Synthesis and Evaluation of Trimetoquinol Derivatives: Novel Thromboxane A_2/Prostaglandin H_2 Antagonists with Diminished β-Adrenergic Agonist Activity

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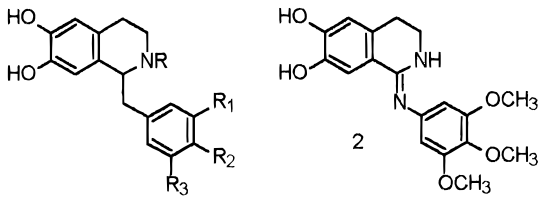
Trimetoquinol (TMQ, **1**) is a unique catecholamine with a strong stereodependence for agonism at β-adrenergic ($S \gg R$) and antagonism at thromboxane A_2/prostaglandin H_2 (TP; $R \gg S$) receptors. Our laboratory has reported the effects of N-alkylation and modification of the trisubstituted benzyl group in these receptor systems. For iodinated derivative **5**, maintaining potency in TP receptor systems (112%) was coupled with maintaining limited potency in β-adrenergic receptor systems (34% for β_1 and 47% for β_2). In this study, several diverse TMQ derivatives were prepared to probe for binding interactions specific to a particular receptor system. Planar amidine **2**, which was designed to explore the importance of TMQ's chiral center, showed a dramatic loss of potency (<1%) in each receptor system. Likewise, the homologation of a previously described *N*-benzyl derivative (**3**) to the *N*-phenylethyl derivative **4** also showed reduced potency (<3%) in both receptor systems. However, modification of the trimethoxybenzyl group of TMQ to a 4-hydroxy-3-nitrobenzyl group (**7**) provided a unique lead for TMQ derivatives with significant potency in TP receptor systems (91%) and reduced potency in β-adrenergic receptor systems (4% for β_1 and 19% for β_2).

Introduction

Trimetoquinol (TMQ, **1**, Chart 1) is a potent, nonselective, nonclassical β-adrenergic agonist[1] and nonprostanoid thromboxane A_2/prostaglandin H_2 (TP) antagonist.[2] This compound contains a trimethoxybenzyl substituent which produces a chiral center that is unique among β-agonists and nonprostanoid TP antagonists. This chiral center shows a strong stereodependence for binding to β-adrenergic ($S \gg R$)[3,4] and TP ($R \gg S$)[5−8] receptor systems. Our laboratory has reported the synthesis and biological evaluation of both N-alkylated[9−12] and modified trisubstituted benzyl TMQ derivatives.[13,14] Of these compounds, only monoiodo (**5**) and diiodo (**6**) analogs retained potency against U46619-induced platelet aggregation. Monoiodo derivative **5**, however, also retained potency for chronotropic responses (34% for β_1) in guinea pig atria[14] and bronchorelaxant responses (47% for β_2) in guinea pig trachea.[14] Therefore, the objective of this research was to design, synthesize, and evaluate unique TMQ derivatives to probe these receptor systems in an effort to identify novel binding interactions to further separate receptor selectivity. These compounds will provide valuable information for the design of future TMQ derivatives.

Planar amidine derivative (**2**) was prepared to investigate the effect of conformationally restricting the trimethoxybenzyl substituent. The amidine derivative lacks a chiral center and tautomerizes between a dihydroisoquinoline and a stilbene system (Chart 2). The planar amidine derivative was synthesized to provide insight on the need for a chiral center for receptor potency and selectivity.

Chart 1



1 R = H $R_1 = R_2 = R_3 = OCH_3$

3 R = CH_2Ph $R_1 = R_2 = R_3 = OCH_3$

4 R = CH_2CH_2Ph $R_1 = R_2 = R_3 = OCH_3$

5 R = H $R_1 = I$ $R_2 = R_3 = OCH_3$

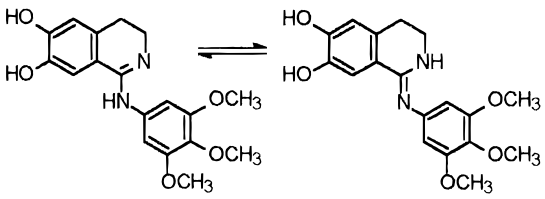
6 R = H $R_1 = R_3 = I$ $R_2 = OCH_3$

7 $R = R_3 = H$ $R_1 = NO_2$ $R_2 = OH$

8 $R = R_1 = R_3 = H$ $R_2 = NO_2$

9 $R = R_1 = R_3 = H$ $R_2 = NH_2$

Chart 2



DIHYDROISOQUINOLINE STILBENE-LIKE

Several N-alkylated TMQ derivatives were also prepared in an effort to separate receptor activity.[9,11] Although many derivatives generally had reduced potency in each system, receptor subtype selectivity in β-adrenergic (β_2 vs β_1 selectivity) and TP (α-subtype vs

