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# (*S*)-4-AMINO-5-CHLORO-3-IODO-2-METHOXY-*N*-(1-AZABICYCLO[2.2.2]OCT-3-YL)BENZAMIDE (TRIZAC), A HIGH-AFFINITY LIGAND FOR THE 5-HT-3 RECEPTOR

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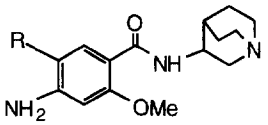
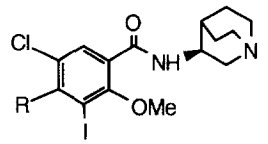
**Abstract** TRIZAC is one of the most potent 5-HT-3 receptor antagonist reported to date, having 20-fold higher affinity than (*S*)-5-iodozacopride. This high affinity ($K_i$ 0.05 ± 0.01 nM) and a moderate apparent lipophilicity (log $P_{app}$ 2.12) makes TRIZAC a promising ligand for studying 5-HT-3 receptors.


5-Hydroxytryptamine (5-HT) is a neurotransmitter that appears to play an important role in modulating the symptoms of psychiatric illness. 5-HT-3 receptor activation has been implicated in the maintenance of certain compulsive behaviors.[1] The 5-HT-3 antagonist ondansetron reduces alcohol consumption in the marmoset and alleviates morphine dependence in the rat.[2] There are few high-affinity ligands for this receptor that are suitable for radiolabeling at high specific activity. Racemic zacopride (**1**) is a potent ($K_D$ 0.6 nM) and selective 5-HT-3 receptor antagonist, and [$^3$H]zacopride has been used to characterize 5-HT-3 receptors in the rat and human brain.[3] The corresponding 5-iodo analogue of zacopride, [$^{125}$I]-**2**, has been useful in identifying rat CNS 5-HT-3 receptors in vitro, despite having 4-7 times lower affinity ($K_D$ 2.6-4.3 nM).[4,5] This moderate affinity of **2** is, in part, due to the fact that the pharmacological activity of zacopride derivatives resides in the *S* enantiomer.[6]




**1**, R = Cl
**2**, R = I

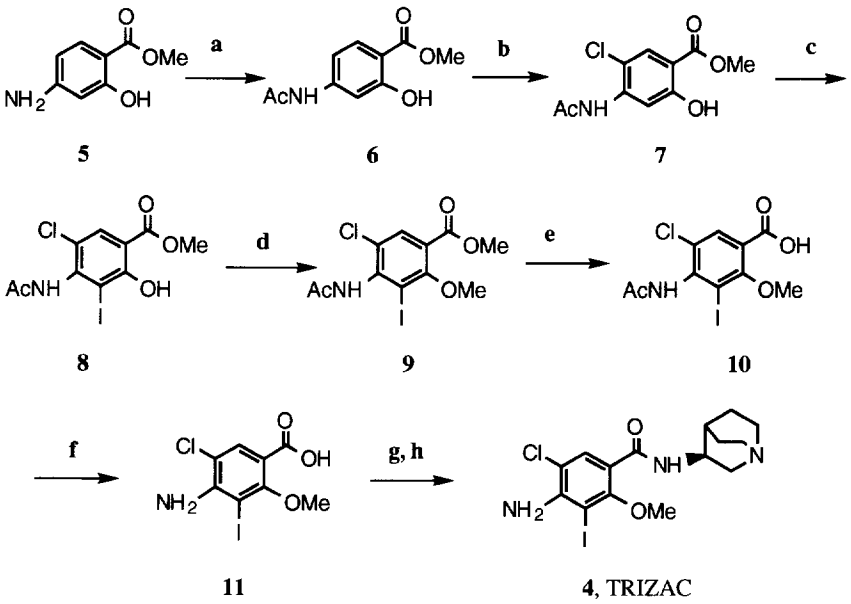



**3**, R = H
**4**, R = $NH_2$

We have shown that the des-4-amino-3-iodo analogue of (*S*)-zacopride, DAIZAC (**3**), is a potent and selective 5-HT-3 receptor antagonist with a $K_D$ of 0.15 nM.[7] In the development of DAIZAC, we found that a chlorine atom in the aromatic 5-position and a large substituent in the aromatic 3-position are prerequisites for high affinity for the 5-HT-3 receptor.[8] We have confirmed the finding by others[9] that the

