As a consequence of this work, it may be possible to isolate the serotoninergic receptor by the covalent binding of a labeled serotonin under oxidative conditions (e.g., H_2O_2/horseradish peroxidase). The results reported here with ceruloplasmin offer new perspectives on the possible role of this oxidizing protein in neurophysiological disorders.

Experimental Section

Proton nuclear magnetic resonance spectra were recorded on a Bruker WM500 spectrometer using tetramethylsilane as an internal standard. Mass spectra were performed on a VG instrument with glycerol matrix. All chromatographic separations were performed on Merck silica gel (Kieselgel 60, 230–400 mesh, ASTM). *N*-Dansylcadaverine, N^{ϵ}-dansyl-L-lysine, and human ceruloplasmin (5% solution in 0.25 M sodium chloride and 0.05 M sodium acetate) were purchased from Sigma. Bufotenine was prepared by demethylation[16] of 5-methoxy-3-[2-(dimethylamino)ethyl]indole.[17]

2-[4-(Dansylamino)butyl]-7-[2-(dimethylamino)ethyl]-5*H*-pyrrolo[2,3-*f*]benzoxazole (4): Oxidation of Bufotenine (2) with MnO_2. To a suspension of bufotenine (2) (100 mg, 0.48 mmol) in water (500 μL) was added a solution of dansylcadaverine (400 mg, 1.19 mmol) in dichloromethane (5 mL) and methanol (5 mL). After addition of MnO_2 (167 mg, 1.91 mmol), the reaction mixture was stirred at room temperature for 24 h and then filtered through Celite. The filtrate was concentrated in vacuo and the residue was purified by two columns [first, eluent, gradient from CH_2Cl_2 to CH_2Cl_2/MeOH (85/15); second, eluent, CH_2Cl_2/MeOH/NH_4OH (25/5/3) organic phase] and then by HPLC (Lichrosorb NH_2 7-μm Merck column, 250 × 10 mm) with a linear gradient, MeOH/$CHCl_3$, 0.1 to 10 in 20 min, flow 5.5 mL/min, to give a yellow oil: yield 18 mg (7%); NMR (Me_2SO + D_2O) δ 8.43 (d, 1 H), 8.28 (d, 1 H), 8.07 (d, 1 H), 7.55 (m, 2 H), 7.31 (s, 2 H), 7.20 (s, 1 H), 7.22 (d, 1 H), 3.05 (t, 2 H), 2.85 (m, 2 H), 2.75 (m, 8 H), 2.26 (s, 6 H), 1.71 (m, 2 H), 1.45 (m, 2 H); MS (FAB$^+$), 534.55 (M^+); UV (EtOH, 96%) λ_{max} 335, nm. 305, 296 ; HPLC (Lichrosorb NH_2 5-μm Merck column, 250 × 4 mm) with the linear gradient MeOH/$CHCl_3$, 0 to 10 in 20 min, t_R = 11.72 min, flow 1 mL/min.

To a solution of bufotenine (2) (2 mg, 0.010 mmol) in water (100 μL) was added a solution of N^{ϵ}-dansyl-L-lysine (11 mg, 0.03 mmol) in dichloromethane (10 mL). After addition of MnO_2 (2.6 mg, 0.03 mmol), the reaction mixture was stirred at room temperture for 48 h. After usual purifications, we obtained a yellow oil, which exhibited the same retention time (HPLC) and gave one single peak when mixed with compound 4 isolated from dansylcadaverine.

Compound 4: Oxidation of Bufotenine with Ceruloplasmin. To a solution of bufotenine (2) (100 mg, 0.48 mmol) in 0.2 M acetate buffer, pH 5.40 (5 mL), and methanol (2 mL) was added a solution of dansylcadaverine (400 mg, 1.19 mmol) in dichloromethane (5 mL) and methanol (5 mL). The reaction mixture was vigorously stirred and human ceruloplasmin (2 mL), 4900 units/mL was added, by portion of 100 μL over 8 h. The suspension was stirred overnight and concentrated in vacuo. The residue was extracted four times with dichloromethane (30 mL). After drying (Na_2SO_4) and evaporation, the residue was purified as previously described for the oxidation by MnO_2: yield 1.5 mg (0.6%); MS (FAB$^+$), 534.16 (M^+); UV (EtOH, 96%) λ_{max} 335 nm, 305, 296; HPLC, Lichrosorb NH_2 5-μm Merck column, 250 × 4 mm) with a linear gradient, MeOH/$CHCl_3$, 0 to 10 in 20 min, t_R = 11.85 min, flow 1 mL/min, the mixture with the compound obtained from oxidation with MnO_2 gave one single peak.

