# Synthesis and Human β-Adrenoceptor Activity of 1-(3,5-Diiodo-4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol Derivatives in Vitro

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Trimetoquinol (**1**, TMQ) is a potent nonselective β-adrenergic receptor (AR) agonist and a thromboxane $A_2$/prostaglandin endoperoxide (TP) receptor antagonist, while 3′,5′-diiodo-TMQ (**2**) exhibits $\beta_3$-AR selectivity. In search of selective $\beta_3$-AR agonists as potential drugs for the treatment of human obesity and type II diabetes mellitus, a series of 1-(3,5-diiodo-4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ols has been prepared and evaluated for their biological activities at human $\beta_1$-, $\beta_2$-, and $\beta_3$-ARs expressed in Chinese hamster ovary (CHO) cells. The compounds have been synthesized by the Bischler−Napieralski cyclization of corresponding amides followed by $NaBH_4$ reduction, and the halogens in the aromatic ring A were introduced by direct halogenation of protected compound **11**. Whereas halogen substitution in ring A reduced either potency or intrinsic activity on $\beta_3$-AR, the non-halogen-substituted compounds **8** and **10** were potent, selective, nearly full agonists for $\beta_3$-AR.

## Introduction

Obesity results from a chronic imbalance between energy intake from ingestion of food and energy expenditure by the body.[1] The $\beta_3$-adrenergic receptor ($\beta_3$-AR) plays a major role in mediating adipocyte lipolysis (breakdown of fat) in white adipocyte tissue (WAT) and thermogenesis in brown adipocyte tissue (BAT).[2−6] Thermogenesis in BAT is initiated by the sympathetic release of noradrenaline from sympathetic nerve endings or treatment with $\beta_3$-AR agonists which act predominantly via $\beta_3$-ARs, to cause an activation of adenylyl cyclase which, by increasing the concentration of cAMP, regulates protein kinase A (PKA) and protein phosphorylation. Active PKA stimulates a hormone-sensitive lipase thereby releasing necessary free fatty acids (FFAs) from the triglyceride stores in BAT and WAT and phosphorylates cAMP response element binding protein (CREB) to increase uncoupling protein (UCP)[7] gene transcription.[8,9] The FFAs act not only as substrates for β-oxidation but also to overcome the restraint on respiration exerted by purine nucleotides, such as GDP, binding to UCP. Activation of UCP leads to dissipation of the hydrogen gradient across the inner mitochondial membrane and conversion of the gradient energy to heat as a byproduct rather than ATP, thereby uncoupling ATP synthesis from respiration.[10,11]

The identification of the $\beta_3$-AR[2,12] in 1983 led to intense interest in developing selective $\beta_3$-AR agonists[13,14] for the treatment of various metabolic diseases, such as obesity and non-insulin-dependent diabetes mellitus (NIDDM).[15,16] Most of the previously developed $\beta_3$-AR compounds have suffered from one or more unacceptable pharmacokinetic or pharmacodynamic problems, including lack of β-AR selectivity, tissue specificity, full agonist activity, drug toxicity, and a short plasma half-life.[17−19] Continuing to search for potent and selective $\beta_3$-AR agonists as potential drugs for the treatment of obesity and NIDDM is a desirable therapeutic goal.

Our aim is to develop a compound of the tetrahydroisoquinoline class which possesses a high degree of potency and selectivity for $\beta_3$-AR and possesses minimal side effects associated with an activation of the $\beta_1$- and $\beta_2$-ARs. The lead compound, trimetoquinol (TMQ, **1**; Figure 1) is a potent nonselective β-AR agonist clinically used in Japan as a bronchorelaxant.[20−23] 3′,5′-Diiodo-TMQ (**2**; Figure 1) retains potent activity on β-AR subtypes[24] and exhibits selectivity for human $\beta_3$-AR versus $\beta_1$- and $\beta_2$-ARs.[25] To improve the stability of diiodo-TMQ, we focused on the modification of the catechol moiety. The catechol portion is easily oxidized in air, and the catechol moiety of these tetrahydroisoquinolines is also enzymatically unstable, being metabolized by catechol *O*-methyltransferase (COMT). The present study examines the structural requirements of the aromatic A ring of diiodo-TMQ for optimum activity at the $\beta_3$-AR and investigates the effects of halogen substituents at the 5- and/or 7-position of the 6-monophenolic diiodo-TMQ system on β-AR subtypes. Compound **10** and its halogen derivatives were designed, synthesized, and evaluated for their functional activities on human β-AR subtypes. Non-catechol compound **8** (no substituent at aromatic A ring of tetrahydroisoquinoline system) was also synthesized and examined on β-AR subtypes.

## Chemistry

The synthesis of compounds **8** and **10** is outlined in Scheme 1. The starting materials (phenethylamines **3**

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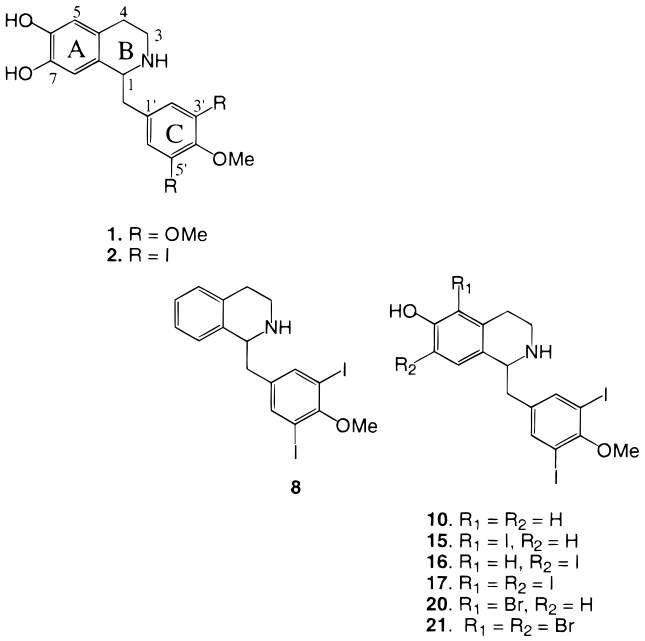



**Figure 1.** Molecular structures of trimetoquinol (**1**, TMQ), diiodo-TMQ (**2**), and compounds **8**, **10**, **15**−**17**, **20**, and **21**.

and **4**) required for compounds **6** and **7**, respectively, are commercially available, while 3,5-diiodo-4-methoxyphenylacetyl chloride **(5)** was synthesized in four steps from *p*-hydroxyphenylacetic acid.[26] The phenethylamine **3** or **4** was condensed with 3,5-diiodo-4-methoxyphenylacetyl chloride **(5)** to afford the corresponding phenylacetamides **6** and **7**, respectively. Bischler−Napieralski[27] cyclization of phenylacetamide (**6** and **7**) with phosphorus oxychloride in acetonitrile resulted in the dihydroisoquinoline, which was reduced with sodium borohydride in methanol without purification to afford compound **8** and the benzyloxy-protected tetrahydroisoquinoline **9**, respectively. Deprotection of **9** with a refluxing equivolume mixture of concentrated hydrochloride acid and methanol gave compound **10**.