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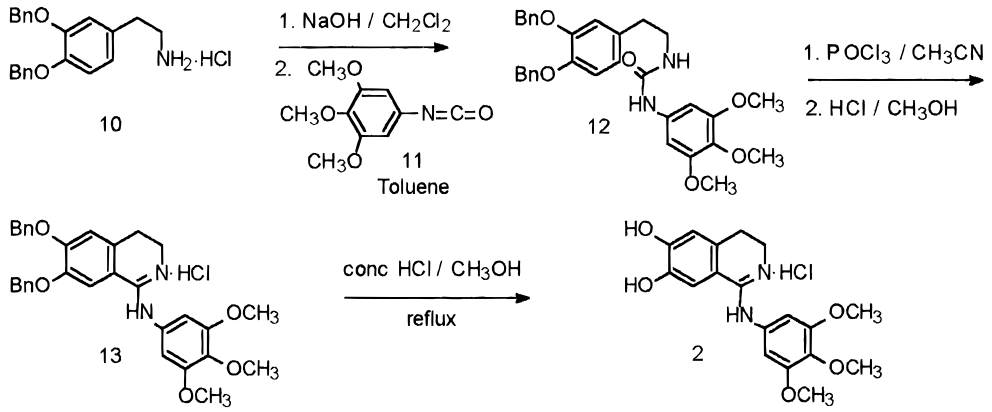
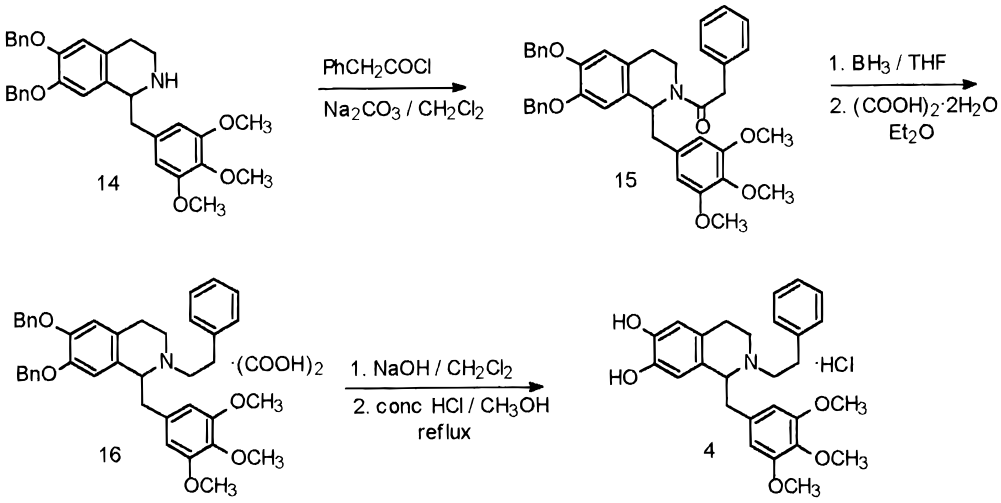
Scheme 1



Scheme 2



τ-subtype) receptor systems was observed.[10,12] In an attempt to restore either β-adrenergic or TP receptor affinity, the N-substituent of *N*-benzyl TMQ (**3**) was elongated to a phenylethyl homolog (**4**). This modification was designed to probe for a potential binding site unique to one of these receptor systems.

The aforementioned trimethoxybenzyl substituent is apparently important for receptor affinity and receptor activation. It is not found in classical β-adrenergic agonists or nonprostanoid TP antagonists and may be significant in identifying important portions of the receptor(s) for enhanced ligand affinity and/or function. Several TMQ analogs have been prepared,[13] but only two iodinated derivatives (**5** and **6**) have similar or enhanced affinity for human β_1- and β_2-adrenergic and/or TP receptors.[15,16] Additional derivatives (**7**−**9**) which differ significantly in their physical parameters have been prepared to probe for unique binding interactions of TMQ analogs with β-adrenergic and/or TP receptors.

Chemistry

Phenylethylamine hydrochloride **10** was converted to its free base, mixed with a toluene solution of isocyanate[17] **11** at room temperature, and subsequently heated to reflux to produce urea **12** (Scheme 1). The urea was cyclized to produce a mixture of compounds which were separated by silica gel chromatography. (Benzyloxy)-amidine **13** was isolated as its hydrochloride salt and converted to catechol **2** with concentrated hydrochloric acid in methanol (1:1).

Bisbenzyloxy-protected TMQ[9] **14** was acylated with phenylacetyl chloride to give amide **15** which was reduced with borane/tetrahydrofuran complex to form amine **16**. The benzyl ethers were cleaved with hydrochloric acid to yield catecholamine **4** (Scheme 2).

The general preparation of compounds **7**−**9** begins with the acylation of the free base of phenylethylamine **10** with the appropriate phenylacetic acid **17** or **18** to form amides **19** and **20** (Scheme 3). Amides **19** and **20** were cyclized, reduced with sodium borohydride, and converted to their hydrochloride salts to form tetrahydroisoquinolines **21** and **22**. The *p*-nitro derivative **22** was reduced in the presence of the benzyloxy ethers by catalytic hydrogenation with Raney nickel[18] to provide *p*-amino derivative **23**. The benzyloxy ethers were removed by heating in concentrated hydrochloric acid in methanol (1:1) to afford catecholamines **7**−**9**.

Biological Results and Discussion

Each of the catecholamine derivatives described above has been evaluated for β-adrenergic and TP receptor activities. β-Adrenergic evaluation was performed in guinea pig atria (β_1) and tracheal (β_2) strips to evaluate increased heart rate and smooth muscle relaxant properties respectively. TP antagonist properties (α-subtype)[19] were determined from the ability of these catecholamines to inhibit agonist U46619-induced platelet activation (aggregation and serotonin secretion) and to inhibit [^{3}H]SQ 29,548 binding to TP receptors. Within each set of experiments, EC_{50} and IC_{50} values

Scheme 3

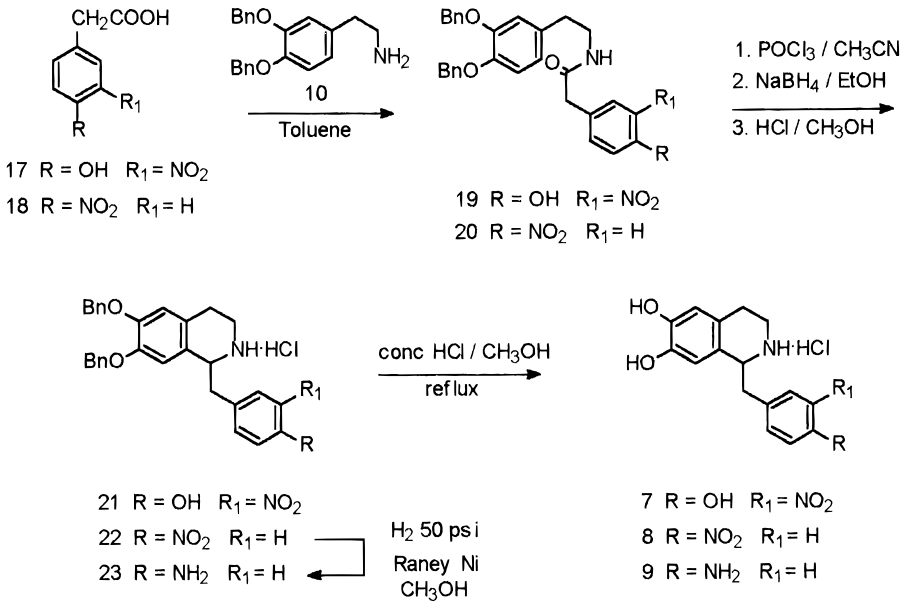


Table 1. Comparative Adrenergic Agonist Activities of Trimetoquinol Analogs on Guinea Pig Right Atrial (β_1) and Tracheal (β_2) Tissues

	pD_2[a]	
compd	β_1 ($n = 3-9$)	β_2 ($n = 4-9$)
1	7.36 ± 0.14	7.26 ± 0.08
2	ND	3.95 ± 0.12
4	4.62 ± 0.36	4.64 ± 0.23
7	5.91 ± 0.31	6.55 ± 0.15
8	7.26 ± 0.26	7.49 ± 0.12
9	6.21 ± 0.04	6.75 ± 0.10

[a] pD_2 = −log EC_{50}. ND = not determined.

