N-Fluoroalkylated and N-Alkylated Analogues of the Dopaminergic D-2 Receptor Antagonist Raclopride

G. S. Lannoye,[†,‖] S. M. Moerlein,*[,†,‡] D. Parkinson,[§] and M. J. Welch[†]

The Edward Mallinckrodt Institute of Radiology, Department of Biochemistry and Molecular Biophysics, and Department of Cell Biology and Physiology, Washington University School of Medicine, 510 South Kingshighway Boulevard, Saint Louis, Missouri 63110. *Received November 20, 1989*

A series of raclopride [(*S*)-2-[(3,5-dichloro-6-methoxy-2-hydroxybenzamido)methyl]-1-ethylpyrrolidine] derivatives bearing pyrrolidino *N*-fluoroalkyl or -alkyl substituents were synthesized and evaluated as potential dopaminergic receptor-based positron tomography radiopharmaceuticals. Radiosynthetic procedures for producing the corresponding N-[^{18}F]fluoroalkylated analogues of raclopride from $^{18}F^-$ (β^+, $t_{1/2}$ = 110 min) in high specific activity were also developed. In vitro binding assays using competitive displacement of [^{3}H]spiperone from primate caudate tissue indicated that the N-alkylated analogues of raclopride had K_i values of 5–40 nM, whereas the corresponding values for analogous N-fluoroalkylated derivatives ranged from 90–160 nM. The relatively low D-2 binding affinity of these fluorinated salicylamides was corroborated by in vivo tissue biodistribution results in rodents. On the basis of structure–binding correlations, the impact of intramolecular hydrogen bonding, ligand basicity, and steric bulk on the affinity of the benzamides for D-2 receptor binding are discussed. Strategies are presented for the development of alternative fluorinated salicylamides that are both receptor active and metabolically stable.

Introduction

The primary pharmacological action of neuroleptics is thought to be blockade of cerebral dopaminergic D-2 receptors,[1,2] and there has been significant effort dedicated to the development of new drugs that interact with these binding sites.[3-6] This approach to drug design has therapeutic relevance since central dopaminergic receptor densities are elevated in schizophrenic patients.[7,8] Measurement of such changes in cerebral dopaminergic receptor numbers is possible via positron emission tomography (PET), an imaging technique that enables the in vivo study of physiology and biochemistry in a noninvasive manner after the administration of positron-emitting radiotracers.[9] PET not only provides important data regarding the neurophysiology of the human brain, but, in addition, makes possible the clinical monitoring of in vivo receptor binding by neuroleptics and other drugs during pharmacotherapy.[10-13]

Most tomographic imaging studies of cerebral D-2 receptors have employed analogues of the butyrophenone neuroleptic spiperone labeled with the positron-emitting nuclides ^{11}C ($t_{1/2}$ = 20 min),[14,15] ^{18}F ($t_{1/2}$ = 110 min),[16-20] ^{75}Br ($t_{1/2}$ = 98 min),[21] or ^{76}Br ($t_{1/2}$ = 16 hr).[22] In addition to the butyrophenone class of neuroleptics, a series of substituted benzamides has been identified with atypical dopamine antagonistic properties.[23-27] One such benzamide, raclopride (1a) has unique antipsychotic effects,[27,28] and in vitro studies with the salicylamide indicate that this ligand has high affinity (K_d = 0.81–1.20 nM) and great specificity for binding to cerebral dopaminergic D-2 receptors.[29] [^{3}H]Raclopride localizes in D-2 receptor-rich areas of the brain in vitro[29-31] and in vivo,[32] which suggests that the benzamide may have potential as a receptor-based radiopharmaceutical. [^{11}C]Raclopride has been successfully employed in PET studies of central D-2 receptors in human and non-human primates,[33-36] and differences between radiolabeled raclopride and spiperone analogues are apparent. There is less nonspecific binding of raclopride in vivo, so dopaminergic D-2 receptor-rich areas of the brain are rapidly visualized with use of this radioligand. Moreover, endogenous dopamine can decrease D-2 receptor binding by raclopride,[37] and differences in B_{max} values as measured with [^{11}C]raclopride and 3-*N*-[^{11}C]methylspiperone in schizophrenics have been noted.[38]

† The Edward Mallinckrodt Institute of Radiology.
‡ Department of Biochemistry and Molecular Biophysics.
§ Department of Cell Biology and Physiology.
‖ Present address: Abbott Laboratories, North Chicago, IL 60864.

(1) Creese, I.; Burt, D. R.; Snyder, S. H. *Science* **1976**, *192*, 481.
(2) Seeman, P.; Lee, T.; Chan-Wong, M.; Wong, K. *Nature* **1976**, *261*, 717.
(3) Vinick, F. J.; Heyn, J. H. *Annu. Rep. Med. Chem.* **1987**, *22*, 1.
(4) Schaus, J. M.; Clemens, J. A. *Annu. Rep. Med. Chem.* **1985**, *20*, 41.
(5) Nichols, D. E. In *Dopamine Receptors*; Kaiser, C., Kebabian, J. W., Eds.; ACS Symposium Series 224; American Chemical Society: Washington, DC, 1983; pp 201–218.
(6) Creese, I.; Burt, D. R.; Snyder, S. H. In *Handbook of Psychopharmacology*; Iverson, L. L., Iverson, S. D., Snyder, S. H., Eds.; Plenum Press: New York, NY, 1978; Vol. 10, pp 37–89.
(7) Lee, T.; Seeman, P.; Tourtellotte, W. W.; Farley, I. J.; Hornykeiwicz, O. *Nature* **1978**, *274*, 897.
(8) Owen, F.; Cross, A. J.; Crow, T. J.; Longden, A.; Poulter, M.; Riley, G. J. *Lancet* **1978**, No. 2, 223.
(9) Phelps, M. E., Mazziotta, J. C., Schelbert, H. R., Eds. *Positron Emission Tomography and Autoradiography*; Raven Press: New York, NY, 1986.
(10) Perlmutter, J. S.; Kilbourn, M. R.; Welch, M. J.; Raichle, M. E. *J. Neurosci.* **1989**, *9*, 2344.
(11) Smith, M.; Wolf, A. P.; Brodie, J. D.; Arnett, C. D.; Barouche, F.; Shiue, C.-Y.; Fowler, J. S.; Russell, J. A. G.; MacGregor, R. R.; Wolkin, A.; Angrist, B.; Rotrosen, J.; Peselow, E. *Biol. Psychiatry* **1988**, *23*, 653.
(12) Cambon, H.; Baron, J. C.; Boulenger, J. P.; Loc'h, C.; Zarifian, E.; Maziere, B. *Br. J. Psychiatry* **1987**, *151*, 824.
(13) Wong, D. F.; Gjedde, A.; Wagner, H. N.; Dannals, R. F.; Douglass, K. H.; Links, J. M.; Kuhar, M. J. *J. Cereb. Blood Flow Metabol.* **1986**, *6*, 147.
(14) Wagner, H. N.; Burns, H. D.; Dannals, R. F.; Wong, D. F.; Langström, B.; Duelfer, T.; Frost, J. J.; Ravert, H. T.; Links, J. M.; Rosenbloom, S. B.; Linkas, S. E.; Kramer, A. V.; Ravert, M. J. *Science* **1983**, *221*, 1264.
(15) Arnett, C. D.; Fowler, J. S.; Wolf, A. P.; Logan, J.; MacGregor, R. R. *Biol. Psychol.* **1984**, *19*, 1365.
(16) Mintun, M. A.; Raichle, M. E.; Kilbourn, M. R.; Wooten, G. F.; Welch, M. J. *Ann. Neurol.* **1984**, *15*, 217.
(17) Arnett, C. D.; Shiue, C.-Y.; Wolf, A. P.; Fowler, J. S.; Logan, J.; Watanabe, M. *J. Neurochem.* **1985**, *44*, 835.
(18) Coenen, H. H.; Wienhard, K.; Stöcklin, G.; Laufer, P.; Hebold, I.; Pawlik, G.; Heiss, W.-D. *Eur. J. Nucl. Med.* **1988**, *14*, 80.
(19) Welch, M. J.; Katzenellenbogen, J. A.; Mathias, C. J.; Brodack, J. W.; Carlson, K. E.; Chi, D. Y.; Dence, C. S.; Kilbourn, M. R.; Perlmutter, J. S.; Raichle, M. E.; Ter-Pogossian, M. M. *Nucl. Med. Biol.* **1988**, *15*, 83.
(20) Huang, S.-C.; Bahn, M. M.; Barrio, J. R.; Hoffman, J. M.; Satyamurthy, N.; Hawkins, R. A.; Mazziotta, J. C.; Phelps, M. E. *J. Cereb. Blood Flow Metabol.* **1989**, *9*, 850.
(21) Moerlein, S. M.; Laufer, P.; Stöcklin, G.; Pawlik, K.; Heiss, W.-D. *Eur. J. Nucl. Med.* **1986**, *12*, 211.

