already used compound 7 to mask out the A_1 component of [^{3}H]NECA binding in tissues such as dog or human brain where CPA does not appear to be sufficiently selective for this purpose.[24]

Separation by cyclodextrin HPLC of the individual diastereomers of 7 afforded compounds 8 and 9 (compound 9 eluted before 8). Compound 8 is the most potent (K_i = 0.24 nM) and selective (16 000-fold) agonist for the A_1 receptor to be reported to date. As anticipated, the other diastereomer (9) is less active and less selective for the A_1 receptor.

In summary, in this study we have identified novel N^6-[2.2.1]bicycloalkyladenosines with unusually high potency and selectivity for the adenosine A_1 receptor. Compounds 4, 7, and 8 should serve as important tools for further characterization of subpopulations of adenosine receptor subtypes in various tissues.

Acknowledgment. We thank James Fergus for [^{3}H]-CHA and [^{3}H](*S*)-ENBA binding data, Gina Lu for [^{3}H]NECA binding data, and Jon Hartman and Che Huang for purification of [^{3}H](*S*)-ENBA.

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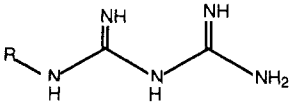
Perfluoro-*N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]-alkanamides. A New Class of Oral Antidiabetic Agents§

Sir:

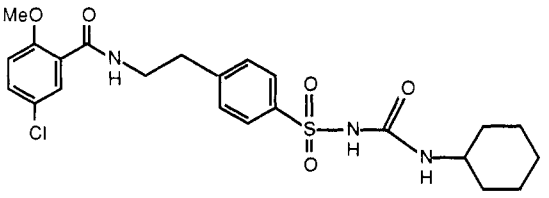
The withdrawal of the biguanides from the U.S. market in 1977 has left only one class of oral hypoglycemic agent, the sulfonylureas, for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) in this country.[1] Despite an improvement of 250-fold in potency over the last 33 years (e.g. glibenclamide), the sulfonylureas are still afflicted with the serious and sometimes fatal problem of drug-induced hypoglycemia,[2] apparently the result of hyperinsulinemia.

In 1982 Takeda revealed a potential alternative to the insulin-releasing sulfonylureas. Ciglitazone[3] represents a series of lipophilic benzylthiazolidinediones that lower plasma glucose in NIDDM but not insulin-dependent (IDDM) or nondiabetic animal models. Unlike insulin secretagogues such as glibenclamide, ciglitazone attenuates hyperinsulinemia and does not promote hypoglycemic action beyond normalization.[4] In addition, ciglitazone improves oral glucose tolerance (OGT) and effects positive changes in lipid metabolism. Recently, Takeda,[5] Sankyo,[6] Wyeth,[7] and Pfizer[8] have disclosed variants of the lipophilic portion of the thiazolidinediones.

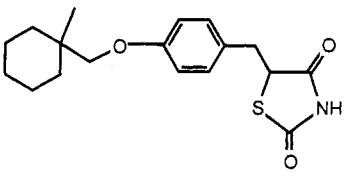


biguanides: R = Me; metformin

R = $Ph(CH_2)_2$; phenformin

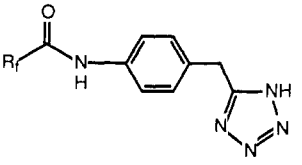


glibenclamide



ciglitazone

We report here the first example of a non-thiazolidinedione-containing oral antidiabetic series, perfluoro anilides 1, that possess a pharmacologic profile similar to that of ciglitazone in two genetic animal models of NIDDM: obese (ob/ob) and diabetic (db/db) mice.[9]



1 (R_f = C_1-C_{10} perfluoroalkyl)

The perfluorinated anilides 1 (**a**–**j**, Table I) were readily prepared in a two-step procedure (Scheme I). 4-Aminobenzyl cyanide (Aldrich) and the corresponding commercially available perfluoro acid or derivative were coupled to give the 4-cyanomethyl perfluoro anilides. Treatment of the nitriles with sodium azide and ammonium chloride in hot DMF gave tetrazoles 1.