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Radioiodinated *p*-Iodoclonidine: A High-Affinity Probe for the α_2-Adrenergic Receptor

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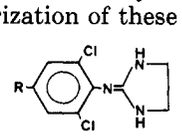
The chemical synthesis of 2-[(2,6-dichloro-4-iodophenyl)imino]imidazolidine (PIC) and its radioiodinated analogue [^{125}I]PIC is described. PIC was synthesized from 2,6-dichloroaniline in five synthetic steps. This agent displayed a high affinity for the α_2-adrenergic receptor (IC_{50} = 1.5 nM) in competitive binding assays conducted with purified human platelet plasma membrane fractions. For the synthesis of radioiodinated PIC the triazene intermediate 11 was synthesized from 2,6-dichloro-4-nitroaniline in five synthetic steps. Acid-catalyzed decomposition of 11 with no-carrier-added Na^{125}I afforded high specific activity [^{125}I]PIC. In view of its high affinity for the α_2-adrenergic receptor, [^{125}I]PIC is a potentially useful probe for studies in adrenergic pharmacology.

Clonidine (1) is a potent antihypertensive drug whose mechanism of action is believed to be via stimulation of centrally located α_2-adrenergic receptors.[1] The commercial availability of tritiated α_2-adrenergic probes, such as [^{3}H]clonidine and [^{3}H]*p*-aminoclonidine ([^{3}H]PAC), has proved to be extremely valuable for the identification and characterization of these receptors in various tissues.[2]



1 R = H ; Clonidine

2 R = I ; PIC

As part of our studies in the area of receptor-specific ligands, we were interested in the development of an ^{125}I-labeled clonidine analogue as a suitable α_2-adrenergic receptor probe. In contrast to tritiated tracers, a radioiodinated probe would have several advantages such as a capability to achieve higher specific activity, an increased counting efficiency, and the opportunity to perform in vivo scintigraphic analyses. Moreover, the ability to achieve high specific activity with an ^{125}I-labeled probe would make it possible to analyze those tissues having very low densities of α_2-adrenergic receptors. A recent paper reports the synthesis and characterization of a radioiodinated analogue of rauwolscine, an α_2 antagonist.[3] We report the first

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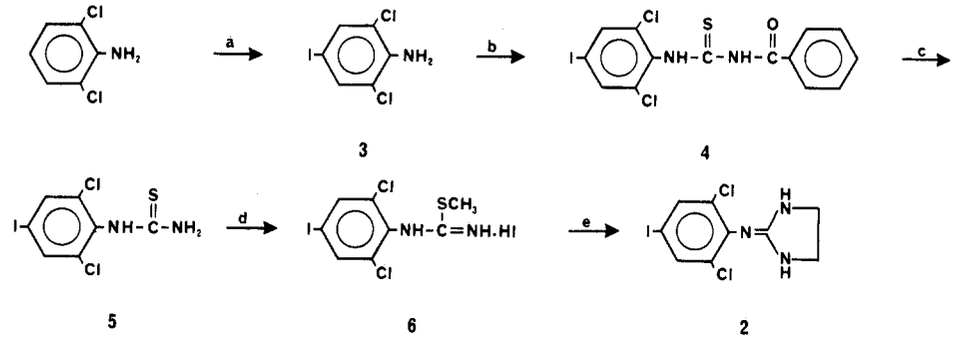
Scheme I



Key: (a) ICl/CH_3COOH; (b) C_6H_5CONCS; (c) 10% NaOH; (d) CH_3I; (e) $H_2NCH_2CH_2NH_2$/EtOH, 140°C.

synthesis of an ^{125}I-labeled analogue in the clonidine series of α_2 agonists.

We chose [^{125}I]*p*-iodoclonidine ([^{125}I]PIC) as our target compound since there was literature precedence to indicate that the para position of clonidine could accommodate lipophilic substituents without a significant loss of affinity for α_2-adrenergic receptors.[4] Since triazenes have been shown to be very useful intermediates for the preparation of high specific activity receptor probes,[5,6] our synthetic strategy envisaged the intermediacy of a suitable triazene analogue of clonidine that would undergo decomposition in the presence of $Na^{125}I$ to afford [^{125}I]PIC.