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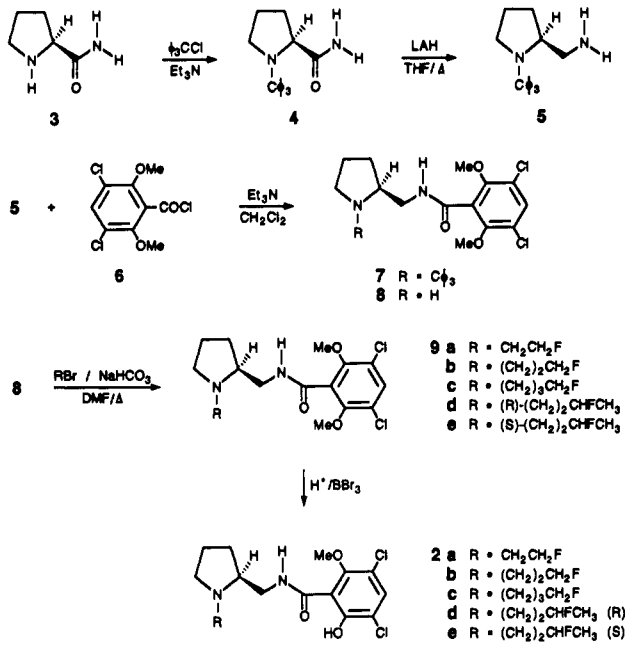
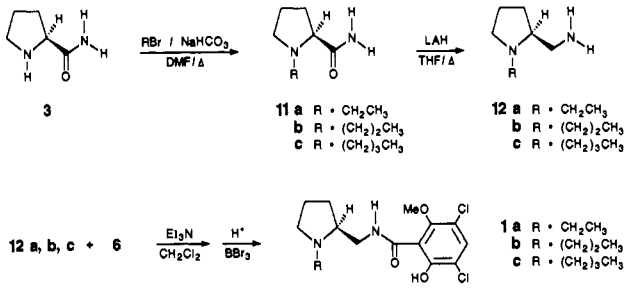
Scheme I

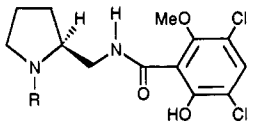


Scheme II



Because raclopride and structurally related[26,27,39,40] *o*-anisamide derivatives exhibit a high affinity and specificity for binding to dopaminergic D-2 receptors, it may be possible to develop fluorinated analogues of raclopride which demonstrate similar receptor-binding characteristics. Such a fluorinated ligand would be advantageous for radiopharmaceutical applications, since the relatively long half-life of ^{18}F would permit PET imaging over extended intervals, as well as allow for convenience in the analysis of blood metabolites for dynamic modeling of in vivo receptor binding.[36,41,42] A further advantage to the relatively long-lived ^{18}F-labeled ligand would be the potential for distribution to PET units remotely located from the cyclotron production center.

Table I. In Vitro Inhibition of [^{3}H]Spiperone Binding to *Macaca nemestrina* Striatal Membranes by Benzamide Derivatives



compd[a]	N-substituent: R	substituent: log P[b]	$K_i \pm$ SE, nM[c]
1a	CH_2CH_3	1.22	8.7 ± 1.1[d]
			5.1 ± 0.5[e]
1b	$CH_2CH_2CH_3$	1.75	17.3 ± 2.5
1c	$CH_2(CH_2)_2CH_3$	2.28	41.1 ± 4.5
2a	CH_2CH_2F	0.55	159 ± 25
2b	$CH_2CH_2CH_2F$	1.07	130 ± 18
2c	$CH_2(CH_2)_2CH_2F$	1.60	88.5 ± 13.5
2d	$(CH_2)_2CHFCH_3$ (*R*)	1.50	2620 ± 550
2e	$(CH_2)_2CHFCH_3$ (*S*)	1.50	2610 ± 280

[a] Unless otherwise indicated, the hydrochloride salt was used for each K_i determination. [b] Calculated from data given in ref 49. [c] Values determined as outlined in the Experimental Section. [d] L-Tartrate salt of raclopride synthesized as described in this work. [e] L-Tartrate salt of authentic raclopride from Astra Läkemedel AB, Södertälje, Sweden.

In the course of experiments to identify an effective fluorinated D-2 receptor-binding benzamide ligand, we have synthesized several N-fluoroalkylated derivatives of raclopride (**1a**). The radiosynthesis of one of these derivatives, 2-[^{18}F]fluoroethyl raclopride ((*S*)-2-[(3,5-dichloro-6-methoxy-2-hydroxybenzamido)methyl]-1-(2-[^{18}F]fluoroethyl)pyrrolidine, **2a**) has previously been reported by this group[43] and others.[44-47] In addition to these benzamides, the corresponding N-alkylated analogues were

(22) Martinot, J.-L.; Peron-Magnan, P.; Huret, J.-D.; Mazoyer, B.; Baron, J.-C.; Boulenger, J.-P.; Loc'h, C.; Maziere, B.; Caillard, V.; Loo, H.; Syrota, A. *Am. J. Psychiatry* **1990**, *147*, 44.
(23) de Paulis, T. *Acta Pharm. Suec., Suppl. 1* **1985**, 405.
(24) Jenner, P.; Marsden, C. D. *Acta Psychiatr. Scand., Suppl.* **1984**, *69*, 109.
(25) Florvall, L.; Ögren, S.-O. *J. Med. Chem.* **1982**, *25*, 1280.
(26) de Paulis, T.; Kumar, Y.; Johansson, L.; Rämsby, S.; Florvall, L.; Hall, H.; Ängeby-Möller, K.; Ögren, S.-O. *J. Med. Chem.* **1985**, *28*, 1263.
(27) de Paulis, T.; Kumar, Y.; Johansson, L.; Rämsby, S.; Hall, H.; Sällemark, M.; Ängeby-Möller, K.; Ögren, S.-O. *J. Med. Chem.* **1986**, *29*, 61.
(28) Hillegart, V.; Ahlenius, S. *Pharmacol. Toxicol.* **1987**, *60*, 350.
(29) Hall, H.; Wedel, I. *Acta Pharmacol. Toxicol.* **1986**, *58*, 368.
(30) Köhler, C.; Radestäter, A.-C. *Neurosci. Lett.* **1986**, *66*, 85.
(31) Dewar, K. M.; Montreuil, B.; Grondin, L.; Reader, T. A. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 696.
(32) Köhler, C.; Hall, H.; Ögren, S.-O.; Gawell, L. *Biochem. Pharmacol.* **1985**, *34*, 2251.
(33) Farde, L.; Ehrin, E.; Eriksson, L.; Greitz, T.; Hall, H.; Hedström, C.-G.; Litton, J.-E.; Sedvall, G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3863.
(34) Farde, L.; Hall, H.; Ehrin, E.; Sedvall, G. *Science* **1986**, *231*, 258.
(35) Farde, L.; Halldin, C.; Stone-Elander, S.; Sedvall, G. *Psychopharmacology* **1987**, *92*, 278.
(36) Farde, L.; Eriksson, L.; Blomquist, G.; Halldin, C. *J. Cereb. Blood Flow Metabol.* **1989**, *9*, 696.
(37) Seeman, P.; Guan, H.-C.; Niznik, H. B. *Synapse* **1989**, *3*, 96.
(38) Andreasen, N. C.; Carson, R.; Diksic, M.; Evans, A.; Farde, L.; Gjedde, A.; Hakim, A.; Lal, S.; Nair, N.; Sedvall, G.; Tune, L.; Wong, D. *Schizophr. Bull.* **1988**, *14*, 471.
(39) Köhler, C.; Hall, H.; Gawell, L. *Eur. J. Pharmacol.* **1986**, *120*, 217.
(40) Ögren, S. O.; Hall, H.; Köhler, C.; Magnusson, O.; Lindbom, L.-O.; Ängeby, K.; Florvall, L. *Eur. J. Pharmacol.* **1984**, *102*, 459.
(41) Perlmutter, J. S.; Larson, K. B.; Raichle, M. E.; Markham, J. R.; Mintun, M. A.; Kilbourn, M. R.; Welch, M. J. *J. Cereb. Blood Flow Metabol.* **1986**, *6*, 154.
(42) Logan, J.; Wolf, A. P.; Shiue, C.-Y.; Fowler, J. S. *J. Neurochem.* **1987**, *48*, 73.
(43) Lannoye, G. S.; Moerlein, S. M.; Welch, M. J. *J. Labelled Compd. Radiopharm.* **1989**, *26*, 340.
(44) Kiesewetter, D. O.; Brücke, T.; Finn, R. D. *Appl. Radiat. Isot.* **1989**, *40*, 455.
(45) Kiesewetter, D.; Finn, R. *J. Labelled Compd. Radiopharm.* **1989**, *26*, 14.
(46) Halldin, C.; Högberg, T.; Stone-Elander, S.; Farde, L.; Hall, H.; Printz, G.; Solin, O.; Sedvall, G. *J. Labelled Compd. Radiopharm.* **1989**, *26*, 338.
(47) Halldin, C.; Högberg, T.; Stone-Elander, S.; Farde, L.; Hall, H.; Printz, G.; Solin, O.; Sedvall, G. *J. Nucl. Med.* **1988**, *29*, 767.