Initially we found that perfluorobutyramide **1c** (Table I), like ciglitazone, normalized plasma glucose and insulin

§ This work was presented in part before the Division of Medicinal Chemistry, 196th National Meeting of the American Chemical Society, Los Angeles, CA, September 1988.

(1) The National Diabetes Data Group estimates that 90–95% of the ~5.8 million diagnosed diabetics in the USA are NIDDM; *Diabetes in America, 1985*, National Institutes of Health: Bethesda, MD.
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(9) Renold, A. E. *Adv. Metab. Disord.* **1968**, *3*, 49 and references therein.
(10) (a) Control (glucose (mg/dL); insulin (μunit/mL)), 194 ± 9, 139 ± 6; ciglitazone, 114 ± 9, 50 ± 5; **1c**, 117 ± 7, 48 ± 7. (b) At 300 mg/kg per day × 4 days; control, 178 ± 18, 206 ± 16; ciglitazone, 92 ± 8, 116 ± 18; **1c**, 90 ± 4, 61 ± 5.

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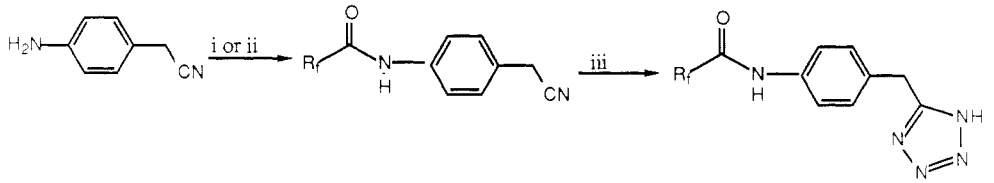
Table I. Effects of Perfluoro Anilides 1 on Plasma Glucose and Insulin in Obese Mice[a]

drug[b]	R_f	mp, °C	exp 1: 20 mg/kg plasma glucose, mg/dL	exp 1: 20 mg/kg plasma insulin, μunit/mL	exp 2: 75 mg/kg plasma glucose, mg/dL	exp 2: 75 mg/kg plasma insulin, μunit/mL
vehicle (0.5% methylcellulose)			256 ± 11	140 ± 15	191 ± 25	161 ± 11
reference (ciglitazone)			191 ± 20	94 ± 12*[c]	107 ± 19*	117 ± 15*
compd 1						
a	CF_3	203–206	273 ± 20	170 ± 20	138 ± 22*	102 ± 13*
b	C_2F_5	183–185 dec	198 ± 23	123 ± 15	120 ± 10*	77 ± 8*
c	C_3F_7	176–178	213 ± 23	181 ± 16	100 ± 4*	71 ± 7*
d	C_4F_9	173–174	207 ± 24	197 ± 14	170 ± 23	167 ± 13
e	C_6F_{13}	186–188 dec	231 ± 27	162 ± 5	162 ± 31	125 ± 14*
f	C_7F_{15}	193–194	111 ± 8*	55 ± 9*	87 ± 3*	28 ± 6*
g	C_8HF_{16}	187.5–190.5	108 ± 8*	65 ± 13*	93 ± 4*	29 ± 2*
h	C_8F_{17}	195–198 dec	128 ± 14*	82 ± 9*	106 ± 9*	45 ± 4*
i	C_9F_{19}	205–209 dec	133 ± 13*	144 ± 14	144 ± 24	107 ± 21
j	$C_{10}HF_{20}$	199.5–202	123 ± 13*	123 ± 12	118 ± 14*	76 ± 11*

[a] Compounds were adminstered at the indicated dose once daily po for 4 days. Blood samples were obtained on the fifth day. Data represent the mean ± SEM. The number of test animals in all cases was 8. [b] Analyses (C, H, N) were within ±0.4% of theoretical values. [c] (*) $p < 0.05$ when compared to vehicle control.