Preparation of iodinated compounds **15**−**17** is outlined in Scheme 2. The synthesis first involved the protection of secondary amine in tetrahydroisoquinoline **10** with trifluoroacetic anhydride (TFAA) to provide compound **11**. After the protection, compound **11** was directly iodinated with benzyltrimethylammonium dichloroiodate ($BTMACl_2I$)[28,29] to afford the iodinated derivatives which were separated by silica gel chromatography using hexanes−ethyl acetate (8:1 to 6:1) as eluent to give compounds **12** (69%), **13** (10%), and **14** (12%). Cleavage of the TFA protecting group in compounds **12**−**14** with potassium carbonate and then making HCl salts gave the desired iodinated compounds **15**−**17**, respectively. The TFA protecting group in compound **11** is necessary; otherwise, direct iodination with $BTMACl_2I$ will form oxidation products instead of the desired iodinated derivatives (data not presented).

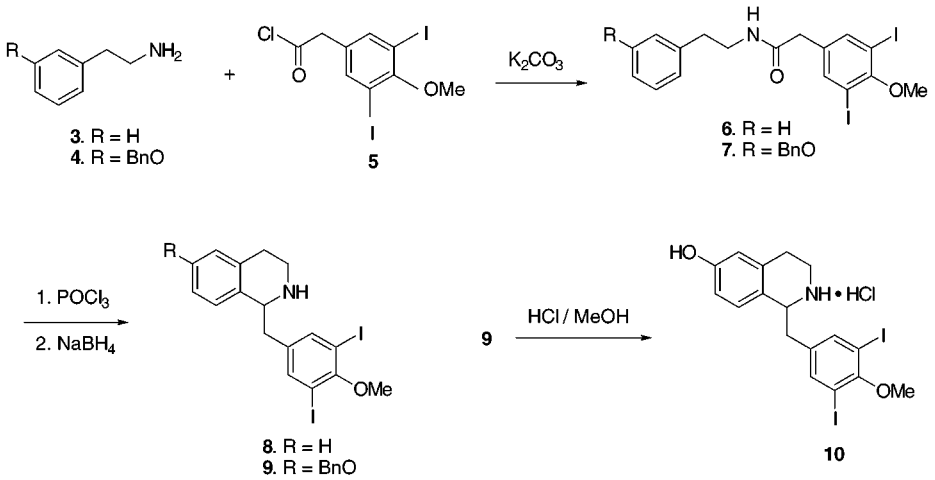
The synthesis of compounds **20** and **21** is outlined in Scheme 3. Direct bromination of protected compound **11** with pyridium tribromide ($PyHBr_3$)[30,31] provided the brominated derivatives which were separated by silica gel chromatography using hexanes−chloroform (2:1 to 1:2) as eluent to afford compounds **18** (76%) and **19** (16%). Deprotection of compounds **18** and **19** with potassium carbonate and making the oxalic acid salts gave the desired brominated compounds **20** and **21**, respectively. We also conducted the alternative route for the synthesis of compound **19** from **11**. Direct bromination of compound **11** with bromine in glacial acetic acid formed compound **19** in nearly quantitative (99%) yield.

## Biological Results and Discussion

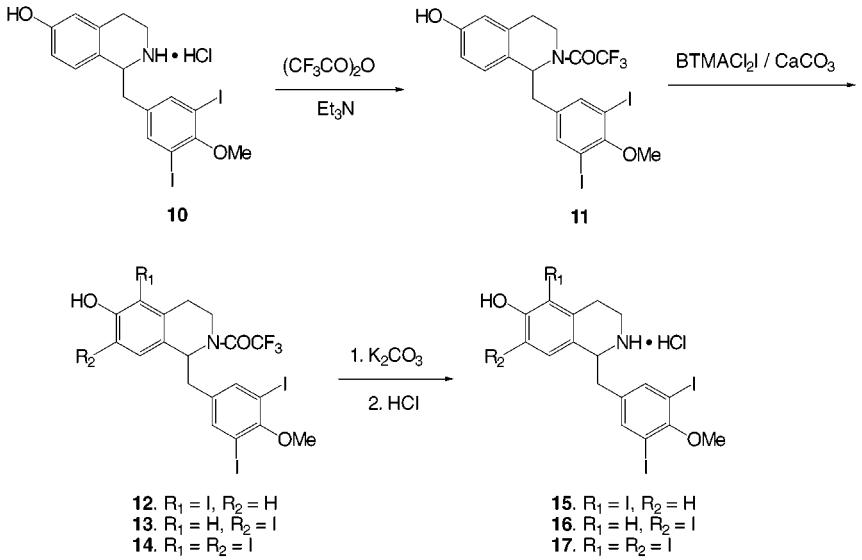
Chinese hamster ovary (CHO) cells expressing human $\beta_1$-, $\beta_2$-, and $\beta_3$-ARs and cAMP response element (CRE)−luciferase (LUC) receptor gene (CRE−LUC) assay (see Experimental Section) were employed to evaluate the receptor functional activities of tested compounds. Figure 2 shows the functional activities of compounds **8** (panel A) and **10** (panel B) on human $\beta_1$-, $\beta_2$-, and $\beta_3$-ARs.