presence of an amino group in the aromatic 4-position also enhances the affinity.[10] These structure-activity relationships predict that the combination of optimal aromatic substituents (i.e. 3-I, 4-$NH_2$, 5-Cl) and the *S* enantiomer of 3-aminoquinuclidine[11] should produce a selective, high-affinity ligand for the 5-HT-3 receptor. Consequently, we now report the synthesis of (*S*)-4-amino-5-chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide (TRIZAC, **4**), and its ability to displace (*S*)-5-[$^{125}$I]iodozacopride and [$^{125}$I]DAIZAC binding in rat brain homogenates.

**Scheme 1**




**5** **6** **7**

**8** **9** **10**

**11** **4**, TRIZAC

**Reagents:** (a) AcCl/pyridine. (b) $SO_2Cl_2/CHCl_3$. (c) NaI, Chloramine-T /DMF. (d) $Me_2SO_4/K_2CO_3$/acetone. (e) 0.5 N NaOH/EtOH, 1 h. (f) 5 N NaOH/EtOH, 16 h. (g) CDI/DMF. (h) (*S*)-3-AQN/MeCN.

TRIZAC (**4**) was prepared in seven steps from 4-aminosalicylic acid as shown in Scheme 1. Fisher esterification of 4-aminosalicylic acid with 18 M $H_2SO_4$ gave the methyl ester **5**.[12] The amino group of **5** was protected by reaction with acetyl chloride in pyridine to give the corresponding *N*-acetyl derivative **6**.[12] Chlorination of **6** with sulfuryl chloride in chloroform gave compound **7**. Subsequent iodination in the 3-position of **7** by the method used for the synthesis of DAIZAC (**3**)[8] resulted in methyl 4-acetylamino-5-chloro-3-iodosalicylate (**8**). Compound **8** was alkylated with dimethyl sulfate in acetone to give compound **9**. Use of excess alkylating reagent and long reaction times produced considerable amounts of the *N*-methylacetylamine derivative of **9**. Hydrolysis of the ester **9** in dilute sodium hydroxide gave 4-acetylamino-5-chloro-3-iodo-2-methoxybenzoic acid (**10**). Attempted removal of the acetyl group with concentrated hydrochloric acid failed, and using forced reaction conditions produced deiodination.

Presumably, the presence of halogen atoms in both 3- and 5-*ortho*-positions of compound **10** caused steric hindrance for hydrolysis of the acetyl group. In fact, the $^1$H NMR spectra at room temperature of both compounds **9** and **10** revealed a broad signal of the acetyl group protons, indicating a high energy barrier for rotation around the bond between the nitrogen and carbon atoms of the acetamido group. Use of 5 N sodium hydroxide in the hydrolysis of **10** and refluxing conditions for 16 h gave the acid **11**. It was coupled with 1,1'-carbonyldiimidazole to the corresponding *N*-imidazolobenzamide (not isolated) and reacted with (*S*)-3-aminoquinuclidine to give TRIZAC (**4**) in 12% overall yield.[13]