Table 2. Comparative Potencies of Trimetoquinol Derivatives for the Inhibition of U46619-Induced (1 μM) Human Platelet Activation (Aggregation and Serotonin Secretion) and [H^3]SQ 29,548 Binding (5 nM) to TP Receptors

	pIC_{50}[a]			
			binding [H^3]SQ 29,548	
compd	platelet aggregation	serotonin secretion	pIC_{50}[a]	pK_i[b]
1	5.94 ± 0.21	5.88 ± 0.13	6.35 ± 0.10	6.76 ± 0.09
2	3.70 ± 0.12	3.69 ± 0.19	3.56 ± 0.03	3.92 ± 0.07
4	4.42 ± 0.12	4.46 ± 0.36	4.38 ± 0.18	4.79 ± 0.18
7	5.90 ± 0.10	5.95 ± 0.19	6.06 ± 0.19	6.47 ± 0.19
8	4.35 ± 0.05	4.22 ± 0.07	4.60 ± 0.15	5.02 ± 0.15
9	3.89 ± 0.04	3.84 ± 0.04	4.05 ± 0.04	4.46 ± 0.04

[a] pIC_{50} = −log IC_{50}. [b] pK_i = −log K_i.

were standardized to TMQ to minimize the variation of experimental results over time. For comparison purposes, the potency and selectivity ratios for new and previously reported compounds[9,14] are listed in Table 3.

Amidine TMQ **2** is considerably less active than TMQ in both β-adrenergic and TP receptor systems. This planar analog is a very weak β_2-agonist with a pD_2 value of 3.95 (Table 1). Since this compound was such a weak β_2-agonist, the β_1 properties were not evaluated. This compound also showed very weak TP antagonism with a pIC_{50} of 3.70 for U46619-induced platelet aggregation and 3.69 for U46619-induced serotonin secretion and a pK_i of 3.92 for competitive binding of [^{3}H]SQ 29,548 to human platelets (Table 2). Thus, the conversion of the chiral center of TMQ (**1**) into a planar amidine provides additional support for the importance of the chiral center for stereoselective interaction of TMQ with the appropriate receptor system.

Homologation of *N*-benzyl TMQ (**3**) to the *N*-phenylethyl derivative **4** further reduced β-adrenergic activity. The *N*-phenylethyl homolog was a weak agonist in both β_1 and β_2 with pD_2 values of 4.62 and 4.64, respectively (Table 1), and had similar or 10-fold loss of β-adrenergic activity when compared to *N*-benzylTMQ (**3**) (Table 3). This homolog was also a weak TP antagonist with a pIC_{50} of 4.42 for U46619-induced platelet aggregation and 4.46 for U46619-induced serotonin secretion and a pK_i of 4.79 for competitive binding of [^{3}H]SQ 29,548 to human platelets (Table 2). *N*-Phenylethyl TMQ (**4**) has similar potency to the previously reported *N*-benzyl derivative **3** in U46619-induced platelet aggregation studies (Table 3) but provides no significant improvement in the overall biological profile when compared directly to TMQ (**1**).

Several 1-benzyl derivatives **7**−**9** were evaluated for their β-adrenergic activity in guinea pig atria and trachea. Modification of the trimethoxybenzyl substituent in TMQ (**1**) to either a mono- or disubstituted benzyl group provided nitrophenol (**7**), nitrophenyl (**8**), and aniline (**9**) derivatives with significant β-adrenergic agonist activity for at least one β-adrenergic receptor (Table 1). In each case, monosubstitution or disubstitution of the 1-benzyl group provided β_2-adrenergic selectivity greater than that of the prior trisubstituted derivatives (Table 3). *p*-Nitrobenzyl derivative **8** was the most potent compound with a pD_2 value of 7.49 for β_2-receptors but was the least selective compound of the new analogs (Table 3). The rank order of potency of increased heart rate (β_1) in comparison to TMQ (**1**) is TMQ (**1**) ≥ nitrophenyl (**8**) > aniline (**9**) ≥ nitrophenol (**7**), while the rank order of potency of tracheal smooth muscle (β_2) relaxation is nitrophenyl (**8**) ≥ TMQ (**1**) > aniline (**9**) ≥ nitrophenol (**7**).

The comparative potencies of modified benzyl derivatives **7**−**9** to inhibit U46619-induced platelet activation and [^{3}H]SQ 29,548 binding to TP receptors is shown in Table 2. The rank order of potency of TP antagonist properties for the inhibition of U46619-induced platelet aggregation and serotonin secretion is nitrophenol (**7**)

Table 3. Comparative Selectivities of Trimetoquinol and Modified Derivatives on Guinea Pig Heart and Trachea and Human Platelet Aggregation

	potency ratio[a]			selectivity ratio[b]		
compd	β_1	β_2	platelet aggregation	β_2/β_1	aggregation/β_1	aggregation/β_2
1	1.00	1.00	1.00	1.00	1.00	1.00
2	ND	4.84×10^{-4}	5.75×10^{-3}	ND	ND	11.9
3	1.74×10^{-3}	2.40×10^{-2}	3.31×10^{-2}	13.8	19.1	1.38
4	1.80×10^{-3}	2.37×10^{-3}	3.02×10^{-2}	1.32	16.8	12.7
5	0.34	0.47	1.12	1.38	3.31	2.40
6	7.07×10^{-2}	3.16×10^{-2}	0.76	0.45	10.7	24.0
7	3.50×10^{-2}	0.19	0.91	5.50	26.0	4.73
8	0.79	1.68	2.57×10^{-2}	2.14	3.3×10^{-2}	1.53×10^{-2}
9	7.00×10^{-2}	0.31	8.91×10^{-3}	4.37	0.13	2.92×10^{-2}

[a] Potency ratio = EC_{50}(TMQ)/EC_{50} (drug). [b] Selectivity ratio = potency ratio(β_2 or aggregation)/potency ratio(β_1 or β_2). ND = not determined.