In the present study, we describe the chemical synthesis of PIC (Scheme I) and its specificity for the α_2 receptor. Although radioiodinated PIC was prepared directly from **2** by isotopic exchange with $Na^{125}I$, the high specific activity required for receptor studies was not achievable by this method. Thus, an alternate approach via a triazene intermediate (Scheme II) was employed to synthesize high specific activity [^{125}I]PIC.

Chemistry

Our initial attempts at preparing **2** by direct iodination of clonidine with ICl/HOAc were unsuccessful. The observed nonreactivity of clonidine to electrophilic attack by iodine can be attributed to the presence of the positively charged guanidinium-like moiety. An alternative multistep approach, similar to one reported by Rouot and co-workers,[7] proved successful and is illustrated in Scheme I.

Benzoyl isothiocyanate (prepared in situ by reaction of benzoyl chloride and ammonium thiocyanate) was treated with 2,6-dichloro-4-iodoaniline (**3**) to afford crystalline 1-benzoyl-3-(2,6-dichloro-4-iodophenyl)thiourea (**4**). Without further purification, **4** was saponified to furnish (2,6-dichloro-4-iodophenyl)thiourea (**5**) in good overall yield (81%). Condensation of **5** with CH_3I in refluxing CH_3OH afforded the corresponding *S*-methylisothiourea hydroiodide salt **6** in essentially quantitative yield. The desired product (**2**) was obtained by treatment of **6** with ethylenediamine in EtOH at 145 °C. Radioiodination of PIC by isotope exchange with iodide-125 in pivalic acid as previously described[8] afforded [^{125}I]PIC of low specific activity (320 mCi/mmol) in 45% radiochemical yield.

Table I. Inhibition of [^3H]PAC Binding to Purified Platelet Plasma Membranes by Various Ligands[a]

competing ligand	IC_{50}, M
yohimbine	8.0×10^{-9} [b]
epinephrine	8.0×10^{-9} [b]
clonidine	2.1×10^{-8} [b]
p-aminoclonidine (**10**)	4.0×10^{-9} [b]
UK 14,304	3.0×10^{-9} [b]
p-iodoclonidine (**2**)	1.5×10^{-9}
p-nitroclonidine (**9**)	1.0×10^{-5}
p-[(1,4-butanediyl)triazeno]clonidine (**11**)	2.0×10^{-6}

[a] The data are means of several individual determinations each performed in triplicate. The IC_{50} values somewhat underestimate the true affinity of the ligands because no correction has been made for the concentration of radioligand used.[11] We present uncorrected IC_{50}'s rather than calculated K_i's because [^3H]PAC binding does not follow a simple mass action mechanism.[12] [b] Taken from ref 12.

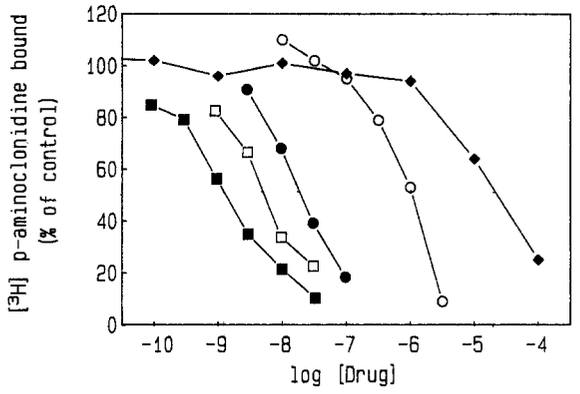


Figure 1. Competition of clonidine analogues for specific [^3H]PAC binding. Equilibrium binding of 3 nM [^3H]PAC was measured in the presence of various concentrations of (♦) *p*-nitroclonidine, (○) *p*-[(1,4-butanediyl)triazeno]clonidine, (●) clonidine, (□) *p*-aminoclonidine, and (■) *p*-iodoclonidine. The final volume was 1.0 mL and incubation was at 23 °C for 30–45 min. Data points are the means of triplicate determinations of a single experiment.