We know that the catechol moiety of TMQ is important for the receptor functional activities on both human $\beta_1$- and $\beta_2$-ARs[32] but is not indispensable for the activity on human $\beta_3$-AR.[25] By removal of the catechol moiety of diiodo-TMQ (**2**), compound **8** shows a significant partial agonist activity on human $\beta_3$-AR in both CRE−LUC and cAMP−RIA assays and shows low intrinsic activities on human $\beta_1$- and $\beta_2$-ARs. Compound **10** which bears only a hydroxyl group at 6-position, lacking the catechol moiety of diiodo-TMQ (**2**), shows full agonist activity on human $\beta_3$-AR and exhibits moderate selec-

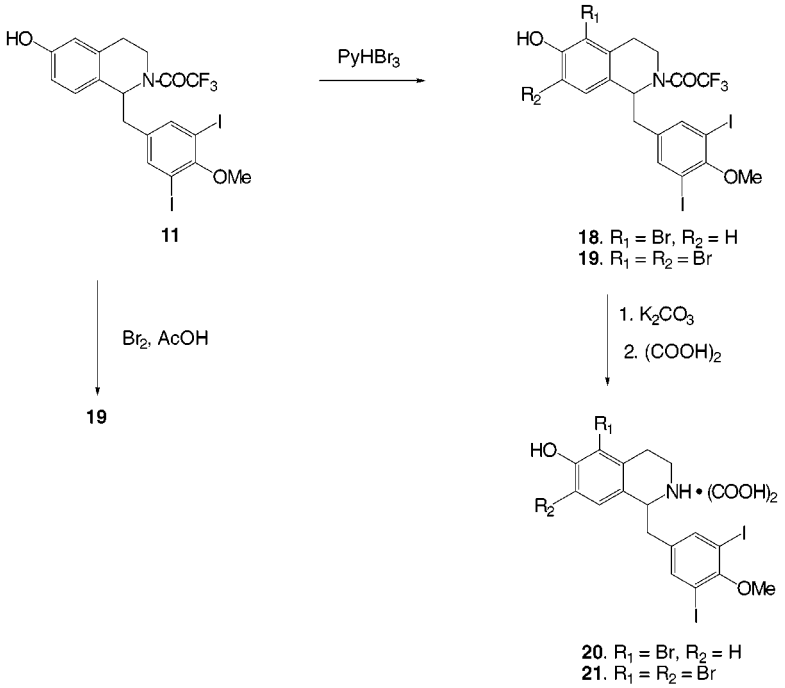
**Scheme 1**



**Scheme 2**




**Scheme 3**




tivity for receptor functional activity of human $\beta_3$-AR versus human $\beta_1$- and $\beta_2$-ARs (Figure 2, Table 1). These results are consistent with our previous findings[25,32] and also indicate that $\beta_3$-AR agonists and new lead compounds can be designed and synthesized by the modifications of the aromatic A ring of diiodo-TMQ.

The biological results in Table 1 show that most of the halogen substituents made on the aromatic A ring of compound **10** decreased human $\beta_2$- and $\beta_3$-AR functional activities in the cAMP−RIA assay, thus providing very interesting findings. By introducing an iodo group at the 5-position of compound **10**, compound **15** increases receptor functional activity on human $\beta_2$-AR and abolishes human $\beta_3$-AR functional activity. Compound **20** bearing a bromine atom at the 5-position decreases the receptor functional activity on both human $\beta_2$- and $\beta_3$-ARs. These results indicate that introduction of different halogen atoms at the 5-position (for example, an iodo group in compound **15**) provides a selective $\beta_2$-AR partial agonist. The introduction of an iodo group at the 7-position of compound **10** provided compound **16** which results in a decrease in the functional activity on human $\beta_3$-AR, while it abolishes human $\beta_2$-AR functional activity. This result indicates that the binding site of human $\beta_3$-AR can tolerate a more bulky group, like an iodo group, at the 7-position than that of $\beta_2$-AR. Therefore compound **16** is a partial agonist on the human $\beta_3$-AR and shows selectivity for the human $\beta_3$-AR versus human $\beta_2$-AR (Table 1).

The substitutions of an iodo or bromo group at both 5- and 7-positions of compound **10** afforded compounds **17** and **21**, respectively, which proved to be without any significant agonist property on both human $\beta_2$- and $\beta_3$-ARs, in comparison to those of compound **10** (Table 1). This phenomenon is likely because the functional active conformation on $\beta_2$- or $\beta_3$-AR could not be achieved by

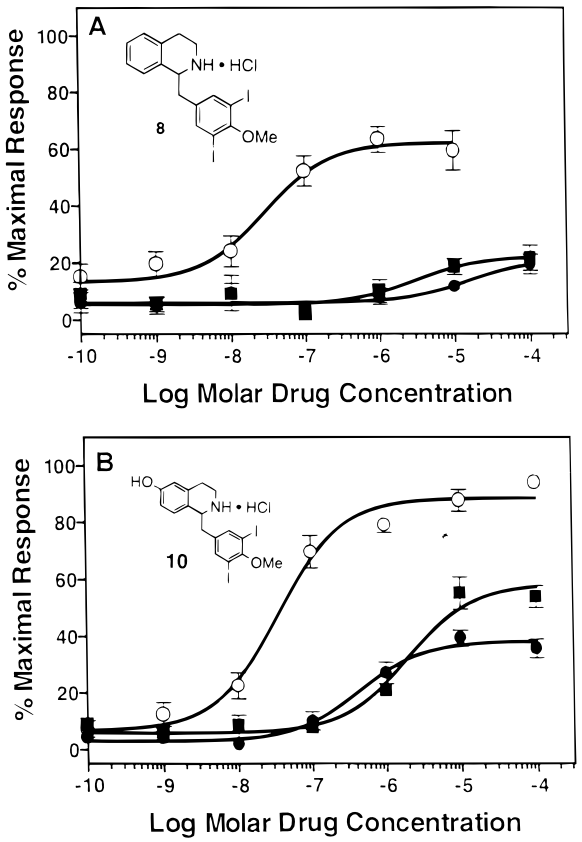



**Figure 2.** Concentration−response curves of compounds **8** (panel A) and **10** (panel B) on human $\beta_1$-AR (■ lines), $\beta_2$-AR (● lines), and $\beta_3$-AR (○ lines) expressed in CHO cells. Drug-induced changes in cellular cAMP were determined by the CRE−LUC assay (see Experimental Section). Responses are expressed relative to the maximal response produced by $10^{-6}$ M isoproterenol, and data represent the mean percent response ± SEM of 6−10 experiments.

steric perturbation on the receptor binding site corresponding to the aromatic A ring portion of diiodo-TMQ analogues. These results indicate that marked differences exist in the receptor binding site or pocket of $\beta_1$-, $\beta_2$-, and $\beta_3$-ARs which interact with halogen substituents at the 5- and/or 7-position of compound **10**. Our findings indicate that the $\beta_3$-AR selectivity of diiodo-TMQ is improved by removal of one or both hydroxyl groups of the catechol nucleus and that halogen substitution of the 6-hydroxy compound **10** (at the 5-, 7-, or 5,7-positions) reduced agonist activity and/or maximal effects for activation of this receptor.