Scheme I



i) R_fCOCl or $(R_fCO)_2O$, EtN(<)$_2$, CH_2Cl_2, 0°C; ii) R_fCO_2H, EtO—≡—H, AcOEt, 85°C, 15h; iii) NaN_3, NH_4Cl, DMF, 135°C

levels in postprandial ob/ob mice after 2 days of oral administration (75 mg/kg per day[10a]). When the mice were dosed at either 75 mg/kg per day for 9 days or at 300 mg/kg per day for 4 days with these compounds, glucose and insulin lowering beyond normalization was not seen.[10b] Body weight and food and water intake were unchanged by drug administration. In the normal rat, neither **1c** nor ciglitazone changed glucose, insulin, or free fatty acid levels relative to control animals (data not shown). The favorable biological profile displayed by **1c** prompted us to seek more potent analogues by varying the perfluoroacyl chain length.[11]

The results obtained from subacute dosing with the series **1a–j** in the ob/ob mouse are given in Table I. Drug was administered orally once daily for 4 days. On the morning of the fifth day the mice were sacrificed by decapitation and plasma glucose and insulin levels were determined.

Inspection of the data in Table I reveals that potency in the series is related to the length of the perfluorinated chain. At a dose of 75 mg/kg significant glucose lowering activity is demonstrated by most members of the series.[12] The largest decrease in glucose and insulin occurred with the perfluorooctanoyl (C_8) and perfluorononanoyl (C_9) chains. Compounds **1f**, **1g**, and **1h** normalized the glucose and insulin levels in the ob/ob mouse. The superior profile of the C_8 and C_9 perfluoroacyl chains is evident when a lower dose (20 mg/kg, Table I) of anilides **1** was administered. Although the glucose-lowering activity of the C_{10} (**1i**) and C_{11} (**1j**) chains are not statistically different from that of **1f**, **1g**, and **1h**, the C_{10} and C_{11} chains do not attenuate the hyperinsulinemia of the ob/ob mouse.

Table II. Effects of Perfluoroanilides 1 on Plasma Glucose and Insulin in Diabetic Mice

compound	N[a]	dose,[b] mg/kg	days	plasma glucose, mg/dL	plasma insulin, μunit/mL
control	8		10	433 ± 15	16 ± 2
ciglitazone	9	20	10	320 ± 23*[c]	20 ± 3
1c	10	20	10	307 ± 23*	19 ± 2
1f	9	20	10	256 ± 37*	14 ± 1
control	9		4	354 ± 26	36 ± 8
ciglitazone	9	75	4	348 ± 34	25 ± 4
1f	9	75	4	163 ± 14*	32 ± 7
1g	9	75	4	124 ± 11*	34 ± 7

[a] N = number of animals. [b] Animals were given either drug or vehicle orally once daily for the indicated number of days. Data represent the mean ± SEM. [c] (*)$p < 0.05$ when compared to control.

OGT experiments conducted in the obese mouse have shown that perfluoro anilides **1f** and **1h** were effective in ameliorating an oral glucose load while **1c** was not. These data will be detailed elsewhere.§

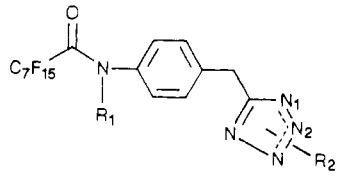
The effects of **1c**, **1f**, and **1g** on plasma glucose and insulin were also determined in the insulin-resistant diabetic (db/db) mouse. Experiments were performed in the manner described for the obese mouse. The results (Table II) demonstrate the greater efficacy of the longer chain perfluoro anilides **1f** and **1g** relative to ciglitazone in this model. In these experiments water-intake measurements indicated that the polydipsia normally observed with diabetic mice was significantly reduced by perfluoro anilide treatment. At 75 mg/kg, water intake was normalized by both **1f** and **1g**.

In order to establish the influence of the acidic proton(s)

(11) The corresponding hydrocarbon of **1c**, *N*-[4-(1*H*-tetrazol-5-ylmethyl)phenyl]butamide, was not active.

(12) Although not active in this screening result, compound **1d** has shown activity in other experiments, e.g., at 75 mg/kg per day × 4 days; control (glucose (mg/dL); insulin (μunit/mL)), 122 ± 13, 192 ± 12; ciglitazone, 78 ± 5, 54 ± 5; **1d**, 81 ± 9, 97 ± 16.

