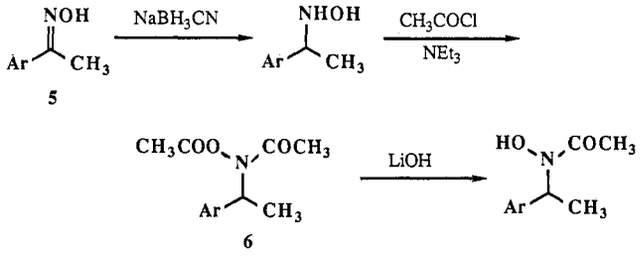
**Scheme I**



to those of their type A congeners (Table I). The greatest distinction between the two classes of compounds is observed in vivo. Whereas the type A hydroxamates are rapidly converted to inactive carboxylic acids, type B compounds are generally resistant to this route of metabolism. As a result, the type B hydroxamates circulate in the plasma longer than their type A congeners. For example, **4b** (Abbott-63162) exhibited a plasma half-life of about 2.5 times that of its type A analogue **4a** (1.1 and 0.4 h, respectively, following a 20 mg/kg dose, iv). Even more impressive is the difference between **4a** and **4b** in plasma concentrations when orally dosed at 100 mg/kg. Type A analogue **4a** reached peak plasma levels of approximately 15 $\mu$M at about 30 min, while **4b** reached its maximum concentration of 140 $\mu$M between 2 and 3 h (Figure 1). The low levels of **4a** could not be detected in the plasma more than 5 h after dosing, while **4b** was still circulating after 15 h. While **4a** is rapidly converted to the corresponding carboxylic acid, the major route of metabolism for **4b** is glucuronide conjugation followed by biliary excretion.

The superior pharmacokinetic properties of type B hydroxamates relative to their type A counterparts can result in greater in vivo potency. For example, in the rat peritoneal anaphylaxis model, **4b** was fivefold more potent in inhibiting leukotriene biosynthesis than **4a**. Type B hydroxamic acid **4b** had an $ED_{50}$ in this model of 8 mg/kg when administered orally. The duration of this inhibition was consistent with the duration of **4b** in the plasma. A 30 mg/kg oral dose of the compound produced 67% and 57% inhibition of leukotriene biosynthesis 2 and 4 h after administration, respectively. At 8 h, this dose no longer produced significant inhibition (36%).

This difference between the in vivo properties of type A and type B hydroxamic acids has been a consistent observation. In each of the examples listed in Table I, the type B hydroxamate exhibited higher plasma concentrations and longer duration than its type A counterpart. The type B configuration consistently produced orally active inhibitors of leukotriene biosynthesis. Although the difference in the two series can be attributed to different rates of metabolism, the underlying molecular basis for this remains to be determined.

The type B hydroxamic acids described here can be prepared as illustrated in Scheme I. Oxime **5** is reduced with sodium cyanoborohydride (methanol, pH 3, 3 h) and converted to the diacetate **6** (2.2 equiv of acetyl chloride and 3 equiv of $Et_3N$ in $CH_2Cl_2$, 30 min). The *O*-acetate is then removed with lithium hydroxide (5 equiv in 2:1 2-propanol–water, 30 min).

On the basis of these results, hydroxamic acids of structural type B appear to be valuable tools for the evaluation of the role of leukotrienes in animal models and human disease.

**Acknowledgment.** We thank Jennifer Barlow-Donnelly, Dirk Bornemeier, Thomas Clark, Wendy Rach, Ellen Roberts, and Sheryl Steihr for technical assistance.

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## [$^{125}$I]-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane: An Iodinated Radioligand That Specifically Labels the Agonist High-Affinity State of 5-$HT_2$ Serotonin Receptors

*Sir:*

The neurotransmitter serotonin (5-HT) is currently receiving renewed interest and widespread attention due to the recent identification of multiple populations of 5-HT binding/receptor sites (see ref 1 for a review). In order to further characterize these sites and to determine their physiological/pharmacological significance, it is necessary to develop site-selective agonists and antagonists. We have demonstrated that certain phenalkylamine derivatives possess a high affinity and selectivity for a particular population of 5-HT sites (i.e., 5-$HT_2$ sites); such agents include 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropane where X = Br (DOB; 1, R = Br) and X = iodo (DOI; 1, R = I).[2] Recently, we reported that [$^3$H]DOB specifically labels a guanine nucleotide-sensitive state of the 5-$HT_2$ receptor in rat brain homogenates.[3] However, because the agonist high-affinity state of the 5-$HT_2$ receptors labeled by [$^3$H]DOB represents only about 5% of the total 5-$HT_2$ receptor population in rat frontal cortical homogenates,[4] it is necessary to use a relatively large amount of tissue (20 mg wet weight) to produce a reliable signal. Furthermore, no specific signal was detectable when [$^3$H]DOB was incubated with 10-$\mu$m slices of rat frontal cortex (ca. 1 mg wet weight of tissue); this precludes the use of [$^3$H]DOB as a useful tool for autoradiographic studies. In order to use less tissue in the binding studies, and because one of the ultimate goals of this work is to perform autoradiographic studies on 5-$HT_2$ receptors, it became necessary to prepare a radioligand that would overcome these problems. Radioiodinated ligands are generally far superior to tritiated ligands because of their extremely high specific activities; such ligands allow for the use of small amounts of tissue and far shorter time periods to produce significant grain densities in autoradiographic studies.[5] We report here the synthesis and preliminary evaluation of a radioiodinated ligand, [$^{125}$I]DOI (1, R = $^{125}$I), that should prove useful for future 5-$HT_2$ studies.