## Conclusions

Functional activity assays in vitro have demonstrated that compound **10** is a potent full agonist while compound **8** is a partial agonist at the human $\beta_3$-AR. Both compounds are selective for the $\beta_3$-AR versus $\beta_1$- and $\beta_2$-ARs. It has also been demonstrated that halogen substitution on the aromatic A ring of compound **10** has a role in the type and potency of biological activity expressed. The introduction of an iodo substituent at the 5-position of compound **10** converts it from a $\beta_3$- into a $\beta_2$-AR agonist (compound **15**), while compound **16** bearing an iodo group at the 7-position remains a partial $\beta_3$-AR agonist. A bromo substitution at the 5-position (compound **20**) results in the decrease of functional activity on both $\beta_2$- and $\beta_3$-ARs. Iodo or bromo substitutions at both 5- and 7-positions abolish nearly completely $\beta_2$- and $\beta_3$-AR intrinsic activity. We believe that these new $\beta_3$-AR agonists could offer therapeutic potentials for treatment of obesity and NIDDM and serve as lead compounds in our future research.

## Experimental Section

**Chemistry.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer system 2000 FT-IR. Proton and carbon-13 magnetic resonance spectra were obtained on a Bruker AX 300 spectrometer (300 and 75 MHz for $^1$H and $^{13}$C, respectively). Chemical shift values are reported as parts per million ($\delta$) relative to tetramethylsilane (TMS). Spectral data are consistent with assigned structures. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, and found values are within 0.4% of the theoretical values. Routine thin-layer chromatography (TLC) was performed on silica gel on aluminum plates (silica gel 60 F 254, 20 × 20 cm; Aldrich Chemical Co. Inc., Milwaukee, WI). Flash chromatography was performed on silica gel (Merck; grade 60, 230−400 mesh, 60 Å). Tetrahydrofuran (THF) was dried by distillation from sodium metal. Acetonitrile (MeCN) and methylene chloride ($CH_2Cl_2$) were dried by distillation from $P_2O_5$.

**Table 1.** Agonist Potency (p$K_{act}$) and Intrinsic Activity (IA) of TMQ Analogues[a]

| | human $\beta_1$-AR | | human $\beta_2$-AR | | human $\beta_3$-AR | |
|---|---|---|---|---|---|---|
| compd | p$K_{act}$ ± SEM[b] | IA ± SEM[c] | p$K_{act}$ ± SEM | IA ± SEM | p$K_{act}$ ± SEM | IA ± SEM |
| | | | 1. CRE−LUC Assay[d] | | | |
| ISO | 8.81 ± 0.12 | 100 | 9.46 ± 0.09 | 100 | 7.74 ± 0.07 | 100 |
| **8** | MA[e] | 22 | MA | 21 | 7.54 ± 0.22 | 62 |
| **10** | 5.71 ± 0.14 | 58 | 6.36 ± 0.15 | 38 | 7.45 ± 0.09 | 90 |
| | | | 2. cAMP−RIA Assay[d] | | | |
| TMQ | 8.70 ± 0.11 | 109 | 8.33 ± 0.24[f] | 95 | 8.60 ± 0.15 | 95 |
| diiodo-TMQ | 8.11 ± 0.13 | 103 | 8.84 ± 0.07 | 52 | 8.76 ± 0.20 | 120 |
| **8** | ND[g] | ND | MA | 15 | 8.20 ± 0.05 | 52 |
| **10** | ND | ND | 6.87 ± 0.07 | 44 | 7.88 ± 0.24 | 106 |
| **15** | ND | ND | 6.77 ± 0.18 | 65 | MA | 8 |
| **16** | ND | ND | MA | 4 | 7.92 ± 0.32 | 35 |
| **17** | ND | ND | MA | 6 | MA | 18 |
| **20** | ND | ND | MA | 26 | 8.25 ± 0.47 | 31 |
| **21** | ND | ND | MA | 3 | MA | 20 |

[a] Human $\beta_1$-, $\beta_2$-, and $\beta_3$-ARs were expressed in CHO cells. Data are expressed as means ± SEM. [b] p$K_{act}$ is −log of the molar concentration of the drug which produces a cAMP response equal to 50% of its maximal response. [c] IA, intrinsic activity, expressed as the percentage of a maximal analogue response relative to the maximal response (100%) of (*R*)-(−)-isoproterenol (ISO). [d] See Experimental Section. [e] MA, minimal activity. $EC_{50}$ values were incalculable for compounds exhibiting IA less than 26%, and the observed IA values were determined at 100 $\mu$M. [f] Data for (*S*)-(−)-TMQ. [g] ND, not determined.

***N*-Phenylethyl-3,5-diiodo-4-methoxyphenylacetamide (6).** 3,5-Diiodo-4-methoxyphenylacetyl chloride[25] (0.52 g, 1.2 mmol) in $CH_2Cl_2$ (8 mL) was added dropwise to a stirred mixture of phenethylamine (0.15 g, 1.2 mmol) in $CH_2Cl_2$ (3 mL) and $K_2CO_3$ (0.50 g, 3.6 mmol) in $H_2O$ (8 mL). The resulting mixture was stirred for additional 1.5 h. The organic layer was separated and the $H_2O$ layer was extracted with $CH_2Cl_2$ (10 mL × 2). The combined organic layer was washed successively with 10% HCl solution (20 mL), saturated $Na_2CO_3$ solution (20 mL), and brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to give an oily residue. The title product was crystallized from ethyl acetate−hexanes to give 0.42 g (67%) of white needles: mp 157−158 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.61 (s, 2H, ArH), 7.28 (m, 3H, ArH), 7.11 (m, 2H, ArH), 5.35 (br s, 1H, NH), 3.86 (s, 3H, MeO), 3.51 (q, $J$ = 6.1 Hz, 2H, $NCH_2$), 3.37 (s, 2H, $COCH_2$), 2.79 (t, $J$ = 6.1 Hz, 2H, $ArCH_2$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.5 (C=O), 158.2 (C−O, Ar), 140.5, 138.6, 134.6, 128.8, 128.7, 126.7, 90.8 (C−I, Ar), 60.7 (MeO), 41.7 ($ArCH_2CO$), 40.8 ($CH_2N$), 35.4 ($ArCH_2$); IR (KBr) 3285 (NH), 1633 (C=O), 1549, 1497 (C=C, Ar) cm$^{-1}$. Anal. ($C_{17}H_{17}I_2NO_2$) C, H, N.