p-Nitroclonidine[9] and *p*-aminoclonidine,[10] key intermediates required for the synthesis of the triazene analogue **11**, were prepared according to literature procedures (Scheme II). Accordingly, 2,6-dichloro-4-nitroaniline was formylated with HCOOH/Ac_2O to afford the formanilide derivative **7**, which upon treatment with $SO_2Cl_2/SOCl_2$ was converted to the dichloro imine **8**. Treatment of **8** with ethylenediamine gave *p*-nitroclonidine (**9**), which was

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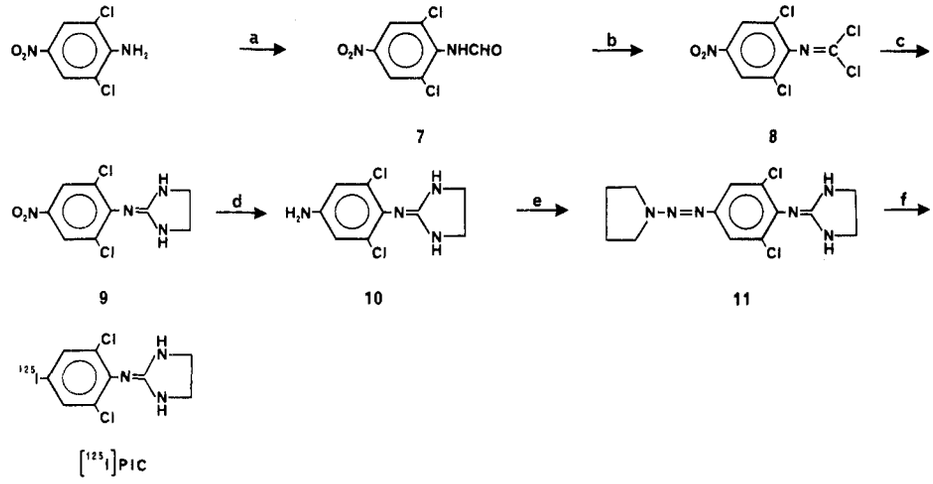
Scheme II



Key: (a) $HCOOH/Ac_2O$; (b) $SO_2Cl_2/SOCl_2$; (c) $H_2NCH_2CH_2NH_2/Et_3N$; (d) Fe/HCl; (e) 1. HNO_2 2. pyrrolidine/OH^- (f) $CF_3COOH/Na^{125}I$

subsequently reduced with Fe/HCl to afford *p*-aminoclonidine (**10**). Diazotization of **10** and treatment of the diazonium intermediate with pyrrolidine afforded the triazene analogue **11** in 69% yield.

Radioiodinated **2** was prepared in high specific activity by decomposition of the triazene **11** with trifluoroacetic acid in the presence of no-carrier-added $Na^{125}I$. This procedure afforded [^{125}I]PIC in 9% radiochemical yield after purification by column chromatography.

Pharmacology

The binding characteristics of PIC to α_2-adrenoceptors were evaluated with the aid of a membrane preparation obtained from human platelets. Table I shows the IC_{50} values for ligand competition with the tritiated α_2 agonist radioligand [^{3}H]PAC (3 nM) obtained for PIC and several other routinely used agonists and antagonists. As seen from these data and those in Figure 1, PIC (IC_{50} = 1.5 nM) is the most potent in terms of binding to α_2-adrenoceptors. Moreover, its affinity even surpasses that found for PAC and UK 14,304, two tritiated agonist radioligands currently in use. It is notable that the precursors in the synthesis of PIC, namely **9** and **11**, fail to exhibit any appreciable affinity for the α_2-adrenergic receptor (Figure 1). Further studies on the pharmacological characterization of stable and radioiodinated PIC are in progress.

Experimental Section

Melting points were obtained in open capillary tubes with a Thomas-Hoover or Mel-Temp melting point apparatus and are uncorrected. NMR spectra were obtained on a Varian EM360 A spectrometer in the indicated solvents. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane, which was used as the internal standard. IR spectra were determined as thin KBr disks with a Perkin-Elmer 281 spectrophotometer. All spectral results are in agreement with the assigned structures. Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, IN, and the analytical values obtained were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was done on E. Merck F-254 plastic-backed silica gel plates. TLC's of the radiolabeled compounds were scanned with a Vanguard 930 Autoscanner. Flash column chromatography[13] was done with E. Merck Kieselgel 60 (230–400 mesh). Sodium iodide-125 was obtained from Amersham as a no-carrier-added (pH 7–11) aqueous solution (reductant-free) at a specific activity of ca. 537 mCi/mL.