= TMQ (**1**) ≫ nitrophenyl (**8**) > aniline (**9**), while the rank order of potency of TP antagonist properties for the inhibition of [^{3}H]SQ 29,548 binding is TMQ (**1**) ≥ nitrophenol (**7**) > nitrophenyl (**8**) > aniline (**9**). The modification of the 1-benzyl substituent to the nitrophenol derivative **7** retained significant potency for TP antagonism that is comparable to previously synthesized[14] monoiodo TMQ derivative **5**. The potency of nitrophenol **7** in a TP receptor system with a concurrent loss of activity in β-adrenergic receptor systems suggests that this compound may be a potential new lead compound for optimization of TP antagonist selectivity. This compound's affinity for TP receptors may be due to an ionic interaction between the phenolic hydroxyl group and a complementary cationic group on the TP receptor. The lack of significant potency for aniline **9** reduces the importance of potential hydrogen-bonding interactions of nitrophenol **7** with this receptor.

In summary, several unique TMQ analogs have been prepared to study interactions with β-adrenergic and TP receptor systems. The synthesis and biological evaluation of amidine TMQ **2** supports the results of prior studies[4,6−8] which demonstrate the importance of a chiral center for affinity in each receptor system. Homologation of *N*-benzyl TMQ **3** to *N*-phenylethyl TMQ **4** did not provide additional receptor affinity in either receptor system. Nitrophenol **7**, however, did provide comparable affinity to TMQ (**1**) in the TP receptor system with a concurrent reduction of affinity in the β-adrenergic system. This disubstituted 1-benzyl derivative provides our group with a new lead for optimizing interactions with TP receptors since previously studied trisubstituted 1-benzyl derivatives displayed significant β-adrenergic affinity.

Experimental Section

Melting points are uncorrected and were determined with a Thomas-Hoover melting point apparatus. NMR spectra were obtained at The Ohio State University College of Pharmacy, with either an IBM NR-250 FTNMR or an IBM AF-270 FTNMR spectrometer, and are reported in parts per million relative to tetramethylsilane. Mass spectra were obtained at The Ohio State University College of Pharmacy with a Kratos MS25RFA mass spectrometer or at The Ohio State University Chemical Instrument Center with either a VG 70-250S, Nicolet FTMS-2000, or Finnigan MAT-900 mass spectrometer. Fast atom bombardment mass spectroscopy (FAB MS) utilized 3-nitrobenzyl alcohol as solvent unless otherwise noted. Infrared spectra were obtained at The Ohio State University College of Pharmacy with an Analect RFX-40 FTIR spectrometer. Elemental analyses were performed by Oneida Research Services, Inc. (Whitesboro, NY), or by Galbraith Laboratories, Inc. (Knoxville, TN), within ±0.4% of the theoretical values. Anhydrous tetrahydrofuran was dried and stored over sodium with benzophenone as an indicator. Dry toluene was stored over anhydrous magnesium sulfate and filtered prior to use. Anhydrous toluene was stored over sodium and distilled prior to use. Anhydrous acetonitrile was heated to reflux for at least 3 h with phosphorus pentoxide, distilled, and stored over 4A molecular sieves. Brine was diluted with distilled water to prepare one-fourth strength and one-half strength brine. All other reagents were used as received from commercial suppliers. All work was completed under an inert argon atmosphere whenever possible.

***N*-(2-(3,4-Bis(benzyloxy)phenyl)ethyl)-*N*′-(3,4,5-trimethoxyphenyl)urea (12).** 3,4-Bis(benzyloxy)phenethylamine hydrochloride (**10**) (3.0 g, 8.1 mmol) was converted to its free base with 5% aqueous NaOH (100 mL) and CH_2Cl_2 (100 mL). The organic layer was washed with one-fourth strength brine (100 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The resulting oil was dissolved in dry toluene (75 mL). Isocyanate **11** (1.8 g, 8.6 mmol) was dissolved in dry toluene (25 mL) and added to the amine solution. The resulting mixture was heated to reflux for 30 min. The condenser was removed, and the mixture was concentrated to ca. 50 mL and allowed to cool. The deposited crystals were collected by vacuum filtration to yield 3.05 g (69%) of a white solid, mp 160−162 °C. ^{1}H NMR ($CDCl_3$/TMS): δ 7.44−7.28 (m, 10 H, ArH), 6.85 (d, J = 8.1 Hz, 1H, ArH), 6.80 (d, J = 2.0 Hz, 1H, ArH), 6.68 (dd, J = 8.1, 2.0 Hz, 1H, ArH), 6.48 (s, 2H, ArH), 6.12 (s, 1H, ArNH), 5.11 (s, 4H, 2 x $ArCH_2O$), 4.63 (t, J = 5.8 Hz, 1H, $NHCH_2$), 3.79 (s, 3H, CH_3O), 3.76 (s, 3H, CH_3O), 3.47−3.39 (m, 2H, $NHCH_2$), 2.23 (t, J = 6.7 Hz, 2H, $ArCH_2$). FAB MS: m/z 543.3 (MH^+). Anal. ($C_{32}H_{34}N_2O_6$) C, H, N.