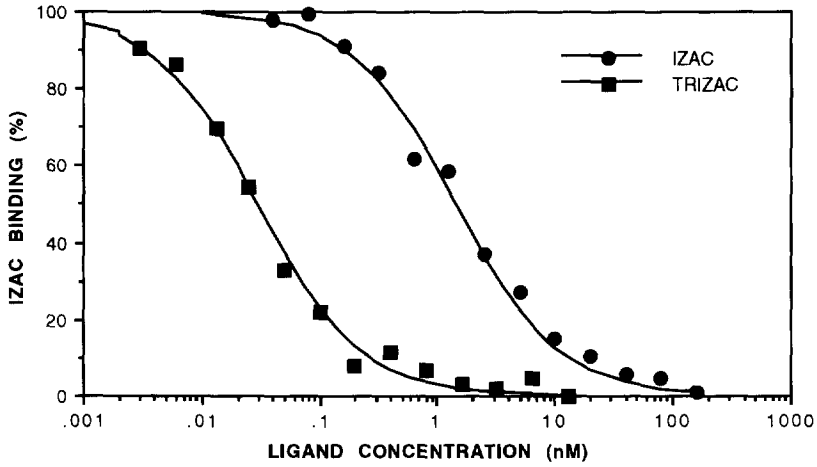



**Figure 1.** Displacement of 5-HT-3-specific (*S*)-[$^{125}$I]-**2** binding (0.2 nM) to whole rat brain by (*S*)-**2** (IZAC) and **4** (TRIZAC) giving $K_i$ 1.07 nM and $K_i$ 0.052 nM, respectively.

Receptor binding was performed using a modification[14] of the method of Kilpatrick et al.[15] The affinity of (*S*)-5-iodozacopride and TRIZAC for the 5-HT-3 receptor was determined by displacement of (*S*)-5-[$^{125}$I]iodozacopride as described by Ponchant et al. for the racemic radioligand.[5] Nonspecific binding was defined by co-incubation with 4 μM bemesetron (MDL-72222).[15] Nonlinear analysis of the binding data (Figure 1) showed a 20-fold difference between the affinity of (*S*)-5-iodozacopride and TRIZAC for the 5-HT-3 receptor (i.e. $K_i$ 1.07 ± 0.08 and 0.052 ± 0.012 nM, respectively). This result was confirmed by displacement of [$^{125}$I]DAIZAC binding[7,8] giving $K_i$ 1.37 and 0.075 nM, respectively (data not shown). Thus, the corresponding active enantiomer of the positional isomer of 5-iodozacopride has 50 times higher affinity for the 5-HT-3 receptor than racemic 5-iodozacopride.[4,5] This makes TRIZAC one of the most potent 5-HT-3 receptor antagonists known.

In consideration that a number of novel 5-HT-3 antagonists currently are undergoing clinical evaluation for treatment of psychiatric disorders, a selective and potent radioligand would be a valuable tool for studying their effects on the 5-HT-3 receptor in the human brain. The availability of a high-

affinity ligand offers several advantages in the study of 5-HT-3 receptors. An I-125 labeled derivative of TRIZAC should permit autoradiographic characterization of receptor distributions at receptor populations as low as 0.1 fmol/mg tissue. Further, the high affinity of TRIZAC predicts that TRIZAC should be useful in animal behavioral studies of the effects of selective 5-HT-3 receptor blockade.

Finally, no radioligand has yet proven to be a useful imaging agent of the 5-HT-3 receptor,[16] primarily because of the low receptor density in human brain.[17] Evidence from SPECT studies of dopamine D-2 receptors in human thalamus using [$^{123}$I]epidepride,[18,19] a structurally related substituted benzamide with optimal affinity ($K_D$ 0.03 nM) and apparent lipophilicity (log $P_{app}$ 2.05) for imaging purposes,[20] suggests that both C-11 and I-123 radiolabeled derivatives of TRIZAC could be used as imaging agents, because of its similar properties ($K_i$ 0.05 nM, log $P_{app}$ 2.12) as those of epidepride. Since the dopamine D-2 receptor density in human thalamus ($B_{max}$ 1.0 fmol/mg)[21] is comparable to that found in 5-HT-3 receptor-rich regions of the brain ($B_{max}$ 1.3 fmol/mg wet tissue),[17] PET or SPECT images with regional contrast[19,22] sufficient to quantify 5-HT-3 receptor densities in human hippocampus or amygdala should be obtainable with appropriately radiolabeled TRIZAC. Thus, provided that receptor selectivity proves to be sufficient and efficient radiolabeling can be acheived, the novel 5-HT-3 receptor antagonist TRIZAC has significant potential to become a tool for in vitro and in vivo studies of this serotonin receptor subtype.