2,6-Dichloro-4-iodoaniline (3). This was prepared in 84% yield following a procedure described in the literature;[14] mp 97–98 °C (lit.[14] mp 96–97 °C); IR (KBr) 3430, 3300 cm^{-1} (NH_2); ^{1}H NMR ($CDCl_3$) δ 7.51 (s, 2, Ar H), 4.53 (br s, 2, NH_2).

1-Benzoyl-3-(2,6-dichloro-4-iodophenyl)thiourea (4). A vigorously stirred hot solution of anhydrous ammonium thiocyanate (1.18 g, 15.48 mmol) in dry acetone (30 mL) was treated dropwise with benzoyl chloride (1.82 g, 12.90 mmol). The reaction mixture was refluxed 5 min at which point a solution of 2,6-dichloro-4-iodoaniline (3.71 g, 12.90 mmol) in dry acetone (40 mL) was added dropwise. The reaction mixture was heated for 1 h, reduced to half its volume in vacuo, and diluted with 400 mL of water. Refrigeration afforded a crude product, which was filtered, dried, and used without further purification in the next step.

(2,6-Dichloro-4-iodophenyl)thiourea (5). The crude product **4** was heated to reflux with 10% aqueous NaOH (20 mL) for 10 min. The cooled reaction mixture was treated with concentrated HCl until acidic to precipitate both benzoic acid and (2,6-dichloro-4-iodophenyl)thiourea. The mixture was then made basic (pH 9) with concentrated NH_4OH to dissolve the benzoic acid. The thiourea was filtered and recrystallized from 95% EtOH (100 mL) to afford 3.65 g (81%); mp 212–214 °C dec; IR (KBr) 3347, 3270, 3165, 3055 cm^{-1} (NH); ^{1}H NMR (Me_2SO-d_6) δ 9.32 (s, 1, NH), 7.94 (s, 2, Ar H), 7.57 (br s, 2, NH_2). Anal. ($C_7H_5Cl_2IN_2S$) C, H, N, I.

***N*-(2,6-Dichloro-4-iodophenyl)-*S*-methylisothiourea Hydriodide (6).** A solution of **5** (1.25 g, 3.60 mmol) in freshly distilled dry CH_3OH (10 mL) was treated with CH_3I (0.51 g, 3.60 mmol). The solution was refluxed for 2 h, cooled, and evaporated to dryness in vacuo. The crystalline product was washed with several portions of Et_2O and dried to afford 1.72 g (98%) of **6**; mp 191–193 °C dec; IR (KBr) 3260, 3050 cm^{-1} (NH); ^{1}H NMR (Me_2SO-d_6) δ 9.31 (br s, 1, NH), 8.14 (s, 2, Ar H), 2.63 (s, 3, SCH_3). Anal. ($C_8H_8Cl_2I_2N_2S$) C, H, N.

2-[(2,6-Dichloro-4-iodophenyl)imino]imidazolidine (2). A mixture of **6** (1.0 g, 2.04 mmol), ethylenediamine (0.41 mL, 6.13 mmol), and absolute EtOH (3 mL) was heated with stirring in a steel bomb at 145 ± 5 °C for 18 h. The oily residue was solubilized in EtOH (2 mL) and treated with 50% aqueous KOH (5 mL). Extraction of the alkaline solution with Et_2O (4 × 50 mL) followed by drying ($MgSO_4$) and evaporation in vacuo afforded a pale yellow oil. Column chromatography (silica gel, Et_2O) furnished **2** as an oil, which was converted to the hydrochloride salt by dissolution in absolute EtOH (1 mL) and treatment with ethereal HCl to afford 58 mg (7%); mp 270–272 °C dec; ^{1}H NMR

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(free base in $CDCl_3$) δ 7.66 (s, 2, Ar H), 4.83 (br s, 2, NH), 3.58 (s, 4, imidazolidine CH_2). Anal. ($C_9H_9Cl_3IN_3$) C, H, N, I.