***N*-(3-Benzyloxyphenylethyl)-3,5-diiodo-4-methoxyphenylacetamide (7).** In the same manner as compound **6**, the title compound was prepared from 3,5-diiodo-4-methoxyphenylacetyl chloride (1.29 g, 3 mmol), NaOH (0.36 g, 9 mmol), and 2-(3-benzyloxyphenyl)ethylamine (0.78 g, 3 mmol) which in turn was obtained by reducing its corresponding nitro precusor[33] with LAH. Recrystallization from ethyl acetate−hexanes affored 1.60 g (86%) of title compound as white crystals: mp 117−119 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.60 (s, 2H, ArH), 7.45−7.19 (m, 5H, ArH), 6.85 (dd, $J$ = 7.5 Hz, $J$ = 2.1 Hz, 1H, ArH), 6.77 (d, $J$ = 2.1 Hz, 1H, ArH), 6.67 (d, $J$ = 7.5 Hz, 1H, ArH), 5.42 (br s, 1H, NH), 5.05 (s, 2H, $ArCH_2O$), 3.83 (s, 3H, MeO), 3.49 (q, $J$ = 6.2 Hz, 2H, CH), 3.33 (s, 2H, $ArCH_2C$=O), 2.75 (t, $J$ = 6.2 Hz, 2H, CH); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 169.5 (C=O), 159.1 (C−O, Ar), 158.2 (C−O, Ar), 140.5, 140.1, 137.0, 134.6, 129.7, 128.6, 128.0, 127.5, 121.3, 115.3, 112.9, 90.7 (C−I, Ar), 70.0 ($ArCH_2O$), 60.7 (MeO), 41.6 ($CH_2NH$), 40.6 ($ArCH_2C$=O), 35.4 ($ArCH_2$); IR (KBr) 3316 (NH), 1635 (C=O), 1596, 1549, 1497 (C=C, Ar) cm$^{-1}$. Anal. ($C_{24}H_{23}I_2NO_3$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinoline, Hydrochloride (8)**. A mixture of the corresponding amide **6** (0.35 g, 0.67 mmol) and $POCl_3$ (0.52 g, 3.36 mmol) in anhydrous $CH_3CN$ (20 mL) was heated at reflux for 4 h. Following cooling, the solvent and excess $POCl_3$ were removed under vacuum to dryness. The residue was dissolved in $CH_2Cl_2$ (20 mL), washed successively with brine (15 mL), saturated $Na_2CO_3$ solution (2 × 15 mL), and brine (2 × 15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to give an oily residue. The oil was dissolved in MeOH (20 mL) and cooled to 0 °C in an ice bath. A large excess $NaBH_4$ (0.25 g, 6.27 mmol) was added slowly in portions, and the resulting suspension was stirred overnight at room temperature. After the end of the reaction was established by TLC, the solvent was removed under vacuum to dryness which was dissolved in $CH_2Cl_2$ (20 mL), washed successively with 10% NaOH solution (2 × 15 mL) and brine (2 × 15 mL), dried over anhydrous $MgSO_4$, and filtered; then 1 mL of 1 M HCl in $Et_2O$ was added to make HCl salt. The title product was crystallized from MeOH−$Et_2O$ to give 0.19 g (55%) of white crystals: mp 199−200 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.10 (br s, 2H, NH), 7.91 (s, 2H, ArH), 7.27 (m, 4H, ArH), 4.77 (dd, 1H, ArCHN), 3.75 (s, 3H, MeO), 3.43−2.91 (m, 6H, $ArCH_2$ and CH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 157.5 (C−O, Ar), 140.7, 136.2, 132.1, 132.1, 128.9, 127.7, 126.8, 126.5, 60.2 (MeO), 55.2 (ArCHN), 39.0, 37.2, 24.9 ($ArCH_2$); IR (KBr) 3436 (NH), 1587, 1532, 1495 (C=C, Ar) cm$^{-1}$. Anal. ($C_{17}H_{17}I_2NO$·HCl) C, H, N.

**6-Benzyloxy-1-(3,5-diiodo-4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinoline, Oxalate (9).** The title compound was prepared from compound **7** (3.19 g, 5.09 mmol) in the same manner as compound **8**. Rerystallization from MeOH−$Et_2O$ affored 1.26 g (76%) of compound **9** as white crystals: mp 197−198 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.84 (s, 2H, ArH), 7.41 (m, 5H, ArH), 7.18 (m, 1H, ArH), 6.88 (m, 2H, ArH), 5.10 (s, 2H, $ArCH_2$), 4.63 (dd, 1H, ArCHN), 3.74 (s, 3H, MeO), 3.35 (m, 2H, CH), 3.17−2.92 (m, 4H, $ArCH_2$ and CH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 164.2 (HOOC−COOH), 157.5 (C−O, Ar), 157.4 (C−O, Ar), 140.6, 137.0, 136.6, 133.8, 128.4, 128.0, 127.8, 127.6, 124.9, 114.3, 113.6, 91.5, 69.2 ($ArCH_2O$), 60.2 (MeO), 54.9 (ArCHN), 38.7, 37.5, 25.5 ($ArCH_2$); IR (KBr) 3300−2400 (COOH, and NH), 1724 (C=O, acid), 1614, 1504 (C=C, Ar) cm$^{-1}$. Anal. ($C_{24}H_{23}I_2NO_2$·0.5(COOH)$_2$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol, Hydrochloride (10).** A solution of protected isoquinoline **9** (0.88 g, 1.45 mmol) in an equivolume mixture of MeOH and concentrated HCl (40 mL) was heated at reflux for 5 h. After the end of the reaction was established by TLC, the solvent was removed under vacuum to dryness which was dissolved in MeOH (30 mL) and evaporated under vacuum three times. The title product was crystallized from MeOH−$Et_2O$ to give 0.45 g (56%) of white crystals: mp 233−234 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.58 (s, 1H, ArOH), 9.16 (br s, 1H, NH), 9.11 (br s, 1H, NH), 7.89 (s, 2H, ArH), 7.19 (d, $J$ = 8.6 Hz, 1H, ArH), 6.65 (dd, $J$ = 8.6 Hz, $J$ = 2.4 Hz, 1H, ArH), 6.60 (d, $J$ = 2.4 Hz, 1H, ArH), 4.61 (dd, 1H, ArCHN), 3.75 (s, 3H, MeO), 3.36 (m, 2H, CH), 3.15−2.82 (m, 4H, $ArCH_2$ and CH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 157.5 (C−O, Ar), 156.7 (C−O, Ar), 140.7, 136.4, 133.4, 127.9, 122.4, 114.8, 114.1, 60.2 (MeO), 55.1 (ArCHN), 39.0 (CHN, or $ArCH_2$), 37.3 ($ArCH_2$, or CHN), 25.1 ($ArCH_2$); IR (KBr) 3600−2400 (br, OH and NH), 1614 (NH bend), 1589, 1534, 1505 (C=C, Ar) cm$^{-1}$. Anal. ($C_{17}H_{17}I_2NO_2$·HCl) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (11).** To a solution of isoquinoline **10** (1.95 g, 3.5 mmol) in anhydrous THF (80 mL) was added $Et_3N$ (2.44 mL, 17.5 mmol) and the resulting solution was cooled to 0 °C in ice bath. Trifluoroacetic anhydride (3.68 g, 17.5 mmol) was added dropwise to the above solution, and the mixture was stirred overnight at room temperature. After the end of the reaction was established by TLC, the solvent was removed under vacuum to dryness. The residue was dissolved in $CH_2Cl_2$ (30 mL), washed successively with brine (20 mL), 1 N HCl solution (20 mL), and brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to give a residue. The title compound was crystallized from ethyl acetate−hexanes to give 1.90 g (88%) of white crystals: mp 185.5−186.5 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.37 (s, 1H, ArOH), 7.72 (s, 2H, ArH), 7.23 (d, $J$ = 8.5 Hz, 1H, ArH), 6.65 (d, $J$ = 8.5 Hz, 1H, ArH), 6.57 (s, 1H, ArH), 5.46 (dd, 1H, ArCHN), 3.82 (m, 2H, CH), 3.69 (s, 3H, MeO), 3.00 (m, 2H, CH), 2.80 (m, 2H, $ArCH_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 156.9 (C−O, Ar), 156.1 (C−O, Ar), 154.4 (q, $J$ = 34.5 Hz, $CF_3$-CO), 140.4, 137.8, 133.9, 128.4, 125.3, 116.2 (q, $J$ = 287.3 Hz, $CF_3$), 114.6, 113.9, 90.5, 60.2 (MeO), 54.3 (ArCHN), 38.9, 38.9, 28.8 ($ArCH_2$); IR (KBr) 3388 (OH), 1679 (C=O), 1612, 1512 (C=C, Ar) cm$^{-1}$. Anal. ($C_{19}H_{16}F_3I_2NO_3$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5-iodo-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (12), 1-(3,5-Diiodo-4-methoxybenzyl)-7-iodo-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (13), and 1-(3,5-Diiodo-4-methoxybenzyl)-5,7-diiodo-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (14).** A mixture of protected isoquinoline **11** (1.24 g, 2 mmol), benzyltrimethylammonium dichloroiodate ($BTMACl_2I$) (0.77 g, 2.2 mmol), and $CaCO_3$ (1.40 g, 14 mmol) in $CH_2Cl_2$ (20 mL) and MeOH (8 mL) was stirred for 5 h at room temperature. After the starting material was consumed nearly completely checked by TLC, the mixture was filtered, and precipitate was washed with $CH_2Cl_2$ (2 × 10 mL). The combined organic solvent was successively washed with 0.5 N $Na_2S_2O_3$ solution (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to dryness. The title products were separated by silica gel chromatography using hexanes−ethyl acetate (8:1−6:1) as eluent to afford **14** (0.25 g, 12%), **12** (1.01 g, 69%), and **13** (0.12 g, 10%).