Table II. In Vivo Tissue Localization of No-Carrier-Added ^{18}F-Labeled Salicylamides

time,[a] min	ligand	tissue concentration, % injected dose/g[b] striatum	cerebellum	whole brain	blood	bone
5	15a	0.62 ± 0.10	0.42 ± 0.09	0.52 ± 0.11	0.16 ± 0.01	0.20 ± 0.02
	15b	1.50 ± 0.23	0.98 ± 0.17	1.16 ± 0.16	0.26 ± 0.02	0.28 ± 0.03
	15c	0.87 ± 0.20	0.58 ± 0.12	0.71 ± 0.13	0.21 ± 0.04	0.22 ± 0.03
30	15a	0.09 ± 0.05	0.05 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.23 ± 0.03
	15b	0.13 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.10 ± 0.01	0.34 ± 0.10
	15c	0.12 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.24 ± 0.02
60	15b	0.07 ± 0.03	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.54 ± 0.06
	15c	0.09 ± 0.02	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.39 ± 0.05

[a] Time postinjection of radiolabeled compound. [b] Data is the mean and standard deviation for 4–5 test animals.

also prepared for comparison with the fluorine-bearing structures. In this work is described the synthesis of this series of salicylamides, as well as the results of in vitro binding assays and in vivo tissue biodistribution experiments using these ligands.

Results

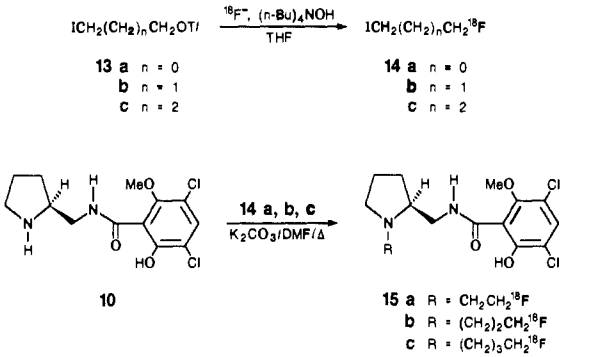
Chemistry. N-Fluoroalkylated derivatives of raclopride were prepared as depicted in Scheme I. By use of an approach similar to that employed for the radiosynthesis of [^{11}C]raclopride,[48] *N*-trityl prolinamide **4** was prepared from the hydrochloride salt of (*S*)-prolinamide **3** via treatment with trityl chloride in the presence of 2 equiv of triethylamine. Treatment of the protected amino acid derivative with excess lithium aluminum hydride (LAH) provided the diamine **5**, which was immediately coupled to the freshly prepared acid chloride **6** in high yield (>60%). The protected benzamide **7** was immediately converted to the free amine **8** via treatment with aqueous HCl. N-Alkylation of free amine **8** was achieved in relatively high yield (50–60%) when conducted in DMF in the presence of $NaHCO_3$. Comparable yields were obtained when the iodo- or bromoalkanes were used as alkylating agents. The (*S*)-N-fluoroalkylated salicylamides (**2a–e**) were obtained in excellent yield by treatment of the corresponding benzamide salt (HCl) with 1 equiv of BBr_3 in the presence of gaseous HCl. These fluoroalkylated derivatives of raclopride were converted into either the HCl or tartrate salt for in vitro binding studies.

For comparative purposes, corresponding nonfluorinated ligands were also prepared, as outlined in Scheme II. The chemistry employed for the preparation of these compounds was essentially the same as that employed for the production of the fluorinated analogues. The only exception was that the protection/deprotection sequence was unnecessary for the former, since the alkyl substituent was insensitive to the LAH reduction reaction. It was therefore possible to initiate the synthetic sequence with the N-alkylation of the prolinamide **3**. All steps in this synthetic pathway were achieved in high yield.

In Vitro Binding Assays. The in vitro binding constants were obtained for the series of fluorinated and non-fluorinated derivatives of raclopride and are given in Table I. These binding assays were conducted with use of caudate tissue dissected from the brains of adult *Macaca nemestrina*, and values were determined via competitive displacement of [^{3}H]spiperone.

Radiochemistry. The radiosynthesis of high specific activity ^{18}F-labeled **15a–c** was accomplished via the two-step procedure outlined in Scheme III. No-carrier-added [^{18}F]fluoride was resolubilized into tetrahydrofuran with

Scheme III



use of tetra(*n*-butyl)ammonium hydroxide, and subsequently used in nucleophilic substitution reactions with alkyl triflates **13a–c**.[50] The resulting [^{18}F]fluoroiodoalkanes were employed in a second reaction step that involved the [^{18}F]fluoroalkylation of *des*-ethyl raclopride (**10**).[48] The ^{18}F-labeled salicylamides were isolated in high specific activity using semipreparative HPLC for in vivo tissue biodistribution experiments. The nonoptimized overall radiochemical yields for **15a–c** were 5–10% for an overall radiosynthesis time of 120 min. This corresponded to a radiochemical yield of 30–40% for production of the respective [^{18}F]fluoroalkyl halide from aqueous [^{18}F]-fluoride, and 15–25% for the subsequent [^{18}F]fluoroalkylation step. The specific activity of the final products as determined chromatographically exceeded 1000 Ci/mmol.

In Vivo Tissue Biodistribution Studies. The in vivo tissue biodistribution behavior of high specific activity **15a–c** was determined in 200–250 g female Sprague-Dawley rats, and the results are reported in Table II. These results show the localization of the ^{18}F-labeled salicylamides in the whole brain, blood, and bone, as well as the regional cerebral accumulation of radioactivity in the striatum and cerebellum. The data is given in terms of percent injected dose per gram of tissue, and represents the mean from 4 to 5 test animals.

Discussion

Chemistry. At the onset of this research, the alkyl substituent was selected as a potential site for the introduction of fluorine, since it is relatively easy to produce N-[^{18}F]fluoroalkylated receptor-binding radioligands in high effective specific activity and useful radiochemical yields.[50–55] In pursuit of this goal, the production of the

(48) Ehrin, E.; Farde, L.; de Paulis, T.; Eriksson, L.; Greitz, T.; Johnström, P.; Litton, J.-E.; Nilsson, J. L. G.; Sedvall, G.; Stone-Elander, S.; Ögren, S.-O. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 269.
(49) Nys, G. G.; Rekker, R. F. *Chim. Ther.* **1973**, *5*, 521.
(50) Chi, D. Y.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Welch, M. J. *J. Org. Chem.* **1987**, *52*, 658.
(51) Chi, D. Y.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Brodack, J. W.; Welch. M. J. *Appl. Radiat. Isot.* **1986**, *37*, 1173.

title salicylamides required three different synthetic pathways for the generation of raclopride derivatives bearing alkyl, fluoroalkyl, or [^{18}F]fluoroalkyl substituents on the pyrrolidino nitrogen atom. There was good overall yield of the target compounds for each of these synthetic pathways.

The preparation of the N-alkylated benzamides **1a–c** was most straightforward, being based directly on the procedure reported for the synthesis of raclopride[27] (Scheme II). In this approach, prolinamide was alkylated then reduced prior to conjugation to the acyl chloride **6** and monodemethylation. Salicylamides **1a–c** were recovered with good overall yield.