**Acknowledgment**

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**References and Notes**

1. Costall, B.; Naylor, R. J. *Arzneim.-Forsch.* **1992**, *42*, 246.
2. Hui, S.-C. G.; Sevilla, E. L.; Ogle, C. W. *Br. J. Pharmacol.* **1996**, *118*, 1044.
3. Barnes, J. M.; Barnes, N. M.; Costall, B.; Ironside, J. W.; Naylor, R. J. *J. Neurochem.* **1989**, *53*, 1787.
4. Laporte, A. M.; Koscielniak, T.; Ponchant, M.; Verge, D.; Hamon, M.; Gozlan, H. *Synapse* **1992**, *10*, 271.
5. Ponchant, M.; Koscielniak, T; Hamon, M.; Gozlan, H. *J. Label. Compds Radiopharm.* **1991**, *29*, 1147.
6. Pinkus, L. M.; Sarbin, N. S.; Gordon, J. C.; Munson, R. H. *Eur. J. Pharmacol.* **1990**, *179*, 231.
7. Hewlett, W. A.; de Paulis, T.; Schmidt, D. E.; Trivedi, B. L.; Ebert, M. H. *Soc. Neurosci. Abstr.* **1995**, *21*, 776 (abstr).
8. Mason, N. S.; Schmidt, D. E.; Trivedi, B. L.; Hewlett, W. A.; Ebert, M. H.; de Paulis, T. *J. Label. Compds Radiopharm.* **1996** (in press).
9. King, F. D.; Dabbs, S.; Bermudez, J; Sanger, G. J. *J. Med. Chem.* **1990**, *33*, 2942.
10. de Paulis, T.; Hewlett, W. A.; Schmidt, D. E.; Mason, N. S.; Trivedi, B. L.; Ebert, M. H. *Eur. J. Med. Chem.* (submitted)

11. Lopez-Rodrigez, M. L.; Morcillo, M. J.; Benhamu, B.; Riaguas, M. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1195.

12. Drain, D. J.; Martin, D. D.; Mitchell, B. W.; Seymour, D. E.; Spring, F. S. *J. Chem. Soc.* **1949**, 1498.

13. 4-Aminosalicylic acid (21 g, 0.14 mol) was converted to its methyl ester by dropwise addition of 18 M sulfuric acid (14 mL, 0.25 mol) to a solution in MeOH (800 mL) and heating to refluxing temperature for 36 h. Evaporation of the solvent, addition of ice-water (500 mL), neutralization to pH 5 with 10 N NaOH (50 mL), and filtration gave 11.6 g (53%) of precipitated *methyl 4-amino-salicylate* (**5**), mp 119 °C. Lit[12] mp 119-121 °C.

Dropwise addition of acetyl chloride (5.4 g, 0.07 mol) in $CHCl_3$ (25 mL) to a chilled solution of **5** (10.5 g, 0.06 mol) in pyridine (75 mL) and stirring for 2 h gave 7.2 g (58%) of *methyl 4-acetyl-aminosalicylate* (**6**), after evaporation of the solvent and recrystallization of the residue from 100 mL EtOAc-*i*-$Pr_2O$ (1:1). Mp 144-146 °C, lit[12] mp 150 °C. $^1H$ NMR ($CDCl_3$) δ 10.85 (s, 1, OH), 7.76 (d, $J$ = 8.7 Hz, 1, C-6 H), 7.64 (b, 1, NH), 7.17 (d, $J$ = 1.8 Hz, 1, C-3 H), 7.08 (dd, $J$ = 1.8 Hz and 8.7 Hz, 1, C-5 H), 3.92 (s, 3, $OCH_3$), 2.19 ppm (s, 3, $COCH_3$).