2,6-Dichloro-4-nitroformanilide (7). The acetic–formic anhydride was prepared by heating acetic anhydride (102 mL, 1.08 mol) and formic acid (43 mL, 1.08 mol) at an internal temperature of 60 °C for 15 min followed by immediate cooling to 0 °C. This solution was then treated in one portion with 2,6-dichloro-4-nitroaniline (10.3 g, 0.05 mol) and the mixture heated at 60 °C for 6 h. Evaporation under reduced pressure followed by trituration of the residual oil with anhydrous Et_2O afforded a straw-colored solid. The crude product was purified by column chromatography on silica gel (EtOAc–hexanes (1:5)) to afford 7.48 g (64%); mp 158.5–159.5 °C; IR (KBr) 1670 (C=O), 1530, 1350 cm^{-1} (NO_2); ^1H NMR (Me_2SO-d_6) δ 10.54 (br s, 1, NH), 8.44 (s, 2, Ar H), 8.40 (s, 1, CHO). Anal. ($C_7H_4Cl_2N_2O_3$) C, H, N.

2,6-Dichloro-1-(dichloroisocyano)-4-nitrobenzene (8). A mixture of $SOCl_2$ (27.14 g, 228 mmol) and SO_2Cl_2 (4.10 g, 30.40 mmol) was cooled in an ice bath to 10 °C and treated quickly with the formanilide derivative **7** (7.15 g, 30.40 mmol). The reaction was heated at 60 °C with stirring under a N_2 atmosphere for 16 h. Evaporation under reduced pressure afforded an oil from which residual $SOCl_2$ was removed by azeotropic distillation with freshly distilled dry benzene (5 × 15 mL). A pale yellow gum (8.28 g) was obtained following drying overnight under high vacuum. This reactive intermediate, which was relatively pure by TLC (silica gel, Et_2O), was used directly without further purification.

2-[(2,6-Dichloro-4-nitrophenyl)imino]imidazolidine (9). The isocyano dichloride **8** in EtOAc (15 mL) and anhydrous ethylenediamine (3.45 g, 57.5 mmol) in EtOAc (15 mL) were added simultaneously and dropwise with vigorous stirring to anhydrous Et_3N (16.7 g, 165 mmol) in EtOAc (18 mL). The mixture was stirred for 3 h, diluted with concentrated brine (500 mL), and extracted into EtOAc. The EtOAc extracts were evaporated to dryness, the residue solubilized in 4 N HCl and extracted with several portions of Et_2O, and the Et_2O layers were discarded. The aqueous solution was neutralized with 25% aqueous NaOH (slow addition with cooling in an ice bath) to precipitate the product. Recrystallization from 95% EtOH afforded 3.85 g (46%) of analytically pure material; mp 283–285 °C dec; IR (KBr) 1555, 1325 cm^{-1} (NO_2); ^1H NMR (Me_2SO-d_6) δ 8.23 (s, 2, Ar H), 6.76 (br s, 2, NH), 3.43 (s, 4, imidazolidine CH_2). Anal. ($C_9H_8Cl_2N_4O_2$) C, H, N.

2-[(2,6-Dichloro-4-aminophenyl)imino]imidazolidine (10). The synthesis was performed by employing a modification of the procedure of Rouot and Leclerc.[10] A vigorously stirred suspension of **9** (0.77 g, 2.80 mmol) in 50% aqueous EtOH (7.0 mL) was initially heated to reflux. Powdered Fe (0.47 g, 8.37 mmol) was then added in one portion followed by the dropwise addition of a solution of concentrated HCl (0.7 mL) in 50% aqueous EtOH (3.6 mL). The mixture was refluxed for 3 h and the pH of the hot solution was adjusted to neutrality with 2.5 N KOH. The reaction mixture was then filtered immediately through Celite to remove the precipitated $Fe(OH)_3$. The filter cake was washed with hot EtOH (4 × 25 mL), and the combined filtrates evaporated under reduced pressure. The residue was reconstituted in aqueous NH_4OH (10 mL) and extracted with EtOAc (3 × 50 mL), and the combined extracts were dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a yellow solid, which was flash chromatographed on silica gel ($CHCl_3$–benzene–EtOH–concentrated NH_4OH, 4:2:1:0.1, as eluant) to afford 0.53 g (77%) of analytically pure material melting at 227–229 °C (lit.[10] mp 230 °C).