6,7-Bis(benzyloxy)-1-((3,4,5-trimethoxyphenyl)amino)-3,4-dihydroisoquinoline Hydrochloride (13). Urea **12** (500 mg, 0.92 mmol) was dissolved in $POCl_3$ (5 mL) and anhydrous CH_3CN (50 mL) and heated to reflux for 4.5 h. After cooling to room temperature, solvent was removed *in vacuo*. The residue was dissolved in $CHCl_3$ (50 mL), washed with 5% aqueous NaOH (50 mL) and one-half strength brine (50 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The resulting oil was subjected to silica gel chromatography with CH_3OH/benzene/Et_3N (5/95/0.5) as the eluent. Appropriate fractions were pooled and evaporated *in vacuo*. Anhydrous HCl(g) was bubbled into a solution of the residue in $CHCl_3$. The addition of Et_2O resulted in precipitation of the product. The solid was collected and recrystallized from saturated HCl(g)/CH_3OH to yield a white solid, mp 154−155 °C. ^{1}H NMR ($CDCl_3$/TMS): δ 7.96 (s, 1H, $=N^+HR$), 7.51−7.27 (m, 11H, ArH), 6.74 (s, 1H, ArH), 6.22 (s, 2H, ArH), 5.21 (s, 2H, $ArCH_2O$), 5.20 (s, 2H, $ArCH_2O$), 5.02 (bs, 1H, ArNHR), 3.84 (s, 9H, 3 × CH_3O), 3.37 (t, J = 6.3 Hz, 2H, N^+CH_2), 2.85 (t, J = 6.3 Hz, 2H, $ArCH_2$). FAB MS: m/z 525.4 (MH^+ − HCl). Anal. ($C_{32}H_{33}N_2O_5Cl_1 \cdot {}^1/_4H_2O$) C, H, N.

6,7-Dihydroxy-1-((3,4,5-trimethoxyphenyl)amino)-3,4-dihydroisoquinoline Hydrochloride (2). Amidine **13** (500 mg, 0.89 mmol) was dissolved in a mixture of CH_3OH (20 mL) and concentrated HCl (20 mL). The mixture was heated to reflux for 5 h, cooled to room temperature, and evaporated *in vacuo*. The residue was rinsed with IpOH (20 mL) and

evaporated *in vacuo*. The resulting oil was recrystallized from CH_3OH/Et_2O to yield 300 mg (89%) of an off-white solid, dp 169−171 °C (with darkening). ^{1}H NMR (D_2O): δ 7.28 (s, 1H, ArH), 6.76 (s, 1H, ArH), 6.63 (s, 2H, ArH), 3.70 (s, 6H, 2 × CH_3O), 3.67 (s, 3H, CH_3O), 3.33 (t, J = 6.7 Hz, 2H, N^+CH_2), 2.74 (t, J = 6.7 Hz, 2H, $ArCH_2$). FAB MS: m/z 345.1 (MH^+ − HCl). Anal. ($C_{18}H_{21}N_2O_5Cl_1 \cdot H_2O$) C, H, N.

6,7-Bis(benzyloxy)-2-(phenylacetyl)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (15). Amine oxalate **14** (500 mg, 0.81 mmol) was converted to its free base with 10% aqueous NaOH (50 mL) and CH_2Cl_2 (50 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic extracts were combined, washed with 10% aqueous NaOH (100 mL) and one-fourth strength brine (100 mL), and dried over anhydrous $MgSO_4$. The organic solution was charged with anhydrous $MgSO_4$ (5.0 g) and anhydrous Na_2CO_3 (5.0 g). The suspension was vigorously stirred for 15 min before a solution of phenylacetyl chloride (0.2 mL, 1.51 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 5 min at room temperature. The suspension was stirred for 30 min and filtered. The organic layer was washed successively with 1.2 N HCl (100 mL), one-fourth strength brine (100 mL), 5% aqueous NaOH (2 × 100 mL), and one-fourth strength brine (100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo*. The clear white oil was dissolved in a minimal amount of hot CH_3OH. After cooling to room temperature, the flask was placed in the freezer. The deposited solid was collected after 72 h to yield 374 mg (72%) of a white solid, mp 122−123 °C. The compound is a mixture of two conformations in solution providing a complex ^{1}H NMR spectrum. Temperature elevation studies in $CDCl_3$ to 323 K and DMSO-d_6 to 357 K did not simplify the ^{1}H NMR spectrum in order to assign proton resonances. FAB MS: m/z 644.3 (MH^+). Anal. ($C_{41}H_{41}N_1O_6$) C, H, N.

6,7-Bis(benzyloxy)-2-(2-phenylethyl)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Oxalate Salt (16). A solution of BH_3/THF complex (5.0 mL, 5.0 mmol) was added to a solution of amide **15** (900 mg, 1.40 mmol) in anhydrous THF (25 mL). The resulting solution was heated to reflux for 2.5 h and subsequently cooled to room temperature. Excess reagent was quenched by the addition of EtOH (5 mL), and solvent was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2 (50 mL) and 5% aqueous NaOH (100 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (50 mL). The organic extracts were combined, successively washed with 5% aqueous NaOH (100 mL), and one-fourth strength brine (2 × 100 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The resulting oil was dissolved in Et_2O (25 mL) and added dropwise to a solution of oxalic acid dihydrate (197 mg, 1.40 mmol) in Et_2O (25 mL). After standing overnight, the precipitate was collected to yield 845 mg (84%) of a white solid, mp 214−216 °C. ^{1}H NMR (free base, $CDCl_3$/TMS): δ 7.42−7.15 (m, 15H, ArH), 6.66 (s, 1H, ArH), 6.29 (s, 2H, ArH), 6.19 (s, 1H, ArH), 5.10 (s, 2H, $ArCH_2O$), 4.87 (ABq, J = 13.7 Hz, $\Delta\upsilon$ = 20.5 Hz, 2H, $ArCH_2O$), 3.82 (s, 3H, CH_3O), 3.77 (s, 6H, 2 × CH_3O), 3.25−3.15 (m, 1H, ArCHN), 3.09−2.66 (m, 8H, 4 × CH_2), 2.52−2.43 (m, 2H, CH_2). FAB MS: m/z 630.3 ($MH^+ - C_2H_2O_4$). Anal. ($C_{43}H_{45}N_1O_9$) C, H, N.