**14:** mp 114−115 °C; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.49 (s, 2H, ArH), 7.13 (s, 1H, ArH), 5.93 (br s, 1H, ArOH), 5.47 (dd,

1H, ArCHN), 4.04 (m, 2H, CH), 3.86 (s, 3H, MeO), 3.61 (m, 1H, $ArCH_2$), 3.11−2.76 (m, 3H, $ArCH_2$ and CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 158.3 (C−O, Ar), 155.7 (q, $J$ = 36.0, $COCF_3$), 153.0 (C−O, Ar), 140.8, 137.8, 137.8, 136.0, 129.7, 116.2 (q, $J$ = 286.5 Hz, $CF_3$), 90.3 (C−I, Ar), 89.8 (C−I, Ar), 79.6 (C−I, Ar), 60.8 (MeO), 54.2 (ArCHN), 40.1 ($ArCH_2$), 40.0 (q, $J$ = 3.8 Hz), 30.9 ($ArCH_2$); IR (KBr) 3425 (OH), 1683 (C=O), 1535 (C=C, Ar) $cm^{-1}$. Anal. ($C_{19}H_{14}F_3I_4NO_3$) C, H, N.

**12**: mp 80−81 °C; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.47 (s, 2H, ArH), 6.88 (d, $J$ = 8.4 Hz, 1H, ArH), 6.82 (d, $J$ = 8.4 Hz, 1H, ArH), 5.54 (br s, 1H, ArOH), 5.45 (dd, 1H, ArCHN), 4.06 (m, 1H, CH), 3.93 (m, 1H, CH), 3.78 (s, 3H, MeO), 3.55 (m, 1H, CH), 2.93−2.78 (m, 3H, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 158.1 (C−O, Ar), 155.8 (q, $J$ = 36.0 Hz, $COCF_3$), 154.4 (C−O, Ar), 140.7, 136.6, 136.3, 129.0, 128.1, 116.3 (q, $J$ = 285.8 Hz, $CF_3$), 113.5, 92.9 (C−I, Ar), 90.2 (C−I, Ar), 60.8 (MeO), 54.8 (ArCHN), 40.3 (q, $J$ = 3.8 Hz), 40.2 ($ArCH_2$), 35.5 ($ArCH_2$); IR (KBr) 3374 (OH), 1681 (C=O), 1603, 1533 (C=C, Ar) $cm^{-1}$. Anal. ($C_{19}H_{15}F_3I_3NO_3 \cdot 0.15C_6H_{14}$) C, H, N.