**Chemistry.**[6] Synthesis of [$^{125}$I]DOI was achieved by using the triazene method[7] (Scheme I). Direct nitration

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(3) Titeler, M.; Herrick, Lyon, R. A.; McKenney, J. D.; Glennon, R. A. *Eur. J. Pharmacol.* **1985**, *117*, 145.
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(6) Proton NMR and infrared spectra are consistent with assigned structures. Satisfactory (±0.4%) elemental analyses were obtained for compounds **4–7** (Atlantic Microlab; Atlanta, GA).

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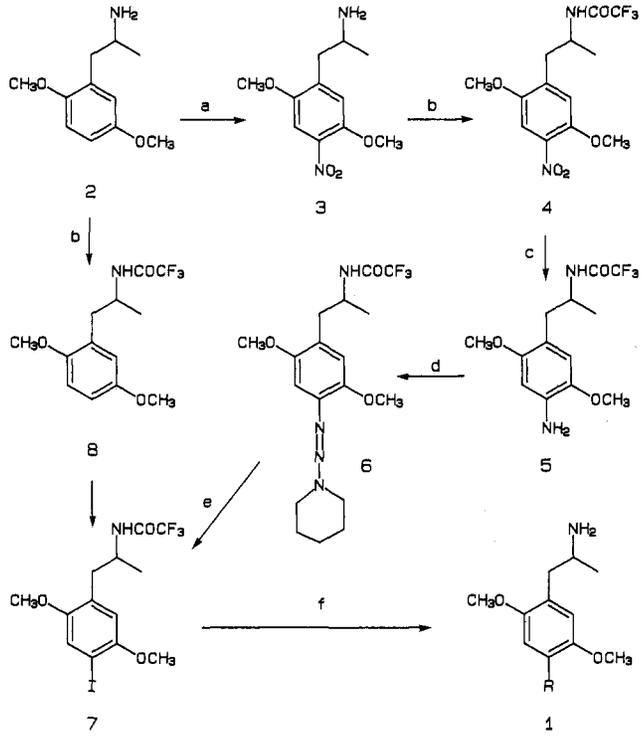
**Scheme I**[a]




[a] (a) $HNO_3$; (b) trifluoroacetic anhydride; (c) $H_2$, Pd/C; (d) $HCl/NaNO_2$; piperidine; (e) NaI; (f) NaOH.

of **2** afforded the 4-nitro derivative **3**,[8] which was protected by reaction with trifluoroacetic anhydride (room temperature, 3 h) to afford **4** (mp 158.5–160 °C; 81% yield). Catalytic reduction of **4** (absolute EtOH, 10% Pd/C, room temperature, 2 h) gave amine **5** (mp 181–183 °C; 82% yield), which was converted to the stable triazene **6** (mp 126–128 °C; 83% yield) via the diazonium salt (**5**, concentrated HCl, $NaNO_2$, 0–5 °C, 30 min; piperidine, <5 °C, 30 min). Treatment of **6** (5 mg) with NaI (MeCN, 0 °C, 30 min; room temperature, 20 h) afforded, **7**, the trifluoroacetyl derivative of DOI. [This product was identical (TLC, HPLC, GC/MS) with the product, i.e., **7** (mp 162–164 °C; 58% yield), obtained upon iodination of the *N*-trifluoroacetyl derivative of **2** i.e., **8** (mp 101–103 °C; 74% yield), using a previously published[8] iodination procedure.] Deprotection of **7** was accomplished by base hydrolysis (15% NaOH, room temperature, 14 h) followed by treatment with HCl to yield DOI·HCl (**1**, R = I) (mp 196–198 °C; lit.[8] mp 198–200 °C) in 65% yield. Treatment of **6** with $Na^{125}I$ in place of NaI gave the $^{125}I$ derivative of **7**. The labeled trifluoroacetyl compound was twice purified by chromatography on a 25-cm semipreparative C-18 reverse-phase column (70% MeOH/30% $H_2O$). The resolution ($R_S$) of **7** from *N*-(trifluoroacetyl)-1-(2,5-dimethoxyphenyl)-2-aminopropane (**8**), a side product of this reaction, was 7.3 and separation, $\alpha_{7/8} = 0.57$. After deprotection with NaOH, [$^{125}$I]DOI·HCl (**1**, R = $^{125}$I) (sp act. = 1625 Ci/mmol, no carrier added) was obtained in *ca.* 3% radiochemical yield. [$^{125}$I]DOI·HCl was determined to be 98% radiochemically pure by using multiple thin-layer chromatography systems [optimal system for resolving **7** ($R_f$ 0.66) from **1** ($R_f$ 0.4): EtOH/EtOAc/$NH_4OH$, 80/80/3].