Table III. Effects of Perfluoro Anilides **2** on Plasma Glucose and Insulin in Obese Mice[a]



2

drug[b]	R_1	R_2	mp, °C	plasma glucose, mg/dL	plasma insulin, μunit/mL
vehicle (0.5% methylcellulose)				258 ± 28	232 ± 22
reference (ciglitazone)				107 ± 5*[c]	166 ± 7*
reference (**1f**; see Table I)				119 ± 4*	140 ± 15*
compd **2**					
a	H	$PhCH_2$-N_1	160-163	152 ± 8*	249 ± 12
b	H	$PhCH_2$-N_2	109-113	119 ± 4*	204 ± 9
c	Me	Me-N_1	80-82	119 ± 7*	204 ± 18
d	Me	Me-N_2	76-78	128 ± 11*	205 ± 18

[a] Compounds were administered at 20 mg/kg once daily po for 4 days. Data represent the mean ± SEM. [b] Analyses (C, H, N) were within ±0.4% of theoretical values. [c] (*) $p < 0.05$ when compared to vehicle control.

present in anilides **1** on their pharmacologic activity, tetrazole **1f** was selectively alkylated (benzyl bromide (1 equiv), K_2CO_3, acetone, 50 °C) to give a mixture (~1:1) of N_1- an N_2-alkylated tetrazoles (**2a** and **2b**, Table III), easily separable by chromatography. Reaction of **1f** with excess methyl iodide (K_2CO_3, acetone, reflux) similarly gave a mixture of dialkylated materials **2c** and **2d** (Table III).

Glucose lowering activity was retained by all the alkylated compounds (Table III) when tested in the ob/ob mouse at 20 mg/kg (once daily, 4 days, po). This result contrasts with the ciglitazone series, where alkylation of the thiazolidinedione ring abolishes hypoglycemic activity.[13]

Of further interest in Table III is that none of these alkylated materials effect insulin lowering after 4 days. These results suggest that either perfluoro anilides **1** lower plasma insulin and glucose levels by independent mechanisms[14] or that with **2** insulin lowering occurs secondarily and much slower than glucose lowering. Recently, the Upjohn group has reported that with ciglitazone administration in the ob/ob mouse, circulating insulin levels are decreased significantly prior to observed reductions in plasma glucose levels.[14] Since ciglitazone requires an acidic heterocycle for its pharmacological activity while perfluoroanilide **1f** does not, these compounds probably alter carbohydrate metabolism by different mechanisms or act at different sites. The results suggest, however, that like ciglitazone the perfluoro anilides **1** act by enhancing tissue responsiveness to insulin and that the glucose- and insulin-lowering activities may result from independent mechanisms.

Due to decreased food consumption induced by the C_9 chain in the normal rat, compounds **1c** and **1f** have been selected for evaluation in a chronic (45 days) study in genetically obese and diabetic mice. The results of these studies and further structure-activity relationships developed in the series will be the subject of a forthcoming paper.

Acknowledgment. We thank Bruce Hofmann (NMR), Marie Politowski (IR, CHN), Charles Kuhlman (HPLC), and their staffs for obtaining the analytical data. Paula Carabelli and Cheryl Delfino prepared the manuscript.

Supplementary Material Available: Experimental details for compounds **1a-j** and **2a-d** (6 pages). Ordering information is given on any current masthead page.

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Benzodiazepine Gastrin and Brain Cholecystokinin Receptor Ligands: L-365,260†

Sir:

The benzodiazepines assumed medicinal importance over 25 years ago with their discovery as anxiolytic, anticonvulsant, and sedative drugs.[1] These agents bind with high affinity to specific receptors in the brain, and numerous agonist, antagonist, and partial agonist structural variants have been developed. Evidence has been advanced that the endogenous benzodiazepine receptor ligands are peptidal in nature.[2] The scope of biological activity of benzodiazepines has been expanded in recent years with the discovery that certain substitutions on the core structure produce specific ligands for other peptide receptors. For example, the 2-substituted benzodiazepine tifluadom is a potent κ opiate agonist[3] and a lower affinity

† Dedicated to Professor Richard K. Hill with gratitude, admiration, and respect on the occasion of his 60th birthday.

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