity) for $C_{21}H_{22}IN_5O_3$: 520 (M+1, 100%).

4-Amino-6,7-dimethoxy-2-[4-(4-iodobenzoyl)piperazinyl]quinoline, 5d: A slurry of **4** (30 mg, 0.083 mmol) in methylene chloride (1 mL) was treated

with triethylamine (24 μL, 0.174 mmol), cooled to 0°C and treated with 4-iodobenzoyl chloride (22 mg, 0.083 mmol). The reaction mixture was stirred as the ice bath warmed to room temperature. After stirring overnight at room temperature, the reaction mixture was concentrated to give 47 mg of a tan solid. Column chromatography (2% MeOH:$CHCl_3$ saturated with NH_3) followed by radial chromatography (1% MeOH:$CHCl_3$ saturated withNH_3) gave 8 mg (19%) of **5d** as a tan solid: mp 182-183.5°C; ^{1}H NMR (δ, MeOH-d_4): 7.87 (2H, m), 7.25 (3H, m), 7.02 (1H, s), 6.15 (1H, s), 4.89 (6H, s), 3.6 (8H, m); MS m/z (relative intensity) for $C_{22}H_{23}IN_4O_3$: 519 (M+1, 85%).

N-Hydroxysuccinimidyl-5-tributylstannyl-2-furoate and N-hydroxysuccinimidyl-3-tributylstannyl-2-furoate, 6a, 6b: A clear brown solution of **2** (100 mg, 0.52 mmol) in THF (10 mL) was cooled to -78°C and treated with t-butyllithium (1.7M in pentane, 1.5 mL, 2.5 mmol). After stirring the mixture at -78°C for 30 minutes, nBu_3SnCl (0.350 mL, 1.29 mmol) was added *via* syringe over several minutes and the reaction was stirred as the cold bath warmed to ambient temperature. After stirring overnight the clear tan solution was quenched with aqueous saturated NH_4Cl, diluted with H_2O and placed in a separatory funnel. The aqueous layer was extracted with ethyl acetate. The ethyl acetate layers were combined, dried over magnesium sulfate, filtered and concentrated *in vacuo* to give 417 mg of an orange oil: This material was dissolved in methylene chloride (24 mL) and treated with N-hydroxysuccinimide (104 mg, 0.9 mmol) and dicyclohexylcarbodiimide (186 mg, 0.9 mmol) and stirred at ambient temperature overnight. The opaque white mixture was filtered through celite, rinsed with methylene chloride and concentrated *in vacuo* to give 615 mg of an oily yellow solid. This material was adsorbed onto silica gel and purified by column chromatography by eluting with 5% to 10% ethyl acetate/hexane. The fractions containing material with R_f 0.14 (20 % ethyl acetate/hexane) were pooled and concentrated *in vacuo* to give 16.5 mg (6%) of **6b** as a clear colorless oil, and fractions containing material with R_f 0.08 (20% ethyl acetate:hexane) were pooled and concentrated to give 53 mg (20%) of **6a** as a clear colorless oil:

N-hydroxysuccinimidyl-3-tributylstannyl-2-furoate: ^{1}H NMR (δ, $CDCl_3$): 7.74 (1H, d, J = 1.5 Hz), 6.60 (1H, d, J = 1.5 Hz), 2.90 (4H, s), 1.6-0.8 (27H, m); MS m/z (relative intensity) for $C_{21}H_{33}NO_5Sn$: 522 (M+1+Na, 65%).

N-hydroxysuccinimidyl-5-tributylstannyl-2-furoate: ^{1}H NMR (δ, $CDCl_3$): 7.48 (1H, d, J = 3.4 Hz), 6.70 (1H, d, J = 3.65 Hz), 2.90 (4H, s), 1.6-0.8 (27H, m); MS m/z (relative intensity) for $C_{21}H_{33}NO_5Sn$: 522 (100%, M+1+Na, 100%).

4-Amino-6,7-dimethoxy-2-[4-(5-tributylstannyl-2-furoyl)piperazinyl]quinoline, 7a: A slurry of **4** (22 mg, 0.061 mmol) in methylene chloride (2 mL) containing triethylamine (18 μL, 0.13 mmol) was cooled to 0°C and treated with **6a** (33 mg, 0.066 mmol) in methylene chloride (1 mL). The resulting mixture was stirred as the ice bath was slowly warmed to ambient temperature. After stirring overnight the tan slurry was concentrated *in vacuo*. The residue was dissolved in methanol and purified using radial chromatography eluting with NH_3 saturated $CHCl_3$ and 1% MeOH/NH_3 saturated $CHCl_3$. The fractions containing material with R_f 0.6 (5% MeOH/NH_3 saturated $CHCl_3$) were combined to give 21.2 mg (52%) of **7a** as a yellow oil. ^{1}H NMR (δ, $CDCl_3$): 7.1 (2H, m), 6.85 (1H, s), 6.62 (1H, d, 3.4Hz), 6.12 (1H, s), 4.39 (2H, br s), 3.99 (3H, s), 3.97 (3H, s), 4.1-3.9 (4H, m), 1.58 (6H, m), 1.34 (6H, m), 1.12 (6H, m), 0.91 (9H, t, J=7.3Hz); MS m/z (relative intensity) for $C_{32}H_{48}N_4O_2Sn$: 673 (M+1, 100%).