(7) Goddard, C. P.; Law, B.; Mason, P. A.; Stead, A. H. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 383.
(8) Glennon, R. A.; Young, R.; Benington, F.; Morin, R. D. *J. Med. Chem.* **1982**, *25*, 1163.

**Table I.** Binding Data with [$^{125}$I]DOI as Radioligand

| agent | $K_i$,[a] nM | $N_H$[a] | $K_i$, nM, for [$^3$H]DOB-labeled sites[b] | $K_i$, nM, for [$^3$H]KET-labeled sites[b] |
|---|---|---|---|---|
| ketanserin | 2.4, 1.9 | 0.87, 0.79 | 1.3 | 1.2 |
| spiperone | 1.2, 1.3 | 0.75, 0.78 | 1.8 | 0.5 |
| cinanserin | 5.0, 7.7 | 0.76, 0.81 | 3.8 | 4.5 |
| serotonin | 10.6 (±3.4) | 0.90 (±0.10) | 6.1 | 600 |
| R(−)DOB | 1.9 (±0.1) | 0.84 (±0.04) | 0.4 | 25 |
| (±)DOI | 2.8 (±0.4) | 0.89 (±0.13) | 0.7 | 20 |

[a] $K_i$ values (affinity constants) and $N_H$ (Hill coefficients) for 5-HT, R(−)DOB, and DOI represent the mean (±SEM) of three separate experiments each performed in triplicate. $K_i$ and $N_H$ values for ketanserin, spiperone, and cinanserin represent two individual determinations performed in triplicate. [b] Binding data previously reported;[3] included for comparison. DOB = 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane, and KET = ketanserin.

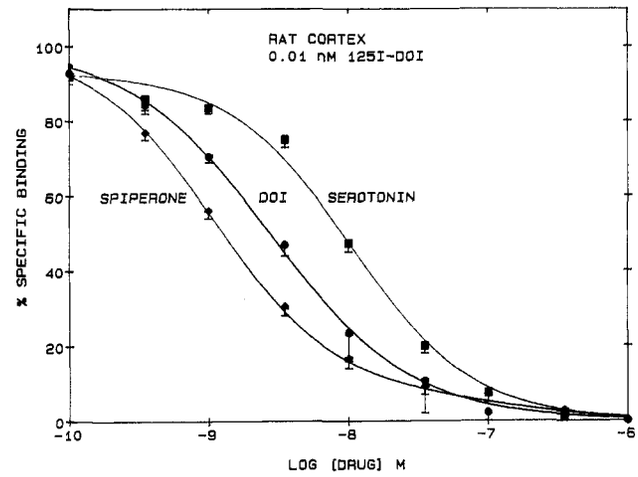



**Figure 1.** Competition of spiperone, DOI, and 5-HT for [$^{125}$I]DOI binding in rat cortex homogenates. Data represent the mean (±SEM) of two (spiperone) or three (DOI, 5-HT) separate experiments each performed in triplicate. Computer-generated curves are the best fit of the binding data (RS/1).

**Pharmacology.** Radioligand binding assays were conducted as previously described.[2,4,9] Specific [$^{125}$I]DOI binding was found to be saturable and of high affinity. The $B_{max}$ was 4.5 ± 0.2 pmol/g wet weight, and the $K_d$ was 2.2 (±0.2) × $10^{-9}$ M. The $B_{max}$ was about 5 times that determined for specific [$^3$H]DOB binding, and the $K_d$ was similar to the $K_i$ determined for DOI in competition experiments for specific [$^3$H]DOB binding.[4] Competition experiments with three 5-$HT_2$ antagonists (ketanserin, spiperone, cinanserin) and three agonists (5-HT, R(−)DOB, DOI) (Table I) revealed a distinctive 5-$HT_2$ receptor pharmacology. That is, we have previously shown that 5-HT agonists compete more potently for the high-affinity state of 5-$HT_2$ receptors (labeled by [$^3$H]DOB) than the