A solution of **6** (3.5 g, 17 mmol) in $CHCl_3$ (150 mL) was mixed with sulfuryl chloride (2.3 g, 17 mmol) at 25 °C and stirred for 3 h, followed by evaporation and recrystallization from EtOAc (60 mL), to give 3.27 g (80%) of *methyl 4-acetylamino-5-chlorosalicylate* (**7**), mp 163-165 °C. $^1H$ NMR ($CDCl_3$) δ 10.73 (s, 1, OH), 8.17 (s, 1, C-6 H), 7.82 (s, 1, C-3 H), 7.75 (b, 1, NH), 3.94 (s, 3, $OCH_3$), 2.25 ppm (s, 3, $COCH_3$). Analysis ($C_{10}H_{10}ClNO_4$) calcd; found: C, 49.30; 49.39. H, 4.14; 4.11. N, 5.75; 5.75.

To a mixture of **7** (2.44 g, 10 mmol) and NaI (1.8 g, 12 mmol) in DMF (50 mL), chloramine-T (2.7 g, 12 mmol) was added in portions at 10 °C. After 1 h, the mixture was diluted with water (400 mL), neutralized with 12 N HCl (2 mL), decolorized with sodium metabisulfite (0.2 g), and the product was extracted with ether (2 x 100 mL). Drying ($Na_2SO_4$) and evaporation gave 2.83 g (77%) of *methyl 4-acetylamino-5-chloro-3-iodosalicylate* (**8**), after recrystallization from EtOAc (150 mL). Mp 219-221 °C. $^1H$ NMR ($CDCl_3$) δ 11.70 (s, 1, OH), 7.95 (s, 1, C-6 H), 7.18 (b, 1, NH), 4.00 (s, 3, $OCH_3$), 2.23 ppm (s, 3, $COCH_3$).

Compound **8** (1.82 g, 5 mmol) was treated with dimethyl sulfate (0.64 g, 5 mmol) in refluxing acetone (100 mL) for 2 h to give 1.18 g (62%) of *methyl 4-acetylamino-5-chloro-3-iodo-2-methoxybenzoate* (**9**) as crystals. Mp 171-173 °C from EtOAc (25 mL). $^1H$ NMR ($CDCl_3$) δ 7.93 (s, 1, C-6 H), 7.18 (b, 1, NH), 3.94 (s, 3, $OCH_3$), 3.89 (s, 3, $OCH_3$), 2.24 ppm (b, 3, $COCH_3$, the signal broadened by hindered rotation).

A solution of the ester **9** (1.0 g, 2.6 mmol) was heated to 80 °C in 0.5 N NaOH (15 mL) in EtOH (15 mL) for 0.5 h. Neutralization with 12 N HCl (0.7 mL) gave 0.67 g (70%) of *4-acetylamino-5-chloro-3-iodo-2-methoxybenzoic acid* **10** as crystals. Mp 234-235 °C ($H_2O$). $^1H$ NMR ($CDCl_3$) δ 8.18 (s, 1, C-6 H), 7.03 (b, 1, NH), 3.99 (s, 3, $OCH_3$), 2.24 ppm (b, 3, $COCH_3$, the signal broadened by hindered rotation).

A solution of **10** (0.74 g, 2.0 mmol) in a mixture of 5 N NaOH (2 mL) and EtOH (10 mL) was heated to reflux temperature for 16 h. The solvent was evaporated and replaced with water (15 mL), followed by neutralization with 12 N HCl (0.1 mL) to give 0.48 g (73%) of *4-amino-5-chloro-3-iodo-2-methoxybenzoic acid* **11** as crystals from EtOAc. Mp 193-195 °C. $^1$H NMR ($CDCl_3$) δ 8.06 (s, 1, C-6 H), 5.20 (b, 2, $NH_2$), 3.94 ppm (s, 3, $OCH_3$). Analysis ($C_{10}H_9ClINO_4$) calcd; found: C, 32.50; 32.52. H, 2.45; 2.43. N, 3.79; 3.73.