6,7-Dihydroxy-2-(2-phenylethyl)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (4). Amine **16** (200 mg, 0.28 mmol) was converted to its free base with 5% aqueous NaOH (50 mL) and CH_2Cl_2 (50 mL). The layers were separated, and the organic layer was washed with one-fourth strength brine (50 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was dissolved in a mixture of CH_3OH (10 mL) and concentrated HCl (10 mL) and subsequently heated to reflux for 24 h. After cooling to room temperature, solvent was evaporated *in vacuo*. The resulting oil was rinsed three times with EtOH (10 mL) and evaporated *in vacuo*. The resulting oil was recrystallized from $EtOH/Et_2O$ to yield 91 mg (67%) of a light brown solid, dp 118−120 °C (with darkening), which was subjected to silica gel chromatography with $CH_3OH/Et_2O/NH_4OH/CHCl_3$ (10/10/1/79) as the eluent. The pooled fractions were acidified with 1.2 N HCl, evaporated *in vacuo*, rinsed with EtOH (10 mL), filtered, and evaporated *in vacuo*. Recrystallization from minimal hot absolute $EtOH/Et_2O$ yielded 55 mg (60%) of a tan solid, dp 122−125 °C (with darkening). ^{1}H NMR (D_2O): δ 7.20−7.08 (m, 5H, ArH), 6.64 (s, 1H, ArH), 6.24 (s, 2H, ArH), 5.87 (s, 1H, ArH), 4.40−4.38 (m, 1H, ArCHN), 3.60 (s, 9H, 3 × CH_3O), 3.38−3.31 (m, 4H, 2 × CH_2), 3.30−2.92 (m, 6H, 3 × CH_2). FAB MS: m/z 450.2 (MH^+ − HCl). Anal. ($C_{27}H_{32}N_1O_5Cl_1 \cdot 2H_2O$) C, H, N.

N-((3,4-Bis(benzyloxy)phenyl)ethyl)(4-hydroxy-3-nitrophenyl)acetamide (19). 3,4-Bis(benzyloxy)phenethylamine hydrochloride (**10**) (10.0 g, 27.0 mmol) was converted to its free base with 5% aqueous NaOH (100 mL) and CH_2Cl_2 (100 mL). The layers were separated, and the organic layer was washed with brine (100 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The light brown oil was dissolved in toluene (125 mL) and charged with 4-hydroxy-3-nitrophenylacetic acid **17** (5.86 g, 29.7 mmol). The suspension was heated to reflux for 72 h with azeotropic removal of water by a Dean−Stark trap. After cooling to room temperature, solvent was removed *in vacuo*. The residue was dissolved in dichloromethane (100 mL), washed with a solution of NH_4HCO_3 (5 g/100 mL), saturated NH_4Cl (100 mL), and brine (100 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The product was recrystallized in two crops from a minimal amount of hot toluene to yield 9.3 g (67%) of a bright yellow solid, mp 135−137 °C. ^{1}H NMR ($CDCl_3$): δ 10.51 (bs, 1H, OH), 7.91 (d, J = 2.2 Hz, 1H, ArH ortho to NO_2), 7.48−7.29 (m, 11H, 10 × ArH, ArH para to NO_2), 7.08 (d, J = 8.7 Hz, 1H, ArH meta to NO_2), 6.82 (d, J = 8.2 Hz, 1H, ArH), 6.71 (d, J = 2.0 Hz, 1H, ArH), 6.58 (dd, J = 8.2, 2.0 Hz, 1H, ArH), 5.33 (bt, 1H, NH), 5.13 (s, 2H, $ArCH_2O$), 5.12 (s, 2H, $ArCH_2O$), 3.47−3.39 (m, 2H, NCH_2), 3.39 (s, 2H, $ArCH_2CO$), 2.67 (t, J = 6.7 Hz, 2H, $ArCH_2$). FAB MS: m/z 513.4 (MH^+). Anal. ($C_{30}H_{28}N_2O_6$) C, H, N.

6,7-Bis(benzyloxy)-1-(4-hydroxy-3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (21). Amide **19** (3.3 g, 6.4 mmol) was suspended in anhydrous CH_3CN (30 mL), charged with $POCl_3$ (15 mL), and heated to reflux for 3.5 h. After cooling to room temperature, solvent was removed *in vacuo*. The residue was rinsed with anhydrous CH_3CN (30 mL) and evaporated *in vacuo*. The dark green oil was suspended in EtOH (40 mL) and cooled to 0 °C. $NaBH_4$ (1.21 g, 32 mmol) was added portionwise to avoid excessive foaming. The suspension was stirred overnight with warming to room temperature. Solvent was evaporated *in vacuo*, and the residue was dissolved in a mixture of EtOAc (100 mL) and 5% NH_4OH (100 mL). The layers were separated, and the organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue was dissolved in CH_3OH (30 mL) and charged with 3 N HCl/CH_3OH. After stirring at room temperature overnight, the solid was collected by vacuum filtration to yield 2.21 g (64%) of an off-white solid, mp 204−207 °C. ^{1}H NMR (CD_3OD/acetone): δ 8.03 (d, J = 2.0 Hz, 1H, ArH ortho to NO_2), 7.57 (dd, J = 8.6, 2.0 Hz, 1H, ArH para to NO_2), 7.44−7.24 (m, 10H, ArH), 7.14 (d, J = 8.6 Hz, ArH meta to NO_2), 6.93 (s, 1H, ArH), 6.79 (s, 1H, ArH), 5.11 (s, 2H, $ArCH_2O$), 4.97 (s, 2H, $ArCH_2O$), 4.96 (t, J = 6.0 Hz, 1H, ArCHRN), 3.62−3.34 (m, 4H, CH_2), 3.08−3.03 (m, 2H, CH_2). EI MS: m/z 496.3205 (M^+). Anal. ($C_{30}H_{29}N_2O_5Cl_1 \cdot {}^1/_2H_2O$) C, H, N.

6,7-Dihydroxy-1-(4-hydroxy-3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (7). Isoquinoline **21** (200 mg, 0.37 mmol) was dissolved in a mixture of concentrated HCl (4 mL) and CH_3OH (4 mL) and heated to reflux overnight. After cooling to room temperature, solvent was evaporated *in vacuo*. The residue was rinsed twice with CH_3OH (10 mL) and evaporated *in vacuo*. The resulting solid was dissolved in excess EtOH, filtered, and concentrated by evaporation on a hot plate to about 5 mL. Et_2O was added until the mixture became cloudy. The deposited solid was collected by vacuum filtration, rinsed with Et_2O, and dried under vacuum at 56 °C for 36 h to yield 106 mg (82%) of a rust-colored solid, dp 253−255 °C (with bubbling). ^{1}H NMR (CD_3OD): δ 8.05 (d, J = 2.1 Hz, 1H, ArH ortho to NO_2), 7.56 (dd, J = 8.6 and 2.1 Hz, 1H, ArH para to NO_2), 7.18 (d, J = 8.6 Hz, 1H, ArH meta to NO_2),

6.63 (s, 1H, ArH), 6.54 (s, 1H, ArH), 4.66 (t, J = 7.2 Hz, 1H, ArCHRN), 3.51−3.41 (m, 2H, CH_2), 3.31−3.23 (m, 1H, CH_2), 3.14−3.05 (m, 1H, CH_2), 3.01−2.92 (m, 2H, CH_2). FAB MS: m/z 317.1 (MH^+ − HCl). Anal. ($C_{16}H_{17}N_2O_5Cl_1$) C, H, N.