When this synthetic procedure was applied to the production of N-fluoroalkylated analogues of raclopride, the reduction of N-fluoroalkylated prolinamide reduced not only the amide functional group, but the alkyl fluoride as well. It was thus necessary to fluoroalkylate the pyrrolidine nitrogen atom in a step subsequent to the reduction of prolinamide by lithium aluminum hydride (Scheme I). The resulting N-fluoroalkylated derivatives of prolinamide were then used in the ether cleavage reaction sequence. The fluorinated benzamides **2a–e** were thereby generated in reasonable overall yield.

Due to the relatively short half-life of ^{18}F (110 min), there were special synthetic constraints incurred in the production of [^{18}F]fluoroalkylated salicylamides. It was essential that as many of the above synthetic steps as possible be completed prior to initiation of the radiofluorination process to avoid unnecessary decay losses. This goal was accomplished via use of the desethyl raclopride derivative (**10**) as a substrate for [^{18}F]fluoroalkylation (Scheme III). In this way, acylation and monodemethylation preceded the fluoroalkylation step, and the radiosynthesis of [^{18}F]fluoroalkylated derivatives of raclopride was achieved by using two radiosynthetic steps: (a) production of the [^{18}F]fluoroiodoalkane reagent, and (b) [^{18}F]fluoroalkylation to generate the target radioligand. The synthesis of [^{18}F]fluoroiodoalkanes was readily accomplished via nucleophilic displacement of the triflic group of the respective iodoalkyl triflate **13** by no-carrier-added [^{18}F]fluoride resolubilized into acetonitrile with use of the base tetra(*n*-butyl)ammonium hydroxide.[50] Overall radiochemical yields of 5–10% were achieved, with final specific activities exceeding 1000 Ci/mmol. These radiochemical yields were not further optimized, as they were sufficient to carry out the in vivo experiments described herein.

In Vitro Binding Assays. The affinity of these N-alkylated and N-fluoroalkylated derivatives of raclopride for binding to dopaminergic D-2 receptor sites was determined via the in vitro displacement of [^{3}H]spiperone binding to primate striatal membranes. As shown in Table I, our experimental results for the receptor affinity of raclopride (K_i = 5.1–8.7 nM) were within the same order of magnitude as values previously reported for the in vitro binding of this benzamide to receptor sites in rat striata (K_i = 1.8–3.0 nM).[29] The slight difference in K_i values may be attributed in part to the fact that primate tissues were used in the receptor-binding experiments, whereas the earlier[29] data was based on experiments that employed striatal tissue from rodents. Although alterations in the magnitude of K_i may also arise from impurity (≤2%), D-prolinamide, and/or racemization during the synthetic sequences of Schemes I and II, these effects were probably minor as evidenced by the close similarity in the inhibition constants that were obtained for our synthesized raclopride (K_i = 8.7 nM) and for authentic raclopride from Astra Läkemedel AB (K_i = 5.1 nM). Thus, our synthetic procedure and evaluative in vitro receptor-binding technique can be considered to be internally consistent.

Perusal of the data in Table I allows for conclusions to be drawn concerning the effect of N-substituents on the affinity of the salicylamides for binding to cerebral D-2 receptors. The most noteworthy generalization that can be made is that the receptor affinities of N-alkylated analogues greatly exceeded those of the N-fluoroalkylated derivatives. Thus, whereas the N-alkylated benzamides were characterized by a K_i of ca. 5–40 nM, the corresponding N-fluoroalkylated raclopride structures had inhibition constants ranging from 90 to 160 nM.

A second remarkable characteristic of the data shown in Table I concerns the effect of the substituent chain length on the receptor-binding affinity of the benzamide ligands. For the case of the N-alkylated salicylamides, the inhibition constants increased as the chain length was made greater. Thus, the D-2 receptor-binding affinities decreased in the rank order $C_2H_5 > C_3H_7 > C_4H_9$ for compounds **1a–c**, respectively. This contrasts with the relationship between the length of the N-fluoroalkyl substituent chain and the inhibition constant K_i. For N-fluoroalkylated raclopride, the affinity for binding to dopaminergic D-2 receptors increased as $CH_2CH_2F \approx CH_2CH_2CH_2F < CH_2(CH_2)_2CH_2F$ for compounds **2a–c**, although the receptor affinity of the latter derivative was still low (K_i = 88.5 nM). Thus, there were distinct differences in the D-2 receptor recognition of this series of fluorinated and non-fluorinated salicylamides.

In Vivo Biodistribution Studies. In vivo biodistribution experiments were performed with use of no-carrier-added ^{18}F-labeled **15a–c** in rats, and the results tabulated in Table II corroborate with in vitro binding data of Table I. These in vivo experiments also give supplemental information concerning the kinetic and metabolic behavior of the test compounds. Upon examination of the results for the global brain and blood localization, it is seen in Table II that there was rapid clearance of **15a–c** from the central compartment. The relative brain-to-blood concentration ratio decreased over time for the benzamides from ca. 3–5 at 5 min postinjection to less than unity after 60 min. This rapid rate of washout for nonspecifically bound radioligand was anticipated from the rapid cerebral clearance kinetics of [^{11}C]raclopride in primates.[33-36] Also worthy of mention are the relative rates of brain extraction of the three salicylamides, which seems to be a function of compound lipophilicity. The in vivo penetration of the blood–brain barrier (BBB) occurred with the rank order **15a < 15b > 15c**, which differs from the relative lipophilicities **15a < 15b < 15c**. Similar parabolic relationships between brain uptake and lipophilicity have been noted for radiolabeled spiperone analogues[56] and were explained by the opposing effects of increased BBB penetration and increased binding to plasma protein and/or precipitation as the lipophilicity of the butyrophenone is enhanced.

(52) Satyamurthy, N.; Bida, G.; Barrio, J. R.; Luxen, A.; Mazziotta, J. C.; Huang, S. C.; Phelps, M. E. *Nucl. Med. Biol.* **1986**, *13*, 617.
(53) Kiesewetter, D. O.; Eckelman, W. C.; Cohen, R. M.; Finn, R. D.; Larson, S. M. *Appl. Radiat. Isot.* **1986**, *37*, 1181.
(54) Coenen, H. H.; Laufer, P.; Stöcklin, G.; Wienhard, K.; Pawlik, G.; Böcher-Schwarz, H. G.; Heiss, W. D. *Life Sci.* **1987**, *40*, 81.
(55) Shiue, C.-Y.; Bai, L.-Q.; Teng, R. R.; Wolf, A. P. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 55.
(56) Moerlein, S. M.; Laufer, P.; Stöcklin, G. *Int. J. Nucl. Med. Biol.* **1985**, *12*, 353.

These arguments may also apply to this series of atypical neuroleptics, on the basis of the relative log P values calculated for the fluoroalkyl substituents.

An important characteristic of radioligands to be employed for receptor-binding studies with PET is the degree to which metabolism of the tracer takes place within the imaging interval. This is because the in vivo biodistribution of labeled metabolites impacts on both image quality and kinetic modeling. An important metabolite for ^{18}F-labeled cerebral D-2 receptor-binding radiopharmaceuticals is [^{18}F]fluoride ion, because its great propensity[57] for localization as fluoroapatite in bone results in high skull sequestration of radioactivity and difficulty in the tomographic quantification of tracer kinetics in brain tissue. For the [^{18}F]salicylamides examined, in vivo defluorination took place to a significant degree over the 60-min interval. As indicated in Table II, the relative bone-to-blood radioactivity concentration ratios were about 1 at 5 min postinjection, but increased to values exceeding 10 after 60 min. Note that there was greater sequestration of radioactivity by the bone following administration of compound **15b**. This may be due to the catabolism of the N-ω-[^{18}F]fluoropropyl substituent to [^{18}F]fluoride rather than to the [^{18}F]fluoroorganic metabolites that are associated with N-ω-fluoroethyl or N-ω-fluorobutyl substituents.[19]

To evaluate the potential of these ^{18}F-labeled benzamides as D-2 receptor-based radiopharmaceuticals, it was necessary that the regional in vivo localization of the radioligands in cerebral tissue be examined. In this regard, the ratio R of radioactivity localized within the striatum relative to that in the cerebellum is an appropriate index for in vivo D-2 receptor-binding, since the rat striatum has a high density of dopaminergic receptors, whereas that in the cerebellum is negligible.[58] Thus, R is large for radioligands with high specificity for localizing to D-2 receptor-rich areas, and the value of R is near 1 for radiotracers in which non-receptor-specific localization predominates in vivo. Referral to the data in Table II indicates that for ^{18}F-labeled ligands **15a–c**, R ranged from 1.5 at 5 min postinjection to 1.8–1.9 after 60 min. This contrasts with the relative striatum-to-cerebellum ratio of 10 that is achieved by [^{18}F]spiperone under similar conditions.[59] Thus, the high in vivo specificity for central D-2 receptors that is characteristic of [^{11}C]raclopride in the primate[33-35] was not seen with these ^{18}F-labeled derivatives of the benzamide. It has been shown that in vivo D-2 receptor-specific localization of radioligands involves a complex interplay between receptor affinity and lipophilicity,[21] and it can be surmised that the low R values achieved by compounds **15a–c** is due primarily to suboptimal K_i values, since the rapid cerebral clearance rate (Table II) is not characteristic of highly lipophilic, nonspecifically bound radioligands.