(9) Briefly, male Zivik–Miller Sprague–Dawley rat parieto-frontal cortices were homogenized in buffer (50 mM Tris·HCl, 0.5 mM $Na_2EDTA$, 10 mM $MgSO_4$; pH 7.4 at 37 °C), and the pellet obtained after centrifugation (24000*g* for 15 min) was resuspended in buffer at a concentration of 15 mg/mL. The final incubation volume (2 mL) contained 1 mL of membrane suspension (added last), 0.01 nM [$^{125}$I]DOI·HCl, and the appropriate amount of competing or excess ligand. Cinanserin ($10^{-6}$ M) was used to define specific binding; 0.01 nM [$^{125}$I]DOI produced a specific binding signal of 65%. Assay tubes were incubated at 37 °C for 20 min. Competition experiments were analyzed by using the nonlinear regression program EBDA[11] to obtain $IC_{50}$, $K_d$, and $B_{max}$ values. Affinity constants ($K_i$ values) were calculated by using the Cheng–Prusoff equation.[12]

low-affinity state (labeled by [$^3$H]ketanserin), whereas 5-HT antagonists compete with equal affinity for the high- and low-affinity states of the receptor.[2,4,10] Ketanserin, spiperone, and cinanserin competed for [$^{125}$I]DOI binding with high affinity, produced Hill coefficients of 0.75–0.87, and competed for 65% of total [$^{125}$I]DOI binding. Serotonin and the putative 5-HT agonists R(−)DOB and DOI also competed for [$^{125}$I]DOI with high affinity, produced competition curves with Hill coefficients of 0.84–0.90, and competed for 65% of total [$^{125}$I]DOI binding (Table I). (Representative competition curves are shown in Figure 1.) The affinities ($K_i$ values) of the six agents examined parallel the results from studies where [$^3$H]DOB was employed as the radioligand (Table I). The observations that all competing ligands reduced [$^{125}$I]DOI binding to the same extent and produced similar Hill coefficients indicate that in this tissue preparation [$^{125}$I]DOI is principally labeling one site but that there is a minor amount of some other site being labeled. This slight contamination with a second labeled site should not significantly deter from the utility of [$^{125}$I]DOI.

The results described herein indicate that [$^{125}$I]DOI, like [$^3$H]DOB, labels the agonist high-affinity state of 5-HT$_2$ receptors (i.e., 5-HT$_{2H}$ receptors) in a saturable, displaceable, and specific manner. We have found the signal to be stable and reliable (presumably due to the very high affinity of the radioiodinated ligand for the receptor). We anticipate that the greater specific activity of [$^{125}$I]DOI relative to [$^3$H]DOB (i.e., 1625 vs 16–40 Ci/mmol) should result in [$^{125}$I]DOI being a useful radioligand for subsequent binding and autoradiographic studies of the agonist high-affinity state of 5-HT$_2$ receptors.

**Registry No.** 1 (R = $^{125}$I), 111381-00-1; 1 (R = $^{125}$I)·HCl, 111381-06-7; 1 (R = T)·HCl, 42203-78-1; **2**, 2801-68-5; **3**, 67460-68-8; **4**, 111381-01-2; **5**, 111381-02-3; **6**, 111381-03-4; **7**, 111381-04-5; [$^{125}$I]-**7**, 111381-05-6; **8**, 79315-43-8; (5-HT), 50-67-9.

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# *Articles*

## Substituted 1,3,4-Thiadiazoles with Anticonvulsant Activity. 4. Amidines

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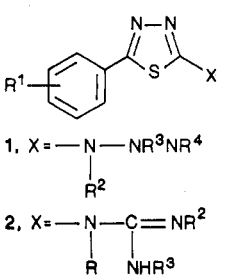
Two different structural types of 2-aryl-1,3,4-thiadiazole amidines were synthesized and evaluated for anticonvulsant activity. Enhancement of the inherent anticonvulsant activity therein and separation of this activity from the accompanying sedative action of these compounds were attempted. The most potent compounds occurred in the 2-(trifluoromethyl)phenyl series of type **3** amidines, but they also possessed a relatively high level of neurotoxicity and sedation as demonstrated in the rotorod test.

Previous papers[1,2] in this series have described 2-aryl-5-hydrazino- and 2-aryl-5-guanidino-1,3,4-thiadiazoles (**1**, **2**) as potential anticonvulsants. The most encouraging results were obtained within the hydrazine series, but it was recognized that the presence of a hydrazine group in these compounds was potentially undesirable because of the side effects associated with the bioisosterically related compound hydralazine.[3] It was felt, therefore, that the closely related amidines could perhaps offer an attractive alternative series to the hydrazines **1**.[1]




The thiadizole amidines studied can be divided into two structural types, **3** and **4**. Although a range of amidines of type **3** have been disclosed previously[4] and claimed to possess herbicidal and fungicidal properties, the derivatives

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