***N*-((3,4-Bis(benzyloxy)phenyl)ethyl)(4-nitrophenyl)-acetamide (20).** 3,4-Bis(benzyloxy)phenethylamine hydrochloride (**10**) (10.0 g, 27.0 mmol) was converted to its free base with 5% aqueous NaOH (250 mL) and CH_2Cl_2 (250 mL). The layers were separated, and the organic layer was washed with brine (250 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The light brown oil was dissolved in toluene (150 mL) and charged with 4-nitrophenylacetic acid (**18**) (7.2 g, 39.8 mmol). The suspension was heated to reflux for 72 h with azeotropic removal of water by a Dean−Stark trap. After cooling to room temperature, solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 (250 mL), washed with saturated $NaHCO_3$ (2 × 125 mL) and brine (100 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The product was recrystallized in two crops from a minimal amount of hot toluene to yield 10.06 g (75%) of a pale yellow solid, mp 132−133.5 °C. ^{1}H NMR ($CDCl_3$): δ 8.13 (AA′XX′, J = 6.8, 2.0 Hz, ArH ortho to NO_2), 7.48−7.27 (m, 12H, ArH, meta to NO_2), 6.80 (d, J = 8.1 Hz, 1H, ArH), 6.72 (d, J = 2.0 Hz, 1H, ArH), 6.53 (dd, J = 8.1, 2.0 Hz, 1H, ArH), 5.32 (bt, 1H, NH), 5.14 (s, 2H, $ArCH_2O$), 5.12 (s, 2H, $ArCH_2O$), 3.51 (s, 2H, $ArCH_2CO$), 3.44 (m, 2H, $NHCH_2$), 2.66 (t, 2H, $ArCH_2CH_2$). EI MS: m/z 496 (M^+). Anal. ($C_{30}H_{28}N_2O_5$) C, H, N.

6,7-Bis(benzyloxy)-1-(4-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (22). To a suspension of amide **20** (5.0 g, 10.0 mmol) in anhydrous CH_3CN (20 mL) at room temperature was added $POCl_3$ (5 mL), and the resulting solution was heated to reflux for 4.5 h. The solution was cooled to room temperature, and solvent was removed *in vacuo*. The oily residue was dissolved in $CHCl_3$ (75 mL) and diluted with an ice slurry (40 mL). The biphasic solution was vigorously stirred while a 5% solution of NaOH was added dropwise until the aqueous layer was basic to pH paper. The layers were separated, and the organic layer was washed with 5% aqueous NaOH (50 mL) and brine (30 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was suspended in EtOH (50 mL) and cooled to 0 °C. $NaBH_4$ (0.95 g, 25 mmol) was added portionwise with vigorous stirring over 10 min to prevent excessive foaming. The mixture was allowed to warm to room temperature with stirring overnight. Solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (100 mL) and water (100 mL). The organic layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was dissolved in CH_3OH (30 mL) and stirred overnight with a solution of saturated HCl(g)/CH_3OH (10 mL). Solvent was removed *in vacuo*. The residue was dissolved in hot EtOH, filtered, and concentrated by evaporation on a hot plate. After cooling, the deposited product was collected by vacuum filtration and washed with Et_2O to yield 2.37 g (46%) of a light yellow solid, mp 213−214.5 °C. The mother liquor was evaporated and a second crop of crystals was produced from hot EtOH to yield an additional 1.36 g (total yield 72%) of a pale yellow solid, mp 210−213 °C. ^{1}H NMR (CD_3OD): δ 8.23 (d, J = 8.7 Hz, 2H, ArH ortho to NO_2), 7.52 (d, J = 8.7 Hz, 2H, ArH meta to NO_2), 7.46−7.26 (m, 10H, ArH), 6.91 (s, 1H, ArH), 6.61 (s, 1H, ArH), 5.14 (s, 2H, $ArCH_2O$), 4.95 (s, 2H, $ArCH_2O$), 4.80 (t, J = 7.2 Hz, 1H, ArCHRN), 3.58−3.49 (m, 2H, 4-NO_2ArCH_2), 3.39−3.22 (m, 2H, NCH_2), 3.06−2.98 (m, 2H, $ArCH_2CH_2$). FAB MS: m/z 481.4 (MH^+ − HCl). Anal. ($C_{30}H_{29}N_2O_4Cl_1 \cdot H_2O$) C, H, N.

6,7-Dihydroxy-1-(4-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8). Amine **22** (500 mg, 0.97 mmol) was dissolved in a mixture of concentrated HCl (4 mL) and CH_3OH (4 mL) and heated to reflux overnight. After cooling to room temperature, solvent was evaporated *in vacuo*. The residue was rinsed twice with CH_3OH (10 mL) and evaporated *in vacuo*. The resulting solid was recrystallized from EtOH/Et_2O to yield 264 mg (81%) of a golden solid, mp 178−179 °C. ^{1}H NMR (CD_3OD) : δ 8.26 (d, J = 8.7 Hz, 2H, ArH ortho to NO_2), 7.56 (d, J = 8.7 Hz, 2H, ArH meta to NO_2), 6.64 (s, 1H, ArH), 6.44 (s, 1H, ArH), 4.74 (t, J = 7.4 Hz, 1H, ArCHRN), 3.60−3.47 (m, 2H, NO_2ArCH_2), 3.34−3.21 (m, 2H, CH_2N), 3.02−2.94 (m, 2H, $ArCH_2$). FAB MS: m/z 301.2 (MH^+ − HCl). Anal. ($C_{16}H_{17}N_2O_4Cl_1 \cdot {}^1/_2H_2O$) C, H, N.