**13:** mp 151−152 °C; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.48 (s, 2H, ArH), 7.06 (s, 1H, ArH), 6.81 (s, 1H, ArH), 5.45 (dd, 1H, ArCHN), 4.14 (m, 1H, CH), 3.95 (m, 1H, CH), 3.87 (s, 3H, MeO), 3.65 (m, 1H, $ArCH_2$), 2.95 (m, 2H, CH), 2.78 (m, 1H, $ArCH_2$); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 158.1 (C−O, Ar), 156.0 (q, $J$ = 36.0 Hz, $COCF_3$), 154.1 (C−O, Ar), 140.9, 137.2, 136.3, 135.5, 128.2, 116.3 (q, $J$ = 286.0 Hz, $CF_3$), 114.5, 90.2 (C−I, Ar), 83.1 (C−I, Ar), 60.8 (MeO), 54.7 (ArCHN), 40.2 (q, $J$ = 3.8 Hz), 40.2, 28.7 ($ArCH_2$); IR (KBr) 3396 (OH), 1682 (C=O), 1599, 1533 (C=C, Ar) $cm^{-1}$. Anal. ($C_{19}H_{15}F_3I_3NO_3 \cdot 0.11C_6H_{14}$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5-iodo-1,2,3,4-tetrahydroisoquinolin-6-ol, Hydrochloride (15).** A mixture of protected isoquinoline **12** (0.62 g, 0.83 mmol) in MeOH (30 mL) and $K_2CO_3$ (1.50 g, 10.90 mmol) in $H_2O$ (10 mL) was heated at reflux for 1 h. After the end of reaction was established by TLC, the solvent was removed under reduced pressure to dryness which was extracted with $CH_2Cl_2$ (3 × 20 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and filtered, and then 1 mL of 1 M HCl in $Et_2O$ was added to make HCl salt. The title product was crystallized from MeOH−$Et_2O$ to give 0.40 g (70%) of white crystals: mp 235−236 °C; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.60 (s, 1H, ArOH), 9.21 (br s, 2H, $NH_2$), 7.88 (s, 2H, ArH), 7.09 (d, $J$ = 8.7 Hz, 1H, ArH), 6.82 (d, $J$ = 8.7 Hz, 1H, ArH), 4.66 (dd, 1H, ArCHN), 3.75 (s, 3H, MeO), 3.36 (m, 2H, CH), 3.01 (m, 2H, CH), 2.92 (m, 2H, $ArCH_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$ 157.5 (C−O, Ar), 156.3 (C−O, Ar), 140.7, 136.2, 135.8, 127.9, 124.4, 113.0, 91.6 (C−I, Ar), 91.6 (C−I, Ar), 60.2 (MeO), 54.8 (ArCHN), 38.8, 37.2, 32.2 ($ArCH_2$); IR (KBr) 3431 (OH), 1595, 1563, 1534 (C=C, Ar) $cm^{-1}$. Anal. ($C_{17}H_{16}I_3NO_2 \cdot HCl$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-7-iodo-1,2,3,4-tetrahydroisoquinolin-6-ol, Hydrochloride (16).** In the same manner as compound **15**, the title compound was prepared from the protected isoquinoline **13** (0.30 g, 0.40 mmol). Recrystallization from MeOH−$Et_2O$ affored 0.14 g (51%) of compound **16** as white crystals: mp 231−232 °C; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 10.38 (s, 1H, ArOH), 9.11 (br s, 2H, $NH_2$), 7.81 (s, 2H, ArH), 7.43 (s, 1H, ArH), 6.65 (s, 1H, ArH), 4.54 (dd, 1H, ArCHN), 3.74 (s, 3H, MeO), 3.31 (m, 2H, CH), 3.11 (m, 1H, $ArCH_2$), 2.99 (m, 2H, CH), 2.84 (m, 1H, $ArCH_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$ 157.4 (C−O, Ar), 155.8 (C−O, Ar), 140.83, 137.1, 136.3, 133.6, 124.6, 114.2, 91.4 (C−I, Ar), 82.5 (C−I, Ar), 60.2 (MeO), 54.3 (ArCHN), 38.7, 38.4, 37.2 ($ArCH_2$); IR (KBr) 3600−2400 (br, OH and NH), 1592, 1534, 1502 (C=C, Ar) $cm^{-1}$. Anal. ($C_{17}H_{16}I_3NO_2 \cdot HCl$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5,7-diiodo-1,2,3,4-tetrahydroisoquinolin-6-ol, Hydrochloride (17).** In the same manner as compound **15**, the title compound was prepared from the protected isoquinoline **14** (0.19 g, 0.22 mmol). Recrystallization from MeOH−$Et_2O$ affored 0.15 g (86%) of compound **17** as white crystals: mp 233−234 °C; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 9.72 (s, 1H, NH), 9.65 (s, 1H, ArOH), 9.58 (s, 1H, NH), 7.88 (s, 2H, ArH), 7.49 (s, 1H, ArH), 4.61 (dd, 1H, ArCHN), 3.76 (s, 3H, MeO), 3.42 (m, 2H, CH), 3.17 (m, 2H, CH), 2.92 (m, 2H, $ArCH_2$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$ 157.5 (C−O, Ar), 154.8 (C−O, Ar), 140.9, 137.3, 136.6, 136.2, 127.7, 94.8 (C−I, Ar), 91.5 (C−I, Ar), 84.3 (C−I, Ar), 60.2 (MeO), 54.0 (ArCHN), 38.3, 37.0 ($ArCH_2$), 32.5 ($ArCH_2$); IR (KBr) 3437 (OH), 1585, 1534 (C=C, Ar) $cm^{-1}$. Anal. ($C_{17}H_{15}I_4NO_2 \cdot HCl$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5-bromo-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (18).** A solution of pyridium tribromide ($PyHBr_3$) (0.96 g, 3 mmol) in anhydrous THF (50 mL) was added dropwise to a solution of isoquinoline **11** (1.24 g, 2 mmol) in THF (50 mL) over a 45-min period. The resulting solution was stirred for an additional 4 h at room temperature. After the starting material was consumed, the solution was filtered, treated with 2% $NaHSO_3$ solution, and concentrated under reduced pressure to dryness. The title product was purified by silica gel chromatography using hexanes−chloroform (2:1−1:2) as eluent and crystallized from ethyl acetate−hexanes to give 0.94 g (76%) of white crystals. Meanwhile, compound **19** (0.25 g, 16%) was also obtained from this procedure: mp 162−163 °C; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.48 (s, 2H, ArH), 6.92 (d, $J$ = 8.7 Hz, 1H, ArH), 6.81 (d, $J$ = 8.7 Hz, 1H, ArH), 5.72 (br s, 1H, ArOH), 5.56 (dd, 1H, ArCHN), 4.00 (m, 2H, CH), 3.84 (s, 3H, MeO), 3.59 (m, 1H, $ArCH_2$), 2.93 (m, 3H, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 158.1 (C−O, Ar), 155.8 (q, $J$ = 36.0 Hz, $COCF_3$), 151.7 (C−O, Ar), 140.7, 136.3, 133.3, 128.0, 127.8, 116.3 (q, $J$ = 288.8 Hz, $CF_3$), 114.4, 112.4, 90.2 (C−I, Ar), 60.8 (MeO), 54.7 (ArCHN), 40.1 ($ArCH_2$), 39.7 (q, $J$ = 3.7 Hz), 30.1 ($ArCH_2$); IR (KBr) 3430 (OH), 1692 (C=O), 1581, 1534 (C=C, Ar) $cm^{-1}$. Anal. ($C_{19}H_{15}F_3BrI_2NO_3$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5,7-dibromo-2-trifluoroacetyl-1,2,3,4-tetrahydroisoquinolin-6-ol (19).** A solution of $Br_2$ (0.26 g, 1.62 mmol) in AcOH (5 mL) was slowly added to an ice-bath-cooled solution of protected isoquinoline **11** (0.50 g, 0.81 mmol) in AcOH (10 mL). The mixture was allowed to warm to room temperature and stirred for 4 h. After the end of the reaction was established by TLC, the solution was concentrated to 2 mL and crystals were precipitated. The acrystals was filtered and recrystallized from ethyl acetate−hexane to afford 0.11 g (86%) of title compound as white crystals: mp 191−192 °C; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 7.58 (s, 2H, ArH), 6.95 (s, 1H, ArH), 5.97 (s, 1H, ArOH), 5.50 (dd, 1H, ArCHN), 4.40 (m, 2H, CH), 3.83 (s, 3H, MeO), 3.57 (m, 1H, CH), 2.90 (m, 3H, CH); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 158.3 (C−O, Ar), 155.8 (q, $J$ = 36.0 Hz, $COCF_3$), 148.8 (C−O, Ar), 140.8, 135.9, 133.4, 130.5, 129.0, 116.3 (q, $J$ = 286.5 Hz, $CF_3$), 112.2, 107.8, 90.3 (C−I, Ar), 60.8 (MeO), 54.3 (ArCHN), 40.0 ($ArCH_2$), 39.4 (q, $J$ = 3.8 Hz), 30.1 ($ArCH_2$); IR (KBr) 3380 (OH), 1689 (C=O), 1603, 1533 (C=C, Ar) $cm^{-1}$. Anal. ($C_{19}H_{14}F_3Br_2I_2NO_3 \cdot 1.3C_6H_{14}$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5-bromo-1,2,3,4-tetrahydroisoquinolin-6-ol, Oxalate (20).** A mixture of the protected isoquinoline **18** (0.30 g, 0.43 mmol) in MeOH (25 mL) and $K_2CO_3$ (1.2 g, 8.68 mmol) in $H_2O$ (10 mL) was stirred overnight at room temperature. The resulting solution was concentrated under vacuum to 5 mL, and crystals were precipitated which were filtered, washed with $H_2O$ and $CHCl_3$, and dissolved in MeOH; then 1.1 equiv of oxalic acid was added to make salt. The title compound was crystallized from MeOH−$Et_2O$ to give 0.28 g (99%) of white crystals: mp 227.5−228 °C; $^1H$ NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.74 (s, 2H, ArH), 6.98 (d, $J$ = 8.5 Hz, 1H, ArH), 6.80 (d, $J$ = 8.5 Hz, 1H, ArH), 4.60 (dd, 1H, ArCHN), 3.73 (s, 3H, MeO), 3.40 (m, 2H, CH), 3.24 (m, 2H, CH), 2.92 (m, 2H, CH); $^{13}C$ NMR (75 MHz, DMSO-$d_6$) $\delta$ 163.9 (HOOC−COOH), 157.4 (C−O, Ar), 153.7 (C−O, Ar), 140.6, 136.3, 132.9, 126.8, 124.8, 114.2, 111.4, 91.5 (C−I, Ar), 60.2 (MeO), 54.7 (ArCHN), 38.1 ($ArCH_2$), 37.4, 26.9 ($ArCH_2$); IR (KBr) 3600−2400 (br, NH and OH), 1712 (C=O), 1645, 1611 (NH bend), 1534 (C=C, Ar) $cm^{-1}$. Anal. ($C_{17}H_{16}NBrI_2O_2 \cdot 1.75(COOH)_2$) C, H, N.