Structure–Binding Relationships. Although the in vitro and in vivo avidity of these fluorinated salicylamides for binding to cerebral D-2 receptors was suboptimal for application as receptor-based PET radiopharmaceuticals, the results of this work give insight into the structure–binding relationships of the benzamides, as well as help to identify new target structures with potential for radiopharmaceutical development. Table I shows the impact of the pyrrolidino N-substituent on the D-2 receptor affinity. Previous workers have shown that in vitro binding of benzamides to D-2 receptors is influenced by the lipophilicity of aromatic substituents, and that the IC_{50} did not differ greatly when the pyrrolidine nitrogen bears an ethyl or propyl substituent.[23,26] Our results for the N-alkylated derivatives **1a–c** show similar trends, in which there was a slight increase in K_i as the log P of the substituent was increased. This decrease in affinity as the substituent was altered ($C_2H_5 > C_3H_7 > C_4H_9$) may be attributed to steric hindrance at the receptor site that is responsible for binding to this portion of the benzamide molecule. However, lipophilic and/or steric effects alone do not explain the structure–binding relationships of the N-fluoroalkylated analogues **2a–c**. In contrast to that of the N-alkylated derivatives, the D-2 affinity of N-fluoroalkylated salicylamides increased as the log P of the N-substituent was increased ($CH_2CH_2F \approx CH_2CH_2CH_2F > CH_2(CH_2)_2CH_2F$). Moreover, the K_i values for **1a–c** as a group were less than those for **2a–c**; this relatively low D-2 receptor affinity was even true for fluorinated benzamide **2c**, which has a substituent log P approximately equal to that of **1b**, but a much higher inhibition constant.

Structure–binding studies[27,60-62] indicate that D-2 receptor binding by benzamide ligands is centered on a six-membered pseudo-ring system formed by hydrogen bonding between the amide hydrogen and the oxygen atom of the aromatic methoxy substituent (Figure 1, ring a). This planar conformation is stabilized by ancillary hydrogen bonding between the aromatic hydroxyl group and the carbonyl oxygen atom (Figure 1, ring b).[62] The N-substituents of **1a–c** do not critically affect receptor affinity; the conformational flexibility of the side chain[62] apparently allows substitution with functionalities as large as the n-butyl group. Although one may envision hydrogen bonding by fluorine to create additional perturbative intramolecular effects in benzamides **2a–e**, this would require formation of an 8–10 membered ring which is less favorable than the 6-membered ring formed via hydrogen bonding between the amide hydrogen and methoxyl oxygen.

The influence of fluoroalkyl substitution may reside in its effect on ligand basicity or steric effects. For example, inductive effects of the fluorine atom on the basicity of the amine[69] would also decrease the receptor-binding affinities of compounds **2a–e** in the same rank order as shown in Table I. In addition, steric bulk may hinder binding of the ligand to D-2 sites. This may explain why compound **2c** displayed relatively low affinity for receptor binding even though hydrogen bonding and/or inductive effects by the fluorine atom are minimized. Steric effects probably also induce the in vitro binding results for compounds **2d** and **2e**. The N-fluoroalkyl substituents of these ligands have the same hydrogen bonding/inductive potential as compound **2b**, but in addition have steric bulk as great as for raclopride analogue **2c**. The combination of these perturbing effects (intramolecular hydrogen bonding, inductive/pK_a, steric bulk) thus resulted in salicylamide structures that were practically without affinity for cerebral D-2 receptors.

Conclusions

To summarize, in this work we have described the synthesis of new derivatives of the dopaminergic D-2 receptor-binding ligand raclopride in which various alkyl and

(57) Wallace-Durbin, P. *J. Dent. Res.* **1954**, *33*, 789.
(58) List, S. J.; Seeman, P. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2620.
(59) Welch, M. J.; Kilbourn, M. R.; Mathias, C. J.; Mintun, M. A.; Raichle, M. E. *Life Sci.* **1983**, *33*, 1687.
(60) van de Waterbeemd, H.; Testa, B. *J. Med. Chem.* **1983**, *26*, 203.
(61) Blaney, F. E.; Clark, M. S. G.; Gardner, D. V.; Hadley, M. S.; Middleton, D.; White, T. J. *J. Med. Chem.* **1983**, *26*, 1747.
(62) Högberg, T.; Norinder, U.; Rämsby, S.; Stenslund, B. *J. Pharm. Pharmacol.* **1987**, *39*, 787.

fluoroalkyl substituents were introduced onto the pyrrolidino nitrogen atom of the benzamide. The radiosynthesis of corresponding [^{18}F]fluoroalkylated analogues of raclopride was also accomplished in high specific activity. In vitro binding assays and in vivo biodistribution experiments indicated that these fluorinated salicylamides had D-2 receptor affinities that were diminished relative to the native raclopride structure. An explanation was presented for these results in which intramolecular hydrogen bonding, inductive effects, or steric bulk from the N-fluoroalkyl substituent perturbs interaction of the salicylamides with D-2 receptors. On the basis of these results, future development of fluorinated benzamides for use as receptor-based ^{18}F-labeled radiopharmaceuticals will target molecular entities in which fluorine is attached to aromatic sites that are metabolically stable, as well as unable to disrupt the receptor-active conformation or basicity of the salicylamides.

Experimental Section

1-Bromo-2-fluoroethane and 1-bromo-3-fluoropropane were purchased from Columbia Organic Chemical Co. (Camden, SC). (*S*)-Prolinamide was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other reagents were purchased from either Aldrich or Fisher Chemical Co. (St. Louis, MO). Tetrahydrofuran (THF) was distilled from sodium and benzophenone before use. Type 150Å (230–425 Mesh) silica gel was employed for flash column chromatography,[63] and the thin-layer chromatography plates (Chromatogram 13181) were purchased from Eastman Kodak (Rochester, NY). [^{3}H]Spiperone for in vitro binding assays was purchased with a specific activity of 77 Ci/mmol from NEN Research Products, Boston, MA, and was used without further examination of radiochemical purity or specific activity. Authentic raclopride was a gift from Astra Läkemedel AB, Södertälje, Sweden.

Melting point determinations were performed with use of open capillary tubes with an Electrothermal melting point apparatus (Gillete, NJ) and were uncorrected. ^{1}H NMR spectra were obtained on Varian XL 300 and Varian EM 360 spectrophotometers. ^{13}C NMR and two-dimensional spectra were obtained on a Varian XL 300 spectrophotometer at 75.4 MHz. Low-resolution mass spectra were obtained on a Finnegan 3200 VG-ZAB-3F GC–mass spectrometer and processed with a VG-11-250 data system. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

(*S*)-2-[(3,5-Dichloro-2,6-dimethoxybenzamido)methyl]-pyrrolidine (8). (*S*)-Prolinamide **3** was converted to the *N*-trityl derivative **4** and subsequently reduced to (*S*)-*N*-trityl prolinamine (**5**) by using lithium aluminum hydride.[25] On the basis of the same report, the protected amine **5** was benzoylated with use of acid chloride **6** to provide benzamide **7**, which was treated with hydrochloric acid to generate compound **8** in 66% yield, mp 142–143 °C (lit.[48] mp 145 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.2–1.4 (1 H, m), 1.5–1.8 (3 H, m), 2.6–2.8 (2 H, m), 3.0–3.5 (4 H, m), 3.8 (6 H, s), 7.2 (1 H, s), 7.4 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 25.5, 28.8, 44.0, 46.1, 57.6, 62.4, 123.6, 129.4, 130.9, 151.9, 163.7.