1-(4-Aminobenzyl)-6,7-bis(benzyloxy)-1,2,3,4-tetrahydroisoquinoline Dihydrochloride (23). *p*-Nitrobenzyl derivative **22** (500 mg, 0.97 mmol) was dissolved in CH_3OH (20 mL) in a Parr bottle. The solution was charged with a slurry of Raney nickel (0.25 mL) and hydrogenated at 50 psi for 2 h. The mixture was double filtered and evaporated *in vacuo*. The oily residue was dissolved in CH_3OH (5 mL) and charged with a solution of 3 N HCl/CH_3OH (1 mL). After stirring for 4 h, solvent was evaporated *in vacuo*. The solid residue was dissolved in minimal hot CH_3OH and diluted with Et_2O until the mixture became cloudy. The solid was collected by vacuum filtration to yield 408 mg (81%) of an off-white solid, mp 211−213 °C. ^{1}H NMR (CD_3OD): δ 7.48−7.25 (m, 14H, ArH), 6.91 (s, 1H, ArH), 6.65 (s, 1H, ArH), 5.13 (s, 2H, $ArCH_2O$), 4.97 (s, 2H, $ArCH_2O$), 4.75 (t, J = 7.5 Hz, 1H, ArCHRN), 3.54−3.16 (m, 4H, 2 × CH_2), 3.06−2.98 (m, 2H, CH_2). FAB MS: m/z 451.4 (MH^+ − 2HCl). Anal. ($C_{30}H_{32}N_2O_2Cl_2 \cdot {}^1/_4H_2O$) C, H, N.

1-(4-Aminobenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Dihydrochloride (9). Amine **23** (200 mg, 0.38 mmol) was dissolved in a mixture of concentrated HCl (4 mL) and CH_3OH (4 mL) and heated to reflux overnight. After cooling to room temperature, solvent was evaporated *in vacuo*. The residue was rinsed twice with CH_3OH (10 mL) and evaporated *in vacuo*. The resulting solid was recrystallized from EtOH/Et_2O. The deposited solid was collected by vacuum filtration, rinsed with Et_2O, and dried under vacuum for 36 h at 56 °C to yield 88 mg (66%) of a tan solid, mp 260−262 °C. ^{1}H NMR (CD_3OD): δ 7.52−7.40 (AA′BB′, 4H, ArH ortho and meta to NH_2) 6.63 (s, 1H, ArH), 6.47 (s, 1H, ArH), 4.70 (t, J = 7.3 Hz, 1H, ArCHRN), 3.53−3.43 (m, 2H, CH_2), 3.33−3.28 (m, 1H, CH_2), 3.26−3.17 (m, 1H, CH_2), 3.02−2.93 (m, 2H, CH_2). FAB MS: m/z 271.1 (MH^+ − 2HCl). Anal. ($C_{16}H_{20}N_2O_2Cl_2 \cdot {}^1/_2H_2O$) C, H, N.

***β*-Adrenergic Receptor Studies.** Male albino Hartley guinea pigs weighing 300−400 g were used. Isolation of tissues and preparation of spontaneously beating right atria and carbachol (0.3 μM)-contracted tracheal strips were done as described previously.[10,12] Cumulative concentration−response curves to each drug were determined. Each successive drug addition was added only after the effects of the previous concentration had reached a maximal response, and the results are expressed relative to the maximal response to 10^{-5} M isoproterenol added after the completion of each concentration−response curve.

Platelet Inhibition Studies. Blood was collected from normal human volunteers who reported to be free of medication for at least 10 days. Platelet-rich plasma (PRP) was prepared and used for studies for inhibition of aggregation and serotonin secretion by drugs in the presence of U46619, a TP agonist as described previously in our laboratory.[10,11] Platelet aggregation experiments were performed according to a turbidometric method in dual-channel aggregometers interfaced to computers for the acquisition, quantification, presentation, and management of aggregation data.[20] [^{14}C]Serotonin (0.03 μCi/mL) was added to PRP for 20 min prior to the start of the experiment to allow for uptake of label into dense granules. U46619 was used at the minimum concentration required to stimulate maximal aggregation. Aspirin (1 mM) was added to PRP to block the formation of endogenous prostaglandins. After a 1 min incubation of PRP samples with aspirin and drug, U46619 (0.3−1 μM) was added and the aggregation response monitored for 4 min. Samples were centrifuged, and a 0.1 mL aliquot of the supernatant was mixed into an emulsion-type scintillation cocktail for liquid scintillation spectrometry. Background counting rates (BKG cpm) were determined from the supernatants of unstimulated samples and was less than 10% of that released into the supernatants by U46619 alone. The percent secretion of serotonin was calculated by the following formula: percent serotonin secretion = [(sample cpm − BKG cpm)/(total cpm − BKG cpm)] × 100%, where cpm = counts per min and total cpm = amount of radioactivity in an uncentrifuged sample.

Radioligand Binding Studies with Thromboxane A_2/Prostaglandin H_2 Receptor Sites in Human Platelets. For

binding experiments, human PRP was centrifuged and platelets were resuspended in 50 mM Tris saline buffer, pH 7.2.[16] Platelets (1×10^8) were incubated with 5 nM [^{3}H]SQ 29,548 in a final volume of 0.5 mL as modified from Hedberg *et al*.[21] Unlabeled SQ 29,548 (50 μM) was used to determine nonspecific binding. Varying concentrations of each drug were used to quantify the inhibition of specific [^{3}H]SQ 29,548 binding. Samples were incubated for 30 min at room temperature, and rapidly filtered by vacuum through Whatman GF/B glass fiber filters on a Brandel cell harvester, and washed for 10 s with ice cold Tris saline buffer. Filters were placed in plastic scintillation vials containing 10 mL of an emulsion-type scintillation mixture, and radioactivity was measured by liquid scintillation spectrometry. Specific binding to human platelets varied between 88% and 95%.

Data Analysis. Effective concentration-50 (EC_{50}) and inhibitory concentration-50 (IC_{50}) values of each drug were determined graphically from individual plots of percent response versus log drug concentration on β-adrenoceptors (guinea pig atria and trachea) and human platelets, respectively. Individual drug responses were expressed as pD_2 ($-\log EC_{50}$) and pIC_{50} ($-\log IC_{50}$) values. Dissociation constants (K_i) for competing drugs in the displacement of specific SQ 29,548 binding were calculated using the equation: $K_i = IC_{50}/(1 + [L]/K_L)$, where [L] = the radioligand concentration (5 nM), K_L = the affinity constant of the radioligand (3.1 nM), and $pK_i = -\log K_i$ values.[16]

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