4-Amino-6,7-dimethoxy-2-[4-(3-tributylstannyl-2-furoyl)piperazinyl]quinoline, 7b: Prepared on the same scale as compound **7a**. After warming to ambient temperature, the reaction was heated at 35°C overnight. The reaction was concentrated *in vacuo* and purified using radial chromatography (2% MeOH/NH_3 saturated $CHCl_3$). The fractions containing material with R_f 0.7 (5% MeOH/NH_3 saturated $CHCl_3$) were pooled to give 24 mg (59%) of **7b** as a yellow oil. ^{1}H NMR (δ, MeOH-d_4): 7.73 (1H, d, J=1.4Hz), 7.27 (1H, s), 7.04 (1H, s), 6.54 (1H, d, J=1.4Hz), 6.16 (1H, s), 4.1-3.7 (4H, m), 3.92 (3H, s), 3.91 (3H, s), 3.62 (4H, m), 1.53 (6H, m), 1.33 (6H, m), 1.08 (6H, m), 0.87 (9H, t, J=7.3Hz); MS m/z (relative intensity) for $C_{32}H_{48}N_4O_4Sn$: 673 (M+1, 70%).

4-Amino-6,7-dimethoxy-2-[4-(5-tributylstannylnicotinoyl)piperazinyl]quinoline, 9: Prepared as **7a** starting with 23 mg (0.064 mmol) of **4** and 32 mg (0.064 mmol) of **8** to give 18 mg (42%) of **9** as a clear, colorless oil: ^{1}H NMR (δ, MeOH-d_4): 8.63 (1H, d, J=1.2Hz), 8.57 (1H, d, J=2.2Hz), 7.89 (1H, m), 7.27 (1H, s), 7.02 (1H, s), 6.16 (1H, s), 3.91 (6H, s), 3.7-3.5 (8H, m), 1.59 (6H, m), 1.37 (6H, m), 1.20 (6H, m), 0.91 (9H, t, J=7.3Hz).

[^{125}I]4-Amino-6,7-dimethoxy-2-[4-(5-iodo-2-furoyl)piperazinyl]quinoline, [^{125}I]5a: A 0.3 mL Wheaton reaction vial was charged with 14 μL of a solution of **7a** (14 mg/mL $CHCl_3$), concentrated to dryness and treated with 40 μL of 30% HOAc/MeOH, 10 μL of Dulbecco's PBS buffer and 10 μL of an N-chlorosuccinimide solution (2 mg/mL 2% HOAc/MeOH). The resulting solution was added to a Na^{125}I (5 mCi)

shipping vial containing a stir bar. The reaction was stirred at ambient temperature for about 5 minutes and was then quenched with 100 μL of $Na_2S_2O_5$ (5 mg/mL H_2O). Purification by HPLC (C-18 Vydac, 4.6 x 250 mm, 25% MeCN:H_2O(0.1% TFA), 1 mL/min, retention time 14 min), removal of HPLC eluant *in vacuo* and dilution with ethanol gave 600 μCi of [^{125}I]**5a** with a radiochemical purity of >96%.

[^{125}I]4-Amino-6,7-dimethoxy-2-[4-(3-iodo-2-furoyl)piperazinyl]quinoline, [^{125}I]5b: Prepared using the same procedure as [^{125}I]**5a** starting with **7b** to give 850 μCi of [^{125}I]**5b** (retention time 12 min) with a radiochemical purity of >97%.

[^{125}I]4-Amino-6,7-dimethoxy-2-[4-(5-iodonicotinoyl)piperazinyl]quinoline, [^{125}I]5c Prepared as [^{125}I]**5a** starting with **9b** and 1 mCi of Na^{125}I to give 100 μCi of [^{125}I]**5c** (C-18 Vydac, 4.6 x 250 mm, 30 minute linear gradient 20% to 90% MeCN:H_2O(0.1% TFA), 1 mL/min, retention time 11 min) with a radiochemical purity of >98%.

Binding Affinity: Cloned Human α_1-Adrenergic Receptor Subtypes. The data in Table 1 was obtained *via* competitive binding with [^{125}I]HEAT using membranes containing the cloned, expressed α_{1a}, α_{1b}, and α_{1d}-adrenergic receptor (15). The K_i values were calculated using an iterative curve-fitting program INPLOT. The data in Table 2 was generated using the same membrane preparations, with increasing concentrations of radioligands to provide saturation of the receptor population. Nine concentrations of each radioligand was incubated with membranes containing the adrenergic receptor subtype in Tris-buffered (50 mM, pH 7.4) normal saline containing 0.5 mM EDTA and 0.1% bovine serum albumin, in triplicate, for two hours at ambient temperatures (20-21°C). Blockade of specific binding was effected by the addition of 10^{-6}M unlabeled prazosin. The samples were filtered over GF/B filters, air dried, and counted in a scintillation counter (for [^{3}H]**1a**) or autogamma counter for the radioiodinated radioligands. Data were reduced and curve fitted using a non-linear regression routine in RS1 (Cambridge). Each value is an average of at least two separate determinations.

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11. If **3a** and **3b** are not separated at this stage, the mixture can be carried through to the end and **5a** and **5b** can be separated using HPLC.

12. Prepared from 5-bromonicotinic acid as described in reference 13 for the synthesis of **8**, quenching with I_2 rather than nBu_3SnCl.

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