(*S*)-2-[(3,5-Dichloro-2,6-dimethoxybenzamido)methyl]-1-(2-fluoroethyl)pyrrolidine (9a). In a typical N-fluoroalkylation procedure, benzamide **8** (0.26 g, 0.79 mmol) was dissolved in anhydrous DMF (2 mL) and placed into a 5-mL Reacti-vial equipped with a magnetic stirring bar. $NaHCO_3$ (0.07 g, 0.82 mmol) and 1-bromo-2-fluoroethane (0.133 g, 0.95 mmol) were added, the vial was sealed, and the contents were stirred at 80–90 °C for 3 h. The reaction mixture was then cooled to room temperature, and the solvent was removed under reduced pressure to provide a semisolid product, which was purified by flash column chromatography (SiO_2, $MeOH/CH_2Cl_2$, 5:95) to provide white solid **9a** (0.163 g, 0.41 mmol, 52%). This material was used in the demethylation reactions described below without further purification, mp 122–124 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.6–2.0 (4 H, m), 2.3 (1 H, q), 2.5–2.7 (2 H, m), 3.0–3.3 (3 H, m), 3.7 (1 H, m), 3.8 (6 H, s), 4.5 (2 H, m, J = 47 Hz), 6.7 (1 H, br), 7.4 (1 H, s). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 22.9, 27.6, 41.0, 53.6 (J = 56.5 Hz), 54.5, 61.9, 62.4, 82.7 (J = 167.3 Hz), 123.3, 129.0, 130.6, 151.7, 163.4. MS (FAB, glycerol): m/e (relative intensity) 379 (M + 1, 58), 381 (M + 3, 38), 383 (M + 5, 8), 233 (75), 235 (45), 130 (20), 116 (100).

(*S*)-2-[(3,5-Dichloro-2,6-dimethoxybenzamido)methyl]-1-(3-fluoropropyl)pyrrolidine (9b). Oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.6–1.9 (6 H, m), 2.1 (1 H, m), 2.3 (1 H, m), 2.6 (1 H, br), 2.9 (1 H, m), 3.1 (1 H, br), 3.2 (1 H, complex doublet), 3.7 (1 H, m), 3.8 (6 H, s), 4.4 (2 H, m, J = 47 Hz), 6.4 (1 H, br), 7.3 (1 H, s). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 22.7, 27.8, 29.5 (J = 19.6 Hz), 40.8, 49.8, 53.8, 62.1, 62.5, 82.0 (J = 166.1 Hz), 122.5, 129.1, 130.8, 151.9, 163.5. MS (FAB, glycerol): m/e (relative intensity) 393 (M + 1, 52), 395 (M + 3, 34), 397 (M + 5, 8), 347 (7), 333 (25), 335 (20), 233 (91), 235 (48), 218 (18), 220 (14), 144 (14), 130 (20).

(*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-(3-fluoropropyl)pyrrolidine (2b). In a typical procedure for monodemethylation of aryl dimethoxy ethers, the *N*-(3-fluoropropyl)benzamide **9b** (0.16 g, 0.41 mmol) was dissolved in CH_2Cl_2 (50 mL) and hydrogen chloride gas was bubbled into the well-stirred solution for 7–10 min. The reaction mixture was cooled to 0 °C and 1.0 N BBr_3 (0.6 mL, 0.60 mmol) in CH_2Cl_2 was slowly added to the reaction solution. The reaction mixture was subsequently stirred at ambient temperature for 1 h. Aqueous NH_4OH (1.0 N) was added dropwise to the reaction mixture until it became slightly basic, and the organic products were extracted with CH_2Cl_2 (3 × 75 mL). The organic layer was dried (Na_2SO_4), and the solvents were removed under reduced pressure to provide a light-orange oil. This material was purified via flash column chromatography (SiO_2; $MeOH/CH_2Cl_2$, 3:97) to provide a light-yellow oil **2b** (0.14 g, 0.38 mmol, 93%). The product was converted into the HCl salt and recrystallized from an EtOH/hexane mixture to yield 0.15 g (88%) of **2b** as a white solid, mp (HCl salt) 134–136 °C. Free base **2b**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.5–2.1 (6 H, m), 2.2–2.4 (2 H, m), 2.7 (1 H, br), 2.9–3.0 (1 H, m), 3.1–3.4 (2 H, m), 3.8 (1 H, two sets of doublets), 3.9 (3 H, s), 4.5 (2 H, m, J = 48 Hz), 7.5 (1 H, s), 8.8 (1 H, br). Free base **2b**. ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 22.8, 28.1, 29.8 (J = 20.6 Hz), 40.4, 49.8 (J = 5.0 Hz), 53.9, 61.7, 62.4, 82.0 (J = 164.2 Hz), 109.0, 116.6, 119.1, 133.5, 153.6, 158.0, 168.5. MS (FAB, glycerol, HCl salt): m/e (relative intensity) 379 (M + 1, 63), 381 (M + 3, 48), 383 (M + 5, 18), 345 (24), 219 (14), 221 (9), 144 (19), 130 (100), 110 (10). Anal. ($C_{16}H_{21}O_3N_2Cl_2F$·HCl): C, H, N.

(*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-(2-fluoroethyl)pyrrolidine (2a), mp (HCl salt) 148–150 °C. Free base **2a**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.5–2.0 (4 H, m), 2.3–2.8 (4 H, m), 3.0–3.3 (2 H, m), 3.8 (1 H, two sets of doublets), 3.9 (3 H, s), 4.5 (2 H, m, J = 47 Hz), 7.4 (1 H, s), 8.8 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 23.0, 27.9, 40.4, 53.7 (J = 20.3 Hz), 54.6, 61.6, 62.2, 82.7 (J = 168.3 Hz), 109.4, 116.5, 118.9, 133.4, 153.5, 157.9, 168.4. MS (FAB, glycerol, HCl salt): m/e (relative intensity) 365 (M + 1, 98), 367 (M + 3, 54), 369 (M + 5, 10), 219 (23), 221 (18), 130 (20), 116 (100). Anal. ($C_{15}H_{19}O_3N_2Cl_2F$·HCl): C, H, N.

(*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-(4-fluorobutyl)pyrrolidine (2c), mp (HCl salt) 124–125 °C. Free base **2c**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.5–2.0 (8 H, m), 2.1–2.3 (2 H, m), 2.6–2.7 (2 H, m), 3.1–3.3 (2 H, m), 3.7 (1 H, q of doublets), 3.9 (3 H, s), 4.4 (2 H, two sets of triplets, J = 46 Hz), 7.4 (1 H, s), 8.8 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 22.6, 24.7 (J = 4.5 Hz), 28.1, 28.3 (J = 20.4 Hz), 40.3, 53.6, 53.7, 61.6, 62.1, 83.8 (J = 164.8 Hz), 109.5, 116.5, 118.7, 133.4, 153.4, 157.9, 168.4. MS (FAB, glycerol, HCl salt): m/e (relative intensity) 393 (M + 1, 54), 395 (M + 3, 23), 397 (M + 5, 7), 373 (11), 219 (18), 221 (13), 173 (10), 158 (16), 144 (100), 124 (20). Anal. ($C_{17}H_{23}O_3N_2Cl_2F$·HCl): C, H, N.

(2*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-[(*R*)-3-fluorobutyl]pyrrolidine (2d), mp (HCl salt) 169–171 °C dec. Free base **2d**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.9 (3 H, d), 1.5–2.3 (8 H, m), 2.4–2.6 (2 H, m), 3.1–3.3 (2 H, m), 3.8 (1 H, two sets of doublets), 3.9 (3 H, s), 4.2 (2 H, m, J = 48 Hz), 7.4 (1 H, s), 8.7 (1 H, s). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 13.9 (J = 6.6 Hz), 22.6, 27.6, 33.8 (J = 18.2 Hz), 40.0, 54.0, 56.7 (J = 6.6 Hz), 61.4, 62.7, 86.7 (J = 168.6 Hz), 109.2, 116.2, 118.6, 133.2, 153.2, 157.7, 168.2. MS (FAB, glycerol, HCl salt) m/e

(63) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(relative intensity) 393 (M + 1, 100), 395 (M + 3, 70), 397 (M + 5, 15), 219 (10), 221 (8), 158 (10), 144 (34). Anal. ($C_{17}H_{23}O_3N_2Cl_2F$·HCl): C, H, N.

(2*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-[(*S*)-3-fluorobutyl]pyrrolidine (2e), mp (HCl salt) 180–182 °C dec. ^{1}H NMR (CD_3OD, 60 MHz): δ 1.0 (3 H, m), 1.8–2.4 (7 H, m), 3.0–3.2 (2 H, m), 3.4–3.7 (4 H, m), 3.8 (3 H, s), 4.2 (2 H, m, J = 48 Hz), 7.4 (1 H, s). Anal. ($C_{17}H_{23}O_3N_2Cl_2F$·HCl): C, H, N.