2-[[2,6-Dichloro-4-[3,3-(1,4-butanediyl)triazeno]phenyl]-imino]imidazolidine (11). To a stirred solution of **10** (0.15 g, 0.61 mmol) in 6 M H_2SO_4 (0.39 mL) at 0 °C was added dropwise an aqueous solution (0.30 mL) of $NaNO_2$ (0.046 g, 0.67 mmol) which had been previously cooled to 0 °C. The resulting pale yellow solution was stirred an additional 15 min at 0 °C and treated dropwise with a precooled (0 °C) solution of pyrrolidine (0.048 g, 0.67 mmol) in 1 M KOH (5 mL). Stirring was continued for 15 min with gradual warming to room temperature. The basic reaction mixture was then extracted with benzene (4 × 25 mL), and the combined extracts were dried ($MgSO_4$). Removal of the solvent under reduced pressure afforded a dark oil. Flash chromatography on silica gel ($CHCl_3$–benzene–EtOH–concentrated NH_4OH, 4:2:1:0.1, as eluant) followed by recrystallization of the product from EtOH–H_2O (1:1) afforded 138 mg (69%) of analytically pure material: mp 214–215.5 °C; IR (KBr) 2870, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.38 (s, 2, Ar H), 3.74 (pentet, 4, pyrrolidine α-CH_2), 3.56 (s, 4, imidazolidine CH_2), 2.03 (pentet, 4, pyrrolidine β-CH_2). Anal. ($C_{13}H_{16}Cl_2N_6$) C, H, N.

Radioiodinated 2. A. By Isotope Exchange. Radioiodination was accomplished by isotope exchange in pivalic acid as previously described.[8] The free base of **2** (1.0 mg) and THF (300 μL) were placed in a vial and treated with 2.0 mCi of $Na^{125}I$ (in 50 μL of 0.1 N NaOH). The reaction vial was sealed, and the solvents were evaporated to dryness under a stream of nitrogen. Pivalic acid (35 mg) was then added, and the vial was resealed and heated at 155 °C for 2.0 h in an oil bath. The vial was allowed to cool and the contents were taken up in THF (300 μL) and chromatographed on silica gel with EtOAc as eluant. A total of 0.9 mCi (45% radiochemical yield) of radioiodinated **2** was isolated having a specific activity of at least 320 mCi/mmol. Radiochemical purity (99%) was established by chromatographic comparison (silica gel, EtOAc) with the unlabeled material.

B. By Triazene Decomposition. Aqueous $Na^{125}I$ (10 μL or 1.0 mCi in dilute NaOH, pH 7–11, Amersham) was injected into a 1-mL vial (Supelco) crimped with a Teflon seal and aluminum cap. The contents were evaporated to dryness under a gentle stream of nitrogen. The triazene analogue **11** (1.0 mg) was added, followed by dry CH_3OH (100 μL) and trifluoroacetic acid (5 μL). The vial was resealed, half immersed in an oil bath, and heated at 55 to 60 °C for 1.0 h. The vial was allowed to cool, its contents were evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in EtOAc (1.0 mL). Aqueous NaOH (50%, 1.0 mL) was then added, and the layers were thoroughly mixed with the aid of a small pipet. The EtOAc layer was separated, the aqueous layer was extracted further with EtOAc (2 × 1.0 mL), and the combined organic layers were dried ($MgSO_4$). Purification was achieved by column chromatography on silica gel (CH_3OH–EtOAc, 1:6, as eluting solvent), which afforded 89 μCi (9% radiochemical yield) of radioiodinated **2** (specific activity of 2200 Ci/mmol based on the initial specific activity of $Na^{125}I$ used and the radiochemical and chemical purity of the isolated product). The product comigrated with unlabeled standard in the following TLC systems: [silica gel; CH_3OH–EtOAc (1:4)] R_f 0.48, [silica gel; $CHCl_3$–benzene–EtOH–concentrated NH_4OH (4:2:1:0.1)] R_f 0.72.

Preparation of Human Platelet Plasma Membranes. This was carried out as previously described.[15]

[^{3}H]*p*-Aminoclonidine Competition Assay. The ability of analogues to compete with the binding of 3 nM [^{3}H]*p*-aminoclonidine at equilibrium was measured as described.[12] IC_{50} values were determined from plots of specific [^{3}H]PAC binding vs. log of inhibitor concentration. Values are geometric means of duplicate or triplicate determinations.

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