The acid **11** (0.33 g, 1.0 mmol) was converted to the *N*-imidazolobenzamide (not isolated) by mixing with 1,1'-carbonyldiimidazole (0.18 g, 1.1 mmol) in THF (15 mL) and stirring for 1 h at 20 °C. (*S*)-3-Aminoquinuclidine (0.2 g, 1.6 mmol) was added and the mixture was heated to reflux temperature for 5 h. The solvent was evaporated and the residue was purified on a $SiO_2$ column in EtOAc - EtOH - $NH_4OH$ (60:40:1). Fractions showing one spot at $R_f$ 0.07 were combined and the solvent was removed to give 43 mg of *(S)-4-amino-5-chloro-3-iodo-2-methoxy-N-(1-azabicyclo-[2.2.2]oct-3-yl)benzamide* (**4**) as an oil. UV $\lambda_{max}$ 275 nm (ε 15,600), $\lambda_{min}$ 252 nm (ε 9,800), $\lambda_{max}$ 223 nm (ε 45,000). Rotation: $[\alpha]^{20}_D$ -28° (*c* 0.44, EtOH). $^1$H NMR ($CDCl_3$) δ 8.03 (s, 1, C-6 H), 7.85 (b, 1, NH), 4.99 (b, 2, $NH_2$), 4.18 (m, 1, C-3' H), 3.83 (s, 3, $OCH_3$), 3.45 (dd, 1, C-2' H), 2.87 (m, 4, C-6' and C-7' H), 2.59 (dd, 1, C-2' H), 2.00 (q, 1, C-4' H), 1.5-1.7 ppm (m, 4, C-5' and 8' H).


14. Whole frozen rat brains (minus cerebellum) were homogenized, the membranes isolated by centrifugation (2 x 12,000 *g*), and incubated in HEPES buffer[15] (15 mg tissue/0.4 mL) with 0.20 nM (*S*)-5-[$^{125}$I]iodozacopride ((*S*)-[$^{125}$I]-**2**), or 0.14 nM [$^{125}$I]DAIZAC ([$^{125}$I]-**3**), for 1h at 20 °C. Unlabeled (*S*)-**2** and **4** (TRIZAC) were added in varying concentrations (0.04-180 nM and 0.003-12 nM, respectively). Nonspecific binding was defined with 4 μM bemesetron (MDL-72222). Bound and free (*S*)-[$^{125}$I]-**2** or ([$^{125}$I]-**3**) were separated by filtration through Schleicher & Schuell glass filters No. 32, presoaked in 0.3% polyethylenimide, in a Brandel M-24R cell harvester. The filters were rinsed 3 x 10 s with cold phosphate buffer and subjected to gamma spectrometry with an ICN Isomatic 4/600 HE instrument at 80% efficiency.
15. Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. *Nature* **1987**, *330*, 746.
16. Pike, V. W. *Nucl. Med. Biol.* **1995**, *22*, 1011.
17. Abi-Dargham, A.; Laurelle, M.; Wong, D. T.; Robertson, D. W.; Weinberger, D. R.; Kleinman, J. E. *J. Neurochem.* **1993**, *60*, 730.
18. Kessler, R. M.; Mason, S. M.; Votaw, J. R.; de Paulis, T.; Clanton, J. A.; Ansari, M. S.; Schmidt, D. E.; Manning, R. G.; Bell, R. L. *Eur. J. Pharmacol.* **1992**, *223*, 105.
19. Kessler, R. M.; Ansari, M.S.; de Paulis, T.; Schmidt, D. E.; Clanton, J. A.; Ebert, M. H.; Smith, H. E.; Manning, R. G.; Gillespie, D. *J. Nucl. Med.* **1991**, *32*, 1593.
20. Schmidt, D. E.; Votaw, J. R.; Kessler, R. M.; de Paulis, T. *J. Pharm. Sci.* **1994**, *83*, 305.
21. Kessler, R. M.; Whetsell, W. O.; Ansari, M. S.; Votaw, J. R.; de Paulis, T.; Clanton, J. A.; Schmidt, Mason, N. S.; Manning, R. G. *Brain Res.* **1993**, *609*, 237.
22. Manning, R. G.; Mason, N. S.; de Paulis, T.; Kessler, R. M. In *Chemist's Views of Imaging Centers*; Emran, A.M., Ed.; Plenum: New York, **1995**, pp 257-264.


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