(*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]pyrrolidine (10). The desethyl raclopride (10) was prepared from the dimethoxy analogue 8 as described elsewhere.[48]

(*S*)-*N*-Ethylprolinamide (11a). In a typical N-alkylation procedure, a solution of (*S*)-prolinamide (3) (0.701 g, 6.1 mmol) in anhydrous DMF (4.0 mL) was placed into a 5-mL Reacti-vial containing a magnetic stirring bar. Bromoethane (0.6 mL, 8.0 mmol) and $NaHCO_3$ (0.57 g, 6.8 mmol) were also added to the vial, which was then sealed tightly, and the reaction mixture heated to 90 °C for 3 h. The solvent was subsequently removed under reduced pressure to provide a solid residue that was washed with warm CH_2Cl_2 (150 mL). The organic solution was dried (Na_2SO_4), and the solvent was removed under reduced pressure to provide the alkylated product. This material was passed through a column of silica (CH_3OH/CH_2Cl_2, 1:99) and provided *N*-ethylprolinamide (11a) (0.554 g, 3.9 mmol, 64%), mp 220–222 °C. ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.0 (3 H, t), 1.7–2.0 (4 H, m), 2.1–3.2 (5 H, m), 7.2 (H, br). Anal. ($C_7H_{14}N_2O$): C, H, N.

(*S*)-*N*-Propylprolinamide (11b), mp (HCl salt) >320 °C. Free base. ^{1}H NMR (CD_3CN, 60 MHz): δ 1.0 (3 H, t), 1.7 (2 H, m), 2.0 (2 H, m), 2.2 (1 H, m), 2.6 (1 H, m), 3.1–3.3 (3 H, m), 3.7 (1 H, br), 4.3 (1 H, br). Anal. ($C_8H_{16}ON_2$·HCl): C, H, N.

(*S*)-*N*-Butylprolinamide (11c), mp 182–184 °C. ^{1}H NMR ($CDCl_3$, 60 MHz): δ 1.0 (3 H, t), 1.6 (2 H, m), 1.9 (4 H, m), 2.1 (1 H, m), 2.3 (1 H, m), 2.5–2.8 (3 H, m), 3.1 (1 H, br), 3.3 (1 H, br), 3.7 (2 H, br). Anal. ($C_9H_{18}N_2O$): C, H, N.

(*S*)-2-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-ethylpyrrolidine (1a, Raclopride). *N*-Ethylprolinamide (11a) was reduced with a solution of lithium aluminum hydride and benzoylated with acid chloride 6 as previously reported.[27] The dimethoxybenzamide was converted into raclopride (1a) via treatment with BBr_3, mp (tartrate salt) 143–146 °C dec (lit.[27] mp 141–142 °C). Free base. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.0 (3 H, t), 1.5–1.9 (5 H, m), 2.1 (2 H, m), 2.6 (1 H, br), 2.8 (1 H, m), 3.1–3.3 (2 H, m), 3.6 (1 H, two sets of doublets), 3.8 (3 H, s), 7.4 (1 H, s), 8.8 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 10.9, 22.5, 28.2, 40.4, 50.1, 53.5, 61.8, 65.2, 108.7, 116.4, 118.2, 133.0, 153.4, 157.2, 168.9. MS (FAB, glycerol): *m/e* (relative intensity) 347 (M + 1, 100), 349 (M + 3, 73), 351 (M + 5, 13), 219 (9), 221 (4), 112 (17), 98 (40). Anal. ($C_{15}H_{20}O_3N_2Cl_2$·HCl): C, H, N.

(*S*)-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-propylpyrrolidine (1b), mp (HCl salt) 127–130 °C dec. Free base. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.9 (3 H, t), 1.5–2.0 (7 H, m), 2.2 (2 H, m), 2.7 (2 H, m), 3.3 (2 H, m), 3.8 (1 H, two sets of doublets), 3.9 (3 H, s), 7.5 (1 H, s), 8.9 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 12.1, 22.1, 22.7, 28.2, 40.4, 53.9, 56.2, 61.7, 62.2, 109.6, 116.6, 119.0, 133.5, 153.6, 158.0, 168.5. MS (FAB glycerol): *m/e* (relative intensity) 361 (M + 1, 100), 363 (M + 3, 63), 365 (M + 5, 20), 219 (22), 221 (17), 126 (17), 112 (37). Anal. ($C_{16}H_{22}O_3N_2Cl_2$·HCl): C, H, N.

(*S*)-[(3,5-Dichloro-6-methoxy-2-hydroxybenzamido)-methyl]-1-butylpyrrolidine (1c), mp (HCl salt) 113–116 °C. Free base. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.9 (3 H, t), 1.3–1.9 (9 H, m), 2.1 (2 H, br), 2.5–2.7 (2 H, m), 3.1–3.3 (2 H, m), 3.7 (1 H, m), 3.8 (3 H, s), 7.3 (1 H, s), 8.8 (1 H, br). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 13.6, 20.3, 22.2, 27.6, 30.6, 39.8, 53.3, 56.9, 61.1, 61.7, 109.0, 115.9, 118.3, 132.8, 153.1, 157.6, 167.9. MS (FAB, glycerol): *m/e* (relative intensity) 375 (M + 1, 100), 377 (M + 3, 68), 379 (M + 5, 13), 219 (7), 221 (4), 154 (11), 140 (12), 126 (44). Anal. ($C_{17}H_{24}O_3N_2Cl_2$·HCl): C, H, N.

In Vitro Binding Assays. Brains from adult *Macaca nemestrina* (both sexes) were obtained from the Tissue Distribution Program of the Regional Primate Center at the University of Washington, Seattle, WA and stored at −70 °C. Prior to dissection, brains were warmed to −20 °C overnight. Brains were hemisected, cut coronally just in front of the temporal pole and then the caudate nuclei were dissected with the aid of a stereotaxic atlas.[64] Tissue samples were homogenized in 20 volumes (w/v) of ice-cold 50 mM potassium phosphate, pH 7.4. The homogenates were centrifuged for 30 minutes at 30000*g*. The resultant pellets were resuspended in the same buffer and recentrifuged. The pellets were then suspended in 10 mM potassium phosphate, pH 7.4, to a protein concentration of 3–5 mg/mL and stored in small aliquots at −70 °C.

The binding assays were performed in a final volume of 0.5 mL which contained 25–50 μg of protein in a buffer composed of 50 mM TRIS·HCl, 120 mM NaCl, 5 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4. The concentration of [^{3}H]spiperone (77 Ci/mmol) was 0.2 nM. After 30 min at 37 °C, the samples were filtered under reduced pressure on glass fiber filters (Whatman GF/A) which were then washed twice with 8 mL of ice-cold buffer. Nonspecific binding was measured in the presence of 1 μM *cis*-flupenthixol.[65] Tritium content of the filters was determined by liquid scintillation spectroscopy (Packard 1500 counter) not sooner than 4 h after addition of scintillation fluid. The efficiency of counting was about 45%, and each vial was individually corrected for quenching.

The amount of specific binding was the same when measured in the presence of either raclopride (1 μM) or *cis*-flupenthixol (1 μM) and represented 65–70% of total binding. The binding was potently inhibited by *cis*-flupenthixol (K_i = 0.5 nM) but not by *trans*-flupenthixol (K_i = 3 μM). There was no inhibition by ketanserin at concentrations lower than 100 nM. These results show that [^{3}H]spiperone was binding to D-2 receptors under these conditions.

All competition experiments were analyzed by computer-assisted least-squares nonlinear regression analysis with the equations given elsewhere.[66]

Radiochemistry. [^{18}F]Fluoride was produced with use of an enriched [^{18}O]water target and the ^{18}O(p,n)^{18}F nuclear reaction.[67] Iodoalkyl triflates 13a–c were prepared from the corresponding alcohols and triflic anhydride as previously described.[50] [^{18}F]-Fluoride (200–300 mCi) in 0.3–0.5 mL of aqueous solution and 5 μmoles of tetra(*n*-butyl)ammonium hydroxide were brought to dryness by azeotropic evaporation with acetonitrile and resolubilized into 200 μL tetrahydrofuran. The resolubilized [^{18}F]-fluoride was then reacted with 3–5 μmol of triflates 13a–c for 2 min at ambient temperature, and the resulting [^{18}F]fluoroalkyl iodides 14a–c were purified via passage through a short (50 mg) column of silica. These [^{18}F]fluoroalkyl iodides were then used to fluoroalkylate desethyl raclopride (10) by heating in 500 μL of dimethylformamide containing 5 mg of potassium carbonate at 100 °C for 30 min. The resulting *N*-(ω-[^{18}F]fluoro)alkylated salicylamides 15a–c were isolated with use of reversed-phase HPLC (Whatman Partisil M9 10/50 ODS-2; 70% acetonitrile/4 mM ammonium phosphate, pH 7.8; 5 mL/min). The eluted product fractions were brought to dryness on a rotary evaporator, reconstituted in lactate-buffered isotonic saline solution (pH 4.5), and sterile-filtered through a 0.22 μm Millex filter (Millipore Corp., Bedford, MA). The nonoptimized overall radiochemical yields were 5–10% following an overall preparation time of 2 h. The specific activity of the final products was determined by injection of an aliquot onto an HPLC column and comparison of the integrated sample UV signal with a calibrated mass/UV absorbance curve for the respective fluorinated salicylamide. The specific activity thus determined exceeded 1000 Ci/mmole at end of synthesis for compounds 15a–c.