**1-(3,5-Diiodo-4-methoxybenzyl)-5,7-dibromo-1,2,3,4-tetrahydroisoquinolin-6-ol, Oxalate (21).** In the same manner as compound **20**, the title compound was prepared from the protected isoquinoline **19** (0.28 g, 0.36 mmol) and $K_2$-

$CO_3$ (1.2 g, 8.68 mmol). Recrystallization from MeOH−$Et_2O$ generated 0.24 g (92%) of white crystalline title compound: mp 220−221 °C; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.82 (s, 2H, ArH), 7.40 (s, 1H, ArH), 4.58 (dd, 1H, ArCHN), 3.75 (s, 3H, MeO), 3.42 (m, 2H, CH), 3.22 (m, 2H, CH), 2.91 (m, 2H, $ArCH_2$); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$ 164.0 (HOOC−COOH), 157.4 (C−O, Ar), 150.1 (C−O, Ar), 140.8, 136.6, 133.0, 130.3, 127.6, 114.4, 109.5, 91.3 (C−I, Ar), 60.2 (MeO), 54.4 (ArCHN), 37.8, 37.4 ($ArCH_2$), 27.4 ($ArCH_2$); IR (KBr) 3600−2400 (br, NH and OH), 1724 (C═O), 1694 (NH bend), 1534 (C═C, Ar) cm$^{-1}$. Anal. ($C_{17}H_{15}Br_2I_2NO_2$·0.5$(COOH)_2$) C, H, N.

**cAMP Response Element (CRE)−Luciferase (LUC) Receptor Gene (CRE−LUC) Assay.** CHO cells stably expressing human $\beta_1$-, $\beta_2$-, or $\beta_3$-AR populations were transfected with a 6 CRE−LUC plasmids (gift from Dr. Himmler, Vienna, Austria) using electroporation with a single 70-ms, 150-V pulse.[34] The transfected CHO cells were seeded at a density of 40 000/well in 96-well microtiter plates (culturplate, Packard) and allowed to grow for 20 h. After 20 h, the cells were treated with varying drug concentrations ($10^{-11}$−$10^{-4}$ M) for 4 h. Following drug exposures, the cells were lysed and luciferase activity was measured using the LucLite assay kit (Packard). Changes in light production were measured by a Topcount luminometer (Packard). Data were analyzed in duplicate at each concentration and expressed as a percent luciferase response relative to the maximum response to (−)-isoproterenol ($10^{-6}$ M). Results are expressed as the mean ± SEM of $n = 5$−16.

**cAMP Radioimmunoassay (cAMP−RIA Assay).** CHO cells expressing human $\beta_1$-, $\beta_2$-, or $\beta_3$-AR subtypes were used as previously described.[35] These cells were grown to confluence in 60-mm dishes, washed with Hank's balanced salt solution, and then incubated with Hank's balanced salt solution (pH = 7.4) containing 20 mM HEPES, 1 mM 3-isobutyl-1-methylxanthine (IBMX), and 1 mM L-ascorbic acid for 30 min at 37 °C. Varying concentrations ($10^{-11}$−$10^{-4}$ M) of the compounds were added with incubation of an additional 30 min. After removal of the Hank's buffer, the cAMP generated within the cells was extracted by the addition of trichloroacetic acid (6% w/v). Protein content was determined by the method of Lowry et al.[36] using bovine serum albumin as a standard. cAMP levels in CHO cells were determined using the radioimmunoassay technique of Brooker et al.[37] The amount of cAMP was measured as the amount of $^{125}$I-labeled succinyl-cAMP tyrosine methyl ester/antibody precipitated

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