In Vivo Biodistribution Studies. For the determination of the in vivo biodistribution behavior of compounds 15a–c, 50–100 μCi of each ^{18}F-labeled raclopride analogue in 0.2 mL buffered saline solution was injected into the femoral vein of 200–250-g, female, Sprague-Dawley rats under light ether anesthesia. Following intervals ranging in length from 5 to 60 min during which the animals were allowed free access to food and water, the rats

(64) Snider, R. S.; Lee, J. C. *A Stereotaxic Atlas of the Monkey Brain*; University of Chicago Press: Chicago, IL, 1961.
(65) Howlett, D. R.; Morris, H.; Nahorski, S. R. *Mol. Pharmacol.* **1979**, *15*, 506.
(66) Molinoff, P. B.; Wolfe, B. B.; Weiland, G. *Life Sci.* **1981**, *29*, 427.
(67) Kilbourn, M. R.; Jerabek, P. A.; Welch, M. J. *Int. J. Appl. Radiat. Isot.* **1985**, *27*, 235.

were sacrificed in groups of five animals. The brain of each animal was immediately excised, and the striatum and cerebellum were dissected over an ice-cold plate.[68] A blood sample was obtained via cardiac puncture immediately preceeding decapitation. The femur was also dissected from each test animal following sacrifice. The tissue samples were weighed, and the radioactivity content assayed by counting in a NaI(Tl) well-type γ scintillation counter. The results were calculated in terms of percent injected dose per gram of tissue by comparison of the data to that of a standard sample of the injectate.

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(68) Glowinsky, J.; Iverson, L. L. *J. Neurochem.* **1966**, *13*, 655.
(69) Henne, A. L.; Stewart, J. J. *J. Am. Chem. Soc.* **1955**, *77*, 1901.

A Novel Series of Selective Leukotriene Antagonists: Exploration and Optimization of the Acidic Region in 1,6-Disubstituted Indoles and Indazoles[1]

Ying K. Yee,* Peter R. Bernstein,*,‡ Edward J. Adams, Frederick J. Brown, Laura A. Cronk, Kevin C. Hebbel, Edward P. Vacek, Robert D. Krell,† and David W. Snyder†,§

Departments of Medicinal Chemistry and Pharmacology, ICI Pharmaceuticals Group, A Business Unit of ICI Americas Incorporated, Wilmington, Delaware 19897. *Received October 16, 1989*

A systematic structure–activity exploration of the carboxylic acid region in a series of indole- or indazole-derived leukotriene antagonists **1** led to several discoveries. Use of the 3-methoxy-*p*-tolyl fragment (illustrated in acid **1**) for connecting the indole and the acidic site provides the most potent carboxylic acids **1**, tetrazoles **20**, and aryl sulfonimides **21** (Figure 1). The aryl sulfonimides are 5–500 times more potent (in vitro and/or in vivo) than the corresponding carboxylic acids **1**. The *o*-tolyl sulfonimides such as **114** (Table VII) show greater oral potency than the phenyl sulfonimides at a given level of in vitro activity. Acidic keto sulfone derivatives **10** (Nu = $CH(CO_2CH_3)SO_2Ph$) mimic the activity of the sulfonimides.

The peptidoleukotrienes (LTC_4, LTD_4, and LTE_4) have been the focus of intense research efforts for the past 10 years,[2] following the discovery by Samuelsson,[3] that the leukotrienes were the active components of the "slow reacting substance of anaphylaxis" (SRS-A). SRS-A is believed to be an important biological mediator in several disorders, especially human allergic diseases. A major pharmaceutical goal has been the discovery and development of novel, selective antagonists of the leukotrienes as potential therapeutic agents for the treatment of asthma. An earlier paper from these laboratories described the discovery of a novel series of indole and indazole carboxylic acids **1** which are leukotriene antagonists.[4] Herein we describe the detailed exploration of the carboxylic acid region in this series of antagonists. These efforts led to novel aryl sulfonimides **21** which show increased potency both in vitro (pK_B up to 10.9 against LTE_4 on guinea pig trachea)[5] and in vivo (oral $ED_{50} < 1$ mg/kg against LTD_4 induced labored abdominal breathing in the guinea pig) (Figure 1).[5]

Chemistry

General Routes and Syntheses of the Aryl Acids 8. The general synthetic routes to the indole/indazole carboxylic acids **8**, sulfonimides **10** (Nu = $-NHSO_2R''$) and many of the acid mimics **3**[6] listed in Tables I, II, and III are presented in Schemes I and II. Our first route (1.a, Scheme I) began with 6-aminoindazole **2a** or with catalytic hydrogenation of 6-nitroindole **32b** to afford 6-aminoindole **2b**. The amines **2** were acylated by treatment either with a carboxylic acid or water soluble carbodiimide (WSCDI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride) and with an acid chloride and triethylamine.[7] These reactions provided amides and urethanes **3** which retained a reactive hydrogen at position N-1′ in the indole/indazole fragment. Therefore, compounds **3** were convenient intermediates for series of acids **8** in which only the acidic region was to be varied (e.g. Tables I and II).

Alkylation of the acylated intermediates **3** to afford esters **4** was achieved by sequential treatment with sodium hydride and halo-ester **7** in dimethylformamide.[8] The halo esters **7** were prepared by bromination of toluate esters.[9,10]

† Department of Pharmacology.
‡ To whom reprint requests should be addressed.
§ Present address: Eli Lilly and Co., Indianapolis, IN.

(1) Portions of this work have been previously presented: (a) Matassa, V. G.; Bernstein, P. R.; Brown, F. J.; Hesp, B.; Yee, Y. K.; Krell, R. D.; Giles, R. E.; Snyder, D. W. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Denver, CO, April, 1987; American Chemical Society: Washington, DC, 1987; Abstract 87. (b) Yee, Y. K.; Bernstein, P. R.; Brown, F. J.; Matassa, V. G. *Ibid.* Abstract 88.
(2) Chakrin, L. W., Bailey, D. M., Eds. *The Leukotrienes, Chemistry and Biology*; Academic: Orlando, FL, 1984.
(3) Hammarstrom, S.; Murphy, R. C.; Clark, D. A.; Mioskowski, C.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 1266.
(4) Brown, F. J.; Yee, Y. K.; Cronk, L. A.; Hebbel, K. C.; Snyder, D. W.; Krell, R. D. *J. Med. Chem.* **1990**, *33*, 1771.
(5) The biological tests are described in the Experimental Section of the text and have been discussed in refs 4 and 10.
(6) These synthetic sequences, where the halo ester **7** was methyl 4-(bromomethyl)-3-methoxybenzoate, have been described in ref 4.
(7) As exemplified in the Experimental Section in the preparation of tetrazole **80** by the conversion of 5-nitroindole (**32b**) to 6-aminoindole (**2b**) and its subsequent acylation to 6-hexanamidoindole (**3b**, R = pentyl).
(8) As exemplified in the Experimental Section under acid **42** by conversion of 6-hexanamidoindole (**3b**, R = pentyl) to ester **4b** (R = pentyl, C and D = hydrogen, CO_2R_1 is at position C-1 and R_1 = methyl).
(9) The syntheses of all the benzylic bromides utilized in this paper are described: (a) Bernstein, P. R.; Willard, A. K. U.S. Patent 4,499,299, 1985. (b) Brown, F. J.; Bernstein, P. R.; Yee, Y. K. European Patent Application, Publication Number 0179 619 A1, published April 30, 1986. (c) Reference 10.

0022-2623/90/1833-2437$02.50/0